Carbon and nitrogen dynamics of native *Leymus chinensis* grasslands along a 1000-km longitudinal precipitation gradient in northeast China

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Abstract

Understanding how ecosystem carbon (C) and nitrogen (N) cycles respond to the variability of precipitation can help us assess the effects of global climate change on terrestrial ecosystem structure and function. We investigated the contributions of aboveground biomass, litter, root, soil and microbial communities to ecosystem C and N processes at 14 sites along a 1000-km precipitation gradient in native *Leymus chinensis* grasslands of northeastern China. The results show that aboveground biomass C and N increased gradually, while no significant regional trends in litter and root biomass were found with increasing mean annual precipitation (MAP) along the gradient. Soil respiration increased first and then decreased from the dry to mesic sites, which could be ascribed to the relative changes in temperature, soil fungal: bacterial biomass and N availability. Surprisingly, N mineralization varied only slightly along the gradient, likely due to the decreases of soil organic matter quality (i.e. C: N). Stepwise regression models indicated regional soil C and N content positively correlated with MAP and clay content. Overall, C and N sequestration enhanced 3.2- and 1.8-fold with increasing MAP in terms of C and N storage in aboveground biomass, root, litter and soil. It was concluded from the current study that regional precipitation variability strongly influences ecosystem C and N dynamics. The ecosystem C and N sequestration are primarily modulated by annual precipitation and soil texture, while the C and N turnover are largely controlled by microbial community composition, temperature and soil quality in *L. chinensis* grasslands across the large-scale precipitation gradient.
1 Introduction

It is well known that precipitation has an important impact on ecosystem C and N cycles, especially in grasslands and water-limited regions, which cover c. 45% of the global terrestrial area (Saco et al. 2006; Zhou et al. 2009). Previous studies have demonstrated that ecosystem aboveground primary production (Barrett et al. 2002; Huxman et al. 2004; Hsu 2012), litter mass (Zhou et al. 2009), soil C and N dynamics (Porporato et al. 2003; Wang et al. 2008; Baumann et al. 2009) and trace gas flux (McCulley et al. 2005; Niu et al. 2009) are strongly influenced by mean annual precipitation (MAP) and water availability at local, regional and global scales. Therefore, teasing apart the contributions of aboveground biomass, litter, root, soil and microbial communities to C and N dynamics across a precipitation gradient can help us assess the effects of global climate change on terrestrial ecosystem structure and function.

Primary production is an important component of the global C cycles (Hsu et al. 2012). Recent large-scale transect studies evaluated the spatial variability of climatic factors and annual aboveground net primary production (ANPP) in many terrestrial ecosystems, and indicated that nonlinear and saturating relationships between regional precipitation and ANPP (Huxman et al. 2004; Yang et al. 2008; Hsu et al. 2012). A 1% increase in MAP caused a -0.2% to 1.8% change in ANPP, and the sensitivities to precipitation peaked at c. 500 mm (Hsu et al. 2012). In temperate grasslands, root production is also an important resource of organic matter, which represents 50-80% of total biomass (Tomaškin 2007). In terms of C sequestration, it is likely that belowground production is a more important soil C source than aboveground production (McCulley et al. 2005), and thus a further understanding of total plant production across a
Another component of ecosystem C and nutrient cycles is litter (Zhou et al. 2009), because it not only contributes to aboveground and belowground C and N sequestration (Rinnan et al. 2008), but also closely relates to element fluxes (Michelsen et al. 2007) and hydrological cycle (Willms and Chanasyk 2006). Available results show that litter mass is positively correlated with precipitation in water-limited regions, and the mass reaches a threshold after which it declines with increasing precipitation in mesic regions (Zhou et al. 2009; Wang et al. 2011). In the temperate grasslands of northeastern China, soil fertility and productivity are maintained primarily by recycling of nutrients through litter decomposition, with little fertilization or N deposition (Liu et al. 2006), whereas observations of litter dynamic are lacking across a large-scale precipitation gradient in grassland ecosystems.

In grassland ecosystems, about 50-70 % of organic C and N sequestered in topsoil (Jobbagy and Jackson 2000; Zhou et al. 2007). The main factor determining the amount of soil C and N storage is decomposition of organic matter (i.e. soil respiration and N mineralization). Soil organic matter are mineralized to inorganic forms by microbial communities (Paul 2007), and result in releasing CO₂ to the atmosphere and providing inorganic nutrient for plant growth (Manzoni and Porporato 2009). Higher mineralization processes typically indicate instability of soil organic matter (Trumbore et al. 1997). There is plenty of evidence to demonstrate that soil respiration and net N mineralization increased with precipitation and water availability in water-limited regions (Sierra et al. 1997; Barrett et al. 2002; Paul et al. 2003; McCulley et al. 2004).
According to Stanford and Epstein (1974), the maximum net N mineralization rate occurs when soil moisture is close to field capacity. However, few studies have looked at regional patterns of soil respiration, N mineralization and soil C and N storage with the variability of precipitation.

The use of naturally occurring climatic gradients is an invaluable approach for examining ecosystem processes responses to climate variability (Austin and Sala 2002). Northeast China Transect (NECT) is identified as a mid-latitude semiarid terrestrial transect and the vegetation shifts from desert steppes to typical steppes and moist meadows along a west-east precipitation gradient. Owing to relatively uniform soil origin and dominant species of the NECT, it provides an ideal setting to investigate grassland ecosystem C and N dynamics along a precipitation gradient (Wang et al. 2003). Our work specially aimed at teasing apart the contributions of aboveground biomass, litter, root, soil and microbial communities to ecosystem C and N dynamics along a 1000-km precipitation gradient in *Leymus chinensis* grasslands.

### 2 Materials and methods

#### 2.1 Study sites

The field study was conducted at 14 perennial grass *Leymus chinensis* grasslands spanning a 1000 km-long transect from west to east (114°E – 124°E), located in northeastern plain and Inner Mongolia plateau of China (Table 1, Fig. 1). The transect has a continental monsoon climate, with large seasonal temperature and precipitation variations. Long-term (1950 – 2000) mean annual temperature, mean annual precipitation and aridity index along the transect range from...
approximately 1.3 – 6.8 °C, 237 – 472 mm and 0.91 – 1.44, respectively (http://www.worldclim.com; Zhang et al. 1997; Table 1). The intra-annual precipitation in the sampling year 2012 increased from 246 mm in the west to 491 mm in the east (http://cdc.cma.gov.cn; Table 1). The elevation gradients range from 140 – 1309 m. Because the transect runs parallel to a latitude line (43° 30’ N), it has approximately uniform theoretical radiation.

The *L. chinensis* community is the most widely distributed in this transect (He et al. 2008). Precipitation gradient is the main critical factor controlling vegetation zonality and plant distributions (Zhang et al. 1997). Following an order of increasing MAP, vegetation shifts from desert steppes (dominate by perennial grass *L. chinensis* and with companion species *Agropyron cristatum, Stipa grandis*) in the west to typical steppes (dominate by *L. chinensis* accompanying with *A. cristatum, Artemisia frigida, Cleistogenes squarrosa, S. grandis, Setaira viridis*) and moist meadows (dominate by *L. chinensis* and *Lathyrus quinquenervius, Artemisia scoparia, Carex duriuscula, Phragmites australis* as companion species) in the east (Table 1). Most of sites have dark meadow soil and chernozem in the east, while steppes and desert steppes have chernozem and chestnut in the west. The sampling sites selected for the study have not been disturbed for at least 10 years (Wang et al. 2011). A detailed description of vegetation types, climate factors and soil properties can be found in Table 1, Ni and Zhang (2000).

**2.2 Plant biomass and litter mass**

About 1 - 2 hm² native *L. chinensis* grassland was selected for field sampling at each site. Aboveground living biomass, standing litter and surface litter were harvested by 10 – 12
randomly 0.5 × 0.5 m quadrats of each site in mid-August 2012, which is the period of aboveground biomass attains peak value. Root biomass was measured by soil coring method sampling to a depth of 30 cm using a cylindrical root sampler (8 cm inner diameter). All samples of aboveground living tissue, litter and root were oven-dried at 65 °C to constant weight. C and N content of shoot, root and litter were determined by elemental analyzer (Elemetaranalysator vario Max CN, Germany).

2.3 Soil property

Soil samples from 14 sites were collected along the systematically located transect lines in 12–18 July 2012. 8 – 16 soil core samples were collected randomly per site (100 × 100 m) for determination of soil properties. The samples were taken to a depth of 15 cm soil layer with a cylindrical soil sampler (5 cm inner diameter), and then immediately preserved at 4 °C in a cooler for transport to the laboratory within one week of collection. The fresh samples were processed using a 2 mm sieve and cleared of visible plant roots and organic debris.

Soil pH was measured with a pH electrode (PHS 29, China). Soil inorganic N content (NH$_4^+$-N and NO$_3^-$-N) was determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer, Denmark). Soil total C and N content were measured by elemental analyzer (Elemetaranalysator vario Max CN, Germany). Soil light and heavy fractions of C and N content were measured by density fractionation method (Sollins et al. 1984). Soil texture was determined by the optical size analyzer (Mastersizer 2000, England). Gravimetric soil water content was measured in mid-July and mid-August by oven-drying samples at 105 °C for 24 h.
2.4 C and N mineralization and microbial community

Soil respiration was measured in the field by the alkaline absorbance method (Meyer et al. 2006). Soil sample (20 g on an oven-dry soil basis) was incubated with 50 ml of 0.5 M NaOH in a closed vessel at ambient soil temperature over 4-week incubation period from 12–18 July to 8–14 August 2012. The NaOH solution was titrated to quantify the amount of CO$_2$ evolution. Soil respiration rate was expressed as mg g$^{-1}$ d$^{-1}$.

Soil net N mineralization was determined by intact core procedure (Raison et al. 1987). A pair PVC tubes (20 cm in length, 5 cm in diameter) was inserted vertically into a depth of 15 cm soil layer. One soil core from each pair was taken as the unincubated sample to measure initial inorganic N content ($\text{NH}_4^+$-N and $\text{NO}_3^-$-N) by a flow injection autoanalyzer (FIAstar 5000 Analyzer, Denmark). The other core was incubated in situ lasting 4-week in capped tubes to prevent leaching. Soil inorganic N content was also measured after incubation. Net N mineralization rate was calculated as the changes in inorganic N content in the initial and incubated samples (Wang et al., 2006), and was expressed as mg g$^{-1}$ d$^{-1}$.

Phospholipid fatty acids (PLFAs) analysis was used to quantify microbial community composition. PLFAs were extracted from soil samples following a procedure described by Bossio et al. (1998). The separation and identification of extracted PLFAs were carried out using a Microbial Identification System (Newark, DE, USA). Methyl nonadecanoate fatty acid (19:0) was used as internal standard. (1) Bacteria: a13: 0, i14: 0, i15: 0, i16: 0, i17: 0, a17: 0, 16: 1ω7c, 17: 1ω8c, 18: 1ω5c, 18: 1ω9t, 17: 0cy and 19: 0cy; (2) Fungi: 18: 2ω6c, 18: 1ω9c (Frostegård et
al., 2011). The ratio of fungal to bacterial PLFAs has been used as the indicator of change in the soil microbial community composition (Fierer et al. 2002).

2.5 Statistical analyses

Data management and regression analyses were performed using the packages for R (R Development Core Team, USA). The AIC criterion was used to select a best model for each data set. We fit a linear or segmented linear model to each data set using least squares regression.

Regression analyses were conducted on the relationships of shoot and root biomass, shoot biomass C and N, root biomass C and N, litter mass, litter biomass C and N, soil respiration and N mineralization rates, soil moisture and clay content, soil total C and N, soil heavy fractions of C and N, inorganic N, ecosystem C and N sequestration with MAP.

Stepwise linear regression analyses were used to determine the relationships between ecosystem C or N processes with environmental factors. If the several independent variables are existed higher correlations, which may contribute to overfitting correlation, stepwise regression can delete one or more correlated independent variables to lest including complexity such as interactions. Significant differences among the sites in measured variables were determined using One-way ANOVA.

3 Results

3.1 Plant and litter properties

Aboveground biomass increased from 62 to 332 g m⁻² (R² = 0.73, P = 0.003), but plant N content
dropped from 3.99 to 1.75 % ($R^2 = 0.64$, $P = 0.01$) with increasing mean annual precipitation (MAP) along the gradient. Root biomass showed no remarkable differences among all sites (Fig. 2A, B). Similarity, aboveground biomass C and N changed significantly with precipitation variability, whereas root biomass C and N remained nearly constant. Aboveground biomass C and N, on average, increased from 33 to 150 g m$^{-2}$ ($R^2 = 0.74$, $P = 0.004$; Fig. 2C) and 2.3 to 6.9 g m$^{-2}$ from the dry to mesic sites ($R^2 = 0.60$, $P = 0.014$; Fig. 2D). Litter N content decreased from 0.35 to 0.25 % ($R^2 = 0.65$, $P = 0.009$), and litter mass ($R^2 = 0.40$, $P = 0.05$) and litter biomass C and N ($R^2 = 0.41$, $P = 0.047$; $R^2 = 0.45$, $P = 0.035$) only increased marginally with increasing MAP along the gradient (Fig. 2E-H; $P > 0.05$, One-way ANOVA).

### 3.2 Soil microbial community, C and N mineralization

The viable microbial biomass (*i.e.* total PLFAs) presented to an increase trend ($R^2 = 0.73$, $P < 0.0001$; Fig. 3A), but soil fungal: bacterial biomass increased first ($R^2 = 0.83$, $P = 0.005$) and then decreased with water availability from the dry to mesic sites along the gradient ($R^2 = 0.86$, $P = 0.001$; Fig. 3B). The meadows had the highest microbial biomass (c. 33.8 nmol g$^{-1}$), having 1.8-fold greater than the desert steppes, which had the lowest value (c. 19.2 nmol g$^{-1}$).

The pattern of soil respiration rate at the depth of 0-15 cm increased at first ($R^2 = 0.73$, $P = 0.006$), reached the highest values, and then decreased with MAP along the gradient ($R^2 = 0.73$, $P = 0.029$; Fig. 4C). The highest respiration rate from the typical steppe (site No. 7) was 28 % higher than the values from the driest and wettest sites, respectively ($P < 0.05$; One-way ANOVA). Inconsistent with soil respiration, soil net N mineralization rate increased marginally
with increasing MAP from the dry to mesic sites (One-way ANOVA, $P > 0.05$; Fig. 4D).

3.3 Soil properties

In general, soil moisture and clay content, soil total C and N content, heavy fractions of C and N, soil C: N and inorganic N differed significantly along the large-scale precipitation gradient (Fig. 4). Soil moisture and clay content linearly increased with MAP ($R^2 = 0.75$, $P < 0.0001$; $R^2 = 0.74$, $P < 0.0001$), and varied 7.4-fold and 1.7-fold along the gradient (Fig. 4A, B). Soil total C and N and heavy fractions of C and N content to a depth of 15 cm layer increased from 1316 to 4232 g m$^{-2}$ ($R^2 = 0.86$, $P < 0.0001$; Fig. 4C), 196 to 438 g m$^{-2}$ ($R^2 = 0.49$, $P = 0.003$; Fig. 4D), 1034 to 3188 g m$^{-2}$ ($R^2 = 0.84$, $P < 0.0001$; Fig. 4E), 177 to 305 g m$^{-2}$ ($R^2 = 0.48$, $P = 0.0036$; Fig. 4F) with MAP along the gradient, respectively. Similarly, soil C: N ($R^2 = 0.43$, $P = 0.006$; Fig. 3G) linearly increased with MAP, and varied 2.2-fold along the gradient. In contrast, soil inorganic N content increased first ($R^2 = 0.77$, $P = 0.001$) and then decreased ($R^2 = 0.46$, $P = 0.209$; Fig. 3H) from the dry to mesic sites. Overall, ecosystem C and N sequestration enhanced with increasing precipitation in terms of C and N storage in shoot, root, litter and soil in *L. chinensis* grasslands, and varied 3.2-fold and 1.8-fold along the gradient, respectively (Fig. 4I,J).

3.4 Relationship among ecosystem C and N dynamics and environmental factors

Across the 14 sites, stepwise multiple regression analyses demonstrated that 73 % and 61 % of the spatial variation in above biomass C and N could be only explained by MAP (Table 2). Similarity, MAP alone contributed to 43 % and 46 % of the variation in litter biomass C and N.
MAP (partial $r^2 = 0.71$) and soil clay content (partial $r^2 = 0.21$) were responsible for 92% of the spatial variation in soil total C content. In contrast, seventy-seven percent of the spatial variation in soil total N content could be explained by MAP (partial $r^2 = 0.57$), clay content (partial $r^2 = 0.12$) and soil C: N (partial $r^2 = 0.08$). Along the precipitation gradient, soil F: B (partial $r^2 = 0.52$), mean monthly temperature (partial $r^2 = 0.30$) and inorganic N content (partial $r^2 = 0.10$) together accounted for 92% of the spatial variation in soil respiration rate. Seventy-nine percent of the spatial variability in net N mineralization rate could be attributable to the combination of mean monthly temperature (partial $r^2 = 0.50$), soil F: B (partial $r^2 = 0.15$) and soil C: N (partial $r^2 = 0.14$).

4 Discussion

Recent researches have indicated that the variability of precipitation is an important driver to impact ecosystem C and nutrient cycles directly through C sequestration, mineralization, and uptake as well as controlling inputs and outputs of nutrients at global and regional extent (Austin and Sala 2002). In this study, aboveground biomass C and N increased linearly with MAP along the gradient (Fig. 2C, D), suggesting that primary production in the native *L. chinensis* grasslands is limited, at least in part, by water availability. The positive precipitation-aboveground production relationship is similar with that reported in grasslands (Barrett et al. 2002; Wang et al. 2003; Zhou et al. 2009), forests (Austin et al. 2002; Schuur and Matson 2001), woodlands (Zerihun et al. 2006) with the MAP range of 125 to 1200 mm. Compared to aboveground biomass, we found that litter mass (standing plus surface litter) only
slightly enhanced with increasing MAP along the gradient (Fig. 2E). This trend likely attributes
to the enhancement of litter decomposition from the dry to the mesic sites in the *L. chinensis*
grasslands. Across the precipitation gradient, MAP and mean annual temperature (MAT) are
highly correlated, whereas stepwise multiple regression analyses demonstrated that MAT did not
significantly contribute to the variation in aboveground biomass and litter mass (Table 2).
Therefore, temperature may not confound the effects of precipitation on aboveground C and N
storage in *L. chinensis* grasslands along the gradient.

It has been known that belowground C allocation to roots and soil organic matter may serve as
important fraction of the C sink in grassland ecosystems (Scurlock and Hall 1998). In this study,
however, root biomass C and N remained relatively constant across the precipitation gradient
(Fig. 2C, D). These trends have also been observed at the local and regional scales (Ma et al.
2012; Zhou et al. 2009), in which plants respond to increased precipitation by decreasing the
photosynthates allocation to root system relative to the aboveground parts (to enhance light
capture). Such lack of response of the root biomass to precipitation variability was likely because
nutrients limit root growth along the gradient. Although we cannot prove this point, manipulating
N and water experiments demonstrated that N addition accelerated plant growth, especially root
growth, whereas no effect of water addition on root biomass in this study region (Lü et al. 2010;

There has been a surge of research showed that soil respiration and N mineralization rate were
positively related to water availability in arid and semiarid ecosystems (McCully et al. 2005;
Xiao et al. 2007; Kim et al. 2011). In contrast, the positive water effects on soil respiration was
inconsistent, with an increase first and then a decrease with increasing water availability ($R^2 = 0.35$, $R^2 = 0.37$; Fig. 3C). This trend could have been attributed to the relative changes in microbial community composition (i.e. soil F: B), mean monthly temperature (MMT) and soil inorganic N content (IN) along the gradient, because stepwise multiple regression analyses showed that soil respiration rates were positively correlated with MMT and IN, and negatively correlated with soil F: B (Table 2). In this study, the shifts in soil F: B probably controls the decomposition process of organic matter. Soil fungal hyphae can enmesh and entangle soil microaggregates into macroaggregates, which favor stabilization and protection of soil organic matter (Tisdall et al. 1997; Rillig 2004). Therefore, the increases in soil F: B in mesic sites (MAP: 415-472 mm) may alter the soil respiration from a faster bacterial-based channel to a slower fungal-based channel. Another possible explanation is that the declines in MMT and soil N availability in the mesic sites (MAP: 422-472mm) likely result in temperature and nutrient limitation of microbial activity.

Surprisingly, soil net N mineralization rate increased marginally with increasing MAP along the gradient (Fig. 4D), which could be largely explained by the changes in organic matter quality. The significant decrease in plant N content (Fig. 2B) with increasing MAP could contribute to large quantities of lower C: N residues incorporating into soil organic matter, and then relatively declined N release during decomposition process (Aber et al. 1998; Boggs et al. 2000). Additionally, positive correlations between MMT and soil respiration or N mineralization indicate that temperature is a more important driver to impact the decomposition of soil organic matter than precipitation across this longitudinal transect.
Soil C and N storage is the result of a balance between inputs from plant production and outflows from organic matter decomposition (Zhou et al. 2009). Many studies have demonstrated that the responses of topsoil C and N content (0-30 cm) to increased precipitation represent increases (Burke et al. 1989; Zhou et al. 2002), decreases (Meersmans et al. 2009) and no changes (Zhou et al. 2009) at regional scales in grassland ecosystems. In this study, our results showed that soil C and N content, especially soil heavy fraction of C and N, increased significantly with MAP along the gradient (Fig. 4C-F) and the enhancements of aboveground biomass and clay content to increased precipitation may account for these trends. These findings have some important implications to soil C and N storage in the long-term. In natural ecosystems, turnover times of soil light fraction of organic matter typically vary with climate and vegetation, and range from months to several years. In contrast, turnover times of soil heavy fraction of organic matter vary with mineralogy and maturity, and range from millennia and longer (Trumbore 1997). The positive relationship between heavy fraction of organic matter content and MAP in this study suggests that the soils of native L. chinensis grassland have a high potential for C and N storage. Totally, ecosystem C and N sequestration enhanced with increasing precipitation in terms of C and N storage in aboveground biomass, root, litter, and soil in the L. chinensis grasslands, and varied approximately 3.2-fold and 1.8-fold along the gradient (Fig. 4I, J). Similar results have been reported in several researches at local and regional scales, in which McCulley et al. (2009) and Lü et al. (2010) observed that increasing precipitation stimulated ecosystem C and N sequestration.

Although this study suffers from several caveat that limit a complete understanding of how the
variability of precipitation affect ecosystem C and N processes, these results may not alter the overall patterns of C and N dynamics along the precipitation gradient according to several reasons. Firstly, while the use of climatology averaged data with field observations on the sampling year to address climate control on plant production may not be the ideal choice, whereas the plant production were also strongly correlated to MAP in this study. So while seasonal representations of precipitation were better predictors of plant production, they were only marginally so (Appendix 1). We attempt to capture the variation of plant production resulting from the climatic fluctuations and to represent the “average” condition of plant production through wide–area samplings (1-2 hm²) in each site. Secondly, soil microbial community composition (i.e. F: B) is strongly controlled by climate history and un-respond to seasonal or annual climate variability (Drenovský et al. 2010).

In conclusion, the variability of precipitation significantly affected ecosystem C and N dynamics. Aboveground biomass and soil C and N content gradually increased with MAP, while litter mass and root biomass varied slightly along the gradient. Soil respiration increased first and then decreased from the dry to mesic sites, but no significant regional trend in N mineralization was found. Increasing precipitation favors ecosystem C and N sequestration in terms of C and N storage in aboveground biomass, root, litter, and soil along the gradient. Our findings indicate that MAP and soil texture (i.e. clay content) primarily regulate ecosystem C and N sequestration, while microbial community composition, seasonal temperature and soil quality (i.e. C: N) mainly contribute to the C and N turnover across the large-scale precipitation gradient in *L. chinensis* grasslands of northeastern China.
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References


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Table 1. Locations of the study site (1 – 14, see Fig. 1), vegetation type, climatic indice, soil type, pH, dominant and companion species. MAP, mean annual precipitation; MAT, mean annual temperature; AP, annual precipitation in 2012; MMT, mean monthly temperature; DI, aridity index; ELE, elevation.

<table>
<thead>
<tr>
<th>Site</th>
<th>No.</th>
<th>Location</th>
<th>Vegetation type</th>
<th>MAP (mm)</th>
<th>MAT (°C)</th>
<th>AP (°C)</th>
<th>MMT (°C)</th>
<th>DI</th>
<th>ELE (m)</th>
<th>Soil type</th>
<th>pH</th>
<th>Dominant and companion species</th>
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</thead>
<tbody>
<tr>
<td>Baogedawula</td>
<td>1</td>
<td>43°56′N, 114°34′E</td>
<td>Desert steppe</td>
<td>237</td>
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<td>7.7</td>
<td><em>L. chinensis, S. grandis</em></td>
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<td>284</td>
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<td>1.37</td>
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<td>24.0</td>
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<td>0.97</td>
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<td>44°35′N, 123°30′E</td>
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<td>430</td>
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<td>11</td>
<td>44°34′N, 123°31′E</td>
<td>Meadow</td>
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<td>5.4</td>
<td>435</td>
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<td>0.97</td>
<td>165</td>
<td>Dark meadow</td>
<td>8.1</td>
<td><em>L. chinensis, L. quinquefolium</em></td>
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<td>44°36′N, 123°48′E</td>
<td>Meadow</td>
<td>442</td>
<td>5.3</td>
<td>432</td>
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<td>0.93</td>
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<td><em>L. chinensis, P. australis</em></td>
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Table 2 Results of stepwise multiple regression analyses. Dependent variables: aboveground biomass C (AB-C, g m$^{-2}$), litter biomass C (LB-C, g m$^{-2}$), Soil C (g m$^{-2}$), soil respiration (SR, mg kg$^{-1}$ d$^{-1}$), aboveground biomass N (AB-N, g m$^{-2}$), litter biomass N (LB-N, g m$^{-2}$), Soil N (g m$^{-2}$), net N mineralization (NMR, mg kg$^{-1}$ d$^{-1}$); Independent variable: mean annual precipitation (MAP, mm), soil clay content (%), soil fungal: bacterial biomass (F: B), mean monthly temperature (MMT, °C), soil inorganic N (mg kg$^{-1}$), soil C: N. Negative values of parameter estimate refer negative relationships between the examined dependent variables and the independent variables.

<table>
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<th>Variable entered</th>
<th>Parameter estimate</th>
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<th>Probability</th>
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Figure legends

Fig. 1. Study sites (1 – 14; see Table 1) along the 1000-km longitudinal transect in native *Leymus chinensis* grasslands of northeastern China.

Fig. 2. Patterns of aboveground and root biomass (A), plant N content (B), aboveground and root biomass C (C), aboveground and root biomass N (D), litter mass (E), litter N content (F), and litter biomass C and N (G, H) along the precipitation gradient in native *Leymus chinensis* grasslands of northeastern China.

Fig. 3. Patterns of soil microbial biomass (PLFAs) (A), soil fungal: bacterial biomass (F: B) (B), soil respiration rate (C) and soil net N mineralization rate (D) across the precipitation gradient in *Leymus chinensis* grasslands of northeastern China.

Fig. 4. Soil properties and C and N sequestration along the large-scale precipitation gradient in *Leymus chinensis* grasslands of northeastern China. Soil moisture (A), clay content (B), soil total C and N (C, D), soil heavy fractions of C and N (E, F), soil C: N (G), inorganic N (H), ecosystem C and N sequestration (I, J).
Support information

Appendix 1 Aboveground and root biomass, plant N content, aboveground and root biomass C, aboveground and root biomass N, litter mass, litter N content, litter biomass C and N, soil microbial biomass (PLFAs), soil fungal: bacterial biomass (F: B), soil respiration rate and soil net N mineralization rate versus annual precipitation in 2012 across the longitudinal transect.
Fig. 1
Fig. 3
Fig. 4

(A) $R^2 = 0.75, P < 0.0001$
(B) $R^2 = 0.74, P < 0.0001$

(C) $R^2 = 0.86, P < 0.0001$
(D) $R^2 = 0.49, P = 0.003$

(E) $R^2 = 0.84, P < 0.0001$
(F) $R^2 = 0.48, P = 0.0036$

(G) $R^2 = 0.43, P = 0.0059$
(H) $R^2 = 0.77, P = 0.001$

(I) $R^2 = 0.72, P = 0.0039$
(J) $R^2 = 0.67, P = 0.0077$
Appendix 1