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Responses of nitrification and ammonia oxidizers to a range of background and adjusted pH in purple soils

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ABSTRACT

Soil pH is often changed by anthropogenic activities such as agronomic management, land use or acidifying pollution, and is widely considered to be a dominant factor affecting soil nitrogen cycling. In this study, three purple soils originating from similar parent materials but varying in pH (5.7, 7.3, and 8.0), representing acidic, neutral and alkaline soils, were selected to determine the effect of background pH on net nitrification rate (NNR) and ammonia oxidizers (AOA and AOB). The background pH of each soil was modified to the pH of the other soils to investigate the effect of adjusted pH on NNR and ammonia oxidizers. Net nitrification rates varied significantly with adjusted pH in neutral soils but did not change in acidic and alkaline soils, suggesting that soil at neutral pH is more sensitive to changes in nitrification. The AOB abundance increased in neutral soils adjusted to high pH, whereas AOA decreased with increased pH in acidic and neutral soils, which indicated that the activity and abundance of AOA and AOB is the more important factor affecting nitrification in neutral soils. The ratios of AOA to AOB in the unmodified acidic, neutral and alkaline soils were 120, 1.55 and 0.07, respectively. The highest AOA and AOB abundances occurred in unmodified pH neutral soil. However, the highest NNR was found in alkaline soils (7.04 mg N kg^{-1} dry soil d^{-1}), which was significantly higher than that in neutral and acidic soils (2.31 and − 0.23 mg N kg^{-1} dry soil d^{-1}, respectively). These results indicate that substrate competition between AOA and AOB exists in neutral soils, which can provide insight and improve our understanding of microbial regulation of N cycling in terrestrial ecosystems.

1. Introduction

Soil pH can dramatically influence soil nitrification (Strayer et al., 1981; Cheng et al., 2013). For example, it has been reported that raising pH in soils with a range of 4.8–8.5 significantly stimulated soil nitrification in field studies (Ste-Marie and Pare, 1999) and microcosm incubations (Cheng et al., 2013). As soil pH increases it approaches the pKa of ammonium resulting in an increase in ammonia availability, the substrate for nitrification (Burton and Prosser, 2001). Increasing evidence shows that soil pH plays an essential role in shaping the active ammonia oxidizer communities of ammonia-oxidizing archaea and bacteria (AOA and AOB) in distinct soils (Nicol et al., 2008; Lehtovirta-Morley et al., 2009; Hu et al., 2014; Jiang et al., 2015; Xi et al., 2017). Ammonia-oxidizing archaea and bacteria likely occupy different soil niches: AOA dominates nitrification activity in acidic soils while AOB dominates in alkaline soils (Stopnišek et al., 2010; Gubry-Rangin et al., 2011; Taylor et al., 2012; Ai et al., 2013; Jiang et al., 2015).

Soil management and anthropogenic activities such as fertilization, lime application, and alkaline or acidifying pollution (Kemmitt et al., 2006; Nugroho et al., 2007), can change soil pH and therefore influence soil nitrification processes (Bäckman et al., 2003; Nugroho et al., 2007; Yao et al., 2011). Previous studies have found that increasing soil pH through liming enhances the net nitrification rate (NNR) in acid forest soils (Carnol et al., 2002; Bäckman et al., 2003; Nugroho et al., 2007). However, lime application did not enhance nitrification activity in a highly acid tea plantation soil (Hayatsu and Kosuge, 1993). Yao et al. (2011) also reported that lime addition significantly decreased NNR in an acidic soil. These contrasting results on the effect of liming acidic soil on NNR indicate that other factors besides substrate availability are likely involved in influencing the nitrification process.

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Soil pH is widely accepted as one of the dominant factors that regulates soil microbial diversity and composition (Zhalnina et al., 2015). The abundance and community composition of active ammonia oxidizers may account for the discrepancies in nitrification in different soils (Alves et al., 2013; Xiao et al., 2017). The abundance and diversity of particular groups of ammonia oxidizers vary in soils with different background pH (Shen et al., 2012). We hypothesized that the NNR and ammonia oxidizers in soils with different background pH values would respond differently to adjusted pH. In this study, three purple soils originating from similar parent materials (purple sandy mudstone) but with different background pH (ranging from acid (pH 5.7) to alkaline (8.0)) were used to investigate how soil background pH affects NNR and different ammonia oxidizers. We were also interested in determining the effect of adjusting the background pH of these soils from acid to alkaline to investigate adjusted pH effects.

2. Materials and methods

2.1. Site description and soil collection

Purple soils (Eutric Regosol, FAO/UNESCO, 1988) are developed from purple rocks with intense eluviation of geologic materials dating to the Trias-Cretaceous. They are characterized by featureless pedogenic horizons mainly distributed in the Sichuan Basin of southwest China, which produce about 10% of China's feed and food production for its 1.36 billion citizens. Three purple soils developed from similar parent materials have different background pH values (acidic, pH = 5.7; neutral, pH = 7.3; alkaline, pH = 8.0) as a result of long-term differences in vegetation and topography. The acidic and neutral soils were collected from Yongchuan (29°11′ N and 105°47′ E, 582 m altitude, and 29°23′ N and 105°59′ E, 387 m altitude, respectively), Chongqing, in southwest China. The site has a subtropical monsoon climate with an average annual temperature of 19.7 °C and a mean annual precipitation of 1400 mm. The acidic soils are planted with pear (*Pyrus* spp.) trees. The neutral soil are planted with maize (*Zea mays* L.) and intercropped with sweet potato (*Ipomoea batatas* L.). The alkaline soil was collected from the Yanting Agro-Ecological Station of Purple Soil (31°16′ N, 105°28′ E, 470 m altitude), Sichuan, in southwestern China. The site has an altitude of 400–600 m a.s.l., and a subtropical climate with an annual mean temperature of 17.3 °C and a mean annual precipitation of 826 mm. The crops in the alkaline soil were the same as the neutral soil. Soil properties at each site are listed in Table 1.

At each soil site, three plots were selected randomly and were treated as three replicates of each soil. The size of each plot was 4 m × 5 m and the distance between each plot was 20 m. In each plot, one composite soil sample was collected by taking five soil cores from 0 to 20 cm in depth using a 13-cm diameter soil auger and mixing these soil cores thoroughly. The composite soil samples were then air-dried. For each composite soil sample, a portion of it was sieved to pass a 1 mm mesh for chemical analysis and the remaining was passed through a 2 mm mesh for laboratory incubations.

2.2. Preparation of pH adjusted soils

For each sieved composite sample, 500 g of dry soil was added to a 1-L pot for a total of three pots. One pot served as a control (without chemical addition), named the natural pH treatment. The other two pots received either 1 M H_2SO_4 or NaOH solutions to adjust the soil pH value to span the range of pH found in the other soils. A pipette was used for adding solutions to ensure a uniform distribution, and all the soil samples were adjusted with distilled water to 30% gravimetric moisture (equals to 60% of water-holding capacity). All the pots were kept open and in the dark at 28 °C for 10 d. Each pot was supplemented with distilled water to compensate for the loss of water during the incubation, and soil pH in each pot was monitored and readjusted once every two days. After the pre-incubation, soil pH in all the treatments reached equilibrium and remained stable: the acidic soils included the background pH (designated as Acidic-acidic) and two-pH adjusted treatments (Acidic-neutral and Acidic-basic); the neutral soils included the background pH treatment (Neutral-neutral) and two pH-adjusted treatments (Neutral-acidic and Neutral-basic); the alkaline soils included the background pH treatment (Basic-basic) and two pH-adjusted treatments (Basic-acidic and Basic-neutral).

2.3. Incubation

Each pH treated soil was weighed (20 g dry mass) into a 150-ml flask (in total there were 18 flasks for each treatment, comprising three replications and six sampling events). One milliliter of $(NH_4)_2SO_4$ solution (112 mg N kg^{-1} dry soil) was added to each flask at day 0 and soil moisture in each flask was adjusted to 60% of water-holding capacity using distilled water. All treatment flasks were incubated at 28 °C under dark and aerobic conditions for 9 days. The flasks were weighed every other day and distilled water was added to keep soil moisture constant during the entire incubation time.

2.4. Soil chemical analyses and NNR calculation

On days 0, 1, 3, 5, 7 and 9, all the treatments were sampled for ammonium and nitrate content using colorimetric methods on a SKLAR continuous-flow analyzer (SKLAR San++, Netherland, 2003). Soil organic matter was determined using acid-dichromate wet oxidation method (Nelson and Sommers, 1996) and total nitrogen was determined by micro-Kjeldahl (Bremner, 1996). Net nitrification rate (mg N kg^{-1} dry soil d^{-1}) in each treatment was calculated as the difference between the final and initial NO_3^--N contents divided by the incubation time (Robertson et al., 1999).

2.5. DNA extraction and q-PCR assay

From 3 of the 6 sampling dates (0, 5 and 9 d), 0.50 g fresh soil was removed from each incubation flask to extract DNA with the FastDNA Spin Kit (MP Biomedicals, United States). The DNA extracts were stored at −20 °C until the follow-up real-time q-PCR was performed on a Bio-Rad CFX-96 thermal cycler (Bio-Rad, Inc., Hercules, CA, USA) with SYBR Premix Ex Taq™ (Takara Bio, Otsu, Shiga, Japan). The q-PCR was conducted in biological triplicates with three analytical replicates. Bacterial and archaeal *amoA* genes were amplified by primer pairs amoA-1F/amoA-2R (Rotthauwe et al., 1997) and Arch-amoAF/Arch-amoAR (Francis et al., 2005), respectively. Standard curves and q-PCR

Table 1
Physical and chemical properties of the three original soils.

Soil type	pH 1:2.5 in H_2O	SOM (g kg^{-1})	TN (g kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	C/N
Acidic soil	5.7	24.82 ± 0.32 a	1.51 ± 0.02 a	14.95 ± 1.63 a	2.18 ± 0.60 b	9.55 ± 0.06 a
Neutral soil	7.4	12.97 ± 0.28 b	0.98 ± 0.03 b	1.18 ± 0.74 b	3.16 ± 0.69 b	7.69 ± 0.09 b
Alkaline soil	8.1	8.56 ± 0.22 c	0.65 ± 0.02 c	4.06 ± 1.32 b	6.49 ± 0.86 a	7.69 ± 0.02 b

SOM soil organic matter; TN total N.
Mean values (n = 3) within each column with the different letter are significantly different, $P < 0.05$.