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Dense macrophyte cover has significant structural and functional influence on planktonic microbial communities leading to bacterial success

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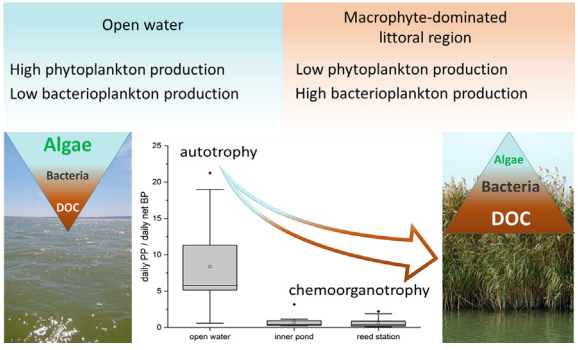
HIGHLIGHTS

- Dense macrophyte cover shifts planktonic processes toward chemoorganotrophy.
- High organic carbon content favours heterotrophic and photoheterotrophic bacteria.
- Phytoplankton in humic-rich areas had lower photosynthetic competence.
- Bacterial production was much higher as a result of dense macrophyte cover.

GRAPHICAL ABSTRACT



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ABSTRACT

We intend to assess how macrophyte cover affects planktonic microbial communities by changing the physical and chemical environment, and how macrophyte-derived DOC affects the balance between autotrophy and heterotrophy/chemoorganotrophy in a shallow lake. The structure and production of phytoplankton and bacterioplankton in the open water of a large shallow lake and in the littoral zone were compared at two sampling stations with different macrophyte cover. According to the obtained results, uncoupling between bacterioplankton and phytoplankton was observed due to the high content of organic carbon of emergent macrophyte origin. While phytoplankton were regulated by TSS, bacterioplankton (in both heterotrophic and photoheterotrophic forms) were determined by dissolved organic carbon. As a result of these processes, the littoral and pelagic zones in the lake are completely separated from each other. In open water the autotrophic processes dominated, but at the sampling stations inside the reed belt, the metabolic processes shifted in the direction of chemoorganotrophy. Our results suggest that increase of macrophyte cover in shallow water bodies will increase the significance of microbe-based carbon pathways and weakens the efficiency of carbon transport from primary producers to higher trophic levels through the planktonic food chain.

1. Introduction

Despite their small size (0.5–10 μm), bacteria form a significant biomass component in both marine and freshwater ecosystems and play a particularly important role in biogeochemical processes (Azam, 1998; Kirchman, 2000; Williams and del Giorgio, 2005; Williams and Ducklow, 2019). In the oceans and deep lakes they not only contribute to production, but also utilize half of the planktonic primary production. Of all free-living freshwater organisms, they are the most abundant, reaching as much as 10^8 cells ml^{-1}. Being the main heterotrophic microorganisms in aquatic habitats, lake bacterial communities form a heterogeneous group in terms of both taxon diversity and metabolic activities. They also represent a key element in the microbial food web by recycling dissolved organic carbon (DOC) released by algae to higher tropic levels via the microbial loop (Fenchel, 2008; Williams and Ducklow, 2019).

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In contrast to their remarkable ecological significance, there is a considerable lack of information on the detailed function of bacterioplankton in numerous aquatic habitats. This lack of knowledge is especially apparent in the case of shallow lakes, where the production of phytoplankton (eukaryotic algae and cyanobacteria, representing the majority of autotrophic microorganisms) and heterotrophic bacterioplankton (heterotrophic prokaryotes) are often uncoupled due to external DOC loading (del Giorgio and Cole, 1998; Jansson et al., 2000; Zingel et al., 2006; Munawar et al., 2018; Kim et al., 2020). An increase in DOC concentration can result in a decrease in the phytoplankton/heterotrophic bacterioplankton production ratio, which causes a shift from a photoautotrophic toward a chemoorganotrophic (net heterotrophic) system and consequently changes the structure and productivity of aquatic communities (Jansson et al., 2000; Boulion, 2020). Increasing plant cover, therefore, may also play a role in a shift toward heterotrophic processes as a result of macrophyte-originated DOC increase, but we have only a little information on its significance. Nevertheless, a comparison of the pelagic and littoral regions of shallow lakes offers a good opportunity to study the effect of plant cover on planktonic processes, as the latter is often characterized by macrophyte dominance.

While in pelagic ecosystems the relationship between planktonic primary production and bacterioplankton production is well-known and clearly demonstrated (Cole et al., 1988; Kopylov et al., 2018; Kopylov et al., 2020), the relative importance of the heterotrophic bacterioplankton in macrophyte-dominated littoral systems is largely unknown and the few papers published on the subject so far have yielded contrasting results. It is generally assumed that heterotrophic bacterial production in the littoral zone can be much (up to 120 times) higher than in the pelagic zone (Reitner et al., 1999; Likens, 2010 and references therein). Mesocosm experiments have demonstrated that macrophyte-derived detritus supply resulted in higher bacterial production than that originating from algae (Wehr et al., 1999). In contrast, lower bacterial production was observed in the littoral zone of some subtropical shallow lakes than in the pelagic zone despite higher DOC concentrations (They et al., 2010). These differences may be due to the fact that an increase in macrophyte dominance not only means an increase in DOC supply (and changing DOC pool composition), but also inevitably modifies many environmental factors, primarily in terms of light environment and nutrient availability.

Aquatic macrophytes could prevent wind-induced mixing of the water column and thus suppress sediment resuspension (Pujol et al., 2010). Suspended solids (TSS) affect microbial communities in several ways. For instance, both organic particles and bacterial cells can attach to inorganic particles and thus remain suspended in the water column. Therefore, being suspended, benthic bacteria attached to sediment particles have better access to utilizable organic matter, nutrients, and oxygen (Eiler et al., 2003). Another important fact is that TSS strongly restricts the availability of PAR in shallow lakes (Kirk, 1994; Scheffer, 1998) and can significantly reduce zooplankton grazing (G.-Tóth et al., 1986; Hart, 1988; Kirk, 1991; Pfandl and Boenigk, 2006; Jin et al., 2022). Thus, water inside macrophyte bands can exhibit higher transparency and increased top-down control than in open-water areas. Besides this, emergent macrophytes shade the water column and their decomposition results in a considerable amount of humic substances (V.-Balogh et al., 2006), which also have a light reducing effect in water (Kirk, 1994) and serve as a food source for bacteria (Kritzberg et al., 2005; Logue et al., 2016). Macrophytes can also affect nutrient availability in the water column, influence algal growth through allelopathic exudates, and harbour high densities of phytoplankton eating cladocerans (Zingel et al., 2006; Ferreira et al., 2018).

Thus, the aim of the present work was to explore how aquatic vegetation affects the phytoplankton-heterotrophic bacterioplankton relationship and the intensity of photoautotrophic and chemoorganotrophic processes directly (through increased DOC load) or indirectly (by changing the physical environment). Our hypothesis was that a dense macrophyte cover is beneficial for heterotrophic bacteria, shifting planktonic metabolic processes toward chemoorganotrophy. Our study site was a large shallow lake with significant (>50%) macrophyte (*Phragmites australis*) cover, where bacterioplankton relie not only on planktonic algae but also on organic matter production of macrophytes (Reitner et al., 1999). We intend to compare the abundance and role of photoautotrophic, photoheterotrophic and heterotrophic microorganisms in the pelagic and littoral zone of the lake and to identify those factors that determine their prevalence.

2. Material and methods

2.1. Study site and sampling

Lake Neusiedl/Lake Fertő (Austria-Hungary) is a wind-exposed, extremely shallow steppe lake (~1.3 m), straddling the Austrian-Hungarian border (Löffler, 1979; Dokulil and Herzig, 2009). The lake has an elongated shape with a maximum length of 36 km and a maximum width of 12 km (Löffler, 1979). More than half (ca. 55%, i.e. 171 km^2) of the surface area is covered by emergent vegetation, mainly reed (*Phragmites australis* (Cav.) Trin. ex Steud). In Hungary, reed occupy a significantly larger area along the shoreline (up to a width of >5 km), than in Austria (up to 2 km). There are numerous reedless brown-water ponds (inner lakes) of variable size within the reed belt, which are intersected with artificial canals connecting the inner ponds with the open water areas. Although the depth of the water column is fairly uniform throughout the open lake, submerged macrophyte growth is restricted to a narrow zone following the outer contour of the reed fringe (Löffler, 1979; Wolfram et al., 2014). The central lake zone is free of macrophytes with the exception of a few scattered *Potamogeton* rings (Löffler, 1979). Floating macrophytes are found only in the open water patches of (inland ponds and canals) the reed belt (Löffler, 1979; Wolfram et al., 2014).

Lake Neusiedl is a natron lake with sodium bicarbonate ($NaHCO_3$) dominance, electric conductivity of about 2200 μS cm^{-1} and pH around 9 (Wolfram et al., 2014). As a result of wind-induced sediment resuspension, the open water of the lake is characterized by high inorganic turbidity and usually low Secchi-disk transparency (Löffler, 1979). Within the reed cover, the water is not turbid due to less exposure to wind and brown in colour due to humic substances (Löffler, 1979; Wolfram et al., 2014). Three sampling stations were chosen representing different water bodies within the lake: an open water sampling station (47.73459 N 16.71941 E) and two sampling stations within the reed belt: a large inner pond with submerged macrophyte cover (Kis-Herlakni; 47.68460 N 16.70272 E; dominated by *Najas marina* with 590 g m^{-2} maximum dry biomass, see details in Szabó-Tugyi and Tóth, 2020) and a small open water area (reed station) within the reed belt (*P. australis* with 1030 g m^{-2} maximum dry biomass, see details in Szabó-Tugyi and Tóth, 2020), which is accessible from the artificial canal system (reed station; 47.65432 N 16.72517 E). The selection of the sites was based on a comprehensive study of the physical, chemical and biological variables of more than 100 sampling station (Boros et al., 2020). The location of the sampling stations is shown on Fig. S1.

Water samples were taken with a 2 m long tube sampler as described in Szuróczki et al. (2020) on a monthly basis between October 2015 and September 2016. Water temperature, depth, Secchi-disk transparency, pH, electrical conductivity (EC) and dissolved oxygen (DO) concentration were determined on-site at the time of sampling. The latter three were determined using a multiline P8211 portable meter (WTW, Weilheim, Germany). Photosynthetically active radiation (PAR, 400–700 nm) in the water column was measured at 0.25 m increments with a Li-COR underwater radiometer, using a flat (2π) quantum sensor. The spectral composition of the underwater light was also determined between 380 and 900 nm in the water column at 0.25 m increments with an Ocean Optics HR2000 + radiometer equipped with a 5 m long optical fiber and a cosine corrector.

2.2. Analytical methods

The concentration of total suspended solid (TSS) was measured gravimetrically according to Eaton et al. (1995). Briefly, water samples were filtered onto pre-dried and pre-weighed glass fiber filters (MN GF-5) and the filters were re-weighted after drying at 105 °C for 24 h. To determine the coloured dissolved organic matter (CDOM), samples were filtered (cellulose

acetate filter with a pore size of 0.45 μm) and buffered with borate buffer. After that, the absorbance of the samples was measured using a Hitachi U-2900 spectrophotometer at 440 nm and 750 nm. CDOM was expressed in Pt (platina) units (mg Pt L^{-1}) and calculated according to Cuthbert and del Giorgio (1992). The DOC concentration was measured using a High-TOC analyser (Elementar Analysensysteme, Hanau, Germany) according to V.-Balogh et al. (2009). Nitrogen forms (ammonium, nitrate, and urea) (Newell et al., 1967; Elliott and Porter, 1971; Mackereth et al., 1978), total phosphorus (TP) (Menzel and Corwin, 1965) and total soluble phosphorus (SRP) (Gales et al., 1966) were determined. Chlorophyll a concentration was measured in freshly collected samples. Briefly, 200–1000 mL of lake water was concentrated on a MN GF-5 filter, from which pigments were extracted using a hot methanol method. Chlorophyll *a* concentration was determined spectrophotometrically according to Iwamura et al. (1970).

2.3. Microscopy

Bacteriochlorophyll-containing cells were identified according to Jiao et al. (2007). Briefly, fresh water samples were concentrated onto a 0.2 μm white polycarbonate filter (Millipore) and excited at 350–550 nm using an Olympus BX51 epifluorescence microscope. Bacteriochlorophyll-containing bacteria were identified at 1000× magnification using a 780 nm long-pass filter and a monochrome CCD camera (Olympus XM10). To detect picophytoplankton cells, the same field was photographed using violet-blue (400–440 nm) and green (520–550 nm) excitation at 1000× magnification according to MacIsaac and Stockner (1993) using a colour CCD camera (Olympus DP71). Bacteria were detected by 4′,6-diamidino-2-phenylindolestaining, DAPI staining (Porter and Feig, 1980). Briefly, formaldehyde-fixed water samples were filtered onto 0.2 μm pore-sized black polycarbonate filters (Millipore) and the cells were identified using 350–450 nm excitation by a colour CCD camera (Olympus DP71). Bacterial abundance and biovolume were determined using Yet Another Bacterial Biovolume Algorithm (YABBA) software (Zeder et al., 2011). The total biomass was calculated based on cell volume and abundance assuming specific gravity of 1.0 g cm^{-3}.

Nano- and microphytoplankton samples were fixed with Lugol's solution, their abundance and composition were determined using an inverted microscope (Utermöhl, 1958). The cell volume of the observed taxa was calculated using the formulas of Hillebrand et al. (1999). Biomass (wet weight) was estimated from the total biovolume (calculated on the basis of cell volume and abundance values) of the fractions assuming a specific gravity of 1.0 g cm^{-3}.

2.4. Photosynthesis measurement and primary production estimation

Photosynthesis of the phytoplankton community was measured using the ^{14}C-technique (Steemann-Nielsen, 1952) according to Somogyi et al. (2016). Briefly, after the addition of $NaH^{14}CO_3$, fresh water samples were incubated in a photosynthetron in 3 parallel (Üveges et al., 2011). At the end of the incubation, the samples were filtered and - after removing the remaining ^{14}C - the radioactivity of the filters were measured with a TRI-CARB 2100TR liquid scintillation counter. Photosynthetic carbon assimilation was calculated based on the ratio of ^{14}C uptake and DIC availability using an isotope discrimination factor of 1.05 (Steemann-Nielsen, 1952). The obtained results were normalized to chlorophyll *a*. Photosynthesis-irradiance (PI) curves were fitted using the model of Eilers and Peeters (1988) with the OriginPro 2020 software.

Primary production was estimated using the PI curves and the light intensity profile of the water column at 0.1 m increments up to the bottom, taking into account the current water depth. Underwater light intensity was calculated for each 0.1 m of the water column using the vertical light attenuation coefficient determined by field measurements and the hourly average ambient light intensity provided by the Hungarian Meteorological Service assuming that PAR accounts for 47% of global radiation (Wetzel and Likens, 2000).

The Fv/Fm parameter was measured in the field with a portable fluorometer (AquaPen AP-100, P.S.I. Ltd. Brno, Czech Republic) that featured a measuring chamber with a 10 mm diameter cuvette after 10 min of dark adaptation. Fv/Fm provides an evaluation of the maximum photochemical efficiency of PSII reaction centers of darkness-adapted algal cells.

2.5. Bacterial production

Bacterial production (BP) was determined at each sampling station using radiolabelled leucine L-[4,5-3H] incorporation method (Kirchman et al., 1985; Gasol, 1999) as described by Szabó-Tugyi and Tóth (2020). Briefly, a mixed leucine solution (radiolabeled leucine:normal leucine = 1:10) were added to fresh and TCA-killed water samples at a saturating concentration of 200 nM 3H-leucine and the samples were incubated for 30 min at in situ water temperature under dark conditions. Unincorporated radiolabelled leucine was separated by centrifugation and radioactivity of the samples were measured using a TRI-CARB 2100TR liquid scintillation counter. Radioisotope-uptake calculations were made as explained in Kirchman et al. (1985) and bacterial biomass production was calculated according to Simon and Azam (1989). Bacterial growth efficiency was calculated based on the bacterial production measurements using the model proposed by del Giorgio and Cole (1998):

$$BGE = (0.037 + 0.54 \times BP)/(1,8 + BP)$$

2.6. Statistical analysis

Relationships between environmental parameters and biological variables were studied using Spearman's rank correlation with OriginPro 2021 (OriginLab, Northampton, MA, http://www.originlab.com). The relationship was considered to be significant at $p < 0.01$. Site comparison was performed by Kruskal-Wallis ANOVA test with OriginPro 9 (OriginLab, Northampton, MA) software with significance levels of $p < 0.05$. In order to detect any possible relationship between the parameters, Principal Component Analysis (PCA) were carried out in OriginPro 2021 by involved multiply variables with significance levels of $p < 0.05$. To do this, all the involved variables were cubic root-transformed, in order to normalise their distribution, and unit variance standardization was used.

3. Results

3.1. Influence of aquatic macrophytes on physical and chemical environment

The physical and chemical environment differed significantly between the open water and the two sampling stations inside the reed belt (Table 1 and Table S1), except for the ionic composition and electrical conductivity. Aquatic macrophytes had a significant effect on sediment resuspension, significantly reducing TSS content inside the reed belt (Fig. 1). As a result, reed areas have greater transparency and a better underwater light climate compared to open water, which is well shown by the vertical attenuation coefficient of the photosynthetically active radiation (Table 1). A significant redshift of the underwater light climate was observed both in open water and in reed areas. In open water, this was primarily due to the high TSS content, while in reed belt it was due to the high CDOM concentration (Fig. 1). Therefore, K_d in the reed areas was higher in the blue region but lower in the red and infrared regions than in open water (Fig. S2). Thus, the water inside the reed belt was relatively enriched in red and infrared radiation.

Another important effect of aquatic plants was the increase of TOC and DOC in the reed areas (Table 1 and Table S1). Consistent with this, pH and dissolved oxygen concentrations were also lower. The amounts of TDP, NH_4-N and Urea-N were higher in the reed areas than in the open water (Table 1 and Table S1).

3.2. Changes of planktonic microbial communities

Chlorophyll *a* concentration and phytoplankton biomass were significantly higher in the open water than in the reed areas (Fig. 2, Table 2,

Table 1
Physical and chemical environmental parameters at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016. Abbreviations: T (temperature) DO (dissolved oxygen), EC (electrical conductivity), TSS (total suspended solids), CDOM (coloured dissolved organic matter), K_d (vertical attenuation coefficient), DOC (dissolved organic carbon), TOC (total organic carbon), TP (total phosphorous), TDP (total dissolved phosphorous), SRP (soluble reactive phosphorous), TN (total nitrogen), TDN (total dissolved nitrogen), NH_4-N (ammonia-nitrogen), NO_3-N (nitrate-nitrogen), urea-N (urea-nitrogen).

	Open water		Inner pond		Reed station	
	Min-Max	Average	Min-Max	Average	Min-Max	Average
Depth (m)	1–1.5	1.3	0.6–1.2	1	1.1–1.5	1.3
T (°C)	5–23	13	5–23	14	5–23	14
DO (mg L^{-1})	8.1–12	9.6	5.3–7.9	6.2	0.3–11.2	6.3
DO (%)	94–100	97	55–97	66	4–110	66
pH	8.8–9.7	9.4	8.4–9.2	8.9	8.0–9.2	8.8
EC (μS cm^{-1})	1700–2040	1900	1700–2500	2100	1400–2450	1900
TSS (mg L^{-1})	16–120	50	1–7	3	1–14	5
CDOM (mg Pt L^{-1})	14–39	23	121–175	142	87–260	158
$K_{d\ PAR}$ (m^{-1})	2.3–9.7	4.7	2.1–3.6	2.7	2.0–5.8	3.0
$K_{d\ 870–890\ nm}$ (m^{-1})	5–11	7	3–7	5.5	5–6	5.6
TOC (mg L^{-1})	10–17	14	24–40	30	22–45	30
DOC (mg L^{-1})	7–16	13	24–39	27	20–42	28
TP (μg L^{-1})	20–94	49	19–36	28	19–39	28
TDP (μg L^{-1})	10–14	12	11–26	18	14–24	17
SRP (μg L^{-1})	4–12	9	2–23	10	2–17	10
TN (μg L^{-1})	1000–1700	1300	1550–2250	1900	1500–2500	1900
TDN (μg L^{-1})	770–1560	1000	1400–2200	1700	1100–2400	1600
NH_4-N (μg L^{-1})	7–60	50	80–200	130	60–420	200
NO_3-N (μg L^{-1})	45–230	130	46–400	190	120–440	240
Urea-N (μg L^{-1})	17–50	32	34–80	52	32–92	53

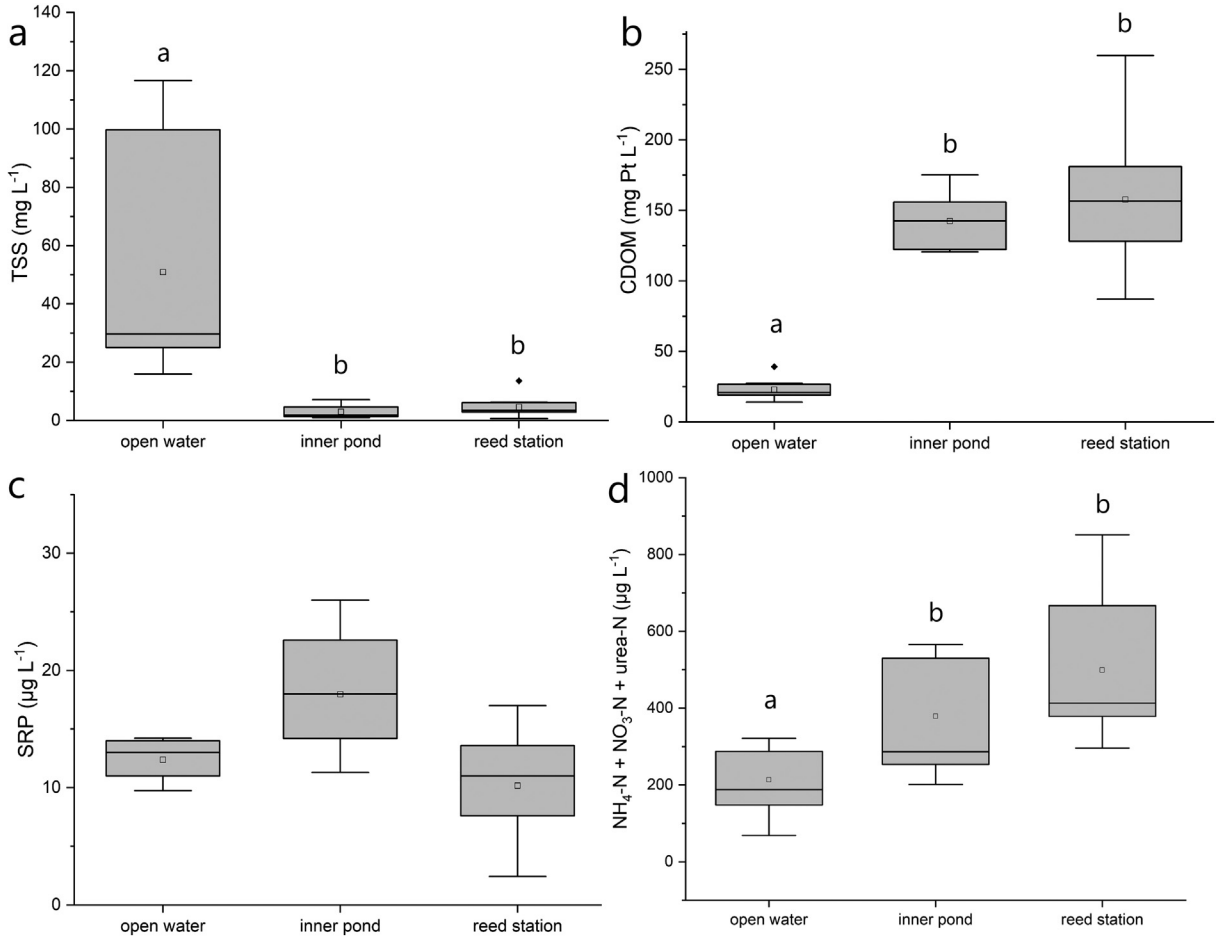

Fig. 1. Selected physical and chemical environmental parameters at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016. Abbreviations: TSS (total suspended solids), CDOM (coloured dissolved organic matter), SRP (soluble reactive phosphorous), NH_4-N (ammonia-nitrogen), NO_3-N (nitrate-nitrogen), urea-N (urea-nitrogen). The average values are represented as black line. Out-of-range values are shown as dots. The letters present statistical significance ($p < 0.05$) of differences between the sites.

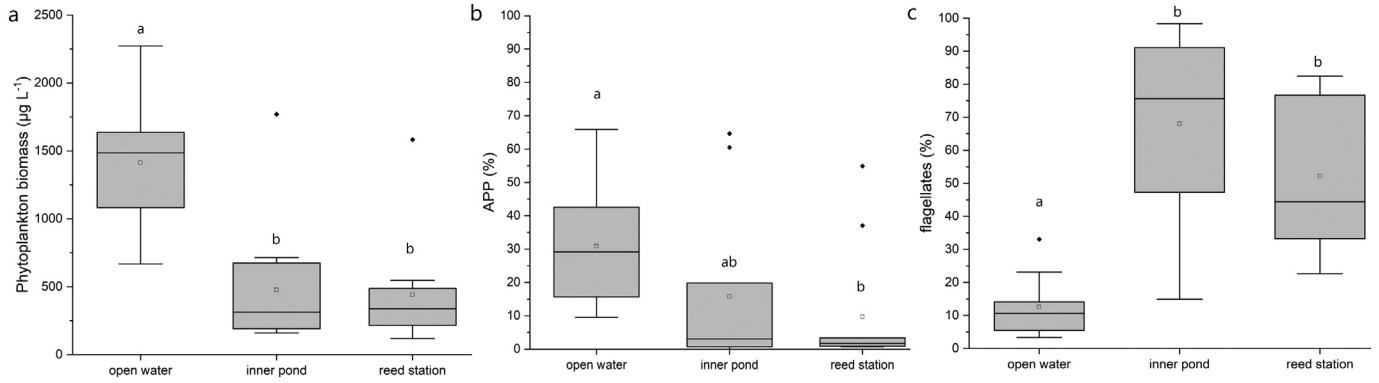

Fig. 2. Phytoplankton biomass, and contribution of the dominant (>50% of the total biomass) groups (picoalgae (APP) and flagellated algae) at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016. The letters present statistical significance ($p < 0.05$) of differences between the sites.

Table S1), despite of better light and nutrient conditions in the latter. The composition of the phytoplankton also differed significantly throughout the year. In the open water, picoalgae (mainly picocyanobacteria) were represented in large numbers (abundance: 1–9 × 10^5 cells mL^{-1}, biomass: 80 - –800 µg L^{-1}), but in the reed areas their quantities was approximately one order of magnitude lower (inner pond: abundance: 0–2 × 10^5 cells mL^{-1}, biomass: 80–150 µg L^{-1}, reed station - abundance: 0.1–2 × 10^5 cells $mL^{-}1$, biomass: 0–160 µg L^{-1}). In the open water, approximately half of the picoplankton biomass was composed of characteristic, *Aphanothece*-like colonial picocyanobacteria (accurate taxonomic determination cannot be performed without DNA-based molecular biology methods).

In open water, phytoplankton was dominated by two groups: picocyanobacteria and chlorophytes, both with an average share of 30% (Fig. 2, Fig. S3). In addition, flagellated algae (mainly cryptophytes) and benthic diatoms (*Campylodiscus clypeus*, *Surirella peisonis*, *Fragilaria* spp.) also occurred, with an average share of 10% (Fig. 2, Fig. S3). The contribution of picocyanobacteria and flagellates was higher in winter (up to 66% and 27%, respectively), while that of green algae was higher in early summer (up to 50%, Fig. S3).

In contrast, the proportion of groups dominant in open water (picoalgae and green algae) in the reed areas was negligible (except for two dates, when picoalgal contribution was higher, see Fig. S3) and phytoplankton was dominated by flagellated algae (*Cryptomonas* spp. and *Rhodomonas lacustris* var. *nannoplanctica*) with no clear seasonal trend (Fig. 2, Fig. S3). In the inner pond, flagellates accounted for 15–90% of the phytoplankton biomass (average contribution: 68%) with periphytic diatoms occurring at an average of 10% (Fig. S3). At the reed station, the contribution of periphytic diatoms was higher, accounting for 1–50% (average 20%) of phytoplankton biomass, while flagellates were responsible for 30–80% (average 50%).

The abundance and biomass of heterotrophic bacteria was nearly identical in the open water (1–9 × 10^6 cells mL^{-1}; 150–1800 µg L^{-1}) and in the reed areas (1–7 × 10^6 cells mL^{-1}, 350–1600 µg L^{-1}; Fig. 3, Table 2). The abundance of bacteriochlorophyll-containing bacteria in the reed areas was on average four times higher (2 × 10^5 cells mL^{-1}) than in the open water (5 × 10^4 cells mL^{-1}; Fig. 3, Table 2, Table S1).

3.3. Influence of aquatic macrophytes on lake metabolisms

The maximum photosynthetic rate, P_{max} of the total phytoplankton varied between 15 and 110 µg C L^{-1} h^{-1} in the open water. A significant seasonal variation was found with summer maximum and winter minimum values (Fig. S4). Based on the PI curves and the average ambient light intensities, the depth-integrated primary production (PP) varied between 50 and 460 mg C m^{-2} day^{-1} (Table 2). Lower P_{max} and PP values were measured in the reed areas: in the inner pond, P_{max} varied between 2 and 22 µg µg C L^{-1} h^{-1} and the PP ranged from 10 to 160 mg C m^{-2} day^{-1}, while at the reed station, the P_{max} varied between 1 and 53 µg C L^{-1} h^{-1} and the PP between 6 and 290 mg C m^{-2} day^{-1} (Table 2). Primary production in the reed areas were higher in summer and autumn and remained low during winter and spring (Fig. S4). The average biomass-specific maximum photosynthetic rate (P^B_{max}) of the total phytoplankton was also higher in the open water (3.6 µg C µg Chl $^{-1}$ h^{-1}), than in the reed areas (inner pond: 2.5 µg C µg Chl $^{-1}$ h^{-1}, reed station: 2 µg C µg Chl $^{-1}$ h^{-1}). There was no difference

Table 2
Characteristics of phytoplankton and bacterioplankton abundance, biomass and production at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016. Abbreviations: APP (autotrophic picoplankton), P_{max} (maximum photosynthetic rate), P^B_{max} (biomass-specific maximum photosynthetic rate), I_k (light saturation parameter), α^B (biomass-specific light utilization parameter), PP (primary production), HB (heterotrophic bacterioplankton), BChl (Bacteriochlorophyll-containing bacteria), BGE (bacterial growth efficiency).

	Open water		Inner pond		Reed station	
	Min-Max	Average	Min-Max	Average	Min-Max	Average
APP abundance (cells mL^{-1})	0.8–9*10^5	4*10^5	0–2*10^5	3*10^4	0.1–2*10^5	4*10^4
Chlorophyll *a* (µg L^{-1})	7–19	12	1.5–8	3.8	1.6–20	5.6
Phytoplankton biomass (µg L^{-1})	670–2270	1400	160–1770	480	120–1580	440
P_{max} (µg C L^{-1} h^{-1})	15–110	48	2–22	10	1–53	14
P^B_{max} (µg C µgChl $^{-1}$ h^{-1})	1.8–5.8	3.6	1.4–5.6	2.5	0.5–4	2
I_k (µmol m^{-2} s^{-1})	55–240	145	90–290	150	70–170	130
α^B (P_{max} / I_k)	0.02–0.03	0.02	0.01–0.03	0.02	0.01–0.03	0.02
PP (mg C m^{-2} day^{-1})	50–460	210	10–160	50	6–290	90
HB abundance (cells mL^{-1})	1–9*10^6	4*10^6	2–5*10^6	3*10^6	1–7*10^6	4*10^6
BChl abundance (cells mL^{-1})	0–3*10^5	5*10^4	1–2*10^5	2*10^5	1–3*10^5	2*10^5
HB biomass (µg L^{-1})	150–1800	680	350–1600	1000	670–1600	1030
HB production (µg C L^{-1} h^{-1})	0.4–3	1.2	1–10	5	1–11	5
BGE	0.58–0.64	0.61	0.60–0.64	0.63	0.62–0.65	0.64
HB production (mg C m^{-2} day^{-1})	14–92	37	23–195	104	40–337	165

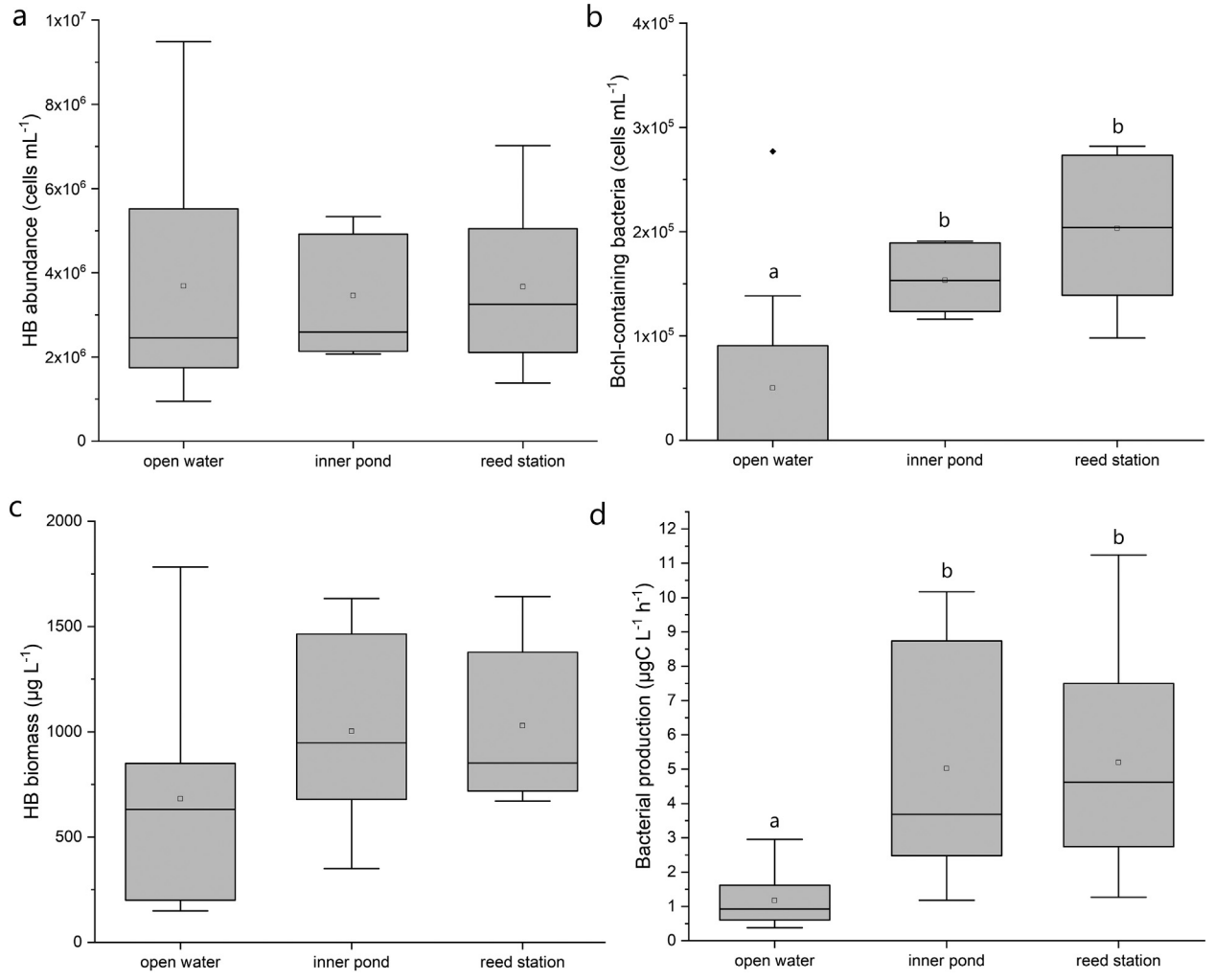

Fig. 3. Heterotrophic bacterial (HB) abundance, biomass and production and abundance of bacterioclorophyll-containing bacteria at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016. The letters present statistical significance ($p < 0.05$) of differences between the sites.

in the light saturation parameter between the different sampling stations despite of clear differences in light availability of the sites (Table 2).The primary annual production of phytoplankton was 82 g C m^{-2} $year^{-1}$ in the open water, 21 g C m^{-2} $year^{-1}$ in the inner pond and 35 g C m^{-2} $year^{-1}$ at the reed station (Fig. 4).

Similar results were obtained when measuring chlorophyll fluorescence as when measuring ^{14}C uptake (Fig. S4, S6). The algal community of the two habitats showed significant metabolic differences: Fv/Fm values in open water ranged from 0.44 to 0.66 (0.5 + 0.07, mean + SD), which was significantly higher than that measured in reed areas (Table S1), where Fv/Fm ranged from 0.26 to 0.52 (inner pond: 0.37 + 0.08, mean + SD; reed station: 0.37 + 0.08, mean + SD).

Bacterial production among the sampling stations showed the exact opposite trend than phytoplankton production (Fig. 3): much lower values were measured in the open water (0.4 and 3 µg C L^{-1} h^{-1}), than in the reed areas (1 and 11 µg C L^{-1} h^{-1}). However, the seasonal dynamics were similar to those observed for phytoplankton (Fig. S5). Bacterial growth efficiency was higher in the reed areas than in the open water (Fig. S6, Table S1). The daily net bacterial production in the reed areas was about three to four times higher than in the open water (Table 2). The ratio of PP to net BP ranged from 0.6 to 22 in the open water with an average value of 8 (Fig. S7). In contrast, this ratio remained below 1 in the reed areas (averaging 0.5 at both study sites) indicating that PP was unable to meet bacterial carbon demand. Hence, other allochtonous substrate (in our case, presumably humic substances and exudates deriving from aquatic plants) must support bacterial growth in the reed areas. On an annual basis, gross bacterioplankton production was 22 g C m^{-2} $year^{-1}$ in the open water, 57 g C m^{-2} $year^{-1}$ in the inner pond and 89 g C m^{-2} $year^{-1}$ at the reed station (Fig. 4).

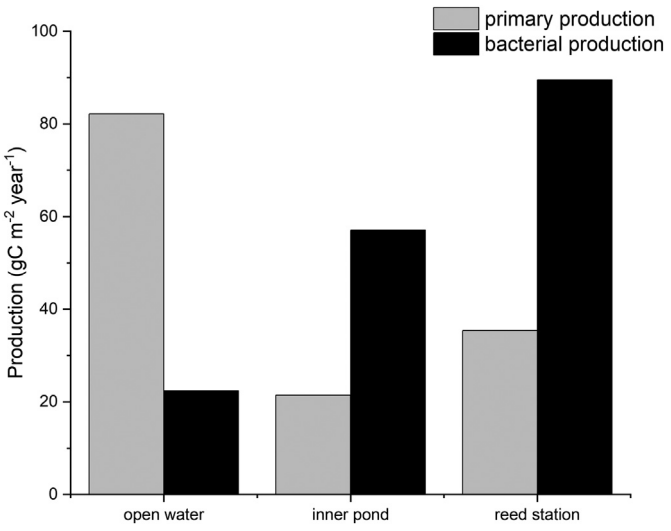

Fig. 4. Yearly primary production and yearly gross bacterial production at different habitats (open water, inner pond and reed station) of Lake Neusiedl in 2015–2016.

3.4. Phytoplankton, bacterioplankton and environmental variables

A significant positive correlation was found between TSS and K_d, indicating that this parameter is primarily responsible for light absorption in Lake Neusiedl, at least in the open water (Table 3). The majority of total phosphorus was associated with suspended particles: a strong correlation was found between TP and TSS and K_d, respectively. However, TSS changed as opposed to DOC, CDOM, TN and available nitrogen forms, and, in line with this, there was a negative correlation between CDOM and TP (Table 3). A significant positive correlation was also found between DOC and CDOM and between both parameters and TN and available nitrogen forms. There was a significant positive correlation between chlorophyll *a* and TSS and K_d, respectively. The production of the phytoplankton was also related to water temperature (Table 3). The production of heterotrophic bacteria was significantly correlated with CDOM, DOC, temperature, and TN (Table 3). Similarly, the abundance of bacteriochlorophyll-containing bacteria was also associated with CDOM, DOC, and TN, in addition to the available nitrogen forms (Table 3). However, no correlation was found between phytoplankton and bacterioplankton in Lake Neusiedl.

Spatial variations in 10 physical and chemical parameters and 9 biological parameters were analysed by PCA to identify differences among habitat types in Lake Neusiedl (Fig. 5). The first two principal components described 39.3% and 20% of total environmental variation, respectively. As a result, open water was prominently separated from reed areas (inner pond and reed station). The concentration of chlorophyll *a* showed a positive correlation with water temperature. The contribution of APP was positively correlated with TSS, while phytoplankton biomass and primary production were correlated with water temperature. Heterotrophic bacterial abundance, biomass and production were positively correlated with DOC and CDOM (Fig. 5, Table S2).

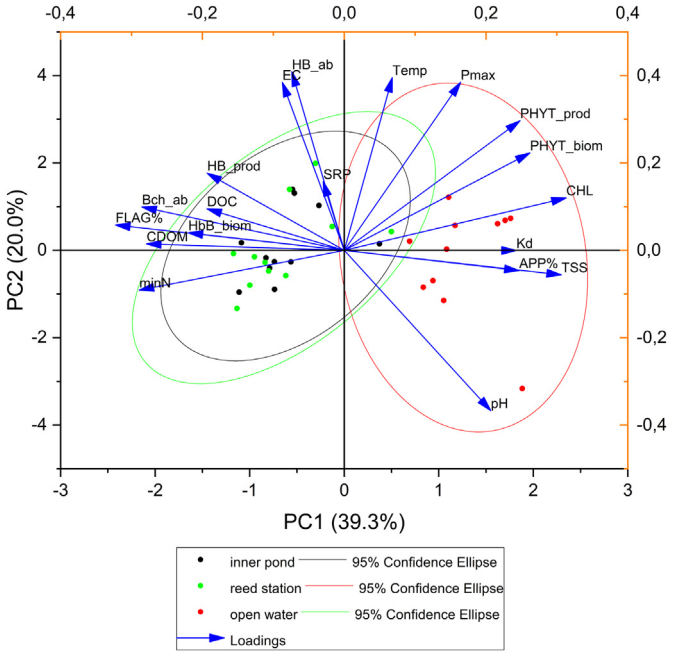

Fig. 5. Principal component analysis (PCA) biplot based on physical, chemical and biological parameters where Temp (temperature), EC (electrical conductivity), TSS (total suspended solids), CDOM (coloured dissolved organic matter), K_d (vertical attenuation coefficient), DOC (dissolved organic carbon), SRP (soluble reactive phosphorous), minN (available N forms), P_{max} (biomass-specific maximum photosynthetic rate), PHYT_biom (phytoplankton biomass), APP% (contribution of autotrophic picoplankton to phytoplankton biomass), FLAG% (contribution of flagellates to phytoplankton biomass), PHYT_prod (primary production), HB_ab (abundance of heterotrophic bacterioplankton), HB_biom (biomass of heterotrophic bacterioplankton), HB_prod (production of heterotrophic bacterioplankton), BChl_ab (abundance of bacteriochlorophyll-containing bacteria).

4. Discussion

4.1. Disadvantage of phytoplankton in the reed areas

Uncoupling between bacterioplankton and phytoplankton in Lake Neusiedl was due to the high organic carbon content of emergent macrophyte origin. While phytoplankton was determined by TSS, bacterioplankton (both heterotrophic and photoheterotrophic forms) was determined by dissolved organic carbon and nitrogen (TN or available N forms), although the production of both groups was also affected by temperature. As a result of these processes, the littoral and pelagic zones are completely separated from each other.

In Lake Neusiedl, phytoplankton behaved just the opposite in terms of underwater light conditions than we could have inferred from the field data. Although we found more light-limited conditions (higher K_d values) in the open water than in the reed belt, phytoplankton biomass and primary production were significantly higher in the former. The average biomass-specific maximum photosynthetic rate (P^B_{max}) of the total phytoplankton was also higher in the open water, than in the reed areas. In agreement with this, Fv/Fm measurements of the phytoplankton in Lake Neusiedl also showed that a highly active phytoplankton community was found in the open water. In contrast, phytoplankton communities in the reed areas had lower photosynthetic competence. According to the literature, estimation of the degree of potential photosynthetic competence and/or condition of cells is possible on the basis of the measured Fv/Fm of phytoplankton (Goto et al., 2008; Falkowski et al., 2017). Regardless of antenna pigmentation, the maximum photosynthetic energy conversion efficiency in photosystem II (PSII), obtained when grown under optimal conditions in cultures, is remarkably constant about 0.65 (Falkowski et al., 2017). In open oceans, nutrient limitation can lead to marked reductions in Fv/Fm, but this is certainly not the case in Lake Neusiedl. Another explanation could be the high concentration of humic substances (characterized here as CDOM content) in the reed areas. Although the role of humic substances in freshwaters is not yet sufficiently known, they appear to play an important role, especially in polyhumic lakes. This is because, in addition to serving as a source of energy or carbon, they appear to cause mild chemical

Table 3
Spearman's rank correlation coefficients between biological, physical and chemical variables (*** $p < 0.001$, ** $p < 0.01$). Abbreviations as in Tables 1 and 2.

Variable 1	Variable 2	Correlation coefficient
TSS (mg L^{-1})	$K_{d\ PAR}$ (m^{-1})	0.583***
TSS (mg L^{-1})	CDOM (mg Pt L^{-1})	−0.677***
TSS (mg L^{-1})	DOC (mg L^{-1})	−0.686***
TSS (mg L^{-1})	TP (μg L^{-1})	0.631***
CDOM (mg Pt L^{-1})	DOC (mg L^{-1})	0.924***
$K_{d\ PAR}$ (m^{-1})	TP (μg L^{-1})	0.489**
CDOM (mg Pt L^{-1})	TN (μg L^{-1})	0.708***
DOC (mg L^{-1})	TN (μg L^{-1})	0.617***
TSS (mg L^{-1})	NH_4-N + NO_3-N + Urea-N (μg L^{-1})	−0.499**
CDOM (mg Pt L^{-1})	NH_4-N + NO_3-N + Urea-N (μg L^{-1})	0.489**
$K_{d\ PAR}$ (m^{-1})	NH_4-N + NO_3-N + Urea-N (μg L^{-1})	−0.656**
$K_{d\ PAR}$ (m^{-1})	Chlorophyll *a* (μg L^{-1})	0.733***
TSS (mg L^{-1})	Chlorophyll *a* (μg L^{-1})	0.668***
CDOM (mg Pt L^{-1})	BChl abundance (cells mL^{-1})	0.568***
DOC (mg L^{-1})	BChl abundance (cells mL^{-1})	0.552**
NH_4-N + NO_3-N + Urea-N (μg L^{-1})	BChl abundance (cells mL^{-1})	0.574***
TN (μg L^{-1})	BChl abundance (cells mL^{-1})	0.652***
Temperature (°C)	PP (mg C m^{-2} day^{-1})	0.482**
NH_4-N + NO_3-N + Urea-N (μg L^{-1})	PP (mg C m^{-2} day^{-1})	−0.628***
TN (μg L^{-1})	HB biomass (μg L^{-1})	0.499**
Temperature (°C)	HB production (μg C L^{-1} h^{-1})	0.498**
CDOM (mg Pt L^{-1})	HB production (μg C L^{-1} h^{-1})	0.737***
DOC (mg L^{-1})	HB production (μg C L^{-1} h^{-1})	0.707***
TN (μg L^{-1})	HB production (μg C L^{-1} h^{-1})	0.579***

stress for microorganisms in a number of ways (Steinberg et al., 2006). It has been described, that these substances may directly quench electrons or bind to the bioquinones in PSII and thereby block electron transfer (Steinberg et al., 2006). As PSII is responsible for the cleavage of water and subsequent release of molecular oxygen, a significant reduction of photosynthetic oxygen release was described in algae in the presence of humic substances (Steinberg et al., 2006). A recent study described a decrease in the Fv/Fm ratio for a filamentous green alga as a result of leaf litter treatment (Shao et al., 2020). In addition, cyanobacteria were more sensitive to oxidative stress (as a result of UV irridation of humic substances) than green algae (Leunert et al., 2014). Based on previous studies, cyanobacteria appear to be more sensitive to the presence of humic substances than eukaryotic algae, but even closely related species within a taxonomic group may behave differently. Therefore, humic substances may also play a role in shaping the composition of phytoplankton and regulation of photosynthetic activity (Steinberg et al., 2006). This is in good agreement with the differences in phytoplankton composition between open water and reedbeds: in the former colonial picocyanobacteria were present in high numbers, while in the latter eukaryotic flagellates are usually present and picocyanobacteria were almost absent.

4.2. Effect of suspended solids on the microbial community

There is another important regulatory factor that is fundamentally different in the above habitats: the TSS content, which can affect the microbial community in a number of ways. Water inside the macrophyte bands can exhibit higher transparency and much lower TSS content as compared to the open-water areas. According to our previous field survey, TSS plays an important role in determining size distribution of phytoplankton in shallow lakes: high TSS regularly leads to a selective advantage of picoalgae over larger ones (Somogyi et al., 2010; Somogyi et al., 2017). The explanation of this effect is unknown; however, two major hypothesis was stated. According the first hypothesis, success of picoalgae besides high TSS relates to their better acclimation to low light environment while the second is that success of picoalgae besides high inorganic turbidity relates to their reduced grazing control (see details in Somogyi et al., 2017). The significant role of zooplankton grazing in reed areas may also be indicated by the fact that phytoplankton biomass and production was significantly lower there than in the open water. The phytoplankton can be effectively controlled by top-down effects, the main element of which is the zooplankton grazing. No zooplankton study was performed in the present work, but previous data show that with increasing macrophyte cover, both zooplankton diversity and abundance increases. According to Dinka et al. (2010), zooplankton density was surprisingly small (25 rotatoria/ 10 L; 140 crustacean/ 10 L) in the open water of Lake Neusiedl, while the inner lakes harboured a rich and abundant zooplankton community (2250 rotatoria/ 10 L; 810 crustacean/ 10 L). A subsequent study (Kiss et al., 2014) found similar results with higher crustacean density in the inner ponds and canal sites (130–150 crustacean/ 10 L) than in the open water (80 crustacean/ 10 L).

Similar results were found for planktonic ciliates: while the maximum abundance in the open water was 17 cells mL^{-1}, in the inner pond it was 203 cells mL^{-1}, which is high for a mesotrophic lake (Schönberger, 1994). The proportion of 'jumper' ciliates was significantly higher in the inner pond than in the open water, indicating that the ciliate community was exposed to an intensive predation pressure (Schönberger, 1994). In terms of the feeding strategies, small-sized herbivore and bacterivore ciliates appeared in large numbers in the inner pond, which can be related to the larger biomass of available food organisms (small flagellates and bacteria). In addition, the large number of ciliates, flagellated algae and zooplankton capable of active movement may also be related to the reduced turbulence as a result of wind-shielding effect of the macrophytes.

A comprehensive study conducted in 2019 found an inverse, non-linear relationship between zooplankton and phytoplankton. Zooplankton reached a high density in the open water patches of the reed zone, strongly controlling planktonic algae (Wolfram et al., 2020).

4.3. Selective advantage of bacteriochlorophyll containing bacteria in the reed belt

By their structuring effect, macrophytes created an environment that is fundamentally different from that of the open water. This was also true for light availability, which limits the occurrence of photoautotrophic microorganisms, presumably causing a shift toward photoheterotrophic life forms. In the reed areas of Lake Neusiedl, the relative enrichment of near infrared radiation (NIR) was observed (Fig. S2). These modified underwater light conditions facilitate the predominance of photoheterotrophic bacteria (e.g. aerobic anoxygenic phototrophs, purple and green bacteria), which are able to survive at light intensities that are too low for photoautotrophic algae and cyanobacteria, and can utilize near-infrared radiation (Kirchman, 2000). Mesocosm experiments have demonstrated that the presence of macrophyte cover can lead to the dominance of photoheterotrophic bacteria over photoautotrophic picoplankton (Izaguirre et al., 2010). These photoheterotrophs may have selective advantage over heterotrophic bacteria as well, because they can synthesize ATP via both phototrophic and chemotrophic mechanisms (Kirchman, 2000). In Lake Neusiedl, bacteriochlorophyll-containing bacteria in the reed areas had much higher abundance values than in the open water accounting for about 5–7% of heterotrophic bacteria.

4.4. Success of bacterioplankton in the reed areas and prominence of chemoorganotrophic processes

Although previous studies have already drawn attention to the significant role of heterotrophic bacteria in Lake Neusiedl, we did not have accurate information on the metabolic relationship between phytoplankton and bacterioplankton, especially for reed belt. Bacterial production in the lake (mainly in the open water) has been measured from the beginning of the 1970s (Dokulil, 1975; Dokulil, 1984) and later in the late 90s (Reitner et al., 1997; Reitner et al., 1999). Reitner et al. (1999) compared the relationship between phytoplankton and bacterioplankton production in the open water and the reed areas of Lake Neusiedl, but due to several factors the measured values should be treated with caution. In their work, the production of both phytoplankton and bacterioplankton was significantly underestimated. In the case of bacterioplankton, production was calculated using a BGE value (0.16) determined in an earlier winter study, which can be considered quite low comparing to direct measurements on freshwater lakes (Del Giorgio & Cole, 1998). In the case of phytoplankton, the photosynthesis of only the fraction smaller than 62 μm was measured, not the whole algal community which resulted in a significant underestimation of primary production. In Reitner et al. (1999), mean primary production was 16.6 mg C m^{-2} day^{-1} in the open water of Lake Neusiedl. These values were an order of magnitude lower than our values (Table 2) and than those (100–500 mg C m^{-2} day^{-1}) measured earlier or even later in the open water of the lake (Dokulil, 1984; Dokulil, 2013). The present work provided an opportunity to compare photoautotrophic and chemoorganotrophic processes in the pelagic and macrophyte-dominated littoral region of the lake and, as a result, we established that the trophic balance differs significantly in the two lake areas.

In the open water of Lake Neusiedl, the autotrophic processes dominated: the PP/net BP ratio was above 1 and the yearly PP was almost four times higher than the gross BP. In contrast, a shift toward chemoorganotrophic (heterotrophic, photoheterotrophic) processes was observed in the reed areas. At the sampling stations inside the reed belt, the PP/net BP ratio was the inverse, well above one and the annual gross BP was about two and a half times higher than the PP. Although only emergent macrophytes were present in one of the two reed sampling stations and both emergent and submergent macrophytes were present in the other, the two habitats were not separated based on the planktonic microbial metabolic processes (see PCA results). Quantification of bacterial parameters (production and respiration) and their coupling to phytoplankton parameters are required to evaluate the carbon flux through the microbial food web and to elucidate the changes in trophic balance in response to any environmental changes at local or global

scales (Jahnke and Craven, 1995; Ducklow et al., 2002; Robinson, 2008; Kim et al., 2020). Jansson et al. (2000) defined net heterotrophy so that the PP/ bacterial respiration (BR) ratios <1 (del Giorgio et al., 1997), and chemoorganotrophy (system production mainly depends on energy mobilization from organic compounds) so that the PP/net BP ratios <1. However, for interactions between different trophic levels in the food web/food chain, the ratio between primary production and bacterial net production is of greater interest (Jansson et al., 2000). Stimulation (via allochtonous DOC or DOC originating from macrophytes) of heterotrophic processes in an autotrophic system can have considerable effects on the biostructure and function of the system even if it does not turn out to be chemoorganotrophic (Jansson et al., 2000). The relative proportions of two major trophic pathways shift: the phytoplankton-based food chain, which is channelled via protozoa and zooplankton to higher trophic levels weakens; while the DOC-based food chain, which is channelled via heterotrophic bacteria to higher trophic levels, strengthens. Based on our results, high macrophyte cover in Lake Neusiedl resulted in high DOC content, high bacterial biomass and production, which shifted the otherwise dominant autotrophic processes toward chemoorganotrophic direction.

5. Conclusions

Dense emergent aquatic vegetation creates an environment in the littoral zone of shallow lakes that is completely different from the open water region: i.) turbidity decreases and thus water transparency increases ii) concentration of organic carbon forms (TOC, DOC, CDOM) and available nitrogen forms (NH4-N, NO3-N, Urea-N) increases, iii) the underwater light climate is relatively enriched in infrared radiation, and iv) the dissolved oxygen saturation decreases. Due to these changes in the physical and chemical environment, significant changes are also taking place in the structure and function of microbial communities. In the littoral zone picoalgae disappear, flagellated algae occur instead, and the biomass of heterotrophic and photoheterotrophic bacteria increases. This environment is not favourable for planktonic algae, as shown by their lower photosynthetic competence, probably due to the high concentration of humic substances. Unlike algae, the high humic content is particularly beneficial for bacteria, both directly as a carbon source and indirectly by altering the underwater light climate, which is beneficial for photoheterotrophs. As a result of all these processes, the open water and the littoral region become completely different, the former being dominated by autotrophic and the latter by chemoorganotrophic processes. In other words, the microbial food web in the littoral zone is not based on planktonic algae but on heterotrophic bacteria.

CRediT authorship contribution statement

Boglárka Somogyi: Conceptualization, Methodology, Field measurements, Experimental design, Data curation, Data analyses, Writing original draft, Editing, Supervision.

Emil Boros: Data analyses, Editing.

Nóra Szabó-Tugyi: Experimental design, Laboratory measurements, Editing.

Attila W. Kovács: Experimental design, Laboratory measurements, Editing.

Lajos Vörös: Conceptualization, Methodology, Data curation, Data analyses, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154576.

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