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

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Summary

Aquatic macrophytes in shallow lakes control habitats through local turbulence, water transparency, nutrients and oxygen concentrations. As engineer species, they structure these ecosystems, increasing the biodiversity. While many studies focused on submerged macrophyte, 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, but with greater shading.

In this study, we showed how macrophytes structure phytoplankton assemblages and allow the coexistence of different assemblages in the same shallow lake. During summer 2018, we characterized the phytoplankton assemblages in 9 stations covered by water lilies and 6 stations in open water, in a large shallow water lake. The lake is 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 taxa of phytoplankton from almost all classes were in higher abundance in stations covered by macrophytes, while cyanobacteria showed a higher biomass and richness in open water. Unicellular mixotrophic flagellates predominated in macrophytes habitat, with all representatives of the classes Euglenophyceae and Cryptophyceae.

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 & 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, which isolates water surface from nutrient-replete sediment. Shallow lakes on the contrary have a low thermal inertia, making them more sensitive to meteorological extremes (rain, wind, and heatwaves). Bottom nutrients released during 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, so that their bottom can be colonized by submerged macrophytes (Scheffer, 2004). These macrophytes control bottom light climate by limiting the resuspension of sediment (James et al., 2004), but they can be outcompeted by phytoplankton and the associated turbidity. Aquatic macrophytes in ponds and shallow lakes are particularly affected by eutrophication (Sayer et al., 2010; Labat et al., 2020). Submerged macrophytes disappear above a threshold of turbidity and strongly declined 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, the macrophytes dominance (clear state) and the phytoplankton dominance (turbid state) (Scheffer & Jeppesen, 2007). Macrophytes are thus in competition with phytoplankton for light and nutrient 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 achieve,

they can control biogeochemical cycles through absorption of nutrients and transitory storage (Carpenter & Lodge, 1986; Teubner et al., 2022). Macrophytes act as a sink of nutrients during the growing season, but they are a net source of dissolved organic carbon, with the release of 1-10% of their photosynthetically-fixed carbon released 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 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 steep vertical gradient and potentially anoxia (Carpenter & Lodge, 1986).

Secondly, they provide support and habitats for a 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 this periphyton (Masclaux et al., 2012).

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

However all these above effects and interactions were mainly described for submerged macrophytes and 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 the increase in turbidity (de Nie et al., 1987; Bornette &

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

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

Two main habitats are expected in lakes partly colonized by rooted macrophytes with surface leaves: an open water area which is turbulent, turbid, and warm, and macrophytes zone which is cooler, poor in light and nutrients but rich in dissolved organic carbon. In the latter habitat, mixing is controlled by night cooling and convection. These habitats should select for different strategies (resource acquisition, grazer avoidance) reflected in phytoplankton assemblages. By decreasing light availability, shade-adapted species should be favored in macrophytes. Reduced turbulence favours 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 turbulent tolerant cyanobacteria. These strategies adopted by phytoplankton can be related to morphological and physiological

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

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

Methods

Lake parameters and sampling stations

The lake of Grand-Lieu, classified as a natural reserve forbidden to the public, 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 has a catchment area of 700km² from which it receives water from two main tributaries: the Boulogne and the Ognon rivers. It then evacuates its waters through the Acheneau channel into the Loire River. In summer, its surface is about 2500 hectares for a depth of less than 1 meter. The lake can be divided into several zones: open water zones (absence of macrophytes), floating-leaf macrophyte beds (*Nuphar lutea, Nymphae alba, Trapa natans, Nymphoides peltata*), wooded reedbeds (Caricaies, Phragmitaies, Saulaies, Aulnaies) and wet meadows (Paillisson & Marion, 2005). The lake experienced hypereutrophication since the 1970s, leading to many different policies regarding the management of its water levels. See Table 1 for limnological characteristics of the lake.

Sampling of Lake Grand-Lieu was conducted in July and August 2018. 15 monitoring stations were distributed over the lake including the mouths of the two tributaries of the lake (Fig. 1). Nine stations (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 habitat (OW). A few stations were not sampled in July, being difficult to access because of a water level lower than 30 cm. This monitoring is part of a larger survey of physico-chemical and biological parameters at the 15 stations from February 2018 to November 2019.

Macrophytes cover

To estimate the presence of macrophytes at each station, vegetation cover was estimated using a 1 m^2 wooden quadrat. The quadrat is thrown five times randomly around the boat. The percentages are then 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 the Secchi depth, measured on the shaded side of the boat. Light profiles could not be done in the lake, because of the height of the probe (approximately 50 cm for a maximal 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 water level was recorded every ten minutes at the mouth of the two tributaries (stations 7 and 13), with Solinst DIVER (LTC Levelogger® from Solinst®).

Water sampling

For water chemistry and plankton, particular attention must be paid in order not to resuspend the sediments, 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, it was chosen to gently sample the water at sub-surface (10-15 cm below the surface) using a 1 L Nalgene bottle, and to test the presence of vertical gradients by specific sampling and probe profiles. The bottle was rinsed three times with lake water prior to sampling and opening and closing is done below the water surface to prevent leaves or branches from contaminating the sample. This technique was already used to sample a shallow fluvial lake similar to the Grand-Lieu Lake (Cattaneo et al., 2013).

Nutrients 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 acid 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 long-term at -20°C, except for silica which remains stored at 4°C.

Total phosphorus (TP) and total nitrogen (TN) were measured colorimetrically after digestion with persulfate (Grasshoff, 1983), with a detection limit of 6 µg P L^{-1} and 50 µg N L^{-1}. Orthophosphate was analyzed by the ammonium molybdate method, according to USEPA protocol (USEPA 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^- were measured calorimetrically with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride, according to USEPA protocol (USEPA Method 365.2, 1993), with a detection limit of 50 µg N L^{-1}. Colorimetric measurements were performed with a Gallery Photometric Analyser Gallery Plus (Thermo Fisher).

Phytoplankton biomass and assemblages

Total phytoplankton biomass and cyanobacteria 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 the laboratory. The same day, these samples were fixed with Lugol's solution and stored at 4°C in the dark. Samples were identified and counted under an inverted microscope using identification keys (Komárek, 1983; Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b).

Zooplankton abundances

Zooplankton were sampled at each station for abundance and diversity for macro-zooplankton, with copepods and cladocerans. Surface water was sampled with a 2L bottle and filtered through a 60 µm zooplankton filter. 20 to 30 L were passed through a 60 µm net depending on zooplankton abundance and filter saturation. We used a 2 L bottle instead of a10 L bucket to prevent sediment resuspension. The sample was then collected on a 60 µm sieve and the zooplankton was anaesthetized by carbonated water (perrier type). The zooplankton was then transferred to a 50 mL tube with 80% ethanol. The samples were kept at 4°C until identification and counting under a microscope and binocular magnifying glass, based on identification keys (Dussart, 1967; Amoros, 1984; Bledzki & Rybak, 2016). Samples were counted in a Bogorov counting chamber, after subsampling if necessary, with a Hensen-Stempel pipette. Total abundance of cladocerans and of copepods are used here.

Statistical analysis

To test the effect of macrophytes on phytoplankton assemblage 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 Eveness diversity indices were calculated with *diversity* function of the ‘vegan’ library (Oksanen et al., 2013). To test for the effect of macrophytes on 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 species that are specific to macrophytes and open water habitats, Venn diagrams were made using the library 'ggvenn' (Yan 2021) in Rstudio, on presence – absence of phytoplankton taxa in July 2018 and August 2018.

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

Indicator species were highlighted with 'indicspecies' library (De Caceres et al., 2016) and the function *multipatt*, which allows determining 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, the probability that the sampled site belongs to the target group (here M or OW habitat) knowing that the species was observed (specificity of the species) and the probability of finding the species when sampling the target group (fidelity of the species) (Dufrêne & Legendre, 1997).

To prioritize environmental factors controlling 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 effect of month. The significance of the model has been tested through a permutation test, while environmental variables were tested individually with an ANOVA, which allowed us to remove the less significant parameters. A classification and regression tree (CART) analysis was then performed with the 'rpart' library, to identify environmental variables importance (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 depth,

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

Results

Effect of macrophytes on habitats

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

Thus, the OW stations were significantly warmer than the stations in the M habitat, 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 compared with the M habitat during summer (Fig. 3b). The pH measured in the middle of the day showed values above 9 in summer in the open water, while it remained below 8 in the M habitat (Fig. 3c). Dissolved oxygen, which was about 100% in winter, was closed to 50% in the M habitat in spring and summer, while very high DO concentration was observed in the OW habitat (Fig. 2c and 3d). The high pH coupled with high oxygen concentrations indicated high primary production in open water compared to the M habitat.

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

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

The concentration of chlorophyll *a* showed a seasonal pattern, with high summer concentrations in OW compared with the M habitat (Fig. 2k and 7F; KW= 9.07; p=0.003). Cyanobacteria biomass represented a large part of the phytoplankton biomass in open water (Fig. 2l; KW=14.8; p<0.001). Higher abundance of both copepods and cladocerans zooplankton groups was observed in the M habitat compared with OW (Fig. 2i, j; p<0.05).

Finally, during summer, OW habitat was characterized by higher temperature (+3-4°C), higher DO (at least factor 2), higher pH (at least +1), higher PO4 (at least factor 2) and TP (+50%), higher DSi (at least +2 mg Si/L), and lower abundances of zooplankton (at least factor 2), compared with stations in M covered by floating-leaf macrophytes. The higher photosynthetic activity resulted in higher biomass of cyanobacteria (factor 5).

Phytoplankton assemblages according to habitats

The phytoplankton assemblages at stations covered with floating-leaf macrophytes (habitat M) differed from assemblages at open-water stations (habitat OW), 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 assemblages in July (ANOSIM: R=0.254; p=0.006; Fig. 4).

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

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

Species richness in samples from the M habitat varied between 72 and 110 in July, and between 57 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 Zygophyceae. 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

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

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

Prioritizing environmental parameters in the control of phytoplankton assemblages

To link phytoplankton assemblages with environmental factors, physical and chemical parameters were used as explanatory parameters of taxa abundances, after removing the effect of the month (9.8% of total variance; Fig. 7). 61% of total variance in phytoplankton assemblages were explained by the environmental parameters (p=0.001 based on 999 permutations) independently of the month. The first axis, which represented 15.2% of constrained variance (9.4% of total variance), contrasted stations from the M habitat on the left from the OW stations on the right of the plot (Fig. 7). SiO2 concentration (r=0.75; p=0.026 based on permutation test by terms), water temperature (r=0.43; 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) and total chlorophyll *a* concentration (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),

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 fist 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 NH_4 L^{-1} and 2,574 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

forcing was particularly visible in that lake by Langmuir cells, which were 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 explained the difference of turbidity between the two habitats (Madsen et al., 2001). Light may thus have been limiting for phytoplankton growth in both habitats, because of shading by leaves in the M and of turbidity in the OW.

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

Phosphates (and total phosphorus) were significantly lower in the M habitat (factor 2). Several non-exclusive processes explain the difference between habitats: First, a difference in pH and redox potential between the two zones can lead to a different adsorption and chemosorption rates (Bostrom, 1982; Sondergaard et al., 2001). P bound to redox-sensitive iron compounds can be indeed quickly released to the water column, if redox changes (Mortimer, 1941; Bostrom, 1982). Secondly, the P in the pore water 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 rate to the water column, as for ammonium (Jiang et al., 2019). Fourthly, P storage in phytoplankton cells lasts a few days and P remained easily remobilized when cells die (high turnover). Macrophytes on the contrary store P for a few months and act as a net sink during their active growth (Carpenter & Lodge, 1986; Teubner et al., 2022). The M habitat acted as a P summer sink area with slower metabolism compared with the OW habitat (high production and regenerating rates).

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

No effect of plant could be observed on nitrates and ammonium. Nitrates in the lake were controlled by winter recharge and increased when river flows restart in fall, while the concentration remained below the detection threshold in summer. Nitrogen-fixing cyanobacteria with numerous heterocysts were observed throughout the summer, supporting nitrogen limitation at this time. Ammonium concentration 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 and consumption processes associated with bacteria and primary producers (Jiang et al., 2019).

Phytoplankton assemblages depending on habitats

We showed here that rooted floating-leaf macrophytes (habitat M), by modifying the physical and chemical parameters and biotic interactions, allowed the spatial coexistence of several phytoplankton assemblages within the lake. The assemblages of phytoplankton 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 effects of plants remained. Based on presence/absence data, three times more taxa were associated with the M habitat (one third of the total) compared with the OW habitat (10-13%). Consistently, the diversity of the phytoplankton was higher in the M habitat than in the OW habitat, while the total biomass of phytoplankton was about twice lower in M habitat than in OW. The presence of water lilies was unfavorable especially to cyanobacteria (such as those observed in high biomass in open water), and allowed the maintenance of rare species and a greater diversity in M habitat.

Most of taxa and some entire classes of phytoplankton showed higher abundances in stations in M compared with stations in OW, which was favorable to cyanobacteria. Phytoplankton assemblage in the M habitat were mostly unicellular flagellates, tolerant to low light, with many mixotroph (combining photosynthesis and ingestion of particulate organic matter) known to interact with organic matter (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 water lilies compared with open water. Three of the seven indicator species of the M habitat were mixotrophic *Euglenophyceae* (*Euglena sp., Phacus sp., Trachelomonas volvocina*), typical of organic ponds (Reynolds et al., 2002). *Chrysophyceae* can also be found in heterotrophic ponds according to the Reynolds functional classification and are known to be tolerant to low nutrients with potential use of mixotrophy (Reynolds et al., 2002; Padisak et al., 2009). *Cryptophyceae* are tolerant to low light conditions (Reynolds et al., 2002) and the main representative (*Cryptomonas sp*.) also has mixotroph ability (Princiotta et al., 2019). These three classes are therefore related to organic carbon and potential mixotrophic activity, which is advantageous in light limiting 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 of diatoms were also in higher abundance in the M habitat. *Aulacoseira granulata, A. ambigua, Cyclotella meneghiniana* and the M indicator species *Thalassiosira 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* (20 times more abundant in the M habitat 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

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

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

The structuring role of macrophytes by controlling environmental parameters

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

our study, water temperature was warmer in macrophytes, but the effects on DO, conductivity, TP and phosphates were the same than in our study, even if we are 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 have advantage in low turbulent condition, because they are adapted to better exploit small-scale heterogeneous environment 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 the dominance of a few ones (Cunha et al., 2012). Moreover, many of these flagellates are mixotroph. The subsidy of dissolved organic carbon derived from macrophytes and from the associated periphyton, coupled with the low light availability, promoted mixotrophic species in that habitat (Søndergaard & Moss, 1998). We thus highlighted a similar effect of water lilies on phytoplankton assemblages than 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 cyanobacteria growth (Paerl & Huisman, 2008; Paerl, 2017). The most dominant species was the cyanobacteria *Dolichospermum flos-aquae,* which formed huge colonies rolled up on itself with numerous heterocysts, confirming N summer 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* and *Dolichospermum*, which are among the most toxic genera, separated spatially between the OW and the M habitats. They are already known to co-occur spatially (Zhang et al., 2016), while they most often succeed each other in reservoirs (Soares et al., 2009; Wu et al., 2016). These studies showed that warmer temperature favors *Microcystis*, which should have been present in higher abundance in open water. However, these species have also different P requirement : *Dolichospermum* needs higher P level than *Microcystis*, because heterocysts' formation consume lot of energy (Wan et al., 2019). *Microcystis* can be competitive at low P concentrations, because of its ability for rapid P uptake and

storage (Wan et al., 2019). However, neither species was really blooming during our study, with density lower than 10,000 cells mL^{-1}.

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

An effect of the large size of the lake can be pointed out. The phytoplankton species-lake area relationship is debated since a long time, due to multiple co-factors controlling phytoplankton richness (Borics et al., 2021). The large lake effect (LLE) predicts a decrease of diversity in large lakes, because of the habitat homogenization by wind in pelagic area (Várbíró et al., 2017). Shallow lakes, especially those with large fetch like here (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 played a key role in maintaining habitats. A recent study comparing shallow lakes with and without water lilies showed differences in biogeochemistry and microbial assemblages in lakes with more than 10% of the 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 for the westernmost stations. The small-scale spatial heterogeneity of physical and chemical parameters generated by macrophytes, coupled with biotic interactions, may have promote a higher diversity in the lake, in particular of *Euglenophyceae* (Várbíró et al., 2017), counteracting 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 relations) 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 and nutrient-rich areas to the downstream stable pelagic area (Bortolini et al., 2017; Yang et al., 2018).

The connected habitats may support a set of metacommunities, ie a set of local communities linked by dispersal of multiple potentially interacting species (Leibold et al., 2004). Metacommunities have already been demonstrated within a shallow lake for bacterioplankton (Wu et al., 2007) and for zooplankton (Cottenie & De Meester, 2003). To go further, it would be interesting to couple the sink-source dynamic of C,N,P with the metacommunity approach in the broader meta-ecosystem concept (Loreau et al., 2003), using landscape ecology tools. However, a major lever remains with the lack of knowledge of 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 thus dispersion.

Conclusion

We showed that floating-leaf macrophytes in shallow lakes act as submerged macrophytes in structuring habitats and phytoplankton assemblages, with increase of small mixotrophic flagellates that better exploit the small-scale heterogeneous environment. Macrophytes promote locally the microbial loop and heterotrophic processes. Mixotrophy is very little considered in the carbon cycle and little is known about the flows associated with these organisms (Beisner et al., 2019). Next step would be to directly measure grazing and photosynthetic performances of mixotrophs at small scale in natural macrophytes habitat (Beisner et al., 2019). While macrophytes are important for aquatic biodiversity in ponds, their degradation leads to the homogenization of the biota and contribute to the loss in freshwater biodiversity. If floating-leaf decline, the lake will shift in summer cyanobacteria 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.

Data Availability Statement

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

Declarations

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Author Contributions

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

Conflicts of Interest

The authors declare no conflict of interest.

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FIGURE CAPTIONS

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). Kruskall-Wallis tests are shown (***: $p<0.001$; **: $p<0.01$; *: $p<0.05$). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 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 ± 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). Kruskall-Wallis tests are shown, while letters indicate the significativity of the post-hoc Dunn's 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 ± 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). Kruskall-Wallis tests are shown, with asterisks showing p values (***: $p<0.001$; **: $p<0.01$; *: $p<0.05$). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 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.

Tables:

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 $P\text{-}PO_4$ L^{-1}	0.079 ± 0.116 (0.01 - 0.66)
Nitrates	mg $N\text{-}NO_3$ 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)

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

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

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

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

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

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

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 : 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 : OW habitat					
Merismopedia punctata	0.79	**1.00**	0.89	0.00	***
Nitzschia fruticosa	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	*

Table 4: result of the pCCA, with the correlations of the environmental and biological parameters to the axes, their significance tested by ANOVA and their importance calculated by 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
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			

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

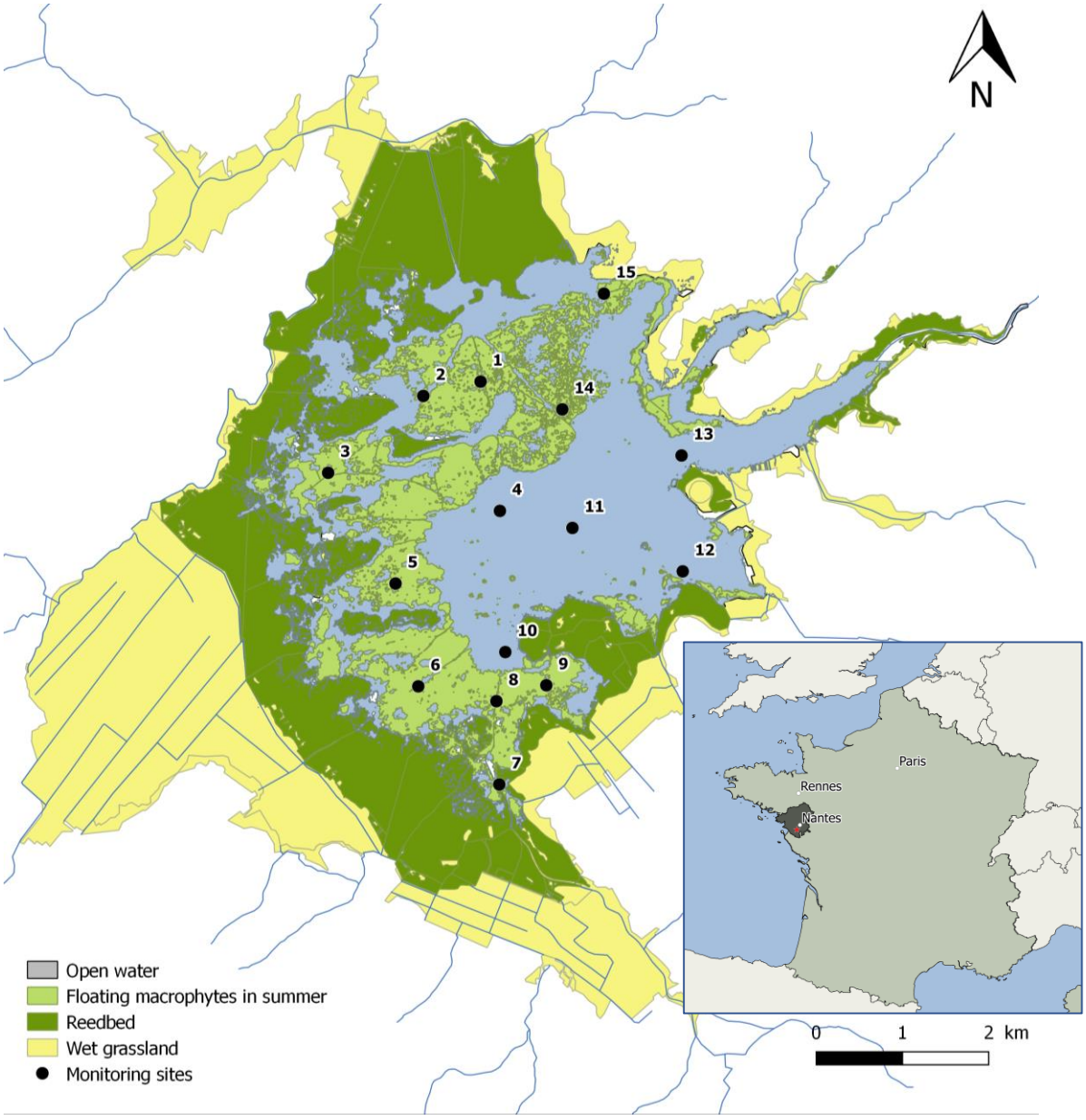

Figure 2: Boxplots of physical, chemical and biological parameters depending on habitats (the M habitat in green and the OW habitat in blue). Kruskall-Wallis tests are shown, with asterics show p value (***: p<0.001; **: p<0.01; *: p<0.05). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 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.

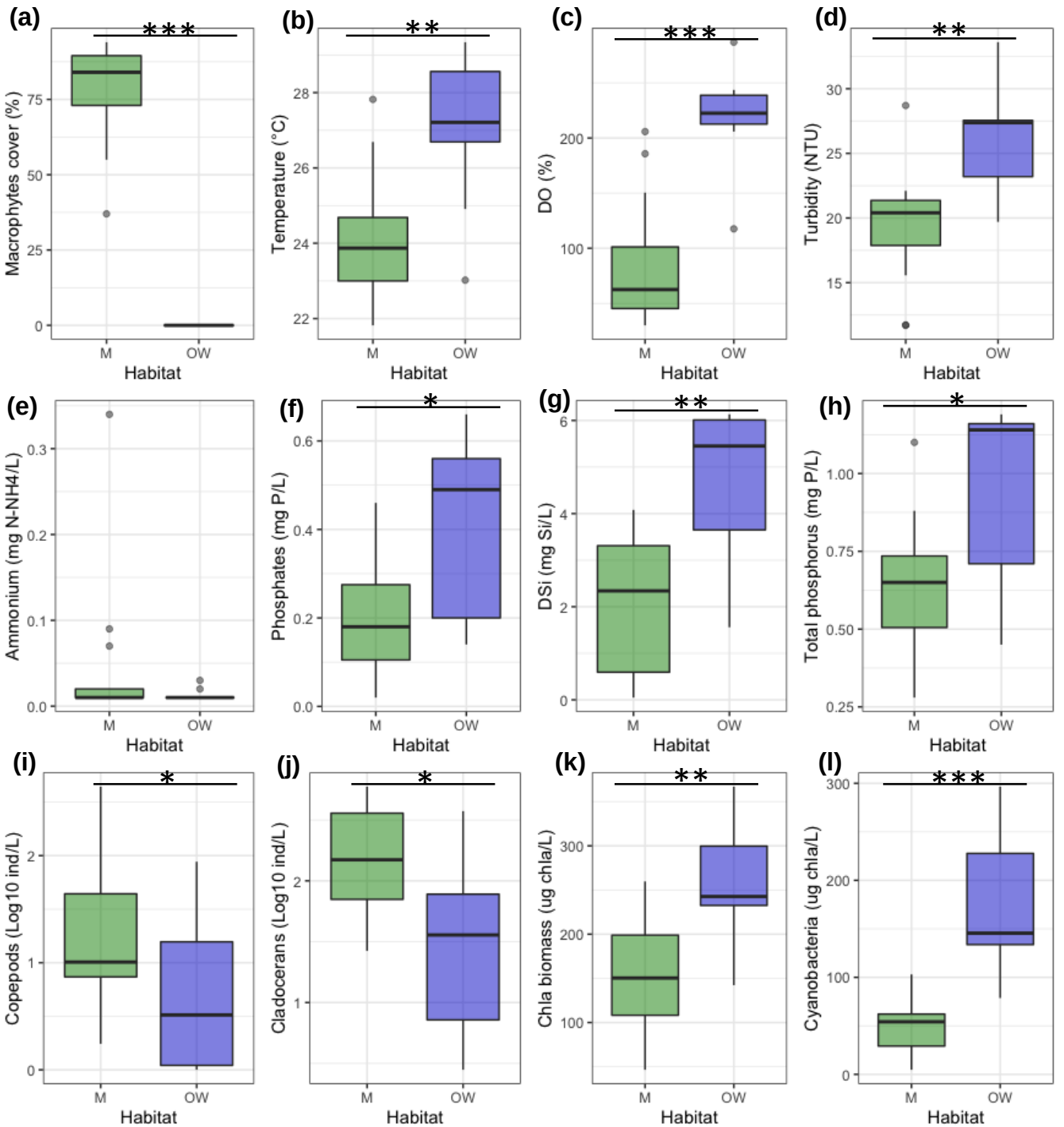

Figure 3: Time series of macrophytes cover (%), water temperature, pH, dissolved oxygen concentration (%), phosphates concentration and Chlorophyll *a* concentration. Means ± standard error are shown. Mean values are averaged from vertical profiles performed in the macrophytes habitats (9 stations) and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

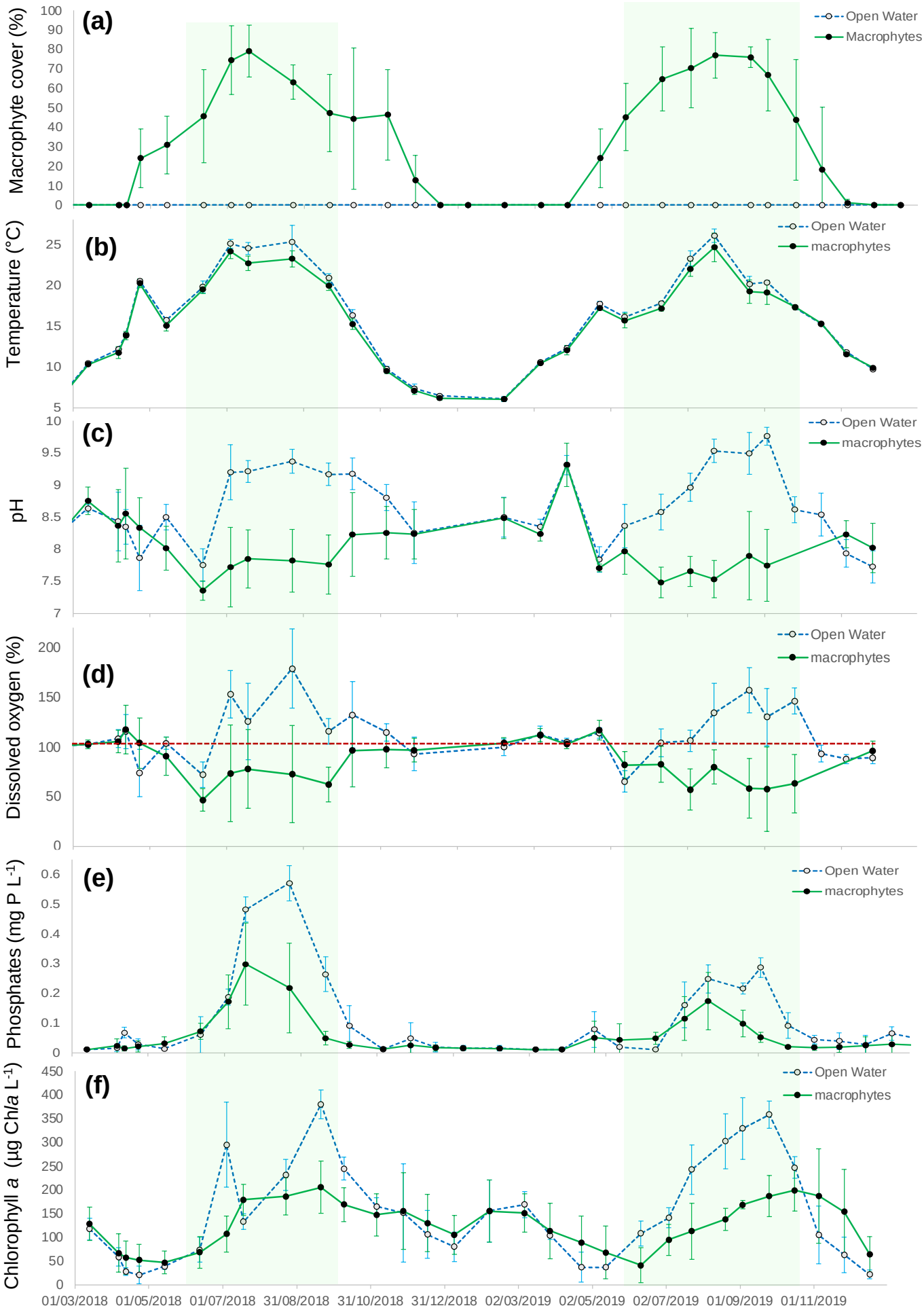

Figure 4: NMDS plot based on Bray-Curtis similarity analysis performed on the relative abundances of phytoplankton taxa. The red dotted ellispse indicates the August samples. The result of the ANOSIM test between habitats is shown.

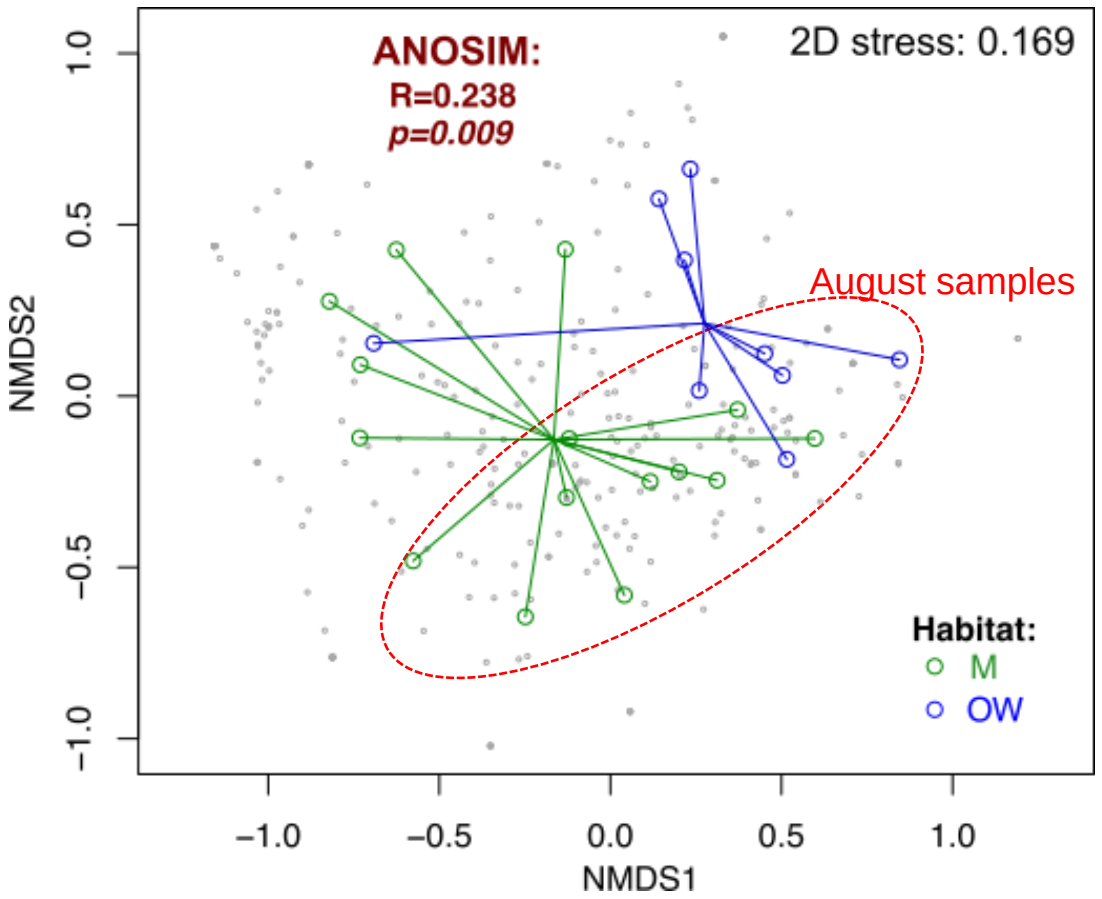

Figure 5 : Venn-diagrams on presence – absence of phytoplankton taxa in macrophytes (M) and open water (OW) habitats in July and August.

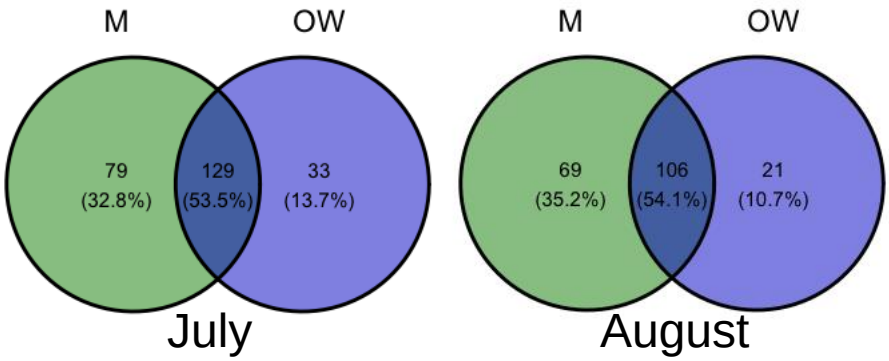

Figure 6: Boxplots of richness and diversity indices depending on habitats (the M habitat in green and the OW habitat in blue). Kruskall-Wallis tests are shown, while letters indicates the significativity of the post-hoc dunn test (a ≠ b ≠ c). The boxplots show the distribution of each parameter, with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

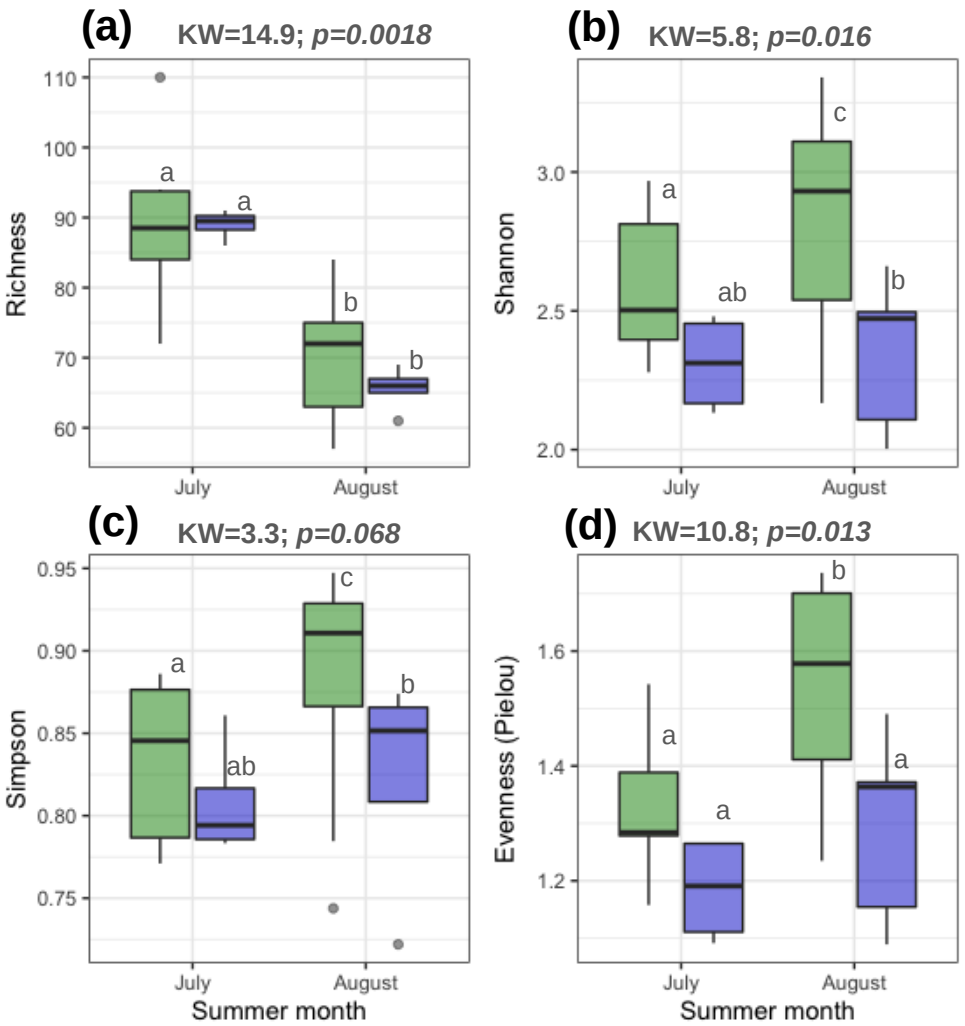

Figure 7: pCCA performed in July and August 2018 (effect of month removed, representing 9.8% of variance), linking taxa abundances with environmental parameters in grey. Samples were then grouped by habitat and mean position of phytoplankton classes are shown. Permutation test was significant (p=0.001 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.

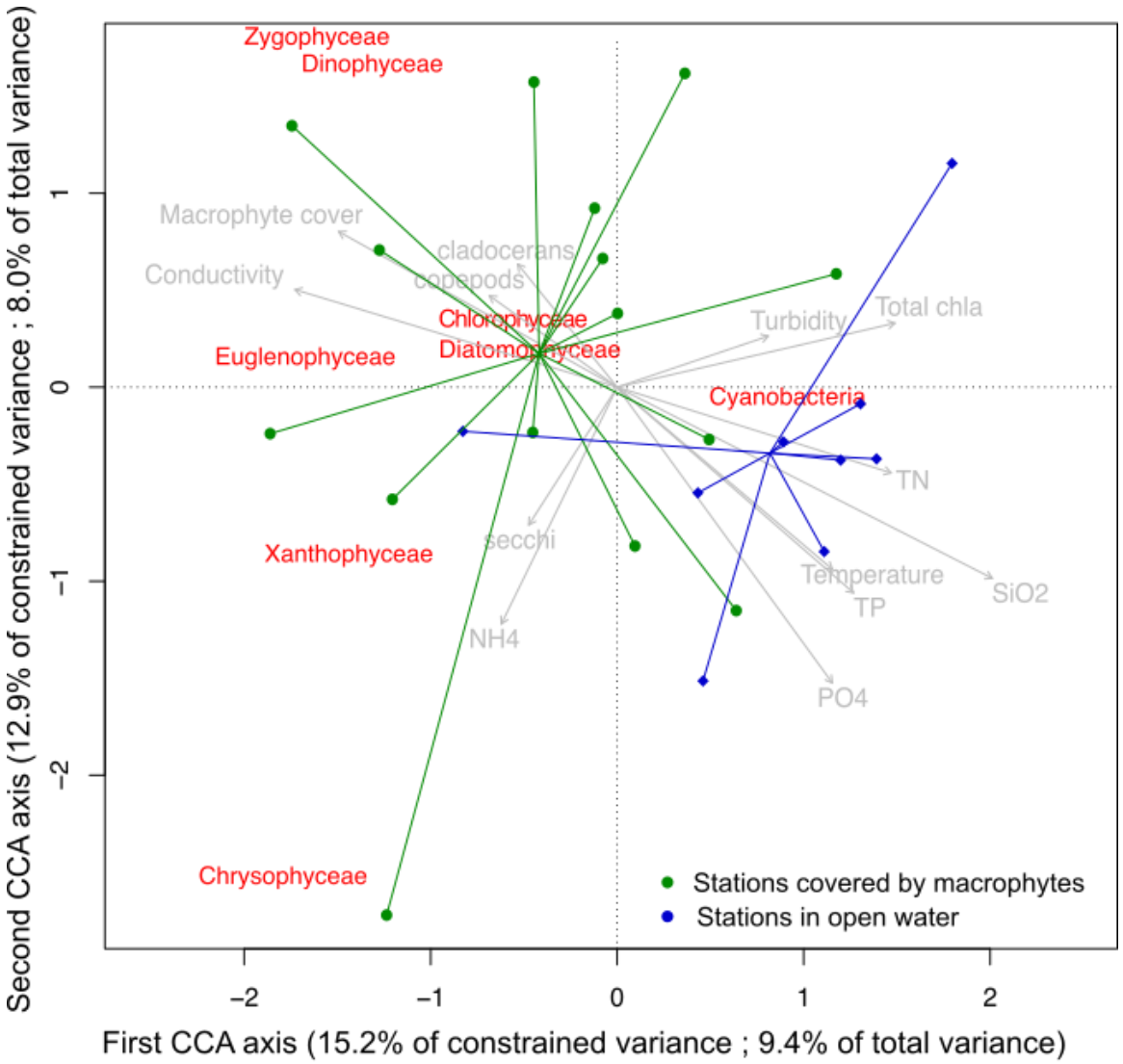

Figure S1: Boxplots of species abundances depending on habitats (the M habitat in green and the OW habitat in blue). Kruskall-Wallis tests are shown, with asterics show p value (***: p<0.001; **: p<0.01; *: p<0.05). The boxplots show the distribution of each parameter, with five summary statistics: the median, the first and third quartiles, the median ± 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.