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Relationships between Environmental Factors and Functional Traits of Macrophyte Assemblages in Running Waters of Greece

Konstantinos Stefanidis [1,*], Anthi Oikonomou [2], Georgios Dimitrellos [1], Dionysios Tsoukalas [1] and Eva Papastergiadou [1]

[1] Department of Biology, University of Patras, University Campus Rio, GR 26500 Patras, Greece; dimitrg@upatras.gr (G.D.); denkaklis@gmail.com (D.T.); evapap@upatras.gr (E.P.)
[2] Hellenic Centre for Marine Research, Institute of Marine Biological Resources and Inland Waters, 46.7 km of Athens—Sounio Ave., 19013 Anavyssos, Attiki, Greece; anthioikonomou@hcmr.gr
* Correspondence: kstefani@upatras.gr

Abstract: The analysis of plant trait composition has raised significant interest among freshwater ecologists as a complementary approach for assessing the effects of environmental change on ecosystem functions. In this study, we investigated patterns of functional traits of the aquatic macrophyte assemblages of 74 lotic ecosystems of Greece, and we identified associations between species traits and environmental variables (hydromorphological and physicochemical parameters) through testing the hypothesis that the environmental features determine the spatial structure of traits. We allocated 12 traits to a total of 39 hydrophyte species, and we conducted RLQ and fourth corner analysis to explore relationships between species, trait composition, and environmental gradients. Based on the results of the RLQ, a hierarchical cluster analysis was conducted to identify groups of plants that share common trait characteristics. Plants were discriminated into five discrete groups based mostly on their life form (e.g., free-floating, rooted submerged etc.) and their ecological preference for nitrogen levels. Hydromorphological parameters had a higher contribution than physicochemical variables in explaining the total variance of the trait data, with water abstraction, channel substrate, and hydrologic alteration being the most important. Our analysis did not reveal significant bivariate relationships between single traits and environmental parameters, although the five groups of macrophyte assemblages appeared to associate with certain environmental gradients. Free-floating and emergent plants were related to higher concentrations of nutrients, whereas rooted submerged plants were related to higher oxygen concentration and increased pH. In addition, free-floating plants were highly associated with metrics of hydromorphological change. Our findings showed clear discrimination of macrophytes based on their functional composition and association of traits with environmental gradients. Thus, further research could explore whether macrophyte functional groups can serve as indicators of environmental change and the overall ecosystem health.

Keywords: aquatic macrophytes; plant traits; RLQ; functional composition; life forms; rivers

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1. Introduction

Aquatic macrophytes have been widely used as indicators of ecosystem health and ecological integrity [1–3] in freshwater ecosystems. There are many studies that have shown the importance of aquatic macrophytes in enhancing ecosystem functioning, providing essential ecosystem services, and regulating the abiotic environment [4–6]. Macrophytes are also known to provide foraging and reproduction habitats for fish, amphibia, and invertebrates [7,8], promoting aquatic biodiversity. Particularly in rivers, macrophytes can influence nutrient uptake and mediate downstream transport to the coastal zone [9], reduce sediment transport into the watercourse [10–12], and regulate flow characteristics within the channel [13–15]. Thus, macrophyte communities are a fundamental component of rivers that respond to both anthropogenic and natural disturbances. In addition to species composition, plant trait composition can show strong linkages with environmental changes [16–19]. For instance, macrophytes show a variety of growth forms that reflect adaptations to their physical habitat with various implications on ecosystem processes [20]. Macrophyte growth forms show varying responses to environmental gradients [21] and have a variable tolerance to eutrophication [22], whereas increased diversity of growth forms has been shown to promote nutrient cycling in freshwater ecosystems [23].

Because of their significance, aquatic macrophytes are one of the four biological quality elements that are used for the ecological classification of streams and rivers in Europe following the implementation of the Water Framework Directive [24]. Ecological assessments are often using the taxonomic composition of the aquatic plant communities [2,25] to monitor and quantify the impact of anthropogenic environmental perturbation on the ecosystem functions and overall quality [26–30]. However, aquatic ecologists have been exploring the relationship between functional composition and abiotic environment and have acknowledged the potential benefits of incorporating trait-based approaches in ecosystem health assessment systems [31–34]. Firstly, the trait-based approaches provide scientists with complementary information about the functions of species within communities but also across communities. For instance, scientists can explore how environmental filtering, dispersal limitation, and species interactions define the community composition by determining which traits, and consequently which species, can persist in the environmental conditions at a given site. Variations in trait composition may reflect species adaptations on resource use and other habitat requirements [35]. Previous research has identified the effects of ecological processes on the composition of morphological, physiological, and life history traits of aquatic macrophytes [36,37]. Trait responses to eutrophication have been documented in several studies [38,39], indicating that functional composition can be used to examine the response of communities to environmental problems. As a result of the increased interest in the potential use of functional diversity and trait composition in ecological monitoring and river management, there is a growing number of studies that investigate various aspects of the trait distribution and/or the functional diversity of macrophyte communities across environmental gradients [16,28,39,40].

The main objective of this study is to investigate the patterns of aquatic macrophyte trait composition recorded in communities of lotic ecosystems in Greece. Our main hypothesis is that certain environmental variables associated with the trophic state and the hydromorphological habitat conditions would have a significant influence on determining functional trait composition. We applied a trait analysis framework using RLQ and fourth-corner analysis to identify discrete groups of macrophyte assemblages that share common functional traits and then to explore preliminary relationships between these functional groups and key environmental variables. We investigated the effects of water chemistry and hydromophological features on functional trait composition, and we identified macrophyte traits that are associated with the analyzed environmental factors. The results of our work can provide useful insights into distinguishing macrophyte assemblages and traits that can be used as indicators of water quality and hydromorphology with implications for the improvement of ecological assessment systems.

2. Materials and Methods

2.1. Field Samplings and Data Compilation

Presence/absence data of macrophytes from 74 river and stream sites were collected during the summer of 2021 and 2022 (Figure 1). The sampled sites belong to the national monitoring network for the ecological quality assessment of inland waters in line with the implementation of the Water Framework Directive (WFD) [41]. The sampling of macrophytes followed national protocols harmonized with European standards.

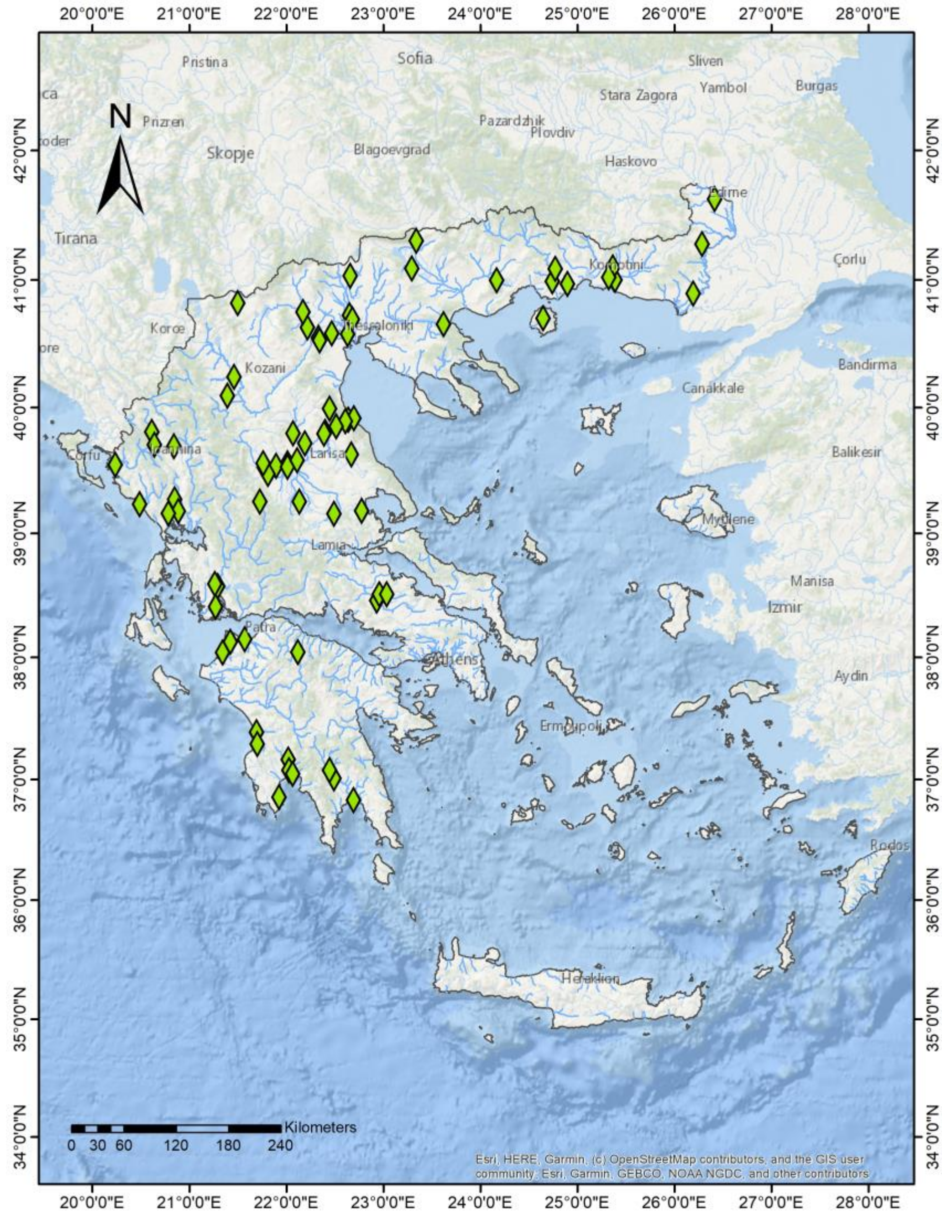

Figure 1. Map with the location of the surveyed river reaches (green squares) in mainland Greece that are part of the National Monitoring Network.

In order to assess relationships between macrophyte traits and environmental variables, we used water quality parameters and specifically measurements of physicochemical variables (electrical conductivity, pH, dissolved oxygen concentration, and total dissolved solids) and nutrients (nitrate, ammonium, and total phosphorus). Furthermore, hydromorphological characteristics at the reach scale, such as channel cross-section alteration, water abstraction, presence of dykes, hydrological alteration, etc., were assessed according to Feio et al. [42] and Stefanidis et al. [25] (Table 1). Hydromorphological parameters were then transformed into continuous variables with optimal scaling and the aspect package [43] in the R environment. For more details on samplings, see our previous publications [25,26].

Table 1. Description of the environmental variables that were considered in the present study. For hydromorphological variables, shorter names that are used in figures are given in parentheses.

Category	Variable Name	Description
Physicochemical	EC	Electrical conductivity [µS/cm]
	pH	Sorensen scale
	DO	Concentration of dissolved oxygen [mg/L]
	Nitrate	Nitrate concentration in the water [mg/L NO_3^-]
	Ammonium	Ammonia concentration in the water [mg/L NH_4^+]
	TP	Concentration of total phosphorus in the water [mg/L P]
	TDS	Concentration of total dissolved solids [mg/L]
Hydromorphological	Channel substrate (Substrate)	Prevailing channel substrate, three levels: Fine (<2 mm), medium (2–64 mm), coarse (>64 mm)
	Bed stability (Stability)	Stability of the riverbed using four levels: Solid (e.g., bedrock), stable, unstable, soft (e.g., mud)
	Shade	Channel shade using three levels: Absence of shade, semi-continuous shade, full shade
	Habitats	Type of river habitat: Pool, riffle, run, slack
	Channel profile alteration (Profile alt.)	Degree of channel profile modification present at the site/cross-section alteration
	Morphology alteration (Morphology alt.)	Degree of the morphological modification of the channel present at the site
	Habitat alteration (Habitat alt.)	Alteration of instream habitats
	Stream hydrology alteration (Hydrology)	Degree of the hydrological alteration present at the site
	Water abstraction (Abstraction)	Influence of water abstraction at the site
	Dykes	Influence of dykes at the site

2.2. *Allocation of Traits*

We allocated 12 traits to a total of 39 hydrophyte species (Table 2). We chose traits that could be extracted from the literature [44–46] and covered various macrophyte features, including life form, morphology, dispersal, and ecological preference. The life forms were divided into six categories according to Wilby et al. [45]: free-floating on the surface (ffsur), free-floating submerged (ffsub), anchored with floating leaves (afl), anchored with submerged leaves (asl), and amphibious species with emergent leaves (ael) and heterophyllous emergent leaves (ahet). Then scores were assigned to each life-form trait using a 0–3 coding scheme based on a fuzzy-coding approach [45,47]. For ecological preference, we used ecological indicator values for nitrogen and light (Ellenberg N; EN, Ellenberg L; EL). The leaf and fruit sizes were classified into three categories (small, moderate, and large) [45]. Reproduction by rhizome was included as a dispersal-related trait (presence, absence). The morphology index that is based on the height and lateral extension of the canopy was also used.

Table 2. Overview of the aquatic macrophyte traits used in the present study.

Trait Code	Trait Name	Category	Values
EN	Ellenberg N—nitrogen preference	Ecological preference	1: low nutrients, 5: intermediate levels of nutrients, 9: rich conditions of nutrients
EL	Ellenberg L—light preference	Ecological preference	1: deep shade, 5: semi-shade, 9: full light
ffsur	Free-floating, surface	Life form	0: no affinity to trait, 1: low affinity, 2: high affinity, 3: exclusive affinity to trait
ffsub	Free-floating submerged	Life form	
afl	Anchored floating leaves	Life form	
asl	Anchored submerged	Life form	
ael	Anchored emergent	Life form	
ahet	Anchored, heterophylly	Life form	
LS	Leaf size	Morphology	1: <1 cm^2, 2: 1–20 cm^2, 3: 20–100 cm^2, 4: >100 cm^2
FS	Fruit size	Morphology	1: <1 mm, 2: 1–3 mm, 3: > 3 mm
MI	Morphology Index	Morphology	1: low, 5: high
rhiz	Reproduction by rhizome	Dispersal	0: absence, 1: presence

2.3. RLQ and Fourth-Corner Analysis

We conducted RLQ analysis to explore the relationships among macrophyte species composition, species traits, and environmental variables [48]. RLQ is an extension of co-inertia analysis that searches for a combination of traits and environmental variables of maximal co-variance, which is weighted by the presence/absence or the abundance of the species in plots [49]. This analysis provides a more general co-variation pattern between traits and environment without any a priori assumption regarding explanatory variables. The method is based on a three-step ordination procedure in which the relationship between environmental variables (R), species presence/absence or abundances (L), and associated traits (Q) are combined into major linear correspondence axes. First, a correspondence analysis (CA) was computed on the species matrix (L). Then, a Hill–Smith ordination was conducted for traits (Q) since traits were considered as a mix of quantitative and factor variables, while the environmental variables (R) were ordinated using a principal component analysis. Both Hill–Smith and PCA ordinations were constrained by the axis of the CA (rows for R and columns for Q). The overall significance of this relationship was tested using a global Monte-Carlo test of the table rows of R and those of Q. The contribution of each trait and environmental parameter to total inertia was used as a measure of relative importance and a criterion to identify the most important traits and environmental factors.

In addition, we performed a fourth corner analysis to test for significant bivariate associations between individual traits and environmental variables. The analysis was conducted based on 10,000 permutations and using model 6, a combination of models 2 and 4, as suggested by Dray and Legendre [50]. *P* values were adjusted with the false discovery rate method, according to Dray et al. [51]. Fourth corner analysis was also conducted on the RLQ results to test for significant relationships between the first two RLQ axes for environmental gradients (AxR1/AxR2) and traits and between the first two RLQ axes for trait syndromes (AxQ1 and AxQ2) and environmental variables.

Finally, hierarchical clustering was conducted on the RLQ species scores to identify functional groups of species. The optimum number of clusters (5 in our case) was selected after performing the Kelley–Gardner–Sutcliffe penalty function [52]. The relationships between the functional groups and the environment were assessed considering the results of the prior analyses (RLQ and fourth corner). All analyses were conducted using the ade4 package in the R environment [53].

3. Results

3.1. RLQ and Fourth Corner Analysis

The first two axes of the RLQ summarize the relationships between traits and environment, explaining approximately 71 and 10% of the cross-variance between traits and water quality parameters. The analysis yielded a *p*-value ≤ 0.005, which indicates that the links between the species, traits, and environment matrices are statistically significant. The position of the plants (points) on the plots of Figure 2A highlights the associations between plants, traits, and the water quality variables. Plants that are free-floating at the surface and have a low morphology index (e.g., *Lemna minor, Lemna gibba, Spirodela polyrhiza*) are located at the bottom left side of the plot. Rooted plants with either submerged or floating leaves and high MI are mostly located at the top and right side of the plot (e.g., *Potamogeton natans, Potamogeton perfoliatus, Myriophyllum spicatum*). Plants with emergent leaves (helophytes), such as *Oenanthe aquatica, Mentha aquatica* and *Nasturtium officinale*, and rooted floating-leaved plants (*Nymphaea alba* and *Nuphar lutea*) with high values of EL and leaf size, are located at the bottom part of the plot (Figure 2B).

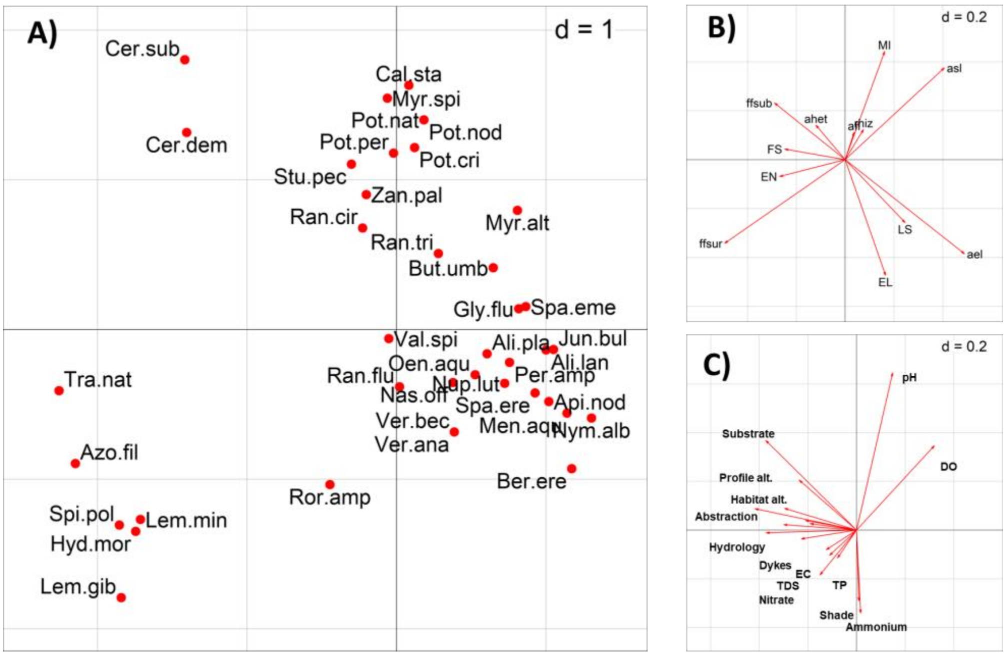

Figure 2. Results of the first two axes of RLQ analysis: (**A**) species scores, (**B**) coefficients for the traits, and (**C**) coefficients of the environmental variables. The 'd' values give the grid size for scale comparison across the three figures. Trait abbreviations are given in Table 2. Species codes are given in Appendix A.

The first RLQ axis was related strongly to hydromorphological variables, namely water abstraction, substrate type, hydrological alteration, and habitat alteration. The second axis was mostly associated with DO, pH, and ammonium concentration (Figure 2C). Regarding the relative contribution of the environmental variables, water abstraction and the channel substrate type had the largest contribution to the total inertia, followed by hydrological alteration and dissolved oxygen concentration (Table 3). Among macrophyte traits, the life forms rooted with emergent leaves, free-floating at the surface and rooted submerged contributed the most to total inertia (Table 4). Thus, combining the results of Figure 2 and Table 4, we see that the first axis of Panel C represents a strong gradient of hydromorphological change, whereas the second axis reflects changes in water quality. Species that are tolerant to hydromorphological alterations are located at the left part of the plot, and species that are tolerant to high nutrients and turbidity are positioned to the bottom part of the plot.

Table 3. RLQ analysis summary outputs: eigenvalues and percentage of total co-inertia, the ratio of inertia and co-inertia for R (the environmental variable matrix), Q (the species traits matrix) and correlation with the L matrix (species), for Axis 1 and Axis 2.

	Axis 1	**Axis 2**
Eigenvalues decomposition	0.71	0.10
% of total co-inertia	76.86	10.39
Inertia and co-inertia R (env)	3.79	5.33
Inertia and co-inertia Q (trait)	2.55	4.99
Correlation L (sp)	0.27	0.15

Table 4. Percentages of the contribution of the environmental variables and traits to the RLQ analysis.

Environmental Variable	**Contribution to Total Inertia (%)**	**Macrophyte Trait**	**Contribution to Total Inertia (%)**
Water abstraction	13.84	Anchored emergent leaves	20.34
Channel substrate	12.78	Free-floating, surface	20.23
Hydrological alteration	11.04	Anchored submerged	14.96
Dissolved oxygen	9.57	Ellenberg N	8.02
Habitat type	7.41	Free-floating submerged	7.33
Habitat alteration	7.16	Fruit size	6.92
pH	6.30	Leaf size	5.86
Nitrate	5.32	Morphology index	4.82
Channel profile alteration	5.11	Ellenberg Light	4.71
Dykes influence	4.27	Anchored floating leaves	2.52
Channel morphological alteration	4.04	Anchored, heterophylly	2.22
Stability	3.48	Rhizome	2.03
Total phosphorus	3.18		
Ammonium	1.91		
Channel shade	1.65		
Conductivity	1.56		
Total dissolved solids	1.36		

The fourth-corner analysis did not reveal any significant bivariate relationships between individual environmental parameters and traits after adjusting p-values with the false discovery rate method. However, RLQ axis 1 was significantly related positively with the rooted submerged, rooted emergent, and the leaf size and negatively with the free-floating life forms (surface and submerged), the fruit size, and the Ellengberg nitrogen indicator. The second axis was significantly positively associated with the rooted submerged life form and the morphology index and negatively with the emergent leaves' life form and the Ellenberg light indicator. Concerning the relationships between the two axes and the environmental variables, the fourth-corner results showed significant negative relationships between axis 1 and almost all the hydromorphological parameters, whereas axis 2 was positively associated only with pH (Figure 3). These findings corroborate the RLQ analysis suggesting that axis 1 reflects a strong hydromorphological gradient and axis 2 a weaker physicochemical gradient.

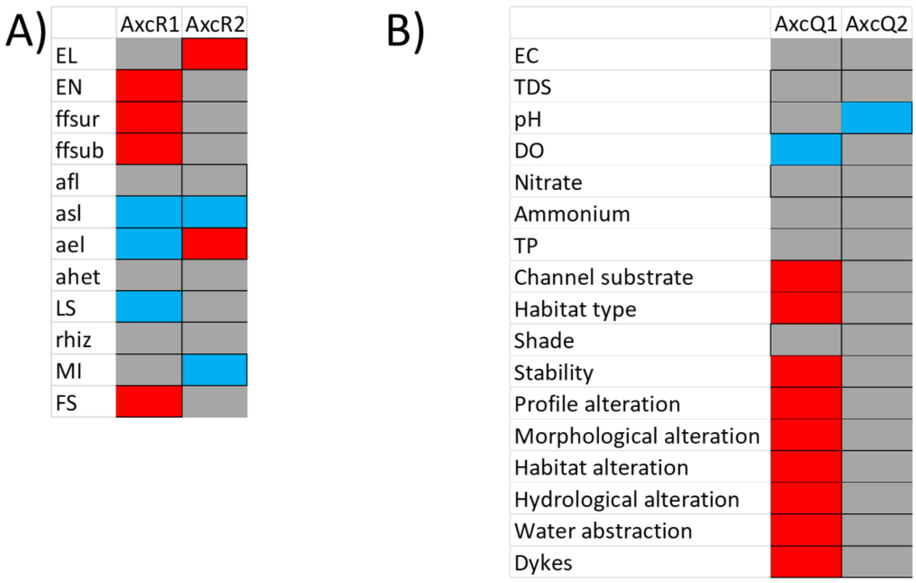

Figure 3. Results of fourth-corner tests for the first two RLQ axes and (**A**) traits and (**B**) environmental variables. Blue color indicates significant positive associations, and red indicates significant negative associations at 0.05 level of significance.

3.2. Macrophyte Functional Groups

The 39 aquatic macrophytes were classified into five discrete functional groups of macrophyte assemblages according to the hierarchical clustering of the RLQ species scores (Figure 4). Group A includes macrophytes of the pondweed family (Potamogetonaceae) with three rooted submerged plants with high MI (*Potamogeton perfoliatus, Potamogeton crispus, Stuckenia pectinata*) and two species with floating leaves plants (*Potamogeton natans* and *Potamogeton nodosus)*. Another group (Group B) consists of just two species (*Ceratopyllum demersum*, *Ceratophyllum submersum*) which are free-floating submerged plants, commonly found in many freshwater ecosystems. The largest group (Group C) consists of emergent plants (e.g., *Mentha aquatica, Alisma lanceolatum, Apium nodiflorum, Persicaria amphibia*) and plants with large floating leaves (*Nymphaea alba* and *Nuphar lutea*). Two submerged rooted species, *Ranunculus trichophyllus* and *Myriophyllum alterniflorum*, also belong to this group. Five species are grouped together into a discrete group (Group D) and are positioned closely across the 2nd axis (bottom part). These are three emergent and two submerged macrophytes (*Vallisneria spiralis* and *Ranunculus fluitans*). Finally, a well-separated group (Group E) consists of five small-sized, surface free-floating plants (*Lemna minor, Lemna gibba, Salvinia natans*, *Hydrocharis morsus-ranae,* and *Azolla filiculoides*) and *Trapa natans*, a larger plant usually found free-floating. The biplot in Figure 5 shows the relationships between the five functional groups of macrophyte assemblages and the vectors of the environmental variables. Group A is associated negatively with the vectors of hydromorphological alteration and the substrate type indicating a preference of these plants for less hydromorphologically disturbed conditions and coarser substrates. They also occur in oxygenated waters with low content of nitrogen and phosphorus, which suggests that group A taxa are more likely to occur in good water quality conditions, although *Stuckenia pectinata* can be found in various habitats ranging from low-flowing eutrophic waters to fast-flowing clear waters. Group B, which consists of *Ceratophyllum* taxa, shows an affinity to sites that are influenced by hydromorphological changes and are characterized by finer substrates (e.g., mud or silt). Plants that belong to groups C and D seem to require better hydromorphological conditions, with coarser substrates but are more tolerant to adverse physicochemical conditions than the taxa of group A. Possibly, these assemblages contain plants that are found in sites that are less disturbed and may preserve a diverse riparian flora (e.g., emergent plants) but also hydrophytes (floating-leaved and submerged) that are less tolerant to hydrological and habitat degradation. For instance, *Myriophyllum alterniflorum*, *Ranunculus fluitans,* and

Vallisneria spiralis usually grow in lotic habitats with low turbidity, good light conditions, and flowing waters. Finally, group E plants, which are free-floating plants, are related to impaired sites in terms of both hydromorphology and water quality.

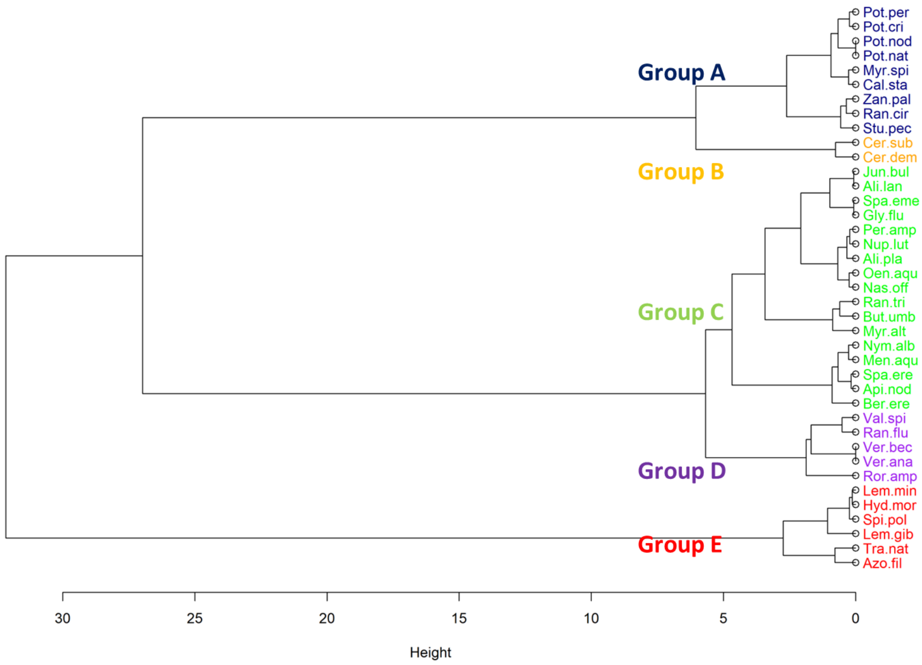

Figure 4. Dendrogram obtained from hierarchical clustering based on the species distances. Colors correspond to the five functional groups (A, B, C, D, and E) derived after the calculation of the Kelley–Gardner–Sutcliffe penalty.

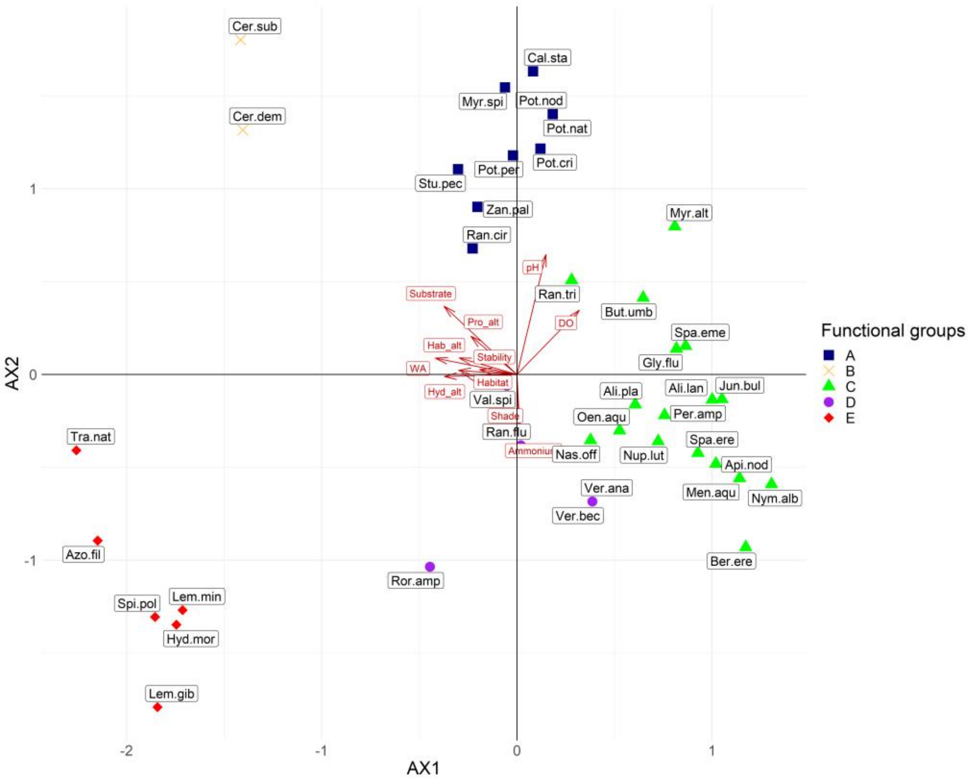

Figure 5. Biplot shows the relationships between the functional macrophyte groups (A, B, C, D, and E) derived from hierarchical clustering and the environmental parameters.

4. Discussion

In this study, we employed a combined analytical framework using RLQ and fourth-corner analysis, and we found indications of a clear separation of macrophytes assemblages based on the associations between traits and environmental variables. Our findings showed clear discrimination of macrophytes into five groups where free-floating and emergent plants were associated with high nutrients and submerged macrophytes were associated with high oxygen concentration. In addition, free-floating plants showed an affinity for sites with hydromorphological alterations (e.g., channel profile and habitat alteration). Although the fourth-corner analysis did not reveal significant bivariate associations between individual traits and environmental variables, our results showed significant relationships between the matrices of traits and environment. We also found that hydromorphological parameters had a very high contribution to explaining the total variance of the trait data. However, these parameters were qualitative and included mainly assessments of hydromorphological features and modifications (e.g., profile alteration, habitat alteration, channel shade, bed stability, and others). Expanding the environmental dataset with additional quantitative parameters on the river sediment geochemistry and sediment size distribution could reveal further significant relationships between macrophyte traits and the lotic environment.

Nevertheless, the significant associations that we found between hydromorphological variables, certain water quality variables (namely dissolved oxygen and pH), and the RLQ axes indicate environmental gradients that influence the position of the species based on their traits along the two axes. Hydromorphological modifications and hydrological alterations are known to influence various aspects of aquatic plant diversity, including traits and life forms [26,28,30,54]. In this study, we found that water abstraction and hydrological alteration were the parameters with the highest contribution in explaining the total variation of the trait data, which indicates a strong influence of the flow regime on plant communities. Interestingly, free-floating plants (submerged and surface) were associated with a high degree of hydrological and habitat alteration, along with changes in channel profile. One possible explanation is that river simplification, which means that rivers lose their natural planform and complex physical structures (e.g., meanders and floodplain lakes), transforms the natural river corridors into slow-flowing linear deep channels that favor the overgrowth of free-floating lemnids and ceratophyllids. Recently, a study by Gebler and Szoszkiewicz [55] highlighted that *Lemna minor* and *Spirodela polyrhiza* were among the most frequent plants in heavily hydromorphologically modified systems, which was attributed to a combination of factors including low water level and accumulation of nutrients which often coincides with hydromorphological modifications. In Greece, river resectioning and overdeepening are common practices that aim to protect adjacent agricultures from floods and are considered the main source of hydromorphological modifications [56]. Apparently, agriculture is the main driver of river habitat degradation that impairs rivers through water abstraction for irrigation, morphological modifications for flood control, and nutrient enrichment with the use of fertilizers. These pressures shape favorable conditions for pleustophytes, such as nutrient-rich waters and low flows, which allow these plants to thrive and dominate within the channel.

In addition, our results showed that free-floating plants are less likely to occur in rich oxygenated waters. On the contrary, rooted submerged plants were highly associated with high concentrations of oxygen and pH levels. These findings highlight the contrasting effect of different life forms on water chemistry and vice versa, as has been documented in previous studies [57–59]. Oxygen dynamics are associated with metabolic processes and the balance between productivity and respiration rates [60]. For instance, there are studies that have shown significant variations in metabolic rates and oxygen levels among macrophyte stands from different habitats [58,61,62]. More specifically, macrophyte stands with floating-leaved plants can limit the light availability in deeper water, hampering productivity and oxygen levels [61,63]. An experimental study by De Tezanos Pinto et al. [64] showed that shading caused by free-floating leaved plants had a substantial effect on lowering oxygen concentration significantly, even at hypoxic levels. Furthermore, dense beds of submerged plants can significantly raise pH levels during daylight due to CO_2 depletion caused by photosynthesis. These examples show that aquatic plants act as ecosystem engineers altering the physicochemical properties of the water, among other things [62,65]. Bearing this in mind, the interpretation of significant relationships between plants and water physicochemical variables should not be limited to environmental filtering that may drive the community assembly, but it should be extended to include the capability of aquatic plants to actively interact with the environment and modify the water chemistry. Free-floating and several emergent plants were additionally related to nitrate and TP, which implies an occurrence of these species in waters that are affected by nutrient pollution (e.g., lowland rivers in agricultural landscapes). In general, emergent and pleustophytes are usually found in eutrophic waters, as opposed to submerged species that require increased water clarity [57]. Interestingly, a similar analysis conducted for the lakes of Greece [27] revealed phosphorus as a more important driver than nitrogen in shaping macrophyte trait composition. Hence, our findings highlight that in the lotic ecosystems of Greece, nitrogen influents from sources of diffuse pollution (e.g., agriculture) are of key importance as opposed to the role of phosphorus which is more important in lakes [32,66]. Furthermore, turbidity in lakes plays a major role in filtering macrophyte communities by promoting plants that withstand growth in limited light conditions [34,67], whereas in our case, it had a small contribution to the total inertia and a non-significant association with the first axis of the RLQ analysis. Probably, due to the dynamic nature of lotic ecosystems, biotic turbidity is not as likely to occur and persist as it is for lentic ecosystems. In addition, most of the studied systems are relatively shallow, allowing the light to reach the bottom.

Apart from the life forms, other trait characteristics, such as Ellenberg N, Ellenberg L, morphology index, and fruit size, presented significant associations with the RLQ axes. High values of MI appeared to relate with rooted submerged plants, a finding that could indicate that these plants were more likely to form extensive mats (higher height and lateral extension of the canopy). These extensive macrophyte stands could also explain the higher pH and concentration of oxygen due to the increased photosynthetic activity.

Based on the functional trait composition and their relationship with the environmental variables, macrophytes were successfully distinguished into five groups. The macrophyte assemblage with the surface free-floating plants was probably the most discrete, and it was the one that showed a clear association with both hydromorphological and water quality parameters. Thus, we can assume that free-floating plants can occur in heavily impaired systems in terms of hydromorphological and physicochemical conditions, which provides a very useful conclusion for assessment purposes. Lemnids, for instance, are known to dominate in eutrophic waters, covering large areas and causing oxygen depletion [68] due to their very high nutrient uptake and growth rate [69]. Probably, anthropogenic interventions in the natural shape and the hydrology of the river may promote low-level and stagnant conditions that boost the aggregation of the pleustophytes. Rooted submerged plants were also well separated into a group by a high morphology index and an association with oxygenated waters and low nutrient content. Overall, the results of the trait-based analysis herein, besides their ecological importance, can be used to classify macrophytes into groups that act as indicators of water quality. Thus, this study incorporating traits has utility from both an ecological and an applied perspective and can serve as the basis for developing a macrophyte trait-based index that reflects the community responses to environmental changes.

5. Conclusions

In this article, we investigated the associations between the traits of macrophytes and environmental gradients. Plants were discriminated against in their life form, ecological preference for nitrogen and light, morphology index, fruit size, and leaf size into five discrete groups of macrophyte assemblages that were associated strongly with hydromorphological variables. Free-floating plants were clearly related to hydromorphological alterations, high nutrient, and low oxygen conditions, whereas rooted submerged plants preferred rich oxygenated waters with lower nutrient content and less disturbed hydromorphological conditions. By applying a trait-based approach to explore macrophyte community responses to both hydromorphological and water quality parameters, our results showed that macrophyte functional groups have the potential to be used as indicators of overall environmental impairment. Further research can focus on developing a macrophyte trait-based index that reflects the ecological interactions and processes that affect assemblages' structure along environmental gradients in riverine ecosystems and the community responses to environmental changes. By broadening the environmental gradients and including more metrics of environmental pressure, it is more likely to identify processes that filter aquatic macrophytes through the selection of traits with a competitive adaptation to certain environmental conditions. In conclusion, our findings suggest that the use of trait-based analyses in ecological monitoring can be effective, and it should be considered at least as a complementary approach to the current ecological assessment methods.

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Appendix A

Table A1. List of aquatic macrophyte species considered in the present study. Species codes that were used for presentation of the results in tables and figures are also given.

Code	Name	Code	Name
Ali.lan	*Alisma lanceolatum* With.	Oen.aqu	*Oenanthe aquatica* L.
Ali.pla	*Alisma plantago-aquatica* L.	Per.amp	*Persicaria amphibia* (L.) Gray
Api.nod	*Apium nodiflorum* (L.) Lag.	Pot.cri	*Potamogeton crispus* L.
Azo.fil	*Azolla filiculoides* Lam.	Pot.nat	*Potamogeton natans* L.
Ber.ere	*Berula erecta* (Huds.) Coville	Pot.nod	*Potamogeton nodosus* Poir.
But.umb	*Butomus umbellatus* L.	Pot.per	*Potamogeton perfoliatus* L.
Cal.sta	*Callitriche stagnalis* Scop.	Ran.flu	*Ranunculus fluitans* Lam.
Cer.dem	*Ceratophyllum demersum* L.	Ran.tri	*Ranunculus trichophyllus* Chaix ex Vill.
Cer.sub	*Ceratophyllum submersum* L.	Ror.amp	*Rorippa amphibia* (L.) Besser
Gly.flu	*Glyceria fluitans* (L.) R.Br.	Sal.nat	*Salvinia natans* (L.) All
Hyd.mor	*Hydrocharis morsus-ranae* L.	Spa.eme	*Sparganium emersum* Rehmann
Jun.Bul	*Juncus bulbosus* L.	Spa.ere	*Sparganium erectum* L.
Lem.gib	*Lemna gibba* L.	Spi.pol	*Spirodela polyrhiza* (L.) Schleid.
Lem.min	*Lemna minor* L.	Stu.pec	*Stuckenia pectinata* (L.) Böerner
Men.aqu	*Mentha aquatica* L.	Tra.nat	*Trapa natans* L.

Table A1. *Cont.*

Code	Name	Code	Name
Myr.alt	*Myriophyllum alterniflorum* DC.	Val.spi	*Vallisneria spiralis* L.
Myr.spi	*Myriophyllum spicatum* L.	Ver.ana	*Veronica anagalis-aquatica* L.
Nas.off	*Nasturtium officinale* W.T.Aiton	Ver.bec	*Veronica beccabunga* L.
Nup.lut	*Nuphar lutea* (L.) Sm.	Zan.pal	*Zannichellia palustris* L.
Nym.alb	*Nymphaea alba* L.		

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