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High pH-enhanced soil nitrification was associated with ammonia-oxidizing bacteria rather than archaea in acidic soils

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ABSTRACT

The integrated effects of environmental factors on soil nitrification are largely unknown. Here, we performed a microcosm experiment to investigate the interactive effects of pH and NH_4^+ on nitrification activity in two acidic soils with different land use patterns (Anhui soil, a forest soil; Jiangxi soil, a brush land soil). Both soils were incubated under native pH and $CaCO_3$-manipulated pH in the presence or absence of added ammonium for 60 days. The addition of $CaCO_3$ alone did not change the nitrification activity of either soil. Ammonium addition stimulated nitrification in Anhui soil, but not in Jiangxi soil, and this stimulation was more pronounced with increased $CaCO_3$. The ammonia monooxygenase (*amoA*) gene copy number of both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) was much higher in Anhui soil than in Jiangxi soil. The *amoA* gene copy number of AOB in Anhui soil was more highly upregulated under $CaCO_3 + NH_4^+$ than NH_4^+ during incubation. In Anhui soil, changes in the denaturing gradient gel electrophoresis (DGGE) fingerprint patterns of bacterial *amoA* genes were parallel to changes in the *amoA* gene copy number of AOB. In Jiangxi soil, DGGE could not be performed because the PCR for bacterial DGGE did not yield any products, while quantitative PCR revealed that the *amoA* gene copy number of AOB changed during incubation. These results suggest that AOB plays an important role in $CaCO_3$-enhanced nitrification of Anhui soil with ammonium addition. The low nitrification rates of Jiangxi soil regardless of $CaCO_3$ with or without NH_4^+ supply may be ascribed to the lower activity of both AOB and AOA, especially AOA.

1. Introduction

Soil nitrification, the oxidation of ammonia to nitrate via nitrite, is a critical step in the nitrogen cycle. The process is usually limited by the first step, ammonia oxidation to nitrite, which is thought to be driven by both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Increasing evidence has demonstrated the dominant role of archaeal ammonia oxidation of acidic soil ecosystems (Gubry-Rangin et al., 2010; Yao et al., 2011; Zhang et al., 2011). Although nitrification was found to be driven by archaea in an acidic forest peat soil in which net nitrification was high, addition of ammonium did not influence its nitrification (Stopnisek et al., 2010). In contrast, in nitrogen-rich grassland soils, nitrification was shown to be driven by bacteria, not archaea, and the rate of nitrification was found to be related to the abundance of AOB rather than AOA (Di et al., 2009). Application of ammonium to an alkaline agricultural soil only increased the abundance of AOB during nitrification (Jia and Conrad, 2009). However, it is still not known if AOA and AOB could be influenced by $CaCO_3$ and/or ammonium amendment in subtropical acidic soils.

Approximately 30% of the world's total land area consists of acidic soils (defined as $pH_{water} < 5.5$), and as much as 54% of the world's potentially arable lands are acidic (von Uexkull and Mutert, 1995). Additionally, great variation of nitrification in acidic soils has been reported (De Boer and Kowalchuk, 2001; Zhao et al., 2007; Nugroho et al., 2009; Zhao and Xing, 2009). Several factors are thought to contribute to such variations, including pH, substrate availability and microorganisms. However, the effects of these factors on nitrification vary. In a laboratory-scale experiment (up to 12 weeks), high pH, rather than N amendment, stimulated the growth of AOB and their associative nitrifying activity in an acidic forest soil (pH around 3.0), suggesting that the low nitrification rates in this acidic soil are due to pH-related factors rather than substrate availability (Nugroho et al., 2007). Another laboratory-based incubation experiment (up to 8 weeks) suggested that NH_4^+ addition stimulated nitrification in two acidic upland soils, but not in an acidic red forest soil based on the concentrations of NH_4^+–N and NO_3^-–N of soils (Zhao and Xing,

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2009). The authors suggested that the physicochemical and biological properties of the upland soils might have been improved by long-term cultivation, which facilitated microbial activity and therefore enhanced nitrification. $CaCO_3$, which is used to counteract acidification, is known to stimulate nitrification (Corre et al., 2003; Islam et al., 2006; Nugroho et al., 2007). Additionally, it has been suggested that AOA prefers low nutrient or low pH soil environments when compared with AOB (Wessén and Hallin, 2011; Hu et al., 2014). However, the exact mechanism of $CaCO_3$-enhanced soil nitrification, especially with respect to nitrifying communities, still remains largely unknown.

Acidic soils are usually characterized by poor fertility and constrained crop production because of the presence of toxic metals and nutrient deficiencies, which may also influence soil microbes including AOA and AOB. For instance, a 3-year field study (Mertens et al., 2009) revealed that bacteria, but not archaea, restored nitrification in a zinc-contaminated soil. Nevertheless, it is still unknown whether soil exchangeable Al can affect the nitrification activity and abundance of AOA and AOB.

In this study, we investigated the interactive effects of pH and NH_4^+ on the nitrification rate of two acidic soils subjected to different land use. To accomplish this, we evaluated the time-course dynamics of NH_4^+ and NO_3^-, soil pH and the *amoA* abundance of both AOA and AOB communities at the microcosm level.

2. Materials and methods

2.1. Soil samples

Two soil samples were collected from brushland and a forest underlain by quaternary red earth. The first site was located in Yingtan, Jiangxi Province, China (28°15′N, 116°55′E), and the second in Langxi, Anhui Province, China (31°04′N, 119°06′E). All soils were taken from the surface layer (0–20 cm), air-dried, ground to pass through a 2 mm sieve, and stored at 4 °C until use. Detailed descriptions of the two studied soils are shown in Table 1.

2.2. Experimental set-up

Soil nitrogen transformation was determined in a 60 day incubation study. Briefly, 50 g aliquots of air-dried soil from Jiangxi and Anhui were put in a series of 250 ml plastic bottles amended with 1.0 g $CaCO_3$ kg^{-1} dry soil. A control treatment without added $CaCO_3$ was also included. Deionized water was then added in a drop-wise manner over the soil surface with a pipette to bring the moisture content to 40% water-holding capacity (WHC), after which the samples were pre-incubated at 28 °C for 1 week in the dark. The ammonium sulfate ($(NH_4)_2SO_4$) solutions were subsequently applied uniformly to the pre-incubated soils at a rate of 250 mg N kg^{-1} dry soil. A control treatment without the addition of ammonium sulfate was also included, in which only deionized water was applied. The final soil moisture content was adjusted to 60% WHC. Four treatments were established, control (no addition), $CaCO_3$ (addition of 1 g $CaCO_3$ kg^{-1} soil), NH_4^+ (addition of 250 mg N kg^{-1}), and $CaCO_3 + NH_4^+$ (addition of 1 g $CaCO_3$ kg^{-1} soil and 250 mg N kg^{-1}). All the bottles were covered with hydrophobic fluorophore membrane (moisture permeability = 4300 g/m^2/24 h) and incubated at 28 °C in the dark. The moisture content of incubated soil was maintained by the addition of deionized water every 3 or 4 days to compensate for water loss through evaporation. Bottles were destructively sampled on day 2, 15, 30, 45, and 60. Three bottles were sampled per treatment and per sampling occasion, at which time 2.0 g of fresh soil was removed from each sample and immediately stored at −20 °C for molecular analysis. The rest of the soil was analyzed for pH, NH_4^+–N, NO_3^-–N, and exchangeable Al and Ca.

2.3. Soil chemical analysis

Soil pH was measured using a pH meter (Model 868, Thermo Orion, USA) after mixing samples with deionized water (soil/water, 1:2.5). Inorganic N (NH_4^+–N and NO_3^-–N) was extracted with 2 M KCl (10:1 solution:soil ratio) by shaking at 200 rpm for 1 h at room temperature. The soil suspensions were then filtered, and the filtered soil extracts were analyzed within 2 days. NH_4^+–N concentrations were measured using the indophenol blue method, while the colorimetric method was used to determine the NO_3^-–N levels based on the difference between the absorbance at 220 and 275 nm (Keeney and Nelson, 1982). To measure the exchangeable Al and Ca, soil samples (0.3 g) were mixed with 15 ml of 1 M KCl and then placed on a shaker at 29 °C for 1 h. The suspensions were then centrifuged at 5000 rpm for 15 min, after which the supernatants were filtered and diluted as necessary for analysis of the exchangeable Al and Ca using inductively coupled plasma atomic emission spectroscopy (Thermo Elemental, Thermo Fisher, Waltham, MA, USA) (Chen et al., 2010).

2.4. Soil DNA extraction

DNA was extracted from soil samples (0.5 g) using a FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The extracted DNA was purified using a PowerClean® DNA Clean-Up Kit (MO BIO, California, USA) as described by the manufacturer. The quality and quantity of DNA were checked using a NanoDrop® ND-1000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.5. Quantitative polymerase chain reaction analysis of amoA gene abundance

The abundance of archaeal *amoA* genes was determined with a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA) using the Arch-amoAF (5′-STAATGGTCTGGCTTAGACG-3′) and Arch-amoAR (5′-GCGGCCATCCATCTGTATGT-3′) primers (Francis et al., 2005). Each reaction was conducted in a 20 μl master mix composed of 10 μl SYBR Premix Ex Taq (Takara Biotechnology, Otsu, Shiga, Japan), 0.2 μl of each primer (20 μM), 1 μl of DNA template (8.6–24.8 ng) and 8.6 μl H_2O. Amplification was conducted using the following thermal program: 95 °C for 30 s, followed by 39 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s, each of which was followed by plate reads at 80 °C. Bacterial *amoA* genes were quantified using

Table 1
Soil properties of Jiangxi and Anhui soils. The mean ± standard deviation of pH, organic matter, total nitrogen (N), available nitrogen, available phosphorus, available potassium, exchangeable aluminium (Al), and exchangeable calcium (Ca) were indicated. Except pH and EC, other soil properties were expressed in soil dry weight. Values within the same column followed by the same letter do not differ at $p < 0.05$.

Site	pH	EC ($\mu s\ cm^{-1}$)	Organic matter ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Available nitrogen ($mg\ kg^{-1}$)	Available phosphorus ($mg\ kg^{-1}$)	Available potassium ($mg\ kg^{-1}$)	Exchangeable Al ($mg\ kg^{-1}$)	Exchangeable Ca ($mg\ kg^{-1}$)
Jiangxi	4.46 ± 0.03a	33.73 ± 3.57b	11.38 ± 0.21b	0.98 ± 0.18a	67.09 ± 2.00b	0.21 ± 0.00b	32.49 ± 2.50b	320.78 ± 20.64a	125.63 ± 43.26b
Anhui	4.64 ± 0.02a	60.23 ± 5.43a	18.24 ± 0.72a	1.04 ± 0.07a	146.53 ± 21.45a	4.98 ± 0.20a	129.98 ± 3.57a	294.92 ± 6.14a	500.19 ± 52.79a