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THEMATIC ISSUE

Size-dependent C, N and P stoichiometry of three submersed macrophytes along water depth gradients

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Abstract Variability in biomass allocation and growth rate of submersed macrophytes along water depth gradients may lead to different carbon (C), nitrogen (N) and phosphorus (P) stoichiometric characteristics. We conducted a field investigation to evaluate long-term effects of water depth on C, N and P stoichiometry of three submersed macrophytes, *Potamogeton maackianus*, *Myriophyllum spicatum* and *Ceratophyllum demersum*. The results indicated that shoot C:N, C:P and N:P of the plants tended to increase with elevated water depths, and patterns of biomass allocation along water depth gradients were more important than biological dilution of increased growth rates in affecting shoot C:N:P stoichiometric characteristics of the plants. Partial correlation analysis using shoot height and biomass as covariates revealed that water depth significantly affected C:P ratios in shoots of *P. maackianus* and *M. spicatum* and C:N ratio in shoots of *M. spicatum*, but did not affect N:P ratios of all the plants. Shoot stoichiometry of *M. spicatum* was most sensitive in response to water depth, followed by *P. maackianus*, and that of *C. demersum* was really unchanged with elevated water depths. Our results suggested that strategies in biomass allocation in organs, which depend largely on the species identity, rather than growth rates of the plants, contributed mainly to variation in the observed element stoichiometry along the water depth gradients.

Keywords Submersed macrophyte · Water depth · Stoichiometry · Biomass · Height

Introduction

Submersed macrophytes are important primary producers and play a key role in maintaining stability of clear water state in shallow lakes (Scheffer et al. 1992; Ni 2001). Submersed macrophytes occupy littoral zone in lakes, where water level fluctuation affects growth and distribution of the plants (Strand and Weisner 2001; Fu et al. 2012; Zhu et al. 2012). Water level fluctuation alters light availability in water column, waves and water–air gas exchange, sediment texture and element composition, which affect physiology, morphology, biomass allocation, growth rate and distribution of submersed macrophytes (Strand and Weisner 2001; Fu et al. 2012; Yuan et al. 2013; Christia et al. 2014). Growth of submersed macrophyte is reduced in deep water due to low light availability (Fu et al. 2012; Zhu et al. 2012; Li et al. 2013). Most submersed macrophytes tend to allocate more biomass to stem and increase shoot height in deep water so as to alleviate low light stress (Strand and Weisner 2001; Fu et al. 2012).

Carbon (C), nitrogen (N) and phosphorus (P) are three essential elements of organisms, and have strong interactions in biological function (Sterner and Elser 2002). Along a water depth gradient, two distinct ways might potentially lead to changes in shoot C:N:P stoichiometric characteristics of submersed macrophytes. Firstly, C, N and P

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concentrations ([C], [N] and [P]) vary considerably in different organs of the plants, e.g., [N] and [P] in leaves were 1.5- to 2-fold higher than those in stems in many plants (Li et al. 2013). In deep water, the plants allocated less biomass to the leaf and more biomass to the stem, and thus led to lower shoot [N] and [P] (higher C:N and C:P ratios) as compared with the plants grown in shallow water. Secondly, shoot [N] and [P] decreased for plants with high growth rate due to dilution of tissue N and P by increased C fixation (Cronin and Lodge 2003; Yan et al. 2006). Submersed macrophytes in deep water had low growth rate (Cao et al. 2011; Fu et al. 2012; Li et al. 2013), which might not dilute [N] and [P] as fast as plants with high growth rate in shallow water. In this point of view, reduced growth rates of submersed macrophytes in deep water may increase shoot [N] and [P] of the plants (thus lower C:N and C:P ratios).

Studies on stoichiometric characteristics of submersed macrophytes are numerous. However, very few studies emphasized effects of water depth. Our previous study hade valuated effects of water depths on C, N and P stoichiometric characteristics of five submersed macrophytes in a short-term (1 month) in situ experiment, and the results implied that water depth significantly affected the plant growth and C:N:P stoichiometry, and its effects were week as compared to the effects induced by plant species and organs (Li et al. 2013). However, the short-term results could not be extrapolated directly to evaluate long-term effects of water depths in natural lakes. In this study, we conducted a field investigation on stoichiometric characteristics of submersed macrophytes growing at natural water depth gradients, with aim to specify effects of biomass allocation versus biological dilution on C:N:P stoichiometry along water depth gradients. As growth of submersed macrophytes was expected to be limited by low light availability at deep water, and then the relative importance of biomass allocation versus biological dilution could be tested by relationships between shoot C:N and C:P of the plants and water depths, with positive correlation indicating biomass allocation more important while negative correlation meaning biological dilution more important. Furthermore, we measured shoot biomass and height of the plants to evaluate whether effects of water depth on C:N:P stoichiometry were size-dependent.

Materials and methods

Field survey of submersed macrophytes

The study was carried out in June, 2010, in the Haichao bay of Lake Erhai (25°52′N, 100°06′E) in subtropic Yunnan Plateau, China. The lake has a mesotrophic status, moderate water depth (max. 20.5 m, mean 10.5 m) and total area of 249.8 km^2 (Wang and Dou 1998). The Haichao bay, which located in the northern part of Lake Erhai, is an important distribution area of submersed macrophytes (He et al. 2015). It has well-developed gentle slopes and fertile sediment in the littoral zone (0.5–5 m water depth) allowing us to sample submersed macrophytes at different water depths.

Three submersed macrophytes, *Potamogeton maackianus*, *Myriophyllum spicatum* and *Ceratophyllum demersum* were chosen in the study. They are canopy formers with similar morphological strategies in response to low light stress that most of their leaves and branches concentrate at or near the water surface (Chambers and Kalff 1987). They occur ubiquitously in the littoral zone (0.5–5 m water depth) of Lake Erhai, and are easily sampled in the field. These three macrophytes differ in leaf morphology that *P. maackianus* has alternate, oblong or linear entire leaves, *M. spicatum* owns divided and feather-like leaves, and *C. demersum* is rootless with whorled palmate dissected leaves (Zhu et al. 2012), giving opportunity for examining effects of different biomass allocations on C:N:P stoichiometry of the plants.

Submersed macrophytes were sampled at intervals of 0.5 m water depth along transects from the shore to the deepest depth where the plants occurred. At transects with a steep lake bottom, submersed macrophytes were sampled at fewer sites. Above-ground parts (i.e. shoots above the surface of sediment) of the above three plant species were collected by an underwater rotatable reaping hook at different water depths. The samples were then put into icebox and taken back to laboratory for further analysis. We also measured water transparency and under water light intensity in several sample sites and collected corresponding water samples for chemical analysis, to get background data.

Water transparency was measured using a secchi's disc. Photosynthetic active radiation (PAR) was measured at subsurface, 1 and 2 m water depths by a Li-COR UWQ-192S sensor coupled with a Li-1400 data logger (Li-Cor, Lincoln, NE, USA). Light extinction coefficient (K_d) was calculated according to the equation: $K_d = (1/z) \ln (I_0/I_z)$ (Krause-Jensen and Sand-Jensen 1998), where I_0 and I_z are values of PAR at subsurface and at depth z (m) below the water surface, respectively.

Measurements of plant traits and water chemicals

Intact individual plants were sorted from each sample by species and water depth, washed carefully with tap water and used for examining the plant height. Then these samples were oven-dried at 80 °C to constant weight and ground into fine powder for analysis of [C], [N] and [P]. In total, we got 28, 24 and 12 samples for *P. maackianus*, *M. spicatum* and *C. demersum* in different water depths, respectively. [C] and [N] were determined using an elemental analyzer (Flash EA 1112 series, CE Instruments, Italy). [P] was measured using sulfuric acid/hydrogen peroxide digest and the ammonium molybdate ascorbic acid methods (Kuo 1996). Total N, P, NH_4-N, NO_3-N and PO_4-P contents in water samples were determined according to standard methods (Huang 2000).

In this survey, water temperature was ca. 24 °C. On average, water transparency was 2.2 m, light extinction coefficient in water was 0.546, and the mean concentrations of total N, P, NH_4-N, NO_3-N and PO_4-P in water column were 1.099 ± 0.167, 0.037 ± 0.007, 0.240 ± 0.057, 0.041 ± 0.034 and 0.024 ± 0.008 mg L^{-1}, respectively.

Statistical analysis

Standardized major axis (SMA) slope-fitting technique was used in this study. It is widely used in ecology and evolution, which summarize the relationship between two variables and can minimize the residuals of variables in both axes. SMA regression analysis was conducted using “smatr” function in R (version 3.1.2) (Warton et al. 2006). Partial correlation analysis for relationships between water depth and plant stoichiometric characteristics (shoot height and biomass as covariates) was carried out with SPSS 16.0 software package (SPSS Inc., Chicago).

Results

In the field investigation, *P. maackianus*, *C. demersum* and *M. spicatum* were found at water depths of 0.4–4.6, 0.4–3.7 and 0.4–4.8 m, respectively, with biomass of the plants ranging from 0.07 to 1.10, 0.44 to 2.51 and 0.32 to 2.88 g, and the plant height ranging from 0.60 to 3.21, 1.03 to 3.46 and 1.16 to 2.94 m. Water depth affected shoot size of the plants significantly as indicated by increased shoot height of the three macrophytes and increased shoot biomass of *P. maackianus* with increasing water depths (Fig. 1). Base on SMA regression analyses, shoot height significantly positively correlated with biomass for *P. maackianus* and *M. spicatum*, but not for *C. demersum* (Fig. 2).

Shoot stoichiometric characteristics of the plants were significantly affected by water depth. They showed clearly increasing tendency along water depth gradient (Fig. 3). However, interspecific differences also existed. For *M. spicatum*, shoot C:N, C:P and N:P were all increased with elevated water depths. For *P. maackianus*, shoot C:P and N:P were increased with elevated water depths, while shoot C:N was uncorrelated with water depth. For *C. demersum*, only shoot C:N was increased with elevated water depths, while both shoot C:P and N:P were uncorrelated with water depth.

Partial correlation analysis using shoot height and biomass as covariates revealed that water depth significantly affected C:P ratios in shoots of *P. maackianus* and *M. spicatum* and C:N ratio in shoots of *M. spicatum*, but did not affect N:P ratios of all the plants. In addition, stoichiometry of *C. demersum* was not affected by water depth according to results of the partial correlation analysis, which indicated that effects of water depth on shoot stoichiometric characteristics of *P. maackianus* and *C. demersum* were size-dependent (Table 1). However, the correlation was still robust for *M. spicatum* whenever shoot height and biomass were used as covariates, which indicated that effects of water depth on shoot stoichiometric characteristics of this species were size-dependent.

Discussion

Shoot C:N, C:P and N:P of *M. spicatum*, *C. demersum* and *P. maackianus* trended to increase with increasing water depth, indicating that different biomass allocation between leaves and stems of the plants induced by various water depths was more important than growth rate-mediated biological dilution in affecting shoot C:N:P stoichiometry. [N] and [P] in leaves were much higher than those in stems of the studied macrophytes (Li et al. 2013), and in deep eutrophic water when the plants experienced low light stress they allocated more biomass to stem for shoot elongation toward water surface (Strand and Weisner 2001; Fu et al. 2012), which leads to decreased [N] and [P] and increased C:N and C:P in the shoots. Furthermore, in eutrophic water submersed macrophytes could uptake nutrients from sediment and water column and accumulate much more N and P in tissues than their requirement for growth (Rattray et al. 1991; Cao et al. 2011), counteracting effects of growth rate-mediated biological dilution on stoichiometry of the studied plants. *C. demersum* is rootless and can float to water surface to alleviate low light stress in eutrophic water, which might have diminished effects of biomass allocation on its stoichiometry.

Generally, plant [N] and [P] increase synergistically because of their closely coupling in various biological functions, leading relatively constant N:P ratios (Güsewell 2004; Yu et al. 2011). However, the present study indicated that shoot N:P followed the same trend with shoot C:N and C:P of the plants i.e. increased with elevated water depth. This may be due to higher variation of [P] than [N], which resulted in P, rather than N, determining variation of shoot N:P. In addition, evidence in our study also shown that shoot N:P of the plants was increased with C:P and

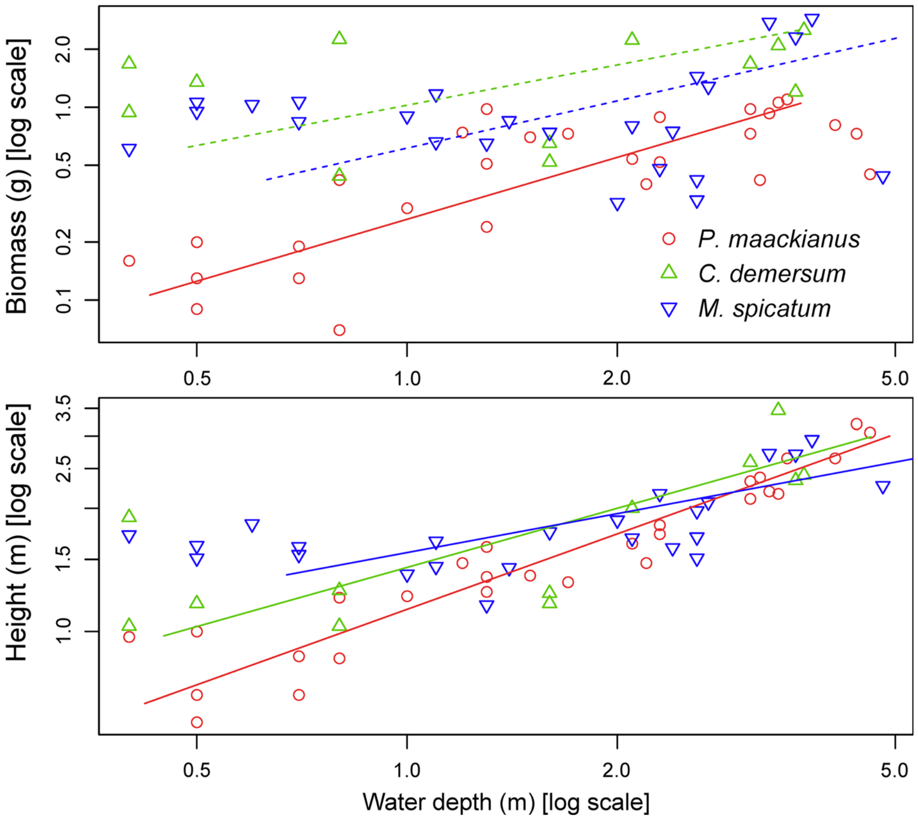

Fig. 1 SMA regression for correlations between water depth and shoot size (height and biomass) of submersed macrophytes *P. maackianus*, *C. demersum* and *M. spicatum*. *Solid fitted lines* indicated the correlations were significant at the level of $p < 0.05$, while *dashed fitted line* indicated the correlations were not significant

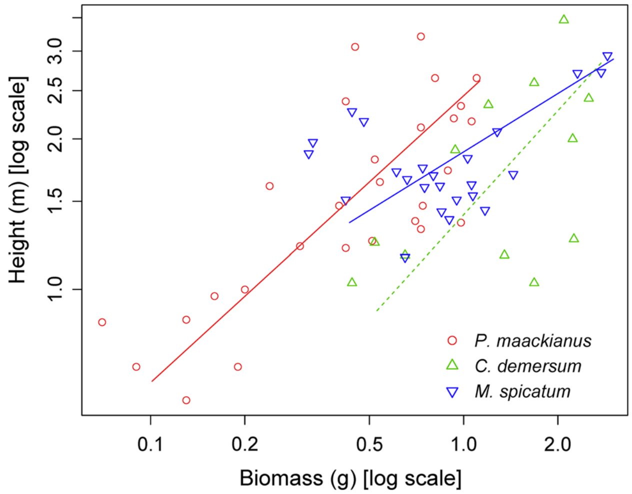

Fig. 2 SMA regression for correlations between shoot biomass and shoot height of submersed macrophytes *P. maackianus*, *C. demersum* and *M. spicatum*. *Solid fitted lines* indicated the correlations were significant at the level of $p < 0.05$, while *dashed fitted line* indicated the correlations were not significant

decreased with [P], while uncorrelated with C:N and [N], which was in consistent with the study of Duarte (1992) and He et al. (2008).

Interspecific differences in stoichiometry in response to water depths were observed in the present study. *M. spicatum* has high shoot [P], low C:P and N:P, possibly due to its much higher affinity and uptake rate of P than many aquatic macrophytes (Zhang et al. 2011). *M. spicatum*can grow in a large range of light regimes (Strand and Weisner 2001; Song et al. 2010; Li et al. unpublished data), but high light availability facilitates its P uptake rate (Zhang et al. 2011), and thus effect of water depth on shoot [N], [P], C:N and C:P were more significant than those of *C. demersum* and *P. maackianus*.

Water depth and its interaction with plant species did affect plant C, N and P stoichiometry more significantly in the natural lake Erhai than in our previous in situ experiment (Li et al. 2013). This is not surprising as nutrients in water and sediment were more homogeneous in the in situ experiment than in natural habitats. Generally, light intensity is sufficient for growth of submersed macrophytes in shallow water, where intensive competition may lead to insufficient resources for plant growth across space and time, while the contrasting situations would be expected in deep water. Submersed macrophytes have large phenotypic plasticity ingrowth rate, biomass allocation and stem elongation in response to different water depths (Strand and Weisner 2001; Fu et al. 2012; Zhu et al. 2012; Bai et al. 2013). However, this phenotypic plasticity did not largely change the internal tissue element ratios of submersed macrophytes as also indicated by the evidence in our study

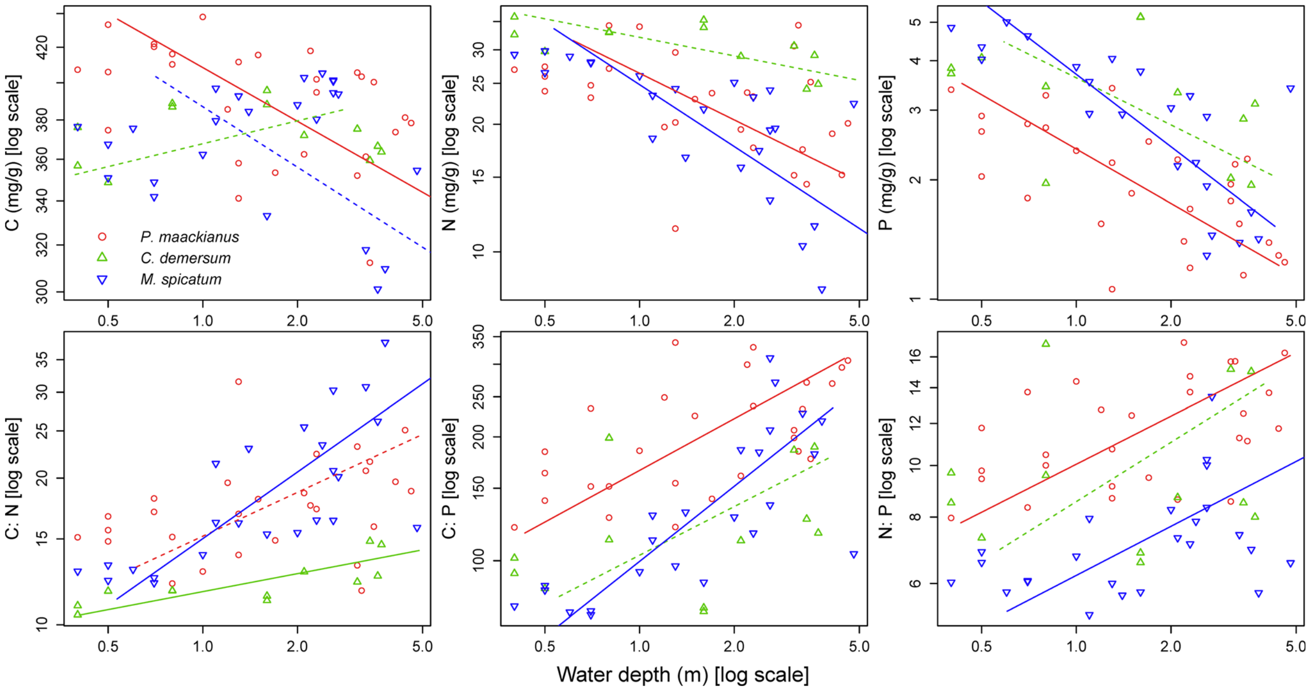

Fig. 3 SMA regression for correlations between water depth and shoot stoichiometric characteristics of submersed macrophytes *P. maackianus*, *C. demersum* and *M. spicatum*. *Solid fitted lines* indicated the correlations were significant at the level of $p < 0.05$, while *dashed fitted line* indicated the correlations were not significant

Table 1 Partial correlation coefficients for relationships between WD and plant traits (plant nutrient concentrations and their ratios) of submersed macrophytes *P. maackianus*, *C. demersum* and *M. spicatum*

Species	C	N	P	C:N	C:P	N:P
P. maackianus	−0.344	−0.313	−0.543*	0.206	0.409*	0.346
C. demersum	0.302	−0.370	−0.084	0.534	0.258	0.152
M. spicatum	0.377	−0.771**	−0.723**	0.698**	0.637**	0.362

Significance is indicated by * and ** at the level of $p < 0.05$ and 0.01, respectively

that responses of shoot stoichiometric characteristics of *P. maackianus* and *C. demersum* to water depths were plant size-dependent.

Conclusions

The present study suggested that shoot C:N:P stoichiometric characteristics of the sampled plants were significantly affected by water depth. They showed clearly increasing tendency along water depth gradient. This was mainly due to biomass allocation between leaf and stem in various water depths. The results also showed inter specific differences of shoot traits in response to water depth. Shoot stoichiometry of *M. spicatum* was most sensitive in response to water depth, followed by *P. maackianus*, and that of *C. demersum* was really unchanged with elevated water depths. Effects of water depth on shoot stoichiometric characteristics of *P. maackianus* and *C. demersum* were size-dependent as indicated by partial correlation analysis that the correlations were disappeared when shoot size (shoot height and biomass) were used as covariates.

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