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SHORT COMMUNICATION

Heterotrophic nitrification is the predominant NO_3^- production pathway in acid coniferous forest soil in subtropical China

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Abstract To date, occurrence and stimulation of different nitrification pathways in acidic soils remains unclear. Laboratory incubation experiments, using the acetylene inhibition and ^{15}N tracing methods, were conducted to study the relative importance of heterotrophic and autotrophic nitrification in two acid soils (arable (AR) and coniferous forest) in subtropical China, and to verify the reliability of the ^{15}N tracing model. The gross rate of autotrophic nitrification was 2.28 mg kg^{-1} day^{-1}, while that of the heterotrophic nitrification (0.01 mg kg^{-1} day^{-1}) was negligible in the AR soil. On the contrary, the gross rate of autotrophic nitrification was very low (0.05 mg kg^{-1} day^{-1}) and the heterotrophic nitrification (0.98 mg kg^{-1} day^{-1}) was the predominant NO_3^- production pathway accounting for more than 95 % of the total nitrification in the coniferous forest soil. Our results showed that the ^{15}N tracing model was reliable when used to study soil N transformation in acid subtropical soils.

Keywords Acetylene inhibitor · Autotrophic nitrification · Coniferous forest soil · Heterotrophic nitrification · ^{15}N tracing model

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Introduction

Nitrification has been one of the most intensively studied processes in forest N cycling (Schimel et al. 1984). Autotrophic nitrification, i.e., the process of ammonia oxidation to nitrate via hydroxylamine and nitrite, is important in arable soils (Wood 1986), and heterotrophic nitrification, the oxidation of organic amino N to nitrate via organic nitroso compounds (Killham 1990), is widespread in acidic forest soils (Kreitinger et al. 1985; Wood 1990; Huygens et al. 2008; Zhang et al. 2011). Killham (1986, 1987) concluded that nitrification in acid arable and grassland soil was largely autotrophic, while in acid coniferous soils it was mediated by heterotrophic fungi. However, De Boer et al. (1992) inhibiting nitrification by acetylene (C_2H_2) showed that nitrification was mainly autotrophic in four acid forest soils. Although it is now accepted that nitrification can occur in a wide range of acid soils, even at pH 3 (De Boer and Kowalchuk 2001), it is still unclear which process occurs in acidic soils. Further studies are needed to clarify the relative importance of heterotrophic and autotrophic nitrification in acid soils.

Humid subtropical forests account for approximately 34 % of China's forest area and play an important role in maintaining local climatic conditions and regulating regional soil-atmospheric exchange of C and N (Fang et al. 2001; Zhou et al. 2006). Generally, forest soils in this region are highly acidic (pH<5; Xu and Cai 2007) and are assumed to have little capacity for the microbiological oxidation of NH_4^+ to NO_3^- (Weber and Gainey 1962; Zhao et al. 2007). Zhang et al. (2011) reported that heterotrophic nitrification was the predominant over autotrophic nitrification in coniferous acid forest soil in subtropical region in China. However, the method used by Zhang et al. (2011) was a ^{15}N tracing model, which presents some problems such as more fluxes than the number of independent variables with uncertainties in measuring the process rate in too complex systems (Luxhøi and Jensen 2005). Therefore, here, we have studied the autotrophic and heterotrophic nitrification rate and their relative contribution in two acid subtropical soils in China, using both C_2H_2 inhibition and ^{15}N tracing methods, with the aims to clarify the relative importance of heterotrophic and autotrophic nitrification in these acid soils, and to verify the reliability of the ^{15}N tracing model.

Materials and methods

Two study sites, an arable planting corn and a coniferous forest located in Shuangzhen Forestry Center in Jiangxi Province China, were selected. Three composite soil samples (0–20 cm) were collected from each site in September 2011. The soil pH were 4.6, and 4.5, soil organic C content were 18.3 and 35.1 $g kg^{-1}$, and total N content were 1.4, and 1.7 for arable (AR) and coniferous forest (CF) soils, respectively. After sampling, the soils were brought immediately to the laboratory, sieved (<2 mm), and stored at 4 °C in sealed plastic bags prior to analyses.

For each soil, a series of 250-ml Erlenmeyer flasks was prepared each containing 30 g of fresh soil (oven-dry basis). Two ^{15}N label treatments were applied (each in triplicate): an ammonium label ($^{15}NH_4NO_3$) and a nitrate label ($NH_4{}^{15}NO_3$) both at 20 atom% ^{15}N excess at a rate of 20 mg NH_4^+–N kg^{-1} soil and 20 mg NO_3^-–N kg^{-1} soil. The soils were incubated at 60 % of the maximum water-holding capacity for 6 days without or with C_2H_2 at 1 KPa (1 %) and 25 °C under dark conditions. The soils were extracted at 0.5, 48, 96, and 144 h after NH_4NO_3 application to determine the NH_4^+ and NO_3^- concentrations and the ^{15}N enrichment by isotope ratio mass spectrometry (IRMS 20–22, SerCon, Crewe, UK). The details were given by Zhang et al. (2011). Gross N transformation rates with or without C_2H_2 were estimated by the ^{15}N tracing model reported by Müller et al. (2007) and by the equation reported by Kirkham and Bartholomew (1954). The gross rate per time interval during the incubation was calculated and the average rate of all time intervals was used in this study.

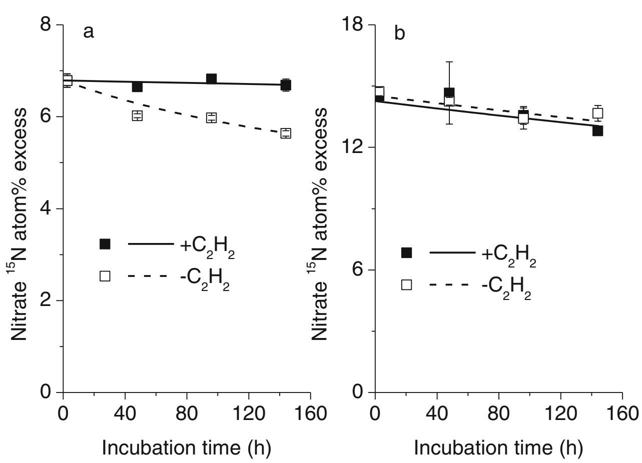

Fig. 1 Measured (*point*) and modeled (*line*) ^{15}N atom% excess of the NO_3^- pools in labeled $^{15}NO_3^-$ treatments for arable (**a**) and coniferous (**b**) soils in subtropical China. *Error bars* SD

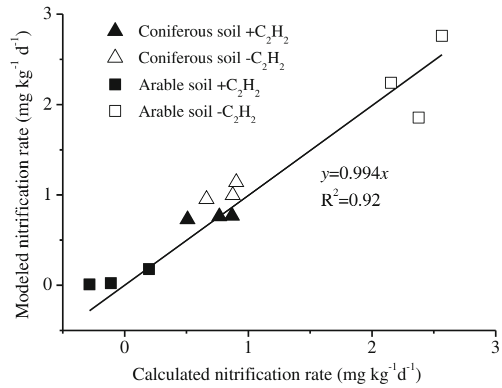

Fig. 2 Comparison of modeled and calculated total nitrification rates (autotrophic nitrification + heterotrophic nitrification; in milligram per kilogram per day). The modeled rates were simulated using the ^{15}N tracing model (Müller et al. 2007), whereas, the calculated rates were determined as reported by Kirkham and Bartholomew (1954). The gross rate per time interval during the incubation was calculated and the average rate of all time intervals was used in figure

Results and discussion

In the acid AR soil, the nitrate ^{15}N atom% excess significantly (p<0.05) decreased during the incubation in the

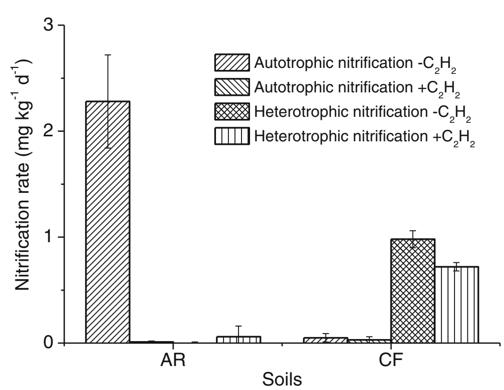

Fig. 3 Gross autotrophic and heterotrophic nitrification rates in the arable and coniferous soil with and without C_2H_2 treatments in subtropical China estimated with the Markov chain Monte Carlo ^{15}N tracing model. *Error bars* SD

$^{15}NO_3^-$ label treatment without C_2H_2, while it remained approximately constant in the $^{15}NO_3^-$ label treatment with C_2H_2, suggesting that autotrophic nitrification was the predominant nitrification process (Fig. 1a). However, the nitrate ^{15}N atom% excess was unaffected by C_2H_2 during the incubation in the acid CF soil (Fig. 1b), suggesting that nitrate production was mainly heterotrophic.

The results showed that the calculated gross nitrification rates (dilution method) and the modeled gross nitrification rates matched very well (Fig. 2). Furthermore, both calculated and modeled rates showed that autotrophic nitrification in the AR soil was effectively inhibited by C_2H_2, suggesting that the ^{15}N tracing model was sensitive to different treatments. Therefore, the ^{15}N tracing model was reliable, when used to study soil N transformation in acid subtropical soils in China.

Autotrophic nitrification (i.e., oxidation of NH_4^+) occurred at a rate of 2.28 $mg kg^{-1} day^{-1}$ in the AR soil, while, in the CF soil it was negligible (0.05 $mg kg^{-1} day^{-1}$; Fig. 3). The heterotrophic nitrification in the AR soil was very low, whereas, it was up to 0.98 $mg kg^{-1} day^{-1}$ in the CF soil. About 95 % of the NO_3^- was produced via heterotrophic nitrification in the CF soil, confirming that heterotrophic nitrification is largely predominant in the acid coniferous forest soil (Zhang et al. 2011).

In conclusion, NO_3^- was mainly produced via autotrophic nitrification in the acid arable soil, while, heterotrophic nitrification was prevalent in the acid coniferous forest soil in subtropical region of China, confirming the results by Killham (1986, 1987). Further studies are needed to estimate the relative importance of heterotrophic and autotrophic nitrification in a broad range of acid soils with the aim of clarifying the microbial mechanisms of heterotrophic nitrification.

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