

Article



Konstantinos Stefanidis ^{1,2,*} and Eva Papastergiadou ²

- Hellenic Centre for Marine Research (HCMR), Institute of Marine Biological Resources and Inland Waters, 46.7 km of Athens—Sounio Ave., 19013 Anavyssos, Attiki, Greece
- ² Department of Biology, School of Natural Sciences, University of Patras, University Campus Rio, GR 26500 Patras, Greece; evapap@upatras.gr
- * Correspondence: kstefanidis@hcmr.gr; Tel.: +30-229-107-6439

Received: 19 April 2019; Accepted: 16 May 2019; Published: 20 May 2019



Abstract: Freshwater ecologists have shown increased interest in assessing biotic responses to environmental change using functional community characteristics. With this article, we investigate the potential of using functional traits of the aquatic plants to assess eutrophication in freshwater lakes. To this end we collected macrophyte and physicochemical data from thirteen lakes in Greece and we applied a trait-based analysis to first identify discrete groups of macrophytes that share common functional traits and then to assess preliminary responses of these groups to water quality gradients. We allocated 11 traits that cover mostly growth form and morphological characteristics to a total of 33 macrophyte species. RLQ and fourth corner analysis were employed to explore potential relationships between species, trait composition and environmental gradients. In addition, a hierarchical cluster analysis was conducted to discriminate groups of plants that share common trait characteristics and then the position of the groups along the environmental gradients was assessed. The results showed total phosphorus, chlorophyll-a, conductivity, pH and Secchi disk depth as main drivers of the environmental gradients. Hierarchical cluster analysis showed a clear separation of macrophyte assemblages with discrete functional characteristics that appeared to associate with different environmental drivers. Thus, rooted submerged plants were related with higher Secchi disk depth, conductivity and alkalinity whereas rooted floating-leaved plants showed a preference for enriched waters with phosphorus and nitrogen. In addition, free-floating plants were related positively with nitrogen and increased pH. Although we did not identify specific trait patterns with environmental drivers, our findings indicate a differentiation of macrophytes based on their functional characteristics along water quality gradients. Overall, the presented results are encouraging for conducting future monitoring studies in lakes focused on the functional plant trait composition, as expanding the current approach to additional lakes and using quantifiable functional characteristics will provide more insight about the potential of trait-based approaches as ecological assessment systems.

Keywords: aquatic macrophytes; functional traits; eutrophication; RLQ analysis; fourth corner analysis; lakes

1. Introduction

Aquatic macrophytes are known to play a key role in lake ecosystems influencing significantly their functioning. There are many studies that have underpinned the importance of aquatic macrophytes in regulating the nutrient availability in the water and enhancing the stability of lakeshores [1,2]. More importantly, species-rich aquatic plant communities are strongly associated with a clear-water state,



particularly in shallow lakes where macrophytes can colonize a large share of the lake bottom [2,3]. Not surprisingly, aquatic macrophytes are used in monitoring the ecological status of surface waters throughout Europe [4–7]. Lately, several studies have developed ecological assessment systems based on compositional characteristics of aquatic plant communities, since certain species seem to correlate directly with environmental gradients and capture changes of trophic status [8–11].

Yet, recently freshwater scientists have gained interest in using functional community characteristics as an alternative or a complement method to compositional characteristics for assessing biotic responses to environmental change [12]. These trait-based approaches use functional trait characteristics, which are defined as the morphological, physiological and phenological features measurable at the individual level [13,14] to identify how environmental factors shape species assemblages. The ecological theory behind the trait-based approaches predicts that strong environmental filters in a freshwater system are likely to constrain the range and variance of certain functional traits favoring the species from the regional pool that have those traits enabling them to persist in the particular site [15,16]. In practice, this implies that changes in community trait composition are likely linked with changes in species composition and richness that indicate the presence of anthropogenic or natural drivers of change. In addition, assessments based on traits can be advantageous over compositional approaches, since the same traits can be applied to species regardless of geographic region and location [17]. To date, traits of freshwater biota have been widely used to describe their response to the environment as different traits may reflect different aspects of the resource use and habitat requirements of the species [18,19]. Regarding plants, research has focused on several traits that are related to life-form characteristics, growth forms, growth rates, leaf morphology and even to photosynthetic and biochemical pathways in order to identify responses to environmental conditions [20,21]. Particularly for aquatic submersed macrophytes, broad morphological, physiological and life history traits have been linked with effects on ecological, biogeochemical and physical processes [12,22]. Examples of such research include the study of aquatic plant communities' responses to eutrophication and habitat degradation [23,24]. These works suggested that eutrophication favors species that concentrate their photosynthetic biomass near the water surface in order to capture light efficiently. Another example is the elongated shoot height and increased specific leaf area to increasing water depth as it has been reported in several articles [15,22,25]. Therefore, examining the potential use of functional trait composition as a response of environmental change becomes essential for understanding the response of communities to change [26,27], as well as identifying key trait characteristics of sensitive species to anthropogenic pressures.

With this article, we aim to investigate whether functional trait composition of the aquatic plant communities can be used to evaluate the water quality degradation in freshwater lakes. Our aim is twofold as we utilize data from thirteen lakes in mainland Greece to first identify discrete groups of macrophytes that share common functional trait characteristics, and second to assess their preliminary responses to water quality gradients. We expect that the results of this work can be used to effectively identify macrophytes that can serve as indicators of water quality degradation, with possible implications for the development or improvement of macrophyte-based assessment systems of the ecological status.

2. Materials and Methods

2.1. Description of Data

The current research included thirteen lakes of mainland Greece (Figure 1), twelve of which are natural, and one is an artificial dam lake /reservoir (Lake Kerkini). Table 1 lists basic information about the morphology and the location of the lakes. All the studied lakes are included in the Pan-European network of Protected Areas "Natura 2000" as Sites of Community Importance (SCI), Special Areas of Conservation (SAC), and Special Protected Areas (SPA) [28]. In addition, the Prespa lakes and the artificial lake Kerkini are characterized as "Wetlands of International Importance under the Ramsar Convention". For a detailed description of the studied areas, see also Stefanidis et al. [28–30].

Water quality, compiled of seven environmental parameters, and macrophyte data, consisted of a species presence/absence, were obtained from published articles and dissertation theses [8,10,28,29,31,32], as well as the Greek National Monitoring Network in line with the implementation of WFD (Table 2). In this work, aquatic macrophytes of the growth form isoetids, elodeids, nymphaeids, and lemnids (hydrophytes) were considered for functional trait analysis. This dataset includes vascular species of macrophytes, as well as, two aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia natans* (L.) All.) which share many similar morphological characteristics with duckweeds.



Figure 1. Map showing the location of the studied lakes in Greece. 1: Lysimachia, 2: Trichonida, 3: Ozeros, 4: Amvrakia, 5: Pamvotida, 6: Kastoria, 7: Zazari, 8: Volvi, 9: Vegoritida, 10: Petron, 11: Mikri Prespa, 12: Megali Prespa, 13: Kerkini.

Table 1. Geographic location, altitude, and morphological descriptors of the studied lakes (Source [29]).

Lake	Longitude	Latitude	Altitude (m a.s.l)	Surface Area (km²)	Mean Depth (m)	Max Depth (m)
Lysimachia	21°28′	38°34′	16	13.5	3.9	9
Trichonida	21°28′	38°34′	18	96.5	30.3	58
Ozeros	21°13′	38°39′	22	9.4	3.1	6.1
Amvrakia	21°11′	38°45′	28	14.5	30	53
Pamvotida	20°53′	39°40′	470	22.0	5.5	11
Kastoria	21°18′	40°30′	629	30.0	4.8	9.1
Zazari	21°33′	40°37′	602	2.0	1.7	5.5
Volvi	23°20′	40°41′	37	68.6	13.5	23.5
Vegoritida	21°45′	40°45′	524	53.0	28.9	70
Petron	21°45′	40°45′	527	14.4	2.6	5
Mikri Prespa	21°05′	40°45′	853	53.0	4.1	8.4
Megali Prespa	21°01′	40°46′	852	266.0	30	55
Kerkini	23°09′	41°11′	30	70	3.1	10

Code	Latin Name	Code	Parameter
Azo.fil	Azolla filiculoides Lam.	Alk.	Alkalinity (mEq/L)
Cal.sta	<i>Callitriche stagnalis</i> Scop.	TP	Total phosphorus (µg/L)
Cer.dem	Ceratophyllum demersum L.	Ν	Nitrate (µg/L)
Cer.sub	Ceratophyllum submersum L.	EC	Electrical conductivity (µS/cm)
Hyd.mor	Hydrocharis morsus-ranae L.	Chla	Chlorophyll-a (µg/L)
Lem.min	Lemna minor L.	pН	pH
Lem.tri	Lemna trisulca L.	SD	Secchi depth (m)
Lem.gib	Lemna gibba L.		-
Myr.spi	Myriophyllum spicatum L.		
Myr.ver	Myriophyllum verticillatum L.		
Naj.mar	Najas marina L.		
Nup.lut	Nuphar lutea (L.) Sm.		
Nym.alb	Nymphaea alba L.		
Nym.pel	<i>Nymphoides peltata</i> (S.G. Gmel.) Kuntze		
Per.amp	Persicaria amphibia (L.) Gray		
Pot.cri	Potamogeton crispus L.		
Pot.luc	Potamogeton lucens L.		
Pot.nat	Potamogeton natans L.		
Pot.nod	Potamogeton nodosus Poir.		
Stu.pec	Potamogeton pectinatus (L.) Böerner		
Pot.per	Potamogeton perfoliatus L.		
Pot.pus	Potamogeton pusillus L.		
Ran.bau	Ranunculus baudotii Godr.		
Ran.cir	Ranunculus circinatus Sibth.		
Ran.flu	Ranunculus fluitans Lam.		
Ran.tri	Ranunculus trichophyllus Chaix ex Vill.		
Sal.nat	Salvinia natans (L.) All		
Spi.pol	Spirodela polyrhiza (L.) Schleid		
Tra.nat	<i>Trapa natans</i> L.		
Utr.aus	Utricularia australis R.Br.		
Utr.vul	Utricularia vulgaris L.		
Val.spi	Vallisneria spiralis L.		
Zan.pal	Zannichellia palustris L.		

Table 2. List of aquatic macrophyte species and environmental variables considered in the present study.

2.2. Functional Traits

We used functional traits of aquatic plants following published studies in the literature [33,34]. We allocated 11 traits to a total of 33 hydrophyte species. The traits covered mostly life-form characteristics and morphology features. Dispersal and ecological preference were represented as reproduction by rhizome and ecological indicator values of nutrients respectively (Ellenberg N; EN). The life-forms were divided into six categories according to Willby et al. [34]: Free floating on the surface (ffsur), free floating submerged (ffsub), anchored with floating leaves (af), anchored with submerged leaves (asub) and amphibious species with homophyllus emergent leaves (aeme) and heterophyllus emergent leaves (ahet). We used a 0–3 coding scheme based on a fuzzy-coding approach [35] for assigning scores in each life-form trait (Table 3). Growth morphology, which described the position of the meristem growth point, was divided into three categories: Single basal, single apical and multi-apical. In addition, the leaf area of the species and a morphology index based on the height and lateral extension of the canopy were included as morphological related traits. For these two traits some species were classified in-between two categories which resulted in in-between scores (i.e., 1.5, 2.5, 3.5, and 4.5).

Trait Code	Trait Name	Category	Values
EN	Ellenberg N—nitrogen preference	Ecological preference	1 = deep shade, 5 = semi shade, 9 = full light
ffsur ffsub af asub aeme ahet	Free-floating, surface Free-floating submerged Anchored floating-leaves Anchored submerged Anchored emergent Anchored, heterophylly	Life form	0 = no affinity to trait, 1 = low affinity, 2 = high affinity, 3 = exclusive affinity to trait
gp leaf MI	Growth morphology Leaf area Morphology Index	Morphology	1 = single basal growth, 2 = single apical growth, 3 = multiple apical growth $1 \le 1 \text{ cm}^2$, 2 = 1-20 cm ² , $3 = 20-100 \text{ cm}^2$, $4 \ge 100 \text{ cm}^2$ $1 = \log 5 = \text{ birb}$
1011	Morphology maex		1 = 10W, 5 = 11gH
rhiz	Reproduction by rhizome	Dispersal	0 = absence, 1 = presence

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

2.3. Data Analysis

We used RLQ analysis to investigate the relationships between species traits and environmental variables constrained by the species abundance [36]. RLQ is an ordination analysis widely applied in functional trait studies that combine separate analyses on multiple datasets to identify the relationships between traits and environmental variables, weighted by the abundances of species [37]. The method is based on a three-step ordination procedure in which a matrix of environmental variables (R) is related to a matrix of associated traits (Q) using a species abundances matrix (L) as a link. First, we conducted a correspondence analysis (CA) 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 (only quantitative variables). 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 for identifying the most important traits and environmental factors.

In addition, we performed a fourth corner analysis on the results of the RLQ analysis that allowed us to investigate significant relationships 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 [38]. The significance of correlations between traits and environmental variables were tested and P values were adjusted with the false discovery rate method after 10,000 permutations according to Dray et al. [39].

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 computing the Kelley-Gardner-Sutcliffe penalty function [40]. The relationships between the functional groups and the environment were assessed taking into account the results of the prior analyses (RLQ and fourth corner). All analyses were conducted with the ade4 package [41] in R environment [42].

3. Results

3.1. Species, Traits and Environment Relationships

The first two axes of the RLQ explained approximately 68% and 16% of the total variance that links the species composition in table L with environmental characteristics in table R and traits in table Q

(Table 4). Among environmental variables, chlorophyll-a concentration had the largest contribution to the total inertia followed by total phosphorus, Secchi disk depth and pH. Regarding macrophyte traits, reproduction by rhizome contributed the most to total inertia followed by anchored emergent and floating-leaved life forms and the morphology index (Table 5). The first RLQ axis represented a gradient of total phosphorus and chlorophyll-a concentration while the second RLQ axis was related positively with pH and nitrogen, and negatively with electrical conductivity, Secchi disk depth and alkalinity (Figure 2A).

Table 4. Summary of the RLQ analysis results. The table presents the 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	0.39	0.09
% of total co-inertia	68.45	15.75
Inertia and co-inertia R (env)	0.93	0.75
Inertia and coinertia Q (trait)	0.74	0.80
Correlation L (sp)	0.26	0.21

Table 5. Percentages of contribution of the environmental variables and functional traits to the RLQ analysis.

Environmental Variable	Contribution to Total Inertia (%)	Macrophyte Trait	Contribution to Total Inertia (%)
Chl-a	30.1	Rhizome	28.6
Total phosphorus	28.9	Anchored emergent	15.5
Secchi disk depth	9.7	Anchored floating-leaves	12.5
pH	9.7	Morphology Index	11.4
Alkalinity	8.7	Anchored submerged	8.1
EC	9.2	Leaf area	5.7
Nitrogen	3.8	Growth morphology	5.5
Ū.		Free-floating submerged	5.4
		Ellenberg N	2.9
		Free-floating, surface	2.6
		Anchored, heterophylly	1.8

Regarding the traits, the first axis differentiated mainly the ability of reproduction by rhizome and morphology index from other macrophyte traits. The second axis clearly discriminated the anchored submerged life-form from the remainder traits. In addition, both free-floating life-forms (ffsur and ffsub) were associated with the second axis of RLQ (Figure 2B).

Figure 2C shows the distribution of plant species in the ordination space. Free-floating species (e.g., *Lemna gibba* and *Salvinia natans*) were placed in the upper left part of the plot, while rooted floating-leaved plants were positioned mostly to the right part. Rooted submerged plants were mostly positioned below the first axis of the ordination plot.

The results of the fourth corner analysis did not show any significant relationships between environmental variables and macrophyte traits after adjusting p-values with the false discovery rate method. This indicates that single environmental drivers were not associated with single traits, but possibly a combination of environmental drivers has an overall effect on a combination of functional traits.



Figure 2. Results of the first two axes of RLQ analysis: (**A**) Coefficients for the environmental variables; (**B**) coefficients for the traits; and (**C**) scores of species. The 'd' values give the grid size for scale comparison across the three figures. Species abbreviations are given in Table 2. Trait abbreviations are given in Table 3.

3.2. Macrophyte Functional Assemblages

The hierarchical clustering of the RLQ species scores suggested five discrete functional assemblages after computing the Kelley-Gardner-Sutcliffe penalty function. The functional assemblages—groups of macrophyte species based on their traits are shown in Figure 3. Groups 1 and 2 are both characterized by a species with anchored submerged life form, but Group 1 contains a species with higher morphology index that are reproduced more often with rhizome than those of Group 2. Group 3 consists of a species with an anchored floating leaved life form, higher values of Ellenberg—N indicator and larger leaf area.

In contrast Groups 4 and 5 consisted of submerged free floating and surface free floating species respectively, with low values of morphology index and smaller leaf area.



Figure 3. Dendrogram obtained from hierarchical clustering of the RLQ species scores based on Euclidean distances using Ward's method. Colors correspond to the five functional groups.

Figure 4 illustrates the relationships between the discrete macrophyte assemblages and the environmental variables. Groups 1 and 2 (anchored submerged macrophytes) are related with high alkalinity, conductivity and Secchi disk depth, but Group 1 appears to show a preference for waters with higher phosphorus content and chlorophyll-a concentration than Group 2.



Figure 4. Biplot showing the relationships between the functional macrophyte assemblages derived from hierarchical clustering and the vectors of environmental parameters

Rooted floating leaved plants in Group 3 were also associated with high total phosphorus, whereas Groups 4 and 5 (free-floating plants) are negatively associated with nutrient enriched waters and with high conductivity. In contrast, floating-leaved rooted plants (Group 3) showed an association with both phosphorus and nitrogen which differentiated them from free-floating plants (Groups 4 and 5) that were related positively with nitrogen concentration.

4. Discussion

The current research is a first attempt to explore how aquatic macrophyte assemblages that share common functional traits respond to water quality environmental drivers in Greek freshwater lakes. Although our results did not reveal significant relationships between single macrophyte traits and environmental variables we found evidence of a discriminating pattern of macrophyte assemblages based on functional traits across environmental gradients.

Our findings noted that total phosphorus and chlorophyll-a concentration contributed the most to the total inertia of the RLQ analysis and indicated a strong gradient along the first axis that was associated with anchored submerged and floating leaved plants of macrophyte assemblages (Groups 1 and 3). This clearly underpins the results of Pan et al. [43] who also found TP as the most important environmental variable to affect the macrophyte traits. The same study also highlighted Secchi disk depth as a more important factor than nitrogen and pH which also agrees with our findings. Concerning the macrophyte traits, our analysis suggested the reproduction by rhizome as the trait with the highest contribution to the total inertia followed by the life forms of anchored emergent and floating-leaved plants and the morphology index. Life forms are known to reflect the prevalence of different trophic states as emergent and floating leaved plants are usually found in eutrophic conditions whereas submerged macrophytes are highly associated with low nutrients and clear waters [44]. As light limitation primarily inhibits submerged macrophyte assemblages [2,45] we expected that submerged functional groups will prefer water clarity indicated by the higher Secchi disk depth. Furthermore, several studies have emphasized the role of light availability in affecting the growth rates and morphology of macrophytes [46]. Here we observed a similar pattern of distribution of macrophytes along environmental gradients of eutrophication where floating leaved plants associated with higher total phosphorus and chlorophyll-a, while anchored submerged plants showed a preference for clearer waters with a lower phosphorus content. Yet, we would expect to observe stronger relationships between submerged plants and light availability (Secchi disk depth).

Furthermore, the analysis showed that species of macrophyte assemblage (Group 3) were characterized by higher morphology index and better dispersal ability with rhizomes than the other groups. This could be associated with the increased nutrient availability (phosphorus in our case) that promotes larger vegetative and reproductive structures as reported recently by Garcia-Giron et al. [47] who attributed larger vegetative and reproductive structures (in their case larger fruit size) to higher concentrations of total phosphorus. In addition, plants with larger morphology index can formulate denser and larger beds inhibiting other species in the competition for light [48]. Baattrip-Pedersen et al [23] recently showed that species with high morphology index were positively associated with high phosphate levels indicating that high nutrient availability promoted those plants. Although leaf area is considered a key characteristic that is linked with ecological strategies [48], we did not find strong evidence that highlights its significance. Leaf area had a small contribution to the total variance although plants of macrophyte assemblages (Groups 1 and 3) were associated with the eigenvector of leaf area, as shown in the RLQ ordination graph. This could be an indication of light limiting conditions favoring plants with large leaf area, such as large leaved or dense-leaved plants, over plants with small leaf area. As a conclusion, rooted macrophytes with floating leaves and large rhizome systems seem to prevail in eutrophicated waters (Group 3). Under eutrophic conditions with high turbidity macrophytes may exhibit various mechanisms to compensate for the low light availability. Most species will develop their photosynthetic biomass on the water surface (e.g., floating-leaved plants) or will utilize light more efficiently [49]. Moreover, studies report that extensive rhizomes may provide a competitive advantage in disturbed environments [50].

Alkalinity and conductivity associated negatively with both axes of the RLQ analysis playing some role in the distribution of aquatic plant assemblages. The importance of alkalinity has been well documented in the functioning of freshwater plants, since it reflects the ability among species to use bicarbonate as a carbon source [51]. Lukacs et al. [48] pinpointed the role of alkalinity in shaping the structure of macrophyte communities based on their physiological ability to use bicarbonates. However, the ability to use bicarbonate in photosynthesis is limited to submerged species that largely depend on inorganic carbon uptake from the water [52] which is in line with the results of the RLQ where anchored submerged plants related with high alkalinity.

Finally, in contrast to phosphorus, nitrogen had a minor role in explaining the total variance (3.8% contribution). Similarly, Ellenberg N score had a very low contribution in the variance in the observed trait composition. Although controversy exists over the role of nitrogen in the eutrophication process [53] our results suggested that phosphorus had the strongest effect than nitrogen on macrophyte assemblages which agrees with the general consensus that phosphorus is the main driver of phytoplankton growth and macrophyte decline in freshwater lakes [54].

5. Conclusions

In this study, we identified distinct macrophytes assemblages, and how the functional traits of macrophytes respond to water quality gradients. Recent studies, that quantify measurable functional traits, may produce more accurate and precise datasets to capture the effects of environmental filtering on trait patterns. Overall, our work demonstrated that multiple environmental stressors contributed to both axes of the RLQ analysis, with phosphorus and chlorophyll-a being the major environmental drivers that explained significant variance in the macrophyte trait composition. Although we did not

find single trait-environmental variable associations, macrophyte functional assemblages differed in response to environmental drivers, implying that functional traits were affected simultaneously by a combination of environmental stressors. Thus, macrophytes were discriminated mainly based on their responses to light utilization and nutrient availability resulting in five discrete assemblages.

We can therefore acknowledge that despite the limited size and the regional character of the data, the applied methodology is robust and suited for the assessment of the relationships between functional traits and the environment. As such, the results of this work can be treated as preliminary findings of a research that attempts to find patterns of macrophyte trait composition along environmental gradients in freshwater lakes of Greece. Further research that will conduct field campaigns in more lakes and will include broader environmental gradients and new traits, is expected to reveal finer responses of traits to abiotic drivers that can be used in novel systems for the evaluation of ecological status.

In conclusion, our findings encourage the conduction of future monitoring studies in lakes focused on the aquatic plant functional trait composition and highlight the possibility of using trait-based approaches in ecological monitoring as they can capture effectively the effects of environmental change on the community composition.

Author Contributions: Conceptualization, K.S. and E.P.; methodology, K.S.; formal analysis, K.S.; investigation, K.S. and E.P.; data curation, K.S. and E.P.; writing—original draft preparation, K.S.; writing—review and editing, K.S. and E.P.; visualization, K.S.

Funding: This research received no external funding.

Acknowledgments: We thank the editor and the reviewers for the valuable comments on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Carpenter, S.R.; Lodge, D.M. Effects of submerged macrophytes on ecosystem processes. *Aquat. Bot.* 1986, 26, 341–370. [CrossRef]
- Blindow, A.; Hargeby, A.; Hilt, S. Facilitation of clear-water conditions in shallow lakes by macrophytes: Differences between charophyte and angiosperm dominance. *Hydrobiologia* 2014, 737, 99–110. [CrossRef]
- Hilt, S.; Alirangues Nuñez, M.M.; Bakker, E.S.; Blindow, I.; Davidson, T.A.; Gillefalk, M.; Hansson, L.-A.; Janse, J.H.; Janssen, A.B.G.; Jeppesen, E.; et al. Response of Submerged Macrophyte Communities to External and Internal Restoration Measures in North Temperate Shallow Lakes. *Front. Plant Sci.* 2018, 9. [CrossRef]
- Stelzer, D.; Schneider, S.; Melzer, A. Macrophyte-based assessment of lakes—A contribution to the implementation of the European Water Framework Directive in Germany. *Int. Rev. Hydrobiol.* 2005, 90, 223–237. [CrossRef]
- Penning, W.E.; Mjelde, M.; Dudley, B.; Hellsten, S.; Hanganu, J.; Kolada, A.; Van Den Berg, M.; Poikane, S.; Phillips, G.; Willby, N.; et al. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. *Aquat. Ecol.* 2008, 42, 237–251. [CrossRef]
- 6. Kolada, A.; Willby, N.; Dudley, B.; Nõges, P.; Søndergaard, M.; Hellsten, S.; Mjelde, M.; Penning, E.; van Geest, G.; Bertrin, V.; et al. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. *Ecol. Indic.* **2014**, *45*, 407–415. [CrossRef]
- Poikane, S.; Portielje, R.; Denys, L.; Elferts, D.; Kelly, M.; Kolada, A.; Mäemets, H.; Phillips, G.; Søndergaard, M.; Willby, N.; et al. Macrophyte assessment in European lakes: Diverse approaches but convergent views of 'good' ecological status. *Ecol. Indic.* 2018, *94*, 185–197. [CrossRef]
- 8. Sarika-Hatzinikolaou, M.; Yannitsaros, A.; Babalonas, D. The macrophytic vegetation of seven aquatic ecosystems of Epirus (NW Greece). *Phytocoenologia* **2003**, *33*, 93–151. [CrossRef]
- 9. Kłosowski, S. The relationships between environmental factors and the submerged Potametea associations in lakes of north-eastern Poland. *Hydrobiologia* **2006**, *560*, 15–29. [CrossRef]
- 10. Stefanidis, K.; Papastergiadou, E. Aquatic vegetation and related abiotic environment in a shallow urban lake of Greece. *Belg. J. Bot.* **2007**, *140*, 25–38.

- Hrivnák, R.; Ot'ahel'ová, H.; Kochjarová, J.; Pal'ove-Balang, P. Effect of environmental conditions on species composition of macrophytes—Study from two distinct biogeographical regions of Central Europe. *Knowl. Manag. Aquat. Ecosyst.* 2013, 9. [CrossRef]
- Fu, H.; Zhong, J.; Yuan, G.; Xie, P.; Guo, L.; Zhang, X.; Xu, J.; Li, Z.; Li, W.; Zhang, M.; et al. Trait-based community assembly of aquatic macrophytes along a water depth gradient in a freshwater lake. *Freshw. Biol.* 2014, *59*, 2462–2471. [CrossRef]
- 13. Diaz, S.; Cabido, M.; Casanoves, F. Plant functional traits and environmental filters at a regional scale. *J. Veg. Sci.* **1998**, *9*, 113–122. [CrossRef]
- 14. McGill, B.J.; Enquist, B.J.; Weiher, E.; Westoby, M. Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* **2006**, *21*, 178–185. [CrossRef]
- Fu, H.; Zhong, J.; Yuan, G.; Ni, L.; Xie, P.; Cao, T. Functional traits composition predict macrophytes community productivity along a water depth gradient in a freshwater lake. *Ecol. Evol.* 2014, *4*, 1516–1523. [CrossRef]
- Fu, H.; Yuan, G.; Lou, Q.; Dai, T.; Xu, J.; Cao, T.; Ni, L.; Zhong, J.; Fang, S. Functional traits mediated cascading effects of water depth and light availability on temporal stability of a macrophyte species. *Ecol. Indic.* 2018, 89, 168–174. [CrossRef]
- 17. Dolédec, S.; Phillips, N.; Scarsbrook, M.; Riley, R.H.; Townsend, C.R. Comparison of structural and functional approaches to determining land use effects on grassland stream invertebrate communities. *J. North Am. Benthol. Soc.* **2006**, *25*, 44–60. [CrossRef]
- 18. Bornette, G.; Henry, C.; Barrat, M.; Amoros, C. Theoretical habitat templets, species traits and species richness: Aquatic macrophytes in the Upper Rhone River and its floodplain. *Freshw. Biol.* **1994**, *31*, 487–505. [CrossRef]
- Suding, K.N.; Lavorel, S.; Chapin, F.S.; Cornelissen, J.H.C.; Diaz, S.; Garnier, E.; Goldberg, D.; Hooper, D.U.; Jackson, S.; Navas, M.-L. Scaling environmental change through the community-level: A trait based response and effect framework for plants. *Glob. Chang. Biol.* 2006, *14*, 1125–1140. [CrossRef]
- 20. Cavalli, G.; Baattrup-Pedersen, A.; Riis, T. The role of species functional traits for distributional patterns in lowland stream vegetation. *Freshw. Sci.* **2014**, *33*, 1074–1085. [CrossRef]
- 21. Violle, C.; Navas, M.-L.; Vile, D.; Kazakou, E.; Fortunel, C.; Hummel, I.; Garnier, E. Let the concept of trait be functional! *Oikos* 2007, *116*, 882–892. [CrossRef]
- Fu, H.; Yuan, G.; Cao, T.; Ni, L.; Zhang, M.; Wang, S. An alternative mechanism for shade adaptation: Implication of allometric responses of three submersed macrophytes to water depth. *Ecol. Res.* 2012, 27, 1087–1094. [CrossRef]
- Baattrup-Pedersen, A.; Göthe, E.; Riis, T.; O'Hare, M.T. Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci. Total Environ.* 2016, 543, 230–238. [CrossRef]
- 24. Mouton, T.L.; Matheson, F.E.; Stephenson, F.; Champion, P.D.; Wadhwa, S.; Hamer, M.P.; Catlin, A.; Riis, T. Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci. Total. Environ.* **2019**, *659*, 1370–1381. [CrossRef]
- 25. Maberly, S.C. Morphological and photosynthetic characteristics of *Potamogeton obtusifolius* from different depths. *J. Aquat. Plant Manag.* **1993**, *31*, 34–39.
- 26. Diaz, S.; Lavorel, S.; de Bello, F.; Quétier, F.; Grigulisand, K.; Robson, T.M. Incorporating plant functional diversity effects in ecosystem service assessments. *PNAS* **2007**, *104*, 20684–20689. [CrossRef]
- 27. Moretti, M.; Legg, C. Combining plant and animal traits to assess community functional responses to disturbance. *Ecography* **2009**, *32*, 299–309. [CrossRef]
- 28. Stefanidis, K.; Sarika, M.; Papastergiadou, E. 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. Mar. Freshw. Ecosyst.* **2019**, 1–16. [CrossRef]
- 29. Stefanidis, K.; Papastergiadou, E. Relationships between Lake Morphometry, water quality and aquatic macrophytes in Greek lakes. *Fresenius Environ. Bull.* **2012**, *21*, 3018–3026.
- 30. Stefanidis, K.; Kostara, A.; Papastergiadou, E. Implications of human activities, land use changes and climate variability in mediterranean lakes of Greece. *Water* **2016**, *8*, 483. [CrossRef]

- 31. Koumpli-Sovantzi, L. The aquatic flora of Aetoloakarnania (W Greece). Willdenowia 1989, 18, 377–385.
- 32. Papastergiadou, E.; Babalonas, D. Aquatic flora of N. Greece I. Hydrophytes. Wildenowia 1993, 23, 137–142.
- 33. Ellenberg, H.; Weber, H.E.; Düll, R.; Wirth, V.; Werner, W.; Paulissen, D. Zeigerwerte von Pflanzen in Mitteleuropa. *Scr. Geobot.* **1991**, *18*, 1–248.
- 34. Willby, N.J.; Abernethy, V.J.; Demars, B.O.L. Attribute-based classification of European hydrophytes and its relationship to habitat utilization. *Freshw. Biol.* **2000**, *43*, 43–74. [CrossRef]
- 35. Chevenet, F.; Dolédec, S.; Chessel, D. A fuzzy coding approach for analysis of long-term ecological data. *Freshw. Biol.* **1994**, *31*, 295–309. [CrossRef]
- 36. Dolédec, S.; Chessel, D.; ter Braak, C.J.F.; Champely, S. Matching species traits to environmental variables: A new three-table ordination method. *Environ. Ecol. Stat.* **1996**, *3*, 143–166. [CrossRef]
- Kleyer, M.; Dray, S.; Bello, F.; Lepš, J.; Pakeman, R.J.; Strauss, B.; Thuiller, W.; Lavorel, S. Assessing species and community functional responses to environmental gradients: Which multivariate methods? *J. Veg. Sci.* 2012, 23, 805–821. [CrossRef]
- Dray, S.; Legendre, P. Testing the species traits-environment relationships: The fourth-corner fourth-corner problem revisited. *Ecology* 2008, *89*, 3400–3412. [CrossRef]
- Dray, S.; Choler, P.; Doledec, S.; Peres-Neto, P.R.; Thuiller, W.; ter Braak, S.; Braak, C.J.F. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* 2014, 95, 14–21. [CrossRef] [PubMed]
- 40. Kelley, L.A.; Gardner, S.P.; Sutcliffe, M.J. An automated approach for clustering an ensemble of NMR-derived protein structures into conformationally-related subfamilies. *Protein Eng.* **1996**, *9*, 1063–1065. [CrossRef]
- Dray, S.; Dufour, A.B. The ade4 package: Implementing the duality diagram for ecologists. *J. Stat. Softw.* 2007, 22, 1–20. [CrossRef]
- 42. R Core Team. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
- Pan, Y.; Zhang, X.; Song, K.; Da, L. Applying trait-based method to investigate the relationship between macrophyte communities and environmental conditions in a eutrophic freshwater lake, China. *Aquat. Bot.* 2017, 142, 16–24. [CrossRef]
- 44. Nurminen, L.; Horppila, J. Life form dependent impacts of macrophyte vegetation on the ratio of resuspended nutrients. *Water Res.* **2009**, *43*, 3217–3226. [CrossRef] [PubMed]
- 45. Zhang, Q.; Dong, B.; Li, H.; Liu, R.; Luo, F.; Zhang, M.; Lei, G.; Yu, F. Does light heterogeneity affect structure and biomass of submerged macrophyte communities? *Bot. Stud.* **2012**, *3*, 377–385.
- Riis, T.; Olesen, B.; Clayton, J.S.; Lambertini, C.; Brix, H.; Sorell, B.K. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. *Aquat. Bot.* 2012, 102, 56–64. [CrossRef]
- 47. García-Girón, J.; Wilkes, M.; Fernández-Aláez, M.; Fernández-Aláez, C. Processes structuring macrophyte metacommunities in Mediterranean ponds: Combining novel methods to disentangle the role of dispersal limitation, species sorting and spatial scales. *J. Biogeogr.* **2019**, (in press). [CrossRef]
- Lukács, B.A.; Vojtkó, A.E.; Mesterházy, A.; Molnár, A.; Süveges, K.; Végvári, Z.; Brusa, G.; Cerabolini, B.E.L. Growth-form and spatiality driving the functional difference of native and alien aquatic plants in Europe. *Ecol. Evol.* 2017, 7, 950–963. [CrossRef] [PubMed]
- 49. Hilton, J.; O'Hare, M.; Bowes, M.J.; Jones, I. How green is my river? A new paradigm of eutrophication in rivers. *Sci. Total Environ.* **2006**, *365*, 66–83. [CrossRef] [PubMed]
- 50. Wiegleb, G.; Bröring, U.; Filetti, M.; Brux, H.; Herr, W. Long-termdynamics of aquatic plant dominance and growth-form types in two north-west German lowland streams. *Freshw. Biol.* **2014**, *59*, 1012–1025. [CrossRef]
- 51. Vestergaard, O.; Sand-Jensen, K. Aquatic macrophyte richness in Danish lakes in relation to alkalinity, transparency, and lake area. *Can. J. Fish Aquat. Sci.* **2000**, *57*, 2022–2031. [CrossRef]
- 52. Maberly, S.C.; Madsen, T.V. Freshwater angiosperm carbon concentrating mechanisms: Processes and patterns. *Funct. Plant Biol.* **2002**, *29*, 393–405. [CrossRef]

- Yu, Q.; Wang, H.Z.; Li, Y.; Shao, J.C.; Liang, X.M.; Jeppesen, E.; Wang, H.J. Effects of high nitrogen concentrations on the growth of submersed macrophytes at moderate phosphorus concentrations. *Water Res.* 2015, *83*, 385–395. [CrossRef] [PubMed]
- 54. Sand-Jensen, K.; Pedersen, N.L.; Thorsgaard, I.; Moeslund, B.; Borum, J.; Brodersen, K.P. 100 years of vegetation decline and recovery in Lake Fure, Denmark. *J. Ecol.* **2008**, *96*, 260–271. [CrossRef]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).