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Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment

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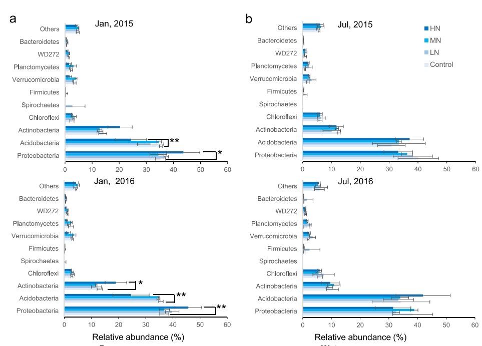
HIGHLIGHTS

- High nitrogen (N) addition reduced bacterial diversity in a tropical forest soil.
- High N addition also altered the soil bacterial community composition (BCC).
- The relative abundance of copiotrophic bacteria was enhanced by the addition of N.
- NH_4^+-N content was a dominant predictor for BCC in the severely acidic forest soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil pH is a dominant factor affecting bacterial community composition in acidic, neutral, and alkaline soils but not in severely acidic soils (pH < 4.5). We conducted a nitrogen (N) addition experiment in the field in severely acidic forest soil to determine the response of the soil bacterial community and identified the dominant factor in determining community composition. Using a high-throughput Illumina HiSeq sequencing platform, we found that high levels of N addition significantly decreased soil bacterial diversity and altered the composition of the soil bacterial community. The addition of nitrogen increased the relative abundance of copiotrophic taxa (*Proteobacteria* and *Actinobacteria* phyla) but decreased the relative abundance of oligotrophic taxa (*Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, and *WD272*). In particular, the relative abundance of N-cycling-related microbes (e.g., *Burkholderia* and *Rhizomicrobium* genera) also increased upon addition of N. Our correlation analysis showed that soil ammonium nitrogen concentration, rather than pH or nitrate nitrogen concentration, was a key environmental parameter determining the composition of the soil bacterial community. However, these bacterial response behaviors were observed only in the dry season and not in the wet season, indicating that high temperature and precipitation in the wet season may alleviate the impact of the addition of N

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on soil bacterial diversity and community composition. These results suggest that the soil bacterial community shifted to copiotrophic taxa with higher N demands under increased N addition in severely acidic forest soil.

1. Introduction

Globally, elevated atmospheric nitrogen (N) deposition resulting from anthropogenic activities has ranged from 15 to 187 Tg N yr^{-1} over the past 145 years, and it is predicted to reach 200 Tg N yr^{-1} over the next 30 years (Galloway et al., 2008). Excessive N input has many adverse effects on the structure and functioning of terrestrial ecosystems, such as soil acidification (Van breemen et al., 1984; Bouwman et al., 2002; Lu et al., 2014), plant growth restriction (Berendse et al., 2001), plant diversity reduction (Stevens et al., 2004; Lu et al., 2010), and alterations in the composition of the soil microbial community (Fierer et al., 2012; Ramirez et al., 2012). Soil microorganisms play a critical role in the maintenance of soil fertility and ecosystem function (Falkowski et al., 2008), and thus monitoring the responses of the soil microorganisms to N deposition is conducive to evaluation of variations in ecosystem processes driven by soil microbiota (Li et al., 2017).

Various responses to exogenous N enrichment have been observed in soil microbial communities. Some studies have shown that addition of N altered the composition of the soil microbial community (Nemergut et al., 2008; Fierer et al., 2012; Ramirez et al., 2012; Yao et al., 2014; Li et al., 2016). Some studies found N enrichment to have no impact on the soil microbial community (Freedman et al., 2015; McHugh et al., 2017). For instance, Freedman et al. (2015) reported that 20 years of experimental N addition (30 kg N ha^{-1} yr^{-1}) did not change the relative abundance of bacteria phyla in northern hardwood forest stands. Fierer et al. (2012) documented that N enrichment significantly decreased the soil bacterial diversity in farmland but not in grassland. Relatively few studies on the responses of soil microbial community composition to elevated N deposition have been done in tropical and subtropical forests (Liu et al., 2013; Li et al., 2015; Fang et al., 2011). In one such study, Li et al. (2015) reported that short-term N addition did not change the fungi/bacteria ratio (F/B) in the soil (2015), whereas Liu et al. (2013) found that addition of N significantly increased the F/B ratio in the tropical forest. In contrast, Tian et al. (2017) showed that addition of N decreased the F/B ratio in the subtropical forest. Results have been contradictory with respect to the pronounced variation in microbial responses across different soil and vegetation types.

Within a soil microbial community, different taxonomic or functional microbial groups may also exhibit differentiated responses. It has been hypothesized that elevated N addition should reduce the abundance of oligotrophic taxa but increase the abundance of copiotrophic taxa (Fierer et al., 2007; Ramirez et al., 2010; Fierer et al., 2012; Ramirez et al., 2012; Li et al., 2016; Ling et al., 2017). Generally, decreased relative abundance of oligotrophic taxa is represented by taxonomic groups of *Acidobacteria*, *Verrucomicrobia*, *Nitrospira*, and *Chloroflexi* phyla, and increased relative abundance of copiotrophic taxa are represented by *Proteobacteria*, *Actinobacteria*, and *Firmicutes* with added N (Ramirez et al., 2012; Ling et al., 2017). However, Li et al. (2016) found that added N to have no effect on the relative abundance of *Actinobacteria* and *Verrucomicrobia* in a natural steppe system. The mechanistic links are still unclear with respect to the responses of soil bacteria to N enrichment at phylogenetic, taxonomic, and community levels.

Many environmental factors have been found to be correlated with variations in soil microbial community composition, such as soil moisture, pH, total organic carbon, total nitrogen, inorganic nitrogen content, climate change, and aboveground vegetation (Högberg et al., 2007; Yang et al., 2013; Yao et al., 2014; Lladó et al., 2017). Of these, pH was considered the most important factor in predicting the alteration of soil bacterial community composition (Lauber et al., 2009). A previous study found that N addition resulted in a lowered soil pH in a tropical forest after long-term N addition (Lu et al., 2014). It is widely accepted that soil bacterial community composition is affected by soil pH across biomes and regions, with soil pH values ranging from 4.5 to 8.5 (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Fernandez-Calvino et al., 2012; Yun et al., 2016; Wu et al., 2017). However, no relationship between soil bacterial community composition and soil pH has been found when the pH is below 4.5 (Rousk et al., 2010).

In this study, we conducted a field N addition experiment in severely acidic tropical forest soil (pH < 4.0). Throughout the first two years of the experiment, bacterial diversity and community composition were examined using 16S rRNA gene-based barcoded pyrosequencing analysis, and soil physicochemical properties were assessed periodically. We aimed to address the following questions: 1) Would copiotrophic bacteria be stimulated and oligotrophic bacteria be suppressed by N addition in the tropical forest soil? 2) How would different taxonomic and phylogenetic bacterial groups respond to the addition of N? 3) Which factors determine the composition of the bacterial community in severely acidic forest soil?

2. Materials and methods

2.1. Site description

Dinghushan Biosphere Reserve (DBR) is located in the city of Zhaoqing, Guangdong Province, in southern China (112°10′ E, 23°10′ N), where we conducted an N addition experiment to simulate N deposition in a tropical forest soil. Evergreen broad-leaved forest >400 years old is the typical zonality vegetation of DBR, with an annual average temperature of 20.9 °C and mean annual precipitation of 1956 mm (Yan et al., 2006). The study site is arranged along an altitudinal gradient from 300 to 355 m above sea level, consists of *Castanopsis chinensis* as the obviously dominant tree species and is influenced by a typical south tropical monsoon climate. At this site, the wet season is concentrated between April and September (approximately 80% rainfall). In contrast, the dry season lasts from October to March (approximately 20% rainfall). In addition, the soil of this region has a pH below 4.0 and is a severely acidic lateritic red loam (Zhang et al., 2008).

2.2. Experimental design and soil sampling

Gradient concentrations of NH_4NO_3 were applied at four levels: Control (ambient: 33.5 kg N ha^{-1} yr^{-1}); LN (low nitrogen: ambient + 35 kg N ha^{-1} yr^{-1}), MN (medium nitrogen: ambient +70 kg N ha^{-1} yr^{-1}), and HN (high nitrogen: ambient + 105 kg N ha^{-1} yr^{-1}). NH_4NO_3 was applied to simulate N deposition because the nitrogen deposited from the atmosphere onto the earth surface is mainly composed of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^-N) all over the world (Fierer et al., 2012; Ramirez et al., 2012). The four N addition levels was applied to simulate the doubled (LN), tripled (MN) and quadrupled ambient N deposition rate (33.5 kg N ha^{-1} yr^{-1}) in the region (Fang et al., 2015). A total of 12 (3 replicates per treatment × 4 treatments) randomly scattered plots (15 m × 15 m per plot) were established beginning in October 2013. Different doses of N (NH_4NO_3) solution were sprayed over the plots at the end of every month beginning in September 2014. From each plot, two layers of soil samples (0–10 cm and 10–20 cm in depth) were collected using 5-core soil