AN EXPERIMENTAL STUDY ON THE GROWTH AND PHYSIOLOGICAL RESPONSES OF *CAREX SCHMIDTII* TO EXCESS NITROGEN AND PHOSPHORUS

ZHANG, D. J.* – LIU, X. P. – TIAN, J. P. – WANG, Z. – WEI, Y. X. – WANG, H. – SHI, X. X. – HE, W. J.

Shandong Key Laboratory of Eco-Environmental Science for the Yellow River Delta, Binzhou University, Binzhou 256600, Shandong Province, China

**Corresponding author*
e-mail: zhangdongjie14@126.com

(Received 19th Jun 2023; accepted 11th Aug 2023)

Abstract. Wastewater from agricultural source carries excess nitrogen and phosphorous into wetlands and causes eutrophication in waterbody. Enrichment of nitrogen and phosphorous affects the natural nutrient removal and plant growth in wetlands. We conducted a greenhouse experience to explore the effects of excess N and P on the growth of *Carex schmidtii*, so as to provide evidence for wetland phytoremediation. The results showed that high concentration of N had negative effects on the plant height and growth rate of *C. schmidtii*. Excess N and P significantly reduced the leaf morphology and mass per plant or leaf but increased the length and the ratio of withered leaf. Furthermore, the chlorophyll content and lid peroxidation indicated that high concentrations of N and P exerted much injury to the blades of *C. schmidtii*. These results help in understanding the responses of *C. schmidtii* to agricultural wastewater and suggest the limited capacity of *C. schmidtii* tussock for the phytoremediation of agricultural wastewater in wetlands.
Keywords: *nutrient enrichment, sedge, tussock wetland, wetland phytoremediation, Momoge wetland*

Introduction

Anthropogenic nitrogen and phosphorous, primarily in agricultural wastewater, are the main causes of nutrient enrichment in wetland (Carpenter et al., 1998; Verhoeven et al., 2006; Chen et al., 2017). For the purification, wetland vegetation has been used successfully in nutrient removal and wetland restoration (Yang et al., 2017). However, excess nitrogen and phosphorous will still affect the natural removal efficiency for nitrogen and phosphorous, and cause eutrophication of wetlands (Tanner et al., 1995; Finlay et al., 2013). Furthermore, high concentrations of nitrogen and phosphorous change the characteristics and blades function in plant, and thereby affect plant growth in wetlands (Liao et al., 2017; Liu et al., 2017). Therefore, exploration of the responses of wetland plants to agricultural wastewater is urgently needed.

Momoge wetland is an internationally important wetland that suffered intensive anthropogenic disturbance, especially agricultural activities (Wang et al., 2005; Zhang et al., 2019). As a major wetland water source, agricultural wastewater brought excess nitrogen and phosphorous into wetlands. Unfortunately, long-term drought exacerbated the enrichment of nitrogen and phosphorus in wetlands. According to investigation, TN was about 6.95 mg/L, and some region even reached 9.85 mg/L in waterbody. Kou (2012) reported that TN increased by 1.78 times from the wetland entrance (2.57 mg/L) to outlet (4.57 mg/L), and TP also showed a slight increase. The environmental problems drawn from enrichment of nitrogen and phosphorous have been gradually recognized.

Tussock sedge meadow is a typical wetland ecosystem in Momoge wetland, which is widespread in the riparian along the Nenjiang River (Pan et al., 2006; Zhang et al., 2021).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

Its abundant root system and biodiversity provide advantages for nutrient uptake and utilization (Costello, 1936; Lawrence et al., 2011, 2013a; Zhang et al., 2022). Simultaneously, the microtopography created by tussock help in increasing the surface area for element absorption and preventing the nutrient loss with water flow (Opdekamp et al., 2012; Lawrence et al., 2013b). Moreover, tussock wetland has an excellent removal capacity for nitrogen and phosphorus (Bernard et al., 1988; Silvan, 2004). For these advantages, tussock was recognized as a promising candidate for agricultural wastewater purification in wetlands. However, the responses of *Carex schmidtii* tussock to high concentrations of nitrogen and phosphorus are still unknown.

Considering the field investigation, we conducted a greenhouse experiment to investigate the height, biomass as well as morphological and physiological responses of leaves in *C. schmidtii* to excess nitrogen and phosphorous in wetland. The purpose of this study was to elucidate the effects of high concentrations of nitrogen and phosphorus from agricultural wastewater on plant growth and physiological characteristics of *C. schmidtii* and attempt to evaluate the feasibility of tussocks in phytoremediation of wetland with agricultural wastewater.

Materials and Methods

Study area

Momoge wetland is located in the Western Jilin province, Northeast China (45°42′-46°.18′N, 122°27′-124°04′E) and covers a total area of 1.44×10^3 km^2. The area is characterized by a semi-arid continental climate with a mean annual temperature of 4.2°C. The mean annual precipitation is approximately 412 mm, with 70-80% focusing on July and August. Typical marsh plants along the Nenjiang River are *C. schmidtii*, *Deyeuxia purpurea* and *Phragmites australis* (Zhang et al., 2019, 2021).

Plant cultivation

In November 2016, *C. schmidtii* tussocks were collected from the riparian wetland along Nenjiang River, Northeast China (45°53′51.97″N, 123°55′58.57″E). Tussocks without above-ground biomass were taken to a greenhouse at the Northeast Institute of Geography and Agroecology, Chinese Academy of Science. The tussocks were cut into uniform size (20 cm in height and 15 cm in diameter, named small tussock), and then planted in plastic buckets (35 cm in height and 30 cm in diameter, one tussock per bucket). Each bucket was filled with soil to keep half of small tussock below the soil surface and half above the soil surface. Twelve plastic buckets were randomly placed in greenhouse and moved every week. Planting tussocks were irrigated by tap water. Soil and water properties used in the experiment were shown in *Table S1*. The temperature of air, soil and water during experiment could be found in *Fig. S1*.

Experiment design and laboratory analyses

Experiment was conducted at the fifth day after planting. Considering the purification of tussock wetlands, the solutions containing 5 mg P/L (P treatment, supplied with KH_2PO_4), 10 mg N/L (N treatment, supplied with NH_4NO_3), 5 mg P/L +10 mg N/L (N+P treatment, supplied with KH_2PO_4 and NH_4NO_3) were watered into planting tussocks, respectively. Control treatment (CK) was irrigated by tap water. Each treatment was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

replicated three times. In the following days, we supply 500 ml tap water for all treatments on the twentieth day.

Height of seeding was measured every six days. Absolute growth rate (AGR) and relative growth rate (RGR) of seeding height were calculated according to the formulas: $AGR=(H_{i+1}-H_i)/(T_{i+1}-T_i)$, $RGR=[ln(H_{i+1})-ln(H_i)]/ (T_{i+1}-T_i)$, where H_{i+1} and H_i were the seeding height of *C. schmidtii* in the date T_{i+1} and T_i, respectively (Ramírez-Valiente et al., 2017). At the end of experiment, leaf area, length, width, leaf shape (the ratio of length to width), the length of withered leaf (LWL) and the ratio of withered leaf (RWL, the ratio of withered leaf length to leaf length) were measured. Plant and leaves were harvested to obtain fresh mass per plant, dry mass per plant, fresh mass per leaf, dry mass per leaf. Dry-fresh mass ratio per plant, dry-fresh mass ratio per leaf and specific leaf area were estimated to indicate blade function. Part of fresh samples was used to determine chlorophyll content and lid peroxidation. The averaged attribute values of ecological indicators in each bucket were used for final analysis.

Chlorophyll content was determined according to Zhang (1986). Fresh leaves (0.2 g) were soaked in 10 mL of ethanol acetone (1:1) for 2 days in 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). Malondialdehyde (MDA) was measured with thiobarbituric acid to estimate lipid peroxidation (Schmedes and Hølmer, 1989).

Statistical analysis

Date analyses were performed in SPSS20.0 and Origin9.3. One-way ANOVA was used to test the effects of excess nitrogen and phosphorus on the growth and physiological characteristics of *C. schmidtii*. Differences were compared by Duncan’s test at $p<0.05$.

Results

Effects on plant height and growth rate

During 12^{th} day to 50^{th} day, plant height was significantly affected by high concentrations of nitrogen and phosphorus in farmland water (*Table 1 and Fig. 1*). Seeding height with treatments by CK and P increased over time, while N and N+P had a negative effect on early growth of *C. schmidtii* tussocks. Moreover, high concentrations of nitrogen and phosphorus have significant effects on the AGR and RGR of *C. schmidtii* during early growth stage (5^{th} day to 23^{rd} day) and 33^{rd} day to 39^{th} day. Both AGR and RGR curves under CK and P decreased and then remained stable as *C. schmidtii* grew. Specifically, the AGR and RGR with treatment N+P had no obvious change until the end of the experiment.

Leaf morphology under different treatments

Results showed as *Fig. 2*, excess nitrogen and phosphorus had significant effects on leaf area, length, and shape of *C. schmidtii* tussock, but not on leaf width. The maximum of leaf area (3.91 cm^2), length (24.55 cm) and leaf shape (99.43) were found in CK treatment. However, the negative effects of N+P on leaf morphology were notable.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

Table 1. *Results (F values) of one-way ANOVA about the effects of excess nitrogen and phosphorus on the growth and physiological traits of Carex schmidtii*

Traits	**5th day**	**12th day**	**17th day**	**23rd day**	**28th day**	**33rd day**	**39th day**	**50th day**
Plant height	0.51	36.02***	73.57***	119.37***	36.68***	239.60***	156.63***	436.01***
Traits	5-12th day	12-17th day	17-23rd day	23-28th day	28-33rd day	33-39th day	39-50th day	
Absolute growth rate	29.72***	26.29***	11.41***	0.85	0.64	8.01***	1.16	
Relative growth rate	13.56***	12.15***	4.65***	0.52	0.04	28.11***	2.14	
Traits	F	Traits	F	Traits	F	Traits	F	
Leaf area	11.88**	The ratio of withered leaf	291.00***	Specific leaf area	7.12*	chlorophyll b content	44.71***	
Leaf length	29.12***	Fresh mass per plant	14.06**	Plant dry matter content	1.81	chlorophyll a:b	2.70	
Leaf width	3.44	Dry mass per plant	19.83**	Leaf dry matter content	0.54	MDA	3.38	
Leaf shape	15.05**	Fresh mass per leaf	12.36**	Chlorophyll content	60.38***			
The length of withered leaf	52.70***	Dry mass per leaf	16.87**	Chlorophyll a content	43.52***			

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

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

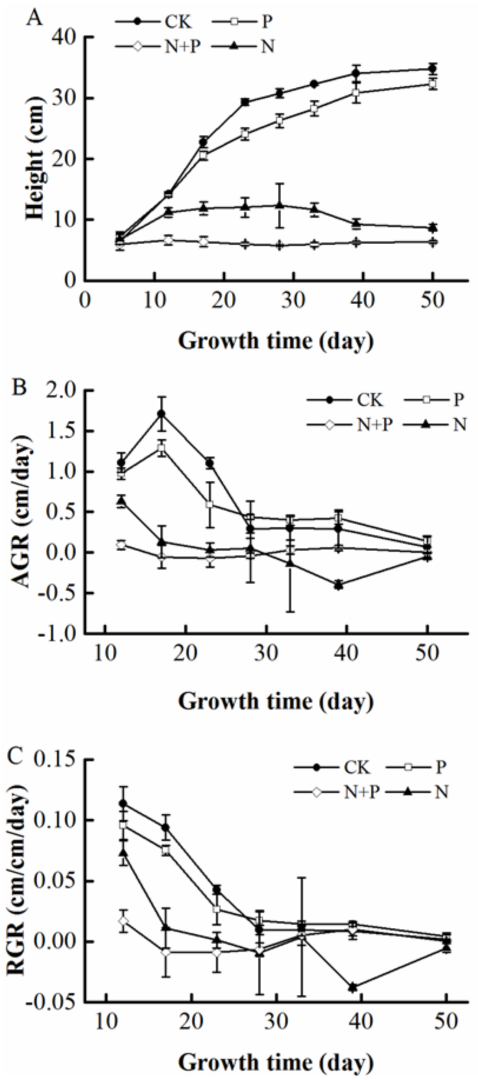

Figure 1. *Plant height, AGR and RGR of Carex schmidtii tussock under different treatments*

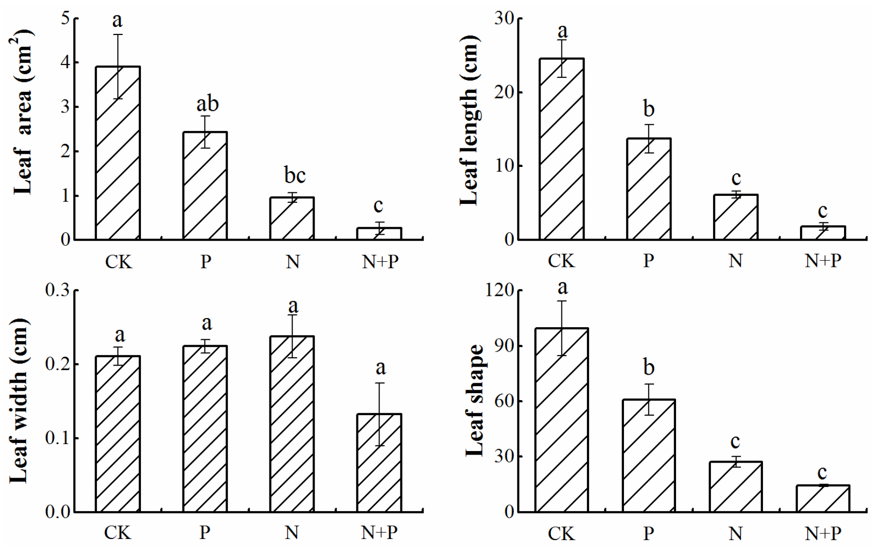

Figure 2. *Leaf morphology of Carex schmidtii tussock under different treatments*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

The length of withered leaf (LWL) and the ratio of withered leaf (RWL) responded to enrichment of nitrogen and phosphorus (*Fig. 3*). All plants treated with N and P showed significant difference from CK. The minimums of LWL and RWL of *C. schmidtii* were found in CK. Although the LWL of N treatment was longer than that in N+P treatment, no significant differences in RWL were recorded between N+P treatment and N treatment.

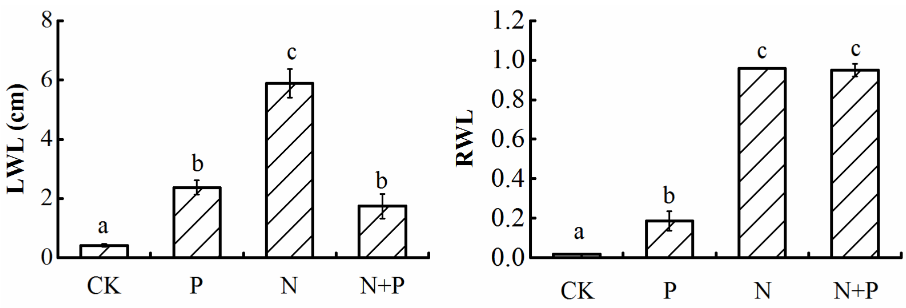

Figure 3. *The length of withered leaf (LLW) and the ratio of withered leaf (RWL) of Carex schmidtii tussock under different treatments*

Plant mass and leaf mass of Carex schmidtii under different treatments

Nitrogen and phosphorus treatments have significant effects on the fresh and dry mass per plant/leaf (*Table 2*). Dry mass per plant and dry mass per leaf decreased followed order: CK> P> N or N+P. Specific leaf area with treatment by N+P showed significant difference from CK treatment and P treatment. However, dry matter content of plant and dry matter content of leaf had no significant difference among the four treatments (*Fig. 4*).

Table 2. *Mass per plant/leaf and specific leaf area under different treatments*

Treatments	Plant		Leaf		
	Fresh mass per plant (g)	**Dry mass per plant (g)**	**Fresh mass per leaf (g)**	**Dry mass per leaf (g)**	**Specific leaf area (m^2/kg)**
CK	0.2579 ± 0.0184 a	0.0759 ± 0.0080 a	0.0703 ± 0.0087 a	0.0227 ± 0.0028 a	17.782 ± 1.908 ab
P	0.1757 ± 0.0183 b	0.0500 ± 0.0023 b	0.0459 ± 0.0047 b	0.0130 ± 0.0006 b	20.405 ± 3.615 a
N	0.0995 ± 0.0179 c	0.0276 ± 0.0052 c	0.0288 ± 0.0027 bc	0.0107 ± 0.0004 bc	10.569 ± 2.080 bc
N+P	0.0979 ± 0.0317 c	0.0211 ± 0.0023 c	0.0245 ± 0.0047 c	0.0066 ± 0.0008 c	3.728 ± 2.102 c

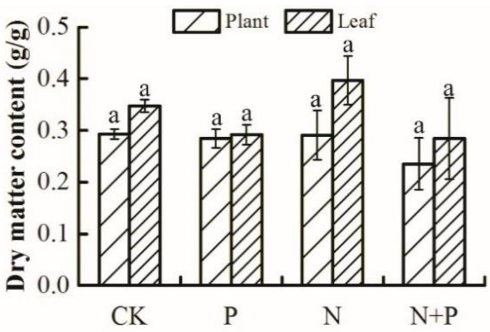

Figure 4. *Dry matter content of Carex schmidtii tussock under different treatments*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

Chlorophyll content and lid peroxidation

There were significant differences in chlorophyll content, chlorophyll a content, chlorophyll b content among the four treatments (*Fig. 5*). The value of chlorophyll content, chlorophyll a content, chlorophyll b content in CK treatment and P treatment were higher than other two treatments. The lowest value of chlorophyll content was recorded in N+P treatment, being similar to that in N treatment. Chlorophyll a: b ratio had no significant difference among the treatments and varied from 2.28 to 3.76. The responses of malondialdehyde (MDA) to the four treatments were not significant. The value of MDA recorded in P treatment was 0.04 μmol/g.

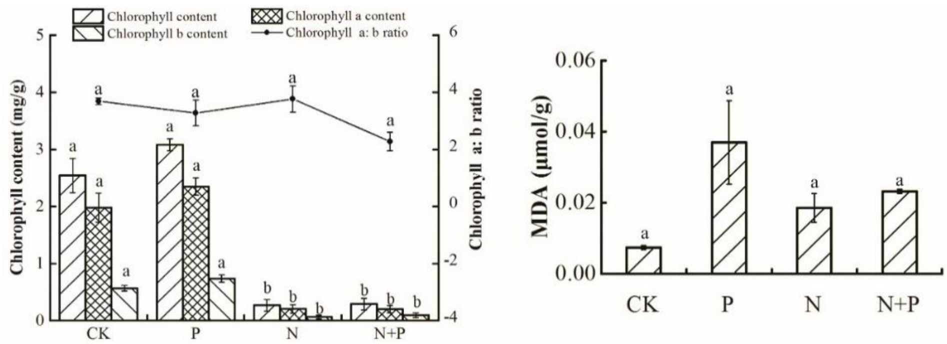

Figure 5. *Chlorophyll content in leaves of Carex schmidtii tussock under different treatments*

Discussion

Reduction of plant size

Nitrogen and phosphorus are key nutrients influencing plant growth and development (Aerts and Chapin, 1999; Richardson et al., 2009). However, excess nutrients from agricultural wastewater caused eutrophication in waterbody and lead to inhibiting the growth of wetland species (Cao et al., 2010; Chen et al., 2015). In our study, excess N and P resulted in plant dwarf of *C. schmidii*, which was closely related to plant growth rate. In a comparison of AGR and RGR of seeding height among the four treatments, high concentrations of N and P, particularly a combination of them, had negative effects on *C. schmidii*. According to *Fig. 1*, the seeding under N+P treatment was faced with harsh habitats at the beginning of experiment. Compared to CK, the N treatment gradually has a negative effect on plant growth, which is much more serious than P treatment over time. Consistently with other studies, plant growth rate and biomass of several species reduced when plants were feed with excess N (Clarke and Baldwin, 2002; Chen et al., 2004; Saunkaew et al., 2011; Jampeetong et al., 2012). Additionally, high concentration of N destroyed the absorption function of plant by influencing the osmotic pressure of root cells, and then contributed to the deficiency of nutrient from soil (González et al., 1968; Ranathunge et al., 2016). However, the slow release of P survived the root of seeding from dehydration and ensured adequate nutrients for growth.

Leaf morphological traits

Leaves are important organs for photosynthesis, which are sensitive to environmental change (Evans and Poorter, 2001; Xu et al., 2009). As responses to abiotic stress, the phenotypic plasticity of plant blades indicates the adaptability to environmental variation

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

(Kessler and Sinha, 2004; Barkoulas et al., 2007). In general, plants alter their leaf morphological and physiological traits for the survival and growth (Guo et al., 2017). For the blades of *C. schmidtii* tussocks, there was a significant reduction of leaf morphology under excess nitrogen and phosphorus from agricultural wastewater, both with respect to leaf area for photosynthesis and leaf shape for leaf dimension. The length of withered leaf (LWL) and the ratio of withered leaf (RWL) indicated that excess nitrogen and phosphorus exerted much injury to the leaf morphology, affecting leaf elongation and expansion of leaf area.

Specific leaf area (SLA), mass per leaf and mass per plant are important proxies for the amount of light absorbed and the biomass stored in leaf (Wilson et al., 1999; Dawson et al., 2017). High SLA and mass per leaf (or plant) represent a rapid production of biomass (Garnier et al., 2001). For example, Hayes et al. (2017) found SLA and leaf mass ratio of plans increased in high nutrient treatment. However, in our study, nutrient enrichment from N was responsible for the decrease of SLA and mass per leaf (or plant). A low SLA in N and N+P treatment indicated a conservation of nutrients in plant under environmental press (Poorter, 1999). Conversely, excess P helped in promising the SLA for photosynthesis. For the dry matter content, there were no significant differences among the four treatments in spite of the rapid growth in CK and P treatment. Considering the dying state of plants under N and N+P treatment in the end of experiment, we can’t help but early growth contributes to their high dry matter content.

Monitoring chlorophyll content of *C. schmidtii* tussock at different nutrient treatments found that excess P contributed to high concentration of photosynthetic pigments, though there were no significant differences with CK treatment (*Fig. 5*). Jeppesen et al. (2005) found chlorophyll a content in lakes decreased with the reduction of external TP loading. However, enrichment of N from agricultural wastewater led to a drop of chlorophyll content due to dying state of *C. schmidtii* tussock. This was consistent with Pincam (2017) who found low pigment concentrations of Hybrid Napier grass at high N concentrations in a flooding environment, but the study by Hu et al. (2008) who reported *Ipomoea aquatical* had increased chlorophyll a content in leaves when it was cultivated with enrichment of N and P. Responses of photosynthetic pigments in plants to excess nutrient varied from different species, but long-term exposure to high concentration of N was toxic to plants (Clemens, 2006; Collos and Harrison, 2014). It was inevitable that the lowest value of MDA was recorded in CK treatment, suggesting that plants suffered less injury. Nevertheless, the MDA peaked in P treatment. Previous studies found that MDA concentration decreased under environmental press due to an overall inhabitation of plant metabolism (Gossett et al., 1994; Luna et al., 2000).

Conclusions

Agricultural wastewater has a negatively effects on the growth of *Carex schmidtii* tussock. Excess N decreased the AGR and RGR of seeding, and reduced the leaf morphology, mass per plant (or leaf) of *C. schmidtii*. Comparing to P treatment, chlorophyll content of *C. schmidii* under different treatments indicated that N exerted much injury to the growth of *C. schmidii* tussock. The inhibition of plant growth of *C. schmidii* tussock due to agricultural wastewater will affect the structure and function of Momoge wetland. Further field studies about *C. schmidii* tussocks will help in understanding the responses of *C. schmidii* to agricultural wastewater.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

Acknowledgements. This research was supported by the Shandong Provincial Natural Science Foundation (No. ZR2021QD101); the PhD research startup foundation of Binzhou University (No. 2021Y14); and College Student Innovation Training Program Plan (S202210449026; X2023104491102).

REFERENCES

[1] Aerts, R., Chapin, F. S. (1999): The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. – Advances in Ecological Research 30: 1-67.

[2] Barkoulas, M., Galinha, C., Grigg, S. P., Tsiantis, M. (2007): From genes to shape: regulatory interactions in leaf development. – Current opinion in plant biology 10: 660-666.

[3] Bernard, J. M., Solander, D., Květ, J. (1988): Production and nutrient dynamics in Carex wetlands. – Aquatic Botany 30: 125-147.

[4] Cao, H., Ge, Y., Liu, D., Cao, Q., Chang, S. X., Chang, J., Song, X. L., Lin, X. (2010): Nitrate/ammonium ratios affect ryegrass growth and nitrogen accumulation in a hydroponic system. – Journal of Plant Nutrition 34: 206-216.

[5] Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., Smith, V. H. (1998): Nonpoint pollution of surface waters with phosphorus and nitrogen. – Ecological Applications 8: 559-568.

[6] Chen, B. M., Wang, Z. H., Li, S. X., Wang, G. X., Song, H. X., Wang, X. N. (2004): Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. – Plant Science 167: 635-643.

[7] Chen, B. Q., Liu, D., Han, W. J., Fan, X., Cao, H. Q., Jiang, Q. S., Liu, Y., Chang, J., Ge, Y. (2015): Nitrogen-removal ability and niche of Coix lacryma-jobi and Reineckia carnea in response to NO_3^-/NH_4^+ ratio. – Aquatic Botany 120: 193-200.

[8] Chen, B., Chang, S. X., Lam, S. K., Erisman, J. W., Gu, B. (2017): Land use mediates riverine nitrogen export under the dominant influence of human activities. – Environmental Research Letters 12: 094018.

[9] Clarke, E., Baldwin, A. H. (2002): Responses of wetland plants to ammonia and water level. – Ecological Engineering 18: 257-264.

[10] Clemens, S. (2006): Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. – Biochimie 88: 1707-1719.

[11] Collos, Y., Harrison, P. J. (2014): Acclimation and toxicity of high ammonium concentrations to unicellular algae. – Marine Pollution Bulletin 80: 8-23.

[12] Costello, D. F. (1936): Tussock meadows in southeastern Wisconsin. – Botanical Gazette 97: 610-648.

[13] Dawson, S. K., Warton, D. I., Kingsford, R. T., Berney, P., Keith, D. A., Catford, J. A. (2017): Plant traits of propagule banks and standing vegetation reveal flooding alleviates impacts of agriculture on wetland restoration. – Journal of Applied Ecology 54(6): 1907-1918.

[14] Evans, J., Poorter, H. (2001): Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. – Plant, Cell & Environment 24: 755-767.

[15] Finlay, J. C., Small, G. E., Sterner, R. W. (2013): Human influences on nitrogen removal in lakes. – Science 342: 247-250.

[16] 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 15: 688-695.

[17] González-Bernáldez, F., López-Sáez, J. F., Garcia-Ferrero, G. (1968): Effect of osmotic pressure on root growth, cell cycle and cell elongation. – Protoplasma 65: 255-262.

[18] Gossett, D. R., Millhollon, E. P., Lucas, M. (1994): Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. – Crop Science 34: 706-714.

[19] Guo, C., Ma, L., Yuan, S., Wang, R. (2017): Morphological, physiological, and anatomical traits of plant functional types in temperate grasslands along a large-scale aridity gradient in northeastern China. – Scientific reports 7.
[20] Hayes, M. A., Jesse, A., Tabet, B., Reef, R., Keuskamp, J. A., Lovelock, C. E. (2017): The contrasting effects of nutrient enrichment on growth, biomass allocation and decomposition of plant tissue in coastal wetlands. – Plant and Soil 416: 193-204.
[21] Hu, M. H., Ao, Y. S., Yang, X. E., Li, T. Q. (2008): Treating eutrophic water for nutrient reduction using an aquatic macrophyte (Ipomoea aquatica Forsskal) in a deep flow technique system. – Agricultural Water Management 95: 607-615.
[22] Jampeetong, A., Brix, H., Kantawanichkul, S. (2012): Response of *Salvinia cucullata* to high NH_4^+ concentrations at laboratory scales. – Ecotoxicology and Environmental Safety 79: 69-74.
[23] Jeppesen, E., Søndergaard, M., Jensen, J. P., Havens, K. E., Anneville, O., Carvalho, L., Coveney, M., Deneke, R., Dokulil, M., Foy, B., Gerdeaux, D., Hampton, S. E., Hilt, S., Kangur, K., Köhler, J., Lammens, E. H. H. R., Lauridsen, T. L., Manca, M., Miracle, M. R., Moss, B., Nõges, P., Persson, G., Phillips, G., Portielje, R., Romo, S., Schelske, C. L., Straile, D., Tatrai, I., Willén, E., Winder, M. (2005): Lake responses to reduced nutrient loading–an analysis of contemporary long-term data from 35 case studies. – Freshwater Biology 50: 1747-1771.
[24] Kessler, S., Sinha, N. (2004): Shaping up: the genetic control of leaf shape. – Current opinion in plant biology 7: 65-72.
[25] Kou, Y. Q. (2012): Urification of farmland back water in Jilin western wetland-A case of Momoge National Nature Reserve. – Dissertation, Northeast Normal University. (in Chinese).
[26] Lawrence, B. A., Zedler, J. B. (2011): Formation of tussocks by sedges: effects of hydroperiod and nutrients. – Ecological Applications 21: 1745-1759.
[27] Lawrence, B. A., Fahey, T. J., Zedler, J. B. (2013a): Root dynamics of Carex stricta-dominated tussock meadows. – Plant and Soil 364: 325-339.
[28] Lawrence, B. A., Jackson, R. D., Kucharik, C. J. (2013b): Testing the stability of carbon pools stored in tussock sedge meadows. – Applied Soil Ecology 71: 48-57.
[29] Liao, J., Zhang, D., Mallik, A., Huang, Y., He, C., Xu, G. (2017): Growth and nutrient removal of three macrophytes in response to concentrations and ratios of N and P. – International journal of phytoremediation 19: 651-657.
[30] Liu, C., Dai, Z., Sun, H. (2017): Potential of duckweed (Lemna minor) for removal of nitrogen and phosphorus from water under salt stress. – Journal of Environmental Management 187: 497-503.
[31] Luna, C., Garcia-Seffino, L., Arias, C., Taleisnik, E. (2000): Oxidative stress indicators as selection tools for salt tolerance. – Plant Breeding 119: 341-345.
[32] Opdekamp, W., Teuchies, J., Vrebos, D., Chormański, J., Schoelynck, J., Van Diggelen, R., Meire, M., Struyf, E. (2012): Tussocks: biogenic silica hot spots in a riparian wetland. – Wetlands 32: 1115-1124.
[33] Pan, X. L., Zhang, D. Y., Quan, L. (2006): Interactive factors leading to dying-off Carex tato in Momoge wetland polluted by crude oil, Western Jilin, China. – Chemosphere 65: 1772-1777.
[34] Pincam, T., Brix, H., Eller, F., Jampeetong, A. (2017): Hybrid Napier grass as a candidate species for bioenergy in plant-based water treatment systems: Interactive effects of nitrogen and water depth. – Aquatic Botany 138: 82-91.
[35] Poorter, H., De Jong, R. O. B. (1999): A comparison of specific leaf area, chemical composition, and leaf construction costs of field plants from 15 habitats differing in productivity. – The New Phytologist 143: 163-176.
[36] Ramírez-Valiente, J. A., Center, A., Sparks, J. P., Sparks, K. L., Etterson, J. R., Longwell, T., Pilz, G., Cavender-Bares, J. (2017): Population-level differentiation in growth rates and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118

leaf traits in seedlings of the neotropical live oak Quercus oleoides grown under natural and manipulated precipitation regimes. – Frontiers in plant science 8.
[37] Ranathunge, K., Schreiber, L., Bi, Y. M., Rothstein, S. J. (2016): Ammonium-induced architectural and anatomical changes with altered suberin and lignin levels significantly change water and solute permeabilities of rice (*Oryza sativa* L.) roots. – Planta 243(1): 231-249.
[38] Richardson, A. E., Barea, J. M., McNeill, A. M., Prigent-Combaret, C. (2009): Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. – Plant and Soil 321: 305-339.
[39] Saunkaew, P., Wangpakapattanawong, P., Jampeetong, A. (2011): Growth, morphology, ammonium uptake and nutrient allocation of Myriophyllum brasiliense Cambess. under high NH_4^+ concentrations. – Ecotoxicology 20: 2011-2018.
[40] Schmedes, A., Hølmer, G. (1989): A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. – Journal of the American oil chemists' society 66: 813-817.
[41] Silvan, N. (2004): Nutrient retention in a restored peatland buffer. – HELDA.
[42] Tanner, C. C., Clayton, J. S., Upsdell, M. P. (1995): Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands-II. Removal of nitrogen and phosphorus. – Water Research 29: 27-34.
[43] Verhoeven, J. T., Arheimer, B., Yin, C., Hefting, M. M. (2006): Regional and global concerns over wetlands and water quality. – Trends in Ecology & Evolution 21: 96-103.
[44] Wang, Z. Q., Zhang, B., Yang, G., Wang, Z. M., Zhang, S. Q. (2005): Responses of wetland eco-security to land use change in Western Jilin Province, China. – Chinese geographical science 15: 330-336.
[45] Wilson, P. J., Thompson, K. E. N., Hodgson, J. G. (1999): Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. – The New Phytologist 143: 155-162.
[46] Xu, F., Guo, W., Xu, W., Wei, Y., Wang, R. (2009): Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves? – Progress in Natural Science 19: 1789-1798.
[47] Yang, T. X., Sheng, L. X., Zhuang, J., Lv, X. G., Cai, Y. P. (2017): Function, restoration, and ecosystem services of riverine wetlands in the temperate zone. – Ecological Engineering 137: 46-52.
[48] Zhang, X. Z. (1986): Determination of chlorophyll content in plant by acetone ethanol mixture method. – Liaoning Agricultural Sciences 3: 26-28. (in Chinese).
[49] Zhang, D., Qi, Q., Tong, S., Wang, X., An, Y., Zhang, M., Lu, X. (2019): Soil degradation effects on plant diversity and nutrient in tussock meadow wetlands. – Journal of Soil Science and Plant Nutrition 19: 535-544.
[50] Zhang, D., Sun, J., Cui, Q., Jia, X., Qi, Q., Wang, X., Tong, S. (2021): Plant growth and diversity performance after restoration in *Carex schmidtii* tussock wetlands, Northeast China. – Community Ecology 22(3): 391-401.
[51] Zhang, D. J., Xia, J. B., Sun, J. K., Ma, J. Z., Zhao, D. D., Duan, D. X., Wang, X. H., Tong, S. Z. (2022): Nutrient responses of *Carex schmidtii* to consecutive drought and re-flooding episodes in semi-aried wetlands: Implications for tussock restoration. – Applied Ecology and Environmental Research 20(5): 4039-4060.

APPENDIX

Table S1. *Soil and Water Properties*

Soil properties		Water properties	
pH	6.50	pH	7.28
BD (g/cm^3)	95.44	TDS (g/L)	0.46
EC (ms/cm)	100.25	EC (ms/cm)	0.61
OM (%)	3.07	Sal (‰)	0.35
TN (g/kg)	1.63	Do (mg/L)	10.26
TP (g/kg)	0.45	TN (mg/L)	0.46
δ^{13}C (‰)	-26.72	TP (mg/L)	0.02

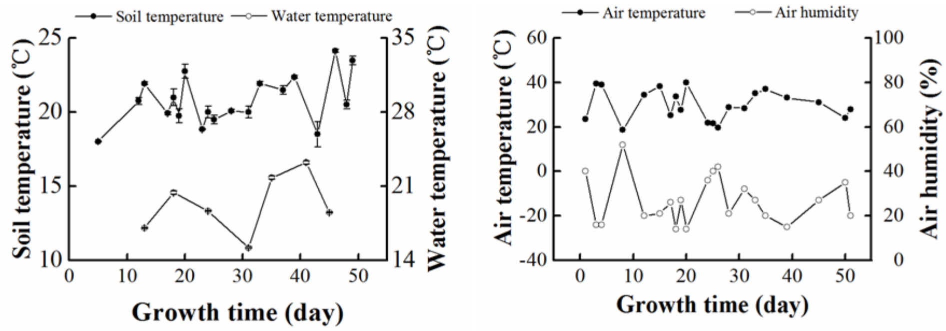

Figure S1. *Air, soil, and water temperature during experiment*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_51075118