

# Complementarity in water sources among dominant species in typical steppe ecosystems of Inner Mongolia, China

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**Abstract** Water is the most important factor controlling plant growth, primary production, and ecosystem stability in arid and semi-arid grasslands. Here we conducted a 2-year field study to explore the contribution of winter half-year (i.e. October through April) and summer precipitation (May through September) to the growth of coexisting plant species in typical steppe ecosystems of Inner Mongolia, China. Hydrogen stable isotope ratios of soil water and stem water of dominant plant species, soil moisture, and plant water potential were measured at three steppe communities dominated by *Stipa grandis*, *Caragana microphylla*, and *Leymus chinensis*, respectively. The fraction of water from winter half-year precipitation was an important water source, contributing 45% to plant total water uptake in a dry summer after a wet winter period (2005) and 15% in a summer where subsoil moisture had been exploited in the previous year (2006). At species level, *Caragana microphylla*

exhibited a complete access to deep soil water, which is recharged by winter precipitation, while *Cleistogenes squarrosa* completely depended on summer rains. *Leymus chinensis*, *Agropyron cristatum*, and *Stipa grandis* showed a resource-dependent water use strategy, utilizing deep soil water when it was well available and shifting to rain water when subsoil water had been exploited. Our findings indicate that differentiation of water sources among plants improves use of available soil water and lessens the interspecific competition for water in these semi-arid ecosystems. The niche complementarity in water sources among coexisting species is likely to be the potential mechanism for high diversity communities with both high productivity and high resilience to droughts.

**Keywords** Water source · Hydrogen stable isotope · Soil moisture · Winter half-year precipitation · Plant water potential · Functional traits

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## Introduction

Water is the most limiting factor influencing species richness, productivity, and stability of grassland ecosystems (Bai et al. 2004, 2008; Knapp et al. 2001; Sala et al. 1988). In arid and semi-arid ecosystems, coexisting plant species may utilize water from different sources (Ehleringer and Dawson 1992). For instance, the early spring species mainly use

water resulting from snowmelt, while most warm-season species take advantage of the rain when the rainy season comes (Ehleringer et al. 1991). This may also cause a systematic variation among photosynthetic types, as  $C_4$  plants dominate more in summer vegetation due to their preference for higher temperatures, while  $C_3$  plants are more efficient at cooler temperatures as found in spring (Ehleringer and Björkman 1977).

For different species, the ability of extracting water from different soil layers depends mainly on their active rooting depth (Flanagan et al. 1992; Leffler and Caldwell 2005; Schwinning et al. 2005), which can vary with the vertical distribution of water contents (Dodd et al. 1998; Leffler and Caldwell 2005). The water source can shift from shallow soil water to greater depth when the shallow soil water is depleted (Nippert and Knapp 2007). Plants that have the ability to shift among different water sources might have competitive advantages in arid and semi-arid environments, because they can exploit water resources to maintain their growth under fluctuating environments (Ehleringer and Dawson 1992). However, Bazzaz (1996) argued that most of the grassland plants could only use limited water resources due to the intrinsic nature of rooting depth and life history strategies. Empirical test of these hypotheses and predictions in semi-arid grassland ecosystems is still lacking, as most studies have been conducted in deserts, savannahs, and mesic grassland ecosystems (Dawson et al. 2002; Nippert and Knapp 2007). Thus, a comprehensive understanding of water use strategies of coexisting species in semi-arid grassland ecosystems is needed, particularly in the Eurasian steppe.

The grassland ecosystems in the Inner Mongolia steppe region of northern China are representatives of the Eurasian steppe (Bai et al. 2007; Chinese Academy of Sciences Integrative Expedition Team to Inner Mongolia and Ningxia 1985). In this semi-arid grassland with evapotranspiration exceeding rainfall during summer, summer rains mainly recharge the topsoil except for macropore flow, while deeper soil layers can only be recharged during the winter-half year, especially by snowmelt, which also drives catchment runoff (Schneider et al. 2007). Consequently, water from winter half-year precipitation (mostly snow water) can be better used by deep rooted plants while shallow rooted plants are restricted to summer rains. When topsoil water is limiting, high fraction of winter precipitation in total water

uptake of some species may mitigate the interspecific competition for water. Based on the theory of niche complementarity (Loreau et al. 2001), the partitioning of water sources among coexisting species will improve the use of available soil water and thus increase ecosystem resilience to droughts.

To test the above hypothesis, we conducted a 2-year field study at three typical steppe communities dominated by *Stipa grandis*, *Caragana microphylla*, and *Leymus chinensis*, respectively. Specifically, we want to address the following three research questions: First, do the dominant plant species differ in water use originating from either the meteorological winter half-year (i.e. October through April) or summer (May through September) precipitation? Second, how does the water source of plants vary with seasonal and yearly changes in precipitation? Third, at community level, what is the relative contribution of winter precipitation to plant total water uptake?

## Materials and methods

### Study site

The study was carried out at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), which is located in the Xilin River Basin (116°42'E, 43°38'N), Inner Mongolia Autonomous Region of China (Bai et al. 2004). The continental temperate semi-arid climate is characterized by a cold and dry winter but a warm and moist summer (Chen 1988). The mean annual temperature in the study area is 0.6°C with mean monthly temperatures ranging from -21°C in January to 19°C in July (Bai et al. 2008). The growing season begins in the end of April and ends in early October, lasting about 150 days. The mean annual precipitation is 341 mm with 60–80% falling during the growing season. Actual evapotranspiration (ET) nearly equals the precipitation within one single year (Wen 2006). Winter precipitation (snow) begins to melt and penetrates to deep soil layers when temperature increases rapidly in April. The river discharge hence peaks in April exceeding the discharge during the precipitation peak in June and July (Schneider et al. 2007).

Three typical steppe communities were selected as our experiment plots, which belong to the permanent field sites of IMGERS. The first plot was dominated

by *S.* and was fenced from animal grazing since 1979. The second plot was dominated by *C. microphylla* and was fenced since 1983. The third plot was dominated by *L. chinensis* and was fenced since 2001. More details about communities and soils are given in Table 1.

#### Field sampling and stable isotope analysis

To explore the water sources of dominant species, we selected six species, i.e., *S. grandis*, *L. chinensis*, *Cleistogenes squarrosa*, *Agropyron cristatum*, *Carex korshinskyi* (only in *L. chinensis* plot) and *C. microphylla* (only in *C. microphylla* and *L. chinensis* plots). All of them are perennials and together account for more than 80% of the total aboveground biomass. Both *S. grandis* and *A. cristatum* are  $C_3$  bunchgrasses, *L. chinensis* is a  $C_3$  rhizome grass, and *C. microphylla* is a  $C_3$  legume shrub. *C. korshinskyi* is a  $C_3$  sedge. *C. squarrosa* is a  $C_4$  short bunchgrass characterized by a shallow rooting system (Chen et al. 2001; Jigjidsuren and Johnson 2003). All species start the growing season in April except for  $C_4$  species like *C. squarrosa*, which begins the growing season after May.

A 100-m transect was established within each plot. Samples were collected in early May (May 7–8) and mid August (August 17–20) respectively in both 2005 and 2006. Plant samples from the non-photosynthetic tissues at the interface between shoot and root systems, often at or just below the soil surface, were collected for analyzing the hydrogen stable isotope ratios (Thorburn and Walker 1993). For each species, the non-photosynthetic tissues from at least 20

individuals were collected as one replicate, enclosed in a screw-capped glass vial, and sealed immediately using Parafilm. Soil samples at different depths (i.e., 0–5 cm, 5–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm) were also collected by a 5-cm diameter soil auger to analyze hydrogen stable isotope ratios of soil water. There were three replicates for each plant species and soil depth at each plot. Soil moisture at different depths (0–20 cm, 20–40 cm, and 40–110 cm,  $N=3$ ) for each plot were measured gravimetrically every 10 days from early May to mid of October in 2005 and 2006, and then converted to volumetric basis. For each soil depth, plant available water was calculated as the difference between volumetric soil water content and unavailable soil water. For each site, the unavailable soil water was the average of the lowest water contents from three replicates at the top (0–20), middle (20–40 cm), and bottom (>40 cm) layer that were measured in both years. These values, which were used to approximate the wilting points, should best reflect the plant–water relations under the local pedoclimatological situation (Li and Chen 1999). At the *Stipa* site, for instance, these values (6% vol.) were close to those values (5.5–7.5% vol.) determined by laboratory methods (Li and Chen 1999).

Summer rain in Inner Mongolia usually occurs in short, high-intensity events. Rains occurring within 4 days to 5 days before the plant sampling were collected at IMGERS with a dry bowl, which was checked for dryness every morning and sampled immediately after a rain as it was continuously under control at the research station and could be reached

**Table 1** Characteristics of the plant communities and soils (mean±SE)

Item	<i>Stipa grandis</i> plot		<i>Caragana microphylla</i> plot		<i>Leymus chinensis</i> plot	
	2005	2006	2005	2006	2005	2006
Species richness ( $N=10$ )	10.5±0.5	11.1±0.7	11.2±0.7	11.8±0.7	15.1±0.6	16.6±1.1
Total aboveground biomass ( $\text{g m}^{-2}$ ) ( $N=10$ )	98.1±5.1	135.2±3.9	101.1±5.6	211.6±12.8	153.5±6.5	135.1±5.0
Litter weight ( $\text{g m}^{-2}$ ) ( $N=10$ )	110.4±8.4	147.2±12.6	104.9±7.4	120.5±11.8	172.2±17.0	234.1±31.6
Bulk density ( $\text{g cm}^{-3}$ ) (0–20 cm)	1.30±0.03		1.41±0.00		1.29±0.04	
Calcic layer	Yes (30–40 cm)		Yes (>50 cm)		No	
Soil texture (%) (0–20 cm)	Clay	7.44	7.65		11.61	
	Silt	23.46	11.29		18.85	
	Sand	69.10	81.06		69.54	

The particle size is 0.05–2.0 mm for sand, 0.002–0.05 mm for silt, and <0.002 mm for clay

within <2 min. Rainwater samples were immediately enclosed in air-tight glass vials and stored in a refrigerator at 4°C. The plant tissue and soil samples were kept frozen in the laboratory until water was extracted using a cryogenic vacuum distillation apparatus (Ehleringer and Osmond 1989). The water samples were pyrolyzed into CO and H<sub>2</sub> in an elemental analyzer (TCEA; Thermo Finnigan) interfaced (ConFlo III; Thermo Finnigan MAT) to a Thermo Finnigan Delta<sup>plus</sup>XP isotope ratio mass spectrometer (Thermo, Waltham, USA) in the Institute of Botany, Chinese Academy of Sciences (Beijing, China). All samples were measured against laboratory working standard gases, which were previously calibrated against IAEA standards (IAEA SMOW for D, accuracy of calibration ± 0.10‰ SD). Two kinds of laboratory working standard water, which were also previously calibrated against IAEA standards (IAEA SMOW), were measured additionally after every twenty samples against these references. The precision for sample repeats was better than 1‰ for δD. The stable isotope ratio of hydrogen in water is expressed using standard delta notation (δ) in parts per thousand (‰) as:

$$\delta D = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of D/H of the sample and standard water (V-SMOW), respectively.

#### Water potential of plant leaves

To explore the relationship between plant water potential and the δD value of plant water, leaf samples of four dominant species, *L. chinensis*, *S. grandis*, *A. cristatum* and *C. squarrosa*, were collected at the *S. grandis* plot during August 20–24, 2005. For each species, the whole aboveground plant tissues were taken, sealed in plastic bags, and stored immediately in an icebox. Predawn (05:00) water potential ( $\psi_{\text{pd}}$ ) and midday (12:00) water potential ( $\psi_{\text{md}}$ ) from three fully matured leaves of each species were measured shortly afterwards (within 2 h) by an HR33-T Dewpoint Potential Meter (Wescor, Logan, USA).

#### Aboveground biomass

Aboveground biomass was sampled within a period of August 17 to September 2, when the standing crop

of the communities reached their annual peak in both 2005 and 2006. For each plot, ten 1 × 1 m<sup>2</sup> sampling quadrats were arranged randomly. All living biomass within each quadrat was collected by clipping to the soil surface, separated to species, oven dried at 65°C to constant mass, and weighed. The height, number of individuals, and coverage of each species were also recorded within each quadrat. Aboveground biomass and species richness for each community were estimated by averaging the ten quadrats. We approximated ANPP with the annual peak community biomass as is commonly done for semi-arid grassland communities (Bai et al. 2004, 2008; Sala et al. 1988) as the plots were fenced.

#### Data analyses

We used simple mass balance (Ehleringer et al. 1991; Williams and Ehleringer 2000) between lower and upper limit δD of possible water sources to calculate the fraction of total plant water uptake constituted by winter precipitation ( $f_w$ ) based on the following reasoning. In the semi-arid Inner Mongolian grassland, the deep soil water is only recharged by precipitation after the growing season and by snowmelt in the early spring because of potential evapotranspiration exceeding rainfall in summer. Therefore, the δD value of deep soil water is a good indicator of winter half-year water although without measurement we do not know how much fractionation occurred during the snowmelt and soil water evaporation (Li et al. 2006; Winograd et al. 1998). The δD value of soil water at 100 cm depth then approximates the lower limit of δD value of plants because the maximum rooting depth for most perennial grasses and forbs is less than 100 cm (Cheng et al. 2006). Even for the deep rooting species, *C. microphylla*, the δD value of 100 cm soil water can also be used for calculating  $f_w$  because the δD value below this depth is less variable (Brunel et al. 1995; Cook and O'Grady 2006). To further validate the above assumption, we measured δD values of soil samples in the early May and mid August in both 2005 and 2006 across the three plots. The δD of 100 cm depth was relative stable ( $\delta D_{\text{mean}} = -94.2$  ‰,  $SE = 1.4$  ‰,  $N = 6$ ) (see results), which was very close to another measurement in 2003 in *L. chinensis* plot ( $\delta D = -94$  ‰, depth = 90 cm;  $\delta D = -96$  ‰, depth = 120 cm) (Qing, unpublished data). Thus, the average value of δD at 100 cm soil depth was used for estimating the  $f_w$  of plants.

We used the  $\delta D$  value of last rain before each sampling date as the upper limit of plant xylem water (Williams and Ehleringer 2000). At the *C. microphylla* plot, no effective rainfall occurred within 2 weeks before plant sampling in August, 2006. Thus, we used the average  $\delta D$  at 2–10 cm soil depth ( $\delta D_{\text{mean}} = -37.18\text{‰}$ ,  $SE = 1.25\text{‰}$ ,  $N = 3$ ) for calculating the  $f_w$  because the  $\delta D$  value of soil water at 0–2 cm depth was highly enriched due to strong evaporation.

The fraction of plant total water uptake constituted by winter precipitation ( $f_w$ ) can then be estimated:

$$f_w = \frac{\delta D_P - \delta D_R}{\delta D_{DSW} - \delta D_R} \quad (2)$$

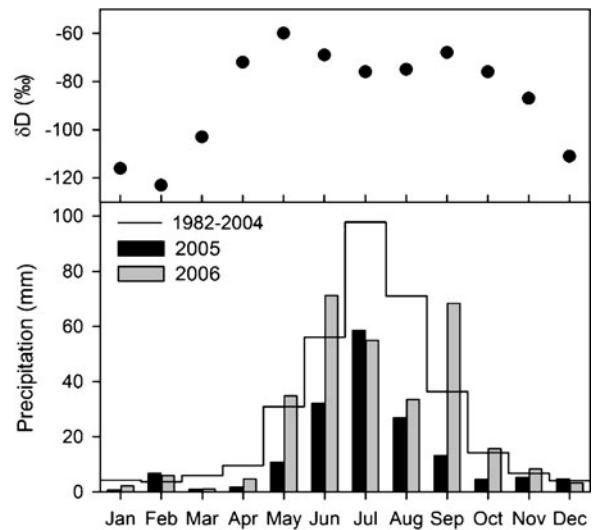
with the indices  $P$  for the water from non-photosynthetic tissues at the interface between shoot and root,  $R$  for last rain water, and  $DSW$  for deep soil water (100 cm). The  $f_w$  was set to 0 when the  $\delta D$  value of plant was above the  $\delta D$  value of rain probably attributed to using enriched dew or surface soil water, particularly in the arid and semi-arid ecosystems (Cheng et al. 2006; Li et al. 2006; Zhao et al. 2009). When the  $\delta D$  value of plants was below the  $\delta D$  value of 100 cm soil water, the  $f_w$  was set to 1 (Cheng et al. 2006).

Statistical analyses for isotope data and volumetric soil water were performed by using general linear model (Univariate analysis) in SPSS Version 16.0 (SPSS Inc., Chicago, USA, 2007). Experimental factors, including plot, species, year, season, and all interactions were treated as fixed effects. One-Way ANOVA, followed by a least-significant difference (LSD) multiple-range test, was applied to compare the predawn water potential, midday water potential, and the diurnal range of water potential for the four species. Independent-samples  $t$ -test for the  $f_w$  was used to examine the statistic significance at  $P < 0.05$  and  $P < 0.01$  levels between May (early growing season) and August (peak growing season) in both 2005 and 2006.

## Results

### Precipitation, soil moisture, and soil water $\delta D$

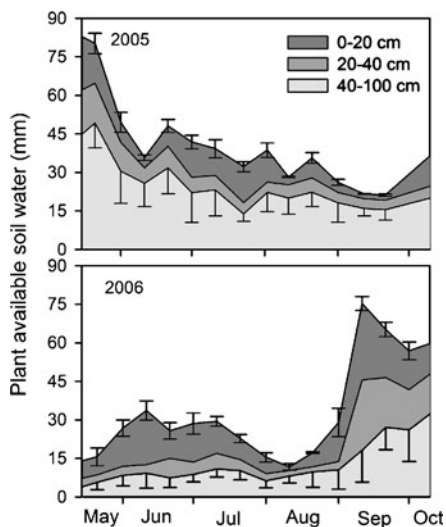
In comparison with the long-term average (1982–2006), annual precipitation was 51% and 11% lower in 2005 and 2006, respectively (Fig. 1). The main



**Fig. 1** The monthly distribution of precipitation for 2005 and 2006 compared to the long-term mean (1982–2004) (*bottom panel*) and predicted hydrogen stable isotope ratio ( $\delta D$ ) for the study area according to [www.waterisotopes.org](http://www.waterisotopes.org). (*top panel*)

growing season precipitation from April to August was 53% higher in 2006 (199 mm) than that in 2005 (130 mm). In 2005 the main share of rain fell in July, while rain distribution was bimodal in 2006 with two peaks appearing in June and September, respectively. Hence precipitation in 2006 was about normal in May and June but also below average in July and August.

For all three plots, soil moisture within 0–20 cm fluctuated more than at other depths (Fig. 2). It was continuously depleted by evapotranspiration and recharged by rain in the top layer while the soil moisture at greater depth—especially  $>40$  cm—only became depleted because rain was not sufficient to cause percolation to that depth. At the end of the 2005 growing season, almost all plant available soil water had been removed from all depths. Precipitation during the winter half-year 2005/2006 was only 25 mm and thus not sufficient to recharge soil moisture at  $>20$  cm depths. Consequently, the depleted subsoil was inherited in the winter 2005/2006. Thus 2005 had a dry summer but soil moisture derived from previous winter precipitation was available below 20 cm depth, while 2006 was a normal year regarding early summer precipitation but it was dry in terms of deep soil moisture originated from the previous winter precipitation. Rains at the end of the growing season 2006 exceeded the already low



**Fig. 2** Plant available soils moisture (volumetric water content minus unavailable water) accumulated over three depths during the growing seasons in 2005 and 2006 and averaged over the *Stipa grandis* plot, the *Caragana microphylla* plot, and the *Leymus chinensis* plot. The total annual precipitation was 166 mm in 2005 and 304 mm in 2006. Vertical bars denote the standard deviation ( $N=9$ ). For readability, error bars are not given for the 20–40 cm layer (on average half of the standard deviation in the <20-cm layer) and only the lower half error bar is shown for the >40-cm layer

evapotranspiration at that time and started to recharge the plant available water >40 cm depth.

Soil water  $\delta D$  (Fig. 3) decreased with increasing depth at all the plots and across sampling times. Greater variation was found at the topsoil (20 cm) while the  $\delta D$  values were less variable at the deep soil layers. For all the three plots, the  $\delta D$  value of the 100 cm soil was most negative, similar for all profiles and similar to what can be expected for the precipitation during the winter-half year (Fig. 1). In the dry summer 2005 with large contribution of cool-season precipitation,  $\delta D$  was lower than that in 2006 when soil water mainly resulted from summer rains.

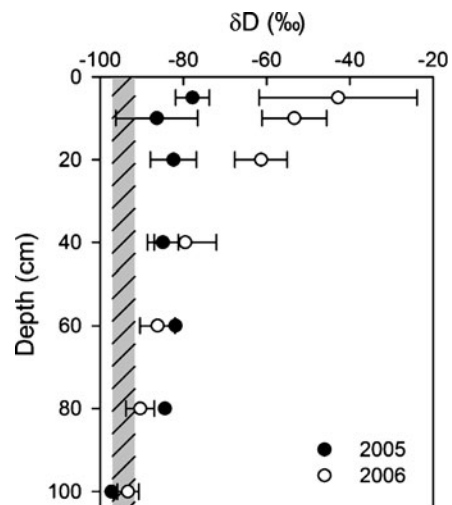
#### $\delta D$ in plant water at the stem base

There were significant differences in  $\delta D$  values among species (S), plots (P), years (Y), and months (M) and the interactions of  $S \times P$ ,  $S \times Y$ ,  $S \times M$ ,  $P \times Y$ ,  $Y \times M$ , and  $S \times P \times Y$  were also significant (Table 2). The main effects (S, P, Y, M) had by far the largest influence except for M (see mean square in Table 2) while the interactions of two factors explained one order of magnitude less except for  $Y \times M$  indicating

that the influence of M in contrast to the other three main factors differed strongly between the years. The interaction of three factors explained another order of magnitude less. Among species, the most distinct differences were found between *C. microphylla* and *C. squarrosa* in their utilization of winter precipitation (see below).

#### Fraction of winter precipitation to plant water uptake (fw)

The  $\delta D$  of rain (Fig. 4) was within the expected range (Fig. 1) and differed significantly from  $\delta D$  at 100 cm soil depth in both the early growing season and peak growing season and across the 2 years ( $P < 0.05$ ). In 2005 the plant stem water was intermediate indicating that the plants took up rain water from the topsoil but also subsoil water (Fig. 4) with two exceptions. *C. microphylla* only used deep soil water while *C. squarrosa* completely depended on shallow water from summer rains. In 2006, when deep soil water was low due to the previous dry year  $\delta D$  in plant stem water was close to or even above precipitation water for most species; this suggested that the plants were mainly using rain water or even the water in the very top soil, which was enriched by evaporation. Again, there were two exceptions. *L. chinensis*, which had already used slightly more subsoil water in 2005, still used some subsoil water in May 2006. *C. microphylla*



**Fig. 3** Hydrogen stable isotope ratio ( $\delta D$ ) of soil water. Gray area indicates the range of the  $\delta D$  values at 100 cm soil depth. Horizontal bars denote the standard deviation of means ( $N=6$ ) for each data point

**Table 2** Univariate analyses of stem base  $\delta D$  values using Species, Plot, Year, Month, and all interactions as fixed-effects

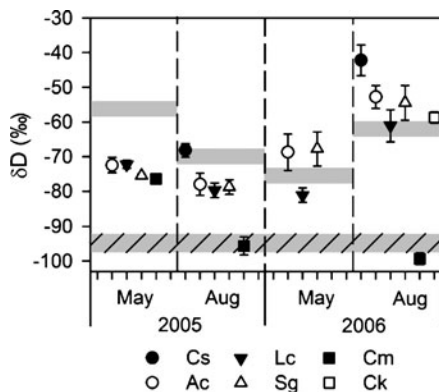
Source	Type III Sum of Squares	df	Mean Square	F	P
Species (S)	9655.34	5	1931.07	82.60	0.000
Plot (P)	3335.66	2	1667.83	71.34	0.000
Year (Y)	3648.33	1	3648.33	156.05	0.000
Month (M)	139.08	1	139.08	5.95	0.018
S $\times$ P	808.33	7	115.48	4.94	0.000
S $\times$ Y	3182.77	4	795.69	34.03	0.000
S $\times$ M	646.29	3	215.43	9.21	0.000
P $\times$ Y	1518.30	1	1518.30	64.94	0.000
Y $\times$ M	765.25	1	765.25	32.73	0.000
S $\times$ P $\times$ Y	243.67	3	81.22	3.47	0.021
S $\times$ Y $\times$ M	42.10	2	21.05	0.90	0.412
Error	1449.53	62	23.38		
Total	456318.08	93			
Corrected total	27905.82	92			

did not switch to rain water; its stem water even was slightly below  $\delta D$  in 100 cm depth, implying that *C. microphylla* accessed even deeper soil water.

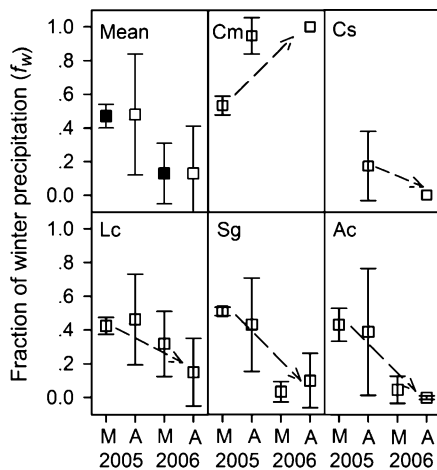
The large contrast between  $\delta D$  in rain and subsoil water allowed calculating the  $f_w$ . In the dry summer year with sufficient subsoil moisture (2005),  $f_w$  was three times larger than that in 2006 when averaged across all species and seasons (2005: mean=0.45, SE=0.05,  $N=42$ ; 2006: mean=0.15, SE=0.04,  $N=51$ ). Less water was delivered by rain while winter-derived water was available in 2005. However, in 2006 more

rain but only little winter-derived water was available. Thus,  $f_w$  was lowest in May 2006. But it increased slightly again in August 2006 when there was enough moisture from the rain though available moisture was low in the subsoil (Fig. 5). The dry July and August in 2006 then forced the plants to extract some subsoil moisture even though the subsoil was largely depleted by the previous year (Fig. 2).

The species differed in their access to water. *C. microphylla* always had access to deep soil water and even increased this access during dry conditions. In contrast, *C. squarrosa* completely depended on shallow water from summer rains even in 2005 when winter moisture was available (Fig. 5). This is not only due to the fact that *C. squarrosa* appears after May and could only be sampled in August. Even in 2006, when there was more water in the topsoil due to more rain, *C. squarrosa* even restricted water uptake to the very top 10 cm, where the soil water was enriched by evaporation compared to rain (compare Figs. 3 and 4). All other species showed a more resource-dependent water use strategy. They used subsoil water in the early growing season 2005 when there was still enough water in the subsoil and then gradually shifted to rain water as the subsoil became depleted. In 2006, when there was not much winter moisture stored in the subsoil, they almost entirely used rain water. Among the species with resource dependent strategy, *L. chinensis* tended to use more winter-derived water than *S. grandis* and *A. cristatum*. This was especially evident in 2006 and caused a



**Fig. 4** Hydrogen stable isotope ratios ( $\delta D$ ) of the non-photosynthetic plant tissues (the interface between the shoot and root systems). Upper gray area indicates the range of rain  $\delta D$  values. Lower gray diagonally hatched area indicates the range of the  $\delta D$  values at 100 cm soil depth. Vertical bars denote the standard deviations. ( $N=3-9$ ). Cs, *C. squarrosa*; Lc, *L. chinensis*; Cm, *C. microphylla*; Ac, *A. cristatum*; Sg, *S. grandis*; and Ck, *C. korshinskyi*



**Fig. 5** Fraction of total plant water uptake constituted by winter precipitation ( $f_w$ ) calculated from the  $\delta D$  values of plant water in non-photosynthetic tissues. Vertical bars denote the standard deviation for each data point. Mean is the average over all species. Species are abbreviated as in Fig. 4. Note: *C. squarrosa* could only be sampled in August due to its late onset of growth. M = May and A = August

slower shift between water sources than with the two other species (see slope of the arrows in Fig. 5).

#### Plant leaf water potential

Both the predawn leaf water potential ( $\psi_{pd}$ ) and midday leaf water potential ( $\psi_{md}$ ) differed significantly among the four species examined (Table 3). For a given species, the mean  $\psi_{md}$  was more negative than that of the  $\psi_{pd}$  ( $P < 0.01$ ). As compared to the other three species, *C. squarrosa* showed the highest diurnal variation of  $\psi_{md}$  and  $\psi_{pd}$ , which was mainly due to a pronounced recovery overnight (Table 3). Among the four species, *L. chinensis* exhibited the least negative  $\psi_{pd}$ , which was followed by *C. squarrosa*, *A. cristatum*, and *S. grandis* (Table 3). In addition, the  $\delta D$  value increased significantly with less negative  $\psi_{pd}$  across all species except for *L. chinensis* ( $R^2 = 0.73$ ,  $P < 0.003$ ;  $N = 9$ ), indicating that the use of deep water increased with increasing plant water stress.

## Discussion

### Hydrology

Both years differed considerably in precipitation. Summer precipitation alone was, however, not suffi-

cient to characterize the water supply to the vegetation, because a considerable amount of the property of the antecedent year was carried over. After a wet antecedent year the subsoil supplied moisture whereas after a dry year the vegetation had to rely solely on summer precipitation. Our assessment based on soil moisture measurements is corroborated by eddy covariance measurements, on the *L. chinensis* plot, which showed that evapotranspiration was about 50 mm larger than summer rainfall in 2005 whereas it equaled precipitation in the growing season of 2006 until soil recharge began at the end of August 2006 and precipitation exceeded evapotranspiration (Wang et al. 2010). The huge inter-annual variability in summer precipitation, typical for this area (Wittmer et al. 2008), is thus attenuated if the soil is recharged before a growing season. Several dry years in succession, even if only moderately dry, should be more severe than a dry year after a wet year. Strategies to switch water use between topsoil and subsoil should hence be advantageous for the plants as none of both sources is reliable.

On average, the  $\delta D$  values of precipitation in the early growing season and peak growing season closely followed the predictions by [www.waterisotopes.org](http://www.waterisotopes.org) with a mean deviation of 2‰, but the individual months of both years varied considerably with a mean absolute deviation from the prediction of 9‰. Summer rains could not recharge the deeper soil depths and the  $\delta D$  values of 100 cm soil water varied in a narrow range ( $\delta D_{\text{mean}} = -94.24\text{‰}$ ,  $SE = 1.40\text{‰}$ ,  $N = 6$ ) while the variation of rain was larger (mean  $-70.03\text{‰}$ ,  $SE = 3.30\text{‰}$ ,  $N = 11$ ). Thus, the fraction of winter precipitation contributing to plant water uptake

**Table 3** Predawn ( $\psi_{pd}$ ) and midday ( $\psi_{md}$ ) leaf water potentials of the four dominant species at the *S. grandis* plot in August, 2005

Species	$\psi_{pd}$	$\psi_{md}$	$\Delta\psi_{pd-\psi_{md}}$
<i>C. squarrosa</i>	$-3.44 \pm 0.10a$	$-7.02 \pm 0.40a$	$3.58 \pm 0.49a$
<i>S. grandis</i>	$-4.71 \pm 0.10b$	$-7.04 \pm 0.21a$	$2.33 \pm 0.15b$
<i>A. cristatum</i>	$-3.96 \pm 0.24c$	$-5.58 \pm 0.09b$	$1.62 \pm 0.64b$
<i>L. chinensis</i>	$-3.33 \pm 0.04a$	$-4.93 \pm 0.13b$	$1.60 \pm 0.17b$

Data in columns are shown with mean  $\pm$  SE ( $N = 3$ ). The different letters indicate a significant difference ( $P < 0.05$ ) in a Least-Significant Difference (LSD) test for species within each column. The statistical difference between  $\psi_{pd}$  and  $\psi_{md}$  within a row was examined using *t*-test; it was  $P < 0.01$  in all cases



could be calculated. The  $\delta D$  of dew was  $-40\text{‰}$  on average (unpublished data), which was above the  $\delta D$  of plant water. The extremely negative  $\psi_{pd}$ , however, indicated that in our case the contribution of dew to total plant water uptake was too small even to cause a significant relaxation of leaf water potential during the night. Previous studies have proposed that some halophytes and woody xerophytes discriminate against deuterium during water uptake, which seems to be caused by the specific root morphology of such plants (Ellsworth and Williams 2007). Based on the measurement of  $\delta D$  and  $\delta^{18}O$  in stem water for one sampling date in 2005, we found that there was a significant correlation between  $\delta D$  and  $\delta^{18}O$  in stem water ( $R^2=0.77$ ,  $N=18$ ), which followed the mixing line between soil water and groundwater with no species exhibiting more negative  $\delta D$ . This indicates that no discrimination against deuterium occurred during plant water uptake.

#### Relationship between $\delta D$ and leaf water potential

The  $\psi_{pd}$  provides a good indicator for leaf water stress and recovery during night when leaf transpiration is small. The  $\psi_{pd}$  was extremely negative and considerably lower than what was found in a South American grassland (Colabelli et al. 2004) but within the range reported in Inner Mongolia (Liu et al. 2004), indicating a pronounced cell wall rigidity (Kramer and Boyer 1995).

The close correlation between predawn water potential and  $\delta D$  (respective  $f_w$ ) indicates that with increasing water stress the plants increasingly exploit winter moisture stored at greater depth. Among the four species, the  $\psi_{pd}$  value of *S. grandis* was most negative and that of *L. chinensis* was the least negative, while those of *A. cristatum* and *C. squarrosa* were intermediate. Similar to the South American grassland (Colabelli et al. 2004), there was a large variation between species but this did not relate to the photosynthetic pathway.

Root architecture seems to be responsible for the large variation of leaf water potential among species. Chen et al. (2001) have reported that the roots of *C. squarrosa* only extend to 14 cm depth and that of *A. cristatum* to 25 cm depth, while *S. grandis* and *L. chinensis* have deeper roots than the former two species. Thus, both the lowest and the highest leaf water potential occur with deep rooted species, while

the shallow rooted species are intermediate. The discrepancy in between the deep rooted species presumably relates to the fact that *L. chinensis* seems to be able to better buffer diurnal fluctuations in water deficit due to its rhizomes and thus exhibits the lowest range in leaf water potential. Also, *L. chinensis*, which extracted winter moisture from below 70 cm depth as indicated by higher  $f_w$ , seems to be competitively superior to other perennial grasses in exploiting the subsoil moisture.

#### Possible relationship between the water-use strategy and species coexistence

The survival of perennial species through extended drought periods depends on both the ability of roots to acquire limited soil water and the ability of shoots to tolerate water stress (MacMahon and Schimpf 1981), and the differences in rooting niche separation among species contribute to minimize competition for water during prolonged drought periods when upper soil layers become dry (Mooney et al. 1980). In this study, we found that plants made use of soil moisture at greater depth when it was available (early growing season of the dry 2005) and otherwise shifted to the upper layer. This suggests that plant competition for water is intensified when water is limited.

Among the species, which were able to shift water extraction between topsoil and subsoil, *L. chinensis* made more use of subsoil water than the others. The large share of *L. chinensis* in total aboveground biomass (Chen et al. 2005) supports the notion that deep rooted species, which use the deep soil water, had competitive advantage in these steppe ecosystems. First, the rhizomes may take up water from soil and serve as a water storage organ (Wang et al. 2003) and buffer diurnal variation in water potential leading to the narrow range of diurnal leaf water potential. Second, the leaf water potential of *L. chinensis* was less negative than those of the other species. Third, even without a large water stress *L. chinensis* extracted much more winter moisture than the other species. *C. squarrosa* does not seem to have the plasticity to shift among water sources as it only used topsoil moisture, which is reasonable as it is the last species to start growth (June). During dry summer years it may even delay the start of growing season (until early July) and thus compensate for the lacking plasticity in water extraction depth.

Our findings support the general predictions of niche complementarity theory that communities with high plant diversity tend to be more productive because of fuller resource utilization (Loreau et al. 2001). Furthermore, our study gives more insights into the relationship between plant water sources, species coexistence and productivity. The niche complementarity in water sources among the coexisting species may lead to better use of available soil water. This is likely to be the potential mechanism for high diversity communities with both high productivity and high resilience to droughts (Bai et al. 2004). Similarly, the reduced community productivity and the intensified severity of droughts in the overgrazed community may be attributable to both the losses of biodiversity and diminishing complementarity in plant water use (unpublished data). Our general conclusion was also corroborated by recent studies (Bai et al. 2004, 2007, 2008; Cui et al. 2005; Querejeta et al. 2007).

Long-term observations showed that frequent drought and warming together with overgrazing in this region, which decrease subsoil water recharge and promote shallow rooting species, are likely to be key drivers for the shifts in species composition, i.e., steppe communities originally dominated by *L. chinensis* and *S. grandis* were replaced by *C. squarrosa*, *Artemisia frigida* but also by *Caragana microphylla* (Li 1989; Wittmer et al. 2010; Xiong et al. 2003). Thus the dominant species that utilize subsoil moisture in the undisturbed communities become less abundant in the degraded communities. This may decrease the water use efficiency and further intensify the impacts of drought, overgrazing, and regional warming.

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