This is a post-peer-review, pre-copyedit version of an article published in Luo, W., et al. *Higher capability of C₃ than C₄ 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

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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Abstract

Background and Aims The nitrogen isotope composition ($\delta^{15}N$) of plants in arid and

semiarid grasslands is affected by environmental factors, especially water availability.

Nevertheless, it is unclear whether the response of $\delta^{15}N$ to water availability differs

between C3 and C4 photosynthetic pathways.

Methods We investigated plant $\delta^{15}N$ of coexisting C3 and C4 species as a function of

aridity along a 3200 km aridity gradient across the arid and semi-arid grasslands of

northern China.

Results Aridity was positively correlated with plant $\delta^{15}N$ values in both C3 and C4

plants and also in the entire plant community, whereas soil bulk $\delta^{15}N$ values increased

first and then decreased along the aridity gradient. The N uptake by C4 plants

appeared to be more affected by competition pressure of neighboring plants and soil

microbes than for C3 plants along the transect.

Conclusions The decoupled relationship between plant and soil $\delta^{15}N$ values indicated

that variations in vegetation and soil $\delta^{15}N$ values were driven by differential

biogeochemical processes, while different soil N sources were used for plant growth

along the climatic gradient. The advantage of C3 plants in the use of N may

counteract the competitive advantage that C4 plants have over C3 plants due to their

higher water use efficiency under drier conditions. These findings can help understand

why C4 plants do not completely replace C3 plants in drier environments despite their

higher water use efficiency.

Keywords

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

Introduction

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

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

Relationships between plant $\delta^{15}N$ values and precipitation are a product of water availability and soil N sources during plant growth (Handley et al. 1999). The general pattern that soil and plant $\delta^{15}N$ 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}N in a

66   more closed N cycle (Amundson et al. 2003; Aranibar et al. 2004). However, the

67   responses of plant δ^{15}N to environmental changes are also dependent on the

68   photosynthetic pathways (Murphy and Bowman 2009). The response of plant δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}N values by delivering ^{15}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 δ^{15}N values than those plants that prefer ammonium (Houlton

82   et al. 2007). Moreover, changes in soil water availability may also alter δ^{15}N values in

83   C3 and C4 plants by changing their rooting depth and N availability with soil depth

84   and thereby the ^{15}N signature of plant N sources (Kahmen et al. 2008), because nitrate

85   and ammonium sources at different soil depths can vary in δ^{15}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 δ^{15}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 δ^{15}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

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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

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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 $\delta^{15}N$

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‰. $\delta^{15}N$ values are expressed in per mil (‰) unit, relative to the

163   atmospheric 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°×0.0083° (approximately 1 km^2 at the

9

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 $\delta^{15}N$

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 $\delta^{13}C$ values (Cerling et al. 1997).

187   Plant community $\delta^{15}N$ values were defined as the overall mean of $\delta^{15}N$ 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 $\delta^{15}N$= (biomass$_1$×$\delta^{15}N_1$+ biomass$_2$×$\delta^{15}N_2$+……biomass$_n$×$\delta^{15}N_n$) /total

192 biomass.

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

194   community $\delta^{15}N$ values to increasing aridity. Binary regression was conducted to

195   analyze the relationship between aridity and soil $\delta^{15}N$ values. Then, OLS regression

196   was also used to examine the correlation between mean community and soil $\delta^{15}N$

197   values. To further analyze patterns of plant $\delta^{15}N$ values, OLS regression was applied

198   to test the relationships of plant $\delta^{15}N$ 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     $\delta^{15}N$ 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 $\delta^{15}N$

203   value in this plant type in response to aridity.

204   To examine the underlying mechanism under the different responses of $\delta^{15}N$ 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     $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values (Table 1). Soil $\delta^{15}N$ values increased first and then

225   reduced with increasing aridity (Figure S1) and was nonlinearly associated with plant

226   community $\delta^{15}N$ values (Figure 3). Removing three sites with soil $\delta^{15}N$ values >10 ‰

227   (outliers) did not change the results (see the insets in Figures 3 and S1).

228   Plant $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$

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 $\delta^{15}N$ values was significantly

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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 $\delta^{15}N$ values in both C3 (Figure 5a) and C4 plants (Figure 5b). Aridity

245   indirectly affected plant $\delta^{15}N$ 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 $\delta^{15}N$ values in C4 plants, respectively (Figure 5b). Aridity

249   indirectly affected plant $\delta^{15}N$ values via the negative effects on soil total N and MBN

250   concentrations for C4 plants (Figure 5b).

251

Discussion

N stable isotopes in plants along the aridity gradient

Consistent with our hypothesis, plant $\delta^{15}N$ values of both the C3 and C4 functional

groups and the entire community increased towards the dry end of the climatic

gradient across northern China's grasslands, where N and water availability are two of

the most constraining factors limiting plant growth and microbial activity. This

finding is similar to that reported in other continents (Aranibar et al. 2008; Austin and

Sala 1999; Heaton 1987; Swap et al. 2004) and in an extensive global synthesis

(McCulley et al. 2009; Murphy and Bowman 2009; Schulze et al. 1998) conducted at

the level of species rather than the entire plant community. Plant $\delta^{15}N$ values also

increased with aridity in our study in the entire plant community, similar to individual

species, which was due to the substitution of plant species/functional groups with

lower $\delta^{15}N$ by those with higher $\delta^{15}N$ when aridity rose along the climatic gradient

(see Table 1). Higher plant $\delta^{15}N$ values indicate a lower capacity of plants to retain N,

because the lighter ^{14}N isotope is more easily cycled and lost (Dalal et al. 2013;

McCulley et al. 2009). Plant species that are less efficient in retaining N are favored

when aridity increases along the climatic gradient. This result suggests that water

conservative mechanisms constitute a trade off with the capacity to retain and use N.

This trade-off has been observed and commented in previous studies (Dijkstra et al.

2016). Thus, the biological response to drier conditions when water limitation is the

main driver of adaptive responses provokes a decrease in the capacity to retain N in

the plant community.

274   The increased plant $\delta^{15}N$ 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 $\delta^{15}N$ rather than plant $\delta^{15}N$ 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 $\delta^{15}N$ values

279   increased with increasing aridity along an elevational/climatic gradient in northern

280   Chile, as expected, whereas plant $\delta^{15}N$ values had a hump-shaped relationship with

281   increasing aridity. The decoupled relationship between the $\delta^{15}N$ values of plants and

282   soils indicates the differences in biogeochemical processes underlying N dynamics

283   between vegetation and soil. An increase in plant $\delta^{15}N$ values with aridity,

284   independent of soil bulk $\delta^{15}N$ 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    $\delta^{15}N$ values than species that prefer ammonium (Houlton et al. 2007). In our previous

288   study along the same climatic gradient, we found that $\delta^{15}N$ values of ammonium

289   consistently increased, while $\delta^{15}N$ 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 $\delta^{15}N$ values and a decoupled relationship between plant and soil bulk

293    $\delta^{15}N$ 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 $\delta^{15}N$ values from drier to wetter sites in tropical forest ecosystems.

15

N isotope and N use in C4 versus C3 plants

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

This advantage of C3 plants in the use of N may improve their competitive ability 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 $\delta^{15}N$ values of both the C3

333 and C4 functional groups and the entire community increased towards drier

334 conditions due to an increase in $\delta^{15}N$ of each individual species, and also to an

335 increased dominance of species with higher $\delta^{15}N$ values. Along the aridity gradient,

336 plant $\delta^{15}N$ values did not covary with soil bulk $\delta^{15}N$ 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 $\delta^{15}N$

339 patterns should be carefully applied to plant $\delta^{15}N$ patterns in arid and semiarid

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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 be C4

345 plants in drier and warmer conditions.

346 **Acknowledgments**

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References

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A,

Brenner D, Baisden WT (2003) Global patterns of the isotopic composition of

soil and plant nitrogen. Glob Biogeochem Cy 17: 1031.

doi:10.1029/2002GB001903.

Aranibar JN, Anderson IC, Epstein HE, Feral CJW, Swap RJ, Ramontsho J, Macko

SA (2008) Nitrogen isotope composition of soils, C3 and C4 plants along land

use gradients in southern Africa. J Arid Environ 72: 326-337.

Aranibar JN, Otter L, Macko SA, Feral CJW, Epstein HE, Dowty PR, Eckardt F,

Shugart HH, Swap RJ (2004) Nitrogen cycling in the soil-plant system along a

precipitation gradient in the Kalahari sands. Glob Chang Biol 10: 359-373.

Austin AT, Sala OE (1999) Foliar δ^{15}N is negatively correlated with rainfall along the

IGBP transect in Australia. Aust J Plant Physiol 26: 293-295.

Bai Y, Han X, Wu J, Chen Z, Li L (2004) Ecosystem stability and compensatory

effects in the Inner Mongolia grassland. Nature 431: 181-184.

Brown R (1978) A difference in N use efficiency in C3 and C4 plants and its

implications in adaptation and evolution. Crop Sci 18: 93-98.

Cai J, Weiner J, Wang R, Luo W, Zhang Y, Liu H, Xu Z, Li H, Zhang Y, Jiang Y

(2017) Effects of nitrogen and water addition on trace element stoichiometry

in five grassland species. J Plant Res 4: 659-668.

Cerling TE, Harris JM, MacFadden BJ, Leakey MG, Quade J, Eisenmann V,

Ehleringer JR (1997) Global vegetation change through the Miocene/Pliocene

382   boundary. Nature 389: 153-158.

383 Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E,

384   Wang L (2015) Ecological interpretations of nitrogen isotope ratios of

385   terrestrial plants and soils. Plant Soil 396: 1-26.

386 Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA,

387   Kahmen A, Mack MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo

388   LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA,

389   Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and

390   their relationships with climate, mycorrhizal fungi, foliar nutrient

391   concentrations, and nitrogen availability. New Phytol 183: 980-992.

392 Cramer MD, Hawkins HJ, Verboom GA (2009) The importance of nutritional

393   regulation of plant water flux. Oecologia 161: 15-24.

394 Dalal RC, Strong WM, Cooper JE, King AJ (2013) Relationship between water use

395   and nitrogen use efficiency discerned by ^{13}C discrimination and ^{15}N isotope

396   ratio in bread wheat grown under no-till. Soil Till Res 128: 110-118.

397 Díaz FP, Frugone M, Gutiérrez RA, Latorre C (2016) Nitrogen cycling in an extreme

398   hyperarid environment inferred from δ^{15}N analyses of plants, soils and

399   herbivore diet. Sci Rep 6, 22226. doi: 10.1038/srep22226

400 Dijkstra FA, Carrillo Y, Aspinwall MJ, Maier C, Canarini A, Tahaei H, Choat B,

401   Tissue DT (2016) Water, nitrogen and phosphorus use efficiencies of four tree

402   species in response to variable water and nutrient supply. Plant Soil 406: 187-

403   199.

404   Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope

405   composition. Trends Plant Sci 6: 121-126.

406   Handley LL, Austin AT, Robinson D, Scrimgeour CM, Raven JA, Heaton THE,

407   Schmidt S, Stewart GR (1999) The ^{15}N natural abundance (δ^{15}N) of ecosystem

408   samples reflects measures of water availability. Aust J Plant Physiol 26: 185-

409   199.

410   Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by

411   coexisting plant species and soil microbes. Ecology 88: 989-999.

412   Hartman G, Danin A (2010) Isotopic values of plants in relation to water availability

413   in the Eastern Mediterranean region. Oecologia 162: 837-852.

414   Heaton TH (1987) The ^{15}N/^{14}N ratios of plants in South Africa and Namibia:

415   relationship to climate and coastal/saline environments. Oecologia 74: 236-

416   246.

417   Hilbig W (1995) Vegetation of Mongolia. SPB Academic Pubishing.

418   Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal

419   fungi correlate with nitrogen isotope patterns in plants. New Phytol 157: 115-

420   126.

421   Hobbie EA, Högberg P (2012) Nitrogen isotopes link mycorrhizal fungi and plants to

422   nitrogen dynamics. New Phytol 196: 367-382.

423   Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar δ^{15}N and

424   nitrogen concentrations may indicate plant-mycorrhizal interactions.

425   Oecologia 122: 273-283.

426   Hobbie EA, Ouimette AP (2009) Controls of nitrogen isotope patterns in soil profiles.

427   Biogeochemistry 95: 355-371.

428   Houlton BZ, Sigman DM, Schuur EA, Hedin LO (2007) A climate-driven switch in

429   plant nitrogen acquisition within tropical forest communities. P Natl Acade Sci

430   USA 104: 8902-8906.

431   Kahmen A, Wanek W, Buchmann N (2008) Foliar δ^{15}N values characterize soil N

432   cycling and reflect nitrate or ammonium preference of plants along a

433   temperate grassland gradient. Oecologia 156: 861-870.

434   Kichenin E, Wardle DA, Peltzer DA, Morse CW, Freschet GT (2013) Contrasting

435   effects of plant inter- and intraspecific variation on community-level trait

436   measures along an environmental gradient. Funct Ecol 27: 1254-1261.

437   Kolb K, Evans R (2002) Implications of leaf nitrogen recycling on the nitrogen

438   isotope composition of deciduous plant tissues. New Phytol 156: 57-64.

439   Liu D, Zhu W, Wang X, Pan Y, Wang C, Xi D, Bai E, Wang Y, Han X, Fang Y (2017)

440   Abiotic versus biotic controls on soil nitrogen cycling in drylands along a

441   3200km transect. Biogeosciences 14: 989-1001.

442   Liu Q, Qiao N, Xu X, Xin X, Han JY, Tian Y, Ouyang H, Kuzyakov Y (2016)

443   Nitrogen acquisition by plants and microorganisms in a temperate grassland.

444   Sci Rep 6: 22642. doi: 10.1038/srep22642.

445   Luo W, Elser JJ, Lü XT, Wang Z, Bai E, Yan C, Wang C, Li MH, Zimmermann NE,

446   Han X (2015) Plant nutrients do not covary with soil nutrients under changing

447   climatic conditions. Glob Biogeochem Cy 29: 1298-1308.

448   Luo W, Sardans J, Dijkstra FA, Peñuelas J, Lü XT, Wu H, Li MH, Bai E, Wang Z,

449   Han X, Jiang Y (2016) Thresholds in decoupled soil-plant elements under

450   changing climatic conditions. Plant Soil 409: 159-173.

451   Mariotte P, Vandenberghe C, Kardol P, Hagedorn F, Buttler A (2013) Subordinate

452   plant species enhance community resistance against drought in semi-natural

453   grasslands. J Ecol 101: 763-773.

454   McCulley RL, Burke IC, Lauenroth WK (2009) Conservation of nitrogen increases

455   with precipitation across a major grassland gradient in the Central Great Plains

456   of North America. Oecologia 159: 571-581.

457   Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ^{15}N natural

458   abundance in heath and forest tundra ecosystems is closely correlated with

459   presence and type of mycorrhizal fungi in roots. Oecologia 115: 406-418.

460   Murphy BP, Bowman D (2009) The carbon and nitrogen isotope composition of

461   Australian grasses in relation to climate. Funct Ecol 23: 1040-1049.

462   Ni J (2003) Plant functional types and climate along a precipitation gradient in

463   temperate grasslands, north-east China and south-east Mongolia. J Arid

464   Environ 53: 501-516.

465   Ogaya R, Peñuelas J (2008) Changes in leaf $\delta^{13}C$ and $\delta^{15}N$ for three Mediterranean

466   tree species in relation to soil water availability. Acta Oecol 34: 331-338.

467   Ouyang S, Tian Y, Liu Q, Zhang L, Wang R, Xu X (2016) Nitrogen competition

468   between three dominant plant species and microbes in a temperate grassland.

469   Plant Soil 408: 121-132.

470   Pardo L, Templer P, Goodale C, Duke S, Groffman P, Adams M, Boeckx P, Boggs J,

471   Campbell J, Colman B (2006) Regional Assessment of N Saturation using

472   Foliar and Root δ^{15}N. Biogeochemistry 80: 143-171.

473   Peri PL, Ladd B, Pepper DA, Bonser SP, Laffan SW, Amelung W (2012) Carbon

474   (δ^{13}C) and nitrogen (δ^{15}N) stable isotope composition in plant and soil in

475   Southern Patagonia's native forests. Glob Chang Biol 18: 311-321.

476   Pyankov VI, Gunin PD, Tsoog S, Black CC (2000) C4 plants in the vegetation of

477   Mongolia: their natural occurrence and geographical distribution in relation to

478   climate. Oecologia 123: 15-31.

479   Robinson D (2001) δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol Evol 16:

480   153-162.

481   Sage RF, Pearcy RW (1987a) The nitrogen use efficiency of C$_3$ and C$_4$ plants I. Leaf

482   nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and

483   *Amaranthus retroflexus* (L.). Plant Physiol 84: 954-958.

484   Sage RF, Pearcy RW (1987b) The nitrogen use efficiency of C$_3$ and C$_4$ plants II. Leaf

485   nitrogen effects on the gas exchange characteristics of *Chenopodium album*

486   (L.) and *Amaranthus retroflexus* (L.). Plant Physiol 84: 959-963.

487   Schulze ED, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker

488   BH (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition

489   of trees along a rainfall gradient in northern Australia. Aust J Plant Physiol 25:

490   413-425.

491   Swap RJ, Aranibar JN, Dowty PR, Gilhooly WP, Macko SA (2004) Natural

492   abundance of ^{13}C and ^{15}N in C_3 and C_4 vegetation of southern Africa: patterns

493   and implications. Glob Chang Biol 10: 350-358.

494 Takebayashi Y, Koba K, Sasaki Y, Fang Y, Yoh M (2010) The natural abundance of

495   ^{15}N in plant and soil-available N indicates a shift of main plant N resources to

496   NO_3^- from NH_4^+ along the N leaching gradient. Rapid Commun Mass Sp 24:

497   1001-1008.

498 Wang C, Wang X, Liu D, Wu H, Lu X, Fang Y, Cheng W, Luo W, Jiang P, Shi J, Yin

499   H, Zhou J, Han X, Bai E (2014) Aridity threshold in controlling ecosystem

500   nitrogen cycling in arid and semi-arid grasslands. Nat Commun 5: 499. doi:

501   10.1038/ncomms5799.

502 Wang R, Tian Y, Ouyang S, Xu X, Xu F, Zhang Y (2016) Nitrogen acquisition

503   strategies used by *Leymus chinensis* and *Stipa grandis* in temperate steppes.

504   Biol Fert Soils 52: 951-961.

505 Watson L, Dallwitz MJ (1992) Grass genera of the world: descriptions, illustrations,

506   identification, and information retrieval; including synonyms, morphology,

507   anatomy, physiology, phytochemistry, cytology, classification, pathogens,

508   world and local distribution, and references. http://delta-

509   intkey.com/grass/www/index.htm.

510 Wittmer MH, Auerswald K, Bai Y, Schaeufele R, Schnyder H (2010) Changes in the

511   abundance of C_3/C_4 species of Inner Mongolia grassland: evidence from

512   isotopic composition of soil and vegetation. Glob Chang Biol 16: 605-616.

513 Wooller MJ, Johnson BJ, Wilkie A, Fogel ML (2005) Stable isotope characteristics

514   across narrow savanna/woodland ecotones in Wolfe Creek Meteorite Crater,

515   Western Australia. Oecologia 145: 100-112.

516   Xue D, Botte J, De Baets B, Accoe F, Nestler A, Taylor P, Van Cleemput O, Berglund

517   M, Boeckx P (2009) Present limitations and future prospects of stable isotope

518   methods for nitrate source identification in surface-and groundwater. Water

519   Res 43: 1159-1170.

520 **Table 1** The most abundant species of each community type. The mean N isotopic signature (δ^{15}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)		
Achnatherum sibiricum	0.69‰	4.10%	*Allium mongolicum*	2.14‰	2.57%	*Nitraria tangutorum*	3.13‰	14.40%
Agropyron cristatum	1.75‰	4.80%	*Allium polyrhizum*	4.17‰	2.90%	*Reaumuria songarica*	1.99‰	31.70%
Allium bidentatum	2.14‰	1.90%	*Allium ramosum*	2.95‰	0.15%	*Salsola passerina*	5.44‰	13.50%
Allium mongolicum	2.88‰	1.10%	*Allium spp.*	5.33‰	0.08%	*Suaeda microphylla*	5.81‰	11.50%
Allium ramosum	2.95‰	0.93%	*Allium tenuissimum*	1.74‰	0.08%	*Calligonum mongolicum*	5.84‰	3.80%
Allium tenuissimum	1.74‰	1.86%	*Artemisia scoparia*	1.86‰	0.46%	*Eragrostis pilosa*	2.59‰	4.80%
Artemisia frigida	2.28‰	3.43%	*Artemisia spp.*	1.24‰	0.15%			
Asparagus cochinchinensis	2.50‰	1.40%	*Cleistogenes spp.*	1.85‰	5.30%			
Carex korshinskii	0.89‰	4.98%	*Cleistogenes squarrosa*	0.46‰	3.60%			
Chenopodium aristatum	1.61‰	3.11%	*Eragrostis pilosa*	2.59‰	5.30%			
Chenopodium glaucum	4.66‰	2.80%	*Reaumuria songarica*	1.99‰	5.36%			
Cleistogenes squarrosa	0.46‰	10.1%	*Kochia prostrata*	5.75‰	3.57%			
Kochia prostrata	5.75‰	2.49%	*Salsola collina*	4.61‰	7.70%			
Leymus chinensis	1.26‰	9.35%	*Calligonum mongolicum*	5.44‰	5.60%			
Potentilla acaulis	0.99‰	0.93%	*Oxytropis aciphylla*	2.28‰	16%			
Potentilla tanacetifolia	0.25‰	1.86%	*Corispermum mongolicum*	5.84‰	2.60%			
Salsola collina	4.61‰	7.17%	*Caragana stenophylla*	0.33‰	2.09%			

Serratula centauroides	0.34‰	7.62%
Stipa spp.	2.27‰	7.62%

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

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

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

523

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 five1 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   ($\delta^{15}N$) along the grassland transect in northern China. Plant community $\delta^{15}N$ values

536   were defined as the overall mean of $\delta^{15}N$ values across all species weighted by the

537   relative contribution of each species to the overall biomass. Aridity was calculated as

538   1-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 $\delta^{15}N$ along the

541   grassland transect in northern China. Plant community $\delta^{15}N$ values were defined as

542   the overall mean of $\delta^{15}N$ values across all species weighted by the relative

543   contribution of each species to the overall biomass. When the three sites with soil

544   $\delta^{15}N$ values higher than 10 ‰ were removed, the non-linear relationship remained

545   between plant and soil $\delta^{15}N$ values.

546   **Figure 4** Correlation between aridity and N isotopic signature (δ^{15}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 (δ^{15}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-

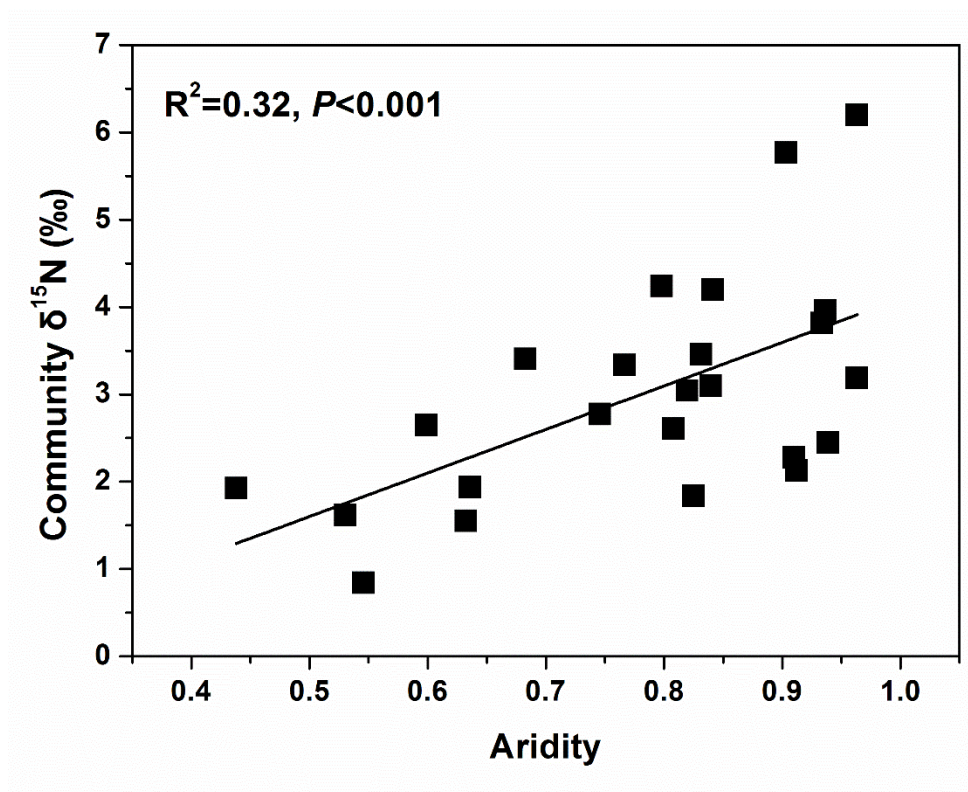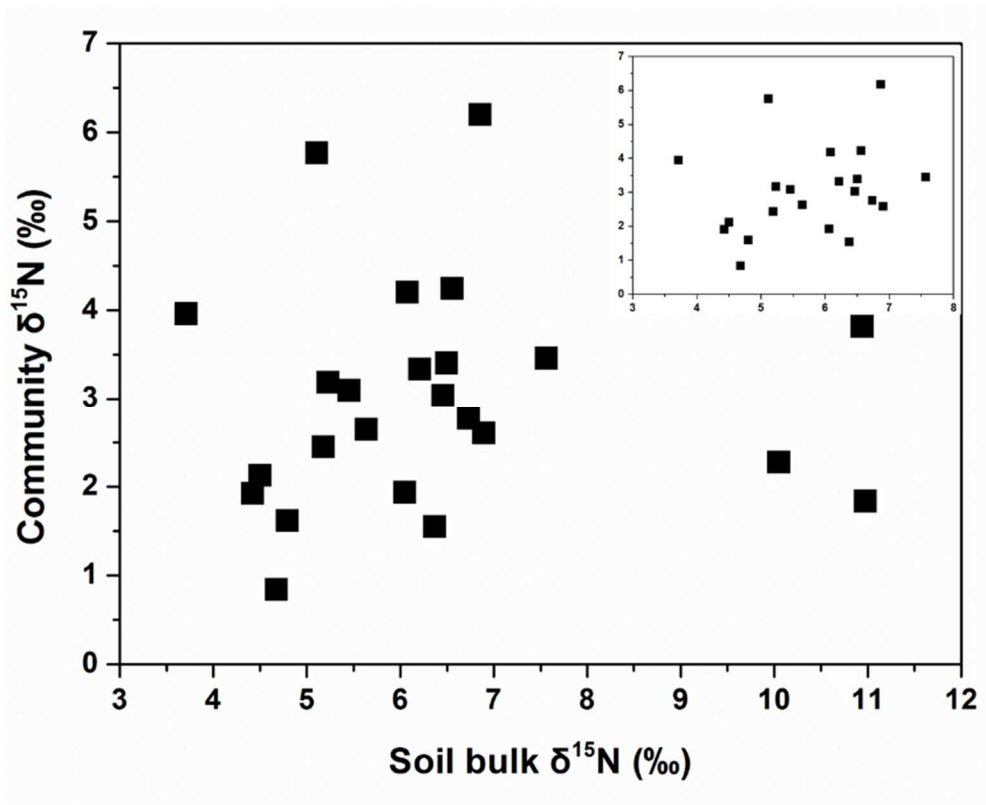558   AI, where AI, the ratio of precipitation to potential evapotranspiration, is the aridity

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Desert grassland   Meadow grassland   Meadow grassland
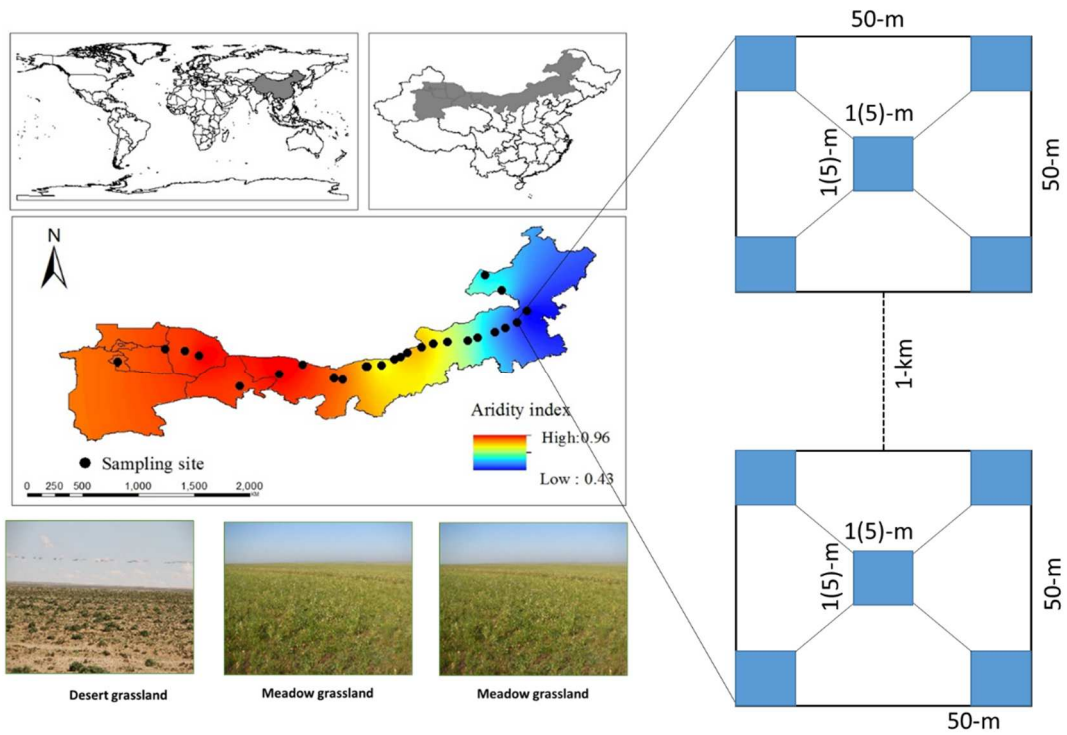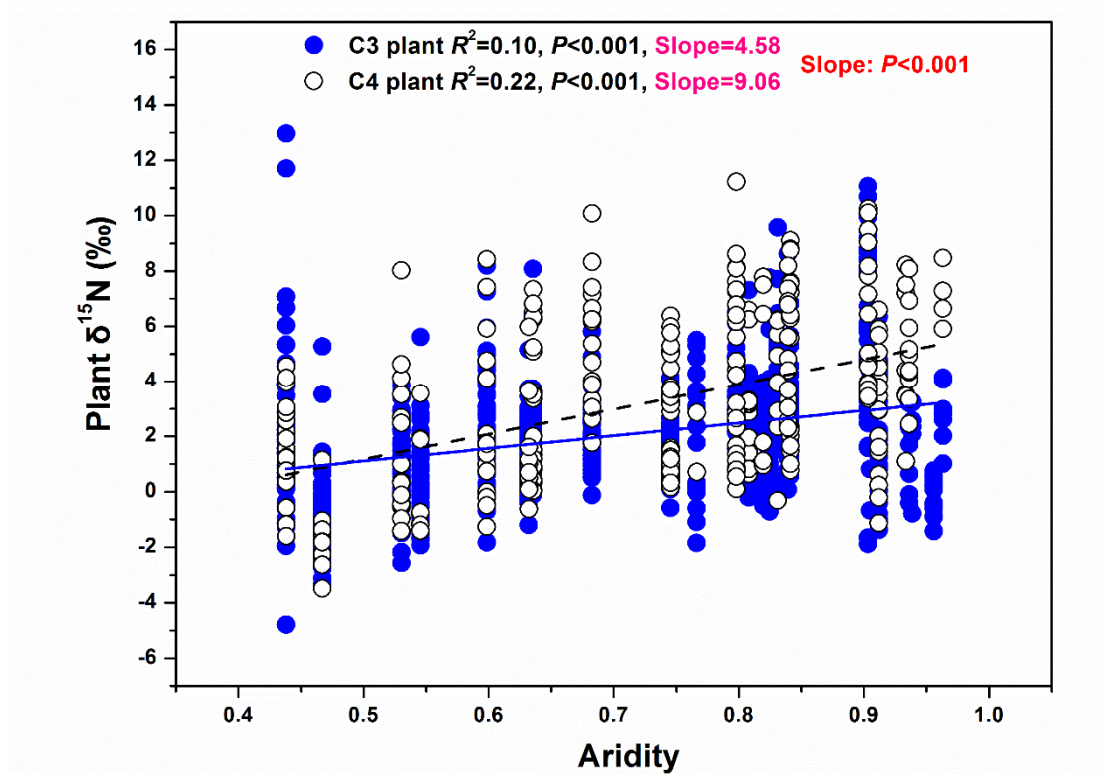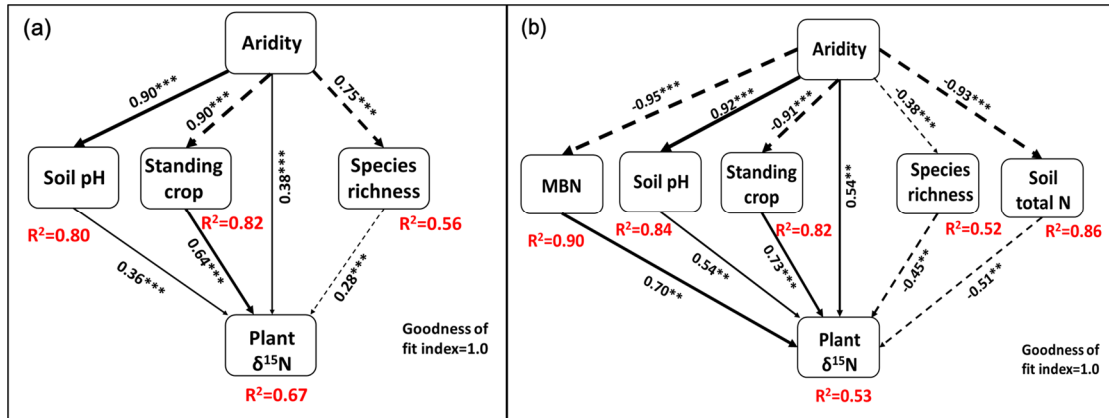
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561 **Figure 1**

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Figure 2

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Figure 3

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570 **Figure 4**

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Figure 5