



Research article

Eutrophication triggers the shift of nutrient absorption pathway of submerged macrophytes: Implications for the phytoremediation of eutrophic waters



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ARTICLE INFO

Keywords:

Eutrophication

Nutrient

Potamogeton crispus

Absorption pathway

Phytoremediation

ABSTRACT

Ecologically restoring eutrophic water bodies by using submerged macrophytes is an economical, effective and sustainable technology worldwide. However, current understanding on the nutrient absorption pathway of submerged macrophytes in freshwater ecosystems, especially under different trophic states, is still limited. In this study, two strategically designed systems were established to form isolated units for preventing nutrient exchange amongst *Potamogeton crispus*, water column and sediments. Results showed that, in oligotrophic state, *P. crispus* mainly relied on their roots to absorb nutrients from sediments for maintaining stable growth, with the maximum average height, fresh weight and relative growth rate of 12.85 cm, 4.86 g ind⁻¹ and 0.062, respectively. However, the eutrophic conditions (TN of 4 mg L⁻¹ and TP of 0.3 mg L⁻¹) triggered the shift of the nutrient absorption pathway from the roots to the shoots to some extent, that is, the shoots of *P. crispus* gradually became a remarkable pathway to directly absorb nutrients from the water column. Approximately 49.85% and 18.35% of total nitrogen (TN) and total phosphorus (TP) from overlying water were allocated to the shoots of *P. crispus*, but had no effects on the growth, photosynthesis and ecological stoichiometric differences ($p > 0.05$). Sediments acting as a nitrogen (N) source supported nearly 11.56% of TN for shoot uptake and simultaneously received around 13.33% of TP subsidy from the overlying water. The no longer sole dependence of submerged macrophytes on their root system to absorb N and phosphorus nutrients indicated that the ability of shoots to absorb nutrients increased with the gradual increase in nutrients in water column. These findings imply that the large specific surface area of shoots is beneficial for restoring eutrophic waters.

1. Introduction

Nitrogen (N) and phosphorus (P) are fundamental elements for organisms (Liu et al., 2013; Li et al., 2017). However, overloading of anthropogenic nutrients, particularly N and P, in shallow lakes can drive regime shifts from a clear state to a turbid state; this shift causes subsequent destruction of aquatic habitats and decrease in biodiversity (Scheffer et al., 1993; Perrow et al., 1997; Scheffer and Nes, 2007). The natural microorganisms can remove nutrients through several pathways (Sepehri and Sarrafzadeh, 2018), while they are not enough to

control the excess nutrients without artificial enhancement. Replanting of submerged macrophytes to restore water quality and improve ecological functions is a useful strategy for lakes that have gone through eutrophication processes (Coops and Doef, 1996; Lau and Lane, 2002; Ciarli et al., 2009). Phytoremediation plays a fundamental role in water purification via competing with microalgae for nutrients, which leads to the control of excessive phytoplankton development and the prevention re-suspension of sediments (Lau and Lane, 2002; Horppila and Nurminen, 2003; Scheffer and Nes, 2007). Therefore, ecologically restoring eutrophic water bodies by using submerged macrophytes is an

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<https://doi.org/10.1016/j.jenvman.2019.03.069>

Received 27 December 2018; Received in revised form 25 February 2019; Accepted 14 March 2019

Available online 26 March 2019

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emerging cost-effective, eco-friendly and stable technology worldwide.

Submerged macrophytes can tolerate a certain degree of nutritional loadings (Smith and Adams, 1986; Li et al., 2008; Zhou et al., 2017). They frequently occupy the mosaic niches in freshwater ecosystems because the available nutrients are spatiotemporally heterogeneous in natural habitats even on a small scale. The lack or excess of any elements can cause imbalance of internal element proportions of organisms and affect their growth. For example, high nutrient loadings can considerably affect the growth of submerged macrophytes and reduce their density (Li et al., 2008; Cai et al., 2012; Olsen et al., 2015; Zhao et al., 2016). In Danish lakes, the density of submerged macrophytes is increased by reducing the nutrient loadings (Lauridsen et al., 2003). In the recent 30 years, plant density has gradually declined in Taihu Lake, and the decreasing trend continues to date (Zhao et al., 2017). In addition, the physiological characteristics of submerged macrophytes, such as photosynthetic rate, enzyme activity and ecological stoichiometry, may be considerably affected (Pflugmacher, 2004; Méndez and Karlsson, 2005). These observations elucidate the considerable eco-physiological effects of the change in nutrient concentrations on submerged macrophytes. However, the source of required nutrients for submerged macrophytes in different trophic states is still unclear.

Submerged macrophytes rely on surrounding sediments and water to satisfy their N and P requirements (Vindbæk and Nina, 2002). Root uptake is the dominant pathway for nutrient acquisition from sediments in general (Carignan and Kalff, 1980; Ratray et al., 1991; Crossley et al., 2002; Vindbæk and Nina, 2002). However, under nutrient-rich situations, ammonia-N is primarily absorbed by the shoots of submerged macrophytes rather than their roots without consideration of microbial transformation; as a result, their growth is sensitive to the concentration in the water given that ammonia-N concentration in sediments is generally high (Jin, 1994; Liu et al., 2017; Sepehri and Sarrafzadeh, 2018). In particular, our previous findings revealed that submerged macrophyte *Potamogeton crispus* can independently uptake nutrients by shoots (Zhou et al., 2017). These observations presumably reflect differences in the relative importance of nutrient utilisation between roots and shoots under different trophic states. In the meantime, a quantification of the relative contribution of water and sediments providing nutrients, especially N, to submerged macrophytes is still insufficient (Carignan and Kalff, 1980; Xie et al., 2005). Present studies are intensively focused on the purification ability of water by aquatic macrophytes (Souza et al., 2013; Paice et al., 2016; Mcandrew et al., 2017). The neglected changes in nutrient absorption pathways under different nutritional conditions and the relative importance of shoots and roots in nutrient absorption pathway need further study.

P. crispus, a rhizomatous perennial herb producing a flattened, branching stem up to a meter long, is widely distributed in shallow freshwater lakes (Kunii, 1982; Qian et al., 2014). It sprouts in autumn, grows throughout winter and increases its biomass rapidly in the following spring (Nichols and Shaw, 1986). *P. crispus* is often used as an indicator of water quality because of its strong purification ability (Mi et al., 2013). In this study, *P. crispus* was chosen and replanted in two parallel-operated mesocosms. The nutrient dynamics in overlying water and sediments were investigated by isolating the different exchange pathways of nutrients amongst *P. crispus*, water column and sediments. Simultaneously, physiological and stoichiometric parameters of *P. crispus* were observed to monitor its growth status. This study hypothesised that enriched nutrient conditions trigger the change in the nutrient absorption pathway between roots and shoots and that the shoots play an important role in direct nutrient uptake from nutrient-rich water.

2. Materials and methods

2.1. Experimental setup

The manipulative experiment was established at Experimental

Platform for Ecological Remediation in Nanjing Normal University (32°6'27"N, 118°54'19"E) in Eastern China on January 2016. The platform was a large glass greenhouse with abundant light supply. The incubation sediments and *P. crispus* were collected from Taihu Lake, China (31°24'42"N, 120°00'38"E) and transported to the laboratory in January 2016. Then, the large particles and residues were removed from sediments using a sieve, and the *P. crispus* individuals were scrubbed with a soft brush and washed with the distilled water to remove adhesive materials on the leaf surface. Seven *P. crispus* individuals of similar size were planted in plastic pots filled with sediments (top diameter of 12 cm, low diameter of 8 cm and height of 10 cm), and each treatment contained 7 pots. The incubation water, which was injected into each treatment of 75 L, was collected from the pond (original total nitrogen (TN) of 0.3 mg L⁻¹ and total phosphorus (TP) of 0.01 mg L⁻¹) and then adjusted with KNO₃ and KH₂PO₄ to the final concentrations of 2 mg L⁻¹ NO₃⁻-N and 0.3 mg L⁻¹ TP to mimic hypereutrophic lakes. The anti-leakage transparent polyethylene membrane was applied to cover sediments and roots or leaves of *P. crispus* for keeping *P. crispus* intact and avoiding the exchange in nutrients amongst plant, sediment and water column. Six treatments, namely, covering sediments with low nutrient addition (LCS), covering leaves (CL), covering sediments (CS), covering roots (CR), natural (NA) and control, were setup with three replicates (Fig. S1). In particular, LCS and CS treatments used anti-leakage membrane to cover sediments. NA treatment showed the natural growth of *P. crispus*. CL treatment used anti-leakage membrane to cover the shoots, through internal and external loading with the same amount of water. CR treatment used anti-leakage membrane to cover the roots through internal and external loading with the same amount of sediments. Except for LCS treatment, others were added with high amounts of nutrient. These treatments were divided into two systems. System I, which included CS, LCS and control was designed to reveal the trophic influence (oligotrophic and eutrophic conditions) on the nutrient absorption pathway of *P. crispus*. System II, which included CS, CR, NA, CL and control was designed to quantitatively estimate the relative contributions of shoots and roots to the nutrient uptake. The experiments considered the effects of trophic dynamics on the nutrient absorption pathway of *P. crispus* and were divided into two stages: the first stage was from day 0 to day 42, and the second stage with nutrient re-addition was from day 42 to day 92. Water temperature was changed by the weather during the experiment, as shown in Fig. S2. Deionised water was added for the balance of water volume throughout the experiment.

2.2. Sample preparation and analyses

The water samples for chemical analyses, including TN, TP, nitrate nitrogen (NO₃⁻-N), ammonium nitrogen (NH₄⁺-N) and chlorophyll-*a* (chl-*a*), were collected with a tube sampler and mixed thoroughly with three replicates. The samples of sediments were freeze-dried and grounded with a mortar into fine powders for analysis of TN, NO₃⁻-N, NH₄⁺-N and TP concentrations. The test methods of water and sediment samples were described in detail in Zhou et al. (2017). The removal rate of nutrients was calculated with the following formula: $(C_0 - C_i)/C_0 \times 100\%$, where C_0 is the initial nutrient concentration and C_i is the nutrient concentration on the *i*th day. Notably, total organic nitrogen (TON) = TN - (NO₃⁻-N + NH₄⁺-N).

The actual photochemical fluorescence yield (Y) (the fraction of absorbed light energy attributed to photosynthesis) of *P. crispus* leaves was measured *in situ* (Zhou et al., 2018) using underwater saturation pulse leaves of green fluorescence (Diving-PAM, Germany). The length and biomass (fresh weight) of all individual plants were obtained and measured by destructive sampling (Zhao et al., 2016). The relative growth rate (RGR) was calculated using the following formula: $RGR = (H_d - H_i)/(H_i \times \text{Days})$, where H_d is the plant height on the day *d* and H_i is the initial plant height. The *P. crispus* individuals were washed to remove adhesive materials. Thereafter, the plants were dried at 105 °C

for 15 min and then at 70 °C for 48 h. After being grounded into fine powders by using the ceramic mortar, the C concentrations were determined by an elemental analyser (Flash EA 1112, CE Instruments, Italy). The N and P concentrations of *P. crispus*, fully digested by sulphuric acid/hydrogen peroxide at 450 °C, were measured by ultraviolet spectrophotometry and ammonium molybdate ascorbic acid method (Li et al., 2013).

2.3. Data analysis

Statistical analysis was conducted with the SPSS 19.0 software package (SPSS Inc., Chicago). The picture was drawn by Adobe Illustrator (AI CS6 Inc., Ireland). Significant differences amongst treatments were tested by one-way ANOVA. The criteria of $p < 0.05$ and $p < 0.01$ were used to determine the statistical significance at the 0.05 and 0.01 levels (two-tailed). The statistical significance of the results was analysed by Student–Newman–Keuls test at the 5% level.

3. Results

3.1. Physiological and stoichiometric parameters of *P. crispus* in system I

In system I, the average height and fresh weight of *P. crispus* gradually increased throughout the experiment (Fig. 1a and b). On day 92, their maximum values in CS and LCS treatments were insignificantly different (average height, $p = 0.346$; fresh weight, $p = 0.415$). The maximum RGR values in CS and LCS treatments were 0.064 and 0.062, respectively, on day 18 (Fig. 1c). Thereafter, they approximated to 0.04 and then kept a stable level with no significant difference. In addition, the Y values of CS and LCS treatments were insignificantly different ($p > 0.05$) (Fig. S3).

Fig. 2a, b and 2c shows the ecological stoichiometric characteristics of carbon (C), N and P of *P. crispus* under different nutrient conditions. The contents of TC, TN and TP of *P. crispus* were 387.67, 27.10 and 2.82 mg g⁻¹, respectively, in the initial stage. The TN and TP contents of *P. crispus* increased gradually in CS and LCS treatments. However, no significant difference was found in the TC content of *P. crispus* during growth. In particular, the maximum TN contents were 32.01 and 35.60 mg g⁻¹ on day 42, respectively, with no significant difference in CS and LCS treatments ($p = 0.135$). In CS and LCS treatments, the N content of *P. crispus* began to decrease on day 42, and the TN content of *P. crispus* on day 92 was significantly lower than that on day 42 ($p < 0.05$). The TP contents reached the maximum on day 62 and then declined with no significant difference on day 42 ($p = 0.121$). Therefore, the TC, TN and TP of *P. crispus* in CS and LCS treatments displayed the same variation trend. The original mean values of C:N, C:P and N:P ratios were 14.32, 138.77 and 9.72, respectively (Fig. 2d, e and 2f). The C:N and C:P ratios decreased firstly and then increased. The C:N ratio started to rise on day 42 and reached the maximum on day 92, but the

ratios showed no significant difference between CS and LCS treatments ($p = 0.101$). The lowest C:P ratios were 95.15 and 94.27 on day 62. The N:P ratio decreased gradually with no significant difference at different stages.

3.2. Physical and chemical parameters of water in system I

The TN concentrations in the water column significantly decreased in CS and control treatments, except for LCS treatment (Fig. 3a). In particular, the TN concentrations in CS and Control treatments were 2.72 and 3.18 mg L⁻¹ on day 3, respectively. Given that the sediments released nutrients into the overlying water, the nutrients absorbed by the *P. crispus* decreased. The TN concentration in LCS treatment kept at a low level without nutrient addition. On day 52, nutrients of approximately 3 mg L⁻¹ were added in CS and control treatments in the second stage. On day 55, the TN and dissolved inorganic nitrogen (DIN) concentrations in CS treatment were increased to 2.76 and 2.10 mg L⁻¹, and those in control treatment were increased to 3.10 and 2.48 mg L⁻¹. The DIN removal rates in CS and control treatments were more than 90% on day 62. However, the TN concentration in control treatment was more than 2.0 mg L⁻¹ on day 62. Evidently, the *P. crispus* significantly absorbed the nutrients from the overlying water. The NH₄⁺-N concentrations in CS and LCS treatments kept at a low level until the end of the experiment (Fig. 3e). The NO₃⁻-N concentration in CS treatment rapidly declined at the beginning of the experiment, whereas that in control treatment began to decline on day 18 (Fig. 3c). The similar declining trend of NO₃⁻-N was observed after re-adding nutrients in CS and control treatments, but NO₃⁻-N in CS treatment declined faster than that in control treatment. The change in TP was similar to that in TN in system I (Figs. 3a and 4). In the initial three days, the TP concentration was approximately 0.25 mg L⁻¹ in CS and control treatments, except for LCS treatment (Fig. 4). The TP reduction rate in CS treatment declined faster than that in control treatment. A significantly negative correlation was observed between TN and TP concentrations of overlying water and N and P concentrations of *P. crispus* ($p < 0.05$), and a significantly positive correlation was found between TN and TP concentrations of overlying water and C:P ratio of *P. crispus* in CS treatment ($p < 0.05$) (Table S2). However, no significant correlation was detected between TN and TP of overlying water and C, N and P concentrations of *P. crispus* in LCS treatment.

In system I, all treatments were free of phytoplankton at the beginning of the experiment (day 3) as evidenced by the chl-*a* concentration below the detection limit, and phytoplankton occurred on day 18 (Fig. 5). The chl-*a* concentration in LCS treatment was significantly lower than those in CS and control treatments on day 18 ($p < 0.05$). After nitrate was added into CS and control treatments on day 52, the chl-*a* concentration in the control treatment was more than the previously reached maximum on day 62 and then gradually decreased. The chl-*a* concentrations in CS treatment on days 18 and 62

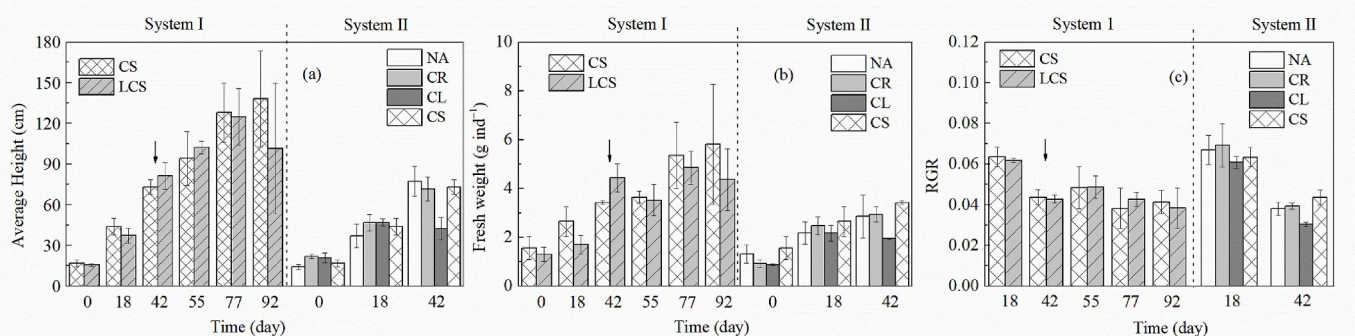


Fig. 1. Average height, fresh weight and relative growth rate (RGR) of *P. crispus* in the different control groups. The arrows indicate that the nutrient is added to the water body again, and the bars denote standard errors ($n = 3$).

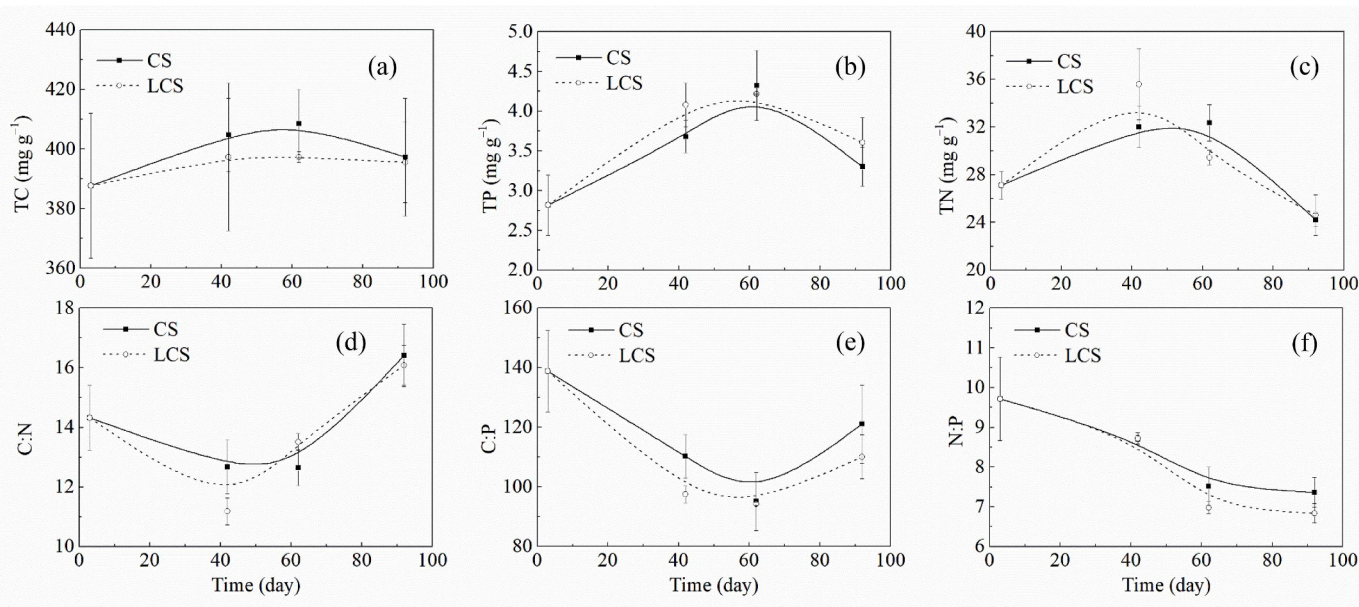


Fig. 2. C, N and P concentrations and C:N, C:P and N:P stoichiometries of the *P. crispus* during the course of the experiment in the system I. The bars denote standard errors ($n = 3$).

were insignificantly different ($p = 0.278$). Evidently, the low nutrient concentrations and submerged macrophytes were the main factors to inhibit the algae growth.

3.3. Plant growth in system II

The average height and fresh weight gradually increased and reached the maximum on day 42 (Fig. 1a and b). However, no significant difference was observed in the average height and fresh weight in CL treatment between day 18 and 42 (average height, $p = 0.352$; fresh weight, $p = 0.202$). The average height and fresh weight in CL treatment were significantly lower than those in NA, CS and CR treatments on day 42 ($p < 0.05$). Those in NA, CS and CR treatments were insignificantly different. The RGR values in all treatments reached the maximum on day 18 with no significant difference. The RGR value in CL treatment was significantly lower than those in other treatments on day 42 ($p < 0.05$). However, the other treatments showed no significant difference.

3.4. Physical and chemical parameters of water in system II

The TN concentrations in the water column significantly decreased in all treatments in system II (Fig. 3a and b). After three days of adding nutrients, the TN concentrations in NA, CR, CL and control treatments were from 3 mg L^{-1} to 4 mg L^{-1} , and that in CS treatment was 2.72 mg L^{-1} . However, the decrease rate of TN concentrations in CS was faster than that in other treatments. On day 18, the removal rates of TN and DIN in CS were 58.45% and 71.20%, whereas those in other treatments did not exceed 30% and 50%. At the end of the experiment, the TN concentrations in all treatments were nearly 1.00 mg L^{-1} .

The $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ concentrations in water were significantly decreased in each treatment (Fig. 3c, d and 3e). The $\text{NH}_4^+ \text{-N}$ concentrations in NA, CR, CL and control treatments were 1.44, 1.20, 1.15 and 0.65 mg L^{-1} on day 3, respectively. The main source of $\text{NH}_4^+ \text{-N}$ in overlying water was released from sediments. By contrast, the $\text{NH}_4^+ \text{-N}$ concentrations in CS treatment remained at a low level, and all treatments decreased to 0.1 mg L^{-1} or less on day 18. Thereafter, the $\text{NH}_4^+ \text{-N}$ concentrations in all treatments remained at a low level (Fig. 3e). On day 18, the $\text{NO}_3^- \text{-N}$ concentrations in NA, CR, CL and control treatments remained at around 2.0 mg L^{-1} and then

decreased (Fig. 3c and d). However, $\text{NO}_3^- \text{-N}$ in CS treatment rapidly declined at the beginning of the experiment. The reduction rate of $\text{NO}_3^- \text{-N}$ in CS treatment declined faster than that in other treatments, and CR treatment displayed the slowest decrease.

The TP concentrations in water were significantly decreased in each treatment (Fig. 4). In the initial three days, the TP concentrations were approximately 0.25 mg L^{-1} in all treatments. The change in TP was similar to that in TN in system II (Fig. 3a and b and 4). The TP reduction rate in CS treatment declined faster than that in other treatments. At the end of the experiment, the maximum TP removal rates in NA, CR, CL, CS and control treatments were 60.60%, 65.89%, 55.96%, 84.41% and 86.94%, respectively. The TP concentrations in all treatments were lower than 0.05 mg L^{-1} .

In system II, the phytoplankton in all treatments was detected on day 18 (Fig. 5). The chl-*a* concentrations of CL and control treatments were insignificantly different ($p = 0.813$) with their maximum concentrations of 38.31 and $37.29 \mu\text{g L}^{-1}$, respectively. On day 42, the chl-*a* concentrations in NA and CS treatments insignificantly differed ($p = 0.164$), and the maximum chl-*a* concentration in control treatment was $30.75 \mu\text{g L}^{-1}$. In addition, the chl-*a* concentration was significantly correlated with TON concentration ($R^2 = 0.5416$, $p = 0.015$) (Fig. S4).

3.5. Nutrients absorption of *P. crispus* from surrounding environments

On the basis of variations in N and P concentrations in overlying water and *P. crispus*, the N and P absorption of *P. crispus* from overlying water and sediments was calculated (Table 1). The relative absorption proportion of *P. crispus* of CL treatment was the lowest with the N (36.21%) and P (8.69%) from the overlying water. The relative absorption proportion was 38.29% for N and 31.68% for P in CS treatment, and these concentrations were evidently higher than those in CL treatment. The sediments acted as a sink for P in the water-sediment interface. Correspondingly, the *P. crispus* absorbed more than 50% N and P from the sediments. Our experimental results showed a quantification of the relative contribution of roots and shoots to nutrient uptake by *P. crispus* (Fig. 6). As shown in Fig. 6, the absorbed nutrients by *P. crispus* in oligotrophic state were mainly from sediments. However, in eutrophic state, the related ratios of absorbed N by *P. crispus* from overlying water and sediments were 49.85% and 50.15%, respectively. Correspondingly, the ratios of absorbed P by *P. crispus* from

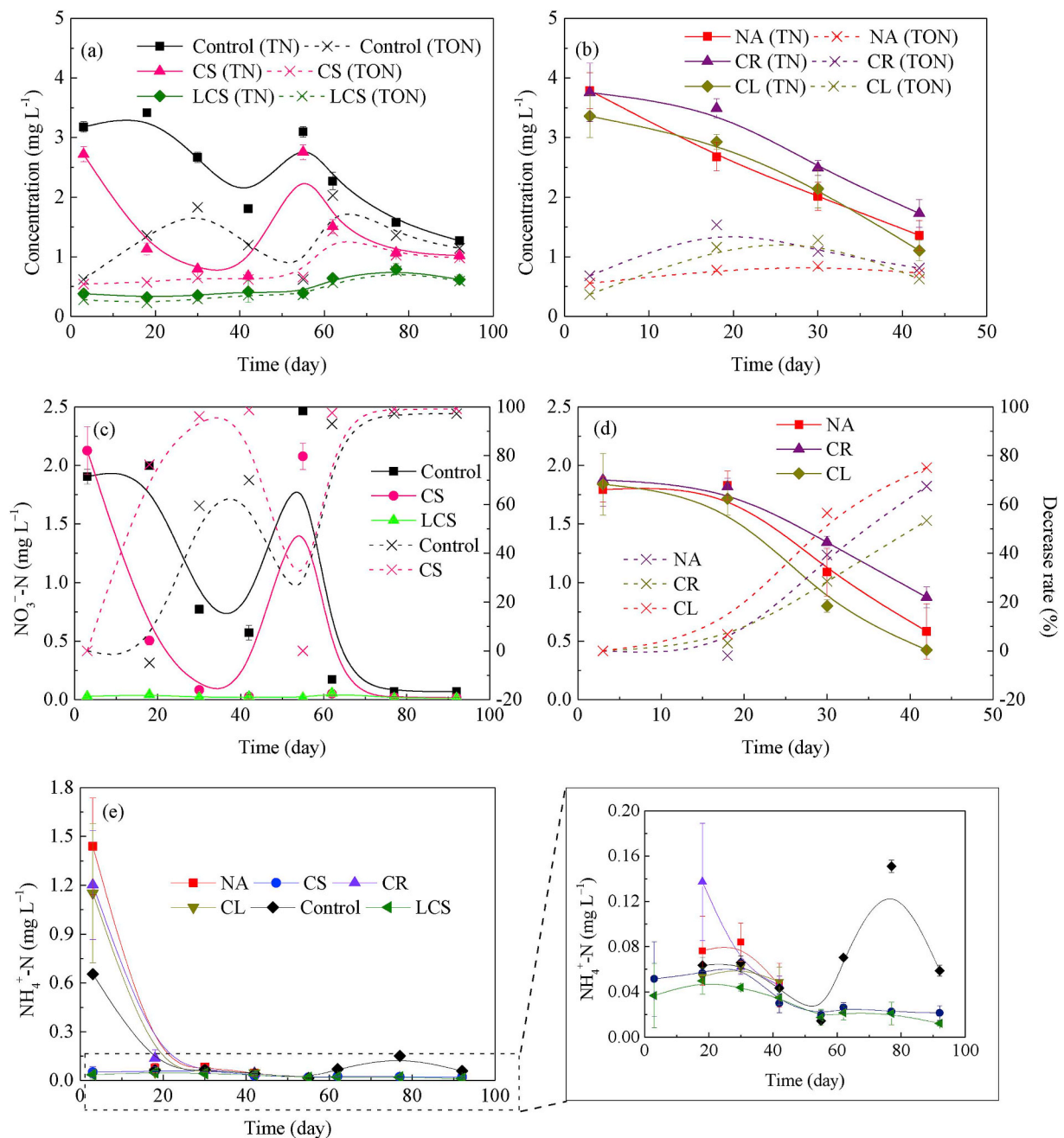


Fig. 3. Variation in N concentrations in different treatments during the course of the experiment; (a) and (b) represent the change in TN and TON in different treatments. The solid and dotted lines represent TN and TON, respectively; (c) and (d) represent the change in NO_3^- -N and decreased rate of NO_3^- -N in different treatments. The solid and dotted lines represent NO_3^- -N and decrease rate of NO_3^- -N, respectively; (e) represents the change in NH_4^+ -N in different treatments. The bars denote standard errors ($n = 3$).

overlying water and sediment were 18.35% and 81.65%, respectively. Approximately 11.50% (released) and –13.33% (deposited) of TN and TP were exchanged from sediments to overlying water.

4. Discussion

Nutrient acquisition by root or shoot is the two dominant pathways for the growth of submerged macrophytes (Rattray et al., 1991; Crossley et al., 2002). Our experiments demonstrated that the nutrient absorption pathway was altered by the nutrient levels in water column. Under oligotrophic state, aquatic macrophytes mainly uptake nutrients from sediments during growth according to previous and our studies (Carignan and Kalff, 1980). Despite the available low nutrient

concentration in overlying water, the nutrient demand of submerged macrophytes *P. crispus* could be satisfied by root uptake, which acted as a pump, from relative nutrient-abundant sediments. With nutrient enrichment, the shift in the relative contribution to nutrient absorption was away from roots and towards shoots. That is, the leaf nutrient uptake gradually became an important role of *P. crispus* in nutrient-rich water column. This deduction was confirmed by the rapid nutrient decline to the minimum of overlying water in the system with high nutrient addition (Figs. 3 and 4). A negative correlation was found amongst tissue-N, P and water nutrient concentrations in high-nutrient state, but no relation was observed in low-nutrient state during the incubation period. This result supported the potential importance of leaf nutrient uptake (Table S2). This phenomenon was parallel to the

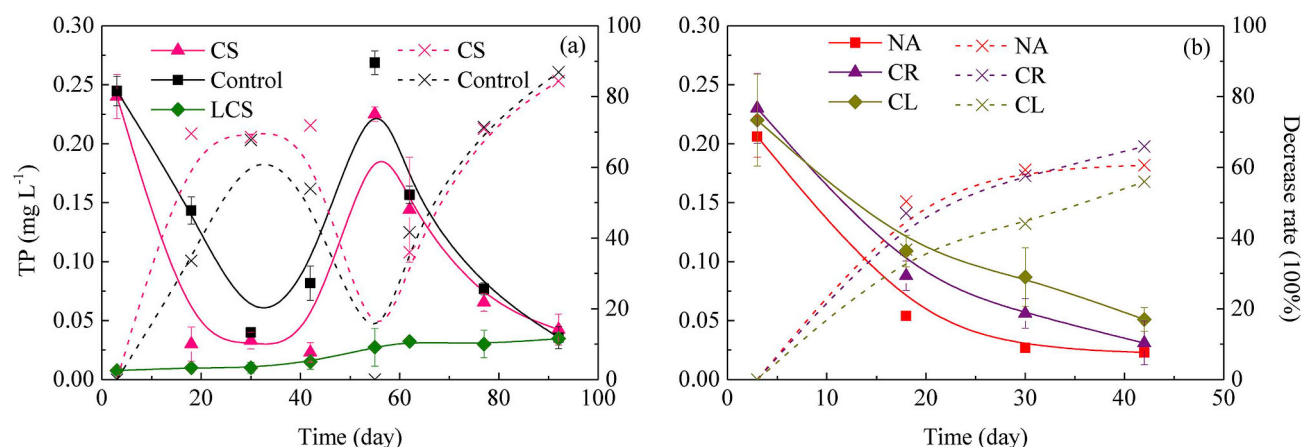


Fig. 4. Variations in TP contents and decrease rate of TP in different treatments during the course of the experiment. The solid and dotted lines representation TP contents and decrease rate of TP, respectively. The bars denote standard errors (n = 3).

test performed by Madsen and Cedergreen (2002), who removed the roots of submerged macrophytes to observe their nutrient absorption pathway. Our previous manipulative experiments also demonstrated that *P. crispus* fragments can independently rely on shoots to absorb nutrients and satisfactorily grow on the surface water for a long time (Zhou et al., 2017). However, these harmed samples may be physiologically and morphologically influenced, and devious explanations are required to evaluate their nutrient absorption pathway. By contrast, our current intact plants in strategical designed mesocosms (system I), forming isolated units to prevent the nutrient exchange between water column and sediments, could effectively *in situ* track the nutrient uptake dynamics and validate the simultaneous absorption of nutrients by shoots and roots.

Notably, the aforementioned conclusions were based on the negligible effects of nutrient enrichment on the RGR, the ecological stoichiometric characteristics and the leaf photosynthetic maximum quantum yield (Y) of *P. crispus* (Figs. 1 and 2 and S3). Nutrients, which

are regarded as a limiting factor, considerably impact the growth state and eco-physiology characteristics in general (Li et al., 2008). The lack of response to nutrient enrichment observed in the current study indicated that the nutrient availability was sufficient to cover the needs for growth in low nutrient concentrations. In theory, the plants with nutrient enrichment should exhibit a relatively high growth rate. A previous field survey demonstrated that the growth of submerged macrophytes is regulated by factors other than nutrient availability in nutrient saturated streams (Kernhansen and Dawson, 1978). Changes in the supply of nutrients may affect the demand for other resources, such as light and inorganic matter (Madsen and Cedergreen, 2002). In addition to growth rate, environmental nutrient elements can strongly affect the stoichiometry of an organism (Aring and Gren, 2004). This deduction was reflected by the dynamic optimal nutrient stoichiometries in different life stages of *P. crispus* in the current study. However, the sediment compensation and foliage uptake in low and high nutrient conditions removed these effects to display their insignificant

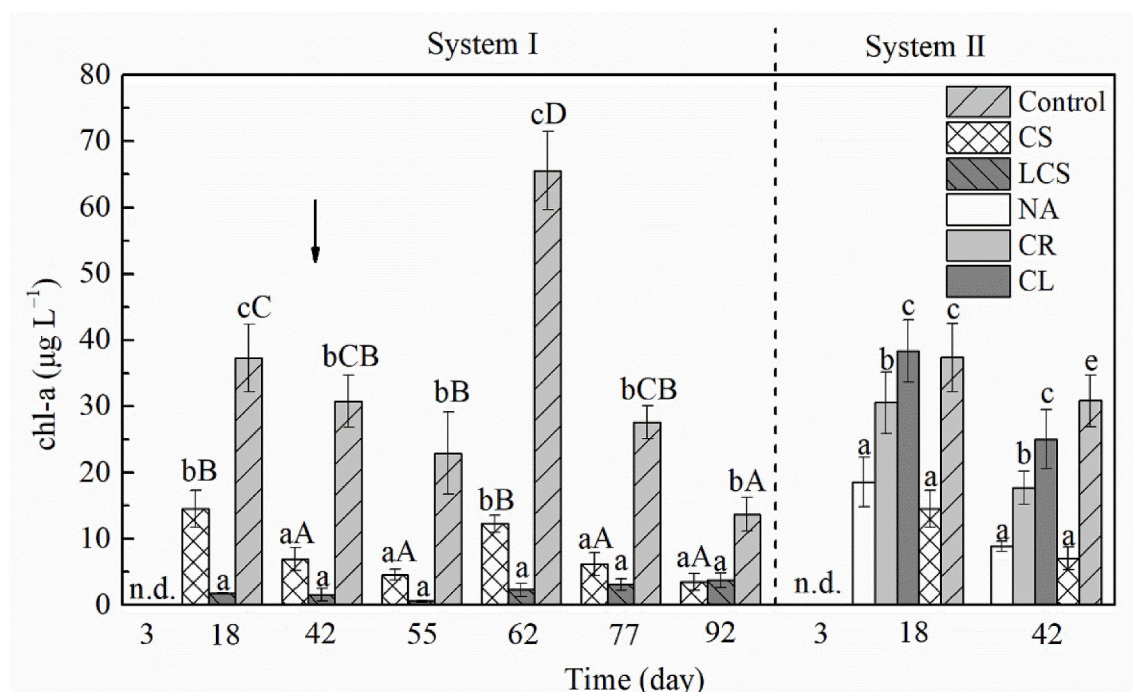


Fig. 5. Variations in chl-a concentration of overlying water in experience. The arrows indicate that the nutrient is added to the water body again. The bars denote standard errors (n = 3). The lowercase indicates significant differences of each groups according to Duncan test at the 5% level. The uppercase indicates significant differences of CS and CK treatments according to Duncan test at the 5% level. The abbreviation of n. d. indicates 'not detected'.

Table 1Dry weight, TN and TP contents of *P. crispus* and relative proportion of shoot and root of nutrient absorption in system II.

Treatment	Dry weight (g)	Total N (mg g ⁻¹)	Total P (mg g ⁻¹)	LBN/PN (%)	LBP/PP (%)	RBN/PN (%)	RBP/PP (%)	Water-sediment exchange flux (N)	Water-sediment exchange flux (P)
NA	1.21	32.89	4.32	49.85	18.35	50.15	81.65	+11.56%	-13.33%
CL	0.79	34.51	4.66	36.21	8.69	63.79	91.31	-19.36%	-5.17%
CR	1.23	30.61	4.21	48.94	14.28	51.06	85.72	+10.65%	-17.40%
CS	1.31	32.01	3.68	38.29	31.68	61.71	68.32	0	0

Herein, LBN represents the shoot uptake of DIN from overlying water, LBN = DIN – TON (algae) – Control; LBP represents the shoot uptake of P from overlying water, LBP = TP – P (algae) – Control; RBN represents the root uptake of DIN from sediments, RBN = 100% – LBN; RBP represents the root uptake of P from sediments, RBP = 100% – LBP; PN and PP represent the N and P contents of *P. crispus*; plus means the flux direction from sediment to overlying water, and minus is opposite.

differences and further confirmed the shift of the nutrient absorption pathway under the same photosynthesis of *P. crispus*.

Although the experiments showed that the nutrient requirements of the plants could be met by leaf uptake, nutrients could also be up taken by algae. Our re-addition of nutrients to the water column for testing the dynamic effects of trophic levels on the absorption pathway of *P. crispus* exhibited a higher chl-*a* concentration in the control treatment than at the first stage (Fig. 5). At the same time, the algae biomass of nutrient treatments at both stages was significantly lower than that in the control due to the inhibition by *P. crispus*. Evidently, the low algae biomass but fast nutrient reduction rate after adding or re-adding nutrients could effectively exclude the influence of algae and verify the nutrient absorption pathway of shoots.

Ultimately, a quantification of the relative contribution of root and shoots to nutrient uptake by submerged macrophytes was tested in another parallel-operated mesocosms (system II). Under eutrophic state, all nutrients in water column were assumed to maintain the regular growth of *P. crispus*, and the relatively nutrient uptakes by shoots were 49.85% and 18.35% for TN and TP, respectively (Table 1). Herein, *P. crispus* maintained the consistent growth in all treatments, as evidenced by the average height, fresh weight and RGR (Fig. 1). This finding was similar to that observed in system I and other systems (Pistori et al., 2004; Zhu et al., 2016). However, the treatment of leaf cover (CL) significantly affected the growth of *P. crispus*. Thus, its weight was eliminated when we expressed the nutrient uptake by roots or shoots. Although the leaf nutrient uptake became an important role with nutrient enrichment, the absolute acquisition by the roots was still the dominant pathway. However, this phenomenon might further increase with the increase in the nutrient level of foliage uptake, particularly for TP, of up to 28% under hypereutrophic conditions (Carignan and Kalff, 1980). The treatment of sediment cover (CS) with high available P in overlying water without deposition also confirmed that 31.68% of TP was allocated to shoots.

The nutrient exchange between sediment and overlying water was simultaneously estimated via these treatments of leaf or root cover.

Approximately 11.50% of TN was released from sediments to overlying water, whereas 13.33% of TP was deposited to the sediments. The P fluxes in all treatments were negative because they easily fixed and deposited (Wodka, 1985), but the foliage uptake was relatively limited. However, the released form of N from sediments was mainly ammonia-N, which was approximately 66.55% of DIN to the water column. This phenomenon was related to the high proportion of ammonia-N in sediments (Fig. S5), and its diffusion direction was generally accepted from sediments to overlying water (Wang et al., 2015; Yang et al., 2015; Lu et al., 2018). In addition, *P. crispus*, as a nutrient pump, preferentially absorbed ammonia compared with nitrate-N when the ammonia concentration of water was higher than 0.35 mg L⁻¹ (Jin, 1994). The released ammonia-N from sediments might mostly be absorbed by *P. crispus* because the high DO concentration in the water at more than 10 mg L⁻¹ (Fig. S1) inhibited the denitrification process. Therefore, sediments not only acted as a N source supporting the relatively high N uptake by shoots but also a P sink for root uptake.

To clarify the influence of trophic states on nutrient absorption pathway of submerged macrophytes and quantitatively estimate their relative contributions, a conceptual diagram was introduced (Fig. 6). Although the absolute nutrient acquisition adsorbed by shoots of submerged macrophytes was not dominant, we verified that its role gradually became crucial with nutrient enrichment. This pathway was no longer solely dependent on the root system to absorb N and P nutrients from the surrounding environments. Therefore, our results have important implications for lacustrine restoration via replanting submerged macrophytes. The relatively large specific surface area of shoots of submerged macrophytes is recommended to absorb N and P nutrients. Interestingly, floating fragments with leaves, easily produced by water current, catastrophic typhoon, animal bite, or anthropological harvest, are frequently observed in freshwater lakes (Xie and Yu, 2011). Our previous findings showed that the separated shoots not only can absorb N and P nutrients in the water column but also can compete with algae for resources and inhibit algal blooms (Zhou et al., 2017). Therefore, the foliage uptake with high N and P nutrients should be given

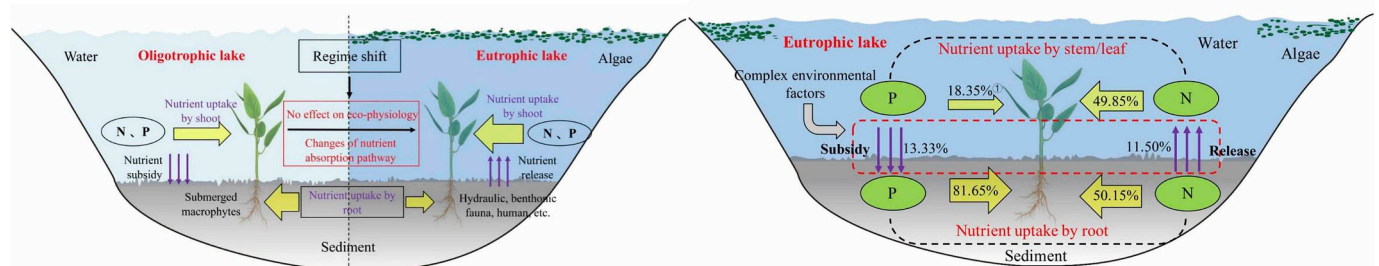


Fig. 6. Simplified schematic view of the trophic influence on nutrient absorption pathway of *P. crispus* and quantitative estimation of the relative contributions of shoot and root to nutrient uptake. The conceptual figure illustrates two key points. One is that the eutrophic conditions trigger the shift of the nutrient absorption pathway from the root to the shoot (left figure), and the other is that the absolute acquisition by the roots is still the dominant pathway (right figure). ^①The absolute nutrient acquisition by the shoot may further increase with the nutrient enrichment, particularly under hypereutrophic conditions. The arrows indicate the maximal nutrient uptake concentration by *P. crispus*.

attention. For specific pollutants, such as heavy metals, the accumulation capability is related to certain species (Xing et al., 2013). The proper choice of submerged macrophytes and further exploration of the phytoremediation technology are necessary for the ecological restoration of eutrophic lakes.

5. Conclusion

The nutrient absorption pathway of submerged macrophytes was tested via isolating plant, water column and sediments. We found that enrichment in water and/or sediment did not affect the RGR, the ecological stoichiometric characteristics and the leaf photosynthetic maximum quantum yield of *P. crispus*. Despite the lack of an ecophysiological response, the shift in the relative contribution to nutrient uptake was observed away from root and towards shoots triggered by the nutrient enrichment. A parallel quantification estimation revealed that the absolute acquisition by the roots was still the dominant pathway; however, it might be weakened by the foliage uptake with the increase in nutrient levels. The nutrient absorption capacity of shoots should be increased in applying phytoremediation on eutrophic water bodies.

Conflict of interest

All authors claim no any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

Acknowledgement

We thank Yawen Huang Ph. D (Nanjing Institute of Geography & Limnology, Chinese Academy of Sciences), Xiaolu Li Ph. D (College of Resources and Environment, Huazhong Agriculture University) and Wenqi Zhang for their stimulating remarks on the manuscript. This work was supported by the Major Science and Technology Programme for Water Pollution Control and Treatment (2017ZX07203-003), the Technology Project of Water Resources Department of Jiangsu Province (2016050), the National Natural Science Foundation of China (41703105, 41877344 and 41773081), and the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (17KJB170009).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2019.03.069>.

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