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The substrate is an important factor in controlling the significance of heterotrophic nitrification in acidic forest soils

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ABSTRACT

In this study, a ^{15}N tracer experiment was carried out to investigate the relative availability of different nitrogen (N) substrates for heterotrophic nitrifiers and to determine the significance of heterotrophic nitrification in two acidic forest soils in eastern China. Five ^{15}N labeled substrates were applied, i.e. Glycine (4.90 atom% ^{15}N excess), L-glutamic acid (4.89 atom% ^{15}N excess), maize straw (3.63 atom% ^{15}N excess), $(NH_4)_2SO_4$ (4.97 atom% ^{15}N excess), and control and were incubated for 4 days without or with C_2H_2 at 1 KPa (1%) in laboratory. The results showed that accumulation of ^{15}N-NO_3^- significantly increased with the incubation time in all ^{15}N labeled treatments in the presence of acetylene, indicating that NO_3^- produced from heterotrophic nitrification convincingly occurred and heterotrophic nitrifiers could use both ammonium and organic N compounds substrates for nitrification in the studied soils. The ^{15}N-NO_3^- production in the Glycine and L-glutamic acid treatments in the presence of acetylene was obviously higher than that in the $(NH_4)_2SO_4$ and maize straw treatments ($p < 0.05$), indicating availability of N substrates for heterotrophic nitrifiers was different. The heterotrophic nitrification of amino organic N compounds could occur via a combined organic and inorganic pathway. However, for complicated organic N substrate, e.g. maize straw in this study, almost all ^{15}N-NO_3^- was produced by organic N pathway. The contribution of heterotrophic nitrification to total nitrification varied from 23% to 93% in the different N substrate treatments as follows: $(NH_4)_2SO_4$ < amino acid < maize straw. The substrate was an important factor in controlling the significance of heterotrophic nitrification in acidic forest soils.

1. Introduction

It is widely accepted that nitrate (NO_3^-) could be produced by two pathways in soils (Pedersen et al., 1999; De Boer and Kowalchuk, 2001; Huygens et al., 2008; Zhang et al., 2013a,b). One is the oxidation of ammonia to NO_3^- driving by chemo-autotrophic nitrifiers, i.e. autotrophic nitrification. The other is the heterotrophic nitrification, which is driven by heterotrophic nitrifying bacteria or fungal. Because autotrophic nitrifiers are sensitive to low pH (Weber and Gainey, 1962), it is thought that NO_3^- is produced predominantly via heterotrophic nitrification process in acidic soils (Kreitinger et al., 1985; Killham, 1990; Wood, 1990; Huygens et al., 2008; Zhang et al., 2011, 2013a,b). However, the significance of heterotrophic nitrification process in soil N transformation is largely unknown (Killham, 1986; De Boer and Kowalchuk, 2001), although the presence of this process has been demonstrated in some pure culture experiments for several decades (Schmidt, 1960; Killham, 1986; Honda et al., 1998).

Some studies, using inhibition methods (mostly acetylene), have provided quantitative data on the relative importance of heterotrophic and autotrophic nitrification in acidic pasture or forest soils (Barraclough and Puri, 1995; Pedersen et al., 1999; Zhang et al., 2013b). Previous investigations showed a very large variation of the relative contribution of heterotrophic to nitrification in acidic soils. Barraclough and Puri (1995) reported that about only 8% of nitrification could come from heterotrophic nitrification in an acidic woodland soil (pH 3.8). However, Pedersen et al. (1999) observed that the importance of heterotrophic nitrification varied in the different land use soils, which was responsible for about 18%, 67%, 78%, 92% of total nitrification in Clear cut area soil (pH 5.7), mature forest organic horizon (pH 5.2), young forest (pH 5.9) and

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mature forest mineral soil (pH 5.8), respectively. Similarly, Zhang et al. (2013b) reported autotrophic nitrification was the predominant nitrification process (more than 99%) in acidic arable soils (pH 4.6), while, the heterotrophic nitrification accounted for more than 95% of the total nitrification in the acidic coniferous forest soil (pH 4.5). Above mentioned results suggested that low soil pH could not be the only factor controlling the importance of heterotrophic nitrification. For modeling nitrification in acidic soils accurately, more studies on the relative importance of heterotrophic and autotrophic nitrification and its controlling factors are needed.

Previous investigation has suggested that the character and availability of substrates may be more important than pH in affecting heterotrophic nitrification process in soils (Killham, 1986). Many reports have suggested that heterotrophic nitrifiers can use both inorganic and organic substrates for nitrification (Honda et al., 1998; De Boer and Kowalchuk, 2001). Pure culture experiments in cuvette have showed that various N compounds can act as substrates for heterotrophic nitrification (Focht and Verstraete, 1977; Rho, 1986; Stroo et al., 1986). However, some investigations observed that unidentified organic N compounds, not NH_4^+, were the substrate for heterotrophic nitrification in acidic soils (Barraclough and Puri, 1995; Pedersen et al., 1999; Islam et al., 2007). To date, very few studies were carried out to investigate the relative availability of N substrates for heterotrophic nitrification in the acidic soils.

The main objective of this study was to investigate the relative availability of different N substrates for heterotrophic nitrifiers and to determine the significance of heterotrophic nitrification in two acidic forest soils in eastern China.

2. Materials and methods

2.1. Study site and soil sample

The study site was located in Wanmulin Nature Reserve in Fujian province, in eastern China (118°09′E, 27°03′). The study region has a middle sub-tropical monsoon climate, with a mean annual air temperature of 19.4°°C and 277 days annual frost-free period. The mean annual precipitation is 1731 mm, most of which falls between March to August. The soil parent material is granite and soils are classified as red soils (humic Planosols, FAO). Soil depth exceeds 1.0 m, and the depths of the O and A horizons are about 4 cm and 10 cm, respectively (Lin et al., 2011). The evergreen broadleaved forest covers approximately 189 ha in the Wanmulin Nature Reserve. Two sites different in dominant tree species were selected in the present investigation, one site was dominated by *Cinnamomum chekiangense* (CI), and the other was *Castanopsis fargesii* (CA). Soil samples were collected in July 2013. Five grids (about 4 m × 4 m) were randomly staked out at each site. From each grid, the O horizon was removed firstly and three subsamples were collected from the 0–10 cm zone. All subsamples at the same site were put together, and passed through a 2 mm sieve. From the sieved sample two subsamples were collected for 1) incubation experiment and 2) analysis of soil properties. Two soils have acidic pH (4.4 and 4.7 for CI and CA, respectively). Soil organic C content was 31.5 g kg^{-1} and 30.2 g kg^{-1} and C to N ratio was 13.6, and 16.1, for CI and CA, respectively.

2.2. ^{15}N labeled N substrates experiments

For each soil, a series of 250 ml Erlenmeyer flasks, each containing 20 g of soil, was prepared. Five ^{15}N labeled substrates were applied: Glycine (4.90 atom% ^{15}N excess), L-glutamic acid (4.89 atom% ^{15}N excess), maize straw (3.63 atom% ^{15}N excess, C/N ratio 55), $(NH_4)_2SO_4$ (4.97 atom% ^{15}N excess) and control (CK, added water). One mL of inorganic or organic N (amino acid) solution was added to each of the conical flask at a rate of 240 mg N kg^{-1} soil. The powder of maize straw was added to the flask and well mixed with soil. Because the turnover of amino acids is very rapid in soil (Levičnik-Höfferle et al., 2012), the soils were incubated at 25° C and 60% of the water-hold capacity for 4 days without or with C_2H_2 at 1 KPa (1%) under dark conditions. Previous investigations, using the method of DNA-based stable isotope probing (SIP), have shown that low concentration of acetylene 10 Pa (0.01%) can inhibit autotrophic nitrification completely in the same soil samples (Wang and Zhong, unpublished data) and in acidic soils in the same region (Lu and Jia, 2013). To make sure that autotrophic nitrification can be inhibited completely in the studied soils, soil samples for the acetylene treatment was previously exposed to 1 KPa (1%) acetylene for one day (De Boer and Kowalchuk, 2001). The acetylene (1%) was kept continuously in the headspace during the experiment.

The samples were collected at 2 and 4 days after substrates application. Three flasks were randomly selected from each substrate treatment, and the soil was extracted using 2 M KCl to determine the NH_4^+ and NO_3^- concentrations by a continuous-flow analyzer (Skalar, Breda, Netherlands) and their ^{15}N enrichment by Isotope Ratio Mass Spectrometry (IRMS 20–22, SerCon, Crewe, UK). The details were given by Zhang et al. (2011).

Three flasks were immediately, randomly selected from each substrate treatment after addition of substrates. Soil samples were immediately mixed with deionized water at a soil:water ratio of 1:2.5 (v/v) and shook on a mechanical shaker for 5 min at 300 rpm at 25° C. Soil pH was immediately measured in soil-water suspension using a DMP-2 mV/pH detector (Quark Ltd, Nanjing, China).

2.3. ^{15}N pool dilution experiment

Gross nitrification rate in the studied soils was determined using the ^{15}N pool dilution technique (Kirkham and Bartholomew, 1954). Briefly, the nitrate pool was labeled using $K^{15}NO_3$ (10.12 atom% excess). For each soil, six 250 ml Erlenmeyer flasks were prepared with 20 g of fresh soil (oven-dry basis). Two ml of $K^{15}NO_3$ solution was added to each of the flasks at a rate of 2 mg NO_3^--N kg^{-1} soil. The soil was adjusted to 60% water hold capacity and incubated for 24 h at 25 °C. The soils (three replications) were extracted at 0.5 and 24 h after $K^{15}NO_3$ application to determine the concentration and isotopic composition of the NO_3^-. The gross nitrification rate was calculated by the ^{15}N isotope pool dilution method (Kirkham and Bartholomew, 1954).

2.4. Calculation and statistical analyses

Based on the assumption that autotrophic nitrifiers were completely inhibited by 1 KPa acetylene, the ratio of the production of ^{15}N-NO_3^- in the presence of acetylene to the production of ^{15}N-NO_3^- in the absence of acetylene was defined as the contribution of heterotrophic nitrification to total nitrification.

One-way ANOVA was carried out to compare the results among the substrate treatments using SPSS 17.0 software (where $p < 0.05$, the difference was considered significant).

3. Results

3.1. Change of soil pH after addition of substrates

Soil pH was immediately measured after addition of substrates in the different treatments (Fig. 1). There were significant changes in pH after adding substrates, which ranged from 3.9 to 5.2 and were likely to affect nitrification process. Comparing with the

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