

Reviewer 1

Water use efficiency is an important parameter reflects plant's adaptation to changes in soil water availability. This study assessed whether or not there are linkage between leaf carbon isotope composition and water use efficiency in a C4 shrub, *Haloxylon ammodendron* growing under different water and nitrogen conditions. The authors reported that leaf photosynthetic parameters, but not carbon isotope composition, were affected by water and nitrogen addition treatments. However, carbon isotope composition of assimilating branch was not correlated with water use efficiency, which may have resulted from bundle sheath leakiness and lower activity of carbonic anhydrase. The topic of the study is very interesting and the results are useful for the prediction of plant drought adaptation. However, lack of correlation between carbon isotope composition of assimilating branch and water use efficiency may have also resulted from differences in temporal scale. Carbon isotope composition was determined by the growing condition and photosynthetic discrimination of the period of formation (days); whereas WUE was calculated based on short-term gas exchange measurements (mins). In another word, the measured branch may have emerged a few weeks or even months before the leaf gas exchange measurements. Lack of treatment effects on carbon isotope composition of assimilating branch may associate with rooting depth and water sources. The studied C4 shrub has the ability to access and uptake groundwater, which will reduce its dependency on precipitation and water addition treatment. The authors need to provide more information on plant water sources. Meteorological information is required for the experimental year. L189, using of 450 mmol mol⁻¹ CO₂ need to be justified. L191, why the authors used 1000 umol m⁻² s⁻¹, but not higher photon flux density, such as 1500 or 2000? L191, what are the meanings of "kept stable"? This sentence need to be described more clearer.

Response:

Thank you for your comments! Indeed, we also recognized that differences in temporal scale may lead the carbon isotope composition ($\delta^{13}\text{C}$) to be irrelevant to water use efficiency (WUE). Yet we think our conclusion should be believable, because both water addition and N addition changed the WUE of *H. ammodendron*, but $\delta^{13}\text{C}$ did not show variability across water and N treatments. We have revised the discussion, please see line 401-420.

We agreed with your comments that utilization of groundwater leads to the insensitivity of *H. ammodendron* to rainfall and water addition. In fact, we have found that the root of *H. ammodendron* can be inserted into the soil layer deeper than 3 m, which makes it easier to absorb groundwater. Thus, this is one of the mechanisms behind our observed results. However, the shallow soil water (0-40 cm) and

groundwater are two important water sources for *H. ammodendron* (Dai et al., 2014), and water addition resulted in an increase of soil water contents in shallow soil layer. Moreover, gas exchange changed across treatments in the present study (Fig. 2). Thus, we believed that the utilization of groundwater by *H. ammodendron* may be one of the reasons why its $\delta^{13}\text{C}$ is not sensitive to water and N addition, but it should not be the main reason. The discussion above has been added, please see line 380-390. In addition, the meteorological information in the sampling year have been added, please see line 147-149.

The photo flux density is $1600 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in our measurement. We considered that it is more suitable for measuring gas exchange in *H. ammodendron*, which grew up in a desert area with high light intensity. The $1000 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ written in the previous version is our clerical error. Thank you for picking out our errors. The meaning of “kept stable” is that leaf temperatures were within the range of $29.5 \text{ }^{\circ}\text{C}$ to $30.5 \text{ }^{\circ}\text{C}$ during the entire period of gas exchange measurements. We have revised these error, please see 190-194.

Reviewer 2

Authors have investigated the effect of precipitation change and increasing atmospheric N deposition on $\delta^{13}\text{C}$ of *H. ammodendron*. Numerous studies have doubted the ability of the carbon isotopic composition of C4 plants as a tracer of water-use efficiency. The existing observations on positive/negative correlation between $\delta^{13}\text{C}$ of C4 plants and precipitation change are extremely scanty, and most of the global records advocate for neutral relationship between them. I have some serious doubts on the experimental procedures which are as follows: (I) the age of the sampled leaves have not been mentioned in the manuscript. Usually, plants attain the final $\delta^{13}\text{C}$ at the end of the growing season, and there is a considerable difference between buds, young and matured leaves. The time-lag between actual $\delta^{13}\text{C}$ fixation in the plant and duration of the experimental process could be a valid reason for the correlation obtained in this work. (II) some of the experimental results do not give any definite conclusion. For example, authors have calculated the leakiness values, but at

later stage discarded the values citing the no correlation between $\delta^{13}\text{C}$ and WUE. Rather than simply rejecting their measured leakiness values, authors should provide alternative concepts. Otherwise, the whole exercise becomes futile and should not be incorporated in the main text. Furthermore, desert environments are home for extreme climatic conditions; high day-time temperature, very low precipitation, strong difference between day and night time temperatures. Therefore, the $\delta^{13}\text{C}$ value of modern desert plants cannot be used as an analogue of all the C4 plants, and this work has very limited application. In terms of the presentation, the manuscript is poorly organised, difficult to follow at places, includes wrong information (example line 93) and does not provide any new insight into the subject. As per my opinion, the manuscript, at its current form, does not meet the standard for the publication in Biogeosciences. As authors have conducted tedious experiments, I am open to review the revised manuscript if they substantially modify the manuscript after addressing all the afore-mentioned points.

Response:

Thank you for your comments! Leaf is most effective for the assessment of plant $\delta^{13}\text{C}$. However, the leaves of *H. ammodendron* are degenerated due to extreme drought, we had to collect the assimilating branches of *H. ammodendron*, which was its prime assimilating organ. We know that there is a considerable difference in carbon isotope composition ($\delta^{13}\text{C}$) between buds, young and matured leaves, so we collected the matured assimilation branches at the top of the treetops in each individual, which were synthesized in the year of sampling, to minimize the effect of the age on $\delta^{13}\text{C}$. We have added the information about the sampling, please see line 196-198.

In addition, we also recognized that differences in temporal scale may lead the $\delta^{13}\text{C}$ to be irrelevant to water use efficiency (WUE). Yet we think our conclusion should be believable, because both water addition and N addition changed the WUE of *H. ammodendron*, but $\delta^{13}\text{C}$ did not show variability across water and N treatments. We have revised the discussion, please see line 401-420.

Thanks for your suggestion on the ϕ value, The definite conclusion obtained from

this study is that the ϕ value of *H. ammodendron* does not change with water and N addition, implying that environmental conditions may have no influence on ϕ value, which is still inconclusive. We have added this conclusion, please see line 435. In addition, since we found that the mean ϕ value is 0.45, it seems that ϕ is not the driver of the observed $\delta^{13}\text{C}$ pattern in *H. ammodendron*. However, due to the time-lag of measured $\delta^{13}\text{C}$ and c_i/c_a , there were some uncertainties in the calculation of ϕ value based on the measured $\delta^{13}\text{C}$ and c_i/c_a . Therefore, we considered that the special ϕ value (0.37) may be one of the mechanisms behind the unchanged $\delta^{13}\text{C}$ in *H. ammodendron*. We have revised the discussion about the ϕ value, please see line 356-362.

The results gained from the present study are not necessarily analogous to all C_4 plants due to the extreme climatic conditions in desert ecosystem. Yet we believed that this work has important application for enhancing our understanding of physiological responses of desert plants to future changes in precipitation and atmospheric N deposition. This is because *H. ammodendron* is a dominant species in desert regions, especially in Asia desert, which has a great effect on the stabilization of sand dunes, the survival and development of understory plants and the structure and function of desert ecosystems (Sheng et al., 2005; Su et al., 2007; Cui et al., 2017). Thus, the prediction of plant drought adaptation in *H. ammodendron* is crucial in desert ecosystem. We have added the significance of this study, please see line 421-429.