

Diversity and stability in aquatic plant communities

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Diversity and stability in aquatic plant communities

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Editorial: Diversity and stability in aquatic plant communities

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aquatic plants, macrophytes, wetland plants, rivers, lakes, wetlands, ponds, diversity

Editorial on the Research Topic

Diversity and stability in aquatic plant communities

Freshwater ecosystems represent a small proportion of the terrestrial surface but host a very high proportion of biodiversity (Dudgeon et al., 2006). Nonetheless, these are facing a high rate of decline, both in biodiversity and in area (Zelnik and Germ, 2023). Biodiversity increases the temporal stability of communities and characteristics of the ecosystems through multiple mechanisms (Downing et al., 2014). Aquatic plants are fundamental for aquatic ecosystems (Zhou et al., 2023) and often govern the diversity and functionality of rivers, lakes, and wetlands (Figure 1). The mentioned issues can be found in the papers gathered in this Research Topic. Their findings deepen our understanding of freshwater ecosystem responses to environmental factors at different scales and gather important knowledge about management and climate change effects on the aquatic plant communities, their diversity and stability, protection and biological control of alien species.

Yang et al. showed temporal niche partition and traits tradeoff theory as primary strategies. The majority of the species pairs were niche partitioned and occurred either in spring or autumn. An approach using functional diversity provides quantitative information to explain macrophyte ecology more effectively than traditional methods based on taxonomy (Dalla Vecchia et al., 2020), since it is more resistant to environmental variations (Liu and Wang, 2018). The importance of functional diversity of aquatic plant communities in freshwater monitoring and conservation plans was highlighted by Stefanidis et al., quantifying this for Greek rivers while examining relationships with ecological factors. Growth forms and light preference were important traits that explained a large share of the total variance of functional composition. Functional richness was significantly higher at fine substrate systems and deep waters with low flow habitats.

Analogously, He et al. investigated the composition and diversity relationships between bacteria, fungi, and plant communities along a successional gradient behind receding glaciers. Taxonomic groups predicted the community composition more accurately than environmental factors, unlike taxa diversity, suggesting that the composition of one taxonomic group is not a strong driver of the diversity of another group. Additionally, Toth investigated the effect of epiphyton on foliar traits of a submerged rooted macrophyte *Potamogeton perfoliatus*, in a shallow lake, showing that epiphytic algal biomass influenced photophysiological traits of submerged macrophyte leaves. The complex interactions between epiphytes and submerged rooted macrophytes play an important role in habitat variability and overall ecosystem stability in littoral zones and should be considered in lake management. Klančnik et al. (2015) also showed the important role of the epiphytic



FIGURE 1
Diverse community of aquatic plants in the Struga river.

diatoms in the prevention of adverse effects of short-wave radiation on submersed leaves of *Potamogeton perfoliatus*.

Furthermore, the spatiotemporal variability of the communities' dynamics also influences coexistence, providing a biodiversity increase in variable conditions (Hallett et al., 2023). Xing et al. monitored the spatiotemporal changes in average wetland Normalised Difference Vegetation Index (NDVI) during the annual growing season in the Amur River basin for four decades, analysing wetland vegetation responses to climatic change. Under climate warming scenarios, the NDVI of a wetland will continue to increase, often resulting in lower diversity of plant communities. This is of great applicability as NDVI has been widely utilized for monitoring wetland vegetation vitality and distribution (Ojdanič et al., 2025).

Various factors shape the structure and diversity of aquatic plant communities, including several anthropogenic pressures, and should be considered in restoration planning. For instance, Svitok et al. explored the diversity of aquatic plants in rivers, streams, ponds and ditches in Central Europe and observed that ponds and ditches support higher macrophyte diversity than running waters. This highlights the conservation value of such artificial habitats and underscores the need to prioritize small waterbodies in conservation strategies. Another example is the effect of light conditions in the distribution of macrophytes (Liu et al., 2016), or water depth, determined by Yu et al. as the best predictor of nutrient threshold of submerged macrophytes collapse and recovery in lakes, further interacting with turbidity. Canopy-forming submerged macrophyte *Myriophyllum spicatum* had a higher resistance to high nutrients and turbidity, whereas submerged macrophyte species richness had a significantly negative response to water depth. These results might provide quantitative guidance for lake restoration of diverse water depths.

Finally, environmental conditions changes influence the coexistence of plants, as Edwards et al. observed with the two common European wetland species *Carex acuta* and *Glyceria maxima*. Specifically, *C. acuta* was more affected by hydrologic changes, growing better in dry and saturated conditions, while *G. maxima* had a more positive response to fertilization. This confirms that it is crucial to maintain stable and diverse wetland plant communities to sustain ecosystem services like carbon sequestration and water purification. Furthermore, Toth recorded gradual changes within a season in the common reed *Phragmites australis* photosynthetic traits, comparing degraded and stable stands, suggesting a universal response to changing environmental conditions. Reed plants exposed to different levels of degradation showed comparable physiological plasticity, without a difference in trait variability between stands. This is likely to contribute to the resilience of reed plants by providing a wider range of adaptive traits under different conditions. The common reed is a very adaptable perennial grass growing in different wetlands, developing dense stands that enable soil stabilization and habitat provision (Ojdanič et al., 2025). Its dieback is a worldwide phenomenon occurring mainly due to water regulation and inadequate reed management practices.

Sediment accretion and nutrient addition also influence the growth of wetland plants and vegetative propagation, as Guo et al. observed, studying *Phalaris arundinacea* within a *Carex thunbergii* stand in the Yangtze River. An increased sedimentation rate facilitated the invasion of *P. arundinacea* into *Carex* stands, further enhanced by nutrient enrichment. Accordingly, management measures should consider sediment loads and nutrient inputs to prevent species invasion and maintain the ecological function of floodplain wetlands.

In conclusion, this Research Topic underpins the key role of aquatic plants in ecosystem stability and support of diversity in other biotic communities (O'Hare et al., 2018). Maintaining the diversity and stability of aquatic plant communities is paramount for the sustainability of freshwater ecosystems, and river restoration actions should not disregard this.

Author contributions

IZ: Conceptualization, Writing – original draft, Writing – review & editing. RR: Writing – review & editing. MG: Conceptualization, Writing – original draft, Writing – review & editing.

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References

- Dalla Vecchia, A., Villa, P., and Bolpagni, R. (2020). Functional traits in macrophyte studies: Current trends and future research agenda. *Aquat. Bot.* 167, 103290. doi: 10.1016/j.aquabot.2020.103290
- Downing, A. L., Brown, B. L., and Leibold, M. A. (2014). Multiple diversity–stability mechanisms enhance population and community stability in aquatic food webs. *Ecology* 95, 173–184. doi: 10.1890/12-1406.1
- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., et al. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.* 81, 163–182. doi: 10.1017/S1464793105006950
- Hallett, L. M., Aoyama, L., Barabás, G., Gilbert, B., Larios, L., Shackelford, N., et al. (2023). Restoration ecology through the lens of coexistence theory. *Trends Ecol. Evol.* 38, 1085–1096. doi: 10.1016/j.tree.2023.06.004
- Klančnik, K., Gradinjan, D., and Gaberščik, A. (2015). Epiphyton alters the quantity and quality of radiation captured by leaves in submerged macrophytes. *Aquat. Bot.* 120, 229–235. doi: 10.1016/j.aquabot.2014.07.007
- Liu, X., and Wang, H. (2018). Contrasting patterns and drivers in taxonomic versus functional diversity, and community assembly of aquatic plants in subtropical lakes. *Biodiv. Conserv.* 27, 3103–3118. doi: 10.1007/s10531-018-1590-2
- Liu, X., Zhang, Y., Shi, K., Lin, J., Zhou, Y., and Qin, B. (2016). Determining critical light and hydrologic conditions for macrophyte presence in a large shallow lake: The ratio of euphotic depth to water depth. *Ecol. Indic.* 71, 317–326. doi: 10.1016/j.ecolind.2016.07.012
- O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lázár, A. N., et al. (2018). Responses of aquatic plants to eutrophication in rivers: A revised conceptual model. *Fron. Plant Sci.* 9. doi: 10.3389/fpls.2018.00451
- Ojdanič, N., Gaberščik, A., Zelnik, I., and Golob, A. (2025). The impact of environmental variables on reed stands of the intermittent Lake Cerknica, Slovenia: 40 years of change. *Ecol. Indic.* 170, 113101. doi: 10.1016/j.ecolind.2025.113101
- Zelnik, I., and Germ, M. (2023). Diversity of inland wetlands: important roles in mitigation of human impacts. *Diversity* 15, 1050. doi: 10.3390/d15101050
- Zhou, J., Li, Y., Lyu, T., Yu, H., Meng, J., Song, W., et al. (2023). Environmental determinants of aquatic plant diversity differ between growth forms and range sizes. *Ecolog. Indic.* 157, 111280. doi: 10.1016/j.ecolind.2023.111280

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Accuracy of mutual predictions of plant and microbial communities vary along a successional gradient in an alpine glacier forefield

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Receding glaciers create virtually uninhabited substrates waiting for initial colonization of bacteria, fungi and plants. These glacier forefields serve as an ideal ecosystem for studying transformations in community composition and diversity over time and the interactions between taxonomic groups in a dynamic landscape. In this study, we investigated the relationships between the composition and diversity of bacteria, fungi, and plant communities as well as environmental factors along a successional gradient. We used random forest analysis assessing how well the composition and diversity of taxonomic groups and environmental factors mutually predict each other. We did not identify a single best indicator for all taxonomic and environmental properties, but found specific predictors to be most accurate for each taxon and environmental factor. The accuracy of prediction varied considerably along the successional gradient, highlighting the dynamic environmental conditions along the successional gradient that may also affect biotic interactions across taxa. This was also reflected by the high accuracy of predictions of plot age by all taxa. Next to plot age, our results indicate a strong importance of pH and temperature in structuring microbial and plant community composition. In addition, taxonomic groups predicted the community composition of each other more accurately than environmental factors, which may either suggest that these groups similarly respond to other not measured environmental factors or that direct interactions between taxa shape the composition of their communities. In contrast, diversity of taxa was not well predicted, suggesting that community composition of one taxonomic group is not a strong driver of the diversity of another group. Our study provides insights into the successional development of multidiverse communities shaped by complex interactions between taxonomic groups and the environment.

KEYWORDS

bacteria, environment, fungi, plants, predictive models, succession

1 Introduction

Local microclimatic conditions, soil properties as well as the tight interactions between plants and belowground microbes shape the communities in natural ecosystems (Zak et al., 2003; Mouhamadou et al., 2013; Navratilova et al., 2019; Harrison et al., 2020; Ohler et al., 2020). pH, temperature and soil chemical properties have been shown to affect the plant and microbial composition by defining the niches available in a given location (Darcy et al., 2018; Dastogeer et al., 2020; Harrison et al., 2020; Davison et al., 2021; Junker et al., 2021). Additionally, the interactions between bacteria, fungi and plants strongly affect local communities. The interactions between plants and microbes, for instance, are mediated through plant root exudates and litter input that offer the carbon sources and provide various niches for microbes (Knelman et al., 2012; Lopez-Angulo et al., 2020). Likewise, microbes decompose the carbon and affect plants through the supply of available soil nutrients to plants such as nitrogen fixation (Schmidt et al., 2008; van der Putten et al., 2013) and the interplay of mutualistic and antagonistic effects determine if they will maintain plant community diversity or cause community convergence (Wardle et al., 2004; Bever et al., 2012; van der Putten et al., 2013; Teste et al., 2017; van der Putten, 2017). The outcome of pairwise interactions between bacterial, fungal and plant species is highly context dependent and may be modulated by the presence of other taxa as well as environmental conditions (David et al., 2020; Raza et al., 2020). For instance, environmental conditions such as temperature and soil moisture affect plant and microbes and can regulate plant-microbe associations (Rasmussen et al., 2019; Rudgers et al., 2020; Robroek et al., 2021), and increasing environmental stress alters microbial facilitation of plant germination or biomass production (David et al., 2020). This may complicate predictions on the composition and diversity of communities based on the knowledge of other taxa or environmental factors in natural ecosystems where environmental conditions strongly vary and thus may modulate the interactions between taxonomic groups. Nonetheless, the interdependencies between plants, bacteria and fungi may leave a signal in community composition and diversity within the taxonomic groups and thus these properties may be mutually predictable (Horn et al., 2017; Leff et al., 2018).

Successional gradients with considerable variation in soil properties and climatic conditions are an ideal study system to reveal how the interdependencies between taxonomic groups change along a temporal and environmental gradient (Cannone et al., 2008; Walker et al., 2010; Chang & HilleRisLambers, 2016). Glacier forefields are prime examples of primary successions and for studies on the assembly of multidiverse communities (Ficetola et al., 2021; Hanusch et al., 2022). Receding glaciers provides barren substrates waiting for the successive colonization of organisms such as plants and soil

biota (Bernasconi et al., 2011; Burga et al., 2010; Ficetola et al., 2021). The time for space substitution of chronosequences allows to track soil development and the processes that shape biotic communities (Walker et al., 2010; Chang & HilleRisLambers, 2016). Multiple studies described the successional trajectories of plant, bacteria and fungi communities and the development of soil conditions along glacier forefields (Bernasconi et al., 2011; Zumsteg et al., 2012; Buma et al., 2017; Buma et al., 2019; De Vries et al., 2021). However, these studies usually focus on one or rarely two specific taxonomic groups (Ficetola et al., 2021), thus a full consideration of multidiverse communities and environmental conditions may facilitate a general evaluation of associations between different biotic and abiotic parts. In this context, we applied random forest analysis to evaluate the interdependences between taxonomic groups and with environmental conditions to reveal the strength of mutual influences along a successional gradient. Machine learning algorithms have been increasingly applied for predictions using complex ecological data. For instance, random forest analysis has been used to explore the links between soil bacterial community composition and environmental factors (Hermans et al., 2020) and in predictions of species interactions based on traits for understanding interaction networks (Pichler et al., 2019). The high performance of machine learning algorithms and especially random forest is obtained by their ability to model non-linear combinations of numerical and categorical data without complex transformations resulting in estimates of the accuracy of predictions (Breiman, 2001; Ghannam & Techtmann, 2021; Goodswen et al., 2021).

Empirical studies have shown that plants and abiotic factors affect microbial communities at the same time but in different ways: they explain different parts of variation in soil microbial communities and different studies have shown contrasting results with either plants or environmental factors being more important in shaping microbial communities (Mitchell et al., 2011; Kruger et al., 2017; Leff et al., 2018; Reese et al., 2018; Cheng et al., 2020). In addition, plant species composition, functional identity, Shannon and phylogenetic diversity have been reported to show different associations with microbial communities (Dassen et al., 2017; Chen et al., 2018; Leff et al., 2018) and the relationships may change at different successional stages (Porazinska et al., 2018; Hanusch et al., 2022). Along the successional gradient of Ödenwinkel glacier forefields in the Austrian Alps, we considered a range of taxonomic groups (bacteria, fungi and plants) and environmental variables (plot age, temperature, and soil pH) to determine how well bacteria, fungi, and plant communities as well as environmental conditions serve as indicator for the composition and diversity of other taxonomic groups as well as environmental factors at different successional stages. We used multivariate datasets on plant species, bacterial operational taxonomic units (OTU), and fungi OTU composition as well as several environmental factors

as explanatory variables. These variables were used to predict univariate data informing about the composition, functional composition and Shannon diversity of plant, bacteria, fungi, plant phylogenetic diversity, plant functional diversity and environmental factors. We aim to address the following three questions: 1) Are bacterial, fungal, and plant communities as well as environmental conditions good predictors for the composition and diversity of the other taxonomic groups or environmental factors? 2) Is the accuracy of prediction variable along the successional gradient? 3) Are there single most important predictors for all taxonomic groups? Our study will reveal the relative importance of interactions across taxa and abiotic factors in shaping the diversity and composition of multidiverse communities along a successional gradient and will thus motivate future studies on the mechanisms underlying community assembly.

2 Materials and methods

2.1 Data collection

Plots set up - Our study site is located at the forefield of the Ödenwinkelkees glacier (Stubachtal valley, Hohe Tauern National Park, Austria; Dynamic Ecological Information Management System – site and dataset registry: <https://deims.org/activity/fefd07db-2f16-46eb-8883-f10fbc9d13a3>, last access: August 2021) (Junker et al., 2020). The Ödenwinkelkees glacier was covered by ice at the latest glacial maximum in the Little Ice Age (LIA; around 1850) but the glacier retreat released a transect of around 1.7 km long with the elevation between 2070 and 2170 m. In summer 2019 (26 June - 16 September), we established 135 permanent plots that were evenly distributed between the LIA glacier maximum and the current extent of the glacier (glacier tongue) within the glacier forefield, representing a chronosequence of succession with high temporal resolution. Each plot is a square with 1 m side length (resulting in an area of 1 m²) and a ground anchor is marking the center of the plot. Plot age was estimated according to its relative position compared with historical records of eight deglaciating periods (year 1850, 1890, 1929, 1969, 1977, 1985, 1998, 2013) (Junker et al., 2020).

Plant survey and functional traits - We identified all vascular plant species ($n = 99$) occurring at every plot and recorded the coverage of every species. We measured the plant height, leaf area, leaf weight and calculated the specific leaf area (SLA) for those 48 plant species that occurred in 10 or more plots. For three focus species we phenotyped up to three individuals on every plot where they occurred: *Oxyria digyna* as representative of early succession, *Trifolium badium* as representative of late succession, and *Campanula scheuchzeri* which occurred all along the successional gradient. For the other $n = 45$ species, up to five individuals per plot were phenotyped on the youngest, the oldest, and the intermediate plot where they occurred.

Additionally, we obtained the functional traits of the plant species from Bioflor database (<https://www.ufz.de/bioflor/index.jsp>) for 92 species out of 99 plant species occurring in the field. We used nine functional traits which have been shown to be response traits to environmental changes at the community level (Kahmen & Poschlod, 2004; Bernhardt-Römermann et al., 2008; Aguiar et al., 2013; Hintze et al., 2013), including reproduction, diaspore type, leaf persistence, life form, life span, pollen vector, strategy type, type of reproduction, dispersal of diaspores.

Soil bacteria and fungi sampling and sequencing - We also characterized the soil microbiome (bacteria and fungi) of each of the plots. We sampled soil from each plot at two locations at a depth of 3 cm, soil from two locations per plot were pooled to one sample for further analysis. We extracted microbial DNA from soil samples following the protocol of the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). Microbiome analysis was performed by Eurofins Genomics (Ebersberg, Germany) using the company's standard procedure. Sequencing was done using Illumina MiSeq and the sequenced regions were V3-V4 region of the 16S rRNA gene to identify bacterial OTUs and the ITS2 region for fungal OTUs following the standard procedure "InView - Microbiome Profiling 3.0 with MiSeq" (for detailed methods see Junker et al., 2020). Abundances of bacterial and fungal taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates (Angly et al., 2014). Prior to the random forest analysis of microbial communities, we performed a cumulative sum scaling (CSS) normalization (R package metagenomeSeq v1.28.2) on the count data to account for differences in sequencing depth among samples.

Soil temperature and pH - To record the seasonal mean temperature, we buried temperature loggers with a resolution of 0.5°C (MF1921G iButton, Fuchs Elektronik, Weinheim, Germany) 10 cm north of each plot center, at a depth of 3 cm below ground (Junker et al., 2020; Ohler et al., 2020) during field work in 2019. The thermo loggers were set to start on 13th August 2019 and were stopped on 9th August 2020 with a total of 2048 measurements recorded on 362 days. Seasonal mean temperature was calculated on the basis of the recordings between 13th August to 16th of September 2019 and 26th June to 9th August 2020 representing the period in which the plots were free of permanent snow cover before and after the winter 2019/2020. In 2020 (25 July - 21 August), we took additional soil samples from all plots to measure soil pH. Samples were sent to AGROLAB Agrar und Umwelt GmbH (Sarstedt, Germany) for analysis.

2.2 Data analysis

To test the predictability of the diversity and composition of each of the taxonomic group by the composition of other

taxonomic groups as well as by environmental parameters, we used the machine learning algorithm random forest with R package *randomForest* (Liaw and Wiener, 2002). We used the 4 sets of variables as explanatory variables: community tables of plants, bacteria, and fungi with plots as rows and the abundance of the species or OTUs as columns (Supplementary Table S1, S2, S3), and environmental conditions of each plot with plots as rows and environmental variables as columns (Supplementary Table S4). As dependent variables we used univariate variables including the composition (principal components PCs), functional composition (PCs) and Shannon diversity of plant, bacteria, fungi, plant phylogenetic diversity, plant functional diversity as well as soil seasonal mean temperature, pH, plot age, resulting in 20 variables in total (Supplementary Table S5; Supplementary Figure S1, S2). Each set of explanatory variables were used separately to predict the variables related with other taxonomic groups or environmental factors. As random forest analysis can only deal with univariate dependent variables, we conducted the principal component analysis (PCA) using R package *vegan* (Oksanen et al., 2013) and used the first two PC axis which carry most information of the composition to refer to plant species composition (15.3% + 11.2%), bacteria composition (6.4% + 4.6%) and fungi composition (4.1% + 3.2%). Plant functional composition matrix includes the categorical functional traits obtained from BiolFlor database and the community weighted means of filed measured traits (plant height, leaf area, leaf weight and SLA) (Supplementary Table S6). For each category of each categorical trait, we calculated the total coverage of species belonging to the category, and this was done for all the 9 traits and all 9 traits were merged to a single table, thus generating the functional composition table with plots name as rows and 39 trait categories as columns, i.e. each categorical trait had two or more categories resulting in a total of 39 categories. Plant functional composition was represented by the first two PCAs, too (63.1% + 12.1%). Plant Shannon diversity was calculated from the compositional dataset using the R package *vegan*. Plant phylogenetic diversity was calculated using the R package *picante* (Kembel et al., 2010). We extracted a phylogenetic tree using the R package *pez* (Pearse et al., 2015) for species existing in our field site from a dated molecular phylogeny tree (32,223 species) for land plants (Zanne et al., 2014). In cases where species were not included in the tree, it was substituted by species from the same genus. Among 99 species existing in our plots, we were able to match and build a tree with 96 species and we used it for the calculation of phylogenetic diversity. We used 'Functional dispersion' calculated from the R package *FD* (Laliberté & Legendre, 2010) as the index for plant functional diversity. The BiolFlor traits and field measured traits of every species were used for the trait table identically for every plot, and for the community table the species with a low occurring frequency along the successional gradient (not included in the 48 species with traits measured) were ignored in the calculation

of functional diversity. For bacteria and fungi, the Shannon diversity was calculated based on the OTU composition (without CSS normalization) after rarefying the data to the minimum number of reads (bacteria: 2117; fungi: 1420) available in the samples (repeats = 999). Additionally, we obtained bacteria functional composition represented by MetaCyc pathway abundances with PICRUST2 (Langille et al., 2013), and fungi functional composition with FUNGuild to assign fungal OTU to different functional groups (Nguyen et al., 2016). Bacteria functional composition (Supplementary Table S7) was represented by the first two PCAs (78.0% + 7.2%), and fungi functional composition (Supplementary Table S8) was represented by the first two PCAs (33.2% + 17.0%), too.

Using all combinations of explanatory and dependent variables, we performed random forest analyses with 10-fold cross validations to quantify the performance of the predictive model, a total of 60 models. Specifically, for each prediction, 80% of the plots were randomly selected as the training dataset and the remaining 20% of the plots were used as test dataset. The predictive model resulting from the training dataset was applied to the test data and the predicted values of the plots in the test dataset were correlated with the observed values of these plots. This process was repeated for ten times, and then we defined the mean Pearson's *r*-value of ten correlations as 'accuracy of prediction' and used the proportion of statistically significant correlations (*p*-value < 0.05) out of the 10 correlations as 'significance frequency'. Additional to random forest analysis using all the plots for a global impression on the predictability of dependent variables, we also employed a moving frame approach to detect how the predictabilities change along the successional gradient. With the 135 plots, we grouped every 45 plots into one frame and used the median plot as identifier of the frame. Thus, the first frame included plots 1 to 45, the second 2 to 46, and so forth. This approach led to a set of 91 moving frames whose identifiers ranged from plot 23 to plot 113. Using the same proportion of training and test dataset, for every 45 plots in each frame, data of 36 (80%) randomly selected plots was used as training dataset, and the other 9 (20%) plots were used as test dataset. The accuracy of prediction and significance frequency were calculated for every frame as stated before. We fitted a linear or quadratic regression with the accuracy of prediction of every variable along the successional gradient as independent variable and the frame number as explanatory variable. The model with a higher *r*² value was chosen and the statistically significant relationships were shown as a regression line. We compared for each group how well they predicted every variable as well as for each variable how well they were predicted by every other taxonomic group or environmental factors along the successional gradient (except for the group that was considered in the dependent variable) using the Tukey Test. Note that our results do not imply a direction of effects in the sense that the dependent variable is affected by the explanatory variable. For

instance, if bacterial communities statistically predict soil temperature it does not mean bacterial communities affect the soil temperature but rather are affected by this environmental parameter.

3 Results

In total we obtained soil bacteria and fungi composition data from $n = 127$ and 130 plots after excluding the plots with missing data, respectively; $n = 4986$ bacteria OTUs and $n = 5701$ fungi OTUs were detected in all the soil samples. A total of 99 plant species were identified from 133 plots as plot 1 and plot 6 were unvegetated. Raw sequences of next-generation 16S rRNA gene amplicon sequencing are available at the NCBI Sequence Read Archive (SRA) under the BioProject accession PRJNA701884 and PRJNA701890. The mean accuracy of prediction of each pair of explanatory variables and dependent variables did usually not strongly differ between the global analysis considering all plots and the mean values of the frame-wise analyses, indicating the validity of using moving frames for random forest predictions. Most of the predictions fit a quadratic regression, indicating a non-monotonic change of the accuracy of prediction along the successional gradient (Figures 1–4).

Bacterial communities as predictors (Figures 1, 5A) – Bacterial communities (quantitative OTU tables) most accurately predicted the taxonomic composition of fungal communities (PC1 and PC2), followed by plant functional composition. Among the environmental parameters, plot age was most accurately predicted by bacterial communities, followed by pH and temperature. Fungi Shannon diversity and plant functional diversity were least accurately predicted among all the variables. Accuracy of prediction by bacterial communities of target variables associated with plant communities mostly decreased with plot age, whereas accuracy of prediction of fungi and most environmental target variables were less variable along the successional gradient or even increased along the age gradient in most cases.

Fungal communities as predictors (Figures 2, 5B) – Fungal communities (quantitative OTU tables) most accurately predicted the functional composition of bacterial communities (PC2), followed by bacterial taxonomic composition (PC1 and PC2) and plant functional composition (PC1). Plot age was the environmental factor most accurately predicted by fungal communities, followed by pH and temperature. Bacteria Shannon diversity was least accurately predicted among all the variables. Accuracy of prediction of target variables associated with plant communities mostly decreased with plot age, and variables associated with bacterial communities mostly remained constant

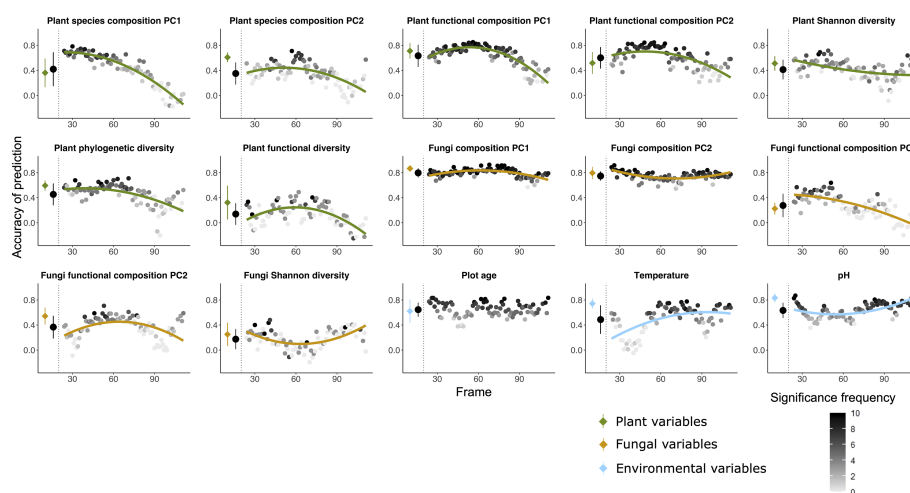


FIGURE 1

Random forest predictions using the community table of soil bacterial communities (OTU table) as explanatory variable to predict seven variables of plant (green), five variables of fungi (orange) as well as three variables of environmental factors (blue) as dependent variables. The prediction was done both using all the 135 plots and using a moving frame approach. For the moving frame approach, every 45 plots were grouped into one frame and the median plot was used as identifier of the frame. Thus, the first frame included plots 1 to 45, the second 2 to 46, and so forth. The x axis represents the median plot of every frame whose identifiers ranged from plot 23 to plot 113 (i.e. from young successional plots to old plots). The colored circles at the left of each plot denote the mean \pm standard deviation of the accuracy of prediction (Pearson's r) using the full dataset (results of 10-fold cross validation), and the black circles denote the mean \pm standard deviation of the accuracy of prediction for all the frames. Each grey to black circle on the right of each plot represents the mean accuracy of prediction of each frame and the color gradient is showing how many correlations of the 10-fold cross-validation were significant with lighter colors indicating less frequent significant predictions. A quadratic or linear regression (the model with higher adjusted r^2 value) is fit for the gradient if it is significant, showing a change of the accuracy of prediction along the successional gradient.

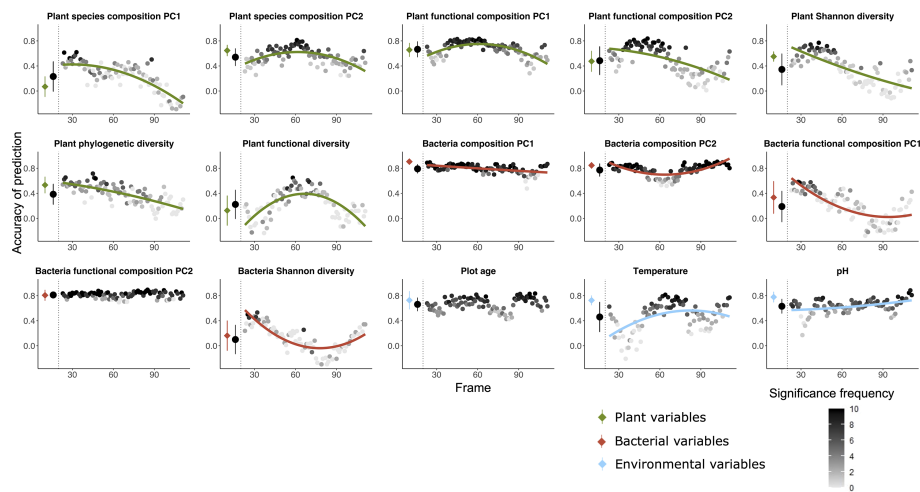


FIGURE 2

Random forest predictions using the community table of soil fungal communities (OUT table) as explanatory variable to predict seven variables of plant (green), five variables of bacteria (red) as well as three variables of environmental factors (blue) as dependent variables. The prediction was done both using all the 135 plots and using a moving frame approach. For the moving frame approach, every 45 plots were grouped into one frame and the median plot was used as identifier of the frame. Thus, the first frame included plots 1 to 45, the second 2 to 46, and so forth. The x axis represents the median plot of every frame whose identifiers ranged from plot 23 to plot 113 (i.e. from young successional plots to old plots). The colored circles at the left of each plot denote the mean \pm standard deviation of the accuracy of prediction (Pearson's r) using the full dataset (results of 10-fold cross validation), and the black circles denote the mean \pm standard deviation of the accuracy of prediction for all the frames. Each grey to black circle on the right of each plot represents the mean accuracy of prediction of each frame and the color gradient is showing how many correlations of the 10-fold cross-validation were significant with lighter colors indicating less frequent significant predictions. A quadratic or linear regression (the model with higher adjusted r^2 value) is fit for the gradient if it is significant, showing a change of the accuracy of prediction along the successional gradient.

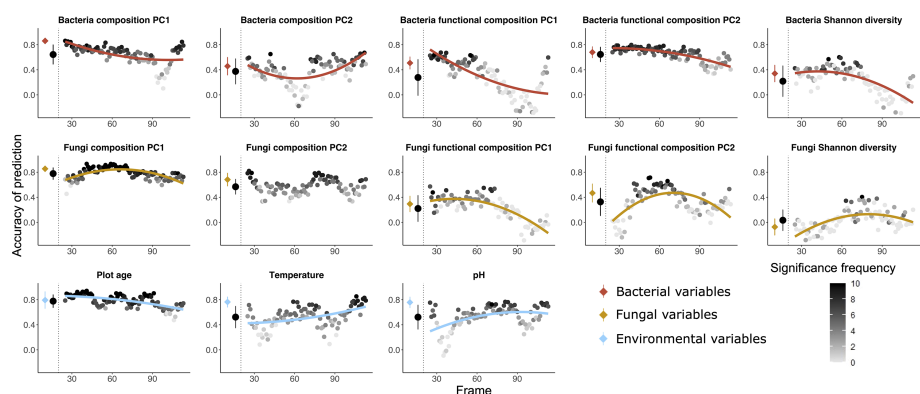


FIGURE 3

Random forest predictions using the community table of plant communities as explanatory variable to predict five variables of bacteria (red), five variables of fungi (orange) as well as three variables of environmental factors (blue) as dependent variables. The prediction was done both using all the 135 plots and using a moving frame approach. For the moving frame approach, every 45 plots were grouped into one frame and the median plot was used as identifier of the frame. Thus, the first frame included plots 1 to 45, the second 2 to 46, and so forth. The x axis represents the median plot of every frame whose identifiers ranged from plot 23 to plot 113 (i.e. from young successional plots to old plots). The colored circles at the left of each plot denote the mean \pm standard deviation of the accuracy of prediction (Pearson's r) using the full dataset (results of 10-fold cross validation), and the black circles denote the mean \pm standard deviation of the accuracy of prediction for all the frames. Each grey to black circle on the right of each plot represents the mean accuracy of prediction of each frame and the color gradient is showing how many correlations of the 10-fold cross-validation were significant with lighter colors indicating less frequent significant predictions. A quadratic or linear regression (the model with higher adjusted r^2 value) is fit for the gradient if it is significant, showing a change of the accuracy of prediction along the successional gradient.

or decreased, whereas accuracy of prediction of environmental target variables increased along the age gradient in most cases.

Plant communities as predictors (Figures 3, 5C) – Plant communities (quantitative plant community table) predicted fungi composition (PC1) most accurately, followed by plot age, bacteria functional composition (PC2) and bacteria composition (PC1). Bacteria and fungi Shannon diversity were least accurately predicted among all the variables. The predictions of variables concerning bacteria and fungi were mostly decreasing with increasing plot age. For environmental variables, the accuracy of prediction for temperature and pH increased and for plot age slightly decreased along the successional gradient.

Environmental factors as predictors (Figures 4, 5D) – Environmental factors (multivariate table of environmental parameters) predicted the fungi composition PC1 and bacteria composition PC1 with the highest accuracy, followed by bacteria functional composition (PC2) and plant species composition (PC2). Fungi Shannon diversity and fungi functional composition (PC1) was least accurately predicted among all the variables. Accuracy of prediction for plant and bacterial variables were often decreasing along the gradient, and for fungal variables they mostly increased with plot age.

4 Discussion

Our results showed that the composition and diversity of plant, bacteria, and fungi is overall well predicted by the composition of the respective other taxonomic groups as well as by environmental factors. The accuracy of prediction, however, varied along the successional gradient of the forefield of the Ödenwinkelkees glacier.

Taxonomic groups and environmental factors differed in the ability to accurately predict the composition of the respectively other taxonomic groups. Bacterial and fungal community compositions was the best predictor for each other's composition, while plant community composition was well predicted both by microbial community composition and environmental factors. In addition, plot age was best associated with plant community composition, followed by fungal and bacterial composition (Supplementary Figure S3). This confirms that plant communities represent an ecological succession with age-specific composition, while microbial communities may be predominantly shaped by biotic interactions that are modulated by community age to a lesser extent. In addition, although the PC axes of community composition do not represent the full composition of a

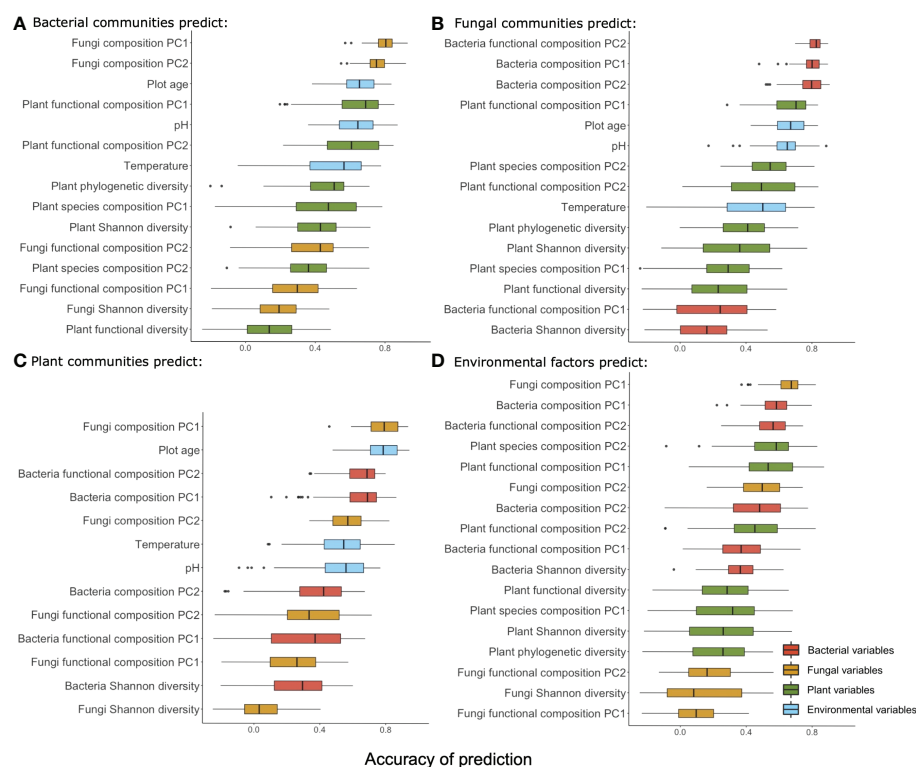


FIGURE 4

Summary of the accuracy of prediction using taxonomic groups (bacteria (A), fungi (B), plant (C)) and environmental factors (D) to predict variables from the other three groups along the successional gradient. Variables from each group are color-coded (red: bacteria, orange: fungi, green: plant, blue: environment) and ranked by the mean accuracy of prediction.

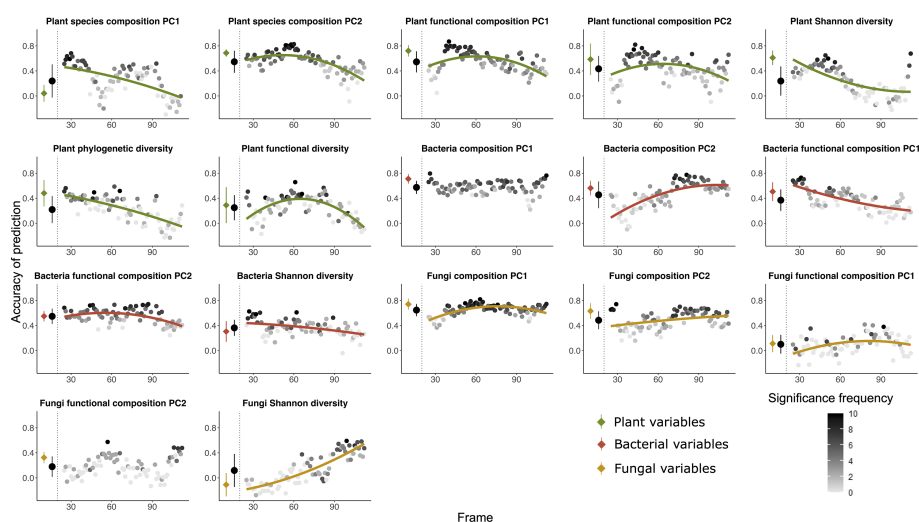


FIGURE 5

Random forest predictions using all the environmental factors as explanatory variable to predict seven variables of plant (green), five variables of bacteria (red) as well as five variables of fungi (orange) as dependent variables. The prediction was done both using all the 135 plots and using a moving frame approach. For the moving frame approach, every 45 plots were grouped into one frame and the median plot was used as identifier of the frame. Thus, the first frame included plots 1 to 45, the second 2 to 46, and so forth. The x axis represents the median plot of every frame whose identifiers ranged from plot 23 to plot 113 (i.e. from young successional plots to old plots). The colored circles at the left of each plot denote the mean \pm standard deviation of the accuracy of prediction (Pearson's r) using the full dataset (results of 10-fold cross validation), and the black circles denote the mean \pm standard deviation of the accuracy of prediction for all the frames. Each grey to black circle on the right of each plot represents the mean accuracy of prediction of each frame and the color gradient is showing how many correlations of the 10-fold cross-validation were significant with lighter colors indicating less frequent significant predictions. A quadratic or linear regression (the model with higher adjusted r^2 value) is fit for the gradient if it is significant, showing a change of the accuracy of prediction along the successional gradient.

taxonomic group, the successful mutual prediction between bacteria and fungi does show that the PC axes are containing a signal of the effects of other taxonomic groups. As stated above, our approach is not implying a direction of effects, which means that it is more likely that the environmental factors affect the composition and diversity of the taxonomic groups and not *vice versa*. Our predictive models may either indicate direct interactions between taxa or common other effects on taxa, as statistical associations between taxa may also suggest that both taxa respond similarly to a third taxonomic group or an environmental factor (Blanchet et al., 2020). In our case, the two environmental factors pH and temperature both have strong associations with all the taxonomic groups after plot age, suggesting their role as important environmental factors defining the community composition of all the taxonomic groups. In addition, temperature was equally well associated with all the taxa and pH had better association with bacterial and fungal composition than with plant composition (Supplementary Figure S3), indicating that pH is affecting soil microbes more than plants, which coincides with previous studies illustrating the importance of pH in affecting microbial communities (Knelman et al., 2012; Glassman et al., 2017; Shen et al., 2020). The decrease in soil pH with increasing successional age at our study site confirms similar patterns in other earlier deglaciation chronosequences, where this soil acidification

mainly results from increased soil organic matter degradation and the associated leaching of organic acids in late succession (Bernasconi et al., 2011). Compared with the studies conducted in other similar glacier forefields such as Morteratsch glacier and Damma glacier in Switzerland (Burga et al., 2010; Bernasconi et al., 2011), Rotmoosferner glacier in Austria (De Vries et al., 2021), or Hailuoguo glacier in China (Jiang et al., 2018), our study aimed at considering the interplay between plants, bacteria, and fungi as well as environmental factors to detect mutual influences in the assembly of multidiverse communities.

Despite the strong associations between pH and temperature with all the taxonomic groups, predictions between the composition of taxonomic groups usually showed better performance than environmental factors. This suggests that apart from the common influence of abiotic factors in affecting the occurrence of different organisms, there is a strong effect of direct interactions between bacteria, fungi and plants which leads to high mutual predictabilities between the taxonomic groups. The importance of biotic interactions in the successional assembly of multidiverse communities is confirmed by previous findings where soil microbial composition was more closely associated to plant communities than to environmental factors (Knelman et al., 2012; Junker et al., 2021). In addition, we found that bacterial and fungal composition serve as better predictors for plant functional composition than for plant species composition, which supports

the notion that the functional composition of plant communities strongly impacts its biotic environment (Dassen et al., 2017). Meanwhile, bacterial functional composition was also well predicted by plant and fungi composition, and these together suggest the important role of functional properties for both plant and microbial community.

Predictabilities for biotic and environmental variables varied with successional age. Among all the environmental factors, the associations between pH and temperature with bacterial, fungal and plant community composition changed in a consistent pattern along the successional gradient (Supplementary Figure S3). This suggests that in the successional stages where these environmental factors were accurately predicted by all the taxonomic groups, they may have strong positive or negative effects on the occurrence of species of different taxonomic groups, while at the stages where they are not important predictors, other factors may act as the main drivers shaping the communities. Under the dynamic environmental conditions, the mutual predictabilities between plant and microbes at early and late successional stages clearly differed. Plant taxonomic and functional composition were well predicted by bacteria and fungi at early while not at late succession. The change of predictive signal may be caused by the increasing complexity of late successional communities, which may prevent the detection of predictabilities between plants and microbes as noise increases in the data. In our previous study at the same site we found a stronger interdependence between taxonomic groups at late succession (Hanusch et al., 2022), in accordance with an earlier study showing that microbial communities utilize mainly ancient carbon in the first decades after deglaciation while plant-derived carbon becomes a major source for microbes after 50 years of succession (Bardgett et al., 2007). These results suggest that in early successional stages, plants may directly leave a signal of bacterial communities that consume plant derived carbon next to those consuming ancient carbon, which infers the importance of species interactions (Ficetola et al., 2021). In later successional stages, further carbon sources such as decomposed soil organic matters may accumulate, which sustains microbial communities not directly related to plant species diversity and composition, resulting in a decreasing signal of interactions between plants and microbial species pairs with a poor prediction, and this is consistent with the results of glacier succession of Green Lakes Valley in Colorado, USA (Porazinska et al., 2018). Finally, age (inferred from distance to glacier) is not the only factor that is affecting the successional age of plots in glacier forefields, instead allogenic factors may reset successions or at least slow down successional progress in community development (Wojcik et al., 2021). These allogenic factors, such as geomorphic events, accumulate over time and thus may lead to outliers in community composition. If these outlier plots are part of test dataset but not the training dataset, they cannot be predicted on models as predictions are only possible in the range of the training dataset.

Variables describing the composition of taxonomic groups (e.g. PC axis of community composition) were mostly more

precisely predicted by other taxonomic groups than diversity indices. Particularly, the community composition of bacteria and fungi mutually predicted each other most precisely, which confirms previous studies demonstrating the interdependences between bacteria and fungi (Miransari, 2011; Deveau et al., 2018). Fungal composition was better predicted by plant composition than bacterial composition, which may reflect the tight interaction between plants and fungi, especially mycorrhiza (Millard & Singh, 2009; Horn et al., 2017; Sweeney et al., 2021). In contrast to the high mutual predictabilities of the composition between the taxonomic groups, the Shannon diversity of all the taxa and the phylogenetic and functional diversity of plants were not well predicted by the composition of other taxonomic groups, suggesting that diversity is not a direct function of community composition. Though plants or microbes may affect the diversity of each other by facilitative or antagonistic effects, the interactions may be regulated by environment and the existence of other taxa as was shown in previous studies (Bennett et al., 2017; Teste et al., 2017; van der Putten, 2017; Raza et al., 2020). In the soil environment multiple bacterial, fungal and plant species interact at the same time with different environmental conditions, and the interplay of these interactions may lead to a hardly predictable complexity of interdependencies and influences between the diversity and composition of different organisms.

Our results demonstrate the concerted development of plants and microbial communities regulated by environmental factors along an alpine glacier chronosequence. We identified how environmental factors define the niches of the organisms at different successional stages and the strongest biotic relationships between taxa in primary succession, revealing the strong interdependencies between taxonomic groups and the dynamic importance of biotic and abiotic factors in shaping natural communities. Our approaches to identify indicators and environmental variables that inform best about the diversity and composition of ecosystems may stimulate the exploration of mechanisms underlying community assembly in future studies, generate hypothesis that can be tested in lab experiments and facilitate monitoring and conservation efforts.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, PRJNA701884; <https://www.ncbi.nlm.nih.gov/>, PRJNA701890.

Author contributions

RJ conceived the study. XH, MH, VR-H, and RJ designed the study and collected the data. XH and RJ analyzed the data. XH

and RJ wrote the manuscript with critical input from MH and VR-H. All authors contributed to the manuscript and approved the final version.

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References

- Aguiar, F. C., Cerdeira, J. O., Martins, M. J., Ferreira, M. T., and Pillar, V. (2013). Riparian forests of southwest Europe: are functional trait and species composition assemblages constrained by environment? *J. Veg. Sci.* 24, 628–638. doi: 10.1111/jvs.12009
- Angly, F. E., Dennis, P. G., Skarshewski, A., Vanwongerghem, I., Hugenholtz, P., and Tyson, G. W. (2014). CopyRighter: a rapid tool for improving the accuracy of microbial community profiles through lineage-specific gene copy number correction. *Microbiome* 2, 11. doi: 10.1186/2049-2618-2-11
- Bardgett, R. D., Richter, A., Bol, R., Garnett, M. H., Baumler, R., Xu, X., et al. (2007). Heterotrophic microbial communities use ancient carbon following glacial retreat. *Biol. Lett.* 3, 487–490. doi: 10.1098/rsbl.2007.0242
- Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., and Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* 355, 181–184. doi: 10.1126/science.aai8212
- Bernasconi, S. M., Bauder, A., Bourdon, B., Brunner, I., Bünemann, E., Chris, I., et al. (2011). Chemical and biological gradients along the damma glacier soil chronosequence. Switzerland. *Vadose Zone J.* 10, 867–883. doi: 10.2136/vzj2010.0129
- Bernhardt-Römermann, M., Römermann, C., Nuske, R., Parth, A., Klotz, S., Schmidt, W., et al. (2008). On the identification of the most suitable traits for plant functional trait analyses. *Oikos* 117, 1533–1541. doi: 10.1111/j.0030-1299.2008.16776.x
- Bever, J. D., Platt, T. G., and Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annu. Rev. Microbiol.* 66, 265–283. doi: 10.1146/annurev-micro-092611-150107
- Blanchet, F. G., Cazelles, K., and Gravel, D. (2020). Co-Occurrence is not evidence of ecological interactions. *Ecol. Lett.* 23, 1050–1063. doi: 10.1111/ele.13525
- Breiman, L. (2001). Random forests. *Mach. Learn.* 45, 5–32. doi: 10.1023/a:1010933404324
- Buma, B., Bisbing, S., Krapek, J., and Wright, G. (2017). A foundation of ecology rediscovered: 100 years of succession on the William s. cooper plots in glacier bay, Alaska. *Ecology* 98, 1513–1523. doi: 10.1002/ecy.1848
- Buma, B., Bisbing, S. M., Wiles, G., and Bidlack, A. L. (2019). 100 yr of primary succession highlights stochasticity and competition driving community establishment and stability. *Ecology* 100, e02885. doi: 10.1002/ecy.2885
- Burga, C. A., Krüsi, B., Egli, M., Wernli, M., Elsener, S., Ziefle, M., et al. (2010). Plant succession and soil development on the foreland of the morteratsch glacier (Pontresina, Switzerland): straight forward or chaotic? *Flora* 205, 561–576. doi: 10.1016/j.flora.2009.10.001
- Cannone, N., Diolaiuti, G., Guglielmin, M., and Smiraglia, C. (2008). Accelerating climate change impacts on alpine glacier forefield ecosystems in the European Alps. *Ecol. Appl.* 18, 637–648. doi: 10.1890/07-1188.1
- Chang, C., and HilleRisLambers, J. (2016). Integrating succession and community assembly perspectives. *F1000Research* 5, 1–10. doi: 10.12688/f1000research.8973.1
- Cheng, J., Zhao, M., Cong, J., Qi, Q., Xiao, Y., Cong, W., et al. (2020). Soil pH exerts stronger impacts than vegetation type and plant diversity on soil bacterial community composition in subtropical broad-leaved forests. *Plant Soil* 450, 273–286. doi: 10.1007/s11104-020-04507-2
- Chen, W., Xu, R., Wu, Y., Chen, J., Zhang, Y., Hu, T., et al. (2018). Plant diversity is coupled with beta not alpha diversity of soil fungal communities following n enrichment in a semi-arid grassland. *Soil Biol. Biochem.* 116, 388–398. doi: 10.1016/j.soilbio.2017.10.039
- Darcy, J. L., Schmidt, S. K., Knelman, J. E., Cleveland, C. C., Castle, S. C., and Nemergut, D. R. (2018). Phosphorus, not nitrogen, limits plants and microbial primary producers following glacial retreat. *Sci. Adv.* 4, eaaq0942. doi: 10.1126/sciadv.aaq0942
- Dassen, S., Cortois, R., Martens, H., de Hollander, M., Kowalchuk, G. A., van der Putten, W. H., et al. (2017). Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. *Mol. Ecol.* 26, 4085–4098. doi: 10.1111/mec.14175
- Dastogeer, K. M. G., Tumpa, F. H., Sultana, A., Akter, M. A., and Chakraborty, A. (2020). Plant microbiome—an account of the factors that shape community composition and diversity. *Curr. Plant Biol.* 23, 100161. doi: 10.1016/j.cpb.2020.100161
- David, A. S., Thapa-Magar, K. B., Menges, E. S., Searcy, C. A., and Afkhami, M. E. (2020). Do plant-microbe interactions support the stress gradient hypothesis? *Ecology* 101, e03081. doi: 10.1002/ecy.3081
- Davison, J., Moora, M., Semchenko, M., Adenan, S. B., Ahmed, T., Akhmetzhanova, A. A., et al. (2021). Temperature and pH define the realised niche space of arbuscular mycorrhizal fungi. *New Phytol.* 231, 763–776. doi: 10.1111/nph.17240

Conflict of interest

The authors declare that the research 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

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.1017847/full#supplementary-material>

- Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., et al. (2018). Bacterial-fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiol. Rev.* 42, 335–352. doi: 10.1093/femsre/fuy008
- De Vries, F. T., Thion, C., Bahn, M., Bergk Pinto, B., Cécillon, S., Frey, B., et al. (2021). Glacier forelands reveal fundamental plant and microbial controls on short-term ecosystem nitrogen retention. *J. Ecol.* 109, 3710–3723. doi: 10.1111/1365-2745.13748
- Ficetola, G. F., Marta, S., Guerrieri, A., Gobbi, M., Ambrosini, R., Fontaneto, D., et al. (2021). Dynamics of ecological communities following current retreat of glaciers. *Annu. Rev. Ecol. Syst.* 52, 405–426. doi: 10.1146/annurev-ecolsys-010521-040017
- Ghannam, R. B., and Techtman, S. M. (2021). Machine learning applications in microbial ecology, human microbiome studies, and environmental monitoring. *Comput. Struct. Biotechnol. J.* 19, 1092–1107. doi: 10.1016/j.csbj.2021.01.028
- Glassman, S. I., Wang, I. J., and Bruns, T. D. (2017). Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Mol. Ecol.* 26, 6960–6973. doi: 10.1111/mec.14414
- Goodswen, S. J., Barratt, J. L. N., Kennedy, P. J., Kaufer, A., Calarco, L., and Ellis, J. T. (2021). Machine learning and applications in microbiology. *FEMS Microbiol. Rev.* 45, fuab015. doi: 10.1093/femsre/fuab015
- Hanusch, M., He, X., Ruiz-Hernandez, V., and Junker, R. R. (2022). Succession comprises a sequence of threshold-induced community assembly processes towards multidiversity. *Commun. Biol.* 5, 424. doi: 10.1038/s42003-022-03372-2
- Harrison, S., Spasojevic, M. J., and Li, D. (2020). Climate and plant community diversity in space and time. *Proc. Natl. Acad. Sci. U.S.A.* 117, 4464–4470. doi: 10.1073/pnas.1921724117
- Hermans, S. M., Buckley, H. L., Case, B. S., Curran-Cournane, F., Taylor, M., and Lear, G. (2020). Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome* 8, 79. doi: 10.1186/s40168-020-00858-1
- Hintze, C., Heydel, F., Hoppe, C., Cunze, S., König, A., and Tackenberg, O. (2013). D3: The dispersal and diaspore database – baseline data and statistics on seed dispersal. *Perspect. Plant Ecol. Evol. Syst.* 15, 180–192. doi: 10.1016/j.ppees.2013.02.001
- Horn, S., Hempel, S., Verbruggen, E., Rillig, M. C., and Caruso, T. (2017). Linking the community structure of arbuscular mycorrhizal fungi and plants: a story of interdependence? *ISME J.* 11, 1400–1411. doi: 10.1038/ismej.2017.5
- Jiang, Y., Lei, Y., Yang, Y., Korpelainen, H., Niinemets, Ü., and Li, C. (2018). Divergent assemblage patterns and driving forces for bacterial and fungal communities along a glacier forefield chronosequence. *Soil Biol. Biochem.* 118, 207–216. doi: 10.1016/j.soilbio.2017.12.019
- Junker, R. R., Hanusch, M., He, X., Ruiz-Hernández, V., Otto, J.-C., Kraushaar, S., et al. (2020). Ödenwinkel: an alpine platform for observational and experimental research on the emergence of multidiversity and ecosystem complexity. *Web Ecol.* 20, 95–106. doi: 10.5194/we-20-95-2020
- Junker, R. R., He, X., Otto, J. C., Ruiz-Hernandez, V., and Hanusch, M. (2021). Divergent assembly processes? a comparison of the plant and soil microbiome with plant communities in a glacier forefield. *FEMS Microbiol. Ecol.* 97, fiab135. doi: 10.1093/femsec/fiab135
- Kahmen, S., and Poschlod, P. (2004). Plant functional trait responses to grassland succession over 25 years. *J. Veg. Sci.* 15, 21–32. doi: 10.1111/j.1654-1103.2004.tb02233.x
- Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., et al. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464. doi: 10.1093/bioinformatics/btq166
- Knelman, J. E., Legg, T. M., O'Neill, S. P., Washenberger, C. L., González, A., Cleveland, C. C., et al. (2012). Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. *Soil Biol. Biochem.* 46, 172–180. doi: 10.1016/j.soilbio.2011.12.001
- Kruger, C., Kohout, P., Janouskova, M., Puschel, D., Frouz, J., and Rydlova, J. (2017). Plant communities rather than soil properties structure arbuscular mycorrhizal fungal communities along primary succession on a mine spoil. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00719
- Laliberte, E., and Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305. doi: 10.1890/08-2244.1
- Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., et al. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* 31, 814–821. doi: 10.1038/nbt.2676
- Leff, J. W., Bardgett, R. D., Wilkinson, A., Jackson, B. G., Pritchard, W. J., De Long, J. R., et al. (2018). Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME J.* 12, 1794–1805. doi: 10.1038/s41396-018-0089-x
- Liaw, A., and Wiener, M. (2002). Classification and regression by random forest. *R News* 2, 18–22. <http://CRAN.R-project.org/doc/Rnews/>.
- Lopez-Angulo, J., de la Cruz, M., Chacon-Labela, J., Illuminati, A., Matesanz, S., Pescador, D. S., et al. (2020). The role of root community attributes in predicting soil fungal and bacterial community patterns. *New Phytol.* 228, 1070–1082. doi: 10.1111/nph.16754
- Millard, P., and Singh, B. K. (2009). Does grassland vegetation drive soil microbial diversity? *Nutr. Cycl. Agroecosyst.* 88, 147–158. doi: 10.1007/s10705-009-9314-3
- Miransari, M. (2011). Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Appl. Microbiol. Biotechnol.* 89, 917–930. doi: 10.1007/s00253-010-3004-6
- Mitchell, R. J., Hester, A. J., Campbell, C. D., Chapman, S. J., Cameron, C. M., Hewison, R. L., et al. (2011). Explaining the variation in the soil microbial community: do vegetation composition and soil chemistry explain the same or different parts of the microbial variation? *Plant Soil.* 351, 355–362. doi: 10.1007/s11104-011-0968-7
- Mouhamadou, B., Puissant, J., Personeni, E., Desclos-Theveniau, M., Kastl, E. M., Schlöter, M., et al. (2013). Effects of two grass species on the composition of soil fungal communities. *Biol. Fertil. Soils.* 49, 1131–1139. doi: 10.1007/s00374-013-0810-x
- Navratilova, D., Tlaskalova, P., Kohout, P., Drevojan, P., Fajmon, K., Chytrý, M., et al. (2019). Diversity of fungi and bacteria in species-rich grasslands increases with plant diversity in shoots but not in roots and soil. *FEMS Microbiol. Ecol.* 95, fty208. doi: 10.1093/femsec/fty208
- Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* 20, 241–248. doi: 10.1016/j.funeco.2015.06.006
- Ohler, L. M., Lechleitner, M., and Junker, R. R. (2020). Microclimatic effects on alpine plant communities and flower-visitor interactions. *Sci. Rep.* 10, 1366. doi: 10.1038/s41598-020-58388-7
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2013). *vegan: Community Ecology Package*, version 2.0 – 7. R package. <http://CRAN.R-project.org/package=vegan>.
- Pearse, W. D., Cadotte, M. W., Cavender-Bares, J., Ives, A. R., Tucker, C. M., Walker, S. C., et al. (2015). Pez: phylogenetics for the environmental sciences. *Bioinformatics* 31, 2888–2890. doi: 10.1093/bioinformatics/btv277
- Pichler, M., Boreux, V., Klein, A. M., Schleuning, M., Hartig, F., and Carvalheiro, L. (2019). Machine learning algorithms to infer trait-matching and predict species interactions in ecological networks. *Methods Ecol. Evol.* 11, 281–293. doi: 10.1111/2041-210x.13329
- Porazinska, D. L., Farrer, E. C., Spasojevic, M. J., Bueno de Mesquita, C. P., Sartwell, S. A., Smith, J. G., et al. (2018). Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem. *Ecology* 99, 1942–1952. doi: 10.1002/ecy.2420
- Rasmussen, P. U., Bennett, A. E., Tack, A. J. M., and Singh, B. (2019). The impact of elevated temperature and drought on the ecology and evolution of plant–soil microbe interactions. *J. Ecol.* 108, 337–352. doi: 10.1111/1365-2745.13292
- Raza, W., Wang, J., Jousset, A., Friman, V. P., Mei, X., Wang, S., et al. (2020). Bacterial community richness shifts the balance between volatile organic compound-mediated microbe-pathogen and microbe-plant interactions. *Proc. Biol. Sci.* 287, 20200403. doi: 10.1098/rspb.2020.0403
- Reese, A. T., Lulow, K., David, L. A., and Wright, J. P. (2018). Plant community and soil conditions individually affect soil microbial community assembly in experimental mesocosms. *Ecol. Evol.* 8, 1196–1205. doi: 10.1002/ece3.3734
- Robroek, B. J. M., Martí, M., Svensson, B. H., Dumont, M. G., Veraart, A. J., and Jassey, V. E. J. (2021). Rewiring of peatland plant–microbe networks outpaces species turnover. *Oikos* 130, 339–353. doi: 10.1111/oik.07635
- Rudgers, J. A., Afkhami, M. E., Bell-Dereske, L., Chung, Y. A., Crawford, K. M., Kivlin, S. N., et al. (2020). Climate disruption of plant–microbe interactions. *Annu. Rev. Ecol. Syst.* 51, 561–586. doi: 10.1146/annurev-ecolsys-011720-090819
- Schmidt, S. K., Reed, S. C., Nemergut, D. R., Grandy, A. S., Cleveland, C. C., Weintraub, M. N., et al. (2008). The earliest stages of ecosystem succession in high-elevation, (5000 metres above sea level), recently deglaciated soils. *Proc. Biol. Sci.* 275, 2793–2802. doi: 10.1098/rspb.2008.0808
- Shen, C., Gunina, A., Luo, Y., Wang, J., He, J. Z., Kuzyakov, Y., et al. (2020). Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient. *Environ. Microbiol.* 22, 3287–3301. doi: 10.1111/1462-2920.15090
- Sweeney, C. J., de Vries, F. T., van Dongen, B. E., and Bardgett, R. D. (2021). Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytol.* 229, 1492–1507. doi: 10.1111/nph.16976
- Teste, F. P., Kardol, P., Turner, B. L., Wardle, D. A., Zemanek, G., Renton, M., et al. (2017). Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* 355, 173–176. doi: 10.1126/science.aai8291
- van der Putten, W. H. (2017). Belowground drivers of plant diversity. *Science* 355, 134–135. doi: 10.1126/science.aal4549
- van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., et al. (2013). Plant-soil feedbacks: the past, the present and future challenges. *J. Ecol.* 101, 265–276. doi: 10.1111/1365-2745.12054

- Walker, L. R., Wardle, D. A., Bardgett, R. D., and Clarkson, B. D. (2010). The use of chronosequences in studies of ecological succession and soil development. *J. Ecol.* 98, 725–736. doi: 10.1111/j.1365-2745.2010.01664.x
- Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., and Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science* 304, 1629–1633. doi: 10.1126/science.1094875
- Wojcik, R., Eichel, J., Bradley, J. A., and Benning, L. G. (2021). How allogenic factors affect succession in glacier forefields. *Earth-Sci. Rev.* 218, 103642. doi: 10.1016/j.earscirev.2021.103642
- Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., and Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: Are there any links? *Ecology* 84, 2042–2050. doi: 10.1890/02-0433
- Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., et al. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature* 506, 89–92. doi: 10.1038/nature12872
- Zumsteg, A., Luster, J., Göransson, H., Smittenberg, R. H., Brunner, I., Bernasconi, S. M., et al. (2012). Bacterial, archaeal and fungal succession in the forefield of a receding glacier. *Microb. Ecol.* 63, 552–564. doi: 10.1007/s00248-011-9991-8



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Beyond taxonomic diversity patterns – investigating how α and β components of macrophyte functional diversity respond to environmental gradients in lotic ecosystems of Greece

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In addition to quantifying the taxonomic diversity of aquatic communities, understanding the patterns of alpha functional diversity (α -diversity) and exploring changes in functional dissimilarity (β -diversity) can improve our understanding on how ecosystems respond to environmental changes. In this study, we quantified functional alpha (α) and beta (β) diversity of macrophytic assemblages from river sites in Greece and then, examined relationships with water quality parameters and hydromorphological factors. We assigned 6 traits (Ellenberg nutrients indicator, Ellenberg light indicator, growth form, leaf size, leaf type, fruit size) to a total of 36 hydrophyte species and calculated three indices of functional diversity (functional richness, functional dispersion and functional evenness). We also estimated the total β -functional diversity and its' main components, turnover and nestedness. To assess the effects of water quality (including physical and chemical variables) we used Generalized Additive Models (GAM) for alpha functional diversity indices and Generalized Dissimilarity Models (GDM) for beta functional diversity. We performed Kruskal-Wallis tests and permutational multivariate analysis of variance (PERMANOVA) to search for significant variations of α - and β -diversity among the hydromorphological factors. Our results showed that macrophyte growth form and light preference were important trait characteristics that explained a large share of the total variance of functional composition. We also found relatively low taxonomic and functional richness, whereas taxonomic and functional dissimilarity were mostly attributed to species turnover, which expresses the changes in taxonomic and functional composition. We found significant relationships between functional dispersion and functional evenness with pH and oxygen saturation, whereas functional dissimilarity was driven only by geographic distance, although the GDM explained a small portion of the total variance. Functional richness, dispersion and evenness were significantly higher at systems with fine

substrates and deep waters with low or high flow compared to systems with coarser substrates and riffle habitats. We also found significant variation in functional dissimilarity among the hydromorphological factors, although much of the total variance remained unexplained. Overall, our study highlights the importance of considering the functional diversity of aquatic plant assemblages within the frame of freshwater monitoring and conservation plans.

KEYWORDS

aquatic macrophytes, functional richness, functional beta diversity, generalized dissimilarity models, rivers

Introduction

Aquatic macrophytes provide numerous functions in lotic ecosystems. For instance, plants that grow within the channel and along the banks are known to mediate nutrient and sediment transport from the land into the watercourse (Valkama et al., 2019; Walton et al., 2020), while at the same time they stabilize the channel and the banks preventing erosion. Most importantly, aquatic plants can provide foraging and reproduction habitats for fish, amphibians and invertebrates (Lind et al., 2019; Cole et al., 2020). They can also influence the physical, chemical and flow characteristics within the channel (Gurnell, 2015; Preiner et al., 2020), which in turn may affect fish and invertebrate communities.

Because of their importance for stream and riverine ecosystems, aquatic macrophytes have been widely used as indicators of ecosystem health and ecological integrity (Aguir et al., 2014; Rodrigues et al., 2019; Szoszkiewicz et al., 2020). Aquatic macrophytes are one of the four biological quality elements that are used for the assessment of ecological status of streams and rivers of Europe, following the implementation of the Water Framework Directive 2000/60, and several national assessment approaches have been developed by the EU Member States (Birk and Willby, 2010). Most ecological assessment schemes consider the composition of aquatic macrophytic communities (Szoszkiewicz et al., 2020; Stefanidis et al., 2022) since the increased occurrence and abundance of certain plants is related with environmental factors that indicate anthropogenic degradation of aquatic ecosystems (e.g. eutrophication and hydromorphological alteration) (Szoszkiewicz et al., 2014; Stefanidis and Papastergiadou, 2019; Manolaki et al., 2020; Stefanidis et al., 2021). However, there are studies that have shown that the responses of macrophytes to environmental gradients can be complex and difficult to decipher (Steffen et al., 2014; O'Hare et al., 2018; Son et al., 2018; Gyosheva et al., 2020). Thus, freshwater ecologists have shown increased interest in studying multiple facets of aquatic biodiversity, including aquatic macrophytes (Fu et al., 2014a; Schneider et al., 2015; Alahuhta et al., 2017; Elo et al., 2018; Son et al., 2018; Stefanidis et al., 2019).

The functional diversity which Tilman (2001) defined as 'those components of biodiversity that influence how an ecosystem

operates or functions', has emerged as a facet of biodiversity and a step beyond species richness. It has become a powerful tool to link community composition to ecosystem properties and then to ecosystem services by quantifying the value and range of functional characteristics and thus ecosystem functioning (Díaz et al., 2007). In addition, the impact of the global loss of biodiversity is increasingly attributed to the loss of functional rather than taxonomic groups (Bellwood et al., 2002). Further, species are not equal in their effects on ecosystem functioning since their functional traits matter to ecosystem processes (Mouchet et al., 2010). The study of trait distributions can be used as a more powerful conceptual model for understanding broad-scale patterns in assemblage structure since organisms with similar traits will share similar niche requirements and will select the same habitat (Olden et al., 2010).

Besides species richness (alpha diversity) and changes in species composition among communities (beta diversity), investigating patterns of functional alpha (α) and beta (β) diversity may provide invaluable information and a better understanding on how environmental gradients affect aquatic biodiversity processes (Zhang et al., 2018; Wang et al., 2021). Considering that assembly processes influencing natural communities may differ depending on the spatial scale considered, separating functional diversity in within-community (α) and among-community (β) components will improve the detection of all processes influencing community assembly. Furthermore, trait-based approaches are more likely to indicate an early response to environmental change than community-based approaches because a change in the functional structure is easier to detect than a change to community composition (Degen et al., 2018). In addition, such approaches can be applied to all species regardless of geographic region and location (Dolédec et al., 2006).

Previous studies have linked morphological and life history traits of aquatic plants with ecological and biogeochemical processes showing the response of aquatic plant functional structure to eutrophication and other environmental changes (Fu et al., 2014b; Baattrup-Pedersen et al., 2016; Stefanidis and Papastergiadou, 2019). Therefore, exploring the patterns of functional trait composition in relation to environmental change

is crucial for a better understanding of the response of aquatic communities to pressure and identifying key trait characteristics that can serve as indicators of anthropogenic pressures. Although there is a general consensus that taxonomic diversity is shaped by environmental filters, including anthropogenic changes (Bornette and Puijalon, 2011; Dybkjaer et al., 2012), there are relatively few studies that have explored the patterns of functional α - and β -diversity of aquatic macrophytes in lotic ecosystems.

The main objective of this study is to investigate the patterns of functional alpha and beta diversity of aquatic macrophytic assemblages in Greek lotic ecosystems and to assess whether these patterns are subject to environmental change. Our hypotheses are that a) indices of α - functional diversity decline with increased levels of nutrient pollution and degraded water quality, b) the change of functional compositional structure increases with water quality impairment and c) α - and β - functional diversity show significant variations among different types of hydromorphological conditions and different degrees of hydromorphological modification.

Material and methods

Macrophyte samplings

Field samplings were conducted in summer of 2021 and 2022 as part of the ecological monitoring program for the assessment of the ecological status of rivers of Greece in line with the EU Water Framework Directive (WFD) (Skoulidakis et al., 2021). We used presence-absence data of hydrophytes, plants that grow exclusively in water, from 74 river sites that belong to the national monitoring network for the ecological quality assessment of inland waters. The sampled sites extend across several biogeographic regions of Greece ranging from 37°N to 41°N and 20°E to 25°E and at altitudes spanning from sea level to 765 m a.s.l (Figure 1).

Macrophytes were sampled in accordance with national protocols harmonized with European standards (CEN, 2003; CEN, 2006). Sampling was conducted by wading into the water, following a zigzag pattern upstream for a 100 meter-long section of

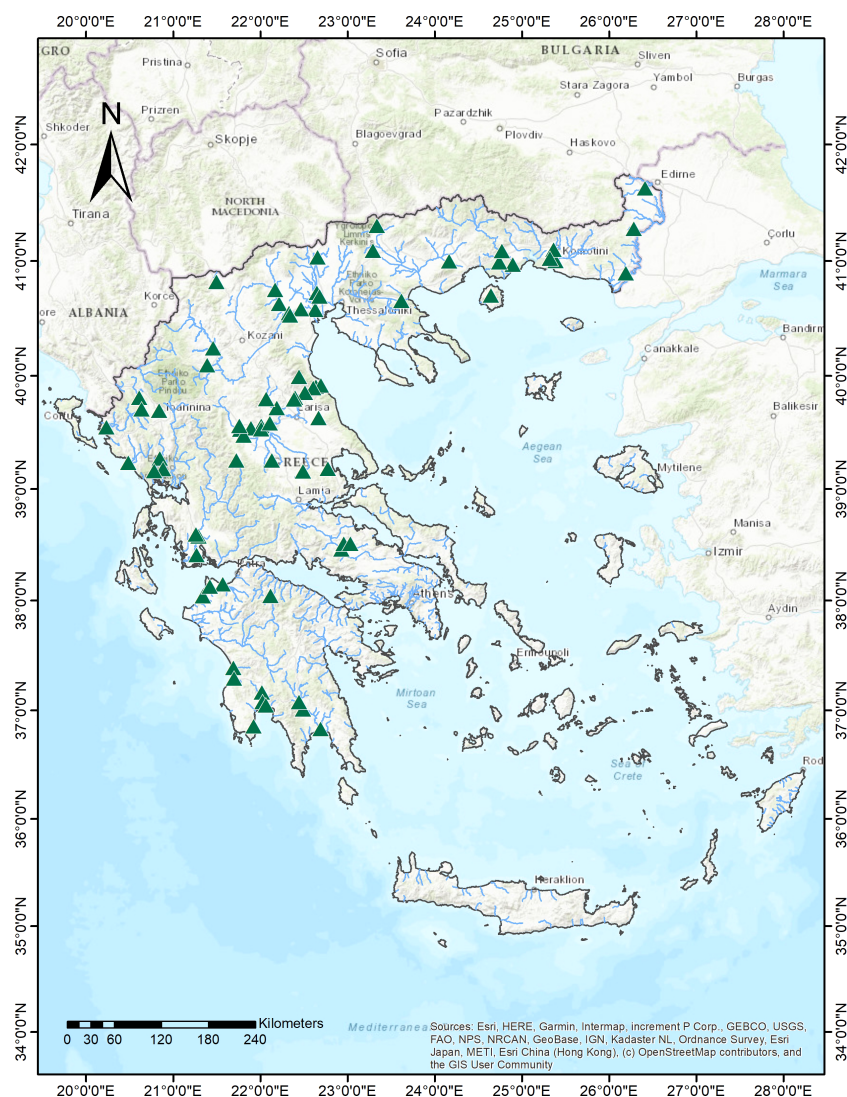


FIGURE 1

Map showing the location of sampling sites (N=74) of the National Monitoring network, across running waters of mainland Greece.

the river channel. Unidentified specimens were collected and transferred at the laboratory for identification.

Environmental parameters

We used geographical variables (latitude, longitude and altitude), physical and chemical parameters (electrical conductivity, total dissolved solids, pH, dissolved oxygen concentration, dissolved oxygen saturation and biochemical oxygen demand, nitrate, nitrite, ammonium, total nitrogen and total phosphorus) and hydromorphological characteristics in order to identify links between facets of plant diversity and environmental characteristics. Water physicochemical parameters and water samples for the determination of nutrients were obtained during samplings that were conducted during the same period with the macrophyte surveys. Hydromorphological features (e.g. type of channel substrate, effects of hydrological and morphological modifications, bed stability, channel shade) were assessed during the plant sampling according to Stefanidis et al. (2022). A description of the environmental variables that were considered in this study is listed in Table 1. For further details on field samplings, characteristics of river reaches, macrophyte identification and quantification of physicochemical and geomorphological variables see our publications (Stefanidis et al., 2021; Papastergiadou, 2022; Stefanidis et al., 2022).

Trait-based analysis and calculation of functional diversity indices

Patterns of functional composition and diversity of aquatic macrophytes were investigated following a methodological framework that is based on the construction of multidimensional functional space using community and trait data (Magneville et al., 2022). First, we created a matrix with presence-absence data of macrophyte species for the 74 river sites. Then, we constructed a matrix with functional community characteristics based on six nominal and ordinal plant traits allocated to a total of 36 hydrophyte species (Table 2). The traits that we used were the Ellenberg indicator values for nitrogen and light preference (Tichý et al., 2023), the life-form according to Wilby et al. (Wilby et al., 2000), the leaf size and fruit size classified into three categories (small, moderate, and large) (Wilby et al., 2000), and the leaf type classified into three types (entire, capillary and tubular) (Wilby et al., 2000). Table 3 includes a list with the allocated traits that we used.

A functional distance matrix that contains the functional distances for each pair of species was calculated using the Gower distance, since all traits are categorical. Then, a hierarchical cluster analysis was conducted on the distance matrix to obtain groups of plants with similar functional assemblages. Following Kelley et al. (1996), we employed the Kelley–Gardner–Sutcliffe penalty function (KGS) to identify distinct clusters of the dendrogram. This method maximises differences between groups and cohesiveness within groups. The minimum of the KGS function corresponds to the

optimal number of clusters. Functional space was then constructed based on the functional dissimilarity matrix using a principal coordinates analysis (PCoA) and functional diversity indices were calculated using the species coordinates on the first three principal components (Maire et al., 2015). Correlations between traits and the functional axes were identified with a Kruskal-Wallis test to help understand how plant groups are distributed across the functional space with regard to their trait composition.

Concerning the functional indices, Mouillot et al. (Mouillot et al., 2013) has proposed the use of several indices that act complementary and can provide useful insights about the functional community structure. Here we calculated three of these indices. Functional dispersion, which shows the deviation of species traits values from the center of the functional space filled by the assemblage, the functional richness which represents the amount of functional space occupied by a species assemblage, and the functional evenness which corresponds to how regularly species abundances are distributed in the functional space (Mason et al., 2005; Mouillot et al., 2013).

Beta (β) diversity was assessed as differences between all pairs of sites using the Sørensen index (β_{sor}). The taxonomic and functional β dissimilarities are consistent with two additive components: the turnover component (replacement of species or functional space not shared by communities) and the nestedness-resultant component (difference in species or functional space filled by communities) (Baselga, 2010; Villéger et al., 2013). The turnover (β_{sim}) and nestedness (β_{sne}) components were quantified in accordance with the β -diversity partitioning framework proposed by Baselga (2010) and Villéger et al. (2013). The indices of β -diversity were calculated using the R package betapart (Baselga and Orme, 2012). These indices range from 0 to 1, where higher values indicate greater dissimilarities among sites.

By examining both taxonomic and functional β -diversity we intended to draw useful conclusions about the changes in taxonomic and functional composition and to associated them further with environmental characteristics. For the calculation of all functional diversity indices, we used the package “mFD” in R environment (Magneville et al., 2022). The correlations between taxonomic and functional β -diversity as well as between their respective components were tested using Mantel permutational tests.

Functional diversity and environmental parameters

We used Generalized Additive Models (GAM) to investigate the relationships between the alpha functional diversity indices, taxonomic diversity (species richness) and the water quality predictors (physical and chemical). GAMs have been commonly used in ecology for fitting non-linear relationships between species and environmental predictors (Guisan et al., 2002; Leathwick et al., 2006). Models were fitted with the “mgcv” package in R environment (Wood, 2020) using cubic smoothing splines. In order to assess the variation of alpha diversity indices among the levels of hydromorphological factors, we conducted Kruskal-Wallis tests.

TABLE 1 Details and brief description of the environmental variables that were considered in this study.

Category	Variable name	Description	Variable type	Mean value
Water quality/ physicochemical	EC	Electrical Conductivity [$\mu\text{S}/\text{cm}$]	Numerical continuous	693.52
	pH	Sorensen scale	Numerical continuous	7.91
	DO	Concentration of dissolved oxygen [mg/l]	Numerical continuous	7.59
	BOD	Biochemical oxygen demand [mg/l]	Numerical continuous	7.13
	Nitrate	Nitrate concentration in the water [$\text{mg}/\text{l NO}_3^-$]	Numerical continuous	1.27
	Nitrite	Nitrite concentration in the water [$\text{mg}/\text{l NO}_2^-$]	Numerical continuous	0.04
	Ammonium	Ammonia concentration in the water [$\text{mg}/\text{l NH}_4^+$]	Numerical continuous	0.39
	TN	Total Nitrogen [$\text{mg}/\text{l N}$]	Numerical continuous	1.92
	Phosphate	Concentration of Orthophosphates in the water [$\text{mg}/\text{l PO}_4^{3-}$]	Numerical continuous	0.16
	TP	Concentration of total phosphorus in the water [$\text{mg}/\text{l P}$]	Numerical continuous	0.17
	TDS	Concentration of total dissolved solids [mg/l]	Numerical continuous	366.21
Hydromorphological/ habitat	Channel substrate	Prevailing channel substrate, three levels: Fine ($<2\text{mm}$), medium (2 – 64 mm), coarse ($>64\text{ mm}$)	Ordinal factor	NA
	Bed stability	Stability of riverbed, four levels: Solid (e.g. bedrock), stable, unstable, soft (e.g. mud)	Ordinal factor	
	Shade	Channel shade, three levels: Absence of shade, semi-continuous shade, full shade	Ordinal factor	
	Habitats	Type of river habitat: Pool, riffle, run, slack	Nominal factor	
	Land Uses	Type of prevailing land use within the adjacent area, four levels: Artificial, Agriculture, Natural, Wetland	Nominal factor	
	Channel profile alteration	Degree of channel profile modification present at the site/cross section alteration	Ordinal factor	
	Morphology alteration	Degree of the morphological modification of the channel present at the site	Ordinal factor	
	Habitat alteration	Alteration of instream habitats	Ordinal factor	
	Stream hydrology alteration	Degree of the hydrological alteration present at the site	Ordinal factor	
	Water abstraction	Influence of water abstraction at the site	Ordinal factor	
	Dykes (flood protection)	Influence of dykes at the site	Ordinal factor	

NA, not applicable.

Prior to the model fitting, predictors were tested for collinearity by calculating the variance inflation factor (VIF) with the vifstep function of the “usdm” package (Naimi et al., 2014) in R environment. Environmental variables with $\text{VIF} > 3$ were excluded from further analysis (Vittinghoff et al., 2012). The other variables were used for fitting full models with functional richness, functional dispersion and functional evenness as response

variables. Then, using the dredge function from package “MuMIN” (Barton, 2020), the model with the lowest Akaike information criterion (AICc) value was selected as the final model.

Furthermore, we calculated Moran’s coefficients based on the geographical coordinates of the sites, to evaluate the spatial autocorrelation in each final model. Calculations were made with R package “ape” (Paradis and Schliep, 2019).

TABLE 2 List of aquatic macrophyte species considered in the present study.

Code	Name	Code	Name
Ali.lan	<i>Alisma lanceolatum</i> With.	Oen.aqu	<i>Oenanthe aquatica</i> L.
Ali.pla	<i>Alisma plantago-aquatica</i> L.	Per.amp	<i>Persicaria amphibia</i> (L.) Gray
Api.nod	<i>Apium nodiflorum</i> (L.) Lag.	Pot.cri	<i>Potamogeton crispus</i> L.
Azo.fil	<i>Azolla filiculoides</i> Lam.	Pot.nat	<i>Potamogeton natans</i> L.
Ber.ere	<i>Berula erecta</i> (Huds.) Coville	Pot.nod	<i>Potamogeton nodosus</i> Poir.
But.umb	<i>Butomus umbellatus</i> L.	Pot.per	<i>Potamogeton perfoliatus</i> L.
Cal.sta	<i>Callitriche stagnalis</i> Scop.	Ran.cir	<i>Ranunculus circinatus</i> Sibth.
Cer.dem	<i>Ceratophyllum demersum</i> L.	Ran.flu	<i>Ranunculus fluitans</i> Lam.
Cer.sub	<i>Ceratophyllum submersum</i> L.	Ran.tri	<i>Ranunculus trichophyllum</i> Chaix ex Vill.
Gly.flu	<i>Glyceria fluitans</i> (L.) R.Br.	Ror.amp	<i>Rorippa amphibia</i> (L.) Besser
Hyd.mor	<i>Hydrocharis morsus-ranae</i> L.	Sal.nat	<i>Salvinia natans</i> (L.) All
Jun.Bul	<i>Juncus bulbosus</i> L.	Spa.eme	<i>Sparganium emersum</i> Rehmann
Lem.gib	<i>Lemna gibba</i> L.	Spa.ere	<i>Sparganium erectum</i> L.
Lem.min	<i>Lemna minor</i> L.	Stu.pec	<i>Stuckenia pectinata</i> (L.) Böerner
Men.aqu	<i>Mentha aquatica</i> L.	Tra.nat	<i>Trapa natans</i> L.
Myr.alt	<i>Myriophyllum alterniflorum</i> DC.	Val.spi	<i>Vallisneria spiralis</i> L.
Myr.spi	<i>Myriophyllum spicatum</i> L.	Ver.ana	<i>Veronica anagalis-aquatica</i> L.
Nas.off	<i>Nasturtium officinale</i> W.T.Aiton	Ver.bec	<i>Veronica beccabunga</i> L.
Nup.lut	<i>Nuphar lutea</i> (L.) Sm.	Zan.pal	<i>Zannichellia palustris</i> L.
Nym.alb	<i>Nymphaea alba</i> L.		

To investigate how β -diversity changes across water quality gradients, we used Generalized Dissimilarity models. Generalized Dissimilarity Models (GDMs) model the dissimilarity in species composition as a function of environmental and geographical parameters using dissimilarity matrices (Fitzpatrick et al., 2013). In our analysis, we used the default three I-spline basis functions per predictor was used and we plotted the I-splines to visually assess how magnitudes and rates of the total functional dissimilarity, functional turnover and nestedness change along the environmental gradients. The variable importance and significance of each environmental

predictor were estimated based on matrix permutation. Specifically, the environmental data were permuted 50 times and for each permuted matrix a new model was fitted. The model significance was estimated by comparing the global deviance of the GDM fit to un-permuted data with that of permuted data. Then the same process was repeated for each predictor separately to assess the variable significance and importance. The GDMs were fitted with the package “gdm” in R environment (Fitzpatrick et al., 2020). Finally, we conducted a permutational multivariate analysis of variance (PERMANOVA) to assess whether functional dissimilarity

TABLE 3 Overview of the aquatic macrophyte traits used in the present study.

Trait Code	Trait Name	Category	Values
EIV N	Ellenberg N—nutrients preference	Ecological preference	1: low nutrients, 5= intermediate levels of nutrients, 9= rich conditions of nutrients
EIV L	Ellenberg L—light preference	Ecological preference	1 = deep shade, 5 = semi shade, 9 = full light
GF	Growth form	Life form	AEL: anchored with emergent leaves, AFL: anchored with floating leaves, ASUB: anchored submerged plants, FFSUR: free floating on surface, FFSUB: free floating submerged
LS	Leaf size	Morphology	SMALL: $\leq 1\text{cm}^2$, MODERATE: $1\text{--}20\text{ cm}^2$, LARGE: $\geq 20\text{ cm}^2$
FS	Fruit size	Morphology	SMALL: $\leq 1\text{cm}^2$, MODERATE: $1\text{--}20\text{ cm}^2$, LARGE: $\geq 20\text{ cm}^2$
LT	Leaf type	Morphology	ENT: entire, CAP: capillary, TUB: tubular

differs among levels of hydromorphological factors. PERMANOVA was run with the `adonis2` function of the “vegan” package in R environment.

Results

Patterns of trait composition

The hierarchical cluster analysis based on the functional distance matrix revealed five distinct groups of plants (Figure 2). The first group (I) consists of nine species of macrophytes, four of which are rooted floating leaved, another four are rooted submerged and one (*Sparganium emersum*) can be found with either emergent leaves or floating on the surface. The second (II) functional group of macrophytes consists of four species that are exclusively free-floating and *Trapa natans* which is a floating-leaved plant usually anchored at the sediment. The third group includes emergent macrophytes growing their stems and leaves above the water surface (e.g. *Alisma plantago-*

aquatica, *Mentha aquatica*, *Veronica anagalis-aquatica*). The two remaining groups (IV, V) include submerged species that seem to vary because of their leaf type. Group IV consists of plants that are fine-leaved (e.g. *Myriophyllum spicatum*, *Ranunculus trichophyllus*, *Ceratophyllum demersum*), whereas Group V is characterized by a mix of aquatic macrophytes that can have submerged leaves (fine-leaves or entire leaves), floating leaves or both.

The first two principal components of the PCA performed on trait data accounted for more than 60% of the variance among species characteristics (Figure 3). The first component (PC1) explained 53% of the total variance of the data, while the PC2 contributed with another 15%. Based on the boxplots of Figure 4, the first PC is mostly related with the traits Ellenberg light (EIV L), growth form (GF), leaf type (LT) and leaf size (LS), whereas the second PC is correlated significantly with Ellenberg light (EIV L), growth form (GF) and fruit size (FS), (Figure 4). Hence, the position of aquatic plants and functional groups along the two axes can indicate a strong affinity with specific functional traits and reflect specific ecological preferences. Plants that are more shade tolerant

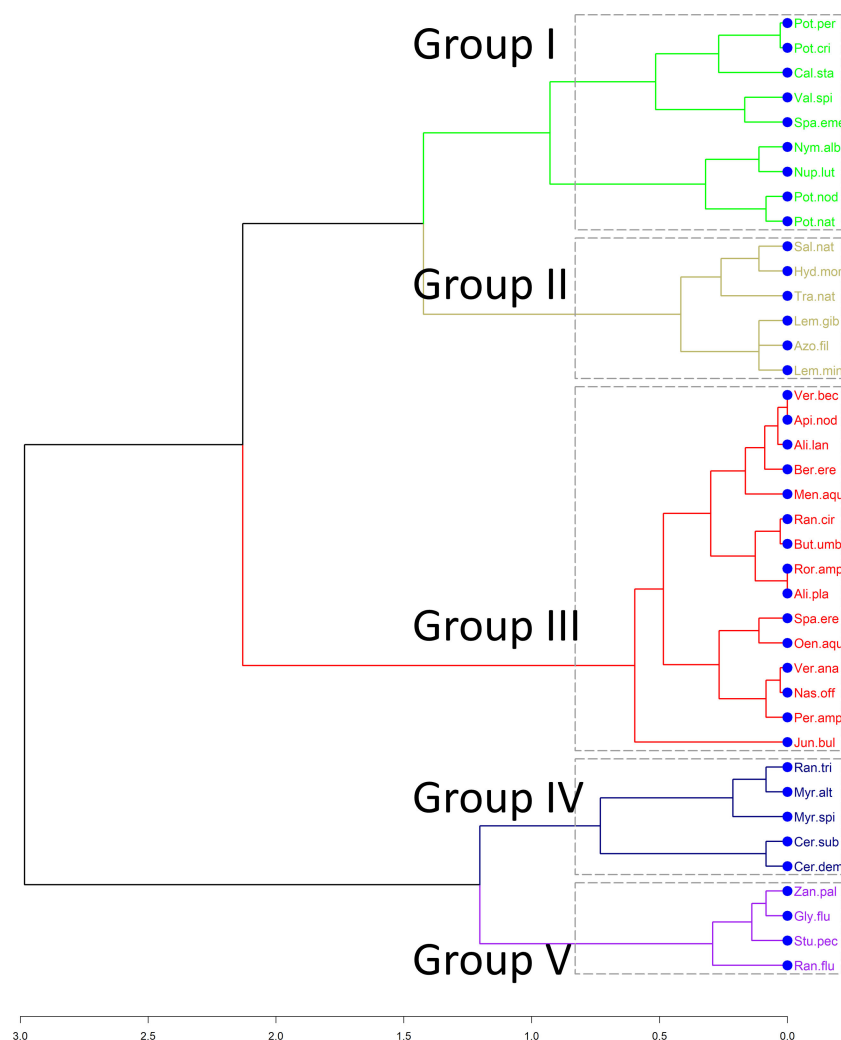


FIGURE 2

Dendrogram obtained from hierarchical clustering based on the species distances. Colors correspond to the five functional groups (I, II, III, IV, V) derived after the calculation of the Kelley-Gardner-Sutcliffe penalty.

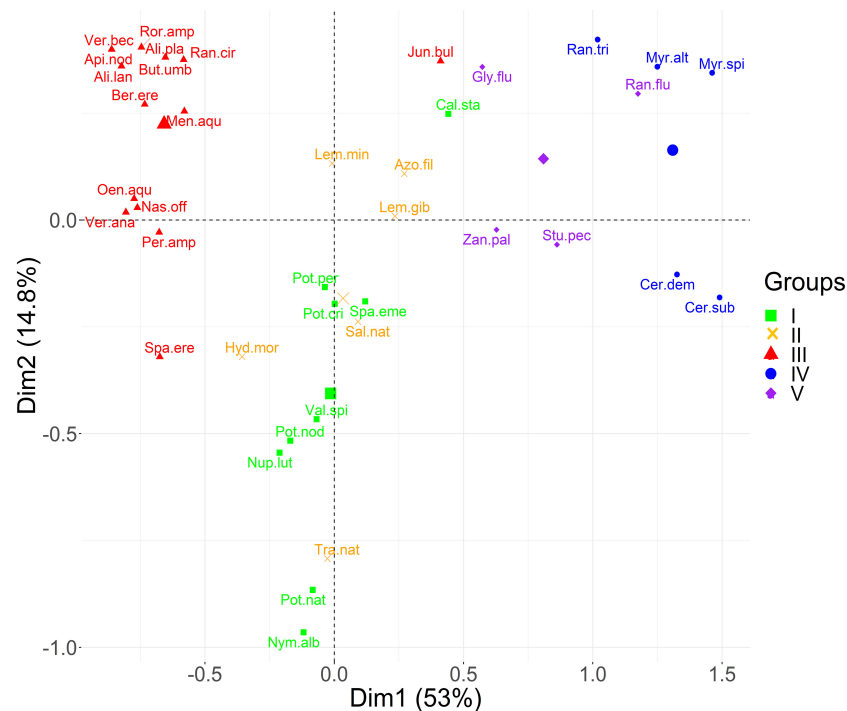


FIGURE 3

Principal component analysis plot of the species scores for the first two components. Colors correspond to the five functional groups (I, II, III, IV, V) derived after the calculation of the Kelley-Gardner-Sutcliffe penalty.

(lower values of EL) are positioned across the right part of the PCA plot (Groups IV and V) whereas plants that prefer good light conditions are clustered across the left part of the PC1 (mostly Group III), **Figure 3**. Macrophytes from Groups IV and V are also characterized by small and fine leaves opposed to Group III which consists of plants that have larger leaves and can be either emergent or floating-leaved. The position of the macrophytes across PC2 appears to relate mostly with the fruit size and the floating-leaved growth form, since macrophytes with floating leaves and larger fruits are positioned at the top part of the plot (**Figures 3, 4**).

Alpha (α -) and beta (β -) functional diversity patterns

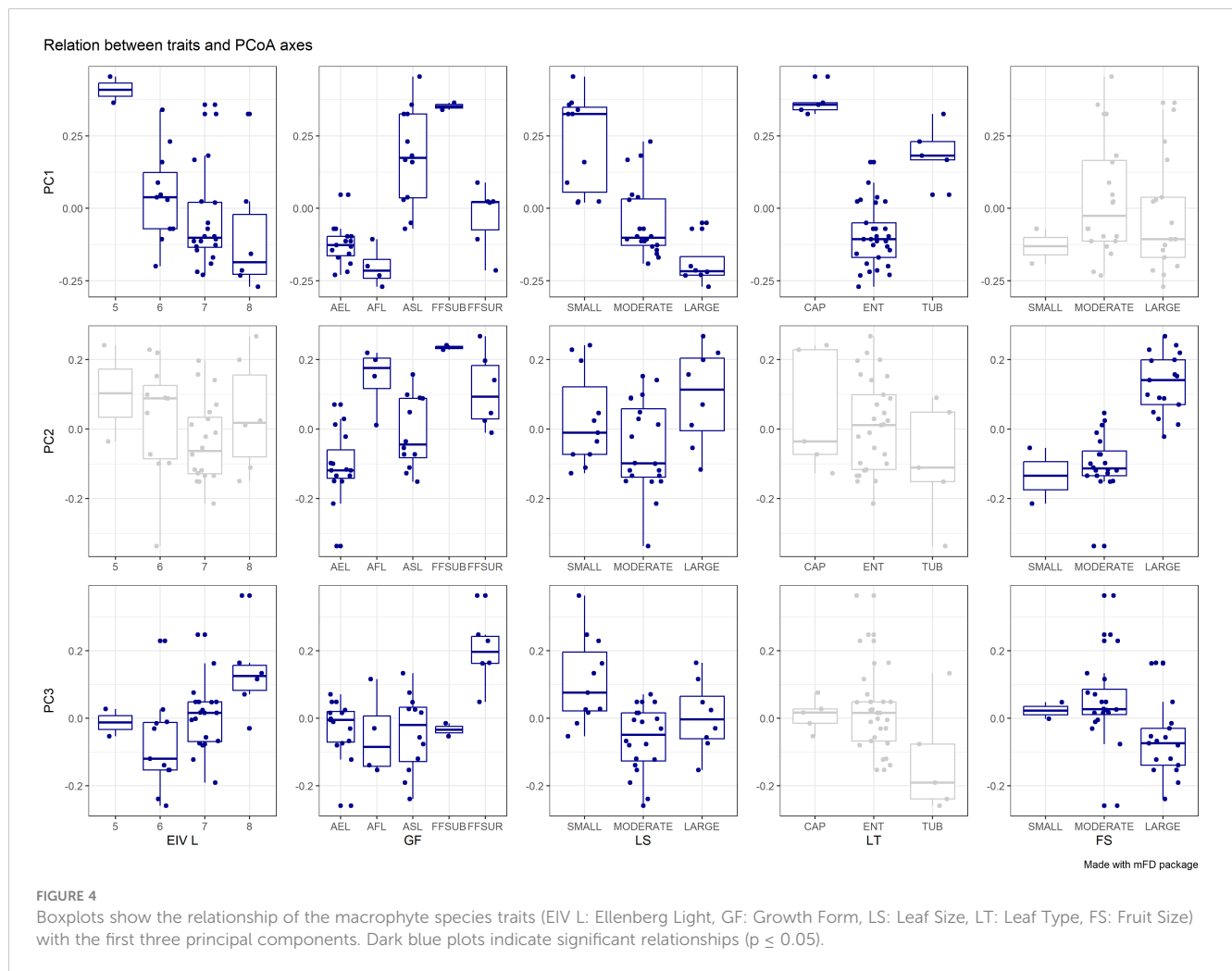
Species richness was relatively low, ranging by a minimum of 4 to a maximum of 15 species per site. Alpha functional diversity was described by three indices: functional richness, dispersion and evenness. The average value of functional richness for all sites was 0.11, with a maximum of 0.39. Functional dispersion and evenness were higher ranging from 0.23 to 0.6 and 0.38 to 0.71 respectively. Additionally, we found that functional diversity was positively correlated ($r=0.72$) to taxonomic diversity (**Figure 5**). However, we have to note here that this result was influenced by a community found in a relatively pristine site, which showed remarkably higher species and functional richness than most communities. Excluding this community from our dataset yields a correlation coefficient $r=0.59$ (significant at p -value ≤ 0.001), which is still relatively high, but considerably lower than 0.72.

Taxonomic β diversity ranged from 0.08 to 1 with a mean value of $0.72 (\pm 0.19)$. The turnover (β_{sim} , 0.65 ± 0.23) was higher than the nestedness-resultant component (β_{sne} , 0.07). Functional dissimilarity based on Sørensen among the aquatic plant communities was high (0.85 ± 0.20), with the functional turnover (i.e. the communities host different functional strategies) accounting for 62% (0.62 ± 0.34) and dissimilarity due to difference in functional richness (nestedness) accounting for 23% of the total variation.

Functional β diversity was significantly correlated with taxonomic β diversity (Mantel test, $r = 0.48$, $p < 0.001$), with functional turnover and taxonomic turnover being also strongly correlated ($r = 0.54$, $p < 0.001$). Nestedness-resultant components of taxonomic and functional β diversity were also correlated ($r = 0.48$, $P < 0.001$).

Relationships between functional diversity indices and environmental descriptors

We tested for relationships between α functional diversity indices and water quality variables with the use of GAMs (**Figure 6**). The results of the best model fits showed that a relatively small share of variance of functional dispersion and functional evenness (25.6 and 25.3%) was explained by environmental variables. For functional richness the percentage of variance explained by the environment was even lower (approximately 11%). The best model for functional dispersion retained four predictors, with altitude, pH and oxygen saturation being significant (**Table 4**). For functional evenness the



model retained five predictors with pH and oxygen saturation being significant (Table 4). The best model for functional richness, which had the lowest R^2 among the three functional indicators, retained two variables (altitude and BOD) with neither being statistically significant.

The results of the Kruskal-Wallis tests indicated significant variations of α functional diversity indices (Table 5) among the channel bed stability (stable, solid, soft, unstable) and habitat type (pool, rifle, run, slack) (Figure 7). In addition, functional evenness and functional dispersion showed statistically significant differences among the channel substrate and the degree of hydrological alteration respectively. Concerning the species richness, we found less significant differences ($p \leq 0.1$) among the habitat type, the adjacent land uses and the effect of water abstraction (Table 5).

The results of the generalized dissimilarity modelling showed that geographical distance was the sole significant predictor for the Sørensen functional dissimilarity, although the model explained just the 8.5% of the total deviance. The models for the components turnover and nestedness explained even lower shares of total deviance (3.9 and 2.1) with none of the environmental variables being significant. We found similar results for the taxonomic dissimilarity with geographic distance being a significant predictor for Sørensen dissimilarity and the turnover component, but both models explained 6.9 and 7.4 of the total deviance

respectively. The GDM for the taxonomic nestedness explained 3.2% of the total deviance, with none of the environmental variables being significant.

PERMANOVA showed significant variations of the Sørensen functional dissimilarity and the turnover component among most hydromorphological factors ($p \leq 0.1$), but with low R^2 ranging between 0.05 and 0.15 (Table 6).

Discussion

The study of plant ecomorphological traits attempts to link morphological characteristics (e.g. habitus size, shape and morphology) with species functions. In this article, we assessed functional diversity patterns and changes of functional composition of aquatic macrophytic communities of riverine ecosystems in Greece. We identified notable variations of the functional composition in terms of key trait characteristics, such as life growth form and preference to light conditions. We also managed to distinguish a few significant relationships between functional diversity (alpha and functional dissimilarity) and environmental variables.

Our results indicated that growth form and light preference are key trait characteristics that grouped the aquatic macrophytes into

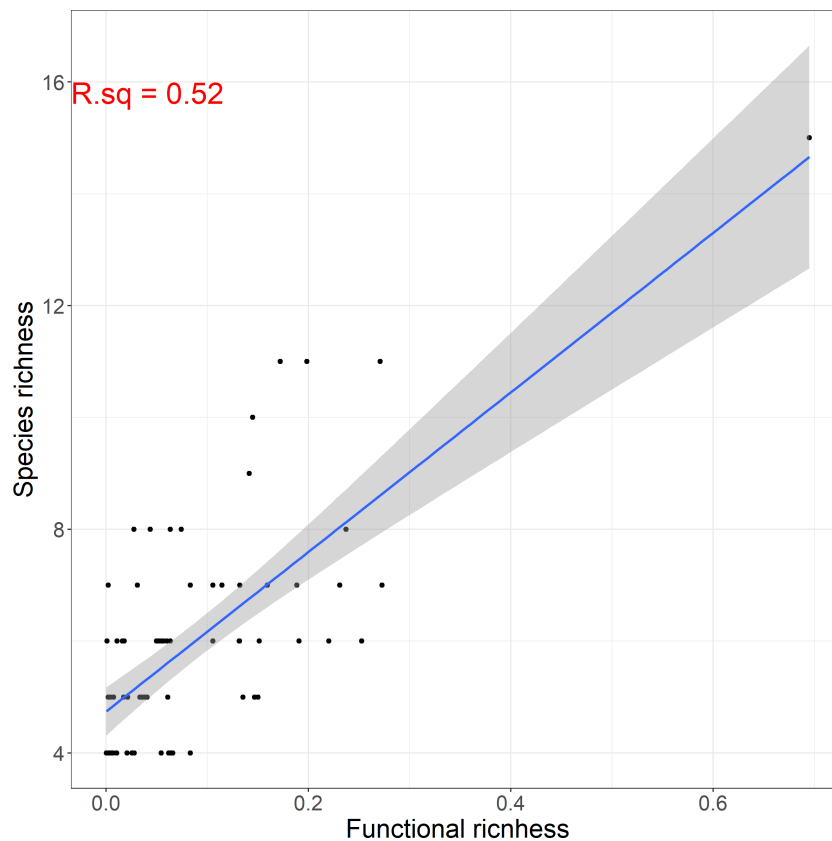


FIGURE 5

Linear relationship between functional and taxonomic macrophyte species richness among sites.

five discrete groups based on their functional trait composition. Macrophytes were distinguished along a gradient that reveals light availability as a key driver that shapes macrophytic communities in the investigated river reaches. The PC1 has a high affinity with rooted submerged macrophytes and macrophytes with high EIV L value (which indicates preference to higher light intensity) and thus plants that were positioned at the left part of the PCA plot are those that could require waters with high clarity and undisturbed conditions (e.g. oxygenated waters and less turbidity). Previous studies have highlighted the role of light availability as an important environmental filter that restricts certain functional traits of aquatic macrophytes (Fu et al., 2014b; Su et al., 2019) and promoting others that can help plants to persist to the environmental conditions. However, when we examined the relationship between functional diversity indices and environmental variables, including water quality features that are related with light availability (e.g. turbidity and nutrient concentrations), we did not find evidence that could explain a possible effect of water clarity on functional diversity. Specifically, we did not find any significant effect of water quality parameters on functional richness, but only a significant effect of pH and oxygen saturation on both functional dispersion and evenness. Similarly, we did not find any significant effect of water quality variables on the taxonomic richness. Previous studies in Greek rivers have noted relatively moderate and high levels of nutrients in several sites, indicating signs of eutrophication (Stefanidis et al., 2021). Narrow gradients of nutrient concentrations and high occurrence of

macrophytes that can be found in a wide range of trophic conditions (e.g. *Myriophyllum spicatum*, *Potamogeton nodosus*, *Stuckenia pectinata*) may explain the difficulty in finding specific patterns of responses along water quality gradients. These results are similar with those published by Zelnik et al. (2021), who also found *Myriophyllum spicatum* as the most common aquatic macrophyte species in watercourses in Slovenia, despite relatively different environmental conditions from those in Greece. Among the submerged macrophytes or macrophytes with floating leaves, the most common species were: *Myriophyllum spicatum*, *Potamogeton nodosus*, *Potamogeton perfoliatus*, *Elodea canadensis*, *Potamogeton crispus*, *Stuckenia pectinata*. This is another evidence for wide ecological amplitudes of these species, which make the interpretations more difficult. Furthermore, excessive nutrients are related with eutrophication processes that reduce light availability and may have substantial effects on taxonomic and functional diversity in standing waters (Stefanidis and Papastergiadou, 2019; Lindholm et al., 2020). However, water transparency in rivers can be affected by geohydromorphological factors such as erosion, sediment load, geology, land uses and rainfall intensity (Chalov and Prokopenva, 2022; Lu et al., 2023) that are not necessarily related with water chemistry (e.g. nutrients). Our findings also showed a significant differentiation of the functional diversity indices among the types of bed stability (stable, solid, soft and unstable) and the types of habitat (pool, rifle, run and slack), which suggests that hydromorphological conditions (e.g. substrate rigidity, depth and flow type) are

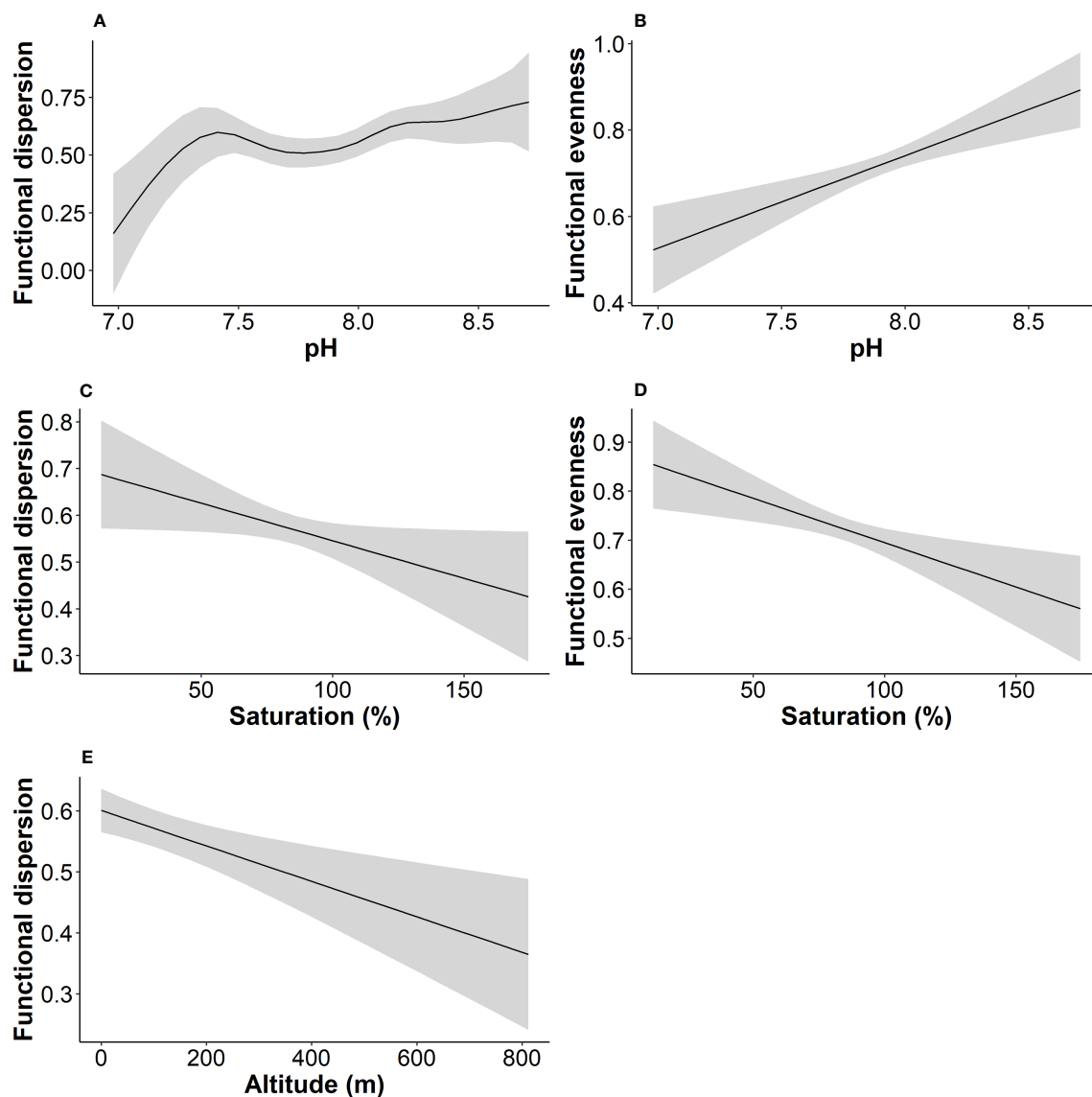


FIGURE 6

Fitted functions of functional dispersion (A, C, E), and functional evenness (B, D) to partial effects of statistically significant environmental variables. Line represents GAM smoothing curve while the grey area depicts the standard error.

important drivers of certain plant traits. Functional richness, dispersion and evenness were higher at slack and run habitats, which are characterized by deeper waters and slow or fast water flow respectively. All three indices were also higher at soft substrates (mostly sand, silt and mud) than more stable substrates (e.g. bedrock, gravel, cobbles and boulders). Similarly, functional evenness was higher at fine substrates ($p=0.002$) which confirms a possible association of increased functional diversity with soft and fine river substrates. Previous studies have shown that geomorphological features such as river bottom type, substrate structure and riverbank stability are important factors for explaining macrophyte composition in lotic ecosystems (Pernel et al., 2022). Rooted aquatic plants in particular, prefer fine sediments (Willby et al., 2000; Hrivnák et al., 2010) while bryophytes usually occur at coarser substrates, such as boulders and cobbles. Thus, life forms are differentiated among various types of substrates. Moreover,

macrophytes are considered to be ecological engineers because macrophyte assemblages have a positive impact on fine sediment accumulation on the river bottom, modifying the channel bed and facilitating plant colonization (Jones et al., 2012). We did not find any other significant differentiation of α diversity indices among the remainder hydromorphological factors, except for a significant effect of hydrological alteration and water abstraction on functional dispersion and evenness respectively. Although aquatic plants are known to respond to hydromorphological changes (Szoszkiewicz et al., 2014; Baattrup-Pedersen et al., 2016; Turunen et al., 2016; Birk et al., 2020; Gyosheva et al., 2020), only a few studies have highlighted the role of hydromorphology as an important driver of aquatic macrophytic diversity, including functional diversity (Manolaki et al., 2020; Stefanidis et al., 2021; Vukov et al., 2022). Besides substrate, hydrology plays a major role in promoting species with traits that enable them to persist droughts and low flow

TABLE 4 Summary of GAMs fitted to the functional richness, functional dispersion, functional evenness and species richness.

Response variable	Adj.R ²	% Deviance explained	Retained explanatory variables	Significance (p value)
Functional richness	0.11	14.9	BOD	0.075
			Altitude	0.062
Functional dispersion	0.26	34.1	Altitude	0.001
			pH	0.020
			Ammonium	0.060
			Oxygen saturation	0.042
Functional evenness	0.25	31.8	Turbidity	0.076
			Oxygen saturation	0.004
			pH	<0.001
			BOD	0.198
			TDS	0.275
Species richness	0.08	11.5	Ammonium	0.054
			TDS	0.069

The retained explanatory variables for each model along with p-values are shown. Adjusted R² and percentage of deviance explained are also shown.

conditions in Mediterranean rivers (Manolaki et al., 2020). In our case, we used nominal and ordinal hydromorphological variables that limited our capability to fully explore how functional diversity changes across hydromorphological gradients (e.g. hydrological alteration). Still, we were able to capture the effect of different substrates and river habitats and extract useful conclusions on the conditions that favor increased functional diversity.

Another issue that might explain why we did not find significant relationships between certain environmental parameters and diversity indices, is the intraspecific trait variability that many plants exhibit, including aquatic plants (Fu et al., 2014b). Some plants may show high phenotypic plasticity that provides them with various adaptations to large environmental changes (Fu et al., 2018;

Lindholm et al., 2020). For instance, a submerged macrophyte in China, *Potamogeton maackianus*, can form large monospecific beds across wide environmental gradients (Fu et al., 2018). Similar macrophyte assemblages with a few species that occur across wide ranges of nutrients and physico-chemical gradients are common in Greece (Stefanidis et al., 2019), such as *Myriophyllum spicatum* and *Potamogeton nodosus*. Other plants can show adaptations to water level fluctuations showing various growth forms (e.g. emergent, rooted with floating-leaved plants, or rooted submerged) that can help them offset environmental limitations such as limited light availability. It is likely that the inclusion of intraspecific trait variability in trait-based studies could further elucidate the functional responses to environment gradients.

TABLE 5 Results of Kruskal-Wallis tests showing significant differences (values in bold) of the functional diversity indices among the levels of hydromorphological factors.

	Functional richness	Functional dispersion	Functional evenness	Species richness
Channel substrate	NS	NS	P=0.002	NS
Bed stability	P=0.037	P=0.034	P=0.006	NS
Shade	NS	NS	NS	NS
Habitats	P ≤ 0.001	P ≤ 0.001	P=0.078	P=0.053
Land Uses	NS	NS	NS	P=0.067
Channel profile alteration	NS	NS	NS	NS
Morphology alteration	NS	NS	NS	NS
Habitat alteration	NS	NS	NS	NS
Stream hydrology alteration	NS	P=0.019	NS	NS
Water abstraction	NS	NS	NS	P=0.037
Dykes (flood protection)	NS	NS	NS	NS

NS, non-significant.

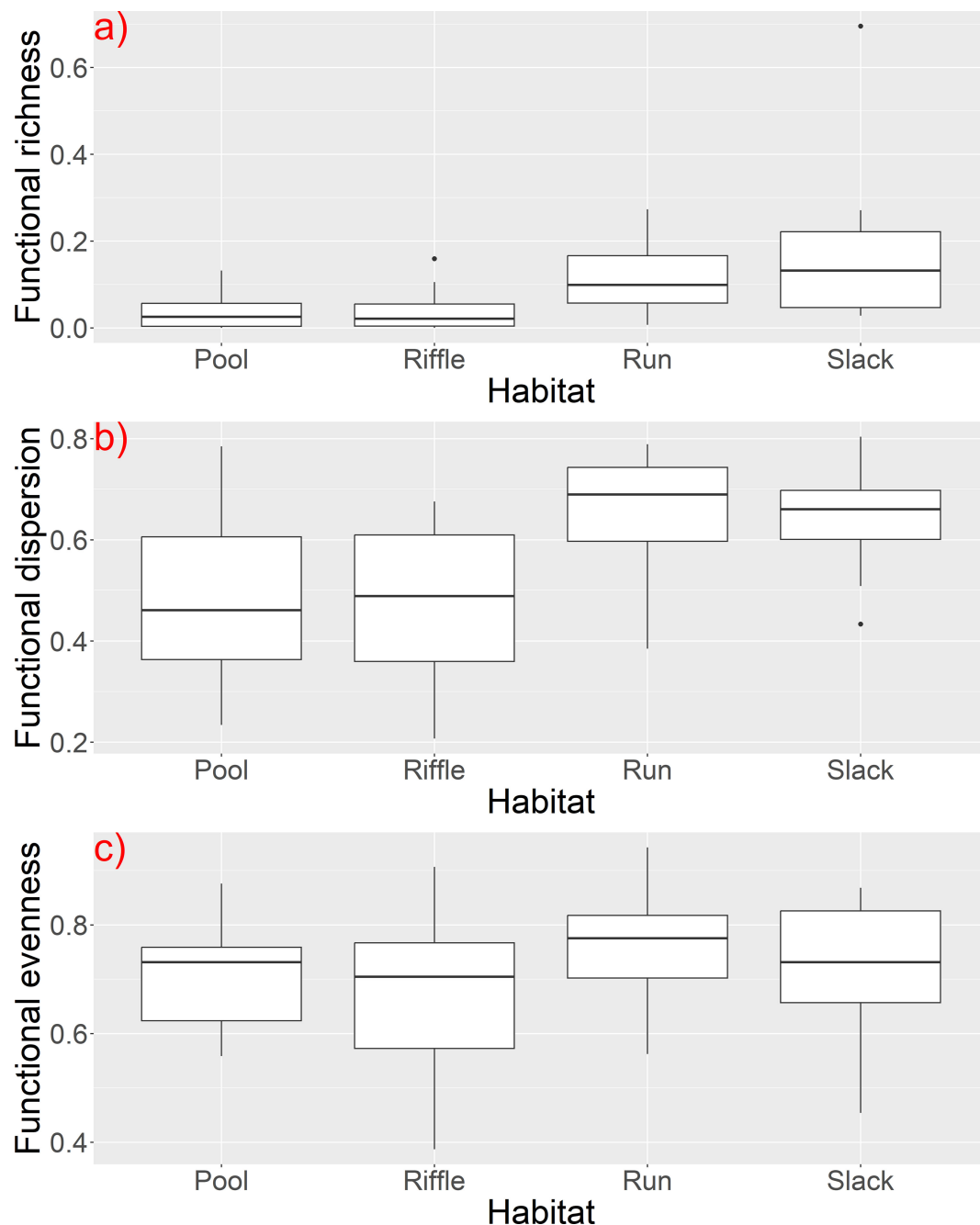


FIGURE 7

Boxplots of (A) functional richness, (B) dispersion and (C) evenness among river habitat types.

An additional finding of the current research concerns the relationship between taxonomic and functional diversity. The relationship between taxonomic and functional richness has been previously used to investigate the functional redundancy of communities (Ricotta et al., 2016; da Silva Camilo et al., 2018). In this study, we found a positive significant relationship between functional and taxonomic richness that indicates low functional redundancy. This means that the loss of a few species from the macrophyte communities is more likely to lead to loss of certain functions that are strictly related with those species. This finding is

particularly important for conservation scientists and environmental managers because it emphasizes the need to include the monitoring of functional diversity (besides taxonomic diversity) in order to better assess the impact of environmental changes (Ricotta et al., 2016; Biggs et al., 2020).

Separating turnover and nestedness-resultant contributions to the overall β -diversity could provide further insights into mechanisms shaping community composition with respect to β -diversity. We found that the taxonomic β -diversity was mainly governed by turnover, i.e., replacement of disappearing species by

TABLE 6 Results of PERMANOVA showing significant variations of the functional dissimilarity matrices among the levels of hydromorphological factors.

	Total Sørensen dissimilarity	Turnover	Nestedness
Channel substrate	P=0.031, R.sq=0.04	P=0.021, R.sq=0.07	NS
Bed stability	P=0.002, R.sq=0.07	P=0.002, R.sq=0.12	NS
Shade	P=0.003, R.sq=0.07	P=0.003, R.sq=0.09	NS
Habitats	P=0.001, R.sq=0.11	P=0.001, R.sq=0.15	NS
Land Uses	NS, R.sq=0.05	P=0.099, R.sq=0.06	NS
Channel profile alteration	NS, R.sq=0.05	NS, R.sq=0.05	NS
Morphology alteration	P=0.011, R.sq=0.06	P=0.008, R.sq=0.10	NS
Habitat alteration	P=0.001, R.sq=0.08	P=0.001, R.sq=0.14	NS
Stream hydrology alteration	P=0.001, R.sq=0.08	P=0.001, R.sq=0.13	NS
Water abstraction	P=0.002, R.sq=0.08	P=0.001, R.sq=0.14	NS
Dykes (flood protection)	P=0.046, R.sq=0.05	P=0.028, R.sq=0.08	NS

R squared values are also shown. NS, non-significant.

new emerging species along the gradient, which is a typical finding in freshwater studies (Perez Rocha et al., 2019). Studies that explore the relationship between taxonomic and functional beta diversity in freshwaters are not common, and their results are rather ambiguous (Perez Rocha et al., 2019; Teittinen and Virta, 2021). In the current research, we found that functional β -diversity was higher than taxonomic β -diversity. The high functional dissimilarity may partly stem from low number of taxonomic species versus considerably higher number of functional traits or functions. A discrepancy was also revealed in the decomposition of functional β -diversity, where the higher levels of functional β -diversity were mainly due to a higher nestedness-resultant component compared to taxonomic β -diversity decomposition, where the taxonomic and functional β -diversity turnover were at similar levels. Thus, replaced species in functionally poor assemblages held traits already included in the functional space of functionally rich ones, resulting in increased functional nestedness. The results of the GDM did not show strong indications of environmental effects on the taxonomic and functional dissimilarity rate and thus our study does not provide support for the role of environmental filtering as a driver of neither functional nor community dissimilarities. The PERMANOVA results showed significant variation of the overall β -diversity and the turnover among the majority of hydromorphological factors but the R^2 values were quite low indicating a large share of unexplained variation. It is likely that regional spatial processes (dispersal limitation with increasing geographic distance) are more important factors than local environmental descriptors for aquatic

macrophyte taxonomic and functional composition changes among sites (Oikonomou and Stefanidis, 2020; Stefanidis et al., 2021).

Conclusions

This article provides new information filling the gap of knowledge of the functional responses of aquatic macrophytic assemblages to environmental gradients in an extended network of running waters from mainland Greece. With this article we quantified the α - and β -functional diversity of aquatic macrophytic communities of river reaches and we attempted to look for significant responses to environmental parameters related with water quality gradients and hydromorphological factors. We found that the trait characteristics that contributed most to explaining the total variance of the functional space were the macrophyte growth form and the preference to light conditions which indicates that light availability plays a major role in filtering traits of aquatic plants. We did not find any clear indication of strong relationships between functional diversity and water quality gradients. We found significant variations of alpha and beta functional diversity among hydromorphological factors - mainly substrate and river habitat - which suggested that lotic systems with fine substrates and deep waters (run and slack habitats) promoted functional diversity. We consider likely that further studies to explore the effects of additional hydromorphological gradients could reveal significant responses of functional plant communities. An important finding was the positive relationship between species richness and functional

richness which implies that the loss of taxonomic richness could lead to a loss of functions. Overall, our study provides useful insights and recommendations concerning the study of functional diversity of aquatic plant assemblages within the frame of freshwater monitoring and conservation.

Data availability statement

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

Author contributions

KS, AO and EP contributed to the conception and design of the study. EP, GD, MS, DT and KS contributed to data compilation. KS and AO analysed the data and wrote the manuscript. KS, AO and EP authors contributed to the final version of the manuscript.

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References

- Aguiar, F. C., Segurado, P., Urbanič, G., Cambra, J., Chauvin, C., Ciadamidaro, S., et al. (2014). Comparability of river quality assessment using macrophytes: A multi-step procedure to overcome biogeographical differences. *Sci. Total Environ.* 476–477, 757–767. doi: 10.1016/j.scitotenv.2013.10.021
- Alahuhta, J., Toivanen, M., Hjort, J., Ecke, F., Johnson, L. B., Sass, L., et al. (2017). Species richness and taxonomic distinctness of lake macrophytes along environmental gradients in two continents. *Freshw. Biol.* 62, 1194–1206. doi: 10.1111/fwb.12936
- Baatrup-Pedersen, A., Göthe, E., Riis, T., and O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci. Total Environ.* 543, 230–238. doi: 10.1016/j.scitotenv.2015.11.027
- Barton, K. (2020). *Package “MuMIn”: Multi-Model Inference*.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecol. Biogeogr.* 19, 134–143. doi: 10.1111/j.1466-8238.2009.00490.x
- Baselga, A., and Orme, C. D. L. (2012). Betapart: An R package for the study of beta diversity. *Methods Ecol. Evol.* 3, 808–812. doi: 10.1111/j.2041-210X.2012.00224.x
- Bellwood, D. R., Wainwright, P. C., Fulton, C. J., and Hoey, A. (2002). Assembly rules and functional groups at global biogeographical scales. *Funct. Ecol.* 16, 557–562. doi: 10.1046/j.1365-2435.2002.00655.x
- Biggs, C. R., Yeager, L. A., Bolser, D. G., Bonsell, C., Dichiera, A. M., Hou, Z., et al. (2020). Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. *Ecosphere* 11, e03184. doi: 10.1002/ecs2.3184
- Birk, S., Chapman, D., Carvalho, L., Spears, B. M., Andersen, H. E., Argillier, C., et al. (2020). Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* 4, 1060–1068. doi: 10.1038/s41559-020-1216-4
- Birk, S., and Willby, N. (2010). Towards harmonization of ecological quality classification: Establishing common grounds in European macrophyte assessment for rivers. *Hydrobiologia* 652, 149–163. doi: 10.1007/s10750-010-0327-3
- Bornette, G., and Puijalon, S. (2011). Response of aquatic plants to abiotic factors: A review. *Aquat. Sci.* 73, 1–14. doi: 10.1007/s00027-010-0162-7
- CEN. (2003). *EN 14184:2003 - Water quality - Guidance standard for the surveying of aquatic macrophytes in running waters* (European Committee for Standardization (CEN)).
- CEN. (2006). *EN 14996:2006 - Water quality - Guidance on assuring the quality of biological and ecological assessments in the aquatic environment*. Available at: <https://standards.iteh.ai/catalog/standards/cen/804ddce8-966c-4ee9-99ea-b18798e71526/en-14996-2006> (Accessed October 6, 2020).
- Chalov, S., and Prokopenko, K. (2022). Sedimentation and erosion patterns of the Lena river anabranching channel. *Water (Switzerland)* 14, 3845. doi: 10.3390/w14233845
- Cole, L. J., Stockan, J., and Helliwell, R. (2020). Managing riparian buffer strips to optimise ecosystem services: A review. *Agric. Ecosyst. Environ.* 296, 106891. doi: 10.1016/j.agee.2020.106891
- da Silva Camilo, G., de Freitas Terra, B., and Araújo, F. G. (2018). Using the relationship between taxonomic and functional diversity to assess functional redundancy in streams of an altered tropical watershed. *Environ. Biol. Fishes* 101, 1395–1405. doi: 10.1007/s10641-018-0786-3
- Degen, R., Aune, M., Bluhm, B. A., Cassidy, C., Kędra, M., Kraan, C., et al. (2018). Trait-based approaches in rapidly changing ecosystems: A roadmap to the future polar oceans. *Ecol. Indic.* 91, 722–736. doi: 10.1016/j.ecolind.2018.04.050
- Díaz, S., Lavorel, S., De Bello, F., Quétier, F., Grigulis, K., and Robson, T. M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20684–20689. doi: 10.1073/PNAS.0704716104/SUPPL_FILE/DIAZ_FINAL.MP3
- Dolédéc, S., Phillips, N., Scarsbrook, M., Riley, R. H., and Townsend, C. R. (2006). Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. *J. North Am. Benthol. Soc.* 25, 44–60. doi: 10.1899/0887-3593(2006)25[44:COFAFA]2.0.CO;2

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- Dybkaer, J. B., Baattrup-Pedersen, A., Kronvang, B., and Thodsen, H. (2012). Diversity and distribution of riparian plant communities in relation to stream size and eutrophication. *J. Environ. Qual.* 41, 348–354. doi: 10.2134/jeq2010.0422
- Elo, M., Alahuhta, J., Kanninen, A., Meissner, K. K., Seppälä, K., and Mönkkönen, M. (2018). Environmental characteristics and anthropogenic impact jointly modify aquatic macrophyte species diversity. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01001
- Fitzpatrick, M., Mokany, K., Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., et al. (2020). Package “*gdm*”: Generalized Dissimilarity Modeling.
- Fitzpatrick, M. C., Sanders, N. J., Normand, S., Svenning, J. C., Ferrier, S., Gove, A. D., et al. (2013). Environmental and historical imprints on beta diversity: Insights from variation in rates of species turnover along gradients. *Proc. R. Soc. B: Biol. Sci.* 280, 20131201. doi: 10.1098/rspb.2013.1201
- Fu, H., Yuan, G., Lou, Q., Dai, T., Xu, J., Cao, T., et al. (2018). Functional traits mediated cascading effects of water depth and light availability on temporal stability of a macrophyte species. *Ecol. Indic.* 89, 168–174. doi: 10.1016/j.ecolind.2018.02.010
- Fu, H., Zhong, J., Yuan, G., Ni, L., Xie, P., and Cao, T. (2014a). Functional traits composition predict macrophytes community productivity along a water depth gradient in a freshwater lake. *Ecol. Evol.* 4, 1516–1523. doi: 10.1002/ece3.1022
- Fu, H., Zhong, J., Yuan, G., Xie, P., Guo, L., Zhang, X., et al. (2014b). Trait-based community assembly of aquatic macrophytes along a water depth gradient in a freshwater lake. *Freshw. Biol.* 59, 2462–2471. doi: 10.1111/fwb.12443
- Guisan, A., Edward, T. C., and Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecol. Modell.* 157, 89–100. doi: 10.1016/S0304-3800(02)00204-1
- Gurnell, A. (2015). Plants as river system engineers: Further comments. *Earth Surf. Process. Landf.* 40, 135–137. doi: 10.1002/esp.3671
- Gyosheva, B., Kalchev, R., Beshkova, M., and Valchev, V. (2020). Relationships between macrophyte species, their life forms and environmental factors in floodplain water bodies from the Bulgarian Danube River Basin. *Ecophysiol. Hydrobiol.* 20, 123–133. doi: 10.1016/j.ecophys.2019.06.003
- Hrivnák, R., Otáhel'ová, H., Valachovič, M., Paľove-Balang, P., and Kubinská, A. (2010). Effect of environmental variables on the aquatic macrophyte composition pattern in streams: A case study from Slovakia. *Fundam. Appl. Limnol.* 177, 115–124. doi: 10.1127/1863-9135/2010/0177-0115
- Jones, J. I., Collins, A. L., Naden, P. S., and Sear, D. A. (2012). The relationship between fine sediment and macrophytes in rivers. *River Res. Appl.* 28, 1006–1018. doi: 10.1002/rra.1486
- Kelley, L. A., Gardner, S. P., and Sutcliffe, M. J. (1996). An automated approach for clustering an ensemble of NMR-derived protein structures into conformationally related subfamilies. *Protein Eng. Des. Sel.* 9, 1063–1065. doi: 10.1093/protein/9.11.1063
- Leathwick, J. R., Elith, J., and Hastie, T. (2006). Comparative performance of generalized additive models and multivariate adaptive regression splines for statistical modelling of species distributions. *Ecol. Modell.* 199, 188–196. doi: 10.1016/j.ecolmodel.2006.05.022
- Lind, L., Hasselquist, E. M., and Laudon, H. (2019). Towards ecologically functional riparian zones: A meta-analysis to develop guidelines for protecting ecosystem functions and biodiversity in agricultural landscapes. *J. Environ. Manage.* 249, 109391. doi: 10.1016/j.jenvman.2019.109391
- Lindholm, M., Alahuhta, J., Heino, J., Hjort, J., and Toivonen, H. (2020). Changes in the functional features of macrophyte communities and driving factors across a 70-year period. *Hydrobiologia* 847 (18), 3811–3827. doi: 10.1007/s10750-019-04165-1
- Lu, Y., Chen, J., Xu, Q., Han, Z., Peart, M., Ng, C. N., et al. (2023). Spatiotemporal variations of river water turbidity in responding to rainstorm-streamflow processes and farming activities in a mountainous catchment, Lai Chi Wo, Hong Kong, China. *Sci. Total Environ.* 863, 160759. doi: 10.1016/j.scitotenv.2022.160759
- Magneville, C., Loiseau, N., Albouy, C., Casajus, N., Claverie, T., Escalas, A., et al. (2022). mFD: an R package to compute and illustrate the multiple facets of functional diversity. *Ecography* 2022. doi: 10.1111/ecog.05904
- Maire, E., Grenouillet, G., Brosse, S., and Villéger, S. (2015). How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Global Ecol. Biogeogr.* 24, 728–740. doi: 10.1111/geb.12299
- Manolaki, P., Guo, K., Vieira, C., Papastergiadou, E., and Riis, T. (2020). Hydromorphology as a controlling factor of macrophytes assemblage structure and functional traits in the semi-arid European Mediterranean streams. *Sci. Total Environ.* 703, 134658. doi: 10.1016/j.scitotenv.2019.134658
- Mason, N. W. H., Moullot, D., Lee, W. G., and Wilson, J. B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111, 112–118. doi: 10.1111/j.0030-1299.2005.13886.X
- Mouchet, M. A., Villéger, S., Mason, N. W. H., and Moullot, D. (2010). Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. *Funct. Ecol.* 24, 867–876. doi: 10.1111/j.1365-2435.2010.01695.X
- Moullot, D., Graham, N. A. J., Villéger, S., Mason, N. W. H., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* 28, 167–177. doi: 10.1016/j.tree.2012.10.004
- Naimi, B., Hamm, N. A. S., Groen, T. A., Skidmore, A. K., and Toxopeus, A. G. (2014). Where is positional uncertainty a problem for species distribution modelling? *Ecography* 37, 191–203. doi: 10.1111/j.1600-0587.2013.00205.x
- O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lázár, A. N., et al. (2018). Responses of aquatic plants to eutrophication in rivers: A revised conceptual model. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00451
- Oikonomou, A., and Stefanidis, K. (2020). α - and β -diversity patterns of macrophytes and freshwater fishes are driven by different factors and processes in lakes of the unexplored Southern Balkan biodiversity hotspot. *Water (Basel)* 12, 1984. doi: 10.3390/w12071984
- Olden, J. D., Kennard, M. J., Leprieux, F., Tedesco, P. A., Winemiller, K. O., and García-Berthou, E. (2010). Conservation biogeography of freshwater fishes: recent progress and future challenges. *Divers. Distrib.* 16, 496–513. doi: 10.1111/j.1472-4642.2010.00655.X
- Papastergiadou, E. (2022). Samplings and analyses of aquatic macrophytes in Greek rivers (in Greek).
- Paradis, E., and Schliep, K. (2019). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. doi: 10.1093/bioinformatics/bty633
- Perez Rocha, M., Bini, L. M., Grönroos, M., Hjort, J., Lindholm, M., Karjalainen, S. M., et al. (2019). Correlates of different facets and components of beta diversity in stream organisms. *Oecologia* 191, 919–929. doi: 10.1007/s00442-019-04535-5
- Peternel, A., Gaberščik, A., Zelnik, I., Holcar, M., and Germ, M. (2022). Long-term changes in macrophyte distribution and abundance in a lowland river. *Plants* 11, 1–14. doi: 10.3390/plants11030401
- Preiner, S., Dai, Y., Pucher, M., Reitsem, R. E., Schoelynck, J., Meire, P., et al. (2020). Effects of macrophytes on ecosystem metabolism and net nutrient uptake in a groundwater fed lowland river. *Sci. Total Environ.* 721, 137620. doi: 10.1016/j.scitotenv.2020.137620
- Ricotta, C., de Bello, F., Moretti, M., Caccianiga, M., Cerabolini, B. E. L., and Pavoine, S. (2016). Measuring the functional redundancy of biological communities: a quantitative guide. *Methods Ecol. Evol.* 7, 1386–1395. doi: 10.1111/2041-210X.12604
- Rodrigues, C., Alves, P., Bio, A., Vieira, C., Guimarães, L., Pinheiro, C., et al. (2019). Assessing the ecological status of small Mediterranean rivers using benthic macroinvertebrates and macrophytes as indicators. *Environ. Monit. Assess.* 191, 1–23. doi: 10.1007/s10661-019-7766-8
- Schneider, B., Cunha, E. R., Marchese, M., and Thomaz, S. M. (2015). Explanatory variables associated with diversity and composition of aquatic macrophytes in a large subtropical river floodplain. *Aquat. Bot.* 121, 67–75. doi: 10.1016/j.aquabot.2014.11.003
- Skoulidakis, N. T., Karaouzas, I., Amaxidis, Y., and Lazaridou, M. (2021). Impact of EU environmental policy implementation on the quality and status of Greek rivers. *Water* 13, 1858. doi: 10.3390/w13131858
- Son, D., Cho, H., and Lee, E. J. (2018). Determining factors for the occurrence and richness of submerged macrophytes in major Korean rivers. *Aquat. Bot.* 150, 82–88. doi: 10.1016/j.aquabot.2018.07.003
- Stefanidis, K., Dimitrellos, G., Sarika, M., Tsoukalas, D., and Papastergiadou, E. (2022). Ecological quality assessment of Greek lowland rivers with aquatic macrophytes in compliance with the EU water framework directive. *Water (Basel)* 14, 2771. doi: 10.3390/w14182771
- Stefanidis, K., Oikonomou, A., and Papastergiadou, E. (2021). Responses of different facets of aquatic plant diversity along environmental gradients in Mediterranean streams: Results from rivers of Greece. *J. Environ. Manage.* 296, 113307. doi: 10.1016/j.jenvman.2021.113307
- Stefanidis, K., and Papastergiadou, E. (2019). Linkages between macrophyte functional traits and water quality: insights from a study in freshwater lakes of Greece. *Water (Basel)* 11, 1047. doi: 10.3390/w11051047
- Stefanidis, K., Sarika, M., and Papastergiadou, E. (2019). Exploring environmental predictors of aquatic macrophytes in water-dependent Natura 2000 sites of high conservation value: Results from a long-term study of macrophytes in Greek lakes. *Aquat. Conserv.* 29, 1133–1148. doi: 10.1002/aqc.3036
- Steffen, K., Leuschner, C., Müller, U., Wiegand, G., and Becker, T. (2014). Relationships between macrophyte vegetation and physical and chemical conditions in northwest German running waters. *Aquat. Bot.* 113, 46–55. doi: 10.1016/j.aquabot.2013.10.006
- Su, H., Chen, J., Wu, Y., Chen, J., Guo, X., Yan, Z., et al. (2019). Morphological traits of submerged macrophytes reveal specific positive feedbacks to water clarity in freshwater ecosystems. *Sci. Total Environ.* 684, 578–586. doi: 10.1016/j.scitotenv.2019.05.267
- Szozkiewicz, K., Ciecierska, H., Kolada, A., Schneider, S. C., Szewabińska, M., and Ruszczyńska, J. (2014). Parameters structuring macrophyte communities in rivers and lakes – results from a case study in North-Central Poland. *Knowl. Manag. Aquat. Ecosyst.* (415), 08. doi: 10.1051/kmae/2014034
- Szozkiewicz, K., Jusik, S., Pietruczuk, K., and Gebler, D. (2020). The macrophyte index for rivers (MIR) as an advantageous approach to running water assessment in local geographical conditions. *Water (Switzerland)* 12, 108. doi: 10.3390/w12010108
- Teittinen, A., and Virta, L. (2021). Exploring multiple aspects of taxonomic and functional diversity in microphytobenthic communities: effects of environmental gradients and temporal changes. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.668993
- Tichý, L., Axmanová, I., Dengler, J., Guarino, R., Jansen, F., Midolo, G., et al. (2023). Ellenberg-type indicator values for European vascular plant species. *J. Vegetation Sci.* 34, e13168. doi: 10.1111/jvs.13168

- Tilman, D. (2001). Functional diversity. *Encyclopedia Biodiver.*, 109–120. doi: 10.1016/B0-12-226865-2/00132-2
- Turunen, J., Muotka, T., Vuori, K. M., Karjalainen, S. M., Rääpysjärvi, J., Sutela, T., et al. (2016). Disentangling the responses of boreal stream assemblages to low stressor levels of diffuse pollution and altered channel morphology. *Sci. Total Environ.* 544, 954–962. doi: 10.1016/j.scitotenv.2015.12.031
- Valkama, E., Usva, K., Saarinen, M., and Uusi-Kämpä, J. (2019). A meta-analysis on nitrogen retention by buffer zones. *J. Environ. Qual.* 48, 270–279. doi: 10.2134/jeq2018.03.0120
- Villéger, S., Grenouillet, G., and Brosse, S. (2013). Decomposing functional β -diversity reveals that low functional β -diversity is driven by low functional turnover in European fish assemblages. *Global Ecol. Biogeogr.* 22, 671–681. doi: 10.1111/GEB.12021
- Vittinghoff, E., Glidden, D., Shiboski, S. C., and McCulloch, C. E. (2012). *Regression Methods in Biostatistics* (Boston, MA: Springer US). doi: 10.1007/978-1-4614-1353-0
- Vukov, D., Ilić, M., Čuk, M., and Igić, R. (2022). The effect of hydro-morphology and habitat alterations on the functional diversity and composition of macrophyte communities in the large river. *Front. Environ. Sci.* 10. doi: 10.3389/fenvs.2022.863508
- Walton, C. R., Zak, D., Audet, J., Petersen, R. J., Lange, J., Oehmke, C., et al. (2020). Wetland buffer zones for nitrogen and phosphorus retention: Impacts of soil type, hydrology and vegetation. *Sci. Total Environ.* 727, 138709. doi: 10.1016/j.scitotenv.2020.138709
- Wang, H., Fu, H., Wen, Z., Yuan, C., Zhang, X., Ni, L., et al. (2021). Seasonal patterns of taxonomic and functional beta diversity in submerged macrophytes at a fine scale. *Ecol. Evol.* 11, 9827–9836. doi: 10.1002/ece3.7811
- Willby, N. J., Abernethy, V. J., and Demars, B. O. L. (2000). Attribute-based classification of European hydrophytes and its relationship to habitat utilization. *Freshw. Biol.* 43, 43–74. doi: 10.1046/J.1365-2427.2000.00523.X
- Wood, S. (2020). Package “mgcv”: Mixed GAM Computation Vehicle with Automatic Smoothness Estimation. doi: 10.1201/9781315370279
- Zelnik, I., Kuhar, U., Holcar, M., Germ, M., and Gaberščik, A. (2021). Distribution of vascular plant communities in Slovenian watercourses. *Water (Switzerland)* 13, 1071. doi: 10.3390/w13081071
- Zhang, M., Molinos, J. G., Zhang, X., and Xu, J. (2018). Functional and taxonomic differentiation of macrophyte assemblages across the yangtze river floodplain under human impacts. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00387



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Heterogeneous responses of wetland vegetation to climate change in the Amur River basin characterized by normalized difference vegetation index from 1982 to 2020

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Climate change affects wetland vegetation dramatically in mid- and high-latitudes, especially in the Amur River basin (ARB), straddling three countries and distributing abundance wetlands. In this study, spatiotemporal changes in average normalized difference vegetation index (NDVI) of wetland during the annual growing season were examined in the ARB from 1982 to 2020, and the responses of wetland vegetation to climatic change (temperature and precipitation) in different countries, geographic gradients, and time periods were analyzed by correlation analysis. The NDVI of wetland in the ARB increased significantly ($p < 0.01$) at the rate of 0.023 per decade from 1982 to 2020, and the NDVI on the Russian side (0.03 per decade) increased faster than that on the Chinese side (0.02 per decade). The NDVI of wetland was significantly positively correlated with daily mean temperature ($p < 0.05$, $r = 0.701$) and negatively correlated with precipitation, although the correlation was not significant ($p > 0.05$, $r = -0.12$). However, the asymmetric effects of diurnal warming on wetland vegetation were weak in the ARB. Correlations between the NDVI of wetland and climatic factors were zonal in latitudinal and longitudinal directions, and 49°N and 130°E were the points for a shift between increasing and decreasing correlation coefficients, closely related to the climatic zone. Under climate warming scenarios, the NDVI of wetland is predicted to continue to increase until 2080. The findings of this study are expected to deepen the understanding on response of wetland ecosystem to global change and promote regional wetland ecological protection.

KEYWORDS

wetlands, vegetation change, NDVI, climate change, Amur River basin

1 Introduction

Vegetation responded dramatically to global climate change in terrestrial ecosystems (Cramer et al., 2001). Wetlands, one of the most important ecosystems, are critical to biodiversity conservation, carbon sequestration, and hydrological and climate regulation (Mao et al., 2021; Salimi et al., 2021). In addition to various ecological functions, wetlands also have certain socioeconomic and cultural values, such as recreation, tourism, and scientific research (Pedersen et al., 2019). However, wetlands are the most vulnerable ecosystems and changes in hydrology, soils, climate, and anthropogenic disturbances all affect the ecological stability of wetlands (Bansal et al., 2019). Wetland vegetation, the main component of wetland ecosystems, is particularly susceptible to dramatic global climate change (Erwin, 2009). Compared with vegetation in other ecosystems, the unique growth environment of wetland vegetation results in obvious differences in the responses of wetland vegetation to climate change (Pang et al., 2017; Boulanger et al., 2018; Shen et al., 2022). Therefore, understanding how wetland vegetation changes in response to climatic change is essential for the adaptive management and conservation of wetlands.

Climate change affects the growth of wetland vegetation especially at the mid- and high-latitudes (Peng et al., 2020; Yuan et al., 2020). The global climate is undergoing a change characterized mainly by warming. Specifically, warming climate has advanced spring phenology and delayed autumn phenology, thereby extending the growing season of wetland vegetation (Herfindal et al., 2012; Crabbe et al., 2016). Although increases in precipitation can increase photosynthetic activity and promote wetland vegetation growth (Peng et al., 2013; Chen et al., 2020), seasonal increases in precipitation can adversely affect the reproduction of wetland vegetation by raising water levels and submerging vegetation (Bardecki, 1991). In previous studies, changes in wetland vegetation in response to climate change in several regions of the Northern Hemisphere were examined by using vegetation indices (Herfindal et al., 2012; Liu et al., 2022; Ren et al., 2022), such as normalized difference vegetation index (NDVI) and enhanced vegetation index. However, due to geographical differences, global climate change is spatially heterogeneous, and different climatic factors have varying impacts on wetland vegetation (Shen et al., 2022). Because of the heterogeneous response of wetland vegetation to climate change, additional research is needed that focuses on detailed analyses in order to develop adaptive management and future conservation strategies, especially in mid- and high latitudes.

As an important indicator of wetland vegetation health and growth, NDVI has been widely used in regional monitoring of changes in wetland vegetation and vegetation feedback on regional climate (Di et al., 1994; Meneses-Tovar, 2011; Wei et al., 2022). Because climate change is a long-term process, applying long time series data to investigate the responses of wetland vegetation to climate change can benefit understanding of processes of change in wetland vegetation and future wetland adaptive management (Hasselman et al., 2003). Currently, data from sensors such as the Advanced Very High Resolution Radiometer (AVHRR) onboard the National Oceanic and Atmospheric Administration

(NOAA) and Moderate Resolution Imaging Spectroradiometer (MODIS) are widely used in analyzing the NDVI of wetland dynamics (Fensholt and Sandholt, 2005; Albarakat and Lakshmi, 2019). Due to the limitation of data sources, in previous studies based on MODIS NDVI, changes in wetland vegetation in response to climate change were analyzed from 2000. However, it is difficult to fully understand longer term changes in wetland vegetation on the basis of those studies (Wang et al., 2020; Chen et al., 2023). Continuous long time series of NDVI can accurately reflect long-term trends in changes in wetland vegetation and abrupt changes (Guo et al., 2021). The longest time series is the NOAA Climate Data Record (CDR) of AVHRR NDVI data set, which is therefore the most suitable data source to analyze wetland vegetation change at both large scale and long time series (Peng et al., 2012). Moreover, with advantages such as free, huge computing power and rapid batch processing of data, the Google Earth Engine (GEE) cloud platform allows acquisition and rapid processing of large-scale long time series NDVI data sets. (Liu et al., 2018; Phan et al., 2020). Therefore, the use of NOAA CDR of AVHRR NDVI data set and GEE processing platform provides a possibility for long-term large-scale wetland analysis.

The Amur River basin (ARB), the world's ninth largest river basin, is an area of wetland concentration in mid- and high-latitude zones, where wetlands are particularly sensitive to global climate change (Wang et al., 2019; Han et al., 2020). Compared with North America and Europe in the same latitudes, wetland research is limited in the ARB (Wang Y. et al., 2020; Mao et al., 2021). China has higher temperatures and less precipitation, but more anthropogenic activity than Russia, and as a result, responses of wetland vegetation to climate change also differ (Chu et al., 2019). Nevertheless, there is a lack of comparative studies that explore changes in wetland vegetation and its response to climate change on Chinese and Russian sides of the ARB. A comparative analysis of environments in China and Russia would help provide a more comprehensive understanding of wetland changes in the ARB and support wetland conservation policies in both countries. In addition, temperature data show faster warming during the night than during the day in the past few decades in the ARB (Peng et al., 2013), and asymmetric effects of monthly average daily maximum temperature (TMX) and monthly average daily minimum temperature (TMN) on the NDVI of wetland were observed on Songnen (Wang Y. et al., 2020) and Sanjiang plains (Liu et al., 2022), which are part of the basin. However, the asymmetric effects differed on these plains. The NDVI of wetland was negatively correlated with TMX and positively correlated with TMN during the growing season on Songnen Plain (Wang Y. et al., 2020), but increasing TMN was more effective in promoting wetland vegetation growth than increasing TMX on Sanjiang Plain (Liu et al., 2022). Until recently, previous studies have been limited to small-scale analyses, and it has remained unclear whether there are long-term asymmetric effects of diurnal warming on wetland vegetation in the entire ARB. To accurately predict future changes in wetland vegetation, it is also necessary to explore the responses of wetland vegetation to changes in diurnal temperatures.

In this paper, therefore, the heterogeneous responses of wetland vegetation to climate factors in the ARB were examined from 1982

to 2020. The specific objectives were to (1) explore spatiotemporal changes in wetland vegetation in the ARB during the growing season from 1982 to 2020; (2) examine the responses of wetland vegetation to climate change in different countries, geographic gradients, and time periods; and (3) predict the effects of future climate change on wetland vegetation in the ARB. The study is expected to provide theoretical support for the conservation and restoration of wetlands.

2 Materials and methods

2.1 Study area

The ARB spans latitudes from 41.72°N to 55.90°N and longitudes from 108.05°E to 141.13°E. It is a transboundary region of three countries (Figure 1), with 48% of the basin in Russia, 43% in China, and 9% in Mongolia, and a total area of 2.08 million km² (Mao et al., 2021). The Greater Khingan Mountains divide the ARB into two relatively different climates. The western ARB is relatively dry, with annual precipitation of only 250 mm–400 mm, whereas the eastern ARB is relatively wet, with annual precipitation of 400 mm–700 mm. The ARB passes through humid, semi-humid and semi-arid zones, with boundaries between the three zones at 130°E and 120°E, respectively, which is continental monsoon climate. Annual average temperature is between –8°C and 6°C, decreasing gradually from south to north, with clear spatial variability. The basin straddles two temperature zones from south to north, mid-temperate and frigid-temperate zones, with the boundary between the two zones at 50°N. There are many rivers and developed water systems that crisscross the basin, interacting to form the Songnen and Sanjiang plains in the east, with lower elevations than those of the mountains in the east. The ARB has a large wetland area that is relatively concentrated in the Greater

Khingan Mountains and Songnen and Sanjiang plains. The main wetland vegetation types common in this basin are *Typha orientalis*, *Phragmites australis*, and *Scirpus triquetus*, and the water level of suitable wetlands is gradually declining. The wetlands provide important resting places for multitudes of migratory waterfowl on the East Asian–Australasia flyway (Jia et al., 2020). The total wetland area of the ARB is 0.17 million km², accounting for 8.1% of the ARB (Yang et al., 2020). However, there were fewer wetlands located in Mongolia, and almost no unchanged wetlands, extracted by the wetland data, were located in Mongolia, so we mainly analyzed the changes of wetlands in the Chinese and Russian sides.

2.2 Data source

2.2.1 NDVI data

The NOAA CDR of AVHRR NDVI version 5 was used in this study. The resolution was 0.05°, and the interval was 1 day. Free images were collected in the growing season (May–September) from 1982 to 2020 on the GEE cloud platform (<https://earthengine.google.com/>). Monthly NDVI data were obtained by the maximum value composite method, which chose the largest value of each pixel in the multitemporal data to reduce the interference of atmospheric and solar zenith angle (Wang et al., 2005), and projection processing and clipping were processed on GEE.

2.2.2 Climatic data

Climatic data were acquired from the monthly gridded Climatic Research Unit Time-Series Data version 4.05 (CRU TS 4.05, available at <https://crudata.uea.ac.uk/cru/data/hrg/>). The CRU TS data were produced by the CRU at the University of East Anglia with a resolution of 0.5° and a long time series from 1901 to 2020. The data have been widely used in studies on climate change and on

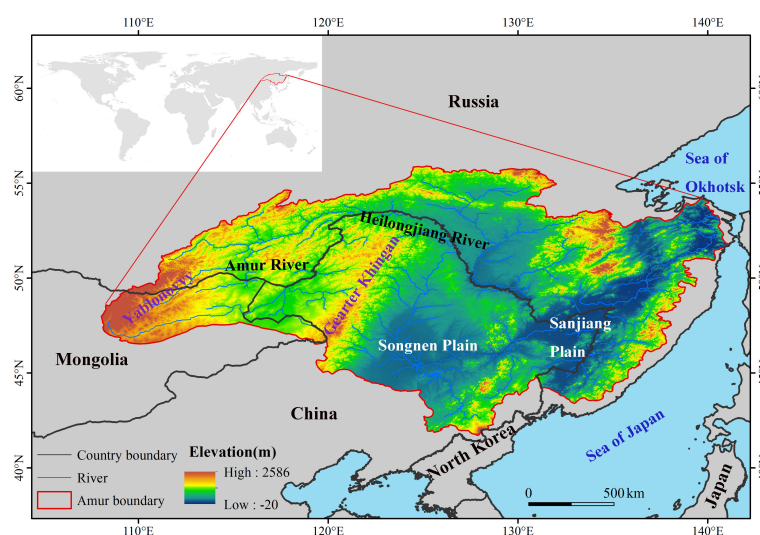


FIGURE 1
Location of the Amur River basin and its spatial terrain pattern.

vegetation growth (Chu et al., 2019; Meng et al., 2019). In this study, four types of climatic factors acquired from CRU TS 4.05 were used to analyze the effect of climate on wetland vegetation change, including daily mean temperature (TMP), TMX, TMN, and precipitation (PRE). To study asymmetric effects of diurnal warming on wetland vegetation in the ARB, TMX and TMN were used to represent daytime and nighttime temperatures, respectively. To coordinate data sets, the spatial resolution of temperature and precipitation data was resampled to 0.05° by using the nearest neighbor method.

2.2.3 Wetland data

Wetland distribution data were extracted from up-to-date land cover data in the ARB in 1980 and 2016 (Mao et al., 2020). Natural wetlands in this study were vegetated wetlands, including marsh, swamp, bog, and fen. Land cover data were classified from Landsat TM/OLI images by an object-based image analysis and hierarchical decision-trees classification method, including multiresolution segmentation, designing place-based hierarchical decision trees, and rule-based object identification (Mao et al., 2020). The data were obtained from the Northeast branch of the National Earth System Science Data Center (<http://wetland.igad.cn/>). Accuracy of the data was verified by field investigations and Google Earth images with overall classification accuracy values larger than 90% (Mao et al., 2021), which showed that the wetland data were reliable to explore the responses of wetland vegetation to changes. For the analysis of NDVI trends and its relations with climatic factors, wetlands existing both in 1980 and 2016 were extracted as unchanged wetlands from 1980 to 2016 in the ARB, with a total area of 0.12 million km².

2.2.4 Future climate data

The future temperature and precipitation data used in this study were produced from the CNRM-ESM2-1 global climate model of the Scenario Model Intercomparison Program (ScenarioMIP) under International Coupled Model Intercomparison Program Phase 6 (CMIP6, available at <https://esgf-node.llnl.gov/search/cmip6/>). The CNRM-ESM2-1 model provided by CMIP6 was derived from the French National Meteorological Center, with a spatial resolution of 1.4° × 1.4°. The combination scenarios of Shared Socioeconomic Pathways (SSPs) and Representative Concentration Pathways (RCPs) incorporates the impact of socioeconomic development (Su et al., 2021), which are widely used in regional climate change prediction (Yang et al., 2020; Ren et al., 2023). SSP1-RCP2.6 is a low-forcing scenario, and SSP5-RCP8.5 is a high-forcing scenario.

2.3 Methods

2.3.1 Sen+Mann–Kendall method

In this study, we have used Sen+Mann–Kendall method based on the R 4.2.1 to analyze trends from 1982 to 2020 for the NDVI of wetland vegetation and four climatic factors (PRE, TMP, TMX, and

TMN). Due to the strong resistance to measurement errors and abnormal data, the Sen+Mann–Kendall method has been increasingly used to analyze trends in long term series data for vegetation (Li et al., 2021; Wu et al., 2021), (Li et al., 2021).

Theil–Sen median trend analysis is a non-parametric statistical trend calculation method (Sen, 1968). The formula is as follows:

$$X(t) = Qt + B$$

where $X(t)$ is the time series data of climate and NDVI, Q is the slope of the data, and B is a constant.

$$Q_{median} = Median\left(\frac{X_j - X_i}{j - i}\right)$$

where Q_{median} is the median of Q sorted from smallest to largest. When $Q_{median} > 0$, the climate or NDVI time series has an increasing trend, whereas when $Q_{median} < 0$, a decreasing trend is indicated.

The Mann–Kendall trend test is a non-parametric statistical test method that determines the significance of trends (Kendall, 1948). The formula is as follows:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^n \text{sgn}(X_j - X_i)$$

where n is the length of the time series (39 years in this study), and $\text{sgn}(X_j - X_i)$ is the sign function, defined as follows:

$$\text{sgn}(X_j - X_i) = \begin{cases} -1 & \text{if } X_j - X_i < 0 \\ 0 & \text{if } X_j - X_i = 0 \\ 1 & \text{if } X_j - X_i > 0 \end{cases}$$

For the time series data $X(t)$, the statistic Z is defined as follows:

$$Z = \begin{cases} \frac{S+1}{\sqrt{\text{Var}(S)}} & S < 0 \\ 0 & S = 0 \\ \frac{S-1}{\sqrt{\text{Var}(S)}} & S > 0 \end{cases}$$

where $\text{Var}(S) = \frac{n(n-1)(2n+5)}{18}$. When $Z > 0$, the variable shows an upward trend; when $Z < 0$, the variable shows a downward trend. In addition, when $|Z| > 1.96$, the time series has significant variation at the level of 0.05 (Liu et al., 2019). According to the significance of trends, variations in NDVI trends were classified into three types: significant increase (slope > 0 , $p < 0.05$), significant decrease (slope < 0 , $p < 0.05$), and nonsignificant change ($p > 0.05$).

2.3.2 Hurst exponent

We have used Hurst exponent to investigate whether trends of the NDVI of wetland vegetation was sustainable in the ARB from 1982 to 2020. The method was proposed by Hurst (1951) in the analysis of hydrological data and then improved by Mandelbrot and Wallis (1969). The method has been successfully applied in studies investigating vegetation changes (Jiang et al., 2017). The basic calculations are as follow:

1) Divide the time series $\{NDVI(\tau)\}(\tau = 1, 2, \dots, n)$ into τ sub series $X(t)$, for each series $t = 1, 2, \dots, \tau$.

- 2) Define the mean sequence of the time series,

$$\overline{NDVI}_{(\tau)} = \frac{1}{\tau} \sum_{t=1}^{\tau} NDVI_{(t)}$$

- 3) Calculate the accumulated deviation,

$$X_{(t)} = \sum_{t=1}^t (NDVI_{(t)} - \overline{NDVI}_{(\tau)}) \quad 1 \leq t \leq \tau$$

- 4) Create the range sequence,

$$R(t) = \max_{1 \leq t \leq \tau} X_{(t,\tau)} - \min_{1 \leq t \leq \tau} X_{(t,\tau)} \quad \tau = 1, 2, \dots, n$$

- 5) Create the standard deviation sequence,

$$S_{(\tau)} = \sqrt{\frac{1}{\tau} \sum_{t=1}^{\tau} (NDVI_{(t)} - \overline{NDVI}_{(\tau)})^2} \quad \tau = 1, 2, \dots, n$$

- 6) Calculate the Hurst exponent

$$\frac{R_{(\tau)}}{S_{(\tau)}} = (c\tau)^H$$

The value of H is obtained by fitting the equation $\log(R/S)_n = a + H \times \log(n)$, which can be used to determine whether the time series $\{NDVI(\tau)\}$ is completely random or persistent. According to previous studies, the value of the Hurst exponent, ranging from 0 to 1, is classified into three types. When $0.5 < H < 1$, the trend of change in NDVI in the future will be consistent with that in the past. When $H = 0.5$, the NDVI time series is random and there is no long-term correlation. When $0 < H < 0.5$, the trend in the future will be the opposite of that in the past.

2.3.3 Sequential Mann–Kendall test

The sequential Mann–Kendall (SQMK) test was used to identify the change point of NDVI of wetland vegetation and four climatic factors (PRE, TMP, TMX, and TMN) from 1982 to 2020. The SQMK test, developed by Sneyers (1991), is used to detect abrupt change points in long-term data series. The steps of the SQMK test are the following:

- 1) For a time series with n sample sizes $X \{X_1, X_2, \dots, X_n\}$, a rank sequence is constructed as follows:

$$S_k = \sum_{i=1}^k R_i \quad (k = 2, 3, \dots, n)$$

where R_i is the cumulative number of samples when $X_i > X_j (1 \leq j \leq i)$.

- 2) Under the assumption of random independence of time series, the statistics are defined as follows:

$$UF_k = \frac{S_k - E(S_k)}{\sqrt{Var(S_k)}} \quad (k = 1, 2, \dots, n)$$

where $UF_1 = 0$, $E(S_k)$ and $Var(S_k)$ are the mean and variance of the cumulative number S_k , respectively. When $X \{X_1, X_2, \dots, X_n\}$ is independent of the others and has the same continuous distribution, $E(S_k) = \frac{n(n-1)}{4}$, and $Var(S_k) = \frac{n(n-1)(2n+5)}{72}$. For a given significance level α , $UF_k > UF_{\alpha}$ indicates a significant trend change in the sequence of X .

- 3) The time series data are arranged in reverse order, and the above calculation is repeated to obtain UB_k , indicated as follows:

$$UB_k = -UF_k$$

- 4) When the significance level α is set to 0.05, as in this study, the value of $U_{1-\alpha/2}$ is ± 1.96 (Wang R. et al., 2020). The intersection points of UF and UB curves indicate the abrupt change year in a time series trend (Mosmann et al., 2004).

2.3.4 Correlation analysis method at the scale of pixels

A correlation coefficient method at the scale of pixels was used to evaluate correlations between NDVI and climatic factors (TMP, TMX, TMN, and PRE) from 1982 to 2020. The method was processed in ArcGIS based on the ArcPy tool, which can investigate possible effects of climate change on NDVI. The formula is as follows:

$$r_{xy} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}}$$

where r_{xy} is the correlation coefficient between variables x and y , ranging from -1 to 1 , n is the number of years, x_i is the value of the NDVI for year i , y_i is the value of the climatic factors (TMP, TMX, TMN, and PRE) for year i , and \bar{x} and \bar{y} are the averaged NDVI and the mean of the climatic factors, respectively, which were obtained from the averages of all pixels assigned to wetlands (Chen et al., 2018). When $r_{xy} > 0$, the NDVI of wetland vegetation and the climatic factors were positively correlated. When $r_{xy} < 0$, the two variables were negatively correlated.

Moreover, the correlations of NDVI with climatic factors were classified into four major categories: significant positive correlations ($p < 0.05$), nonsignificant positive correlations ($p > 0.05$), significant negative correlations ($p < 0.05$), and nonsignificant negative correlations ($p > 0.05$). In addition, we considered the lag effects of precipitation, so we calculated the correlation coefficients between the NDVI of the current year and the precipitation of the previous year and the previous 2 years.

2.3.5 Partial correlation analysis

Partial correlation analysis was used to calculate the partial correlation coefficients between PRE, TMP, and NDVI, respectively, to examine interaction term between temperature and precipitation. When the dependent variable is correlated with two or more independent variables, partial correlation analysis is used to investigate the correlation between one independent variable and the dependent variable by excluding the influence of another independent variable (Cao et al., 2014).

The formula is as follows:

$$r_{xy.z} = \frac{r_{xy} - r_{xz}r_{yz}}{\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)}}$$

where r_{xy} , r_{xz} , and r_{yz} is the correlation coefficient between variables x and y , x and z , and y and z , respectively, ranging from -1 to 1 , $r_{xy.z}$ is

the partial correlation coefficient between r_{xy} and r_{xz} fixed r_{yz} . The value of the partial correlation coefficient ranges from +1 to -1.

3 Results

3.1 Spatiotemporal changes in the NDVI of wetland in the ARB

At the basin scale, the mean NDVI of wetland in the growing season showed a significantly increasing trend ($p < 0.01$) from 1982 to 2020 with a growth rate of 0.023 per decade, although there was a minimum value in 2003 (Figure 2A). At the pixel scale, relatively large the NDVI of wetland values were mainly distributed in the eastern and central parts of the ARB, such as the Greater and Lesser Khingan mountains and the eastern Russian side. Relatively small NDVI values were mainly distributed in the southwestern part of the basin.

According to Sen+Mann–Kendall analysis, variations in the NDVI of wetland from 1982 to 2020 in the ARB had apparent spatial heterogeneity (Figure 2B). With the Greater Khingan Mountains as a border, the NDVI of wetland primarily decreased in the relatively dry western region but increased in the relatively wet eastern region. In the ARB, 89.85% of pixels experienced significant increases ($p < 0.05$) in NDVI in the growing season (Figure 2C).

The mean value of the NDVI Hurst index for wetland vegetation was 0.71. As shown in Figure 2D, the Hurst index exceeded 0.5 in much of the ARB. Pixels with Hurst index values between 0.5 and 0.7 and greater than 0.7 accounted for 25.58% and 74.21%, respectively.

On the Chinese side of the ARB, the average the NDVI of wetland was 0.59, the trend of increase in average NDVI was 0.02 per decade, and the average Hurst index was 0.68. However, on the Russian side, the average the NDVI of wetland was 0.65, the trend of increase in average NDVI was 0.03 per decade, and the average Hurst index was 0.73. Thus, in the comparative analysis of Chinese and Russian sides, values on the Russian side were all higher than those on the Chinese side.

According to the SQMK test, the NDVI of wetland values in the ARB from 1982 to 2020 changed abruptly in 2000 (Figure 3A). During 1982–2000, the average NDVI fluctuated between 0.55 and 0.65, and the trend in the NDVI of wetland in the ARB was clearly one of increase. However, during 2000–2020, although the average NDVI fluctuated between 0.60 and 0.70, no significant trend of increase was observed (Figure 3B).

3.2 Spatiotemporal change in climatic factors in the Amur River basin

From 1982 to 2020, increasing trend of PRE, TMP, TMX, and TMN were observed at different levels (Figure 4). The PRE had a growth rate of 1.5 mm per decade, and temperature (TMP, TMX, and TMN) increased significantly with a growth rate of 0.2°C per decade. In terms of spatial changes, PRE showed a downward trend in the west, mainly in the Songnen Plain, but an upward trend in the east, mainly in the Sanjiang Plain, with the reversal line at approximately 130°E. The temperature increase was higher in the western ARB than in the eastern ARB. On the Chinese side, PRE showed a downward trend, whereas on the Russian side, it showed

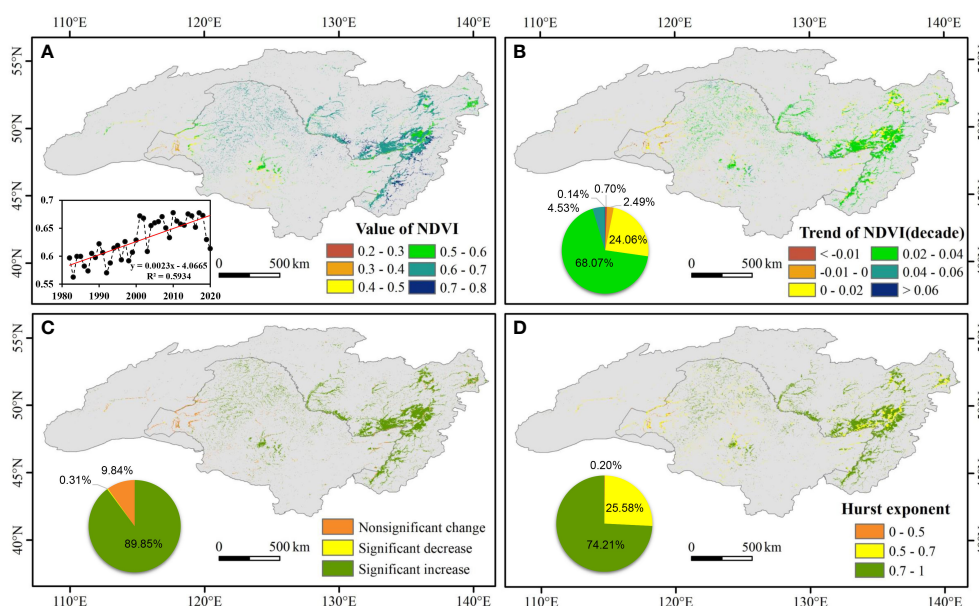


FIGURE 2

Spatial pattern of wetland normalized difference vegetation index (NDVI) in the Amur River basin from 1982 to 2020. (A) Spatial distribution of the average NDVI of wetland during the annual growing season, inset shows the temporal changes of NDVI; (B) spatial pattern in temporal trend (per decade) of NDVI; (C) spatial distribution of trend types; (D) spatial pattern in Hurst exponent of NDVI. The pie charts illustrate the area percentage of NDVI trends and sustainable characteristics.

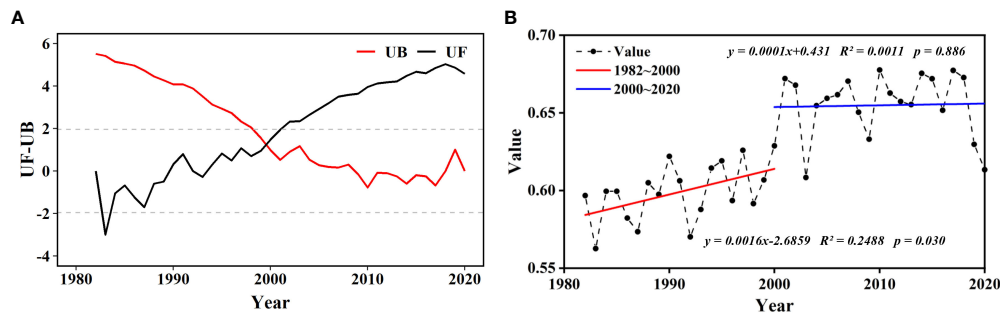


FIGURE 3

Abrupt and temporal changes in wetland normalized difference vegetation index (NDVI) in the Amur River basin (ARB) from 1982 to 2020: **(A)** abrupt change of the NDVI of wetland using SQMK test. Dotted horizontal straight lines indicate the lower limit and upper limit of 95% confidence interval. **(B)** temporal changes of the NDVI of wetland in the ARB during 1982–2000 and 2000–2020. $p < 0.05$ and $p < 0.01$ indicate significance at the 95% and 99% levels.

an upward trend, indicating clear spatial heterogeneity. The magnitude of temperature increase in China was significantly higher than that in Russia.

From 1982 to 2020, an SQMK test revealed the abrupt change point in PRE occurred in 2019 (Figure 5). During the 4 decades, two break points in TMP occurred in 2000 and 2019, one break point in TMX occurred in 1998, and three break points in TMN occurred in 2006, 2018, and 2019.

Because abrupt changes in both NDVI and TMP occurred in 2000, the interannual variation in each climatic factor was analyzed in two stages separated by 2000 (Figure 5). Precipitation showed an overall decreasing trend from 1982 to 2000 but an increasing trend

from 2000 to 2020. Daily mean temperature exhibited an increasing trend from 1982 to 2000 but a decreasing trend from 2000 to 2020. The trends in TMX were almost the same as those in TMP, but the trend in TMN was continuous increase in both periods.

According to the CNRM-ESM2-1 model, the climate will become warmer and drier under the SSP126 scenario (low-emission scenario) in the ARB in the growing season from 2021 to 2080, but under the SSP585 scenario (high-emission scenario), it will become warmer and wetter (Figure 6). In addition, the growth rate in TMN will be higher than that in TMX under the SSP585 scenario, whereas the difference will be not obvious under the SSP126 scenario.

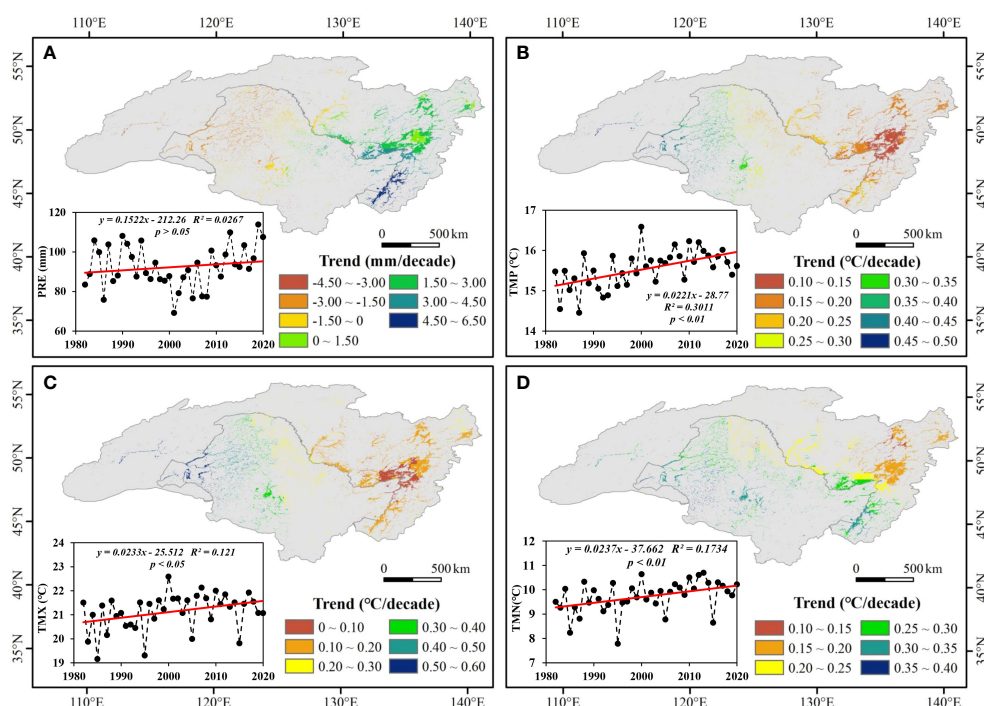


FIGURE 4

Spatiotemporal changes in **(A)** precipitation (PRE), **(B)** daily mean temperature (TMP), **(C)** monthly average daily maximum temperature (TMX), and **(D)** monthly average daily minimum temperature (TMN) in the Amur River basin from 1982 to 2020. $p < 0.05$ and $p < 0.01$ indicate significance at the 95% and 99% levels.

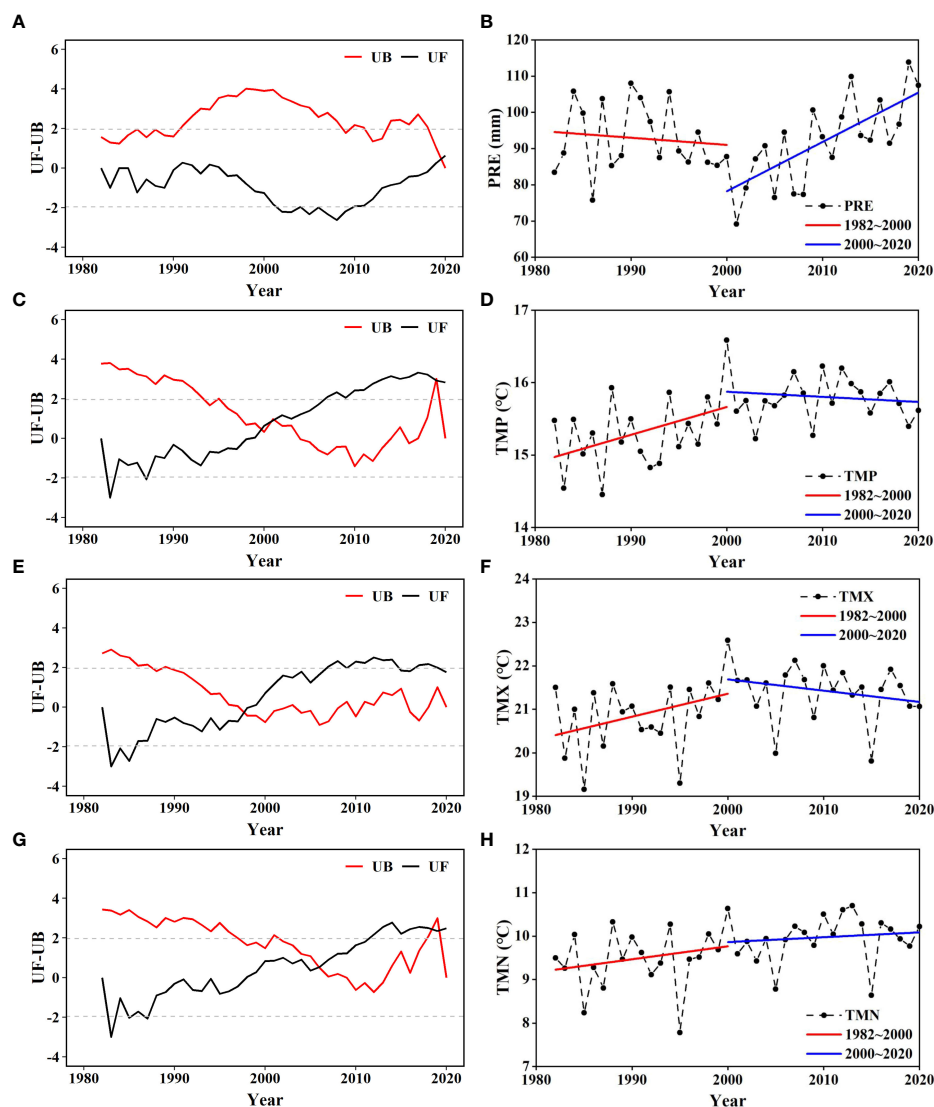


FIGURE 5

Abrupt change in (A, B) precipitation (PRE), (C, D) daily mean temperature (TMP), (E, F) monthly average daily maximum temperature (TMX), and (G, H) monthly average daily minimum temperature (TMN) in the Amur River basin.

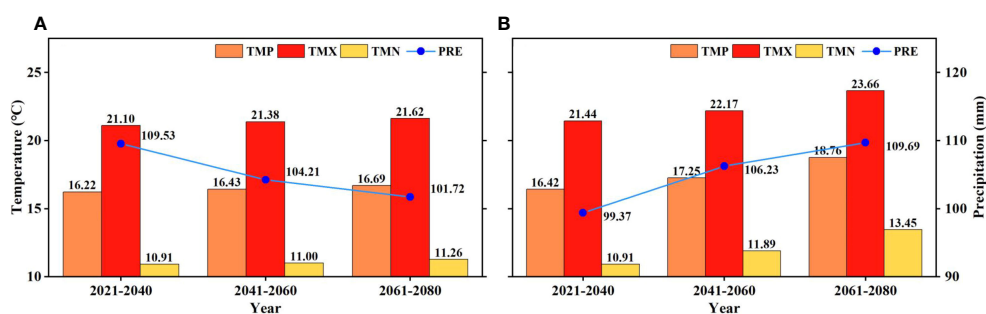


FIGURE 6

Future climate under (A) SSP126 and (B) SSP585 scenarios in the growing season from 2021 to 2080 in the Amur River basin.

3.3 Spatiotemporal patterns of correlations between the NDVI of wetland and climatic factors

Table 1 shows the correlations between the NDVI of wetland and the four climatic factors throughout the ARB. In terms of temporal correlation, the NDVI of wetland in the ARB was significantly and positively correlated with TMP, TMX, and TMN ($p < 0.05$) and negatively correlated with PRE ($p > 0.05$). The highest correlation was with TMP ($r = 0.701$). On both Chinese and Russian sides of the ARB, the NDVI of wetland was significantly and positively correlated with temperature ($p < 0.05$) but negatively correlated with PRE. In addition, partial correlation analysis revealed that TMP remained significantly and positively correlated with NDVI, while PRE was negatively correlated with NDVI except for Chinese side. In terms of the lag effects of precipitation and NDVI, the correlation coefficients between the NDVI of the current year and the precipitation of the previous year and the previous 2 years were -0.7 ($p > 0.05$) and 0.157 ($p > 0.05$), respectively.

Spatially, there was a weak negative correlation between the NDVI of wetland and PRE in the central ARB, whilst the NDVI of wetland was positively correlated with PRE in the eastern and western ARB (Figure 7). At the pixel scale, 86.66% of the ARB showed significant positive correlations between the NDVI of wetland and TMP ($p < 0.05$), distributed throughout the ARB except in the west. Positive correlations between NDVI and TMX and between NDVI and TMN were mainly in the central and eastern ARB. On the Chinese side of the ARB, correlations between the NDVI of wetland and PRE were primarily negative, whereas on the Russian side, there were both positive and negative correlations between the NDVI of wetland and PRE. The NDVI of wetland was significantly and positively correlated with temperature on both Chinese and Russian sides of the ARB.

According to the analysis of the two stages with 2000 as the node, correlations between the NDVI of wetland and the four climatic factors varied in different time periods and different countries (Table 2). There was a weak asymmetric effect of diurnal warming on wetland vegetation in the ARB during 2000–2020, which means Pearson correlation coefficients between NDVI and TMX and TMN are smaller than 0.2 and significances are greater than the 0.05 level. The NDVI of wetland was positively correlated with daily maximum temperature but negatively correlated with daily minimum temperature. On Chinese and

Russian sides of the ARB during the two periods, the NDVI of wetland was primarily negatively correlated with PRE and significantly and positively correlated with TMP ($p < 0.01$). Nevertheless, on the Russian side, NDVI and PRE were positively correlated during 1982–2000, and NDVI and TMP were negatively correlated during 2000–2020.

Pronounced latitudinal and longitudinal zonal variability was observed among correlation coefficients (Figure 8). With an increase in latitude, correlation coefficients between NDVI and TMP increased before decreasing, whereas those between NDVI and PRE decreased before increasing. However, in the longitudinal direction, trends in correlation coefficients between NDVI and TMP and PRE were opposite to those in the latitudinal direction. Notably, 49°N and 130°E were apparent points for the change between increasing and decreasing correlation coefficients.

4 Discussion

4.1 The NDVI of wetland dynamics and its relations with climatic factors

The NDVI of wetland showed a significant upward trend during 1982–2020, which was primarily attributed to increases in temperature and precipitation. A warm and humid environment is conducive to the growth of wetland vegetation (Burkett and Kusler, 2000). Higher temperature can increase greater photosynthetic intensity, which played an important role for the growth of vegetation (Sharma et al., 2020). The percentage increase in the NDVI trend and the Hurst index indicated good overall growth of wetland vegetation during the four decades examined in this study. In addition, according to the results of Hurst exponent, the predicted future change in the NDVI of wetland trend in the ARB was consistent with that of the past trend (Figure 2D). The minimum point of the NDVI of wetland occurred in 2003 but temperature, affected vegetation growth strongly, was not the lowest value, and precipitation was not strongly correlated with wetland vegetation. However, the minimum point of precipitation occurred in 2001, so we considered whether the minimum NDVI in 2003 could be caused by the lagging effect of precipitation and spring drought led to a large reduction. The correlation coefficients were -0.7 ($p > 0.05$) and 0.157 ($p > 0.05$), respectively, which indicated that there were lag effects of PRE on the NDVI of wetland in the ARB.

TABLE 1 Pearson correlation coefficients between NDVI and temperature (daily mean temperature, TMP; monthly average daily maximum temperature, TMX; monthly average daily minimum temperature, TMN) and precipitation (PRE) and partial correlation coefficients between NDVI and TMP and PRE in the Amur River basin and different nations during 1982–2020.

Study area	Pearson correlation coefficients				Partial correlation coefficients	
	R_{NDVI_PRE}	R_{NDVI_TMP}	R_{NDVI_TMX}	R_{NDVI_TMN}	R_{NDVI_PRE}	R_{NDVI_TMP}
ARB	-0.12	0.701^{**}	0.423^{**}	0.386^{*}	-0.016	0.648^{**}
Chinese side	-0.224	0.728^{**}	0.413^{**}	0.487^{**}	0.018	0.655^{**}
Russian side	-0.052	0.608^{**}	0.350^{*}	0.340^{*}	-0.044	0.556^{**}

* and ** represent significance at the 0.05 level and the 0.01 level, respectively.

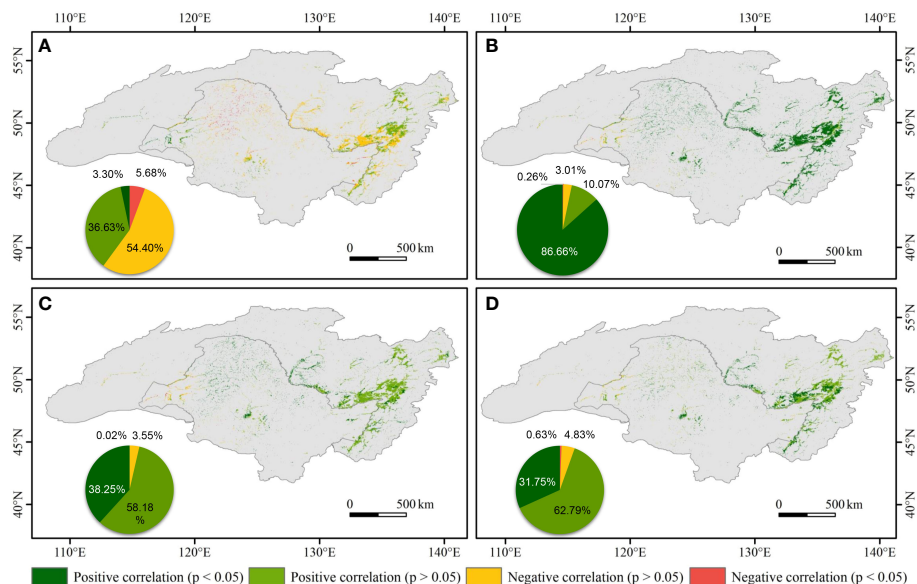


FIGURE 7 Spatial patterns of correlations between normalized difference vegetation index (NDVI) and (A) precipitation (PRE), (B) daily mean temperature (TMP), (C) monthly average daily maximum temperature (TMX), and (D) monthly average daily minimum temperature (TMN) in the Amur River basin during 1982–2020. The pie charts illustrate the area percentage of different spatial patterns of the correlations.

According to the SQMK test, average the NDVI of wetland in the ARB increased after 2000. The increase in average NDVI was probably attributed to recognition of the importance of wetlands beginning in the late 1990s and China’s promulgation of the China Action Plan for Wetland Conservation in 2000 (Chen et al., 2018). In addition, Northeast China responded positively to the national policy. For example, Heilongjiang Province promulgated the *Decision on Wetland Protection* in 1998 and the *Heilongjiang Wetland Protection Regulations* in 2003 (Cui, 2006). However, the rate of increase in the NDVI of wetland slowed after 2000, which was likely associated with the decline in TMP during 2000–2020. The global warming interruption from 1999 onward resulted in a weak cooling trend in TMP (Fyfe et al., 2013; Karl et al., 2015), also called the warming interval (Li et al., 2015). Because the abrupt changes in both NDVI and TMP occurred in 2000, it also confirmed that the NDVI of wetland in the ARB was mostly influenced by TMP. Increases in temperature

lead to increases in photosynthetic intensity of plants (Sharma et al., 2020), thus promoting the growth of vegetation in the growing season, according to Yang et al. (2020). In further analysis of the relations between NDVI and daytime and nighttime temperatures in the ARB, weak asymmetric effects of diurnal warming on wetland vegetation were detected during 2000–2020. The growing season NDVI of wetland vegetation was positively correlated with TMX and negatively correlated with TMN, which is consistent with the Peng et al. (2013). Wetland vegetation perform photosynthesis during the daytime, and increasing TMX can enhance photosynthetic enzyme activity and carbon dioxide concentration, thus promoting vegetation growth (Lucht et al., 2002; Beer et al., 2010). Respiration in plants occurs mainly at night, via enhanced autotrophic respiration and produced a compensatory stimulation of photosynthesis the next day (Wan et al., 2009), which could partly explain the positive correlation between NDVI and TMN.

TABLE 2 Pearson correlation coefficients between normalized difference vegetation index (NDVI) and four climatic factors in different regions during two periods.

Periods	Study area	PRE	TMP	TMX	TMN
1982–2000	ARB	0.178	0.642**	0.302	0.291
	Chinese side	−0.009	0.602**	0.325	0.245
	Russian side	0.178	0.622**	0.27	0.291
2000–2020	ARB	−0.354	0.351	0.14	−0.012
	Chinese side	−0.168	0.259	0.07	0.011
	Russian side	−0.391	−0.011	0.15	0.319

* and ** represent significance at the 0.05 level and the 0.01 level, respectively.

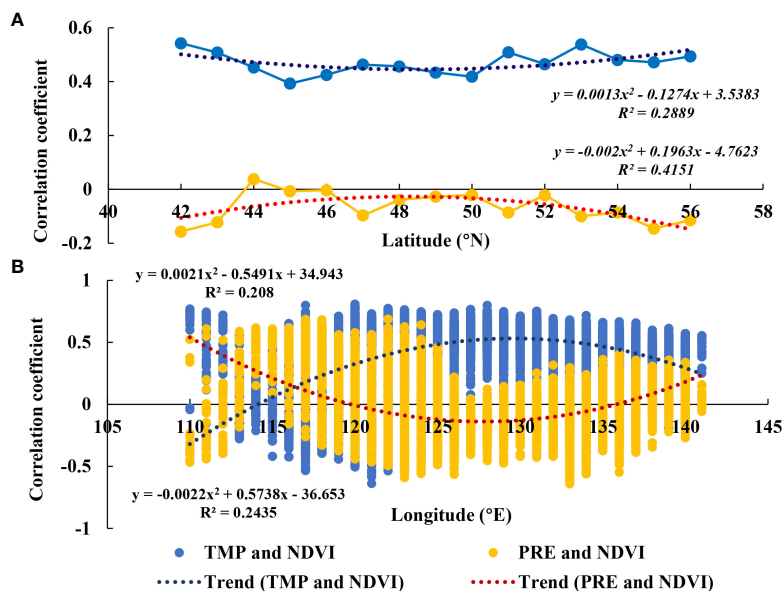


FIGURE 8

Changes in correlation coefficients between normalized difference vegetation index (NDVI) and temperature and precipitation (PRE) in (A) latitudinal and (B) longitudinal directions.

4.2 Wetland vegetation changes in response to climate change at multiple scales

The growing season trends in the NDVI of wetland and its responses to climate change on Chinese and Russian sides of the ARB had obvious spatial heterogeneity. The NDVI of wetland was primarily negatively correlated with PRE and significantly positively correlated with TMP on the Chinese and Russian sides. Nevertheless, PRE had a positive effect on the NDVI of wetland in Russia during 1982–2000, possibly because adequate water promoted wetland vegetation growth. Russia is geographically closer to the sea than China; affected by the position of sea and land, there was more precipitation in Russia, especially in the east of Russia (Figure 4). During 2000–2020, the NDVI of wetland and TMP were negatively correlated on the Russian side, which might be associated with permafrost degradation under a warming climate. Permafrost covers 60% of the entire basin (Avis et al., 2011), and many wetlands in cold regions live in symbiosis with permafrost. Permafrost prevents precipitation and runoff from percolating into the ground, and excessive surface moisture inhibits aerobic bacterial activity, and promotes peat accumulation, which contributes to the growth of wetland vegetation (Jin et al., 2008). China is located in a mid-temperate region with higher temperature, which is the strong climatic factor affecting vegetation growth. However, the growth of wetland vegetation on the Russian side was better than that on the Chinese side. This discrepancy could be attributed to not only the influence of climate but also the different intensity of human activity (Mao et al., 2021). In addition, over-cultivation of rice in northeastern China, an important grain production base,

competes with wetlands for water (Zou et al., 2018). Surface water is reintroduced from the subsurface to irrigate farmland in some areas due to excessive oxidative enrichment, resulting in a decline in wetland water levels (Zhou et al., 2021).

Notably, correlation coefficients between NDVI and PRE and TMP in the ARB were zonal in latitudinal and longitudinal directions, and 49°N and 130°E were the apparent turning points between increasing and decreasing correlation coefficients. In the latitudinal direction, 49°N is roughly consistent with the boundary between mid-temperate and frigid-temperate zones (Wang et al., 2021). In the frigid-temperate zone, relatively high temperatures are associated with increases in effective soil nitrogen, which promote wetland vegetation growth by increasing photosynthetic intensity of the vegetation (Amundson et al., 2003). In addition, increases in rainfall are associated with an increase in cloud cover and a decrease in sunlight, which may limit vegetation growth (Mao et al., 2012). In the longitudinal direction, 130°E is roughly consistent with the boundary between humid and semi-humid zones (Zhang et al., 2019). The eastern part of the ARB is primarily in the humid region, whereas the western part is in the semi-humid region. In semi-humid areas, wetland vegetation is hydrophilic, and PRE is the main factor influencing its growth (Li et al., 2013). However, with an increase in longitude in the humid area, excessive PRE leads to the drowning and death of some wetland vegetation (Figure 9), including *Phragmites australis* and *Scirpus triquetus*, which are the dominant wetland vegetation in the ARB (Xie et al., 2008). Except for the drowning of wetland vegetation, the increase of clouds and the reduction of sunshine caused by excessive PRE inhibits the growth of wetland vegetation (Yang et al., 2020). Therefore, correlation coefficients between PRE and NDVI decreased, and TMP was a major factor affecting vegetation growth.

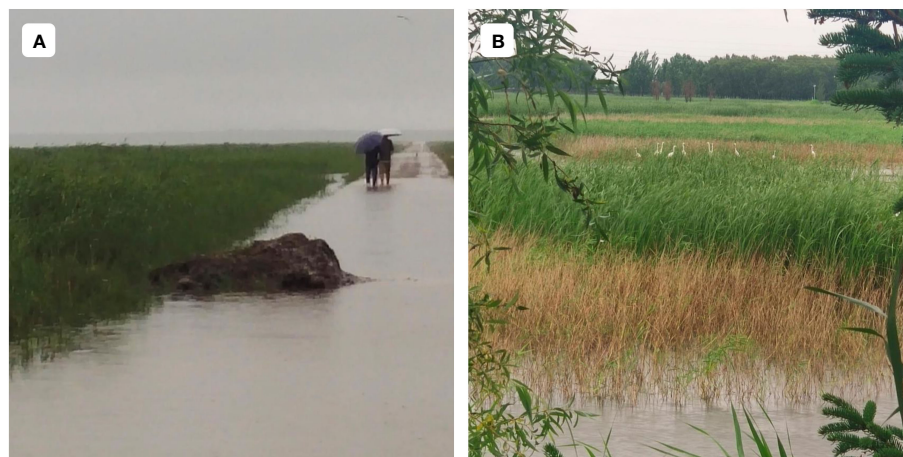


FIGURE 9
Excessive PRE condition in the ARB (A) and the brown parts indicate the death of the wetland vegetation (B).

4.3 Projection of future climate change effects on wetland vegetation in the Amur River basin

The warming and wetting in the ARB during the 4 decades of this study are consistent with results of previous researches (Yu et al., 2013; Chu et al., 2019). According to the observations and the analysis of data by various organizations, global climate change is dramatic (IPCC, 2007; Potter et al., 2017). In the future, the climate warming trend will continue in the Northern Hemisphere (Salinger, 2005). In the ARB, temperature and precipitation are predicted to increase by the end of the 21st century under the SSP585 scenario (Yang et al., 2021), and the area of marshland is predicted to increase on the Songnen and Sanjiang plains (Chen et al., 2018). Compared with Canada in the same latitudes, the future warmer and wetter climate will favor increased wetland abundance in the western prairies (Zhang et al., 2021). Future climate change (i.e., warming, variation in precipitation) will affect the growth of wetland vegetation through photosynthesis, respiration and transpiration. In order to predict future climate change conditions, CMIP6 developed different emission scenarios to simulate different possible socioeconomic developments (O'Neill et al., 2016). SSP-RCP was widely used in regional climate change prediction (Yang et al., 2020; Ren et al., 2022). To further explore the future growth of wetland vegetation in the entire ARB, the NDVI of wetland from 2021 to 2080 was predicted selecting SSP1-RCP2.6 (SSP126) and SSP5-RCP8.5 (SSP585).

Under the SSP126 scenario, warming in the future will increase the NDVI of wetland (Sharma et al., 2020), and the relatively dry but still humid conditions will encourage growth of wetland vegetation in the future (Xie et al., 2008). Under the high-emission scenario, the NDVI of wetland will also show an upward trend from 2021 to 2080, because temperature will have a greater effect on the growth of wetland vegetation than that of

precipitation in the ARB (Table 1). Notably, in the humid area, an increase of precipitation will cause a decrease in NDVI (Mao et al., 2012). In terms of diurnal temperature, the negative effect of the rapid rise in TMN on the NDVI of wetland will gradually increase and might even exceed the positive effect of TMX in the future.

5 Conclusions

Based on NDVI and CRU data, this study investigated spatiotemporal changes in the NDVI of wetland and its responses to climate change in the ARB during 1982–2020 in different countries, geographic gradients, and time periods. From 1982 to 2020, the NDVI of wetland increased significantly ($p < 0.01$) at the rate of 0.023 per decade, but the increasing rate gradually slowed. Approximately 89.85% of the total basin experienced significant increases in the NDVI of wetland in the growing season ($p < 0.05$). Correlation analysis and SQMK method both indicated that the NDVI of wetland responded most strongly to TMP. Asymmetric diurnal warming was detected during 2000–2020 by comparing the trend in TMX with that in TMN. The NDVI of wetland was positively correlated with TMX ($r = 0.14$, $p > 0.05$), but it was negatively correlated with TMN ($r = -0.012$, $p > 0.05$). The influence of PRE on wetland was weak and not significant. In addition, correlations between the NDVI of wetland and climatic factors were zonal in latitudinal and longitudinal directions. Under the SSP126 and SSP585 scenarios, the climate will become warmer, and the NDVI of wetland in the ARB is predicted to increase until 2080. Note that the negative effect of the rapid rise in TMN on the NDVI of wetland will gradually increase and might even exceed the positive effect of TMX in the future. The climatic factors affecting wetland NDVI are not only temperature and precipitation, but also solar radiation and wind speed et al. In addition to climate factors, human activities also have a great impact on wetlands, and human

factors were rarely discussed in this study. The findings of this study are expected to provide support for wetland conservation and sustainable management in the ARB.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

ZX: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. DM: Conceptualization, Funding acquisition, Software, Supervision, Writing – review & editing. XL: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. LL: Data curation, Formal Analysis, Methodology, Writing – review & editing. ZW: Conceptualization, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research 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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References

- Albarakat, R., and Lakshmi, V. (2019). Comparison of normalized difference vegetation index derived from Landsat, MODIS, and AVHRR for the Mesopotamian marshes between 2002 and 2018. *Remote Sens.* 11 (10), 1245. doi: 10.3390/rs11101245
- Amundson, R., Austin, A. T., Schuur, E. A., Yoo, K., Matzek, V., Kendall, C., et al. (2003).). Global patterns of the isotopic composition of soil and plant nitrogen. *Global biogeochemical cycles* 17 (1), 1031. doi: 10.1029/2002GB001903
- Avis, C. A., Weaver, A. J., and Meissner, K. J. (2011). Reduction in areal extent of high-latitude wetlands in response to permafrost thaw. *Nat. Geosci.* 4 (7), 444–448. doi: 10.1038/ngeo1160
- Bansal, S., Lishawa, S. C., Newman, S., Tangen, B. A., Wilcox, D., Albert, D., et al. (2019). Typha (Cattail) invasion in North American wetlands: Biology, regional problems, impacts, ecosystem services, and management. *Wetlands* 39, 645–684. doi: 10.1007/s13157-019-01174-7
- Bardecki, M. J. (1991). Wetlands and climate change: a speculative review. *Can. Water Resour. J.* 16 (1), 9–22. doi: 10.4296/cwrj1601009
- Beer, C., Reichstein, M., Tomelleri, E., Ciais, P., Jung, M., Carvalhais, N., et al. (2010). Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. *Science* 329 (5993), 834–838. doi: 10.1126/science.1184984
- Boulanger, Y., Taylor, A. R., Price, D. T., Cyr, D., and Sainte-Marie, G. (2018). Stand-level drivers most important in determining boreal forest response to climate change. *J. Ecol.* 106 (3), 977–990. doi: 10.1111/1365-2745.12892
- Burkett, V., and Kusler, J. (2000). Climate change: Potential impacts and interactions in wetlands of the united states. *J. Am. Water Resour. Assoc.* 36 (2), 313–320. doi: 10.1111/j.1752-1688.2000.tb04270.x
- Cao, R., Jiang, W., Yuan, L., Wang, W., Lv, Z., and Chen, Z. (2014). Inter-annual variations in vegetation and their response to climatic factors in the upper catchments of the Yellow River from 2000 to 2010. *J. Geographical Sci.* 24, 963–979. doi: 10.1007/s11442-014-1131-1
- Chen, H., Zhang, W., Gao, H., and Nie, N. (2018). Climate change and anthropogenic impacts on wetland and agriculture in the Songnen and Sanjiang Plain, Northeast China. *Remote Sens.* 10 (3), 356. doi: 10.3390/rs10030356
- Chen, Y., Sun, L., Xu, J., Liang, B., Wang, J., and Xiong, N. (2023). Wetland vegetation changes in response to climate change and human activities on the Tibetan Plateau during 2000–2015. *Front. Ecol. And Evol.* 11. doi: 10.3389/feco.2023.1113802
- Chen, Z., Wang, W., and Fu, J. (2020). Vegetation response to precipitation anomalies under different climatic and biogeographical conditions in China. *Sci. Rep.* 10 (1), 1–16. doi: 10.1038/s41598-020-57910-1
- Chu, H., Venevsky, S., Wu, C., and Wang, M. (2019). NDVI-based vegetation dynamics and its response to climate changes at Amur-Heilongjiang River Basin from 1982 to 2015. *Sci. Total Environ.* 650, 2051–2062. doi: 10.1016/j.scitotenv.2018.09.115
- Crabbe, R. A., Dash, J., Rodriguez-Galiano, V. F., Janous, D., Pavelka, M., and Marek, M. V. (2016). Extreme warm temperatures alter forest phenology and productivity in Europe. *Sci. Total Environ.* 563, 486–495. doi: 10.1016/j.scitotenv.2016.04.124
- Cramer, W., Bondeau, A., Woodward, F. I., Prentice, I. C., Betts, R. A., Brovkin, V., et al. (2001). Global response of terrestrial ecosystem structure and function to CO2 and climate change: results from six dynamic global vegetation models. *Global Change Biol.* 7 (4), 357–373. doi: 10.1046/j.1365-2486.2001.00383.x
- Cui, M. (2006). Status quo of wetlands of Heilongjiang river valley and the protection (in Chinese). *Forest Inventory and Planning* 1, 75–78. doi: 10.3969/j.issn.1671-3168.2006.01.020
- Di, L., Rundquist, D. C., and Han, L. (1994). Modelling relationships between NDVI and precipitation during vegetative growth cycles. *Int. J. Remote Sens.* 15 (10), 2121–2136. doi: 10.1080/01431169408954231
- Erwin, K. L. (2009). Wetlands and global climate change: the role of wetland restoration in a changing world. *Wetlands Ecol. Manage.* 17 (1), 71–84. doi: 10.1007/s11273-008-9119-1

- Fensholt, R., and Sandholt, I. (2005). Evaluation of MODIS and NOAA AVHRR vegetation indices with *in situ* measurements in a semi-arid environment. *Int. J. Remote Sens.* 26 (12), 2561–2594. doi: 10.1080/01431160500033724
- Fyfe, J. C., Gillett, N. P., and Zwiers, F. W. (2013). Overestimated global warming over the past 20 years. *Nat. Climate Change* 3 (9), 767–769. doi: 10.1038/nclimate1972
- Guo, Y., Zeng, J., Wu, W., Hu, S., Liu, G., Wu, L., et al. (2021). Spatial and temporal changes in vegetation in the Ruogai Region, China. *Forests* 12 (1), 76. doi: 10.3390/f12010076
- Han, D., Gao, C., Liu, H., Yu, X., Li, Y., Cong, J., et al. (2020). Vegetation dynamics and its response to climate change during the past 2000 years along the Amur River Basin, Northeast China. *Ecol. Indic.* 117, 106577. doi: 10.1016/j.ecolind.2020.106577
- Hasselmann, K., Latif, M., Hooss, G., Azar, C., Edenhofer, O., Jaeger, C. C., et al. (2003). The challenge of long-term climate change. *Science* 302 (5652), 1923–1925. doi: 10.1126/science.1090858
- Herfindal, I., Drever, M. C., Høgda, K. A., Podrutzny, K. M., Nudds, T. D., Grøtan, V., et al. (2012). Landscape heterogeneity and the effect of environmental conditions on prairie wetlands. *Landscape Ecol.* 27, 1435–1450. doi: 10.1007/s10980-012-9798-0
- Hurst, H. E. (1951). Long-term storage capacity of reservoirs. *Trans. Am. Soc. civil engineers* 116 (1), 770–799. doi: 10.1061/TACEAT.0006518j.rse.2004.10.006
- IPCC. (2007). Climate change 2007: The physical science basis (Cambridge: Cambridge University Press). Available at: <https://www.ipcc.ch>
- Jia, M., Mao, D., Wang, Z., Ren, C., Zhu, Q., Li, X., et al. (2020). Tracking long-term floodplain wetland changes: A case study in the China side of the Amur River Basin. *Int. J. Appl. Earth Observation Geoinf.* 92, 102185. doi: 10.1016/j.jag.2020.102185
- Jiang, L., Bao, A., Guo, H., and Ndayisaba, F. (2017). Vegetation dynamics and responses to climate change and human activities in Central Asia. *Sci. Total Environ.* 599, 967–980. doi: 10.1016/j.scitotenv.2017.05.012
- Jin, H., Sun, G., Yu, S., Jin, R., and He, R. (2008). Symbiosis of marshes and permafrost in Da and Xiao Hinggan Mountains in northeastern China. *Chin. Geographical Sci.* 18, 62–69. doi: 10.1007/s11769-008-0062-0
- Karl, T. R., Arguez, A., Huang, B., Lawrimore, J. H., McMahon, J. R., Menne, M. J., et al. (2015). Possible artifacts of data biases in the recent global surface warming hiatus. *Science* 348 (6242), 1469–1472. doi: 10.1126/science.aaa5632
- Kendall, M. G. (1948) *Rank correlation methods*. Available at: <https://psycnet.apa.org/record/1948-15040-000>.
- Li, F., Zhao, W., and Liu, H. (2013). The response of aboveground net primary productivity of desert vegetation to rainfall pulse in the temperate desert region of northwest China. *PloS One* 8 (9), e73003. doi: 10.1371/journal.pone.0073003
- Li, P., Wang, J., Liu, M., Xue, Z., Bagherzadeh, A., and Liu, M. (2021). Spatiotemporal variation characteristics of NDVI and its response to climate on the Loess Plateau from 1985 to 2015. *Catena* 203, 105331. doi: 10.1016/j.catena.2021.105331
- Li, Q., Yang, S., Xu, W., Wang, X. L., Jones, P., Parker, D., et al. (2015). China experiencing the recent warming hiatus. *Geophys. Res. Lett.* 42 (3), 889–898. doi: 10.1002/2014GL062773
- Liu, S., Li, W., Qiao, W., Wang, Q., Hu, Y., and Wang, Z. (2019). Effect of natural conditions and mining activities on vegetation variations in arid and semiarid mining regions. *Ecol. Indic.* 103, 331–345. doi: 10.1016/j.ecolind.2019.04.034
- Liu, X., Hu, G., Chen, Y., Li, X., Xu, X., Li, S., et al. (2018). High-resolution multi-temporal mapping of global urban land using Landsat images based on the Google Earth Engine Platform. *Remote Sens. Environ.* 209, 227–239. doi: 10.1016/j.rse.2018.02.055
- Liu, Y., Shen, X., Wang, Y., Zhang, J., Ma, R., Lu, X., et al. (2022). Spatiotemporal variation in aboveground biomass and its response to climate change in the marsh of Sanjiang Plain. *Front. Plant Sci.* 13, 920086. doi: 10.3389/fpls.2022.920086
- Lucht, W., Prentice, I. C., Myneni, R. B., Sitch, S., Friedlingstein, P., Cramer, W., et al. (2002). Climatic control of the high-latitude vegetation greening trend and Pinatubo effect. *Science* 296 (5573), 1687–1689. doi: 10.1126/science.1071828
- Mandelbrot, B. B., and Wallis, J. R. (1969). Robustness of the rescaled range R/S in the measurement of noncyclic long run statistical dependence. *Water Resour. Res.* 5 (5), 967–988. doi: 10.1029/WR005i005p0967
- Mao, D., Tian, Y., Wang, Z., Jia, M., Du, J., and Song, C. (2021). Wetland changes in the Amur River Basin: Differing trends and proximate causes on the Chinese and Russian sides. *J. Environ. Manage.* 280, 111670. doi: 10.1016/j.jenvman.2020.111670
- Mao, D., Wang, Z., Du, B., Li, L., Tian, Y., Jia, M., et al. (2020). National wetland mapping in China: A new product resulting from object-based and hierarchical classification of Landsat 8 OLI images. *ISPRS J. Photogrammetry Remote Sens.* 164, 11–25. doi: 10.1016/j.isprsjprs.2020.03.020
- Mao, D., Wang, Z., Luo, L., and Ren, C. (2012). Integrating AVHRR and MODIS data to monitor NDVI changes and their relationships with climatic parameters in Northeast China. *Int. J. Appl. Earth Observation Geoinf.* 18, 528–536. doi: 10.1016/j.jag.2011.10.007
- Meneses-Tovar, C. L. (2011). NDVI as indicator of degradation. *Unasylva* 62 (238), 39–46. Available at: <https://www.fao.org/docrep/015/i2560e/i2560e07.pdf>.
- Meng, M., Huang, N., Wu, M., Pei, J., Wang, J., and Niu, Z. (2019). Vegetation change in response to climate factors and human activities on the Mongolian Plateau. *PeerJ* 7, e7735. doi: 10.7717/peerj.7735
- Mosmann, V., Castro, A., Fraile, R., Dessens, J., and Sánchez, J. L. (2004). Detection of statistically significant trends in the summer precipitation of mainland Spain. *Atmospheric Res.* 70 (1), 43–53. doi: 10.1016/j.atmosres.2003.11.002
- O'Neill, B. C., Tebaldi, C., Van Vuuren, D. P., Eyring, V., Friedlingstein, P., Hurtt, G., et al. (2016). The scenario model intercomparison project (ScenarioMIP) for CMIP6. *Geosci. Model Dev.* 9 (9), 3461–3482. doi: 10.5194/gmd-9-3461-2016
- Pang, G., Wang, X., and Yang, M. (2017). Using the NDVI to identify variations in, and responses of, vegetation to climate change on the Tibetan Plateau from 1982 to 2012. *Quaternary Int.* 444, 87–96. doi: 10.1016/j.quaint.2016.08.038
- Pedersen, E., Weisner, S. E., and Johansson, M. (2019). Wetland areas' direct contributions to residents' well-being entitle them to high cultural ecosystem values. *Sci. Total Environ.* 646, 1315–1326. doi: 10.1016/j.scitotenv.2018.07.236
- Peng, J., Liu, Z., Liu, Y., Wu, J., and Han, Y. (2012). Trend analysis of vegetation dynamics in Qinghai–Tibet Plateau using Hurst Exponent. *Ecol. Indic.* 14 (1), 28–39. doi: 10.1016/j.ecolind.2011.08.011
- Peng, S., Piao, S., Ciais, P., Myneni, R. B., Chen, A., Chevallier, F., et al. (2013). Asymmetric effects of daytime and night-time warming on Northern Hemisphere vegetation. *Nature* 501 (7465), 88–92. doi: 10.1038/nature12434
- Peng, X., Zhang, T., Frauenfeld, O. W., Wang, S., Qiao, L., Du, R., et al. (2020). Northern Hemisphere greening in association with warming permafrost. *J. Geophys. Res.: Biogeosci.* 125 (1), e2019JG005086. doi: 10.1029/2019JG005086
- Phan, T. N., Kuch, V., and Lehnert, L. W. (2020). Land cover classification using Google Earth Engine and random forest classifier—The role of image composition. *Remote Sens.* 12 (15), 2411. doi: 10.3390/rs12152411
- Potter, K. M., Crane, B. S., and Hargrove, W. W. (2017). A United States national prioritization framework for tree species vulnerability to climate change. *New Forests* 48, 275–300. doi: 10.1007/s11056-017-9569-5
- Ren, Y., Mao, D., Li, X., Wang, Z., Xi, Y., and Feng, K. (2022). Aboveground biomass of marshes in Northeast China: spatial pattern and annual changes responding to climate change. *Front. Ecol. Evol.* 10, 1043811. doi: 10.3389/fevo.2022.1043811
- Ren, Y., Mao, D., Wang, Z., Yu, Z., Xu, X., Huang, Y., et al. (2023). China's wetland soil organic carbon pool: New estimation on pool size, change, and trajectory. *Global Change Biol.* 29 (21), 6139–6156. doi: 10.1111/gcb.16923
- Salimi, S., Almkutur, S. A., and Scholz, M. (2021). Impact of climate change on wetland ecosystems: A critical review of experimental wetlands. *J. Environ. Manage.* 286, 112160. doi: 10.1016/j.jenvman.2021.112160
- Salinger, M. J. (2005). Climate variability and change: past, present and future—an overview. Increasing Climate Variability and Change. *Reducing Vulnerability Agric. Forestry* 70 (1–2), 9–29. doi: 10.1007/1-4020-4166-7_3
- Sen, P. K. (1968). Estimates of the regression coefficient based on Kendall's tau. *J. Am. Stat. Assoc.* 63 (324), 1379–1389. doi: 10.1080/01621459.1968.10480934
- Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G. P., Bali, A. S., et al. (2020). Photosynthetic response of plants under different abiotic stresses: a review. *J. Plant Growth Regul.* 39, 509–531. doi: 10.1007/s00344-019-10018-x
- Shen, X., Liu, Y., Zhang, J., Wang, Y., Ma, R., Liu, B., et al. (2022). Asymmetric impacts of diurnal warming on vegetation carbon sequestration of marshes in the Qinghai Tibet Plateau. *Global Biogeochemical Cycles* 36 (7), e2022GB007396. doi: 10.1029/2022GB007396
- Sneyers, R. (1991) *On the statistical analysis of series of observations* (No. 143). Available at: <https://www.cabdirect.org/cabdirect/abstract/19912451385>.
- Su, B., Huang, J., Mondal, S. K., Zhai, J., Wang, Y., Wen, S., et al. (2021). Insight from CMIP6 SSP-RCP scenarios for future drought characteristics in China. *Atmos. Res.* 250, 105375. doi: 10.1016/j.atmosres.2020.105375
- Wan, S., Xia, J., Liu, W., and Niu, S. (2009). Photosynthetic overcompensation under nocturnal warming enhances grassland carbon sequestration. *Ecology* 90 (10), 2700–2710. doi: 10.1890/08-2026.1
- Wang, Q., Adiku, S., Tenhunen, J., and Granier, A. (2005). On the relationship of NDVI with leaf area index in a deciduous forest site. *Remote Sens. Environ.* 94 (2), 244–255. doi: 10.1016/j.rse.2004.10.006
- Wang, R., Xia, H., Qin, Y., Niu, W., Pan, L., Li, R., et al. (2020). Dynamic monitoring of surface water area during 1989–2019 in the hetao plain using landsat data in Google Earth Engine. *Water* 12 (11), 3010. doi: 10.3390/w12113010
- Wang, Y., Gao, R., Wang, X., Duan, L., Liu, T., and Li, D. (2021). Characterizing the properties of daily precipitation concentration in Amur River Basin of northeast China. *E3S Web of Conf.* (EDP Sciences), 117, 00003. doi: 10.1051/e3sconf/201911700003
- Wang, Y., Guan, J., Zhang, X., Du, P., Zhang, L., and Luo, M. (2019). “Characterizing the properties of daily precipitation concentration in Amur River Basin of northeast China,” in *E3S Web of Conferences*, Vol. 117. 00003 (EDP Sciences). doi: 10.1051/e3sconf/201911700003
- Wang, Y., Shen, X., Jiang, M., and Lu, X. (2020). Vegetation change and its response to climate change between 2000 and 2016 in marshes of the Songnen Plain, Northeast China. *Sustainability* 12 (9), 3569. doi: 10.3390/su12093569
- Wei, Y., Lu, H., Wang, J., Wang, X., and Sun, J. (2022). Dual influence of climate change and anthropogenic activities on the spatiotemporal vegetation dynamics over the Qinghai-Tibetan Plateau from 1981 to 2015. *Earth's Future* 10 (5), e2021EF002566. doi: 10.1029/2021EF002566

- Wu, N., Liu, A., Ye, R., Yu, D., Du, W., Chaolumeng, Q., et al. (2021). Quantitative analysis of relative impacts of climate change and human activities on Xilingol grassland in recent 40 years. *Global Ecol. Conserv.* 32, e01884. doi: 10.1016/j.gecco.2021.e01884
- Xie, Y., Luo, W., Wang, K., and Ren, B. (2008). Root growth dynamics of *Deyeuxia angustifolia* seedlings in response to water level. *Aquat. Bot.* 89 (3), 292–296. doi: 10.1016/j.aquabot.2008.03.003
- Yang, R., Li, X., Mao, D., Wang, Z., Tian, Y., and Dong, Y. (2020). Examining fractional vegetation cover dynamics in response to climate from 1982 to 2015 in the Amur river basin for SDG 13. *Sustainability* 12 (14), 5866. doi: 10.3390/su12145866
- Yang, X., Zhou, B., Xu, Y., and Han, Z. (2021). CMIP6 evaluation and projection of temperature and precipitation over China. *Adv. Atmospheric Sci.* 38, 817–830. doi: 10.1007/s00376-021-0351-4
- Yu, L. L., Xia, Z. Q., Li, J. K., and Cai, T. (2013). Climate change characteristics of Amur River. *Water Sci. Eng.* 6 (2), 131–144. doi: 10.3882/j.issn.1674-2370.2013.02.002
- Yuan, F., Liu, J., Zuo, Y., Guo, Z., Wang, N., Song, C., et al. (2020). Rising vegetation activity dominates growing water use efficiency in the Asian permafrost region from 1900 to 2100. *Sci. Total Environ.* 736, 139587. doi: 10.1016/j.scitotenv.2020.139587
- Zhang, K., Wang, Q., Chao, L., Ye, J., Li, Z., Yu, Z., et al. (2019). Ground observation-based analysis of soil moisture spatiotemporal variability across a humid to semi-humid transitional zone in China. *J. Hydrol.* 574, 903–914. doi: 10.1016/j.jhydrol.2019.04.087
- Zhang, Z., Bortolotti, L. E., Li, Z., Armstrong, L. M., Bell, T. W., and Li, Y. (2021). Heterogeneous changes to wetlands in the Canadian prairies under future climate. *Water Resour. Res.* 57 (7), e2020WR028727. doi: 10.1029/2020WR028727
- Zhou, S., Zhang, W., Wang, S., Zhang, B., and Xu, Q. (2021). Spatial-temporal vegetation dynamics and their relationships with climatic, anthropogenic, and hydrological factors in the Amur River basin. *Remote Sens.* 13 (4), 684. doi: 10.3390/rs13040684
- Zou, Y., Duan, X., Xue, Z., Mingju, E., Sun, M., Lu, X., et al. (2018). Water use conflict between wetland and agriculture. *J. Environ. Manage.* 224, 140–146. doi: 10.1016/j.jenvman.2018.07.052



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Sediment accretion and nutrient enrichment enhance the growth and vegetative propagation of *Phalaris arundinacea* growing within a *Carex thunbergii* stand

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Sediment accretion (burial) and nutrient enrichment may exert a synergistic influence on the growth and distribution of macrophytes in floodplain wetlands; however, this phenomenon has rarely been examined. In this study, we investigated the effects of sediment accretion and nutrient enrichment on the growth and vegetative propagation of *Phalaris arundinacea* within a *Carex thunbergii* stand (one *P. arundinacea* ramet within 25 *C. thunbergii* ramets) using a factorial sediment burial (0, 3, and 6 cm) and nutrient addition (low, medium, and high) experimental design. High sediment burial (6 cm) without nutrient addition decreased the aboveground and total biomass of *C. thunbergii* but did not affect *P. arundinacea*, indicating that *P. arundinacea* is more tolerant to sediment burial than *C. thunbergii*. Moderate sediment burial (3 cm) with nutrient addition enhanced the aboveground and total biomass of *P. arundinacea* but did not affect *C. thunbergii*, indicating that *P. arundinacea* may gain a growth advantage over *C. thunbergii* under moderate sedimentation. High sediment burial with nutrient addition increased the number of rhizomes and ramets produced by *P. arundinacea* but did not affect *C. thunbergii*, indicating that the relative abundance of *P. arundinacea* may increase within the *Carex* community under high sedimentation conditions. Based on these results, it can be concluded that an increased sedimentation rate facilitates the invasion of *P. arundinacea* into *Carex* grasslands, and this invasion is further enhanced by nutrient enrichment. Therefore, management measures should be taken to reduce the sediment load and nutrient input to prevent *Carex* grasslands invasion by *P. arundinacea* and maintain the ecological function of floodplain wetlands.

KEYWORDS

Carex grasslands, floodplain wetlands, macrophyte distribution, sediment deposition, vegetative propagation

1 Introduction

Macrophyte vegetation in floodplain wetlands plays crucial ecological roles such as controlling sediment nutrients and providing food sources and habitats for wildlife (Dar et al., 2020; Dou et al., 2023). Macrophytic species are typically distributed along hydrological gradients and exhibit dynamic zonal patterns (Zhang et al., 2020). Hydrological conditions and sediment properties influence their growth and distribution, thereby influencing the ecological function of floodplain wetlands (Chen et al., 2014; Liu et al., 2018; Zhang et al., 2020; Jing et al., 2021; Moritz et al., 2022).

Flood regimes and sediment properties have changed considerably in many freshwater environments because of climatic changes and anthropogenic activities, which may influence the distribution patterns of macrophytes (Blakey et al., 2017; Chen et al., 2018; Ji et al., 2021; Huang et al., 2022). In floodplain wetlands, sedimentation co-occurs with flooding (Maun, 1998). After flooding, sediments are deposited on floodplain wetlands, ranging in depth from a few millimeters to several centimeters per year (Ou et al., 2019; Baniya et al., 2020; Geng et al., 2021; Kretz et al., 2021; Huang et al., 2022; Zeng et al., 2023). Wetland macrophytes with different growth forms respond differently to sediment accretion and have evolved various morphological and physiological strategies to acclimate to sediment burial stress (Li et al., 2015). Caulescent macrophytes, such as *Phalaris arundinacea* and *Polygonum hydropiper*, elongate the internode length, release dominant buds on their stems, and increase stem biomass to escape sediment accretion (Chen et al., 2014, 2017a). Non-caulescent macrophytes, such as *Carex brevicuspis* and *Vallisneria spiralis*, may not respond as successfully to sediment accretion as caulescent macrophytes (Pan et al., 2012). Thus, the differential responses to sediment accretion among macrophyte species may alter macrophyte distribution (Owen et al., 2004).

Nutrients in floodwater, such as nitrogen and phosphorus, may be retained in the sediment after flooding, facilitating the acclimation of wetland macrophytes to sediment stress (Ou et al., 2019; Huang et al., 2022; Zeng et al., 2023). Nutrients in the sediment may play an important role in stimulating plant growth following sediment burial. However, their effects on macrophyte growth vary from positive to negative, particularly following deep sediment burial (Chen et al., 2017b). Therefore, the effects of sediment nutrient enrichment on growth and vegetative reproduction may differ among macrophyte species.

Moreover, sediment accretion and nutrient enrichment are concurrent processes that may exert a synergistic influence on the growth and distribution of macrophytes but have rarely been examined. In this study, we investigated the effects of sediment accretion and nutrient enrichment on the growth and propagation of *Phalaris arundinacea* in a *C. thunbergii* stand. *Carex* is the dominant species in the floodplain wetlands of the Yangtze River basin, with *P. arundinacea* sparsely distributed within the *Carex* vegetation (Jing et al., 2017). Recently, the abundance and distribution of *P. arundinacea* has increased, especially in

lacustrine wetlands with considerable sedimentation (Nelson and Anderson, 2015). Sediment accretion and nutrient enrichment are potential drivers of these changes, although empirical evidence supporting this is limited. We tested two hypotheses: (1) *P. arundinacea* can tolerate higher sediment accretion than *C. thunbergii* because it can escape sediment burial more efficiently than *C. thunbergii*, and (2) *P. arundinacea* may benefit more from nutrient enrichment than *C. thunbergii* because it can utilize nutrients more efficiently than *C. thunbergii* (Wetzel and van der Valk, 1998; Nelson and Anderson, 2015).

2 Materials and methods

2.1 Study site

Shengjin Lake (30°15'–30°30'N, 116°55'–117°15'E), located in the lower reaches of the Yangtze River, was listed as an internationally important wetland in 2015 (Wang et al., 2018). Influenced by the subtropical monsoon climate, wetlands tend to be inundated from June to October and are exposed from November to May. *Carex thunbergii* is the dominant plant community in the water-level fluctuation zone of this wetland.

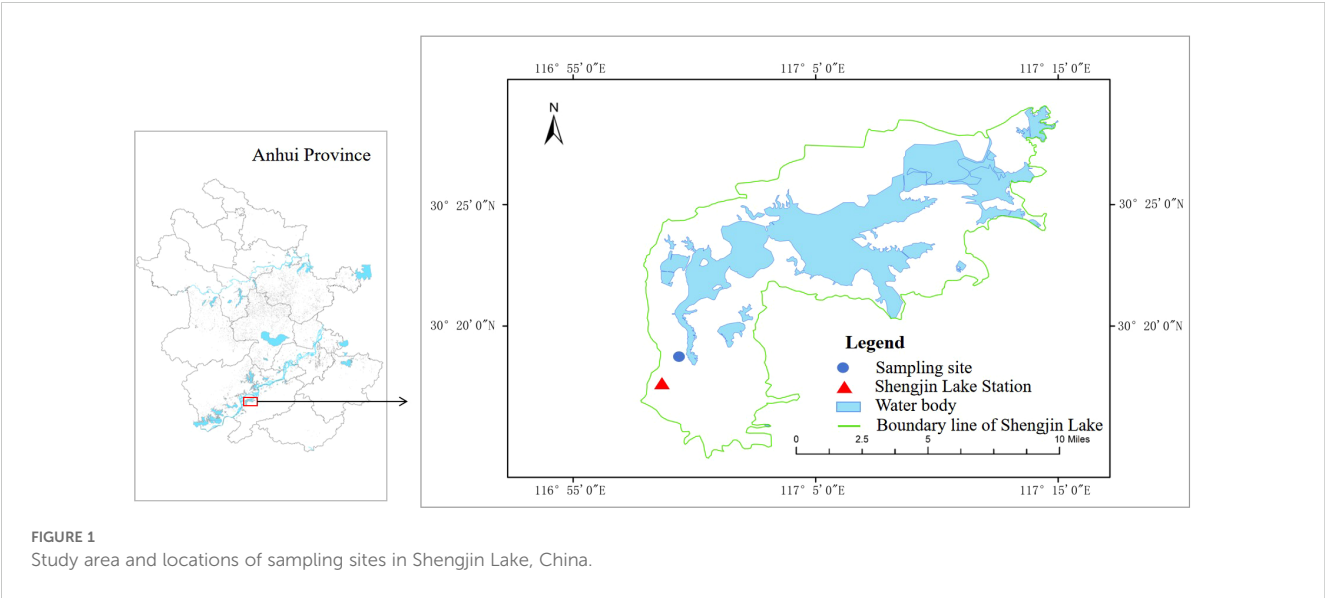
2.2 Study species

Carex thunbergii is a rhizomatous sedge widely distributed in lakes and wet grasslands of eastern Asia (Dai et al., 2010). The pseudoculms of *C. thunbergii* have overlapping sheaths and are usually 40–100 cm high. *Carex thunbergii* often forms a monodominant community with a coverage of approximately 100% (Jia et al., 2020). The aboveground shoots of *C. thunbergii* are submerged and decompose during the flooding season, and new shoots emerge from the belowground rhizome buds immediately after the floodwaters recede (Chen et al., 2014).

Phalaris arundinacea is widely distributed in riparian and lacustrine wetlands in the subtropical and temperate regions of the Northern Hemisphere (Wu and Phillips, 2006). The erect culms are reed-like and 60–150 cm in height. They produce extensively spread rhizomes, enabling them to reproduce vigorously and spread aggressively (Wu and Phillips, 2006; Chen et al., 2017a). In the *Carex* community, *P. arundinacea* is a commonly observed companion species; however, its relative abundance and importance have increased considerably in recent times, which may affect the structure and function of *Carex* grasslands (Chen et al., 2017a).

2.3 Experimental design

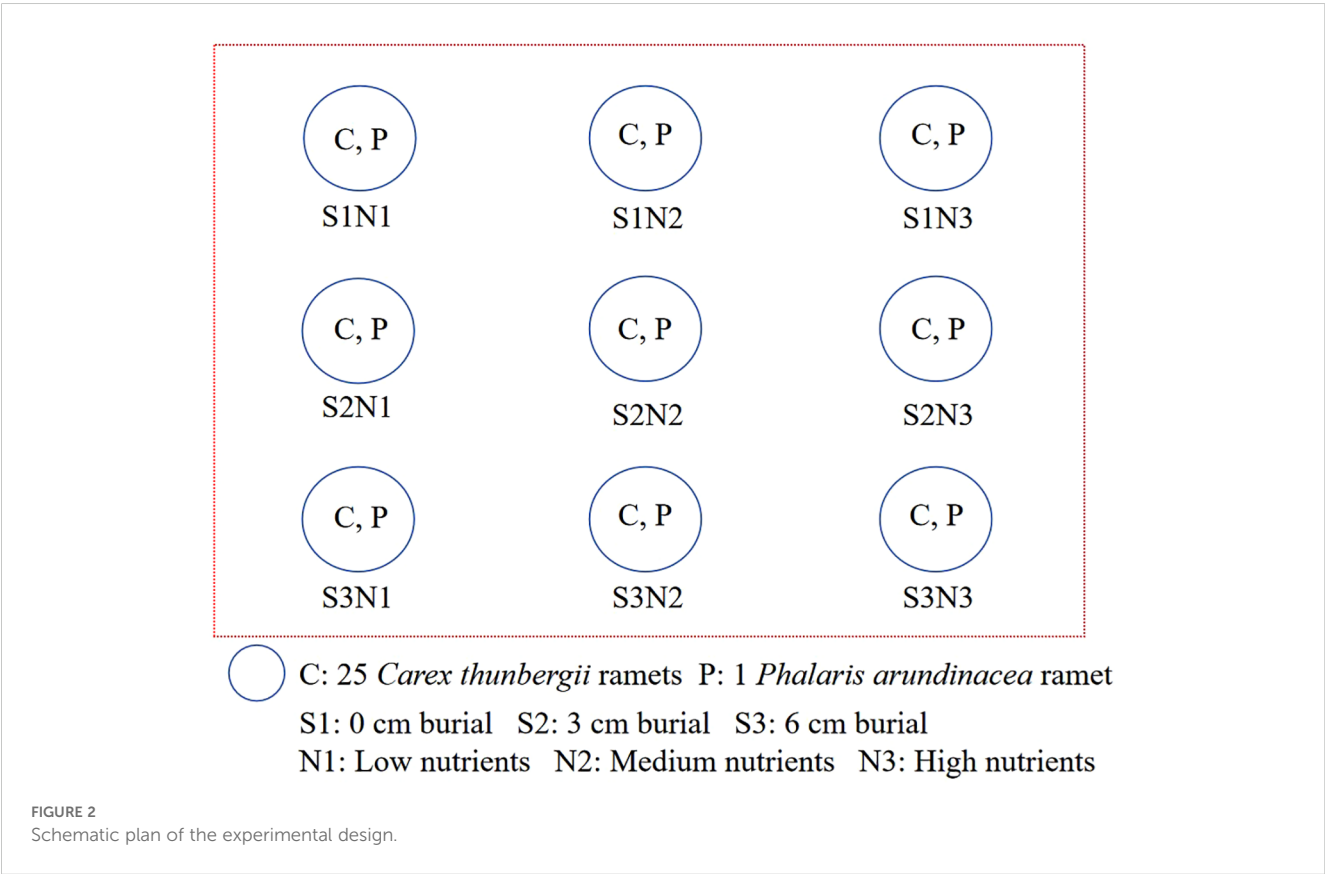
The experiment was conducted at the Shengjin Lake Station for Wetland Ecosystem Research, Dongzhi County, Anhui Province, China (Figure 1). Before the experiment, we surveyed the ramet density of *P. arundinacea* in *Carex* grasslands in Shengjin Lake. The ramet density of *P. arundinacea* ranges from 1 to 112 ramets m⁻²,



and density of *C. thunbergii* ranges from 400 to 2240 ramets m^{-2} . Then, young ramets with soil from the Yaozui section of the lake were dug up and transported to the research station on April 3, 2023. Ramets were cultivated in a nursery bed containing a 10 cm soil/sand mixture (1:1 v/v) sourced from the Shengjin Lake. On April 10, one *P. arundinacea* ramet and 25 *C. thunbergii* ramets of similar heights (4–6 leaves and 14–20 cm in height) were planted in plastic buckets containing wetland soil 15 cm deep (each plastic bucket was 20 cm in diameter and 30 cm in height). There are many small holes (< 3 mm

at the bottom of the buckets, which allowed water to penetrate. The densities of *C. thunbergii* and *P. arundinacea* in the experiment were 796 and 32 ramets m^{-2} , respectively, which were within the density range observed in the field.

The experimental design was a randomized block with five replicates (Figure 2). The experiment was conducted in separate outdoor water tanks (98 × 76 × 68 cm). Three sediment burial depths (0, 3, and 6 cm) and three levels of nutrient addition (low, medium, and high) were used in the experiment. The low-nutrient



sediment was sand collected from Shengjin Lake, which contained 0.03 mg kg⁻¹ total nitrogen, 0.02 mg kg⁻¹ total phosphorus, and 0.48 mg kg⁻¹ total potassium. The medium- and high-nutrient sediments were created by mixing lake sand homogeneously with 1 or 2 g of Osmocote slow-release fertilizer (501 Osmocote Plus [N-P-K, 15-10-12 + 2 MgO + TE ICL], Belgium N.V.). For the 3 and 6 cm sediment accretion with nutrient addition treatments, medium- or high-nutrient sand was added to the container to the corresponding depth. For the 0 cm sediment accretion with nutrient addition treatment, 1 or 2 g of slow-release fertilizer was added to the soil surface and covered with a thin layer of sand (< 0.2 cm). The water level in the tanks was maintained at 15 cm (0 cm for each plant) during the experiment. Plants were monitored weekly, and new ramets were marked with plastic labels.

2.4 Harvest and measurement

Plants were harvested on August 29, 2023, 20 weeks after treatment. The plants were carefully removed from the plastic containers to ensure the integrity of the aboveground and belowground parts. The plants were washed with tap water to remove attached sediment. Ramet height, root length, and number of ramets and rhizomes of each *P. arundinacea* parent ramet were measured. We also measured the number of rhizomes and ramets produced by each *C. thunbergii* parent ramet in the plastic containers. We then selected five *C. thunbergii* parent ramets from each container to measure the ramet height and root length. The ramets of *P. arundinacea* and *C. thunbergii* were separated into roots, shoots, and leaves. The biomass of each plant part was measured after drying at 85°C for 48 h in an oven.

2.5 Data analysis

Two-way ANOVA was performed to evaluate the main effects and interactions of sediment burial depth and nutrient addition levels on biomass accumulation, biomass allocation, ramet height, root length, and the number of new ramets and rhizomes. Multiple

comparisons of the means were performed using Tukey's test at a significance level of 0.05. Data were log¹⁰-transformed, if necessary, to reduce the heterogeneity of variance, and homogeneity was confirmed using Levene's test. All analyses were performed using the "car" and "multcomp" package in R program (v. 4.1.2; R Core Team 2020).

3 Results

3.1 Biomass accumulation and allocation

Aboveground, belowground, and total biomass of *P. arundinacea* were significantly affected by burial depth and nutrient levels ($P < 0.01$; Table 1). Sediment accretion increased the aboveground, belowground, and total biomass of *P. arundinacea* growing in medium- and high-nutrient sediments (Figures 3A–C). Nutrient addition increased the aboveground and total biomass of *P. arundinacea* growing in the 3 and 6 cm sediment burial treatments (Figures 3A, B).

The total and belowground biomass of *C. thunbergii* were only affected by the nutrient levels (Table 1). Nutrient addition increased the total and belowground biomass of *C. thunbergii* growing in the 6 cm sediment burial treatment (Figures 3D, F). The aboveground biomass of *C. thunbergii* was significantly affected by burial depth and nutrient levels ($P < 0.05$; Table 1). When growing in low-nutrient sediment, the aboveground biomass of *C. thunbergii* was higher in the 3 cm sediment layer than in the 6 cm sediment layer (Figure 3E). Nutrient addition increased the aboveground biomass of *C. thunbergii* growing in the 6 cm sediment burial treatment (Figure 3E).

The ratio of shoot biomass of *P. arundinacea* to the shoot biomass of *C. thunbergii* ranges from 9.04% to 48.47% across treatments (Figure 4A). The ratio of root biomass of *P. arundinacea* to the root biomass of *C. thunbergii* ranges from 2.23% to 7.28% across treatments (Figure 4B). The ratio of total biomass of *P. arundinacea* to the total biomass of *C. thunbergii* ranges from 5.17% to 28.78% across treatments (Figure 4C). The ratios of the shoot, root, and total biomass of *P. arundinacea* to the shoot, root, and total biomass of *C. thunbergii* were higher in the 6 cm sediment burial treatment with high nutrient addition than that in the 0 cm burial depth treatment without nutrient addition (Figures 4A–C).

TABLE 1 Summary of two-way ANOVA (F -values) for total biomass, aboveground biomass, belowground biomass, ramet height, root length, and number of rhizomes and ramets in *Phalaris arundinacea* and *Carex thunbergii* grown at three sedimentation depths and three nutrient levels.

	<i>Phalaris arundinacea</i>			<i>Carex thunbergii</i>		
	Burial depth (B)	Nutrient level (N)	B × N	Burial depth (B)	Nutrient level (N)	B × N
Total biomass	28.117***	26.301***	5.864**	2.337 ^{ns}	4.662*	1.286 ^{ns}
Aboveground biomass	28.059***	22.675***	5.937***	5.659**	10.340***	3.042*
Belowground biomass	13.392***	23.024***	2.726*	1.091 ^{ns}	6.220**	1.369 ^{ns}
Ramet height	14.291***	9.566***	1.807 ^{ns}	4.715*	0.459 ^{ns}	3.889*
Root length	5.606**	4.095*	0.998 ^{ns}	0.592 ^{ns}	13.204***	0.514 ^{ns}
No. of rhizomes	5.283**	2.768 ^{ns}	3.013*	3.766*	0.265 ^{ns}	0.089 ^{ns}
No. of ramets	10.088***	11.192***	5.672**	1.027 ^{ns}	0.995 ^{ns}	0.501 ^{ns}

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ^{ns} $P > 0.05$.

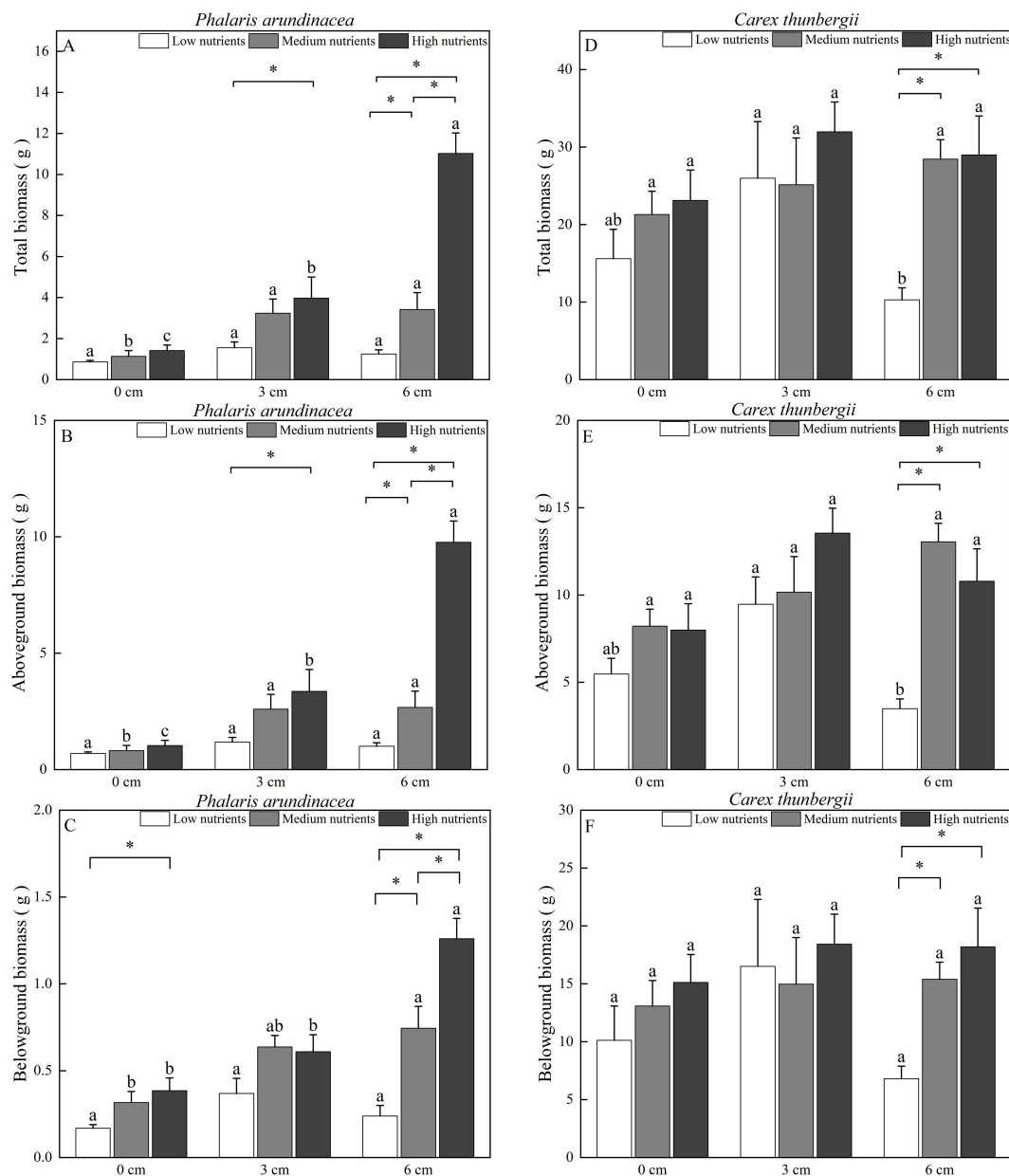


FIGURE 3

Biomass accumulation in *Phalaris arundinacea* and *Carex thunbergii* at three sediment burial depths with three nutrient levels. Total biomass, Aboveground biomass and Belowground biomass of *Phalaris arundinacea* (A–C), Total biomass, Aboveground biomass and Belowground biomass of *Carex thunbergii* (D–F). Different lowercase letters indicate significant differences among burial depth treatments at the same nutrient level ($P < 0.05$). * indicates significant differences among different nutrient level treatments at the same sediment burial depth ($P < 0.05$).

3.2 Ramet height and root length

The ramet height of *P. arundinacea* was affected by burial depth and nutrient levels ($P < 0.05$; Table 1). When growing in high-nutrient sediments, the ramet height of *P. arundinacea* increased with sediment depth. Nutrient addition increased the ramet height of *P. arundinacea* in both the 3 and 6 cm burial depth treatments (Figure 5A). The ramet height of *C. thunbergii* was affected by burial depth and had a significant interaction with nutrient levels (Table 1). Without nutrient addition, the ramet height of *C.*

thunbergii was shorter in the 6 cm burial depth treatment than that in the 3 cm burial depth treatment (Figure 5C). At a sediment burial depth of 6 cm, addition of nutrients increased the ramet height of *C. thunbergii* (Figure 5C).

The root length of *P. arundinacea* was affected by burial depth and nutrient levels ($P < 0.05$; Table 1). Without nutrient addition, the root length of *P. arundinacea* was greater in the 6 cm sediment burial treatment than that in 0 cm burial depth treatment (Figure 5B). Without sediment burial, nutrient addition increased the root length of *P. arundinacea* (Figure 5B). The root length of *C.*

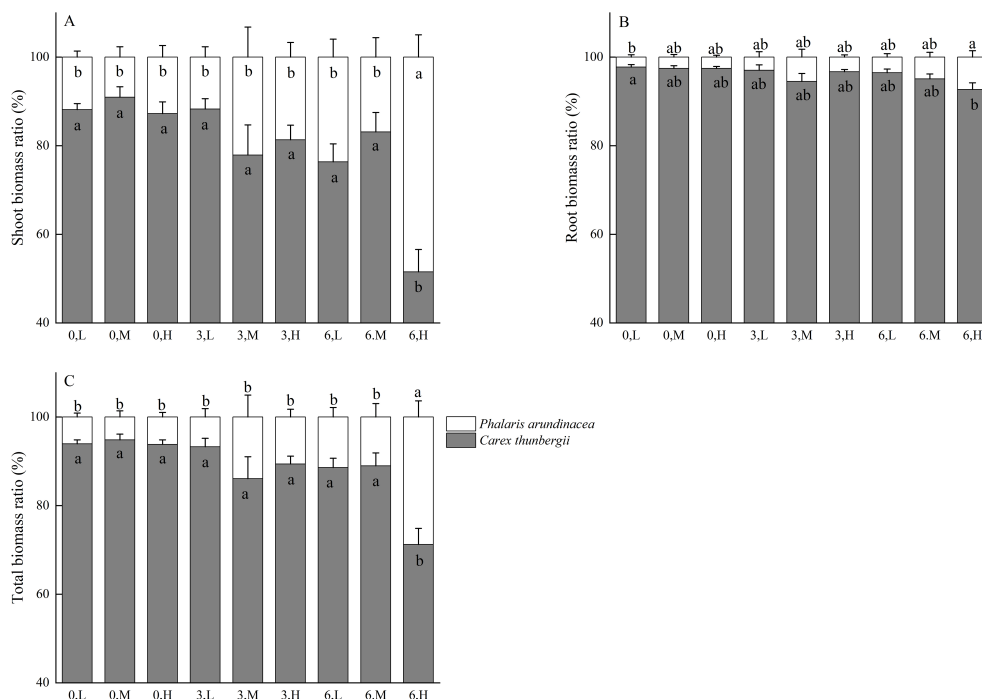


FIGURE 4

Shoot, root, and total mass ratio of *Phalaris/Carex* at three burial depths and three nutrient levels (A–C). Different lowercase letters indicate significant differences among the treatments ($P < 0.05$). Treatments: 0 cm burial depth with low nutrients (0, L); 0 cm burial depth with medium nutrients (0, M); 0 cm burial depth with high nutrients (0, H); 3 cm burial depth with low nutrients (3, L); 3 cm burial depth with medium nutrients (3, M); 3 cm burial depth with high nutrients (3, H); 6 cm burial depth with low nutrients (6, L); 6 cm burial depth with medium nutrients (6, M); and 6 cm burial depth with high nutrient levels (6, H).

thunbergii was only affected by nutrient levels (Table 1). At a sediment burial depth of 6 cm, nutrient addition increased the root length of *C. thunbergii* (Figure 5D).

3.3 Number of rhizomes and ramets

The number of rhizomes produced by *P. arundinacea* was significantly affected by the sediment burial depth, with significant interactions with nutrient levels ($P < 0.05$; Table 1). When grown under medium nutrient conditions, *P. arundinacea* produced more rhizomes in the 6 cm burial depth treatment than in the 0 cm burial depth treatment (Figure 6A). Nutrient addition increased the number of rhizomes produced by *P. arundinacea* when subjected to 3 and 6 cm sediment burial (Figure 6A). The number of rhizomes and ramets produced by *C. thunbergii* was neither affected by burial depth or nutrient levels (Figures 6C, D).

The number of ramets produced by *P. arundinacea* was significantly affected by burial depth and nutrient levels ($P < 0.05$; Table 1). With the addition of nutrients, the number of ramets produced by *P. arundinacea* was higher in the 6 cm burial depth treatment than in the 0 cm burial depth treatment (Figure 6B). At a burial depth of 6 cm, addition of nutrients increased the number of

ramets produced by *P. arundinacea* (Figure 6B). The number of ramets produced by *C. thunbergii* was neither affected by burial depth or nutrient levels (Table 1; Figure 6D).

4 Discussion

4.1 High sediment accretion inhibited *C. thunbergii* growth but did not affect *P. arundinacea*

In our study, 6 cm sediment burial without nutrient addition decreased the aboveground and total biomass of *C. thunbergii* but had no effect on *P. arundinacea* (Figures 3A–D). As a non-stem sedge, *C. thunbergii* can escape sediment burial by producing spreading ramets via rhizome elongation (Li et al., 2015). However, projecting new ramets onto a sediment surface consumes large quantities of carbohydrate reserves (Pan et al., 2012; Chen et al., 2017b). Consequently, the biomass of *C. thunbergii* decreases with heavy sediment burial. In contrast, *P. arundinacea* can escape sediment burial through rapid stem growth and internode elongation (Chen et al., 2014, 2017a). Therefore, *P. arundinacea* can tolerate higher sediment accretion than *C. thunbergii*, which is consistent with hypothesis 1. Hypothesis 1 was

also supported by the higher proportional biomass of *P. arundinacea* to *C. thunbergii* in the heavy sedimentation treatment (Figure 4C).

4.2 Moderate sedimentation enhanced *P. arundinacea* growth but did not affect *C. thunbergii*

Our results showed that moderate sediment burial with nutrients enhanced the aboveground and total biomass of *P. arundinacea* but had no effect on *C. thunbergii*. Studies have indicated that moderate sedimentation stimulates macrophyte growth (Maun, 1998; Gilbert and Ripley, 2010; Chen et al., 2017a; Fan et al., 2018). For example, moderate sedimentation increases the growth of *Spartina patens*, *Scirpus marigueter*, and *S. alterniflora* (Halun et al., 2002; Matzke and Elsey-Quirk, 2018; Xiao et al., 2023). The stimulation of macrophyte growth upon sedimentation may be attributed to increased soil volume, nutrients, moisture, and microbial activity (Maun, 1998). For *P. arundinacea*, increased nutrient availability may have been the primary

contributor stimulating growth during sediment burial (Katagiri et al., 2011; Chen et al., 2017a).

Nevertheless, we did not observe a stimulatory effect of moderate sedimentation on *C. thunbergii* growth. Non-caulescent *Carex* species adapt to sedimentation by changing their clonal growth from economically clumped ramets to costively spreading ramets (Bernard, 1990; Chen et al., 2010; Li et al., 2015). Even with moderate sediment burial (3 cm), the proportion of spreading ramets produced by *C. brevicuspis* increased significantly, indicating that *Carex* species were sensitive to sediment burial. Furthermore, the increased growth of *P. arundinacea* after sediment deposition may have suppressed the growth of native species through light interception (Kercher and Zedler, 2004). *Phalaris arundinacea* can effectively utilize resources such as light and sediment nutrients to produce additional ramets, enhancing its competitive capacity (Martina and von Ende, 2012; Chen et al., 2017a; Winikoff et al., 2020). Therefore, *P. arundinacea* may have a growth advantage over *C. thunbergii* under moderate sedimentation conditions.

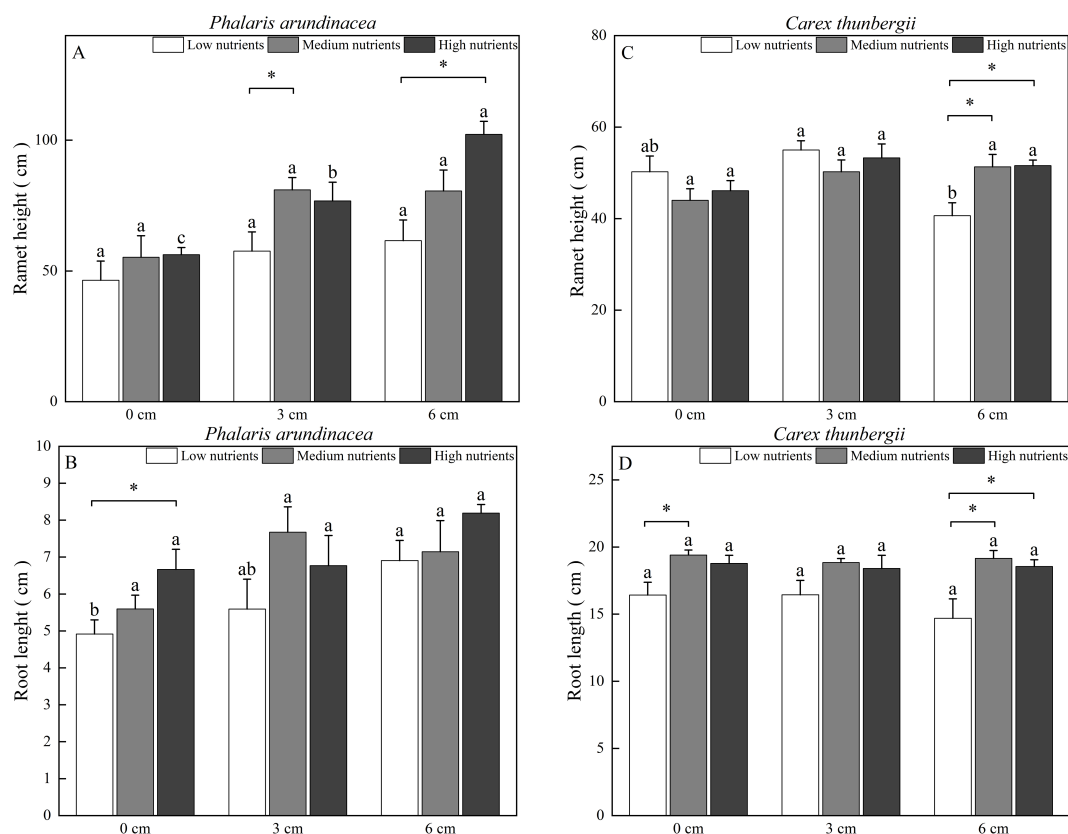


FIGURE 5

Ramet height and root length of *Phalaris arundinacea* (A, B) and *Carex thunbergii* (C, D) growing at three sediment burial depths with three nutrient levels. Different lowercase letters indicate significant differences among burial depth treatments at the same nutrient level ($P < 0.05$). * indicates significant differences among different nutrient level treatments at the same sediment burial depth ($P < 0.05$).

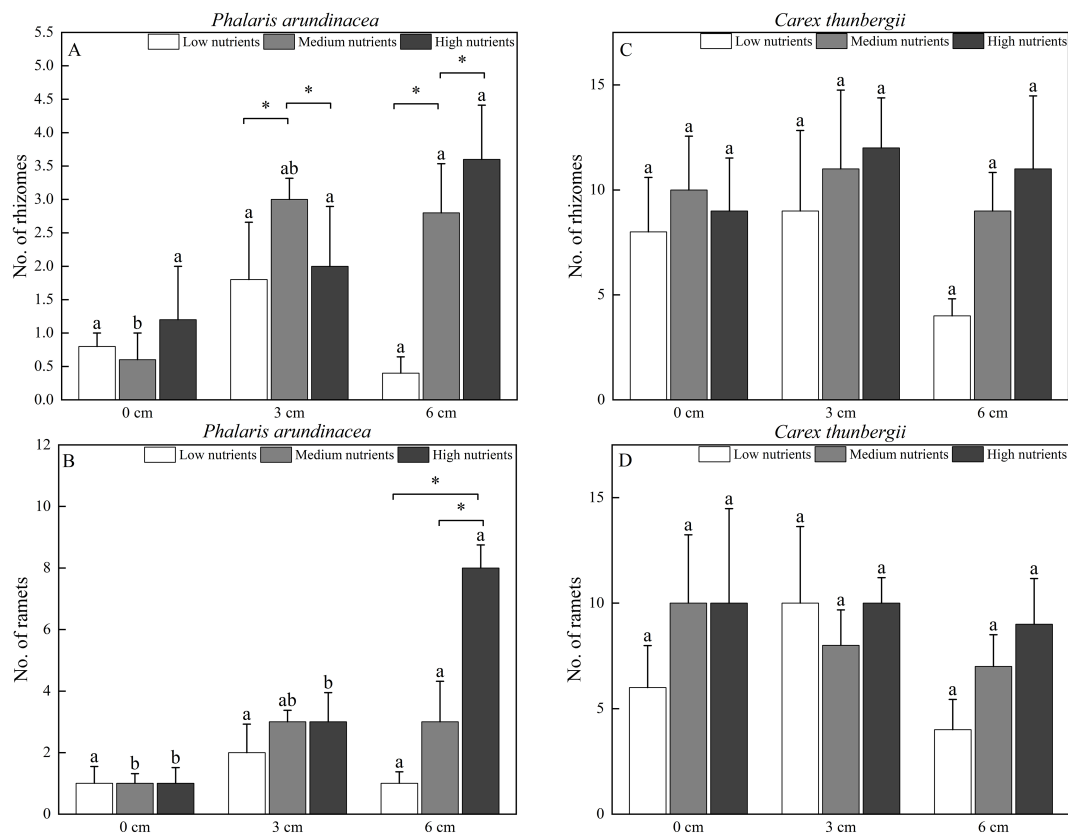


FIGURE 6

The number of rhizomes and ramets of *Phalaris arundinacea* (A, B) and *Carex thunbergii* (C, D) growing at three sediment burial depths with three nutrient levels. Different lowercase letters indicate significant differences among burial depth treatments at the same nutrient level ($P < 0.05$). * indicates significant differences among different nutrient level treatments at the same sediment burial depth ($P < 0.05$).

4.3 Nutrient enrichment increased the vegetative propagation of *P. arundinacea* but not *C. thunbergii*

Our results indicated that high sediment accretion with nutrients increased the number of rhizomes and ramets produced by *P. arundinacea* but did not affect *C. thunbergii*. This result is consistent with hypothesis 2: *P. arundinacea* benefits more from nutrient enrichment than *C. thunbergii* does. Other studies also found that nutrient addition increased the abundance and production of *P. arundinacea* in wet prairie assemblages during *P. arundinacea* invasion (Kercher and Zedler, 2004; Weston et al., 2021). As an opportunistic invader, *P. arundinacea* may efficiently utilize fluctuating resources and produce additional ramets to occupy its habitat (Davis et al., 2000; Kettenring et al., 2019). In contrast, *Carex* species may adopt a conservative reproductive strategy to manage resource availability, that is, produce a relatively constant number of rhizomes and ramets but change individual size (Chen et al., 2016). Therefore, with increasing

nutrient input in floodplain wetlands, the abundance of *P. arundinacea* may increase in *Carex* grasslands.

5 Conclusion

Our study indicated that high sediment accretion inhibited the growth of *C. thunbergii* but had no effect on *P. arundinacea*. Moderate sedimentation enhanced the growth of *P. arundinacea* but did not affect *C. thunbergii*. Nutrient enrichment of sediments increased the vegetative propagation of *P. arundinacea* but did not affect *C. thunbergii*. From these results, we inferred that in floodplain wetlands, *P. arundinacea* may increase in abundance and coverage when growing in *Carex* grasslands subjected to sediment accretion and nutrient enrichment. Therefore, to maintain the ecological function of floodplain wetlands, management measures should be implemented to reduce sediment load and nutrient input to protect *Carex* grasslands from *P. arundinacea* invasion.

Data availability statement

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

Author contributions

XG: Data curation, Writing – original draft. JZ: Methodology, Writing – review & editing. WS: Data curation, Writing – review & editing. XS: Data curation, Investigation, Writing – review & editing. JL: Methodology, Writing – review & editing. XC: Conceptualization, Methodology, Writing – review & editing.

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References

- Baniya, M. B., Asaeda, T., Fujino, T., Jayasanka, S., Muhetaer, G., and Li, J. H. (2020). Mechanism of riparian vegetation growth and sediment transport interaction in floodplain: A dynamic riparian vegetation model (DRIPVEM) approach. *Water* 12, 77. doi: 10.3390/w12010077
- Bernard, J. M. (1990). Life history and vegetative reproduction in *Carex*. *Botany* 68, 1441–1448. doi: 10.1139/B90-182
- Blakey, R. V., Kingsford, R. T., Law, B. S., and Stoklosa, J. (2017). Floodplain habitat is disproportionately important for bats in a large river basin. *Biol. Conserv.* 215, 1–10. doi: 10.1016/j.biocon.2017.08.030
- Chen, X. S., Deng, Z. M., Xie, Y. H., Li, F., Hou, Z. Y., Li, X., et al. (2014). Effects of sediment burial disturbance on the vegetative propagation of *Phalaris arundinacea* with different shoot statuses. *Aquat. Ecol.* 48, 409–416. doi: 10.1007/s10452-014-9493-0
- Chen, X. S., Deng, Z. M., Xie, Y. H., Li, F., Hou, Z. Y., and Wu, C. (2016). Consequences of repeated defoliation on belowground bud banks of *Carex brevicuspis* (Cyperaceae) in the dongting lake wetlands, China. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.01119
- Chen, M. S., Ding, S. M., Chen, X., Sun, Q., Fan, X. F., Lin, J., et al. (2018). Mechanisms driving phosphorus release during algal blooms based on hourly changes in iron and phosphorus concentrations in sediments. *Water Res.* 133, 153–164. doi: 10.1016/j.watres.2018.01.040
- Chen, J. S., Lei, N. F., and Dong, M. (2010). Clonal integration improves the tolerance of *Carex praecleara* to sand burial by compensatory response. *Acta Oecologica-International J. Ecol.* 36, 23–28. doi: 10.1016/j.actao.2009.09.006
- Chen, X. S., Liao, Y. L., Xie, Y. H., Li, F., Deng, Z. M., Hou, Z. Y., et al. (2017b). Concurrent effects of sediment accretion and nutrient availability on the clonal growth strategy of *Carex brevicuspis*-A wetland sedge that produces both spreading and clumping ramets. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01685
- Chen, X. S., Liao, Y. L., Xie, Y. H., Wu, C., Li, F., Deng, Z. M., et al. (2017a). The combined effects of sediment accretion (burial) and nutrient enrichment on the growth and propagation of *Phalaris arundinacea*. *Sci. Rep.* 7, 39963. doi: 10.1038/srep39963
- Dai, L. K., Liang, S. Y., Zhang, S. R., Tang, Y. C., Koyama, T., Tucker, G. C., et al. (2010). *Flora of China (Cyperaceae)* Vol. 23 (Beijing: Science Press).
- Dar, S. A., Bhat, S. U., Rashid, I., and Dar, S. A. (2020). Current status of wetlands in srinagar city: threats, management strategies, and future perspectives. *Front. Environ. Sci.* 7. doi: 10.3389/fenvs.2019.00199
- Davis, M., Grime, J., and Thompson, K. (2000). Fluctuating resources in plant communities: A general theory of invasibility. *J. Ecol.* 88, 528–536. doi: 10.1046/j.1365-2745.2000.00473.x
- Dou, X. Y., Guo, H. D., Zhang, L., Liang, D., Zhu, Q., Liu, X. T., et al. (2023). Dynamic landscapes and the influence of human activities in the Yellow River Delta wetland region. *Sci. Total Environ.* 899, 166239. doi: 10.1016/j.scitotenv.2023.166239
- Fan, B. L., Zhao, C. M., Zhang, X. W., and Sun, K. (2018). Impacts of sand burial and wind erosion on regeneration and growth of a desert clonal shrub. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01696
- Geng, M. M., Wang, K. L., Yang, N., Li, F., Zou, Y. A., Chen, X. S., et al. (2021). Spatiotemporal water quality variations and their relationship with hydrological conditions in Dongting Lake after the operation of the Three Gorges Dam, China. *J. Cleaner Production* 283, 124644. doi: 10.1016/j.jclepro.2020.124644
- Gilbert, M. E., and Ripley, B. S. (2010). Resolving the differences in plant burial responses. *Austral Ecol.* 35, 53–59. doi: 10.1111/j.1442-9993.2009.02011.x
- Halun, Z., Terrados, J., Borum, J., Kamp-Nielsen, L., Duarte, C. M., and Fortes, M. D. (2002). Experimental evaluation of the effects of siltation-derived changes in sediment conditions on the Philippine seagrass *Cymodocea rotundata*. *J. Exp. Mar. Biol. Ecol.* 279, 73–87. doi: 10.1016/S0022-0981(02)00366-0
- Huang, Y., Chen, X. S., Li, F., Hou, Z. Y., Li, X., Zeng, J., et al. (2022). Concurrent effects of flooding regimes and floodwater quality on sediment properties in a Yangtze River-connected floodplain wetland: Insights from field investigations during 2011–2020. *Sci. Total Environ.* 827, 154225. doi: 10.1016/j.scitotenv.2022.154225
- Ji, Z. H., Long, Z. W., Zhang, Y., Wang, Y. K., Qi, X. Y., Xia, X. H., et al. (2021). Enrichment differences and source apportionment of nutrients, stable isotopes, and trace metal elements in sediments of complex and fragmented wetland systems. *Environ. pollut.* 289, 117852. doi: 10.1016/j.envpol.2021.117852
- Jia, X., Huangfu, C. H., and Hui, D. F. (2020). Nitrogen uptake by two plants in response to plant competition as regulated by neighbor density. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.584370
- Jing, L., Lu, C., Xia, Y., Shi, L. L., Zuo, A. J., Lei, J. L., et al. (2017). Effects of hydrological regime on development of *Carex* wet meadows in East Dongting Lake, a Ramsar Wetland for wintering waterbirds. *Sci. Rep.* 7, 41761. doi: 10.1038/srep41761
- Jing, C., Yunliang, L., Junfeng, Z., Jingyuan, L., Li, W., and Yuyin, G. (2021). Assessing surface water-groundwater interactions in the seasonal lake-wetland system of Lake Poyang. *J. Lake Sci.* 33, 842–853. doi: 10.18307/2021.0317
- Katagiri, K., Yabe, K., Nakamura, F., and Sakurai, Y. (2011). Factors controlling the distribution of aquatic macrophyte communities with special reference to the rapid expansion of a semi-emergent *Phalaris arundinacea* L. @ in Bibi River, Hokkaido, northern Japan. *Limnology* 12, 175–185. doi: 10.1007/s10201-010-0335-z
- Kercher, S. M., and Zedler, J. B. (2004). Multiple disturbances accelerate invasion of reed canary grass (*Phalaris arundinacea* L.) in a mesocosm study. *Oecologia* 138, 455–464. doi: 10.1007/s00442-003-1453-7

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- Kettenring, K. M., Menuz, D. R., and Mock, K. E. (2019). The nativity and distribution of the cryptic invader *phalaris arundinacea* (Reed canarygrass) in riparian areas of the Columbia and missouri river basins. *Wetlands* 39, 55–66. doi: 10.1007/s13157-018-1074-x
- Kretz, L., Bondar-Kunze, E., Hein, T., Richter, R., Schulz-Zunkel, C., Seele-Dilbat, C., et al. (2021). Vegetation characteristics control local sediment and nutrient retention on but not underneath vegetation in floodplain meadows. *PloS One* 16, e0252694. doi: 10.1371/journal.pone.0252694
- Li, F., Xie, Y. H., Zhu, L. L., Jiang, L., Chen, X. S., Pan, B. H., et al. (2015). Changed clonal growth form induced by sand burial facilitates the acclimation of *Carex brevicuspis* to competition. *PloS One* 10, e0121270. doi: 10.1371/journal.pone.0121270
- Liu, Z. Y., Zhao, L. N., Xu, T., Bu, F., Liu, X. M., and Zhou, D. M. (2018). Quantification of potential flood inundation areas in the marsh wetland of Honghe National Natural Reserve, Northeast China. *Ecology Hydrobiology* 18, 355–364. doi: 10.1016/j.ecohyd.2018.10.005
- Martina, J. P., and von Ende, C. N. (2012). Highly plastic response in morphological and physiological traits to light, soil-N and moisture in the model invasive plant, *Phalaris arundinacea*. *Environ. Exp. Bot.* 82, 43–53. doi: 10.1016/j.envexpbot.2012.03.010
- Matzke, S., and Elsey-Quirk, T. (2018). *Spartina patens* productivity and soil organic matter response to sedimentation and nutrient enrichment. *Wetlands* 38, 1233–1244. doi: 10.1007/s13157-018-1030-9
- Maun, M. A. (1998). Adaptations of plants to burial in coastal sand dunes. *Can. J. Bot.* 76, 713–738. doi: 10.1139/b98-058
- Moritz, C. M., Vepraskas, M. J., and Ricker, M. C. (2022). Hydrology and vegetation relationships in a Carolina Bay Wetland 15 years after restoration. *Wetlands* 42, 8. doi: 10.1007/s13157-022-01530-0
- Nelson, M. F., and Anderson, N. O. (2015). Variation among genotypes and source habitats in growth and fecundity of the wetland invasive plant *Phalaris arundinacea* L. *Wetlands* 35, 1175–1184. doi: 10.1007/s13157-015-0704-9
- Ou, Y., Rousseau, A. N., Wang, L. X., Yan, B. X., Gumiere, T., and Zhu, H. (2019). Identification of the alteration of riparian wetland on soil properties, enzyme activities and microbial communities following extreme flooding. *Geoderma* 337, 825–833. doi: 10.1016/j.geoderma.2018.10.032
- Owen, N. W., Kent, M., and Dale, D. M. (2004). Plant species and community responses to sand burial on the machair of the Outer Hebrides, Scotland. *J. Vegetation Sci.* 15, 669–678. doi: 10.1111/j.1654-1103.2004.tb02309.x
- Pan, Y., Xie, Y. H., Chen, X. S., and Li, F. (2012). Effects of flooding and sedimentation on the growth and physiology of two emergent macrophytes from Dongting Lake wetlands. *Aquat. Bot.* 100, 35–40. doi: 10.1016/j.aquabot.2012.03.008
- R Core Team (2020). R: A Language and Environment for Statistical Computing. In: R Version, 4.0.1. R Foundation for Statistical Computing, Vienna, Austria.
- Wang, C., Dong, B., Zhu, M., Huang, H., and Zhao, K. K. (2018). Habitat selection of wintering crane in Shengjin Lake wetland. *Chin. J. Ecol.* 37, 810. doi: 10.13292/j.1000-4890.201803.020
- Weston, L. M., Mattingly, K. Z., Day, C. T. C., and Hovick, S. M. (2021). Potential local adaptation in populations of invasive reed canary grass (*Phalaris arundinacea*) across an urbanization gradient. *Ecol. Evol.* 11, 11457–11476. doi: 10.1002/ece3.7938
- Wetzel, P. R., and van der Valk, A. G. (1998). Effects of nutrient and soil moisture on competition between *Carex stricta*, *Phalaris arundinacea*, and *Typha latifolia*. *Plant Ecol.* 138, 179–190. doi: 10.1023/A:1009751703827
- Winikoff, S. G., Larkin, D. J., Meier, S. L., and Finlay, J. C. (2020). Vegetation trajectories of restored agricultural wetlands following sediment removal. *Restor. Ecol.* 28, 612–622. doi: 10.1111/rec.13128
- Wu, Z. L., and Phillips, S. (2006). *Phalaris linnaeus*, sp. Pl. 1:54. 1753. (Flora of China) 22, 335–336.
- Xiao, M., Cai, T. L., Wang, X. K., Cheng, J., Liu, B., Xia, X. M., et al. (2023). Response of native and exotic saltmarsh species to sediment deposition addition. *Sci. Total Environ.* 888, 164271. doi: 10.1016/j.scitotenv.2023.164271
- Zeng, L. H., Ji, J., Xu, S. Y., Cao, Y. M., and Chen, X. (2023). Decoupling of nitrogen, phosphorus and biogenic silica in floodplain sediments in response to land use change and hydrological alterations. *J. Hydrology* 623, 129833. doi: 10.1016/j.jhydrol.2023.129833
- Zhang, Z. Q., Bianchette, T. A., Meng, C. H., Xu, Q. H., and Jiang, M. (2020). Holocene vegetation-hydrology-climate interactions of wetlands on the Heixiazhi Island, China. *Sci. Total Environ.* 743, 140777. doi: 10.1016/j.scitotenv.2020.140777



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Photosynthetic traits of *Phragmites australis* along an ecological gradient and developmental stages

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Common reed (*Phragmites australis*) is a cosmopolitan species, though its dieback is a worldwide phenomenon. In order to assess the evolutionary role of phenotypic plasticity in a successful plant, the values and plasticity of photophysiological traits of *Phragmites australis* were investigated in the Lake Fertő wetlands at 5 sites with different degrees of reed degradation and along a seasonal sequence. On the one hand, along the established ecological degradation gradient, photophysiological traits of *Phragmites* changed significantly, affecting plant productivity, although no consistent gradient-type trends were observed. Gradual changes within a season in the values of photosynthetic traits were observed that were recorded in both degraded and stable stands, suggesting a universal response to seasonally changing environmental conditions that could not be overridden by the ecological gradient. On the other hand, reed plants exposed to different levels of degradation showed comparable physiological plasticity; there was no difference in trait variability between stable and degraded stands. This relatively uniform plasticity is likely to contribute to the resilience of reed plants by providing a wider range of adaptive traits under different conditions. In contrast, the 150–200% gradual change in photophysiological trait plasticity with senescence in *Phragmites* was also demonstrated, reflecting a more dynamic response of the photosynthetic apparatus to seasonal changes. Senescence affected the plasticity of plant traits independently of their degradation status, suggesting a more universal nature of seasonal changes. This research shows that under conditions of conservative resource use determined by stressful habitats, trait values respond to conditions, while trait plasticity shows minimal changes. Furthermore, phenological sequence significantly influenced both the values and the plasticity of the photosynthetic traits studied. Our results underline the impact of ecological degradation on reed physiology and highlight the importance of understanding both trait values and plasticity in plant responses to environmental and seasonal change.

KEYWORDS

phenotypic plasticity, *Phragmites* degradation, reed photophysiology, trait variability, wetlands

1 Introduction

The natural world exhibits considerable temporal and spatial variability, and in order to be successful organisms must evolve the ability to adapt to these changing conditions. Common reed (*Phragmites australis* (Cav.) Trin ex Steud – referred to in this text as *Phragmites* and reed), is a widespread perennial emergent aquatic plant that can dominate vast areas by forming extensive monocultures in its habitats and can be found in wetland and aquatic habitats around the world. Its cosmopolitan distribution highlights its remarkable ability to acclimate and adapt to a wide range of environmental conditions, from pristine wetlands to anthropogenically altered ecosystems. *Phragmites* not only possesses a wide range of competitive traits (Kettenring et al., 2012; Eller et al., 2014), but also a significant plasticity of these traits that determines the success of the species under very different environmental conditions (Vretare et al., 2001; Mozdzer and Zieman, 2010; Eller et al., 2017). This success of the common reed in different habitats underlines the importance of understanding the trait variability of successful species in relation to environmental gradients and developmental stages (Clevering and Lissner, 1999; Meyerson et al., 2016; Eller et al., 2017), thus making it an interesting subject to study the adaptability of plants as well as facilitating its effective conservation and management.

While genetic diversity remains the key component of biodiversity, structuring, functioning and stabilising ecosystems (Salo and Gustafsson, 2016; Carvalho et al., 2019), knowledge of the effects of phenotypic plasticity (trait variability) on growth and functioning of wetland plants remains limited (Chambers et al., 2008). The plastic response of *Phragmites* to various extreme conditions serves to increase the success of the species (Clevering et al., 2001; Vretare et al., 2001; Engloner, 2009), although the functional value of this plasticity, its evolutionary role, has not been fully explored and thus understood. The importance and role of phenotypic plasticity in organismal adaptation to environmental change is probably related to its dynamic nature. Researchers have investigated various mechanisms underlying phenotypic plasticity, and experimental evidence has shown that phenotypic plasticity can enable organisms to adjust their morphology and physiology in response to environmental cues, thereby enhancing their fitness and survival (Ghalambor et al., 2007; Valladares et al., 2007; Nicotra et al., 2010).

Although phenotypic plasticity is intrinsic to organisms, it is not static and can be modulated by various factors. Research has shown that phenotypic plasticity can change over time, even within weeks, with factors such as senescence or seasonal variation influencing its expression (Nicotra et al., 2010; Stotz et al., 2021). Studies have shown that as organisms age, their capacity for phenotypic plasticity may decrease due to physiological changes or reduced environmental sensitivity. In addition, phenotypic plasticity can vary within a year in response to seasonal cues such as changes in temperature, photoperiod or resource availability (Nicotra et al., 2010; Stotz et al., 2021). These environmental fluctuations can trigger phenotypic adaptations, resulting in temporal shifts in the

expression of plastic traits. Thus, phenotypic plasticity exhibits a dynamism that is shaped by both intrinsic and extrinsic factors, highlighting its adaptability in facilitating organismal responses to changing environmental conditions.

Studying functional traits and their plasticity in an otherwise successful plant could be useful in unravelling the mechanisms behind its adaptability and ecological success (Ackerly et al., 2000; Reich et al., 2003). Photosynthesis is a fundamental physiological process that directly influences plant growth and productivity, and patterns of intraspecific trait variation could provide valuable insights into the role of photosynthetic traits in evolutionary adaptation (Arntz and Delph, 2001; Maire et al., 2015). By studying how photosynthetic traits vary along ecological gradients and across phenological sequences, we can gain insights into the adaptive strategies used by populations to cope with different environmental challenges (McKown et al., 2013; Fajardo and Siefert, 2016).

Understanding the dynamics of photosynthetic traits in *Phragmites australis* populations with different ecological and phenological backgrounds contributes to the understanding of how divergence in photosynthetic traits could lead to evolutionary advantages for species and, consequently, have broader ecological and conservation goals (Lessmann et al., 2001; Mészáros et al., 2003; Tóth, 2016). Common reed has been shown to be able to adapt to very different environments by maintaining a higher photosynthetic capacity in different habitats compared to other species (Lessmann et al., 2001; Engloner, 2009; Eller et al., 2017). This higher rate allows reeds to fix more carbon, giving them a significant advantage in terms of biomass production and species expansion. Chlorophyll fluorescence techniques are convenient, fast and important tools in plant physiology studies, as they provide a non-invasive way to monitor the photosynthetic performance of plants (Roháček et al., 2008; Kalaji et al., 2016; Tóth, 2018; Tóth et al., 2019). As an early indicator of stress manifestation in plants, it can be used to determine and understand heterogeneity in leaf photochemical efficiency (Li et al., 2004; Stratoulis et al., 2015; Tóth, 2016, 2018) and can provide useful information on leaf photosynthetic performance.

The phenomenon of reed die-back has been observed across numerous lakes throughout Europe, with over 35 cases documented (Ostendorp, 1989; van der Putten, 1997), though some areas of North America experiencing similar phenomena (Reed and Cahoon, 1992; Visser et al., 1999). The die-back was primarily attributed to human interventions that altered the natural environments of these aquatic ecosystems. The common reed has experienced large-scale declines, especially in areas where hydrological regimes, water quality, or land use have been modified by anthropogenic activities. The most pronounced impacts have been observed in lakes that have undergone water-level regulation, eutrophication, and habitat fragmentation, with reed beds breaking up, reduced vitality, and eventual loss of large sections of reed cover.

In response to the alarming prevalence and severity of reed die-back, the European project EUREED was initiated (van der Putten, 1997; Brix, 1999). The project's objective was to analyse the mechanisms regulating the growth dynamics and stability of reed-

dominated ecosystems, develop models and predictions of the impact of human activities and climate change, and devise management strategies for reed die-back (van der Putten, 1997; Brix, 1999). The project identifies a number of factors contributing to the dieback of *Phragmites* in Europe, including eutrophication, water management practices, genetic diversity constraints, mechanical disturbances, pollution and climate change. It seems important that these factors are addressed through integrated management strategies if reed ecosystems across the continent are to be conserved and restored (van der Putten, 1997; Brix, 1999, 1999; Čížková et al., 2000).

Hungarian lakes have not been exempt from the die-back tendency, as Lake Fertő and other lakes and wetlands in Hungary experienced significant reed disappearance during the same period (Dinka et al., 2010; Tóth, 2016). Human activities, including water regulation, urbanisation and inadequate reed management practices, have been identified as contributing factors for Hungarian lakes too. The loss of reed stands in these areas has not only resulted in a reduction in habitat availability for a diverse range of species, thereby impacting the ecosystem services they provide, but it remains a significant challenge for the conservation of European wetlands. This research aimed to understand changes in photosynthetic traits along an ecological gradient and developmental stages within a season. This study proposes a hypothesis that differences between the studied reed stands (ecological gradient) will have a significant effect on the values

(a^1) and plasticity (a^2) of the studied photosynthetic traits. It further hypothesised that seasonal changes (phenological sequence) will have a significant effect on the values (b^1) and plasticity (b^2) of the studied photosynthetic traits.

2 Materials and methods

2.1 Study area

Lake Fertő/Neusiedl is a large water body (309 km²) on the border of Hungary and Austria (N47.71, E16.73 - Figure 1). It is an endorheic lake with a relatively small catchment area of 1120 km². The lake is shallow: the average depth is 0.7 metres, while the average depth of the pelagic parts is 1.4 metres. Lake Fertő can be divided into two distinct parts: the pelagic (open water) zone and the wetland area; the water quality and environmental conditions in each zone are unique. The 85% of the Hungarian part of the lake is covered with reeds, although the overall coverage of the lake is lower (55% or about 170 km²). Over time, the ecological status of the reed beds has deteriorated, particularly in the Hungarian section (Dinka et al., 2004, 2010). This deterioration has been caused by both natural and anthropogenic factors. Natural causes include senescence (most reed stands are more than a decade old and may be subject to dieback), zonation related to water depth (areas of waterlogged reed stands without direct freshwater inflow often have

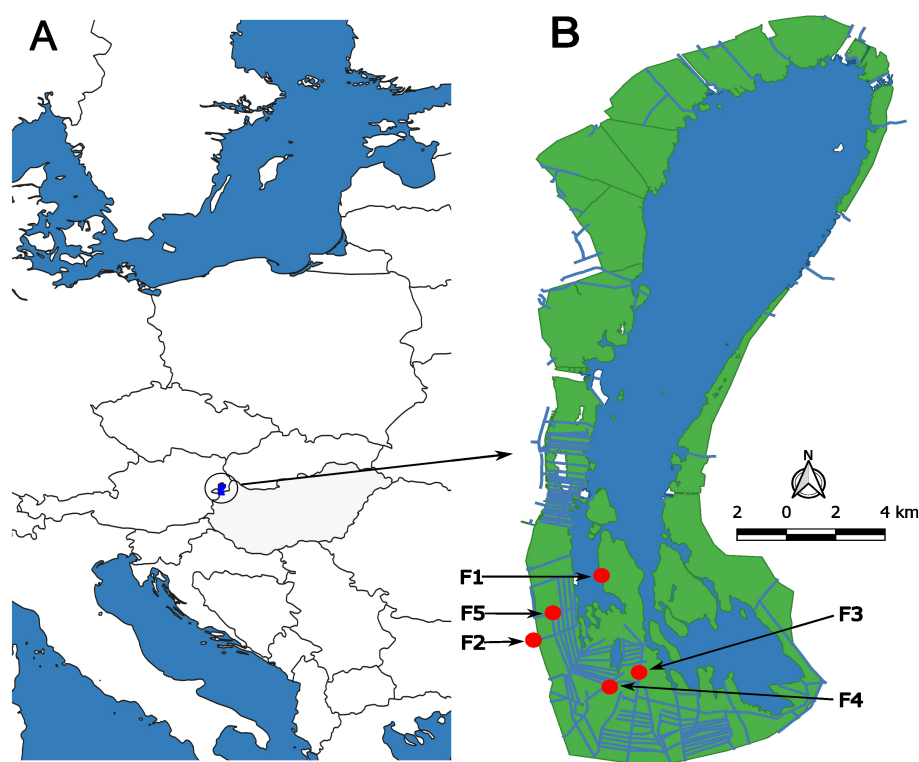


FIGURE 1

(A) Location of Lake Fertő (blue polygon) in Central Europe between Hungary (grey colour) and Austria. (B) Position of sampling points in the reed beds (green colour) of Lake Fertő. The numbering indicates the degree of degradation of the reed stands: F1 - stable site, F2 - terrestrial stable site, F3 - moderately degraded site, F4 - severely degraded site, F5 - dieback site. The artificial canal structure of the wetland is indicated by blue lines within the green reed stands.

high dissolved organic matter content) and successional changes (drying up of parts of the wetland and transition to grassland). However, anthropogenic factors such as inappropriate reed management practices and infrastructure development are likely to be the main drivers of this degradation.

Over the past 20 years, local water authorities have attempted to rehabilitate the reed stands by reconstructing canals within the wetlands to improve water supply to isolated reed stands (Figure 1). Unfortunately, the technology used in these reconstructions has been ineffective. The combination of these factors has resulted in significant habitat variability, with reed stands in the Hungarian part of Lake Fertő now showing varying degrees of degradation.

The aim of this manuscript is not to detail the factors causing this degradation, but rather to quantify its effects using *Phragmites* morphology as a proxy to encapsulate the effects of degradation. Although the quality of the reed beds has changed over the study period, the data presented are of scientific value not only at a local scale but also to macrophyte ecologists worldwide.

2.2 Macrophyte morphology and study site characterisation

To accurately categorise the selected sites ecologically, the morphological characteristics of the reed plants at each site were assessed during the peak vegetation period of the previous year (August 2019) and used as a proxy to describe the degree of degradation at these sites. At each sampling site 15 *Phragmites* plants were randomly collected cutting them either at water surface or sediment level. Stem height was determined from cut surface to the tip of the top leaf with a measuring tape. Water depth was measured at each site and added to plant height. Diameter in the middle of the basal internode of each reed stem was measured with a vernier calliper, green and dry leaves of each plant were counted, number of nodes was determined. Plant density was measured three times at each site using a 50 x 50 cm quadrat. The initial point of quadrat placement was randomly selected and subsequent quadrats were placed at 6 m intervals along a marked rope. All green *Phragmites* plants within each quadrat were counted.

Based on these data and experience of previous years reed stands of Lake Fertő were divided into 5 degradational categories (F1-F5) described as follows:

- The stable reed stand (F1) in Lake Fertő was highly monospecific, with vegetation that was tall, dense, and homogeneous (Figure 1). The stand was continuously exposed to wave action. The water depth on the water side of the stand ranged from 50 to 100 cm, and new reed vegetation grew vigorously along the water's edge.
- The sampling site at the terrestrial edge of Lake Fertő's wetland (Figure 1 - F2) contained approximately 5-10% non-*Phragmites* species. The reed plants within this stand exhibited significant height, density, and uniformity. The water depth throughout the extent of the reed stand at the sampling site was uniform, ranging from 10 to 30 cm.
- The mildly degraded sampling site F3 was located in the centre of the wetland of Lake Fertő and was characterised by its monospecific nature, consisting mainly of *Phragmites* species (Figure 1). The reed plants within this stand exhibited remarkable height and density. However, small patches with no reed growth were observed in the otherwise homogeneous reed stand. The landscape was further marked by the construction of water management canals by the water authorities around the sampling site. At the sampling site, the reed stand maintained a consistent water depth of 0-20 cm throughout its extent.
- The moderately degraded sampling site F4 was situated in the centre of the wetland of Lake Fertő and had a species composition of 5-10% other than *Phragmites* (Figure 1). Within this stand, the reed plants were smaller and thinner, and the area was less densely populated, with frequent 10-20 square metre patches without vegetation. Water management canals were also present in the vicinity of the site. The water depth at the site ranged from 20-40 cm.
- The die-back reed stand of the wetland of Lake Fertő (Figure 1 - F5) was result of a high level ecological degradation. Approximately 70% of the area was without any vegetation (0 plants m⁻²), while the remaining area was covered by 0.1-0.5 m² patches of clumped reeds of extreme density of approximately 300-400 individuals per square metre. The reed plants within these patches were small and thin. Near the sampling site, artificial canals were reconstructed 10 years ago to rehabilitate the area, and the water depth remained consistently low, ranging from 0 to 30 cm throughout the affected area.

2.3 Macrophyte photophysiological measurements

Using the above site categorisations, chlorophyll fluorescence parameters were measured in 2020 and 2021 using a chlorophyll fluorometer (PAM-2500, Heinz Walz GmbH, Germany) between 9:00 and 15:00. Measurements were performed at least once a month between April and October. Plants were randomly selected using a marked rope with evenly spaced knots at 3 metre intervals, and a plant was selected nearest to the knot. However, preference was given to selecting average looking, intact plants. For sites F1-F4, measurements were taken along the waterward part of the reed stand, ~4 metres into the stand. At site F5, the reeds were clumped at varying distances, so it was decided to select the six closest clumped reeds within a manageable distance (less than 30 by 30 metres). The study areas at each site were deliberately minimised to reduce environmental variability and ensure more uniform conditions. At each site chlorophyll fluorescence measurements were made on the youngest, largest intact leaves. During this, light response curves [i.e., the electron transport rate (ETR) of the photosystem II (PSII) as a function of photosynthetically active radiation (PAR)] were measured. After dark adaptation (20

minutes), emitted initial fluorescence yield (F_0) and maximal fluorescence yield (F_m) resulting from a pulse of a saturated light (630 nm, intensity $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$) were determined. From these, the photochemical PSII efficiency (F_v/F_m), coefficient of photochemical quenching (qP), and coefficient of non-photochemical quenching (qN) were calculated (Table 1). The measured leaves were exposed to 11 actinic lights for a duration of 15 seconds, at 630 nm, with an intensity of between 5 and $787 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the ETR values were measured after each illumination step with a new pulse of saturated ($3000 \mu\text{mol m}^{-2} \text{s}^{-1}$) light. Exponentially saturating curves (Eilers and Peeters, 1988) were fit to the light response data, and the maximum ETR (ETR_{max}), theoretical saturation light intensity (I_k), and maximum quantum yield for whole chain electron transport (α) were retrieved using formula from this study (Genty et al., 1989).

2.4 Statistics

Several statistics were used in this study using R statistical software (R Development Core Team, 2012). Descriptive statistics, including means, standard deviations and ranges, were calculated to provide a general overview of the data. One-way ANOVA was used to compare the means of photosynthetic traits between reed stands classified into different degradation levels (F1-F5). The assumptions of normality and homogeneity of variances were tested using Shapiro-Wilk and Levene's tests. Two-way ANOVA was used to analyse the effects of two factors (e.g. degradation level and seasonality) on photosynthetic traits. The assumptions of normality and homogeneity of variances were also tested using Shapiro-Wilk and Levene's tests. Pearson product-moment correlation was used to examine relationships between photosynthetic traits and their plasticity, and between site degradation eigenvalue and month of the year, assuming normality of residuals (tested with the Shapiro-Wilk test) and homoscedasticity (checked visually with residual plots). Where assumptions of ANOVA or Pearson correlation were violated, log transformations were used to normalise the data and stabilise variances.

The plasticity of photophysiological responses in this study was quantified using the coefficient of variance (CV), which is a statistical measure of the relative variability of a parameter. The CV expresses the extent of variability in relation to the mean of the parameter. For each photophysiological trait measured at a specific spot (F1-F5) on a given date (month), the CV was calculated as follows:

$$CV = \left(\frac{\sigma}{\mu} \right),$$

where σ - is the standard deviation of the measured parameters, while μ - is the mean value of the measured parameter (Schlichting and Levin, 1984).

3 Results

3.1 Site characterisation

The selected reed stands of Lake Fertő were arranged along a degradation gradient based on their morphological parameters (Table 2; Figure 2). Some of the recorded traits showed clear signs of *Phragmites* degradation, especially height, diameter, biomass and leaf biomass (Table 2), while other parameters were not affected by degradation. The changes in morphological parameters across the degradation gradient were not equidistant or linear, as F1, F2 and F3 showed significant similarity, whereas F4 and F5 were more affected by degradation. For example, the average stem height of reeds in F1, F2 and F3 stands was 326, 295 and 280 cm respectively, while plants in degraded (F4) and dieback (F5) stands were 34% and 48% smaller than those in F1 (Table 2). Similar trends were observed for basal diameter, number of nodes, and average plant biomass (Table 2).

The variation in plant density between sampling sites also reflected the response of plants to the different ecological conditions of their stands. Higher densities (F1 = 67 plants m^{-2} , F2 = 79 plants m^{-2}) indicated optimal conditions and more established stands, whereas the lower densities of site F3 (61 plants m^{-2}) indicated an established, but slightly disturbed and less dense reed stand compared to F1 and F2. The F4 and F5 sites (48 and 7 plants m^{-2} , respectively) indicated that reed plants were responding to developing challenges and significant ecological degradation.

The dry biomass of green *Phragmites* plants in the wetlands of Lake Fertő varied significantly between 0.2 and 1.9 kg m^{-2} . Higher biomass values (F1 = 1.6, F2 = 1.9, F3 = 1.8 kg m^{-2}) indicate more productive areas in the favourable conditions of the stable stands, while in the degraded and dieback areas of F4 and F5 the lower values (0.7 and 0.2 kg m^{-2} , respectively) indicated a substantial decrease in the overall productivity of the plants due to the suboptimal conditions.

The principal component analysis showed that the reed stands at sampling sites F1, F2 and F3 were grouped together, while F4 and

TABLE 1 Fluorescence parameters derived from PAM fluorometry, including equations for minimum (F_0) and maximum (F_m) fluorescence yields, apparent (F_s) and maximum (F_m) fluorescence values, irradiance (I) and empirical absorption factor ($AF=0.84$).

Parameter	Name	Equation	Reference
F_v/F_m	maximum quantum efficiency of PSII	$(F_m - F_0)/F_m$	(Schreiber, 1998)
qP	photochemical quenching	$(F_m - F_s)/(F_m - F_0)$	(Titus and Adams, 1979)
qN	non-photochemical quenching	$1 - (F_m - F_0)/(F_m - F_0)$	(Titus and Adams, 1979)
ETR	electron transport rate	$(F_m - F_s)/(F_m) \cdot I \cdot AF \cdot 0.5$	(Schreiber et al., 1995)

Further details can be found in the cited literature.

TABLE 2 Biometric properties of *Phragmites australis* plants from the studied reed stands in Lake Fertő in August of 2019 (n=15).

	Water depth (cm)	Height (cm)	Diameter (mm)	Green leaf	Node number	Biomass (g)	Leaf biomass (%)
F1	74 ± 13	252.7 ± 17.1	7.2 ± 1.8	11.6 ± 0.6	20.6 ± 5.4	23.5 ± 3.4	32.1 ± 0.8
F2	27 ± 34	268.6 ± 44.5	7.7 ± 0.6	9.5 ± 2.7	19.7 ± 8.1	24.1 ± 5.2	27.5 ± 5.0
F3	11 ± 7	269.9 ± 57.5	7.6 ± 1.1	12.4 ± 4.3	22.0 ± 4.9	29.4 ± 11.6	31.4 ± 3.1
F4	23 ± 18	190.2 ± 16.7	7.1 ± 0.4	12.5 ± 3.8	19.1 ± 5.2	14.3 ± 3.9	32.1 ± 5.8
F5	6 ± 8	163.05 ± 8.1	5.1 ± 0.6	11.0 ± 2.6	16.9 ± 4.5	9.6 ± 2.0	29.6 ± 4.9
ANOVA							
	F	30.6	15.8	2.4	1.6	25.1	3.2
	P	<0.001	<0.001	0.059	0.180	<0.001	0.019

F1 and F2 are stable aquatic and terrestrial stands, F3 and F4 are stands at different degree of degradation, while F5 is a die-back stand.

F5 were separated from this group to varying degrees, although only sampling site F5 exhibited difference (Figure 2). The eigenvalues using Component 1 of the PCA of each site with the assigned degradation level (F1 - stable reed stand, etc., F5 - dying reed stand) were used in the correlation analysis (Figure 2; Table 3).

3.2 Photophysiology data

The photophysiological traits of *Phragmites* plants were significantly affected by both their ecological (level of degradation) and phenological status, as well as their interaction (Figure 3; Tables 3, 4). The studied photophysiological traits described the potential photosynthetic efficiency of reed plants from different stands as it

was affected by the process of reed degradation: minor, though significant effects were connected to the degradation of the reed stands (Figure 3; Supplementary Figure S1; Table 4), suggesting a reduction in the efficiency of light absorption and electron transport and resulting in lower potential photosynthetic rates. Parallel to this, gradual increase in qN (non-photochemical quenching) showed the increased disbalance in photochemical and non-photochemical processes of the degraded stands, for example increased intensity of photoprotection processes (Figure 3D).

The photophysiological parameters also exhibited significant seasonal changes that exceeded the effects caused by degradation (Figure 3; Supplementary Figure 2; Tables 3, 4). Except for qN, all major photophysiological parameters started from high values in spring and gradually decreased towards the end of the season, as

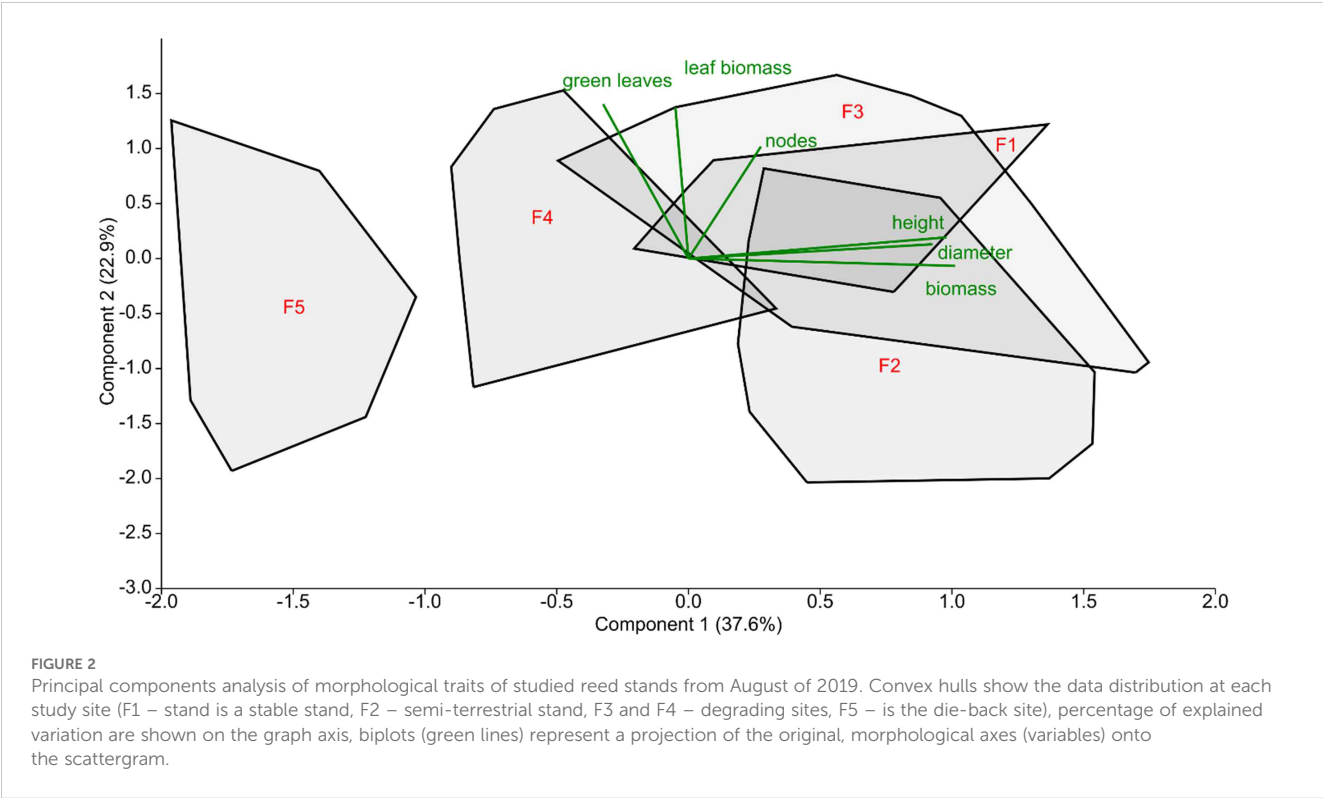


TABLE 3 Pearson product moment correlation (correlation coefficient and its significance - r^2) between the studied photophysiological parameters (data) of *Phragmites australis* plants and their variability (cv) with the site eigenvalue (site, $n=84$) and the month of year ($n=12$).

	Data		CV	
	Site	Month	Site	Month
α	0.366	-0.74	-0.767	0.758
ETR_{max}	0.892	-0.968	0.713	0.691
I_k	0.486	-0.978	0.872	0.964
qP	0.484	-0.927	0.718	0.898
qN	-0.783	0.891	0.539	-0.841
F_v/F_m	-0.090	-0.809	0.375	0.866

The shown parameters are the maximum electron transport capacity (ETR_{max}), the theoretical saturation light intensity (I_k), the non-photochemical quenching (qN), the photochemical quenching (qP), maximum quantum efficiency of PSII (F_v/F_m) of *Phragmites australis* plants. The significance of the correlations.

seasonal senescence caused a decrease in light absorption and electron transport efficiency of the plants (Figures 3B, C, E). Specifically, ETR_{max} , I_k and qP decreased by 57%, 51% and 45%, respectively, regardless of the ecological status of the reed stands (Figures 3B, C, E;

Supplementary Figure 2; Tables 3, 4). The likelihood of experiencing oxidative stress increased with plant age, resulting in a decrease in the maximum quantum efficiency of PS II (F_v/F_m) from 0.80 to 0.76 (a 5% decrease) (Figure 3F). To protect against this oxidative stress and to mitigate the excess energy absorption resulting from the decreased light absorption efficiency, non-photochemical quenching (qN) increased by approximately 23% (Figure 3D).

3.3 Plasticity of photophysiological data

Plasticity of the studied photophysiological traits (Figure 4; Supplementary Figure S3; Table 5) were not affected by the different degradation level of the reeds stands. Generally, sites F1, F2 and F3 exhibited the highest variability in most cases, while plants in the degraded reed stands (F4 and F5) had the lowest variability (Figure 4). It is worth noting that ETR_{max} and I_k exhibited the highest variability overall, at approximately 0.3, while F_v/F_m showed the lowest variability, at approximately 0.05.

The plasticity of the photophysiological traits mostly showed an increasing seasonal trend (Figure 4; Supplementary Figure S4; Table 5). In most cases, trait variability within the season nearly

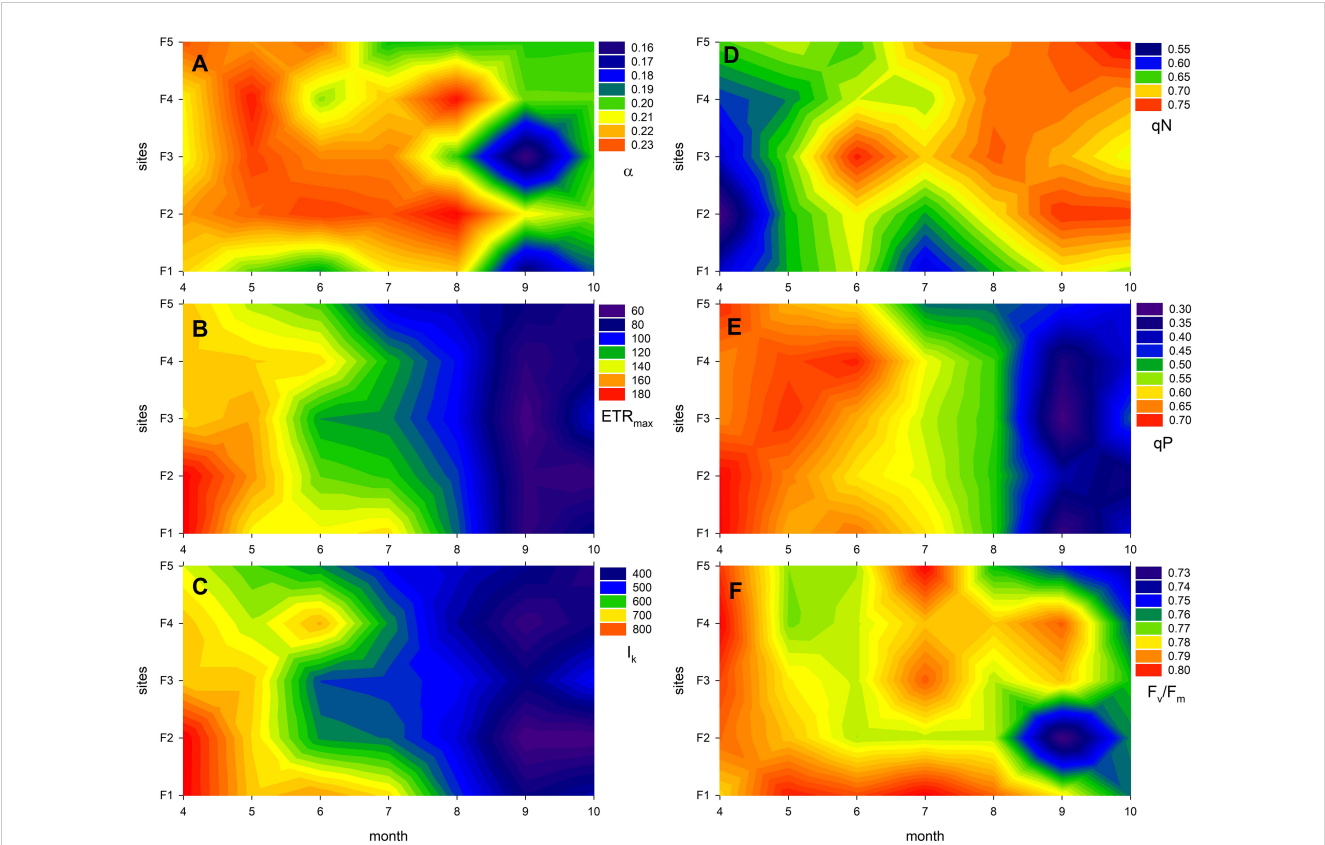


FIGURE 3 Expression of photophysiological traits of *Phragmites australis* in Lake Fertő during the study period (2020–2021) at sites with different degrees of degradation (F1 – stable to F5 – dying reeds). The figure shows the monthly averages of twelve measurements per site. Panel (A) shows the photosynthetic efficiency of photosystem II (α , the initial slope of the light curve), (B) shows the maximum electron transport rate (ETR_{max}), (C) shows the light saturation point (I_k , the light intensity at which ETR_{max} is reached), (D) shows the non-photochemical quenching (qN, indicating thermal dissipation of excess light energy), (E) shows photochemical quenching (qP, reflecting the fraction of open reaction centres), and panel (F) shows the maximum quantum yield of photosystem II (F_v/F_m , a measure of photosynthetic performance and stress level).

TABLE 4 Two-way ANOVA of photophysiological traits of *Phragmites australis* plants in the wetlands of Lake Fertő.

	Site	Month	Interaction
α	8.17***	11.88***	4.12***
ETR _{max}	8.30***	88.98***	2.79***
I _k	10.13***	59.56***	2.15**
qP	3.20*	90.60***	2.16**
qN	7.52***	14.58***	1.92*
F _v /F _m	4.15**	9.35***	1.91*

F-test values and significance are shown (F^p). The measured traits are the maximum electron transport capacity (ETR_{max}), the theoretical saturation light intensity (I_k), the non-photochemical quenching (qN), the photochemical quenching (qP), maximum quantum efficiency of PSII (F_v/F_m) of *Phragmites* plants. The significance of the correlations: **p*<0.05, ***p*<0.01, ****p*<0.001. Main factors: sites (degradation level) and months.

doubled, although the increase in photochemical quenching and the maximum quantum efficiency of PS II was even higher (qP – 172%, F_v/F_m – 143%). The plasticity of non-photochemical quenching (qN) decreased, indicating a significant reduction in variability of this parameter by the end of vegetation season (Figure 4D). In the

background of majority of temporal variability were the seasonal changes of ETR_{max}, qP, I_k and qN (Supplementary Figures S5–S9). The stability in annual mean photophysiological traits is shown on Supplementary Figure S10.

4 Discussion

The symptoms of reed dieback around the world are very similar, i.e. retreat from deep water, increased inhomogeneity and clumping, reduction in stem density, size and diameter, and premature senescence of plants (Armstrong et al., 1996; van der Putten, 1997; Armstrong and Armstrong, 1999; Brix, 1999; Tóth, 2016). In this work, the studied reed stands of Lake Fertő were categorised into stages of degradation based on their morphological appearance, plant density and stand homogeneity. The result of the categorisation was not equidistant, as e.g. the stable (F1 - deep water stand and F2 - terrestrial stand) and the moderately degraded (F3) reed stands were somewhat similar and were grouped in a related ecotype. The F3 stand provided an excellent example of the resilience of *Phragmites*, i.e. how reed stands can recover under the right conditions: the previously degraded (inhomogeneous, smaller) F3 reed stand changed its appearance due to the low water

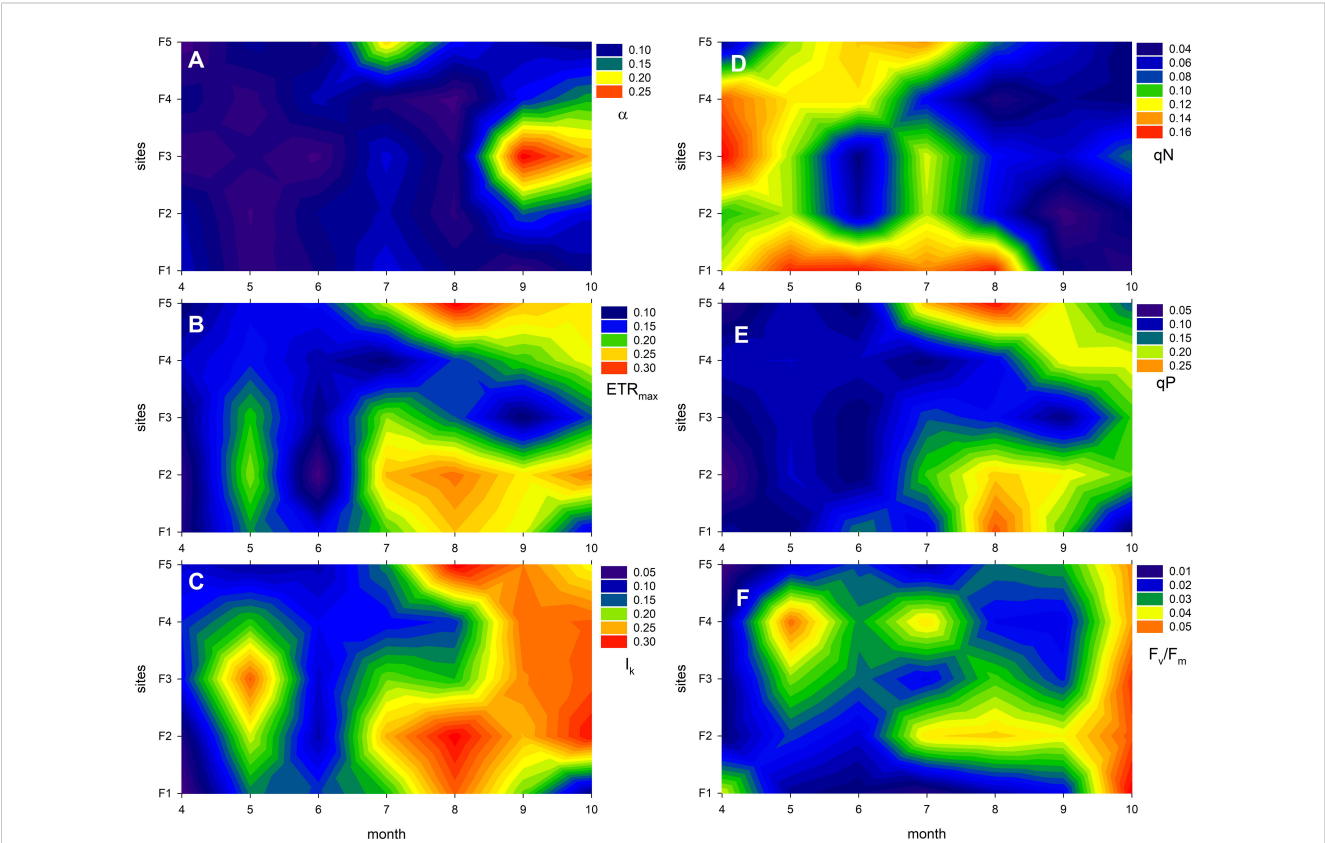


FIGURE 4 Plasticity of photophysiological traits of *Phragmites australis* in Lake Fertő during the study period (2020–2021) at sites with different degrees of degradation (F1 – stable to F5 – dying reeds). Plasticity was calculated as coefficient of variation (cv). Figures show average of 2 sets of measurements (2020 and 2021) per site per month. Panel (A) shows the cv of the photosynthetic efficiency of photosystem II (α , the initial slope of the light curve), (B) shows the cv of the maximum electron transport rate (ETR_{max}), (C) shows the cv of the light saturation point (I_k, the light intensity at which ETR_{max} is reached), (D) shows the cv of the non-photochemical quenching (qN, indicating thermal dissipation of excess light energy), (E) shows the cv of photochemical quenching (qP, reflecting the fraction of open reaction centres), and panel (F) shows the cv of the maximum quantum yield of photosystem II (F_v/F_m, a measure of photosynthetic performance and stress level).

TABLE 5 Two-way ANOVA of plasticity of photophysiological traits of *Phragmites australis* plants in the wetlands of Lake Fertő.

	Site	Month
α	0.51	1.26
ETR _{max}	0.55	3.57*
I _k	1.33	3.12*
qP	0.32	2.87*
qN	1.74	4.85*
F _v /F _m	1.73	5.01*

Plasticity was calculated as coefficient of variation. Main factors: sites (degradation level, n=84) and months (n=12). F-test values and significance are shown (F^p). The measured traits are the maximum electron transport capacity (ETR_{max}), the theoretical saturation light intensity (I_k), the non-photochemical quenching (qN), the photochemical quenching (qP), maximum quantum efficiency of PSII (F_v/F_m) of *Phragmites* plants. The significance of the correlations: *P<0.05.

levels of Lake Fertő in 2020 and 2021. This led to an improvement of the ecological conditions in the surroundings of F3 and consequently to an improvement of the photophysiological parameters measured. Although these environmental conditions led to an improvement in the moderately degraded reed stands, the degraded (F4) and dying (F5) stands were not affected and showed no signs of regeneration in 2020 and 2021.

Along the established ecological gradient, photophysiological traits of *Phragmites* changed in a well-defined pattern making a¹ hypothesis supported. The differences between sites were significant, but not equidistant, thus no prominent trends along the studied ecological gradient were identified, except for maximum electron transport capacity (ETR_{max}). This apparent stability of photophysiological traits and their independence from reed stand degradation may be due to the large genetic variation of the *Phragmites* (Lambertini et al., 2008; Engloner and Major, 2011; Eller et al., 2017) paired with a high trait plasticity of plants (Clevering et al., 2001; Eller and Brix, 2012; Tóth, 2016), while data of the research suggest also a compensatory effect of phenological changes in reed populations.

On the contrary, notable seasonal variations in the photosynthetic traits were recorded (b¹ - supported), and these trends were consistent both in degraded and stable reed stands. Along the growing season, significant decrease (or increase in the case of non-photochemical quenching - qN) in monthly averages, regardless of degradation level were observed, suggesting a universal response of reed photophysiological traits to changing seasonal environmental conditions. Although *Phragmites* plants in the Fertő wetlands have been exposed to very similar dynamic changes in environmental conditions from April to October for millennia, the autumn changes cause stress that is compensated by adjustments in photosynthetic parameters in all reed stands regardless of the degree of degradation. The pattern of changes may be driven by seasonal shift of environmental factors such as temperature, light quantity and quality, etc., which have a strong influence on photophysiological traits.

Despite differences in habitat quality and structural characteristics between degraded and stable stands, reed plants of Lake Fertő exhibited considerable and comparable photophysiological plasticity (a² - not

supported), allowing them to adapt to different environments and maintain a relatively constant level of photosynthetic performance under changing ecological conditions. The photophysiological plasticity of *Phragmites* plants often involves mechanisms such as altering photosynthetic rates and/or modulating enzyme activities to optimise resource use efficiency (Lessmann et al., 2001; Pagter et al., 2005; Tóth, 2016, 2018). In addition, it cannot be excluded that genetic diversity within the reed population of Lake Fertő may increase resilience by providing a wider range of adaptive traits. This genetic variation allows some clones to possess traits that confer resilience to specific environmental stressors associated with degradation, such as changes in sediment redox potential, permanent inundation, or other stresses.

A significant increase in the plasticity of photophysiological parameters with seasonal senescence in *Phragmites* plants in Lake Fertő suggests a dynamic response of the photosynthetic apparatus and makes the b² hypothesis of this study true. Senescence is a natural physiological process in plants in which older tissues degrade or transform during the later stages of the plant life cycle (Gan and Amasino, 1997; Liu et al., 2016; Woo et al., 2019). During senescence, stochastic changes may occur in the chloroplasts and photosynthetic system of older leaves, leading to the increased plasticity in photochemical parameters observed in this study, although the nature of the observed trend in plasticity (Supplementary Figure S4) may imply an unknown deterministic background. This, combined with seasonal changes in environmental conditions (such as lower temperatures in autumn and changes in optical properties), may contribute to the increased plasticity observed with senescence. Meanwhile, other environmental, plant physiological, genetic and phenotypic factors that may be associated with degraded *Phragmites* stands do not significantly affect the plastic response of reed plants. This suggests a very specific mechanism of regulation of *Phragmites* plasticity, primarily driven by plant senescence and independent of the degradation status of reed stands.

5 Conclusion

It is consistent with the literature suggesting that certain groups of *Phragmites* are able to acclimate to specific environmental changes due to the increase of their phenotypic plasticity (Eller et al., 2017; Ren et al., 2020). The data from the study showed that the values of the studied photosynthetic traits of reed were indeed lower in the degraded and die-back sites. The observed reduction of photosynthetic trait values in degraded reed stands underlines the detrimental effects of ecological degradation on plant physiological processes. Contrary to the initial hypothesis, the analysis showed that the plasticity of the traits studied were not statistically significantly different between the degraded and more stable reed stands. The lack of significant differences in plasticity between degraded and stable reed stands suggests that phenotypic flexibility may not vary significantly with ecological stability. These findings highlight the refined responses of common reed to ecological gradients, and emphasise the importance of considering both trait values and plasticity in understanding plant responses to environmental change.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the author, upon reasonable request.

Author contributions

VT: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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References

- Ackerly, D. D., Dudley, S. A., Sultan, S. E., Schmitt, J., Coleman, J. S., Linder, C. R., et al. (2000). The evolution of plant ecophysiological traits: recent advances and future directions: new research addresses natural selection, genetic constraints, and the adaptive evolution of plant ecophysiological traits. *Bioscience* 50, 979–995. doi: 10.1641/0006-3568(2000)050[0979:TEOPET]2.0.CO;2
- Armstrong, J., and Armstrong, W. (1999). Phragmites die-back: toxic effects of propionic, butyric and caproic acids in relation to pH. *New Phytol.* 142, 201–217. doi: 10.1046/j.1469-8137.1999.00395.x
- Armstrong, J., Armstrong, W., and Putten, W. H. (1996). Phragmites die-back: bud and root death, blockages within the aeration and vascular systems and the possible role of phytotoxins. *New Phytol.* 133, 399–414. doi: 10.1111/j.1469-8137.1996.tb01907.x
- Arntz, M. A., and Delph, L. F. (2001). Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127, 455–467. doi: 10.1007/s004420100650
- Brix, H. (1999). The European research project on reed die-back and progression (EUREED). *Limnologia-Ecology Manage. Inland Waters* 29, 5–10. doi: 10.1016/S0075-9511(99)80033-4
- Carvalho, Y. G., Vitorino, L. C., de Souza, U. J., and Bessa, L. A. (2019). Recent trends in research on the genetic diversity of plants: implications for conservation. *Diversity* 11, 62. doi: 10.3390/d11040062
- Chambers, P. A., Lacoul, P., Murphy, K. J., and Thomaz, S. M. (2008). Global diversity of aquatic macrophytes in freshwater. *Hydrobiologia* 595, 9–26. doi: 10.1007/s10750-007-9154-6
- Čížková, H., Brix, H., and Herben, T. (2000). Ecology of Phragmites populations in the changing landscape. *Folia Geobotanica* 35, 351–351. doi: 10.1007/BF02803547
- Clevering, O. A., Brix, H., and Lukavská, J. (2001). Geographic variation in growth responses in Phragmites australis. *Aquat. Bot.* 69, 89–108. doi: 10.1016/S0304-3770(01)00132-2
- Clevering, O. A., and Lissner, J. (1999). Taxonomy, chromosome numbers, clonal diversity and population dynamics of Phragmites australis. *Aquat. Bot.* 64, 185–208. doi: 10.1016/S0304-3770(99)00059-5
- Dinka, M., Ágoston-Szabó, E., Berczik, Á., and Kutrucz, G. (2004). Influence of water level fluctuation on the spatial dynamic of the water chemistry at Lake Fertő/Neusiedler See. *Limnologia* 34, 48–56. doi: 10.1016/S0075-9511(04)80021-5
- Dinka, M., Ágoston-Szabó, E., and Szeglet, P. (2010). Comparison between biomass and C, N, P, S contents of vigorous and die-back reed stands of Lake Fertő/Neusiedler See. *Biologia* 65, 237–247. doi: 10.2478/s11756-010-0006-x
- Eilers, P. H. C., and Peeters, J. C. H. (1988). A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol. Model.* 42, 199–215. doi: 10.1016/0304-3800(88)90057-9
- Eller, F., and Brix, H. (2012). Different genotypes of Phragmites australis show distinct phenotypic plasticity in response to nutrient availability and temperature. *Aquat. Bot.* 103, 89–97. doi: 10.1016/j.aquabot.2012.07.001
- Eller, F., Lambertini, C., Nguyen, L. X., and Brix, H. (2014). Increased invasive potential of non-native Phragmites australis: elevated CO₂ and temperature alleviate salinity effects on photosynthesis and growth. *Global Change Biol.* 20, 531–543. doi: 10.1111/gcb.2014.20.issue-2
- Eller, F., Skálová, H., Caplan, J. S., Bhattacharai, G. P., Burger, M. K., Cronin, J. T., et al. (2017). Cosmopolitan species as models for ecophysiological responses to global change: the common reed Phragmites australis. *Front. Plant Sci.* 8, 1833. doi: 10.3389/fpls.2017.01833
- Engloner, A. I. (2009). Structure, growth dynamics and biomass of reed (Phragmites australis) – A review. *Flora - Morphology Distribution Funct. Ecol. Plants* 204, 331–346. doi: 10.1016/j.flora.2008.05.001
- Engloner, A. I., and Major, Á. (2011). Clonal diversity of Phragmites australis propagating along water depth gradient. *Aquat. Bot.* 94, 172–176. doi: 10.1016/j.aquabot.2011.02.007
- Fajardo, A., and Siefert, A. (2016). Phenological variation of leaf functional traits within species. *Oecologia* 180, 951–959. doi: 10.1007/s00442-016-3545-1
- Gan, S., and Amasino, R. M. (1997). Making sense of senescence (molecular genetic regulation and manipulation of leaf senescence). *Plant Physiol.* 113, 313. doi: 10.1104/pp.113.2.313
- Genty, B., Briantais, J.-M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta (BBA)-General Subj.* 990, 87–92. doi: 10.1016/S0304-4165(89)80016-9
- Ghalambor, C. K., McKay, J. K., Carroll, S. P., and Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21, 394–407. doi: 10.1111/j.1365-2435.2007.01283.x
- Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., et al. (2016). Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta physiologiae plantarum* 38, 1–11. doi: 10.1007/s11738-016-2113-y
- Kettenring, K. M., de Blois, S., and Hauber, D. P. (2012). Moving from a regional to a continental perspective of Phragmites australis invasion in North America. *AoB Plants* 2012, pls040. doi: 10.1093/aobpla/pls040
- Lambertini, C., Gustafsson, M. H. G., Frydenberg, J., Speranza, M., and Brix, H. (2008). Genetic diversity patterns in Phragmites australis at the population, regional and continental scales. *Aquat. Bot.* 88, 160–170. doi: 10.1016/j.aquabot.2007.10.002

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2024.1476142/full#supplementary-material>

- Lessmann, J. M., Brix, H., Bauer, V., Clevering, O. A., and Comin, F. A. (2001). Effect of climatic gradients on the photosynthetic responses of four *Phragmites australis* populations. *Aquat. Bot.* 69, 109–126. doi: 10.1016/S0304-3770(01)00133-4
- Li, S., Pezeshki, S. R., and Goodwin, S. (2004). Effects of soil moisture regimes on photosynthesis and growth in cattail (*Typha latifolia*). *Acta Oecologica* 25, 17–22. doi: 10.1016/j.actao.2003.10.004
- Liu, Q., Fu, Y. H., Zeng, Z., Huang, M., Li, X., and Piao, S. (2016). Temperature, precipitation, and insolation effects on autumn vegetation phenology in temperate China. *Global Change Biol.* 22, 644–655. doi: 10.1111/gcb.2016.22.issue-2
- Maire, V., Wright, I. J., Prentice, I. C., Batjes, N. H., Bhaskar, R., van Bodegom, P. M., et al. (2015). Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecol. Biogeography* 24, 706–717. doi: 10.1111/geb.2015.24.issue-6
- McKown, A. D., Guy, R. D., Azam, M. S., Drewes, E. C., and Quamme, L. K. (2013). Seasonality and phenology alter functional leaf traits. *Oecologia* 172, 653–665. doi: 10.1007/s00442-012-2531-5
- Mészáros, I., Veres, S., Dinka, M., and Lakatos, G. (2003). Variations in leaf pigment content and photosynthetic activity of *Phragmites australis* in healthy and die-back reed stands of Lake Fertő/Neusiedlersee. *Hydrobiologia* 506, 681–686. doi: 10.1007/BF03335350
- Meyerson, L. A., Cronin, J. T., and Pyšek, P. (2016). *Phragmites australis* as a model organism for studying plant invasions. *Biol. Invasions* 18, 2421–2431. doi: 10.1007/s10530-016-1132-3
- Mozdzer, T. J., and Zieman, J. C. (2010). Ecophysiological differences between genetic lineages facilitate the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. *J. Ecol.* 98, 451–458. doi: 10.1111/j.1365-2745.2009.01625.x
- Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., et al. (2010). Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692. doi: 10.1016/j.tplants.2010.09.008
- Ostendorp, W. (1989). Die-back” of reeds in Europe – a critical review of literature. *Aquat. Bot.* 35, 5–26. doi: 10.1016/0304-3770(89)90063-6
- Pagter, M., Bragato, C., and Brix, H. (2005). Tolerance and physiological responses of *Phragmites australis* to water deficit. *Aquat. Bot.* 81, 285–299. doi: 10.1016/j.aquabot.2005.01.002
- R Development Core Team (2012). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: <http://www.R-project.org/>.
- Reed, D. J., and Cahoon, D. R. (1992). The relationship between marsh surface topography, hydroperiod, and growth of *Spartina alterniflora* in a deteriorating Louisiana salt marsh. *J. Coast. Res.* 77–87. Available online at: <https://www.jstor.org/stable/4297954>.
- Reich, P. B., Wright, I. J., Cavender-Bares, J., Craine, J., Oleksyn, J., Westoby, M., et al. (2003). The evolution of plant functional variation: traits, spectra, and strategies. *Int. J. Plant Sci.* 164, S143–S164. doi: 10.1086/374368
- Ren, L., Guo, X., Liu, S., Yu, T., Guo, W., Wang, R., et al. (2020). Intraspecific variation in *Phragmites australis*: Clinal adaption of functional traits and phenotypic plasticity vary with latitude of origin. *J. Ecol.* 108, 2531–2543. doi: 10.1111/1365-2745.13401
- Roháček, K., Soukupová, J., and Barták, M. (2008). Chlorophyll fluorescence: a wonderful tool to study plant physiology and plant stress. *Plant Cell Compartments-Selected Topics*. Research Signpost, Kerala, India, 41, 104.
- Salo, T., and Gustafsson, C. (2016). The effect of genetic diversity on ecosystem functioning in vegetated coastal ecosystems. *Ecosystems* 19, 1429–1444. doi: 10.1007/s10021-016-0014-y
- Schlichting, C. D., and Levin, D. A. (1984). Phenotypic plasticity of annual *Phlox*: tests of some hypotheses. *Am. J. Bot.* 71, 252–260. doi: 10.1002/j.1537-2197.1984.tb12511.x
- Schreiber, U. (1998). “Chlorophyll fluorescence: new instruments for special applications,” in *Photosynthesis: Mechanisms and Effects: Volume I–V: Proceedings of the XIth International Congress on Photosynthesis, Budapest, Hungary, August 17–22, 1998*. (Netherlands: Springer), 4253–4258. doi: 10.1007/978-94-011-3953-3_984
- Schreiber, U., Endo, T., Mi, H., and Asada, K. (1995). Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol.* 36, 873–882. doi: 10.1093/oxfordjournals.pcp.a078833
- Stotz, G. C., Salgado-Luarte, C., Escobedo, V. M., Valladares, F., and Gianoli, E. (2021). Global trends in phenotypic plasticity of plants. *Ecol. Lett.* 24, 2267–2281. doi: 10.1111/ele.v24.10
- Stratoulas, D., Balzter, H., Zlinszky, A., and Tóth, V. R. (2015). Assessment of ecophysiology of lake shore reed vegetation based on chlorophyll fluorescence, field spectroscopy and hyperspectral airborne imagery. *Remote Sens. Environ.* 157, 72–84. doi: 10.1016/j.rse.2014.05.021
- Titus, J. E., and Adams, M. S. (1979). Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria spiralis* Michx. *Oecologia* 40, 273–286. doi: 10.1007/BF00345324
- Tóth, V. R. (2016). Reed stands during different water level periods: physico-chemical properties of the sediment and growth of *Phragmites australis* of Lake Balaton. *Hydrobiologia* 778, 193–207. doi: 10.1007/s10750-016-2684-z
- Tóth, V. R. (2018). Monitoring spatial variability and temporal dynamics of *Phragmites* using unmanned aerial vehicles. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00728
- Tóth, V. R., Villa, P., Pinardi, M., and Bresciani, M. (2019). Aspects of invasiveness of *Ludwigia* and *Nelumbo* in shallow temperate fluvial lakes. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00647
- Valladares, F., Gianoli, E., and Gomez, J. M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytol.* 176, 749–763. doi: 10.1111/j.1469-8137.2007.02275.x
- van der Putten, W. H. (1997). Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on Reed Die-back and Progression, (1993–1994). *Aquat. Bot.* 59, 263–275. doi: 10.1016/S0304-3770(97)00060-0
- Visser, J. M., Sasser, C. E., Chabreck, R. H., and Linscombe, R. (1999). Long-term vegetation change in Louisiana tidal marshes 1968–1992. *Wetlands* 19, 168–175. doi: 10.1007/BF03161746
- Vretare, V., Weisner, S. E. B., Strand, J. A., and Granéli, W. (2001). Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. *Aquat. Bot.* 69, 127–145. doi: 10.1016/S0304-3770(01)00134-6
- Woo, H. R., Kim, H. J., Lim, P. O., and Nam, H. G. (2019). Leaf senescence: systems and dynamics aspects. *Annu. Rev. Plant Biol.* 70, 347–376. doi: 10.1146/annurev-arplant-050718-095859



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Integrating water depth to predict the threshold of collapse and recovery of submerged macrophytes for lakes with large depth gradients

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Introduction: The nutrient threshold of collapse and recovery of submerged macrophytes have been widely reported for shallow lakes. However, understanding the threshold variation for lakes with water depth (Z) gradients remains limited.

Methods: In this study, based on a field investigation of 9 lakes with varying water depths and nutrient levels in the Yunnan Plateau, southwest of China, we integrated water depth to predict the nutrient threshold of collapse and recovery of submerged macrophytes in lakes.

Results: Our results showed that: 1) Canopy-forming submerged macrophytes, i.e. *Potamogeton pectinatus* and *Myriophyllum spicatum*, had a higher resistance to high nutrients and turbidity; 2) Submerged macrophyte species richness had a significantly negative response to water depth, while biomass did not; 3) A multiplication of turbidity (Turb) with water depth provided the best explanation for the collapse and recovery of submerged macrophytes for lakes with large depth gradients compared to the single variables; 4) The thresholds of Z_{SD}/Z were 0.06 for the collapse of submerged macrophytes and 0.53 for the recovery of submerged macrophytes; the corresponding thresholds were 81.6 and 9.92 NTU m for $Turb \times Z$, respectively.

Discussion: Our findings on the thresholds of macrophyte collapse and recovery are expected to provide quantitative guidance for lake restoration of diverse water depths.

KEYWORDS

submerged macrophytes, thresholds, water depth, nutrient, turbidity, transparency

1 Introduction

The extinction of submerged macrophytes in lakes has become a global ecological problem (Dakos et al., 2019; Carpenter et al., 2022), and the explored triggering factors varied from nutrient loading (Ibelings et al., 2007; Shang et al., 2023), fish introduction (Zambrano et al., 2001; Hobbs et al., 2016), changes in hydrological regime (Søndergaard et al., 1992; Wu et al., 2013), to climate changes (Scheffer et al., 2001b; Moss et al., 2011; Zhang et al., 2021). The disappearance of submerged macrophytes is often unpredictable, catastrophic, and irreversible (Scheffer et al., 2001a; Dakos et al., 2015; Janssen et al., 2021), posing a great threat to water quality and the survival of other aquatic organisms for a long time (Carpenter and Lodge, 1986; Jeppesen et al., 1998; Scheffer and Carpenter, 2003).

The growth of submerged macrophytes is often affected by multiple factors (Middelboe and Markager, 1997; Azzella et al., 2014; Zhang et al., 2017; Ren et al., 2022). Light has been most frequently reported as the main limiting factor affecting submerged macrophyte growth (Madsen et al., 2001). Generally, submerged macrophytes can only survive at depths where light intensity reaches at least 1% of that on the water surface (Sculthorpe, 1967). In many eutrophic lakes, the lack of light at the lake bottom and competition for light in the water column by phytoplankton resulted in the disappearance of submerged macrophytes (Blindow et al., 2006). Water depth affected the distribution of submerged macrophytes (May and Carvalho, 2010). Many studies have shown that deep water can attenuate underwater light intensity and inhibit the growth and spread of submerged macrophytes (Søndergaard et al., 2013). The abundance and maximum distribution depth of submerged macrophytes in deeper lakes were strongly related to light conditions (Chambers and Kalf, 1985; Sand-Jensen and Madsen, 1991). Besides, submerged macrophytes with different morphologies and physiologies are distributed in different water depth ranges (Fu et al., 2014; Wen et al., 2022). Excessive nutrient input caused the overgrowth of phytoplankton. The latter reduced the underwater light through the shading effect, thus impeding the survival of submerged macrophytes (Madsen et al., 2001; Sayer et al., 2010; Yu et al., 2015; van Wijk et al., 2023).

The nutrient thresholds of collapse and recovery of submerged macrophytes were originally proposed and widely studied for shallow lakes. Studies included multi-lake comparison (Jeppesen et al., 1990; Kosten et al., 2009; Wang et al., 2014), long-term monitoring (Jeppesen et al., 1999; Ibelings et al., 2007), and paleolimnological studies (Yang et al., 2006). A long-term observation of Lake Veluwe in the Netherlands showed that the coverage of aquatic plants decreased gradually when the total phosphorus (TP) increased to 0.15 mg L⁻¹, and disappeared completely at a TP concentration higher than 0.20 mg L⁻¹ (Ibelings et al., 2007). Data on water parameters and biotic factors from the 1950s to 2009 in Lake Dianchi identified that the TN and TP concentration thresholds at which the collapse of submerged macrophytes occurred were 1.2 mg L⁻¹ and 0.13 mg L⁻¹, respectively (Wang et al., 2018). A multi-lake analysis including empirical data from subtropical lakes on the Yangtze floodplain found that the TP thresholds of collapse and recovery of submerged macrophytes were 0.08–0.12 mg L⁻¹ and 0.04–0.06 mg L⁻¹, respectively (Wang et al., 2014). Besides, other factors, such as

transparency and water depth, also had limited impact on submerged macrophytes. An empirical model between submerged macrophytes and the ratio of Secchi depth (Z_{SD}) to mean depth (Z_M) in Yangtze lakes found that when the Z_{SD}/Z_M threshold was less than 0.45, the disappearance of submerged macrophytes may occur (Wang et al., 2005). Although most of these thresholds were widely based on shallow lakes, understanding of nutrient thresholds for lakes with large depth gradients is still very limited.

Studies based on simulation models suggested that thresholds of nutrients and critical turbidity for the thresholds of collapse and recovery of submerged macrophytes generally decreased with water depth (Genkai-Kato and Carpenter, 2005; Scheffer and van Nes, 2007; Janse et al., 2008). An empirical study based on multi-lake comparison and long-term monitoring for the Yangtze shallow lakes revealed that TP thresholds vary little at moderate depths, with an assumed notable decrease when depth exceeds a level of probably 3–4 m (Wang et al., 2014). The distribution and growth of submerged macrophytes are closely linked to water depth, it normally acted together with underwater light conditions and nutrients to influence the growth of submerged macrophytes (Middelboe and Markager, 1997; Baastrop-Spoehr et al., 2016; Chou et al., 2022; Zhang et al., 2022a). At specific eutrophication status, deeper distribution means stronger stress of light limitation on submerged macrophytes (Chen et al., 2023). Therefore, compared to the single variables of nutrient or light conditions, an integration with water depth may provide a better explanation on changes in the collapse and recovery of submerged macrophytes for lakes with large depth gradients.

To explore the thresholds of the collapse and recovery of submerged macrophytes for lakes under large depth gradients, nine lakes with a wide range of water depth and nutrient status in Yunnan Plateau, southwestern China, were investigated. Our purposes were fourfold: 1) To compare the responses of submerged macrophyte biomass and species richness to single variables of nutrients (or turbidity) or transparency and their integration with water depth; 2) To compare the single variable of nutrients (or turbidity) or transparency, a multiplication of nutrients (or turbidity) with water depth and a division of transparency by water depth on the explanation of changes in submerged macrophytes species richness and biomass for lakes with large depth gradients; 3) To explore the thresholds of collapse and recovery of submerged macrophytes for lakes with large depth gradients. The findings of this study can provide threshold conditions for lake managers to restore submerged macrophytes for lakes with large depth gradients.

2 Materials and methods

2.1 Study area

The Yunnan Plateau is located in the southwest of China, dominated by a subtropical highland monsoon climate with an average annual temperature of 15–18 °C and an annual precipitation of 1,000–1,200 mm. Nine plateau lakes (Lake Luguhu, Lake Chenghai, Lake Yangzonghai, Lake Erhai, Lake Dianchi, Lake Fuxianhu, Lake Xingyunhu, Lake Qiluhu, and Lake Yilonghu)

(Figure 1) were investigated from October to November (a season with high biomass), 2021. The total water area of these nine plateau lakes is approximately 1,021 km², and the total drainage area is 8,110 km². The lake area and mean water depth range from 31.7 to 297.9 km² and 2.2 to 38.6 m, respectively. The area of Lake Dianchi, Lake Fuxianhu, and Lake Erhai are larger than 200 km², and the remaining 6 lakes are smaller than 80 km². Besides Lake Luguhu and Lake Chenghai, all the other lakes have inflowing and outflowing rivers (Yang et al., 2023; Fan et al., 2023).

2.2 Field sampling and analysis

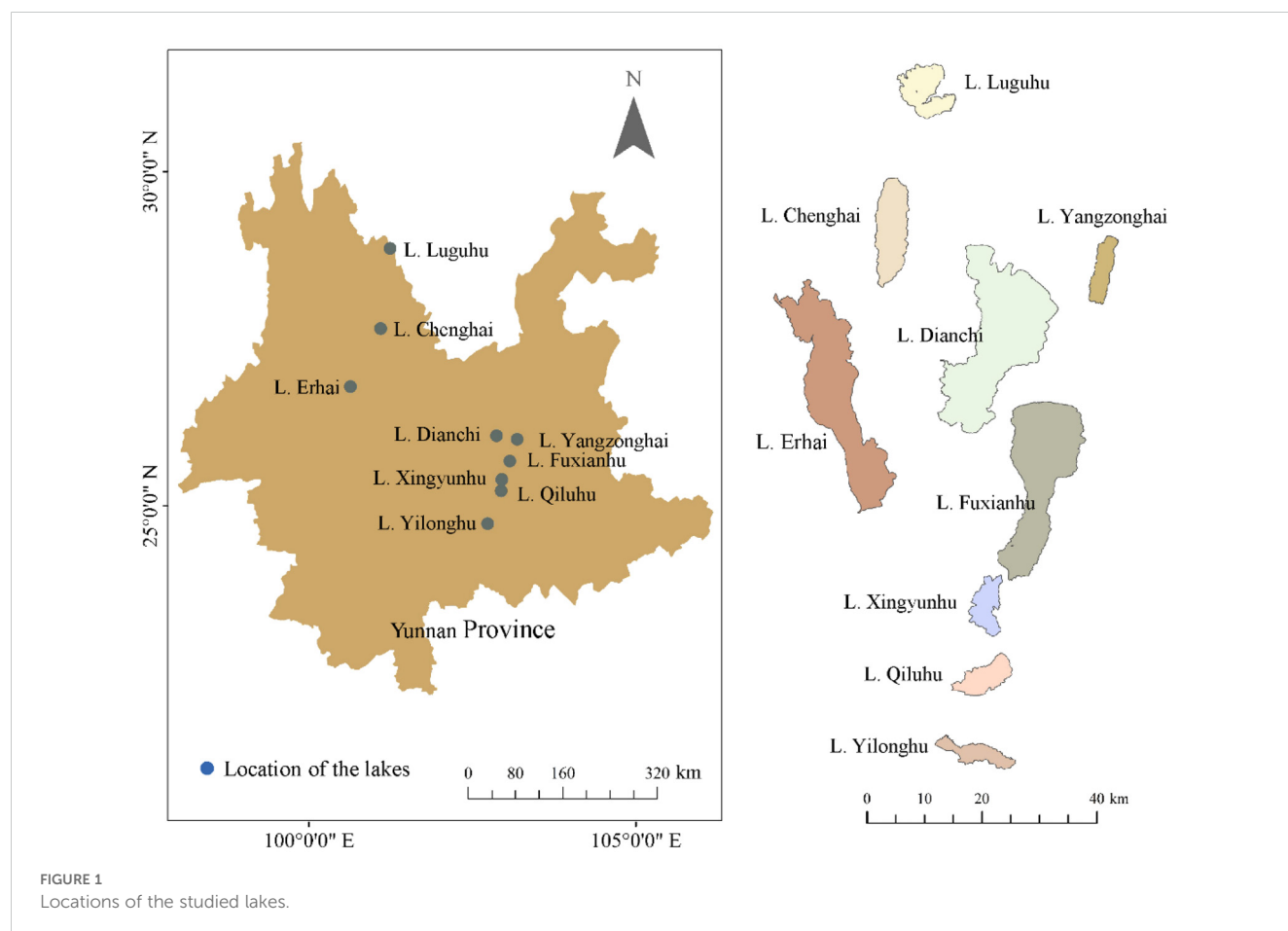
The field survey included 226 sampling sites, ranging from 17 to 50 for each lake according to their surface area. Water temperature (WT), dissolved oxygen (DO), conductivity (Cond), and pH were measured *in situ* using a YSI Pro Plus (Yellow Spring Inc, USA). Turbidity (Turb) was measured using a turbidimeter (2100 Q, HACH, Loveland, CO, USA). Water depth (Z) was measured using a depth sounder (Speedtech, SM-5A, USA). Transparency was measured with a Secchi Disc. Depth-integrated water samples were taken from three layers (surface, middle, and bottom water depth) within each site using a 5 L polymethyl methacrylate water sampler and then pooled into a bucket. One liter of well-mixed water was taken back to the laboratory and stored at 0°C for analysis of TN, TP, and phytoplankton Chlorophyll *a* (Chl *a*). TN was

determined by using an alkaline potassium persulfate digestion-UV spectrophotometric method, and TP was determined through an ammonium molybdate-UV spectrophotometric method (PERSEE, TU-1810, Beijing, China) (Huang et al., 1999). Chl *a* was extracted in 90% acetone at 4°C for 24 h after filtering the water sample through GF/C filters (Whatman, GE Healthcare UK Limited, Buckinghamshire, UK) (Huang et al., 1999). The absorbance values of the acetone extract were then measured at 665 nm and 750 nm by employing a spectrophotometer, both before and after acidification with 10% HCl. Main limnological characteristics (mean ± SD) of the sampling sites with and without macrophytes were showed in Supplementary Table A1.

Submerged macrophytes were randomly collected (2-4 replicates) with a grab-type sampler (0.2 m² in sampling area). Samples were rinsed to remove extraneous material such as sticks, macroinvertebrates, and substrates, and the macrophytes were identified to species and counted, to obtain the species richness (SR). The water on surface was wiped off, and the wet weight was measured. All the samples were immediately brought back to the lab for further analysis.

2.3 Data processing and analysis

The biomass of submerged macrophytes (B_{Mac}) was expressed by the dry biomass of leaves and stems for vascular plants (known as above-ground biomass). To prevent invalid values when taking



logarithms, 0.1 was added to the biomass and species richness of submerged macrophytes (Bachmann et al., 2002). The ‘diffslope’ function of the ‘Simba’ package in R was used to calculate the differences in slope and intercept between the two linear regressions of sites with and without macrophytes (Nekola and White, 1999). Mantel tests were used to explore the potential link between the submerged macrophyte biomass and species richness and nutrients or light conditions and their multiplication with water depth. Spearman’s correlation analysis identified the relevance between nutrients, light conditions, and their multiplications with water depth. Analysis of these processes was completed through the ‘linkET’ package in R. Redundancy discriminant analysis (RDA) and Monte Carlo permutation tests (permutations = 999) were used to analyze the effects of environmental factors on submerged macrophyte biomass and species richness using the ‘vegan’ package (Oksanen et al., 2016). All data analyses were conducted in R 4.1.3 (R Core Team, 2021).

3 Results

3.1 Environmental variables and submerged macrophytes

The nutrient concentrations of the studied lakes formed a clear gradient (Table 1). The water depth of the sampling sites ranged from 1.8 m to 24.2 m. Submerged macrophytes occurred in 74 of the 226 sampling sites. In total 17 species of submerged macrophytes were identified (Figure 2), with 8 species having a frequency higher than 20 and 4 lakes with ≥ 10 species, Charophyta the highest (33) while *Utricularia aurea* the lowest (1) according to their occurrence frequency of sampling sites. The occurrence frequencies of canopy-forming submerged macrophytes were higher than others. Species richness of submerged macrophytes in deep lakes was higher than that in shallow lakes. According to the numbers of distributed lakes, *Potamogeton pectinatus* and *Myriophyllum spicatum* were most widely distributed in 8 lakes (Figure 2).

3.2 The environment ranges of eight frequent submerged macrophytes

The distribution of submerged macrophytes is wide across environmental gradients. The maximum growing depth of Charophyte was the deepest (12.5 m) and the distribution was the widest (0.48–12.5 m), there were no significant differences for other species (Figure 3A). Besides, the maximum transparency of the distribution of Charophyte was the largest and the distribution range was also the widest. However, when integrated with water depth, the maximum Z_{SD}/Z of the distribution of *Potamogeton wrightii* was the largest among the eight frequent submerged macrophytes and the distribution range of *P. pectinatus* along the gradients of Z_{SD}/Z was the widest range (Figures 3B, C). In general, the distributions of the canopy-forming submerged macrophytes, i.e. *Potamogeton pectinatus* and *Myriophyllum spicatum* had larger and wider range of Turb, TN, and TP, which were the lowest for Charophyte. When integrated with water depth, the distribution range of the eight frequently submerged macrophytes along the gradients of environmental factors changed greatly. For example, When Z_{SD} was combined with water depth, the distribution range of *Potamogeton maackianus* along the Z_{SD}/Z gradients expanded notably. Conversely, when Turb, TN, and TP were integrated with water depth, this range contracted significantly. *Vallisneria natans* had no greater changes when Z_{SD} integrated with water depth, but increased a lot when Turb, TN, and TP integrated with water depth (Figure 3).

3.3 Relationships of Turb, Z_{SD} , and Chl *a* with nutrients for sampling sites with and without macrophytes

The ecosystem states, i.e. Turb, Z_{SD} , and Chl *a*, all showed significantly positive relationships with TN and TP for sampling sites with and without macrophytes ($p < 0.001$). Besides, Turb and Z_{SD} also showed significantly positive relationships with Chl *a* ($p <$

TABLE 1 Main limnological characteristics (mean \pm SD) of the sampling sites in nine studied lakes.

Studied lakes	Area (km ²)	Z_{Max} (m)	Z_{Min} (m)	Z_M (m)	TN (mg/L)	TP (mg/L)	Chl <i>a</i> (μg/L)	Turb (NTU)
L. Fuxianhu	216.6	14.3	1.9	9.1 \pm 4.5	0.13 \pm 0.04	0.004 \pm 0.002	1.93 \pm 0.46	0.6 \pm 0.2
L. Luguhu	48.5	69.4	1.3	24.2 \pm 22.0	0.11 \pm 0.02	0.006 \pm 0.002	0.76 \pm 0.18	0.7 \pm 0.2
L. Chenghai	77.2	26.8	4.7	19.7 \pm 7.2	0.56 \pm 0.12	0.03 \pm 0.01	8.54 \pm 5.55	2.0 \pm 0.8
L. Yangzonghai	31.7	27.5	0.5	14.4 \pm 9.8	0.77 \pm 0.06	0.03 \pm 0.006	6.58 \pm 3.96	2.1 \pm 0.7
L. Erhai	249.0	20.0	0.8	7.2 \pm 4.9	0.99 \pm 0.85	0.05 \pm 0.06	32.54 \pm 31.54	3.5 \pm 3.1
L. Dianchi	297.9	6.7	0.4	4.5 \pm 1.7	2.00 \pm 0.43	0.06 \pm 0.04	44.07 \pm 25.81	27.4 \pm 7.2
L. Xingyunhu	34.7	10.3	1.3	5.9 \pm 3.0	1.81 \pm 0.40	0.08 \pm 0.03	51.91 \pm 46.86	18.6 \pm 14.8
L. Yilonghu	38.0	4.5	0.7	1.8 \pm 0.9	3.22 \pm 0.21	0.10 \pm 0.01	84.00 \pm 10.72	48.6 \pm 11.1
L. Qiluhu	36.9	6.0	1.4	3.5 \pm 4.6	4.00 \pm 0.87	0.14 \pm 0.01	83.06 \pm 14.60	21.5 \pm 13.0

Area, the area of the studied lakes; Z_M , the mean water depth; Z_{Max} , the maximum water depth; Z_{Min} , the minimum water depth; TN, total nitrogen; TP, total phosphorus; Chl *a*, phytoplankton chlorophyll *a*; Turb, turbidity.

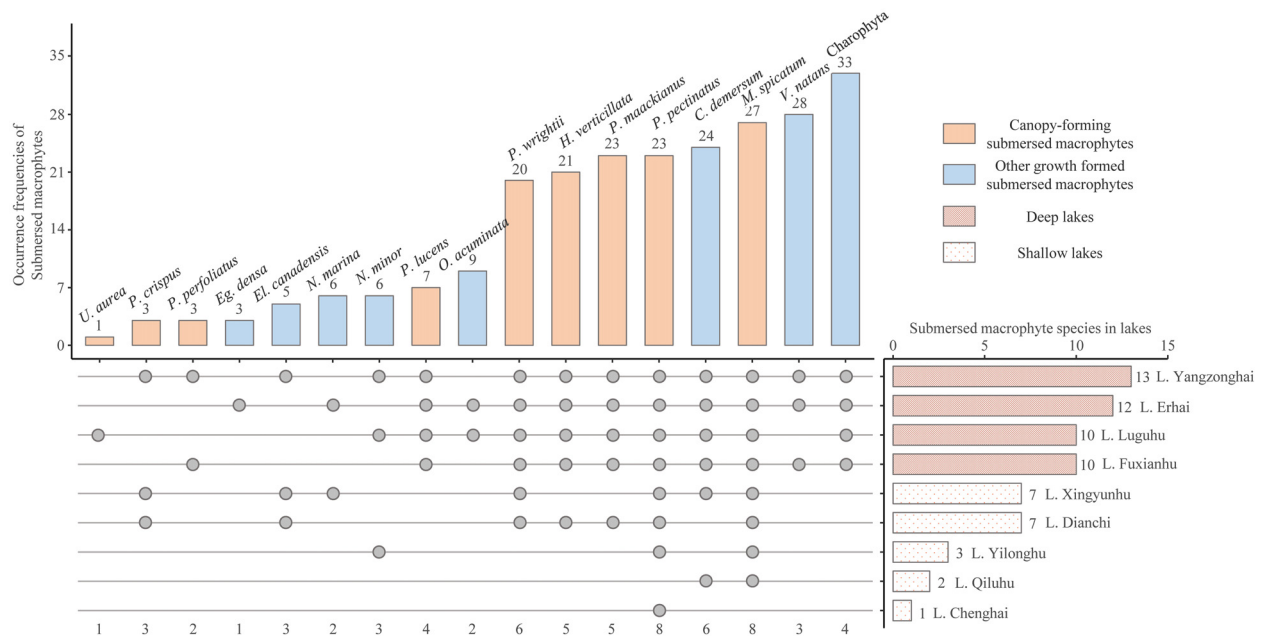


FIGURE 2

The occurrence frequencies of identified submerged macrophytes in the nine studies lakes (The points indicate the presence of submerged macrophyte species in the lakes. *U. aurea*, *Utricularia aurea*; *P. crispus*, *Potamogeton crispus*; *P. perfoliatus*, *Potamogeton perfoliatus*; *Eg. densa*, *Egeria densa*; *El. canadensis*, *Eloдея canadensis*; *N. marina*, *Najas marina*; *N. minor*, *Najas minor*; *P. lucens*, *Potamogeton lucens*; *O. acuminata*, *Ottelia acuminata*; *P. wrightii*, *Potamogeton wrightii*; *H. verticillata*, *Hydrilla verticillata*; *P. maackianus*, *Potamogeton maackianus*; *P. pectinatus*, *Potamogeton pectinatus*; *C. demersum*, *Ceratophyllum demersum*; *M. spicatum*, *Myriophyllum spicatum*; *V. natans*, *Vallisneria natans*).

0.001). But there were no notable differences in the relationships between sampling sites with and without macrophytes, only found significant difference in the relationship of Turb with TN between sampling sites with and without macrophytes. The scatterplots for sampling sites with and without macrophytes overlapped along the full gradients of x-variables; the pairs of sampling sites mixed together for the relations of $\log_{10}\text{Turb}$, $\log_{10}Z_{SD}$, and $\log_{10}\text{Chl } a$ against the nutrients and the relations of $\log_{10}\text{Turb}$ and $\log_{10}Z_{SD}$ against $\log_{10}\text{Chl } a$ (Figure 4).

3.4 Relationships between submerged macrophyte biomass, species richness and environmental factors

Mantel analysis showed that the biomass of submerged macrophytes (B_{Mac}) had highly positive correlations with Z and negative correlations with TN, TP, and Chl *a* ($p < 0.05$, Figure 5), while the species richness of submerged macrophytes (SR) indicated positive correlations with Z_{SD}/Z and negative correlations with Z, TN^*Z , TP^*Z , and $Turb^*Z$ ($p < 0.05$, Figure 5).

As for regression analysis, the biomass of submerged macrophytes increased remarkably with increasing Z_{SD} ($p < 0.05$, Figure 6), while decreased significantly with growing Turb, TN, and TP ($p < 0.05$, Figure 6). However, it showed no relationships with Z. The biomass of submerged macrophytes also indicated same trends with multiplications of nutrient concentrations (or turbidity) with water depth and a division of transparency by water depth, although only obvious relationships were found with TN^*Z and

TP^*Z ($p < 0.05$, Figure 6). The species richness of submerged macrophytes showed no relationships with Z_{SD} , Turb, TN, and TP, while increased significantly with growing Z_{SD}/Z ($p < 0.01$, Figure 6) and saw a notable decline with increasing Z, $Turb^*Z$, TN^*Z , and TP^*Z ($p < 0.01$, Figure 6).

The histograms showed the correlation coefficient (*R*) of biomass and species richness with Z, Z_{SD} , Turb, TN, TP, Z_{SD}/Z , $Turb^*Z$, TN^*Z , and TP^*Z . The absolute values of *R* between B_{Mac} and Z_{SD} , Turb, TN, and TP were higher than that when Z_{SD} , Turb, TN, and TP integrated with water depth, however, the absolute values of *R* between SR and Z_{SD} , Turb, TN, and TP were less than that when Z_{SD} , Turb, TN, and TP integrated with water depth (Figure 6).

The redundancy analysis revealed that 48.07% of the overall variability was explained by the first two principal components (RDA1 and RDA2) (Figure 7). Among the assessed physicochemical factors, Z was the most significant variable that influenced submerged macrophyte biomass and species richness ($p < 0.001$).

3.5 The thresholds of collapse and recovery of submerged macrophytes

When plotting the submerged macrophyte biomass and species richness against Z_{SD} and Turb, no folded bifurcation was found. The scatterplots for sampling sites with and without macrophytes overlapped along the full gradients of x-variables (Figures 8A, C, E, G). While multiplying Z_{SD} and Turb by water depth, a clear folded bifurcation emerged for their relations with $\log_{10}(B_{Mac}+0.1)$ and $\log_{10}(SR+0.1)$ (Figures 8B, D, F, H).

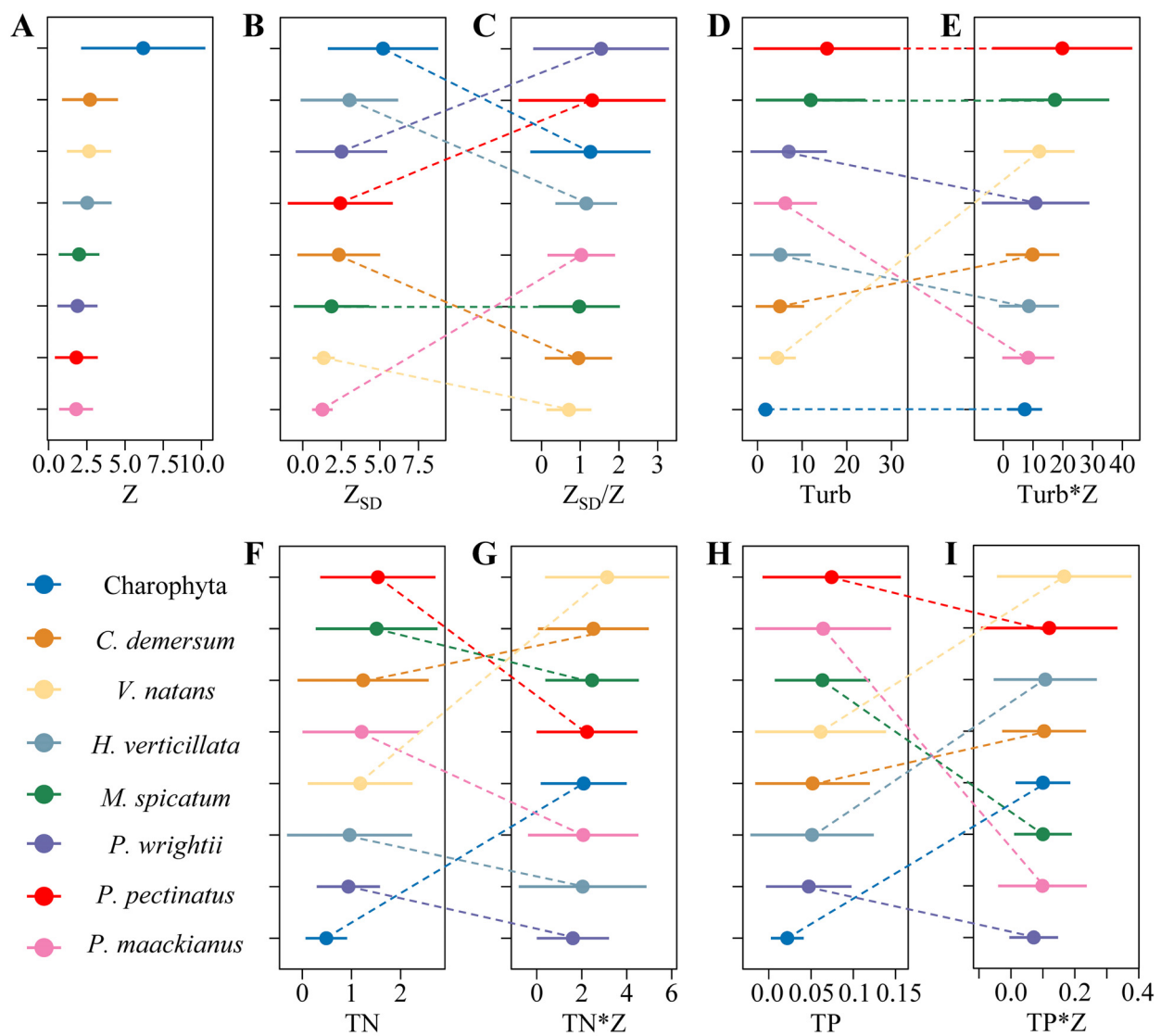


FIGURE 3

The distribution ranges of the eight frequent submerged macrophytes along the gradients of (A) water depth, (B) transparency, (D) turbidity, (F) TN - total nitrogen, (H) TP - total phosphorus and along most of them integrated with water depth e.g., (C) transparency, (E) turbidity, (G) TN, (I) TP (*C. demersum*, *Ceratophyllum demersum*; *V. natans*, *Vallisneria natans*; *H. verticillata*, *Hydrilla verticillata*; *M. spicatum*, *Myriophyllum spicatum*; *P. wrightii*, *Potamogeton wrightii*; *P. pectinatus*, *Potamogeton pectinatus*; *P. maackianus*, *Potamogeton maackianus*).

The thresholds of collapse and recovery of submerged macrophytes in lakes with large water depth spans were 0.06 and 0.53 for Z_{SD}/Z , 81.6 and 9.92 NTU m for Turb*Z, respectively.

4 Discussion

The study found that *Potamogeton pectinatus* and *Myriophyllum spicatum* were most widely distributed in the investigated lakes, with a larger nutrient and turbidity ranges. In other words, they had a higher resistance to high nutrients and turbidity, which was consistent with other survey (Li et al., 2017). *Potamogeton pectinatus* and *Myriophyllum spicatum* both belongs to canopy-forming submerged macrophytes with long branches and vigorous crown. Due to the high growth rate, canopy-forming submerged macrophytes can

concentrate at or near the water surface and have strong competition ability for light and space (Chambers and Kalff, 1987; James et al., 2004). Compared with other growth forms, canopy-forming macrophytes have a high tolerance to eutrophication (Zhang et al., 2022b). As a result, when the water quality deteriorates, canopy-forming submerged macrophytes gradually replace other growth forms as the main dominant species (Jeppesen et al., 2000; Sand-Jensen et al., 2017; Murphy et al., 2018).

Our study further found that submerged macrophyte species richness was more susceptible to the combinations of nutrient (or turbidity) or light conditions with water depth than single variables of nutrient or light conditions, while biomass showed the opposite patterns. The main reason is that water depth had a substantial negative effect on species richness, with no impact on biomass, which was in accordance with other previous study (Fu et al., 2014).

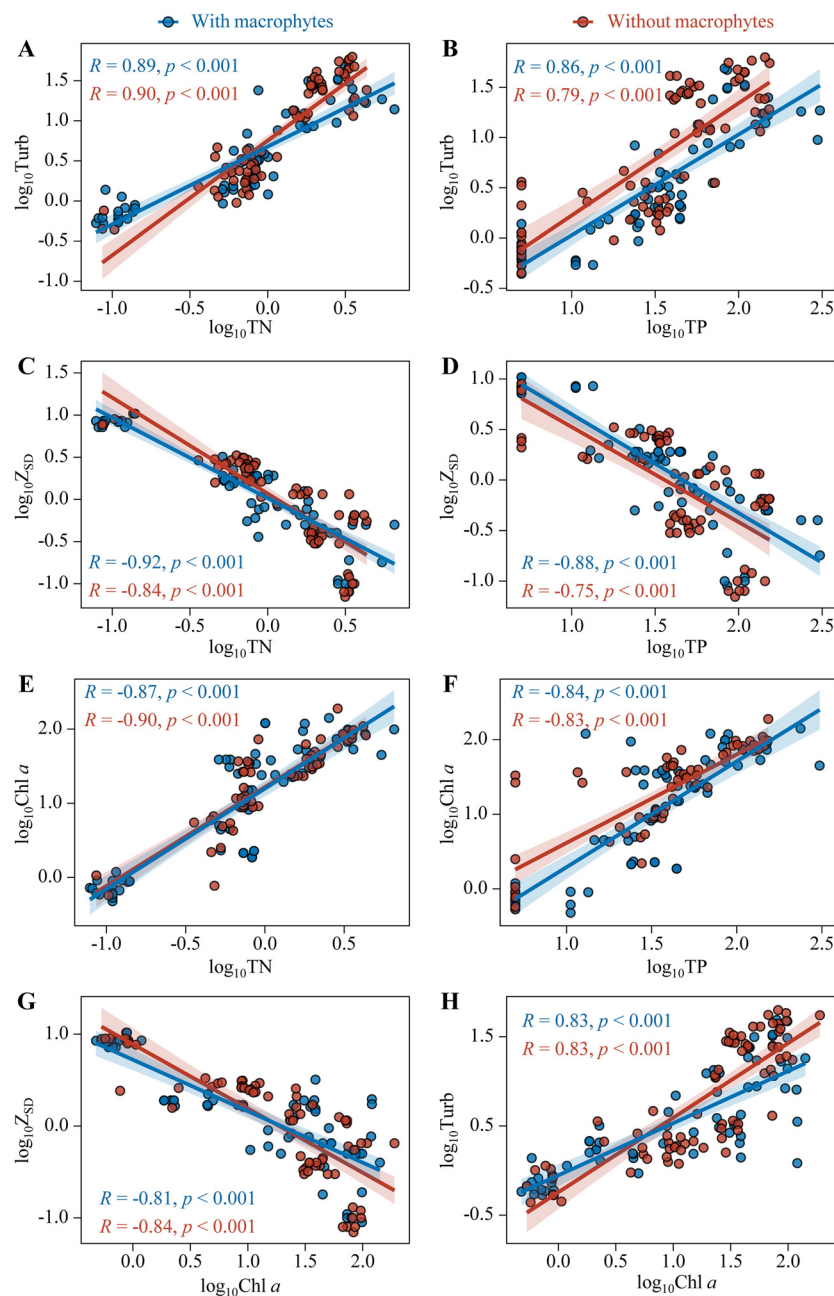


FIGURE 4

Relationships of Turb (A, B), Z_{SD} (C, D), and Chl *a* (E, F) with TN and TP and relationships of Z_{SD} (G) and Turb (H) with Chl *a* for sampling sites with (Blue points) and without macrophytes (Red points).

Water depth can more directly bring about light reduction and the stress of water pressure of submerged macrophytes (Schwarz et al., 1996; Søndergaard et al., 2013). With the increase of water depth and the attenuation of light, canopy-forming species, i.e. *P. pectinatus* and *P. maackianus* with higher light demand cannot survive and are replaced by bottom-dwelling and rosette-forming species with low light requirement. In our study, Charophyta was the only taxon where the water was deeper than 7 m. Due to the absence of competition from other species for space and light in

deep water, charophytes were able to proliferate in large numbers and formed dense and extensive “underwater meadows” with enormous biomass (Middelboe and Markager, 1997). Therefore, although the numbers of species in submerged macrophyte community decreased as the water depth increased, the overall biomass showed no obvious reduction.

Our study found that the thresholds of collapse and recovery of submerged macrophytes in deep lakes were 0.06 and 0.53 for Z_{SD}/Z, 81.6 and 9.92 NTU m for Turb*Z, respectively. Compared to Yangtze

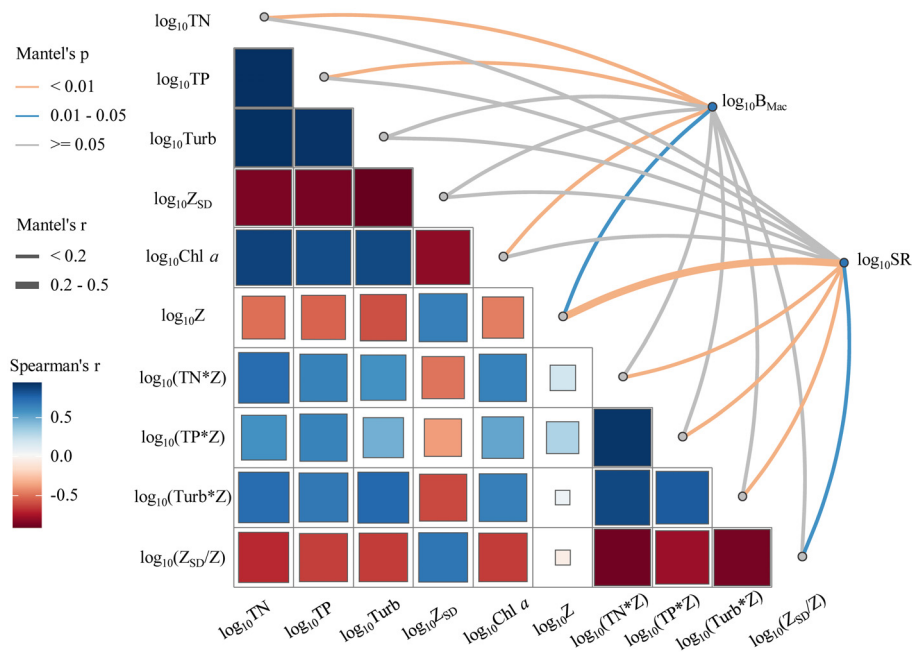


FIGURE 5

Pairwise correlations of environmental factors are shown with a color gradient denoting Spearman's correlation coefficient. Submerged macrophytes biomass (B_{Mac}) and species richness (SR) were related to each environmental factor by Mantel tests (The size of the square stands for the size of the Spearman's correlation coefficient).

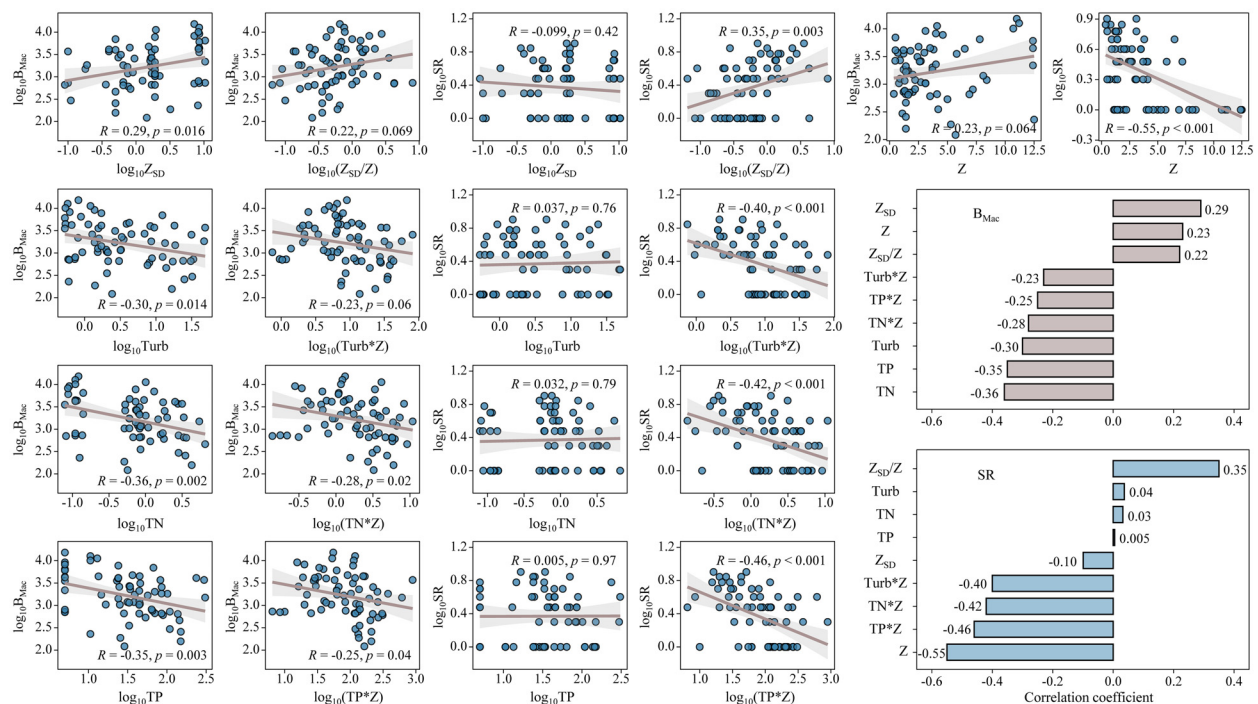


FIGURE 6

Linear regressions between submerged macrophyte biomass (B_{Mac}) and species richness (SR) and Z_{SD} , $Turb$, TN , TP , Z_{SD}/Z , $Turb*Z$, $TN*Z$, and $TP*Z$. Histograms showed the values of correlation coefficient (R) of biomass (B_{Mac}) and species richness (SR) with Z_{SD} , $Turb$, TN , TP , Z_{SD}/Z , $Turb*Z$, $TN*Z$, and $TP*Z$.

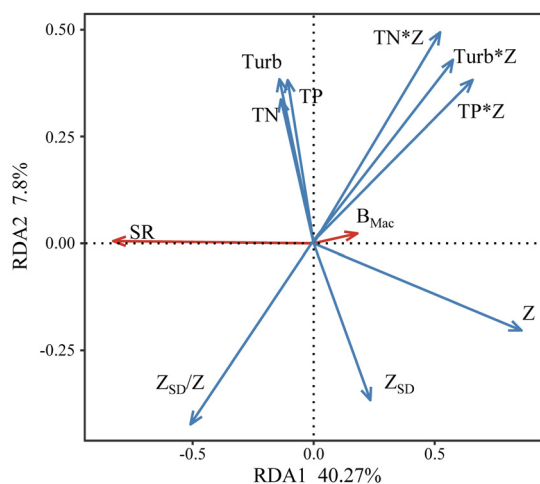


FIGURE 7
Redundancy analysis (RDA) of environmental factors and submerged macrophyte biomass (B_{Mac}) and species richness (SR).

River Plain lakes, the thresholds for Z_{SD}/Z of collapse of submerged macrophytes in Yunnan Plateau lakes were much lower than that in Yangtze River Plain lakes (Yunnan Plateau lakes: 0.06 and Yangtze River Plain lakes: 0.45). It seemed that the submerged macrophytes in Yunnan Plateau lakes had a higher resistance to low light than those in Yangtze River Plain lakes. The higher stoichiometric characteristics of submerged macrophytes in Yunnan Plateau lakes can best explain it (Xing et al., 2013; Li et al., 2017). For instance, the contents of C, N, and P of *M. spicatum* in Yangtze River Plain lakes were 359.87, 12.09, and 2.92 mg g^{-1} , respectively, while the contents were 425.11, 22.84, and 3.06 mg g^{-1} in Yunnan Plateau lakes. The similar values were also found for *P. malaianus* (Xing et al., 2013; Li et al., 2017). Under stresses, plants can produce various kinds of primary and secondary metabolites, i.e. enzyme to protect themselves, while, C, N, and P are the basic elements for the synthesis of various enzymes. Submerged macrophytes may accumulate additional C, N, and P to improve their resistance to low light. Besides, the difference in the UV part of the spectrum between the Yunnan Plateau and Yangtze plain may also influence the distribution of submerged macrophytes. UV-B radiation,

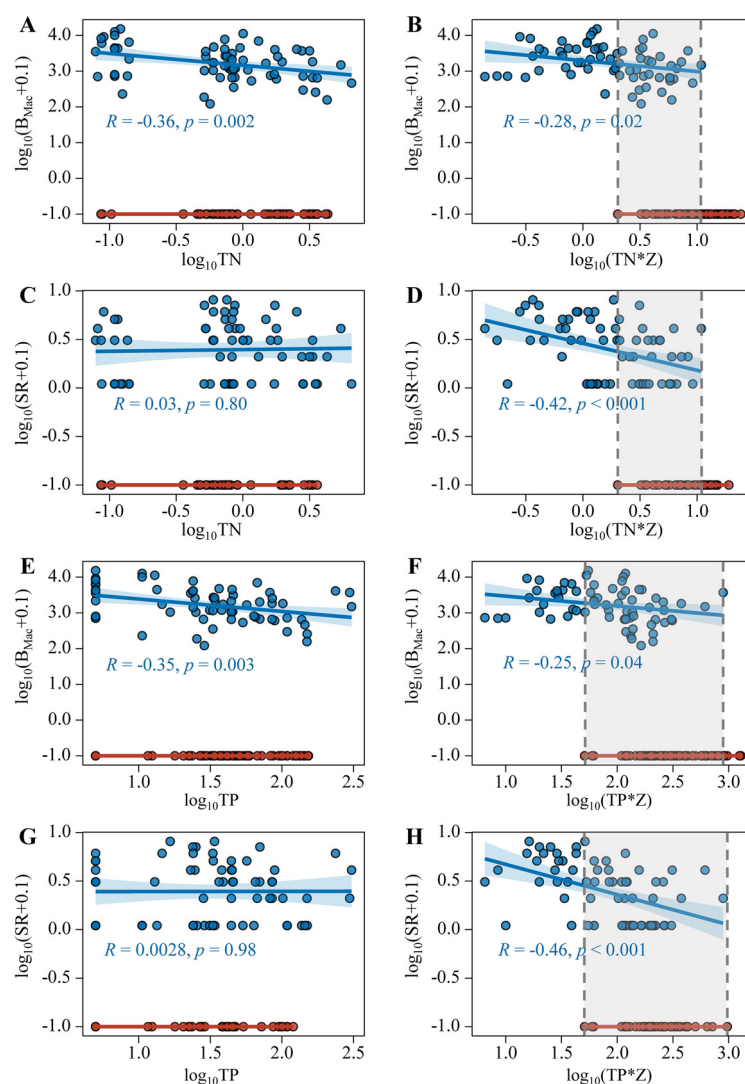


FIGURE 8
Relationships between $\log_{10} \text{TN}$ (A, C), $\log_{10}(\text{TN} \cdot \text{Z})$ (B, D), $\log_{10} \text{TP}$ (E, G), $\log_{10}(\text{TP} \cdot \text{Z})$ (F, H) with $\log_{10}(B_{Mac}+0.1)$ and $\log_{10}(\text{SR}+0.1)$ (Blue points indicate the sampling sites with macrophytes, red points indicate the sampling sites without macrophytes).

which is harmful to organisms, can penetrate several meters or even tens of meters into the water column (Smith et al., 1992). Due to the negative effects of strong UV-B on the physiological characteristics of submerged macrophytes, macrophytes may shift to greater depths to avoid damage (Yuan et al., 2019). Therefore, the collapse thresholds for Z_{SD}/Z of submerged macrophytes in Yunnan Plateau lakes were much lower than those in Yangtze River Plain lakes.

Previous studies on the thresholds of disappearance of submerged macrophytes was commonly focused on shallow lakes (Bayley and Prather, 2003; Ibelings et al., 2007; Wang et al., 2014). It had been identified turbidity and transparency as key environmental factors, which influenced the survival of submerged macrophytes in shallow lakes (Scheffer et al., 1993; Wang et al., 2014; van Wijk et al., 2023). However, due to nonnegligible effect of water depth on the survival of submerged macrophytes in deep lakes (Jeppesen et al., 2007; Kosten et al., 2009), the thresholds in deep lakes were different from shallow lakes (Jeppesen et al., 1990; Bachmann et al., 2002; Kosten et al., 2009; Wang et al., 2014), which may mainly depend on the differences of lake morphometry (Genkai-Kato and Carpenter, 2005; Kosten et al., 2009). For instance, the average water depth in this study was 10.03 m (1.79–24.19 m), which is much deeper than other lakes such as Lake Væng (1.2 m), Lake Veluwe (1.5 m), and Lake Baoan (1.9 m) (Jeppesen et al., 1999; Ibelings et al., 2007; Wang et al., 2014). Because of the good variation of B_{Mac} along the gradients of Z_{SD}/Z and $Turb*Z$ (contrary to the situation when water depth was removed), the thresholds of collapse and recovery of submerged macrophytes in deep lakes had a great dependence on the water depth. However, there was also a certain relevance between the thresholds for the two types of lakes. Turb and nutrients (promoted the growth of phytoplankton and then had a shading effect) can affect underwater light conditions and thus affect the distribution of submerged macrophytes (Schelske et al., 2010; Arthaud et al., 2012; Olsen et al., 2015; Zhang et al., 2016), but water depth can more directly bring about light reduction and the stress of water pressure (Schwarz et al., 1996; Søndergaard et al., 2013). It seemed that underwater light conditions were the most important factors affecting the survival of submerged macrophytes. Turb, Z_{SD} , Chl *a*, nutrient, and water depth all restricted the maximum growing depth of submersed macrophytes (Chambers and Kalfj, 1985; Søndergaard et al., 2013). Therefore, the survival of submerged macrophytes in lakes with different depths mainly depended on the demand of light.

5 Conclusions

In the present study, we found that: 1) Canopy-forming submerged macrophytes, i.e. *Potamogeton pectinatus* and *Myriophyllum spicatum*, were most widely distributed in the investigated lakes, with a higher resistance to high nutrients and

turbidity; 2) Submerged macrophyte species richness had significant negative response to water depth, while biomass did not; 3) Compared to the single variables, a multiplication of turbidity and a division of transparency with water depth provided a better explanation on predicting the thresholds of collapse and recovery of submerged macrophytes for lakes with large depth gradients.; 4) The thresholds of Z_{SD}/Z were 0.06 for the collapse of submerged macrophytes and 0.53 for the recovery of submerged macrophytes; the corresponding thresholds were 81.6 and 9.92 NTU m for $Turb*Z$, respectively. Our findings demonstrate that the role of water depth should be taken into account when restoring submerged macrophytes in the management of lakes with large depth gradients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

YHY: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. YLi: Formal analysis, Investigation, Resources, Writing – original draft. HW: Formal analysis, Funding acquisition, Supervision, Writing – review & editing. HS: Formal analysis, Writing – review & editing. QR: Supervision, Writing – review & editing. YLiu: Investigation, Writing – review & editing. PX: Project administration, Supervision, Writing – review & editing.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1541394/full#supplementary-material>

References

- Arthaud, F., Mousset, M., Vallod, D., Robin, J., Wezel, A., and Bornette, G. (2012). Effect of light stress from phytoplankton on the relationship between aquatic vegetation and the propagule bank in shallow lakes. *Freshw. Biol.* 57, 666–675. doi: 10.1111/j.1365-2427.2011.02730.x
- Azzella, M. M., Bolpagni, R., and Oggioni, A. (2014). A preliminary evaluation of lake morphometric traits influence on the maximum growing depth of macrophytes. *J. Limnol.* 73, 400–406. doi: 10.4081/jlimnol.2014.932
- Baastrup-Spohr, L., Moller, C. L., and Sand-Jensen, K. (2016). Water-level fluctuations affect sediment properties, carbon flux and growth of the isoetid *Littorella uniflora* in oligotrophic lakes. *Freshw. Biol.* 61, 301–315. doi: 10.1111/fwb.12704
- Bachmann, R. W., Horsburgh, C. A., Hoyer, M. V., Mataraza, L. K., and Canfield, D. E. (2002). Relations between trophic state indicators and plant biomass in Florida lakes. *Hydrobiologia* 470, 219–234. doi: 10.1023/A:1015660922494
- Bayley, S. E., and Prather, C. M. (2003). Do wetland lakes exhibit alternative stable states? Submersed aquatic vegetation and chlorophyll in western boreal shallow lakes. *Limnology Oceanography* 48, 2335–2345. doi: 10.4319/lo.2003.48.6.2335
- Blindow, I., Hargeby, A., and Schubert, J. M. (2006). Primary production in two shallow lakes with contrasting plant form dominance: a paradox of enrichment? *Limnol. Oceanogr.* 51, 2711–2721. doi: 10.4319/lo.2006.51.6.2711
- Carpenter, S. R., Arani, B. M. S., Van Nes, E. H., Scheffer, M., and Pace, M. L. (2022). Resilience of phytoplankton dynamics to trophic cascades and nutrient enrichment. *Limnology Oceanography* 67, S258–S265. doi: 10.1002/lno.v67.S1
- Carpenter, S. R., and Lodge, D. M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquat. Bot.* 26, 341–370. doi: 10.1016/0304-3770(86)90031-8
- Chambers, P. A., and Kalf, J. (1985). Depth distribution and biomass of submersed aquatic macrophyte communities in relation to Secchi depth. *Can. J. Fish. Aquat. Sci.* 42, 701–709. doi: 10.1139/f85-090
- Chambers, P. A., and Kalf, J. (1987). Light and nutrients in the control of aquatic plant community structure. I. *In situ* experiments. *J. Ecol.* 75, 611–619. doi: 10.2307/2260193
- Chen, S. W., Jiang, L., Ma, S. Z., Wu, Y., Ye, Q., Chang, Y. H., et al. (2023). Response of a submersed macrophyte (*Vallisneria spiralis*) to water depth gradients and sediment nutrient concentrations. *Sci. Total Environ.* 912, 169154–169154. doi: 10.1016/j.scitotenv.2023.169154
- Chou, Q., Zhang, W., Chen, J., Ren, W., Yuan, C., Wen, Z., et al. (2022). Phenotypic responses of a submersed macrophyte (*Vallisneria spiralis*) to low light combined with water depth. *Aquat. Bot.* 176, 103462. doi: 10.1016/j.aquabot.2021.103462
- Dakos, V., Carpenter, S. R., van Nes, E. H., and Scheffer, M. (2015). Resilience indicators: prospects and limitations for early warnings of regime shifts. *Phil. Trans. R. Soc. B* 370, 20130263. doi: 10.1098/rstb.2013.0263
- Dakos, V., Matthews, B., Hendry, A. P., Levine, J., Loeuille, N., Norberg, J., et al. (2019). Ecosystem tipping points in an evolving world. *Nat. Ecol. Evol.* 3, 355–362. doi: 10.1038/s41559-019-0797-2
- Fan, X., Yang, K., Yang, R. B., and Zhao, L. (2023). Changes in meteorological elements and its impacts on Yunnan Plateau lakes. *Appl. Sci.* 13, 2881. doi: 10.3390/app13052881
- Fu, H., Zhong, J. Y., Yuan, G. X., Xie, P., Guo, L. G., Zhang, X. L., et al. (2014). Trait-based community assembly of aquatic macrophytes along a water depth gradient in a freshwater lake. *Freshw. Biol.* 59, 2462–2471. doi: 10.1111/fwb.2014.59.issue-12
- Genkai-Kato, M., and Carpenter, S. R. (2005). Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. *Ecology* 86, 210–219. doi: 10.1890/03-0545
- Hobbs, J. M. R., Hobbs, W. O., Edlund, M. B., Zimmer, K. D., Theissen, K. M., Hoidal, N., et al. (2016). The legacy of large regime shifts in shallow lakes. *Ecol. Appl.* 26, 2662–2676. doi: 10.1002/eap.2016.26.issue-8
- Huang, X. F., Chen, W. M., and Cai, Q. M. (1999). *Standard methods for observation and analysis in Chinese ecosystem research network-survey, observation, and analysis of lake ecology* (Beijing: Standards Press of China).
- Ibelings, B. W., Portielje, R., Lammens, E. H. R. R., Noordhuis, R., van den Berg, M. S., Joosse, W., et al. (2007). Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: Lake Veluwe as a case study. *Ecosystems* 10, 4–16. doi: 10.1007/s10021-006-9009-4
- James, W. F., Barko, J. W., and Butler, M. G. (2004). Shear stress and sediment resuspension in relation to submersed macrophyte biomass. *Hydrobiologia* 515, 181–191. doi: 10.1023/B:HYDR.0000027329.67391.c6
- Janse, J. H., Domis, L. N. D. S., Scheffer, M., Lijklema, L., Van Liere, L., Klinge, M., et al. (2008). Critical phosphorus loading of different types of shallow lakes and the consequences for management estimated with the ecosystem model PCLake. *Limnologia* 38, 203–219. doi: 10.1016/j.limno.2008.06.001
- Janssen, A. B. G., Hilt, S., Kosten, S., de Klein, J. J. M., Paerl, H. W., and Van de Waal, D. B. (2021). Shifting states, shifting services: Linking regime shifts to changes in ecosystem services of shallow lakes. *Freshw. Biol.* 66, 1–12. doi: 10.1111/fwb.13582
- Jeppesen, E., Jensen, J. P., Kristensen, P., Søndergaard, M., Mortensen, E., Sortkjær, O., et al. (1990). Fish manipulation as a lake restoration tool in shallow, eutrophic, temperate lakes 2: threshold levels, long-term stability and conclusions. *Hydrobiologia* 200, 219–227. doi: 10.1007/BF02530341
- Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., and Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in Danish lakes: Changes along a phosphorus gradient. *Freshw. Biol.* 45, 201–218. doi: 10.1046/j.1365-2427.2000.00675.x
- Jeppesen, E., Meerhoff, M., Jacobsen, B. A., Hansen, R. S., Søndergaard, M., Jensen, J. P., et al. (2007). Restoration of shallow lakes by nutrient control and biomanipulation: the successful strategy varies with lake size and climate. *Hydrobiologia* 581, 269–285. doi: 10.1007/s10750-006-0507-3
- Jeppesen, E., Søndergaard, M., and Christoffersen, K. (1998). *The structuring role of submersed macrophytes in lakes*. (New York, NY, USA: Ecological Studies, Springer), 421.
- Jeppesen, E., Søndergaard, M., Kronvang, B., Jensen, J. P., Svendsen, L. M., and Lauridsen, T. L. (1999). Lake and catchment management in Denmark. *Hydrobiologia* 395, 419–432. doi: 10.1023/A:1017071602549
- Kosten, S., Kamarainen, A., Jeppesen, E., van Nes, E. H., Peeters, E. T. H. M., Mazzeo, N., et al. (2009). Climate-related differences in the dominance of submersed macrophytes in shallow lakes. *Glob. Change Biol.* 15, 2503–2517. doi: 10.1111/j.1365-2486.2009.01969.x
- Li, W., Zhong, J. Y., Yuan, G. X., Fu, H., Fan, H. B., Ni, L. Y., et al. (2017). Stoichiometric characteristics of four submersed macrophytes in three plateau lakes with contrasting trophic statuses. *Ecol. Eng.* 99, 265–270. doi: 10.1016/j.ecoleng.2016.11.059
- Madsen, J. D., Chambers, P. A., James, W. F., Koch, E. W., and Westlake, D. F. (2001). The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia* 444, 71–84. doi: 10.1023/A:1017520800568
- May, L., and Carvalho, L. (2010). Maximum growing depth of macrophytes in Loch Leven, Scotland, United Kingdom, in relation to historical changes in estimated phosphorus loading. *Hydrobiologia* 646, 123–131. doi: 10.1007/s10750-010-0176-0
- Middelboe, A. L., and Markager, S. (1997). Depth limits and minimum light requirements of freshwater macrophytes. *Freshw. Biol.* 37, 553–568. doi: 10.1046/j.1365-2427.1997.00183.x
- Moss, B., Kosten, S., Meerhoff, M., Battarbee, R. W., Jeppesen, E., Mazzeo, N., et al. (2011). Allied attack: climate change and eutrophication. *Inland Waters* 1, 101–105. doi: 10.5268/IW-1.2.359
- Murphy, F., Schmieder, K., Baastrup-Spohr, L., Pedersen, O., and Sand-Jensen, K. (2018). Five decades of dramatic changes in submerged vegetation in Lake Constance. *Aquat. Bot.* 144, 31–37. doi: 10.1016/j.aquabot.2017.10.006

- Nekola, J. C., and White, P. S. (1999). The distance decay of similarity in biogeography and ecology. *J. Biogeography* 26, 867–878. doi: 10.1046/j.1365-2699.1999.00305.x
- Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., and McGlinn, D. (2016). *Vegan: Community Ecology Package. R Package Version 2.4-0*. Available online at: <https://CRAN.R-project.org/package=vegan>.
- Olsen, S., Chan, F., Li, W., Zhao, S. T., Søndergaard, M., and Jeppesen, E. (2015). Strong impact of nitrogen loading on submerged macrophytes and algae: a long-term mesocosm experiment in a shallow Chinese lake. *Freshw. Biol.* 60, 1525–1536. doi: 10.1111/fwb.2015.60.issue-8
- R Core Team. (2021). R: A language and environment for statistical computing. (Vienna, Austria: R Foundation for Statistical Computing). Available online at: <https://www.R-project.org/>.
- Ren, W. J., Wen, Z. H., Cao, Y., Wang, H., Yuan, C. B., Zhang, X. L., et al. (2022). Cascading effects of benthic fish impede reinstatement of clear water conditions in lakes: A mesocosm study. *J. Environ. Manage.* 301, 113898. doi: 10.1016/j.jenvman.2021.113898
- Sand-Jensen, K., Bruun, H. H., and Bastrup-Spohr, L. (2017). Decade-long time delays in nutrient and plant species dynamics during eutrophication and re-oligotrophication of Lake Fure 1900–2015. *J. Ecol.* 105, 690–700. doi: 10.1111/1365-2745.12715
- Sand-Jensen, K., and Madsen, T. V. (1991). Minimum light requirements of submerged freshwater macrophytes in laboratory growth experiments. *J. Ecol.* 79, 749–764. doi: 10.2307/2260665
- Sayer, C. D., Davidson, T. A., and Jones, J. I. (2010). Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: a Eutrophication-driven pathway from plants to plankton? *Freshw. Biol.* 55, 500–513. doi: 10.1111/j.1365-2427.2009.02365.x
- Scheffer, M., and Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends Ecol. Evol.* 18, 648–656. doi: 10.1016/j.tree.2003.09.002
- Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., and Walker, B. (2001a). Catastrophic shifts in ecosystems. *Nature* 413, 591–596. doi: 10.1038/35098000
- Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., and Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends Ecol. Evol.* 8, 275–279. doi: 10.1016/0169-5347(93)90254-M
- Scheffer, M., Straile, D., van Nes, E. H., and Hosper, H. (2001b). Climatic warming causes regime shifts in lake food webs. *Limnol. Oceanogr.* 46, 1780–1783. doi: 10.4319/lo.2001.46.7.1780
- Scheffer, M., and van Nes, E. H. (2007). Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584, 455–466. doi: 10.1007/s10750-007-0616-7
- Schelske, C. L., Lowe, E. F., Kenney, W. F., Battoe, L. E., Brenner, M., and Coveney, M. F. (2010). How anthropogenic darkening of Lake Apopka induced benthic light limitation and forced the shift from macrophyte to phytoplankton dominance. *Limnol. Oceanogr.* 55, 1201–1212. doi: 10.4319/lo.2010.55.3.1201
- Schwarz, A. M., Hawes, I., and Howard-Williams, C. (1996). The role of photosynthesis light relationships in determining lower depth limits of Characeae in South Island, New Zealand lakes. *Freshw. Biol.* 35, 69–79. doi: 10.1046/j.1365-2427.1996.00481.x
- Sculthorpe, D. (1967). *The Biology of Aquatic Vascular Plants* (New York: St. Martin's Press).
- Shang, J., Zhang, W., Li, Y., Zheng, J., Ma, X., Wang, L., et al. (2023). How nutrient loading leads to alternative stable states in microbially mediated N-cycle pathways: A new insight into bioavailable nitrogen removal in urban rivers. *Water Res.* 236, 119938. doi: 10.1016/j.watres.2023.119938
- Smith, R. C., Prezelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., et al. (1992). Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* 225, 952–959. doi: 10.1126/science.1546292
- Søndergaard, M., Kristensen, P., and Jeppesen, E. (1992). Phosphorus release from resuspended sediment in the shallow and wind-exposed Lake Arreso, Denmark. *Hydrobiologia* 228, 91–99. doi: 10.1007/BF00006480
- Søndergaard, M., Phillips, G., Hellsten, S., Kolada, A., Ecke, F., Maemets, H., et al. (2013). Maximum growing depth of submerged macrophytes in European lakes. *Hydrobiologia* 704, 165–177. doi: 10.1007/s10750-012-1389-1
- van Wijk, D., Chang, M., Janssen, A. B. G., Teurlincx, S., and Mooij, W. M. (2023). Regime shifts in shallow lakes explained by critical turbidity. *Water Res.* 242, 119950. doi: 10.1016/j.watres.2023.119950
- Wang, H. Z., Wang, H. J., Liang, X. M., Ni, L. Y., Liu, X. Q., and Cui, Y. D. (2005). Empirical modelling of submersed macrophytes in Yangtze lakes. *Ecol. Model.* 188, 483–491. doi: 10.1016/j.ecolmodel.2005.02.006
- Wang, H. J., Wang, H. Z., Liang, X. M., and Wu, S. K. (2014). Total phosphorus thresholds for regime shifts are nearly equal in subtropical and temperate shallow lakes with moderate depths and areas. *Freshw. Biol.* 59, 1659–1671. doi: 10.1111/fwb.2014.59.issue-8
- Wang, M., Wu, J., Chen, H., Yu, Z., Zhu, Q. A., Peng, C., et al. (2018). Temporal-spatial pattern of organic carbon sequestration by Chinese lakes since 1850. *Limnol. Oceanogr.* 63, 1283–1297. doi: 10.1002/lno.10771
- Wen, Z. H., Wang, H., Zhang, Z. H., Cao, Y., Yao, Y. Q., Gao, X. Y., et al. (2022). Depth distribution of three submerged macrophytes under water level fluctuations in a large plateau lake. *Aquat. Bot.* 176, 103451. doi: 10.1016/j.aquabot.2021.103451
- Wu, Z., Cai, Y., Liu, X., Xu, C. P., Chen, Y., and Zhang, L. (2013). Temporal and spatial variability of phytoplankton in Lake Poyang: The largest freshwater lake in China. *J. Great Lakes Res.* 39, 476–483. doi: 10.1016/j.jglr.2013.06.008
- Xing, W., Wu, H. P., Hao, B. B., and Liu, G. H. (2013). Stoichiometric characteristics and responses of submerged macrophytes to eutrophication in lakes along the middle and lower reaches of the Yangtze River. *Ecol. Eng.* 54, 16–21. doi: 10.1016/j.ecoleng.2013.01.026
- Yang, X. D., Shen, J., Dong, X. H., Liu, E. F., and Wang, S. M. (2006). Historical trophic evolutions and their ecological responses from shallow lakes in the middle and lower reaches of the Yangtze River: case studies on Longgan Lake and Taibai Lake. *Sci. China Ser. D* 49, 51–61. doi: 10.1007/s11430-006-8105-9
- Yang, L. J., Tao, Y., Jiang, X., Wang, Y., Li, Y. H., Zhou, L., et al. (2023). Interactive effects of nutrients and salinity on zooplankton in subtropical plateau lakes with contrasting water depth. *Front. Environ. Sci.* 11, 1110746. doi: 10.3389/fenvs.2023.1110746
- Yu, Q., Wang, H. J., Li, Y., Shao, J. C., Liang, X. M., Jeppesen, E., et al. (2015). Effects of high nitrogen concentrations on the growth of submersed macrophytes at moderate phosphorus concentrations. *Water Res.* 83, 385–395. doi: 10.1016/j.watres.2015.06.053
- Yuan, C. B., Zhu, T. S., Cao, T., Xi, Y. L., Zhang, X. L., and Ni, L. Y. (2019). Antioxidant systems of aquatic macrophytes in three life forms: a case study in Lake Erhai. *J. Ocean. Limnol.* 37, 665–674. doi: 10.1007/s00343-019-8025-0
- Zambrano, L., Scheffer, M., and Martinez-Ramos, M. (2001). Catastrophic response of lakes to benthivorous fish introduction. *Oikos* 94, 344–350. doi: 10.1034/j.1600-0706.2001.940215.x
- Zhang, H., Huo, S., Xiao, Z., He, Z., Yang, J., Yeager, K. M., et al. (2021). Climate and nutrient-driven regime shifts of cyanobacterial communities in low-latitude plateau lakes. *Environ. Sci. Technol.* 55, 3408–3418. doi: 10.1021/acs.est.0c05234
- Zhang, Y. L., Jeppesen, E., Liu, X. H., Qin, B. Q., Shi, K., Zhou, Y. Q., et al. (2017). Global loss of aquatic vegetation in lakes. *Earth-Sci. Rev.* 173, 259–265. doi: 10.1016/j.earscirev.2017.08.013
- Zhang, Y. L., Liu, X. H., Qin, B. Q., Shi, K., Deng, J. M., and Zhou, Y. Q. (2016). Aquatic vegetation in response to increased eutrophication and degraded light climate in eastern Lake Taihu: implications for lake ecological restoration. *Sci. Rep.* 6, 23867. doi: 10.1038/srep23867
- Zhang, C. X., Pei, H. C., Lu, C., Liu, P. Z., Liu, C. Q., and Lei, G. C. (2022b). Eutrophication drives regime shift via changes in stoichiometric homeostasis-based submerged macrophyte assemblages. *NPJ Clean Water* 5, 17. doi: 10.1038/s41545-022-00161-6
- Zhang, M., Wen, S. L., Wu, T., Wang, S. M., Li, X., Gong, W. Q., et al. (2022a). Patterns of internal nitrogen and phosphorus loadings in a cascade reservoir with a large water level gradient: Effects of reservoir operation and water depth. *J. Environ. Manage.* 320, 115884. doi: 10.1016/j.jenvman.2022.115884



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Comparative diversity of aquatic plants in three Central European regions

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Freshwaters are among the most threatened ecosystems globally, with biodiversity declining at far greater rates than the biodiversity of the most affected terrestrial ecosystems. There is an urgent need for accurate information on spatial patterns of freshwater biodiversity, a first step in effective conservation planning and management of these ecosystems. We explored patterns of aquatic macrophyte diversity in four waterbody types, rivers, streams, ponds and ditches, across three Central European regions. By analyzing local (α), among-site (β) and regional (γ) diversity, we assessed the roles of these ecosystems as biodiversity hotspots, particularly for red-listed species. Sampling 220 sites across Slovakia and Slovenia, we recorded 113 macrophyte taxa (31% of which were red-listed), with ponds and ditches consistently supporting higher α and γ diversity than running waters. β diversity was primarily driven by species turnover, with ponds displaying high heterogeneity linked to environmental variability. Our findings highlight the conservation value of artificial habitats like ditches and ponds, harbouring significant macrophyte diversity, including unique and threatened species. These results underscore the need to prioritize small waterbodies in biodiversity conservation strategies within agricultural landscapes.

KEYWORDS

α , β , γ diversity, turnover, nestedness, meta-analysis, ditches, ponds

Introduction

Freshwaters are among the most threatened ecosystems on Earth (Dudgeon et al., 2006). Intensification of land use, eutrophication, flow modification, expanding hydropower exploitation, pollution, changes in biotic interactions due to the introduction of invasive alien species, and climate change are the main direct drivers

of freshwater degradation (Sala et al., 2000; Millennium Ecosystem Assessment, 2005; Williams-Subiza and Epele, 2021). The degradation and loss of freshwater habitats occur more rapidly than in other ecosystems, and the adverse effects of these changes on freshwater biodiversity are considerable (Dudgeon et al., 2006; Lacoul and Freedman, 2006; Reid et al., 2019). As a result of all these impacts, freshwater biodiversity is declining at far greater rates than the biodiversity of the most affected terrestrial ecosystems, and this trend is expected to continue (Ricciardi and Rasmussen, 1999). There is an urgent need for accurate information on freshwater biodiversity, as the state of knowledge regarding biodiversity threats is unsatisfactory for many freshwater habitat types (Millennium Ecosystem Assessment, 2005), and inventories of freshwater biodiversity are far from complete in many regions (Dudgeon et al., 2006).

While preserving intact freshwater ecosystems and their biodiversity remains a conservation priority, the call for recognition of the important potential of human-modified habitats to maintain freshwater biodiversity appeared relatively recently (Dudgeon et al., 2006). Regardless of habitat type, identifying biodiversity hotspots is still mandatory for effectively protecting freshwater biodiversity. Early studies in the United Kingdom compared freshwater biodiversity across various waterbodies, from natural to human-modified and even artificial (Williams et al., 2004; Biggs et al., 2007). Davies et al. (2008a, b) expanded the geographic scope by comparing aquatic diversity in agricultural landscapes across Denmark, France, Germany and the United Kingdom. The results of recent studies highlight the role of small waterbodies as hotspots for plant and macroinvertebrate biodiversity (Biggs et al., 2017; Zelnik et al., 2018). In particular, ponds have been shown to support more rare and red-list species than other waterbody types. The high biodiversity values of both natural and artificial ponds have also been demonstrated by other studies (e.g., Boix et al., 2012; Lukács et al., 2013; Bubíková and Hrivnák, 2018a). However, ponds remain a low priority in national and international conservation and environmental legislation in most countries (Hill et al., 2018, 2021).

In addition to ponds, ditches – shallow channels found in agricultural landscapes – can play a key role in preserving biodiversity in human-dominated environments (Svitok et al., 2016; Bubíková and Hrivnák, 2018b). Historic ditches can contribute to cultural heritage and biodiversity conservation (Lin et al., 2020). These habitats are also considered hotspots for macrophyte diversity (Verdonschot et al., 2011; Dorotovičová, 2013; Clarke, 2015). However, other specific habitat type, lakes, can have higher or equal alpha diversity in landscapes with various dominant land use compared to ponds, canals and ditches (Law et al., 2024). Given the importance of spatial processes in community structuring (Wiens et al., 1993), it is unlikely that findings from a limited number of case studies can be easily extrapolated to other geographical and ecological contexts. The diversity of local communities is influenced by the regional species pool, local biotic interactions and abiotic factors, with different environmental variables potentially controlling community structure in different regions. Specifically, freshwater diversity patterns are regionally context-dependent (Heino, 2011). In

macrophyte communities, where the significance of spatial processes and environmental factors can vary unpredictably by location, it may be unwise to draw broad conclusions from a few geographical regions (Alahuhta and Heino, 2013; Alahuhta et al., 2017).

In this study, we assessed patterns of macrophyte diversity across a range of aquatic ecosystems in three Central European regions, which primarily differ in altitude and associated climate but share a large majority of species. Compared to studies conducted in Western Europe, these Central European regions were historically shaped by socialist-style agriculture characterized by large-scale monocultural farming and state-directed practices that frequently neglected ecological considerations (Bezák and Mitchley, 2014). Information on the comparative diversity of macrophytes in Central Europe is limited; there is only one study from the Váh River Valley in Slovakia (Bubíková and Hrivnák, 2018b).

The geographic proximity of these regions and the strong dispersal abilities of aquatic plants are advantageous, as they significantly reduce the effect of dispersal limitation on our results. Specifically, we aimed to compare local (α), among-site (β), and regional (γ) diversity of aquatic macrophytes in ponds, ditches, streams, and rivers across Slovakia and Slovenia. More broadly, we tested the generality of conclusions from previous comparative diversity studies within a Central European context. Based on earlier research (e.g., Williams et al., 2004; Davies et al., 2008a; Bubíková and Hrivnák, 2018b), we hypothesize that ponds and ditches serve as hotspots of macrophyte biodiversity in these Central European regions, despite their artificial origin.

To inform conservation planning, we specifically focused on the diversity of red-list species. Aquatic macrophytes are of particular conservation importance, as they include many threatened species (Bilz et al., 2011; Bolpagni et al., 2018) and are relatively easy to identify, making them a valuable proxy group of organisms that are less challenging to distinguish (Gioria et al., 2012; Law et al., 2019). Despite these advantages, aquatic macrophytes remain insufficiently studied across large spatial scales (Alahuhta and Heino, 2013). We believe this research will help establish practical conservation priorities for freshwater habitats in Central Europe.

Study sites

The comparative diversity study was conducted in three model regions of Central Europe: the Turčianska kotlina Basin (TKB) in northern Slovakia (centered around 48.988°N, 18.883°E) within the Continental biogeographical region (see Cervellini et al., 2020; hereafter referred to as bioregion), the Borská nížina Lowland (BNL) in south-western Slovakia (~48.490°N, 17.072°E) within the Pannonian bioregion, and Northeast Slovenia (NESLO, ~46.566°N, 16.002°E), which lies at the transition between the Pannonian and Continental bioregions (Figure 1). These model areas mainly differ in altitude (mean altitude: TKB 437 m, min–max 383–576; BNL 161 m, 142–233; NESLO 226 m, 164–374) and associated climate. The mean annual temperature is lowest in TKB (7.4°C, 6.7–7.8), while the other two regions have the same temperature of about 9.8°C (BNL 9.3–10.1 and NESLO 9.2–10.3).

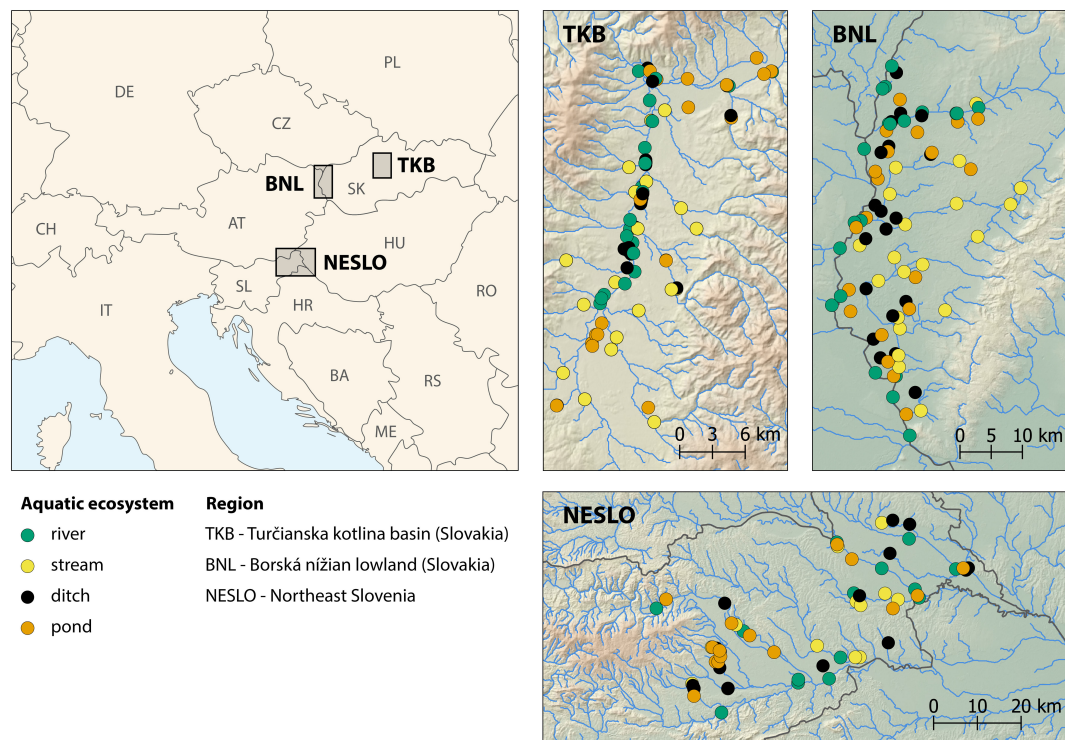


FIGURE 1

Location of the three studied regions in Central Europe and the positions of individual waterbodies within these regions.

Mean annual precipitation totals increase in the following order: BNL (695 mm, 641–823), TKB (805 mm, 794–838) and NESLO (944 mm, 772–1097). Additional details are given in [Supplementary Table S2](#).

Methods

Sampling

Four types of aquatic ecosystems were explored within the studied regions: rivers, streams, ditches and ponds. We used classification criteria for waterbody types similar to those in previous studies on comparative diversity ([Williams et al., 2004](#); [Bubíková and Hrivnák, 2018b](#)) (for details, see [Supplementary Table S1](#)). A total of 220 sites were randomly selected from all potential sites. Sampling sites were stratified by region (80 in Slovakia and 60 in Slovenia) and waterbody type (20 sites per waterbody type in Slovakia and 15 in Slovenia). Waterbodies were sampled for macrophytes during the early summer (June) and late summer (August and September) of 2011 in Slovakia and 2016 in Slovenia to minimize the effect of vegetation seasonality.

At each sampling site, an area of 100 m² was assessed, and the presence of all macrophytes (cf. [Janauer and Dokulil, 2006](#)) was recorded by walking or boat. The sampling area in rivers, streams and ditches was determined by the length × width of the watercourse. In ponds, the area was approximately triangular, with the apex at the centre of the waterbody and the base

following the waterbody's margin. The smallest lentic waterbodies were excluded from potential sampling sites based on the specified sampling area (cf. [Williams et al., 2004](#)).

For the identification of red-list species, we used the lists published by [Eliáš et al. \(2015\)](#) for Slovakia and those presented in “https://www.uradni-list.si/files/RS_-2002-082-04055-OB~P001-0000.PDF” for Slovenia. Species nomenclature was standardized according to the Euro+Med PlantBase (<https://euromedplus.org>; for full names see [Supplementary Table S3](#)).

Data analyses

Plant incidence data were summarized in species presence/absence matrices. We compared the diversity across the four waterbody types mentioned above within the three regions, examining three spatial scales: local (α) diversity, among-site (β) diversity, and regional (γ) diversity ([Magurran, 2003](#)). We conducted separate analyses on matrices for all recorded macrophyte species (total species), true aquatic plants (hydrophytes) and species of conservation concern (red-listed species).

Local (α) diversity

This study defined α diversity as the species richness within individual waterbodies. Differences in local diversity were assessed using generalized linear models (GLMs) with a Poisson error distribution and a logarithmic link function ([McCullagh and](#)

Nelder, 1989). The GLMs included fixed effects for waterbody type (four levels: river, stream, ditch and pond), region (three levels: TKB, BNL and NESLO) and their interaction (waterbody \times region). Model assumptions were examined using residual diagnostics. Due to considerable overdispersion, GLMs were refitted using a negative binomial distribution for overall α diversity and α diversity of hydrophytes (Hilbe, 2011). Statistically significant results from the overall GLM tests were followed by pairwise Tukey-type comparisons (Lenth, 2016).

Among-site (β) diversity

We defined β diversity as a variation in the species composition among particular sites. However, two different ecological phenomena can produce differences in species composition among sites: spatial turnover of species and nestedness of assemblages (Baselga, 2010, 2012). Species spatial turnover implies the replacement of species by others from site to site due to environmental sorting or spatial and historical constraints. In contrast, nestedness of species assemblages occurs when the species composition of poorer assemblages are nested subsets of richer assemblages as a consequence of various processes (e.g., Wright et al., 1997; Ulrich et al., 2009; Leprieur et al., 2011). Thus, we disentangled the turnover and nestedness component of β diversity following Baselga (2010, 2012). Specifically, we calculated Sørensen dissimilarity among sampling sites and additively partitioned this total dissimilarity measure (β_{SOR}) to dissimilarity due to species replacement (a turnover component of dissimilarity: β_{STU}) and dissimilarity due to nestedness (nestedness-resultant component of dissimilarity: β_{SNE}). Differences in the total, turnover and nestedness-resultant components of β diversity among habitats within regions were assessed using distance-based tests for homogeneity of multivariate dispersion with 10,000 permutations (Anderson, 2006). Pairwise Tukey comparisons followed significant overall tests.

Regional (γ) diversity

We expressed γ diversity as the total number of species per habitat within each region. Randomization tests were employed to assess the null hypothesis that there are no differences in the total number of species among waterbody types within a given region. We used the differences in total species counts as the test statistic, comparing the observed values against a null distribution generated from 10,000 randomly reshuffled datasets (Manly, 2007). We calculated the probabilities of detecting differences greater than or equal to the observed value from these comparisons. Due to the unequal number of sites sampled in Slovakia ($n = 20$ per habitat) and Slovenia ($n = 15$), we employed sample-based rarefaction analysis to estimate the total number of taxa expected across fifteen sites per habitat and region. Ninety-five percent confidence intervals for each estimate were calculated using the analytical formulas provided by Colwell et al. (2004).

Analyses were performed in R (R Core Team, 2022), using the libraries *betapart* (Baselga et al., 2023), *ggplot2* (Wickham, 2016), *emmeans* (Lenth, 2023), *iNEXT* (Hsieh et al., 2022), *MASS* (Venables and Ripley, 2002) and *vegan* (Oksanen et al., 2022).

Results

Altogether, 113 macrophyte taxa were identified across the study regions. Vascular plants comprised 86.1% of these taxa, followed by bryophytes – *Fontinalis antipyretica*, *Rhynchostegium riparioides* and *Riccia fluitans* – at 2.7% and macroscopic algae (identified to genus level as *Chara* and *Nitella*) at 1.8%. The highest number of macrophytes was detected in NESLO (94 taxa), followed by BNL (51) and TKB (43). All three regions had similarly high numbers of hydrophytes NESLO (24), THB (26) and BNL (22). The number of red-listed plants was comparable across the regions, with NESLO having 24, TKB 19 and BNL 17. The studied waterbodies were relatively species-poor, with macrophyte counts ranging from 0 to 22 in NESLO, 0 to 14 in BNL, and 0 to 10 in TKB. The most common plants in TKB were hydrophytes, with *Fontinalis antipyretica* at 30% and *Myriophyllum spicatum* at approximately 22%. In BNL, hydrophytes were also most frequent, with *Lemna minor* at around 42% and *Ceratophyllum demersum* at about 29%. In contrast, helophytes were most prevalent in NESLO, where *Phalaris arundinacea* reached 55% and *Agrostis stolonifera* approximately 37%. The most frequently found red-listed species were *Ranunculus aquatilis* in TKB (21%), *Potamogeton nodosus* in BNL (20%) and *Myriophyllum spicatum* and *Carex riparia* in NESLO (each at 25%).

Each waterbody type supported unique species that were not found in any other type, with the highest numbers in ponds and ditches and considerably fewer in streams and rivers. The number of species unique to ponds, ditches, rivers and streams was as follows: TKB – 13, 10, 3, 0; BNL – 7, 7, 2, 2; NESLO – 13, 11, 6, 6 (Supplementary Table S4). Ponds played an especially important role in supporting unique red-listed species. We recorded 4, 5 and 7 unique threatened species in TKB, BNL and NESLO ponds, respectively. Other aquatic ecosystems supported substantially fewer unique red-list species: in TKB, ditches had 2, rivers 3 and streams none; in BNL 3, 0 and 1; and in NESLO 2, 1 and 1 (for details see Supplementary Table S4).

Local (α) diversity

We did not find evidence for a general pattern of differences in α diversity among the aquatic ecosystems across the studied regions, as indicated by a significant interaction between region and habitat for all species ($\chi^2 = 19.7$, $df = 6$, $p = 0.003$), hydrophytes ($\chi^2 = 21.2$, $df = 6$, $p = 0.002$) and red-listed species ($\chi^2 = 30.5$, $df = 6$, $p < 0.001$). Regarding all macrophyte species, ditches in NESLO supported significantly more species than rivers (Figure 2a). In TKB, rivers had significantly fewer species than the other waterbodies, while in BNL, streams had significantly fewer species than ditches and ponds.

For hydrophytes, no significant differences were observed in NESLO (Figure 2b). However, in TKB, ponds supported significantly more species than streams, while in BNL, ponds and ditches hosted more hydrophytes than rivers.

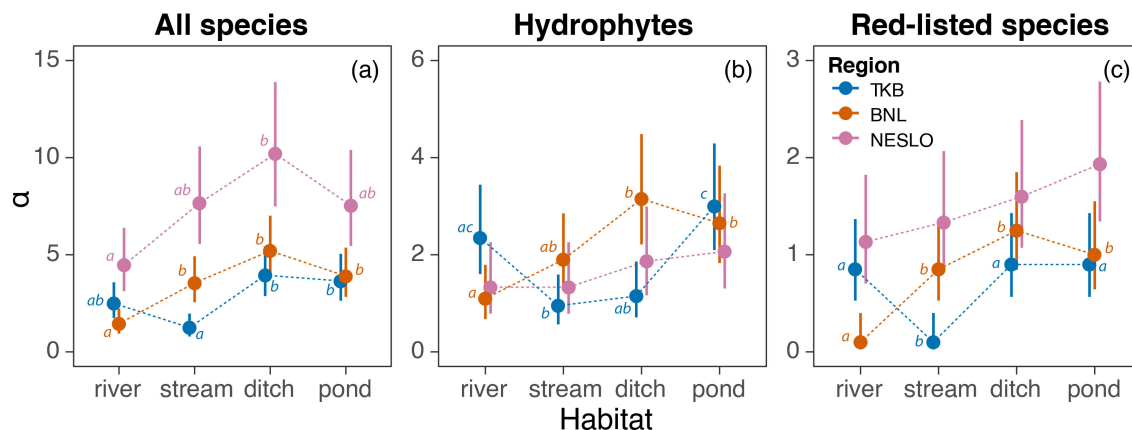


FIGURE 2

Comparison of macrophyte α diversity among aquatic ecosystems in the studied regions. Mean species richness values for all taxa (a), hydrophyte species (b), and red-listed species (c) are displayed (circles) along with 95% confidence intervals (error bars). Different lowercase letters indicate significant differences among habitat means within each region.

The diversity of red-listed species was comparable among the waterbodies in NESLO (Figure 2c). Nevertheless, rivers and streams harboured significantly fewer species of conservation concern than the other aquatic ecosystems in BNL and TKB, respectively.

Among-site (β) diversity

For all macrophyte species, total β diversity was statistically comparable among habitats in NESLO (pseudo- $F = 0.36$, $p = 0.78$), BNL (pseudo- $F = 2.15$, $p = 0.086$), and TKB (pseudo- $F = 2.08$, $p = 0.107$) (Figure 3a). A more detailed examination of β diversity through additive partitioning revealed marginally significant differences in the turnover component in TKB (pseudo- $F = 3.04$, $p = 0.031$), where ditches and ponds showed greater heterogeneity due to species replacement compared to rivers (Supplementary Figure

S1a). No significant differences were detected in the turnover components of β diversity for NESLO (pseudo- $F = 0.50$, $p = 0.685$) and BNL (pseudo- $F = 2.73$, $p = 0.053$), nor were there significant changes in the nestedness-related components of β diversity (NESLO: pseudo- $F = 2.01$, $p = 0.119$; BNL: pseudo- $F = 2.01$, $p = 0.126$; TKB: pseudo- $F = 0.45$, $p = 0.731$) (Supplementary Figure S1b).

We found significant differences in total β diversity among aquatic ecosystems in BNL (pseudo- $F = 3.04$, $p = 0.033$), TKB (pseudo- $F = 4.78$, $p = 0.011$) but not in NESLO (pseudo- $F = 0.15$, $p = 0.926$). In BNL, hydrophyte communities in ditches were significantly more homogeneous than those in ponds and streams (Figure 3b). In contrast, streams in TKB exhibited lower β diversity than ditches and ponds, while pond communities were more heterogeneous than rivers. This overall pattern of hydrophyte β diversity was driven by species turnover among waterbodies rather than by community nestedness (Supplementary Figures S1c, d).

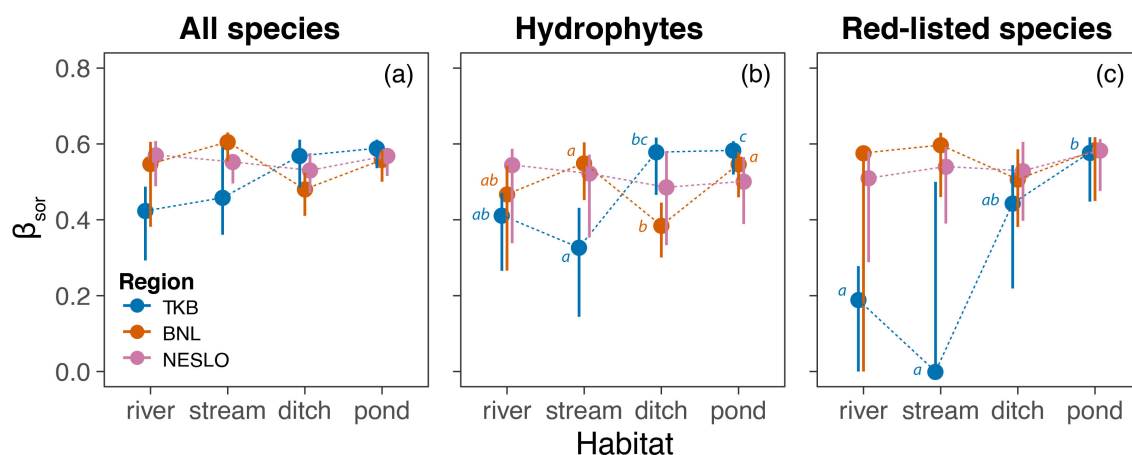


FIGURE 3

Comparison of macrophyte β diversity among aquatic ecosystems in the studied regions. Total β diversity (β_{SOR}) of whole communities (a), hydrophytes (b) and red-listed species (c) is displayed as distance to centroids in multivariate space (circles), along with 95% bootstrap confidence intervals (1,000 resamples). Different lowercase letters indicate significant differences among habitat means within each region. Results for the turnover and nestedness-resultant components of β diversity are given in Supplementary Figure S1.

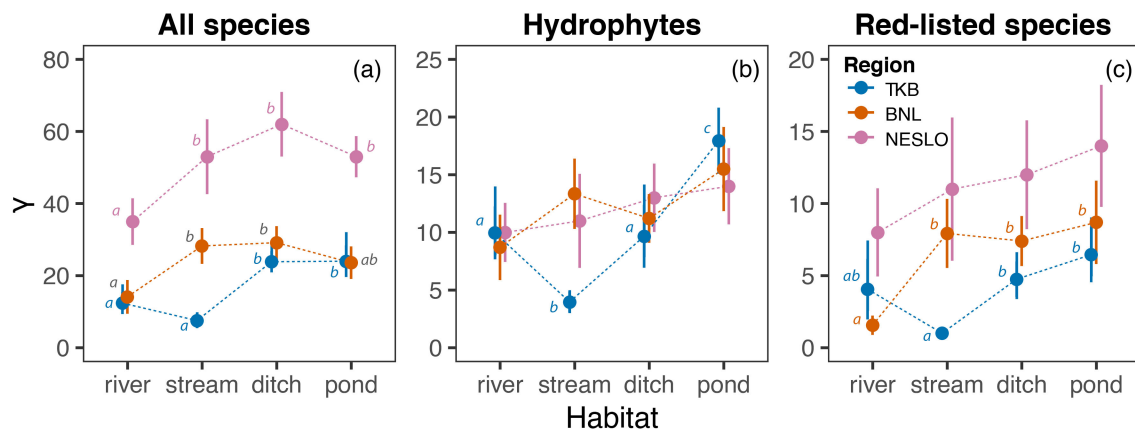


FIGURE 4

Comparison of aquatic macrophyte γ diversity among aquatic ecosystems in the studied regions. Overall γ diversity (a), γ diversity of hydrophytes (b) and γ diversity of red-listed species (c) are estimated as the expected number of species in 15 sites. The estimates (circles) are shown along their 95% confidence intervals (error bars). Different lowercase letters indicate significant differences among habitat means within each region.

The analysis of red-listed species revealed significant differences among aquatic ecosystems in TKB (pseudo- $F = 5.07$, $p = 0.008$) but not in BNL (pseudo- $F = 0.57$, $p = 0.637$) and NESLO (pseudo- $F = 0.32$, $p = 0.818$). Ponds in TKB showed significantly higher heterogeneity of red-listed species than rivers and streams (Figure 3c). Again, these differences in total β diversity were driven by species turnover among individual waterbodies, not by nestedness (Supplementary Figures S1e, f).

Regional (γ) diversity

Ditches supported significantly more macrophyte species than rivers across all studied regions, with a similar trend observed in ponds, except in BNL (Figure 4a). Also, streams in NESLO and BNL harboured a high number of species, but their γ diversity was very low in TKB.

We did not find any significant differences in hydrophyte regional diversity among habitats in NESLO and BNL. In TKB, ponds supported significantly more species than the other habitats, while rivers and ditches hosted more hydrophytes than streams (Figure 4b).

Although the total number of red-listed species was similar across aquatic ecosystems in NESLO, ponds and ditches harboured more species of conservation concern than streams in TKB and rivers in BNL, respectively (Figure 4c). The relative importance of streams and rivers interchanged between TKB and BNL.

Discussion

Local (α) diversity

Ponds and ditches exhibited higher α diversity than running waters across all regions, while the role of streams and rivers varied geographically. Ponds are generally known to support the high local

diversity of macrophyte species (Williams et al., 2004; Biggs et al., 2005, 2007, 2017; Ořaheřová et al., 2007; Davies et al., 2008a, b; Fernández-Aláez et al., 2020). Based on these studies, local macrophyte diversity appears to decline in the following order: ponds (lakes) > rivers > streams > ditches. However, a comparative study of macrophyte richness across various waterbody types in Central Europe found that ditches, followed by rivers, exhibited species richness comparable to that of ponds (Bubíková and Hrivnák, 2018b). We conducted a meta-analysis using a random-effects model to synthesize findings from multiple comparative studies on macrophyte local diversity. The results indicate that ponds have significantly higher local diversity than both ditches and streams (Figure 5). No strong evidence was found for differences in local diversity among the other waterbody types. However, all comparisons exhibited considerable heterogeneity, as shown by significant Cochran's Q-test results (all p-values < 0.01), elevated heterogeneity indices (all I^2 values > 50%) and broad prediction intervals for effect sizes that included zero, suggesting a wide range of possible outcomes in future studies comparing the local diversity of these waterbodies. All studies confirmed higher diversity in ponds than in streams, but the comparison between ponds and ditches varied geographically. Ponds in Western Europe exhibited generally higher diversity than ditches (but see Law et al., 2024), while in Central and South-Eastern Europe, ditches had similar or even slightly higher diversity than ponds (see Figure 2; Bubíková and Hrivnák, 2018b). Our results and the meta-analysis highlight the role of ponds as macrophyte local diversity hotspots. High local diversity, along with a significant number of threatened species and species uniquely found in ponds – both in Central Europe and other regions (e.g., Linton and Goulder, 2000; Rhazi et al., 2012; Fois et al., 2024; Germ et al., 2024) – makes ponds habitats of high conservation priority within the European agricultural landscape.

We found that ditches supported the greatest number of macrophyte species recorded at individual sites in the studied regions. The high macrophyte diversity in ditches observed here and in Bubíková and Hrivnák (2018b) deviates markedly from the

findings of many other comparative studies reporting lower macrophyte diversity in ditches and higher diversity in rivers (e.g., Williams et al., 2004; Biggs et al., 2007; Davies et al., 2008a). Moreover, the number of species unique to studied ditches was relatively high in all three regions. What explains the high diversity observed in artificial, man-made habitats such as ditches? In Central Europe, many ditches are historical structures built primarily in lowlands and basins during the intensification of agriculture and industrialization in the former Austro-Hungarian monarchy at the turn of the 19th to 20th century. These ditches were used for flood protection, agricultural drainage and/or as irrigation channels and are typical for relatively large size and permanent water levels (Dulovičová and Velísková, 2010). Due to political and economic changes in post-communist countries at the beginning of the 1990s, the current use of agricultural landscapes is much less intensive. Extensive soil fertilization and herbicide application were economically constrained, and many ditches were left to undergo the process of succession in areas of de-intensified or abandoned land (Bezák and Mitchley, 2014). As a result, these ditches have become species-rich habitats, supporting a relatively high proportion of endangered macrophyte species (Oťahelová and Valachovič, 2002; Sipos et al., 2003; Dorotovičová, 2013). Other studies from European regions have shown that ditches can be diverse and provide exceptional conditions for aquatic plants (e.g., Armitage et al., 2003; Milsom et al., 2004; Biggs et al., 2007; Law et al., 2024). However, in several regions of Western Europe, ditches are often small, highly seasonal, located away from floodplain areas and close

to intensively cultivated agricultural land. Using agrochemicals, such as herbicides, in these areas is likely to reduce macrophyte richness. Suboptimal hydrological conditions due to low water retention capacity and huge differences in run-off from cultivated areas, siltation and intensive agriculture may result in low biodiversity in these ditches (Williams et al., 2004; Davies et al., 2008a). Nevertheless, ditches have been shown to provide valuable wet, vegetated, non-cultivated habitats for both aquatic and terrestrial taxa, offer food resources and facilitate connectivity within the broader landscape (Herzon and Helenius, 2008). Despite their artificial origin, ditches play an important role in maintaining aquatic macrophyte diversity and supporting a large number of unique species in otherwise dry and intensively cultivated agricultural landscapes.

The comparison of local macrophyte diversity between and within regions revealed that diversity patterns are region-specific. The diversity trends in NESLO and BNL are similar, while they differ in TKB, particularly in streams. This pattern was observed across all studied species groups (Figure 2). Carpathian streams (TKB), in contrast to (sub)Pannonian streams (NESLO, BNL), retain a near-natural character but their macrophyte diversity is not equally high. Higher flow velocity, dominance of coarse sediments and heavy shading from riparian vegetation likely contribute to the naturally lower macrophyte species richness in these streams (Svitok et al., 2016). In general, only bryophytes and a few vascular aquatic plants are adapted to the conditions of European (sub-) mountain streams (Baatrup-Pedersen et al., 2006; Hrivnák et al., 2010).

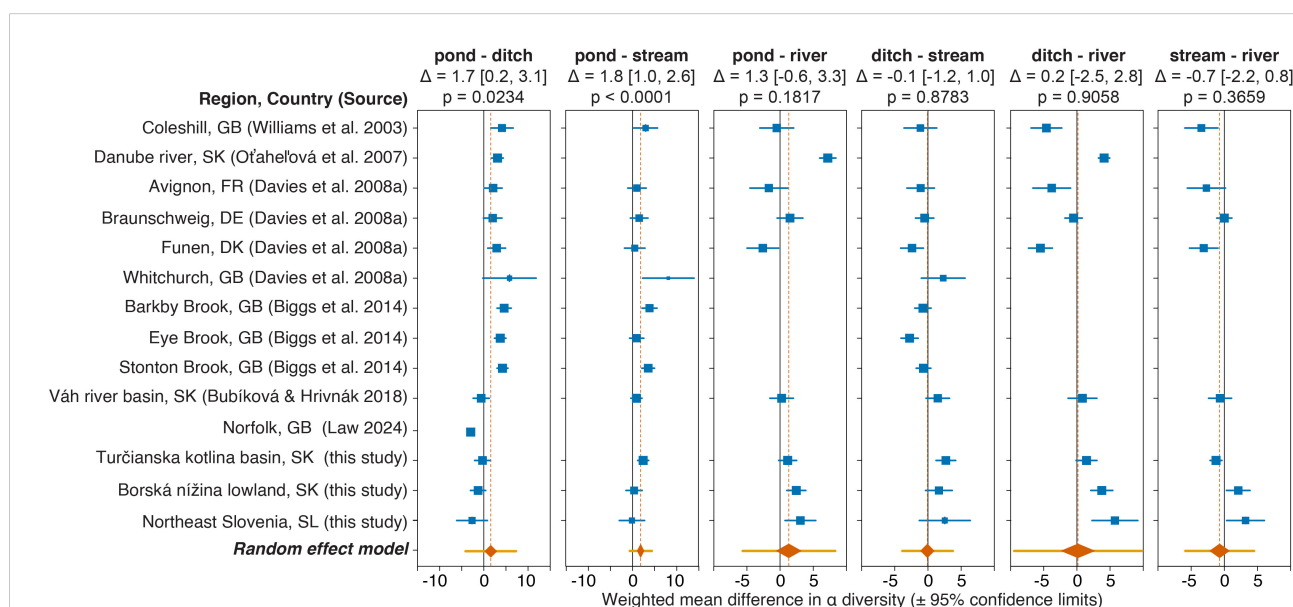


FIGURE 5

Forest plots showing differences in local (α) diversity of aquatic macrophytes among various habitats in Great Britain (GB), Denmark (DK), France (FR), Germany (DE), Slovenia (SL) and Slovakia (SK). Squares indicate the mean difference for each study, with their size proportional to the study's weight in the meta-analysis. Diamonds represent pooled estimates of mean differences based on random-effects models. Blue horizontal error bars and the sides of the diamonds denote 95% confidence intervals for individual studies and pooled estimates, respectively. Orange error bars indicate 95% prediction intervals for the random-effects models. Pooled estimates of mean differences (Δ) and their 95% confidence intervals (in square brackets) are displayed above each plot, along with the test results (p-values) for each meta-analytical model. Note that in Davies et al. (2008a), standard deviations were approximated from ranges using the normal distribution (two standard deviation rule, Higgins and Green, 2011).

Among-site (β) diversity

Ponds consistently showed high β diversity, while the contribution of other aquatic ecosystems varied idiosyncratically across studied regions. In general, β diversity, or the differences in community composition among sites, increases either with dispersal limitations along spatial gradients or with species sorting along environmental gradients (Heino, 2011). Given the strong dispersal abilities of aquatic macrophytes (Santamaría, 2002) and considering that, despite several artificial barriers (Jones et al., 2020), the flat landscapes of the studied regions (max. altitudinal range of sites < 200 m) and relatively short distances between sites within regions, it is unlikely that contrasting β diversity patterns result from different dispersal processes between regions. Presumably, the contrasting patterns between regions are linked to habitat heterogeneity, often considered a key driver of β diversity (Suurkuikka et al., 2012; Astorga et al., 2014; Hamerlík et al., 2014). Most biotic communities are strongly influenced by environmental factors, and thus, habitat heterogeneity and the associated species sorting dynamics are thought to be the dominant mechanisms structuring communities (Cottenie, 2005). Based on the test of homogeneity of multivariate dispersion, the variability of habitat characteristics was comparable among waterbodies in NESLO (pseudo-F = 0.05, p = 0.99), while TKB and BNL exhibited significant heterogeneity (pseudo-F = 4.77, p = 0.007 and pseudo-F = 6.68, p = 0.002, respectively). These differences in habitat heterogeneity result in macrophyte communities with varying similarities, a pattern reflected in the differing β diversity among regions. Our findings suggest that β diversity patterns depend on region-specific environmental heterogeneity, precluding broader generalizations of the comparison.

In TKB, ponds exhibited a higher turnover component of β diversity than streams and rivers, regardless of whether the analysis included entire communities or focused on subsets such as hydrophytes and red-listed species. This pattern aligns with similar findings from other Western and Central European studies (Williams et al., 2004; Davies et al., 2008a; Bubíková and Hrivnák, 2018b). The consistency of these results suggests that common factors may sustain the high β diversity observed in ponds. The underlying mechanisms are likely related to habitat heterogeneity and connectivity, with plausible explanations of turnover species-sorting and patch-dynamics (Leibold et al., 2004; Fernández-Aláez et al., 2020). Ponds often have small catchment areas (Novikmec et al., 2016), resulting in highly variable physicochemical conditions that can differ significantly even across short distances (Svitok et al., 2011; Hamerlík et al., 2014). In contrast, rivers and streams typically have larger catchments, and the homogenizing effect of flowing water generally leads to more stable physical and chemical conditions than ponds. Analysis of physicochemical data from the TKB showed that ponds have significantly higher environmental heterogeneity than rivers (p = 0.004) and streams (p = 0.023). Sufficient dispersal within a heterogeneous environment and associated niche differences are

expected to promote species sorting along resource gradients (Cottenie, 2005). As a result, this environmental heterogeneity may exert a niche-related influence on macrophyte communities, helping to sustain the high β diversity observed in ponds. Jeffries (2008) demonstrated that deterministic (i.e., niche-related) factors affecting macrophyte community variability are relevant even at very small spatial scales (much smaller than those investigated here), with the immediate surroundings of ponds playing a crucial role in shaping pond communities.

Spatial isolation may also contribute to the high β diversity of macrophytes in ponds. Unlike rivers and streams, which are highly connected water bodies where species dispersal is facilitated by fluvial action, ponds are more isolated, reducing species exchange and potentially enhancing β diversity. We observed a trend of increasing β diversity from fast-flowing to stagnant waters for all species and hydrophytes. However, this pattern was not evident for red-listed species, which were relatively scarce in the studied habitats (Figure 2 and Figure 3). This is consistent with previous studies showing that high connectivity of lotic waterbodies may lead to more uniform vegetation and overall lower diversity of macrophytes (Tockner et al., 1998; Bornette et al., 1998, 2001). In contrast, small lentic habitats like ponds scattered within a hostile terrestrial matrix face a higher risk of local extinction and a lower likelihood of colonization (Wright et al., 1997). These stochastic processes are thought to contribute to the high heterogeneity observed in pond communities (Williams et al., 2004; Scheffer et al., 2006). However, empirical evidence directly linking isolation to increased variability in wetland plant communities remains limited. In fact, the effect of connectivity is one aspect of more complex metacommunity dynamics where habitat patches undergo both stochastic and deterministic extinctions that are counteracted by dispersal and where the environmental heterogeneity and inter-specific interactions shape species composition (Leibold et al., 2004; Scheffer et al., 2006). Even within strictly controlled experimental microcosms, aquatic plant communities did not display entirely deterministic behaviour, exhibiting a strong stochastic component in community assembly (Weiher and Keddy, 1995). We suggest that greater environmental heterogeneity and the small size and spatial isolation of ponds likely contribute to their elevated β diversity.

We demonstrated that macrophyte β diversity was predominantly driven by species turnover rather than nestedness. Our findings are consistent with global patterns in the β diversity of lake macrophytes, suggesting that natural environmental heterogeneity is the primary influence on macrophyte β diversity, with nestedness accounting for only a small fraction of the overall β diversity (Alahuhta et al., 2017). Nestedness, where communities with fewer species are subsets of richer communities, may arise due to factors like habitat size and isolation but these effects are often weak in freshwater systems (Heino, 2011). Given the strong dispersal abilities of macrophytes and the relatively small spatial scales explored in our study, it is unsurprising that species turnover is the dominant component of β diversity.

Regional (γ) diversity

We have found that ponds and ditches harboured a high γ diversity of macrophytes, including red-listed species, across all studied regions, while the relative importance of ponds was especially pronounced in hydrophytes. The high regional diversity observed for ponds and ditches is likely related to their relatively high β diversity caused by higher heterogeneity of their environmental conditions and stochastic events (see above). Ponds, for example, often have small catchment areas (Novikmec et al., 2016), with great differences in land use, bedrock, management, and purpose resulting in significant gradients of physical and chemical conditions across the region (Svitok et al., 2011) that may promote greater biodiversity at the regional scale (Williams et al., 2004; Zelnik et al., 2012). Compared to other aquatic ecosystems, ponds have been shown to support the highest plant γ diversity across Europe (e.g., Williams et al., 2004; Biggs et al., 2005; Davies et al., 2008b; Lukács et al., 2013). In contrast, identifying ditches as the habitats with the highest regional diversity in Central Europe contradicts findings from Western Europe, where ditches differ in ecological characteristics, history and land use, leading to divergent results (Williams et al., 2004; Dorotovičová, 2013; Bubíková and Hrivnák, 2018b). Nevertheless, ponds exhibited the highest regional diversity of hydrophytes and red-listed plant species, highlighting their role as biodiversity hotspots with significant conservation value.

Regarding ponds, our results fully confirm the importance of these habitats for the maintenance of macrophyte diversity in Europe (Biggs et al., 2005, 2017). In contrast, ditches, which are considered relatively poor for plant species in Western Europe (Williams et al., 2004; Davies et al., 2008a), are among the richest in Central Europe for both wetland and Red List species (see also Bubíková and Hrivnák, 2018b).

Conclusions

Ponds and ditches are critical habitats for biodiversity conservation within the European agricultural landscape. Despite their artificial origins, these small aquatic habitats support high local diversity, host a significant number of threatened and regionally unique species, and serve as vital refuges in predominantly dry and intensively cultivated areas. Ponds, in particular, stand out as biodiversity hotspots due to their high regional diversity of hydrophytes and red-listed plant species. Their small size, spatial isolation, and environmental heterogeneity likely contribute to their elevated β diversity, underscoring the importance of maintaining a network of these habitats to promote ecological connectivity and species persistence. Given their conservation value, creating, protecting and restoring ponds and ditches should be prioritized in agricultural land management strategies to preserve aquatic macrophyte biodiversity and the ecosystem services they provide.

Data availability statement

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

Author contributions

MS: Formal Analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. IZ: Funding acquisition, Investigation, Writing – original draft. KB: Investigation, Writing – original draft. MG: Investigation, Writing – original draft. AG: Writing – original draft. JK: Investigation, Writing – original draft. HO: Conceptualization, Investigation, Writing – original draft. PP: Investigation, Writing – original draft. RH: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research 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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References

- Alahuhta, J., and Heino, J. (2013). Spatial extent, regional specificity and metacommunity structuring in lake macrophytes. *J. Biogeogr.* 40, 1572–1582. doi: 10.1111/jbi.2013.40.issue-8
- Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M. M., Bolpagni, R., et al. (2017). Global variation in the beta diversity of lake macrophytes is driven by environmental heterogeneity rather than latitude. *J. Biogeogr.* 44, 1758–1769. doi: 10.1111/jbi.2017.44.issue-8
- Anderson, M. J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62, 245–253. doi: 10.1111/j.1541-0420.2005.00440.x
- Armitage, P. D., Szoszkiewicz, K., Blackburn, J. H., and Nesbitt, I. (2003). Ditch communities: a major contributor to floodplain biodiversity. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 13, 165–185. doi: 10.1002/aqc.v13:2
- Astorga, A., Death, R., Death, F., Paavola, R., Chakraborty, M., and Muotka, T. (2014). Habitat heterogeneity drives the geographical distribution of beta diversity: the case of New Zealand stream invertebrates. *Ecol. Evol.* 4, 2693–2702. doi: 10.1002/ece3.2014.4.issue-13
- Baatrup-Pedersen, A., Szoszkiewicz, K., Nijboer, R., O'Hare, M., and Ferreira, T. (2006). Macrophyte communities in unimpacted European streams: variability in assemblage patterns, abundance and diversity. *Hydrobiologia* 566, 179–196. doi: 10.1007/s10750-006-0096-1
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Glob. Ecol. Biogeogr.* 19, 134–143. doi: 10.1111/j.1466-8238.2009.00490.x
- Baselga, A. (2012). The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. *Glob. Ecol. Biogeogr.* 21, 1223–1232. doi: 10.1111/j.1466-8238.2011.00756.x
- Baselga, A., Orme, D., Villeger, S., De Bortoli, J., Leprieux, F., Logez, M., et al. (2023). *betapart: Partitioning Beta Diversity into Turnover and Nestedness Components* (R package version 1), 6.
- Bezák, P., and Mitchley, J. (2014). Drivers of change in mountain farming in Slovakia: from socialist collectivisation to the Common Agricultural Policy. *Regional Environ. Change* 14, 1343–1356. doi: 10.1007/s10113-013-0580-x
- Biggs, J., Von Fumetti, S., and Kelly-Quinn, M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: Implications for policy makers. *Hydrobiologia* 793, 3–39. doi: 10.1007/s10750-016-3007-0
- Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Brown, C., Hollis, J., et al. (2007). The freshwater biota of British agricultural landscapes and their sensitivity to pesticides. *Agric. Ecosyst. Environ.* 122, 137–148. doi: 10.1016/j.agee.2006.11.013
- Biggs, J., Williams, P., Whitfield, M., Nicolet, P., and Weatherby, A. (2005). 15 years of ponds assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 15, 693–714. doi: 10.1002/aqc.745
- Bilz, M., Kell, S. P., Maxted, N., and Lansdown, R. V. (2011). *European Red List of Vascular Plants* (Luxembourg: Publications Office of the European Union).
- Boix, D., Biggs, J., Hull, A. P., Kalettka, T., and Oertli, B. (2012). Pond research and management in Europe: "Small is Beautiful". *Hydrobiologia* 689, 1–9. doi: 10.1007/s10750-012-1015-2
- Bolpagni, R., Laini, A., Stanzani, C., and Chiarucci, A. (2018). Aquatic Plant Diversity in Italy: Distribution, Drivers and Strategic Conservation Actions. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00116
- Bornette, G., Amoros, C., and Lamouroux, N. (1998). Aquatic plant diversity in riverine wetlands: The role of connectivity. *Freshw. Biol.* 39, 267–283. doi: 10.1046/j.1365-2427.1998.00273.x
- Bornette, G., Piegay, H., Citterio, A., Amoros, C., and Godreau, V. (2001). Aquatic plant diversity in four river floodplains: A comparison at two hierarchical levels. *Biodiv. Conserv.* 10, 1683–1701. doi: 10.1023/A:1012090501147
- Bubiková, K., and Hrivnák, R. (2018a). Artificial ponds in Central Europe do not fall behind the natural ponds in terms of macrophyte diversity. *Knowl. Manage. Aquat. Ecosyst.* 419, 8. doi: 10.1051/kmae/2017055
- Bubiková, K., and Hrivnák, R. (2018b). Comparative Macrophyte Diversity of Waterbodies in the Central European landscape. *Wetlands* 38, 451–459. doi: 10.1007/s13157-017-0987-0
- Cervellini, M., Zannini, P., Di Musciano, M., Fattorini, S., Jimenez-Alfaro, B., Rocchini, D., et al. (2020). A grid-based map for the Biogeographical Regions of Europe. *Biodiv. Data J.* 8, 53720. doi: 10.3897/BDJ.8.e53720
- Clarke, S. J. (2015). Conserving freshwater biodiversity: the value, status and management of high-quality ditch systems. *J. Nat. Conserv.* 24, 93–100. doi: 10.1016/j.jnc.2014.10.003
- Colwell, R. K., Mao, C. X., and Chang, J. (2004). Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85, 2717–2727. doi: 10.1890/03-0557
- Cottenie, K. (2005). Integrating environmental and spatial processes in ecological community dynamics. *Ecol. Lett.* 8, 1175–1182. doi: 10.1111/j.1461-0248.2005.00820.x
- Davies, B. R., Biggs, J., Williams, P. J., Lee, J. T., and Thompson, S. (2008b). A comparison of the catchment sizes of rivers, streams, ponds, ditches and lakes: implications for protecting aquatic biodiversity in an agricultural landscape. *Hydrobiologia* 597, 7–17. doi: 10.1007/s10750-007-9227-6
- Davies, B., Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Sear, D., et al. (2008a). Comparative biodiversity of aquatic habitats in the European agricultural landscapes. *Agric. Ecosyst. Environ.* 125, 1–8. doi: 10.1016/j.agee.2007.10.006
- Dorotovičová, C. (2013). Man-made canals as a hotspot of aquatic macrophyte biodiversity in Slovakia. *Limnologia* 43, 277–287. doi: 10.1016/j.limno.2012.12.002
- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., et al. (2006). Freshwater biodiversity: importance, threats status and conservation challenges. *Biol. Rev.* 81, 163–182. doi: 10.1017/S1464793105006950
- Dulovičová, R., and Velísková, Y. (2010). Aggradation of the irrigation canal network in Žitný Ostrov, Southern Slovakia. *J. Irrig. Drain. Eng.* 136, 421–428. doi: 10.1061/(ASCE)IR.1943-4774.0000190
- Eliš, P. J., Dítě, D., Kliment, J., Hrivnák, R., and Feráková, V. (2015). Red list of ferns and flowering plants of Slovakia, 5th edn (October 2014). *Biologia* 70, 218–228. doi: 10.1515/biolog-2015-0018
- Fernández-Aláez, M., García-Criado, F., García-Girón, J., Santiago, F., and Fernández-Aláez, C. (2020). Environmental heterogeneity drives macrophyte beta diversity patterns in permanent and temporary ponds in an agricultural landscape. *Aquat. Sci.* 82, 20. doi: 10.1007/s00027-020-0694-4
- Fois, M., Cuena-Lombrana, A., Artufel, M., Attard, V., Cambria, S., Farrugia, K., et al. (2024). Plant distribution and conservation in mediterranean islands' lentic wetlands: there is more than aquatic species. *Biodivers. Conserv.* 33, 1373–1392. doi: 10.1007/s10531-024-02803-2
- Germ, M., Bajc Tomšič, M., Zelnik, I., Ojdanič, N., and Golob, A. (2024). Aquatic Plants in Ponds at the Brdo Estate (Slovenia) Show Changes in 20 Years. *Plants* 13, 2439. doi: 10.3390/plants13172439
- Gioria, M., Schaffers, A., Bacaro, G., and Feehan, J. (2012). The conservation value of farmland ponds: Predicting water beetle assemblages using vascular plants as a surrogate group. *Biol. Conserv.* 143, 1125–1133. doi: 10.1016/j.biocon.2010.02.007
- Hamerlik, L., Svitok, M., Novikmec, M., Očadlík, M., and Bitušik, P. (2014). Local, among-site, and regional diversity patterns of benthic macroinvertebrates in high-altitude waterbodies: Do ponds differ from lakes? *Hydrobiologia* 723, 41–52. doi: 10.1007/s10750-013-1621-7
- Heino, J. (2011). A macroecological perspective of diversity patterns in the freshwater realm. *Freshw. Biol.* 56, 1703–1722. doi: 10.1111/j.1365-2427.2011.02610.x
- Herzon, I., and Helenius, J. (2008). Agricultural drainage ditches, their biological importance and functioning. *Biol. Conserv.* 141, 1171–1183. doi: 10.1016/j.biocon.2008.03.005
- Higgins, J. P. T., and Green, S. (2011). *Cochrane Handbook for Systematic Reviews of Interventions* (The Cochrane Collaboration).
- Hilbe, J. M. (2011). *Negative binomial regression* (Cambridge University Press).
- Hill, M. J., Greaves, H. M., Sayer, C. D., Hassall, C., Milin, M., Milner, V. S., et al. (2021). Pond ecology and conservation: research priorities and knowledge gaps. *Ecosphere* 12, 03853. doi: 10.1002/ecs2.3853
- Hill, M. J., Hassall, C., Oertli, B., Fahrig, L., Robson, B. J., Biggs, J., et al. (2018). New policy directions for global pond conservation. *Conserv. Lett.* 11, 12447. doi: 10.1111/conl.12447
- Hrivnák, R., Oťaheľová, H., Valachovič, M., Paľove-Balang, P., and Kubinská, A. (2010). Effect of environmental variables on the aquatic macrophyte composition pattern in streams: a case study from Slovakia. *Fundam. Appl. Limnol.* 177, 115–124. doi: 10.1127/1863-9135/2010/0177-0115
- Hsieh, T. C., Ma, K. H., and Chao, A. (2022). *iNEXT: iNterpolation and EXTrapolation for species diversity* (R package version 3.0.0).

- Janaauer, G. A., and Dokulil, M. (2006). "Macrophytes and algae in running waters," in *Biological monitoring of rivers*. Eds. G. Ziglio, M. Siligardi and G. Flaim (John Wiley & Sons, Ltd), 89–109.
- Jeffries, M. (2008). The spatial and temporal heterogeneity of macrophyte communities in thirty small, temporary ponds over a period of ten years. *Ecography* 31, 765–775. doi: 10.1111/j.0906-7590.2008.05487.x
- Jones, P. E., Consuegra, S., Börger, L., Jones, J., and Garcia de Leaniz, C. (2020). The effectiveness of aquatic plants as surrogates for wider biodiversity in standing fresh waters: A critical review. *Freshw. Biol.* 65, 1165–1180. doi: 10.1111/fwb.13493
- Lacoul, P., and Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environ. Rev.* 14, 89–136. doi: 10.1139/a06-001
- Law, A., Baker, A., Sayer, C. D., Foster, G., Gunn, I. D. M., Macadam, C. R., et al. (2024). Repeatable patterns in the distribution of freshwater biodiversity indicators across contrasting landscapes. *Landsc. Ecol.* 39, 195. doi: 10.1007/s10980-024-01992-z
- Law, A., Baker, A., Sayer, C., Foster, G., Gunn, I. D. M., Taylor, P., et al. (2019). The effectiveness of aquatic plants as surrogates for wider biodiversity in standing fresh waters. *Freshw. Biol.* 64, 1664–1675. doi: 10.1111/fwb.13369
- Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., et al. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecol. Lett.* 7, 601–613. doi: 10.1111/j.1461-0248.2004.00608.x
- Lenth, R. V. (2016). Least-squares means: the R package lsmeans. *J. Stat. Softw.* 69, 1–33. doi: 10.18637/jss.v069.i01
- Lenth, R. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (R package version 1.8), 4–1.
- Leprieux, F., Tedesco, P. A., Huguency, B., Beauchard, O., Dürr, H. H., Brosse, S., et al. (2011). Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. *Ecol. Lett.* 14, 325–334. doi: 10.1111/j.1461-0248.2011.01589.x
- Lin, H.-Y., Cooke, S. J., Wolter, C., Young, N., and Bennett, J. R. (2020). On the conservation value of historic canals for aquatic ecosystems. *Biol. Conserv.* 251, 108764. doi: 10.1016/j.biocon.2020.108764
- Linton, S., and Goulder, R. (2000). Botanical conservation value related to origin and management of ponds. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 10, 77–91.
- Lukács, B. A., Sramkó, G., and Molnár, A. V. (2013). Plant diversity and conservation value of continental temporary pools. *Biol. Conserv.* 158, 393–400. doi: 10.1016/j.biocon.2012.08.024
- Magurran, A. E. (2003). *Measuring biological diversity* (John Wiley & Sons).
- Manly, B. F. (2007). *Randomization, bootstrap and Monte Carlo methods in biology, 3rd edition* (Chapman and Hall/CRC).
- McCullagh, P., and Nelder, J. A. (1989). *Generalized Linear Models, 2nd edition* (Boca Raton: Chapman & Hall/CRC).
- Millennium Ecosystem Assessment (2005). *Ecosystems and human well-being – synthesis* (Washington DC: Island Press).
- Milsom, T. P., Sherwood, A. J., Rose, S. C., Town, S. J., and Runham, S. R. (2004). Dynamics and management of plant communities in ditches bordering arable fenland in eastern England. *Agric. Ecosyst. Environ.* 103, 85–99. doi: 10.1016/j.agee.2003.10.012
- Novikmec, M., Hamerlik, L., Kočík, D., Hrivnák, R., Kochjarová, J., Otaheľová, H., et al. (2016). Ponds and their catchments: Size relationships and influence of land use across multiple spatial scales. *Hydrobiologia* 774, 155–166. doi: 10.1007/s10750-015-2514-8
- Oksanen, J., Simpson, G., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., et al. (2022). *vegan: Community Ecology Package* (R package version 2), 6–4.
- Otaheľová, H., and Valachovič, M. (2002). Effects of the Gabčíkovo hydroelectric station on the aquatic vegetation of the Danube River (Slovakia). *Preslia* 74, 323–331.
- Otaheľová, H., Valachovič, M., and Hrivnák, R. (2007). The impact of environmental factors on the distribution pattern of aquatic plants along the Danube River corridor (Slovakia). *Limnologica* 37, 290–302. doi: 10.1016/j.limno.2007.07.003
- R Core Team (2022). *R: A language and environment for statistical computing* (Vienna: R Foundation for Statistical Computing).
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., et al. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94, 849–873. doi: 10.1111/brev.2019.94.issue-3
- Rhazi, L., Grillas, P., Saber, E. R., Rhazi, M., Brendonck, L., and Waterkeyn, A. (2012). Vegetation of Mediterranean temporary pools: a fading jewel? *Hydrobiologia* 689, 23–36. doi: 10.1007/s10750-011-0679-3
- Ricciardi, A., and Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conserv. Biol.* 13, 1220–1222. doi: 10.1046/j.1523-1739.1999.98380.x
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, R., Bloomfield, J., Dirzo, R., et al. (2000). Global biodiversity scenarios for the year 2100. *Science* 287, 1770–1774. doi: 10.1126/science.287.5459.1770
- Santamaría, L. (2002). Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecol.* 23, 137–154. doi: 10.1016/S1146-609X(02)01146-3
- Scheffer, M., Van Geest, G. J., Zimmer, K., Jeppesen, E., Søndergaard, M., Butler, M. G., et al. (2006). Small habitat size and isolation can promote species richness: second-order effects on biodiversity in shallow lakes and ponds. *Oikos* 112, 227–231. doi: 10.1111/j.0030-1299.2006.14145.x
- Sipos, V. K., Kohler, A., Köder, M., and Janaauer, G. (2003). Macrophyte vegetation of Danube canals in Kiskunság (Hungary). *Arch. Hydrobiol. Suppl.* 14, 143–166. doi: 10.1127/lr/14/2003/143
- Suurkuukka, H., Meissner, K. K., and Muotka, T. (2012). Species turnover in lake littorals: Spatial and temporal variation of benthic macroinvertebrate diversity and community composition. *Divers. Distrib.* 18, 931–941. doi: 10.1111/j.1472-4642.2012.00889.x
- Svitok, M., Hrivnák, R., Kochjarová, J., Otaheľová, H., and Paľove-Balang, P. (2016). Environmental thresholds and predictors of macrophyte species richness in aquatic habitats in central Europe. *Folia Geobot.* 51, 227–238. doi: 10.1007/s12224-015-9211-2
- Svitok, M., Hrivnák, R., Otaheľová, H., Dúbravková, D., Paľove-Balang, P., and Slobodník, V. (2011). The importance of local and regional factors on the vegetation of created wetlands in Central Europe. *Wetlands* 31, 663–674. doi: 10.1007/s13157-011-0182-7
- Tockner, K., Schiemer, F., and Ward, J. V. (1998). Conservation by restoration: The management concept for a river-floodplain system on the Danube River in Austria. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 8, 71–86. doi: 10.1002/(SICI)1099-0755(199801/02)8:1<71::AID-AQC265>3.0.CO;2-D
- Ulrich, W., Almeida-Neto, M., and Gotelli, N. J. (2009). A consumer's guide to nestedness analysis. *Oikos* 118, 3–17. doi: 10.1111/j.1600-0706.2008.17053.x
- Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics with S, 4th ed.* (New York: Springer).
- Verdonschot, R. C., Keizer-vlek, H. E., and Verdonschot, P. F. (2011). Biodiversity value of agricultural drainage ditches: a comparative analysis of the aquatic invertebrate fauna of ditches and small lakes. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 21, 715–727. doi: 10.1002/aqc.1220
- Weier, E., and Keddy, P. A. (1995). The assembly of experimental wetland plant communities. *Oikos* 73, 323–335. doi: 10.2307/3545956
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (New York: Springer-Verlag).
- Wiens, J. A., Stenseth, N. C., Van Horne, B., and Ims, R. A. (1993). Ecological mechanisms and landscape ecology. *Oikos* 66, 369–380. doi: 10.2307/3544931
- Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., et al. (2004). Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biol. Conserv.* 115, 329–341. doi: 10.1016/S0006-3207(03)00153-8
- Williams-Subiza, E. A., and Epele, L. B. (2021). Drivers of biodiversity loss in freshwater environments: A bibliometric analysis of the recent literature. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 31, 2469–2480. doi: 10.1002/aqc.3627
- Wright, D. H., Patterson, B. D., Mikkelsen, G. M., Cutler, A., and Atmar, W. (1997). A comparative analysis of nested subset patterns of species composition. *Oecologia* 113, 1–20. doi: 10.1007/s004420050348
- Zelnik, I., Gregorič, N., and Tratnik, A. (2018). Diversity of macroinvertebrates positively correlates with diversity of macrophytes in karst ponds. *Ecol. Eng.* 117, 96–103. doi: 10.1016/j.ecoleng.2018.03.019
- Zelnik, I., Potisek, M., and Gaberščik, A. (2012). Environmental conditions and macrophytes of karst ponds. *Polish J. Environ. Stud.* 21, 1911–1920.



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Niche partitioning and trait tradeoff strategies enable plants to coexist under interspecific competition in restored wetlands

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Background: Niche partition and traits tradeoff theory were primary strategies for plants coexistence. However, specific strategies of plants remained to be verified to guide community configuration and biodiversity maintenance in ecological restoration.

Methods: The variation of plants composition and niche breath were utilized to examine the temporal and spatial niche partition strategies, respectively. Meanwhile, the chi-square (χ^2), Spearman rank correlation coefficient (r_{ij}), Ochiai index (OI) were employed to analyze the interspecific relationship of 30 predominant species from species pool of 220 vascular plants. Besides, the Lotka-Volterra model was utilized to reveal the traits tradeoff strategies of predominant species from five vegetation formations.

Results: About 62.41% pairs of wetland species were niche partitioned while 37.58% of species pairs were niche overlapped. In temporal scale, 60.5% of species occurred either in spring or autumn while 39.5% occurred in both seasons. Meanwhile, significant change of relative height (RH) and relative coverage (RC) were observed in constructive species and auxiliary species. Height tradeoff strategy ($\frac{ARH}{ARC} > 1$), coverage enlarge strategy ($\frac{ARH}{ARC} < 1$), or both strategies ($\frac{ARH}{ARC} = 1$) observed in wetland plants.

Discussion: Our finding testified that the temporal niche partition and traits tradeoff strategies are objectively observable in wetland plants. These findings on coexistence strategies can be used in the configuration of plants communities and the biological control of alien invasive plants.

KEYWORDS

interspecific competition, coexisting strategies, niche partitioning, traits tradeoff, vascular plants

1 Introduction

Restored or created wetlands have been established globally to promote biodiversity conservation, but often end up with undesirable outcomes due to unpredictable ecological succession trajectories in changing environments (Baumane et al., 2021; Funk and Wolf, 2016; Jenkins et al., 2021; Salaria et al., 2018). For these factors such as biological invasions, unreasonable configuration and interspecific competition between native and non-native species can lead to the exclusion of less competitive species (Adler et al., 2018; Carboni et al., 2021; Tilman, 1990). The exclusion of species is mainly attributed to the competition for resources such as light, nutrient, and water (Silvertown, 2004). Traditional theories about the mechanisms of species coexistence include environmental heterogeneity, niche and resource partitioning (Adler et al., 2013; Aguiar et al., 2001; Fargione and Tilman, 2005; Gaudet and Keddy, 1988; Meilhac et al., 2019). The partition of different species depended on fluctuation of plants population densities and resource in space and time (Chesson, 2000). Besides, the phenology as the avenue of niche partition supplemented the high biodiversity of wetlands (Levine et al., 2022; Pak et al., 2023). Although the mechanisms for plants coexistence had been developed, the processes and strategies of plants in managing interspecific competition among heterospecific species remained unclear.

Previous studies assumed that coexistence functioned in two ways: the decrease of fitness difference or the increase of negative intraspecific interactions (Chesson, 2000). The fitness of plants could be revealed by its functional traits which were highly correlated with its competitive ability (Gaudet and Keddy, 1988). These traits of plants including canopy height, leaf size, growth rate, plant lifespan, aboveground and belowground biomass that could be employed to assess plant competitiveness (Adler et al., 2013; Grime, 1974; Lhotsky et al., 2016; Puglielli et al., 2024). In natural wetlands, the environmental heterogeneity acted as environmental filter that led to the convergence of plants traits between native and exotic species (Cleland et al., 2011). On the contrary, niche-based community theory predicted that competitive exclusion contributed to the divergence of traits between species pools (Cleland et al., 2011). Trait-based community assembly theory suggested that plants functional traits could be used to reveal the biotic and abiotic processes of plant coexistence (de Bello et al., 2012).

But there remained challenges in how to quantify the variance of trait-based characters. In stabilizing process, species inclined to offset disadvantages or enhance certain traits to protect themselves from being excluded by intraspecific competition (Adler et al., 2013; Chesson, 2000; Leibold et al., 2022). The trait-based tradeoff strategies of plant can provide insights into plant behaviors when competing with neighbor species (Cleland et al., 2011; de Bello et al., 2013; Kempel et al., 2011; Puglielli et al., 2024). Additionally, mathematical methods like interspecific correlation index and Lotka–Volterra competition models had been developed to quantify the competition between heterospecific species (Broekman et al., 2019; Chesson, 2000). However, the variables in the Lotka–Volterra competition model were morphologically, physiologically and behaviorally unrealistic (Tilman, 1990). Thus, simplified and easy measured variables are urgently needed to explain the mechanism underlying plant coexisting.

Light, as an essential resource for plant photosynthesis, tended to be the primary limiting factor for plant coexistence when nutrient supply was relatively constant in the same macrohabitat (Eskelinen et al., 2022). The functional traits of plants are primarily linked to their competitive abilities for light (Gaudet and Keddy, 1988). As plant competitiveness for light and soil space is often size-asymmetric, morphological traits can be used to understand plants' strategies (Aschehoug et al., 2016; Craine et al., 2013; Öpik et al., 2013). For example, the alien invasive plant species (*Solidago canadensis*, *Bidens frondosa* and *Erigeron canadensis*) obtained greater phenological plasticity like phenology and reproduction than native species (Cao et al., 2018; Ren et al., 2023). Thus, morphological parameters such as plants' phenology, relative height, and coverage can be utilized as proxies to quantify the variation plants traits under interspecific competition (Aschehoug et al., 2016).

To deepen our understanding of the impacts of interspecific competition on plant diversity and coexistence, empirical studies on plants coexisting strategies are necessary. This involves analyzing pairwise interactions between native and non-native species within regional species pools among 19 lakeside wetlands. Additionally, spatial and temporal niches, along with phylogenetic information, are studied to uncover niche partitioning strategies. Morphological metrics are also incorporated into Lotka–Volterra competition models to visualize the tradeoff strategies of plants in competitive

environments. The results of this study can provide a theoretical basis in the guide of plants conservation in restored or created wetlands.

2 Materials and methods

2.1 Study regions

This study focused on plants diversity and stability of aquatic communities in 19 restored lakeside wetlands of Chaohu Lake which is the fifth largest freshwater lake in China (Chen et al., 2023). The studied 19 restored lakeside wetlands located in the First Level Protected Area (FLPA) with a scope of 1 kilometer extended the shoreline of Chaohu Lake. The completed date of the 19 restored wetlands was listed in [Supplementary Table S1](#). This region experiences a subtropical monsoon climate, with an annual precipitation of 1,124.4 mm and a temperature of 16.7°C (Yang et al., 2024). Meanwhile, the studied 19 restored lakeside wetlands encompassed a variety of microhabitats such as plains, hills, marshes, and ponds (Figure 1). The combination of diverse geological conditions and suitable climate in this area facilitated the colonization of abundant vascular plants. According to the lifeform and ecological types of vascular plants, lakeside wetlands vegetation was classified into five vegetation formations, including upland plants, wet grasslands, emergent plants, floating-leaved plants, and submerged plants (Jenkins et al., 2021; Yang et al., 2024).

2.2 Data sources

To reveal plants coexisting strategies, a number of 19 sampling transects were conducted among 19 lakeside wetlands of Chaohu

Lake in the spring (April 20th to May 12th) and autumn (September 23rd to October 12th) of 2023. Each sampling-transect of restored wetland traversed five vegetation formation with a length ranged from 0.5 to 2 kilometers that was positively correlated with the width of wetland. Meanwhile, a number of 8-15 herbaceous plant quadrats (1×1m) were set at each transect and a total of 427 samples were conducted in this study. Plant characteristics including abundance, height, coverage, phenology, above ground biomass of plants were recorded to analyze the spatial niche and interspecific competition among vascular plants (Yang et al., 2024). Additionally, 10 traits encompassing functional, morphological, and phenological parameters of plants were examined to elucidate the competitive strategies of plants (Wang et al., 2012). Specifically, the content of the 10 traits were listed in [Supplementary Table S2](#). The data of morphological, and phenological parameters of vascular plants were collected from field observation and *Flora of China* (Wu and Zhou, 2001).

2.3 Data analysis

The chi-square (χ^2) statistics were utilized to examine the interspecific associations of functional groups (Hurlbert, 1969). The χ^2 value was calculated according to the following formula (Gu et al., 2017; Jin et al., 2022).

$$\chi^2 = \frac{(|ad - bc| - 0.5N)^2 N}{(a + b)(a + c)(b + d)(c + d)} \quad (1)$$

Where a represents the number of quadrats where both species i and j occurred. b represents the number of quadrats where only species i occurred. c represents the number of quadrats where only species j occurred. d represents the number of quadrats where



FIGURE 1
Location of studied 19 restored wetlands in the lakeside of Chaohu Lake basin. (A, B) Geographical position of Chaohu Lake Basin.

neither species i nor j occurred, and N represents the total number of quadrats. When $\chi^2 < 3.841$, there is no interspecific association between species; when $3.84 \leq \chi^2 \leq 6.635$, there is a moderate association between species; and when $\chi^2 > 6.635$, there is significant association between species (Gu et al., 2017).

The Ochiai index (OI) was used to assess the degree of isolation between species. The OI index was calculated according to the following equation (Hu et al., 2022).

$$OI = \frac{a}{\sqrt{(a+b)(a+c)}} \quad (2)$$

Where the value of a , b , and c in Equation 2 are the same as those in Equation 1. When OI value is 0, indicating that the species are completely independent. The closer the OI value is to 1 and $\chi^2 > 0$, the higher the probability of two species cooccur in the same habitat.

The Spearman rank correlation coefficient (r_{ij}) was used to assess the level of linear correlation between independent species (Gauthier, 2001). The calculation of r_{ij} was based on the following equations:

$$r_{ij} = \frac{\sum_{k=1}^N (x_{ik} - \bar{x}_i)(x_{jk} - \bar{x}_j)}{\sqrt{\sum_{k=1}^N (x_{ik} - \bar{x}_i)^2 \sum_{k=1}^N (x_{jk} - \bar{x}_j)^2}} \quad (3)$$

$$r_{(ij)} = 1 - \frac{6 \sum_{k=1}^N d_k^2}{N^3 - N} \quad (4)$$

$$d_k = X_{ik} - X_{jk} \quad (5)$$

Where $r_{(ij)}$ is the rank correlation analysis between species i and j . N is the total number of quadrats. X_{ik} and X_{jk} are the rank vector of i and j that were converted from the quantity of species i and j in k th quadrat. d_k is the difference of the rank vector of species i and j . The value of $r_{(ij)}$ is ranged from -1 to 1. If $r_{(ij)} > 0$, the species have a positive correlation, and if $r_{(ij)} < 0$, species have a negative correlation.

The niche breadth (B) was utilized to analyze the niche of plants (Colwell and Futuyma, 1971; Jin et al., 2022).

$$B = \frac{1}{\sum p_j^2} = \frac{N_T^2}{\sum N_j^2} \quad (6)$$

The N_T is the total number of species i among five vegetations formations while N_j is the number of species i in each vegetation formation.

Morphological traits of vascular plants, such as relative average height (RH), relative coverage (RC), and relative abundance (RA), were used to assess plant competitiveness. The important value (IV), calculated as the average of relative abundance (RA), relative frequency (RF), and relative coverage (RC), was used to determine species significance in wetland ecosystems. Dominance (D) was employed to indicate plant prevalence among numerous species. These traits were calculated as the following equations (Gu et al., 2017; Jin et al., 2022).

$$RH = H_i / \sum_{i=1}^S H_i \times 100 \% \quad (7)$$

$$RC = C_i / \sum_{i=1}^S C_i \times 100 \% \quad (8)$$

$$RA = A_i / \sum_{i=1}^S A_i \times 100 \% \quad (9)$$

$$RF = F_i / \sum_{i=1}^S F_i \times 100 \% \quad (10)$$

$$RW = W_i / \sum_{i=1}^S W_i \times 100 \% \quad (11)$$

$$IV = (RA + RC + RF) / 3 \quad (12)$$

$$D = (RH + RC + RA + RF + RW) / 5 \quad (13)$$

The height of individuals of the i th species is represented by H_i . C_i signifies the total coverage of the i th species, while A_i represents the total number of individuals of the i th species. S indicates the total number of species in the quadrat, and F_i denotes the total number of quadrats in which the i th species appears.

The variation of relative height (ΔRH) and relative coverage (ΔRC) were used to evaluate the growth rate of plants. The calculation of ΔRH and ΔRC was based on the following equations:

$$\Delta RH = RH_{t2} - RH_{t1} \quad (14)$$

$$\Delta RC = RC_{t2} - RC_{t1} \quad (15)$$

Where RH_{t2} is the relative height of plants at time t_2 . RH_{t1} is the relative height of plants at time t_1 . Analogously, RC_{t2} is the relative coverage of plants at time t_2 while RC_{t1} is the relative coverage of plants at time t_1 .

All the data were calculated with Excel and analyzed via Origin 2024 pro.

3 Results

3.1 Species pool in this region

A total of 220 vascular plants from 64 families and 168 genera were identified in 19 restored wetlands around Chaohu Lakeside (Figure 2). The proportion of local species in the regional species pool varied among different plant taxa. Among them, the species from Poaceae, Asteraceae, Cyperaceae, Polygonaceae, and Fabaceae contributed to 15%, 10.91%, 5.91%, 5.45%, and 4.55% of the total species, respectively. Poaceae, particularly annual and biennial weeds, were more dominant than forbs in wetlands, that embraced 33% of richness and 38.76% of abundance (Sutherland, 2004). Poaceae, Cyperaceae, and Polygonaceae were major contributors to the weed list in this region, characterized by fast growth rate, high adaptability, and rapid reproduction (Baker, 1974).

Annual species primarily propagated through seeds, while most perennial species spread through asexual reproduction. The total mass of annual species' seeds was twice as much as that of perennial plants in the soil of emergent communities (Drahota and Reichart,

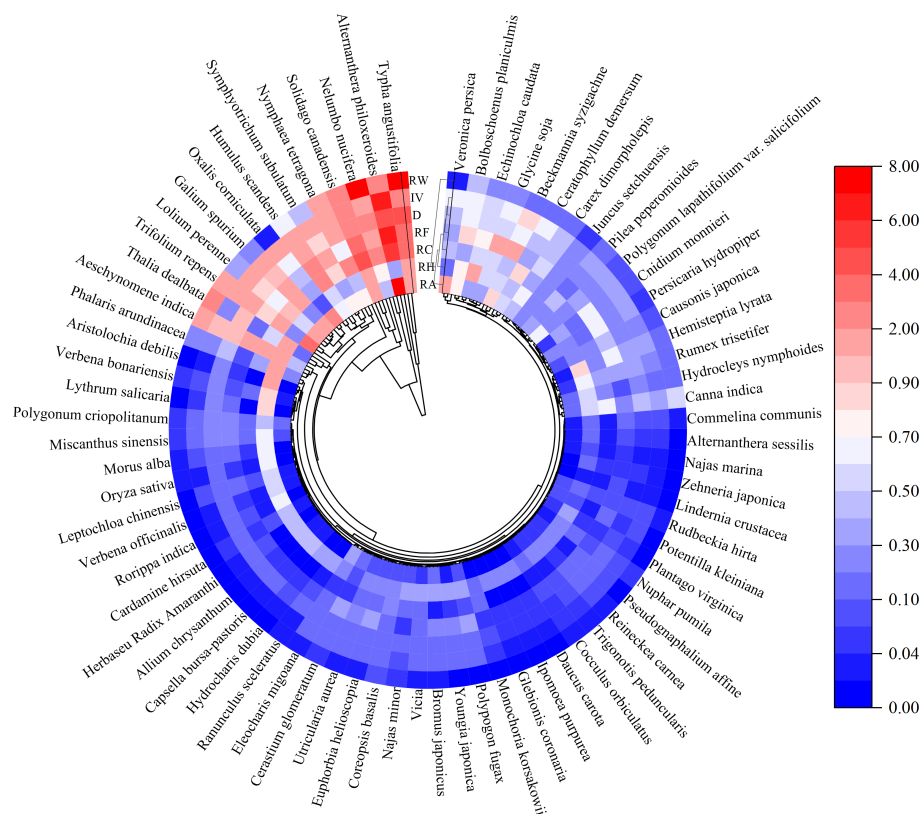


FIGURE 2

The phylogenetic tree ($n=220$ species) was derived from the sequence number of species in *Flora of Anhui Province*. Cells from the inside to the outside of the phylogeny are plant parameters: RA, relative abundance; RH, relative height; RC, relative coverage; RF, relative frequency; D, dominance; IV, important value; and RW, relative weight.

2015). Aquatic vegetation restoration provided high-energy food sources (seeds, tubers, and submerged aquatic plants) tend to enhance waterfowl diversity of wetlands (Lishawa et al., 2020; Zhou et al., 2020). Species like *Beckmannia syzigachne* (*B. syzigachne*) and *Echinochloa caudata* (*E. caudata*), prevalent in abandoned farmland, were often used as pioneer species for wetland restoration (Yan et al., 2023). However, perennial species such as *S. canadensis*, *P. australis*, and *T. angustifolia* are not edible by birds, and their proliferation can reduce bird diversity in restored wetlands. This condition can be revised by controlling invasive plants (Lishawa et al., 2020).

The predominant species of upland vegetation formation was mainly originated from the Asteraceae and Fabaceae families, such as *Solidago canadensis* (*S. canadensis*), *Erigeron canadensis* (*E. canadensis*), *Sonchus oleraceus* (*S. oleraceus*), *Glycine soja* (*G. soja*) and *Vicia sativa* L. (*V. sativa*). *S. canadensis* was initially introduced as an ornamental plant, but escaped and became pervasive in wetlands (Wang et al., 2021). The shading tolerance capacity of *S. canadensis* enabled it adapted to various environmental variables (Wu et al., 2020). On the other hand, *G. soja*, a native species under state protection (category II), is known for its climbing and strangling behavior towards other species. While Cyperaceae and Polygonaceae were mainly obligate or

facultative wetland species like *Bolboschoenus planiculmis*, *Cyperus difformis*, *Persicaria lapathifolia*, and *Persicaria orientalis* which mainly distributed in wet grassland and emergent vegetation formations.

According to the plant source, the proportion of native species, artificially planted species, alien species, and alien invasive species accounted for 63.64%, 21.82%, 3.18%, and 11.36%, respectively. The top five native species with higher important value were *Phragmites australis* (*P. australis*), *Paspalum distichum* (*P. distichum*), *Cynodon dactylon* (*C. dactylon*), *Potamogeton crispus* (*P. crispus*), and *T. angustifolia*, with IV of 0.054, 0.043, 0.042, 0.030, and 0.026 respectively. Among many invasive plants, *A. philoxeroides*, *S. canadensis*, and *E. canadensis* tended to be more pervasive and predominant in lakeside wetlands with IV values of 0.064, 0.029, and 0.026, respectively. *A. philoxeroides*, as an invasive species, can spread quickly through vegetative propagation and adapt well to diverse habitats under different light regimes conditions (Pan et al., 2006). *S. canadensis* exhibits high drought stress tolerance, low nutrient stress tolerance, high fecundity, and diffusion ability, making it the most threatened invasive species in wetlands (Wang et al., 2023). Another invasive species *Erigeron canadensis* (*E. canadensis*) with high growth rate that can secrete allelochemicals to inhibit the growth of neighboring plants (Yan et al., 2020).

3.2 Interspecific correlation of plants

To determine interspecific relationship among vascular plants, a total of 30 predominant species and 435 pair of relation were analyzed in the spring and autumn, respectively (Figure 3). In spring, alien invasive plants such as *A. philoxeroides*, *G. carolinianum*, *E. canadensis*, and *S. canadensis* exhibited higher dominance. *A. philoxeroides*, as semiaquatic species showed a positive correlation with hydrophyte and macrophyte like *Rumex japonicus* (*R. japonicus*), *Leersia japonica*, and *T. angustifolia* with OI exceed 0.35. Conversely, *A. philoxeroides* exhibited a negative correlation with xerophytes and mesophytes like *G. carolinianum*, *E. canadensis*, and *S. oleraceus*, with OI value of 0.31, 0.07, and 0.03, respectively. On the other hand, *G. carolinianum*, *E. canadensis*, and *S. canadensis* are xerophilous or mesic plants mainly found in upland vegetation formation. Furthermore, the *G. carolinianum* and *E. canadensis* showed positively correlated with most xerophilous or mesic species in spring but negative correlations in autumn. Specifically, *S. canadensis* exhibited a positive correlation

with vine and herbaceous plants with larger size while it obtained a negative correlation with herbaceous plants with smaller size.

The dominant native species identified in this study were *P. australis*, *Vicia sativa*, and *T. angustifolia*. Among them, *P. australis* showed a positive interspecific correlation with *T. angustifolia*, with an OI of 0.34 and χ^2 of 13.64, indicating a significant overlap in their ecological niche. Both *P. australis* and *T. angustifolia*, known as obligate wetland species, are widely utilized in wetland restoration projects. The positive correlation observed in invasive plants suggested their superior competitive ability over native species. Furthermore, the intensity of interspecific competition between invasive and native plants was more intense than that among different invasive species. Interestingly, xerophilous or mesic species such as *Humulus scandens* (*H. scandens*), *Nelumbo nucifera* Gaertn (*N. nucifera*) and *Hydrilla verticillata* (*H. verticillata*) showed no correlation with macrophytes, for that most macrophyte were monocultured. Additionally, the presence of hydrophytic habitats have effectively restricted the spread of xerophilous and mesic invaders (Dou et al., 2022).

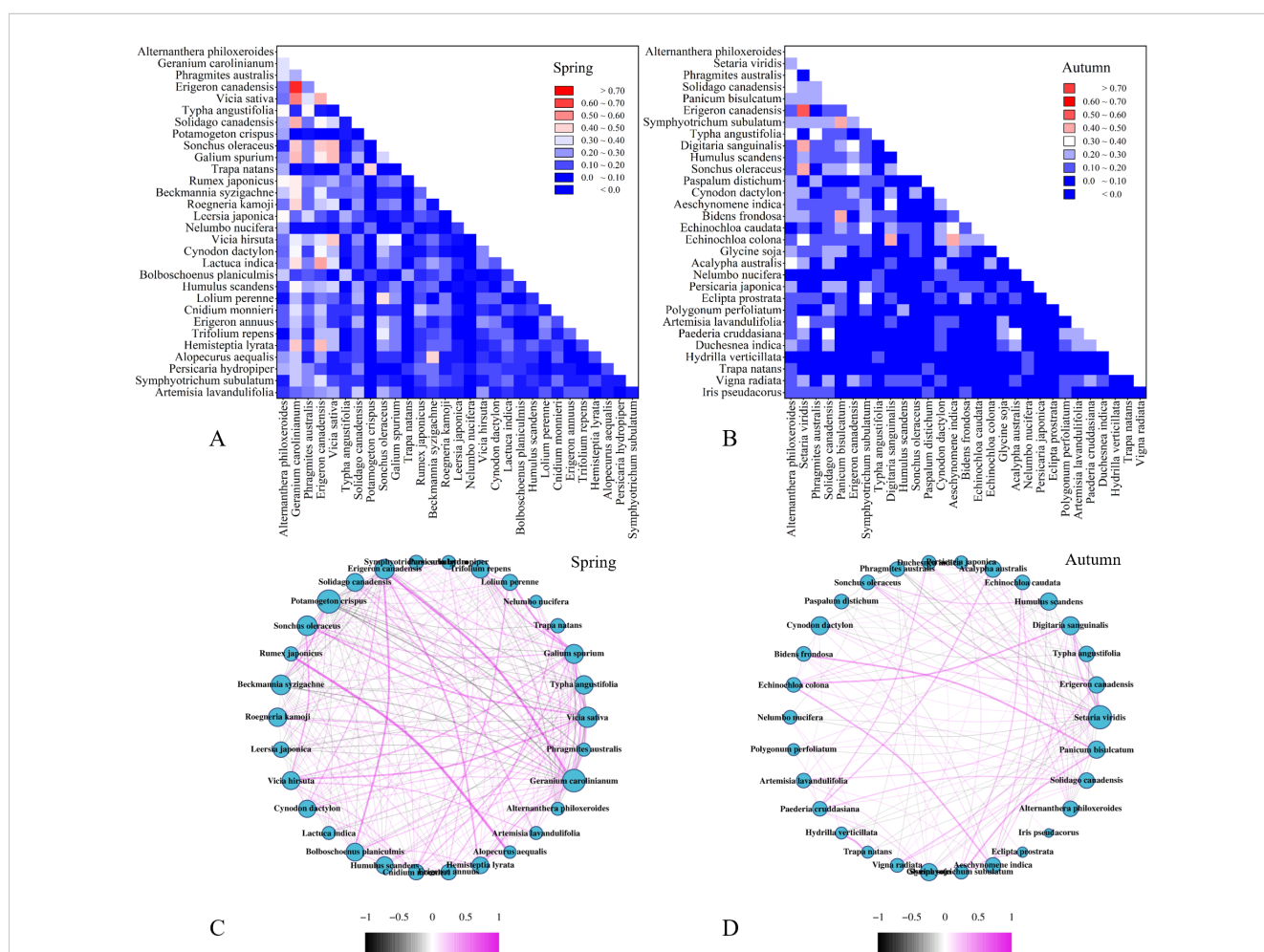


FIGURE 3

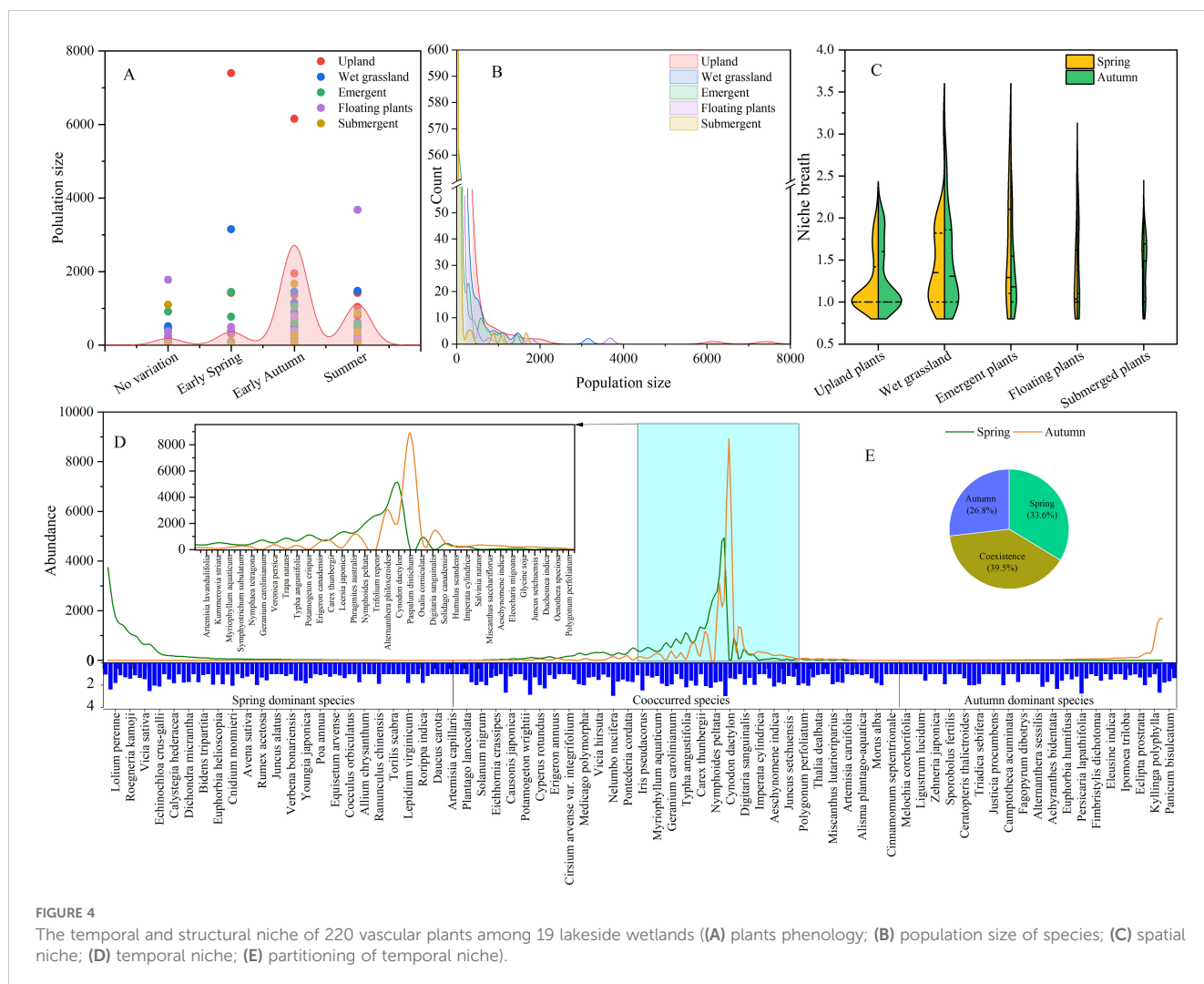
Interspecific relationship among the top thirty predominant species in the regional species pool of the Chaohu lakeside wetlands. (A, B) Ochiai index. From up to bottom and left to right, species quoted by its frequency from high to low. (C, D) Spearman rank correlation coefficient (r_{ij}) of species in the spring and autumn, respectively. The purple curve represents positive correlation while the black curve represents negative correlation. The absolute value of r_{ij} under 0.1 were neglected.

3.3 Niche partition of plants

The strategies employed by plants to alleviate competition involves niche partitioning in both space and time (Figure 4). The distribution of vascular plants within various vegetation formations such as upland plants, wet grassland plants, emergent plants, floating-leaved plants, and submerged plants was 51.36%, 31.36%, 8.64%, 4.55%, and 4.09%, respectively (Yang et al., 2024). As shown in Figure 4C, the average niche breadth upland plants, wet grassland plants, emergent plants, floating-leaved plants, and submerged plants were 1.28 ± 0.36 , 1.53 ± 0.52 , 1.67 ± 0.60 , 1.48 ± 0.52 , and 1.58 ± 0.49 , respectively. This result supported the hypothesis that the niche of functional groups in wetlands was predominantly influenced by hydrological conditions (Deane et al., 2017). Meanwhile, species of wet grassland and emergent vegetation formations usually obtained higher tolerance to hydrological changes. Specifically, species like *A. philoxeroides*, *N. nucifera*, and *Rumex japonicus* exhibited wider niche breadth exceeding 2.85 compared with *S. canadensis* of 1.34. Due to the coexistence of these adaptive species that contributed to the ecotone's higher biodiversity (Yang et al., 2024).

Another factor that influences species fluctuations is the annual climate variability. A previous study by Zheng et al. (2024) highlighted the significant impact of temporal shifts and functional traits on grassland productivity. In contrast, Usinowicz et al. (2017) found that the benefits of asynchronous coexistence were more pronounced in tropical and subtropical forests compared to temperate forests. Figure 4D illustrates the explicit partition of plants' temporal niche. The result indicated that 160 species were observed in spring and 148 species in autumn. The distribution of species along the X-axis indicates that 33.6% of investigated species occurred in spring, 26.8% in autumn, and 39.5% in both seasons. Wang et al. (2012) noted that 25.45% of species matured from late spring to summer, with 8.18% achieving the highest biomass in early spring and 4.09% maintaining consistent biomass throughout the year. Additionally, 62.27% of plants matured in early autumn (Figure 4A). The partition of plants reproductive phenology can effectively enrich the seed bank of wetland as well as facilitate the survive rate of plants.

According to the reproductive capacity of plants, annual species generally embraced shorter germination times compared to perennial species (Shipley and Parent, 1991). In lakeside wetlands,



the species pool consisted of 97 annual plants (44.09%) and 123 perennial herbs (55.91%). Spring-dominant species were typically annual herbs with smaller size and biomass, such as *Azolla pinnata*, *Spirodela polyrhiza*, and *Galium spurium*. Annual herbs tend to spread quickly due to shorter generation times or higher fecundity, giving them a selective advantage over biennials and perennials (Sutherland, 2004). On the other hand, perennial hygrophytes like Phragmites, Typhaceae and Cyperaceae embrace well-developed rhizomes or corms that provide flooding tolerance (Wang et al., 2022). In the initial stages of wetland restoration, annuals typically dominate the species composition, but there is a gradual shift towards perennials in long-term succession (Jenkins et al., 2021).

Species predominant in autumn were mainly from the Poaceae family, such as *Echinochloa colona*, *E. caudata*, and *Setaria viridis*, with seeds maturing in autumn. These species were often accompanied by planted species like *N. nucifera*, *Nymphaea tetragona*, and *T. angustifolia*. Notably, *G. carolinianum* matured in spring and completed its life cycle in summer. While most species withered in autumn, *Panicum bisulcatum* bloomed and outperformed others in terms of height and coverage. Species coexisting in spring and autumn were characterized by perennial macrophytes with greater competitiveness in terms of height and biomass. Additionally, species with wider temporal niche tended to possess higher niche breadth as well (Figure 4D).

3.4 Traits tradeoff of plants

Ultimately, the interspecific competition among plants is a competition for resources. The competitiveness of plants is

usually size asymmetric that the larger plants typically receiving a greater share of light resources which may suppress neighboring plants' fitness (Aschehoug et al., 2016). In this study, relative height (RH) and relative coverage (RC) were introduced into the Lotka-Volterra model to assess the competitiveness and strategies for plant coexistence in a large community (Silvertown, 2004; Tilman, 1990). Because majority (62.27%) of plants in this region reached maturity in the autumn, leading to the use of autumnal traits to determine stability conditions, while variations of traits between spring and autumn were used to assess the strategies of plants (Chesson, 2000).

The coverage and height tradeoff are the most common strategies for plant coexistence in the presence of interspecific competition. If $\frac{\Delta RH}{\Delta RC} > 1$, the species is determined by the height tradeoff strategy. If $\frac{\Delta RH}{\Delta RC} < 1$, the species is determined by the coverage tradeoff strategy. When $\frac{\Delta RH}{\Delta RC} \approx 1$, the species is determined by both coverage and height tradeoff strategies. When the $\frac{\Delta RH}{\Delta RC}$ is negative, the equation is substituted by $\frac{RH}{RC}$. Plants applied height tradeoff strategy are these species like *E. canadensis*, *Miscanthus lutarioriparius*, *E. caudata*, and *N. nucifera*, while the coverage tradeoff strategy is observed in *G. carolinianum*, *A. philoxeroides*, *Trapa natans*, and *P. crispus* (Figure 5).

Our results indicated that plants in different vegetation formations may adopt disparate strategies. In upland vegetation formation, *S. canadensis* was predominant by height and coverage while *E. canadensis* and *G. carolinianum* were driven by height and coverage strategies, respectively. Additionally, plants in different habitat may also adopt different strategies. For example, *P. australis* adopted coverage strategy in hygrophilous habitat while it employed height strategy in aquatic habitat. According to the Lotka-Volterra model, species with trading strategies tend to

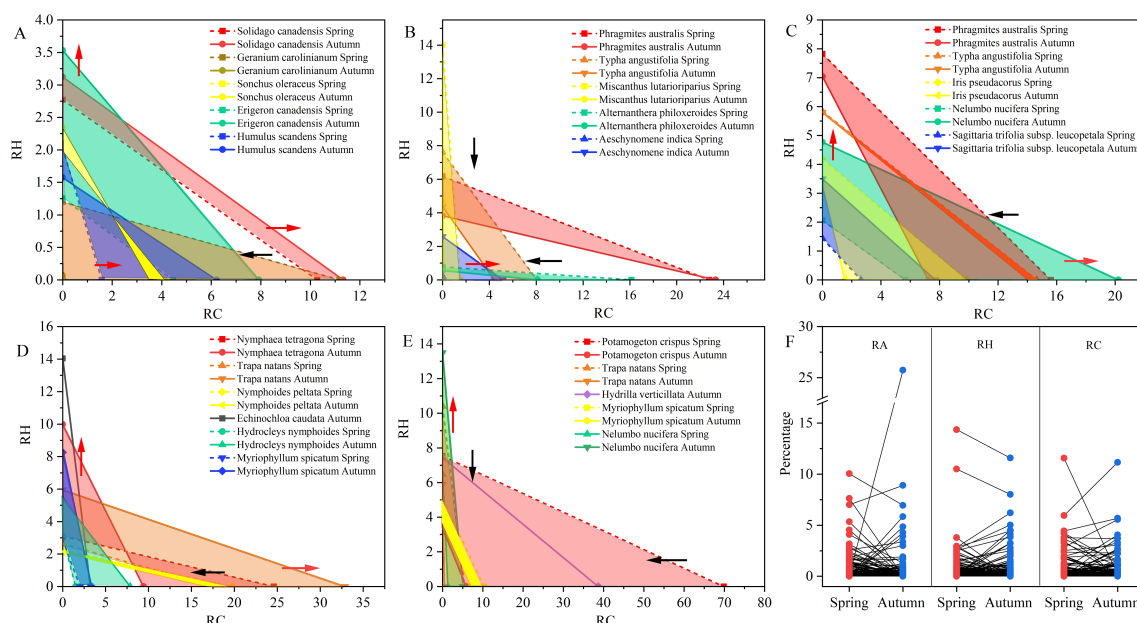


FIGURE 5

Variation of plants functional traits in different vegetation formations: (A) upland vegetation formation; (B) wet grassland vegetation; (C) emergent plants; (D) floating-leaved plants; (E) submerged plants; (F) seasonal change of plants' traits; RA, relative abundance; RH, relative height; RC, relative coverage; The red arrows mean the expansion of traits while the black arrows represent the shrink of traits.

coexist, while non-trading strategy species are more likely to be excluded by competitive species (De Mazancourt and Schwartz, 2010). The weaker competitors increase their fitness via traits trade off while stronger competitors are suppressed by their conspecific neighbors (Stoll and Prati, 2001). The increase in dominance of a species in one resource comes at the expense of competitiveness in another resource.

4 Discussion

Plant strategies in coping with competition can be summed up as spatial and temporal niche partitioning and trait tradeoffs (Figure 6). Temporal niche differentiation is primarily achieved through phenology partitioning, which reduces competition intensity and promotes species coexistence (Pak et al., 2023). As the resources including light, water, soil nutrient availability variate during the year, the partitioning of plant phenology allowing

competitors to coexist (Chesson, 1994). Short-lived species tend to complete their life cycles under favorable environmental conditions to avoid dreadful environment (Hereford et al., 2017). Our findings revealed that 33.6% of species occurred in spring, 26.8% in autumn, and 39.5% in both seasons. Specifically, 25.45% of species matured in early summer, while 62.27% matured in autumn. Species that matured in early spring were typically small-sized, such as *Vicia hirsuta* and *Medicago polymorpha*, with lower competition, leading to variations in plant maturity timing. Notably, the endangered fern *Ceratopteris chingii* completed its life cycle in autumn (Guan et al., 2019). Special attention should be given to these annual species with specific and narrower temporal niches.

Among the five vegetation formations of restored wetlands, emergent plants had the widest niche breadth of 1.67 ± 0.60 , while upland vegetation species had the lowest niche breadth of 1.28 ± 0.36 . The emergent plant vegetation formation was predominantly composed of facultative species that can survive in both upland and wet grassland of wetlands. On the other hand, upland vegetation was

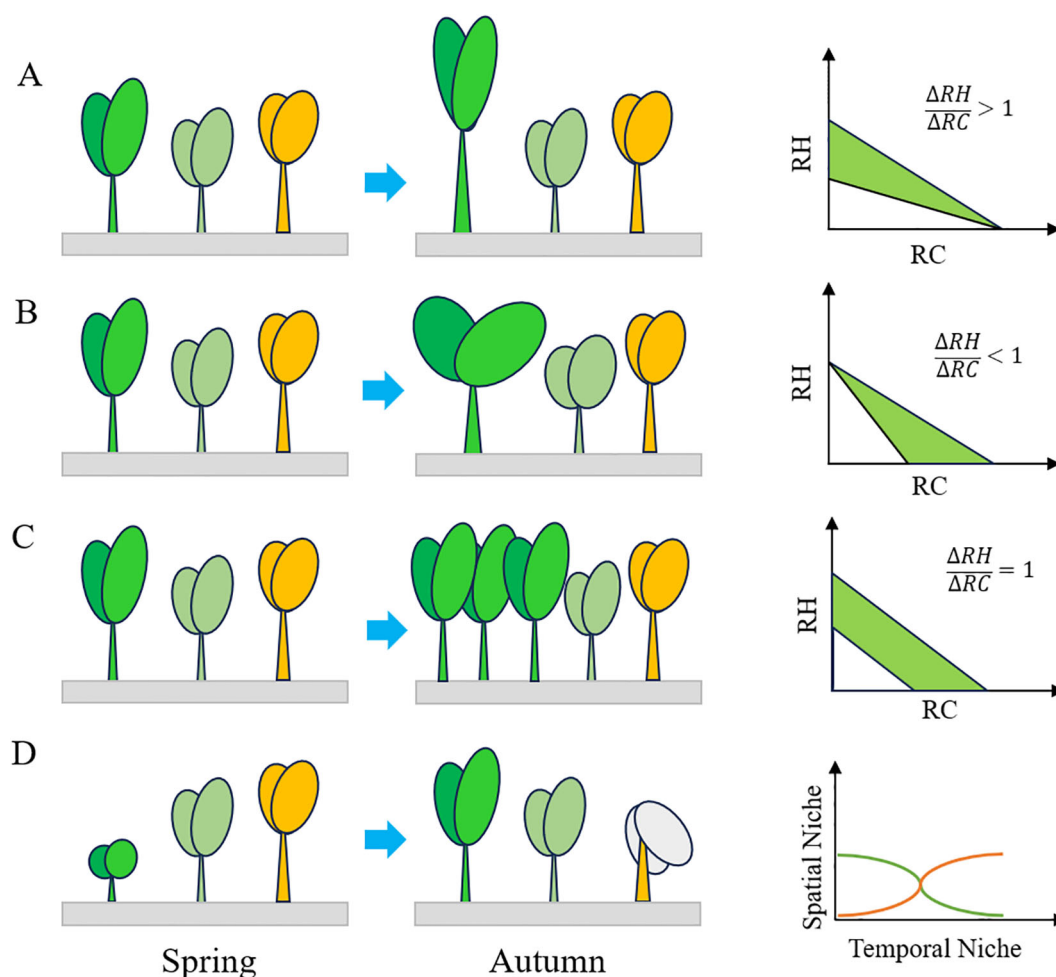


FIGURE 6

The strategies of plants coexistence under competition: (A) height tradeoff strategy; (B) coverage tradeoff strategy; (C) height and coverage tradeoff strategy; (D) niche partitioning strategy; RH, relative height; RC, relative coverage; ΔRH , variation of relative height; ΔRC , variation of relative coverage.

dominated by xerophyte and mesophyte species, resulting in higher plant richness of 81 species but a lower niche breadth of 1.28. There was no correlation between upland species and most hygrophytes, indicating that habitat heterogeneity promoted vegetation partitioning. The vegetation formations in wetlands were primarily influenced by soil moisture, with the wetland hydrology regime playing a key role in regulating plant communities (Regmi et al., 2021). In particular, the inundation time of wetlands was found to influence species niche differentiation in wetlands (Foti et al., 2012). By managing the hydrological regime of seasonal flooding lakeside wetlands, it was possible to control the spread of xerophytic invasive species such as *G. carolinianum*, *E. canadensis*, and *S. canadensis*.

Traits tradeoff in height and coverage were primary strategies of plants to acquire more resources under interspecific competition. The established species like *P. australis*, *T. angustifolia*, and *S. canadensis* are usually taller in the communities, while subordinate species inclined to pursue for a height tradeoff to compete for light resources when there was little height difference. Less competitive species turned to pursue for a coverage tradeoff, investing more nutrients in their leaves to maximize light absorption. For instance, plants with high specific leaf area tended to occupy areas with higher resources, whereas those with lower specific leaf area tended to establish in areas with lower resources (Adler et al., 2013). Plants adopting coverage tradeoff strategy are prostrate plants like *Trifolium repens*, *H. scandens*, *Kummerowia striata*, *Actinostemma tenerum*, *Causonis japonica* which incline to expand their coverage when competed with other species (Eskelinen et al., 2022). However, the pervasive of these species may lead to the exclusion of other species due to light shading effects. Another way for plants to alleviate interspecific competition is the tradeoff height and coverage strategies. This strategy was mainly achieved through the increase in individual numbers, which could, in turn, intensify intraspecific competition. Species commonly found in this scenario are most Cyperaceae, Poaceae, and Juncus species like *Carex brachyathera*, *Cynodon dactylon*, *P. distichum*, and *Juncus effusus*.

To recover and maintain plant diversity in restored wetlands, wetland conservators can adopt the following strategies: Firstly, protect annual species and control perennial plants to ensure the sustainability of annual species propagule. Secondly, clear invasive species or replace them with native plants which have highly competitive capacities that can coexist with alien invasive plants through height, coverage tradeoff strategy, or both of it. Thirdly, establish a biological defense line by safeguarding the hydrology regime and reducing the diffusion risk of non-native species (Deane et al., 2017; Raulings et al., 2010). In general, priority should be given to plant functional traits, landscape configuration, and hydrology regime in the restoration and maintenance of wetlands.

5 Conclusion

To investigate the coexistence mechanisms and strategies of plants under interspecific competition, a comprehensive study was

conducted among 220 vascular plants from 19 restored wetlands. The OI index indicated that 62.41% of species pairs embraced a negative correlation while 37.59% species pairs were positive. Notably, invasive species such as *A. philoxeroides*, *G. carolinianum*, *S. canadensis*, and *E. canadensis*, along with native species like *V. sativa* and *S. viridis*, embraced higher competitiveness than neighboring species. The primary strategies for different plant coexisted including spatial and temporal niche partitioning, as well as tradeoffs in height and coverage. Emergent plants obtained a wider niche breadth of 1.67 ± 0.60 compared to upland species with the narrowest niche of 1.28 ± 0.36 . In temporal scale, species occurred in spring, autumn, and both spring and autumn reached 33.6%, 26.8% and 39.5%, respectively. Phenology character of plants indicated that the proportion of species matured in spring, summer and autumn accounted for 8.18%, 25.45% and 62.27%, respectively. Height tradeoff strategies were predominantly observed in communities of *P. australis*, *T. angustifolia*, and *S. canadensis*, while coverage tradeoff strategies were more prevalent in *Trifolium repens*, *H. scandens*, and *Kummerowia striata* communities. Overall, the coexistence strategies including spatial and temporal niche partitioning, as well as tradeoffs in height and coverage traits among vascular plants that hold promise for biodiversity conservation and the management of alien invasive species.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://datadryad.org/stash/share/hlC-twvPymbqGh2wsUbZwp6tu6TBAbTtTjodHjSo5tc>.

Author contributions

SY: Investigation, Software, Writing – original draft. ZY: Data curation, Writing – original draft. BY: Data curation, Investigation, Writing – review & editing. FZ: Methodology, Software, Writing – original draft. XT: Project administration, Supervision, Writing – review & editing. RG: Project administration, Supervision, Writing – original draft. ZC: Conceptualization, Funding acquisition, Writing – review & editing. XL: Funding acquisition, Investigation, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

References

- Adler, P. B., Fajardo, A., Kleinhesselink, A. R., Kraft, N. J. B., and Scherber, C. (2013). Trait-based tests of coexistence mechanisms. *Ecol. Lett.* 16, 1294–1306. doi: 10.1111/ele.12157
- Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., et al. (2018). Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecol. Lett.* 21, 1319–1329. doi: 10.1111/ele.13098
- Aguiar, M. R., Lauenroth, W. K., and Peters, D. P. (2001). Intensity of intra- and interspecific competition in coexisting shortgrass species. *J. Ecol.* 89, 40–47. doi: 10.1046/j.1365-2745.2001.00523.x
- Aschehoug, E. T., Brooker, R., Atwater, D. Z., Maron, J. L., and Callaway, R. M. (2016). The mechanisms and consequences of interspecific competition among plants. *Annu. Rev. Ecology Evolution Systematics* 47, 263–281. doi: 10.1146/annurev-ecolsys-121415-032123
- Baker, H. G. (1974). The evolution of weeds. *Annu. Rev. Ecol. Systematics* 5, 1–24. doi: 10.1146/annurev.es.05.110174.000245
- Baumane, M., Zak, D. H., Riis, T., Kotowski, W., Hoffmann, C. C., and Baatrup-Pedersen, A. (2021). Danish wetlands remained poor with plant species 17-years after restoration. *Sci. Total Environ.* 798. doi: 10.1016/j.scitotenv.2021.149146
- Broekman, M. J. E., Muller-Landau, H. C., Visser, M. D., Jongejans, E., Wright, S. J., de Kroon, H., et al. (2019). Signs of stabilisation and stable coexistence. *Ecol. Lett.* 22, 1957–1975. doi: 10.1111/ele.13349
- Cao, Y., Xiao, Y. A., Zhang, S., and Hu, W. (2018). Simulated warming enhances biological invasion of *Solidago canadensis* and *Bidens frondosa* by increasing reproductive investment and altering flowering phenology pattern. *Sci. Rep.* 8. doi: 10.1038/s41598-018-34218-9
- Carboni, M., Livingstone, S. W., Isaac, M. E., and Cadotte, M. W. (2021). Invasion drives plant diversity loss through competition and ecosystem modification. *J. Ecol.* 109, 3587–3601. doi: 10.1111/1365-2745.13739
- Chen, P., Luo, J., Xiong, Z., Wan, N., Ma, J., Yuan, J., et al. (2023). Can the establishment of a protected area improve the lacustrine environment? A case study of Lake Chaohu, China. *J. Environ. Manage.* 342. doi: 10.1016/j.jenvman.2023.118152
- Chesson, P. (1994). Multispecies competition in variable environments. *Theor. Population Biol.* 45, 227–276. doi: 10.1006/tpbi.1994.1013
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Systematics* 31, 343–366. doi: 10.1146/annurev.ecolsys.31.1.343
- Cleland, E. E., Clark, C. M., Collins, S. L., Fargione, J. E., Gough, L., Gross, K. L., et al. (2011). Patterns of trait convergence and divergence among native and exotic species in herbaceous plant communities are not modified by nitrogen enrichment. *J. Ecol.* 99, 1327–1338. doi: 10.1111/j.1365-2745.2011.01860.x
- Colwell, R. K., and Futuyma, D. J. (1971). On the measurement of niche breadth and overlap. *Ecology* 52, 567–576. doi: 10.2307/1934144
- Craine, J. M., Dybzinski, R., and Robinson, D. (2013). Mechanisms of plant competition for nutrients, water and light. *Funct. Ecol.* 27, 833–840. doi: 10.1111/1365-2435.12081
- Deane, D. C., Nicol, J. M., Gehrig, S. L., Harding, C., Aldridge, K. T., Goodman, A. M., et al. (2017). Hydrological-niche models predict water plant functional group distributions in diverse wetland types. *Ecol. Appl.* 27, 1351–1364. doi: 10.1002/eap.1529
- de Bello, F., Price, J. N., Münkemüller, T., Liira, J., Zobel, M., Thuiller, W., et al. (2012). Functional species pool framework to test for biotic effects on community assembly. *Ecology* 93, 2263–2273. doi: 10.1890/11-1394.1
- de Bello, F., Vandewalle, M., Reitalu, T., Lepš, J., Prentice, H. C., Lavorel, S., et al. (2013). Evidence for scale- and disturbance-dependent trait assembly patterns in dry semi-natural grasslands. *J. Ecol.* 101, 1237–1244. doi: 10.1111/1365-2745.12139
- De Mazancourt, C., and Schwartz, M. W. (2010). A resource ratio theory of cooperation. *Ecol. Lett.* 13, 349–359. doi: 10.1111/j.1461-0248.2009.01431.x
- Dou, Z., Cui, L., Li, W., Lei, Y., Zuo, X., Cai, Y., et al. (2022). Effect of freshwater on plant species diversity and interspecific associations in coastal wetlands invaded by *Spartina alterniflora*. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.965426
- Drahota, J., and Reichart, L. M. (2015). Wetland seed availability for waterfowl in annual and perennial emergent plant communities of the rainwater basin. *Wetlands* 35, 1105–1116. doi: 10.1007/s13157-015-0698-3
- Eskelinen, A., Harpole, W. S., Jessen, M.-T., Virtanen, R., and Hautier, Y. (2022). Light competition drives herbivore and nutrient effects on plant diversity. *Nature* 611, 301–305. doi: 10.1038/s41586-022-05383-9
- Fargione, J., and Tilman, D. (2005). Niche differences in phenology and rooting depth promote coexistence with a dominant C4 bunchgrass. *Oecologia* 143, 598–606. doi: 10.1007/s00442-005-0010-y
- Foti, R., del Jesus, M., Rinaldo, A., and Rodriguez-Iturbe, I. (2012). Hydroperiod regime controls the organization of plant species in wetlands. *Proc. Natl. Acad. Sci.* 109, 19596–19600. doi: 10.1073/pnas.1218056109
- Funk, J. L., and Wolf, A. A. (2016). Testing the trait-based community framework: Do functional traits predict competitive outcomes? *Ecology* 97, 2206–2211. doi: 10.1002/ecy.1484
- Gaudet, C. L., and Keddy, P. A. (1988). A comparative approach to predicting competitive ability from plant traits. *Nature* 334, 242–243. doi: 10.1038/334242a0
- Gauthier, T. (2001). Detecting trends using spearman's rank correlation coefficient. *Environ. Forensics* 2, 359–362. doi: 10.1006/enfo.2001.0061
- Grime, J. P. (1974). Vegetation classification by reference to strategies. *Nature* 250, 26–31. doi: 10.1038/250026a0
- Gu, L., Gong, Z.-W., and Li, W.-Z. (2017). Niches and interspecific associations of dominant populations in three changed stages of natural secondary forests on loess plateau, P.R. China. *Sci. Rep.* 7. doi: 10.1038/s41598-017-06689-9

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1539136/full#supplementary-material>

- Guan, B.-c., Liu, X., Gong, X., Cai, Q.-Y., and Ge, G. (2019). Genetic landscape and landscape connectivity of *Ceratopteris thalictroides*, an endangered aquatic fern. *Ecol. Inf.* 53. doi: 10.1016/j.ecoinf.2019.100973
- Hereford, J., Schmitt, J., Ackerly, D. D., and Satake, A. (2017). The seasonal climate niche predicts phenology and distribution of an ephemeral annual plant, *Mollugo verticillata*. *J. Ecol.* 105, 1323–1334. doi: 10.1111/1365-2745.12739
- Hu, Y., Wang, H., Jia, H., Pen, M., Liu, N., Wei, J., et al. (2022). Ecological niche and interspecific association of plant communities in alpine desertification grasslands: A case study of qinghai lake basin. *Plants* 11. doi: 10.3390/plants11202724
- Hurlbert, S. H. (1969). A coefficient of interspecific association. *Ecology* 50, 1–9. doi: 10.2307/1934657
- Jenkins, D. G., McKown, J. G., Moore, G. E., Payne, A. R., White, N. A., and Gibson, J. L. (2021). Successional dynamics of a 35 year old freshwater mitigation wetland in southeastern New Hampshire. *PLoS One* 16. doi: 10.1371/journal.pone.0251748
- Jin, S.-S., Zhang, Y.-Y., Zhou, M.-L., Dong, X.-M., Chang, C.-H., Wang, T., et al. (2022). Interspecific association and community stability of tree species in natural secondary forests at different altitude gradients in the southern taihang mountains. *Forests* 13. doi: 10.3390/f13030373
- Kempel, A., Schädler, M., Chrobok, T., Fischer, M., and van Kleunen, M. (2011). Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proc. Natl. Acad. Sci.* 108, 5685–5689. doi: 10.1073/pnas.1016508108
- Leibold, M. A., Govaert, L., Loeuille, N., De Meester, L., and Urban, M. C. (2022). Evolution and community assembly across spatial scales. *Annu. Rev. Ecology Evolution Systematics* 53, 299–326. doi: 10.1146/annurev-ecolsys-102220-024934
- Levine, J. I., Levine, J. M., Gibbs, T., Pacala, S. W., and Knopss, J. (2022). Competition for water and species coexistence in phenologically structured annual plant communities. *Ecol. Lett.* 25, 1110–1125. doi: 10.1111/ele.13990
- Lhotsky, B., Kovács, B., Ónodi, G., Csecserits, A., Rédei, T., Lengyel, A., et al. (2016). Changes in assembly rules along a stress gradient from open dry grasslands to wetlands. *J. Ecol.* 104, 507–517. doi: 10.1111/1365-2745.12532
- Lishawa, S. C., Dunton, E. M., Pearsall, D. R., Monks, A. M., Himmler, K. B., Carson, B. D., et al. (2020). Wetland waterbird food resources increased by harvesting invasive cattails. *J. Wildlife Manage.* 84, 1326–1337. doi: 10.1002/jwmg.21912
- Meilhac, J., Deschamps, L., Maire, V., Flajoulot, S., and Litrico, I. (2019). Both selection and plasticity drive niche differentiation in experimental grasslands. *Nat. Plants* 6, 28–33. doi: 10.1038/s41477-019-0569-7
- Öpik, M., de Bello, F., Price, J. N., and Fraser, L. H. (2013). New insights into vegetation patterns and processes. *New Phytol.* 201, 383–387. doi: 10.1111/nph.12606
- Pak, D., Swamy, V., Alvarez-Loayza, P., Cornejo-Valverde, F., Queenborough, S. A., Metz, M. R., et al. (2023). Multiscale phenological niches of seed fall in diverse Amazonian plant communities. *Ecology* 104. doi: 10.1002/ecy.4022
- Pan, X., Geng, Y., Zhang, W., Li, B., and Chen, J. (2006). The influence of abiotic stress and phenotypic plasticity on the distribution of invasive *Alternanthera philoxeroides* along a riparian zone. *Acta Oecologica* 30, 333–341. doi: 10.1016/j.actao.2006.03.003
- Puglielli, G., Bricca, A., Chelli, S., Petruzzellis, F., Acosta, A. T. R., Bacaro, G., et al. (2024). Intraspecific variability of leaf form and function across habitat types. *Ecol. Lett.* 27. doi: 10.1111/ele.14396
- Raulings, E. J., Morris, K. A. Y., Roache, M. C., and Boon, P. I. (2010). The importance of water regimes operating at small spatial scales for the diversity and structure of wetland vegetation. *Freshw. Biol.* 55, 701–715. doi: 10.1111/j.1365-2427.2009.02311.x
- Regmi, T., Shah, D. N., Doody, T. M., Cuddy, S. M., and Tachamo Shah, R. D. (2021). Hydrological alteration induced changes on macrophyte community composition in sub-tropical floodplain wetlands of Nepal. *Aquat. Bot.* 173. doi: 10.1016/j.aquabot.2021.103413
- Ren, J., Chen, P., Shen, C., Tao, Z., Huang, W., and Nie, M. (2023). Functional and phylogenetic similarities of co-occurring invaders affect the growth of an invasive forb. *J. Plant Ecol.* 16. doi: 10.1093/jpe/rtad007
- Salaria, S., Howard, R., Clare, S., and Creed, I. F. (2018). Incomplete recovery of plant diversity in restored prairie wetlands on agricultural landscapes. *Restor. Ecol.* 27, 520–530. doi: 10.1111/rec.12890
- Shipley, B., and Parent, M. (1991). Germination responses of 64 wetland species in relation to seed size, minimum time to reproduction and seedling relative growth rate. *Funct. Ecol.* 5. doi: 10.2307/2389561
- Silvertown, J. (2004). Plant coexistence and the niche. *Trends Ecol. Evol.* 19, 605–611. doi: 10.1016/j.tree.2004.09.003
- Stoll, P., and Prati, D. (2001). Intraspecific aggregation alters competitive interactions in experimental plant communities. *Ecology* 82, 319–327. doi: 10.1890/0012-9658(2001)082[0319:laacii]2.0.Co;2
- Sutherland, S. (2004). What makes a weed a weed: life history traits of native and exotic plants in the USA. *Oecologia* 141, 24–39. doi: 10.1007/s00442-004-1628-x
- Tilman, D. (1990). Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* 58. doi: 10.2307/3565355
- Usinowicz, J., Chang-Yang, C.-H., Chen, Y.-Y., Clark, J. S., Fletcher, C., Garwood, N. C., et al. (2017). Temporal coexistence mechanisms contribute to the latitudinal gradient in forest diversity. *Nature* 550, 105–108. doi: 10.1038/nature24038
- Wang, C., Cheng, H., Wang, S., Wei, M., and Du, D. (2021). Plant community and the influence of plant taxonomic diversity on community stability and invasibility: A case study based on *Solidago canadensis* L. *Sci. Total Environ.* 768. doi: 10.1016/j.scitotenv.2020.144518
- Wang, L., Dronova, I., Gong, P., Yang, W., Li, Y., and Liu, Q. (2012). A new time series vegetation–water index of phenological–hydrological trait across species and functional types for Poyang Lake wetland ecosystem. *Remote Sens. Environ.* 125, 49–63. doi: 10.1016/j.rse.2012.07.003
- Wang, X., Wang, H., Wang, H., Guo, W., Zhai, H., and Zhang, X. (2022). Responses of lakeshore herbaceous plant guilds to altered water level fluctuations in Yangtze floodplain lakes, China. *Ecol. Indic.* 145. doi: 10.1016/j.ecolind.2022.109714
- Wang, W., Zhu, Q., Dai, S., Meng, L., He, M., Chen, S., et al. (2023). Effects of *Solidago canadensis* L. @ on mineralization-immobilization turnover enhance its nitrogen competitiveness and invasiveness. *Sci. Total Environ.* 882. doi: 10.1016/j.scitotenv.2023.163641
- Wu, M., Li, Z., and Wang, J. (2020). Transcriptional analyses reveal the molecular mechanism governing shade tolerance in the invasive plant *Solidago canadensis*. *Ecol. Evol.* 10, 4391–4406. doi: 10.1002/ece3.6206
- Wu, Z., and Zhou, Z. (2001). *Flora of China* (Beijing: Science Press).
- Yan, H., Feng, L., Zhao, Y., Feng, L., Zhu, C., Qu, Y., et al. (2020). Predicting the potential distribution of an invasive species, *Erigeron canadensis* L., in China with a maximum entropy model. *Global Ecol. Conserv.* 21. doi: 10.1016/j.gecco.2019.e00822
- Yan, P., Lu, X., Li, W., Zhang, J., Li, P., Li, Y., et al. (2023). Seasonal variations in plant species diversity and phylogenetic diversity in abandoned farmland of China's huang-huai plain. *Diversity* 15. doi: 10.3390/d15080922
- Yang, S., Yuan, Z., Ye, B., Zhu, F., Chu, Z., and Liu, X. (2024). Impacts of landscape pattern on plants diversity and richness of 20 restored wetlands in Chaohu Lakeside of China. *Sci. Total Environ.* 906. doi: 10.1016/j.scitotenv.2023.167649
- Zheng, L., Barry, K. E., Guerrero-Ramírez, N. R., Craven, D., Reich, P. B., Verheyen, K., et al. (2024). Effects of plant diversity on productivity strengthen over time due to trait-dependent shifts in species overyielding. *Nat. Commun.* 15. doi: 10.1038/s41467-024-46355-z
- Zhou, J., Zhou, L., and Xu, W. (2020). Diversity of wintering waterbirds enhanced by restoring aquatic vegetation at Shengjin Lake, China. *Sci. Total Environ.* 737. doi: 10.1016/j.scitotenv.2020.140190



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The impact of epiphytic algae on the foliar traits of *Potamogeton perfoliatus*

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This study investigated the effect of epiphyton on foliar traits of a submerged rooted macrophyte, *Potamogeton perfoliatus*, in a shallow freshwater lake, highlighting its influence on the ecological dynamics of littoral zones in aquatic ecosystems. It was shown that the limnological characteristics of the sampling sites (water chlorophyll-a, total suspended matter and coloured dissolved organic matter content) had no significant effect on the average values of epiphytic algal content found on pondweed leaves, while influencing the plasticity of these data. The responses of morphological and physiological traits of submerged macrophytes to accumulated epiphyton demonstrate the complexity of their relationship: epiphyton colonisation had no relevant effect on leaf morphology (except leaf length) and leaf pigment content (except Chl-a/Chl-b ratio), however, this study highlights the significant influence of epiphytic algal biomass on photophysiological traits of submerged macrophyte leaves, as 5 out of 6 photophysiological traits were affected. The results highlight the importance of considering epiphyte colonisation when seeking to understand the ecological functioning of littoral aquatic ecosystems. Furthermore, the complex interactions between epiphytes and submerged rooted macrophytes should be considered in integrated lake management and environmental protection policies. These interactions play an important, though ambiguous role in shaping habitat variability and overall ecosystem health in littoral zones.

KEYWORDS

macrophyte, epiphyton, foliar morphology, foliar photophysiology, littoral zone, lake Balaton

1 Introduction

The dynamic relationship between epiphytes – a community of algae, microbes and fungi that colonise macrophyte surfaces – and submerged macrophytes is complex, and goes beyond the simple competition (Sand-Jensen and Søndergaard, 1981; Köhler et al., 2010; Wijewardene et al., 2022). It affects the structure and functioning of the littoral zone and can even influence the balance of the whole aquatic ecosystem, especially in shallow lakes. While both macrophytes and the autotrophic part of the epiphytic community play key roles by contributing to primary production, nutrient cycling, and habitat complexity

(McAbendroth et al., 2005; Kovalenko et al., 2012; Tóth, 2024), their coexistence is often characterised by competition, notably for light (Sand-Jensen and Søndergaard, 1981; Vis et al., 2006; Sultana et al., 2010). Light is a primary resource required for photosynthesis, and in aquatic systems, it becomes increasingly limited with depth, turbidity, abundance of phytoplankton, epiphytes and macrophytes (Wetzel, 1975; Kirk, 1994; Tóth, 2013, 2024). Within macrophyte stands, light is attenuated by several factors (Kirk, 1994; Hill, 1996; Wijewardene et al., 2022). In particular, a significant proportion of the light attenuation reaching the leaf surface of submerged macrophytes is attributed to epiphytic algae (Tóth, 2013). Furthermore, the pigment systems of periphytic algae not only absorb light, thereby altering its quantity, but also do so selectively at specific wavelengths, thereby affecting the quality of the optical environment (Tóth, 2013, 2024; Klančnik et al., 2015; Tóth and Palmer, 2016). Individually, these phenomena could alter the morphology and physiology of aquatic plants, but their combined effects have the potential to induce significant phenotypic variability. Epiphytes often form dense layers on the leaves and stems of submerged plants, reducing the amount and quality of light reaching the macrophytes' photosynthetic tissues, leading to physiological and morphological adjustments to compensate for these changes (Asaeda et al., 2004; Tóth, 2013, 2024).

For epiphytes, submerged macrophytes are primarily a substrate that maintains the community in an optimal environment, as aquatic plants provide essential habitat and structural complexity for various aquatic organisms (Kuczynska-Kippen, 2008; Thomaz et al., 2008). The presence of epiphytes on macrophyte surfaces further increases habitat complexity by providing additional food sources for micro- and macro-organisms (McAbendroth et al., 2005; Kovalenko et al., 2012; Tóth, 2013). However, when epiphyte growth becomes excessive, it can negatively affect macrophytes and reduce their capacity to perform their ecosystem services (Strand and Weisner, 1996; Köhler et al., 2010; Tóth, 2013) and thus could play a pivotal role in determining water clarity (Takamura et al., 2003; Thomaz, 2023). While macrophytes help to maintain clear water by reducing wave action, stabilising sediments, and sequestering nutrients (Søndergaard et al., 2010; Blindow et al., 2014), the weakened aquatic plants due to excessive epiphyte growth could disappear (Tóth, 2013; Wijewardene et al., 2022), and, over time, shift ecosystems towards a turbid, phytoplankton-dominated state (Scheffer et al., 1993; Scheffer and van Nes, 2007). Under conditions of nutrient enrichment, such as those seen in eutrophic systems, the increased growth of epiphytes can tip the balance against macrophytes, resulting in a shift towards degraded ecosystem states (Duffy et al., 2007; Özen et al., 2018). Conversely, stable macrophyte populations can help buffer ecosystems against the effects of eutrophication by sequestering nutrients and maintaining water clarity, demonstrating their role in ecosystem resilience (Duffy et al., 2007).

Competition between these two groups of autotrophs (epiphytes and submerged macrophytes) is particularly complex (Liboriussen and Jeppesen, 2006; Dos Santos et al., 2013; Tóth and Palmer, 2016), and while both require light and occupy nearly the

same niche, the epiphytes have a significant advantage. The epiphytes can quickly respond to nutrient pulses and proliferate within days, while macrophytes, due to their complex structure and morphogenesis are slower to adjust to the changing conditions (Worm and Sommer, 2000; Yuan et al., 2023). There is an additional element to the temporal aspect of the interaction between macrophytes and their epiphytes. As epiphytic colonisation is a time-consuming process (the average colonisation time in Lake Balaton is around 8-9 days), the younger parts of the macrophytes are largely free of epiphytic cover (Tóth, 2013). Consequently, leaf morphogenesis in submerged macrophytes is initiated without the influence of epiphytes, and only gradually, over a period of time, does the epiphyte biomass begin to affect plant development. As epiphytes accumulate on the adaxial surface of leaves, their presence reduces the quantity and changes the quality of light to the photosynthetic tissues of the aquatic plant. To compensate for these changes, macrophytes may adjust their leaf morphology, physiology and resource allocation, potentially altering their vertical structure by promoting vertical expansion and branching in the apical parts (Tóth, 2024). In nutrient-rich conditions, where epiphyte loads are higher, macrophytes may show even more drastic changes, leading to the deterioration of basal parts of the plant.

Light perception involves different parts of the plant (Neff et al., 2000; Kami et al., 2010). However, the local information about the exact quantity and quality of light is perceived, expressed and only affects the growth and morphogenesis of the corresponding plant module at the specific leaf that sensed it (Quail, 2002; de Kroon et al., 2005; Tóth and Palmer, 2016). Consequently, submerged rooted macrophytes develop a morphological and physiological profile typical for their specific environment (Tóth, 2024).

The reduction in macrophyte abundance due to epiphyte overgrowth can have cascading effects on ecosystem services (Strand and Weisner, 1996; Köhler et al., 2010; Tóth, 2013). Submerged macrophytes play a critical role in stabilising sediments, enhancing water clarity, and providing habitat for a range of aquatic organisms. Their decline can lead to increased turbidity, altered nutrient cycling, and the loss of biodiversity, further pushing the system towards a turbid, phytoplankton-dominated state - a hallmark of advanced eutrophication.

Studying the interactions between epiphytes and macrophytes, therefore, provides insight into the broader processes that drive aquatic ecosystem change. As climate change exacerbates eutrophication by increasing nutrient loads and altering hydrological regimes, the need to understand these interactions becomes more urgent. By examining how epiphytes affect macrophyte foliar traits, physiological processes, and ecosystem functions, researchers can identify key factors that influence the resilience of aquatic plant communities. This knowledge is crucial for developing effective management strategies aimed at mitigating the impacts of eutrophication, preserving aquatic biodiversity, and maintaining the ecosystem services provided by submerged macrophytes.

Understanding the response of this underwater biotic framework (scaffold) to environmental conditions is crucial not only for comprehending the resilience and survival of macrophytes

but also for understanding the ecology, interaction and phenology of organisms connected to these macrophytes. In order to assess the effect of epiphytic algae on the foliar traits of *Potamogeton perfoliatus* L. and their plasticity, the morphological and photophysiological characteristics of leaves at a certain depth were determined in different parts of Lake Balaton with different water quality parameters. The main questions were

- which morphological leaf traits of *P. perfoliatus* are affected by epiphytic algae?
- which photophysiological leaf traits of *P. perfoliatus* are affected by epiphytic algae?
- how do studied limnological parameters affected leaf traits of *P. perfoliatus*?

2 Materials and methods

2.1 Lake Balaton

Lake Balaton (Figure 1) is a large (596 km²), shallow (mean depth 3.7 m) freshwater lake located in Central Europe (N 46.83°, E 17.71°). Due to its prevailing length (78 km), the lake can be divided into four basins, each of which has different limnological characteristics. The westernmost, Keszthely basin, historically has experienced eu-hypertrophic conditions at the end of summer, mainly due to considerable nutrient loading in the past. The easternmost, Siófok basin, is predominantly meso-oligotrophic. The prevailing north-westerly wind direction makes the southern shore exposed to waves, while the northern shore remains relatively calm. For convenience and feasibility of the sampling, observations and measurements were made in calm weather with only moderate wavelets on Lake Balaton.

2.2 Study areas

Experiments were conducted at five sites on the northern shore of Lake Balaton (Figure 1) at the phenological peak of the dominant species of Lake Balaton, the claspingleaf pondweed, *Potamogeton perfoliatus* L. (second half of July) in 2022. Site 1 is located in the easternmost meso-oligotrophic basin of Lake Balaton. Sites 2 and 3 are situated in the oligotrophic middle basin of the lake, while sites 4 and 5 are located in the eutrophic western and westernmost basins of the lake.

2.3 Water chemistry analyses

From each site, in the close vicinity of the studied pondweed stands depth-integrated water column samples were collected into one-litre bottles (3 from each site). The bottles were kept in a cooling box to maintain their original temperature and brought to the laboratory for further analysis within 20 minutes.

Total suspended matter (TSM) was measured gravimetrically after filtering the water through a previously dried and pre-weighed GF-5 fibreglass filter (nominal pore size = 0.4 µm), drying for 2 h at 105°C, then weighing again and subtracting the filter weight from the total weight. TSM concentration was calculated based on the predetermined volume of the filtered sample.

Coloured dissolved organic matter (CDOM) concentration was measured spectrophotometrically at 440 nm (Hitachi, U-2900) after filtering the water through a cellulose acetate filter (pore size 0.45 µm), and was expressed as Pt (platinum) units (mg Pt l⁻¹) (Cuthbert and Del Giorgio, 1992).

Chlorophyll-*a* of the water column was determined from freshly collected samples. 1 litre of sampled water was filtered through Whatman GF-5 filter papers, and pigments were analysed following extraction in 60°C methanol and clarification by centrifugation (10 000 rpm for 10 min) (Iwamura et al., 1970).

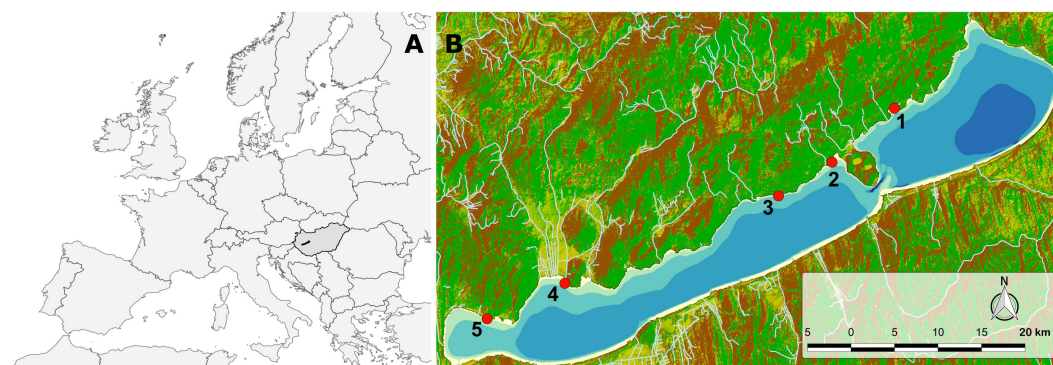


FIGURE 1

(A) Map of Europe showing Hungary (dark grey) and Lake Balaton (black polygon). (B) Topographic map of Lake Balaton and its surroundings, with sampling sites (red dots) along the northern shore. Green areas represent lowlands, yellow areas represent highlands, and brown areas represent mountains. Small tributaries of the lake are shown with white lines. The bathymetric areas of Lake Balaton are depicted with a 1-meter step. The darkest blue polygon in the easternmost part of the lake represents an area with waters deeper than 5 meters.

2.4 Plant measurements

To ensure that the plants collected were of similar ecological status and age, a number of criteria were considered during sampling. At each site, 3–4 relatively large monospecific patches of clasping-leaf pondweed were selected along a 100-metre shoreline. To avoid self-shading, only patches with low to moderate densities (~5–6 plants per m²) were selected. Also, all selected stands were located in wave-exposed areas to reflect typical lake conditions. Sampled plants were located near the maximum depth of colonisation (1.8 m) and to maintain uniformity in plant age, all sampled pondweeds were 1.8 m long, i.e. their apical parts were at the water surface. Only individuals that were at least 1 metre from the edge of their macrophyte patch were sampled.

The sampling was carried out on foot. At each sampling site (as shown in Figure 1B), ~20 intact plants were chosen. One intact leaf was carefully collected from each of these *P. perfoliatus* plants at a depth of approximately 35 cm, roughly corresponding to the apical 25th leaf.

Leaves at a depth of 35 cm were chosen to ensure consistency of developmental stage and to minimise confounding variables related to plant physiology and environmental exposure. At this depth, the leaves sampled were among the youngest mature leaves, not yet entering senescence, but old enough for epiphytic algae to influence their morphological properties during morphogenesis. Previous experience in Lake Balaton has shown that epiphytic colonisation and foliar morphogenesis occurs in the same time: although epiphytic colonisation peaks after about 8 days (Tóth, 2013), leaves at 35 cm depth are 2 weeks old, i.e. biofilm accumulation was in its early stages, and these leaves were without epiphyton for part (probably half) of their development. Furthermore, the selected leaves were not deep enough to be affected by plant self-shading, ensuring that light availability remained a constant factor.

The stem was cut under and over the leaf, and the leaf then was carefully placed in a 20 ml glass vial not fully filled with purified water and sealed tightly. The vials were transported to the laboratory within an hour in the dark.

Upon arrival, in the laboratory, to remove any epiphyton adhering to the leaf surfaces the vials were shaken for 15 minutes. The leaves were then additionally gently washed and then transferred to 20 ml vials filled with filtered Lake Balaton water. The epiphyton removed from the leaves was filtered onto GFC filters (Whatman, USA). The filters were cut into small pieces to enhance the effectiveness of epiphytic algae pigment extraction. Epiphyton pigments present on these filters were extracted with an 80% acetone solution overnight at 4°C. To ensure that all pigments were removed from the filters, two additional extractions were performed in the morning. The supernatant's absorbance was measured with a Shimadzu UV1600 spectrophotometer from Japan, and pigment concentrations were calculated using empirical equations (Wellburn, 1994).

The cleaned leaves were used first for photophysiological measurements and latter to determine their morphological properties. Chlorophyll fluorescence measurements were performed on these leaves within 1 hour of arrival to the

laboratory. During this time the leaves were mostly kept in darkness. It started with the determination of the minimum fluorescence yield (F_0) of the dark-adapted leaves. Subsequently, the maximum fluorescence yield (F_m) was determined in leaves exposed to pulse-saturated light ($\lambda=630$ nm, $I_s=3000$ $\mu\text{mol m}^{-2} \text{s}^{-1}$) using a chlorophyll fluorimeter (PAM-2500, Heinz Walz GmbH, Germany). Following this initial assessment, each leaf was exposed to a sequence of 11 actinic illumination of known intensity ranging from 5 to 787 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($t=15$ s, $\lambda=630$ nm). After each illumination step, both the apparent (F_s) and light-adapted maximum (F_m') fluorescence values were measured with a new saturation pulse ($\lambda=630$ nm, $I_s=3000$ $\mu\text{mol m}^{-2} \text{s}^{-1}$). The apparent electron transport rate (ETR) was then calculated from the data obtained (Table 1) (Bilger and Schreiber, 1987; Schreiber et al., 1995; Schreiber, 1998).

Following the fluorescence measurement, each leaf was digitised using a scanner (CanoScan LiDE 60). Subsequently, from each digitised leaf, a 1 cm (diameter) disc was excised using a core borer. Leaf pigments were then extracted from the leaf discs, utilizing an 80% acetone solution overnight at 4°C. To ensure that all pigments were removed from the leaf disc, two additional extractions were performed in the morning. The absorption of the resulting supernatant was quantified using a spectrophotometer (UV1600, Shimadzu, Japan), and pigment concentrations were calculated by using empirical equations (Wellburn, 1994). The digital scans were processed to extract morphological traits of the leaves. This was done using ImageJ software (<http://rsbweb.nih.gov/ij/>), during which the length, width, area, perimeter, and circularity (ranging from 0 for an infinitely elongated polygon to 1 for a perfect circle) of the leaves were determined.

2.5 Mathematical and statistical analysis

The light response of the ETR was modelled with an exponential saturation curve (Eilers and Peeters, 1988). From the fitted model, the maximum electron transport capacity (ETR_{max}),

TABLE 1 Fluorescence parameters calculated from PAM fluorometry.

parameter	name	equation	reference
F_v/F_m	maximum quantum efficiency of PSII	$(F_m - F_0)/F_m$	(Schreiber, 1998)
qP	photochemical quenching	$(F_m - F_s)/(F_m - F_0)$	(Bilger and Schreiber, 1987)
qN	non-photochemical quenching	$1 - (F_m - F_s)/(F_m - F_0)$	(Bilger and Schreiber, 1987)
ETR	electron transport rate	$(F_m - F_s)/(F_m) \cdot I \cdot AF \cdot 0.5$	(Schreiber et al., 1995)

The equations contain the minimum (F_0) and the maximum (F_m) fluorescence yields, the apparent (F_s) and maximum (F_m') values of fluorescence, the irradiance value (I), and empirical absorption factor ($AF=0.84$). For more information please see referenced literature.

the theoretical saturation light intensity (I_k) and the maximum quantum yield for the entire chain of electron transport (α) were retrieved.

The coefficient of variation was calculated for each specific trait at each sampling site to estimate trait plasticity. This parameter was considered equally important as the trait mean, as it provides insight into the extent of phenotypic flexibility, which is a crucial characteristic from an evolutionary perspective.

Statistical analyses were conducted using the R programming environment (R Development Core Team, 2012). Pearson product-moment correlation was applied to evaluate linear correlations between normally distributed variables, following the verification of normality through Shapiro-Wilk tests, with epiphytic algal chlorophyll concentration as an independent variable and morphological (length, width, area and circularity of leaves), photophysiological (ETR_{max} , I_k , α , F_v/F_m , qP and qN), and pigment (total chlorophyll concentration, chlorophyll a to b ratio, total chlorophyll to carotenoid ratio) parameters as dependent variables. When necessary, transformations were applied.

In preparing the Principal Component Analysis (PCA) plot, the data were first checked for normality and then normalised by centering each variable on its mean and scaling to unit variance. Following this normalisation, PCA was performed to extract the principal components that captured the majority of the variance in the data set. The first two components were then plotted in a biplot to visually represent the relationships between the morphological, photophysiological, pigmentation and limnological parameters. This procedure ensured that the influence of variables with different scales was minimised, thus accurately reflecting the inherent structure of the data.

Generalized additive models (GAM) were used to determine the effects of explanatory variables on epiphyton biomass. For one of the explored relationships the dependent variable was foliar epiphyton, while water chlorophyll-a, TSM, CDOM, and their interaction were used as continuous independent variables. For another explored relationship epiphyton (epi-chla), water chlorophyll-a content (w-chla), water total suspended matter (w-TSM), and water coloured dissolved organic matter (w-CDOM) were the independent, while leaf area, perimeter, circularity, length and width of leaves, the maximum quantum yield for whole-chain electron transport (α), maximum electron transport capacity (ETR_{max}), the theoretical saturation light intensity (I_k), maximum quantum efficiency of PSII (F_v/F_m), non-photochemical quenching (qN), photochemical quenching (qP), total chlorophyll (total chl), chlorophyll a to b ratio (chl_a/chl_b), total chlorophyll to carotenoid ratio (chl/car) were the dependent variables. The models used identity link functions with a Gaussian error distribution, as the data did not exhibit linear relationships. The Gaussian distribution was chosen to account for the continuous nature of the response variables, while allowing flexibility to capture non-linear patterns in the data (Hastie and Tibshirani, 1987; Wood, 2017). Prior to conducting the tests, normality of the data was assessed using the Shapiro-Wilk test. When necessary, transformations were applied. GAM was executed using the mgcv package (Wood, 2017).

3 Results

3.1 Effect of limnological parameters on epiphytic algal content

The water quality of the studied areas, including the amount of phytoplankton, had no significant effect on the amount of epiphytic algal biomass found on *P. perfoliatus* leaves (Table 2, Figure 2). Each parameter had a non-linear, bitonic relationship with the epiphytic algal content, with the highest epiphytic chlorophyll-a content measured at 10.8 $\mu\text{g l}^{-1}$ water chlorophyll-a content, at 12.6 mg l^{-1} total suspended matter and 10.7 mg l^{-1} coloured dissolved organic matter (Figure 2, white circles).

Contrary to this, plasticity of the epiphytic algal content showed monotonic relationship with the studied limnological parameters (Figure 2, red squares). The highest plasticity of the epiphytic algal content was recorded at the lowest values of the studied limnological parameters and the plasticity was gradually decreasing with increase of every studied limnological parameter resulting in Pearson product-moment correlation of $r=-0.94$ ($p=0.019$), $r=-0.84$ (no significance), and $r=-0.97$ ($p=0.005$) for water chlorophyll-a content, total suspended matter and coloured dissolved organic matter, respectively (Figure 2, red squares).

3.2 Effect of epiphytic algal biomass on foliar traits

The coefficient of variation (CV) data obtained from the samples were very different, providing some background information on the relationship between the epiphyton and the morphological and photophysiological parameters studied. The chlorophyll-a content of the epiphyton had the highest CV ($67.2 \pm 14.4\%$), indicating considerable plasticity in the epiphytic algal biomass within the study sites, while the morphological, photophysiological and pigment parameters varied much less. The morphological parameters

TABLE 2 Summary results of the generalized additive models (GAM) for amount of epiphyton (epiphytic algal chlorophyll-a) in the studied sites described by chlorophyll-a content (chl-a), total suspended matter (TSM), and coloured dissolved organic matter (CDOM) of the water.

	Estimate	Std. Error	t value	Pr(> t)
Intercept	4.6313	0.2765	16.75	$<2 \times 10^{-16}$
	edf	Ref.df	F	p-value
s(chl-a)	1.056	1.062	0.012	0.980
s(TSM)	1.056	1.062	0.409	0.598
s(CDOM)	1.783	1.865	2.362	0.111
R-sq.(adj)	0.18			
Deviance explained	21.1%			
GCV	8.4228			

The GCV score is the minimised generalised cross-validation (GCV) score of the GAM fitted.

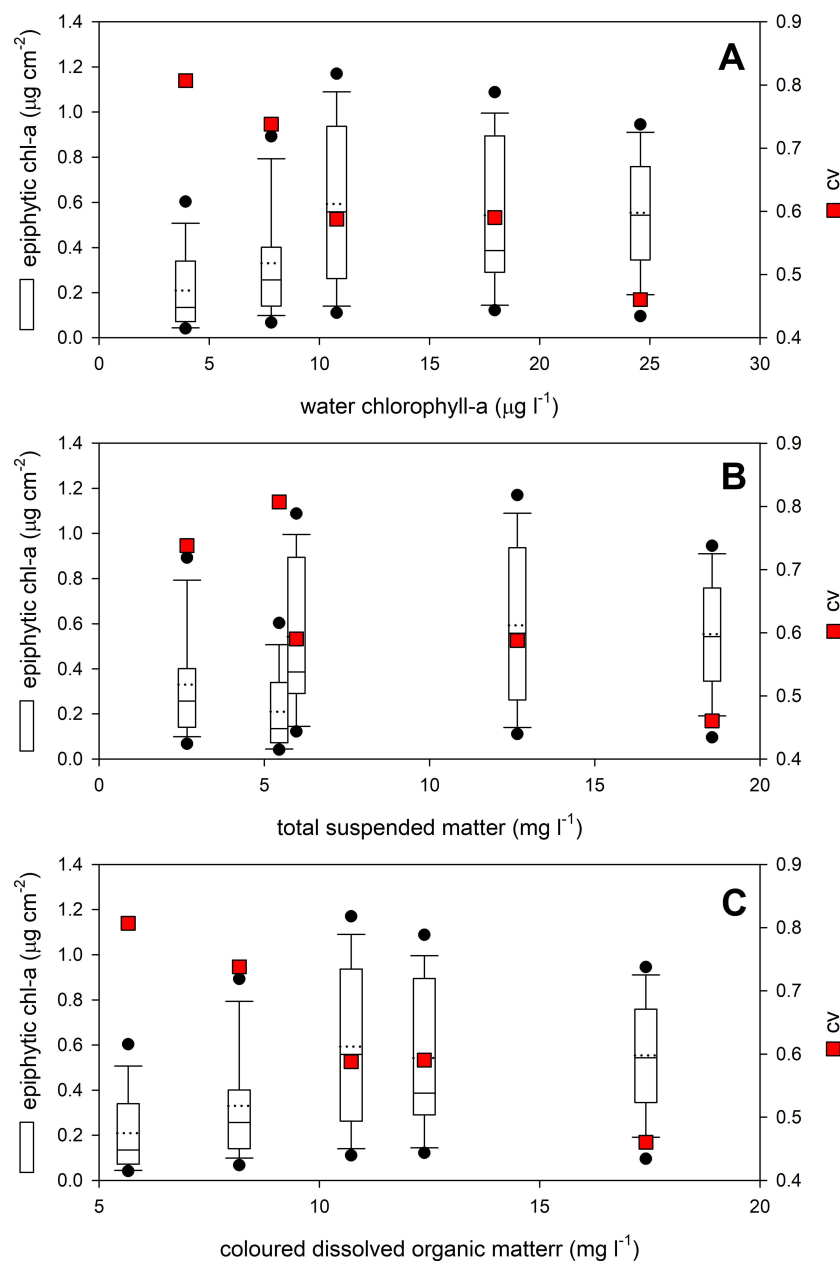


FIGURE 2

Effect of (A) phytoplankton (water chlorophyll-a), (B) total suspended matter and (C) coloured dissolved organic matter on the epiphytic content of *Potamogeton perfoliatus* leaves collected from 35 cm water depth. Data are shown with boxplots, while plasticity of data (cv - coefficient of variation) is shown as red squares.

showed the lowest CV ($17.6 \pm 5.8\%$) plasticity, indicating a more stable response, a consistent structural development across sampling sites, regardless of the differences in epiphytic biomass. The physiological parameters, with a CV of $18.8 \pm 8.4\%$, showed a similar plasticity as the morphological characteristics. Foliar pigment parameters had a CV of $25.3 \pm 7.5\%$ (mainly due to the high plasticity of the chlorophyll:carotenoid ratio ($\sim 32\%$)). This suggests that pigment adjustments, possibly related to changes in light conditions due to epiphyton shading, were somewhat variable, but less so than the epiphyton biomass itself.

Epiphyton biomass accumulated on the of *P. perfoliatus* leaves had little effect on leaf morphology (Figure 3, Table 3). The data indicate a moderate positive linear relationship between epiphyton biomass and leaf length in *P. perfoliatus* (Pearson product moment correlation coefficient $r=0.397$, $p<0.001$), resulting in 23% increase of leaf length, suggesting that the conditions of epiphyton biomass accumulation are also favourable for leaf longitudinal growth, while other morphological parameters (leaf width, leaf area, leaf circularity and leaf perimeter) were not affected in the same way (Figure 3).

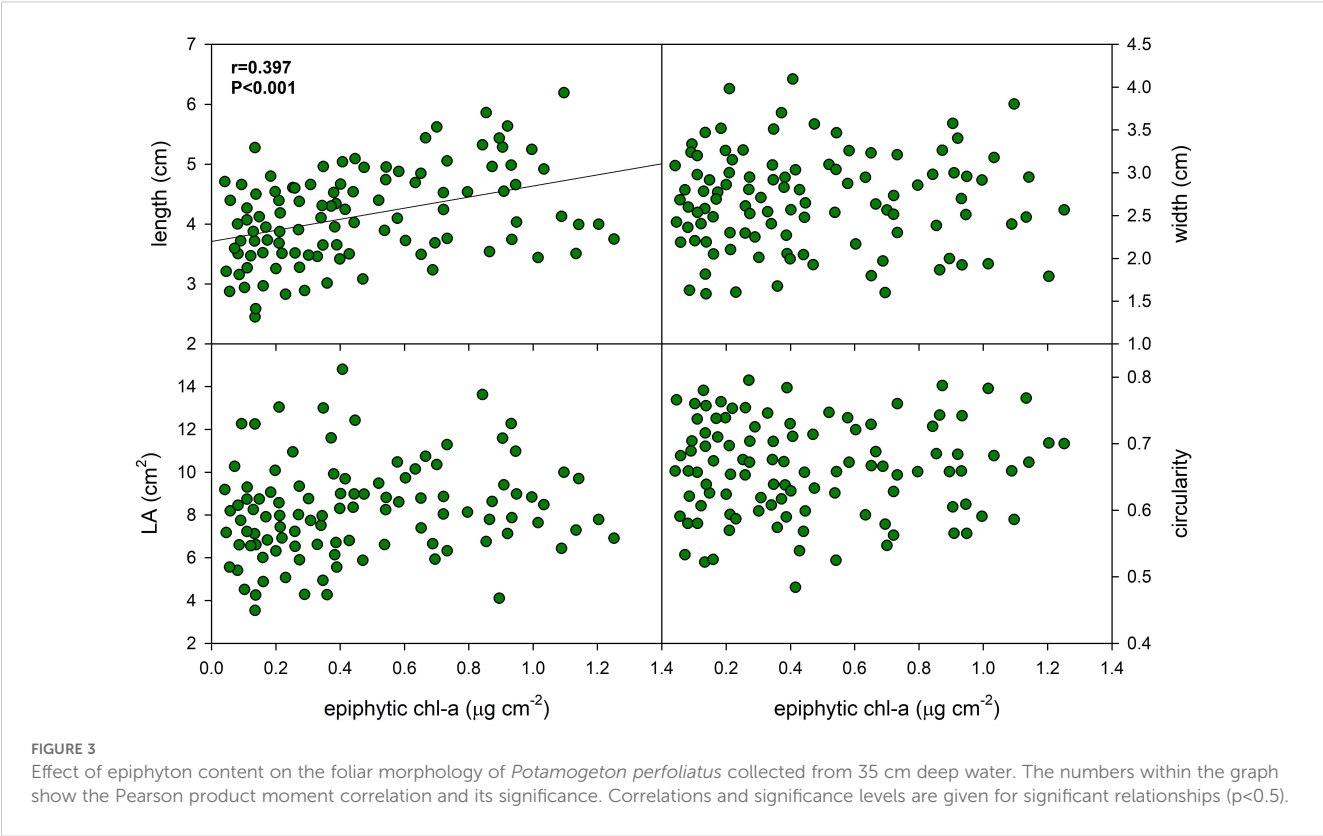


TABLE 3 Summary results of the generalized additive models (GAM) for the foliar morphological and photophysiological properties of *Potamogeton perfoliatus* at 35 cm depth affected by the amount of epiphyton (epi-chla), water chlorophyll-a content (w-chla), water total suspended matter (w-TSM), and water coloured dissolved organic matter (w-CDOM) of the studied sites.

	intercept	epi-chla	w-chla	w-TSM	w-CDOM
leaf area	2.92**	2.02*	-1.67	-0.14	1.14
perimeter	6.56***	1.34	-0.38	-0.81	0.29
circularity	8.27***	0.27	-0.22	0.28	0.02
length	4.64***	4.20***	-0.93	-0.52	0.79
width	4.08***	-0.01	-0.67	-0.84	0.74
α	5.36***	4.86***	0.05	-0.27	-0.03
ETR _{max}	2.62*	-3.72***	-2.87**	-3.03**	2.92**
I _k	0.85	-4.17***	-3.22**	-3.34**	3.34**
F _v /F _m	10.75***	2.93**	1.03	0.29	-0.87
qN	11.73***	-1.18	0.65	0.22	-0.58
qP	1.83	-4.52***	-3.09**	-2.63**	3.03**
total chl	6.35***	0.73	1.51	1.78	-1.64
chl _a /chl _b	3.29**	-3.32**	-1.00	-0.47	0.93
chl/car	3.13**	-1.87	-0.45	0.12	0.47

Data showed the t value of the test and its significance. The shown parameters are the leaf area, perimeter, circularity, length and width of leaves, the maximum quantum yield for whole-chain electron transport (α), maximum electron transport capacity (ETR_{max}), the theoretical saturation light intensity (I_k), maximum quantum efficiency of PSII (F_v/F_m), non-photochemical quenching (qN), photochemical quenching (qP), total chlorophyll (total chl), chlorophyll a to b ratio (chl_a/chl_b), total chlorophyll to carotenoid ratio (chl/car). The significance of the GAM tests: * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

The results suggest that epiphyton biomass had a significant, but moderate effect on several aspects of the photophysiology of *P. perfoliatus* leaves, affecting the initial slope of the photosynthesis-light curve (α), the minimum saturation irradiance (I_k) and the maximum relative electron transport rate (ETR_{max}), the maximum efficiency of PSII (F_v/F_m) and the photochemical quenching (qP) of PSII (Figure 4, Table 3). ETR_{max} , I_k and qP were negatively correlated, indicating a decrease in maximum electron transport rate (37.5%), minimum saturation irradiance (62.7%) and photochemical quenching (59.4%) with increasing epiphyton biomass. Meanwhile, α and F_v/F_m showed a positive increase of 24.5% and 9.7%, respectively, with an increase in leaf epiphyton biomass (Figure 4).

Epiphyton biomass exhibited a low, though statistically significant negative correlation (Pearson product moment correlation: $r = -0.294$, $p = 0.002$) with the Chl-a/Chl-b ratio in *P. perfoliatus* leaves, indicating a decrease in the ratio of chlorophyll-a to chlorophyll-b with higher epiphyton biomass (Figure 5, Table 3). Removing the outliers (chl a/b > 4.5 and < 1.5) did not substantially reduce the Pearson product-moment correlation, which remained significant ($r = -0.246$, $p = 0.015$). However, epiphyton biomass did not show a statistically significant effect on total chlorophyll content or the chlorophyll:carotenoid ratio in the data set examined.

Principal component analysis (Figure 6) revealed that the differentiation between sampling sites was primarily driven by

differences in periphyton biomass, total suspended matter (TSM) and coloured dissolved organic matter (CDOM). These factors had the greatest influence on site separation. In addition, physiological parameters such as maximum electron transport rate (ETR_{max}), light saturation point (I_k) and photochemical quenching (qP), as well as pigment properties, in particular the chlorophyll a:b (Chl-a/Chl-b) ratio and the chlorophyll:carotenoid ratio, contributed to the differentiation. These physiological and pigmentary traits, although secondary in influence to periphyton and limnological parameters, also played a role in the observed separation of the sites, indicating their relevance in characterising foliar traits and environmental conditions across sites (Figure 6).

The PCA performed only on the morphological, photophysiological and pigment content parameters of the studied *P. perfoliatus* leaves did not significantly distinguish the studied sites in Lake Balaton (Figure 7), suggesting that the lack of territorial response shows a core species-level response to epiphytic colonisation.

The relations shown on Table 3 suggest that epiphyton accumulated on the leaf surface of *P. perfoliatus* is associated with certain aspects of leaf morphology (weakly positive with leaf length and area). Certain photophysiological parameters (positive with α , F_v/F_m and negative with ETR_{max} , I_k and qP) and certain parameters of leaf pigment content (negative with Chl-a/Chl-b

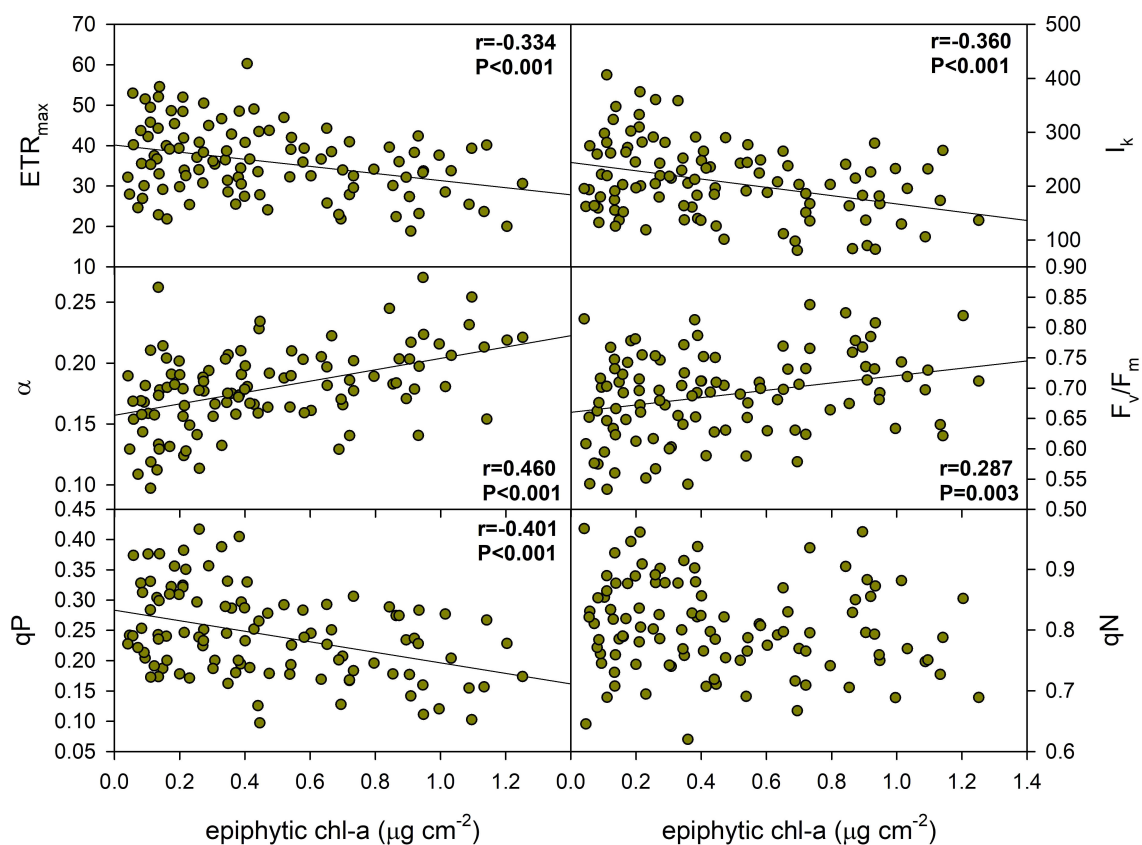


FIGURE 4

Effect of epiphyton on the photochemical properties of *Potamogeton perfoliatus* collected from 35 cm deep water. The numbers within the graph show the Pearson product moment correlation and its significance. Correlations and significance levels are given for significant relationships ($p < 0.5$).

ratio) were also associated by the GAM with epiphyton biomass (Table 3).

4 Discussion

From a meteorological and limnological point of view, the study year (2022) at Lake Balaton was not considered exceptional. No

local algal blooms were observed at the study sites in the weeks prior to the survey, and no major storms occurred in July. While environmental background of epiphyton accumulation is complex and is often influenced by factors such as nutrient levels, light availability, and the composition of dissolved organic matter (DOM) (Aizaki and Sakamoto, 1988; Frost et al., 2007), this work found no significant correlation between the major limnological parameters, including the amount of phytoplankton in the water,

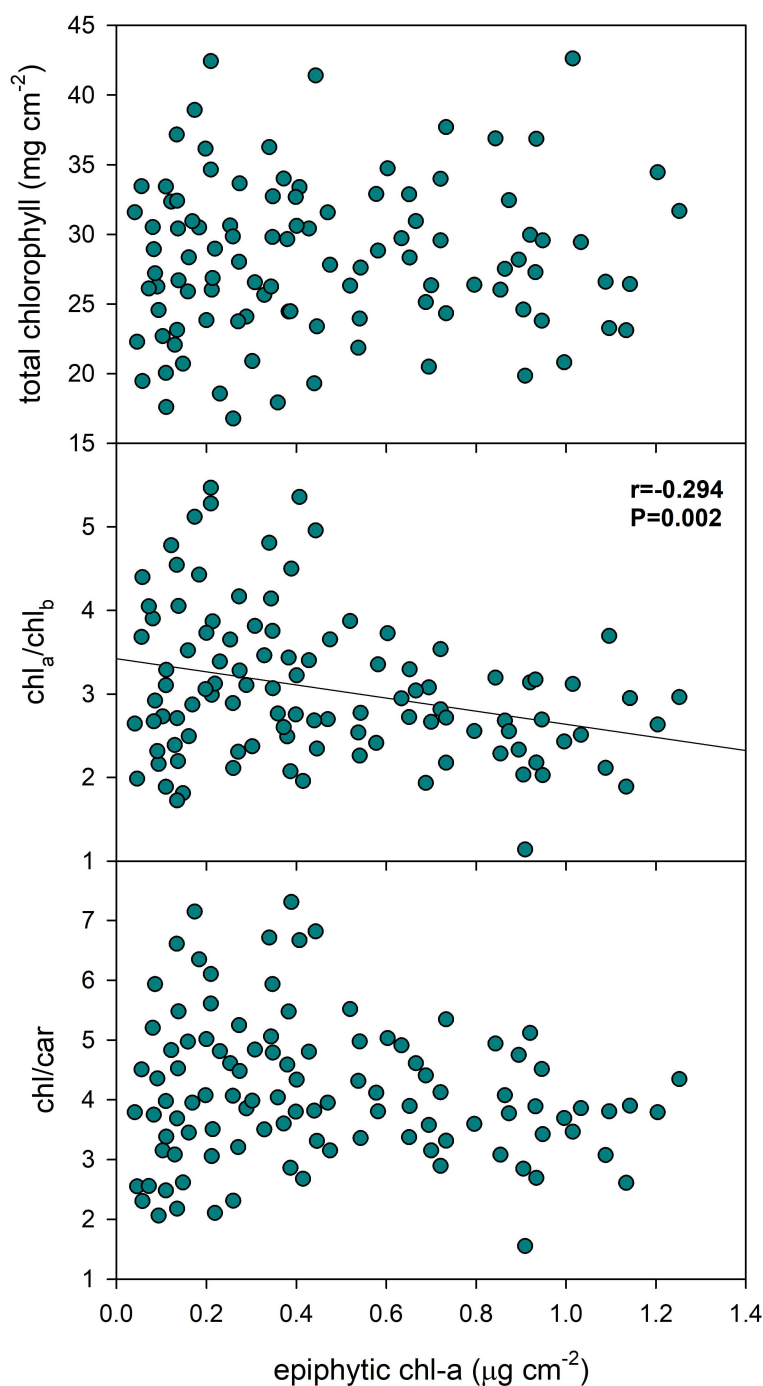


FIGURE 5

Effect of epiphyton on the pigment content of *Potamogeton perfoliatus* leaves collected from 35 cm deep water. The numbers within the graph show the Pearson product moment correlation and its significance. Correlations and significance levels are given for significant relationships ($p < 0.5$).

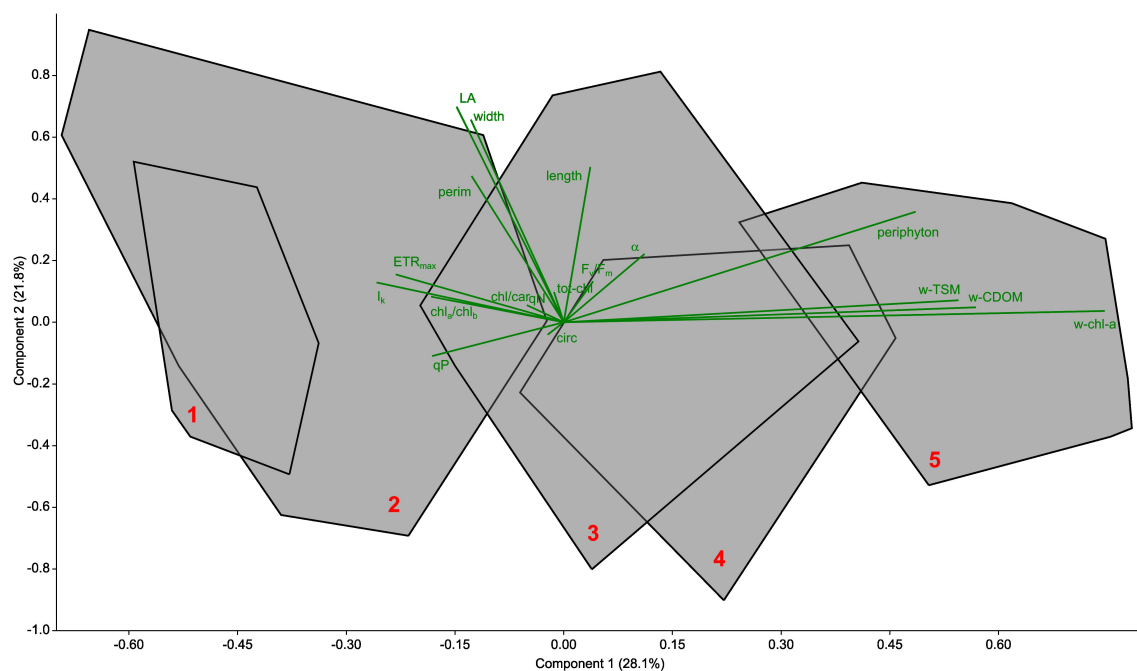


FIGURE 6

Principal component analysis (PCA) biplot of all parameters determined from the Lake Balaton sampling. Data from each site are grouped into a convex hull (data points not shown) and marked with a red number. The biplot is shown in green. The biplot shows the studied limnological (wTSM – total suspended matter of the water, wCDOM – coloured dissolved organic matter of the water, wchl-a – chlorophyll-a content of the water), morphological (length, width, LA – area, perim – perimeter and circ – circularity of the leaves), photophysiological (ETR_{max} – the maximum electron transport capacity, I_k – the theoretical saturation light intensity, the α – maximum quantum yield for the entire chain of electron transport, F_v/F_m – maximum quantum efficiency of PSII, qP – photochemical quenching, qN – non-photochemical quenching), pigment (totchl – total chlorophyll concentration, chl_a/chl_b – chlorophyll a to b ratio, chl/car – total chlorophyll to carotenoid ratio) and periphytic (periphyton – epiphytic chlorophyll-a content) parameters.

and epiphytic algal biomass in the studied sites of Lake Balaton. This lack of relationship further confirmed the non-linear association between water quality and periphyton accumulation as previously reported (Hansson, 1992; Lowe, 1996; Liboriussen and Jeppesen, 2003). At low values of the limnological parameters studied, the amount of epiphytic algae remained low and increased only up to a certain threshold (water chl-a at $18 \mu\text{g l}^{-1}$, TSM at 15 mg l^{-1} and CDOM at 14 mg l^{-1}), beyond which other factors, such as light attenuation, influenced the relationship and changed its nature.

For the epiphyte, the submerged macrophyte is mostly a substrate, an available and useful structure to be exploited. The accumulation of epiphyton on the surface of broad-leaved submerged macrophyte leaves can have both direct (light attenuation, nutrient competition) and indirect (community restructuring, light quality filtering, etc.) effects (Brandt and Koch, 2003; Tóth, 2013; Tóth and Palmer, 2016; Wijewardene et al., 2022). Although only one component of the epiphyton, the epiphytic algae, was quantified in this study, the relatively young age of the leaves was chosen on the assumption that only algae had colonised the leaves. In reality, no prior information on this aspect was available at the time, as studies on the dynamics of epiphyton accumulation in Lake Balaton are still lacking. Nevertheless, this study showed direct evidence that epiphytic algal content is affecting foliar traits of pondweeds in Lake Balaton. Although the

observed relationships were weak, some correlations were as high as 0.45 and explained nearly 25% of variance, a clear association between epiphytic algal content and the studied leaf traits of pondweed was evident.

It was shown that, apart from contributing to an increase in leaf length, epiphytic biofilms had no significant effect on the leaf morphology of the studied pondweeds. This finding is partially consistent with previous studies indicating that low light conditions can induce compensatory changes in leaf morphology (Goldsborough and Kemp, 1988; Tóth et al., 2011). The robust and time-intensive nature of leaf photomorphogenesis may underlie this phenomenon, as the younger, developing leaves – located at the apical part of the pondweed – were able to respond to changing environmental conditions. Over time, epiphyte colonisation probably altered the initial conditions, leading to secondary morphogenetic transformations. These secondary changes may have counteracted the primary responses, ultimately resulting in the observed weak correlations between epiphyton density and leaf morphological traits.

Pigment photomorphogenesis is a rapid but long-lasting (from days to weeks) response to various environmental signals (Barko and Filbin, 1983; Horppila et al., 2022; Tóth, 2024): chlorophyll molecules are continuously synthesised and degraded within chloroplasts, while their lifetime depends on factors such as light exposure, temperature and plant age. This study found no

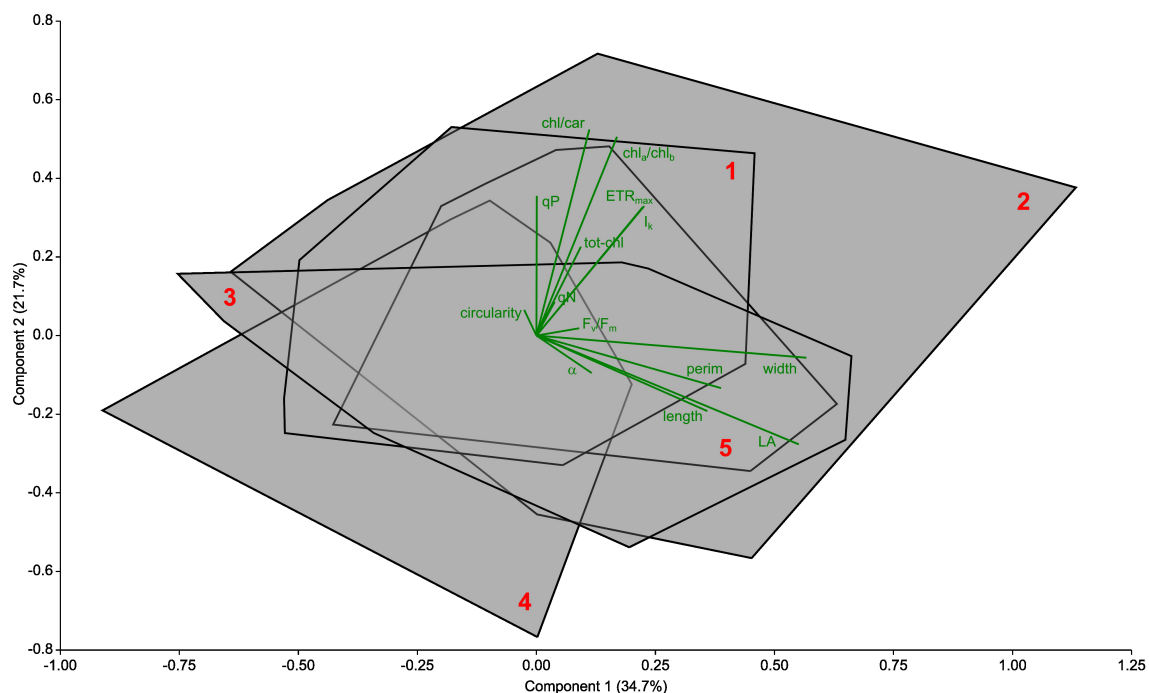


FIGURE 7

Principal component analysis (PCA) biplot of the determined foliar morphological, photochemical and pigment parameters collected from *Potamogeton perfoliatus* plants at 35 cm deep waters of Lake Balaton. Data from each site are grouped into a convex hull (data points not shown) and marked with red number. Trait biplot show in green colour. The biplot shows the studied morphological (length, width, LA – area, perim – perimeter and circ – circularity of the leaves), photophysiological (ETR_{max} – the maximum electron transport capacity, I_k – the theoretical saturation light intensity, the α – maximum quantum yield for the entire chain of electron transport, F_v/F_m – maximum quantum efficiency of PSII, qP – photochemical quenching, qN – non-photochemical quenching) and pigment (totchl – total chlorophyll concentration, chl_a/chl_b – chlorophyll a to b ratio, chl/car – total chlorophyll to carotenoid ratio) parameters. No significant differences detected ($P > 0.05$).

correlation between the amount of epiphytic algae and the total chlorophyll content or chlorophyll to carotenoid ratio of the leaves. However, a weak negative correlation was observed between the epiphyton found on the leaves and the Chl-a/Chl-b ratio of these leaves: the leaves with no, or minimal epiphyton had 25% more chlorophyll-a molecules, compared to leaves with high epiphytic algal biofilm. This response mechanism of leaf pigments to epiphyton could be connected to adaptive biochemical adjustments in pigment composition as a response to spectral changes of light reaching the photosystem of leaves (Tóth and Palmer, 2016; Tóth, 2024), since in low light environments, plants often increase the size of their light-harvesting antennae to capture more light, thereby adjusting the chlorophyll a:b ratio (Dale and Causton, 1992; Murchie and Horton, 1997; Beneragama and Goto, 2010).

Changes in foliar photophysiological traits require several complex metabolic pathways, but are easily reversed and thus may be part of the short-term, fast-response acclimation tactics of submerged macrophytes. The photophysiological responses to epiphytic algae were more pronounced, as 83% of the traits studied showed a relationship to epiphytic algae, whereas only 40% of morphological and 33% of pigment traits responded to the same conditions, although it cannot be excluded that these effects result from the combined influence of the entire epiphyton

community. The negative correlations observed for ETR_{max} , I_k and qP and the positive correlations observed for α and F_v/F_m suggest that higher epiphyton biomass may affect the photosynthetic efficiency and capacity of leaves, possibly due to light attenuation. Not only the magnitude of photophysiological responses (83%) to epiphyton was substantial, but the photophysiological traits studied also showed high plasticity of responses (27% on average) to epiphytic algal content compared to the plasticity of morphological (11% on average) and pigment (13% on average) traits, demonstrating the immediate involvement of the Photosystem II complex in the process. Therefore, the morphological and pigment responses could be estimated to be used as an adaptive response of plants, whereas photophysiological responses are faster, more plastic and cost-effective way to acclimate to changing environmental conditions. The whole framework for understanding the differential responses of macrophytes to epiphytic algal abundance is based on the interplay between immediate plasticity and strategic adaptive responses in shaping macrophyte fitness and ecological resilience.

Regarding the mechanism of action, in the case of submerged rooted macrophytes, which take up nutrients mainly from the sediment and where the effect of epiphyton on the nutrient content of the foliage could be considered negligible, the primary mechanism of action of epiphyton in the case of pondweeds is

through reduced light penetration, potentially limiting the photosynthetically available leaf area, limiting gas exchange and affecting photosynthesis in general (Tóth, 2013; Klančnik et al., 2015). Epiphyton especially its algal component can impair leaf physiology, thus changing the survival strategies of macrophytes, and could be the first factor resulting in the disappearance of macrophyte during eutrophication (Scheffer and van Nes, 2007; Tóth and Palmer, 2016). Epiphyton may also affect macrophytes by altering the spectral composition of light reaching the macrophyte leaf surface. Biofilm structure and pigmentation selectively filter incident light, potentially attenuating more ultraviolet (UV) radiation while allowing the transmission of photosynthetically active radiation (PAR) (Tóth, 2013). This selective filtering could act as a protective mechanism, reducing photoinhibition and photodamage under high light conditions, which may be relevant in shallow and near-surface waters. In addition to modulating light availability, epiphytes may also influence the local availability of inorganic carbon to macrophytes. The presence of epiphytic biofilms could modify the local carbon environment by affecting diffusion dynamics and altering the carbonate balance at the leaf surface. This modification could occur through metabolic activities such as respiration and photosynthesis within the biofilm matrix, which could create microgradients of dissolved inorganic carbon (DIC) (Sand-Jensen et al., 1985; Madsen et al., 1993). These complex interactions should also be considered when assessing the ecological role of epiphyton in macrophyte physiology and growth.

This study investigated the effect of epiphyton on foliar characteristics of submerged rooted macrophytes. Despite the influence of other seemingly more important factors such as herbivory, light availability, water clarity, species composition and macrophyte biomass, the weak but significant effects of epiphyton on plant physiology and morphology highlight its importance in the functioning and biodiversity of the littoral zone. Although epiphyton encompasses more than photosynthetic algae, this study specifically assessed the effects of epiphytic algae and may have underestimated the wider importance of epiphyton. It was shown that aquatic plants can employ a wide range of adaptive responses to ensure their survival, depending on the prevailing conditions. The response to epiphyton colonisation varied from significant to negligible, highlighting the need to consider this interaction to fully understand the littoral zone. These results highlight the importance of integrated lake management and environmental protection strategies that take into account the complex interactions between epiphytes and submerged rooted macrophytes. Recognising the dynamic nature of these interactions and their effects on habitat structure, biodiversity and ecosystem functioning could guide management efforts to mitigate

anthropogenic impacts and enhance the resilience and sustainability of aquatic environments.

Data availability statement

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

Author contributions

VT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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References

- Aizaki, M., and Sakamoto, K. (1988). Relationship between water quality and periphyton biomass in several streams in Japan: With 5 figures and 3 tables in the text. *Intern. Vereinigung für theoretische und angewandte Limnol.: Verhandlungen* 23, 1511–1517. doi: 10.1080/03680770.1987.11898054

- Asaeda, T., Sultana, M., Manatunge, J., and Fujino, T. (2004). The effect of epiphytic algae on the growth and production of *Potamogeton perfoliatus* L. @ in two light conditions. *Environ. Exp. Bot.* 52, 225–238. doi: 10.1016/j.envexpbot.2004.02.001
- Barko, J. W., and Filbin, G. J. (1983). Influences of light and temperature on chlorophyll composition in submersed freshwater macrophytes. *Aquat. Bot.* 15, 249–255. doi: 10.1016/0304-3770(83)90072-4
- Beneragama, C., and Goto, K. (2010). Chlorophyll a: b ratio increases under low-light in 'shade-tolerant' *Euglena gracilis*. *Trop. Agric. Res.* 22, 12–25. doi: 10.4038/tar.v22i1.2666
- Bilger, W., and Schreiber, U. (1986). Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. *Photosynth. Res.* 10, 303–308. doi: 10.1007/BF00118295
- Blindow, I., Hargeby, A., and Hilt, S. (2014). Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. *Hydrobiologia* 737, 99–110. doi: 10.1007/s10750-013-1687-2
- Brandt, L. A., and Koch, E. W. (2003). Periphyton as a UV-B filter on seagrass leaves: a result of different transmittance in the UV-B and PAR ranges. *Aquat. Bot.* 76, 317–327. doi: 10.1016/S0304-3770(03)00067-6
- Cuthbert, I. D., and Del Giorgio, P. (1992). Toward a standard method of measuring color in freshwater. *Limnol. Oceanogr.* 37, 1319–1326. doi: 10.4319/lo.1992.37.6.1319
- Dale, M., and Causton, D. (1992). Use of the chlorophyll a/b ratio as a bioassay for the light environment of a plant. *Funct. Ecol.* 6, 190–196. doi: 10.2307/2389754
- de Kroon, H., Huber, H., Stuefer, J. F., and van Groenendaal, J. M. (2005). A modular concept of phenotypic plasticity in plants. *New Phytol.* 166, 73–82. doi: 10.1111/j.1469-8137.2004.01310.x
- Dos Santos, T. R., Ferragut, C., and de Mattos Bicudo, C. E. (2013). Does macrophyte architecture influence periphyton? Relationships among *Utricularia foliosa*, periphyton assemblage structure and its nutrient (C, N, P) status. *Hydrobiologia* 714, 71–83. doi: 10.1007/s10750-013-1531-8
- Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thébault, E., and Loreau, M. (2007). The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecol. Lett.* 10, 522–538. doi: 10.1111/j.1461-0248.2007.01037.x
- Eilers, P. H. C., and Peeters, J. C. H. (1988). A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol. Model.* 42, 199–215. doi: 10.1016/0304-3800(88)90057-9
- Frost, P. C., Cherrier, C. T., Larson, J. H., Bridgman, S., and Lamberti, G. A. (2007). Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton. *Freshw. Biol.* 52, 319–330. doi: 10.1111/j.1365-2427.2006.01696.x
- Goldsborough, W. J., and Kemp, W. M. (1988). Light responses of a submersed macrophyte: implications for survival in turbid tidal waters. *Ecology* 69, 1775–1786. doi: 10.2307/1941156
- Hansson, L.-A. (1992). Factors regulating periphytic algal biomass. *Limnol. Oceanogr.* 37, 322–328. doi: 10.4319/lo.1992.37.2.0322
- Hastie, T., and Tibshirani, R. (1987). Generalized additive models: some applications. *J. Am. Stat. Assoc.* 82, 371–386. doi: 10.1080/01621459.1987.10478440
- Hill, W. (1996). "Effects of Light," in *Algal Ecology* (Academic Press, San Diego), 121–148.
- Horppila, J., Pippingsköld, E., and Estlander, S. (2022). Effects of water colour on the pigment content of a floating-leaved macrophyte—Implications of lake brownification. *Aquat. Bot.* 181, 103540. doi: 10.1016/j.aquabot.2022.103540
- Iwamura, T., Nagai, H., and Ichimura, S. E. (1970). Improved methods for determining contents of chlorophyll, protein, ribonucleic acid, and deoxyribonucleic acid in planktonic populations. *Intern. Rev. der gesamten Hydrobiol. und Hydrogr.* 55, 131–147. doi: 10.1002/iroh.19700550106
- Kami, C., Lorrain, S., Hornitschek, P., and Fankhauser, C. (2010). Chapter two-light-regulated plant growth and development. *Curr. topics Dev. Biol.* 91, 29–66. doi: 10.1016/S0070-2153(10)91002-8
- Kirk, J. T. O. (1994). *Light and Photosynthesis in Aquatic Ecosystems* (Cambridge: Cambridge University Press).
- Klančnik, K., Gradinjan, D., and Gaberščik, A. (2015). Epiphyton alters the quantity and quality of radiation captured by leaves in submersed macrophytes. *Aquat. Bot.* 120, 229–235. doi: 10.1016/j.aquabot.2014.07.007
- Köhler, J., Hachol, J., and Hilt, S. (2010). Regulation of submersed macrophyte biomass in a temperate lowland river: Interactions between shading by bank vegetation, epiphyton and water turbidity. *Aquat. Bot.* 92, 129–136. doi: 10.1016/j.aquabot.2009.10.018
- Kovalenko, K. E., Thomaz, S. M., and Warfe, D. M. (2012). Habitat complexity: approaches and future directions. *Hydrobiologia* 685, 1–17. doi: 10.1007/s10750-011-0974-z
- Kuczynska-Kippen, N. (2008). Spatio-temporal segregation of cladocerans within a *Chara hispida* bed. *J. Freshw. Ecol.* 23, 643–650. doi: 10.1080/02705060.2008.9664252
- Liboriussen, L., and Jeppesen, E. (2003). Temporal dynamics in epipellic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. *Freshw. Biol.* 48, 418–431. doi: 10.1046/j.1365-2427.2003.01018.x
- Liboriussen, L., and Jeppesen, E. (2006). Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. *Freshw. Biol.* 51, 95–109. doi: 10.1111/j.1365-2427.2005.01481.x
- Lowe, R. (1996). "Periphyton patterns in lakes," in *Algal Ecology: Freshwater benthic ecosystems*. Eds. R. Stevenson, M. Bothwell and R. Lowe (San Diego: Academic Press), 57–76.
- Madsen, T., Sand-Jensen, K., and Beer, S. (1993). Comparison of photosynthetic performance and carboxylation capacity in a range of aquatic macrophytes of different growth forms. *Aquat. Bot.* 44, 373–384. doi: 10.1016/0304-3770(93)90078-B
- McAbendroth, L., Ramsay, P. M., Foggo, A., Rundle, S. D., and Bilton, D. T. (2005). Does macrophyte fractal complexity drive invertebrate diversity, biomass and body size distributions? *Oikos* 111, 279–290. doi: 10.1111/j.0030-1299.2005.13804.x
- Murchie, E., and Horton, P. (1997). Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. *Plant Cell Environ.* 20, 438–448. doi: 10.1046/j.1365-3040.1997.d01-95.x
- Neff, M. M., Fankhauser, C., and Chory, J. (2000). Light: an indicator of time and place. *Genes Dev.* 14, 257–271. doi: 10.1101/gad.14.3.257
- Özen, A., Tavşanoğlu, Ü. N., Çakıroğlu, A. İ., Levi, E. E., Jeppesen, E., and Beklioğlu, M. (2018). Patterns of microbial food webs in Mediterranean shallow lakes with contrasting nutrient levels and predation pressures. *Hydrobiologia* 806, 13–27. doi: 10.1007/s10750-017-3329-6
- Quail, P. H. (2002). Phytochrome photosensory signalling networks. *Nat. Rev. Mol. Cell Biol.* 3, 85–93. doi: 10.1038/nrm728
- R Development Core Team (2012). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: <http://www.R-project.org/> (Accessed April 14, 2025).
- Sand-Jensen, K., Revsbech, N. P., and Barker Jørgensen, B. (1985). Microprofiles of oxygen in epiphyte communities on submersed macrophytes. *Mar. Biol.* 89, 55–62. doi: 10.1007/BF00392877
- Sand-Jensen, K., and Søndergaard, M. (1981). Phytoplankton and epiphyte development and their shading effect on submersed macrophytes in lakes of different nutrient status. *Intern. Rev. der gesamten Hydrobiol. und Hydrogr.* 66, 529–552. doi: 10.1002/iroh.19810660406
- Scheffer, M., Hosper, S., Meijer, M., Moss, B., and Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends Ecol. Evol.* 8, 275–279. doi: 10.1016/0169-5347(93)90254-M
- Scheffer, M., and van Nes, E. H. (2007). Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584, 455–466. doi: 10.1007/s10750-007-0616-7
- Schreiber, U. (1998). Chlorophyll fluorescence: new instruments for special applications. *Photosynth. Mech. Effects* 5, 4253–4258. doi: 10.1007/978-94-011-3953-3_984
- Schreiber, U., Endo, T., Mi, H., and Asada, K. (1995). Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol.* 36, 873–882. doi: 10.1093/oxfordjournals.pcp.a078833
- Søndergaard, M., Johansson, L. S., Lauridsen, T. L., Jørgensen, T. B., Liboriussen, L., and Jeppesen, E. (2010). Submersed macrophytes as indicators of the ecological quality of lakes. *Freshw. Biol.* 55, 893–908. doi: 10.1111/j.1365-2427.2009.02331.x
- Strand, J. A., and Weisner, S. E. B. (1996). Wave exposure related growth of epiphyton: implications for the distribution of submersed macrophytes in eutrophic lakes. *Hydrobiologia* 325, 113–119. doi: 10.1007/BF00028271
- Sultana, M., Asaeda, T., Ekram Azim, M., and Fujino, T. (2010). Morphological responses of a submersed macrophyte to epiphyton. *Aquat. Ecol.* 44, 73–81. doi: 10.1007/s10452-009-9291-2
- Takamura, N., Kadono, Y., Fukushima, M., Nakagawa, M., and Kim, B.-H. (2003). Effects of aquatic macrophytes on water quality and phytoplankton communities in shallow lakes. *Ecol. Res.* 18, 381–395. doi: 10.1046/j.1440-1703.2003.00563.x
- Thomaz, S. M. (2023). Ecosystem services provided by freshwater macrophytes. *Hydrobiologia* 850, 2757–2777. doi: 10.1007/s10750-021-04739-y
- Thomaz, S. M., Dibble, E. D., Evangelista, L. R., Higuity, J., and Bini, L. M. (2008). Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshw. Biol.* 53, 358–367. doi: 10.1111/j.1365-2427.2007.01898.x
- Tóth, V. R. (2013). The effect of periphyton on the light environment and production of *Potamogeton perfoliatus* L. in the mesotrophic basin of Lake Balaton. *Aquat. Sci.* 75, 523–534. doi: 10.1007/s00027-013-0297-4
- Tóth, V. R. (2024). Vertical optical complexity shaped by submersed macrophytes. *Sci. Rep.* 14, 5100. doi: 10.1038/s41598-024-55824-w
- Tóth, V. R., and Palmer, S. C. (2016). Acclimation of *Potamogeton perfoliatus* L. @ to periphyton accumulation-induced spectral changes in irradiance. *Hydrobiologia* 766, 293–304. doi: 10.1007/s10750-015-2462-3
- Tóth, V. R., Vári, Á., and Luggosi, Sz. (2011). Morphological and photosynthetic acclimation of *Potamogeton perfoliatus* to different environments in Lake Balaton. *Oceanol. Hydrobiol. Stud.* 40, 43–51. doi: 10.2478/s13545-011-0028-1
- Vis, C., Hudon, C., and Carignan, R. (2006). Influence of the vertical structure of macrophyte stands on epiphyte community metabolism. *Can. J. Fish. Aquat. Sci.* 63, 1014–1026. doi: 10.1139/f06-021

Wellburn, A. R. (1994). The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol. Jena* 144, 307–313. doi: 10.1016/S0176-1617(11)81192-2

Wetzel, R. G. (1975). *Limnology* (Philadelphia, London, and Toronto: Saunders Philadelphia).

Wijewardene, L., Wu, N., Fohrer, N., and Riis, T. (2022). Epiphytic biofilms in freshwater and interactions with macrophytes: Current understanding and future directions. *Aquat. Bot.* 176, 103467. doi: 10.1016/j.aquabot.2021.103467

Wood, S. N. (2017). *Generalized additive models: an introduction with R* (New York: CRC Press).

Worm, B., and Sommer, U. (2000). Rapid direct and indirect effects of a single nutrient pulse in a seaweed-epiphyte-grazer system. *Mar. Ecol. Prog. Ser.* 202, 283–288. doi: 10.3354/meps202283

Yuan, G., Tan, X., Guo, P., Xing, K., Chen, Z., Li, D., et al. (2023). Linking trait network to growth performance of submerged macrophytes in response to ammonium pulse. *Water Res.* 229, 119403. doi: 10.1016/j.watres.2022.119403



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Changing environmental conditions impact the phenotypic plasticity of *Carex acuta* and *Glyceria maxima*, two common wet grassland species

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Introduction: Maintenance of species coexistence is an important and on-going subject of plant ecology. Here, we aimed to determine how *Carex acuta* and *Glyceria maxima*, two common, co-occurring plant species in European wet grasslands, respond to changing environmental conditions and what these changes portend for coexistence of these two species. Such studies are important for predicting and modelling the effects of management and climate change on wet grassland plant species composition and for maintaining the ability of wet grasslands to provide their important ecosystem services including carbon sequestration and water purification. Based on past studies, we hypothesized that both species would be affected by hydrologic changes but that these effects would be modified by nutrient conditions with fertilization having a more positive impact on *G. maxima*.

Methods: We established a mesocosm to distinguish the effect of hydrology and nutrients on the biomass allocation patterns of these two species to determine how environmental conditions may impact the life history traits of these two species, which would influence their ability to co-exist. Plants were grown in pots from late May to early September 2019 and subjected to two nutrient and three water level treatments. Half of the plants were harvested in July while the other half were harvested in early September and their biomass allocation patterns calculated. Univariable and multivariable analyses were conducted to determine the effects of the environmental treatments on the measured parameters. In addition, we determined the phenotypic plasticity of the two species and whether these showed allometric relationships to plant size.

Results and discussion: *C. acuta* was affected more by hydrologic changes, growing better in dry and saturated conditions, while fertilization had a more positive effect on *G. maxima*. Both species were stressed when flooded, but *C. acuta* more so than *G. maxima*. Contrary to our predictions, *C. acuta* produced more ramets and was taller than *G. maxima*. Both species showed plastic responses to changing nutrient and water conditions, but only some were

related to plant size. Our results indicate that *C. acuta* and *G. maxima* are more likely to co-exist in oligo- to mesotrophic wet grasslands with fluctuating water levels.

KEYWORDS

allometry, biomass allocation, coexistence, niche differences, phenotypic plasticity, wet grasslands

1 Introduction

Wet grasslands are semi-natural, graminoid-dominated habitats that are maintained through some sort of disturbance, usually grazing or mowing (Tallowin and Jefferson, 1999; Joyce, 2014). These grasslands can be highly diverse ecological systems that usually are found in agricultural landscapes, especially in Europe (Garcia, 1992; Klimkowska et al., 2007; Zelnik and Čarni, 2013). Both natural wet grasslands, such as those associated with prairie potholes, or those created and maintained by human activities, have similar hydrologic characteristics, being flooded periodically or having a high-water level, which influences their plant species composition (Joyce et al., 2016). Mowing provides not only fodder or bedding for livestock (Tasset et al., 2019), but, with the removal of plant biomass, allows for less-competitive plant species to survive and co-exist with more competitive species (Berg et al., 2012; Tardella et al., 2020).

Wet grasslands provide many important ecosystem services including nutrient removal, carbon sequestration, various hydrologic services such as flood attenuation and groundwater recharge, being important bird habitats (Joyce, 2014; Manton and Angelstam, 2021), as well as having a special microclimate that, through the cooling effect of evapotranspiration, can impact on the local and regional climate (Harding and Lloyd, 2008; Dietrich and Behrendt, 2022).

Native to Europe and Asia, *Carex acuta* and *Glyceria maxima* are two common plant species in Central European wet grasslands (Grootjans and ten Klooster, 1980; Hroudová et al., 1988; Nurminen, 2003; Tylová-Munzarová et al., 2005). *G. maxima* is considered to be an invasive species in North America, Australia and New Zealand, and South Africa (Mugwedi et al., 2014) where it may suppress native wetland plant species and affect the hydrology of whole wetland ecosystems (Sorrell et al., 2012). Therefore, it is important to study changes to the life history characteristics of these two plant species in more detail to understand how they spread, co-exist or outcompete other plant species.

Both species are emergent macrophytes which can form extensive, monospecific stands on gleyed clays or waterlogged organogenic soils (Hroudová and Zákravský, 2002). *C. acuta* is considered to be a slower growing, more conservative species which allocates more biomass to belowground structures, while growth of *G. maxima* is more in keeping with a competitive strategy,

seemingly doing better in nutrient-rich conditions and allocating more biomass to aboveground structures. Both species are tolerant of moist conditions, as indicated by them having mean Ellenberg indicator values for moisture of 8 (Chytrý et al., 2018), but the species differ in their response to nutrient conditions, with *G. maxima* being associated with nutrient-rich wetland habitats while *C. acuta* is restricted to nutrient-poorer areas (mean Ellenberg nutrient values of 9 and 4, respectively). Prach (1993, 2008) found that *C. acuta* will replace *C. nigra* in formerly oligotrophic wet grasslands subject to fertilization, but it is outcompeted by reeds, including *G. maxima*, with increasing eutrophication, in agreement with the Ellenberg values.

However, there are situations when these two species can co-exist in the field (De Deyn, 2017; Edwards and Čížková, 2020; Glocker et al., 2024). Under what environmental conditions such long-term co-existence can arise and be maintained, and how changes in life history characteristics underlie this co-existence, is an important and on-going topic of plant ecology (Chesson, 2018; Bowler et al., 2022). Co-existence is thought to occur when two or more species occupy different niches, which may arise due to the partitioning of resources or by spatial and/or temporal heterogeneity (Chesson, 2000; Silvertown, 2004; Adler et al., 2010).

Edwards and Čížková (2020) proposed that spatial and/or temporal differences in site hydrology and nutrient availability would likely lead to *C. acuta* and *G. maxima* co-existing in wet grasslands. These authors predicted that co-existence of *C. acuta* and *G. maxima* would occur under moist, but not long-term flooded, conditions or in nutrient-rich but dry (un-flooded) habitats (Edwards and Čížková, 2020). However, it is difficult to separate the effect of these environmental factors in the field. In addition, their study only considered aboveground production and nutrient contents, but did not include belowground structures, nor did they take into account biomass allocation patterns or phenotypic plasticity, two parameters that are important in determining a species niche (Bowler et al., 2022).

Here, we established a mesocosm experiment to determine the effects of different nutrient and water level conditions on the growth of *C. acuta* and *G. maxima*, in particular the biomass allocation patterns of the two species. Our study thus expands on the study by Edwards and Čížková (2020) in order to answer some of the open questions left by their earlier, more limited study. In addition, we wanted to determine whether this plasticity is related to plant size, in other

words whether there was an allometric relationship inherent in the observed biomass allocation patterns and vegetative spread of these clonal species (Jameson et al., 2022). Based on the literature and our past results (Edwards and Čížková, 2020), we predicted that 1) *G. maxima* plants would have greater biomass in nutrient-rich conditions, with greater allocation to aboveground structures, compared to *C. acuta* but 2) that this nutrient effect would be altered by hydrology thus indicating important interactive relationships between these two environmental factors. Additionally, 3) allocation patterns and vegetative reproduction would be dependent on plant size with larger plants having a greater number of new daughter shoots, being most notable in *G. maxima*. Therefore, based on these predictions, 4) *G. maxima* should be the more plastic species compared to *C. acuta* and should be favored over a wider range of environmental conditions.

2 Methods

2.1 Mesocosm set-up

Details of the mesocosm set-up are given in Glocker et al. (2024). Briefly, plants of *C. acuta* and *G. maxima* were collected from monospecific patches in the same wet grassland that was the focus of the study by Edwards and Čížková (2020). Plants, consisting of 2–3 shoots with attached roots, were transplanted into pots (9×9×14 cm; L×W×D) which were filled with a 2:1 sand/peat (by volume) mixture. Soil inocula were added to each specific pot using soil collected from patches of the two species which were assumed to contain the soil microbiome for each species. Pots with plants were then randomly distributed to different basins (187×120×45 cm; L×W×D) in order to better control water and nutrient levels. Thus, a basin would contain either *C. acuta* or *G. maxima* but never both.

The experiment consisted of a split-plot design with water levels nested within the nutrient treatment. Nutrient treatments were assigned randomly to each basin. Pots within a basin either received no added nutrients or were fertilized with 350 kg NPK fertilizer ha⁻¹ yr⁻¹ of an inorganic solution (Lovofert 15:15:15 NPK, Lovochemie, a.s.), added in two half-doses (mid-May and mid-July). The amount of added nutrients is the mid-point of the fertilizer application range recommended by the agri-chemical company. A micronutrient solution (“BioNova MicroMix”, BIONOVA, CR) was applied to the leaves of all plants at two-week intervals to insure that there was no micronutrient limitation. To minimize the chance of nutrient leakage from the pots, all pots were maintained at the low water level for three days to allow for nutrient mineralization and plant uptake. Also, past studies with *C. acuta* (Edwards et al., 2023, 2025) found rapid nutrient uptake by the plants. Therefore, any nutrient leaching from the pots to the water was assumed to be minimal. The pots within each basin were then subjected to three different water levels (dry = -15 cm below the soil surface; saturated = water level maintained at the soil surface; flooded = 15 cm above the soil surface) using wooden constructions. Overall, there were eight pots with plants per basin

for each water level treatment for a total of 24 pots per basin. There were three replicate basins for each treatment combination (species × nutrient with water level nested within each basin).

2.2 Plant measurements

The plants grew from late May to early September 2019. During this time, plant height (height of longest leaf) and shoot number were measured at two-week intervals. Half of the plants were harvested in mid-July while the remaining pots were harvested in early September. Aboveground structures of the harvested plants were divided into leaves and stems, while belowground structures were carefully cleaned of soil and then separated into roots, rhizomes and rootstocks. In this case, rhizomes represent underground stems which lead to the production of new aboveground shoots, while the rootstocks are the belowground portions from which aboveground shoots arise, but not including the underground stems. Rhizomes were separated from the rootstock since rhizomes may be important storage structures which may have different nutrient composition than roots or rootstocks (Lubbe et al., 2023). All plant structures were placed into separate labelled paper bags, dried at 65°C for at least 48 hours and weighed. From these, we calculated dry weight (g, DW) of each plant part per pot. These data were then used for the subsequent analyses.

2.3 Data analyses

To answer the first two questions, we ran split-plot ANOVAs to test the effect of nutrient addition and water level on maximum plant height, plant modular dry weight (DW) (leaves, stems, roots, rhizomes and rootstocks) as well as the respective biomass allocation ratios (plant structure DW/total plant DW) and shoot number, following natural logarithmic or square root transformations if needed. These analyses were conducted in R v 4.4 (“Puppy Love”) using the nlme (Pinheiro et al., 2016), car (Fox and Weisberg, 2023) and emmeans (Lenth, 2024) packages. Nutrient, water level, species and time period (biweekly for shoot number and plant height; month of harvest for the DW measures) were the fixed effects while basin was considered as a random factor. In all cases, there was never a significant basin effect thus only the results of the linear models are shown.

In addition, changes in the mean DW of each plant module across the nutrient and water level treatments were determined as mean reaction norms. From these, we calculated whether these reaction norms followed linear or non-linear (power equation) trajectories (Gomulkiewicz and Stinchcombe, 2022). Since there were only two nutrient level treatments, these automatically followed a linear path. Thus, any analysis incorporating non-linear equations could only be conducted on the water level treatment data. These analyses were run in R v. 4.4 using the nlme package (Pinheiro et al., 2016).

Changes in plant biomass allocation patterns may be actual responses to different environmental conditions (real phenotypic plasticity) but may also only be related to changes in plant size (allometric relationship = apparent phenotypic plasticity; Weiner,

2004). We followed the procedure outlined by Jameson et al. (2022) to determine whether biomass allocation was related to plant size. First, we performed the traditional size-independent analysis determining the effect of the treatment factors on the biomass allocation ratios. For this, we conducted the aforementioned split-plot analyses on leaf, stem and root weight ratios (LWR, SWR, RWR, respectively). Then we conducted size-dependent analyses, for which the DW of the particular plant structure was divided by the total DW of the plant minus the DW of that structure, for example, $LWR = \text{leaf DW} / (\text{total plant DW} - \text{leaf DW})$ since using total plant DW would result in loss of independence between the tested factors (Jameson et al., 2022). For this analysis, we compared both methods of calculating the biomass allocation ratios.

Size-dependent allocation relationships were analyzed by fitting three possible allometric equations to the data relating plant structure DW to plant size based on graphical inspection of this relationship. The three models used were:

- 1) an isometric equation ($R=cV$, where R = plant structure DW, V = total plant DW excluding the DW of the particular plant structure, c = a scaling factor).

A good fitting isometric model would indicate that the allometric relationship is not affected by changing environmental conditions. In addition, we used two non-linear allometric models:

- 2) a power equation ($R=cV^\alpha$, where α is an allocation coefficient that is influenced by the treatment conditions) and
- 3) a hump equation ($R=cV^{\alpha V}$) (Oddi et al., 2019; Jameson et al., 2022).

For comparison, we also included a non-allometric, null model ($R=c$; Equation 4) in which plant size was not included. Analyses of these models were run in R v 4.4 using the `nls` function in the `nlme` package (Pinheiro et al., 2016) following natural logarithmic or square root data transformations based upon the results of bivariate normal analyses (Legendre, 2022).

Species plasticity was determined using the mean ratio values for each nutrient and water level combination separately for the July and September harvests. Plasticity was determined by subtracting the ratio values of the unfertilized samples from those that were fertilized within each water level. A positive value would show an increase in allocation to that particular structure while a negative value would indicate decreased allocation. The percent change was calculated by dividing that difference by the unfertilized value and then multiplying by 100 (Jameson et al., 2022).

Because none of our plants flowered, we were limited to using a measure of vegetative reproduction to describe the treatment effects on plant fitness (Shipley et al., 2016). Since both species are capable of producing ramets, we used shoot number as this measure. Treatment effects on the biweekly measures of shoot number were analyzed in the same manner as for the other data (split-plot ANOVA; the size-dependent models). In the case of the size-dependent analyses, we analyzed the relationship between shoot number and total plant DW.

3 Results

3.1 Environmental effects on plant biomass and allocation ratios

As expected, plant height and total biomass increased throughout the growing period, with maximum plant height occurring in late July, followed by a slight decrease to the end of the experiment. Biomass, especially root DW and total belowground biomass, reached their peak in September compared to July (Figures 1, 2). Ramet production, our measure of vegetative reproduction, was greatest at the end of the experiment with *C. acuta* producing more new shoots than *G. maxima* (Figure 3). There was a significant basin effect ($p < 0.001$) with this spatial effect being greater earlier in the growing season but disappearing by the time of the two harvests. The production of new shoots had a linear relationship over time for both species but showed a significant increase for *C. acuta* while *G. maxima* had a more constant number of ramets except when fertilized or under dry conditions (Figure 3).

Nutrient addition resulted in taller plants (Figure 1) while, except for roots, the mass of all plant parts were significantly greater with fertilization (Figure 2; Table 1). This positive nutrient effect was seen for both species, which is counter to our first prediction, but it also depended on the particular plant part. *G. maxima* had greater stem biomass, both in absolute (stem DW) and relative (SWR) terms in agreement with our first hypothesis. However, *C. acuta* had significantly more leaf DW and a greater allocation to leaves (LWR), especially in September (Figure 4B). In fact, *C. acuta* live leaf mass did not differ between July and September while there was a large decrease for *G. maxima* by September, which coincided with a significant increase in dead leaf DW for the latter species (Table 1), indicating that leaf senescence in *G. maxima* was greater and started earlier than in *C. acuta*. Also, in keeping with our first hypothesis, live leaf DW for *G. maxima* was greater in fertilized conditions in September while there was no such nutrient effect on *C. acuta* (month * species * nutrient interaction, $p < 0.01$). Because of these between-species differences in leaf and stem biomass, the species had similar total live aboveground biomass (Table 1). Also, due to greater rhizome and rootstock masses, *G. maxima* plants allocated more biomass belowground (higher live belowground DW and R:S ratio), also opposite to what we predicted (hypothesis 1), even though *C. acuta* allocated more biomass to roots (Table 1; Figures 4, 5). Overall, there was little evidence that fertilization favored *G. maxima*, which would be noted as a significant species * fertilization interaction and which was found only for rhizomes.

While leaf DW was affected more by fertilization (Table 1; Figures 4A, B), water level had a greater impact on both stem and root DW (Figures 4E, F). Of the three water level treatments, flooding appeared to be the more stressful condition, while results were similar for the dry and saturated treatments. Flooding led to greater stem growth, but significantly diminished ramet production in *G. maxima* (species * water interaction, $p < 0.001$; Figures 4C, D) while it resulted in decreased root mass in *C. acuta* (Figures 4E, F). There were fewer new shoots when no additional nutrients were applied (nutrient * water interaction, $p < 0.001$) as predicted

(hypothesis 2). As a result, more biomass was allocated aboveground when the plants were flooded, especially in September (Figure 5H). Also, there was little evidence to support our contention that hydrology would alter any nutrient effect on biomass and the allocation pattern (second hypothesis) with only rhizome DW and RWR having significant nutrient * water interactions (Table 1).

3.2 Allometric relationships

Both species showed positive plastic responses to fertilization, with the exceptions of root DW in July and *C. acuta* leaf and root DWs in September (Figure 4), and to changing hydrology with *C. acuta* having greater plasticity in belowground structures (Figure 5D), and possibly leaf DW, while there was larger plasticity in aboveground structures shown by *G. maxima* (Figure 5B). Further analyses were then conducted to determine whether this plasticity was only due to changes in plant size.

3.2.1 Size independent analyses

Overall, the allocation ratios and non-linear power models gave the best results, based on AIC, while the null and isometric models never fit well with the data (Supplementary Table S1). All of the experimental factors (month, species, water level, nutrient addition) significantly affected the biomass allocation ratios, usually singly although there were important month × species interactions especially with LWR and RWR for *C. acuta* plants in July and September respectively, while *G. maxima* SWR was much greater in July compared to the other combinations (Table 2). For both species, nutrient addition tended to positively affect LWR and SWR plasticity but had a negative impact on RWR plasticity.

Both species showed high plasticity for the size-independent analyses, but this depended on season as well as plant part. Both species had similar leaf plasticity in July, with leaf DW increasing with fertilization, with maximum leaf plasticity occurring in the saturated treatment for *C. acuta* but dry conditions for *G. maxima* (Table 3). Fertilization had a negative impact on leaf DW for *C. acuta* in September while it still had a positive effect on *G. maxima*.

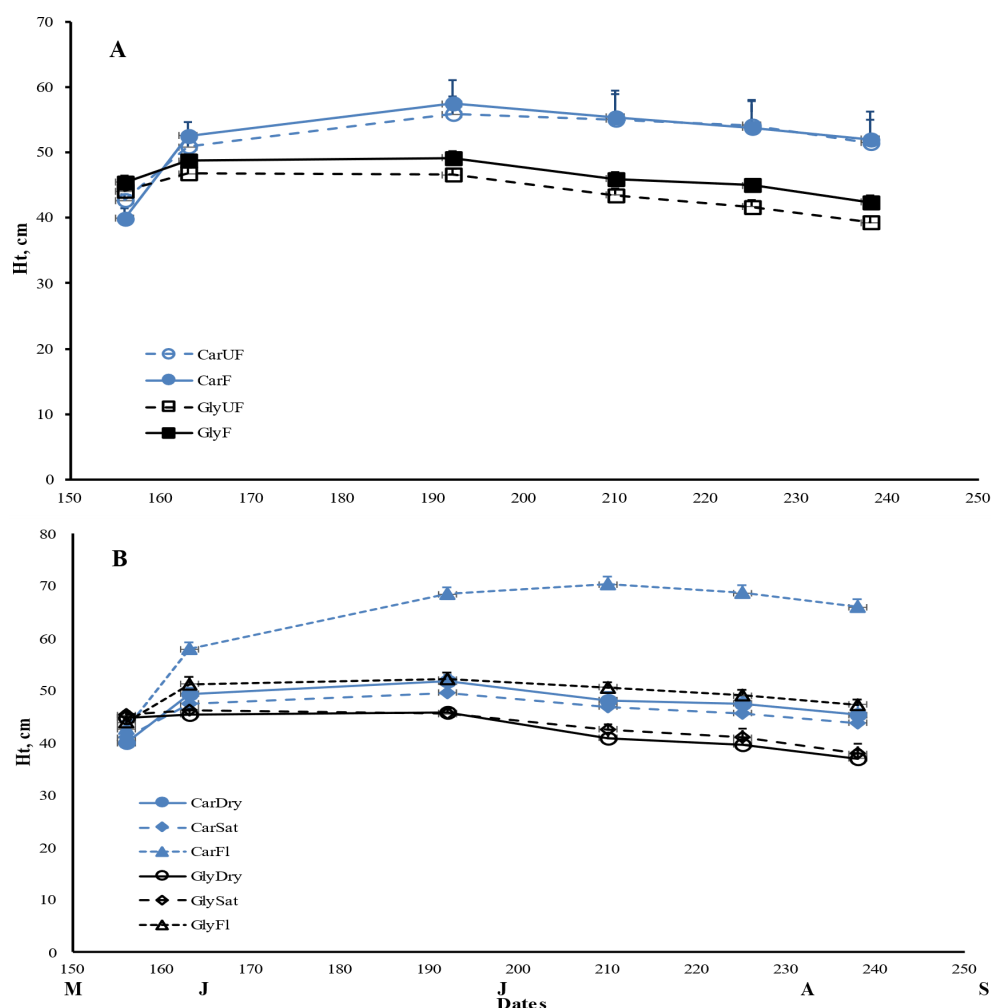


FIGURE 1

Mean maximum plant height (\pm 1SE) for *Carex acuta* (Car) and *Glyceria maxima* (Gly) in relation to (A) nutrient addition and (B) water level over the experimental growing period, early June to early September. Treatments: Nutrient addition – UF=unfertilized; F = fertilized (350 kg NPK ha⁻¹ yr⁻¹)/ Water level – Dry=15 cm below the soil surface; Sat=saturated (water level at the soil surface); Fl=flooded (15 cm above the soil surface).

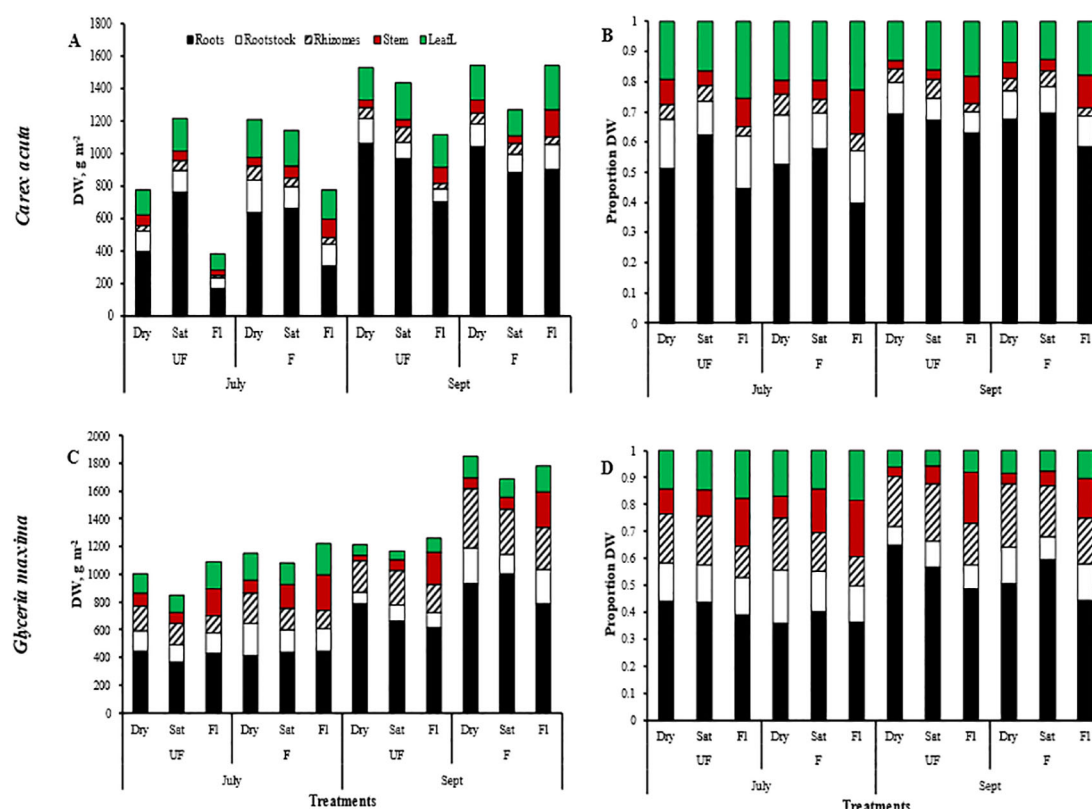


FIGURE 2

Plant module dry weights (DW; A, C) and DW proportions (B, D) for *Carex acuta* (A, B) and *Glyceria maxima* (C, D). Treatments: Month = harvest times/Nutrient addition – UF=unfertilized; F = fertilized (350 kg NPK ha⁻¹ yr⁻¹)/Water level – Dry=15 cm below the soil surface; Sat=saturated (water level at the soil surface); Fl=flooded (15 cm above the soil surface).

Overall, *G. maxima* was the more plastic species for stem and root DW, consistent with our prediction (hypothesis 4). The plasticity of vegetative reproduction was also positively impacted by nutrient addition in all water level treatments with the exception of flooded *G. maxima* plants in July (Table 3).

3.2.2 Size-dependent analyses

Similarly as with the size independent analyses, leaf, stem and root masses were significantly affected by all of the treatment factors (Table 4). The main difference was that there were more treatment interactions in the size dependent analyses, notably species \times water interactions. For example, *C. acuta* leaf and root DWs, as well as stem number, were greater than those of *G. maxima* especially in dry and saturated conditions. On the contrary, *G. maxima* had the highest stem DW when flooded while that of *C. acuta* was the lowest in dry and saturated conditions. In addition, the water level effect on stem and root DWs differed between July and September (season \times water interaction) with stem DW being the lowest under dry conditions in September while it was greater when flooded in both months. Likewise, root DW was the highest in September, especially in dry conditions, but the lowest when flooded in July.

Contrary to our third hypothesis, we found no consistent indication for allometric effects in any of the measured parameters (Supplementary Tables S2–S4). Stem DW and number

had the fewest such relationships especially when flooded (Table 4; Supplementary Table S3). Leaf DW also showed no allometric relationships in September for both species, notably when saturated or flooded (season \times water interaction; Supplementary Table S2). However, allometric relationships were most common for root DW (Supplementary Table S4), especially for *C. acuta* in July, in which all three size-dependent models could sufficiently represent the results, while this was the case for *G. maxima* only in July under dry conditions. This differed in September, when the power and hump models (Equations 2, 3; Supplementary Table S4) could adequately express the root DW to total plant weight minus root weight relationship.

4 Discussion

The aim of our study was to describe the niches of two common wet grassland species, *C. acuta* and *G. maxima*, by investigating changes in their life history characteristics as a result of changing environmental conditions (fertilization, water level). Determining how the species respond to these environmental changes, and where and by how much their respective niches may overlap, would help us to understand how these two species can sometimes co-exist in wet grasslands. Our initial hypotheses could be divided into two

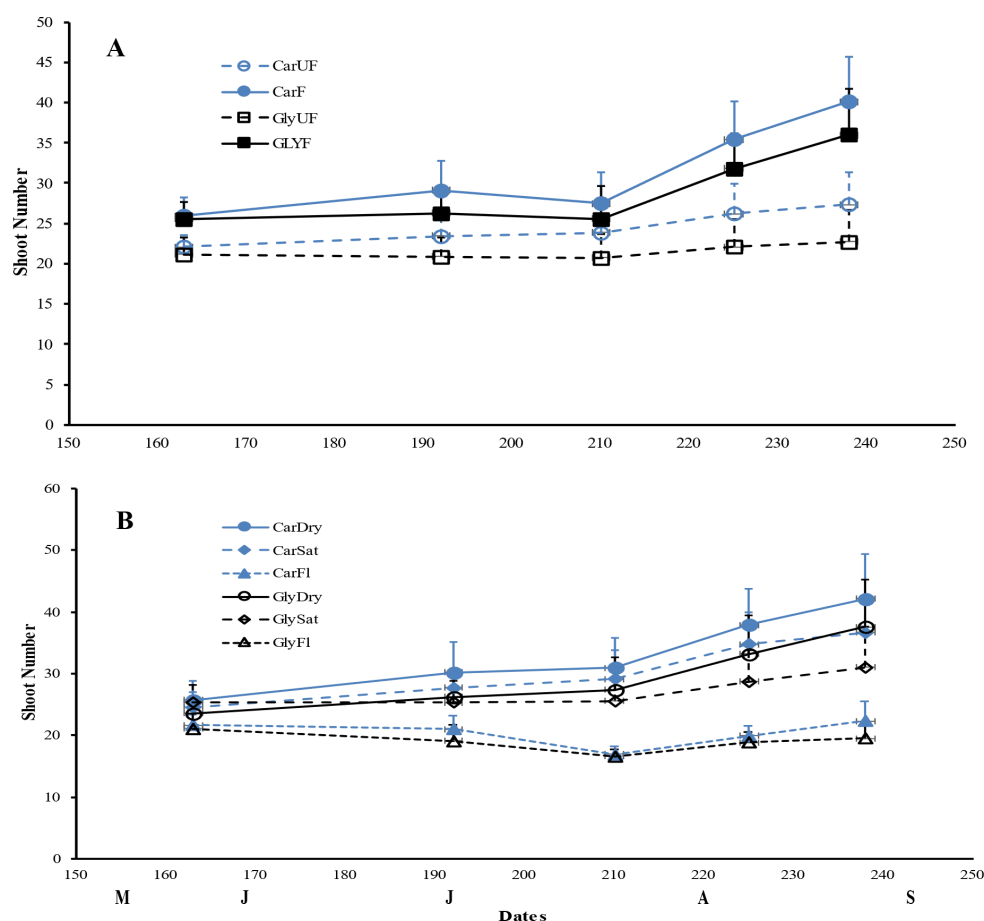


FIGURE 3

Mean shoot number ($\pm 1SE$) for *Carex acuta* (Car) and *Glyceria maxima* (Gly) in relation to (A) nutrient addition and (B) water level over the experimental growing period, early June to early September. Treatments: Nutrient addition – UF=unfertilized; F = fertilized (350 kg NPK ha⁻¹ yr⁻¹)/ Water level - Dry=15 cm below the soil surface; Sat=saturated (water level at the soil surface); FI=flooded (15 cm above the soil surface).

groups, the first dealing with nutrient and water level effects on plant growth and biomass allocation patterns (hypotheses 1, 2) while the second group of hypotheses were more concerned with whether the observed phenotypic plasticity followed an allometric relationship (hypotheses 3, 4). While some of our results supported our hypotheses, many other results were quite the opposite of what was expected.

4.1 Environmental effects greatly impact plant traits

According to the “fast-slow” plant economic spectrum (Reich, 2014), more competitive plant species should dominate in higher resource conditions, having faster growth and more biomass allocated to aboveground structures, while stress tolerators should have traits indicative of the conservative plant strategy, namely slower growth with greater biomass allocation to belowground structures (De Deyn, 2017). At the onset of our study, we considered *G. maxima* to be the more competitive species since it is often found in nutrient-rich habitats. The high nutrient

contents found in its plant structures indicate a high uptake of available nutrients, which make it a valuable fodder plant (Lamber, 1947; Mugwedi et al., 2014). These traits have also resulted in *G. maxima* becoming a very invasive species not only in areas outside of its native range in temperate Europe and Asia (North America, Australia, New Zealand, South Africa) but even in more northern locations in Europe (Ireland and Scandinavia; Mugwedi et al., 2014; NOBANIS, 2018). Therefore, we expected that *G. maxima* would act more like a competitive species, having greater growth in fertilized conditions with more biomass allocated to aboveground structures, with a concomitant lower R:S ratio, than *C. acuta*, which should have features characteristic of stress-tolerators (Menges and Waller, 1983; Edwards and Čížková, 2020). Our results only partially supported our first hypothesis. As predicted, the *C. acuta* plants in our study had greater root DW than *G. maxima*, while the latter species had greater aboveground biomass when fertilized. However, contrary to the first hypothesis, total live belowground DW was greater in *G. maxima*, due to this species having significantly larger rootstock and rhizome masses. This resulted in *G. maxima* having larger R:S ratios, notably in unfertilized conditions (Table 1). Nutrient addition did result in greater

TABLE 1 Results of split-plot ANOVAs (F statistic) determining the effect of the experimental treatments on (A) plant module dry weight (DW) and (B) shoot number and plant height.

A										
Parameter/Factor	Month	Species	Fert	Water	M*S	M*F	M*W	S*F	S*W	F*W
Leaf DW – live	3.92 * J > S	17.50 *** Car > Gly	12.85 *** F > UF	4.58 + F > D > S	18.83 ***				9.42 **	
Leaf DW - dead	239.87 *** S > J	227.73 *** Gly > Car	6.72 * F > UF		6.11 *	4.08 *				
Stem DW		41.39 *** Gly > Car	13.49 *** F > UF	101.20 *** F > S > D	4.61 *		8.92 *		11.32 **	
Root DW	249.53 *** S > J	3.33 + Car > Gly		40.96 *** D > S > F			5.27 +		22.48 ***	
Rootstock DW		4.61 * Gly > Car	14.66 *** F > UF							
Rhizomes DW	34.64 *** S > J	391.82 *** Gly > Car	6.12 * F > UF	10.23 *** D > S > F	15.77 ***			5.37 *	3.52 *	
Above DW	4.95 * J > S		42.84 *** F > UF	50.65 *** F > S > D	26.35 ***		7.82 **	3.13 +	17.85 ***	
Below DW	196.59 *** S > J	17.76 *** Gly > Car	23.44 *** F > UF	19.86 *** D > S > F					7.46 **	2.65 +
R:S	112.00 *** S > J	3.51 + Gly > Car	2.58 + UF > F	172.56 *** D > S > F	18.99 ***					
B										
Parameter/Factor	Time	Species	Fert	Water	T*S	T*F	T*W	S*F	S*W	F*W
Shoot Density	43.01 ***	135.18 *** Car > Gly	27.03 ** F > UF	141.51 *** D > S > F	10.37 **	33.93 ***	49.01 ***		21.99 ***	12.59 ***
Height	53.16 ***	47.70 *** Car > Gly	7.95 *** F > UF	180.61 *** F > S > D	109.95 ***		67.11 ***		7.80 ***	

Only significant effects shown. Treatments: Month (A): July (J); September (S). Species: *Carex acuta* (Car); *Glyceria maxima* (Gly). Fert: nutrient addition = unfertilized (UF); fertilized (F = 350 kg NPK ha⁻¹ yr⁻¹). Water: water level = dry (D = 15 cm below the soil surface); saturated (S = at the soil surface); flooded (F1 = 15 cm above the soil surface). Time (B): biweekly intervals. R:S, root-to-shoot ratio; P values: + < 0.10; * < 0.05; ** < 0.01; *** < 0.001.

allocation of biomass to aboveground structures in *G. maxima* compared to unfertilized plants (Table 1), while there was no nutrient effect on the R:S ratio of *C. acuta*. This greater impact of fertilization on *G. maxima* is in agreement with the first hypothesis. Overall, however, these biomass allocation results only partially support our first hypothesis.

Also contrary to expectations, *C. acuta* produced a greater number of new ramets, having a more compact growth form than *G. maxima*, which produced fewer but more spatially separated ramets, by which it could grow into bare areas within or between *C. acuta* clumps (Glocker et al., 2024). Since shoot density is a proxy measure of plant fitness (Shipley et al., 2016), the greater number of ramets would indicate that *C. acuta* is more fit especially in dry and saturated conditions. In addition, the greater production of ramets by *C. acuta* later in the growing season is likely the reason why live leaf DW did not differ between July and September for this species, even though the amount of dead leaf DW did increase near the end of the growing season. Thus, *G. maxima* starts to die back and senesce earlier than *C. acuta*. Both species were stressed when flooded, with *C. acuta* and *G. maxima* responding to prolonged flooding by increasing their allocation to stem and leaf growth,

which is a well-known response of wetland plants (Pezeshki, 2001). However, counter to expectations, this increase in shoot height was much greater for *C. acuta* than that of the supposedly more competitive species. Plant height has often been linked to competitive ability (Tilman, 1982; Givnish, 1982), thus, by that metric, *C. acuta* should be considered as the better competitor than *G. maxima* if only above ground structures are considered. However, a different picture emerges when belowground structures are included. The R:S ratio decreased in both harvests for both species when flooded (Figure 5H), but this reduction was due to different growth patterns in the two species, especially at the peak time of the growing season in July. At that time, leaf and stem growth increased for *C. acuta*, but the overall mass of the aboveground structures remained the same. This stability is likely associated with the smaller number of ramets produced by *C. acuta* when flooded so that, on an area basis, there were larger but fewer leaves, which led to the stable aboveground biomass unaffected by water level changes. Concurrently, root growth decreased in *C. acuta* which, associated with the lack of change in aboveground DW, resulted in the decreased R:S ratio. *G. maxima* showed the opposite trends at the same point of the growing season, with this

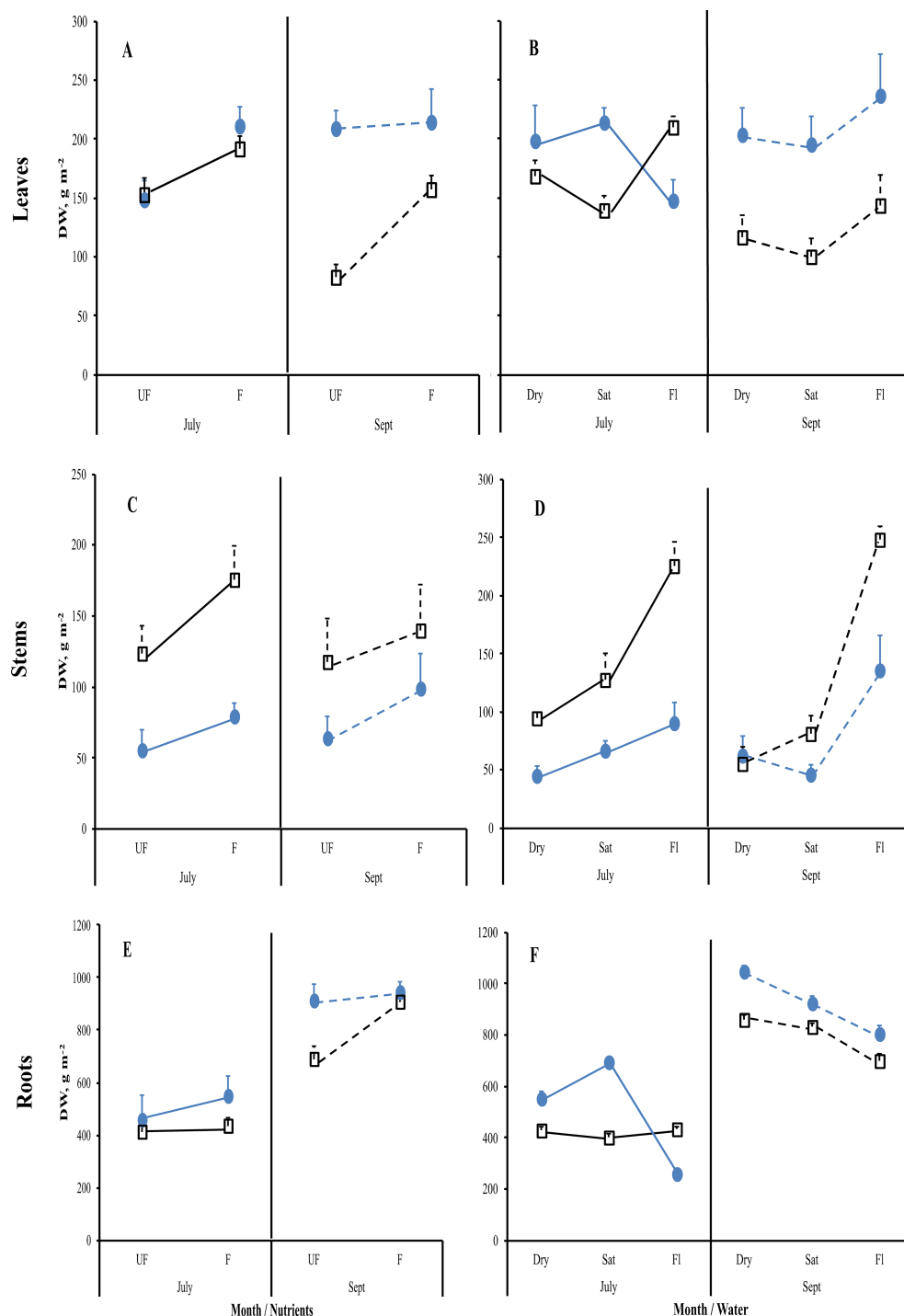


FIGURE 4

Mean reaction norms (\pm 1SE) of leaf (A, B), stem (C, D), and root (E, F) dry weights for *Carex acuta* (blue circles) and *Glyceria maxima* (open black squares) in relation to nutrient addition (A, C, E) and water level (B, D, F) for the July (solid lines) and September (Sept; dashed lines) harvests. Treatments: Nutrient addition – UF=unfertilized; F = fertilized ($350 \text{ kg NPK ha}^{-1} \text{ yr}^{-1}$)/Water level – Dry=15 cm below the soil surface; Sat=saturated (water level at the soil surface); FI=flooded (15 cm above the soil surface).

species allocating more biomass to above ground structures while allocation to root mass was unaffected by flooding. Also, while the new ramet production by *G. maxima* was reduced when flooded, it was not as affected by changing water level conditions as *C. acuta*. By September, both species responded in a similar manner, with

both allocating more mass to above ground and less to below ground structures. These results indicate that *C. acuta* will be more stressed under prolonged flooding thereby likely reducing its competitive ability. Unfortunately, direct competition between the two species was not measured in our study so we cannot

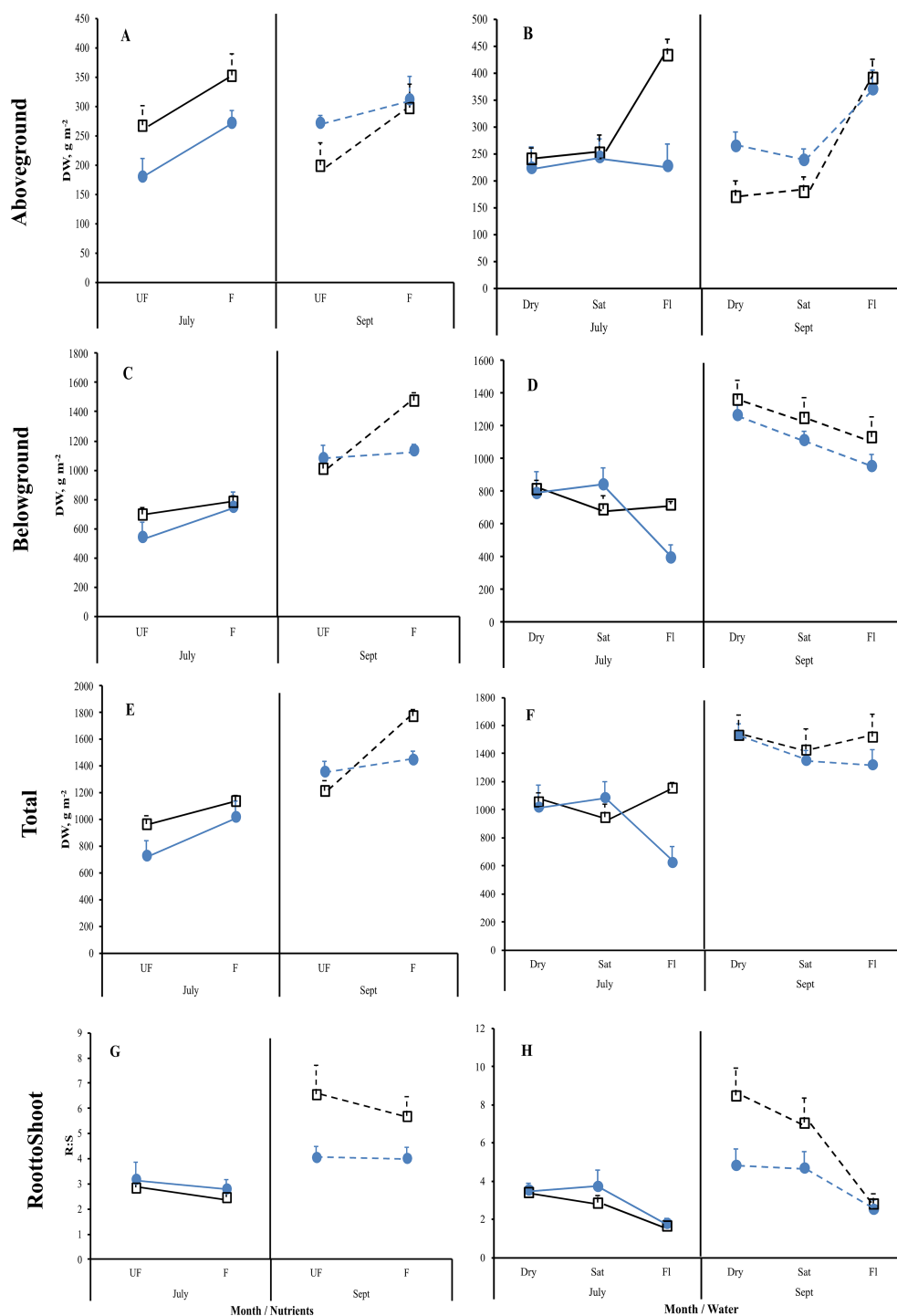


FIGURE 5

Mean reaction norms (\pm 1 SE) of aboveground (A, B), belowground (C, D) and total plant (E, F) dry weights (DW), and the root-to-shoot (R:S) ratio (G, H) for *Carex acuta* (blue circles) and *Glyceria maxima* (open black squares) in relation to nutrient addition (A, C, E, G) and water level (B, D, F, H) for the July (solid lines) and September (Sept; dashed lines) harvests. Treatments: Nutrient addition – UF=unfertilized; F = fertilized ($350 \text{ kg NPK ha}^{-1} \text{ yr}^{-1}$)/Water level – Dry=15 cm below the soil surface; Sat=saturated (water level at the soil surface); FI=flooded (15 cm above the soil surface).

categorically state whether *C. acuta* may be able to outcompete *G. maxima* under the experimental conditions used here.

Two or more plant species may co-exist if they inhabit different niches (Silvertown, 2004). In turn, biomass allocation patterns are influenced externally by these environmental factors and internally

by the life history strategy of the particular species (Metcalf et al., 2006; De Deyn, 2017). While there are areas of overlap in the niches of the two species (similar total above and belowground live DW), there are also important differences (allocation ratios, stem number, height) which would make co-existence more likely under certain

environmental conditions (Chesson, 2000). Future studies incorporating direct species interactions are needed to test this idea.

Our results indicate that *G. maxima* should dominate in nutrient-rich wet grasslands and at times of prolonged flooding and in slight depressions, while *C. acuta* would be favored at times of lower water levels regardless of the nutrient condition of the site, which is, in fact, the zonation observed in the field (Hroudová et al., 1988; Edwards et al., 2024). This prediction differs from that of Edwards and Čížková (2020), who predicted that, while *G. maxima* should dominate in nutrient-richer conditions as in our study, flooding would favor *C. acuta*. This discrepancy in the results of the two studies may be due to the fact that Edwards and Čížková (2020) only considered net annual aboveground production of these two species in years differing in hydrologic conditions, but did not incorporate belowground biomass as we did here. As stated above, even in our study, flooding led to greater leaf and shoot growth in *C. acuta*, which likely led to the greater above ground production noted by Edwards and Čížková (2020). Therefore, our results emphasize the importance of including belowground plant structures in such studies (also see De Kroon et al., 2012; Bardgett et al., 2014).

4.2 Possible allometric relationships

Phenotypic plasticity is the ability of a plant to produce different phenotypes under changing environmental conditions (Pigliucci, 2001), with plant modular units being the level at which plasticity is expressed and analyzed (De Kroon et al., 2005). Biomass allocation ratios and reaction norms are common methods for analyzing phenotypic plasticity. Here, both species showed plastic responses to changing nutrient and water level conditions, which was opposite of what we initially predicted (hypothesis 4), with these changes having a seasonal aspect. However, while some of these changes were also related to plant size (Schneider, 2022; Gomulkiewicz and

Stinchcombe, 2022), our overall results were inconsistent with other responses having no allometric relationship. Therefore, our third hypothesis that most of the plastic responses would be attributed to changes in plant size cannot be supported.

Counter to our prediction, in only a few cases there was a clear relationship between the production of new ramets and plant size, with the fewest occurring when the plants were flooded. Most such relationships were found for *C. acuta* (Table 4). This is in contrast to Jameson et al. (2022) who found a larger number of size-dependent relationships. However, these authors used sexual reproduction as their measure of fitness. Given that none of our plants flowered, we had to use vegetative spread as our measure of fitness. Therefore, it is not known whether sexual reproduction would show such allometric relationships.

A similar lack of an allometric relationship was noted in the size-dependent model analyses for leaves and stems but such relationships were more common with roots. Again, *C. acuta* had more size-dependent relationships than *G. maxima*. In addition, time of the growing season determined whether size-dependent relationships were possible. For example, the response of leaf DW to flooding did show a clear allometric relationship in July for both species, but this had largely disappeared by September, notably for *C. acuta*, likely reflecting plant development patterns in that the plants had begun to senesce by early September (Schneider, 2022). However, there was no such seasonal effect for *G. maxima* (Supplementary Table S1). In fact, while leaf DW decreased from July to September, nutrient addition maintained these structures, likely prolonging the *C. acuta* growing season.

5 Conclusions

In our study, *C. acuta* and *G. maxima* did not behave as expected according to the general predictions about conservative and competitive species. We found that:

TABLE 2 Results of size-independent split-plot ANOVAs (F values) determining the effect of the experimental treatments on the biomass allocation ratios.

Parameter/ Factor	Month	Species	Fert	Water	M*S	M*W	S*W	S*F	F*W	AIC
LWR	147.01 *** J > S	134.61 *** Car > Gly	5.31 * F > UF	19.17 *** F > S > D	16.70 ***					-444.64
SWR	49.12 *** J > S	19.37 *** Gly > Car	4.34 * F > UF	72.92 *** F > S > D	4.03 *					-308.18
RWR	296.42 *** S > J	189.52 *** Car > Gly	16.62 *** UF > F	49.84 *** S > D > F				8.23**	4.40*	-475.78
StockWR	47.35 *** J > S		6.95 ** F > UF	2.87 + D > F > S						181.66
RhizWR		403.74 *** Gly > Car		10.35 *** D > S > F	5.16*					-253.68

Only significant effects shown. Treatments: Month (M): July (J); September (S). Species (S): *Carex acuta* (Car); *Glyceria maxima* (Gly). Fert (F): nutrient addition = unfertilized (UF); fertilized (F = 350 kg NPK ha⁻¹ yr⁻¹). Water (W): water level = dry (D = 15 cm below the soil surface); saturated (S = at the soil surface); flooded (Fl = 15 cm above the soil surface). LWR, leaf weight ratio; SWR, stem weight ratio; RWR, root weight ratio; StockWR, rootstock weight ratio; RhizWR, rhizome weight ratio; AIC, Akaike information criteria. P values: + < 0.10; * < 0.05; ** < 0.01; *** < 0.001.

TABLE 3 Plasticity in allocation of plant modular structures measured as the difference in mean allocation ratios (Ratio) for *Carex acuta* (Car) and *Glyceria maxima* (Gly).

Ratio	Month	Species	Water	F-UF	% Change
LWR	July	Car	Dry	0.021	9.06
			Sat	0.055	26.61
			Flood	0.006	1.79
		Gly	Dry	0.038	23.48
			Sat	0.028	17.40
			Flood	0.012	5.76
	September	Car	Dry	0.009	5.57
			Sat	-0.044	-22.80
			Flood	-0.002	-0.79
		Gly	Dry	0.027	40.38
			Sat	0.024	38.30
			Flood	0.023	24.92
SWR	July	Car	Dry	-0.004	-7.78
			Sat	0.017	31.07
			Flood	0.035	19.58
		Gly	Dry	-0.005	-5.07
			Sat	0.082	88.64
			Flood	0.052	23.98
	September	Car	Dry	0.023	68.89
			Sat	0.009	29.27
			Flood	0.021	20.24
		Gly	Dry	0.006	18.32
			Sat	-0.002	-2.81
			Flood	-0.028	-14.32
RWR	July	Car	Dry	-0.130	-10.36
			Sat	-0.259	-16.21
			Flood	-0.200	-23.36
		Gly	Dry	-0.275	-31.84
			Sat	-0.305	-34.61
			Flood	-0.087	-13.22
	September	Car	Dry	-0.266	-11.02
			Sat	0.206	9.63
			Flood	-0.307	-17.60
		Gly	Dry	-0.880	-45.57
			Sat	0.056	3.92
			Flood	-0.174	-17.69

(Continued)

TABLE 3 Continued

Ratio	Month	Species	Water	F-UF	% Change
Shoot No	July	Car	Dry	12.417	34.98
			Sat	1.000	3.18
			Flood	6.583	27.53
		Gly	Dry	6.500	36.79
			Sat	4.917	28.37
			Flood	-0.083	-0.52
	September	Car	Dry	25.083	64.18
			Sat	10.667	26.28
			Flood	9.667	46.40
		Gly	Dry	13.333	62.26
			Sat	12.583	81.18
			Flood	6.667	51.95

Differences between the two nutrient treatments (UF = unfertilized; F = fertilized (350 NPK kg ha⁻¹ yr⁻¹) calculated for month (July, September), species and water levels (Dry = 15 cm below the soil surface; Sat = saturated (water level at the soil surface); flood = 15 cm above the soil surface). LWR, leaf weight ratio; SWR, stem weight ratio; RWR, root weight ratio; Shoot No, shoot density.

1. as predicted, *G. maxima* was more affected by fertilization than *C. acuta*. However, counter to our prediction, *G. maxima* allocated more biomass to below ground than above ground structures having higher R:S ratios than *C. acuta*. Therefore, our results emphasize the importance of including belowground plant structures in such studies;

2. counter to our second hypothesis, changes in site hydrology affected *C. acuta* more than *G. maxima*, especially regarding stem DW, plant height and ramet production. In addition, water level had a stronger effect on the R:S ratio than fertilization in both species with below ground biomass decreasing for both species when flooded as compared to drier conditions;

TABLE 4 Comparison of three size dependent (1: isometric; 2: power; 3: hump) equations and a size-independent null model (4) for shoot number as a measure of vegetative reproduction.

Water	Equations	July				September			
		UF		F		UF		F	
		Carex	Glyceria	Carex	Glyceria	Carex	Glyceria	Carex	Glyceria
Dry	1) R=cV	7.67 (6.15)	2.40 (0.42)	6.14 (5.30)	3.51	15.46	1.18 (0)	0	6.82
	2) R=cV ^α	1.52 (0)	1.98 (0)	0.85 (0)	0.52	0	1.92 (0.74)	1.44	0
	3) R=cVe ^{αV}	3.43 (1.91)	2.23 (0.25)	1.41 (0.57)	0	17.44	1.90 (0.71)	1.44	NA
	4) R=c	0	0	0	0.77	16.84	0	1.31	4.75
Sat	1) R=cV	5.18	0.78	2.15	2.47 (1.55)	10.89	21.63	6.26 (4.40)	2.69
	2) R=cV ^α	0	0	0.01	1.02 (0.10)	0.79	4.85	1.98 (0.13)	0
	3) R=cVe ^{αV}	2.71	0.48	0	0.92 (0)	0	0	1.86 (0)	0.16
	4) R=c	3.77	4.58	1.47	0	18.32	9.50	0	0.17
Flood	1) R=cV	0.61	3.07 (1.22)	5.32 (3.91)	0	3.24 (1.60)	4.70 (3.86)	0	4.22 (2.47)
	2) R=cV ^α	0	1.84 (0)	1.42 (0)	1.95	1.64 (0)	0.84 (0)	1.60	1.75 (0)
	3) R=cVe ^{αV}	0.52	1.79 (0.05)	2.48 (1.07)	1.95	1.89 (0.24)	20.3 (1.19)	1.61	2.02 (0.27)
	4) R=c	1.15	0	0	5.68	0	0	3.03	0

Differences in Akaike information criteria (AIC) values are shown. Equations with a zero value had the lowest AIC score. Values in parentheses represent AIC differences when comparing the three size-dependent models only. Comparisons > 2 are significantly different. Equations: R = shoot number; V = plant dry weight; c, α = scaling factors. Nutrient addition: UF = unfertilized; F = fertilized (350 NPK kg ha⁻¹ yr⁻¹)/Water level: Dry = 15 cm below the soil surface; Sat = saturated (water level at the soil surface); flood = 15 cm above the soil surface. NA = samples which did not converge with the model. Based on [Oddi et al. \(2019\)](#) and [Jameson et al. \(2022\)](#).

3. both species showed plastic responses to changing environmental conditions but only some were related to plant size. *C. acuta* produced more daughter shoots than *G. maxima*. Thus, we could only partially support our last two predictions that phenotypic plasticity followed an allometric relationship (hypotheses 3) and *G. maxima* was the more plastic species (hypothesis 4);
4. *C. acuta* would likely be favored in drier, lower nutrient habitats while *G. maxima* would prefer growing in wetter, nutrient-rich sites. With warmer and drier conditions predicted for central Europe because of climate change (IPCC, 2023), such a future would favor *C. acuta* to the detriment of *G. maxima*.

This information may be important for predicting possible sites that would be prone to *G. maxima* invasion as well as developing management and control plans to combat the spread and establishment of invasive populations of this species (Mugwedi et al., 2014). Overall, the allocation patterns and vegetative reproduction of the two species were often different than expected, with both species having characteristics of both the conservative and competitive plant functional types (De Deyn, 2017). Our results imply that *C. acuta* and *G. maxima* may be able to co-exist in oligo- to mesotrophic wet grasslands with fluctuating water levels as well as more heterogeneous habitats. Further studies including direct species interactions would be needed to determine the validity of this conjecture. Still, our study provides important information which would be useful for predicting and modelling the effects of management and climate change on wet grassland plant species composition as well as maintaining the ability of wet grasslands to provide their important ecosystem services.

Data availability statement

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

Author contributions

KE: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision,

Writing – original draft, Writing – review & editing. BG: Data curation, Investigation, Writing – review & editing. JM: Data curation, Investigation, Methodology, Writing – review & editing. TP: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research 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

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1542907/full#supplementary-material>

References

- Adler, P. B., Ellner, S. P., and Levine, J. M. (2010). Coexistence of perennial plants: an embarrassment of niches. *Ecol. Letts.* 13, 1019–1029. doi: 10.1111/j.1461-0248.2010.01496.x
- Bardgett, R. D., Mommer, L., and De Vries, F. T. (2014). Going underground: root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692–699. doi: 10.1016/j.tree.2014.10.006
- Berg, M., Joyce, C., and Burnside, N. (2012). Differential responses of abandoned wet grassland plant communities to reinstated cutting management. *Hydrobiologia* 692, 83–97. doi: 10.1007/s10750-011-0826-x
- Bowler, C. H., Weiss-Lehman, C., Towers, I. R., Mayfield, M. M., and Shoemaker, L. G. (2022). Accounting for demographic uncertainty increases predictions for species coexistence: A case study with annual plants. *Ecol. Letts.* 25, 1618–1628. doi: 10.1111/ele.14011
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* 31, 343–366. doi: 10.1146/annurev.ecolsys.31.1.343
- Chesson, P. (2018). Updates on mechanisms of maintenance of species diversity. *J. Ecol.* 106, 1773–1794. doi: 10.1111/1365-2745.13035
- Chytrý, M., Tichý, L., Dřevojan, P., Šádlo, J., and Zelený, D. (2018). Ellenberg-type indicator values for the Czech flora. *Preslia* 90, 83–103. doi: 10.23855/preslia.2018.083

- De Deyn, G. B. (2017). Plant life history and above-belowground interactions: missing links. *Oikos* 126, 497–507. doi: 10.1111/oik.03967
- De Kroon, H., Hendriks, M., van Ruijven, J., Ravenek, J., Padilla, F. M., Jongejans, E., et al. (2012). Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. *J. Ecol.* 100, 6–15. doi: 10.1111/j.1365-2745.2011.01906.x
- De Kroon, H., Huber, H., Stuefer, J. F., and van Groenendael, J. M. (2005). A modular concept of phenotypic plasticity in plants. *New Phytol.* 166, 73–82. doi: 10.1111/j.1469-8137.2004.01310.x
- Dietrich, O., and Behrendt, A. (2022). Wet grassland sites with shallow groundwater conditions: Effects on local meteorological characteristics. *Water* 14, 3560. doi: 10.3390/w14213560
- Edwards, K. R., Bárta, J., Mastný, J., and Pícek, T. (2023). Multiple environmental factors, but not nutrient addition, directly affect wet grassland soil microbial community structure: a mesocosm study. *FEMS Microbiol. Ecol.* 99, 1–15. doi: 10.1093/femsec/fiad070
- Edwards, K. R., and Čížková, H. (2020). Nutrient inputs and hydrology interact with plant functional type in affecting plant production and nutrient contents in a wet grassland. *Wetlands* 40, 707–719. doi: 10.1007/s13157-019-01216-0
- Edwards, K. R., Kaštovská, E., Bárta, J., Pícek, T., and Šantrůčková, H. (2025). Multiple environmental factors interact to affect wet grassland ecosystem functions. *Ecol. Eng.* 212, 107511. doi: 10.1016/j.ecoleng.2024.107511
- Edwards, K. R., Květ, J., Ostrý, I., Zákravský, P., and Hroudová, Z. (2024). Peak-season and off-season distribution of mineral nutrients in littoral vegetation of an ancient shallow reservoir. *Hydrobiologia* 851, 1593–1606. doi: 10.1007/s10750-022-05079-1
- Fox, J., and Weisberg, S. (2023). *An R Companion to Applied Regression* (Thousand Oaks CA: Sage). Available online at: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/> (Accessed June 3, 2024).
- García, A. (1992). Conserving the species-rich meadows of Europe. *Agric. Ecosyst. Environ.* 40, 219–232. doi: 10.1016/0167-8809(92)90094-R
- Givnish, T. J. (1982). On the adaptive significance of leaf height in forest herbs. *Am. Nat.* 120, 353–381. doi: 10.1086/283995
- Glocker, B., Mastný, J., Pícek, T., and Edwards, K. R. (2024). Environmental effects on assimilated carbon quantity and quality in two different wet grassland plants. *Wetlands* 44, 91. doi: 10.1007/s13157-024-01853-0
- Gomulkiewicz, R., and Stinchcombe, J. R. (2022). Phenotypic plasticity made simple, but not too simple. *Am. J. Bot.* 109, 1519–1524. doi: 10.1002/ajb2.16068
- Grootjans, A. P., and ten Klooster, W. P. (1980). Changes of groundwater regime in wet meadows. *Acta Bot. Neerl.* 29, 541–554. doi: 10.1111/j.1438-8677.1980.tb01257.x
- Harding, R. J., and Lloyd, C. R. (2008). Evaporation and energy balance of a wet grassland at Tadham Moor on the Somerset Levels. *Hydrol. Process.* 22, 2346–2357. doi: 10.1002/hyp.6829
- Hroudová, Z., Hejny, S., and Zákravský, P. (1988). “Littoral vegetation of the Rožmberk fishpond,” in *Littoral Vegetation of the Rožmberk Fishpond and Its Mineral Nutrient Economy. Studie ČSAV, 88/9*. Ed. Z. Hroudová (Academia, Praha, Czech Republic), 23–60.
- Hroudová, Z., and Zákravský, P. (2002). “Littoral plant communities and soils,” in *Freshwater Wetlands and Their Sustainable Future*, vol. 28. Eds. J. Květ, J. Jeník and L. Soukupová (Mann and the biosphere series (Parthenon publishing, Boca Raton, Florida, USA)), 195–210.
- IPCC and Core Writing Team (2023). *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Eds. H. Lee and J. Romero (Geneva, Switzerland: IPCC). doi: 10.59327/IPCC/AR6-9789291691647
- Jameson, E. E., Elgersma, K., Martina, J. P., Currie, W. S., and Goldberg, D. E. (2022). Size-dependent analyses provide insights into the reproductive allocation and plasticity of invasive and native *Typha*. *Biol. Invasions* 24, 3799–3815. doi: 10.1007/s10530-022-02881-9
- Joyce, C. B. (2014). Ecological consequences and restoration potential of abandoned wet grasslands. *Ecol. Eng.* 66, 91–102. doi: 10.1016/j.ecoleng.2013.05.008
- Joyce, C. B., Simpson, M., and Casanova, M. (2016). Future wet grasslands: ecological implications of climate change. *Ecosyst. Health Sustain* 2, e01240. doi: 10.1002/ehs2.1240
- Klimkowska, A., van Diggelen, R., Bakker, J. P., and Grootjans, A. P. (2007). Wet meadow restoration in Western Europe: A quantitative assessment of the effectiveness of several techniques. *Biol. Conserv.* 140, 318–328. doi: 10.1016/j.biocon.2007.08.024
- Lamber, J. M. (1947). Biological flora of the British Isles: *Glyceria maxima* (Hartm.) Holmb. *J. Ecol.* 34, 310–344. doi: 10.2307/2256721
- Legendre, P. (2022). *Model II regression user's guide, R edition. lmodel2: Model II Regression. R package version 1.7-3*. Available online at: <https://CRAN.R-project.org/package=lmodel2> (Accessed May 24, 2024).
- Lenth, R. (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.2*. Available online at: <https://CRAN.R-project.org/package=emmeans> (Accessed June 3, 2024).
- Lubbe, F. C., Bitomský, M., Bartoš, M., Marešová, I., Martinková, J., and Klimešová, J. (2023). Trash or treasure: Rhizome conservation during drought. *Funct. Ecol.* 37, 2300–2311. doi: 10.1111/1365-2435.14385
- Manton, M., and Angelstam, P. (2021). Macroecology of north European wet grassland landscapes: Habitat quality, waders, avian predators and nest predation. *Sustainability* 13, 8138. doi: 10.3390/su13158138
- Menges, E. S., and Waller, D. M. (1983). Plant strategies in relation to elevation and light in floodplain herbs. *Am. Nat.* 122, 454–473. doi: 10.1086/284149
- Metcalfe, C. J. E., Rees, M., Alexander, J. M., and Rose, K. (2006). Growth-survival trade-offs and allometries in rosette-forming perennials. *Funct. Ecol.* 20, 217–225. doi: 10.1111/j.1365-2435.2006.01084.x
- Mugwedi, L. F., Goodall, J., Witkowski, E. T. F., and Byrne, M. J. (2014). The role of reproduction in *Glyceria maxima* invasion. *Afr. J. Range For. Sci.* 1–8. doi: 10.2989/10220119.2014.929177
- NOBANIS, European Network on Invasive Alien Species (2019). *Glyceria maxima*. Available online at: <https://www.nobanis.org/species-info/?taxaId=1940> (Accessed February 20 2025).
- Nurminen, L. (2003). Macrophyte species composition reflecting water quality changes in adjacent water bodies of Lake Hiidenvesi, SW Finland. *Annales Bot. Fenn.* 40, 199–208.
- Oddi, F. J., Miquez, F. E., Ghermandi, L., Bianchi, L. O., and Garibaldi, L. A. (2019). A nonlinear mixed-effects modelling approach for ecological data: Using temporal dynamics of vegetation moisture as an example. *Ecol. Evol.* 9, 10225–10240. doi: 10.1002/ecs3.5543
- Pezeshki, S. R. (2001). Wetland plant responses to soil flooding. *Environ. Exper. Bot.* 46, 299–312. doi: 10.1016/S0098-8472(01)00107-1
- Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture* (Baltimore: Johns Hopkins University Press).
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2016). *nlme: linear and nonlinear mixed effects models*. Available online at: <http://CRAN.R-project.org/package=nlme> (Accessed June 17 2024).
- Prach, K. (1993). Vegetational changes in a wet meadow complex, South Bohemia, Czech Republic. *Folia Geobot. Phytotax* 28, 1–13. doi: 10.1007/BF02853197
- Prach, K. (2008). Vegetation changes in a wet meadow complex during the past half-century. *Folia Geobot. Phytotax* 43, 119–130. doi: 10.1007/s12224-008-9011-z
- Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Schneider, H. M. (2022). Characterization, costs, cues and future perspectives of phenotypic plasticity. *Ann. Bot.* 130, 131–148. doi: 10.1093/aob/mcac087
- Shipley, B., De Bello, F., Cornelissen, J. H. C., Laliberté, E., Laughlin, D. C., and Reich, P. B. (2016). Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia* 180, 923–931. doi: 10.1007/s00442-016-3549-x
- Silvertown, J. (2004). Plant coexistence and the niche. *Trends Ecol. Evol.* 19, 605–611. doi: 10.1016/j.tree.2004.09.003
- Sorrell, B. K., Brix, H., Fitridge, I., Konnerup, D., and Lambertini, C. (2012). Gas exchange and growth responses to nutrient enrichment in invasive *Glyceria maxima* and native New Zealand *Carex* species. *Aquat. Bot.* 103, 37–47. doi: 10.1016/j.aquabot.2012.05.008
- Tallowin, J. R. B., and Jefferson, R. G. (1999). Hay production from lowland seminatural grasslands: a review of implications for ruminant livestock systems. *Grass Forage Sci.* 54, 99–115. doi: 10.1046/j.1365-2494.1999.00171.x
- Tardella, F. M., Bricca, A., Goia, I. G., and Catorci, A. (2020). How mowing restores montane Mediterranean grasslands following cessation of traditional livestock grazing. *Agric. Ecosyst. Environ.* 295, 106880. doi: 10.1016/j.agee.2020.106880
- Tasset, E., Boulanger, T., Diquélou, S., Laine, P., and Lemauiel-Lavenant, S. (2019). Plant trait to fodder quality relationships at both species and community levels in wet grasslands. *Ecol. Indic.* 97, 389–397. doi: 10.1016/j.ecolind.2018.10.035
- Tilman, D. (1982). *Resource Competition and Community Structure* (Princeton: Princeton University Press).
- Tylová-Munzarová, E., Lorenzen, B., Brix, H., Vojtková, L., and Votrubová, O. (2005). Effect of NH₄⁺/NO₃⁻ availability on nitrate reductase activity and nitrogen accumulation in wetland helophytes *Phragmites australis* and *Glyceria maxima*. *Aquat. Bot.* 55, 49–60. doi: 10.1016/j.aquabot.2005.01.006
- Weiner, J. (2004). Allocation, plasticity and allometry in plants. *Persp. Plant Ecol. Evol. Syst.* 6, 207–215. doi: 10.1078/1433-8319-00083
- Zelnik, I., and Čarni, A. (2013). Plant species diversity and composition of wet grasslands in relation to environmental factors. *Biodivers. Conserv.* 22, 2179–2192. doi: 10.1007/s10531-013-0448-x

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