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Water use strategies of natural *Pinus sylvestris* var. *Mongolica* trees of different ages in Hulunbuir Sandy Land of Inner Mongolia, China, based on stable isotope analysis

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Abstract

Key message Natural Mongolian pine trees of different ages consistently use shallow water throughout the main growing season; therefore, water stored in the shallow soil layer is vital for maintaining their viability.

Abstract Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantations in sandy regions often experience dieback after 30–35 years of growth due to water deficiency, whereas natural Mongolian pine forests remain healthy during the same growth stage. However, the water use strategies of natural Mongolian pines remains unclear. Therefore, δ^2H and $\delta^{18}O$ in twig xylem water, soil water and groundwater were analyzed in 10–20, 20–30 and 30–50-year-old natural Mongolian pine trees to identify their water sources. In addition, needle $\delta^{13}C$ was measured simultaneously to assess water use efficiency. Results showed that pine trees of different ages utilized soil water from the same depth. During the growing season (June–August), all pine trees utilized water from 0 to 20 cm soil depth, regardless of the soil water condition. During the end of growing season (September and October), even though soil moisture content in the 0–20 cm depth was higher, pine trees of different ages utilized water from the 0–60 cm soil depth in September and switched to utilize water from the 20–80 cm soil depth in October. There were no significant differences in needle $\delta^{13}C$ among the sampling dates for trees in each age group, indicating that pine trees can absorb sufficient water to satisfy their water requirements regardless of age. These findings suggest that water stored in the shallow soil layer (0–20 cm) plays an important role in supporting tree transpiration during the growing season (June–August). Therefore, the stability of shallow soil is vital for maintaining the viability of natural Mongolian pine forests.

Keywords Stable isotopes · Deuterium and oxygen isotopic composition · Carbon isotopic composition · Soil water content · Groundwater

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Introduction

Mongolian pine (*Pinus sylvestris* var. *mongolica*), a regional variety of Scots pine (*Pinus sylvestris*), is naturally distributed in the Daxinganling Mountains of China, in the Hulunbuir sandy plain of China, and in parts of Russia and Mongolia (46°30′–53°59′N, 118°00′–130°08′E) (Zhu et al. 2003, 2005; Song et al. 2014). A strongly cold- and drought-resistant tree species, the Mongolian pine, was first introduced in 1955 to reduce the wind speed and enhance sand fixation in the southeast Keerqin Sandy Land, China (42°43′N, 122°22′E) (Zhu et al. 2003, 2008). The Mongolian pine plantations in the Keerqin Sandy Land were afforested with an analog of natural Mongolian pine forests (pure forest) from the Hulunbuir Sandy Land. However, dieback often appears in Mongolian pine plantations after

30–35 years of afforestation in the Keerqin Sandy Land (Jiao 2001; Zhu et al. 2008), whereas the natural Mongolian pine forests in the Hulunbuir Sandy Land remain healthy at the same growth stage (Zhu et al. 2005, 2008). Although the dieback of Mongolian pine plantations has occurred, large-scale afforestation with Mongolian pine is still under way in arid and semiarid sandy regions of northern China (Song et al. 2017). The dynamics of water availability in soils and the utilization of water sources by trees are critical for tree survival and growth as well as stability of forest ecosystems (Xu et al. 2011; Song et al. 2016a; Liu et al. 2017). Previous studies also have indicated that water deficiency was the main reason for Mongolian pine plantation dieback. Therefore, it is urgent to determine the water use strategies of natural Mongolian pine forests in semiarid regions, which contribute to understand the mechanisms underlying the stability of natural forests and then provide references for plantation forest management to avoid dieback.

For the Mongolian pine trees in the plantation forests, 98% of roots were distributed within 1.0 m, but only 3–5 taproots can reach several meters in depth (4–5 m) (Zhu et al. 2008; Song et al. 2016a). However, for the Mongolian pine trees in the natural forests, the taproot is undeveloped with a maximum rooting depth of approximately 1.5 m in the soil layer, and it has a strong lateral root system with a depth of 30–40 cm in the top soil layer, with the majority of roots only growing in the top 20 cm soil layer (Zhao and Li 1963; Zhu et al. 2005). This indicates that the Mongolian pine trees in the natural forests should utilize shallower water source compared with the Mongolian pine trees in the plantation forests. In addition, Song et al. (2016a) reported that the 10- and 22-year-old Mongolian pine plantation trees only used soil water (0–100 cm), whereas the 32- and 42-year-old Mongolian pine plantation trees utilized both soil water (0–100 cm) and groundwater in the Keerqin Sandy Land. Moreover, Song et al. (2015) reported that the 21-year-old Mongolian pine plantation trees had the highest water use efficiency, followed by the 41-year-old plantation trees and the lowest in the 9-year-old plantation trees. However, it is still unknown how water sources and water use efficiency vary with tree age in natural Mongolian pine forests. Furthermore, the upper layer of soil in the Mongolian pine plantation has been continually disturbed by human disturbance compared with the natural Mongolian pine forests (Jiang et al. 2002; Zhu et al. 2003), which influences the soil water regime and nutrient availability in plantation forests. Therefore, investigation into soil moisture dynamics in the upper soil layer and its association with water uptake sources by the natural Mongolian pine trees of different ages are imperative to learn how this species responds to variations in water availability.

Stable isotope ratios of hydrogen and oxygen in water within the plants could provide information about water sources under natural conditions (Huang and Zhang 2015; Zhou et al. 2015; Song et al. 2016a; Wu et al. 2016). The stable isotope ratios of hydrogen and oxygen in xylem water remain unaltered from that of the soil during water transport between roots and shoots (Ehleringer and Dawson 1992), except for some coastal wetland species (Lin and Sternber 1993) and woody xerophytes (Ellsworth and Williams 2007), where isotope fractionation occurs for hydrogen but not for oxygen during plant water uptake. Therefore, the sources of water used by plants can be identified by comparing the stable isotope ratios of potential water sources with those of xylem water, based on the assumption that no isotopic fractionation occurs during water uptake by plant roots (Dawson et al. 2002). In addition, leaf carbon isotope composition ($\delta^{13}C$) provides useful information on time-integrated water use efficiency (Farquhar et al. 1989), which is an important indicator for detecting seasonal plant water status (Song et al. 2015; Wu et al. 2016). Extensive studies have been conducted to examine the water use strategies in plantation forests by analyzing the stable isotope ratios of xylem water and leaf (Huang and Zhang 2015; Zhou et al. 2015; Song et al. 2016a; Wu et al. 2016). Yet, little is known about the water use strategies of natural forests, especially in semiarid sandy regions.

The objective of the present study was to determine the water sources and water use efficiency for the natural Mongolian pine trees of different ages (NMPDA) in Hulunbuir Sandy Land of Inner Mongolia, China, which could then serve as a reference for the management of Mongolian pine plantation. Based on the available background information, we hypothesized that (1) the NMPDA utilize water from different soil layers, and (2) the NMPDA use shallower water sources compared with the plantation trees. Therefore, the isotopic ratios of twig xylem water, soil water, and groundwater were analyzed to identify the sources of water utilized by NMPDA, and needle $\delta^{13}C$ was measured simultaneously to assess the water use efficiency.

Materials and methods

Study site

The present study was conducted in natural Mongolian pine forests in a sandy region in Honghuaerji (120°03′N, 48°15′E, 789 m above sea level), which is a part of the Hulunbuir Sandy Land in the Inner Mongolia Autonomous Region, China. The natural Mongolian pine forests on sandy land in the Honghuaerji became a National Nature Reserve Area (NNRA) in 1998, with a total extent of 20,085 km^2 (Zhu et al. 2005). Forest coverage in this region is 83.5%. The climate is semiarid with cool and short summers, cold and long winters (Zhu et al. 2005; Yu et al. 2009). The annual mean

precipitation is 391 mm (mean value for 1963–2009) and the pan evaporation is 1174 mm. Annual mean temperature is − 1.8 °C, with annual extremes of − 49.3 and 35 °C, with a growing season mainly between June and August (Zhao and Li 1963; Zhu et al. 2005). The general topography of the NNRA is relatively flat and the major soil type is sandy soil. Fraction of soil particles with diameter ≥ 0.06 mm account for 90.2%, whereas those with diameter < 0.06 mm account for 9.8% (Zhao and Li 1963). The available phosphate and nitrogen contents are 0.02–0.03 and 0.010–0.015%, respectively (Zhu et al. 2003). The average soil depth is approximately 90 cm. In addition, the thickness of litter and humus layers in the study region is approximately 5 and 20–40 cm, respectively (Mao et al. 2009). In the study region, the maximum depth of frozen soil is approximately 40 cm in January, and it starts thawing from the mid-April (Chen et al. 2012). The herbaceous vegetation species belong to the Davuri–Mongolian flora, which are typically patchily distributed in forest glades and edges (Mao et al. 2009).

The natural distribution of Mongolian pine forests in the sandy region includes Honghuaerji, Haila'er, Wangong, Cuogang, He'erhongde, Hunhe, and Ha'erhahe areas in the Inner Mongolia Autonomous Region, China, which are located in semiarid and arid climate zones with sandy soil type (Zhao and Li 1963). Among these regions, the largest area of natural forests in a sandy region, Honghuaerji, is approximately 200 km long and 14–20 km wide (Zhu

et al. 2005). The studied natural Mongolian pine forest was approximately 70 years (2012), with a mean diameter at breast height (DBH, 1.3 m) and height of 23.5 cm and 18.6 m, respectively. The stand density (DBH > 6 cm) of the natural Mongolian pine forest was 424 trees ha^{-1}. The studied forest had a broad tree age distribution. Four plots (400 m^2) were selected randomly in the studied natural Mongolian pine forest. The distance between any two plots was greater than 20 m. In each plot, three sample trees of different ages were selected randomly within a circle (2.5 m diameter) for stable isotope ratios measurements. The age groups of the three sampling trees were 10–20, 20–30, and 30–50 years (determined by tree rings) (Table 1). The natural Mongolian pine trees obtained water mainly from the soil layer via their roots. The groundwater was treated as a potential water source for the natural pine trees because the roots of pine trees are more likely to reach the saturated capillary zone above the water table (2–5 m) and take up groundwater (Zhao and Li 1963; Jiang et al. 2002). Therefore, we focused on the analysis of stable isotopic compositions of the twig xylem water, soil water, and groundwater in the present study.

Plant, soil and water sampling

Plant and soil samples were collected over a 3-year period (2012, 2013 and 2014) on the following dates: 16 August 2012, 6 June 2013, 5 October 2013, 17 August 2014, 15 September 2014, and 5 October 2014 (Fig. 1). Twig xylem samples were collected between 10:00 a.m. and 12:00 p.m. when the trees were actively transpiring on each sampling date. Lignified twigs were cut from the base of the canopy in the four cardinal directions on each sampling tree, and all leaves and green stem tissue were removed from the twigs to avoid contamination of xylem water by isotopically enriched water (Ehleringer and Dawson 1992). Clipped twigs were immediately placed in a capped glass vial, wrapped in parafilm, and

Table 1 Characteristics of the sampling NMPDA in the study region

Tree age (year)	Stand density (trees ha^{-1})	Mean diameter at breast height (cm)	Mean height (m)	Mean crown diameter (m)
10–20	424	6.2	2.6	0.9
20–30	424	14.5	14.9	4.1
30–50	424	26.1	17.6	4.9

Fig. 1 Monthly precipitation and air temperature in 2012, 2013, 2014 and long-term mean (1963–2009) in the study region. Long-term mean soil temperature at 10 cm depth (2001–2011) is also shown. Vertical arrows indicate the sampling dates
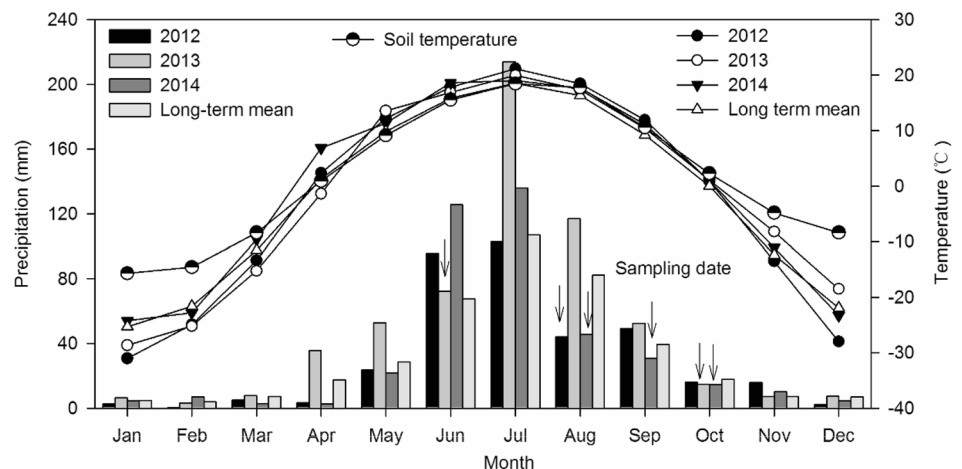
placed in a cooler with ice for transportation to the laboratory. In the laboratory, samples were frozen and stored (− 20 °C). A total of 72 twig samples were collected. Soil samples beneath the three sampling trees in each plot were collected at depths of 0–20, 20–40, 40–60 and 60–80 cm using a soil auger (5 cm diameter). The soil sampling point was established in the center of the three sampling trees in each plot circle. Therefore, there were four soil sampling points on each sampling date and corresponding four replicates per soil layer. We sampled the soil only until 80 cm depth in the present study based on the roots distribution of natural Mongolian pine trees. The soil samples were separated into two parts for stable isotope analyses and gravimetric water content determination. Soil samples for stable isotope analyses were placed into capped vials, wrapped in parafilm, and stored in the freezer (− 20 °C) until water extraction. A total of 96 soil samples were used for isotopic analysis. Soil samples for gravimetric water content determination were placed into aluminum boxes (8 cm diameter and 5 cm height) and sealed.

Groundwater samples were collected on each sampling date from a drilling well (approximately 3.0 km away from the study site), which represent the groundwater for pine trees. Groundwater samples were immediately enclosed in air-tight glass vials, wrapped in parafilm and stored (4 °C) until stable isotope analysis. Three groundwater samples were collected on each sampling date. A total of 18 groundwater samples were taken.

Needle sampling

On each sampling date, current-year needles (the newly emerging needles of the current year) were selected from the middle of the crown (heights) toward the sunlit side of each sampling tree using pruning shears. A total of four replicates per tree age groups were collected at each sampling date. Needles were placed into paper bags and transported to the laboratory for stable carbon isotope measurement.

Stable isotopic composition measurement

Water was extracted from twig xylem and soil samples using a cryogenic vacuum distillation line (100 °C, 3 h) (Ehleringer et al. 2000). Water samples were analyzed in an isotope ratio mass spectrometer (Finnigan MAT Delta V advantage; Thermo Finnigan Inc., Austin, TX, USA) interfaced with an elemental analyzer (Flash EA1112 HT; Thermo Finnigan Inc.). Isotopic ratios of hydrogen and oxygen are expressed in delta notation (δ) using Eq. (1):

$$\delta^2H \text{ or } ^{18}O \ (\text{‰}) = \left(R_{sample}/R_{standard} - 1 \right) \times 1000, \quad (1)$$

where R_{sample} and $R_{standard}$ are the molar ratios of $^2H/^1H$ and $^{18}O/^{16}O$ of the samples and standard water (Vienna Standard Mean Ocean Water), respectively. The analytical errors for δ^2H and $\delta^{18}O$ were ± 1.5 and ± 0.2‰, respectively.

Needle samples were oven-dried to a constant mass at 75 °C for 48 h, finely ground, and then sub-sampled for stable carbon isotope analysis. The needle $\delta^{13}C$ values were measured using a continuous-flow isotope ratio mass spectrometer (Delta Plus XP; Thermo Finnigan Inc.). $\delta^{13}C$ was expressed in delta notation relative to an accepted international standard (Pee Dee Belemnite). The analytical error for $\delta^{13}C$ was ± 0.2‰.

Soil water content measurement

The soil samples in aluminum boxes were weighed to determine fresh weight, and then oven-dried at 105 °C for 24 h. Soil samples were weighed again to determine dry weight, and then, gravimetric water content was calculated using Eq. (2):

$$\text{Soil water content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100. \quad (2)$$

Meteorological data measurement

Long-term precipitation and temperature (air and soil) data were obtained from the Honghuaerji weather station (approximately 3.5 km away from the study site). During the study period, precipitation was sampled from the Honghuaerji weather station, and the air temperature was measured using a HOBO data logger near the study site.

Data analysis

To identify the sources of water utilized by the natural Mongolian pine trees, the isotopic ratios of twig xylem water were compared with the isotopic ratios of soil water at different depths and groundwater. As contributions of water from different water sources could account for the same stem water isotope values, actual plant water sources may be misinterpreted if only the direct inference approach was used (Asbjornsen et al. 2007; Nie et al. 2012). Therefore, the IsoSource mixing model (Phillips and Gregg 2003; Phillips et al. 2005) was used to calculate the mean and possible range of contributions from different water sources, which were treated as supplementary information to identify the water uptake depths. Both $\delta^{18}O$ and δ^2H values were used for the calculations. In this method, all possible combinations of each source contribution (0–100%) were examined in small increments (e.g., 1%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g., 0.2‰) were considered feasible solutions, using which the frequency and range of potential water source contributions could be determined (Phillips and Gregg

2003; Phillips et al. 2005). The minimum and maximum proportions for each source were reported; therefore, the relative importance of individual sources could be assessed by comparing the range of their proportional contributions to the mixture (Phillips and Gregg 2003). In general, a low maximum value for a source would indicate relatively low importance, whereas a high minimum value for a source could indicate a significant contribution. If the maximum value of the source was not high and the minimum value was not low, these sources might be important (Nie et al. 2012). Furthermore, additional non-isotopic constraints were also used to further rule out certain sources.

One-way analysis of variance (ANOVA) was used to detect significant differences in soil water content among the different soil depths on each sampling date, the needle $\delta^{13}C$ among the different-aged pine trees at each sampling date, and the needle $\delta^{13}C$ among sampling dates for trees of each age. The effects of soil depth and sampling date on soil water content on all the sampling dates and the effects of tree age and sampling date on twig xylem water δ^2H and $\delta^{18}O$ on all the sampling dates were examined using two-way ANOVA. Statistical analyses were performed using SPSS 16.0 software. The level of significance was set at 0.05.

Results

Precipitation, temperature and soil water content

Total precipitation during 2012, 2013 and 2014 was 361.8, 591.2 and 406.1 mm, respectively, which was 92.5, 151.2 and 103.9% of the long-term mean annual precipitation (391 mm, Fig. 1), respectively. Variations in monthly mean air temperatures in 2012, 2013 and 2014 (-2.2, -1.5, and -0.5 °C, respectively) were consistent with the variation in the 57-year mean (Fig. 1). In addition, the long-term mean monthly soil temperature at 10 cm depth (1.9 °C) had a similar pattern with the long-term mean monthly air temperature (Fig. 1).

Soil water content significantly decreased with soil depth on each sampling dates (one-way ANOVA, $P < 0.05$, Fig. 2). In addition, the two-way ANOVA results showed that the soil water content (0–80 cm depth) significantly differed among different sampling dates ($F = 13.56$, $P < 0.05$, Fig. 2), with higher values on 5 October 2013 (12.0%), 15 September 2014 (11.3%) and 5 October 2014 (11.6%) than on 16 August 2012 (8.9%), 6 June 2013 (7.8%) and 17 August 2014 (8.6%). The two-way ANOVA results also showed the mean soil water content was also significantly different across soil depths on all the sampling dates ($F = 83.82$, $P < 0.05$, Fig. 2), decreasing from 13.9% in 0–20 cm depth to 5.8% in 60–80 cm depth. Moreover, significant interaction effect between sampling date and soil depth was observed
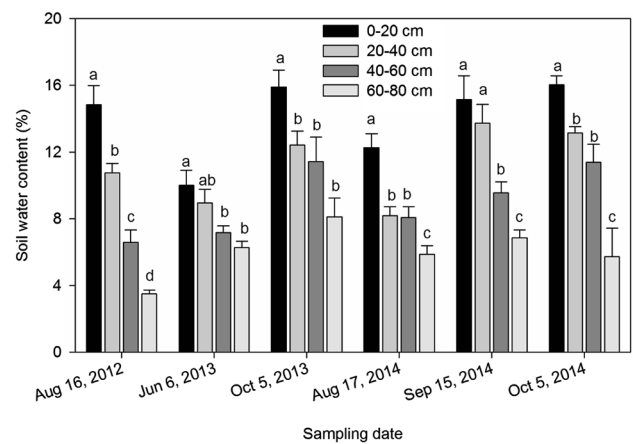

Fig. 2 Variations in soil water content of the different soil depths in the natural Mongolian pine forest during the measurement period. Error bars represent SE ($n = 4$). Different letters indicate significant differences in soil water content among soil depths on each sampling date at $P < 0.05$

during the entire measurement period (two-way ANOVA, $F = 2.57$, $P < 0.05$).

Isotopic compositions of soil water, groundwater and twig xylem water

Average $\delta^{18}O$ and δ^2H of twig xylem water for the natural pine trees, soil water and groundwater were located along or below the local meteoric water line ($\delta^2H = 7.59\ \delta^{18}O$ -0.14; Gao et al. 2017) (Fig. 3), possibly due to evaporation. The isotopic ratios of soil water ranged between -22.2 and $-8.71‰$ for $\delta^{18}O$ and between -167.9 and $-75.3‰$ for δ^2H for all sampling dates and soil depths, and were higher in the 0–20 cm depth compared with the deeper soil layers on most sampling dates (Fig. 3). Compared with the soil water, the groundwater exhibited relatively stable isotope values ($-14.9 \pm 0.2‰$ for $\delta^{18}O$, $-113.3 \pm 1.0‰$ for δ^2H). $\delta^{18}O$ and δ^2H of the twig xylem water varied with sampling date during the measurement period (Fig. 3), ranging from -19.9 to $-8.4‰$ for $\delta^{18}O$ and from -155.7 to $-75.1‰$ for δ^2H. Significant differences were observed in twig xylem water $\delta^{18}O$ and δ^2H across the sampling dates (two-way ANOVA, $P < 0.05$), with the lowest values on 6 June 2013 (Table 2; Fig. 3). In addition, only δ^2H (two-way ANOVA, $P < 0.05$) but not $\delta^{18}O$ (two-way ANOVA, $P > 0.05$) of twig xylem water differed significantly among tree ages, with higher values in the 10–20- and 30–50-year-old age groups (Table 2), possibly due to higher experimental error ($\pm 2.5\%$) and variation of the biological fractionation factors for δ^2H.

The average $\delta^{18}O$ and δ^2H of twig xylem water for different-aged natural pine trees overlapped with those of soil water in the 0–20 and 20–40 cm depths on 6 June 2013 and 17 August 2014 (Fig. 3a, c), although the error
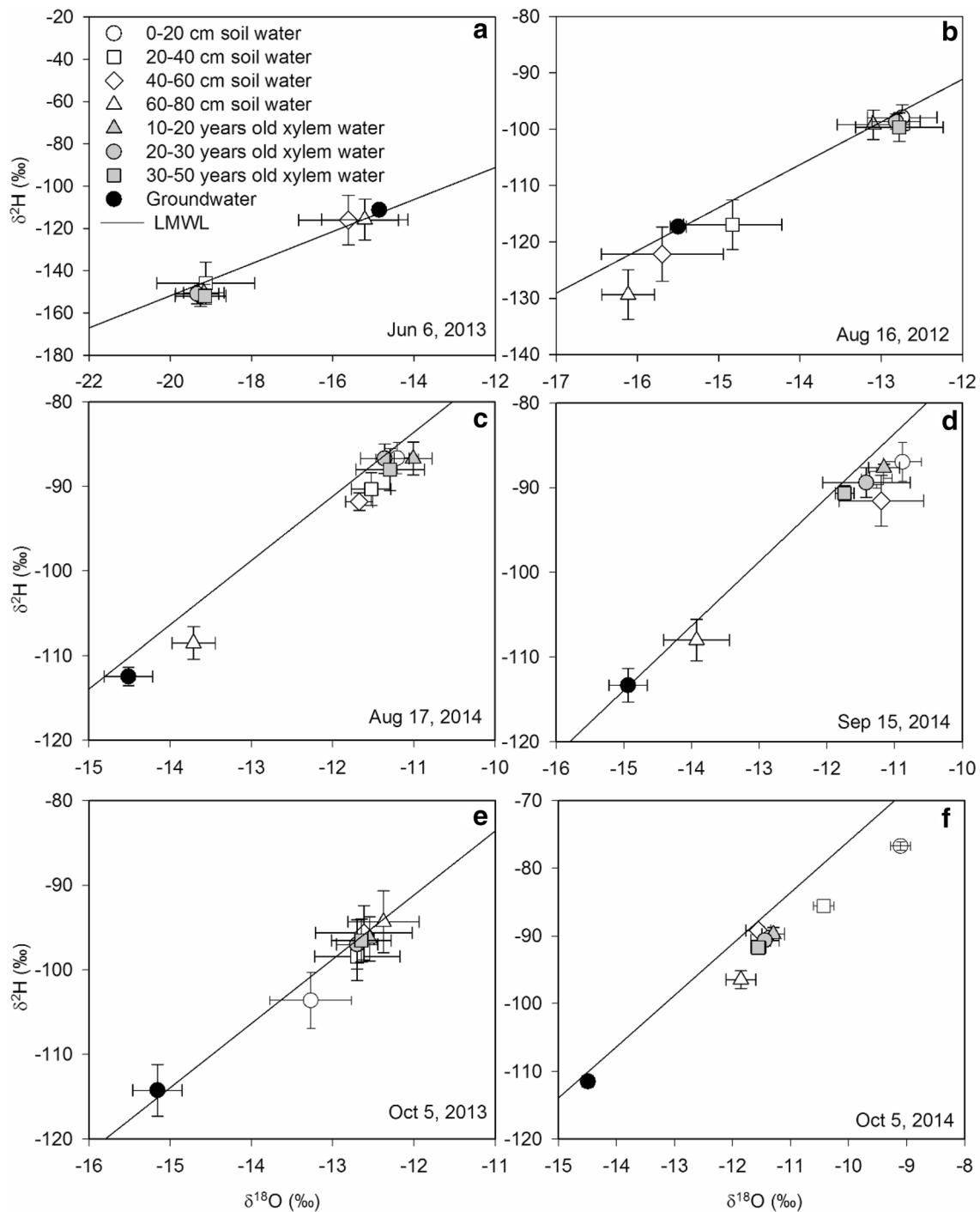
Fig. 3 Average δ^2H and $\delta^{18}O$ values for water extracted from tree twig xylem and soil over the six sampling dates, as well as groundwater collected on tree sampling dates. The fitted line is the Local Meteoric Water Line (LMWL: $\delta^2H = 7.59\ \delta^{18}O - 0.14$, Gao et al. 2017). Error bars represent SE ($n = 4$)

range of isotopic ratios for twig xylem water also overlapped with those of soil water at 40–60 cm depth on 17 August 2014. However, the average $\delta^{18}O$ and δ^2H of twig xylem water for different-aged natural pine trees typically overlapped with those of soil water in the 0–20 cm depths on 16 August 2012 (Fig. 3b). On 15 September 2014, the average $\delta^{18}O$ and δ^2H of twig xylem water for the natural pine trees overlapped with those of soil water at 0–20, 20–40 and 40–60 cm depths (Fig. 3d); however, these values were often intermediate to those of soil water at

Table 2 Effect of tree age and sampling date on the δ^2H and $\delta^{18}O$ of the twig xylem water of NMPDA

Sources	df	δ^2H		$\delta^{18}O$	
		F	P	F	P
Tree age	2	5.61	<0.05	2.63	>0.05
Sampling date	5	280.46	<0.05	183.00	<0.05
Tree age × sampling date	10	4.15	<0.05	3.41	<0.05

20–40, 40–60 and 60–80 cm depths on 5 October 2013 and 2014 (Fig. 3e, f).

IsoSource estimation of feasible contributions of potential water sources

Results from the mixing IsoSource model show that the fraction of five potential water sources typically varied among sampling dates for the pine trees of different ages. In June (2013) and August (2012 and 2014), the contributions of 0–20 cm soil water occupied a large percentage of water usage for the pine trees in each age group (Table 3). However, by September (2014), the contributions of 0–20 cm soil water decreased, while those of 20–40 and 40–60 cm soil water increased, except for the 40–60 cm soil water in 30–50-year-old pine trees (Table 3). From September to October (2013 and 2014), the usage of 0–20 and 20–40 cm

soil water decreased, except for the 0–20 cm soil water in 30–50-year-old pine trees and 20–40 cm soil water in 10–20-year-old pine trees, whereas the usage of 40–60 and 60–80 cm soil water increased (Table 3).

Needle $\delta^{13}C$ for NMPDA

The needle $\delta^{13}C$ for the pine trees varied with sampling dates (Fig. 4). Needle $\delta^{13}C$ ranged from −31.52 to −28.52‰ (mean value: −29.88‰) for the 10–20-year-old trees, from −30.15 to −26.29‰ (mean value: −29.06‰) for the 20–30-year-old trees, and from −29.84 to −27.10‰ (mean value: −28.57‰) for the 30–50-year-old trees. No significant differences in $\delta^{13}C$ were observed among the sampling dates for the trees in each age group ($F = 0.63$, $P > 0.05$ for 10–20 years; $F = 0.42$, $P > 0.05$ for 20–30 years; and $F = 0.56$, $P > 0.05$ for 30–50 years; Fig. 4). In addition, the needle $\delta^{13}C$ was significantly greater in older trees on each sampling date ($P < 0.05$, Fig. 4).

Discussion

Sources of water used by the NMPDA

$\delta^{18}O$ and δ^2H of twig xylem water and the results of mixing IsoSource model indicated that the natural Mongolian pine

Table 3 Proportions of feasible water sources (%) for NMPDA over six sampling dates

Sampling date	Tree age (year)	Potential water sources				
		0–20 cm soil water	20–40 cm soil water	40–60 cm soil water	60–80 cm soil water	Groundwater
16 Aug 2012	10–20	93.6 (93–95)	1.4 (0–5)	1.5 (0–6)	0.6 (0–4)	2.8 (0–7)
	20–30	96.6 (96–98)	1.1 (0–4)	0.7 (0–3)	0.5 (0–2)	1.1 (0–4)
	30–50	92.3 (90–95)	2.5 (0–10)	1.7 (0–7)	1.3 (0–6)	2 (0–8)
6 Jun 2013	10–20	88.7 (71–96)	8.3 (0–29)	1.0 (0–5)	1.0 (0–5)	0.9 (0–4)
	20–30	91.7 (79–97)	6.4 (0–21)	0.7 (0–3)	0.7 (0–3)	0.5 (0–3)
	30–50	98.0 (96–100)	2.0 (0–4)	0	0	0
5 Oct 2013	10–20	5.8 (0–23)	13.7 (0–53)	32.9 (0–97)	45.1 (0–90)	2.4 (0–10)
	20–30	8.4 (0–31)	18.5 (0–70)	32.7 (0–93)	36.6 (0–87)	3.7 (0–14)
	30–50	6.5 (0–25)	15.0 (0–58)	33.7 (0–92)	42.1 (0–89)	2.8 (0–12)
17 Aug 2014	10–20	98.4 (97–100)	1 (0–3)	0.6 (0–2)	0	0
	20–30	96.3 (93–100)	2.2 (0–7)	1.4 (0–1)	0.1 (0–1)	0 (0–1)
	30–50	79.1 (58–95)	10.8 (0–42)	7.5 (0–30)	1.4 (0–7)	1.1 (0–6)
15 Sep 2014	10–20	71.7 (39–96)	22.3 (0–61)	4.1 (0–18)	1.1 (0–5)	0.8 (0–4)
	20–30	29.1 (0–82)	59.7 (6–98)	6.8 (0–30)	2.3 (0–10)	2.1 (0–9)
	30–50	4.7 (4–5)	95.0 (94–96)	0.3 (0–1)	0	0
5 Oct 2014	10–20	18.8 (0–62)	22.6 (0–82)	32.2 (0–84)	14.4 (0–52)	11.9 (0–37)
	20–30	16.8 (0–60)	20.8 (0–79)	32.4 (0–84)	15.9 (0–55)	14.1 (0–40)
	30–50	16.0 (0–57)	20.8 (0–77)	26.7 (0–76)	19.3 (0–64)	17.3 (0–43)

Both δ^2H and $\delta^{18}O$ were used for the calculations. Average source proportions calculated by the model are shown, as well as range of minimum and maximum source proportions
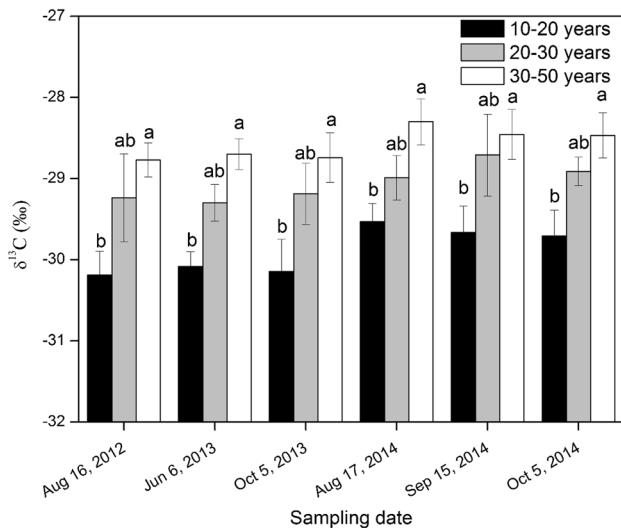
Fig. 4 Variations in $\delta^{13}C$ of the current-year-old needles for NMPDA during the measurement period. Error bars represent SE ($n=4$). Different letters indicate significant differences in needle $\delta^{13}C$ among different aged pine trees on each sampling date at $P<0.05$

trees of different ages used soil water from the same depth (Fig. 3; Table 3), which was not consistent with our hypothesis (1), but supported our hypothesis (2). Similar results have been reported in other environments (Rong et al. 2011; Goldsmith et al. 2012; Penna et al. 2013), which showed that the tree size or age does not affect water uptake. For example, Goldsmith et al. (2012) reported that plant species at different sizes (ages) used water from the same soil layer in a seasonally dry tropical montane cloud forest, owing to relatively high soil water availability and root distribution in relation with nutrient availability. Our findings were in contrast to those obtained from other forests, where a clear partitioning of soil water resources among plants of different ages (sizes) was exhibited (Su et al. 2014; Liu et al. 2015). For instance, Su et al. (2014) reported that the 2–5-year-old saplings and juvenile trees of *Ulmus pumila* utilized water from the soil layer at depth > 40 cm; however, mature trees mainly utilized very deep, stable groundwater (> 2 m) in a semiarid sandy region in northern China. In the present study, pine trees utilized water from the 0–20 soil depth in June and August (Fig. 3), as indicated by $\delta^{18}O$ and $\delta^{2}H$ of twig water and the results of the mixing IsoSource model (Table 2). Utilization of water from the 0–20 cm soil depth for the pine trees was possibly due to relatively higher soil water availability in the upper soil layer and root distribution related to nutrient availability (Zhao and Li 1963). In June (2013) and August (2012 and 2014), the soil moisture in the 0–20 cm depth was high and was significantly higher than at other depths (Fig. 2). Therefore, the natural pine trees were easy to obtain enough water from the 0–20 cm soil depth. Li et al. (2007) also reported that the most water was absorbed

from depths with relatively high soil water content. In addition, for natural Mongolian pine trees, most of their roots are distributed within the top 20 cm soil layer (Zhao and Li 1963; Zhu et al. 2005). In fact, nitrogen and phosphorus, two primary limiting macronutrients, have been demonstrated to have the highest concentration at soil depths < 20 cm in Mongolian pine ecosystems (Zhu et al. 2005). Therefore, the utilization of soil water at 0–20 cm depth during June and August may be beneficial for nutrient uptake. Retzlaff et al. (2001) also reported that water uptake from the upper soil profile during the growing season could be attributed to increased fine root production and root system function related to nutrient uptake.

In September (2014), although the soil water content in the shallow layer (0–20 cm) was higher (Fig. 2), the natural pine trees mainly obtained soil water from the 0–60 cm depth, as demonstrated by the isotopic ratios of twig xylem water and the results of the IsoSource model. This might be because shallow soils (0–20 cm) become colder than deeper soils in September (Zhang et al. 2014). Lower soil temperature in the 0–20 cm depth could reduce the absorption of water directly by decreasing the permeability of roots to water and indirectly by increasing the viscosity of water (Kozlowski 1987). Therefore, the natural pine trees reduced the proportion of water usage from the 0–20 cm soil depth and switched to partial use water from the 20–60 cm soil depth (Table 3). However, in October (2013 and 2014), the natural pine trees used water from the 20–80 cm soil depth (Fig. 3; Table 3). Although the results of the IsoSource model showed relatively high proportions of 0–20 cm soil water contribution for the pine trees in October 2014, the upper 0–20 cm soil started to freeze due to low and more extreme soil temperature (Fig. 1). Therefore, the natural pine trees could not use the 0–20 cm soil water in October. In agreement with our findings, Eggemeyer et al. (2009) reported that *Pinus ponderosa* trees extracted the majority of their water from the deeper soil layer during winter in the semiarid Sandhills grasslands of Nebraska, USA, which could be attributed to low and more extreme soil temperatures in the upper soil profiles, reducing the shoot demand or changing root system function.

The $\delta^{13}C$ of the NMPDA

At the leaf level, $\delta^{13}C$ of recently assimilated carbohydrates is a useful integrator of the long-term balance between photosynthetic capacity and stomatal conductance and of intrinsic water use efficiency (Farquhar and Richards 1984; Brendel 2001; Ogée et al. 2009). Therefore, in the present study, we assumed that the current-year needles were mainly formed from recently assimilated carbohydrates. No significant differences in $\delta^{13}C$ of the current-year needles among the sampling dates for the trees in each age group indicated

that the natural trees maintained a constant water use efficiency during the measurement period. Similar results have been reported in other ecosystems (Schulze et al. 1996; Song et al. 2014). Li and Zhang (2003) reported that the leaf $\delta^{13}C$ of several dominant species was relatively stable during the growing season in the southern margin of the Taklimakan desert, suggesting that they did not suffer from water stress under current water conditions. Song et al. (2014) reported that the needle $\delta^{13}C$ for Mongolian pine trees in a sparse wood grassland did not differ between months and years in a semiarid sandy region of northeast China, suggesting that the water obtained by the Mongolian pine trees in a sparse wood grassland might basically satisfy their water requirements during the measurement period. In the present study, no significant differences in needle $\delta^{13}C$ among the sampling dates for the natural Mongolian pine trees of different ages might be related to high soil water availability during the measurement period (Fig. 2). In the study region, in addition to precipitation, the melt water from accumulated snow could supply water for pine growth as well as contribute to soil moisture (Zhu et al. 2005; Chen et al. 2012; Song et al. 2017). Furthermore, the temperature in the study region was low, leading to low soil evaporation and transpiration demand (Zhu et al. 2005; Goldsmith et al. 2012). These data suggest that natural pine trees could obtain enough water to satisfy their relatively low transpiration demand, and thus, natural pine trees could maintain constant water use efficiency during the measurement period.

Moreover, the significant higher needle $\delta^{13}C$ in the older pine trees indicates that the older natural pine trees had a higher water use efficiency, which is consistent with the tree age and height-related increase in water use efficiency resulting from increased hydraulic limitations (McDowell et al. 2005). Similar results have also been reported for *Pinus sylvestris* in North Scotland (Martínez-Vilalta et al. 2007) and for *Pseudotsuga menziesii* in America (McDowell et al. 2005). For example, McDowell et al. (2005) reported that foliar $\delta^{13}C$ of *Pseudotsuga menziesii* significantly increased with tree size (age) in wet and mild climatic conditions due to high soil water availability. In the present study, the soil water was plentiful; thus, significant higher needle $\delta^{13}C$ in the older pine trees was mainly due to tree age and height-related hydraulic limitation.

Implication

Our results indicate that the natural Mongolian pine trees of different ages maintained static water use efficiency and static use of shallow water (0–20 cm) during the growing season over multiple years. It was concluded that this is a temporally consistent behavior. This was consistent across different age cohorts and also coherent with the knowledge of rooting depths, which was linked with high water and nutrient availability in shallow soils by the previous studies (Goldsmith et al. 2012; Penna et al. 2013). This indicated that the tree size or age does not affect water uptake behavior. Only during the end of growing season did we observe a shift towards use of deeper soil water, because shallow soils became colder than deeper soils. Therefore, water stored in the shallow soil layer plays an important role in maintaining the stability of the natural Mongolian pine.

Our findings have important implications for the management of Mongolian pine plantation. Compared with the water source utilization of natural forests, Mongolian pine plantation trees used groundwater besides soil water when their age was greater than 30 years (Song et al. 2016a, b). This indicates that any sudden groundwater level decline, due to water uptake, may lead to additional tree water stress, crown dieback, or even tree death in the Mongolian pine plantation (Zheng et al. 2012; David et al. 2013; Song et al. 2016b). In these conditions, thinning has been reported to be the most effective management practice. By reducing stand density, soil water availability to the remaining trees could be increased, thus reducing its dependence on groundwater (Giuggiola et al. 2013; Song et al. 2016a). Therefore, thinning is recommended in Mongolian pine plantations. In addition, the upper layers of soil in plantations have been continually disturbed by human disturbance, such as grazing and needle litter collection for firewood (Jiang et al. 2002; Zhu et al. 2005). Grazing and trampling can reduce infiltration of water and increase runoff and erosion, and alter spatial arrangement of nutrients, thus reducing the water and nutrient availability to tree roots (Yusuf et al. 2015). Meanwhile, needle litter collection for firewood not only removes large quantities of nutrients from the forest ecosystem, but also increases the soil evaporation and reduces soil water availability for plantation trees (Jiang et al. 2002). Therefore, countermeasures for reducing human disturbances (e.g., prohibiting grazing and needle litter collection) should be undertaken to maintain the stability of Mongolian pine plantations in semiarid regions (Jiang et al. 2002; Zhu et al. 2005).

Conclusion

In the present study, we first use stable isotope techniques to determine the water utilization pattern of the natural Mongolian pine trees of different ages in the Hulunbuir Sandy Land. The natural pines of different ages used soil water from the same depth. During the growing season (June–August), the natural trees used water from the 0–20 cm soil depth. During the end of growing season (September and October), they switched the water source from the 0–60 cm soil depth in September to the 20–80 cm soil depth in October. In addition, no significant differences in needle $\delta^{13}C$ among the

sampling dates for the trees in each age group were found, which suggested a constant water use efficiency during the measurement period. These findings indicate that shallow soil water was the main water source for transpiration in the natural Mongolian pine trees during the growing season. Therefore, shallow soil might play an important role in maintaining natural Mongolian pine stability. Countermeasures for reducing human disturbances and thinning should be undertaken to maintain the stability of Mongolian pine plantation in semiarid sandy land.

Author contribution statement LS: writing the paper and running the data analysis. JZ: designing the experiment and writing the paper. ML: designing the experiment. JZ: running the data analysis. DL: running the data analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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