



## Agricultural land use affects nitrate production and conservation in humid subtropical soils in China

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### ABSTRACT

To date, very few studies have been conducted to investigate the characteristics of gross nitrogen (N) transformations in subtropical agricultural soils. In this study, 12 natural woodland and 10 agricultural soils were collected to investigate the effects of land use on soil gross N transformations in the humid subtropical zones in China. The results showed that gross autotrophic nitrification rates (average 0.19 mg N kg<sup>-1</sup> d<sup>-1</sup>) in the woodland soils were significantly lower than those determined in the agricultural soils (average 1.81 mg N kg<sup>-1</sup> d<sup>-1</sup>) ( $p < 0.01$ ). However, the NO<sub>3</sub><sup>-</sup> immobilization rates (average 0.10 mg N kg<sup>-1</sup> d<sup>-1</sup>) in the agricultural soils were significantly lower than in the woodland soils (average 0.47 mg N kg<sup>-1</sup> d<sup>-1</sup>) ( $p < 0.01$ ). On average, 98% of the total NO<sub>3</sub><sup>-</sup> produced could be immobilized into organic-N in the woodland soils, while, it accounted for only 10% in the agricultural soils. These differences in gross N transformations resulted in the inorganic N being dominated by NH<sub>4</sub><sup>+</sup> in the woodland soils; however, NO<sub>3</sub><sup>-</sup> dominated the inorganic N in the agricultural soils. The risk of N leaching and runoff from soil sharply increases after woodland soils are converted to agricultural soils. Application of organic fertilizers with high C/N ratios to agricultural soils in subtropical regions to increase soil organic C content and the C/N ratio is expected to improve NO<sub>3</sub><sup>-</sup> immobilization capacity and reduce the risk of N leaching and runoff from soil.

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### 1. Introduction

Nitrogen (N) is one of the key elements required for biomass growth. Previous investigations have suggested that in some natural ecosystems (e.g. forest ecosystems), in particular under conditions of high rainfall, most available N may be conserved successfully in soils through inherent soil N conservation mechanisms, e.g. i) low mineral N production and low NH<sub>4</sub><sup>+</sup> oxidation rates; ii) a combination of high mineral N production with efficient N immobilization; and iii) NO<sub>3</sub><sup>-</sup> retention via processes such as dissimilatory NO<sub>3</sub><sup>-</sup> reduction to ammonium (DNRA) (Huygens et al., 2007; Rütting and Müller, 2007, 2008). Zhang et al. (2011a,b) and Zhu et al. (2012) have reported that the autotrophic nitrification (i.e. NH<sub>4</sub><sup>+</sup> oxidation) rate was low, resulting in the inorganic N being

dominated by NH<sub>4</sub><sup>+</sup>, and produced NO<sub>3</sub><sup>-</sup> could be conserved efficiently in soils by NO<sub>3</sub><sup>-</sup> immobilization processes in the humid, acidic subtropical forest soils in China.

Over the past 60 years, diverse land use patterns have been developed to meet an increasing demand for food, cash crops, and fiber, thus making the subtropical region of China important agriculturally. The arable area in the humid subtropical region of China is about 446,890 km<sup>2</sup>, which accounts for approximately 4% of the world's subtropical arable land surface or 37% of China's arable land (Zhao, 2002). Previous observations have suggested that the organic carbon content in subtropical agricultural soils in China is generally low due to intensive land use and poor management (Zhao et al., 1988). Land use and management practices, such as the use of inorganic N fertilizer, organic manure, and lime, could markedly influence the physical and chemical properties of the soil, which would in turn affect soil N cycling. Therefore, the characteristics of soil N transformations in agricultural soils would be expected to differ from those in forest soils. However, very few studies have been conducted to investigate the characteristics of

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soil gross N transformations in subtropical and tropical agricultural soils (Booth et al., 2005). It is currently unknown whether the mechanisms for conserving inorganic N in humid subtropical soils (i.e.  $\text{NO}_3^-$  immobilization and DNRA) could be maintained after woodlands are converted for agricultural use. Understanding soil gross N transformations in subtropical soils and how it is affected by land use would provide a scientific basis for rationally applying and appropriately assessing the environmental impacts of N fertilizers.

The objectives of this study were to investigate the effects of land use on soil gross N transformations. Our hypothesis is that soil N conservation mechanisms in humid subtropical forest soils would be destroyed by intensive land use and practice management such as fertilization and liming. Large amounts of mineral N fertilization and liming could stimulate  $\text{NO}_3^-$  production, while a low organic carbon content and C/N ratio could result in a decrease in the  $\text{NO}_3^-$  immobilization capacity, inducing  $\text{NO}_3^-$  dominance in the inorganic N present in agricultural soils, thus increasing N losses through  $\text{NO}_3^-$  leaching and runoff. Twelve natural woodland and 10 agricultural soils were collected from typical subtropical zones in China to test the above hypothesis.

## 2. Material and methods

### 2.1. Soil samples

Twelve natural woodland soils and ten agricultural soils (one orange grove, three rice paddies, three peanut fields, and three maize fields) were collected from the typical subtropical zones in China. The annual precipitation is approximately 1,700 mm, and the mean annual temperature is 18.4–19.4 °C. All agricultural sites were established after clearing the native woodland, and had received lime amendments at the beginning of establishment. The orange orchard was approximately 10 years old, and mineral N fertilizer was applied at a rate of approximately 80 kg N ha<sup>-1</sup> y<sup>-1</sup>. The peanut sites were approximately 20 years old, and were fertilized with mineral N at a rate of approximately 200 kg N ha<sup>-1</sup> y<sup>-1</sup>. Both the rice paddies and maize fields were established more than 100 years ago, and ~300 kg N ha<sup>-1</sup> y<sup>-1</sup> N fertilizer had been applied in the most recent 10 years. The majority of crop residues were removed after harvest. At each site, soil samples were taken from three grids (approximately 4 m × 4 m) that were randomly selected from a representative 100 m × 100 m plot. From each grid, the O horizon, if present, was removed, and one sample was then taken from the mineral A horizon (0–20 cm). Three samples were pooled together, sieved (2 mm), homogenized and subsequently split into two subsamples. One sub-sample was stored at 4 °C for the incubation studies. The other sub-sample was air-dried for analysis of soil properties.

### 2.2. <sup>15</sup>N tracing experiment

In the present investigation, the combination of an <sup>15</sup>N tracing experiment and full process-based N cycle models was used to quantify simultaneously occurring gross N transformations in soil, mineralization of recalcitrant organic-N to  $\text{NH}_4^+$ , mineralization of labile organic-N to  $\text{NH}_4^+$ , immobilization of  $\text{NH}_4^+$  to labile organic-N, immobilization of  $\text{NH}_4^+$  to recalcitrant organic-N, release of adsorbed  $\text{NH}_4^+$ , adsorption of  $\text{NH}_4^+$  on cation exchange sites, oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (autotrophic nitrification), oxidation of recalcitrant organic-N to  $\text{NO}_3^-$  (heterotrophic nitrification), immobilization of  $\text{NO}_3^-$  to recalcitrant organic-N, and dissimilatory  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  (DNRA) (Müller et al., 2007). The transformation rates were calculated using zero-order, first-order, or Michaelis–Menten kinetics. In the present study several model modifications, varying in

the number of considered N transformation, kinetic settings and considered N pools, were tested to find the model which best describes the measured N dynamics (Rütting et al., 2008). The final model was selected according to Akaike's Information Criterion (AIC) (Cox et al., 2006). Parameter optimization was carried out with the Markov chain Monte Carlo Metropolis algorithm (MCMC-MA). The steps in model development and the optimization algorithm are described in detail by Müller et al. (2007) (Müller et al., 2007). The misfit function  $f(\mathbf{m})$  between the simulation output and observations (see Equation (3) in Müller et al., 2007) takes into account the variance of the individual observations. Analyses using this parameter optimization concept in previous studies have shown that the mineralization of two conceptual organic-N pools produced realistic  $\text{NH}_4^+$  dynamics (Huygens et al., 2007; Müller et al., 2009). The MCMC-MA routine was used in the MatLab software package (Version 7.2, The MathWorks Inc.), which calls models that are separately set up in Simulink (Version 6.4, The MathWorks Inc.). Initial concentrations of the mineral N pools were determined according to Müller et al. (2004). Briefly, concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are estimated for time zero by back-extrapolation to  $t = 0$ . The difference between  $\text{NH}_4^+$  applied and  $\text{NH}_4^+$  determined was considered to be the amount of  $\text{NH}_4^+$  that was immediately adsorbed on  $\text{NH}_4^+$  exchange sites ( $\text{NH}_4^+$ ads).

There were two  $\text{NH}_4\text{NO}_3$  treatments (each in three replicates): one contained <sup>15</sup>N labeled ammonium (<sup>15</sup> $\text{NH}_4\text{NO}_3$ ) while the other contained <sup>15</sup>N labeled nitrate ( $\text{NH}_4^{15}\text{NO}_3$ ) (labeled with <sup>15</sup>N at 20 atom percentage excess). For each soil, a series of 250-ml conical flasks was prepared with 30 g of fresh soil (oven-dry basis). Two ml of <sup>15</sup> $\text{NH}_4\text{NO}_3$  or  $\text{NH}_4^{15}\text{NO}_3$  solution was added to each of the conical flasks at a rate of 20 mg  $\text{NH}_4^+$ -N kg<sup>-1</sup> soil and 20 mg  $\text{NO}_3^-$ -N kg<sup>-1</sup> soil. The soil was adjusted to 60% water holding capacity (WHC) and incubated for 144 h at 25 °C. The conical flasks were sealed with silicone rubber stoppers. The soils (three replicates for each <sup>15</sup>N label treatment) were extracted at 0.5, 24, 72, and 144 h after fertilizer application for the determination of the concentration and isotopic composition of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The detailed principle of the model and the <sup>15</sup>N incubation experiment were from Müller et al. (2004, 2007).

### 2.3. Analyses

Soil properties were determined following the Soil Agro-Chemical Analysis procedures of Lu (2000). Soil pH was measured in a 1:2.5 (v/v) soil to water ratio using a DMP-2 mV/pH detector (Quark Ltd, Nanjing, China). Soil organic carbon (SOC) was analyzed by wet-digestion with  $\text{H}_2\text{SO}_4$ – $\text{K}_2\text{Cr}_2\text{O}_7$ , and total nitrogen was determined by semi-micro Kjeldahl digestion using Se,  $\text{CuSO}_4$  and  $\text{K}_2\text{SO}_4$  as catalysts. Ammonium and  $\text{NO}_3^-$  were extracted with 2 M KCl at a soil/solution ratio of 1:5 on a mechanical shaker for 60 min at 300 rpm at 25 °C. The extracts were filtered through filter paper (Qualitative Filter Paper, BH92410262) and the concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were determined with a continuous-flow analyzer (Skalar, Breda, Netherlands).

For isotopic analysis,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were separated by distillation with magnesium oxide and Devarda's alloy (Feast and Dennis, 1996; Zhang et al., 2011a,b). In detail, a portion of the extract was steam-distilled with MgO to separate  $\text{NH}_4^+$  on a steam distillation system; thereafter, the sample in the flask was distilled again after addition of Devarda's alloy to separate  $\text{NO}_3^-$ . The liberated  $\text{NH}_3$  was trapped using boric acid solution. The trapped N was acidified and converted to  $(\text{NH}_4)_2\text{SO}_4$  using 0.02 mol L<sup>-1</sup>  $\text{H}_2\text{SO}_4$  solution. The  $\text{H}_2\text{SO}_4$  solution containing  $\text{NH}_4^+$  was then evaporated to dryness at 65 °C in an oven and analyzed for <sup>15</sup>N abundance. Before separating  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the extract using the steam distillation system, the recovery of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in a standard solution (1 g  $\text{NH}_4^+$ -N L<sup>-1</sup>

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