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Effects of water depth on carbon, nitrogen and phosphorus stoichiometry of five submersed macrophytes in an *in situ* experiment

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ABSTRACT

The distribution of submersed macrophytes is wide across environmental gradient, e.g. depth profile. Submersed macrophytes develop great phenotypic plasticity in response to increasing water depth. However, the effects of water depth on carbon (C), nitrogen (N) and phosphorus (P) stoichiometry of submersed macrophytes are not very clear. In this study, five submersed macrophyte species (*Potamogeton malaianus*, *Potamogeton maackianus*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Hydrilla verticillata*) were cultured at three water depths (1 m, 2.5 m and 4 m). The C, N and P concentrations and C:N:P stoichiometry were examined in leaves, stems and roots (underground parts) of the plants. We found that the increased water depth significantly inhibited growth of all the plants, but relatively less affect the C, N and P concentrations and C:N:P stoichiometry of the plants. The organs and the species together explained more than 65% of the total variance in the C, N and P concentrations and C:N:P stoichiometry, but water depth contributed to less than 2% of the variance. Significant effects of water depth were only observed for the leaf N and P concentrations of *C. demersum* and the leaf P concentrations of *P. maackianus* and *P. malaianus*. Strong stoichiometric homeostasis was found in submersed macrophytes. Our results imply that the C:N:P stoichiometry of submersed macrophytes is affected not by water levels directly but the species identity instead.

1. Introduction

Ecological stoichiometry, which balances multiple elements across ecological scales, has greatly advanced our understanding of how elemental composition of an organism couples its growth with inorganic environment and cascades to food-web dynamics and nutrient cycling in an ecosystem (Sterner and Elser, 2002; Vanni, 2002; Andersen et al., 2004; Lü Reed et al., 2013). Nitrogen (N) and phosphorus (P) are two major nutrients limiting growth of aquatic primary producers (Elser et al., 2007). Over loading of anthropogenic N and P have altered the carbon (C):N:P stoichiometry, productivity and assemblage of primary producers, and consequently affected behavior of herbivores and predators in aquatic systems (Elser et al., 2000a; Zhang et al., 2011).

Submersed macrophytes rely upon the surrounding sediment and water to satisfy their N and P requirements (Rattray et al., 1991; Madsen and Cedergreen, 2002; Cao et al., 2011). However, the relationship between external N and P availability and the tissue N and P concentrations of plant is often weak (Güsewell and Koerselman, 2002), implying that processes other than N and P availability contribute to variation of N and P concentration of plant. Plant identity associating with growth rate, nutrient allocation and storage (Ågren, 2004; Yu et al., 2012), and plant size, taxa and life forms (He et al., 2006; Zhang et al., 2013) as well as temperature and light availability (Cronin and Lodge, 2003; Cao et al., 2011) cause great variation in the N and P concentrations and C:N:P stoichiometry of plant. For instance, plants with high relative growth rate (RGR) and small size were reported to have high N and P concentrations and low C:P and N:P ratios in photosynthetic tissues (Elser et al., 2000b, 2010; Sterner and Elser, 2002). Other studies have revealed unimodal relationships between RGR and the N:P ratio or no relationship in plants (Matzek and Vitousek, 2009; Yu et al., 2012). This inconsistency indicates that plant stoichiometry is far from clear. While rapid growth plant may allocate more N and P to photosynthetic tissue to support high CO_2 assimilation

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(Elser et al., 2000b), a dilution effect of more C gain of the plant decreases N and P concentrations of the whole plant (Cronin and Lodge, 2003; Yan et al., 2006). Plants accumulate N and P in the tissues more than growth requirements when their growth is limited by factors other than N and P availability (Demars and Edwards, 2007; Cao et al., 2011). Therefore, some methodological problems that different tissues were used for C, N and P examination or plants were collected from different habitats with varying light and temperate have obscured the relationships among external nutrient availability, RGR and C:N:P stoichiometry of plants.

Light and nutrient availability are quite different between aquatic and terrestrial habitats. While growth of terrestrial plants is generally limited by N and P availability (Güsewell, 2004; Reich and Oleksyn, 2004), growth of submersed macrophytes is additionally limited by underwater light intensity (Cronin and Lodge, 2003; Cao et al., 2011). These differences between aquatic and terrestrial habitats may contribute to fundamental differences in C:N:P stoichiometry of plants, as previously revealed by comparative studies among terrestrial plants, seagrasses, phytoplanktons, and freshwater submersed macrophytes (Duarte, 1992; Elser et al., 2000a; Demars and Edwards, 2007; Xing et al., 2013). Different submersed macrophytes show different strategies in nutrient metabolism in response to varying light and nutrient availability (Cao et al., 2011; Yuan et al., 2013). Changes in water level alter the nutrient availability, the growth and species composition of submersed macrophytes in the littoral zone of lakes (Chambers and Kalff, 1985; Geest et al., 2005; Zhu et al., 2012; Fu et al., 2013). Human activities such as overgrazing are expected to increase the probability of flooding (Blom and Voesenek, 1996), and coincident with anthropogenic hydraulic adjustment to alter the previous regular water level fluctuation in many lakes (Hu et al., 2010). Though effects of water depth on morphology of submersed macrophytes have been studied extensively (Strand and Weisner, 2001; Fu et al., 2012; Zhu et al., 2012), few studies focus on the C:N:P stoichiometry of submersed macrophytes (Xing et al., 2013).

In this study, we cultured five submersed macrophyte species at three water depths and examined the C, N and P concentrations in leaves, stems and roots (we defined "roots" as underground parts of plants in this paper, the same below) of the plants in a mesotrophic lake. The plants experienced different underwater light intensity but relatively homogenous nutrient availability at various water depths. Specifically, we test two assumptions: (1) the N and P concentrations and C:N:P stoichiometry were mainly affected by the species, particularly for the plants grown at deeper water, because the underwater light intensity, rather than the nutrient availability controlled growth of the plants; and (2) the N and P concentrations and C:N:P stoichiometry of the leaves and roots were more responsive to changes in water depth than those of the stems, because the leaves and roots renewed more rapidly.

2. Materials and methods

2.1. Experimental site and submersed macrophyte species

The experiment was conducted on a floating platform (26 m × 21 m) 1 km offshore in Lake Erhai (25°52′ N, 100°09′ E, Fig. 1), a mesotrophic lake (max. water depth 21 m, mean water depth 10 m) in the southwestern subtropical plateau of China. The species composition and area of submersed vegetation has changed greatly in the past 30 years when eutrophication and increased water level occurred in the lake. In field survey in 2009, we found submersed macrophytes colonizing at water depth of max. 6 m, with the highest biomass at ca. 3 m water depth.

Five submersed macrophytes, *Potamogeton malaianus*, *Potamogeton maackianus*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Hydrilla verticillata*, were used in this study due to (i) their wide distribution in China and the world (Liang et al., 1996), (ii) their dominance in mesotrophic and eutrophic lakes and Lake Erhai, and (iii) their differences in taxonomy, morphology and growth forms (Chambers and Kalff, 1987; Chambers, 1987).

2.2. Experiment design and procedures

In the experiment, approximately 25-cm long apical shoots (similar in size, healthy in appearance and without branch) of *M. spicatum*, *H. verticillata*, *P. maackianus*, *C. demersum* and *P. malaianus* were collected from Lake Erhai. The shoots were transplanted into 60 sediment-filled plastic containers (43 cm in diameter, 30 cm in depth), with the lower 15 cm of the shoots buried in the sediment to avoid uprooting, and 10 and 6 shoots per container for *P. maackianus* and the other four species, respectively. All the containers were filled by the same fully mixed sediment that was collected from Lake Erhai. The nitrogen, phosphorus and inorganic matter concentrations of the sediment were 2.979 ± 0.116, 0.869 ± 0.015 and 50.62 ± 1.64 mg g^{-1} dry weight, respectively (n = 6).

The containers with the plants were tied by ropes and fastened to the floating platform, and immersed at various water depths by adjusting the length of the ropes (Fig. 1). The experiment was carried out on 26 October 2010. The containers with

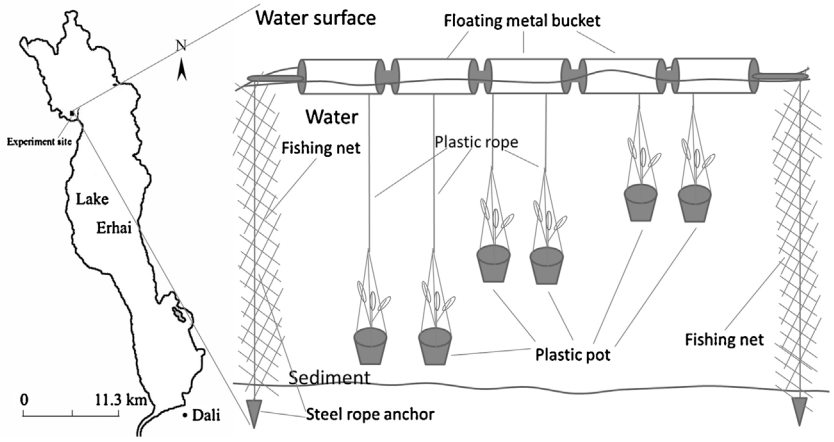

Fig. 1. Experimental site and design of the *in situ* experiment.

Table 1
Physic–chemical characteristics of the water at different water depths. Values are mean ± SE (n = 9). Different letters indicate Duncan's test at the 0.05 significance level.

Physiochemical parameters	Water depth			
	Surface	1.0 m	2.5 m	4.0 m
TN (mg L^{-1})	0.510 ± 0.017^a	0.475 ± 0.016^a	0.482 ± 0.032^a	0.493 ± 0.034^a
TP (mg L^{-1})	0.021 ± 0.003^a	0.021 ± 0.002^a	0.024 ± 0.002^a	0.020 ± 0.003^a
NO_3-N (mg L^{-1})	0.230 ± 0.003^a	0.225 ± 0.003^a	0.223 ± 0.003^a	0.224 ± 0.003^a
NH_4-N (mg L^{-1})	0.064 ± 0.014^a	0.063 ± 0.013^a	0.101 ± 0.017^a	0.067 ± 0.014^a
PO_4-P (mg L^{-1})	0.008 ± 0.001^a	0.009 ± 0.001^a	0.010 ± 0.001^a	0.009 ± 0.001^a
Chl-a (μg L^{-1})	21.99 ± 2.91^a	23.73 ± 2.56^a	26.62 ± 2.14^a	27.12 ± 2.22^a
DO (mg L^{-1})	8.695 ± 0.382^b	7.765 ± 0.158^a	7.812 ± 0.153^a	7.682 ± 0.201^a
PAR (μmol m^{-2} s^{-1})	807.0 ± 72.7^c	290.3 ± 28.0^b	69.4 ± 7.3^a	15.4 ± 1.7^a

Table 2
Variance contribution of organs (leaf, stem and underground part), species (*C. demersum*, *H. verticillata*, *M. spicatum*, *P. maackianus* and *P. malaianus*) and water depth (WD, 1.0 m, 2.5 m and 4.0 m) for tissue C, N and P concentrations and their ratios.

	Percentage of total sum of squares (%)							
	Organs (O)	Species (S)	WD	O × S	O × WD	S × WD	O × S × WD	Error
C	47.7***	28.9***	0.3	5.4***	0.5	2.2**	3.2**	11.8
N	72.6***	15.2***	0.3**	3.7***	0.7***	2.1***	1.2**	4.1
P	18.8***	48.0***	2.0***	13.1***	0.6	0.8	5.2***	11.5
C:N	38.5***	33.2***	1.1***	11.6***	0.9**	4.9***	2.0**	7.8
C:P	11.6***	61.1***	1.6***	7.6***	0.2	2.7***	3.2**	11.9
N:P	42.3***	27.2***	0.4	12.2***	1.0*	0.9	2.8*	13.3

* Significance is indicated by <0.05.
** Significance is indicated by <0.01.
*** Significance is indicated by <0.001.

the plants were immersed at 0.8 m water depth for acclimation of 15 days at the beginning of the experiment, and then immersed at 1.0, 2.5 and 4 m water depths for another 15 days. Four replicates were conducted for each species at each water depth.

2.3. Measurements of the environmental indices, the growth and nutrient concentrations of the macrophytes

At the end of the experiment, all the plants were harvested, clearly washed with distill water, divided into leaves, stems and roots, oven-dried at 80 °C for 48 h and weighted, and ground into fine powder for analysis of C, N and P concentrations. The C and N concentrations were determined by an elemental analyzer (Flash EA 1112, CE Instruments, Italy). The P concentrations were measured by the ammonium molybdate ascorbic acid method after the plant samples were fully digested by sulfuric acid/hydrogen peroxide at 400–500 °C (Kuo, 1996). RGR of the plants was calculated by a formula: $\mu = \ln(M_2/M_1)/dt$, where M_1 and M_2 were the fresh weight of the plants at the beginning and the end of the experiment, respectively, and dt was the experimental time (days).

During the experimental period, water samples were collected at water depths of 1.0, 2.5 and 4.0 m every 3 days to measure the concentrations of NH_4-N, NO_3-N, PO_4-P, total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), and chlorophyll a (Chl-a) according to standard methods (Huang, 2000). Photosynthetically active radiation (PAR) in the water column (at water depths of 1.0, 2.5 and 4.0 m, respectively) was measured every 3 days by a Li-COR UWQ-192S sensor coupled with a Li-1400 data logger (Li-Cor, Lincoln, NE, USA). Sediment samples were air dried and ground into fine powder for the analysis of TN, TP and total organic carbon (TOC) concentrations. TN and TOC concentrations in the sediment were simultaneously determined by an elemental analyzer (Thermo Flash 2000, Cambridge, UK). TP concentration in the sediment was determined following the method of Kuo (1996).

2.4. Statistical analysis

Statistical analysis was conducted with SPSS 16.0 software package (SPSS Inc., Chicago). Analysis of variance (ANOVA) was used to test statistical significance of the effects of water depth. The effects of water depth on the RGR, the C, N and P concentrations and C:N:P stoichiometry were evaluated for each species by one-way ANOVA. We used variance partitioning based on the sum of squares (SS) of three-way ANOVA with organ (O), species (S) and water depth (WD) as factors to indicate their contribution to the variance in the C, N and P concentrations and C:N:P stoichiometry (Güsewell and Koerselman, 2002). The total SS of the ANOVA was decomposed as: $SS_{total} = SS_O + SS_S + SS_{WD} + SS_{O\times S} + SS_{O\times WD} + SS_{S\times WD} + SS_{O\times S\times WD} + SS_{Error}$. Variance contribution of each factor was then expressed as percentage of total SS.

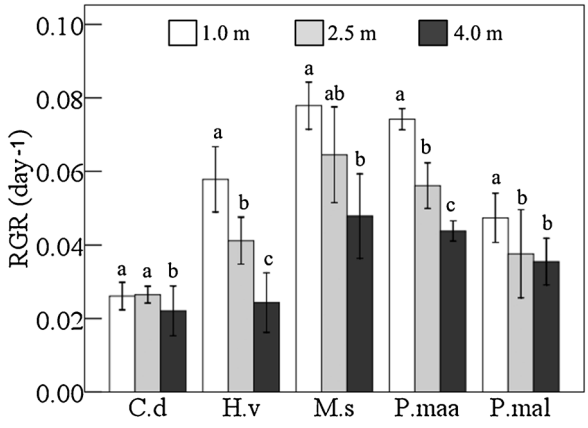

Fig. 2. The relative growth rate (RGR, day^{-1}) of *Ceratophyllum demersum* (C.d), *Hydrilla verticillata* (H.v), *Myriophyllum spicatum* (M.s), *Potamogeton maackianus* (P.maa) and *P. malaianus* (P.mal) grown at different water depths. The vertical bars are standard deviations, and the different letters are significantly different at $P < 0.05$ level among the different water depths.

3. Results

During the experimental period, the average water temperature was 21.4 °C, Secchi depth of water transparency was 1.52 m, and PAR was 290, 70 and 15 μmol m^{-2} s^{-1} at water depths of 1.0, 2.5 and 4.0 m, respectively. The concentrations of TN, TP, NO_3-N, NH_4-N, PO_4-P, DO and Chla in water column did not differ significantly among the three water depths (Table 1).

The increased water depth significantly decreased the plant RGR. Compared to the 1.0 m water depth, the RGR decreased by −1.45%, 28.73%, 17.19%, 24.41% and 20.65% at 2.5 m water depth, and by 15.45%, 57.89%, 38.15%, 40.93% and 25.08% at 4.0 m water depth for *C. demersum*, *H. verticillata*, *M. spicatum*, *P. maackianus* and *P. malaianus*, respectively (Fig. 2).

At the 1.0 m water depth, the N concentrations in leaves of *C. demersum*, *H. verticillata*, *M. spicatum*, *P. maackianus* and *P. malaianus* were 16.44, 39.50, 39.15, 44.65 and 38.33 mg g^{-1},

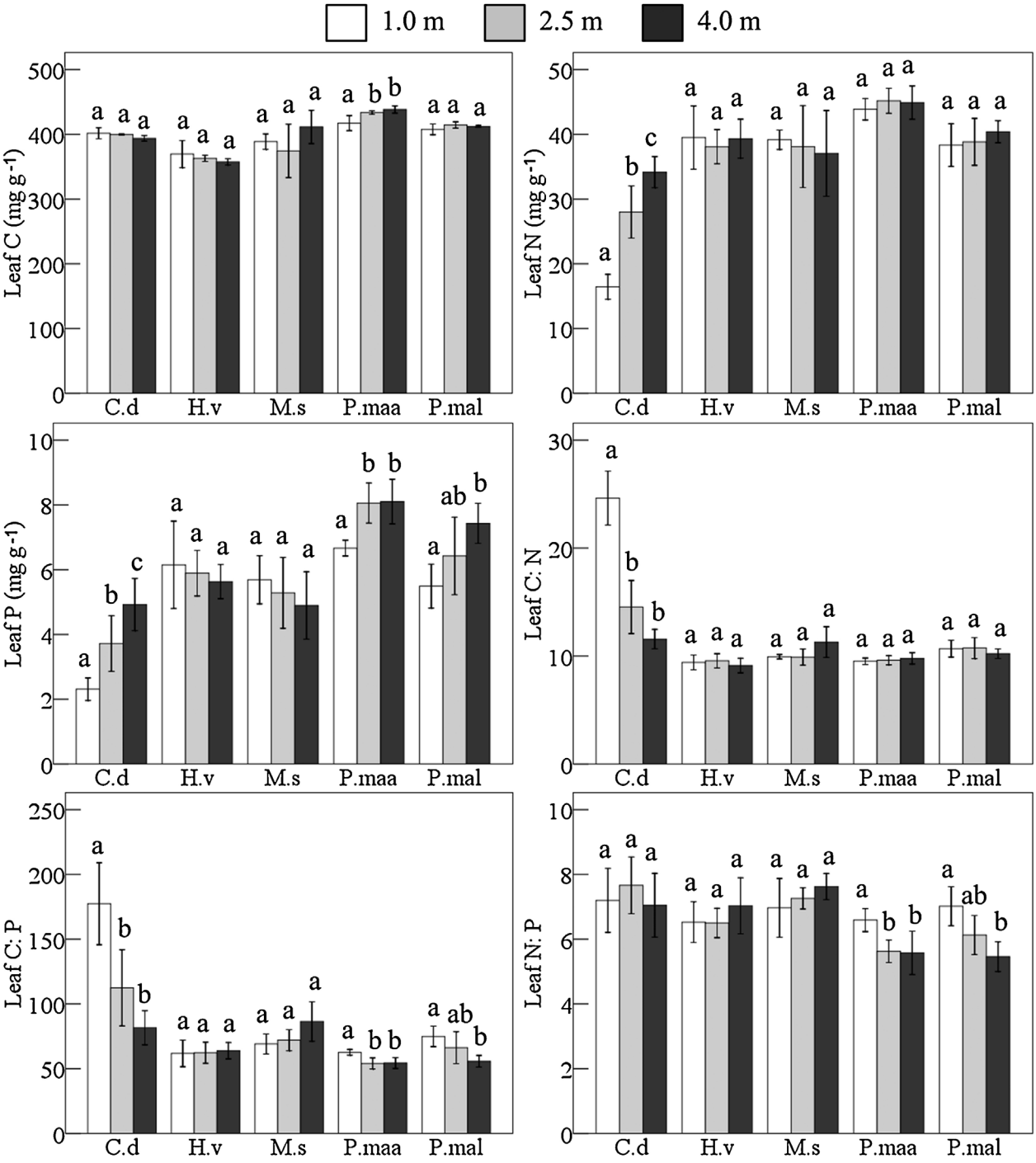

Fig. 3. The C, N and P concentrations and C:N:P stoichiometry in the leaves of *C. demersum* (C.d), *H. verticillata* (H.v), *M. spicatum* (M.s), *P. maackianus* (P.maa) and *P. malaianus* (P.mal) grown at different water depths. The vertical bars are standard deviations, and the different letters are significantly different at $P < 0.05$ level among the different water depths.

respectively, and the P concentrations were 2.31, 6.15, 5.69, 6.67 and 5.49 mg g^{-1}, respectively.

The species and their organs were two key factors significantly affecting the C, N and P concentrations of the submersed macrophytes. They together explained more than 65% of the total variance in the nutrient concentrations and stoichiometry (Table 2). The organs contributed more variation to the C, N concentrations and C:N and N:P ratios than the species, while the species contributed more variation to the P concentrations and C:P ratios than the organs. Although water depth had a marginal contribution to the variation (<2%) relative to the organs and the species, it significantly affected N, P concentrations and C:N, C:P ratios.

The increased water depth enhanced the leaf N concentrations of *C. demersum* and the leaf P concentrations of *C. demersum*, *P. maackianus* and *P. malaianus* (Fig. 3). The C concentrations were approximately 400 mg g^{-1} in leaves of all the macrophytes and were not affected by the various water depths. Therefore, the water depth affected the C:N and C:P ratios inversely to the tendency of the N and P concentrations. The N:P ratios in leaves of *C. demersum*, *H. verticillata* and *M. spicatum* were 7.20, 6.52 and

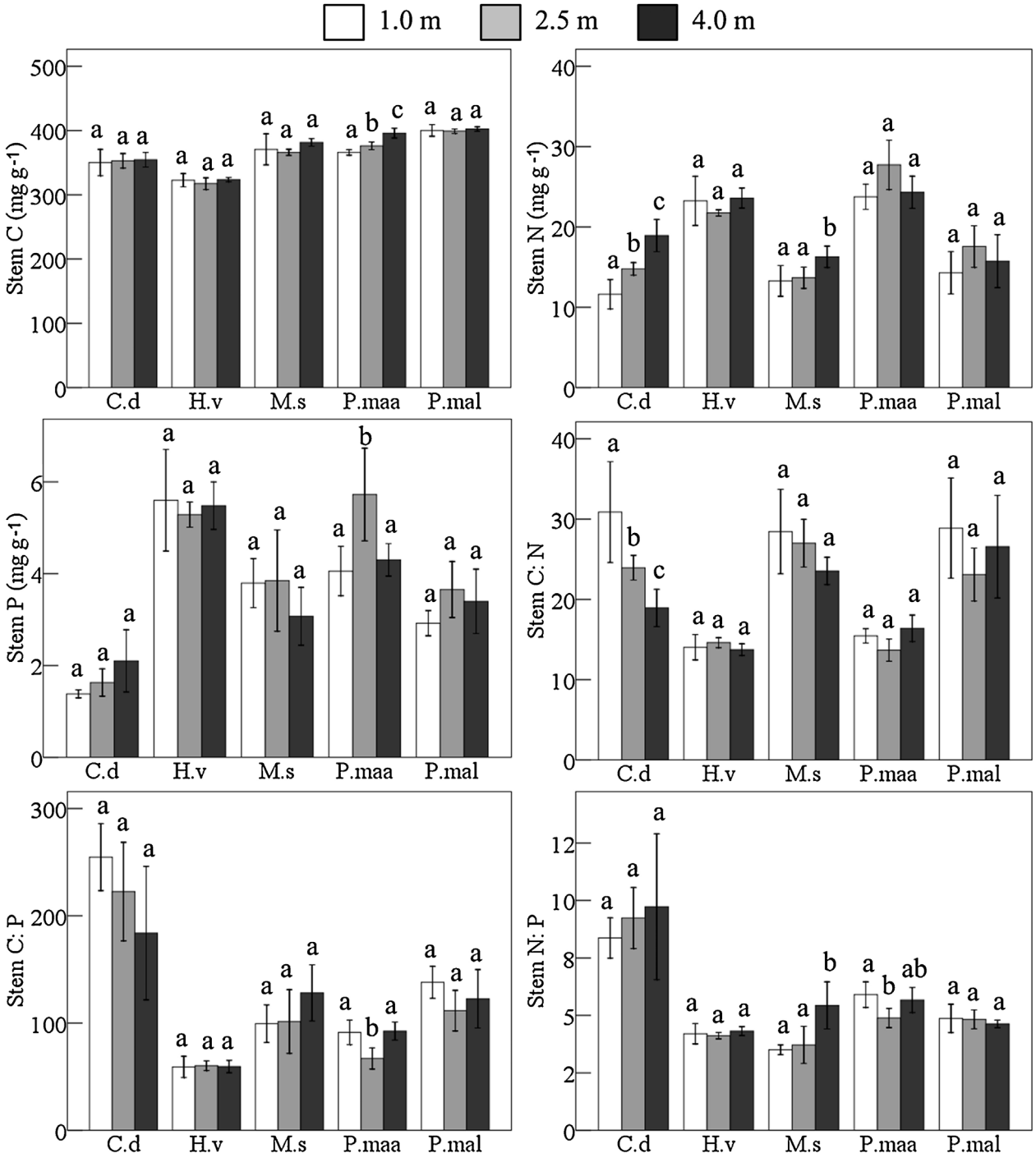

Fig. 4. The C, N and P concentrations and C:N:P stoichiometry in the stems of *C. demersum* (C.d), *H. verticillata* (H.v), *M. spicatum* (M.s), *P. maackianus* (P.maa) and *P. malaianus* (P.mal) grown at different water depths. The vertical bars are standard deviations, and the different letters are significantly different at $P < 0.05$ level among the different water depths.

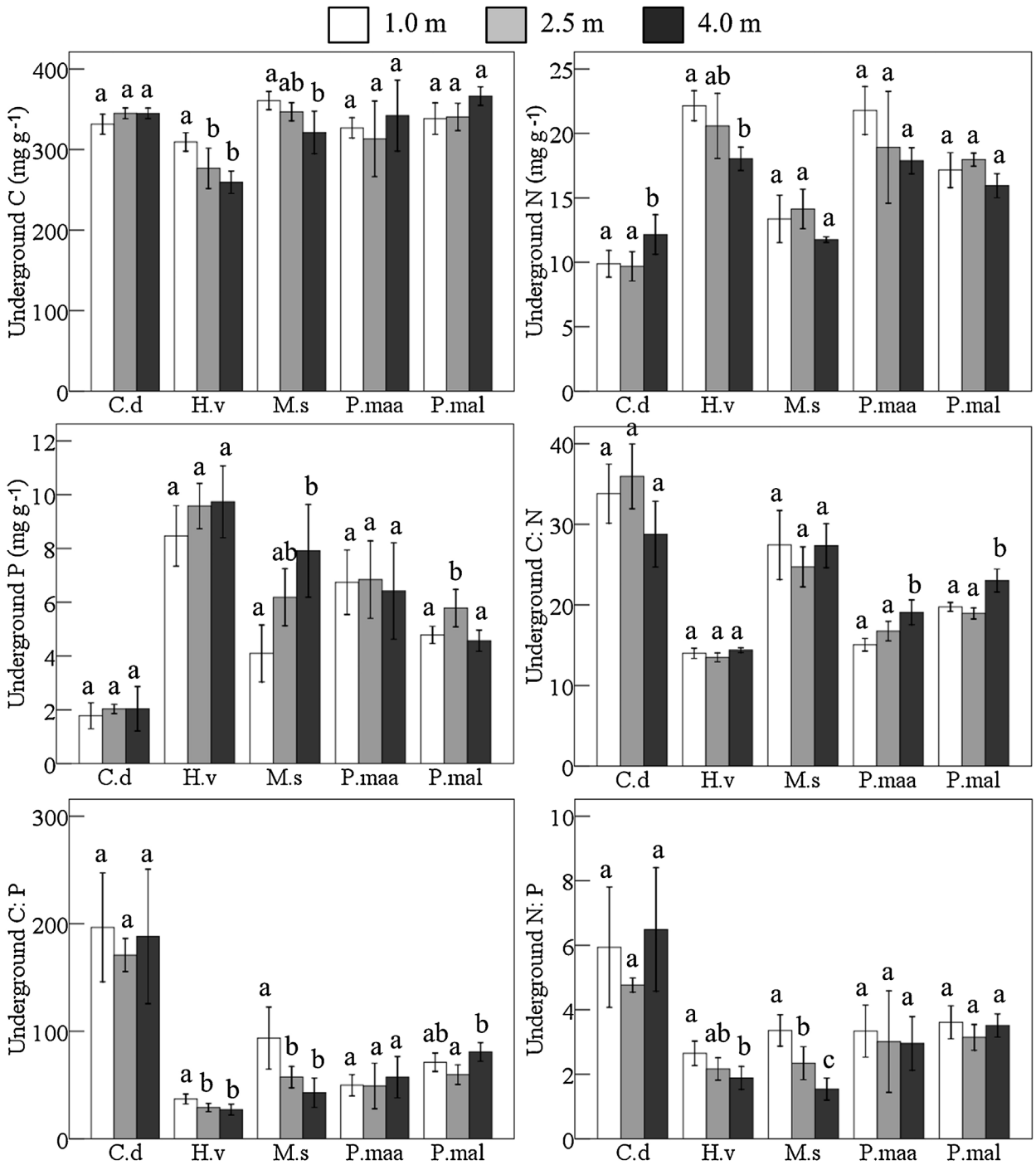

Fig. 5. The C, N and P concentrations and C:N:P stoichiometry in the underground parts of *C. demersum* (C.d), *H. verticillata* (H.v), *M. spicatum* (M.s), *P. maackianus* (P.maa) and *P. malaianus* (P.mal) grown at different water depths. The vertical bars are standard deviations, and the different letters are significantly different at $P < 0.05$ level among the different water depths.

6.97 at 1.0 m water depth, respectively, and did not differ from those at 2.5 and 4.0 m water depths. The N:P ratios in leaves of *P. maackianus* and *P. malaianus* were 6.59 and 7.01 at water depth of 1.0 m, respectively, and decreased with the increased water depth (Fig. 3).

The various water depths did not affect the C, N and P concentrations and C:N, C:P and N:P ratios in stems of all the macrophytes except for enhancing the N and P concentrations and decreasing the C:N ratios in stems of *C. demersum* (Fig. 4). As for the underground parts of the plants, the increased water depth decreased the C and N concentrations of *H. verticillata*, increased the N concentrations of *C. demersum* and P concentrations of *M. spicatum*, increased the C:N ratios of *P. malaianus*, and decreased the C:P and N:P ratios of *H. verticillata* and *M. spicatum* (Fig. 5). Irrespective of the differences in water depth, the C and N concentrations in the plant organs were ranked by leaf > stem > underground part, and the P concentrations were lowest in the stems of all the macrophytes (Fig. 6).

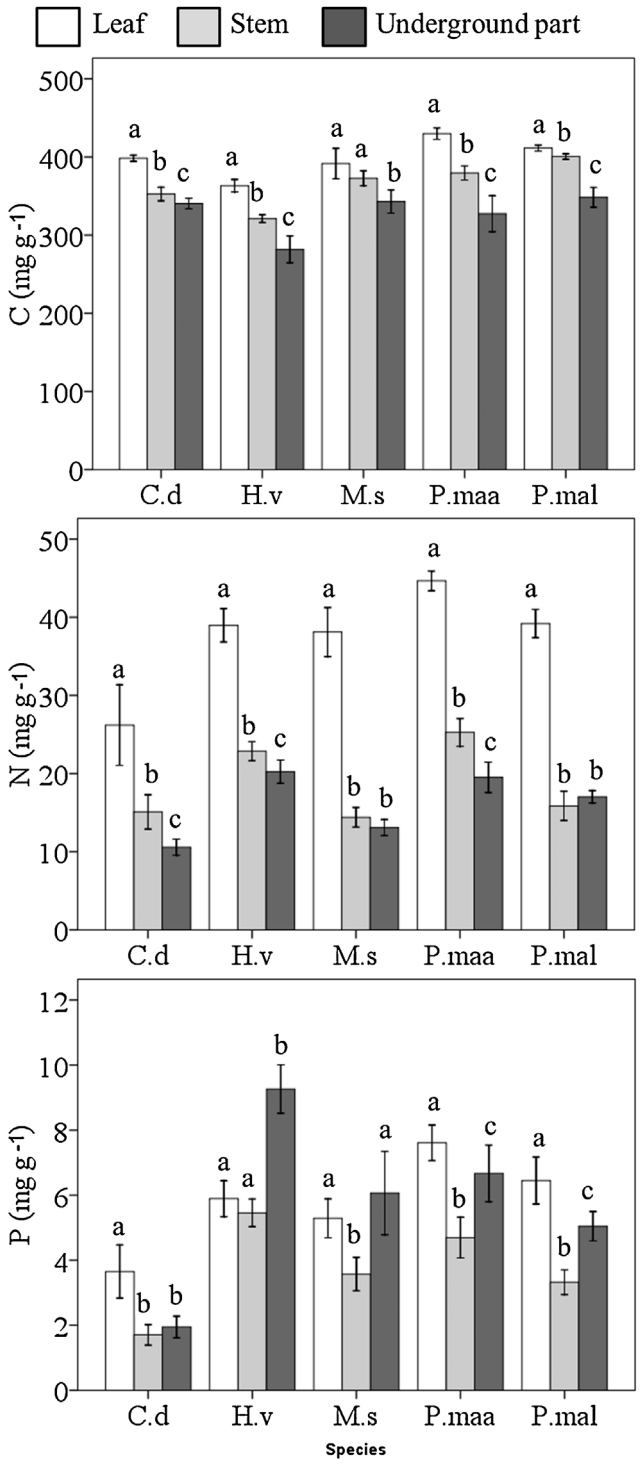

Fig. 6. The mean C, N and P concentrations in the leaves, stems and underground parts of *C. demersum* (C.d), *H. verticillata* (H.v), *M. spicatum* (M.s), *P. maackianus* (P.maa) and *P. malaianus* (P.mal) grown at different water depths. The vertical bars are standard deviations, and the different letters are significantly different at $P < 0.05$ level among the different water depths.

4. Discussion

The present study revealed that most of the variance in the examined C, N and P concentrations and C:N:P stoichiometry were due to the inter-species and the organ-specific differences of the five submersed macrophytes, and the differences in water depth made relatively small contributions to the C:N:P stoichiometric variances. However, growth of the plants was significantly affected by the water depth, indicating that the submersed macrophytes were capable of maintaining stoichiometric homeostasis in response to varying water levels. Many studies which focused on terrestrial plant stoichiometry revealed that the N and P stoichiometry is affected by many factors, such as soil fertility, temperature, developmental stage and herbivores (Güsewell, 2004; Reich and Oleksyn, 2004; Yu et al., 2011; Zhang et al., 2011). Plant growth is generally inhibited by external N and P availability in infertile environments, and thus rapid growth of the plants dilutes the N and P concentrations in the plant tissues (Cronin and Lodge, 2003; Yan et al., 2006). The present study found that the increased water depth enhanced the N and P concentrations in leaves and stems of *C. demersum* and did not affect its growth. Yet, the increased water depth reduced the RGR of the other four macrophytes and did not affect their N and P concentrations, indicating that the N and P stoichiometry of the five macrophytes were weakly coupled to their RGR. Unlike previous observations in fast growing plants, the effects of water depth on the N and P stoichiometry could not result from the dilution effects of the plant growth. In eutrophic lakes, submersed macrophytes can absorb nutrients from both the surrounding water and the sediments, and the water depth-dependent light availability is the primary factor affecting growth of the plants. In this experiment, the homogeneous nutrient concentrations in the water column and the sediments, coupled with the limited growth of the plant, might have diminished the stoichiometric variance of the plants at the various water depths.

In the present study, *C. demersum* responded to the increased water depth contrast with the responses of the other four macrophytes in term of N and P concentrations in the leaves and stems and the plant growth, indicating species-specific responses to water depth. The average values of N:P ratios of the five macrophytes in our experiment were 6.68 in the leaves, 5.56 in the stems and 3.38 in the underground parts, respectively. These values are lower than the critical N:P ratios (<14) indicating N limitation of many plants (Koerselman and Meuleman, 1996; Tessier and Raynal, 2003). In our experiment, the N and P concentrations in leaves and stems of *H. verticillata*, *M. spicatum*, *P. maackianus* and *P. malaianus* were relatively stable, and growth of the plants differed greatly at the various water depths, with *C. demersum* showing contrast responses with the other four macrophytes, suggesting that the N:P ratios could not indicate N or P limitation of the plants when their growth was primarily limited by the low light availability in eutrophic lakes. Our results are consistent with the results of Madsen and Cedergreen (2002) and Cao et al. (2011) when submersed macrophytes were cultured in fertile environments, but inconsistent with the results of Olff (1992) and Cronin and Lodge (2003), who found that low light availability increased the N concentrations of submersed macrophytes. These conflicting results imply that water depth-dependent light availability affected the N and P concentrations of submersed macrophytes, but the effects were species-specific and varied along environmental fertility.

Consistent with some previous findings in wetland plants (McJannet et al., 1995; Güsewell and Koerselman, 2002) and seagrass (Duarte, 1990), submersed macrophytes had considerable variation in C, N and P concentrations, C:N, C:P and N:P ratios in different organs and higher C and N concentrations in leaves in the present study. The variation of C, N and P concentrations and C:N, C:P, N:P ratios was highest in the underground part, middle in the stems, and lowest in the leaves, indicating that submersed macrophytes could maintain relatively stable foliar nutritional stoichiometry by modulating the stoichiometric composition of other parts in different environments, and did support our second assumption. These results were consistent with those of Yu

et al. (2011), who examined vascular plants in the Inner Mongolia grassland.

5. Conclusions

This study is one of a few investigations that use *in situ* experiment to evaluate the effects of water depth on C, N and P stoichiometry of submersed macrophytes. We found that the plant species and their organs explained most of the variance in the C:N:P stoichiometry, indicating strong stoichiometric homeostasis in the five submersed macrophytes. The increased water depth significantly inhibited growth of the plants but weakly affected the plant C, N and P stoichiometry.

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References

Ågren, G.I., 2004. The C:N:P stoichiometry of autotrophs – theory and observations. Ecol. Lett. 7, 185–191.
Andersen, T., Elser, J.J., Hessen, D.O., 2004. Stoichiometry and population dynamics. Ecol. Lett. 7, 884–900.
Blom, C.W.P.M., Voesenek, L.A.C.J., 1996. Flooding: the survival strategies of plants. Trends Ecol. Evol. 11, 290–295.
Cao, T., Ni, L.Y., Xie, P., et al., 2011. Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. Freshwater Biol. 56, 1620–1629.
Chambers, P.A., 1987. Light and nutrients in the control of aquatic plant community structure, II. In situ observation. J. Ecol. 75, 621–628.
Chambers, P.A., Kalff, J., 1985. Depth distribution and biomass of submersed aquatic macrophyte communities in relation to secchi depth. Can. J. Fish. Aquat. Sci. 42, 701–709.
Chambers, P.A., Kalff, J., 1987. Light and nutrients in the control of aquatic plant community structure, I. In situ experiments. J. Ecol. 75, 611–619.
Cronin, G., Lodge, D.M., 2003. Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. Oecologia 137, 32–41.
Demars, B.O.L., Edwards, A.C., 2007. Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biol. 52, 2073–2086.
Duarte, C.M., 1990. Seagrass nutrient content. Mar. Ecol. Prog. Ser. 67, 201–207.
Duarte, C.M., 1992. Nutrient concentration of aquatic plants: patterns across species. Limnol. Oceanogr. 37, 882–889.
Elser, J.J., Fagan, W.F., Dobberfuhl, D.R., et al., 2000a. Nutritional constrains in terrestrial and freshwater food webs. Nature 408, 578–580.
Elser, J.J., Sterner, R.W., Gorokhova, E., et al., 2000b. Biological stoichiometry from genes to ecosystems. Ecol. Lett. 3, 540–550.
Elser, J.J., Fagan, W.F., Kerkhoff, A.J., et al., 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytol. 186, 593–608.
Elser, J., Bracken, M.E.S., Cleland, E.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.
Fu, H., Yuan, G., Cao, T., et al., 2012. An alternative mechanism for shade adaptation: implication of allometric responses of three submersed macrophytes to water depth. Ecol. Res. 27, 1087–1094.
Fu, H., Yuan, G., Zhong, J., et al., 2013. Environmental and ontogenetic effects on intraspecific trait variation of a macrophyte species across five ecological scales. PLoS ONE 8 (4), e62794.
Geest, G.J., Wolters, H., Roozen, F.C.J.M., et al., 2005. Water-level fluctuations affect macrophyte richness in floodplain lakes. Hydrobiologia 539, 239–248.
Güsewell, S., Koerselman, W., 2002. Variation in nitrogen and phosphorus concentrations of wetland plants. Perspect. Plant Ecol. 5, 37–61.
Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243–266.
He, J.S., Fang, J.Y., Wang, Z., et al., 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grasslands of China. Oecologia 149, 115–122.
Hu, L., Hu, W., Zhai, S., et al., 2010. Effects on water quality following water transfer in Lake Taihu, China. Ecol. Eng. 36, 471–481.
Huang, X., 2000. Survey, Observation and Analysis of Lake Ecology. Standards Press of China, Beijing (in Chinese).
Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33, 1441–1450.
Kuo, S.,1996. Phosphorus. In: Methods of Soil Analysis. Part 3: Chemical Methods. Soil Science Society of America, Madison, WI, USA, pp. 869–919.
Liang, X.Q., Fang, J.Z., Yang, H.Q., 1996. Hydrobiology: Morphology and Taxology. China Agriculture Press, Beijing (in Chinese).
Reed, LüX.T., Yu, S.Q., et al., 2013. Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semi-arid grassland. Global Change Biol., http://dx.doi.org/10.1111/gcb.12235.
Madsen, T.V., Cedergreen, N., 2002. Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. Freshwater Biol. 47, 283–291.
Matzek, V., Vitousek, P.M., 2009. N:P stoichiometry and protein: RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. Ecol. Lett. 12, 765–771.
McJannet, C.L., Keddy, P.A., Pick, F.R., 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. Funct. Ecol. 9, 231–238.
Olff, H., 1992. Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species. Oecologia 89, 412–421.
Rattray, M.R., Howard-Williams, C., Brown, J.M.A., 1991. Sediment and water as sources of nitrogen and phosphorus for submerged rooted aquatic macrophytes. Aquat. Bot. 40, 225–237.
Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proc. Natl. Acad. Sci. U.S.A. 101, 11001–11006.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, NJ.
Strand, J.A., Weisner, S.E.B., 2001. Morphological plastic responses to water depth and wave exposure in an aquatic plant (*Myriophyllum spicatum*). J. Ecol. 89, 166–175.
Tessier, J.T., Raynal, D.J., 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40, 523–534.
Vanni, M.J., 2002. Nutrient cycling by animals in freshwater ecosystems. Annu. Rev. Ecol. Syst. 33, 341–370.
Xing, W., Wu, H.P., Hao, B.B., et al., 2013. Stoichiometric characteristics and responses of submerged macrophytes to eutrophication in lakes along the middle and lower reaches of the Yangtze River. Ecol. Eng. 54, 16–21.
Yan, X., Yu, D., Li, Y.K., 2006. The effects of elevated CO_2 on clonal growth and nutrient content of submerge plant *Vallisneria spinulosa*. Chemosphere 62, 595–601.
Yu, Q., Elser, J.J., He, N., et al., 2011. Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland. Oecologia 166, 1–10.
Yu, Q., Wu, H., He, N., et al., 2012. Testing the growth rate hypothesis in vascular plants with above- and below-ground biomass. PLoS ONE 7 (3), e32162.
Yuan, G., Cao, T., Fu, H., et al., 2013. Linking carbon and nitrogen metabolism to depth distribution of submersed macrophytes using high ammonium dosing tests and a lake survey. Freshwater Biol., http://dx.doi.org/10.1111/fwb.12230.
Zhang, G., Han, X., Elser, J.J., 2011. Rapid top–down regulation of plant C:N:P stoichiometry by grasshoppers in an Inner Mongolia grassland ecosystem. Oecologia 166, 253–264.
Zhang, H., Wu, H., Yu, Q., et al., 2013. Sampling date, leaf age and root size: implications for the study of plant C:N:P stoichiometry. PLoS ONE 8 (4), e60360.
Zhu, G., Li, W., Zhang, M., et al., 2012. Adaptation of submerged macrophytes to both water depth and flood intensity as revealed by their mechanical resistance. Hydrobiologia 696, 77–93.