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Response of soil microbial communities and nitrogen thresholds of *Bothriochloa ischaemum* to short-term nitrogen addition on the Loess Plateau

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ABSTRACT

China is becoming the world's third largest area of nitrogen (N) deposition, which is attracting increasing attention. Understanding how N affects soil microbial communities and determining the thresholds for the effect of N on microorganisms and ecosystems are critical. We investigated the changes to the characteristics of microbial communities after two years of adding N to soil with the perennial grass *Bothriochloa ischaemum*, simulating N deposition on the Loess Plateau, at four rates of N addition (0 (CK), 2.5 (N1), 5.0 (N2), and 10.0 (N3) g N m^{-2} y^{-1}) and a control BL (bare land without vegetation or N addition). Soil microbial biomass carbon (C) and N contents and microbial activity increased in N1. The lowest rate of N addition (N1) increased soil total, bacterial, fungal, and actinomycetic phospholipid fatty acid (PLFA) contents, but excessive N addition decreased bacterial and actinomycetic PLFA contents. N addition did not alter microbial-community structure. The effect of N addition on soil microbial properties was influenced by soil C content (SOC and DOC), increased the diversity and evenness of the microbial community and decreased the diversity of the bacterial community. Soil microbial biomass and activity increased in N1, which was beneficial to the stability of the soil ecosystem on the plateau and defined the threshold of N addition for microorganisms and the ecosystem. More attention should thus be paid to depositional level represented by N2 (5 g N m^{-2} y^{-1}), which might limit microbial communities. The microbial community was inhibited, the diversity decreased, and the ecological system was affected by the level represented over N2.

1. Introduction

Nitrogen (N) is an important limiting element in most terrestrial ecosystems, and increased N contents can increase food production, plant diversity and plant coverage in degraded areas and improve the function of ecosystems (Isbell et al., 2013). The burning of fossil fuels, the production and use of chemical fertilizers, and the influence of human activities and animal husbandry are increasing atmospheric N deposition (Holland et al., 1999). And soil N content is close to or even beyond the threshold of ecosystems in some areas, which is causing various serious ecological environmental problems such as a decrease in plant diversity (Clark and Tilman, 2008; Stevens et al., 2004) and soil acidification caused by changes to the physical and chemical environment (Phoenix et al., 2012). These issues have drawn the widespread attention of the scientific community and are being actively studied for their contributions to global climate change.

The response of microbial communities to N in terrestrial ecosystems is mainly influenced by the duration and content of N inputs and is weakly affected by N type and mode of application (Treseder, 2008). Changes to a microbial community can be obvious within the first five years of N addition (Treseder, 2008), and long-term N addition can reduce microbial biomass, inhibit respiration, and decrease microbial diversity (Janssens et al., 2010; Liu and Greaver, 2010; Zhong et al., 2015). The results of experimental short-term N addition, however, have not been inconsistent. Zhang et al. (2005) indicated that short-term N addition significantly increased grassland microbial biomass in the dry, hot valleys along the Jinsha River in China, and some studies have shown that short-term N addition can significantly increase soil respiration (Zong et al., 2013). A study in New Zealand indicated that N addition decreased microbial biomass (Sarathchandra et al., 2001), and Ramirez et al. (2012) reported that microbial biomass and respiration intensity decreased in a short-term N-addition incubation experiment. Johnson et al. (2005) reported that N addition did not change microbial biomass in Scotland. The response of microorganisms

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to N deposition in a region thus likely depends on soil N content and the rate and duration of N deposition. N content is relatively low in some areas, and an appropriate amount of N addition can mitigate N limitation in an ecosystem, increase the activity of microorganisms (Yao et al., 2014), and change community structure (Bai et al., 2010). N input beyond saturation will inhibit soil microorganisms. Analyzing the effect of N addition on microbial characteristics and understanding the threshold of the effect of N addition on microbial communities in terrestrial ecosystems are therefore very important.

Thresholds are critical values beyond which ecosystemic functions will change. When the contents of N deposition exceed the tolerance of a system, the function of the system will change in an unpredictable manner (Wei et al., 2013). The threshold of N addition that different ecosystems can sustain in different regions is not consistent. A farmland ecosystem had a threshold of 180 kg ha^{-1} y^{-1} (Zhong et al., 2015), a grassland in Inner Mongolia had a threshold of 56 kg ha^{-1} y^{-1} (Wei et al., 2013), and a forest in the USA had a threshold of 19 kg ha^{-1} y^{-1} (Fenn et al., 2010). The N-saturation concentrations that plants, soil microbes, and soil physical and chemical properties can endure are also inconsistent, even within the same ecosystem. For example, Wei et al. (2013) found that N-deposition rates > 112 kg ha^{-1} y^{-1} significantly decreased microbial biomass, while the two main functional communities of plant have inconsistent threshold (56 kg ha^{-1} y^{-1} for perennial bunch grass; no specific threshold for perennial rhizome grasses), and soil pH significantly changed at 56 kg ha^{-1} y^{-1}. Understanding the thresholds of different components in the ecosystem of the hilly-gully region of the Loess Plateau in China is extremely important, so comprehensively determining the threshold of N deposition for regional environmental protection and for developing policies and regulations that inhibit N deposition is necessary.

The impact of N on microorganisms can be divided into direct and indirect effects by soils and plants. The effects of N on microorganisms are likely associated with the supply of plant carbon (C) and productivity even though the forms of N added to the soil may differ (Mooshammer et al., 2014) and are also likely associated with the production and efficiency of enzymes that decompose organic matter. N can also decrease soil pH and thus has an indirect influence on soil microbial communities (Mooshammer et al., 2014; Vitousek et al., 1997) by inhibiting bacterial diversity (Zhang and Han, 2012), and directly lower the C:N ratio that can increase the relative abundance of fungi, and significantly decrease the bacteria: fungi ratio (Yevdokimov et al., 2008), thus changing the structure of microbial communities.

Soil erosion is a serious problem on the Loess Plateau, which is a typical ecologically fragile area in the country. Low effective N contents in the soil and serious soil and water losses make this area the most representative grassland ecosystem affected by N deposition. The amount of N deposition has recently increased dramatically, so conducting experiments to analyze the impact of N addition on microbial characteristics and to determine the N threshold for the ecosystem is particularly urgent. We therefore used an area of the plateau with native vegetation (*Bothriochloa ischaemum* (L.) Keng, a perennial grass) as experimental materials, testing various levels of N addition to simulate N deposition. Our aim was to determine the effect of short-term N addition on soil microbial activity and community structure, identify the N threshold and mechanism of N deposition on typical grassland soil microorganisms, and estimate the threshold for the ecosystem.

2. Material and methods

2.1. Site description

The experiment was conducted at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau in Yangling (34°27′N, 108°7′E; 530 m a.s.l.). This region is characterized by a temperate continental monsoon climate with a mean annual temperature of 13.2 °C and a mean annual precipitation of 674.3 mm. Homemade soil bins with a slope of 15° were used to simulate the natural slope where natural *B. ischaemum* communities live. The bins were 2.0 m long, 1.0 m wide, and 0.5 m deep. Seeds of *B. ischaemum* were acquired in autumn 2012 from the experimental fields at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′30″N, 109°19′23″E; 1068–1309 m a.s.l.). The loessial soil used in our experiment was obtained from the upper 20 cm of an arable field at ARS. The soil had a bulk density of 1.2 g cm^{-3}, organic-matter content of 1.3 g kg^{-1}, and total N (TN) and phosphorus (TP) contents of 0.19 and 0.27 g kg^{-1}, respectively. Soil was added to the bins in 10-cm layers to a depth of 40 cm, with a bulk density of about 1.2 g m^{-3}. The soil was well watered before sowing to ensure seedling establishment. The seeds were sown at a density of 10 × 10 cm. Excess grass plants and weeds were manually removed during the experiment to restrict plants of the same size to one per hole.

2.2. Experimental design

The experiment had four levels of N addition, based on the global N sedimentation levels (Bobbink et al., 2010) and the amounts of N addition in experiments in China and other countries, and five treatments: bare land (BL) with no vegetation or N addition, CK (0 g N m^{-2} y^{-1}) with vegetation but no N addition, N1 with vegetation and 2.5 g N m^{-2} y^{-1}, N2 with vegetation and 5 g N m^{-2} y^{-1}, and N3 with vegetation and 10 g N m^{-2} y^{-1}. Three replicates of the five treatments received additional N in the form of urea ($CO(NH_2)_2$). The experiment ran for two years. The seed were sown in June 2013. N was applied in August 2013 (the amount of N for one year) and in May, June, July, and August in 2014 as a solution of urea in 1 l of deionized water (equivalent to the amount of N in a year divided into four applications; CK and BL received the same volume of water).

2.3. Soil sampling and analysis

Soil was sampled in September 2014. Soil cores (20 × 20 cm quadrat) were collected to a depth of 20 cm from six randomly selected locations in each bin and combined into one composite sample. This sample was sieved through a 2-mm mesh after the stones and roots were manually removed. The sieved samples were divided into two subsamples. One subsample was air-dried and then divided into two parts. One part was sieved through a 0.25-mm mesh for the determination of soil total organic C (SOC), TN, TP, nitrate N, and ammonium N contents, and the other part was sieved through a 1-mm mesh for the determination of pH and available P (aP) content. The other subsample was also further divided into two parts. One part was stored at 4 °C for measuring soil microbial biomass C (SMBC), soil microbial biomass N (SMBN), basal respiration (BR), and soil-induced respiration (SIR). The other part was stored at − 80 °C for the determination of phospholipid fatty acid (PLFA) contents.

The chemical and physical properties of the soil were determined using standard procedures. SOC was measured with the H_2SO_4-$K_2Cr_2O_7$ method. TN was measured using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil TP was determined colorimetrically after digestion with H_2SO_4 and $HClO_4$ (Schade et al., 2003). Soil NH_4^+-N and NO_3^--N in filtered 2.0 mol l^{-1} extracts of fresh soil sample were measured with a flow injection autoanalyzer. Soil pH was determined in 1:2.5 (w:v) solutions. Soil aP was measured by molybdenum-antimony colorimetry with $Na(HCO_3)_2$ extracts. Dissolved nutrients were extracted with deionized water after shaking 1 h and then filtering through prewashed cellulose acetate filters (0.45 μm pore size). TDN (total dissolved N), DOC, N-NH_4^+, N-NO_3^- were measured. The DOC concentrations were determined using TOC analyzer (liqui TOC II, elementar, Germany). The TDN concentrations were determined using alkaline digestion-UV spectrophotometric method. Dissolved organic nitrogen (DON) was calculated as TDN- ($NH_4^+ + NO_3^-$). Table 1 shows the basic physical and chemical properties of the soil, And C:N is the