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## Seasonal water use by Larix principis-rupprechtii in an alpine habitat

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## ABSTRACT

Understanding the seasonal variation of source-water utilization by trees is important to implement effective methods for forest water management. Here, we studied water-source variations of Larix principis-rupprechtii throughout the entire growing season in an alpine habitat, Luya Mountain (North-Central China). Approximately 2000 sample measurements of precipitation, snow water, soil water, and tree xylem water were collected at weekly/biweekly intervals from September 2013 to October 2014, and water isotopes ( $\delta D$  and  $\delta^{18}O$ ) were analyzed. The contribution of soil water in different depths to trees was estimated by using a Bayesian mixing model. We simultaneously monitored leaf phenology and cambial activity throughout the growing season. The latter was observed by the micro-core method based on the characteristics of xylem cells, to identify the specific dates of onset and cessation of the tree's growth. Our analysis showed several interesting results. First, before needle unfolding (May 16), xylem water loss occurred even in cold-moist alpine conditions. Second, around late May/early June, winter snowmelt water seemed to play an important role for growth onset. Finally, during the growing season (June to August), larch depended on waters from deep soil (below 10 cm), which could be explained by their deep root system, and the maximum soil water content being at a soil depth of 10-20 cm. The mixing water, in addition to summer rainfall, was predominantly recharged by previous-year autumn/winter precipitation (rainfall/snowmelt). This study clearly demonstrates the variation of source-water in different phases, and it emphasizes the contribution of previous-year precipitation for tree growth. Therefore, it is essential to distinguish the timing of tree leaf phenology and growing season when exploring the inter- and intraseasonal changes in water-source use.

## 1. Introduction

In arid or semi-arid environments, temporal and spatial variations in water use by trees has been widely reported (Meinzer et al., 1999; Battipaglia et al., 2013). In humid ecosystems, it might be assumed that variation in water use is of less significance to trees, as water is not a limiting factor (Cui et al., 2009). However, some studies have shown that there is global convergence in the vulnerability of forests to drought, which occurs not only in arid regions but also in wet forests (Choat et al., 2012). As trees growing at high altitudes/latitudes are adapted to moist conditions, even a moderate water deficit might lead to a strong reduction in transpiration (Anfodillo et al., 1998). Therefore, qualitative and quantitative information about seasonal water sources on high- altitude trees hinges on an understanding of the adaptive mechanisms governing drought stress in moist conditions (Anderson et al., 2003). Numerous studies have used isotopic analysis to describe water sources for trees during the growing season. It is well known that trees access water from different soil layers through different root depths and respond differently to water pulses, as suggested by Walter's two-layer model (Jackson et al., 1999; Eggemeyer et al., 2009; Ward et al., 2013). Depending on their active root depth (Schwinning et al., 2005) and the vertical distribution of soil moisture (Liu et al., 2011), trees have the ability to shift their primary water source from shallow soil to a deeper layer, when the shallow soil moisture is depleted (Nippert and Knapp, 2007). However, water utilization by trees may present plastic changes between the growing and non-growing seasons. During the nongrowing season, when leafless, the transport of water from root to tree stem may slow down or even be stagnant. Therefore, source water (soil/ precipitation) entered into tree xylem can be uncoupled, slowed or even masked (Treydte et al., 2014).

On one hand, an increasing number of recent studies have found

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that tree xylem water and soil water were uncoupled in winter (Brandes et al., 2007; Ellsworth and Williams, 2007). By conducting a defoliation experiment on woody species, Ellsworth and Sternberg (2014) proved that xylem water evaporated through the bark when trees were leafless. As a result, suberized stems displayed isotopic enrichment, and the xylem isotopic value diverged from the soil value. These evaporative water losses through the stem surface are not negligible and will weaken until trees leaf out (Catinon et al., 2012). On the other hand, it is accepted that the responses of trees to precipitation have a certain time lag (Vicente-Serrano et al., 2013; Wu et al., 2015). Numerous studies on dendroclimatology and dendroecology have emphasized the importance of precipitation legacies (e.g. rainfall and snowmelt water), which revealed that tree-ring width was particularly sensitive to water recharge in the preceding autumn (Mäkinen et al., 2003; Zhang et al., 2017). However, knowledge of the seasonal variations of water source throughout the whole year and the timing and magnitude of different source water (soil/precipitation) in different seasons used by trees remains fragmentary.

In this study, we simultaneously used the micro-core method, leaf phenology observation and stable hydrogen and oxygen isotope analysis to explore seasonal variation water use by larch trees at a weekly interval in an alpine habitat. Here, the micro-core method is an effective technique that can capture the timing of the growing season (divided by radial growth of stems) based on the analysis of intra-annual xylem differentiation (Rossi et al., 2006; Jiang et al., 2015). Our main objectives were threefold: (1) to quantify tree water uptake from soil layers during the growing season; (2) to test whether water loss by evaporation of xylem also exists before needle unfolding in a humid alpine habitat, because no related study on evaporation of suberized twigs has been reported in monsoon climatic regions; and (3) to explore the magnitude and timing of the contribution of previous-year precipitation (rainfall/snowmelt) for tree growth.

#### 2. Materials and methods

#### 2.1. Study site and species description

The study was conducted at Luya Mountain, North-Central China (38° 40′ N–38° 50′ N, 111° 50′ E–112° 00′ E, Fig. 1). The mean annual temperature at the base of the mountain is approximately 5.2 °C. The annual precipitation is approximately 473 mm (1957–2013), and over 60% of it falls between June and September. The year 2014 was a moist

year with 20.4% higher annual precipitation than the long-term average (1957–2013). These data were obtained from the nearest State standard meteorological station in Wuzhai ( $38^{\circ}$  55' N, 111° 49' E, 1401 m a.s.l., linear distance *c*.20 km from the study site).

Our study site was located near the summit of Mt. Luya (2740 m a.s.l.), which is in the transition zone from cold coniferous forest (1850–2700 m a.s.l.) to sub-alpine meadow (2700–2780 m a.s.l.) (Ma, 2001). *Larix principis-rupprechtii* Mayr. and *Picea meyeri* Rehd. et Wils. are two dominant species. These trees are very sparse and isolated, as the study site was near the treeline ecotone (Fig. 1). The soil is well-drained sandy loam with 12–18% clay and 50–60% fine sand, which is defined as mountainous brown forest soil (National Soil Survey Office, 1995). The soils are generally not more than 50 cm deep, overlying the fractured parent material. The pH of the topsoil ranged between 6.4 and 7.3, based on measurements taken in August and September of 2009 (Jiang et al., 2014).

As a dominant species in cold-temperature coniferous forests, *L. principis-rupprechtii* is distributed mainly at middle and high elevations (1800–2800 m a.s.l.) of North-Central China (Wang et al., 1992). Mt. Luya is located at the center of this species' distribution range by latitude and in the west margin by longitude (Wu, 1980). Based on our field investigation in September 2014, the fine root distribution (< 2 mm) of larch trees was concentrated primarily in the 10–30 cm soil layers (58.5%), which was calculated from 30 soil cores (see Supplement 1).

Meteorological factors, including precipitation (measured with a Davis 7852 instrument, Hayward, USA), air temperature, soil temperature, and soil volumetric water content (SWC) were measured at an automatic weather station in the study site (2740 m a.s.l.). The air temperature probe (HOBO Pro V2, Onset Computer Corp., USA) was fixed at a height of 1.5 m above the ground and the soil temperature probe (ST-05, Delta-T, UK) was buried at a depth of 10–20 cm. The soil volumetric water content was observed (with a PR2 instrument, Delta-T, UK) at depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm. Data were recorded at 30 min intervals.

#### 2.2. Isotopic sampling of xylem, soil, and precipitation

Xylem and soil were simultaneously sampled over a 9-month period during September– October 2013 and April–October 2014. The sampling frequency was once a month in 2013 and increased to weekly (at midday) from April 2014. To determine the onset of the growing season



Fig. 1. (a) Location of study area in North-Central China. (b and c) The study area landscape in summer (b) and winter (c).



Fig. 2. Division of growing and non-growing seasons by monitoring the cambial zone and xylem cells of *Larix principis-rupprechtii* under alpine habitat (Luya Mountain, China) in 2014. (a) Before the growing season, the cambial zone was dormant on April 24; no new xylem cell production was observed. (b and c) During the growing season, xylem cells were under cell differentiation on July 25; shown as observed under bright field (b) and polarized (c) light, respectively. (d) After the growing season, cambial zone and xylem cells stop cell differentiation and finish intra-annual growth on September 18. *CZ*: cambial zone, *CD*: cell differentiation, *M*: mature.

accurately, at the beginning of sample processing, we took samples at intervals of 3–5 day. Once the full elongation of needles (95% needles finished 95% length growth) in trees finished at the end of June, we sampled at 7–10 day intervals. In autumn, the growing season stops; therefore, we processed samples less frequently (González-González et al., 2013).

Five larch trees were chosen, with similar age (57  $\pm$  6 yr), diameter (21  $\pm$  3 cm), and tree height (8  $\pm$  2 m). For each tree, three 5 cm-long twigs from the sun-exposed crown at heights above 2 m were obtained. For each twig, the phloem tissue was removed to avoid contamination by isotopic enriched water. Samples were placed in vials, sealed with parafilm (BEMIS Company Inc., USA), and stored in a freezer (-10 °C) until water extraction using cryogenic vacuum distillation (West et al., 2006).

Soil water samples beneath the larch trees were collected at the same time that xylem material was collected. Using a hand auger, four soil profiles were dug. At each soil profile, samples were collected from depths of 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm with two replicates per depth. It was impractical to obtain soil samples from a depth greater than 40 cm because of the underlying rock at most sampling sites. A total of 1344 soil samples were obtained, sealed in glass bottles and frozen until isotope analysis.

Precipitation sampling was conducted during April–October 2014. Thirty-two rain samples were obtained during each sample interval with five replicates. A funnel and a polyethylene bottle were connected together as a rain collector. A ping-pong ball was placed in the funnel to avoid isotopic fractionation by the evaporation of rainwater (Liu et al., 2014). Snow in spring is a more representative water source for trees. Therefore, the observation of snow cover was also conducted from early April to May 23, 2014, until the snow thawed and disappeared completely. Entire layers of snow cover were taken, transferred into plastic bags, and kept in a warm room for thawing without evaporation. A total of 165 snow samples were collected. There is no groundwater in this study because the site was located at the summit of Mt. Luya.

#### 2.3. Isotopic analysis

Before the isotope analysis, water was extracted from xylem or soils using the cryogenic vacuum distillation method (West et al., 2006). Generally, this would require 0.5–1.5 h, depending on the water content of samples (Meißner et al., 2014). All water analyses were conducted at the State Key Laboratory of Earth Surface Process and Resource Ecology, Beijing Normal University, China, using an Isotopic Ratio Infrared Spectroscopy (IRIS) system (Model DLT-100, Los Gatos Research, USA). The precision of the liquid water isotope analyzer was typically better than  $\pm 1.2\%$  for  $\delta$ D and  $\pm 0.3\%$  for  $\delta^{18}$ O. Due to the presence of the organic contaminants in cryogenically extracted water samples from plant tissues, some stable isotopic ratios of xylem water as measured by the LGR system were corrected following the procedure of Schultz et al. (2011) and Wu et al. (2016). The correction procedures are given in detail in Supplement 2.

Monthly weighted means of  $\delta D_w$  and  $\delta^{18}O_w$  for precipitation were calculated as:

$$\delta p = \frac{\sum_{i=1}^{n} \delta_i \times P_i}{\sum_{i=1}^{n} P_i}$$

where  $\delta_i$  is the  $\delta$ D and  $\delta^{18}$ O of the *i*th precipitation, and  $P_i$  is the amount of the *i*th precipitation.

2.4. Intra-annual growing season monitoring and leaf phenology observations

The intra-annual growing season (by radial growth of stem) can be



Fig. 3. Temporal variation of precipitation amount and its isotope values under alpine habitat (Luya Mountain, China) in 2014.

determined by monitored xylem cell growth using the micro-core method on a weekly or biweekly basis. This micro-core method is an effective technique that has been widely accepted and used in dendroecology (Cuny et al., 2015).

In our study, the growing season was monitored on the same dates and in the same trees where the tree xylem water was sampled. Each time, five micro-cores per tree were collected from stems at breast height (1.3 m) using a Trephor tool (Jiang et al., 2015). Intra-annual stem radial growth is the process of cambial cell division and new xylem cell formation. In the cambial zone, before the growing season, cambial cells have small radial diameters and thin cell walls (Fig. 2a). In spring, the first appearance of an enlarging cell signifies the onset of the growing season. In late summer/autumn, the empty lumen and red cell walls in the last-formed tracheid indicate the end of the growing season (Fig. 2c).

Based on weekly micro-core sampling, the initiation and cessation of the growing season was determined to be in early June (June 4) and early September (September 4), respectively. Therefore, the observation periods were divided into three phases: before the growing season (April–May), the growing season (June–August), and after the growing season (September–October). In addition, the growth initiation period was also fixed from late May to early June (May 25–June 4).

The development of leaf phenological stages was monitored for the same trees by recording the times of bud break, needle unfolding, yellowing and fall (Du and Fang, 2014). Phenological observations were carried out weekly (3–5 days shorter intervals after bud break), a frequency suited for phenological studies at the forest stand scale. Bud break was marked as the time when the first emerging needles were clearly visible (larch needles length < 1 mm); the needle unfolding was the time when the needles had been fully expanded and elongated (needles length > 3 mm); and the needle fall were defined as the time of complete defoliation when 90% needles on trees dropped (Migliavacca et al., 2008). Larch trees did not begin budburst until early May (May 5) and soon started to unfold needles in mid-May (May 16). After 5.5 months of leaf lifespan, larch trees dropped their leaves in mid-November (November 19).

#### 2.5. Water source model

The contributions of different soil layers to trees were analyzed using SIAR (Stable Isotope Analysis in R, version 4.2). SIAR is a Bayesian calibrated mixing model that can incorporate the uncertainty of multiple sources, the variability in isotope signatures, and isotope fractionation (Parnell et al., 2010). Compared with the widely used IsoSource model, the Bayesian mixing model can incorporate the variation in isotope signatures (Phillips and Gregg, 2003).

To rule out the possible effect of hydrogen fractionation on the assessment of water use, we selected  $\delta^{18}$ O to calculate tree water sources (Lin and Sternberg, 1993). The input data for SIAR included  $\delta^{18}$ O data of xylem water and  $\delta^{18}$ O data of soil water with 10 cm intervals (0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm). The fractionation value was set to zero, because there was no isotopic fractionation of  $\delta^{18}$ O during plant water uptake in the growing season. Model predictions were presented as the 50th, 75th, and 95th confidence percentile values (Moore and Semmens, 2008), and were combined into three source endpoints (0–10 cm, 10–20 cm, and 20–40 cm).

## 2.6. Statistic analyses

All statistical analyses were performed in SPSS 20.0 (SPSS Inc., Chicago, USA). A one-way analysis of variance (ANOVA) was used to assess the temporal patterns in  $\delta^{18}$ O of precipitation and xylem water across the three phases (before, during, and after growing season), after testing for the normality of residuals and homogeneity of variances. The Fisher's Least Significant Difference (LSD) test was used for post hoc multiple comparisons. A Kruskal-Wallis non-parametric rank test was used to compare the SWC and  $\delta^{18}$ O values across soil depths and sampling phases. For detecting the differences among soil depths, multiple comparisons were made using the Mann-Whitney *U* test. ANOVA could not be used here, because neither SWC nor isotopic composition data had homogeneity in variance.

## 3. Results

## 3.1. Isotopic variation in precipitation (rainwater and snowmelt water)

The annual precipitation amount was 541.2 mm in 2014, and 70.4% of the precipitation events less than 5 mm (Fig. 3). The  $\delta$ D and  $\delta^{18}$ O values of rainwater fluctuated largely during our sampling period;  $\delta$ D ranged from -18.50% to -185.61%, and  $\delta^{18}$ O ranged from -4.59% to -25.51%, with a monthly weighted average of -77.41% and -11.69%, respectively. Among the three phases, the  $\delta^{18}$ O value of rainwater was obviously depleted after the growing season, which was significantly different than that of other two phases (p < .05, Table 1).

The snow depth was approximately 20 cm and  $\delta D$  and  $\delta^{18}O$  of snowmelt were -96.52% and -14.00%, respectively. The period of snow thawing was from May 5 to May 23, during which the soil temperature reached 0 °C (Fig. 4).

#### Table 1

Mean $\delta^{18}$	O of precipitation,	soil water and xylem wat	er during different p	hases of the growing	season from September	2013 to October 2014.
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Source	2013	2014			
	Phase 3	Phase 1	Phase 2	Phase 3	
Precipitation 0–10 cm Soil water 10–20 cm Soil water 20–40 cm Soil water <i>Larix principis – rupprechtii</i> xylem water	NA -11.07 $\pm$ 2.05 -12.08 $\pm$ 1.82 -12.26 $\pm$ 1.71 -10.77 $\pm$ 1.54	$\begin{array}{l} -10.58^{a}\\ -10.61\ \pm\ 1.83^{ab}\\ -11.26\ \pm\ 1.48^{a}\\ -11.25\ \pm\ 1.04^{a}\\ -7.03\ \pm\ 1.51^{a} \end{array}$	$\begin{array}{r} -10.62^{a} \\ -9.20 \ \pm \ 2.34^{a} \\ -10.18 \ \pm \ 1.53^{b} \\ -10.11 \ \pm \ 1.47^{b} \\ -10.37 \ \pm \ 1.13^{b} \end{array}$	$\begin{array}{r} -14.76^{b} \\ -14.08 \pm 5.48^{b} \\ -11.58 \pm 2.46^{a} \\ -11.14 \pm 2.07^{a} \\ -10.05 \pm 0.56^{b} \end{array}$	

Phase 1 = before growing season (April–May), Phase 2 = growing season (June–August), Phase 3 = after growing season (September–October);  $\delta^{18}$ O values of precipitation were weighted by volumetric precipitation; letters (a, b) indicate significant differences between three phases in 2014 based on Fisher's Least Significant Difference (LSD) Test or Mann–Whitney rank-sum Test (p < .05).

## 3.2. Soil water content (SWC) and isotopic variations

For temporal variations, the SWC of each depth all changed significantly across different months (p < .001, Kruskal-Wallis nonparametric rank test, Fig. 4b). In late May, by infiltration of snowmelt water, SWC<sub>10</sub> and SWC<sub>20</sub> (0–10 cm and 10–20 cm) reached their yearly maximum values of 38.2% on May 24 and 46.0% on June 7, respectively. From June to October, SWC showed high values with small variation, and SWC<sub>10</sub>, SWC<sub>20</sub>, SWC<sub>30</sub> and SWC<sub>40</sub> were 26.6  $\pm$  3.1%, 40.7  $\pm$  2.4%, 26.1  $\pm$  3.7% and 10.2  $\pm$  2.0%, respectively (Figs. 4b, 5a). However, SWC displayed a decreasing trend around mid-August due to 33.1% less monthly precipitation than the mean value (1957–2013). For spatial variations, maximum SWC occurred at the depth of 10–20 cm during the sampling period, except for late May, when there was a percolation of snowmelt water at the 0–10 cm depth (Fig. 4).

The  $\delta^{18}$ O values of soil water changed considerably with both soil depth and phase (p < 0.05).  $\delta^{18}$ O in the 0–10 cm soil layer ( $\delta^{18}O_{\text{soil}10}$ ) was most variable and strongly reflected the seasonal variation of  $\delta^{18}$ O in precipitation ( $\delta^{18}O_{\text{spt}}$ ) (r = 0.663, p < .01, N = 28). Among soil layers,  $\delta^{18}O_{\text{soil}10}$  was significantly different from isotopic values in the 20–40 cm layer ( $\delta^{18}O_{\text{soil}40}$ ) (p = .015). However,  $\delta^{18}O_{\text{soil}20}$  did not differ significantly from either the  $\delta^{18}O_{\text{soil}10}$  or the  $\delta^{18}O_{\text{soil}40}$  layer (p = .076 and p = .352, respectively). Across three phases,  $\delta^{18}O_{\text{soil}}$ 

differed significantly between the growing and non-growing seasons for both the 10–20 cm and the 20–40 cm layer, but not for the 0–10 cm layer (Table 1).

A strong linear relationship between  $\delta D$  and  $\delta^{18}O$  was observed in both local precipitation and soil water. The  $\delta D$ - $\delta^{18}O$  relationship for Soil Water Line (SWL,  $\delta D = 7.06\delta^{18}O - 2.09$ ,  $R^2 = 0.943$ , N = 615, p < .001) was below the Local Meteoric Water Line (LMWL,  $\delta D = 7.95\delta^{18}O + 15.79$ ,  $R^2 = 0.988$ , N = 32, p < .001), indicating that the soil water had undergone slight evaporative enrichment (Fig. 6).

## 3.3. Isotopic variations in xylem water

Xylem water  $\delta^{18}$ O ( $\delta^{18}$ O<sub>xylem</sub>) showed pronounced seasonal trends. Particularly, before the growing season (April–May),  $\delta^{18}$ O<sub>xylem</sub> displayed high values and decreased markedly after needle unfolding (May 16). Compared with  $\delta^{18}$ O<sub>xylem</sub> before the growing season, significant depletions with offsets of 3.34‰ and 3.02‰ were found for the growing season (June–August) and after the growing season (September–October), respectively (p < .001, Table 1).

This initial trend with high  $\delta^{18}$ O values was regarded as winter evaporative enrichment in twigs caused by leaflessness.  $\delta$ D and  $\delta^{18}$ O in xylem water ( $\delta$ D = 3.04 $\delta^{18}$ O - 37.71,  $R^2$  = 0.664, N = 205, p < .001, Fig. 6) also supported this explanation that xylem water underwent

**Fig. 4.** Meteorological factors, including the mean daily (a) air and soil temperature, (b) soil water content (SWC) of different layer depths under alpine habitat (Luya Mountain, China) in 2014. Grey area represents the start and end of growing season on both panels.





**Fig. 5.** Monthly values of (a) soil water content (SWC) and (b)  $\delta^{18}$ O of soil water in vertical profile under alpine habitat (Luya Mountain, China) from September 2013 to October 2014. Values are average  $\pm$  standard error (N > 8).

isotopic fractionation. Therefore, early values during the trees' leafless period should be omitted for the determination of the water source.

From needle unfolding to the growing season onset (mid-May to beginning of June), correlations between  $\delta^{18}O_{xylem}$  and  $\delta^{18}O_{soil}$  were clearly negative ( $\delta^{18}O_{soil,10}$ : r = -0.862, p < .05;  $\delta^{18}O_{soil,20}$ : r = -0.924, p < .01; N = 7, Fig. 7). During the growing season (June-August),  $\delta^{18}O_{xylem}$  followed the seasonal patterns of  $\delta^{18}O_{soil,20}$  (r = 0.521, p < .05, N = 20). This  $\delta^{18}O_{xylem} - \delta^{18}O_{soil,20}$  relationship disappeared both after the growing season (September–October) and the whole phase (April–October). Moreover, the seasonal patterns of  $\delta^{18}O_{ppt}$  did not fingerprint in the  $\delta^{18}O_{xylem}$  either at the single phase or through the whole phase.

## 3.4. Water-source utilization during growing season

In June, the highest probabilities (mode values) of the soil layer

contributing to plant water uptake were found below 10 cm, with mode values of 38% and 40% for the 10–20 cm and 20–40 cm layers, respectively (Fig. 8). The contribution of the top layer (0–10 cm) to tree water uptake was less likely, at 14%. In the monsoon season, the water uptake in July was generally equally distributed along the soil profile, with the 0–10 cm, 10–20 cm and 20–40 cm layers contributing 30%, 38%, and 38% of water, respectively, similar to the values obtained in August. Thus, during the growing season (June–August), larch depended less on the 0–10 cm soil layer (14–32%) and stably relied on the deeper layers below 10 cm (38–41%).

## 4. Discussion

## 4.1. Water loss by evaporation of xylem before needle unfolding

Our results showed that  $\delta^{18}O_{xylem}$  values were often much higher

**Fig. 6.** Relationship between  $\delta D$  and  $\delta^{18}O$  of precipitation, soil water, and *Larix principis-rupprechtii* xylem water under alpine habitat (Luya Mountain, China) from September 2013 to October 2014. Blue solid and black dashed lines represent Local Meteoric Water Line (LMWL) and Soil Water Line (SWL), respectively, in Luya Mountain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)





**Fig. 7.** Seasonal variation in  $\delta^{18}$ O of precipitation, snow water, soil water (0–10 cm, 10–20 cm and 20–40 cm depths), and *Larix principis-rupprechtii* xylem water during the growing and non-growing seasons. Standard error bars for snow water (N = 5) and xylem water (N = 5) are also shown. For clearly display, standard error bars for soil water in three vertical depths are not shown. Instead, horizontal bands (brown) depict the standard error ranges for soil water  $\delta^{18}$ O values (N = 16) of three depths during sampling period. Dashed vertical lines represent the transition between the growing and non-growing seasons. Green triangles represent the dates of leaf phenology. bb, bud break; nu, needle unfolding; nf, needle fall. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 8.** Proportional source contributions of different soil depths to *Larix principis-rupprechtii* xylem water, derived by SIAR (Stable Isotope Analysis in R, version 4.2) based on the Bayesian model. Boxplots illustrate the 50th, 75th, and 95th percentile confidence intervals from dark to light. Under the 95th percentile confidences interval, the mean and median values are indicated by black and red lines, respectively. Number of replicates in June, July and August are N = 10, N = 6, and N = 5, respectively.

than the highest soil water  $\delta^{18}$ O values before needle unfolding. This isotopic enrichment in xylem is regarded as a result of evaporation enrichment, which has been reported by other studies (Brandes et al., 2007; Treydte et al., 2014). Moreover, xylem stem water evaporative enrichment is not only an issue for deciduous species, but could also occur in evergreen species, such as *Pinus sylvestris* L. (Martin-Gomez et al., 2017), *Pinus halepensis* Mill. (del Castillo et al., 2016), and *Picea meyeri* (our unpublished data).

Although water loss in the suberized stem from its surface is considered minimal (Schönherr, 1982), evaporation over a period of 1 month was sufficient to isotopically enrich stem water (~ +4‰ in  $\delta^{18}$ O) (Ellsworth and Sternberg, 2014). In our study, due to the much colder weather, enriched xylem water was *ca.* ~ +2‰ (in  $\delta^{18}$ O), suggesting that the water loss in tree xylem also occured in the moist condition of a cold alpine habitat and might lead to water stress of trees in early spring. These water deficits in trees may be aggravated in a dryer future, especially at predicted continuation warming in earlier spring. Therefore, besides temperature, water might be a limiting factor that affects the distribution of alpine trees in treeline.

## 4.2. Winter snowfall for the growth initiation period

During the period of the growing season initiation (around late-May/early June), winter snowfall (November to April) might play an important role for the onset of larch tree growth. After needle unfolding, sap flows recover by transpiration. In addition to low evaporation,  $\delta^{18}O_{xylem}$  displayed a marked decreasing trend, which was consistent with the trend expected to result from the influence of isotopic depleted snowmelt water (-14‰). The influence of soil water, especially thawed topsoil water (0–10 cm), can be excluded, since  $\delta^{18}O_{soil}$  values appeared with an opposite and increasing trend. Therefore, snowmelt water rather than immobile soil water appears to be the source water for growth onset.

Several studies also support the effect of previous-year precipitation

for tree growth. Sugimoto et al. (2002) observed that more than 50% of snowmelt infiltrated into soil and was absorbed by *Larix gmelinii*. The  $\delta^{18}$ O of tree-ring cellulose suggested that larch trees mostly use the snow-melt water of the previous winter (November to March) through the whole growing season (Leonelli et al., 2017). The lasting time-extent which snow can be used by trees might be not for only the current year but also for two consecutive years (Reichmann et al., 2013). These convergent results suggest winter snowfall probably plays an important role for tree growth (Blok et al., 2015).

As a result of global temperatures increase, a reduction in snowpack depths is expected. Numerous studies have reported that shallower snowpack had earlier snowmelt timing, and in turn resulted in an earlier start of season, and a longer growing season length (GSL) in the mid and higher latitude areas (Hu et al., 2010). However, our study and some other studies in Trans Himalayan region both show that the reduced winter snowpacks would increase the risk of water shortage for trees, especially in areas where the trees mainly rely on the winter snowfall for their growth onset (Paudel and Andersen, 2013). Hence, the management actions should be taken, to create a longer residence time for snowmelt water in stand and to augment the infiltration of snow water into soil layers. Reduction of stand density to decrease interception of snowfall (Kerhoulas et al., 2013), and retention a thick litter layer to slow down surface runoff (Xu et al., 2011) are a few good examples among many practices.

## 4.3. Water utilization during growing season

The results showed that larch preferentially acquired the relatively consistent water from deep soil (below 10 cm) (Fig. 8). Similarly, Li et al. (2007) found that *L. sibirica* in cold Mongolia mainly used water from a depth of 10–30 cm during a wet summer. This water-use pattern of *L. principis-rupprechtii* might be related to its root distribution depth, which was 80.0% of fine roots (< 2 mm) at depths below 10 cm. Similar deeper-root distribution characteristics were also reported for other *Larix* species, such as *L. gmelinii* (Rupr.) Kuzen. near Tura (Saurer et al., 2016) and *L. decidua* Mill. in the Italian Alps (Valentini et al., 1994). Larches have deep root systems, which might allow them access to additional soil water resources when competition is high (King et al., 2013).

In addition to the root morphological characteristics, the data for soil water availability also supported the larch water utilization pattern (Sánchez-Pérez et al., 2008), as maximum soil water appeared at the 10–20 cm (Figs. 4, 5). Unlike the high variation at surface soil (0–10 cm),  $\delta^{18}O_{soil_20}$  did not differ significantly with either the  $\delta^{18}O_{soil_10}$  or  $\delta^{18}O_{soil_40}$  layer. The presence of an efficient mixing process and long water residence time were found at this soil depth. In agreement with our results, previous studies at the same site showed that soil in this ecosystem has a soil texture transition layer at the depth of 10–20 cm, with a low bulk density, high total porosity, and maximum soil organic carbon (SOC) storage (Wu et al., 2011). All these soil characteristics result in a lasting residence time for the mixing of new and old water in the 10–20 cm layer.

Thus, based on the characteristics of larch roots and soil, it is probably true that *L. principis-rupprechtii* used the mixed water of summer precipitation and previous-year autumn/winter precipitation (rainfall/snowmelt) during the entire growing season. Reliance on a mixture water rather than only summer rainfall could be attributed to three reasons. First, larches were tapping into a deeper and more consistent water source (> 10 cm depth) during the growing season. Our isotopic results from no link between  $\delta^{18}O_{xylem}$  and  $\delta^{18}O_{ppt}$  provide the evidence that lower reliance on summer rainfall may much safer (Hu et al., 2010). Second, the rain events, although frequent during the summer, are too small (70% of all summer rain event were < 5 mm) to penetrate deeply into the soils (Cheng et al., 2006). While, after some larger rain events (> 10 mm) in July and August, trees turned to utilize more summer rainfall from the shallow soil (0–10 cm), which contributed to 30% of water compared with 14% in June (Fig. 8). Third, the soil layer in 10–20 cm depth was a transition layer that allowed rain water for long residence and good mixing. After the growing season, the precipitation in September was extremely depleted with low  $\delta^{18}$ O value (Table 1), recorded in  $\delta^{18}O_{soil}$  but not in  $\delta^{18}O_{xylem}$  (Fig. 7). This implied that precipitation water (rainfall/snowmelt) after the growing season probably had infiltrated into deeper soils and be stored there, until next year for trees to use these mixture waters for growing. Reliance on precipitation left over from last year (precipitation legaies) could explain why, in some dendroecological studies, tree-ring width and previous-year autumn/winter precipitation have a positive correlation (Lévesque et al., 2013; Zhang et al., 2017).

#### 5. Conclusions

Our results indicated that source-water utilization by L. principisrupprechtii may change throughout the whole year. Before needle unfolding, xylem water loss by evaporation could occur even in alpine conditions. Evaporation over 1 month was sufficient to enriched stem water (~ +2‰ in  $\delta^{18}O_{xylem}$ ), and this water loss should not be neglected. Therefore, forest water management is likewise fundamentally important in wet forests as well as in arid regions. During the initiation of the growing season, winter snowmelt water (November to April) might be an important water source for larch. During the growing season (June-August), larch relied mainly on the mixture water from deep soil (below 10 cm), which is consistent with its deep root system and the occurrence of the maximum SWC at the soil depth of 10-20 cm. The mixture water, in addition to summer rainfall, was predominantly recharged by previous-year autumn/winter precipitation (rainfall/ snowmelt). The results further suggest that since the period during which precipitation affects tree growth is extended, water distribution studies should focus not only on the current year but also on previousyears.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.11.009.

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