

Article

Principal Determinants of Aquatic Macrophyte Communities in Least-Impacted Small Shallow Lakes in France

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Abstract: Small shallow lakes (SSL) support exceptionally high and original biodiversity, providing numerous ecosystem services. Their small size makes them especially sensitive to anthropic activities, which cause a shift to dysfunctional turbid states and induce loss of services and biodiversity. In this study we investigated the relationships between environmental factors and macrophyte communities. Macrophytes play a crucial role in maintaining functional clear states. Better understanding the factors determining the composition and richness of aquatic plant communities in least-impacted conditions may be useful to protect these shallow lakes. We inventoried macrophyte communities and collected chemical, climatic, and morphological data from 89 least-impacted SSL widely distributed in France. SSL were sampled across four climatic ecoregions, various geologies, and elevations. Hierarchical cluster analysis showed a clear separation of four macrophyte assemblages strongly associated with mineralization. Determinant factors identified by distance-based redundancy analysis (db-RDA) analysis were, in order of importance, geology, distance from source (DIS, a proxy for connectivity with river hydrosystems), surface area, climate, and hydroperiod (water permanency). Surprisingly, at a country-wide scale, climate and hydroperiod filter macrophyte composition weakly. Geology and DIS are the major determinants of community composition, whereas surface area determines floristic richness. DIS was identified as a determinant in freshwater lentic ecosystems for the first time.



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Keywords: depressional wetlands; ponds; alkalinity; geology; distance from source; connectivity; climate; altitude; hydroperiod

1. Introduction

Human activities such as fish stocking, impoundment, or mineral extraction have induced the creation of numerous small shallow lakes (hereafter SSL) [1,2]. The term SSL includes both man-made and natural waterbodies. These freshwater ecosystems are the most abundant on Earth [3]. The mean depth of SSL in temperate regions is less than 3 m, but can reach 7 m [4], and their surface area ranges from 1 m² to 100 ha [5]. In this study we focused on waterbodies with a surface area less than 50 ha. Waterbodies with a surface area greater than 50 ha are usually monitored in France according to system A from the EU Water Framework Directive (Annex II, 2000/60/EC). SSL can be largely colonized by macrophytes, and do not have persistent stratification for long periods in summer [6]. Obviously, intense sediment–water interaction and the potentially large impact of aquatic vegetation make the functioning of SSL different from that of their deeper counterparts [7].

SSL provide many economically valuable services and long-term benefits to society, such as drinking-water supply, irrigation, and aquaculture, and are often used for different types of recreation, such as angling, boating and swimming, or are built for amenity value [8]. They provide habitats for rich and distinct aquatic fauna and flora [9], and also contribute to the preservation of terrestrial biodiversity, such as birds and bats, by providing habitats and food [10,11]. SSL play a role in regional carbon processing, with burial in sediments and the emission of natural greenhouse gases [12], and are useful for carbon

sequestration [13]. They retain part of watershed nutrients and contaminants [14,15], and influence river hydrology and hydromorphology [16]. Despite their economic importance and conservation value, SSL are largely neglected by the scientific community [17,18]. In particular, SSL remain little studied in many European countries, including France, although pond loss has reached 90% in many regions [19], due to agricultural intensification, urbanization and, probably, global warming [20,21]. Human pressures on freshwater ecosystems are increasing, through eutrophication and climate change [22], or by the introduction of exotic or native species [23]. Indeed, climatic disruption induces changes in stratification and mixing regime that could increase the frequency of harmful algal blooms, favoring cyanobacteria and altering nutrient and light availability [24]. Furthermore, extensive nutrient loading increases phytoplankton and periphyton growth, increasing turbidity and limiting access to light for macrophytes [25]. When a critical level of turbidity is exceeded, submerged macrophytes disappear, producing shifts from clear to turbid states [26].

The decline of macrophyte beds in these freshwater ecosystems induces a reduction in biodiversity, and has a negative impact on their function since macrophytes play a key role in SSL. When they are least impacted, many SSL are characterized by clear waters with abundant submerged vegetation. Macrophytes have a positive feedback on transparency, maintaining clear-water conditions through: (1) competition with microalgae and excretion of allelochemicals; (2) providing habitats and refuges from fish predation for zooplankton and invertebrates; and (3) providing habitat for piscivorous fish, limiting zooplanktivorous fishes [27]. Macrophytes also provide habitat for numerous other living organisms, such as periphyton [28,29], water birds [30], and amphibians [31]. As primary producers, they often dominate the production of organic matter, absorbing nutrients, ions, and aerial carbon dioxide [32]. Finally, they also strongly attenuate light and wind-driven turbulence, and separate warm surface water and colder bottom waters [33].

In Europe, most studies on the structure and composition of aquatic plant communities have been conducted in Central, Northern, or Mediterranean countries, within an area defined by homogeneous climatic conditions [34]. The aim of this study was to identify the environmental drivers of aquatic macrophyte assemblages in French least-impacted SSL. France is characterized by four of the five European climatic ecoregions and by a high altitudinal gradient. The hydrogeochemistry of SSL in France also varies quite substantially, from mountainous hard rocks to regions dominated by low-lying alluvial deposits. SSL can be found on non-calcareous and calcareous sandy deposits, in bogs, and on calcareous bedrocks. The baseline hydrogeochemical conditions play a fundamental role in regulating the diversity of macrophyte communities [35].

The target waterbodies of this study occupy a wide range of environmental conditions (altitude, climate, geology), although the latitudinal range is relatively small. Our first hypothesis was that the main driver of aquatic plant communities and richness is the biogeochemistry of these lakes, and in particular the mineralization (water mineral content), because nutrient concentrations are generally low in least-impacted conditions. In particular, we expected a lower richness in non-calcareous lakes, because such lakes may support only carbon-limited species. Our second hypothesis was that the distribution is also explained by climatic gradient, because temperature is a determinant factor for macrophyte metabolism [36].

2. Materials and Methods

2.1. Site Selection

We selected 89 SSL ranging from 3 to 3340 m above sea level (asl), and differing in their geology (calcareous to siliceous), substrate type (sand, clay, rock), water supply (rainfall, groundwater, river flow), and surface area (from 1 m² to 41.4 ha). These SSL included both permanent, semi-permanent (dry only exceptionally, in drought years), and temporary waters [37]. They were selected from four different biogeographical regions: Alpine, Mediterranean, Continental, and Atlantic (Figure 1). They were of natural origin

(glacial, alluvial) or the result of human activity. SSL characterized by shading equal or higher to 75% were not included in the analysis, because of their very low floristic richness [38].

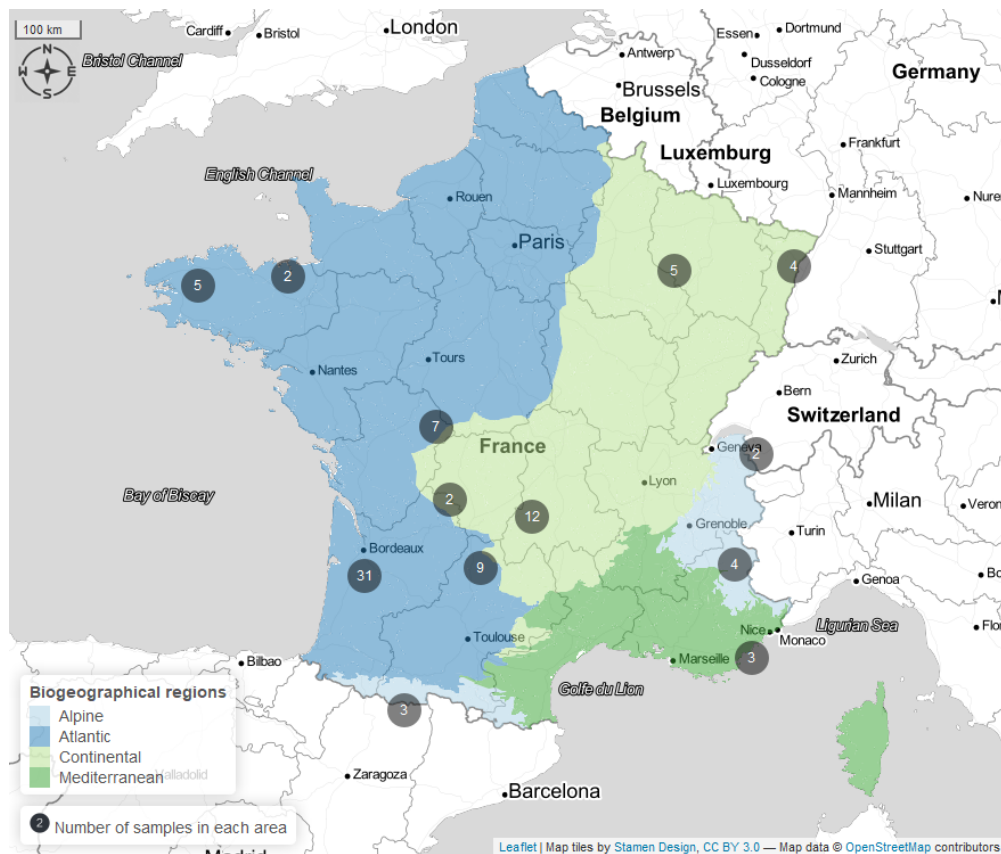


Figure 1. Localization of the sampling sites. The number in the circles indicates the number of samples of small shallow lakes in each area.

2.2. Environmental Data

Environmental data, except for GIS analyses, were collected the same or the year following the macrophyte survey. For estimation of hydroperiod, each site was prospected at least three times at different seasons during a minimum of two years. GIS analyses were conducted mainly in 2020.

The size, altitude, average depth (estimated), maximum depth, shoreline index [39], and % shading were noted for each sampling site; whereas other factors were coded into four or five classes: slope of banks (class 1: <5%; 2: 5%–25%; 3: 25%–50%; 4: 50%–75%; 5: >75%), and hydroperiod (0: Permanent waters; 1: exceptional pluri-annual droughts; 2: short annual droughts; 3: long annual droughts). Distance from source (DIS), was measured using French National Geography Institute topographic maps; this is a proxy for the degree of connectivity between the SSL and a river hydrosystem [40]. If DIS = 0, the SSL was outside a river floodplain. If DIS > 0, the SSL is crossed by a river or located in its floodplain. Where the SSL was itself a source, DIS = length of the longest distance from the banks to the SSL outlet. Where the SSL was a river impoundment, DIS = length from the source of the river to the SSL outlet. Where the SSL was on a river floodplain, DIS = length from the source to the perpendicular formed by the line between the river and the SSL. So, higher values of DIS denote connections with a larger river. Climatic factors, defined by mean annual air temperature, mean annual air temperature amplitude, and mean annual precipitations, were extracted from the French National Institute for

Agronomic and Environmental Research INRAE reanalysis [41] of the SAFRAN/France database [42] for the years 2010–2016.

We collected water and sediment samples once only, in the winter following the macrophyte surveys when biological activity is lower and the concentration of inorganic nutrients is potentially the highest [43]. The water and sediments were sampled at the deepest point of the waterbody, with the exception of the impoundments. In this last case, three sediment samples were taken along an upstream-downstream gradient and were mixed to form a single aggregate sample. The top centimeters of sediment were sampled with a sediment corer or with an Ekman grab, depending on the water depth. pH, conductivity, and water color were measured directly in the water column with probes (Multi-parameter WTW 3430, WTW 3630, Hydrolab, YSI, and Exo3 for the usual parameters, and Lovibond MD100 for water color). Additional chemical parameters (Kjeldhal N, ammonium, nitrite, nitrates, total phosphorus, soluble reactive phosphorus, calcium, alkalinity) were analyzed from the water, whereas Kjeldhal N, total phosphorus, soluble reactive phosphorus, organic carbon, and loss on ignition were analyzed from the sediment samples according to French norms by an accredited laboratory.

2.3. Macrophyte Community

Macrophytes were studied during the period of vegetation growth from 2013 to 2018. We sampled all sites once. All macrophytes were surveyed according to a newly-adapted method from the French standard method XPT90-328 [44] and the PSYM protocol [45]. Terrestrial plants and wetland plants growing outside the outer edge of the waterbodies were not recorded. The vegetation abundance was assessed using the XPT90-328 classes (class 1: few individuals; 2: isolated small patches; 3: numerous small patches; 4: large discontinuous patches; 5: large continuous patches). Macrophytes at the outer edge and the shallow part of the site were surveyed while walking or wading in a zig-zag pattern. Deeper water zones were point-sampled from a boat following a zig-zag pattern with a grapnel or a rake.

Samples such as species of Characeae, *Ranunculus* subg. *Batrachium*, and mosses were kept in alcohol or dried for identification in the laboratory. We identified all macrophytes (spermatophytes, bryophytes, and Characeae) at species level when it was possible.

The study of the macrophyte community was realized by calculation of four biodiversity indices (taxonomic richness, Shannon–Wiener (1), Pielou's (2), and Simpson (3) diversity indices) [46]. The Shannon and Simpson diversity indices are the most widely used indices based on species richness, and provide a synthetic image of richness and species distribution. The Simpson and Pielou's indices are independent of species richness, and depend solely on species distribution [47].

$$H' = - \sum_{i=1}^S p_i \log_2 p_i, \quad (1)$$

$$J = \frac{H'}{\log S}, \quad (2)$$

$$D1 = 1 - \sum_{i=1}^S p_i^2, \quad (3)$$

where p_i is the proportion of species (i), and S is the number of species.

2.4. Data Analysis

For multivariate analysis, taxa found at one station only were discarded. Singleton macrophyte species were removed to prevent random and uninterpretable fluctuations, and only taxa identified at species level were retained in the analysis [48].

Square-rooted Bray–Curtis dissimilarity was calculated for the abundance-based taxonomic matrix. The dissimilarity matrix was then subjected to a hierarchical cluster analysis using Ward's minimum variance method, which seeks to define well-delimited groups by minimizing the within-group sum of squares [49].

Community groups were then determined by comparing the distance matrix obtained with dendrogram and binary matrices representing partitions [50]. A category label (Soft-Water, Soft-Water Marginal, Mixed, and Calcareous) was attributed to each community group in accordance with the boundaries of the physico-chemical conditions. Strictly-defined, soft-water lakes are those with Ca <3 mg/L, and with very low alkalinity, whereas soft-water-marginal lakes are characterized by Ca 10–15 mg/L, and higher pH [35]. Calcareous lakes are those with Ca >20 mg/L and alkalinity >1 meq/L [51].

Finally, indicator species for each group were identified using the IndVal index from the indicpecies package [52,53].

IndVal index (4) is a measure of association between a taxon (i) and a group (j) of stations, and is calculated as the product of specificity, A (5) (mean abundance of species i across sites of group j compared to the mean abundance of the species over all groups), and fidelity, B (6) (proportion of sites of a group j with species i). IndVal is maximal (=100%) when a given taxon is observed at all stations of one community group only, and in none of the other groups.

$$\text{IndVal}_{ij} = A_{ij} \times B_{ij} \times 100 \quad (4)$$

$$A_{ij} = \frac{a_{ij}}{a_i} \quad (5)$$

$$B_{ij} = \frac{N_{ij}}{N_j} \quad (6)$$

Relationships between environmental factors and groups were explored using pairwise comparisons with Dunn's tests and boxplots for groups with significant shifts.

Major environmental determinants for macrophyte abundance-based taxonomic composition and groups were identified with distance-based redundancy analysis (db-RDA) [54] combined with parametric forward selection [55], and tested by an ANOVA-like permutation test [56].

Correlations between community characteristics and quantitative environmental variables were assessed using Spearman rank correlations to investigate the intensity of all possible relations following a positive or negative monotonic trend [57]. We used R software [58] for statistical analysis and data plotting. Most of the analysis was processed with the R Vegan package, except Dunn's test, where the FSA R package was used, and forward selection, which was conducted with the adespatial R package.

3. Results

A total of 183 plant taxa, reduced to 145 after singleton removal, were identified across all 89 stations.

3.1. Community Groups and Environmental Patterns

Ward clustering analysis resulted in four defined plant community groups (Figure 2, Table 1) based on the major variables identified by Dunn's test (alkalinity, calcium, pH, Figure 3, Table S1). The complete record of indicator species for each group, with fidelity, specificity, and indicator values, are provided in Table S2.

The first group of stations corresponded to acid (pH = 5.9 ± 0.8) soft-water lakes (median (calcium) = 1.83 ± 3.25 mg/L, median alkalinity = 0.18 ± 0.21 meq/L). This group pooled together 23 SSL, mostly located at low altitude (<138 m asl), with the exception of three outliers (around 800 m asl). DIS values (median DIS = 0.0 ± 0.8 km) indicated that most of these lakes were not connected to a stream. The indicator species were helophytes, such as *Eleocharis multicaulis*, hydrophytes, such as *Potamogeton polygonifolius*, or bryophytes, such as *Sphagnum auriculatum* (Table 1). The median macrophyte richness was low (n = 11 ± 5).

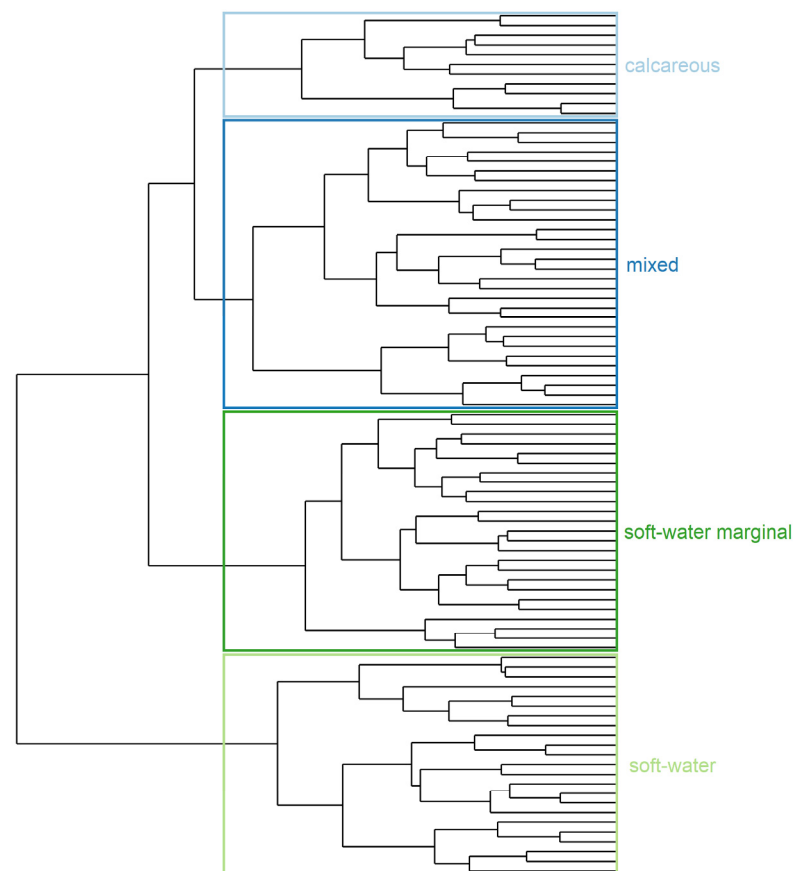


Figure 2. Community group partition. Ward's minimum variance cluster analysis based on Square-rooted Bray–Curtis matrix from abundance-classes taxonomic composition at 89 sites sampled between 2013–2018. Groups are labelled according to variables identified by Dunn's test (alkalinity, calcium, pH).

Table 1. Floristic community group characteristics and their respective median values (\pm SD). Best indicator taxa are noticed for each biological form (helophyte, hydrophyte, Characeae, and bryophyte). Complete list of indicator taxa and corresponding index values (specificity, fidelity, indicator value) are noted in Table S2. asl = above sea level. DIS = distance from source.

Community Group	No. of Station	Richness	Calcium mg/L	Alkalinity Meq/L	pH	DIS in km	Surface in m ²	Altitude in m (asl)	Significant Indicator Taxa
Soft-water	23	11 (\pm 5)	1.8 (\pm 3.3)	0.18 (\pm 0.21)	5.9 (\pm 0.81)	0 (\pm 0.8)	242 (\pm 2585)	68 (\pm 257)	<i>Eleocharis multicaulis</i> <i>Potamogeton polygonifolius</i> <i>Sphagnum auriculatum</i>
Soft-water-marginal	25	22 (\pm 12)	3.7 (\pm 16.3)	0.24 (\pm 0.91)	7.5 (\pm 0.97)	0.4 (\pm 22)	9855 (\pm 93,324)	857 (\pm 109)	<i>Carex rostrata</i> <i>Sphagnum fallax</i> <i>Ranunculus peltatus</i>
Mixed	30	22 (\pm 9)	31.4 (\pm 38.8)	1.85 (\pm 1.89)	7.93 (\pm 0.62)	5.2 (\pm 238)	1975 (\pm 23,841)	30 (\pm 109)	<i>Phragmites australis</i> <i>Leptodyctium riparium</i> <i>Potamogeton lucens</i>
Calcareous	11	9 (\pm 4)	63.0 (\pm 24.5)	2.68 (\pm 1.17)	8.3 (\pm 0.36)	0 (\pm 2.8)	195 (\pm 2146)	371 (\pm 908)	<i>Juncus articulatus</i> <i>Ranunculus trichophyllus</i> <i>Chara contraria</i> <i>Drepanocladus aduncus</i>

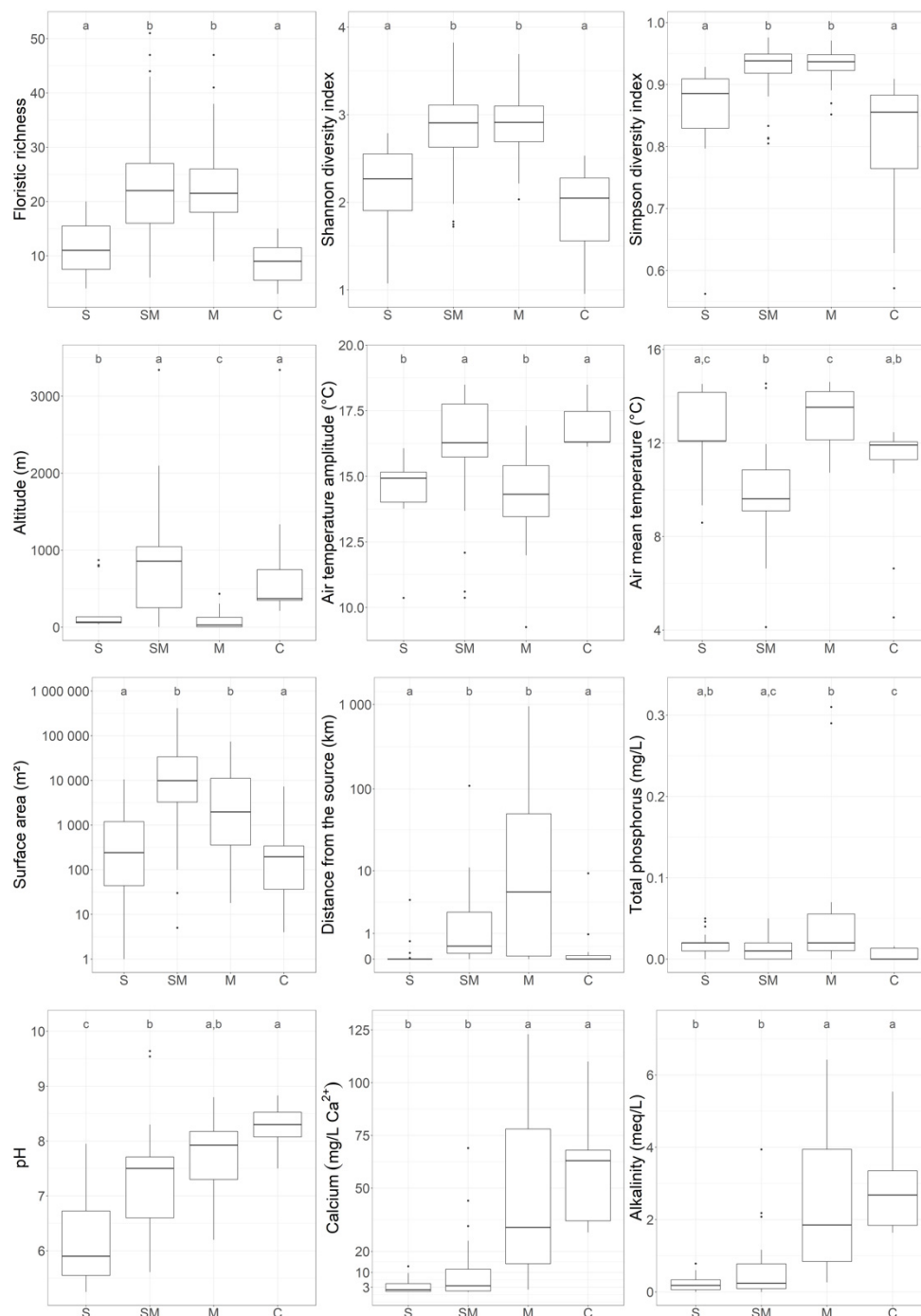


Figure 3. Box-plots of richness and diversity indices (Shannon’s index, Simpson’s index) and determinant environmental variables (pH, calcium concentration, alkalinity, surface area, distance from the source, total phosphorus). S = soft-water, SM = soft-water marginal, M = mixed, C = calcareous group. Small letters (a, b, c) indicated significant differences between the groups (Dunn’s test).

The second group of stations corresponded to neutral (median pH = 7.5 ± 0.96) soft-water marginal lakes. It comprised 25 SSL. The mineralization level was higher than in the soft-water lake group (median (calcium) = 3.70 ± 16.28 mg/L, median alkalinity 0.24 ± 0.91 meq/L). Most of the rare calcareous fens sampled were strongly associated with this group, and explained the outliers found for calcium, alkalinity, pH, and altitude. SSL from Group 2 were frequently larger than those of the first group. The DIS values (median DIS = 0.4 ± 21.6 km) were higher in this group than those of Group 1, because

most of these SSL were upland lakes located close to the source of a stream. This group was defined by numerous helophyte species, such as *Carex rostrata* or *Equisetum fluviatilis*, by bryophyte species of the *Sphagnum* Section *cuspidata* (mostly *S. fallax*) and by hydrophyte *Ranunculus peltatus*. It was characterized by a higher median richness ($n = 22 \pm 12$) than that of the first group.

The third group of stations was the mixed group, with slightly alkaline conditions (median pH = 7.9 ± 0.6). It was characterized by higher mineralization (median (calcium) = 31.35 ± 39.82 mg/L; and alkalinity = 1.85 ± 1.89 meq/L). It grouped together 30 intermediate-hardwater lakes, located in ancient polders, estuaries, or rare geological formations, such as limestone lens on siliceous bedrock. The DIS of these sites showed wide variation (from 0 to 950 km, with median = 5.2 km). The main indicator species were the hydrophyte *Potamogeton lucens*, the helophyte *Phragmites australis*, and the bryophyte *Leptodictyum riparium*. The median richness was similar to that of Group 2 ($n = 22 \pm 9$).

The fourth group of stations, called the calcareous group, comprised 11 alkaline SSL (pH = 8.3 ± 0.36). The group was characterized by its high mineralization level (median (calcium) = 63.00 ± 24.52 mg/L; alkalinity = 2.68 ± 1.17 meq/L), DIS was generally low (DIS = 0.0 ± 2.8 km, but with two outliers). Altitude ranged from 213 to 3340 m asl, with a median of 371 m asl. The outlier at 3340 m asl corresponded to a rare calcareous fen with low floristic richness. Group 4 was mostly characterized by submerged macrophyte species, such as *Chara contraria*, *Ranunculus trichophyllus*, mosses, such as *Drepanocladus aduncus*, and by helophyte species such as *Juncus articulatus*. Species richness was low (median $n = 9 \pm 4$).

3.2. Environmental Drivers of Macrophyte Communities

The db-RDA analysis indicated four determinant axes according to the results of the ANOVA-like permutation test ($P < 0.001$). Two db-RDA biplots, respectively, on the 1–2 axes, and 3–4 axes, are illustrated in Figure 4. In these two biplots, macrophyte communities were significantly influenced by a set of environmental variables that explained a total variation of 26.48%, with the first two axes explaining 15.02%, and the two later axes contributing 11.46% of the db-RDA analysis. Ten variables were retained in the forward selection of the db-RDA model (Table 2).

Table 2. Results of correlations between axes and environmental variables and ANOVA-like permutation test performed on db-RDA computed with macrophyte abundance, based on taxonomic composition against forward selected environmental variables. $\text{Pr}(>f) = p$ values of the permutation tests. Best correlation values for each axis are bolded.

Factors	Correlation with Axes				ANOVA Test	
	Axis 1	Axis 2	Axis 3	Axis 4	F	Pr (>f)
Variance Explained (%)	9.38	5.93	6.49	4.45		
Ca ²⁺ concentration	−0.80	−0.17	0.24	0.09	2.51	0.002
pH	−0.76	0.20	−0.05	0.04	1.67	0.028
Alkalinity	−0.79	−0.14	0.20	0.11	1.52	0.063
Distance from source	−0.41	−0.28	−0.40	−0.31	2.73	0.001
Total water phosphorus	−0.18	−0.20	−0.17	−0.17	2.20	0.004
Altitude	−0.03	0.87	0.08	0.10	2.28	0.006
Air mean temperature	0.10	−0.81	0.11	0.02	2.15	0.004
Air temperature amplitude	−0.18	0.62	0.04	−0.34	1.64	0.045
Surface area	−0.14	−0.01	−0.67	0.11	3.21	0.001
Mean depth	−0.01	0.24	0.33	−0.18	1.42	0.100
Hydroperiod	−0.07	−0.27	0.08	0.37	1.54	0.049

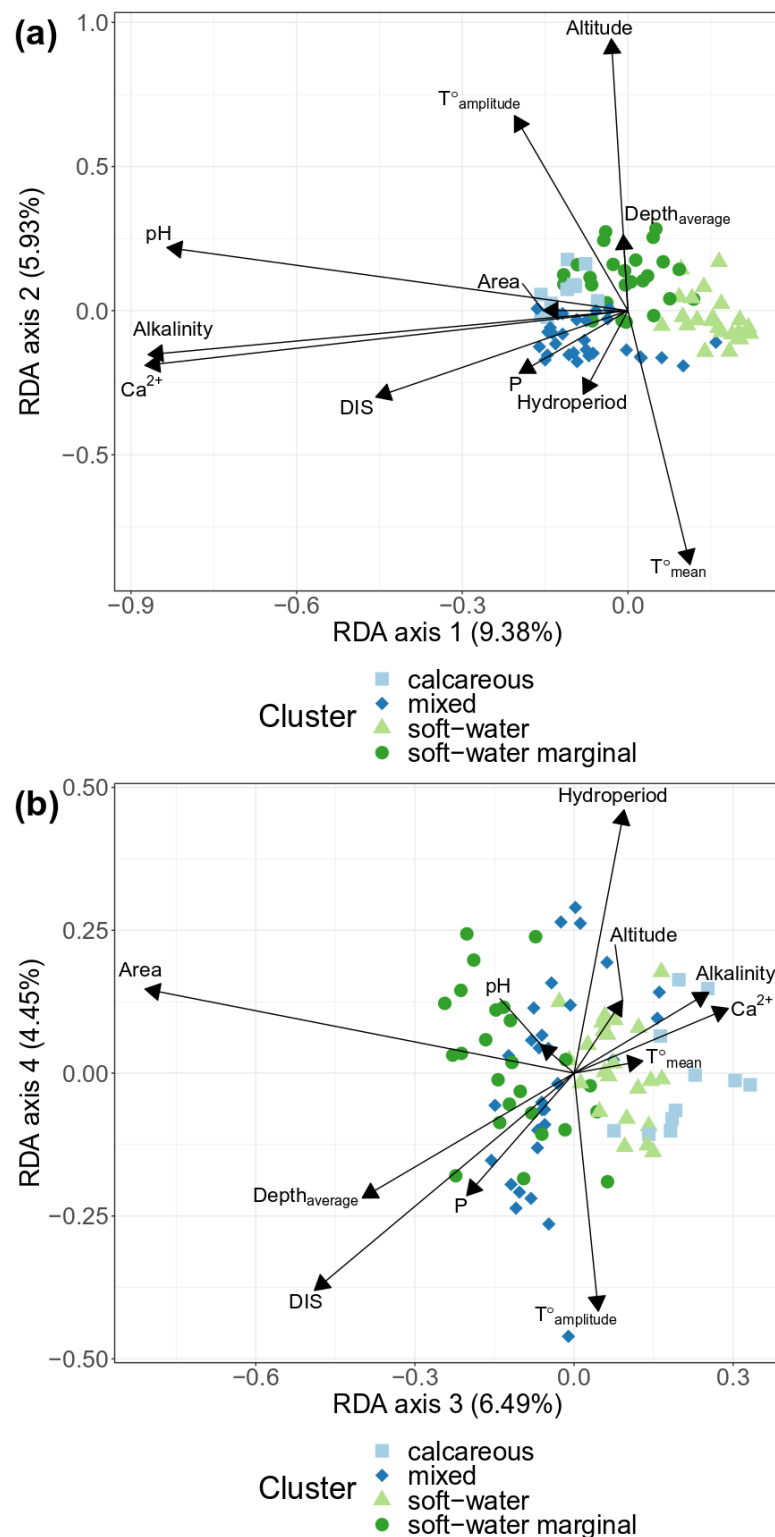


Figure 4. Distance-based redundancy analysis (db-RDA) ordination plots of macrophyte abundance based on taxonomic composition against forward selected environmental variables (black arrows) at 89 stations sampled from 2013 to 2018. **(a)** = Axis 1 and Axis 2, **(b)** = Axis 3 and Axis 4. Explained variance ($Pr < 0.001$) for each axis are indicated in parenthesis at each legend axis. Colors and point shape represent the 4 groups defined by cluster partition (soft-water, soft-water marginal, mixed, and calcareous). Area = surface area, DIS = distance from the source, P = total phosphorus in water, T amplitude = air annual temperature amplitude, T mean = air mean annual temperature.

Variance explained by db-RDA axes are in order of importance, axis 1 (9.38%), axis 3 (6.49%), axis 2 (5.93%), and axis 4 (4.45%).

According to correlations test with db-RDA axes (Table 2), alkalinity ($r = -0.79$), calcium concentration ($r = -0.80$), pH ($r = -0.76$), and DIS ($r = -0.41$) were negatively correlated with the first db-RDA axis. Surface area ($r = -0.67$) and DIS ($r = -0.40$) were negatively correlated with the third db-RDA axis. Altitude ($r = 0.87$) and air temperature amplitude ($r = 0.62$) were positively correlated with the second db-RDA axis, whereas mean annual air temperature ($r = -0.81$) was negatively correlated with this second axis. Hydroperiod ($r = 0.37$) and air temperature amplitude ($r = -0.34$) were the environmental factors best correlated with the fourth db-RDA axis (respectively $r = 0.37$ and $r = -0.34$).

Total phosphorus was weakly correlated with the fourth db-RDA axis, but showed a strong correlation with DIS ($r = 0.70$). Finally, mean depth was weakly correlated with the third db-RDA axis ($r = 0.33$), and a moderate correlation with surface area ($r = 0.46$) was established.

According to these observations, the ANOVA-like permutation test, and the correlations test with db-RDA axes (Table 2), the best explanatory variables may be classified, in order of importance: (1) Calcium concentration ($F = 2.51$, $P = 0.002$) and intercorrelated variables pH and alkalinity; (2) surface area ($F = 3.21$, $P = 0.001$); (3) DIS ($F = 2.73$, $P = 0.001$) and total phosphorus ($F = 2.20$, $P = 0.004$); (4) altitude ($F = 2.28$, $P = 0.006$), mean annual air temperature ($F = 2.15$, $P = 0.004$), and air temperature amplitude ($F = 1.64$, $P = 0.045$); and (5) hydroperiod ($F = 1.54$, $P = 0.049$). The influence of mean depth was not significant ($P = 0.1$).

3.3. Community Characteristics: Biological and Environmental Links

Pielou's index was not strongly correlated with any environmental variables (Table S3). Among the relationships tested with environmental variables (Table S3, Figure 5), Floristic Richness and Shannon H' index were strongly positively correlated with surface area ($r = 0.73$) and perimeter ($r = 0.74$). Simpson D1 index was strongly negatively correlated with surface area ($r = -0.74$) and perimeter ($r = -0.74$).

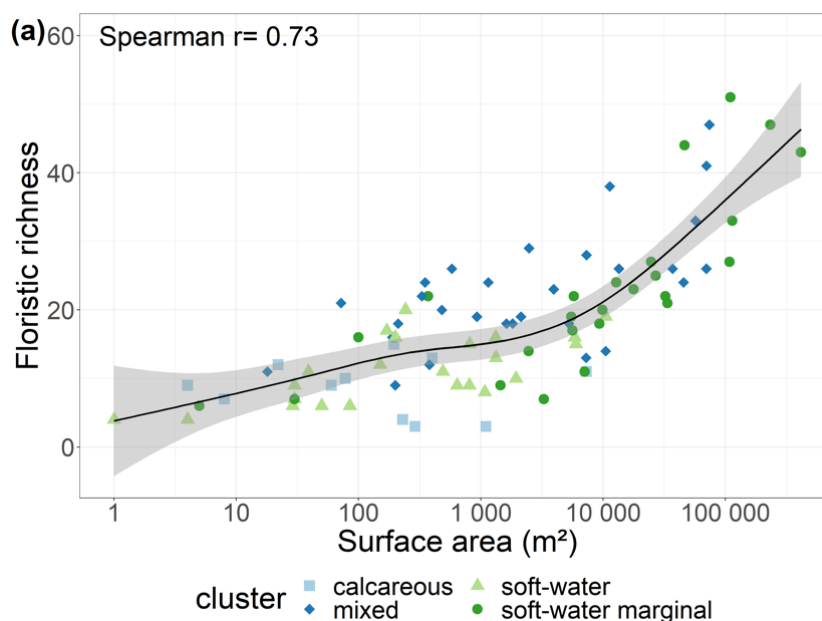


Figure 5. Cont.

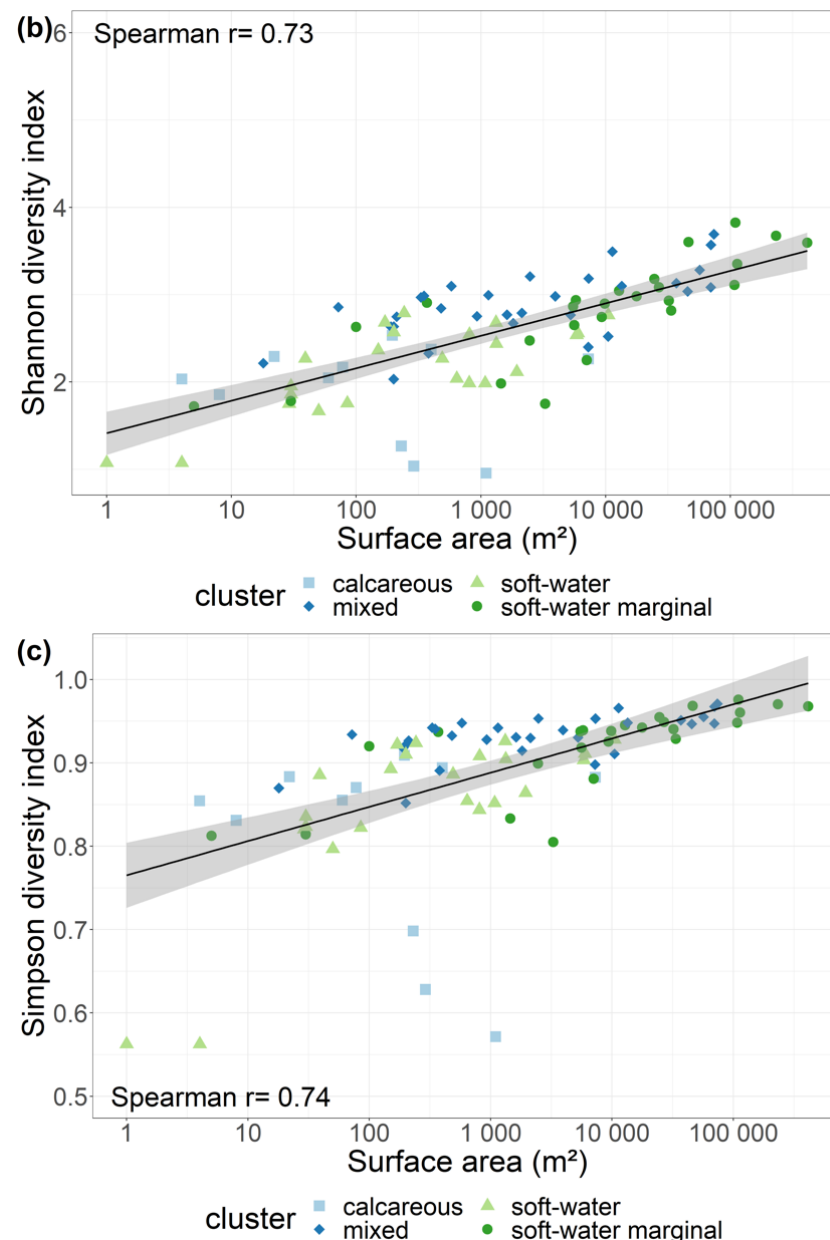


Figure 5. Relationships between the surface area of each SSL and: (a) floristic richness, (b) Shannon diversity index, and (c) Simpson diversity index. Community groups (soft-water, soft-water marginal, mixed, and calcareous) are indicated.

4. Discussion

4.1. Drivers of Aquatic Plant Community Composition

Our results suggest that mineralization (i.e., alkalinity, calcium concentration) may have a stronger influence than climate on plant distribution and on their abundance at a French scale. The influence of alkalinity on macrophyte species originates from their varying ability to use bicarbonate or carbon dioxide as a source of carbon in photosynthesis [59]. For the first time DIS was found to be among the best explanatory variables for aquatic community composition. DIS is a proxy for connectivity between SSL and river hydrosystems. Being close to a source (DIS close to 0) indicates that SSL are more isolated and have a water chemistry mostly influenced by geology. Increasing values of DIS indicates increased inputs from the catchment area, and a higher chance of an SSL receiving propagules.

The influence of regional, spatial, and environmental drivers can vary according to geographic region [60]. Continental France is subject to highly heterogeneous climatic conditions in a very small area (less than 600,000 km²), excluding most of the influence of latitude, which may have strong effects on macrophyte communities [61]. Climate and altitude seem to influence macrophyte community composition and not the richness, e.g., filtering some species that are especially adapted to high elevations, such as *Philonotis seriata*, or endemic species, such as *Caropsis verticillato-inundata* in the south-west lowlands. Climate and altitude can also exceed geological influence in littoral macrophyte composition. In France, beyond a certain climatic threshold (e.g., cold temperatures, heavy rainfalls), peat seems to have formed, whatever the geological nature of the rocks [62]. As a consequence, communities of peaty formations associated with soft-water marginal SSL are mostly independent of the water composition of the lakes [63], with numerous acidophilous species, even in calcareous fens [64]. This phenomenon can explain the chemical outliers (calcareous fens) associated with soft-water marginal lakes group.

Hydroperiod is another driver highlighted by our results. This factor influenced floristic composition, affecting hydrophyte species [65], and favoring therophyte species, or species adapted to drought or frequent water level fluctuations, such as helophytes. Helophytes are related to hydroperiod. Their adaptations can explain their distribution in the four groups of plants. Water fluctuations imply a discontinuity of the habitat conditions suitable for aquatic plant species, for shorter or longer periods, and induced stress for most aquatic plants, whereas helophytes are well-adapted to these conditions.

The influences of hydroperiod can be observed in our dataset through species with strong affinities with temporary systems, such as the occurrence of *Sphagnum inundatum* in the soft-water SSL, or *Calliergonella cuspidata* in the mixed SSL.

4.2. Drivers of Macrophyte Species Richness

Contrary to what was predicted, richness was not correlated with calcium concentration, nor with alkalinity. This result is surprising because other studies have found a major effect of alkalinity and correlated chemical parameters on macrophyte richness in varying regions (e.g., [35,66,67]). We also expected to find higher macrophyte richness in calcareous SSL than in soft-water SSL. Soft-water stations (Group 1) had a moderate richness (n = 11). The highest richness was observed in the soft-water marginal lakes (Group 2: n = 22) and in the mixed group (Group 3: n = 22). If we have established that soft-water marginal lakes are richer in species than soft-water or calcareous SSL (Group 2 versus Groups 1 and 4), this is clearly linked to the higher surface area of the lakes. The richness in Group 3 (mixed) could be explained by the presence of some large sites. In contrast, the calcareous Group (Group 4) had the lowest richness (n = 9). Most of the larger calcareous lakes in France, and more broadly in Europe, are impacted [51]. In our study, most of the calcareous SSL were small, with rocky substrata, factors which do not favor the establishment of numerous plants, and lead to low floristic richness.

Hydroperiod was not found to be an important predictor of floristic richness. These results were surprising, since moderate water level fluctuations may increase floristic richness [68], and severe droughts may also reduce it [69]. The effects of water fluctuation in the investigated SSL are unclear, because: (1) most of the temporary SSL were very small, making it difficult to disentangle the influence of surface area from that of hydroperiod; and (2) water level fluctuations were not quantified.

Thus, our first hypothesis was only partially validated. Floristic richness appears to be influenced mostly by surface area. Lack of evidence of the influence of alkalinity may be explained by the dominance in SSL of helophytes species, which are less sensitive to carbon dioxide availability.

Similarly to the case of alkalinity, the influence of nutrients on macrophyte community richness is well-known [36]. Nitrogen concentrations may be negatively correlated with macrophyte richness [70]. The response of macrophyte richness to total phosphorus is frequently unimodal, decreasing [66] or increasing with eutrophication [71], which is

probably hiding a more general hump-shaped relationship (low richness in oligotrophic, high richness in mesotrophic, and low richness in hypereutrophic lakes) [72]. These relations can also vary across regions or contexts [73] and macrophyte growth-forms [67]. Our results showed no correlation between total phosphorus and macrophyte species richness, probably due to the low phosphorus concentrations found in most of our SSL.

Contrary to our second hypothesis, we did not establish a correlation between aquatic plant richness and climate (neither with mean annual temperature nor with elevation). Several studies have demonstrated a linear decrease or negative correlation of macrophyte species richness with altitude [67,74,75], probably depending on the strength of elevational gradient [76]. However, some other studies failed to demonstrate a clear pattern between floristic richness and altitude [34,77]. The generality of a decrease in species richness with elevation has been more and more criticized [78]. The decrease is not necessarily uniform, nor similar for all groups of organisms. Indeed, Rahbek [79] showed that a decrease in species richness was not the rule, and that approximately half of the studies had a mid-elevation peak in species richness, because mountains may represent an attractive ecosystem for certain organisms. There are several environmental constraints that change with elevation [80]: those directly linked to the physical elevation above sea level (e.g., temperature, oxygen), and those determined by local characteristics (e.g., geology, land use). Confounding the former with the latter introduces confusion into studies of elevational gradients. The floristic richness of our high-altitude SSL was sometimes lower than the richness in other SSL, but some sites had levels of floristic richness that were similar to those observed at mid- or low-altitudes. Differences in macrophyte species richness between high altitude SSL probably reflect morphological features of the lakes (steep slopes versus flat banks, depth, type of substrate) more than climatic limitations [81].

We found a strong positive correlation between macrophyte species richness and surface area. Relationships between surface area and floristic richness are generally accepted [34,82]. For example, macrophyte species richness has been found to respond positively to increasing surface area in Norway [68], in Finland, and in Minnesota [66], or in the study by Rørslett [66] in North Europe. Some conflicting results [83,84] were obtained in studies that were not focused on least-impacted sites. Disturbances such as eutrophication and stress, well known as factors reducing floristic richness [45], could explain the contrasting results in richness–area relationships. Another possible cause of confusion is the contrasting responses of particular floristic groups in SSL, with helophyte richness clearly being sensitive to surface area but not hydrophyte richness, because the hydrophyte species pool would be too small in SSL, and the deepest zones and bottom sediments of larger SLL are frequently unfavorable to hydrophytes [68].

5. Conclusions

To conclude, our study highlights that factors influencing macrophyte abundance and composition in French least-impacted conditions are mainly geology and DIS, which determine soft-water, mixed, and calcareous communities. In particular, DIS separates most of the mixed communities from the soft-water or calcareous groups. DIS is also an indication of a major connectivity pattern. However, our findings suggest that mineralization and, to a lesser extent, total phosphorus concentrations were determinant variables of SSL macrophyte community composition, but not of their richness, which is mostly correlated with surface area. Finally, climate and hydroperiod marginally filter macrophyte composition. The drivers of aquatic plant community composition differed from the drivers of aquatic plant richness in least-impacted small lakes. Contrary to Edvardsen and Økland [65], who suggested aquatic communities in SLL are unpredictable, we expect that the combination of geology and DIS may be useful in predicting floristic composition. Stochastic events determining macrophyte composition, in particular randomness in dispersal and establishment success, might be largely compensated by a generalized dispersal pattern, where macrophyte dispersal originates from the nearest river hydrosystem, a parameter

represented by DIS. On the other hand, global optimal floristic richness could be predicted with high reliability from surface area.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4441/13/5/609/s1>, Table S1: Dunn’s test results for 33 environmental parameters between the 4 groups obtained with cluster partition from abundance-based taxonomic matrix. S = soft-water, SW = soft-water marginal, M = mixed, C = calcareous. Table S2: Complete results of Indicator species analysis Indval performed on the 4 groups obtained with cluster partition from abundance-based taxonomic matrix. Table S3: complete results of the correlation analysis between environmental parameters floristic richness and diversity indices.

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