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Effect of Nitrogen and Phosphorus on Tissue Nutrition and Biomass of Freshwater Wetland Plant in Sanjiang Plain, Northeast China

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Abstract: Nitrogen (4, 10, 20 and 40g/m^2) and phosphorus (1.2, 4.8 and 9.6g/m^2) were applied to tanks to evaluating the effects of N and P additions on plant tissue nutrition and the biomass of two freshwater wetland plants in the Sanjiang Plain of Northeast China, namely *Carex lasiocarpa* and *Carex meyeriana*. For *C. lasiocarpa*, the total N concentration (TN) of plant tissues under the treatment of 10g/m^2 was lower compared with the other N treatments. Initially, *C. lasiocarpa* exhibited a significant increase of biomass as compared with the control value, reaching the maximum of 31.20±4.01g/tank under the treatment of 10g/m^2, and then dropped to 18.02±1.53g/tank under the treatment of 40g/m^2. For *C. meyeriana*, TN generally increased with increasing amount of N applied. High N applied produced more aboveground biomass than low N applied. *C. meyeriana*, as the accompanying species, can adapt itself to the wetland enriched by N, and it may replace *C. lasiocarpa* as the dominant species of wetland. The total P concentration (TP) in tissues of *C. lasiocarpa* increased with P addition. The aboveground biomass of *C. lasiocarpa* increased with P addition, and it changed from 18.77±3.29g/tank to 46.03±3.95g/tank. However, TP of tissue may accelerate the *C. meyeriana* development under the treatment of 1.2g/m^2. P accumulation contributes to the dominance of *C. lasiocarpa* but limits the production of *C. meyeriana*, and the latter may disappear gradually from the wetland enriched by P. Increased input of N and P might have an influence on wetland plant community composition and structure, so the effects of nutrient inputs and accumulation should be considered to protect the freshwater wetland.

Keywords: freshwater wetland; nitrogen; phosphorus; *Carex lasiocarpa*; *Carex meyeriana*; Sanjiang Plain

1 Introduction

It has been reported that habitat nutrient availability frequently limited plant growth and determined species dominance and abundance in natural communities (Miao et al., 2000). Nutrient availability is also a main regulator of aquatic primary production. Human-induced nutrient enrichment results in die-back of native vegetation and alteration of species dominance in various aquatic ecosystems (Miao et al., 2000; Green and Galatowitsch, 2002). Particularly, nutrient enrichment may adversely impact plant species richness in wetlands and enhance their susceptibility to colonization and dominance by invasive species. Miao et al. (2000) and Johnson (2004) observed that increased input of N and P had caused the alteration of wetland plant community structure and composition in a variety of habits worldwide. So far, many studies have reported the detailed response of wetland plants to nutriment enrichments and additions and mostly focused on cattail (*Typha domingensis* Pers), bulrush (*Phalaris arundinacea*), *Eleocharis cellulose* and *Rhynchospora tracyi*, algal, and so on (Davis, 1991; Miao and Sklar, 1998, Miao et al., 2000; Chiang et al., 2000; Green and Galatowitsch, 2002; Johnson, 2004; Busch et al., 2004). In these studies, P or N addition had a significant influence upon the wetland plant growth (Davis, 1991; Miao and Sklar, 1998). Excessive N and P would adversely affect the structure and constitution of wetland vegetation (Lenssen et al., 1999).

Carex spp. are typical in wetland, and previous studies have focused on the external factors of germination of *Carex* spp. (Budelsky and Galatowitsch, 1999; Van Der Valk et al., 1999) and the hydro-chemical range of *C. lasiocarpa* (Gorham, 1950). And there were some studies that analyzed the life history and the methane emissions and oxidation from freshwater marsh of *C. lasiocarpa* (Bedfod et al., 1988; Ding et al., 2004). The wetland of Sanjiang Plain in Northeast China is one of the large and

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typical freshwater wetlands in China. Marshes in this area are mainly divided into four types according to vegetation type, i.e. *C. lasiocarpa*, *C. psudocuraica*, *C. meyeriana* and *Deyeuxia angustifolia* (Zhao, 1999). The accumulation, distribution and allocation of the nutrient element were analyzed in *C. lasiocarpa* wetland in Sanjiang Plain (Sun et al., 2000; He and Zhao, 2001; He, 2002). However, few study assumed that nutrient addition would cause a change of *C. lasiocarpa* and *C. meyeriana* growth compared to the natural environment in Sanjiang Plain. In this study, the effects of nutrient application on plant tissue nutrition, biomass and its allocation were tested in a simulation experiment. If the above assumption was proved to be true, a set of guidelines can be developed to conserve and restore the native wetland for resources managers.

2 Material and Methods

2.1 Study area

Experimental site is located at the Sanjiang Wetland Ecological Experimental Station, Chinese Academy of Sciences, in Heilongjiang Province, China (47˚35′N, 133˚31′E) (Fig. 1). The average elevation is 56m, and the mean annual precipitation is 600mm and the mean annual temperature is 1.9℃. Water and soil in the wetland are completely frozen in October and begin to melt in late April. The highest and lowest temperatures occur in July and January, respectively.

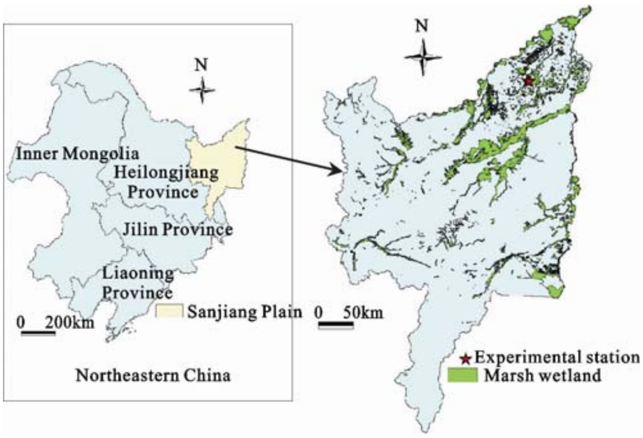

Fig. 1 Location of experimental site

2.2 Experimental design

On May 27, 2004, the dominant plant species of *C. lasiocarpa* communities were collected in the field. Approximately 60 seedlings of dominant species of *C. lasiocarpa* and 40 seedlings of companion of *C. meyeriana* were planted in each polyethylene tank. The dimension of the tank was 40cm×35cm×60cm. The profile is composed of the water layer, root layer, peat layer and gley layer (Ding, 2004). The undisturbed soil was collected from different layers of soil profile in the marsh wetland. Soil profile was repacked with peat soil and gley soil to a depth of 28cm by successive filling in each tank. Specifically, the undisturbed gley soil, composed of 341.65mg/kg of total N and 214.59mg/kg of total P, was added to structure a profile of 16cm depth, which is similar to the natural soil environment. Next, the undisturbed peat soil, composed of 5154.77mg/kg of TN and 882.45mg/kg of TP with sedge remains, was added to structure a layer of 12cm thickness. Then an additional root layer of 20cm thickness was added to the soil surface. Tanks were initially irrigated with freshwater from the wetland to make water level about 10cm. After a 20d of plant acclimation period, the experiment was performed. Seven levels of nutrient treatments and one control were designed in the experiment, and three replicates were set up under each treatment (Table 1). N was applied in the form of ammonium sulphate. P was applied in the form of sodium dihydrogen phosphate. Nutrient was applied once more on May 30, 2005. The experiment lasted two growing seasons beginning on June 17, 2004 and ending on August 25, 2005. During the experiment, the soil tanks were placed in the field without additional nitrogen and phosphorus application.

The aboveground parts of the plants were harvested on August 25, 2005. Then root was harvested in the same day. Aboveground part of plant was classified as stem, leaves, leaf sheath. Both aboveground and underground parts were washed out with tap water to remove soil and dust, and then rinsed with deionized water and dried at 80℃ to constant weight and weighed to determine the biomass. The dried plants were ground through a 2mm-diameter mesh screen to analyze N and P concentration of plant tissue. Total nitrogen (TN) was determined by indigotin colorimetry, and total phosphorus (TP) by ammonium molybdate colorimetry (State Forestry Administation, P. R. China, 2000).

2.3 Data processing

The data were analyzed using the software of Statistical Package for the Social Science (SPSS 12.0). Data from the harvest were analyzed with analysis of variance (ANOVA). In two-way analysis of variance (two-way ANOVA) with plant tissue, N or P as main factor was used for plant tissue nutrient concentration. Multiple comparisons were performed using the Tukey test at the 0.05 significant level. To further visualize the effects of N and P additions on plant growth, nonlinear regression was used (SigmaPlot Version 9.0).

3 Results and Discussion

3.1 Nutrient concentration in plant tissue

The N concentration (TN) of plant tissue generally increased with increasing amount of nutrients applied (Table 1). For *C. lasiocarpa,* TN of plant tissues under the treatment of 10g/m^2 was lower compared with the other N treatments. N addition had a significant impact on TN in plant tissue of two species over the range of concentration studied ($P<0.05$), but they were similar between treatment of 4g/m^2 and the control and between treatment of 10g/m^2 and the control ($P>0.05$). Plant tis-

Table 1 TN and TP in different tissues of *C. lasiocarpa* and *C. meyeriana* under different treatments

Species	Treatment (g/m^2)		Nutrient	Stem (g/kg)	Leaves (g/kg)	Sheath (g/kg)	Root (g/kg)
C. lasiocarpa	Control	0	TN	5.05	10.09	5.52	10.23
	N addition	4		5.37	10.44	5.71	12.01
		10		5.22	9.66	4.63	11.09
		20		5.42	12.50	6.81	15.05
		40		11.37	22.23	8.21	23.71
	Control	0	TP	0.68	0.67	0.53	1.26
	P addition	1.2		0.77	0.75	0.61	1.54
		4.8		0.93	0.91	0.84	1.80
		9.6		1.15	1.14	1.13	1.86
C. meyeriana	Control	0	TN	4.81	12.72	4.83	7.55
	N addition	4		5.04	11.41	4.92	11.10
		10		5.46	12.98	5.57	11.54
		20		5.41	16.38	7.42	14.54
		40		12.60	23.63	11.01	15.36
	Control	0	TP	0.85	0.96	0.74	0.73
	P addition	1.2		1.05	1.22	1.02	0.45
		4.8		1.32	1.21	1.09	0.63
		9.6		1.05	1.31	1.13	1.03

sues also significantly affected N allocation ($P<0.05$), but N concentrations were similar between stems and sheathes ($P>0.05$). In the case of *C. lasiocarpa*, TN decreased in the order of root>leaves>stem≈sheath. In the case of *C. meyeriana*, TN decreased in the order of leaves>root>stem≈sheath. TN in stem and sheath of the two wetland plants were lower than other plant tissues. Normally, increased N tissue concentrations were observed after N application, particularly the significant elevated foliar N concentrations. Several other researchers (Sun et al., 2000; He and Zhao, 2001) also reported similar results. According to He (2002), comparatively high N in leaves resulted from the photosynthesis. However, N mainly accumulated in the root of *C. lasiocarpa*, and leaf is the second important N-rich tissue in this study. *C. lasiocarpa* is perennial by root reproduction, the enrichment of N in root provide the necessary nutrient for plant revival in the next spring. In contrast, *C. meyeriana* leaves showed higher N concentrations compared to roots. For *C. meyeriana*, nutrient is returned to the soil through litter-fall in the next spring, which is necessary for plant growth.

The total P concentrations (TP) in tissues of *C. lasiocarpa* increased with P addition. P addition had a significant influence upon TP in the tissue of *C. lasiocarpa* ($P<0.05$). Plant tissues also significantly affected P allocation ($P<0.05$). TP in root was higher compared to that in the other tissues. It was similar between stem and leaves ($P>0.05$), but still decreased as the order of stem>leaves>sheath. Effect of P addition on TP in the stem, leaves and sheathes of *C. meyeriana* was not significant ($P>0.05$) (Table 1). TP of aboveground plant tissue after P addition were greater than the control value. TP of *C. meyeriana* was almost evenly distributed among aboveground plant tissues under the same P treatment ($P>0.05$), although foliar P concentration was greater than that in the other tissues under all treatments excepting the treatment of 4.8g/m^2. TP was significantly lower in the root compared to the other tissue under all treatments.

3.2 Plant biomass

Nutrient applied has an impact on the plant biomass. According to the analysis, N addition had a significant impact on the aboveground biomass of the *C. lasiocarpa* ($P<0.01$) and *C. meyeriana* ($P<0.01$) over the range of concentration studied. Aboveground biomass of *C. lasiocarpa* under the treatment of 10g/m^2 was significantly different with the other treatments ($P<0.05$), whereas similar biomass was found among the other treatments ($P>0.05$). For *C. meyeriana*, the aboveground biomass was significantly different in response to varying N additions ($P<0.05$).

From Fig. 2, both *C. lasiocarpa* (r^2=0.81) and *C. meyeriana* (r^2=0.96) exhibited relationships between biomass and N addition. Initially, *C. lasiocarpa* exhibited a significant increase of biomass compared with the control value, reaching the maximum of 31.20±4.01 g/tank under the treatment of 10g/m^2, and then dropped to 18.02±1.53g/tank under the treatment of 40g/m^2. As far as *C. meyeriana* was concerned, a positive correlation has been found between the biomass and N addition. High N applied produced more aboveground biomass than low N applied.

In the case of the underground biomass, *C. lasiocarpa* showed significant difference under all treatments except between 4g/m^2 and 20g/m^2 and between 10g/m^2 and 40g/m^2 (Table 2). For *C. meyeriana*, there was no sig-

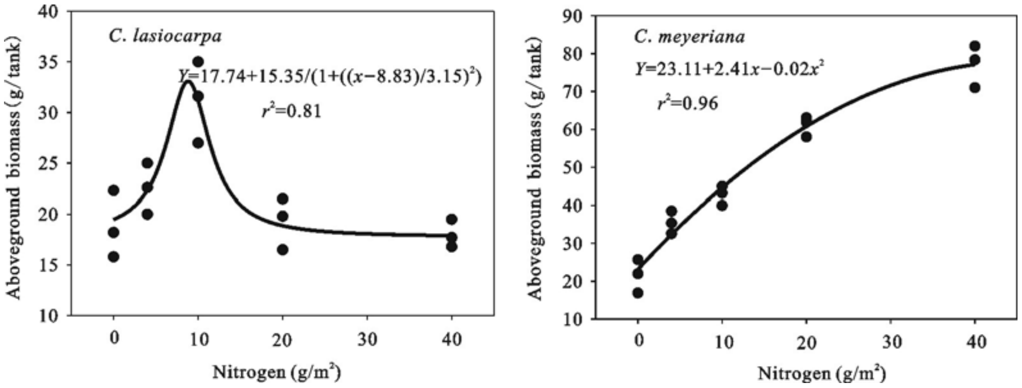

Fig. 2 Aboveground biomass of *C. lasiocarpa* and *C. meyeriana* under different N treatments

nificant difference for underground biomass, except between 4g/m^2 and the other treatments. Maximum underground biomass of two wetland plants appeared under the control treatment (Table 2). Under the N treatments, the change trend of total biomass of two species consisted with underground biomass. For *C. lasiocarpa,* the ratio of UGB (underground biomass) to AGB (aboveground biomass) has reached its maximum under the control treatment. For *C. meyeriana*, UGB/AGB decreased with increasing N applied (Table 2). N application influenced the biomass reallocation. For *C. lasiocarpa*, UGB/AGB increased with increased N addition from 10g/m^2 to 40g/m^2. So, more biomass was allocated to the underground part with increased N addition. For *C. meyeriana*, UGB/AGB decreased with increased N addition, more biomass was allocated to the aboveground part with increasing N addition (Table 2).

Table 2 Biomass of *C. lasiocarpa* and *C. meyeriana* under varied N treatments (mean ± standard deviation)

Species	Biomass	Control	4g/m^2	10g/m^2	20g/m^2	40g/m^2
C. lasiocapa	Underground (g/tank)	492.57±16.38a	312.75±13.26b	409.28±28.08c	330.95±23.40b	380.60±20.28c
	Total (g/tank)	511.62±11.79a	336.55±14.96b	442.58±30.49ac	349.10±25.74b	398.75±22.19bc
	UGB/AGB	26.2	13.9	13.1	17.2	21.1
C. meyeriana	Underground (g/tank)	405.97±24.96a	298.40±21.84b	354.01±14.82a	373.42±22.62a	404.31±7.02a
	Total (g/tank)	427.26±31.19a	333.40±21.28b	395.61±12.56a	434.97±20.43a	480.46±6.53a
	UGB/AGB	18.0	8.4	8.3	6.1	5.3

Notes: The different letters of a, b and c indicate the difference at significant level of $P< 0.05$ in the same row;
UGB: underground biomass (dry weight); AGB: aboveground biomass (dry weight)

P addition also had a significant impact on the aboveground biomass of *C. lasiocarpa* and *C. meyeriana* over the range of concentrations studied ($P<0.05$). The aboveground biomass of *C. lasiocarpa* increased with increasing P addition, and it changed from 18.77 ± 3.29g/tank to 46.03 ± 3.95g/tank (Fig. 3). Compared with *C. lasiocarpa*, *C. meyeriana* had a totally different growth character and P limited its production, and the biomass declined from 30.53 ± 2.45g/tank to 5.47 ± 0.70g/tank as P treatment changed from 1.2g/m^2 to 9.6g/m^2. Both *C. lasiocarpa* ($r^2=0.98$) and *C. meyeriana* ($r^2=0.93$) (Fig. 3) exhibited relationships between biomass and P addition.

Underground biomass showed no significant difference when varied P was applied for *C. lasiocarpa.* However, there was significant difference between control and the other treatments ($P<0.05$). For *C. meyeriana*, significant difference only appeared between 1.2g/m^2 and control treatment ($P<0.05$) (Table 3). Similarly, the change trend of total biomass consisted with underground biomass under the P treatments. Similarly, P also induced the biomass reallocation. For *C. lasiocarpa*, the ratio of UGB/AGB decreased with increasing P addition. So, more biomass was allocated to the aboveground with increasing P addition. For *C. meyeriana,* the ratio of UGB/AGB increased with increased P addition from 1.2g/m^2 to 9.6g/m^2 (Table 3). More biomass was allocated to the underground with increased P addition. When varied levels of P were applied, difference of underground biomass was not significant for the two plants.

C. lasiocarpa was totally different with *C. meyeriana* in response to N addition. Leaf TN of *C. meyeriana* generally increased with increasing amount of N applied. *C. meyeriana* can utilize high N supplies to facilitate plant photosynthesis and growth. In contrast, *C. lasiocarpa* reached its maximum biomass under the treatment of 10g/m^2. Moreover, TN of plant tissues under the treatment of 10g/m^2 was lower compared with the other N treatments. It can be concluded that *C. meyeriana*, as the accompanying species, can adapt itself to the wetland

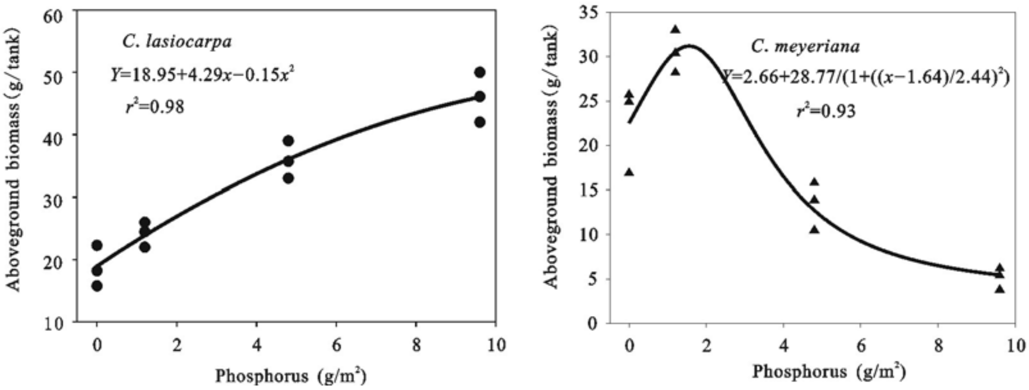

Fig.3 Aboveground biomass of *C. lasiocarpa* and *C. meyeriana* under different P treatments

Table 3 Biomass of *C. lasiocarpa* and *C. meyeriana* under varied P treatments (mean±standard deviation)

Species	Biomass	Control	1.2g/m^2	4.8g/m^2	9.6g/m^2
C. lasiocarpa	Underground (g/tank)	492.57±16.38a	385.01±29.64b	392.18±14.82b	415.90±9.36b
	Total (g/tank)	511.62±11.79a	408.26±10.26b	426.53±16.73b	463.95±12.12ab
	UGB/AGB	26.2	15.9	10.9	9.0
C. meyeriana	Underground (g/tank)	405.97±24.96a	357.43±14.04b	382.2±17.10ab	363.50±8.58ab
	Total (g/tank)	427.26±31.19a	389.13±15.88a	394.40±11.21a	368.60±9.00a
	UGB/AGB	18.0	11.7	30.0	66.5

Notes: The different letters of a, b and c indicate the difference at significant level of P< 0.05 in the same row;
UGB: underground biomass (dry weight); AGB: aboveground biomass (dry weight)

enriched by N, and it may replace *C. lasiocarpa* as the dominant species of wetland. And P also played an important role in plant production in controlling the structure of wetland plants communities. Under P-rich conditions, both tissue TP and aboveground biomass of *C. lasiocarpa* increased with increasing P applied. However, tissue TP concentration may accelerate the *C. meyeriana* development under the treatment of 1.2g/m^2. P accumulation can contribute to the dominance of *C. lasiocarpa* but limit the production of *C. meyeriana*, and the latter may disappear gradually from the wetland enriched by P. Some authors have reported similar results, and they observed that additions of N and P to mesocosms led to dramatic changes in community structure and that new plants replaced native plants as the dominant primary producers (Mcdougal et al., 1997; Havens et al., 1999). The physiological characters of *C. lasiocarpa* and *C. meyeriana* may be different from each other, and thus evolved differently in response to changing environment.

4 Conclusions

The results of this study have shown that nutrient addition significantly affects the wetland ecosystem of *C. lasiocarpa* and *C. meyeriana*. Plant tissue nutrient concentration reflects the amount of nutrient in plant, and they are directly related with the production. The results are applicable to the long-term freshwater wetland eutrophication studies, in that the response of native vegetation, both in terms of productivity and species composition, would become obvious after long-term experiments. Hence, it provides an appreciated ecological basis for decision-making to protect and utilize this delicate wetland. In addition, plant growth and distribution in the wetland are affected by many other factors besides nutrients, which should be thorough studied in the future to better understand the complicated mechanism of wetland degradation.

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