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Decoupling of soil microbes and plants with increasing anthropogenic nitrogen inputs in a temperate steppe

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ABSTRACT

Plant growth and soil microbial activity are intrinsically correlated. Numerous evidence shows that nitrogen (N) deposition can greatly alter both processes. However, it is unknown whether such changes caused by N deposition can create new dynamics between plants and soil microbes. This study was conducted with an attempt to examine the plant–microbe relationship along an N addition gradient. Eight levels of N addition (0, 1, 2, 4, 8, 16, 32, 64 g N m^{-2}) were applied annually in a temperate steppe in northern China since 2003. Plant and soil samples were collected from 2005 to 2007. We found that N addition acidified soil significantly. Both plant aboveground biomass and dissolved organic carbon (DOC) increased with increasing N input. However, soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and (soil) microbial respiration showed nonlinear responses to N input. Low levels of N inputs stimulated MBC, MBN and microbial respiration, whereas high levels of N input suppressed them. Although MBC and MBN were both positively correlated with aboveground biomass at each level of N treatments, the dependence of such biomass on MBC and MBN declined with the increase in N addition, as indicated by the exponential decreases in the regression coefficients. The weakened linkage between aboveground biomass and MBC was mostly attributed to soil acidification. The decrease in soil pH caused by elevated N inputs reduced soil microbial activities, but not plant growth. Overall, our results revealed a trend of shifting plant–microbe relationship from coupling to decoupling with the increase of N input. The divergent responses of plants and soil microbial activities under intensified N addition could have consequent impacts on ecosystem function and services.

1. Introduction

Plants and soil microbes are critical to maintain the functions of natural terrestrial ecosystems (Reynolds et al., 2003; Wardle et al., 2004; Wagg et al., 2011). Plant productivity and soil microbial activities often tightly coupled, especially in nutrient poor ecosystems (Bardgett et al., 1999; Paterson, 2003; van der Heijden et al., 2008). Plants depend upon nutrient supply mediated by soil microbes, which mineralize nutrients from organic to inorganic forms that can be utilized by plants (De Deyn et al., 2004; Bardgett et al., 2005; van der Heijden et al., 2008). On the other hand, soil microbes rely on plants for carbon (C) substrates in the form of litter and root exudates (Paterson, 2003; Wardle et al., 2004; Bardgett et al., 2005).

Atmospheric nitrogen (N) deposition, mainly from fossil fuel combustion and fertilizer applications, has risen dramatically since the Industrial Revolution and is projected to continually increase in the future (Erisman et al., 2011). Such global N enrichment has profoundly altered biogeochemical cycles in both aquatic and terrestrial biospheres (Gruber and Galloway, 2008). N is often the most limiting nutrient for plant growth (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). Plant growth and productivity generally increased with the increase of N deposition across various terrestrial plant species and ecosystems (Elser et al., 2007; Xia and Wan, 2008). The stimulation of plant growth and primary productivity induced by enhanced N availability will increase aboveground litter inputs to soil (Liu and Greaver, 2010), and may also result in greater belowground C input by increasing root growth and root exudates, especially in nutrient poor sites (Bradford et al., 2008). Consequently, growth and activity of soil microorganisms are expected to increase with the increase in C supply by plants. However, an increasing body of evidence has demonstrated

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reductions in soil microbial biomass and respiration under N enrichment across various terrestrial ecosystems (Treseder, 2008; Liu and Greaver, 2010; Ramirez et al., 2012). Such negative impacts were exacerbated with increasing amount and duration of N addition (Treseder, 2008). The differential responses of plants and soil microbes to N addition point to the possibility that the linkages between plant–microbe may be weakened under high anthropogenic N inputs.

The relationships between terrestrial plants and soil microorganisms are crucial for maintaining the functions and stability of the terrestrial ecosystems. If the coupling between plants and soil microbes is disrupted, it could have unexpected impacts on the ecological goods and services. However, few studies have been conducted to examine whether environmental changes, such as increasing in anthropogenic N input, will result in a decoupling of plants and soil microorganisms.

A field manipulative experiment with eight N addition levels (0, 1, 2, 4, 8, 16, 32, 64 g N m^{-2} y^{-1}) has been conducted in a temperate steppe in northern China since 2003, which is experiencing increasing N deposition because of nearby intensive agriculture and industry (Liu et al., 2011). We measured plant and microbial growth from 2005 to 2007, and aimed to address two hypotheses: 1) plant growth and soil microbial activities will show differential responses to N addition; 2) the coupling between plant growth and soil microbial activities will be disrupted with the increase in N input.

2. Materials and methods

2.1. Experimental design

The study site is located in a semiarid grassland (42°02′N, 116°17′E and 1324 m a.s.l) in Duolun County, Inner Mongolia, China. Long-term (1953–2007) mean annual temperature and precipitation are 2.1 °C and 383.5 mm, respectively. The soil is Haplic Calcisols (FAO classification) with mean bulk density of 1.31 g cm^{-3} and pH of 6.84. The vegetation is typical steppe dominated by perennial herbs, including *Stipa krylovii* Roshev., *Artimesia frigida* Willd., *Potentilla acaulis* L., *Cleistogenes squarrosa* (Trin.) Keng., *Allium bidentatum* Fisch. Ex Prokh., and *Agropyron cristatum* (L.) Gaertn.

Sixty-four 10 × 15 m plots were established in 8 rows and 8 columns in 2003. Each of the 8 plots in each row was randomly assigned with one of the 8 levels of N addition treatments (0, 1, 2, 4, 8, 16, 32 and 64 g N m^{-2} y^{-1} in the form of urea). Four rows (one in each two rows) were clipped once a year since 2005. N was applied once a year in July since 2003.

2.2. Sampling and measurements

Peak aboveground biomass was estimated by clipping living biomass in the middle of August each year from 2005 to 2007. All living plant tissues were harvested from a 1 × 1 m quadrat in each plot, and ground litter in the same quadrat was also collected. All plant samples, including litter, were oven-dried at 70 °C for 48 h and weighed to determine biomass.

Soil samples were taken from each of the 64 plots on 20 August 2005 and 21 August 2007 coinciding with maximal aboveground biomass. Soil samples were collected from the 32 plots of clipping control on 19 August 2006. In each plot, five soil cores (15 cm in depth and 5 cm in diameter) were randomly taken and completely mixed to obtain a composite sample. After removing roots or stones by sieving with a 2 mm mesh size sieve, the soil samples were stored in ice boxes and subsequently transferred to the lab for dissolve organic C (DOC), inorganic N concentration, and microbial analysis. Subsamples of each soil sample were obtained to measure gravimetrical water content and soil chemical properties (air-dried, finely ground and sieved with mesh < 250 μm). Soil inorganic N (NH^{4+}–N and NO^{3-}–N) was extracted with 2 M KCl solution and the concentrations of NH^{4+}–N and NO^{3-}–N in the extracts were subsequently measured using a flow injection analyzer (SAN-System, Netherlands). The pH values were determined with a combination glass electrode (soil:water W/V ratio 1:2.5).

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were estimated using the chloroform fumigation-extraction method (Vance et al., 1987). MBC and MBN were calculated from the differences between extractable C and N contents, respectively, in the fumigated and the unfumigated samples using conversion factors of 0.45. Microbial respiration was measured by alkali absorption of CO_2 evolved at 25 °C for 14 days, followed by titrating the residual OH^- with a standardized acid (Hu and Bruggen, 1997).

2.3. Data analysis

Three-way factorial analyses of variance (ANOVA) were used to examine the effects of year, N addition and clipping on soil chemistry and microbial variables. Due to the lack of main effects of clipping or its interactions with N addition on the measured parameters, the data under clipping treatment were combined with that under control. Two way ANOVA was then used to determine the effects of year and N addition on these parameters. Differences in the mean of soil chemical and microbial measurements along the N addition gradient treatments were evaluated using one-way ANOVA for each year from 2005 to 2007.

To test changes in temporal relationships between soil microbial variables with aboveground biomass under N addition, simple linear regressions were used to determine the relationships between soil MBC and MBN with aboveground biomass across the 3 years at each N treatment levels (1, 2, 4, 8, 16, 32, 64 g m^{-2} N y^{-1}), respectively. The exponential regressions were used to examine the relationships between correlation slopes and corresponding *r* square values with N addition rates. To examine whether N addition rates affected the relationships of soil microbes with plant and soil properties, the data in each plot across the 3 years were averaged. Given that the microbial variable usually peaked at middle level of N addition rate (4 g m^{-2} N y^{-1}), simple linear regressions were used to determine relationships of microbial variable with aboveground biomass and pH value at low to middle (including 0, 1, 2, 4 g N m^{-2}) and middle to high N addition treatments (including 4, 8, 16, 32 and 64 g N m^{-2}). Stepwise multiple regressions were used to examine relationships between soil microbial biomass and respiration with aboveground biomass, litter biomass, DOC, inorganic N concentration and pH value below 4 g m^{-2} N addition (including 0, 1, 2, 4 g N m^{-2}) and above 4 g m^{-2} N addition treatments (including 4, 8, 16, 32 and 64 g N m^{-2}), respectively. All statistical analyses were performed using SAS V.8.1 (SAS Institute Inc., Cary, NC, USA). Significance was accepted at the $P < 0.05$ level of probability.

3. Results

3.1. Soil chemistry, plant growth and microbial activities

Soil inorganic N concentration increased with increase in N supply (Table S1; Fig. 1a). N addition acidified the soil significantly. Soil pH decreased with the increase in N input, and the magnitudes of pH decline increased with the experimental duration (Fig. 1b).

Aboveground biomass increased along the N addition gradient in 2005 and 2006 (Table S1; Fig. 1c). However, the stimulation of