Catena 137 (2016) 24–30

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena

Manganese oxide affects nitrification and ammonia oxidizers in subtropical and temperate acid forest soils

Xiaoping Xin [a], Xianjun Jiang [a,*], Jing Su [a], Xiaojuan Yan [a], Jiupai Ni [a], Sarwee J. Faeflen [a], Xueru Huang [a], Alan L. Wright [b]

[a] College of Resources and Environment, Southwest University, 2 Tiansheng Road, Beibei, Chongqing 400715, China
[b] Everglades Research & Education Center, University of Florida, Belle Glade, FL 33430, United States

ARTICLE INFO

Article history:
Received 24 May 2015
Received in revised form 1 September 2015
Accepted 6 September 2015
Available online xxxx

Keywords:
Nitrification
Ammonia oxidizing bacteria
Ammonia oxidizing archaea
Manganese

ABSTRACT

Variations in nitrification dynamics were observed both in temperate and subtropical acidic forest soils. The effect of Mn on nitrification was studied to explain variation in nitrification between different soil types. Weakly and highly acidic soils in subtropical and temperate forests were treated with 0% or 3% birnessite. The nitrification process was simulated by kinetic model. Dynamic changes of *amoA* gene abundance of ammonia monooxygenase (AMO) for ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were estimated by qPCR. Nitrification rates were significantly decreased by Mn addition in 3 days both for the weakly acidic subtropical and temperate soils. However, the total nitrification rate did not change for either soil by Mn addition after 10 days' incubation. Nitrification was best fitted by a first-order kinetic model for both weakly acidic soils. However, it was best fitted with a zero-order model after MnO_2 addition. Numbers of AMO *amoA* gene copy decreased after Mn addition. No significant nitrification was observed for highly acidic soils and Mn had a minimal effect. Soil nitrification was retarded by 3% MnO_2 for both subtropical soil and temperate soils. Nitrification dynamics were altered by MnO_2 in weakly acidic soils, probably due to Mn toxicity.

1. Introduction

Great variation of nitrification potential in acid soils has been reported (Compton et al., 2004; De Boer and Kowalchuk, 2001; Nugroho et al., 2009) due to numerous biotic and abiotic factors. It is now accepted that nitrification can occur in a wide range of acid soils (De Boer and Kowalchuk, 2001), but there are also many acid soils for which nitrification appears to be minimal or absent (Robertson, 1982). Biotic and abiotic factors such as pH, N availability and microbial community structure are responsible in part but not completely for the variation in nitrification rates in acid soils (Bäckman and Klemedtsson, 2003; Compton et al., 2004; Nugroho et al., 2007; Tolli and King, 2005). For example, it was reported that net nitrification rates could vary 3-fold in two acid soils within a given pH (Nugroho et al., 2007). NH_4^+-N content also did not constrain net nitrification, as some temperate acidic forest soils having high NH_4^+-N concentrations did not experience net nitrification (Nugroho et al., 2007; Zhao et al., 2007). Nugroho et al. (2009) also concluded that low net nitrification rates in acid Scots pine forest soils cannot be solely explained by unfavorable abiotic soil conditions after observing the bacterial community structure and interactions between members of the bacterial community. Therefore, there are still uncaptured biotic factors or abiotic factors that contribute to suppression of nitrification in acid soils.

Manganese is one of the elements released during the weathering of primary minerals, which explains its common accumulation in most soils in the range of 0.07–4.0% (Aller, 1990; Fujimoto and Sherman, 1948). Manganese activity is closely related to soil pH, as its concentrations in soil solution increase 100-fold for each pH unit decrease (Hue et al., 2001). Hence, Mn toxicity may be one of the important abiotic stresses on nitrification in acidic soils (Marschner, 1991). Stimulation, retardation, or inhibitation of nitrification by Mn has been observed in different ecosystems and a variety of soil types depending on Mn concentration (Leeper, 1970; Premi and Cornfield, 1969; Wilson, 1977).

Manganese toxicity to microorganisms may be responsible for the nitrification inhibition, but no data are as yet available to substantiate this hypothesis. Furthermore, the mechanistic patterns of nitrification altered by Mn are poorly understood. We hypothesize that the effects of Mn on nitrification occur in different temperate and subtropical soils. Both highly acidic and weakly acidic forest soil samples from temperate area (brown soils) and subtropical area (purple soils) were selected to test this hypothesis by investigating the effect of Mn on the nitrification processes and nitrifying microorganisms.

* Corresponding author.
E-mail address: jiangxj@swu.edu.cn (X. Jiang).

http://dx.doi.org/10.1016/j.catena.2015.09.004
0341-8162/

2. Material and methods

2.1. Site description and soil sampling

The subtropical soil samples (purple soil, Inceptisols) were collected from a subtropical forestland in Yongchuan, Chongqing, southwest of China (29°16′N, 105°84′E). This region has the annual mean temperature 19.7 °C and annual mean rainfall 1400 mm, with middle subtropical evergreen broad-leaved forests. Two sites were chosen to obtain weakly acidic soil and highly acidic soil. Temperate soil samples (brown soil, Luvisols) were collected from Dahei Mountain in Dalian, Liaoning Province, northeast of China (38°43′N, 120°58′E) where the climate is between monsoon and continental with annual mean temperature 10.2 °C (ranging from −19.1 °C to 37.8 °C) and annual mean rainfall 810 mm. The two sampling sites were weakly acidic and highly acidic soils.

Five soil cores (0–20 cm) were collected from a 4 m × 4 m plot using a soil corer (Ø = 13 cm). Portion of the soil samples collected was pooled and homogenized to reduce heterogeneity. The samples were air-dried and separated into two parts. One was ground to pass a 2-mm sieve and stored at 4 °C prior to make subsamples for incubation; another was ground to pass a 1-mm sieve and used for chemical analyses.

2.2. Preparation of birnessite (&-MnO_2) and samples

Birnessite (&-MnO_2) is a naturally occurring, poorly crystalline oxide of tetravalent manganese. It is one of the most common forms of mineralized Mn in soils (Taylor et al., 1964). Birnessite was prepared by dropwise addition of HCl to $KMnO_4$ (McKenzie, 1971). The precipitate was washed, dried, and aged at 60 °C for 12 h, and again washed with water. Obtained birnessite was ground to pass through a 1-mm sieve and stored at 4 °C prior before use. The obtained birnessite was checked by X-ray powder diffractometer (XRD).

Subsamples were prepared by amendment with 0% (unmended) or 3% birnessite by weight. Eight subsamples were obtained as follows: weakly acidic subtropical soil, weakly acidic subtropical soil +3% birnessite, highly acidic subtropical soil, highly acidic subtropical soil +3% birnessite; weakly acidic temperate soil, weakly acidic temperate soil +3% birnessite, highly acidic temperate soil, highly acidic temperate soil +3% birnessite. Each subsample was mixed thoroughly and ground to pass a 1-mm sieve and stored at 4 °C for 2 months before use.

2.3. Physical and chemical analysis

Soil properties were determined following Soil Agro-Chemical Analyses procedures (Lu, 2000). Soil pH was measured in a soil to water ratio of 1:2.5 (*v*/*v*) by a DMP-2 mV/pH detector (Quark Ltd., Chongqing, China). Soil organic matter (SOM), total N and kjeldahl N contents were determined by a Macro Elemental Analyzer (Elementary Analysensysteme GmbH, Hanau, Germany). Total soil Fe and Mn were determined by digesting the soil with HNO_3–HF–$HClO_4$, and HCl-extractable Fe and Mn in soil were extracted by shaking 150 g of soil in 300 ml of 0.002 M HCl for 1 h. The concentration of Fe and Mn in the digesters and extracts was determined by atomic absorption spectrophotometry with a graphite furnace (GFAAS) using a model Z-8200 spectrophotometer.

2.4. Incubation

For each subsample, 20 g soil was placed into a 250-ml plastic bottle. Distilled water was added to adjust the moisture content to 20% by weight (50% of water holding capacity). All bottles were covered with polyethylene film punctured with needle holes to maintain aerobic conditions, and pre-incubated at 28 °C in the dark for 7 days. After pre-incubation, 120 mg N kg^{-1} dry soils were added as $(NH_4)_2SO_4$ to assess nitrification rate. The loss of water through evaporation was compensated by addition of distilled water daily. At the interval of 0, 1, 3, 7 and 10 days after incubation days, subsamples in four replicates were taken and extracted with 100 ml of 2 M KCl for 1 h (Keeney and Nelson, 1982). The concentrations of inorganic N forms in the extracts were determined using SKLAR continuous-flow analyzer (SKLAR San++, Netherland, 2003). The net nitrification rate was calculated using the following equation (Robertson et al., 1999):

$$\text{Net nitrification rate} = \left[\left(NO_3^- - N\right)_f - \left(NO_3^- - N\right)_i\right]/T_d$$

where the subscripts i and f indicate concentrations measured before and after aerobic incubation, respectively and T_d indicates incubation time in days. Net ammonification and net nitrification were expressed as ammonia-oxidizing archaea mg N kg^{-1} day^{-1} dry soil per day (Francis et al., 2005).

2.5. DNA extraction and quantitative PCR assay

Right after pre-incubation, 4 replicate bottles of each treatment were randomly selected to extract DNA and analysis of *amoA* genes was performed by quantitative PCR (qPCR). The DNA was extracted from 0.50 g of soil with the Fast DNA Spin Kit for soil (MP Biomedicals, United States), according to the protocol of the manufacturer. The quality and quantity of the extracted DNA were analyzed with a spectrophotometer (Nanodrop, PeqLab, Germany), and pooled and stored at −20 °C until use. Quantitative PCR of *amoA* genes was performed to estimate the abundance of the ammonia-oxidizing bacterial and archaeal communities, respectively. The primers *amoA*-1 F (5′-GGGGTTTCTACTGGTGGT-3′) and *amoA*-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′) were used for ammonia-oxidizing bacteria generating a 491 bp fragment; Arch-*amoA* F (5′-STAATGGTCTGGCTTAGACG-3′) and Arch *amoA* R (5′-GCGG CCATCCATCTGTATGT-3′) were used for generating a 635 bp fragment (Francis et al., 2005). Quantification was based on the fluorescence intensity of the SYBR Green dye and reactions for each sample were carried out in a Bio-Rad CFX-96 thermal cycler. The quantification of *amoA* genes was performed in a total volume of 25 μl reaction mixtures with 12.5 μl of SYBR Premix Ex Taq™ as described by the suppliers (Takara Bio, Otsu, Shiga, Japan), 0.25 μl of each primer (50 μm), 1 μl of soil DNA template, with a final content of 1–10 ng in each reaction mixture, and 11 μl ddH_2O. The fragments for the AOB and AOA were both amplified using an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C for AOB, and 45 s at 72 °C for AOA for the collection of fluorescence data. All reactions were finished with a melting curve starting at 65 °C with an increase of 0.5 °C up to 95 °C to verify amplicon specificity. The PCR reaction runs had an efficiency of 90% and 94% for the AOB and AOA, respectively. Standard curves for the AOB and AOA were obtained using serial dilutions of linearized plasmids (pGEM-T, Promega) containing cloned *amoA* genes amplified from environmental clones ($r^2 > 0.995$ for both standard curves).

2.6. Statistical analysis

The changes in NO_3^- N content with incubation time were modeled with a first-order reaction kinetic model, expressed as $N_{NO3} = N_0 + N_P\,(1 - \exp.(-k_1 t)$, or by a zero-order reaction kinetic model, expressed as $N_{NO3} = N_0 = k_0 t$, where N_{NO3} was NO_3^- N content at incubation time t; N_0 was NO_3^- N content after pre-incubation ($t = 0$); N_p was nitrification potential; and k_1 and k_0 were rate constants of first- and zero-order reactions, respectively. The potential nitrification rate (V_p) was calculated from first-order kenetics as $V_p = k_1 * N_p$ (Oorts et al., 2007). The mean actual net nitrification rate (V_a) was calculated as $V_a = (N_{10} - N_0)/10$, here N_{10} was NO_3^- N content after 10 days' incubation ($t = 10$).