LEAF TRAITS OF SPARTINA ALTERNIFLORA IN RESPONSE TO SOIL SALINITY

ZHANG, D. J.[1*] – SHI, X. X.[1] – LIU, X. P.[1] – WANG, S. Q.[1] – TIAN, J. P. [1] – WANG, T. X.[2] – ZHANG, M. Y.[3*]

[1]*Shandong Key Laboratory of Eco-Environmental Science for the Yellow River Delta, Shandong University of Aeronautics, Binzhou 256600, Shandong Province, China*

[2]*School of Geographical Sciences, Northeast Normal University, Changchun, China*

[3]*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, 130102 Jilin Province, China*

**Corresponding authors*
e-mail: zhangdongjie14@mails.ucas.ac.cn; zhangmingye@iga.ac.cn

(Received 20th Feb 2024; accepted 8th Jul 2024)

Abstract. Salinity is one of the main limiting factors driving the expansion of *Spartina alterniflora* by affecting plant functioning. Comparative studies of plant leaves, under 0 (T0), 5 (T5), 10 (T10), and 20 g/kg (T20) soil salinity, were carried out. Results showed that soil salinity significantly affected most leaf traits of *S. alterniflora*. Total and live leaf numbers first increased and then decreased with increasing soil salinity. Leaf morphology in T5 were substantially larger than another three treatments, and the ratio of leaf length to width considerably decreased with increasing soil salinity. As the soil salinity increased, leaf dry mass and leaf dry matter content (LDMC) displayed a pattern similar to total leaf number. Leaf mass traits indicated that while a more efficient nutrient conservation strategy was adopted in T10, *S. alterniflora* rapidly produced biomass in T0. Strong correlations were observed between leaf morphological and mass traits except for some parts of LDMC. The confidence ellipses of leaf traits indicated significant differences in leaf traits of the four treatments. We believe that insights into the aforementioned aspects can contribute to a better understanding of the responses of leaf traits of *S. alterniflora* and provide valuable information about its adaptation strategy.
Keywords: *functional traits, biological invasion, salt marsh, adaptation strategy, leaf number*

Introduction

Coastal wetlands are typically fragile and ecologically sensitive zones, with wetland structures and functions that are prone to change due to human activities and global changes (Wu et al., 2020; Qu et al., 2020; Jia et al., 2021). Because of the coupling effects of multiple factors, the structure of plant communities in coastal wetlands is simple and highly vulnerable to invasive alien species (Liu et al., 2020a; Ren et al., 2021; Gao et al., 2021; Li et al., 2022). *Spartina alterniflora* Loisel. is a typical invasive species in coastal wetlands. Thus, significant effort has been dedicated to understand its invasion process and mechanism (Han et al., 2023; Pang et al., 2023). The research on the response of *S. alterniflora* to environmental factors has become a hot topic.

Spartina alterniflora is a perennial grass plant with salt tolerance, flood resistance, silt resistance, hypoxia tolerance, and strong reproductive capacity (Lu et al., 2020). These biological properties of *S. alterniflora* lead to its great ecological and economical benefits in terms of protecting beaches and embankments, promoting siltation, improving saline soil, purifying water quality, and enhancing wetland vegetation coverage and productivity (Maricle and Lee, 2002; Chung et al., 2006; Meng et al.,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

2020). However, the invasion of *S. alterniflora* is a global concern. Due to its strong adaptability and reproductive capacity, *S. alterniflora* gradually takes a dominant position in wetland communities after the invasion; and then spreads quickly. This has resulted in a coastal distribution zone along the eastern coast of China from Liaoning to the Leizhou Peninsula, and has caused it to be the most common invasive plant in coastal wetlands in China (Tian et al., 2020; Jia et al., 2021; Li et al., 2022). The invasion of *S. alterniflora* threatens the local plant community and its biodiversity, changes the pattern of macrobenthos, affects the habitat and food source of migratory birds, and has a negative impact on the material cycle, energy flow and socio-economic activities of the wetland ecosystem (Liu et al., 2018; Okoye et al., 2020; Tian et al., 2020; Jia et al., 2022). In increasingly severe situations, scientific prevention and control of *S. alterniflora* invasion are urgently required (Xie et al., 2019; Meng et al., 2020). The prerequisite for addressing the problem is to understand the coupling mechanism between *S. alterniflora* and various processes in coastal wetlands, especially the relationship between leaf functional traits and environmental changes.

Plant leaves are the main place for obtaining light, water, and nutrients, and shaped by surrounding environment (Pan et al., 2019; Garnier et al., 2001; Jia et al., 2021). Leaf traits are closely related to the efficiency of resource utilization and photosynthesis of plants, and can represent the adaptive strategies of plants to the environment (Pan et al., 2019; Zhang et al., 2020a; Liu et al., 2022). Leaf traits are not isolated from each other, and they often coordinate the adaptation of plants to the environment through synergy (Zhang et al., 2021; Mao et al., 2023; Zuo et al., 2020; Jiang et al., 2009). Leaf size and shape have significant impacts on light energy utilization, dry matter accumulation, harvest yield, and economic benefits (Pan et al., 2019; Zhang et al., 2021; Liu et al., 2022). The external morphological characteristics of leaves are closely related to the functions of plants, the community, and even the ecosystem in which plants are located (Fraser et al., 2021; Shao et al., 2020). Leaf number, size, shape, and mass traits are key indicators in many relevant studies because they reflect the growth status of plants and can be measured easily (Hessini et al., 2021; Liu et al., 2022). Leaf number and live leaf number describe plant morphology from the perspective of leaf quantity, and reflected plant growth status under environmental fluctuation (Courtney et al., 2021). Leaf area, leaf length, leaf width, and the RLL focus on leaf morphology in response to environmental presses (Zhang, 2020a; Liu et al., 2022). Additionally, dead leaf number (DLN), the proportion of dead leaf (PDL), the ratio of dead leaf to live leaf (RDL), length of withered leaf tip (LWL), the proportion of withered leaf tip (PWL) were important indicators charactering the state of withered leaves, indicating the degree of environmental damage to plant leaves (Zhang et al., 2019b). The larger the values of these indicators, and the greater the degree of environmental damage is to the leaves. Leaf dry mass, specific leaf area (SLA) and leaf dry matter content (LDMC) are comprehensive indicators of plant leaves, and are related to the efficiency of resource utilization and the prediction of plant adaptation strategy (Zhang et al., 2020; Garnier et al., 2001). In the context of global changes, some plants (e.g. *S. alterniflora*) growing in special habitats are sensitive to environmental changes, and the study of leaf traits of these plants is even more important (Zhang et al., 2023; Hessini et al., 2021; Liu et al., 2022; Mao et al., 2019). The relationships between leaf number, morphological traits and mass traits can help in understanding leaf trait network and trait linkage.

The unique geographical location of the Yellow River Delta wetland endows it with unique salt differentiation patterns (Yu et al., 2014; Guan et al., 2020). Salinity is one of

APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 22(5):4137-4153.
http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

the main driving forces for changes in plant growth and development in the Yellow River Delta wetland. Changing salt dynamics in the water-plant-soil system affects the distribution pattern of soil physicochemical properties and the feedback effect of the plant community, and has an important impact on the wetland function (Pang et al., 2023; Zhang et al., 2019b). Therefore, the effect of salinity changes on the growth and diffusion of *S. alterniflora* in the Yellow River Delta wetland is worthy of in-depth research. Recently, many investigations have been conducted in this region on the invasion mechanism, ecological effects, and comprehensive control measures of *S. alterniflora* (Xie et al., 2019; Yan et al., 2022), but the responses of leaf traits of *S. alterniflora* to soil salinity have not been studied sufficiently.

Because of the invasion trend of *S. alterniflora* and its harmfulness to the ecosystem, most researchers pay attentions to the invasion of *S. alterniflora* and its corresponding plant traits (Liu et al., 2022; Jiang et al., 2009; Shao et al., 2020; Zhang et al., 2021). The impact of the interaction between leaf traits of *S. alterniflora* and environmental factors is an important link for revealing the invasion and adaptation mechanisms of *S. alterniflora*. Currently, many studies have reported the instantaneous growth status of *S. alterniflora*, while some have considered the response dynamics and cycling process of carbon elements in the soil-*S. alterniflora* system (Xie et al., 2019; Zuo et al., 2020; Pang et al., 2023; Matsuda et al., 2002). Few studies have reported the coupling relationship between *S. alterniflora* growth and increasing soil salinity. Besides, an in-depth analysis of the leaf functional traits that are related to matter production and nutrient conservation is lacking in the existing studies.

The Yellow River Delta wetland is an important intersection area between the Yellow River and the Bohai Sea in China. The invasion of *S. alterniflora* seriously threatens the ecological security of the Yellow River Delta wetland and hinders the high-quality and fast development of the regional ecological economy. Therefore, conducting research on (1) the leaf traits of *S. alterniflora* in response to soil salinity, (2) exploring the strategies for balancing material production and nutrient storage of *S. alterniflora*, and (3) revealing the relationships between any two indicators of leaf traits of *S. alterniflora* is of great significance for understanding the physiological and ecological adaptation of *S. alterniflora* and the prevention and control of biological invasion. Such comprehensive research carried out during this study can meet the needs of major national strategies and socio-economic development.

Materials and methods

Sampling site and plant materials

The Yellow River Delta wetland is the youngest and most complete wetland ecosystem in the warm temperate zone of China, with rich biodiversity and strong material production capacity, which provides a good habitat for wetland birds (Han et al., 2023; Cui et al., 2009; Bai et al., 2020). Yellow River Delta wetland plays an important role in responding to environmental changes and biological invasions (Pang et al., 2023; Zhang et al., 2020b). *Spartina alterniflora* was introduced to the Yellow River Estuary zone in 1987, and the distribution area did not change substantially in the following 20 years (Zhang et al., 2021; Liu et al., 2018). After 2010, a large-scale expansion of *S. alterniflora* occurred in the estuary wetland, which posed an increasingly serious threat to the coastal wetland (Zhang et al., 2017; Liu et al., 2018).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

The rapid expansion of *S. alterniflora* may be related to population size, increasing temperature and soil salinity.

Spartina alterniflora was collected in May 2022 from the Yellow River Delta wetland (37°40’N-38°10’N, 118°41’E-119°16’E) in the northeast of Shandong Province, China (*Fig. 1*). *Spartina alterniflora* seedlings (with a plant height of 20-25 cm) were taken to a greenhouse at the Shandong Key Laboratory of Eco-Environmental Science for the Yellow River Delta. The seedlings with a little in-situ soil were cultured with 30 L of 1% saline water for ten days in a plastic water tank (Wang et al., 2022). Experimental soil was collected from non-saline zone near the Yellow River Delta wetland (*Table 1*) and then fine roots and gravel existing in the soil were removed. Afterward, we loaded 7.5 kg of soil mixed with different masses of sea salt into 30 plastic buckets, which were 34 cm in height and 31 cm in diameter. *Spartina alterniflora* seedlings were washed using distilled water, and then planted in plastic buckets (two seedlings per bucket). The lowest end of the seedling roots was 15 cm from the soil surface. An equal amount of distilled water was poured into 30 buckets until the soil surface became moist.

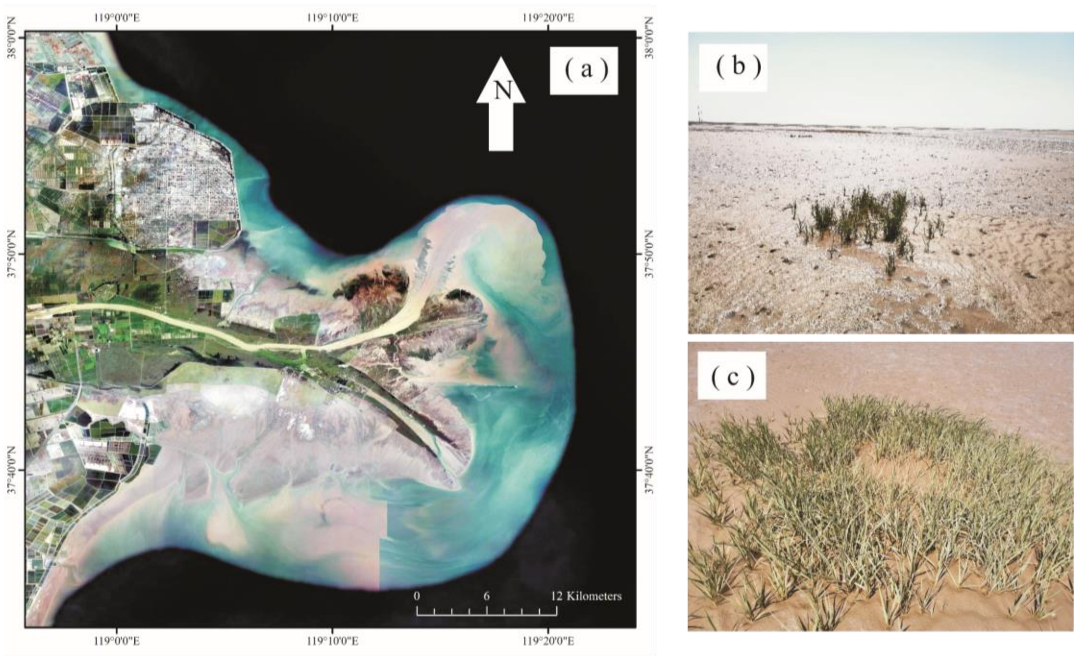

Figure 1. *Spartina alterniflora in the Yellow River Delta wetland*

Table 1. *The physiochemical traits of soil*

Soli physiochemical parameters (mean ± standard error, n = 3)	
pH	7.51 ± 0.12
Soil salinity (%)	0.19 ± 0.03
Soil electrical conductivity (μS/cm)	474 ± 31.18
Total carbon (%)	1.90 ± 0.03
Total nitrogen (%)	0.12 ± 0.00
Available phosphorus (mg/kg)	23.01 ± 0.68
C:N ratio	15.76 ± 0.27
δ^{13}C (‰)	-7.95 ± 0.08
δ^{15}N (‰)	4.06 ± 0.17

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

Initial soil pH, salinity, and electrical conductivity (EC) in experimental plots were 7.51, 0.19%, and 474 $\mu S \cdot cm^{-1}$, respectively. Initial soil total nitrogen, carbon, nitrate nitrogen, ammonium nitrogen, and available phosphorus contents were 1.90%, 0.12%, 23.01 $mg \cdot kg^{-1}$, respectively. The soil have a value of 15.76, -7.95‰ and 4.06‰ for C:N, $\delta^{13}C$ and $\delta^{15}N$, respectively. The temperature of greenhouse ranged from 18°C to 45°C.

Experimental design

Spartina alterniflora was widely distributed in the salt marsh zone of Yellow River Delta wetland, and the soil salinity ranged from 0 to 20 g/kg. Thus, *S. alterniflora* seedlings were exposed to five saline treatments including 0 g/kg (T0, maintaining the original soil salinity), 5 g/kg (T5), 10 g/kg (T10), and 20 g/kg (T20). We considered twelve duplicates for every treatment. The salinity treatments were achieved by adjusting the masses of sea salt (g) and soil (kg). We dissolved salt in water and poured it into the soil. The sea salt used in the experiment was obtained from the region of Yellow River Delta wetland. The experiment started on 1st, June, and lasted for 105 days. During growth stages, the plastic buckets were randomly placed. It should be noted that one plant in T0, two plants in T10, and four plants (1/3) in T20 died before the end of the experiment.

Determination of leaf traits

At the end of the experiment, the best growing and living mature leaves (including some leaves with a withered leaf tip) were acquired from *S. alterniflora* under the four treatments. Total leaf number (TLN), living leaf number (LLN), and DLN of *S. alterniflora* were counted, and the PDL and RDL were calculated by *Equations 1* and *2*, respectively. Two leaves in every plant were employed to measure leaf length (±0.1 cm) and LWL (±0.1 cm) using a meter ruler, and leaf width (±0.1 cm) using a vernier caliper. The PWL was calculated using *Equation 3*. The leaf area (±0.1 cm^2) was scanned using a scanner (HP LaserJet M233sdw) and analyzed using Fiji.app software. The ratio of leaf length to width (RLW) was computed by *Equation 4*. The leaf fresh mass (0.0001 g) of *S. alterniflora* was recorded after harvest. The leaves were killed at 120℃ for 2 h and then dried at 70℃ until a constant weight was achieved. The leaves were re-weighed to obtain leaf dry mass (0.0001 g). LDMC (%) and SLA (cm^2/g) were calculated by *Equations 5* and *6*, respectively.

$$\mathrm{PDL} = \mathrm{DLN}/\mathrm{TLN} \tag{Eq.1}$$

$$\mathrm{RDL} = \mathrm{DLN}/\mathrm{LLN} \tag{Eq.2}$$

$$\mathrm{PWL} = \mathrm{LWL}/\mathrm{Leaf\ length} \tag{Eq.3}$$

$$\mathrm{RLW} = \mathrm{Leaf\ length}/\mathrm{Leaf\ width} \tag{Eq.4}$$

$$\mathrm{LDMC} = \mathrm{Leaf\ dry\ mass} * 100\%/\mathrm{Leaf\ fresh\ mass} \tag{Eq.5}$$

$$\mathrm{SLA} = \mathrm{Leaf\ area}/\mathrm{Leaf\ dry\ mass} \tag{Eq.6}$$

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

Statistical analysis

Statistical analyses were carried out using R 4.1.0 software. Before further analyses, leaf traits of *S. alterniflora* were checked for normality and homogeneity. To meet the assumptions of homoscedasticity, some traits were log-transformed or square root-transformed. When the assumptions of homoscedasticity were also not satisfied, permutational analysis of variance (permutational ANOVA) was implemented in R (package lmPerm). One-Way ANOVA was applied to determine the effects of soil salinity on leaf traits of *S. alterniflora* using Duncan's test, and different letters indicated significant differences at the 0.05 significance level. A box diagram was utilized to describe the data dispersion for every leaf trait under different soil salinity treatments. Polynomial fitting was applied to describe the relationship between leaf number traits and soil salinity. Linear fitting was employed to investigate the relationship between any two indicators in DLN, PDL, RDL, LWL and PWL. Based on the data from the four treatments, linear fitting was performed between SLA and LDMC to examine whether or not *S. alterniflora* could achieve a trade-off between rapid matter production and efficient nutrient conservation. Linear fitting of SLA and LDMC was used to describe the effects of soil salinity on the plant functioning of *S. alterniflora* (Zhang et al., 2020). The correlation analysis for any two indicators of leaf traits of *S. alterniflora* was carried out using "ggplot2" package, "GGally" package, and "graphics" package in R software. To identify the differences in overall leaf traits of *S. alterniflora* in different soil salinity treatments, principal component analysis (PCA) was performed using the "FactoMineR" and "factoextra" packages in R software.

Results

Leaf number of S. alterniflora

Soil salinity greatly affected the total leaf number and live leaf number of *S. alterniflora* (*Table 2; Fig. 2*). The TLN and LLN first increased and then decreased with increasing soil salinity. The greatest total leaf area and LLN were recorded in T10 (13.5 ± 0.4 blades and 9.5 ± 0.7 blades), which were 36.7% and 55.7% larger than those in T20, respectively. There were no significant differences in DLN, PDL, and RDL. The DLN, PDL, and RDL of *S. alterniflora* ranged from 3.5 ± 0.3 (T0) to 4.9 ± 0.2 (T5) blades, 0.29 ± 0.06 (T10) to 0.38 ± 0.04 (T20) blades, and 0.46 ± 0.07 (T0) to 0.67 ± 0.14 (T20) blades, respectively. The RDL positively increased with the PDL (*Fig. 3*).

Leaf morphological traits of S. alterniflora

Substantial differences in leaf area, leaf length, leaf width, the RLW, LWL and PWL were identified between different soil salinity treatments (*Table 2; Fig. 4*). The greatest leaf area was found in T5 (17.41 ± 0.65 cm^2), which was 2.02 times of T20 (8.62 ± 0.57 cm^2). Leaf length increased from 30.55 ± 1.00 cm (T0) to 35.11 ± 0.91 cm (T5) and then decreased to 20.82 ± 0.89 cm (T20). A similar pattern was recorded in leaf width. The RLW considerably decreased from 424.07 ± 9.13 to 303.55 ± 9.86 with increasing soil salinity. In contrast to our expectations, the greatest LWL and PWL were recorded in T5 (5.48 ± 0.72 cm and 0.16 ± 0.02), while the smallest values were 1.40 ± 0.90 cm and 0.06 ± 0.02, respectively, which were recorded in T20. Furthermore, no significant relationships were found between the PWL and RDL, PWL and PDL,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

LWL and PDL, LWL and RDL (*Fig. 3*). Weak positive linear relationships were recorded between DLN and LWL, and between DLN and PWL (*Fig. 3*).

Leaf mass traits of S. alterniflora

Leaf dry mass, LDMC and SLA area displayed significant differences in the four soil salinity treatments (*Table 2; Fig. 5*). The greatest leaf dry mass was recorded in T5 (0.1823 ± 0.0084 g), which was 1.19 times larger than that in T20. The LDMC ranged from 36.51 ± 0.35% (T0) to 39.20 ± 0.51% (T10), and the greatest value was 7.36% larger than the lowest value. Compared to T5 (97.60 ± 1.70 cm^2/g) and T10 (95.67 ± 2.22 cm^2/g), a higher SLA was recorded in T0 (109.731 ± 1.76 cm^2/g) and T20 (105.31 ± 2.34 cm^2/g).

Table 2. *Results (F-values) of ANOVAs regarding the effects of soil salinity on the leaf traits of S. alterniflora in response to soil salinity in the Yellow River Delta wetland, China*

Leaf traits	F	p
Total leaf number	**6.088**	**p < 0.01**
Live leaf number	**3.889**	**p < 0.05**
Dead leaf number	1.768	p > 0.05
Proportion of dead leaf	1.611	p > 0.05
Ratio of dead leaf to live leaf	0.916	p > 0.05
Leaf area	**35.415**	**p < 0.001**
Leaf length	**36.042**	**p < 0.001**
Leaf width	**25.736**	**p < 0.001**
Ratio of leaf length to width	**28.804**	**p < 0.001**
Length of withered leaf tip	**6.489**	**p < 0.001**
Proportion of withered leaf tip	**4.730**	**p < 0.001**
Leaf dry mass	**27.652**	**p < 0.001**
LDMC	**7.049**	**p < 0.001**
SLA	**11.396**	**p < 0.001**

***P < 0.001, **P < 0.01, *P < 0.05

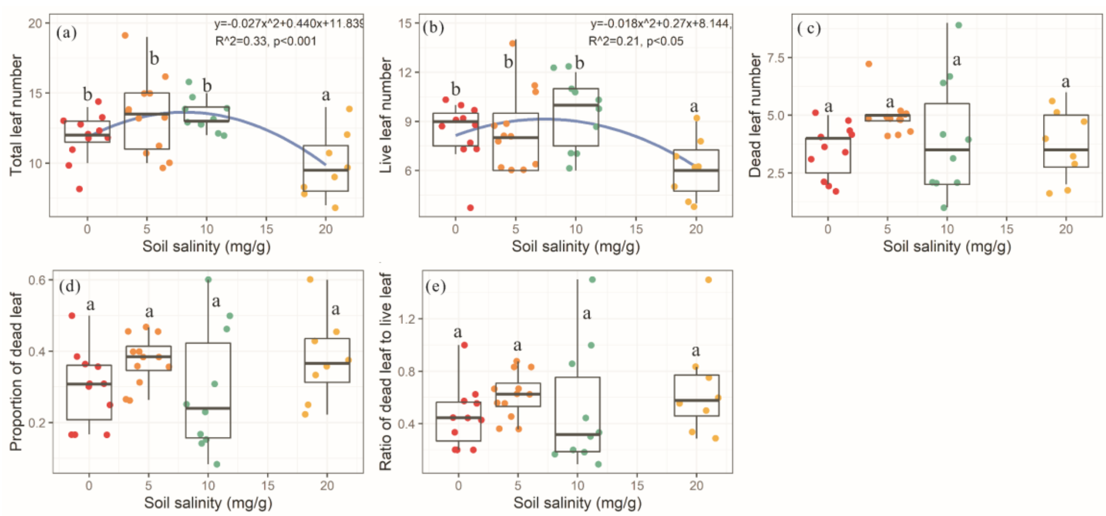

Figure 2. *Leaf number of S. alterniflora in response to soil salinity in the Yellow River Delta wetland, China (means ± standard error (SE), n = 8-12)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

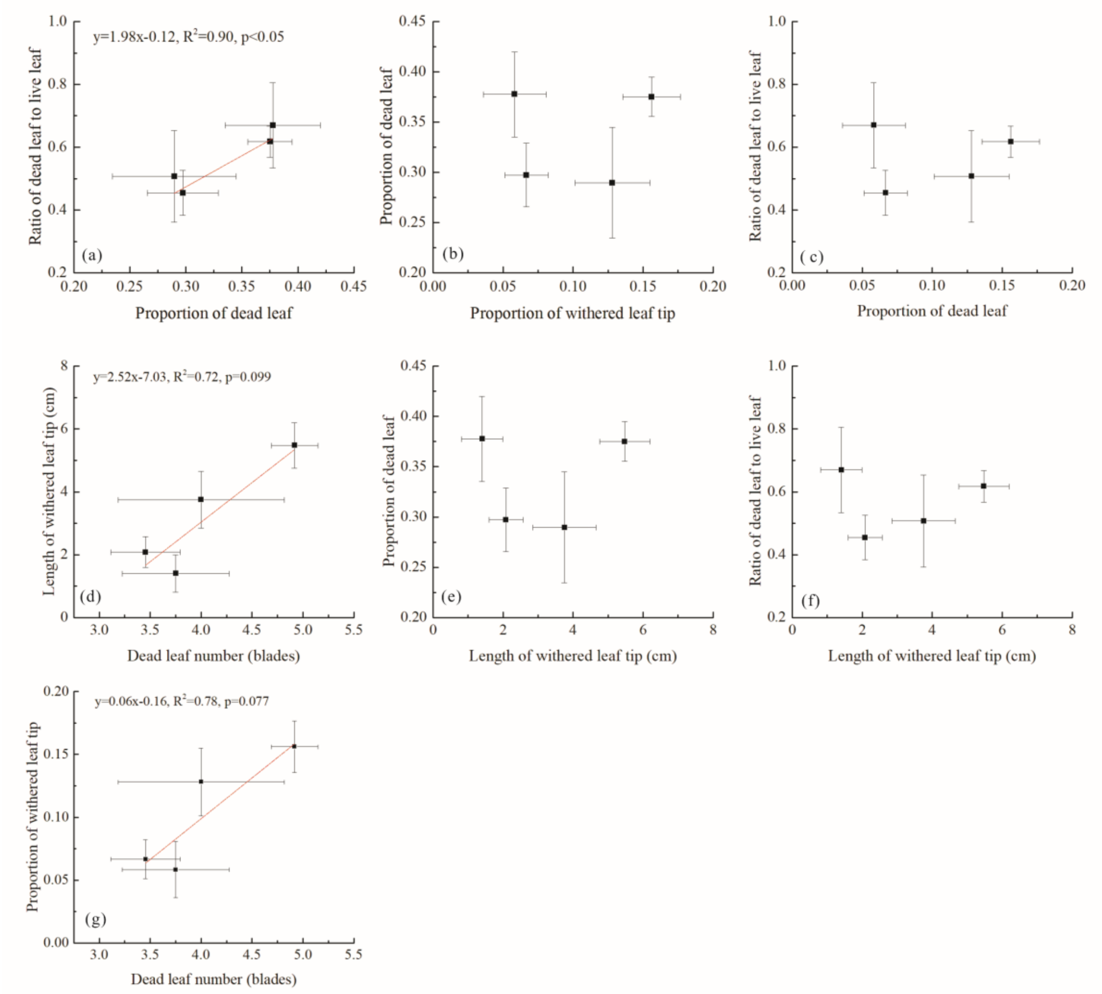

Figure 3. *Relationships between any two indicators of dead leaf number, the proportion of dead leaf, the ratio of dead leaf to live leaf, length of withered leaf tip, and the proportion of withered leaf tip of S. alterniflora in the Yellow River Delta wetland, China*

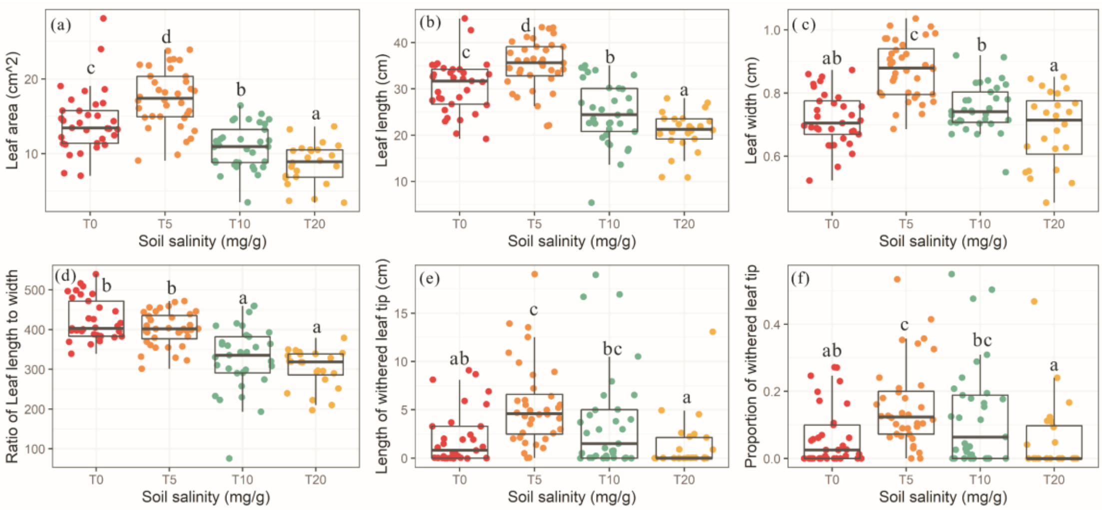

Figure 4. *Leaf morphological traits of S. alterniflora in response to soil salinity in the Yellow River Delta wetland, China (means ± standard error, n = 24-36)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

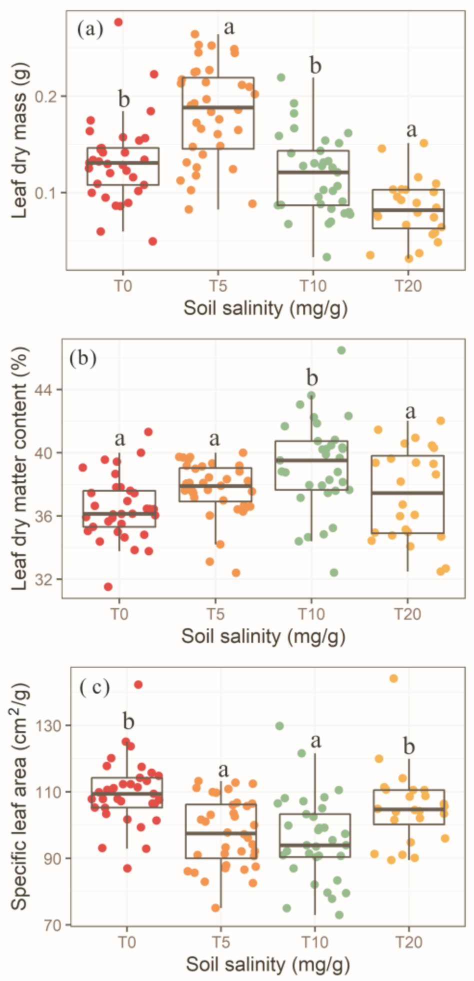

Figure 5. *Leaf mass traits of S. alterniflora in response to soil salinity in the Yellow River Delta wetland, China (means ± standard error, n = 24-36)*

The SLA of *S. alterniflora* substantially decreased with the increasing LDMC based on all data (*Fig. 6*), suggesting the trade-off in leaves between rapid matter production and efficient nutrient conservation. Compared to other treatments, *S. alterniflora* in T0 had a greater rate of biomass production. Moreover, the fundamental trade-off in plant functioning indicated that more efficient nutrient conservation occurred in T10, however, it did not occur for the other three treatments because of a vague relationship at high LDMC.

Correlation analysis and PCA of leaf traits of S. alterniflora

Significant correlations between leaf morphological and mass traits were observed ($0.20 \leq |r| \leq 0.94$, *Fig. 7*). Based on all available data, we could record positive relationships between leaf area, leaf length, leaf width, the RLW, LWL, PWL, and leaf dry mass. The SLA significantly decreased with increasing leaf area, leaf length, leaf width, LWL, PWL, leaf dry mass, and LDMC. Besides, the LDMC had a positive correlation with LWL, PWL, and the leaf dry mass. It is worth mentioning that there was no significant correlation between SLA and PWL in the four treatments.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

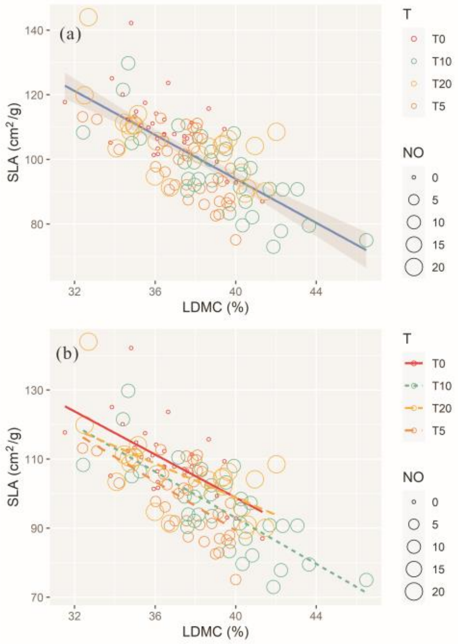

Figure 6. *The relationships of LDMC and SLA of S. alterniflora in response to soil salinity in the Yellow River Delta wetland, China (means ± standard error, n = 24-36). "No" means soil salinity treatments*

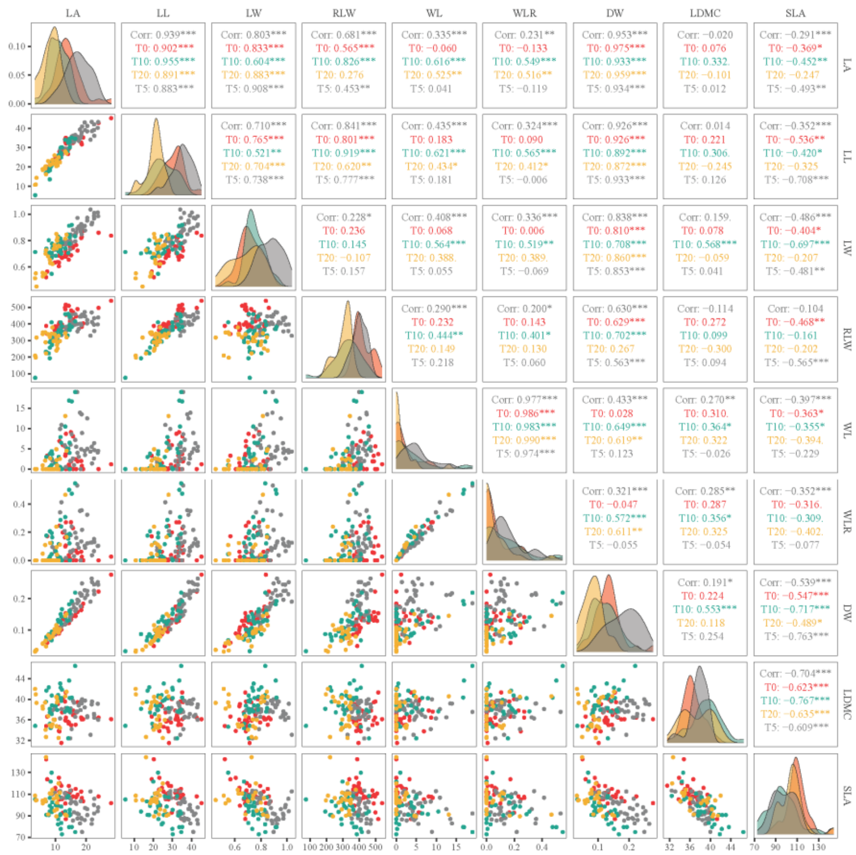

Figure 7. *Correlation analysis of leaf traits of S. alterniflora in the Yellow River Delta wetland, China*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

The first two components (Dim1 and Dim2) revealed 75.1% of the total variability of leaf traits (*Fig. 8*). The Dim1 was formed based on leaf area, leaf length, leaf width, leaf dry mass, the RLW, LWL, and PWL. The SLA, LDMC, and PWL were important contributors to the Dim2. The confidence ellipses of leaf traits occupied different positions, demonstrating significant differences in leaf traits of *S. alterniflora* among the four treatments.

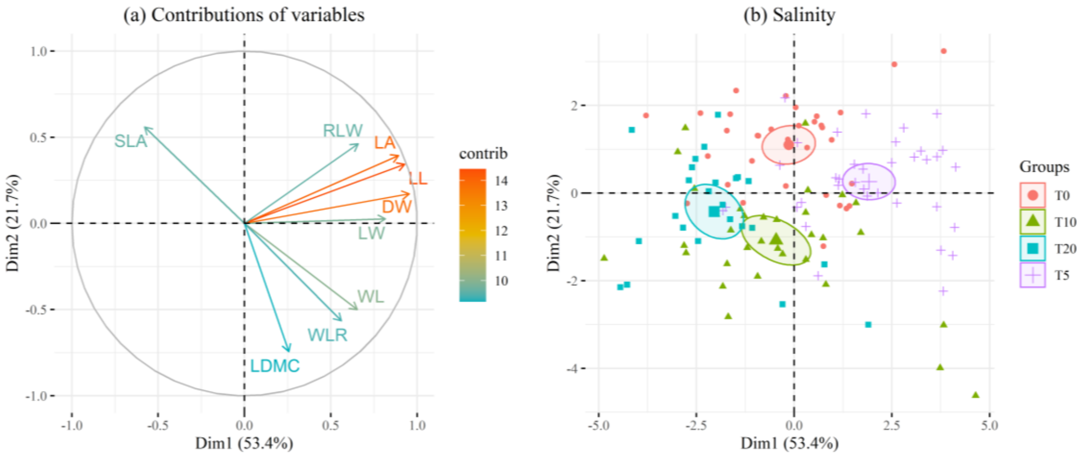

Figure 8. *The PCA plot showing the locations of leaf traits of S. alterniflora under different soil salinity conditions*

Discussion

Leaf traits play an important role in plant functioning by involving strategies for species to acquire and utilize resources (Fajardo and Siefert, 2018). Due to the close relationship between plant leaves and the environment, leaf traits can intuitively reflect the adaptive strategies of plants to environmental changes (He et al., 2020; Li et al., 2021; Zhang et al., 2020). In this study, the rapid spreading of the *S. alterniflora* after 2010 may be related to the adaptation of *S. alterniflora* to the environment. Soil salinity was a controlling factor affecting growth of *S. alterniflora*. The TLN and LLN that are related to plant vitality were substantially changed with increasing soil salinity, and the smallest value of them was recorded in T20. To ensure normal plant growth in a suitable environment, a reasonable canopy leaf number is the primary condition (Qing et al., 2011). A previous study reported that 200 mM NaCl treatment increased the leaf number of *S. alterniflora* compared to the control treatment (0 mM NaCl), but 500 mM NaCl treatment had a positive or negative impact on leaf number due to the supply of different forms of nitrogen element (Hessini et al., 2021). However, Courtney et al. (2016) found that the leaf number of *S. alterniflora* decreased with increasing salinity (0-12 ppt). High salinity inhibited plant growth, affected leaf differentiation and formation of *S. alterniflora*, and even resulted in plant death (e.g., plants in T30). Surprisingly, indicators used to characterize the degree of plant wilt, including DLN, PDL, and RDL of *S. alterniflora*, had no significant differences in the four different treatments. This was attributed to the dead plants (1/3 of 12 duplicates) under T20 and the data dispersion of these three indicators in T10. The death of leaves under high salinity was much more severe, and thus, dead plants under T20 annihilated the information of dead leaves. The coefficient of variance for these corresponding

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

indicators ranged from 60.14%-90.97% in T10 (much larger than that in other treatments), indicating the extensive changes between dead and live leaves of *S. alterniflora*. Leaf withering and green leaf strategy of *S. alterniflora* maintain the normal survival of plants, while regulating the leaf number is also beneficial for the expansion of *S. alterniflora*.

As soil salinity increased in our study, leaf area, length, width first increased and then decreased, and the highest value of these indicators were recorded in T5. A similar pattern of leaf area was reported by Xiao et al. (2005), who found that the leaf area of *S. alterniflora* increased from 2.19 cm^2 in 0‰ to 2.14 cm^2 in 10‰, and decreased to 1.28 cm^2 in 50‰ salinity (salinity: water). Contrary to our results, Zhang et al. (2019a) reported that leaf length and leaf area first decreased and then increased with increasing salinity (0-900 Mm NaCl salinity: water). In our study, a negative linear relationship was found between the RLW to soil salinity, indicating that higher soil salinity (T10, T20) inhibited the leaf size of *S. alterniflora* by affecting the longitudinal elongation rate of blades. All these demonstrated that the leaf plasticity of *S. alterniflora* changed due to salinity. The appropriate salinity threshold is more suitable for the growth and reproduction of *S. alterniflora*, resulting in an expansion of its invasion zone.

The withered state of leaves can characterize the degree of environmental damage to plant leaves (Zhang et al., 2019c, 2020). The larger the DLN and PDL, the greater the LWL and PWL, which all indicate a stronger degree of environmental damage to the leaves. We used the DLN, PDL and RDL to describe the withered state of leaves from a perspective of leaf number and its corresponding ratio. There were no significant effects of salinity on the withered state of leaves. Positive relationships between the PDL and the RDL implied the consistency of dead leaf information. This reflected the damage of the environment to leaves and also demonstrated the trade-off between DLN and LLN of *S. alterniflora* at the population scale. The LWL and the PWL describe the withered state of leaves from the perspective of leaf morphology (Zhang et al., 2019b). Unlike the DLN and its corresponding ratio, substantial differences were recorded among the four treatments in terms of the LWL and the PWL. Surprisingly, a strong degree of salinity damage to the leaf occurred in T5. This was justified by the following reasons: 1) The DLN took a high proportion of the total leaf number in T20; 2) relatively robust or young leaves survived, contributing to a lower degree of the withered state of leaves. Further evidence is needed to prove that the DLN positively increased with the increasing LWL and the increasing PWL. The synchronization of these three indicators is conducive to a deeper understanding of the strategies of Survival First in plants and invasion of *S. alterniflora*.

Higher matter conservation occurred in T5 and T10, and a quicker matter production was recorded in T0 and T20. It should be noted that the strategy of matter production of young leaf in T20 helped the plant to survive the high soil salinity, while that in T0 was conducive to the rapid growth of plants in suitable environments. Compared to T20, we found a higher trade-off between rapid matter production and efficient nutrient conservation in T0 which supported the above growth strategy of *S. alterniflora* (*Fig. 6*). Thus, plants adopt the same growth strategies in response to stress and suitable habitats despite different environmental triggers. At the same time, this strategy ensures that *S. alterniflora* can survive in adverse environment, so as to further achieve the purpose of invasion and expansion.

The response of *S. alterniflora* to soil salinity varied in terms of different leaf traits, and this was summarized as local differences and overall consistency of growth strategy

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

(Zhang et al., 2020; Courtney et al., 2016). Most leaf traits had a significant positive or negative correlation based on all data collected from the four treatments, while weak relationships were exhibited in single treatments, which demonstrated the particularity and universality of plant growth strategy (*Fig. 6*). Local differences in some leaf traits indicated the particularity of plant in response to soil salinity, but overall consistency explained the response strategies commonly found in plants (Hessini et al., 2021). The PCA suggested significant differences in overall leaf traits among the four treatments. Different positions of confidence ellipses for T5 and T20 along the first PC1 demonstrated a significant difference in leaf morphological traits, and those for T10 and T0 along the first PC2 indicated that leaf traits based on SLA and LDMC were significantly different. All of these supported the overall consistency of the plant growth strategy. The local differences and overall consistency of growth strategy about leaf traits ensure the strong adaptability and invasion ability of *S. alterniflora*. However, further investigations on the leaf traits, especially plant phenological and physiological parameters, of *S. alterniflora* are needed to thoroughly explore the structure and function of wetland ecosystems.

Conclusion

Soil salinity has a significant effect on leaf traits of *S. alterniflora*, except for dead leaf number, the proportion of dead leaf, and the ratio of dead leaf to live leaf. As soil salinity increased, total leaf number, live leaf number, leaf area, leaf length, leaf width, leaf dry mass and leaf dry matter content first increased and then decreased, the ratio of leaf length to width decreased from 424.07 to 303.55. The peak value of leaf area, withered leaf tip, proportion of the withered leaf tip, leaf dry mass was recorded in T5. The trade-off of specific leaf area and leaf dry matter content indicated that while a more efficient nutrient conservation strategy was adopted in T10, *S. alterniflora* rapidly produced biomass in T0. Strong correlations were observed between leaf morphological and mass traits except for some parts of leaf dry matter content. The confidence ellipses of leaf traits occupied different positions, which indicated significant differences in leaf traits of the four treatments. The findings help in understanding of the leaf traits of *S. alterniflora* in response to soil salinity and provide valuable information for the adaptation strategy of *S. alterniflora*.

Acknowledgements. This research was supported by the Shandong Provincial Natural Science Foundation (No. ZR2021QD101); the National Natural Science Foundation of China (No. 42101111); the Youth Innovation Support Program of Shandong Universities (No. 2023KJ273); the PhD research startup foundation of Binzhou University (No. 2021Y14); Binzhou Youth Science and Technology Rising Star Program Project (QMX2023001) and College Student Innovation Training Program Plan.

REFERENCES

[1] Bai, J., Yu, L., Ye, X., Yu, Z., Wang, D., Guan, Y., Cui, B., Liu, X. (2020): Dynamics of phosphorus fractions in surface soils of different flooding wetlands before and after flow-sediment regulation in the Yellow River Estuary, China. – Journal of Hydrology 580: 124256.

[2] Chung, C. H. (2006): Forty years of ecological engineering with *Spartina* plantations in China. – Ecological Engineering 27(1): 49-57.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

[3] Courtney, A. J., Xu, J., Xu, Y. (2016): Responses of growth, antioxidants and gene expression in smooth cordgrass (*Spartina alterniflora*) to various levels of salinity. – Plant Physiology and Biochemistry 99: 162-170.
[4] Cui, B., Tang, N., Zhao, X., Bai, J. (2009): A management-oriented valuation method to determine ecological water requirement for wetlands in the Yellow River Delta of China. – Journal for Nature Conservation 17(3): 129-141.
[5] Fajardo, A., Siefert, A. (2018): Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. – Ecology 99(5): 1024-1030.
[6] Fraser, M. R., Winsor, T., Williams, J., Wyeth, R. C., Garbary, D. J. (2021): Assessing the viability of pre-industrial sediment prior to remediation using primary producer (*Zostera marina* and *Spartina alterniflora*) growth and survival. – Canadian Journal of Fisheries and Aquatic Sciences 78(4): 361-370.
[7] Gao, G. F., Peng, D., Zhang, Y., Li, Y., Fan, K., Tripathi, B. M., Adams, J. M., Chu, H. (2021): Dramatic change of bacterial assembly process and co-occurrence pattern in *Spartina alterniflora* salt marsh along an inundation frequency gradient. – Science of the Total Environment 755: 142546.
[8] Garnier, E., Shipley, B., Roumet, C., Laurent, G. (2001): A standardized protocol for the determination of specific leaf area and leaf dry matter content. – Functional Ecology 5(15): 688-695.
[9] Guan, B., Zhang, H., Wang, X., Yang, S., Chen, M., Hou, A., Grace, A., Han, G. (2020): Salt is a main factor shaping community composition of arbuscular mycorrhizal fungi along a vegetation successional series in the Yellow River Delta. – Catena 185: 104318.
[10] Han, G., Song, W., Li, Y., Xiao, L., Zhao., M., Chu, X., Xie, B. (2023): Enhancement of coastal blue carbon: concepts, techniques, and future suggestions. – Bulletin of Chinese Academy of Sciences 38(3): 492-503 (in Chinese).
[11] He, N., Li, Y., Liu, C., Xu, L., Li, M., Zhng, J., He, J., Tang, Z., Han, X., Ye, Q., Xiao, C., Yu, Q., Liu, S., Sun, W., Niu, S., Li, S., Sack, L., Yu, G. (2020): Plant trait networks: improved resolution of the dimensionality of adaptation. – Trends in Ecology & Evolution 35(10): 908-918.
[12] Hessini, K., Jeddi, K., Siddique, K. H., Cruz, C. (2021): Drought and salinity: a comparison of their effects on the ammonium‐preferring species *Spartina alterniflora*. – Physiologia Plantarum 172(2): 431-440.
[13] Jia, M., Wang, Z., Mao, D., Ren, C., Wang, C., Wang, Y. (2021): Rapid, robust, and automated mapping of tidal flats in China using time series Sentinel-2 images and Google Earth Engine. – Remote Sensing of Environment 255: 112285.
[14] Jia, P., Qu, G., Jia, J., Li, D., Sun, Y., Liu, L. (2024): Long term *Spartina alterniflora* invasion simplified soil seed bank and regenerated community in a coastal marsh wetland. – Ecological Applications 34(1): e2754.
[15] Jiang, L. F., Luo, Y. Q., Chen, J. K., Li, B. (2009): Ecophysiological characteristics of invasive *Spartina alterniflora* and native species in salt marshes of Yangtze River estuary, China. – Estuarine, Coastal and Shelf Science 81(1): 74-82.
[16] Li, H., Mao, D., Wang, Z., Huang, X., Jia, M. (2022): Invasion of *Spartina alterniflora* in the coastal zone of mainland China: control achievements from 2015 to 2020 towards the Sustainable Development Goals. – Journal of Environmental Management 323: 116242.
[17] Li, S., Wang, H., Gou, W., White, J. F., Kingsley, K. L., Wu, G., Su, P. (2021): Leaf functional traits of dominant desert plants in the Hexi Corridor, Northwestern China: trade-off relationships and adversity strategies. – Global Ecology and Conservation 28: e01666.
[18] Li, S., Xie, T., Bai, J., Cui, B. (2022): Degradation and ecological restoration of estuarine wetlands in China. – Wetlands 42(7): 90.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

[19] Liu, M., Mao, D., Wang, Z., Li, L., Man, W., Jia, M., Ren, C., Zhang, Y. (2018): Rapid invasion of *Spartina alterniflora* in the coastal zone of mainland China: new observations from Landsat OLI images. – Remote Sensing 10(12): 1933.

[20] Liu, W., Zhang, Y., Chen, X., Maung-Douglass, K., Strong, D. R., Pennings, S. C. (2020): Contrasting plant adaptation strategies to latitude in the native and invasive range of *Spartina alterniflora*. – New Phytologist 226(2): 623-634.

[21] Liu, W., Wang, W., Zhang, Y. (2022): Differences in leaf traits of *Spartina alterniflora* between native and invaded habitats: implication for evolution of alien species competitive ability increase. – Ecological Indicators 138: 108799.

[22] Lu, H. F., Zhang, H. S., Qin, P., Li, X. Z., Campbell, D. E. (2020): Integrated energy and economic evaluation of an ecological engineering system for the utilization of *Spartina alterniflora*. – Journal of Cleaner Production 247: 119592.

[23] Mao, D., Liu, M., Wang, Z., Li, L., Man, W., Jia, M., Zhang, Y. (2019): Rapid invasion of *Spartina alterniflora* in the coastal zone of mainland China: spatiotemporal patterns and human prevention. – Sensors 19(10): 2308.

[24] Mao, L., Mishra, D. R., Hawman, P. A., Narron, C. R., O'Connell, J. L., Cotten, D. L. (2023): Photosynthetic performance of tidally flooded *Spartina alterniflora* salt marshes. – Journal of Geophysical Research: Biogeosciences 128(3): e2022JG007161.

[25] Maricle, B. R., Lee, R. W. (2002): Aerenchyma development and oxygen transport in the estuarine cordgrasses *Spartina alterniflora* and *S. anglica*. – Aquatic Botany 74(2): 109-120.

[26] Matsuda, R., Yamada, K., Hayasaka, D., Henmi, Y. (2023): Effects of salinity, temperature, and immersion conditions on seed germination of invasive *Spartina alterniflora* Loisel (smooth cordgrass) in Japan. – Regional Studies in Marine Science 57: 102738.

[27] Meng, W., Feagin, R. A., Innocenti, R. A., Hu, B., He, M., Li, H. (2020): Invasion and ecological effects of exotic smooth cordgrass *Spartina alterniflora* in China. – Ecological Engineering 143: 105670.

[28] Okoye, O. K., Li, H., Gong, Z. (2020): Retraction of invasive *Spartina alterniflora* and its effect on the habitat loss of endangered migratory bird species and their decline in YNNR using remote sensing technology. – Ecology and Evolution 10(24): 13810-13824.

[29] Pan, Y., Cieraad, E., van Bodegom, P. M. (2019): Are ecophysiological adaptive traits decoupled from leaf economics traits in wetlands? – Functional Ecology 33(7): 1202-1210.

[30] Pang, B., Xie, T., Ning, Z., Cui, B., Zhang, H., Wang, X., Gao, F., Zhang, S., Lu, Y. (2023): Invasion patterns of *Spartina alterniflora*: response of clones and seedlings to flooding and salinity—A case study in the Yellow River Delta, China. – Science of The Total Environment 877: 162803.

[31] Qing, H., Yao, Y., Xiao, Y., Hu, F., Sun, Y., Zhou, C., An, S. (2011): Invasive and native tall forms of *Spartina alterniflora* respond differently to nitrogen availability. – Acta Oecologica 37(1): 23-30.

[32] Qu, W., Han, G., Eller, F., Xie, B., Wang, J., Wu, H., Li, J., Zhao, M. (2020): Nitrogen input in different chemical forms and levels stimulates soil organic carbon decomposition in a coastal wetland. – Catena 194: 104672.

[33] Ren, J., Chen, J., Xu, C., van de Koppel, J., Thomsen, M. S., Qiu, S., Cheng, F., Song, W., Liu, Q., Xu, C., Bai, J., Zhang, Y., Cui, B., Bertness, M., Silliman, B., Li, B., He, Q. (2021): An invasive species erodes the performance of coastal wetland protected areas. – Science advances 7(42): eabi8943.

[34] Shao, D., Zhou, W., Bouma, T. J., Asaeda, T., Wang, Z. B., Liu, X., Sun, T., Cui, B. (2020): Physiological and biochemical responses of the salt-marsh plant *Spartina alterniflora* to long-term wave exposure. – Annals of Botany 125(2): 291-300.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

[35] Tian, Y., Jia, M., Wang, Z., Mao, D., Du, B., Wang, C. (2020): Monitoring invasion process of *Spartina alterniflora* by seasonal Sentinel-2 imagery and an object-based random forest classification. – Remote Sensing 12(9): 1383.
[36] Wang, Q., Shi, H., Yu, Z., Wang, T., Wang, C. (2022): Effects of salinity and interspecific interaction on germination and growth of *Scirpus mariqueter* and *Spartina alterniflora*. – Acta Ecological Sinica 42(20): 8300-8310 (in Chinese).
[37] Wu, H., Guan, Q., Lu, K., Han, G., Li, B., Yang, M. (2020): Effects of hydrological connectivity on snail assemblages in the intertidal zone of coastal wetlands. – Wetlands 40: 1627-1634.
[38] Xiao, Q., Zheng, H., Chen, Y., Huang, W., Zhu, Z. (2005): Effects of salinity on the growth and proline soluble sugar and protein contents of *Spartina alterniflora*. – Chinese Journal of Ecology 24(4): 373-376 (in Chinese).
[39] Xie, B., Han, G., Qiao, P., Mei, B., Wang, Q., Zhou, Y., Zhang, A., Song, W., Guan, B. (2019): Effects of mechanical and chemical control on invasive *Spartina alterniflora* in the Yellow River Delta, China. – PeerJ 7: e7655.
[40] Yan, D., Li, J., Yao, X., Luan, Z. (2022): Integrating UAV data for assessing the ecological response of *Spartina alterniflora* towards inundation and salinity gradients in coastal wetland. – Science of the Total Environment 814: 152631.
[41] Yu, J., Li, Y., Han, G., Zhou, D., Fu, Y., Guan, B., Wang, G., Ning, K., Wu, H., Wang, J. (2014): The spatial distribution characteristics of soil salinity in coastal zone of the Yellow River Delta. – Environmental Earth Sciences 72: 589-599.
[42] Zhang, D., Hu, Y., Liu, M., Chang, Y., Yan, X., Bu, R., Zhao, D., Li, Z. (2017): Introduction and spread of an exotic plant, *Spartina alterniflora*, along coastal marshes of China. – Wetlands 37: 1181-1193.
[43] Zhang, D., Qi, Q., Wang, X., Tong, S., Lv, X., An, Y., Zhu, X. (2019b): Physiological responses of *Carex schmidtii* Meinsh to alternating flooding-drought conditions in the Momoge wetland, northeast China. – Aquatic Botany 153: 33-39.
[44] Zhang, D., Zhang, M., Tong, S., Qi, Q., Wang, X., Lu, X. (2020a): Growth and physiological responses of *Carex schmidtii* to water-level fluctuation. – Hydrobiologia 847: 967-981.
[45] Zhang, G., Bai, J., Zhao, Q., Jia, J., Wang, W., Wang, X. (2020b): Bacterial succession in salt marsh soils along a short-term invasion chronosequence of *Spartina alterniflora* in the Yellow River Estuary, China. – Microbial Ecology 79: 644-661.
[46] Zhang, L., Zhou, Y., Hou, D., Wang, R., Cai, J. (2019a). Effects of different interaction stress of salt and heavy metal Cadmium on the growth of *Spartina alterniflora*. – Journal of Sichuan Agricultural University 37(3): 330-337 (in Chinese).
[47] Zhang, M., Schwarz, C., Lin, W., Naing, H., Cai, H., Zhu, Z. (2023): A new perspective on the impacts of *Spartina alterniflora* invasion on Chinese wetlands in the context of climate change: a case study of the Jiuduansha Shoals, Yangtze Estuary. – Science of the Total Environment 868: 161477.
[48] Zhang, Y., Pennings, S. C., Li, B., Li, B., Wu, J. (2019c): Biotic homogenization of wetland nematode communities by exotic *Spartina alterniflora* in China. – Ecology 100(4): e02596.
[49] Zhang, Y., Pennings, S. C., Liu, Z., Li, B., Wu, J. (2021): Consistent pattern of higher lability of leaves from high latitudes for both native *Phragmites australis* and exotic *Spartina alterniflora*. – Functional Ecology 35(9): 2084-2093.
[50] Zuo, X., Cui, L., Li, W., Lei, Y., Dou, Z., Liu, Z., Cai, Y., Zhai, X. (2020): *Spartina alterniflora* leaf and soil eco-stoichiometry in the Yancheng coastal wetland. – Plants 10(1): 13.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153

APPENDIX

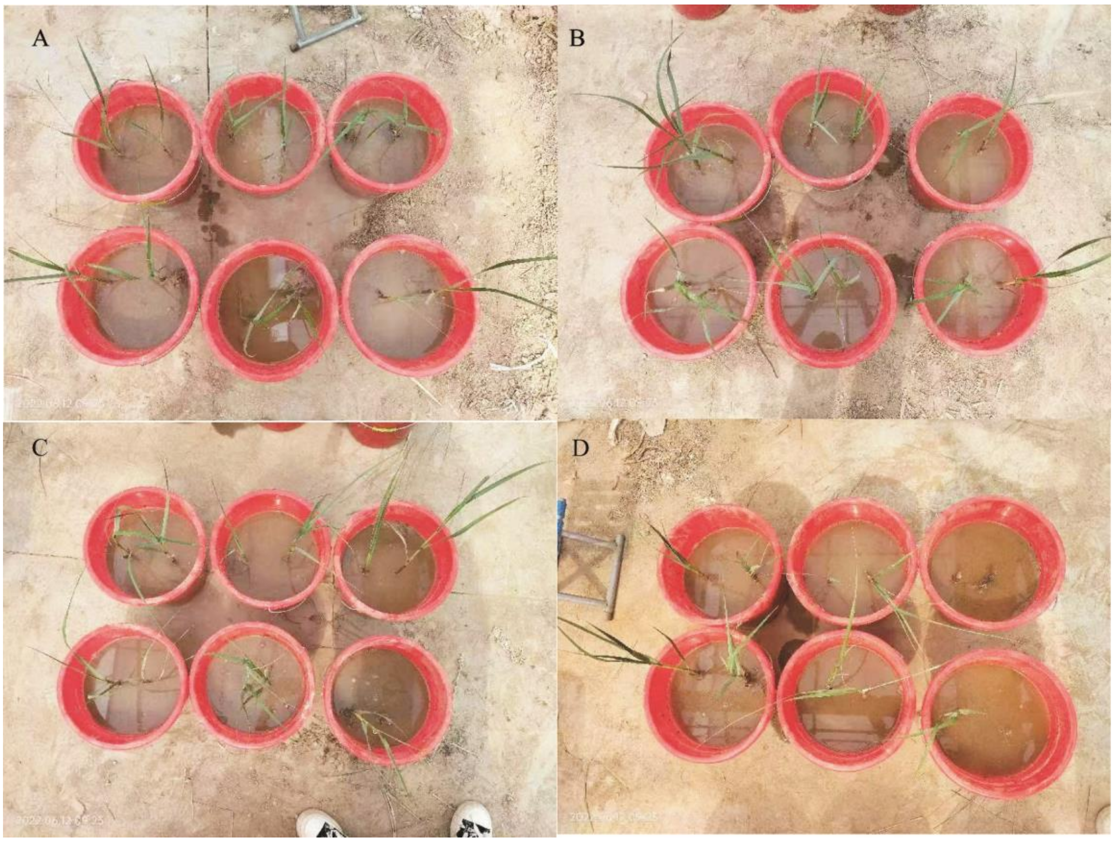

Figure A1. *Experimental treatments on 10th, June*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_41374153