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Highly abundant acidophilic ammonia-oxidizing archaea causes high rates of nitrification and nitrate leaching in nitrogen-saturated forest soils

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ABSTRACT

In southern China, high levels of atmospheric nitrogen (N) are being deposited in forests. Soil acidification and high rates of soil nitrification and subsequent NO_3^- leaching have been observed in the N-saturated forests. We previously did not detect NH_3-oxidizing bacteria in non-N-saturated or N-saturated forest soils. However, NH_3-oxidizing archaea were present in both soils, more in the saturated ones. The purpose of this study was to investigate the roles of autotrophic NH_3-oxidizing archaea and heterotrophic nitrifiers in soil N transformations in N-saturated and non-N-saturated forests in southern China. We investigated the contribution of heterotrophic nitrifiers in the soils by determining the gross nitrification rates with and without an inhibitor of autotrophic nitrification, acetylene (C_2H_2). We also reevaluated nitrification by NH_3-oxidizing archaea by correlating the C_2H_2-inhibited gross nitrification rates with the abundance of the *amoA* transcripts of NH_3-oxidizing archaea. We further measured the gross NH_4^+ production rates and analyzed the community composition of the NH_3-oxidizing archaea. The results suggest that NH_3-oxidizing archaea, rather than heterotrophic nitrifiers and NH_3-oxidizing bacteria, are responsible for the nitrification in the N-saturated forest soils. NH_3-oxidizing archaea in the soils could be acidophilic, having low *amoA* diversity, indicating their strong adaptation to the highly acidified soils. The gross NH_4^+ production rate did not differ between N-saturated and non-N-saturated forests; however, the gross nitrification rate was higher in N-saturated forests. Consequently, the high abundance and NH_3 oxidation activity of NH_3-oxidizing archaea caused the high rates of nitrification and subsequent leaching of NO_3^- in the N-saturated forest. This study suggests that acidophilic NH_3-oxidizing archaea have a great impact on soil N cycling in N-saturated forests.

1. Introduction

By the year 2030, worldwide deposition of N is predicted to increase by 50%–100% relative to 2000, with the largest absolute increases occurring in East and South Asia (Reay et al., 2008). Chronic anthropogenic N deposition into a forest can result in a gradual alteration in N dynamics from a closed internal cycle to an open cycle, where excess N is leached and emitted from the forest. This progressive course was conceptually summarized in a N-saturation model in the 1990s based on observations in temperate forests in North America and Europe (Aber et al., 1998; Galloway et al., 2003). Briefly, net soil nitrification drastically increases, and subsequent leaching of NO_3^- and efflux of NO and N_2O also increase. Nitrification and NO_3^- leaching can accelerate soil acidification, depletion of soil cations, and mobilization of potentially toxic aluminum ions, eventually leading to a decline in the forest (Isobe et al., 2011a). Forests in tropical and subtropical regions may become N saturated earlier than forests in temporal and boreal regions, because the primary production of tropical and subtropical forests is frequently limited by soil phosphorus (P) availability, rather than soil N availability, due to low primary mineral P in highly weathered soils

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(Matson et al., 1999).

Soil nitrification mediated by microbes is the key reaction of forest N saturation (Isobe and Ohte, 2014). Oxidation of NH_3, the rate-limiting step of nitrification, has long been considered to be performed in soils by autotrophic NH_3-oxidizing bacteria. However, because NH_3-oxidizing bacteria can grow only at near-neutral pH in batch culture, the actual mechanism of oxidation of NH_3 in acidic soils is not fully understood (De Boer and Kowalchuk, 2001). The availability of NH_3, the sole substrate for NH_3-oxidizing bacteria, decreases exponentially along with the decrease in pH due to the high pKa of NH_3. Therefore, it has been assumed that heterotrophic microorganisms, which can use both organic and inorganic forms of N as substrates, have importance in nitrification in acidic soils (Jordan et al., 2005; Killham, 1990). Jordan et al. (2005) suggested that the contribution of heterotrophic nitrifiers was larger than that of autotrophic nitrifiers in acidic forest soils receiving high deposition of N in the United States. This view was challenged by the discovery of NH_3-oxidizing archaea, which frequently outnumber NH_3-oxidizing bacteria in acidic soils (He et al., 2012), and the subsequent isolation of the obligatory acidophilic NH_3-oxidizing archaea (Lehtovirta-Morley et al., 2011). Now the idea is widely accepted that nitrification by NH_3-oxidizing archaea, in contrast to nitrification by NH_3-oxidizing bacteria, becomes dominant with decrease in the soil pH level. Nitrification by heterotrophic microbes is now ignored in many studies regarding nitrifiers. However, large contribution of heterotrophic nitrifying microbes to nitrification in acidic forest soil is still proposed or debated (Faeflen et al., 2016; Yu et al., 2017; Zhang et al., 2015, 2014; Zhu et al., 2015).

We have studied nitrification rates and nitrifying microbial communities in subtropical forest soils receiving long-term deposition of atmospheric N located in the Dinghushan Biosphere Reserve in southern China (Fang et al., 2006; Isobe et al., 2012). The pH of forest soils in this region declined significantly from 4.60 to 4.75 in the 1980s to 3.84–4.02 in 2005 (Liu et al., 2010). We observed that the rates of nitrification and subsequent N leaching in N-saturated forest soils were much higher than those in non-N-saturated forest soils in the area, and that the high concentrations of NO_3^- (4.3 mg N/L) in the streams of N-saturated forests were derived mainly from NO_3^- produced by nitrification in the soil rather than from precipitated NO_3^- (Fang et al., 2015). We also found that NH_3-oxidizing archaea were more abundant in N-saturated forest soils than in non-N-saturated forest soils, whereas the *amoA* (ammonia monooxygenase gene) and 16S rRNA genes of NH_3-oxidizing bacteria were not detectable in either N-saturated or non-N-saturated forest soils (Isobe et al., 2012). This was surprising, because both NH_3-oxidizing bacteria and NH_3-oxidizing archaea were detected in many boreal and temperate forest soils, although NH_3-oxidizing archaea frequently outnumbered NH_3-oxidizing bacteria (Boyle-Yarwood et al., 2008; Isobe et al., 2015; Norman and Barrett, 2014). This could provide a new perspective for the paradox in a N-saturation model, i.e., accelerated nitrification in acidified soils. However, heterotrophic nitrification was not investigated in our forest soils, which hampered the evaluation of the contribution of heterotrophs to the nitrification in the N-saturated soils.

The purpose of this study was to investigate the roles of NH_3 oxidizing archaea and heterotrophic nitrifiers in the soil N transformations in N-saturated and non-N-saturated forests. To this end, we investigated the contribution of heterotrophic nitrifiers in the soils by determining the gross nitrification rates with and without C_2H_2 treatment. Acetylene inhibits the activities of NH_3-oxidizing bacteria and archaea (Offre et al., 2009). We also reevaluated (see Isobe et al., 2012) nitrification by NH_3-oxidizing archaea by correlating the C_2H_2-inhibited gross nitrification rates with the abundance of the *amoA* transcripts of NH_3-oxidizing archaea. We further measured the gross NH_4^+ production rates to illustrate the soil N transformations and investigate the regulation factors for nitrification, and analyzed the phylogenies of *amoA* and 16S rRNA of NH_3 oxidizing archaea to characterize their community composition.

2. Materials and methods

2.1. Study sites

Two study sites in subtropical forests in southern China where high levels of N deposition and soil acidification have been observed were selected for the investigations. The sites were the Dinghushan Biosphere Reserve (DHS), located at N23° 10.3′, E112° 32.3′, and the Heishiding Natural Reserve (HSD), located at N23° 27.4′, E111° 53.5'. DHS and HSD are located in Guangdong Province; they are 90 and 160 km west, respectively, of the metropolitan area of Guangzhou (10 million inhabitants), whose emissions of N oxides are among the largest of any city in China (Huang et al., 2010). Both sites have a humid, subtropical, monsoon climate (Fang et al., 2011). The forest soils are lateritic red earth. The sites have received substantial levels of atmospheric N deposition, at 38.2 kg N ha^{-1} yr^{-1} for DHS and 18.1 kg N ha^{-1} yr^{-1} for HSD (Fang et al., 2011). DHS was chosen as the site that receives the highest N deposition in southern China. HSD was chosen as a control site that receives an average level of N deposition.

Forests with different N-saturation status based on the ecosystem N budget and degrees of N deposition were selected within the two sites: two forest types (evergreen broadleaf and pine forests) from DHS and one type (evergreen broadleaf forest) from HSD. The broadleaf forest (more than 400 years old) in DHS is a N-saturated forest with a high rate of leaching of dissolved N (38–43 kg N ha^{-1} yr^{-1}), a level that exceeded the N input. The younger (60–70 years) pine forest is a non-N-saturated forest with the lower rate of leaching (11–17 kg N ha^{-1} yr^{-1}) (Fang et al., 2008). The broadleaf forest also exhibited the larger rate of denitrification (Zhang et al., 2008) and the larger degree of soil acidification (Fang et al., 2006; Liu et al., 2010) than pine forest in DHS. The broadleaf forest (60–70 years old) in HSD is a non-N-saturated forest expected to leach NO_3^- at a lower rate (2–4 kg N ha^{-1} yr^{-1}).

In addition, in the broadleaf and pine forests of DHS, 100 kg N ha^{-1} yr^{-1} as dissolved NH_4NO_3 was sprayed monthly on the forest floor, beginning in July 2003, to predict the ecosystem change that might be expected to occur in the future under more N-saturated conditions (Fang et al., 2009).

2.2. Soil sampling and chemical analysis

Soil sampling was performed in August 2009. Soils were collected from all the plots, the control (non-N-supplemented) and N-supplemented plots in the broadleaf and pine forests in DHS, and the control plot in the broadleaf forest in HSD (Table 1). The layout of the plots was described in Fang et al. (2009). Soil (approximately 600 g per sample) was collected with a small shovel from the upper 0–5 cm of the soil mineral layer (A horizon) at 12 different points in the four replicate plots. The soil samples were sieved through a 2-mm mesh and thoroughly mixed for each replicate plot. The mixed soils were shipped at a cool temperature (approximately 4 °C) to the laboratory in Japan. NH_4^+, NO_3^-, and dissolved organic N (DON) concentrations, total N content, and soil pH were measured as described in Isobe et al. (2012) and Koba et al. (2010), and soil microbial biomass N was measured as described in Brookes et al. (1985), as shown in Table 1.

2.3. Measurements of gross rates of heterotrophic and total nitrification and NH_4^+ production

The gross soil nitrification and NH_4^+ production rates were determined by the isotope dilution method (Hart et al., 1994) as described previously (Isobe et al., 2012). First, we used C_2H_2, which inhibits NH_3 oxidation activity in both NH_3-oxidizing bacteria (Hynes and Knowles, 1983) and NH_3-oxidizing archaea (Lehtovirta-Morley et al., 2011), to determine the heterotrophic nitrification rates. The C_2H_2-insensitive nitrification rates, i.e., the heterotrophic nitrification rates, were determined by measuring the gross nitrification rate with 1% (v/v) C_2H_2.