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Original article

Activity, abundance and structure of ammonia-oxidizing microorganisms in plateau soils

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Abstract

Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) can be involved in biotransformation of ammonia to nitrite in soil ecosystems. However, the distribution of AOA and AOB in plateau soils and influential factors remain largely unclear. In the present study, the activity, abundance and structure of ammonia oxidizers in different soils on the Yunnan Plateau were assessed using potential nitrification rates (PNRs), quantitative PCR assay and clone library analysis, respectively. Wide variation was found in both AOA and AOB communities in plateau soils. PNRs showed a significant positive correlation with AOB abundance. Both were determined by the ratio of organic carbon to nitrogen (C/N) and total phosphorous (TP). AOB could play a more important role in ammonia oxidation. AOB community diversity was likely affected by soil total nitrogen (TN) and total organic carbon (TOC) and was usually higher than AOA community diversity. Moreover, *Nitrososphaera*- and *Nitrosospira*-like organisms, respectively, were the dominant AOA and AOB in plateau soils. AOA community structure was likely shaped by TP and C/N, while AOB community structure was determined by pH.
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Keywords: Nitrification; Acidic soil; *Nitrososphaera*; *Nitrosospira*; Environmental factor

1. Introduction

Soils usually shelter highly diverse bacterial communities which participate in a variety of biogeochemical processes [2,5,19,32]. Microbial transformation of ammonia to nitrite, as the first and rate limiting step in the nitrification process, is of fundamental importance for soil nitrogen cycling. Ammonia oxidation in soil ecosystems is known to be carried out by ammonia oxidizing archaea (AOA) and bacteria (AOB) that carry ammonia monooxygenase encoded by *amoA* gene [18,22,38,43]. The activity, abundance and structure of soil AOA and AOB can be shaped by a number of environmental variables, such as pH [7,20,21], fertility [3,21,40,41], heavy metal [13] and vegetation type [39,43,45]. However, thus far, how multiple factors collectively shape the distribution of soil AOA and AOB remains unclear.

Plateaus are known for their special environmental conditions (e.g. low atmospheric pressure, low oxygen content in air and strong ultraviolet exposure) which might have significant impact on soil microbes and can lead to a distinctive belowground microbial community [4,10,25,26]. A few previous studies have investigated ammonia-oxidizing microorganisms in meadow, forest and farmland soils on the Tibetan Plateau (China) [34,36,47] and in grassland soils on the Inner Mongolia Plateau (China) [3], while factors regulating the distribution of AOA and AOB in alpine soils are not well

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understood. The Yunnan Plateau (southwest China) is located in the subtropical monsoon climate zone and is warmer and has much more precipitation compared to the Tibetan Plateau and the Inner Mongolia Plateau. Thus far, only two recent studies reported a change in the abundance and structure of AOA and AOB communities in agricultural soils on the Yunnan Plateau [4,43], but those studies paid no attention to nitrifier activity. Moreover, information on AOA and AOB in unmanaged soil on this plateau is still lacking. Therefore, the objective of the current study was to investigate the activity, abundance and structure of AOA and AOB in plateau soils. Environmental factors shaping ammonia-oxidizing microorganisms were also investigated.

2. Materials and methods

2.1. Site description and sampling

Eight soil samples (0–5 cm depth) near the Baoxiang River, in triplicate, were collected in August 2014 (Fig. S1). The river is located in Kunming City (on the Yunnan Plateau). Soils R1, R2 and R3, planted with *Cryptomeria fortune*, *Poa annua* L. and *Tripogon bromoides*, respectively, were obtained from a protected reservoir region. Soils V1, V2 and V3, planted with *Eriobotrya japonica*, *Salix cavaleriei* and *Zea mays*, respectively, were collected from a village region. Moreover, soils T1 (*Vitisv inifera*) and T2 (*Brassica oleracea*) were collected from the transitional zone between the reservoir region and village region. The soils in agricultural sites had been linked to a long history of ammonia nitrogen fertilization, while those in the protected reservoir region had no previous exposure to fertilization. These eight soil samples were immediately transported to the laboratory and homogenized and subsampled for further analysis. Soil pH was measured with an IQ150 pH meter (IQ Scientific Instruments, Inc.). Soil total organic carbon (TOC) and total nitrogen (TN) were determined using an Elemental Analyzer (Vario EL III, Elementar, Germany). Total phosphorus (TP) was measured using the ascorbic acid-molybdate blue method [18] after 2 h of combustion (500 °C) and 16 h of extraction with 1 M HCl. Ammonium (NH_4^+–N) and nitrate (NO_3^-–N) contents were extracted from fresh soil samples with 2 M KCl and then determined using a continuous flow Analyzer (SAN++, Skalar, Holland). The geographic features and physicochemical characteristics (pH, TOC, TN, ratio of TOC to TN (C/N), NH_4^+–N, NO_3^-–N and TP) of soil samples are shown in Table 1.

2.2. Potential nitrification rate (PNR)

The PNR of each soil was assessed using the chlorate inhibition method [3,12]. Briefly, fresh soil (5 g) was added to 50-mL centrifuge tubes containing 20 mL of 1 mM phosphate buffer solution (PBS) and 1 mM $(NH_4)_2SO_4$. Potassium chlorate (10 mg L^{-1}) was amended to the tubes to inhibit nitrite oxidation. These tubes were incubated in the dark at 25 °C for 24 h and nitrite was extracted with 5 mL of 2 M KCl for analysis. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test was used to test the difference ($P < 0.05$) in the potential nitrification rates among different soils using software SPSS 20.

2.3. Molecular analyses

Soil genomic DNA was extracted using the Powersoil DNA extraction kit (Mobio Laboratories, USA). Quantitative PCR assay of archaeal and bacterial *amoA* genes was performed using the ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) based on the SYBR Green I method. The primer sets Arch-amoAF/Arch-amoAR and AmoA-1F/AmoA-2R were used for determination of the number of archaeal and bacterial *amoA* genes, respectively [14,39]. Standard curves ranging from 10^3 to 10^7 *amoA* gene copies mL^{-1} were obtained using serial dilutions of linearized plasmids. The average amplification efficiency and coefficient (r^2) for AOA and AOB *amoA* genes were 95% and 0.997, 94% and 0.998, respectively. One-way ANOVA followed by Student–Newman–Keuls test was applied to determine the difference ($P < 0.05$) in the abundance of the AOA or AOB *amoA* gene among different soils.

For construction of AOA and AOB clone libraries, the above-mentioned primer sets were also applied for amplification of archaeal and bacterial *amoA* genes [14,39]. The amplicons from each triplicate soil sample were mixed in equal amounts and then subjected to cloning. Chimera-free sequences were assigned to operational taxonomic units (OTUs) using a 97% similarity cutoff. The Shannon diversity index and rarefaction curve of each soil sample were generated using the MOTHUR program [28]. Phylogeny-based weighted UniFrac environmental clustering was applied to compare the

Table 1
Geographic and physicochemical features of soil sample.

Sample	Longitude	Latitude	Vegetation type	pH	TN (g kg^{-1})	TP (g kg^{-1})	TOC (g kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})	C/N
V1	102°51′36″	25°02′17″	*Eriobotrya japonica*	5.40 ± 0.05	1.41 ± 0.12	1.00 ± 0.06	12.81 ± 0.22	27.00 ± 0.18	4.59 ± 0.08	9.09 ± 0.13
V2	102°52′20″	25°02′41″	*Salix cavaleriei*	4.82 ± 0.31	2.8 ± 0.09	1.06 ± 0.08	18.80 ± 0.19	17.10 ± 0.27	5.75 ± 0.12	6.71 ± 0.11
V3	102°53′36″	25°02′48″	*Zea mays*	5.25 ± 0.12	1.53 ± 0.01	0.96 ± 0.08	11.63 ± 0.09	1.07 ± 0.07	4.61 ± 0.11	7.60 ± 0.06
T1	102°54′26″	25°02′52″	*Vitisv inifera*	5.01 ± 0.08	2.05 ± 0.12	0.87 ± 0.11	28.09 ± 0.15	25.30 ± 0.32	4.09 ± 0.05	13.72 ± 0.10
T2	102°54′39″	25°02′34″	*Brassica oleracea*	5.44 ± 0.13	0.71 ± 0.02	0.26 ± 0.03	15.72 ± 0.17	34.20 ± 0.27	3.20 ± 0.10	22.13 ± 0.23
R1	102°54′42″	25°01′37″	*Cryptomeriafortunei*	5.95 ± 0.14	2.84 ± 0.04	0.09 ± 0.01	41.31 ± 0.23	38.71 ± 0.23	7.75 ± 0.05	14.55 ± 0.21
R2	102°55′12″	25°01′14″	*Poa annua* L.	5.09 ± 0.20	1.80 ± 0.11	0.14 ± 0.02	33.70 ± 0.52	0.92 ± 0.02	4.11 ± 0.02	18.72 ± 0.09
R3	102°55′16″	25°01′26″	*Tripogon bromoides*	5.83 ± 0.06	1.34 ± 0.01	0.51 ± 0.04	23.85 ± 0.18	0.46 ± 0.01	3.79 ± 0.06	17.80 ± 0.05

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