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Effect of summer warming on growth, photosynthesis and water status in female and male *Populus cathayana*: implications for sex-specific drought and heat tolerances

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Effects of climate warming on tree growth and physiology may be driven by direct thermal effects and/or by changes in soil moisture. Dioecious tree species usually show sexual spatial segregation along abiotic gradients; however, few studies have assessed the sex-specific responses to warming in dioecious trees. We investigated the sex-specific responses in growth, photosynthesis, nonstructural carbohydrate (NSC), water-use efficiency and whole-plant hydraulic conductance (K_P) of the dioecious tree species *Populus cathayana* Rehd. under +4 °C elevated temperature with and without supplemental water. For both sexes, high-temperature treatments significantly decreased growth (height and biomass), photosynthetic rate (A), the ratio of A to dark respiration rate, stomatal conductance (g_s), transpiration rate, NSC, leaf water potential and K_P, but increased water-use efficiency (estimated from carbon isotope composition). Under warming with supplemental water, most traits of females did not change relative to ambient conditions, but traits of males decreased, resulting in greater sexual differences. Females showed a lower K_P, and their g_s and A responded more steeply with water-related traits than males. These results show that the effect of summer warming on growth and photosynthesis was driven mainly by soil moisture in female *P. cathayana*, while male performance was mainly related to temperature. Females may experience less thermal stress than males due to flexible water balance strategy via stomata regulation and water use.

Keywords: dioecy, poplar, sexual dimorphism, summer heat, water use strategy.

Introduction

For many parts of the planet, climate change projections predict not only increases in surface temperature but also changes in precipitation and evapotranspiration, which affect the soil moisture available to plants (Dai 2013). Evapotranspiration may increase more than precipitation in temperate and boreal forest ecosystems under climate warming, while the reverse may occur in tropical and subtropical forest ecosystems (Sherwood and Fu 2014, Perez and Feeley 2018). Temperature and water

are key drivers of plant growth, survival and distribution across terrestrial ecosystems. Extreme high temperature and/or heat-induced soil moisture changes may threaten tree growth and survivorship (Allen et al. 2010, Park Williams et al. 2013, Grossiord et al. 2017). It is important for studies on the effect of climate warming to consider the effect of soil moisture changes induced by warming, which is crucial for predicting impacts on forest ecosystems and for modeling carbon and water cycles under novel climatic conditions (Allen et al. 2010, Perez and Feeley 2018, Reich et al. 2018).

Under high temperature, plant physiological responses generally minimize heat absorption and maximize dissipation. Higher temperatures inhibit photosynthesis through Rubisco deactivation, photo-oxidation and/or membrane denaturation, while transpiration might be improved as warming usually increases the vapor pressure deficit (VPD) between leaf and air (Rennenberg et al. 2006, Garcia-Forner et al. 2016, Kumarathunge et al. 2019). Since photosynthetic and transpiration processes are shaped by stoma, stomatal regulation may be more important under warming climates (Garcia-Forner et al. 2016). Higher stomatal conductance (g_s) leads to higher rates of photosynthesis and transpiration under adequate water resources, but may increase water loss when water is scarce. There are species-specific adaptive responses in g_s to water loss to VPD (Oren et al. 1999). To maintain function, some species will decrease g_s while increasing VPD to limit transpiration and water use; however, decreasing transpiration and stomatal closure to avoid leaf desiccation can lead to increases in heat risk to leaves when warming beyond the thermal optimum for photosynthesis (Marchin et al. 2016, Vico et al. 2019). Other species will maintain g_s through increasing the leaf water supply to deal with elevated VPD (Wu et al. 2018). Therefore, the mechanism of water balance may mediate the effects of high temperature when soil moisture is low (Rennenberg et al. 2006).

At a given soil moisture, leaf water status is determined by plant hydraulic characteristics and transpiration (Sperry et al. 2002). Some studies in woody plant species found that hydraulic conductance (K_P) can determine leaf water status, influencing gas exchange (Tyree 2003, Brodribb et al. 2007, McCulloh et al. 2016). Hydraulic conductance can also control plant responses to stresses associated with water (Mitchell et al. 2013, Nardini et al. 2013) and temperature (Sellin and Kupper 2007, McCulloh et al. 2016). Recent studies found that elevated temperature can exacerbate the risks of hydraulic failure and carbon starvation when soil moisture is limited (Yan et al. 2020). However, studies on how plants adjust water physiology for maximizing carbon assimilation under warming remain limited (Vico et al. 2019). More intra- and inter-species studies of whole-plant vascular systems under warming conditions are needed to predict the responses of whole plants to changes in atmospheric (e.g., temperature) and soil conditions (Way and Oren 2010).

Despite making up only 5–6% of total plant species (Renner 2014), dioecious plant species (those with separate female and male individuals) play important pioneer roles in the structure and function of forest ecosystems (such as *Salix*, *Ilex* and *Populus* species). Previous studies have found tree species show sexual dimorphism in plant morphology, reproductive allocation, resource efficiency and even sexual spatial segregation along environmental gradients (Li et al. 2007, Hultine et al. 2013, Lei et al. 2017, Melnikova et al. 2017, Zhang et al. 2020). How these sex-specific responses could change under climate change is still an open question (Xu et al. 2008b, Tognetti 2012,

Hultine et al. 2013, Munné-Bosch 2015). To compensate for higher reproductive costs, females might be more efficient than males in photosynthesis and water use (Tognetti 2012, Hultine et al. 2016). However, studies that contrast the hydraulic response of females and males are rare. One study of the dioecious tree species *Juniperus thurifera* L. (evergreen conifer) showed that females have a greater hydraulic efficiency than males (Olano et al. 2017). Compared with evergreen trees, deciduous species usually have a higher growth rate, resulting in more susceptibility to warming-induced responses in growth (Way and Oren 2010, Dusenge et al. 2020). To the best of our knowledge, no previous studies have investigated the impact of warming on hydraulic conductance of dioecious deciduous trees.

Populus cathayana Rehd. is a fast-growing, dioecious tree that is widely distributed in the northern hemisphere. During recent decades, this species' geographic range has experienced high summer temperatures, which results in declining growth and increasing mortality (Zhou and Ren 2011, Liu et al. 2013). Previous studies have found that *P. cathayana* is responsive to environmental stress and that females usually suffer from more negative effects in growth and physiology than do males with increasing drought stress (Xu et al. 2008a, 2008b, Zhang et al. 2010, 2012, Li et al. 2015). Moreover, differences in *P. cathayana* photosynthesis between sexes under warming may be related to soil moisture and/or warming strength (Xu et al. 2008b, Zhao et al. 2012) and warming season (Yu et al. 2018). However, it is still unclear whether the effects on growth, photosynthesis and hydraulic characteristics of *P. cathayana* result from thermal effects or from changes in soil moisture induced by increased temperature. Here, we hypothesized that there are sex-specific responses to warming in *P. cathayana* growth, photosynthesis and hydraulic characteristics. We investigated the sex-related responses in growth, photosynthesis and hydraulic conductance to summer high temperature with and without supplemental water, allowing us to distinguish between the direct effects of temperature alone and the joint effects of elevated temperature and reduced soil moisture. We ask the following questions. (i) Are there sex differences in plant growth, carbon assimilation and hydraulic conductance under elevated temperature (no supplemental water)? (ii) Does elevated soil moisture (supplemental water) modify the effect of elevated temperature on sex-specific growth and physiology? (iii) Do female and male plants have different sensitivities in balances of carbon and water to warming? Our results have significance for predicting physiological responses of dioecious species to global climate change.

Materials and methods

Study species and experimental design

Healthy shoots of female and male *P. cathayana* were collected from 30 different trees (~15 years old) from five populations

in the Qinghai Province, China (36°31′ N, 102°28′ E) at the beginning of mid-March. Populations were at least 400 m apart, and we took six cuttings from each population from at least three individuals (some cuttings were genetic clones). The cuttings (10 cm in length) were planted in a glasshouse at China West Normal University (30°48′ N, 106°03′ E) that blocked ambient rainfall but otherwise maintained ambient light and temperature (light transition rate was over 90% and temperature inside and outside the glasshouse differed by <0.3 °C). Summer climate in this site is typically hot and wet; the average rainfall total and temperature from June to September are 141 mm and 29.9 °C, respectively. Over recent decades, the frequency of high temperature (∼40 °C) and low precipitation during the mid-summer season has increased (from Meteorological Bureau of Nanchong (http://sc.cma.gov.cn/ds/nc/)). After 8 weeks of clonal propagation, 180 cuttings (90 males and 90 females) of similar size (15 cm in length and 0.5 cm in diameter) were chosen randomly from all cuttings across populations and transplanted to plastic pots (15 cm × 20 cm) filled with an 8-kg homogenized soil (1 cutting per pot). The surface soil (0–20 cm) was collected from farmland near the experimental site. The soil was sifted with a 0.35-mm-diameter sieve. The soil was a Cambisol (pH 7.9), which contained 12.22 g kg^{-1} organic carbon, 0.88 g kg^{-1} total nitrogen, 0.92 g kg^{-1} total phosphorus and 74.8 mg kg^{-1} available potassium.

Experimental treatments were applied to potted individuals in a completely randomized design. There were three treatments applied to each sex: (i) CK (control, where temperature and soil moisture matched ambient conditions (average 31% soil moisture by irrigation with 600 ml pot^{-1} day^{-1})), (ii) WA (+4 °C warming with the same amount of irrigated water as CK (600 ml pot^{-1} day^{-1}; no supplemental water)) and (iii) WM (+4 °C with supplemental water to match the average 31% soil moisture of the CK treatment). This design allows us to separate the effects of warming that are due to increased temperature versus decreased soil moisture. A ∼4.0 °C air temperature increase (widely used to study the effect of warming in plants (e.g., Duan et al. 2018, Hoeppner and Dukes 2012, Dusenge et al. 2020)) was accomplished with infrared lamp heaters placed ∼1 m above the plant canopy. Warming and control plants were separated by 10 m. The positions of pots were rotated weekly to ensure equal light availability and minimize differences under heaters. Elevated air temperature was maintained throughout the growing season (from 1 May to 1 September). The air temperature of warming plants or ambient plants was monitored by two TP-2000-W1 Temperature Data loggers (Anfu Electronic Technique Co., Ltd Beijing, China), which were placed 0.2 m above the plant canopy. All plants were watered each day of the experiment, and the soil moisture content was measured after watering using a time-domain reflectometer (Dong et al. 2016).

Measurement of morphology and biomass

At the end of experiment, four cuttings from each treatment were randomly selected to measure height, total leaf area (TA) and biomass accumulation. The trees were harvested and their biomass was separated into leaves, stem, fine roots (<2 mm in diameter) and coarse roots (>2 mm in diameter). The area of total leaves was measured using a leaf area meter (LI-COR 3000C, LI-COR Inc., Lincoln, NE, USA). Dry mass of all biomass was measured after oven-drying at 70 °C to a constant mass. Plant total dry mass (TM) was calculated as the sum of dry masses of all tissue. The specific leaf area (SLA) was estimated as the ratio of leaf area to leaf dry mass.

Measurement of photosynthesis

One individual with healthy, fully expanded, exterior leaves in each treatment was chosen to measure gas exchange and chlorophyll fluorescence characteristics. Leaf gas exchange rates were measured using a LI-6400 portable photosynthesis system (LI-COR Inc.) with a standard LED leaf chamber (2 × 3 cm^2). The measurement conditions were leaf temperature of 28 °C; relative air humidity of 60%; CO_2 concentration of 400 ± 5 μmol mol^{-1} and photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}. Once steady-state gas exchange rates were observed at these conditions, light-saturated photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration (E) were recorded. The leaf dark respiration rate (R_d) was measured under the same conditions except for the absence of light after the leaves were darkened for at least 5 min before recording the rates (Dong et al. 2019).

The response of A to changing CO_2 was measured at 400 μmol mol^{-1}, which was decreased to 300, 200, 150, 100 and 50 μmol mol^{-1}, then returned to 400 and subsequently increased to 500, 600, 800, 1000 and 1200 μmol mol^{-1} under saturating irradiance (1500 μmol m^{-2} s^{-1} PPFD). The maximum rate of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) were estimated according to Long and Bernacchi (2003).

Chlorophyll fluorescence measurements were performed on the same leaves used for gas exchange observation with a Junior-PAM chlorophyll fluorometer (Walz, Effeltrich, Germany). A measurement leaf was dark-adapted for at least 30 min prior to the measurements by an aluminum foil cover, and the minimum dark-adapted fluorescence yield (F_0) was measured. Thereafter, a saturating white light pulse of 8000 μmol m^{-2} s^{-1} was applied for 0.8 s to measure the maximum dark-adapted fluorescence yield (F_m). Then, the leaf was illuminated with an actinic light at an intensity of 600 μmol m^{-2} s^{-1} corresponding to the ambient light intensity at the time of measurements, and a saturating white light pulse was applied again to measure the light-adapted maximum fluorescence yield (F_m'). Then, the actinic light was further switched off,
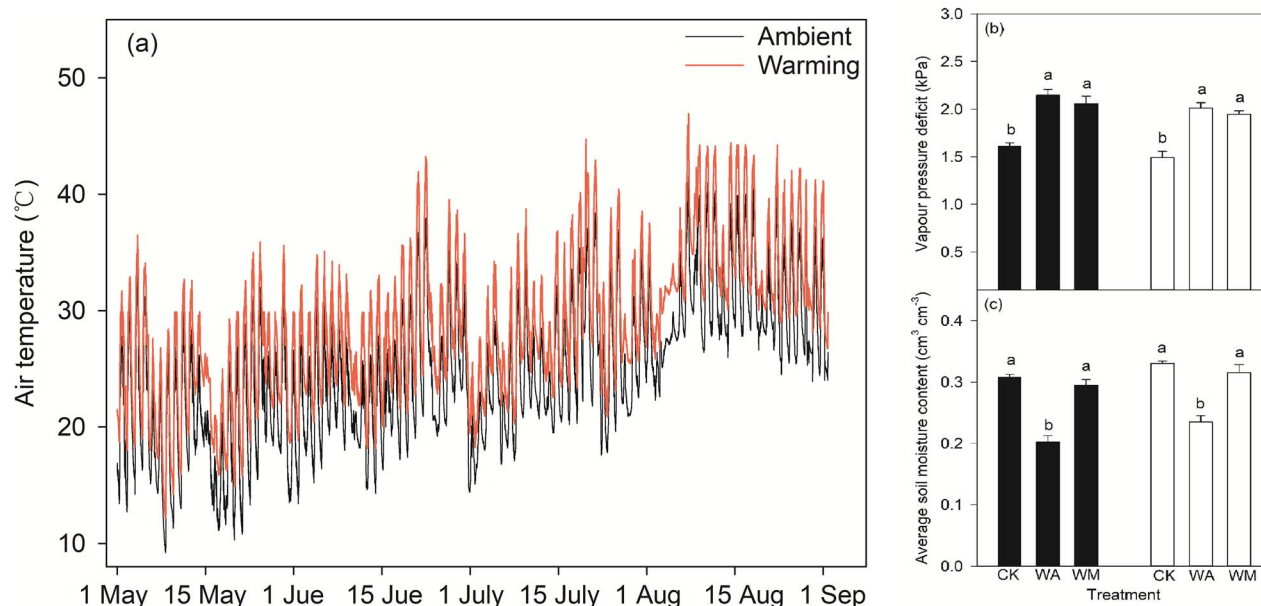
Figure 1. Air temperature during warming treatment (a) and differences in vapor pressure deficit (b) and average soil moisture content (c) in female (black bar) and male (white bar) *P. cathayana* cuttings under warming with water supply. CK, ambient temperature and irrigated to match the ambient soil moisture; WA, +4 °C keeping the same amount of irrigated water as CK (no supplemental water); WM, +4 °C and keeping the same soil moisture content as CK (with supplemental water). The different letters above the bars indicate the significant difference among treatments at $P < 0.05$ according to Tukey's HSD test.

the leaf was illuminated for 3 s with far-red light, and the minimal fluorescence yield (F_O') was determined. From these measurements, the maximum photochemical quantum yield of photosystem II (PSII) (F_v/F_m), effective photochemical quantum yield of PSII (Φ), coefficient of photochemical fluorescence (qP) and non-photochemical fluorescence quenching (NPQ) were calculated. These were calculated as follows: $F_v/F_m = (F_m - F_O)/F_m$; $\Phi = (F_m' - F')/F_m'$; qP = $(F_m' - F')/(F_m' - F_O')$; NPQ = $F_m/F_m' - 1$.

Measurement of soil-to-leaf hydraulic conductance

Whole-plant hydraulic conductance (K_P) corresponds to the following equation (Tyree 2003): $K_P = E_L/(\Psi_{soil} - \Psi_{leaf})$, where E_L is the whole-plant transpiration (mmol m^{-2} s^{-1}), Ψ_{soil} (MPa) is bulk soil water potential and Ψ_{leaf} (MPa) is leaf water potential. Ψ_{soil} are Ψ_{leaf} estimated from predawn (06:00–7:00 h) and midday (13:00–14:00 h) leaf water potential, respectively (Mitchell et al. 2013). The leaf water potential was determined with a thermocouple psychrometer (Wescor PSYPRO, Logan, UT, USA). E_L was calculated as the weight lost over the measurement interval at midday according to Mitchell et al. (2013).

Carbon isotope composition and carbohydrate analyses

Dried samples of leaf, stem, coarse root and fine root were then ground in a ball mill. We determined the foliar carbon isotope composition (δ^{13}C) and nonstructural carbohydrate (NSC) content of leaves, stems, coarse roots and fine roots according to Dong et al. (2019).

Statistical analysis

We used a two-way analysis of variance (ANOVA) to test the effects of sex, warming treatments and their interaction on morphological, biomass and physiological parameters. Trait differences among the treatments were tested by Tukey's honest significant difference (HSD) tests. Simple linear regressions were used to assess the relationships between A, A/R_d or g_s and water-related (leaf water potential, leaf water content, leaf water-use efficiency and whole-plant hydraulic conductance) or leaf temperature variables. The effects were considered significant if $P < 0.05$. Principal component analysis (PCA) of ecophysiological traits was also undertaken to examine the effects of warming in each sex. All data were analyzed with SPSS (Chicago, IL, USA) version 16.0.

Results

From May to September, the average ambient air temperature was 31.10/20.31 °C (day/night; varied from 9.2 to 41.6 °C across the growing season), and the warming treatment had an average 4.04 °C increase (Figure 1a). The mean vapor pressure deficits (VPD; Figure 1b) under ambient and warming treatments were 1.55 ± 0.04 kPa and 2.00 ± 0.03 kPa, respectively. Warming significantly increased VPD, but VPD was similar between WA (warming with same irrigation amount as the control) and WM (warming with same soil moisture as the control) treatments. During the growing season, the average soil moisture content under CK and WM was, respectively, 0.312 ± 0.01 (~81% soil field capacity) cm^3 cm^{-3} and 0.219 ± 0.02

Table 1. Effect of warming on morphology and biomass accumulation and allocation in female and male *P. cathayana* cuttings under different water supply.

Sex	Treatment	Height (cm)	TA (cm² plant⁻¹)	SLA (cm² mg⁻¹)	TM (mg plant⁻¹)	R/S	FR/TR
Female	CK	55.55 ± 5.42b	689.41 ± 40.65a	135.1 ± 0.4bc	11.86 ± 0.75a	0.43 ± 0.02ab	0.31 ± 0.01b
	WA	43 ± 4.21c	376.03 ± 41c	122.17 ± 2.47cd	7.21 ± 0.57c	0.53 ± 0.04a	0.58 ± 0.03a
	WM	58.18 ± 2.93b	681.58 ± 24.43a	143.9 ± 2.05b	11.16 ± 0.24ab	0.34 ± 0.02bc	0.4 ± 0.02b
Male	CK	81.58 ± 3.73a	530.16 ± 16.33b	151.22 ± 4.94b	9.72 ± 0.31b	0.3 ± 0.02c	0.39 ± 0.03b
	WA	59.13 ± 3.23b	227.59 ± 28.9d	114.09 ± 5.81d	6.38 ± 0.31c	0.41 ± 0.02b	0.51 ± 0.01a
	WM	92.58 ± 1.57a	404.92 ± 24.72c	172.59 ± 6.55a	7.66 ± 0.3c	0.26 ± 0.02c	0.37 ± 0.03b
	S	71.048***	60.506***	12.099**	34.259***	33.699***	0.256ᴺˢ
	T	22.885***	55.843***	44.182***	40.313***	26.479***	37.299***
	S × T	3.044ᴺˢ	2.688ᴺˢ	9.392**	4.371*	0.798ᴺˢ	4.807*

TA, total leaf area; SLA, specific leaf area; TM, total dry mass; R/S, root-to-shoot ratio; FR/TR, the ratio of fine root mass to total root mass. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The value is mean ± SE ($n = 4$). Different lowercases in the same column indicate a significant difference at 0.05 level according to Turkey's HSD test. F value and P value are shown. NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

($\sim$55% soil field capacity) cm³ cm⁻³, and there was a significant 29.86% decrease under the warming without water supplement treatment (Figure 1c).

Sex-specific effects of warming on morphology, biomass accumulation and allocation under water supply

After the 4-month warming treatment, we found that plant height, total leaf area (TA), specific leaf area (SLA), total dry mass (TM; including leaf, stem, coarse root and fine root dry mass; Table S1 available as Supplementary Data at *Tree Physiology* Online) and root-to-shoot ratio (R/S) were significantly affected by sex and warming treatments (Table 1). Compared with the control (CK), warming without water supplement (WA) decreased leaf area and dry mass in both sexes, but increased the fine root dry mass to total root dry mass ratio (FR/TR). When water was supplied to match the soil moisture of CK (WM treatment), female height, TA, SLA, TM, R/S and FR/TR were similar to the CK, while male TA and TM were lower than the CK. Female height was always lower than that of males in each treatment, but TA and leaf dry mass of females were always higher. Under CK and WM treatments, female TM and coarse root dry mass were higher than males. The highest fine root mass, R/S and FR/TR but the lowest TM (including leaf mass, stem mass, coarse root mass) and TA among all treatments were found in WA treatment (Tables 1 and S1 available as Supplementary Data at *Tree Physiology* Online).

Sex-specific effects of warming on gas exchange and chlorophyll fluorescence with water supply

Warming treatment significantly influenced gas exchange and chlorophyll fluorescence (Figure 2 and Table 2). The light-saturated photosynthetic rate (A), stomatal conductance (g_s), leaf dark respiration rate (R_d), transpiration (E) and effective photochemical quantum yield of PSII (Φ) were significantly influenced by sex and the interaction of sex and warming. Compared with CK, there was significantly lower A, g_s, E, maximum photochemical quantum yield of PSII (F_v/F_m), Φ and coefficient of photochemical fluorescence (qP) under WA

treatment in both sexes, while most of these traits were similar across sexes; we observed even higher g_s, R_d, E, J_{max} and non-photochemical fluorescence quenching (NPQ) in females under WM treatment. Warming treatment did not influence the maximum rate of Rubisco carboxylation (V_{cmax}), but lowered the ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i/C_a) of females under WA treatment and raised the C_i/C_a ratio of male under WM treatment compared with controls. In addition, warming always decreased male F_v/F_m and NPQ but increased R_d. Compared with females, male g_s and NPQ were lower in each treatment, A and E in males were higher under WA treatment, and E, J_{max}, F_v/F_m and Φ of males were lower under WM treatment.

In addition, warming with and without supplemental water always decreased the ratio of net photosynthetic rate to dark respiration rate (A/R_d) in each sex (Figure 3). The A/R_d of females under WM treatment was higher than under WA treatment, but A/R_d of males was similar between WM and WA treatments. Compared with females, male A/R_d was higher than females under control, but it was lower than females under WM treatment.

Sex-specific effects of warming on nonstructural carbohydrates with water supply

Whole-plant soluble sugars (SS_P), starch (ST_P) and total nonstructural carbohydrate (NSC_P) (including the stem and coarse root) were significantly influenced by sex (except NSC_P) and warming treatments (except NSC in coarse root) (Tables 3 and S2 available as Supplementary Data at *Tree Physiology* Online). The highest values of SS_P, NSC_P, leaf SS and NSC were found in females under WA treatment, while the lowest values of SS_P and NSC_P were found in males under WM treatment. In females, warming with water supplement decreased the contents of SS_P and NSC_P. Female ST_P was similar among the three treatments, whereas male ST_P under WA or WM treatment was lower than its control. Warming did not influence the leaf SS and NSC and fine root SS contents, but decreased the coarse root SS, ST and NSC contents in both sexes (Table S2 available as Supplementary
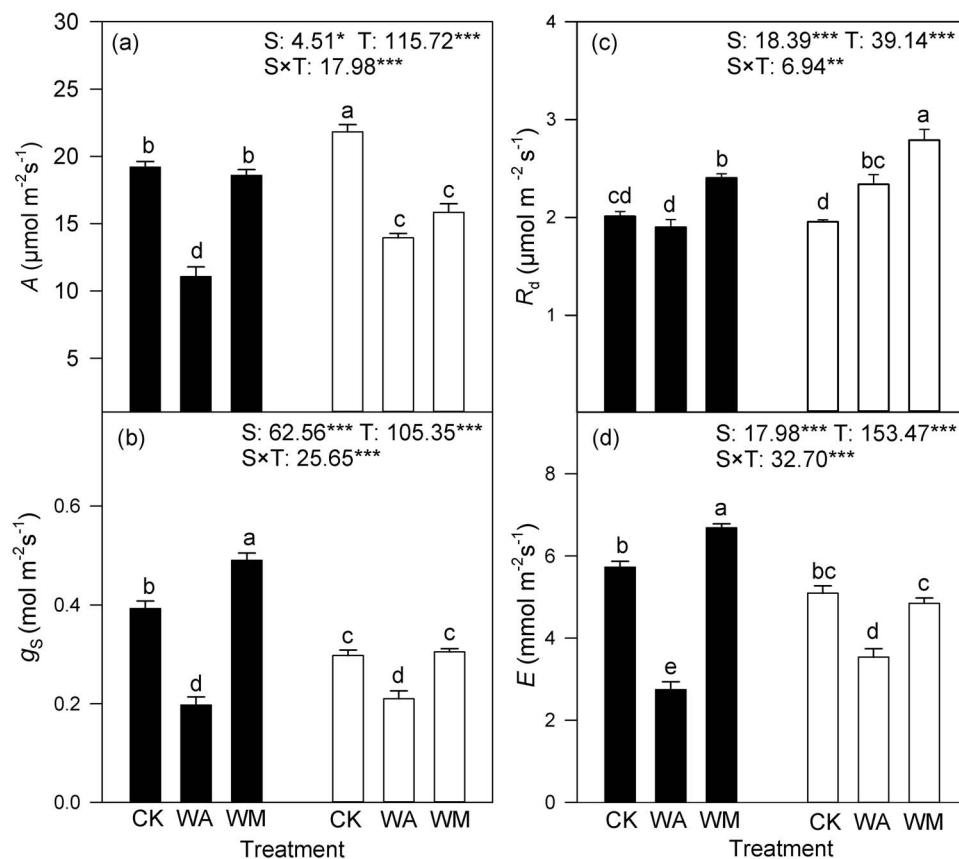
Figure 2. Effect of warming on light-saturated photosynthetic rate (A; a), stomatal conductance (g_S; b), the leaf dark respiration rate (R_d; c) and transpiration (E; d) in female (black bar) and male (white bar) *P. cathayana* cuttings under different water supply levels. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The different letters above the bars indicate the significant difference among treatments at $P < 0.05$ according to Tukey's HSD test. F value and P value are shown. *$P < 0.05$; **$P < 0.01$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

Table 2. Effect of warming on traits of photosynthetic-related biochemistry and chlorophyll fluorescence in female and male *P. cathayana* cuttings under different water supply.

Sex	Treatment	C_i/C_a	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	F_v/F_m	Φ	qP	NPQ
Female	CK	0.68 ± 0.01ab	75.78 ± 1.38	96.82 ± 1.61c	0.78 ± 0.02ab	0.48 ± 0.02a	0.68 ± 0.02a	0.34 ± 0.02c
	WA	0.57 ± 0.01d	72.07 ± 1.87	91.92 ± 2.36c	0.61 ± 0.02c	0.33 ± 0.01bc	0.59 ± 0.01b	0.49 ± 0.01a
	WM	0.63 ± 0.02bc	76.12 ± 4.51	119.51 ± 4a	0.75 ± 0.01b	0.50 ± 0.01a	0.72 ± 0.02a	0.41 ± 0.02b
Male	CK	0.63 ± 0.01c	80.26 ± 1.12	108.56 ± 3.24ab	0.84 ± 0.01a	0.50 ± 0.01a	0.73 ± 0.02a	0.26 ± 0.01d
	WA	0.61 ± 0.02cd	72.61 ± 0.99	88.41 ± 1.06c	0.66 ± 0.01c	0.33 ± 0.01c	0.56 ± 0.01b	0.38 ± 0.02c
	WM	0.7 ± 0.01a	75.06 ± 0.91	98.57 ± 2.22bc	0.65 ± 0.02c	0.39 ± 0.01b	0.66 ± 0.01ab	0.33 ± 0.01c
	S	2.474^{NS}	0.544^{NS}	3.974^{NS}	0.068^{NS}	6.260*	0.418^{NS}	70.801***
	T	20.296***	3.38^{NS}	27.223***	54.777***	68.138***	29.777***	52.782***
	S × T	11.922**	0.845^{NS}	19.736***	14.537***	11.026***	5.161*	0.891^{NS}

C_i/C_a, the ratio of intercellular CO_2 concentration to ambient CO_2 concentration; V_{cmax}, the maximum rate of Rubisco carboxylation; J_{max}, maximum electron transport rates; F_v/F_m, maximum photochemical quantum yield of PSII; Φ, effective photochemical quantum yield of PSII; qP, coefficient of photochemical fluorescence; NPQ, non-photochemical fluorescence quenching. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The value is mean ± SE ($n = 4$). Different lowercases in the same column indicate a significant difference at 0.05 level according to Turkey's HSD test. F value and P value are shown. NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

Data at *Tree Physiology* Online). Although male ST_P and NSC_P under control conditions were similar to females, these traits were lower than females in the warming treatment.

Sex-specific effects of warming on water use and leaf temperature with water supply

Predawn leaf water potential (Ψ) and whole-plant hydraulic conductance (K_P) under WA treatment decreased in both

sexes, relative to the ambient control (CK), while under WM treatment they increased in females and were similar in males (Figure 4a and d). The leaf carbon isotope composition increased under WA treatment in both sexes, while this trait under WM treatment was lower in females and higher in males than the control (Figure 4c). Male K_P was higher than females in the CK and WA treatment, but was similar between females and males under the WM treatment. The leaf
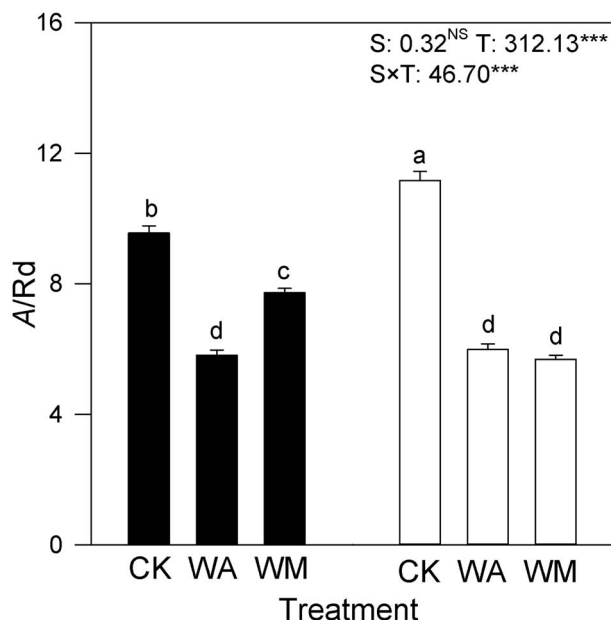
Figure 3. Effect of warming on the ratio of leaf photosynthetic rate to dark respiration rate (A/R_d) in female (black bar) and male (white bar) *P. cathayana* cuttings under different water supply. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The different letters above the bars indicate the significant difference among treatments at $P < 0.05$ according to Tukey's HSD test. F value and P value are shown. NS, $P > 0.05$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

Sex-specific relationships between A or g_s and water-related traits among treatments

In both sexes, we observed positive correlations between A and g_s; Ψ, leaf water content and g_s; and Ψ and leaf water content (except in males) and negative correlations between A or g_s and leaf carbon isotope composition ($\delta^{13}C$) across all treatments (Figure 6). Female A was higher than males at a given g_s (Figure 6a) or $\delta^{13}C$ (Figure 6d). We saw steeper relationships between A or g_s and water-related traits (Ψ, leaf water content or $\delta^{13}C$) in females than that in males, especially the relationship of g_s with Ψ, leaf water content or $\delta^{13}C$ (Figure 6b–g).

In addition, A and g_s were positively related with whole-plant hydraulic conductance (K_P) in both sexes (Figure 7a and b). Both of their relationships were steeper in females than in males.

Sex-specific relationships between A, A/R_d or g_s and leaf temperature among treatments

There were negative correlations between A, A/R_d or g_s and leaf temperature in both sexes (Figure 8a–c). We observed a steeper negative relationship between A or A/R_d and leaf temperature in males than in females, especially on A/R_d and leaf temperature. Males had higher A than females at a given leaf temperature (Figure 8a). Female g_s showed a steeper negative relationship with leaf temperature than in males (Figure 8c).

Relationships among all traits in each sex under different treatments

The PCA showed clear delineation based on trait combinations in the different treatments (Figure 9a). Warming with water supply treatments was well separated from each other in females (Figure 9b) and males (Figure 9c). The two-component PCA models explained 73.79%, 82.97% and 86.68% of the observed total variance in total individuals, female plants and male plants, respectively. Principal component 1 (PC1) was strongly influenced by leaf area, total dry mass, photosynthetic

water content was similar between sexes in each treatment (Figure 4b).

Leaf temperature was significantly influenced by sex and warming treatments (Figure 5). Warming treatment always increased leaf temperature. Female leaf temperature under warming decreased with water supplement, but water supplement had no effect on leaf temperature in males. Female leaf temperature was lower than that of males in each treatment.

Table 3. Effect of warming on whole-plant soluble sugar (SS_P), starch (SS_P) and total nonstructural carbohydrate (NSC_P) in female and male *P. cathayana* cuttings under different water supply.

Sex	Treatment	SS_P (mg g^{-1})	ST_P (mg g^{-1})	NSC_P (mg g^{-1})
Female	CK	62.52 ± 1.22ab	60.72 ± 1.64a	123.24 ± 0.83ab
	WA	67.59 ± 2.3a	56.97 ± 2.08a	124.56 ± 2.45a
	WM	54.74 ± 1.8cd	55.63 ± 1.23a	110.37 ± 1.6bcd
Male	CK	57.34 ± 1.25bc	59.83 ± 0.75a	117.17 ± 1.03bc
	WA	60.12 ± 1.56bc	44.77 ± 0.81b	104.89 ± 1.42de
	WM	49.24 ± 1.32d	49.62 ± 0.72b	98.86 ± 1.79e
	S	20.86***	27.775***	0.291^{NS}
	T	35.607***	29.303***	9.392***
	S × T	89.138***	48.237***	9.026***

S, sex effect; T, treatment effect; S × T, sex × treatment effect. The value is mean ± SE ($n = 4$). Different lowercases in the same column indicate a significant difference at 0.05 level according to Turkey's HSD test. F value and P value are shown. NS, $P > 0.05$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.
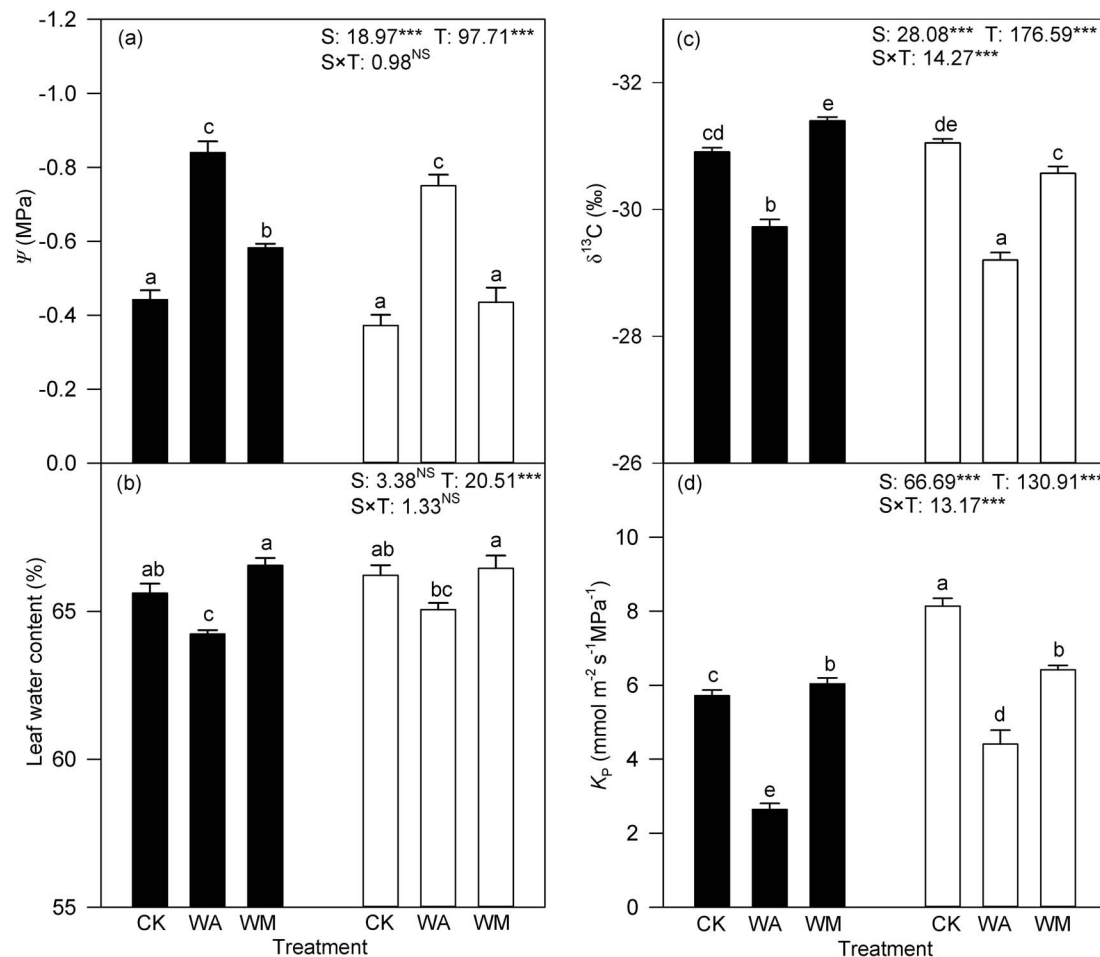
Figure 4. Effect of warming on predawn leaf water potential (Ψ; a), leaf water content (b), leaf carbon isotope composition ($\delta^{13}C$; c) and whole-plant hydraulic conductance (K_P; d) of female (black bar) and male (white bar) *P. cathayana* cuttings under different water supply. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The different letters above the bars indicate the significant difference among treatments at $P < 0.05$ according to Tukey's HSD test. *F* value and *P* value are shown. NS, $P > 0.05$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

rate, transpiration rate, water potential, K_P, g_s, F_v/F_m, $\delta^{13}C$, NPQ and leaf temperature. PC2 was strongly influenced by leaf dark respiration rate and whole-plant soluble sugar contents.

Discussion

In this study, we demonstrated the sex differences in growth, photosynthesis and water use of a dioecious tree species, *P. cathayana*, under an elevated temperature, and that the effect of warming was related to soil moisture. Warming without supplemental water treatment decreased plant growth, photosynthesis and water status in both sexes. However, adding supplemental water alleviated the effects of warming on females but not males. In addition, warming always decreased the ratio of photosynthetic rate to dark respiration rate (A/R_d) and plant NSC content, especially in coarse roots. Across all treatments, female stomatal conductance (g_s) and light-saturated photosynthetic rate (A) showed steeper relationships with water-related traits

than males, while A/R_d showed steeper relationships with leaf temperature in males than in females. These results suggest that the effect of high temperature in *P. cathayana* was mainly determined by soil moisture for females, while males were more sensitive in carbon assimilation and water balance to the direct effects of temperature per se. Sex differences in adaptive responses to heat may be related to differing water use strategies and thermal sensitivities.

Male growth and photosynthesis suffered more than females under heat

In this study, we found that most traits involved in growth, photosynthesis and water use of *P. cathayana* were similar between the sexes under ambient conditions (CK), but most of these traits were lower in males than in females under +4 °C warming with similar soil moisture (WM). Previous studies in this species usually showed that plant responses to warming can promote growth and biochemical processes (Xu et al. 2008b, Zhao et al.
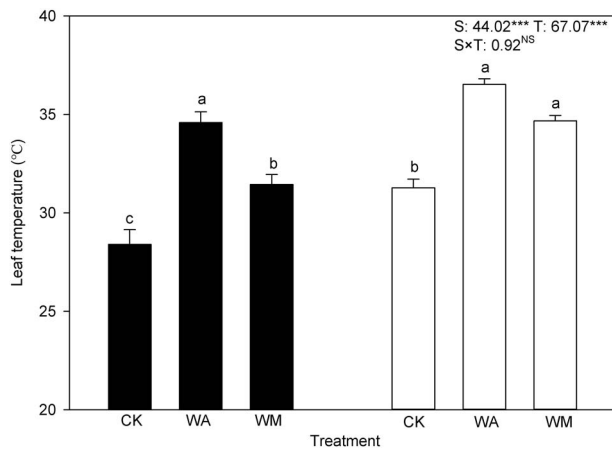
Figure 5. Effect of warming on leaf temperature of female (black bar) and male (white bar) *P. cathayana* cuttings under different water supply. S, sex effect; T, treatment effect; S × T, sex × treatment effect. The different letters above the bars indicate the significant difference among treatments at $P < 0.05$ according to Tukey's HSD test. F value and P value are shown. NS, $P > 0.05$; ***$P \leq 0.001$. Treatments are as defined in Figure 1.

2012, Yu et al. 2018). The apparent discrepancy of current and previous results reflects the differences in absolute temperature in the warming treatments. Plant responses to warming in growth and biochemical processes are often parabolic, where warming will promote photosynthesis when the temperature is below the optimum and impede it above the optimum (Saxe et al. 2001, Lin et al. 2012, Reich et al. 2018). Also, rising temperature often results in increased rates of respiratory and photorespiratory CO_2 release exceeding carboxylation rates, which impedes gains in biomass (Ameye et al. 2012, Zhu et al. 2018).

Our warming treatment led to leaf temperature increases in both sexes (Figure 5). The average temperatures of air and leaves (33.2 and 35.6 °C, respectively) were beyond optimal photosynthetic temperatures for *P. cathayana* (~28 °C; Figure S1 available as Supplementary Data at *Tree Physiology* Online), and there was a negative relationship between A or g_s and leaf temperature, such that A was reduced in response to heat stress (Vico et al. 2019, Winkler et al. 2019). Moreover, warming increased the leaf dark respiration rate (Figure 2c) and decreased the ratio of leaf photosynthetic rate to dark respiration rate (Figure 3), and this led to decreases in biomass accumulation. In addition, our present results show that the optimum temperature for photosynthesis in males was lower than in females (27.1 versus 28.9 °C; Figure S1 available as Supplementary Data at *Tree Physiology* Online), and we found higher leaf temperature (Figure 8a) for males than for females at a given A, which explains why males suffered more heat stress than females. Thus, the sex-specific responses in growth and photosynthesis to heat stress were mainly caused by a decrease in males rather than in females under heat. These results are
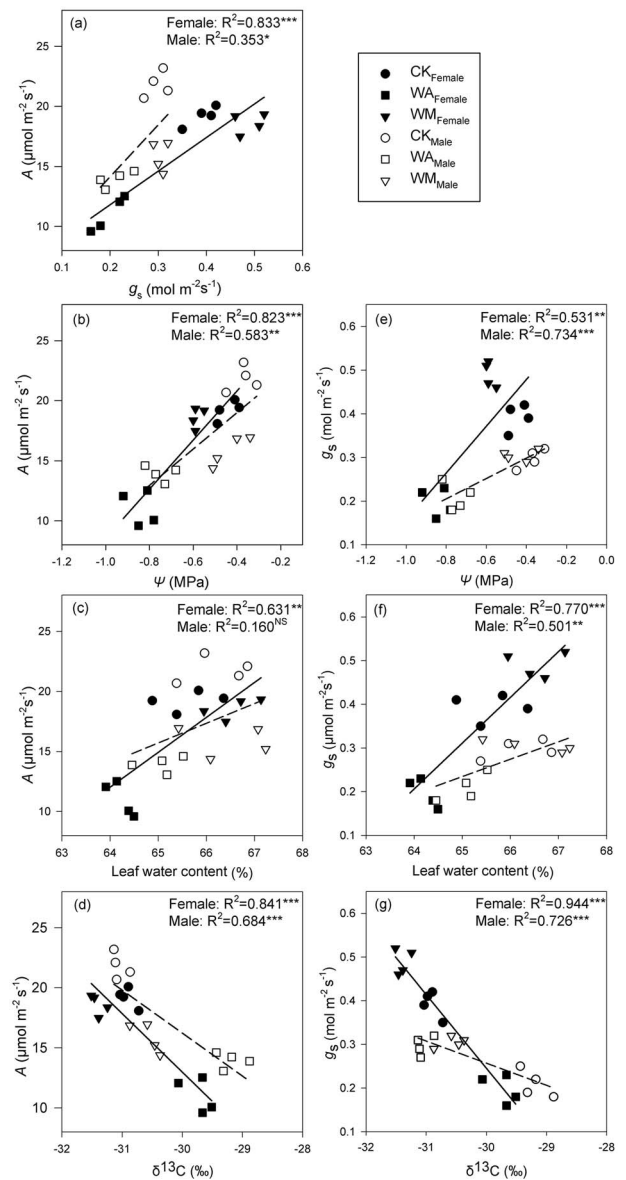

Figure 6. Relationship between light-saturated photosynthetic rate (A) and stomatal conductance (g_s; a), predawn leaf water potential (Ψ; b), leaf water content (c) and leaf carbon isotope composition ($\delta^{13}C$; d); and between stomatal conductance (g_s) and predawn leaf water potential (Ψ; e), leaf water content (f) and leaf carbon isotope composition ($\delta^{13}C$; g) across treatments in each sex. F value and P value are shown. NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P \leq 0.001$.

consistent with the previous studies in *Populus* showing that environmental stress (e.g., drought, nutrient deficiency, heavy metals) usually magnifies differences in growth and photosynthesis between the sexes (Xu et al. 2008a, Zhang et al. 2012, Melnikova et al. 2017, Liu et al. 2020, Xia et al. 2020).

Heat stress can directly affect plant metabolism, and leaves can minimize heat absorption and maximize dissipation of latent heat through biochemistry and stoma adjustment (Rennenberg et al. 2006). We found that warming always increased non-photochemical fluorescence quenching (NPQ). Higher NPQ can
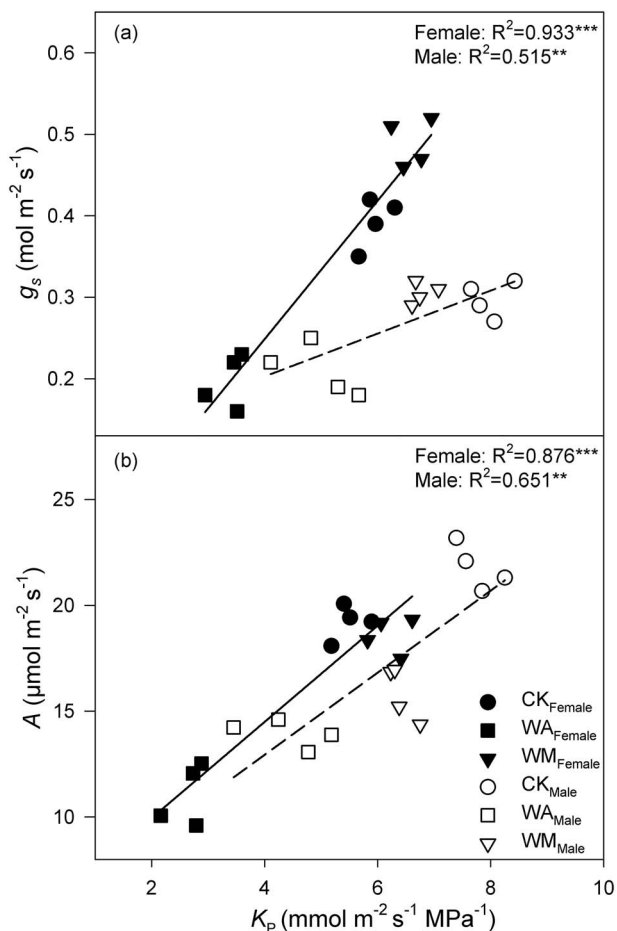
Figure 7. Relationship between stomatal conductance (g_s; a) and light-saturated photosynthetic rate (A; b) and whole-plant hydraulic conductance (K_P) across treatments in each sex. F value and P value are shown. **$P < 0.01$; ***$P \le 0.001$.

transfer and dissipate excitation energy, which can protect plants against heat and photoinhibition (Ruban 2016). On the other hand, heat stress from exceeding photosynthetic optima usually decreases the maximum rate of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) (Rennenberg et al. 2006, Way and Yamori 2014, Kumarathunge et al. 2019). However, we found that V_{cmax} and J_{max} of P. cathayana were not significantly affected by WM treatment, which suggests that the decrease in photosynthesis in males was not driven by photosynthetic biochemical limitation.

Besides biochemistry, stoma adjustment is also crucial for plant responses to heat, as stoma regulates CO_2 uptake and controls transpiration. We found that warming increased transpiration rate (E) as well as stomatal conductance (g_s) in females, but these traits in males were not affected (Figure 2b and d). Higher g_s in females may have two benefits: (i) for plant carbon assimilation by decreasing limitation of CO_2 entering cells and (ii) for heat tolerance by decreasing leaf temperature, which is important for responses to high temperature stress (Grossiord

et al. 2017). So, sex-specific differences in photosynthesis under heat may be mainly affected by stoma adjustment. Male P. cathayana suffered more suppression in growth and photosynthesis than females under heat with no soil water limitation, which may explain observations of female bias in lower elevation riparian populations (Meng et al. 2018).

Female responses were related with soil moisture rather than air temperature

Heat-induced declines in soil moisture usually increase plants' water deficit (Grossiord et al. 2017). In this study, we found a decrease in the average soil moisture content under warming without supplemental water (WA) treatment, and plant predawn leaf water potential and leaf water content were lower than under control conditions. These results indicate that plants suffered a water deficit under WA treatment, which supports the previous conclusion that P. cathayana is very responsive to soil moisture (Xu et al. 2008a, 2008b, Zhang et al. 2012). Xu et al. (2008b) found that female P. cathayana suffered a more negative effect on growth than do males under a combination of +4 °C warming and drought, similar to our findings that females had a lower A and that their biomass decreased more than males under WA treatment. Therefore, our results suggest that suppressed growth and leaf carbon assimilation of P. cathayana under WA treatment may be induced by heat and the concurrent water deficit (Hoeppner and Dukes 2012). Moreover, high-temperature treatments (including WA and WM) always decreased the ratio of leaf A to dark respiration rate by nearly 50% (A/R_d; Figure 3), and significantly negative relationships between A or A/R_d and leaf temperature could result in a higher risk of carbohydrate depletion and even mortality (Allen et al. 2010, Drake et al. 2016, Duan et al. 2018).

However, when growing under +4 °C with the same soil moisture content as CK (WM), female growth and photosynthetic rate were similar to those under CK, and they were higher than under WA treatment. These results indicate that the decrease of growth and photosynthesis of females under warming was related with soil moisture rather than air temperature. Higher temperatures will increase water transfer requirements at a given soil moisture (Vico et al. 2019). Our results showed that WM treatment led to a higher whole-plant hydraulic conductance (K_P) of females (Figure 4d). Increased K_P may directly increase leaf water supply and leaf water status (Sperry et al. 2002). Higher female K_P under WM treatment may compensate for higher E. These results indicate that females adjust water balance mechanisms under warming through changing water uptake, hydraulic transfer and use (Marchin et al. 2016).

Sex-specific water balance with warming

Previous findings have found that female A or g_s was more sensitive to changes in leaf water status than males (Xu et al.
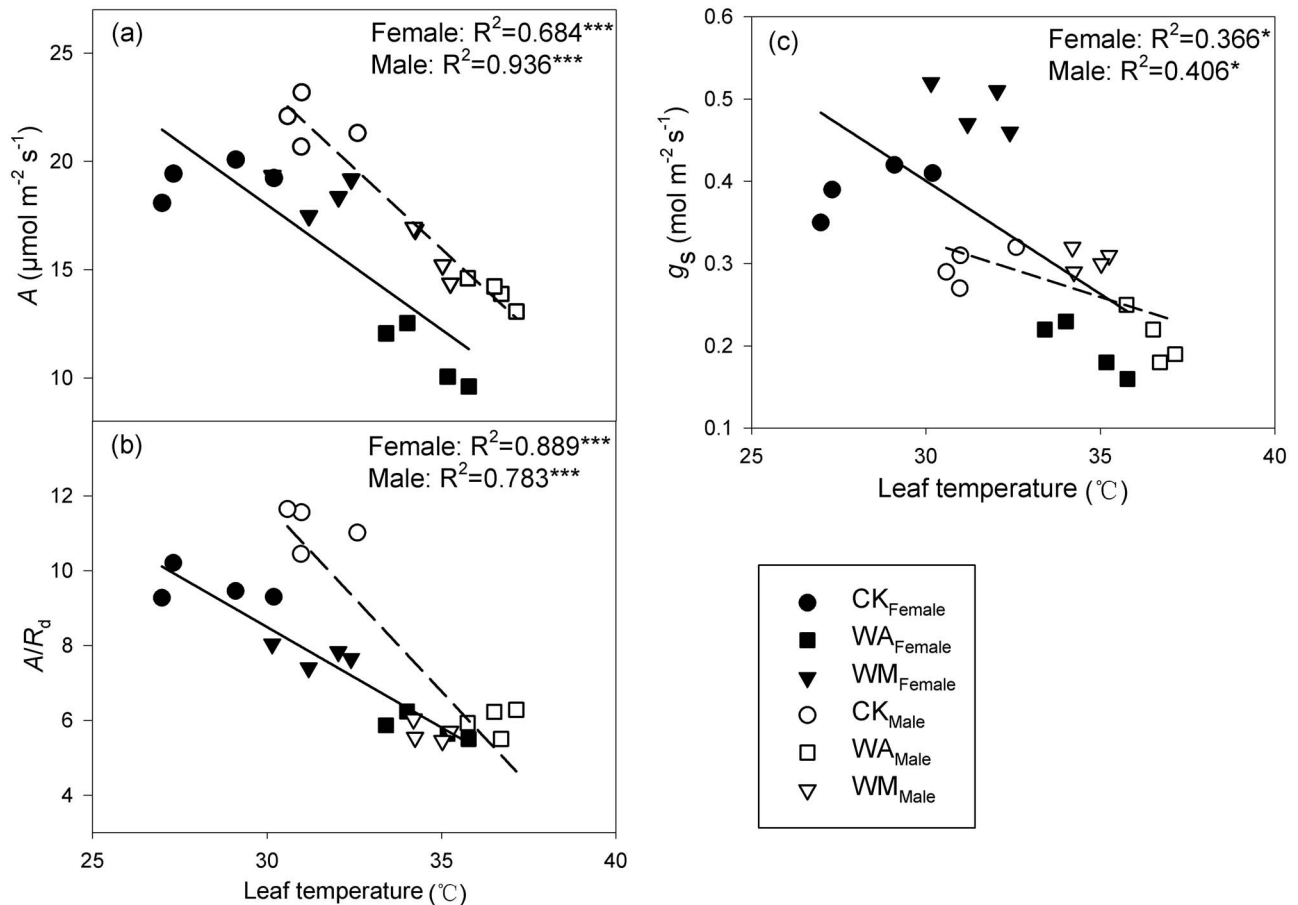
Figure 8. Relationship between light-saturated photosynthetic rate (A; a), carbon accumulation efficiency (A/R_d; b), stomatal conductance (g_s; c) and leaf temperature across treatments in each sex. F value and P value are shown. $*P < 0.05$; $***P \leq 0.001$.
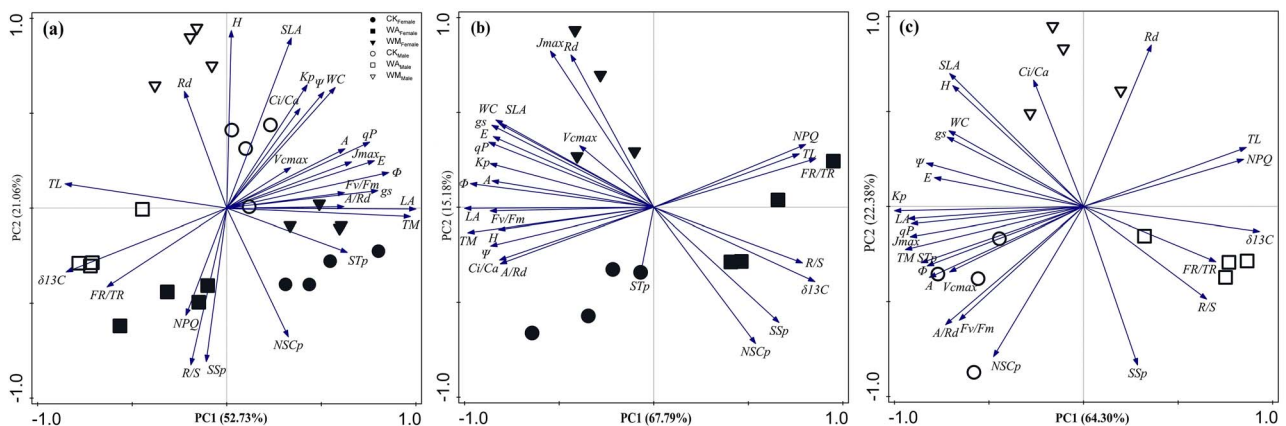

Figure 9. PCA based on the growth, photosynthesis and water use traits in each treatment (as defined in Figure 2; CK, circle; WA, square; WM, rhombus) in total (a), female (b) and male (c) *P. cathayana* cuttings. H, height; TA, total leaf area; *SLA*, special leaf area; TM, total dry mass; R/S, root-to-shoot ratio; FR/TR, the ratio of fine root mass to total root mass; A, photosynthetic rate; g_s, stomatal conductance; R_d, dark respiration rate of leaf; E, transpiration rate; SS$_P$, ST$_P$ and NSC$_P$, soluble sugars, starch and NSC concentration of whole plant; Ψ, leaf predawn water potential; C_i/C_a, the ratio of intercellular CO_2 concentration to ambient CO_2 concentration; V_{cmax}, the maximum rate of Rubisco carboxylation; J_{max}, maximum electron transport rates; F_v/F_m, maximum photochemical quantum yield of PSII; Φ, effective photochemical quantum yield of PSII; qP, coefficient of photochemical fluorescence; NPQ, non-photochemical fluorescence quenching; TL, leaf temperature; $\delta^{13}C$, carbon isotope composition; K_P, whole-plant hydraulic conductance.

2008*a*, 2008*b*, Zhang et al. 2012). In this study, we observed steeper positive relationships between *A* or g_s and leaf water status in females than in males (Figure 6b–g). These results support the previous findings. However, we also found that there were positive relationships between K_P and g_s or *A*, and females showed higher g_s or *A* at a given K_P than males. Moreover, WM treatment led to higher K_P of females but lower K_P of males, while male K_P was higher than females under CK and WA condition. Hydraulic conductance is a crucial trait for leaf water status, as it may decrease the stomatal sensitivity to water loss via transpiration (Sperry et al. 2002, Mitchell et al. 2013). These results suggest that sex-specific adaptive responses in hydraulic characteristics contribute to plant g_s or *A* adjustments in varied temperature and soil moisture environment. Up to now, there has been no study of sex-specific responses in hydraulic characteristic in dioecious woody trees to warming. Further study is needed to investigate the hydraulic characteristics of water transfer efficiency, which may help explain sex-related ecological strategies related to water sensitivity and population distributions, especially in a warming world (Olano et al. 2017).

On the other hand, we further found that leaf carbon isotope composition ($\delta^{13}C$, widely used to represent plant long-term water-use efficiency) in females was lower under CK but higher under WA or WM treatment than in males (Figure 4c). These results suggest that females and males responding to heat in water-use strategies are different, and female strategies may be more variable, whereas male strategies may be more conservative (Tognetti 2012, Hultine et al. 2016). Thus, our results collectively suggest that the differences in water balance strategies through the regulation of stomatal aperture, water-use efficiency and efficiency of the hydraulic system between sexes may affect their drought and heat resistances (Grossiord et al. 2017). Female *P. cathayana* responses showed greater sensitivity in water balance and a more liberal water-use efficiency than males, which may help compensate for female costs of reproduction (Barrett and Hough 2012, Lei et al. 2017).

Conclusions

In summary, we found that the effects of warming on growth and physiology were mediated by soil moisture. Carbon assimilation in female plants was more affected by water-related traits (e.g., leaf potential, whole-plant hydraulic conductance) than that of males, while males were more affected by leaf temperature. Sexually divergent responses of *P. cathayana* to warming and a flexible water balance strategy via stomatal regulation and water use mean that females show a higher heat tolerance than males. Future studies on sexual responses to environmental changes should consider traits in plant hydraulic characteristics. The predicted warmer conditions in wet areas are likely to amplify differences between sexes in dioecious species, which could lead to skewed sex ratios, sexual spatial segregation and potentially less successful reproduction.

Authors' contributions

J.L., R.Z. and T.D. had the main responsibility for data collection, analysis and writing, X.X. had a significant contribution to experimental design, J.F. and T.E.X.M. had significant contribution to the interpretation of data and manuscript preparation, and T.D. (the corresponding author) had the overall responsibility for experimental design and project management.

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