

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

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

40

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

43

43 **1. Introduction**

44 Ecological stoichiometry is the study of the balance of multiple chemical elements in
45 ecological interactions (Elser et al., 2000, 2010; Elser and Hamilton, 2007). Carbon (C),
46 nitrogen (N) and phosphorus (P) are key elements in terrestrial ecosystems (Daufresne and
47 Loreau, 2001; Elser et al., 2010; Hessen et al., 2013), and the C:N:P stoichiometry reflects
48 complex interactions between evolutionary processes coupled to phenotypic plasticity. These
49 complex interactions are, at least partially, controlled by patterns of element supply from the
50 environment (Hessen et al., 2004). Ecological stoichiometry provides a valuable approach in
51 assessing possible changes in C, N and P cycling (Hessen et al., 2013). Over the past three
52 decades ecological stoichiometry has expanded greatly (Hessen et al., 2013), and a lot of
53 studies have concentrated on understanding the variation in plant C, N and P concentrations
54 among and within species (Schmidt et al., 1997; Aerts and Chapin, 2000; Güsewell and
55 Koerselman, 2002; Augustine et al., 2003; Güsewell 2004; Frank, 2008). However,
56 relationships among soil nutrient availability, plant growth and ecological C:N:P
57 stoichiometry of plants, litter, soil and soil microbes under global climate change in terrestrial
58 ecosystems is not well understood.

59 Litter is an important nutrient pool which strongly alters nutrient availability and
60 therefore affects cycling of C, N, and P in terrestrial ecosystems with significant feedback on
61 plant growth and on climate (Hungate et al., 2003; Subke et al., 2004; Ryan and Law, 2005;
62 Sayer, 2006; Cornelissen et al., 2007; Villalobos-Vega et al., 2011). The anticipated doubling
63 of atmospheric carbon dioxide (CO₂) concentration within the next 100 years (Houghton et

64 al., 2001) due to continued anthropogenic carbon emissions is generally predicted to increase
65 net primary production (NPP) of most terrestrial ecosystems (IPCC, 2013). Nevertheless such
66 effects do not scale linearly with increases in atmospheric CO₂ because productivity is also
67 partially controlled by climate. Regions with dryer climates are therefore likely to present
68 reduced net primary production in the next decades (IPCC, 2013). Although uncertainties
69 exists in the magnitude of the changes (e.g. Campbell et al., 1991; Arnone and Körner, 1995;
70 Gill et al., 2002), increases in net primary production are probably predominant and will
71 simultaneously increase litter inputs to soils. Modification of atmospheric CO₂, of climate
72 and of nutrient cycling may also modify the chemical composition of the litter (Cotrufo et al.,
73 1999). Elevated CO₂ generally increases the lignin content and reduces the N concentration
74 of plant tissues, although Norby et al., (2001) showed that this response depends on the
75 experimental system used (open top chamber, free air CO₂ enrichment, etc.).

76 In past decades, the effects of litter addition on plant growth, soil C content and cycling,
77 microbial biomass and priming of decomposition rates have been reported and confirmed (eg.
78 Sulzman et al., 2005; Guenet et al., 2010; Jin et al., 2010; Chemidlin Prévost-Bouré et al.,
79 2010; Sayer et al., 2011; Villalobos-Vega et al., 2011; Ma et al., 2012, Xiao et al., *in press*).
80 However, the impacts of litter addition on soil nutrients, plant growth and ecological C:N:P
81 stoichiometry of plants, litter, soil and soil microbes remain highly uncertain. “Moreover,
82 climate change and increased atmospheric CO₂ would not only affect NPP but also
83 environmental conditions for decomposers in soils (soil moisture, temperature), inducing
84 modification of microbial community structure and activity (Singh et al., 2010). Associated

85 to litter additions, these effects would disturb the C cycle in soil, affecting the net ecosystem
86 exchange particularly in grasslands where the vast majority of the C stock is stored
87 belowground, due to their high root:shoot biomass and productivity ratios (Mokany et al.,
88 2006).

89 Grassland is one of the most important global terrestrial ecosystems, covering about 25%
90 of the global terrestrial area and 40% of the land area in China (Kang et al., 2006). The
91 semi-arid and temperate grasslands of northern China account for about 78% of the national
92 grassland area, where the native vegetation is predominantly characterized by the abundance
93 of grass species such as *Stipa* spp. and *Leymus chinensis* (Trin) Tzvel., with *Stipa krylovii*
94 Roshev. being well-represented as one of the major steppe community types (Zhao et al.,
95 2003). Plant recruitment, growth and nutrient cycling of the region often are limited by soil
96 water, nitrogen and phosphorus, and the regional soil fertility and productivity are maintained
97 by recycling of nutrients through plant litter decomposition as an essential mechanism (Liu et
98 al., 2006) with little natural nitrogen deposition. A better understanding of the effects of litter
99 addition on plant growth and ecological C:N:P stoichiometry of plants, litter, soil and soil
100 microbes could help to reduce uncertainties in our predictions of C, N and P balance as well
101 as cycling, and structure and function in grassland ecosystems under global climate change.
102 We conducted a field experiment in which we artificially added litter inputs to subsoils (i.e.
103 10-20 cm) under a *S. krylovii* steppe community in a temperate grassland of northern China
104 to assess the effects on soil inorganic N and available P, plant growth, litter, C, N and P pools
105 and the C:N:P stoichiometry of plant, litter, soil and soil microbes. The primary objectives of

106 our study were to determine whether litter addition would increase soil inorganic N and
107 available P and thereby enhance soil nutrient availability for plant growth and if it would
108 affect plant growth, litter, and the C, N, P pools and the C:N:P stoichiometry of plants, litter,
109 soil and soil microbes.

110 We assumed that litter additions could increase nutrient release through a priming effect
111 on decomposition rate, thereby provoking an increase in plant biomass. Priming is defined
112 here as a modification of the soil organic matter decomposition rate induced by an input of
113 litter and mediated by the altered activity of the microbial community. Priming effects can be
114 negative (reduction of the decomposition rate), but are typically positive (increase of the
115 decomposition rate) (Blagodatskaya and Kuzyakov, 2008). Nevertheless, regarding the
116 complexity of the mechanisms implied, we expected a non-linear relationship between litter
117 additions and the plant biomass response. The objective of this study was to estimate such
118 relationship in the temperate grassland of northern China.

119

120 **2. Materials and Methods**

121 **2.1 Study site**

122 This study was conducted at a field site of the Duolun Restoration Ecology Experimentation
123 and Demonstration Station of the Institute of Botany, the Chinese Academy of Sciences,
124 located in south-eastern Inner Mongolia, northern China (Latitude 42°02'N, longitude
125 116°16'E, elevation 1350 m a.s.l.). The climate is temperate and semiarid with a dry spring
126 and a wet summer. The long-term mean annual temperature at the site is 2.1°C, with monthly

127 mean temperatures ranging from -17.5°C in January to 18.9°C in July. Mean annual
128 precipitation is about 380mm, with 90% of the precipitation falling in the growing season
129 between May and October.

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

134 Soil type was classified as chestnut soil (Chinese classification) or Calcic Luvisols
135 according to the FAO Classification (FAO, 1974). The soil characteristics were measured
136 ofr the 0-30cm layer. Soils are composed of 63% sand, 20% silt, and 17% clay (Niu et al.,
137 2010), with concentrations of soil organic C, N and P of 1.55%, 0.17% and 0.03%,
138 respectively. The C:N:P ratio of soil is about 51.7:5.7:1. Compared to the global database of
139 Xu et al., (2013), the soil of the study site is a nutrient rich soil. Mean bulk density is 1.31g
140 cm^{-3} , and the soil pH is 7.7. The native vegetation is represented by typical steppe
141 communities, where *Stipakrylovii* Roshev., a perennial bunchgrass, dominates. Other
142 common species include *Leymus chinensis* (Trin) Tzvel, *Cleistogenes squarrosa* (Trin.) Keng,
143 *Agropyroncristatum* (L.) Gaertner, *Artemisia frigid* Willd., *Potentillaacaulis* L., and
144 *Carexduriuscula* CA Mey. Total vegetation cover was relatively sparse, ranging from 85 to
145 90%. Annual plant biomass production at the site was $c.1000\text{g}$ (dry mass) $\text{m}^{-2}\text{year}^{-1}$ (Li et al.,
146 2004).

147

148 2.2 Experimental design

149 On October 1st 2008, we established twenty-five 2m ×1m treatment plots. Treatments
150 involved the addition of fresh organic matter to the soil in the 10-20 cm soil layer. The
151 applied fresh organic matter consisted of senescent above-ground tissues from an
152 abundance-weighted mix of plant species occurring at the site. For this purpose, senescent
153 plant biomass was harvested from an adjacent field, air-dried, fragmented, and passed
154 through a sieve with a 2-mm mesh size. The additions were equivalent to 0 (control
155 treatment), 150, 300, 600 and 1200 g (dry mass) m⁻², with all arranged as a complete
156 randomized-block design including five replications corresponding to litter input increases of
157 0, 15, 30, 60 and 120% respectively. It must be noted that net primary production is assumed
158 to increase between 10 and 60% at the end of the 21st century (Arora and Boer, 2014;
159 Todd-Brown et al., 2014), which means that the first three addition amounts are very realistic.
160 The plant litter used had a C concentration of 400.8 mg g⁻¹ (standard error (SE) =1.3 mg g⁻¹,
161 n=5), an N concentration of 9.72 mg g⁻¹ (SE =0.04 mg g⁻¹, n=5), and a P concentration of
162 0.768 mg g⁻¹ (SE =0.01 mg g⁻¹, n=5), corresponding to 41.3, 521.9 and 12.6 for C:N, C:P and
163 N:P ratio, respectively. Lignin concentration in litter was 190.9 mg g⁻¹ (SE =0.9 mg g⁻¹, n=5).

164 Adding litter to the uppermost soil layers is impossible without drastically disturbing the
165 soils. To minimize disturbance, we carefully removed the top 10-cm soil blocks, containing
166 60% of the root system (Zhou et al., 2007), with a sharp spade, keeping the soil blocks and
167 vegetation as intact as possible. The soil underneath was loosened to a depth of 20 cm, and a
168 predetermined quantity of plant litter was mixed homogeneously with the soil in the 10-20

169 cm layer. The surface soil blocks were then placed back into their original positions.
170 Remaining fissures between the soil blocks were carefully filled with soil from the 0-10 cm
171 soil layer and gently compacted by hand. To create consistent soil disturbance across
172 treatments, the plots with zero litter addition were processed in the same manner as the plots
173 that received plant litter. We did not add the litter at the soil surface, but inserted it in the soil
174 to reduce export due to wind or rain and thereby better control the amount of litter added.

175

176 **2.3 Field sampling and measurements**

177 Field sampling of above-ground biomass, root biomass, litter and soils was conducted from
178 August 1th to 3st in 2009, 2010 and 2011.

179 Above-ground biomass and litter were simultaneously sampled in each plot using a 1 m ×
180 0.3 m quadrat. Live and dead above-ground biomass were measured by clip-harvesting and
181 dead parts were combined with the litter. Below-ground biomass in the 0-20 cm soil layer
182 was determined by soil coring to a depth of 20 cm using a cylindrical root sampler (8-cm
183 inner diameter). Roots were manually removed from the soil samples. All samples of
184 above-ground biomass, below-ground biomass and litter were oven-dried at 65°C to constant
185 mass.

186 Soils were sampled in three different points of each plot within the 0-20cm soil layer with a
187 soil sampler of 3 cm inner diameter. The samples were pooled and mixed to produce one
188 composite sample. The fresh samples were sieved using a 2 mm sieve and visible plant
189 tissues were removed. Then the samples were divided into two groups. The first group was

190 immediately transported to the laboratory with a portable ice box and stored at 4 °C before
191 microbial biomass analysis and the second group was dried by air in the shade. Additionally,
192 soil bulk densities of the 0-20 cm soil layers of each plot were determined concurrently with
193 soil sampling by a special coring device (volume = 100.0 ml).

194 In the lab, chemical analysis was performed on samples of above-ground biomass,
195 below-ground biomass, litter and soil in the 0-20 cm soil layer for organic C and total N
196 using an automatic elemental analyzer Vario EL III (Elementar Analysensysteme Comp.,
197 Hanau, Germany). Total P was determined by the H₂SO₄-HClO₄ fusion method (Sparks *et al.*,
198 1996). Soil microbial C and N biomass was measured by the fumigation-extraction method
199 (Vance *et al.*, 1987). Briefly, the fresh soil samples were adjusted to approximate 60 % of
200 water holding capacity and then incubated for one week in dark at 25 °C to reactivate soil
201 microbes. Next 20 g (dry weight equivalent) of fumigated with CH₃Cl for 24 hours and
202 non-fumigated soil samples were both extracted with 0.5 M K₂SO₄. The extracts were filtered
203 through 0.45-µm filters and the extractable C and N was analysed by dichromate and
204 Kjeldahl digestion as described by Lovell *et al.* (1995). Soil microbial C and N biomass was
205 calculated as the difference in extractable C and N contents between the fumigated and the
206 unfumigated samples using conversion factors (k_{ec} and k_{en}) of 0.38 and 0.45 (Lovell *et al.*,
207 1995), respectively. Mass ratios of C:N, C:P and N:P in plant, litter and soil samples and of
208 C:N in soil microbial biomass were calculated and used to facilitate comparisons with
209 previous studies (He *et al.*, 2008). Additionally, 10 g dry soil samples in the 0-20 cm layer
210 were extracted with 50 ml of 2 M KCl. Inorganic N (NH₄⁺-N and NO₃⁻-N) of the filtered

211 extracts of soils in the 0-20 cm soil layer were determined using a flow injection autoanalyzer
212 (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Available soil P in soils in the 0-20 cm soil
213 layers was measured using the Olsen method (Olsen et al., 1954).

214

215 **2.4 Statistical analysis**

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

223

224 **3. Results**

225 **3.1 Soil inorganic N and available P**

226 Litter addition significantly enhanced soil inorganic N and available P in 2009, 2010 and
227 2011 ($P < 0.05$; Fig. 2), and there were significant differences in soil inorganic N and
228 available P among different years ($P < 0.01$; Fig. 2). Nevertheless, these effects were mainly
229 due to the highest input treatments. Indeed, for the years 2009 and 2011, only the highest
230 litter inputs, corresponding to 1200 g DM m^{-2} , induced significant highest inorganic N and
231 available P contents in soils. For the year 2010, the two highest litter inputs treatments

232 induced significant increases of the inorganic N and available P contents in soils. There were
233 no significant interactions between litter addition and year for soil inorganic N and available
234 P ($P > 0.05$; Fig. 2).

235

236 **3.2 Plant biomass, carbon allocation and litter production**

237 Significant effects of litter addition were observed for above-ground biomass and litter in
238 2009, 2010 and 2011 as well as for below-ground biomass and total biomass in 2010 and
239 2011 ($P < 0.05$; Fig. 3). In our study site, the belowground biomass was 6 times higher than
240 the aboveground biomass. The ratio of belowground biomass to aboveground biomass in
241 2010 was significantly affected by litter inputs ($P < 0.05$; Fig. S1). However, it must be noted
242 that the Duncan post hoc test showed that the effects described above are mainly due the
243 highest input treatments, which is generally the sole treatment significantly different from the
244 control when significant effects were detected by the two-ways ANCOVA. The highest litter
245 addition increased biomass in all the compartments except for the belowground biomass and
246 the total biomass in 2009. Furthermore, the highest litter addition treatment decrease the ratio
247 of belowground biomass to aboveground biomass in 2010, while others treatments produced
248 no effect. There were no significant interactions between litter addition and year on
249 aboveground biomass, belowground biomass, total biomass, litter and ratio of belowground
250 biomass to aboveground biomass ($P > 0.05$; Fig. 3 and 4).

251

252 **3.3 C, N and P pools in plants, litter, soil and soil microbial biomass**

253 Litter addition did not affect significantly the C, N and P pools of aboveground biomass and
254 litter as well as soil C pools in 2009, 2010 and 2011 for all but the highest treatment. The C,
255 N and P pools of belowground biomass in 2010 and 2011, and the C and N pools of soil
256 microbial biomass were also not affected by litter addition except for the highest treatment (P
257 < 0.05 ; Fig. 4). There were no significant differences in the C, N and P pools of aboveground
258 biomass, the N and P pools of litter, the C pool of soil and the C and N pools of soil microbial
259 biomass among different years for all treatment but the highest treatment ($P < 0.05$; Fig. 4).
260 There were no significant interactions between litter addition and year on the C, N and P
261 pools of aboveground biomass, belowground biomass, litter, soil and the C and N pools of
262 soil microbial biomass ($P > 0.05$; Fig. 4).

263

264 **3.4 C, N and P stoichiometry in plant, litter, soil and soil microbe**

265 Litter addition did not significantly modified C:N and C:P ratios of aboveground biomass and
266 litter in 2010 and 2011 and the C:N ratio of soil microbial biomass in 2009, 2010 and 2011
267 for all but the highest treatment where a decrease was observed. The highest treatment
268 significantly increased soil C:N and C:P ratios in 2009, 2010 and 2011 ($P < 0.05$; Fig. 5). But
269 no effect was detected for the other treatments. Litter addition did not affect N:P of
270 aboveground biomass, belowground biomass, litter and soil ($P > 0.05$; Fig. 5). There was
271 significant difference in the C:N of aboveground biomass and soil microbial biomass among
272 different years ($P < 0.05$; Fig. 5). There were no significant interactions between litter
273 addition and year in effects on the C:N:P stoichiometry of aboveground biomass,

274 belowground biomass, litter, soil and soil microbial biomass ($P > 0.05$; Fig. 5).

275

276 **4. Discussion**

277 **4.1 Effect on litter additions on plant growth**

278 Plant growth is limited by the rate of resource supply, for example nutrients and water
279 (Enquist et al., 2003). Furthermore, soil N and P are the main nutrient sources for plant
280 growth (Elser et al., 2007; Vitousek et al., 2010; Alvarez-Clare et al., 2013; Fageria et al.,
281 2013). The NPP in our study sites is expected to increase between 10 and 60% (Arora and
282 Boer, 2014; Todd-Brown et al., 2014). Moreover, temperature is also expected to increase in
283 China during the next decades (Piao et al., 2010), likely accelerating litter and soil organic
284 matter decomposition and nutrient release. Future predictions about the evolution of
285 precipitation are still highly uncertain (Piao et al., 2010). The litter amendments we applied
286 are substantial supply of nutrients, suggested to release nutrients during decomposition.
287 Results show, that availability of N and P, were only modified for the two highest inputs
288 treatment. Additionally, high litter addition also greatly increased soil microbial biomass C
289 and N, indicating that soil microbial biomass does plays an active role in nutrient
290 transformation, conservation, and availability to plants (Wardle, 1992; Zaman et al., 1999; Tu
291 et al., 2003). Notably, for more moderate litter additions, the observed effect on plant
292 biomass was quite limited, suggesting that only the plant did not benefit from these inputs.
293 Indeed, litter addition significantly increased aboveground biomass in 2009, 2010 and 2011,
294 and belowground biomass and total biomass in 2010 and 2011 but only for the highest input

295 level.

296 In spite of the important nutrient inputs through litter additions, the observed effect on
297 plant biomass is quite limited, suggesting that the plant did not benefit from these inputs.
298 Indeed, litter addition significantly increased aboveground biomass in 2009, 2010 and 2011,
299 and belowground biomass and total biomass in 2010 and 2011 but only for the highest input
300 level. One plausible explanation for the lack of effect on plant biomass in spite of the
301 important amount of organic nutrients added might be the imbalance between the C:N:P ratio
302 of the litter added and the C:N:P ratio of the soil, the plants and the microbial community.
303 For instance, the C:N and C:P ratios of the added litter were 41.3 and 521.9, respectively.
304 Compared to the C:N ratio of microbial biomass around 12 and the C:N and C:P ratio of soil
305 around 9 and 55, respectively, added litter decomposition might immobilize available mineral
306 nutrients. As a consequence, the competition between plants and microorganisms for mineral
307 N and available P would increase. Since plant biomass only responded to the highest litter
308 treatments, it suggests that, in our case, microorganisms might be more efficient than plants
309 in using nutrient resources and plant growth might be still nutrient limited. Nevertheless, soil
310 microorganisms benefited only slightly from the litter inputs as suggested by the small
311 observed increase in microbial biomass (Fig. 4m, 4n). Therefore, carbon use efficiency
312 probably decreased when litter was added, increasing heterotrophic respiration. It must be
313 noted that the litter used here was harvested under natural conditions. Thus, the modifications
314 of the litter chemical composition expected under climate change are not taken into account
315 here. Litter C:N ratio may increase in the future (Norby et al., 2001) inducing a higher

316 microbial N demand. Microorganisms may still decrease their carbon use efficiency as
317 assumed here or may increase the N uptake. Since the microbial N uptake in our experiment
318 only increased for very high and unrealistic litter inputs, we assume that, at our site,
319 microorganisms will likely modify their carbon use efficiency in response to the modification
320 of litter C:N ratio instead of competing with plants for N. Furthermore, when litter additions
321 significantly affected plant biomass, aboveground biomass and belowground biomass were
322 higher in 2010 than in 2009 and 2011. Those more favourable soil moisture conditions may
323 have caused the higher soil nutrient availability via accelerated litter decomposition. Indeed,
324 vegetation invested and allocated more biomass toward shoots than roots biomass allocation.
325 In our study, high litter addition decreased the ratio of belowground biomass to aboveground
326 biomass, and the decrease reached a significant level in 2010.

327

328 **4.2 Biomass allocation**

329 Biomass allocation is often affected by factors such as soil nutrient conditions and plant
330 habitat (Vogt et al., 1983; Schmid, 2002; Mokany et al., 2006). Plants respond to lower
331 nutrient supply by increasing allocation of photosynthates to their root system resulting in
332 higher root biomass (Vogt et al., 1983; Schmid, 2002). In our study, high litter addition
333 decreased the ratio of belowground biomass to aboveground biomass, and the decrease
334 reached a significant level in 2010. The reason for this decrease may be that litter addition
335 greatly increased soil organic N and available P and soil microbial biomass C and N,
336 allowing the plant to invest more photosynthates in aboveground biomass in 2010. Sims et al.

337 (2012) found that adding nitrogen increased plant growth and allocated more biomass toward
338 shoots than roots. The plastic response of increased allocation to shoots corresponds to
339 theoretical predictions of increased aboveground competition when nutrient availabilities are
340 high (Tilman, 1988). Such an increase in photosynthates concentration is also explained by the
341 decline in C : N and C : P concentrations in aboveground biomass and litter upon high litter
342 addition, but not for belowground biomass.

343

344 **4.3 Effect of litter addition on plant N:P ratio**

345 Plant N and P are essential nutrients for primary producers and decomposers in terrestrial
346 ecosystems, and N:P ratios of plant biomass or litter have been widely used as indicators of
347 nutrient limitation for primary production (Koerselman and Meuleman, 1996; Tessier and
348 Raynal, 2003; Güsewell, 2004; Güsewell and Verhoeven, 2006). In our study, litter addition
349 did not affect the N:P of aboveground biomass, belowground biomass and litter. Our results
350 showed that the N:P of aboveground biomass (ranging from 13.3 to 13.9 under different litter
351 additions) is higher than that of belowground biomass (ranging from 11.6 to 12 under
352 different litter additions). Similar results were observed in the study of Xu et al., (2010). The
353 N:P of aboveground biomass and belowground biomass is lower than 14 on a community
354 level, suggesting that our *S. krylovii* steppe community was N limited (Koerselman and
355 Meuleman, 1996). Additionally, the N:P of litter ranged from 12.1 to 12.5 after different litter
356 treatments and were much lower than 25 (the threshold between N and P limitation for
357 graminoid leaf litter; Güsewell and Verhoeven 2006), also indicating that our *S. krylovii*

358 steppe community is subject to N limitation. This result is consistent with the conclusion of
359 Bai et al. (2012) who found that meadow steppe, typical steppe and desert steppe
360 communities of temperate grasslands in northern China are N-limited systems. Nevertheless,
361 the lack of response for realistic litter additions suggested CO₂ fertilization might not change
362 the plant-limiting factor in our site.

363

364 **5. Conclusion**

365 Anthropogenic modifications of the C, N and P cycles affect the natural ecological
366 stoichiometry and causes imbalances that will have consequences for biogeochemical cycles
367 including C-sequestration and long term structure and function of ecosystems (Lambers et al.,
368 2010; Vitousek et al., 2010; Peñuelas et al., 2013). Surprisingly, in our study, litter addition
369 significantly affected the stoichiometry of the systems only when it was quite high (twice the
370 natural inputs). Previous modeling exercises have not predicted an increase of primary
371 production sufficient to double litter inputs (Arora and Boer 2014; Todd-Brown et al., 2014).
372 This suggests that, the grassland studied here is quite resilient in terms of stoichiometry. This
373 resilience is the result of complex interactions between C and nutrients cycles as well as
374 between plants and microbial biomass.

375 In conclusion, our results showed that very high litter addition increased soil inorganic N
376 and plant available P, the C and N pools of soil microbial biomass, aboveground and
377 belowground plant biomass. On the other hand, realistic additions according to the models
378 predictions had no effect. This suggests that the expected increase of NPP associated with

379 nutrients imbalance may not have important consequences on the stoichiometric functioning
380 for some particular ecosystems, such as grasslands in northern China. Nevertheless, it must
381 be noted that climate change will also affect temperature and soil moisture, which will largely
382 affect the response of plants to modifications of NPP due to the atmospheric CO₂ increase.

383

384 **Acknowledgments**

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

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597

597 **Figure captions**

598

599 Fig. 1 Seasonal variations of daily mean air temperature and daily precipitation during the
600 experimental period from 1 October 2008 to 31 September 2011.

601

602 Fig. 2 Soil inorganic N (a) and available P (b) in 2009, 2010 and 2011 under different
603 amounts of litter addition in a steppe community of northern China. Vertical bars indicate one
604 standard error about the mean (n=5). A significant year effect ($p < 0.01$) was detected by
605 ANCOVA but no interaction between litter addition and year. Treatments with different
606 letters are significantly different ($P < 0.05$) according to the Duncan test, small letters, capital
607 letters and Greek letters correspond to the year 2009, 2010 and 2011, respectively. 'ns'
608 implies that no significant differences were detected.

609

610 Fig. 3 Aboveground biomass (a), belowground biomass (b), total biomass (c) and litter (d) in
611 2009, 2010 and 2011 under different amounts of litter addition in a steppe community of
612 northern China. Vertical bars indicate one standard error about the mean (n=5). A significant
613 year effect ($p < 0.05$) was detected by ANCOVA except for the below ground biomass.
614 Interactions between litter addition and year were not significant. Treatments with different
615 letters are significantly different ($P < 0.05$) according to the Duncan test, small letters, capital
616 letters and Greek letters correspond to the year 2009, 2010 and 2011, respectively. 'ns'
617 implies that no significant differences were detected.

618

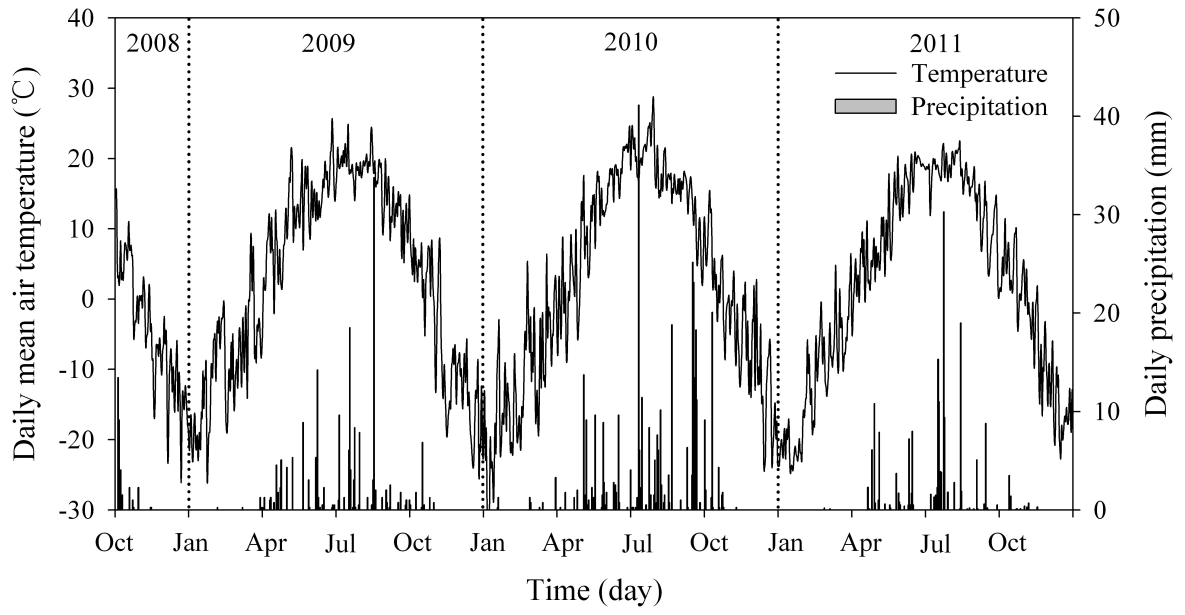
619 Fig 4. The C, N, and P pools of plant, litter and soil and the C and N pools of soil microbial
620 biomass in 2009, 2010 and 2011 under different amounts of litter addition in a steppe
621 community of northern China. Vertical bars indicate one standard error about the mean (n=5).
622 A significant year effect ($p < 0.05$) on the C, N and P content was detected by ANCOVA
623 except for the below ground biomass, the soil. No year effect for litter and soil microbial
624 biomass C content was detected. Interactions between litter addition and year were not
625 significant. Treatments with different letters are significantly different ($P < 0.05$) according
626 to the Duncan test, small letters, capital letters and Greek letters correspond to the year 2009,
627 2010 and 2011, respectively. 'ns' implies that no significant differences were detected.

628

629 Fig. 5 The C:N, C:P and N:P ratio of plant, litter and soil and C:N ratio 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 ratio of above ground biomass and soil
633 microbial biomass was detected by ANCOVA. Interactions between litter addition and year
634 were not significant. Treatments with different letters are significantly different ($P < 0.05$)
635 according to the Duncan test, small letters, capital letters and Greek letters correspond to the
636 year 2009, 2010 and 2011, respectively. 'ns' implies that no significant differences were
637 detected.

638

639 Figure 1

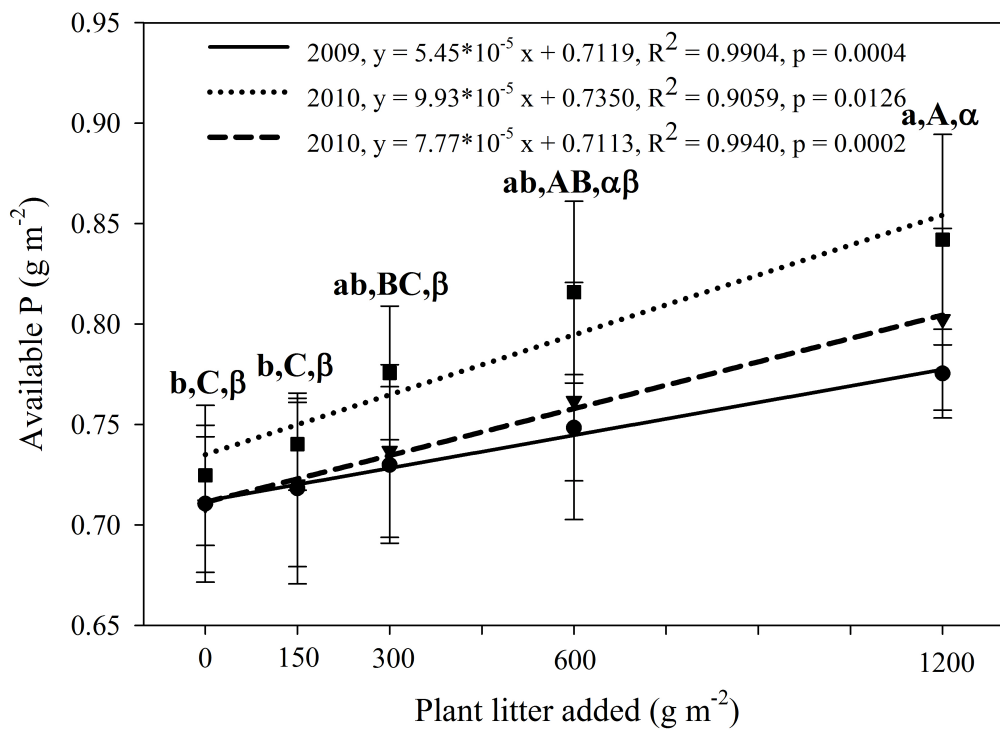
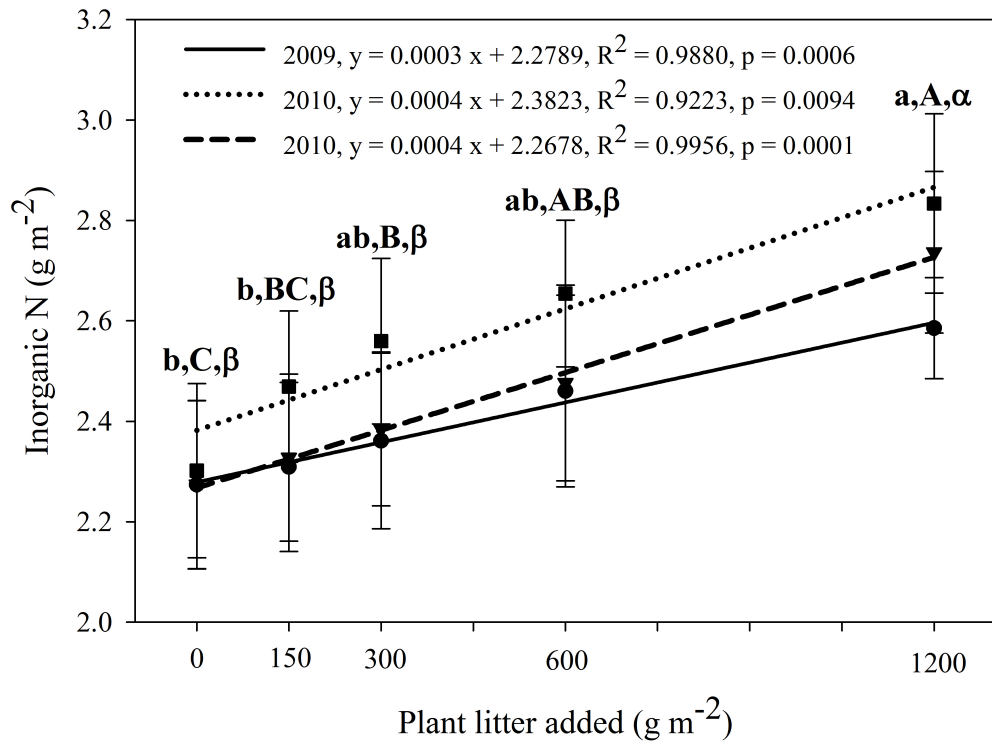


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642 Figure 2

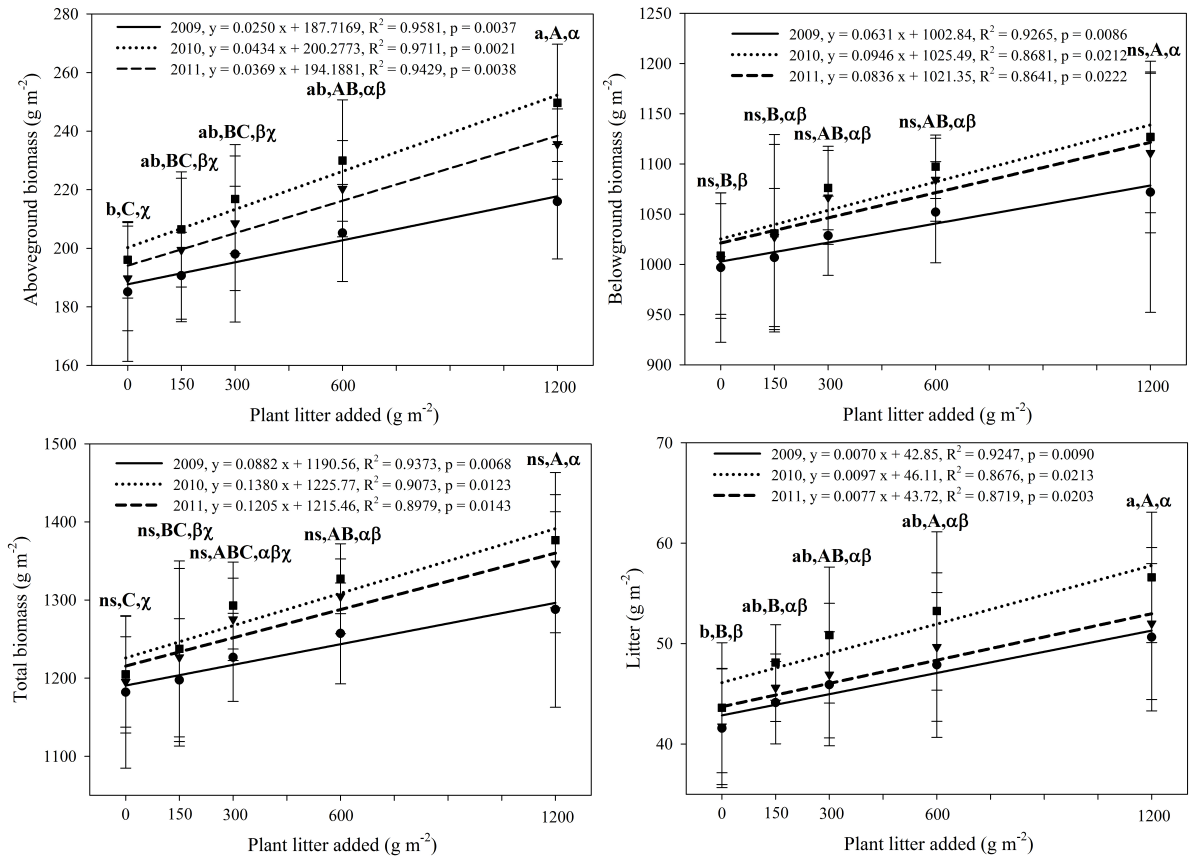


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646 Figure 3



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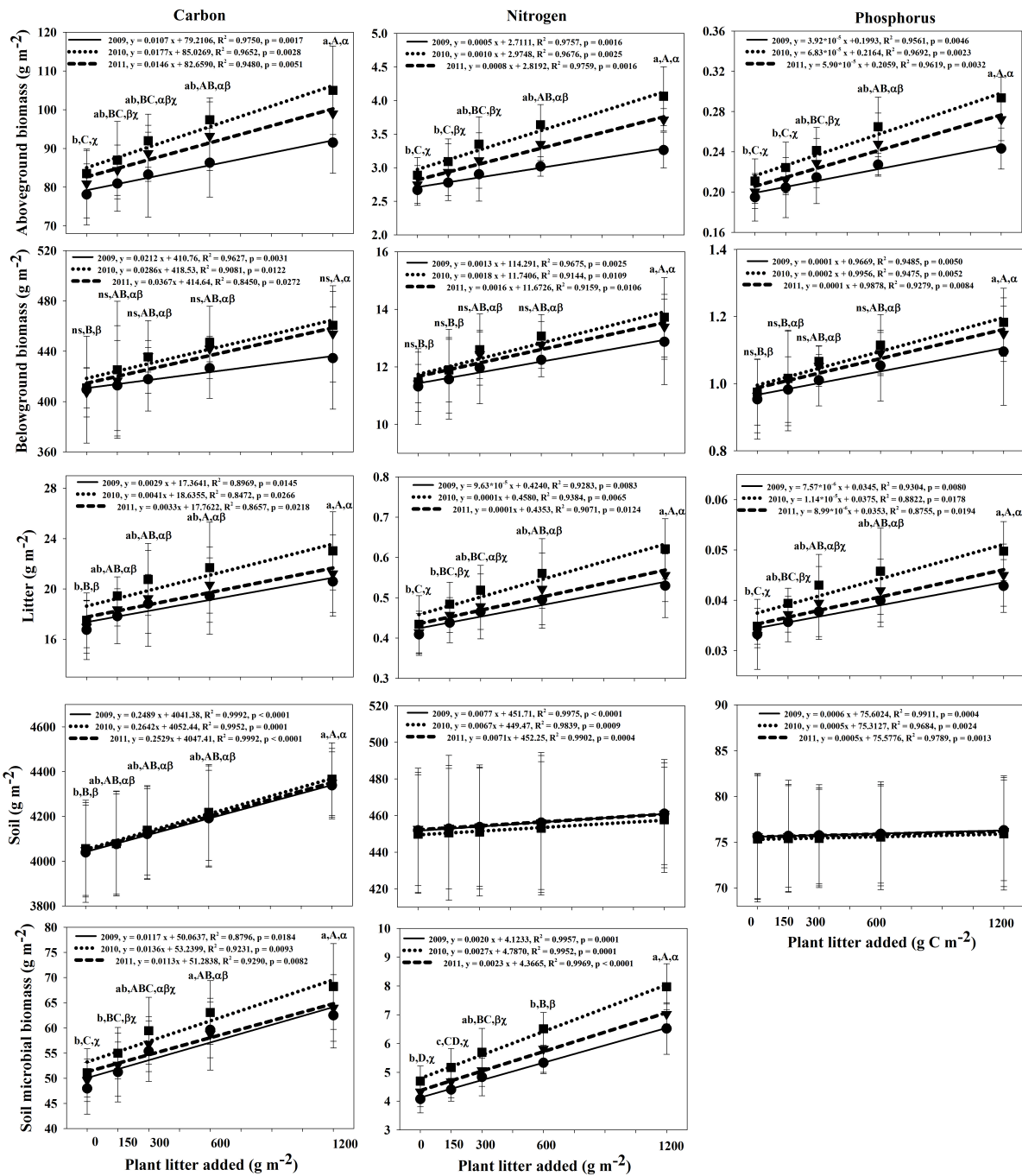
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651 Figure 4



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