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Water using source of a perennial semi-shrub *Reaumuria soongorica* as measured by water isotopes in Gobi Desert, Inner Mongolia, China

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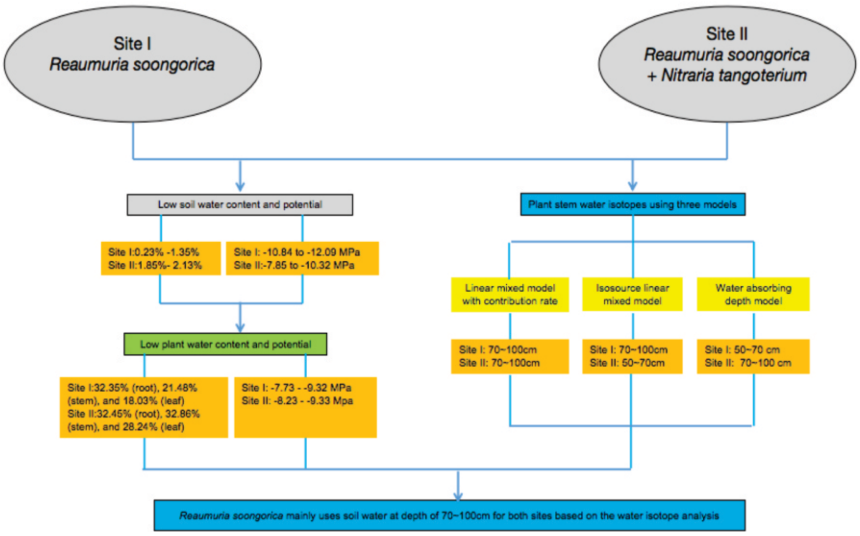
Abstract

Aim: *Reaumuria soongorica* plays significant role in wind induced soil erosion and combating desertification due to its unique adaptation mechanisms in the Gobi Desert. This study was conducted to investigate its water using sources revealed by hydrogen and oxygen stable isotope as one of its adaptive strategies in Gobi Desert.

Methodology: Field experiment was designed in Alashan Desert, China to evaluate the soil and plant water conditions from two *R. soongorica* sampling sites. Plant and soil water potential were measured by using the Psypro dew point water potential meter equipped with C-52 sample chamber. Water isotopes was measured based on hydrogen (Δ^2H) and oxygen (δ^{18}O) stable isotopes.

Results: The results showed that the highest soil moisture content in both sites at 70~100 cm was 2.08% and 1.28%, respectively. The average leaf water content of *R. soongorica* ranged from 18.032% to 38.063% and the average water potential of its root, stem, and leaf was -8.89 and -9.71 MPa for site I and II, respectively. Δ^2H and δ^{18}O stable isotope analysis showed that soil water source for *R. soongorica* for both sites was from layer of 70~100 cm.

Interpretation: *R. soongorica* uses water mainly from the medium soil layer and the results uncover its water using strategies as one of adaptive mechanism in Gobi Desert.



Key words: Gobi desert, Isotopes, *Reaumuria soongorica*, Water content, Water potential, Water using sources

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Introduction

Alashan Plateau lies between the border of China and the Hexi Corridor at an elevation of 1000-1500 m. Its south is higher than the north and is surrounded by Mazong Mountains and the Helan Mountains from west to east, respectively. The landscape of the plateau is desert and desert steppe with moving sand dunes (Li *et al.,* 2015). Due to wind erosion and low groundwater level (10 m below ground) in Alashan Desert, the surface soil layer is desalted and the subsoil is accumulated with high level of salt which creates difficulties for plant survival (Yu and Chen 1999). For different species, rooting depth (Flanagan *et al.,* 1992; Leffler and Caldwell 2005) and vertical distribution of water contents (Dodd *et al.,* 1998; Leffler and Caldwell 2005) play an important role in its ability of extracting water from different soil layers. For instance, *Sabina vulgaris* and *Salix matsudana* in Maowusu Desert depend mainly on deep soil water and groundwater for their survival, while *Artemsia ordosica* relies on shallow soil water to survive (Ohte *et al.,* 2003).

The potential available water sources to plants of different species vary on precipitation, soil water, and groundwater. Plants can use primarily groundwater, precipitation recharged soil water, or a mixture of two (Chimner and Cooper 2004). If plants use primarily groundwater, a water table decline may reduce water availability and lead to high plant mortality. However, if plants can acquire precipitation recharged soil water, then groundwater decline can have less impact on plants (Chimner and Cooper, 2004). *R. soongorica* is a xerophyte and constituent species for desert vegetation which distributes mainly in Alashan Desert of China (Huang *et al.,* 1987; Ma and Kong, 1998). The length of the main root of *R. soongorica* in Alashan Desert is approximately one meter, whereas water table at the sampling location is at a depth of 2~3 m and deep groundwater is generally at a depth of 10~15 m. Due to high evaporation of the desert, it is difficult for *R. soongorica* to absorb soil water or groundwater (Li and Yan, 2008). “How does *R. soongorica* uptake water from desert environment” is the question that to be answered. Therefore, investigating the water using sources of *R. soongorica* will help in understanding its survival mechanism and adaptability as well as its cultivation in combating desertification.

Soil and plant tissue water can be sampled to estimate the abundance of hydrogen or oxygen stable isotope ratios of water to compare the possible water sources in the root zone to indicate the origin of water in plant (Phillips *et al.,* 2005) since soil water moves from soil into roots and then to stems of plants without fractionation or isotopic contamination (Dawson and Elheringer 1991; Dawson, 1993; Thorburn *et al.,* 1993; Thorburn and Mensforth, 1993; White *et al.,* 1985). The commonly used method is via measuring hydrogen or oxygen stable isotope signatures in the fields of environment, plant, hydrology, and agriculture to study water using strategy and determine the sources of plant water (Phillips and Gregg, 2003). For instance, two-sources or three-sources linear mixed model (Thorburn *et al.,* 1993; Zhang *et al.,* 2012), IsoSource linear model (Phillips and Ehleringer 1995), and water absorption depth model (Romero-Saltos *et al.,* 2005) are the main methods of using stable isotope techniques to study the contribution rate of water sources to plants. Due to extreme drought and high evaporation in Alashan Desert, investigating the potential water sources of *R. soongorica* plays important role in its survival and adaptation. Therefore, this study was designed to characterize the soil profile and *R. soongorica* distributed from sampling sites of Alashan Desert and to estimate its water using sources based on hydrogen and oxygen stable isotopic analysis.

Materials and Methods

Experimental design and sampling sites: In July 2019, two representing *R. soongorica* communities with different ground water level in Ejina Qi, Alashan, Inner Mongolia, People’s Republic of China were selected as sampling sites. Site I is located in the north of Swan Lake Road where *R. soongorica* serves as dominant species and the main soil type is sand with ground water locates 1.0 m below ground. Its soil pH is 8.2 and soil salinity is 0.89 ms.cm^{-1} (Liu *et al.,* 2018). Site II is located in the north-east of the railway station where *R. soongorica* is mixed with *Nitraria tangoterium* and the main soil type is loam and sand with ground water level of 1.0 m below ground. Its soil pH is 8.5 and salinity is 0.87 ms.cm^{-1} (Liu *et al.,* 2018).

Soil sampling and analysis: The soil profile samples for water content were made under the middle of plant crown at soil surface for both sties. Soil samples were also taken every 30 cm between 70 and 100 cm for site I and II, respectively. One part of the soil samples was used for measuring soil water content. The other part was stored in a screw-vial with the cap tightened and sealed with parafilm stored in a cooler with ice package in the field until transferred to 4°C refrigerator in the laboratory for determination of hydrogen and oxygen isotope. Three technical replications were conducted for each site.

Plant sampling and water content analysis: Six *R. soongorica* plants with same growing stage were selected from each site for plant sampling. In order to exclude the effect of external conditions such as light intensity which may affect the results of isotope analysis, the sampling was completed between 9:00 and 11:00 a.m. A twig from a one-year-old branch on a two-year-old branch was selected and the leaf was simultaneously excised from the twig. The leaf and stem samples were placed in a 4-ml screw-vial with cap tightened and parafilm sealed in a cooler with ice package in the field until transferred to -20 °C in laboratory for measurement. Roots were dug out from ground and collected and processed the same as stem and leaf from the chosen *R. soongorica* plants in each site. Three technical replications were conducted for each time. Leaf relative water content was measured and calculated according to Barrs and Weatherley (1962).

Plant water potential: Soil and plant water potential was determined by using the Psypro dew point water potential meter equipped with C-52 sample chamber (Wescor, USA). The plant water potential of *R. soongorica* in predawn was measured at 6:00 a.m. and in midday was measured at 13:00 p.m. Three replications were conducted for each measurement.

Isotope analysis: The isotope content of water in soil and plant samples was extracted by automatic ultra-low-pressure vacuum condensation equipment (Li-2100, Lijia United, Beijing, China). The hydrogen and oxygen isotope ratios of xylem water and soil water were determined by liquid water isotope analyzer of LGR (LWIA-30d, Los Gatos Research). The stable isotope compositions were expressed using the standard denotation (δ^2H and δ^{18}O) and were reported in per mil (%) after normalization to Vienna Standard Mean Ocean Water (VSMOW) scale (Dawson, 1993).

Statistical analysis: Linear mixed model with contribution rate (Dawson, 1991), water absorbing depth model (Romero-Saltos *et al.,* 2005), and Isosource linear mixed model were used to quantify the relative magnitude of multiple soil layers and predict water using sources. Excel 2007 was used to organize data and basic calculations. Two-way ANOVA was analyzed in SAS (SAS 9.0, Windows). Origin software (Origina 8.0) was used for graphing.

Results and Discussion

The structure and ecosystem of desert plant community is severely affected by drought stress due to low rainfall and high evaporation (Hou *et al.,* 2013). The study found that soil moisture content of *R. soongorica* communities from two sampling sites increased with the increase in soil depth (Fig. 1). The soil moisture content of site I ranged from 0.23% to 1.35% between 0 to 100 cm of soil depth whereas at 70~100 cm it was 1.28% (Fig. 1A). For site II, it ranged from 1.85% to 2.13% between 0 to 100 cm of soil depth whereas at 70~100 cm it was 2.08% (Fig. 1B). Such low soil water content leads to low soil water potential at different depth of soil layers and low *R. soongorica* water potential in Alashan Desert. The soil water potential decreased with increase in soil depth and the deep soil water potential was significantly lower than the surface soil water potential (Fig. 1C-D). The soil water potential of site I ranged from -10.84 to -12.09 MPa between 0 to 100 cm soil depth (Fig. 1C). For site II, it ranged from -7.85 to -10.32 MPa (Fig. 1D). This type of low soil water potential may result in difficulty for plants to absorb soil water. This study found that the average root, stem, and leaf water content of site I was 32.35%, 21.48%, and 18.03%. (Fig. 2A-C), while, for site II, it was 32.45%, 32.86% and 28.24%, respectively (Fig. 2A-C). The

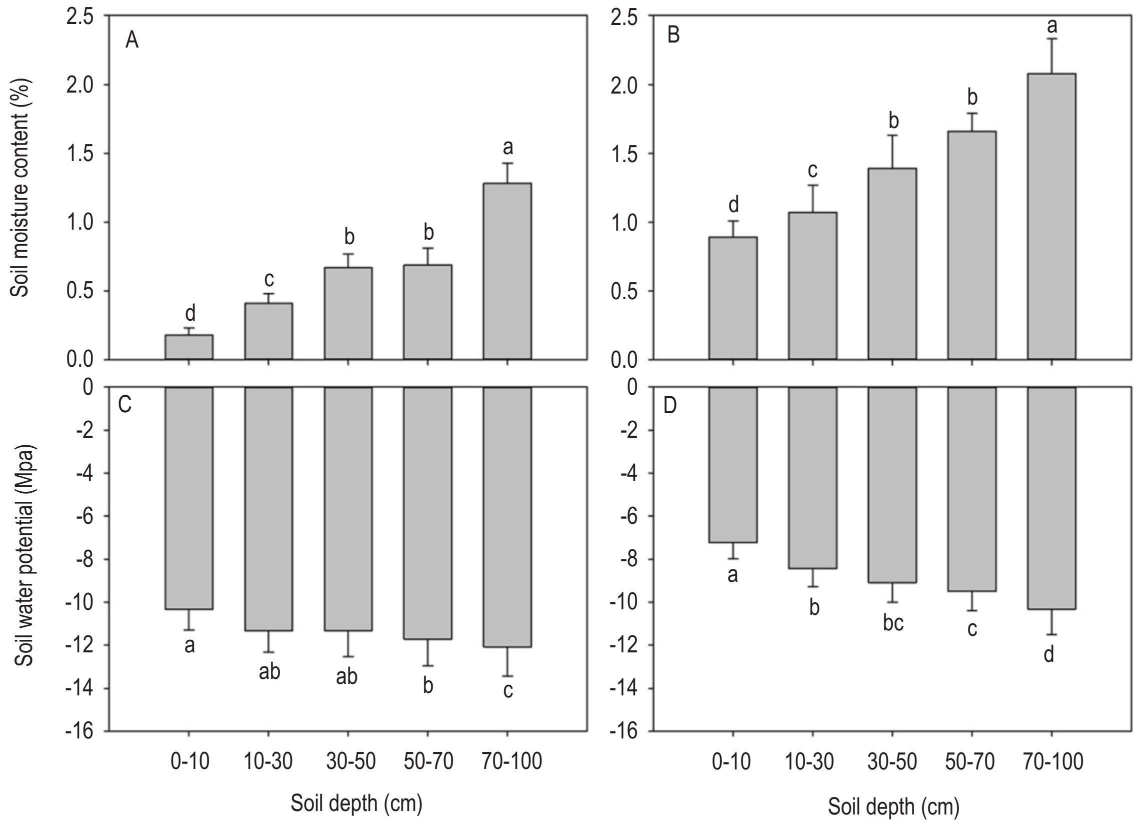

Fig. 1: Dynamic changes of soil moisture content at site I (A) and II (B) at different soil depth; soil water potential in site I (C) and II (D) at different soil depth. Note: a, b and c on top of or below the figure bar represent a significant difference (P<0.05).

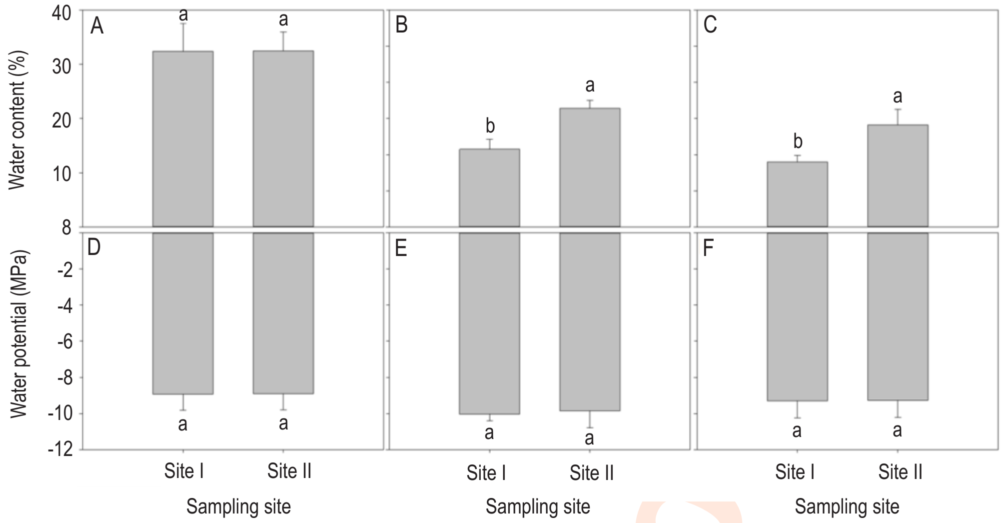

Fig. 2: Changes in plant water content in root (A), stem (B), and leaf (C) as well as plant water potential in root (D), stem (E), and leaf (F) from two sampling sites. Note: a, b and c on top of or below the figure bar represent significant difference ($P<0.05$).

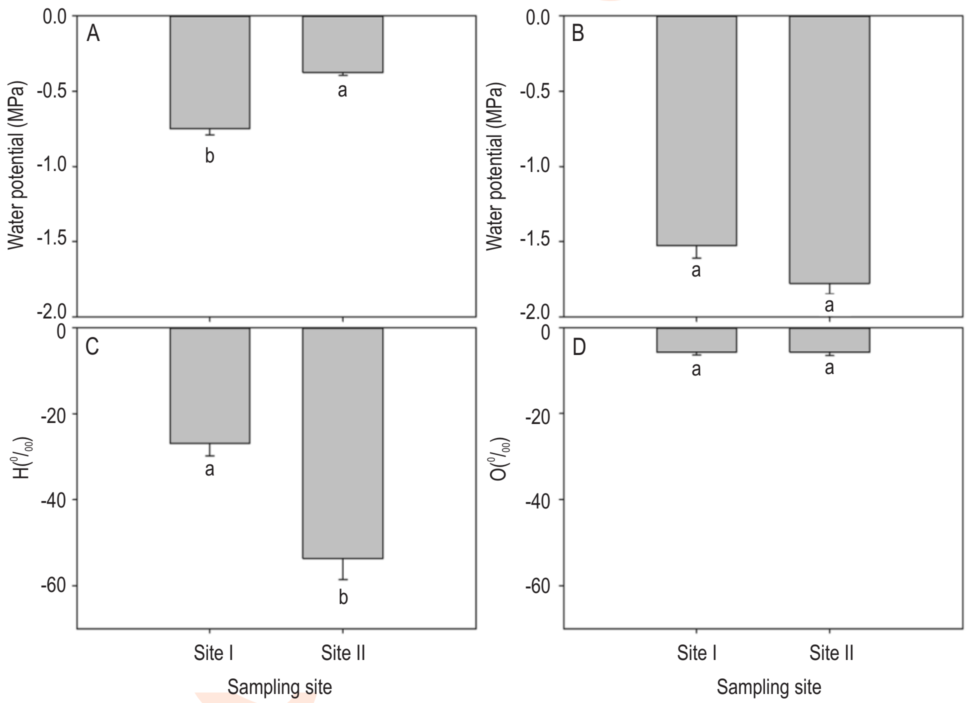

Fig. 3: Changes of plant stem water potential at predawn (A) and midday (B) from two sampling sites; hydrogen (C) and oxygen (D) isotope signatures as unit of ‰ for plant stem water from two sampling sites.

overall water content of *R. soongorica* decreased in the following order as root > stem > leaf for both sampling sites (Fig. 2A-B), which indicate that the roots of *R. soongorica* is the main water storage organ. The level of plant water potential also reflects its ability to absorb water from the surrounding environment which shows close relationship with plant water absorption. The average leaf, stem, and root water potential of *R. soongorica* of site I, ranged from -7.73 to -9.32 MPa (Fig. 2D-F). For site II, it ranged from -8.23 to -9.33 MPa (Fig. 2D-F). The lower the water potential of the plant, the stronger is its water absorption capacity.

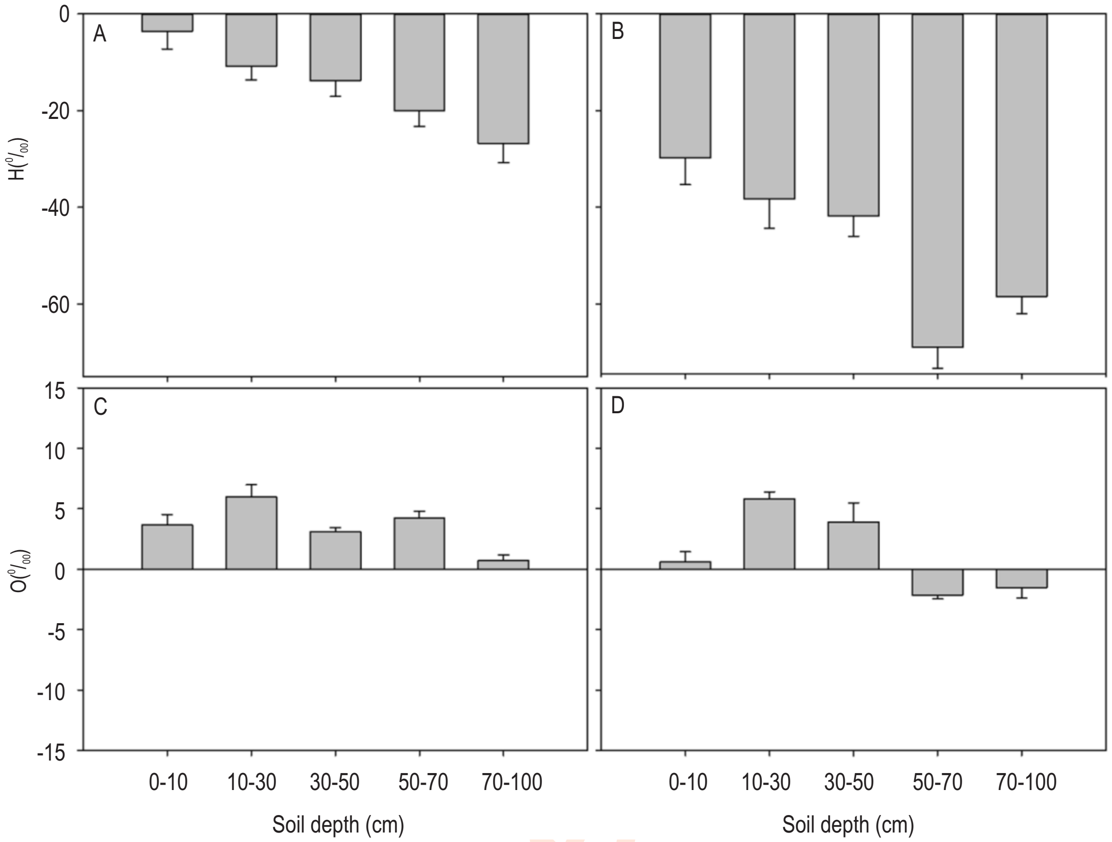

Fig. 4: Hydrogen isotope signatures of xylem water from *R. soongorica* at site I (A) and II (B); oxygen isotope signatures of xylem water from *R. soongoricain* site I (C) and II (D) as unit of ‰.

Predawn and midday plant leaf, stem, and root water content and potential were also low and affected by low soil water content and high temperature. The stem water potential of both sampling sites in predawn (-0.43 to -0.75 MPa) was slightly higher than that of midday (-1.73 to -1.85 MPa; Fig. 3A-B), which indicates water loss due to increased midday temperature. It also implies that plant stem plays an important role in buffering water movement from root to leaf for both sites. The significantly decreased water potential during midday indicates that transpiration tension increases when temperature rises and *R. soongorica* can only continue to absorb water from soil by lowering its water potential. Soil water potential in predawn indicates the soil moisture status at that time (Atklnson *et al.,* 2000; Drake and Franks 2003; Hochberg *et al.,* 2018). This is similar to the assumption that plant water potential in midday measured at the highest temperature throughout the day reflects the plant water potential and drought level at high temperature (Xavier *et al.,* 2001). It is thus known that *R. soongorica* is subjected to severe water stress during midday.

The stable isotope ratios ($\delta^{18}O$) of water derived from plant stems reflect various potential water sources that plant uses (Grieu *et al.,* 2001; Jackson *et al.,* 1999; Adams and Grierson 2001; Duan and Ouyang, 2007). Since no fractionation or contamination of water occurs during root uptake process, $\delta^{18}O$ and $\delta^{2}H$ values of xylem water can provide an integrated estimation of water uptake by roots (Dawson and Ehleringer, 1991). Thus, the main water source used by plants can be determined by comparing $\delta^{18}O$ and $\delta^{2}H$ signatures of potential water sources (Jackson *et al.,* 1999). For instance, isotopic signatures of soil water can be measured to indicate plant water using sources (Flanagan *et al.,* 1992; Gat 1996). Here, the stem water $\delta^{2}H$ and $\delta^{18}O$ isotope signatures in predawn (-23‰-52‰) were significantly lower than midday (-8.2‰) from both sampling sites (Fig. 3C-D) and precipitation is not considered as the main water source for *R. soongorica* (Fig 3A-D). Therefore, a relatively stable water source from deeper soil profiles under such extreme drought region is necessary for exploring its survival and adaptation under such conditions in combating desertification and future landification.

The plant stem water $\delta^{2}H$ and $\delta^{18}O$ signatures from both sampling sites had similar trend while the values in site I was slightly higher than in site II at different soil depths (Fig. 4A-D). By comparing the $\delta^{2}H$ and $\delta^{18}O$ values of the stem water of *R. soongorica* with each potential water source, the $\delta^{2}H$ and $\delta^{18}O$

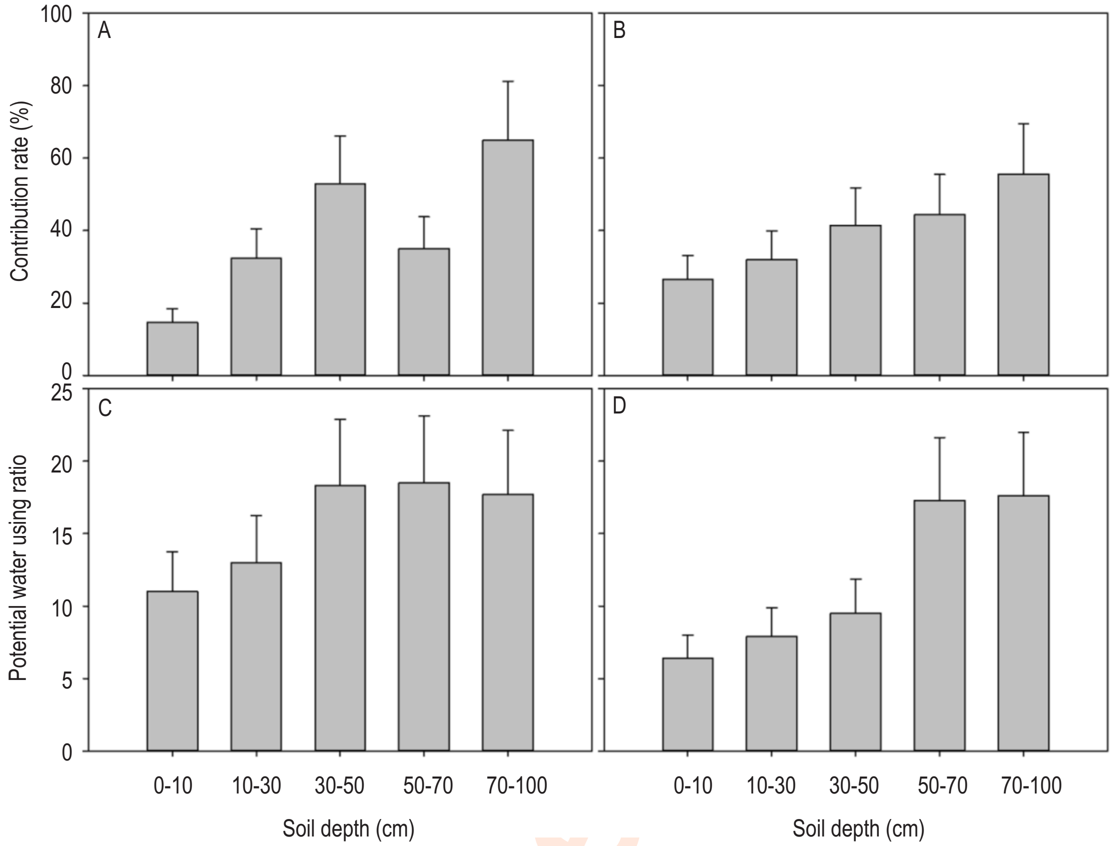

Fig. 5: Contribution rate of isotope to *R. soongorica* in each soil layer at site I (A) and II (B); potential water sources of *R. soongorica* in each soil layer at site I (C) and II (D) based on the water absorbing depth model.

values of stem water of *R. soongorica* of both sites were close to those of soil water at 70~100 cm, implying that *R. soongorica* of two sites mainly uses soil water at 70~100cm (Fig. 4). These results confirmed the assumption that plant may utilize the water if the stable isotope values of the xylem water of a plant stem are roughly in the same range as those of the potential water source (Brunel *et al.,* 1995; Asbjornsen *et al.,* 2007; Li *et al.,* 2007). Three years of field observations conducted at three sites along aridity gradient from the middle to lower reaches of the Heihe River basin, northwestern China using stable oxygen composition ($\delta^{18}O$) from plant xylem water showed that *R. soongorica* relies on groundwater rather than precipitation-derived water (Zhang *et al.,* 2017) which is consistent with what was reported in this study. A similar study (Gat *et al.,* 2007) found that trees and large shrubs of Tamarix can take up water below 40 cm based on similar isotope signatures to the value of soil water approximately.

Recently, Zhang *et al.* (2020) reported that *R. soongorica* progressively switched to suck up deeper soil water and increased the water use proportion from 0.5% to 84.4% as the seasons changed, indicating a greater degree of ecological plasticity and enhanced adaptability to arid environments. In order to identify the most probable sources of water transpired by plants, contribution rate of isotopic analysis plays an important role in determining the source of water use by a plant using IsoSource model (Phillips and Ehleringer 1995). The isotope values from xylem water of *R. soongorica* and the soil water were almost in the same range, and combining it with the soil moisture content, can initially obtain the main water source of *R. soongorica* from different sites. Greater the contribution rate of soil water to *R. soongorica*, the higher the frequency and more likely *R. soongorica* absorbs that soil water.

For both sites, the contribution rate of soil water to *R. soongorica* was highest at 70~100 cm which were 64.97% and 55.61% for site I and II, respectively, indicating that soil water at 70~100 cm was the main water source (Fig 5A-B). Lin *et al.,* (1993) showed some salt-tolerant and drought-tolerant plants may undergo isotopic fractionation in their roots during the process of absorbing water to the stem. The vibration caused by ^{1}H instead of ^{2}H in water molecules is much larger than that caused by ^{16}O instead of ^{18}O, indicating that the isotopic fractionation of H is more obvious than that of O. Therefore, the $\delta^{18}O$ value of xylem of plant stem is more accurate and should mainly be considered while using the IsoSource model. However, the $\delta^{18}O$ value for *R. soongorica* for both sites was lower than $\delta^{2}H$

which implies that *R. soongorica* may not discriminate for water absorption for salt tolerance. We recently found that it could secrete salt using a salt gland present in the leaf (Liu *et al.,* 2021). According to the contribution rate, the water using source for site I and II were 70~100 cm and 50~70 cm, respectively.

Plants tend to have deep roots and extract water from deep soil layers or groundwater in water-limited environment where water evaporation exceeds precipitation in which water are likely to be unavailable in upper soil layers (Romero-Saltos *et al.,* 2005; West *et al.,* 2008; Wu *et al.,* 2016). On the basis of water absorbing depth model, the average water absorption depth by *R. soongorica* was calculated by δ^2H and δ^{18}O isotope values and their occurring frequency (Fig. 5C-D). The average water absorption depths of *R. soongorica* in site I and II were 72 cm and 92 cm, respectively (Fig. 5C-D). The average depth of soil water absorbed by *R. soongorica* calculated by δ^{18}O and δ^2H value can also be confirmed by the distribution of its root system in the soil. In this study, the maximum soil moisture content of site I and II was 1.28% (70~100 cm) and 2.08% (70~100 cm), respectively. This is consistent with the phenomenon of higher soil moisture content of the corresponding root system which is an important feature of *R. soongorica* adapted to extreme arid environment of the desert. The water absorption depth is considered as a reference to the above mentioned methods. Based on the water absorbing depth model, the water absorption depths of *R. soongorica* in site I and II were 50~70 cm and 70~100 cm, respectively.

In summary, the soil water content and potential in Gobi desert in Inner Mongolia was extremely low which leads to low plant leaf, stem, and root water content and potential in R. soongorica. Based on three calculation methods, the water isotopics of soil at the depth of 70-100 cm correlates to the plant water isotopes in *R. soongorica*. This indicates that *R. soongorica* uses water mainly from the medium soil layer and the results uncover its water using strategies as one of adaptive mechanisms in Gobi Desert.

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Add-on Information

Authors' contribution: **R. X. Liu:** Consived and designed the research and provided funding support from her research grant. Revised and approved manuscript; **B. Liu:** Conducted the research work, analyzed the data; **Y. M. Ma:** Wrote and revised the manuscript; **S. C. Chen, H. F. Hu and Y. J. Mi:** Revised and approved the final manuscript.

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