Soil water use sources and patterns in shrub encroachment in semiarid grasslands of Inner Mongolia

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ABSTRACT

Soil water sources for shrub encroachment in arid and semiarid regions have not been fully understood. This study used stable δ^2H and $\delta^{18}O$ isotope ratios of plants, soil water and precipitation, coupled with proportional similarity index of species to identify relations between shrub encroachment and water sources for four shrub and grass species at various shrub encroachment stages in a semiarid grassland of Inner Mongolia. We found that shallow rooted grasses (*Agriophyllum squarrosum* and *Stipa bungeana*) predominantly used shallow soil water (0–20 cm) during growing season. In contrast, deeply-rooted semi-shrubs (*Artemisia ordosica* and *Artemisia sphaerocephala*) used middle and deep soil water in July but shallow and middle soil depth water in May and September. Plants competition for soil water in different shrub encroachment stages were different depending on plant water use sources. The highest plant competition existed in the *A. ordosica* community in semi-fixed sandy land, followed by *A. squarrosum* community in shifting sandy land, *A. ordosica* community in fixed sandy land, and *S. bungeana* community in fixed sandy land. Competition for soil water and differences of water use sources of grass and shrub species were crucial in driving shrub encroachment in semiarid grasslands. Communities dominated by *A. ordosica* were not stable, with an increase in surface soil water availability, the dominance was replaced by *S. bungeana*. Our findings on the linkage between plant water use sources and patterns and shrub encroachment shall help future arid and semiarid grassland management under changing climate conditions.

1. Introduction

Shrub encroachment is a global phenomenon of the proliferation and range expansion of woody plant species in arid and semiarid grassland ecosystems (Maestre et al., 2009). Shrub encroachment can be defined as the increase in density, cover and biomass of native or non-native woody plants (Archer et al., 2017; Van Auken, 2000). This phenomenon is likely due to multiple interacting factors at multiple scales, such as fluctuating climatic conditions (Knapp et al. 2008), increasing atmospheric CO_2 concentration (Archer, 2010), overgrazing, reduced fire frequency (Scholes and Archer 1997; Van Auken, 2009) and historical land management practices (Archer et al., 2017). Encroachment of woody species induces vegetation change and landscape fragmentation (Scholtz et al., 2018), reduces herbaceous productivity, increases the amount of bare ground, and is considered one of the most threatening forms of rangeland degradation in arid and semiarid areas (Eldridge et al., 2011). Global climate models and the observation data indicated that drylands will likely continue to expand during the twenty-first century and that woody plant encroachment will increase under climate change (Huang et al., 2016; Knapp et al., 2008). Accordingly, understanding shrub encroachment is important to ecology.

Water is the most crucial limiting factor that determines the evolutionary trends of vegetation in arid and semiarid ecosystems (Li et al. 2013; Reynolds et al., 2007). Plant water use patterns are affected by numerous physiological and physical characteristics (Volkmann et al., 2016), such as soil water availability (Gow et al, 2018) and distribution of fine roots (Lanning et al., 2020; Wang et al., 2017, 2021). When shallow soil water is unavailable in dry period, species with deep roots

use deeper soil water or groundwater than shallow-rooted species (McCole and Stern, 2007; Wang et al., 2019). This capacity to shift water use along with soil water availability is crucial for plant to survive in water-limited areas (Wu et al., 2016). Shrubs have deeper rooting systems compared with grasses that have different water use patterns (Schenk and Jackson, 2002). The absorbing roots of shallow-rooted grasses are restricted to topsoil profile. Therefore, they are sensitive to rainfall pulses and exhibit a heightened ability to exploit water stored in shallow soil layers (Wu et al., 2019a). There are some hypotheses about water use for shrubs and grasses. For example, Walker et al. (1981) suggested a two-layer hypothesis that shrubs and grasses exploit different hydrological niches, with deep-rooted shrubs using deeper soil layer water and shallow-rooted grasses using shallow soil layer water. Nevertheless, these water source hypotheses stated that growth pool (shallow soil water) is rapidly absorbed with high competition among plants, while maintenance pool (deeper soil water) maintains physiological activities and survival of deep-rooted plants with low competition (Ryel et al., 2008). In addition, stress gradient hypothesis indicates that in mixed tree-grass systems, trees facilitate grass growth in drier regions and suppress grass growth in wetter regions (Dohn et al., 2013) due to hydraulic lift. In contrast, Barron-Gafford et al. (2017) suggested that hydraulic redistribution regime is characterized predominantly by hydraulic descent relative to hydraulic lift. D'Odorico et al., (2007) found that positive feedback could exist between soil moisture and woody vegetation caused two stable states corresponding to conditions with and without tree canopy cover in arid and semiarid ecosystems. However, there is no universal hypothesis to explain water use patterns of woody species and grasses. The possible reason for the difference among various researches and hypotheses might be due to the difference of woody species, such as tree versus shrub or semi-shrub that is similar to shrub, but twigs at top positions died annually. Trees not only use soil water from different layers, but also have larger effects on soil moisture because of different soil evaporation induced from larger and higher canopy coverage compared with shrubs (Barron-Gafford et al., 2017; D'Odorico et al., 2007; Walker et al., 1981). Therefore, exploring water use patterns between co-occurring grass species, and woody species that belong to same functional group, such as shrub species, is critical to improving the knowledge related to hydrological niche differentiation in water-limited shrub encroached grasslands.

The stable isotope technique provides an effective and powerful method for revealing and partitioning the different potential water sources used by plants (Dawson et al., 2002; Rothfuss and Javaux, 2017). Previous studies have proven that no isotopic fractionation of water occurs during water absorption by roots and transportation along shoot before transpiration in terrestrial plants (Dawson et al., 2002; Ehleringer and Dawson, 1992) except for some coastal wetland species and woody xerophytes, and these coastal wetland species and woody xerophytes seem to fractionate H (but not O) due to symplastic movement of water during uptake (Ellsworth and Williams, 2007; Lin and Sternber, 1993). In addition, this method is effective only when there is a gradient in water isotope ratios with soil depth caused by evaporative enrichment at soil surface (Brum et al., 2018). Eggemeyer et al. (2009) and Nippert and Knapp (2007) suggested that grasses extract most of water from upper soil layers but woody species exhibit obvious plasticity in water uptake according to soil water availability by stable isotopic ratios of hydrogen and oxygen. However, most of these researches focused on seasonal variations in water sources use, and insufficient attention was paid on hydrological niche differentiation between co-occurring species. Enabled by method advances, new insights into plant water use can yield important information with which to predict the effect of future climate change on hydrological niche differentiation between shrubs and grasses and its relevance to shrub encroachment in arid and semiarid areas.

The grasslands of Inner Mongolia, a central part of the Eurasian steppe, account for approximately 24% of total grassland area in China and have experienced prevalent shrub encroachment due to climate warming and livestock overgrazing during recent decades (Peng et al., 2013). The Ordos Plateau located in the southern part of Inner Mongolia is one of the desertification centers of China. It was historically a grassland with abundant grass before the Yuan dynasty (1271–1368) (Zheng et al., 2020). Due to anthropogenic activities, such as overgrazing and land-use change over a long period, regional grassland dominated by a native perennial grass, *Stipa bungeana*, has been replaced by a native semi- shrub species, *Artemisia ordosica*. Some researchers have shown the relationship between shrub encroachment and soil water content changes in different soil layers and water use patterns of dominant species (Yang et al., 2019; Zheng et al., 2020). For example, Yang et al. (2019) found that with its high enzyme activities, *S. bungeana*, developed well comparing with semi-shrubs when soil water content improved in shallow soil layer. After grazing, increase of soil fine particles and moisture in top soil layer induces native grassland restoration from shrub encroachment grasslands (Zheng et al., 2020). However, water use sources from different soil layers for shrub and grass species during shrub encroachment are still under debate.

We hypothesize that *S. bungeana* utilizes top layer soil water, but *A. ordosica* uses deeper layer soil water, and these source uses vary with seasons. The objectives of this research are to examine what the seasonal water use patterns of shrub and grass species are and how these water source uses for shrub and grass species will change at different shrub encroachment stages.

2. Materials and methods

2.1. Study area

This study was conducted in town of Ejin Horo in the middle of the Ejin Horo Banner, on the Ordos Plateau (37°35′24″–39°29′37.6″ N, 106°42′40″–111°27′20″ E), a typical semiarid region located in the southern Inner Mongolia (Yang et al., 2019). The study area is influenced by a typical semiarid continental climate, with an annual average temperature of 6.3 °C and annual precipitation of 346 mm. Most of precipitation is from July to September, accounting for more than half of the annual precipitation. The average annual sunshine time is 3011 h, and the frost-free period generally lasts 137–154 days. The altitude is approximately 1350 m. Local soil types include loam, chestnut and sandy soil. The climatic climax vegetation is a warm-temperate *S. bungeana* steppe. At present, *A. ordosica* is the dominant species of the most widely distributed sandy plant community.

2.2. Experimental design and sample collection

Field experiments were conducted in May, July and September 2019 during growth period of plants, which represented spring, summer and autumn, respectively. Landscapes on the Ordos Plateau influenced by long-term shrub encroachment can be divided into shifting sandy land, semifixed sandy land and fixed sandy land (Cai et al., 2018). Different shrub encroachment stages were determined based on landscapes and dominant species (Table 1), i.e., extremely severe: shifting sandy land with annual species (*Agriophyllum squarrosum*) (SA), severe: semifixed sandy land dominated by semi shrub species (*A. ordosica*) (SFA), moderate: fixed sandy land dominated by semi shrub species (*A. ordosica*) (FA) and slight: fixed sandy land dominated by perennial grass species (*S. bungeana*) (FS) (Ding et al., 2011; Yang et al., 2019). Sample sites were set up in four types of plant communities (SA, SFA, FA and FS), and there were three replicates for each type of plant community. In each replicate, one 20 × 20 m plot was established to collect soil, roots and isotope samples.

Two days after precipitation, at each plot, $\delta^{18}O$ and δ^2H isotope samples from plants, soil and groundwater were collected at the same time. Dominant species in SA (*A. squarrosum* and *A. sphaerocephala*), SFA (*A. ordosica* and *A. sphaerocephala*), FA (*A. ordosica* and *S. bungeana*) and FS (*A. ordosica* and *S. bungeana*) were selected in May, July and

Table 1

Characteristics of plant communities in different shrub encroachment stages (Mean ± SD). Abbreviations: shifting sandy land with annual species (*Agriophyllum squarrosum*) (SA), semifixed sandy land dominated by semi-shrub species (*Artemisia ordosica*) (SFA), fixed sandy land dominated by semi-shrub species (*A. ordosica*) (FA) and fixed sandy land dominated by perennial grass species (*Stipa bungeana*) (FS).

Plant community	Shrub encroachment Stage	Landscape type	Species (plant coverage (%), Mean ± SD)
SA	Extremely severe	Shifting sandy land	*Agriophyllum squarrosum* (8 ± 2.7) *Artemisia sphaerocephala* (5 ± 2.7)
SFA	Severe	Semifixed sandy land	*Artemisia ordosica* (26 ± 4.2) *A. sphaerocephala* (17 ± 2.7)
FA	Moderate	Fixed sandy land	*A. ordosica* (64 ± 5.5) *Stipa bungeana* (10 ± 6.1)
FS	Slight	Fixed sandy land	*S. bungeana* (56 ± 4.2) *A. ordosica* (13 ± 5.7)

September. *A. squarrosum* had not yet grown in May, isotope samples were not collected for this species in May (see Yang et al., 2019 for pictures of species). For semi-shrub and shrubs, lignified twigs 3–5 mm in diameter and 5 cm in length were collected from south-facing side of three individuals of each species, and phloem tissue was removed to avoid isotopic fractionation of xylem water (Martín-Gómez et al., 2017). For grass, nongreen tissue of root crown was collected in three individuals of each species because it correlates with source water (Barnard et al., 2006).

Soil isotope samples were collected at layers of 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm by manually excavating soils with three replicates at each plot in four communities and three months (total 108 soil pits). Groundwater samples were collected with three replicates immediately from two drinking wells (5 m from water surface to ground) in SA and FA after plant and soil isotope samples were collected. Rainwater samples were collected with a polyethylene bottle and funnel before plant isotope samples were collected when there were plenty precipitation events (> 5 mm in May and September, > 10 mm in July) (Wang et al., 2017).

All isotope samples were immediately placed into glass sample vials that were sealed with parafilm and stored in a refrigerator at 4 °C. Isotope analysis for these samples were conducted using a cryogenic vacuum distillation system. Our extraction process relied on water content of the samples. Extraction efficiency needed to be over 98% in order to obtain unfractionated water samples. Hydrogen and oxygen isotope ratios of xylem water were determined with an isotope ratio mass spectrometer system (MAT253, Thermo Fisher Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash 2000 HT, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Measurement precision was consistently ±1‰ for δ^2H and ± 0.2‰ for δ^{18}O.

Precipitation, soil water and groundwater isotope ratios were analyzed with an isotopic ratio infrared spectroscopy system (DLT-100; Los Gatos Research, Mountain View, USA). Measurement precision was consistently ±1.2‰ for δ^2H and ± 0.3‰ for δ^{18}O.

Calculation of isotopic ratios was expressed as:

$$\delta^2H\left(\delta^{18}O\right) = \left(R_{sample} / R_{standard} - 1\right) \times 1000 \tag{1}$$

where R_{sample} and $R_{standard}$ are ^{2}H/^{1}H and ^{18}O/^{16}O molar abundance ratios of the samples and the standard (V-SMOW, Standard Mean Ocean Water), respectively.

At each plot, soil samples were collected randomly at layers of 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm using a cutting ring (height is 5 cm, volume is 100 cm^3) from six soil depths (2.5–7.5, 12.5–17.5, 22.5–27.5, 37.5–42.5, 57.5–62.5 and 82.5–87.5 cm) with three

replicates after removing any rocks and litter during the experimental period. After collecting soil samples, fresh weight was measured immediately, and samples were then taken to laboratory and oven dried at 105 °C to a constant weight to measure dry weight. Soil water content was converted into volumetric soil water content by multiplying it by soil bulk density.

Root biomass was measured by manually excavating soils. Soils and roots were collected for a 50 cm × 50 cm area at 10 cm depth intervals to a depth where roots were obviously sparse, and then these soil samples with roots were brought to laboratory. Each soil core was first sieved with a 2 mm mesh to collect fine roots (< 2 mm diameter), and then all root samples were separated from soils by carefully washing them with water. Dead roots were separated and discarded based on color by hand. Live roots were bagged, oven dried to a constant weight (80 °C), and weighed to calculate root biomass and fine root biomass (Valverde-Barrantes et al., 2015).

Root and fine root biomass fractions were calculated using a nonlinear function (Gale and Grigal, 1987):

$$Y\left(Y_1\right) = 1 - \beta^d\left(\beta_1{}^d\right) \tag{2}$$

where Y and Y_1 are cumulative root and fine root fractions from topsoil to depth d in centimeters calculated based on root and fine root biomass, respectively, and β and β_1 are estimated parameters. High β and β_1 values indicate that a large proportion of roots or fine roots are in deeper soil layers, and low β and β_1 values indicate a large proportion near soil surface.

Climate data, including precipitation and temperature data were obtained from the National Meteorological Science Data Center (http://data.cma.cn/) (Ejin Horo weather station, 109°43′59″ E, 39°33′57″ N).

2.3. Statistical analysis

Two methods were used to identify sources of water used by plants. First, direct inference approach method was applied by comparing isotopic compositions of stem water with those of potential water sources (e.g., soil water at different depths and groundwater) (Ehleringer and Dawson, 1992). Second, a Bayesian mixing model MixSIAR (version 3.1.7) was applied to determine relative proportional contributions of different water sources (Rothfuss and Javaux, 2017; Stock and Semmens, 2013). Raw xylem isotope values δ^2H and δ^{18}O of species were used as mixture data input into MixSIAR. Average and standard errors of isotope values (δ^2H and δ^{18}O) from each potential water source were used as source data and input into MixSIAR. Source data had no concentration dependence. Individual effects as a random occurrence were included in all analyses. Discrimination data were set to zero for both δ^2H and δ^{18}O because there was no fraction during water uptake from soil by roots (Ehleringer and Dawson, 1992).

It shall be noted that there would be potential errors in stem water cryogenic extraction for deuterium (^{2}H) due to dynamic exchanges between organically bound deuterium and liquid water during water extraction (Chen et al., 2020). Based on differences in distribution of soil water isotope values across soil depth (Phillips et al., 2005), soil profile was divided into several soil depths, and four potential water sources were determined:

(1) Shallow soil water (0–20 cm): δ^{18}O varied significantly with season and depth and was susceptible to precipitation pulse input and evaporation;
(2) Middle soil water (20–50 cm): δ^{18}O varied mildly with seasonal changes;
(3) Deep soil water (50–100 cm): δ^{18}O changed little across growing season;
(4) Groundwater: relatively constant δ^{18}O throughout growing season.

Hydrological niche overlap refers to the joint use of water resources by two or more species, which is often used to evaluate the water competition between different species. It can be calculated as proportional similarity index (PS) (Colwell and Futuyma, 1971), to account for a proportional contribution to water uptake from potential water sources (Hoekstra et al, 2014):

$$PS = 1 - 0.5 \sum_{i=1}^{n=4} |p1i - p2i| \qquad (5)$$

where $p1i$ and $p2i$ are contribution ratios of coexisting species (e.g., species 1 and 2) absorbed from same water source i, calculated by MixSIAR model, and $n = 4$ means there were 4 potential water sources. PS ranges from 0 to 1 where a high PS suggests an obvious hydrological niche overlap and high competition for water sources, and a low PS suggests that coexisting species uptake from different water sources.

$\Delta\delta^{18}O$ of different species was calculated as:

$$\Delta\delta^{18}O = |\delta^{18}O_1 - \delta^{18}O_2| \qquad (6)$$

$\Delta\delta^{18}O$ is the difference between coexisting species' xylem water $\delta^{18}O$ (e.g., $\delta^{18}O_1$ for specie 1 and $\delta^{18}O_2$ for specie 2).

To investigate the differences of volumetric soil water content, $\delta^{18}O$ values of soil water and root biomass between four shrub encroachment stages (SA, SFA, FA and FS), observed data from different seasons and soil depths were analyzed using the repeated measures ANOVA of General Linear Model in SPSS (Statistical Package for the Social Sciences version 20.0). In analyses, the dependent variables were volumetric soil water content, $\delta^{18}O$ values of soil water and root biomass, the between-subject factors were shrub encroachment stage and soil depth, and the within-subject factor was time. For each dependent variable, the effects estimated were treatment (four shrub encroachment stages), soil depth, time and their two-way and three-way interactions. A linear regression was used to analyze the relationship between proportional similarity index and $\delta^{18}O$ values of xylem water.

3. Results

3.1. Site microclimate and local meteoric water line

Total precipitation was 381.9 mm in the year of study (2019), and 77.45% occurred during growing season (May to September) (Fig. 1). Precipitation in July (61.5 mm) in 2019 was lower than average from 1980 to 2018 (91.9 mm). Rainfall amount in 2019 was 8.1% greater than that of multiyear mean in this region. Monthly variation was consistent with that of multiyear mean (1980–2018). Over the course of collection period, precipitation isotope values for δ^2H and $\delta^{18}O$ in May, July, and September were –27.25‰ and –4.95‰, –15.38‰ and –3.16‰ and –28.31‰ and –4.91‰, respectively.

Local meteoric water line was fitted for δ^2H and $\delta^{18}O$ values of precipitation (Fig. 2), and slope and intercept of fitted lines were lower than those of the global meteoric water line, indicating that evaporative
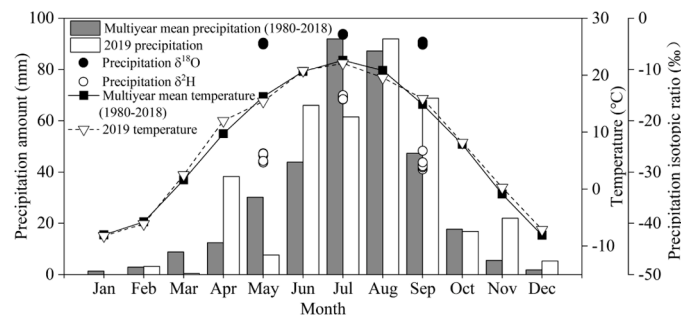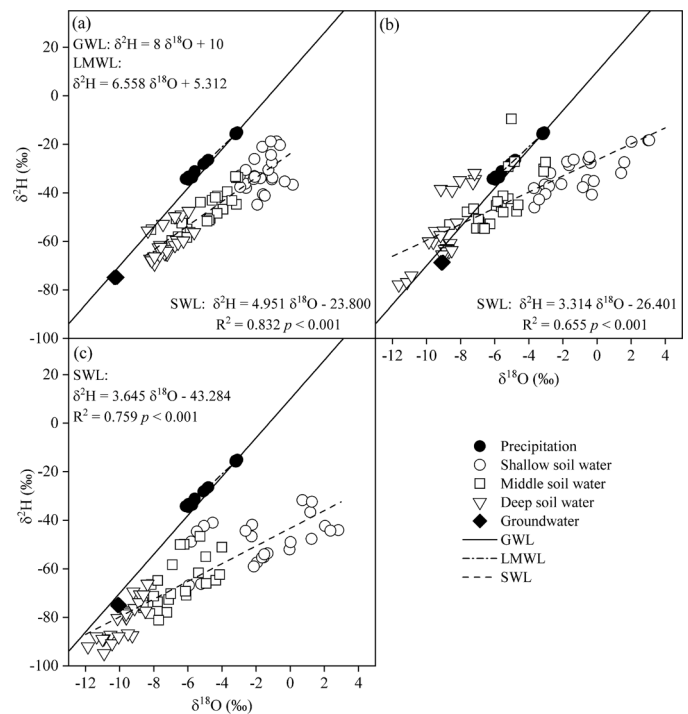
Fig. 2. Relationships between δ^2H and $\delta^{18}O$ values in precipitation and soil water in May (a), July (b) and September (c). Abbreviations: Global water line (GWL), local meteoric water line (LMWL), soil water line (SWL).

enrichment occurred at the study sites. Most of isotope values of soil water during growing season were located to right side of local meteoric water line, indicating that soil water was derived from precipitation and experienced enrichment related to evaporation in this area. The slope of soil water line in July was lower than that in May and September, suggesting that soil experienced a higher evaporation in July than in the other two months.

3.2. Seasonal changes in soil moisture and root biomass

Shrub encroachment stage, soil depth, time, and their two-way interactions had significant effects on volumetric soil water content and root biomass, their three-way interactions had significant effects on volumetric soil water content, but not on root biomass (Tables 2, 3). In SA, volumetric soil water content was the highest at 20–30 cm in May (12.30 ± 1.75%), but lowest at 0–10 cm in July (3.43 ± 1.27%) (Fig. 3).

Table 2
Results of repeated measures ANOVA, Mauchly's test of sphericity indicated that assumption of sphericity had been violated, Pillai's trace in multivariate test was used. Volumetric soil water content was analyzed as dependent variables, shrub encroachment stage (SA, SFA, FA and FS), soil depth as between-subject factors, and time as within-subject factor. Abbreviations are shown in Table 1.

Multivariate Tests				
Effect	F	Hypothesis df	Error df	p
Time (T)	50.599	2	47	<0.001
Time × Shrub encroachment stage (SS)	12.531	6	96	<0.001
Time × Soil depth (S)	3.252	10	96	0.001
T × SS × S	3.939	30	96	<0.001
Test of between-subject effects				
Source	Df	Mean square	F	p
Shrub encroachment stage (SS)	3	53.514	27.456	<0.001
Soil depth (S)	5	36.653	18.805	<0.001
SS × S	15	8.172	4.193	<0.001
Error	48	1.949		

Fig. 1. Temporal variation in monthly precipitation, temperature, δ^2H and $\delta^{18}O$ of precipitation in 2019 and mean monthly temperature and precipitation.

Table 3
Results of repeated measures ANOVA, Mauchly's test of sphericity indicated that assumption of sphericity had been violated, Pillai's trace in multivariate test was used. Root biomass was analyzed as dependent variables, shrub encroachment stage (SA, SFA, FA and FS), soil depth as between-subject factors, and time as within-subject factor. Abbreviations are shown in Table 1.

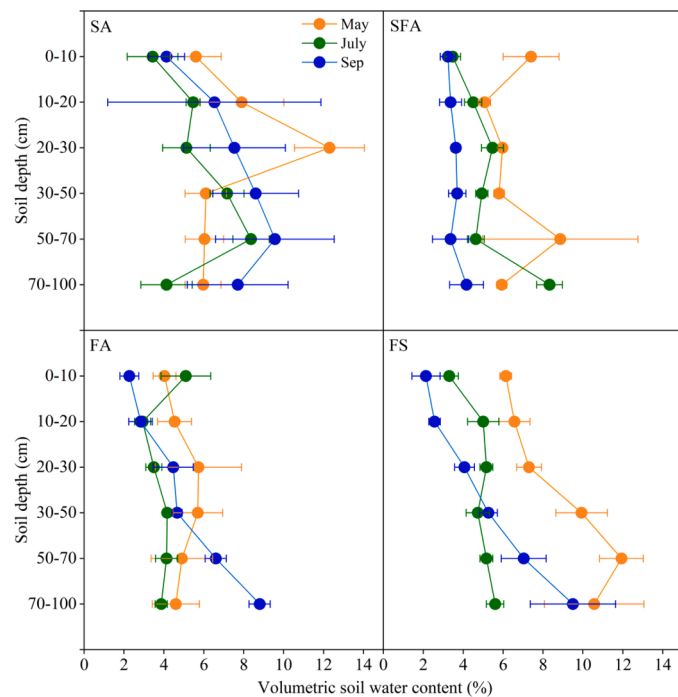| Multivariate Tests				
Effect	F	Hypothesis df	Error df	p
Time (T)	28.398	2	47	<0.001
Time × Shrub encroachment stage (SS)	2.578	6	96	0.023
Time × Soil depth (S)	6.646	10	96	<0.001
T × SS × S	1.277	30	96	0.186
Test of between-subject effects				
Source	Df	Mean square	F	p
Shrub encroachment stage (SS)	3	1.235	10.079	<0.001
Soil depth (S)	5	5.914	48.264	<0.001
SS × S	15	0.665	5.424	<0.001
Error	48	0.123		

Fig. 3. Volumetric soil water content in four shrub encroachment stages in May, July and September. Error bars represent standard deviation. Abbreviations are shown in Table 1.

In SFA, volumetric soil water content was the highest in May, followed by July and September. In FA and FS, volumetric soil water content was the highest in May, followed by September and July. Volumetric soil water content generally increased with soil depth in FS during growing season, while volumetric soil water content in SA increased and then decreased with soil depth.

Generally, communities dominated by grass had more root biomass in shallow soil layers compared with communities dominated by semi shrub species, e.g. the ratio of root biomass in 0–10 cm soil layer to total root biomass were 75.9%, 59.8%, 76.0%, and 81.3% in May in SA, SFA, FA, FS, respectively.

β values were 0.886, 0.906, 0.891 and 0.899 in SA, SFA, FA and FS, respectively (Fig. 4a), and more root biomass was distributed in deep soil layer in SFA than in other stages. *A. ordosica* had the highest β_1 value (0.904), followed by *A. sphaerocephala* (0.899), *A. squarrosum* (0.893) and *S. bungeana* (0.875) (Fig. 4b), and *A. ordosica* had higher fine root
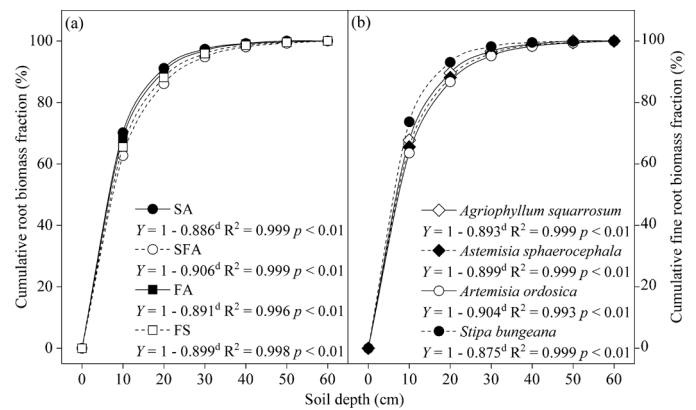

Fig. 4. Root vertical distribution in four shrub encroachment stages (a), and fine root vertical distribution of four dominant species (b). Abbreviations are shown in Table 1.

fractions in deeper soil layers than other species.

3.3. Seasonal changes in soil water and plant xylem water $\delta^{18}O$ values

$\delta^{18}O$ values of soil water were significantly affected by shrub encroachment stage, soil depth, time, and their interactions (Table 4). Mean values of $\delta^{18}O$ in soil water decreased from SA, SFA, FA to FS, with values of –4.41‰, –5.31‰, –5.65‰ and –5.74‰, respectively. $\delta^{18}O$ values of soil water in shallow and middle (0–50 cm) layers varied much more than those in deep layers (50–100 cm) (Fig. 5).

Isotopic values in xylem water varied among different shrub encroachment stages and seasons (Fig. 5). In SA, average xylem water isotope values of *A. sphaerocephala* were similar to shallow soil (0–20 cm) water in May but similar to middle soil (20–50 cm) water in July and September; average xylem water isotope values of *A. squarrosum* were similar to shallow soil (0–20 cm) water in July and Sep (Fig. 5). In SFA, *A. sphaerocephala* and *A. ordosica* had similar $\delta^{18}O$ values in xylem water, and they were both close to that of middle soil (20–50 cm) water during growing seasons. In FA and FS, average xylem water isotope values of *A. ordosica* were similar to that of middle soil (20–50 cm) water in May and July but similar to shallow soil (0–20 cm) water in September; average xylem water isotope values of *S. bungeana* were similar to shallow soil (0–20 cm) water during growing seasons (Fig. 5).

Table 4
Results of repeated measures ANOVA, Mauchly's test of sphericity indicated that assumption of sphericity had been violated, Pillai's trace in multivariate test was used. $\delta^{18}O$ of soil water in different soil layers was analyzed as dependent variables, shrub encroachment stage (SA, SFA, FA and FS), soil depth as between-subject factors, and time as within-subject factor. Abbreviations are shown in Table 1.

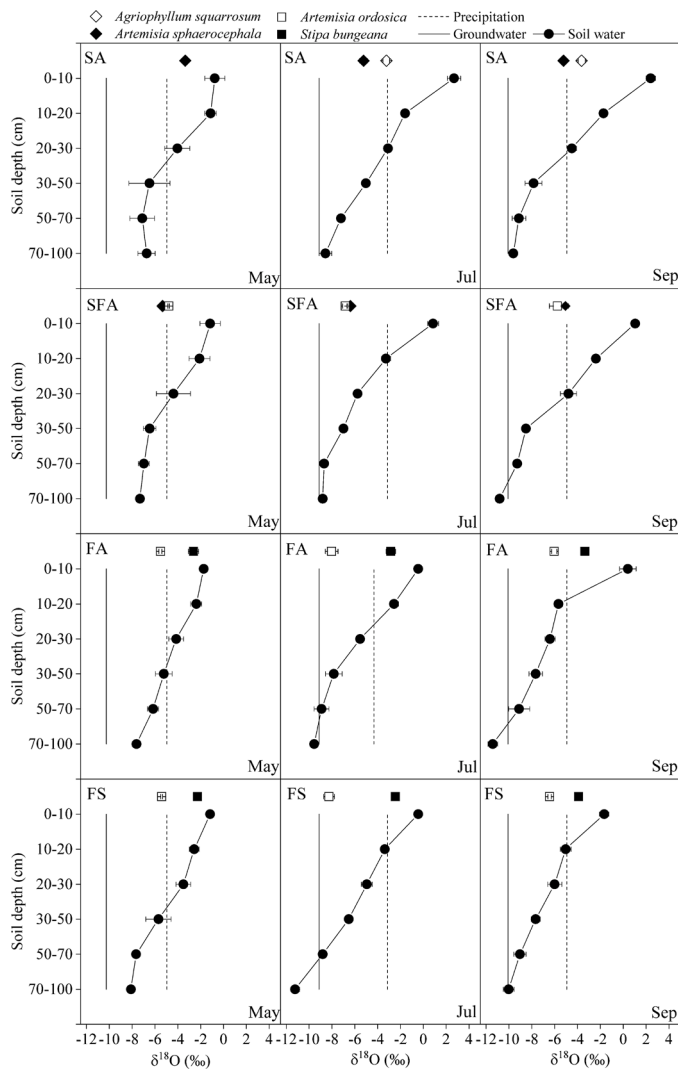| Multivariate Tests				
Effect	F	Hypothesis df	Error df	p
Time (T)	76.296	2	47	<0.001
Time × Shrub encroachment stage (SS)	8.831	6	96	<0.001
Time × Soil depth (S)	9.341	10	96	<0.001
T × SS × S	3.949	30	96	<0.001
Test of between-subject effects				
Source	df	Mean square	F	p
Shrub encroachment stage (SS)	3	20.066	79.889	<0.001
Soil depth (S)	5	431.955	1719.732	<0.001
SS × S	15	1.939	7.720	<0.001
Error	48	0.251		

Fig. 5. δ18O values of soil water and xylem water of different species in four shrub encroachment stages in May, July and September. Error bars represent standard deviation. Vertical black dash lines and vertical black lines represent δ18O values of precipitation and groundwater, respectively. Abbreviations are shown in Table 1.

3.4. Seasonal change in water use sources predicted by MixSIAR model

Water uptake fractions of plants predicted by MixSIAR model varied between different species and seasons (Fig. 6). In SA, *A. squarrosum* mainly used shallow soil water in July and September (48.5 ± 3.5% and 61.8 ± 2.9%, respectively), while *A. sphaerocephala* mainly used shallow soil water in May (42.5 ± 4.5%) and September (40.4 ± 6.1%), but changed to middle soil water in July (54.8 ± 1.3%). In SFA, *A. sphaerocephala* and *A. ordosica* used more shallow soil water in September (50.1 ± 11.4% and 35.9 ± 7.6%), but middle soil water in July (60.4 ± 3.0% and 58.8 ± 4.0%). In FA, *A. ordosica* mainly used shallow soil water in May and September (28.5 ± 3.4% and 43.4 ± 3.0%), but deep soil water in July (50.2 ± 16.9%), while *S. bungeana* mainly used shallow soil water in growing season (85.8 ± 5.6%). In FS, *A. ordosica* mainly used middle soil water (43.6 ± 8.0% and 41.4 ± 8.5%, respectively) in May and September, and deep soil water (58.8 ± 7.8%) in July, while *S. bungeana* used water from shallow soil throughout growing season (90.6 ± 3.6%).
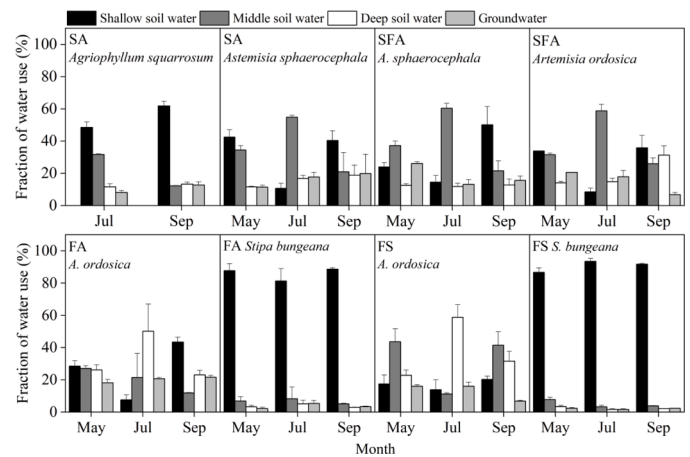

Fig. 6. Fractions of water use from different soil layers of different grass and semi-shrub species in four shrub encroachment stages in May, July and September. Error bars represent standard deviation. Abbreviations are shown in Table 1.

3.5. Seasonal changes in hydrologic niche overlap

Proportional similarity index was highest between two semi-shrub species *A. sphaerocephala* and *A. ordosica* in SFA (0.846), followed by SA (0.696), FA (0.407) and FS (0.266) across growing seasons (Fig. 7a). From May to September, proportional similarity between species increased and then decreased in SFA, decreased and then increased in FA and FS, and increased from July to September in SA. There was a strong negative linear relationship (*p* < 0.001) between proportional similarity and variation in δ18O in xylem water of coexisting species (Fig. 7b).

4. Discussion

4.1. Variation in soil water isotope composition

Isotopic compositions of soil water are simultaneously influenced by evaporation and infiltration, with mixing water from precipitation (Wang et al., 2017). In our study, δ18O values of soil water became more negative as soil depth increased (Fig. 5). Vertical fluctuations in topsoil may be due to precipitation input and strong evaporation in water-limited regions (Tiemuerbieke et al., 2018). δ18O values in water
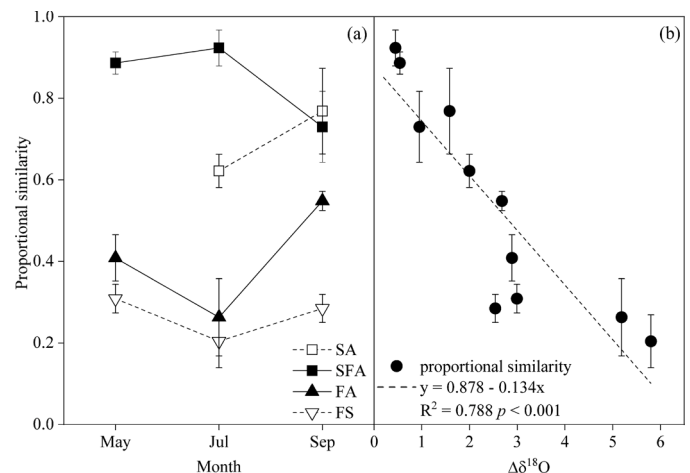

Fig. 7. Hydrologic niche overlap (proportional similarity) of different species in four shrub encroachment stages in May, July and September (a). Linear regression of hydrologic niche overlap and difference of coexisting species' xylem water δ18O (b). Error bars represent standard deviation. Abbreviations are shown in Table 1.

from 50 to 100 cm soil layers varied less with both depth and season, and was lighter than that of precipitation (Table 4, Fig. 5). There are some possible explanations for vertical variations. Normally, precipitation infiltrates through soil pores that bypass topsoil will loss heavier isotopologues and subsequently mix with stationary soil water, resulting in higher accumulation of ^{16}O in deeper soil layers (Berry et al., 2017; Wang et al., 2017). Water from intense precipitation events can infiltrate to deeper soil layers, and the dominant control on $\delta^{18}O$ values is the precipitation amount. Indeed, there is an inverse correlation between rainfall amount and $\delta^{18}O$ values (Lachniet and Patterson, 2009). In addition, groundwater with more negative $\delta^{18}O$ values recharges soil water via capillary rise (Wu et al., 2019b). Therefore, $\delta^{18}O$ variation in soil water was affected by precipitation input, soil evaporation and groundwater recharge.

4.2. Interspecific differences in spatial-temporal water use

Our study investigated spatial-temporal water source use in both semi-shrubs and grasses during shrub encroachment. Consistent with our hypothesis, MixSIAR model estimated that semi-shrubs and grasses had different water use patterns during the study period (Fig. 6). *S. bungeana* and *A. squarrosum* predominately used water from shallow soil layers (0–20 cm) across growing seasons. Plant water use patterns are associated with vertical distribution of fine roots (Wang et al., 2017). *S. bungeana* and *A. squarrosum* had more fine roots mainly distributed in shallow soil layers (Fig. 4b), which played a crucial role in their water use. Unlike grasses, *A. sphaerocephala* and *A. ordosica* had more fine roots in deeper soil layers and opportunistically shifted between possible water sources. In SFA, two *Artemisia* species showed a very similar water use pattern. In summer (July), they used middle soil water as their major water source, but in September, they responded rapidly by using shallow soil water. This was likely due to trade-off between activity of shallow and deep roots as well as soil water availability (Williams and Ehleringer, 2000). Although precipitation in May and September of 2019 was lower and higher than that in May and September of multiyear mean precipitation (Fig. 1), temperature in May and September was much lower than that in July, indicating there was less water penetrating into deep soil layers and more available shallow soil water. Low precipitation coupled with high temperature in July of 2019, may cause less shallow soil water. Grasses with shallow root systems mostly use topsoil water and exhibit a more conservative water use patterns (Hoekstra et al., 2014; Pierce et al., 2018). Shrubs shift water sources from shallow to deep soil layers and exhibit a flexible water use patterns, indicating a great ecological plasticity (Wang et al., 2017). This may be due to functional root system which lateral roots use shallow soil water and taproots use water from deeper soil layers or groundwater (Ehleringer and Dawson, 1992; Pierce et al., 2018; Yang et al., 2015). However, fine root fractions of two semi-shrub species were concentrated in upper layers of 0–20 cm (Fig. 4). Namely, water use dynamics are determined by root activity and distribution rather than root presence and biomass (Rothfuss and Javaux, 2017; Wu et al., 2014), and root presence may not be a reliable indicator of actual water uptake dynamics in either time or space compared with fine root distribution (Ehleringer and Dawson, 1992).

Semi-shrub species reduced the use of shallow soil water in FA and FS where semi-shrub and grass species co-occurred, because much more grass presented in these two stages compared with in SA and SFA (Fig. 6). Grasses with their shallow and dense root systems could compete with shrubs for upper soil resources (Kambatuku et al., 2013). In addition, grasses tend to respond to precipitation more quickly than shrubs (Jobbagy and Sala, 2000), which could become a competitive advantage for grasses under highly variable precipitation in drylands (Pierce et al., 2018). Schenk (2008) found that plants use water from shallow soil layers (when available) due to that energy costs are lower and both water and nutrient availability are generally high in upper layers. Soil moisture and root distribution are related with each other,

thus plant water uses are affected (Williams and Ehleringer, 2000). This was also true in our study, soil moisture and root distribution in different soil layers were affected by shrub encroachment stages and seasons (Tables 2, 3), this complex relationship and difference of water use patterns between co-occurring species might trigger plant community succession in different shrub encroachment stages.

4.3. Variations in interspecific relationship of water use

Interspecific interactions between co-occurring species include competition, facilitation and neutral interactions (Pierce et al., 2018). In SFA, *A. sphaerocephala* and *A. ordosica* had an obvious hydrological niche overlap due to their similar water use patterns (Fig. 6), suggesting a net competitive interaction for water between them. With the increase in semi-shrub cover, the rates of semi-shrub proliferation decreased and reinforced notion that competition between two semi-shrubs was an important factor influencing structure and stability of semi-shrub communities in arid ecosystems (Axelsson and Hanan, 2018). In SA, *A. squarrosum* and *A. sphaerocephala* had the second highest niche overlap, reflecting shrub-grass competition. In FA and FS, *A. ordosica* and *S. bungeana* had distinct water use patterns and exhibited an obvious hydrological niche segregation, avoiding competition for water. Segregation of hydrological niche among different species has been suggested to serve as an adaptation to minimize competition for water during prolonged periods of less precipitation and when upper soil layers become extremely dry, thereby promoting their coexistence (Silvertown et al., 2015). From spring to autumn, hydrological niche overlap decreased and then rose to its highest, suggesting that *A. ordosica* and *S. bungeana* facilitated each other's growth under drought stress but competed in May and September. Nippert and Knapp (2007) suggested that C_3 forbs and shrubs show distinct species strategies to avoid competition with grasses for water in surface soil layers, when this resource is limiting. Dohn et al. (2013) also showed that there may be a transition from net facilitation of grass by shrubs in drier regions to net competitive interactions in mixed shrub-grass ecosystems in more mesic regions. Plant communities in fixed sandy land with obvious hydrological niche segregation were more stable than ones in shifting sandy land.

$\delta^{18}O$ values of xylem water reflect water use and hydrological niche overlap of co-occurring species (Brum et al., 2018). Species with same xylem water $\delta^{18}O$ values (*A. sphaerocephala* and *A. ordosica*) may originate from same hydrological niche with high competition for water. Species with distinct xylem water $\delta^{18}O$ values (*A. ordosica* and *S. bungeana*) had different water use patterns. Coexisting plant species in ecosystem segregate along a wide ecophysiological spectrum of contrasting water use strategies ranging from "opportunistic" to "conservative", this segregation is reflected in their $\delta^{18}O$ values of xylem water (Moreno-Gutierrez et al., 2012). Brum et al. (2018) also found hydrological niche segregation by water stable $\delta^{18}O$ from tree xylem: species with lower stable $\delta^{18}O$ values in xylem water has a large stem size with deep root systems and use deep permanent water to resist drought stress throughout dry season. In general, $\delta^{18}O$ values of xylem water and hydrological niche overlap can help clarify process of shrub encroachment.

4.4. Shrub encroachment, water use sources and implications for restoration of shrub-encroached grassland

Arid and semiarid regions are sensitive to changes in climate and hydrological cycles. Altering water use patterns according to soil water availability is crucial for plants to withstand water stress in water-limited ecosystems (Williams and Ehleringer, 2000). Semi-shrub species (*A. sphaerocephala* and *A. ordosica*) exhibited flexible water use patterns that modulated their water sources between shallow, middle, deep soil water and groundwater during growing seasons (Fig. 6). Perennial grass (*S. bungeana*) had a conservation water use strategy that mainly used water in shallow soil layer (Fig. 6). Plant water use strategies can be identified by exploring isotopic compositions of xylem

water and all available water sources (Wu et al. 2019a), providing vital support for grassland revegetation. To restore degraded grasslands, deeply-rooted *A. sphaerocephala* and *A. ordosica* can be used in their extremely severe and severe stages because these species had flexible water use patterns. Shallow-rooted grass species *S. bungeana* can be used in moderate and slight stages because this species mainly used shallow soil water.

5. Conclusions

Separation of water sources of dominant semi-shrub and grass species in different shrub encroachment stages by stable isotope values of soil water revealed that precipitation and plant xylem water was crucial for driving process of shrub encroachment in semiarid grasslands in Inner Mongolia. From extremely severe to slightly shrub-encroached grasslands, four dominant species (*A. squarrosum, A. sphaerocephala, A. ordosica,* and *S. bungeana*) had different water use sources and hydrological niches during growing season. Shallow rooted species (*A. squarrosum* and *S. bungeana*) mainly used shallow soil water (0–20 cm), while deeply-rooted species (*A. sphaerocephala* and *A. ordosica*) had shifts in water use sources caused by seasonal changes in soil water availability. Extremely severe and severe shrub encroachment stages had an obvious hydrological niche overlap with high interspecies competition for soil water and low community stability. Moderate and slight shrub encroachment stages had obvious hydrological niche segregation caused by different water sources of coexisting species with high community stability.

CRediT authorship contribution statement

Xin Liu: . **Qianlai Zhuang:** Writing – original draft. **Liming Lai:** Data curation. **Jihua Zhou:** Data curation. **Qinglin Sun:** Data curation. **Sangui Yi:** Data curation. **Benben Liu:** Data curation. **Yuanrun Zheng:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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