

## Research Article

# 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 tabulaeformis*, 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 ( $WUE_{\text{WP}}$ ). In contrast, intrinsic water-use efficiency ( $WUE_i$ ) 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  $\text{CO}_2$  concentration ( $C_i$ ) probably suggested that stomatal limitations were the main cause of the decreased  $A_{\text{sat}}$ . However, all the measured variables from the HP were affected less by drought compared with 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  $WUE_{\text{WP}}$ . 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 tabulaeformis*; 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

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of climate change on the 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; Foito et al. 2009; Gao 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 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 (Eric et al. 2010).

Water-use efficiency (WUE) is one of the most important indicators for evaluating the tolerance of plants to water stress (Kozłowski 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  $WUE_i$  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 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). Water-use efficiency at the whole-plant level, defined as the ratio of actual dry matter production to water consumption ( $WUE_{wp}$ ), 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 which is one of the most economically and ecologically important tree species in the northern part of the country and covers a total area of  $228.10 \times 10^4$  ha. Particularly in 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 occurred 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 (HP) having a higher drought tolerance than the low-elevation population (LP), 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 ( $35^{\circ}33.85'E$ ,  $102^{\circ}13.60'N$ , 2810 m Alt.; HP) and Zhengning ( $35^{\circ}31.18'E$ ,  $108^{\circ}29.51'N$ , 1444 m Alt.; LP). The corresponding mean annual rainfall values in the two areas are 516 and 623 mm, while the mean annual temperatures (MATs) are 3.6 and 9.6 °C, respectively. These seeds were germinated and grown indoors for 1 year in a tree nursery, and 112 seedlings of each population with no statistical differences in height and size were transferred to Yuzhong, Gansu Province ( $35^{\circ}56.61'N$ ;  $104^{\circ}09.07'E$ ; 1750 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 FC for 2 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, the roof of which 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 outside ambient 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 7 days from the beginning of the experiment. Soil water content was maintained by weighing the pots every 2 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 3 sunny days (15 August, 15 September and 15 October) during the experiment, the light-saturated photosynthetic rate ( $A_{\text{sat}}$ ), stomatal conductance ( $g_s$ ) and intercellular  $\text{CO}_2$  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  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  using an LI-6400-02B LED light source (10 % blue light) and the external  $\text{CO}_2$  concentration was maintained at  $370 \mu\text{mol mol}^{-1}$  using a  $\text{CO}_2$  injector (LI-6400-01). The ambient and internal temperatures and vapour pressure deficits were  $31.03 \pm 1.18 \text{ }^\circ\text{C}$ ,  $3.18 \pm 0.53 \text{ kPa}$  and  $31.50 \pm 0.11 \text{ }^\circ\text{C}$ ,  $3.35 \pm 0.20 \text{ kPa}$  on 15 August;  $27.00 \pm 1.08 \text{ }^\circ\text{C}$ ,  $2.52 \pm 0.17 \text{ kPa}$  and  $27.67 \pm 0.35 \text{ }^\circ\text{C}$ ,  $2.83 \pm 0.32 \text{ kPa}$  on 15 September and  $21.30 \pm 0.83 \text{ }^\circ\text{C}$ ,  $2.20 \pm 0.17 \text{ kPa}$  and  $21.89 \pm 0.22 \text{ }^\circ\text{C}$ ,  $2.31 \pm 0.14 \text{ kPa}$  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 an LI-COR-3000A planimeter (LI-COR, Lincoln, NE, USA). The  $\text{WUE}_i$  was defined as the ratio of  $A_{\text{sat}}$  to  $g_s$ .

The mean values of  $A_{\text{sat}}$ ,  $g_s$ ,  $C_i$  and  $\text{WUE}_i$  measured on 3 days are presented in this paper.

### Growth and water use

Due to possible within pot effects, such as competition 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  $80 \text{ }^\circ\text{C}$  in an oven, weighted and then the weights were divided by four to determine per plant values from the per pot values. The relative growth rate (RGR) was calculated using the following formula:  $\text{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ , where  $W_1$  and  $W_2$  are the dry weights per plant at Day  $t_1$  and Day  $t_2$ . The root/shoot (R/S) ratio was also calculated. The  $\text{WUE}$  at the whole-plant level was calculated as  $\text{WUE}_{\text{WP}} \text{ per plant} = (W_2 - W_1)/T$ , 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 (Retsch, Haan, Germany), and the carbon isotope composition of the needles ( $\delta^{13}\text{C}$ ) was determined by combusting the samples in an elemental analyser EA1108 (Carlo Erba, Milano, Italy) coupled to a Finnigan Delta Plus isotope mass spectrometer (Thermo Finnigan MAT GmbH, Bremen, Germany) at the Key Laboratory of Western China's Environmental Systems (Ministry of Education), Lanzhou University. The carbon isotope composition was calculated relative to the Pee Dee Belemnite (PDB) standard as the ratio (‰):  $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the ratios of  $^{13}\text{C}/^{12}\text{C}$  in the sample and the standard, respectively.

### Statistical analyses

The variables including LDM, SDM, RDM, TDM, RGR, R/S ratio, TWU,  $\text{WUE}_{\text{WP}}$  and  $\delta^{13}\text{C}$  were analysed using the general linear model (Proc GLM) to test the effect of the populations, water treatments and their interactions. Leaf gas exchange parameters, including  $A_{\text{sat}}$ ,  $g_s$ ,  $C_i$  and  $\text{WUE}_i$ , were analysed by the GLM with the measurement time as a covariate. When the differences were significant, a multiple comparison of means (post-hoc Tukey's honestly significant difference test) was carried out. Before the statistical tests were performed using the

SPSS software package (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 leads 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 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

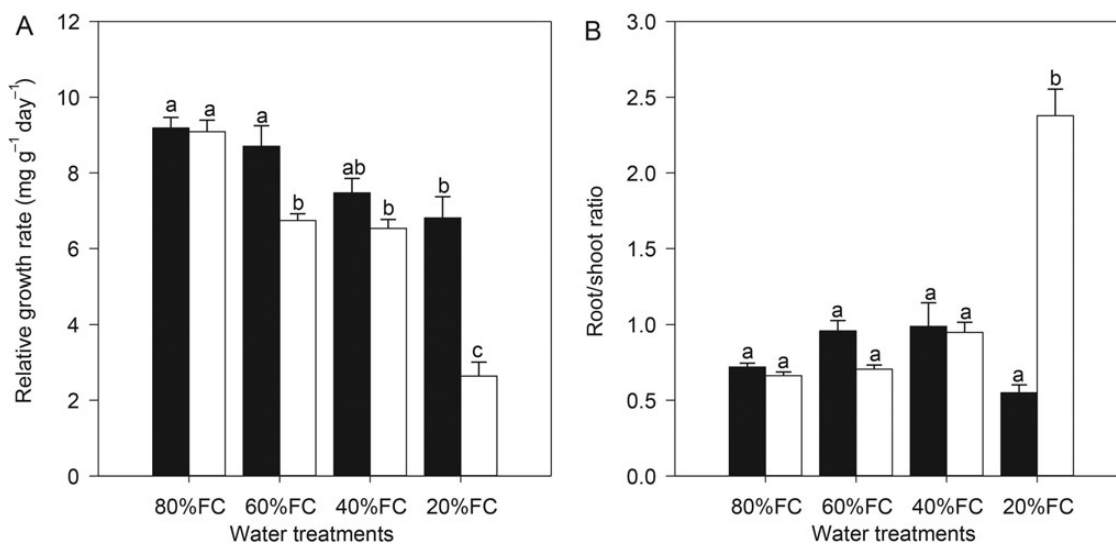
An increased water stress resulted in a significantly reduced  $A_{sat}$ ,  $g_s$  and  $C_i$  in both populations (Fig. 2, Table 2). However, the reductions in  $A_{sat}$ ,  $g_s$  and  $C_i$  followed different patterns for the different populations investigated. Much of the decline of  $A_{sat}$ ,  $g_s$  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  $A_{sat}$  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 with  $A_{sat}$  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 those variables (Table 2). In addition, for both populations, there were strong positive correlations for the  $A_{sat}$  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

**Table 1.** Growth, biomass production and allocation as well as water use of *Pinus tabulaeformis* from a HP and a LP under various soil water conditions (80 % of maximal FC, 60 % FC, 40 % FC and 20 % FC). Each point represents mean  $\pm$  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
Leaf dry mass (LDM) (g)				
HP	2.00 $\pm$ 0.04 <sup>a</sup>	1.81 $\pm$ 0.26 <sup>ab</sup>	1.20 $\pm$ 0.12 <sup>b</sup>	1.67 $\pm$ 0.28 <sup>ab</sup>
LP	2.02 $\pm$ 0.11 <sup>a</sup>	1.24 $\pm$ 0.11 <sup>b</sup>	1.04 $\pm$ 0.18 <sup>b</sup>	0.13 $\pm$ 0.03 <sup>c</sup>
Stem dry mass (SDM) (g)				
HP	1.23 $\pm$ 0.09 <sup>ab</sup>	0.83 $\pm$ 0.11 <sup>bc</sup>	0.82 $\pm$ 0.16 <sup>bc</sup>	0.59 $\pm$ 0.07 <sup>cd</sup>
LP	1.44 $\pm$ 0.14 <sup>a</sup>	0.84 $\pm$ 0.02 <sup>bc</sup>	0.70 $\pm$ 0.03 <sup>c</sup>	0.18 $\pm$ 0.04 <sup>d</sup>
Root dry mass (RDM) (g)				
HP	2.33 $\pm$ 0.17 <sup>ab</sup>	2.46 $\pm$ 0.17 <sup>ab</sup>	1.93 $\pm$ 0.15 <sup>bc</sup>	1.19 $\pm$ 0.08 <sup>de</sup>
LP	2.29 $\pm$ 0.14 <sup>ab</sup>	1.46 $\pm$ 0.04 <sup>cd</sup>	1.64 $\pm$ 0.03 <sup>cd</sup>	0.72 $\pm$ 0.10 <sup>e</sup>
TDM (g)				
HP	5.55 $\pm$ 0.29 <sup>a</sup>	5.10 $\pm$ 0.54 <sup>ab</sup>	3.95 $\pm$ 0.33 <sup>bc</sup>	3.45 $\pm$ 0.42 <sup>c</sup>
LP	5.75 $\pm$ 0.34 <sup>a</sup>	3.54 $\pm$ 0.14 <sup>c</sup>	3.38 $\pm$ 0.18 <sup>c</sup>	1.04 $\pm$ 0.17 <sup>d</sup>
TWU (g)				
HP	1.34 $\pm$ 0.06 <sup>a</sup>	1.53 $\pm$ 0.14 <sup>ac</sup>	1.63 $\pm$ 0.08 <sup>ac</sup>	0.85 $\pm$ 0.04 <sup>bd</sup>
LP	1.79 $\pm$ 0.05 <sup>c</sup>	1.25 $\pm$ 0.08 <sup>ab</sup>	1.57 $\pm$ 0.04 <sup>ac</sup>	0.85 $\pm$ 0.12 <sup>d</sup>
$WUE_{WP}$ (g kg <sup>-1</sup> )				
HP	4.13 $\pm$ 0.04 <sup>a</sup>	3.33 $\pm$ 0.04 <sup>bc</sup>	2.42 $\pm$ 0.09 <sup>cd</sup>	4.00 $\pm$ 0.33 <sup>ab</sup>
LP	3.21 $\pm$ 0.12 <sup>bc</sup>	2.86 $\pm$ 0.29 <sup>cd</sup>	2.15 $\pm$ 0.06 <sup>d</sup>	1.21 $\pm$ 0.03 <sup>e</sup>



**Figure 1.** Relative growth rate and R/S ratio in two populations of *Pinus tabuliformis* from a HP (black bars) and a LP (white bars) under various soil water conditions (80 % of maximal FC, 60 % FC, 40 % FC and 20 % FC). Scale bars represent mean  $\pm$  SE. The letters indicate statistical differences ( $P < 0.05$ ) for the water treatments, populations and the interactions between them.

**Table 2.** Comparison of all variables measured in the experiment. The  $P$ -values are presented for the watering treatments, populations and their interactions. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Variables	Treatment (T)	Population (P)	T × P
LDM	20.52***	26.86***	10.45***
SDM	32.73***	1.36	3.67*
RDM	44.33***	27.00***	5.55**
TDM	38.92***	22.84***	6.32**
RGR	49.63***	47.07***	11.31***
R/S ratio	27.68***	33.12***	57.88***
$A_{sat}$	101.09***	69.00*	2.57***
$g_s$	102.77***	131.99***	13.88***
$C_i$	103.80***	253.01***	12.06***
$WUE_i$	37.55**	146.34***	13.49***
TWU	36.10***	0.194	6.97**
$WUE_L$	35.67***	119.75***	12.23***
$\delta^{13}C$	39.11***	25.84***	7.51**

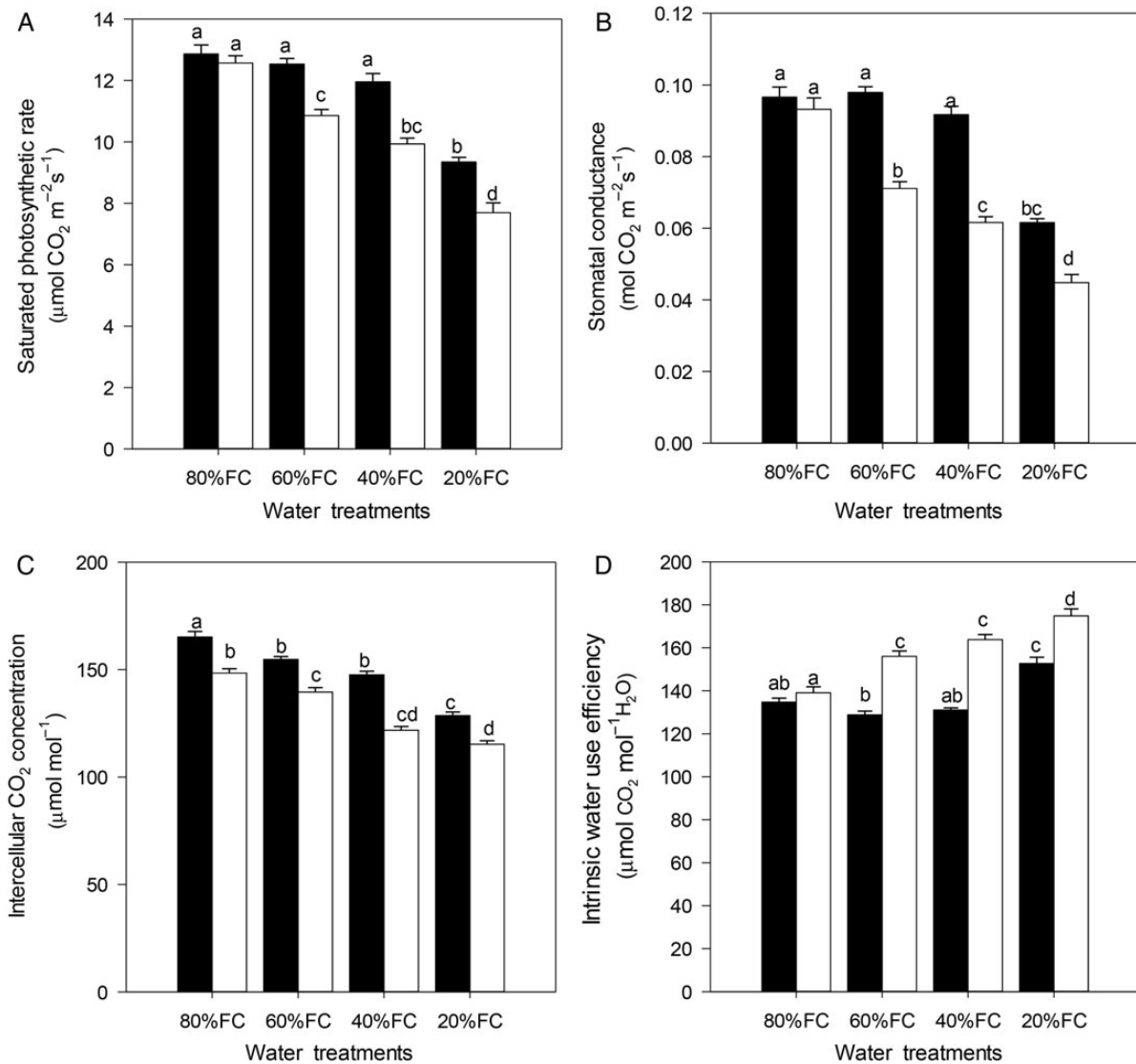
moderate stress, a decline in  $WUE_{WP}$  was observed in both populations. Severe water stress saw a further decrease in the LP but an increase in the HP. However, the HP exhibited a higher  $WUE_{WP}$  than the LP in all the water level treatments and significant differences were observed in the low and severe stress treatments (Table 1). The  $\delta^{13}C$  gradually increased as the water stress increased in the LP, while only the severe water stress induced an increase in  $\delta^{13}C$  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 the 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,  $g_s$  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).

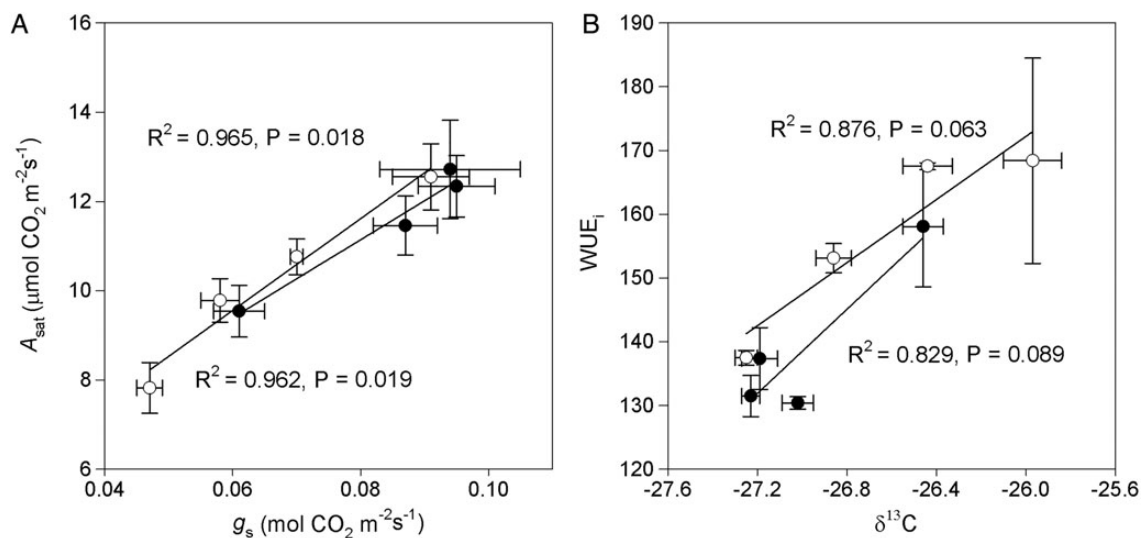


**Figure 2.** Means of light-saturated photosynthetic rate ( $A_{sat}$ ), stomatal conductance ( $g_s$ ), intercellular  $CO_2$  concentration ( $C_i$ ) and intrinsic water-use efficiency ( $WUE_i$ ) measured on 3 days (15 August, 15 September and 15 October) in two populations of *Pinus tabuliformis* from a HP (black bars) and a LP (white bars) under various soil water conditions (80 % of maximal FC, 60 % FC, 40 % FC and 20 % FC). Each bar represents mean  $\pm$  SE. The letters indicate statistical differences ( $P < 0.05$ ) for the water treatments, populations and the interactions between them.

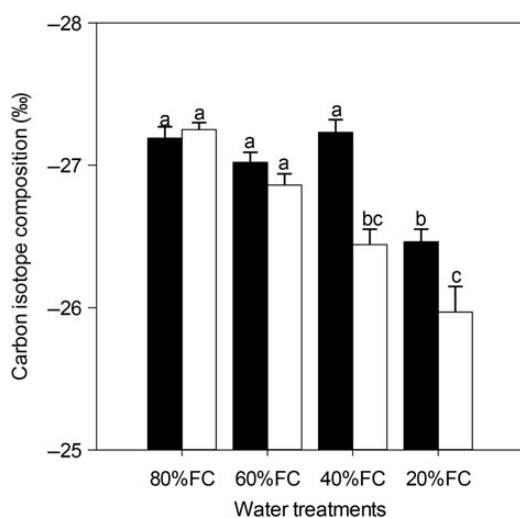
From this it was possible to surmise that stomatal closure caused by drought stress resulted in the  $A_{sat}$  being reduced under drought conditions (Fig. 3), and the  $C_i$  in both populations being reduced at the same time supports this conclusion (Michelozzi et al. 2011). However, compared with 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  $\delta^{13}C$  (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 severe stress treatment (Table 2). The higher  $WUE_i$  and  $\delta^{13}C$  values in the LP than in the HP under mild, moderate and



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



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

severe water stress treatments were mainly due to the relatively small changes of  $A_{sat}$  and  $g_s$  in the HP under 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 the HP than in the LP.

With respect to the  $WUE$  at the whole-plant level, the  $WUE_{wp}$  showed an opposite trend to the  $WUE_i$  and  $\delta^{13}\text{C}$ ,

with both populations recording a significant drop between the low and moderate stress treatments, and a further significant drop between the moderate and severe stress 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 survive 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 favour 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 HP and LP. 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<sub>L</sub> 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 the Authors

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

## Conflicts of Interest Statement

None declared.

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