

Sex-specific responses of bud burst and early development to nongrowing season warming and drought in *Populus cathayana*

Lei Yu, Yan Han, Yonglei Jiang, Tingfa Dong, and Yanbao Lei

Abstract: Bud burst is one of the most observable phenological stages in tree species, and its responses to environmental factors are found to be species-specific. Nevertheless, for dioecious plants, whether the bud burst responses are sex specific remains an open question, as do the underlying physiological mechanisms. Here, we investigated the effect of elevated temperature (+2 °C) and drought (30% field capacity) during December–March on bud development, gas exchange, water and nitrogen status, and carbohydrate metabolism in female and male *Populus cathayana* to understand how nongrowing season warming and drought modifies physiological and phenological traits. Our results showed that at ambient temperature, males experienced earlier bud burst than females. Winter warming significantly delayed bud burst and even synchronized it for both sexes because of the greater responsiveness of males. Although drought exerted little effect on the timing of bud burst, it significantly reduced bud fresh mass and limited bud growth by decreasing gas exchange capacity and nonstructural carbohydrate (NSC) accumulation; moreover, females were more affected by drought stress. The significant sex × watering × temperature interactions for $\delta^{13}\text{C}$ and NSC indicate that sexual dimorphism in these condition-specific traits would increase along the environmental gradients, implying contrasting life history strategies in different ecological scenarios. The convergence in the time for bud burst caused by elevated temperature might exaggerate the competition among males, thus influencing the sex ratio, structure, and functioning of *P. cathayana* populations.

Key words: bud burst, nongrowing season, warming and drought, dioecious plant.

Résumé : Parmi les stades phénologiques, le débournement est un des plus facilement observables chez les arbres et ses réactions aux facteurs environnementaux sont propres à chaque espèce. Néanmoins des questions non résolues subsistent chez les plantes dioïques, à savoir le lien potentiel entre ces réactions et le sexe des individus, de même que la nature des mécanismes physiologiques sous-jacents. Nous avons étudié l'effet d'une augmentation de la température (+2 °C) et de la sécheresse (30 % de la capacité au champ) durant les mois de décembre à mars sur le développement des bourgeons, les échanges gazeux, les bilans hydrique et azoté ainsi que le métabolisme des hydrates de carbone chez les individus mâles et femelles du *Populus cathayana*. Le but était de comprendre comment le réchauffement et la sécheresse durant la saison dormante modifient les traits physiologiques et phénologiques. À la température ambiante, nos résultats montrent que le débournement survient plus tôt chez les individus mâles que femelles. Le réchauffement durant l'hiver a significativement retardé le débournement et l'a même synchronisé pour les individus des deux sexes étant donné la plus forte réponse des individus mâles. Même si la sécheresse avait peu d'effet sur le moment du débournement, elle a significativement réduit le poids frais des bourgeons et limité leur croissance en réduisant la capacité des échanges gazeux et l'accumulation des hydrates de carbone non structuraux (HCNS). De plus, le stress hydrique causé par la sécheresse a davantage nui aux individus femelles. Les interactions significatives entre le sexe, l'arrosage et la température dans le cas du $\delta^{13}\text{C}$ et des HCNS indiquent que le dimorphisme sexuel en lien avec les traits propres à ces conditions devrait augmenter le long des gradients environnementaux, ce qui implique des stratégies de cycle vital contrastées dans différents scénarios écologiques. La convergence dans le moment du débournement causée par l'augmentation de la température pourrait exacerber la compétition entre les individus mâles, ce qui influencerait par conséquent le rapport des sexes, la structure et le fonctionnement des populations de *P. cathayana*. [Traduit par la Rédaction]

Mots-clés : débournement, saison dormante, réchauffement, sécheresse, plante dioïque.

Introduction

The phenology of bud burst is fundamental to tree survival and growth in temperate and boreal regions of the world (Sakai and Larcher 1987) and is highly sensitive to climate change (Menzel et al. 2006; Schwartz et al. 2006; Morin et al. 2009; Yu et al. 2010; Jeong et al. 2011). Because the timing of phenological events has an important effect on plant mortality, annual growth, and repro-

ductive success (Fu et al. 2012; Chung et al. 2013), proper timing of plant phenology plays an important role in terrestrial ecosystems. Perennial plants have to synchronize physiological processes to seasonal changes through accurate sensing of environmental cues, including the length of photoperiod and light quality, as well as temperature fluctuation (Strømme et al. 2015). Therefore, detecting and understanding differential budburst responses to the environment among co-occurring species, together with the

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mechanisms involved, can shed light on community responses to environmental changes.

Land surface temperature is increasing, particularly in the northern hemisphere (IPCC 2014), where temperature is widely considered to be a major factor controlling phenology of temperate and boreal tree species (Schwartz et al. 2006). Thus, warming-induced phenological changes can affect ecosystem structure and functioning by extending the length of the growing season (Cleland et al. 2007; Piao et al. 2008; Fu et al. 2014), changing species composition (Chuine 2010), or altering the interaction between plants and animals (Memmott et al. 2007) and even between plants and climate (Peñuelas et al. 2009). Previous studies have showed that the timing of bud burst was often advanced by increasing temperature (Hollister et al. 2005; Kilpeläinen et al. 2006; Menzel et al. 2006); however, some evidence suggested that warming does not always produce a typical response but, rather, has no effect on phenological timing (Kuster et al. 2014) or even has a delaying effect (Heide 2003; Norby et al. 2003; Yu et al. 2010).

Several reasons have been suggested for the contrasting observations. Firstly, northern and southern ecotypes may react differently to autumn warming (Dormling et al. 1968), and temperature effects on tree phenology have been found to interact with photoperiod (Tanino et al. 2010; Olsen et al. 2014). Secondly, temperature increases are often associated with an increase in drought stress (Williams et al. 2013). No significant changes (Sherry et al. 2007), including earlier bud break (Sanz-Pérez and Castro-Díez 2010), were found under conditions of soil drought stress. Given the variation in temperature and drought effects on tree growth and development, investigation of the physiological causes of phenological responses may be a useful approach for improving climate change predictions (Schaber and Badeck 2003). More importantly, temperatures are predicted to increase disproportionately, being typically greater in winter and spring than in other seasons (Robeson 2004; Gu et al. 2008). However, most studies have focused on growing season, and only a few warming experiments were conducted in the nongrowing season. Among them, Yu et al. (2010) found that the spring phenology on the Tibetan Plateau was delayed mainly because of winter and spring warming. Pagter et al. (2015) insisted that even mild winter warming (October–April) could alter carbohydrate metabolism and modify phenological traits of *Ribes nigrum* L., but the extents were tightly dependent on genotype-specific differences in the chilling requirement. Therefore, the relationships between budburst phenology and multiple environmental factors, including warming and drought stress, especially in the nongrowing season, should be further tested under natural conditions by gradually altering environmental conditions.

The responses of bud burst to environmental factors have been proven to be species specific; nevertheless, whether there are sex-specific responses in dioecious plants remain an open question. Dioecious plants play an important role in maintaining the structure and function in terrestrial ecosystems (Wang and Curtis 2001), because they constitute nearly 6% of extant angiosperm species and are distributed among 959 genera of angiosperms (Renner and Ricklefs 1995). The sexual dimorphism in morphology, reproductive allocation, and resource-use efficiency can entail differential responses to environmental factors and result in niche divergence, variation in sex ratios, and sexual spatial segregation across environmental gradients (Bertiller et al. 2002; Li et al. 2007). Female-biased sex ratios appear to occupy moister and more nutrient-rich habitats, whereas males are more abundant than females under adverse environmental conditions (Ortiz et al. 2002). Greater responsiveness in females and therefore inferior performance under various abiotic and biotic environmental stresses has been observed for several species belonging to Salicaceae (Xu et al. 2008; Han et al. 2013; Chen et al. 2014; Zhang et al. 2014; Juvany and Munné-Bosch 2015; Dong et al. 2017).

Populus cathayana, an endemic dioecious tree species with fast growth, is distributed in the northern, central, and southwestern regions of China and plays an important role in maintaining ecological stability in these regions. Previous studies have shown sex-related differences of expanded leaves in responses to environmental stresses in protein composition, gene transcription, and morphological and physiological processes in *P. cathayana* in the growing season (Xu et al. 2008; Zhang et al. 2011; Han et al. 2013; Chen et al. 2014). However, much less attention has been paid to sex-related bud development and environmental changes (e.g., warming and drought) at the beginning of the growing season than leaf responses in the growing season, which is crucial to improve our predictions of gender-specific phenology responses to future climate change. Therefore, in this study, female and male *P. cathayana* were exposed to winter warming, drought, and their combination, with the goal to ascertain sexual differences in bud burst phenology and physiological mechanisms by assessing a suite of physiological responses, including gas exchange, water and nitrogen status, and carbohydrate metabolism. We hypothesized that (i) female *P. cathayana* are more responsive to nongrowing season environmental changes for bud development; (ii) compared with drought stress, warming exhibits stronger effects on bud burst and early development; (iii) furthermore, the two sexes differ in the magnitude and (or) direction of phenological responses to drought and warming; therefore, significant interactions existed among sex, temperature, and watering factors; and (iv) a suite of physiological processes, including gas exchange, water and nitrogen status, and carbohydrate metabolism, are involved in the contrasting phenological responses.

Materials and methods

Plant materials and experimental design

The experiment was carried out using 1-year-old female and male *P. cathayana* plants. Plants were produced from cuttings, which were grown in the field from 20 April to 29 November 2015. The day before initiation of the warming and drought treatment, 80 healthy cuttings (40 females and 40 males) with the same crown size and equal height were chosen and replanted into 10 L plastic pots filled with 8 kg homogenized soil (one cutting per pot), which was sieved surface sandy soil (0–30 cm) taken from the experimental site. Then, the cuttings were put in a greenhouse at the Chengdu Institute of Biology (CIB), the Chinese Academy of Sciences (CAS), on 30 November 2015.

On 1 December 2015, after growing for about 7 months, the 80 healthy cuttings (40 females and 40 males) of *P. cathayana* (naturally leaf free at that time of year) were subjected to warming and drought treatments. The experimental layout was completely randomized with three factors (sex, temperature, and watering regime). Two sexes (females and males), two temperature conditions (ambient temperature; +2 °C compared with ambient temperature), and two watering regimes (well-watered, 100% field capacity; drought, 30% field capacity) were employed. Ten replicates per treatment were included in the experiment. All plants were watered with varying amounts of water every other day. Measurements of soil water content (SWC) by a time-domain reflectometer (Robinson et al. 2003) demonstrated that the average SWC was maintained at 38.5% in the well-watered treatment and at 12.9% in the drought treatment. Evaporation from the soil surface was prevented by enclosing all pots in plastic bags sealed at the base of the stem of each cutting. In elevated temperature treatments, four climate-controlled chambers (south facing) were employed to warm treatments about 2 °C above ambient air temperature (Naudts et al. 2011, 2013; Yu et al. 2016). The interior surface area was 2 m × 2 m; the height at the north side was 2 m, and at the south side, it was 1.5 m. Two small electric fans were installed at the height of 1.5 m on east and west sides to keep the CO₂ concentrations in the chambers the same as the outside concentration.

The top of the chambers consisted of a colorless polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film (200 μm thick), with both being UV transparent. Supplementary Figure S1¹ presents the elevated air temperature and the ambient air temperature from December 2015 to March 2016. In addition, the day length in the treatments from 1 December 2015 to 31 March 2016 ranged from 10.2 to 12.5 h in our study region. The experimental treatments lasted 16 weeks; they started on 1 December 2015 and were harvested on 1 April 2016. Thus, a total of four treatments were included: (i) ambient temperature and well-watered (AW); (ii) ambient temperature and drought (AD); (iii) elevated temperature and well-watered (EW); (iv) elevated temperature and drought (ED). The height and bud number per plant ranges were 85–95 cm and 30–36 buds, respectively, and did not show significant differences among treatments.

Determination of bud burst

Bud burst was observed every other day, and the date was recorded. Bud burst was defined by the appearance of the first green leaf tip (Basler and Körner 2014). Five stages (dormant, swollen, bud burst, leaf unfolding, and juvenile leaves) for bud burst and early development were observed in *P. cathayana* in our study. At the end of March, when gas exchange, bud fresh mass, and other parameters were measured, buds were roughly at the juvenile leaf stage under the AW treatment, leaf unfolding stage under AD and EW treatments, and bud burst stage under the ED treatment. Therefore, broken buds, unfolding leaves, and juvenile leaves were used for measurements in our study. At the end of the experiment, five cuttings from each treatment were selected randomly to measure bud fresh mass. Then, bud samples were dried at 70 °C for 48 h.

Determination of bud gas exchange

The bud net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and dark respiration (R_d) were measured from five randomly chosen individuals in each treatment using the LI-COR 6400 portable photosynthesis measuring system (LI-COR, Lincoln, NE, USA) on 26 and 27 March 2016. Gas exchange measurements were taken between 08:00 and 11:30, and carbon dioxide gas cylinders were used to provide a constant and stable CO_2 concentration. A conifer type chamber (PLC-broad, PP Systems) was used for P_n , g_s , and E measurements under the following conditions: leaf temperature, 25 °C; leaf air vapour pressure deficit, 1.5 ± 0.5 kPa; relative humidity, 50%; $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD; and CO_2 concentration, $400 \pm 5 \mu\text{mol}\cdot\text{mol}^{-1}$. Once an apparent steady gas exchange was achieved, the steady-state data were recorded. Measurements of R_d were obtained through gas-exchange measurements after covering the leaf chamber for 15 min; P_n , g_s , E , and R_d were calculated on mass basis. The bud nitrogen (N) content (mass basis, $\text{mg}\cdot\text{g}^{-1}$) was determined using the semi-micro Kjeldahl method (Mitchell 1998). Then, the ratio of P_n to N was regarded as photosynthetic nitrogen use efficiency (PNUE).

Determination of bud nonstructural carbohydrate content

Soluble sugar and sucrose were extracted from dried buds in 80% (v/v) ethanol. The soluble sugar was detected colorimetrically (using a Multiskan GO 1510 spectrophotometer, Thermo Fisher Scientific, Finland) at 625 nm following the anthrone–sulphuric acid method (Yemm and Willis 1954). Sucrose was also detected colorimetrically at 480 nm following the modified resorcinol method (Murata 1968). Then, the starch content was determined for the pellet of plant material that remained after the removal of ethanol. The solutions were filtered through Whatman GF/C filters and diluted in 10 mL volumetric flasks. The concentrations of starch as glucose equivalents were determined colorimetrically at

620 nm. The absorption of an enzyme blank was subtracted from each sample's absorbance prior to the calculation of the sugar content. The amount of total nonstructural carbohydrates (NSC) was calculated as the sum of soluble sugar and starch.

Determination of bud water potential

On 30 March 2016, the intact buds from five randomly chosen cuttings in each treatment were cut and sealed immediately in small plastic bags containing moist paper towels. Their predawn bud water potential was then determined with WP4C Dew point Potentiometer (Decagon Devices, Inc., Pullman, WA, USA) before sunrise.

Determination of bud carbon isotope composition

The carbon isotope composition ($\delta^{13}\text{C}$) was determined for the same buds that had been used for net photosynthetic rates measurements. Bud samples were oven-dried at 70 °C for 48 h. Dried buds were ground, and ^{13}C to ^{12}C ratios were determined with an isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., USA). The carbon isotope composition was expressed as $\delta^{13}\text{C}$ values. The overall precision of the δ values was better than 0.1‰, as determined by repetitive samples. The $\delta^{13}\text{C}$ values were reported relative to the international standard, Pee Dee Belemnite (PDB). The entire analysis was performed in the Stable Isotope Laboratory for Ecological and Environmental Research, Chinese Academy of Forestry.

Statistical analysis

The statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0. Before the statistical analyses, data were checked for normality and the homogeneity of variances and log transformed to correct deviations from these assumptions when needed. Individual differences among means were determined by Tukey's HSD tests of one-way ANOVA. Three-way ANOVAs were used to test the effects of sex, temperature, watering, and their interactions. All statistical analyses were set at a significance level of $P < 0.05$. Principal component analysis (PCA) of ecophysiological traits was used to identify the most discriminatory effects of elevated temperature and drought. PCA analyses were performed using Canoco 5.0 (Microcomputer Power, USA).

Results

Sexual differences in bud development under nongrowing season warming and drought

Single factors (sex or temperature) or their interactions significantly influenced the time of bud burst (Fig. 1A). At ambient temperature, compared with female individuals, male individuals displayed earlier bud burst under both well-watered and drought treatments. Winter warming significantly delayed bud burst; moreover, warming synchronized bud break, as there were no significant differences for time of bud burst under warming conditions regardless of sex and watering (Fig. 1A). In addition, the sex \times watering and sex \times temperature interactions significantly affected bud fresh mass (Fig. 1B). Drought stress and elevated temperature significantly reduced bud mass, and only male individuals showed higher bud weight under drought stress.

Sexual differences in bud gas exchange under nongrowing season warming and drought

The sex \times watering interaction significantly affected bud P_n , g_s , E , and R_d (Fig. 2). Drought significantly decreased bud P_n , g_s , E , and R_d of both sexes; meanwhile, an elevated temperature enhanced g_s , E , and R_d (Figs. 2A–2D). There was no significant sexual difference under ambient temperature, except for E (Fig. 2C). Under

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2017-0259>.

Fig. 1. Bud development in female and male individuals of *Populus cathayana*, as affected by elevated temperature, drought, and their combination. (A) time for bud burst and (B) bud fresh mass. Each value is the mean \pm standard error ($n = 5$). Different lowercase letters indicate significant differences according to Tukey's HSD test at a significance level of $P < 0.05$. Three-way analyses of variance (ANOVA) were applied to evaluate the effects of different factors and their interactions. S, sex effect; T, temperature effect; W, watering effect; interactions between any of these three effects are indicated by an "x". ns, not significant; *, $0.01 < P < 0.05$; **, $0.001 < P \leq 0.01$; and ***, $P \leq 0.001$. Time for bud burst, days after 1 January. AW, ambient temperature and well-watered; AD, ambient temperature and drought; EW, elevated temperature and well-watered; ED, elevated temperature and drought.

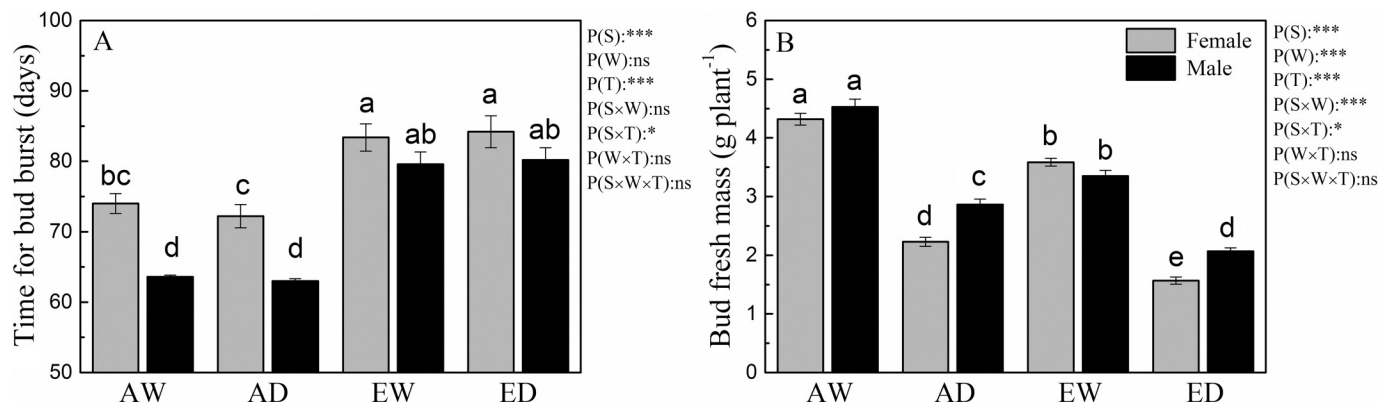
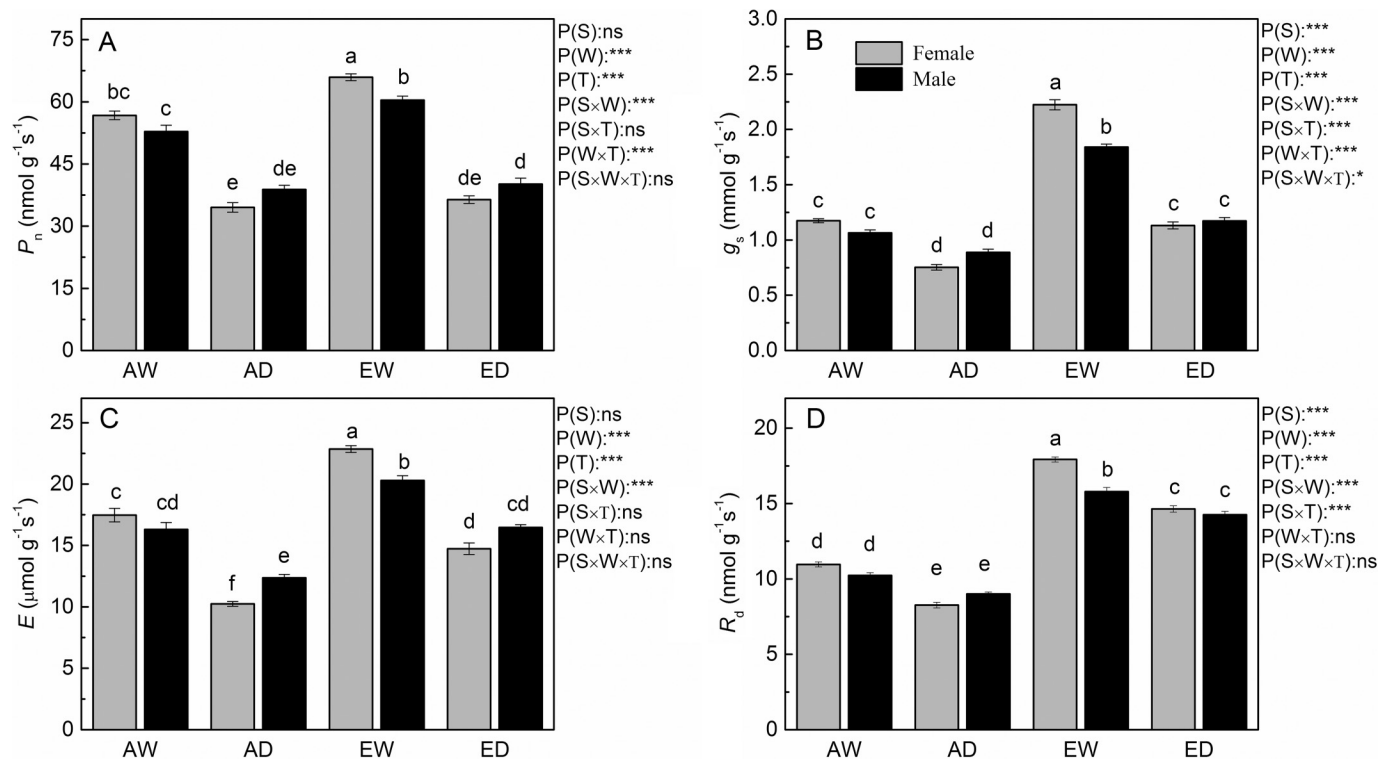


Fig. 2. Gas exchange characteristics of buds in female and male individuals of *Populus cathayana*, as affected by elevated temperature, drought, and their combination. (A) Net photosynthesis rate per dry mass (P_n); (B) stomatal conductance (g_s); (C) transpiration (E); and (D) dark respiration (R_d). Each value is the mean \pm standard error ($n = 5$). Treatment codes and statistical analyses are as in Fig. 1.



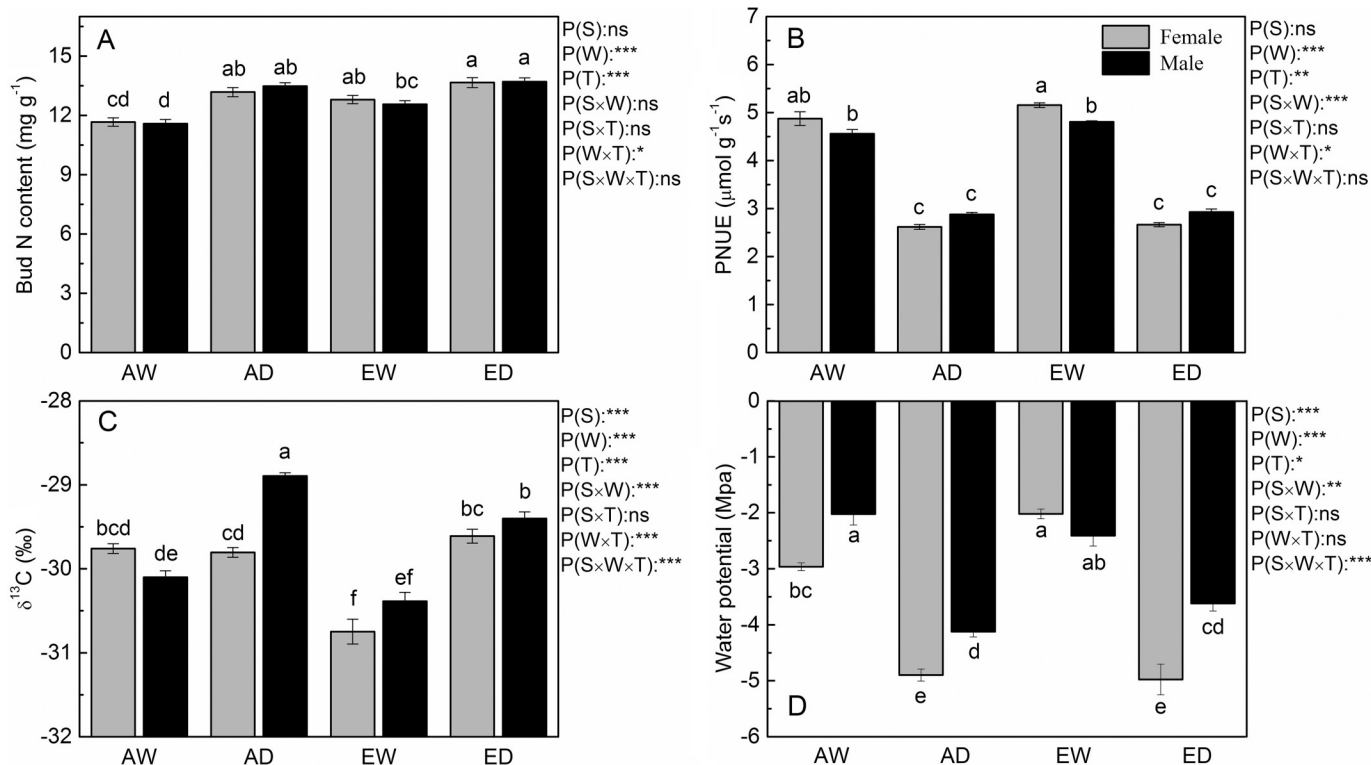
elevated temperature, females showed higher gas exchange potentials than males under well-watered conditions but showed no differences under drought stress. These findings implied that females were more responsive to watering.

Sexual differences in bud water and nitrogen status under nongrowing season warming and drought

The temperature \times watering interaction significantly affected bud N content (Fig. 3A). Elevated temperature and drought stress increased bud N content of both sexes; moreover, the enhanced magnitude was more pronounced under ambient temperature.

Drought stress significantly decreased PNUE, whereas sex and temperature had little effect on PNUE (Fig. 3B). The sex \times watering and sex \times watering \times temperature interactions significantly affected bud $\delta^{13}C$ and water potential (Figs. 3C and 3D). Drought increased the $\delta^{13}C$ value of both sexes, except females kept at ambient temperature. In contrast, elevated temperature decreased the value of $\delta^{13}C$ of both sexes, except females in drought conditions. Moreover, warming caused greater decreases for females under well-watered conditions, whereas greater decreases occurred for males under drought stress (Fig. 3C). For water

Fig. 3. Bud water and nitrogen status in female and male individuals of *Populus cathayana*, as affected by elevated temperature, drought, and their combination. (A) Bud N content; (B) photosynthetic N use efficiency (PNUE); (C) carbon isotope compositions ($\delta^{13}\text{C}$); and (D) water potential. Each value is the mean \pm standard error ($n = 5$). Treatment codes and statistical analyses are as in Fig. 1.



potential, warming exerted little effect under drought stress, whereas under the well-watered conditions, contrasting directions for sexes were found, namely increases in females and decreases in males (Fig. 3D).

Sexual differences in carbohydrate concentrations under nongrowing season warming and drought

Generally, sucrose concentrations were higher in females compared with males, whereas soluble sugars, starch, and total NSC were higher in males, except for starch under combined warming and drought (Figs. 4A–4D). Sucrose concentrations changed slightly among different treatments (Fig. 4A). Compared with ambient temperature conditions, starch and NSC contents of both sexes were decreased by elevated temperature and drought, as well as their combination (Figs. 4C and 4D). The sex \times watering \times temperature interactions significantly affected NSC. Under well-watered conditions, elevated temperature caused more of an NSC decrease in females, whereas under drought stress, NSC decreased more in males (Fig. 4D).

Relationships among physiological traits under different sex, temperature, and watering regimes

The first two axes of the PCA explained 89.2% of the total variation of the physiological traits in *P. cathayana*'s response to warming and drought treatments (Fig. 5). PC1 explained 62.3% of the observed variance and was strongly correlated with NSC, starch, g_s , E , and R_d . Alternatively, 27.0% of the variance could be explained by PC2, which was strongly influenced by bud fresh mass, PNUE, P_n , sucrose, and N content (Fig. 5). Ambient and elevated temperature treatments were separated along the first axis of the PCA and grouped on the left- and right-hand sides of PCA, respectively. Well-watered and drought stress treatments were separated by PC2, whereas sexual effects showed modest differentiation (Fig. 5).

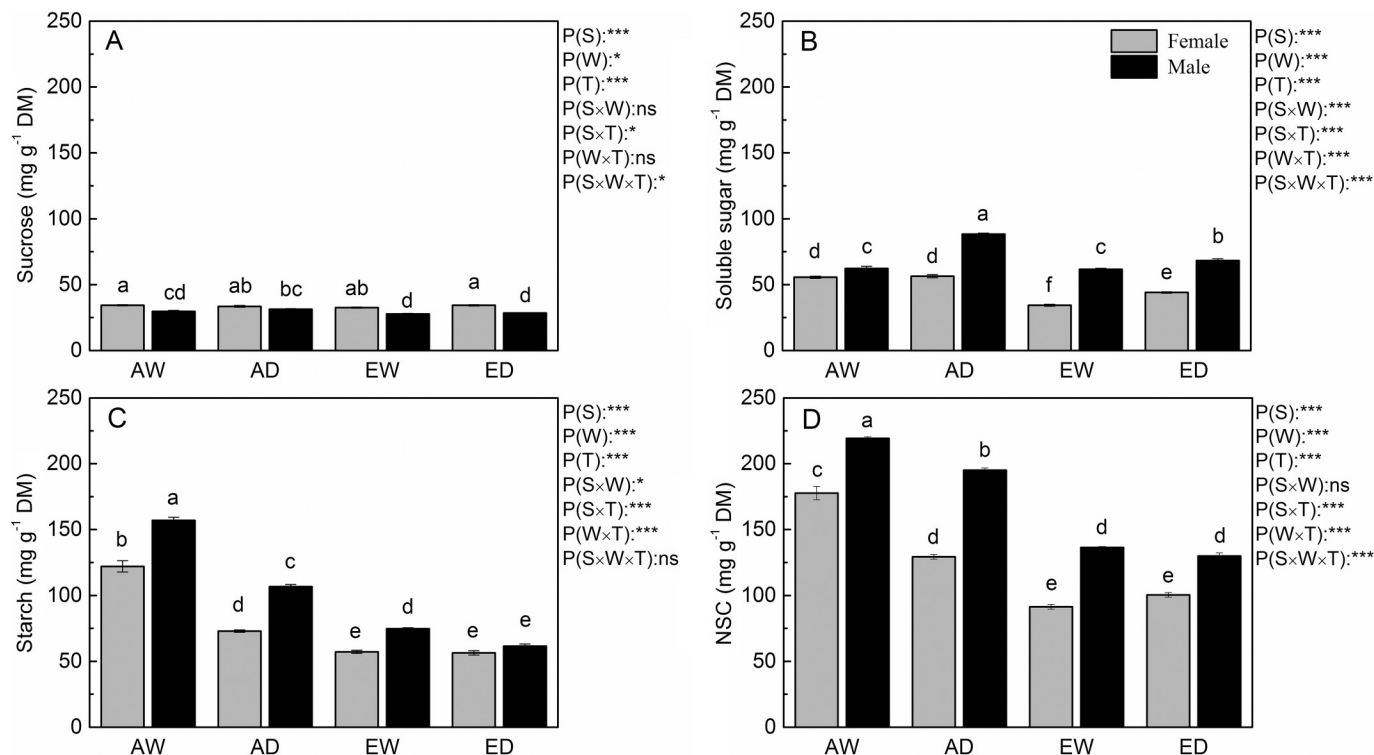
Discussion

Responses of bud burst phenology to nongrowing season warming and drought

At ambient temperatures, compared with female individuals, male individuals displayed earlier bud burst under both well-watered and drought treatments (Fig. 1A). It is generally assumed that females, which produce both flowers and fruits and allocate more resources to reproduction, will require more time before leafing, flowering, and fruiting (Obeso 2002; Juvany and Munné-Bosch 2015). Conversely, consistent with the earlier bud burst, protandry, the maturation, arrival or emergence of males before conspecific females, is common in many taxa (Morbey and Ydenberg 2001). One hypothesis is that competition for access to high-quality unfertilized ovules favors early-flowering males; especially when females mate only once, the optimal and evolutionarily stable male strategy is to emerge somewhat earlier than females (Forrest 2014).

Elevated temperatures in winter significantly delayed bud burst for male and female individuals, which was mainly attributed to the insufficient chilling to meet normal phenological requirements. Chilling has been reported to affect the subsequent temperature response of bud growth in most boreal and temperate plants (Badeck et al. 2004). Thus, the chilling requirement is an adaptation to prevent early onset of growth, minimizing the risk of frost damage because of early and temporary spring warming episodes (Cannell and Smith 1983). Our results are in accordance with earlier studies that reported that inadequate chilling in warmer winter may delay bud burst of *Pseudotsuga menziesii* (Mirb.) Franco (Harrington et al. 2010) and of *Betula pubescens* Ehrh. and *Populus tremula* L. (Pletsers et al. 2015). Moreover, males were more responsive to winter warming, because more delayed effects in males caused a convergence in bud burst phenology in male and

Fig. 4. Bud nonstructural carbohydrate concentrations in female and male individuals of *Populus cathayana*, as affected by elevated temperature, drought, and their combination. (A) sucrose; (B) soluble sugar; (C) starch; and (D) NSC. Each value is the mean \pm standard error ($n = 5$). Treatment codes and statistical analyses are as in Fig. 1.



female *P. cathayana* under an elevated temperature (discussed below). Meanwhile, our study showed that watering conditions did not affect the timing of bud burst (Fig. 1A). This was in agreement with Sherry et al. (2007) who also found that a double precipitation treatment had little effect on flowering and fruiting phenology for 12 species in a tallgrass prairie in North America.

Physiological mechanisms for bud development under environmental changes

In the present study, we discovered that gas exchange, water and nitrogen status, and nonstructural carbohydrate content in female and male *P. cathayana* seedlings were affected by nongrowing season warming and drought treatments. Water use efficiency (WUE) is considered an important component of adaptation to water stress, with stable carbon isotope composition ($\delta^{13}\text{C}$) an indicator of plant long-term water use efficiency (Li 1999; Guo et al. 2016). It has been speculated that a decrease in g_s caused by drought is responsible for the significant increase in WUE (Zhang et al. 2005), and the decrease in WUE resulting from an increase in the E rate is mainly attributed to high temperature (Kellomäki and Wang 2001). Furthermore, a greater $\delta^{13}\text{C}$ caused by drought may result in decreased E , the closure of stomata, and increased fixation of ^{13}C . Some studies have also detected a trade-off between PNUE and WUE, which may partially explain the distribution of plants in relation to moisture and nutrient availability (Field et al. 1983; Martin et al. 2010). Thus, the trade-off between WUE and PNUE reflects the ability of the tree to maximize the efficiency of use of limited resources.

Bud burst requires a carbon supply for metabolic reactivation and leaf primordial growth. Previous studies showed that carbohydrate availability is critical to bud burst (Maurel et al. 2004; Bonhomme et al. 2010). Similar patterns for starch and NSC were observed as pronounced decreases under winter warming (Fig. 4), which coincided with delayed bud burst (Fig. 1). These results were consistent with the source-sink dynamics concept, in which

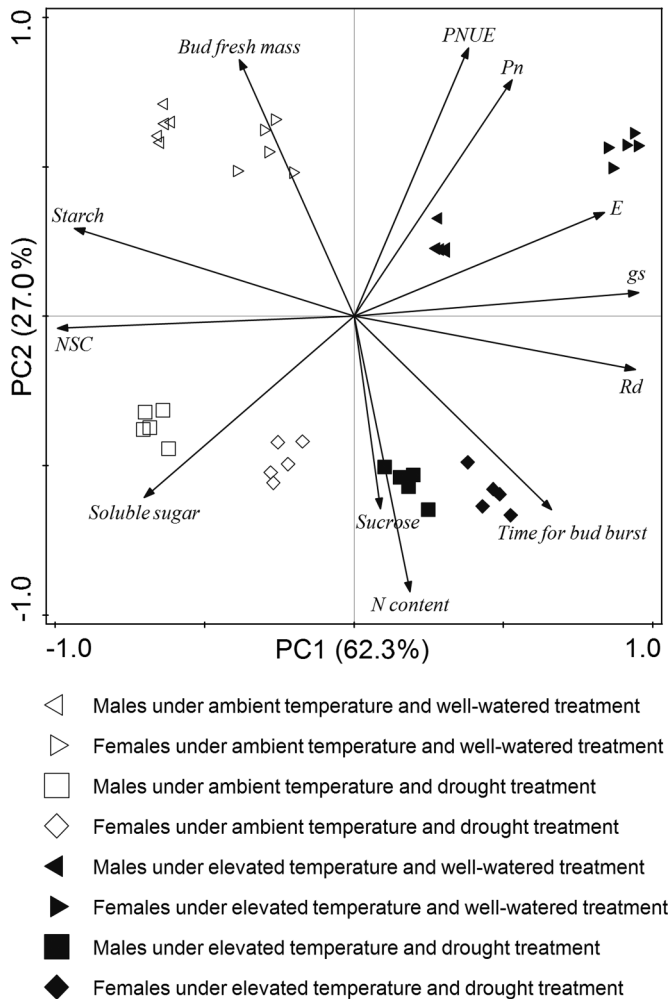
stored NSC was a reservoir of carbon that fluctuated depending on the balance between supply via photosynthesis and demand for growth and respiration (Martínez-Vilalta et al. 2016). The modest changes in sucrose and soluble sugar indicated that developing leaves already exhibited a positive carbon balance, which was supported by earlier models that predicted a transition from carbon sinks to sources for developing leaves of deciduous trees before they reached 50% of their final size (Marchi et al. 2005). Unfortunately, because of single sampling, our study did not capture the dynamic changes in NSC concentrations before and after bud burst.

Complex interactions among sex, watering, and temperature on bud development

According to the PCA, ambient and elevated temperature treatments were separated along the first axis of the PCA, which were strongly correlated with NSC, starch, g_s , E , and R_d and accounted for 62.3% of the observed variance. Well-watered and drought stress treatments were separated by PC2, which was mostly influenced by bud fresh mass, PNUE, P_n , sucrose, and N content; meanwhile, sexual effects showed modest differentiation (Fig. 5). As suggested by our hypothesis, these results implied that 'compared with drought stress, winter warming caused stronger effects on bud burst and early development in *P. cathayana*, which was also supported by the fact that all studied parameters were significantly affected by temperature, whereas there was no watering effect on bud burst time and no sexual difference on P_n , E , N, and PNUE (Figs. 1–4).

There were complex interactions among the three factors (sex, watering, and temperature). For example, significant interactive effects of temperature \times watering for many studied parameters, including P_n , g_s , PNUE, N, and $\delta^{13}\text{C}$, indicated that the responsiveness was environment dependent, i.e., plants were more affected by winter warming when combined with well-watered conditions (Figs. 2–4). For bud burst time, our results showed that males were

Fig. 5. Principal component analysis (PCA) based on eco-physiological traits of female and male *Populus cathayana* under elevated temperature and drought treatments. P_n , net photosynthesis rate; g_s , stomatal conductance; E , transpiration rate; and R_d , dark respiration.



more delayed by winter warming compared with females (Fig. 1). The greater responsiveness in males was in line with the findings of Strømme et al. (2015) who also found that male *P. tremula* were more strongly affected by UVB and temperature on bud maturation, which could potentially yield sex-related differences in performance, sex ratios, and survival of *Populus*. Similarly, Wang and Curtis (2001) claimed that male saplings of *P. tremuloides* had higher photosynthesis than females throughout the growing season regardless of CO_2 concentrations and sexual difference in photosynthesis were greater at elevated than ambient CO_2 concentrations, as males were more responsive to CO_2 enrichment. However, for other parameters, females were more affected, especially by drought stress, including bud mass and photosynthetic capacity (P_n , g_s , E , R_d), as indicated by the significant sex \times watering interaction (Figs. 1 and 2).

For some parameters, there were even sex \times watering \times temperature interactions. For $\delta^{13}C$, warming caused greater decreases in females under well-watered conditions but greater decreases in males under drought stress, thus resulting in significant interacting effects of sex \times watering \times temperature (Fig. 3C). Similarly, for NSC, under well-watered conditions, elevated temperature caused greater decreases in females; nevertheless, under drought stress, greater decreases in males occurred, which was further supported by the significant interactive effect among sex \times watering \times

temperature (Fig. 4D). The interactions suggest that sexual dimorphism in these condition-specific traits would increase along the environmental gradients, implying contrasting life history strategies in different ecological scenarios. Consequently, these findings may have significance in predicting the potential response of dioecious species to global environmental changes.

In our study, winter warming synchronized the bud burst in male and female *P. cathayana* (Fig. 1). Opposite to our findings, Sherry et al. (2007) claimed that warming resulted in a phenological divergence in reproductive phenology among 12 monocious grassland species. Consequently, they speculated that those variations in the direction and magnitude of their responses to warming caused compression and expansion of the reproductive periods of different species and created possibilities for an altered selective environment to reshape communities in a future warmed world. The convergence in the bud burst time may exaggerate the competitions among male *P. cathayana* for pollination and may also alter the population sex ratios, structures, and productivities.

Conclusions

Our results showed that at ambient temperature, males experienced earlier bud burst than females. Timing for bud burst was significantly delayed by winter warming and even synchronized for male and female individuals because of the greater responsiveness in males. A suite of physiological processes, including gas exchange, water and nitrogen status, and carbohydrate metabolism, contributed to the contrasting phenological responses. There were complex interactions among the three factors (sex, watering, and temperature) on the studied parameters. The sex \times watering interaction significantly affected bud mass and photosynthetic capacity and indicated that females were more sensitive to drought. The significant sex \times watering \times temperature interactions for $\delta^{13}C$ and NSC suggest that the sexual dimorphism in these condition-specific traits would increase along these environmental gradients, implying contrasting life history strategies in different ecological scenarios. The convergence in the bud burst time may exaggerate the competition among male *P. cathayana* individuals for pollination, especially in extreme years, and may also alter the population sex ratios, structures, and productivities. Consequently, these findings clearly showed the complex responses to global environmental changes in a dioecious species. Thus, comparisons of the performance between sexes on long-term growth and reproduction will be critical to interpret the responses to global change.

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