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The effects of N and P additions on microbial N transformations and biomass on saline-alkaline grassland of Loess Plateau of Northern China

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ABSTRACT

Microbial nutrient transformation plays an important role in regulating nitrogen (N) and phosphorus (P) cycling in terrestrial ecosystems. Soil N and P contents also control microbial nutrient transformations. However, there is still dispute on how N and P additions affect microbial activity and N transformations. A field experiment was conducted to examine the effects of N and P on microbial N transformations and biomass in saline-alkaline grassland in Loess Plateau of northern China during growing season in 2009. N was added at a rate of 10 g N m^{-2} y^{-1} in the form of NH_4NO_3. P was added at a rate of 5 g P m^{-2} y^{-1} in the form of $P_2O_5^-$. We measured the *in situ* net ammonification rate (R_{amm}), and nitrification rate (R_{nit}) once a month from May to October; we also measured potential soil microbial biomass carbon (MBC), nitrogen (MBN), and potential microbial respiration (MR) once a month in laboratory.

Results: During the whole growing seasons, P addition significantly stimulated soil inorganic N pool, soil extractable C, soil extractable N pool, R_{min}, and the metabolic quotient (qCO_2) from the estimates of microbial respiration and microbial biomass carbon, and there was no effect on peak aboveground biomass, MBC, MBN and MR during the whole growing seasons in 2009. N addition significantly increased peak aboveground biomass, inorganic N pool, R_{min}, MBN, MR, and qCO_2, decreased soil extractable C and the ratio of MBC/MBN, and there was no effect on soil extractable N and MBC during the growing season in 2009. P addition increased the soil net N mineralization rate and N addition not only increased the soil net N mineralization rate but also increased microbial biomass N. We observed that P induced a decreased soil inorganic N pool, but N addition directly increased soil inorganic N pool, how to balance the quantity of N and P additions in agriculture system is an important technique in agriculture harvest in the future in Loess Plateau of Northern China.

1. Introduction

Human activities have altered global nutrient cycles and caused nutrient enrichment, especially N and phosphorus (P) in different ecosystems (Mahowald et al., 2008; Vitousek and Farrington, 1997). Nitrogen is an important growth-limiting nutrient in terrestrial ecosystems (Vitousek and Howarth, 1991). However, in addition to increasing N availability, N deposition has increased the mobility of N (Vitousek and Farrington, 1997), and it can alter the rates and the pathways of N cycling and loss (Aber et al., 1989). Nitrogen enrichment can influence composition of biota and processes of the ecosystem. Matson et al. (2002) reviewed the consequences of N deposition for terrestrial ecosystems, and they suggested that not all ecosystems respond to N deposition similarly. In addition, P fertilization can lead to eutrophication of water by P runoff and leaching (Schoumans and Groenendijk, 2000), but few studies have examined the ecological effects of P addition on terrestrial ecosystems (Stocklin et al., 1998). Moreover, the effects of simultaneous N and P additions on terrestrial ecosystems remain uncertain (Elser et al., 2007) because of the limited number of studies. Soil quality is an important indicator in ecosystem management and sustainability (Masto et al., 2008), and thus, changes in soil quality due to nutrient additions need to be evaluated (Malý et al., 2009), so as to develop effective strategies for the management and sustainability of ecosystems under nutrient additions.

Soil microorganisms play a key role in sustaining soil quality, and soil microbial properties have been proposed as sensitive indicators of changes in soil quality (Filip, 2002; Jenkinson, 1988; Nannipieri et al., 2003). Soil microbial biomass serves as a source and sinks of plant available nutrients and is closely related to soil quality (Kaschuk et al., 2010). Soil microbial activity controls soil nutrient availability and cycling (Jonasson et al., 1996; Schmidt et al., 1997; Singh et al., 1989), and plays an important role in the nutrient storage capability of soils (Jonasson et al., 1996; Schmidt et al., 1997; Writer and Kanal, 1998). On the contrary, microbial activity is strongly influenced by nutrient availability. For example, some results have shown that the addition of N and P can increase the rate of soil C mineralization,

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microbial biomass and enzymatic activity (Chantigny et al., 1999; Joergensen and Scheu, 1999; Jonasson et al., 1996). Thus, we hypothesize that soil microbial activity was enhanced by N addition.

Most of the studies of the effect of fertilization on soil microbial activity were conducted in boreal and temperate ecosystems (i.e. Chantigny et al., 1999; Dilly and Nannipieri, 2001; Joergensen and Scheu, 1999; Jonasson et al., 1996; Schmidt et al., 1997). Despite the severity of soil degradation in semi-arid regions of northern China little is known about the effects of fertilization in saline–alkaline soils in this area. Understanding the effects of fertilization on soil fertility is important to evaluate the ecological consequences of land use change in saline–alkaline grassland. Grasslands that are characterized by saline–alkaline soils constitute a large percentage of natural vegetation in arid and semi-arid regions of northern China. Located at the ecotone of agro-pastoral systems, Youyu saline–alkaline grasslands are one of the biggest saline–alkaline lands in China and are characterized by nutrient-poor soils. Rapid economic development in China has increased nutrient addition to soil throughout the country (Li and Chen, 2004; Lü and Tian, 2007). Availability of soil N and P limited growth at the low-stress site for plant, but P and Mg limited growth at the high-stress site (James et al., 2005). To date, however, few studies about the effects of nutrient addition on soil quality are available for this saline–alkaline. In this study, we have evaluated changes in soil microbial and chemical properties after 1 year of N and P additions in a saline–alkaline grassland in loess plateau of northern China.

Although much research has been conducted on the responses of microbial activity to N addition that has considered the implications for ecosystem N cycling, there has been little research effort devoted to the changes in microbial N transformation, which includes ammonification, nitrification and mineralization etc., in response to N addition in saline–alkaline grassland ecosystems in northern China.

This research work was designed to characterize soil microbial responses to changes in N and P availability due to N and P fertilization. We hypothesized that P addition will further enhance N addition effects on microbial processes. The specific objectives of this study were to evaluate the individual and combined effects of N and P addition on, (1) *in situ* net N nitrification and mineralization; and (2) microbial biomass and activities.

2. Materials and methods

2.1. Site description

The experimental site is located in Youyu county (42°02′N and 116°17′E), a semi-arid area in north of Shanxi, China. The mean annual precipitation in this region is about 350 mm. The average annual temperature is about 2 °C, with monthly mean temperatures ranging from the lowest (−18 °C) in January to the highest (22 °C) in July. The soil at our sites is chestnut soil (Chinese classification) or Calcic Luvisol. Soil bulk density and pH values are 1.33 ± 0.04 cm^{-3} and 9.98 ± 0.15, respectively. Soil organic C and total N contents in the topmost 10 cm of the mineral soil are 12.3 ± 0.9, and 1.25 ± 0.12 g kg^{-1}, respectively.

2.2. Experimental design

Twenty four 6 m × 6 m plots were established in Aug. 2008. Four different treatments with six spatially randomly selected replicates were conducted as follows: a) control, b) N addition, c) P addition d) N + P addition. Nitrogen (NH_4NO_3) and phosphorus (KH_2PO_4) addition was applied before it rained in mid-July 2008, and 2009 at a rate of 10 g N m^{-2} performed and 5 g P m^{-2} in early 2008, and 2009.

Topsoil (0–10 cm mineral soil) samples were taken at monthly intervals from all 24 plots for the determination of net N turnover and microbial activity. MBC and MBN, as well as MR were measured from May to October in 2009. Soil moisture (0–10 cm) was measured using drying method in each plot once a month from May to October.

2.3. Soil sampling and field incubation

For measurements of *in situ* net N mineralization during the growing season in 2009, we used the buried soil core technique, as described by Raison et al. (1987). At each sampling date and in each plot, four sharpened PVC cylinders (5 cm in diameter and 12 cm in length) were randomly driven 10 cm into the soil adjacent to each other (<5 cm in distance) after the removal of aboveground living plants and litter. Two cylinders were immediately removed, and the other two remained for 28 days in the field. Cores were covered with parafilm to minimize evaporation during the 28-day *in-situ* incubation. Following sampling, soil cores were immediately processed at a laboratory located at the Youyu research station: the soil was mixed, stones and coarse roots removed and, finally, soil was sieved through 2-mm mesh and stored at 4 °C until further analyses were conducted (<48 h) in the laboratory of the Institute of Botany in Beijing.

To analyze inorganic N, a 10-g aliquot was taken from each sieved soil sample and extracted with 50 ml of 2 mol l^{-1} KCl solution. The soil suspension was rigorously shaken for 1 h in a reciprocal shaker and then filtered through Whatman No. 1 filter paper (12.5 cm in diameter). Soil solutions were immediately analyzed for NH_4^+-N and NO_3^--N on a FIAstar 5000 Analyzer (Foss Tecator, Denmark). Net N mineralization/nitrification rates were calculated from differences in soil NH_4^+ and NO_3^- concentrations between day 0 and day 28, and mineralization/nitrification rates were expressed on a dry mass basis.

Microbial biomass was measured using the fumigation-extraction method (Vance et al., 1987). Briefly, the fresh soil samples were carefully adjusted to about 60% of field water-holding capacity. 60 grams soil were put into plastic and covered with parafilm in the dark and incubated for 10 days at 25 °C. Then, 20 g moisture sample was as control, another 20 g moist samples were fumigated for 24 h with ethanol-free $CHCl_3$ (Vance et al., 1987), the last soil samples were left for soil moisture content. Soil extracts from control and the fumigated samples were obtained by shaking soil samples with 50 ml of 0.5 M K_2SO_4 for 30 min. Extracts were filtered through 0.45-μm filters and frozen at −20 °C before analysis of extractable C and N by dichromate digestion and Kjeldahl digestion, as described by Lovel et al. (1995). Microbial biomass C and N were calculated from the difference between extractable C and N contents in the fumigated and control samples using conversion factors (k_{EC} and k_{EN}) equal to 0.38, and 0.45 (Lovel et al., 1995), respectively. All results were expressed on an oven-dried soil basis (105 °C, 24 h).

Microbial respiration was measured by alkali absorption of CO_2 evolved at 25 °C and optimal soil moisture for 10 days, followed by titrating the residual OH^- with standardized acid (Hu and Bruggen, 1997). Briefly, 20 g fresh soil was placed evenly in a 500-ml glass flask. The glass flask was connected with a glass tube (6 cm in diameter) in which 5 ml of 0.05 M NaOH solution was injected to capture the CO_2 that was produced by the soil microbes. The metabolic quotient (qCO_2) was calculated as follows: [(milligrams CO_2-C evolved in 10 days kg^{-1} soil)/(milligram microbial biomass C kg^{-1} soil)/(10 days × 24 h) × 1,000], and thus, the qCO_2 was expressed as mgCO_2-C g^{-1} $C_{microbial}$ h^{-1} (Wardle and Ghani, 1995). All microbial indices were calculated on the basis of the mass of oven-dried soil.

2.4. Statistical analysis

The seasonal mean values used in this study were calculated from the monthly mean values, which were first averaged from all measurements in the same month. We included values both before and after P and N addition to evaluate the P and N effects. Two-way ANOVA was used to examine the effects P addition, and N addition, and their possible interactions on microbial N transformation, biomass and respiration. After observing that the interaction between our collected data and