

This is a post-peer-review, pre-copyedit version of an article published in Luo, W., et al. *Higher capability of C<sub>3</sub> than C<sub>4</sub> plants to use nitrogen inferred from nitrogen stable isotopes along an aridity gradient* in Plant and soil, vol. 428, issue 1-2 (Jul. 2018), p. 93-103. The final authenticated version is available online at DOI [10.1007/s11104-018-3661-2](https://doi.org/10.1007/s11104-018-3661-2)

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1 **Higher capability of C3 than C4 plants to use nitrogen inferred from nitrogen**  
2 **stable isotopes along an aridity gradient**

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15

16 **Abstract**

17 *Background and Aims* The nitrogen isotope composition ( $\delta^{15}\text{N}$ ) of plants in arid and  
18 semiarid grasslands is affected by environmental factors, especially water availability.  
19 Nevertheless, it is unclear whether the response of  $\delta^{15}\text{N}$  to water availability differs  
20 between C3 and C4 photosynthetic pathways.

21 *Methods* We investigated plant  $\delta^{15}\text{N}$  of coexisting C3 and C4 species as a function of  
22 aridity along a 3200 km aridity gradient across the arid and semi-arid grasslands of  
23 northern China.

24 *Results* Aridity was positively correlated with plant  $\delta^{15}\text{N}$  values in both C3 and C4  
25 plants and also in the entire plant community, whereas soil bulk  $\delta^{15}\text{N}$  values increased  
26 first and then decreased along the aridity gradient. The N uptake by C4 plants  
27 appeared to be more affected by competition pressure of neighboring plants and soil  
28 microbes than for C3 plants along the transect.

29 *Conclusions* The decoupled relationship between plant and soil  $\delta^{15}\text{N}$  values indicated  
30 that variations in vegetation and soil  $\delta^{15}\text{N}$  values were driven by differential  
31 biogeochemical processes, while different soil N sources were used for plant growth  
32 along the climatic gradient. The advantage of C3 plants in the use of N may  
33 counteract the competitive advantage that C4 plants have over C3 plants due to their  
34 higher water use efficiency under drier conditions. These findings can help understand  
35 why C4 plants do not completely replace C3 plants in drier environments despite their  
36 higher water use efficiency.

37 **Keywords**

38 Competition, grassland transect, photosynthetic pathway, precipitation, trade-off.

39 **Introduction**

40 The relative proportions of the stable isotopes of nitrogen ( $^{14}\text{N}$  and  $^{15}\text{N}$ , expressed as  
41  $\delta^{15}\text{N}$ ) in ecosystem components can serve as a proxy of N dynamics and as a  
42 nondestructive indicator of how plants respond to environmental changes in terrestrial  
43 ecosystems (Amundson et al. 2003; Díaz et al. 2016; Robinson 2001). The two stable  
44 isotopes of N are discriminated in several fundamental biogeochemical processes that  
45 in turn are sensitive to environmental conditions (Amundson et al. 2003; Robinson  
46 2001), so  $\delta^{15}\text{N}$  values have been widely used to reflect how environmental changes  
47 alter the ecosystem N-cycles over large scales (Craine et al. 2009; Ogaya and  
48 Peñuelas 2008; Peri et al. 2012; Swap et al. 2004).

49 The values of  $\delta^{15}\text{N}$  in individual plants are determined by the isotopic ratio of the  
50 external source and the redistribution of N within the plant (Evans 2001; Kolb and  
51 Evans 2002), while plant community-level  $\delta^{15}\text{N}$  values are also controlled by the  
52 relative abundance of plant species (Craine et al. 2015; Peri et al. 2012). Previous  
53 studies have shown different  $\delta^{15}\text{N}$  values between C3 and C4 photosynthetic  
54 pathways (Brown 1978; Sage and Percy 1987a; b). For instance, higher  $\delta^{15}\text{N}$  values  
55 in C4 plants than their C3 neighbors were found in western Australia (Wooller et al.  
56 2005) and the Mediterranean region (Hartman and Danin 2010), but C3 plants had  
57 higher  $\delta^{15}\text{N}$  values than C4 plants in southern Africa (Aranibar et al. 2008).

58 Relationships between plant  $\delta^{15}\text{N}$  values and precipitation are a product of water  
59 availability and soil N sources during plant growth (Handley et al. 1999). The general  
60 pattern that soil and plant  $\delta^{15}\text{N}$  values decrease with increased precipitation has been

61 demonstrated at both regional and global scales and suggests different biogeochemical  
62 processes and cycles of N induced by increased aridity, producing more open cycles  
63 in drier regions (Amundson et al. 2003; Aranibar et al. 2004; Craine et al. 2009;  
64 Ogaya and Peñuelas 2008). In less stressed environments the higher plant uptake  
65 allows for a greater N retention in the plant-soil system and reduced loss of  $^{15}\text{N}$  in a  
66 more closed N cycle (Amundson et al. 2003; Aranibar et al. 2004). However, the  
67 responses of plant  $\delta^{15}\text{N}$  to environmental changes are also dependent on the  
68 photosynthetic pathways (Murphy and Bowman 2009). The response of plant  $\delta^{15}\text{N}$   
69 values to increasing water availability was more positive in C3 than in C4 grasses in  
70 Australian grasslands (Murphy and Bowman 2009). It has been argued that plant  $\delta^{15}\text{N}$   
71 values are inversely correlated with precipitation for C3 but not for C4 plants in  
72 southern Africa (Swap et al. 2004).

73 The differential responses of  $\delta^{15}\text{N}$  values between the coexisting C3 and C4 plants  
74 to climate changes are caused by their different N sources (Wang et al. 2016;  
75 Michelsen et al. 1998; Pardo et al. 2006). It has been well known that the N sources  
76 from mycorrhizal fungi and direct root uptake from soils can vary isotopically as a  
77 result of local environmental conditions (Hobbie et al. 2000). The enhanced  
78 dependence of C3 or C4 plants on mycorrhizal fungi generally reduces their  
79 corresponding  $\delta^{15}\text{N}$  values by delivering  $^{15}\text{N}$ -depleted N to host plants (Hobbie and  
80 Colpaert 2003; Hobbie and Högberg 2012), and C3 or C4 plants that prefer nitrate are  
81 predicted to have lower  $\delta^{15}\text{N}$  values than those plants that prefer ammonium (Houlton  
82 et al. 2007). Moreover, changes in soil water availability may also alter  $\delta^{15}\text{N}$  values in

83 C3 and C4 plants by changing their rooting depth and N availability with soil depth  
84 and thereby the  $^{15}\text{N}$  signature of plant N sources (Kahmen et al. 2008), because nitrate  
85 and ammonium sources at different soil depths can vary in  $\delta^{15}\text{N}$  signature (Hobbie  
86 and Ouimette 2009; Xue et al. 2009). Until recently, however, a lack of available  
87 ecological data has limited our ability to determine the underlying mechanisms for the  
88 differential responses of C3 and C4 photosynthetic pathways to climatic variables  
89 (Hartman and Danin 2010).

90 To address this knowledge gap, we investigated plant  $\delta^{15}\text{N}$  of all the species in 26  
91 plant communities across a 3200 km climatic gradient in arid and semiarid grasslands  
92 of northern China, a suitable study region because the impact of climatic factors on  
93 ecosystem N cycles is particularly strong as water stress and N availability are the  
94 main constraints limiting plant growth and microbial activity in these areas (Bai et al.  
95 2004; Cai et al. 2017). The C3 and C4 species are widely distributed and coexist  
96 across this transect. The dominant plant growth forms gradually changed from grasses  
97 and forbs to low shrubs with increasing aridity from the east to the west (Hilbig 1995;  
98 Ni 2003; Pyankov et al. 2000). The unique features of this region encompass  
99 relatively gentle geographical relief, distinct patterns of precipitation and temperature,  
100 and relatively low N deposition levels. We hypothesized that I) plant  $\delta^{15}\text{N}$  values  
101 would increase towards the dry end of the climatic gradient for both C3 and C4 plants  
102 and the whole plant community, and II) the response sensitivity would differ between  
103 the coexisting C3 and C4 plants given the differences in N metabolism and the large  
104 fractionations within plants (Robinson 2001). Specifically, we expected that C3 plants

105 would be more sensitive to aridity than C4 plants, consistent with previous results in  
106 southern Africa (Swap et al. 2004) and Australian grasslands (Murphy and Bowman  
107 2009).

## 108 **Material and Methods**

### 109 **Study sites**

110 In early August 2012, our study was conducted along an east-west transect across arid  
111 and semiarid grasslands in northern China, which has been previously described  
112 (Wang et al. 2014; Luo et al. 2016). This transect is approximately 3200 km long and  
113 covers approximately 10° latitude and 33° longitude (39.8-50.5°N and 87.7-120.5°E)  
114 (Figure 1). The topography of the study area consists of gently rolling hills and  
115 tablelands, with elevations ranging from 700 m in the east to 1500 m above sea level  
116 in the west. The arid and semiarid grasslands are far from human perturbations,  
117 subjected to minimal animal grazing and other anthropogenic disturbances. This  
118 region has a dry, continental climate with marked annual variation in both temperature  
119 and precipitation. Mean annual precipitation (MAP) ranges from 450 mm (east) to 50  
120 (west) mm, and mean annual temperature (MAT) ranges from -1.5°C (east) to 9.5°C  
121 (west). The interaction of increasing MAP and decreasing MAT is closely tracked by  
122 species richness and vegetation cover (%), which both increase with increased water  
123 availability from the west to the east across this transect. A total of 26 sites at intervals  
124 of *ca.* 150 km were selected along the transect. The latitude, longitude and elevation  
125 of each sampling site were recorded by GPS (eTrex Venture, Garmin, USA).

126 Four representative types of vegetation can be found along the transect: desert

127 steppe, typical steppe and meadow steppe, which are characterized by increasing  
128 precipitation and decreasing temperature from the west to the east (Figure 1). The  
129 desert steppe, at the dry end of the gradient, is dominated by low shrubs *Calligonum*  
130 *mongolicum* (C3) and *Suaeda microphylla* (C4), with low species richness and soil  
131 organic matter content. The typical steppe, in the central part of the gradient, is  
132 dominated by *Salsola collina* (C4) and *Reaumuria soongarica* (C3). The meadow  
133 steppe, at the wet end of the gradient, is dominated by *Leymus chinensis* (C3), *Stipa*  
134 *grandis* (C3) and *Cleistogenes squarrosa* (C4) and has relatively high species richness  
135 and soil organic matter content. Related soil types of this region are gray-brown desert  
136 soils, brown calcic soils and chestnut soils distributed from west to east, belonging to  
137 the Kastanozems in the classification system of the Food and Agriculture  
138 Organization and Mollisol order of the US Soil Taxonomy.

### 139 **Sampling and measurement**

140 At each site, two 50 m × 50 m main plots were established and five 1 m × 1 m  
141 sampling subplots (or 5 m × 5 m sampling subplots in site dominated by low shrub)  
142 were placed within each main plot at the four corners and the center (Figure 1). Plant  
143 species presence were measured in each subplots, and from these data species  
144 richness (number of plant species per subplot) were calculated. Standing crop was  
145 estimated from the dry biomass of the aboveground living parts. Aboveground  
146 biomass was sampled by clipping all plants at ground level within each sampling  
147 subplot. All living plants were sorted to species and then stored in paper bags. Plant  
148 materials were dried at 105 °C for 30 min in a portable drying oven to minimize

149 respiration and decomposition and were later completely oven dried at 65°C to  
150 constant weight in the laboratory. After removal of surface litter, one composite soil  
151 sample (0-10 cm depth) was randomly collected from each sampling subplot using a  
152 soil corer (2.5 cm diameter). Soil samples was carefully removed from the plant  
153 material and then separated into two sub-samples: one was stored in a cloth bag at  
154 room temperature (air-dried soils); the other one was stored in a plastic bag in a  
155 refrigerator at 4°C (fresh soils). A detailed description of the vegetation and soil  
156 survey was documented in Luo et al. (2015; 2016).

157 Dried plant and soil materials were ground in a ball mill (NM200, Retsch, Haan,  
158 Germany) and stored in a plastic bag until further analysis. Plant and soil bulk  $\delta^{15}\text{N}$   
159 values and soil total N concentrations were determined using an elemental analyzer  
160 (Elementar Vario Micro Cube, Elementar, Germany) connected to an isotope ratio  
161 mass spectrometer (IsoPrime100, Isoprime Ltd., UK), with an overall precision  
162 better than 0.2‰.  $\delta^{15}\text{N}$  values are expressed in per mil (‰) unit, relative to the  
163 atmospheric  $\text{N}_2$  standards.

164 Methods for the determination of soil pH and microbial-biomass N (MBN) has  
165 been described previously (Luo et al. 2016). Briefly, soil pH was measured using a pH  
166 electrode (S210 SevenCompact™, Mettler, Germany) in a 1: 2.5 mixture of soil:  
167 water. The concentration of MBN was measured with the fumigation-extraction  
168 method.

169 The MAT and MAP data (data range 1950-2000) were extracted from a global  
170 climate dataset with a resolution of  $0.0083^\circ \times 0.0083^\circ$  (approximately 1 km<sup>2</sup> at the

171 equator), obtained from <http://www.worldclim.org>. The potential evapotranspiration  
172 (PET) data (data range 1950-2000) were extracted from the CGIAR-CSI Global  
173 Aridity Index and Global Potential Evapo-Transpiration Climate Database  
174 (<http://www.cgiar-csi.org/data/global-aridity-and-pet-database>). Aridity (unitless) was  
175 calculated as 1-AI, where AI, the ratio of MAP to PET, is the aridity index (Luo et al.  
176 2016). Aridity therefore increased with increasing MAT and decreasing MAP. Across  
177 this transect, aridity ranged from 0.45 to 0.95, equivalent to a range in MAP of 450-50  
178 mm, and a range in MAT ranged from -1.5 to 9.5 °C. The aridity was applied to  
179 incorporate MAP and MAT into one parameter to assess the variations in plant  $\delta^{15}\text{N}$   
180 values along the climatic gradient due to the strongly positive correlation between  
181 PET and MAT.

## 182 **Data analysis**

183 All sampled plant species were classified into C3 or C4 photosynthetic pathways. If  
184 the specimen could be assigned to a genus, classification was made using the  
185 identification in Watson and Dallwitz (1992). If the specimen could not be identified  
186 to generic level, classification was made by the  $\delta^{13}\text{C}$  values (Cerling et al. 1997).

187 Plant community  $\delta^{15}\text{N}$  values were defined as the overall mean of  $\delta^{15}\text{N}$  values  
188 across all species (n, species richness) weighted by the relative (fractional)  
189 contribution of each species to the overall biomass at each quadrat (Kichenin et al.  
190 2013):

191 Community  $\delta^{15}\text{N} = (\text{biomass}_1 \times \delta^{15}\text{N}_1 + \text{biomass}_2 \times \delta^{15}\text{N}_2 + \dots + \text{biomass}_n \times \delta^{15}\text{N}_n) / \text{total}$   
192 biomass.

193 Ordinary least squares (OLS) regression was used to analyze the responses of plant

194 community  $\delta^{15}\text{N}$  values to increasing aridity. Binary regression was conducted to  
195 analyze the relationship between aridity and soil  $\delta^{15}\text{N}$  values. Then, OLS regression  
196 was also used to examine the correlation between mean community and soil  $\delta^{15}\text{N}$   
197 values. To further analyze patterns of plant  $\delta^{15}\text{N}$  values, OLS regression was applied  
198 to test the relationships of plant  $\delta^{15}\text{N}$  values and N concentrations with aridity for both  
199 C3 and C4 plants. Then, OLS regression was applied to test the relationships of plant  
200  $\delta^{15}\text{N}$  values with species richness for both C3 and C4 plants. We conducted analysis  
201 of covariance to identify the differences in the slopes of the regression lines between  
202 C3 and C4 photosynthetic pathways. Steeper slope means greater sensitivity of  $\delta^{15}\text{N}$   
203 value in this plant type in response to aridity.

204 To examine the underlying mechanism under the different responses of  $\delta^{15}\text{N}$  values  
205 to aridity between coexisting C3 and C4 plants, structural equation modeling (SEM)  
206 was applied to examine the interactive effects of climatic and soil variables on the  
207  $\delta^{15}\text{N}$  values in C3 and C4 plants, respectively. In the SEM analysis, we compared the  
208 model-implied variance-covariance matrix against the observed variance-covariance  
209 matrix. Data were fitted to the models using the Akaike information criterion and the  
210 goodness of fit index. For simplicity, the least significant path was deleted and the  
211 model was re-estimated; then the next least significant path was removed, and so on,  
212 until the paths that remained in the final SEM were all significant. Standard errors and  
213 the significance level ( $P$  value) were calculated using bootstrapping (1200  
214 repetitions).

215 All statistical analyses were carried out using the statistical package of SPSS 13.0

216 for Windows® (SPSS Inc., Chicago, IL, USA, 2004) and the *sem* function in the *sem*  
217 package of R-project (R i386 3.1.1).

## 218 **Results**

219 Plant  $\delta^{15}\text{N}$  values significantly increased with increasing aridity at the community  
220 level ( $P < 0.001$ , Figure 2). Removing the leguminous species from this analysis did  
221 not change the results (data not shown). The relative biomass of leguminous species  
222 (%) showed no significant relationship with aridity (data not shown). Plant  
223 communities in drier sites contained a greater percentage of total plant biomass of  
224 species with higher  $\delta^{15}\text{N}$  values (Table 1). Soil  $\delta^{15}\text{N}$  values increased first and then  
225 reduced with increasing aridity (Figure S1) and was nonlinearly associated with plant  
226 community  $\delta^{15}\text{N}$  values (Figure 3). Removing three sites with soil  $\delta^{15}\text{N}$  values  $> 10 \text{ ‰}$   
227 (outliers) did not change the results (see the insets in Figures 3 and S1).

228 Plant  $\delta^{15}\text{N}$  values also significantly increased with aridity in both C3 and C4 plants  
229 at the level of individual species ( $P < 0.001$ , Figure 4). The interaction between aridity  
230 and type of photosynthetic pathway was significant, *i.e.*, the slope of the regression  
231 line for aridity and  $\delta^{15}\text{N}$  values was significantly steeper for C4 than C3 plants  
232 ( $P < 0.001$ ). When the specific-biomass weighting factor was considered, the response  
233 of  $\delta^{15}\text{N}$  values to increasing aridity was also more positive in the C4 than in the C3  
234 plant group (Figure S2). Plant N content did not show any significant relationships  
235 with aridity for both C3 and C4 plants along the transect (Figure S3). Plant  $\delta^{15}\text{N}$   
236 values reduced with increasing species richness in both C3 and C4 plants, and the  
237 slope of the regression line for species richness and  $\delta^{15}\text{N}$  values was significantly

238 steeper for C4 than C3 plants ( $P < 0.001$ , Figure S4). The proportional contribution of  
239 C3 plants to total biomass reduced and that of C4 plants increased with the increase in  
240 aridity (Figure S5). Soil pH increased with increasing aridity, while standing crop,  
241 species richness, soil total N concentration, soil C:N ratio, and MBN concentration all  
242 reduced with increasing aridity along the transect (Figure S6).

243 The SEM analyses showed that the total effects of aridity, soil pH and ANPP were  
244 positive on  $\delta^{15}\text{N}$  values in both C3 (Figure 5a) and C4 plants (Figure 5b). Aridity  
245 indirectly affected plant  $\delta^{15}\text{N}$  values via the positive effect on soil pH and the negative  
246 effects on ANPP and species richness for both C3 (Figure 5a) and C4 plants (Figure  
247 5b). The total effects of soil total N and MBN concentrations were negatively and  
248 positively correlated with  $\delta^{15}\text{N}$  values in C4 plants, respectively (Figure 5b). Aridity  
249 indirectly affected plant  $\delta^{15}\text{N}$  values via the negative effects on soil total N and MBN  
250 concentrations for C4 plants (Figure 5b).

251

252 **Discussion**

253 **N stable isotopes in plants along the aridity gradient**

254 Consistent with our hypothesis, plant  $\delta^{15}\text{N}$  values of both the C3 and C4 functional  
255 groups and the entire community increased towards the dry end of the climatic  
256 gradient across northern China's grasslands, where N and water availability are two of  
257 the most constraining factors limiting plant growth and microbial activity. This  
258 finding is similar to that reported in other continents (Aranibar et al. 2008; Austin and  
259 Sala 1999; Heaton 1987; Swap et al. 2004) and in an extensive global synthesis  
260 (McCulley et al. 2009; Murphy and Bowman 2009; Schulze et al. 1998) conducted at  
261 the level of species rather than the entire plant community. Plant  $\delta^{15}\text{N}$  values also  
262 increased with aridity in our study in the entire plant community, similar to individual  
263 species, which was due to the substitution of plant species/functional groups with  
264 lower  $\delta^{15}\text{N}$  by those with higher  $\delta^{15}\text{N}$  when aridity rose along the climatic gradient  
265 (see Table 1). Higher plant  $\delta^{15}\text{N}$  values indicate a lower capacity of plants to retain N,  
266 because the lighter  $^{14}\text{N}$  isotope is more easily cycled and lost (Dalal et al. 2013;  
267 McCulley et al. 2009). Plant species that are less efficient in retaining N are favored  
268 when aridity increases along the climatic gradient. This result suggests that water  
269 conservative mechanisms constitute a trade off with the capacity to retain and use N.  
270 This trade-off has been observed and commented in previous studies (Dijkstra et al.  
271 2016). Thus, the biological response to drier conditions when water limitation is the  
272 main driver of adaptive responses provokes a decrease in the capacity to retain N in  
273 the plant community.

274 The increased plant  $\delta^{15}\text{N}$  values with aridity, however, do not agree with our  
275 previous study along the same gradient, which found that the ecosystem N-cycles,  
276 based on soil bulk  $\delta^{15}\text{N}$  rather than plant  $\delta^{15}\text{N}$  signals, were more closed at the two  
277 ends of the aridity gradient and more open in the middle of the aridity gradient (Wang  
278 et al. 2014). Similarly, Díaz et al. (2016) recently reported that soil  $\delta^{15}\text{N}$  values  
279 increased with increasing aridity along an elevational/climatic gradient in northern  
280 Chile, as expected, whereas plant  $\delta^{15}\text{N}$  values had a hump-shaped relationship with  
281 increasing aridity. The decoupled relationship between the  $\delta^{15}\text{N}$  values of plants and  
282 soils indicates the differences in biogeochemical processes underlying N dynamics  
283 between vegetation and soil. An increase in plant  $\delta^{15}\text{N}$  values with aridity,  
284 independent of soil bulk  $\delta^{15}\text{N}$  values, may be caused by changes in the uptake of  
285 nitrate versus ammonium along the aridity gradient (Houlton et al. 2007; Takebayashi  
286 et al. 2010). Species that prefer nitrate relative to ammonium generally have lower  
287  $\delta^{15}\text{N}$  values than species that prefer ammonium (Houlton et al. 2007). In our previous  
288 study along the same climatic gradient, we found that  $\delta^{15}\text{N}$  values of ammonium  
289 consistently increased, while  $\delta^{15}\text{N}$  values of nitrate increased first and then decreased  
290 with increasing aridity (see Liu et al. 2017). These results suggest a shift of dominant  
291 inorganic N sources for plants with aridity, resulting in a positive relationship between  
292 aridity and plant  $\delta^{15}\text{N}$  values and a decoupled relationship between plant and soil bulk  
293  $\delta^{15}\text{N}$  values. Similarly, Houlton et al. (2007) demonstrated that increased aridity  
294 resulted in a switch in the dominant N source for plant growth, leading to a reduction  
295 in plant  $\delta^{15}\text{N}$  values from drier to wetter sites in tropical forest ecosystems.

296 **N isotope and N use in C4 versus C3 plants**

297 In line with our hypothesis, the responses of plant  $\delta^{15}\text{N}$  values to environmental  
298 changes differed between the coexisting C3 and C4 photosynthetic pathways (Figures  
299 5 and 6). The  $\delta^{15}\text{N}$  values in C4 plants were positively correlated with soil MBN  
300 concentration and negatively correlated with soil total N concentration but not in C3  
301 plants (Figure 5). This suggests a stronger competition intensity of N uptake between  
302 soil microbes and C4 plants when soil N content becomes scarcer under drier  
303 conditions (Liu et al. 2016; Ouyang et al. 2016). The increases in plant  $\delta^{15}\text{N}$  values in  
304 C4 plants along the aridity gradient therefore corresponded to a large proportion of  
305 soil N incorporated into microbial biomass, suggesting that C4 plants competed with  
306 soil microbes for N less strongly than C3 plants (Liu et al. 2016; Ouyang et al. 2016)).  
307 Moreover, our results showed that the reduction in species richness with increasing  
308 aridity directly resulted in a reduction in plant  $\delta^{15}\text{N}$  values in both C3 and C4 plants,  
309 with the effects being stronger in C4 than C3 plants (Figure S4). These results suggest  
310 that C4 plants were more sensitive to plant neighborhood competition with respect to  
311 N uptake than C3 plants (Harrison et al. 2007; Mariotte et al. 2013). Taken together,  
312 C4 plants appeared more affected by competition pressure of neighboring plants and  
313 soil microbes than C3 plants with respect to N uptake and C3 plants had an apparent  
314 advantage in the use and retention of N compared to C4 plants in arid and semiarid  
315 grasslands.

316 This advantage of C3 plants in the use of N may improve their competitive ability  
317 and thus their survival capacity under dry conditions. Previous studies have reported

318 that C3 plants would be replaced by C4 plants due to their lower water-use efficiency  
319 if the global climate becomes drier in the near future (Pyankov et al. 2000; Wittmer et  
320 al. 2010). However, this higher N-use efficiency we observed in C3 plants could  
321 counteract the competitive advantage of the more water-use efficient C4 plants under  
322 drier conditions, thereby partially avoiding the total replacement of C3 by C4 plants.  
323 A reduction in transpiration can further decrease the capacity of C4 plants to take up  
324 and compete for nutrients (Cramer et al. 2009). Our results provide new evidence of a  
325 trade-off between N-use and water-use efficiencies for plants with different  
326 photosynthetic pathways (Dijkstra et al. 2016). The higher ability of C3 plants to  
327 absorb, retain and use N could, at least partially, explain why C3 plants are not fully  
328 replaced by the more water-use efficient C4 plants under arid conditions when N  
329 supply decreases.

### 330 **Conclusion**

331 Our results have two important implications for predicting the responses of vegetation  
332 and biogeochemical cycles to climate change. First, plant  $\delta^{15}\text{N}$  values of both the C3  
333 and C4 functional groups and the entire community increased towards drier  
334 conditions due to an increase in  $\delta^{15}\text{N}$  of each individual species, and also to an  
335 increased dominance of species with higher  $\delta^{15}\text{N}$  values. Along the aridity gradient,  
336 plant  $\delta^{15}\text{N}$  values did not covary with soil bulk  $\delta^{15}\text{N}$  values, resulting from a shift in  
337 dominant inorganic N sources for plant growth along the aridity gradient. The  
338 decoupled relationships suggest that the mechanisms underlying soil bulk  $\delta^{15}\text{N}$   
339 patterns should be carefully applied to plant  $\delta^{15}\text{N}$  patterns in arid and semiarid

340 ecosystems. Second, our results suggest that competition pressure for N by  
341 neighboring plants and soil microbes became more intense for C4 than C3 plants,  
342 thereby partly counteracting the competitive advantage of C4 plants due to their  
343 higher water use efficiencies under drier and warmer conditions. These findings  
344 provide new hypotheses to explain why C3 plants are not completely replaced by C4  
345 plants in drier and warmer conditions.

### 346 **Acknowledgments**

347 We thank all members of the Field Expedition Team from the Institute of Applied  
348 Ecology, Chinese Academy of Sciences, for assistance with the collection of the field  
349 data. We thank three anonymous referees as well as the Handling Editor for  
350 constructive comments on the manuscript. This work was supported by the National  
351 Basic Research Program of China (2016YFC0500601, 2016YFC0500700 and  
352 2015CB150802), National Natural Science Foundation of China (41600302,  
353 3231470505 and 41273094), Strategic Priority Research Program of the Chinese  
354 Academy of Sciences (XDB15010403), Youth Innovation Promotion Association  
355 CAS (2014174), and the Key Research Program from CAS (KFZD-SW-305-002). JP  
356 and JS were funded by the European Research Council Synergy grant ERC-SyG-  
357 2013-610028, IMBALANCE-P, the Spanish Government grant CGL2013-48074-P  
358 and the Catalan Government grant SGR 2014-274.

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520 **Table 1** The most abundant species of each community type. The mean N isotopic signature ( $\delta^{15}\text{N}$ ) of the species along the gradient and the  
 521 percentage of total individual plant biomass that the species represents in the corresponding community are indicated in parentheses in red and  
 522 black, respectively.

Most abundance species in each community type								
Meadow steppe (low aridity)			Typical steppe (medium aridity)			Desert steppe (high aridity)		
<i>Achnatherum sibiricum</i>	0.69‰	4.10%	<i>Allium mongolicum</i>	2.14‰	2.57%	<i>Nitraria tangutorum</i>	3.13‰	14.40%
<i>Agropyron cristatum</i>	1.75‰	4.80%	<i>Allium polyrhizum</i>	4.17‰	2.90%	<i>Reaumuria songarica</i>	1.99‰	31.70%
<i>Allium bidentatum</i>	2.14‰	1.90%	<i>Allium ramosum</i>	2.95‰	0.15%	<i>Salsola passerina</i>	5.44‰	13.50%
<i>Allium mongolicum</i>	2.88‰	1.10%	<i>Allium spp.</i>	5.33‰	0.08%	<i>Suaeda microphylla</i>	5.81‰	11.50%
<i>Allium ramosum</i>	2.95‰	0.93%	<i>Allium tenuissimum</i>	1.74‰	0.08%	<i>Calligonum mongolicum</i>	5.84‰	3.80%
<i>Allium tenuissimum</i>	1.74‰	1.86%	<i>Artemisia scoparia</i>	1.86‰	0.46%	<i>Eragrostis pilosa</i>	2.59‰	4.80%
<i>Artemisia frigida</i>	2.28‰	3.43%	<i>Artemisia spp.</i>	1.24‰	0.15%			
<i>Asparagus cochinchinensis</i>	2.50‰	1.40%	<i>Cleistogenes spp.</i>	1.85‰	5.30%			
<i>Carex korshinskii</i>	0.89‰	4.98%	<i>Cleistogenes squarrosa</i>	0.46‰	3.60%			
<i>Chenopodium aristatum</i>	1.61‰	3.11%	<i>Eragrostis pilosa</i>	2.59‰	5.30%			
<i>Chenopodium glaucum</i>	4.66‰	2.80%	<i>Reaumuria songarica</i>	1.99‰	5.36%			
<i>Cleistogenes squarrosa</i>	0.46‰	10.1%	<i>Kochia prostrata</i>	5.75‰	3.57%			
<i>Kochia prostrata</i>	5.75‰	2.49%	<i>Salsola collina</i>	4.61‰	7.70%			
<i>Leymus chinensis</i>	1.26‰	9.35%	<i>Calligonum mongolicum</i>	5.44‰	5.60%			
<i>Potentilla acaulis</i>	0.99‰	0.93%	<i>Oxytropis aciphylla</i>	2.28‰	16%			
<i>Potentilla tanacetifolia</i>	0.25‰	1.86%	<i>Corispermum mongolicum</i>	5.84‰	2.60%			
<i>Salsola collina</i>	4.61‰	7.17%	<i>Caragana stenophylla</i>	0.33‰	2.09%			

<i>Serratula centauroides</i>	0.34‰	7.62%
<i>Stipa</i> spp.	2.27‰	7.62%

---

Represents 71% of total plant biomass in typical grasslands, with a weighted average of  $\delta^{15}\text{N} = 1.38\text{‰}$

Represents 77% of total plant biomass in desert grasslands, with a weighted average of  $\delta^{15}\text{N} = 2.75\text{‰}$

Represents 80% of total plant biomass in shrublands, with a weighted average of  $\delta^{15}\text{N} = 6.01\text{‰}$

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524 **Figure captions**

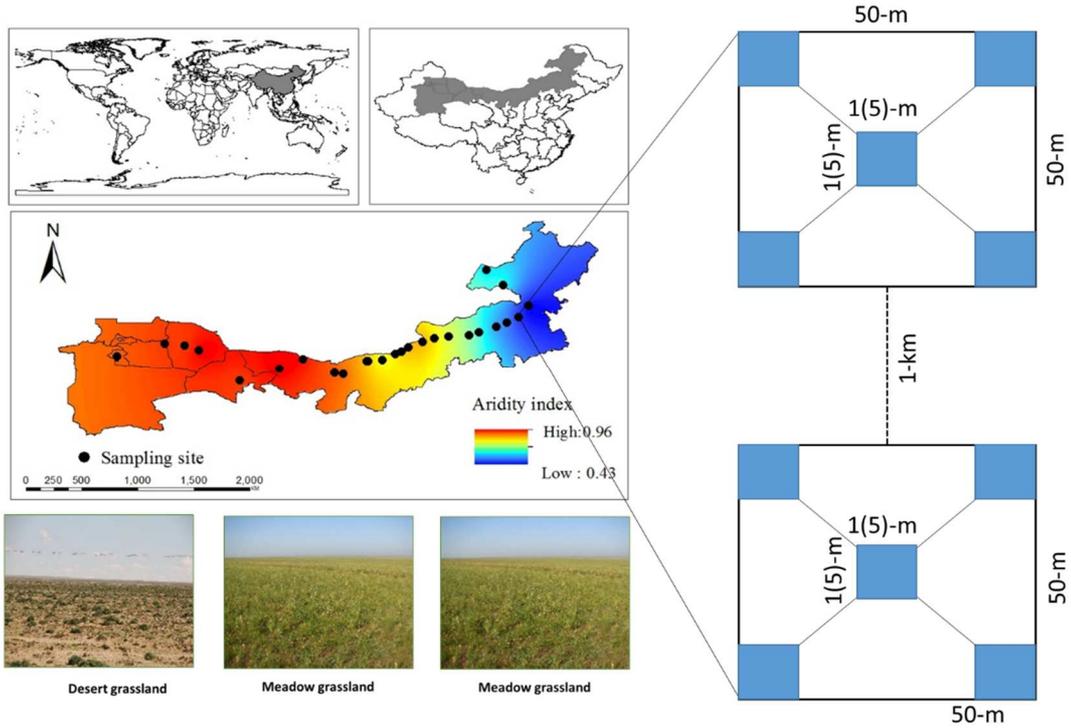
525 **Figure 1** A 3200-km long transect in arid and semiarid grasslands of northern China. A  
526 total of 26 sampling sites from west to east were selected along the aridity gradient.  
527 Two 50 m × 50 m plots were selected at each site, and five 1 m × 1 m sampling subplots  
528 (or 5 m × 5 m sampling subplots in site dominated by low shrubs) were placed within  
529 each plot. Three typical vegetation types are distributed with increasing aridity:  
530 meadow steppe, typical steppe, and desert steppe. The dominant plant growth forms  
531 change gradually from perennial grasses (*Leymus chinensis* (C3), *Stipa grandis* (C3)  
532 and *Cleistogenes squarrosa* (C4)) to low shrubs (*Calligonum mongolicum* (C3) and  
533 *Suaeda microphylla* (C4)).

534 **Figure 2** Correlation between aridity and plant community N isotopic signature  
535 ( $\delta^{15}\text{N}$ ) along the grassland transect in northern China. Plant community  $\delta^{15}\text{N}$  values  
536 were defined as the overall mean of  $\delta^{15}\text{N}$  values across all species weighted by the  
537 relative contribution of each species to the overall biomass. Aridity was calculated as  
538  $1-\text{AI}$ , where AI, the ratio of precipitation to potential evapotranspiration, is the aridity  
539 index.

540 **Figure 3** Relationships between plant community and soil bulk  $\delta^{15}\text{N}$  along the  
541 grassland transect in northern China. Plant community  $\delta^{15}\text{N}$  values were defined as  
542 the overall mean of  $\delta^{15}\text{N}$  values across all species weighted by the relative  
543 contribution of each species to the overall biomass. When the three sites with soil  
544  $\delta^{15}\text{N}$  values higher than 10 ‰ were removed, the non-linear relationship remained  
545 between plant and soil  $\delta^{15}\text{N}$  values.

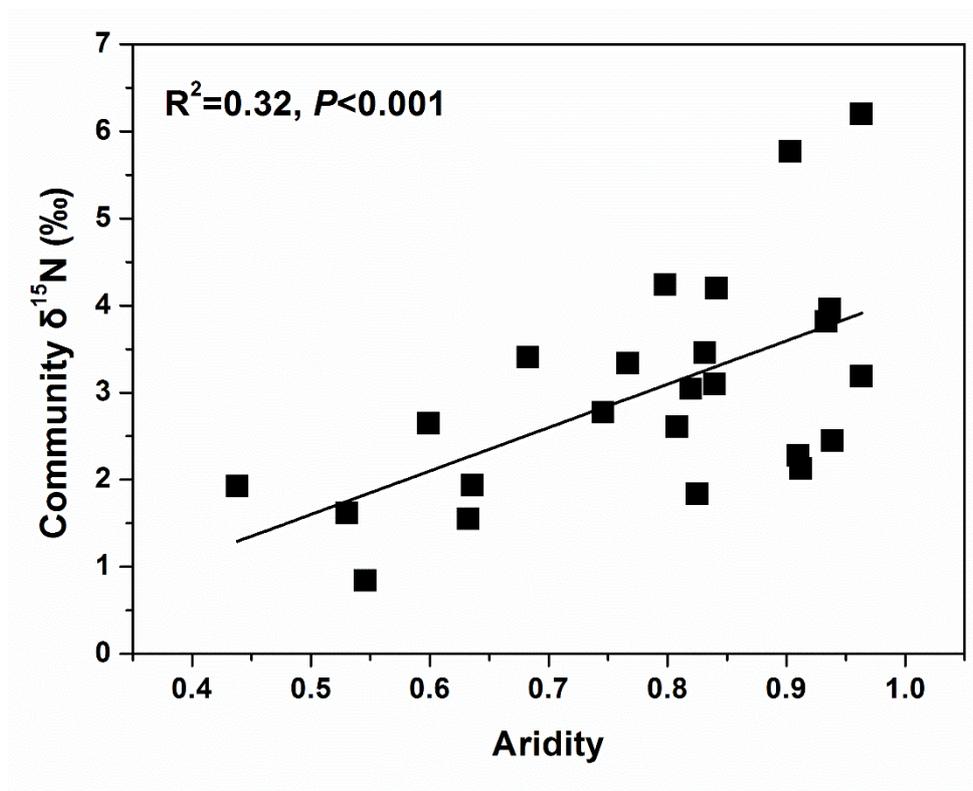
546 **Figure 4** Correlation between aridity and N isotopic signature ( $\delta^{15}\text{N}$ ) for C3 and C4  
547 plants along the grassland transect in northern China. Aridity was calculated as 1-AI,  
548 where AI, the ratio of precipitation to potential evapotranspiration, is the aridity index.

549 **Figure 5** Diagram of the structural equation modeling (SEM) that best explain the  
550 maximum variance of (a) C3 and (b) C4 plant N isotopic signatures ( $\delta^{15}\text{N}$ ) along the  
551 environmental gradient in northern China. Numbers adjacent to arrows are  
552 standardized path coefficients, analogous to relative regression weights, and indicative  
553 of the effect size of the relationship. Dashed and continuous arrows indicate negative  
554 and positive relationships, respectively. Arrow width is proportional to the strength of  
555 the relationship. Goodness-of-fit statistic for each model are shown in the lower right  
556 corner. The proportion of variance explained ( $R^2$ ) appears alongside every response  
557 variable in the model. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Aridity was calculated as 1-  
558 AI, where AI, the ratio of precipitation to potential evapotranspiration, is the aridity  
559



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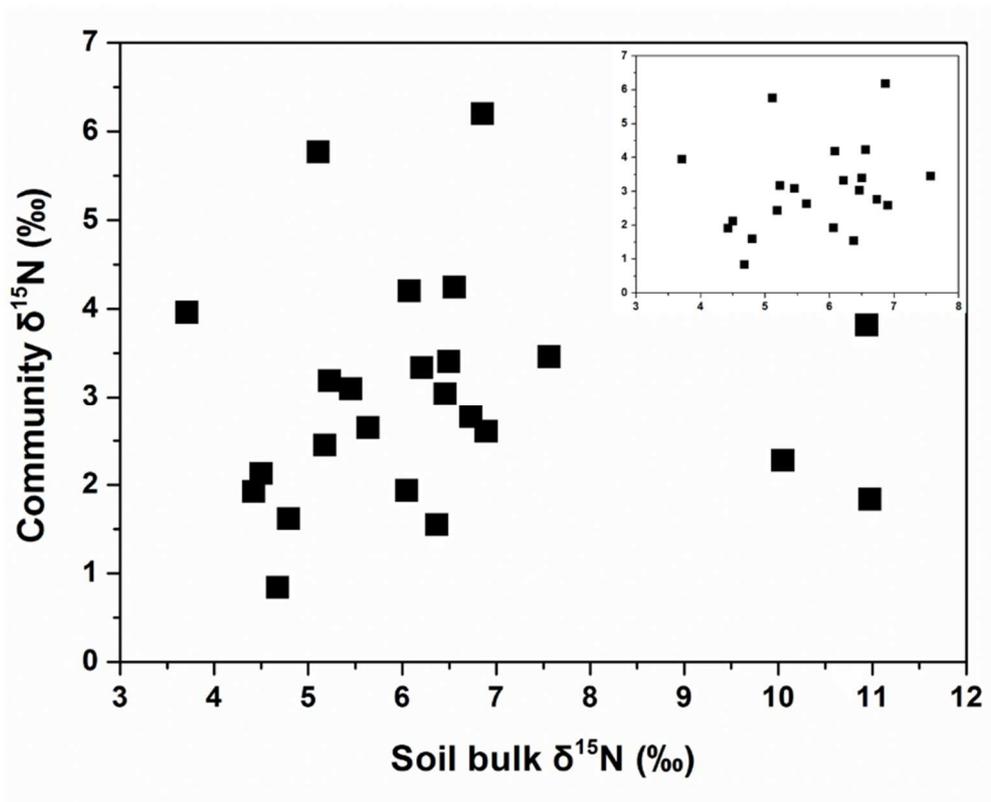
561 **Figure 1**



563

564 **Figure 2**

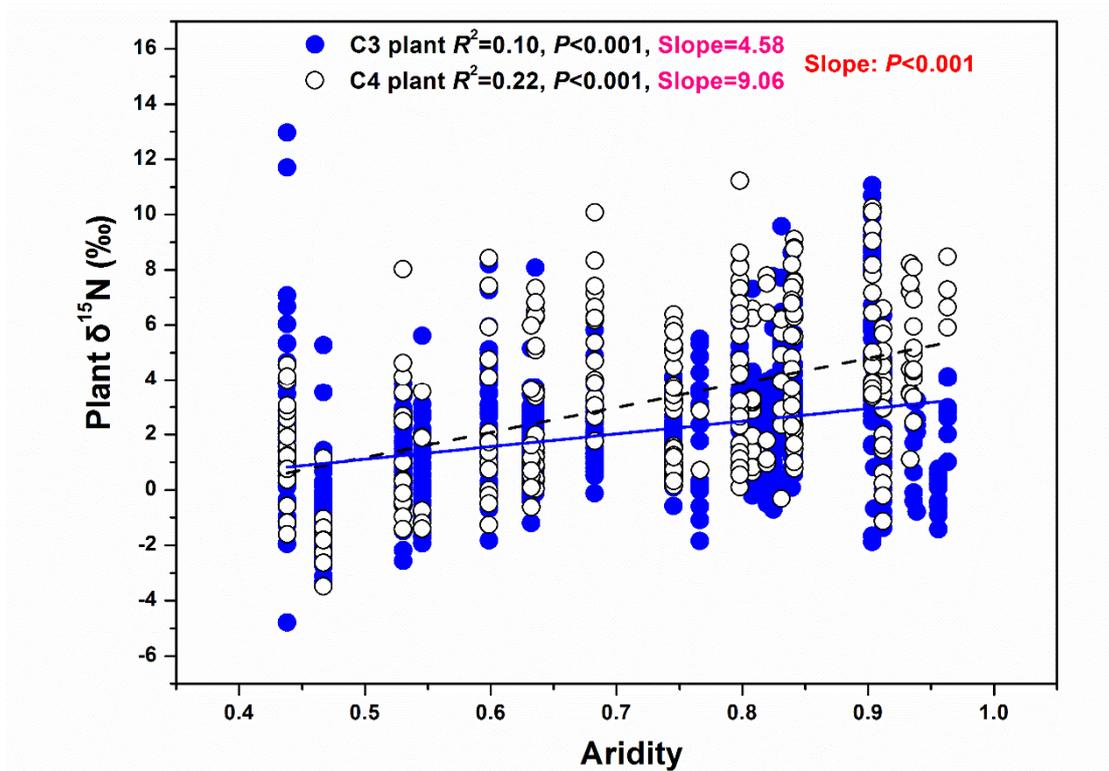
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567 **Figure 3**

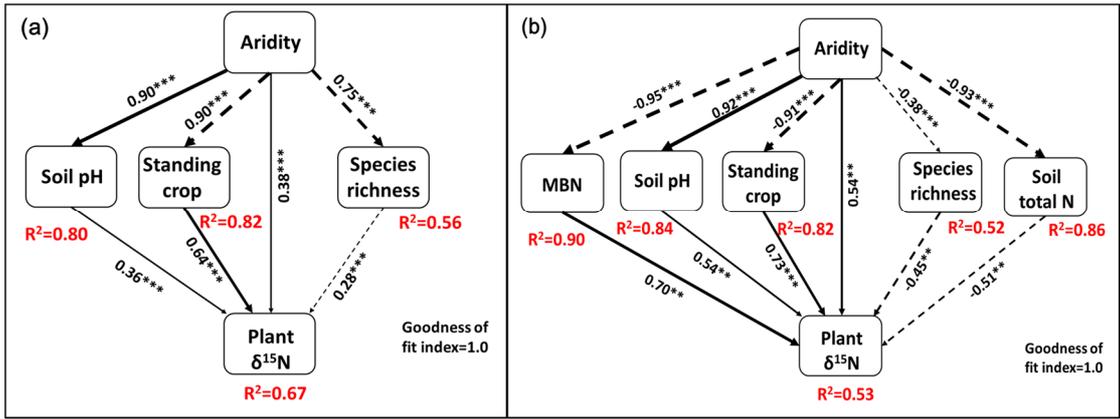
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570 **Figure 4**

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572

573 **Figure 5**