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3   Species-specific responses to drought, salinity and their interactions

4   in *Populus euphratica* and *P. pruinosa* seedlings

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21   **Running Head**: Species-specific variations in two desert poplars

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Abstract

Aims Drought and salinity are severe abiotic stress factors, which limit plant growth and productivity, particularly in desert regions. In this study, we employed two desert poplars, *Populus euphratica* Oliver and *P. pruinosa* Schrenk seedlings, to compare their tolerance to drought, salinity and combined stress.

Methods We investigated species-specific responses of *P. euphratica* and *P. pruinosa* in growth, photosynthetic capacity and pigment contents, nonstructural carbohydrate concentrations, Cl^- allocation, osmotic regulation and the accumulation of reactive oxygen species under drought, salinity and the combined stress.

Important Findings *P. pruinosa* exhibited greater growth inhibitory effects, photosynthesis decline, stomatal closure and reactive oxygen species accumulation, and lower antioxidant enzyme activities and osmotic regulation compared with *P. euphratica* under drought, salinity and especially under their combined stress. On the other hand, salt-stressed *P. euphratica* plants restricted salt transportation from roots to leaves, and allocated more Cl^- to coarse roots and less to leaves, whereas salt-stressed *P. pruinosa* allocated more Cl^- to leaves. It was shown that there is species-specific variation in these two desert poplars, and *P. pruinosa* suffers greater negative effects compared with *P. euphratica* under drought, salinity and especially under the combined stress. Therefore, in ecological restoration and afforestation efforts, species-specific responses and tolerances of these two poplar species to drought and salinity should be considered under climate change with increasing drought and soil salinity developing.

Keywords: desert poplars, drought and salinity, Cl^- allocation and transportation, coarse

45   and fine roots, tolerance

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66   **Introduction**

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Drought will become more frequent and severe in the future along with global climate

change (Trenberth et al. 2014; Cook et al. 2015; Choat et al. 2018). Salinity is another

severe abiotic stress factor, which limits plant growth and production worldwide. In dry

areas, evapotranspiration transports salt to the surface, which causes salt accumulation

to toxic concentrations. Additionally, irrigation water may dissolve salt from mineral

stocks, and it may be transported back to the surface by water evaporation and plant

uptake (Rengasamy 2006). Therefore, increasing water irrigation taking place under

climate change may potentially result in further salinization (Polle and Chen 2015). In

natural ecosystems, plants are commonly exposed to different abiotic stresses and their

interactions, such as the interaction between drought and salinity. Although many

experiments have been conducted on plant responses to drought or salinity stress alone,

only a few studies have investigated the combination of drought and salinity, which can

induce unique physiological and biochemical responses (Brown et al. 2006; Mittler

2006; Chen et al. 2010).

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Salinity, like drought, represents osmotic stress, and plants may have similar responses

to salinity and drought (Hu et al. 2006; Chen et al. 2010; Polle and Chen 2015). To

mitigate the damage caused by salinity and drought stress, tree species have different

developmental and defense responses, visible in morphological and physiological traits,

to cope with osmotic stress. For instance, it is known that trees can shift biomass

allocation and change the root to shoot ratio under stress (Brunner et al. 2015; Song et

89 al. 2017; Yu et al. 2019). Additionally, stomatal closure minimizes water loss by

90 transpiration, which decreases photosynthetic carbon assimilation (Chen et al. 2010;

91 Adams et al. 2013; Choat et al. 2018), and affects osmotic regulation (Hartmann and

92 Trumbore 2016; Karst et al. 2017) and reactive oxygen species (ROS) accumulation

93 (Xu et al. 2008; Chen et al. 2010; Cao et al. 2014).

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95 Nonstructural carbohydrates (NSC), such as soluble sugars and starch, have received

96 an increasing attention in plants as means to resist stress (Martínez-Vilalta et al. 2016;

97 Choat et al. 2018; Tomasella et al. 2019). In fact, stored NSC can provide a buffer to

98 enhance plant survival under periods of stress conditions (e.g. drought, salinity, shade

99 and disturbances) (Chen et al. 2010; McDowell et al. 2011; Martínez-Vilalta et al. 2016).

100 Soluble sugars play critical roles in osmoregulation, signaling and xylem repair (Sala

101 et al. 2010; Secchi and Zwieniecki 2011; Martínez-Vilalta et al. 2016), while starch is

102 regarded as an important energy supply to enhance plant survival (Niinemets 2010;

103 Dietze et al. 2014; Hesse et al. 2019).

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105 Previous studies have revealed that the Cl$^-$ exclusion mechanism plays a key role in salt

106 tolerance in *Populus*, which is able to restrict salt transportation from roots to leaves

107 (Chen et al. 2001, 2002, 2010; Polle and Chen 2015; Li et al. 2016). Consequently, a

108 large proportion of Cl$^-$ may accumulate in roots, when *Populus* is exposed to salinity

109 stress. However, to our knowledge, few studies have investigated and compared Cl$^-$

110 allocation into coarse and fine roots. It is well known that coarse and fine roots have

distinct structures and functions. Coarse roots are longer-lived and involved in NSC storage, while fine roots are analogous to leaves, more ephemeral and critical for physiological functions, such as osmoregulation, and water and nutrient uptake (Kong et al. 2014; Iversen et al. 2017; Kannenberg et al. 2017). Yet, improved understanding of how *Populus* excludes Cl⁻ and allocates Cl⁻ into coarse and fine roots under salinity stress is desirable.

P. euphratica Oliver and *P. pruinosa* Schrenk are two desert poplars, which can grow in barren or semi-barren desert regions worldwide. The natural *P. euphratica* forest in the Tarim River watershed accounts for about 90% of its total area in China and 55% of the species' distribution worldwide (Wang et al. 1995). In China, *P. pruinosa* mainly occurs along rivers (e.g. Tarim, Yarkand, Kashgar and Hotan rivers) in the Xinjiang province. *P. pruinosa* grows usually as a pure forest or as a mixed forest with *P. euphratica.* It is one of the dominant species in the riparian zone of the arid desert in Xinjiang (Zheng et al. 2016). There are morphological differences between *P. euphratica* and *P. pruinosa*. For instance, young individuals and twigs of *P. euphratica* have stripped leaves, while the leaves are lanceolate and ovate in mature trees (Zhai et al. 2020). In contrast, *P. pruinosa* has ovate leaves with thick hair. Furthermore, *P. euphratica* has been used as an important model species for studying abiotic responses to drought or salinity stress (Wang et al. 2008; Ding et al. 2010; Zhang et al. 2013; Polle and Chen 2015; Ye et al. 2019). In arid and semi-arid regions of NW China, both *P. euphratica* and *P. pruinosa* play crucial roles in ecological conservation, stabilizing the

133 ecosystem balance and sand dunes, and in shelterbelts for agriculture.

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135 In this study, we investigate the species-specific responses of *P. euphratica* and *P.*

136 *pruinosa* in growth, photosynthetic capacity and pigments, NSC concentration, Cl^-

137 allocation, osmotic regulation, and the accumulation of reactive oxygen species (ROS)

138 under drought, salinity and the combined stress. We hypothesized that: (1) *P. euphratica*

139 and *P. pruinosa* show different adaptive responses (e.g. in growth and physiological

140 traits) to drought, salinity and combined stress; (2) Cl^- allocation and accumulation are

141 different in coarse and fine roots; (3) *P. euphratica* exhibits stronger resistance to

142 drought, salinity and especially to the combined stress.

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152 **Materials and methods**

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154 *Plant material and experimental design*

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This study was conducted at the gardening station of the Tarim University, located on

the north edge of the Taklimakan desert, upriver of the Tarim River (elevation 1009 m

above sea level, 40°54′N, 81°30′E). The conditions at the station represent warm

temperate, continental dry climate. The annual sunshine duration, mean annual total

solar radiation, mean annual rainfall and mean annual evaporation are 2750-3029 h,

5.89×10^5 J cm^{-2}, <50 mm and > 2500 mm, respectively.

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In summer 2015, *P. euphratica* and *P. pruinosa* seeds were sown and germinated in a

nursery near the gardening station. After more than two years of growth with normal

management, 120 uniform-size seedlings (60 seedlings of *P. euphratica* and 60

seedlings of *P. pruinosa*) with a height of approximately 120 cm were used for the study

in a common garden experiment. In early April 2018, healthy *P. euphratica* and *P.

pruinosa* seedlings were planted in 30-L plastic pots (one seedling per pot) filled with

homogenized soil. The planting soil, was obtained from a mixed forest dominated by *P.

euphratica* and *P. pruinosa* near the experimental site, had a pH of 8.75 ± 0.04, soil

organic matter content of 26.42 ± 0.74 mg g^{-1}, total N content of 0.88 ± 0.07 mg g^{-1} and

total P content of 1.12 ± 0.01 mg g^{-1}. After one-month adaptation to the environment,

all seedlings were subjected to drought and salinity treatments in early May 2018, and

the plants were harvested at the end of August 2018.

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The experiment was a completely randomized design with the following factors: two

species (*P. euphratica* and *P. pruinosa*), two watering regimes (well-watered and drought), and two levels of salinity (no salt and added salt). Both *P. euphratica* and *P. pruinosa* were divided into two batches. The first batch was irrigated with 100-mM NaCl solution (Janz et al. 2012) every other day, five times in total, while the second batch was irrigated with water. Then, these two batches of seedlings were divided into two further batches exposed to drought stress or to well-watered conditions. A time-domain reflectometer (Robinson et al. 2003; Yu et al. 2018) was employed for the determination of the soil water content (SWC) to demonstrate that SWC in the well-watered treatments ranged between 28-32% and SWC in the drought treatments ranged between 8-12% (Fig. S1). There were four treatments in total as follows: well-watered (W), drought (D), salinity (S) and drought and salinity (DS). All seedlings were watered with varying amounts of water every day. Fifteen replicates per treatment were included in the experiment.

Growth and relative water content of leaves

At the end of the experiment, five seedlings were selected randomly from each treatment to measure biomass. All harvested plants were divided into leaves, stems, fine roots (< 2 mm) and coarse roots (> 2 mm), then dried at 70 °C for 72 h to a constant weight and weighed. The root/shoot ratio (R/S ratio) was calculated as total root biomass / (leaf biomass + stem biomass).

199   A fully expanded leaf from each of the five randomly chosen seedlings in each

200   treatment was used to determine the relative water content (RWC). We measured fresh

201   mass (FM), turgid mass (TM) and dry mass (DM) of 10 leaf discs (0.8 cm in diameter)

202   collected from the middle part of each leaf. Then, RWC was calculated as follows: RWC

203   = 100(FM − DM) / (TM − DM).

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205   *Determination of gas exchange and pigment contents*

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207   A LI-COR 6400 portable photosynthesis measuring system with the standard leaf

208   chamber (2×3 cm^2 window area; LI-COR, Lincoln, NE, USA) was used to determine

209   the net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) of the

210   fourth fully expanded and intact leaf from five randomly chosen seedlings in each

211   treatment between 08:00 a.m. and 11:30 a.m. in early August 2018. Additional details

212   concerning the measurement procedures are described by Song et al. (2017). The leaves

213   used for the gas exchange determination were sampled for the measurement of leaf

214   pigment concentrations. We used a UV-330 spectrophotometer (Unicam, Cambridge,

215   UK) to determine chlorophyll concentrations according to the protocol of Lichtenthaler

216   (1987). The total chlorophyll content (*TChl ab*) was the sum of chlorophyll *a* and *b*.

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218   *Determination of carbon isotope composition*

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220   Neighboring leaves used for the P_n determination were randomly sampled and analyzed

221 for the C isotopic composition, expressed as $\delta^{13}C$ values (relative to Pee Dee Belemnite).

222 The $^{13}C/^{12}C$ ratios of the leaf samples were measured using a DELTA V Advantage

223 Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA)

224 according to the method of Chen et al. (2014).

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226 *Determination of NSCs*

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228 At the end of the experiment, five randomly chosen plant samples (leaf, stem, fine root

229 and coarse root) were sampled from each treatment for the NSC analysis. In brief, about

230 50 mg of powdered plant samples were placed into 10-ml centrifuge tubes, and then

231 extracted in 80% (v/v) ethanol at 80 °C for 30 min. The extract was used to determine

232 soluble sugars, and the residue was used for starch measurements (Yemm and Willis

233 1954). Additional details are described by Song et al. (2017).

234

235 *Determination of Cl⁻ concentration*

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237 Five randomly chosen dry powdered plant samples (leaf, stem, fine root and coarse root)

238 were sampled from each treatment for Cl^- analyses according to the modified silver

239 titration method (Chen et al. 2001).

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241 *Determination of lipid peroxidation and antioxidant enzyme activities*

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243   For the determination of lipid peroxidation and antioxidant enzyme activities, fully

244   expanded and intact leaves were selected from five randomly chosen individuals from

245   each treatment. The concentrations of superoxide radicals (O_2^-) and malondialdehyde

246   (MDA) were measured according to the methods of Lei et al. (2006) and Kramer et al.

247   (1991), respectively. For the analysis of peroxidase (POD) and superoxide dismutase

248   (SOD) activities, about 0.5 g fresh leaves were ground in liquid nitrogen and extracted

249   with 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 1% (w/v)

250   PVP, 0.1 mM PMSF and 0.1% (v/v) Triton X100. Additional methodological details are

251   described in Li et al. (2013) and Liu et al. (2020).

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253   *Statistical analyses*

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255   Statistical analyses were performed using the Statistical Package for the Social Sciences

256   (SPSS, Chicago, IL, USA) version 18.0. All data were checked for normality and the

257   homogeneity of variances and log-transformed to correct deviations from these

258   assumptions when necessary. Tukey's HSD tests were conducted to detect significant

259   differences among treatments. Three-way analyses of variance (ANOVA) were

260   performed to analyze the effects of species, salinity, drought and their interactions. All

261   statistical effects were considered significant at $P<0.05$. Principal component analysis

262   (PCA) was carried out with Canoco 5.0 (Microcomputer Power, USA).

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Results

Differences in growth traits

Drought, salinity and combined stress significantly decreased leaf, stem, total root, fine

287   root and total biomass of both poplar species, whereas they increased their R/S ratio

288   (Fig. 1a-f). *P. euphratica* possessed a higher leaf, total root and total biomass than *P.*

289   *pruinosa* under drought, salinity and combined stress. In addition, the interactions

290   between species, drought and salinity for leaf, total root and total biomass were

291   significant. Thus, the results indicate that under drought stress, salinity caused greater

292   decreases in the studied traits in *P. pruinosa* (Table S1).

293

294   *Differences in gas exchange and pigments*

295

296   Drought, salinity and combined stress significantly decreased P_n, *TChl ab*, g_s, E and

297   RWC of both poplar species, whereas they increased $\delta^{13}C$ (Fig. 2a-f). In addition, *P.*

298   *euphratica* had higher P_n, *TChl ab*, g_s, E and $\delta^{13}C$ under salinity, and higher P_n, *TChl*

299   *ab*, g_s, E and RWC under combined stress. Furthermore, P_n, *TChl ab*, g_s and $\delta^{13}C$ were

300   significantly affected by species × salinity and drought × salinity interactions. Thus,

301   those traits decreased more under salinity and combined stress in *P. pruinosa* (Table

302   S1). These findings implied that *P. pruinosa* may suffer greater inhibitory effects under

303   salinity and combined stress.

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305   *Differences in soluble sugar and starch concentrations*

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307   *P. euphratica* showed higher concentrations of leaf soluble sugars compared to *P.*

308   *pruinosa* both in control and stress conditions (Table 1). Soluble sugar concentrations

of all organs in *P. euphratica* showed increasing trends under stress, especially under

combined stress, whereas they decreased (except for leaves) in *P. pruinosa* under stress.

In addition, starch concentrations of all organs in both poplar species decreased under

drought, salinity and combined stress. Furthermore, starch concentrations of stems, fine

roots and coarse roots were significantly affected by species × drought and species ×

salinity interactions (Table 1).

Differences in Cl⁻ concentration

Under salinity, Cl⁻ concentrations of *P. euphratica* leaves, stems and coarse roots

increased approximately 36.8, 43.0 and 81.4%, respectively, but 44.3, 60.9 and 104.9%

under combined stress, respectively (Fig. 3). In addition, Cl⁻ concentrations of leaves,

stems and coarse roots increased 72.7, 28.2 and 32.4% under salinity in *P. pruinosa*,

respectively, while 78.2, 40.9 and 54.0% under combined stress, respectively. Under

salinity and the combination of drought and salinity, *P. euphratica* showed significantly

higher Cl⁻ concentrations in stems and coarse roots, whereas *P. pruinosa* allocated more

Cl⁻ into leaves. Furthermore, the Cl⁻ concentrations of all organs were significantly

affected by the interaction of species × salinity (except for fine roots), which indicated

that salinity caused greater increases in leaf Cl⁻ concentrations, and greater decreases

in stem and coarse root Cl⁻ concentrations of *P. pruinosa* (Table S2).

Differences in oxidative stress and antioxidants

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Concentrations of O_2^-, MDA, POD and SOD significantly increased in both poplar

species under all stress conditions (Fig. 4). Compared with *P. pruinosa*, *P. euphratica*

had lower O_2^- and higher POD under combined stress, and lower MDA and higher SOD

under all stress conditions. In addition, these four parameters were all significantly

affected by the species × salinity interaction (Table S2).

Relationships among studied traits under drought and salinity

The two main components of the principal component analysis (PCA) explained 86.1%

of the total variation in the studied traits in *P. euphratica* and *P. pruinosa*, as affected

by drought, salinity and combined stress (Fig. 5). Control, drought and salinity

treatments, especially the combined stress, were well separated from each other. In

addition, *P. euphratica* and *P. pruinosa* were separated along the second PCA axis (Fig.

5). PC1 was strongly influenced by leaf, stem, fine root, total root and total biomass,

P_n, *TChl ab*, g_s, *E*, stem starch, RWC, leaf, stem, fine root and coarse root Cl^-, POD,

SOD, O_2^- and MDA, R/S ratio, and leaf, fine root and coarse root soluble sugars. PC2

was strongly influenced by stem soluble sugars, and fine root and coarse root starch.

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Discussion

Drought and salinity affect plant growth

Our results revealed that drought and salinity decreased the growth of both poplar

species, but they also showed the presence of species-specific responses to stress

conditions, particularly the significant interactions of drought and salinity on the growth

traits of *P. euphratica* and *P. pruinosa* (Fig. 1, Table S1). Compared with *P. pruinosa*,

P. euphratica possessed a higher biomass under drought, salinity and combined stress.

These results demonstrated that *P. euphratica* had a better tolerance under drought and

salinity. Our results are consistent with previous studies suggesting that drought and

salinity tolerance in poplars varies considerably among species (Yang et al. 2010; Polle

and Chen 2015; Rancourt et al. 2015). In addition, the significant interactive effects of

species × drought × salinity on the total biomass (Table S1) indicated that under drought,

salinity caused greater decreases in the total biomass of *P. pruinosa* (Fig. 1d). These

interactions showed that the two poplar species possess different growth strategies

under changing conditions. Our findings also allow predictions of the potential

responses of these two desert poplar species to increasing drought and soil salinity

under global climate change.

Previous studies have reported that plants can shift biomass allocation and change

root/shoot ratios to cope with varied environmental conditions, such as drought

(Brunner et al. 2015; Phillips et al. 2016; Zhou et al. 2018). Our results are in line with

previous studies suggesting that both poplar species possess a higher R/S ratio under

drought, salinity and combined stress (Fig. 1f), thus optimizing water uptake. A greater

root system is usually associated with a greater capacity of nutrient and water uptake,

as confirmed in many studies (Shipley and Meziane 2002; Brunner and Godbold 2007;

Portmuth and Niinemets 2007). In our study, *P. euphratica* had a higher total root

397   biomass compared with *P. pruinosa* under drought, salinity and combined stress (Fig.

398   1c), which indicated that *P. euphratica* may have a greater root nutrient and water

399   uptake capacity under stress conditions.

400

401   *Drought and salinity affect photosynthesis and nonstructural carbohydrates*

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403   Many studies have revealed that drought and salinity have inhibitory effects on

404   photosynthesis and growth (Xu et al. 2008; McDowell et al. 2011, 2013; Chen et al.

405   2014; Polle and Chen 2015). Our results were consistent with these observations

406   indicating that drought, salinity and combined stress significantly decrease P_n, *TChl ab*,

407   g_s, E and RWC of *P. euphratica* and *P. pruinosa* (Fig. 2a-e). However, we also found

408   species-specific differences under stressful conditions. For example, *P. euphratica* had

409   higher P_n, *TChl ab*, g_s, E and δ^{13}C under salinity stress. This result is probably due to

410   the greater tolerance of *P. euphratica* when exposed to salinity stress (Chen et al. 2001,

411   2002; Chen and Polle 2010). There were positive associations among leaf, stem, root

412   and total biomass, P_n, *TChl ab*, g_s, E, RWC, and leaf, stem and coarse root starch

413   concentrations according to PCA analysis. Moreover, *P. euphratica* and *P. pruinosa*

414   were separated along the second PCA axis (Fig. 5).

415

416   Our results indicate that the growth and physiological trait responses to drought, salinity

417   and combined stress were species-specific, and further supported by significant species

418   × drought × salinity interactions on many studied parameters (leaf, total root and total

419　biomass, g_s, $\delta^{13}C$, leaf Cl^- concentration, leaf and stem starch concentrations). In

420　addition, the carbon isotope composition ($\delta^{13}C$) of leaves normally acts as an indicator

421　of the long-term water use efficiency (WUE) of plants (Dawson et al. 2004; Li et al.

422　2007). Greater $\delta^{13}C$ caused by drought and salinity may result in decreased E and

423　stomatal closure, and increased ^{13}C fixation(Zhang et al. 2005; Chen et al. 2010). In the

424　present study, drought and salinity increased WUE (e.g. $\delta^{13}C$) of both poplar species,

425　which corroborated previous findings in *Populus* (Xu et al. 2008; Chen et al. 2010,

426　2014; Li et al. 2016).

427

428　It has been suggested that plants with higher soluble sugar and starch contents have a

429　better tolerance and survival under stressful environments (Dietze et al. 2014; Martínez-

430　Vilalta et al. 2016; Kannenberg et al. 2017). In this study, drought, salinity and

431　combined stress decreased starch concentrations of all organs in both poplar species

432　(Table 1). This result was similar to other studies suggesting that stress decreases the

433　content of starch (Hartmann et al. 2013; Mitchell et al. 2013; Dai et al. 2017), which

434　may convert into soluble sugars (McDowell et al. 2011; García-Forner et al. 2016).

435　Previous studies have demonstrated that soluble sugars contribute to osmotic

436　adjustment and play crucial roles in maintaining the soil-to-plant water potential

437　gradient during drought and osmotic stress, and in later embolism repair (Dietze et al.

438　2014; Martínez-Vilalta et al. 2016; De Baerdemaeker et al. 2017).

439

440　In our study, there were species- and organ- specific responses to drought and salinity.

441 *P. euphratica* showed significantly higher soluble sugar concentration in leaves than *P.*

442 *pruinosa* in both control and stress conditions (Table 1). The high soluble sugar

443 concentration in the leaves of *P. euphratica* indicates that the protective effect and

444 osmoregulation may be already in place before the stress conditions begins (Chen et al.

445 2001; Janz et al. 2010), whereas *P. pruinosa* may lag too much behind to prevent

446 effectively drought and salinity imposed problems. Previously, Karst et al. (2017) have

447 reported that soluble sugars in fine roots, analogous to leaves, can also serve as

448 osmoregulatory compounds. Therefore, the higher soluble sugar concentration in the

449 fine roots of *P. euphratica* relative to *P. pruinosa* under stress (Table 1) indicates that *P.*

450 *euphratica* may have a greater capacity of osmotic regulation in fine roots when

451 exposed to stress.

452

453 *Drought and salinity affect Cl⁻ allocation and accumulation*

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455 Previous studies have revealed that Cl⁻ is mainly allocated into the leaf tissue of poplars

456 (Chen et al. 2002, 2010; Zalesny et al. 2007; Chen and Polle 2010). Comparably, our

457 results suggested that both poplar species show highest Cl⁻ concentrations in leaves

458 exposed to salinity and combined salinity and drought stress (Fig. 3). Moreover, the Cl⁻

459 concentration of *P. pruinosa* leaves increased approximately 72.7% and 78.2% under

460 salinity and combined drought and salinity stress, respectively. In comparison to *P.*

461 *pruinosa*, *P. euphratica* accumulated less Cl⁻ in leaves and allocated more Cl⁻ into

462 coarse roots, where Cl⁻ concentration increased 81.4% and 104.9% under salinity and

combined drought and salinity stress, respectively (Fig. 3). Therefore, the higher

resistance to salinity of *P. euphratica* appears to be linked to the restriction of salt

transport from roots to leaves. These results are consistent with previous studies

suggesting that the exclusion of Cl^- is the most important mechanism of salt tolerance

in *P. euphratica* (Chen et al. 2001, 2002; Polle and Chen 2015). Leaves and fine roots

are sensitive plant organs and indicate the carbon assimilation capacity, and the

absorption of nutrients and water (Brunner et al. 2015; McCormack et al. 2015). In the

present study, although salinity caused *P. euphratica* to allocate more Cl^- into coarse

roots, the Cl^- allocation into fine roots increased only little, approximately 11.1% and

25.3% under salinity and combined salinity and drought stress, respectively (Fig. 3).

Thus, our results demonstrated that *P. euphratica* possesses a Cl^- exclusion mechanism

that allocates more Cl^- into coarse roots and less into leaves and fine roots, which may

minimize ion toxicity to these more sensitive organs under salinity stress.

Drought and salinity affect oxidative stress and antioxidants

MDA is a product of lipid peroxidation and a key indicator of membrane damage caused

by oxidative stress. Previous studies have reported that a lower MDA level reflects a

higher anti-oxidative capacity, which represents higher resistance to stress (Xu et al.

2008; Li et al. 2011; Liu et al. 2020). In the present study, MDA significantly increased

in both poplar species, but *P. pruinosa* showed a higher MDA concentration than *P.*

euphratica under drought, salinity and especially under combined stress (Fig. 4b).

485 Furthermore, under stressful conditions, O_2^- significantly increased in both species, but

486 *P. euphratica* had a lower O_2^- level than *P. pruinosa* under combined stress (Fig. 4a).

487 These results demonstrated that drought, salinity and especially the combined stress

488 induced damage to membrane lipid peroxidation that could be lower in *P. euphratica*

489 than in *P. pruinosa*.

490

491 Foliar antioxidant enzyme activities (e.g. POD and SOD) play crucial roles in oxygen-

492 scavenging activities and, thereby, represent an important tolerance mechanism to deal

493 with abiotic stress (Petrov et al. 2015; Li et al. 2016). In our study, there were

494 significantly higher activities of SOD and POD isoenzymes in *P. euphratica* than *P.*

495 *pruinosa* under drought, salinity and especially under combined stress (Fig. 4). Our

496 result is consistent with previous studies suggesting that *P. euphratica* could maintain

497 the activities of POD and SOD isoenzymes under stress (Wang et al. 2007, 2008; Chen

498 and Polle 2010). Thus, *P. euphratica* possesses a more effective antioxidant defense

499 system to cope with stressful conditions.

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501 **Conclusions**

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503 In the present study, we discovered that *P. euphratica* and *P. pruinosa* have different

504 morphological, physiological and biochemical responses to drought, salinity and

505 combined stress. In addition, *P. euphratica* exhibited stronger resistance to drought,

506 salinity and especially to combined stress, visible as a higher biomass, photosynthetic

capacity and long-term water use efficiency (δ^{13}C) under stress conditions. It is possible that the stronger resistance of *P. euphratica* is mainly associated with higher antioxidant enzyme activities, better ROS scavenging and osmotic regulation, as well as with Cl$^-$ being allocated less into leaves and more into coarse roots. Significant species × drought × salinity interactions in many studied parameters (biomass, g_s, δ^{13}C, leaf Cl$^-$ concentration, etc.) suggest that species-related differences in these traits would increase along environmental gradients, implying different life history strategies. Considering increasing drought and soil salinity developing under climate change, knowledge of species-specific stress responses and tolerances in these two poplar species is crucial for ecological restoration and afforestation efforts in arid areas.

Author contributions Lei Yu had the main responsibility for data collection, analysis and writing, Haojie Dong, Zhijun Li and Zhanjiang Han contributed to data analysis, 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.

529   **Conflict of interest** The authors declare that they have no conflict of interest.

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Table 1. Soluble sugar and starch concentrations of different organs (mean ± SE) in *P. euphratica* and *P. pruinosa*, as affected by drought, salinity and combined stress.

Species	Treatment	Leaf soluble sugar (mg g⁻¹)	Stem soluble sugar (mg g⁻¹)	Fine root soluble sugar (mg g⁻¹)	Coarse root soluble sugar (mg g⁻¹)	Leaf starch (mg g⁻¹)	Stem starch (mg g⁻¹)	Fine root starch (mg g⁻¹)	Coarse root starch (mg g⁻¹)
P. euphratica	W	116.98±3.43c	79.15±1.47b	56.53±1.87e	65.58±1.72c	31.19±0.81a	13.98±0.43a	20.28±0.47a	48.49±1.62a
	D	123.56±2.14bc	70.44±1.60c	82.59±1.43b	86.89±1.73b	22.03±0.97b	9.85±0.45c	16.95±0.63b	35.73±1.17b
	S	126.39±3.75bc	73.40±1.71bc	80.45±0.78bc	87.29±1.07b	24.38±0.89b	12.22±0.50b	17.84±0.57b	33.98±1.40b
	DS	161.25±3.04a	89.85±2.69a	95.40±1.36a	106.04±1.92a	17.30±0.58c	6.73±0.33d	16.71±0.33b	26.56±1.37c
P. pruinosa	W	80.38±1.57e	72.59±1.51bc	75.33±1.97c	105.81±4.42a	25.54±1.40b	14.44±0.55a	20.59±0.71a	44.23±1.61a
	D	96.39±1.37d	59.12±1.81d	63.51±1.18d	86.03±2.55b	14.44±0.26c	9.33±0.39c	17.24±0.56b	35.13±1.10b
	S	88.76±1.45de	55.05±2.08d	68.20±1.48d	84.64±2.38b	13.70±0.83cd	8.64±0.39c	17.66±0.46b	34.76±1.17b
	DS	130.99±2.99b	53.02±2.06d	63.64±2.06d	86.53±2.13b	10.36±0.62d	6.41±0.30d	17.08±0.48b	26.98±0.75c
	$P{:}F_{Sp}$	**0.000**	**0.000**	**0.000**	**0.017**	**0.000**	**0.002**	0.605	0.329
	$P{:}F_{D}$	**0.000**	0.161	**0.000**	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**
	$P{:}F_{S}$	**0.000**	0.074	**0.000**	**0.006**	**0.000**	**0.000**	**0.001**	**0.000**
	$P{:}F_{Sp \times D}$	**0.030**	**0.000**	**0.000**	**0.000**	0.461	0.067	0.726	0.376
	$P{:}F_{Sp \times S}$	0.584	**0.000**	**0.000**	**0.000**	0.080	**0.003**	0.785	0.110
	$P{:}F_{D \times S}$	**0.000**	**0.000**	0.390	**0.009**	**0.000**	0.207	**0.003**	0.079
	$P{:}F_{Sp \times D \times S}$	0.782	**0.016**	**0.000**	**0.001**	**0.025**	**0.001**	0.715	0.282

Different letters denote significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. W, well-watered; D, drought; S, salinity; DS, drought and salinity.

Three-way analyses of variance (ANOVA) were applied to evaluate the effects of different factors and their interactions. F_{Sp}, species effect; F_{D}, drought effect; F_{S}, salinity effect; $F_{Sp \times D}$, species × drought interaction effect; $F_{Sp \times S}$, species × salinity interaction effect; $F_{D \times S}$, drought × salinity interaction effect; $F_{Sp \times D \times S}$, species × drought × salinity interaction effect.

Figure legends

Figure 1. (a) Leaf biomass, (b) stem biomass, (c) total root biomass, (d) total biomass, (e) fine root biomass, and (f) root to shoot (R/S) ratio in *P. euphratica* and *P. pruinosa,* as affected by drought, salinity and combined stress. Each value is the mean ± SE (n = 5). Different letters above bars denote significant differences according to Tukey's HSD test at a significance level of $P < 0.05$. W, well-watered; D, drought, S, salinity; DS, drought and salinity.

Figure 2. (a) Net photosynthetic rate (P_n), (b) total chlorophyll content (*TChl ab*), (c) stomatal conductance (g_s), (d) transpiration (E), (e) relative water content (RWC) and (f) carbon isotope composition ($\delta^{13}C$) in *P. euphratica* and *P. pruinosa,* as affected by drought, salinity and combined stress. Each value is the mean ± SE (n = 5). Treatment codes and statistical analyses as in Figure 1.

Figure 3. (a) Leaf Cl^- concentration, (b) stem Cl^- concentration, (c) fine root Cl^- concentration and (d) coarse root Cl^- concentration in *P. euphratica* and *P. pruinosa,* as affected by drought, salinity and combined stress. Each value is the mean ± SE (n = 5). Treatment codes and statistical analyses as in Figure 1.

Figure 4. (a) Superoxide radicals (O_2^-), (b) malondialdehyde (MDA) (c) peroxidase (POD) and (d) superoxide dismutase (SOD) in *P. euphratica* and *P. pruinosa,* as

23   affected by drought, salinity and combined stress. Each value is the mean $\pm$ SE ($n = 5$).

24   Treatment codes and statistical analyses as in Figure 1.

25

26   **Figure 5.** Principal component analysis (PCA) based on eco-physiological traits in *P.*

27   *euphratica and P. pruinosa*, as affected by drought, salinity and combined stress. Open

28   symbols: *P. euphratica*, filled symbols: *P. pruinosa*. Circles, up triangle arrows, down

29   triangle arrows and squares indicate W, D, S and DS treatments, respectively. P_n, net

30   photosynthetic rate; g_s, stomatal conductance; E, transpiration; LM, leaf biomass; SM,

31   stem biomass; FRM, fine root biomass; TRM, total root biomass; TM, total biomass;

32   leaf ST, leaf starch; stem ST, stem starch; FR ST, fine root starch; CR ST, coarse root

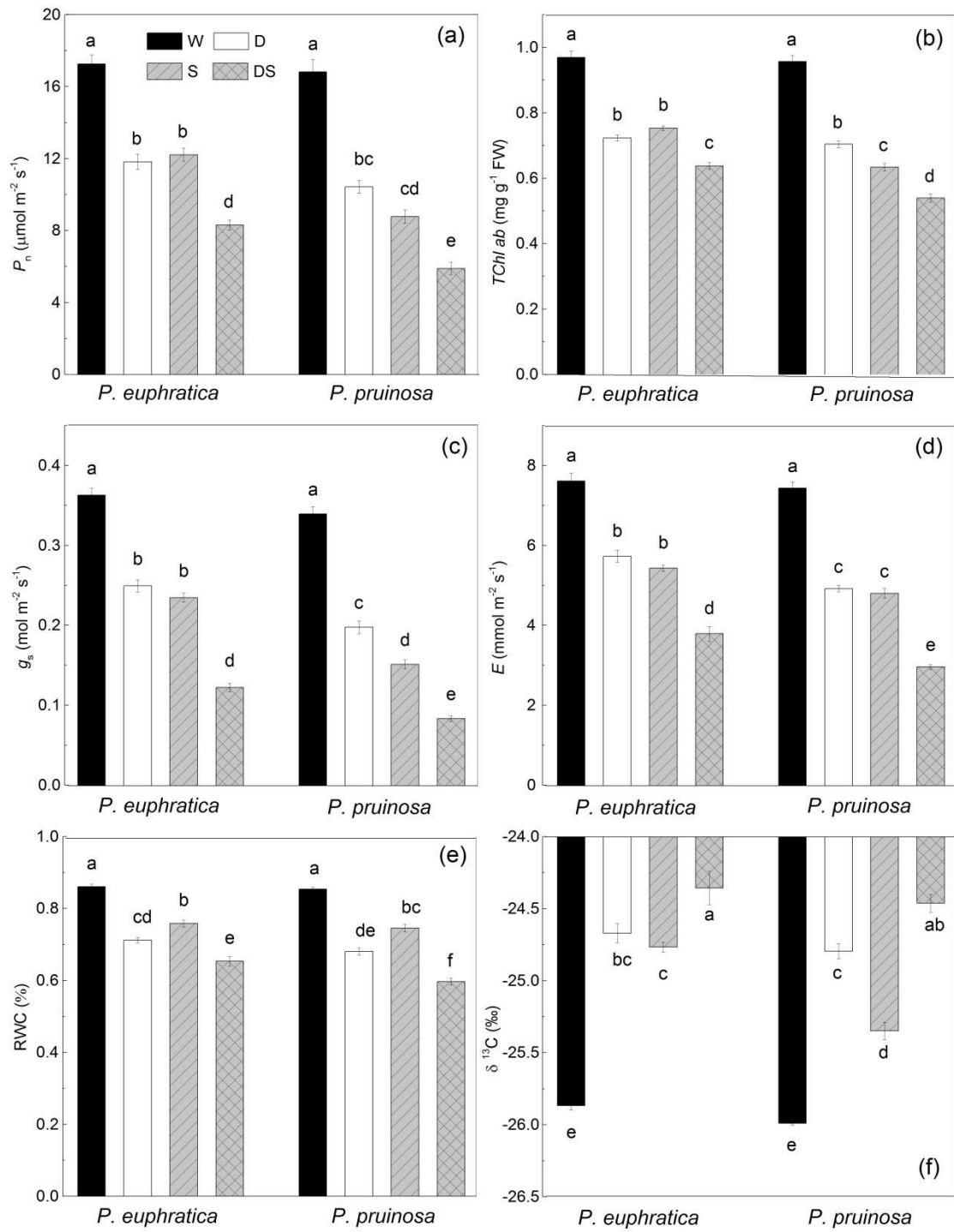33   starch; leaf SS, leaf soluble sugar; stem SS, stem soluble sugar; FR SS, fine root soluble

34   sugar; CR SS, coarse root soluble sugar; FR Cl$^-$, fine root Cl$^-$; CR Cl$^-$, coarse root Cl$^-$.
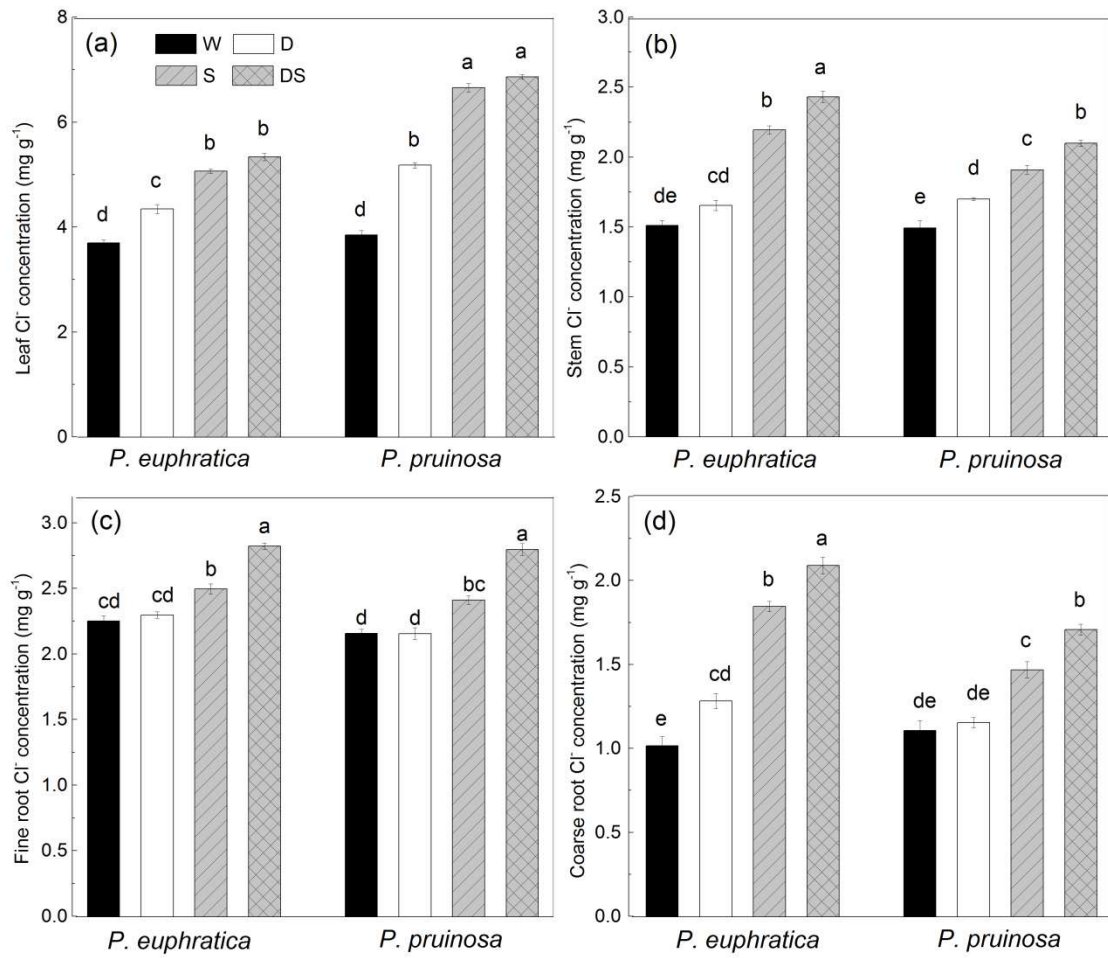
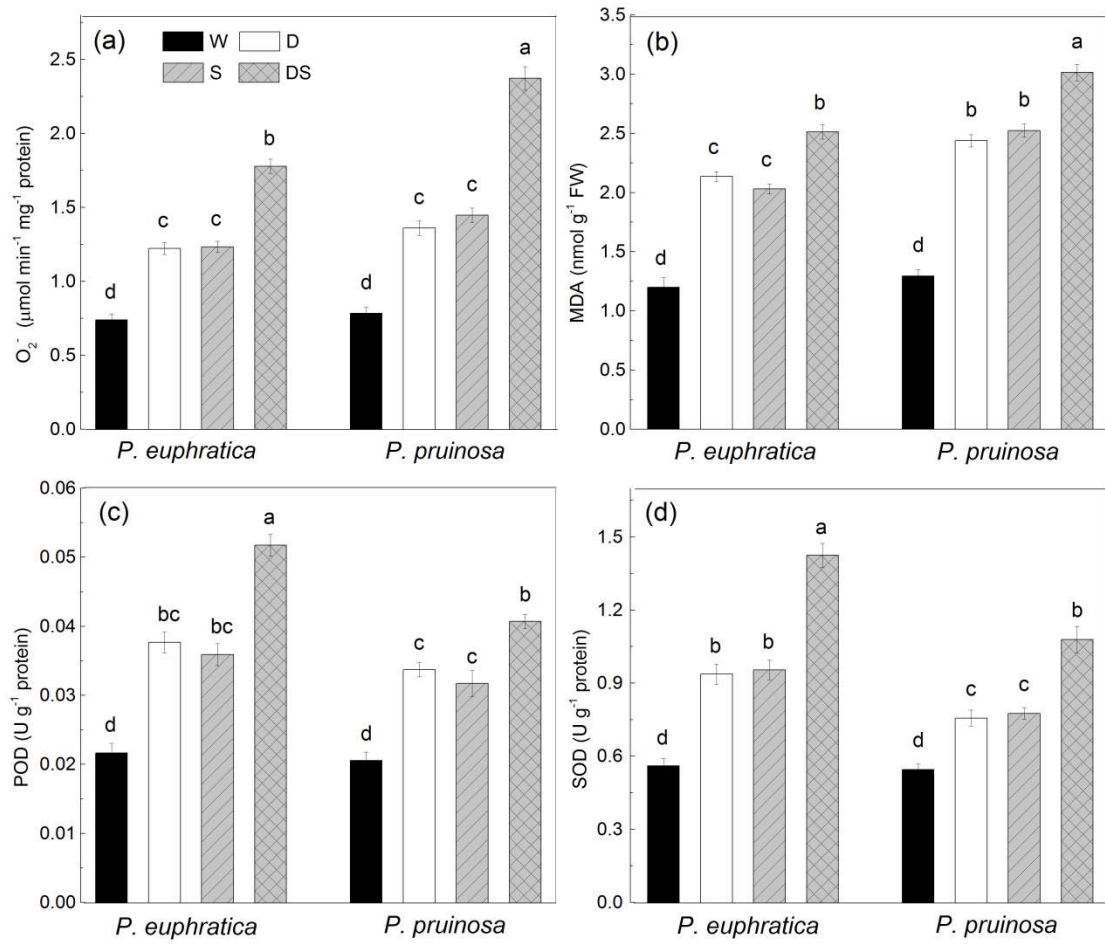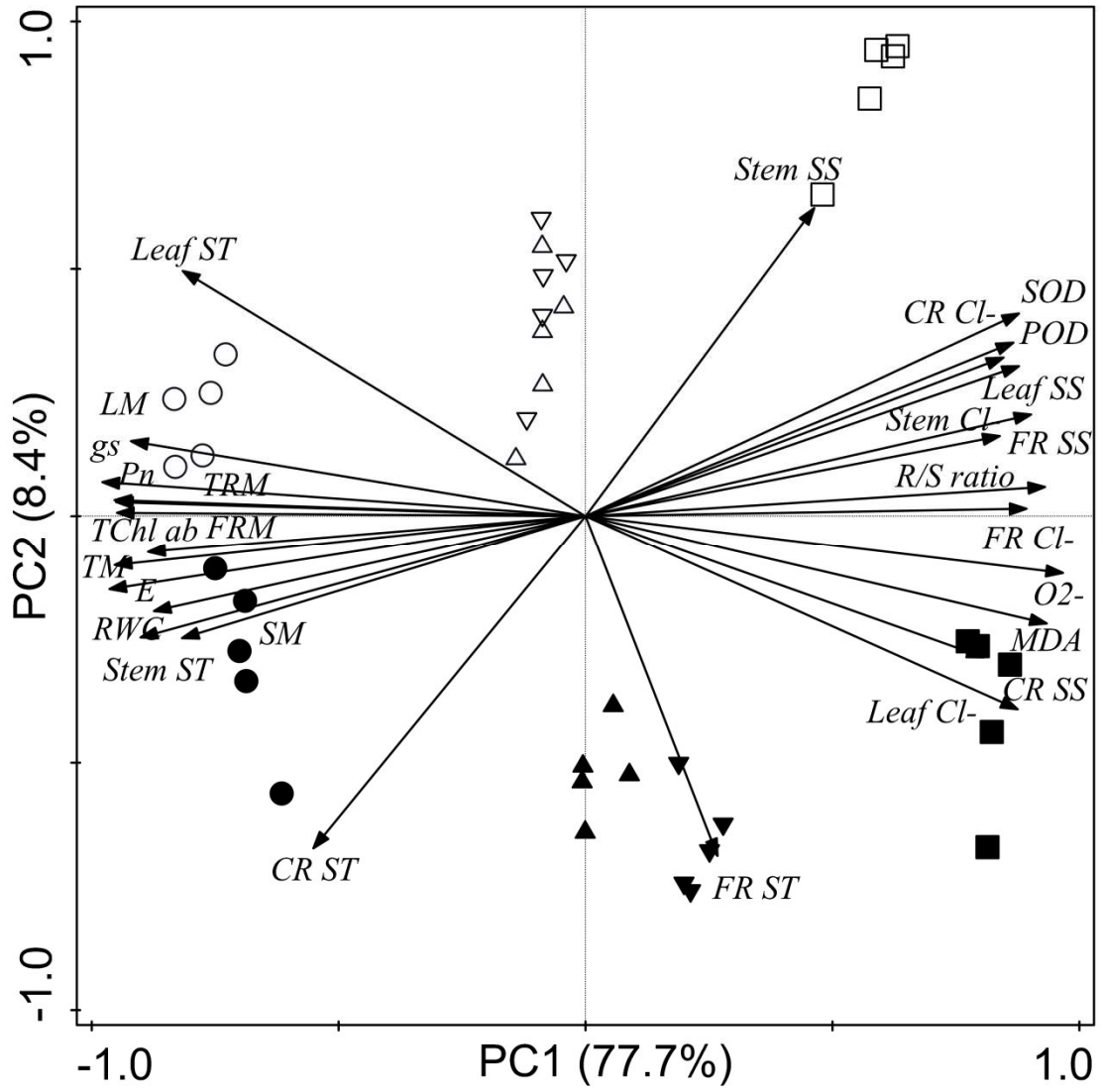35   Treatment codes as in Figure 1.

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Figure 1

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Figure 2

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Figure 3

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Figure 4

Figure 5