Land-use type affects nitrate production and consumption pathways in subtropical acidic soils

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ABSTRACT

The production and consumption pathways of nitrate (NO_3^--N) are key factors for NO_3^--N retention capacity (NR) in soils, especially in subtropical regions with high precipitation. This study was designed to examine the effect of land-use type on NO_3^--N production and consumption pathways, in acidic soils in a subtropical region. Soils were collected from 5 differing land-use types: shrubland (SB), a eucalyptus plantation (ET), a sweet potato farm (SP), a citrus orchard (CO) and a mixed-vegetable farm (VE), in southeast China. In our study, SB was considered a natural system, ET was considered an artificial plantation system, and SP, CO and VE were considered agricultural systems. An incubation experiment was conducted with two ^{15}N tracing treatments, and a numerical modeling method was applied to the experimental data to quantify gross N transformation rates. The results showed that 76% and 69% of NO_3^--N was produced by heterotrophic nitrification (O_{Nrec}) in SB and ET respectively. Whereas, O_{Nrec} was negligible in SP, CO and VE, with > 99% of NO_3^--N produced by autotrophic nitrification. Dissimilatory NO_3^--N reduction to NH_4^+-N (DNRA) had an important role in soil NO_3^--N consumption in SB and ET, with > 90% of NO_3^--N produced in SB being simultaneously consumed via DNRA. However, DNRA was unimportant in SP, CO and VE. Nitrate immobilization and denitrification (I_{NO3}) was negligible for all land-use types. Soil NR under different land-use types was in the order SB > ET > SP ≈ CO ≈ VE. Although N fertilizer was applied at low rates, for only the following two years after planting eucalyptus saplings, the NR in ET was significantly lower than in SB (natural system). Nitrate retention capacity was negatively correlated with soil organic carbon (SOC) and total nitrogen (TN) concentrations, and positively correlated soil C:N and C: NO_3^--N ratios. The lower NR in SP, CO and VE may therefore be due to higher SOC and TN concentrations and lower C:N and C:NO_3^--N ratios in those soils relative to SB and ET soils. Our observations imply that O_{Nrec} and DNRA play an important role in soil NO_3^--N production and consumption in SB and ET land-uses, but not in agricultural land-use (SP, CO and VE), within the subtropical region of China studied. These findings could contribute to a theoretical and practical framework for managing NO_3^--N in subtropical acidic soils.

1. Introduction

It is widely accepted that NO_3^--N can be produced through autotrophic or heterotrophic nitrification pathways in soils (Islam et al., 2007; Kuroiwa et al., 2011; Liu et al., 2015; Müller et al., 2004; Zhang et al., 2014). However, the predominant NO_3^--N production pathway in acidic soils is still under debate (Kuroiwa et al., 2011; Liu et al., 2015). It has been shown that low soil pH inhibits autotrophic nitrification (Hanan et al., 2016; Ste-Marie and Paré, 1999), whereas, heterotrophic nitrification has been shown to continue under acidic

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conditions (De Boer and Kowalchuk, 2001). Thus, several authors have proposed that heterotrophic nitrification is the main NO_3^--N production pathway in acidic soils (Huygens et al., 2008; Liu et al., 2015; Y. Zhang et al., 2013; Zhang et al., 2014). However, other studies have indicated that nitrification in acidic soils could also be attributed to acid-tolerant autotrophs (Hayatsu et al., 2017; He et al., 2012), and high autotrophic nitrification rates have been reported in two agricultural soils with low pH (Gubry-Rangin et al., 2010). Islam et al. (2007) showed that the gross rates of autotrophic nitrification in two acidic pasture soils (pH 4.8 and 5.3) were 0.16 and 0.12 μg N g^{-1} h^{-1}, which were significantly higher than heterotrophic nitrification rates (0.04 and 0.01 μg N g^{-1} h^{-1}).

These contrasting results could be due to the processes of autotrophic and heterotrophic nitrification being affected by additional factors other than pH, such as N availability (Ste-Marie and Paré, 1999), C:N ratio (J. Zhang et al., 2013), and soil temperature (Fungo et al., 2017). Additionally, high heterotrophic nitrification rates accompanied with low autotrophic nitrification rates have been observed in forest soils (Huygens et al., 2008; Liu et al., 2015; Y. Zhang et al., 2013; Zhang et al., 2014), whereas low heterotrophic nitrification rates accompanied with high autotrophic nitrification rates have been observed in agricultural soils (Gubry-Rangin et al., 2010; Islam et al., 2007). Consequently, land-use and management influences NO_3^--N production pathways. However, the full impact of land-use and management practices on the mechanisms of NO_3^--N production in terrestrial ecosystems is not fully understood.

Unlike ammonium (NH_4^+-N), NO_3^--N is readily leached to groundwater, especially in regions with high precipitation rates. Therefore, the dominant pathways of NO_3^--N production and consumption are important factors to understand for better managing N in subtropical soils. There are two different processes of NO_3^--N consumption in soils, denitrification and DNRA (Rütting et al., 2011). During denitrification, N is released from soil via the gaseous forms N_2O, N_2 and NO_x; during DNRA, soil NO_3^--N is reduced to NH_4^+-N. Nitrogen losses via leaching and gaseous emissions can be reduced by decreasing the size of the NO_3^--N pool in soil and increasing the NH_4^+-N pool, therefore providing additional NH_4^+-N for immobilization or for uptake and assimilation by primary producers (Minick et al., 2016; Silver et al., 2005). Traditionally, NO_3^--N reduction via DNRA was thought to be carried out primarily by facultative and obligatory fermentative bacteria, as it was found in highly reduced and carbon (C) rich environments (Bonin, 1996; Fazzolari et al., 1998; Nijburg et al., 1997), and was affected by C:N and C:NO_3^--N ratios (Chen et al., 2015). However, studies have found that DNRA is performed by obligate anaerobes (e.g. *Clostridium* spp.), facultative anaerobes (e.g. *Enterobacter* spp.) and aerobes (e.g. *Bacillus* spp.) (Kraft et al., 2014; Medinets et al., 2015). The process of DNRA is also considered to be a significant NO_3^--N consumption process in forest soils, under not fully anoxic conditions (Huygens et al., 2007; Silver et al., 2005). This suggests that DNRA may not be restricted to high reducing conditions, and is likely to occur at different soil redox conditions, including oxic conditions (Chen et al., 2015; Schmidt et al., 2011; Takaya et al., 2002; Zhou et al., 2001). Additionally, previous investigations showed that the optimum pH for NO_2^- and NO_3^- reduction was 6.5 and 7.5 respectively, and great DNRA was generally observed in alkaline environments (Rütting et al., 2011; Stevens et al., 1998). Recent studies have demonstrated high rates of DNRA in acidic soils (pH < 5; Lu et al., 2013; Minick et al., 2016). Thus, DNRA may be an important NO_3^--N consumption pathway in acidic soils. However, further research is required to determine the role of DNRA in acidic soils.

The aim of this study was to examine the impact of land-use and management practices on NO_3^--N production pathways, and to determine the role of DNRA in NO_3^--N consumption in acidic soils. We hypothesized that (1) the dominant NO_3^--N production pathway in natural soils and agricultural soils differs, due to stimulation of autotrophic nitrification in agricultural soils; (2) the role of DNRA in NO_3^--

N consumption varies with different land-use types, due to differences in C:N and C:NO_3^--N ratios. To test the hypotheses, soil samples were collected from shrubland (SB), a eucalyptus plantation (ET), a sweet potato farm (SP), a citrus orchard (CO) and a mixed vegetable farm (VE) in southeast China. A ^{15}N tracing experiment was combined with a numerical model to quantify specific gross N transformation rates, to determine the dominant NO_3^--N production pathway in these subtropical-acidic soils, and to estimate the functional role of DNRA under different land-uses.

2. Materials and methods

2.1. Site description and soil collection

Soils were collected from Yongchun County, Fujian Province, China, which has a typical subtropical monsoon climate. Yongchun County has annual precipitation of 1700 mm and a mean annual air temperature of 20.3 °C (30 year mean). Five land-uses were selected including SB, ET, SP, CO and VE, all with the same Ultisol soil type (Soil Survey Staff, 2014). All selected sites were, or had been, converted from similar, secondary woodland dominated by *Castanopsis, Lithocarpus* and *Cyclobalanopsis*. The SB had never been ploughed nor received any fertilizer. The ET was a cultivation of eucalyptus species (*E. saligna*) for timber production, which had received N fertilizer at ~50 kg N ha^{-1} year^{-1} (as urea or compound fertilizer) for the first 2 years after planting. The ET had been cultivated for > 15 years and the eucalyptus had been replanted 7 years prior to the time of soil sampling. The SP was under continuous sweet potato cultivation for > 20 years, which was ploughed annually and received ~220 kg N ha^{-1} year^{-1} (as urea or compound fertilizer). The CO had received ~300 kg N ha^{-1} year^{-1} for > 15 years. The VE was ploughed 5–8 times every year (under multi-cropping cultivation of vegetable crops) and received ~600 kg N ha^{-1} year^{-1} (as urea or compound fertilizer) for > 20 years. In our study, SB was considered a natural system, ET was considered an artificial plantation system, and SP, CO and VE were considered agricultural systems.

To minimize the effect of fertilization, soil samples were collected in May 2015, > 1 month after fertilizer amendments. For each land-use type, 3 sites (under different ownership) were selected as 3 replicates. At each site, 4 plots (~3 m × 3 m) were selected randomly and soil samples taken to a depth of 20 cm were pooled together as one soil sample. Soil samples were passed through a 2 mm sieve, split into 2 subsamples, and stored in the dark at 4 °C prior to use. The first sub-sample was used to characterize background soil properties (Table 1), and the second sub-sample was retained for the ^{15}N tracing experiment (carried out within 2 weeks of sampling).

2.2. ^{15}N tracing experiment

The methods and model reported in Müller et al. (2007) was used to investigate the pathways of NO_3^--N production and consumption in the collected soils. This ^{15}N tracing with laboratory incubation approach has been tested extensively and applied to studies on soils from orchard, paddy, peanut, vegetable, and maize fields, as well as forest soils (Huygens et al., 2008; Liu et al., 2017; Wang et al., 2016; J. Zhang et al., 2015; J. Zhang et al., 2013; Y. Zhang et al., 2015). In brief, 24 conical flasks were prepared for each land-use soil with each flask containing 30 g dry weight equivalent (DWE) of fresh soil. Ammonium nitrate (NH_4NO_3) was applied to each jar at a rate of 60 μg N g^{-1} DWE soil (comprising 30 μg NH_4^+-N g^{-1} soil and 30 μg NO_3^--N g^{-1} soil). To 12 of the flasks for each land-use soil this was applied as $^{15}NH_4NO_3$ (9.85 atom% excess), and to the other 12 as $NH_4^{15}NO_3$ (9.82 atom% excess). After the labeled NH_4NO_3 application, the soil moisture concentration was adjusted to 60% of water holding capacity (WHC) using deionized water and incubated for a total of 144 h at 25 °C, in the dark. The conical flasks were sealed with rubber stoppers, which were