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Review Paper

Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition

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ABSTRACT

While aboveground biodiversity has been widely studied, how microbial biodiversity responds to increasing nitrogen (N) deposition is still unclear. Here we conducted a meta-analysis to investigate the responses of soil microbial diversity and composition to N addition. Overall, we found N addition decreased both soil microbial diversity and the relative abundance of Actinobacteria and Nitrospirae, although the effect may vary among different ecosystems. The effect size on microbial Shannon index was positively correlated with the changes in soil microbial biomass under N addition. The initial soil conditions, the duration of treatment, the N addition rate and changes in soil organic carbon under N addition all affected the effect sizes of N addition on microbial Shannon index, while changes in soil pH played a minor role. Overall, our results suggest that the losses of microbial diversity with increasing N deposition rate would alter ecosystem functions and may have profound feedbacks to global climate change.

1. Introduction

Anthropogenic inputs of reactive nitrogen (N) have increased by two times since the industrial revolution (Vitousek et al., 1997; Galloway et al., 2008; Gruber and Galloway, 2008). While the increase of N deposition could relieve N limitation and increase net primary productivity in some ecosystems (Quinn Thomas et al., 2009), it may also cause negative ecological effects (Erisman et al., 2013). Our understanding of the ecosystem responses to increasing N deposition has mainly been limited to aboveground plant communities and soil nutrient cycling processes (Stevens et al., 2004; Xia and Wan, 2008; Bobbink et al., 2010; Lu et al., 2011; Simkin et al., 2016; Deng et al., 2017). Belowground soil microorganisms may also be sensitive to N availability, but have been much less studied. Soil microorganisms play an important role in nutrient cycling, soil organic matter decomposition, and other ecosystem functions and services (Brussaard, 1997; Van Der Heijden et al., 2008), and their responses to increasing N deposition may have serious consequences to global carbon (C) and N cycling and climate changes.

Recently, a few attempts of meta-analysis have been made to assess how microbial biomass would respond to N addition, and they found N addition generally suppresses microbial biomass (Treseder, 2008; Liu and Greaver, 2010; Zhou et al., 2017), but the effects may vary among different ecosystems (Geisseler et al., 2016). However, no meta-analysis is available to reveal the general responses of soil microbial diversity and composition to N addition. Microbial diversity and composition are key determinants of their ecological functions (Brussaard, 1997; Nannipieri et al., 2003; Philippot et al., 2013). Soil microbes are also potential resources of antibiotics and other drugs (Daniel, 2004). Besides, the diverse microorganisms have complex interactions among each other and have both redundant and specific functions. Therefore, microbial diversity and the related process level responses such as biomass, respiration rates, and enzyme activities should be examined simultaneously under the context of global changes.

Nitrogen addition could directly affect soil microorganisms due to the high osmotic potential and ion toxicity (Eno et al., 1955; Omar and Ismail, 1999) and the changes in N availability. On one hand, N addition may alleviate N limitation to some microbes, especially nitrifiers and denitrifiers who use inorganic N as their energy sources or electron acceptors. On the other hand, due to the high energy cost, N_2-fixing microbes may decline with increasing N availability (Berthrong et al., 2014) and microbes less tolerant to high osmotic potential may be killed, both resulting in the decline of microbial biodiversity.

Nitrogen addition could also indirectly affect soil microorganisms due to its influences on soil C availability, soil pH, and plant species richness (Zak et al., 2003; Treseder, 2008). For soil C availability, on

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one hand, N addition may increase net primary productivity (LeBauer and Treseder, 2008) and the quality of plant C (Liu et al., 2016b), which would result in the increase in labile C inputs into soils. Because soil microbes are mainly C limited (Fierer et al., 2009), the increase in labile C inputs is expected to increase microbial biomass. However, the biomass of different groups of microbes may all increase, resulting in unchanged microbial diversity. On the other hand, N addition may decrease fine roots production (Peng et al., 2017) and increase the fraction of recalcitrant compounds such as melanins and lignin (Liu et al., 2016b), resulting in less C availability to soil microbes. Therefore, microbial species specializing in the utilization of recalcitrant compounds may increase, while other species may decline or disappear. Furthermore, N addition has been found to decrease soil pH (Tian and Niu, 2015), enhancing the leaching of magnesium and calcium and mobilization of aluminum. Aluminum poisoning and magnesium and calcium limitation to microbes under N addition may also decrease the diversity of soil microbes and change microbial community composition (Bowman et al., 2008).

With the development of high-throughput sequencing technique, the determination of soil microbial community structure becomes more convenient and less expensive (Mardis, 2008). The number of studies on the effects of N addition on microbial diversity has increased lately, especially in the past five years (Allison et al., 2007; Freedman et al., 2015; Wang et al., 2015). A meta-analysis on these results is necessary in order to better understand the general pattern of N addition effects on soil microbial diversity. Additionally, knowledge on which microbial groups increase and which microbial groups decline under N addition could also help our understanding of the mechanisms of N addition effects on soil microbes. In this study, we used a meta-analysis approach to answer three questions: 1) How did soil bacterial and fungal diversity change under N addition? 2) How did the dominant phyla of soil microorganisms change under N addition? and 3) What are the underlying mechanisms of N addition effects on soil microorganisms? The Shannon index (Shannon, 1948) and Chao1 (Chao, 1984) were selected as our metrics for microbial diversity and richness because they are highly recommended when analyzing microbial alpha diversity (Lemos et al., 2011; He et al., 2013; Delgado-Baquerizo et al., 2016). Based on previous findings, we hypothesized that (i) N addition would decrease soil microbial diversity, and cause an overall reduction in microbial biomass; (ii) N addition would change the abundance of different functional groups; (iii) N addition would affect microbial richness (Chao1) more than Shannon diversity index due to the loss of some rare species with low tolerance to N additions; and (iv) Changes in soil organic carbon (SOC), soil N and soil pH under N addition treatments would drive the observed changes in soil microbial diversity.

2. Materials and methods

2.1. Selection criteria

To explore the effects of N addition on soil microbial diversity, we analyzed results from published data using a meta-analysis method (Hedges et al., 1999). We searched the online database (ISI Web of Science, Science Direct, Google and Google Scholar) using the following keyword combinations: “microbial diversity” or “alpha diversity” and “N addition” or “fertilization”. The following criteria were used to select appropriate studies: (1) Only field N addition studies were selected and laboratory incubation studies were not included; (2) At least one microbial diversity metric (Shannon or Chao1) was reported; (3) The N addition and control plots were established to have the same ecosystem types, dominant plant species, and soil types; (4) When studies reported data from several soil layers, only data from the upper 20 cm were included; (5) Treatments which differed in their N application rates and types of N fertilizers were entered as individual treatments. Some studies with treatments of different P or K fertilizers were also included.

2.2. Data extraction

A total of 55 studies across the world met our criteria and were included in this meta-analysis (Fig. S1, Table S1). For each study in our database, we noted the experiment location (latitude and longitude), climatic factors (mean annual temperature and precipitation), ecosystem types, durations of experiment (years), nitrogen fertilizer types, nitrogen application rate (g N m^{-2} yr^{-1}), microbial diversity metrics (Shannon or Chao1) and relative abundance of major soil microbial community members (ten major phyla for bacteria and two phyla for fungi). Data were extracted from tables and figures. The standard deviation was either reported or calculated from the standard error and sample size. When data were presented in the form of figures, they were extracted by Engauge Digitizer (Free Software Foundation, Inx., Boston, MA, USA). In addition, soil properties including SOC, soil total nitrogen (TN), C/N ratio, pH value, and microbial biomass carbon (MBC) were included if they could be obtained. For those studies (6 out of 55 studies), which did not report climate data, we used the coordinates of the study sites to extract climate data from the WorldClim database (http://www.worldclim.com) using ArcGIS (Version 10.0, ESRI, Redlands, CA).

Finally, we found a total of 198 data points, with 171 observations on Shannon index and 102 observations on Chao1 (Table S1). These data covered a wide gradient of climatic conditions and soil properties. For example, the altitude, mean annual temperature and precipitation ranged from 2 to 3500 m, from −7 to 20.6 °C, and from 164 to 1749 mm, respectively. Soil properties such as SOC (0.19–45.7%), C/N ratio (5.8–24.7) and pH (4.0–9.0) also showed wide ranges. The duration of the N addition ranged from 1 to 110 years, averaging 15 years. The annual N application rate ranged from 0.001 to 80 g N m^{-2}, averaging 13.7 g N m^{-2}. In addition, urea and ammonium nitrate (NH_4NO_3) were the most commonly used fertilizers. The fertilizer combinations included in our database were N alone (66.3%), NP (12.6%), NK (2%) and NPK (19.1%). The microbial analysis methods were generally divided into two groups: sequencing (72%, including Roche 454, Illumina MiSeq, Illumina HiSeq, Mega BACE and PacBio RS) and molecular fingerprinting (28%, including denaturing gradient gel electrophoresis (DGGE) and terminal-restriction fragment length polymorphism (T-RFLP)).

2.3. Statistical analyses

Meta-analyses were conducted on microbial diversity (Shannon index, H) and richness (Chao1), which were calculated using the following equations:

$$\text{Shannon index (H)} = -\sum_{i=1}^{s} p_i \ln p_i \quad (1)$$

where p_i is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), and S is the number of species.

$$\text{Chao1} = S_{obs} + \frac{F_1^2}{2F_2} \quad (2)$$

where S_{obs} is the total number of species observed in a sample; F_1 is the number of singleton species and F_2 is the number of doubleton species. Chao1 represents microbial richness, while Shannon index considers both richness and the relative abundance of different groups. Therefore, Chao1 is more sensitive to rare species in the community. It could be possible that Shannon index increases while Chao1 decreases under the same treatment, which generally would suggest potential loss of rare species.

The response ratio statistic was used to estimate effect size (Hedges et al., 1999). The response ratio ($\log_e R$) is the difference between the mean $\log_e$-transformed treatment value ($\overline{X}_t$) and the mean $\log_e$-transformed control value ($\overline{X}_c$):