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MICROALGAL FUNCTIONAL TRAITS

Rooted floating-leaf macrophytes structure the coexistence of different phytoplankton assemblages within a shallow lake

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Abstract Aquatic macrophytes in shallow lakes control habitats through local turbulence, water transparency, nutrient, and oxygen concentrations. As engineer species, they structure these ecosystems and increase biodiversity. Many studies have focused on submerged macrophytes, but research on habitats created by rooted floating-leaf macrophytes is scarcer. Macrophytes such as water lilies should have the similar ecological consequences as submerged macrophytes do, but with a greater shading effect. In this study, we show how macrophytes structure phytoplankton assemblages and allow different assemblages to coexist in the same shallow lake. During the summer of 2018, we characterized the phytoplankton assemblages in 9 stations covered by water lilies and 6 stations in open water, all in a large shallow lake. The lake was colonized on a third of its surface by water lilies from April to October. We showed an effect of waterlilies on temperature, oxygen, pH, turbidity, phosphates, and dissolved silicon. Many phytoplankton taxa from almost all classes were in higher abundance in the stations covered by macrophytes, while cyanobacteria showed a higher biomass and richness in open water. Unicellular mixotrophic flagellates predominated in the macrophyte habitats, where all representatives of the classes Euglenophyceae and Cryptophyceae were present.

Keywords Floating plant · Grand-Lieu nature reserve · Temperate lake · Phytoplankton ecology · Functional traits

Introduction

Freshwater covers 3% of continental land surface, with small shallow lakes and ponds largely dominating this global surface (Downing et al., 2006). However, their ecological functioning has been widely endangered by water pollution, eutrophication (Khan & Ansari, 2005), climate change (Paerl &

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Huisman, 2008; Moss, 2011), and invasive species (Reynolds & Aldridge, 2021).

Because of light dependency and wind energy transfer, the depth of a lake is critical to its functioning (Wetzel, 2001; Scheffer, 2004). Deep lakes are characterized by the presence of seasonal thermal stratification that isolates the water surface from nutrient-replete sediment. On the contrary, shallow lakes have a low thermal inertia that makes them more sensitive to meteorological extremes (rainfall, wind, and heatwaves). Bottom nutrients released under windy conditions are beneficial for pelagic phytoplankton (Sondergaard et al., 1992; Carrick et al., 1993). In shallow lakes, light is likely to reach the bottom of the lake, which can be colonized by submerged macrophytes (Scheffer, 2004). These macrophytes drive the light climate at the bottom of the lake by limiting the resuspension of sediment (James et al., 2004), but they can be outcompeted by phytoplankton and their associated turbidity.

Aquatic macrophytes in ponds and shallow lakes are particularly affected by eutrophication (Sayer et al., 2010; Labat et al., 2021). Submerged macrophytes disappear above a threshold of turbidity and strongly decline with eutrophication (Sand-Jensen et al., 2000; Phillips et al., 2016). This disequilibrium is well described in Scheffer's model with the two stable ecological states of shallow lakes—macrophyte dominance (clear state) and phytoplankton dominance (turbid state) (Scheffer & Jeppesen, 2007). Macrophytes compete with phytoplankton for light and nutrients in a complex interaction known to be a good ecological example of hysteresis (Scheffer, 2001).

Aquatic macrophytes are engineer species of shallow lakes, with many direct and indirect effects on the physical and chemical parameters of water, also on the production of bio-surface and habitats, and on aquatic communities themselves (Teubner et al., 2022). Depending on the biomass they reach, they can control biogeochemical cycles by absorbing nutrients and transiently storing them (Carpenter & Lodge, 1986; Teubner et al., 2022). Macrophytes act as a nutrient sink during the growing season, but they are a net source of dissolved organic carbon through the release of 1–10% of their photosynthetically fixed carbon into the water column (Carpenter & Lodge, 1986). Rooted macrophytes stabilize the sediment and dissipate the kinetic energy of waves and wind (Beklioglu & Moss, 1996; Madsen et al., 2001). They control gas exchanges with the atmosphere and the underwater oxygen concentration, with cascading effects on nutrient and water chemistry (Caraco et al., 2006). Daily thermal stratification can be observed in summer (Andersen et al., 2017), with a steep vertical gradient and potential anoxia (Carpenter & Lodge, 1986).

Macrophytes provide support and habitats for diverse epiphytic algae (Wijewardene et al., 2022), littoral zooplankton (Bolduc et al., 2016, 2020), and macro-invertebrates (Misteli et al., 2022, 2023). They fuel a high secondary production (from bacteria to macro-invertebrates) directly through organic carbon release (Søndergaard et al., 1998; de Kluijver et al., 2015). Periphyton growing on macrophytes provides an additional resource compared with open water (Vadeboncoeur et al., 2008; Jaschinski et al., 2011). Plant-associated cladocerans are observed in higher density in macrophytes and can feed on periphyton (Masclaux et al., 2012).

Macrophytes can also drive the trophic networks of lakes, from bacteria to birds (Jeppesen et al., 1998). They stabilize biotic interactions in the trophic network by providing refuge and habitats (Diehl, 1993). They provide refuge for intermediate predators such as macro-zooplankton (Jeppesen et al., 1998; Bertolo et al., 1999). Zooplankton taxa like *Daphnia* spp. move to macrophytes during the day through a process known as diel horizontal migration (DHM), and can preserve the clear-water state by grazing on phytoplankton (Perrow et al., 1999; Bertolo et al., 2000). Habitats generated by macrophytes support a large functional and specific diversity (Søndergaard & Moss, 1998).

All these effects and interactions have mainly been described for submerged macrophytes, but less is known about the other morphotypes of macrophytes, such as rooted macrophytes with surface leaves (Cazzanelli et al., 2008). Floating-leaf aquatic macrophytes are less diversified, but they are less affected by P eutrophication and increased turbidity (de Nie et al., 1987; Bornette & Puijalon, 2011). They can maintain their biomass in shallow lakes, particularly in littoral areas, under the form of more or less developed macrophyte beds. Moreover, even if most of the known effects of emerged macrophytes should be similar to those of submerged ones, we expect their direct (e.g., light) and indirect (e.g., temperature) shading effects to be stronger.

Studies highlighting the effects of macrophytes on phytoplankton have compared different shallow lakes with different levels of vegetation cover (Takamura et al., 2003; Hornbach et al., 2020). However, macrophyte beds create complex landscapes in lakes, with areas dominated by well-mixed turbid open water and areas more or less colonized by macrophytes. In summer, despite the high passive dispersive nature of phytoplankton, assemblages should differ between open-water and waterlily areas within a shallow lake if a large standing crop is achieved. Phytoplankton species sorting along an environmental gradient has been observed in a large tropical reservoir (Yang et al., 2018). While a change in zooplankton assemblages is expected with a higher density of littoral species (Cazzanelli et al., 2008; Bolduc et al., 2016), the effect on phytoplankton assemblages is barely documented (Gebrehiwot et al., 2017).

Two main habitats are expected in lakes partly colonized by floating-leaf rooted macrophytes: a turbulent, turbid and warm open-water area, and a cooler macrophyte zone, poor in light and nutrients but rich in dissolved organic carbon where mixing is driven by night cooling and convection. These habitats should select for different strategies (resource acquisition, grazer avoidance) reflected in the phytoplankton assemblages. By decreasing light availability, shade-adapted species should be favored in macrophyte zones. Reduced turbulence favors motile species. Low nutrient concentrations should favor nutrient-competitive species such as small cells. The release of dissolved organic carbon in parallel with low light availability should favor mixotrophic species. A higher zooplankton biomass is expected in macrophytes used as daytime refuge, so that strategies against grazing should be observed (Lürling, 2021). In open water, high nutrient availability and warm turbulent conditions should favor turbulence-tolerant cyanobacteria. These strategies adopted by phytoplankton can be related to morphological and physiological differences in traits (size, shape, motility, and nutritional status) and drive growth, sedimentation, and resource acquisition (Margalef, 1978; Reynolds et al., 2002).

We hypothesized that macrophytes modify the physical, chemical, and biological parameters compared to open-water stations, leading to different phytoplankton assemblages. By driving the local environment, plants structure phytoplankton assemblages and allow for the coexistence of different assemblages within a lake despite the strong dispersing power of phytoplankton. The three aims of the study were (1) to show the effect of plants on physical, chemical, and biological parameters, (2) to highlight different community structures between rooted floating-leaf macrophyte stations and open-water stations, and (3) to highlight the main environmental drivers of phytoplankton assemblages in a lowland shallow lake. We took samples in summer from a large shallow lake colonized by water lilies on a third of its surface. Water lilies (nymphaeid water plants) are common in alkaline waters in northern Europe (Smits et al., 1988) and represented a good model to test our hypotheses.

Methods

Lake parameters and sampling stations

The lake of Grand-Lieu is located in the west of France, about ten kilometers south-west of Nantes and 20 km from the Atlantic coast (47 4′59.999"N–1°40′0.001"W). It is classified as a natural reserve forbidden to the public. It has a catchment area of 700 km^2 that receives water from two main tributaries: the Boulogne and the Ognon rivers. Then, its waters are evacuated through the Acheneau channel into the Loire River. In summer, its surface is about 2,500 hectares for a depth of less than 1 m. The lake can be divided into several zones: open-water zones (absence of macrophytes), floating-leaf macrophyte beds (*Nymphaea alba* Linn., 1753*; Nuphar lutea* (L.) Smith, 1809*; Trapa natans* Linn., 1753*; Nymphoides peltata* (S.G.Gmel.) Kuntze, 1891), wooded reedbeds (carex beds, reed beds, willow groves, alder groves), and wet meadows (Paillisson & Marion, 2005). The lake has experienced hypereutrophication since the 1970s, leading to many different policies for the management of its water levels. See Table 1 for its limnological characteristics.

Sampling was conducted in July and August 2018. Fifteen monitoring stations were distributed across the lake, including the mouths of its two tributaries

Table 1 Limnological characteristics of Grand-Lieu Lake

Characteristic	Units	Mean value ± standard deviation (min–max)
Geographical coordinates	–	47° 4′ 59.999" N / 1° 40′ 0.001" W
Catchment area	km^2	700
Surface area	km^2	51 (25–65)
Depth	m	1.6 (0.8–4)
Residence time	days	219 (40–3,000)
Tributaries	–	Ognon & Boulogne
Conductivity	mS/cm	380 ± 58 (250–527)
pH	–	8.4 ± 0.7 (7.1–10.1)
Dissolved oxygen	%	98.9 ± 38 (13–287)
Secchi depth	cm	43 ± 27 (9–192)
Phosphates	mg P-PO$_4$ l^{-1}	0.079 ± 0.116 (0.01–0.66)
Nitrates	mg N-NO$_3$ l^{-1}	1.35 ± 2.26 (0.01–12.9)
Dissolved silicon	mg Si l^{-1}	1.9 ± 1.8 (0.01–7.6)
Total phosphorus	mg P l^{-1}	0.269 ± 0.266 (0.04–1.19)
Total nitrogen	mg N l^{-1}	4.09 ± 2.16 (1.28–13.6)
Total chlorophyll *a*	µg Chl*a* l^{-1}	128.7 ± 83 (5.8–415.6)

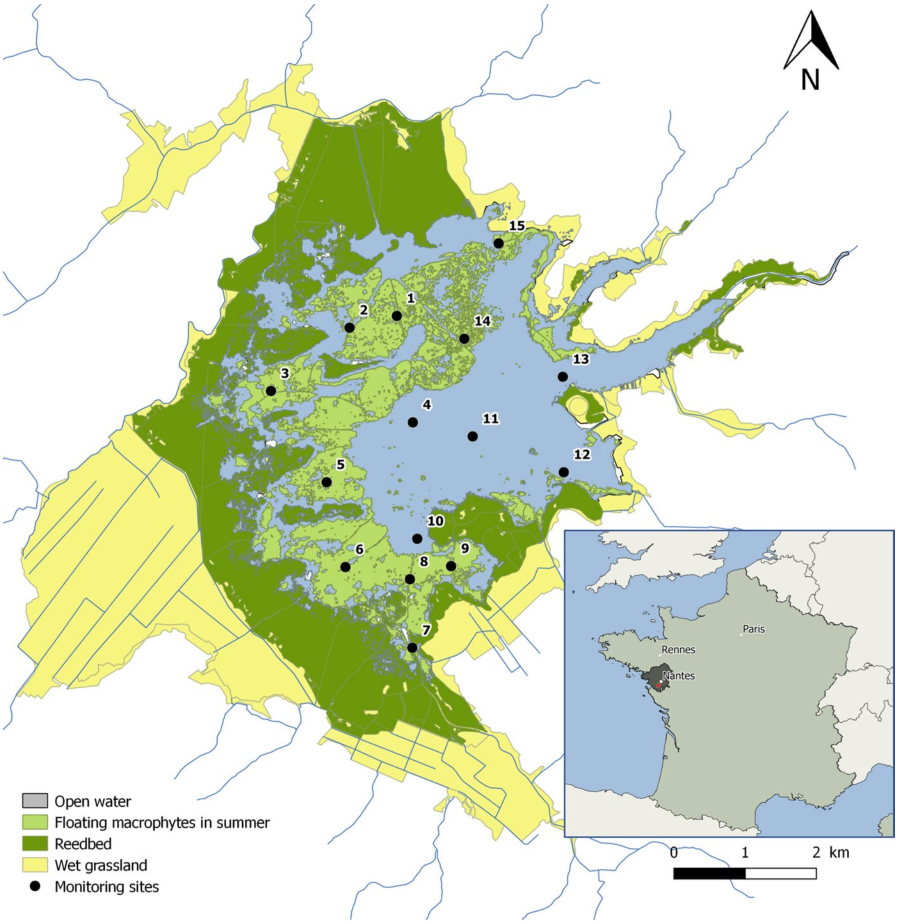


Fig. 1 Map of Grand-Lieu Lake and localization of the stations and aquatic vegetation (source: S.N.P.N.)

(Fig. 1). Nine stations (1,2,3,5,6,8,9,14,15) were in macrophyte habitats (M), while 6 (4,7,10,11,12,13) were in open-water habitats (OW). A few stations were not sampled in July because they were difficult to access because of a water level below 30 cm. This monitoring was part of a larger survey of physico-chemical and biological parameters at the 15 stations from February 2018 to November 2019.

Macrophyte cover

To estimate the presence of macrophytes at each station, the vegetation cover was estimated using a 1 m^2 wooden quadrat. The quadrat was thrown randomly five times around the boat. The percentages were averaged and ranked according to the Braun-Blanquet scale (0%, < 5%, 5–25%, 25–50%, 50–75%, > 75%).

Physical and chemical parameters

Water transparency was measured with a Secchi disc, on the shaded side of the boat. Light profiles were not feasible in the lake because of the height of the probe (approximately 50 cm for a maximum depth of less than one meter) and because the sensor was at the top. Water temperature, pH, conductivity, and dissolved oxygen were measured at each station using a multiparameter probe (Idronaut Ocean Seven 316Plus CTD, Milan, Italy). Turbidity was measured with a BBE AlgaeTorch fluorescence probe (BBE moldaenke GmbH, Germany).

As part of the two-year monitoring, water temperature was also measured at ten stations every ten minutes with HOBO Temperature Pro v2 Loggers (U22-001), and the water level was recorded every ten minutes at the mouths of the tributaries (stations 7 and 13), with Solinst DIVER (LTC Levelogger® from Solinst®).

Water sampling

For water chemistry and plankton, special attention must be paid not to resuspend the sediment in a water column that is mostly less than 1 m deep. After trials with a rigid integrating water sampler like the Bailer water sampler, the subsurface water (10–15 cm below the surface) was gently sampled using a 1 l Nalgene bottle, and the presence of vertical gradients was tested by specific sampling and probe profiles. The bottle was rinsed three times with lake water prior to sampling and was opened and closed below the water surface to prevent leaves or branches from contaminating the sample. This technique was used to sample a shallow fluvial lake similar to Grand-Lieu Lake (Cattaneo et al., 2013).

Nutrient concentrations

Filtrations for nutrients were performed immediately on the boat: a first 10 ml sample was collected and transferred into a 15-ml tube for total nitrogen (N) and phosphorus (P) measurements. Using a 0.45 µm filter (filtropur) and a 30-ml syringe (previously washed with 10% HCl and rinsed three times with the sample), four aliquots were divided into 15-ml tubes for silica (SiO_2), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), and ammonium (NH_4^+) analyses. All 15 ml samples were kept in a cooler and stored at − 20 °C, except silica (4 °C).

Total phosphorus (TP) and total nitrogen (TN) were measured by colorimetry after digestion with persulfate (Grasshoff, 1983), with detection limits of 6 µg P l^{-1} and 50 µg N l^{-1}. Orthophosphate was analyzed by the ammonium molybdate method, according to the US EPA protocol (US EPA Method 365.1, 1993), with a detection limit of 3 µg P l^{-1}. After reduction of nitrate (NO_3^-) to nitrite (NO_2^-) with vanadium chloride, NO_2^- was measured by calorimetry with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride, according to the US EPA protocol (US EPA Method 365.2, 1993), with a detection limit of 50 µg N l^{-1}. Colorimetry measurements were performed with a Gallery Photometric Analyser Gallery Plus (Thermo Fisher).

Phytoplankton biomass and assemblages

Total phytoplankton biomass and cyanobacterial biomass were measured using a BBE AlgaeTorch fluorescence probe (bbe moldaenke GmbH, Germany). Three measurements were taken at the subsurface at each station.

A 100 ml surface water sample was collected at each station and kept cool until storage in the laboratory. On the same day, these samples were fixed with Lugol's solution and stored at 4 °C in the dark. The samples were identified and counted under an inverted microscope using identification keys (Komárek, 1983; Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b).

Zooplankton abundance

Zooplankton were sampled at each station for macrozooplankton abundance and diversity, together with copepods and cladocerans. Surface water was sampled with a 2-L bottle and filtered through a 60 µm zooplankton filter. Twenty to 30 l were passed through a 60 µm net depending on zooplankton abundance and filter saturation. A 2-L bottle was used instead of a 10-L bucket to prevent sediment resuspension. The sample was collected on a 60 µm sieve, and zooplankton was anaesthetized with carbonated water (Perrier® type). Zooplankton was transferred to a 50-ml tube containing ethanol 80%. The samples were kept at 4 °C until identification and counted under a microscope and a binocular microscope, based on identification keys (Dussart, 1967; Amoros, 1984; Bledzki & Rybak, 2016). The samples were counted using a Bogorov counting chamber, after subsampling if necessary using a Hensen-Stempel pipette. The total abundances of cladocerans and copepods were used.

Statistical analysis

To test for the effect of macrophytes on phytoplankton assemblages compared with open water, we performed a nonmetric multidimensional scaling (NMDS) using the Bray–Curtis dissimilarity distance calculated on phytoplankton relative abundances. In complement of the NMDS plot, we performed an ANOSIM test on Bray–Curtis dissimilarity distances. The 'vegan' library in Rstudio was used for both NMDS and ANOSIM tests (Oksanen et al., 2013).

Species richness, Shannon, Simpson, and evenness diversity indices were calculated with the *diversity* function of the 'vegan' library (Oksanen et al., 2013). To test for the effect of macrophytes on the indices, we performed boxplots and Kruskal–Wallis tests with the *kruskal.test* function, using 'ggplot2' and 'cowplot' libraries (Wickham et al., 2016; Wilke et al., 2019).

To quantify the species specific to M and OW habitats, Venn diagrams were drawn using the 'ggvenn' library (Yan, 2021) in Rstudio on the presence/absence of phytoplankton taxa in July and August 2018.

To identify the phytoplankton species contributing to the differentiation of assemblages by macrophytes, a partial canonical correspondence analysis (pRDA) was performed on Hellinger-transformed phytoplankton abundances, with the macrophyte cover as the only explanatory factor. The pRDA removes time dependency associated with the sampling month through multiple regression. The pRDA was used to reposition species along Axis 1 (habitat) and identify those that contributed most to the assemblages. The link between assemblages and the macrophyte cover was tested through a Monte Carlo permutations test. The pRDA was performed using the 'vegan' library in Rstudio (Oksanen et al., 2013). The effect of the habitat was also tested independently of the pRDA by a PERMANOVA on Bray–Curtis distances with the *adonis* function from the 'vegan' library.

Indicator species were highlighted with the 'indicspecies' library (De Caceres et al., 2016) and the *multipatt* function, which determines lists of species associated with groups of sites, here the presence/absence of macrophytes (Dufrêne & Legendre, 1997). The indicator value associated with the statistical analysis is the average of two probabilities: (1) the probability for the sampled site to belong to the target group (here M or OW habitats), knowing that the species was observed (specificity of the species), and (2) the probability of finding the species when sampling the target group (fidelity of the species) (Dufrêne & Legendre, 1997).

To prioritize the environmental factors that drive phytoplankton assemblages in the two habitats independently of the sampling month, we performed a pCCA with the physical, chemical, and biological parameters as explanatory variables, and after removing the 'month' effect. The significance of the model was tested through a permutation test, while environmental variables were tested individually with an ANOVA that removed the least significant parameters.

A classification and regression tree (CART) analysis was performed with the 'rpart' library to determine the importance of environmental variables (Therneau et al., 1997). The response variable was the position of the samples along the first axis (constrained weighted site scores) of the pCCA performed just before (Chen et al., 2019). The initial model included temperature, turbidity, Secchi

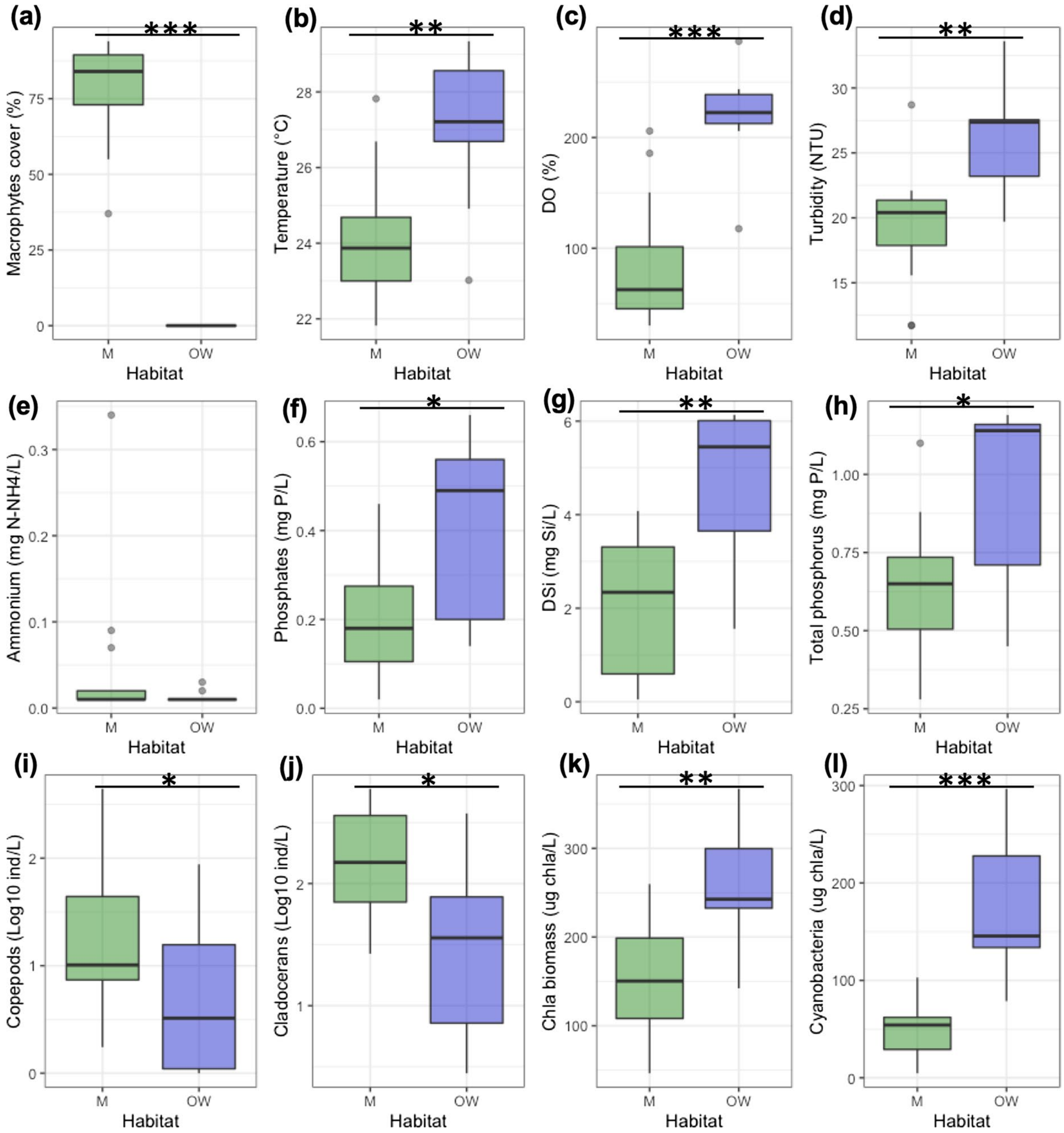
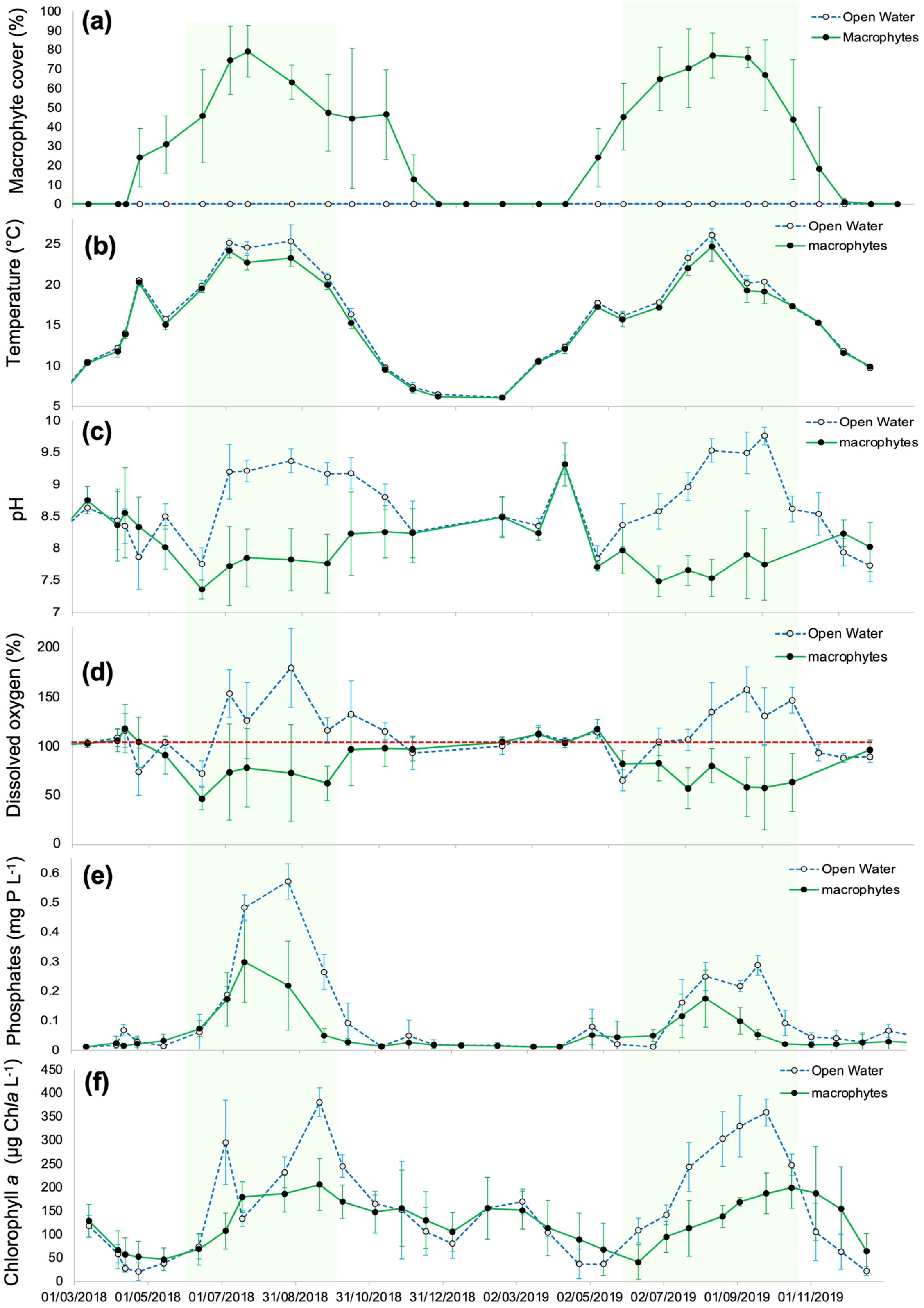


Fig. 2 Boxplots of physical, chemical and biological parameters depending on habitats (green, M; blue, OW); Kruskal–Wallis tests: *** $P<0.001$; ** $P<0.01$; *$P < 0.05$. Boxplots, distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 1.5 * IQR (corresponding to the inter-quartile range, i.e., the distance between the first and third quartiles). Data outside the 1.5*IQR interval (outliers) are plotted individually


(a)
Macrophyte cover (%)
100
90
80
70
60
50
40
30
20
10
0
Open Water
Macrophytes
(b)
Temperature (°C)
25
20
15
10
5
Open Water
macrophytes
(c)
pH
10
9.5
9
8.5
8
7.5
7
Open Water
macrophytes
(d)
Dissolved oxygen (%)
200
150
100
50
0
Open Water
macrophytes
(e)
Phosphates (mg P L-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
Open Water
macrophytes
(f)
Chlorophyll a (µg Chla L-1)
450
400
350
300
250
200
150
100
50
0
Open Water
macrophytes
01/03/2018
01/05/2018
01/07/2018
31/08/2018
31/10/2018
31/12/2018
02/03/2019
02/05/2019
02/07/2019
01/09/2019
01/11/2019

◂**Fig. 3** Time series of the macrophyte cover (%), water temperature, the pH, the dissolved oxygen concentration (%), the phosphate concentration, and the chlorophyll *a* concentration. Means ± standard errors. Mean values are averaged from vertical profiles performed in the macrophyte habitat (9 stations) and in the open-water stations (5 stations). Green colored area, period with macrophytes in the lake

depth, concentrations of phosphates, ammonium, dissolved silicon, total phosphorus and total nitrogen, cladoceran and copepod abundances, and the percentage of macrophyte cover.

Results

Effect of macrophytes on habitats

All stations from habitat M but station 9 exceeded 70% of surface cover (Fig. 2a). An effect of macrophytes on different physico-chemical parameters was observed in summer (Fig. 2), consistent with the seasonal divergence of the mean values of the two habitats (Fig. 3). At the seasonal scale, the mean macrophyte cover increased between spring and summer, with the highest cover in July and August (Fig. 3a). The greatest divergence in physical and chemical parameters was observed during the period with the maximum cover (Fig. 3).

The OW stations were significantly warmer than the M stations, with + 2–3 °C (up to 8 °C) during the day (Fig. 2b; KW = 8.54; $P = 0.003$). Water temperature followed the season, with higher temperature in OW than in M in summer (Fig. 3b). The mid-day pH was above 9 in summer in OW, while it remained below 8 in habitat M (Fig. 3c). Dissolved oxygen was about 100% in winter in M and OW. In spring and summer, low DO concentration was observed with about 50% in habitat M, while a very high DO concentration was observed in OW (Fig. 2c and 3d). The high pH coupled with high oxygen concentrations indicated high primary production in OW compared to M.

Water conductivity was higher (+ 9%) in M (399 μS cm^{-1}) than in OW (367 μS cm^{-1}) (KW = 6.12; $P = 0.01$). Phosphate concentrations also followed a strong seasonal pattern, with low values in winter and very high values in summer (Fig. 3e), and concentrations twice as high in OW as in M (Fig. 2f). A significant 'habitat' effect was observed in summer (KW = 4.75; $P = 0.03$; Fig. 2f). A similar pattern of higher concentrations in OW was observed for dissolved silicon (DSi) (KW = 10.56; $P = 0.001$; Fig. 2g) and total phosphorus (KW = 5.98; $P = 0.015$; Fig. 2h). The DSi concentration was at least twice as high in OW as in M, and was significantly correlated with phosphates ($r = 0.530$; $P < 0.001$). Ammonium was slightly but not significantly higher in summer in M than in OW ($P = 0.48$; Fig. 2e). The nitrate concentration was below the detection limit in all stations in summer (data not showed).

The chlorophyll *a* concentration followed a seasonal pattern, with higher summer concentrations in OW than in M (Fig. 2k and 7F; KW = 9.07; $P = 0.003$). The cyanobacterial biomass represented a large part of the phytoplankton biomass in OW (Fig. 2l; KW = 14.8; $P < 0.001$). Both the copepod and cladoceran zooplankton groups were more abundant in M than in OW (Fig. 2i, j; $P < 0.05$).

Finally, in summer, OW was characterized by higher temperature (+ 3–4 °C), higher DO (at least × 2), a higher pH (at least + 1 unit), higher PO_4 (at least × 2) and TP (+ 50%), higher DSi (at least + 2 mg Si/l), and lower abundances of zooplankton (at least:2) compared with the M stations. The higher photosynthetic activity (×5) resulted in a higher cyanobacterial biomass.

Phytoplankton assemblages according to habitats

The phytoplankton assemblages in M stations differed from those in OW stations, as shown by the ANOSIM analysis (R = 0.238; $P = 0.009$) and the NMDS plot (Fig. 4). The assemblages in August also differed from the assemblages in July (ANOSIM: R = 0.254; $P = 0.006$; Fig. 4).

Over the two summer months, 264 phytoplankton taxa were observed in M, and 197 in OW. Venn diagrams were used to depict the number of taxa common to the two habitats and the habitat-specific ones *per* month (Fig. 5). Fifty-three point four percent and 54.1% of the taxa were present in both habitats in July and August, respectively (Fig. 5). Ten point seven percent to 13.7% were observed only in OW, while one-third (32.8% to 35.2%) was observed in M. Although the total number of taxa differed between the two months, the proportions in the Venn diagrams remained the same.

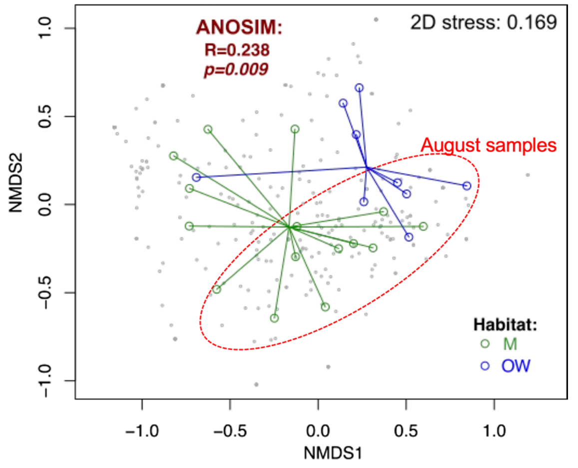


Fig. 4 NMDS plot based on Bray–Curtis similarity analysis performed on the relative abundances of phytoplankton taxa. Red dotted ellipse, samples taken in August. Result of the ANOSIM test between habitats

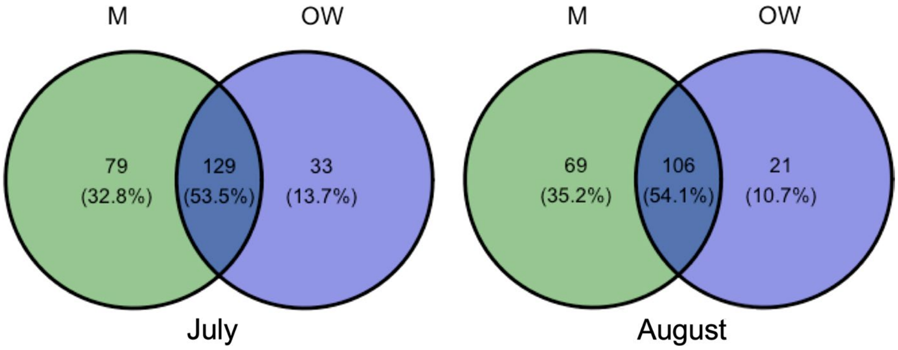
Consistent with the Venn diagrams, species richness was higher in July than in August.

It varied in M between 72 and 110 in July, and between 57 and 84 in August (Fig. 6a). In OW, it ranged from 86 to 91 in July, and from 61 to 69 in August (Fig. 6a). The OW samples were more homogenous than the M samples, in view of their quartiles on the boxplots (Fig. 6a) and their standard deviations (less than 3 for OW and more than 9 for M, both in July and August). An effect of 'month' on richness was observed, but no effect of 'habitat' was demonstrated (KW = 14.9; $P = 0.0018$). Shannon, Simpson, and evenness indices showed similar patterns, with an effect of 'month' in M and a higher diversity in M than in OW in August only (Fig. 6b,c,d). Some variability was observed among M stations, with Shannon values fluctuating from 2.3 to 3.3 for a same date (Fig. 6b). OW showed values ranging from 2.0 to 2.7.

The partial RDA relating phytoplankton assemblages to macrophyte cover highlighted that 7.6% of the total variance of phytoplankton assemblages was explained by the macrophyte cover, and 14.5% was explained by the sampling month. The use of a single explanatory parameter (percent of macrophyte cover) allowed the positioning of 'responsive' species along the first axis. The taxa located at the ends of axis 1 are showed *per* habitat and *per* class in Table 2. First, more taxa were observed in M (Table 2a), with all taxa in the classes Chrysophyceae, Euglenophyceae, Cryptophyceae, Ulothricophyceae, Xanthophyceae, and Zygophyceae. Only cyanobacteria had a higher number of contributing taxa in OW (Table 2b). Chlorophyceae and Diatomophyceae had a higher number of contributing taxa in M, and responses to macrophytes varied among species of the same genus (e.g., *Coelastrum* spp., *Pediastrum* spp., and *Scenedesmus* spp.). Among Euglenophyceae, several species of the same genus were observed with all the same preferred habitat, e.g., *Phacus acuminatus* Stokes, *P. costatus* Conrad, *P. pyrum* (E.) Stein, *P. raciborskii* Drezepolski, *P. skujae* Skvortzov, *P. suecicus* Lemmermann, and *P. tortus* (Lemmermann) Skv. (not showed). *Trachelomonas* genera were also well represented by several species.

Habitat preference was very clear for most classes, but also outside these classes for several genera, e.g., the Chlorophyceae *Monoraphidium* spp., the cyanobacteria *Microcystis* spp. and *Cyanogranis* spp., and the large diatoms *Aulacoseira* spp. in M (Table 2a). Several species of the cyanobacterial genera *Chroococcus*, *Coelosphaerium*,

Fig. 5 Venn-diagrams of the presence/absence of phytoplankton taxa in macrophyte (M) and open-water (OW) habitats in July and August



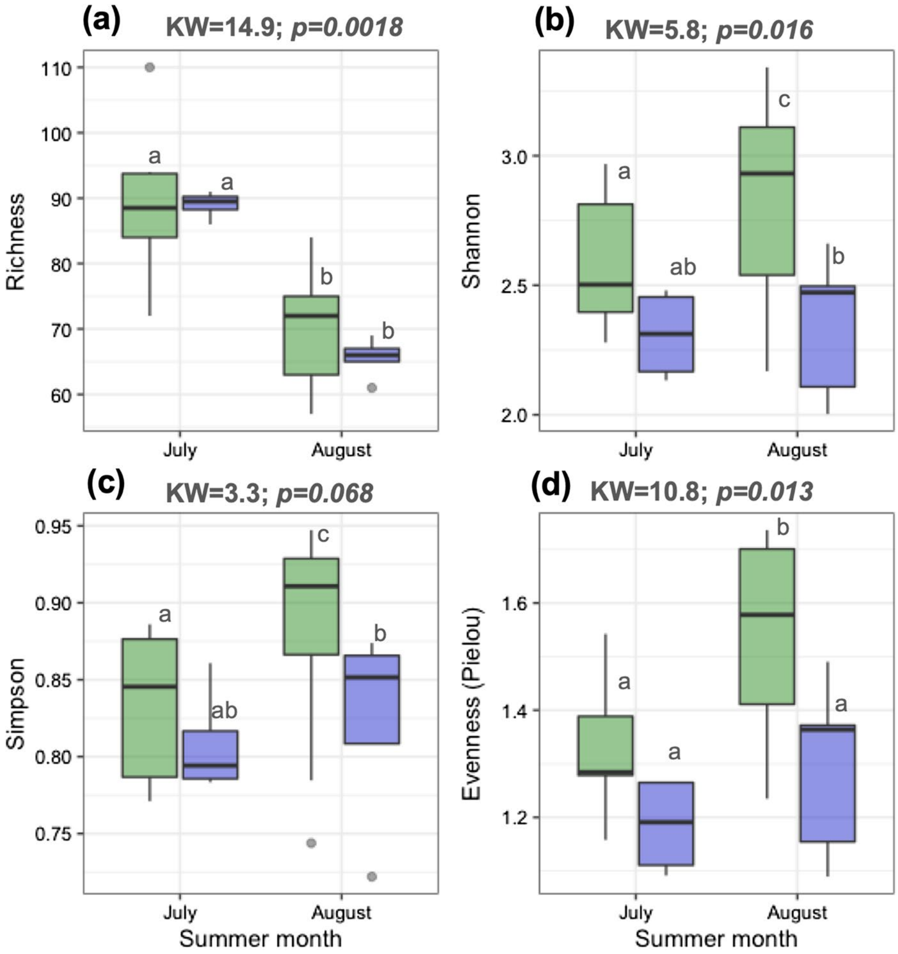


Fig. 6 Boxplots of richness and diversity indices depending on habitats (green, M; blue, OW). Kruskal–Wallis tests are shown. Letters, significance of Dunn's *post-hoc* test (a ≠ b ≠ c). Boxplots, distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 1.5 * IQR (corresponding to the inter-quartile range, *i.e.,* the distance between the first and third quartiles). Data outside the 1.5*IQR interval (outliers) are plotted individually

Dolichospermum, and *Merismopedia* showed a preference for OW.

Seventeen species were particularly related to a habitat and were identified as indicator species (seven in M; ten in OW) (Table 3). Among them, the Euglenophyceae *Phacus* spp. Dujardin (Fig. S1a; KW = 7.75, *P* = 0.005), *Euglena* spp. Ehrenberg (Fig. S1b; KW = 4.23; *P* = 0.04), and *Trachelomonas volvocina* (Ehrenberg) Ehrenberg (Fig. S1c; KW = 8.98; *P* = 0.003) were significantly more abundant in M, whereas they were nearly absent in OW. On the contrary, the cyanobacteria *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (Fig. S1d; KW = 4.28; *P* = 0.04), and *Romeria leopoliensis* (Raciborski) Koczwara (Fig. S1e; KW = 7.10; *P* = 0.008) were in higher abundance in OW, and so was the diatom *Nitzschia fruticosa* Hustedt (Fig. S1f; KW = 7.0; *P* = 0.008).

Based on the indicator values (Table 3), the probability of sampling M stations was highest when the Euglenophyceae *Phacus* spp. (*P* = 1) or *Trachelomonas* spp. (*P* = 1) were observed, as well as the green alga *Siderocelis ornata* (Fott) Fott (*P* = 1) or the diatom *Thalassiosira duostra* Pienaar (*P* = 0.97). The probability of sampling an OW station was highest when the green alga *Pediastrum boryanum* (Turpin) Meneghini was observed (*P* = 0.94) or the cyanobacterium *C. microscopicus* Komárková-Legnerová & Cronberg (*P* = 0.94) (Table 3). When sampling OW stations, the probability of finding the cyanobacterium *M. punctata* Meyen in the sample was maximum (*P* = 1.00), followed by the two diatoms *N. fruticosa* (*P* = 0.89) and *Staurosira venter* (Ehrenberg) Cleve & Moeller (*P* = 0.89). When sampling M stations, the probability was highest for the green algae *Crucigenia tetrapedia* (Kirchner) West (*P* = 0.93) and *Micractinium pusillum* Fresenius (*P* = 0.87).

Table 2 Taxa contributing to the first axis of the pRDA linking taxa with the macrophyte cover as an explanatory factor, with (a) preference for the microphyte (M) habitat (negative correlation with the first axis) and (b) preference for the open-water (OW) habitat (positive correlation with the first axis)

Classes	*taxa*	RDA1	Domi-nance	M	OW
(a) Preference for the macrophyte (M) habitat (negative correlation with axis 1):					
Chlorophyceae	*Actinastrum hantzschii* Lagerheim	− 0.21	++	**7,594 ± 4,124**	1,279 ± 431
	Crucigenia tetrapedia (Kirchner) West	− 0.24	++	**4,898 ± 1,095**	537 ± 291
	C. crucifera (Wolle) Komárek	− 0.10	+	**1,118 ± 406**	193 ± 97
	Crucigeniella rectangularis (Nägeli) Komárek	− 0.03	–	**88 ± 59**	0 ± 0
	Didymogenes palatina Schmidle	− 0.04	–	**215 ± 136**	0 ± 0
	Diplochloris raphidioides F. Fott	− 0.09	+	**2,301 ± 1,335**	71 ± 54
	Golenkinia radiata Chodat	− 0.08	+	**1,631 ± 594**	369 ± 171
	Kirchneriella microscopica G. Nygaard	− 0.03	–	**191 ± 142**	163 ± 163
	Micractinium pusillum Fresenius	− 0.17	++	**2,581 ± 1,037**	581 ± 304
	Monoraphidium komarkovae Nygaard	− 0.06	–	**281 ± 88**	80 ± 53
	M. arcuatum (Korshikov) Hindák	− 0.05	–	**383 ± 107**	114 ± 53
	Nephrochlamys willeana (Printz) Korshikov	− 0.03	–	**101 ± 73**	0 ± 0
	Pediastrum duplex Meyen	− 0.06	+	**462 ± 204**	194 ± 137
	Scenedesmus gr. Armati	− 0.28	++	**10,564 ± 3,132**	4,771 ± 1,178
	Scenedesmus gr. Abundantes/ Spinosi Meyen	− 0.04	–	**375 ± 130**	245 ± 104
	Schroederia setigera (Schroeder) Lemmermann	− 0.04	–	**140 ± 60**	18 ± 18
	Selenodictyon brasiliense Uherkovich et Schmidt	− 0.04	–	**196 ± 171**	0 ± 0
	Siderocelis ornata (Fott) Fott	− 0.04	–	**94 ± 30**	0 ± 0
	Tetraedron triangulare Korshikov	− 0.03	–	**314 ± 59**	209 ± 39
	Tetrastrum staurogeniaeforme (Schroeder) Lemmermann	− 0.04	–	**281 ± 101**	180 ± 114
	T. punctatum (Schmidle) Ahlstr. & Tiff	− 0.03	–	**85 ± 45**	73 ± 73
	Treubaria triappendiculata Bernard	− 0.03	–	**324 ± 100**	165 ± 61
Cyanobacteria	*Aphanocapsa* sp. Nägeli	− 0.14	++	**9,348 ± 3,982**	4,675 ± 3,470
	Cuspidothrix issatschenkoi (Usachev) Rajaniemi, Komárek, Willame, Hrouzek, Katovská, Hoffmann & Sivonen	− 0.06	++	**6,519 ± 1,781**	5,065 ± 1,360
	Cyanogranis ferruginea (Wawrik) Hindák	− 0.09	+	**1,945 ± 812**	123 ± 123
	C. irregularis Joosten	− 0.03	–	**166 ± 160**	0 ± 0
	Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju	− 0.08	++	**7,101 ± 2,697**	5,709 ± 2,012
	Jaaginema sp. Anagnostidis & Komárek	− 0.04	+	**1,260 ± 824**	0 ± 0
	Microcystis aeruginosa (Kützing) Kützing	− 0.08	+	**1,471 ± 1,424**	0 ± 0

Table 2 (continued)

Classes	taxa	RDA1	Domi-nance	M	OW
	Microcystis flos-aquae (Wittrock) Kirchner	– 0.04	+	**1,226 ± 827**	0 ± 0
	Tychonema sequanum (Couté) Anagnostidis & Komárek	– 0.04	–	**163 ± 157**	0 ± 0
Chrysophyceae	*Mallomonas* sp. Perty	– 0.04	–	**68 ± 24**	18 ± 14
	Synura sp. Ehrenberg	– 0.03	–	**167 ± 159**	0 ± 0
Cryptophyceae	*Cryptomonas* sp. Ehrenberg	– 0.07	+	**766 ± 131**	573 ± 180
	Plagioselmis nannoplanctica (Skuja) Novarino, Lucas & Morrall	– 0.03	–	**272 ± 89**	253 ± 117
Diatomophyceae	*Aulacoseira ambigua* (Grunow) Simonsen	– 0.09	+	**1,055 ± 326**	290 ± 138
	Aulacoseira granulata (Ehrenberg) Simonsen	– 0.05	+	**728 ± 157**	424 ± 146
	Aulacoseira granulata var. angustissima (O.F.Müller) Simonsen	– 0.04	–	**435 ± 108**	430 ± 80
	Centriques (d = 8–15 µm)	– 0.14	+	**2,426 ± 510**	1304 ± 522
	Cyclotella meneghiniana Kützing	– 0.04	+	**841 ± 376**	451 ± 152
	Praestephanos triporus (Genkal&Kuzmin) Tuji & Ki	– 0.14	+	**4,704 ± 2,224**	246 ± 173
	Thalassiosira duostra Pienaar	– 0.10	+	**1,730 ± 640**	62 ± 44
Euglenophyceae	*Cryptoglena pigra* Ehrenberg	– 0.03	–	**53 ± 23**	14 ± 14
	Euglena spp. Ehrenberg	– 0.03	–	**94 ± 32**	12 ± 12
	Phacus spp. Dujardin	– 0.06	–	**145 ± 58**	0 ± 0
	Trachelomonas volvocina (Ehrenberg) Ehrenberg	– 0.06	–	**195 ± 66**	0 ± 0
	Trachelomonas hispida (Perty) F.Stein	– 0.03	–	**75 ± 27**	20 ± 20
Ulothricophyceae	*Gloeotila contorta* (Lemmermann) Chodat	– 0.04	–	**570 ± 298**	0 ± 0
Xanthophyceae	*Centritractus belonophorus* Lemmermann	– 0.03	–	**74 ± 24**	12 ± 12
Zygophyceae	*Closterium* sp. J. Ralfs	– 0.04	–	**102 ± 43**	4 ± 4
(b) Preference for the open-water (OW) habitat (positive correlation with axis 1):					
Chlorophyceae	*Coelastrum reticulatum* (Dang.) Senn	0.05	–	1,018 ± 0	**82 ± 0**
	Dichotomococcus curvatus Korshikov	0.07	–	3,858 ± 0	**178 ± 0**
	Dictyosphaerium pulchellum Wood	0.03	–	3,396 ± 0	**0 ± 0**
	Diplochloris decussata Korshikov	0.03	–	1,925 ± 0	**806 ± 0**
	D. raphidioides F. Fott	0.11	+	13,020 ± 1	**1,346 ± 1**
	Pediastrum boryanum (Turpin) Meneghini	0.06	–	1,605 ± 0	**71 ± 0**
	Scenedesmus gr. Scenedesmus sensu stricto Meyen	0.03	–	585 ± 0	**64 ± 0**
Cyano-bacteria	*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault	0.07	–	3,677 ± 0	**237 ± 1**

Table 2 (continued)

Classes	*taxa*	RDA1	Domi-nance	M	OW
	Aphanocapsa elegans (Lemmermann) Joosten	0.09	++	40,161 ± 4	**6,656 ± 4**
	Aphanocapsa nubila Komárek & Kling	0.11	+	46,585 ± 0	**670 ± 2**
	Aphanothece smithii Komárková -Legnerová & Cronberg	0.07	++	61,511 ± 1	**3,303 ± 1**
	Chroococcus minutus (Kützing) Nägeli	0.03	–	3,677 ± 0	**0 ± 0**
	C. microscopicus Komárková-Legnerová & Cronberg	0.34	++	186,821 ± 2	**2,940 ± 5**
	Coelosphaerium kuetzingianum Nägeli	0.04	–	4,902 ± 0	**0 ± 0**
	Coelosphaerium minutissimum Lemmermann	0.17	++	91,543 ± 3	**4,515 ± 6**
	Dolichospermum compactum (Nygaard) Wacklin, Hoffmann & Komárek	0.09	–	10,260 ± 0	**518 ± 1**
	Dolichospermum flos-aquae (Brébisson ex Bornet & Flahault) Wacklin, Hoffmann & Komárek	0.09	+++	103,522 ± 19	**7,261 ± 23**
	Merismopedia warmingiana Lagerheim	0.03	–	3,040 ± 0	**0 ± 0**
	Merismopedia punctata Meyen	0.15	++	14,082 ± 2	**1,640 ± 3**
	Merismopedia tenuissima Lemmermann	0.46	+++	263,167 ± 14	**20,355 ± 24**
	Pannus planus Hindák	0.10	++	48,102 ± 1	**3,962 ± 1**
	Planktothrix agardhii (Gomont) Anagnostidis & Komárek	0.20	++	21,350 ± 2	**2,235 ± 4**
	Pseudanabaena catenata Lauterborn	0.05	–	8,943 ± 0	**0 ± 0**
	Pseudanabaena sp. Lauterborn	0.06	+	12,941 ± 0	**618 ± 1**
	Romeria leopoliensis (Raciborski) Koczwara	0.07	–	2,474 ± 0	**197 ± 0**
Chrysophyceae					
Cryptophyceae					
Diatomophyceae	*Staurosira venter* (Ehrenberg) Cleve & Moeller	0.03	–	1,228 ± 0	**114 ± 0**
Euglenophyceae					
Ulothricophyceae					
Xanthophyceae					
Zygophyceae					

Abundances in bold indicate the abundance of species in their preferred habitat

The ‘sampling month’ effect, which explained 14.5% of taxon abundance, was removed. Species scores are showed, as well as their dominance: -, less than 0.2% of mean frequency; +, 0.2% < mean frequency < 1%; ++, 1% < mean frequency < 10%; +++ mean frequency > 10%. The abundance (mean ± standard deviation) in each habitat is showed

Table 3 Indicator values found for the indicator species, with detailed probabilities of specificity and fidelity to the habitat

	Specificity prob	Fidelity prob	Stat	P value	
Group: macrophyte (M) habitat					
Crucigenia tetrapedia	**0.90**	**0.93**	0.92	0.00	***
Thalassiosira duostra	**0.97**	0.73	0.84	0.04	*
Micractinium pusillum	0.82	**0.87**	0.84	0.03	*
Trachelomonas volvocina	**1.00**	0.67	0.82	0.01	*
Phacus sp.	**1.00**	0.60	0.78	0.01	*
Siderocelis ornata	**1.00**	0.60	0.78	0.01	*
Euglena sp.	**0.88**	0.53	0.69	0.05	*
Group: open-water (OW) habitat					
Merismopedia punctata	0.79	**1.00**	0.89	0.00	***
Nitzschia fruticosa Hustedt	0.83	**0.89**	0.86	0.00	**
Pediastrum boryanum	**0.94**	0.78	0.85	0.00	**
Chroococcus microscopicus	**0.93**	0.78	0.85	0.01	**
Dichotomococcus curvatus	0.89	0.78	0.83	0.02	*
Staurosira venter	0.77	**0.89**	0.83	0.02	*
Romeria leopoliensis	0.87	0.78	0.82	0.01	**
Coelosphaerium minutissimum	0.86	0.78	0.82	0.02	*
Planktothrix agardhii	0.81	0.78	0.79	0.03	*
Scenedesmus gr. scenedesmus	0.84	0.56	0.69	0.02	*

Fig. 7 pCCA performed in July and August 2018 ('month' effect removed, representing 9.8% of variance), linking taxon abundances with environmental parameters in gray. Samples were grouped *per* habitat, and the mean positions of phytoplankton classes are shown. Permutation tests were significant ($P = 0.001$ based on 999 permutations). Sixty-one percent of total variance was explained by environmental parameters. The importance of the variables based on the classification and the regression tree on the sample coordinates of the pCCA are shown in Table 4

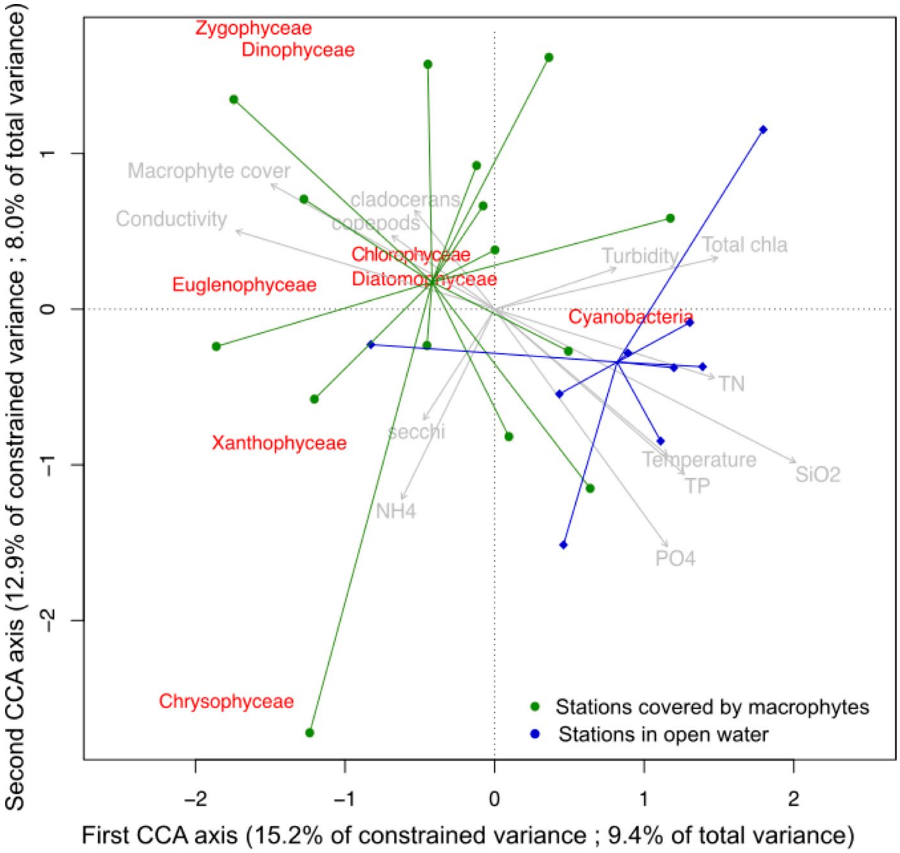


Table 4 Results of the pCCA, with correlations of the environmental and biological parameters with the axes, their significance tested by ANOVA and their importance calculated by a classification and regression tree (CART) analysis

Parameters	CCA1	CCA2	Df	ChiSquare	F	Pr(> F)	Importance from CART
Turbidity	0.30	0.10	1	0.089	1.12	0.278	7.37
Temperature	0.43	− 0.35	1	0.107	1.34	**0.049**	4.73
Total nitrogen	0.55	− 0.16	1	0.139	1.75	**0.002**	3.41
Macrophyte cover	− 0.55	0.30	1	0.111	1.39	**0.046**	3.20
Conductivity	− 0.64	0.19	1	0.155	1.95	**0.002**	2.66
Total phosphorus	0.47	− 0.39	1	0.126	1.671	**0.005**	2.51
SiO_2	0.75	− 0.37	1	0.117	1.47	**0.026**	2.45
PO_4	0.43	− 0.57	1	0.181	2.40	**0.001**	1.31
Secchi depth	− 0.18	− 0.26	1	0.124	1.56	**0.009**	0.77
Sampling month	Removed by pCCA		1	0.232	2.91	**0.001**	–
Total chl *a*	0.55	0.12	1	0.120	1.51	**0.012**	
NH_4	− 0.23	− 0.45	1	0.157	1.97	**0.004**	
Copepod abundance	− 0.25	0.18	1	0.071	0.89	0.641	
Cladoceran abundance	− 0.20	0.24	1	0.065	0.81	0.827	
Residuals			11	0.875			

Probabilities significant at the 5% level are shown in bold

Prioritizing the environmental parameters that drive phytoplankton assemblages

Physical and chemical parameters were used as explanatory parameters of taxon abundances to link phytoplankton assemblages with environmental factors, after removing the ‘month’ effect (9.8% of total variance; Fig. 7). Sixty-one percent of total variance in phytoplankton assemblages was explained by the environmental parameters ($P = 0.001$ based on 999 permutations) independently of the month. The first axis represented 15.2% of constrained variance (9.4% of total variance); it separated M stations on the left of the plot from OW stations on the right (Fig. 7). SiO_2 concentrations ($r = 0.75$; $P = 0.026$ based on permutation tests by terms), water temperature ($r = 0.43$; $P = 0.049$), TP ($r = 0.47$; $P = 0.005$), phosphate concentrations ($r = 0.43$; $P = 0.001$), TN ($r = 0.55$; $P = 0.012$), and total chlorophyll *a* concentrations ($r = 0.55$; $P = 0.012$) correlated positively with the first axis, indicating higher values in open water (Fig. 7 and Table 4). Conductivity ($r = -- 0.64$; $P = 0.002$), the macrophyte cover ($r = - 0.55$; $P = 0.046$), ammonium ($r = - 0.23$; $P = 0.004$), and Secchi depth ($r = - 0.18$; $P = 0.009$) correlated negatively with the fist axis, toward M. Copepod ($r = - 0.25$) and cladoceran ($r = - 0.20$) abundances also correlated negatively, but they were not significant explanatory parameters.

The mean positions of the phytoplankton classes were added on the pCCA plot (Fig. 7). Cyanobacteria were associated with OW, while all other classes were on the left side of the plot. Chlorophyceae and Diatomophyceae remained near the plot center, while the other classes were distributed along axis 2. Chrysophyceae in the bottom part (Fig. 7) were correlated with ammonium ($r = 0.95$; $P < 0.001$), mainly because of station 5 in July, with 0.34 mg N-NH_4 l^{-1} and 2,574 cells ml^{-1}.

We used the classification and regression tree (CART) model to prioritize the importance of environmental variables in the scores of the sites on the first pCCA axis (Table 4). Turbidity was not significant in the pCCA, but was the most important parameter explaining site scores, with an importance value of 7.37 and a threshold at 19.5 NTU. Temperature was the second most important parameter (4.73), with a threshold at 23.3 °C, followed by TN (3.41) and the macrophyte cover (3.2).

Discussion

Effect of plants on abiotic parameters

Water lilies modified the habitat and generated small-scale spatial heterogeneity favorable to motile taxa. First, they decreased water temperature compared with open water. Macrophytes strongly decrease the depth down to which both wind mixing energy and solar radiation penetrate, leading to lower temperature and turbulence (Andersen et al 2017 Aquatic Science). The average difference of 3 °C between M and OW can have important repercussions on biological activities and competition among species. Water temperature and mixing are indeed key drivers of the biogeochemical and ecological functioning of lakes because they drive biological activities and gas exchanges (Woolway et al., 2016).

In open water, the lake was highly turbid, hence superficial heating (Persson & Jones, 2008). Conditions were also more turbulent, thanks to a large fetch. The sensitivity of the lake to wind forcing was evidenced by the presence of Langmuir cells regularly observed in the open water only as soon as wind was above a threshold (Wetzel, 2001).

Plants decreased turbidity compared with open water, consistently with lower phytoplankton biomass. Lower sediment resuspension associated with lower mixing can also explain the difference in turbidity between the two habitats (Madsen et al., 2001). Light may have been limiting for phytoplankton growth in both habitats because of shading by water lily leaves in M and turbidity in OW.

Plants also decreased the pH and DO compared with open water. High pH, DO saturation above 150%, and high cyanobacterial biomass characterized OW in summer and indicated a high photosynthetic activity, probably favored by the phosphate concentration and high temperature. A pH close to 7.5 and DO saturation below 50% characterized the macrophyte area, dominated by heterotrophic processes. Each habitat had its own functioning: OW was autotrophic (gross primary production (GPP) over ecosystem respiration ratio > 1), whereas M was heterotrophic ($GPP/R < 1$). Spatial zonation of the metabolism of a lake has already been observed, with heterotrophy in the area of *Trapa natans* macrophytes compared with submerged macrophytes in a shallow lake (Stefanidis & Dimitriou, 2019). The authors suggest that allochthonous organics fuel heterotrophic processes in macrophyte areas. Unfortunately, dissolved organic carbon was not measured in our study. However, it should have been higher in the macrophyte area compared with open water, as actively growing macrophytes release 1 to 10% of their primary production (Carpenter & Lodge, 1986). Contrary to nutrients, macrophytes are considered as a net source of DOC for lakes.

Phosphates (and total phosphorus) were significantly lower in M (:2) than in OW. Several non-exclusive processes explain the difference between habitats: First, a difference in pH and redox potential between the two zones can lead to different adsorption and chemosorption rates (Bostrom & Pettersson, 1982; Sondergaard et al., 2001). P bound to redox-sensitive iron compounds can indeed be quickly released into the water column if the redox status changes (Mortimer, 1941; Bostrom & Pettersson, 1982). Secondly, the pore water P of the sediment can be mobilized in the water column during episodes of sediment resuspension (Sondergaard et al., 1992). Thirdly, warmer temperature may also contribute to accelerated P recycling to the water column, in the same way as for ammonium recycling (Jiang et al., 2019). Fourthly, P storage in phytoplankton cells lasts a few days and P remains easily remobilized when cells die (high turnover). On the contrary, macrophytes store P for a few months and act as a net sink during their active growth (Carpenter & Lodge, 1986; Teubner et al., 2022). M acted as a P summer sink with a slower metabolism than OW (high production and regenerating rates).

Water lilies also decreased dissolved silicon concentrations by twofold compared with open water.

A higher consumption of silica was expected in M because diatoms were in higher density. Periphyton growing on macrophytes may also have absorbed it. Lastly, storage of biogenic silica (BSi) by macrophytes can explain the strong difference. *N. lutea* indeed contains 8 mg BSi g^{-1} DW (Schoelynck et al., 2010) and may also have acted as a Si summer sink. In OW, higher mineralization of organic matter may also have increased the DSi concentration. Biogenic silica originated from the dissolution of dead diatom frustules accumulated in the sediment and was easily mobilizable (Sarazin et al., 1995).

No 'plant' effect was observed on nitrates and ammonium. Nitrates in the lake were driven by winter recharge and increased when river flows resumed in fall, while the concentration remained below the detection threshold in summer. Nitrogen-fixing cyanobacteria with numerous heterocysts were observed throughout summer, supporting nitrogen limitation at this time. The ammonium concentrations remained lower than 20 μg N l^{-1} most of the year, but peaks in ammonium were observed in some of the M stations. Ammonium in eutrophic lakes is highly dynamic depending on coupled production-consumption processes associated with bacteria and primary producers (Jiang et al., 2019).

Phytoplankton assemblages depending on habitats

By modifying the physical and chemical parameters and biotic interactions, rooted floating-leaf macrophytes allowed the spatial coexistence of several phytoplankton assemblages in the lake. The assemblages changed between the beginning of July and the end of August, in accordance with the seasonal dynamics (Sommer et al., 1986; Pannard et al., 2008), but the 'plant' effects remained. Based on the presence/absence data, three times more taxa were associated with M (one-third of the total) than with OW (10–13%). Consistently, phytoplankton diversity was higher in M than in OW, while the total phytoplankton biomass was about twice lower in M than in OW. The presence of water lilies was especially unfavorable to cyanobacteria (such as those observed in high biomass in open water), and allowed for the maintenance of rare species and a greater diversity in M.

Most of the taxa and some entire classes of phytoplankton were more abundant in M than in OW, which was favorable to cyanobacteria. Phytoplankton assemblages in M were mostly composed of unicellular flagellates tolerant to low light, with many mixotrophs (combining photosynthesis and ingestion of particulate organic matter) known to interact with organic matter in heterotrophic ponds. All taxa from the unicellular flagellate classes *Euglenophyceae, Chrysophyceae,* and *Cryptophyceae* showed a habitat preference for macrophytes, with a fivefold higher abundance in M compared with OW. Three of the seven indicator species of M were mixotrophic *Euglenophyceae* (*Euglena* spp., *Phacus* spp., *T. volvocina*) typical of organic ponds (Reynolds et al., 2002). *Chrysophyceae* can also be found in heterotrophic ponds according to Reynolds' functional classification and are tolerant to low nutrients, with potential use of mixotrophy (Reynolds et al., 2002; Padisák et al., 2009). *Cryptophyceae* are tolerant to low light conditions (Reynolds et al., 2002), and their main representative (*Cryptomonas* sp.) also has mixotrophic ability (Princiotta et al., 2019). Therefore, three classes are related to organic carbon and potential mixotrophic activity, which is advantageous in light-limited environments.

For other classes with habitat preference for M (*Chlorophyceae, Ulothricophyceae, Xanthophyceae,* and *Zygophyceae*), lifeforms were more variable, from unicellular flagellates to simple colonial and filamentous forms. Most diatoms were also more abundant in M. *Aulacoseira granulata* (Ehrenberg) Simonsen, *A. ambigua* (Grunow) Simonsen, *Cyclotella meneghiniana* Kützing, and the M indicator species *T. duostra* are all planktonic diatoms found in mixed eutrophic lakes, with tolerance to low light and C deficiency (Reynolds et al., 2002). Similarly, *Praestephanos triporus* (Genkal&Kuzmin) Tuji & Ki (20 times more abundant in M than in OW) is a planktonic diatom found in shallow turbid water (Padisák et al., 2009). A few free-floating colonies of cyanobacteria showed a preference for M, in particular the toxic blooming species *Microcystis aeruginosa* (Kützing) Kützing and *M. flos-aquae* (Wittrock) Kirchner, typical of shallow nutrient-rich waters (Padisák et al., 2009).

Cyanobacteria were the only class showing a habitat preference for OW, with a threefold increase in biomass and dominance of filamentous N-fixing cyanobacteria and picocyanobacteria. One of the two dominant taxa (relative frequency > 10%) was *Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) Wacklin, Hoffmann & Komárek, a buoyant N-fixing filament. A similar lifeform was observed in lower abundance, represented by *Dolichospermum compactum* (Nygaard) Wacklin, Hoffmann & Komárek, *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, and *Pseudanabaena catenate* Lauterborn. The second dominant taxon was the mat-forming *Merismopedia tenuissima* Lemmermann, a flat rectangular colony of small cells arranged in rows within a mucilaginous matrix, co-occurring with *M. warmingiana* Lagerheim and

M. punctata Meyen. Many other picocyanobacteria were observed in OW, such as *Aphanocapsa elegans* (Lemmermann) Joosten, *A. nubila* Komárek & Kling, *Aphanothece smithii* Komárková -Legnerová & Cronberg, *Chroococcus minutus* (Kützing) Nägeli, *C. microscopicus* Komárková-Legnerová & Cronberg, *Coelosphaerium kuetzingianum* Nägeli, *C. minutissimum* Lemmermann, and *Pannus planus* Hindák (Callieri et al., 2012). Small-size cells are more efficient in absorbing light and nutrients (Finkel & Irwin, 2000; Finkel et al., 2009) in the turbid N-depleted open water. The cyanobacteria observed here have also been found in a low-N and turbid mixed layer (Reynolds et al., 2002; Padisák et al., 2009). A single diatom—*Staurosira venter*—was indicator of OW with a threefold increase in biomass. These lanceolate cells (5 µm wide and 5–26 µm long) can be attached to the substratum by a mucilage pad or be planktonic, and are found in turbid and frequently mixed shallow lakes (Padisák et al., 2009).

Cyanobacteria form large colonies, so it is no surprise that they contribute more than the other classes in terms of relative frequency. We could have calculated the biovolume of each species, but not all species could be measured. We would have lost taxa in the analysis, knowing that what we are interested in anyway is the difference between habitats.

The ‘submerged macrophyte’ effect on zooplankton assemblages is well known: the differentiation of assemblages is driven by active dispersal to benefit from the refuge effect, and by the presence of plant-associated species feeding on periphyton (Jeppesen et al., 1998; Bertolo et al., 1999). A horizontal diel migration between M and OW can be expected for the non-littoral species of large zooplankton (Lauridsen et al., 1998). However, it probably remained limited to the edges of the macrophyte beds because several hundred meters had to be covered. The effect of surface-leaf macrophytes is lesser than that of submerged macrophytes, but this habitat also hosts a higher biomass, abundance, and richness of zooplankton than OW (Carpenter & Lodge, 1986). Similarly, M phytoplankton could have been enriched with meroplanktic and epiphytic species benefiting from biological support. However, there were no more benthic or meroplanktic taxa in M than in OW, while genera such as *Fragilaria* and *Nitzschia* were observed in both habitats. A response of pelagic phytoplankton was observed. The species that could benefit from benthic growth were characteristic of OW, with the genus *Merismopedia* and the diatom *S. venter*, showing a potential role of meroplankton in increasing diversity in the pelagic zone.

The difference in phytoplankton biomass and assemblages between M and OW could also have been explained by differences in zooplankton grazing. However, zooplankton was more abundant in M than in OW, as were most phytoplankton groups, while less edible species (cyanobacteria) were more abundant in OW. Despite more abundant zooplankton in M, sensitive taxa such as unicellular flagellates kept a higher biomass in M than in OW. Zooplankton did not explain the differences in phytoplankton taxa between the two habitats.

Structuring role of macrophytes through environmental parameters

The floating-leaf macrophytes changed the phytoplankton assemblages—lower total biomass but more diverse microalgal assemblages—by driving the physical and chemical parameters. Our findings are consistent with a previous study on the tropical lake Ziway (Ethiopia) colonized by *Typha latifolia* Linn. and *Phragmites australis* (Cav.) Trin. ex Steud. (Gebrehiwot et al., 2017). The authors observed more diverse phytoplankton assemblages in the emerged macrophyte beds, with more *Bacillariophyceae* and *Euglenophyceae* species. As in our study, the cyanobacterium *Microcystis spp*. was associated with M, while *Merismopedia punctata* was associated with OW. Contrary to our study, water temperature was warmer in the macrophyte beds, but the effects on DO, conductivity, TP, and phosphates were the same as in our study, even if it was conducted in a temperate lake.

A greater dominance of flagellates in the presence of submerged macrophytes has already been pointed out (Søndergaard & Moss, 1998). The flagellated shape better counteracts sedimentation losses in low-turbulence environments (Margalef, 1978). Despite a greater vulnerability to zooplankton grazing, flagellates may be advantaged in low turbulent conditions because they are adapted to better exploit small-scale heterogenous environments in terms of nutrients and organic matter associated with macrophytes (Sommer, 1988; Søndergaard & Moss, 1998).

This heterogenous environment also limits competition among species and prevents dominance of a few (Cunha et al., 2012). Moreover, many of these flagellates are mixotrophic. The supply of dissolved organic carbon derived from macrophytes and from the associated periphyton, coupled with the low light availability, promoted mixotrophic species in M (Søndergaard & Moss, 1998). We highlighted a similar effect of water lilies on phytoplankton assemblages as that of submerged macrophytes, with local promotion of the microbial loop and heterotrophic processes.

In open water, the high biomass of cyanobacteria was expected owing to the hypereutrophic state of the lake (Huisman et al., 2018). The high phosphate concentration and warm temperature synergically promoted cyanobacterial growth (Paerl & Huisman, 2008; Paerl, 2017). The most dominant species was *D. flos-aquae,* which formed huge colonies rolled up on themselves with numerous heterocysts, confirming summer N limitation. Many co-occurring blooming genera were observed simultaneously in the lake, while eutrophic shallow lakes generally experience alternating blooms of a few dominant species (Wu et al., 2016; Le Moal et al., 2021). *Microcystis* spp. and *Dolichospermum* spp., which are among the most toxic genera, were spatially distributed between OW and M. They are known to co-occur spatially (Zhang et al., 2016), and most often follow each other in reservoirs (Soares et al., 2009; Wu et al., 2016). These studies show that warmer temperature favors *Microcystis*, which should have been more abundant in OW. However, these species also have different P requirements: *Dolichospermum* spp. needs a higher P concentration than *Microcystis* spp. because heterocyst formation consumes a lot of energy (Wan et al., 2019). *Microcystis* can be competitive at low P concentrations, thanks to its ability for rapid P uptake and storage (Wan et al., 2019). However, neither species really bloomed during our study: their densities remained lower than 10,000 cells ml^{-1}.

Allelopathic effects of macrophytes on phytoplankton could partly explain the changes in assemblages and the limited dominance of cyanobacteria in M. Floating-leaf macrophytes like water lilies produce hydrolysable polyphenols whose algaecide activity is not yet proven (Gross, 2003). Macrophytes produce phenolic compounds involved in defense against herbivores, and *N. alba* and *N. lutea* are among the biggest producers of these substances (Smolders et al., 2000). However, to our knowledge, the inhibiting effect of these water lilies on growth has only been demonstrated on *Lemna minor* Linn. (Elakovich & Wooten, 1991).

An effect of the large size of the lake can be pointed out. The 'phytoplankton species-lake area' relationship has been debated for a long time, due to multiple co-factors driving phytoplankton richness (Borics et al., 2021). The large lake effect (LLE) predicts a decrease of diversity in large lakes because of habitat homogenization by wind in the pelagic area (Várbíró et al., 2017). Shallow lakes—especially those with large fetch like Grand-Lieu Lake (5 km^2) —are exposed to strong horizontal mixing by wind, which homogenizes water masses and suspended communities. However, the maintenance of macrophytes on one-third of the lake surface plays a key role in maintaining habitats. A recent study comparing shallow lakes harboring water lilies or not showed differences in biogeochemistry and microbial assemblages in the lakes with more than 10% of their surface covered by water lilies (DeWolf et al., 2022).

Water may have been isolated below the water lilies located on the wind-protected west side of the lake, especially in the westernmost stations. The small-scale spatial heterogeneity of physical and chemical parameters generated by macrophytes, coupled with biotic interactions, may have promoted a higher diversity in the lake, in particular of *Euglenophyceae* (Várbíró et al., 2017), and counteracted the mass effect and species sorting (Leibold et al., 2004; Yang et al., 2018). When horizontal mixing of water masses is low, environmental filters and biotic interactions (competition, predator–prey relationships) predominate in the structuring of local communities. The higher conductivity and the establishment of a horizontal gradient attest to a low horizontal mixing in this lake in summer. Spatial heterogeneity in phytoplankton assemblages has already been demonstrated in reservoirs from upstream, turbulent, nutrient-rich areas to downstream, stable, pelagic areas (Bortolini et al., 2017; Yang et al., 2018).

Connected habitats may support a set of metacommunities, i.e., a set of local communities linked by dispersal of multiple potentially interacting species (Leibold et al., 2004). Metacommunities of bacterioplankton (Wu et al., 2007) and zooplankton (Cottenie & De Meester, 2003) have been demonstrated in a shallow lake. To go further, it would be interesting to couple the sink-source dynamic of C, N, and P with the metacommunity approach in the broader meta-ecosystem concept (Loreau et al., 2003), using landscape ecology tools. However, a major knowledge gap remains as to the aquatic ’landscape’ and local residence times of water, in particular how water masses flow within a lake partially colonized by macrophytes and how the underwater shape (submerged *versus* rooted floating-leaf macrophytes) impacts these water flows and dispersal.

Conclusion

Floating-leaf macrophytes in shallow lakes act as submerged macrophytes when it comes to structuring habitats and phytoplankton assemblages, with an increase of small mixotrophic flagellates that better exploit the small-scale heterogenous environment. Macrophytes promote the microbial loop and heterotrophic processes locally. Mixotrophy is very little considered in the carbon cycle, and little is known about the flows associated with these organisms (Beisner et al., 2019). The next step would be to directly measure the grazing and photosynthetic performances of mixotrophs at a small scale in natural macrophyte habitats (Beisner et al., 2019). While macrophytes are important for aquatic biodiversity in ponds, their degradation leads to a more homogenous biota and contributes to a loss in freshwater biodiversity. If floating-leaf macrophytes decline, the lake will shift to summer cyanobacterial blooms and lose at least one-third of its phytoplankton diversity. The conservation of macrophytes in sufficient biomass is essential for the maintenance of habitats and diversity in shallow lakes, even in turbid eutrophic lakes.

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Author contributions AP obtained the funding. SMa, AP, SL, GB and J-MG performed the field sampling. ML and SMo performed phytoplankton identification and counting. SMa and SL performed zooplankton identification and counting. AP performed the data analyses and statistics. AP and CP wrote the manuscript, with substantial contributions from all authors.

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Data Availability The data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

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