

1 Strong stoichiometric resilience after litter manipulation experiments;  
2 a case study in a Chinese grassland  
3

4 Authors: Chunwang Xiao<sup>1</sup>, Ivan A. Janssens<sup>2</sup>, Yong Zhou<sup>1</sup>, Jiaqi Su<sup>1,3</sup>, Yun Liang<sup>1,3</sup>,  
5 Bertrand Guenet<sup>2,4</sup>  
6

7 <sup>1</sup>State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, the  
8 Chinese Academy of Sciences, Beijing 100093, China.

9 <sup>2</sup>Department of Biology, Research Group of Plant and Vegetation Ecology, University of  
10 Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium.

11 <sup>3</sup>University of Chinese Academy of Sciences, Beijing, 100049, China

12 <sup>4</sup>CNRS-CEA-UVSQ, Laboratoire des Sciences du Climat et de l'Environnement, UMR 8212,  
13 91191 Gif-sur-Yvette, France  
14

15 Correspondence to: Bertrand Guenet ([Bertrand.guenet@lsce.ipsl.fr](mailto:Bertrand.guenet@lsce.ipsl.fr)) and Chunwang Xiao  
16 ([cwxiao@ibcas.ac.cn](mailto:cwxiao@ibcas.ac.cn))  
17  
18  
19  
20  
21

22 **Abstract**

23 Global climate change has generally modified net primary production (NPP) which leads to  
24 increasing litter inputs in some ecosystems. Therefore, assessing the impacts of increasing  
25 litter inputs on soil nutrients, plant growth and ecological carbon (C):nitrogen  
26 (N):phosphorus (P) stoichiometry is critical for an understanding of C, N and P cycling and  
27 their feedback processes to climate change. In this study, we added plant above ground litter,  
28 harvested near the experimental plots, to the 10-20 cm subsoil layer of a steppe community at  
29 rates equivalent to annual litter input of 0, 15, 30, 60 and 120%, respectively, covering the  
30 entire range of the expected NPP increases in this region due to climate change (10-60%).  
31 We measured the resulting C, N and P content of different pools (above and below ground  
32 plant biomass, litter, microbial biomass). Small litter additions, which are more plausible  
33 compared to the expected increase predicted by Earth system models, had no effect on the  
34 variables examined. Nevertheless, high litter addition (120% of the annual litter inputs)  
35 significantly increased soil inorganic N and available P, aboveground biomass, belowground  
36 biomass and litter. Our results suggest that while very high litter addition can strongly affect  
37 C:N:P stoichiometry, the grassland studied here is resilient to more plausible inputs in terms  
38 of stoichiometric functioning.

39

40 Keywords: litter addition; C, N and P pools; C:N:P stoichiometry; soil microorganisms;  
41 temperate grassland

42

## 42 **1. Introduction**

43 Ecological stoichiometry is the study of the balance of multiple chemical elements in  
44 ecological interactions (Elser et al., 2000, 2010; Elser and Hamilton, 2007). Carbon (C),  
45 nitrogen (N) and phosphorus (P) are key elements in terrestrial ecosystems (Daufresne and  
46 Loreau, 2001; Elser et al., 2010; Hessen et al., 2013), and the C:N:P stoichiometry reflects  
47 complex interactions between evolutionary processes coupled to phenotypic plasticity  
48 (Sardans et al., 2012). These complex interactions are, at least partially, controlled by  
49 patterns of element supply from the environment (Hessen et al., 2004). Ecological  
50 stoichiometry provides a valuable approach in assessing possible changes in C, N and P  
51 cycling (Hessen et al., 2013). Over the past three decades ecological stoichiometry has  
52 expanded greatly (Hessen et al., 2013), and many studies have concentrated on understanding  
53 the variation in plant C, N and P concentrations among and within species (Schmidt et al.,  
54 1997; Aerts and Chapin, 2000; Güsewell and Koerselman, 2002; Augustine et al., 2003;  
55 Güsewell 2004; Frank, 2008). However, relationships among soil nutrient availability, plant  
56 growth and ecological C:N:P stoichiometry of plants, litter, soil and soil microbes under  
57 global climate change in terrestrial ecosystems remain poorly understood.

58 Litter is an important nutrient pool and litter decomposition rates strongly affect nutrient  
59 availability in terrestrial ecosystems with significant feedback on plant growth and on climate  
60 (Subke et al., 2004; Ryan and Law, 2005; Sayer, 2006; Cornelissen et al., 2007;  
61 Villalobos-Vega et al., 2011). The increase of atmospheric carbon dioxide (CO<sub>2</sub>)  
62 concentration within the next 100 years (Meinshausen et al., 2011) due to continued

63 anthropogenic carbon emissions is generally predicted to increase net primary production  
64 (NPP) (Todd-Brown et al., 2014). Nevertheless such effects do not scale linearly with  
65 increases in atmospheric CO<sub>2</sub> because productivity is also strongly controlled by climate.  
66 Regions with drying climates are likely to present reduced net primary production in the next  
67 decades (IPCC, 2013). Although uncertainties exists in the magnitude of the changes (e.g.  
68 Campbell et al., 1991; Arnone and Körner, 1995; Gill et al., 2002), increases in net primary  
69 production are probably predominant and will simultaneously increase litter inputs to soils.  
70 Modification of atmospheric CO<sub>2</sub>, of climate and of nutrient cycling may also modify the  
71 chemical composition of the litter (Cotrufo et al., 1999). Elevated CO<sub>2</sub> generally increases the  
72 lignin content and reduces the N concentration of plant tissues, although Norby et al., (2001)  
73 showed that this response depends on the experimental system used (open top chamber, free  
74 air CO<sub>2</sub> enrichment, etc.).

75 In past decades, the effects of litter addition on plant growth, soil C content and cycling,  
76 microbial biomass and priming of decomposition rates have been reported and confirmed (eg.  
77 Sulzman et al., 2005; Guenet et al., 2010; Jin et al., 2010; Chemidlin Prévost-Bouré et al.,  
78 2010; Sayer et al., 2011; Villalobos-Vega et al., 2011; Ma et al., 2012, Xiao et al., 2014).  
79 However, the impacts of litter addition on soil nutrients, plant growth and ecological C:N:P  
80 stoichiometry of plants, litter, soil and soil microbes remain highly uncertain. Moreover,  
81 climate change and increased atmospheric CO<sub>2</sub> would not only affect NPP but also  
82 environmental conditions for decomposers in soils (soil moisture, temperature), inducing  
83 modification of microbial community structure and activity (Singh et al., 2010). Associated



84 to litter additions, these effects would disturb the C cycle in soil, affecting the net ecosystem  
85 exchange particularly in grasslands where the vast majority of the C stock is stored  
86 belowground, due to their high root:shoot biomass and productivity ratios (Mokany et al.,  
87 2006).

88 Grassland is one of the most important global terrestrial ecosystems, covering about 25%  
89 of the global terrestrial area and 40% of the land area in China (Kang et al., 2006). The  
90 semi-arid and temperate grasslands of northern China account for about 78% of the national  
91 grassland area, where the native vegetation is predominantly characterized by the abundance  
92 of grass species such as *Stipa* spp. and *Leymus chinensis* (Trin) Tzvel., with *Stipa krylovii*  
93 Roshev. being well-represented as one of the major steppe community types (Zhao et al.,  
94 2003). Plant recruitment, growth and nutrient cycling of the region often are limited by soil  
95 water, nitrogen and phosphorus, and the regional soil fertility and productivity are maintained  
96 by recycling of nutrients through plant litter decomposition as an essential mechanism (Liu et  
97 al., 2006) with little natural nitrogen deposition. A better understanding of the effects of litter  
98 addition on plant growth and ecological C:N:P stoichiometry of plants, litter, soil and soil  
99 microbes could help to reduce uncertainties in our predictions of C, N and P balance as well  
100 as cycling, and structure and function in grassland ecosystems under global climate change.

101 We conducted a field experiment in which we artificially added litter inputs to subsoils (i.e.  
102 10-20 cm) under a *S. krylovii* steppe community in a temperate grassland of northern China  
103 to assess the effects on soil inorganic N and available P, plant growth, litter, C, N and P pools  
104 and the C:N:P stoichiometry of plant, litter, soil and soil microbes. The litter added was

105 obtained under current atmospheric CO<sub>2</sub> concentration and therefore, the impact of elevated  
106 CO<sub>2</sub> on the litter stoichiometry (Cotrufo et al., 1999) was not represented in our case. The  
107 primary objectives of our study were to determine whether the studied grassland was resilient  
108 to litter addition or if litter additions would increase soil inorganic N and available P and  
109 thereby affect plant growth, litter, and the C, N, P pools and the C:N:P stoichiometry of  
110 plants, litter, soil and soil microbes. Here, we define the term resilience as the absence of a  
111 significant effect of litter additions on the measured variables (plant biomass, C:N:P ratio,  
112 etc.).

113 In a previous study (Xiao et al 2014), we observed that litter additions in the temperate  
114 grassland of northern China accelerate decomposition of soil organic matter through a  
115 priming effect. Priming is defined here as a modification of the soil organic matter  
116 decomposition rate induced by an input of litter and mediated by the altered activity of the  
117 microbial community (Blagodatskaya and Kuzyakov, 2008). The main objective of this  
118 study was to analyze how additional litter inputs and associated priming of soil organic  
119 matter decomposition alter nutrient availability and thereby plant stoichiometry and  
120 productivity. .

121

## 122 **2. Materials and Methods**

### 123 **2.1 Study site**

124 This study was conducted at a field site of the Duolun Restoration Ecology Experimentation  
125 and Demonstration Station of the Institute of Botany, the Chinese Academy of Sciences,

126 located in south-eastern Inner Mongolia, northern China (Latitude 42°02'N, longitude  
127 116°16'E, elevation 1350 m a.s.l.). The climate is temperate and semiarid with a dry spring  
128 and a wet summer. The long-term mean annual temperature at the site is 2.1°C, with monthly  
129 mean temperatures ranging from -17.5°C in January to 18.9°C in July. Mean annual  
130 precipitation is about 380mm, with 90% of the precipitation falling in the growing season  
131 between May and October.

132 Total precipitation was 196, 369, and 187 mm in 2009, 2010 and 2011, respectively, and  
133 the precipitation between May 1st and September 30th was 164, 314, and 159 mm in 2009,  
134 2010 and 2011, respectively. Mean annual air temperature was 2.96, 2.43 and 2.11°C in 2009,  
135 2010 and 2011, respectively (Fig. 1).

136 Soil type was classified as chestnut soil (Chinese classification) or Calcic Luvisols  
137 according to the FAO Classification (FAO, 1974). The soil characteristics were measured  
138 in the 0-30cm layer. Soils are composed of 63% sand, 20% silt, and 17% clay (Niu et al.,  
139 2010), with concentrations of soil organic C, N and P of 1.55%, 0.17% and 0.03%,  
140 respectively. The C:N:P ratio of soil is about 51.7:5.7:1. Compared to the global database of  
141 Xu et al., (2013), the soil of the study site is a nutrient rich soil. Mean bulk density is 1.31g  
142 cm<sup>-3</sup>, and the soil pH is 7.7. The native vegetation is represented by typical steppe  
143 communities, where *Stipakrylovii* Roshev., a perennial bunchgrass, dominates. Other  
144 common species include *Leymus chinensis* (Trin) Tzvel, *Cleistogenes squarrosa* (Trin.) Keng,  
145 *Agropyroncristatum* (L.) Gaertner, *Artemisia frigid* Willd., *Potentillaacaulis* L., and  
146 *Carexduriuscula* CA Mey. Total vegetation cover was relatively sparse, ranging from 85 to

147 90%. Annual plant biomass production at the site was *c.*1000g (dry mass) m<sup>-2</sup> year<sup>-1</sup> (Li et al.,  
148 2004).

149

## 150 **2.2 Experimental design**

151 On October 1<sup>st</sup> 2008, at the end of the growing season, we established twenty-five 2m ×1m  
152 treatment plots. Treatments involved the addition of fresh organic matter to the soil in the  
153 10-20 cm soil layer. The applied fresh organic matter consisted of senescent above-ground  
154 tissues from an abundance-weighted mix of plant species occurring at the site. For this  
155 purpose, senescent plant biomass was harvested from an adjacent field, air-dried, fragmented,  
156 and passed through a sieve with a 2-mm mesh size. We used fragmented and sieved litter  
157 mainly to standardize the litter area available to microbes. The additions were equivalent to 0  
158 (control treatment), 150, 300, 600 and 1200 g (dry mass) m<sup>-2</sup>, with all arranged as a complete  
159 randomized-block design including five replications corresponding to litter input increases of  
160 0, 15, 30, 60 and 120% respectively. It must be noted that net primary production is assumed  
161 to increase between 10 and 60% at the end of the 21<sup>st</sup> century (Arora and Boer, 2014;  
162 Todd-Brown et al., 2014), which means that the first three addition amounts are in the range  
163 of the expected increase. The plant litter used had a C concentration of 400.8 mg g<sup>-1</sup> (standard  
164 error (SE) =1.3 mg g<sup>-1</sup>, n=5), an N concentration of 9.72 mg g<sup>-1</sup> (SE =0.04 mg g<sup>-1</sup>, n=5), and a  
165 P concentration of 0.768 mg g<sup>-1</sup> (SE =0.01 mg g<sup>-1</sup>, n=5), corresponding to a C:N:P ratio of  
166 521.9:12.6:1. Lignin concentration in litter was 190.9 mg g<sup>-1</sup> (SE =0.9 mg g<sup>-1</sup>, n=5).

167 Adding litter to the uppermost soil layers is impossible without drastically disturbing the

168 soils. To minimize disturbance, we carefully removed the top 10-cm soil blocks, containing  
169 60% of the root system (Zhou et al., 2007), with a sharp spade, keeping the soil blocks and  
170 vegetation as intact as possible. The soil underneath was loosened to a depth of 20 cm, and a  
171 predetermined quantity of plant litter was mixed homogeneously with the soil in the 10-20  
172 cm layer. The surface soil blocks were then placed back into their original positions.  
173 Remaining fissures between the soil blocks were carefully filled with soil from the 0-10 cm  
174 soil layer and gently compacted by hand. To create consistent soil disturbance across  
175 treatments, the plots with zero litter addition were processed in the same manner as the plots  
176 that received plant litter. We did not add the litter at the soil surface, but inserted it in the soil  
177 to reduce export due to wind or rain and thereby better control the amount of litter added.

178

### 179 **2.3 Field sampling and measurements**

180 Field sampling of above-ground biomass, root biomass, litter and soils was conducted from  
181 August 1<sup>st</sup> to 3<sup>rd</sup> in 2009, 2010 and 2011. We choose this period because August is the peak  
182 of the growing season.

183 Above-ground biomass and litter were simultaneously sampled in each plot using a 1 m ×  
184 0.3 m quadrat. Live and dead above-ground biomass were measured by clip-harvesting and  
185 dead parts were combined with the litter. Below-ground biomass in the 0-20 cm soil layer  
186 was determined by soil coring to a depth of 20 cm using a cylindrical root sampler (8-cm  
187 inner diameter). Roots were manually removed from the soil samples. All samples of  
188 above-ground biomass, below-ground biomass and litter were oven-dried at 65°C to constant

189 mass.

190 Soils were sampled in three different points of each plot within the 0-20cm soil layer with a  
191 soil sampler of 3 cm inner diameter. The samples were pooled and mixed to produce one  
192 composite sample. The fresh samples were sieved using a 2 mm sieve and visible plant  
193 tissues were removed. Then the samples were divided into two groups. The first group was  
194 immediately transported to the laboratory with a portable ice box and stored at 4 °C before  
195 microbial biomass analysis and the second group was dried by air in the shade. Additionally,  
196 soil bulk densities of the 0-20 cm soil layers of each plot were determined concurrently with  
197 soil sampling by a special coring device (volume = 100.0 ml).

198 In the lab, chemical analysis was performed on samples of above-ground biomass,  
199 below-ground biomass, litter and soil in the 0-20 cm soil layer for organic C and total N  
200 using an automatic elemental analyzer Vario EL III (Elementar Analysen systeme Comp.,  
201 Hanau, Germany). Total P was determined by the H<sub>2</sub>SO<sub>4</sub>-HClO<sub>4</sub> fusion method (Sparks *et al.*,  
202 1996). Soil microbial C and N biomass was measured by the fumigation-extraction method  
203 (Vance *et al.*, 1987). Briefly, the fresh soil samples were adjusted to approximate 60 % of  
204 water holding capacity and then incubated for one week in the dark at 25 °C to reactivate soil  
205 microbes following the original procedure published by Vance *et al.* (1987). Next 20 g (dry  
206 weight equivalent) of fumigated with CH<sub>3</sub>Cl for 24 hours and non-fumigated soil samples  
207 were both extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>. The extracts were filtered through 0.45-µm filters and  
208 the extractable C and N was analysed by dichromate and Kjeldahl digestion as described by  
209 Lovell *et al.* (1995). Soil microbial C and N biomass was calculated as the difference in

210 extractable C and N contents between the fumigated and the unfumigated samples using  
211 conversion factors ( $k_{ec}$  and  $k_{en}$ ) of 0.38 and 0.45 (Lovell et al., 1995), respectively. Mass  
212 ratios of C:N, C:P and N:P in plant, litter and soil samples and of C:N in soil microbial  
213 biomass were calculated and used to facilitate comparisons with previous studies (He et al.,  
214 2008). Additionally, 10 g dry soil samples in the 0-20 cm layer were extracted with 50 ml of  
215 2 M KCl. Inorganic N ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) of the filtered extracts of soils in the 0-20 cm  
216 soil layer were determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss  
217 Tecator, Denmark). Available soil P in soils in the 0-20 cm soil layers was measured using  
218 the Olsen method (Olsen et al., 1954).

219

## 220 **2.4 Statistical analysis**

221 Data management and statistical analyses were performed using the SPSS software package  
222 (SPSS, Chicago, IL, USA). Two-way analysis of covariance (ANCOVA) was used to detect  
223 the effects of litter addition and year (sampling time) on soil inorganic N and available P,  
224 aboveground and belowground biomass, total biomass, litter, the C, N, P pools and C:N:P  
225 stoichiometry of plant, litter and soil, and C and N pools and C:N of soil microbial biomass.  
226 Multiple comparisons were also performed to permit separation of effect means using the  
227 Duncan test at a significance level of  $P < 0.05$ .

228

## 229 **3. Results**

### 230 **3.1 Soil inorganic N and available P**

231 Litter addition significantly enhanced soil inorganic N and available P in 2009, 2010 and  
232 2011 ( $P < 0.05$ ; Fig. 2), and there were significant differences in soil inorganic N and  
233 available P among different years ( $P < 0.01$ ; Fig. 2). Nevertheless, these effects were mainly  
234 due to the highest input treatments. Indeed, for the years 2009 and 2011, only the highest  
235 litter inputs, corresponding to  $1200 \text{ g DM m}^{-2}$ , induced significant highest inorganic N and  
236 available P contents in soils. For the year 2010, the two highest litter inputs treatments  
237 induced significant increases of the inorganic N and available P contents in soils. There were  
238 no significant interactions between litter addition and year for soil inorganic N and available  
239 P ( $P > 0.05$ ; Fig. 2) indicating that the effect of treatment was conservative over the year.

240

### 241 **3.2 Plant biomass, carbon allocation and litter production**

242 Significant effects of litter addition were observed for above-ground biomass and litter in  
243 2009, 2010 and 2011 as well as for below-ground biomass and total biomass in 2010 and  
244 2011 ( $P < 0.05$ ; Fig. 3). In our study site, the belowground biomass was 6 times higher than  
245 the aboveground biomass. The ratio of belowground biomass to aboveground biomass in  
246 2010 was significantly affected by litter inputs ( $P < 0.05$ ; Fig. S1). However, it must be noted  
247 that the Duncan post hoc test showed that the effects described above are mainly due the  
248 highest input treatments, which is generally the sole treatment significantly different from the  
249 control when significant effects were detected by the two-ways ANCOVA. The highest litter  
250 addition increased biomass in all the compartments except for the belowground biomass and  
251 the total biomass in 2009. Furthermore, the highest litter addition treatment decreased the



252 ratio of belowground biomass to aboveground biomass in 2010, while others treatments  
253 produced no effect. There were no significant interactions between litter addition and year on  
254 aboveground biomass, belowground biomass, total biomass, litter and ratio of belowground  
255 biomass to aboveground biomass ( $P > 0.05$ ; Fig. 3 and 4). Therefore, for all these variables  
256 the treatment was conservative over the years.

257

### 258 **3.3 C, N and P pools in plants, litter, soil and soil microbial biomass**

259 Litter addition did not affect significantly the C, N and P pools of aboveground biomass and  
260 litter as well as soil C pools in 2009, 2010 and 2011 for all but the highest treatment. The C,  
261 N and P pools of belowground biomass in 2010 and 2011, and the C and N pools of soil  
262 microbial biomass were also not affected by litter addition except for the highest treatment ( $P$   
263  $< 0.05$ ; Fig. 4). There were no significant differences in the C, N and P pools of aboveground  
264 biomass, the N and P pools of litter, the C pool of soil and the C and N pools of soil microbial  
265 biomass among different years for all treatment but the highest treatment ( $P < 0.05$ ; Fig. 4).  
266 There were no significant interactions between litter addition and year, indicating a  
267 conservative treatment effect over the year on the C, N and P pools of aboveground biomass,  
268 belowground biomass, litter, soil and the C and N pools of soil microbial biomass ( $P > 0.05$ ;  
269 Fig. 4). Even though C, N and P contents were significantly different for the highest  
270 treatment, for almost all the pools, they tended to increase when litter was added.

271

### 272 **3.4 C, N and P stoichiometry in plant, litter, soil and soil microbe**

273 Litter addition did not significantly modified C:N and C:P ratios of aboveground biomass and  
274 litter in 2010 and 2011 and the C:N ratio of soil microbial biomass in 2009, 2010 and 2011  
275 for all but the highest treatment where a decrease was observed. The highest treatment  
276 significantly increased soil C:N and C:P ratios in 2009, 2010 and 2011 ( $P < 0.05$ ; Fig. 5). But  
277 no effect was detected for the other treatments. Litter addition did not affect N:P of  
278 aboveground biomass, belowground biomass, litter and soil ( $P > 0.05$ ; Fig. 5). There was  
279 significant difference in the C:N of aboveground biomass and soil microbial biomass among  
280 different years ( $P < 0.05$ ; Fig. 5). There were no significant interactions between litter  
281 addition and year in effects on the C:N:P stoichiometry of aboveground biomass,  
282 belowground biomass, litter, soil and soil microbial biomass ( $P > 0.05$ ; Fig. 5) indicating that  
283 the effect of treatment was conservative over the year.

284

## 285 **4. Discussion**

### 286 **4.1 Effect on litter additions on plant growth**

287 Plant growth is limited by the rate of resource supply, for example nutrients and water  
288 (Enquist et al., 2003). Furthermore, soil N and P are the main nutrient sources for plant  
289 growth (Elser et al., 2007; Vitousek et al., 2010; Alvarez-Clare et al., 2013; Fageria et al.,  
290 2013). The NPP in our study sites is expected to increase between 10 and 60% due to climate  
291 change and atmospheric CO<sub>2</sub> increase (Arora and Boer, 2014; Todd-Brown et al., 2014).  
292 Moreover, temperature is also expected to increase in China during the next decades (Piao et  
293 al., 2010), likely accelerating litter and soil organic matter decomposition and nutrient release.

294 Future predictions about the evolution of precipitation are still highly uncertain (Piao et al.,  
295 2010). The litter amendments we applied are substantial supply of nutrients, suggested to  
296 release nutrients during decomposition. Results show that availability of N and P were  
297 significantly modified only for the two highest inputs treatment. Additionally, high litter  
298 addition also greatly increased soil microbial biomass C and N, indicating that soil microbial  
299 biomass does plays an active role in nutrient transformation, conservation, and availability to  
300 plants (Wardle, 1992; Zaman et al., 1999; Tu et al., 2003). Notably, for more moderate litter  
301 additions, the observed effect on plant biomass was limited, suggesting that the plant did not  
302 benefit from these inputs. Litter addition significantly increased aboveground biomass in  
303 2009, 2010 and 2011, and belowground biomass and total biomass in 2010 and 2011 only for  
304 the highest input level.

305 One plausible explanation for the lack of effect on plant biomass in spite of the important  
306 amount of organic nutrients added might be the imbalance between the C:N:P ratio of the  
307 litter added and the C:N:P ratio of the soil, the plants and the microbial community. For  
308 instance, the C:N and C:P ratios of the added litter were 41.3 and 521.9, respectively.  
309 Compared to the C:N ratio of microbial biomass around 12 and the C:N and C:P ratio of soil  
310 around 9 and 55, respectively, added litter decomposition might immobilize available mineral  
311 nutrients. As a consequence, the competition between plants and microorganisms for mineral  
312 N and available P would increase. Furthermore, a priming effect was observed during this  
313 experiment (Xiao et al., 2014) but the nutrients release associated might be not sufficient to  
314 increase the plant growth. Since plant biomass only responded to the highest litter treatments,

315 it suggests that, in our case, microorganisms might be more efficient than plants in using  
316 nutrient resources and plant growth might be still nutrient limited. Nevertheless, soil  
317 microorganisms benefited only slightly from the litter inputs as suggested by the small  
318 observed increase in microbial biomass (Fig. 4m, 4n). Therefore, carbon use efficiency  
319 probably decreased when litter was added, increasing heterotrophic respiration. Such  
320 modification of the carbon use efficiency might be due to physiological modifications at the  
321 individual level, to a modification of the microbial community structure or to both  
322 mechanisms combined. With our data, we can't estimate the carbon use efficiency at the  
323 individual level but based on the same experiment, Xiao et al., (2014) showed that the  
324 microbial community structure was different between treatments. It must be noted that the  
325 litter used here was harvested under natural conditions. Thus, the modifications of the litter  
326 chemical composition expected under climate change are not taken into account here. Litter  
327 C:N ratio may increase in the future (Norby et al., 2001) inducing a higher microbial N  
328 demand. Microorganisms may still decrease their carbon use efficiency as assumed here or  
329 may increase the N uptake. Since the microbial N uptake in our experiment only increased  
330 for very high and unrealistic litter inputs, we assume that, at our site, microorganisms will  
331 likely modify their carbon use efficiency in response to the modification of litter C:N ratio  
332 instead of competing with plants for N. Furthermore, when litter additions significantly  
333 affected plant biomass, aboveground biomass and belowground biomass were higher in 2010  
334 than in 2009 and 2011. The years 2009 and 2011 were dry compared to the year 2010. Thus,  
335 a water stress may have limited the plant growth in 2009 and 2011. Moreover, soil moisture

336 was likely higher in 2010. Such more favourable soil moisture conditions may have caused  
337 the higher soil nutrient availability via accelerated litter decomposition.

338

#### 339 **4.2 Biomass allocation**

340 Biomass allocation is often affected by factors such as soil nutrient conditions and plant  
341 habitat (Vogt et al., 1983; Schmid, 2002; Mokany et al., 2006). In our study, high litter  
342 addition decreased the ratio of belowground biomass to aboveground biomass, and the  
343 decrease reached a significant level in 2010. The reason for this decrease may be that litter  
344 addition greatly increased soil organic N and available P and soil microbial biomass C and N,  
345 allowing the plant to invest more photosynthates in aboveground biomass in 2010 as already  
346 observed (Vogt et al., 1983; Schmid, 2002). Sims et al. (2012) found that adding nitrogen  
347 increased plant growth and allocated more biomass toward shoots than roots. The plastic  
348 response of increased allocation to shoots corresponds to theoretical predictions (Tilman,  
349 1988). Such an increase in photosynthates concentration is also explained by the increase in C :  
350 N and C : P ratio in aboveground biomass and litter upon high litter addition, but not for  
351 belowground biomass.

352

#### 353 **4.3 Effect of litter addition on plant N:P ratio**

354 Plant N and P are essential nutrients for primary producers and decomposers in terrestrial  
355 ecosystems, and N:P ratios of plant biomass or litter have been widely used as indicators of  
356 nutrient limitation for primary production (Koerselman and Meuleman, 1996; Tessier and

357 Raynal, 2003; Güsewell, 2004; Güsewell and Verhoeven, 2006 ). In our study, litter addition  
358 did not affect the N:P of aboveground biomass, belowground biomass and litter. Our results  
359 showed that the N:P of aboveground biomass (ranging from 13.3 to 13.9 under different litter  
360 additions) is higher than that of belowground biomass (ranging from 11.6 to 12 under  
361 different litter additions). Similar results were observed in the study of Xu et al., (2010). The  
362 N:P of aboveground biomass and belowground biomass is lower than 14 on a community  
363 level, suggesting that our *S. krylovii* steppe community was N limited (Koerselman and  
364 Meuleman, 1996). Additionally, the N:P of litter ranged from 12.1 to 12.5 after different litter  
365 treatments and were much lower than 25 (the threshold between N and P limitation for  
366 graminoid leaf litter; Güsewell and Verhoeven 2006), also indicating that our *S. krylovii*  
367 steppe community is subject to N limitation. This result is consistent with the conclusion of  
368 Bai et al. (2012) who found that meadow steppe, typical steppe and desert steppe  
369 communities of temperate grasslands in northern China are N-limited systems.

370

## 371 **5. Conclusion**

372 In our study, litter addition significantly affected the stoichiometry of the systems only when  
373 it was quite high (twice the natural inputs). Previous modeling exercises have not predicted  
374 an increase of primary production sufficient to double litter inputs (Arora and Boer 2014;  
375 Todd-Brown et al., 2014). This suggests that, the grassland studied here is quite resilient in  
376 terms of stoichiometry. This resilience is the result of complex interactions between C and  
377 nutrients cycles as well as between plants and microbial biomass.

378 In conclusion, our results showed that very high litter addition increased soil inorganic N  
379 and plant available P, the C and N pools of soil microbial biomass, aboveground and  
380 belowground plant biomass. On the other hand, plausible additions according to the models  
381 predictions had no effect. This suggests that the expected increase of NPP may not have  
382 important consequences on the stoichiometric functioning for some particular ecosystems,  
383 such as grasslands in northern China. Nevertheless, it must be noted that climate change will  
384 also affect temperature and soil moisture, which will largely affect the response of  
385 ecosystems to modifications of NPP due to the atmospheric CO<sub>2</sub> increase.

386

### 387 **Acknowledgments**

388 This study was financially supported by “Strategic Priority Research Program — Climate  
389 Change: Carbon Budget and Related Issues” of the Chinese Academy of Sciences  
390 (XDA05050407), the National Natural Science Foundation of China (No. 31370462), and  
391 State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, the  
392 Chinese Academy of Sciences. We thank Prof. Li LH for providing weather data, and Dr. Pei  
393 ZQ, Dr Xia JY and Dr. Zhang JL for help with field sampling. We gratefully acknowledge  
394 the Duolun Restoration Ecology Experimentation and Demonstration Station for help with  
395 logistics and access permission to the study site. IAJ and BG acknowledge support from the  
396 Flemish National Science Foundation, from the European Community’s Seventh Framework  
397 Programme (FP7/2007-2013) under grant agreement nr. 226701 (CARBO-Extreme), from  
398 the Cost Action Terrabites (ES-0805) and the European Research Council Synergy grant

399 ERC-2013-SyG-610028 IMBALANCE-P. The authors acknowledge Matthew Macgrath for

400 his valuable comments on the manuscript.

401

402

403

404



404 **References**

- 405 Aerts, R. and Chapin, F.S. III.: The mineral nutrition of wild plants revisited: a re-evaluation  
406 of processes and patterns, *Adv. Ecol. Res.*, 30, 1-67, 2000.
- 407 Ågren, G.I.: The C:N:P stoichiometry of autotrophs - theory and observations, *Ecol. Lett.*, 7,  
408 185-191, 2004.
- 409 Alvarez-Clare, S., Mack, M.C., Brooks, M.A.: Direct test of nitrogen and phosphorus  
410 limitation to net primary productivity in a lowland tropical wet forest, *Ecology*, 94,  
411 1540-1551, 2013.
- 412 Arora, V.K. and Boer, G.J.: Terrestrial ecosystems response to future changes in climate and  
413 atmospheric CO<sub>2</sub> concentration. *Biogeosciences Discussions*, 11, 3581–3614, 2014.
- 414 Arnone, J.A. III. and Körner, C.: Soil and biomass carbon pools in model communities of  
415 tropical plants under elevated CO<sub>2</sub>, *Oecologia*, 104, 61-71, 1995.
- 416 Bai, Y.F., Wu, J.G., Clark, C.M., Pan, Q.M., Zhang, L.X., Chen, S.P. et al.: Grazing alters  
417 ecosystem functioning and C:N:P stoichiometry of grasslands along a regional  
418 precipitation gradient, *J. Appl. Ecol.*, 49, 1204-1215, 2012.
- 419 Blagodatskaya, E., Kuzyakov, Y.: Mechanisms of real and apparent priming effects and  
420 their dependence on soil microbial biomass and community structure: critical review. *Biol.*  
421 *Fert. Soils* 45, 115 - 131, 2008.
- 422 Campbell, C.A., Lafond, G.P., Zentner, R.P., Biederbeck, V.O.: Influence of fertilizer and  
423 straw baling on soil organic matter in a thick black chernozem in western Canada, *Soil*  
424 *Biol. Biochem.*, 23, 443-446, 1991.

425 Chemidlin Prévost-Bouré, N., Soudani, K., Damesin, C., Berveiller, D., Lata, J.-C., Dufrêne,  
426 E.: Increase in aboveground fresh litter quantity over-stimulates soil respiration in a  
427 temperate deciduous forest. *App. Soil Ecol.* 46, 26–34, 2010.

428 Cotrufo, M., Raschi, A., Lanini, M., Ineson, P.: Decomposition and nutrient dynamics of  
429 *Quercus pubescens* leaf litter in a naturally enriched CO<sub>2</sub> Mediterranean ecosystem.  
430 *Func.Ecol.* 13, 343–351, 1999.

431 Cornelissen, J.H.C., Van Bodegom, P.M., Aerts, R., Callaghan, T.V., Van Logtestijn, R.S.P.,  
432 Alatalo, J. et al.: Global negative vegetation feedback to climate warming responses of  
433 leaf litter decomposition rates in cold biomes, *Ecol. Lett.*, 10, 619-627, 2007.

434 Daufresne, T. and Loreau, M.: Ecological stoichiometry, primary producer-decomposer  
435 interactions, and ecosystem persistence, *Ecology*, 82, 3069-3082, 2001.

436 Elser, J.J. and Hamilton, A.L.: Stoichiometry and the new biology: the future is now, *PLoS.*  
437 *Biol.*, 5: e181, 2007.

438 Elser, J.J., Fagan M W.F., Kerkhoff, A.J., Swenson, N.G., Enquist, B.J.: Biological  
439 stoichiometry of plant production: metabolism, scaling and ecological response to global  
440 change, *New Phytol.*, 186, 593-608, 2010.

441 Elser, J.J., Bracken, M.E.S, Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H. et al.:  
442 Global analysis of Nitrogen and Phosphorus limitation of primary producers in freshwater,  
443 marine and terrestrial ecosystems, *Ecol. Lett.*, 10, 1135-1142, 2007.

444 **Elser, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B. et al.:**  
445 **Biological stoichiometry from genes to ecosystems, *Ecol. Lett.*, 3, 540-550, 2000.**

446 Enquist, B.J., Economo, E.P., Huxman, T.E., Allen, A.P., Ignace, D.D., Gillooly, J.F.:  
447 **Scaling metabolism from organisms to ecosystems, *Nature*, 423, 639-642, 2003.**

448 Fageria, N.K., Baligar, V.C., Moreira, A., Moraes, L.A.C.: Soil phosphorous influence on  
449 **growth and nutrition of tropical legume cover crops in acidic soil, *Commun. Soil Sci. Plan.***  
450 **44, 3340-3364, 2013.**

451 FAO-UNESCO.: Soil map of the world 1:5 000 000. Paris, France:UNESCO,1974.

452 Frank, D.A.: Ungulate and topographic control of nitrogen: phosphorus stoichiometry in a  
453 temperate grassland; soils, plants and mineralization rates, *Oikos*, 117, 591-601, 2008.

454 Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherall, H., Jackson, R.B.:  
455 Nonlinear grassland responses to past and future atmospheric CO<sub>2</sub>, *Nature*, 417, 279-282,  
456 2002.

457 Güsewell, S.: N:P ratios in terrestrial plants: variation and functional significance, *New*  
458 *Phytol.*, 164, 243-266, 2004.

459 Güsewell, S. and Koerselman ,W.: Variation in nitrogen and phosphorus concentrations of  
460 wetland plants, *Perspect. Plant Ecol.*, 5, 37-61, 2002.

461 Güsewell, S. and Verhoeven, J.T.A.: Litter N:P ratios indicate whether N or P limits the  
462 decomposability of graminoid leaf litter, *Plant Soil*, 287, 131-143, 2006.

463 Guenet, B., Neill, C., Bardoux, G., Abbadie, L.: Is there a linear relationship between priming  
464 effect intensity and the amount of organic matter input? *Appl. Soil Ecol.*, 46, 436-442,  
465 2010.

466 He, J.S., Wang, L., Flynn, D.F.B., Wang, X.P., Ma, W.H., Fang, J.Y.: Leaf

467 nitrogen:phosphorus stoichiometry across Chinese grassland biomes, *Oecologia*, 155,  
468 301-310, 2008.

469 Henrot, J. and Robertson, G.P.: Vegetation removal in two soils of the humid tropics: effect  
470 on microbial biomass, *Soil Biol. Biochem.*, 26, 111-116, 1994.

471 Herrick, J.E. and Wander, M.: Relationships between SOC and soil quality in cropped and  
472 rangeland soils: the importance of distribution, composition, and soil biological activity. In:  
473 Lal R, Kimble JM, Follett RF, Stewart BA (Eds.), *Soil Processes and the Carbon Cycle*,  
474 CRC Press, Boca Raton, pp. 405-425, 1997.

475 Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., de Ruiter, P.: Carbon sequestration in  
476 ecosystems: the role of stoichiometry, *Ecology*, 85, 1179-1192, 2004.

477 Hessen, D.O., Elser, J.J., Sterner, R.W., Urabe, J.: Ecological stoichiometry: An elementary  
478 approach using basic principles, *Limnol. Oceanogr.*, 58, 2219-2236, 2013.

479 Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K.,  
480 Johnson, C.A.: *IPCC 2001: Climate Change 2001: The Scientific Basis*. Cambridge  
481 University Press, New York, 2001.

482 Jin, H., Sun, O.J., Liu, J.: Changes in soil microbial biomass and community structure with  
483 addition of contrasting types of plant litter in a semiarid grassland ecosystem, *J. Plant*  
484 *Ecol.*, 3, 209-217, 2010.

485 Kang, L., Han, X., Zhang, Z., Sun, O.J.: Grassland ecosystems in China: review of current  
486 knowledge and research advancement, *Phil. Trans. R. Soc. B.*, 1482, 997-1008, 2007.

487 Koerselman, W. and Meuleman, A.F.M.: The vegetation N:P ratio: a new tool to detect the

488 nature of nutrient limitation, *J. Appl. Ecol.*, 33, 1441-1450, 1996.

489 IPCC.: Fifth Assessment Report: Climate Change. The physical science basis, 2013.

490 Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D.: Plant mineral nutrition in ancient  
491 landscapes: high plant species diversity on infertile soils is linked to functional diversity  
492 for nutritional strategies, *Plant Soil*, 334, 11-31, 2010.

493 Lambert, A.M., Dudley, T.L., Robbins, J.: Nutrient enrichment and soil conditions drive  
494 productivity in the large-statured invasive grass *Arundo donax*, *Aquat. Bot.*, 112, 16-22,  
495 2014.

496 Li, C.P. and Xiao, C.W.: Above- and belowground biomass of *Artemisia ordosica*  
497 communities in three contrasting habitats of the Mu Us desert, northern China. *J. Arid*  
498 *Environ.*, 70, 195-207, 2007.

499 Li, L., Li, X., Bai, W.M., Wang, Q.B., Yan, Z.D., Yuan, Z.Y., Dong, Y.S.: Soil carbon  
500 budget of a grazed *Leymus chinensis* steppe community in the Xilin River Basin of Inner  
501 Mongolia, *Acta Phytocologica Sinica*, 28, 312-317, 2004. (In Chinese)

502 Liu, P., Huang, J., Han, X., Sun, O.J., Zhou, Z.: Differential responses of litter decomposition  
503 to increased soil nutrients and water between two contrasting grassland plant species of  
504 Inner Mongolia, China, *Appl. Soil Ecol.*, 34, 266-275, 2006.

505 Lovell, R.D., Jarvis, S.C., Bardgett, R.D.: Soil microbial biomass and activity in long-term  
506 grassland: effects of management changes, *Soil Biol. Biochem.*, 27, 969-975, 1995.

507 Luo, Y.Q., Field, C.B., Jackson, R.B.: Do nitrogen constrain carbon cycling, or does carbon  
508 input stimulate nitrogen cycling? *Ecology*, 87, 3-4, 2006.

509 Ma, L.N., Huang, W.W., Guo, C.Y., Wang, R.Z., Xiao, C.W.: Soil microbial properties and  
510 plant growth responses to carbon and water addition in a temperate steppe: the importance  
511 of nutrient availability, PLoS ONE, 7, e35165, 2012.

512 Meinshausen, M., Smith, S.J., Calvin, K., Daniel, J.S., Kainuma, M.L.T., Lamarque, J.-F.,  
513 Matsumoto, K., Montzka, S. a., Raper, S.C.B., Riahi, K., Thomson, a., Velders, G.J.M.,  
514 Vuuren, D.P.P.: The RCP greenhouse gas concentrations and their extensions from 1765  
515 to 2300. Climatic Change 109, 213–241, 2011.

516 Mokany, K., Raison, R.J., Prokushkin, A.S.: Critical analysis of root:shoot ratios in terrestrial  
517 biomass, Global Change Biol., 12, 84-96, 2006.

518 Norby, R.J., Cotrufo, M.F., Ineson, P., O’Neill, E.G., Canadell, J.G.: Elevated CO<sub>2</sub>, litter  
519 chemistry, and decomposition: a synthesis. Oecologia 127, 153–65, 2001.

520 Niu, S., Wu, M., Han, Y., Xia, J., Zhang, Z., Yan, H., Wan, S.: Nitrogen effects on net  
521 ecosystem carbon exchange in a temperate steppe, Global Change Biol., 16,144-155,  
522 2010.

523 Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A.: Estimation of available phosphorus in  
524 soils by extraction with sodium bicarbonate. Circular No. 939. USDA, 1954.

525 **Padgett, P.E., Allen, E.B., Bytnerowicz, A., Minich, R.A.: Changes in soil inorganic nitrogen**  
526 **as related to atmospheric nitrogenous pollutants in southern California, Atmos. Environ.,**  
527 **33, 769-781, 1999.**

528 Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O.,  
529 Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens,

530 I.A.: Human-induced nitrogen-phosphorus imbalances alter natural and managed  
531 ecosystems across the globe. *Nature communications* 4, 2934, 2013.

532 Piao, S., Ciais, P., Huang, Y., Shen, Z., Peng, S., Li, J., Zhou, L., Liu, H., Ma, Y., Ding, Y.,  
533 Friedlingstein, P., Liu, C., Tan, K., Yu, Y., Zhang, T., Fang, J.: The impacts of climate  
534 change on water resources and agriculture in China. *Nature* 467, 43–51, 2010.

535 Ryan, M.G. and Law, B.E.: Interpreting, measuring, and modeling soil respiration,  
536 *Biogeochemistry*, 73, 3-27, 2005.

537 Sayer, E.J.: Using experimental manipulation to assess the roles of leaf litter in the  
538 functioning of forest ecosystems, *Biol. Rev.*, 81, 1-31, 2006.

539 Sayer, E.J., Heard, M.S., Grant, H.K., Marthews, T.R., Tanner, E.V.: Soil carbon release  
540 enhanced by increased tropical forest litterfall, *Nat. Clim. Change*, 1, 304-307, 2011.

541 Sardans, J., Rivas-Ubach, a., Peñuelas, J.: The C:N:P stoichiometry of organisms and  
542 ecosystems in a changing world: A review and perspectives. *Perspectives in Plant Ecology,*  
543 *Evolution and Systematics* 14, 33–47, 2012.

544 Schmid, I.: The influence of soil type and interspecific competition on the fine root system of  
545 Norway spruce and European beech, *Basic Appl. Ecol.*, 3, 339-346, 2002.

546 Schmidt, I.K., Michelsen, A., Jonasson, S.: Effects of labile soil carbon on nutrient  
547 partitioning between arctic graminoid and microbes, *Oecologia*, 112, 557-565, 1997.

548 Sims, L., Pastor, J., Lee, T., Dewey, B.: Nitrogen, phosphorus and light effects on growth and  
549 allocation of biomass and nutrients in wild rice, *Oecologia*, 170, 65-76, 2012.

550 Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S.: Microorganisms and climate change:

551 terrestrial feedbacks and mitigation options. *Nature reviews. Microbiology* 8, 779–90,  
552 2010.

553 Sparks, D.L., Page, A.L., Loeppert, P.A., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T.,  
554 Sumner, M.E.: *Methods of Soil Analysis Part 3: Chemical methods*. Soil Science Society  
555 of America and American Society of Agronomy, Madison, WI, 1996.

556 Subke, J.A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N., Cotrufo, M.F.: Feedback  
557 interactions between needle litter decomposition and rhizosphere activity, *Oecologia*, 139,  
558 551-559, 2004.

559 Sulzman, E.W., Brant, J.B., Bowden, R.D., Lajtha, K.: Contribution of aboveground litter,  
560 belowground litter, and rhizosphere respiration to total soil CO<sub>2</sub> efflux in an old growth  
561 coniferous forest, *Biogeochemistry*, 73, 231-256, 2005.

562 Tessier, J.T. and Raynal, D.Y.: Use of nitrogen to phosphorus ratios in plant tissue as an  
563 indicator of nutrient limitation and nitrogen saturation, *J. Appl. Ecol.*, 40, 523-534, 2003.

564 Tilman, D.: *Plant strategies and the dynamics and structure of plant communities*. Princeton  
565 University Press, Princeton, 1988.

566 Todd-Brown, K.E.O., Randerson, J.T., Hopkins, F., Arora, V., Hajima, T., Jones, C.,  
567 Shevliakova, E., Tjiputra, J., Volodin, E., Wu, T., Zhang, Q., Allison, S.D.: Changes in  
568 soil organic carbon storage predicted by Earth system models during the 21st century.  
569 *Biogeosciences* 11, 2341–2356, 2014.

570 Tu, C., Koenning, S.R., Hu, S.: Root-parasitic nematodes enhance soil microbial activities  
571 and nitrogen mineralization, *Microb. Ecol.*, 46, 134–144, 2003.



572 Villalobos-Vega, R., Goldstein, G., Haridasan, M., Franco, A.C., Miralles-Wilhelm, F.,  
573 Scholz, F.G., Bucci, S.J.: Leaf litter manipulations alter soil physicochemical properties  
574 and tree growth in a Neotropical savanna, *Plant Soil*, 346, 385-397, 2011.

575 Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A.: Terrestrial phosphorus limitation:  
576 mechanisms, implications, and nitrogen-phosphorus interactions, *Ecol. Appl.*, 20: 5-15,  
577 2010.

578 Vance, E.D., Brookes, P., Jenkinson, D.S.: An extraction method for measuring soil  
579 microbial biomass C, *Soil Biol. Biochem.*, 19, 703-707, 1987.

580 Vogt, K.A., Moore, E.E., Vogt, D.J., Redlin, M.J., Edmonds, R.L.: Conifer fine root and  
581 mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages  
582 and productivities, *Can. J. For. Res.*, 13, 429-437, 1983.

583 Wardle, D.A.: A comparative assessment of factors which influence microbial biomass  
584 carbon and nitrogen levels in soil, *Biol. Rev. Camb. Philos. Soc.*, 67, 321-358, 1992.

585 Xiao, C., Guenet, B., Zhou, Y., Su, J., Janssens, I. A.: Priming of soil organic matter  
586 decomposition scales linearly with microbial biomass response to litter input in steppe  
587 vegetation. *Oikos*, doi: 10.1111/oik.01728, 2014.

588 Xu, B., Cheng, Y.X., Gan, H.J., Zhou, W.J., He, J.S.: Correlations between leaf and fine root  
589 traits among and within species of typical temperate grassland in Xilin River Basin, Inner  
590 Mongolia, China, *Chinese J. Plant Ecol.*, 34, 29-38, 2010.

591 Xu, X., Thornton, P.E., Post, W.M.,: A global analysis of soil microbial biomass carbon,  
592 nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biog.* 22, 737-749, 2013.

593 Yano, Y., Shaver, G.R., Rastetter, E.B., Giblin, A.E., Laundre, J.A.: Nitrogen dynamics in  
594 arctic tundra soils of varying age: differential responses to fertilization and warming,  
595 *Oecologia*, 173, 1575-1586, 2013.

596 Zaman, M., Di, H.J., Cameron, K.C.: A field study of gross rates of N mineralization and  
597 nitrification and their relationships to microbial biomass and enzyme activities in soils  
598 treated with dairy effluent and ammonium fertilizer, *Soil Use Manage.*, 15, 188-194, 1999.

599 Zeng, S.C., Jacobs, D.F., Sloan, J.L., Xue, L., Li, Y., Chu, S.S.: Split fertilizer application  
600 affects growth, biomass allocation, and fertilizer uptake efficiency of hybrid Eucalyptus,  
601 *New Forest*, 44, 703-718, 2013.

602 Zhang, C., Tian, H.Q., Liu, J.Y., Wang, S.Q., Liu, M.L., Pan, S.F., Shi, X.Z.: Pools and  
603 distributions of soil phosphorus in China, *Global Biogeochem. Cy.*, 19, GB1020, 2005.

604 Zhao, M.L., Han, B., Wu, J.F., Zhang, H.L., Zhen, H., Suo, P.F.: A preliminary study on the  
605 population variation of *Stipa krylovii*, *Acta Agrestia Sinica*, 11, 163-169, 2003.

606 Zhou, Z., Sun, O.J., Huang, J., Li, L., Liu, P., Han, X.: Soil carbon and nitrogen stores and  
607 storage potential as affected by land-use in an agro-pastoral ecotone of northern China,  
608 *Biogeochemistry*, 82,127-138, 2007.

609

609 **Figure captions**

610

611 Fig. 1 Seasonal variations of weekly mean air temperature and weekly precipitation during  
612 the experimental period from 1 October 2008 to 31 September 2011.

613

614 Fig. 2 Soil inorganic N (a) and available P (b) in 2009, 2010 and 2011 under different  
615 amounts of litter addition. Vertical bars indicate one standard error about the mean (n=5).  
616 The soil inorganic N and the available P contents were different each year ( $p < 0.01$ ) but no  
617 interaction occurred between litter addition and year. Treatments with different letters are  
618 significantly different ( $P < 0.05$ ) according to the Duncan test, small letters, capital letters  
619 and Greek letters correspond to the year 2009, 2010 and 2011, respectively. 'ns' implies that  
620 no significant differences were detected.

621

622 Fig. 3 Aboveground biomass (a), belowground biomass (b), total biomass (c) and litter (d) in  
623 2009, 2010 and 2011 under different amounts of litter addition in a steppe community of  
624 northern China. Vertical bars indicate one standard error about the mean (n=5). A significant  
625 year effect ( $p < 0.05$ ) was detected by ANCOVA except for the below ground biomass.  
626 Interactions between litter addition and year were not significant. See Fig. 2 legend for letter  
627 signification.

628

629 Fig 4. The C, N, and P pools of plant, litter and soil and the C and N pools of soil microbial

630 biomass in 2009, 2010 and 2011 under different amounts of litter addition in a steppe  
631 community of northern China. Vertical bars indicate one standard error about the mean (n=5).  
632 A significant year effect ( $p<0.05$ ) on the C, N and P content was detected by ANCOVA  
633 except for the below ground biomass, the soil. No year effect for litter and soil microbial  
634 biomass C content was detected. Interactions between litter addition and year were not  
635 significant. See Fig. 2 legend for letter signification. Letters are only given where statistically  
636 significant differences are observed.

637

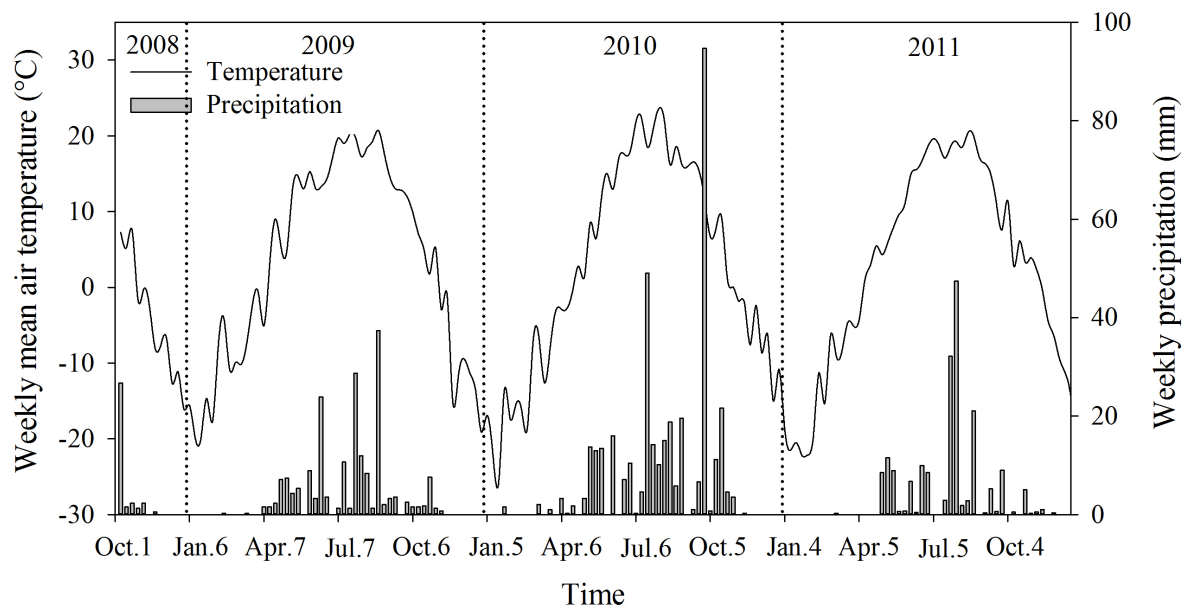
638 Fig. 5 The C:N, C:P and N:P ratio of plant, litter and soil and C:N ratio of soil microbial  
639 biomass in 2009, 2010 and 2011 under different amounts of litter addition in a steppe  
640 community of northern China. Vertical bars indicate one standard error about the mean (n=5).  
641 A significant year effect ( $p<0.05$ ) on the C:N ratio of above ground biomass and soil  
642 microbial biomass was detected by ANCOVA. Interactions between litter addition and year  
643 were not significant. See Fig. 2 legend for letter signification. Letters are only given where  
644 statistically significant differences are observed.

645

646

646

647 Figure 1

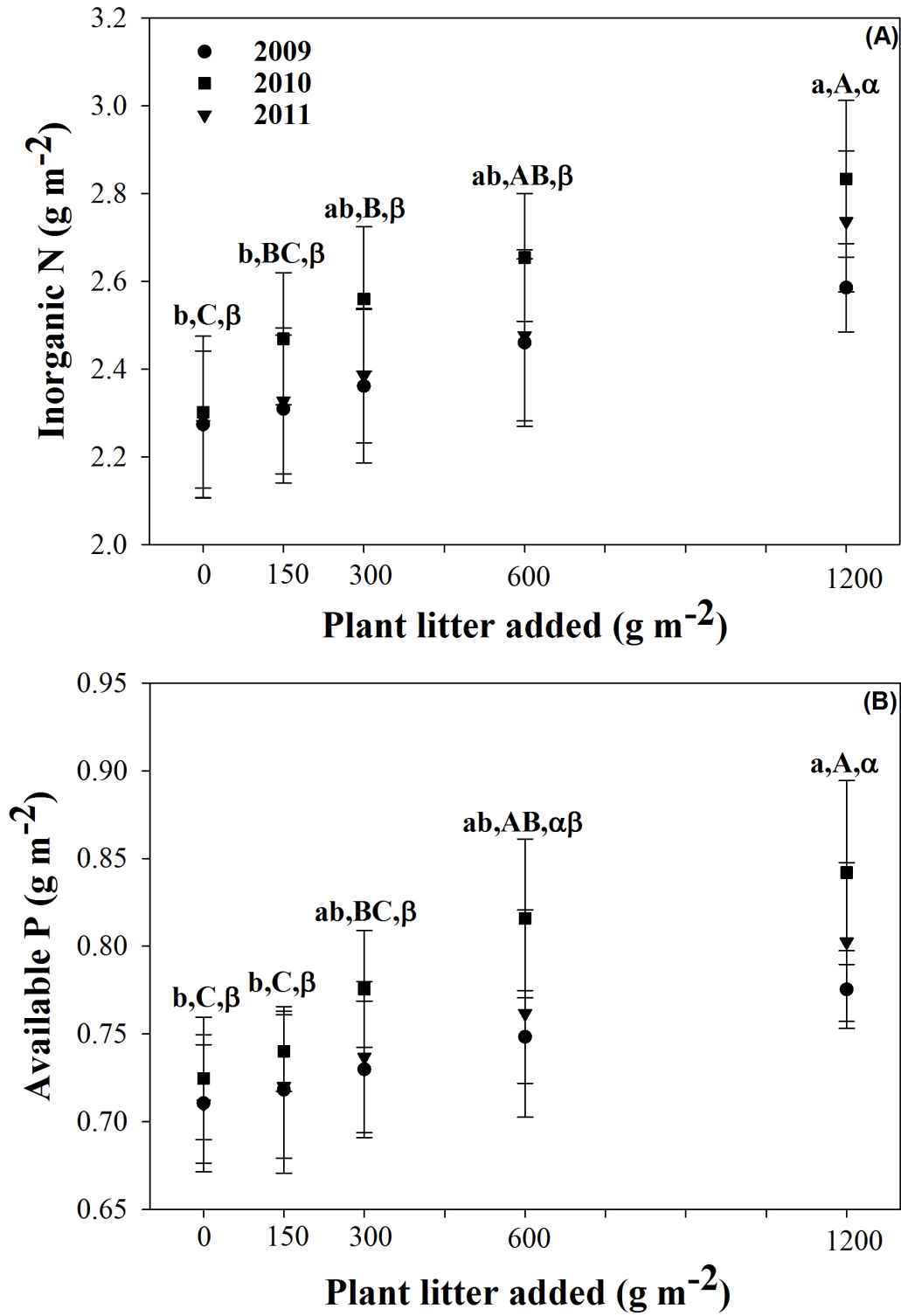


648

649

649

650 Figure 2

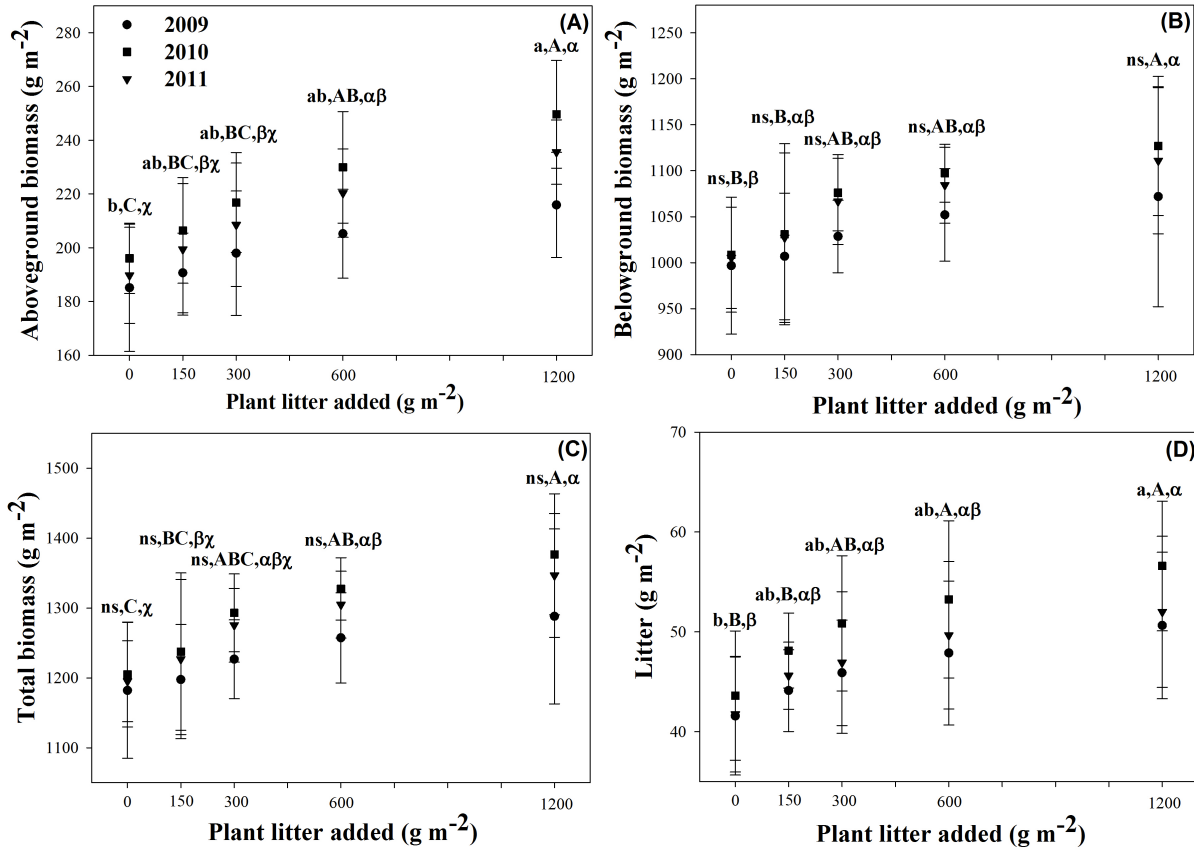


651

652

652

653 Figure 3

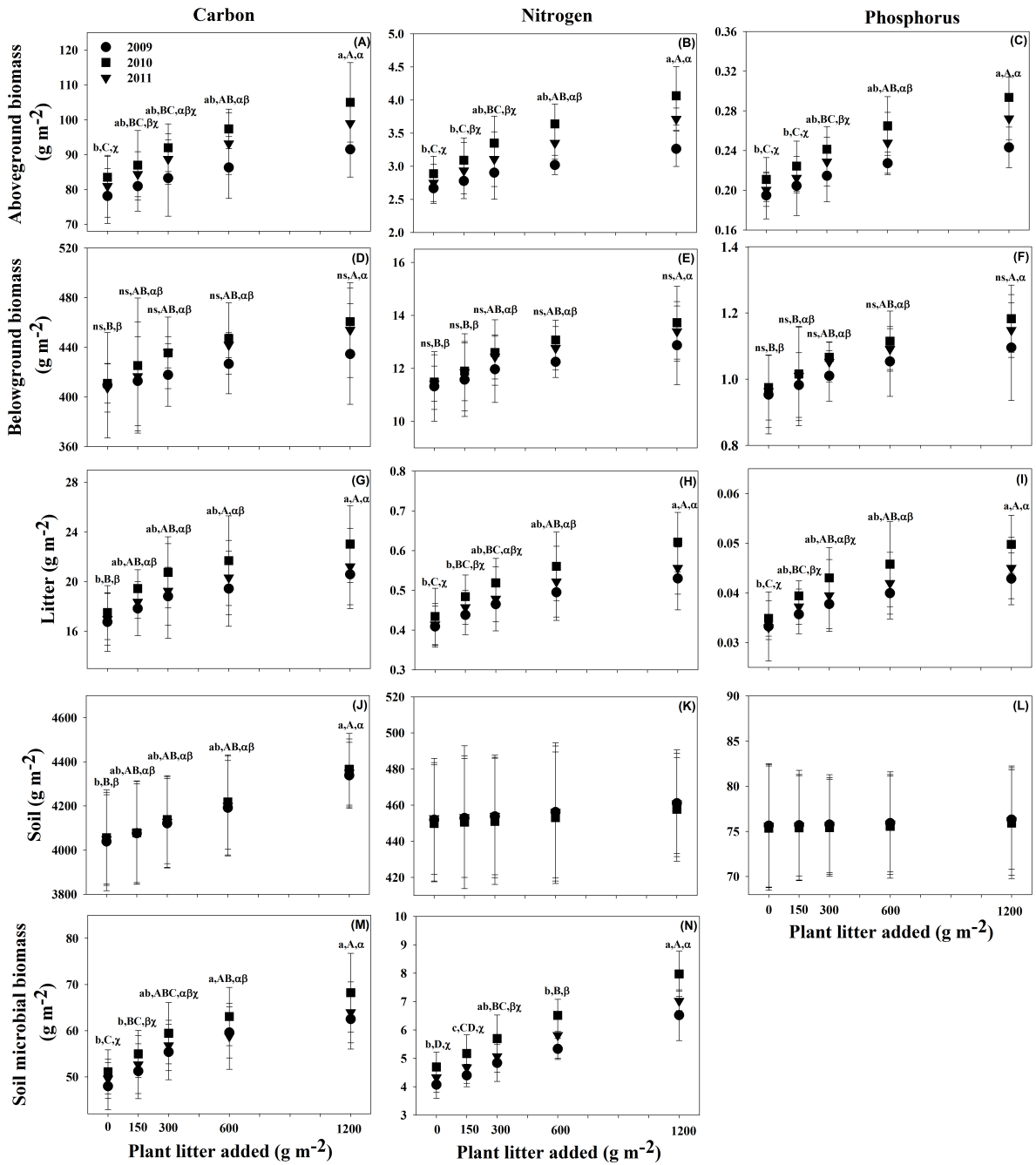


654

655

655

656 Figure 4



657

658



