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1 **Rooted floating-leaf macrophytes structure coexistence of different phytoplankton**
2 **assemblages within a shallow lake?**

3 Alexandrine Pannard^{1,2}, Stéphanie Massé¹, Stéphanie Llopis¹, Maria Leitao³, Sara Morata³, Guillaume
4 Bouger¹, Jean-Marc Gillier⁴ and Christophe Piscart¹

5
6 1. University of Rennes, UMR CNRS 6553 ECOBIO, Rennes, France

7 2. INRAE Rennes, UMR 1069 SAS, Rennes, France

8 3. Bi-Eau, Angers, France

9 4. Société nationale de protection de la nature, Réserve naturelle nationale du Lac de Grand-Lieu, La
10 Chaussée 44830 Bouaye

11 **Corresponding author:** alexandrine.pannard@univ-rennes1.fr

12

13 **Summary**

14 Aquatic macrophytes in shallow lakes control habitats through local turbulence, water transparency,
15 nutrients and oxygen concentrations. As engineer species, they structure these ecosystems, increasing
16 the biodiversity. While many studies focused on submerged macrophyte, research on habitats created
17 by rooted floating-leaf macrophytes is scarcer. Macrophytes, such as water lilies, should have the similar
18 ecological consequences as submerged macrophytes, but with greater shading.

19 In this study, we showed how macrophytes structure phytoplankton assemblages and allow the
20 coexistence of different assemblages in the same shallow lake. During summer 2018, we characterized
21 the phytoplankton assemblages in 9 stations covered by water lilies and 6 stations in open water, in a
22 large shallow water lake. The lake is colonized on a third of its surface by water lilies from April to
23 October. We showed an effect of waterlilies on temperature, oxygen, pH, turbidity, phosphates and
24 dissolved silicon.

25 Many taxa of phytoplankton from almost all classes were in higher abundance in stations covered by
26 macrophytes, while cyanobacteria showed a higher biomass and richness in open water. Unicellular
27 mixotrophic flagellates predominated in macrophytes habitat, with all representatives of the classes
28 Euglenophyceae and Cryptophyceae.

29 **Keywords:** floating plant; Grand Lieu nature reserve; temperate lake; phytoplankton ecology;
30 functional traits.

31

32 **Introduction**

33 Freshwater covers 3% of continental land surface, with small shallow lakes and ponds largely
34 dominating this global surface (Downing et al., 2006). However, their ecological functioning has been
35 widely endangered by water pollution, eutrophication (Khan & Ansari, 2005), climate change (Paerl &
36 Huisman, 2008; Moss, 2011) and invasive species (Reynolds & Aldridge, 2021).

37 Because of light dependency and wind energy transfer, the depth of a lake is critical to its functioning
38 (Wetzel, 2001; Scheffer, 2004). Deep lakes are characterized by the presence of seasonal thermal
39 stratification, which isolates water surface from nutrient-replete sediment. Shallow lakes on the
40 contrary have a low thermal inertia, making them more sensitive to meteorological extremes (rain,
41 wind, and heatwaves). Bottom nutrients released during windy conditions are beneficial for pelagic
42 phytoplankton (Sondergaard et al., 1992; Carrick et al., 1993). In shallow lakes, light is likely to reach
43 the bottom of the lake, so that their bottom can be colonized by submerged macrophytes (Scheffer,
44 2004). These macrophytes control bottom light climate by limiting the resuspension of sediment
45 (James et al., 2004), but they can be outcompeted by phytoplankton and the associated turbidity.

46 Aquatic macrophytes in ponds and shallow lakes are particularly affected by eutrophication (Sayer et
47 al., 2010; Labat et al., 2020). Submerged macrophytes disappear above a threshold of turbidity and
48 strongly declined with eutrophication (Sand-Jensen et al., 2000; Phillips et al., 2016). This
49 disequilibrium is well described in Scheffer's model with the two stable ecological states of shallow
50 lakes, the macrophytes dominance (clear state) and the phytoplankton dominance (turbid state)
51 (Scheffer & Jeppesen, 2007). Macrophytes are thus in competition with phytoplankton for light and
52 nutrient in a complex interaction known to be a good ecological example of hysteresis (Scheffer,
53 2001).

54 Aquatic macrophytes are engineer species of shallow lakes, with many direct and indirect effects on
55 the physical and chemical parameters of water, also on the production of bio-surface and habitats, and
56 on aquatic communities themselves (Teubner et al., 2022). Depending on the biomass they achieve,

57 they can control biogeochemical cycles through absorption of nutrients and transitory storage
58 (Carpenter & Lodge, 1986; Teubner et al., 2022). Macrophytes act as a sink of nutrients during the
59 growing season, but they are a net source of dissolved organic carbon, with the release of 1-10% of
60 their photosynthetically-fixed carbon released into the water column (Carpenter & Lodge, 1986).
61 Rooted macrophytes stabilize the sediment and dissipate the kinetic energy of waves and wind
62 (Beklioglu & Moss, 1996; Madsen et al., 2001). They control gas exchanges with the atmosphere and
63 underwater oxygen concentration, with cascading effects on nutrient and water chemistry (Caraco et
64 al., 2006). Daily thermal stratification can be observed in summer (Andersen et al., 2017), with steep
65 vertical gradient and potentially anoxia (Carpenter & Lodge, 1986).
66 Secondly, they provide support and habitats for a diverse epiphytic algae (Wijewardene et al., 2022),
67 littoral zooplankton (Bolduc et al., 2016, 2020), and macro-invertebrates (Misteli et al., 2022, 2023).
68 They fuel a high secondary production, from bacteria to macro-invertebrates, directly through organic
69 carbon release (Søndergaard et al., 1998; de Kluijver et al., 2015). Periphyton growing on
70 macrophytes provides an additional resource compared with open water (Vadeboncoeur et al., 2008;
71 Jaschinski et al., 2011). Plant-associated cladocerans are observed in higher density in macrophytes
72 and can feed on this periphyton (Masclaux et al., 2012).
73 Macrophytes may also control the trophic network of the lakes from bacteria to birds (Jeppesen et al.,
74 1998). They stabilize biotic interactions in the trophic network by providing refuge and habitat (Diehl,
75 1993). Macrophytes provide refuge for intermediate predators, such as macro-zooplankton (Jeppesen
76 et al., 1998; Bertolo et al., 1999). Zooplankton taxa like *Daphnia* move to macrophytes during the day,
77 a process known as the diel horizontal migration (DHM), and can preserve the clear water state by
78 grazing phytoplankton (Perrow et al., 1999; Bertolo et al., 2000). Habitats generated by macrophytes
79 support a large functional and specific diversity (Søndergaard & Moss, 1998).
80
81 However all these above effects and interactions were mainly described for submerged macrophytes
82 and less is known about the other morphotypes of macrophytes, such as rooted macrophytes with
83 surface leaves (Cazzanelli et al., 2008). Floating-leaf aquatic macrophytes are less diversified, but they
84 are less affected by P eutrophication and the increase in turbidity (de Nie et al., 1987; Bornette &

85 Puijalon, 2011). They can maintain their biomass in shallow lakes, particularly in littoral areas, with
86 more or less developed macrophytes beds. Moreover, even if most of the known effect of emerged
87 macrophyte should be similar to those of submerges ones, we suppose that their direct (e.g. light) and
88 indirect (e.g. temperature) effects of shading are expected to be stronger.

89 Studies highlighting the effects of macrophytes on phytoplankton compare different shallow lakes
90 with different level of vegetation cover (Takamura et al., 2003; Hornbach et al., 2020). However,
91 within a lake, macrophyte beds create a complex landscape, with areas dominated by well-mixed
92 turbid open-water and areas more or less colonized by macrophytes. During summer, despite the high
93 passive dispersive nature of phytoplankton, assemblages should differ within a shallow lake between
94 open water and waterlilies area, if large standing crop is achieved. Phytoplankton species sorting along
95 environmental gradient has already been observed in a large tropical reservoir (Yang et al., 2018).
96 While a change in zooplankton assemblage is expected with higher density of littoral species
97 (Cazzanelli et al., 2008; Bolduc et al., 2016), the effect on phytoplankton assemblage is almost not
98 studied (Gebrehiwot et al., 2017).

99

100 Two main habitats are expected in lakes partly colonized by rooted macrophytes with surface leaves:
101 an open water area which is turbulent, turbid, and warm, and macrophytes zone which is cooler, poor
102 in light and nutrients but rich in dissolved organic carbon. In the latter habitat, mixing is controlled by
103 night cooling and convection. These habitats should select for different strategies (resource
104 acquisition, grazer avoidance) reflected in phytoplankton assemblages. By decreasing light
105 availability, shade-adapted species should be favored in macrophytes. Reduced turbulence favours
106 motile species. Low nutrient concentrations should favor nutrient competitive species, such as small
107 cells. The release of dissolved organic carbon in parallel with low light availability should favor
108 mixotrophic species. A higher zooplankton biomass is expected in macrophytes used as daytime
109 refuge, so that strategies against grazing should be observed (Lüring 2021). In open water, high
110 nutrient availability and warm turbulent conditions should favor turbulent tolerant cyanobacteria.
111 These strategies adopted by phytoplankton can be related to morphological and physiological

112 differences in traits (size, shape, motility, nutritional status) and control growth, sedimentation and
113 resources acquisition (Margalef, 1978; Reynolds et al., 2002).

114 We hypothesize that plants modify the physical, chemical and biological parameters compared to open
115 water stations, leading to a change in phytoplankton assemblages. Plants, by controlling the local
116 environment, thus structure phytoplankton assemblages and allow the coexistence of different
117 assemblages within a lake, despite the strong dispersing power of phytoplankton. The three aims of the
118 study are (1) to show the effect of plants on physical, chemical and biological parameters, (2) to
119 highlight a different community structure between stations covered by rooted floating-leaf
120 macrophytes and those in open water, and (3) to highlight the main environmental drivers of
121 phytoplankton assemblages in a lowland shallow lake. We sampled during a summer a large shallow
122 lake, colonized on a third of its surface by water lilies. Water lilies (Nymphaeid water plants) are
123 common in alkaline water in North Europe (Smits et al., 1988) and represent a good model to test for
124 our hypotheses.

125

126

127 **Methods**

128 *Lake parameters and sampling stations*

129 The lake of Grand-Lieu, classified as a natural reserve forbidden to the public, is located in the west of
130 France about ten kilometers south-west of Nantes and 20 km from the Atlantic coast (47°4'59.999"N -
131 1°40'0.001"W). It has a catchment area of 700km² from which it receives water from two main
132 tributaries: the Boulogne and the Ognon rivers. It then evacuates its waters through the Acheneau
133 channel into the Loire River. In summer, its surface is about 2500 hectares for a depth of less than 1
134 meter. The lake can be divided into several zones: open water zones (absence of macrophytes),
135 floating-leaf macrophyte beds (*Nuphar lutea*, *Nymphae alba*, *Trapa natans*, *Nymphoides peltata*),
136 wooded reedbeds (Caricaies, Phragmitaies, Saulaies, Aulnaies) and wet meadows (Paillisson &
137 Marion, 2005). The lake experienced hypereutrophication since the 1970s, leading to many different
138 policies regarding the management of its water levels. See Table 1 for limnological characteristics of
139 the lake.

140 Sampling of Lake Grand-Lieu was conducted in July and August 2018. 15 monitoring stations were
141 distributed over the lake including the mouths of the two tributaries of the lake (Fig. 1). Nine stations
142 (1,2,3,5,6,8,9,14,15) were in macrophytes habitat (M), while 6 (4,7,10,11,12,13) were in open water
143 habitat (OW). A few stations were not sampled in July, being difficult to access because of a water
144 level lower than 30 cm. This monitoring is part of a larger survey of physico-chemical and biological
145 parameters at the 15 stations from February 2018 to November 2019.

146

147 ***Macrophytes cover***

148 To estimate the presence of macrophytes at each station, vegetation cover was estimated using a 1 m²
149 wooden quadrat. The quadrat is thrown five times randomly around the boat. The percentages are then
150 averaged and ranked according to the Braun-Blanquet scale (0%, <5%, 5-25%, 25-50%, 50-75%,
151 >75%).

152

153 ***Physical and chemical parameters***

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

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

164

165 ***Water sampling***

166 For water chemistry and plankton, particular attention must be paid in order not to resuspend the
167 sediments, in a water column that is mostly less than 1 m deep. After trials with a rigid integrating

168 water sampler like the Bailer water sampler, it was chosen to gently sample the water at sub-surface
169 (10-15 cm below the surface) using a 1 L Nalgene bottle, and to test the presence of vertical gradients
170 by specific sampling and probe profiles. The bottle was rinsed three times with lake water prior to
171 sampling and opening and closing is done below the water surface to prevent leaves or branches from
172 contaminating the sample. This technique was already used to sample a shallow fluvial lake similar to
173 the Grand-Lieu Lake (Cattaneo et al., 2013).

174

175 *Nutrients concentrations*

176 Filtrations for nutrients were performed immediately on the boat: a first 10 mL sample was collected
177 and transferred into a 15 mL tube for total nitrogen (N) and phosphorus (P) measurements. Using a
178 0.45 µm filter (filtropur) and a 30 mL syringe (previously washed with 10% HCl acid and rinsed three
179 times with the sample), four aliquots were divided into 15 mL tubes for silica (SiO₂), nitrate (NO₃⁻),
180 orthophosphate (PO₄³⁻) and ammonium (NH₄⁺) analyses. All 15 mL samples were kept in a cooler and
181 stored long-term at -20°C, except for silica which remains stored at 4°C.

182 Total phosphorus (TP) and total nitrogen (TN) were measured colorimetrically after digestion with
183 persulfate (Grasshoff, 1983), with a detection limit of 6 µg P L⁻¹ and 50 µg N L⁻¹. Orthophosphate was
184 analyzed by the ammonium molybdate method, according to USEPA protocol (USEPA Method 365.1,
185 1993), with a detection limit of 3 µg P L⁻¹. After reduction of nitrate (NO₃⁻) to nitrite (NO₂⁻) with
186 vanadium chloride, NO₂⁻ were measured calorimetrically with sulfanilamide and N-1-
187 naphthylethylenediamine dihydrochloride, according to USEPA protocol (USEPA Method 365.2,
188 1993), with a detection limit of 50 µg N L⁻¹. Colorimetric measurements were performed with a
189 Gallery Photometric Analyser Gallery Plus (Thermo Fisher).

190

191 *Phytoplankton biomass and assemblages*

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

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

199

200 ***Zooplankton abundances***

201 Zooplankton were sampled at each station for abundance and diversity for macro-zooplankton, with
202 copepods and cladocerans. Surface water was sampled with a 2L bottle and filtered through a 60 µm
203 zooplankton filter. 20 to 30 L were passed through a 60 µm net depending on zooplankton abundance
204 and filter saturation. We used a 2 L bottle instead of a 10 L bucket to prevent sediment resuspension.
205 The sample was then collected on a 60 µm sieve and the zooplankton was anaesthetized by carbonated
206 water (perrier type). The zooplankton was then transferred to a 50 mL tube with 80% ethanol. The
207 samples were kept at 4°C until identification and counting under a microscope and binocular magnifying
208 glass, based on identification keys (Dussart, 1967; Amoros, 1984; Bledzki & Rybak, 2016). Samples
209 were counted in a Bogorov counting chamber, after subsampling if necessary, with a Hensen-Stempel
210 pipette. Total abundance of cladocerans and of copepods are used here.

211

212 ***Statistical analysis***

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

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

222 To quantify species that are specific to macrophytes and open water habitats, Venn diagrams were
223 made using the library ‘ggvenn’ (Yan 2021) in Rstudio, on presence – absence of phytoplankton taxa
224 in July 2018 and August 2018.

225 To identify phytoplankton species contributing to the differentiation of assemblages by macrophytes, a
226 partial canonical correspondence analysis (pRDA) was performed on Hellinger-transformed
227 phytoplankton abundances, with macrophytes cover as the only explanatory factor. The pRDA allows
228 to remove time dependency associated with sampling month through multiple regression. The pRDA
229 was used to reposition species along Axis 1 (habitat) and identified those that contributed most to the
230 assemblages. The link between assemblages and macrophyte cover was tested through a Monte Carlo
231 permutations test. The pRDA was performed using ‘vegan’ library in Rstudio (Oksanen et al., 2013).

232 The effect of habitat has also been tested independently of the pRDA by a PERMANOVA on Bray-
233 Curtis distances with *adonis* function from ‘vegan’ library.

234 Indicator species were highlighted with ‘indicspecies’ library (De Caceres et al., 2016) and the
235 function *multipatt*, which allows determining lists of species associated with groups of sites, here the
236 presence/absence of macrophytes (Dufrêne & Legendre, 1997). The indicator value associated with
237 the statistical analysis is the average of two probabilities, the probability that the sampled site belongs
238 to the target group (here M or OW habitat) knowing that the species was observed (specificity of the
239 species) and the probability of finding the species when sampling the target group (fidelity of the
240 species) (Dufrêne & Legendre, 1997).

241 To prioritize environmental factors controlling phytoplankton assemblages in the two habitats
242 independently of the sampling month, we performed a pCCA, with the physical, chemical and
243 biological parameters as explanatory variables, and after removing the effect of month. The
244 significance of the model has been tested through a permutation test, while environmental variables
245 were tested individually with an ANOVA, which allowed us to remove the less significant parameters.

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

250 concentrations of phosphates, ammonium, dissolved silicon, total phosphorus and total nitrogen,
251 cladoceran and copepods abundances, and the percentage of cover by macrophytes.

252

253 **Results**

254 *Effect of macrophytes on habitats*

255 Except station 9, all stations from M habitat exceeded 70% of surface cover (Fig. 2a). An effect of
256 macrophytes on different physico-chemical parameters has been observed in summer (Fig. 2), and is
257 consistent with the observed seasonal divergence of the means values of the two habitats (Fig. 3). At
258 the seasonal scale, we observed an increase in the mean macrophytes cover between spring and
259 summer, with highest cover in July and August (Fig. 3a). The greatest divergence in physical and
260 chemical parameters was observed during the period with maximum cover (Fig. 3).

261 Thus, the OW stations were significantly warmer than the stations in the M habitat, with +2-3°C (up to
262 8°C) during the day (Fig. 2b; KW = 8.54; p=0.003). Water temperature followed the season, with
263 higher temperature in OW compared with the M habitat during summer (Fig. 3b). The pH measured in
264 the middle of the day showed values above 9 in summer in the open water, while it remained below 8
265 in the M habitat (Fig. 3c). Dissolved oxygen, which was about 100% in winter, was closed to 50% in
266 the M habitat in spring and summer, while very high DO concentration was observed in the OW
267 habitat (Fig. 2c and 3d). The high pH coupled with high oxygen concentrations indicated high primary
268 production in open water compared to the M habitat.

269 A higher conductivity of water (+9%) was observed in the M habitat ($399 \mu\text{S cm}^{-1}$) compared with the
270 OW habitat ($367 \mu\text{S cm}^{-1}$) (KW=6.12; p=0.01). Phosphates concentration also showed a strong
271 seasonal pattern, with low values in winter and very high value in summer (Fig. 3e), and
272 concentrations twice as high in the OW habitat compared to the M habitat (Fig. 2f). A significant
273 habitat effect was observed in summer (KW=4.75; p=0.03; Fig. 2f). Similar pattern of higher
274 concentration in OW was observed for dissolved silicon DSi (KW=10.56; p=0.001; Fig. 2g) and total
275 phosphorus (KW=5.98; p=0.015; Fig. 2h). We observed DSi concentration at least twice higher in OW
276 compared with the M habitat, with DSi concentration correlating significantly with phosphates ($r =$
277 0.530; $p < 0.001$). Ammonium was slightly higher in summer in M habitat compared with OW, but

278 this was not significant ($p=0.48$; Fig. 2e). The concentration of nitrates was below detection limits in
279 all stations in summer (data not shown).

280 The concentration of chlorophyll *a* showed a seasonal pattern, with high summer concentrations in
281 OW compared with the M habitat (Fig. 2k and 7F; $KW=9.07$; $p=0.003$). Cyanobacteria biomass
282 represented a large part of the phytoplankton biomass in open water (Fig. 2l; $KW=14.8$; $p<0.001$).

283 Higher abundance of both copepods and cladocerans zooplankton groups was observed in the M
284 habitat compared with OW (Fig. 2i, j; $p<0.05$).

285 Finally, during summer, OW habitat was characterized by higher temperature ($+3-4^{\circ}\text{C}$), higher DO (at
286 least factor 2), higher pH (at least +1), higher PO_4 (at least factor 2) and TP (+50%), higher DSi (at
287 least +2 mg Si/L), and lower abundances of zooplankton (at least factor 2), compared with stations in
288 M covered by floating-leaf macrophytes. The higher photosynthetic activity resulted in higher biomass
289 of cyanobacteria (factor 5).

290

291 *Phytoplankton assemblages according to habitats*

292 The phytoplankton assemblages at stations covered with floating-leaf macrophytes (habitat M)
293 differed from assemblages at open-water stations (habitat OW), as shown by the ANOSIM analysis
294 ($R=0.238$; $p=0.009$) and the NMDS plot (Fig. 4). The assemblages in August also differed from
295 assemblages in July (ANOSIM: $R=0.254$; $p=0.006$; Fig. 4).

296 Over the two summer months, a total of 264 phytoplankton taxa were observed in habitat M, and 197
297 in stations in habitat OW. Venn diagrams were used to see month by month the number of taxa
298 common between the two habitats and those specific to one of the habitats (Fig. 5). 53.5% and 54.1%
299 of taxa were present in both habitat in July and August, respectively (Fig. 5). 10.7% to 13.7% were
300 observed only in the OW habitat, while one-third (32.8% to 35.2%) was observed in the M habitat.

301 Although the total number of taxa differed between the two months, the proportions in Venn diagrams
302 remained the same.

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

304 Species richness in samples from the M habitat varied between 72 and 110 in July, and between 57
305 and 84 in August (Fig. 6a). In the OW habitat, it ranged from 86 and 91 in July, and from 61 and 69 in

306 August (Fig. 6a). In terms of richness, samples from OW stations were more homogeneous than those
307 in M, in view of their quartiles on the boxplots (Fig. 6a) and their standard deviations (less than 3 for
308 OW and more than 9 for M, in both July and August). While an effect of month on richness was
309 observed, no effect of habitat could be demonstrated here (KW=14.9; p=0.0018). Shannon, Simpson
310 and Evenness showed a similar pattern, with an effect of month in habitat M and a higher diversity in
311 habitat M compared to habitat OW in August only (Fig. 6b,c,d). Some variability was observed
312 between stations in the M habitat, with Shannon values fluctuating from 2.3 to 3.3 for the same date
313 (Fig. 6b). The OW habitat showed values ranging from 2.0 to 2.7.

314 In the partial RDA relating phytoplankton assemblages to macrophyte cover, 7.6% of the total
315 variance in phytoplankton assemblage was explained by the macrophyte cover and 14.5% was
316 explained by sampling month. The use of a single explanatory parameter (percent macrophyte cover)
317 allowed the positioning of ‘responsive’ species along the first axis. Taxa located at the extremities of
318 axis 1 are shown by habitat and by class in Table 2. First, more taxa were observed in the M habitat
319 (Table 2a), with all taxa in the classes Chrysophyceae, Euglenophyceae, Cryptophyceae,
320 Ulothricophyceae, Xanthophyceae and Zygothricophyceae. Only cyanobacteria had a higher number of
321 contributing taxa in the OW habitat (Table 2b). Chlorophyceae and Diatomophyceae showed a higher
322 number of contributing taxa in the M habitat, with responses to macrophytes varying among species of
323 the same genus (for example, *Coelastrum*, *Pediastrum* and *Scenedesmus*). In Euglenophyceae, several
324 species of the same genus were observed with all the same preferred habitat, with for example, *Phacus*
325 *acuminatus*, *P. costatus*, *P. pyrum*, *P. raciborskii*, *P. skujae*, *P. suecicus* and *P. tortus* (not shown).
326 Genera of *Trachelomonas* were also well represented by several species.

327 Thus, habitat preference was very clear for most classes, but also outside these classes, for several
328 genera: for example, in the M habitat, the Chlorophyceae *Monoraphidium*, the cyanobacteria
329 *Microcystis*, and *Cyanogranis*, and the large diatom *Aulacoseira* (Table 2a). The cyanobacteria genera
330 *Chroococcus*, *Coelosphaerium*, *Dolichospermum* and *Merismopedia* showed several species with a
331 preference for the OW habitat.

332 Seventeen species were particularly related to a habitat and were identified as indicator species. Seven
333 species were highlighted as indicator species of the M habitat, and ten species of the OW habitat

334 (Table 3). Among the indicator species, the euglenophyceae *Phacus sp.* (Fig. S1a; KW = 7.75, p =
335 0.005), *Euglena sp.* (Fig. S1b; KW = 4.23; p=0.04) and *Trachelomonas volvocina* (Fig. S1c; KW =
336 8.98; p=0.003) showed a significantly higher abundance in the M habitat, whereas they were close to
337 zero in the OW habitat. On the contrary, the cyanobacteria *Planktothrix agardhii* (Fig. S1d; KW =
338 4.28; p=0.04) and *Romeria leopoliensis* (Fig. S1e; KW = 7.10; p=0.008) were in higher abundance in
339 OW habitat, as well as the diatom *Nitzschia fruticosa* (Fig. S1f; KW = 7.0; p=0.008).

340 Based on the indicator values (Table 3), the probability of sampling stations in the M habitat was the
341 highest when the euglenophyceae *Phacus sp.* (p=1) or *Trachelomonas sp.* (p=1) were observed, as
342 well as the green algae *Siderocelis sp.* (p=1) or the diatom *Thalassiosira duostra* (p=0.97). The
343 probability of sampling an OW station was the highest when the green algae *Pediastrum boryanum*
344 was observed (p=0.94) or the cyanobacteria *Chroococcus microscopicus* (p=0.94) (Table 3). When
345 sampling the OW habitat, the probability of having the cyanobacteria *Merismopedia punctata* in the
346 sample was maximum (p=1.00), followed by the two diatoms *Nitzschia fruticosa* (p=0.89) and
347 *Stauriosira venter* (p=0.89). When sampling the M habitat, the probability was the highest for the
348 green algae *Crucigenia tetrapedia* (p= 0.93) and *Micractinium pusillum* (p=0.87).

349

350

351 ***Prioritizing environmental parameters in the control of phytoplankton assemblages***

352 To link phytoplankton assemblages with environmental factors, physical and chemical parameters
353 were used as explanatory parameters of taxa abundances, after removing the effect of the month (9.8%
354 of total variance; Fig. 7). 61% of total variance in phytoplankton assemblages were explained by the
355 environmental parameters (p=0.001 based on 999 permutations) independently of the month. The first
356 axis, which represented 15.2% of constrained variance (9.4% of total variance), contrasted stations
357 from the M habitat on the left from the OW stations on the right of the plot (Fig. 7). SiO₂
358 concentration (r=0.75; p=0.026 based on permutation test by terms), water temperature (r=0.43;
359 p=0.049), TP (r=0.47; p=0.005), phosphates concentration (r=0.43; p=0.001), TN (r=0.55; p=0.012)
360 and total chlorophyll *a* concentration (r=0.55; p=0.012) correlated positively with the first axis,
361 indicating higher values in open water (Fig. 7 and Table 4). Conductivity (r=-0.64; p=0.002),

362 macrophyte cover ($r = -0.55$; $p = 0.046$), ammonium ($r = -0.23$; $p = 0.004$) and Secchi depth ($r = -0.18$;
363 $p = 0.009$) correlated negatively with the first axis, thus in direction to the M habitat. Copepods ($r = -$
364 0.25) and cladocerans ($r = -0.20$) abundances also correlated negatively, but they were not significant
365 explanatory parameters.

366 The mean position of phytoplankton classes has been added on the pCCA plot (Fig. 7). Cyanobacteria
367 was associated with the OW habitat, while all other classes were on the left side of the plot.

368 Chlorophyceae and Diatomophyceae remained close to the plot center, while the other classes were
369 distributed along the axis 2. Chrysophyceae in the bottom part (Fig. 7) were correlated with
370 ammonium ($r = 0.95$; $p < 0.001$), mainly because of the station 5 in July, which had with 0.34 mg N-
371 $\text{NH}_4 \text{ L}^{-1}$ and $2,574 \text{ cells mL}^{-1}$.

372 We used the classification and regression tree (CART) model to prioritize environmental variables
373 importance in sites scores of the first pCCA axis (Table 4). Turbidity, which was not significant in the
374 pCCA, was the most important parameter explaining site scores, with an importance value of 7.37 and
375 a threshold at 19.5 NTU. Temperature was the second important parameter (4.73), with a threshold at
376 23.3°C , followed by TN (3.41) and macrophytes cover (3.2).

377

378 **Discussion**

379 *Plant effect on abiotic parameters*

380 Water lilies modified the habitat and generated small-scale spatial heterogeneity, favorable to motile
381 taxa. They first decreased water temperature compared with open water. Macrophytes are known to
382 strongly decrease depth penetration of both wind mixing energy and solar radiations, leading to lower
383 temperature and turbulence (Andersen et al 2017 Aquatic Science). The average difference of 3°C
384 between the two habitats M and OW can have important repercussions on biological activities and
385 competition between species. Water temperature and mixing are indeed key drivers of the
386 biogeochemical and ecological functioning of lakes, controlling biological activities and gases
387 exchanges (Woolway et al 2016).

388 In open water, the lake being highly turbid, it causes superficial heating (Persson & Jones, 2008).

389 Conditions were also more turbulent, thanks to a large fetch. The sensitivity of the lake to wind

390 forcing was particularly visible in that lake by Langmuir cells, which were regularly observed in the
391 open water only as soon as wind was above a threshold (Wetzel, 2001).

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

397 Plants also decreased pH and DO compared with open water. High pH, DO saturation above 150%
398 and high cyanobacteria biomass characterized the open water in summer and indicated a high
399 photosynthetic activity, probably favored by phosphates concentration and high temperature. A pH
400 close to 7.5 and DO saturation below 50% characterized macrophytes area, thus dominated by
401 heterotrophic processes. We thus observed contrasted functioning areas, with autotrophic area (Ratio
402 between gross primary production GPP and ecosystem respiration $R > 1$) in OW habitat and
403 heterotrophic area ($GPP/R < 1$) in M habitat. Such spatial zonation in lake metabolism has already been
404 observed with a net heterotrophy in the macrophytes *Trapa natans* compared with submerged
405 macrophytes within a shallow lake (Stefanidis & Dimitriou, 2019). The authors suggest that
406 allochthonous organics fuel heterotrophic processes in macrophytes area. Unfortunately, dissolved
407 organic carbon was not measured in our study. However, it should have been higher in macrophytes
408 compared with open water, as actively growing macrophytes release 1 to 10% of their primary
409 production (Carpenter & Lodge, 1986). Contrary to nutrients, macrophytes are considered as net
410 source of DOC for lakes.

411 Phosphates (and total phosphorus) were significantly lower in the M habitat (factor 2). Several non-
412 exclusive processes explain the difference between habitats: First, a difference in pH and redox
413 potential between the two zones can lead to a different adsorption and chemisorption rates (Bostrom,
414 1982; Sondergaard et al., 2001). P bound to redox-sensitive iron compounds can be indeed quickly
415 released to the water column, if redox changes (Mortimer, 1941; Bostrom, 1982). Secondly, the P in
416 the pore water of the sediment can be mobilized in the water column during episodes of sediment
417 resuspension (Sondergaard et al., 1992). Thirdly, warmer temperature may also contribute to

418 accelerated P recycling rate to the water column, as for ammonium (Jiang et al., 2019). Fourthly, P
419 storage in phytoplankton cells lasts a few days and P remained easily remobilized when cells die (high
420 turnover). Macrophytes on the contrary store P for a few months and act as a net sink during their
421 active growth (Carpenter & Lodge, 1986; Teubner et al., 2022). The M habitat acted as a P summer
422 sink area with slower metabolism compared with the OW habitat (high production and regenerating
423 rates).

424 Water lilies also decreased dissolved silicon concentrations by a factor two compared with open water.
425 A higher consumption of silica can be expected in the M habitat, diatoms being in higher density.
426 Periphyton growing on macrophytes may also have absorbed it. Lastly, storage of biogenic silica (BSi)
427 by macrophytes can explained the strong difference. *Nuphar lutea* contains indeed 8 mg BSi g⁻¹ DW
428 (Schoelynck et al., 2010) and may also have acted as a Si summer sink. In open water, a higher
429 mineralization of organic matter may also increase DSi concentration. Biogenic silica originated from
430 the dissolution of frustules of dead diatoms accumulated in the sediment and is easily mobilizable
431 (Sarazin et al., 1995).

432 No effect of plant could be observed on nitrates and ammonium. Nitrates in the lake were controlled
433 by winter recharge and increased when river flows restart in fall, while the concentration remained
434 below the detection threshold in summer. Nitrogen-fixing cyanobacteria with numerous heterocysts
435 were observed throughout the summer, supporting nitrogen limitation at this time. Ammonium
436 concentration remained lower than 20 µg N L⁻¹ most of the year, but peaks in ammonium were
437 observed in some of the M stations. Ammonium in eutrophic lakes is highly dynamic depending on
438 coupled production and consumption processes associated with bacteria and primary producers (Jiang
439 et al., 2019).

440

441 ***Phytoplankton assemblages depending on habitats***

442 We showed here that rooted floating-leaf macrophytes (habitat M), by modifying the physical and
443 chemical parameters and biotic interactions, allowed the spatial coexistence of several phytoplankton
444 assemblages within the lake. The assemblages of phytoplankton changed between the beginning of
445 July and the end of August, in accordance with the seasonal dynamics (Sommer et al., 1986; Pannard

446 et al., 2008), but the effects of plants remained. Based on presence/absence data, three times more taxa
447 were associated with the M habitat (one third of the total) compared with the OW habitat (10-13%).
448 Consistently, the diversity of the phytoplankton was higher in the M habitat than in the OW habitat,
449 while the total biomass of phytoplankton was about twice lower in M habitat than in OW. The
450 presence of water lilies was unfavorable especially to cyanobacteria (such as those observed in high
451 biomass in open water), and allowed the maintenance of rare species and a greater diversity in M
452 habitat.

453 Most of taxa and some entire classes of phytoplankton showed higher abundances in stations in M
454 compared with stations in OW, which was favorable to cyanobacteria. Phytoplankton assemblage in
455 the M habitat were mostly unicellular flagellates, tolerant to low light, with many mixotroph
456 (combining photosynthesis and ingestion of particulate organic matter) known to interact with organic
457 matter (heterotrophic ponds). All taxa from the unicellular flagellate classes *Euglenophyceae*,
458 *Chrysophyceae* and *Cryptophyceae* showed a habitat preference for macrophytes with a fivefold
459 higher abundance in water lilies compared with open water. Three of the seven indicator species of the
460 M habitat were mixotrophic *Euglenophyceae* (*Euglena sp.*, *Phacus sp.*, *Trachelomonas volvocina*),
461 typical of organic ponds (Reynolds et al., 2002). *Chrysophyceae* can also be found in heterotrophic
462 ponds according to the Reynolds functional classification and are known to be tolerant to low nutrients
463 with potential use of mixotrophy (Reynolds et al., 2002; Padisak et al., 2009). *Cryptophyceae* are
464 tolerant to low light conditions (Reynolds et al., 2002) and the main representative (*Cryptomonas sp.*)
465 also has mixotroph ability (Princiotta et al., 2019). These three classes are therefore related to organic
466 carbon and potential mixotrophic activity, which is advantageous in light limiting environments.

467 For other classes with habitat preference for M (*Chlorophyceae*, *Ulothricophyceae*, *Xanthophyceae*
468 and *Zygothricaceae*), lifeforms were more variable from unicellular flagellates to simple colonial and
469 filamentous forms. Most of diatoms were also in higher abundance in the M habitat. *Aulacoseira*
470 *granulata*, *A. ambigua*, *Cyclotella meneghiniana* and the M indicator species *Thalassiosira duostra*
471 are all planktonic diatoms found in mixed eutrophic lakes, with tolerance to low light and C deficiency
472 (Reynolds et al., 2002). Similarly, *Praestephanos triporus* (20 times more abundant in the M habitat
473 than the OW) is a planktonic diatom found in shallow turbid water (Padisak et al., 2009). A few free-

474 floating colonies of cyanobacteria showed a preference for the M habitat, in particular the toxic
475 blooming species *Microcystis aeruginosa* and *Microcystis flos-aquae*, typical of shallow nutrient-rich
476 water (Padisak et al., 2009).

477 Cyanobacteria were the only class showing a habitat preference for the OW habitat, with a threefold
478 increase in biomass and dominance of filamentous N-fixing cyanobacteria and of picocyanobacteria.
479 One of the two dominant taxa (relative frequency > 10%) was thus *Dolichospermum flosaquae*, a
480 buoyant N-fixing filament. In lower abundances was observed a similar lifeform, with
481 *Dolichospermum compactum*, *Aphanizomenon flosaquae*, and *Pseudanabaena catenata*. The second
482 dominant taxa was the mat-forming *Merismopedia tenuissima*, a flat rectangular colony of small cells
483 arranged in rows within a mucilaginous matrix, co-occurring with *M. warmingiana* and *M. punctata*.
484 Many other picocyanobacteria were observed in the OW, such as *Aphanocapsa elegans*, *A. nubile*,
485 *Aphanothece smithii*, *Chroococcus minutus*, *C. microscopicus*, *Coelosphaerium kuetzingianum*, *C.*
486 *minutissimum* and *Pannus planus* (Callieri et al., 2012). Small-size cell makes species more efficient
487 to absorb light and nutrients (Finkel & Irwin, 2000; Finkel et al., 2009) in the turbid N-depleted open
488 water. Cyanobacteria observed here are thus found in low nitrogen and turbid mixed layer (Reynolds
489 et al., 2002; Padisak et al., 2009). A single diatom, *Staurosira venter*, was indicator of the OW habitat
490 with a threefold increase in biomass. These lanceolate cells (5 µm wide and 5-26 µm long) can be
491 attached to the substratum by a mucilage pad or be planktonic, and is found in turbid and frequently
492 mixed shallow lakes (Padisak et al., 2009).

493 Since cyanobacteria form large colonies, it is not surprising that they contribute more in terms of
494 relative frequency, compared with the other classes. We could have calculated biovolumes for each
495 species, but not all species could be measured here. We would therefore have lost taxa in the analysis,
496 knowing that what we are interested in anyway is the difference between habitats.

497

498 For zooplankton assemblage, the submerged macrophyte effect is well known, with differentiation of
499 assemblages driven by active dispersion to benefit for the refuge effect, and by the presence of plant
500 associated species feeding on periphyton (Jeppesen et al., 1998; Bertolo et al., 1999). A horizontal diel
501 migration between the M and OW stations can be expected for the non-littoral species of large

502 zooplankton (Lauridsen et al. 1998). However, it probably remained limited to the edges of the
503 macrophyte beds, because of the distances of several hundred meters to cover. The effect of surface-
504 leaf macrophytes is less strong than those of submerged macrophytes, but this habitat also host a
505 higher biomass, abundance and richness of zooplankton compared with open water (Carpenter &
506 Lodge, 1986). Similarly, the phytoplankton in the M habitat could have been enriched with
507 meroplanktic and epiphytic species, benefiting from the biological support. However, there were no
508 more benthic or meroplanktic taxa in the M habitat than in OW, while genera such as *Fragilaria* and
509 *Nitzschia* were observed in both habitats. It is indeed a response of the pelagic phytoplankton that has
510 been observed here. The species that could benefit from benthic growth were characteristics of the
511 OW habitat, with the genus *Merismopedia* and the diatom *Staurosira venter*, showing a potential role
512 of meroplankton in increasing diversity in the pelagic zone.

513 The difference in phytoplankton biomass and assemblage between M and OW could also have been
514 explained by differences in zooplankton grazing. However, zooplankton was in higher abundance in
515 M compared with OW, as were most phytoplankton groups, while less edible species (cyanobacteria)
516 were more abundant in OW. Despite higher zooplankton in M habitat, sensitive taxa such as
517 unicellular flagellates kept a higher biomass in M compared with OW. Zooplankton do not explain
518 here the differences in phytoplankton taxa between the two habitats.

519

520

521 ***The structuring role of macrophytes by controlling environmental parameters***

522 The floating-leaf macrophytes by controlling the physical and chemical parameters changed the
523 phytoplankton assemblage, with lower total biomass but more diverse microalgae assemblage. Our
524 findings are consistent with the previous study on the tropical lake Ziway (Ethiopia) colonized by
525 *Typha latifolia* and *Phragmites australis* (Gebrehiwot et al., 2017). The authors observed a more
526 diverse phytoplankton assemblages within the emergent macrophytes beds, with more species of
527 Bacillariophyceae and *Euglenophyceae*. Like in our study, the cyanobacteria *Microcystis spp.* was
528 associated to the M habitat, while *Merismopedia punctata* was associated with open water. Contrary to

529 our study, water temperature was warmer in macrophytes, but the effects on DO, conductivity, TP and
530 phosphates were the same than in our study, even if we are in a temperate lake.

531 A greater dominance of flagellates in the presence of submerged macrophytes has already been
532 pointed out (Søndergaard & Moss, 1998). The flagellated shape better counteracts sedimentation
533 losses in low turbulence environments (Margalef, 1978). Despite a greater vulnerability to
534 zooplankton grazing, flagellates may have advantage in low turbulent condition, because they are
535 adapted to better exploit small-scale heterogeneous environment in terms of nutrients and organic
536 matter, associated with macrophytes (Sommer, 1988; Søndergaard & Moss, 1998). This heterogenous
537 environment also limits competition among species and prevents the dominance of a few ones (Cunha
538 et al., 2012). Moreover, many of these flagellates are mixotroph. The subsidy of dissolved organic
539 carbon derived from macrophytes and from the associated periphyton, coupled with the low light
540 availability, promoted mixotrophic species in that habitat (Søndergaard & Moss, 1998). We thus
541 highlighted a similar effect of water lilies on phytoplankton assemblages than submerged
542 macrophytes, with local promotion of the microbial loop and heterotrophic processes.

543 In open water, the high biomass of cyanobacteria was expected owing to the hypereutrophic state of
544 the lake (Huisman et al., 2018). The high phosphate concentration and warm temperature synergically
545 promoted cyanobacteria growth (Paerl & Huisman, 2008; Paerl, 2017). The most dominant species
546 was the cyanobacteria *Dolichospermum flos-aquae*, which formed huge colonies rolled up on itself
547 with numerous heterocysts, confirming N summer limitation. Many co-occurring blooming genera
548 were observed simultaneously in the lake, while eutrophic shallow lakes generally experience
549 alternating blooms of a few dominant species (Wu et al., 2016; Le Moal et al., 2021). *Microcystis* and
550 *Dolichospermum*, which are among the most toxic genera, separated spatially between the OW and the
551 M habitats. They are already known to co-occur spatially (Zhang et al., 2016), while they most often
552 succeed each other in reservoirs (Soares et al., 2009; Wu et al., 2016). These studies showed that
553 warmer temperature favors *Microcystis*, which should have been present in higher abundance in open
554 water. However, these species have also different P requirement : *Dolichospermum* needs higher P
555 level than *Microcystis*, because heterocysts' formation consume lot of energy (Wan et al., 2019).
556 *Microcystis* can be competitive at low P concentrations, because of its ability for rapid P uptake and

557 storage (Wan et al., 2019). However, neither species was really blooming during our study, with
558 density lower than 10,000 cells mL⁻¹.

559 Allelopathic effects of macrophytes on phytoplankton could partly explain the changes in assemblages
560 and the limiting the dominance of cyanobacteria in the habitat M. Floating-leaf macrophytes such as
561 water lilies produce hydrolysable polyphenols, the algaecide activity of which is not yet proven (Gross
562 2003). Macrophytes produce phenolic compounds, involved in the defence against herbivores, and
563 *Nymphaea alba* and *Nuphar lutea* are among the biggest producers of these substances (Smolders et
564 al. 2000). However, to our knowledge, the inhibitory effect on growth for these water lilies has only
565 been proven on *Lemna minor* (Elakovich and Wooten, 1991).

566 An effect of the large size of the lake can be pointed out. The phytoplankton species-lake area
567 relationship is debated since a long time, due to multiple co-factors controlling phytoplankton richness
568 (Borics et al., 2021). The large lake effect (LLE) predicts a decrease of diversity in large lakes,
569 because of the habitat homogenization by wind in pelagic area (Várbíró et al., 2017). Shallow lakes,
570 especially those with large fetch like here (5 km²), are exposed to strong horizontal mixing by wind,
571 which homogenizes water masses and suspended communities. However, the maintenance of
572 macrophytes on one third of the lake surface played a key role in maintaining habitats. A recent study
573 comparing shallow lakes with and without water lilies showed differences in biogeochemistry and
574 microbial assemblages in lakes with more than 10% of the surface covered by water lilies (DeWolf et
575 al., 2022).

576 Water may have been isolated below the water lilies located on the wind-protected west side of the
577 lake, especially for the westernmost stations. The small-scale spatial heterogeneity of physical and
578 chemical parameters generated by macrophytes, coupled with biotic interactions, may have promote a
579 higher diversity in the lake, in particular of *Euglenophyceae* (Várbíró et al., 2017), counteracting the
580 mass effect and species sorting (Leibold et al., 2004; Yang et al., 2018). When horizontal mixing of
581 water masses is low, environmental filters and biotic interactions (competition, predator-prey
582 relations) predominate in the structuring of local communities. The higher conductivity and the
583 establishment of a horizontal gradient attest to a low horizontal mixing in this lake in summer. Spatial
584 heterogeneity in phytoplankton assemblages has already been demonstrated in reservoirs from

585 upstream turbulent and nutrient-rich areas to the downstream stable pelagic area (Bortolini et al.,
586 2017; Yang et al., 2018).

587 The connected habitats may support a set of metacommunities, ie a set of local communities linked by
588 dispersal of multiple potentially interacting species (Leibold et al., 2004). Metacommunities have
589 already been demonstrated within a shallow lake for bacterioplankton (Wu et al., 2007) and for
590 zooplankton (Cottenie & De Meester, 2003). To go further, it would be interesting to couple the sink-
591 source dynamic of C,N,P with the metacommunity approach in the broader meta-ecosystem concept
592 (Loreau et al., 2003), using landscape ecology tools. However, a major lever remains with the lack of
593 knowledge of the aquatic 'landscape' and local residence times of water, in particular how water
594 masses flow within a lake partially colonized by macrophytes and how the underwater shape
595 (submerged versus rooted floating-leaf macrophytes) impacts these water flows and thus dispersion.

596

597 **Conclusion**

598 We showed that floating-leaf macrophytes in shallow lakes act as submerged macrophytes in
599 structuring habitats and phytoplankton assemblages, with increase of small mixotrophic flagellates
600 that better exploit the small-scale heterogeneous environment. Macrophytes promote locally the
601 microbial loop and heterotrophic processes. Mixotrophy is very little considered in the carbon cycle
602 and little is known about the flows associated with these organisms (Beisner et al., 2019). Next step
603 would be to directly measure grazing and photosynthetic performances of mixotrophs at small scale in
604 natural macrophytes habitat (Beisner et al., 2019). While macrophytes are important for aquatic
605 biodiversity in ponds, their degradation leads to the homogenization of the biota and contribute to the
606 loss in freshwater biodiversity. If floating-leaf decline, the lake will shift in summer cyanobacteria
607 blooms and lose at least one third of its phytoplankton diversity. The conservation of macrophytes in
608 sufficient biomass is essential for the maintenance of habitats and diversity in shallow lakes, even in
609 turbid eutrophic lakes.

610

611 **Data Availability Statement**

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

613

614 **Declarations**

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618

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624

625 **Author Contributions**

626 AP obtained the funding. S.Ma, AP, S.L. G.B and J-M.G. performed the field sampling. ML and S.Mo
627 performed phytoplankton identification and counting. S.Ma and S.L. performed zooplankton
628 identification and counting. AP performed the data analyses and statistics. AP and CP wrote the
629 manuscript, with substantial contributions from all authors.

630

631 **Conflicts of Interest**

632 The authors declare no conflict of interest.

633

634

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891 **FIGURE CAPTIONS**

892 **Figure 1.** Map of Grand-Lieu Lake with location of stations and aquatic vegetation.

893 **Figure 2.** Boxplots of physical, chemical and biological parameters depending on habitats (the M
894 habitat in green and the OW habitat in blue). Kruskal-Wallis tests are shown (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$). The boxplots show the distribution of each parameter, with five summary
895 statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to the inter-
896 quartile range *ie* the distance between the first and third quartiles). Data outside the interval of
897 1.5*IQR (outliers) are plotted individually.

899 **Figure 3.** Time series of macrophytes cover (%), water temperature, pH, dissolved oxygen
900 concentration (%), phosphates concentration and Chlorophyll a concentration. Means \pm standard error
901 are shown. Mean values are averaged from vertical profiles performed in the macrophytes habitats (9
902 stations) and in the open-water stations (5 stations). Green colored area indicates the period with
903 macrophytes in the lake.

904 **Figure 4.** NMDS plot based on Bray-Curtis similarity analysis performed on the relative abundances
905 of phytoplankton taxa. The red dotted ellipse indicates the August samples. The result of the ANOSIM
906 test between habitats is shown.

907 **Figure 5.** Venn-diagrams on presence – absence of phytoplankton taxa in macrophytes (M) and open
908 water (OW) habitats in July and August.

909 **Figure 6.** Boxplots of richness and diversity indices depending on habitats (the M habitat in green and
910 the OW habitat in blue). Kruskal-Wallis tests are shown, while letters indicate the significativity of
911 the post-hoc Dunn's test ($a \neq b \neq c$). The boxplots show the distribution of each parameter, with five
912 summary statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to
913 the inter-quartile range *ie* the distance between the first and third quartiles). Data outside the interval
914 of 1.5*IQR (outliers) are plotted individually.

915 **Figure 7.** pCCA performed in July and August 2018 (effect of month removed, representing 9.8% of
916 variance), linking taxa abundances with environmental parameters in grey. Samples were then
917 grouped by habitat and mean position of phytoplankton classes are shown. Permutation test was
918 significant ($p=0.001$ based on 999 permutations). 61% of total variance explained by environmental
919 parameters. Importance of variables based on classification and regression tree on samples coordinates
920 of the pCCA are shown in table 4.

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923 **Figure S1.** Boxplots of species abundances depending on habitats (the M habitat in green and the OW
924 habitat in blue). Kruskal-Wallis tests are shown, with asterisks showing p values (***: $p<0.001$; **:
925 $p<0.01$; *: $p<0.05$). The boxplots show the distribution of each parameter, with five summary
926 statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to the inter-
927 quartile range *ie* the distance between the first and third quartiles). Data outside the interval of
928 $1.5*IQR$ (outliers) are plotted individually.

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932 **Tables:**

933 Table 1: Limnological characteristics of Lake Grand-Lieu.

Characteristic	Units	Mean value ± standard deviation (min -
		max)
Location	-	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 – 3000)
Affluents	-	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 Chla L ⁻¹	128.7 ± 83 (5.8 - 415.6)

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936 Table 2: taxa contributing to the first axis of the pRDA linking taxa with macrophyte cover as
 937 explanatory factor, with (a) preference for the M habitat (negative correlation with the first axis) and
 938 (b) preference for the OW habitat (positive correlation with the first axis). The effect of sampling
 939 month, which explained 14.5% of taxa abundance, has been removed. Species scores are shown as
 940 well as their dominance: « - » means less than 0.2% of mean frequency, « + » means between 0.2%
 941 and 1%, « ++ », between 1% and 10% and « +++ » means >10%. The abundance (mean ± standard
 942 deviation) for each habitat is shown.

943

944 (a) preference for the M habitat (negative correlation with axis 1):

Classes	taxa	RDA1	dominance	M	OW
	<i>Actinastrum hantzschii</i>	-0.21	++	7594 ± 4124	1279 ± 431
	<i>Crucigenia tetrapedia</i>	-0.24	++	4898 ± 1095	537 ± 291
	<i>C. crucifera</i>	-0.10	+	1118 ± 406	193 ± 97
	<i>Crucigeniella rectangularis</i>	-0.03	-	88 ± 59	0 ± 0
	<i>Didymogenes palatina</i>	-0.04	-	215 ± 136	0 ± 0
	<i>Diplochloris raphidioides</i>	-0.09	+	2301 ± 1335	71 ± 54
	<i>Golenkinia radiata</i>	-0.08	+	1631 ± 594	369 ± 171
	<i>Kirchneriella microscopica</i>	-0.03	-	191 ± 142	163 ± 163
	<i>Micractinium pusillum</i>	-0.17	++	2581 ± 1037	581 ± 304
	<i>Monoraphidium komarkovae</i>	-0.06	-	281 ± 88	80 ± 53
Chlorophyceae	<i>M. arcuatum</i>	-0.05	-	383 ± 107	114 ± 53
	<i>Nephrochlamys willeana</i>	-0.03	-	101 ± 73	0 ± 0
	<i>Pediastrum duplex</i>	-0.06	+	462 ± 204	194 ± 137
	<i>Scenedesmus gr. Armati</i>	-0.28	++	10564 ± 3132	4771 ± 1178
	<i>Scenedesmus gr. Abundantes/ Spinosi</i>	-0.04	-	375 ± 130	245 ± 104
	<i>Schroederia setigera</i>	-0.04	-	140 ± 60	18 ± 18
	<i>Selenodictyon brasiliense</i>	-0.04	-	196 ± 171	0 ± 0
	<i>Siderocelis ornata</i>	-0.04	-	94 ± 30	0 ± 0
	<i>Tetraedron triangulare</i>	-0.03	-	314 ± 59	209 ± 39
	<i>Tetrastrum staurogeniaeforme</i>	-0.04	-	281 ± 101	180 ± 114
	<i>T. punctatum</i>	-0.03	-	85 ± 45	73 ± 73
	<i>Treubaria triappendiculata</i>	-0.03	-	324 ± 100	165 ± 61
	<i>Aphanocapsa sp.</i>	-0.14	++	9348 ± 3982	4675 ± 3470
Cyanobacteria	<i>Cuspidothrix issatschenkoi</i>	-0.06	++	6519 ± 1781	5065 ± 1360
	<i>Cyanogranis ferruginea</i>	-0.09	+	1945 ± 812	123 ± 123
	<i>Cyanogranis irregularis</i>	-0.03	-	166 ± 160	0 ± 0

	<i>Cylindrospermopsis raciborskii</i>	-0.08	++	7101 ± 2697	5709 ± 2012
	<i>Jaaginema</i>	-0.04	+	1260 ± 824	0 ± 0
	<i>Microcystis aeruginosa</i>	-0.08	+	1471 ± 1424	0 ± 0
	<i>Microcystis flos-aquae</i>	-0.04	+	1226 ± 827	0 ± 0
	<i>Tychonema sequanum</i>	-0.04	-	163 ± 157	0 ± 0
Chrysophyceae	<i>Mallomonas</i>	-0.04	-	68 ± 24	18 ± 14
	<i>Synura</i>	-0.03	-	167 ± 159	0 ± 0
Cryptophyceae	<i>Cryptomonas</i>	-0.07	+	766 ± 131	573 ± 180
	<i>Plagioselmis nannoplanctica</i>	-0.03	-	272 ± 89	253 ± 117
	<i>Aulacoseira ambigua</i>	-0.09	+	1055 ± 326	290 ± 138
	<i>Aulacoseira granulata</i>	-0.05	+	728 ± 157	424 ± 146
	<i>Aulacoseira granulata var. angustissima</i>	-0.04	-	435 ± 108	430 ± 80
Diatomophyceae	<i>Centriques (d = 8-15 µm)</i>	-0.14	+	2426 ± 510	1304 ± 522
	<i>Cyclotella meneghiniana</i>	-0.04	+	841 ± 376	451 ± 152
	<i>Praestephanos triporus</i>	-0.14	+	4704 ± 2224	246 ± 173
	<i>Thalassiosira duostra</i>	-0.10	+	1730 ± 640	62 ± 44
	<i>Cryptoglana pigra</i>	-0.03	-	53 ± 23	14 ± 14
	<i>Euglena</i>	-0.03	-	94 ± 32	12 ± 12
Euglenophyceae	<i>Phacus</i>	-0.06	-	145 ± 58	0 ± 0
	<i>Trachelomonas volvocina</i>	-0.06	-	195 ± 66	0 ± 0
	<i>Trachelomonas hispida</i>	-0.03	-	75 ± 27	20 ± 20
Ulothricophyceae	<i>Gloeotila contorta</i>	-0.04	-	570 ± 298	0 ± 0
Xanthophyceae	<i>Centritractus belonophorus</i>	-0.03	-	74 ± 24	12 ± 12
Zygophyceae	<i>Closterium</i>	-0.04	-	102 ± 43	4 ± 4

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948 **(b) preference for the OW habitat (positive correlation with axis 1):**

Classes	taxa	RDA1	dominance	M	OW
Chlorophyceae	<i>Coelastrum reticulatum</i>	0.05	-	1018 ± 0	82 ± 0
	<i>Dichotomococcus curvatus</i>	0.07	-	3858 ± 0	178 ± 0
	<i>Dictyosphaerium pulchellum</i>	0.03	-	3396 ± 0	0 ± 0
	<i>Diplochloris decussata</i>	0.03	-	1925 ± 0	806 ± 0
	<i>D. decussata</i>	0.11	+	13020 ± 1	1346 ± 1
	<i>Pediastrum boryanum</i>	0.06	-	1605 ± 0	71 ± 0
	<i>Scenedesmus gr. Scenedesmus sensu stricto</i>	0.03	-	585 ± 0	64 ± 0
Cyanobacteria	<i>Aphanizomenon flos-aquae</i>	0.07	-	3677 ± 0	237 ± 1
	<i>Aphanocapsa elegans</i>	0.09	++	40161 ± 4	6656 ± 4
	<i>Aphanocapsa nubila</i>	0.11	+	46585 ± 0	670 ± 2
	<i>Aphanothece smithii</i>	0.07	++	61511 ± 1	3303 ± 1
	<i>Chroococcus minutus</i>	0.03	-	3677 ± 0	0 ± 0
	<i>Chroococcus microscopicus</i>	0.34	++	186821 ± 2	2940 ± 5
	<i>Coelosphaerium kuetzingianum</i>	0.04	-	4902 ± 0	0 ± 0
	<i>Coelosphaerium minutissimum</i>	0.17	++	91543 ± 3	4515 ± 6
	<i>Dolichospermum compactum</i>	0.09	-	10260 ± 0	518 ± 1
	<i>Dolichospermum flos-aquae</i>	0.09	+++	103522 ± 19	7261 ± 23
	<i>Merismopedia warmingiana</i>	0.03	-	3040 ± 0	0 ± 0
	<i>Merismopedia punctata</i>	0.15	++	14082 ± 2	1640 ± 3
	<i>Merismopedia tenuissima</i>	0.46	+++	263167 ± 14	20355 ± 24
	<i>Pannus planus</i>	0.10	++	48102 ± 1	3962 ± 1
	<i>Planktothrix agardhii</i>	0.20	++	21350 ± 2	2235 ± 4
	<i>Pseudanabaena catenata</i>	0.05	-	8943 ± 0	0 ± 0
	<i>Pseudanabaena</i>	0.06	+	12941 ± 0	618 ± 1
<i>Romeria leopoliensis</i>	0.07	-	2474 ± 0	197 ± 0	
Chrysophyceae					
Cryptophyceae					
Diatomophyceae	<i>Staurosira venter</i>	0.03	-	1228 ± 0	114 ± 0
Euglenophyceae					
Ulothricophyceae					
Xanthophyceae					
Zygothryxaceae					

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953 Table 3: Indicator values found for the indicator species, with detailed probabilities of specificity and

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fidelity to the habitat.

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	specificity prob.	fidelity prob.	stat	p.value	
group : M habitat					
<i>Crucigenia tetrapedia</i>	0.90	0.93	0.92	0.00	***
<i>Thalassiosira duostra</i>	0.97	0.73	0.84	0.04	*
<i>Micractinium pusillum</i>	0.82	0.87	0.84	0.03	*
<i>Trachelomonas volvocina</i>	1.00	0.67	0.82	0.01	*
<i>Phacus sp.</i>	1.00	0.60	0.78	0.01	*
<i>Siderocelis ornata</i>	1.00	0.60	0.78	0.01	*
<i>Euglena sp.</i>	0.88	0.53	0.69	0.05	*
group : OW habitat					
<i>Merismopedia punctata</i>	0.79	1.00	0.89	0.00	***
<i>Nitzschia fruticosa</i>	0.83	0.89	0.86	0.00	**
<i>Pediastrum boryanum</i>	0.94	0.78	0.85	0.00	**
<i>Chroococcus microscopicus</i>	0.93	0.78	0.85	0.01	**
<i>Dichotomococcus curvatus</i>	0.89	0.78	0.83	0.02	*
<i>Staurisira venter</i>	0.77	0.89	0.83	0.02	*
<i>Romeria leopoliensis</i>	0.87	0.78	0.82	0.01	**
<i>Coelosphaerium minutissimum</i>	0.86	0.78	0.82	0.02	*
<i>Planktothrix agardhii</i>	0.81	0.78	0.79	0.03	*
<i>Scenedesmus gr. scenedesmus</i>	0.84	0.56	0.69	0.02	*

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961 **Table 4:** result of the pCCA, with the correlations of the environmental and biological parameters to

962 the axes, their significance tested by ANOVA and their importance calculated by classification and

963 regression tree (CART) analysis.

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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
SiO2	0.75	-0.37	1	0.117	1.47	0.026	2.45
PO4	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 chla	0.55	0.12	1	0.120	1.51	0.012	
NH4	-0.23	-0.45	1	0.157	1.97	0.004	
copepods abundance	-0.25	0.18	1	0.071	0.89	0.641	
cladocerans abundance	-0.20	0.24	1	0.065	0.81	0.827	
residuals			11	0.875			

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Figure 1: Map of Grand-Lieu Lake with location of stations and aquatic vegetation.

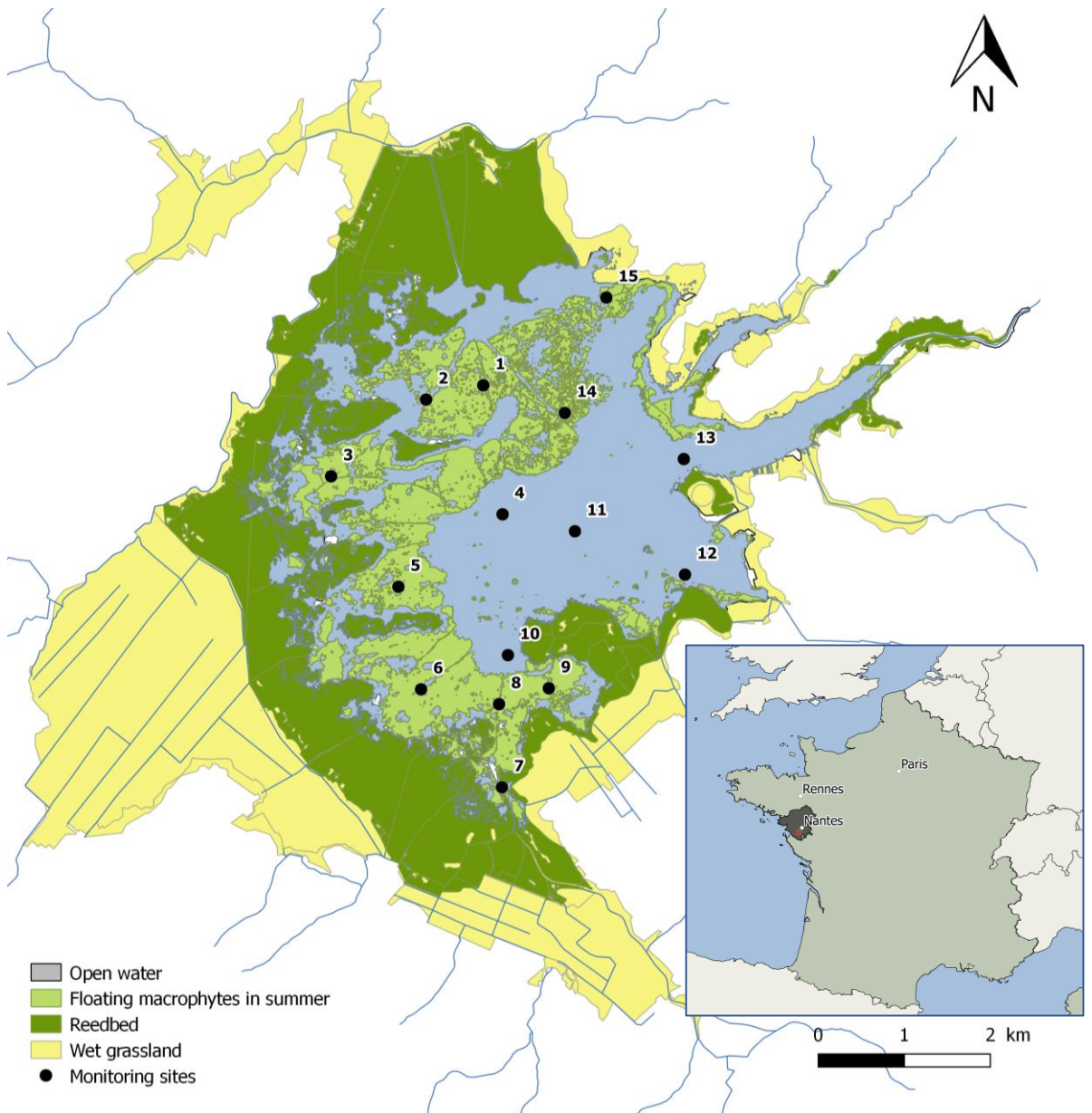


Figure 2: Boxplots of physical, chemical and biological parameters depending on habitats (the M habitat in green and the OW habitat in blue). Kruskal-Wallis tests are shown, with asterics show p value (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to the inter-quartile range ie the distance between the first and third quartiles). Data outside the interval of $1.5 * IQR$ (outliers) are plotted individually.

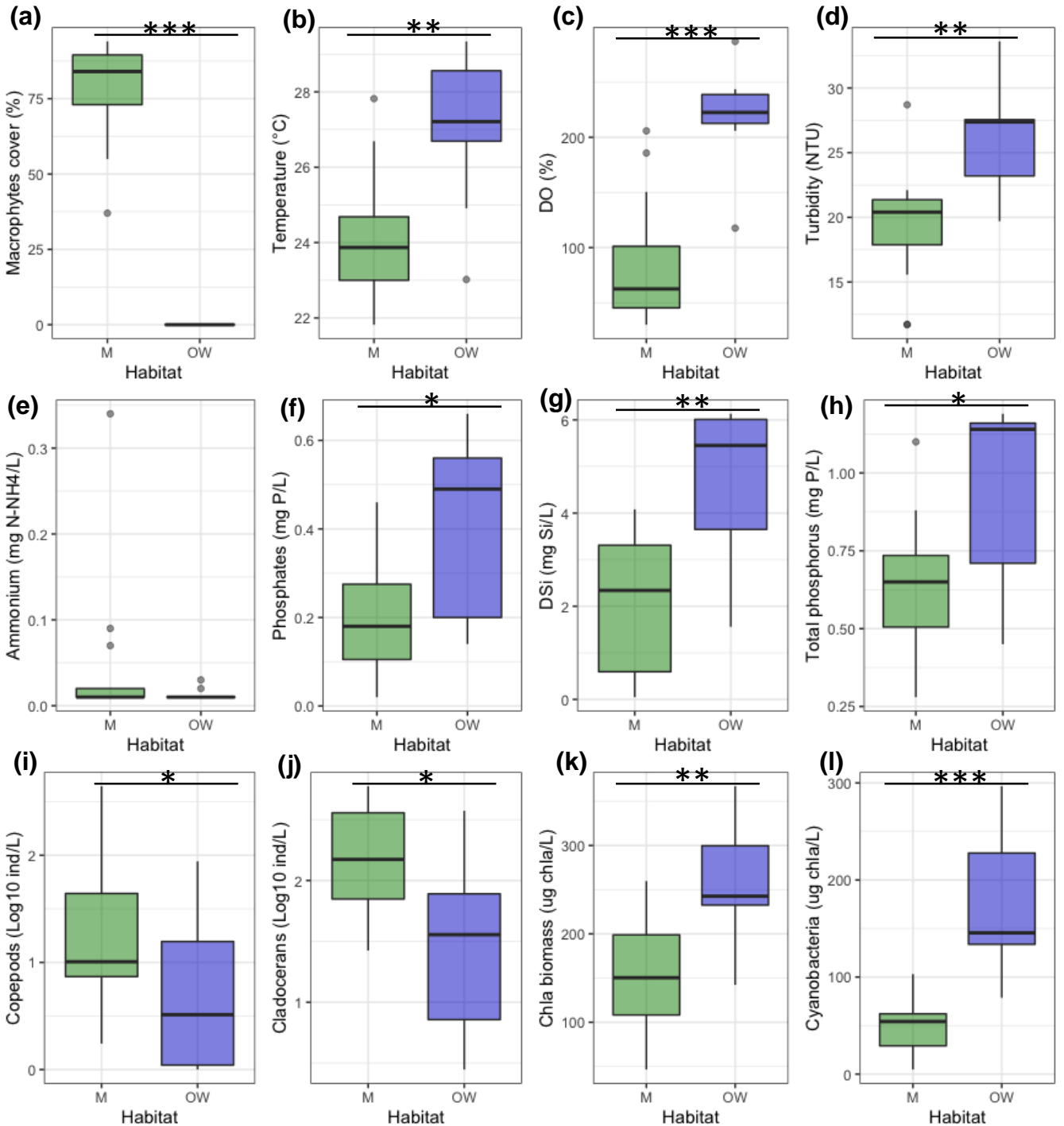


Figure 3: Time series of macrophytes cover (%), water temperature, pH, dissolved oxygen concentration (%), phosphates concentration and Chlorophyll a concentration. Means \pm standard error are shown. Mean values are averaged from vertical profiles performed in the macrophytes habitats (9 stations) and in the open-water stations (5 stations). Green colored area indicates the period with macrophytes in the lake.

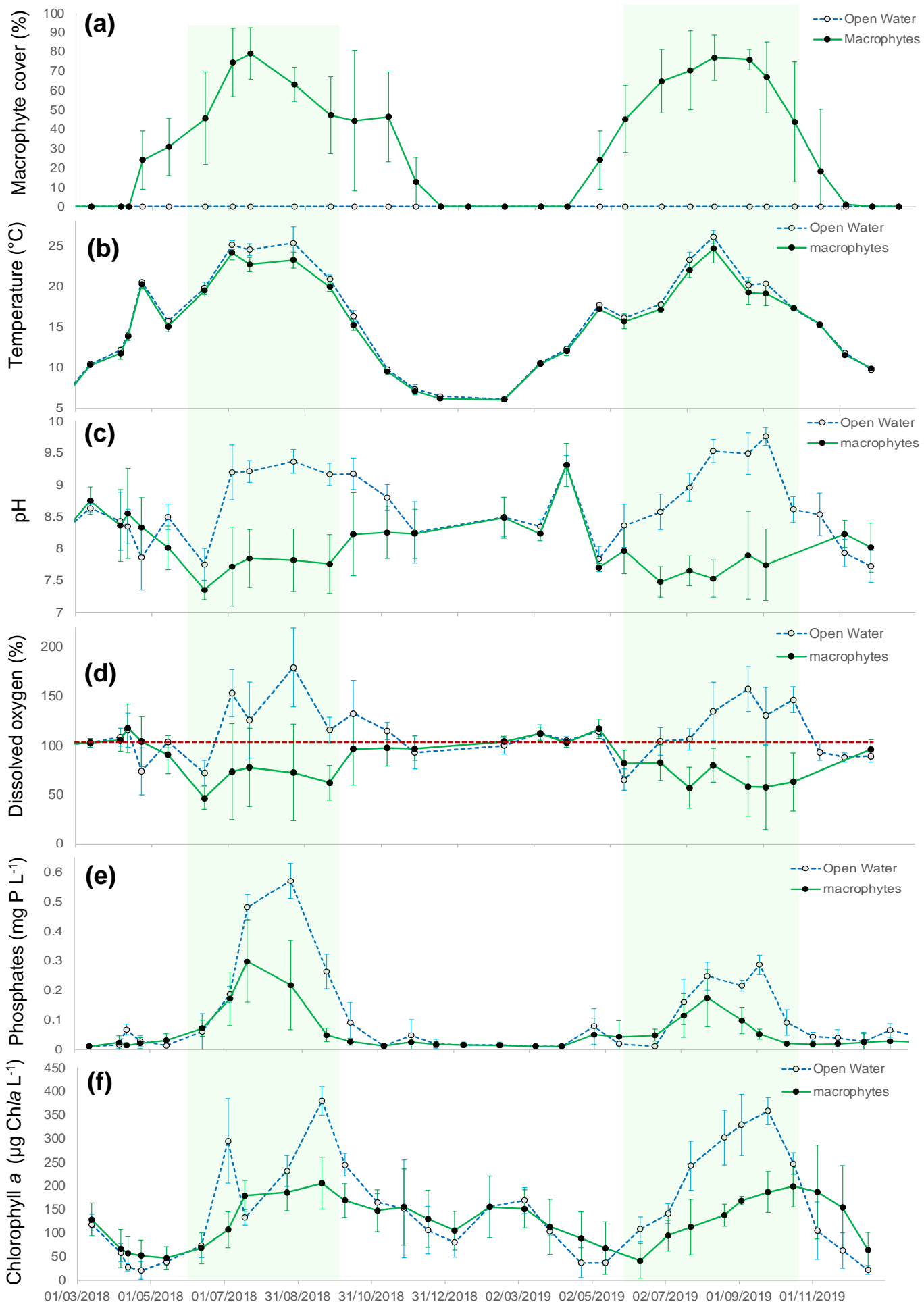


Figure 4: NMDS plot based on Bray-Curtis similarity analysis performed on the relative abundances of phytoplankton taxa. The red dotted ellipse indicates the August samples. The result of the ANOSIM test between habitats is shown.

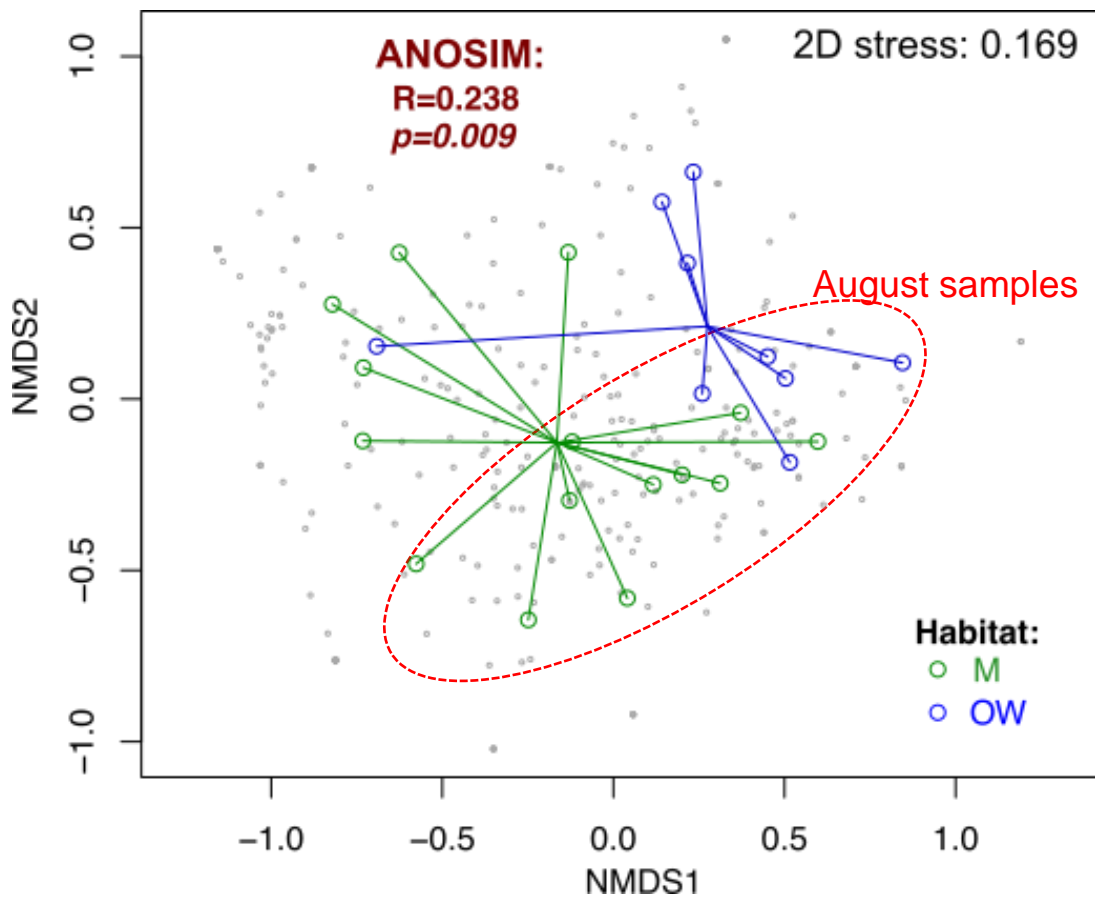


Figure 5 : Venn-diagrams on presence – absence of phytoplankton taxa in macrophytes (M) and open water (OW) habitats in July and August.

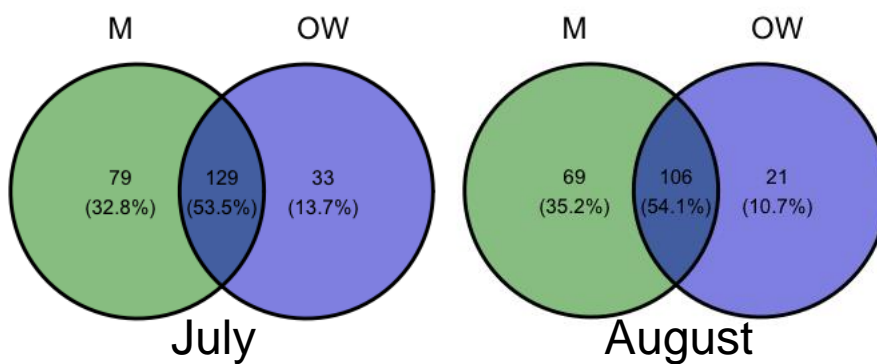


Figure 6: Boxplots of richness and diversity indices depending on habitats (the M habitat in green and the OW habitat in blue). Kruskal-Wallis tests are shown, while letters indicates the significance of the post-hoc dunn test ($a \neq b \neq c$). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to the inter-quartile range ie the distance between the first and third quartiles). Data outside the interval of $1.5 * IQR$ (outliers) are plotted individually.

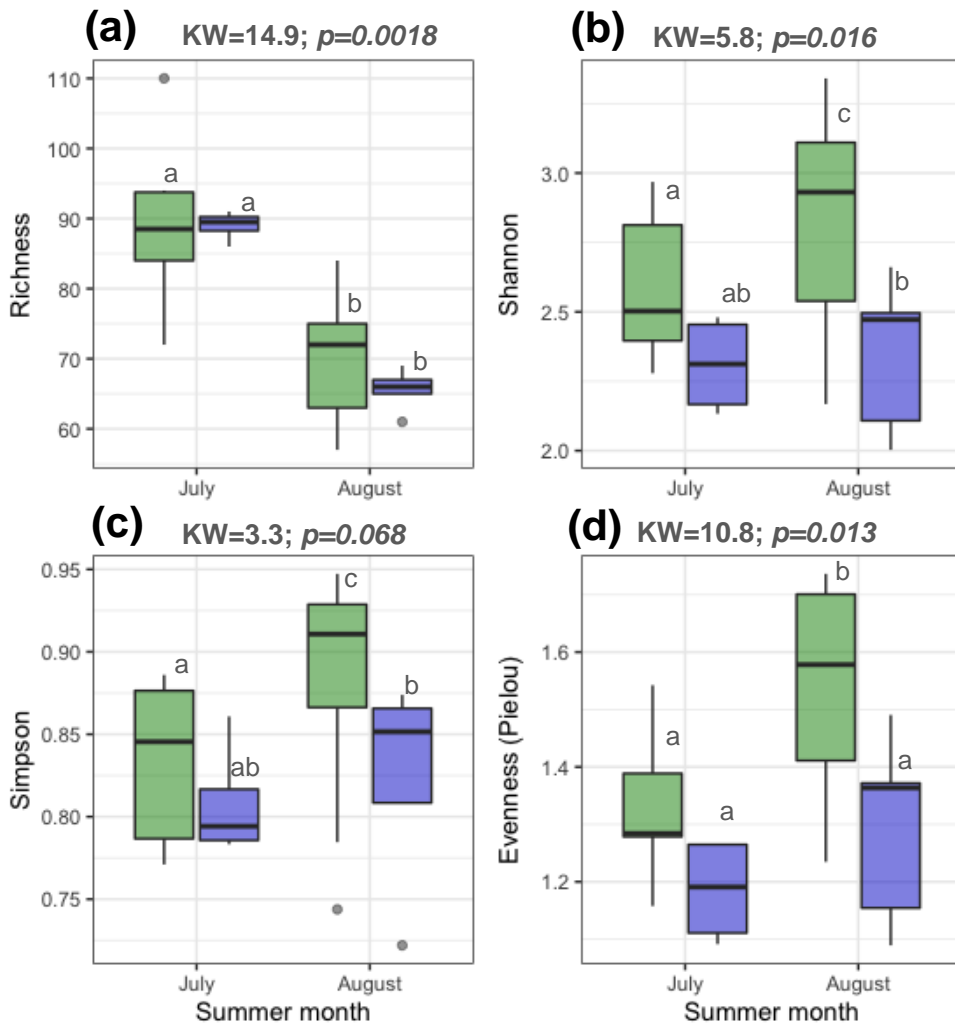


Figure 7: pCCA performed in July and August 2018 (effect of month removed, representing 9.8% of variance), linking taxa abundances with environmental parameters in grey. Samples were then grouped by habitat and mean position of phytoplankton classes are shown. Permutation test was significant ($p=0.001$ based on 999 permutations). 61% of total variance explained by environmental parameters. Importance of variables based on classification and regression tree on samples coordinates of the pCCA are shown in table 4.

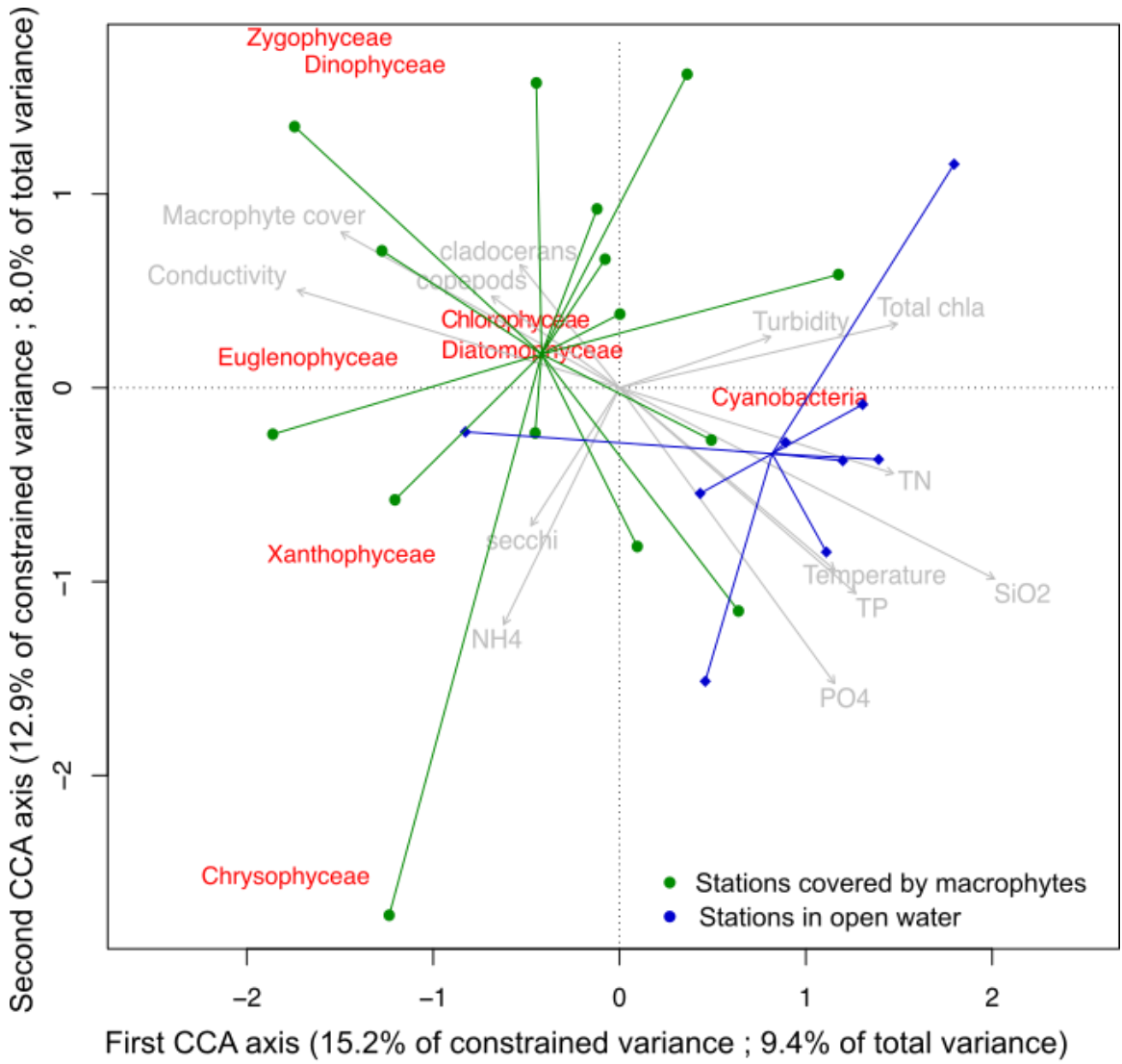


Figure S1: Boxplots of species abundances depending on habitats (the M habitat in green and the OW habitat in blue). Kruskal-Wallis tests are shown, with asterics show p value (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median $\pm 1.5 * IQR$ (corresponding to the inter-quartile range ie the distance between the first and third quartiles). Data outside the interval of $1.5 * IQR$ (outliers) are plotted individually.

