WETLAND ECOSYSTEMS

Differences in relative air humidity affect responses to soil salinity in freshwater and salt marsh populations of the dominant grass species *Phragmites australis*

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Abstract Soil salinity diminishes the dominance of species and affects their distribution. *Phragmites australis* is a dominant ecosystem engineer with broad distribution, high intraspecific variation and great socio-economic importance. Coastal ecosystems inhabited by *P. australis* are threatened by salinization. Here, we investigated salinity tolerance of freshwater and salt marsh population of the species, grown under two soil salinities and in two common gardens. Salinity significantly affected the growth, biomass, leaf physiological parameters and ion concentration. Climate altered morphology and ion concentration of *P. australis*. In contrast to our hypothesis, the salt marsh population was not more salt tolerant than the freshwater population, and both showed a low degree of salt stress, maintaining their photosynthesis and chlorophyll concentration, and only showing small decreases in biomass and height when salt-treated. We therefore ruled out local adaptation to soil salinity. Instead, both populations acclimated by phenotypic plasticity of biomass, root: shoot ratio, stomatal conductance and ion content. The salinity tolerance strategy of both populations was ion concentration by tissue desiccation, which was most efficient in the drier climate common garden. Hence, plants utilizing tissue desiccation as salinity tolerance strategy will have an advantage in areas where climate change leads to drier air humidity.

Keywords Compatible osmolytes · Intraspecific diversity · Local adaptation · Phenotypic plasticity · Salinity tolerance · Yellow River Delta

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Introduction

Salt marshes are valued for their ecosystem services such as carbon sequestration, erosion control, regulated water flows, nutrient retention and generally improved water quality (Himes-Cornell et al., 2018). Humans use salt marshes for grazing of creatures and agriculture but also leisure and recreation. However, salt marshes are threatened by overutilization and climate change, in particular salinization. Salinization occurs mainly due to water extraction by humans, which reduces freshwater flow to the ocean, while the predicted changes in temperature and air humidity will affect evapotranspiration. When soil water evaporates, dissolved ions such as sodium (Na^+) and chloride (Cl^-) are retained in the ground, thereby increasing the salt concentration in the soil. Climate change is expected to exacerbate soil salinities because air humidity will likely decrease in the future (Byrne & O'Gorman, 2018), which could lead to higher salt concentrations in the soil, caused by increased evapotranspiration. High soil salinity lowers the soil water potential and can be toxic to the vegetation of the marsh, thereby primarily restricting plant water uptake and growth (Munns & Tester, 2008). Increasing soil salinity in salt marshes is a problem, as it can reduce the primary productivity (Curco et al., 2002) and negatively affect plant species composition (Pennings et al., 2005), consequences which both are known to decrease the value of the ecosystem services provided to humans (Costanza et al., 1998, 2007).

Salt marshes often consist of few dominant plant species distributed in distinct patches. Historically, several studies have focused on how soil salinity in interaction with other abiotic factors, such as flooding, determined plant species composition (Stephenson & Stephenson, 1949; Adams, 1963; Snow & Vince, 1984). Biotic interactions like competition were also recognized as factors governing plant biodiversity (Bertness, 1991; Emery et al., 2001; Pennings et al., 2005). However, studies concerning plant species composition in salt marshes have so far primarily focused on interspecific and intergeneric diversity, although intraspecific variation has been shown to have a large effect on ecological and spatial differences (see citations in Eller et al., 2017). For example, differences in salinity tolerance can result in different distribution of different genotypes of the same species (Gao et al., 2012; Achenbach & Brix, 2014). Also, the

prevailing climate can have an immediate effect on salinity tolerance. For example, high temperature has been reported to increase the salinity tolerance (Lissner et al., 1999), while low air humidity can reduce plant growth in saline soils (Pasternak, 1987).

Phragmites australis (Cav.) Trin. ex Steud. (Poaceae) is an example of a dominating plant species with high intraspecific genotypic and phenotypic variation. It is a tall, fast-growing and highly productive perennial grass (Haslam, 1973; Graneli, 1984). This species reproduces sexually by seeds and also asexually by clonal production from the belowground rhizomes, which also function as storage and overwintering organs. Due to its vigorous growth, tall stature and clonal growth form, it is capable of forming large and dense patches of often monotypic stands in wetlands. These large stands are very important in structuring the ecosystem and its function, such as habitat for wildlife, sediment stabilization, nutrient retention and storm buffering. In China, large native *P. australis* stands are economically important, as *P. australis* is managed and harvested for paper production (Brix et al., 2014; Kobbing et al., 2016). The high intraspecific variation also results in a large variation in flooding and salinity tolerance within the species (White & Ganf, 2002; Achenbach & Brix, 2014). The great intraspecific variation of *P. australis* has in certain parts of the globe resulted in severe consequences for the ecosystems the species inhabits (Windham & Ehrenfeld, 2003; Mueller et al., 2016; Eller et al., 2017). One such example is a cryptic invasion of *P. australis* in North America (Vasquez et al., 2005; Mozdzer et al., 2013) where a distinct type of *P. australis* with origin in Europe has been introduced and is outcompeting the native vegetation, including native north American *P. australis* (Saltonstall, 2002). The invasive type's greater salinity tolerance compared to native North American *P. australis* has been proposed as one mechanism, which can explain the vigorous growth of *P. australis* from Europe compared to its native North American congener (Chambers et al., 1999). The invasive European type is now considered a nuisance, as it has had major impacts on ecosystem services and functions (Caplan et al., 2015; Mozdzer et al., 2016). These studies highlight the importance of intraspecific differences in salinity tolerance, which can have major effects on the ecosystem.

Intraspecific differences can arise due to local adaptation or phenotypic plasticity. Local adaptation is found in ecotypes within a species that are genetically adapted to their particular habitat and environmental conditions, where those ecotypes have the highest fitness. Phenotypic plasticity is the capacity of a genotype to produce different phenotypes as acclimation to different environmental conditions (Bradshaw, 1965). Phenotypic plasticity is highly likely to be genetically determined (Latzel & Klimesova, 2010; Münzbergová et al., 2017). A highly plastic genotype will have similar fitness in different habitats due to environmental induced variation in functional traits (e.g., leaf nitrogen content, photosynthesis and leaf thickness). Elucidating to what degree either phenotypic plasticity or local adaptation contributes to intraspecific differences is important when predicting species responses to climate change, since that will affect the ecosystem and its functioning and services (Ellison et al., 2005; Valladares et al., 2014). This fact is true particularly for foundation species, which have a strong role in structuring a community (Ellison, 2019).

In China, *P. australis* can be considered a foundation species, as it is native and very abundant, constitutes a large part of the standing biomass and is placed near the bottom of wetland food webs (Kiviat, 2013). Several salt marshes with high abundance of *P. australis* are associated with the Yellow River Delta. The Yellow River Delta is part of the Ramsar convention (Xing et al., 2016; Liang et al., 2018), but the delta is highly threatened by salinization (Fan et al., 2012). Due to its ecological value and threats to the Yellow River Delta, the main focus of a large wetland restoration project has focused on establishing conditions that allow *P. australis* to revegetate these areas (Cui et al., 2009). To elucidate the optimal growing conditions for *P. australis*, several studies have manipulated soil salinity and water level (Cui et al., 2011; Yuan et al., 2013; Guan et al., 2017), which are known to affect the growth of *P. australis*, but none of these considered how intraspecific variation affected the outcome. Additionally, *P. australis* in Asia has received little attention compared to Europe and North America despite it being a hotspot of *P. australis* diversity (Eller et al., 2017; Pyšek et al., 2019).

Therefore, we aim to assess the salinity tolerance of *P. australis* from Chinese wetlands, which differed in soil salinity, and investigate whether *P. australis* is locally adapted to high salinity or whether it relies on phenotypic plasticity. More specifically, we compared salinity tolerance of *P. australis* collected from different Chinese freshwater wetlands (freshwater population) to *P. australis* from the Yellow River Delta salt marshes (salt marsh population) (Fig. 1). We compared salinity tolerance by growing the plants at 0 ppt (control) and 20 ppt sea salt. We used two common gardens (Kawecki & Ebert, 2004) located in different climatic regions to understand how climate affects the plants' salinity tolerance. To quantify the salinity tolerance, we assessed the relative salinity tolerance, defined as the biomass difference between salt-affected plants and the control (Munns et al., 2019), and the absolute salinity tolerance, defined as the total biomass produced in salt-affected plants irrespective of a control (Lissner et al., 1999). Additionally, we assessed the tissue tolerance, defined as the ability to maintain physiological integrity in the presence of Na^+. We did so by assessing nutrient concentrations in the plant tissue and the ability to maintain the K^+/Na^+ ratio, which is important under long-term salt stress (Asch et al., 2000). For example, the K^+/Na^+ ratio was correlated to salt-tolerance and grain yield in different grass species (Azooz et al., 2004), and is likely to be correlated to biomass production as fitness proxy also in *P. australis*. Finally, morphological traits, such as shoot height and density, as well as different physiological leaf parameters were evaluated as performance indicators. We hypothesized that (1) the freshwater population would perform better (greater biomass, shoot height and density) than the salt marsh population at the control salinity (0 ppt), but this pattern would be opposite at 20 ppt, due to local adaptation; and (2) at 20 ppt, the salt marsh population would have a greater capacity to maintain its K^+/Na^+ ratio and tissue nutrient concentration compared to the freshwater population.

Materials and methods

Plant material and experimental setup in common gardens

Twenty-two clones of native Chinese *P. australis* from two original populations were used in this study
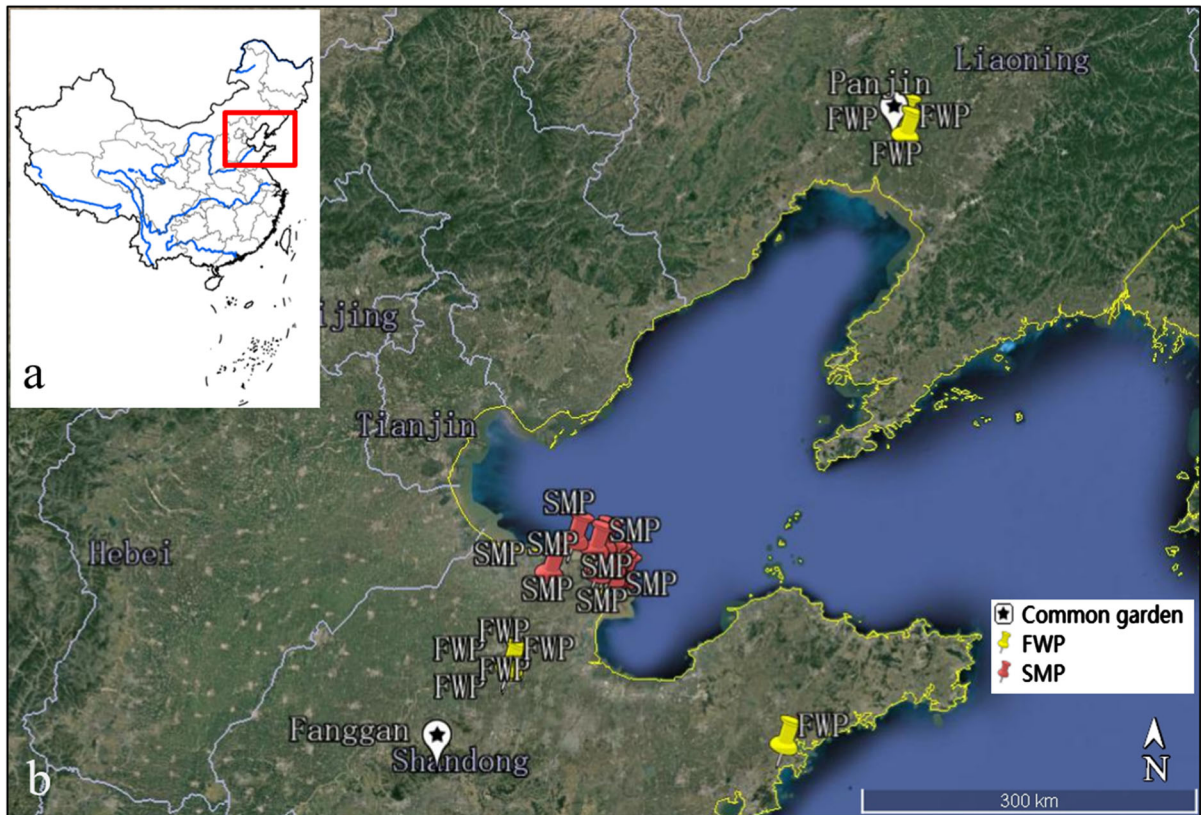
Fig. 1 Map of China (**a**) highlighting the location of the freshwater (FWP) and salt marsh populations (SMP) of *P. australis* (**b**) collected and used in our experiment

(Fig. 1). Eleven rhizomes of *P. australis* were collected from saltmarsh wetlands of the Yellow River Delta, another eleven rhizomes were collected from inland freshwater areas (Xixi Wetland, Mata Lake, riverine wetlands in Qingdao and Panjin). Each clone was collected at least 10 m apart, and thus the progeny of a single plant represented a different genotype.

We conducted the experiment in two common gardens. One garden was located in Fanggan ecological research station, Shandong University (36° 26′ N; 117° 27′ E). The annual average temperature here is about 13°C and the annual average precipitation is 600–830 mm. The second garden was located in Panjin Wetland Research Institute (41° 12′ N; 122° 1′ E). The annual average temperature here is 9.3°C and the average annual precipitation is 623.2 mm. The experimental sites were chosen to represent two distinct climates. The temperature in Fanggan during the experiment ranged from 5.5 to 42.8°C, 9 days exceeded 40°C, with an average temperature of

24.4 ± 6.1°C. The average relative air humidity (± S.D.) during the experimental period was 81.3 ± 24.7%, ranging from 11.4 to 100%. The air temperature of Panjin during the experiment ranged from 9.5 to 34.9°C, with an average temperature of 24.3 ± 4.5°C. The relative air humidity ranged from 4.8% to 100%, on average it was 41.6 ± 22.1%.

Each rhizome of the 22 clones was divided into two parts, resulting in two replicate plants per clone in both gardens. Each clone was planted in its own 20 l PVC pot (diameter 30 cm, height 38 cm) containing commercial sand with a slow-release fertilizer (50 g per pot, Peters Professional NPK 20-20-20 + Fe, The Scotts Company, USA) and a chelated iron Fe-EDDHA (0.5 g per pot) mixed into the sand. The plants were sufficiently watered twice a week with tap water. To assure sufficient nutrient availability, every plant was also fertilized twice a month with 1 l of a nutrient solution prepared from tap water containing 5 g of a commercial macronutrient solution and

micronutrients (in mM: 8 S, 2.5 B, 2.7 Fe, 2.9 Mn, 0.7 Cu, 0.01 Mo and 1.4 Zn) from a commercial micronutrient stock solution (Peters Professional, The Scotts Company, USA). The plants were placed such as to largely avoid shading by adjacent taller plants. The plants were rotated regularly and randomly throughout the experimental period, to avoid placement effects.

In both gardens, one of each of the 22 divided clones was grown in a pot receiving 20 ppt of formulated sea salt (salt treatment), while the other clone was grown without added salt (0 ppt, control). The salinity in the salt treatment was increased stepwise, to allow the plants to acclimate to the salinity. Thus, the initial salinity was 10 ppt, which after 1 week was increased to 15 ppt, and after another week to 20 ppt. To confirm that the salinity had the desired concentration in the salt treatment, we measured the electrical conductivity of the upper and bottom sand at the end of the experiment, where the average salinity was about 21.1 ppt in Fanggan and 19.7 ppt in Panjin. The experimental setup was a $2 \times 2 \times 2$ fully factorial design with the factors being 'Population' (salt marsh population versus freshwater population), 'Salinity' (control versus salt treatment) and 'Garden' (Fanggan garden versus Panjin garden). The experiment was run at the full final salinity for 100 days from May 21 to August 30 in 2017.

Plant growth and biomass

Plant height (from the soil surface to the tip including apical leaves) of the five tallest shoots in each pot was measured every second week. The shoot elongation rate (SER) was calculated from the height difference between two successive samplings after the shoot elongation had ceased. The number of live shoots was counted twice, in the first week of May and by the end of the growing season in September. The difference between the two shoot counts was expressed as the shoot density per area. At the final harvest, plants were separated into stems (including the leaf sheaths), leaf blades and the belowground (roots and rhizomes) biomass fraction. The fractions were dried to constant weight at 80°C to determine their final biomass. Since we needed to keep the live belowground biomass for further experimental studies, the fresh and dry mass of only a subsample of the belowground biomass was determined, the rest of it was weighed as fresh mass.

The fresh: dry mass ratio from the belowground subsample was used to determine the dry mass of the total belowground biomass. Allocation to the different biomass fractions was calculated as the ratio of the respective fraction to the total biomass.

Leaf parameters

After approximately 2 months of growth, the quantum efficiency of PSII (F_v/F_m) and the maximum electron transport rate (J_{max}) were measured on the third or fourth youngest fully developed leaf of each replicate using a portable fluorometer (MINI-PAM-II, Walz, Germany). The leaves were dark-covered with leaf-clamps covered by tin foil at least 30 min before measurements were conducted. The stomatal conductance (g_s) of three young but fully developed leaves per replicate was measured with a Leaf Porometer SC-1 (Decagon, USA) and their average value was calculated. The average chlorophyll concentration (Chl) of three leaves was measured with a chlorophyll meter (CCM-300, Opti-Sciences, USA). Three fully developed leaves were harvested and scanned and their leaf area was determined using ImageJ ver. 1.52 (Abràmoff et al., 2004). The same three leaves were dried and their specific leaf area (SLA) was calculated as leaf area per dry mass.

Ion concentrations

Leaves and roots of a random subset of the dried biomass were ground to fine powder. We selected 2–5 mg dry mass to analyze total carbon (C) and nitrogen (N) of leaves and roots, using a CHNS analyzer (Vario EL Cube, Elementar, Langenselbold, Germany). Another 0.5 g dry mass of leaves and roots was acid-digested with 4 ml 65% HNO_3 and 2 ml 30% H_2O_2 in a microwave digestion system (Multiwave 3000, Anton Paar GmbH, Graz, Austria). The concentrations of Na, K, P, Ca, Mg, S, Fe and Mn in the digest were determined by ICP-OES (Optima 2000 DV, Perkin Elmer Instruments Inc., Shelton, CT, USA).

Statistical analyses

We investigated the existence of significant differences ($P < 0.05$) among plant biomass, shoot height and density, leaf parameters and ion concentration

(response variables) in the different populations, salinity levels and common gardens (categorical predictors) by three-way analysis of variance (ANOVA). Beforehand, we assured that the conditions of normal distribution and homoscedasticity were met. A linear correlation analysis between total biomass and leaf K^+/Na^+ ratio of both populations and treatments was conducted for each common garden. In Fanggan garden, two of the salt marsh population clones in the control and one in the salt treatment grew very poorly or died and were therefore removed from all statistical analyses. Statistical analyses were done using the software JMP ver. 14.0.0 (SAS Institute Inc.). Graphics were drafted with Origin 9.0.

Results

Plant growth and biomass allocation

All biomass and growth parameters, except shoot density, were affected by a Salinity × Garden interaction (Table 1). Opposite to our expectations, the two populations responded similarly to salinity, except for total biomass and belowground biomass allocation, for which we observed a Garden × Population and Salinity × Population interaction, respectively (Table 1). Specifically, total biomass differed with respect to salinity and population in Fanggan garden, but not in Panjin garden. In Fanggan garden, biomass was lower in the salt marsh population compared to the freshwater population and in salinity treatment compared to the control. There was a tendency of similar responses in Panjin garden, but not to a significant degree (Fig. 2). Belowground biomass allocation was higher in the salt treatment than in the control, which was most pronounced in the salt marsh population in Fanggan garden (Salinity × Population and Salinity × Garden interaction, Table 1). Biomass allocation to leaves and stems was higher in the salt marsh population than in the freshwater population, and reduced by the salt treatment, compared to the control, especially in Fanggan garden (Table 1, Fig. 2b).

Shoot density was similar in both populations and at both treatments, but shoots in Panjin garden had higher density than in Fanggan garden (Table 2). The shoot elongation rate (SER) was higher in the salt marsh than in the freshwater population. SER was reduced by the

salt treatment in both populations, which was most pronounced in Fanggan garden (Salinity × Garden interaction, Table 2). The final shoot height was decreased in both populations due to the salt treatment. Also, this observation was strongest in Fanggan garden, where shoots were 20% lower compared to only 8% in Panjin garden (Fig. 3a).

Leaf parameters

There was a significant interaction between Salinity and Garden for SLA. The SLA in the control treatment was higher than in the salt treatment in Panjin garden, while the opposite was observed in Fanggan garden (Table 1, Fig. 2b). Stomatal conductance (g_s) was significantly affected by all three main factors, but no interactions were detected (Table 1). In the salt treatment, g_s was significantly lower compared to the control. The salt marsh population had significantly higher g_s than the freshwater population, and g_s was significantly lower in the humid climate in Fanggan garden, compared to Panjin garden (Fig. 4a). The chlorophyll concentration (Chl) of both populations was higher in the salt treatment compared to the control in Panjin garden, while there was no significant difference between salt treatments in Fanggan garden (Salinity × Garden interaction, Table 1, Fig. 4b). The quantum efficiency of PSII (F_v/F_m) significantly differed between gardens and was higher in Panjin than in Fanggan garden (Table 1, Fig. 4c). However, the rather small difference was of no biological relevance for plant performance, which indicated no stress or PSII damage. The maximum electron transport rate (J_{max}) was significantly higher in the salt marsh population than in the freshwater population. This difference was slightly more pronounced in Fanggan than in Panjin garden, although this interaction was not significant (Table 1, Fig. 4d).

Ion concentrations

Root N and leaf N concentrations were higher in salt-treated plants and in Panjin garden, compared to the control treatment and to plants in Fanggan garden. Leaf N also differed between populations and was higher in the salt marsh population (Fig. 4c, d). Leaf C concentration showed rather small differences between salinities in Fanggan but not in Panjin garden, where salt treatment resulted in lower leaf C in both

Table 1 F-ratios of three-way Analysis of Variance for phenotypic parameters of two *Phragmites australis* populations from salt or freshwater marshes in China, grown without salt or with 20 ppt soil salinity in a common garden at Panjin and Fanggan, respectively

Parameter	Source of variation						
	Salinity	Garden	Population	Salinity × garden	Salinity × population	Garden × population	Garden × salinity × population
Total biomass	75.5***	2.4	32.3***	24.4***	0.3	8.9**	1.9
Allocation to leaf biomass	7.8**	19.2***	25.5***	5.0*	0.7	3.3	3.8
Allocation to stem biomass	41.5***	0.8	8.0**	14.3***	3.0	0.4	0.0
Allocation to belowground biomass	25.0***	8.2**	25.7***	6.0*	4.4*	0.0	1.2
Final shoot height	33.6***	68.7***	0.2	10.7**	0.1	2.6	0.0
SER	36.9***	13.3***	18.1***	5.4*	2.3	0.2	0.1
Shoot density	1.6	7.9**	2.6	0.0	0.4	0.8	0.0
SLA	0.0	0.0	0.6	13.1***	0.2	1.0	0.1
Chl	9.5**	19.5***	0.8	9.1**	0.0	0.1	0.5
g_s	32.0***	6.0*	14.2***	0.5	0.8	0.7	0.4
F_v/F_m	0.0	11.5**	1.2	1.0	0.0	0.1	0.3
J_{max}	0.8	0.5	4.1*	0.1	0.0	3.0	0.3
Leaf N %	6.1*	11.3**	6.6*	0.1	2.0	1.2	0.8
Leaf C %	1.4	2.6	0.0	9.6**	1.7	0.1	0.0
Leaf C/N	8.1**	15.7***	10.1**	0.9	3.8	1.8	1.2
Leaf P	4.4*	5.8*	1.8	6.9*	1.5	0.1	0.1
Leaf S	0.5	0.5	11.5***	0.0	0.0	3.4	0.2
Leaf K	21.5***	8.2**	3.8	4.3*	3.6	0.2	0.0
Leaf Ca	1.2	0.9	13.9***	12.4***	1.8	0.3	2.7
Leaf Na	154.4***	12.5***	4.0*	0.8	0.0	2.1	1.8
Leaf Mg	4.8*	18.3***	0.2	0.9	0.0	8.4**	0.0
Leaf K/Na ratio	204.6***	22.7***	12.8***	4.6*	0.7	0.2	0.2
Leaf cation pool	0.0	28.9***	1.2	14.2***	1.1	1.0	0.2
Leaf Fe	15.7***	0.7	1.5	0.1	0.1	0.2	2.0
Leaf Mn	61.2***	40.4***	8.0**	8.0**	0.0	4.6*	0.1
Root N %	84.7***	5.3*	0.8	2.0	2.8	0.0	0.0
Root C %	44.0***	4.8*	0.4	0.9	0.1	1.9	0.2
Root C/N	96.7***	2.8	1.1	2.0	2.5	0.0	0.0
Root P	24.8***	84.2***	2.0	2.0	1.8	6.5*	0.3
Root S	0.1	36.1***	1.3	0.3	3.1	0.1	2.3
Root K	17.0***	5.1*	2.7	0.4	0.0	16.0***	0.0

Table 1 continued

Parameter	Source of variation						
	Salinity	Garden	Population	Salinity × garden	Salinity × population	Garden × population	Garden × salinity × population
Root Ca	0.2	0.3	0.7	2.8	0.3	0.3	0.0
Root Na	**488.0***	**20.5***	0.2	0.7	0.3	**7.1**	1.5
Root Mg	**15.8***	**71.2***	1.6	1.4	0.1	0.4	0.7
Root K/Na ratio	**84.3***	**16.9***	0.8	1.9	0.0	1.4	0.0
Root cation pool	**207.5***	**4.3***	2.4	0.1	0.5	**13.1***	0.0
Root Fe	0.1	**11.8**	1.5	3.9	1.3	0.4	1.6
Root Mn	**4.3***	**28.7***	**11.6**	2.5	0.3	**5.0***	1.0

SER shoot elongation rate, *SLA* specific leaf area, *Chl* chlorophyll concentration, g_s stomata conductance, F_v/F_m maximum quantum yield, J_{max} maximum electron transport rate, *Leaf X* nutritional elements and ions concentrations in leaf, *Root X* nutritional elements and ions concentrations in root. Statistically significant values in bold. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

populations (Table 2). Root C concentrations of both populations were significantly higher in the control treatment compared to the salt treatment, and in Panjin garden, compared to Fanggan garden (Table 2).

Leaf Na^+ concentration was significantly higher in salt-treated plants compared to the control, in the salt marsh population compared to the freshwater population, and in Panjin garden compared to Fanggan garden (Fig. 5a). Leaf K^+ concentration barely differed between salt treatments in Fanggan garden but was higher in control plants of both populations in Panjin garden (Salinity × Garden interaction, Table 1, Fig. 5b). The leaf K^+/Na^+ ratio was, hence, higher in control plants than in salt-treated plants, which was more pronounced in Fanggan, compared to Panjin garden. The freshwater population had overall higher K^+/Na^+ ratio in leaves than the salt marsh population (Fig. 5c). The leaf total cation pool was somewhat higher in the control than in the salt treatment in Panjin garden, while the opposite was observed in Fanggan garden (Fig. 5d). Root K^+, root Na^+ and the root cation pool were all higher in the salt treatment than in the control and affected by a Population × Garden interaction (Table 1). The freshwater population had higher root concentrations of these in Panjin garden, while the salt marsh population had higher concentrations in Fanggan garden (Fig. 6). The root K^+/Na^+ ratio was higher in the control than salt-treated plants and higher in Fanggan compared to Panjin garden (Fig. 6c).

Other ions such as Fe, Mg, Mn, P and S in leaves and roots were also affected by Salinity and Garden, but their responses followed no consistent pattern. Only leaf and root Mn, leaf Ca and leaf S were overall higher in the salt marsh population than the freshwater population (Table 1, 2). We found that biomass and leaf K^+/Na^+ ratio were significantly positively correlated in Fanggan garden, but not in Panjin garden (Fig. 7).

Discussion

We here tested the hypothesis that a freshwater population of *P. australis* would perform better than the salt marsh population at the control salinity (0 ppt), which was confirmed. However, opposite to our expectation, this pattern was not reversed at 20 ppt, which showed that the salt marsh population was not
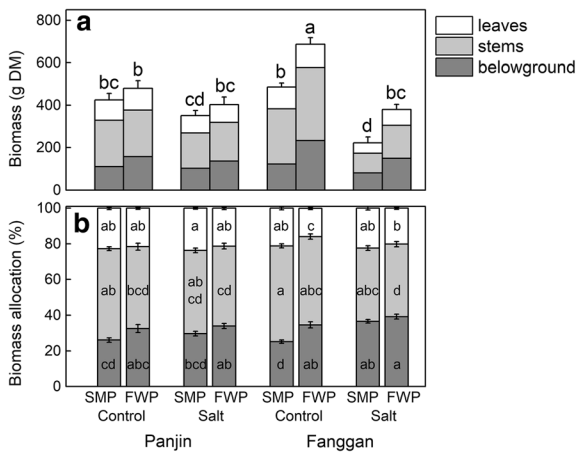
Fig. 2 Dry mass (DM; **a**) and biomass allocation (**b**) of two *P. australis* populations (*SMP* salt marsh population, *FWP* freshwater population) grown without salt ("control") and with 20 ppt soil salinity ("salt") in a common garden in Panjin and Fanggan, respectively. Mean ± SE, *n* = 11. Bars sharing the same letters are not different at *P* > 0.05. Different letters in (**a**) indicate significant differences between treatments for the total biomass, different letters inside bars in (**b**) indicate significant differences between treatments for each plant part

more salt tolerant than the freshwater population at 20 ppt. Hence, our observations could be explained not by local adaptation, but rather phenotypic plasticity, rejecting our first hypothesis. The salt treatment induced a biomass reduction that was of similar magnitude for the two populations combined with an overall higher biomass production in the freshwater population. This indicated that both populations performed similarly under different soil salinity and that the freshwater population generally produced more biomass, but not that its relative salt tolerant was higher. Judging by the absolute salinity tolerance, the freshwater population was the somewhat more salt tolerant of the two populations as it produced more biomass than the salt marsh population at 20 ppt salinity. Additionally, we detected large variation in biomass production in both the freshwater and the salt marsh population, indicating large variation in salinity tolerance between the genotypes within each population. High intra-population variability is crucial for a population's range shift under changing environmental conditions (Valladares et al., 2014). Reduced intra-population diversity may be the consequence of increased soil salinity, if less salt tolerant genotypes are eliminated (Eller et al., 2017). Low genetic diversity in general can be problematic (Frankham,

2005), and reed die back in Europe (Brix, 1999) has been associated with low genetic diversity by some scientists (Neuhaus et al., 1993), while others disagree (Coppi et al., 2018).

We observed higher allocation to belowground tissues of both populations in the salt treatment, which may have contributed to a positive water balance. Higher biomass allocation to the roots can assist in maintaining adequate water uptake under saline conditions (Poorter et al., 2012). In our study, the belowground fraction represented both roots and rhizomes, but only roots are responsible for water uptake. The rhizomes are primarily storage organs, and it seems unlikely that the here observed increased allocation in belowground biomass can mainly be explained by allocation to storage organs. Belowground biomass allocation was the only parameter (besides some ion tissue concentrations), where the response strength, and hence, phenotypic plasticity, differed between populations (interaction Salinity × Population, Table 1). Salt marsh *P. australis* has been shown to have higher rhizome: root ratios under oligohaline conditions compared to more saline conditions (Moore et al., 2012).

An effect of climate, here evaluated as the two different common gardens, on salinity tolerance was also detected in our study. The largest difference between both common gardens was the considerably higher relative air humidity in Fanggan garden, compared to Panjin garden. The humidity gradient between the internal airspaces inside the leaf and the external air is the driving force of transpirational water loss through stomata (Jarvis & Davies, 1998). This gradient, and hence, g_s, will be higher with drier air. This was confirmed by lower g_s in Fanggan than Panjin garden, which strongly suggests concomitantly lower transpiration rates (Jarvis & Davies, 1998). The transpiration stream facilitates uptake of mobile ions in the soil solution (Matimati et al., 2014), and the presumably lower transpiration rates in Fanggan garden therefore led to a lower cation pool in the control treatment compared to Panjin garden. Although the total biomass of control plants was highest in Fanggan garden, salt-treated plants produced higher biomass in Panjin garden, with a salinity-induced biomass reduction of about 50% in Fanggan but only 20% in Panjin. It may seem counterintuitive that the much drier climate in Panjin would increase the relative salinity tolerance and growth in both

Table 2 Mean ± standard error of leaf and root parameters of two *Phragmites australis* populations (SMP: salt marsh population, FWP: freshwater population) grown without salt (control) or with 20 ppt soil salinity (salt treatment) in a common garden at Panjin and Fanggan, respectively

Parameter	Panjin				Fanggan			
	Control		Salt treatment		Control		Salt treatment	
	SMP	FWP	SMP	FWP	SMP	FWP	SMP	FWP
SER (cm d^{-1})	1.34 ± 0.08^{bc}	1.29 ± 0.07^{bcd}	1.26 ± 0.05^{bcd}	1.02 ± 0.05^{d}	1.72 ± 0.10^{a}	1.47 ± 0.05^{ab}	1.33 ± 0.06^{bc}	1.07 ± 0.05^{cd}
Shoot density (cm^{-2})	0.22 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.16 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.02
Leaf C (%)	46.57 ± 0.19	46.92 ± 0.28	47.39 ± 0.23	47.29 ± 0.22	47.43 ± 0.13	47.38 ± 0.13	47.20 ± 0.11	47.07 ± 0.21
Leaf C:N	15.93 ± 0.46^{b}	17.14 ± 0.82^{b}	15.48 ± 0.42^{b}	15.91 ± 0.53^{b}	17.03 ± 0.72^{b}	20.38 ± 0.70^{a}	16.70 ± 0.63^{b}	17.35 ± 0.68^{b}
Leaf P (mg g^{-1})	2.50 ± 0.10^{a}	2.37 ± 0.10^{ab}	2.13 ± 0.08^{ab}	2.12 ± 0.08^{ab}	2.21 ± 0.10^{ab}	2.01 ± 0.09^{b}	2.15 ± 0.07^{ab}	2.14 ± 0.10^{ab}
Leaf S (mg g^{-1})	5.97 ± 0.22^{b}	4.69 ± 0.44^{ab}	5.68 ± 0.32^{ab}	4.50 ± 0.29^{a}	5.24 ± 0.45^{ab}	4.90 ± 0.31^{ab}	5.06 ± 0.21^{ab}	4.94 ± 0.61^{ab}
Leaf Ca (mg g^{-1})	6.58 ± 0.46^{a}	5.26 ± 0.50^{abc}	5.18 ± 0.37^{abc}	4.04 ± 0.27^{c}	4.67 ± 0.28^{bc}	4.63 ± 0.47^{bc}	6.22 ± 0.45^{ab}	4.47 ± 0.33^{bc}
Leaf Mg (mg g^{-1})	2.07 ± 0.15^{a}	1.80 ± 0.08^{ab}	1.76 ± 0.14^{abc}	1.57 ± 0.09^{abc}	1.37 ± 0.08^{bc}	1.63 ± 0.11^{abc}	1.27 ± 0.07^{c}	1.56 ± 0.15^{bc}
Leaf Fe (mg g^{-1})	0.16 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.12 ± 0.01	0.12 ± 0.01
Leaf Mn (mg g^{-1})	0.43 ± 0.03^{bc}	0.29 ± 0.04^{c}	0.58 ± 0.06^{b}	0.44 ± 0.04^{bc}	0.45 ± 0.04^{bc}	0.46 ± 0.05^{b}	0.96 ± 0.09^{a}	0.90 ± 0.09^{a}
Root C (%)	42.22 ± 0.43^{a}	42.21 ± 0.48^{a}	40.19 ± 0.40^{abc}	39.61 ± 0.40^{bc}	41.30 ± 0.95^{ab}	42.10 ± 0.50^{a}	38.19 ± 0.73^{c}	39.05 ± 0.69^{bc}
Root C:N	41.06 ± 2.23^{a}	45.44 ± 2.17^{a}	29.66 ± 1.38^{b}	28.42 ± 2.22^{b}	46.24 ± 2.42^{a}	50.95 ± 3.23^{a}	29.82 ± 2.95^{b}	29.22 ± 1.97^{b}
Root P (mg g^{-1})	1.40 ± 0.12^{a}	1.41 ± 0.10^{a}	1.61 ± 0.12^{a}	1.79 ± 0.14^{ab}	2.66 ± 0.28^{bc}	1.91 ± 0.23^{ab}	3.54 ± 0.28^{c}	3.20 ± 0.35^{c}
Root S (mg g^{-1})	5.40 ± 0.33^{c}	5.53 ± 0.22^{bc}	5.30 ± 0.25^{c}	5.53 ± 0.25^{bc}	6.92 ± 0.42^{ab}	6.52 ± 0.35^{abc}	6.35 ± 0.27^{abc}	7.44 ± 0.46^{a}
Root Ca (mg g^{-1})	4.12 ± 0.26	3.69 ± 0.34	3.73 ± 0.26	3.48 ± 0.37	3.49 ± 0.22	3.25 ± 0.22	3.83 ± 0.35	3.94 ± 0.55
Root Mg (mg g^{-1})	2.90 ± 0.15^{abc}	2.83 ± 0.12^{abc}	3.17 ± 0.12^{a}	3.11 ± 0.19^{ab}	1.98 ± 0.11^{de}	1.85 ± 0.08^{e}	2.55 ± 0.16^{bcd}	2.30 ± 0.13^{cde}
Root Fe (mg g^{-1})	6.00 ± 0.50^{ab}	5.23 ± 0.66^{ab}	5.03 ± 0.49^{ab}	4.14 ± 0.32^{b}	6.92 ± 0.85^{ab}	5.60 ± 0.65^{ab}	6.57 ± 0.56^{ab}	7.37 ± 0.88^{a}
Root Mn (mg g^{-1})	0.71 ± 0.05^{abc}	0.51 ± 0.05^{a}	0.78 ± 0.08^{bc}	0.51 ± 0.06^{ab}	0.88 ± 0.10^{c}	0.70 ± 0.06^{abc}	1.05 ± 0.09^{c}	1.22 ± 0.25^{c}

Different letters indicate significant differences ($p \leq 0.05$) with Tukey's test

SER shoot elongation rate
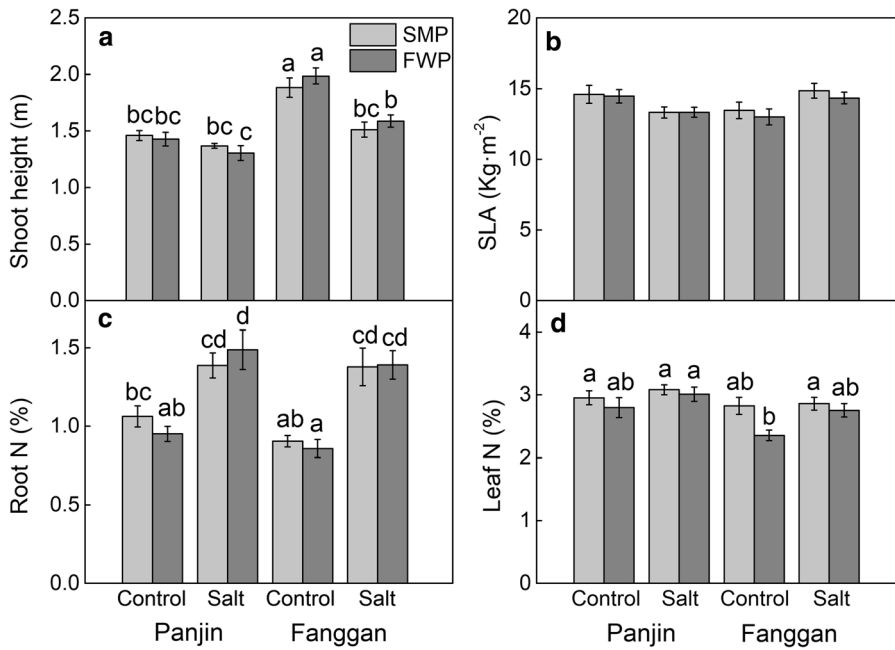
Fig. 3 Shoot height (**a**), specific leaf area (SLA, **b**), root N concentration (**c**) and leaf N concentration (**d**) of two *P. australis* populations (*SMP* salt marsh population, *FWP* freshwater population) grown without salt ("control") and with 20 ppt soil salinity ("salt") in a common garden at Panjin and Fanggan, respectively. Mean ± SE, $n = 11$. Different letters indicate significant differences ($p \leq 0.05$) with Tukey's test
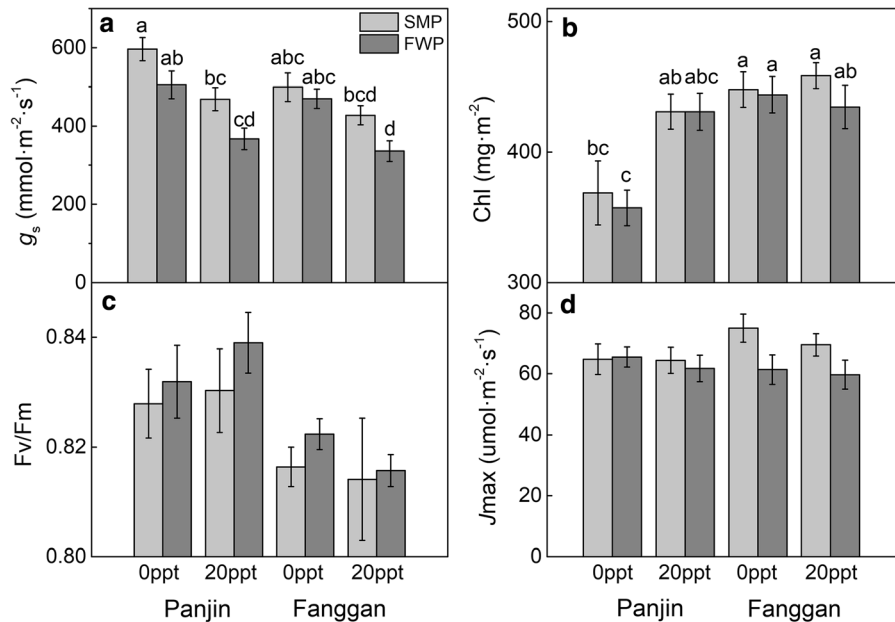

Fig. 4 Leaf stomatal conductance (g_s; a), chlorophyll concentration (Chl; **b**), quantum efficiency of PSII (F_v/F_m; **c**), maximum electron transport rate (**d**) of two *P. australis* populations (*SMP* salt marsh population, *FWP* freshwater population) grown without salt ("control") and with 20 ppt soil salinity ("salt") in a common garden at Panjin and Fanggan, respectively. Mean ± SE, $n = 11$. Different letters indicate significant differences ($p \leq 0.05$) with Tukey's test
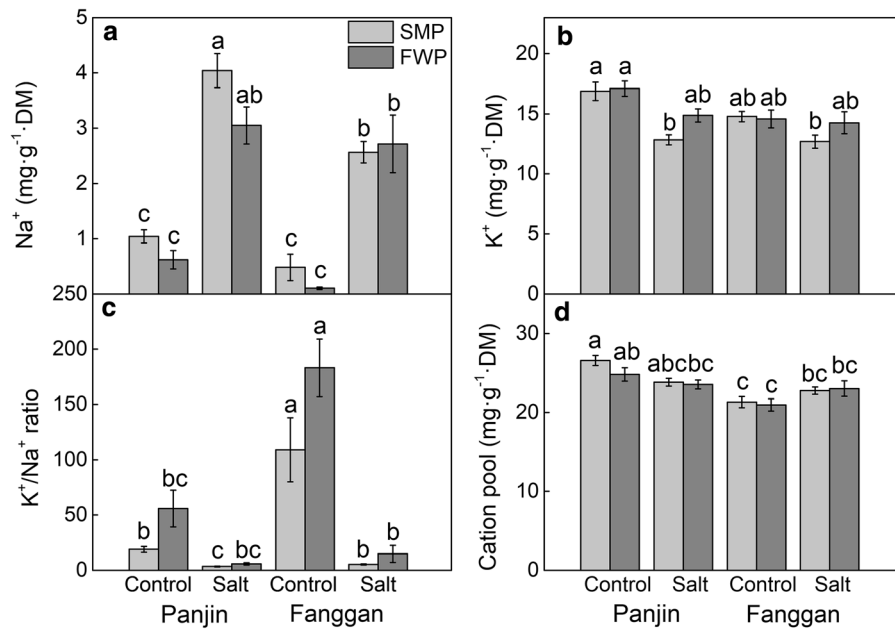
Fig. 5 Leaf concentrations of Na$^+$ (**a**) and K$^+$ (**b**), the leaf K$^+$/Na$^+$ ratio (**c**) and the pooled content of all leaf cations (**d**) of two *P. australis* populations (*SMP* salt marsh population, *FWP* freshwater population) grown without salt ("control") and with 20 ppt soil salinity ("salt") in a common garden at Panjin and Fanggan, respectively. Mean ± SE, $n = 11$. Different letters indicate significant differences ($p \leq 0.05$) with Tukey's test. *DM* dry mass
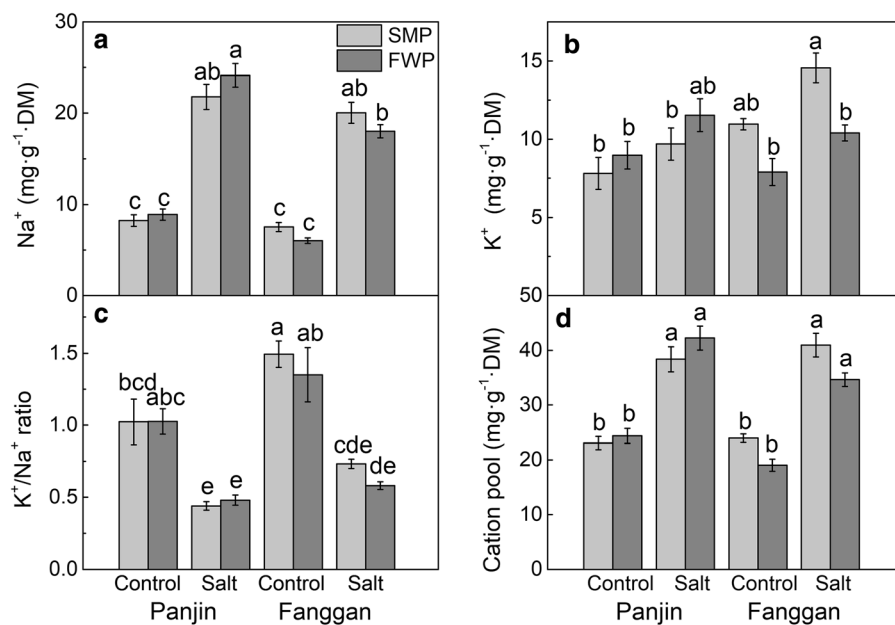
Fig. 6 Root concentrations of Na$^+$ (**a**) and K$^+$ (**b**), the leaf K$^+$/Na$^+$ ratio (**c**) and the pooled content of all leaf cations (**d**) of two *P. australis* populations (*SMP* salt marsh population, *FWP* freshwater population) grown without salt ("control") and with 20 ppt soil salinity ("salt") in a common garden at Panjin and Fanggan, respectively. Mean ± SE, $n = 11$. Different letters indicate significant differences ($p \leq 0.05$) with Tukey's test. *DM* dry mass
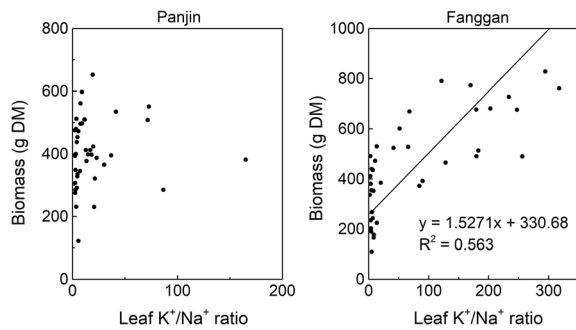
Fig. 7 Linear correlation between total biomass and leaf K^+/Na^+ ratio of two *P. australis* populations grown without salt or with 20 ppt soil salinity in a common garden at Panjin and Fanggan, respectively. Significant correlation in Fanggan is indicated by regression line. $n = 44$

populations, since low air humidity increases transpiration rates and high soil salinity poses a limitation to water uptake by roots. Nonetheless, *P. australis* displayed higher relative salinity tolerance in the drier climate, very likely due to leaf dehydration as a means of osmoregulation (Lissner et al., 1999). The difference in osmoregulation therefore decoupled the relationship between growth and K^+/Na^+ ratio in Panjin but not in Fanggan garden (Fig. 7). As water is lost from living plant tissue, the concentration of ions acting as osmolytes increases, thereby maintaining leaf turgor without major effects on photosynthetic rates (Matoh et al., 1988; Lissner et al., 1999). Hence, due to the higher relative air humidity in Fanggan garden, leaf dehydration was lower compared to Panjin garden, and an osmolyte concentration was not achieved to the same extend. If dehydration was prevented, osmotic adjustment could either be achieved by increased ion uptake from the soil or elevated production of compatible solutes. Compared to dehydration, these processes are energetically costly and occur at the expense of growth.

An important trait in salt acclimation is the ability to maintain turgor by adjusting osmotic compatibility. In *P. australis*, the K^+/Na^+ ratio is generally increased by active exclusion of Na^+ from the leaf tissue and maintaining high K^+ concentrations in the living tissue (Takahashi et al., 2007). We therefore hypothesized that the salt marsh population would have a greater capacity to maintain its K^+/Na^+ ratio and tissue nutrient concentration compared to the freshwater population. However, the freshwater population had 1.7 to 3.0 times higher leaf K^+/Na^+ ratio in the

salinity treatment compared to the salt marsh population. This was caused by higher leaf Na^+ concentrations in the salt marsh population, as no differences in leaf K^+ were observed for the two populations. Even though biomass and leaf K^+/Na^+ ratio decreased due to the salt treatment, neither population showed any sign of stress in the measured leaves, as their F_v/F_m was very close to 0.8, indicating a healthy green leaf (Demmig Adams & Adams, 1992). This suggests that the salt marsh population was highly efficient at sequestering Na^+ in the vacuole, due to a higher Na^+ tissue tolerance, since we observed no negative impacts on most measured parameters, despite the higher Na^+ concentration (Flowers & Colmer, 2008). High tissue tolerance is due to effective storage of Na^+ in the vacuole and higher production of compatible osmolytes located in the cytoplasm, thereby minimizing the negative effects on enzymatic processes such as photosynthesis (Flowers & Colmer, 2008; Roy et al., 2014). Species with high Na^+ tissue tolerance can use Na^+ as osmolyte in a similar manner as seen in true halophytes, when K^+ is not readily available (Zhao et al., 1999; Munns & Tester, 2008; Wakeel et al., 2011; Mateus et al., 2019). The two populations might also differ in Na^+ and K^+ allocation to young and old tissue, as intraspecific variation in Na^+ and K^+ allocation has been found previously for North American *P. australis* (Achenbach & Brix, 2014).

The leaves used for leaf trait measurements were green and healthy, supported by the fact that J_{max} and Chl were similar in the control and salinity treatment. Despite lower stomatal conductance in salt-treated plants, g_s was still above 0.34 mol m^{-2} s^{-1} in all treatments and therefore high enough to facilitate substantial gas diffusion for photosynthetic rates. Other studies found that photosynthesis was largely unaffected in *P. australis* grown at salinities of 20–28 ppt, despite a substantial biomass reduction (Pagter et al., 2009). It is therefore likely that the reduced biomass production under the salt treatment in both populations was caused by increased energetic demands required to maintaining ion homeostasis, rather than decreased assimilation rates (Munns et al., 2019). Moreover, we detected high N concentrations in tissues with high Na^+ concentration, such as roots, to a similar magnitude in both populations. Metabolic costs are associated with an adjustment of N concentration in leaves and roots (Crain, 2007) to increase the production of N-containing compatible osmolytes,

such as proline, to balance the osmotic potential between the cytoplasm and the vacuole where most Na^+ is stored (Pagter et al., 2009; Flowers et al., 2015).

A common effect of salt stress is a lower specific leaf area (SLA), which is thought to assist in water conservation (Munns & Tester, 2008; Wellstein et al., 2017) and which we observed in Panjin garden. In Fanggan garden, however, SLA in salt-treated plants was higher than in the control. The relative humidity was close to 100% for 2–3 h after sunrise on several days and we observed dew on the leaves in several instances. A probable explanation for this observation is high relative air humidity that has previously been described to facilitate water uptake directly from the atmosphere into the leaves (Reef & Lovelock, 2015). An advantage of higher SLA is increased light capturing for higher assimilation rates, thus increasing the energy available for ion uptake and Na^+ exclusion.

Opposite to our hypothesis, the freshwater population seemed to perform slightly better than the salt marsh population under higher soil salinity, although only the salt marsh population was sampled from a saline habitat. Hence, we could not confirm local adaptation, as both populations adjusted through phenotypic plasticity. The lack of local adaptation could be explained by the very dynamic nature of the Yellow River preventing evolutionary adaptation. Due to the high silt content in the river water, large amounts of sediment are deposited in the riverbed and this has changed the course of the Yellow river and the habitats associated with it on several instances (Cong et al., 2019). This has been exacerbated by human activities that heavily impact the environment of the Yellow River (Li et al., 2017). Moreover, high gene flow and migration between Chinese populations of *P. australis* (Gao et al., 2012) could interfere with local adaptation, while the changing habitats and frequent disturbances would put rapidly changing multidirectional selective pressures on the salt marsh populations.

In conclusion, we found that both populations were able to grow and survive in saline conditions, and we did not detect strong signs of salt stress. Under conditions of high air humidity, water loss is restricted and therefore generally ameliorating salt stress. Nonetheless, we found that relative salinity tolerance was lower in the humid climate in Fangang garden, as osmotic adjustment in *P. australis* involved leaf dehydration, which was only possible in the drier climate in Panjin garden. In contrast to active ion

uptake and Na^+ exclusion, leaf dehydration requires no energy input, which was evident in the higher biomass production in Panjin, compared to Fanggan garden. Climate change is expected to lower the relative air humidity (Byrne & O'Gorman, 2018) and will thereby increase the salinity tolerance of *P. australis* and other species utilizing the same salt-stress coping strategy, which could influence species composition and zonation patterns in salt marshes (Engels & Jensen, 2010).

References

Abràmoff, M. D., P. J. Magalhães & S. J. Ram, 2004. Image processing with ImageJ. Biophotonics International 11(7): 36–42.

Achenbach, L. & H. Brix, 2014. Can differences in salinity tolerance explain the distribution of four genetically distinct lineages of *Phragmites australis* in the Mississippi River Delta? Hydrobiologia 737(1): 5–23.

Adams, D. A., 1963. Factors influencing vascular plant zonation in North Carolina salt marshes. Ecology 44(3): 445–456.

Asch, F., M. Dingkuhn, K. Dörffling & K. Miezan, 2000. Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. Euphytica 113(2): 109.

Azooz, M., M. Shaddad & A. Abdel-Latef, 2004. Leaf growth and K^+/Na^+ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. Acta Agronomica Hungarica 52(3): 287–296.

Bertness, M. D., 1991. Interspecific interactions among high marsh perennials in a New-England salt-marsh. Ecology 72(1): 125–137.

Bradshaw, A. D., 1965. Evolutionary significance of phenotypic plasticity in plants. Genetics 13(1): 115–155.

Brix, H., 1999. The European research project on reed die-back and progression (EUREED). Limnologica 29(1): 5–10.

Brix, H., S. Y. Ye, E. A. Laws, D. C. Sun, G. S. Li, X. G. Ding, H. M. Yuan, G. M. Zhao, J. Wang & S. F. Pei, 2014. Large-scale management of common reed, *Phragmites australis*, for paper production: a case study from the Liaohe Delta, China. Ecological Engineering 73: 760–769.

Byrne, M. P. & P. A. O'Gorman, 2018. Trends in continental temperature and humidity directly linked to ocean warming. Proceedings of the National Academy of Sciences of the United States of America 115(19): 4863–4868.

Caplan, J. S., R. N. Hager, J. P. Megonigal & T. J. Mozdzer, 2015. Global change accelerates carbon assimilation by a wetland ecosystem engineer. Environmental Research Letters. https://doi.org/10.1088/1748-9326/10/11/115006.

Chambers, R. M., L. A. Meyerson & K. Saltonstall, 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Botany 64(3–4): 261–273.

Cong, P. F., K. X. Chen, L. M. Qu & J. B. Han, 2019. Dynamic changes in the wetland landscape pattern of the Yellow River Delta from 1976 to 2016 based on satellite data. Chinese Geographical Science 29(3): 372–381.

Coppi, A., L. Lastrucci, D. Cappelletti, M. Cerri, F. Ferranti, V. Ferri, B. Foggi, D. Gigante, R. Venanzoni, D. Viciani, R. Selvaggi & L. Reale, 2018. AFLP approach reveals variability in *Phragmites australis*: implications for its dieback and evidence for genotoxic effects. Frontiers in Plant Science 9: 386–386.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton & M. van den Belt, 1998. The value of ecosystem services: putting the issues in perspective. Ecological Economics 25(1): 67–72.

Costanza, R., B. Fisher, K. Mulder, S. Liu & T. Christopher, 2007. Biodiversity and ecosystem services: a multi-scale empirical study of the relationship between species richness and net primary production. Ecological Economics 61(2): 478–491.

Crain, C. M., 2007. Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. Estuaries and Coasts 30(1): 26–34.

Cui, B., Q. Yang, Z. Yang & K. Zhang, 2009. Evaluating the ecological performance of wetland restoration in the Yellow River Delta, China. Ecological Engineering 35(7): 1090–1103.

Cui, B. S., Q. A. He & Y. A. An, 2011. Community structure and abiotic determinants of salt marsh plant zonation vary across topographic gradients. Estuaries and Coasts 34(3): 459–469.

Curco, A., C. Ibanez, J. W. Day & N. Prat, 2002. Net primary production and decomposition of salt marshes of the Ebre delta (Catalonia, Spain). Estuaries 25(3): 309–324.

Demmig Adams, B. & W. W. Adams, 1992. Carotenoid composition in sun and shade leaves of plants with different life forms. Plant, Cell and Environment 15(4): 411–419.

Eller, F., H. Skalova, J. S. Caplan, G. P. Bhattarai, M. K. Burger, J. T. Cronin, W. Y. Guo, X. Guo, E. L. G. Hazelton, K. M. Kettenring, C. Lambertini, M. K. McCormick, L. A. Meyerson, T. J. Mozdzer, P. Pysek, B. K. Sorrell, D. F. Whigham & H. Brix, 2017. Cosmopolitan species as models for ecophysiological responses to global change: the common reed *Phragmites australis*. Frontiers in Plant Science 8: 1833.

Ellison, A. M., 2019. Foundation species, non-trophic Interactions, and the value of being common. Science 13: 254–268.

Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V. Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. Von Holle & J. R. Webster, 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3(9): 479–486.

Emery, N. C., P. J. Ewanchuk & M. D. Bertness, 2001. Competition and salt-marsh plant zonation: stress tolerators may be dominant competitors. Ecology 82(9): 2471–2485.

Engels, J. G. & K. Jensen, 2010. Role of biotic interactions and physical factors in determining the distribution of marsh species along an estuarine salinity gradient. Oikos 119(4): 679–685.

Fan, X., B. Pedroli, G. Liu, Q. Liu, H. Liu & L. Shu, 2012. Soil salinity development in the yellow river delta in relation to groundwater dynamics. Land Degradation & Development 23(2): 175–189.

Flowers, T. J. & T. D. Colmer, 2008. Salinity tolerance in halophytes. New Phytologist 179(4): 945–963.

Flowers, T. J., R. Munns & T. D. Colmer, 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of Botany 115(3): 419–431.

Frankham, R., 2005. Genetics and extinction. Biological Conservation 126(2): 131–140.

Gao, L. X., S. Q. Tang, L. Q. Zhuge, M. Nie, Z. Zhu, B. Li & J. Yang, 2012. Spatial genetic structure in natural populations of *Phragmites australis* in a mosaic of saline habitats in the Yellow River Delta, China. PLoS ONE. https://doi.org/10.1371/journal.pone.0043334.

Graneli, W., 1984. Reed *Phragmites-Australis* (Cav) Trin Ex Steudel as an energy-source in Sweden. Biomass 4(3): 183–208.

Guan, B., J. B. Yu, A. X. Hou, G. X. Han, G. M. Wang, F. Z. Qu, J. B. Xia & X. H. Wang, 2017. The ecological adaptability of *Phragmites australis* to interactive effects of water level and salt stress in the Yellow River Delta. Aquatic Ecology 51(1): 107–116.

Haslam, S. M., 1973. Some aspects of the life history and autecology of *Phragmites* communis Trin: a review. Polskie Archiwum Hydrobiologii 20(1): 79–100.

Himes-Cornell, A., L. Pendleton & P. Atiyah, 2018. Valuing ecosystem services from blue forests: a systematic review of the valuation of salt marshes, sea grass beds and mangrove forests. Ecosystem Services 30: 36–48.

Jarvis, A. J. & W. J. Davies, 1998. The coupled response of stomatal conductance to photosynthesis and transpiration. Journal of Experimental Botany 49: 399–406.

Kawecki, T. J. & D. Ebert, 2004. Conceptual issues in local adaptation. Ecology Letters 7(12): 1225–1241.

Kiviat, E., 2013. Ecosystem services of *Phragmites* in North America with emphasis on habitat functions. Aob Plants 5: 1–29.

Kobbing, J. F., V. Beckmann, N. Thevs, H. Peng & S. Zerbe, 2016. Investigation of a traditional reed economy (*Phragmites australis*) under threat: pulp and paper market, values and Netchain at Wuliangsuhai Lake, Inner Mongolia, China. Wetlands Ecology and Management 24(3): 357–371.

Latzel, V. & J. Klimešova, 2010. Transgenerational plasticity in clonal plants. Evolutionary Ecology 24(6): 1537–1543.

Li, B., C. Y. Li, J. Y. Liu, Q. Zhang & L. M. Duan, 2017. Decreased streamflow in the Yellow River Basin, China: Climate change or human-induced? Water. https://doi.org/10.3390/w9020116.

Liang, J., W. L. Xing, G. M. Zeng, X. Li, Y. H. Peng, X. D. Li, X. Gao & X. Y. He, 2018. Where will threatened migratory birds go under climate change? Implications for China's national nature reserves. Science of the Total Environment 645: 1040–1047. https://doi.org/10.1016/j.scitotenv.2018.07.196.

Lissner, J., H. H. Schierup, F. A. Comin & V. Astorga, 1999. Effect of climate on the salt tolerance of two *Phragmites australis* populations. I. Growth, inorganic solutes, nitrogen relations and osmoregulation. Aquatic Botany 64(3–4): 317–333.

Mateus, N. D., E. V. D. Ferreira, J. C. Arthur, J. C. Domec, L. Jordan-Meille, J. L. D. Goncalves & J. Lavres, 2019. The ideal percentage of K substitution by Na in *Eucalyptus* seedlings: evidences from leaf carbon isotopic composition, leaf gas exchanges and plant growth. Plant Physiology and Biochemistry 137: 102–112.

Matimati, I., G. A. Verboom & M. D. Cramer, 2014. Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients. Journal of Experimental Botany 65: 159–168.

Matoh, T., N. Matsushita & E. Takahashi, 1988. Salt tolerance of the reed plant *Phragmites*-communis. Physiologia Plantarum 72(1): 8–14.

Moore, G. E., D. M. Burdick, C. R. Peter & D. R. Keirstead, 2012. Belowground biomass of *Phragmites australis* in coastal marshes. Northeastern Naturalist 19(4): 611–626.

Mozdzer, T. J., J. Brisson & E. L. G. Hazelton, 2013. Physiological ecology and functional traits of North American native and Eurasian introduced *Phragmites australis* lineages. Aob Plants. https://doi.org/10.1093/aobpla/plt048.

Mozdzer, T. J., J. S. Caplan, R. N. Hager, C. E. Proffitt & L. A. Meyerson, 2016. Contrasting trait responses to latitudinal climate variation in two lineages of an invasive grass. Biological Invasions 18(9): 2649–2660.

Mueller, P., R. N. Hager, J. E. Meschter, T. J. Mozdzer, J. A. Langley, K. Jensen & J. P. Megonigal, 2016. Complex invader-ecosystem interactions and seasonality mediate the impact of non-native *Phragmites* on CH_4 emissions. Biological Invasions 18(9): 2635–2647.

Munns, R. & M. Tester, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59: 651–681.

Munns, R., D. A. Day, W. Fricke, M. Watt, B. Arsova, B. J. Barkla, J. Bose, C. S. Byrt, Z. H. Chen, K. J. Foster, M. Gilliham, S. W. Henderson, C. L. D. Jenkins, H. J. Kronzucker, S. J. Miklavcic, D. Plett, S. J. Roy, S. Shabala, M. C. Shelden, K. L. Soole, N. L. Taylor, M. Tester, S. Wege, L. H. Wegner & S. D. Tyerman, 2019. Energy costs of salt tolerance in crop plants. New Phytologist. https://doi.org/10.1111/nph.15864.

Münzbergová, Z., V. Hadincová, H. Skálová, V. Vandvik & S. Bonser, 2017. Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change. Journal of Ecology 105(5): 1358–1373.

Neuhaus, D., H. Kühl, J. G. Kohl, P. Dörfel & T. Börner, 1993. Investigation on the genetic diversity of *Phragmites* stands using genomic fingerprinting. Aquatic Botany 45(4): 357–364.

Pagter, M., C. Bragato, M. Malagoli & H. Brix, 2009. Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquatic Botany 90(1): 43–51.

Pasternak, D., 1987. Salt tolerance and crop production - a comprehensive approach. Annual Review of Phytopathology 25: 271–291.

Pennings, S. C., M. B. Grant & M. D. Bertness, 2005. Plant zonation in low-latitude salt marshes: disentangling the roles of flooding, salinity and competition. Journal of Ecology 93(1): 159–167.

Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot & L. Mommer, 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193(1): 30–50.

Pyšek, P., H. Skálová, J. Čuda, W. Y. Guo, J. Doležal, O. Kauzál, C. Lambertini, K. Pyšková, H. Brix & L. A. Meyerson, 2019. Physiology of a plant invasion: biomass production, growth and tissue chemistry of invasive and native *Phragmites australis* populations. Preslia 91(1): 51–75.

Reef, R. & C. E. Lovelock, 2015. Regulation of water balance in mangroves. Annals of Botany 115(3): 385–395.

Roy, S. J., S. Negrao & M. Tester, 2014. Salt resistant crop plants. Current Opinion in Biotechnology 26: 115–124.

Saltonstall, K., 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proceedings of the National Academy of Sciences of the United States of America 99(4): 2445–2449.

Snow, A. A. & S. W. Vince, 1984. Plant zonation in an Alaskan salt marsh: II. An experimental study of the role of edaphic conditions. Journal of Ecology 72(2): 669–684. https://doi.org/10.2307/2260075.

Stephenson, T. A. & A. Stephenson, 1949. The universal features of zonation between tide-marks on rocky coasts. Journal of Ecology 37(2): 289–305.

Takahashi, R., T. Nishio, N. Ichizen & T. Takano, 2007. Salt-tolerant reed plants contain lower Na^+ and higher K^+ than salt-sensitive reed plants. Acta Physiologiae Plantarum 29(5): 431–438.

Valladares, F., S. Matesanz, F. Guilhaumon, M. B. Araujo, L. Balaguer, M. Benito-Garzon, W. Cornwell, E. Gianoli, M. van Kleunen, D. E. Naya, A. B. Nicotra, H. Poorter & M. A. Zavala, 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecology Letters 17(11): 1351–1364.

Vasquez, E. A., E. P. Glenn, J. J. Brown, G. R. Guntenspergen & S. G. Nelson, 2005. Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). Marine Ecology Progress Series 298: 1–8.

Wakeel, A., M. Farooq, M. Qadir & S. Schubert, 2011. Potassium substitution by sodium in plants. Critical Reviews in Plant Sciences 30(4): 401–413.

Wellstein, C., P. Poschlod, A. Gohlke, S. Chelli, G. Campetella, S. Rosbakh, R. Canullo, J. Kreyling, A. Jentsch & C. Beierkuhnlein, 2017. Effects of extreme drought on specific leaf area of grassland species: Aa meta-analysis of experimental studies in temperate and sub-Mediterranean systems. Global Change Biology 23(6): 2473–2481.

White, S. D. & G. G. Ganf, 2002. A comparison of the morphology, gas space anatomy and potential for internal aeration in *Phragmites australis* under variable and static water regimes. Aquatic Botany 73(2): 115–127.

Windham, L. & J. G. Ehrenfeld, 2003. Net impact of a plant invasion on nitrogen-cycling processes within a brackish tidal marsh. Ecological Applications 13(4): 883–896.

Xing, Y. C., C. G. Zhang, E. Y. Fan & Y. H. Zhao, 2016. Freshwater fishes of China: species richness, endemism, threatened species and conservation. Diversity and Distributions 22(3): 358–370.

Yuan, Y., K. Y. Wang, D. Z. Li, Y. Pan, Y. Y. Lv, M. X. Zhao & J. J. Gao, 2013. Interspecific interactions between *Phragmites australis* and *Spartina alterniflora* along a tidal gradient in the Dongtan Wetland, Eastern China. PLoS ONE 8(1): e53843.

Zhao, K. F., L. T. Feng & S. Q. Zhang, 1999. Study on the salinity-adaptation physiology in different ecotypes of *Phragmites australis* in the Yellow River Delta of China: Osmotica and their contribution to the osmotic adjustment. Estuarine Coastal and Shelf Science 49: 37–42.