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Sex-specific nitrogen allocation tradeoffs in the leaves of *Populus cathayana* cuttings under salt and drought stress

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ABSTRACT

Nitrogen (N) partitioning within a leaf affects leaf photosynthesis and adaptation to environmental fluctuations. However, how plant sex influences leaf N allocation and its tradeoffs in acclimation to drought, excess salt and their combination remains unknown. Here, leaf N allocation between the photosynthetic and non-photosynthetic apparatus and among the components of the photosynthesis in *Populus cathayana* Rehder females and males were investigated under drought, salt and their combination to clarify the underlying mechanism. We found that males with a lower leaf N allocation (N_L) into non-protein N (N_{np}), showed a greater leaf N allocation into photosynthetic apparatus, especially into the carboxylation component under all treatments, and a greater leaf N allocation into cell wall under drought and salt stress alone, consequently causing higher photosynthetic N use efficiency (*PNUE*) and tolerance to stresses. Conversely, females had a greater leaf N allocation into N_{np} under all treatments than males and a lower leaf photosynthetic N (N_P) allocation. There was a tradeoff in leaf N allocation among photosynthetic apparatus (N_P/N_L), cell wall (N_{CW}/N_L) and N_{np}, which explained plant responses to drought, salt and their combination. Moreover, the leaf N allocation into the carboxylation component could explain the intersexual difference in responses to all treatments, while leaf cell wall N (N_{CW}) and N_{np} reflected intrasexual differences among treatments in both sexes. These findings indicate sex-specific strategies in coping with drought, salt and their combination that relate to leaf N allocation, which may contribute to sex-specific photosynthesis and niche segregation.

1. Introduction

Plants are often subjected to drought and salt stress during their life cycle. It is estimated that more than 10 percent of arable land suffers from drought and salinity, especially in arid or semi-arid regions influenced by global climate change (Wicke et al., 2011). Photosynthetic capacity and plant growth are critical processes affected by drought and salt stress. Therefore, understanding the photosynthetic limitation and the regulatory mechanism under drought and salt stress is especially important for alleviating the negative impact of such stressful factors on agroforestry production and ecosystem functioning (Chaves et al., 2011).

N is one of the most nutrient elements determining leaf functional trait via the formation of the photosynthetic machinery, functional proteins and structural compounds (Takashima et al., 2004; Ghimire et al., 2017; Liu et al., 2018). Leaf N not only adjusts the mesophyll cell thickness and permeability that affects CO_2 diffusion within leaves, but

also changes photosynthetic enzyme contents and activities (Zhong et al., 2019). The capacity of leaf photosynthesis is associated with leaf N content, especially the relative allocation of leaf N to the photosynthetic apparatus (Liu et al., 2018; Zhong et al., 2019). In leaves, approximately half of the total leaf N is allocated into the photosynthetic apparatus (Hikosaka and Shigeno, 2009). Therefore, some authors proposed that the photosynthetic capacity of leaves could be predicted by the N content (Evans and Clarke, 2019; Zhong et al., 2019). Besides photosynthetic N allocation, large proportions of leaf N are generally allocated into cell walls, where they act as a structural component of plant defense and chemical defense (Onoda et al., 2017). The increased N allocation to cell walls would reduce the allocation of N into the photosynthetic apparatus, and vice versa (Takashima et al., 2004; Onoda et al., 2017). Additionally, it has been reported that c. 50% of leaf N exists elsewhere than in cell walls or photosynthetic proteins. Among those, non-protein N (N_{np}) plays important roles as allelopathic chemicals and/or metabolites in signaling, nutrient acquisition and stress responses (Huang

et al., 2011; Vranova et al., 2011). Besides that, leaf photosynthesis is also predicted by the leaf dry mass based on leaf area (*LMA*) (Evans and Clarke, 2019). *LMA* is a multiple structural parameters related to plant the energy trade-off between leaf structure and chemical composition investment. It has been suggested that the higher *LMA* was associated with greater energy investment into dry mass was but negatively associated with leaf photosynthesis (Tomás et al., 2013; Onoda et al., 2017). Moreover, *LMA* is associated with leaf N content and allocation in plants (Zong et al., 2021). However, it is unclear how the tradeoff of leaf N allocation and *LMA* affected leaf photosynthesis and in acclimation to environmental factors.

It should be pointed out that the links between leaf N and photosynthetic capacity vary among plant species and environmental conditions. N utilization efficiency may vary in poplar with different genus under the same N levels (Zabek, 1995). Plants could change their N allocation strategy within leaves in response to environmental changes, and even small adjustments in N allocation influence photosynthetic N use efficiency (*PNUE*) and photosynthesis, and plant survival (Feng et al., 2009; Mu et al., 2018; Park et al., 2019). Drought and salt stress induce stomatal closure, declined photosynthetic enzyme abundance and activity, photosynthetic protein degradation and disintegration of thylakoids, thus leading to the declined photosynthesis capacity of leaves in plants, including poplar (Yoon et al., 2014; Zhong et al., 2019). However, plants trigger N-containing osmolytes or regulators related to redox homeostasis, such as soluble proteins, glutathione and free amino acids, in response to drought and salt stress (Zhong et al., 2019). The lower leaf photosynthesis under drought and salt stress probably increased N allocation into defense against environmental stresses (Hessini et al., 2019). In addition, leaf N allocation into the photosynthetic apparatus affects leaf N tradeoff between photosynthesis and environmental adaptability (Mu et al., 2016; Evans and Clarke, 2019). More leaf N allocation into electron transport and photophosphorylation induced by low N reduces light energy capture in maize (Mu et al., 2018). However, leaf N tradeoffs between photosynthesis and non-photosynthesis components and among the components of the photosynthetic apparatus are not well understood in dioecious plant species.

Poplar species are fast-growing woody plants with high N requirements and are widely distributed around the world (Isebrands and Richardson, 2014; Hu et al., 2020). Some studies suggested that drought and/or salt stress reduced leaf Rubisco content and *PNUE* but increased leaf cell wall thickness in poplar (Durand et al., 2011; Liu et al., 2021b). Moreover, the increased N-containing compounds in poplar also elevated plant tolerance to drought/or salt stress (Arndt et al., 2004; Dluzniewska et al., 2007). *Populus cathayana* is a typical dioecious plant. Dioecious plant species usually exhibit sexual dimorphism in terms of primary sexual traits (sexual organs) and secondary sexual traits (Liu et al., 2021a). Many studies have suggested that males of *Populus* species show sexual differences in morphological and physiological responses to environmental stresses, including drought and salt stress, and males usually show less damage, better growth, higher photosynthesis and antioxidative ability compared to females (Liu et al., 2020, 2021b). Some studies suggested that poplar females increased N allocation into soluble proteins, while males kept a stable N allocation pattern under N enrichment in P*opulus cathayana* (Chen et al., 2014; Wu et al., 2021). However, links between sex-specific differences in photosynthesis and biomass, and N allocation within leaves have been rarely reported.

In this study, we employed *Populus cathayana* to examine sex-specific adaptive strategies based on N allocation within leaves under salt stress and drought. We aim to answer the following questions: (1) Is there a sex-specific tradeoff between leaf N allocation into photosynthetic and non-photosynthetic apparatus under salt stress and drought stress? (2) How does leaf N allocation tradeoff drive sex-specific responses to salt and drought stress and their combination?

2. Materials and methods

2.1. Plant material and experimental design

P. cathayana cuttings were chosen from different trees of each sex from populations in riparian and valley flat habitats in the Qinghai Province, China (30° 67′ N, 104° 06′ E, 3160 m Alt), as described by Liu et al. (2020). Briefly, *P. cathayana* cuttings were obtained from 25 different trees of each sex, sampled from 5 populations of each sex (five adult trees per population of each sex). We selected the annual vegetative branches of adult trees at the similar height and position. Cuttings with similar size were planted on March 2019 in a semi-controlled greenhouse of the Hangzhou Normal University, China, (30° 01′ N, 104° 07′ E). The growth conditions were as follows: a night-time temperature of 15–18 °C, a photoperiod of 12–14 h, a relative humidity of 76–81%, and a daytime temperature of 21–25 °C. After growth for 4 weeks, uniform and healthy seedlings with *c.* 20 cm heights were transplanted into 10-l plastic pots with 10 kg soils containing 1.75 g kg^{-1} total N, 122.56 mg kg^{-1} available P, 476.46 mg kg^{-1} available K, 106.33 mg kg^{-1} NO$_3^-$-N, 52.69 mg kg^{-1} NH$_4^+$-N, 33.32 g kg^{-1} soil organic matter. The experiment was completely randomized including 2 sexes (female, male) × 2 drought treatments (well-watered, drought) × 2 salt treatments (0, 150 mM NaCl). Each treatment was replicated four times. After 8 weeks, the seedlings were treated with or without salt and drought. For well-watered plants, the pots were weighed each day to maintain 80% field capacity. For drought treatments, the pots were weighed each day to maintain 30% field capacity. For salt stress treatments, the seedlings were treated with 200 ml NaCl (150 mM) administered every day until the final NaCl concentration reached 150 mM kg^{-1} dry soil.

2.2. Gas exchange measurements

The gas exchange of fully expanded leaves was measured using a portable photosynthesis measuring system (Ll-6800; Li-Cor Inc., Lincoln, NE, USA) at 09:00 h and 16:30 h. The leaf chamber parameters were as follows: 75% relative humidity of the air, 25 °C leaf temperature, 500 μmol s^{-1} air flow rate, 400 μmol mol^{-1} CO$_2$ concentration, and 1200 μmol m^{-2} s^{-1} saturating photon quantum flux density (with 90% red light and 10% blue light). After these measurements, net photosynthesis rate/CO$_2$ (A_n/C_i) curves were measured with the above leaf chamber parameters with a series of CO$_2$ concentration gradients (400–300, 200, 100 and 50 μmol mol^{-1}, 600, 800 and 1000 μmol mol^{-1}). The maximum electron transport (J_{max}) and the maximum carboxylation rate (V_{cmax}) were calculated according to the model of Farquhar et al. (1980). The *PNUE* was calculated as the ratio of A_{max} to N_{area}.

2.3. Plant harvesting and sampling

After the measurement of leaf photosynthesis, leaves were harvested, weighed and divided into four subsamples. A part of the subsample was dried at 75 °C for 96 h after scanning the leaves with the Image J software (version 1.52, Wayne Rasband, NIH USA). *LMA* was calculated as the ratio of leaf dry weight to its area. One part of fresh samples was used to measure the leaf chlorophyll concentration. One part of fresh samples was stored at −80 °C to measure the leaf N allocation. One part of fresh samples was dried at 75 °C for 96 h to measure the leaf N levels.

2.4. Total leaf N and chlorophyll measurements

The dried leaves were ground finely in a ball mill. The leaf N concentration was measured with an elemental analyser (FLASH EA1112, Thermo). The leaf N content per unit area (N_{area}) was calculated as the ratio of leaf N mass per leaf dry mass to *LMA*. The leaf chlorophyll content was extracted with 80% acetone and determined with a spectrophotometer at 663 nm according to the method of Porra et al. (1989).

2.5. Calculation of N partitioning within leaves

The leaf N partitioning in the photosynthetic apparatus was divided into three categories: carboxylation system, proteins involved in bioenergetics and light-harvesting proteins. The fractions of leaf N partitioning in the carboxylation system (P_C), light-harvesting system (P_L) and bioenergetics (P_B) were calculated as follows (Niinemets and Tenhunen, 1997; Ghimire et al., 2017):

$$P_C = V_{cmax}/(6.25 \times V_{cmax} \times N_{area})$$

$$P_B = J_{max}/(8.06 \times J_{max} \times N_{area})$$

$$P_L = C_{chl}/(N_{mass} \times C_B)$$

$$P_P = P_B + P_C + P_L$$

where V_{cmax} is the maximum carboxylation rate; J_{max} is the maximum electron transport rate; C_{chl} is the leaf chlorophyll content. The leaf N content in the carboxylation system (N_C), light-harvesting system (N_L) and bioenergetics (N_B) were calculated as the products of N_{area} and P_C, P_L and P_B, respectively. All leaf components of photosynthetic apparatus (N_P) were the sums of P_C, P_L and P_B.

The water-soluble, membrane-bound and cell wall protein fractions were measured according to the method of Wu et al. (2017) and Liu et al. (2018). The leaves were finely ground in liquid N and homogenized with 1 ml of 100 mM sodium phosphate buffer (containing 2 mM MgCl$_2$, 10 mM NaCl, 5 mM iodoacetate, 0.4 M sorbitol, 1% (v/v) polyvinylpyrrolidone, 5 mM dithiothreitol, 5 mM phenylmethyl sulfonyl fluoride, pH 7.5). The homogenate was centrifuged at 12000 g, 4 °C for 20 min. The supernatants were collected and regarded as water-soluble proteins. The water-soluble proteins were quantified with the ninhydrin method. Bovine serum albumin was used as standard. Afterwards, 1 ml of phosphate buffer containing 3% SDS was added to the residuals, heated at 90 °C for 5 min. The mixture was centrifuged at 4500 g for 10 min. This procedure was replicated six times. The supernatant (regarded as SDS-soluble protein) was collected and quantified with the ninhydrin method. The residue (regarded as cell wall protein) was washed with ethanol and determined with an element analyser (FLASH EA1112, Thermo). The N_{np} was calculated as total leaf N minus the water-soluble N, SDS-soluble N and SDS-insoluble N.

2.6. Statistical analyses

Statistical analyses were conducted using the SPSS software (version 22.0, Chicago, IL, USA). All data were subjected to a two-way analysis of variance (ANOVAs). The differences between mean values were compared by Duncan's tests at a significance level of $P < 0.05$. Graphics and regression analysis were performed with the Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. N levels of anatomic and photosynthetic apparatus

When compared to controls, the levels of leaf net photosynthetic rate (A_n), photosynthetic N utilization efficiency ($PNUE$), leaf area-based leaf N content (N_{mass}), leaf non-protein N content (N_{np}) and the proportion of leaf N allocated to the photosynthetic machinery (N_P/N_L) in female leaves decreased by 54%, 42%, 34%, 59% and 53%, respectively, by salt stress alone, by 34%, 42%, 27%, 32% and 29%, respectively, by drought stress alone, and by 83%, 85%, 44%, 74% and 74%, respectively, by the combination of two stresses (Table 1). In contrast, in males, when compared to controls, the levels of leaf A_n, $PNUE$, N_{mass}, N_{np} and N_P/N_L decreased by 38%, 33%, 29%, 34% and 24%, respectively, by salt stress alone, by 17%, 22%, 2%, 32% and 33%, respectively, by drought stress alone, and by 49%, 50%, 23%, 47% and 49%, respectively, by the combination of two stresses (Table 1). Drought, salt and their combination in females increased LMA by 36%, 42% and 67%, respectively, and N_{CW}/N_L by 35%, 89% and 226%, respectively, when compared to controls (Table 1). In males, the levels of LMA and N_{CW}/N_L increased by 15% and 31%, respectively, by drought alone, 23% and 55%, respectively, by salt alone, and 33% and 103%, respectively, by the combination of salt and drought (Table 1). Drought, salt and their combination did not affect the leaf N content based on leaf area (N_{area}) in females. In contrast, N_{area} increased by drought stress, decreased by salt stress, and remained stableunder the combined stress (Table 1).

The values of P_B, P_C, P_L, N_B, N_C and N_L in female leaves decreased by 28%, 33%, 36%, 28%, 32% and 38%, respectively, by drought alone, by 38%, 40%, 68%, 49%, 54% and 70%, respectively, by salt alone, and by 70%, 75%, 66%, 74%, 78% and 70%, respectively, by their combined stress (Table 2). In males, the values of P_B, P_C, P_L, N_B, N_C and N_L in leaves decreased by 29%, 32%, 37%, 22%, 17% and 34%, respectively, by drought alone, by 16%, 30%, 22%, 20%, 36% and 37%, respectively, by

Table 1

Leaf net photosynthetic rate (A_n, μmol g^{-1} s^{-1}), leaf photosynthetic nitrogen-use efficiency ($PNUE$, μmol g^{-1} s^{-1}g^{-1}), leaf dry mass per unit area (LMA, g m^{-2}), leaf area-based N content (N_{area}, g m^{-2}), leaf mass-based N content (N_{mass}, g g^{-1}), non-protein N content (N_{np}, g g^{-1}), the ratio of the proportion of leaf nitrogen allocated to total photosynthetic apparatus (N_P/N_L, g g^{-1}) and the proportion of leaf N allocated to cell wall (N_{CW}/N_L, g g^{-1}) in leaves of *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. P_{sex}, sex effect; P_{salt}, salt effect; P_D, drought effect; $P_{sex \times salt}$, the interactive effect of sex and salt stress; $P_{sex \times D}$, the interactive effect of sex and drought effect; $P_{salt \times D}$, the interactive effect of salt stress and drought stress; $P_{sex \times salt \times D}$, the interactive effect of sex, salt stress and drought stress. Different letters in the column indicate significant differences between treatments ($P < 0.05$, Duncan's test). Values are expressed as means ± SE ($n = 4$). The significant values of variance are shown as follows: ns, not significant; * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; ***$P \leq 0.001$.

Sex	Treatment	A_n	$PNUE$	LMA	N_{area}	N_{mass}	N_{np}	N_P/N_L	N_{CW}/N_L
F	CK	14.12 ± 0.96b	9.08 ± 0.38bc	38.2 ± 2.33f	1.56 ± 0.09 ab	40.3 ± 1.40a	22.10 ± 1.89a	0.298 ± 0.008b	0.160 ± 0.01e
F	D	9.35 ± 0.48d	5.23 ± 0.80d	52.1 ± 0.44cd	1.55 ± 0.07 ab	29.4 ± 1.28c	17.08 ± 0.74b	0.213 ± 0.027d	0.216 ± 0.03d
F	S	6.52 ± 0.56e	5.19 ± 0.57d	54.3 ± 1.48cd	1.44 ± 0.10b	26.4 ± 1.31d	13.48 ± 1.18c	0.139 ± 0.011f	0.303 ± 0.02c
F	DS	2.36 ± 1.07f	1.40 ± 0.44e	63.8 ± 2.80a	1.44 ± 0.09b	22.6 ± 0.67e	7.13 ± 1.32e	0.077 ± 0.007g	0.521 ± 0.05a
M	CK	16.81 ± 0.43a	12.27 ± 1.34a	44.5 ± 1.56e	1.48 ± 0.11b	33.2 ± 1.56b	17.12 ± 1.07b	0.343 ± 0.012a	0.249 ± 0.01d
M	D	13.94 ± 0.63b	9.52 ± 0.64b	51.2 ± 1.45d	1.65 ± 0.09a	32.6 ± 2.44b	13.74 ± 1.16c	0.230 ± 0.013d	0.326 ± 0.03c
M	S	10.49 ± 0.58c	8.19 ± 0.51c	54.8 ± 2.41c	1.28 ± 0.09c	23.5 ± 1.94e	9.85 ± 0.40d	0.259 ± 0.018c	0.385 ± 0.03b
M	DS	8.64 ± 0.54d	6.18 ± 0.34c	59.2 ± 2.76b	1.52 ± 0.03 ab	25.7 ± 0.79d	8.33 ± 1.03de	0.174 ± 0.008e	0.505 ± 0.02a
P_{sex}		***	***	ns	ns	***	***	***	***
P_{salt}		***	***	***	***	***	***	***	***
P_D		***	***	***	***	**	***	***	***
$P_{sex \times salt}$		***	ns	***	ns	***	***	***	***
$P_{sex \times D}$		***	**	***	**	ns	**	*	ns
$P_{salt \times D}$		*	ns	*	ns	***	***	*	***
$P_{sex \times salt \times D}$		ns	ns	ns	ns	ns	ns	ns	**

Table 2

The fraction of leaf N partitioning in the bioenergetics (P_B, g g^{-1}), carboxylation system (P_C, g g^{-1}) and light-harvesting system (P_L, g g^{-1}), the leaf N content in bioenergetics (N_B, g m^{-2}), carboxylation (N_C, g m^{-2}) and light-harvesting systems (N_L, g m^{-2}), the leaf mass-based cell wall N content ($N_{CW-mass}$, g g^{-1}), the leaf area-based cell wall N content (N_{CW}, g g^{-1}) in leaves of *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. P_{sex}, sex effect; P_{salt}, salt effect; P_D, drought effect; $P_{sex \times salt}$, the interactive effect of sex and salt stress; $P_{sex \times D}$, the interactive effect of sex and drought effect; $P_{salt \times D}$, the interactive effect of salt stress and drought stress; $P_{sex \times salt \times D}$, the interactive effect of sex, salt stress and drought stress. Different letters in the column indicate significant differences between treatments ($P < 0.05$, Duncan's test). Values are expressed as means ± SE ($n = 4$). The significant values of variance are shown as follows: ns, not significant; * $0.01 < P \le 0.05$; ** $0.001 < P \le 0.01$; ***$P \le 0.001$.

Sex	Treatment	P_B	P_C	P_L	N_B	N_C	N_L	$N_{CW-mass}$	N_{CW}
F	CK	0.061 ± 0.001a	0.190 ± 0.003b	0.047 ± 0.004a	0.094 ± 0.006a	0.292 ± 0.018b	0.074 ± 0.004a	1.35 ± 0.16e	7.76 ± 1.13e
F	D	0.044 ± 0.002c	0.128 ± 0.008d	0.030 ± 0.002c	0.068 ± 0.001c	0.199 ± 0.016cd	0.046 ± 0.002b	2.78 ± 0.48bc	14.60 ± 0.69d
F	S	0.038 ± 0.002d	0.114 ± 0.013d	0.0153 ± 0.001e	0.048 ± 0.005d	0.133 ± 0.007e	0.022 ± 0.002d	2.16 ± 0.31d	13.28 ± 0.88d
F	DS	0.018 ± 0.004f	0.048 ± 0.009e	0.016 ± 0.003e	0.024 ± 0.005e	0.065 ± 0.009f	0.022 ± 0.002d	4.49 ± 0.43a	19.99 ± 0.38b
M	CK	0.058 ± 0.003a	0.231 ± 0.015a	0.049 ± 0.002a	0.086 ± 0.003b	0.324 ± 0.035a	0.076 ± 0.011a	2.47 ± 0.20cd	7.64 ± 0.88e
M	D	0.041 ± 0.003cd	0.158 ± 0.012c	0.031 ± 0.004c	0.067 ± 0.003c	0.268 ± 0.013b	0.050 ± 0.005b	2.86 ± 0.52bc	15.92 ± 1.15c
M	S	0.049 ± 0.003b	0.162 ± 0.012c	0.038 ± 0.005b	0.069 ± 0.003c	0.208 ± 0.014c	0.048 ± 0.003b	3.22 ± 0.29b	13.91 ± 0.33d
M	DS	0.031 ± 0.003e	0.117 ± 0.010d	0.022 ± 0.001d	0.047 ± 0.004d	0.178 ± 0.009d	0.033 ± 0.001c	4.59 ± 0.47a	22.09 ± 1.16a
P_{sex}		***	***	***	***	***	***	ns	**
P_{salt}		***	***	***	***	***	***	***	***
P_D		***	***	***	***	***	***	***	***
$P_{sex \times salt}$		***	**	***	***	**	***	ns	ns
$P_{sex \times D}$		ns	ns	***	ns	**	ns	ns	*
$P_{salt \times D}$		ns	ns	***	ns	ns	***	*	ns
$P_{sex \times salt \times D}$		ns	*	***	ns	ns	*	ns	ns

salt alone, and by 47%, 49%, 55%, 45%, 45% and 57%, respectively, by their combined stress (Table 2). When compared to controls, drought, salt stress and their combined stress increased the levels of leaf mass-based cell wall N ($N_{CW-mass}$) by 106%, 60% and 233%, respectively, in females, and by 16%, 30% and 86%, respectively, in males. The levels of leaf area-based cell wall N (N_{CW}) were 88%, 71% and 158% of N_{CW} in control females, and 108%, 82% and 189% of $N_{CW-area}$ in control males subjected to drought, salt and their combination (Table 2).

3.2. Correlations of LMA with leaf photosynthetic N allocation

LMA exhibited a significant negative correlation with A_n and *PNUE* (in males $R^2 = 0.81$ and $R^2 = 0.84$, respectively; in females $R^2 = 0.80$ and $R^2 = 0.82$, respectively) in both sexes under all treatments (Fig. 1a and b). The relationship between *LMA* and leaf A_n was similar in both sexes, as well as the relationship between *LMA* and *PUNE* (Fig. 1a and b). On average, *LMA*, A_n and *PNUE* were greater in males than in females (Fig. 1a and b). Both sexes showed an increasing trend in *LMA* and N_P, and this relationship was weaker in males than in females under all
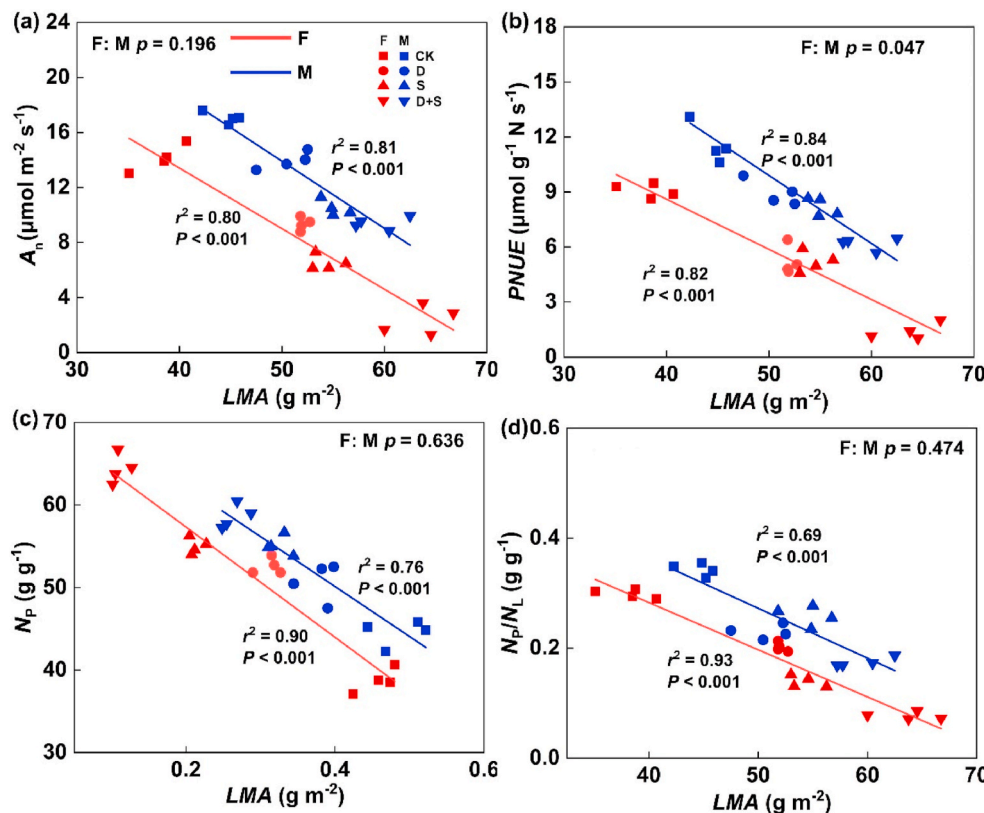

Fig. 1. Relationships between the leaf dry mass per unit area (*LMA*) and net photosynthetic rate (A_n) (a), photosynthetic nitrogen-use efficiency (*PNUE*) (b), nitrogen content in total photosynthetic apparatus (N_P) (c), and proportion of leaf nitrogen allocated to total photosynthetic apparatus (N_P/N_L) (d) in *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. *P* after F:M indicates indicate the statistical differences between sexes.

treatments ($R^2 = 0.76$ in males; $R^2 = 0.90$ in females) (Fig. 1c). Similarly, both sexes showed a decreasing trend in *LMA* with N_P/N_L ($R^2 = 0.69$ in males; $R^2 = 0.93$ in females), and this trend was weaker in males than in females under all treatments (Fig. 1d). At a given *LMA*, males had greater N_P/N_L than females under all treatments, except for drought alone.

3.3. Correlations of LMA and cell wall-related parameters

Leaf mass-based cell wall dry weight (CW_{mass}) and leaf area-based cell wall dry weight (CW_{area}) increased linearly with *LMA* in both sexes ($R^2 = 0.64$ and $R^2 = 0.74$, respectively, in males; $R^2 = 0.74$ and $R^2 = 0.86$, respectively, in females) under all treatments. The correlations between *LMA* and CW_{mass}, and *LMA* and CW_{area} were similar in females and males. On average, males exhibited a stronger increasing trend in CW_{mass} and CW_{area} than females when *LMA* increased from 38.2 to 63.8 g m^{-2}. Similarly, both sexes had an increasing trend in *LMA* and P_{CW} ($R^2 = 0.80$ in males and $R^2 = 0.57$ in females) and *LMA* and N_{CW}/N_L ($R^2 = 0.88$ in males and $R^2 = 0.74$ in females) under all treatments.

3.4. Leaf N allocation into photosynthetic apparatus

Strong positive correlations between P_B and *PNUE* ($R^2 = 0.69$ in males, $R^2 = 0.90$ in females), P_C and *PNUE* ($R^2 = 0.85$ in males, $R^2 = 0.93$ in females), P_L and *PNUE* ($R^2 = 0.53$ in males, $R^2 = 0.81$ in females) and N_P/N_L and *PNUE* ($R^2 = 0.85$ in males, $R^2 = 0.98$ in females) were observed in both sexes under all treatments (Fig. 3). Females had higher P_C and N_P/N_L than males under drought, salt and their combination (Fig. 3a, d). The slopes of *PNUE* vs P_C, *PNUE* vs P_B, and *PNUE* vs N_P/N_L were similar in both sexes, but because of drought, salt and their combination caused lower P_C and N_P/N_L, the lines of *PNUE* vs P_C and *PNUE* vs N_P/N_L shifted due to lower P_C and N_P/N_L in females. At a given P_B and P_L, *PNUE* was higher in males than in females under all treatments.

3.5. Leaf N allocation into cell walls

PNUE increased with CW_{mass} ($R^2 = 0.75$ in males, $R^2 = 0.79$ in females), CW_{area} ($R^2 = 0.82$ in males, $R^2 = 0.92$ in females), P_{CW} ($R^2 = 0.71$ in males, $R^2 = 0.86$ jn females) and N_{CW}/N_L ($R^2 = 0.72$ in males, $R^2 = 0.78$ in females) under all treatments in both sexes (Fig. 4). The relationships between CW_{mass} and *PNUE*, and N_{CW}/N_L and *PNUE* were similar in both sexes. The lines of *PNUE* vs CW_{mass} shifted due to lower *PNUE* under drought, salt and their combination, *PNUE* being lower in females than in males. The relationship between P_{CW} and *PNUE* was stronger in females than in males under all treatments (Fig. 4c). At a given CW_{mass}, CW_{area}, P_{CW} and N_{CW}/N_L, females had a lower *PNUE* than males under all treatments.

3.6. Correlations of leaf N allocation between photosynthetic apparatus and cell wall

P_T had a negative correlation with P_{CW} and N_{CW}/N_L in females and males under all treatments (Fig. 5a and b). P_T had a higher correlation with P_{CW} in females than in males under all treatments. The slope of P_T vs N_{CW}/N_L shifted toward higher N_{CW}/N_L values along the common slope (Fig. 5b). Similarly, N_P/N_L decreased with P_{CW} and N_{CW}/N_L in both sexes under all treatments, and females showed a stronger decreasing trend in N_P/N_L when P_{CW} and N_{CW}/N_L increased (Fig. 5c and d). The relationship between N_P/N_L and P_{CW} under all treatments was higher in males than in females. A similar relationship between N_P/N_L and N_{CW}/N_L was observed between females and males under all treatments (Fig. 5c and d).

3.7. A tradeoff between leaf N in cell wall and photosynthesis

N portioning clearly altered the treatment factors in females and males (Fig. 6a and b). Under control conditions, females increased N
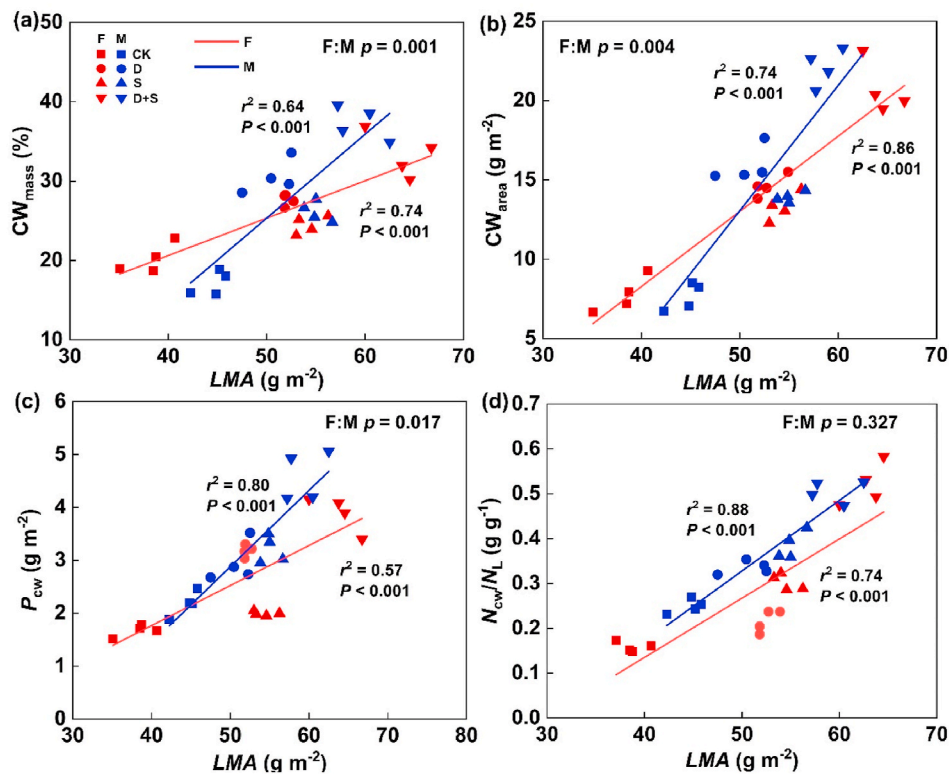

Fig. 2. Relationships between the leaf dry mass per unit area (*LMA*) and leaf mass-based cell wall dry weight (CW_{mass}), leaf area-based cell wall dry weight (CW_{area}), leaf mass-based cell wall protein content (P_{CW}) and ratio of leaf N allocated to cell wall (N_{CW}/N_L) in *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combination stress (DS) conditions. *P* after F:M indicates indicate the statistical differences between sexes.
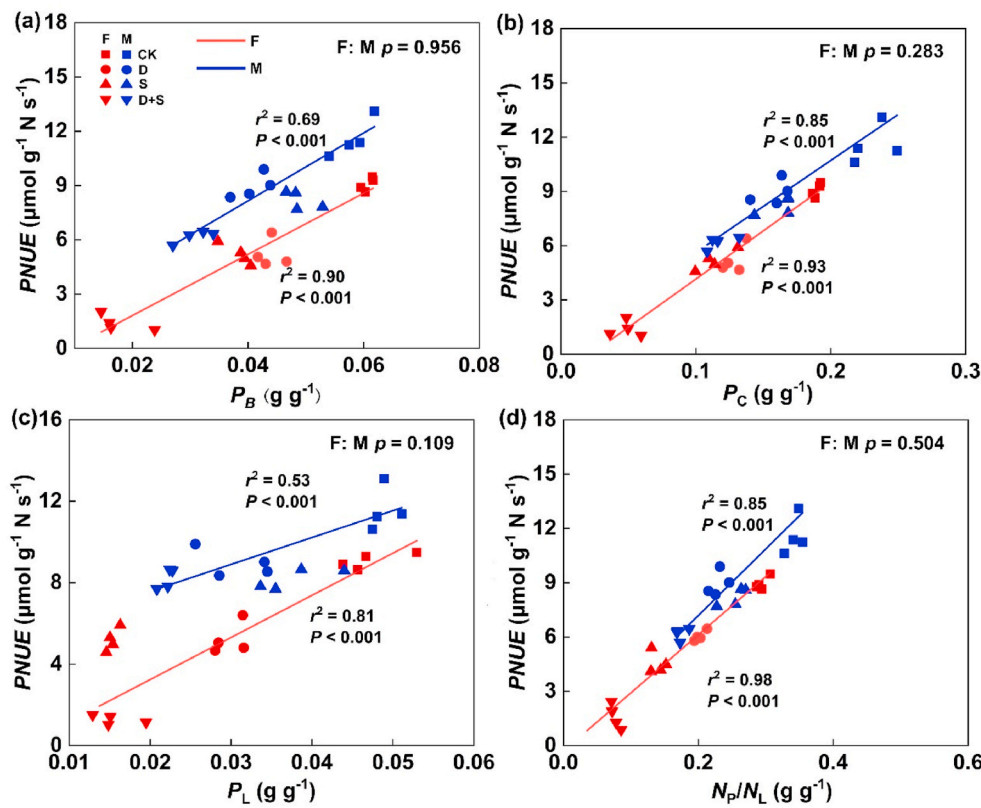
Fig. 3. Relationships between the photosynthetic nitrogen-use efficiency (*PNUE*) and fraction of leaf N allocated to bioenergetics (P_B) (a), fraction of leaf N allocated to carboxylation (P_C) (b), fraction of leaf N allocated to light-harvesting components (P_L) (c) and proportion of leaf nitrogen allocated to total photosynthetic apparatus (N_P/N_L) in *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. *P* after F:M indicates indicate the statistical differences between sexes.
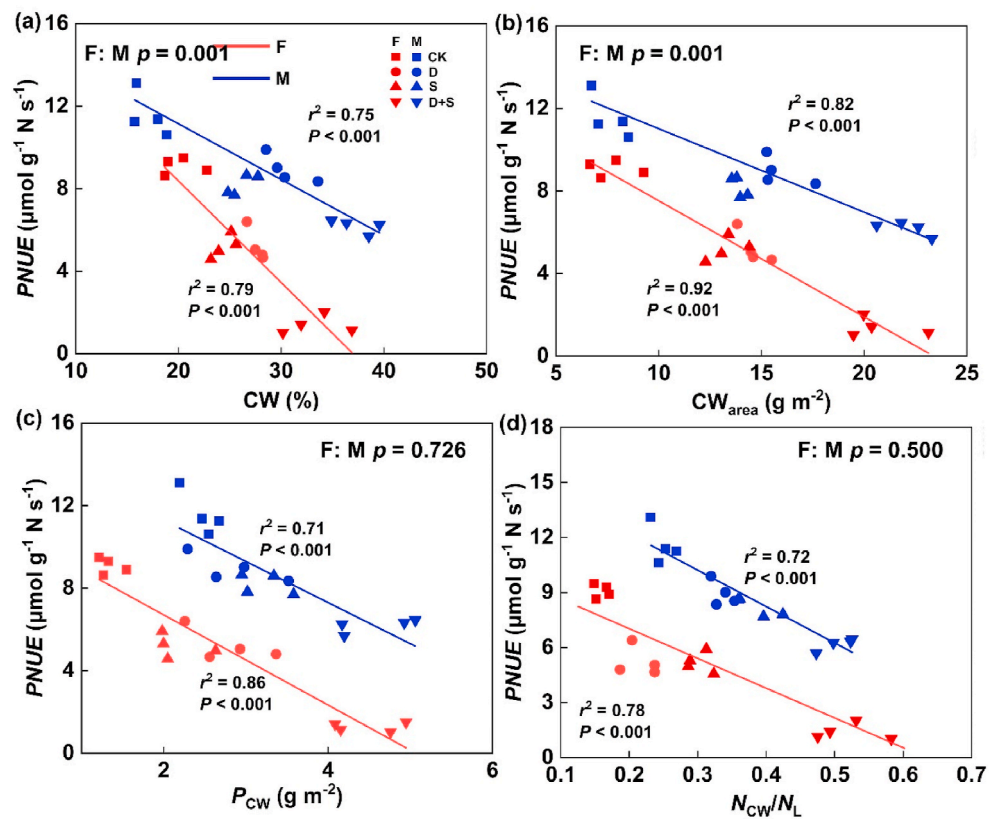

Fig. 4. Relationships between the photosynthetic nitrogen-use efficiency (*PNUE*) and leaf mass-based cell wall dry weight (CW_{mass}) (a), leaf area-based cell wall dry weight (CW_{area}) (b), leaf mass-based cell wall protein content (P_{CW}) (c) and ratio of leaf N allocated to cell wall (N_{CW}/N_L) in *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. *P* after F:M indicates indicate the statistical differences between sexes.
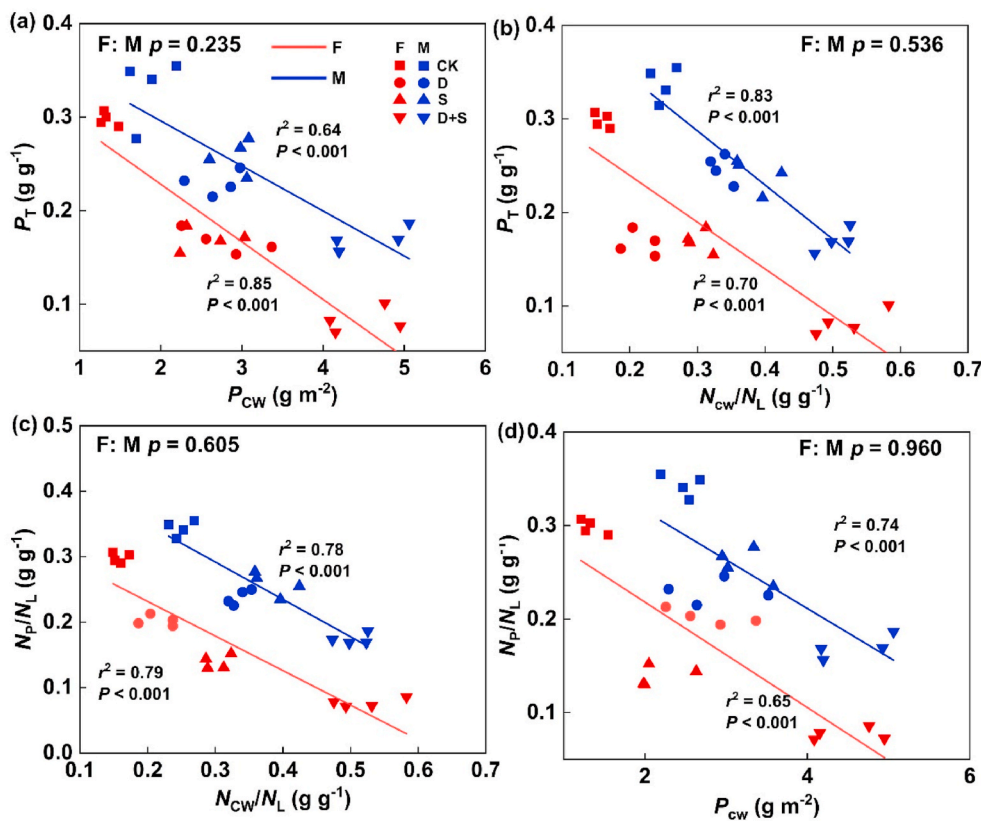
Fig. 5. Relationships between the fraction of leaf N allocated to total photosynthetic apparatus (P_T) and leaf mass-based cell wall protein content (P_{CW}) (a), proportion of leaf N allocated to cell wall (N_{CW}/N_L) (b), and the relationships between the ratio of the proportion of leaf nitrogen allocated to total photosynthetic apparatus (N_P/N_L) and leaf N allocated to cell wall (N_{CW}/N_L) and leaf mass-based cell wall protein content (P_{CW}) in *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and combined stress (DS) conditions. *P* after F:M indicates indicate the statistical differences between sexes.
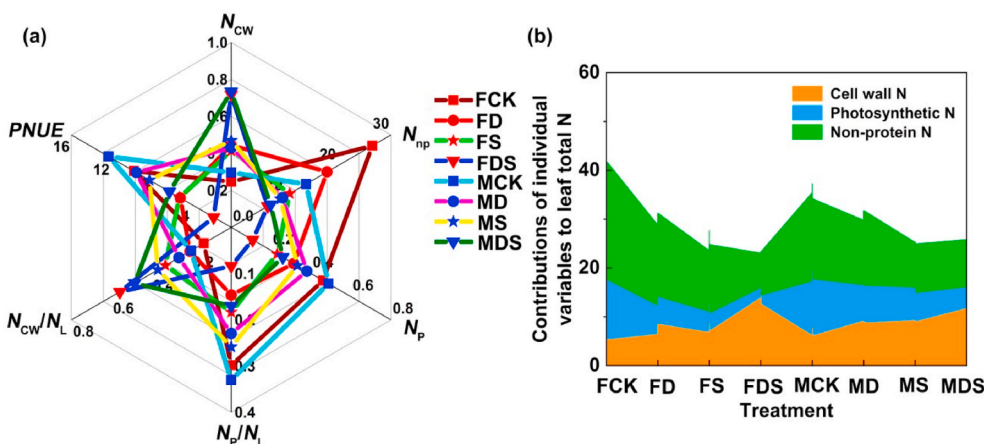

Fig. 6. Partitioning of N between photosynthetic apparatus and non-photosynthetic apparatus (a), and the contributions of cell wall N, photosynthesis N and non-protein N to total N in leaves of *P. cathayana* females (F) and males (M) under control (CK), drought stress (D), salt stress (S) and ombined stress (DS) conditions. *PNUE*, the photosynthetic nitrogen-use efficiency; N_{CW}, leaf N content in cell wall; N_{CW}/N_L, the proportion of leaf N allocated to cell wall; N_P/N_L, the proportion of leaf nitrogen allocated to total photosynthetic apparatus; N_P, nitrogen content in total photosynthetic apparatus, N_{np}, non-protein N content.

partitioning into N_{np} relative to males, while N increased in the photosynthetic apparatus more in males than in females. Salt or drought alone significantly reduced N allocation into N_P/N_L in males, but increased N in N_{np} in females. The combined salt and drought stress similarly increased leaf N partitioning into cell wall but reduced N partitioning into N_{np} and the photosynthetic apparatus in both sexes. However, the decrease in the N content of the photosynthetic apparatus was more significant in females than in males under the combination of salt and drought stress.

4. Discussion

The present study demonstrates that drought, salt and their combination impose sex-specific restraints on leaf photosynthesis, mainly deriving from the adjustment of leaf N allocation and *PNUE*. Compared

to females, males show a better tradeoff in the leaf N allocation between photosynthesis and non-photosynthesis, and, consequently, higher *PNUE* and tolerance to drought and salt stress, and their combination.

4.1. Leaf morphological adjustments reflected the sexual difference in photosynthetic ability in both sexes under drought, salt and their combination

It has been widely recognized that morphological adjustments of leaves are more relevant determinants of photosynthetic adaptation to the environment than their biochemical properties (Terashima et al., 2011; Ren et al., 2019). In this study, morphological traits and N allocation of leaves reflected sex-specific photosynthesis and tolerance under all treatments. In previous studies (Liu et al., 2021b), changes in leaf photosynthesis induced by morphological and anatomical traits of

leaves were found in *Poplar* females and males under salt stress. Leaf N content and allocation are associated with the morphological and anatomic properties of leaves (Hu et al., 2020; Liu et al., 2018). *LMA*, which is a structural parameter concerning plant physiology and ecology, is strongly associated with other functional traits of leaves, such as photosynthetic capacity based on leaf area and N_{mass} (Dong et al., 2020; Lu et al., 2020). Consistently, the decreased photosynthesis under drought, salt and their combination could be explained by increased *LMA* in both sexes, and females were more sensitive to these stresses than males (Fig. 1; Table), which may be associated with the leaf N allocation into photosynthesis (Fig. 1). We further found that the increased *LMA* under all stressors could be contributable to the increased N investment into cell walls in both sexes (Figs. 2 and 4). Under such conditions, females tend to invest more N into the cell walls and increase the *LMA*, thus causing the induction of leaf N into photosynthesis relative to males (Figs. 2 and 4). However, according to the relationship between *LMA* and *PNUE*, and N_{CW}/N_L and *PNUE*, in both sexes under all treatments, *LMA* and N allocation into cell wall did not completely explain sex-specific *PNUE*.

4.2. Tradeoffs between photosynthesis and non-photosynthetic apparatus reflected the sex-specific responses to drought and salt stress and their combination

In addition to leaf dry mass, leaf N allocation appears to be a critical factor that predicts the photosynthetic capacity of leaves. We found that leaf N_{area} was not correlated with leaf *PNUE* and P_n in either sex under any treatments. Instead, N_P/N_L was closely associated with leaf photosynthesis in both sexes under all treatments. Thus, relative to the leaf N content, leaf N allocation into the photosynthetic apparatus is especially important for the photosynthetic capacity of leaves in both sexes under all treatments. These results were consistent with previous studies, which proposed that the allocation of leaf N into photosynthesis is more important than total leaf N_{area} (Zhong et al., 2019). Importantly, we found that the leaf photosynthetic N allocation better explained the sexual difference in *PNUE*. The higher N_P/N_L in poplar males relative to females under all stress treatments could indicate an efficient strategy to maintain higher *PNUE*.

In addition, photosynthetic N allocation into the light-harvesting system, Rubisco and electron transport affects leaf *PNUE* and environmental adaptability (Mu et al., 2018). Previous studies have suggested that poplar males have higher photosynthesis and tolerance than females under abiotic stress conditions (Liu et al., 2020, 2021b), which is consistent with our results. Moreover, this study found that higher A_n and tolerance in males compared to females are mainly associated with photosynthetic N in three photosynthetic components under all treatments. Importantly, among the three photosynthetic N components, N allocation into the carboxylation component determined the sex difference in *PNUE* in response to drought, salt and their combination, as reflected by their relationships (Fig. 3). Rubisco is the primary limitation of light-saturated photosynthesis, which constitutes about half of photosynthesis-related N (Suganami et al., 2021). The higher leaf N allocation into the carboxylation component in males relative to females would be an important strategy to maintain higher *PNUE* under salt, drought and their combination. These results suggest that leaf N allocation into photosynthesis, especially into the carboxylation component, determines the sexual difference in *PNUE* under all treatments.

Growth-defense tradeoffs are reported to occur in plants due to resource restrictions, and they function in optimizing growth and defense depending on internal and external factors (Huot et al., 2014). Leaf N partitioning between photosynthesis and non-photosynthesis could reflect the tradeoff between growth and environmental adaptability (Feng et al., 2009; Yao et al., 2018; Zhong et al., 2019). Cell wall occupies a large fraction of leaf mass, which largely affects plant responses to environmental stresses, such as osmotic stress (De Lorenzo et al., 2019; Rui and Dinneny, 2020). In this study, we found that the increased cell wall mass in both sexes under drought, salt stress and their combination decreased *PNUE* (Fig. 4). Moreover, the correlation between CW_{mass} and *PNUE* was similar to the relationships between N_{CW}/N_L and *PNUE*, suggesting that drought, salt stress and their combination increase leaf N allocation into the cell wall. It is noteworthy that the relationship between N_{CW}/N_L and *PNUE* was present among all treatments, but not between females and males. These results suggested that, unlike leaf N allocation into photosynthesis, leaf N allocation into cell wall determines intrasexual differences in *PNUE* under all treatments, rather than the intersexual difference in *PNUE*.

According to the N allocation principle, it is expected that males with higher leaf N allocation into the photosynthetic apparatus have a lower leaf N investment into the cell wall under all treatments when compared to females. However, we found that males with higher leaf N allocation into the photosynthetic apparatus have similar or higher cell wall N partitioning relative to females (Table 2). These results suggested that there might be other N-containing compounds involved in the leaf N allocation. It has been suggested that *c.* 48% of leaf N allocation would go into neither cell wall nor photosynthesis (such as nucleic acids, N-rich defensive compounds) and that the leaf N tradeoff between cell wall and photosynthesis varies among different plant species and different genotypes within species (Onoda et al., 2017). In this study, the similar and/or higher N allocation of leaf N into the cell wall and photosynthesis in males relative to females resulted in a lower leaf N allocation into N_{np} in males under all treatments (Fig. 6; Table 2). N_{np}, including osmotic regulation compounds and secondary metabolites, plays important roles in plants as protectant molecules in chemical defense, oxidative damage and tolerance to abiotic stresses, such as drought and salt stress (Batista-Silva et al., 2019; Rodrigues-Corrèa and Fett-Neto, 2019). Female plants usually have an increased carbon investment into carbon-based secondary metabolism, which also increases N demand for synthesizing these metabolic substances, such as alkaloids and cyanogenic glycosides (Tsuji and Sota, 2010). The increased N_{np} in females detected in the present study probably reduces the damage from drought, salt and their combination. However, more data are needed to confirm these views.

Considering the relationships between *PNUE* and N_P/N_L, and N_{CW}/N_L and N_{np}, we found that the tradeoff of leaf N allocation between leaf photosynthesis and cell wall did not completely explain sex-specific differences in responses to drought, salt and their combination. Instead, sex-specific N allocation among leaf photosynthesis, cell wall and N_{np} determined sex-specific *PNUE* and tolerance under all treatments. Thus, at a given N_P/N_L, N_{CW}/N_L competes directly with N_{np}, and an increase in N allocated to one would result in a decrease in leaf N allocated to the other(s) in both sexes under all treatments. These results also suggested that females and males adopt different strategies to cope with drought, salt and their combination, even though males balance growth and defense better than females.

4.3. Sexual difference in response to drought, salt and their combination

Drought and salt stress represent osmotic stress but have different effects on plant growth and N allocation in females and males. For example, the inhibition of leaf A_n was greater under salt stress than drought in both sexes although the inhibiting effect on A_n was greatest under the combination of salt and drought stress. Moreover, lower leaf A_n was probably associated with the increased leaf N investment into cell wall and N_{np} in females, and into cell wall in males under salt stress relative to drought stress. Plants' response to salt stress follows a biphasic model, i.e., an early similarity with drought and long-term ion toxicity (Chaves et al., 2009). The long-term salt treatment probably induced females to cope with both osmotic stress and ion stress, leading to an increased N investment into the cell wall and N_{np} and lower photosynthesis. These results confirmed that sex-specific differences in leaf photosynthesis and tolerance are context-dependent, which is consistent with a previous study (Chen et al., 2010).

5. Conclusions

The present study clearly showed a tradeoff between N allocation among photosynthesis, cell wall and non-protein components in *Populus* females and males under salt and drought stress, and their combination. Females tended to invest more N into N_{np} and cell wall but less N into the photosynthetic apparatus, while males preferred to invest more N into photosynthesis and cell wall but less N into N_{np}, under all stress treatments. These results suggested that leaf N allocation tradeoff largely determined sex-specific responses to drought, salt stress and their combination, and males have a better capacity to adjust the tradeoff between growth and defense, thus facilitating growth and survival in stressful environments.

Author contributions

Miao Liu had the main responsibility for data collection, analysis and writing, Xiucheng Liu and Yang Zhao contributed to data collection, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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