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Nonlinear responses of forest soil microbial communities and activities after short- and long-term gradient nitrogen additions

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ABSTRACT

To compare the responses of forest soil microorganisms to short-term and long-term nitrogen (N) addition, temperate forest soils were fertilized with gradient N (0, 2.4, 4.8, 9.6, 14.4 and 19.2 g N m^{-2} y^{-1}). Soil microbial biomass and extracellular enzymatic activities (EEAs) were assayed after 1 and 5 years of the field experiment. Results showed that microbial biomass and EEAs responded nonlinearly to gradient N addition. The addition of N with limited amount alleviated the N limitation of the soil and increased soil microbial biomass and EEAs. However, soils converted to N saturation and even N inhibition when the N amount was substantial, thereby inducing a significant decrease in soil microbial biomass and EEAs. Furthermore, the nonlinear responses differed significantly between the short- and long-term treatment groups. After the short-term N addition, soil microbial biomass and EEAs substantially changed and rapidly converted to N inhibition with the increase in the amount of N. After long-term N addition, however, the changes were less pronounced. The threshold values of added N amount, which microbial biomass and EEAs reached the greatest values, significantly increased. Soil microbial communities are dynamic and self-adjustable systems. After the addition of N, microorganisms (*e.g.* fungi), which cannot survive in soils with lower ratio of carbon to nitrogen (C/N), significantly decreased. Bacteria, which are considerably tolerant to high N conditions, survived. Over the long term, soil microbial community became less sensitive to high N deposition and even might convert to N stimulation from N inhibition.

1. Introduction

Atmospheric deposition of nitrogen (N) compounds increased substantially in the second half of the 20th century, mainly because of the increased emissions from fossil fuel combustion and agricultural fertilizers (Galloway et al., 2004). This resulted in ecological consequences, including changes in soil fertility, shifts in microbial biomass, vegetation types, influences in litter decomposition and soil extracellular enzymatic activities (EEAs) (Baron et al., 2000; Carreiro et al., 2000; Guo et al., 2011a, 2017; Saiya-Cork et al., 2002).

Many studies have focused on the changes in soil microbial biomass after the addition of Sparrius and Kooijman (2013) and Paredes et al. (2011) suggested that N could accelerate microbial biomass, particularly in N-limited ecosystems (Johnson et al., 1998). However, several studies have also determined that N fertilizers, such as NH_4NO_3 and $(NH_4)_2SO_4$ have suppressive effects on microbial biomass, particularly in low-fertility sites (Liu and Greaver, 2010; Thirukkumaran and Parkinson, 2000). Nevertheless, Allison et al. (2008) determined no suppressive effect of N addition on microbial biomass. The deposition of N on fungi and bacteria also has numerous effects. Zheng et al. (2015) revealed that N addition had no effect on fungal biomass but significantly decreased soil bacterial biomass. However, Zhang et al. (2016) showed that N addition did not affect either fungi or bacteria in soils. Rousk et al. (2011) showed that N fertilization did not affect bacterial growth and marginally affected fungal growth. de Vries et al. (2006) suggested that N fertilization reduced fungal biomass more than it did bacterial biomass.

Litter decomposition and nutrient cycling are primarily biochemical processes, which are dependent on the action of extracellular enzymes. Previous studies revealed that soil EEAs showed significant responses to N fertilization (Weand et al. (2010) [β-glucosidase and polyphenol oxidase]; Michel and Matzner (2003) [polyphenol oxidase]); Geisseler and Horwath (2009) [cellulase and β-glucosidase]; Wang et al. (2008) [invertase]; Marklein and Houlton (2012) [phosphatase]; Currey et al. (2010) [*N*-acetyl-glucosaminidase and cellobiohydrolase]). However, other studies have shown that soil EEAs did not respond significantly to

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N fertilization (Thomas et al. (2012) [β-glucosidase, cellobiohydrolase]; Lucas et al. (2007) [phenol oxidase]). Sinsabaugh et al. (2002) reported that N deposition affected litter decomposition by increasing the degradation of cellulose, repressing the oxidative activities associated with recalcitrant litter.

Although strong N deposition is expected to alter the diversity and activities of soil microorganisms, the conclusions have also varied. One reason for such variety may be the duration of the N stimulated experiment. Magill et al. (2000) found that foliar N content reached a maximum after a four-year N addition and did not increase through six years. Fang et al. (2009) and Lu et al. (2013) reported that no changes were observed in the dissolved organic carbon (C) concentrations after a two-year addition of N; however, the concentrations for the same site significantly decreased after six years. Magill and Aber (1998) demonstrated that the addition of N did not consistently increase the decay rates in the first year; the addition of N actually reduced the decay rates over the long-term (six years). However, the changes in soil microbial biomass and EEAs were not determined. In this work, a temperate forest ecosystem was selected, which has been constantly and regularly fertilized with different amounts of N for five years. We compared the changes in soil microbial biomass and EEAs between short-term (after one year) and long-term (after five years) N additions. We hypothesized that the composition and activities of the soil microbial community may change to adapt to the high N conditions.

2. Material and methods

2.1. Site description and experiment design

This study was conducted in Wuyuezhai National Forest Park (38°41′ N, 113°52′ E), which is located in Shijiazhuang, China. This park encompasses 120 km^2 and has a maximum elevation of 1946 m. It has a temperate, humid climate with an annual mean temperature of 12.1 °C and annual precipitation of 800 mm. The main tree species in this forest include *Betula platyphylla*, *Populus davidiana*, *Pinus tabulaeformis*, and *Platycladus orientalis*. The total N deposition level is approximately 2.35 g N m^{-2} y^{-1} (Zhang et al., 2006).

The study site was selected where *Betula platyphylla* was the dominant tree species. The soil in the study site is slightly acidic and brown, with total N of 1.48–1.91 g kg^{-1}, total organic C of 18.5–24.1 g kg^{-1}, and phosphorus (P) of 0.16–0.22 g kg^{-1}. Four 10.0 × 15.0 m plots with a buffer zone of 50 m between plots were randomly established. Each plot was subdivided into 6 subplots (5.0 m × 5.0 m) by embedded plats to avoid interference when N solutions (NH_4NO_3) of different amounts were applied. Each of the 6 subplots received one of the following treatments: N1 (2.4 g N m^{-2} y^{-1}), N2 (4.8 g N m^{-2} y^{-1}), N4 (9.6 g N m^{-2} y^{-1}), N6 (14.4 g N m^{-2} y^{-1}), N8 (19.2 g N m^{-2} y^{-1}), and N0 (deionized water only), which were equivalent to 1, 2, 4, 6, 8, and 0 times respectively of the annual atmospheric N deposition for the study site. N fertilizer was sprayed on each subplot every 3 months. The study started in September 2010.

2.2. Sampling, storage, and microbial biomass and EEA assays

In September 2011 and September 2015, soil was sampled by using a 5 cm diameter metal corer and cores were randomly sampled to a depth of 5 cm from each plot. Soil samples were stored in sealed bags and transported immediately to the laboratory. In the laboratory, fresh soil samples were passed through 2-mm mesh to eliminate leaves, roots, and gravel. Immediately after sieving, 5.0 g of each fresh soil sample was used for soil microbial biomass determination. The rest of the soils were used for enzymatic activity assays.

Soil microbial biomass was measured using the substrate-induced respiration (SIR) method (Guo et al., 2011b; Kooijman et al., 2016). During the determination, bacteria and fungi were selectively inhibited with streptomycin (4 mg g^{-1} soil) and cycloheximide (3 mg g^{-1} soil) (Kooijman et al., 2016). Enzymes involved in litter decomposition such as invertase, β-galactosidase, polyphenol oxidase, and cellulase were assayed spectrophotometrically using a UV/Vis spectrophotometer. Supplemental material lists the methods of enzymatic assay and International Unit (IU) definition. All data were expressed using soil dry weight.

2.3. Statistical analyses

All data were statistically analyzed by analysis of variance (ANOVA). Significant differences were accepted at the $p < 0.05$ level of probability. Correlations between measured variables were analyzed using the Spearman rank correlation test. Regression analysis was used to analyze the relationships between the amount of N and each data (*i.e.*, microbial biomass and EEAs). The regression model design is illustrated as follows:

$$y = a\,(x + b)^2 + c$$

where x is the added N amount, and a, b, and c are the constants. In this model, the N addition was assumed to exhibit positive effects on each dataset. All statistical analyses were performed using SPSS 17.0 for Windows.

3. Results

In this work, soil microbial biomass responded differently to gradient N addition. In 1-year N addition treatment groups, compared with N0 (0.1951 μmol CO_2 h^{-1} g^{-1} soil), soil fungal biomass (determined using SIR) was higher in N1 and N2 but lower in N4, N6, and N8 (0.2101, 0.2114, 0.1926, 0. et al., 17541833, and 0.1754 μmol CO_2 h^{-1} g^{-1} soil, respectively). Bacterial biomass showed similar results, but it declined only in N6 and N8 (0.2029 and 0.1955 *vs.* 0.2121 μmol CO_2 h^{-1} g^{-1} soil). Meanwhile, compared with N0 (0.9197), the fungi:bacteria ratio (F:B ratio) increased in N1 and N2 (0.9777 and 0.9678) and decrease in N4, N6, and N8 (0.9076, 0.9043, and 0.8992). In the 5-year N addition treatment groups, microbial biomass showed similar changes, but the increases or decreases were less pronounced (−10.08 to 8.34% in the 1-year N addition treatment groups *vs.* −6.47 to 5.80% in the 5-year N addition treatment groups). Compared to N0 (0.1953 μmol CO_2 h^{-1} g^{-1} soil), Soil fungal biomass increased in N1, N2, and N4 (0.1949, 0.2002, and 0.2062 μmol CO_2 h^{-1} g^{-1} soil) but declined in N6 and N8 (0.1853 and 0.1822 μmol CO_2 h^{-1} g^{-1} soil). Bacterial biomass and F:B ratio showed similar trends as fungal biomass (Fig. 1). Regression analysis revealed that both fungal and bacterial biomass primitively increased with the increase of x (N amount) but

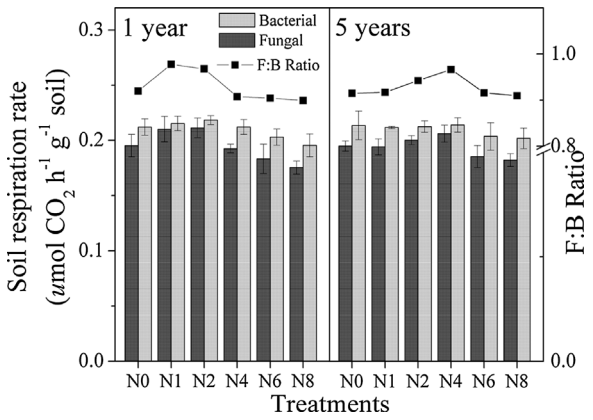

Fig. 1. Responses of soil fungal, bacterial biomasses and F:B Ratios to short-term (1 year) and long-term (5 years) gradient N fertilization treatments. The treatments were N0 (deionized water), N1 (2.4 g N m^{-2} y^{-1}), N2 (4.8 g N m^{-2} y^{-1}), N4 (9.6 g N m^{-2} y^{-1}), N6 (14.4 g N m^{-2} y^{-1}) and N8 (19.2 g N m^{-2} y^{-1}). Error bars indicate standard deviation (SD, $n = 4$).