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Macrophyte community structure across Serbian water-body types: foundations for developing bioassessment tools

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Macrophytes are widely used as biological quality elements in ecological assessment, yet the extent to which they reflect water-body typology remains insufficiently evaluated in Serbia. We analyzed macrophyte assemblages from 99 sites representing nine nationally designated water-body types to determine (i) whether assemblages differ among types, (ii) which environmental gradients structure these differences, (iii) which species contribute most strongly to typological separation, and (iv) how accurately typology can be predicted from macrophyte composition. Presence-absence and abundance data were assessed using incidence-based rarefaction/extrapolation (iNEXT), non-metric multidimensional scaling (NMDS) with fitting of environmental vectors (envfit), community differentiation analysis (PERMANOVA/PERMDISP), distance-based redundancy analysis (dbRDA), Indicator Value Analysis (IndVal), dissimilarity analysis (SIMPER), and Random Forest (RF) classification. Diversity patterns, constrained ordination, and multivariate tests revealed clear typological structure, with conductivity, water temperature, and elevation emerging as the strongest predictors of community composition. IndVal, SIMPER, and RF consistently identified a small suite of submerged, floating, and emergent species that characterized individual types or shared ecological gradients. The RF classifier achieved 72.7% out-of-bag accuracy, demonstrating strong predictive signal despite heterogeneity in some modified or sparsely sampled types. Overall, macrophyte assemblages responded coherently to the interacting hydro-morphological and physicochemical gradients that define Serbia's surface-water typology. These findings establish a robust ecological foundation for the future development of macrophyte-based bioassessment tools and support the implementation of WFD-compliant ecological status classification in Serbia.

KEYWORDS

aquatic macrophytes, community structure, ecological assessment, environmentalgradients, indicator species, random forest classification, water-body typology, Water Framework Directive (WFD)

1 Introduction

Aquatic macrophytes are key structural and functional components of freshwater ecosystems, contributing to primary production, nutrients cycling, sediment stabilization, and habitat provisioning for invertebrates and fish (Chambers et al., 2008). Their distribution reflects the interplay among hydro-morphological conditions, physicochemical gradients, light availability, and disturbance regimes, making them sensitive indicators of ecological change (Riis and Biggs, 2003; Lacoul and Freedman, 2006; Baattrup-Pedersen et al., 2006; O'Hare et al., 2006). Because of their strong responses to eutrophication, hydrological alteration, and habitat degradation, macrophytes constitute a core biological quality element in ecological assessment under the EU Water Framework Directive (WFD; European Commission, 2000; Hering et al., 2010; Aguiar et al., 2011a; Birk et al., 2012).

Substantial progress has been made toward harmonizing macrophyte-based assessment across Europe (Birk et al., 2006; Birk and Willby, 2010; Aguiar et al., 2014). However, challenges persist in regions where hydrological conditions are diverse and where natural rivers, modified channels, reservoirs, and artificial canals coexist. In such settings, variable flow regimes, sediment dynamics, and nutrient pressures interact with broader environmental gradients, complicating efforts to define type specific reference conditions. These challenges are further exacerbated where biological quality element datasets are incomplete, particularly with respect to type-specific macrophyte assemblages needed to refine WFD-compliant typology. Serbia represents one such case. Its freshwater network spans lowland rivers, regulated and impounded water bodies, and an extensive Pannonian canal system. These systems differ markedly in hydro-morphology, nutrient enrichment, sediment characteristics, land-use intensity - environmental factors known to structure macrophyte assemblages (Riis and Biggs, 2003; Aguiar et al., 2011b; Alahuhta et al., 2012; Baláži and Hrivnák, 2017; Stefanidis et al., 2023; Budka et al., 2024). Despite this environmental diversity and the importance of typology-driven classification for national WFD implementation, empirical evaluations of typology-macrophyte correspondence in Serbia remain scarce.

Ecological mechanisms strongly suggest that macrophyte communities should reflect water-body typology. Hydro-morphological attributes–such as flow velocity, substrate composition, and bank slope–influence species establishment, growth forms, and competitive interactions (Riis and Biggs, 2003; O'Hare et al., 2006; Szoszkiewicz et al., 2014; Kaijser et al., 2022). Physicochemical gradients, including nutrients, temperature, and conductivity, shape tolerance limits and successional patterns (Baattrup-Pedersen et al., 2006; Navarro Law et al., 2024), while landscape context modulates shading, sedimentation, and disturbance (Aguiar et al., 2011b; Lemm et al., 2021). Therefore, water-body types that differ consistently in these conditions are expected to support distinct macrophyte assemblages, whereas transitional or highly heterogeneous types may show weaker differentiation.

Studies from Central and Eastern Europe report varying levels of typology-macrophyte correspondence, depending on gradient magnitude, hydro-morphological alteration, and spatial scale (Hrivnák et al., 2013; Jusik et al., 2015; Bubíková and Hrivnák, 2018a; Szoszkiewicz et al., 2017; Halabowski and Lewin, 2020). However, no integrated, quantitative assessment has yet examined how macrophyte assemblages vary across Serbia's water-body typology.

To address this, we analyzed macrophyte assemblages from 99 sites representing nine Serbian water-body types, applying combined framework of unconstrained and constrained ordination, sample completeness and diversity estimation, indicator species analysis, and machine-learning classification. Specifically, we asked: (i) Do macrophyte assemblages differ significantly among Serbian water-body types? (ii) To what extent do environmental gradients explain these differences? (iii) Which species contribute most strongly to typological separation or act as reliable indicators? and (iv) How accurately can typology be predicted from macrophyte assemblage composition? By addressing these questions, our aim is to strengthen the ecological basis of water-body typology in Serbia and to evaluate the capacity of macrophytes to support WFD-compliant ecological assessment across natural, modified, and artificial water bodies.

2 Materials and methods

2.1 Study area

The study was conducted on the territory of the Republic of Serbia, encompassing aquatic environments representative of the country's officially designated surface-water typology (Republic of Serbia, 2011). The region spans the Pannonian lowland plain, dominated by warm, mineral-rich watercourses, to the foothills of the Carpathian and Balkan Mountains, where cooler, dilute upland reservoirs occur. This physiographic gradient corresponds to major ecological differences in hydrology, conductivity, nutrient status, channel morphology, and catchment land use–factors known to structure macrophyte assemblages.

A total of 99 sites were sampled across nine nationally defined water-body types (Figure 1; Table 1), including natural rivers (T_1, T_2, T_3, T_5), artificial canals (T_8), and heavily modified water bodies such as reservoirs (R_1, R_3, R_4, R_6). Natural lowland rivers are characterized by warm temperatures, high conductivity, and strong agricultural influence. Artificial canals form extensive, hydrologically regulated network with engineered banks and elevated mineral content. Heavily modified types included the impounded Danube main stem (R_1) and mid-altitude to upland reservoirs (R_3, R_4, R_6), which exhibit cooler temperatures, lower conductivity, and simplified littoral zones. The diversity of hydrological and landscape settings provides a robust environmental template for evaluating typology-macrophyte relationships.

2.2 Study sites, macrophyte and environmental data

Macrophyte surveys were conducted in 2017, 2019, and 2024 within Serbia's National Monitoring Programme for ecological classification of surface waters, following WFD requirements (European Commission, 2000). Sampling followed the European standard EN 141484 for aquatic macrophyte assessment in running waters (CEN, 2014). Macrophytes were surveyed in longitudinal transects–survey units that were 100, 500, or 1,000 m long, depending on water-body size and morphology. At each site a minimum of three survey units were sampled on each bank. Species abundance was estimated using five-level Kohler scale (Kohler, 1978). Across the 99 sites, a total of

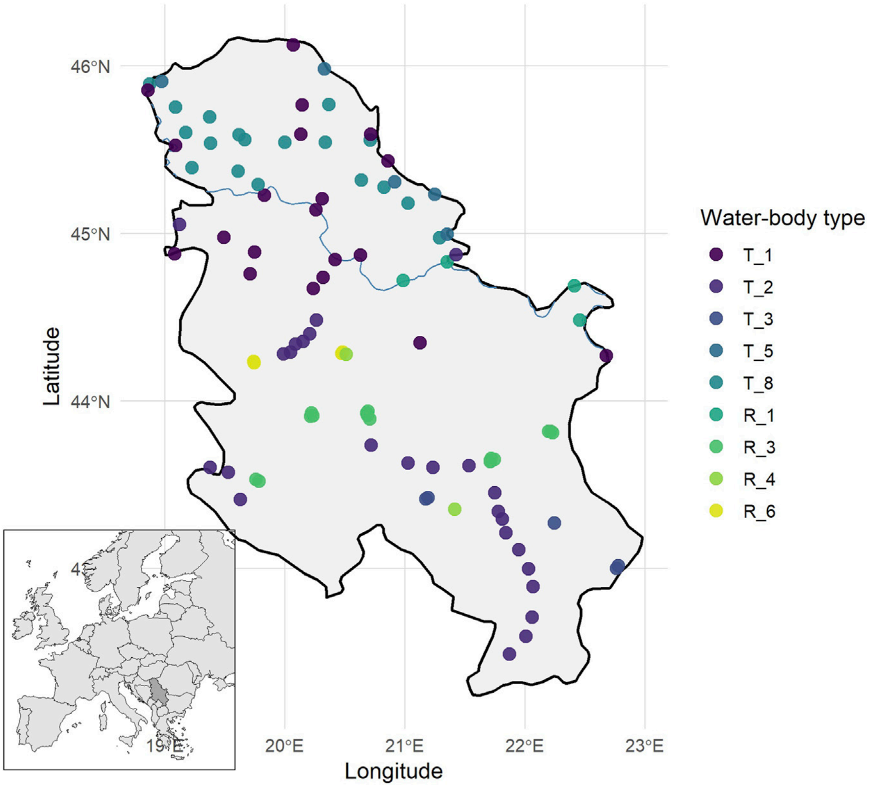

FIGURE 1
Location of 99 sampling sites across nine Serbian water-body types. Sites are mapped according to GPS coordinates and colored by typology.

TABLE 1 Typology of water bodies used in this study. Typological groups are based on the official WFD-compliant national classification criteria (Republic of Serbia, 2011).

Code	Description
T_1	Large lowland rivers with domination of fine sediment
T_2	Large rivers dominated by medium-sized sediment (except pannonian rivers)
T_3	Small and medium watercourses (<500 m a.s.l.) with coarse substrate
T_5	Pannonian watercourses not assigned to T_1
T_8	Artificial water bodies – canals
R_1	Reservoirs on T_1 rivers
R_3	Reservoirs on T_3 rivers
R_4	Reservoirs on T_4 rivers (>500 m a.s.l., small/medium coarse substrate watercourses)
R_6	Reservoirs on T_6 rivers (small non-pannonian watercourses not included in T_3 and T_4)

81 aquatic plant species were recorded (Supplementary Table S1), representing submerged, floating-leaved, free-floating, and emergent growth forms.

Physicochemical variables included water temperature (w_t), dissolved oxygen (dis_O), pH, electrical conductivity (cond), ammonium (NH4_N), nitrite (NO2_N), nitrate (NO3_N), total nitrogen (total_N), orthophosphate (PO4_P) and total phosphorus (total_P). Annual mean values were obtained from the Serbian Environmental Protection Agency (SEPA, www.sepa.gov.rs).

Hydro-morphological and landscape variables included bank slope (bnk_slp; 1 = gentle; 2 = intermediate; 3 = steep), elevation (elev) channel width (chn_w), riparian zone width (rip_w), number of land use types (No_LUt) and the average degree of hemeroby (avg_hmrb). CORINE Land Cover (CLC) classes were recorded in the field following Copernicus guidelines (https://land.copernicus.eu/en/products/corine-land-cover), and the number of land use types and the average degree of hemeroby were derived from CLC categories (Walz and Stein, 2014). Channel and riparian zone widths were measured from high-resolution Google Earth imagery. Descriptive statistics for all environmental variables were provided in Supplementary Table S2.

Spatial data for mapping (Figure 1) were created using site-level GPS coordinates and publicly available Natural Earth datasets for national boundaries and major river networks.

2.3 Data analysis

Pre-processing. Species abundance values were Hellinger-transformed prior to multivariate analyses to reduce dominance effects and improve sensitivity to compositional gradients (Legendre and Gallagher, 2001). For diversity estimation, presence-absence matrices were derived. Environmental variables were checked for outliers, normality, and missing values, and standardized (mean = 0, SD = 1) before multivariate modelling.

2.3.1 Diversity estimation

Macrophyte diversity and sampling completeness were assessed using *iNEXT* (Chao et al., 2014; Hsieh et al., 2016), which implements rarefaction and extrapolation based on Hill numbers. For each site we calculated: q0 – species richness (observed and Chao2-estimated); q1 – Shannon diversity (effective number of common species); q2 – Simpson diversity (effective number of dominant species). Because abundances were not measured as individual counts, we used incidence-based iNEXT formulation, treating each site as sampling unit. This allows standardized comparison of diversity across water-body types with unequal sampling effort.

2.3.2 Ordination and environmental fitting

Community structure was explored using non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarities (Clarke, 1993), as implemented in the *vegan* package (Oksanen et al., 2025). Multiple random starts ensured solution stability. Environmental correlates were assessed using environmental vector fitting (envfit) procedure with 999 permutations. Only variables with significant associations ($p < 0.05$) were retained for interpretation (Supplementary Table S4).

2.3.3 Multivariate tests of community differences

Differences in macrophyte composition among water-body types were evaluated using Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) with 999 permutations. Assumptions of homogeneity of multivariate dispersion were tested using Permutational Analysis of Multivariate Dispersions (PERMDISP; Anderson, 2006). Significant PERMANOVA results were interpreted with dispersion differences (Supplementary Table S5), as recommended for community-level comparisons.

2.3.4 Distance-based redundancy analysis (dbRDA)

To quantify environmental control of community structure, we performed dbRDA using Bray-Curtis dissimilarities, implemented in *vegan*'s capscale function (McArdle and Anderson, 2001). Significance of the overall model, individual predictors and canonical axes was assessed using 999 permutations. To avoid multicollinearity, we computed variance inflation factors (VIF) via *vegan* (Dixon, 2003; Oksanen et al., 2025). Variables with VIF >5 were removed iteratively until all remaining predictors met VIF <5. Both VIF screening and envfit converged on water temperature (w_t), conductivity (cond), and elevation (elev) as independent, ecologically relevant predictors. These were used in the final dbRDA (Supplementary Table S6).

2.3.5 Species-level analyses: SIMPER and IndVal

Similarity Percentage Analysis (SIMPER; Clarke, 1993) was used to identify species contributing most to compositional dissimilarities among water-body types. For each pairwise contrast, species were ranked by contribution, and those cumulatively contributing to 70% dissimilarity were retained. Only contrasts with significant overall dissimilarity ($p < 0.05$) were included in Supplementary Table S12.

Indicator species were identified using IndVal method (Dufrêne and Legendre, 1997) with 999 permutations (*indispecies* package; De Cáceres and Legendre, 2009; De Cáceres et al., 2025). Significant species ($p \leq 0.05$) were assigned to either single water-body types (Supplementary Table S10) or multi-type combinations (Supplementary Table S11).

2.3.6 Random forest classification

To evaluate predictive power of macrophyte assemblages for water-body typology discrimination, we applied Random Forest (RF) classifier (Breiman, 2001) (via *randomForest*; Breiman et al., 2025). The response variable was the nine-level typology factor. Model performance was assessed using the out-of-bag (OOB) error rate, providing internal cross-validation; and 10-fold cross-validation to optimize mtry tuning parameter (Supplementary Table S8). Species importance was quantified using: MeanDecreaseAccuracy (reduction in classification accuracy when species is permuted) and MeanDecreaseGini (total decrease in node impurity attributable to each species). Higher values indicate stronger discriminatory influence (Supplementary Table S9). Classification accuracy per type was evaluated using confusion matrices (Supplementary Table S7) and visualized alongside community ordinations (Figure 3C).

2.3.7 Software

All analyses were performed in R (version 4.5.2) using the packages: *vegan* (NMDS, PERMANOVA, PERMDISP, dbRDA); *iNEXT* (rarefaction/extrapolation); *indispecies* (IndVal); *randomForest* (classification and variable importance). Script used for analyses is provided in the Supplementary Material.

3 Results

3.1 Environmental context and typology

A total of 99 sites were surveyed across nine water-body types, including natural rivers (T_1, T_2, T_3, T_5), heavily modified water bodies (i.e., reservoirs; R_1, R_3, R_4, R_6), and artificial canal systems (T_8) (Figure 1).

Annual mean environmental data indicated generally warm, well-oxygenated, and slightly alkaline conditions across all types (Supplementary Table S2; Supplementary Figure S1). Water temperature ranged from ~12 °C–13 °C in lowland rivers (T_2,

TABLE 2 Observed and asymptotic macrophyte diversity estimates across water-body types. Diversity metrics include: q0 = species richness, q1 = Shannon diversity (effective number of common species), q2 = Simpson diversity (effective number of dominant species) Estimates are derived from incidence-based rarefaction/extrapolation using iNEXT framework. Values represent effective number of species; asymptotic richness values are shown with 95% confidence intervals in parentheses.

Type	n	Richness (q0)		Shannon (q1)		Simpson (q2)	
		Observed	Asymptotic	Observed	Asymptotic	Observed	Asymptotic
T_1	20	42	42 (42–45.92)	29.76	31.53	21.94	23.27
T_2	25	37	47 (37–70.12)	21.39	22.79	16.19	16.84
T_3	5	24	25.11 (24–35.17)	19.53	22.69	16.53	20.11
T_5	5	38	38.64 (38–47.12)	31.78	36.47	27.16	33.19
T_8	18	44	45.78 (44–58.47)	24.7	25.75	18.15	18.7
R_1	4	35	37.24 (35–49.65)	27.14	30.51	22.98	26.44
R_3	16	29	29 (29–32.96)	21.23	22.13	16.9	17.72
R_4	2	2	2.33 (2–4.51)	1.89	2.52	1.8	3
R_6	4	9	10.96 (9–16.82)	6.9	7.71	6.02	6.72

T_5) to >15 °C in canals and impounded systems (T_8, R_1, R_3). Dissolved oxygen concentrations were consistently high (typically 8–10 mg L^{-1}), and pH showed little variation around alkaline values (~8.0). In contrast, conductivity and nutrients displayed pronounced typological patterns: conductivity was lowest in upland reservoirs (R_4, R_6; ~130–190 µS cm^{-1}) and highest in lowland rivers (T_1, T_3, T_5; 450–700 µS cm^{-1}), while canals (T_8) also exhibiting elevated values. Nutrient enrichment was strongest in modified lowland types (T_2, T_5, T_8), whereas reservoirs (R_1, R_4, R_6) generally showed lower nutrient concentrations.

Hydro-morphological and landscape metrics further differentiated types. Channel width ranged from narrow streams (T_5 ~10 m; T_2 ~35 m) to the very wide Danube main stem (R_1 > 1 km). Natural lowland rivers supported broad riparian zones, whereas canals and reservoirs typically had steep, engineered banks and restricted littoral habitats. Hemeroby scores and land-use richness indicated strong anthropogenic influence around T_2, T_3, T_5, and T_8, while upland reservoirs, though less impacted by land use, exhibited structurally simplified shorelines.

Together, these gradients in trophic state, mineral content, hydro-morphology, and surrounding landscape form a clear environmental template structuring macrophyte assemblages across water-body types and underpin the community differences observed in multivariate analyses.

3.2 Macrophyte diversity and sampling completeness across water-body types

Rarefaction and asymptotic richness estimates revealed marked variation in macrophyte diversity among water-body types, reflecting both underlaying environmental contrasts and differences in sampling completeness. Types with the largest number of sampled sites (T_2: 25; T_1: 20; T_8: 18; Table 2) displayed well-saturated rarefaction curves (Figure 2). Observed richness closely matched asymptotic values (e.g. T_1: 42 observed vs. 42 estimated; T_8: 44 vs. 46; Table 2; Supplementary Table S3), indicating high sampling completeness.

Differences in sampling effort among water-body types should be considered when interpreting diversity estimates. Types represented by larger number of sites (e.g., T_1, T_2, T_8) exhibited well saturated rarefaction curves and narrow confidence intervals, whereas sparsely sampled types (notably R_4 and R_6) showed wide confidence intervals and unstable asymptotic estimates. For these latter types, diversity values are therefore best interpreted as indicative rather than exhaustive representations of macrophyte assemblages.

Moderately sampled types–R_3 (16 sites); T_3 and T_5 (five sites each); R_1 and R_6 (four sites each) – showed stable but less precise asymptotes. T_2 exhibited the largest gap between observed and estimated richness (37 observed vs. ~48 estimated), suggesting the presence of additional undetected rare species. R_4, represented by only two sites, showed extremely low richness and wide confidence intervals, consistent with under-sampling rather than ecological simplicity.

Diversity patterns were consistent across richness (q0), common-species diversity (q1), and dominance-weighted diversity (q2). High-diversity types (T_1, T_5, T_8, R_1) supported the greatest richness (35–44 species observed; asymptotic ~46–56) and higher evenness (Table 2; Supplementary Figures S2-S3), characteristic for wide, mineral rich, structurally heterogeneous habitats. Intermediate-diversity types (R_3, T_3, T_2) displayed moderate richness and substantial gamma diversity. Low-diversity types (R_4, R_6) were species-poor and uneven, reflecting strong hydro-morphological and physicochemical constraints such as steep banks, narrow littoral zones, and low conductivity.

These patterns, combined with the environmental gradients described in Section 3.1, indicate that high-diversity types occur in environmentally heterogeneous, nutrient- and mineral-rich settings, whereas constrained hydro-morphology and low mineral content limit macrophyte diversity in upland and modified lentic systems.

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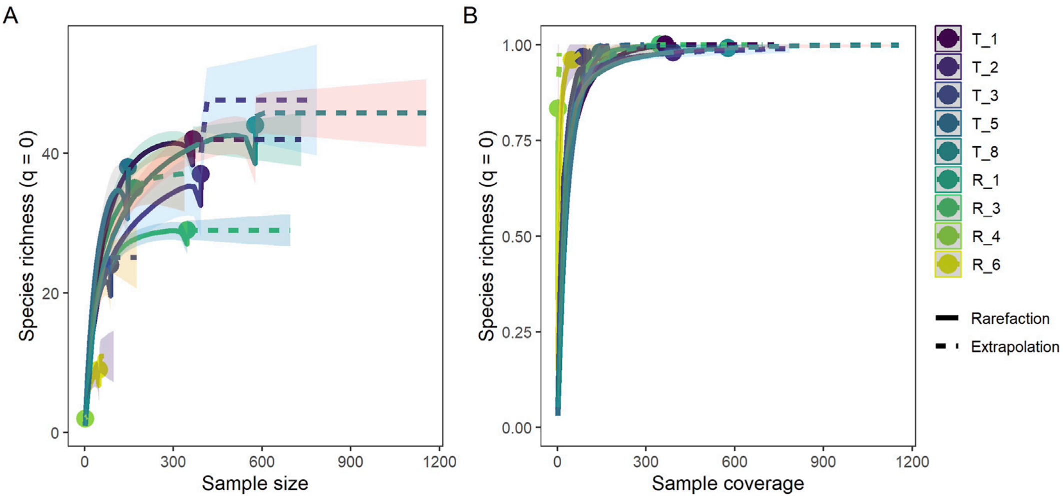

FIGURE 2
Sample-size- and sample-coverage-based rarefaction/extrapolation of macrophyte species richness (q0) across water-body types. **(A)**. Sample-size-based rarefaction/extrapolation curves showing relationship between the number of samples and estimated species richness. Solid lines indicate rarefaction, dashed lines indicate extrapolation, shaded ribbons represent 95% confidence intervals, and points mark observed richness. **(B)** Sample-coverage-based rarefaction/extrapolation curves depicting estimated richness at standardized levels of sample completeness, enabling direct comparison among types, regardless of unequal sampling effort. Together, panels A and B illustrate differences among water-body types in both estimated species richness and sampling completeness.

3.3 Environmental drivers of macrophyte community structure and typological differentiation

Non-metric multidimensional scaling (NMDS) of Hellinger-transformed data (Bray-Curtis; stress = 0.165) revealed moderate but interpretable separation among water-body types (Figure 3A). Environmental vector fitting (envfit) identified water temperature (w_t), conductivity (cond), and elevation (elev) as significant correlates of community structure ($p < 0.05$; Supplementary Table S4). Conductivity and temperature aligned with gradients distinguishing nutrient-enriched lowland rivers and canals from more diluted systems, whereas elevation primarily separated upland reservoirs from lowland types.

PERMANOVA confirmed significant differences in species composition among types ($R^2 = 0.319$, $F = 5.27$, $p = 0.001$; Supplementary Table S5a). PERMDISP revealed significant differences in within-type dispersion ($F = 3.94$, $p = 0.001$; Supplementary Table S5b,c), indicating that both centroid shifts and dispersion heterogeneity contributed to the observed typological structure. These dispersion differences indicate that significant PERMANOVA results reflect both shifts in average community composition among types and variation in within-type heterogeneity. In particular, higher dispersion in certain modified and lowland systems suggests increased ecological variability rather than weak typological signal.

Variance inflation factor (VIF) screening identified water temperature, conductivity, and elevation as independent predictors for constrained ordination. Distance-based redundancy analysis (dbRDA) explained 13.3% of total variation (constrained inertia = 5.132; Figure 3B). The full model and all predictors were highly significant ($p = 0.001$; Table 3; Supplementary Table S6). First constrained axes (CAP1) represented a combined elevation-conductivity gradient; CAP2 distinguished lowland from upland types; CAP3 captured additional minor structure. Convex-hull overlays highlighted clear separation between upland reservoirs and several lowland modified systems.

Random Forest (RF) classification further evaluated typological structure. The model achieved an out-of-bag (OOB) accuracy of 72.7%, with cross-validated accuracies ranging from 0.58–0.68 across tested mtry values; mtry = 81 performed best (Supplementary Table S8). Classification accuracy was highest for T_8 (94.4%), T_2 (88.0%), T_1 (85.0%), and R_3 (75.0%), while types represented by few sites (R_1, R_4, T_5, T_3) showed poorer classification (Supplementary Table S7). These outcomes corresponded to PERMDISP patterns and reflect ecological variability across types (Figure 3C).

Overall, macrophyte community structure responded predictably to gradients in water temperature, conductivity, and elevation. NMDS, PERMANOVA, dbRDA, and RF analyses all demonstrated clear typological differentiation shaped by environmental heterogeneity and hydro-morphological modification.

3.4 Species contributions to typological differentiation

Indicator species analysis (IndVal) identified 29 species significantly associated with individual water-body types or type combinations ($\alpha = 0.05$). Strong single-type indicators (Figure 4A; Supplementary Table S10) included *Fontinalis antipyretica* and *Epilobium palustre* (T_3), *Lycopus europaeus* (T_5), *Cabomba caroliniana* (T_8), *Potamogeton gramineus, P. lucens*, and *P. perfoliatus* (R_1), *Eleocharis palustris* (R_4), and *Najas minor* and

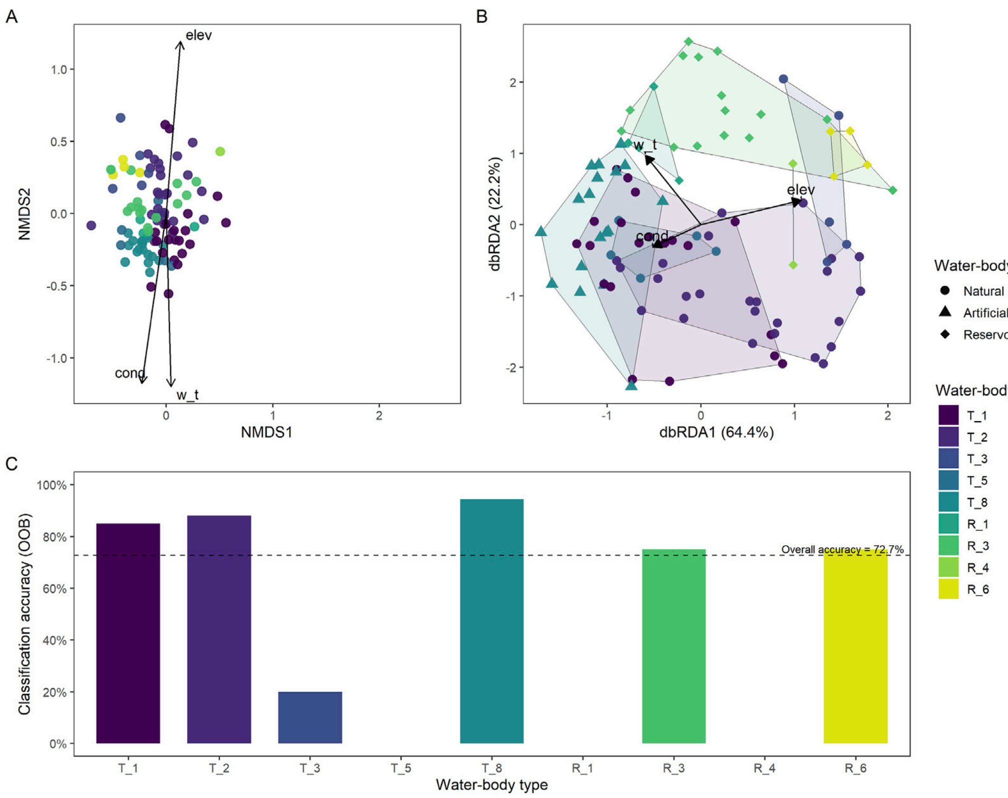

FIGURE 3
Panels A, B, and C depict the multivariate structure of macrophyte communities, the environmental gradients driving community turnover, and the extent to which assemblages predict water-body typology. **(A)** Non-metric multidimensional scaling (NMDS) ordination (Bray-Curtis; stress = 0.165) of Hellinger transformed macrophyte assemblages across water-body types. Significant environmental variables identified by *envfit* ($p < 0.05$) are shown as vectors. Targeted axis scaling was applied to improve readability of the NMDS ordination plot. **(B)** Distance-based redundancy analysis (dbRDA) constrained by water temperature (w_t), conductivity (cond), and elevation (elev), illustrating principal environmental gradients separating water-body types. Distinct point shapes indicate broader water-body categories (circle–natural rivers; triangle–artificial canals; lozenge–reservoirs). **(C)** Out-of-bag (OOB) classification accuracy of the Random Forest model for each water-body type based on macrophyte assemblages.

Chara sp. (R_6). Multi-type indicators (Supplementary Table S11) reflected shared ecological conditions across typological groups; for example, *Sparganium emersum, Mentha aquatica, Phragmites australis. Hydrocharis morsus-ranae,* and *Typha angustifolia* were broadly associated with lowland modified and transitional systems.

SIMPER analysis identified species contributing most strongly to pairwise dissimilarities among types. Key discriminating taxa included submerged species (*Myriophyllum spicatum, Ceratophyllum demersum, Potamogeton crispus, Stuckenia pectinata*), floating-leaved species (*Trapa natans, Hydrocharis morsus-ranae*), and emergent dominants (*Phragmites australis, Typha angustifolia*) (Figure 4B). Because SIMPER varies in informativeness across contrasts, only those with significant overall dissimilarity ($p < 0.05$) were retained. Species contributing up to 70% of cumulative dissimilarity are summarized in Supplementary Table S12.

Random Forest (RF) variable importance metrics (Supplementary Table S9) recovered many of the same diagnostic species. The highest MeanDecreaseAccuracy and MeanDecreaseGini values corresponded to *Phragmites australis, Phalaris arundinacea, Myriophyllum spicatum,* and *Salvinia natans*, indicating strong discriminatory power (Figure 4C). Additional influential taxa included *Potamogeton natans, Typha angustifolia, Ceratophyllum demersum, Elodea canadensis, Hydrocharis morsus-ranae, Butomus umbellatus, Stuckenia pectinata, Potamogeton crispus,* and *P. perfoliatus*.

The convergence among IndVal, SIMPER, and RF analyses demonstrates that a relatively small suite of species consistently drives typological differentiation. These taxa represent ecologically informative components of assemblages shaped by hydro-morphological modification, nutrient status, and water temperature.

4 Discussion

This study provides the first comprehensive assessment of macrophyte community structure across Serbia's nationally designated water-body types and represents a unified evaluation of typology-macrophyte relationships in a hydrologically and environmentally diverse region. By combining diversity estimation, multivariate ordination, indicator species analysis, and machine-learning classification we show that macrophyte assemblages respond strongly and predictably to the gradients that underpin Serbia's freshwater typology. These findings support the ecological validity of the typology and demonstrate the capacity of macrophytes to serve as reliable indicators for WFD-compliant assessment across natural, modified, and artificial systems.

TABLE 3 Distance-based redundancy analysis (dbRDA) of Hellinger-transformed macrophyte assemblages constrained by water temperature (w_t), conductivity (cond), and elevation (elev), using Bray-Curtis dissimilarities. The table reports: (a) overall model significance; (b) sequential contributions of individual predictors; and (c) significance of constrained axes (999 permutations). Complete dbRDA results are provided in Supplementary Table S6.

Component	Df	Eigenvalue	Sum of squares	F	p-value
(a) Overall dbRDA model					
Model	3	—	5.132	4.860	0.001
Residuals	95	—	33.441	—	—
(b) Sequential tests for individual predictors					
w_t	1	—	1.707	4.850	0.001
cond	1	—	1.371	3.894	0.001
elev	1	—	2.054	5.835	0.001
Residuals	95	—	33.441	—	—
(c) Tests for constrained axes					
CAP1	1	3.303	3.303	9.384	0.001
CAP2	1	1.138	1.138	3.266	0.001
CAP3	1	0.691	0.691	2.005	0.009
Residuals	95	—	33.441	—	—

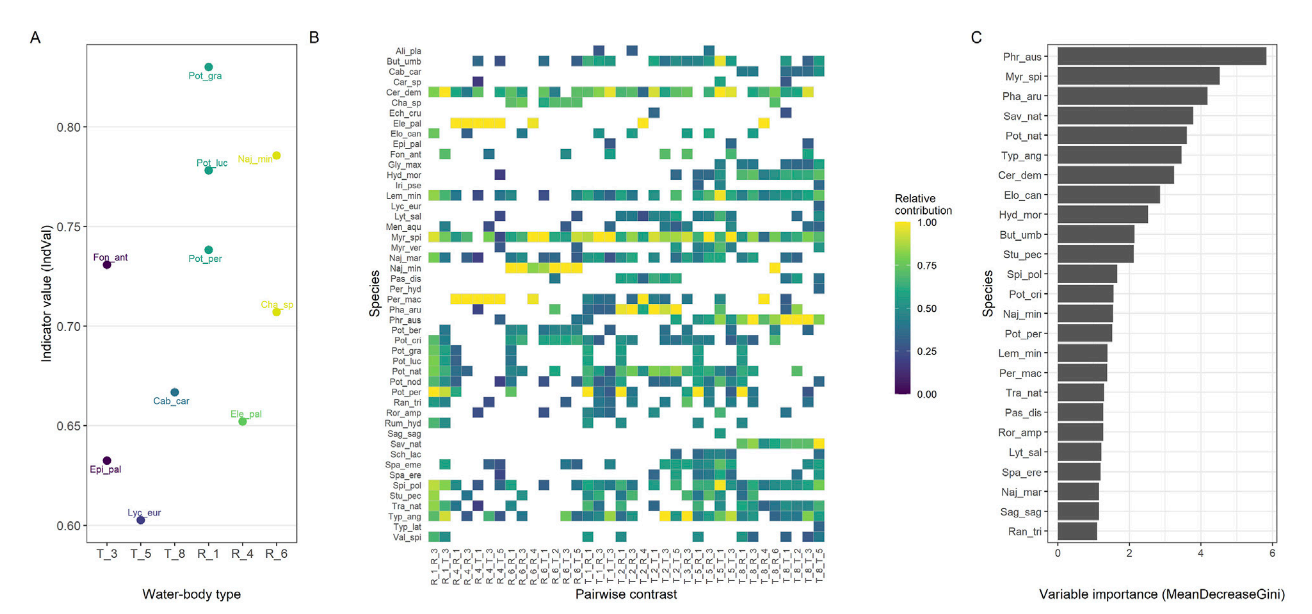

FIGURE 4
Diagnostic macrophyte species identified by Indicator Value Analysis (IndVal), SIMPER, and Random Forest (RF) classification. **(A)** Single-type indicator species (IndVal) showing significant associations ($p < 0.05$) with individual water-body types. **(B)** SIMPER heatmap showing species contributions to pairwise typological contrasts (scaled to the maximum contribution within each contrast). **(C)** Random Forest species importance (MeanDecreaseGini) for the 25 most discriminating taxa.

4.1 Environmental gradients structuring macrophyte assemblages

Environmental variation across the study area followed gradients well documented to structure aquatic vegetation in European fresh waters, including hydro-morphology, mineral content, and land-use modification (Riis and Biggs, 2003; Alahuhta et al., 2012; Baláži and Hrivnák, 2017). Water temperature, conductivity, and elevation consistently emerged as the strongest predictors of macrophyte community structure, a pattern confirmed across NMDS, envfit, dbRDA, and VIF screening. Although the dbRDA model explained a relatively modest proportion of total community variation (13.3%), values in this range are commonly reported for macrophyte assemblages at regional scales, where fine-scale habitat heterogeneity, dispersal processes, and unmeasured local drivers account for substantial variation (Svitok et al., 2016; Alahuhta et al., 2018). A substantial fraction of unexplained variation likely reflects drivers not captured in the available dataset, including sediment characteristics, light availability, water-level fluctuations, shoreline management, biotic interactions, and stochastic dispersal. As such, the unexplained component should be interpreted as ecologically meaningful rather than indicative of analytical limitation.

The strong role of conductivity matches findings that mineral content integrates both natural geology and anthropogenic influence, shaping macrophyte functional composition and species turnover (Baattrup-Pedersen et al., 2006; Demars and Edwards, 2009; Chappuis et al., 2014; Szoszkiewicz et al., 2014; Manolaki and Papastergiadou, 2015; Svitok et al., 2016). Elevation, capturing the transition from Pannonian lowlands to upland reservoirs, is also a well-known determinant of species pools, life-form spectra, and trait composition along river networks (Jones et al., 2003; Alahuhta et al., 2012; Akasaka and Takamura, 2011; Hrivnák et al., 2013; Fernández-Aláez et al., 2018). Water temperature, affected by channel width, depth, and hydrological alteration, governs growth phenology and habitat suitability for warm-adapted or cold-restricted species (Thiébaut and Muller, 1998; Kosten et al., 2009), reinforcing the separation of reservoirs and canals from natural rivers. Together, these gradients represent key ecological filters shaping macrophyte distribution across Central European rivers (Hrivnák et al., 2013; Szoszkiewicz et al., 2014; Svitok et al., 2016) and are consistent with mechanisms observed at continental scales (Alahuhta et al., 2018).

Importantly, the aim of the constrained ordination was not to exhaustively explain macrophyte community variation, but to test whether a limited set of typology-relevant environmental gradients yields a coherent and interpretable ecological signal. The consistent importance of temperature, conductivity, and elevation across multiple analytical approaches supports their relevance for typology-level differentiation and bioassessment under the Water Framework Directive.

4.2 Typology-level differences in richness, diversity, and community composition

Rarefaction and asymptotic estimators revealed clear differences in macrophyte diversity among water-body types. High diversity types (T_1, T_5, T_8, R_1) corresponded to wide channels, heterogeneous habitats, and enriched mineral conditions, which support greater species richness and evenness. Low-diversity types (R_4, R_6) exhibited strong morphological constraints, including steep banks, reduced littoral zones, and low-conductivity consistent with patterns observed in hydrologically constrained or oligotrophic upland systems. Similar relationships between habitat heterogeneity, nutrient status, and macrophyte richness have been frequently reported (French and Chambers, 1996; Jones et al., 2003; Bubíková and Hrivnák, 2018b; Jiang et al., 2025).

PERMANOVA indicated significant compositional differences among types, while PERMDISP revealed that certain lowland, human-modified systems show high internal heterogeneity. High internal heterogeneity observed in reservoirs and large impounded rivers is ecologically expected, as these systems integrate longitudinal, lateral, and vertical gradients within single typological units. Water-level fluctuations, variable littoral development, and spatially heterogeneous substrate conditions can promote pronounced within-type variability in macrophyte assemblages. Rather than undermining typological classification, this heterogeneity reflects complex ecological character of heavily modified water bodies under the WFD framework. This agrees with studies demonstrating that altered rivers and canal networks often blur typological boundaries due to disturbance regimes and homogenization (Szoszkiewicz et al., 2017; Lemm et al., 2021). The dbRDA further confirmed that a small set of environmental factors explains a substantial share of community turnover–typical for macrophyte assemblages shaped by few strong environmental filters (Alahuhta et al., 2018).

4.3 Diagnostic species and typological differentiation

Indicator species (IndVal), SIMPER, and Random Forest (RF) analyses converged on a robust set of taxa characterizing each typology. These diagnostic species correspond closely to ecological gradients and are widely recognized in literature. For instance, *Fontinalis antipyretica,* typical of cooler, higher-elevation, faster-flowing systems (French and Chambers, 1996; Lang and Murphy, 2011) was indicative of T_3; *Cabomba caroliniana,* an invasive warm-water species, characterized T_8 canals after its recent establishment in Serbia (Vukov et al., 2013); and *Potamogeton* species in R_1 reflected stable lentic-lotic transition zones in impounded river sections (Janauer et al., 2021). High typological predictability driven by invasive or disturbance-tolerant species highlights an important distinction between typology discrimination and ecological status/potential assessment. While such species can provide strong signal for identifying water-body types, their dominance may simultaneously indicate deviation from reference conditions. This underscores the need to interpret indicator species roles within the broader context of WFD objectives, particularly in artificial and heavily modified systems.

SIMPER results highlighted a small set of species driving most compositional dissimilarity. Submerged taxa such as *Myriophyllum spicatum, Ceratophyllum demersum, Potamogeton crispus,* and *Stuckenia pectinata* are well-known indicators of nutrient enrichment, slow-flowing conditions, and hydro-morphological alteration, while *Phragmites australis* and *Typha angustifolia* typify disturbed lowland littoral zones with elevated nutrient loads (Mason and Bryant, 1975; Lacoul and Freedman, 2006; O'Hare et al., 2006 Hilt et al., 2018; Stefanidis et al., 2023). These same species emerged as dominant predictors in RF importance metrics.

Such congruence across three analytical approaches underscores their role as core diagnostic taxa within Serbian typology.

4.4 Predictive power of macrophyte assemblages for typology discrimination

The Random Forest classifier achieved strong predictive performance (out-of-bag accuracy of 72.7%), comparable to accuracies reported in similar studies (Jusik et al., 2015; Svitok et al., 2016; Van Echelpoel and Goethals, 2018). High classification success for types T_1, T_2, T_8, and R_3, reflects their distinct environmental settings and coherent floristic signatures. Poorer classification of R_1, R_4, T_5, and T_3 relates to low sample size, mixed hydrological regimes, or genuinely high heterogeneity–patterns frequently observed in reservoir and canal systems (Riis and Biggs, 2003; Tutova et al., 2025; Alahuhta et al., 2025). Lower classification accuracy for sparsely sampled types is consistent with limited representation of within-type variability and should therefore not be interpreted as absence of ecological structure. Rather, these results emphasize the combined effect of sample size and internal heterogeneity on predictive performance.

The correspondence between RF- identified taxa and those identified by IndVal and SIMPER demonstrates the utility of machine-learning approaches in complementing classical community analyses and reinforces the ecological validity of typological signals.

4.5 Implications for WFD assessment and typology refinement

The study's findings carry several implications for ecological assessment and WFD implementation:

Strong ecological basis for the national typology. The clear correspondence between typology, environmental gradients, and macrophyte assemblages supports the continued use of Serbia's existing classification framework.

Macrophytes as reliable indicators across diverse systems. Macrophyte assemblages captured both broad-scale physicochemical gradients and local hydro-morphological conditions, reaffirming their value for WFD-compliant assessment.

Need to strengthen representation of certain types. Water-body types showing high internal heterogeneity or low sample size (e.g., R_1, R_4, T_5) may benefit from refined delineation or expanded monitoring.

Diagnostic taxa as practical tools for assessment. The species consistently identified as indicators across analyses provide clear guidance for field diagnostics and for refining macrophyte-based indices.

4.6 Final synthesis

Overall, our results demonstrate that macrophyte communities in Serbia respond coherently to the environmental gradients underpinning national surface-waters typology. Diversity patterns, multivariate and indicator species analyses, and machine-learning classification all converged on a consistent ecological signal, confirming that typology reflects meaningful variation in habitat conditions and biological composition across natural rivers, modified channels, and artificial canals. These findings provide a robust ecological foundation for WFD-compliant assessment and highlight the potential for integrating traditional ecological tools with modern analytical approaches to support effective monitoring and management of freshwater ecosystems. Taken together, these results demonstrate that macrophyte assemblages provide robust typological signal despite unequal sampling effort and inherent heterogeneity, particularly pronounced in modified systems, reinforcing their value for large-scale freshwater assessment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

DV: Writing – original draft, Conceptualization, Investigation, Formal Analysis. MĆ: Investigation, Data curation, Writing – original draft. MI: Data curation, Investigation, Writing – original draft.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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