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Differential drought tolerance in tree populations from contrasting elevations

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ABSTRACT

To predict the ecological consequences of climate change for a widely distributed tree species, it is essential to develop a deep understanding of the ecophysiological responses of populations from contrasting climates to varied soil water availabilities. In the present study, we focused on Pinus tabuliformis, one of the most economically and ecologically important tree species in China. In a greenhouse experiment, we exposed trees from high-elevation (HP) and low-elevation (LP) populations to low (80% of field capacity, FC), mild (60% FC), moderate (40% FC) and severe (20% FC) water stresses. Leaf gas exchange, biomass production and allocation, as well as water use efficiency were measured during the experiment. Increasing soil water stress clearly decreased the relative growth rate (RGR), total dry mass (TDM), light-saturated photosynthetic rate ($A_{\text{sat}}$), stomatal conductance ($g_s$), total water use (TWU) and whole-plant water use efficiency ($\text{WUE}_{WP}$). In contrast, intrinsic water use efficiency ($\text{WUE}_i$) and carbon isotope composition ($\delta^{13}\text{C}$) both increased significantly with increasing soil water stress for both populations. Only in the LP did the root/shoot ratio (R/S ratio) significantly increase when the water stress increased. A strong positive correlation between $A_{\text{sat}}$ and $g_s$ coupled with a reduced intercellular CO$_2$ concentration ($C_i$) probably suggested stomatal limitations were the main cause for the decreased $A_{\text{sat}}$. However, all the measured variables from the HP were affected less by drought compared to those of the LP, and most aspects of the HP were canalized against drought stress, which was reflected by the relatively higher RGR, TDM and $\text{WUE}_{WP}$. Overall, the results suggest that the two populations responded differentially to drought stress with the HP showing higher drought tolerance than the LP, which was reflected by its faster seedling growth rate and more efficient water use under drought conditions.

Keywords: Carbon isotope composition; drought tolerance; growth; leaf gas exchange; Pinus tabuliformis; water use efficiency.
INTRODUCTION

Water availability is a crucial factor that limits the growth, development and distribution of all plants (Chaves et al. 2003; Ordoñez et al. 2009; Wu et al. 2010), and its importance will only become more pronounced in the future due to human-caused climate change resulting in more frequent and severe drought events (IPCC 2007). Therefore, to predict the ecological consequences of climate change on widely distributed tree species, detailed knowledge on their ability to cope with varied water availability is needed within and among populations.

Low water availability (drought) affects the performance of plants by affecting their morphological, physiological and biochemical, as well as transcriptomic and proteomic processes (Anyia and Herzog 2004; González-Rodríguez et al. 2005; Dias et al. 2007; Gao et al. 2009; Foito et al. 2009; Ma et al. 2010; Tomlinson et al. 2012). A gradual depletion of the soil water leads to the stomatal ($g_s$) and mesophyll ($g_m$) conductance being reduced, and this is believed to be the primary drought stress response (Flexas et al. 2008; Chaves et al. 2009; Galmés et al. 2011; Warren et al. 2011), which causes the water loss to be reduced, but this also results in the rate of photosynthesis being reduced due to reduced CO$_2$ in the chloroplasts (Flexas et al. 2008). Photosynthesis can be further limited by metabolic impairment due to increasing drought stress (Flexas et al. 2008). Drought can also lead to reduced growth and biomass production, while also altering the allocation pattern of biomass (Erice et al. 2010).

Water use efficiency (WUE) is one of the most important indicators for evaluating the tolerance of plants to water stress (Kozlowski and Pallardy 1997). At the leaf level, WUE can be defined as the ratio of the net photosynthetic rate ($A_n$) to $g_s$ (WUE$_i$, intrinsic water use efficiency), and an integrated measurement of WUE, can be reliably assessed by the carbon isotope composition ($\delta^{13}C$, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared with the air) as it has a linear relationship with the intercellular to ambient CO$_2$ ratio ($C_i/C_a$) (Farquhar et al. 1989; Brodribb and Hill 1998). Factors that affect $g_s$ and $g_m$ can thus influence $C_i$ and subsequently the relationship between WUE$_i$ and $\delta^{13}C$ (Flexas et al. 2008; Seibt et al. 2008; Fleck et al. 2010). WUE
at the whole-plant level, defined as the ratio of actual dry matter production to water consumption (WUE\textsubscript{wp}), represents a large spatial (whole plant) and temporal scale (whole growth period) water use that is closely associated with the physiological processes of plants, such as photosynthesis, respiration and transpiration (Flexas et al. 2010). If a plant has a greater WUE it is expected to be able to survive environments that are more arid better than a plant with a lower WUE (Jones 1992; Ares et al. 2000; Franco et al. 2005).

*Pinus tabuliformis* is an endemic pine species from China that is one of the most economically and ecologically important tree species in the northern of the country and covers a total area of $228.10 \times 10^4$ ha. Particularly in the arid and semi-arid areas, it plays an important role in reforestation (Zhao and Zhou 2005). Due to its wide geographical distribution and long life span, populations of *P. tabuliformis* are exposed to a wide variety of drought stresses that has likely led to the adaptation of natural populations to locally distinct environments. Therefore, determining how populations have adapted to varied soil water conditions will enable a greater understanding of past differentiation while also enabling better forest management and restoration in the future (Yong et al. 2000). In the present study, two populations of *P. tabuliformis* from contrasting elevations were selected and subjected to a gradient of soil water contents, due to the species having occurring over a wide range of elevations from 100 to 2800 m above sea level (Chen et al. 2008). Relative to populations growing at lower elevations, tree populations from higher elevations generally exhibit reduced growth, smaller and thicker leaves, higher leaf nutrient content per unit area, higher fine root production and higher allocation of biomass to roots (Oleksyn et al. 1998; Körner 1999; Zhao et al. 2008; Bresson et al. 2011; Petit et al. 2011). The differentiation in these physiological and morphological traits has been thought to be an adaptation to enhance photosynthesis and water use efficiency while increasing the resistance to the limited water availability (Oleksyn et al. 1998; Körner 1999; Bresson et al. 2011). Therefore, we expected that the two populations would show differential responses to varied soil water availabilities, with the population from the high-elevation having a higher drought tolerance than the low-elevation
population, which would result in a higher growth rate, biomass production and water use efficiency under limited water conditions.

METHODS

Plant material and experimental design
Seeds of *P. tabuliformis* for use in the present study were collected from two locations: Xiahe (35°33.85’ E, 102°13.60’ N, 2,810 m Alt.; high-elevation, HP) and Zhengning (35° 31.18’ E, 108° 29.51’ N, 1,444 m Alt.; low-elevation, LP). The corresponding mean annual rainfall (MAR) values in the two areas are 516 and 623 mm, while the mean annual temperatures (MAT) are 3.6 and 9.6 °C, respectively. These seeds were germinated and grown indoors for 1 yr in a tree nursery, and 112 seedlings of each population with no statistical differences in height and size were transferred to Yuzhong, Gansu Province (35°56.61’ N; 104°09.07’ E; 1,750 m Alt.), and immediately replanted into 6-l plastic pots (28 pots, four seedlings per pot) filled with the same weight of a homogeneous mixture (peat and perlite, 1:1 by volume). Another 12 pots were prepared in the same way but without seedlings and these were used to determine the evaporation of water from the soil. The soil surface in all the pots was covered with a small quantity (c. 2 cm) of Perlite to minimize evaporation. The maximum field capacity (FC) for watering the pots was determined gravimetrically according to Shou et al. (2004) with some modifications. All pots were periodically watered to field capacity for two months after repotting to allow the seedlings to become established. The seedlings were grown for the rest of the study in a canopied and naturally lit glasshouse, whose roof was closed at night and on rainy days, but opened during any day it was not raining. The sides of the glasshouse were always open for aeration during the whole experiment, so that the temperature inside the glasshouse was closely linked to the ambient outside temperature.

For each population 20 pots were selected and divided into four lots of five pots each (low, mild, moderate and severe water stress treatments). The remaining pots were used to determine the initial biomass. Water stress treatments were achieved by watering to 80% of maximum FC,
60% FC, 40% FC and 20% FC. All water stress treatments reached the target FC in seven days from the beginning of the experiment. Soil water content was maintained by weighing the pots every two days, recording the water loss and re-watering to the designated water level immediately. The soil water contents before and after watering were maintained at 54-60%, 45-50%, 34-40% and 22-25% for the treatments respectively. The experiment lasted for 134 days from July to November, and during the whole experiment no fertilizer was added at any point and no plants died.

**Leaf gas exchange**

On three sunny days (15th August, 15th September and 15th October) during the experiment, the light-saturated photosynthetic rate ($A_{sat}$), stomatal conductance ($g_s$) and intercellular CO$_2$ concentration ($C_i$) were measured on sun-adapted needles using an LI-COR 6400 infrared gas-analyzer (IRGA, LI-COR Lincoln, NE, USA). The light level was maintained at 1500 μmol m$^{-2}$ s$^{-1}$ using a LI-6400-02B LED light source (10% blue light) and the external CO$_2$ concentration was maintained at 370 μmol mol$^{-1}$ using a CO$_2$ injector (LI-6400-01). The ambient and internal temperatures and vapor pressure deficits (VPD) were 31.03 ± 1.18 °C, 3.18 ± 0.53 kPa and 31.50 ± 0.11 °C, 3.35 ± 0.20 kPa on 15 August, 27.00 ± 1.08 °C, 2.52 ± 0.17 kPa and 27.67 ± 0.35 °C, 2.83 ± 0.32 kPa on 15 September and 21.30 ± 0.83 °C, 2.20 ± 0.17 kPa and 21.89 ± 0.22 °C, 2.31 ± 0.14 kPa on 15 October, respectively. At least four replicates for each treatment per population were measured and measurements of two individual seedlings in one pot were considered as one replicate. Needles were marked and cut after the last measurement for area determination using a LI-COR-3000A planimeter (LI-COR Lincoln, NE, USA). The intrinsic water use efficiency (WUE$_i$) was defined as the ratio of $A_{sat}$ to $g_s$. The mean values of $A_{sat}$, $g_s$, $C_i$ and WUE$_i$ measured on three days are presented in this paper.

**Growth and water use**

Due to possible within pot effects, such as completion for resources, each pot was considered to be a single replicate with the four seedlings’ measurements being combined for determining the growth and water use. To estimate the biomass production during the experiment, three pots (12
seedlings) from each population at the beginning of the experiment \((t_1)\) and four pots \((16\) seedlings) at the end of the experiment \((t_2)\) were harvested. From each pot, the four seedlings were bulked together and divided into three parts: leaves, stems and roots. The three biomass parts were dried for \(48\) h at \(80\) °C in an oven, weighted and then the weights were divide by four to determine per plant values from the per pot values. The relative growth rate \((RGR)\) was calculated using the following formula: \(RGR = (\ln W_2 - \ln W_1)/(t_2-t_1);\) where \(W_1\) and \(W_2\) are the dry weight per plant at day \(t_1\) and day \(t_2.\) The R/S ratio was also calculated. The WUE at the whole-plant level was calculated as \(WUE_{wp}/\) per plant = \((W_2- W_1)/ T,\) where \(T\) is the total transpired water use per plant \((TWU)\) between \(t_1\) and \(t_2.\)

**Carbon isotope composition**

The oven dried needle samples were finely ground with a Tissuelyzer \((\text{Retsch, Haan, Germany}),\) and the carbon isotope composition of the needles \((\delta^{13}C)\) was determined by combusting the samples in an elemental analyzer \(\text{EA1108 (Carlo Erba, Milano, Italy)}\) coupled to a Finnigan Delta Plus isotope mass spectrometer \((\text{Thermo Finnigan MAT GmbH, Bremen, Germany})\) at the Key Laboratory of Western China’s Environmental Systems \((\text{Ministry of Education}, \text{Lanzhou University}).\) The carbon isotope composition was calculated relative to the Pee Dee Belemnate \((\text{PDB})\) standard as the ratio \((\%)\): \(\delta^{13}C = [(R_{\text{sample}}/ R_{\text{standard}})-1] 	imes 1,000,\) where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are the ratios of \(^{13}C/^{12}C\) in the sample and the standard, respectively.

**Statistical analyses**

The variables including \(\text{LDM, SDM, RDM, TDM, RGR, R/S ratio, TWU, WUE}_{wp}\) and \(\delta^{13}C\) were analyzed using the General Linear Model \((\text{Proc GLM})\) to test the effect of the populations, water treatments and their interactions. Leaf gas exchange parameters, including \(A_{\text{sat}}, g_\text{s}, C_\text{i}\) and \(\text{WUE}_i,\) were analyzed by the GLM with the measurement time as a covariate. When the differences were significant, a multiple comparison of means \((\text{post hoc Tukey’s Honestly Significant Difference test})\) was carried out. Before the statistical tests were performed using the SPSS software package \((\text{SPSS Inc., Chicago, IL, USA}),\) the homogeneity of the data was determined.
RESULTS

Plant growth, biomass production and allocation

As the available soil water decreased, the dry mass of leaves (LDM), stems (SDM) and roots (RDM) decreased in both populations, which lead to a decrease in total dry mass (TDM); RGR was also reduced (Table 1, Fig. 1). Compared with the seedlings exposed to the low water stress, the severe water stress resulted in a significant decrease in the TDM by 38% and 82% and the RGR by 26% and 71% for the HP and LP, respectively (Table 1, Fig. 1). The values of the RGR and TDM were higher in the HP than those in the LP across the mild, moderate and severe stress treatments (Table 1, Fig. 1). The dry mass allocation differed significantly between the HP and LP as the water stress increased (Fig. 1). The R/S ratio increased by a factor of 1.54 for the LP from low to severe water stress, but there were only slight changes between the low water stress and the other three treatments in the HP (Fig. 1). The interactions between the populations and treatments for these variables were also highly significant (Table 2).

Leaf gas exchange

An increased water stress resulted in a significantly reduced $A_{\text{sat}}$, $g_s$ and $C_i$ in both populations (Fig. 2, Table 2). However, the reductions in $A_{\text{sat}}$, $g_s$ and $C_i$ followed different patterns for the different populations investigated. Much of the decline of $A_{\text{sat}}$, $g_s$ and $C_i$ occurred under severe water stress in the HP, but for the LP the declines were more gradual (Fig. 2). Severe water stress decreased the $A_{\text{sat}}$ by 27% and 39%, $g_s$ by 36% and 52% and $C_i$ by 22% and 27% for the HP and LP, respectively. The greater decreases in $g_s$ compared to $A_{\text{sat}}$ led to a 15% and 22% increase in the WUE$_i$ for the HP and the LP, respectively (Fig. 2). The effects of the populations, treatments and their interactions were also significant on these variables (Table 2). In addition, for both populations, there were strong positive correlations for the $A_{\text{sat}}$ and $g_s$ variables (Fig. 3).

Water use traits

TWU and WUE$_{WP}$ both decreased significantly with decreasing soil water content (Table 1). From the low to moderate stress, a decline in WUE$_{WP}$ was observed in both populations. Severe water
stress saw a further decrease in the LP but an increase in the HP. However, the HP exhibited a higher WUE_{wp} than the LP in all the water level treatments and significant differences were observed in the low and severe stress treatments (Table 1). The δ^{13}C gradually increased as the water stress increased in the LP, while only the severe water stress induced an increase in the δ^{13}C for the HP (Fig. 4). The interactions between the populations and treatments for these three variables were also highly significant (Table 2).

**DISCUSSION**

Water availability as a growth-limiting factor was demonstrated in the present study, as it caused significant reductions in RGR, TDM, LDM, SDM and RDM in both populations (Table 1, Fig. 1). Comparatively, the HP showed a higher RGR and TDM from the mild to severe water stress treatments than the LP, and the differences were highly significant (Table 1, Fig. 1). These results support previously published work that various growth responses within and between species were due to drought stress (Bacelar et al. 2007; Bruschi 2010; Ma et al. 2010). Research has also revealed that plants with higher drought tolerance exhibit less growth inhibition and had relatively higher growth and biomass production than drought sensitive ones (Loggini et al. 1999; Türkan et al. 2005). Therefore, these results suggested a higher capacity for the HP than for the LP to sustain growth and production under water-limited conditions.

Drought affects plant growth by influencing the leaf gas exchange rates (Zhang and Marshall 1994; Bacelar et al. 2007; Ma et al. 2010; Sapeta et al. 2013). A reduction in g_{s} and g_{m} as well as metabolic impairment are considered to be the main causes of the depression of photosynthesis in the face of drought stress (Flexas et al. 2008). Accordingly, the g_{s} and A_{sat} of the two populations significantly decreased after exposure to drought stress, and A_{sat} was strongly positively correlated with g_{s} (Fig. 2). From this it was possible to surmise that stomatal closure caused by drought stress resulted in the A_{sat} being reduced under drought conditions (Fig. 3), and the C_{i} in both populations being reduced at the same time supports this conclusion (Michelozzi et al. 2011). However, compared to the gradual decrease of g_{s} and A_{sat} in the LP, only severe water
stress induced significant reductions in those two parameters in the HP. Even under extreme water stress conditions, the HP had higher $g_s$ and $A_{sat}$ values than the LP (Fig. 2). These results indicated that the leaf gas exchange in the two populations responded differently to the drought conditions, and that the apparent ability of the HP to maintain higher photosynthetic rates may allow it to grow more rapidly under water-limited conditions. This conclusion is supported by the above results that the HP exhibited a higher growth rate and biomass production than the LP under water-limited conditions (Table 1, Fig. 1).

The WUE$_i$ and $\delta^{13}$C significantly increased in both populations with decreasing water availability, and the WUE$_i$ was positively correlated with the $\delta^{13}$C (Fig. 3), which was similar to the results of previous studies (Farquhar et al. 1989; Jones 1993; Zhang and Marshall 1994). The WUE$_i$ and $\delta^{13}$C of the LP gradually increased from the low to severe water stresses, whereas these two parameters for the HP only showed significant increases under the severe stress treatment (Table 2). The higher WUE$_i$ and $\delta^{13}$C values in the LP than in the HP under the mild, moderate and severe water stress treatments were mainly due to the relatively small changes of $A_{sat}$ and $g_s$ in the HP under the drought conditions (Table 2). These findings support the hypothesis that populations will be less plastic if they come from an environment that is dry (Volis et al. 2002; Heschel et al. 2004). Aranda et al. (2010) also reported lower plasticity to environmental changes in high-elevation populations than in low-elevation ones.

With respect to the WUE at the whole plant level, the WUE$_{wp}$ showed an opposite trend to the WUE$_i$ and $\delta^{13}$C, with both populations recording a significant drop between the low and moderate stress treatments, and a further significant drop between the moderate and severe treatments for the LP (Table 1). These findings confirmed previous observations by Tomás et al. (2014) and Flexas et al. (2010) that there are large discrepancies when scaling-up WUE measurements from the leaf to the whole-plant level. Several structural and physiological processes, such as canopy structure, transpiration by plant organs other than leaves, respiration by leaf during the night and by stem and root during the whole day, will lead to a decrease in the WUE$_{wp}$, but not influence the leaf-level estimates. However, the HP showed a significantly higher
$WUE_{WP}$ than the LP in all water treatments (Table 1), which indicates a higher potential to survival water-limited conditions by efficient water use (Jones 1992).

It is widely accepted that a reduced water supply will result in an increased partitioning of biomass in favor of root growth (Fernández and Reynolds 2000; Khurana and Singh 2004; Nagakura et al. 2004), but not all studies have found this (Osório et al. 1998; Tomlinson et al. 2012). Curiously, in the current study, an increase in the R/S ratio was evident in the LP, whereas in the HP there was no detectable change, which indicates that a loss of plasticity for this character might have been an advantage for existence at higher elevations (Sobrado and Turner 1986; Aranda et al. 2010)

CONCLUSIONS

This study indicated that increasing water stress had a significant effect on leaf gas exchange, biomass production and allocation, carbon isotope composition and water use efficiency in both high-elevation and low-elevation populations. However, the two populations differed significantly in their responses to drought stress: the HP appeared to be less affected by water stress than the LP as far as the examined variables were concerned, as well as the exhibited TDM, RGR and $WUE_{L}$ in the stress treatments. The results supported the hypothesis that there would be different drought tolerance levels in the two populations with the HP having a greater tolerance.

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CONTRIBUTIONS BY AUTHORS

The research design and preparation of the manuscript are credited to F. Ma. T.T. Xu contributed to the data collection and analysis. M.F. Ji mainly contributed to the seedling cultivation. C.M. Zhao contributed to conception of the study and suggestions for writing the manuscript.

CONFLICTS OF INTEREST

No conflicts of interest.

ACKNOWLEDGEMENTS

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LITERATURE CITED


FIGURE LEGENDS

Figure 1. Relative growth rate (RGR) and root/shoot ratio (R/S ratio) in two populations of *Pinus tabuliformis* from a high-elevation (HP, black bars) and a low-elevation (LP, white bars) under various soil water conditions (80% of maximal field capacity (FC), 60% FC, 40% FC and 20% FC). Bars represent mean ± SE. The letters indicate statistical differences (P < 0.05) for the water treatments, populations and the interactions between them.

Figure 2. Means of light-saturated photosynthetic rate ($A_{sat}$), stomatal conductance ($g_s$), intercellular CO$_2$ concentration ($C_i$) and intrinsic water use efficiency (WUE$_i$) measured on three days (Aug 15$^{th}$, Sept 15$^{th}$ and Oct 15$^{th}$) in two populations of *Pinus tabuliformis* from a high-elevation (HP, black bars) and a low-elevation (LP, white bars) under various soil water conditions (80% of maximal field capacity (FC), 60% FC, 40% FC and 20% FC). Each point represents mean ± SE. The letters indicate statistical differences (P < 0.05) for the water treatments, populations and the interactions between them.

Figure 3. Relationships between light-saturated photosynthetic rate ($A_{sat}$) and stomatal conductance ($g_s$) as well as between intrinsic water use efficiency (WUE$_i$) and carbon isotope composition ($\delta^{13}C$) in two populations of *Pinus tabuliformis* from a high-elevation (HP, filled circles) and a low-elevation (LP, empty circles) across water treatments. The coefficient of determination ($R^2$) and significance are shown for each regression.

Figure 4. Carbon isotope composition ($\delta^{13}C$) in two populations of *Pinus tabuliformis* from a high-elevation (HP, black bars) and a low-elevation (LP, white bars) under various soil water conditions (80% of maximal field capacity (FC), 60% FC, 40% FC and 20% FC). Each point represents mean ± SE. The letters indicate statistical differences (P < 0.05) for the water treatments, populations and the interactions between them.
Water treatments

80%FC  60%FC  40%FC  20%FC

Saturated photosynthetic rate

μmol CO₂ m⁻² s⁻¹

0  2  4  6  8  10  12  14

0  20  40  60  80  100  120  140  160  180  200

Intrinsic water use efficiency

(μmol CO₂ mol⁻¹ H₂O)

Stomatal conductance

mol CO₂ m⁻² s⁻¹

0.00  0.02  0.04  0.06  0.08  0.10

Intercellular CO₂ concentration

(μmol mol⁻¹)

0  50  100  150  200

Intrinsic water use efficiency

(μmol CO₂ mol⁻¹ H₂O)

0  20  40  60  80  100  120  140  160  180  200

Water treatments

80%FC  60%FC  40%FC  20%FC

Water treatments

80%FC  60%FC  40%FC  20%FC

Water treatments

80%FC  60%FC  40%FC  20%FC

Water treatments
A: $R^2 = 0.965, P = 0.018$

B: $R^2 = 0.876, P = 0.063$

$A_{sat}$ (mol CO$_2$ m$^{-2}$ s$^{-1}$)

\[ \begin{array}{c|c|c|c|c|c|c}
\text{gs} & 0.04 & 0.06 & 0.08 & 0.10 \\
\hline
\text{Asat} & 6 & 8 & 10 & 12 \\
\hline
\end{array} \]

$R^2 = 0.962, P = 0.019$

$R^2 = 0.829, P = 0.089$

$R^2 = 0.876, P = 0.063$

$R^2 = 0.829, P = 0.089$

WUE

\[ \begin{array}{c|c|c|c|c|c|c}
\delta^{13}C & -27.6 & -27.2 & -26.8 & -26.4 & -26.0 & -25.6 \\
\hline
WUE & 120 & 130 & 140 & 150 & 160 & 170 \\
\hline
\end{array} \]
Table 1. Growth, biomass production and allocation as well as water use of *Pinus tabulaeformis* from a high-elevation (HP, black bars) and a low-elevation (LP, white bars) under various soil water conditions (80% of maximal field capacity (FC), 60% FC, 40% FC and 20% FC). Each point represents mean ± SE. The letters indicate statistical differences (P < 0.05) for the water treatments, populations and the interactions between them.

<table>
<thead>
<tr>
<th>Water treatments</th>
<th>80% FC</th>
<th>60% FC</th>
<th>40% FC</th>
<th>20% FC</th>
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<tbody>
<tr>
<td>Leaf dry mass (LDM) (g)</td>
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<tr>
<td>HP</td>
<td>2.00 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 0.26&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.20 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.67 ± 0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>LP</td>
<td>2.02 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Stem dry mass (SDM) (g)</td>
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<tr>
<td>HP</td>
<td>1.23 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.83 ± 0.11&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.82 ± 0.16&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.59 ± 0.07&lt;sup&gt;cd&lt;/sup&gt;</td>
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<tr>
<td>LP</td>
<td>1.44 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.70 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.18 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Root dry mass (RDM) (g)</td>
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<tr>
<td>HP</td>
<td>2.33 ± 0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.46 ± 0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.93 ± 0.15&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.19 ± 0.08&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP</td>
<td>2.29 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.46 ± 0.04&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.64 ± 0.03&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.72 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Total dry mass (TDM) (g)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>5.55 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.10 ± 0.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.95 ± 0.33&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.45 ± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP</td>
<td>5.75 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.54 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.38 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.04 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total water use (TWU) (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>1.34 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.53 ± 0.14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.63 ± 0.08&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.85 ± 0.04&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP</td>
<td>1.79 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.25 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.57 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.85 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole-plant water use efficiency (WUE&lt;sub&gt;wp&lt;/sub&gt;) (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HP</td>
<td>4.13 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.42 ± 0.09&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.00 ± 0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>LP</td>
<td>3.21 ± 0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.86 ± 0.29&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.15 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.21 ± 0.03&lt;sup&gt;e&lt;/sup&gt;</td>
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</table>
Table 2. Comparison of all measured variables measured in the experiment.

The *P*-values are presented for the watering treatments, populations and their interactions. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment (T)</th>
<th>Population (P)</th>
<th>T × P</th>
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<tr>
<td>LDM</td>
<td>20.52***</td>
<td>26.86***</td>
<td>10.45***</td>
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<tr>
<td>SDM</td>
<td>32.73***</td>
<td>1.36</td>
<td>3.67*</td>
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<tr>
<td>RDM</td>
<td>44.33***</td>
<td>27.00***</td>
<td>5.55**</td>
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<tr>
<td>TDM</td>
<td>38.92***</td>
<td>22.84***</td>
<td>6.32**</td>
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<td>RGR</td>
<td>49.63***</td>
<td>47.07***</td>
<td>11.31***</td>
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<tr>
<td>R/S ratio</td>
<td>27.68***</td>
<td>33.12***</td>
<td>57.88***</td>
</tr>
<tr>
<td>A&lt;sub&gt;sat&lt;/sub&gt;</td>
<td>101.09***</td>
<td>69.00*</td>
<td>2.57***</td>
</tr>
<tr>
<td>g&lt;sub&gt;s&lt;/sub&gt;</td>
<td>102.77***</td>
<td>131.99***</td>
<td>13.88***</td>
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<tr>
<td>C&lt;sub&gt;i&lt;/sub&gt;</td>
<td>103.80***</td>
<td>253.01***</td>
<td>12.06***</td>
</tr>
<tr>
<td>WUE&lt;sub&gt;i&lt;/sub&gt;</td>
<td>37.55**</td>
<td>146.34***</td>
<td>13.49***</td>
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<tr>
<td>TWU</td>
<td>36.10***</td>
<td>0.194</td>
<td>6.97**</td>
</tr>
<tr>
<td>WUE&lt;sub&gt;L&lt;/sub&gt;</td>
<td>35.67***</td>
<td>119.75***</td>
<td>12.23***</td>
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<tr>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>39.11***</td>
<td>25.84***</td>
<td>7.51**</td>
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