

# Changes in water use with growth in *Ulmus pumila* in semiarid sandy land of northern China

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## Abstract

**Key message** *Ulmus pumila* vary its water use strategy from seedling to maturity in a water-limited sandy land by adopting different photosynthetical capacities, water use efficiencies and morphological traits.

**Abstract** Regeneration failure of natural *Ulmus pumila* populations has become a growing concern related to vegetation conservation and prediction of environmental change in the sandy lands of northern China. To better understand the life-history strategies of *U. pumila* and its adaptation to drought in semiarid environments, we studied ecophysiological and morphological traits related to water use in an age sequence of *U. pumila* representing four age classes: current-year seedlings (Uc), age 2- to 5-year-old saplings (Us), juveniles (Uj), and mature trees (Um). A comparison of hydrogen isotope data in xylem sap, soil water in different layers and groundwater showed that Uc relied on the soil water in the topsoil (0–40 cm), Us and Uj absorbed soil water from deeper soil (>40 cm), while Um mainly used stable groundwater with very deep (>2 m) taproots. Significantly lower predawn leaf water potentials were observed in Uc than in Uj or Um, suggesting that Uc experienced more severe water stress and had a weaker capacity to recovery. Moreover, Uc had the highest daily

maximum net assimilation rate, daily maximum transpiration rate and daily maximum stomatal conductance, all of which decreased remarkably at midday. A “go for broke” strategy is probably practiced by Uc which try to provide the growth they need to become established, but with a great risk of mortality. Um used a more conservative strategy by effectively regulating the instantaneous water-use efficiency, and maintaining both stable gas exchange levels and significantly higher long-term water-use efficiency. Uj endured and adapted to drought conditions by developing steeper leaf angles, denser leaf pubescence and more stomata than differently aged plants. Our findings illustrate that significantly different water-use strategies were developed by *U. pumila* trees as they grew from seedlings to maturity, which were based on different water sources.

**Keywords** Arid · Gas exchange · Stable isotope · Water source · Water-use efficiency

## Introduction

Sandy lands in northern China are easily distinguished from typical temperate grasslands by the presence of trees or shrubs (Li et al. 2003; Su et al. 2009; Wang et al. 2011). Woody species not only contribute significantly to biodiversity, structural complexity, and spatial heterogeneity (Belsky 1994), but also play a distinct role in the regulation of water availability among woody plants and herbaceous communities (Jin et al. 2009; Wesche et al. 2011). In arid and semiarid regions, plant species have to contend with water shortages throughout their life cycles (Wesche et al. 2011). A better understanding of the life-history strategies of woody species as they relate to water use will provide

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important information for the prediction of vegetation dynamics under global climate change (Ragab and Prudhomme 2002).

Water availability is the most important abiotic factor in sandy lands because it not only regulates growth, but also limits the distribution and abundance of plant species (Li et al. 2007a). The physiological and morphological responses of plants to water stress have been extensively investigated (Dulamsuren et al. 2009; Fang et al. 2012; Li et al. 2007b). For example, some tree species in Brazilian savanna maintain higher rates of water use during the dry season because they maintain a deep root system and a large capacity to extract water from deep soil layers (Jackson et al. 1999). DeLucia and Heckathorn (1989) suggested that *Pinus ponderosa* uses a conservative eco-physiological strategy by maintaining high levels of water-use efficiency (WUE) in a competitive water-limited environment. Zhang et al. (1997) found that drought-tolerant pine seedlings used water quickly when water was available, but closed their stomata in response to water stress. Stomatal closure is thought to be the earliest response of plants to drought and progressive down-regulation or inhibition of metabolic processes becomes the dominant limitation during severe drought which inhibits photosynthetic CO<sub>2</sub> assimilation (Flexas and Medrano 2002). Leaf pubescence has been reported to be an adaptation to arid environments by reducing the radiant energy absorbed by leaves, affecting their energy balance (Ehleringer 1983) and reducing transpiration while maintaining a favorable leaf temperature and so helping the plant to maintain a favorable water balance (Galmés et al. 2007; Savé et al. 2000). However, the sensitivity of plants to water availability and their need for water may vary during different developmental stages of their lifecycle (Grubb 1977) but relatively few studies have focused on how plant water-use strategies vary throughout their lifecycle.

Natural stands of *U. pumila* were once widely distributed throughout the Mongolian forest-steppe (Dulamsuren et al. 2009), but by the late 1990s only “degraded relic stands” remained (Hilbig 1995) forming scattered and fragmented patches with apparent regeneration failure. Grazing impact has been considered as the most likely reason for this degradation (Guo and Liu 2004; Wesche et al. 2011). However, Dulamsuren et al. (2009) also believed that environmental stress could prevent *U. pumila* from forming closed forests. Seedlings of *U. pumila* often suffer from severe water stress during dry summers caused by repeated cycles of drying in the upper soil layers (Guo and Liu 2004; Wesche et al. 2011) and more than 90 % current-year seedlings in fenced plots died because of their vulnerability to drought (Wang et al. 2011). Dry conditions may be an important factor in lowering levels of successful regeneration in *U. pumila*. Understanding how *U. pumila*

responds to drought through its life-history stages can play a major role in the protection of natural vegetation.

In sandy lands of northern China, use of groundwater for anthropogenic activities has greatly altered the annual hydrologic balance and climate change is expected to further shift the system toward a drier environment (Ding et al. 2007; Zheng et al. 2006). We studied the physiological and morphological traits in *U. pumila* along an age sequence, from current-year seedlings through mature trees during August when competition among plants for available water is at its peak. The main objectives of this study were to clarify how water sources used by *U. pumila* vary with age and to show what functional traits are likely to be involved in the age-dependent regulation of water use. Knowledge of a plant’s life-history strategy as it relates to growth and water use is both key to understand the structural dynamics of *U. pumila* woodlands and is also vital to the conservation of vegetation and sustainable land management for securing ecosystem stability in many arid regions.

## Materials and methods

### Ethics statement

No specific permit was required for the described field study. The study site is not privately owned or protected in any way and the field study did not involve endangered or protected species. The subject of our study, *U. pumila*, is a common, widely distributed plant species in the semiarid region of northern China and is not listed as an endangered or protected species.

### Study site

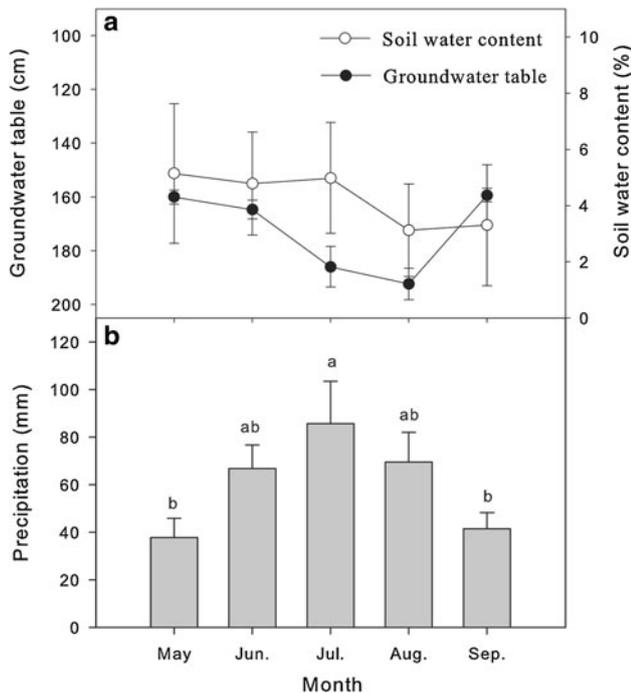
The study was carried out in *U. pumila* stand in the southern edge of Hunshandake sandy land (42°55′01.43″N; 116°02′06.77″E, 2,667 ha, 1,313 m above sea level). Grazing was excluded on the site for 7 years prior to the study. The Hunshandake sandy land lies 180 km north of Beijing and covers an area of about  $7.2 \times 10^4$  km<sup>2</sup> (Yue et al. 2009). Meteorological data were obtained from Zhenglan Qi weather station which is located about 50 km far away. The long-term (1999–2008) annual mean temperature, monthly mean air temperatures of the coldest month (January) and the warmest month (July) are −1.7, −23.4 and 14.7 °C, respectively. Mean annual precipitation is about 367.1 mm (range 250–450 mm), of which 80 % falls as rain between June and August. However, soil water content (0–3 m) was uneven among heterogeneous habitats including fixed dunes (annual mean soil water content of 1.3 %), semi-fixed dunes (3 %), shifting dunes

(6 %) and lowlands (8 %) (Guo and Liu 2004). Lowlands have the best water condition and several ephemeral lakes may form when the water table rises to the surface (Yang et al. 2007). Shrublands dominated by *Salix gordejvii* and *Salix microstachya* occur there. In comparison, fixed dunes have the poorest water conditions, which are significantly influenced by precipitation and vegetation cover (Li et al. 2007a). Dunes are mostly covered by herbaceous plants such as brush and trees including sparsely distributed *Ulmus pumila*, *Pinus tabulaeformis* and *Cerasus tomentosa*.

#### Field sampling and measurements

##### Plot survey and selection of sample plants

Three 50 × 50 m plots were selected in stable flat lands where previous dunes have been stabilized for more than 15 years. Any pair of plots was less than 2 km apart to ensure the same conditions. The soil consists of 21 % clay, 60 % sand and 19 % silt, with a bulk density of 1.46 g cm<sup>-3</sup> and pH at ~7.2. Soil organic C, total N, total P and total K contents are 5.99, 1.36, 0.19 and 17.90 g kg<sup>-1</sup>, respectively. The groundwater level ranges from 1.6 to 2.0 m (Fig. 1a). The water content in the topsoil (0–40 cm) ranged from 1.0 to 8.5 % and is significantly influenced by precipitation (Li et al. 2007a), while



**Fig. 1** Groundwater table (a), soil water content in deep soil layer (40–150 cm) (a) and average monthly precipitation (b) from May to September in the Hunshandake sandy land. Mean ± SE ( $n = 9$ ). Different letters indicate a significant difference ( $P < 0.05$ ) in a least significant difference test

the water content in deeper soil (40–150 cm) remains between 3.0 and 6.0 % (Fig. 1a). Five *U. pumila* plants of each age class, current-year seedlings (Uc), 2 to 5-year-old saplings (Us), juveniles (Uj), and mature (Um), in each plot were marked for measurements of ecophysiological and growth traits and their basic characteristics were recorded (Table 1).

Precipitation statistics collected from 1999 to 2008 indicated that average monthly precipitation remained statistically unchanged during the growing season (June, July and August; Fig. 1b). Also, all plant species grow vigorously during August when competition for available water is the highest; so, we conducted all field studies and measurements in August, 2008.

##### Hydrogen ( $\delta D$ ) stable isotope composition

Twenty non-green branches (at least 20 cm segment) were collected from the marked individuals and enclosed in air-tight glass vials. Soil water samples from seven soil layers (0–20, 20–40, 40–60, 60–80, 80–100, 100–140, 140–180 cm) and groundwater samples were collected near the marked *U. pumila* using a 5-cm diameter soil auger. In the laboratory, plant and soil samples were held at  $-20$  °C in a refrigerator until being processed and analyzed for measurement of the hydrogen stable isotope ratio ( $\delta D$ ).

Yang et al. (2011) described the procedure used for the analysis of  $\delta D$ . For plant and soil samples, water was extracted using the cryogenic vacuum distillation method (Ehleringer and Osmond 1989). All water samples were pyrolyzed into H<sub>2</sub> in an elemental analyzer (TC/EA, Thermo Finnigan, Bremen, Germany) interfaced (ConFloIII; Thermo Finnigan MAT) to a Thermo Finnigan Delta<sup>plus</sup>XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). They were measured against laboratory working standard gases, which were previously calibrated against IAEA standards (IAEA SMOW for D, accuracy of calibration  $\pm 0.10$  ‰ SD). The stable isotope ratio of hydrogen in water is expressed using standard delta notation ( $\delta$ ) in parts per mil (‰) as Yang et al. (2011):

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

**Table 1** Performance of current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juvenile (Uj) and mature (Um) *Ulmus pumila*

Age class	Ages (years)	Height (m)	Diameter (cm)	Density (ind. per ha)	Seed
Uc	1	0.05	<0.1	~140,000	No
Us	2–5	0.05–2	0.1–1	767–2,720	No
Uj	5–20	2–5	1–10	140–640	No
Um	20–70	5–11	10–30	100–780	Yes

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

#### Leaf water potential and gas exchange

For each age class, six of the newest fully developed mature leaves from sun-exposed terminal branches of the marked individuals were selected to measure leaf water potential ( $\Psi_1$ ). Predawn and midday  $\Psi_1$  were measured by an automatic plant water potential system (Psypro, Wescor, Logan, UT, USA) from 04:00 to 05:00 and from 12:00 to 13:00; each test was repeated three times for 3 days.

Daily maximum and midday net  $\text{CO}_2$  assimilation rate ( $A$ ), transpiration rate ( $E$ ) and stomatal conductance ( $g_s$ ) were measured with a portable photosynthesis measurement system (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany) under ambient conditions on typical sunny days. Daily maximum  $A$ ,  $E$  and  $g_s$  were measured at 10:00 to 11:30 h, while minimum/midday  $A$ ,  $E$  and  $g_s$  were measured at 12:30 to 14:00 h. Each measurement was repeated three times for 3 days. Ambient photosynthetically active radiation (PAR) was about  $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the morning, and was about  $1,700 \mu\text{mol m}^{-2} \text{s}^{-1}$  at midday. Ambient temperature was around  $23^\circ\text{C}$  in the morning and  $32^\circ\text{C}$  at midday. Ambient atmospheric vapor pressure deficit was around  $3.3 \text{ kPa}$  in the morning and  $4.0 \text{ kPa}$  at midday.

#### Water-use efficiency

Instantaneous WUE was calculated from gas exchange measurements as the ratio of  $A$  to  $E$ . The foliar stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) was used as a proxy for long-term WUE, because of the well-established empirical correlation between the two variables (Ebdon and Kopp 2004; Farquhar et al. 1989; Johnson et al. 1990). For each age class, five individual plants were randomly selected; five healthy and fully expanded leaves from each individual were collected and oven-dried at  $65^\circ\text{C}$  for at least 48 h to a constant weight. The leaves were grounded with a shaker-type ball mill (MM400, Retsch GmbH, Germany) into uniformly fine powder and finally sieved with a  $150\text{-}\mu\text{m}$  mesh screen. The carbon isotopic analyses were conducted using a Thermo Finnigan Delta<sup>plus</sup>XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) with an elemental analyzer in a continuous flow mode in the State Key Laboratory of Vegetable and Environmental Change, Institute of Botany, Chinese Academy of Science according to Zheng and Shanguan (2007). Values of  $\delta^{13}\text{C}$  were expressed relative to the Pee Dee Belemnite standard and the analytical precision was  $\pm 0.15\%$  (standard deviation).

#### Plant growth traits

Leaf angle of ten fully expanded leaves in the middle of current-year twigs (for Uj and Um) or near the new leaves (for Uc and Us) was measured with a clinometer (PM-5/360PC, Suunto, Vantaa, Finland) while still attached to the plants. Immediately after the measurements, all leaves were collected and taken to laboratory for leaf area measurement. Each leaf lamina was quickly scanned using a desktop scanner and the area of each leaf was calculated using the *ImageJ* software ( $1.38\times$  Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). Leaves were then dried at  $65^\circ\text{C}$  to a constant weight. Specific leaf area (SLA) was calculated as the ratio of leaf area to dry mass (Markestijn et al. 2011). The tap roots were dug up by hand for Uc and Us and by an excavator for Uj and Um. The depth was the distance from ground level to the end of the taproot.

Ten fully expanded sun-lit leaves from the marked individuals were collected early in the morning to avoid dehydration of the tissue and the diurnal swelling/shrinkage cycling (McBurney 1992) following procedures as described in Su et al. (2009). Microscopic pictures were obtained for micro-structure examination with a scanning electron microscope (Hitachi S-570 SEM, Hitachi Ltd., Tokyo, Japan) and analyzed using an image analysis program, *Image-Pro plus* (v. 6.0, Media Cybernetics, Silver Spring, MD, USA). Stomatal size was defined as the length between the junctions of the guard cells at each end of a stoma multiplied by total width of the closed guard cell pair. It may indicate the maximum potential opening of the stomatal pore, but not the aperture of opening that actually occurs (Xu and Zhou 2008). Proportion of stomatal area to leaf area (%) was calculated by the ratio of total stomatal size to leaf area.

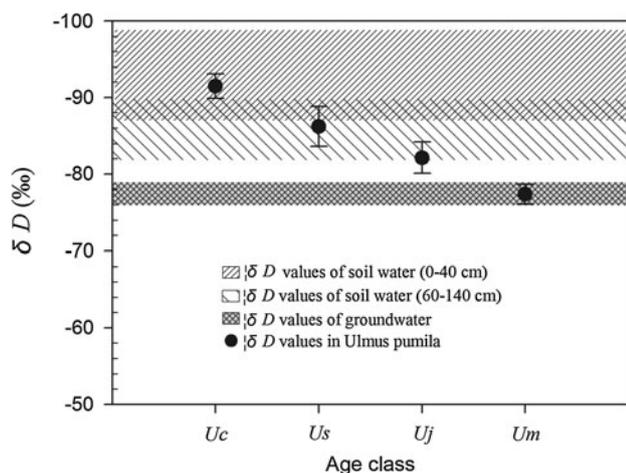
#### Statistical analyses

Data of  $A$ ,  $E$ ,  $g_s$ , instantaneous WUE,  $\delta^{13}\text{C}$ , predawn  $\Psi_1$  and midday  $\Psi_1$  were tested for normality and homogeneity of variance before statistical analysis. Age class was treated as a fixed effect. Statistical analysis was carried out as separate one-way ANOVA, followed by post hoc comparisons among age classes using least significant difference (LSD) test ( $P = 0.05$ ). All analyses were performed with SPSS version 10.0 (SPSS Inc., Chicago, USA).

## Results

#### Water source

The water source of a plant can be obtained by comparing the hydrogen stable isotope level (D) in xylem sap and



**Fig. 2** Hydrogen stable isotopic compositions ( $\delta D$ ) of the nonphotosynthetic tissues in the current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juveniles (Uj) and mature trees (Um) in *Ulmus pumila* relative to the range of  $\delta D$  values in the soil profile. Vertical bars denote the standard errors of means ( $n = 15$ )

potential water sources, because there is no isotopic fractionation of D during water uptake by roots (Ehleringer and Dawson 1992). The  $\delta D$  information from xylem sap, soil water and groundwater indicated that each age class of *U. pumila* plants used distinctly different water sources (Fig. 2); Um mainly used groundwater and others mainly used soil water. However, Uc relied on the soil water in the topsoil (0–40 cm) while Us and Uj absorbed water from deeper soil.

#### Leaf water potential and gas exchange

Predawn  $\Psi_1$  of Uc, Us and Uj were significantly different from each other, with the value in the sequence of  $Uc < Us < Uj$  (Table 2). However, there was no significant difference between the predawn  $\Psi_1$  of Uj and Um. Midday  $\Psi_1$  of Um was highest, followed by Uj, Us and Uc (Table 2). Difference ( $\Delta$ ) calculation from predawn  $\Psi_1$  minus midday  $\Psi_1$  showed the greatest value in Uc (1.88), followed by Us (1.33), Uj (0.25) and Um (0.23).

*U. pumila* significantly down-regulated the leaf net assimilation rate (A), stomatal conductance ( $g_s$ ), transpiration (E) and instantaneous WUE at midday, regardless of the age class (Fig. 3). Uc has a significant maximum and midday A,  $g_s$  and E, followed by Us, Uj and Um, Um has the lowest (Table 3). The  $\Delta$  value was calculated from the maximum value minus the midday value. There was a significant difference of the  $\Delta$  value of A,  $g_s$ , E and WUE between Uc and Um, while an insignificant difference was found between Us and Uj (Fig. 4). Uc showed the largest  $\Delta$  value of A,  $g_s$  and E, while Um was the smallest. However, for instantaneous WUE, the reverse happened (Fig. 4d). No significant correlation was found between daily maximum

**Table 2** Predawn and midday leaf water potentials ( $\Psi_1$ ) of current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juvenile (Uj) and mature (Um) *Ulmus pumila*

Age class	Predawn $\Psi_1$ (kPa)	Midday $\Psi_1$ (kPa)	$\Delta$ of $\Psi_1$ (kPa)
Uc	$-2.46 \pm 0.10^c$	$-4.57 \pm 0.21^d$	$1.88 \pm 0.17^c$
Us	$-1.71 \pm 0.10^b$	$-3.04 \pm 0.13^c$	$1.33 \pm 0.15^b$
Uj	$-1.48 \pm 0.04^a$	$-1.73 \pm 0.09^b$	$0.25 \pm 0.13^a$
Um	$-1.23 \pm 0.03^a$	$-1.46 \pm 0.04^a$	$0.23 \pm 0.06^a$

$\Delta$  was calculated from predawn  $\Psi_1$  minus midday  $\Psi_1$ . Mean  $\pm$  SE ( $n = 30$ ). Different letters indicate a significant difference ( $P < 0.05$ ) in a least significant difference test for age classes within each column

$g_s$  and daily maximum instantaneous WUE across age classes (Fig. 5f). In contrast, a significant, positive correlation was found between midday  $g_s$  and midday instantaneous WUE (Fig. 5c). Both midday A (Fig. 5a) and E (Fig. 5b) were positively correlated with midday  $g_s$ . Positive correlations between daily maximum  $g_s$  and daily maximum A (Fig. 5d) and midday E (Fig. 5e) were also found.

#### Foliar carbon stable isotope

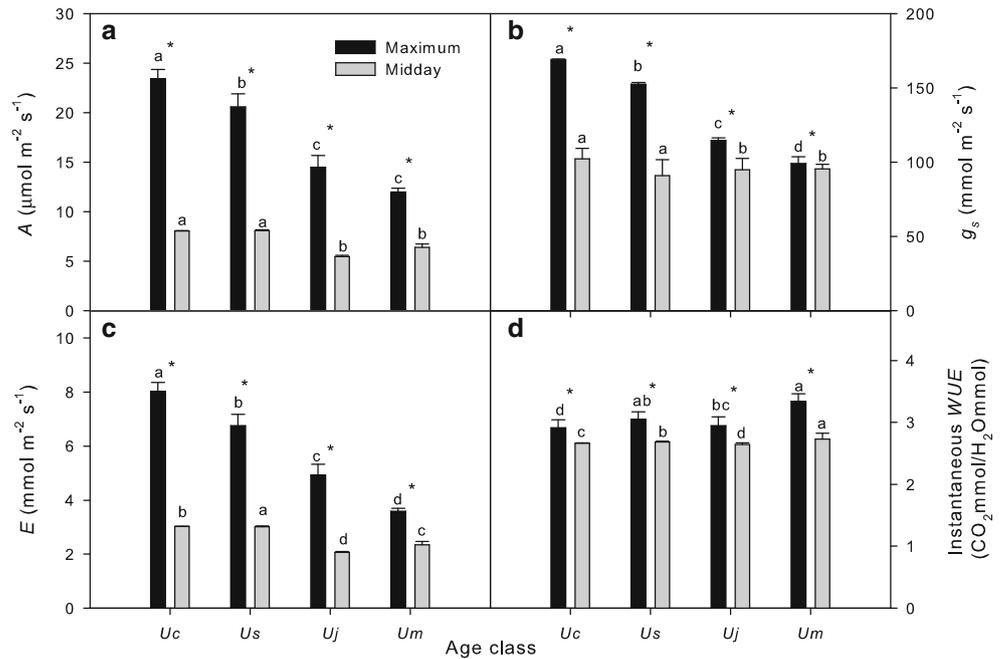
Foliar stable carbon isotopic compositions ( $\delta^{13}C$ ) of Uc was significantly higher than Um ( $P < 0.05$ ) and Uc was found to have more negative  $\delta^{13}C$  (Fig. 6), which reflected significant lower long-term WUE existed for Uc than for Um. No significant difference was found between both Uc and Us, and between Uj and Um.

#### Root and leaf traits

Taproot characteristics varied significantly across age classes, with the length varying 8.64 times from 21.27 cm for Uc to 205.00 cm for Um (Table 4). Leaf morphological traits also varied significantly (Table 4). Leaf size varied from less than  $1 \text{ cm}^2$  for Uc to close to  $10 \text{ cm}^2$  for Um. However, SLA changed in the opposite direction; Uc had the highest SLA while Um had the lowest. Uj had the smallest leaf angle, followed by Um which was significantly smaller than the leaf angle of Uc and Us.

Leaves from the four age class plants adopted significantly different anatomic traits (Table 5). Among the four age classes, the lower leaf surface of Uj had the most leaf pubescence and stomata while Um had the fewest. No significant differences in the density of both leaf pubescence and stomata were found between Uc and Us. Stoma was significantly larger as plants aged from Uc ( $1.35 \times 10^{-3} \mu\text{m}^2$ ) to Um ( $4.44 \times 10^{-3} \mu\text{m}^2$ ). Thicker cuticles and more deeply sunken stomata were found in the leaves of Um than that in the leaves of other age classes (Fig. 7).

**Fig. 3** Daily maximum and midday assimilation rate ( $A$ ) (a), stomatal conductance ( $g_s$ ) (b), transpiration rate ( $E$ ) (c) and instantaneous water-use efficiency (WUE) (d) of current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juveniles (Uj) and mature (Um) *Ulmus pumila*. Vertical bars denote the standard errors of means ( $n = 30$ ). Different letters indicate a significant difference ( $P < 0.05$ ) among the four age classes in a least significant difference test. Star represents significant difference ( $P < 0.05$ ) between maximum and midday values



**Table 3** One-way ANOVA for photosynthetic rate ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ) and instantaneous water-use efficiency (WUE) of *Ulmus pumila* in different age classes ( $n = 30$ )

Dependent variable	df	Mean square	$F$	$P$ value
Mean $A$	3	275.66	36.43	0.000
Mean $g_s$	3	12325.08	26.20	0.000
Mean $E$	3	37.74	46.29	0.000
Mean instantaneous WUE	3	0.36	224.07	0.000
Maximum $A$	3	23.00	38.04	0.000
Maximum $g_s$	3	3765.04	37.42	0.000
Maximum $E$	3	3.23	48.41	0.000
Maximum instantaneous WUE	3	0.015	6.97	0.000
Midday $A$	3	358.45	24.22	0.000
Midday $g_s$	3	9766.52	11.43	0.000
Midday $E$	3	49.09	30.82	0.000
Midday instantaneous WUE	3	0.54	513.62	0.000

Significant difference if  $P < 0.05$

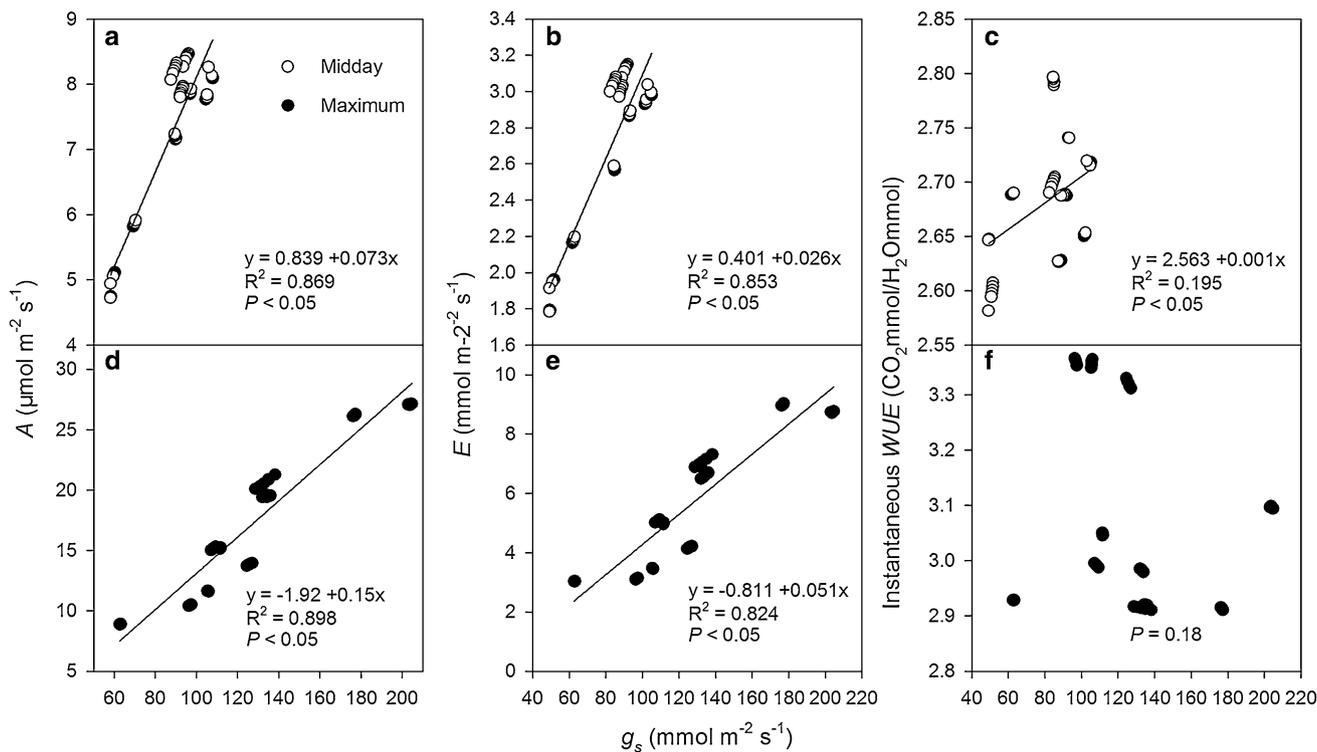
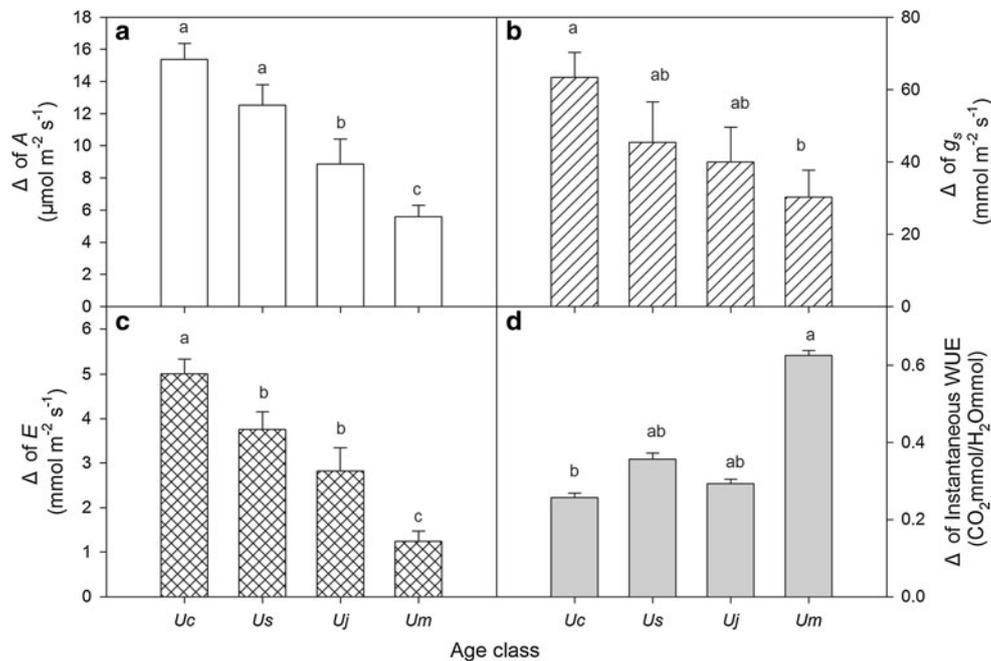
## Discussion

Regeneration failure of *Ulmus pumila* trees in the sandy lands of northern China is an important ecological issue, which is attracting more attention than in the past. Previous studies have focused on the effects of overgrazing (Guo and Liu 2004; Wesche et al. 2011), but few studies on environmental stresses, such as drought. Given the limited precipitation and poor water-holding capacity of the sandy soil in the Hunshandake sandy land, frequent severe drought should be an important reason for the lack of

regeneration in the existing *U. pumila* stands. At our study site, although livestock have been excluded for 7 years and current-year seedlings are abundant, recruitment of older seedling and juveniles is still limited. By studying the differences in the water source,  $\Psi_1$ , gas exchange, WUE and leaf traits of different age classes, i.e., current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juveniles (Uj) and mature (Um) *U. pumila*, our results reveal some insights into concerning water-use strategies and adaptive traits of *U. pumila*. *U. pumila* plants of different age classes may use different water sources. Differential water availability creates differential water conditions, which influence photosynthetic, morphological and anatomical adaptive traits. However, our measurements were performed in August when both soil water content and the groundwater table were at their lowest levels during the growing season (Fig. 1). Measurements were conducted in August avoiding rainfall, allowing the differences in water sources and adaptive traits to explain the different functional traits among the four age classes rather than others sources of variation such as those caused by rainfall.

Arid and semiarid climates require that plants grow in a water-limited environment with frequent severe drought and soil water should be the most important water source for plants (Li et al. 2006). During most of the growing season, water sources used by larch trees in northern Mongolia were from the upper 30 cm surface layer of the soil (Li et al. 2006). *Chrysothamnus Greenei* in south-central Colorado only used soil water recharged by precipitation, while phreatophytic *Chrysothamnus nauseosus* used soil water from the upper 0.5 m of soil and shallow

**Fig. 4** Difference ( $\Delta$ ) from daily maximum minus midday assimilation rate ( $A$ ) (a), stomatal conductance ( $g_s$ ) (b), transpiration rate ( $E$ ) (c) and instantaneous water-use efficiency (WUE) (d) of current-year seedlings ( $Uc$ ), 2- to 5-year-old saplings ( $Us$ ), juveniles ( $Uj$ ) and mature ( $Um$ ) *Ulmus pumila*. Vertical bars denote the standard errors of means ( $n = 30$ ). Different letters indicate a significant difference ( $P < 0.05$ ) among the four age classes in a least significant difference test



**Fig. 5** Midday assimilation rate ( $A$ ) (a), transpiration rate ( $E$ ) (b) and instantaneous water-use efficiency (WUE) (c) in relation to midday stomatal conductance ( $g_s$ ); and daily maximum  $A$  (d),  $E$  (e) and

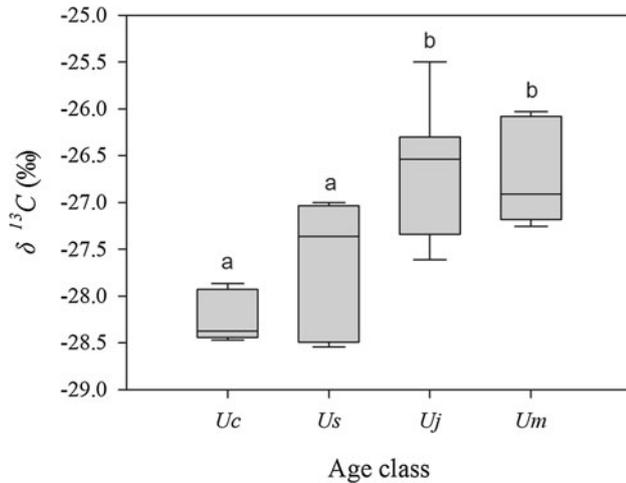
instantaneous WUE (f) in relation to daily maximum  $g_s$  across different age classes of *Ulmus pumila*. Solid lines are linear regressions fitted to the data

groundwater (Chimner and Cooper 2004). In this study,  $Uc$  relied on water in the topsoil (0–40 cm) (Fig. 2). Soil water content at a depth of 0–0.4 m of sandy land varied widely temporally mainly because of infiltrating precipitation and

subsequent evapotranspiration (Li et al. 2007a). As a consequence,  $Uc$  plants frequently experience severe water stress. By contrast,  $Um$  mainly used groundwater (Fig. 2) with very deep (>2 m) taproots (Table 4). Because a stable

groundwater source is available, Um could successfully cope with periodically severe droughts and extend their photosynthetic activity far into the dry season. Us and Uj absorbed soil water from deeper soil (>40 cm) which is relatively stable but remains at a low level (3–6 %).

Leaf water potential is a major factor in stomatal regulation because the stomatal aperture responds directly to guard cell turgor (Franks et al. 1995). Predawn  $\Psi_1$  could



**Fig. 6** Box-plots of foliar stable carbon isotopic compositions ( $\delta^{13}C$ ) of the current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juveniles (Uj) and mature (Um) *Ulmus pumila*. The box shows the upper and lower quartiles and the median. The same lowercase letters above the vertical bars indicate insignificant differences by least significant difference tests ( $P < 0.05$ )

indicate plant capacity to recover from water stress at night (Yang et al. 2011). The results showed that Uc had significantly more negative  $\Psi_1$  values than other age classes (Table 2), reflecting the more significantly severe water stress of Uc, and the weaker capacity of Uc to recover was confirmed by the midday  $\Psi_1$  and the difference ( $\Delta$ ) between predawn and midday  $\Psi_1$ . Comparatively, Um had a significantly higher predawn  $\Psi_1$  and midday  $\Psi_1$  and a significantly smaller  $\Delta$  (Table 2), which indicated the presence of a better hydraulic system in Um plants. The ability of a plant to maintain high  $\Psi_1$  during active transpiration indicates that the plant is able to replace water lost to transpiration quickly and therefore can maintain higher  $\Psi_1$ . (Zhang et al. 2013).

Recent studies have shown that diurnal depression in leaf gas exchange is common in many plant species (González-Rodríguez et al. 2001; González-Rodríguez et al. 2002; Romero and Botía 2006), including *U. pumila* (Li et al. 2007b; Li et al. 2003). Significant differences in the daily maximum assimilation rate ( $A$ ), daily maximum transpiration ( $E$ ) and daily maximum stomatal conductance ( $g_s$ ) among the four age classes were found (Table 3). Uc had the highest daily maximum  $A$ , daily maximum  $E$  and daily maximum  $g_s$  (Fig. 3), with the values of  $23.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $8.0$  and  $169.0 \text{mmol m}^{-2} \text{s}^{-1}$ , respectively, while Um had the lowest, with the values of  $12.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $3.6$  and  $99.2 \text{mmol m}^{-2} \text{s}^{-1}$ , respectively. However, although there were also significant differences of midday  $A$ , midday  $E$  and midday  $g_s$  among the four age classes (Table 3), the differences between Uc and

**Table 4** Leaf and root traits of current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juvenile (Uj) and mature (Um) *Ulmus pumila*

Age class	Leaf size ( $\text{cm}^2$ )	SLA ( $\text{cm}^2 \text{g}^{-1}$ )	Leaf angle ( $^\circ$ )	Taproot depth (cm)
Uc	$0.56 \pm 0.03^a$	$174.17 \pm 27.85^d$	$65.13 \pm 2.29^c$	$21.27 \pm 0.36^a$
Us	$1.00 \pm 0.08^b$	$107.51 \pm 23.00^c$	$64.67 \pm 1.86^c$	$99.07 \pm 2.28^b$
Uj	$2.19 \pm 0.52^c$	$95.89 \pm 23.69^b$	$46.67 \pm 5.89^b$	$151.81 \pm 9.01^c$
Um	$9.49 \pm 2.33^d$	$81.48 \pm 32.81^a$	$55.00 \pm 3.76^a$	$205.00 \pm 17.96^d$

Mean  $\pm$  SE ( $n = 30$ )

Different letters indicate a significant difference ( $P < 0.05$ ) in a least significant difference test for age classes within each column

**Table 5** Anatomical traits of the lower leaf surface of current-year seedlings (Uc), 2- to 5-year-old old saplings (Us), juvenile (Uj) and mature (Um) *Ulmus pumila*

Age class	Leaf hairs density (per $\text{mm}^2$ )	Stoma density (per $\mu\text{m}^2$ )	Mean stomatal size ( $\times 10^{-3} \mu\text{m}^2$ )	Proportion of total stomatal area to leaf area (%)
Uc	$8.4 \pm 1.2^b$	$8.37 \pm 0.16^b$	$1.35 \pm 0.40^a$	$1.13 \pm 0.37^a$
Us	$6.2 \pm 1.6^b$	$8.24 \pm 0.16^b$	$1.65 \pm 0.64^b$	$1.36 \pm 0.50^a$
Uj	$10.6 \pm 1.9^c$	$14.68 \pm 0.28^c$	$2.73 \pm 0.65^c$	$4.02 \pm 0.62^c$
Um	$2.6 \pm 2.5^a$	$4.37 \pm 0.17^a$	$4.44 \pm 0.60^d$	$1.94 \pm 0.12^b$

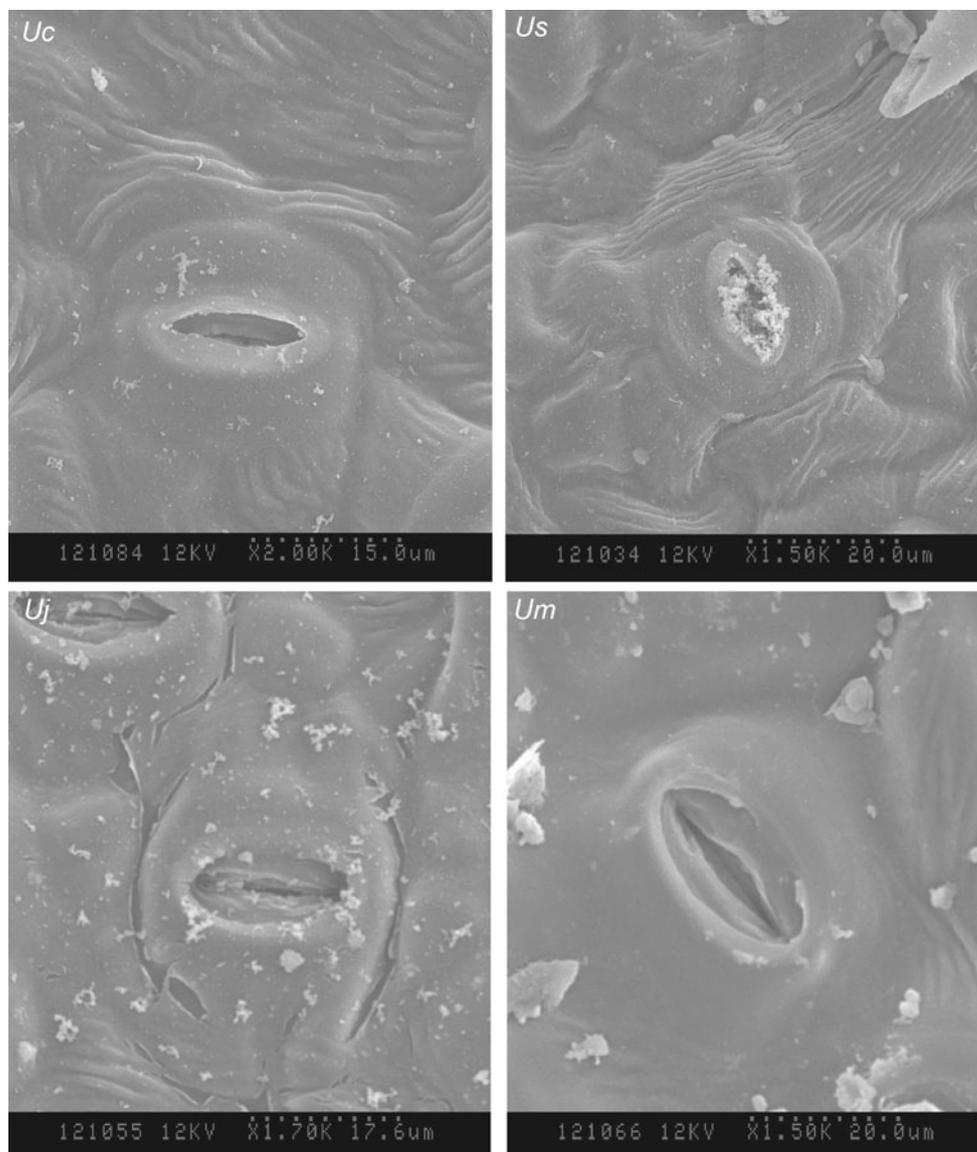
Mean  $\pm$  SE ( $n = 30$ ). The different letters indicate a significant difference ( $P < 0.05$ ) in a least significant difference test for age classes within each column

Um were no longer large (Fig. 3). Uc had such a high daily maximum  $A$  that it should be a positive adaptation to water use. Uc maximized its photosynthetic activities when light and temperature were suitable in the morning, but quickly down-regulated when high light and temperature would induce rapid consumption of water at midday (Fig. 4). Increasing growth should be of primary importance for seedlings because they have to reach deeper water stores as soon as possible by allocating more biomass to roots (Donovan and Ehleringer 1992). Decreased  $g_s$  at midday could slow additional water loss and consequently protect plants from xylem dysfunction (Zhang et al. 2013). In contrast, Um maintained its gas exchange both in the morning or at midday (Fig. 4), which may be a result of its stable water supply (Fig. 2) and relatively large non-structural carbon stocks (Hoch et al. 2003). *Acer*

*saccharum* trees of different sizes have also been reported to have various transpiration rates caused by differential access of large and small trees to groundwater and soil water, respectively (Dawson 1996). Both  $A$  and  $E$ , at both daily maximum and midday, were positively correlated with  $g_s$ , which indicated that *U. pumila* used stomatal regulation (Fig. 5).

Significantly higher instantaneous WUE in the morning was found in Um when compared with Uc (Fig. 3d).  $\Delta$ WUE of Uc was significantly smaller than Um (Fig. 4d), which indicated the flexibility to adjust WUE increased from seedlings to mature trees. Higher instantaneous WUE in mature individuals relative to seedlings has been found by other researchers (Cavender-Bares and Bazzaz 2000; Donovan and Ehleringer 1992; Tanaka-Oda et al. 2010a). Both Uj and Um plants have higher long-term WUE than

**Fig. 7** Representative scanning electron microscopy (SEM) images of stoma located in the lower leaf surface of current-year seedlings (Uc), 2- to 5-year-old saplings (Us), juveniles (Uj) and mature (Um) *Ulmus pumila*



seedlings and saplings (Uc and Us), as indicated by higher  $\delta^{13}\text{C}$  values (Fig. 6). Donovan and Ehleringer (1992) suggested that a lower WUE may be favored by seedlings during establishment, which helps them to increase allocation to roots and increase the probability of reaching deeper water stores sooner, thereby increasing the chances of survival in a water-limited environment. These results agree with the results from gas exchange data. However, a high WUE helps mature trees to maintain high C uptake rates while preventing excessive water loss (Cavender-Bares and Bazzaz 2000). Maintaining a high level of WUE was considered to be conservative strategy (DeLucia and Heckathorn 1989).

Leaf structural characteristics can influence gas exchange through their effects on light absorption, carbon fixation and the control of water loss (Woodruff et al. 2009). Larger leaves and smaller SLA were found as *U. pumila* plants aged (Table 4). A development of low SLA is considered to be a conservative strategy for reducing water stress (Tanaka-Oda et al. 2010b). The narrow leaf angles in Uj (Table 4) may reduce the risk of over-heating and photoinhibition (Valiente-Banuet et al. 2011), help the plants to maintain increased levels of photosynthesis during high radiation periods (Falster and Westoby 2003), and ultimately help plants to increase WUE (King 1997). The seedlings (Uc) and the saplings (Us) had developed a relatively large angle to absorb light as much as possible for immediate photosynthesis and metabolism, which matches the findings related to their gas exchange rate and WUE.

Plant epidermis is a multifunctional tissue playing important roles in water relations (Glover 2000) and epidermis has pubescence, stomata and wax to prevent excessive water loss (Abdulrahman and Oladele 2011). Leaf pubescence was densest in Uj and successively lower in Uc, Us and Um (Table 5). We presumed that *U. pumila* is adapted to a water-limited environment starting with morphological adaptations as seen in Uj. Stomatal density and proportion of stomatal area to leaf area of Uj were also significantly higher than in other age classes (Table 5). Stomata density and distribution may affect gas exchange quite remarkably (Nilson and Assmann 2007), as can be seen in the results of this study (Fig. 5). Our results showed that both stomatal density and size may have a higher plasticity in Uj than other age classes in response to water stress (Table 5). An earlier report has shown an increase in stomatal density under water deficit, indicating that an adaptation to drought could occur (Martínez et al. 2007). Nevertheless, different effects of abiotic factors on stomatal size may depend on plant species (Maherali et al. 2002). A greater stomatal size, which can facilitate the diffusion of  $\text{CO}_2$  into leaves (Parkhurst 1994) and lead to an increased  $g_s$  (Maherali et al. 2002), was found with advancing age class (Table 5). Many studies have shown

that a water deficit leads to an increase in stomatal density (Xu and Zhou 2008) and a decrease in stomatal size, indicating this may enhance the adaptation of plants to drought (Martínez et al. 2007). More wax and sunken stoma were found from Uc to Um (Fig. 7), which further indicates that those age classes have a stronger capacity to decrease water loss.

## Conclusions

In conclusion, we found that significantly different water-use strategies developed as seedlings matured into mature *U. pumila* trees, with differently aged plants using different water sources. For seedlings and saplings, water is a transient resource. A “go for broke” strategy is probably practiced by seedlings which exhibit higher rates of photosynthesis and transpiration once the conditions improve, as they try to provide the growth potential that they need for establishment, but with a high risk of mortality. For Um, which are able to access groundwater, a conservative strategy is probably used by regulating WUE effectively and maintaining stable gas exchange rates. For Uj, an adaptive strategy tends to be used, as plants attempt to endure drought condition by developing a variety of adaptive traits, involving minimizing water loss and maximizing water uptake. Water loss is minimized by closing stomata; by reducing light absorbance through dense pubescence which increases reflectance, or by the use of steep leaf angles or by covering the leaf surfaces with wax. Water uptake is maximized by increasing the investment in the roots.

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