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RESEARCH ARTICLE

Understanding stoichiometric adjustments in a freshwater plant: Responses to sediment and water nutrient dynamics across lake trophic gradients

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Abstract

Despite ongoing efforts to reduce nutrient inputs, eutrophication continues to disrupt biogeochemical cycles and destabilize freshwater food webs. In this study, we examine the stoichiometric responses of the freshwater plant *Myriophyllum spicatum* under varied environmental conditions across lakes of differing trophic status. Specimens were collected from lakes with a wide natural range of macro- (C, N, P) and micronutrient (Fe, Cu, Zn) concentration in both water and sediments. We applied the ecological stoichiometry framework and analyzed the relationship between nutrient availability (water and sediments) and the elemental composition of *M. spicatum*'s organs (leaves, stems, and roots). The C : N : P ratios in organs were not affected by eutrophication. Instead, all macro- and micronutrient concentrations differed between plant organs. N concentration was highest in leaves and roots, indicating uptake from both sources. Furthermore, sediments significantly influenced the plant organs' C, P, and Zn concentration, while nutrients in the water column showed no correlation. Leaves demonstrated flexibility in C and Zn concentrations, negatively correlating with sediment levels of these elements. The concentration of micronutrients was highest in the roots. Our results indicate distinct nutrient allocation strategies for different plant organs: leaves are rich in N to support photosynthesis, stems store C and P, aiding growth and reproduction, and roots accumulate micronutrients Fe, Zn, and Cu. It highlights sediments as a critical nutrient source for *M. spicatum*, shaping its elemental composition. The relationship between organisms' biochemistry, trophic interactions, and their transformation into dead organic matter is crucial for understanding environmental stress impacts on aquatic ecosystems.

Next to the consequences of climate change, and equivalently with them, eutrophication of freshwater ecosystems remains one of the most challenging civilizational problems. Ongoing efforts to reduce anthropogenic nutrient inputs still stand in contrast to phosphorus (P) and nitrogen (N) harvesting from the geosphere and the atmosphere, and employing them for agricultural production intensification (Jenny et al. 2016; Zhang et al. 2021). The increasing amount of reactive P and N in the global budget has led to doubling and tripling global environmental concentrations within the last 60 years (Bouwman et al. 2009; FAO 2023). As a

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consequence, their planetary boundary has already been fairly crossed (Richardson et al. 2023). Their negative impacts on freshwater environments are particularly reflected as biodiversity loss, habitat destruction, harmful phytoplankton blooms, and expanding hypoxia (Søndergaard et al. 2007). Moreover, eutrophication arising from N and P overload has far-reaching consequences for biogeochemical cycles and food web functioning in aquatic environments, and is strictly connected with adjacent terrestrial ecosystems (Sardans et al. 2012; Su et al. 2019).

Submerged macrophytes, keystones in freshwater ecosystems, play a crucial role in the mobilization, accumulation, and transfer of nutrients, while simultaneously acting as an important part of the food web and as a refuge for animals (Joniak et al. 2007; Short et al. 2016). Their specific ability of nutrient uptake via both roots and aboveground parts allows for the presence of many diverse habitats and ecological niches (Carignan and Kalff 1980). Moreover, the spatial heterogeneity in the nutrient concentrations in water and sediments can affect plants' metabolic activity and the presence of intermediate metabolites such as soluble carbohydrates, starch, phenolic compounds, as well as the N- and P-rich compounds such as proteins, RNA, and ATP (Cronin and Lodge 2003; Demars and Edwards 2008). Reallocating previously limited resources is a crucial plant adaptive strategy to endure changing environmental conditions. A combination of evolutionary history, environmental stresses, and trade-offs in functional traits influences this strategy (Xie et al. 2005). To effectively carry out various functions such as growth, reproduction, and nutrient storage, plants have to carefully distribute elements across different organs, especially in the face of diverse environmental challenges (Schreeg et al. 2014). Understanding stoichiometric differences between plant organs is therefore crucial, as it reveals how plants allocate resources, adapt to nutrient limitations and excess, and respond to stress. Additionally, it provides insights into ecosystem dynamics, nutritional quality, and strategies for managing and conserving plant resources. Changes in carbon-to-nutrient ratios, whether increasing or decreasing, have profound effects on the nutritional properties of plants. Such alterations can have a cascading impact on the composition of consumer species and lead to a restructuring of the entire food web within the ecosystem. While nutrient allocation strategies in terrestrial plants have garnered significant attention (e.g., Reich and Oleksyn 2004; Han et al. 2005), there is still a scarcity of knowledge regarding these dynamics in aquatic plants.

The leaves, stems, and roots of aquatic vascular plants function in different ways to form and maintain an organic whole. The distribution of nutrients among different plant organs may reflect the dynamic interaction between plants and their environment. It is the result of harmonizing the functions of various organs, ensuring the satisfaction of nutritional needs and adaptation to a shifting environment by efficiently allocating limited nutrients (Liu et al. 2010). However, the degree to which submerged macrophytes maintain and allocate elements between organs at various nutrient levels is still unclear. Studies on submerged macrophyte stoichiometry have focused on the light and/or selected nutrient availability and concentrations in different organs (Su et al. 2016; Dülger et al. 2017; Rybak et al. 2020, 2023). Insufficient focus has been directed toward changes in nutrient allocation among organs, which, even if analyzed, were still investigated with limited environmental factors and nutrient sources (Yuan et al. 2013).

In this study, we extended the classical carbon–nitrogen–phosphorus (C : N : P) approach to include the elemental composition of microelements: iron (Fe), zinc (Zn), and copper (Cu). These elements are essential micronutrients that play critical roles in various biological processes. They are integral components of many enzymes, including oxidoreductases involved in electron transfer (Fe, Cu), or carbonic anhydrases and superoxide dismutase (Zn). They are also crucial for chlorophyll synthesis (Fe) and protein synthesis (Zn), as well as the regulation of carbohydrate metabolism (Zn) (Kochhar and Gujral 2020). However, as global environmental changes intensify driven by urbanization, eutrophication of aquatic ecosystems, and restoration treatments, the natural cycles and concentrations of these elements are disrupted (Xue et al. 1997; Zhang et al. 2019).

Hence, our aim was to explore the stoichiometric characteristics of the leaves, stems, and roots of *Myriophyllum spicatum* in relation to water and sediment originating from lakes with different levels of nutrient concentration. We assumed that, in increasing lake trophic conditions, *M. spicatum* differentiates the allocation of macro- and micronutrients between organs as an adaptation to the availability of nutrients in the environment. Under higher trophic conditions, we hypothesize a reduction in C : N and C : P ratios due to increased nitrogen and phosphorus accumulation, while carbon concentrations remain stable, alongside greater concentrations of micronutrients (Fe, Zn, Cu). By analyzing the concentrations of these elements in both water and sediment, we address whether there is a relationship between the distribution of elements within the plant and their environmental availability. We chose *M. spicatum* since it meets the requirements of a model species, is cosmopolitan, and has an invasive status in some parts of the world, which increases the interest in this species (Ortiz et al. 2019).

Materials and methods

Field sampling and preparing

Sample collection was conducted during the growing season (1 July to 15 September) of 2022 from 31 lakes located between 52°20′23″–52°29′60″N and 15°49′47″–17°55′49″E (Fig. 1). The lakes represented several levels of trophic states and ecotypes: oligo-mesotrophic, softwater Lobelia-dominated lakes; mesotrophic, hardwater Charophyte-dominated lakes; macrophyte-dominated meso- and eutrophic lakes; and

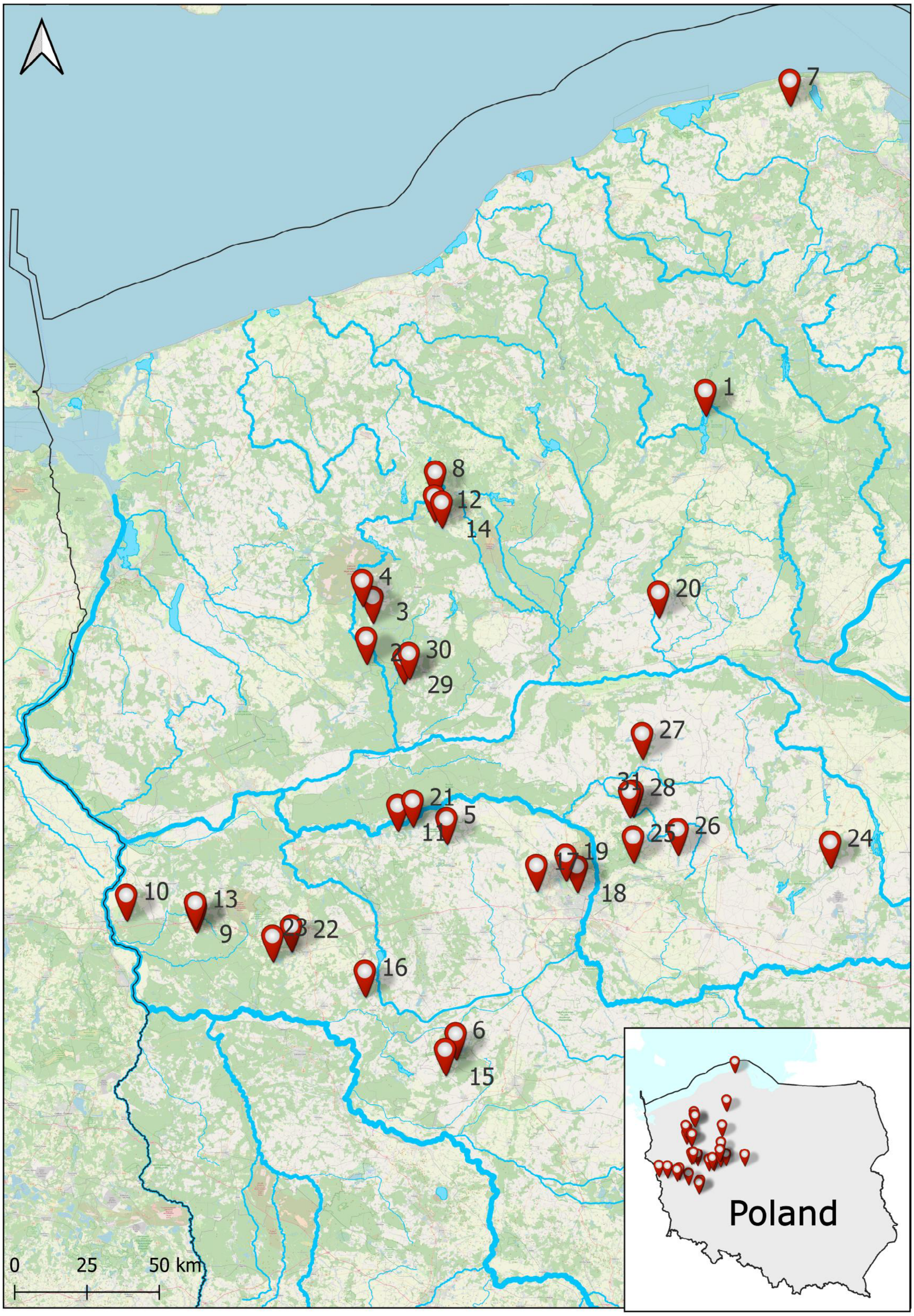

Fig. 1. Sample collection locations (details are presented in Table S1).

hypertrophic lakes with degraded floristic structure (morphometric characteristics of studied lakes are presented in Supporting Information Table S1).

After locating a patch of *M. spicatum* in each lake, pH and electric conductivity were measured (HI 98129, Hanna Instruments). Water samples were taken 50 cm below the surface. Samples for C analysis were preserved using zinc chloride (3.7 M $ZnCl_2$), samples for N and P analysis were preserved using hydrochloric acid (2 M HCl), and samples for metals (Fe, Cu, and Z) were preserved using nitric acid (15.2 M HNO_3) (Śliwka-Kaszyńska et al. 2003; Wilson et al. 2020). All samples were placed in a refrigerator during transportation and then frozen at −20°C. Bottom sediments were collected at the rooting site of *M. spicatum* using a tube scoop, and a 10 cm surface layer was dedicated for nutrient analysis. The sediments were air-dried, ground in a ceramic mortar, and sieved through a 2 mm mesh diameter sieve.

Individual stems of *M. spicatum* were collected with either a macrophyte anchor or by hand in shallow parts. Specimens were accurately rinsed in lake water and transported to the laboratory along with a 5 L of water. Subsequently, macrophytes were thoroughly rinsed twice in tap water and cleaned to completely remove sediments, algae, and invertebrates. Another two rinses were performed in demineralized water, after which the specimens were dried on a paper towel. Prepared plants were manually divided into leaves, stems, and roots. Organoleptic inspection allowed for the precise separation of plant material into individual organs, ensuring that each group was free of any foreign matter. The separated macrophyte samples were then dried at 60°C for 72 h and ground into a fine powder using a tissue mill (A11, IKA) prior to elemental analysis.

Laboratory analysis

Dissolved carbon (DC) concentration was determined as sum of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC), which were determined by infrared spectroscopy by the total organic carbon analyzer (TOC-L CPH/CPN; Shimadzu) according to PN EN 1484:1999. Water samples for dissolved inorganic phosphorus (DIP $= PO_4^{3-}$) and dissolved inorganic nitrogen (DIN $= NO_2^- + NO_3^- + NH_4^+$) were analyzed after filtration through membrane filters (0.45 μm pore size, Whatmann) using a flow injection analyzer (FIA compact, MLE GmbH) according to ISO 15681-1:2005, ISO 13395:1996, ISO 11732:2005, respectively. Further analyses were performed based on these forms since these are more readily available for macrophytes, thus better reflecting stoichiometric reliance (Reynolds and Davies 2007). The C and N concentrations in bottom sediments and plant samples were determined using the Flash 2000 Series elemental analyzer (Thermo Fisher Scientific). The P concentration was determined via the molybdate–ascorbic acid after mineralization with HNO_3 in a Mars 6 Xpress microwave mineralization system (CEM Corporation). The concentrations of Fe, Zn, Cu in water, sediments and plants samples were analyzed using the Spectra 280AA atomic absorption spectrometer with flame atomization (Agilent Technologies). Organic matter content was determined as loss on ignition at 550°C for 4 h.

Statistical analysis

Generalized linear mixed models were used to compare whether elemental concentrations differed between plant parts and to test for the relationships between elemental concentrations in plant organs and their concentration in water and sediment. We fitted six models, in which the concentrations of C, N, P, Fe, Zn, or Cu in plants were response variables. As predictors, we included plant organ (leaves or stems or roots), the concentration of a given element in water and sediment, as well as the interactions between plant organ and elemental concentrations in water and sediment. Moreover, we built three models testing whether C : N : P stoichiometry differed between plant parts and whether it was related to C : N : P stoichiometry in the environment. In these models, we included either C : P, C : N, or N : P as the response, while as predictors we included the respective ratio in the sediment and water and their interaction with plant organ. All models included lake ID as a random intercept to account for multiple plant sampling from the same lakes and were fitted with a Gamma error distribution and log link function to reflect data distribution. For post-hoc comparisons of elemental concentrations and elemental ratios between plant organs (leaves, stems, roots), we used estimated marginal means with Tukey's multiplicity adjustment implemented via the *emmeans* ver. 1.8.5 R package (Lenth 2023). In case of finding a significant interaction between elemental concentration or ratio in the environment and plant organ, we used estimated marginal means of linear trends to compare the trends between different plant organs (Lenth 2023). Model diagnostics was performed using the *DHARMa* ver. 0.4.6 R package (Hartig 2021). All analyses were conducted using R 4.2.2 (R Core Team 2023).

Results

Physicochemical characteristics of water and sediments

The lakes exhibited diverse physicochemical properties of water and bottom sediments. The pH ranged from 7.12 to 8.64, conductivity from 82 to 812 μS cm^{-1}, and concentrations of DC, DIN, and DIP in water were 1.26–32.71 mg L^{-1}, 0.26–3.53 mg L^{-1}, and 0.06–0.17 mg L^{-1}, respectively. Micronutrients like Cu, Zn, and Fe were present in low concentrations (Table 1).

Sediments contained 0.4–23.9% organic matter, with C, N, and P concentrations of 3.08–177.59 mg g^{-1}, 0.07–9.12 mg g^{-1}, and 0.05–1.11 mg g^{-1}, respectively. Micronutrients, particularly Cu and Zn, were low, while Fe showed greater variability (0.30–6.37 mg g^{-1}).

Myriophyllum spicatum elemental composition and their environment

Carbon

Among the analyzed plant organs, C concentration was significantly higher in roots (379.8 ± 17.5 mg g^{-1}, mean ± SD)

Table 1. Physicochemical properties of water and bottom sediments in the studied lakes.

	Water								Sediments						
	DC	DIN	DIP	Cu	Zn	Fe	pH	EC	C	N	P	Cu	Zn	Fe	OM
	mg L^{-1}							μS cm^{-1}	mg g^{-1}						%
Minimum	1.26	0.26	0.06	0.09	0.01	0.01	7.12	82	3.08	0.07	0.05	0.00	0.00	0.30	0.4
Average	8.14	0.60	0.08	0.14	0.02	0.04	8.15	382	34.31	2.23	0.25	0.01	0.01	2.36	3.5
Maximum	32.71	3.53	0.17	0.18	0.06	0.28	8.64	812	177.59	9.12	1.11	0.04	0.03	6.37	23.9

and stems (351.7 ± 19.0 mg g^{-1}) than in leaves (Table 2; Tukey's tests: $t = -7.74$, $p < 0.001$ and $t = -5.72$, $p < 0.001$, respectively), which showed the largest variation in C concentration (285.0 ± 78.3 mg g^{-1}). C concentration did not differ between roots and stems (Fig. 2; $t = 2.04$, $p = 0.11$). C concentration in plants was significantly related to C-sed. (Table 2), and this relationship varied depending on organ. Specifically, C concentration in leaves decreased with increasing C-sed., while there was no relationship between C-sed. and C in roots or stems (Fig. 3; Table 2; Supporting Information Table S2). No relationship between C concentration in plants and the water column was observed (Table 2).

Nitrogen

N concentration differed between organs (Fig. 2; Table 2). Specifically, N concentration was significantly lower in stems as compared to N concentration in leaves ($t = 3.58$, $p = 0.002$) and roots ($t = 2.72$, $p = 0.021$). N concentration in leaves and roots did not differ (Fig. 2; $t = 0.87$, $p = 0.664$). No relationship between N concentration in plants and N-sed. nor DIN was observed (Fig. 3; Table 2).

Phosphorus

P concentration differed significantly between plant organs. To be specific, P concentration was significantly lower in leaves compared to stems (Table 2; $t = -3.24$, $p = 0.005$), while it did not differ between stems and roots ($t = -1.57$, $p = 0.265$) or leaves and roots (Fig. 2; $t = -1.65$, $p = 0.233$). The differences in P concentration between plant organs interacted with the P concentration in the sediment (Table 2). However, when tested individually per plant organ, these relationships were not apparent (Fig. 3; Supporting Information Table S2). No relationship between P concentration in plants and DIP concentration was observed (Table 2).

Iron

Root Fe concentration was significantly higher than in leaves (Table 2; $t = -13.07$, $p < 0.001$) and stems ($t = 19.67$, $p < 0.001$). Moreover, Fe concentration in leaves was also significantly higher than in stems (Fig. 4; $t = 6.32$, $p < 0.001$). Plant Fe concentration neither depended on Fe-sed., nor on Fe-wat. (Fig. 5; Table 2).

Zinc

Root Zn concentration was higher than in leaves (Table 2; $t = -5.27$, $p < 0.001$) and stems ($t = 8.12$, $p < 0.001$). Also, Zn concentration significantly differed between leaves and stems (Fig. 4; $t = 2.93$, $p = 0.012$). Moreover, the differences in Zn concentration between plant organs depended on Zn-sed (Table 2). In particular, Zn concentration in leaves decreased with increasing Zn-sed., while there was no relationship between Zn-sed. and Zn concentration in roots or stems (Fig. 5; Supporting Information Table S2). No relationship between Zn concentration in plants and Zn-wat. was observed (Table 2).

Copper

Similar to the other elements content, Cu concentration differed significantly between plant organs, with higher values in roots compared to leaves (Table 2; $t = -7.20$, $p < 0.001$) and stems ($t = 5.56$, $p < 0.001$). No differences between leaves and stems were observed (Fig. 4; $t = -1.91$, $p = 0.141$), and no relationship between plant Cu concentration and Cu-sed. or Cu-wat. was observed (Fig. 5; Table 2).

C : N : P stoichiometry

Similar to the results for elemental composition described above, C : N : P stoichiometry differed significantly between plant organs (Fig. 6; Table 3). C : P ratios were significantly higher in roots (660.8 ± 320.0 mol mol^{-1}) compared to leaves (570.1 ± 271.0 mol mol^{-1}; $t = -2.64$, $p = 0.027$) and stems (562.1 ± 319.8; $t = 3.32$, $p = 0.004$). There were no differences in plant C : P ratios between leaves and stems ($t = 0.69$, $p = 0.772$). The differences in organ C : P ratios did not interact with sediment or water C : P ratios (Fig. 6; Table 3).

C : N ratios of leaf material were significantly lower (21.2 ± 6.6) than those of stems (34.4 ± 17.5; $t = -9.77$, $p < 0.001$) and roots (28.6 ± 5.0, $t = -6.92$, $p < 0.001$). Moreover, C : N ratios in roots were significantly lower than in stems ($t = -2.82$, $p = 0.016$). There were no relationships between C : N ratios in plants and C : N ratios in sediment and water (Table 3).

Plant N : P ratios differed significantly between organs (Fig. 6; Table 3). Leaves had significantly higher N : P ratios (26.7 ± 7.2) compared to roots (22.4 ± 6.9; $t = 3.87$, $p < 0.001$) and stems (16.4 ± 4.8; $t = 11.04$, $p < 0.001$). Moreover, there were significant differences between stems and roots ($t = 7.04$, $p < 0.001$).

Table 2. Results of generalized linear mixed models testing how elemental concentrations in different plant organs (leaves, stem, and roots) depend on their concentration in water and sediment. Significant *p*-values (< 0.05) are indicated in boldface.

Model term	DF_{num}	DF_{denom}	F ratio	*p*
Carbon ($R^2_{cond} = 0.58$, $R^2_{marg} = 0.50$)				
C-wat.	1	76	0.39	0.534
Organ	2	76	35.93	< **0.001**
C-sed.	1	76	7.65	**0.007**
C-wat. × organ	2	76	1.91	0.155
C-sed. × organ	2	76	7.26	**0.001**
Nitrogen ($R^2_{cond} = 0.48$, $R^2_{marg} = 0.21$)				
N-wat.	1	40	0.002	0.964
Organ	2	40	3.49	**0.04**
N-sed.	1	40	0.66	0.422
N-wat. × organ	2	40	0.17	0.842
N-sed. × organ	2	40	2.76	0.075
Phosphorus ($R^2_{cond} = 0.55$, $R^2_{marg} = 0.15$)				
P-wat.	1	78	0.44	0.508
Organ	2	78	6.76	**0.002**
P-sed.	1	78	0.29	0.592
P-wat. × organ	2	78	0.20	0.817
P-sed. × organ	2	78	5.68	**0.005**
Iron ($R^2_{cond} = 0.88$, $R^2_{marg} = 0.73$)				
Fe-wat.	1	78	2.51	0.117
Organ	2	78	209.15	< **0.001**
Fe-sed.	1	78	0.73	0.396
Fe-wat. × organ	2	78	2.68	0.075
Fe-sed. × organ	2	78	1.11	0.335
Zinc ($R^2_{cond} = 0.85$, $R^2_{marg} = 0.25$)				
Zn-wat.	1	78	0.003	0.956
Organ	2	78	35.88	< **0.001**
Zn-sed.	1	78	3.91	0.052
Zn-wat. × organ	2	78	0.10	0.904
Zn-sed. × organ	2	78	4.21	**0.018**
Copper ($R^2_{cond} = 0.75$, $R^2_{marg} = 0.25$)				
Cu-wat.	1	78	0.68	0.412
Organ	2	78	33.97	< **0.001**
Cu-sed.	1	78	0.37	0.545
Cu-wat. × organ	2	78	1.75	0.181
Cu-sed. × organ	2	78	0.31	0.737

Discussion

Our study demonstrates that the elemental composition of *M. spicatum* varies significantly among leaves, stems, and roots mainly in relation to nutrient availability in sediments, with no correlation to dissolved concentrations in water. C, P, and Zn accumulation patterns indicate that sediments serve as the primary nutrient source for this species, emphasizing the role of belowground uptake in macrophyte nutrient dynamics. These findings also suggest that *M. spicatum* employs a differentiated nutrient allocation strategy.

The observed stoichiometric adjustments emphasize the adaptability of submerged macrophytes to varied nutrient environments. The ongoing global environmental changes, including increased nutrient loading, warming, and pollutants, are considered the main reason for macrophyte decline (Phillips et al. 2016). Consequently, understanding these mechanisms plays a key role in predicting plant responses and their influence on aquatic ecosystems. Among them, N and P enrichment is deemed the primary stressor, as they promote phytoplankton blooms and periphyton overgrowth, ultimately reducing light availability (Zhang et al. 2017). However, the physiological and biochemical adjustments of macrophytes to ambient environmental conditions are very well reflected in their stoichiometry (Hessen et al. 2004).

We collected *M. spicatum* plants from lakes representing its wide range of environmental requirements (Aiken et al. 1979), allowing us to examine nutrient allocation strategies across diverse environmental settings. Consequently, these lakes exhibited variation in macro- and micronutrient concentrations in both water and bottom sediments. These two factors, water and sediment nutrient composition, are often described as a main driver for plant element allocation strategies (Li et al. 2018; Su et al. 2019). Employing *M. spicatum* as a model, our study reveals the strategy on how submerged macrophytes can regulate nutrient uptake and distribution, enhancing the understanding of aquatic plant ecology.

Elemental composition of plant organs

Both above- and belowground organs of submerged plants can acquire nutrients, and the source depends on their relative concentrations in the sediment and water. Moreover, luxury consumption of both N and P is documented in nutrient-rich conditions (Carignan and Kalff 1980; Sterner and Elser 2002). *Myriophyllum spicatum* can meet its N demand both by uptake from the sediment via its roots and by acquisition from the water by stem and leaf tissues. In our study, the highest N concentration was observed in the leaves and roots compared to stems, which is in line with previous studies (Best and Mantai 1978). Interestingly, no relationship between plant N and N sources was observed. Possibly, the plants acquired N simultaneously from water and sediments, significantly reducing transport through the stem and lowering its concentration in this organ. As stems play an essential role in photosynthate loading (Madsen and Sand-Jensen 1991), they may need more N to maintain phloem transport, which may have been reduced in this case. In nutrient-rich environments with low photosynthetic activity due to poor light conditions, the

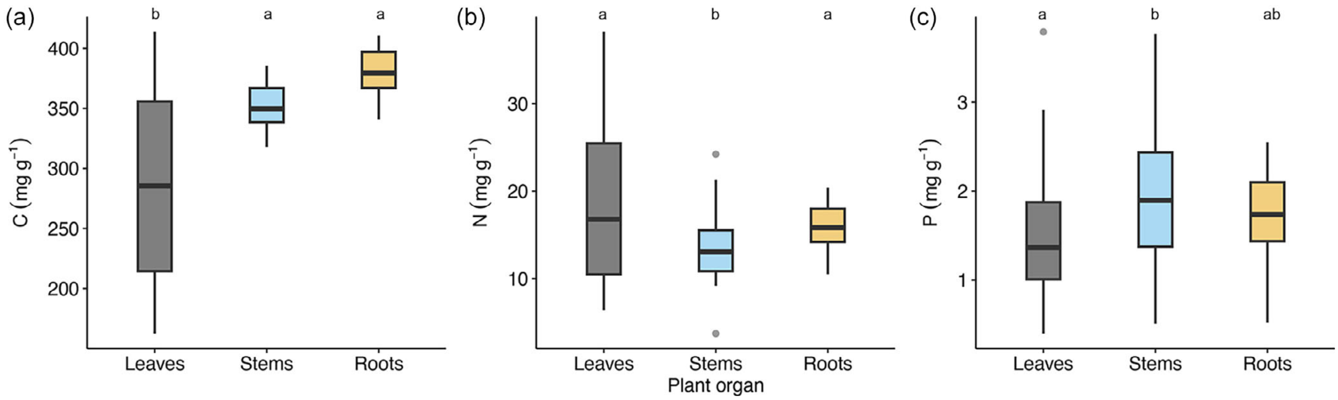

Fig. 2. The concentration of carbon (**a**), nitrogen (**b**), and phosphorus (**c**) in leaves, stems, and roots of *Myriophyllum spicatum*. Different letters denote significant differences.

formation of sugars (C-based) and associated photosynthate transport and nutrient requirements of the phloem are reduced. However, more nutrients can be allocated to the leaves to offset the reduced photosynthetic activity under low-light conditions (Leakey et al. 2009; Rao et al. 2020).

Phosphorus is mainly taken up by the roots from the sediment due to its relatively higher concentration in the sediment than in the water. This assumption was confirmed in our study, as the differences in P concentrations between plant organs interacted with sediment P concentrations. Though not significant for the individual plant organs (Table S2), a tendency for a simultaneous decrease in leaf P concentration and an increase in root P concentration was observed with increasing sediment P concentration. In addition, sediments, through their high spatial heterogeneity and chemical characteristics, determine the distribution of submerged plants in the ecosystem and influence the nutrient allocation strategy in rooted submerged plants (Mackay et al. 2012; Li et al. 2018). The highest P concentration in the stem may be due to its allocation from leaves and roots and be linked to C accumulation and the role of the stem as a reproductive organ in the process of auto-fragmentation. Reproductive organs maintain higher nutrient concentrations to stimulate seedling establishment and reproduction (Kerkhoff et al. 2006). Moreover, *M. spicatum* roots contribute to phosphorus uptake from sediments and transfer to the water, with stems playing a primary role in phosphorus transport (Best and Mantai 1978) and may explain the higher P concentration in the stems than in the leaves. Considering the critical function of the stem in linking leaves and roots and transporting nutrients and photosynthates, plants showed higher C and P allocation in stems and roots than in leaves and higher N allocation in leaves than in stems.

The roots were the main organ responsible for C storage, followed by the stems. Leaves had the lowest concentration of this element. C allocated to stems is used to form structural compounds (primarily lignin and structural polysaccharides) and non-structural carbohydrates (Marschner 2012). C is also a source of energy for the synthesis of amino acids, which in feedback processes play a vital role in the metabolism of C or N. Moreover, C, in the form of soluble carbohydrates and starch, serves as a principal energy storage and a C reservoir as a building material (Cao et al. 2008; Dülger et al. 2017). In aquatic plants, C storage in different structures, such as stems, winter buds, and rhizomes participates in increasing re-growth and reducing offspring mortality in systems with frequent disturbance, as well as after overwintering (Barrat-Segretain and Bornette 2000; James et al. 2006). Nonetheless, *M. spicatum* asexual reproduction takes place predominantly via shoot auto-fragmentation, which provides the species with an efficient mechanism for population expansion and can explain the high stem C concentration. For the same reason, non-structural carbohydrates in roots could account for 20–25% of their dry weight (Aiken et al. 1979) and correspond with total C concentration in our studies, which reach up to average ~ 38%.

Myriophyllum spicatum is characterized by high efficiency in the photosynthetic process. This is due to high tolerance to shading (compensation point is about 1–2% of surface light), utilization of CO_2 and bicarbonate, the ability to recapture respired CO_2, and carbon fixation similarities to the C_4 pathway (Nichols and Shaw 1986; Madsen and Sand-Jensen 1991). *Myriophyllum spicatum* can also supply C to photosynthetic tissue from roots, though this accounts for less than 1.5% of total C in stems (Loczy et al. 1983). Considering these adaptations together with relatively high C-wat. concentrations in the studied lakes (Song et al. 2018) and no relationship between C in sediments and roots, the possibility of a direct transfer from lake sediment to roots is not very plausible. The explanation for this negative relationship could rather be sought in habitat conditions. Since the amount of carbon reflects the lake's trophic state and is connected to all the parameters that characterize it (e.g., light availability, phytoplankton abundance; Anderson et al. 2014), we hypothesize

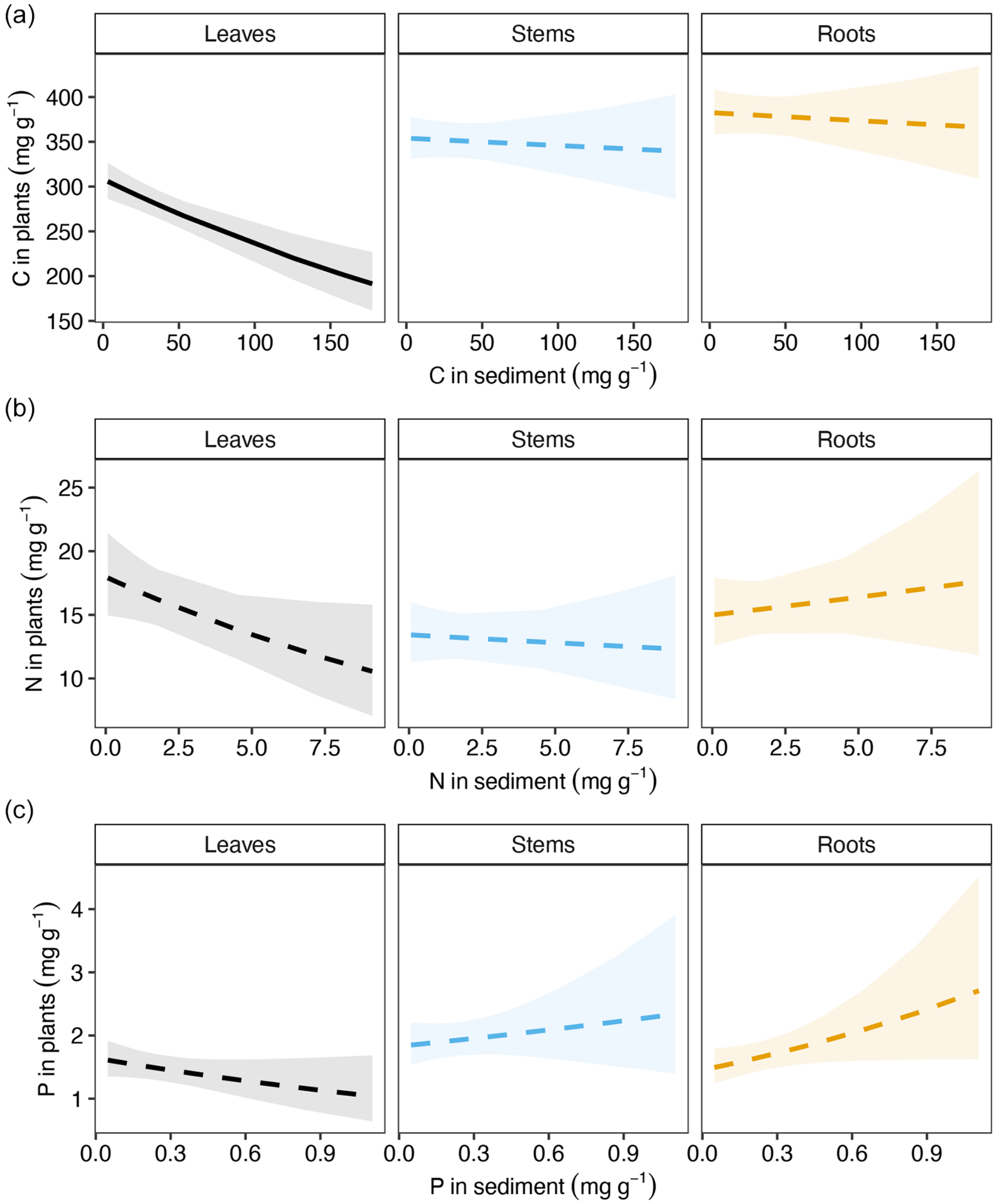

Fig. 3. Relationships between carbon (**a**), nitrogen (**b**), and phosphorus (**c**) concentrations in different *Myriophyllum spicatum* organs and their concentrations in sediment. Line types denote either significant (solid) or insignificant (dashed) relationships, while shaded regions show 95% confidence intervals.

that the higher amount of C in stems and roots, compared to leaves, resulted from its intensified allocation from the binding site to the storage site. This mechanism increases the chances of survival in a situation of sudden environmental deterioration. This also aligns with the strategy to elongate the stem to alleviate low-light stress, which demands more resources (Chen et al. 2016).

The highest accumulation of the micronutrients Fe, Zn, and Cu was in the roots, and the lowest was in the stem. This finding is in line with other studies on *M. spicatum* and aquatic plants generally (Baldantoni et al. 2004; Yabanli et al. 2014) and may be attributed to root uptake and allocation to aboveground parts as the main pathway of metal ion transport (Jackson 1998).

C : N : P stoichiometry

The C : N, C : P, and N : P ratios in *M. spicatum* organs showed a significant increase from leaves to roots, confirming different biomass allocations between these organs. Since the major component of total P biomass in the organism is RNA

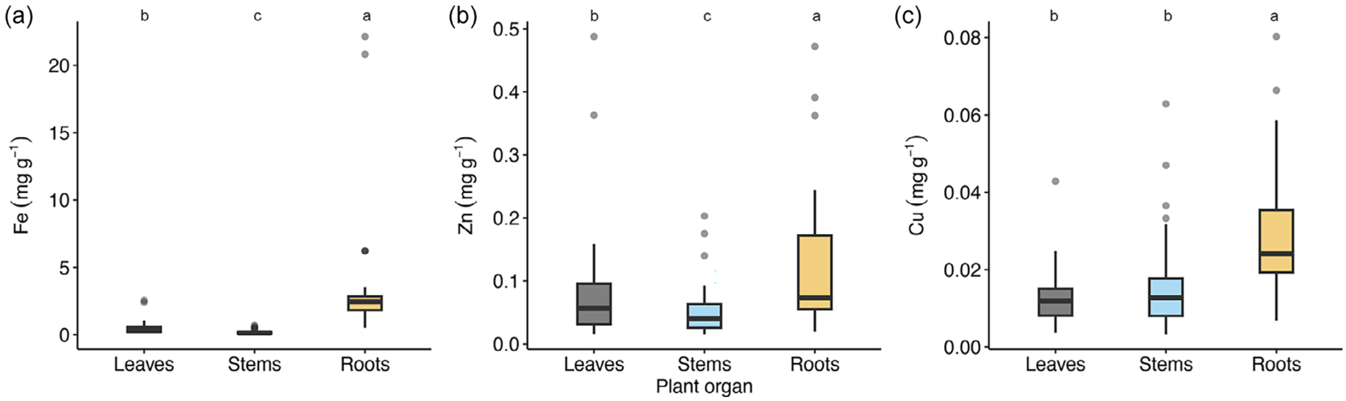

Fig. 4. Iron (**a**), zinc (**b**), and copper (**c**) concentration in leaves, stems, and roots of *Myriophyllum spicatum*. Different letters denote significant differences.

(Elser et al. 1996), the positive relationship between RNA concentration and growth rate indicates that differences in vital strategies are reflected in the P concentration. Therefore, organs with a high P concentration and a lower C : P ratio, as observed in our study, could be displaying a relatively high growth rate (Geider and La Roche 2002; Vrede et al. 2002). This would imply that the leaves of *M. spicatum*, despite the relatively high C : P ratio compared to other studies (Velthuis et al. 2018; Yuan et al. 2022), would display the highest growth rates compared to other plant organs. Leaves are the main organs of photosynthesis, producing the driving force for growth, so an increase in photosynthetic area is a desirable trait under unfavorable light conditions associated with higher trophy (Middelboe and Markager 1997; Owens et al. 2008). Not only do stems transport minerals and carbohydrates (Schutten et al. 2005), but their rapid elongation toward the water surface is a defense mechanism against shading, bringing leaves closer to the water surface (Chen et al. 2016). In addition, their rapid growth allows for the elevation of generative reproduction organs above the water surface, relatively rapid reproduction, and the extension of the diaspore bank.

In the case of N, the dominant pool in autotroph biomass is represented by ribulose bisphosphate carboxylase-oxygenase (RuBisCO), the enzyme that drives the dark phase of photosynthesis, making it the most abundant protein on Earth (Raven 2013). This contribution is used to explain the relationship between biomass N concentration and photosynthetic efficiency (Vose and Ryan 2002). Since the C : N ratio of biomass is related to N use efficiency, in this way it helps to illustrate the mechanisms of allocation between organs and their performance (Vitousek 1982). In our case, changes in N are co-occurring with changes in P, highlighting the higher performance of *M. spicatum* leaves as trophy increases. A decreasing C : N ratio, that is, the highest N concentration in leaves, indicates intensified photosynthetic processes and intensified growth rates (Vrede et al. 2004).

In our study, stoichiometric plasticity was observed for C and P concentration in leaves, and they negatively correlated with C and P concentration of sediments. When considering stoichiometric responses in individual plant organs, leaves were most affected by environmental influences, whereas stem stoichiometry was not affected, and roots were the main part responsible for metal accumulation. Therefore, our studies underscore the importance of sediment as a primary source of elements for freshwater plants.

Implications for nutrient cycling and food quality

Myriophyllum spicatum is known to be a nutrient-tolerant species that thrives across a range of trophic conditions, from mesotrophic to eutrophic lakes. It establishes stable populations in environments with moderate to high nutrient loads, whereas in nutrient-poor or highly eutrophic systems, its presence tends to be more sporadic and less developed (Smith and Barko 1990). Considering its invasive potential in some regions, *M. spicatum* may outcompete native macrophytes, potentially leading to shifts in community composition and ecosystem functioning. Despite its limited palatability, the species plays an important role in nutrient cycling and ecosystem structure. Its ability to tolerate mesotrophic and eutrophic conditions makes it a key player in nutrient retention and redistribution, highlighting the strong interdependence between growth strategy, biomass composition, and biochemical investment in it. In such systems, intense growth requires a close match between the stoichiometry of resources and their consumers, shaping trophic interactions and overall ecosystem functioning (Sterner and Elser 2002). Therefore, the C : N : P stoichiometry of the environment, producers, and consumers affects the structure and function of entire food webs and broadly regulates global processes such as the carbon cycle (Hessen et al. 2004). High C : P and C : N ratios affect consumers, who will have low growth efficiency for C and consequently low biomass growth rates, leading to lower grazing pressure (Cebrián et al. 1998). However, strict matching of C : P ratios in plants and herbivores is

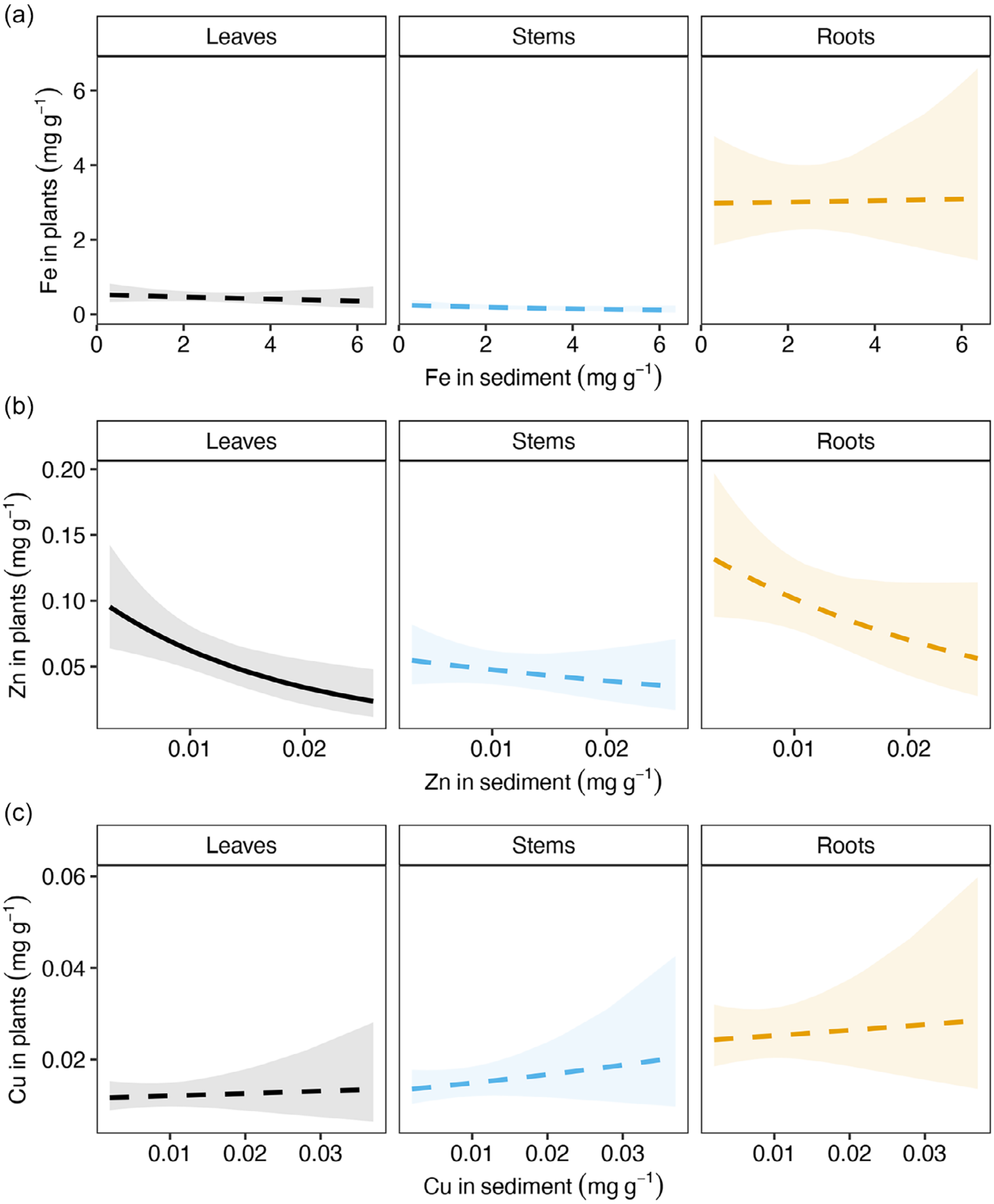

Fig. 5. Relationships between iron (**a**), zinc (**b**), and copper (**c**) concentrations in different *Myriophyllum spicatum* organs and their concentration in sediment. Line types denote either significant (solid) or insignificant (dashed) relationships, while shaded regions show 95% confidence intervals.

not a general rule, either in aquatic or terrestrial habitats, suggesting that stoichiometric limits on herbivore growth are common (Elser et al. 2000). Eutrophication, whether natural or accelerated by anthropogenic changes, affects the supply of nutrients, which consequently determines the biochemical composition and growth rate of primary producers. Autotrophs, here *M. spicatum* as an example, can rapidly produce new biomass characterized by very high C : N and C : P ratios in enriched habitats. This can have further consequences on the trophic structure. By making a trade-off between growth rate and nutrient requirements in herbivores (Branco et al. 2010; Mooney et al. 2010), an increase in C : nutrients could shift herbivores from species with high nutrient requirements and high growth rates to species with low requirements and low growth rates, with possible consequences for herbivore grazing (Teurlincx et al. 2017). Moreover, organic matter built up, while passing into detritus form, can be decomposed by microorganisms at a lower rate (with relatively high nutrient demand), which can slow down the rate of nutrient recycling (Makino et al. 2003).

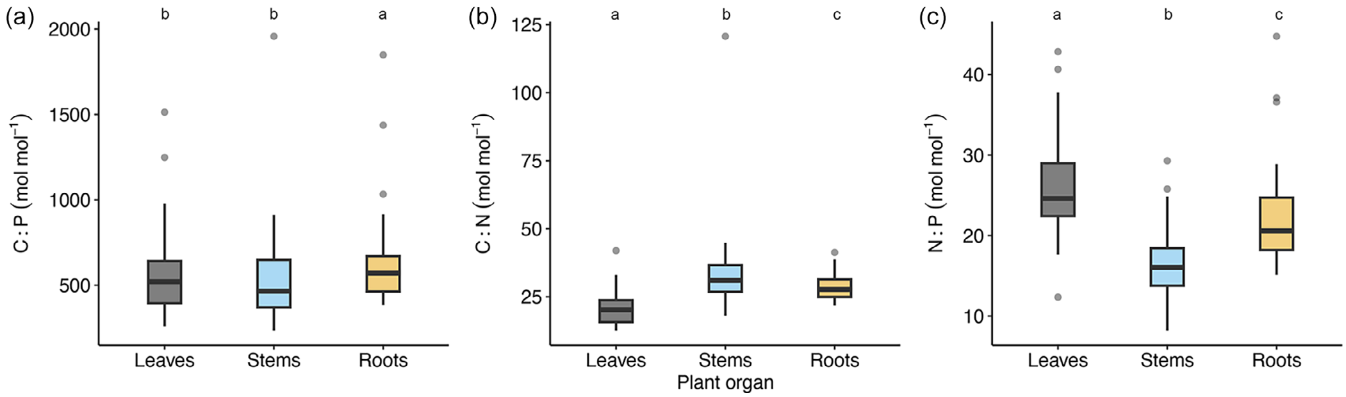

Fig. 6. Ratio of C : P (**a**), C : N (**b**), and N : P (**c**) in leaves, stems, and roots of *Myriophyllum spicatum*. Different letters denote significant differences.

Table 3. Results of generalized linear mixed models testing how C : N : P stoichiometry in different plant organs (leaves, stem, and roots) depend on their ratio in water and sediment. Significant *p*-values (< 0.05) are indicated in boldface.

Model term	DF_{num}	DF_{denom}	F ratio	*p*
Carbon : Phosphorus ($R^2_{cond} = 0.65$, $R^2_{marg} = 0.10$)				
Organ	2	75	7.90	< **0.001**
C : P-sed.	1	75	0.05	0.825
C : P-wat.	1	75	0.55	0.461
C : P-sed. × organ	2	75	0.49	0.615
C : P-wat. × organ	2	75	0.07	0.929
Carbon : Nitrogen ($R^2_{cond} = 0.74$, $R^2_{marg} = 0.55$)				
Organ	2	40	44.86	< **0.001**
C : N-sed.	1	40	1.01	0.320
C : N-wat.	1	40	1.92	0.173
C : N-sed. × organ	2	40	2.94	0.065
C : N-sed. × organ	2	40	0.02	0.977
Nitrogen : Phosphorus ($R^2_{cond} = 0.75$, $R^2_{marg} = 0.38$)				
Organ	2	39	34.49	< **0.001**
N : P-sed.	1	39	0.02	0.882
N : P-wat.	1	39	0.39	0.536
N : P-sed. × organ	2	39	1.21	0.310
N : P-wat. × organ	2	39	0.19	0.830

Conclusions

Although our initial hypothesis predicted a reduction in C : N and C : P ratios with increasing trophic status, the results did not support this prediction. However, the observed differences in elemental concentrations between plant organs were linked to sediment nutrient availability rather than water column concentrations, providing partial support for the proposed adaptive nutrient allocation strategy. These findings emphasize the role of sediments as the primary nutrient source shaping the stoichiometry of *M. spicatum* and highlight organ-specific responses that reflect their distinct functional roles.

Leaves exhibited variability in C and Zn content, with concentrations decreasing as sediment levels of these elements increased. Meanwhile, Fe and Cu were predominantly accumulated in the roots. These patterns suggest a specific nutrient allocation strategy: N enrichment in leaves supports photosynthetic activity, stems function as reservoirs for C and P to facilitate growth and reproduction, and roots serve as the primary sites for micronutrient storage, particularly Fe, Zn, and Cu.

Understanding the biochemical composition of organisms, their trophic interactions, and the transformation of biomass into detritus is essential for assessing how environmental stressors influence nutrient cycling and ecosystem stability, particularly in the context of climate change and ongoing shifts in aquatic environments.

Author Contributions

Conceptualization: Michał Rybak. Formal analysis: Michał Rybak, Jakub Szymkowiak, Magdalena Woźniak, Izabela Ratajczak, and Mandy Velthuis. Funding acquisition: Michał Rybak. Investigation: Michał Rybak, Tomasz Joniak, Piotr Klimaszyk, and Łukasz Wejnerowski. Methodology: Michał Rybak and Jakub Szymkowiak. Software: Jakub Szymkowiak and Mandy Velthuis. Supervision: Michał Rybak. Visualization: Jakub Szymkowiak. Writing – original draft preparation: Michał Rybak, Jakub Szymkowiak, and Mandy Velthuis. Writing – Review & Editing: Michał Rybak, Jakub Szymkowiak, Łukasz Wejnerowski, and Mandy Velthuis. All authors provided feedback on drafts.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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