Higher capability of C3 than C4 plants to use nitrogen inferred from nitrogen 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 $\delta^{15}N$ to environmental changes are also dependent on the
68 photosynthetic pathways (Murphy and Bowman 2009). The response of plant $\delta^{15}N$
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 $\delta^{15}N$
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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values than those plants that prefer ammonium (Houlton
82 et al. 2007). Moreover, changes in soil water availability may also alter $\delta^{15}N$ values in

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

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

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

Material and Methods

Study sites

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

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

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

Sampling and measurement

At each site, two 50 m × 50 m main plots were established and five 1 m × 1 m sampling subplots (or 5 m × 5 m sampling subplots in site dominated by low shrub) were placed within each main plot at the four corners and the center (Figure 1). Plant species presence were measured in each subplots, and from these data species richness (number of plant species per subplot) were calculated. Standing crop was estimated from the dry biomass of the aboveground living parts. Aboveground biomass was sampled by clipping all plants at ground level within each sampling subplot. All living plants were sorted to species and then stored in paper bags. Plant 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 $\delta^{15}N$
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

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

Data analysis

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

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

Community $\delta^{15}N = (biomass_1 \times \delta^{15}N_1 + biomass_2 \times \delta^{15}N_2 + \ldots\ldots biomass_n \times \delta^{15}N_n)$ /total biomass.

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

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

Results

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

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

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

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

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.

The increased plant $\delta^{15}N$ values with aridity, however, do not agree with our previous study along the same gradient, which found that the ecosystem N-cycles, based on soil bulk $\delta^{15}N$ rather than plant $\delta^{15}N$ signals, were more closed at the two ends of the aridity gradient and more open in the middle of the aridity gradient (Wang et al. 2014). Similarly, Díaz et al. (2016) recently reported that soil $\delta^{15}N$ values increased with increasing aridity along an elevational/climatic gradient in northern Chile, as expected, whereas plant $\delta^{15}N$ values had a hump-shaped relationship with increasing aridity. The decoupled relationship between the $\delta^{15}N$ values of plants and soils indicates the differences in biogeochemical processes underlying N dynamics between vegetation and soil. An increase in plant $\delta^{15}N$ values with aridity, independent of soil bulk $\delta^{15}N$ values, may be caused by changes in the uptake of nitrate versus ammonium along the aridity gradient (Houlton et al. 2007; Takebayashi et al. 2010). Species that prefer nitrate relative to ammonium generally have lower $\delta^{15}N$ values than species that prefer ammonium (Houlton et al. 2007). In our previous study along the same climatic gradient, we found that $\delta^{15}N$ values of ammonium consistently increased, while $\delta^{15}N$ values of nitrate increased first and then decreased with increasing aridity (see Liu et al. 2017). These results suggest a shift of dominant inorganic N sources for plants with aridity, resulting in a positive relationship between aridity and plant $\delta^{15}N$ values and a decoupled relationship between plant and soil bulk $\delta^{15}N$ values. Similarly, Houlton et al. (2007) demonstrated that increased aridity resulted in a switch in the dominant N source for plant growth, leading to a reduction in plant $\delta^{15}N$ 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 $\delta^{15}N$ values to environmental
298 changes differed between the coexisting C3 and C4 photosynthetic pathways (Figures
299 5 and 6). The $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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

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

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520 **Table 1** The most abundant species of each community type. The mean N isotopic signature ($\delta^{15}N$) 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

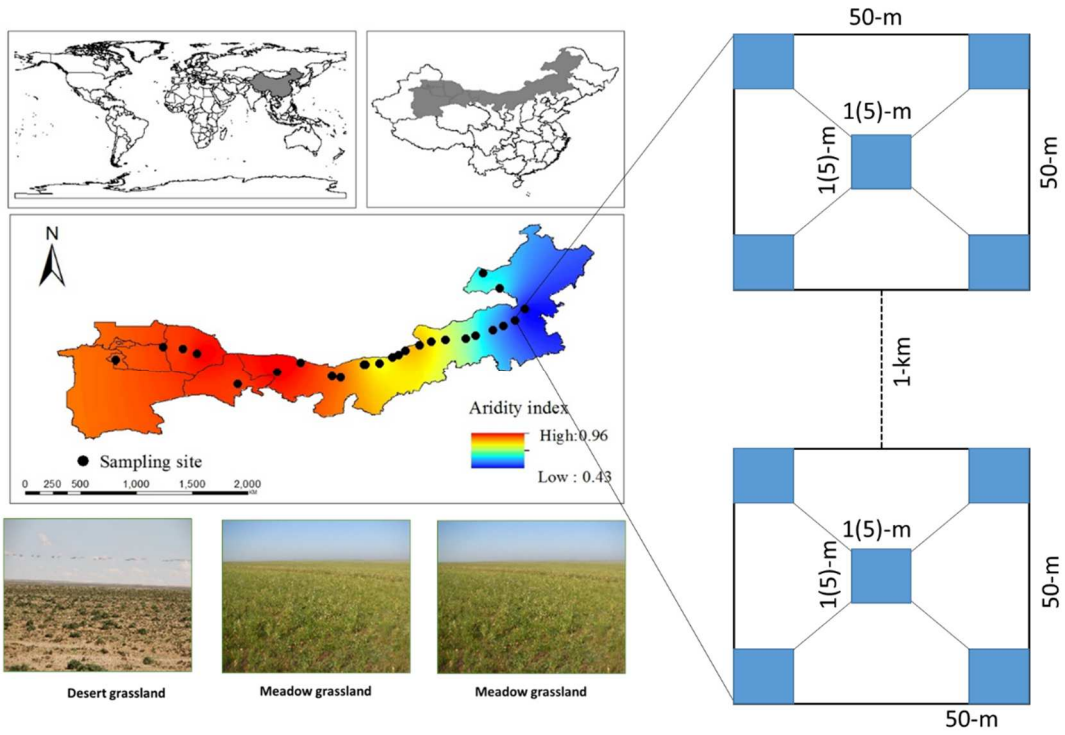
524 **Figure captions**

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 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 ($\delta^{15}N$) 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 ($\delta^{15}N$) 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
559



560

561 **Figure 1**

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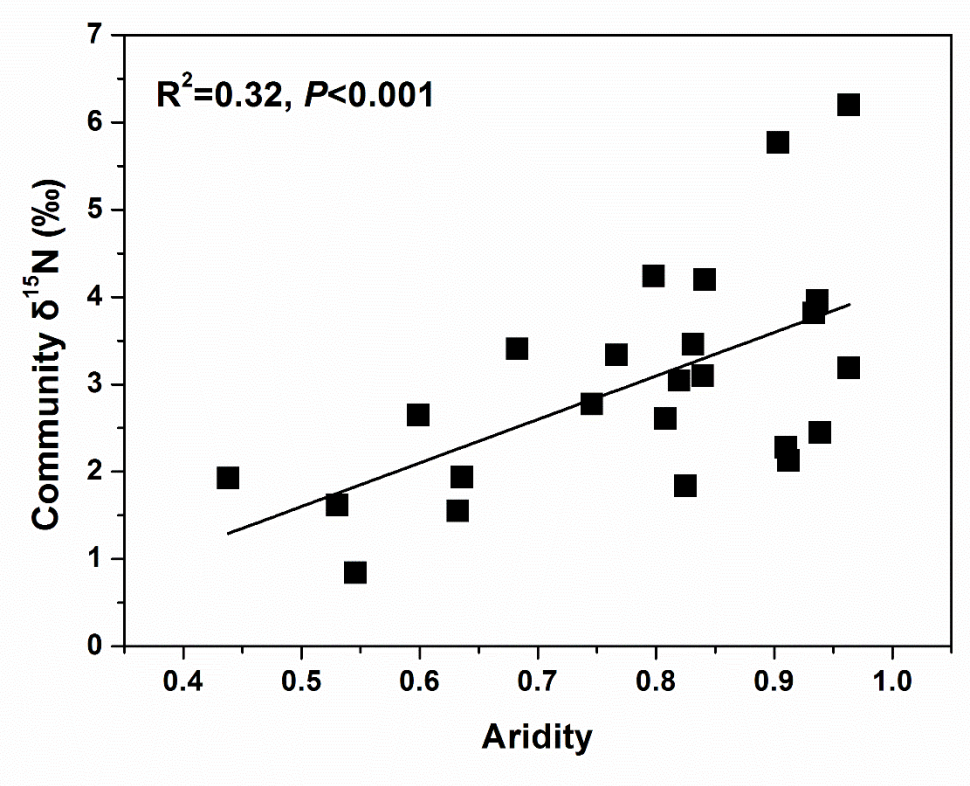

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564 **Figure 2**

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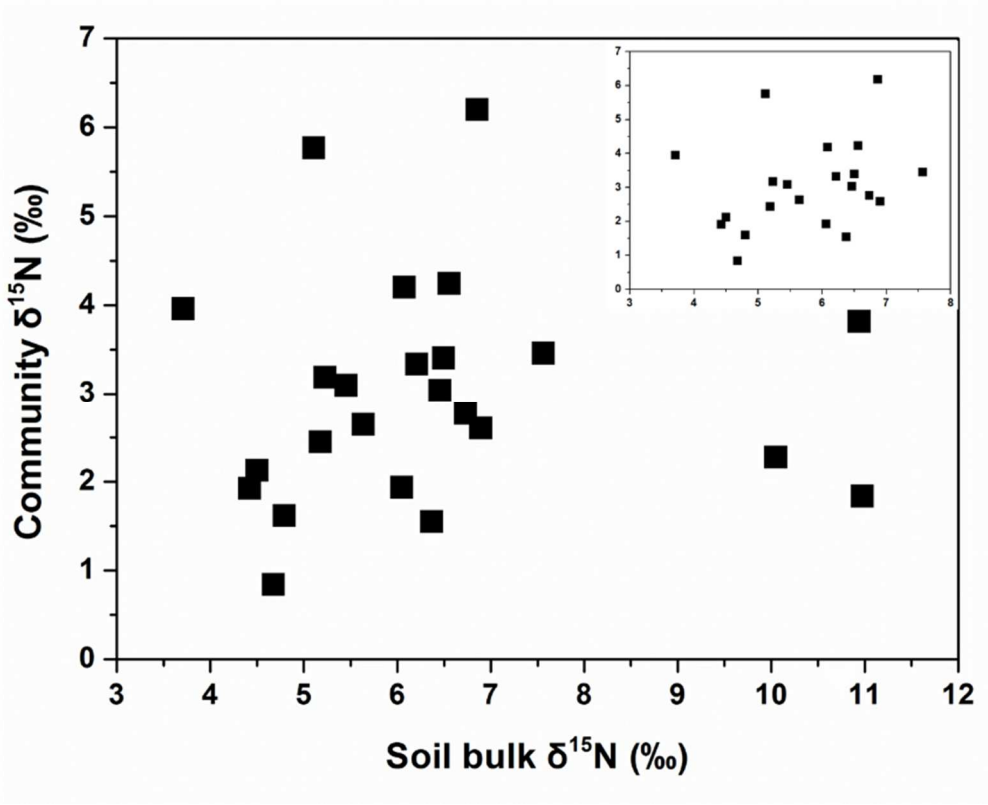

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567 **Figure 3**

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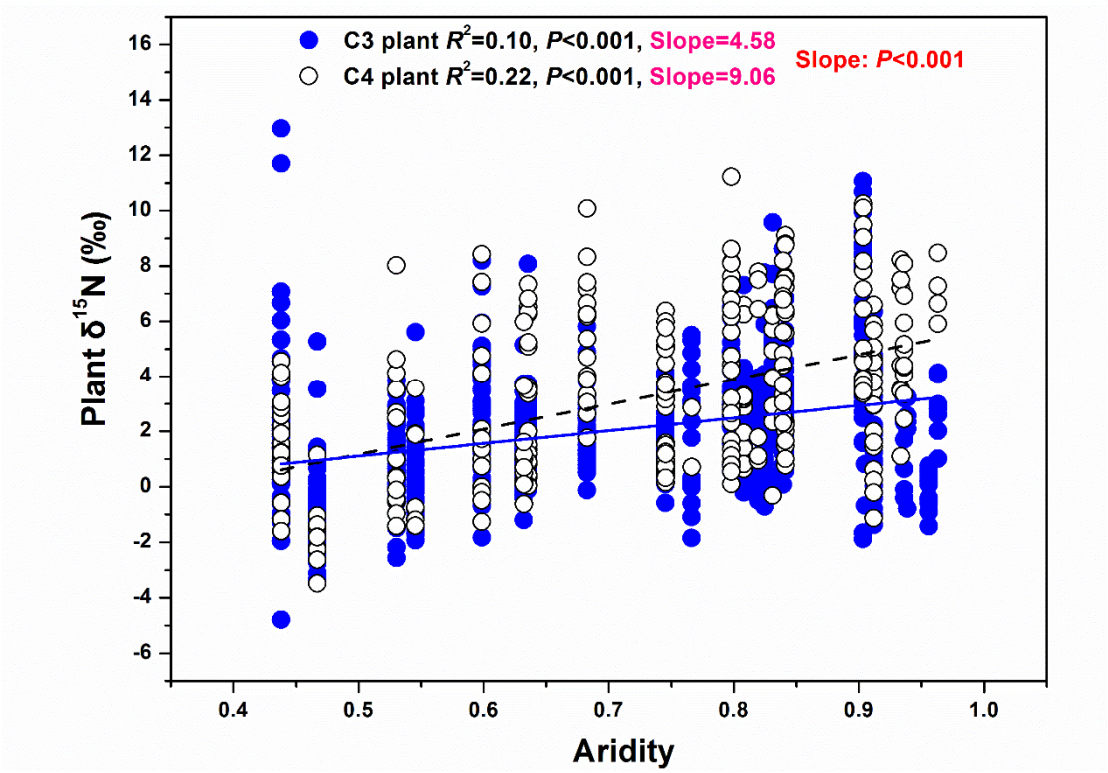

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570 **Figure 4**

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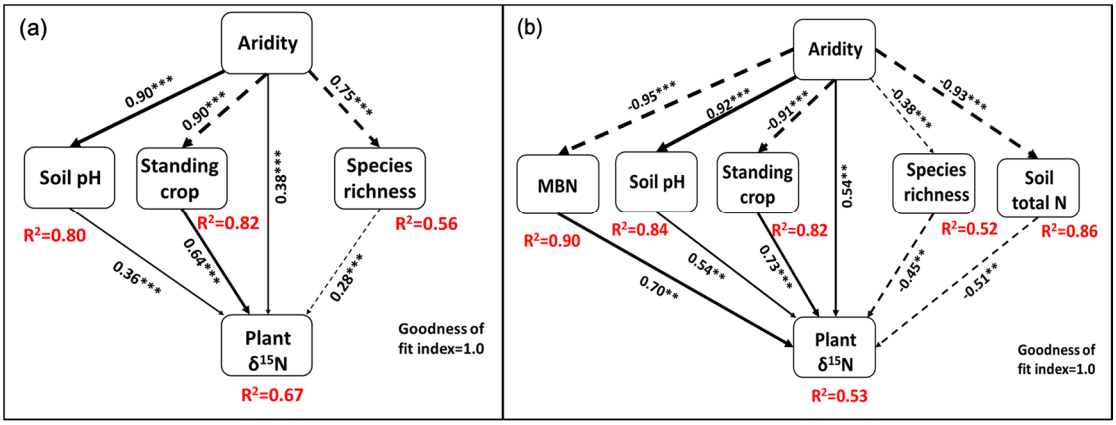

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573 **Figure 5**