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Original Research

Eco-physiological Responses of Carex Schmidtii to Soil Salinization in a Chinese Wetland

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Abstract

Soil salinization, a growing problem in arid and semi-arid areas, significantly influences the ecological dynamics and processes in wetland ecosystems. To fully examine the physiological responses with the aim of wetland protection and management, a laboratory simulation experiment was conducted to study the effects of soil salinization on the growth of *C. schmidtii* tussocks. Plant height and leaf traits, as well as physiological characteristics, were analyzed to explore the responses of *C. schmidtii* to soil salinization. Results showed that the highest value of electrical conductivity (EC) (4.71 mS/cm) recorded in 4000 mg/L treatment was 3.04 times greater than the lowest value (1.55 mS/cm) recorded in 0 mg/L treatment. It was well demonstrated that plant height under the 1000 mg/L treatment was 57.6% greater than that obtained under the 4000 mg/L treatment. Additionally, the growth of plants under the 4000 mg/L treatment achieved significantly higher length and the ratio of leaf withering (by 13.76 and 16.42 times, respectively), compared with those obtained under 0 mg/L treatment. 0 and 1000 mg/L treatments were found to greatly increase chlorophyll content and decrease malondialdehyde. Hence, slight salinization will stimulate the responses of *C. schmidtii* to environmental fluctuation, but the persistent serious salinization can inhibit the growth and physiology of *C. schmidtii*. The optimum ecological threshold of salinity for the growth of *C. schmidtii* was in the range 0~1000 mg/L. Results help in understanding the responses of *C. schmidtii* tussocks to soil salinization, and suggest the vital significance of preventing salinization in the Momoge Wetlands of northeastern China.

Keywords: physiological response, soil salinization, Carex schmidtii, Momoge Wetland, wetland management

Introduction

Soil salinization due to climate change and anthropogenic modification of the hydrological cycle is a growing problem in arid and semi-arid areas [1-2]. Soil salinization increases the environmental variability and results in the structural and functional loss of wetland ecosystem [3-5]. In general, salinization alters the fundamental physicochemical properties of soil and the biogeochemical cycling of nutrient elements, and then affects the growth of wetland vegetation [6]. Additionally, the physiological stress due to soil salinization will induce the large shifts in wetland-

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dominant species and their associated ecosystem functions [7]. Therefore, understanding the responses of wetland plants to soil salinization is important for the protection and restoration of wetlands in arid and semi-arid areas.

Momoge National Nature Reserve (MNNR), located in a semi-arid and soda-salinized region of western Sonnen Plain in China, is an internationally important wetland and a vital resting site for the Siberian Crane (*Grus leucogeranus*) [8-9]. At the same time, this nature reserve also plays an important role in preventing the saline-alkaline desertification of western Sonnen Plain [10]. However, soil salinization due to drought and irrigation has resulted in degradation of wetlands, threatening the persistence of rare bird habitat.

C. schmidtii, a native, tussock-forming species, is wildly distributed in the riparian wetland along the downstream of the Nenjiang River in the MNNR [11]. This species has abundant root systems and a strong accumulation ability, and it is able to support a rich biodiversity [12]. The sponge network structure (hummock) formed by root and peat provides favorable conditions for water conservation and sewage purification in tussock sedge wetlands [13]. Simultaneously, the microtopography created by hummocks not only increases the surface area to support plants being free from inundation, but also expands the living space range of plants to obtain the resources of sunlight, water and nutrients [14]. However, soil salinization of tussock wetland becomes increasingly serious and has resulted in the dying-off of *C. schmidtii* tussocks. Although previous studies have focused on the effects of water depth and flooding-drought conditions on the growth of *C. schmidtii* [12-13], few studies have explained how the physiology of *C. schmidtii* responds to soil salinization.

In this study, we conducted a greenhouse experiment to examine plant height, leaf functional traits (leaf area, length, width, shape, the length of withered leaves, the ratio of withered leaf, leaf mass, dry matter content and specific leaf area) and physiological characteristics (chlorophyll and malondialdehyde) of *C. schmidtii*. The purposes were to (1) explore the growth and physiological responses of *C. schmidtii* to soil salinization, and (2) elucidate the optimum ecological threshold of salinity for the growth of *C. schmidtii*. Results help in understanding the significance of preventing soil salinization of wetland in an arid and semi-arid region, and provide theoretical support for the protection and management for the tussock wetlands in the MNNR.

Materials and Methods

Plant Cultivation

C. schmidtii tussocks (the hummock being above the soil surface) and soil samples from the 0-10 cm soil layer were collected in November 2016 from the riparian wetland along the downstream of the Nenjiang River (45°53′51.97″N, 123°55′58.57″E), which was located in the MNNR of northeastern China. Tussocks and soil were taken to a greenhouse at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. We removed the aboveground biomass of tussocks and cut the hummocks into uniform pieces (20 cm in height and 15 cm in diameter). These small hummocks were then individually planted in plastic buckets (35 cm in height and 30 cm in diameter). Each bucket was filled with 12 kg soil to keep a small hummock half in soil and half above it. The soil type used in the study belongs to meadow bog soil with pH of 7.34, electrical conductivity of 140.50 mS/m, organic carbon content of 33.67%, total nitrogen content of 4.27 g/kg, total phosphorus content of 0.52 g/kg, and a carbon isotope ($\delta^{13}C$) of -27.39‰, respectively. The hummock samples (36 in total) were randomly positioned in the greenhouse and repositioned every week. During the experimental period (50 days), tussocks were irrigated with tap water (1 liter). The water capacity was about 65% after irrigation. The temperature of the greenhouse ranged from 23ºC to 32ºC.

Experiment Design and Laboratory Analyses

The experiment was conducted on the fifth day after the rhizomes sprouting of *C. schmidtii*. In order to stimulate soil salinization, two liters of the solutions containing 0 mg/L, 1000 mg/L, 3000 mg/L and 4000 mg/L salinity, which were prepared by NaCl and $NaHCO_3$ at a ratio of 2:1 and were watered into the planting tussocks, respectively. *E*ach treatment was replicated nine times and lasted 50 days. In the following days, 500 ml tap water was irrigated to all treatments on the 15th and 25th day*s*.

The height of *C. schmidtii* was measured every six days by a meter rule. At the end of the experiment, leaf morphology of the second leaf, including leaf area, length and width, were determined using a leaf area meter (Yaxin-1242, China). Leaf shape was expressed by the ratio of length to width. We also measured the length of withered leaf (LWL) to calculate the ratio of withered leaf (RWL, the ratio of withered leaf length to leaf length). The plant and the second leaves were harvested to obtain fresh and dry masses. Dry matter content and specific leaf area were used to indicate blade function. Part of fresh leaf samples was used to determine chlorophyll a, chlorophyll b [15] and malondialdehyde (MDA) contents [16]. Fresh leaves (0.2 g) were soaked in 10 mL of ethanol-acetone (1:1) for two days in the dark and then centrifuged at 2000 rpm for 15 min. Absorbance of the supernatant was measured at 663 and 645 nm, with an ultraviolet spectrophotometer (Shimadzu UV-2550, China). MDA was measured with thiobarbituric acid to estimate lipid peroxidation. Soil electrical conductivity (EC) was determined by a soil moisture sensor (HH2 W.E.T Sensor, Cambridge, UK) every week.

Statistical Analysis

Data analyses were performed using SPSS 20.0 and Origin 9.2. One-way analysis of variance (ANOVA) was employed to test the effect of salinization on the growth and physiology of *C. schmidtii*. Differences were compared by Duncan's test and different letters indicated significant differences at the 0.05 significance level.

Results and Discussion

Effect of Soda Salinity on Soil EC in *C. schmidtii* Tussock Wetland

As shown in Fig. 1, soil EC significantly increased with increasing salinity concentration from the initiation time of the experiment ($F>17$, $p<0.01$). The soil EC values of 0 mg/L treatment ranged from 1.35 mS/cm to 1.64 mS/cm, this being lower than that of other treatments. Additionally, a significant decrease of EC values from the tenth day was observed under 3000 mg/L and 4000 mg/L treatments. At the end of the experiment, the highest EC value (4.71 mS/cm) was recorded in 4000 mg/L treatment, this being 3.04 times greater than the lowest value recorded in 0 mg/L treatment. Salinization negatively affects the physicochemical nature of soil, especially the structural stability of soil [17-18]. In addition, soil hydraulic conductivity sharply decreases after irrigation with saline-sodic water, this leading to aggregate slaking and then inhibiting the soil nutrient cycle [19-20]. Zhou et al. (2017) found that soil salinization increased plant nitrogen (N) content and soil total N content, but decreased soil NO_3 and microbial biomass N [21]. Thus, preventing soil salinization has become much more important for the protecting wetlands in arid and semi-arid areas.

Effects of Soil Salinization on the Growth of *C. schmidtii*

Effects of Soil Salinization on the Height of C. schmidtii

At the end of the experiment, Significant difference*s* in the height of *C. schmidtii* was identified among the treatments ($F = 15.064$, $p<0.01$; Fig. 2). The height of *C. schmidtii* rapidly increased first, and then reached a stable state in 0 mg/L and 1000 mg/L treatments, while that significantly decreased in 3000 mg/L and 4000 mg/L treatments from the 28th day. The height varied from 24.9 cm to 39.2 cm, peaking in the 1000 mg/L treatment, which had a value 57.59% higher than the lowest value recorded in the 4000 mg/L treatment. Soil salinization affects the growth of *C. schmidtii* by inhibiting the growth rate of plants.

Salinity is common in abiotic factors influencing the growth and development of wetland plants [18]. However, excessive salinity caused soil salinization and then inhibited the growth of wetland plants. The most visual injury of excessive salinity on the *C. schmidii* was plant dwarf, which was closely related to the growth rate of plants. According to Fig. 2, the growth rate of plant height in 3000 mg/L and 4000 mg/L treatments turned to a negative value from the 28th day, and the negative effects of soil salinization on the growth of *C. schmidtii* has become serious over time. This was consistent with other studies that found that growth rate and biomass will be reduced when the plants are faced with high saline alkali stress [23-25]. The salinity threshold of some vegetable crops ranged from about 1 to 2.5 mS/cm and the salt tolerance deceased when the plants were irrigated with saline water [26]. Soil salinization induced by high concentration of ions destroys the absorption function of wetland plants by

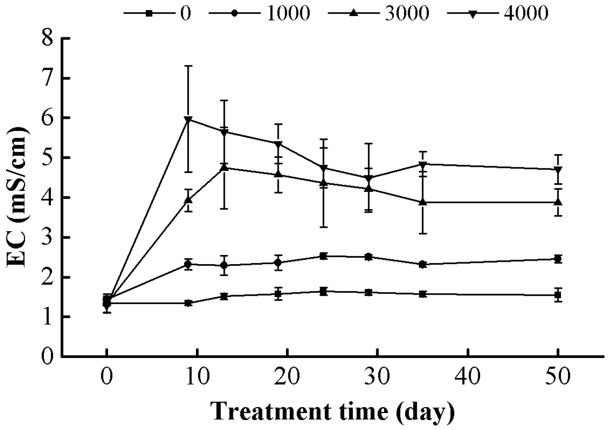

Fig. 1. Variations of soil electrical conductivity (EC) over time (mean±SD, n = 9).

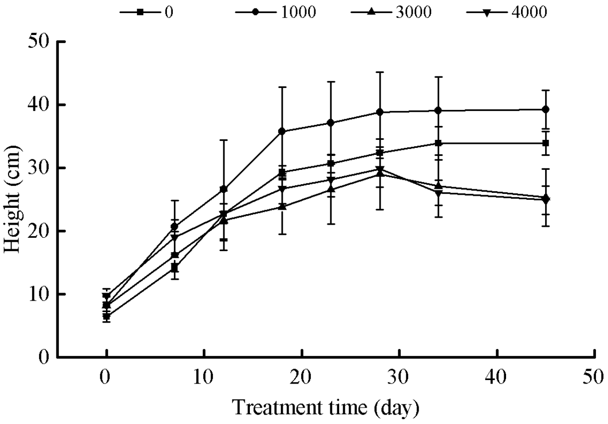

Fig. 2. Variation of the height of *C. schmidtii* over time (mean±SD, n = 9).

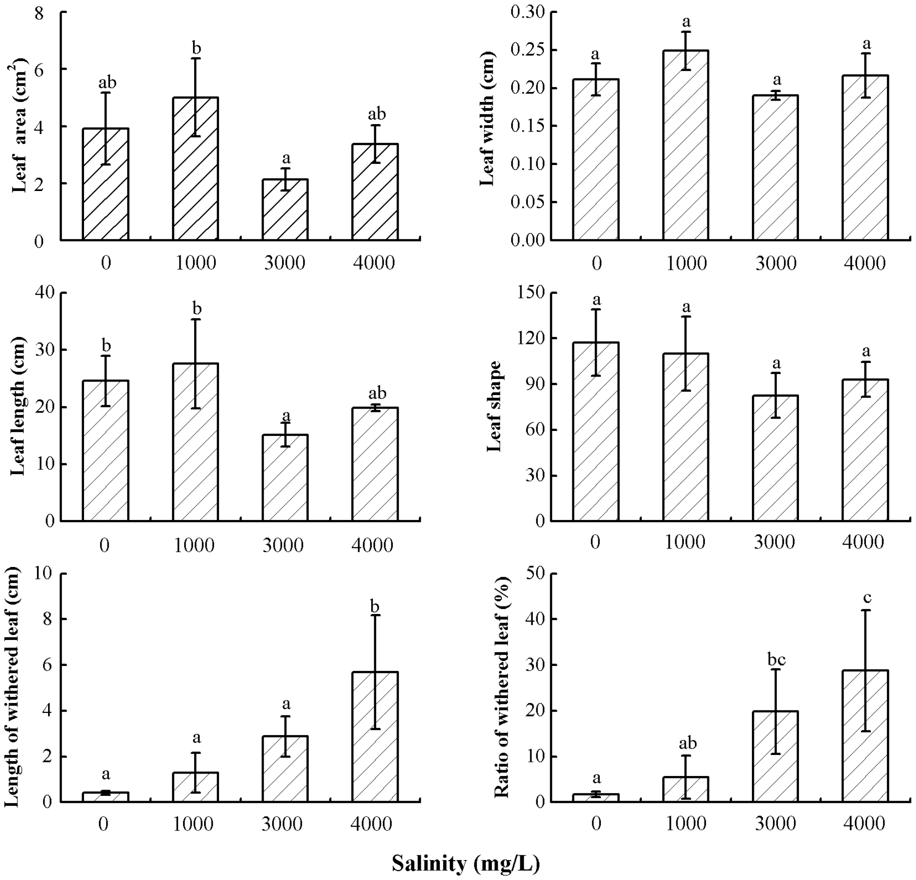

Fig. 3. Effects of soil salinization on leaf morphology of *C. schmidtii* (mean±SD, n = 9).

influencing the osmotic pressure of root cells, resulting in nutrient deficiency for the growth of plants [27]. However, some studies have found that low saline-alkaline stress promoted the growth of wetland plants through enhancing plant absorption of nutrient elements [25, 28].

Effects of Salinization on Leaf Morphology of C. schmidtii

Soil salinization significantly influenced the leaf area (F = 4.244, $p<0.05$) and leaf length (F = 4.200, $p<0.05$) of *C. schmidtii* (Fig. 3). The maximum of leaf area (5.00 cm2), length (27.58 cm) and width (0.25 cm) were all recorded in the 1000 mg/L treatment. Leaf shape, ranging from 82.57 to 117.26 mm, peaked in the 0 mg/L treatment. Additionally, there were significant differences in length of withered leaves (F = 8.271, $p<0.01$) and the ratio of withered leaves (F = 6.733, $p<0.05$). Length of withered leaf and ratio of withered leaf increased with increasing salinity, and reached a peak value in 4000 mg/L. It was noted that the 1000 mg/L treatment also led to the withered leaf of *C. schmidtii.*

Plant leaves, as important organs for photosynthesis, are sensitive to environmental fluctuations. In general, plants adapt to environmental fluctuations by changing leaf morphological and physiological traits [29]. For *C. schmidtii*, soil salinization reduced leaf morphology, both with respect to leaf area for photosynthesis and leaf shape for dimension. This was related to Pan et al., who found that salinity had a negative effect on leaf area and the relative growth rate [30]. Additionally, the length of withered leaves and the ratio of withered leaves indicated that salinization exerted much injury to leaf morphology, affecting elongation and expansion of leaves. Excessive salinity affected the morphological and physiological processes, including plant growth and water and nutrient uptake [31].

Plant Mass and Leaf Mass of C. schmidtii

Table 1 shows no significant differences in plant mass and leaf mass of *C. schmidtii* as identified among treatments. The highest values of plant mass, leaf mass and dry matter content were all observed in 1000 mg/L treatment, while the lowest of those were found in the 3000 mg/L treatment. Specific leaf area, ranging from 14.57 to 17.30 m^2/kg, peaked in the 0 mg/L treatment, which had a value 20.48% higher than that recorded in the 3000 mg/L treatment.

Specific leaf area and dry matter content are vital proxies for the amount of light absorbed and the biomass stored in leaves [32]. High specific leaf area and mass represents a rapid production of biomass. Hayes et al. (2017) found specific leaf area and leaf

Table 1. Effects of salinization on plant mass and leaf mass of *C. schmidtii* (mean±SD, n = 9).

Salinity (mg/L)	Plant			Leaf			
	Fresh mass per plant (g)	Dry mass per plant (g)	Dry matter content per plant (g/g)	Fresh mass per leaf (g)	Dry mass per leaf (g)	Dry matter content per leaf (g/g)	Specific leaf area (m^2/kg)
0	0.2579±0.0318	0.0759±0.0139	0.2931±0.0166	0.0703±0.0150	0.0227±0.0048	0.3261±0.0324	17.3101±2.7614
1000	0.2989±0.0604	0.0931±0.0356	0.3045±0.0527	0.0984±0.0331	0.0355±0.0169	0.3498±0.0529	15.3678±4.1004
3000	0.1946±0.0304	0.0528±0.0069	0.2745±0.0156	0.0518±0.0056	0.0149±0.0016	0.2868±0.0015	14.5712±1.6054
4000	0.2265±0.0857	0.0650±0.0253	0.2855±0.0178	0.0729±0.0185	0.0221±0.0057	0.3022±0.0071	15.7647±2.3284

mass ratio of plans increased when they were faced with environmental press [33]. However, soil salinization due to high saline-alkali press contributed to a moderate SLA and a low dry mass content. The lowest specific leaf area and dry matter content were recorded in 3000 mg/L treatment, indicating a conservation of nutrients in plants. Conversely, 0 mg/L and 1000 mg/L treatments helped in promising the SLA for photosynthesis. Increasing salinity press negatively affected leaf area and dry weight accumulation and then decreased total yield [34].

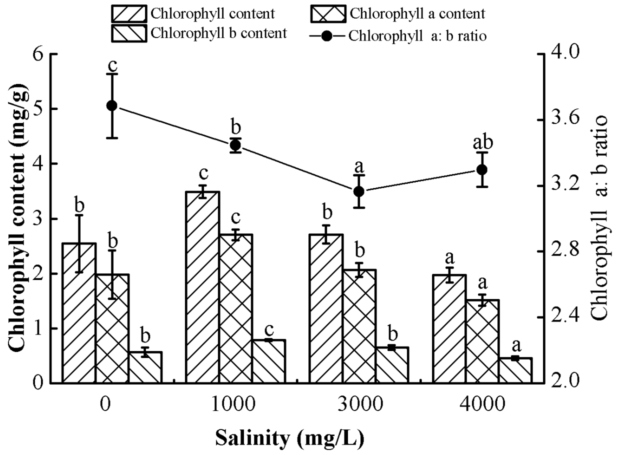

Fig. 4. Effects of soil salinization on chlorophyll content of *C. schmidtii* (mean±SD, n = 9).

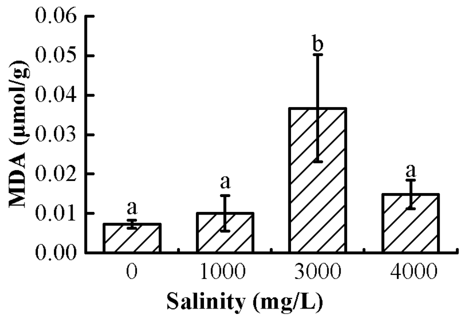

Fig. 5. Effects of soil salinization on malondialdehyde (MDA) content of *C. schmidtii* (mean±SD, n = 9).

Physiological Responses of *C. schmidtii* to Salinization

As shown in Figs 4 and 5, significant differences in chlorophyll (F>9.92, p<0.01) and MDA content (F = 9.76, p<0.01) were found among the treatments. The highest chlorophyll content was recorded in 1000 mg/L treatment (3.49 mg/g), and the lowest was recorded in 4000 mg/L treatment (1.97 mg/g). Moreover, chlorophyll a:b ratio decreased with increasing saline-alkali press. MDA content in 3000 mg/L treatment was 0.04 μmol/g, being 4.99 times greater than the lowest value recorded in 0 mg/L treatment. Soil salinization decreased the photosynthetic pigments. This was consistent with Zhang et al. (2014), who found that Scirpus planiculmis seedlings had low chlorophyll content when the salinity exceeded 3000 mg/L [9]. Salinity affected photosystem II efficiency by reducing leaf area and chlorophyll content [35]. Compared to the 3000 mg/L treatment, MDA has a decrease in 4000 mg/L treatment. Previous studies also found that the overall inhibition of plant metabolism can decrease MDA in plants.

Conclusions

Soil salinization negatively affects the growth and physiological traits of *C. schmidtii*. Soil EC significantly increased with increasing salinity concentration from the initiation time of the experiment. Soil salinization seriously reduced plant height, leaf area and length, and also led to significant leaf withering. However, slight salinization (1000 mg/L) improved the plant mass and leaf mass with a high biomass accumulation. Additionally, chlorophyll and MDA concentration indicated that 0 and 1000 mg/L treatment exerted less injury to the leaves of *C. schmidtii*. Therefore, the optimum ecological threshold of salinity for the growth of *C. schmidtii* was in the range 0~1000 mg/L. However, serious soil salinization inhibited the growth of *C. schmidtii* and resulted in the degradation of tussock wetlands. Preventing soil salinization will be an efficient method for protecting and restoring tussock sedge wetlands in the MNNR.

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Conflict of Interest

The authors have not declared any conflict of interest.

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