

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

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25 **Abstract**

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

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45

## 46 **1 Introduction**

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

57 Primary production is an important component of the global C cycles (Hsu et al. 2012).  
58 Recent large-scale transect studies evaluated the spatial variability of climatic factors and annual  
59 aboveground net primary production (ANPP) in many terrestrial ecosystems, and indicated that  
60 nonlinear and saturating relationships between regional precipitation and ANPP (Huxman et al.  
61 2004; Yang et al. 2008; Hsu et al. 2012). A 1 % increase in MAP caused a -0.2% to 1.8% change  
62 in ANPP, and the sensitivities to precipitation peaked at *c.* 500 mm (Hsu et al. 2012). In  
63 temperate grasslands, root production is also an important resource of organic matter, which  
64 represents 50-80% of total biomass (Tomaškin 2007). In terms of C sequestration, it is likely that  
65 belowground production is a more important soil C source than aboveground production  
66 (McCulley et al. 2005), and thus a further understanding of total plant production across a

67 large-scale precipitation gradient is crucial for predicting grassland ecosystem C and N  
68 dynamics.

69 Another component of ecosystem C and nutrient cycles is litter (Zhou et al. 2009), because it  
70 not only contributes to aboveground and belowground C and N sequestration (Rinnan et al.  
71 2008), but also closely relates to element fluxes (Michelsen et al. 2007) and hydrological cycle  
72 (Willms and Chanasyk 2006). Available results show that litter mass is positively correlated with  
73 precipitation in water-limited regions, and the mass reaches a threshold after which it declines  
74 with increasing precipitation in mesic regions (Zhou et al. 2009; Wang et al. 2011). In the  
75 temperate grasslands of northeastern China, soil fertility and productivity are maintained  
76 primarily by recycling of nutrients through litter decomposition, with little fertilization or N  
77 deposition (Liu et al. 2006), whereas observations of litter dynamic are lacking across a  
78 large-scale precipitation gradient in grassland ecosystems.

79 In grassland ecosystems, about 50-70 % of organic C and N sequestered in topsoil (Jobbagy  
80 and Jackson 2000; Zhou et al. 2007). The main factor determining the amount of soil C and N  
81 storage is decomposition of organic matter (i.e. soil respiration and N mineralization). Soil  
82 organic matter are mineralized to inorganic forms by microbial communities (Paul 2007), and  
83 result in releasing CO<sub>2</sub> to the atmosphere and providing inorganic nutrient for plant growth  
84 (Manzoni and Porporato 2009). Higher mineralization processes typically indicate instability of  
85 soil organic matter (Trumbore et al. 1997). There is plenty of evidence to demonstrate that soil  
86 respiration and net N mineralization increased with precipitation and water availability in  
87 water-limited regions (Sierra et al. 1997; Barrett et al. 2002; Paul et al. 2003; McCulley et al.

88 2005; Ma et al. 2013). According to Stanford and Epstein (1974), the maximum net N  
89 mineralization rate occurs when soil moisture is close to field capacity. However, few studies  
90 have look at regional patterns of soil respiration, N mineralization and soil C and N storage with  
91 the variability of precipitation.

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

101

## 102 **2 Materials and methods**

### 103 **2.1 Study sites**

104 The field study was conducted at 14 perennial grass *Leymus chinensis* grasslands spanning a  
105 1000 km-long transect from west to east (114°E – 124°E), located in northeastern plain and Inner  
106 Mongolia plateau of China (Table 1, Fig. 1). The transect has a continental monsoon climate,  
107 with large seasonal temperature and precipitation variations. Long-term (1950 – 2000) mean  
108 annual temperature, mean annual precipitation and aridity index along the transect range from

109 approximately 1.3 – 6.8 °C, 237 – 472 mm and 0.91 – 1.44, respectively (<http://www.worldclim.com>;  
110 Zhang et al. 1997; Table 1). [The intra-annual precipitation in the sampling year 2012](#)  
111 [increased from 246 mm in the west to 491 mm in the east](#) (<http://cdc.cma.gov.cn>; Table 1). The  
112 elevation gradients range from 140 – 1309 m. Because the transect runs parallel to a latitude line  
113 (43° 30' N), it has approximately uniform theoretical radiation.

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

126

## 127 **2.2 Plant biomass and litter mass**

128 [About 1 - 2 hm<sup>2</sup> native \*L. chinensis\* grassland was selected for field sampling at each site.](#)

129 Aboveground living biomass, standing litter and surface litter were harvested by 10 – 12

130 randomly  $0.5 \times 0.5$  m quadrats of each site in mid-August 2012, which is the period of  
131 aboveground biomass attains peak value. Root biomass was measured by soil coring method  
132 sampling to a depth of 30 cm using a cylindrical root sampler (8 cm inner diameter). All samples  
133 of aboveground living tissue, litter and root were oven-dried at 65 °C to constant weight. C and N  
134 content of shoot, root and litter were determined by elemental analyzer (Elementaranalyser  
135 vario Max CN, Germany).

136

### 137 **2.3 Soil property**

138 Soil samples from 14 sites were collected along the systematically located transect lines in  
139 12–18 July 2012. 8 – 16 soil core samples were collected randomly per site ( $100 \times 100$  m) for  
140 determination of soil properties. The samples were taken to a depth of 15 cm soil layer with a  
141 cylindrical soil sampler (5 cm inner diameter), and then immediately preserved at 4 °C in a  
142 cooler for transport to the laboratory within one week of collection. The fresh samples were  
143 processed using a 2 mm sieve and cleared of visible plant roots and organic debris.

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

151

152 **2.4 C and N mineralization and microbial community**

153 Soil respiration was measured in the field by the alkaline absorbance method (Meyer et al. 2006).

154 Soil sample (20 g on an oven-dry soil basis) was incubated with 50 ml of 0.5 M NaOH in a

155 closed vessel at ambient soil temperature over 4-week incubation period from 12–18 July to

156 8–14 August 2012. The NaOH solution was titrated to quantify the amount of CO<sub>2</sub> evolution.

157 Soil respiration rate was expressed as mg g<sup>-1</sup> d<sup>-1</sup>.

158 Soil net N mineralization was determined by intact core procedure (Raison et al. 1987). A pair

159 PVC tubes (20 cm in length, 5 cm in diameter) was inserted vertically into a depth of 15 cm soil

160 layer. One soil core from each pair was taken as the unincubated sample to measure initial

161 inorganic N content (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) by a flow injection autoanalyzer (FIAStar 5000

162 Analyzer, Denmark). The other core was incubated in situ lasting 4-week in capped tubes to

163 prevent leaching. Soil inorganic N content was also measured after incubation. Net N

164 mineralization rate was calculated as the changes in inorganic N content in the initial and

165 incubated samples (Wang et al., 2006), and was expressed as mg g<sup>-1</sup> d<sup>-1</sup>.

166 Phospholipid fatty acids (PLFAs) analysis was used to quantify microbial community

167 composition. PLFAs were extracted from soil samples following a procedure described by

168 Bossio et al. (1998). The separation and identification of extracted PLFAs were carried out using

169 a Microbial Identification System (Newark, DE, USA). Methyl nonadecanoate fatty acid (19: 0)

170 was used as internal standard. (1) Bacteria: a13: 0, i14: 0, i15: 0, i16: 0, i17: 0, a17: 0, 16: 1ω7c,

171 17: 1ω8c, 18: 1ω5c, 18: 1ω9t, 17: 0cy and 19: 0cy; (2) Fungi: 18: 2ω6c, 18: 1ω9c (Frostegård *et*



172 *al.*, 2011). The ratio of fungal to bacterial PLFAs has been used as the indicator of change in the  
173 soil microbial community composition (Fierer et al. 2002).

174

## 175 **2.5 Statistical analyses**

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

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

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

189

## 190 **3 Results**

### 191 **3.1 Plant and litter properties**

192 Aboveground biomass increased from 62 to 332 g m<sup>-2</sup> ( $R^2 = 0.73$ ,  $P = 0.003$ ), but plant N content

193 dropped from 3.99 to 1.75 % ( $R^2= 0.64, P = 0.01$ ) with increasing mean annual precipitation  
194 (MAP) along the gradient. Root biomass showed no remarkable differences among all sites (Fig.  
195 2A, B). Similarly, aboveground biomass C and N changed significantly with precipitation  
196 variability, whereas root biomass C and N remained nearly constant. Aboveground biomass C  
197 and N, on average, increased from 33 to 150 g m<sup>-2</sup> ( $R^2= 0.74, P = 0.004$ ; Fig. 2C) and 2.3 to 6.9 g  
198 m<sup>-2</sup> from the dry to mesic sites ( $R^2= 0.60, P = 0.014$ ; Fig. 2D). Litter N content decreased from  
199 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  
200 and N ( $R^2= 0.41, P = 0.047$ ;  $R^2= 0.45, P = 0.035$ ) only increased marginally with increasing  
201 MAP along the gradient (Fig. 2E-H;  $P > 0.05$ , One-way ANOVA).

202

### 203 3.2 Soil microbial community, C and N mineralization

204 The viable microbial biomass (*i.e.* total PLFAs) presented to an increase trend ( $R^2= 0.73, P <$   
205  $0.0001$ ; Fig. 3A), but soil fungal: bacterial biomass increased first ( $R^2= 0.83, P = 0.005$ ) and then  
206 decreased with water availability from the dry to mesic sites along the gradient ( $R^2= 0.86, P =$   
207  $0.001$ ; Fig. 3B). The meadows had the highest microbial biomass (*c.* 33.8 nmol g<sup>-1</sup>), having  
208 1.8-fold greater than the desert steppes, which had the lowest value (*c.* 19.2 nmol g<sup>-1</sup>).

209 The pattern of soil respiration rate at the depth of 0-15 cm increased at first ( $R^2= 0.73, P =$   
210  $0.006$ ), reached the highest values, and then decreased with MAP along the gradient ( $R^2= 0.73,$   
211  $P = 0.029$ ; Fig. 4C). The highest respiration rate from the typical steppe (site No. 7) was 28 %  
212 higher than the values from the driest and wettest sites, respectively ( $P < 0.05$ ; One-way  
213 ANOVA). Inconsistent with soil respiration, soil net N mineralization rate increased marginally

214 with increasing MAP from the dry to mesic sites (One-way ANOVA,  $P > 0.05$ ; Fig. 4D).

215

### 216 **3.3 Soil properties**

217 In general, soil moisture and clay content, soil total C and N content, heavy fractions of C and N,

218 soil C: N and inorganic N differed significantly along the large-scale precipitation gradient (Fig.

219 4). Soil moisture and clay content linearly increased with MAP ( $R^2 = 0.75$ ,  $P < 0.0001$ ;  $R^2 = 0.74$ ,

220  $P < 0.0001$ ), and varied 7.4-fold and 1.7-fold along the gradient (Fig. 4A, B). Soil total C and N

221 and heavy fractions of C and N content to a depth of 15 cm layer increased from 1316 to 4232 g

222  $m^{-2}$  ( $R^2 = 0.86$ ,  $P < 0.0001$ ; Fig. 4C), 196 to 438 g  $m^{-2}$  ( $R^2 = 0.49$ ,  $P = 0.003$ ; Fig. 4D), 1034 to

223 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)

224 with MAP along the gradient, respectively. Similarly, soil C: N ( $R^2 = 0.43$ ,  $P = 0.006$ ; Fig. 3G)

225 linearly increased with MAP, and varied 2.2-fold along the gradient. In contrast, soil inorganic N

226 content increased first ( $R^2 = 0.77$ ,  $P = 0.001$ ) and then decreased ( $R^2 = 0.46$ ,  $P = 0.209$ ; Fig. 3H)

227 from the dry to mesic sites. Overall, ecosystem C and N sequestration enhanced with increasing

228 precipitation in terms of C and N storage in shoot, root, litter and soil in *L. chinensis* grasslands,

229 and varied 3.2-fold and 1.8-fold along the gradient, respectively (Fig. 4I,J).

230

### 231 **3.4 Relationship among ecosystem C and N dynamics and environmental factors**

232 Across the 14 sites, stepwise multiple regression analyses demonstrated that 73 % and 61 % of

233 the spatial variation in above biomass C and N could be only explained by MAP (Table 2).

234 Similarly, MAP alone contributed to 43 % and 46 % of the variation in litter biomass C and N.

235 MAP (partial  $r^2 = 0.71$ ) and soil clay content (partial  $r^2 = 0.21$ ) were responsible for 92 % of the  
236 spatial variation in soil total C content. In contrast, seventy-seven percent of the spatial variation  
237 in soil total N content could be explained by MAP (partial  $r^2 = 0.57$ ), clay content (partial  $r^2 =$   
238 0.12) and soil C: N (partial  $r^2 = 0.08$ ). Along the precipitation gradient, soil F: B (partial  $r^2 =$   
239 0.52), mean monthly temperature (partial  $r^2 = 0.30$ ) and inorganic N content (partial  $r^2 = 0.10$ )  
240 together accounted for 92 % of the spatial variation in soil respiration rate. Seventy-nine percent  
241 of the spatial variability in net N mineralization rate could be attributable to the combination of  
242 mean monthly temperature (partial  $r^2 = 0.50$ ), soil F: B (partial  $r^2 = 0.15$ ) and soil C: N (partial  $r^2$   
243 = 0.14).

244

#### 245 **4 Discussion**

246 Recent researches have indicated that the variability of precipitation is an important driver to  
247 impact ecosystem C and nutrient cycles directly through C sequestration, mineralization, and  
248 uptake as well as controlling inputs and outputs of nutrients at global and regional extent (Austin  
249 and Sala 2002). In this study, aboveground biomass C and N increased linearly with MAP along  
250 the gradient (Fig. 2C, D), suggesting that primary production in the native *L. chinensis*  
251 grasslands is limited, at least in part, by water availability. The positive  
252 precipitation-aboveground production relationship is similar with that reported in grasslands  
253 (Barrett et al. 2002; Wang et al. 2003; Zhou et al. 2009), forests (Austin et al. 2002; Schuur and  
254 Matson 2001), woodlands (Zerihun et al. 2006) with the MAP range of 125 to 1200 mm.  
255 Compared to aboveground biomass, we found that litter mass (standing plus surface litter) only

256 slightly enhanced with increasing MAP along the gradient (Fig. 2E). This trend likely attributes  
257 to the enhancement of litter decomposition from the dry to the mesic sites in the *L. chinensis*  
258 grasslands. Across the precipitation gradient, MAP and mean annual temperature (MAT) are  
259 highly correlated, whereas stepwise multiple regression analyses demonstrated that MAT did not  
260 significantly contribute to the variation in aboveground biomass and litter mass (Table 2).  
261 Therefore, temperature may not confound the effects of precipitation on aboveground C and N  
262 storage in *L. chinensis* grasslands along the gradient.

263 It has been known that belowground C allocation to roots and soil organic matter may serve as  
264 important fraction of the C sink in grassland ecosystems (Scurlock and Hall 1998). In this study,  
265 however, root biomass C and N remained relatively constant across the precipitation gradient  
266 (Fig. 2C, D). These trends have also been observed at the local and regional scales (Ma et al.  
267 2012; Zhou et al. 2009), in which plants respond to increased precipitation by decreasing the  
268 photosynthates allocation to root system relative to the aboveground parts (to enhance light  
269 capture). Such lack of response of the root biomass to precipitation variability was likely because  
270 nutrients limit root growth along the gradient. Although we cannot prove this point, manipulating  
271 N and water experiments demonstrated that N addition accelerated plant growth, especially root  
272 growth, whereas no effect of water addition on root biomass in this study region (Lü et al. 2010;  
273 Ma et al. 2012, 2013; Xiao et al. 2007).

274 There has been a surge of research showed that soil respiration and N mineralization rate were  
275 positively related to water availability in arid and semiarid ecosystems (McCully et al. 2005;  
276 Xiao et al. 2007; Kim et al. 2011). In contrast, the positive water effects on soil respiration was

277 inconsistent, with an increase first and then a decrease with increasing water availability ( $R^2 =$   
278  $0.35$ ,  $R^2 = 0.37$ ; Fig. 3C). This trend could have been attributed to the relative changes in  
279 microbial community composition (i.e. soil F: B), mean monthly temperature (MMT) and soil  
280 inorganic N content (IN) along the gradient, because stepwise multiple regression analyses  
281 showed that soil respiration rates were positively correlated with MMT and IN, and negatively  
282 correlated with soil F: B (Table 2). In this study, the shifts in soil F: B probably controls the  
283 decomposition process of organic matter. Soil fungal hyphae can enmesh and entangle soil  
284 microaggregates into macroaggregates, which favor stabilization and protection of soil organic  
285 matter (Tisdall et al. 1997; Rillig 2004). Therefore, the increases in soil F: B in mesic sites  
286 (MAP: 415-472 mm) may alter the soil respiration from a faster bacterial-based channel to a  
287 slower fungal-based channel. Another possible explanation is that the declines in MMT and soil  
288 N availability in the mesic sites (MAP: 422-472mm) likely result in temperature and nutrient  
289 limitation of microbial activity.

290 Surprisingly, soil net N mineralization rate increased marginally with increasing MAP along  
291 the gradient (Fig. 4D), which could be largely explained by the changes in organic matter quality.  
292 The significant decrease in plant N content (Fig. 2B) with increasing MAP could contribute to  
293 large quantities of lower C: N residues incorporating into soil organic matter, and then relatively  
294 declined N release during decomposition process (Aber et al. 1998; Boggs et al. 2000).  
295 Additionally, positive correlations between MMT and soil respiration or N mineralization  
296 indicate that temperature is a more important driver to impact the decomposition of soil organic  
297 matter than precipitation across this longitudinal transect.

298 Soil C and N storage is the result of a balance between inputs from plant production and  
299 outflows from organic matter decomposition (Zhou et al. 2009). Many studies have  
300 demonstrated that the responses of topsoil C and N content (0-30 cm) to increased precipitation  
301 represent increases (Burke et al. 1989; Zhou et al. 2002), decreases (Meersmans et al. 2009) and  
302 no changes (Zhou et al. 2009) at regional scales in grassland ecosystems. In this study, our  
303 results showed that soil C and N content, especially soil heavy fraction of C and N, increased  
304 significantly with MAP long the gradient (Fig. 4C-F) and the enhancements of aboveground  
305 biomass and clay content to increased precipitation may account for these trends. These findings  
306 have some important implications to soil C and N storage in the long-term. In natural ecosystems,  
307 turnover times of soil light fraction of organic matter typically vary with climate and vegetation,  
308 and range from months to several years. In contrast, turnover times of soil heavy fraction of  
309 organic matter vary with mineralogy and maturity, and range from millennia and longer  
310 (Trumbore 1997). The positive relationship between heavy fraction of organic matter content and  
311 MAP in this study suggests that the soils of native *L. chinensis* grassland have a high potential  
312 for C and N storage. Totally, ecosystem C and N sequestration enhanced with increasing  
313 precipitation in terms of C and N storage in aboveground biomass, root, litter, and soil in the *L.*  
314 *chinensis* grasslands, and varied approximately 3.2-fold and 1.8-fold along the gradient (Fig. 4I,  
315 J). Similar results have been reported in several researches at local and regional scales, in which  
316 McCulley et al. (2009) and Lü et al. (2010) observed that increasing precipitation stimulated  
317 ecosystem C and N sequestration.

318 Although this study suffers from several caveat that limit a complete understanding of how the

319 variability of precipitation affect ecosystem C and N processes, these results may not alter the  
320 overall patterns of C and N dynamics along the precipitation gradient according to several  
321 reasons. Firstly, while the use of climatology averaged data with field observations on the  
322 sampling year to address climate control on plant production may not be the ideal choice,  
323 whereas the plant production were also strongly correlated to MAP in this study. So while  
324 seasonal representations of precipitation were better predictors of plant production, they were  
325 only marginally so (Appendix 1). We attempt to capture the variation of plant production  
326 resulting from the climatic fluctuations and to represent the “average” condition of plant  
327 production through wide-area samplings (1-2 hm<sup>2</sup>) in each site. Secondly, soil microbial  
328 community composition (i.e. F: B) is strongly controlled by climate history and un-respond to  
329 seasonal or annual climate variability (Drenovsky et al. 2010).

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



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344

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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.

Site	No.	Location	Vegetation type	MAP (mm)	MAT (°C)	AP	MMT (°C)	DI	ELE (m)	Soil type	pH	Dominant and companion species
Baogedawula	1	43°56'N, 114°34'E	Desert steppe	237	1.7	246	23.8	1.44	1092	Chestnut	7.7	<i>L. chinensis</i> , <i>S. grandis</i>
Dabuxiletu	2	43°55'N, 115°44'E	Desert steppe	276	1.4	284	23	1.37	1158	Chestnut	7.8	<i>L. chinensis</i> , <i>A. critatum</i>
Aqiwula	3	43°33'N, 116°40'E	Steppe	340	1.3	322	22.7	1.33	1239	Chestnut	8.8	<i>L. chinensis</i> , <i>S. grandis</i>
Xinchengzi	4	43°27'N, 118°04'E	Steppe	397	3.5	353	24	1.23	919	Chestnut	7.7	<i>L. chinensis</i> , <i>P. flagellaris</i>
Tianshan	5	43°50'N, 119°55'E	Steppe	386	5.8	380	25.2	1.18	513	Chernozern	8.3	<i>L. chinensis</i> , <i>A. cristatum</i>
Shaogen	6	43°38'N, 120°47'E	Steppe	385	6.8	392	25.4	1.12	270	Chernozern	8	<i>L. chinensis</i> , <i>S. grandis</i>
Baolongshan	7	43°56'N, 122°42'E	Meadow	415	6	400	24.7	1	156	Chernozern	7.9	<i>L. chinensis</i> , <i>P. chinensis</i>
Jiamatu	8	44°01'N, 122°56'E	Meadow	422	6	425	24.5	1	149	Chernozern	8.2	<i>L. chinensis</i> , <i>S. grandis</i>
Taipingchuan	9	44°21'N, 123°14'E	Meadow	428	5.6	410	24.1	0.97	150	Chernozern	8.6	<i>L. chinensis</i> , <i>A. scoparia</i>
Yaojingzi	10	44°35'N, 123°30'E	Meadow	435	5.4	430	23.6	0.97	159	Dark meadow	8.4	<i>L. chinensis</i> , <i>A. scoparia</i>
Yaojingzi	11	44°34'N, 123°31'E	Meadow	434	5.4	435	23.6	0.97	165	Dark meadow	8.1	<i>L. chinensis</i> , <i>L. quinquene</i>
Wulanaodu	12	44°36'N, 123°48'E	Meadow	442	5.3	432	23	0.93	152	Dark meadow	8.1	<i>L. chinensis</i> , <i>C. duriuscula</i>
Chaganhua	13	44°35'N, 124°16'E	Meadow	467	5.1	478	22.5	0.93	202	Dark meadow	8.4	<i>L. chinensis</i> , <i>P. australis</i>
Wulantuga	14	44°28'N, 124°18'E	Meadow	472	5.1	491	22.5	0.91	291	Dark meadow	8.5	<i>L. chinensis</i> , <i>P. australis</i>

**Table 2** Results of stepwise multiple regression analyses. Dependent variables: aboveground biomass C (AB-C, g m<sup>-2</sup>), litter biomass C (LB-C, g m<sup>-2</sup>), Soil C (g m<sup>-2</sup>), soil respiration (SR, mg kg<sup>-1</sup>d<sup>-1</sup>), aboveground biomass N (AB-N, g m<sup>-2</sup>), litter biomass N (LB-N, g m<sup>-2</sup>), Soil N (g m<sup>-2</sup>), net N mineralization (NMR, mg kg<sup>-1</sup>d<sup>-1</sup>); 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<sup>-1</sup>), soil C: N. Negative values of parameter estimate refer negative relationships between the examined dependent variables and the independent variables.

	Variable entered	Parameter estimate	Partial r <sup>2</sup>	Probability
<b>C dynamic</b>				
AB-C	MAP	0.47	0.73	0.004
LB-C	MAP	0.02	0.43	0.043
Soil-C	MAP	12.84	0.71	0.000
	Clay content	1.38	0.21	0.011
SR	F: B	-31.82	0.52	0.000
	MMT	12.73	0.30	0.000
	Inorganic N	0.86	0.10	0.037
<b>N dynamic</b>				
AB-N	MAP	0.74	0.61	0.014
LB-N	MAP	0.01	0.46	0.035
Soil-N	MAP	1.15	0.57	0.000
	Clay content	1.06	0.12	0.028
	Soil C:N	-11.52	0.08	0.046
NMR	MMT	0.05	0.50	0.000
	F: B	-0.93	0.15	0.035
	Soil C:N	-0.07	0.14	0.037



## 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.

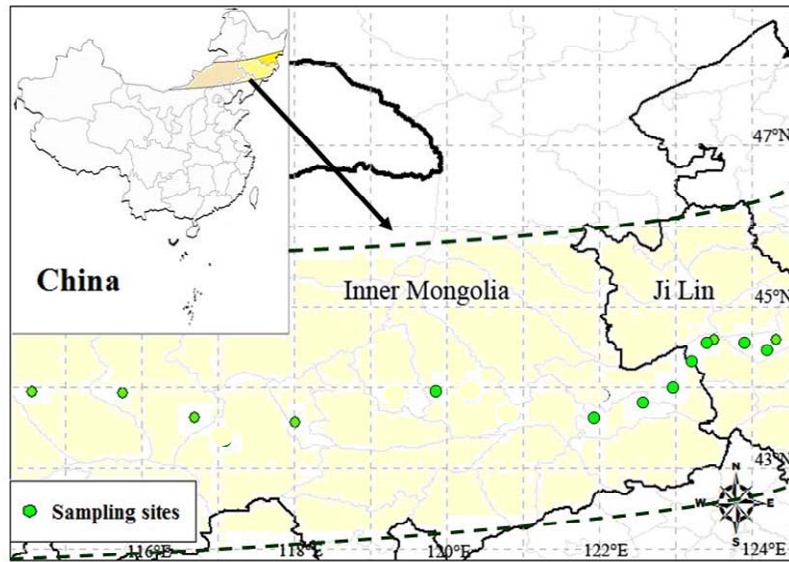
**Fig. 3.** Patterns of soil microbial biomass (PLFAs) (**A**), soil fungal: bacterial biomass (F: B) (**B**), soil respiration rate (**C**) and soil net N mineralization rate (**D**) across the precipitation gradient in *Leymus chinensis* grasslands of northeastern China.

**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. 2**

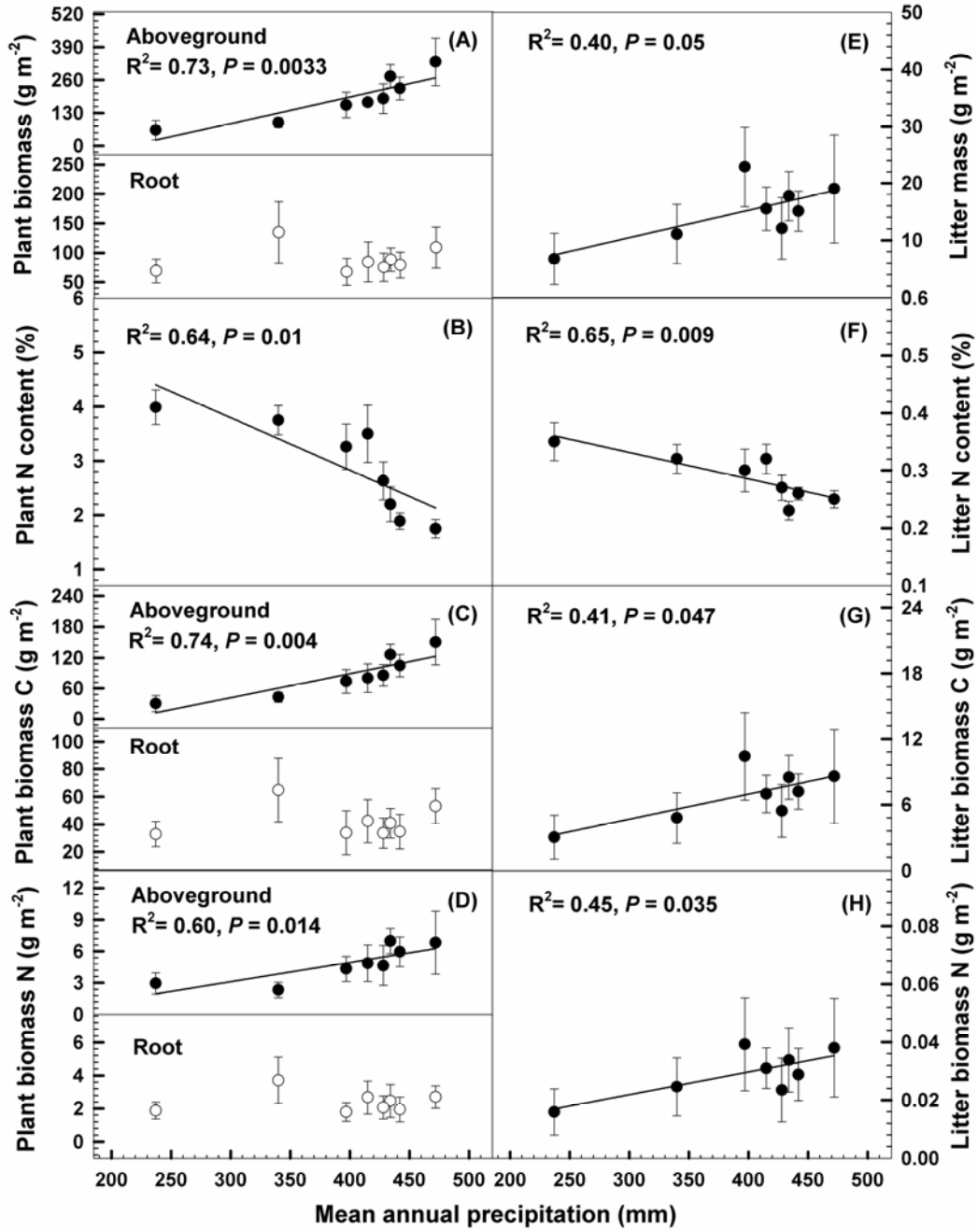
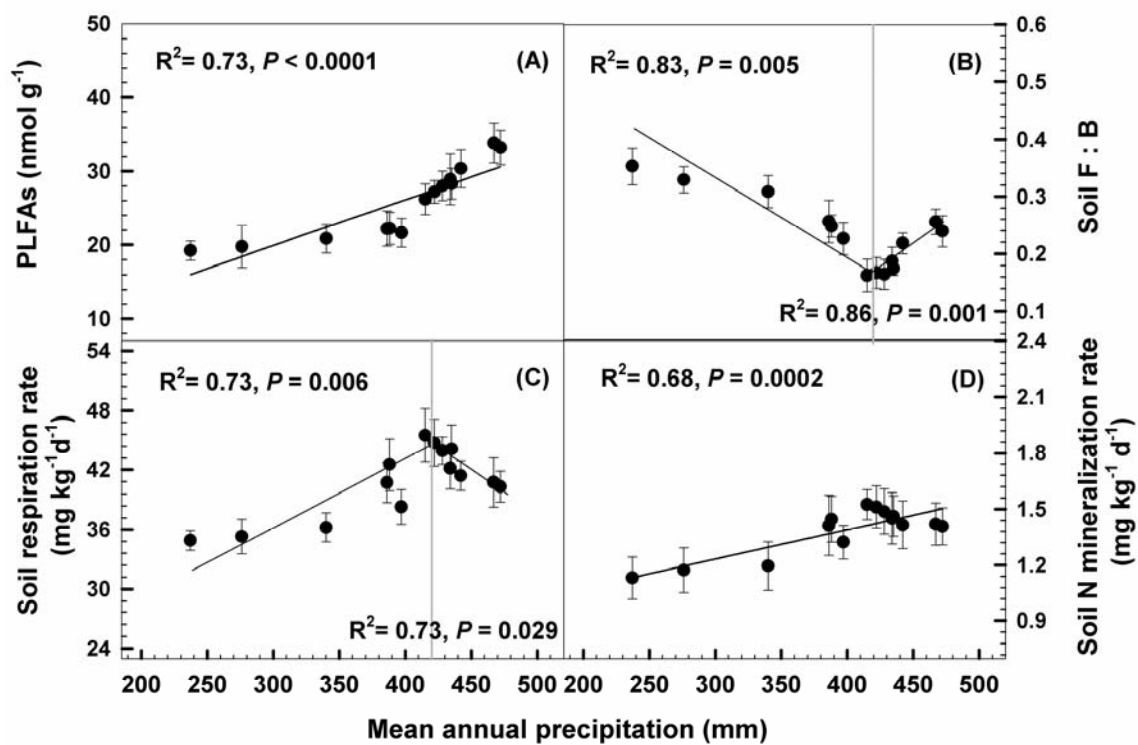
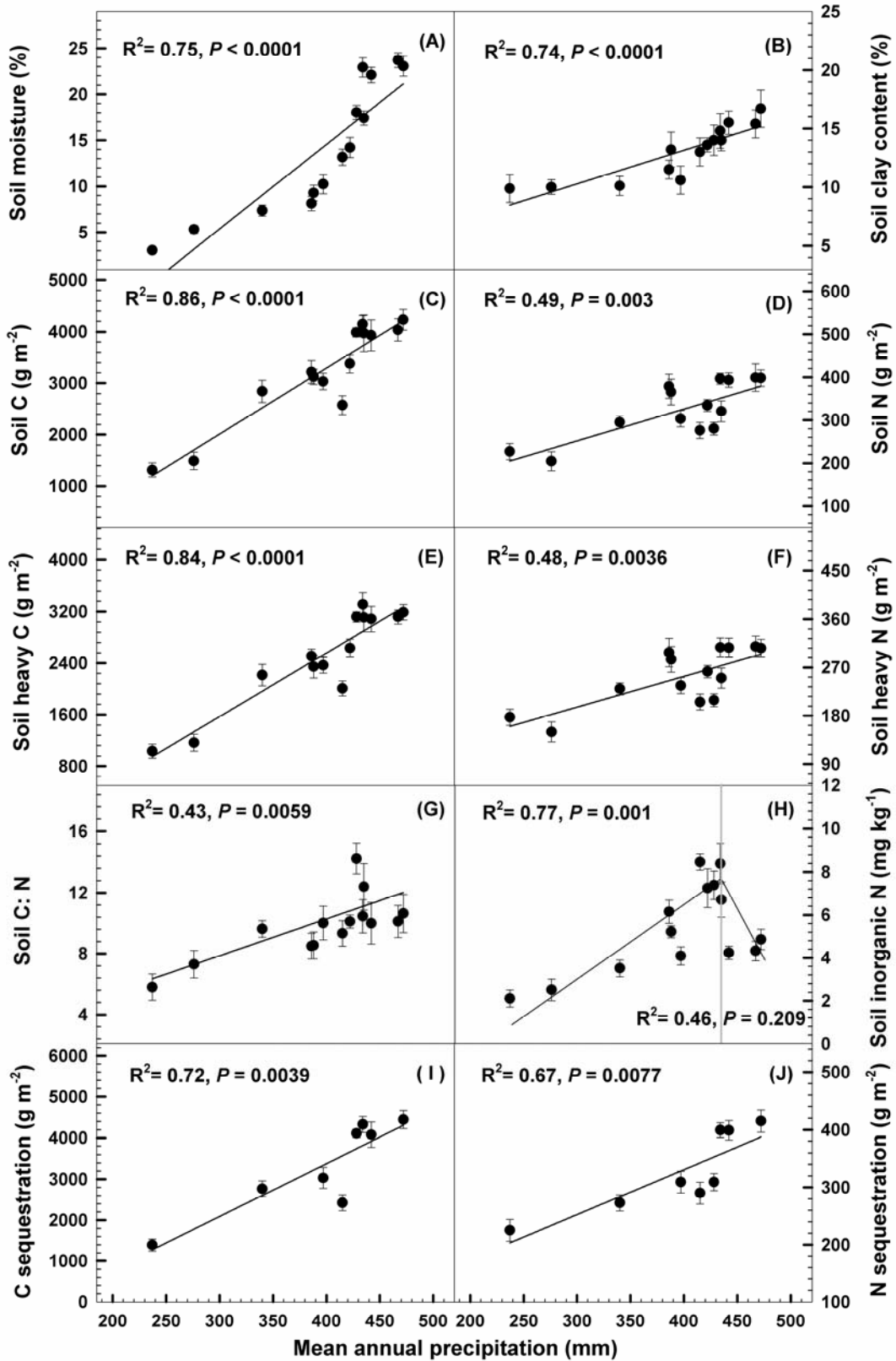


Fig. 3



**Fig. 4**



# Appendix 1

