

# Photosynthetic acclimation to long-term high temperature and soil drought stress in two spruce species (*Picea crassifolia* and *P. wilsonii*) used for afforestation

Xiaowei Zhang<sup>1,2</sup> · Litong Chen<sup>1,3</sup> · Jingru Wang<sup>1</sup> · Minghao Wang<sup>1</sup> · Shuli Yang<sup>4</sup> · Changming Zhao<sup>1</sup>

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**Abstract** *Picea crassifolia* and *P. wilsonii*, commonly used for afforestation in northern China, are increasingly likely to be subjected to high temperatures and soil drought stress as a result of global warming. However, little is known about the effects of these stresses on foliar photosynthesis in the two species. To investigate how photosynthetic characteristics and sensitivity respond to prolonged high temperatures and soil drought, foliar gas exchange and other closely related parameters were recorded from four-year-old seedlings of both species. Seedlings were grown under two temperature treatments (25/15 and 35/25 °C) and four soil water regimes [80, 60, 40 and 20% of maximum field capacity (FC)] for 4 months.

Although all treatments significantly reduced photosynthetic rates ( $P_n$ ) of both species, *P. crassifolia* exhibited greater photosynthetic acclimation than *P. wilsonii*. Differences in photosynthetic acclimation were mainly related to variations in stomatal conductance ( $Cond$ ) and the maximum quantum yield of PSII ( $F_v/F_m$ ) between treatments. Indeed, higher  $Cond$  and  $F_v/F_m$  in all treatments were shown for *P. crassifolia* than for *P. wilsonii*. Moreover, photosynthesis in *P. crassifolia* exhibited inherently lower temperature sensitivities (broader span for the temperature response curves; lower  $b$ ) and higher thermostability (invariable  $b$  between treatments). Further, severe drought stress (20% FC) limited the survival of *P. wilsonii*. Our results indicate that *P. wilsonii* is more susceptible to high temperatures and soil drought stress. Planting *P. crassifolia* would be more expected to survive these conditions and hence be of greater benefit to forest stability if predicted increases in drought and temperature in northern China occur.

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✉ Changming Zhao  
zhaochm@lzu.edu.cn

<sup>1</sup> State Key Laboratory of Grassland Agro-Ecosystems, School of Life Sciences, Lanzhou University, Lanzhou 730000, China

<sup>2</sup> College of Forestry Science, Gansu Agricultural University, Lanzhou 730070, China

<sup>3</sup> Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China

<sup>4</sup> State Key Laboratory of Cryospheric Sciences, Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China

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## Introduction

Water availability and temperature are considered to be the main variables limiting photosynthesis, affecting growth and survival of plants (Niu et al. 2008; Ghannoum and Way 2011; Gago et al. 2013). Photosynthesis is sensitive to environmental variables which will be profoundly affected by future climate change, including elevated air temperatures and decreased water availability (Luo 2007; Gunderson et al. 2010; Lin et al. 2012; Ashraf and Harris 2013). Meanwhile, the ability of plants to modify photosynthesis

in response to high temperatures and/or drought stress has been shown to be species-specific resulting from different photosynthetic acclimation potentials (Way and Oren 2010; Ashraf and Harris 2013; Way and Yamori 2014). Therefore, the potential for photosynthetic acclimation to new growing conditions plays a central role in the effects of climate change on plant growth and survival (Smith and Dukes 2013; Sendall et al. 2015).

Photosynthetic acclimation can alter the short-term abiotic factor-response functions of photosynthesis associated with maintaining leaf gas exchanges under different growing conditions (Smith and Dukes 2013; Zhang et al. 2015; Aspinwall et al. 2016). For example, thermal acclimation of photosynthesis has resulted in a shift in optimum temperature ( $T_{opt}$ ) and/or a change in sensitivity, revealed, for example, by a change in the shape of the temperature response curves (see Berry and Björkman 1980; Hikosaka et al. 2006; Way and Yamori 2014; Sendall et al. 2015). Furthermore, the potential for plants to maintain photosynthetic capacity when water availability decreases depends on the sensitivity of leaf gas exchange to drought (Limousin et al. 2013). However, plants have at least three different strategies for maintaining photosynthesis in response to changing temperatures or water availability. First, photosynthetic capacity is closely linked to stomatal conductance ( $Cond$ ) (Flexas and Medrano 2002; Hikosaka et al. 2006; German and Roberto 2013). Long-term elevated air temperatures or drought may alter the sensitivity of stomatal apertures, thus limiting photosynthesis (Zhang et al. 2001; Reddy et al. 2004; Gao et al. 2009; Greer and Weedon 2012). Second, elevated temperatures or drought may lead to changes in leaf anatomy and density, for example, changing the leaf mass per area ( $LMA$ ) to affect the mesophyll conductance of  $CO_2$  (Poorter et al. 2009; Yamori et al. 2009; Vasseur et al. 2012; Heroult et al. 2013; Drake et al. 2015). These two strategies could influence water loss and constrain  $CO_2$  exchange from leaves. The third, photosynthesis also be limited through biochemical processes which was temperature and water availability dependent (Way and Sage 2008; Lin et al. 2012; Limousin et al. 2013; Aspinwall et al. 2016). In fact, the biochemical processes may be related to photosynthetic attributes such as the amount and/or efficiency of enzymes and photosystem II (PSII) activity. Nitrogen content per mass ( $N_{mass}$ ) is an important feature of photosynthetic apparatus, indicative of the relative proportion of enzymes in photosynthetic processes (Yamori et al. 2009; Aspinwall et al. 2016). In addition, the maximum quantum yield of PSII ( $F_v/F_m$ ) is a reliable diagnostic indicator of photosynthetic activity (Reddy et al. 2004; Gao et al. 2009; Ma et al. 2010; Wang et al. 2014).

*Picea crassifolia* Kom. and *P. wilsonii* Mast. are two endemic species in Western China which often form a

dominant component in coniferous forests (Farjón 2001; Fu et al. 1999; Zhao et al. 2008). Due to their significant commercial and ecological value, *P. crassifolia* and *P. wilsonii* are widely used for afforestation in Northern China. For example, in Qinghai Province alone, there are 14,000 ha of planted spruce forest, accounting for ~5.4% of the total spruce forest areas (Han et al. 2015). However, current climate models suggest increasing temperatures (raising about 10 °C by 2100) and droughts in these regions (Zou et al. 2005; IPCC 2013). Coniferous forests may be particularly sensitive to climate change, which may result in changes in carbon exchange and a serious threat to survival (Zhang et al. 2015; Aspinwall et al. 2016; Kroner and Way 2016). Therefore, the objectives of our study were to examine the effects of long-term high temperatures and drought on photosynthesis of these two important conifers and to determine which was likely to be of more benefit to future forest stability in Northern China.

## Materials and methods

### Plant materials and growing conditions

Seeds of each species were collected within their natural range (*Picea crassifolia* Kom: 30.3–37.8°N, 126.5–130.5°E, Alt.: 2400–3600 m above sea level (a.s.l.); *P. wilsonii* Mast: 33.7–40.8°N, 101.6–116.8°E, Alt.: 1400–2800 m a.s.l.). In 2005, seeds were germinated and grown indoors at Yuzhong campus, Lanzhou University (35°56′37″N, 104°09′05″E, Alt.: 1750 m a.s.l. Temperatures ranged during the growing season from 7.7 to 25.8 °C) for one year; the seedlings received ample water and light. Seedlings were then transplanted into 24 cm (upper diameter) × 16 cm (basal diameter) × 17 cm (depth) pots filled with a homogeneous mixture consisting of equal volumes of peat and perlite. One pot was designed three seedlings. All pots were periodically watered to field capacity (FC) according to Ma et al. (2010). On June 15, 2009 (day  $t_1$ ), 25 pots of each species of uniform growth (c. 20 cm tall *P. crassifolia* and c. 25 cm tall *P. wilsonii*) were selected and divided into two groups: one for the water stress experiment, the other for the high temperature experiment. Control pots (five of each species) were used for both experiments and grown at 25/15 °C and 80% FC conditions.

### Experiment 1: Drought experiment

Each five pots of each species were randomly divided into low [80% of maximum field capacity (FC)], mild (60% FC), moderate (40% FC) and severe (20% FC) water stress treatments. Water stress levels continued until October 16,

2009, and maintained at these levels by weighing the pots every two days. Pots were assigned a random position in an artificial intelligent greenhouse with growth temperatures controlled at 25/15 °C day/night [moderate temperature (MT)] by a temperature control system. All seedlings were grown under 12 h photoperiods with light levels of 300–400  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  at seedling height by artificial light sources for automatic control. Unfortunately, all *P. wilsonii* seedlings grown at 20% FC died during the experimental period. Seedlings of both species in the remaining 35 pots were alive at the end of this experiment (October 15, 2009; day  $t_2$ ).

## Experiment 2: High temperature experiment

The remaining pots (five pots) of each species were placed in another greenhouse with growth temperatures controlled at 35/25 °C [high temperature (HT)]. All seedlings were raised under 12 h photoperiods with light levels of 300–400  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  at seedling height. In both greenhouses, the  $\text{CO}_2$  concentration was maintained at  $\sim 380 \mu\text{mol mol}^{-1}$  and relative humidity at  $50 \pm 5\%$ . Seedlings in the high temperature treatment were watered sufficiently to avoid any effects caused by extreme water deficit. The experimental period continued until October 15, 2009 (day  $t_2$ ).

## Gas exchange and chlorophyll fluorescence measurements

Leaf-level gas exchange ( $P_n$ ,  $Cond$  and  $T_r$ ) measurements were made with a portable open-path gas exchange system and a conifer chamber (Li-6400 and 6400-07, LI-COR Biosciences, Inc.) on fully expanded current year-old twigs for each experiment. Three to five seedlings per treatment and species were randomly selected between 10:00–13:30 h during sunny weather, when the temperatures were 25 or 35 °C, depending on each growth conditions. During photosynthetic measurements,  $\text{CO}_2$  concentration was maintained at  $380 \mu\text{mol mol}^{-1}$  using portable  $\text{CO}_2$ /air mixture tanks with output controlled by a Li-6400-01  $\text{CO}_2$  injector (LI-COR Biosciences, Inc.). Light levels were maintained at approximately  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  (saturated light level) at measurement height provided by an external light source. In addition, photosynthetic temperature curves of seedlings in each temperature treatment at 5 °C intervals from 40 to 15 °C were recorded. To ensure that the whole plant was exposed to the desired temperature settings, temperatures were controlled by changing air temperatures of the growth chamber, and micro-changing with the Li-6400 temperature control model. When measurements at one temperature were complete, the chamber temperature conditions were

adjusted. Seedlings were allowed to equilibrate to chamber conditions for a minimum of 30 min before measuring the same twigs again. All measurements were completed in one day. After measurements had been taken, needles were cut and leaf areas were determined with a LI-3000A portable area meter (LI-COR Biosciences, Inc.) to calculate gas exchange parameters on an area basis. The measured needles were dried at 65 °C for 72 h and dry mass determined ( $LM$ ; Precisa XT120A, Precisa Instruments. Ltd., Switzerland). These  $LM$  and  $LA$  values were used to calculate leaf mass per area ( $LMA$ ).

When gas exchange measurements were taken, the maximum quantum yield of photosystem II (PSII) ( $F_v/F_m = (F_m - F_o)/F_m$ ) was measured for leaves adapted to dark conditions during an acclimatization period of 30 min. Chlorophyll fluorescence measurements were taken with a portable pulse amplitude modulated fluorometer FMS-2 (Hansatech, King's Lynn, Norfolk, UK). At least five replicates from each treatment were taken.

## Leaf nitrogen measurements

We measured the concentration of nitrogen ( $N$ ) using samples used for gas exchange measurements. Dried samples were finely ground with a mortar and pestle, and sent to the Analytical Testing Center, Lanzhou University for analysis using a CHN analyzer (Vario EL, Elementar, Germany).

## Modeling of photosynthetic temperature curves

Photosynthesis data from temperature response curves were used to determine temperature-dependence and fitted to the following quadratic equation (Gunderson et al. 2010; Niu et al. 2008; Sendall et al. 2015):

$$P_T = P_{opt} - b(T - T_{opt})^2 \quad (1)$$

where  $P_T$  represents the mean net photosynthetic rate at temperature  $T$  in °C; and  $P_{opt}$  is the photosynthetic rate at the optimum temperature ( $T_{opt}$ ). Parameter  $b$  describes the spread of the parabola (Battaglia et al. 1996). For a given  $A_{opt}$  and  $T_{opt}$ , parameter  $b$  is smaller and the photosynthetic temperature parabola “broader” if photosynthesis is less sensitive to short-term temperature changes.

## Long-term sensitivity of photosynthesis to temperature

We also calculated an index to quantify the degree of thermal acclimation of  $P_n$  in response to HT (Way and Oren 2010) based on the following:

$$Acclim_{p_n} = \frac{P_n HT}{P_n MT} \quad (2)$$

The index of photosynthesis ( $Acclim_{p_n}$ ) for each species was equal to the ratio of  $P_n$  in HT leaves (35 °C) to MT leaves (25 °C). As an index for the degree of acclimation, if  $Acclim_{p_n}$  is close to 1.0, this indicates that temperature acclimation exhibited is high (Way and Oren 2010; Yamori et al. 2009).

### Statistical analysis

Quadratic fitting was used to estimate temperature response functions for photosynthetic rates (15–40 °C). The experiment was arranged in a completely randomized design with 3–5 replicates. Differences in all traits were determined by analysis of variance (one-way and General Linear Model, Proc GLM) and Tukey's test for multiple comparisons. All data are presented as mean  $\pm$  SE. Differences were considered significant at  $p < 0.05$ . Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Effects of long-term drought stress on photosynthesis

As available soil water decreased,  $P_n$ ,  $Cond$ , and  $T_r$  decreased significantly in both species (Table 1). In the low water treatment,  $P_n$ ,  $Cond$ , and  $T_r$  in *P. wilsonii* were higher than in *P. crassifolia*, particularly in the case of the latter two variables ( $p < 0.05$ , Table 1). In contrast, *P. crassifolia* had significantly higher values of  $P_n$ ,  $Cond$  and  $T_r$  under mild and moderate water conditions, leading to a relatively greater decrease of  $P_n$ ,  $Cond$  and  $T_r$  in *P. wilsonii* with increasing water stress (Table 1). Furthermore, there were significant interactions between species and water treatments for  $P_n$ ,  $Cond$  and  $T_r$ , suggesting that photosynthetic response to water stress was different in the two species (Table 2).

The maximum quantum yield of photosystem II ( $F_v/F_m$ ) was not significantly different between *P. crassifolia* and *P. wilsonii* under the low water treatment. However, as drought stress increased,  $F_v/F_m$  for *P. crassifolia* only decreased significantly in the moderate treatment, while  $F_v/F_m$  for *P. wilsonii* decreased significantly in both mild and medium treatments. Meanwhile, the value of  $F_v/F_m$  in *P. wilsonii* was lower than in *P. crassifolia* for mild and moderate treatments (Fig. 1; Table 2). Hence, *P. wilsonii* also appeared more sensitive to drought stress for this character.

Compared with the low water treatment, increasing soil water stress generated a significant reduction in the value of  $N_{mass}$  for both *Picea* species, with the exception of *P. wilsonii* in the mild treatment. Meanwhile, the variations in  $N_{mass}$  between species were only apparent in the low water treatment (Fig. 2). Therefore,  $N_{mass}$  differed, depending on both the watering treatments and species (Table 2). However,  $LMA$  for both species was not significantly changed in either treatment (data not shown), while the values of  $LMA$  for *P. crassifolia* were obviously larger than those for *P. wilsonii* (see Table 3).

### Effects of long-term high temperatures on photosynthesis

We designed the second experiment to test the effects of temperature as a major limiting factor. As in the low water treatment above,  $P_n$ ,  $Cond$  and  $T_r$  in *P. wilsonii* measured at 25 °C (MT) were significantly higher than in *P. crassifolia*. After long-term 35 °C (HT) treatment,  $P_n$  and  $Cond$  for *P. crassifolia* were significantly higher than for *P. wilsonii*, whereas  $T_r$  measured for *P. crassifolia* was significantly lower with consequences for a relatively greater reduction in  $P_n$  for *P. wilsonii* (Fig. 3). Hence, the value of  $Acclim_{p_n}$  for *P. crassifolia* was about 0.75, higher than for *P. wilsonii* (Fig. 4). Meanwhile, there were significant differences in temperature, species and their interaction (Table 4).

**Table 1** Comparison of gas exchange parameters between *Picea crassifolia* and *P. wilsonii*, across different soil water treatments (80% of maximal field capacity (FC), 60 and 40% FC). Each value

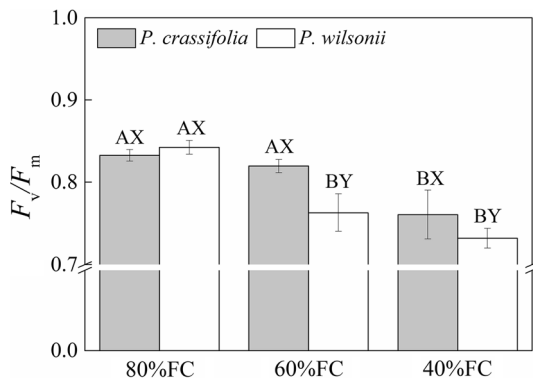
represents a mean and SE. Letters after SE values distinguish between statistically different ( $p < 0.05$ ) values for different water treatments (A, B, C) and between different species (X, Y)

Treatment	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		$Cond$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )		$T_r$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	
	<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>
Low stress (80% FC)	3.71 $\pm$ 0.15 AX	4.34 $\pm$ 0.19 AX	0.025 $\pm$ 0.001 AX	0.038 $\pm$ 0.0001 AY	0.84 $\pm$ 0.05 AX	1.23 $\pm$ 0.003 AY
Mild stress (60% FC)	3.06 $\pm$ 0.06 BX	1.49 $\pm$ 0.16 BY	0.019 $\pm$ 0.0020 BX	0.008 $\pm$ 0.0004 BY	0.79 $\pm$ 0.07 AX	0.25 $\pm$ 0.04 BY
Moderate stress (40% FC)	1.46 $\pm$ 0.02 CX	0.78 $\pm$ 0.03 CY	0.009 $\pm$ 0.0002 CX	0.003 $\pm$ 0.0004 CY	0.49 $\pm$ 0.10 BX	0.11 $\pm$ 0.01 CY

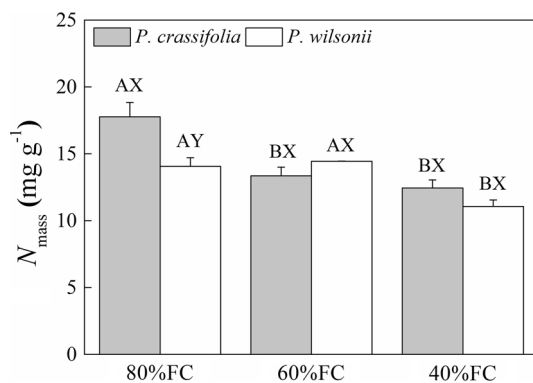
**Table 2** Effects of watering treatment, species and their interaction on five indicators measured during drought treatments

Parameters	Watering treatment	Species	Watering × species interaction
$P_n$	227.73***	22.97***	36.11***
$Cond$	168.63***	1.42	40.91***
$T_r$	90.30***	14.36**	41.47***
$F_v/F_m$	12.63**	2.92	1.93
$N_{mass}$	19.67***	6.03*	6.54**

\*\* Difference is significant at the 0.01 level; \*\*\* Difference is significant at the 0.001 level



**Fig. 1** Maximum quantum yield of PSII ( $F_v/F_m$ ) in seedlings of *Picea crassifolia* and *P. wilsonii* under different soil water conditions (80% of maximal field capacity (FC), 60 and 40% FC). Each value represents a mean and SE. Letters after SE values distinguish between statistically different ( $p < 0.05$ ) values for different water treatments (A, B) and between different species (X, Y)



**Fig. 2** Effects of watering treatments on the nitrogen content per dry mass ( $N_{mass}$ ) in seedlings of *Picea crassifolia* and *P. wilsonii* under different soil water conditions (80% of maximal field capacity (FC), 60 and 40% FC). Each value represents a mean and SE. Letters after SE values distinguish between statistically different ( $p < 0.05$ ) values for different water treatments (A, B) and between different species (X, Y)

At 25 °C (MT), all estimated parameters ( $b$ ,  $T_{opt}$  and  $A_{opt}$ ) of the photosynthetic temperature response curves in *P. wilsonii* were significantly higher than those for *P. crassifolia* (Table 3). Following the 35 °C (HT) treatment, the shapes of the curves were obviously different between species (Fig. 5). Only  $T_{opt}$  for *P. crassifolia* was

significantly greater, whilst  $b$  and  $A_{opt}$  were reduced for *P. wilsonii*. Meanwhile,  $A_{opt}$  at 35 °C in *P. crassifolia* was higher than in *P. wilsonii*; however, there were no significant differences in  $b$  and  $T_{opt}$  between species (Table 3). Interactions between temperature and species for these variables were also highly significant, indicating that temperature treatments in  $b$ ,  $T_{opt}$  and  $A_{opt}$  were different between *P. crassifolia* and *P. wilsonii* (Table 4).

For growth at 25 °C (MT),  $F_v/F_m$  was not significantly different between the two species. In contrast,  $F_v/F_m$  in *P. wilsonii* was significantly lower following the 35 °C (HT) treatment for 4 months, and its value was clearly less than that for *P. crassifolia* (Table 3). Variations in  $LMA$  and  $N_{mass}$  were species-specific following the 25 °C (MT) treatment;  $LMA$  and  $N_{mass}$  were higher in *P. crassifolia* (Table 3). In contrast, there were no significant differences in  $LMA$  and  $N_{mass}$  between species grown at 35 °C (HT) (Table 3). However, there were only significant species effects with respect to  $LMA$ , while the values of  $N_{mass}$  were significantly affected by temperature treatments (Table 4).

### Discussion

Precipitation and temperature are the most important factors affecting plant growth and distribution because of their influence on photosynthesis (Zhang et al. 2009; Way and Oren 2010). In this study, we examined a combination of photosynthetic parameters and leaf morphological characteristics of *P. crassifolia* and *P. wilsonii* grown under two temperature conditions and four water supply regimes. We found different patterns in their long-term response to temperature and drought: *P. crassifolia* exhibited greater photosynthetic acclimation in both treatments as compared with *P. wilsonii*.

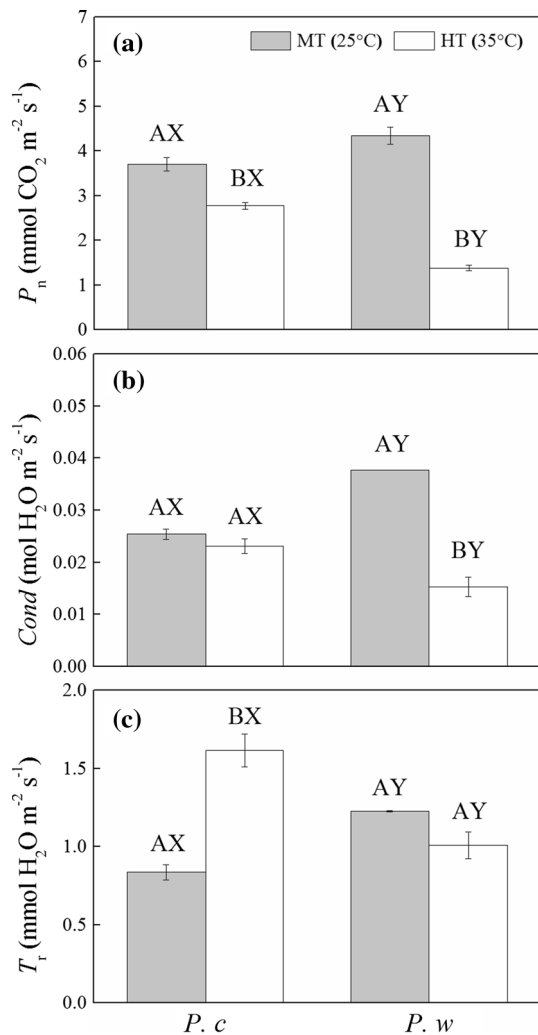
### Photosynthetic acclimation to drought

Drought tolerance is essential for the survival and growth of many plants (Reddy et al. 2004; Mao and Wang 2011).  $P_n$  for *P. crassifolia* and *P. wilsonii* decreased with increasing drought stress (Table 1), suggesting that drought was inhibiting photosynthetic activity (Ashraf and Harris

**Table 3** Effects of growth temperature on several parameters (b, T<sub>opt</sub>, A<sub>opt</sub>, F<sub>v</sub>/F<sub>m</sub>, LMA and N<sub>mass</sub>) for *Picea crassifolia* and *P. wilsonii*

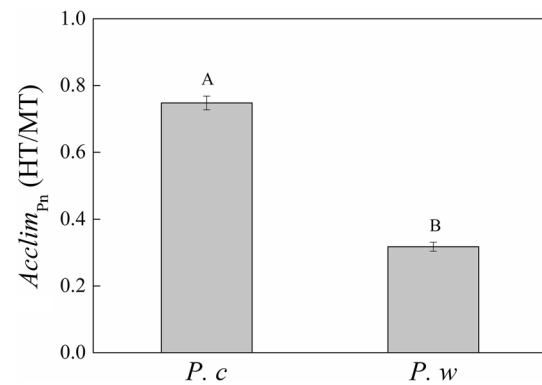
Treatments	b	T <sub>opt</sub> (°C)		A <sub>opt</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )		F <sub>v</sub> /F <sub>m</sub>		LMA (g m <sup>-2</sup> )		N <sub>mass</sub> (mg g <sup>-1</sup> )		
		<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>	<i>P. crassifolia</i>	<i>P. wilsonii</i>	
MT (25 °C)	0.009± 0.001AX	0.015± 0.001AY	23.40± 0.69AX	27.40± 0.75AY	3.74± 0.15AX	4.46± 0.25AY	0.83± 0.007AX	0.84± 0.008AX	244.24± 2.89AX	189.44± 2.84AY	17.77± 1.07AX	14.06± 0.65AY
HT (35 °C)	0.008± 0.001AX	0.007± 0.001BX	28.96± 0.97BX	28.10± 0.41AX	3.10± 0.09BX	1.70± 0.04BY	0.84± 0.007AX	0.67± 0.039BY	226.94± 22.74AX	190.12± 1.63AX	19.74± 0.52AX	20.56± 0.66BX

Values represent the mean ± SE. Letters after SE values distinguish between statistically different ( $p < 0.05$ ) values for different temperature treatments (A, B) and different species in the same temperature treatment (X, Y)



**Fig. 3** Comparison of leaf gas exchange parameters at growth temperature [the net photosynthetic rate ( $P_n$ , a), the stomatal conductance ( $Cond$ , b) and the transpiration rate ( $T_r$ , c)] between *Picea crassifolia* (*P. c*) and *P. wilsonii* (*P. w*) in the MT and HT treatments. Data are presented as mean  $\pm$  SE. Letters distinguish between statistically different ( $p < 0.05$ ) values for two temperature treatments (A, B) and between different species (X, Y)

2013). Similar patterns have been observed in other plants (Ma et al. 2010). However, photosynthetic responses to drought were different between the two *Picea* species in this study: the decrease of  $P_n$  for *P. wilsonii* was much larger than that for *P. crassifolia*, in mild and moderate water treatments, particularly in the mild water treatment (c.  $-65\%$ ; Table 1), suggesting that the photosynthesis of *P. wilsonii* was more prone to drought limitations, whilst the photosynthesis of *P. crassifolia* showed an acclimatory response to drought. In addition, whilst the seedlings of *P. crassifolia* survived the high stress treatment with water supplied at 20% FC, *P. wilsonii* seedlings did not (see *Materials and methods*). This also suggests that high drought stress limits the growth and survival of *P. wilsonii*,



**Fig. 4** Comparison of the temperature acclimation of photosynthesis ( $P_n$ ) between *Picea crassifolia* (*P. c*) and *P. wilsonii* (*P. w*). Letters distinguish between statistically different ( $p < 0.05$ ) values for two temperature treatments (A, B)

while *P. crassifolia* is more resistant to drought, especially under extreme water deficit conditions.

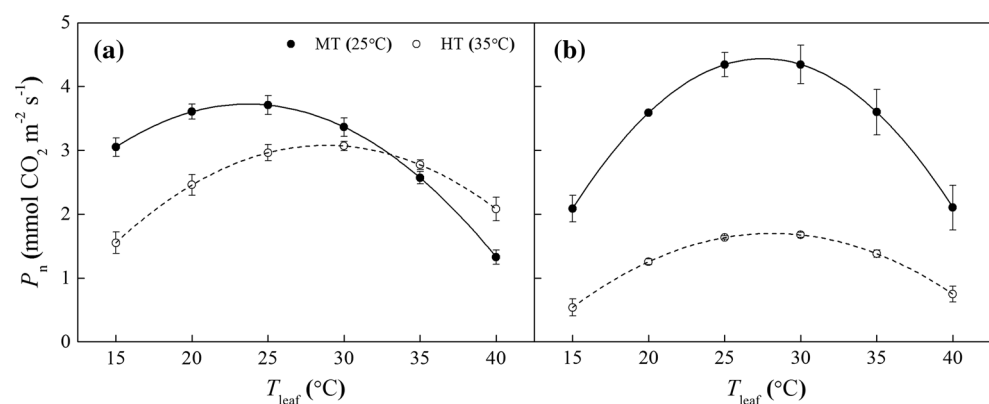
The higher  $P_n$  under drought observed in *P. crassifolia* could be explained by the conditions affecting two processes. First, water supply was reduced with increasing drought stress, progressively inducing stomatal closure (Flexas and Medrano 2002; Reddy et al. 2004; Gao et al. 2009; Matteo et al. 2014). Therefore, photosynthetic reduction in both *Picea* species may be partly explained by stomatal limitation. However, *P. crassifolia* had higher  $Cond$  and  $T_r$  than *P. wilsonii* in the mild and moderate treatments. Moreover, larger decreases in  $Cond$  of *P. wilsonii* (decreasing c. 80%) were observed in the mild water treatment relative to non-stressed seedlings. These results suggest that the leaves of each *Picea* species showed different sensitivities to water deficit (Ashraf and Harris 2013), and that leaves of *P. wilsonii* were more sensitive to drought. Second, with increasing water stress, drought can affect biochemical processes (Way and Sage 2008; Lin et al. 2012; Limousin et al. 2013). Our results reveal that decreases in  $F_v/F_m$  for *P. crassifolia* were smaller in each treatment. There was only a slight reduction under the mild water treatment (Fig. 1), suggesting more stable light capture and utilization in *P. crassifolia* leaves (Evans 1989; Reddy et al. 2004). However, there was a significant decrease in  $N_{mass}$  in the mild water treatment. In contrast, larger decreases in  $F_v/F_m$  were observed in *P. wilsonii*, suggesting that increasing water stress may inhibit or damage photosynthetic process in *P. wilsonii* (Ma et al. 2010; Ashraf and Harris 2013). At the same time, steady values of  $N_{mass}$  in *P. wilsonii* in the mild water treatment did not compensate for a larger reduction in  $F_v/F_m$ . Thus, variations in  $F_v/F_m$  also partly explain different photosynthetic acclimations to drought in both *Picea* species. These results suggest that the photosynthetic process of *P.*

**Table 4** Effects of growth temperature, species and their interaction on 10 measured indicators under the two temperature treatments

Parameters	Temperature treatment	Species	Temperature × species interaction
$P_{\text{growth}}$	230.42***	8.79*	62.36***
$Cond$	78.10***	2.68	51.61***
$T_r$	15.01**	2.23	47.14***
$Fv/Fm$	8.18*	8.53*	11.00**
$b$	18.93**	4.99	11.63**
$T_{\text{opt}}$	20.01**	5.05*	12.11**
$A_{\text{opt}}$	140.58***	5.54*	54.35***
$LMA$	0.72	21.93**	0.85
$N_{\text{mass}}$	9.03*	10.68*	5.06

\* Difference is significant at the 0.05 level; \*\* Difference is significant at the 0.01 level; \*\*\* Difference is significant at the 0.001 level

**Fig. 5** Temperature ( $T_{\text{leaf}}$ ) responses of leaf photosynthetic rates ( $P_n$ ) in *Picea crassifolia* (a) and *P. wilsonii* (b) under the MT and HT treatments. Data are presented as mean ± SE



*crassifolia* has a higher drought tolerance than that of *P. wilsonii*.

### Photosynthetic acclimation to high temperatures

Grown at elevated temperatures,  $P_n$  measured at 35 °C decreased in both *Picea* species compared to seedlings grown at 25 °C, indicating that photosynthesis of these two *Picea* species did not completely acclimatize to elevated temperatures (Berry and Björkman 1980). However, the  $Acclim_{P_n}$  for *P. crassifolia* was higher than that for *P. wilsonii* (Fig. 4), suggesting that thermal acclimation of photosynthesis in *P. crassifolia* is more effective than in *P. wilsonii* (Yamori et al. 2009; Way and Oren 2010). *P. crassifolia* has an inherently lower photosynthetic sensitivity to short-term temperature fluctuations (e.g. parameter  $b$  in Table 3 and Fig. 5; see Battaglia et al. 1996; Niu et al. 2008; Gunderson et al. 2010; Sendall et al. 2015). Photosynthesis of *P. wilsonii* was more sensitive to short-term temperature fluctuations (Table 3; Fig. 5) and therefore the thermal sensitivity of photosynthesis in *P. wilsonii* was limited. Meanwhile, there was an upward shift in the  $T_{\text{opt}}$  of *P. crassifolia* that reduced high temperature stress; this was not observed in *P. wilsonii* (Gunderson et al. 2010; Way

and Yamori 2014; Sendall et al. 2015). Therefore, the photosynthetic process of *P. crassifolia* has a higher thermal tolerance than that of *P. wilsonii*.

The regulation of  $P_n$  is related to changing stomatal conductance, leaf development, and biochemical processes during prolonged exposure to high temperatures (Way and Sage 2008; Lin et al. 2012; Heroult et al. 2013). Changes in stomatal conductance ( $Cond$ ) could reduce  $P_n$  irrespective of biochemical effects (Hamerlynck and Knapp 1996; Zhang et al. 2001; Hikosaka et al. 2006; Greer and Weedon 2012). High temperatures are associated with increasing leaf-to-air vapor pressures leading to increasing drought stress. Potentially plants could limit  $Cond$  to reduce  $T_r$  (Day 2000). Our results show that decreased  $Cond$  for *P. wilsonii* limited  $T_r$  at elevated temperatures (Fig. 3), indicating increased heat-induced physiological drought stress for *P. wilsonii* even though water deficits were avoided by providing abundant water during the experimental period (German and Roberto 2013). In contrast, increased  $T_r$  in *P. crassifolia* meant that *P. crassifolia* plants were exposed to thermal stress as a result of increasing water loss from leaves even when there was abundant soil moisture (Fig. 3c). Hence, variations in  $Cond$  between temperature treatments were important in explaining treatment



differences in  $P_n$  (German and Roberto 2013). On the other hand,  $LMA$  was identical between temperature treatments for the two species (Tables 3, 4), suggesting that high temperatures did not affect leaf structure and density. Leaf developmental processes are therefore unlikely to explain temperature treatment differences in  $P_n$ . In addition, biochemical processes may partly explain the smaller proportional decreases in  $P_n$  at high temperatures. Our results showed that  $F_v/F_m$  was only significantly reduced by high temperatures in *P. wilsonii*, indicating that high temperature inhibits or damages the photosynthetic apparatus in *P. wilsonii* leaves. Hence, increasing  $N_{mass}$  in *P. wilsonii* may be assumed to repair chlorophyll and thylakoids (Evans 1989; Hikosaka et al. 2006; Hikosaka and Shigeno 2009; Wang et al. 2014). This was not compensated for by greater photosynthetic range. These results suggest that thermal acclimation of photosynthesis in *P. crassifolia* is more effective than in *P. wilsonii* (Fig. 4).

## Conclusions

Overall, our results suggest that stress caused by drought and high temperatures reduce the  $P_n$  of the seedlings of both *Picea* species. However, *P. crassifolia* exhibited higher photosynthetic acclimation to both increasing drought and temperature than *P. wilsonii*. In addition, we recorded higher  $Cond$  and  $F_v/F_m$  for *P. crassifolia* with increasing drought and temperature, indicating that these were responsible for the improved acclimation compared to *P. wilsonii*. Moreover, the photosynthetic apparatus in *P. crassifolia* leaves exhibited an inherently lower temperature-sensitivity and higher thermostability (see parameter  $b$ ). Further, severe drought stress (20% FC) killed *P. wilsonii*. In conclusion, our results indicate that *P. wilsonii* is more susceptible to drought and high temperatures; *P. crassifolia* is more appropriate to plant to survive future climate increases and to sequester carbon.

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