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Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest

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ABSTRACT

Intensified anthropogenic activities will increase rates of nitrogen (N) deposition over the next decades, especially in the tropics. There are urgent needs to know how soil microbial community in N-rich tropical forests responds to long-term N deposition. This study examined effects of long-term N additions on soil microbial biomass (determined by chloroform fumigation), microbial community composition (based on phospholipid fatty acids, PLFAs), and microbial enzyme activities, using an ongoing experimental N additions field in an N-rich tropical forest of South China. There were four N additions levels: no additions (Control); 50 kg N ha^{-1} yr^{-1} (Low-N); 100 kg N ha^{-1} yr^{-1} (Medium-N), and 150 kg N ha^{-1} yr^{-1} (High-N). Results showed that long-term N additions significantly decreased microbial biomass carbon (MBC) and nitrogen (MBN), but had little effects on total PLFAs. However, elevated N inputs significantly reduced the relative abundance of bacterial PLFAs, especially gram-negative bacterial PLFAs with higher gram-positive bacteria: gram-negative bacteria ratio in N treatment plots. Although N additions did not change fungi: bacteria ratio, the proportion of arbuscular mycorrhizal fungi increased significantly with N additions. Long-term N additions greatly increased bacterial stress indexes and enhanced specific enzyme activity (activity per unit of microbial biomass) involved in carbon, nitrogen and phosphorus mineralization. Meanwhile, shifts in microbial community composition and specific enzyme activity were correlated well with soil pH and available N. These results suggest that N-mediated environmental stresses can play an important role in shaping microbial community, and that soil microbes will invest more resources on enzyme production in N-rich forest under elevated N deposition.

1. Introduction

Anthropogenic activities have more than doubled atmospheric deposition of reactive nitrogen (N) in terrestrial ecosystems globally, with regional variation resulting from differences in the intensity of fertilizer application, fossil fuels combustion, and cultivation of N-fixing legume (Vitousek et al., 1997; Galloway et al., 2004, 2008). Due to rapid developments of industry and agriculture, tropical areas are expected to face progressive N deposition in the following decades (Galloway et al., 2004). Intensified deposition of reactive N has large effects on terrestrial ecosystems (Vitousek et al., 1997). It has been reported that excess N can acidify soils, deplete soil nutrients, alter quality and quantity of soil organic matter, and negatively affect biodiversity in tropical forests (Matson et al., 1999; Phoenix et al., 2006; Lu et al., 2010; Cusack et al., 2011). All these changes directly or indirectly affect soil microbial communities in terms of population abundance, species diversity, and functional activity (Bengtsson and Bergwall, 1995).

Previous studies over last several decades in temperate and boreal forests suggested that N deposition could decrease microbial biomass and shift microbial community composition (Treseder, 2008; Liu and Greaver, 2010). In temperate and boreal forests, plant growth and microbial activity are generally limited by N supply (Matson et al., 1999). Therefore, amendment in N availability may play an important role in regulating microbial biomass and community composition. Specially, fungal taxa are sensitive to elevated N inputs in temperate and boreal forests, as many studies observed that enhanced N contents mainly decreased fungal abundance and further resulted in a narrower fungi: bacteria (F: B) ratio (Lilleskov et al., 2002; Frey et al., 2004; Wallenstein et al., 2006; Demoling et al., 2008; Zechmeister-Boltenstern et al., 2010; Turlapati et al., 2013). In contrast to temperate and boreal forests, tropical forests are typically N-rich but phosphorus (P) or other nutrient limited (Vitousek et al., 1997; Matson et al., 1999). In addition,

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tropical forest soils are often highly acidic, depleted in base cation, poorly buffered and rich in Al oxides (Matson et al., 1999; Lu et al., 2015). These tropical forests are therefore poorly buffered against external inputs of acidity. Previous studies suggest that N inputs in tropical forests can aggravate soil acidification, with decrease in base cation and P supply but increase in Al mobility (Lu et al., 2014; Huang et al., 2015; Cusack et al., 2016b; Mao et al., 2017). While soil acidification can inhibit microbial growth, fungi are more tolerant to acidic environment than bacteria (Rousk et al., 2010). N-induced soil acidification may therefore decrease microbial biomass but increase F: B ratio by reducing bacterial abundance in tropical forests. Actually, previous studies reported that N additions exerted negative (Peng et al., 2017; Li et al., 2018), neutral (Turner and Wright, 2014; Fanin et al., 2015) or positive (Li et al., 2006; Cusack et al., 2011) effects on microbial biomass C (MBC) in tropical/subtropical forests, and several studies from tropical/subtropical forests observed increase in F: B ratio after short-term N additions (Cusack et al., 2011; Liu et al., 2013a; Li et al., 2015). However, recent meta-analysis found that N additions increased MBC and decreased F: B ratio in tropical/subtropical forests (Zhou et al., 2017). These results indicate that it is still an opening question on how N depositions affect soil microbial biomass and community composition in tropical/subtropical forests. More studies conducted on this topic are needed, especially under long-term N additions.

In general, shifts in abundance and composition of microbial community are accompanied by changes in its functional activity (Cusack et al., 2011; Kaiser et al., 2014). To meet energy and nutrient requirements, soil microbes can produce extracellular enzymes to depolymerize organic matter into assimilable small molecules (Bell et al., 2013). Glycosidases, including cellobiohydrolases (CBH), α-glucosidase (αG), β-glycosidases (βG) and β-xylosidases (βX), are responsible for C acquisition through degrading cellulose and sugar into dissolved organic C. In order to meet N demand, microbes can secrete leucine amino peptidases (LAP) to degrade protein and β-1, 4-N-acetylglucosaminidases (NAG) to degrade chitin. Acid phosphatases (AP) can hydrolyze phospholipid to release available forms of inorganic phosphorus (P). Through extracellular enzymes, soil microbes mediate litter and organic matter decomposition and nutrient releasing (Burns et al., 2002, 2013), affecting soil fertility and plant growth. Extracellular enzyme activities are sensitive to N deposition, although different groups of enzyme may respond differently. For example, Cusack et al. (2011) found that N additions decreased the activities of cellobiohydrolases and β-xylosidases, but had no effects on those of leucine amino peptidases and β-1, 4-N-acetylglucosaminidases in a lower-elevation tropical forest.

The objective of this study is to evaluate how microbial community responds to environmental changes and stresses induced by long-term high N deposition in southern China, where N deposition has been increasing dramatically (Fowler et al., 2013; Liu et al., 2013b). We address this question in an ongoing long-term N additions experimental site in a warm humid tropical forest located at the Dinghushan natural reserve, southern China (Mo et al., 2006). Although underground microbial community is as important as aboveground plant community for ecosystem functioning (Allison and Martiny, 2008), underground microbial community has not been completely investigated in such a long-term N addition site. Based on previous studies that elevated N additions can accelerate soil acidification (Lu et al., 2010, 2014), we hypothesized that (1) long-term N additions could decrease microbial biomass; (2) and shift the microbial community composition, in particular towards a higher F: B ratio, because bacterial growth is more inhibited by an acidic environment than fungi (Bååth, 1998; Pennanen et al., 1998); and (3) alterations of soil chemical characteristics and microbial community would enhance C and P acquiring enzymes and inhibit N acquiring enzymes, because of potential decreased availability of C and P but amendment in N situation (Sinsabaugh et al., 2009).

2. Method

2.1. Study site

The present study was conducted at the Dinghushan Biosphere Reserve (DHSBR) which is an UNESCO/MAB site located in the middle Guangdong Province in southern China (112°10′E, 23°10′N). The area of the reserve approximately covers 1155 ha with a monsoon climate and is located in a subtropical/tropical moist forest biota (Holdridge, 1967). The average annual rainfall is 1927 mm, with 75% of the rainfall distribution in March to August and 6% of the rainfall distribution in December to February (Huang and Fan, 1982). The annual mean temperature is 21.0 °C, and the average temperature of the most coldest (January) and warmest (July) month is 12.6 °C and 28.0 °C, respectively.

Inorganic N deposition in precipitation was 34 kg N ha^{-1} yr^{-1} in 2004 and 32 kg N ha^{-1} yr^{-1} in 2005, among which about 60% was in the form of NH_4^+-N (Fang et al., 2008). According to the results of ^{14}C measurement, the forest has been well protected from anthropogenic land-use disturbance for over 400 years (Shen et al., 1999), and the soil is lateritic red earth (Oxisols) formed from sandstone with a soil depth > 60 cm (Mo et al., 2003).

2.2. Experiment design

Our research site in this monsoon evergreen broadleaf forest was established in 2002, and N additions experiment started in July 2003 (Mo et al., 2006). Four N-additions treatments (with three replicates for each treatment) were established: Control (without N added), Low-N (50 kg N ha^{-1} yr^{-1}), Medium-N (100 kg N ha^{-1} yr^{-1}), and High-N (150 kg N ha^{-1} yr^{-1}). Twelve 20 m × 10 m plots were established with each plot surrounded by a ≥10m wide buffer strip. All plots and treatments were randomly distributed. The form of added N was NH_4NO_3. Monthly applications of N solution were administered by hand to the forest floor. During each application, fertilizer was weighed, mixed with 20 L of water, and applied to each of the 20 m × 10 m plots using backpack sprayers below the canopy. Two passes were made across each plot to ensure an even distribution of fertilizer. Control plots received 20 L of deionized water.

2.3. Soil sampling

Soil sampling was conducted in January 2016. Ten soil cores (2.5 cm inner diameter) at top 10 cm soil layer were randomly collected in each plot and combined into one composite sample. Before sampling, litter layer was carefully removed. Soil samples were sieved to 2 mm mesh size and mixed, after stones and coarse roots were removed. Then, each sample was divided into three subsamples. One subsample was used to measure soil total N, soil organic carbon (SOC), soil electrical conductivity (EC) and soil pH after air dried. One subsample was stored at −20 °C after freeze dry for later measurements of microbial community composition. Another subsample was refrigerated at 4 °C and used to analyze microbial biomass, enzyme activity, available N and available P in two weeks.

2.4. Soil properties

Soil moisture content was measured gravimetrically using 10 g of field moist soil sample oven at 105 °C for 24 h. The pH of the soil sample was measured in a 1:2.5 soil/water suspension. The content of (SOC) and total N concentration was determined by a C/N analyzer (IsoPrime100, Isoprime). Soil electrical conductivity (EC, as an indicator of total dissolved salt, ionic strength and osmotic pressure) was measured at soil/water ratio of 1: 5 (Rhoades, 1996). Soil inorganic N was extracted with 2M KCl and the filtrates were analyzed for NH_4^+-N and NO_3^--N by colorimetric method using a Lachat flow-injection auto-