Optimal balance of water use efficiency and leaf construction cost with a link to the drought threshold of the desert steppe ecotone in northern China

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INTRODUCTION

As aridity increases, drought-resistant plants (hereafter arid plants) tend to have a higher nitrogen content per leaf area (Narea, a ratio of mass-based nitrogen to specific leaf area, Nmass/SLA) (Cunningham et al., 1999; Wright et al., 2005; Cornwell et al., 2007), which can increase the water-use efficiency (WUE) of photosynthesis (Smith et al., 1997; Wright et al., 2001, 2003). While the positive relationship between Nmass and SLA generally exists across species and sites (Reich et al., 1997; Wright et al., 2004), higher Narea (i.e. higher Nmass at a given SLA, and vice versa) in species from low-rainfall areas could be achieved by higher Nmass or lower SLA, or both (Wright et al., 2001, 2003). Such a strategy shift in the SLA–Nmass relationship exists within the widespread species Artemisia ordosica along a rainfall gradient in northern China (Wei et al., 2011). Along a gradient of water availability, variations in leaf traits may arise from changes in leaf-level anatomical structure (Smith et al., 1997) and/or canopy foliage turnover and nitrogen allocation (Field, 1983; Farquhar et al., 2002) to maximize water- and nitrogen-use efficiencies. At the leaf level, the ultimate evolution of leaf form for arid plants tends towards a more cylindrical leaf with low SLA (i.e. high leaf thickness), which maximizes WUE by increasing the overlap area of light and CO₂ inside the leaf with few changes in the mesophyll conductance (Smith et al., 1997). Increased leaf thickness and decreased SLA associated with decreasing rainfall have been observed in previous studies (Wright et al., 2011). At the stand level, the theoretical model for the simultaneous optimization of water- and nitrogen-use efficiencies of photosynthesis suggests that at a given total amount of canopy foliage N-pool, leaf area index (LAI) generally decreases as water becomes less available, resulting in a concomitant increase in Narea (Narea = N-pool/LAI) (Farquhar et al., 2002). Thus, in response to decreased rainfall, higher Narea within a widespread species may result from reduced LAI with unchanged foliage N-pool and SLA (Field, 1983; Farquhar et al., 2002; Wei et al., 2011), or from increased leaf thickness (i.e. lower SLA, Smith et al., 1997; Poorter et al., 2009) when the reduction of LAI can no longer compensate for soil water deficiency at low-rainfall sites. This suggests that maximizing Narea may be a key process.

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in shaping arid species’ distribution and ecosystem function, and, if so, a drought threshold would exist associated with the switch change from allocating canopy leaf nitrogen to altering leaf-level anatomical structure along a rainfall gradient. However, little research has combined both theories to understand the intraspecific continuous variations in leaf traits and stand variables with rainfall. It is still unclear whether a drought threshold exists that causes a shift in controls on \( N_{\text{area}} \) across populations of an arid-adapted, widespread species along a large rainfall gradient. Such knowledge would help to understand the response of arid plants to climate change and to explore a simple predictor of arid vegetation zonation.

Leaf construction cost (CC) is defined as the amount of glucose required for constructing a unit leaf mass or leaf area (Williams et al., 1987). A leaf with low SLA or high \( N_{\text{mass}} \) generally has a high content of lignin or protein to resist environmental stress (Gower et al., 1989; Groeneveld et al., 1998; Zhang et al., 2012). These compounds (lignin and protein) are expensive to produce (Williams et al., 1987; Nagel and Griffin, 2001). To maximize \( N_{\text{area}} \) for high WUE, the induction of a low SLA and/or high \( N_{\text{mass}} \) by drought may also increase CC (Penning de Vries et al., 1974; Williams et al., 1987; Griffin, 1994; Nagel and Griffin, 2001; Nagel et al., 2002; Chen et al., 2006; Zhang et al., 2012). Higher CC is usually associated with lower energy-use efficiency and growth rate (Griffin, 1994; Poorter and Villar, 1997; Baruch and Goldstein, 1999; Nagel et al., 2004; Song et al., 2007), which may hinder plant survival and competition with other species in arid environments. It has been suggested that arid plants have to balance the costs of carbon gain and water transport along a rainfall gradient by altering their leaf traits (Wright et al., 2003; Prentice et al., 2014). The regulation of \( N_{\text{area}} \) along a rainfall gradient should be a process to balance WUE and CC, although it is still unknown how maximizing \( N_{\text{area}} \) could achieve this in arid-adapted species.

In arid and semi-arid sandy lands in northern China (1200–1800 m) and in the southeast Qaidam Basin of Qinghai (3200–3300 m), the deciduous sub-shrub \( A. \) ordosica is widely distributed in mild and moderately disturbed (fixed and semi-fixed, respectively) sandy lands across a broad range of annual rainfall (150–400 mm). The Mu Us Sandy Land is the distribution centre of \( A. \) ordosica, where mean air temperature and soil texture are similar across areas with differing rainfall (Wei et al., 2011). Moreover, there is no significant genetic differentiation among \( A. \) ordosica populations from divergent geographical zones (Wang et al., 2004). Such a species distribution pattern provides an ideal system for identifying the drought threshold and related mechanisms of the intraspecific shift in controls on \( N_{\text{area}} \) along a rainfall gradient. In this study, leaf traits (SLA, \( N_{\text{mass}}, N_{\text{area}}, \text{CC}_{\text{mass}}, \text{CC}_{\text{area}}, \delta^{13}\text{C} \)) and related stand variables (LAI and foliage N-pool) within populations of \( A. \) ordosica were measured across 17 study sites in the Mu Us Sandy Land and its neighbouring areas with annual rainfall ranging from 150 to 370 mm. Our aim was to test the hypothesis that below a climatic drought threshold, arid plants tend to maximize their intrinsic WUE (i.e. high leaf \( \delta^{13}\text{C} \), Farquhar et al., 1989) through increased \( N_{\text{area}} \) at a given \( \text{CC}_{\text{area}} \). We investigated: (1) whether there is a drought threshold determining the significant shift in SLA–\( N_{\text{mass}} \) relationships and, if so, whether this drought threshold also determines the turning point in leaf traits and stand variables along the rainfall gradient; (2) if the positive relationship between \( N_{\text{area}} \) and \( \text{CC}_{\text{area}} \) also shifts between low- and high-rainfall groups consistent with the pattern found in the SLA–\( N_{\text{mass}} \) relationship, and whether the low-rainfall group has higher \( N_{\text{area}} \) and \( \delta^{13}\text{C} \) at a given \( \text{CC}_{\text{area}} \) compared with the high-rainfall group; and (3) if the drought threshold identified by leaf-trait data could indicate the zonal boundary between typical temperate and desert steppes in northern China. Furthermore, we investigated the generality of the low-altitude data from northern China using the high-altitude data from the south-east Qaidam Basin of Qinghai.

**MATERIALS AND METHODS**

**Study sites**

\( A. \) ordosica is adapted to fixed and semi-fixed sandy land habitats across typical temperate steppes, desert steppes and semi-deserts in northern China, where annual rainfall ranges from 150 to 400 mm (Fig. 1) (Cui, 1991; Wang et al., 2002). \( A. \) ordosica is a dominant species that forms a relatively stable community in sub-climax state in the mild and moderately degraded Mu Us Sandy Land and its neighboring areas that are characterized by arid and infertile soils. Soil textures at 0–50 cm depth are similar across different rainfall areas, with sand contents of >96 % and clay contents of <4 % across fixed and semi-fixed sandy lands (Duan and Liu, 1995; Chen et al., 1998; Wang, 2006; Li, 2007). We selected our study sites by overlapping the geographical distribution of \( A. \) ordosica with the map of annual rainfall isolines in northern China. Along a geographical transect from east to west in the Mu Us Sandy Land and its neighbouring areas, we selected 17 study sites (four of which are presented in Wei et al., 2011) to represent 17 different rainfall areas (Fig. 1). Locations and altitudes of the 17 study sites were recorded by a global positioning system, with latitudes of 37°27′40″–39°43′57″N, longitudes of 102°46′33″–109°52′06″E and altitudes of 1210–1783 m (Table 1).

Daily meteorological data (1985–2010) for 142 meteorological stations in northern China were obtained from China’s National Meteorological Bureau. The meteorological data included atmospheric pressure (Pa), vapour pressure (Pa), mean air temperature (°C), maximum air temperature (°C), minimum air temperature (°C), mean relative humidity (%), sunshine duration (h), wind speed (m s\(^{-1}\)) and rainfall (mm). Annual potential evapotranspiration for the 142 meteorological stations were calculated with the Penman–Montieth equation (Allen et al., 1998). Moisture index (MI) was then calculated as the ratio of annual rainfall to annual potential evapotranspiration. The isolate maps of annual mean temperature, rainfall, potential evapotranspiration and MI were produced with the Krigi spatial interpolation method. The climate data (averaged over 1985–2010) of the 17 study sites were estimated according to the geographical locations. Across the 17 study sites, annual mean temperature was 7.5–9.4 °C, annual rainfall 150–370 mm, annual potential evapotranspiration 867–965 mm and MI 0.17–0.41 (Table 1).
Sampling and measurement of leaf traits

At each study site, leaf and soil samples were collected at both fixed and semi-fixed sandy land habitats, which were identified according to differences in vegetation cover (> 40 vs. 20–40 %, respectively) and soil crust thickness (>1 vs. 0.5–1 cm, respectively) (Wu and Ci, 2002). During July and August when leaves were fully expanded, the outer-canopy leaves of nine healthy A. ordosica individuals were sampled from three 5 × 5-m plots per habitat.

For each plant individual sampled, 50 fresh leaves were scanned with a conventional digital scanner (HP Scanjet 2400, Hewlett Packard Company, Palo Alto, CA, USA) and...
calibrated with a square of known surface area. We determined single-sided leaf area from scanned images using Image Pro Plus 6.0 software (Media Cybernetics Inc, New York, USA). SLA was calculated as the fresh leaf area divided by its dry mass (oven-dried for 48 h at 70°C). Leaf N$_{\text{max}}$ was analysed using the Kjeldahl method (Kjeldahl, 1883) and N$_{\text{area}}$ was calculated as the ratio of N$_{\text{max}}$ to SLA. The $\delta^{13}$C ratio of leaf samples, relative to a Pee Dee Belemnite (PDB) standard, was determined as the ratio of leaf $\delta^{13}$C to PDB $\delta^{13}$C.

### Table 1. Climate and soil factors of study sites across northern China and the south-east Qaidam Basin sandy lands

<table>
<thead>
<tr>
<th>Site number</th>
<th>Place name</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Altitude (m)</th>
<th>MAP (mm)</th>
<th>MAE (mm)</th>
<th>MI</th>
<th>MAT (°C)</th>
<th>Total soil N concentration (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yulin</td>
<td>109°52'06&quot;</td>
<td>38°37'18&quot;</td>
<td>1213</td>
<td>367</td>
<td>902</td>
<td>0.41</td>
<td>8.4</td>
<td>0.55±0.064&lt;sup&gt;AB&lt;/sup&gt; 0.17±0.043&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Ejin Horo</td>
<td>109°46'26&quot;</td>
<td>39°21'17&quot;</td>
<td>1350</td>
<td>342</td>
<td>881</td>
<td>0.39</td>
<td>7.5</td>
<td>0.55±0.170&lt;sup&gt;AB&lt;/sup&gt; 0.18±0.016&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Uxin Henan</td>
<td>108°25'36&quot;</td>
<td>37°47'27&quot;</td>
<td>1307</td>
<td>336</td>
<td>946</td>
<td>0.36</td>
<td>9</td>
<td>0.37±0.172&lt;sup&gt;B&lt;/sup&gt; 1.0±0.014&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Uxin</td>
<td>108°38'36&quot;</td>
<td>38°09'28&quot;</td>
<td>1270</td>
<td>331</td>
<td>930</td>
<td>0.36</td>
<td>8.7</td>
<td>0.40±0.013&lt;sup&gt;AB&lt;/sup&gt; 0.17±0.023&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Otog Qian</td>
<td>108°13'26&quot;</td>
<td>38°11'17&quot;</td>
<td>1380</td>
<td>320</td>
<td>947</td>
<td>0.34</td>
<td>8.7</td>
<td>0.41±0.110&lt;sup&gt;AB&lt;/sup&gt; 0.09±0.053&lt;sup&gt;AC&lt;/sup&gt;</td>
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<td>6</td>
<td>Uxin Ju</td>
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<td>39°05'54&quot;</td>
<td>1295</td>
<td>318</td>
<td>903</td>
<td>0.35</td>
<td>7.9</td>
<td>0.51±0.087&lt;sup&gt;AB&lt;/sup&gt; 0.08±0.014&lt;sup&gt;BC&lt;/sup&gt;</td>
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<tr>
<td>7</td>
<td>Otog Sumitu</td>
<td>108°16'34&quot;</td>
<td>38°44'53&quot;</td>
<td>1361</td>
<td>290</td>
<td>929</td>
<td>0.31</td>
<td>8.1</td>
<td>0.45±0.142&lt;sup&gt;AB&lt;/sup&gt; 0.12±0.068&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>8</td>
<td>Otog</td>
<td>108°03'30&quot;</td>
<td>39°03'37&quot;</td>
<td>1419</td>
<td>265</td>
<td>923</td>
<td>0.29</td>
<td>7.8</td>
<td>0.35±0.099&lt;sup&gt;AB&lt;/sup&gt; 0.21±0.017&lt;sup&gt;AB&lt;/sup&gt;</td>
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<tr>
<td>9</td>
<td>Hangjin</td>
<td>108°23'45&quot;</td>
<td>39°43'57&quot;</td>
<td>1410</td>
<td>258</td>
<td>900</td>
<td>0.29</td>
<td>7.8</td>
<td>0.36±0.029&lt;sup&gt;BC&lt;/sup&gt; 0.18±0.014&lt;sup&gt;BC&lt;/sup&gt;</td>
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<tr>
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<td>Otogqian Damiao</td>
<td>107°13'49&quot;</td>
<td>38°29'46&quot;</td>
<td>1431</td>
<td>250</td>
<td>965</td>
<td>0.26</td>
<td>8.7</td>
<td>0.32±0.036&lt;sup&gt;BCD&lt;/sup&gt; 0.21±0.086&lt;sup&gt;BC&lt;/sup&gt;</td>
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<tr>
<td>11</td>
<td>Shapotou</td>
<td>105°00'28&quot;</td>
<td>37°27'40&quot;</td>
<td>1261</td>
<td>271</td>
<td>917</td>
<td>0.23</td>
<td>9.4</td>
<td>0.17±0.051&lt;sup&gt;AB&lt;/sup&gt; 0.07±0.008&lt;sup&gt;BC&lt;/sup&gt;</td>
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<tr>
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<td>Gulan</td>
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<td>37°33'03&quot;</td>
<td>1783</td>
<td>172</td>
<td>851</td>
<td>0.20</td>
<td>9.1</td>
<td>0.20±0.050&lt;sup&gt;BC&lt;/sup&gt; 0.10±0.036&lt;sup&gt;B&lt;/sup&gt;</td>
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<td>38°12'57&quot;</td>
<td>1444</td>
<td>150</td>
<td>867</td>
<td>0.17</td>
<td>8.8</td>
<td>0.13±0.033&lt;sup&gt;AB&lt;/sup&gt; 0.06±0.008&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>Yulin</td>
<td>109°51'57&quot;</td>
<td>38°37'26&quot;</td>
<td>1210</td>
<td>370</td>
<td>898</td>
<td>0.41</td>
<td>8.6</td>
<td>0.28±0.049&lt;sup&gt;AB&lt;/sup&gt; 0.18±0.075&lt;sup&gt;AB&lt;/sup&gt;</td>
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<tr>
<td>15*</td>
<td>Uxin</td>
<td>108°38'30&quot;</td>
<td>39°08'39&quot;</td>
<td>1270</td>
<td>353</td>
<td>931</td>
<td>0.38</td>
<td>8.7</td>
<td>0.24±0.021&lt;sup&gt;AB&lt;/sup&gt; 0.07±0.016&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>16*</td>
<td>Otogqian Chenghuan</td>
<td>108°27'22&quot;</td>
<td>37°42'17&quot;</td>
<td>1320</td>
<td>310</td>
<td>950</td>
<td>0.33</td>
<td>8.8</td>
<td>0.32±0.052&lt;sup&gt;BCD&lt;/sup&gt; 0.19±0.038&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>17*</td>
<td>Otog</td>
<td>108°03'30&quot;</td>
<td>39°03'38&quot;</td>
<td>1420</td>
<td>264</td>
<td>923</td>
<td>0.29</td>
<td>7.6</td>
<td>0.37±0.039&lt;sup&gt;AB&lt;/sup&gt; 0.19±0.018&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters within a row and a column show significant differences between each site’s sandy land habitats (lowercase) and between study sites (uppercase) at a 0.05 level, respectively. MAP, mean annual precipitation; MAE, mean annual potential evapotranspiration; MAT, mean annual temperature; MI = MAP/MAE, moisture index.

*Data from Wei et al. (2011).

### Table 2. Differences in slopes and intercepts of N$_{\text{max}}$–SLA relationships for A. ordosica among 17 study sites and three rainfall groups in northern China sandy lands

<table>
<thead>
<tr>
<th>Site/group</th>
<th>MI</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>FS</td>
<td>SFS</td>
</tr>
</tbody>
</table>

**Note:** MI = moisture index; Total, in pooled data from FS and SFS; FS, fixed sandy land; SFS, semi-fixed sandy land.
determined by combusting samples in an elemental analyser coupled to a stable isotope mass spectrometer (Flash EA + Delta V, Thermo Fisher Scientific Inc., Waltham, MA, USA). The overall precision of the δ13C analysis was 0.1.

For each leaf sample, the heat of combustion (HC) was measured with an oxygen bomb calorimeter (PARR 1281, Parr Instrument Company, Moline, IL, USA). The HC for each sample was determined in triplicate, with the relative differences among the three measurements being < 2%. The ash concentration (AC) was determined by combustion of 1-g leaf samples in a muffle furnace at 550 °C for 4 h until a white–grey residue remained. Mass-based leaf construction cost (CCmass) was calculated by the formula given by Williams et al. (1987):

\[
CC_{\text{mass}} = \left[ (0.06968 \times HC - 0.065) \left( 1 - AC \right) + 7.5062(kN/14 \times 0067) \right]/E_G
\]

where CCmass = construction cost (g glucose g\(^{-1}\)), HC = heat of combustion (kJ g\(^{-1}\)), AC = total ash content (%), k is the oxidation state of the N source (+5 for nitrate or –3 for ammonium), N = total Kjeldahl nitrogen (g g\(^{-1}\)), and E\(_G\) is a constant of 0.89 (Williams et al., 1987). In this study, we calculated CCmass with k = 5, as nitrate is the principal source of nitrogen that is available to terrestrial plants under most field conditions (Taiz and Zeiger, 1991). CCarea was calculated as the ratio of CCmass to SLA.

Measurement of stand and soil variables

Within each of the 102 plots across the 17 study sites, we measured the crown diameters along the maximum and minimum axes for each A. ordosica individual clump and then calculated the projected area of a crown as the elliptical area. At each study site, 18 individual clumps of A. ordosica with different crown areas were harvested for measurements of foliage dry mass per clump. Allometric regression equations were developed between foliage mass and crown area for each study site. The foliage biomass of A. ordosica within each plot was then estimated according to the allometric equations using the clump-specific crown area measurements. LAI and foliage N-pool were calculated as the foliage biomass multiplied by SLA and Nmass, respectively.

For each plot, two soil samples (0–10 and 20 cm in depth) were collected and analysed for soil total nitrogen concentration (STN) using the Kjeldahl method (Kjeldahl, 1883).

Additional data from the south-east Qaidam Basin

Wei et al. (2011) indicated that the drought-induced shift in the SLA–Nmass relationship was also found in the data from the south-east Qaidam Basin of Qinghai. To test the generality of the CC and δ13C data found in the low-altitude regions of northern China, we also measured these two traits in A. ordosica individuals across two high-altitude sandy lands in Dulan and Qinghai Lake within the south-east Qaidam Basin, using the leaf samples collected by Wei et al. (2011). The methods of leaf sampling and measurements were the same as described above. According to climate data obtained from the...
Dulan and Qinghai Lake stations, the calculated MI was 0.25 in Dulan and 0.68 in Qinghai Lake (Table 1).

Data analysis

One-way analysis of variance (ANOVA) was applied to assess differences in leaf traits and STN between the two sandy land habitats per site and between 17 study sites. If the results of the ANOVA were significant, Tukey’s pair-wise comparisons were made.

A simple linear model ($y = a + bx$) was used for analysing bivariate relationships of leaf traits. Analysis of covariance (ANCOVA) in a general linear model framework was applied to test for differences in the slopes and intercepts of SLA–$N_{\text{mass}}$ relationships from different rainfall areas, in which rainfall served as a grouping variable, $N_{\text{mass}}$ as a dependent variable and SLA as a covariate. We tested first for the homogeneity of slopes and then for the difference in intercepts. Data from different rainfall areas were pooled as a rainfall group if there were not significant differences in slopes and intercepts. In this way, a drought threshold was determined by the significant shift in the SLA–$N_{\text{mass}}$ relationship along the rainfall gradient. Accordingly, differences in slopes and intercepts for relationships of $N_{\text{area}}$–$C_{\text{area}}$ and $\delta^{13}C$–$N_{\text{area}}$ among different rainfall groups (identified by the SLA–$N_{\text{mass}}$ relationship) were further tested with ANCOVA in a general linear model framework.

The relationships of leaf traits (SLA, $N_{\text{mass}}$) and stand variables (LAI, foliage N-pool) with MI below and above the drought threshold were also analysed by a simple linear model ($y = a + bx$).
At each of the 17 study sites in northern China, there were significant differences in STN between the two sandy land habitats. STN increased with increasing MI in fixed sandy lands \( (P < 0.01) \) but varied little in semi-fixed sandy lands \( (P = 0.30) \) (Table 1). To examine the effect of STN on the leaf-trait relationships, data analyses were performed for fixed and semi-fixed sandy land habitats and as well as in pooled data. The drought threshold identified by leaf trait data was then compared with the zonal boundary between typical temperate and desert steppes by overlapping the map of vegetation zonations in Zhang (2007) with the map of the MI isolines in northern China.

The statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA), and all significant differences were taken at \( P < 0.05 \).

**RESULTS**

**Drought threshold indicating a shift in the SLA–\( N_{\text{mass}} \) relationship and its link to stand variables along the rainfall gradient**

Across the two sandy land habitats and in pooled data, there were no differences in individual SLA–\( N_{\text{mass}} \) slopes among the 17 study sites \( (P = 0.40–0.66) \) (Table 2). However, the SLA–\( N_{\text{mass}} \) intercepts showed significant differences between the three rainfall groups \( (P < 0.001) \): high-rainfall areas (rainfall group 1, 310–370 mm; MI = 0.33–0.41), mid-rainfall transition (rainfall group 2, 290 mm; MI = 0.31) and low-rainfall areas (rainfall group 3, 150–265 mm; MI = 0.17–0.29) (Fig. 2, Table 2). In general, the SLA–\( N_{\text{mass}} \) relationship shifted significantly

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**Fig. 4. Variations in SLA (A), \( N_{\text{mass}} \) (C), \( N_{\text{area}} \) (E), \( \delta^{13}C \) (B), \( C_{\text{mass}} \) (D) and \( C_{\text{area}} \) (F) for A. ordosica along an MI gradient in northern China sandy lands. Data analyses were performed on pooled data from fixed and semi-fixed sandy land habitats. (A, C) Grey dashed lines indicate a climatic drought threshold with MI = 0.29, and the relationships of SLA (A) and \( N_{\text{mass}} \) (C) to MI differ below and above the drought threshold; the solid trend lines are for the areas with MI \textless{} 0.29 and the dashed trend lines are for the areas with MI \textgreater{} 0.29. Bars indicate mean \pm s.d.**
between low-rainfall areas and high-rainfall areas with a transition in between (Fig. 2).

Regardless of STN variability, the relationship between foliage N-pool and MI differed below and above the drought threshold. Canopy foliage N-pool increased with increasing MI in areas with MI ≤ 0.29 (P < 0.001) but varied little in areas with MI > 0.29 (P = 0.07–0.89) (Fig. 3A, C, E). However, LAI generally increased with increasing MI in a continuously linear model along the entire rainfall gradient (P < 0.001) (Fig. 3B, D, F). In pooled data, leaf-level SLA and Nmass increased with increasing MI in areas with MI ≤ 0.29 (P < 0.01). In areas with MI > 0.29, Nmass decreased with increasing MI (P < 0.01) while SLA varied little (P = 0.09–0.45) (Fig. 3B, D, F). As a result, Narea (Nmass/SLA or N-pool/LAI) decreased continuously with increasing MI along the entire rainfall gradient (P < 0.001) (Fig. 4E). Because Narea and δ13C were positively correlated with CC and its major components of HC and Nmass (Table 3), δ13C, CCmass and CCarea generally showed a continuously decreasing trend with increasing MI (P < 0.05) (Fig. 4B, D, F). The same patterns were also found in fixed and semi-fixed sandy land habitats (Supplementary Data, Figs S1 and S2, respectively).

**Shifts in relationships of Narea to CCarea and δ13C between low- and high-rainfall areas**

Across the sandy land habitats and in the pooled data, the positive relationship of Narea to CCarea also shifted between low-rainfall areas and high-rainfall areas along the rainfall gradient in northern China (Fig. 5A, C, E and Table 4, test for slopes, P = 0.12–0.45; test for intercepts, P > 0.001). The plants in low-rainfall areas had higher Narea at a given CCarea compared with those in high-rainfall areas (Fig. 5A, C, E). Similar patterns were also found in the south-east Qaidam Basin (Fig. 6A, C, E and Table 4, test for slopes, P = 0.71; test for intercepts, P < 0.05).

There was a positive Narea-δ13C relationship with insignificant differences of slopes and intercepts between low- and high-rainfall areas in northern China (Table 5, test for slopes, P = 0.09–0.62; test for intercepts, P = 0.28–0.96). In contrast to strategy shifts in relationships of SLA–Nmass and Narea–CCarea between low- and high-rainfall areas, there was a continuous positive relationship between Narea and δ13C along the entire rainfall gradient (P < 0.001) (Fig. 5B, D, F). Similar patterns were also found in additional data from the south-east Qaidam Basin (P < 0.05) (Fig. 6B, D, F).

**The drought threshold for the boundary between typical and desert steppes**

The drought threshold where MI = 0.29 identified by leaf-trait data of A. ordosica corresponded well to the zonal boundary between typical and desert steppes in northern China (Fig. 7). The sites from the high-rainfall group were distributed in the typical steppe zone in the east, while the sites from the low-rainfall group were from the desert steppe and semi-desert zones in the west (Fig. 7).

**DISCUSSION**

**Maximizing Narea is a process to balance WUE and CC in arid plants along a rainfall gradient**

To the best of our knowledge, few studies have examined the intraspecific continuous variations in leaf traits and related stand variables along a rainfall gradient. Our data demonstrated that a continuous increase in Narea with decreasing rainfall was achieved by a reduced LAI with unchanged foliage N-pool and SLA (higher Nmass and constant SLA) in high-rainfall areas with MI > 0.29, but by an increased leaf thickness (lower SLA and Nmass) in low-rainfall areas with MI ≤ 0.29 (Figs 2–4). The results indicate a drought threshold where MI = 0.29 determines the shift in controls on Narea associated with the switch change from allocating canopy leaf nitrogen to altering leaf-level anatomical structure along a rainfall gradient, which can be explained by the theories of Farquhar et al. (2002) and Smith et al. (1997). Such a drought threshold is close to the reported aridity threshold in controlling ecosystem nitrogen cycling of temperate grasslands in northern China (MI = 0.32, Wang et al., 2014).

Our transect data further indicated that relationships of Narea–CCarea consistently shifted between low-rainfall areas and high-rainfall areas (Fig. 5), which was confirmed by additional data from the south-east Qaidam Basin (Fig. 6). Because there was a continuous positive relationship between Narea and δ13C and both generally increased with decreasing MI along the entire rainfall gradient, the low-rainfall group had higher Narea and δ13C at a given CCarea compared with the high-rainfall group. Our data supported the hypothesis that below a climatic drought threshold, arid plants tend to maximize their intrinsic WUE.
(\^{13}C) through increased \(N_{\text{area}}\) at a given \(CC_{\text{area}}\). Such ecophysiological mechanisms may explain why \(A.\ ordosica\) can be widely distributed in arid sandy lands and how it forms a relatively stable community in a sub-climax state. Our findings suggest that maximizing \(N_{\text{area}}\) for optimal balance of WUE and CC is a key process in shaping arid species distribution and ecosystem function.

Variations of CC along an environmental gradient may be determined by changes in leaf biochemical composition and leaf morphology (Griffin, 1994). There are still ongoing debates about how CC changes in response to environmental stress (Chapin, 1989; Poorter and De Jong, 1999; Villar and Merino, 2001; Martinez et al., 2002). Several studies have suggested that there is an increase in CC under stress conditions (Penning de Vries et al., 1974; Amthor, 1989), while Merino (1987) found that water availability has no effects on the CC of 30 species in the Mediterranean. In this study, the \(\delta^{13}C\), \(CC_{\text{mass}}\) and \(CC_{\text{area}}\) of \(A.\ ordosica\) continuously increased with decreasing MI along the rainfall gradient. This suggests that maximizing \(N_{\text{area}}\) for high WUE inevitably leads to a high CC, which is consistent with the theoretical model prediction of Prentice et al. (2014), suggesting that altering leaf-level anatomical structure might be more costly than allocating canopy leaf nitrogen.

To further investigate the possible effect of soil texture on the shifts in leaf trait relationships between low- and high-rainfall areas, the literature data on soil texture across 23 sandy land sites located in our study areas were obtained from Li (2007).

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**Fig. 5.** Relationships of \(N_{\text{area}}\) to \(CC_{\text{area}}\) (A, C, E) and \(\delta^{13}C\) (B, D, F) for \(A.\ ordosica\) along a rainfall gradient in northern China sandy lands. The \(N_{\text{area}}\)–\(CC_{\text{area}}\) relationships (A, C, E) shift between high-rainfall areas (rainfall group 1, 310–370 mm; MI, 0.33–0.41) and low-rainfall areas (rainfall group 3, 150–265 mm; MI, 0.17–0.29), contrasting with the continuous relationships of \(N_{\text{area}}–\delta^{13}C\) (B, D, F) along the entire rainfall gradient. Data analyses were performed on pooled data (A, B) and for fixed (C, D) and semi-fixed (E, F) sandy land habitats, respectively. Symbols are as in Fig. 2. ANCOVA statistics are given in Tables 4 and 5.
The data indicated that along a rainfall gradient ranging from 210 to 350 mm, sand and clay contents in fixed and semi-fixed sandy lands varied little with rainfall (sand content: $R^2 = 0.001$, $P = 0.89$; clay content: $R^2 = 0.09$, $P=0.16$). Given a soil water content, soil water potential calculated from the soil water retention curves (Saxton et al., 1986) based on soil texture also showed no significant variation along the rainfall gradient ($R^2 = 0.04$, $P = 0.38$). In contrast, leaf traits in this study varied significantly along the rainfall gradient (Figs S1 and S2). Furthermore, the results of ANOVA indicated that there were no significant differences in soil texture or soil water potential between low-rainfall areas (MI $< 0.29$) and high-rainfall areas (MI $> 0.29$) ($P = 0.12–0.59$), while relationships of SLA–Nmass and Narea–CCarea shifted significantly between the two rainfall areas (Figs 2 and 5). Therefore, the variations in leaf traits were mainly driven by rainfall but not by soil texture.

**Table 4. Differences in slopes and intercepts of CCarea–Narea relationships for A. ordosica between low- and high-rainfall groups across northern China and the south-east Qaidam Basin sandy lands**

<table>
<thead>
<tr>
<th>Group/site</th>
<th>MI</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern China sandy lands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1, 0.33–0.41</td>
<td>Total</td>
<td>33.7a</td>
<td>36.4a</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>35.6a</td>
<td>73.1a</td>
</tr>
<tr>
<td></td>
<td>SFS</td>
<td>61.9a</td>
<td>73.8a</td>
</tr>
<tr>
<td>Group 3, 0.17–0.29</td>
<td>Total</td>
<td>47.6a</td>
<td>46.9a</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>49.9a</td>
<td>1.8b</td>
</tr>
<tr>
<td></td>
<td>SFS</td>
<td>7.4b</td>
<td>$-9.7b$</td>
</tr>
<tr>
<td>South-east Qaidam Basin sandy lands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qinghai Lake, 0.68</td>
<td>Total</td>
<td>40.0a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.8a</td>
<td>96.9a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87.3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dulan, 0.25</td>
<td></td>
<td>32.0a</td>
<td>38.3a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92.6a</td>
<td>$-71.9a$</td>
</tr>
</tbody>
</table>

Data analysis was performed by ANCOVA. Different letters within a column show significant differences between rainfall groups at a 0.05 level. MI, moisture index; Total, in pooled data from FS and SFS; FS, fixed sandy land; SFS, semi-fixed sandy land; Group 1, high-rainfall areas (sites 1–6, 14–16); Group 3, low-rainfall areas (sites 8–13, 17).

**Fig. 6.** The relationships of $N_{\text{area}}$–CCarea (A, C, E) and $N_{\text{area}}$–$^{13}$C (B, D, F) for A. ordosica along a rainfall gradient in the Qaidam Basin sandy lands. Empty circles and dashed trend lines are for the high-rainfall area (Qinghai Lake; rainfall, 401 mm; MI, 0.68); filled circles and solid trend lines are for the low-rainfall area (Dulan; rainfall 207 mm; MI, 0.25).
A new method to link leaf functional traits with arid vegetation zonation

According to the Vegetation Divisions of China (Editorial Committee for Vegetation of China, 1980; Zhang, 2007), the ecotone boundary between typical temperate and desert steppe zones is mainly determined by regional differences in annual rainfall, genus and species indicators, soil types, and dryland cropping systems, based on the realistic distribution map of natural and artificial vegetation. It is difficult to use such complicated indicators to predict the boundary change and to further understand the related mechanisms underlying the boundary formation. It has been demonstrated that leaf traits are useful for predicting ecosystem functions and processes at large scales (Schulze et al., 1994; Luo et al., 2009). Along an environmental gradient, variations in leaf traits affect plant adaptations to abiotic factors and therefore play an important role in determining plant species distribution patterns (Maharjan et al., 2011). There is evidence that leaf lifespan is a simple predictor of evergreen forest zonation in China (Zhang et al., 2010), but few studies have examined the linkage between leaf traits and arid vegetation zonation.

In this study, the leaf-trait data of *A. ordosica* indicated that the optimal balance of WUE and CC exists below a common climatic drought threshold (\(MI_{29}\)). This drought threshold of \(MI_{29} = 0.0\) corresponds well to the zonal boundary between typical and desert steppes in northern China (Fig. 7). As it is easy to measure leaf traits with repeatable sampling along a geographical transect, our findings suggest an operational way to link leaf functional traits with arid vegetation zonation. This is especially important to be able to detect and predict the dynamic vegetation change in arid and semi-arid regions due to climate change.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: Variations in SLA, \(N_{area}\), \(\delta^{13}C\), \(CC_{mass}\) and \(CC_{area}\) for *A. ordosica* in fixed sandy land habitats along an MI gradient in northern China. Figure S2: Variations in SLA, \(N_{mass}\), \(N_{area}\), \(\delta^{13}C\), \(CC_{mass}\) and \(CC_{area}\) for *A. ordosica* in semi-fixed sandy land habitats along an MI gradient in northern China.

**TABLE 5. Differences in slopes and intercepts of \(N_{area}\)–\(\delta^{13}C\) relationships for A. ordosica between rainfall groups in northern China sandy lands**

<table>
<thead>
<tr>
<th>Group</th>
<th>MI</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>FS</td>
<td>SFS</td>
</tr>
<tr>
<td>Group 1</td>
<td>0.33–0.41</td>
<td>1.0*</td>
<td>0.71*</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.17–0.29</td>
<td>0.57*</td>
<td>0.51*</td>
</tr>
</tbody>
</table>

Data analysis was performed by ANCOVA. Different letters within a column show significant differences between rainfall groups at a 0.05 level. MI, moisture index; Total, in pooled data from FS and SFS; FS, fixed sandy land; SFS semi-fixed sandy land; Group 1, high-rainfall areas (sites 1–6, 14–16); Group 3, low-rainfall areas (sites 8–13, 17).

**Fig. 7. The drought threshold (MI = 0.29) identified by leaf-trait data of A. ordosica corresponded well to the zonal boundary between typical temperate and desert steppes in Zhang (2007). Empty circles are the sites for high-rainfall areas (rainfall group 1, 310–370 mm; MI, 0.33–0.41); the grey triangle is the site for mid-rainfall transition (rainfall group 2, 290 mm; MI, 0.31); and filled circles are the sites for low-rainfall areas (rainfall group 3, 150–265 mm; MI, 0.17–0.29).**
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LITERATURE CITED


