Nitrite transformations under acidic conditions in temperate and subtropical forest ecosystems

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ABSTRACT

Nitrite (NO_2^-) is an intermediate in a number of soil N transformations, and is a precursor for NO and N_2O emissions. Due to the transient nature of NO_2^- it is often neglected in N cycling research. Thus, the full suite of production and consumption pathways of NO_2^- in terrestrial ecosystems is poorly understood. The primary objectives of this study were to clarify the production and consumption pathways of NO_2^- in the acidic forest soils and compare the NO_2^- dynamics between temperate and subtropical forest soils. A series of ^{15}N tracing studies were performed, where ^{15}N was added as NH_4^+, NO_3^-, or NO_2^- to temperate and subtropical forest soils, and NO_2^- transformations were investigated in these acidic forest soils. The results showed that the average NO_2^- production rates ranged from 4.42 to 5.90 mg $kg^{-1}d^{-1}$ and from 1.29 to 2.77 mg $kg^{-1}d^{-1}$ in the temperate and subtropical forest soils, respectively, within the 1–6 h incubations. Oxidation of soil organic N was the dominant NO_2^- production pathway in the acidic forest soils, which was negatively related to soil pH ($p < 0.01$). The average consumption rates of NO_2^- varied during the incubation period from 12.46 to 14.10 mg $kg^{-1}d^{-1}$ and from 5.84 to 6.74 mg $kg^{-1}d^{-1}$ in the temperate and subtropical forest soils, respectively. The NO_3^- pool recovered 6–44% of added ^{15}N-NO_2^- in the studied forest soils, which was positively related to soil pH ($p < 0.05$). The 32–36% of added ^{15}N-NO_2^- was incorporated into insoluble soil organic N pool (SON) associated with soil organic C concentration. Nitrite incorporation into DON was also important for soil N retention in the subtropical strongly acidic forest soils (pH 4.7), however, in the temperate acidic forest soils, ^{15}N was not detected in the DON pool. Self-decomposition of NO_2^- to NO and NO_2 was a main pathway under subtropical strongly acidic conditions. The NO_2^- turnover was most likely the key driving force for N transformations in the acidic forest soils. Further work regarding the dynamics of NO_2^- and their mechanisms in soils of the different terrestrial ecosystems is important to gain an improved understanding of the global N cycle.

1. Introduction

Process based research in the last years has revealed a number of new processes in the N cycle highlighting that this most important biogeochemical cycle in soil, is more complex and by no means fully understood. Various N forms in soil, such as soil organic N, dissolved organic N (DON), nitrate (NO_3^-), ammonium (NH_4^+), nitrite (NO_2^-), NO, N_2O, N_2 are interacting with each other based on the conditions (Spott et al., 2011; Butterbach-Bahl et al., 2013; Müller and Clough, 2014). Nitrite is not only a key intermediate of several N transformations process but, through process-based ^{15}N tracing studies, it has recently become clear that NO_2^- occurs in the soil as independent N

pools relating to the gaseous N emissions (Russow et al., 2000; Rütting and Müller, 2008; Müller et al., 2014). Nitrite can be produced by soil N transformation processes including: 1) the oxidation of NH_4^+-N (e.g. autotrophic nitrification and heterotrophic nitrification), 2) the reduction of NO_3^--N (e.g. denitrification, assimilatory NO_3^- reduction, and dissimilatory nitrate reduction to ammonium (DNRA)), and 3) the oxidation of soil organic N (i.e. heterotrophic nitrification) (Burns et al., 1995, 1996; Russow et al., 2000; Müller et al., 2006; Rütting and Müller, 2008; Russow et al., 2009; Butterbach-Bahl et al., 2013). Furthermore, the consumption pathways of NO_2^- also include several N transformation processes, such as nitrification ($NO_2^- \rightarrow NO_3^-$), denitrification ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), DNRA ($NO_2^- \rightarrow NH_4^+$),

ANAMMOX ($NO_2^- \rightarrow N_2$), and chemodenitrification ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (Samater and Van Cleemput, 1999; Islam et al., 2008; Russow et al., 2009; Butterbach-Bahl et al., 2013). Thus, the dynamics of NO_2^- are closely linked to the production of NO, N_2O and N_2 (Venterea and Rolston, 2000; Venterea et al., 2003; Venterea, 2007; Isobe et al., 2012; Maharjan and Venterea, 2013). However, despite this importance, NO_2^- is largely ignored in the previous studies of soil N transformations (Lewis and Kaye, 2011), mainly due to the experimental difficulties to capture this transient N pool. In recent years, NO_2^- dynamics in soil have been drawing attention in the study soil N transformations via process-based ^{15}N tracing approaches (Müller et al., 2006; Rütting and Müller, 2008; Su et al., 2011; Isobe et al., 2012; Müller et al., 2014).

All N transformations related to NO_2^- dynamics in soil occur simultaneously in suitable soil microhabitats and the understanding of the NO_2^- dynamics also helps to elucidate the processes for gaseous N emissions. Although there are several NO_2^- production pathways, these pathways are, nevertheless, always associated with the three N pools, i.e. NH_4^+, NO_3^-, and organic N in soil. Thus, it is possible to differentiate the production of NO_2^- at least into three pathways, using stable isotope tracing techniques. Some work has been carried out to quantify the contributions of nitrification and denitrification to NO_2^- production using ^{15}N labelled techniques (Burns et al., 1995, 1996; Russow et al., 2009). A few studies have also considered the contribution of the oxidation of organic N to NO_2^- production. Müller et al. (2006) reported that the pathway of NH_4^+, NO_3^-, and organic N contributed 33, 57, and 10% to the total NO_2^- production, respectively, in grassland soil (organic C 6.6%, pH 6.2) under aerobic conditions. While Rütting and Müller (2008) observed that nitrification of NH_4^+, denitrification, and the oxidation of organic N contributed 18.1, 29.5, and 52.4% to the total NO_2^- production, respectively, in a permanent grassland soil (organic C 4.6%, pH 6.2) under aerobic conditions. To date, the study on the sources of NO_2^-, especially the contribution of heterotrophic nitrification to NO_2^- production in the forest soil, is limited. Consumption of NO_2^- is not only associated with the oxidation and reduction of N compounds, but also with soil organic matter, the mineral, or metallic ions (Van Cleemput and Samater, 1996; Thorn and Mikita, 2000; Davidson et al., 2003). Chemodenitrification of NO_2^- to gaseous N can obviously occur at pH < 5 (Van Cleemput and Samater, 1996; Samater and Van Cleemput, 1999; Islam et al., 2008). Venterea (2007) observed the production of N_2O even in the sterilized uncultivated woodland soil with pH 5.5–5.6 when applied NO_2^-. However, the full suite of production and consumption pathways of NO_2^- in terrestrial ecosystems which are governed by the diversity of soil properties and conditions is poorly understood.

Soil pH appears to be a key factor regulating N transformation processes related to NO_2^- dynamics. In acidic soil environment autotrophic nitrification and heterotrophic denitrification are inhibited, while, heterotrophic nitrification and chemodenitrification may be favoured (Van Cleemput and Samater, 1996; Zhang et al., 2011). Therefore, we hypothesized that the production and consumption pathways of NO_2^- in strongly acidic forest soils should be different from other reported results in the previous studied soils (Müller et al., 2006; Rütting and Müller, 2008), i.e. the contribution of heterotrophic nitrification to NO_2^- production would increase, while the contribution of autotrophic nitrification and denitrification would decrease. The aim of our study was to quantify the contributions of NH_4^+, NO_3^- and organic N pools to total NO_2^- production, and to determine the fates of NO_2^- in the acidic forest soils under aerobic conditions. Thus, in this study, six acidic forest soil samples were collected from temperate (three samples) and subtropical (three samples) regions in China, to study the production and consumption pathways of NO_2^- in these acidic soils via a series of ^{15}N tracing experiments.

2. Materials and methods

2.1. Soil samples

In October 2015 three forest soils were collected in the temperate and subtropical regions of China, respectively. The sample sites of the temperate region were located in the Liangshui National Reserve in Heilong Jiang province, Northeastern China (47°10′N, 128°53′E). The average annual precipitation was 700 mm (30-year average) and the mean annual temperature was 4 °C, and the dominant vegetation type were *Pinus koraiensis*, *Betula platyphylla*, *Larix gmelimi*, and *Picea asperata*. Three sample sites, natural *Pinus koraiensis* forest, plantation *Pinus koraiensis* forest, natural *Betula platyphylla* forest, were selected. The sample sites of the subtropical region were located in Yanjifeng National Reserve in Jiangxi province, South China (27°59′N, 117°25′E). The average annual precipitation was 1785 mm and the mean annual temperature was 18.4 °C. *pinus massoniana* and *Cunninghamia lanceolata* were the dominant vegetation. Three *Pinus massoniana* forest sites were selected. At each site, four plots (~ 4 m × 4 m) were selected randomly. Five surface soil samples (0-20 cm) were collected at each plot after removing the O horizon, pooled together and passed through a 2-mm sieve. Each soil sample was divided into two sub-samples with one stored at 4 °C for the incubation experiments and the other was air-dried for measurement of soil properties.

2.2. The ^{15}N tracing experiment

Two ^{15}N tracing experiments were performed. One was labelled $^{15}NO_2^-$ experiment (with three repetitions) to determine the production and consumption rates of NO_2^- and study the fates of added NO_2^- in soils, in the other experiment, there were three labelled ^{15}N treatments (each with three repetitions), including $^{15}NH_4NO_3$, $NH_4^{15}NO_3$ and $^{15}NH_4^{15}NO_3$ to differentiate the production pathways in soils.

2.2.1. The nitrite addition experiment

In the labelled $^{15}NO_2^-$ experiment, eighteen flasks (250 ml), each containing 20 g (oven-dry basis) fresh soil, were prepared for each soil. Two ml $Na^{15}NO_2$ (^{15}N at 10 atom%) was evenly applied to the surface of soils in each flask with the concentration of 3 mg N kg^{-1} soil (oven-dry basis). Then, flasks were flushed with ambient air, resealed with stoppers and incubated for 24 h at 60% water holding capacity (WHC) and 25 °C conditions. Soil and gas samples (three repetitions) were extracted at 1, 2, 4, 6, 12, 24 h after $^{15}NO_2^-$ application. When gas sampling, 26 ml of gas was collected, 5 ml for determining the N_2O concentration (using a Agilent 7890 gas chromatograph), 20 ml for determining isotopic composition of N_2O (using a Thermo DELTA V PLUS mass spectrometer), and 1 ml for determining isotopic composition of N_2 (using a Thermo DELTA V PLUS mass spectrometer). The isotopic composition of N_2O and N_2 and the concentration of N_2O in the ambient air was also determined. After gas sampling, 2 M KCl solution (solution:soil, 5:1 [volume:mass]) was used to extract NH_4^+, NO_3^-, NO_2^- and DON. The pH of soil extract was adjusted to approximately 7 (Stevens and Laughlin, 1995). The efficiency of NO_2^- extraction was 96.3% in the preliminary experiment, which was used as NO_2^- concentration at 0 h. After extracting, soils were washed using distilled water, oven-dried at 60 °C and sieved (150 μm) to determine the concentration and isotopic composition of soil organic N.

2.2.2. Ammonium nitrate addition experiment

Thirty six flasks (250 ml), each containing 20 g (oven-dry basis) fresh soil, were prepared for each soil. Two ml $^{15}NH_4NO_3$ (^{15}N at 10.12 atom%), $NH_4^{15}NO_3$ (^{15}N at 10.11 atom%) or $^{15}NH_4^{15}NO_3$ (^{15}N at 10.18 atom%) was evenly applied to the surface of soils in each flask at a concentration of 20 mg N kg^{-1} soil (oven-dry basis). Soils were incubated for 6 days at 60% WHC and 25 °C condition. At 0.5, 48, 96 and 144 h after N application, 2 M KCL solution (soil:KCl solution, 1:5