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Effect of Drought and Topographic Position on Depth of Soil Water Extraction of *Pinus sylvestris* L. var. *mongolica* Litv. Trees in a Semiarid Sandy Region, Northeast China

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Abstract: Drought and topographic position are the most important factors influencing tree growth and survival in semiarid sandy regions of Northeast China. However, little is known about how trees respond to drought in combination with topographic position by modifying the depth of soil water extraction. Therefore, we identified water sources for 33-year-old Mongolian pine (*Pinus sylvestris* L. var. *mongolica* Litv.) trees growing at the top and bottom of sand dunes by comparing stable isotopes $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in twig xylem water, soil water at various depths and groundwater during dry and wet periods. Needle carbon isotope composition ($\delta^{13}\text{C}$) was simultaneously measured to assess water use efficiency. Results showed that when soil moisture was low during the dry period, trees at the top used 40–300 cm soil water while trees at the bottom utilized both 40–300 cm soil water and possibly groundwater. Nevertheless, when soil moisture at 0–100 cm depth was higher during the wet period, it was the dominant water sources for trees at both the top and bottom. Moreover, needle $\delta^{13}\text{C}$ in the dry period were significantly higher than those in the wet period. These findings suggested that trees at both the top and bottom adjust water uptake towards deeper water sources and improve their water use efficiency under drought condition. Additionally, during the dry period, trees at the top used shallower water sources compared with trees at the bottom, in combination with significantly higher needle $\delta^{13}\text{C}$, indicating that trees at the bottom applied a relatively more prodigal use of water by taking up deeper water (possibly groundwater) during drought conditions. Therefore, Mongolian pine trees at the top were more susceptible to suffer dieback under extreme dry years because of shallower soil water uptake and increased water restrictions. Nevertheless, a sharp decline in the groundwater level under extreme dry years had a strong negative impact on the growth and survival of Mongolian pine trees at the bottom due to their utilization of deeper water sources (possibly groundwater).

Keywords: stable isotopes; water sources; water use efficiency; soil water; groundwater

1. Introduction

Drought is one of the most important environmental factors limiting tree growth and survival worldwide [1,2]. Under drought condition, trees in arid and semiarid environments could maximize water uptake (e.g., absorbing deep water sources through extensive, deep, or dense root systems) or/and minimize water loss through transpiration (e.g., limiting leaf growth, stomatal closure, etc.) [3,4]. For example, many trees in arid and semiarid regions have a dimorphic root system that enables them to switch water sources from shallow soil layers in the wet season to deep soil layers (e.g., groundwater) in the dry season [5–7]. A similar phenomenon has been also observed for plants in the saline southeast Everglades ecotone [8]. The shift of water sources plays an important role in the survival and growth of trees in arid and semiarid regions [9]. In addition, under drought condition, many trees could improve their water use efficiency by decreasing their stomatal conductance to limit water loss, which is associated with a high capacity for drought resistance [10–12]. However, climate change will increase temperatures and the frequency and severity of drought events in arid and semiarid regions [13]. Furthermore, a large number of episodes of tree dieback and mortality have been shown to be associated with drought [14–16]. Therefore, determining the depth of water extraction by trees under drought condition will improve our understanding of the survival and growth strategies for trees in arid and semiarid regions [17].

Moreover, the topographic position is another factor affecting the tree's survival and growth in semiarid and arid regions [18,19], especially in sandy regions. This is because the sandy regions are characterized by the distribution of sand dunes, which have been shown to provide habitat heterogeneity and to profoundly influence the spatial and temporal distribution of water and the nutrition regime [20,21]. It has been reported that trees at the top of sand dunes had a lower leaf water potential or stronger stomatal regulation than did trees at the bottom of the sand dunes, which may be due to more available water for trees at the bottom of sand dunes [22,23]. Although numerous studies have investigated effective topographic positions in semiarid and arid sandy regions [4,18,24], knowledge about the effects of drought and topographic position on the depth of water extraction of trees is still not well-known, which limits our understanding of the long-term adaptation mechanisms of trees to temporal and spatial changes of water availability in semiarid and arid sandy regions [25].

Mongolian pine (*Pinus sylvestris* var. *mongolica*), a geographical variety of Scots pine (*Pinus sylvestris* L.), is naturally distributed in the Daxinganling Mountains of China (50°10'–53°33' N, 121°11'–127°10' E) and in Honghuaerji, on the sandy plains of Hulunbeier (47°35'–48°36' N, 118°58'–120°32' E) [26]. Due to its hardy nature and suitability for sandy environments, Mongolian pine was first introduced to reduce wind speed and to enhance sand fixation in the 1950s in the southeast of the Keerqin Sandy Land, China [12,27,28]. Until now, the area of the Mongolian pine plantations in the sandy lands covers more than 7.0×10^5 ha in northern China [29]. These Mongolian pine plantations play an important role in the control of desertification in northeast China [28,30,31]. However, the Mongolian pine plantations have frequently suffered from dieback and mortality during extreme drought years when they are more than approximately 30–35 years old [26,32]. Previous studies have indicated that water deficiency was the main cause of the dieback and mortality of Mongolian pine plantations [26,28]. In the Keerqin Sandy Land, precipitation (soil water) and groundwater are the main water sources for the growth and survival of Mongolian pine trees [32,33]. However, extreme drought occurs frequently during the growing season [29], and the groundwater level has decreased linearly in the southeast Keerqin Sandy Land since the introduction of the Mongolian pine plantations [28]. Furthermore, it has been observed that soil moisture at the top of sand dunes was significantly lower than that at the bottom of sand dunes in the Keerqin Sandy Land [31]. Nevertheless, how the depth of water extraction of the Mongolian pine trees respond to the effect of drought and topographic position is still unknown, which limits our understanding of the mechanism underlying the dieback and mortality of the Mongolian pine plantations in semiarid sandy regions.

The objective of this study was to determine the depth of water extraction of the Mongolian pine trees at the top and bottom of sand dunes during dry and wet periods based on their water sources and

water use efficiency. The depth of water extraction by trees can be identified by comparing the stable hydrogen and oxygen isotopes of the potential water sources with those of xylem water [32,34,35]. This is because the isotopic compositions of hydrogen (^2H) and oxygen (^{18}O) in water do not change when they are taken up by roots and transported from the roots to the leaves [36]. The common assumption is that isotope fractionation does not occur during water uptake by roots and transported from the roots to the leaves. Therefore, the isotopic signature of plant xylem water and water in the soil at the site of root uptake are similar [37]. In addition, leaf stable carbon isotope composition ($\delta^{13}\text{C}$) provides a comprehensive insight into tree carbon cycles and water use efficiency (WUE) and has also been widely applied to evaluate the response of trees to environmental change [36]. Due to higher precipitation in the wet period than in the dry period [30], we hypothesized that (1) the Mongolian pine trees use deeper water sources and have higher water use efficiency during the dry period compared to the wet period. In consideration of the known differences in physical and hydraulic properties between the top and the bottom of sand dunes [31], e.g., higher soil water content in the bottom of sand dunes than in the top of sand dunes, we also hypothesized that (2) the Mongolian pine trees growing on the top of sand dunes utilize deeper water sources and have higher water use efficiency compared with those growing on the bottom of sand dunes. In order to verify the above two hypotheses, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of twig xylem water, soil water at 0–300 cm depth, and groundwater were measured and analyzed, in combination with soil moisture, to determine the depth of water extraction by the 33-year-old Mongolian pine trees growing at the top and bottom of sand dunes, during the dry and wet periods of 2017. In addition, the needle carbon isotope composition ($\delta^{13}\text{C}$) was measured simultaneously to assess their WUE. This study would contribute to our understanding of the mechanism underlying the dieback and mortality of the Mongolian pine plantations, which supply some implications for forest management in semiarid sandy regions.

2. Materials and Methods

2.1. Study Site

This study was conducted in the Zhanggutai region (42°43' N, 122°22' E, 226 a.s.l.), Liaoning Province, China, which is located in the southeastern part of Keerqin Sandy Land. This region belongs to the semiarid climatic zone. The mean annual temperature is approximately 6.7 °C with the minimum and maximum air temperatures of −29.5 °C and 37.2 °C, respectively. The annual mean precipitation is 474 mm (mean for the period of 1954–2010), whereas the mean pan evaporation is approximately 1700 mm (an evaporation pan with a diameter of 20 cm) [32]. In the study region, from the beginning of May to late June, the precipitation was low (25% of the mean annual precipitation), but the evaporative demand was high; thus, the soil moisture was low. This period (May and June) was considered to be the dry period (Figure 1a). By July and August, the precipitation was plentiful (approximately 50% of the mean annual precipitation), and the evaporative demand was low; thus, the soil moisture was high. This period was considered to be the wet (rainy) period (Figure 1a, [30]). The major soil type was classified as belonging to the Semiaripsamment taxonomic group that developed from sandy parent material through the action of wind [26]. Based on landforms, vegetation cover types, and soil characteristics, the sandy areas were classified into active and semi-active sand dunes, fixed sand dunes, flat sandy land, and low-lying land [31]. The soil physical and hydraulic properties differ by the different positions of the sand dunes. The particle sizes of soil at the top of the sand dunes were 32.37% coarse (1–0.250 mm), 48.80% fine (0.250–0.06 mm), and 18.83% silt (<0.06 mm); the corresponding values at the bottom of sand dunes were 12.29%, 58.48%, and 29.22% [31]. The organic matter content of the soil at the bottom of sand dunes was 0.61%, whereas it was 0.07% at the top of sand dunes. The total soil nitrogen, phosphorus, and potassium (0–100 cm) at the bottom of the sand dunes were 0.08%, 0.008%, and 1.17%, respectively, whereas they were 0.03%, 0.003%, and 2.01% at the top of sand dunes, respectively [38]. The understory was annual grass, such as *Potentilla anserine* L., *Cleistogenes chinensis* (Maxim.) Keng., *Artemisia frigida* Willd., and *Setaria viridis* (L.) Beauv. [32].

Figure 1. Long-term monthly mean precipitation and monthly precipitation in 2017 (a), and daily precipitation (b) in the growing season of 2017. Arrows indicate sampling dates.

The studied Mongolian pine plantation was 33 years old in 2017. The area of this plantation was 7.8 ha. The topography of this plantation was characteristic by undulating fixation sand dunes. The planting spacing was 3 m by 3 m, and sanitation cutting had been implemented in the plantation over the past years. Four typical fixation sand dunes were selected in this study (Table 1). The mean vertical dimension between the top and bottom of the selected sand dunes was 8.7 ± 1.3 m (Table 1). In each selected sand dune, two 10 m $\times$ 10 m plots were established; one was at the top of the sand dune and the other was at the bottom (west-facing) of the sand dune. In each plot, one representative Mongolian pine tree was selected based on the averaged diameter at breast height and tree height (Table 1). The total number of sampling trees was eight. Although the number of sampling trees (four trees in the bottom of sand dunes and four trees in the top of sand dunes) was small, four replicates could generally meet the demand of statistical analysis, which have been widely applied in determining the water source used by plants because of the expensive test fare [7].

Table 1. Characteristics of the Mongolian pine plantations at the top and bottom of selected sand dunes in the study region. Different letters indicate significant differences between the top and bottom of sand dunes.

No. of Sand Dunes	Position of Sand Dunes	Stand Density (Trees hm ²)	Mean DBH (cm)	Mean Height (m)	Canopy Area (m ²)	Vertical Dimension of Different Positions (m)
1	Top	600	15.2	7.3	10.5	10.0
	Bottom	400	25.3	11.5	26.6	
2	Top	400	23.5	8.2	23.5	12.0
	Bottom	300	27.1	11.3	33.5	
3	Top	500	19.3	6.7	21.6	6.8
	Bottom	500	26.3	11.0	28.8	
4	Top	700	15.2	7.1	12.9	6.0
	Bottom	600	18.7	10.6	15.7	
Mean	Top	550 a	18.3 b	7.3 b	17.1 b	8.7
	Bottom	450 a	23.4 a	11.1 a	26.2 a	

2.2. Twig Xylem, Soil, Precipitation, and Groundwater Sampling

Twig xylem and soil samples were collected on June 9 (dry period) and August 25 (wet period) during the growing season of 2017 (Figure 1b). The twig xylem samples were collected between 8:00 am and 12:00 pm when the trees were actively transpiring on each sampling date. The lignified twigs (diameter 0.2–0.5 cm, length 4–6 cm) were cut from the base of the canopy in each sampling tree, and all leaves and green stem tissue were removed from these twigs to avoid contamination of the xylem water by isotopically enriched water. Clipped twigs were immediately placed in a capped glass vial, wrapped in parafilm, and placed in a cooler with ice for transportation to the laboratory. In the laboratory, twig samples were frozen and stored (−20 °C). One twig sample was taken in each sampling tree, and thus there were four twig samples in the top or bottom of sand dunes at each sampling date. The total number of twig samples was 16. Simultaneously, soil samples beneath each sampling tree were collected at the depth of 0–300 cm by using a soil auger (diameter of 5 cm). The distance from the base of the sampling tree to the soil sampling point was within 1.5 m. Soil samples within 0–100 cm were obtained at 20 cm intervals, and soil samples within 100–300 cm were collected at 50 cm intervals. There were four soil samples for each soil layer in the top or bottom of sand dunes on each sampling date. Soil samples were separated into two parts for stable isotope analyses of soil water and gravimetric water content determination. Soil samples for stable isotope analyses were placed in capped vials (50 mL), wrapped in parafilm and stored in the freezer (−20 °C) until water extraction. Soil samples for gravimetric water content were put into aluminum boxes (diameter of 8 cm and a height of 5 cm) and sealed. In addition, three groundwater samples for each sampling date were collected from a drilling well near the study site. The drilling well was located in the plantation, and the distances between the drilling well and any of the four sites were no more than 500 m. Groundwater samples were enclosed in airtight glass vials, wrapped in parafilm, and stored (4 °C) until stable isotope analysis.

2.3. Needle Sampling

On June 9 (dry period) and August 25 (wet period) of 2017, current- and 1-year needles (more than 100 needles) were sampled from the middle of the crown on the sunlit side of each sampled tree. Two needle samples were taken in each sampling tree on each sampling date, one was the current-year-old needle and the other was 1-year-old needle. Therefore, there were four needle samples per needle age group at the top or bottom of the sand dunes on each sampling date. The total number of needle samples was 32. The needle samples were placed into paper bags and were transported to the laboratory for measurement.

2.4. The $\Delta^2\text{H}$, $\Delta^{18}\text{O}$, and $\Delta^{13}\text{C}$ Measurement

Water from twig xylem and soil samples was extracted with a cryogenic vacuum distillation system [39,40]. Although several recent studies reported that cryogenic vacuum distillation was problematic for dry soils that have a high proportion of bound water [41,42], Koeniger et al. [40] observed that there was not a strong influence of soil water content on the stable isotope composition of the extraction water in the sandy soil. We routinely weighed and oven dried the extracted samples to ensure an extraction time long enough to vaporize the water within the plant tissue and soil (2 h, 100 °C). Water samples were measured in an isotope ratio mass spectrometer (Finnigan MAT Delta V advantage, Thermo Finnigan Inc., Austin, TX, USA). Isotopic ratios of hydrogen and oxygen were expressed in delta notation (δ) as:

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

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

The needle samples were oven dried to a constant mass at 75 °C for 48 h, finely ground, and then subsampled for measurement. The needle samples were contained in folded tin capsules. The measurements of stable carbon isotope ratios were performed using a mass spectrometer (Delta Plus XP, Thermo Electron, Bremen, Germany) coupled with an elemental analyzer (Flash EA 1112, Thermo Electron, Bremen, Germany). The precision of the online procedure was better than $\pm 0.2\text{‰}$ for carbon isotope ratios. The natural abundance of ^{13}C was expressed in per mil (‰) deviation from international standards. Pee Dee Belemnite was used as the international standard.

2.5. Soil Water Content

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

$$\text{SWC (\%)} = (\text{wet weight} - \text{dry weight}) / \text{dry weight} \times 100 \quad (2)$$

2.6. Meteorological Measurements

Meteorological data, such as precipitation (Belfort Instrument, Baltimore, MD, USA), solar radiation (Model 1650), air temperature, and relative humidity (model HMP45C) were measured every 30 min, and then the values were stored using a CR1000 datalogger (Campbell Scientific Inc., Logan, UT, USA) in an automatic weather station installed near the study site (2 km).

2.7. Data Analysis

The local meteoric water line (LMWL, [32]), global meteoric water line (GMWL, [43]), and the relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for soil water, xylem water, and groundwater were fitted using a simple linear regression. To identify the depth of water uptake by the Mongolian pine trees, the isotopic ratios of the twig xylem water were compared with the isotopic ratios of soil water at different depths and in groundwater [7,32,36]. The dual-isotope approach ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) was used in this study. In addition, the IsoSource mixing model [44,45] was used to calculate the range of contribution of each water source to the total tree water uptake. The fractional increment was set at 1% and the mass balance tolerance was set at 0.2. To avoid the misinterpretation of the results from the IsoSource mixing model, the results are shown as the distribution of feasible solutions (i.e., minimally to maximally feasible) rather than focusing on the mean value. A low maximum value for a source generally 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 [46]. In this study, four potential water sources (0–40 cm soil water, 40–100 cm soil water, 100–300 cm soil water, and groundwater) were applied to facilitate the comparison of the potential water sources. This was based on the root distribution (approximately 85% roots distribution within 40 cm soil layer, more than 98% roots distributed within 100 cm of the soil layer, [26]); variations in the gravimetric soil water content and isotopic composition of soil water along the soil profile, although the soil water isotopic composition between 40–100 cm and 100–300 cm layers was similar during the dry period. The isotopic composition of each soil water layer was calculated using the soil water content-weighted mean approach [10,47].

All statistical analyses were performed in R software (R version 3.3.3, a language and environment for statistical computing and graphics) [48].

A mixed linear model was conducted using the lme4 package in R [49] to test the effect of positions of sand dunes and sampling periods on the soil water content and xylem water $\delta^{18}\text{O}$ and $\delta^2\text{H}$. In this model, position, sampling period, and their interaction were included as fixed factors, and plot as a random factor. To analyze the effects of sampling periods, positions of the sand dune and needle ages on the needle $\delta^{13}\text{C}$, another mixed linear model was conducted with these three factors and their interactions as fixed factors and plot as a random factor. In addition, mixed linear models were

performed to test the differences in the isotopic ratios between the twig xylem water and soil water at different depths, in the needle $\delta^{13}\text{C}$ between dry and wet periods on the same topographic position, and in fractional contribution of water sources to total water uptake among different potential water sources in the same sampling period on the same topographic position. In these three models, the plot was included as a random factor, and soil depths, sampling periods, and potential water sources were separately included as fixed factors.

3. Results

3.1. Environmental Factors

The total precipitation in 2017 was 346.0 mm, which accounted for 73% of the long-term mean annual precipitation (474 mm, Figure 1a), and indicated that 2017 was a moderately dry year. Before July, the study area experienced a continuous drought from April to June, and only 33.4 mm of the precipitation in total was received (22.9% of the long-term mean precipitation in the same period). This drought was terminated by precipitation events (32.1 mm in total) between July 1 and 2. In addition, this area received a large amount (250.9 mm) of precipitation from July to August, which accounted for 107% of the long-term mean precipitation in the same period (Figure 1b).

The gravimetric SWC values for both the bottom and top of the sand dunes displayed similar temporal variations in each of the soil depths (Figure 2). The gravimetric SWC was highly variable above the 40 cm soil depth, followed by at the 40–100 cm soil depth, and it was relatively stable over 100 cm soil depth (Figure 2). Additionally, the sampling period and position of sand dunes had a significant effect on the variations in gravimetric SWC, with higher values for the wet period ($4.71\% \pm 0.53\%$) and at the bottom of the sand dunes ($4.38\% \pm 0.67\%$, Figure 2 and Table 2).

Figure 2. Vertical profiles (0–300 cm) of gravimetric soil water content (SWC) during the dry and wet periods at the top (a) and bottom (b) of sand dunes. Error bars represent standard deviation ($n = 4$).

Table 2. Summary results of mixed model analyses in gravimetric soil water content (SWC), xylem water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ by sampling period, and position of sand dune.

Source	df	SWC		$\delta^2\text{H}$		$\delta^{18}\text{O}$	
		F	p	F	p	F	p
Sampling period	1	34.89	<0.001	20.98	0.004	5.85	0.032
Position of sand dune	1	17.98	0.001	3.03	0.132	0.97	0.343
Sampling period $\times$ Position of sand dune	1	2.93	0.113	0.83	0.397	0.01	0.916

3.2. Isotopic Compositions of Soil Water, Groundwater, and Twig Xylem Water

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of soil water, groundwater and twig xylem water followed by global and local meteoric water lines (Figure 3) indicated that all of the sampled water originally came from precipitation [50]. The soil water had isotope values ranging from -99.79‰ to -41.42‰ for $\delta^2\text{H}$ and from -13.73‰ to -0.55‰ for $\delta^{18}\text{O}$ during the measurement period, and the isotope values of the soil water in the upper soil layers became heavier than those of the deeper layers (Figure 4). The isotopic values for 0–40 cm soil water was highly variable with season and depth, whereas 40–100 cm soil water had lower isotopic ratios and exhibited relatively mild seasonal changes than 0–40 cm soil water. However, the isotopic values for the 100–300 cm soil water displayed relatively stable variations compared with those for 0–40 cm and 40–100 cm soil water. Compared with the soil water, the groundwater exhibited relatively stable isotope values, e.g., $-9.62\text{‰} \pm 0.25\text{‰}$ for $\delta^{18}\text{O}$ and $-71.55 \pm 0.52\text{‰}$ for $\delta^2\text{H}$. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of the twig xylem water ranged from -84.18‰ to -60.30‰ and from -11.40‰ to -8.12‰ , respectively. However, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of the twig xylem water significantly differed between the seasons (Figure 4 and Table 2).

Figure 3. Relationships between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for the xylem water, soil water, and groundwater during the measurement period.

In the top of sand dunes, the average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of twig xylem water mainly overlapped with those of soil water at 40–300 cm depth during the dry period (Figure 4a,c), although the error range of isotopic ratios for twig xylem water also overlapped partly with those of soil water at 20–40 cm depth. A significant difference in $\delta^2\text{H}$ or $\delta^{18}\text{O}$ between twig xylem water and soil water at 0–20 cm depth was observed during the dry period ($p < 0.05$). However, during the wet period, the average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of twig xylem water overlapped with those of soil water at 0–100 cm depth (Figure 4a,c). Significant differences between twig xylem water and soil water at 100–150 cm, 150–200 cm, 200–250 cm, and 250–300 cm depths for $\delta^2\text{H}$ or at 100–150 cm, 200–250 cm, and 250–300 cm depths for $\delta^{18}\text{O}$ ($p < 0.05$) were observed during the wet period at the top of sand dunes. At the bottom of sand dunes, the average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of twig xylem water overlapped with those of soil water at 40–300 cm depth and groundwater during the dry period, while they overlapped with those of soil water at 0–100 cm depth during the wet period (Figure 4b,d). Significant differences between the twig xylem water and soil water at 0–20 cm depth for $\delta^2\text{H}$ or at 0–20 cm and 20–40 cm depths for $\delta^{18}\text{O}$ ($p < 0.05$) were found during the dry period, whereas significant differences between twig xylem water and soil water at 80–100 cm,

100–150 cm, 200–250 cm, and 250–300 cm depths for $\delta^2\text{H}$ or at 80–100 cm, 100–150 cm, 150–200 cm, 200–250 cm, and 250–300 cm depths for $\delta^{18}\text{O}$ ($p < 0.05$) were detected during the wet period.

Figure 4. Average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isotopic profile of soil water, twig water, and groundwater during the dry and wet periods for Mongolian pine trees growing on the top (a,c) and bottom (b,d) of the sand dunes. Horizontal crossed bar represents ± 1 SE ($n = 4$).

3.3. Isosource Estimation of the Feasible Contributions of Potential Water Sources

Results from the mixing IsoSource model showed that the contribution of water uptake from four potential water sources (0–40 cm soil water, 40–100 cm soil water, 100–300 cm soil water, and groundwater) typically varied between sampling periods and positions of sand dunes. At the top of the sand dunes, the contributions of the 40–100 cm soil water and 100–300 cm soil water to total water uptake were in the ranges of 48%–78% and 0%–52% during the dry period, respectively, with mean values of 63.5% and 17.5%, whereas the possible ranges of 0–40 cm soil water and groundwater were only 0%–26% and 0%–30%, with the mean values of 9.7% and 9.3%, respectively (Figure 5a). By the wet period, the contribution of the 0–40 cm soil water increased dramatically (0%–72%, with a mean value of 43.3%) while that of the 40–100 cm soil water decreased (0%–100%, with a mean value of 33.8%), whereas the possible ranges of the 100–300 cm soil water and groundwater were only 0%–29% and 0%–46%, with mean values of 9.3% and 13.6%, respectively (Figure 5a).

Figure 5. Percentage contribution of potential water sources during the dry and wet periods for Mongolian pine growing on the top (a) and bottom (b) of the sand dunes. Column heights represent the mean value of relative contributions and bars represent the ranges of minimum and maximum, both were calculated using the IsoSource model [44]. Different letters indicated significant differences among the potential water sources in the same sampling period on the same topographic position ($n = 4$).

At the bottom of the sand dunes, the contribution of the 0–40 cm soil water was only in the range of 0%–4% in the dry period, with a mean value of 1.4%, while the contributions of 40–100 cm soil water, 100–300 cm soil water, and groundwater were in the ranges of 0%–95%, 0%–86%, and 4%–62%, with mean values of 34.8%, 33.9%, and 29.9%, respectively (Figure 5b). However, in the wet period, the contribution of the 0–40 cm soil water increased dramatically at the bottom of the sand dunes (24%–75%, with a mean value of 54.8%), while that of the 40–100 cm soil water decreased slightly (0%–76%, with a mean value of 24.9%), whereas the contribution of the 100–300 cm soil water and groundwater decreased significantly, with the range of 0%–36% and 0%–26% (Figure 5b).

3.4. Needle $\delta^{13}\text{C}$

The needle $\delta^{13}\text{C}$ ranged from -27.36‰ to -23.37‰ during the measurement period, with the mean value of $-25.30\text{‰} \pm 0.20\text{‰}$ (Figure 6a,b). Significant effects of the sampling period, position and needle age on the variations in needle $\delta^{13}\text{C}$ were observed during the measurement, with the higher values in the dry period ($-25.03\text{‰} \pm 0.3\text{‰}$), top of the sand dune ($-24.89\text{‰} \pm 0.3\text{‰}$), and current-year-old needle ($-24.44\text{‰} \pm 0.2\text{‰}$, Table 3), respectively.

Figure 6. Needle $\delta^{13}\text{C}$ of the current- and 1-year-old (a,b) during the dry and wet periods for Mongolian pine trees growing on the top and bottom of the sand dunes. Error bars represent standard deviation ($n = 4$). Different letters indicate significant differences between dry and wet periods on the same topographic position.

Table 3. Summary results of mixed model analyses in the needle $\delta^{13}\text{C}$ by sampling period, the position of the sand dune and needle age.

Source	df	$\delta^{13}\text{C}$	
		F	p
Sampling period	1	7.43	0.011
Position of sand dune	1	17.23	<0.001
Needle age	1	76.37	<0.001
Sampling period $\times$ Position of sand dune	1	0.003	0.961
Sampling period $\times$ Needle age	1	1.36	0.255
Position of sand dune $\times$ Needle age	1	0.79	0.382
Sampling period $\times$ Position of sand dune $\times$ Needle age	1	0.02	0.880

4. Discussion

4.1. Water Uptake and Water Use Efficiency of Trees during the Dry and Wet Periods

The isotopic values of twig xylem water and the results from the mixing IsoSource model indicate that the Mongolian pine trees growing at both the top and bottom of sand dunes took up water from deeper soil layers under drought condition, e.g., the Mongolian pine trees at the top and bottom of sand dunes mainly used 40–300 cm soil water and 40–300 cm soil water as well as possibly groundwater during the dry period, respectively, and switched their water sources to 0–100 cm soil water during the wet period (Figures 4 and 5), which supported our first hypothesis. These results of the present study are consistent with those of previous studies in arid and semiarid regions, in which trees with dimorphic root systems exhibited a shift in water sources from mainly shallow water during the wet season to deep water during the dry season [32,34,51–53]. In the present study, the gravimetric SWC at both the top and bottom of the sand dunes during the dry period were significantly lower than those during the wet period (Figure 2 and Table 2). In addition, the Mongolian pine trees had higher water consumption in June compared with trees in August [32]. Therefore, the Mongolian pine trees would use deeper water sources to support their high transpiration demand during the dry period. Although most of the fine roots (>85%, dry weight) of the Mongolian pine trees were distributed within the 40 cm soil layer determined by auger methods [26], the Mongolian pine trees appeared to scarcely use water from this soil layer during the dry period due to the extremely low gravimetric SWC in this soil layer (2.0% and 1.4% for the bottom and top of sand dunes, respectively, Figure 2). The soil water could not be used by the pine trees in the top of sand dunes when the gravimetric soil water content was <1.7% during the dry period, whereas it was 2.0% in the bottom of sand dunes, possibly due to the fine texture for sandy soil in the bottom of sand dunes with high wilting point. However, several roots were observed at a depth of 40–300 cm soil layer [26,30], but they were possibly essential for water uptake and enabled the trees to survive in drought conditions. Similar to our findings, Sugimoto et al. [54] reported that most of the fine roots (80% in number) of *Larix gmelinii* trees in East Siberian taiga were observed at 0–30 cm depth, but it was difficult for those roots to take up water from this soil layer during drought summer due to the low gravimetric SWC. The ability to switch water sources between the dry and wet periods contributed to alleviating drought stress and to help prevent hydraulic system failures, which appeared to be an important survival strategy for Mongolian pine trees growing in the semiarid sandy land [32,55,56].

Significantly higher needles $\delta^{13}\text{C}$ in the dry period than in the wet period for the Mongolian pine trees growing at both the bottom and top of sand dunes indicated that the Mongolian pine trees could adjust the WUE under drought condition. In general, the WUE can be inferred by using the $\delta^{13}\text{C}$ of plant tissues based on the simpler linear model of Farquhar et al. [57], which makes the assumption that mesophyll conductance to CO_2 is infinite, whereby a higher WUE is indicated by a higher $\delta^{13}\text{C}$ [36,58]. The leaf $\delta^{13}\text{C}$ typically increases with decreasing water availability, as a result of the stomatal closure and reduced transpiration and prevents their hydraulic system from irreversible damage [59]. The

results of this study were consistent with the findings of several other studies, which reported that leaf $\delta^{13}\text{C}$ of plants was generally higher in the dry than the wet periods [60,61]. For example, Ávila-Lovera and Tezara [62] reported that leaf $\delta^{13}\text{C}$ of *Parkinsonia praecox* (Ruiz and Pav. ex Hook.) was significantly higher in the dry season than in the rainy season in tropical semiarid regions of the north of Venezuela due to drought. Therefore, an improvement in the WUE for the Mongolian pine trees under drought condition may be a benefit for tree survival.

4.2. Water Uptake and Water Use Efficiency of Trees at the Top and Bottom of Sand Dunes

Our results showed that the Mongolian pine trees at the top of the sand dunes used a shallower water source than the trees at the bottom of the sand dunes during the dry period. However, during the wet season, Mongolian pine trees at the top and at the bottom of sand dunes used the same water sources. This indicated that the greatest differences in the water sources that were used by trees across the topographic position occurred only during the dry period, which did not support the second hypothesis. In agreement with our findings, Nippert and Knapp [63] reported that plants in the uplands used more shallow soil water compared to those in the lowlands, with the greatest differences across the topographic gradient occurring during dry periods. In the present study, significant differences in the gravimetric SWC at 0–150 cm depth and 0–200 cm depth were observed between the bottom and top of sand dunes during the dry and wet periods, respectively (Figure 2), which indicated that the difference in topographic position (8.7 ± 1.4 m) significantly influenced the water redistribution. In addition, the sandy soil in the bottom of sand dunes had the fine texture compared with that in the top of sand dunes, which also contribute to higher gravimetric SWC in the bottom of sand dunes. The significant difference in the water sources between the top and bottom of sand dunes during the dry period may be associated with differences in their root distribution, water availability, and transpiration demand [30,63]. Zhao and Zhang [64] reported that the Mongolian pine trees at the top of the sand dunes had a more developed root system compared with those at the bottom of the sand dunes in the study region, which would allow them to access subsurface precipitation-derived water reservoirs at the top of sand dunes and thus compensate for inaccessibility to the groundwater. In addition, the Mongolian pine trees at the top of sand dunes had a low transpiration demand compared with those at the bottom of sand dunes due to their significantly smaller tree size (Figure 1, [31]). Therefore, the Mongolian pine trees at the top of the sand dunes, which had a more developed horizontal root system, obtained more water from the shallower soil layer (63.5% from 40–100 cm soil water) to support their low transpiration demand during the dry season. Although approximately 85% of the roots were distributed within 40 cm for Mongolian pine trees, the taproots of the trees (only 3–5 taproots) can reach 4–5 m in depth [30]. However, the groundwater level for the trees at the bottom of the sand dunes averaged 4.6 m during the dry season (data not shown), and the maximum height of the capillary rise from the groundwater was 1.0 m [33]. Therefore, the Mongolian pine trees at the bottom of sand dunes could utilize deeper water sources (possibly groundwater) to support their highest transpiration demand when the upper soil water became scarce during the drought condition. A similar phenomenon was observed by Song et al. [32] for Mongolian pine trees in the study region. However, the lack of significant differences in the depth of soil water extraction for the Mongolian pine trees between the bottom and top of sand dunes during the wet season may be due to abundant water in the 0–100 cm soil layer (4.2% and 7.0% at the top and bottom of the sand dunes, respectively, Figure 4). Li et al. [65] also reported that most water was absorbed from depths with relatively high gravimetric SWC.

Significantly higher needle $\delta^{13}\text{C}$ at the top of sand dunes than at the bottom of sand dunes suggested that the Mongolian pine trees had a higher WUE and suffered from high water stress at the top of sand dunes. Consistent with our results is the finding that *Grevillea stenobotrya* displayed a significantly higher $\delta^{13}\text{C}$ value at the top of sand dunes than that at the bottom of sand dunes in the Great Sandy Desert, northwestern Australia, due to a greater amount of soil water at the bottom of sand dunes [20]. In the present study, the higher needle $\delta^{13}\text{C}$ at the top of the sand dunes than at

the bottom of the sand dunes was associated with a relatively low gravimetric SWC at the top of the sand dunes (Figure 2). Therefore, the Mongolian pine trees at the top of sand dunes used water more efficiently to avoid excessive water loss and to acclimatize to the water shortage.

4.3. Implication for Forest Management

In the study region, extremely low rainfall (approximately half of the long-term mean value) occurred at an interval of 15 years [32]. Additionally, the groundwater level declined at 0.1 m per year following the afforestation of Mongolian pine plantations in 1955, mainly due to the increasing water use for the expansion of agricultural land and broadleaved forests (*Populus* spp.) [28]. Mongolian pines at different topographical positions on the sand dunes should be differently affected by changes in the rainfall regime and by the decline in the groundwater level. The predicted increase in the frequency and severity of drought events would make the Mongolian pine growing at the top of sand dunes more vulnerable to dieback due to the shallower soil water uptake (mainly from 40–100 cm layer) and increased water restrictions. Under this circumstance, thinning, which usually decreases competition among the remaining trees for water in the soil, should be the most effective practice [32,66]. However, any sharp drop in the groundwater level due to groundwater extraction may result in additional water stress for the trees, crown dieback or even tree mortality [32,66]. Therefore, a sharp decline in the groundwater level in extreme drought years might have a strong negative impact on the growth and survival of the Mongolian pine plantation at the bottom of sand dunes due to the utilization of deeper water sources (possibly groundwater). To maintain a stable groundwater level for the Mongolian pine trees at the bottom of sand dunes, the amount of agricultural land and broadleaved forests should be reduced or transformed into other land use types that have lower water consumption, such as coniferous forest, shrubland, and grassland [28,32].

5. Conclusions

Stable isotope technology was applied to determine the effect of drought and topographic positions on the depth of water extraction of Mongolian pine trees in semiarid sandy regions. Mongolian pine trees growing at both the top and bottom of sand dunes adjusted their water uptake towards deeper water sources and used water more efficiently under drought condition. However, Mongolian pine trees at the bottom of the sand dunes employed a relatively prodigal use of water compared with that of the trees at the top of the sand dunes by absorbing deeper water source (possibly groundwater) during drought conditions. Therefore, Mongolian pine trees at the top were more susceptible to dieback in extreme drought years due to shallower soil water uptake and more water restrictions. Nevertheless, a sharp decline in groundwater level under extreme drought years had a strong negative impact on the growth and survival of Mongolian pine trees at the bottom of sand dunes. Therefore, thinning should be adopted to alleviate water stress for trees at the top of sand dunes, whereas land use types with high water consumption should be reduced or transformed into other land use types with lower water consumption to maintain a stable groundwater level for trees at the bottom of sand dunes.

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References

1. Liu, J.F.; Arend, M.; Yang, W.J.; Schaub, M.; Ni, Y.Y.; Gessler, A.; Jiang, Z.P.; Rigling, A.; Li, M.H. Effects of drought on leaf carbon source and growth of European beech are modulated by soil type. *Sci. Rep.* **2017**, *7*, 42462. [[CrossRef](#)] [[PubMed](#)]
2. Zhang, Q.; Shao, M.; Jia, X.; Wei, X. Relationship of climatic and forest factors to drought- and heat-induced tree mortality. *PLoS ONE* **2017**, *12*, e0169770. [[CrossRef](#)]
3. Su, H.; Li, Y.G.; Liu, W.; Xu, H.; Sun, O.J.X. Changes in water use with growth in *Ulmus pumila* in semiarid sandy land of Northern China. *Trees* **2014**, *28*, 41–52. [[CrossRef](#)]
4. Antunes, C.; Barradas, M.C.D.; Zunzunegui, M.; Vieira, S.; Pereira, Â.; Anjos, A.; Correia, O.; Pereira, M.J.; Máguas, C. Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems. *Funct. Ecol.* **2018**, *32*, 1931–1943. [[CrossRef](#)]
5. Asbjornsen, H.; Mora, G.; Helmers, M.J. Variation in water uptake dynamics among contrasting agricultural and native plant communities in the Midwestern US. *Agric. Ecosyst. Environ.* **2007**, *121*, 343–356. [[CrossRef](#)]
6. Prieto, I.; Martínez-Tillería, K.; Martínez-Manchego, L.; Montecinos, S.; Pugnaire, F.I.; Squeo, F.A. Hydraulic lift through transpiration suppression in shrubs from two arid ecosystems: Patterns and control mechanisms. *Oecologia* **2010**, *163*, 855–865. [[CrossRef](#)]
7. Nie, Y.P.; Chen, H.S.; Wang, K.L.; Yang, J. Water source utilization by woody plants growing on dolomite outcrops and nearby soils during dry seasons in karst region of Southwest China. *J. Hydrol.* **2012**, *420–421*, 264–274. [[CrossRef](#)]
8. Ewe, S.M.L.; Sternberg, L.d.S.L.; Childers, D.L. Seasonal plant water uptake patterns in the saline southeast Everglades ecotone. *Oecologia* **2007**, *152*, 607–616. [[CrossRef](#)] [[PubMed](#)]
9. Dawson, T.E.; Pate, J.S. Seasonal water uptake and movement in root systems of Australian phraeatophytic plants of dimorphic root morphology: A stable isotope investigation. *Oecologia* **1996**, *107*, 13–20. [[CrossRef](#)]
10. Zhang, C.C.; Li, X.Y.; Wu, H.W.; Wang, P.; Wang, Y.; Wu, X.C.; Li, W.; Huang, Y.M. Differences in water-use strategies along an aridity gradient between two coexisting desert shrubs (*Reaumuria soongorica* and *Nitraria sphaerocarpa*): Isotopic approaches with physiological evidence. *Plant Soil* **2017**, *419*, 169–187. [[CrossRef](#)]
11. Peters, W.; van der Velde, I.R.; Van Schaik, E.; Miller, J.B.; Ciais, P.; Duarte, H.F.; van der Laan-Luijckx, I.T.; van der Molen, M.K.; Scholze, M.; Schaefer, K.; et al. Increased water-use efficiency and reduced CO₂ uptake by plants during droughts at a continental scale. *Nat. Geosci.* **2018**, *11*, 744–748. [[CrossRef](#)]
12. Song, L.N.; Zhu, J.J.; Li, M.C.; Zhang, J.X.; Zheng, X.; Wang, K. Canopy transpiration of *Pinus sylvestris* var. *mongolica* in a sparse wood grassland in the semiarid sandy region of Northeast China. *Agric. For. Meteorol.* **2018**, *250–251*, 192–201.
13. Naumann, G.; Alfieri, L.; Wyser, K.; Mentaschi, L.; Betts, R.A.; Carrao, H.; Spinon, J.; Vogt, J.; Feyen, L. Global changes in drought conditions under different levels of warming. *Geophys. Res. Lett.* **2018**, *45*, 3285–3296. [[CrossRef](#)]
14. Allen, C.D.; Macalady, A.K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M.; Kitzberger, T.; Rigling, A.; Breshears, D.D.; Hogg, E.H.; et al. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag.* **2010**, *259*, 660–684. [[CrossRef](#)]
15. Greenwood, S.; Ruiz-Benito, P.; Martínez-Vilalta, J.; Lloret, F.; Kitzberger, T.; Allen, C.D.; Fensham, R.; Laughlin, D.C.; Kattge, J.; Bönsch, G.; et al. Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. *Ecol. Lett.* **2017**, *20*, 539–553. [[CrossRef](#)]
16. Choat, B.; Brodribb, T.J.; Brodersen, C.R.; Duursma, R.A.; López, R.; Medlyn, B.E. Triggers of tree mortality under drought. *Nature* **2018**, *558*, 531–539. [[CrossRef](#)]
17. Sun, S.J.; Meng, P.; Zhang, J.S.; Wan, X.C. Variation in soil water uptake and its effect on plant water status in *Juglans regia* L. during dry and wet seasons. *Tree Physiol.* **2011**, *31*, 1–12. [[CrossRef](#)]
18. Wang, B.W.; Zhang, G.H.; Duan, J. Relationship between topography and the distribution of understory vegetation in a *Pinus massoniana* forest in Southern China. *Int. Soil Water Conserv. Res.* **2015**, *3*, 291–304. [[CrossRef](#)]
19. Helman, D.; Osem, Y.; Yakir, D.; Lensky, I.M. Relationships between climate, topography, water use and productivity in two key Mediterranean forest types with different water-use strategies. *Agric. For. Meteorol.* **2017**, *232*, 319–330. [[CrossRef](#)]

20. Grigg, A.M.; Veneklaas, E.J.; Lambers, H. Water relations and mineral nutrition of closely related woody plant species on desert dunes and interdunes. *Aust. J. Bot.* **2008**, *56*, 27–43. [[CrossRef](#)]
21. Li, Q.X.; Jia, Z.Q.; Zhu, Y.J.; Wang, Y.S.; Li, H.; Yang, D.F.; Zhao, X.B. Spatial heterogeneity of soil nutrients after the establishment of *Caragana intermedia* plantation on sand dunes in alpine sandy land of the Tibet Plateau. *PLoS ONE* **2015**, *10*, e0124456. [[CrossRef](#)] [[PubMed](#)]
22. Giordano, C.V.; Guevara, A.; Boccalandro, H.E.; Sartor, C.; Villagra, P.E. Water status, drought responses, and growth of *Prosopis flexuosa* trees with different access to the water table in a warm South American desert. *Plant Ecol.* **2011**, *212*, 1123–1134. [[CrossRef](#)]
23. Dai, Y.; Zheng, X.J.; Tang, L.S.; Li, Y. Stable oxygen isotopes reveal distinct water use patterns of two *Haloxylon* species in the Gurbantonggut Desert. *Plant Soil* **2015**, *389*, 73–87. [[CrossRef](#)]
24. Xu, G.Q.; Yu, D.D.; Xie, J.B.; Tang, L.S.; Li, Y. What makes *Haloxylon persicum* grow on sand dunes while *H. ammodendron* grows on interdune lowlands: A proof from reciprocal transplant experiments. *J. Arid. Land* **2014**, *6*, 581–591. [[CrossRef](#)]
25. Hawthorne, S.; Miniati, C.F. Topography may mitigate drought effects on vegetation along a hillslope gradient. *Ecohydrology* **2018**, *11*, e1825. [[CrossRef](#)]
26. Zhu, J.J.; Li, F.Q.; Xu, M.L.; Kang, H.Z.; Xu, D.Y. The role of ectomycorrhizal fungi in alleviating pine decline in semiarid sandy soil of northern China: An experimental approach. *Ann. For. Sci.* **2008**, *65*, 304. [[CrossRef](#)]
27. Zhu, J.J.; Fan, Z.P.; Zeng, D.H.; Jiang, F.Q.; Takeshi, M. Comparison of stand structure and growth between plantation and natural forests of *Pinus sylvestris* var. *mongolica* on sandy land. *J. For. Res.* **2003**, *14*, 103–111.
28. Zheng, X.; Zhu, J.J.; Yan, Q.L.; Song, L.N. Effects of land use changes on the groundwater table and the decline of *Pinus sylvestris* var. *mongolica* plantations in southern Horqin Sandy Land, Northeast China. *Agric. Water Manag.* **2012**, *109*, 94–106.
29. Song, L.N.; Li, M.C.; Zhu, J.J.; Zhang, J.X. Comparisons of radial growth and tree-ring cellulose $\delta^{13}\text{C}$ for *Pinus sylvestris* var. *mongolica* in natural and plantation forests on sandy lands. *J. For. Res.* **2017**, *22*, 160–168.
30. Zhu, J.J.; Zeng, D.H.; Kang, H.Z.; Wu, X.Y.; Fan, Z.P. *Decline of Pinus sylvestris* var. *Mongolica* Plantation on Sandy Land; Chinese Forestry Press: Beijing, China, 2005. (In Chinese)
31. Jiao, S.R. *Structure and Function of Mongolian Pine Plantation for Sand Fixation in Zhanggutai*; Liaoning Science and Technology Press: Liaoning, China, 1989. (In Chinese)
32. Song, L.N.; Zhu, J.J.; Li, M.C.; Zhang, J.X. Water use patterns of *Pinus sylvestris* var. *mongolica* trees of different ages in a semiarid sandy lands of Northeast China. *Environ. Exp. Bot.* **2016**, *129*, 94–107.
33. Jiang, F.Q.; Cao, C.Y.; Zeng, D.H.; Guan, W.B.; Wu, X.Y.; Zheng, Y.R. *Degradation and Restoration of Ecosystems on Keerqin Sandy Land*; Chinese Forestry: Beijing, China, 2002. (In Chinese)
34. Grossiord, C.; Sevanto, S.; Dawson, T.E.; Adams, H.D.; Collins, A.D.; Dickman, L.T.; Newman, B.D.; Stockton, E.A.; McDowell, N.G. Warming combined with more extreme precipitation regimes modifies the water sources used by trees. *New Phytol.* **2017**, *213*, 584–596. [[CrossRef](#)]
35. Liu, Z.Q.; Yu, X.X.; Jia, G.D. Water utilization characteristics of typical vegetation in the rocky mountain area of Beijing, China. *Ecol. Indic.* **2018**, *91*, 249–258. [[CrossRef](#)]
36. Dawson, T.E.; Mambelli, S.; Plamboeck, A.H.; Templer, P.H.; Tu, K.P. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Evol. Syst.* **2002**, *33*, 507–559. [[CrossRef](#)]
37. Berry, Z.C.; Evaristo, J.; Moore, G.; Poca, M.; Steppe, K.; Verrot, L.; Asbjornsen, H.; Borma, L.S.; Bretfeld, M.; Hervé-Fernández, P.; et al. The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. *Ecohydrology* **2018**, *11*, e1843. [[CrossRef](#)]
38. Li, Y.H.; Jiao, S.R.; Liu, S.L.; Yuan, C.L.; Wang, H.; Ai, T.T.; Cheng, W.B. Afforestation trial on *Populus* × *Xiaozhuanica* on Zhanggutai sandy land. *Prot. For. Sci. Technol.* **2012**, *6*, 12–13, 31. (In Chinese with English abstract)
39. Ehleringer, J.R.; Roden, J.; Dawson, T.E. Assessing ecosystem level water relations through stable isotope analyses. In *Methods in Ecosystem Science*; Sala, O.E., Jackson, R.B., Mooney, H.A., Howarth, R.W., Eds.; Springer: New York, NY, USA, 2000; pp. 181–198.
40. Koeniger, P.; Marshall, J.D.; Link, T.; Mulch, A. An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3041–3048. [[CrossRef](#)]
41. West, A.G.; Patrickson, S.J.; Ehleringer, J.R. Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1317–1321. [[CrossRef](#)] [[PubMed](#)]

42. Orłowski, N.; Frede, H.G.; Brüggemann, N.; Breuer, L. Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *J. Sens. Sens. Syst.* **2013**, *2*, 179–193. [[CrossRef](#)]
43. Rozanski, K.; Araguds-Araguds, L.; Gonfanti, R. Isotopic patterns in modern global precipitation. In *Climate Change in Continental Isotopic Records-Geophysical Monograph*; Swart, P.K., Lohman, K.C., McKenzie, J., Savin, S., Eds.; American Geophysical Union: Washington, DC, USA, 1993; Volume 8, pp. 1–36.
44. Phillips, D.L.; Gregg, J.W. Source partitioning using stable isotopes: Coping with too many sources. *Oecologia* **2003**, *136*, 261–269. [[CrossRef](#)] [[PubMed](#)]
45. Phillips, D.L.; Newsome, S.D.; Gregg, J.W. Combining sources in stable isotope mixing models: Alternative methods. *Oecologia* **2005**, *144*, 520–527. [[CrossRef](#)]
46. Benstead, J.P.; March, J.G.; Fry, B.; Ewel, K.C.; Pringle, C.M. Testing isosource: Stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology* **2006**, *87*, 326–333. [[CrossRef](#)] [[PubMed](#)]
47. Wu, H.W.; Li, X.Y.; Jiang, Z.Y.; Chen, H.Y.; Zhang, C.C.; Xiao, X. Contrasting water use pattern of introduced and native plants in an alpine desert ecosystem, Northeast Qinghai–Tibet Plateau, China. *Sci. Total Environ.* **2016**, *542*, 182–191. [[CrossRef](#)] [[PubMed](#)]
48. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
49. Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [[CrossRef](#)]
50. Rong, L.; Chen, X.; Chen, X.H.; Wang, S.J.; Du, X.L. Isotopic analysis of water sources of mountainous plant uptake in a karst plateau of southwest China. *Hydrol. Process.* **2011**, *25*, 3666–3675. [[CrossRef](#)]
51. Liu, Z.Q.; Yu, X.X.; Jia, G.D.; Jia, J.B.; Lou, Y.H.; Lu, W.W. Contrasting water sources of evergreen and deciduous tree species in rocky mountain area of Beijing, China. *Catena* **2017**, *150*, 108–115. [[CrossRef](#)]
52. Tobella, A.B.; Hasselquist, N.J.; Bazié, H.R.; Nyberg, G.; Laudon, H.; Bayala, J.; Ilstedt, U. Strategies trees use to overcome seasonal water limitation in an agroforestry system in semiarid West Africa. *Ecohydrology* **2017**, *10*, e1808. [[CrossRef](#)]
53. Tiemuerbieke, B.; Min, X.J.; Zang, Y.X.; Xing, P.; Ma, J.Y.; Sun, W. Water use patterns of co-occurring C3 and C4 shrubs in the Gurbantonggut desert in northwestern China. *Sci. Total Environ.* **2018**, *634*, 341–354. [[CrossRef](#)] [[PubMed](#)]
54. Sugimoto, A.; Yanagisawa, N.; Naito, D.; Fujita, N.; Maximov, T.C. Importance of permafrost as a source of water for plants in East Siberian taiga. *Ecol. Res.* **2002**, *17*, 493–503. [[CrossRef](#)]
55. Ehleringer, J.R.; Dawson, T.E. Water uptake by plants: Perspectives from stable isotope composition. *Plant Cell. Environ.* **1992**, *15*, 1073–1082. [[CrossRef](#)]
56. Eggemeyer, K.D.; Awada, T.; Harvey, F.E.; Wedin, D.A.; Zhou, X.; Zanner, C.W. Seasonal changes in depth of water uptake for encroaching trees *Juniperus virginiana* and *Pinus ponderosa* and two dominant C4 grasses in a semiarid grassland. *Tree Physiol.* **2009**, *29*, 157–169. [[CrossRef](#)] [[PubMed](#)]
57. Farquhar, G.D.; O’Leary, M.H.; Berry, J.A. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* **1982**, *9*, 121–137. [[CrossRef](#)]
58. Song, L.N.; Zhu, J.J.; Li, M.C.; Yan, Q.L. Intrinsic water use efficiency in wet and dry years at young and old plantations of *Pinus sylvestris* var. *mongolica* in semiarid China. *J. For. Res.* **2015**, *20*, 263–271.
59. Irvine, J.; Perks, M.P.; Magnani, F.; Grace, J. The response of *Pinus sylvestris* to drought: Stomatal control of transpiration and hydraulic conductance. *Tree Physiol.* **1998**, *18*, 393–402. [[CrossRef](#)]
60. Cavender-Bares, J.; Bazzaz, F.A. Changes in drought response strategies with ontogeny in *Quercus rubra*: Implications for scaling from seedlings to mature trees. *Oecologia* **2000**, *124*, 8–18. [[CrossRef](#)]
61. Hartman, G.; Danin, A. Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. *Oecologia* **2010**, *162*, 837–852. [[CrossRef](#)]
62. Ávila-Lovera, E.; Tezara, W. Water-use efficiency is higher in green stems than in leaves of a tropical tree species. *Trees* **2018**, *32*, 1547–1558. [[CrossRef](#)]
63. Nippert, J.B.; Knapp, A.K. Linking water uptake with rooting patterns in grassland species. *Oecologia* **2007**, *153*, 261–272. [[CrossRef](#)]
64. Zhao, S.J.; Zhang, Y.X. Analysis of growth of *Pinus sylvestris* var. *mongolica* in Zhanggutai sand land. *J. Liaoning Agric. Coll.* **2005**, *7*, 3–6. (In Chinese with English abstract)

65. Li, S.G.; Romero-Saltos, H.; Tsujimura, M.; Sugimoto, A.; Sasaki, L.; Davaa, G.; Oyunbaatar, D. Plant water sources in the cold semiarid ecosystem of the upper Kherlen river catchment in Mongolia: A stable isotope approach. *J. Hydrol.* **2007**, *333*, 109–117. [[CrossRef](#)]
66. Giuggiola, A.; Bugmann, H.; Zingg, A.; Dobbertin, M.; Rigling, A. Reduction of stand density increases drought resistance in xeric Scots pine forests. *For. Ecol. Manag.* **2013**, *310*, 827–835. [[CrossRef](#)]

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