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**Letter to the Editor**

# Autotrophic and Heterotrophic Nitrification in a Highly Acidic Subtropical Pine Forest Soil

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## ABSTRACT

The occurrence of nitrification in some acidic forest soils is still a subject of debate. Identification of main nitrification pathways in acidic forest soils is still largely unknown. Acidic yellow soil (Oxisol) samples were selected to test whether nitrification can occur or not in acidic subtropical pine forest ecosystems. Relative contributions of autotrophs and heterotrophs to nitrification were studied by adding selective nitrification inhibitor nitrapyrin. Soil $NH_4^+$-N concentrations decreased, but $NO_3^-$-N concentrations increased significantly for the no-nitrapyrin control during the first week of incubation, indicating that nitrification did occur in the acidic subtropical soil. The calculated net nitrification rate was 0.49 mg N $kg^{-1}$ $d^{-1}$ for the no-nitrapyrin control during the first week of incubation. Nitrapyrin amendment resulted in a significant reduction of $NO_3^-$-N concentration. Autotrophic nitrification rate averaged 0.28 mg N $kg^{-1}$ $d^{-1}$ and the heterotrophic nitrification rate was 0.21 mg N $kg^{-1}$ $d^{-1}$ in the first week. Ammonia-oxidizing bacteria (AOB) abundance increased slightly during incubation, but nitrapyrin amendment significantly decreased AOB *amoA* gene copy numbers by about 80%. However, the ammonia-oxidizing archaea (AOA) abundance showed significant increases only in the last 2 weeks of incubation and it was also decreased by nitrapyrin amendment. Our results indicated that nitrification did occur in the present acidic subtropical pine forest soil, and autotrophic nitrification was the main nitrification pathway. Both AOA and AOB were the active biotic agents responsible for autotrophic nitrification in the acidic subtropical pine forest soil.

*Key Words*: acidic yellow soil, ammonia-oxidizing archaea, ammonia-oxidizing bacteria, net nitrification rate, nitrapyrin, nitrification inhibitor

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Although low pH does not seem to exclude nitrification in soils, there are many acid soils for which nitrification appears to be absent (Robertson, 1982). Incubation studies have revealed that nitrification occurs readily in some acid pine forest soils, but slowly in others (Nugroho *et al.*, 2005, 2007). In environments unfavorable for autotrophic nitrifying bacteria, nitrification may result from the activity of heterotrophic microorganisms (Brierley and Wood, 2001) since ammonia-oxidizing bacteria (AOB) are poor competitors for $NH_4^+$ relative to ammonia-assimilating heterotrophs when $NH_4^+$ is limited (van Niel *et al.*, 1993; Verhagen *et al.*, 1995). Thus, active nitrification under acid conditions has been suggested as an evidence of heterotrophic nitrification (Papen and von Berg, 1998). However, it has been suggested that acid-tolerant autotrophs could also be responsible for nitrification in such soils (De Boer and Kowalchuk, 2001). Previous studies (Laverman *et al.*, 2000; Nugroho *et al.*, 2005) applying specific inhibitors of autotrophic nitrification have revealed that heterotrophic nitrification did not play a significant role in acid Scots pine forest soils. However, it was also reported that heterotrophic nitrification dominated over autotrophic nitrification in coniferous acid forest soil in subtropical region in China (Zhang *et al.*, 2011). Additionally, heterotrophic nitrifiers can use both inorganic and organic substrates for nitrification (Honda *et al.*, 1998; De Boer and Kowalchuk, 2001), and some heterotrophic nitrifiers can denitrify simultaneously, resulting in little or no nitrite accumulation (Matheson *et al.*, 2003).

Soils in subtropical and tropical forests are generally characterized by rapid N cycling rates and high N availability (Zhang *et al.*, 2013), while most temperate ecosystems are N-limited (Krusche *et al.*, 2003; Chen *et al.*, 2004). Forest soils in these regions are highly acidic

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(pH < 5) (Xu and Cai, 2007) and are assumed to have little capacity for the microbiological oxidation of $NH_4^+$ to $NO_3^-$ (Zhao *et al.*, 2007), but occurrence and stimulation of nitrification in specific subtropical acidic soils remain unclear.

While microbial autotrophic nitrification have been thought to be performed by AOB and only bacteria possess the *amoA* gene for the ammonia monooxygenase (AMO), recent discoveries have suggested that ammonia-oxidizing archaea (AOA) may contribute significantly to nitrification under acidic soil conditions (Prosser and Nicol, 2008). In a forest ecosystem, the AOB and AOA varied from $0.32 \times 10^7$ to $8 \times 10^7$ and $0.66 \times 10^4$ to $6.31 \times 10^4$ copies $g^{-1}$ dry soil, respectively (Long *et al.*, 2012), whereas they varied from $2 \times 10^4$ to $4 \times 10^6$ and $1 \times 10^4$ to $3.4 \times 10^7$ copies $g^{-1}$ dry soil, respectively, in another forest ecosystem (Stempfhuber *et al.*, 2015). These variations imply that the relative importance of AOB and AOA to nitrification may vary depending up environmental and specific site conditions (Erguder *et al.*, 2009; Wessén *et al.*, 2010), which actually widens the debate and increases the need to determine the predominant nitrification pathway for given ecosystems.

Research from different ecosystems is needed to elucidate the mechanisms responsible for nitrification under a variety of environmental and soil conditions. Accordingly, one approach to study the importance of nitrification in soils is the use of chemical inhibitors, which disrupt the first step of the nitrification reaction (conversion of ammonia to nitrite). Nitrapyrin inhibits AMO activities by acting as an alternative substrate (Iizumi *et al.*, 1998), but does not appear to directly affect other microbially-mediated processes of the N cycle (Sahrawat, 1989). Therefore, this study aimed at assessing soil nitrification rates with and without inhibitor to elucidate the occurrence of nitrification and the dominant pathway responsible for nitrification in a highly acidic subtropical forest soil in the southwestern region of China.

## MATERIALS AND METHODS

### *Site description and soil sampling*

Soil samples of a pine forest soil (Oxisol) derived from sandstone were collected in the Jinyun Mountain, Chongqing, China. In this region, the annual temperature ranges from 14 to 19 °C and rainfall from 1 000 to 1 400 mm. The mountain is mainly covered with middle subtropical evergreen broad-leaved forests, together with other vegetation types such as warm coniferous forests, bamboo forests, and evergreen broad-leaved shrubs (Jiang *et al.*, 2011).

Five plots, comprising of pine stands, were established at the study site. Each plot had a dimension of 4 m × 4 m, with a distance of 20 m between the plots. Five soil cores (0–20 cm) were collected from each plot using a soil corer with a diameter of 13 cm. A portion of the soil samples were pooled and homogenized to reduce heterogeneity, ground to pass a 1-mm sieve and used for chemical analyses. The remaining samples were air-dried to pass a 2-mm sieve and separated into two parts for incubation. The soil was sand-loam, with a clay content of 147 g $kg^{-1}$. The clay minerals were kaolinite, illite, and oxidate. Selected soil properties were previously analyzed: cation exchange capacity (CEC), 8.2 cmol $kg^{-1}$; pH ($H_2O$), 5.0; organic C, 22.4 g $kg^{-1}$; total N, 1.7 g $kg^{-1}$.

### *Incubation and analyses*

For each sample, 200 g soil was pre-incubated in a 500-ml beaker for 1 Week, maintaining at 60% water-holding capacity. Immediately after the pre-incubation, pH and nitrate and ammonium concentrations were analyzed prior to the amendment of ammonium sulphate ($(NH_4)_2SO_4$) at a ratio of 100 mg $kg^{-1}$, which was considered as day 0. The soils were then aerobically incubated at 28 °C in the dark for 28 d (Hart *et al.*, 1994). There were two soil treatments, including no-selective nitrification inhibitor (nitrapyrin) control and nitrapyrin-amended treatment (10 μg $C_6H_3Cl_4N$ $g^{-1}$ soil), with three replications for each. During the whole incubation, soil moisture was maintained at 60% water-holding capacity by adding distilled water every three days. Soil samples were collected on days 1, 7, 14, 21, and 28 of incubation.

Soil pH was determined using a pH meter after mixing with distilled water at a ratio of 1:1 (soil/water, weight/volume). Additionally, 10 g soil samples were extracted with 50 mL KCl (2 mol $L^{-1}$) , shaken for 1 h at 250 r $min^{-1}$, and allowed to rest for 30 min before extracting the clear filtrate/supernatant (Keeney and Nelson, 1982). The resultant clear supernatant was colorimetrically analyzed for $NH_4^+$-N and $NO_3^-$-N with the UV 1000 Spectrophometer (Thermo Scientific, Madison, USA). The net nitrification rates (mg N $kg^{-1}$ dry soil $d^{-1}$) were calculated by subtracting the initial concentration of nitrate on day 0 from that measured at the end of the incubation divided by the days of incubation (Nugroho *et al.*, 2005).

The net nitrification rates in the microcosms without nitrification inhibitor represented the pathways of both autotrophs and heterotrophs. The difference in nitrate concentrations between the start and end of the