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Impact of inorganic nitrogen additions on microbes in biological soil crusts

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ABSTRACT

Many studies have shown that changes in nitrogen (N) availability affect the diversity and composition of soil microbial community in a variety of terrestrial systems, but less is known about the responses of microbes specific to biological soil crusts (BSCs) to increasing N additions. After seven years of field experiment, the bacterial diversity in lichen-dominated crusts decreased linearly with increasing inorganic N additions (ambient N deposition; low N addition, 3.5 g N m^{-2} y^{-1}; medium N addition, 7.0 g N m^{-2} y^{-1}; high N addition, 14.0 g N m^{-2} y^{-1}), whereas the fungal diversity exhibited a distinctive pattern, with the low N-added crust containing a higher diversity than the other crusts. Pyrosequencing data revealed that the bacterial community shifted to more Cyanobacteria with modest N additions (low N and medium N) and to more Actinobacteria and Proteobacteria and much less Cyanobacteria with excess N addition (high N). Our results suggest that soil pH, together with soil organic carbon (C), structures the bacterial communities with N additions. Among the fungal communities, the relative abundance of Ascomycota increased with modest N but decreased with excess N. However, increasing N additions favored Basidiomycota, which may be ascribed to increases in substrate availability with low lignin and high cellulose contents under elevated N conditions. Bacteria/fungi ratios were higher in the N-added samples than in the control, suggesting that the bacterial biomass tends to dominate over that of fungi in lichen-dominated crusts after N additions, which is especially evident in the excess N condition. Because bacteria and fungi are important components and important decomposers in BSCs, the alterations of the bacterial and fungal communities may have implications in the formation and persistence of BSCs and the cycling and storage of C in desert ecosystems.

1. Introduction

Nitrogen (N) is a key element controlling the species composition, diversity and productivity of many terrestrial ecosystems (Zechmeister-Boltenstern et al., 2011). Over the past century, atmospheric deposition of reactive N (mainly nitrogen oxide and ammonia) has increased three- to fivefold (IPCC, 2007), and the atmospheric N deposition in terrestrial ecosystems is predicted to further increase by 250% over the next century (Lamarque et al., 2005). It is well established that elevated N additions to ecosystems have wide-ranging consequences for the environment, including climate change, the emissions of greenhouse gases, species loss and even human health threats (Nemergut et al., 2008; Ramirez et al., 2010). The effects of elevated N deposition on altering the primary productivity in ecosystems have been extensively studied, and it is widely accepted that increases in N deposition can lead to increased C storage in the form of plant biomass and that higher N inputs can drive shifts in plant species composition, with growing evidence of a general trend towards a loss of diversity (Clark et al., 2007).

Several studies have suggested that increased N may alter the microbial community structure and diversity and subsequent ecological function (Waldrop et al., 2004; Allison et al., 2008;

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Campbell et al., 2010). Amongst other changes, a shift towards bacteria-dominated microbial communities is expected (Tietema, 1998). Changes in the diversity and composition of soil microbial community after N addition have recently received increased attention in ecosystems, including forests (Entwistle et al., 2013), steppes (Zhang et al., 2008), grasslands and agriculture fields (Ramirez et al., 2010), and alpine tundras (Nemergut et al., 2008). A consensus of recent studies suggests that excess soil N reduces microbial biomass and activity and decreases the soil microbial respiration levels (Ramirez et al., 2010, 2012). To our knowledge, there are few consistent microbial responses to N addition across ecosystems. For example, experimental atmospheric N deposition seems to have no effect on the abundance of *Actinobacteria* on the forest floor (Eisenlord and Zak, 2010), while Ramirez et al. (2010) showed that *Actinobacteria* increased with N addition in both grasslands and agriculture fields. In addition, N manipulation variously reported increases, decreases or no net changes in the diversity and structure of fungal communities (Johnson, 1993; Frey et al., 2004; Jumpponen and Johnson, 2005; Allison et al., 2007). These changes may be ascribed to the different amounts and durations of N treatments, as meta-analyses showed that declines in the abundances of microbes (*e.g.*, bacteria and fungi) and the changes in the microbial community structure were more evident with greater durations and amounts of N added (Treseder, 2008). Alternatively, each soil's unique set of starting characteristics (*e.g.*, distinct edaphic characteristics and microbial communities) may induce very different, unquantifiable impacts of N enrichment on soil responses (Zeglin et al., 2007).

Biological soil crusts (BSCs) are formed by communities of microorganisms that bind together the surface soil. Cyanobacteria, eukaryotic microalgae, fungi, mosses, bacteria and archaea are involved in the assembly of BSCs (Bates et al., 2010). BSCs are widespread in arid and semi-arid lands, and approximately one-third of the Earth's terrestrial surface is arid or semi-arid land where BSC coverage can be as high as 70% in some areas (Belnap and Eldridge, 2001). The ecological roles of BSCs (*e.g.*, soil hydrological, soil biological and geochemical processes and ecological rehabilitation) have been well documented (Belnap, 2006; Bowker, 2007; Li et al., 2007; Moquin et al., 2012).

Regarding the biological process of BSCs formation and persistence, it is crucial to keep a balance between microbial diversity, community structure and microbial abundance in the topsoil layer. If the soil microbes inside are disrupted in some way, it could have unexpected impacts on the ecological role and function of the BSCs. Therefore, our explanatory and predictive abilities with regard to the N management and preservation of BSCs must be based on a thorough mechanistic understanding of microbial responses to N constraints. However, few published studies (Porras-Alfaro et al., 2011) have analyzed BSC microbial community shifts with N addition. As bacteria and fungi are two of the most ecologically important components of BSCs (Gundlapally and Garcia-Pichel, 2006; Green et al., 2008) and most arid and semi-arid ecosystems are N-limited (McCalley and Sparks, 2009), there is good reason to suspect that increased N addition has the potential to alter the diversity and structure of the microbial community (*e.g.*, shifts in the dominant phylotypes of bacteria and fungi) and/or alter the microbial abundance, especially the bacteria to fungi ratio. Either of these changes will very likely affect the microstructure and subsequent functional roles of the BSCs.

To better understand how N additions impact the soil crust microbial diversity and community composition, we performed a manipulative field experiment with four N addition levels (0, 3.5, 7.0 and 14.0 g N m^{-2} y^{-1}) on lichen-dominated crusts in the Tengger desert since 2007. Using N additions that include rates that few, if any, soils are likely to experience, we are able to determine how the possible elimination of N limitation or excess N may alter microbial communities, the threshold of these responses, and possible mechanisms driving these responses (Ramirez et al., 2010). We measured the shifts of bacterial/fungal communities by 454 pyrosequencing and the changes of absolute bacterial/fungal abundance by quantitative real-time PCR (qPCR) and aimed to assess 1) whether the diversity or composition of bacterial and fungal communities in BSCs will show differential responses to N additions 2) whether the shifts of bacteria to fungi ratio are associated with the increasing of N additions and 3) the N addition thresholds of altering microbial communities. Understanding how N additions alter the composition of microbes specific to BSCs is critical to accurately predict terrestrial ecosystem responses and to identify approaches for ameliorating the negative effects of adverse environments.

2. Materials and methods

2.1. Study site and experimental design

A simulated N deposition experiment was conducted starting in 2007 in a field with widespread lichen-dominated crusts as part of an existing experiment for assessing the long-term impacts of N additions on plant species diversity, productivity and dynamics (Su et al., 2013). This field is located in the Cuiliugou region (37°25′N, 104°35′E) on the southeastern fringe of Tengger desert in China) and has a crust coverage of greater than 90% (mainly *Endocarpon pusillum* Hedwig lichen-dominated crusts). The soils are well-buffered alkaline sierozem and are thus only slightly prone to acidification. N in the form of NH_4NO_3 was added homogeneously to plots (1.0 × 1.0 m) covered with *Endocarpon pusillum* Hedwig lichen-dominated crusts without macroscopic mosses at rates of 0 (ambient N deposition, control), 3.5 (low N), 7.0 (medium N) and 14.0 g N m^{-2} y^{-1} (high N). Each plot was surrounded with an at least 1-m buffer zone that received the same N deposition. N was added in solution twice per year, half in mid-May and half in mid-July. Each treatment was repeated eight times (eight plots). To assure that N was the only limiting nutrient (Tilman, 1987), we added phosphorus (5 g P_2O_5 m^{-2} y^{-1}) and some trace elements (Zn, Mn, B, Mo and Co) per year (Su et al., 2013), which are based on the soil census data (the PhD thesis of Jieqiong Su, unpublished data). All 32 plots were distributed randomly across an area of 20 m × 50 m.

2.2. Soil sample collection and soil characterization

In late October of 2013, four soil cores (5-cm depth and 3.5-cm diameter, with crust layers) from each of eight plots were sampled individually using a sterile trowel, and thus 32 soil cores were taken for each N treatment. All soil samples were randomly divided into duplicate aliquots in the field: one for nucleic acid-based molecular analysis and the other for soil physicochemical analysis. For the nucleic acid-based molecular analysis, the upper crust layers of the replicate plots were bulked and thoroughly mixed in the field to form a composite sample. Approximately 5 g of each composited sample was kept in a cooler box for transport to the laboratory and then stored at −70 °C prior to DNA extraction (DNA was extracted within a month after sampling). For the soil physicochemical analysis, the total N was measured with a Kjeltec System 2300 distilling unit (Tecator, Höganäs, Sweden); the soil available N was determined by the alkali diffusion method, the NO_3^-–N and NH_4^+–N contents were analyzed colorimetrically after the extraction of the fresh soil with 2 M KCl; available phosphorus (P) was extracted with 2 M ammonium acetate at pH 7.0; soil organic carbon (C) was measured by the Walkley-Black method; the soil pH

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