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Principal determinants of aquatic macrophyte communities in least-impacted small shallow lakes in France

Frédéric Labat [1,2, *], Gabrielle Thiébaut [2] and Christophe Piscart [2]

[1]   Aquabio, 10 Rue Hector Guimard, 63800 Cournon-d'Auvergne, France; frederic.labat@aquabio-conseil.com
[2]   Univ Rennes, CNRS, Géosciences Rennes - UMR 6118, 263 Avenue Général Leclerc, Campus de Beaulieu, Bâtiment 15, F-35000, Rennes, France
*   Correspondence: frederic.labat@aquabio-conseil.com

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, that causes a shift to dysfunctional turbid states and induces 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 factors determining the composition and richness of aquatic plant communities in least-impacted conditions may be useful to protect them. 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 mineralisation. Determinant factors identified by db-RDA analysis are, 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 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 is identified as determinant in freshwater lentic ecosystems for the first time.

Keywords: 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], which are the most abundant freshwater ecosystems on Earth [3]. The term SSL includes both man-made and natural waterbodies. The mean depth of SSL in temperate regions is less than 3 m, but can reach 7m [4], and their surface area ranges from 1 m² to 100 ha [5]. 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 or bats, by providing habitat or food [10,11]. SSL play a role in regional carbon processing, 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, urbanisation 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, favouring cyanobacteria and altering nutrients and light availability [24]. Furthermore, extensive nutrients 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. In their pristine state, 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, (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 is 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 resistant 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 underlying 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 is 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 is 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), 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 which were characterized by shading equal to, or higher than, 75% were not included in the analysis, because of their very low floristic richness [38].
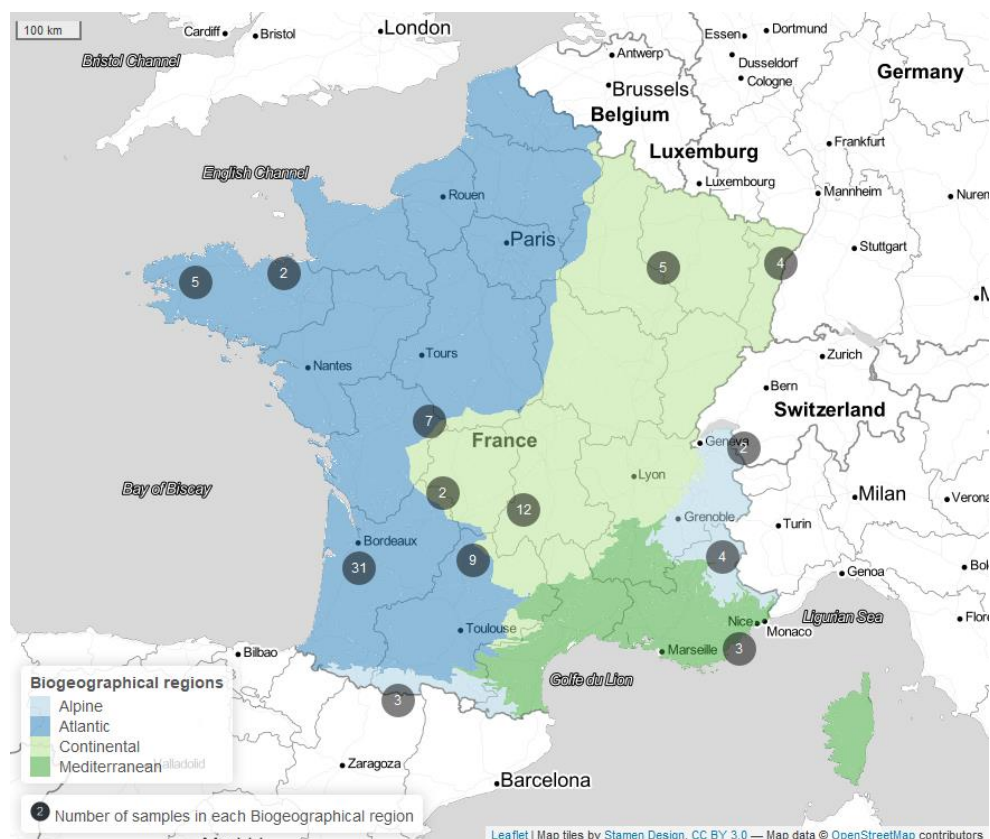
Figure 1. Localization of the sampling sites.

2.2. Environmental data

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 SLL 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 (e.g. mean annual air temperature, mean annual air temperature amplitude, mean annual precipitation) were extracted from an INRAE reanalysis [41] of the SAFRAN/France database [42] for the years 2010-2016, with a grid size of 5 km.

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, 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, 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 at least 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 water-bodies 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].

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

$$J \ = \ \frac{H'}{\log S'} \tag{2}$$

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

where pi 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.

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 within-group sum of squares [48].

Community groups were then determined by comparing the distance matrix obtained with dendrogram and binary matrices representing partitions [49]. 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, with very low alkalinity, whereas soft-water-marginal lakes are characterized by Ca c10 -15 mg/L, and higher pH [35]. Calcareous lakes are those with Ca > 20 mg/L and alkalinity > 1 meq/L [50].

Finally, indicator species for each group were identified using the indicspecies package [51,52].

IndVal is a measure of association between a taxon and a group of stations and is calculated as the product of specificity (mean biomass of a given taxon within one group compared to other groups) and fidelity (taxon occurrence at stations belonging to a group). 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.

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) [53] combined with parametric forward selection [54] and tested by an ANOVA-like permutation test [55].

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 [56]. We used R software [57] 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 the Dunn test and db-RDA analysis (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.
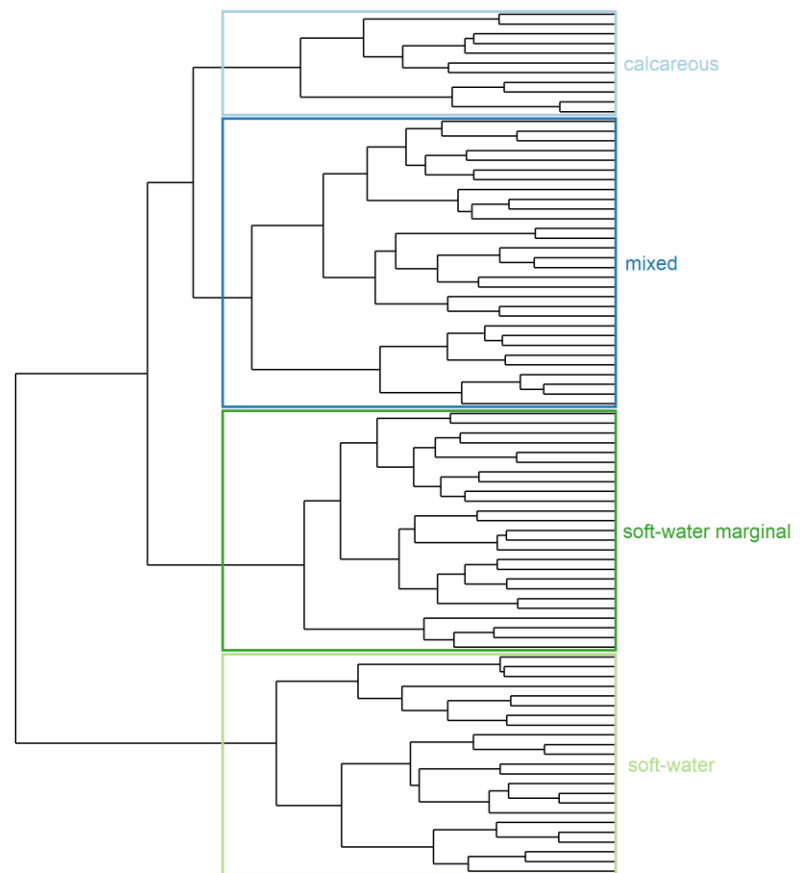
Figure 2. Community group partition. Ward's minimum variance cluster analysis based on Square-rooted Bray-Curtis matrix from abundance-classes taxonomic composition at 89 sites sampled between 2013-2018. Groups are labelled according to the major variables identified by and db-RDA analysis (alkalinity, calcium, pH): calcareous (C), mixed (M), soft-water marginal (SW), soft water (S).

Table 1. Floristic community groups and their respective median values and standard deviation in parenthesis for taxonomic richness, calcium (mg/L), alkalinity (meq/L) pH, DIS (distance from the source, km), surface area (m²), altitude (meters above sea level), and some best indicator taxa for each group. Complete indicators taxa and corresponding index values (specificity, fidelity, indicator value) are precised Table S6.

Community group	N° of station	Richness	Calcium mg/L	Alkalinity Meq/L	pH	DIS in m	Surface in m²	Altitude in m NGF	Significant indicator taxa
Soft-water	23	11 (5)	1.83 (3.25)	0.18 (0.21)	5.9 (0.81)	0 (0.8)	242 (2585)	68 (257)	*Eleocharis multicaulis* *Potamogeton polygonifolius* *Sphagnum auriculatum*
Soft-water-marginal	25	22 (12)	3.7 (16.28)	0.24 (0.91)	7.5 (0.97)	0.4 (22)	9855 (93324)	857 (109)	*Carex rostrata* *Sphagnum fallax* *Ranunculus peltatus*
Mixed	30	22 (9)	31.35 (38.82)	1.85 (1.89)	7.93 (0.62)	5.2 (238)	1975 (23841)	30 (109)	*Phragmites australis* *Leptodyctium riparium* *Potamogeton lucens*
Calcareous	11	9 (4)	63 (24.53)	2.68 (1.17)	8.3 (0.36)	0 (2.8)	195 (2146)	371 (908)	*Juncus articulatus* *Ranunculus trichophyllus* *Chara contraria* *Drepanocladus aduncus*
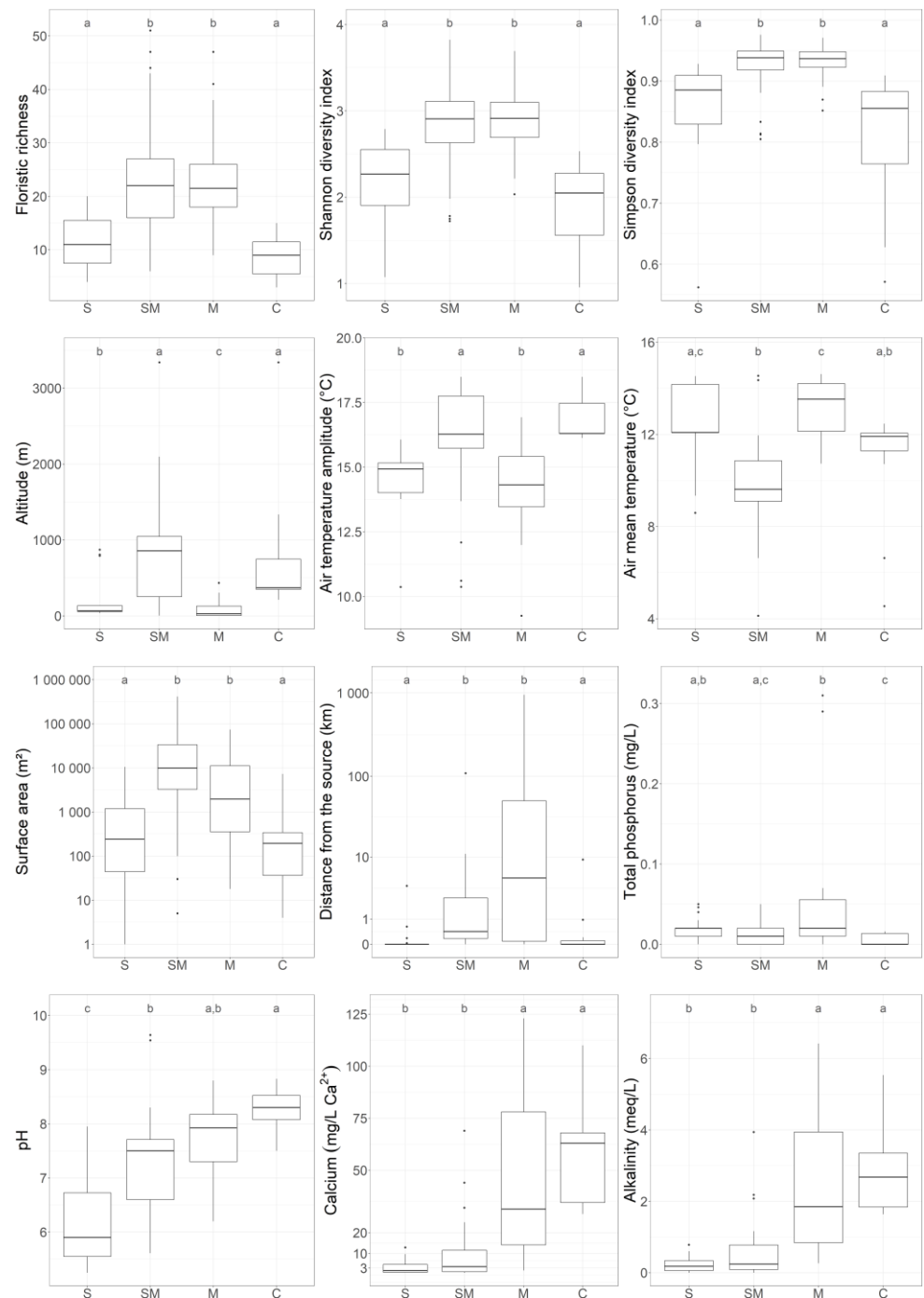
Figure 3. Box-plots of environmental variables that were significantly different among the different types of communities defined by group partition. S = soft-water, SM = soft-water marginal, M = mixed, C = calcareous group. A Dunn's test revealed significance groups for each variable, represented by letters a,b,c.

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 (<138m asl), with the exception of three outliers (around 800m 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).

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 Softwater 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 to this group, and explained most of 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 ± 21.6km) 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 ± 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 ± 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 ± 2.8km, 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 ± 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. Among the 22 explanatory variables employed in the forward selection of the db-RDA model, 10 variables were retained (Table 2).
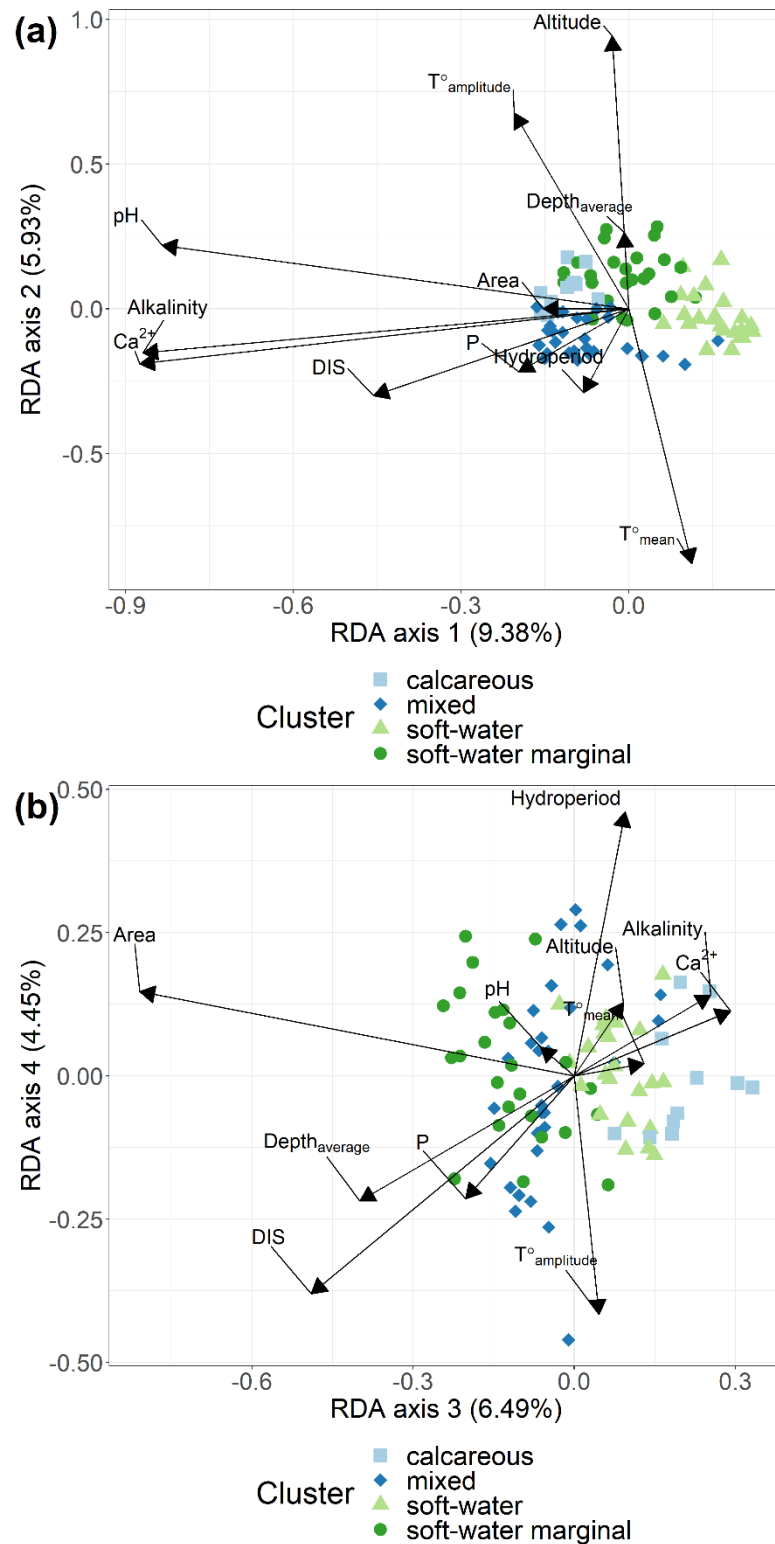
Figure 4. Distance-based redundancy analysis (db-RDA) ordination plots of macrophytes abundance-based taxonomic composition against forward selected environmental variables (black arrows) on 89 stations sampled from 2013 to 2018. **(a)** = Axis 1 & Axis 2, **(b)** = Axis 3 & Axis 4. Explained variance (Pr <0.001) for each axis are indicated in parenthesis at each legend axis. Colors and points 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.

Table 2. Results of correlations between axes and environmental variables and ANOVA-like permutation test performed on db-RDA computed with macrophytes abundance-based taxonomic composition against forward selected environmental variables. Environmental variables are ordered according to the F values obtained with the permutation test. Pr(>f) = p.values of the permutation tests.

| Factors	Correlation with axes				ANOVA test	
	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**	**F**	**Pr (>f)**
Ca^{2+} concentration	**-0,8**	-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

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

According to the ANOVA-like permutation test and the correlations test with db-RDA axes (Table 2), the best explanatory variables were, in order of importance, surface area (F = 3.15, *P* = 0.001), DIS (F = 2.53, *P* = 0.002), Calcium concentration (F = 2.44, *P* = 0.001), altitude (F = 2.21, P = 0.007) and mean annual air temperature (F = 2.14, *P* = 0.005). Alkalinity (r = -0.79), calcium concentration (r = -0.79), pH (r = -0.76) and DIS (r = -0.41) were negatively correlated with the first db-RDA axis. Altitude (r = 0.88) and air temperature amplitude (r = 0.62) were positively correlated with the second db-RDA axis, whereas mean annual air temperature (r = -0.77) was negatively correlated with this second axis. Surface area (r = -0.68) and air temperature amplitude (r = -0.44) were negatively correlated with the third db-RDA axis, and hydroperiod (r = 0.37) with the fourth db-RDA axis. 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, and a moderate correlation with surface area (r = 0.46) was established. However, the influence of mean depth was not significant (P = 0.077).

3.3. Community characteristics: biological and environmental links

Floristic richness (Figure 5) was positively correlated with the Shannon H' and Simpson D1 indices (r = 1 and r = 0.99, respectively). The Shannon H' and Simpson D1 indices were perfectly correlated (r = 1). Pielou's J was not strongly correlated with any community characteristics nor with any environmental variables (best correlation r = -0.58 with Simpson D1) (Table S3).

Among the relationships tested with environmental variables, Floristic Richness and Shannon H' index were strongly positively correlated with surface area (respectively, r = 0.73, r = 0.73) and perimeter (r = 0.74, r = 0.74, respectively). Simpson D1 index was strongly negatively correlated with surface area, r = -0.74) and perimeter (r = -0.74).
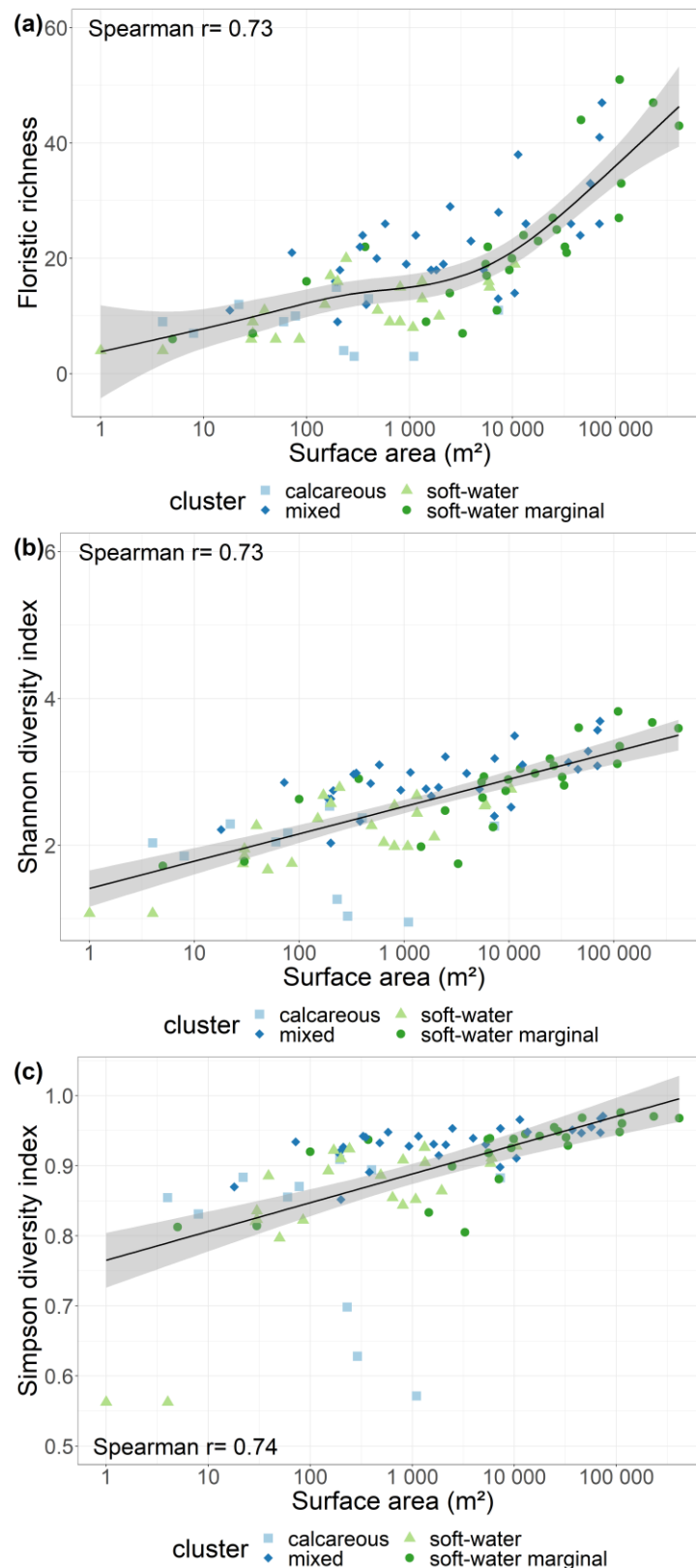
Figure 5. Relationships between surface area of each SSL and **(a)** floristic richness, **(b)** Shannon diversity index, **(c)** Simpson diversity index. Community groups (soft-water, soft-water marginal, mixed and calcareous) are indicated.

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. 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 [58]. 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 [59]. Continental France is subject to highly heterogenous 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 [60]. Climate and altitude seem to have a marginal influence on macrophyte community composition, 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.

Hydroperiod is another driver highlighted by our results. This factor influenced floristic composition, affecting hydrophyte species [61] and favouring 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 [e.g.35,62,63] a major effect of alkalinity and correlated chemical parameters on macrophyte richness in varying regions. We also expected to find higher macrophyte richness in Calcareous SSL than in Soft-Water SSL. Soft-Water Stations (Group 1) had 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 [50]. In our study, most of the Calcareous SSL were small, with calcareous rocky substrata, factors which do not favour the establishment of numerous plants and lead to low floristic richness.

Water fluctuation was not found to be an important predictor of floristic richness. These results were surprising, since moderate water level fluctuations may increase floristic richness [64], and severe droughts may also reduce it [65]. 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, (2) water level fluctuations were not monitored accurately.

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 [66]. The response of macrophyte richness to total phosphorus is frequently unimodal, decreasing [62] or increasing with eutrophication [67], which is probably hiding a more general hump-shaped relationship (low richness in oligotrophic, high richness in mesotrophic, and low richness in hypereutrophic lakes) [68]. These relations can also vary across regions or contexts [69] and macrophyte growth-forms [63]. 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 [63,70,71], probably depending on the strength of elevational gradient [72]. However, some other studies failed to demonstrate a clear pattern between floristic richness and altitude [34,73]. The generality of a decrease in species richness with elevation has been more and more criticized [74]. The decrease is not necessarily uniform, nor similar for all groups of organisms. Indeed, Rahbek [75] 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 [76]: 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 [77].

We found a strong positive correlation between macrophyte species richness and surface area. Relationships between surface area and floristic richness are generally accepted [34,78]. For example, macrophyte species richness has been found to respond positively to increasing surface area in Norway [64], in Finland and in Minnesota [63] or in the study by Rørslett [62] in North Europe. Some conflicting results [79,80] were obtained in studies that were not focused on least-impacted sites. Disturbance 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 unfavourable to hydrophytes [64].

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 & Økland [64], 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.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

References

1. Wood, P.J.; Greenwood, M.T.; Agnew, M.D. Pond biodiversity and habitat loss in the UK. Area 2003, 35, 206–216, doi:10.1111/1475-4762.00249.
2. Downing, J.A.; Prairie, Y.T.; Cole, J.J.; Duarte, C.M.; Tranvik, L.J.; Striegl, R.G.; McDowell, W.H.; Kortelainen, P.; Caraco, N.F.; Melack, J.M.; et al. The global abundance and size distribution of lakes, ponds, and impoundments. Limnol. Oceanogr. 2006, 51, 2388–2397, doi:10.4319/lo.2006.51.5.2388.
3. Downing, J.A. Emerging global role of small lakes and ponds: little things mean a lot. Limnetica 2010, 29, 9–24, doi:10.23818/limn.29.02.

4.   Meerhoff, M.; Jeppesen, E. Shallow Lakes and Ponds. In Lake ecosystem ecology: a global perspective: a derivative of Encyclopedia of inland waters; Likens, G.E., Ed.; Elsevier/Academic Press: Amsterdam ; Boston, 2010; pp. 343–353 ISBN 978-0-12-382002-0.

5.   Moss, B. Ponds and small lakes; Naturalists'Handbooks; Pelagic Publishing, 2017; Vol. 32; ISBN 978-1-78427-138-1.

6.   Padisák, J.; Reynolds, C.S. Shallow lakes: the absolute, the relative, the functional and the pragmatic. Hydrobiologia 2003, 506–509, 1–11, doi:10.1023/B:HYDR.0000008630.49527.29.

7.   Scheffer, M. Alternative Attractors of Shallow Lakes. Sci. World J. 2001, 1, 254–263, doi:10.1100/tsw.2001.62.

8.   O'Sullivan, P.E.; Reynolds, C.S. The Lakes Handbook Volume 2 - Lake restoration and rehabilitation; Blackwell Science, 2005; Vol. 2;.

9.   Biggs, J.; von Fumetti, S.; Kelly-Quinn, M. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. Hydrobiologia 2017, 793, 3–39, doi:10.1007/s10750-016-3007-0.

10.   Salvarina, I.; Gravier, D.; Rothhaupt, K. Seasonal bat activity related to insect emergence at three temperate lakes. Ecol. Evol. 2018, 8, 3738–3750, doi:10.1002/ece3.3943.

11.   Vanausdall, R.A.; Dinsmore, S.J. Habitat Associations of Migratory Waterbirds Using Restored Shallow Lakes in Iowa. Waterbirds 2019, 42, 135–153, doi:10.1675/063.042.0201.

12.   Williamson, C.E.; Dodds, W.; Kratz, T.K.; Palmer, M.A. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front. Ecol. Environ. 2008, 6, 247–254, doi:10.1890/070140.

13.   Mitsch, W.J.; Bernal, B.; Nahlik, A.M.; Mander, Ü.; Zhang, L.; Anderson, C.J.; Jørgensen, S.E.; Brix, H. Wetlands, carbon, and climate change. Landsc. Ecol. 2013, 28, 583–597, doi:10.1007/s10980-012-9758-8.

14.   Zedler, J.B. Wetlands at your service: reducing impacts of agriculture at the watershed scale. Front. Ecol. Environ. 2003, 1, 65–72.

15.   Schmadel, N.M.; Harvey, J.W.; Alexander, R.B.; Schwarz, G.E.; Moore, R.B.; Eng, K.; Gomez-Velez, J.D.; Boyer, E.W.; Scott, D. Thresholds of lake and reservoir connectivity in river networks control nitrogen removal. Nat. Commun. 2018, 9, 1–10, doi:10.1038/s41467-018-05156-x.

16.   Bajkiewicz-Grabowska, E.; Golus, W.; Markowski, M.; Kwidzińska, M. The Role of Lakes in Shaping the Runoff of Lakeland Rivers. In Polish River Basins and Lakes – Part I: Hydrology and Hydrochemistry; Korzeniewska, E., Harnisz, M., Eds.; The Handbook of Environmental Chemistry; Springer International Publishing: Cham, 2020; pp. 175–187 ISBN 978-3-030-12123-5.

17.   Boix, D.; Biggs, J.; Céréghino, R.; Hull, A.P.; Kalettka, T.; Oertli, B. Pond research and management in Europe: "Small is Beautiful." Hydrobiologia 2012, 689, 1–9, doi:10.1007/s10750-012-1015-2.

18.   Beklioğlu, M.; Meerhoff, M.; Davidson, T.A.; Ger, K.A.; Havens, K.; Moss, B. Preface: Shallow lakes in a fast changing world. Hydrobiologia 2016, 778, 9–11, doi:10.1007/s10750-016-2840-5.

19.   Oertli, B. Editorial: Freshwater biodiversity conservation: The role of artificial ponds in the 21st century. Aquat. Conserv. Mar. Freshw. Ecosyst. 2018, 28, 264–269, doi:10.1002/aqc.2902.

20.   Boothby, J.; Hull, A.P. A census of ponds in Cheshire, North West England. 1997, 7, 5.

21.   Declerck, S.; De Bie, T.; Ercken, D.; Hampel, H.; Schrijvers, S.; Van Wichelen, J.; Gillard, V.; Mandiki, R.; Losson, B.; Bauwens, D.; et al. Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. Biol. Conserv. 2006, 131, 523–532, doi:10.1016/j.biocon.2006.02.024.

22.   Moss, B. Allied attack: climate change and eutrophication. Inland Waters 2011, 1, 101–105, doi:10.5268/IW-1.2.359.

23.   Seebens, H.; Blackburn, T.M.; Dyer, E.E.; Genovesi, P.; Hulme, P.E.; Jeschke, J.M.; Pagad, S.; Pyšek, P.; Winter, M.; Arianoutsou, M.; et al. No saturation in the accumulation of alien species worldwide. Nat. Commun. 2017, 8, 14435, doi:10.1038/ncomms14435.

24.   Piccioni, F.; Casenave, C.; Lemaire, B.J.; Le Moigne, P.; Dubois, P.; Vinçon-Leite, B. The response of small and shallow lakes to climate change: new insights from hindcast modelling; Dynamics of the Earth system: models, 2020;

25.   Zhang, C.; Huang, Y.; Špoljar, M.; Zhang, W.; Kuczyńska-Kippen, N. Epiphyton dependency of macrophyte biomass in shallow reservoirs and implications for water transparency. Aquat. Bot. 2018, 150, 46–52, doi:10.1016/j.aquabot.2018.07.001.

26.   Scheffer, M.; van Nes, E.H. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. Hydrobiologia 2007, 584, 455–466, doi:10.1007/s10750-007-0616-7.

27.   Scheffer, M.; Hosper, S.H.; Meijer, M.-L.; Moss, B.; Jeppesen, E. Alternative equilibria in shallow lakes. Trends Ecol. Evol. 1993, 8, 275–279, doi:10.1016/0169-5347(93)90254-M.

28.   Pip, E.; Robinson, G.G.C. A comparison of algal periphyton composition on eleven species of submerged macrophytes. Hydrobiol. Bull. 1984, 18, 109–118, doi:10.1007/BF02257050.

29.   Santos, T.R. dos; Ferragut, C.; Santos, T.R. dos; Ferragut, C. Changes in the taxonomic structure of periphytic algae on a free-floating macrophyte (Utricularia foliosa L.) in relation to macrophyte richness over seasons. Acta Bot. Bras. 2018, 32, 595–601, doi:10.1590/0102-33062018abb0031.

30.   Thomaz, S.M.; Cunha, E.R. da The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. Acta Limnol. Bras. 2010, 22, 218–236, doi:10.4322/actalb.02202011.

31.   Martín, J.; Luque-Larena, J.J.; López, P. Factors affecting escape behavior of Iberian green frogs (Rana perezi). Can. J. Zool. 2005, 83, 1189–1194, doi:10.1139/z05-114.

32.   Wetzel, R.G. Limnology: lake and river ecosystems; 3rd ed.; Academic Press: San Diego, 2001; ISBN 0-12-744760-1.

33. Sand-Jensen, K.; Andersen, M.R.; Martinsen, K.T.; Borum, J.; Kristensen, E.; Kragh, T. Shallow plant-dominated lakes – extreme environmental variability, carbon cycling and ecological species challenges. Ann. Bot. 2019, 124, 355–366, doi:10.1093/aob/mcz084.

34. Fernández-Aláez, C.; Fernández-Aláez, M.; García-Criado, F.; García-Girón, J. Environmental drivers of aquatic macrophyte assemblages in ponds along an altitudinal gradient. Hydrobiologia 2018, 812, 79–98, doi:10.1007/s10750-016-2832-5.

35. Murphy, K.J. Plant communities and plant diversity in softwater lakes of northern Europe. Aquat. Bot. 2002, 73, 287–324, doi:10.1016/S0304-3770(02)00028-1.

36. Lacoul, P.; Freedman, B. Environmental influences on aquatic plants in freshwater ecosystems. Environ. Rev. 2006, 14, 89–136, doi:10.1139/a06-001.

37. Collinson, N.H.; Biggs, J.; Corfield, A.; Hodson, M.J.; Walker, D.; Whitfield, M.; Williams, P.J. Temporary and permanent ponds: An assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. Biol. Conserv. 1995, 74, 125–133, doi:10.1016/0006-3207(95)00021-U.

38. Brian, A.D. The flora of the marl-pits (ponds) in one Cheshire parish. Watsonia 1987, 16, 417–426.

39. Indermuehle, N.; Angélibert, S.; Rosset, V.; Oertli, B. The pond biodiversity index "IBEM": a new tool for the rapid assessment of biodiversity in ponds from Switzerland. Part 2. Method description and examples of application. Limnetica 2010, 29, 105–120, doi:10.23818/limn.29.08.

40. Petts, G.E.; Amoros, C. The Fluvial Hydrosystems; Springer Netherlands: Dordrecht, 1996; ISBN 978-94-009-1491-9.

41. Marzin, A.; Delaigue, O.; Logez, M.; Belliard, J.; Pont, D. Jeux de données de référence pour le calcul de l'IPR+ Available online: http://seee.eaufrance.fr/algos/IPRplus/Documentation/IPRplus_v1.0.3_Import_export.zip (accessed on May 4, 2020).

42. Vidal, J.-P.; Martin, E.; Franchistéguy, L.; Baillon, M.; Soubeyroux, J.-M. A 50-year high-resolution atmospheric reanalysis over France with the Safran system. Int. J. Climatol. 2010, 30, 1627–1644, doi:10.1002/joc.2003.

43. Linton, S.; Goulder, R. Botanical conservation value related to origin and management of ponds. Aquat. Conserv. Mar. Freshw. Ecosyst. 2000, 10, 77–91, doi:10.1002/(SICI)1099-0755(200003/04)10:2<77::AID-AQC391>3.0.CO;2-Y.

44. AFNOR XP T90-328 - Échantillonnage des communautés de macrophytes en plans d'eau; AFNOR: Paris, 2010; p. 33;.

45. Biggs, J.; Williams, P.; Whitfield, M.; Fox, G.; Nicolet, P.; Shelley, H. A new biological method for assessing the ecological quality of lentic waterbodies. In L'eau, de la cellule au paysage; Elsevier: Paris, 2000; pp. 235–250 ISBN 978-2-84299-243-9.

46. Magurran, A.E. Measuring biological diversity; Blackwell Pub: Malden, Ma, 2004; ISBN 0-632-05633-9.

47. Leprêtre, A.; Mouillot, D. A comparison of species diversity estimators. Popul. Ecol. 1999, 41, 203–215, doi:10.1007/s101440050024.

48. Ward, J.H. Hierarchical Grouping to Optimize an Objective Function. J. Am. Stat. Assoc. 1963, 58, 236–244.

49. Borcard, D. Numerical ecology with R; Use R!; Springer: New York, 2011; ISBN 978-1-4419-7975-9.

50. Lyche Solheim, A.; Globevnik, L.; Austnes, K.; Kristensen, P.; Moe, S.J.; Persson, J.; Phillips, G.; Poikane, S.; van de Bund, W.; Birk, S. A new broad typology for rivers and lakes in Europe: Development and application for large-scale environmental assessments. Sci. Total Environ. 2019, 697, 134043, doi:10.1016/j.scitotenv.2019.134043.

51. Dufrene, M.; Legendre, P. Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. Ecol. Monogr. 1997, 67, 345, doi:10.2307/2963459.

52. De Cáceres, M.; Legendre, P.; Moretti, M. Improving indicator species analysis by combining groups of sites. Oikos 2010, 119, 1674–1684, doi:10.1111/j.1600-0706.2010.18334.x.

53. Legendre, P.; Anderson, M.J. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 1999, 69, 1–24, doi:10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2.

54. Dray, S.; Pélissier, R.; Couteron, P.; Fortin, M.-J.; Legendre, P.; Peres-Neto, P.R.; Bellier, E.; Bivand, R.; Blanchet, F.G.; De Cáceres, M.; et al. Community ecology in the age of multivariate multiscale spatial analysis. Ecol. Monogr. 2012, 82, 257–275, doi:10.1890/11-1183.1.

55. Legendre, P.; Oksanen, J.; ter Braak, C.J.F. Testing the significance of canonical axes in redundancy analysis: Test of canonical axes in RDA. Methods Ecol. Evol. 2011, 2, 269–277, doi:10.1111/j.2041-210X.2010.00078.x.

56. Quinn, G.P.; Keough, M.J. Experimental design and data analysis for biologists; Cambridge University Press: Cambridge, UK; New York, 2002; ISBN 0-511-07812-9.

57. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/.

58. Madsen, T.V.; Maberly, S.C.; Bowes, G. Photosynthetic acclimation of submersed angiosperms to CO_2 and HCO^{-}_3. Aquat. Bot. 1996, 53, 15–30, doi:10.1016/0304-3770(95)01009-2.

59. Alahuhta, J.; Lindholm, M.; Bove, C.P.; Chappuis, E.; Clayton, J.; de Winton, M.; Feldmann, T.; Ecke, F.; Gacia, E.; Grillas, P.; et al. Global patterns in the metacommunity structuring of lake macrophytes: regional variations and driving factors. Oecologia 2018, 188, 1167–1182, doi:10.1007/s00442-018-4294-0.

60. García-Girón, J.; Heino, J.; Baastrup-Spohr, L.; Bove, C.P.; Clayton, J.; de Winton, M.; Feldmann, T.; Fernández-Aláez, M.; Ecke, F.; Grillas, P.; et al. Global patterns and determinants of lake macrophyte taxonomic, functional and phylogenetic beta diversity. Sci. Total Environ. 2020, 723, 138021, doi:10.1016/j.scitotenv.2020.138021.

61. Sciandrello, S.; Privitera, M.; Puglisi, M.; Minissale, P. Diversity and spatial patterns of plant communities in volcanic temporary ponds of Sicily (Italy). Biologia (Bratisl.) 2016, 71, doi:10.1515/biolog-2016-0096.

62. Rørslett, B. Principal determinants of aquatic macrophyte richness in northern European lakes. Aquat. Bot. 1991, 39, 173–193, doi:10.1016/0304-3770(91)90031-Y.

63. Alahuhta, J.; Toivanen, M.; Hjort, J.; Ecke, F.; Johnson, L.B.; Sass, L.; Heino, J. Species richness and taxonomic distinctness of lake macrophytes along environmental gradients in two continents. Freshw. Biol. 2017, 62, 1194–1206, doi:10.1111/fwb.12936.

64. Edvardsen, A.; Økland, R.H. Variation in plant species richness in and adjacent to 64 ponds in SE Norwegian agricultural landscapes. Aquat. Bot. 2006, 85, 79–91, doi:10.1016/j.aquabot.2006.01.014.

65. El Madihi, M.; Rhazi, L.; Van den Broeck, M.; Rhazi, M.; Waterkeyn, A.; Saber, E.; Bouahim, S.; Arahou, M.; Zouahri, A.; Guel-mami, A.; et al. Plant community patterns in Moroccan temporary ponds along latitudinal and anthropogenic disturbance gradients. Plant Ecol. Divers. 2017, 10, 197–215, doi:10.1080/17550874.2017.1346716.

66. James, C.; Fisher, J.; Russell, V.; Collings, S.; Moss, B. Nitrate availability and hydrophyte species richness in shallow lakes. Freshw. Biol. 2005, 50, 1049–1063, doi:10.1111/j.1365-2427.2005.01375.x.

67. Srivastava, D.S.; Staicer, C.A.; Freedman, B. Aquatic vegetation of Nova Scotian lakes differing in acidity and trophic status. Aquat. Bot. 1995, 51, 181–196, doi:10.1016/0304-3770(95)00457-B.

68. Azevedo, L.B.; van Zelm, R.; Elshout, P.M.F.; Hendriks, A.J.; Leuven, R.S.E.W.; Struijs, J.; de Zwart, D.; Huijbregts, M.A.J. Species richness-phosphorus relationships for lakes and streams worldwide: Freshwater species richness and phosphorus concentrations. Glob. Ecol. Biogeogr. 2013, 22, 1304–1314, doi:10.1111/geb.12080.

69. Declerck, S.; Vandekerkhove, J.; Johansson, L.; Muylaert, K.; Conde-Porcuna, J.M.; Van der Gucht, K.; Pérez-Martínez, C.; Lauridsen, T.; Schwenk, K.; Zwart, G.; et al. Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. Ecology 2005, 86, 1905–1915, doi:10.1890/04-0373.

70. Lacoul, P.; Freedman, B. Relationships between aquatic plants and environmental factors along a steep Himalayan altitudinal gradient. Aquat. Bot. 2006, 84, 3–16, doi:10.1016/j.aquabot.2005.06.011.

71. Kochjarová, J.; Novikmec, M.; Oľaheľová, H.; Hamerlík, L.; Svitok, M.; Hrivnák, M.; Senko, D.; Bubíková, K.; Matúšová, Z.; Paľove-Balang, P.; et al. Vegetation-Environmental Variable Relationships in Ponds of Various Origins along an Altitudinal Gradient. Pol. J. Environ. Stud. 2017, 26, 1575–1583, doi:10.15244/pjoes/68155.

72. Alahuhta, J. Macroecology of macrophytes in the freshwater realm: Patterns, mechanisms and implications. Aquat. Bot. 2021, 168, 10.

73. Chappuis, E.; Ballesteros, E.; Gacia, E. Aquatic macrophytes and vegetation in the Mediterranean area of Catalonia: patterns across an altitudinal gradient. Phytocoenologia 2011, 41, 35–44, doi:10.1127/0340-269X/2011/0041-0467.

74. Kattan, G.H.; Franco, P. Bird Diversity along Elevational Gradients in the Andes of Colombia: Area and Mass Effects. Glob. Ecol. Biogeogr. 2004, 13, 451–458.

75. Rahbek, C. The Elevational Gradient of Species Richness: A Uniform Pattern? Ecography 1995, 18, 200–205.

76. Körner, C. The use of 'altitude' in ecological research. Trends Ecol. Evol. 2007, 22, 569–574, doi:10.1016/j.tree.2007.09.006.

77. Zelnik, I.; Potisek, M.; Gaberščik, A. Environmental Conditions and Macrophytes of Karst Ponds. Pol. J. Environ. Stud. 2012, 21, 1911–1920.

78. Møller, T.R.; Rørdam, C.P.; Moller, T.R.; Rordam, C.P. Species Numbers of Vascular Plants in Relation to Area, Isolation and Age of Ponds in Denmark. Oikos 1985, 45, 8, doi:10.2307/3565216.

79. Friday, L.E. The diversity of macro invertebrate and macrophyte communities in ponds. Freshw. Biol. 1987, 18, 87–104, doi:10.1111/j.1365-2427.1987.tb01297.x.

80. Oertli, B.; Joye, D.A.; Castella, E.; Juge, R.; Cambin, D.; Lachavanne, J.-B. Does size matter? The relationship between pond area and biodiversity. Biol. Conserv. 2002, 104, 59–70, doi:10.1016/S0006-3207(01)00154-9.