Evaluating the response of $\delta^{13}C$ in *Haloxylon ammodendron,* a dominant C_4 species in Asian desert ecosystem, to water and nitrogen addition as well as the availability of its $\delta^{13}C$ as the indicator of water use-efficiency

Zixun Chen[1,2], Xuejun Liu[2,3], Xiaoqing Cui[2,3], Yaowen Han[2], Guoan Wang[1,2]*, Jiazhu Li[4]*

1. Key Lab of Plant–Soil Interaction, College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China.

2. Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, Department of Environmental Sciences and Engineering, College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China.

3. Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 83011, China

4. Institute of Desertification Studies, Chinese Academy of Forestry, Beijing, 100192, China.

*Corresponding author:

Guoan Wang, gawang@cau.edu.cn

Jiazhu Li, leejzids@caf.ac.cn

Abstract

Variations in precipitation and atmospheric N deposition affect water and N availability in desert, and thus may have significant effects on desert ecosystems. *Haloxylon ammodendron* is a dominant plant in Asian desert, and addressing its physiological acclimatization to the changes in precipitation and N deposition can provide an insight into how desert plants adapt extreme environment by physiological adjustment. Carbon isotope ratio ($\delta^{13}C$) in plants has been suggested as a sensitive long-term indicator of physiological acclimatization. Therefore, this study evaluated the effect of precipitation change and increasing atmospheric N deposition on $\delta^{13}C$ of *H. ammodendron*. Furthermore, *Haloxylon ammodendron* is a C_4 plant, whether its $\delta^{13}C$ can indicate water use-efficiency (WUE) has not been addressed. In the present study, we designed a field experiment with a completely randomized factorial combination of N and water, and measured $\delta^{13}C$ and gas exchange, then we calculated the degree of bundle-sheath leakiness (φ) and WUE of the assimilating branches of *H. ammodendron*. $\delta^{13}C$ and φ in *H. ammodendron* remained stable under N and water supply, while N addition, water addition and their interaction affected gas exchange and WUE in *H. ammodendron*. In addition, $\delta^{13}C$ had no correlation with WUE. These results are associated with the irrelevance between $\delta^{13}C$ and the ratio of intercellular to ambient CO_2 concentration (c_i/c_a), which might be caused by a special value (0.37) of the degree of bundle-sheath leakiness (φ) or a lower activity of carbonic anhydrase (CA) of *H. ammodendron*. In conclusion, $\delta^{13}C$ of *H. ammodendron* is unsensitive to global change in precipitation and atmospheric N deposition, and cannot be used for

45   indicating its WUE.

46

1 Introduction

48   Recently, global precipitation pattern has changed significantly (Frank et al., 2015;

49   Knapp et al., 2015), and atmospheric N deposition has continued to rise (Galloway et

50   al., 2004; Liu et al., 2013; Song et al., 2017). Previous researchers have suggested that

51   arid ecosystems are most sensitive to climate change (Reynolds et al., 2007; Huang et

52   al., 2016), while global change in precipitation and atmospheric N deposition has an

53   important impact on water and N availability in desert (Huang et al., 2018). Thus,

54   these changes may have significant effects on desert ecosystems. *Haloxylon*

55   *ammodendron* is a dominant species in desert regions, especially in Asia. Studying the

56   physiological responses of *H. ammodendron* to global change can provide an insight

57   into how desert plants adapt extreme environment by physiological adjustment.

58   Carbon isotope ratio ($\delta^{13}C$) in plants depends on the ratio of intercellular to ambient

59   CO_2 concentration (c_i/c_a), which reflects the balance between inward CO_2 diffusion

60   rate, regulated by stomatal conductance (g_s), and CO_2 assimilating rate (A) (Farquhar

61   and Richards, 1984), and has been suggested as a sensitive long-term indicator of

62   physiological acclimatization (Battipaglia et al., 2013; Cernusak et al., 2013; Tranan

63   and Schubertt, 2016; Wang and Feng, 2012). Therefore, investigating the variations in

64    $\delta^{13}C$ of *H. ammodendron* under water and nitrogen addition can enhance

65   understanding of physiological responses of desert plants to future changes in

66   precipitation and atmospheric N deposition.

67   A large quantity of works has been devoted to the relationships between C_3 plant

68    $\delta^{13}C$ and water availability or precipitation (e.g., Diefendorf et al., 2010; Kohn, 2010;

69   Liu et al., 2005; Ma et al., 2012; Serret et al., 2018; Stewart et al., 1995; Wang et al.,

70   2005, 2008) and nitrogen availability (e.g. Cernusak et al., 2007; Li et al., 2016;

71   Sparks and Ehleringer, 1997; Yao et al., 2011; Zhang et al., 2015). However, a

72   relatively small amount of research has focused on the responses of C_4 plant $\delta^{13}C$ to

73   water availability or precipitation (Ellsworth et al., 2017; Liu et al., 2005; Rao et al.,

74   2017; Wang et al., 2006) and nitrogen availability (Ma et al., 2016; Schmidt et al.,

75   1993). For C_4 plants, $\delta^{13}C$ is controlled by both the c_i/c_a ratio and the degree of

76   bundle-sheath leakiness (φ), the proportion of CO_2 produced within bundle sheath

77   cells from C_4 acids that leaks back to mesophyll cells (Ellsworth and Cousins, 2016;

78   Ellsworth et al., 2017; Farquhar, 1983). Thus, the responses of C_4 plant $\delta^{13}C$ to water

79   and N availability are also affected by φ. Genetic factors control φ values, which

80   causes the interspecific differences in $\delta^{13}C$, even the responses of plant $\delta^{13}C$ to water

81   and N availability (Gresset et al., 2014). On the other hand, enzymatic activity of

82   carbonic anhydrase (CA) may influence $\delta^{13}C$ in C_4 plants as CA activity is low

83   (Cousins et al., 2006). CA is an enzyme that catalyzes the hydration of CO_2 in

84   mesophyll cells to form bicarbonate (HCO_3^-). Previous studies showed that CA

85   activity in most C_4 plants is usually low, just sufficient to support photosynthesis

86   (Cousins et al., 2006; Gillon and Yakir, 2000, 2001; Hatch and Burnell, 1990). *H.*

87   *ammodendron* is a typical C_4 plant. How its $\delta^{13}C$ responds to water and N availability

88   has never been addressed.

89   Foliar $\delta^{13}C$ in C_3 plants has been considered as a useful indicator of intrinsic water

90   use-efficiency (WUE) (Farquhar, 1983). However, although some studies suggested

91   that $\delta^{13}C$ of C_4 plants could also indicate its WUE (Henderson et al., 1992; Wang et al.,

92   2005; Cernusak et al., 2013; Ellsworth and Cousins, 2016), this statement is still

93   controversial. The relationship between $\delta^{13}C$ and WUE is based on the links between

94   c_i/c_a ratio and $\delta^{13}C$ and between c_i/c_a ratio and WUE (Ehleringer and Cerling, 1995).

95   For C_3 plants, $\delta^{13}C$ always decreases with an increase in c_i/c_a ratio; but for C_4 plants,

96   the correlation between $\delta^{13}C$ and c_i/c_a ratio depends on φ value (Cernusak et al., 2013)

97   and CA activity (Cousins et al., 2006). As mentioned above, φ value is under genetic

98   control, and the CA activity changes across species (Cousins et al., 2006; Gillon and

99   Yakir, 2000, 2001; Hatch and Burnell, 1990), thus, the correlation between $\delta^{13}C$ and

100   c_i/c_a ratio, as well as the relationship between WUE and $\delta^{13}C$, shows interspecific

101   difference. Whether $\delta^{13}C$ of *H. ammodendron* indicates WUE has never been

102   evaluated.

103   In this study, we designed an experiment with multiple water and nitrogen supply in

104   the southern Gurbantunggut Desert in Xinjiang Uygur Autonomous Region, China.

105   We measured the $\delta^{13}C$, gas exchange and WUE of the assimilating branches of *H.*

106   *ammodendron*. We had two objectives. One objective was to evaluate the response of

107   the dominant plant of Asian desert to future changes in precipitation and atmospheric

108   N deposition by revealing the effects of water and N supply on $\delta^{13}C$ of *H.*

109   *ammodendron*. The other was to explore the availability of $\delta^{13}C$ as the indicator of

110   water use-efficiency in *H. ammodendron*.

111

2 Materials and methods

2.1 Definitions and Basic Equations

Stable carbon isotopic ratio ($\delta^{13}C$) of natural materials is expressed as:

$$\delta_{13}C(‰)= \left[\frac{(13C/12C)_{sample}}{(13C/12C)_{standard}} - 1\right] \times 1000 \qquad (1)$$

where the standard is the carbon dioxide obtained from the Peedee belemnite (PDB)

limestone (Craig, 1957). Farquhar (1983) proposed the pattern of carbon isotopic

discrimination (Δ) in C_4 plant:

$$\Delta = \frac{\delta_{13}C_{air} - \delta_{13}C_{plant}}{1 + \delta_{13}C_{plant}/1000} \approx \delta_{13}C_{air} - \delta_{13}C_{plant} = a + [b_4 + \varphi (b - s) - a]\frac{c_i}{c_a} \qquad (2)$$

where $\delta^{13}C_{plant}$ and $\delta^{13}C_{air}$ are the $\delta^{13}C$ values of plants and CO_2 in the ambient air. The

parameter a (= 4.4‰, Craig, 1954) is the carbon isotopic fractionation in the diffusion

of CO_2 into internal leaves; b_4 (= -5.9‰, O'Leary, 1984) is the combined carbon

isotopic fractionations occurring in the processes of gaseous CO_2 dissolution,

hydration/dehydration reactions of CO_2 and HCO_3^- in mesophyll cells, and

HCO_3^- carboxylation by PEP (phosphoenolpyruvate) carboxylase; s (= 1.8‰, O'Leary,

1984) is the carbon isotopic fractionation during diffusion of CO_2 out of the

bundle-sheath cells, and b (= 27‰, Farquhar and Richards, 1984) is the carbon

isotopic fractionation of CO_2 carboxylation by RuBP (ribulose-1,5-bisphosphate)

carboxylase. The variable φ is the proportion of CO_2 producing within bundle sheath

cells from C_4 acids that leaks back to mesophyll cells, and c_i/c_a is the ratio of

intercellular to ambient CO_2 concentration.

Water use-efficiency (WUE) is defined as the amount of assimilated carbon dioxide

133   by plants under the consumption of per unit water. There are two characteristics of

134   WUE, instantaneous WUE (ins-WUE) and intrinsic WUE (int-WUE), respectively.

135   ins-WUE can be calculated by:

136    $$\text{ins-WUE} = A/E = (c_a - c_i)/1.6v = c_a(1 - c_i/c_a)/1.6v \qquad (3)$$

137   where A is photosynthetic rate, E is transpiration rate and v is calculated

138   by:

139    $$v = (e_i - e_a)/p \qquad (4)$$

140   where e_i and e_a are the water vapor pressure inside and outside the leaves, p is the

141   atmospheric pressure.

142   The definition of int-WUE is:

143    $$\text{int-WUE} = A/g_s = (c_a - c_i)/1.6 = c_a(1 - c_i/c_a)/1.6 \qquad (5)$$

144   where g_s is stomatal conductance.

145   **2.2 Study site**

146   This experiment was conducted at the Fukang Station of Desert Ecology, Chinese

147   Academy of Sciences, on the southern edge of the Gurbantunggut Desert (44°26′ N,

148   87°54′ E) in northwestern China. The altitude of the study site is 436.8 m above

149   average sea level (a.s.l.). It is a typical continental arid, temperate climate, with a hot

150   summer and cold winter in the area. The mean annual temperature is 7.1ºC and the

151   mean annual precipitation is 215.6 mm, with a potential evaporation of about 2000

152   mm. The mean annual temperature and the annual rainfall amount in the sampling

153   year are 10.23ºC and 122.7 mm (Cui, 2018). The soil type is grey desert soils

154   (Chinese classification) with aeolian sands on the surface (0-100 cm). The

155 percentages of clay (< 0.005 mm), silt (0.005-0.063 mm), fine sand (0.063-0.25 mm)

156 and medium sand (0.25-0.5 mm) range from 1.63-1.76%, 13.79-14.15%,

157 55.91-56.21% and 20.65-23.23%, respectively (Chen et al., 2007). The soil is highly

158 alkaline (pH = 9.55 ± 0.14) with low fertility. The vegetation is dominated by

159 *Haloxylon ammodendron* and *Haloxylon persicum* with about 30% coverage. Herbs

160 include ephemerals, annuals and small perennials, with a cover of ca. 40% (Fan et al.,

161 2013). Although the coverage of the two *Haloxylon* species is a little lower than that

162 of herbs, the biomass of the former is much larger than that of the latter, because

163 *Haloxylon* plants are shrubs with an average height of 1.5 m whereas the latter are

164 very low herbaceous plants. Biological soil crusts are distributed widely on the soil

165 between the herbs and *Haloxylon*, with almost 40% coverage (Zhang et al., 2007).

166 **2.3 Experimental design**

167 A field experiment with a completely randomized factorial combination of water and

168 nitrogen has been conducted from 2014 to 2017. We designed two water addition

169 levels (0, 60mm·yr^{-1}; W0, W1), since precipitation is predicted to increase by 30% in

170 northern China in the next 30 years (Liu et al., 2010), and three levels of N addition (0,

171 30, 60 kg N·ha^{-1}·yr^{-1}; N0, N1 and N2), because N deposition has reached 35.4 kg

172 N·ha^{-1}·yr^{-1} in the nearby city, Urumqi (Cui et al., 2017) and will double by 2050

173 relative to the early 1990s (Galloway et al., 2008). Therefore, there were six

174 treatments (W0N0, W0N1, W0N2, W1N0, W1N1, W1N2) in this experiment. Four

175 replicates of each treatment were set, making a total of 24 plots with a size of 10 m

176 × 10 m. A small sub-plot with a size of 1.5 m×1.5 m was set in each plot. A

177   well-grown *H. ammodendron* was enclosed in the center of the sub-plot. The average

178   height and coverage of an individual *H. ammodendron* were 1.5 m and 1.9 m^2,

179   respectively, and did not vary significantly across the plots. To simulate natural water

180   and N inputs, the treatments were applied in equal amounts, twelve times, once a

181   week in April, July and September, as 5 mm·m^{-2} of water and 2.5 or 5 kg N·ha^{-1} each

182   week (Cui et al., 2017). Usually, water addition was with a sprinkler kettle, irrigating

183   over the canopy of *H. ammodendron*.

184   **2.4 Measurements of gas exchange traits and WUE**

185   The leaves of *H. ammodendron* have been completely degraded due to the extreme

186   drought, and the assimilation branches perform the same functions as the leaves. Thus,

187   we conducted gas exchange measurements on the assimilation branches of the *H.*

188   *ammodendron* grown in the sub-plots by LI-6400 portable photosynthesis system on

189   27-29, June 2016. The ins-WUE and the int-WUE were calculated based on these

190   measured gas exchange traits by the Eq. (3), and int-WUE by Eq. (5). At each plot,

191   the top assimilating branches of a mature individual was selected randomly for the

192   measurement. About 5s was needed for stability after the assimilating branches was

193   inserted in the cuvette and then the assimilating branches were measured. We

194   repeated 10 times on the same assimilating branches for each measurement. We

195   measured gas exchange with a standard 450 mmol·mol^{-1} CO_2 concentration at a flow

196   rate of 500 mmol·s^{-1} above saturation in photo flux density of 1600 mmol·m^{-2}·s^{-1}. The

197   temperature of the measuring assimilating branches varied from 29.5 °C to 30.5 °C

198   during the entire period of gas exchange measurements.

2.5 Samples collection

Considering that there is a considerable difference in $\delta^{13}C$ between buds, young and matured leaves, so we collected the mature assimilating branches of *H. ammodendron* for the $\delta^{13}C$ measurements. All *H. ammodendron* individuals grown in plots (10 m × 10 m) were sampled. Eight pieces of the mature assimilating branches were collected from each individual, two pieces of assimilating branches were collected at each of the four cardinal directions from the positions of full irradiance. All assimilating branches from the same plot were combined into one sample. All plant samples were air-dried in the field and then in the laboratory. Then the samples were ground into a fine powder using a steel ball mixer mill MM200 (Retsch GmbH, Haan, Germany) for the measurements of $\delta^{13}C$, N contents and chlorophyll contents.

2.6 Measurements of plant $\delta^{13}C$, plant N and chlorophyll contents

The $\delta^{13}C$ and N measurements were performed on a Delta^{Plus} XP mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an automated elemental analyzer (Flash EA1112, CE Instruments, Wigan, UK) in a continuous flow mode, at the Stable Isotope Laboratory of the College of Resources and Environmental Sciences, China Agricultural University. The carbon isotopic ratios were reported in the delta notation relative to the V-PDB standard. For this measurement, we obtained standard deviations low than 0.15‰ for $\delta^{13}C$ among replicate measurements of the same sample. And standard deviations for the N measurements were 0.1%.

The chlorophyll contents of all samples were also determined. The samples were first extracted by 95% ethyl alcohol (0.5 g sample to 25 mL ethyl alcohol), and then

221 the absorbancy was measured under the wave length of 665 and 649 mm by the

222 spectrophotometer. The content of chlorophyll a, b was calculated by the follow

223 equations:

224 $$\text{Chlorophyll a (mg/L)} = 13.95 \times OD665 - 6.88 \times OD649 \tag{6}$$

225 $$\text{Chlorophyll b (mg/L)} = 24.96 \times OD649 - 7.32 \times OD665 \tag{7}$$

226 where OD665 and OD649 are the absorbancy under the wave length of 665 and

227 649mm, respectively.

2.6 Calculation of the degree of bundle-sheath leakiness

229 The degree of bundle-sheath leakiness (φ) was calculated by the transformation of Eq.

230 (2):

231 $$\varphi = \left(\frac{(\delta^{13}C_{air} - \delta^{13}C_{plant})\big/(1+\delta_{13}C_{plant}/1000) - a}{c_i/c_a} + a - b_4 \right)\Big/(b - s) \tag{8}$$

232 In this equation, parameters a, b_4, b and s are constant, while $\delta^{13}C_{plant}$ and c_i/c_a are

233 the measured values of our samples. We did not measure the $\delta^{13}C_{air}$ at our study site,

234 so we had to use an approximation of the $\delta^{13}C_{air}$ to do this φ calculation. The

235 approximated value we used is -9.77‰, which has been measured at Donglingshan

236 Mountain, Beijing, north China in September 2019. We believe that the two sites

237 should have similar $\delta^{13}C_{air}$ because the two sites are located in countryside with less

238 human activities and have a similar distance from the nearest city. The straight line

239 distances between Donglingshan Mountain and the city center of Beijing, and

240 between our study site and Urumqi city are about 90 km.

2.7 Statistical analysis

242 Statistical analyses were conducted using SPSS software (SPSS for Windows, Version

243 20.0, Chicago, IL, United States). One-way analysis of variance (ANOVA) and

244 two-way analysis of variance (ANOVA) were used to compare the difference of $\delta^{13}C$

245 and other physiological traits between each treatment. Pearson analysis was used to

246 determine the correlation among $\delta^{13}C$, WUE and c_i/c_a in *H. ammodendron*.

247

3 Results

3.1 Plant $\delta^{13}C$ under water and nitrogen addition

250 The $\delta^{13}C$ of the assimilating branches of *H. ammodendron* in the six treatments

251 W0N0, W0N1, W0N2, W1N0, W1N1, W1N2 was -14.18 ± 0.19 ‰, -14.71 ± 0.35 ‰,

252 -14.45 ± 0.18 ‰, -14.67 ± 0.40 ‰, -14.65 ± 0.38 ‰, -14.34 ± 0.29 ‰, respectively.

253 One-way ANOVA analyses showed no significant variation in $\delta^{13}C$ across treatments

254 ($p = 0.788$, Fig. 1). Two-way ANOVA analyses suggested that $\delta^{13}C$ was not affected

255 by water addition ($p = 0.678$), N addition ($p = 0.607$) and their interaction ($p = 0.563$,

256 Table 1).

257   Fig.1

258   Table 1

3.2 Gas exchange and WUE under water and nitrogen addition

260 Photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E) and c_i/c_a

261 ranged from 12.11 μmol CO_2 m^{-2} s^{-1} to 39.35 μmol CO_2 m^{-2} s^{-1}, from 0.09 mol H_2O

262 m^{-2} s^{-1} to 0.31 mol H_2O m^{-2} s^{-1}, from 2.87 mmol H_2O m^{-2} s^{-1} to 8.49 mmol H_2O m^{-2} s^{-1}

263 and 0.11 to 0.57, respectively. One-way ANOVA analyses showed significant changes

264 in leaf gas exchange across the six treatments ($p = 0.012$ for A, $p = 0.006$ for g_s, $p =$

265   0.002 for E and c_i/c_a, Fig. 2). Two-way ANOVA analyses suggested that water

266   addition had exerted effect on c_i/c_a ($p = 0.004$), that N additions influenced A ($p =$

267   0.008) and c_i/c_a ($p = 0.009$), and that the interaction between water and N supply

268   played a role in g_s ($p < 0.001$), E ($p < 0.001$) and c_i/c_a ($p < 0.001$, Table 1).

269   Fig. 2

270   Instantaneous WUE (ins-WUE) and intrinsic WUE (int-WUE) ranged from 3.09

271   µmol CO_2 / mmol H_2O to 8.49µmol CO_2 / mmol H_2O and from 93.64µmol CO_2 / mol

272   H_2O to 208.47µmol CO_2 / mmol H_2O, respectively. One-way ANOVA analyses

273   showed significant changes in these two indexes (both $p < 0.001$, Fig. 3). Two-way

274   ANOVA analyses suggested that water addition, N addition and their interaction all

275   have significant effect on these two indexes (all $p < 0.05$, Table 1).

276   Fig. 3

277 **3.3 Correlations among $\delta^{13}C$, WUE and c_i/c_a ratio**

278 In order to test whether $\delta^{13}C$ in *H. ammodendron* can indicate WUE, the relationships

279 among $\delta^{13}C$, ins-WUE, int-WUE and c_i/c_a ratio were revealed in this study. Our

280 results showed no correlation between $\delta^{13}C$ and ins-WUE ($p = 0.229$, Fig. 4a),

281 between $\delta^{13}C$ and int-WUE ($p = 0.229$, Fig. 4c), and between $\delta^{13}C$ and c_i/c_a ratio ($p =$

282 0.183, Fig. 4e). However, there was a negative correlation between ins-WUE and c_i/c_a

283 ratio ($p < 0.001$, Fig. 4b), and between int-WUE and c_i/c_a ratio ($p < 0.001$, Fig. 4d).

284   Fig. 4

285 **3.4 The degree of bundle-sheath leakiness under water and nitrogen addition**

286 The calculated φ ranged from 0.32 to 0.59 with a mean value of 0.45. One-way

287   ANOVA analyses showed no significant variation in φ across treatments (p = 0.768,

288   Fig. 5). Two-way ANOVA analyses suggested that $\delta^{13}C$ was not affected by water

289   addition (p = 0.644), N addition (p = 0.600) and their interaction (p = 0.521, Table 1).

290   Fig. 5

291

4 Discussion

293   The $\delta^{13}C$ of the assimilating branches in *H. ammodendron* did not change across

294   treatments (Fig. 1, Table 1), suggesting that neither water addition nor nitrogen

295   addition influenced the $\delta^{13}C$ of *H. ammodendron*. Previous studies also reported no

296   significant relationship between $\delta^{13}C$ of C_4 plant and water availability (Swap et al.,

297   2004; Wang et al., 2008), and between $\delta^{13}C$ of C_4 plant and nitrogen availability (Yao

298   et al., 2011, Yang et al., 2017).

299   In general, the effects of water availability and nitrogen availability on $\delta^{13}C$ are

300   dependent on c_i/c_a ratio, which reflects the balance between stomatal conductance (g_s)

301   and photosynthetic rate (A) (Farquhar and Richards, 1984). With more water

302   availability under water addition, plants tend to open stomata to absorb more CO_2,

303   leading to an increase in g_s. Two-way ANOVA analyses suggested that water addition

304   had no effect on both A and g_s (Table 1). However, One-way ANOVA analyses

305   showed that g_s was higher in W1N0 than that in W0N0 (Fig. 2b). Thus, at least water

306   addition had a positive effect on g_s under ambient N condition. Increasing g_s under

307   water supply will lead to the rise of intercellular CO_2 because of the decrease of

308   diffusional resistance to CO_2. As the results, c_i/c_a ratio was observed to increase with

309 increasing moisture (Fig. 2d, Table 1). However, $\delta^{13}C$ remained stable under water

310 addition (Fig. 1, Table 1). Thus, c_i/c_a ratio could not explain the observed response of

311 $\delta^{13}C$ to water supply.

312 For most plants in natural ecosystems, nitrogen is the key factor limiting plant

313 growth (Hall et al., 2011). Thus, nitrogen addition usually causes plants to absorb

314 more N. However, extreme drought could prevent plants from absorbing N even

315 under high N supply. In the present experiment, N supply was found to have an effect

316 on N contents in *H. ammodendron*. Relative to the control treatment (W0N0), N

317 contents increased with N supply under low N addition, but kept unchanged under

318 high addition (Table S1, S2). Nitrogen is the main constituent of Rubisco (ribulose‐

319 1,5‐bisphosphate carboxylase oxygenase) and chlorophyll in plants. Thus,

320 chlorophyll a was found to have the similar pattern as N contents under water and N

321 supply. Chlorophyll a was higher in W0N1 than W0N0, and there was no difference in

322 chlorophyll a between W0N0 and W0N2 (Table S1). Increasing chlorophyll contents

323 in W0N1 should lead to the increase in photosynthetic rate (A). However, different

324 from our prediction, one-way ANOVA analyses suggested that A in W0N1 did not

325 differ from that in W0N0, and that A in W0N2 is lower than that in W0N0 (Fig., 2a).

326 Two-way ANOVA analyses showed that N addition had an influence on A (Table 1).

327 Both the analyses suggested that N supply played a negative role in A, and thus the

328 consumption of intercellular CO_2. Consequently, c_i/c_a ratio was found to increase with

329 N supply (Fig. 2d, Table 1). Therefore, the variations in c_i/c_a ratio with N addition

330 could not account for the unchanged pattern in $\delta^{13}C$ under N supply (Fig. 1).

331   The co-application of water and nitrogen was found to have a negative effect on A

332 but no effect on g_s (W0N0 vs. W1N1, W1N2, Fig. 2a, b). The responses of A and g_s to

333 the co-application of water and nitrogen resulted in an increase in c_i/c_a ratio (Fig., 2d).

334 Since $\delta^{13}C$ remained unchanged under the co-application of water and nitrogen (Fig.

335 1), c_i/c_a ratio could not also explain the observed $\delta^{13}C$ response to the co-application

336 of water and nitrogen.

337   In summary, the unchanged $\delta^{13}C$ across treatments did not related to c_i/c_a ratio in *H.*

338 *ammodendron*, and we did find that there is no correlation between $\delta^{13}C$ and the

339 measured c_i/c_a ratio (Fig. 4e). Thereby, the observed $\delta^{13}C$ stability across treatments

340 may be associated with the φ value and carbonic anhydrase (CA) in *H. ammodendron*.

341 For C$_4$ plants, the relationship between carbon isotope discrimination (Δ) and c_i/c_a

342 ratio is dependent on φ values (Ellsworth and Cousins, 2016; Ellsworth et al., 2017;

343 Farquhar, 1983; Wang et al., 2008). Some studies suggested that φ value was stable

344 for a given species under a wide range of environmental conditions (Henderson et al.,

345 1992; Wang et al., 2008; Cernusak et al., 2013). However, other studies had different

346 conclusions that φ value was influenced by irradiation (Bellasio and Griffiths, 2014;

347 Kromdijk et al., 2010; Pengelly et al., 2010; Ubierna et al., 2013), temperature (von

348 Caemmerer et al., 2014), water stress (Fravolini et al., 2002; Gong et al., 2017;

349 Williams et al., 2001; Yang et al., 2017) and nitrogen supply (Fravolini et al., 2002;

350 Meinzer and Zhu, 1998; Yang et al., 2017). In current study, the φ value of *H.*

351 *ammodendron* remained unchanged across six treatments (Fig. 5), and two-way

352 ANOVA analyses suggested that water supply and N supply had no effect on φ (Table

353 1). Therefore, the φ value of *H. ammodendron* was insensitive to water and N addition

354 in this study. Even if the φ value remains stable, the relationship between Δ and c_i/c_a

355 ratio is also associated with the magnitude of the φ value. Cernusak et al. (2013)

356 predicted that when φ value is greater than 0.37, the correlation between Δ and c_i/c_a

357 ratio is positive; conversely, when φ value is less than 0.37, the correlation is negative.

358 In particular, when φ value is equal to 0.37, no significant correlation can be found,

359 because the coefficient ($[b_4 + φ (b - s) – a]$ in Eq. (2)) of c_i/c_a ratio equals to 0

360 (Cernusak et al., 2013). The φ value ranged from 0.32 to 0.59 with a mean value of

361 0.45 in present study. Thus, the correlation between Δ and c_i/c_a in *H. ammodendron*

362 should be positive based on the prediction by Cernusak et al. (2013). It seems that φ is

363 not the driver of the observed $\delta^{13}C$ pattern in *H. ammodendron*. However, there were

364 some uncertainties in the calculation of φ value based on the measured $\delta^{13}C$ and c_i/c_a.

365 The measured $\delta^{13}C$ represents the fixed carbon isotope composition throughout the

366 assimilation branch formation period, which usually spans at least several weeks. And

367 the measured c_i/c_a is a instant indicator. Therefore, the φ value of *H. ammodendron*

368 may be close to 0.37, which leaded to the observed insensitive response of $\delta^{13}C$ to

369 water and N addition.

370 The enzymatic activity of CA may be another mechanism behind the unchanged

371 $\delta^{13}C$ across treatments. Cousins et al. (2006) suggested that enzymatic activity of CA

372 affects carbon isotope discrimination in most C_4 plants because CA can result in the

373 changing of parameter b_4 (see Eq. (2)). But in traditional view, the parameter b_4 was a

374 constant. However, it is only true when the ratio of PEP carboxylation rate to the CO_2

375 hydration rate (V_p/V_h) is equal to zero, which is caused by a high CA activity. If

376 V_p/V_h is not zero, b_4 will change and be controlled by V_p/V_h (Cousins et al., 2006).

377 Previous studies reported that CA activity is low in most C_4 plants (Cousins et al.,

378 2006; Gillon and Yakir, 2000, 2001; Hatch and Burnell, 1990). Thus, CA activity in *H.*

379 *ammodendron* might also be low, leading to the change in b_4 with V_p/V_h, and thus

380 $\delta^{13}C$. Cousins et al. (2006) added V_p/V_h into the discrimination pattern of C_4 plants

381 and predicted that at a given φ value, when the V_p/V_h is 0 or 1, the correlation

382 between Δ and c_i/c_a ratio is negative or positive, respectively. Since CA activity is low

383 in most C_4 plants, and the V_p/V_h always ranges from 0 to 1, we speculate that no

384 correlation between Δ and c_i/c_a ratio may also occur when the V_p/V_h is a certain value

385 between 0 and 1. Thus, the uncorrelated pattern between Δ and c_i/c_a ratio in *H.*

386 *ammodendron* might be related to this specific V_p/V_h value due to low CA activity.

387 In addition, the unchanged $\delta^{13}C$ across treatments may also be controlled by the

388 water sources of *H. ammodendron*. Previous study has found that the root of *H.*

389 *ammodendron* can be inserted into the soil layer deeper than 3 m (Sheng et al., 2004),

390 which made it easy to uptake groundwater. Therefore, *H. ammodendron* may be less

391 sensitive to water addition. However, a study conducted in the same region has found

392 that the shallow soil water (0-40 cm) and groundwater are two important water

393 sources for *H. ammodendron* (Dai et al., 2014), and another study has reported that

394 water addition resulted in an increase of soil water contents in shallow soil layer (Cui,

395 2018). Moreover, gas exchange changed across treatments in the present study (Fig.

396 2). Thus, the utilization of groundwater by *H. ammodendro*n may be one of the

397 reasons why its $\delta^{13}C$ is not sensitive to water and N addition, but it should not be the

398 main reason.

399   Whether foliar $\delta^{13}C$ of C$_4$ plants can indicate their WUE is still controversial.

400 Henderson et al. (1992) found that $\delta^{13}C$ of 10 C$_4$ species has negative correlation with

401 their WUE. Although this result was just opposite to a positive relationship between

402 $\delta^{13}C$ and WUE for C$_3$ plants, it is a proof that $\delta^{13}C$ of C$_4$ plants can indicate their

403 WUE. In the work of Henderson et al. (1992), they found that the φ values in 10 C$_4$

404 species was around 0.21 over a range of irradiance and leaf temperature. According to

405 the suggestion by Cernusak et al. (2013) that Δ is negatively related to c_i/c_a ratio when

406 φ value is less than 0.37, thus, the $\delta^{13}C$ of 10 C$_4$ species has a positive correlation with

407 c_i/c_a ratio. In general, under fixed ambient CO_2 concentration, WUE is always

408 negatively correlated with c_i/c_a ratio (see Eq. (3) and Eq. (5)). This is why a negative

409 relationship between $\delta^{13}C$ and WUE was observed for the 10 C$_4$ species. The present

410 study showed that ins-WUE and int-WUE both had no correlation with $\delta^{13}C$ in *H.*

411 *ammodendron* (Fig. 4a, 4c), which was different from the results published by

412 Henderson et al. (1992). In general, c_i/c_a ratio is the link between WUE and $\delta^{13}C$. As

413 mentioned above, if the φ value equals to 0.37 and/or the activity of CA is very low,

414 $\delta^{13}C$ would not correlate to c_i/c_a ratio, and thus leads to the uncorrelation between

415 $\delta^{13}C$ and WUE. In addition, the different time scales of $\delta^{13}C$, ins-WUE and int-WUE

416 may also result in this uncorrelation. As mentioned above, the measured $\delta^{13}C$

417 represents the long-term fixed carbon isotope composition (at least several weeks).

418 And the value of ins-WUE and int-WUE were calculated from the gas exchange of a

419 short-term measurement, which lasted only a few minutes. Therefore, this difference

420 may also drive the uncorrelation between $\delta^{13}C$ and WUE. In other word, there should

421 be a correlation between WUE and $\delta^{13}C$ in *H. ammodendron*, and the obtained results

422 was only caused by the uncertainty of the experiment. However, $\delta^{13}C$ remained stable

423 under water and nitrogen addition (Fig. 1, Table 1), while the measured ins-WUE and

424 int-WUE was higher in the control treatment (W0N0) than other treatments (Fig. 3),

425 suggesting and water and N supply had a significant effect on WUE (Table 1). As a

426 result, although there are some uncertainties in the relationship between $\delta^{13}C$ and

427 WUE, our results indirectly confirmed that $\delta^{13}C$ of *H. ammodendron* could not

428 indicate its WUE.

429 The present study has found that $\delta^{13}C$ of *H. ammodendron* could not be used as a

430 indicator of its WUE. Although this conclusion cannot be analogous to all C_4 plants,

431 the present study has important implications for the understanding of physiological

432 responses of desert plants to future changes in precipitation and atmospheric N

433 deposition. *H. ammodendron* is a dominant species in Asia desert, which has a great

434 effect on the stabilization of sand dunes, the survival and development of understory

435 plants and the structure and function of desert ecosystems (Sheng et al., 2005; Su et

436 al., 2007; Cui et al., 2017). Thus, *H. ammodendron* is widely distributed in desert

437 areas, and the prediction of its drought adaptation is crucial in desert ecosystem.

438

439 5 Conclusion

440 Global changes including precipitation and atmospheric N deposition have been

441   proved to have an important influence on ecosystems, especially for the arid

442   ecosystems. The present study showed that water and N addition had little effect on

443   the $\delta^{13}C$ values and the degree of bundle-sheath leakiness (φ) of *H. ammodendron*, but

444   played an important role in the change of its gas exchange and water use efficiency

445   (WUE). In addition, different pattern of instantaneous WUE (ins-WUE), intrinsic

446   WUE (int-WUE) and $\delta^{13}C$ across treatment and no correlation between instantaneous

447   WUE (ins-WUE) and $\delta^{13}C$, and between intrinsic WUE (int-WUE) and $\delta^{13}C$ has been

448   found in this study, suggesting that $\delta^{13}C$ of *H. ammodendron* could not indicate its

449   WUE. This result is caused by the lack of the correlation between $\delta^{13}C$ and the ratio

450   of intercellular to ambient CO_2 concentration (c_i/c_a), which might be associated with

451   the degree of bundle-sheath leakiness (φ) or the low activity of carbonic anhydrase

452   (CA). Thus, the current experiment implies that the availability of $\delta^{13}C$ as the

453   indicator of WUE could be not universal for C_4 species.

454

455   **Conflict of interest**

456   None declared.

457

458   **Funding**

459   This research was supported by the Chinese National Basic Research Program (No.

460   2014CB954202 and a grant from the National Natural Science Foundation of China

461   (No. 41772171).

462

Authors' Contributions

G Wang and J Li designed the experiment and modified the manuscripts. Z Chen designed and executed the experiment and wrote the manuscripts. X Liu designed the experiment. X Cui executed the experiment. Y Han executed the experiment.

Acknowledgements

This research was supported by the Chinese National Basic Research Program (No. 2014CB954202 and a grant from the National Natural Science Foundation of China (No. 41772171). We would like to thank the supports from the Fukang Observation Station of Desert Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, and to thank Ma Yan for analyzing stable carbon isotope ratios in the Isotope Lab at the College of Resources and Environment, China Agricultural University.

Data availability

The datasets analyzed in this manuscript are not publicly available. Requests to access the datasets should be directed to gawang@cau.edu.cn.

References

Battipaglia, G., Saurer, M., Cherubini, P., Calfapietra, C., McCarthy, H. R., Norby, R. J., and

Cotrufo, M. F.: Elevated CO_2 increases tree-level intrinsic water use efficiency: insights from

carbon and oxygen isotope analyses in tree rings across three forest FACE sites. New Phytol.,

485   197, 544-554, 2013.

486   Bellasio, C., and Griffiths, H.: Acclimation to low light by C_4 maize: implications for bundle

487   sheath leakiness. Plant Cell Environ., 37, 1046-1058, 2014.

488   Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A. M., Marshall, J.. D., and Farquhar, G. D.:

489   Environmental and physiological determinants of carbon isotope discrimination in terrestrial

490   plants. New Phytol., 200, 950-965, doi: 10.1111/nph.12423, 2013.

491   Cernusak, L. A., Winter, K., Aranda, J., Turner, B. L., and Marshall, J. D.: Transpiration efficiency

492   of a tropical pioneer tree (Ficus insipida) in relation to soil fertility. J. Exp. Bot., 58(13),

493   3549-3566, 2007.

494   Chen, Y., Wang, Q., Li, W., and Ruan, X.: Microbiotic crusts and their interrelations with

495   environmental factors in the Gurbantonggut desert, western China. Environ. Geol., 52, 691-700,

496   2007.

497   Cousins, A. B., Badger, M. R., and von Caemmerer, S.: Carbonic anhydrase and its influence on

498   carbon isotope discrimination during C_4 photosynthesis. Insights from antisense RNA in

499   *Flaveria bidentis*. Plant Physiol., 141, 232-242, 2006.

500   Craig, H.: Carbon-13 in plants and relationships between carbon-13 and carbon-14 variations in

501   nature. J. Geol., 62, 115-149, 1954.

502   Craig, H.: Isotopic standards for carbon and oxygen and correction factors for mass spectrometric

503   analysis of carbon dioxide. Geochim. Cosmochim. Acta, 12, 133-149, 1957.

504   Cui, X. Q., Yue, P., Gong, Y., Li, K. H., Tan, D. Y., Goulding, K., and Liu, X. J.: Impacts of water

505   and nitrogen addition on nitrogen recovery in, *Haloxylon ammodendron*, dominated desert

506   ecosystems. Sci. Total Environ., 601-602, 1280-1288, 2017.

507 Cui, X. Q.: Effects of enhanced precipitation, temperature and nitrogen addition on nitrogen fate

508   and plant stoichiometry in temperate desert ecosystem in Xinjiang. Ph.D thesis, China

509   Agricultural University, 2018.

510 Dai, Y., Zheng, X., Tang, L., and Li, Y.: Dynamics of water usage in *Haloxylon ammodendron* in

511   the southern edge of the Gurbantunggut Desert. Chinese J. Plant Ecol., 38, 1214-1225, 2014.

512 Diefendorf, A. F., Mueller, K. E., and Wing, S. L.: Global patterns in leaf ^{13}C discrimination and

513   implications for studies of past and future climate. Proc. Natl. Acad. Sci. U.S.A., 107,

514   5738-5743. doi: 10.1073/pnas.0910513107, 2010.

515 Ehleringer, J. R., and Cerling, T. E.: Atmospheric CO_2 and the ratio of intercellular to ambient CO_2

516   concentrations in plants. Tree Physiol., 15, 105-111, 1995.

517 Ellsworth, P. Z., Ellsworth, P. V., and Cousins, A. B.: Relationship of leaf oxygen and carbon

518   isotopic composition with transpiration efficiency in the C_4 grasses *Setaria viridis* and *Setaria*

519   *italica*. J. Exp. Bot., 68(13), 3513-3528, 2017.

520 Ellsworth, P. Z., and Cousins, A. B.: Carbon isotopes and water use efficiency in C_4 plants. Curr.

521   Opin. Plant. Biol., 31, 155-161, 2016.

522 Fan, L. L., Li, Y., Tang, L. S., and Ma, J.: Combined effects of snow depth and nitrogen addition

523   on ephemeral growth at the southern edge of the Gurbantunggut Desert, China. J. Arid. Land, 5,

524   500-510, 2013.

525 Farquhar, G. D.: On the nature of carbon isotope discrimination in C_4 species. Aust. J. Plant

526   Physiol., 10(2), 205-226, 1983.

527 Farquhar, G. D., and Richards, P. A.: Isotopic composition of plant carbon correlates with

528   water-use efficiency of wheat gemotypes. Aust. J. Plant Physiol., 11, 539-552, 1984.

529   Frank, D., Reichstein, M., Bahn, M., Thonicke, K., Frank, D., Mahecha, M. D., Smith, P., der

530   Velde, M. V., Vicca, S., Babst, F., Beer, C., Buchmann, N, Canadell, J. C., Ciais, P., Cramar, W.,

531   SIbrom, A., Miglietta, F., Poulter, B., Rammig, A., Seneviratne, S. I., Walz, A., Wattenbach, M.,

532   Zavala, M. A., and Zscheischler, J.: Effects of climate extremes on the terrestrial carbon cycle:

533   Concepts, processes and potential future impacts. Global Chang. Biol., 21(8), 2861-2880, 2015.

534   Fravolini, A., Williams, D. G., and Thompson, T. L.: Carbon isotope discrimination and bundle

535   sheath leakiness in three C_4 subtypes grown under variable nitrogen, water and atmospheric

536   CO_2 supply. J. Exp. Bot., 53(378), 2261-2269, 2002.

537   Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P.,

538   Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter,

539   J. H., Townsend, A. R., and Vörösmarty, C.J.: Nitrogen cycles: past, present, and future.

540   Biogeochem. 70, 153-226, 2004.

541   Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli,

542   L. A., Seitzinger, S. P., and Sutton, M. A.: Transformation of the nitrogen cycle: recent trends,

543   questions, and potential solutions. Science, 320, 889-892, 2008.

544   Gillon, J. S., and Yakir, D.: Naturally low carbonic anhydrase activity in C_4 and C_3 plants limits

545   discrimination against (COO)-O-18 during photosynthesis. Plant Cell Environ., 23, 903-915,

546   2000.

547   Gillon, J. S., and Yakir, D.: Influence of carbonic anhydrase activity in terrestrial vegetation on the

548   O-18 content of atmospheric CO_2. Science, 291, 2584-2587, 2001.

549   Gong, X. Y., Schäufele, R., and Schnyder, H.: Bundle-sheath leakiness and intrinsic water use

550   efficiency of a perennial C_4 grass are increased at high vapour pressure deficit during growth. J.

551   Exp. Bot., 68(2), 321-333, 2017.

552   Gresset, S., Westermeier, P., Rademacher, S., Ouzunova, M., Presterl, T., Westhoff, P., and Schön,

553   C.: Stable carbon isotope discrimination is under genetic control in the C_4 species maize with

554   several genomic regions influencing trait expression. Plant Physiol., 164(1), 131-143, 2014.

555   Hall, S. J., Sponseller, R. A., Grimm, N. B., Huber, D., Kaye, J. P., Clark, C., and Collins, S. L.:

556   Ecosystem response to nutrient enrichment across an urban airshed in the Sonoran Desert. Ecol.

557   Appl., 21, 640-660, 2011.

558   Hatch, M. D., and Burnell, J. N.: Carbonic anhydrase activity in leaves and its role in the first step

559   of C_4 photosynthesis. Plant Physiol., 93, 825-828, 1990.

560   Henderson, S. A., von Caemmerer, S., and Farquhar, G. D.: Short-termmeasurements of carbon

561   isotope discrimination in several C_4 species. Aust. J. Plant Physiol., 19, 263-285, 1992.

562   Huang, J. P., Yu, H. P., Guan, X. D., Wang, G. Y., and Guo, R. X.: Accelerated dryland expansion

563   under climate change. J. Nature Climat. Chang., 6(2), 2016.

564   Huang, J. Y., Wang, P., Niu, Y. B., Yu, H. L., Ma, F., Xiao, G. J., and Xu, X.: Changes in C:N:P

565   stoichiometry modify N and P conservation strategies of a desert steppe species Glycyrrhiza

566   uralensis. Sci. Rep., 8(1), 12668, 2018.

567   Knapp, A. K., Hoover, D. L., Wilcox, K. R., Avolio, M. L., Koerner, S. E., La Pierre, K. J., Loik,

568   M. E., Luo Y. Q., Sala, O. E., and Smith, M. D.: Characterizing differences in precipitation

569   regimes of extreme wet and dry years: Implications for climate change experiments. Glob.

570   Chang. Biol.y 21(7), 2624-2633, 2015.

571   Kohn, M. J.: Carbon isotope compositions of terrestrial C_3 plants as indicators of (paleo) ecology

572   and (paleo) climate. Proc. Natl. Acad. Sci. U.S.A., 107, 19691-19695. doi:

573   10.1073/pnas.1004933107, 2010.

574 Kromdijk, J., Griffiths, H., and Schepers, H. E.:. Can the progressive increase of C_4 bundle sheath

575   leakiness at low PFD be explained by incomplete suppression of photorespiration? Plant Cell

576   Environ., 33, 1935-1948, 2010.

577 Li, J. Z., Wang, G. A., Zhang, R.N., and Li, L.: A negative relationship between foliar carbon

578   isotope composition and mass-based nitrogen concentration on the eastern slope of mount

579   gongga, China. PLoS ONE, 11(11), e0166958, 2016.

580 Liu, W. G., Feng, X. H., Ning, Y. F., Zhang, Q. L., Cao, Y. N., and An, Z. S.: $\delta^{13}C$ variation of C_3

581   and C_4 plants across an asian monsoon rainfall gradient in arid northwestern China. Glob.

582   Chang. Biol., 11(7), 1094-1100, 2005.

583 Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J. W., Goulding, K.,

584   Christie, P., Fangmeier, A., and Zhang, F.: Enhanced nitrogen deposition over China. Nature

585   494, 459-462, 2013.

586 Liu, Y. X., Li, X., Zhang, Q., Guo, Y. F., Gao, G., and Wang, J. P.: Simulation of regional

587   temperature and precipitation in the past 50 years and the next 30 years over China. Quat. Int.,

588   212, 57-63, 2010.

589 Ma, J. Y., Sun, W., Liu, X. N., and Chen, F. H.: Variation in the stable carbon and nitrogen isotope

590   composition of plants and soil along a precipitation gradient in northern China. PLoS ONE,

591   7(12), e51894, 2012.

592 Ma, J. Y., Sun, W., Koteyeva, N. K., Voznesenskaya, E., Stutz, S. S., Gandin, A., Smith-Moritz, A.

593   M., Heazlewood, J. L., and Cousins, A. B.: Influence of light and nitrogen on the photosynthetic

594   efficiency in the c4 plant *Miscanthus×Giganteus*. Photo. Res., 131(1), 1-11, 2016.

595   Meinzer, F. C., and Zhu, J.: Nitrogen stress reduces the efficiency of the C$_4$ CO$_2$ concentrating

596   system, and therefore quantum yield, in *Saccharum (sugarcane)* species. J. Exp. Bot., 49(324),

597   1227-1234, 1998.

598   O' Leary, M. H.: Measurement of the isotopic fractionation associated with diffusion of carbon

599   dioxide in aqueous solution. J. Phys. Chem., 88, 823-825, 1984.

600   Pengelly, J. J. L., Sirault, X. R. R., Tazoe, Y., Evans, J. R., Furbank, R. T., and von Caemmerer, S.:

601   Growth of the C$_4$ dicot Flaveria bidentis: photosynthetic acclimation to low light through shifts

602   in leaf anatomy and biochemistry. J. Exp. Bot., 61, 4109-4122, 2010.

603   Rao, Z. G., Guo, W. K., Cao, J. T., Shi, F. X., Jiang, H., and Li, C. Z.: Relationship between the

604   stable carbon isotopic composition of modern plants and surface soils and climate: A global

605   review. Earth Sci. Rev., 165, 110-119, 2017.

606   Reynolds, J. F., Smith, D. M. S., Lambin, E. F., Turnerll, B. L., Mortimore, M., Batterbury, S. P. J.,

607   Downing, T. E., Dowlatabadi, H., Fernández, R. J., Herrick, J. E., Huber-Sannwald, E., Jiang,

608   H., Leemans, R., Lynam, T., Maestre, F. T., Ayarza, M., and Walker, B.: Global desertification:

609   building a science for dryland development. Science, 316(5826), 847-851, 2007.

610   Saranga, Y., Flash, I., Paterson, A. H., and Yakir, D.: Carbon isotope ratio in cotton varies with

611   growth stage and plant organ. Plant Sci. (Shannon), 142(1), 0-56, 1999.

612   Schmidt, G., Gebauer, G., Widmann, K., and Ziegler, H.: Influence of nitrogen supply and

613   temperature on stable carbon isotope ratios in plants of different photosynthetic pathways (C$_3$,

614   C$_4$, CAM). Isotopenp. Isot. Environ. Heal. Stud., 29(1-2), 9-13, 1993.

615   Serret, M. D., Yousfi, S., Vicente, R., Piñero, M. C., Otálora-Alcón G., del Amor, F. M., and Araus,

616   J. L.: Interactive effects of CO$_2$ concentration and water regime on stable isotope signatures,

617   nitrogen assimilation and growth in sweet pepper. Front. Plant Sci., 8, 2180, 2018.

618   Sheng, J., Qiao, Y., Liu, H., Zhai, Z., and Guo, Y.: A Study on the Root System of *Haloxylon*

619   *Aammodendron (C. A. Mey.) Bunge.* Acta Agrestia Sinica, 12, 91-94, 2004.

620   Song, L., Kuang, F., Skiba, U., Zhu, B., Liu, X., Levy, P., Dore, A., and Fowler, D.: Bulk

621   deposition of organic and inorganic nitrogen in southwest China from 2008 to 2013. Environ.

622   Pollut. 227, 157-166, 2017.

623   Sparks, J. P., and Ehleringer, J. R.: Leaf carbon isotope discrimination and nitrogen content for

624   riparian trees along elevational transects. Oecologia, 109, 362-367. doi:

625   10.1007/s004420050094, 1997

626   Stewart, G. R., Turnbull, M. H., Schmidt, S., and Erskine, P. F.: ^{13}C Natural abundance in plant

627   communities along a rainfall gradient: a biological integrator of water availability. Aust. J. Plant

628   Physiol., 22, 51-55. doi: 10.1071/ PP9950051, 1995.

629   Swap, R. J., Aranibar, J. N., Dowty, P. R., Gilhooly III, W. P., and Macko, S. A.: Natural

630   abundance of ^{13}C and ^{15}N in C_3 and C_4 vegetation of southern africa: patterns and implications.

631   Glob. Change Biol., 10(3), 350-358, 2004.

632   Tranan, M. W., and Schubertt, B. A.: Temperature-induced water stress in high-latitude forests in

633   response to natural and anthropogenic warming. Glob. Change Biol., 22, 782-791, doi:

634   10.1111/gcb.13121, 2016.

635   Ubierna, N., Sun, W., Kramer, D. M., and Cousins, A. B.: The efficiency of C_4 photosynthesis

636   under low light conditions in Zea mays, Miscanthus x giganteus and Flaveria bidentis. Plant

637   Cell Environ., 36, 365-381, 2013.

638   von Caemmerer, S., Ghannoum, O., Pengelly, J. J. L., and Cousins, A. B.: Carbon isotope

639   discrimination as a tool to explore C$_4$ photosynthesis. J. Exp. Bot., 65, 3459-3470, 2014.

640   Wang, G. A., Feng, X., Han, J., Zhou, L., Tan, W., and Su, F.: Paleovegetation reconstruction using

641   δ^{13}C of soil organic matter. Biogeosci., 5, 1325-1337. doi: 10.5194/bg-5-1325-2008, 2008.

642   Wang, G. A., and Feng, X. H.: Response of plants' water use efficiency to increasing atmospheric

643   CO$_2$ concentration. Environ. Sci. Technol., 46, 8610-8620, 2012.

644   Wang, G. A., Han, J. M., Zhou, L. P., Xiong, X. G., and Wu, Z. H.: Carbon isotope ratios of plants

645   and occurrences of C$_4$ species under different soil moisture regimes in arid region of Northwest

646   China. Physiol. Plant., 25, 74-81, 2005.

647   Wang, G. A., Han, J. M., Zhou, L. P., Xiong, X. G., Tan, M., Wu, Z. H., and Peng, J.: Carbon

648   isotope ratios of C$_4$ plants in loess areas of North China. Sci. China Ser. D., 49(1), 97-102,

649   2006.

650   Williams, D. G., Gempko, V., Fravolini, A., Leavitt, S. W., Wall, G. W., Kimball, B. A., Pinter Jr, P.

651   J., LaMorte, R., and Ottman, M.: Carbon isotope discrimination by *Sorghum bicolor* under CO$_2$

652   enrichment and drought. New Phytol., 150, 285-293, 2001.

653   Yang, H., Yu, Q., Sheng, W. P., Li, S. G., and Tian, J.: Determination of leaf carbon isotope

654   discrimination in C$_4$ plants under variable N and water supply. Sci. Rep., 7, 351, 2017.

655   Yao, F. Y., Wang, G. A., Liu, X. J., and Song, L.: Assessment of effects of the rising atmospheric

656   nitrogen deposition on nitrogen uptake and long-term water-use efficiency of plants using

657   nitrogen and carbon stable isotopes. Rapid Commun. Mass Spectrom., 25, 1827-1836, 2011.

658   Zhang, J., Gu, L., Bao, F., Cao, Y., Hao, Y., He, J., Li, J., Li, Y., Ren, Y., Wang, F., Wu, R., Yao, B.,

659   Zhao, Y., Lin, G., Wu, B., Lu, Q., and Meng, P.: Nitrogen control of ^{13}C enrichment in

660   heterotrophic organs relative to leaves in a landscape-building desert plant species. Biogeosci.,

661   12(1), 15-27, 2015.

662   Zhang, Y. M., Chen, J., Wang, L., Wang, X. Q., and Gu, Z. H.: The spatial distribution patterns of

663   biological soil crusts in the Gurbantunggut Desert, Northern Xinjiang, China. J. Arid Environ.,

664   68, 599-610, 2007.

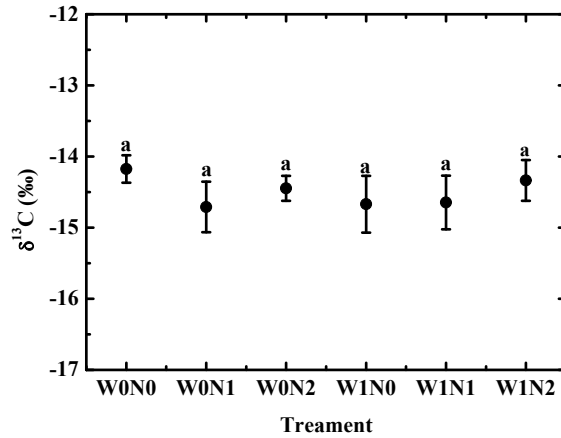
670   Table 1 The p values of all measured and calculated indexs in plants under two-way ANOVA
671   analysis of water (W) and nitrogen (N) additions

	W	N	W*N
$\delta^{13}C$	0.678	0.607	0.563
Photosynthetic rate (A)	0.331	0.008**	0.183
Stomatal conductance (g_s)	0.533	0.871	<0.001***
Transpiration rate (E)	0.622	0.883	<0.001***
c_i/c_a	0.004**	0.009**	<0.001***
ins-WUE	0.002**	<0.001***	<0.001***
int-WUE	0.004**	0.018*	<0.001***
φ	0.644	0.600	0.521

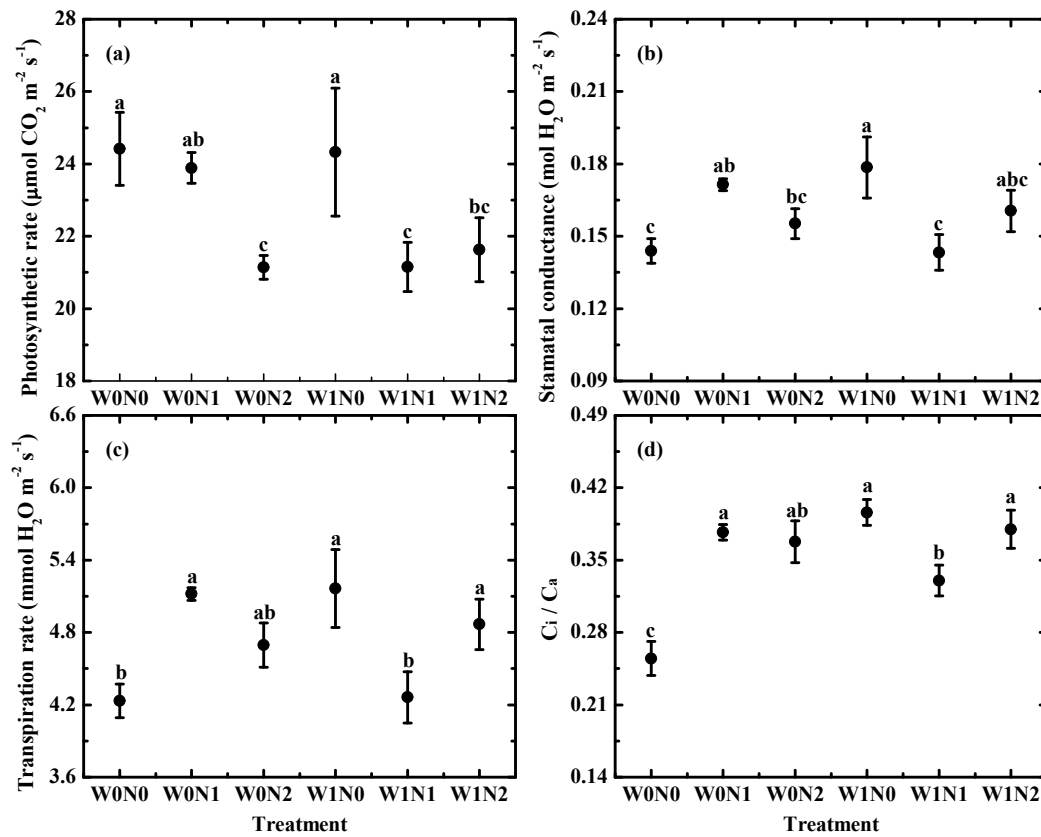
672   Note. *, **, *** indicates a significant influence.

681

682

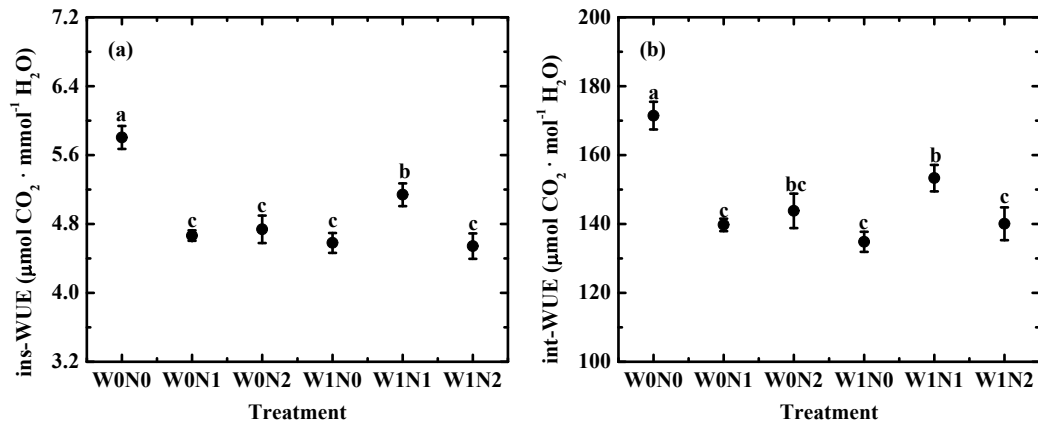
683

684

685 Fig. 1 The $\delta^{13}C$ of assimilating branches of *Haloxylon ammodendron* under water (W) and
686 nitrogen (N) additions. The spot represents the mean value of four replicates with error bars
687 denoting the standard error (SE).

688

689

690 Fig. 2 Variations in photosynthetic rate (a), stomatal conductance (b), water use-efficiency (c) and

691   c_i/c_a (d) across water (W) and nitrogen (N) additions. The spot represents the mean value of four

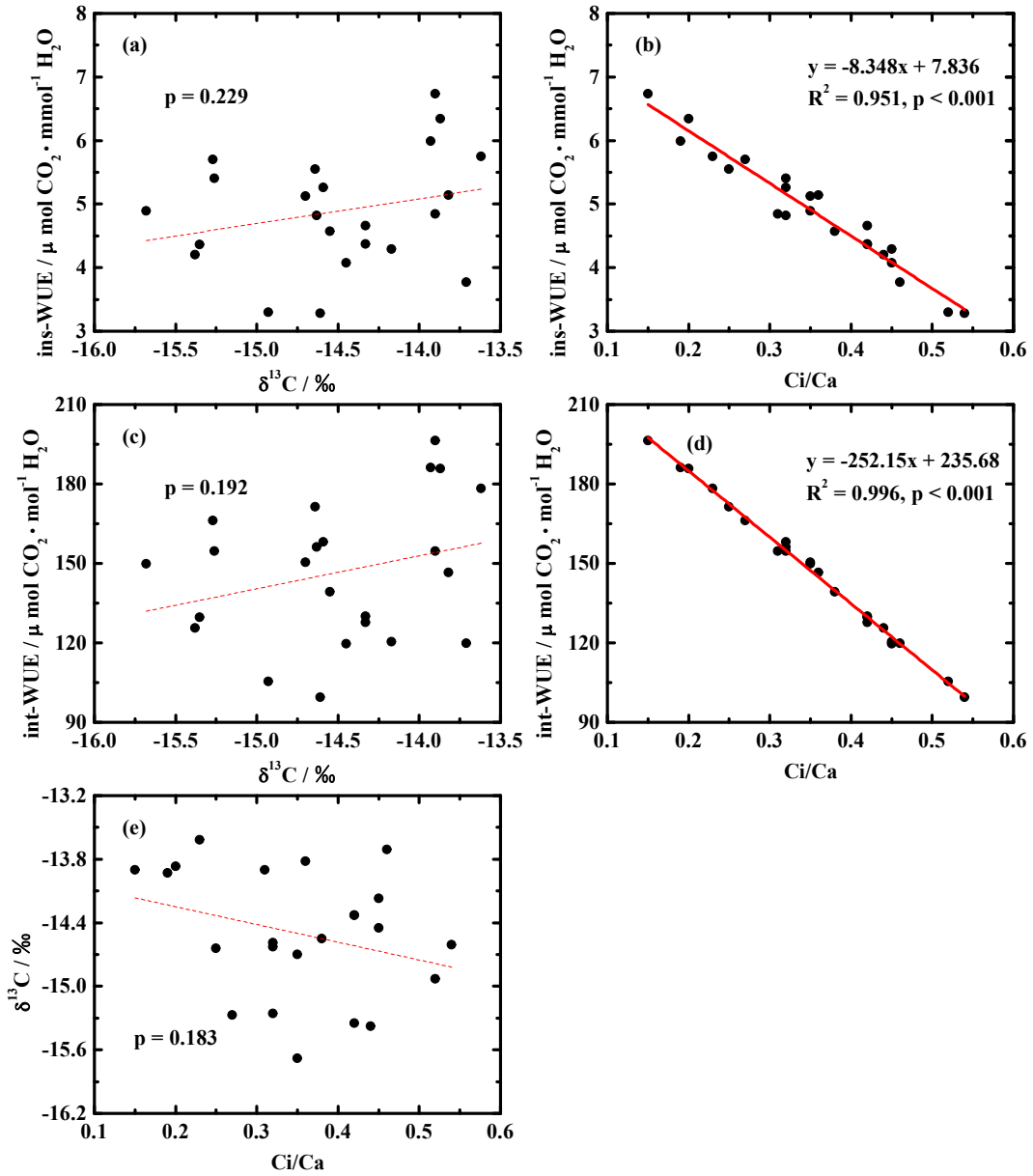
692   replicates with error bars denoting the standard error (SE).

693

694   Fig. 3 Variations in ins-WUE (a) and int-WUE (b) across water (W) and nitrogen (N) additions.

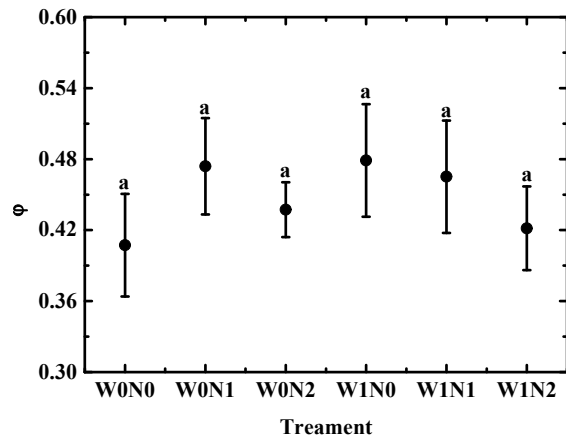
695   The spot represents the mean value of four replicates with error bars denoting the standard error

696   (SE).

697

698

699

700 Fig. 4 Correlations of ins-WUE vs. $\delta^{13}C$ (a), ins-WUE vs. c_i/c_a (b), int-WUE vs. $\delta^{13}C$ (c), int-WUE

701 vs. c_i/c_a (d), and $\delta^{13}C$ vs. c_i/c_a (e) of assimilating branches of *Haloxylon ammodendron*

702
703   Fig. 5 Variations in φ across water (W) and nitrogen (N) additions. The spot represents the mean
704   value of four replicates with error bars denoting the standard error (SE).
705

706