

EFFECT OF WATER LEVEL AND SUBMERGENCE TIME ON LEAF GROWTH, STOICHIOMETRY AND HOMEOSTASIS OF *CAREX BREVICUSPIS*

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Abstract. Water level and submergence time are important factors influencing plant growth and plant C:N:P characteristics in freshwater wetlands. Here, we performed a controlled experiment using water levels and submergence times with the aim of investigating the changes in plant growth and stoichiometric characteristics, as well as the strength of N, P, and N:P homeostasis in *Carex brevicuspis*, China. Results showed that biomass, density, height, and total carbon (TC) decreased significantly with increasing water level; however, total nitrogen (TN) and total phosphorus (TP) increased significantly. The highest TN and TP were at 40 cm water level, while the highest C:N, C:P, and N:P ratios were at 0 cm water level. Furthermore, significant stoichiometric homeostasis of *C. brevicuspis* was found for N (H_N) and P (H_P) except for N:P ($H_{N:P}$). H_N and H_P decreased with increasing submergence time, and H_N was consistently greater than H_P , indicating that *C. brevicuspis* had a weak ability to maintain its internal P balance under long-term submergence. Therefore, the impact of water level and submergence time on plant growth and stoichiometric characteristics in this study are applicable to the conservation and management of the wetlands dominated with *C. brevicuspis*.

Keywords: freshwater wetland, C, N, P stoichiometry, stoichiometric homeostasis, vegetation characteristics, *Carex brevicuspis*

Introduction

Ecological stoichiometry focuses on the mass balance of multiple chemical elements at different scales from individuals to ecosystems, and has been proven useful for understanding the links between ecological processes and nutrient biogeochemistry (Qu et al., 2014; Zhang et al., 2021). Carbon (C), nitrogen (N), and phosphorus (P) are essential nutrient elements and the foundation of the chemical composition of living organisms on earth (Zhang et al., 2019a). The quantity and stoichiometry of these three elements in plants are crucial indicators for revealing various ecological processes, such as decomposition, population stability, nutrient availability and limitation, and the distribution patterns of plants (Yu et al., 2012; Zhang et al., 2013; Chen et al., 2016). Furthermore, they assist with identifying the factors influencing the variation in these elements and the mechanisms affecting ecosystem processes (Li et al., 2017a; Zhang et al., 2019b).

Stoichiometric homeostasis is a foundational concept of ecological stoichiometry and refers to the regulatory ability of organisms to maintain relatively constant element concentrations and ratios under changing environments (Sterner and Elser, 2002). Generally, plants with higher stoichiometric homeostasis have a stable physiological structure and function, and their tissue nutrient concentrations and ratios show little change under a fluctuating environment, while plants with lower stoichiometric homeostasis show rapid changes in their tissue nutrient concentrations and ratios (Harvey and Leffler, 2020; Julian et al., 2020; Yang et al., 2020). Previous research found that *Potamogeton malaianus* and *Vallisneria natans* with a high P stoichiometric homeostasis (H_P) occurred predominantly in relatively infertile soil, and were more stable in the aquatic environment compared to those with a low H_P in shallow lakes in the Yangtze Plain, China (Su et al., 2018). Similarly, a high H_P and compensation ability of substantial leaf tissue may result in a large distribution of macrophytes (Bai et al., 2020). To date, stoichiometric homeostasis in aquatic ecosystems has been used as an indicator for reflecting the response of organisms to environmental fluctuations, and existing studies have primarily focused on different nutrient conditions in aquatic ecosystems (Xing et al., 2016; Su et al., 2018).

In wetlands, water level is one of the main environment factors influencing macrophyte growth and stoichiometry, as this affects light availability (Rybak et al., 2020), microbial activity (Ran et al., 2020), redox potential (Eh), and nutrient cycling in the soil (Shen et al., 2020). For instance, with increasing water level, low light availability usually leads to a decline in the soluble carbohydrate content and C:N ratio in plants, through decreasing carbohydrate production during photosynthesis in Dongting Lake (Qin et al., 2013), which determines plant C, N, and P concentrations and stoichiometry (Li et al., 2015, 2018b). Besides water level, submergence time is another important factor determining plant growth and distribution, especially in river-connected lakes and floodplains (Li et al., 2018a). However, the influence of submergence time on plant growth performance and physiological response varies depending on the water level (Gao et al., 2016; Fu, 2018; Ding et al., 2019). Although many studies have investigated the influence of water level and submergence time on plant growth, propagation, and distribution (Visser et al., 2010; Chen et al., 2019), it is unclear how plant stoichiometry changes under the interactive stress of water level and submergence time fluctuations.

To investigate the interactive influence of water level and submergence time on plant stoichiometric homeostasis, a controlled experiment was performed using *Carex brevicuspis*, a dominant species in the Dongting Lake wetland, China, which was planted under four water levels (0, 10, 20, and 40 cm, relative to the soil surface) and three submergence time (30, 60, and 90 days) treatments in a factorial design with five replicates. The objectives of this study were to 1) elucidate how differences in water level and submergence time influence plant growth and stoichiometry and 2) analyze the link between stoichiometric homeostasis and plant C:N:P ratios under different water levels and submergence times.

Materials and methods

Study site and plant materials

Dongting Lake (28°30'–30°20' N, 111°40'–113°10' E) is the second largest freshwater lake and China's archetypical river-connected lake, and is characterized by

large seasonal fluctuations in water level (Xie and Chen, 2008). The lake's wetlands are usually completely flooded from May to October, but are susceptible to drought from November to April. The mean annual temperature is 16.8 °C, with hot summers (June–August, 27.3 °C) and cold winters (December–February, 5.8 °C). The mean annual precipitation is 1382 mm, with more than 60% of the rain falling from April to August (Li et al., 2017b).

C. brevicuspis is a perennial rhizomatous clonal plant, widely distributed on the lakeshore from adjacent areas to the embankment of Dongting Lake wetland (Fig. 1a). It consists of a series of overlapping leaf sheaths and is usually 20–55 cm in height. The belowground meristems produce 2–25 cm long rhizomes, capable of obtaining resources under stressful conditions, and < 1 cm short rhizomes, better adapted to utilizing resources in favorable patches. *C. brevicuspis* usually has two growing phases according to the flooding patterns in Dongting Lake. Flowers and fruits mature during the first growing phase, which is from April to May before flooding begins. During the flooding season (May to October), the aboveground parts of *C. brevicuspis* die due to them being completely submerged. After flooding, *C. brevicuspis* shoots immediately emerge at the beginning of November and keep growing until January. In January, the aboveground plant parts wither due to cold temperatures. Consequently, new ramets sprout and grow rapidly in February or March (Deng et al., 2013).

Experimental design

The study aims to simulate natural environmental conditions in Dongting lake wetland. Submerge time range from 30 to 90 days aim to simulate an early flooding of the wetland. In the field, most of roots of *C. brevicuspis* are distributed in the 20 cm soil layer, and rarely reach 40 cm below the ground. The ramets of *C. brevicuspis* were completely submerged to the 40 cm water level, and partially submerged to the 20 cm water level. On March 15, 2016, when the new ramets of *C. brevicuspis* emerged and were just beginning to germinate, a total of 60 soil columns of *C. brevicuspis* were collected using PVC tubes (30 cm height and 40 cm diameter) in the field with the plant density of 800 (plants m⁻²). The *C. brevicuspis* in each PVC tube is about 25–30 cm in height with 4–5 leaves and roots reaching 20–40 cm. After sampling, these PVC tubes were transported to an experimental field at the Dongting Lake Station for Wetland Ecosystem Research, Chinese Academy of Sciences. During experimental preparation, 12 soil columns of *C. brevicuspis* (4 water levels and 3 submergence times) were placed into 5 cement pools each (2 m length, 2 m width, and 1 m height), and the experiment began 7 days later. Different water levels were obtained by elevating the PVC tubes with cement bricks (Fig. 1b). The water in the pools was maintained manually by adding tap water (tap water was containing 0.51 mg L⁻¹ NH₄-N, 1.76 mg L⁻¹ NO₃-N, and 0.53 mg L⁻¹ PO₄³⁺-P, pH = 7.2). Water was completely replaced every two weeks to prevent algal growth. The total nitrogen, total phosphorus and total carbon concentrations of the soil were 2.80 ± 0.24, 0.9 ± 0.04 and 34.01 ± 3.81 g kg⁻¹.

Harvest and measurement

The experiment started on March 23, 2016, after which the aboveground parts of *C. brevicuspis* and soil samples at each water level were collected from the PVC tubes after 30, 60, and 90 days of submergence, respectively. The live leaves were cleaned up using tap water and then oven dried at 80 °C for 48 h for laboratory analysis. The roots

were not harvested because it was difficult to distinguish between dead and living roots. Plant biomass (g m^{-2}) was the mass of the aboveground parts of live *C. brevicuspis*. Plant height (cm) was measured from the ground to the top leaf three times with a 0.1 cm steel tape. Mean height was recorded for further analysis. Plant density (plants m^{-2}) was calculated by the number of plants in each PVC tube after 30, 60, and 90 days of submergence, respectively.

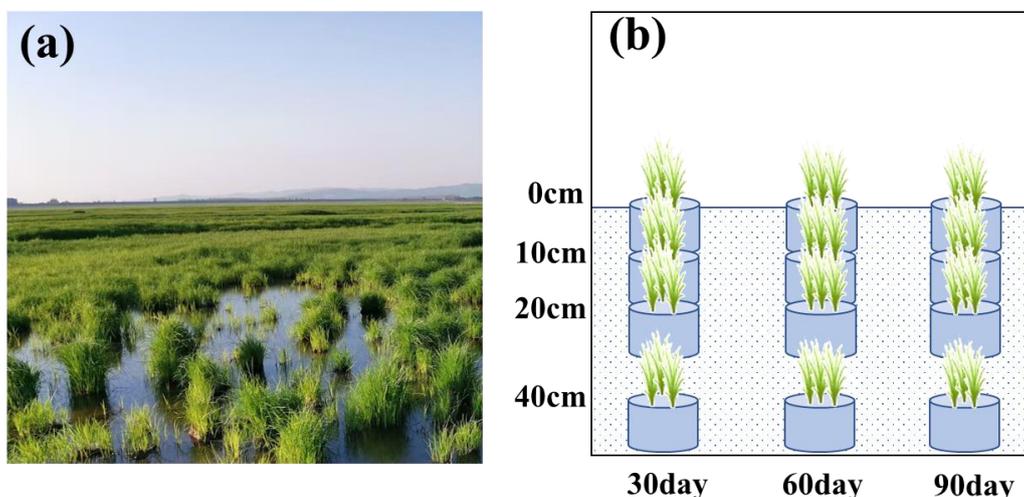


Figure 1. (a) The natural environmental of *C. brevicuspis* in Dongting wetland, (b) the applied experimental design, four water levels (0, 10, 20, and 40 cm, relative to the soil surface) and three submergence times (30, 60, and 90 days)

Laboratory analysis

The total carbon (TC) and total nitrogen (TN) concentrations in the leaves were measured using an elemental analyzer (Vario MAX CN Elemental Analyzer, Elementar, Hanau, Germany). Total phosphorus (TP) concentration in the leaves was measured by colorimetric analysis using a TU-1901 spectrophotometer (Thermo Scientific, Delft, The Netherlands) after being pretreated with $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ digestion (Zhang et al., 2015).

The soil inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) concentration was analyzed using a flow injection autoanalyzer (FIAstar 5000 Analyser; Foss Tecator, Hillerød, Denmark) after the dried and pre-weighed soil samples were extracted using 2 mol L^{-1} KCl. Soil available phosphorus (AP) was extracted with ammonium fluoride (NH_4F , 0.03 mol L^{-1}) and hydrochloric acid (HCl , 0.025 mol L^{-1}), and measured using a UV-Vis spectrophotometer (Spectrum lab 24, Shanghai, China) (Yu et al., 2010). Soil N:P ratio was derived from the measured inorganic N and AP. Three replicates were used to determine C, N, and P concentrations in the samples. The units (g kg^{-1}) of TC, TN, and TP concentrations were transformed to mol kg^{-1} . The ratios in each plant sample were then calculated as molar ratios (atomic ratios) for TC:TN, TC:TP, and TN:TP.

Data analysis and statistics

The stoichiometric homeostasis coefficient (H) for *C. brevicuspis* was calculated using the following equation (Sternner and Elser, 2002):

$$y = c * x^{\frac{1}{H}} \quad (\text{Eq.1})$$

where y is TN or TP concentration (%) or N:P ratio of the plant; x is the inorganic N or AP concentrations (mg kg^{-1}) or N:P ratio in the soil; and c is a constant.

Plant biomass, height, density, concentrations of TC, TN and TP, ratios of C:N, C:P, and N:P were analyzed by using two-way ANOVA, with submergence time and water level as fixed factors. Multiple comparisons of the means were performed using Tukey's test at the 0.05 significance level. All statistical analyses were performed in SPSS 20.0 (SPSS Inc., Chicago, IL).

Results

Vegetation characteristics

Water level, submergence time, and their interaction had a significant effect on plant height and density in *Table 1*. The water level explained the highest total variance (87.05%—91.98%) in plant height, density at highly significant level ($P < 0.001$). Submergence time explained lower total variance (3.28%—7.83%) than the interaction of water level and submergence time (4.74%—7.81%). In addition, the interaction was not significant by the biomass, comparing the water levels between the submergence times is not relevant.

Table 1. Summary of two-way ANOVA on concentrations of TC, TN and TP, and ratios of C:N, C:P and N:P in *C. brevicuspis* growing in four water levels and three submergence times (*F*-values)

	Water level (W)		Submergence time (S)		W*S	
	SS%	F	SS%	F	SS%	F
Height (cm)	88.08	111.82***	4.11	7.83***	7.81	7.83***
Density (plants m^{-2})	87.05	57.75***	6.07	6.04**	6.88	6.04**
Biomass (g m^{-2})	91.98	50.26***	3.28	2.69 ^{ns}	4.74	2.69 ^{ns}
TC (mg g^{-1})	62.98	34.81***	28.10	23.30***	8.92	23.30***
TN (mg g^{-1})	76.39	57.23***	6.53	7.33**	17.08	7.33**
TP (mg g^{-1})	78.83	14.74***	1.00	0.28 ^{ns}	20.17	0.28 ^{ns}
C:N	83.98	80.12***	3.61	5.17**	12.41	5.17**
C:P	76.51	22.98***	1.71	0.77 ^{ns}	21.78	0.77 ^{ns}
N:P	26.03	1.9 ^{ns}	29.27	3.2*	44.70	3.2*

TC, total carbon; TN, total nitrogen; TP, total phosphorus; SS% is the relative variance rate; ns means not significant; The stars indicate the levels of significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 2 showed that the highest values for plant height, density occurred in the 0 cm + 90 days treatment, and they were 4.69, 2.41 times higher than the lowest values, respectively. The plant height and density displayed a similar changing pattern, which decreased with increasing water level. The increased submergence time showed non-significant difference on plant biomass, density and height at the same water level, apart from the plant density at 10 cm of water level and the plant height at 10 cm and 20 cm of water level. The plant density and height significantly decreased with the increased in

submergence time from 30 days to 90 days at 10 cm water level, while the plant height significantly decreased with the submergence time from 30 days to 60 days at 20 cm water level.

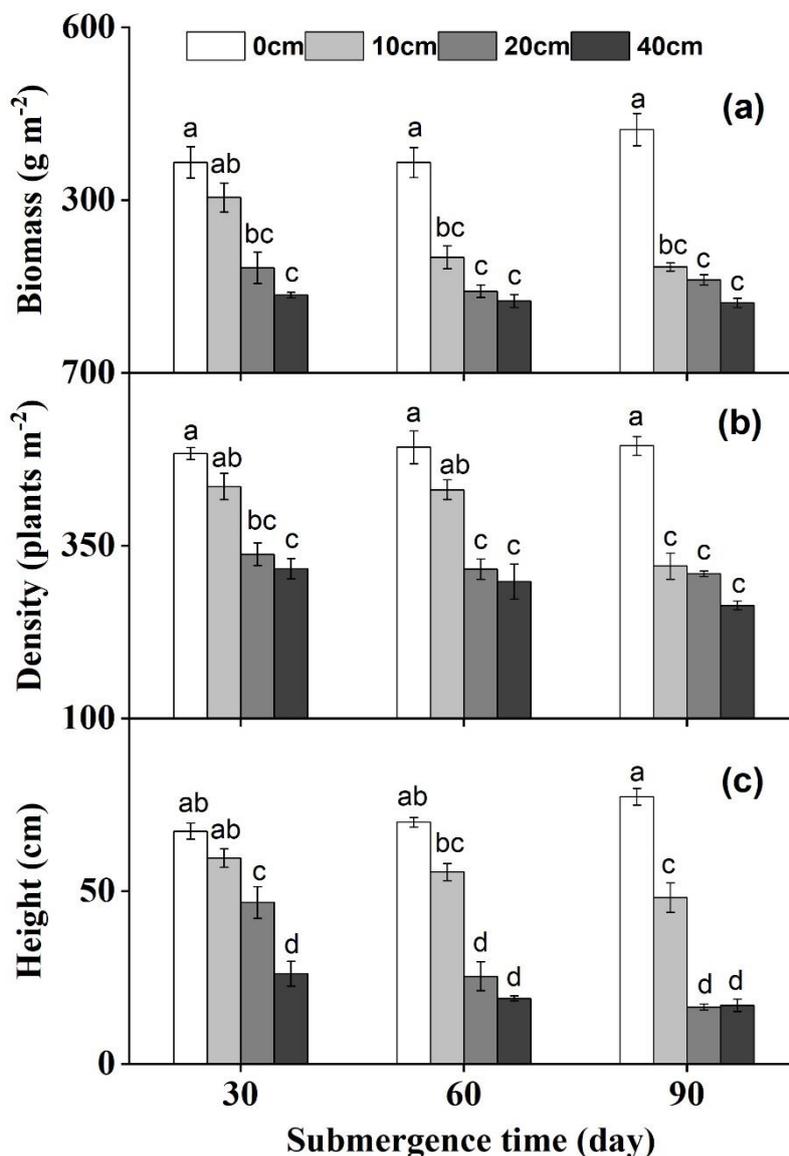


Figure 2. Plant biomass, density, and height (means \pm SE) in leaves of *C. brevicuspis* growing in four water levels (0, 10, 20, and 40 cm, relative to the soil surface) and three submergence times (30, 60, and 90 days). Different letters indicate significant differences among water levels and submergence times, based on Tukey's test ($P < 0.05$)

Total C, N, and P concentrations

Water level, submergence time, and their interaction had a significant effect on TC and TN concentrations in *C. brevicuspis*, with water level and submergence time explaining 62.98% and 28.1% of the total variance in TC and 76.39% and 6.53% of the total variance in TN, respectively ($P < 0.01$, Table 1). In addition, the interaction was not significant by TP, comparing the water levels between the submergence times is not relevant.

In *Figure 3*, TC concentration of the live leaves decreased significantly with increasing water level, while the TN concentration increased significantly. The effect of submergence time on the nutrient concentration of the live leaves were highly inconsistent. The submergence time had significant effect on TC concentration at the 10 cm, 20 cm and 40 cm of water level, and on TN concentration at 0 cm, 20 cm and 40 cm of water level, respectively. However, the influence of submergence time on TP concentration has no significantly. The highest TC concentration ($437.06 \pm 1.63 \text{ mg g}^{-1}$) was in the 10 cm + 30 days treatment. TN concentration increased significantly with increasing water level. The highest TN concentration ($23.29 \pm 0.41 \text{ mg g}^{-1}$) was in the 40 cm + 90 days treatment, which were 1.67 times higher than that in the 0 cm + 90 days treatment, respectively (*Fig. 3*).

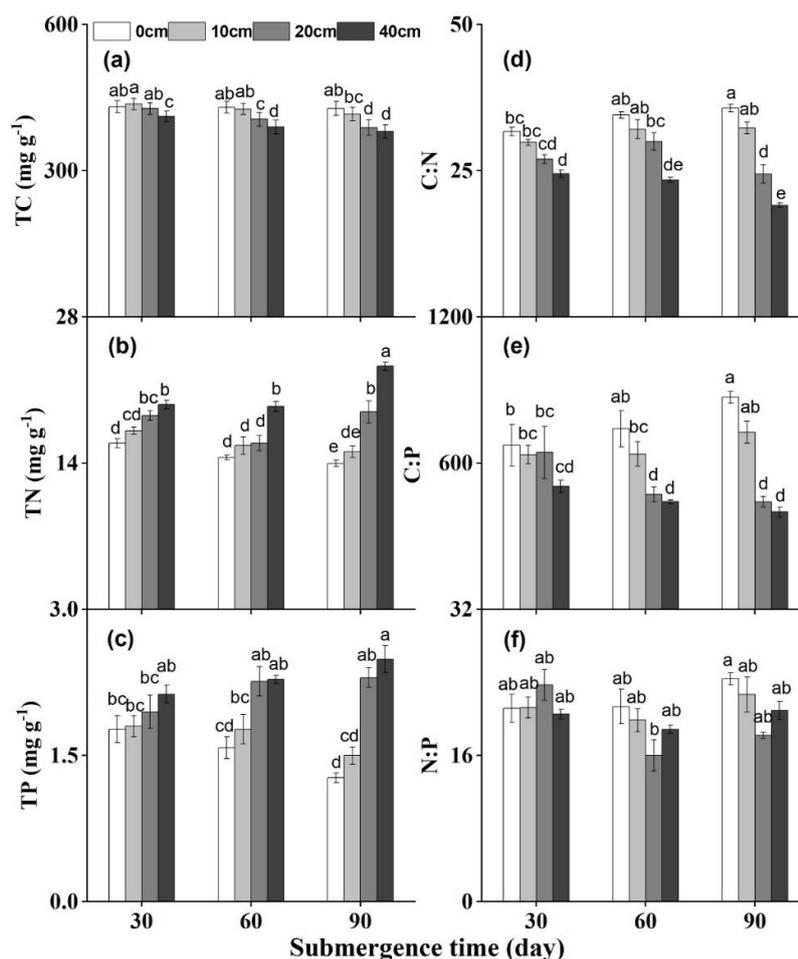


Figure 3. Concentrations of TC, TN and TP, and ratios of C:N, C:P, and N:P (means \pm SE) in leaves of *C. brevicuspis* growing in four water levels (0, 10, 20, and 40 cm, relative to the soil surface) and three submergence times (30, 60, and 90 days). Different letters indicate significant differences among water levels and submergence times, based on Tukey's test ($P < 0.05$)

C, N, and P stoichiometry ratios

Table 1 showed that the water level, submergence time, and their interaction significantly affected the C:N ratio, with explaining 83.98%, 3.61% and 12.41% of the total variance, respectively. Except for the water level, the submergence time and the

interaction of these two hydrological properties had significant effect on the N:P ratio explaining 29.27% and 44.70% of the total variance, respectively. In addition, the interaction was not significant by C:P, comparing the water levels between the submergence times is not relevant (Table 1).

The C:N ratio decreased with increasing water level in Figure 3, while the variation of N:P ratios was inconsistent with increasing water level. The submergence time had significant effect on C:N ratios at 0 cm, 20 cm and 40 cm of water level. However, there was no significant effect of submergence time on N:P ratio. The highest C:N ratio in the 0 cm + 90 days treatment were 1.87 time higher than the lowest ratios in the 40 cm + 90 days treatment, respectively (Fig. 3).

Stoichiometric homeostasis coefficients (H) for N and P

In Figure 4, the homeostasis of N (H_N) at 30, 60, and 90 days of submergence was 2.27, 2.22, and 0.9, respectively. The H of P (H_P) at 30, 60 and 90 days was 0.63, 0.51, and 0.24, respectively. The H_N and H_P values after 30 days (2.27 and 0.63, respectively) of submergence were higher than those of 60 and 90 days.

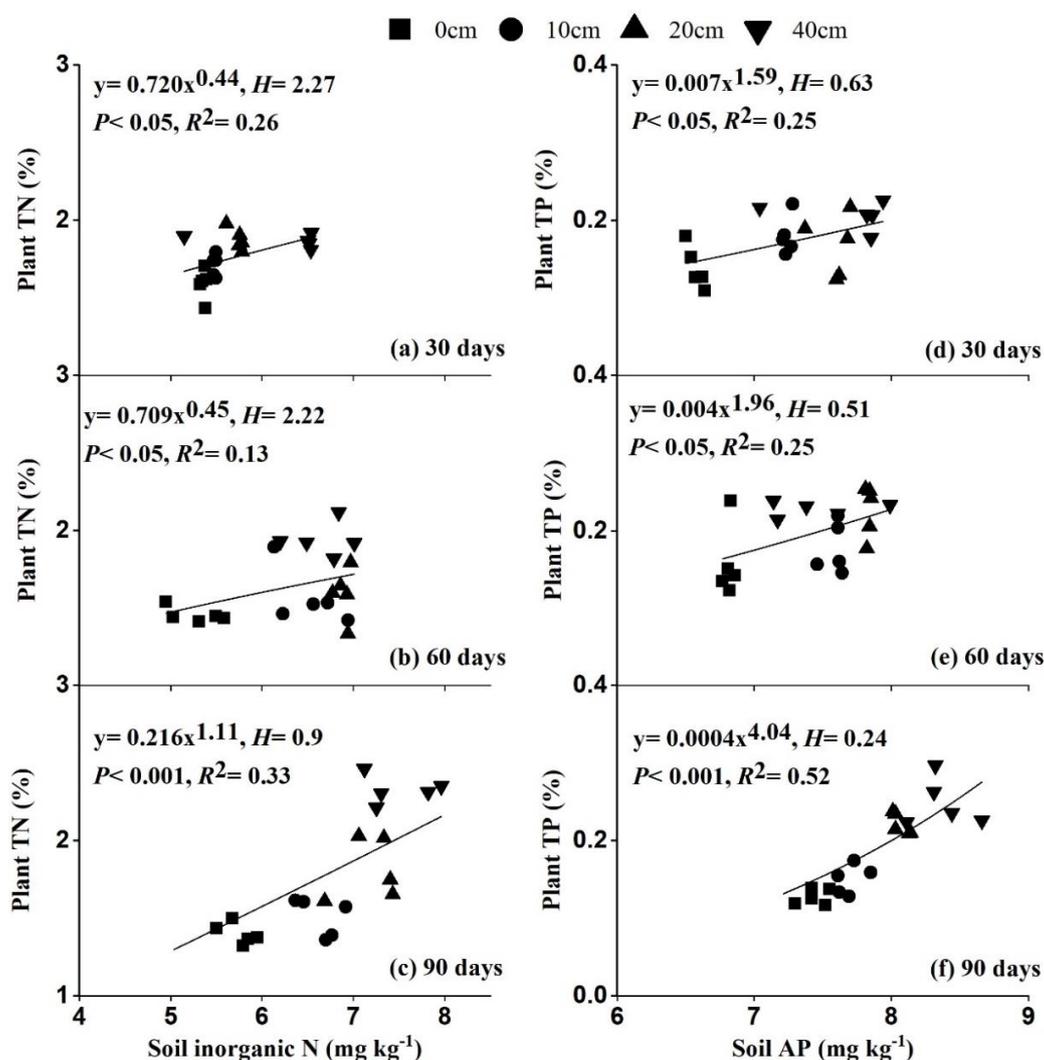


Figure 4. Stoichiometric homeostasis coefficients (H) between concentration of N and P in leaf of *C. brevicuspis*, and soil inorganic N and soil available phosphorus (AP)

Discussion

In wetland ecosystems, water level and submergence time are regarded as the important hydrological factors to determine plant distribution pattern and productivity (Liu et al., 2018a). Our findings showed that plant biomass, height, and density were significantly higher at low water level than that at high water level. These results are consistent with those of a previous study, which showed that high water level caused a significant decrease in the biomass of wetland plant communities (Luo et al., 2016; Li et al., 2018b). Moreover, higher water level stress restricted the biomass of *Carex lasiocarpa* and *Carex pseudocuraica* in Sanjiang Plain (Tan et al., 2020). Chen et al. (2014) also reported that *C. brevicuspis* growth and vegetative reproduction was better at a 0 cm water level than at a 20 cm and 40 cm water level in Dongting Lake. Qin et al. (2013) showed that extended periods of anoxic conditions would eventually inhibit plant growth and biomass accumulation.

Besides affecting plant biomass, height, and density, the hydrological regime (e.g., water level and submergence time) can also affect nutrient transformation and availability by controlling various biogeochemical processes, which then determine plant stoichiometric characteristics (Anderson and Lockaby, 2011; Li et al., 2018c). Our results showed that leaf TC concentration significantly decreased while leaf TN and TP concentrations significantly increased with increasing water level, indicating that water level had a significant effect on leaf nutrient concentrations. This might come from the dead leaves which are decomposed by microorganisms with the increased in water level. In addition, fast-growing *C. brevicuspis* at 0 cm water level, which converts more N and P in its photosynthetic tissues to enzymes, nucleic acids, and ATP (adenosine-triphosphate) for high CO₂ assimilation, also might be the reason of diluting the N and P concentration at shallow water level (Li et al., 2018b). This also was consistent with Miao and Zou (2012), which had the highest concentrations of leaf N and P and accumulated more nutrients of *Cladium jamaicense* at a 60 cm water level than that at a 20 cm water level in a South Florida wetland.

In addition, the water level contributed to the decrease in C:N and C:P ratios. These might be caused by a higher C accumulation of the plant with the increases in N and P consumption (Huang et al., 2019). Li et al. (2018a) also confirmed that a high water level and longer submergence might be the main reason for the reduction in C:N, and C:P ratios for *C. brevicuspis* in the Dongting Lake wetland. In addition, the N:P ratio, which plays a greater role in assessing the nutrient limitations of plants (Güsewell, 2002), was larger than 16 in all experimental treatments in this study. Verhoeven et al. (1996) reported that an N:P ratio of < 14 indicated a N limitation, N:P ratio of > 16 indicated a P limitation, and an N:P ratio between 14 and 16 represented the co-limitation of N and P or non-limitation of N and P. Therefore, this study indicated that the *C. brevicuspis* was limited by P in the Dongting Lake wetland. This might be explained by the fact that prolonged submergence and anaerobic conditions increase iron solubility and P mobilization from soils (Anderson and Lockaby, 2011). In many freshwater wetland systems, P is considered the most limiting nutrient because the amount of P in lakes is largely reliant on P released from sediments or P entering the system via streamflow, with lower internal inputs from weathering (Grimm et al., 2003). For example, high N:P ratios in *Peltandra virginica* and *Pontederia cordata* leaves also suggest that these species were P limited in the Florida Everglades wetland (Daoust and Childers, 1999). In addition, Fe²⁺ can block PO₄³⁻ from diffusing into the overlying water column by combining with PO₄³⁻ to form vivianite or other particulate

ferrous compounds, leading to P limitations in freshwater wetlands (Hartzell and Jordan, 2012).

The stoichiometric homeostasis coefficient (H) represents the ability of a plant to maintain a relatively stable nutrient composition under a changing environment and underpins the interpretation of observed variations in plant elemental stoichiometric ratios (Sterner and Elser, 2002). Previous studies reported that vascular plants had a strong ability to regulate stoichiometric homeostasis in freshwater aquatic ecosystems (Li et al., 2018c; Liu et al., 2018b; Su et al., 2018). However, stoichiometric homeostasis could reflect the variation in species and plant nutrients responding to external environmental fluctuations. In the present study, mean values of N homeostasis (H_N) and P homeostasis (H_P) for *C. brevicuspis* (H_N ranged from 0.9 to 2.27, H_P ranged from 0.24 to 0.63) were weaker than those of submerged macrophytes in the Yangtze Lakes (H_P ranged from 1.29 to 3.73), such as *Potamogeton maackianus*, *Potamogeton malaianus*, and *Myriophyllum spicatum* (Su et al., 2018), and also weaker than some herbaceous species (H_N ranged from 4.45 to 9.76, H_P ranged from 3.14 to 9.6) in a terrestrial environment, such as *Andropogon gerardii*, *Schizachyrium scoparium*, and *Aster ericoides* (Yu et al., 2015). This might be due to the submerged macrophytes having a strong resilience to eutrophication in the Yangtze Lakes and atmospheric N deposition in the terrestrial environment (Yu et al., 2015; Su et al., 2018). In this study, increased submergence time reduced leaf N and P homeostasis in *C. brevicuspis*, indicating that plants might change from being strongly to weakly homeostatic under long-term submergence stress. Meanwhile, H_N was far higher than H_P in our experiment. This was similar with Yu et al. (2010), as *C. brevicuspis* might have the reduced ability to maintain its internal P balance under long-term submergence, and N availability might be a more important driver of ecosystem function than P.

In recent years, *C. brevicuspis* in the Dongting Lake wetland has been seriously degraded, mainly due to changing water levels, longer submergence times, and anthropogenic disturbances. This study showed that water levels, submergence times, and their interaction significantly influenced plant growth and plant stoichiometry of *C. brevicuspis* in the Dongting Lake wetland. Furthermore, we found that H_N and H_P decreased with increasing submergence time, and H_N was consistently greater than H_P . These results indicated that *C. brevicuspis* had a weak ability to maintain its internal P balance under long-term submergence.

Conclusions

Therefore, by exploring the effects of these hydrological variables on plant growth and stoichiometric characteristics, we can contribute to better understanding aquatic macrophyte ecological processes and establishing effective measures for macrophyte protection and biodiversity maintenance. However, our study was primarily based on controlled experimental conditions and further studies are required to test these results in situ.

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Conflict of interests. The authors declare that they have no competing interests.

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