Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification

Dima Chen a,b,*, Zhichun Lan a, Shuixin Hu b, Yongfei Bai a

a State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
b Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, USA

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ABSTRACT

Terrestrial ecosystems worldwide are receiving increasing amounts of biologically reactive nitrogen (N) as a consequence of anthropogenic activities. This intended or unintended fertilization can have a wide range of impacts on the above- and belowground communities. An increase in high N availability has been assumed to be a major mechanism enhancing the abundance of above- and belowground communities. In addition to increasing available N, however, N enrichment causes soil acidification, which may negatively affect above- and belowground communities. The relative importance of increased N availability vs. increased soil acidity for above- and belowground communities in natural ecosystems experiencing N enrichment is unclear. In a 12-year N enrichment experiment in a semi-arid grassland, N enrichment substantially increased both above- and belowground plant biomass mainly via the N availability-induced increase in biomass of perennial rhizome grasses. N enrichment also dramatically suppressed bacterial, fungal, and actinobacteria biomass mainly via the soil acidification pathway (acidification increased concentrations of H+ ions and Al3+ and decreased concentrations of mineral cations). In addition, N enrichment also suppressed bacterial-, fungal-feeding, and omnivorous- carnivorous nematodes mainly via the soil acidification pathway (acidification reduced nematode food resources and reduced concentrations of mineral cations). The positive effects resulting from the increase in belowground carbon allocation (via increase in quantity and quality of plant production) on belowground communities were outweighed by the negative effects resulting from soil acidification, indicating that N enrichment weakens the linkages between aboveground and belowground components of grassland ecosystems. Our results suggest that N enrichment-induced soil acidification should be included in models that predict biota communities and linkages to carbon and nitrogen cycling in terrestrial ecosystems under future scenarios of N deposition.

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1. Introduction

Anthropogenic reactive nitrogen inputs to the terrestrial biosphere, originating mainly from fossil-fuel burning and artificial fertilizer application, has increased three- to five-fold over the past century (Galloway et al., 2008). In many areas of the globe and especially in Asia, nitrogen deposition is expected to continue to increase (Zhao et al., 2009). It is well established that increases in nitrogen inputs stimulate plant growth and change plant community structure (LeBauer and Treseder, 2008; Bai et al., 2010; Bobbink et al., 2010). Nitrogen deposition can also result in soil acidification, leading to the suppression of plant growth in grassland (Van Breemen and Van Dijk, 1988; Chen et al., 2013) or forest ecosystems (Berg and Verhoeof, 1998; Bobbink et al., 2010).

Increased availability of nitrogen for primary production is assumed to greatly increase absolute belowground carbon allocation (Magnani et al., 2007) and therefore to alleviate the carbon limitation for the belowground food web, which relies almost

* Corresponding author. State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, 100093 Beijing, China. Tel.: +86 10 6283 6272; fax: +86 10 8259 5771.
E-mail address: chendima@ibcas.ac.cn (D. Chen).
entirely on plant-derived nutrients in most terrestrial ecosystems (Polrier et al., 2007; Zak et al., 2008; Keith et al., 2009). However, while nitrogen deposition increases nitrogen availability, it simultaneously causes soil acidification, which will also affect carbon allocation to soils (and hence the belowground food web) by changing the concentration of H$^+$ ions and soil base cations (Van Breemen and Van Dijk, 1988; Kuperman and Edwards, 1997). In addition, a number of studies have focused on how the diversity or taxonomic group of belowground communities (mostly soil microbes) changes in response to nitrogen enrichment (Campbell et al., 2010; Ramirez et al., 2010), but the changes lack consistency (Treseder, 2008; Garcia-Palacios et al., 2015). Surprisingly, few studies have simultaneously examined how multiple trophic levels of the belowground food web (e.g., microbes and nematodes) are affected by nitrogen enrichment (Fierer et al., 2009; Eisenhauer et al., 2012). Our incomplete understanding of responses of the belowground food web to nitrogen enrichment limits our ability to predict the impact of future nitrogen deposition on ecosystem productivity and carbon and nitrogen cycling.

Two pathways may primarily determine how nitrogen enrichment affects the belowground food web. In the first pathway, nitrogen enrichment directly increases carbon and nitrogen availability to soil organisms by increasing above- and belowground plant biomass (Ingham et al., 1985; Bardgett and Wardle, 2010). In highly nitrogen-limited environments, this increase in soil nitrogen availability could enhance belowground carbon allocation and thus enhance soil organism activity (LeBauer and Treseder, 2008; Eisenhauer et al., 2012). In the second pathway, nitrogen enrichment alters belowground carbon allocation and soil organisms as a consequence of soil acidification (Van Breemen and Van Dijk, 1988). Soil acidification could suppress plant and belowground communities by increasing concentrations of soil H$^+$ and Al$^{3+}$ (Berg and Verhoef, 1998; Van Den Berg et al., 2005; Rousk et al., 2010) and by decreasing concentrations of base mineral cations (e.g., Ca$^{2+}$, Mg$^{2+}$, and Na$^+$) (Van Breemen and Van Dijk, 1988; Bowman et al., 2008). Although changes in several specific trophic levels of belowground food webs have been documented in response to nitrogen enrichment in natural ecosystems (Treseder, 2008; Campbell et al., 2010; Ramirez et al., 2010), data are lacking concerning the degree to which increased nitrogen availability vs. acidification explains the effects of nitrogen enrichment on multiple trophic groups in natural ecosystems.

Here, we used data from a 12-year nitrogen enrichment experiment to assess the relative roles of nitrogen availability and soil acidification in determining the effects of long-term nitrogen enrichment on belowground communities in a typical semi-arid steppe. First, we determine how nitrogen enrichment affects soil microbes and nematodes, plant variables (aboveground biomass, belowground biomass, and community structure), soil acid cations (H$^+$ and Al$^{3+}$), soil base cations (Ca$^{2+}$, Mg$^{2+}$, and Na$^+$), and nitrogen availability (soil NH$_4^-$N and soil NO$_3^-$N). Second, we determine the relative significance of nitrogen availability and soil acidification in determining nitrogen enrichment-induced changes in components of the belowground food web. Finally, we identify those factors that best explain how nitrogen enrichment affects the belowground food web.

2. Materials and methods

2.1. Study site

This study was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38’N, 116°42’E) of the Chinese Academy of Sciences, which is located in the Xilin River Basin of Inner Mongolia, China, at approximately 1200 m a.s.l. The semi-arid continental climate is characterized by a mean annual precipitation of 334 mm and a mean annual temperature of 0.9°C (1982–2009). Precipitation mainly occurs in the growing season (June–August), which is coincident with the relatively high temperatures. The site has a dark chestnut soil (Calcic Chernozem according to ISSS Working Group RB, 1998), with a loamy-sand texture (Bai et al., 2010). Before the experiment began, the plant community was dominated by Leymus chinensis (Trin.) Tzvel., a C$_3$ perennial rhizomatous grass that is distributed widely in the Eurasia steppe region (Bai et al., 2004).

2.2. Long-term nitrogen enrichment experiment

The establishment of the nitrogen enrichment experiment was described by Bai et al. (2010) and is described briefly here. In 1999, a 120-m x 70-m area with fairly uniform vegetation was designated within the permanent research plots of IMGERS. The area was divided into 162 5-m x 5-m plots with 1-m buffers. These plots were laid out in a randomized block design with nine replicate blocks. Each replicate block included six levels of nitrogen enrichment (0, 1.75, 5.25, 10.50, 17.5, and 28.0 g of N m$^{-2}$ yr$^{-1}$) and nitrogen application in the middle of the growing season (July 1–5). Nitrogen was added as commercial, pelleted NH$_4$NO$_3$ fertilizer. Because of limitations in available labor, we sampled only five of the nine blocks (total plots is 30, 5 blocks x 6 levels of nitrogen enrichment).

2.3. Plant sampling

In late August 2010 and 2011, aboveground vegetation was sampled in a 0.5-m x 0.5-m quadrat in each plot. Living vascular plants were sorted into species by clipping and were oven-dried at 65°C for 48 h and weighed. We classified all plants into five plant functional groups based on life forms as described in Bai et al. (2004): perennial rhizome grasses, perennial bunchgrasses, perennial forbs, shrubs and semi-shrubs, and annuals. Principal component analysis (PCA), based on the biomass of each of the five PFGs, was conducted for each sampling year, and the PC1 scores were used as indicators of plant community structure (Table S1). After the aboveground biomass was sampled, three soil cores (6.5 cm diameter and 0–30 cm depth) were collected in each plot to determine plant belowground biomass. The roots were rinsed from the soil cores under running water, collected on a 1-mm screen, oven-dried at 65°C, and weighed.

2.4. Soil sampling and analysis

Four soil cores (2 cm diameter, 0–15 cm depth) were randomly collected from each plot and were combined to form one composite soil sample per plot. After the soil was gently mixed and roots were removed, the moist soil was passed through a 2-mm-mesh sieve and separated into two parts. One part was maintained fresh for extraction of microorganisms and nematodes. The second part was air-dried for determination of soil pH and extractable cations (Al$^{3+}$, Ca$^{2+}$, Mg$^{2+}$, and Na$^+$). A 20-g subsample of moist soil was oven-dried at 105°C for 24 h to determine soil moisture. Soil pH was measured in a 1:2.5 (soil: water) suspension. NH$_4^-$N and NO$_3^-$N concentrations were determined by extracting inorganic nitrogen at 100 rpm for 2 h from subsamples (10 g) with 100 ml of 2 mol L$^{-1}$ KCl. Extract was subjected to colorimetric determination on a 2300 Kjeltec Analyzer Unit (FOSS, H€ogan€as, Sweden). The extractable cations (Al$^{3+}$, Ca$^{2+}$, Mg$^{2+}$, and Na$^+$) were measured using a modified BCR sequential extraction (Rauret et al., 2000). A 1-g subsample of air-dried soil per sample was placed in a 50-ml polypropylene centrifuge tube. A 20-ml volume of 0.1 mol L$^{-1}$
acetic acid was added to the tube, which was then shaken at 30 rpm for 16 h at 22 °C. The extract was separated from the solid phase by centrifugation at 3000 rpm for 20 min. The contents of the extractable cations were determined with an inductive coupled plasma emission spectrophotometer (Thermo 6300, USA). All results are expressed on the basis of dry soil mass.

2.5. Soil microbial and nematode communities

The microbial community in soil samples was assessed by analysis of phospholipid fatty acids (PLFAs) (Bosio et al., 1998). Qualitative and quantitative fatty acid (FA) analyses were performed with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The abundance of each individual FA (total FAs is 23) in a given sample was expressed as FA nmol g⁻¹ dry soil against an internal standard (methyl ester C19:0, Matreya Inc., State College, PA, USA). FAs specific to bacteria (14:0, a15:0, i15:0, 16:0, a17:0, i17:0, 16:1ω5c, 17:1ω8c, 18:1ω9c, 18:1ω7c, cy17:0, and cy19:0), fungi (18:2ω6,9, and actinobacteria (10Me 16:0, 10Me 17:0, and 10Me 18:0) were used to determine the abundances of these microbial groups and to calculate fungi:bacteria ratios (Frostegård et al., 2011). Like plant community structure, microbial community component was assessed using PCA; the PC1 scores of the three microbial groups were used as indicators of microbial community structure (Table S1).

Nematodes were extracted for 48 h from 50 g of moist soil per sample by the Baermann funnel method (Barker, 1985). Based on feeding habits and life-history characteristics, soil nematodes were assigned to four trophic groups (Bongers, 1990): plant-feeders, bacterial-feeders, fungal-feeders, and omnivores + carnivores. Because carnivorous nematodes were infrequently found, we included carnivorous nematodes in an omnivorous + carnivorous trophic group. The number of genera was used as an indicator of taxon richness (Bongers, 1990). The PC1 scores of the four trophic groups were used as indicators of nematode community structure (Table S1).

2.6. Statistical analyses

Statistical analyses were performed using R version 2.15.1 (R Development Core Team 2009). First, mixed linear models were used to assess how response variables were affected by nitrogen addition treatment, year, and their interactions (as fixed effects) and block (as a random effect). One-way ANOVAs with Duncan’s multiple-range tests were performed across all response variables to compare the effect of each rate of nitrogen addition in 2010 and 2011. Second, structural equation modeling (SEM) was performed to analyze hypothetical pathways that may explain how nitrogen enrichment affects belowground community responses (Fig. S1). Most variables or categories examined in our nitrogen enrichment experiment were correlated with one another, making these data sets appropriate for SEM analysis (Table S2). The SEMs were implemented using the “lavaan” package (Rosseel, 2012). All variables were transformed to natural logarithms before SEM analysis to mitigate departure from normality and linearity. To address the potential problems of nonlinear and remaining univariate non-normality after transformations and to increase the robustness of our SEMs, we used the nonparametric Bollen-Stine bootstrapping estimations (Grace, 2006; Rosseel, 2012). Finally, relationships between potential explanatory variables and soil microbial or nematode variables were further examined using linear regression.

To facilitate our analyses and interpretations, we classified all dependent response variables into the following six groups before SEM analyses were performed (Chen et al., 2013): i) soil nitrogen availability (soil NH₄⁻N and NO₃⁻-N); ii) soil acid cations (H⁺ and Al³⁺); iii) soil base cations (Ca²⁺, Mg²⁺, and Na⁺); iv) plants (aboveground biomass, belowground biomass, and plant community structure); v) soil microorganisms (total biomass, fungi:bacteria ratio, and microbial community structure); and vi) nematodes (total nematode abundance, nematode taxon richness, and nematode community structure). Because variables in each group are often correlated, we used PCAs to create multivariate indices (principal components) for each group (Table S3).

3. Results

3.1. Responses of soil abiotic properties and the plant community

Nitrogen addition increased soil nitrogen availability but reduced soil pH in both years (Table 1). Soil pH decreased across the N-addition gradient by 0.3–1.8 units in 2010 and by 0.1–1.7 units in 2011. With respect to soil base cations, nitrogen addition decreased soil NO₃⁻-N, NH₄⁺, Mg²⁺, and Na⁺ but increased the concentration of Ca²⁺ (Table 1). Soil NO₃⁻-N, NH₄⁺, and Na⁺ concentration were greater in 2010 than in 2011 while pH and Ca²⁺ concentration less in 2010 than in 2011 (Table 1).

Nitrogen addition substantially increased plant productivity (Fig. 1a–c). Relative to the untreated control, nitrogen addition

Table 1

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Year Level of nitrogen addition (g of N m⁻² yr⁻¹)</th>
<th>F</th>
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<tr>
<td></td>
<td>0.00</td>
<td>1.75</td>
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<tr>
<td>NO₃⁻-N (mg kg⁻¹)</td>
<td>2010</td>
<td>1.34(0.14)⁰</td>
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<td>NH₄⁺-N (mg kg⁻¹)</td>
<td>2010</td>
<td>1.05(0.16)⁰</td>
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<td>Soil pH</td>
<td>2010</td>
<td>6.79(0.11)⁰</td>
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<tr>
<td>Al³⁺ (mg kg⁻¹)</td>
<td>2010</td>
<td>43(2)⁰</td>
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<td>Ca²⁺ (mg kg⁻¹)</td>
<td>2010</td>
<td>2231(81)⁰</td>
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<td>Mg²⁺ (mg kg⁻¹)</td>
<td>2010</td>
<td>217(11)⁰</td>
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<td>Na⁺ (mg kg⁻¹)</td>
<td>2010</td>
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*P < 0.05; **P < 0.01; ***P < 0.001. 
increased aboveground biomass by 29–81% in 2010 and by 19–69% in 2011, and increased belowground biomass by 5–35% in 2010 and by 17–67% in 2011. The increase in aboveground biomass mainly resulted from the increase in biomass of perennial rhizome grasses, which was increased from 5 to 91 g m⁻² in 2010 and from 13 to 151 g m⁻² in 2011 (Fig. 2). In contrast, nitrogen enrichment suppressed the biomass of perennial forbs, perennial bunchgrasses, and shrubs and semi-shrubs in both years (Fig. 2). Nitrogen enrichment also substantially altered the plant community structure because of the changes in biomass of the five plant functional groups (Figs. 1c and 2). The aboveground biomass and belowground biomass were higher in 2011 than in 2010 (Fig. 1a, b).

3.2. Responses of soil microbial and nematode communities

Nitrogen enrichment substantially affected soil microbes in both years (Figs. 1d–f). Nitrogen addition decreased total microbial biomass by up to 32% in 2010 and 33% in 2011, bacterial biomass by up to 33% in 2010 and 32% in 2011, fungal
moderate levels of nitrogen addition (0, 1.75, and 10.5 g N m\(^{-2}\) yr\(^{-1}\)) for each year indicated that nitrogen enrichment directly induced changes in soil nitrogen availability and soil acid cations (H\(^{+}\), Al\(^{3+}\)) in both years and that nitrogen enrichment directly explained 85–91% of the total variance in soil nitrogen availability and 82–92% of the total variance in soil acid cations in both years (Fig. 4). The soil acid cations pathway directly explained 77–91% of the total variance in soil base cations (Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{+}\)), and the increases in H\(^{+}\) and Al\(^{3+}\) concentration were associated with decreases in Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{+}\) (Fig. 4). Surprisingly, the effects of soil pH and Al\(^{3+}\) on the plants were not significant in both years, and the observed increases in above- and belowground biomass and changes in plant community structure were mainly attributed to the increase in soil nitrogen availability and changes in soil base cations (Fig. 4). The changes in the soil microbes (decrease in total biomass and increase in the fungi:bacteria ratio values) were evidently affected by both soil acid cations and base cations (Fig. 4). In addition, soil nitrogen availability and plants appeared to have an indirect effect on the microbes (Fig. 4). Soil pH and base

3.3. Pathways determining soil microbial and nematode communities

For each group, the first principal component (PC1), which explained 73–98% of the total variance (Table S3), was used in the subsequent SEM analysis. SEM analyses performed separately for each year indicated that nitrogen enrichment directly induced changes in soil nitrogen availability and soil acid cations (H\(^{+}\) and Al\(^{3+}\)) in both years and that nitrogen enrichment directly explained 85–91% of the total variance in soil nitrogen availability and 82–92% of the total variance in soil acid cations in both years (Fig. 4). The soil acid cations pathway directly explained 77–91% of the total variance in soil base cations (Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{+}\)), and the increases in H\(^{+}\) and Al\(^{3+}\) concentration were associated with decreases in Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{+}\) (Fig. 4). Surprisingly, the effects of soil pH and Al\(^{3+}\) on the plants were not significant in both years, and the observed increases in above- and belowground biomass and changes in plant community structure were mainly attributed to the increase in soil nitrogen availability and changes in soil base cations (Fig. 4). The changes in the soil microbes (decrease in total biomass and increase in the fungi:bacteria ratio values) were evidently affected by both soil acid cations and base cations (Fig. 4). In addition, soil nitrogen availability and plants appeared to have an indirect effect on the microbes (Fig. 4). Soil pH and base

For both years, nitrogen enrichment altered the soil nematode community (Figs. 1g–i and 3d–g). Nitrogen addition decreased the total abundance of soil nematodes due to the decreases in bacterial-feeding, fungal-feeding, and omnivorous + carnivorous nematodes (Fig. 3d–g). In contrast, nitrogen addition did not affect the abundance of plant-feeding nematodes in either year (Fig. 3f). Nitrogen addition decreased the total nematode abundance by up to 56% in 2010 and 43% in 2011. Nematode taxon richness was significantly decreased by up to 24% in 2010 and 20% in 2011 (Fig. 3g). The abundances of total and BF nematodes were lower in 2011 than in 2010, while other nematode variables were similar between the two years (Figs. 1g–i and 3d–g).
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microbial biomass because the negative impact of soil acidification on microbial biomass was greater than the positive impact of the increased carbon allocation to soils. The decline in microbial biomass coincided with substantial declines in pH and in the soil base cations. Previous reports have proposed that an N-induced reduction of carbon input into soil explains the negative effects of nitrogen enrichment on microbial biomass (Janssens et al., 2010). According to this explanation, a large increase in nitrogen availability reduces the need for plants to invest carbon in nitrogen-absorbing fine roots and mycorrhizal fungi, and induces a shift in carbon allocation toward aboveground tissue production (Litton et al., 2007). This reduction in belowground carbon allocation has been shown to directly reduce rhizosphere microbes and, thus, total microbial biomass for forest ecosystem (Phillips and Fahey, 2007). However, our results show that the plant production (both above- and belowground plant biomass) and root nitrogen content of the five dominant plant species (Fig S4) increased with nitrogen enrichment; these changes are consistent with a previous meta-analysis (Xia and Wan, 2008). The observed patterns show that the quantity and quality of carbon allocated to soil in our system increases with increasing nitrogen enrichment, although nitrogen enrichment reduced the proportion of gross primary production allocated to soils, i.e., the belowground:aboveground biomass ratio decreased (data not shown). Because soil microorganisms are often carbon-limited (Treseder, 2008), one might reason that the increase in labile carbon input to soils should lead to higher microbial biomass. However, our results show that nitrogen enrichment significantly reduced microbial biomass in spite of the substantial increase in labile carbon input to soil.

We found that nitrogen enrichment negatively affected total microbial biomass and changed the taxonomic compositions of microbial community components (i.e., bacteria, fungi, and actinobacteria). These results are partly consistent with previous grassland studies at the local scale (Grayston et al., 2001) and global scale (Treseder, 2008). Several mechanisms have been proposed to explain how soil acidification suppresses microbial biomass under nitrogen enrichment. First, the increases in soil H⁺ caused by nitrogen enrichment greatly affect the microbial community component, as evidenced in an acid addition experiment that was conducted at the same site as in the current study; in that experiment, a high level of acid addition also decreased total microbial FAs and bacterial FAs (Chen et al., 2013). Second, loss of base mineral cations and especially of Ca²⁺ and Mg²⁺ has been linked to increased susceptibility of microorganisms to various stresses and to a decline in soil microbial biomass (Grayston et al., 2001; Högberg et al., 2007). Third, acidification could also change the physiological capabilities of microorganisms and decrease extracellular enzyme activity (Waldrop and Zak, 2006). Although nitrogen enrichment may increase litter production and thereby, increase the supply of organic carbon to microorganisms, this potential positive effect on microorganisms was more than offset by the negative effects of soil acidification. We also found that a high level of nitrogen addition increased the fungi:bacteria ratio, although the biomass of both groups dramatically decreased with increasing nitrogen enrichment. This trend was consistent with previous studies in a temperate grassland (Grayston et al., 2001) and a boreal forest (Högb erg et al., 2007) and with the acid addition experiment at the same site (Chen et al., 2013). The increase in the fungi:bacteria ratio may be explained by the fact that fungi are more tolerant than bacteria to Al³⁺ and H⁺ ions (Kuperman and Edwards, 1997). Also, the lower microbial biomass coinciding with lower pH in year of 2010 than year of 2011 confirmed that soil acidification contributed the changes in microbial community. It is important to note that the PLFA results is not equate directly to microbial biomass of specific groups due to that the specific groups have different proportions of PLFA in their real biomass. Therefore, techniques based on nucleic acid extraction and analysis (e.g. high-throughput sequencing) is necessary and warrants further research. Taken together, our results indicate that the nitrogen enrichment-induced decline in microbial biomass and shift in microbial community structure were mainly mediated by the soil acidification pathway and the associated changes in soil base cations.

**Fig. 4.** Structural equation model (SEM) analysis of the effects of nitrogen addition on the belowground food web via pathways of soil nitrogen availability and soil acidification in year (a) 2010 and (b) 2011. Square boxes indicate variables included in the model: AB—aboveground biomass, BB—belowground biomass, PCS—plant community structure, F/B—fungi:bacteria ratio, MCS—microbial community structure, TNA—total nematode abundance, NTR—nematode taxa richness, and NCS—nematode community structure. The symbols “+” and “−” indicate a significant increase or decrease, respectively, in response to nitrogen addition. The number in each square box indicates the response to nitrogen addition (slope of linear model with respect to nitrogen). Values associated with solid arrows represent standardized path coefficients.
4.3. Effects of nitrogen enrichment on the nematode community

Our soil nematode data indicate that the effects of nitrogen addition on nematode trophic groups resulted from reductions in their resources (e.g., total microbial biomass, bacterial biomass, and fungal biomass) and in soil base cations (e.g., \( \text{Ca}^{2+}, \text{Mg}^{2+}, \) and \( \text{Na}^+ \)) through the soil acidification pathway. Nitrogen enrichment had detrimental effects on all nematode trophic groups except plant-feeding nematode in both years. In general, organisms at lower trophic levels in belowground food webs are thought to be controlled by bottom-up forces (Pollierer et al., 2007; Bardgett and Wardle, 2010). The decreasing abundance of bacterial- and fungal-feeding nematodes induced by nitrogen addition probably resulted from the declines in bacterial and fungal biomass, which provides energy sources and nutrients to bacterial- and fungal-feeding nematodes (Ingham et al., 1985; Wardle et al., 2004). Similarly, our results show that the decline in omnivorous + carnivorous was associated with the declines of bacterial- and fungal-feeding nematodes (Ingham et al., 1985; Wardle et al., 2004). Similarly, our results show that the decline in omnivorous + carnivorous was associated with the declines of bacterial- and fungal-feeding nematodes. However, nitrogen addition did not significantly affect plant-feeding nematodes even though it dramatically increased plant production. Perhaps the positive effect of plant production on plant-feeding nematodes was offset by the substantial decrease in soil base cations induced by soil acidification. In addition, the substantial soil base cations were the most plausible explanation for the decreasing abundance of nematode trophic groups. Several studies found that soil acidification (Korthals et al., 1996), by reducing base cations and by otherwise altering the abiotic environment, can affect the physiological condition of soil nematodes.

Our experiment also showed that nitrogen addition reduced soil nematode taxon richness and altered the nematode community structure. This is easily explained by the decrease in all nematode trophic groups (but especially by decreases in nematodes in the higher c-p classes, i.e., omnivores + carnivores) with increasing rates of nitrogen addition. The negative influence of nitrogen addition on taxon richness was due to a reduction in the number of genera of all functional groups of nematodes (data not shown). Previous studies have confirmed that environmental stress can reduce soil community diversity (Nagy, 1999) and that nitrogen enrichment can decrease nematode taxon richness (Suominen, 1999). In the semi-arid grassland, our results with soil nematodes support the idea that nitrogen addition reduces belowground diversity. Surprisingly, soil nematode abundance was less sensitive than soil nematode taxon richness to \( \text{Al}^{3+} \) and \( \text{H}^+ \) concentrations in the current study, although the abundance of nematodes was also associated with \( \text{Al}^{3+} \) and \( \text{H}^+ \) concentrations in previous studies (Nagy, 1999). Overall, our experiment showed that nitrogen...
addition affects soil nematodes through the soil acidification pathway. More specifically, nitrogen addition reduces soil nematode abundance and diversity by reducing soil microbes and soil base cations.

4.4. Implications of nitrogen enrichment-induced soil acidification for semi-arid grasslands

Our results concerning the effects of long-term nitrogen enrichment on the belowground food web have several implications. First, the tendency of sandy soils to release base cations in response to N-induced soil acidification suggests that the semi-arid grasslands may be vulnerable to base cation losses under future nitrogen deposition scenarios. Over the long-term, N-induced soil acidification may enhance base cation depletion in semi-arid ecosystems, which would constrain long-term ecosystem productivity and reduce the nutritional quality of livestock foods (Cheng et al., 2010). Second, an increase in soil acidification is likely to greatly reduce the abundance of organisms at all trophic levels in the belowground food web and is also likely to weaken the linkages between above- and belowground subsystems in grassland ecosystems, suggesting that ecosystem productivity and biodiversity may not be maintained under future scenarios of nitrogen deposition. A lower density of bacterial- and fungal-feeding nematodes, for example, could decrease the turnover in microbial biomass and hence retard soil nitrogen mineralization and plant growth in semi-arid grasslands (Ingham et al., 1985). Third, our results show that soil acidification resulting from long-term nitrogen enrichment may greatly affect the decomposition of plant litter and soil organic matter by altering the decomposer trophic levels of the belowground food web, soil nutrient content, and the plant community (Bowman et al., 2008; Rousk et al., 2010). We therefore suggest that nitrogen-induced soil acidification should be included in models that predict above- and belowground communities and linkages to carbon and nitrogen cycling in terrestrial ecosystems under future scenarios of nitrogen deposition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.06.028.

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