Regional-scale patterns of soil microbes and nematodes across grasslands on the Mongolian plateau: relationships with climate, soil, and plants

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Belowground communities exert major controls over the carbon and nitrogen balances of terrestrial ecosystems by regulating decomposition and nutrient availability for plants. Yet little is known about the patterns of belowground communities and their relationships with environmental factors, particularly at the regional scale where multiple environmental gradients co-vary. Here, we describe the patterns of belowground communities (microbes and nematodes) and their relationships with environmental factors based on two parallel studies: a field survey with two regional-scale transects across the Mongolia plateau and a water-addition experiment in a typical steppe. In the field survey, soils and plants were collected across two large-scale transects (a 2000-km east–west transect and a 900-km south–north transect). At the regional-scale, the variations in soil microbes (e.g. bacterial PLFA, fungal PLFA, and F/B ratio) were mainly explained by precipitation and soil factors. In contrast, the variation in soil nematodes (e.g. density of trophic groups and the bacterial-feeding/fungal-feeding nematode ratio) were primarily explained by precipitation. These variations of microbe or nematode variables explained by environmental factors at regional scale were derived from different vegetation types. Along the gradient from nutrient-poor to nutrient-rich vegetation types, the total variation in soil microbes explained by precipitation increased and that explained by plant and soil decreased, while the opposite was true for soil nematodes. Experimental water addition, which increased rainfall by 30% during the growing season, increased biomass or density of belowground communities, with the nematodes being more responsive than the microbes. The different responses of soil microbial and nematode communities to environmental gradients at the regional scale likely reflect their different adaptations to climate, soil nutrients, and plants. Our findings suggest that the soil nematode and microbial communities are strongly controlled by bottom-up effects of precipitation alone or in combination with soil conditions.

Identifying factors that determine the spatial patterns of communities is an important goal of community ecology (Bell et al. 2009). Although the spatial patterns of aboveground communities have been relatively well documented at regional and global scales (Mittelbach et al. 2001, Bai et al. 2008), the spatial patterns of belowground communities (e.g. soil microbes and nematodes) have received less attention because taxon identification is difficult. Only a few studies have investigated patterns of belowground communities at the local scale (Sessitsch et al. 2001, Jangid et al. 2008, Hawkes et al. 2011) or regional scale (Fierer et al. 2009, de Vries et al. 2012, Chen et al. 2014). As a consequence, we still know little about the regional-scale patterns of belowground communities (e.g. microbes and nematodes) along multiple environmental gradients; we also know little about the relative importance of these environmental factors in controlling the belowground communities. Critical knowledge gaps in our understanding of factors affecting the spatial patterns of belowground communities at the regional scale limit our ability to understand the importance of these communities in regulating interactions in soil food webs, soil nitrogen (N) and carbon (C) cycling, plant species diversity, and net primary production (NPP) (Wardle et al. 2004, Van Der Heijden et al. 2008, Bardgett and Wardle 2010).

The underlying environmental factors that control belowground communities are much debated and dependent on spatial scale and vegetation type (Jangid et al. 2008, Lauber et al. 2008, Fierer et al. 2009). In addition, soil food webs are essentially controlled by bottom-up forces via both abiotic and biotic factors (Hairston et al. 1960, Hunter and Price 1992, Scherber et al. 2010). At the local scale (particular location within vegetation type) or vegetation type level, researchers have suggested that the variation in belowground communities can be explained by soil micro-environment (e.g. pH) and soil substrate (e.g. soil organic C) (Balser and Firestone 2005, Waldrop and Firestone 2006, Rousk et al. 2014).
Growing evidence indicates that plant parameters (e.g. species richness, traits, and biomass) also greatly affect belowground biota at a range of spatial scales (Orwin et al. 2010, de Vries et al. 2012). Together, climate, soil substrate, and plant factors have been shown to shape the structure of belowground communities at both vegetation type level and regional scale. Surprisingly, few studies have simultaneously examined how multiple trophic levels of soil food webs (e.g. microbes and nematodes) are affected by these environmental factors (Fierer et al. 2009, Scherber et al. 2010), and this is especially true for the Mongolian grasslands, which covers distinct environmental gradients (Bai et al. 2012).

We examined the patterns of two important belowground communities (microbes and nematodes) by characterizing the biotic (plant) and abiotic parameters (climate and soil) along two transects (an east–west transect that was 2000 km long, and a south–north transect that was 900 km long) in the Mongolian grassland at a regional scale. The transects also covered a wide range of vegetation types (including desert, desert steppe, typical steppe, and meadow steppe) with distinct abiotic (climate and soil) and biotic (plant) conditions. In addition, we conducted a water-addition experiment in a typical steppe to assess the effects of water availability on the two belowground communities. We attempted to answer two questions. First, how do the structures of soil microbial and nematode communities change along gradients of climate, soil properties, and plant properties at the regional scale? Second, how do the relative effects of climate, soil, and plant factors on the two belowground communities differ at the regional scale (across vegetation types) and vegetation type level (particular vegetation type)? To guide our analyses, we tested the following three hypotheses.

Hypothesis 1: the belowground communities will shift from a fungal-based energy channel in nutrient-poor systems (e.g. desert) towards a bacterial-based energy channel in nutrient-rich systems (e.g. meadow). It is widely documented that, compared to bacterial-based energy channels (dominated by bacteria and bacterial-feeding nematodes), fungal-based energy channels (dominated by fungi and fungal-feeding nematode) are associated with lower nutrient availability and slow-growing plants (Bardgett and Wardle 2010, de Vries et al. 2012).

Hypothesis 2: the regional-scale variations in soil microbial and nematode communities will be explained largely by plants and soils. This is because plants and soil directly provide substrates to belowground communities, e.g. plants determine the quantity and quality of plant litter returned to soil and the quantity of carbon exuded from roots into soil (Yeates 1999, Bardgett and Wardle 2003). We further predict that plants and soils will explain more variation in the structure of the microbial community than of the nematode community because the bottom-up effects are stronger on lower trophic levels (microbes) than on higher trophic levels (nematodes) (Wardle et al. 2003, Scherber et al. 2010).

Hypothesis 3: precipitation will also explain a large proportion of the variation in belowground communities because plant productivity and soil substrate are predominantly limited by water availability in arid and semi-arid grasslands (Bai et al. 2004, 2007). We predict that the relative importance of water limitation on belowground communities will decrease from desert to meadow steppe as mean annual precipitation increases.

**Material and methods**

**Study area and experimental design**

We established two regional-scale transects on the Mongolian grassland in the central part of the Eurasian steppe (Fig. 1). One transect, which was established in 2010, ran east–west for about 2000 km and covered latitudes from 38.9 to 49.9°N and longitudes from 101.6 to 120.4°E, with elevations between 640 and 1692 m. Mean annual precipitation (MAP) along the east–west transect ranges from 104 to 412 mm, and mean annual temperature (MAT) ranges from −2.8 to 8.1°C. The second transect, which was established in 2011, ran south–north for about 900 km and covered latitudes from 43.8 to 49.9°N and longitudes from 105.9 to 111.8°E, with elevations between 721 and 1439 m. MAP along the south–north transect ranges from 122 to 321 mm, and MAT ranges from −1.9 to 4.1°C. Each transect crossed four major vegetation types: meadow steppe, typical steppe, desert steppe, and desert (Fig. 1). The dominate species of each vegetation type were detailed by Bai et al. (2012) and Chen et al. (2014). Soil types for the two transects include dark chestnut, typical chestnut, calcic brown, and light brown soils, corresponding to the different vegetation types from meadow steppe to desert (Bai et al. 2008). Along the east–west and south–north transects, a total of 44 sites that were not grazed by large mammals were selected, including 12 for meadow steppe, 10 for typical steppe, 11 for desert steppe, and 11 for desert. Part data of microbial biomass and environmental factors in the east–west transect were derived from Chen et al. (2014).

**Climate data**

MAP and MAT from 1961 to 2011 were obtained from 756 evenly distributed climate stations across China (Climate Database, National Meteorological Bureau of China). MAP and MAT for each site in our study were interpolated with a Geographic Information System (GIS)-based multiple regression method that used latitude, longitude, and altitude as predictors (Bai et al. 2007). The mean values of MAP and MAT from 1961 to 2011 (n = 51) were used as climate factors.

**Field sampling and measurements**

The east–west transect was sampled in August 2010, and the south–north transect was sampled in August 2011; August corresponds to the annual peak in aboveground standing biomass (Bai et al. 2008). At each site, aboveground standing biomass of herbaceous plants was determined in five 1 × 1 m quadrats located randomly within a 100 × 100 m area. For each quadrat, live and dead aboveground biomass was clipped by species at ground level,
and dead parts were removed and combined with litter. All plant materials in each quadrat were oven-dried at 65°C for 48 h and weighed. Aboveground biomass for shrubs was measured by collecting living leaves and current-year twigs of each species in five 5 × 5 m quadrats. The total dry mass, number of species, and biomass of each species were used to estimate aboveground biomass and species richness for each quadrat.

Belowground plant biomass and soils were sampled within each of the five 1 × 1 m quadrats at each site. Belowground plant biomass was sampled by randomly taking three 7-cm-diameter soil cores from 0 to 40 cm depth in each quadrat; this depth contains about 80–90% of the total root biomass in the top 100 cm of soil (Bai et al. 2012). Roots were cleaned by placing them under running water over a 1-mm screen, and the cleaned roots were oven-dried at 65°C and weighed. Soil samples were collected by taking three 7-cm-diameter soil cores from 0 to 20 cm depth in each of the five 1 × 1 m quadrats. The three cores from each quadrat were mixed in situ to form one composite sample. After gentle homogenization and removal of roots, the moist soil was sieved through a 2-mm mesh and separated into two parts. One part was maintained without drying for measurement of soil moisture, the soil microbial community, and the soil nematode community. The other part was air-dried and used to determine soil pH, soil organic C, and total soil N. A subsample of 20 g of field-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. Soil organic C content was determined using the Walkley–Black modified acid-dichromate FeSO₄ titration method (Sparks et al. 1996). Total soil N was determined by micro-Kjeldahl digestion, followed by colorimetric determination with a 2300 Kjeltec Analyzer Unit (Sparks et al. 1996). All results are expressed on a dry weight basis.

Water addition experiment

To directly assess the effect of water availability on belowground communities, we conducted a water-addition experiment on a typical steppe (116.70°E and 43.63°N). The MAT is 0.3°C and MAP is 346.1 mm (Chen et al. 2014). The experiment was established in 2010 and included two treatments (control and water addition), each with 6 replicates. Twelve 4 × 4 m plots (2 treatments × 6 blocks) were laid out following a randomized block design with a 1-m-wide buffer around each plot and a 2-m-wide buffer zone around each block. The control plots received natural precipitation only, and the water-addition plots received natural precipitation plus a total of 100 mm of water each year in 2010 and 2011. The water was added 10 times (10 mm each time every 2 weeks) during the growing season from June to August. In early September 2011, soil samples were collected by taking three soil cores (3 cm diameter) from 0 to 20 cm depth in each plot. The three cores from each plot were mixed in situ to form one composite sample. To allow direct test the responses of variables in belowground communities to water addition, the response (%) was calculated as the difference between the value of variable in water treatment and control treatment from same block (i.e. Response(%) = 100 × (Value of variablewater – Value of variablecontrol)/Value of variablecontrol).

Soil microbial and nematode communities

In our regional-scale sampling, we assessed the biomass and composition of the soil microbial community by Phospholipid fatty acid (PLFA) method. Compared with
techniques based on nucleic acid extraction and analysis, this PLFA method has several strengths (e.g., rapid, inexpensive, and sensitive to changes in environment) (Frostegård et al. 2011). PLFAs were extracted from soil as described by Bossio et al. (1998). The resultant fatty acid methyl esters were separated, quantified, and identified using capillary gas chromatography (GC). Qualitative and quantitative fatty acid analyses were performed with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) and the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE, USA). The content of each individual fatty acid (FA) (among the 23 most abundant FAs) was expressed as FA nmol g$^{-1}$ dry soil in a given sample against an internal standard (methyl ester C19:0, Sigma-Aldrich). To avoid misuse or misinterpretation of the PFLA method, the assignment of specific FAs to groups of bacteria and fungi was conducted following Frostegård et al. (2011). Bacterial biomass was considered to be indicated by the mass of the following bacteria-specific FAs: i14:0, a15:0, i15:0, i16:0, 10Me16:0, a17:0, i17:0, 16:1(7c), 17:1(9c), 18:1(9c), 18:1(7c), cy17:0, and cy19:0. Fungal biomass was considered to be indicated by the mass of the fungi-specific FA 18:2ω6,9. The ratio of bacterial to fungal FAs (B/F) was considered an indicator of the relative abundance of the two groups. All results are expressed on a dry weight basis.

Nematodes were extracted from 50 g of moist soil per sample by the Baermann funnel method for 48 h (Barker 1985). After fixation in 4% formalin solution, nematodes were counted with an inverted microscope, and the first 100 individuals encountered were identified to genus. Based on morphology, soil nematodes were assigned to five trophic groups (Yeates et al. 1993, Ferris et al. 2001): plant-feeding, bacterial-feeding, fungal-feeding, omnivorous, and carnivorous nematodes. Because carnivorous nematodes were found infrequently, we included carnivorous nematodes in an omnivorous + carnivorous trophic group. The ratio of densities of bacterial-feeding to fungal-feeding nematodes was considered an indicator of the relative abundance of the two groups.

**Statistical analyses**

All statistical analyses were done with R ver. 2.15.1 (R Development Core Team) and figures were plotted with SigmaPlot 11.0 (Systat Software, Chicago, IL, USA). To facilitate our analysis and interpretations, statistical analyses included three groups of explanatory environmental factors: 1) climate (MAP and MAT); 2) soil (pH, soil organic carbon or SOC, and total soil N or TSN); and 3) plant (aboveground biomass or ANPP, belowground biomass or BB, and species richness or PSR). First, all variables were compared among vegetation types using one-way ANOVAs with Tukey’s multiple-range tests. The adjust p values of multiple comparisons were calculated by multiple.correction function in the ‘EMAS’ package: based on Benjamini–Hochberg multiple-testing correction by false discovery rate (FDR). In the water-addition experiment, the belowground community variables were compared between control and water addition plots using one-way ANOVAs. The data of variables were transformed to natural logarithms before one-way ANOVA analysis to improve normality. Second, the relative importance of environmental factors as explanatory variables of the belowground communities at the regional scale (the entire Mongolian plateau) or among the four vegetation types was examined using a linear mixed-effects model with climate, soil, and plant factors as fixed effects and sampling site as a random effect. For each model, environmental factors were added according to a fixed sequential order as climate (MAP and MAP), soils (pH, SOC, and TSN), and then plants (ANPP, PSR, and BB). The proportion of variance (R$^2$) explained by the total fixed, random, and residual effects was estimated using the function of rsquared.lme by Jon Lefcheck (R code available at <http://jonlefcheck.net/2013/03/13/r2-for-linear-mixed-effects-models/>). The total R$^2$ of fixed effects was assigned to each environmental factor using the pamer.fnc function from the ‘LMERConvenienceFunctions’ package. Third, we conducted partial regressions to visualize the relationships between belowground community variables and the statistically significant environmental factors from linear mixed-effects model; the partial residuals of belowground community variables were created using the lm.sumSquares function in the ‘lmSupport’ package. The partial regressions allow us examining the independent influence of each significant environmental factor on the belowground community variables when the effects of other environmental factors were controlled for (Sjöstedt et al. 2014).

**Results**

**Patterns of belowground community components and structures**

The desert and desert steppe had low nutrient soils (as indicated by low soil organic C, low soil total N, and low plant biomass) and a harsh climate (e.g., low MAP), while the typical steppe and meadow steppe had high nutrient soils and a more benign climate (Supplementary material Appendix 1). All of the variables describing the belowground communities differed among the four vegetation types (Table 1). For soil microbes, total biomass increased from 9.6 nmol g$^{-1}$ dry soil in desert to 29.2 nmol g$^{-1}$ dry soil in meadow steppe owing to the increases in bacterial biomass (Ba FAs) and fungal biomass (Fu FAs). Similarly, the B/F ratio increased from desert to meadow steppe along the precipitation gradient. For nematodes, total density increased from 1.1 ind. g$^{-1}$ dry soil in desert to 6.0 ind. g$^{-1}$ dry soil in meadow steppe owing to the increases in density of all nematode trophic groups (Table 1). The BF/FF ratio decreased from desert to meadow steppe (Table 1).

**Relationships between belowground community components and environmental factors**

According to the linear mixed-effect models, climate, soil, and plant factors explained a large percentage of the total variation in each variable describing the belowground communities, but the percentage explained by the three groups differed between the microbial and the nematode communities. For microbe variables, climate (e.g., MAP), soil (e.g.
The results of the water-addition experiment in the typical steppe confirmed that water availability played a key role in regulating the belowground communities in the semi-arid grassland (Fig. 4). Compared to the control, water

SOC and TSN), and plant (e.g. ANPP and BB) explained 12–22, 12–24, and 5–7% of the variation, respectively (Table 2). For soil nematode variables, however, climate, soil, and plant explained 30–67, 3–9, and 0.4–11% of the variation, respectively (Table 2). In contrast, pH did not significantly contribute to the variation in belowground community variables. After controlling for the effect of other environmental factors, the partial residual regression analysis also showed that major variation of microbes was explained by climate (i.e. MAP) and soil factors (i.e. SOC and TSN) while variation of nematodes were mostly explained by climate (i.e. MAP) and plant factors (i.e. BB) (Fig. 2 and Supplementary material Appendix 2–3). For instance, the residuals of total FAs were significant associated with MAP, SOC, and TSN (Fig. 2a–c) while the residuals of total ND were affected by MAP and BB (Fig. 2d–e).

When linear mixed-effect models were separately applied to each vegetation type, the percentage of variation in the belowground communities explained by climate, soil, and plant factors differed among vegetation types (Fig. 3). For microbes, the percentage of variation explained by climate (mostly by precipitation) increased with increasing nutrients, i.e. the percentages were lowest in the desert (11–23%) and were highest in the meadow steppe (39–53%) (Fig. 3). The percentages of variation in microbe community variables explained by soil and plant factors were highest in the desert (22–31% by soil and 12–18% by plant) and were lowest in the meadow steppe (9–13% by soil and 3–12% by plant). For nematodes, in contrast, the percentage of variation in each variable explained by climate (mostly by precipitation) decreased with increasing nutrients, i.e. the percentages were highest in the desert (36–63%) and lowest in the meadow steppe (6–12%) (Fig. 3). In addition, soil and plant factors explained more of the variation of each variable of the soil nematode community in the meadow steppe than in the desert.

The characteristics of the belowground communities (microbes and nematodes) associated with the four vegetation types in the Mongolian grassland. Values are means (SE). Different letters in a row indicate significant differences among the four vegetation types (one-way ANOVA, p < 0.05).

### Table 1. Characteristics of the belowground communities (microbes and nematodes) associated with the four vegetation types in the Mongolian grassland.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Desert 11 sites</th>
<th>Desert steppe 11 sites</th>
<th>Typical steppe 10 sites</th>
<th>Meadow steppe 12 sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbial community</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FAs (ng g⁻¹)</td>
<td>9.64 (0.82)ᵃ</td>
<td>18.77 (0.90)ᵇ</td>
<td>24.71 (1.67)ᶜ</td>
<td>29.22 (2.08)ᶜ</td>
</tr>
<tr>
<td>Ba FAs (ng g⁻¹)</td>
<td>5.33 (0.46)ᵃ</td>
<td>10.01 (0.48)ᵇ</td>
<td>12.97 (0.88)ᶜ</td>
<td>15.57 (1.12)ᶜ</td>
</tr>
<tr>
<td>Fu FAs (ng g⁻¹)</td>
<td>0.45 (0.04)ᵃ</td>
<td>0.54 (0.03)ᵇ</td>
<td>0.61 (0.04)ᵇ</td>
<td>0.72 (0.05)ᶜ</td>
</tr>
<tr>
<td>B/F</td>
<td>12.83 (0.45)ᵃ</td>
<td>19.37 (0.94)ᵇ</td>
<td>21.62 (0.79)ᵇ</td>
<td>22.76 (0.74)ᶜ</td>
</tr>
<tr>
<td><strong>Nematode community</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ND (Ind. g⁻¹)</td>
<td>1.06 (0.13)ᵃ</td>
<td>2.94 (0.30)ᵇ</td>
<td>5.09 (0.35)ᶜ</td>
<td>5.98 (0.23)ᵈ</td>
</tr>
<tr>
<td>BF (Ind. g⁻¹)</td>
<td>0.07 (0.09)ᵃ</td>
<td>1.73 (0.16)ᵇ</td>
<td>2.83 (0.23)ᶜ</td>
<td>3.46 (0.17)ᵈ</td>
</tr>
<tr>
<td>FF (Ind. g⁻¹)</td>
<td>0.08 (0.03)ᵃ</td>
<td>0.66 (0.04)ᵇ</td>
<td>0.99 (0.09)ᶜ</td>
<td>1.07 (0.07)ᶜ</td>
</tr>
<tr>
<td>OC (Ind. g⁻¹)</td>
<td>0.80 (0.019)ᵃ</td>
<td>0.30 (0.051)ᵇ</td>
<td>0.61 (0.067)ᶜ</td>
<td>0.84 (0.067)ᶜ</td>
</tr>
<tr>
<td>BF/FF</td>
<td>7.96 (0.20)ᶜ</td>
<td>4.67 (0.31)ᵇ</td>
<td>2.59 (0.18)ᵇ</td>
<td>2.03 (0.10)ᵃ</td>
</tr>
</tbody>
</table>

Note: Total FAs, total biomass; Ba FAs, bacterial biomass; Fu FAs, fungal biomass; B/F, ratio of Ba FAs to Fu FAs; Total ND, total soil nematode density; BF, bacterial-feeding nematodes; FF, fungal-feeding nematodes; PF, plant-feeding nematodes; OC, omnivore + carnivore nematodes; BF/FF, ratio of bacterial-feeding to fungal-feeding nematode densities.

### Table 2. The percentage (%) of variation (as determined by linear mixed models) in belowground communities explained by climate, plant, and soil factors at the regional scale in the Mongolian grassland.

<table>
<thead>
<tr>
<th>Explanatory factor</th>
<th>Microbial community (SS%)</th>
<th>Nematode community (SS%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climate</td>
<td>22.3</td>
<td>22.3</td>
</tr>
<tr>
<td>MAP</td>
<td>21.2**</td>
<td>21.2**</td>
</tr>
<tr>
<td>MAT</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Soil</td>
<td>24.1</td>
<td>22.8</td>
</tr>
<tr>
<td>pH</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>SOC</td>
<td>9.9**</td>
<td>9.0**</td>
</tr>
<tr>
<td>TSN</td>
<td>10.8**</td>
<td>10.6**</td>
</tr>
<tr>
<td>Plant</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>ANPP</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>PSR</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>BB</td>
<td>4.1*</td>
<td>4.3*</td>
</tr>
<tr>
<td>Random + Residuals</td>
<td>51.2</td>
<td>52.0</td>
</tr>
</tbody>
</table>

Note: MAP, mean annual precipitation; MAT, mean annual temperature; SOC, soil organic carbon; TSN, total soil nitrogen; ANPP, aboveground net primary productivity; PSR, plant species richness; BB, belowground plant biomass; abbreviations of variables in belowground communities are explained in Table 1. Asterisks of ‘*’ and ‘**’, indicate the p < 0.05 and p < 0.01, respectively.
addition increased total microbial biomass by 20% and bacterial biomass by 18%, but it did not alter fungal biomass or the B/F ratio (Fig. 4). Water addition increased total nematode density by 149%, bacteria-feeding nematode density by 110%, fungal-feeding nematode density by 209%, and plant-feeding nematode density by 185% (Fig. 4). As a result, the ratio of bacterial-feeding to fungal-feeding nematodes decreased by 29%. The greater responses of nematodes than microbes to water addition indicated that the nematode community was more sensitive than the microbial community to water availability.

Discussion

Dominance of the bacterial-based vs fungal-based energy channel as affected by the nutrient status of the environment

Our results for soil microbes were partially consistent with our first hypothesis, which stated that nutrient-rich environments (e.g. the meadow) would be characterized by bacterial-based energy channels and that nutrient-poor environments (e.g. the desert) would be characterized by fungal-based energy channels. This hypothesis was based in part on previous findings that low precipitation and slow-growing plants support fungal-dominated microbial communities and that high precipitation and fast-growing plants support bacterial-dominated microbial communities (Moore et al. 2003, de Vries et al. 2012). While our results did show that fungi more dominant at the nutrient-poor end of the gradient and that bacteria dominated at the nutrient-rich end, the results for the nematode community were inconsistent with the first hypothesis in that BF/FF ratios were lower in nutrient-rich environments than in nutrient-poor environments. The results for the nematode community were also inconsistent with previous findings that the BF/FF ratio increased with nutrient availability (Williamson et al. 2005, Doblas-Miranda et al. 2008). The decreasing BF/FF ratio from the nutrient-poor to nutrient-rich vegetation types in the present study could have two explanations:

Figure 2. Partial residual plots of the relationship between variables of belowground communities (total FAs and total ND) and environmental factors at the regional scale in the Mongolian grassland. Abbreviations are explained in Table 1 and 2. Partial regression analysis is indicated by partial r and significant level (*, p < 0.05; **, p < 0.01).
Figure 3. The percentage of variation (as determined by linear mixed models) in soil microbes and nematodes explained by environmental factors for the four vegetation types (a–d). Abbreviations are explained in Table 1 and 2.

Effects of climate, plant, and soil factors on the belowground communities

Our study demonstrates that the variations in belowground communities explained by climate, soil, and plant differed across a broad range of arid and semi-arid grasslands. The variation in each soil microbe variable was largely explained by both precipitation and soil nutrients, and the variation in each soil nematode variable was largely explained by precipitation. These findings are partly inconsistent with our second hypothesis, which stated that soil and plant, as the primary limiting factors in arid and semi-arid grasslands, determine the components of belowground communities. The strong relationships between components of belowground communities and precipitation documented in our study were consistent with the findings of previous studies (Mulder et al. 2005, Williams and Rice 2007, Clark et al. 2009). For example, bacterial biomass (Griffiths et al. 2011), fungal biomass (Drenovsky et al. 2004, Williams and Rice 2007), and densities of plant-feeding and bacteria-feeding nematodes (Todd et al. 1999) increased with precipitation at a regional scale. The increase in the B/F ratio with precipitation in the current study could be due to that bacteria were more sensitive to nutrient conditions than fungi, although both bacterial and fungal biomass increased with precipitation (Moore-Kucera and Dick 2008, Drenovsky et al. 2010). However, our findings are inconsistent with a recent study in a grassland in England, which found that biotic factors (plant functional traits) explained more of the variation in soil microbial communities than abiotic factors (climate and soil properties) (de Vries et al. 2012). This difference may be ascribed to differences in precipitation in Mongolia vs England: the annual precipitation ranges from 104 to 412 mm in Mongolia grasslands and from 595 to 3190 mm in English grasslands (de Vries et al. 2012). Moreover, the
Our research also revealed that precipitation-driven plant community and the density of the soil nematode community are more sensitive to precipitation than the microbial community. Researchers have determined that the spatial distribution of soil organisms is associated with the spatial distribution of plants at a large scale because the spatial distribution of plants affects the quantity and quality of plant litter returned to soil, the rate of root turnover, and the quantity of carbon exuded from roots into soil (Yeates 1999, Bardgett and Wardle 2003, Jones et al. 2004). As a whole, our research in this semi-arid grassland showed that soil microbial communities were strongly controlled by bottom-up effects via soil substrate and climate, and that the nematode community was strongly controlled by bottom-up effects via climate.

How the effect of precipitation on the percentage of variation in belowground communities differs among vegetation types

Our findings indicate that the variations in belowground communities explained by climate, soil, and plant factors differed substantially among vegetation types. In contrast to our third hypotheses, the contribution of precipitation to the variation in soil microbe variables increased from desert to meadow steppe while the contributions of soil and plant factors decreased. The trends, however, were the opposite for soil nematodes in that total variation explained by precipitation decreased from the desert to the meadow steppe. In the desert subjected low precipitation, the contributions of water availability to the variation in soil microbe variables were notably lower than that in nematode variables, suggesting that nematode variables were more sensitive to water availability than microbial variables. While in the meadow steppe subjected abundant precipitation, the contributions in soil microbe variables were notably higher than nematode variables. This opposite contribution between soil microbe and nematode variables in the meadow steppe indicate that water availability was still the primary limiting factor while nutrients provided by soil and plant factors became less limiting (Mulder et al. 2005, Williams and Rice 2007). Although water availability was the primary limiting factor for soil nematodes in the meadow steppe, soil and plant factors became more important as water availability increased (Korthals et al. 1996, Todd et al. 1999).

Looking at the patterns in variation of belowground communities at regional scale, we found variations of different belowground communities explained by environmental factors at regional-scale could be derived from different vegetation types. The relative higher variations of microbes explained by precipitation and soil factor at regional scale probably were mainly derived from nutrient-poor vegetation types (desert and desert steppe). In contrast, the relative higher variations of nematodes explained by climate were mainly derived from nutrient-rich vegetation types (meadow steppe and typical steppe). These inconsistent patterns between regional scale and vegetation type level indicate that the underlying environmental factors controlling belowground communities are dependent on vegetation type or spatial scale (Lauber et al. 2008, Fierer et al. 2009). Taken together, these climate- or nutrient-driven properties (e.g. ANPP and BB) alone explained a small but significant percentage of the variance in belowground communities. Researchers have determined that the spatial distribution of soil organisms is associated with the spatial distribution of plants at a large scale because the spatial distribution of plants affects the quantity and quality of plant litter returned to soil, the rate of root turnover, and the quantity of carbon exuded from roots into soil (Yeates 1999, Bardgett and Wardle 2003, Jones et al. 2004). As a whole, our research in this semi-arid grassland showed that soil microbial communities were strongly controlled by bottom-up effects via soil substrate and climate, and that the nematode community was strongly controlled by bottom-up effects via climate.

![Figure 4. Responses of belowground communities to water addition in the typical steppe. The response (%) = 100 × (Value of variable water – Value of variable control)/Value of variable control. “Control” plots received precipitation only. “Water” plots received precipitation + 100 mm of irrigation per year. Abbreviations are explained in Table 1. Values are mean response + SE (n = 6 blocks). Bars with asterisks of “*” and “**” indicate significant differences between control and water plots at p < 0.05 and p < 0.01 (one-way ANOVA), respectively.](image-url)
shifts in community composition at different vegetation types could be due to the favoring of certain functional groups whose ability to thrive is increased by a particular biotic and abiotic conditions (Evans and Wallenstein 2014). These different responses of soil microbes and nematodes to climate, soil, and plant factors probably reflect differences in their adaptations to climate and nutrients across a range of vegetation types.

Caveats and uncertainties regarding the PLFA method

It is important to note that the PLFA method for assaying composition and biomass of microbial community includes some limitations. One limitation is that the assignment of individual PLFA biomarker is not exclusive to specific group of microorganisms (Frostegård et al. 2011). In another word, a certain PLFA biomarker can represent both fungal and bacterial groups, e.g. 18:206,9 and 18:1609 (Frostegård et al. 2011). Another limitation related to the assignment is that multiple biomarkers are assigned to bacterial group but only one biomarker (18:206,9) to fungal group. In these situations, the PLFA method probably contributed to an overestimation of bacterial biomass and an underestimation of fungal biomass, and hence leading an overestimation of B/F. This uncertainty may be especially strong in the meadow steppe which has relative higher bacterial biomass. Therefore, techniques based on nucleic acid extraction and analysis (e.g. high-throughput sequencing) along with PLFA method for assessing the biomass, phenotype and activity of microbial community for different steppes is necessary and warrants further research in the arid and semi-arid grasslands.

Summary and implications

To our knowledge, the current study is the first to examine the patterns of both soil microbial and soil nematode communities across a broad range of arid and semi-arid grasslands using an integrative approach that included a field survey and a water-addition experiment. We found that the components of the belowground communities were closely associated with environmental factors. The relative dominance of bacterial- and fungal-based energy channels was significantly affected by the nutrient status of the environment. The variation in community components were mainly explained by precipitation alone (soil nematodes) or by a combination of precipitation and soil factors (soil microorganisms). Moreover, from desert to meadow steppe, the percentage of the variation in the community structural parameters explained by precipitation increased in the case of soil microorganisms but decreased in the case of soil nematodes, suggesting that the two communities have different adaptive strategies with respect to environmental gradients and associated changes in plant communities.

Our results demonstrate that the soil nematode community is more sensitive than the microbial community to changes in precipitation in semi-arid grasslands. It is predicted that annual precipitation in the Inner Mongolia steppe may increase by 30–100 mm in the next 100 yr (Ni and Zhang 2000). Such an increase in precipitation is likely to greatly affect nematode and microbial communities and therefore ecosystem productivity and biodiversity. A higher density of microvorous nematodes (i.e. BF and FF), for example, could increase microbial biomass turnover and hence stimulate soil nitrogen mineralization and promote plant growth (Ingham et al. 1985, Bardgett et al. 2005). These findings advance our understanding of the regional-scale patterns of soil microbial and nematode communities, and hence improve our ability to predict the impacts of land-use change, climate change, and other disturbances on ecosystem functions.

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