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Temporal dynamics of planktonic microeukaryotes and bacteria in shallow soda lakes of the Carpathian Basin

Ph.D. Dissertation

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Content

Abbreviations

bOTU	Bacterial operative taxonomic unit
CCA	Canonical correspondence analysis
Chl a	Chlorophyll a
core4	Core community of Böddi-szék, Kelemen-szék, Pan no.60 and Zab-szék
core5	Core community of Böddi-szék, Kelemen-szék, Sós-ér, Pan no.60 and Zab-szék
DAPI	4',4-diamidino-2-phenylindole
DO	Dissolved oxygen
DOC	Dissolved organic carbon
eLSA	Extended local similarity analysis
eOTU	Eukaryotic operative taxonomic unit
GAM	Generalized additive model
HF-HNF	Heterotrophic flagellates and heterotrophic nanoflagellates
LS	Local similarity score
ML	Multiple linear regression
NCBI	National Centre for Biotechnology Information
NMDS	Non-metric multidimensional scaling
non-core4	Not part of the core community of Böddi-szék, Kelemen-szék, Pan no.60 and Zab-szék

non-core5	Not part of the core community of Böddi-szék, Kelemen-szék, Sós-ér, Pan no.60 and Zab-szék
OTU	Operative taxonomic unit
PCR	Polymerase chain reaction
PD	Phylogenetic diversity
PEG	Plankton ecology group
PERMANOVA	Permutational multivariate analysis of variance
SSCC	Spearman's rank correlation coefficients
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
WI	Weighted topological importance

1. Introduction

Soda lakes are a special type of athalassic saline lakes, characterized by carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and sodium (Na^+) as dominant ions, and they have a stable alkaline pH (Boros et al., 2014; Felföldi, 2020). They can be found worldwide but they are less frequent than other naturally saline lakes (Sorokin et al., 2014). In Europe, they predominantly occur as soda pans in the Carpathian Basin (Austria, Hungary, and Serbia) (Boros et al., 2014). These locally confined unique ecosystems serve as important feeding and breeding sites for waterbirds (Szabó et al., 2022) and as refuges for rare and endangered species (Horváth et al., 2013). Due to their uniqueness and their special biota, they are under legal protection, and most of them are located in national parks (Boros et al., 2017, 2013). Human activities, such as poor water management and various water drainages, have led to the disappearance of many soda lakes in the Carpathian Basin (Boros et al., 2013; Felföldi, 2020), which resulted in species loss as well (Horváth et al., 2019). Furthermore, Boros et al., (2020) implicated that the intensifying effects of climate change, such as prolonged desiccations due to decreasing precipitation and increasing temperature are threatening these vulnerable ecosystems.

In spring of 2013-2014, Szabó et al., (2020) observed sudden seasonal changes in the prokaryotic communitiy composition. Previous to that sudden spring community shift, the algae associated prokaryotic community "collapsed", which resulted in the highest relative abundance of actinobacteria so far, based on the literature. In the summer, picocyanobacteria can dominate the water, while below 15 °C picoeukaryotic algae can cause mass production (Pálffy et al., 2014; Somogyi et al., 2009). Sometimes dual blooms of green algae and purple bacteria was also observed in these shallow soda pans (Korponai et al., 2019). But still we have very little information about the seasonality of the planktonic microbes and to what degree is seasonality determined by environmental variables and how much desiccation influence the community composition.

In our work, our goal was to gain knowledge about the planktonic microbial diversity along environmental gradients, and to get a comprehensive picture of the seasonality and community structure of microeukaryotes and prokaryotes of soda pans.

2. Literature review

2.1 General introduction of soda lakes

2.1.1 Classification of salt lakes

According to Hammer's (1986) classification, inland (athalassic) waters with a salt concentration (salinity) below 0.5 g/L are categorized as freshwater, while water bodies with salinity exceeding 3.0 g/L are referred to as salt lakes. The transitional zone between these two environments is named as subsaline water bodies, with salinity ranging from 0.5 to 3.0 g/L (Hammer, 1986). According to a recent update on this classification, water bodies can be defined based on the amount of total dissolved solids in the water (g/L): freshwater (0.02-1.0 g/L), moderately saline 1.1-3 g/L, brackish (3.1-10 g/L), saline (10.1-35 g/L) and hypersaline (> 35 g/L) (Saccò et al., 2021).

Saline lakes can be categorized according to the ionic composition of their water (Boros et al., 2014). The combined dominance of sodium (Na^+) and carbonate (HCO_3^-/CO_3^{2-}) ions in the water creates a permanently alkaline environment. Boros and Kolpakova (2018) conducted a water chemistry analysis of 220 Eurasian lakes to classify alkaline lakes. The main categories and subtypes were separated based on the ranks of the dominant ions. Based on the dominant anions of the water and the molar equivalent according to the separated calculation of total cation or anion pools, three categories were distinguished: saline, soda-saline, and soda. In the soda group, Na^+ is the dominant cation, and HCO_3^-/CO_3^{2-} is the first in rank of anions (> 25 equivalent %), while in the case of the soda-saline type, Na^+ is typically the dominant cation, and HCO_3^-/CO_3^{2-}, but also Cl^- or SO_4^{2-} are often the primary anions. However, the amount of carbonate ions present in these water bodies exceeds 25 equivalent %. Saline lakes are characterized by Cl^- or SO_4^{2-} being the primary anion, with the equivalent % of HCO_3^- and CO_3^{2-} ions typically not exceeding 25 e% (Figure 1.) (Boros and Kolpakova, 2018). In this analysis, the distribution of the identified lake types was heterogeneous across the different geographical regions of Eurasia. The majority of the typical soda lakes were found in the Carpathian Basin (68% in Austria, Hungary, and Serbia), followed by Russia (14%) and Mongolia (13%). Soda-saline lakes were mostly present in Russia (44%) and Mongolia (31%), while the highest concentration of saline lakes was observed in China and Kazakhstan (Boros and Kolpakova, 2018).

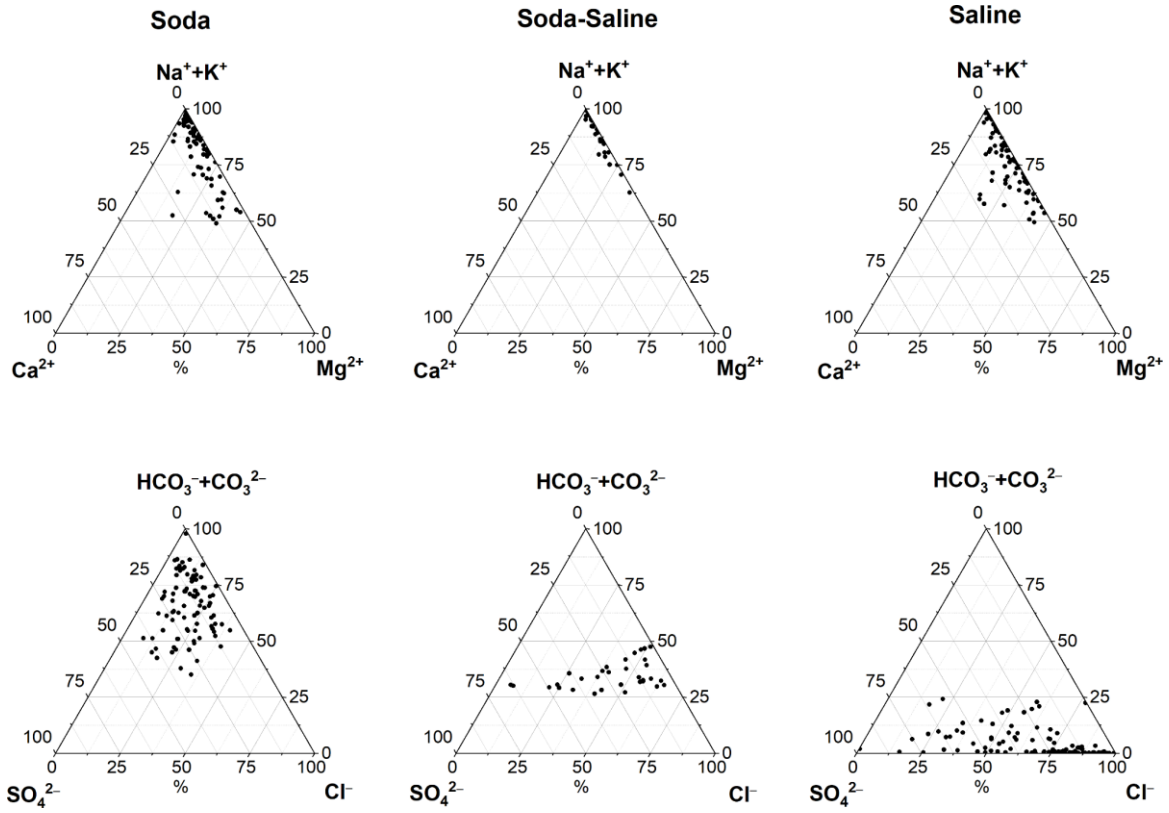

Figure 1. Ion contribution of the identified water chemical types to the equivalent % on ternary diagrams (Boros and Kolpakova, 2018)

2.1.2 Hydrogeology of soda lakes

Most of the soda lakes arose in endorheic (closed) basins that are formed in volcanic terrains. The chemical weathering of these volcanic rocks results in mineral rich solutes that flows into the closed lake basin (Schagerl, 2016; Toner and Catling, 2020). One of the most common chemical weathering processes is silicate hydrolysis when atmospheric or volcanic CO_2 dissolves into the water and forms H_2CO_3, which weathers the volcanic rocks or other silicate minerals. Rain and also the acidity of the soil can intensify these chemical weathering processes. Silicate hydrolysis leads to zeolite and other clay mineral formations which can give the water different coloring. Na^+, K^+, Ca^{2+}, Mg^{2+} cations, and HCO_3^-/CO_3^{2-} are produced during this silicate weathering while the cations can dissolve into the surface and groundwaters, the anions lead to alkaline pH. Most of the Ca^{2+} and Mg^{2+} precipitate as minerals, like calcite and dolomite in the lake basin. The evaporation of this water produces saline alkaline lake water, which is rich in Na^+, HCO_3^-, CO_3^{2-}, and Cl^- ions, while SO_4^{2-} usually is lost by microbial activity (Getenet

et al., 2023; Schagerl, 2016). Due to the local environmental conditions, such as annual precipitation and evaporation, the salinity of the lakes can vary. The hypersaline lakes can precipitate sodium carbonate salts, the most common ones are trona [$Na_3(CO_3)(HCO_3)*2(H_2O)$], natron [$Na_2CO_3*10(H_2O)$], and themonatrite ($Na_2CO_3*H_2O$) (Getenet et al., 2023; Schagerl, 2016). Soda lakes of the Carpathian Basin were formed on different sediments (loess, sand, or alluvial sediment), where the groundwater is constantly close to the surface. In addition to the sediment composition and sodium and carbonate rich groundwater supply, the characteristic continental and subcontinental climates of the Carpathian Basin play a key role in the sodification processes (Figure 2.) (Boros et al., 2014).

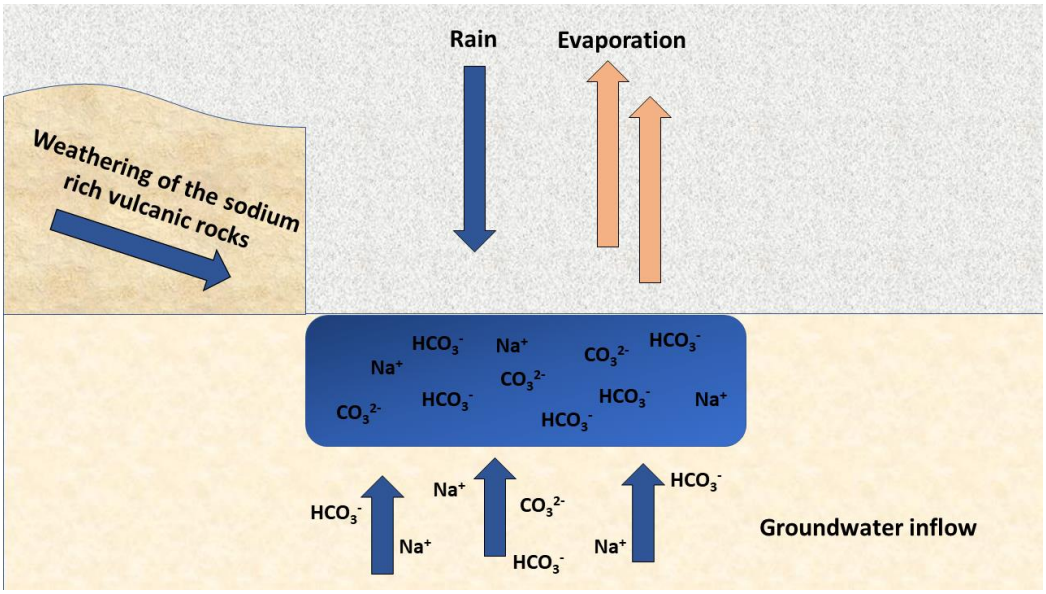

Figure 2. Schematic representation of the chemical origin of the soda lakes (based on Schagerl, 2016)

Microbialites are special benthic sedimentary structures that can be found in soda lakes, like in Lake Van, which is located 1.648 meters above sea level in the eastern part of Anatolia, Turkey. Microbialites are formed through the biological activities of microbial communities. The Nemrut volcanic mountain's eruption gave rise to a lake that boasts impressive dimensions - a surface area of 3.522 km^2, a maximum depth of 450 meters, and a volume of 576 km^3. By volume, Lake Van is the world's largest soda lake and the third largest endorheic (closed basin) lake. A distinct feature that sets Lake Van apart from other soda lakes is the microbialites that can grow up to 70 meters tall (Arslan et al., 2018; Kremer et al., 2019). These microbialites are

sedimentary structures that form when the microbial communities induce mineral precipitation or sediment accumulation. They develop near the shore where groundwater from Ca^{2+}-rich sources meets the alkaline lake water, which is low in Ca^{2+}. The mixing of these two types of water creates a local $CaCO_3$ supersaturation, resulting in a milky solution near the coast. The tower-like microbialite formations are enveloped by a spherical cyanobacterial biofilm that mineralizes with aragonite and precipitated calcite. The formations' interiors are traversed by channels carrying spring water (López-García et al., 2005). These microbialites are taller than the best-known tufa towers in Mono Lake, located in California's Sierra Nevada Range (Kremer et al., 2019).

Mono Lake in California is considered a very important place worldwide for studying the geochemical formation of tufa towers. The tufa towers are located along the fracture lines at the edge of the lake connected to the springs and the rock-compacted tufa slabs. These impressive tower-like structures are the result of recent carbonate deposition that has been observed sporadically in the lake since the last glaciation. Although based on these properties Mono Lake can be classified as an archetypal carbonate-precipitating, hyperalkaline lake, little has been studied about the precipitation processes. One reason for this is that active carbonate precipitation has not been observed since the 1980s and 1990s, when gaylussite [$Na_2Ca(CO_3)_2 \times 2.5\ H_2O$] and the mineral ikaite ($CaCO_3 \times 6\ H_2O$) formed, where the spring water and lake water met (Brasier et al., 2018). They tried to explain the past formation of the tufa with geochemical and geobiological models. According to one of the popular geochemical models, the ikaite ($CaCO_3 \times 6\ H_2O$), the primary carbonate mineral crystallized into gaylussite [$Na_2Ca(CO_3)_2 \times 2.5\ H_2O$], while based on another model the mixing of Ca^{2+} rich spring water and CO_3^{2-} rich lake water led to the precipitation of $CaCO_3$ (Brasier et al., 2018; Council and Bennett, 1993). Early geobiological models were based on microbes or algae observations influencing carbonate precipitation at sites of active tufa formation (Brasier et al., 2018; Scholl and Taft, 1964). Based on geobiological theory, the calcium required for the tufa formation is bound through microbial mechanisms, like the extracellular organic matter produced by cyanobacteria (Emeis et al., 1987).

2.1.3 Occurrence of soda lakes

Soda lakes are distributed globally, although they are primarily located in regions characterized by arid or semiarid climates, such as the Great Rift Valley in East Africa, California, and

Nevada in the United States, and the Eurasian Steppe (Figure 3.) (Sorokin et al., 2014). In Eurasia, soda lakes are commonly found in Austria, Hungary, Serbia, Mongolia, Russia, Kazakhstan, China, and Turkey (Boros et al., 2017; Boros and Kolpakova, 2018). The shallow soda lakes of the Carpathian Basin are known to frequently dry out, due to the high variaton in daily temperature fluctuations of the water, precipiation amount, groundwater upwelling and evaporation and also the characteristic of the lakes (closed basin, no in or outflow) (Boros et al., 2017; Felföldi, 2020). These soda lakes also have a remarkably high concentration of dissolved organic carbon as observed by both the average and maximum values, even compared to other highly productive aquatic ecosystems (Boros et al., 2020).

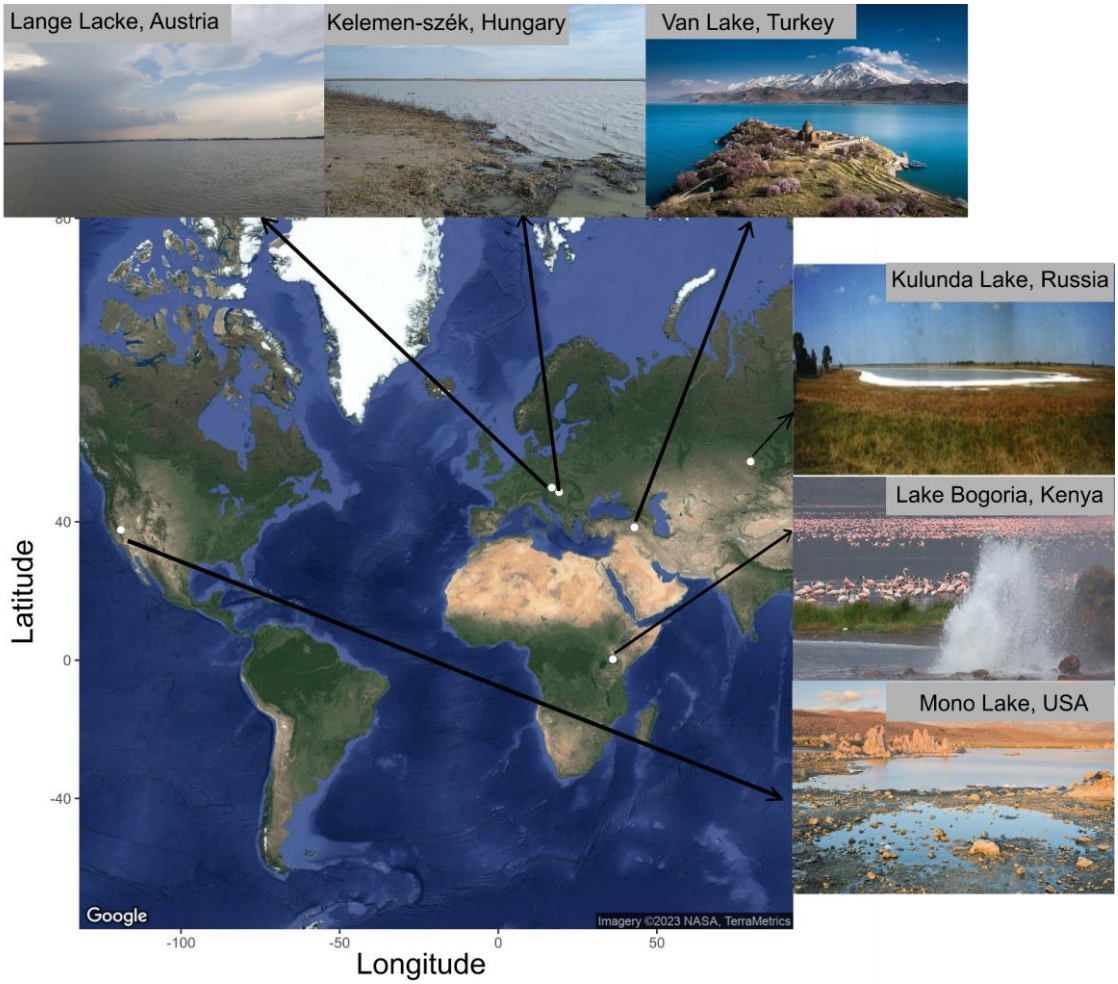

Figure 3. Location of the soda lakes in the world (Map was created with R v. 4.2.2 (R Core Team, 2022)). Pictures: Lange Lacke, Austria (by me); Kelemen-szék, Hungary (by Bianka Csitári); Van Lake, Turkey (https://www.tourcounsel.com/2022/01/lake-van.html); Kulunda Lake, Russia (Sorokin et al., 2006); Lake Bogoria, Kenya (by Steve Garvie); Mono Lake, USA (https://rove.me/to/california/mono-lake-tufa-towers)

Soda lakes found in the Great Rift Valley of Africa were formed by tectonic movements that created indentations in volcanic bedrocks (Schagerl, 2016). These unique environments harbor alkalophilic, halophilic, and thermophilic microorganisms (Jones and Grant, 2000). Some lakes exhibit strong salt gradients that decrease along their shorelines due to water sources, while in the middle salinity increases. Fluctuating water levels also strongly influence salinity in most shallow East African soda lakes.

Soda pans (shallow intermittent water bodies) in the Carpathian Basin can be distinguished into two ecological subtypes based on turbidity, the turbid-white, and the non-turbid-colored type. The turbid-white type pans (referred to as turbid) are characterized by a high amount of suspended inorganic particles, which leads to high turbidity and grayish color. The non-turbid-colored type pans (referred to as colored) are dominated by dissolved humic substances which results in a brownish color of their water (Figure 4.) (Boros et al., 2014).

Figure 4. The two ecological subtypes of soda pans. Left: Turbid type, Kelemen-szék; Right: Colored type, Sós-ér (Photos by Bianka Csitári)

2.1.4 Microeukaryotic and prokaryotic communities of soda lakes

Microbial communities are crucial components of biogeochemical cycles and energy flow in every ecosystem. While research over the last few decades has mainly focused on bacterial communities, microeukaryotes (20-200 μm) (Wu and Liu, 2022), which include protists, fungi, microalgae, and small-sized zooplankton have received relatively little attention (Wu et al.,

2020). Nonetheless, microeukaryotes are essential parts of the food web and play a significant role in the uptake, use, and transformation of organic and inorganic matters (Chen et al., 2021). Their diversity, distribution, and community composition are really important to understand the ecological processes that maintain ecosystem functioning (Kong et al., 2019).

The most prevalent algae are filamentous cyanobacteria, specifically *Arthrospira*, which is dominant in some lakes such as Lake Chitu (Ethiopia), Lake Bogoria (Kenya), and Lake Momela (Tanzania). Other soda lakes have mixed algal communities consisting of *Arthrospira*, *Anabaenopsis*, and *Cyanospira*. Among the East African soda lakes, the phytoplankton of Kenyan lakes (Lake Bogoria, Lake Elmentaita, Lake Nakuru, and Lake Sonachi) have been studied the most. Photosynthesizing organisms had lower abundance in the lakes, but the community composition shifted towards A*nabaenopsis arnoldii*, *Synechococcus* spp., *Monoraphidium minutum*, *Anomoeoneis sphaerophora*, *Navicula elkab*, and *Nitzschia frustulum* dominance in the spring. Members of the genus *Cyanospira* dominate in Lake Magadi, together with *Arthrospira* and *Synechococcus*, but also green algae *Picocystis salinarium* was observed (Krienitz et al., 2012; Krienitz and Schagerl, 2016). Some studies have also reported flagellated algae, such as *Chlamydomonas* and *Ceratium*, in these soda lakes. Ciliophora has been studied in the East African soda lakes since the 1980s and their quantity and biomass production is greater than in freshwater, subtropical, or temperate lakes (Schagerl, 2016). The ciliate community is mostly dominated by planktivorous and bacterivorous taxa, like genera *Halteria*, *Vorticella*, *Uroleptus*, *Phialina*, and *Metopus* (Yasindi and Taylor, 2006). Anoxygenic phototrophic bacteria are also capable of mass production in these soda lakes, genera *Rhodobaca*, *Rhodobacter*, *Pseudorhodobacter* and *Roseibacter* were identified among the purple non-sulfur bacteria, while in case of the purple sulfur bacteria genera *Ectothiorhodospira* and *Halorhodospira* were observed (Lanzén et al., 2013; Schagerl, 2016).

Members of phyla Cyanobacteria and Bacteroidetes were identified on the surface of the microbialites from Lake Van, while Proteobacteria, Actinobacteria, and Firmicutes were detected in the inner parts (López-García et al., 2005). Sadly, we still have very little information about the aquatic microbial community composition of Lake Van.

Studies on the microbial community of soda lakes located in the Kulunda steppe (Altai Region, Russia) commenced in the early 1900s. Based on the phytoplankton and bacterioplankton studies, it was established that the most characteristic filamentous green algae of the community are *Ctenocladus circinnatus*, while *Geitlerinema* spp. és *Nodosilinea* spp. are the most

dominant filamentous cyanobacteria. In addition to *Ctenocladus circinnatus*, green algae *Dunaliella viridis*, *Chlorella minutissima*, and the diatoms *Brachysira zellensis* and *Nitzschia communis* were also members of the phytoplankton community (Samylina et al., 2014).

Hundreds of soda lakes are located in the Onon-Boryza system, which can be found in the Trans-baikal region of Russia (Afonina and Tashlykova, 2020). This area is characterized by a dry continental climate which results in high salinity and alkalinity levels in the local lakes (Sklyarov et al., 2011). The Uldz Gol-Torey basin is the largest endorheic basin of the Onon-Boryza lake system. The most common species identified are the *Oocystis borgei* green algae, *Lemmermannia komarekii* green algae, *Cyclotella* sp. diatom, and *Euglena* spp. species. Among Cyanobacteria taxa A*nabaenopsis* sp., *Merismopedia minima*, *Microcystis aeruginosa* and *Pleurocapsa minor* were found abundant. Zooplankton community consists of many widespread and eurybiont species, like *Daphnia magna*, *Moina brachiata*, *Arctodiaptomus niethammeri* and *Cyclops strenuus*. The community composition and biomass of the phytoplankton and zooplankton communities are similar to other soda lakes (Afonina and Tashlykova, 2020).

The best-known soda lakes in North-America are the meromictic Soap Lake and Mono Lake. Soap Lake with an area of 3 km^2 and 24 m depth can be found in Washington state. The lake has an extremely high sulfide content, reaching 175 mM near the sediment. This is the highest measured sulfide concentration in natural waters (Asao et al., 2011). Periodically picoeukaryotic green algae blooms characterized by the genus *Chlorella* occur in the aerobic water layer, which provides a carbon source for consumers located at different trophic levels (Dimitriu et al., 2008). Subsequent findings showed that the algae previously identified as *Chlorella* sp. is, in fact, a member of the *Chloroparva* genus, which was first described by Somogyi et al. (2011), following research conducted in the Böddi-szék soda lake, Hungary (Tamás Felföldi, personal communication). In addition, the green algae S*phaerocystis schroeteri*, *Amphora salina* and *Nitzschia* diatoms were also present in Soap Lake. *Moina hutchinsoni*, *Diaptomus nevadensis*, and the rotifers *Hexarthra* spp. were also identified within the zooplankton community (Walker, 1975). Mono Lake is located in California, Sierra Nevada and it is 160 km^2, highly stratified, hypersaline, alkaline (pH ~ 9.8), and rich in carbonate (400 mM). The current ecosystem consists of a poor phototrophic community in the mixolimnion but diatoms (mainly *Nitzschia* sp.) and occasionally cyanobacteria can be found. Brine shrimp *Artemia monica*, an abundant species of zooplankton, feeds on these phototrophic organisms,

which, in turn, serve as a vital food source for migratory birds. In the western US, the green algae genus *Picocystis* so far has been only described from Mono Lake. *Picocystis* is capable of photosynthesis under low light and highly saline conditions. Thus, it can undergo significant proliferation at the boundary of the oxic/anoxic zone, which restricts light penetration into deeper layers where sulfur bacteria are found (Roesler et al., 2002). A *Picocystis* algae bloom was observed at Mono Lake in 2016 (Stamps et al., 2018). Most of the bacteria from Mono Lake belonged to five main lineages, α and γ-Proteobacteria, Bacteroidetes, Actinobacteria, and Bacillus/Clostridium based on the 16S rRNA sequences (Melack et al., 2017). Due to the hypersaline and alkaline nature of the lake, methane-oxidizing bacteria affiliated with methanotrophic genera *Methylobacter*, *Methylomicrobium*, *Methylothermus,* and *Methylocystis* taxa were also identified in the planktonic bacterial community (Table 1.) (Lin et al., 2005).

2.1.5 Biota of the Carpathian Basin soda lakes

The biota of the Carpathian Basin's soda lake water and soil are truly unique in the world. The moss vegetation is mostly affected by the water level changes in the soil water. The characteristic moss of the salt marshes and meadows is the Pannonic endemic moss, the *Enthostodon hungaricus*. There are many moss species listed in the Hungarian Red Data Book, like the *Phascum floerkeanum*, *Desmatodon cernuus*, *Pterygoneurum subsessile*, and the *Pseudocrossidium revolutum*, which is considered quite rare in Hungary as well.

Soda lakes serve as migration and resting places for many waterbird species, and around spring the only available macroinvertebrate as food for them is the fairy shrimp. Three fairy shrimps can be found in the soda lakes, *Branchinecta ferox, Branchinecta orientalis,* and *Chirocephalus carnuntanus*, which are all common during the spring migration (Horváth et al., 2013). In addition to the fairy shrimps, waterbirds can feed on the members of mesozooplankton as well (Horváth et al., 2013). The role of waterbirds in biogeochemical cycles and lake fertility has been studied extensively, they found that bird excrement (guanotrofication) helps to enrich the lakes with nutrients and reaccumulate phosphorus (Boros et al., 2016, 2021). Most of the waterbird species that prefer shallow, open water can be found in this area. Characteristic waterbird species of soda lakes are greylag goose, *Anser anser*, but the red-breasted goose, *Banta ruficollis* and the lesser white-fronted goose, *Anser erythropus* can also be observed (Boros et al., 2002; Szabó et al., 2022). Waterbirds can also play an essential role in the dispersal of aquatic microorganisms, which can influence the biodiversity patterns of soda lakes (Szabó et al., 2022).

Table 1. Comparison of planktonic taxa of soda lakes (Africa: Krienitz and Schagerl, 2016; Lanzén et al., 2013; Schagerl, 2016; North America: Dimitriu et al., 2008; Lin et al., 2005; Melack et al., 2017; Roesler et al., 2002; Stamps et al., 2018; Walker, 1975; Eurasia: Afonina and Tashlykova, 2020; López-García et al., 2005; Samylina et al., 2014; Sklyarov et al., 2011; Carpathian Basin: Borsodi et al., 2013; Felföldi, 2020; Felföldi et al., 2009; Horváth et al., 2014; Korponai et al., 2019; Somogyi et al., 2011; Szabó et al., 2020, 2020, 2017)

	Africa	**North-America**	**Eurasia**	**Carpathian Basin**
Bacterioplankton	*Rhodobaca*	*Ectothiorhodospira vacuolata*	*Microcystis aeruginosa*	*Ilumatobacter*
	Rhodobacter	*Halomonas campisalis*	*Pleurocapsa minor*	*Nitriliruptor*
	Pseudorhodobacter	*Nitrincola lacisaponensin*	*Anabaenopsis sp.*	*Limnohabitans*
	Ectorhodospira	*Alkalitalea saponicalus*	*Proteobacteria*	*Hydrogenophaga*
	Halorhodospira	*Methylobacter*	*Actinobacteria*	*acIII-A1*
	Arthrospira	*Methylomicrobium*	*Firmicutes*	*Rhodobaca*
	Cyanospira	*Methylothermus*		*Synechoccus*
Eukaryotic phytoplankton	*Monoraphidium minutum*	*Chlorella*	*Ctenocladus circinnatus*	*Chloroparva*
	Anomoeoneis sphaerophora	*Amphora salina*	*Nitzschia communis*	*Choricystis*
	Navicula elkab	*Nitzschia*	*Dunaliella viridis*	*Nitzschia*
	Nitzschia frustulum		*Chlorella minutissima*	*Nannochloris*
	Picocystis salinarium			*Marvania*
				Wislouchiella
Zooplankton	*Vorticella*	*Diaptomus nevadensis*	*Daphnia magna*	*Daphnia magna*
	Uropleptus	*Moina hutchinsoni*	*Moina brachiata*	*Moina brachiata*
	Phialina	*Artemia monica*	*Arctodiaptomus niethammeri*	*Arctodiaptoms spinosus*
	Metopus		*Cyclops strenuus*	*Arctodiaptomus bacilifer*
	Halteria			*Halteria*
				Vorticella
				Cyclops sp

Due to the usually absent predatory fish population and seaweed vegetation, the trophic network mainly contains (both individually and in terms of biomass) invertebrate zooplankton (Copepoda and Cladocera) (Horváth et al., 2013). However, if the conditions are right, e.g. the lakes don't desiccate for several consecutive years, certain fish can colonize the lakes, such as silver carp or chinese rasbora (Boros et al., 2013). In the case of large soda lakes, like Lake Fertő and Lake Velence, the permanently low salinity and the artificially high water level harbors a steady fish stock (Felföldi, 2020). The most typical zooplankton indicator species (a

species whose absence or presence provides additional information about the state of the habitat) is the *Arctodiaptomus spinosus* (copepoda). However, the cladoceran *Moina brachiata* in the summer can be considered as an indicator species as well, due to its high abundance. *Arctodiaptomus spinosus* and *Moina brachiata* can dominate the zooplankton community together, due to their wide salinity tolerance (Figure 5.) (Horváth et al., 2014).

These soda lakes have a truly unique algae community, one of the typical genus of Cyanobacteria is *Synechococcus*, and in the case of the green algae, genera *Choricystis* and *Chloroparva* (Felföldi, 2020; Somogyi et al., 2011, 2010). Water level fluctuation, salinity, nutrient content, light intensity, and the presence of viruses and parasites can affect the structure of the algal community. However, the high nutrient content and the favorable light conditions can lead to algal blooms as well (Schagerl, 2016). Generally, in the temperate zone picoeukaryotic algae dominates the water body from autumn to spring, while picocyanobacteria are more abundant in the summer (Callieri, 2008; Kellogg et al., 2019).

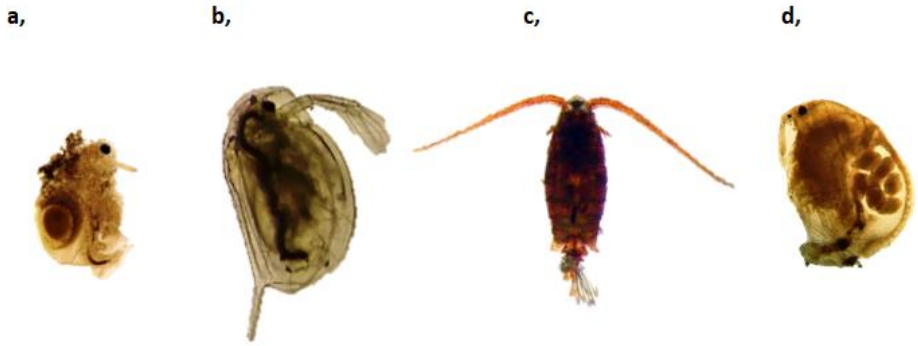

Figure 5. Characteristic zooplankton species of the soda pans of the Carpathian Basin. a) *Moina brachiata*, b) *Daphnia magna*, c) *Arctodiaptomus spinosus*, d) *Macrothrix hirsuticornis* (Picture a, b, d was made by the author; Picture c was taken by Alois Herzig)

Somogyi et al., conducted an experiment in 2009 with algae strains isolated from soda lakes where it was observed that picoeukaryotic algae have a better light utilization and photosynthetic activity under 15 °C than picocyanobacteria. These findings suggest that lower temperature and light intensity can give a competitive advantage to picoeukaryotic algae. The widely distributed diatom genera *Nitzschia* and *Navicula* were found most common in soda lakes (Ács et al., 2017; Stenger-Kovács et al., 2014).

Bacteriological studies of the soda lakes in the Carpathian Basin started several decades ago, but the focus of theses research was mainly biofilm, sediments, or the rhizosphere (Borsodi et al., 1998). It is only in the recent years that the planktonic bacterial community has been the subject of detailed studies. Representatives of Actinobacteria, Bacteroidetes, and Proteobacteria were abundant in the lakes (Borsodi et al., 2013; Korponai et al., 2019; Szabó et al., 2020, 2017, 2015, 2022; Szuróczki et al., 2020). Betaproteobacteria a typical freshwater bacteria, such as *Limnohabitans* and *Hydrogenophaga* (Newton et al., 2011), were found to be common during low salinity (Szabó et al., 2020). The most characteristic planktonic actinobacterial lineages were the uncultured acIII-A1, acSTL, and acTH1, along with genera *Illumatobacter* and *Nitriliruptor* (Szabó et al., 2017, 2020). The abundance of aerobic anoxygenic phototrophs, a group of photoheterotroph bacteria was found to be influenced by phytoplankton biomass, coloured dissolved organic matter and total suspended solids content as well. At high total suspended solid content aerobic anoxygenic phototrophs were abundant due to the decreased grazing pressure (Szabó-Tugyi et al., 2019). It has been shown that the dense macrophyte cover of shallow lakes can create very different environments in the open water area and the littoral zone. This environmental difference between the two area due to turbidity, water transparency, concentration of organic carbons, led to the dominance of flagellated algae, heterotrophic and photoheterotrophic bacteria in the littoral zone (Somogyi et al., 2022a).

2.1.6 Preserving the ecological status of soda lakes in the Carpathian Basin

In the Carpathian Basin, one of the main issues concerning the nature conservation of soda lakes can be traced back to the river regulation projects during the 19th century. Due to the increased use of groundwater resources and inland water management activities, some of the soda lakes were completely drained and used as agricultural land, while others were used as fish ponds. The spring and autumn filling of the lakes was often delayed due to global warming which worsened the water balance of the lakes. The uneven distribution of the precipitation led to empty groundwater reservoirs. The desiccated areas have become targets of agriculture use and invasive weed species (Boros, 2010). Soda lakes are included in the habitat protection guidelines as the Pannonian steppes and salt marshes, and they belong to the highly protected areas of the Natura 2000 network, which is due to their extremities, wildlife, and significantly decreasing area (Boros et al., 2013). Soda lakes are considered as “ex lege” areas (protected by law due to their ecological significance). The Hungarian State Nature Conservancy currently recognizes 397 “ex lege” soda lakes, all of which are situated in National Parks or other

protected areas (https://termeszetvedelem.hu/ex-lege-vedett-szikes-tavak/). Hortobágy National Park launched the LIFE program "Protection of the Carpathian Basin's Saline Lakes" in 2009, in which they carried out an assessment of the natural state of the soda lakes in the area. Based on the results they created reconstruction and rehabilitation plans (Boros, 2010). Many soda lakes are listed as important feeding and breeding bird habitats in the Ramsar Convention. Soda lakes of Austria are part of the UNESCO world heritage (Boros et al., 2017), also some soda lakes are protected in Serbia as well (Gavrilovic et al., 2018). Also throughout history, hypersaline and soda lakes played a vital role in the economy, like fishing, tourism, recreational use, and salt and soda mining for therapeutic and commercial use as well (Saccò et al., 2021; Schagerl, 2016). The extraction of salt and soda, or other minerals, such as lithium, sodium, and magnesium from these lakes had a negative impact on the entire environment. Furthermore, due to the extremities of these lakes, they have a unique flora and fauna and they can provide compounds for industrial use, such as soap, body oil, cosmetics, or battery manufacturing (Cavicchioli et al., 2019; Konkol and Rasmussen, 2015; Saccò et al., 2021; Schagerl, 2016). Due to the lack of long-term data on the provided ecosystem services, their value can not be estimated for conservation plans. However, for the future of these unique lakes it is an urgent matter to develop plans for their sustainable usage and protection (Cunillera-Montcusí et al., 2022; Hassani et al., 2020; Saccò et al., 2021; Schagerl, 2016).

2.2 Methodological development of microbial ecology in recent decades

2.2.1 Genomic methods

Identifying the impacts that alter the community's composition and how microbes react to environmental and spatial variations present the most significant challenge in microbial ecology (Chen et al., 2017). Several recent studies propose a combined investigation of environmental and spatial factors (Chen et al., 2019), nevertheless, disentangling the effects of these factors is challenging since their influence and interplay vary depending on the geographic and environmental variables' scale (Zhang et al., 2018). For most of the twentieth century, studies of microbial diversity were based on microscopic and cultivation methods, and these methods were also used for genetic and phenotypic identification of isolated microbial strains. Although these approaches led to several fundamental discoveries, microbial diversity and the phylogenetic relationships of microorganisms remained poorly understood (Lynch and Neufeld, 2015). Microscopic studies reported a much greater morphological diversity than

studies based on cultivation techniques from the same environment, an observation called the “great plate anomaly” (Harwani, 2012). However, it became possible to detect uncultured taxa with the spread of DNA/RNA-based methods. Norman Pace began to explore the environmental microbial diversity based on the idea proposed by Woese and Fox in 1977, which turned the small subunit of ribosomal RNA (16S rRNA) into a marker gene for uncovering microbial diversity based on DNA (Lynch and Neufeld, 2015). The nowadays commonly used new-generation DNA sequencing has been the breakthrough for studying microbial diversity (Escobar-Zepeda et al., 2015). With these methods, the entire genome of bacterial strains could be explored from several samples in a short time. Microbial community composition can be explored by amplicon sequencing (e.g. amplification of taxonomic marker genes or gene regions and determination of their base sequence) or "shotgun" genome sequencing (fragmentation and sequencing of the genetic material). The presence of extremophiles was detected and connections between microbes and diseases were revealed by the wide application of these techniques. These methods allow us the comprehensive examination of time series of a given environment, revealing changes in the microbial community in relation to trophic interactions and environmental effects (Hiraoka et al., 2016).

2.2.2 Network analyses

In recent years, the successful utilization of biological association networks has enabled the investigation of the co-occurrence patterns and interspecific interactions of microbial communities in various ecosystems (Zhao et al., 2016). An ecological community can be interpreted as a network of species connected by interspecific relationships such as predation, mutualism, or parasitism (Ratzke et al., 2020). Several methods have been developed to construct ecological networks, like regression methods, probabilistic graph-based methods, and the most commonly used correlation-based methods (Li et al., 2020). Amplicon sequencing data based co-occurrence patterns can be used to estimate positive and negative ecological interactions among various species and environmental factors. The advantage of the network analysis is that it enables the modeling of complex microbial interaction, spatial or temporal dynamics, trophic interactions, or ecosystem services (Röttjers and Faust, 2018). Also one of the biggest advantages of the network perspective is the possibility to identify keystone species that play a central role in maintaining the stability of the community (Yang et al., 2020). The definition and statistical analyses of keystone taxa are still not unified, several studies define keystone taxa as hubs (highly associated microbes in the network) or species that have a

disproportionately high importance in the community (removal of the keystone species have a dramatic impact on the network structure and the community) (Banerjee et al., 2018; Berry and Widder, 2014). Most of the studies use the "leave-one-out strategy" (their removal have a destructive effect on the community) (Banerjee et al., 2018; Berry and Widder, 2014), network topological features (degree, betweenness centrality, closeness centrality) (Berry and Widder, 2014) or topological indexes (Jordán et al., 2006) to identify keystone species but it is still not settled to this day which one is the best method in general. However, if the network perspective is not used with caution, it can lead to misinterpretations. The main issues of network analysis of abundance data are normalization, the choice of network method, testing issues, and measuring biases. All of these challenges can affect the end result of the network construction (Berry and Widder, 2014; Faust and Raes, 2012; Röttjers and Faust, 2018).

Network methods have been applied to study microbial communities of lakes (Eiler et al., 2012; Horton et al., 2019), oceans (Aylward et al., 2015; Milici et al., 2016; Wang et al., 2016), soils (De Menezes et al., 2015; Gao et al., 2022) and also the human microbiome (Faust et al., 2012; Mainali et al., 2019). The study of Gao et al. (2022) found that the dynamics of the microbial communities changed due to desiccation and also that microbes could strengthen the drought tolerance of plants. The improved tolerance probably helps the microbial host plant to adapt to desiccation. These findings can help agricultural advancement, if the microbiome of the plant gets disrupted by droughts, it can be rescued by artificial inoculation with desiccation-tolerant members of the networks, like Actinobacteria and Chloroflexi. Wang et al. (2016) revealed based on network analysis that complex interactions (like competition, modularity) between microbial species can improve the resistance and recovery from pH and pCO_2 change in marine environments, which could lead to the explanation of how ocean acidification affects the microbial community.

3. Main objectives

To date, there has not been carried out a comprehensive study focusing on the planktonic microeukaryotes and prokaryotes of shallow soda lakes, which examines both the taxonomic composition, core microbiome (species shared among all sites), and diversity along environmental gradients as well as delving into their seasonal dynamics and ecological interactions. To the best of my knowledge, no study has yet focused simultaneously on the microeukaryotics and prokaryotic communities of these shallow soda lakes. However, soda pans (their community structure quickly responds to rapidly changing environmental parameters) can serve as models for understanding the processes of more complex aquatic ecosystems. Therefore we studied the planktonic microbial communities through time – three seasons: spring, summer, and autumn (by sampling biweekly) and space: twenty-six soda pans (by sampling in two consecutive spring seasons).

Accordingly, the main questions of my doctoral thesis were the following in the two projects:

A. <u>Seasonal changes of the microeukaryotic and bacterial communities</u>

   I.) How similar are the seasonal changes of planktonic microeukaryotic and prokaryotic communities in nearby soda pans?

   II.) How core and non-core microbial taxa contribute to the adaptation of the microbial communities, and how does this contribution vary between microeukaryotic and bacterial communities?

B. <u>Temporal dynamics of microbial diversity along environmental gradients</u>

   III.) Do the identity and strength of the main environmental drivers change between subsequent years?

4. Seasonal changes of the microeukaryotic and bacterial communities

4.1 Introduction

Seasonality induced annual succession dynamics of plankton communities are the focal point of numerous marine and freshwater aquatic microbial ecology studies (Bista et al., 2017; Lambert et al., 2018; Reji et al., 2020). Seasonal changes of major external factors (like temperature, and precipitation) are considered to be primary drivers of community assembly and internal interaction dynamics. As a result, these processes follow characteristic patterns repeated each year that can be generally outlined by ecological models such as the PEG (Plankton Ecology Group) model (Sommer et al., 1986, 2012). The basis of the PEG model was that the seasonal dynamic of the community composition is initiated by the increase of light in spring and ends with the decrease of light in autumn. Also, it was assumed that the reproduction of zooplankton depends on the availability of food and follows the phytoplankton biomass with a shift in time (Sommer, 1985; Sommer et al., 2012, 1986). Departures from such general seasonal patterns identified in long-term time series are usually explained by interannual variations or long-term trends. However, the drivers of the variation in the seasonal succession of plankton communities within the same year and region remain understudied.

Shallow lakes are the most abundant lentic inland water bodies (Jeppesen et al., 2009) and they are particularly affected by seasonal changes as temperature and precipitation dynamics impact them strongly due to their high surface-to-volume ratio (Cobbaert et al., 2014; Jeppesen et al., 2009; Li et al., 2021). In the case of endorheic lakes, the property of shallowness enhances the effect of seasonal variation (precipitation and evaporation) which introduces strong seasonal variation in the concentration of dissolved substances which makes them ideal for studying the seasonality of planktonic communities. Although previous studies already showed the influence of interannual variations on planktonic communities (Afonina and Tashlykova, 2020; García-Ciudad et al., 1997), it is still unknown whether it is driven by interannual variations of the seasonal environmental trends or local stress events.

Microbial communities are highly complex and consist of hundreds or thousands of species (Bengtsson-Palme, 2020). However, only a smaller fraction of the microbial species are shared among the local communities associated with specific habitat types (soda lakes) and can therefore be defined as a core microbiome (Shu et al., 2020). The core microbiome is hypothesized to represent the functionally or ecologically most important taxa (Degenhardt et

al., 2020; Neu et al., 2021). Moreover, it is proposed that microbial adaptation to environmental changes is happening through species sorting via dispersal which operates mainly on the core microbiome as opposed to the rare or periodic community members (Niño-García et al., 2016). Therefore, it can be expected that microbial communities adapt to regular seasonal changes through the core microbiome, while the non-common community members are more important in the response to less predictable environmental changes such as the desiccation events of soda pans.

The highly diverse and complex nature of microbial communities also means that a network perspective is required to study their species interactions (Leibold et al., 2022). Association networks are a popular tool to evaluate the response of communities to environmental changes as they can assess the dynamics of occurrence and abundance patterns of organisms belonging to different domains or trophic levels (Faust et al., 2015; Fuhrman et al., 2015; Röttjers and Faust, 2018). The direction of correlations in species association networks can be positive or negative. Mutualistic and facilitative interactions usually result in positive co-occurrence relationships, while predator-prey relationships are expected to present negative correlations (Fuhrman et al., 2015; Röttjers and Faust, 2018). To understand species interactions association networks time-lagged interactions must be also included when for example the increase of the abundance of a species at a certain point leads to the change of abundance of another species later in time (Faust and Raes, 2012; Fuhrman et al., 2015; Nagpal et al., 2020).

4.2 Aims

Field sampling was carried out to explore the seasonality of planktonic microbial communities of five soda pans from the same region (i.e., Kiskunság National Park) throughout three seasons (spring, summer, autumn). The aims of the study were to understand how microbial community structure and interactions are impacted by seasonality and the extent to which microbial response to seasonal changes is driven by stochastic processes and also the differences in the response of core microbiome members versus the entire community as well as microeukaryotic and bacterial communities. Our primary hypothesis was that shallow soda lakes of the same region follow similar seasonal dynamics in terms of community composition and interactions. We hypothesized that seasonal adaptation happens through the core microbiome, while non-core taxa mainly respond to local sudden environmental events and bacterial communities are more stable through sudden environmental events than microuekaryotic communities.

4.3 Materials and methods

4.3.1 Study area

High sodium and bicarbonate/carbonate content are characteristic of soda pans of the Kiskunság National Park, Hungary, resulting in saline conditions and alkaline pH. Shallowness and high evaporation leads to high salinity and regular dry-ups during summer and autumn. We sampled five characteristic soda pans of the Kiskunság National Park: Böddi-szék (46°46.07′ N, 19°09.007′ E), Kelemen-szék (46°47.893' N, 19°10.440' E), Sós-ér (46°47.341′ N, 19°8.679′ E), Zab-szék (46°50.190′ N, 19°10.283′ E) and an unnamed pan (Pan no. 60 in (Boros et al., 2013) 2013, 46°45.492′ N, 19°10.497′ E) (Figure 6.).

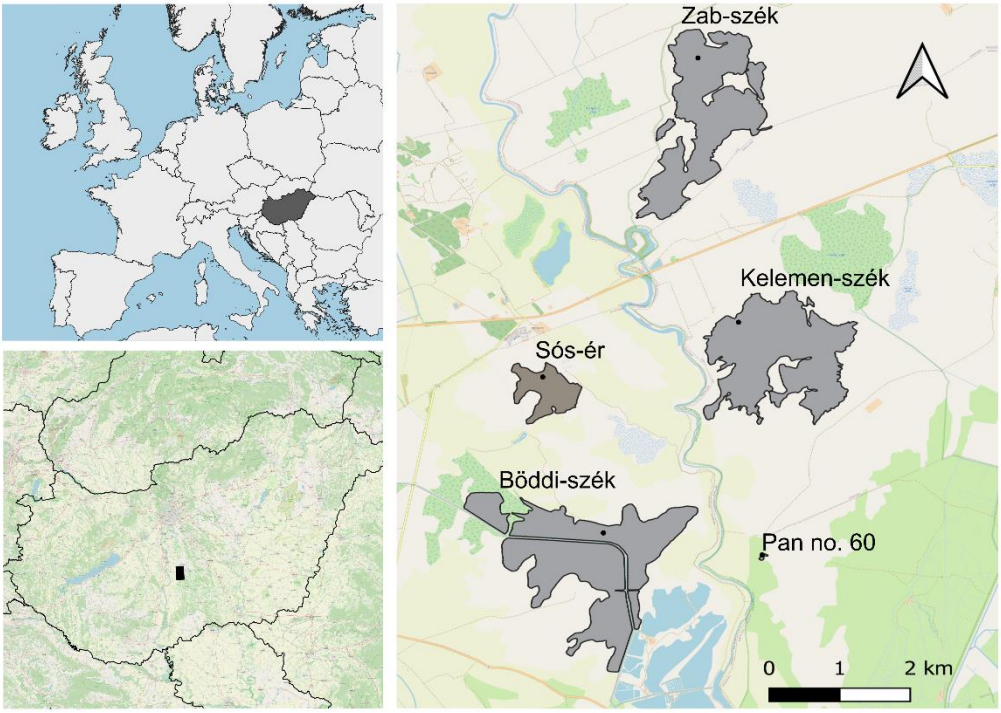

Figure 6. Location of the sampling sites in Kiskunság National Park, Hungary (Map was created with QGIS Geographic Information System v.3.28.1)

Among the sampled pans Sós-ér was the only colored type, the other four pans belonged to the turbid type. The close proximity (within 14 km^2) of these sites means that they are exposed to the same climatic and meteorological conditions. Sampling was carried out biweekly from April 12 to november 14 in 2017, covering three seasons: spring (sampling time 1-4), summer (sampling time 5-10), and autumn (sampling time 11-14).

4.3.2 Sample collection and environmental parameters

At each sampling occasion and sampling site, composite water samples were collected by sampling water from 1 cm below the surface at least five different points near the deepest part of the pans. We collected water samples in a 1-liter sterile beaker for molecular biology and bacterial cell count determination purposes. The water samples were filtered through a clean plankton net (mesh size of 40 μm) on-site to remove large organisms (zooplankton or algae) with their associated microbiota, which could significantly distort the community composition determined by amplicon sequencing. Pre-filtered water samples were transferred to the lab in a cooling box. To determine the bacterial cell count, the water samples were preserved with paraformaldehyde (final concentration 4%) and then incubated overnight at 4°C. Water samples (30 ml from turbid soda pans and 50 ml from the colored type of soda pan) were filtered through a 0.1 μm pore size membrane filter (MF-Millipore) upon return to the laboratory. The filters were stored at −20 °C until further processing.

To determine abundant zooplankton, 20 liters of water were collected randomly at the same time from 20 different points around the deepest part of the open water area and sieved through a plankton net with a mesh size of 40 μm according to Horváth et al., 2014. After collection, all zooplankton samples were preserved in 70% ethanol and stored at room temperature.

We measured temperature, pH (SenTix 41 electrode), conductivity (TetraCon 325 cell) and dissolved O_2 (CellOx-325 electrode) with a MultiLine Handheld Meter model 340i (WTW, Weilheim in Oberbayern, Germany) on site. Conductivity data were converted to salinity with the equation of [Salinity (g/L) = Conductivity (mS/cm) x 0.8] according to Boros et al., 2014.

Further environmental parameters were measured in the laboratory. Total nitrogen (TN) concentration and dissolved organic carbon (DOC) were measured according to Nydahl et al., 2019, while total phosphorus (TP) and chlorophyll a (Chl a) were performed as described previously in Mentes et al., 2018.

The daily temperature and precipitation data for 2017 were provided by the Hungarian Meteorological Service (Soltszentimre automatic meteorological station is within 8 km from all of the sampling sites).

4.3.3 Community analysis

Zooplankton abundances were calculated by subsampling according to Herzig (1984). Identification was carried out microscopically based on the identification key of Einsle (1993) and Gulyás & Forró (1999, 2001).

Bacterial cell number was determined by fluorescence microscopy. The preserved samples were filtered through a nitrocellulose filter with a pore size of 0.22 µm. The filters were stained with DAPI (4′,6-diamidino-2-phenylindole), then washed with alcohol and water according to Vajna et al., 2016. (DAPI is a fluorescent dye that has an absorption maximum of 358 nm and an emission maximum of 461 nm.) The stained filters were examined with an epifluorescence microscope under UV excitation, with a blue filter. For each sample, ten views were counted manually, then the following equation was used to calculate the bacterial cell count [bacterial cell count/ml = (total bacterial cell count of the views×filtering area) / (number of views used×volume of the filtered sample in ml×area of the view)].

DNA of the microbial community was extracted using the DNeasy PowerSoil Kit (QIAGEN) according to the manufacturer's instructions. Extracted DNA was stored at -80 °C until further processing.

The composition of the microeukaryotic and prokaryotic communities was determined based on the V4-V5 region of the 18S rRNA and the V3-V4 region of the 16S rRNA taxonomic marker genes by Illumina amplicon sequencing. Eukaryotic primers 574*F (CGGTAAYTCCAGCTCYAV), 1132R (CCGTCAATTHCTTYAART) (Hugerth et al., 2014), and prokaryotic primers 341F (CCTACGGGNGGCWGCAG) (Herlemann et al., 2011), 805NR (GACTACHVGGGTATCTAATCC) (Apprill et al., 2015) were used for the polymerase chain reactions (PCR). To decrease the stochastic effect of the reaction, all PCR were performed in duplicates in 20 µl of final volume, which contained 4 µl of 5xQ5 reaction buffer, 2 µl of dNTP (2 mM), 0.2 µl of Q5 High Fidelity DNA polymerase (2 U/µl) (New England Biolabs), 0.5 µl of each primer (10 µM), 11.8 µl of nuclease free water and 1 µl of template DNA. The following thermal cycle conditions were used for 18S rRNA amplification: initial denaturation at 98 °C for 1 min and an additional 10 sec, followed by 20 cycles (annealing at 51 °C for 30 sec, extension at 72 °C for 30 sec) and a final elongation step at 72 °C for 2 min. The following thermal cycle conditions were used for 16S rRNA amplification: initial denaturation at 98 °C for 30 sec and 98 °C for 10 sec, followed by 20 cycles (annealing at 48

°C for 30 sec, extension at 72 °C for 30 sec) and a final elongation step at 72 °C for 2 min. Amplicons were pooled before the purification with magnetic beads (Agencourt AMPure XP PCR Purification, 2013). To prepare libraries for Illumina sequencing, primers were prolonged by Illumina handles and index primers. The second PCR reaction contained 4 µl of 5xQ5 Reaction buffer, 2 µl of dNTP (2 mM), 0.2 µl of Q5 High Fidelity DNA polymerase, 1 µl of each index primer (5 µM), 9.8 µl of nuclease free water and 1 µl of template from the first PCR reaction. The following thermal cycle was used for both 18S rRNA and 16S rRNA amplification: initial denaturation 98 °C for 30 sec and an additional 98 °C for 10 sec, followed by 15 cycles (denaturation 98 °C for 10 sec, annealing 66 °C for 30 sec, extension 72 °C for 30 sec/kb) and the final extension 72°C for 2 min. Amplicons were purified again with magnetic beads (Agencourt AMPure). Quantification of the libraries was carried out using a PicoGreen assay (Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen). Sequencing was performed at the SciLifeLab (Uppsala, Sweden) on an Illumina MiSeq platform (Illumina Inc, San Diego, CA, USA).

Bioinformatic analysis of the sequence reads was carried out with mothur v1.41.1 (Schloss, 2021) using the MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP downloaded on 9th July 2018), but adjusting deltaq to 10 in the 'make.contigs' command. Additionally, primers were removed from the start and the end of the sequences, and singletons were also removed from the dataset (Kunin et al., 2010). For the alignment of sequence reads and detection of non-target lineages (e.g. Archaea, Chloroplast, Mitochondria, unknown) the ARB-SILVA SSU Ref NR 132 reference database (Quast et al., 2013) was used. To reveal seasonal community dynamics on a finer scale, operational taxonomic units (OTUs) were assigned at 99% similarity threshold levels. Taxonomic assignment of the 18S rRNA OTUs was carried out using the PR2 v4.10 reference database (Guillou et al., 2013) with a minimum bootstrap confidence score of 80 and applying 1000 iterations, while for the 16S rRNA gene amplicons, the TaxAss software (Rohwer et al., 2017) was used with default parameters based on the FreshTrain (2018 April 30 release) and ARB-SILVA SSU Ref NR 132 databases. The 7th sampling time of Pan no. 60 was discarded from the 18S rRNA gene amplicon dataset due to the low number of high-quality sequences. OTUs assigned to taxa Metazoa, Streptophyta, Basidiomycota, and Ascomycota were excluded from the 18S rRNA gene amplicon dataset due to the prefiltration (through a 40 µm pore sized mesh) of the water samples. For statistical analyses, reads were subsampled to

the read number of the sample having the lowest sequence count (62 samples in the 18S rRNA amplicon set, n = 2407, and 63 samples in the 16S rRNA amplicon set, n = 3188).

OTUs present in all of the five studied soda pans were defined as core5 and OTUs shared between the four turbid soda pans as core4. OTUs not shared between the pans were defined as non-core5 and non-core4, respectively.

4.3.4 Statistical analysis

Statistical analyses were performed using the "vegan" R package (Oksanen, 2017).

The Mantel test was implemented to reveal the similarities between the planktonic microeukaryotic, and prokaryotic communities. Principal component analysis (PCA) was applied to assess the (scaled) environmental variables among samples. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to test the differences of environmental variables between soda pans. To determine the temporal dynamics of community turnover Bray-Curtis dissimilarity between subsequent sampling occasions of each pan was calculated for 18S rRNA and 16S rRNA gene OTUs (from now on eOTUs and bOTUs). One-way permutational multivariate analysis of variance (PERMANOVA, permutations 999) was applied to compare the communities of turbid and colored pans ("adonis" function, permutations = 999) based on Bray-Curtis dissimilarity of the eOTUs and bOTUs. Two-way permutational multivariate analysis of variance (PERMANOVA, permutations = 999) was used to test the seasonality and lake identity of the communities. Non-metric multidimensional scaling (NMDS) was used to visualize microeukaryotic and bacterial plankton communities based on OTU composition using Bray-Curtis distance. The "envfit" function was applied to test the significance of the environmental parameters and plot onto the NMDS ordinations. Mantel test was used to verify the results of the "envfit" analysis. Venn diagrams were generated using jvenn, (Bardou et al., 2014) to visualize the core microbial community of the pans.

4.3.5 Network analysis

Networks were created using the Extended Local Similarity Analysis (eLSA) tool (Ruan et al., 2006; Xia et al., 2013, 2011) based on the parallel time-series data of the five soda pans to reveal microbial associations throughout the study period (i.e., global correlations) and also associations that only occur interim in the time series (i.e., local associations). Furthermore, this

time-series analysis tool can detect associations between coexisting taxa in time (i.e., co-occurrence) and time-shifted correlations also. Two eLSA networks of the microeukaryotic and bacterial communities of each soda pan were created to better understand the synchronous and asynchronous interactions: using the synchronous correlations (i.e., co-occurrence networks, delay 0) and another using only time-shifted correlations (delay 1 or -1). The eLSA was carried out for each pan using the default settings except for adjusting the delay limit to 1 and data normalization with the percentileZ function. To reduce the complexity of the data sets, only microeukaryotic and bacterial OTUs having more than 1% relative abundance in at least one sample and presenting with more than 10 reads in at least three different subsampled samples were included in the network analysis. Only strongly significant ($q < 0.01$ and $p < 0.01$) correlations were included (local similarity scores (LS) and global: Spearman's rank correlation coefficients (SSCC)) as edges of the networks. Network visualization was carried out with Cytoscape v3.8.2. Networks were generated with the edge-weighted spring-embedded layout. In order to identify the keystone OTUs of the networks (i.e., the OTUs that play a key role in the network and their removal would drastically impact the structure of the network) (Berry and Widder, 2014; Jordán et al., 2006), weighted topological importance (WI) measure suggested by Müller et al., 1999 and generalised by Jordán et al., 2006 were used. Weighted topological importance (WI) is an index that calculates the number of neighbours and the number of their neighbours of a given node, while also considering the strength of those interactions. Here we used the indirect interactions up to three steps (WI_i^3). OTUs with $WI_i^3 > 1$ were selected. If less than six OTUs fulfilled this requirement in a network, the selection was expanded to include $WI_i^3 \geq 1$ OTUs. Two heatmaps were generated using "ComplexHeatmap" R package (Gu, 2022) to visualize the clustered key OTUs of each soda pan based on taxonomy and z-score transformed abundance. We evaluated negative keystone OTUs (connected with negative associations to others) and positive keystone OTUs (richly connected with positive associations to others) of the networks.

4.4 Results

4. 4. 1 Environmental parameters

Meteorological data revealed that during the spring period air temperature showed an increasing trend (increase rate: 0.16 °C/day, mean: 14.1 °C), in summer no trend was detected (mean: 22.3

°C), while in autumn there was a clear decreasing trend (decrease rate: 0.17 °C/day, mean: 11.3 °C) (Figure 7.).

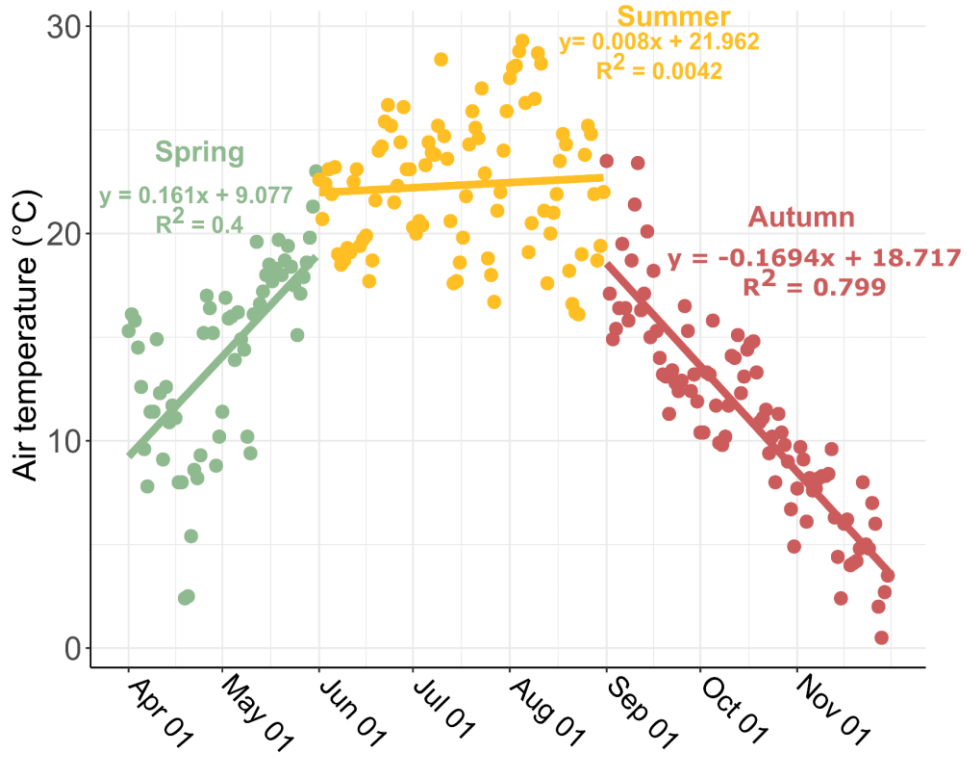

Figure 7. Seasonal changes of air temperature trend in the sampling period in 2017 at Soltszentimre

The limnological parameters measured in this study are given in Supplementary Table S1. Water depth varied greatly (1.5-46.0 cm) during the sampling period with the deepest water levels measured during spring and the lowest in summer and autumn. Zab-szék and Kelemen-szék were completely dried out on some occasions (sampling times 7, 8, 10, 11 and 13 for Kelemen-szék and 11 and 13 for Zab-szék) making sampling impossible. Water temperature increased from 13.7 °C to 27.8 °C during spring (mean: 20.0 °C) and decreased from 24.8 °C to 7.1 °C during autumn (mean: 16.3 °C), while in summer varied between 19.6 and 30.9 °C (mean: 25.2 °C). Salinity values varied between the subsaline (0.9 g/L) and mesosaline (27.8 g/L) categories with the majority of samples (54 out of 63) being hyposaline (3-20 g/L) (Hammer, 1986). The values of DOC, TN and TP varied between 10-3341 mg/L, 1.5-25.7 mg/L and 0.5-25 mg/L, respectively. The pH value remained alkaline during the study period in all pans varying from 8.5 to 10.0. The pans were aerobic at each sampling time (O_2 saturation >79%) and often over-saturated (O_2 saturation >100%). Chlorophyll a concentration ranged

between 1.8 and 696.7 µg/L, with an average of 204.1 µg/L with both the lowest and highest chlorophyll a values measured in Pan no. 60 (Figure 8.).

TN, TP, DO, DOC, salinity, pH and copepoda and cladocera were negatively correlated with water depth, which had the highest values in spring based on the PCA (Figure 9.). Based on the environmental parameters, the colored Sós-ér was clearly separated from the turbid pans with significantly deeper water levels and higher TN values (Figure 8.).

4.4.2 Zooplankton

Copepoda abundance was in general higher in the turbid-white pans (mean: 341 individuals/L) than in the non-turbid, colored Sós-ér (mean: 182 ind/L), although the highest copepod abundance was registered in a summer sample from Sós-ér (7th sampling time: 2043 ind/L). Cladoceran abundance was also higher in the turbid-white pans (mean: 125 ind/L) than in Sós-ér (mean: 55 ind/L) (Figure 10., Table S2.).

The most common copepod species in the turbid-white pans was *Arctodiaptomus spinosus* with the highest abundances in summer and autumn, while in Sós-ér *Arctodiaptomus bacilifer* was the dominant copepod species. The most abundant cladoceran species was *Moina brachiata*, which in average was the most abundant in autumn, although its highest density was measured in summer in Zab-szék (2140 ind/L). The second most abundant cladoceran species was *Daphnia magna* which had the highest abundances in spring (highest abundance 111 ind/L measured in Pan no. 60 at the 4th sampling time) (Figure 11., Table S2.).

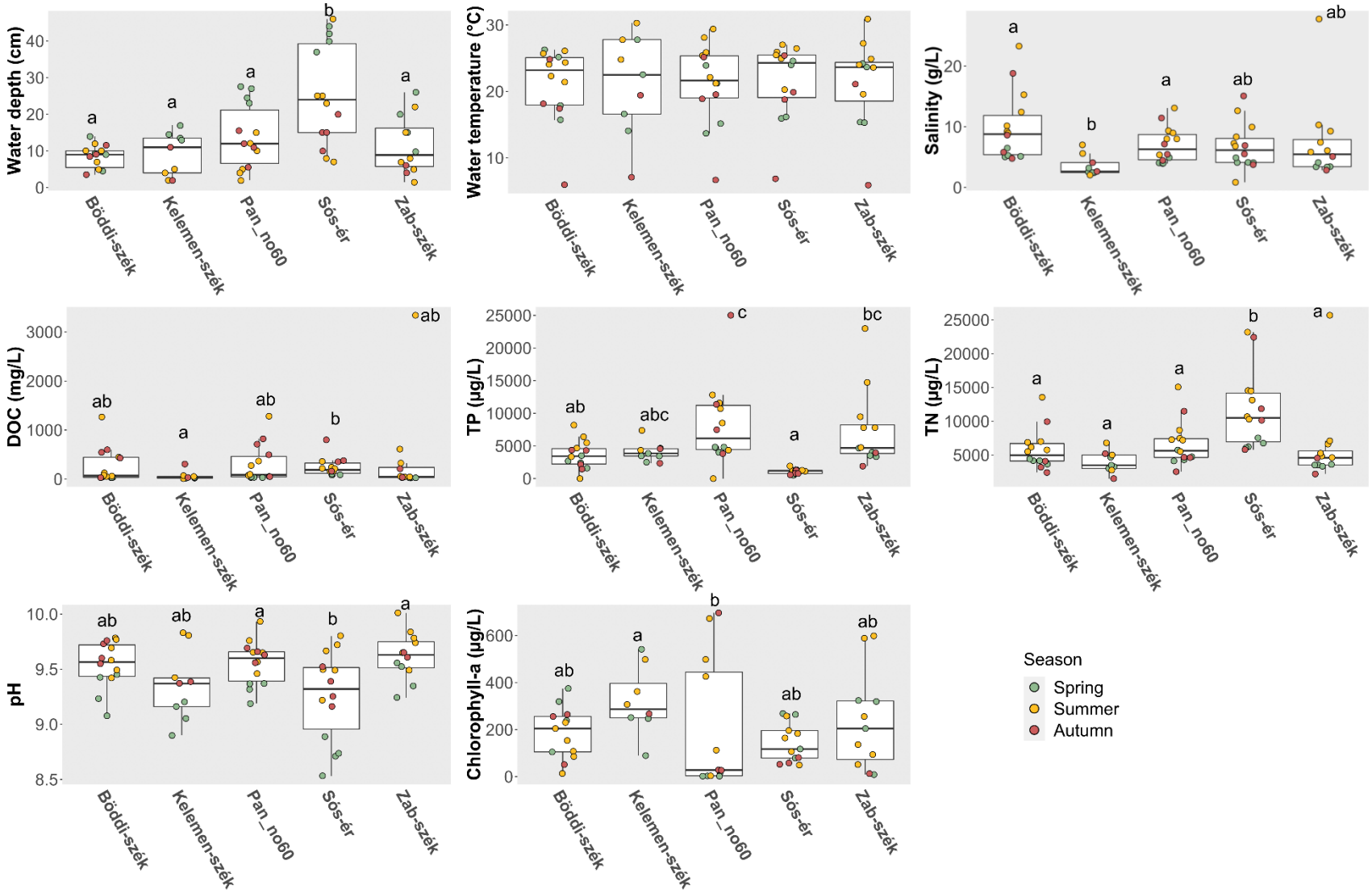

Figure 8. Environmental parameters of the soda pan water samples.

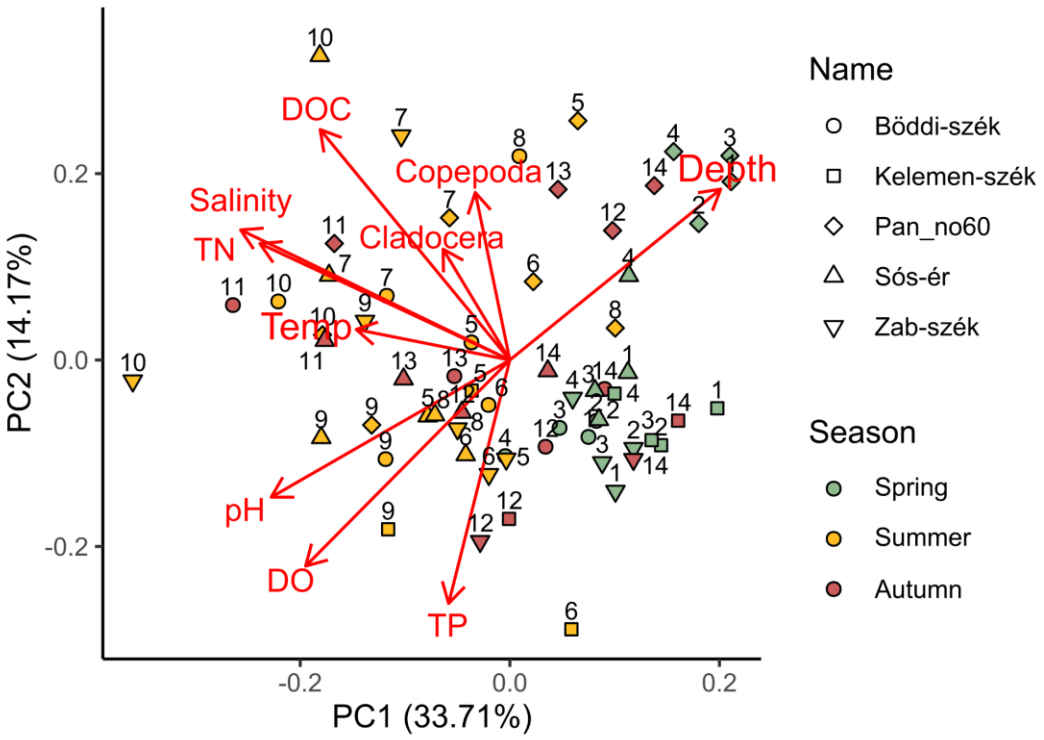

Figure 9. PCA biplot of the environmental variables of the soda pan water samples. Numbers represent the sampling times, different symbol shapes the five pans, while different colours represent the three studied seasons (green for spring, yellow for summer and red for autumn). (Depth = Water depth, Temp = Water temperature, Copepoda = Copepoda abundance, Cladocera = Cladocera abundance)

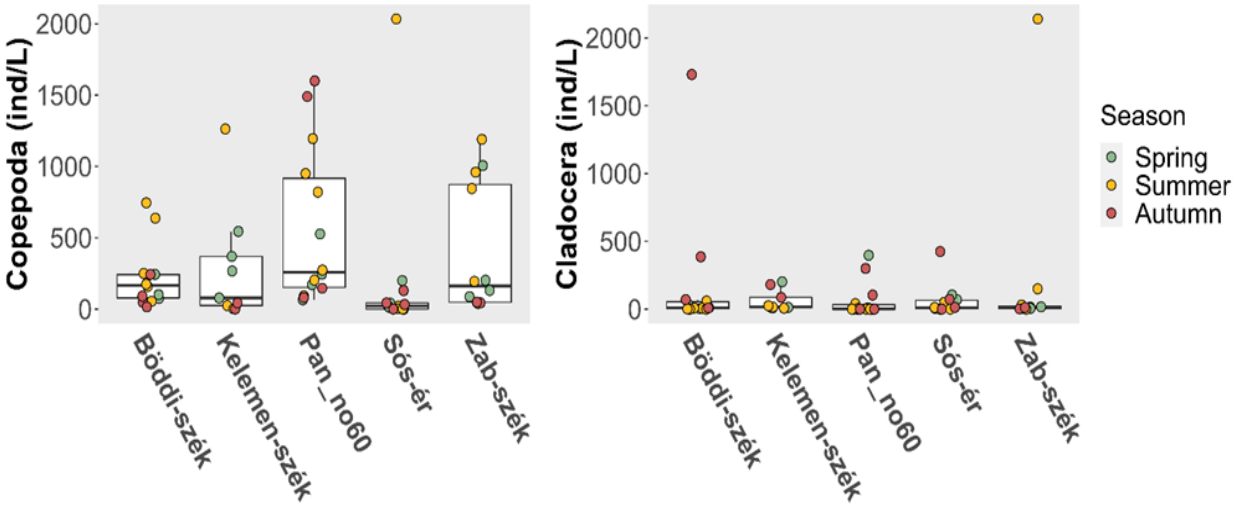

Figure 10. Copepoda and Cladocera abundances in the soda pan water samples

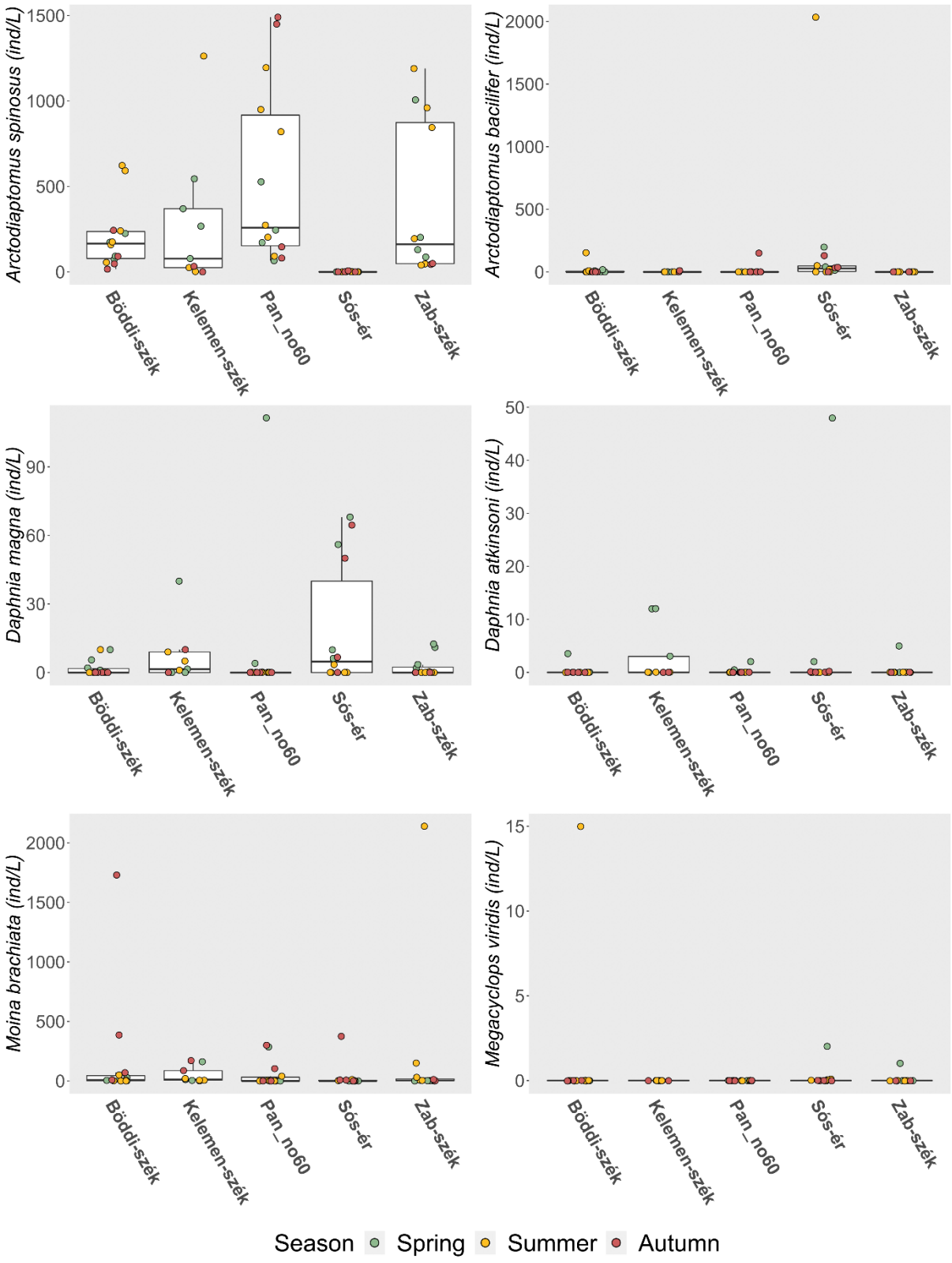

Figure 11. Zooplankton species abundances in the soda pan water samples

4.4.3 Community composition

The three most abundant microeukaryotic phyla in all of the studied pans were Chlorophyta (mean relative abundance 52.5%; 8.1-97.6%), Ochrophyta (14.9%; 0-82.4%) and Fungi (3.9%; 0-59.0%). *Chorycistis* (abbreviated name Ch) and *Chloroparva* (Cl) were the most abundant genera within the phylum Chlorophyta, while *Spumella* and an unidentified Sordariomycetes genera were the most abundant taxa in the phyla Ochrophyta and Fungi, respectively (Figure 12.). Actinobacteria (30.4%; 7.0-69.3%) and Cyanobacteria (10.0%; 0-46.9%) were the most abundant bacterial phyla in all pans. Within the Actinobacteria phylum, sequences assigned to the family Luna1 (Lu), acIII (ac) and Nitriliruptoraceae (Ni) were the most common with the most abundant OTUs belonging to Luna1-A1, Luna1-A2, acIII-A1 and an unclassified Nitriliruptoraceae lineage. Meanwhile Cyanobium_PCC-6307 (Cy) was detected as the most frequent cyanobacterial lineage (Figure 13.).

The single most abundant eOTU in all pans was a green algae affiliated with the *Choricystis* genus with a mean relative abundance of 20.9% (0.1-86.4%). Meanwhile, the mean relative abundance of the second most abundant eOTU (*Chloroparva*) was 5.7% (0-47.3%) and the mean relative abundance of the most abundant bOTU (unclassified Nitriliruptoraceae) was 3.8% (0-29.5%). The most abundant *Choricystis* eOTU showed clear seasonality with a mean relative abundance in spring of 45.7% which decreased to 9.4% in the summer and to only 3.0% in autumn, although by November it increased again to 6.9%. Subsequently, in mid summer the diatom genus *Nitzschia* (Ni) and an unidentified Chrysophyceae (Cy) taxon have the highest relative abundance in Zab-szék and Böddi-szék. During this time in Pan no. 60, heterotrophic nanoflagellates belonging to genus *Andalucia* (Ad) and unidentified Perkinsida (Pe) had high relative abundances. At the beginning of autumn, a heterotrophic nanoflagellate taxa genus *Paraphysomonas* (Pa) became dominant in Pan no. 60, while in Böddi-szék, *Spumella* sp. (Sp), another heterotrophic nanoflagellate had higher relative abundances and were also abundant in Pan no. 60.

There were three desiccation periods in Kelemen-szék, and two in Zab-szék. Following the desiccation/refillment events, drastic shifts in the microeukaryotic community composition were observed. In the case of Kelemen-szék, after the first desiccation, the dominant green algaes (*Choricystis*, *Chloroparva*, unclassified Chlorellales (Cu) and Chlamydomonadales) disappeared and a diatom (genus *Anomoeoneis* (An)) and a ciliate (genus *Halteria* (Hl)) appeared and became dominant. After the second desiccation and refillment genus

Anomoeoneis disappeared, genus *Halteria*'s relative abundance decreased remarkably, green algaes appeared again with genus *Wislouchiella* (Wi) as the dominant one. After the third drought genus *Choricystis* and *Marvania* (Ma) became the dominant green algaes, genus *Halteria* disappeared and another ciliate, unclassified *Hypotrichia* became abundant and a parasite nanoflagellate (genera Pirsonia_Clade_XX (Pi) and unclassified Pirsonia_Clade_XX) appeared in the community. In Zab-szék two drying-out periods occurred, after the first one the dominant green algaes (genera *Choricystis*, *Chloroparva*, unclassified Chlorellales_X, unclassified Chlamydomonadales_X), ciliates (genera *Vorticella* (Vo) and unclassified *Platyophryda* (Pl)) disappeared and a heterotroph nanoflagellate (genus *Spumella*), green algae (genus *Wislouchiella*), parasitic fungi (genus unclassified Chytridiomycetes (Ct)) and unclassified Stramenopiles became dominant. After the second desiccation the heterotroph nanoflagellate (genus *Spumella*), the parasitic fungi (genus unclassified Chytridiomycetes), and the green algae (genus *Wislouchiella*) disappeared. The green algaes (genus *Choricystis*, *Chloroparva*, unclassified Chlorellales_X and *Marsupiomonas*) which were dominant before the first drought became abundant again, but algaes (genus *Diacronema* (Di) and *Nannochloropsis* (Nn), a diatom (genus *Surirella* (Su)), a parasite nanoflagellate (genus unclassified Pirsonia_Clade_XX) and a cerocoza (genus Novel-clade-2X (No)) appeared also in the community. The seasonal community dynamics of Sós-ér differed from those of the other four lakes. Some eOTUs were only dominant (>1%) in Sós-ér, such as the genus *Pythium* (Py), a parasitic fungus, and the green algal genus *Tetracystis* (Te) (Figure 12.).

Regarding phototrophic bacteria, the most abundant lineages were the cyanobacterial groups *Cyanobium*_PCC-6307 (Cy) and *Synechococcus*_MBIC10613 (Sy). While filamentous nitrogen-fixing cyanobacteria belonging to the *Nodularia*_PCC-9350 (No) genus were identified with high relative abundance in July in Sós-ér (Figure 13.).

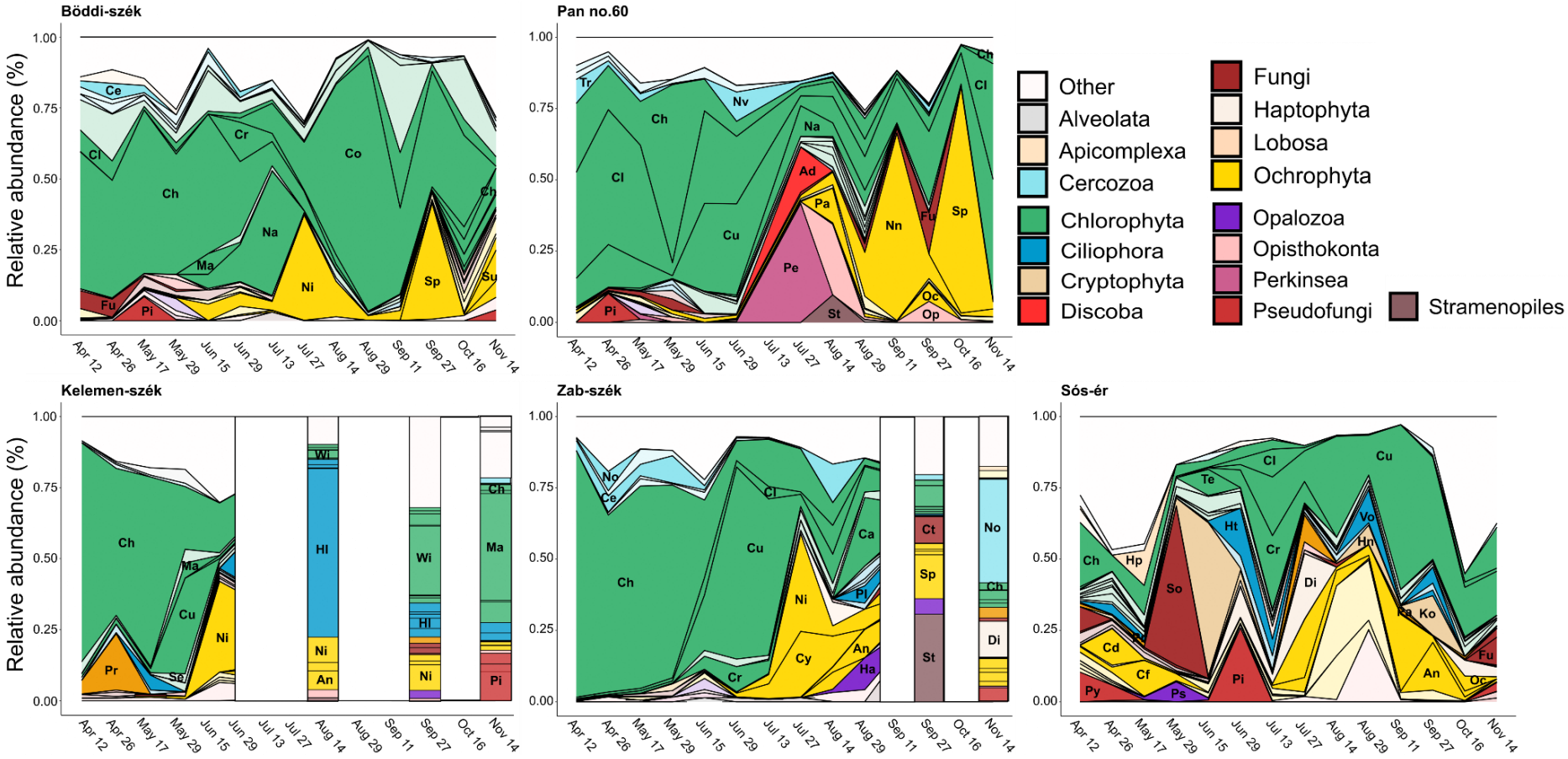

Figure 12. Planktonic microeukaryotic community dynamics of the soda pans based on amplicon sequencing. Figures show eOTUs with > 1% relative abundance in at least one sample of the given soda pan, coloured according to the corresponding phyla. OTUs with > 5% relative abundance in at least one sample are highlighted with vivid colours and indicated by the abbreviated name of the corresponding microeukaryotic genera. Key to the abbreviations can be found in the supplementary material Table S3.

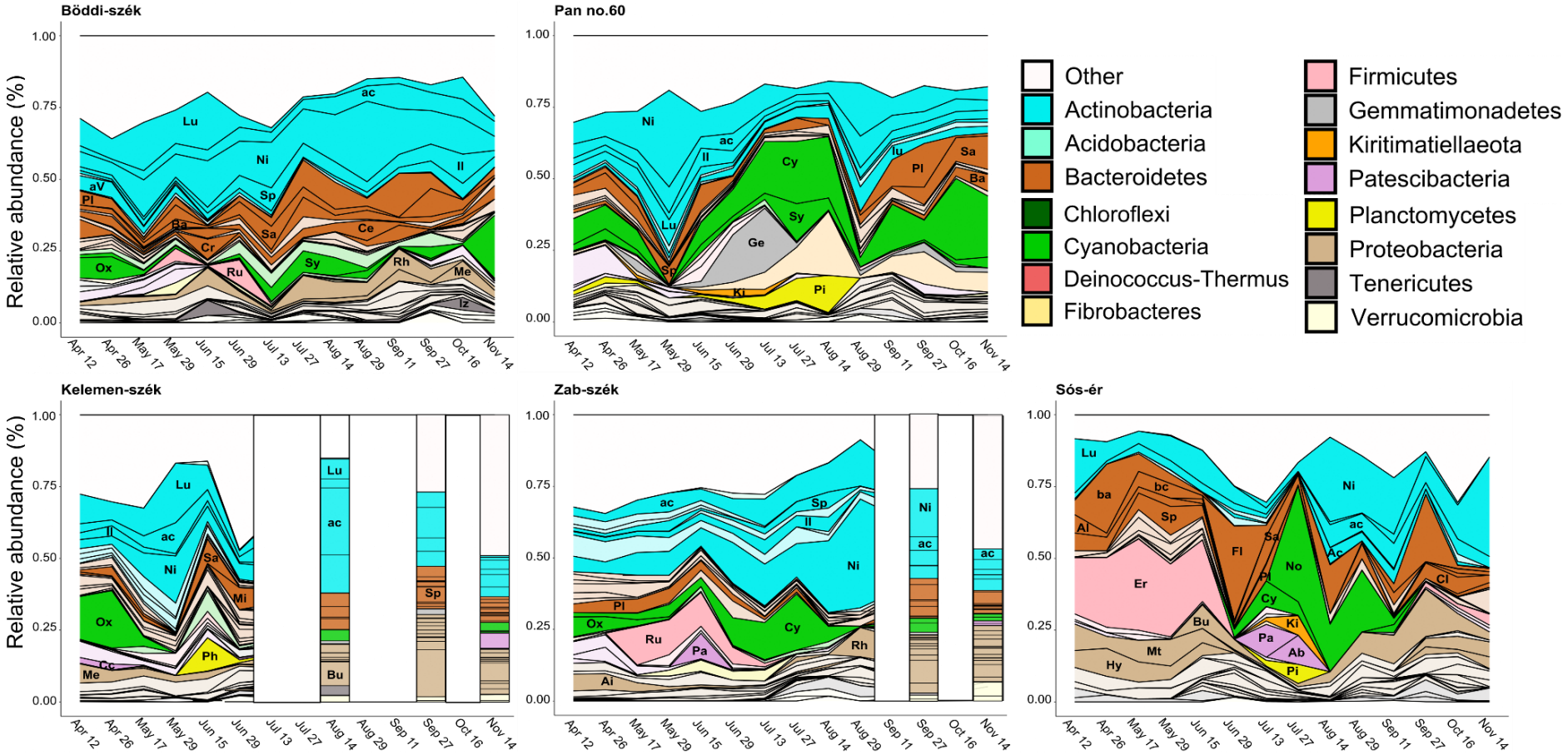

Figure 13. Bacterial community dynamics of the soda pans based on amplicon sequencing. Figures show bOTUs with > 1% relative abundance in at least one sample of the given soda pan, coloured according to the corresponding phyla. OTUs with > 5% relative abundance in at least one sample are highlighted with vivid colours and indicated by the abbreviated name of the corresponding bacterial clades. Key to the abbreviations can be found in the supplementary material Table S3.

4.4.4 Drivers of community changes

The Mantel test detected a moderate significant positive correlation between the bacterial and microeukaryotic community composition as well as between the microeukaryotic and zooplankton communities. There were significant correlations between the environmental parameters and the microbial communities but the correlation was weak for the microeukaryotic communities and very weak for bacteria. No significant correlation was detected between zooplankton and bacterial communities (Table 2.).

Table 2. Mantel test results of the soda pans based on Spearman's rank correlation (rho = 1 "strong positive correlation", rho = -1 "strong negative correlation") (bold: significant)

Mantel test between	Spearman's correlation coefficient (rho)	Significance of the test (p)
Bacterial and microeukaryotic communities	**0.5822**	**0.001**
Microeukaryotic communities and environmental parameters	**0.2781**	**0.001**
Microeukaryotic communities and zooplankton	**0.3969**	**0.001**
Bacterial communities and environmental parameters	0.174	0.011
Bacterial communities and zooplankton	0.0818	0.099

The season of sampling had a significant effect on the communities (PERMANOVA Microeukaryotes: $R^2 = 0.1438$, $p = 0.001$; Bacteria: $R^2 = 0.1149$, $p = 0.001$) with the strongest differentiation between the spring samples from the summer and autumn samples (Table 3.).

Based on the NMDS plots and the PERMANOVA analysis both microeukaryotic and bacterial communities of the colored Sós-ér showed clear separation from the communities of the other four turbid pans (microeukaryotes: $R^2 = 0.0861$, $p = 0.001$; Bacteria: $R^2 = 0.1447$, $p = 0.001$). The envfit analyses of the five lakes significantly ($p < 0.05$) fitted the following environmental

variables on the NMDS plots: salinity, pH, DOC, TN, oxygen, and *Daphnia magna* abundance and water depth for the microeukaryotic and salinity, pH, DOC, TN, TP, oxygen, water temperature and chlorophyll a for the bacterial communities (Figure 14.). The Mantel tests enforced the significance of DOC, TN, and TP for microeukaryotic and bacterial communities, and water temperature for bacterial communities. Although, water temperaturea and DOC for the bacterial communities were only marginally significant (p=0.046), while salinity and DO were only significant for the microeukaryotic communities (Table S4.).

Table 3. Differences of planktonic microbial communities from the five soda pans comparing the three studied seasons. (PERMANOVA test results; number of * indicates the statistical significance with p. 0 '***. 0.001 '**'. 0.01 '*'. 0.05 '·'. 0.1 ' '. 1)

	Spring/Summer (R2)	Spring/Summer (p)	Spring/Autumn (R2)	Spring/Autumn (p)	Summer/Autumn (R2)	Summer/Autumn (p)
Microeukaryotes	0.129	0.001***	0.185	0.001***	0.043	0.014*
Bacteria	0.102	0.001***	0.124	0.001***	0.039	0.045*

Lake identity always explained more variance than the season of sampling according to the two-way PERMANOVAs. Meanwhile, the two-way PERMANOVAs testing only the four turbid pans showed that in the case of all OTUs of microeukaryotic communities, the season of sampling explained more variance (20.6%) than the identity of the pans (14.4%), while for bacterial communities the variances explained by the two factors were similar (16.0% and 17.7%, respectively). The same analyses performed on only the core communities revealed very similar patterns with seasonality having a stronger effect (22.6%) on the microeukaryotic core4 communities than lake identity (14.4%), while for bacterial core communities, the variances explained by the two factors were very similar (16.0% and 17.7%, respectively). However, for the noncore communities lake identity explained more variance than the season of sampling for

both microeukaryotes (13.0% and 8.34%, respectively) and bacteria (16.7% and 10.8%) (Figure 15., Table S5.).

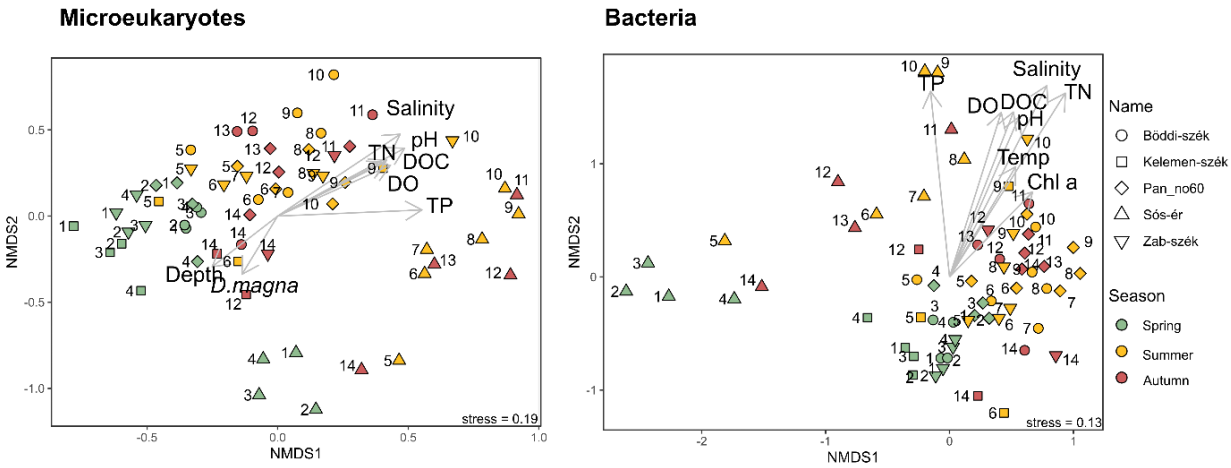

Figure 14. Non-metric multidimensional scaling (NMDS) ordination with the significantly fitted environmental parameters of planktonic microeukaryotic and bacterial communities of five soda pans

4.4.5 Community turnover

Microeukaryotic communities of the turbid pans showed low turnover at the beginning of the sampling, in April-May, according to the low Bray-Curtis dissimilarity (< 0.5). BC dissimilarity of Pan no.60, Zab-szék, Kelemen-szék, and Böddi-szék increased between the late spring and early summer sampling occasions which indicated a shift in the community composition between spring and summer. BC dissimilarity remained high (> 0.5) from mid July till the last sampling occasion in all pans suggesting a higher turnover. Kelemen-szék and Zab-szék dried out during our study period, BC dissimilarity was very high before and after each desiccation, suggesting a drastically different community structure after each refillment. BC dissimilarity trend in Sós-ér was quite different from the turbid pans, it remained high during the sampling period indicating a high turnover (Figure 16.).

Contrary to the microeukaryotic communities bacterial BC dissimilarity did not increase as a consequence of desiccation events in late summer and in autumn. Only Sós-ér and Kelemen-szék BC dissimilarity increased between spring and summer, the other pans BC dissimilarity remained quite low during the summer suggesting a high turnover. Pan no. 60's BC dissimilarity decreased while Böddi-szék's increased and Sós-ér's varied in autumn (Figure 16.).

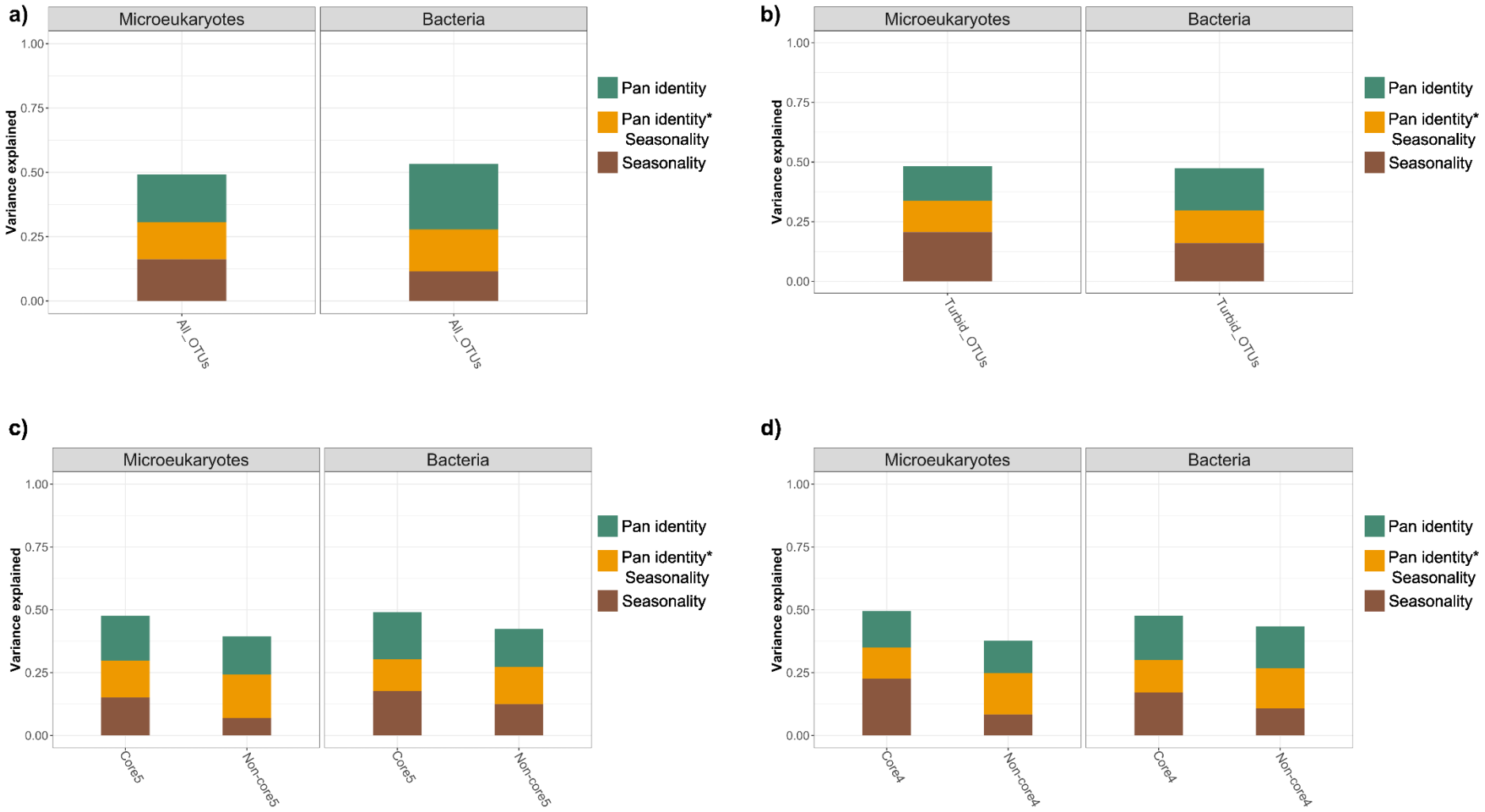

Figure 15. Impact of pan identity and seasonality on the structure of microeukaryotic and bacterial communities based on two-way PERMANOVA analysis

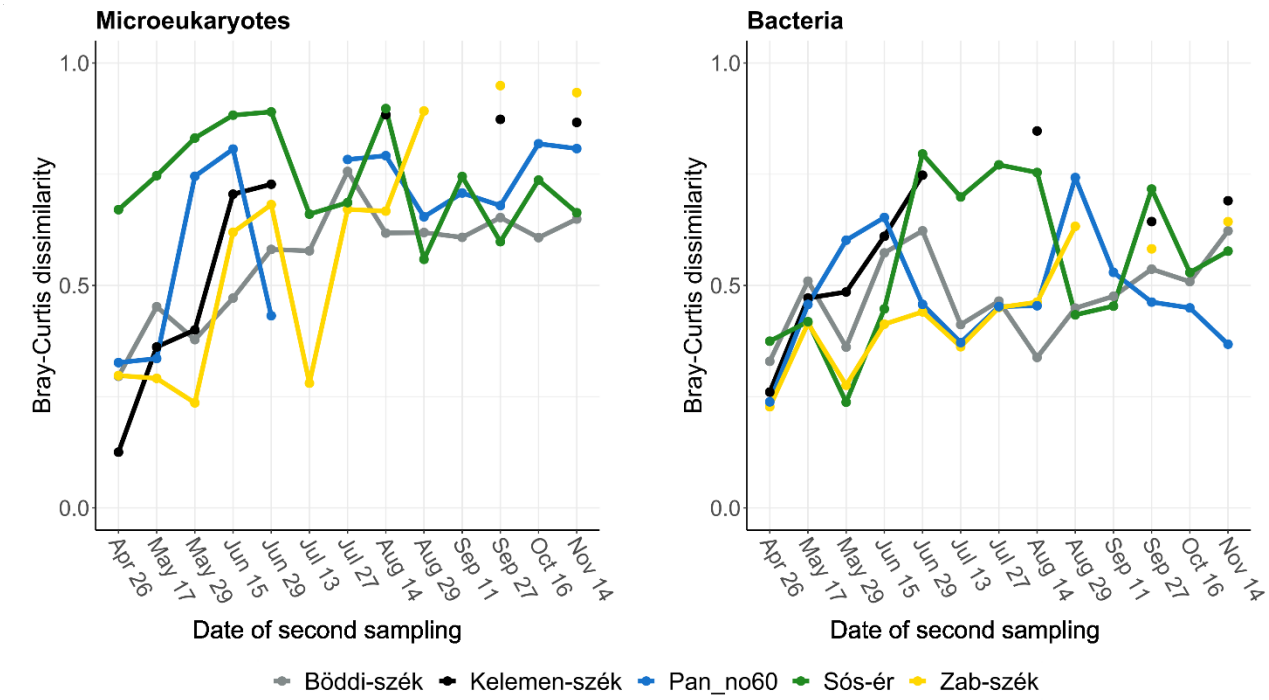

Figure 16. Bray-Curtis dissimilarity index between subsequent samplings as a proxy for planktonic microbial community turnover (the X-axis represents every second date of the sampling)

4.4.6 Core microbial community

Total of 97 eOTUs (which were 61.8% of the 18S rRNA amplicon reads) and 191 bOTUs (which were 66.83% of the 16S rRNA amplicon reads) were shared among the five lakes and was defined as core5 community (Figure 17. a, c). The OTUs of the core5 community were the most dominant OTUs in all of the pans, like primary producer taxa *Choricystis* (eOTU1) *Chloroparva* (eOTU2), *Nannochloropsis* (eOTU9, eOTU38), *Nitzschia* (eOTU11, eOTU21), heterotrophic nanoflagellate (HNF) genera *Spumella* (eOTU6) and *Paraphysomonas* (eOTU50), ciliate genus Halteriidae (eOTU23), photosynthetic primary producers *Cyanobium* (bOTU5, bOTU22), and bacterial heterotrophic groups Nitriliruptoraceae (bOTU1, bOTU6) and Luna1-A2 tribes (bOTU2), acIII-A1 (bOTU3, bOTU10), Luna1-A1 (bOTU9, bOTU35. Zab-szék and Kelemen-szék shared the lowest number of OTUs with 38 eOTUs and 35 bOTUs. The microbial community composition of the colored Sós-ér significantly differed from the other four turbid pans, so we also determined the OTUs shared by the four turbid pans (Figure 17. b, d). (Böddi-szék, Kelemen-szék, Pan no.60 and Zab-szék) (i.e., core4). 952 eOTUs (which represented 80.3% of the 18S rRNA amplicon reads) and 988 bOTUs (which represented 84.4% of the 16S rRNA amplicon reads) belonged to the core4 community. Zab-szék, Böddi-szék and Pan no. 60 shared 432 eOTUs and 568 bOTUs which was the highest amount, 87 eOTUs and

125 bOTUs were shared between Kelemen-szék and Pan no. 60 which was the lowest amount of shared OTUs (Figure 17.).

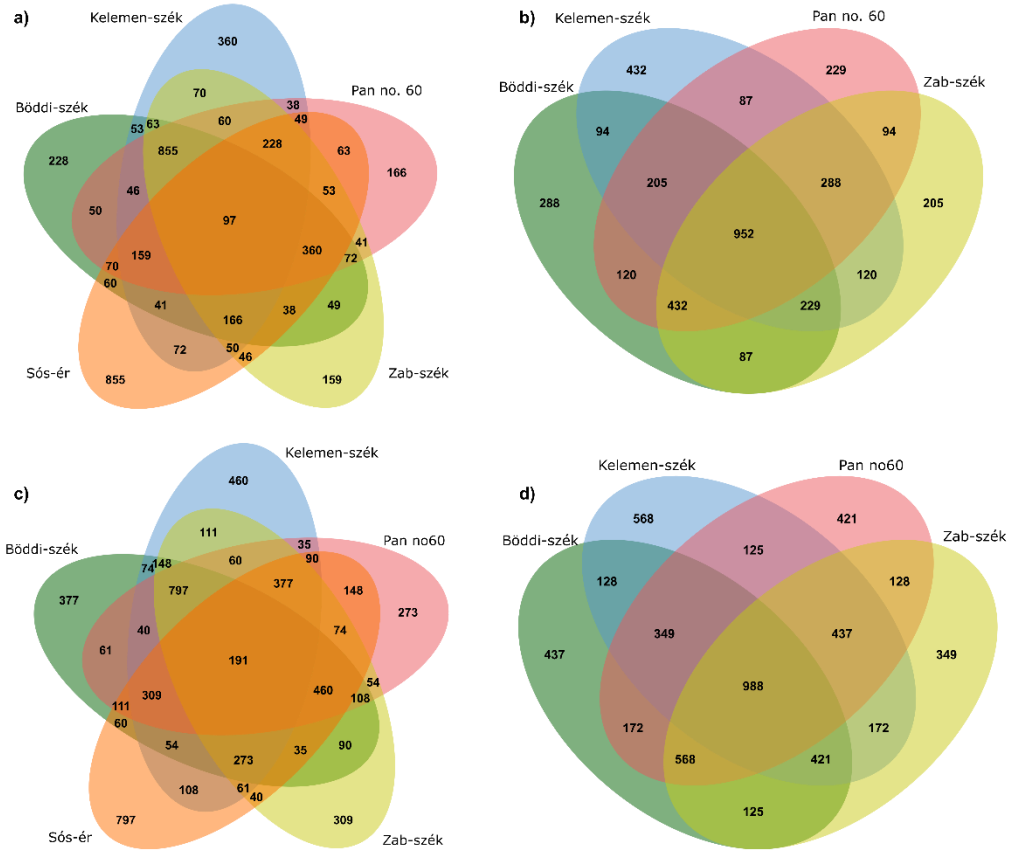

Figure 17. Venn diagrams showing the numbers of shared and unique planktonic microbial OTUs: a) microeukaryotic OTUs of all the five lakes, b) microeukaryotic OTUs of the four turbid type of soda pans, c) bacterial OTUs of all the five lakes, d) bacterial OTUs of the four turbid type of soda pans

Not surprisingly Sós-ér had the lowest contribution of core5 reads to microeukaryotes and bacterial communities as well with 30% and 51%. Sós-ér was followed by Kelemen-szék and Zab-szék (with 58% and 64% of core5 eOTU reads and 65% and 67% of core5 bOTU reads), the pans with occasional desiccations. Böddi-szék and Pan no.60 (permanent turbid pans) had the highest contribution of core5 (with 79% for eOTU reads and 75% for bOTU reads) (Figure 18). The contribution of core4 showed similar patterns to core5 with the lowest ratio in Kelemen-szék (desiccated for the most extended period and most times), 65% eOTU reads and 79% bOTU reads (Figure 18.).

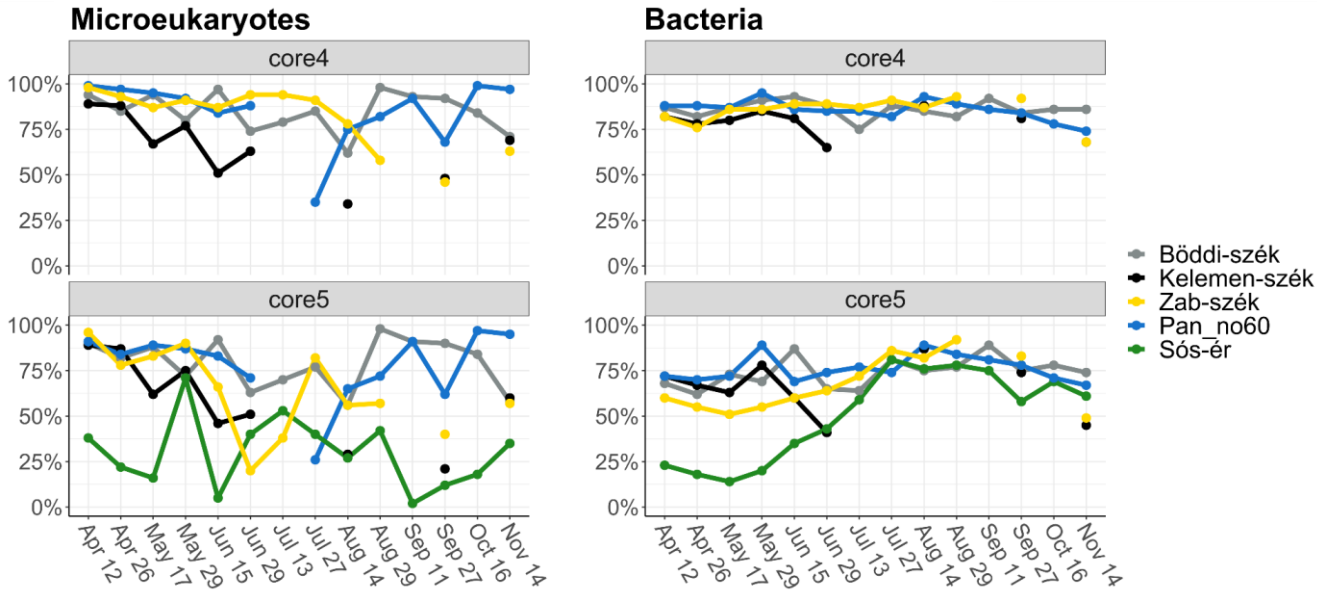

Figure 18. Relative abundance of the core4 and core5 OTUs in the studied pans during the study period

4.4.7 Microbial interactions

We constructed five synchronous and five time-shifted networks based on the significant LS and SSCC correlations. Regardless of the pan (turbid or colored) or type of network (synchronous or time-shifted), all of the networks had more positive correlations than negative (Table 4.). The time-shifted networks had more correlations than the synchronous ones for Böddi-szék and Pan no. 60, while for the other pans, it was the opposite. The number of SSCC correlations was always higher in the time-shifted networks than the LS correlations and the difference between the two correlation types was the largest in Böddi-szék and Pan no.60. In the case of synchronous networks Böddi-szék and Pan no. 60 had more SSCC correlations than LS, while for the others there were more LS correlations. The synchronous network of Kelemen-szék and the time-shifted network of Böddi-szék had the highest number of edges and the average number of neighbours. In contrast, the two networks of Sós-ér had the lowest numbers of these two parameters (Table 4.). The synchronous network of Kelemen-szék and the time-shifted network of Böddi-szék were also the most dense, while the synchronous network of Sós-ér and time-shifted network of Zab-szék had the lowest density. The networks of Sós-ér also had the least amount of nodes. In the case of Böddi-szék and Pan no. 60 the number of nodes was nearly the same in the time-shifted and synchronous networks, while for

the other three pans, the number of nodes of the synchronous networks was higher than for the time-shifted networks.

Based on network topology, the synchronous and time-shifted networks of Böddi-szék and Pan no. 60 formed two distinct hubs connected mainly with SSCC edges (i.e., global correlations). The nodes of the synchronous networks of the desiccated pans Kelemen-szék and Zab-szék were connected mostly with LS edges (i.e., local correlations). Sós-ér and Zab-szék's time-shifted network was the most fragmented. In the case of Sós-ér, an additional hub corresponding to the high water level period in July was distinguishable when a *Nodularia* bloom took place in the community composition (Figure 19.).

Table 4. General network properties of the five synchronous and five time-shifted networks generated from the Network Analyzer of Cytoscape v3.8.2,
a) Synchronous b) Time-shifted, (* Number of edges = Number of negative correlations + Number of positive correlations)

a)	Lake	Number of nodes	Number of edges*	Average number of neighbours	Density	Number of negative correlations	Number of positive correlations
	Böddi-szék	199	1417	14.24	0.07	506	911
	Kelemen-szék	170	2672	31.44	0.19	1020	1652
	Pan no. 60	176	848	9.88	0.06	304	544
	Sós-ér	139	314	5.17	0.05	53	261
	Zab-szék	212	1839	17.35	0.08	549	990
b)							
	Böddi-szék	202	2246	22.24	0.11	911	1335
	Kelemen-szék	147	689	9.37	0.06	207	482
	Pan no. 60	182	1304	14.55	0.08	508	796
	Sós-ér	99	153	4.03	0.07	12	141
	Zab-szék	156	535	7.08	0.05	85	450

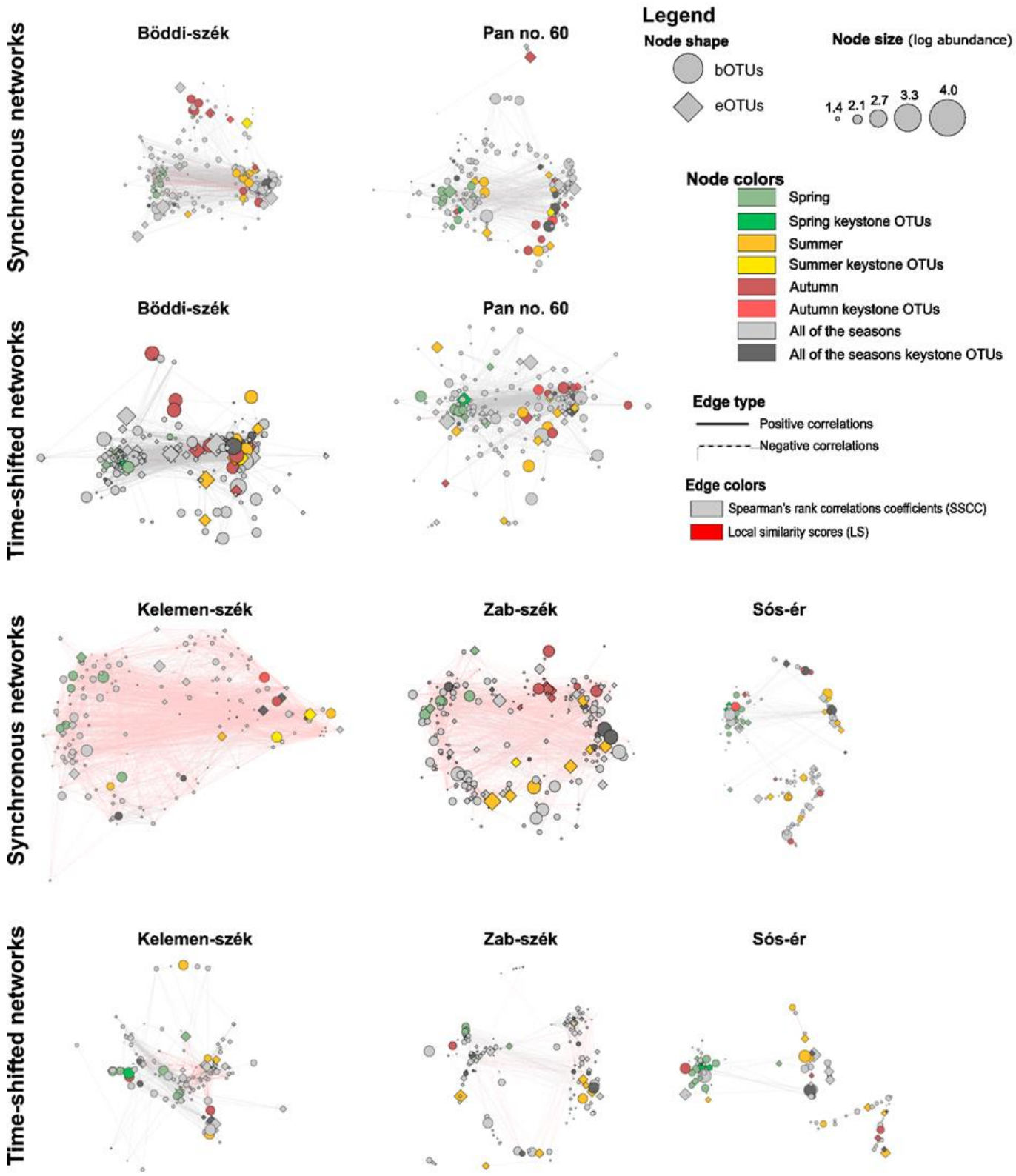

Figure 19. Synchronous and time-shifted networks of planktonic bacterial and microeukaryotic OTUs of the soda pans. The colouring of the network nodes was based on the difference of the mean abundance of the corresponding OTUs in spring, summer or, autumn:

green = deviation of the mean abundance of the sampling period < mean abundance in spring nodes
yellow = deviation of the mean abundance of the sampling period < mean abundance in summer nodes
red = deviation of the mean abundance of the sampling period < mean abundance in autumn nodes
grey = deviation of the mean abundance of the sampling period > mean abundance of spring, summer and autumn nodes. The colouring of the key OTUs is brighter than the other colours.

Most of the keystone OTUs identified were bacteria. Among the studied pans, the colored pan had more microeukaryotic keystone OTUs than the turbid pans (Figure 20.). Only 36% of microuekaryotic keystone OTUs belonged to the core5 and 58% were core4. The majority of the bacterial keystone OTUs (79%) were core5, while almost all of the bacterial keystone OTUs (91%) were core4.

There were taxonomic and seasonal differences among positive and negative keystone OTUs (Figure 20.). Positive keystone OTUs had higher abundance in spring, while in autumn negative keystone OTUs tend to be more abundant. The most common taxa among both positive and negative microeukaryotic keystone OTUs belonged to Archaeplastida (primary Chlorellales) and the SAR-subgroup (Stramenopiles-Alveolata-Rhizaria). Meanwhile, there was one negative Opisthokonta OTU and five positive ones including two assigned to the parasitic Cryptomycotina. There were more taxonomic differences among the positive and negative bacterial keystone OTUs. Among positive keystone OTUs Bacteroidetes (with 10 keystone OTUs) was one of the most common, and then Proteobacteria (with 7 keystone OTUs) and Actinobacteria (with 6 keystone OTUs). Actinobacteria (with 7 keystone OTUs) was the most common among the negative keystone OTUs on the phylum level, followed by Proteobacteria (with 13 keystone OTUs) and Bacteroidetes (with 6 keystone OTUs). Most of the actinobacterial keystone OTUs were abundant in our study period and belonged to Nitriliruptoraceae (with 10 keystone OTUs) and acIII-A1 (with 3 keystone OTUs) lineages. Positive actinobacterial keystone OTUs had higher abundances in spring and belonged to Luna1-A (with 3 keystone OTUs), and acIV-C (with 1 keystone OTUs).

Microeukaryotic keystone OTUs did not have notable taxonomic differences regardless of the type of the network, while actinobacterial keystone OTUs were more common in the synchronous networks (16 vs 8) and Bacteroidetes keystone OTUs were more common in the time-shifted networks (10 vs 6).

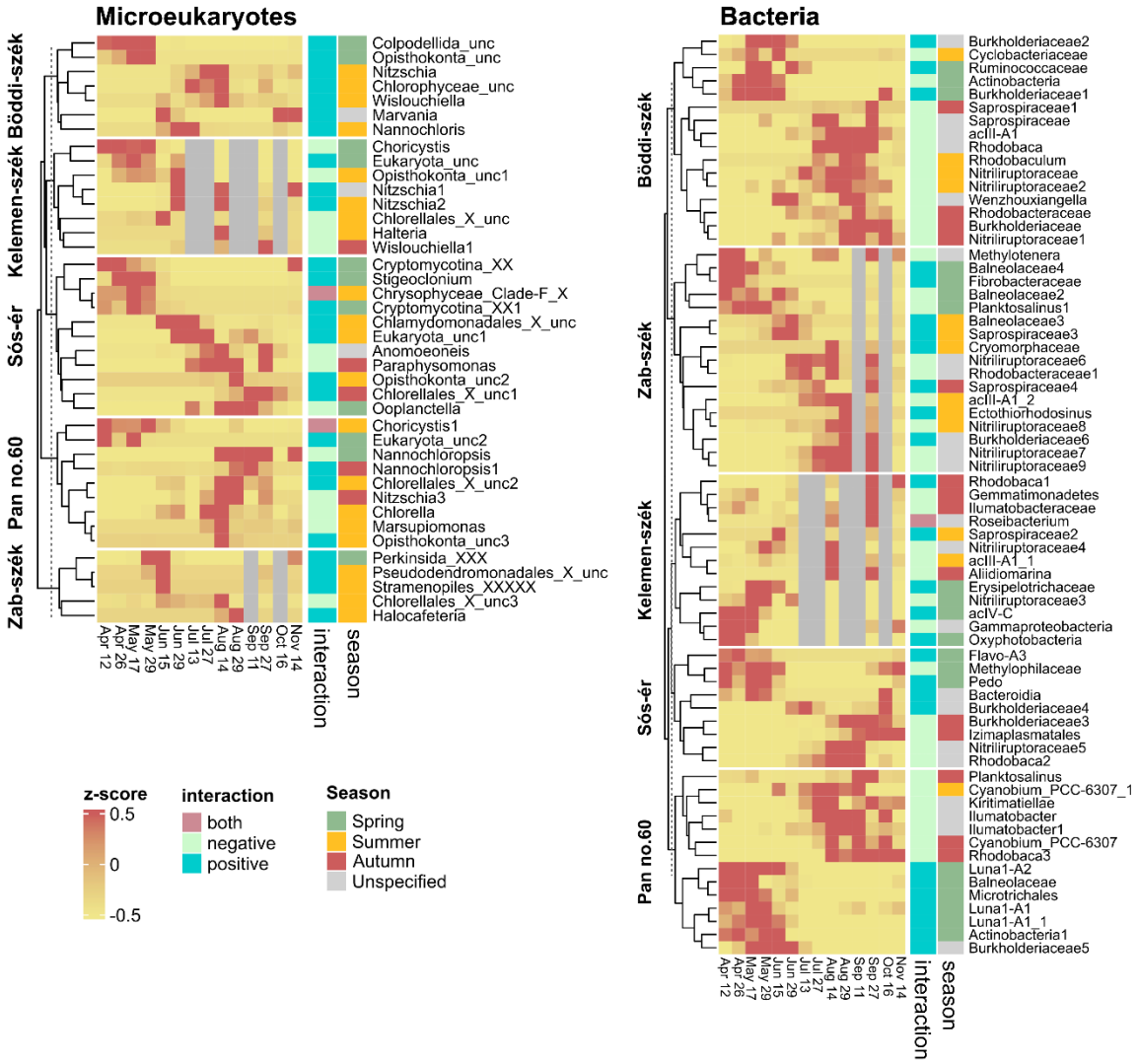

Figure 20. Taxonomic affiliation and distribution of the top keystone planktonic microbial OTUs of the networks based on their z-score transformed abundance. Row annotations represent the keystone OTUs interaction in the network and the season when the keystone OTUs were dominant (their mean z-score transformed abundance was positive).

4.5 Discussion

Our study focused on five parallel time series on the bacterial and microeukaryotic community composition of shallow, nutrient rich, alkaline lakes to disentangle the ecological role of microeukaryotic and bacterial communities and their seasonality. The study sites represented the same aquatic habitat and due to their geographical proximity, they were exposed to identical meteorological conditions during the studied period. The studied pans shared a core microbial community and similar seasonal dynamics, however sudden environmental effects (e.g., desiccation events) resulted in shifts in the microbial composition. Nevertheless, this is the first report that provides detailed information on microeukaryotic community composition of the Carpathian Basin soda pans obtained by eDNA amplicon sequencing.

Although having the same pH, salinity, and chemical characteristics, Sós-ér has non-turbid, brown water with a high amount of CDOM (coloured dissolved organic matter) and DOC (dissolved humic substances) (Boros et al., 2020). The pan also has abundant shoreline vegetation in contrast to the other sites which prevents the wind from stirring up the sediment. Sós-ér differed the most from the other four turbid lakes, due to the significantly deeper water levels, higher TN concentrations, and different microeukaryotic and bacterial communities compositions which was reflected in the lower amount of OTUs in core5 than in core4. The contribution of Sós-ér to core5 eOTUs was low during the study period, also numerous unique microeukaryotic taxa were only abundant in this pan, which suggested that the selection of microeukaryotic taxa was based on the soda pan type. The contribution of core5 bOTUs was high just like in the turbid pans, indicating a similar selection force and no dispersal limitation between the five pans for the bacterial communities. Furthermore, Sós-ér's network topology was different from the other four turbid pans, as well the turnover of eOTUs was quite high, which suggested highly dynamic communities. The turnover of Sós-ér bOTUs was high during summer and autumn, and in spring it was low suggesting a stable spring bacterial community. The sudden summer rainfall could have led to the elevated groundwater levels, which resulted in higher nutrient inflow to the Sós-ér which could explain the summer cyanobacteria bloom in July. However, the neighbouring cattle farm could also be the cause of the higher nutrient levels. The presence of certain bacterial groups (unc. Erysipelotrichaceae, unc. Ruminococcaceae, *Ruminiclostridium*) could be also related to local cattle or buffalo herds which are used for wetland management by the local nature conservation authorities. These ruminants frequently

wallow in the pans. Taxon Erysipelotrichaceae was previously reported from soda pans of the Carpathian Basin (Szabó et al., 2020), however, it has been described mainly from the digestive systems of mammals and insects (Cox et al., 2017; Tegtmeier et al., 2016; Wu et al., 2021).

Seasonality had a great impact on the microeukaryotic and bacterial communities of all pans. Regardless of the differences between Sós-ér and the four turbid pans, we observed various common seasonal dynamics of the microbial succession, like the similarity of spring and autumn communities. Occasional droughts occurred during our sampling period, Kelemen-szék was dried out on five sampling occasions, constituting three distinct desiccation periods altogether, while Zab-szék had two distinct desiccation events. Every desiccation ended with refillment during the study. Desiccation and refillment combine physical and chemical stress on the microorganisms due to drastic changes in the habitat's water, salt, and nutrient content (Schimel, 2018). These stresses can alter the community composition by shifting the dominance and relationships of taxa. Based on our results, the microeukaryotic community was more sensitive to these changes, while the bacterial community remained more stable. This difference between microeukaryotes and bacteria can be driven by trophic interactions or adaptation strategies. Also, desiccation can alter the trophic food web and the length of the food chain (McIntosh et al., 2017). Based on previous studies, microbes inhabitating saline aquatic habitats, with fluctuating salinity prefer the energetically more expensive salt-out strategy (exclusion of salt ions from the cytoplasm) rather than the salt-in strategy (accumulation of inorganic material) as adaptation strategy (Menéndez-Serra et al., 2021). Overall Bray-Curtis dissimilarities in community compositions between sampling times were greater for microeukaryotes on average, assuming the pans' core bacterial community has the ability to withstand sudden changes in environmental conditions. Core communities explained higher seasonal variance than noncore communities, which supported our hypothesis that seasonal adaptation is primarily driven through species recruitment from the core community. Although, the lake identity of the five pans explained higher variation than the lake identity of the four turbid pans.

Based on earlier studies on soda pans of the region, phytoplankton biomass is generally high in these sites and the phytoplankton is dominated by pico-sized algae (> 3 μm) (Boros et al., 2017; Felföldi et al., 2009; Somogyi et al., 2017, 2010, 2009). Planktonic microeukaryotic algae are dominated by genera *Choricystis* and *Chloroparva* and other unclassified/uncultured green

algae taxa which showed seasonal dynamics in previous studies (Somogyi et al., 2016, 2011). Picocyanobacteria community composition is mostly composed of coccoid small cells (Somogyi et al., 2009) belonging to genera *Synechococcus* and *Cyanobium* (Felföldi et al., 2011, 2009). Within the phylum Chlorophyta, genera *Choricystis*, *Chloroparva*, unclassified Chlorophyceae, unclassified Chlorellales, *Marvania*, *Nannochloris*, unclassified Chlorophyta and *Wislouchiella* were the most abundant primary producers in our samples. Eukaryotic OTU1 which was affiliated with genus *Choricystis* was one of the most abundant eOTUs in all of the studied lakes in the sampling period. It is widely distributed in the phytoplankton communities of freshwater lakes, rivers, and ponds. It can be associated with ciliates and metazoans (Kulakova et al., 2020). Genus *Chloroparva* is another dominant picoeukaryotic green algae in soda pans which were first described from Böddi-szék (one of our studied sites) by Somogyi et al. (2011). Eukaryotic OTU2 which belongs to the genus *Chloroparva* was the second most abundant OTUs in all of the pans. Microalgae *Marvania* has an interesting cell reproduction, called "budding", which is a modified version of autosporulation (Yamamoto et al., 2007). Genus *Wislouchiella* is a loricated green algae with flagellates (Hepperle et al., 1998) which was abundant at shallower water depths and higher salinity. In all of the pans, a shift was observed in the dominance of green algae, which showed their seasonality. Genus *Choricystis* had a higher relative abundance than the other green algae during spring, but in summer their relative abundance decreased and unclassified Chlorellales or unclassified Chlorophyceae became abundant. Around autumn another shift occurred when genus *Choricystis'*s relative abundance increased again. Representatives of genus *Nitzschia* were the most abundant diatom in all of the pans; they appeared mainly in summer (June-July). Previous studies recorded the genus *Nitzschia* as the dominant species from soda pans and pools of the region (Ács et al., 2017; Stenger-Kovács and Lengyel, 2015). Representatives of another diatom genus, *Anomoeoneis*, were also abundant in all of the pans at the end of summer.

Protozoa are important consumers of organic debris and microorganisms in freshwater and marine systems. They act as trophic links between primary producers, bacteria, and zooplankton (Burns and Schallenberg, 2001). Heterotrophic flagellates and nanoflagellates as bacterivores are really important links of microbial food webs, especially their role in carbon transfer to higher trophic levels (Kellogg et al., 2019). However, their identification and enumeration with classical microscopic techniques are still challenging, and it can be especially difficult from turbid habitats like soda pans, characterized by a high amount of suspended sediment (Arndt et

al., 2000). Jakobids are a group of bacterivorous flagellate, they harbour the most bacteria like mitochondrial genomes (Burger et al., 2013; Rodríguez-Ezpeleta et al., 2007). The term jakobids have been used to describe the "core jakobids" (*Seculamonas, Reclinomonas, Histiona, and Jakoba*) and malawimonads (Marx et al., 2003). From a phylogenetic point of view, malawimonads share several ultrastructural features with jakobids (Rodríguez-Ezpeleta et al., 2007), so we treated them as two separate groups. In our study, genera *Seculamonas, Reclinomonas,* and *Jakoba* were identified; however, their relative abundance was not detected above 3% within the microeukaryotic community. Genus *Andalucia* also belongs to the group of bacterivorous jakobids and its relative abundance was higher during summer in Pan no. 60. Genera *Halocafeteria, Malawimonas, Ochromonas, Paraphysomonas,* and *Spumella* were present as heterotrophic nanoflagellates in the pans. Genera *Malawimonas* and *Ochromonas* relative abundance didn't reach 3% within the microeukaryotic community. *Spumella* is the most common bacterivorous freshwater heterotrophic nanoflagellate (Matz et al., 2002; Mylnikov et al., 2008). Its relative abundance showed strong seasonality, it was only detected in the autumn samples of the pans. *Paraphysomonas* cells are similar to *Spumella* cells, except for the diverse silica scale (Boenigk et al., 2005). Genus *Paraphysomonas* were abundant in Sós-ér pan and Pan no. 60. Genus *Halocafeteria* had higher relative abundance in Zab-szék at the end of August. Overall, heterotrophic flagellates and nanoflagellates were abundant when the lakes were close to desiccation and mesozooplankton number was low in the water.

The ciliate genera *Vorticella* and *Epistylis* were observed in the soda pan Nagyszék by Stiller in 1963. These genera were also detected by our amplicon sequencing approach, but only eOTUs assigned as unclassified *Sessilida*, unclassified *Hypotrichia*, *Halteria,* and unclassified Halteriidae were detected with higher relative abundances. Genus *Halteria* often dominates the pelagic ciliate community in freshwaters; it has been described as an abundant bacterivore in meso- and eutrophic ponds (Simek et al., 2000). Most of the members of the order Sessilida attach to the surface of a substrate (Zhan et al., 2009). Taxa belonging to the order Hypotrichia are also common in freshwater, sea, and soil (Zhu et al., 2019). The resulting high relative abundance of genera *Halteria*, unclassified *Halteriidae,* and *Hypotrichia* in the community composition after drying-out periods of pan Kelemen-szék could be due to the resuspending force of the wind and their fast reproduction rates. Genera *Halteria* and unclassified Halteriidae were also detected in the Sós-ér pan, especially during summer. Genus *Vorticella* appeared only at one-time point (2017.08.29) in pan Zab-szék and Sós-ér when water depth was very low,

close to desiccation in both pans. Genus *Vorticella* is a common ciliate in many aquatic habitats. Species of *Vorticella* and peritrichs are suspension feeders; they feed on bacteria, phytoplankton, or organic debris (Buhse et al., 2011).

Crustacean zooplankton was dominated by typical soda lake species like *Daphnia magna*, *Arctodiaptomus spinosus* and *Moina brachiata* (Horváth et al., 2014). *Daphnia magna* usually feeds on a wide size range of organisms, such as bacteria, ciliates, HNFs and algae. It could have a key role in altering the food web structure (Berga et al., 2015). *Arctodiaptomus spinosus* is an eurythermic species, it can reproduce at high and low temperature also (Dokulli and Herzig, 2009). They are mainly feeding on algae, rotifers, and protists. One of the most abundant microcrustacean species was *Moina brachiata*. It has advantageous traits in unstable environments such as short egg development time (Nédli et al., 2014). Crustaceae zooplankton grazing pressure can influence the community composition of planktonic bacteria and microeukaryotes. Following *A. spinosus* abundance peaks in the water during late spring and early summer, the relative abundance of dominant green algae decreased and diatoms appeared in the phytoplankton. Diatoms are more resistant to grazing than green algae, due to their thick silicate cell walls (Lürling, 2021). *D. magna* was more abundant during spring when the pans had higher water levels and salinity was lower. Due to the lowest turbidity and salinity of Sós-ér instead of *A. spinosus*, *A. bacilife*r was the dominant copepoda species. *A. spinosus* is an indicator species of high quality soda pans, and it is more dominant in turbid pans, while *A. bacilifer* is more common in degraded turbid pans or colored pans (Tóth et al., 2014).

Parasites have a number of properties that enable them to control the host population, such as reducing the survival rate or the fertility of the host (Hall et al., 2009). Also, parasites are really important top-down controllers in aquatic food webs; they contribute to the transfer of carbon and energy between trophic levels (Frenken et al., 2017). However, planktonic parasites of soda pans of the Carpathian basin still remain understudied. Representatives of class Chytridiomycota have been present in all of the soda pans. Only eOTU75 had a higher relative abundance than 3% (in autumn), after the first desiccation event in Zab-szék. eOTU75 were only detected in Böddi-szék and Zab-szék pans. Chytridiomycota can cause mass mortalities and changes in the phytoplankton community size, distribution, or succession. Chytrids have a free-living motile stage (zoospore) while they search for their host by chemotaxis. After they found their host organism, they develop rhizoids to extract nutrients from it. Zoospores and

chytrids themselves can serve as nutrient rich food sources for zooplankton (Frenken et al., 2017). Although, the abundance of chytrids didn't correlate with the abundance of green algae in any of our studied pans. *Pythium* is a genus of soil-borne saprobes or facultative pathogens of terrestrial plants, marine algae, invertebrates, and mammals (Badis et al., 2020). Several species of *Phytium* were described as pathogens to marine red algae, but only one isolate is known which could infect marine green algae (Ulva species). The infection of *Pythium* has a salinity limitation, high salinity (30 ppt, equivalent to 37 g/L) can delay the infection progress, but it can survive in the infected algae for up to two weeks. Sporulation was not observed on infected algae; probably floating terrestrial debris is the infecting vector (Herrero et al., 2020). *Pythium* was only detected in the Sós-ér pan in spring (May). During this period, the relative abundance of *Choricystis* green algae started to decrease, but we did not find any significant correlation between green algae and genus *Pythium* abundance. Members of the *Pirsonia* genus are well-documented parasitoid nanoflagellates of diatom species. Some previous studies showed that a higher pH level (> 8.7) can increase the diatom's survival by suppressing the parasitic infection (Guinder et al., 2018). The nutrient uptake of genus *Pirsonia* superficially resembles the method of chytrids (Kühn et al., 1996). *Pirsonia* was observed with high relative abundance in our dataset during spring and autumn, while the relative abundance of diatoms was low. One possible explanation for this observation is our biweekly sampling method, which could be not frequent enough to observe the diatom abundance changes in the pans. An alveolate protist, belonging to Perkinsida was also identified from the lakes. It is mainly known to be a parasite of marine oysters and molluscs (Mangot et al., 2011). Molluscs are generally rare in soda pans, but *Anisus spirorbis* is a common species of soda waters (Boda et al., 2019; Boros et al., 2013). With increasing salinity molluscs populations are decreasing, but next to *Anisus spirorbis*, *Planorbanius corneus*, *Valvata cristata*, *Valvata piscinalis*, *Gryalusu crista*, *Gryalus albus* and *Segmentia nitida* can survive a wide range of salinity (Boros et al., 2013). Data on the molluscs community was not available from our sampling period of the studied soda pans.

We identified the same bacterial taxa as studies before (Felföldi, 2020; Korponai et al., 2019; Szabó et al., 2020, 2017) like lineages (i.e., Luna1-A and acIV-C) that are characterized by very small cell-sizes (< 0.1 μm^3, Duda et al., 2012). Also, it has been suggested that these lineages are grazing resistant. Order Rhodobacterales, clade acIII-A, unclassified Nitriliruptoraceae, and genus *Ectothiorhodosinus* were more abundant in the pans when salinity increased and the

water level was shallow. Clade acIV-C had higher relative abundance in spring and the beginning of the summer when the water level was higher in the pans. Genus *Nodularia*_PCC-9350 had a higher relative abundance in July, in only pan Sós-ér, during the surprisingly high water level in the pan. The high water level was probably caused by precipitation and groundwater inflows from the surrounding area. Overall the microeukaryotic and bacterial community composition of the pans showed a clear correlation with seasonality and lake identity as well.

Spring had analogous properties in the five soda pans, although according to the community structure and interactions, it differed the most from summer and autumn. Spring was characterised by positive keystone OTUs. Mutualistic and facilitative interactions usually result in positive synchronous relationships, but parasitism, predation, or similar niche preference can also mean positive associations. However, the direction of correlations in species association networks can be positive or negative and it is not always obvious which one it is, for example, if species have similar niche preferences it can reflect as positive synchronous correlations (coexistence) or it can be also negative due to competition or exclusion. Furthermore, predation can be positive synchronous associations when the prey attracts the predator, but also negative synchronous or time-shifted associations when the predator terminates their prey (Barberán et al., 2011; Faust and Raes, 2012; Röttjers and Faust, 2018). Positive keystone eOTUs abundant in spring belonged to flagellates like Colpodellida (Mylnikov, 2009) or parasitic taxa such as Cryptomycotina (Letcher et al., 2017; Rachik et al., 2018) and Perkinsozoa (Mangot et al., 2011). Positive actinobacterial keystone OTUs were assigned to lineages like Luna1-A and acIV-C that have been identified in different aquatic habitats, including soda lakes (Ghai et al., 2012; Newton et al., 2011; Szabó et al., 2020).

After the analogous trajectory of spring, microbial community turnovers started to increase even for the turbid pans which were driven by the warming temperature of summer, and shrinking habitat size. This was in agreement with previous studies showing that shrinking habitat size modifies community assembly and reduces stability (Bier et al., 2022). Desiccation periods were considered local stressors, due to the fact that desiccation is common in soda pans of this region, but not every pan dries out every year, and also different pans dry out in different years. Microeukaryotic communities turnover increased quite uniformly in the turbid pans and it increased extensively at each drying-rewetting cycle which suggested limited resilience

against local stressors. It was reflected in the increased contribution of non-core4 after desiccation events, supporting the hypothesis that non-core OTUs are more important in the response to sudden environmental events.

The turnover of bacterial communities remained quite similar in the turbid pans throughout the study period regardless of the desiccation events. The turnover of bacteria only increased when water levels were extremely low (≤ 2 cm) suggesting that extremely shallow water levels are a strong selective force on bacterial communities. Otherwise, bacteria were more resistant to desiccation periods, and summer-autumn conditions than microeukaryotes. Negative keystone bOTUs had a high abundance in summer-autumn which indicated that bacterial groups were outcompeting each other under different conditions. The contribution of core4 bOTUs remained very high at each sampling occasion and site suggesting that the core bacterial community of turbid pans is highly resistant to quickly changing conditions and also helps to adapt to seasonal changes. According to previous studies (Fazi et al., 2013, 2008; Székely and Langenheder, 2017) drying-rewetting cycles have a strong selection force on bacterial communities and dispersal is needed for full recovery, which suggests the high contribution of core4 bOTUs regardless of desiccation periods is due to no dispersal limitation between the studied soda pans.

We generated networks to study the interaction of microeukaryotes and bacteria. All of the networks had more positive associations than negative implying the dominance of positive interactions in the communities. Previous studies showed that positive correlations are more common in habitats characterized by high abiotic stress due to a higher number of mutualistic interactions letting species exist in harsher environments than otherwise would be possible (Hernandez et al., 2021; Travis et al., 2005). However, the dominance of positive associations (mutualisms) reduces network stability, especially in the case of low-modularity networks (Hernandez et al., 2021). Based on network topology, there was a clear difference between the colored Sós-ér, the two occasionally desiccated turbid pans (Kelemen-szék and Zab-szék), and the two turbid pans that did not dry out (Böddi-szék and Pan no. 60). The network of the soda pans reflected low community stability in these extreme habitats that was further aggravated by sudden environmental events (desiccation) and cyanobacteria blooms.

5. Temporal dynamics of microbial diversity along environmental gradients

5.1 Introduction

Temporary pans are a widely distributed habitat type that supports diverse aquatic communities and ecological functions (Céréghino et al., 2007; Olmo et al., 2022). While temporary pans are known to be important for a variety of macroorganisms such as amphibians (Boix et al., 2020; Fritz and Whiles, 2021; Griffiths, 1997), and macroinvertebrates (Florencio et al., 2014; Meland et al., 2020), their microbial communities remain understudied (Hahn, 2006). Despite their small size, microorganisms play an essential role in nutrient cycling and biogeochemical processes in temporary aquatic ecosystems (Felföldi, 2020; Grossart et al., 2020). The link between microbial biodiversity and ecosystem functions highlights the importance of identifying the drivers of microbial communities in temporary pans to better understand their functioning and ecosystem services (Bell et al., 2005; Trivedi et al., 2019). However, studies on microbial diversity in temporary pans are still less common than on macroorganisms (Marrone et al., 2022).

Environmental harshness in temporary pans is higher compared to permanent ones due to periodic droughts and seasonal changes in environmental conditions, which makes it challenging for local species to survive (Tweed et al., 2011; Wellborn et al., 1996). Shallow temporary aquatic habitats require species to withstand high UV radiation (Aguilar et al., 2016) and extreme daily fluctuations in temperature (Boros et al., 2017), while saline temporary waters face additional environmental stress caused by high salinity levels and their seasonal fluctuation (Lengyel et al., 2019). Microorganisms have a high physiological and evolutionary adaptation potential, which enables them to sustain large local population sizes even in such extreme conditions. Despite this, little attention has been paid to the diversity of microbial communities in saline inland waters compared to estuaries (Šolić et al., 2015; Traving et al., 2017) and man-made salterns (Ali et al., 2016; Paul et al., 2020; Tkavc et al., 2011). Studies on temporary saline waters are generally limited to specific groups such as diatoms (Stenger-Kovács et al., 2016, 2014; Szabó et al., 2018) or bacteria (Szabó et al., 2020, 2017), based on small spatial scales and a limited number of habitats (Cunillera-Montcusí et al., 2022). Although salinity has been a focal point of studies (Benlloch et al., 2002; Horváth et al., 2014; Mo et al., 2021), there is still limited information about the phylogenetic diversity of microorganisms along salinity gradients in temporary pans and how community composition or the relative share of multiple functional groups changes along these gradients. Regional-scale studies could

provide valuable insights into these patterns and processes, but they are still largely lacking (Cunillera-Montcusí et al., 2022).

5.2 Aims

In this study, we examine the diversity, phylogenetic diversity, and community composition of microeukaryotes and prokaryotes along local environmental gradients in 26 temporary soda pans during two consecutive spring seasons. The pans are located in eastern Austria and have salinities ranging from sub- to hyposaline values. As the pans are in close proximity, they are under similar climatic and geographical conditions, making them a suitable regional case study for examining the environmental drivers of microbial communities. However, the long-term future of these inland saline ecosystems is threatened by the intensifying effects of climate change, which include decreasing precipitation and increasing temperature in the region (as shown in Figure 21.). The primary aim of this study is to identify the main environmental drivers of taxonomic and phylogenetic richness and composition of microorganism communities. Additionally, we compare the results between the dry spring of 2017 and the wetter 2018 and to earlier reference data from 2009-2010 to determine if the identity and strength of the main drivers change with contrasting climatic conditions.

5.3 Materials and methods

5.3.1 Study area

Our study sites are located in the Seewinkel region of Austria, in the national park Neusiedlersee-Seewinkel (Figure 21., Table S6.). Water samples were collected from 26 soda pans in 2017 (3-6 of April, spring) and 2018 (2-4 of April, spring). The sampled two years had very different weather and hydrological conditions. Four months prior to the 2017 sampling, the average monthly precipitation was half of the measured average monthly precipitation in 2018 (Table 5.), while the annual temperature increased in the region (Figure 21.), which can contribute to higher evaporation rates. These led to an overall difference in the hydrological conditions in the region, with one of the soda pans (Kirchsee) being completely dried out in our study period in 2017.

5.3.2 Sample collection and environmental parameters

We measured water depth and Secchi depth in the open water area at each sampling site. 20 L of water was collected from at least 20 different points of the soda pans using a one-liter plastic

beaker and filtered through a plankton net (mesh size of 100 µm) to remove larger zooplankton and filamentous algae and also to prevent sequencing bias in community composition assessments. 1 L of the sieved composite water sample was delivered back to the laboratory in a glass bottle in a cool box for total suspended solids (TSS) measurements and molecular samples. We collected a similar composite water sample (unfiltered) to measure conductivity and pH with a multimeter and to take samples for total nitrogen (TN) and total phosphorus (TP), which later were stored frozen until further processing.

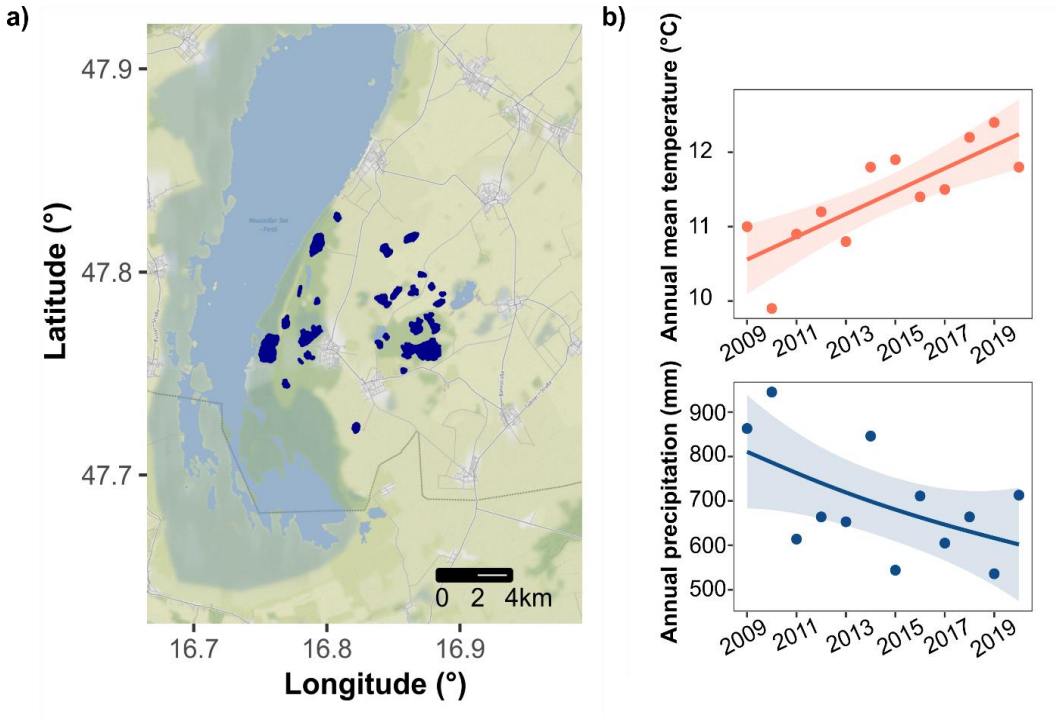

Figure 21. a) Location of the sampled soda pans (dark blue) in the Neusiedlersee-Seewinkel National Park in eastern Austria (Map was created with R v. 4.2.2 (R Core Team, 2022) b) Temporal trends in annual mean temperature and precipitation since 2009 based on meteorological data from the Seewinkel region (Station Eisenstadt)

For TSS measurements, water samples were filtered through a pre-weighted GF/F filter (Ø 24 mm, 0.7 µm pore size) until it was clogged. Afterward, the filters were incubated at 60 °C for desiccation and their weight was measured to calculate the TSS concentration. TN and TP were measured according to Clesceri et al., (1999) (Hansen and Koroleff, 1999).

We filtered 1-50 ml of the composite water samples (depending on turbidity) through a nitrocellulose membrane filter (Ø 47 mm, 0.22 µm pore size) for molecular biology purposes. Filters were stored at -20 °C until further processing.

The annual temperature and precipitation data from 2009 till 2020 were downloaded on 2022.01.12. from the ZAMG - Zentralanstalt für Meteorologie und Geodynamik, Station Eisenstadt.

Table 5. Meteorological data of the Seewinkel region four months prior to the sampling in April 2017 and April 2018 (source: ZAMG - Zentralanstalt für Meteorologie und Geodynamik, Station Eisenstadt)

Year	Month	Average temperature (°C)	Amount of precipitation (mm)	Sunshine duration (hour)	Average precipitation in the four months (mm)
2016	December	1.2	20	90	
2017	January	-3.7	14	95	
2017	February	3.0	44	83	26
2017	March	9.2	34	196	
2017	December	3.0	57	82	
2018	January	3.8	33	58	47
2018	February	-0.8	45	75	
2018	March	3.2	53	117	

5.3.3 Community analysis

DNA for the microbial community was isolated from the filters with PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The isolated DNA was stored at -20 °C until shipping to LGC Genomics (Berlin, Germany) for amplicon sequencing.

Amplification of the the V7 region of 18S rRNA and the V4 region of the 16S rRNA genes was carried out by LGC Genomics (Berlin, Germany), using eukaryotic primers UnivF-1183mod (AATTTGACTCAACRCGGG) – UnivR-1443mod (GRGCATCACAGACCTG) (Ray et al., 2016) and prokaryotic primers EMBf 515F (GTGYCAGCMGCCGCGGTAA) (Parada et al., 2016) – EMBr 806R (GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015). PCR contained 20 μL of 1 x MyTaq buffer, 1.5 units of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 2 μl of BioStabII PCR Enhancer (Sigma-Aldrich Co.), 15 pmol of each primer and 1 μl of template DNA. The following thermal cycle conditions were used: initial denaturation at 96 °C for 1 min and an additional 15 sec, followed by 30 cycles (annealing at 55 °C for 30 sec, extension at 70 °C for 30 sec). PCR products were pooled and purified with Agencourt AMPure XP beads (Beckman Coulter, Inc., IN, USA) to remove primer dimer and also with an additional purification on MiniElute columns (QIAGEN GmbH, Hilden, Germany). Illumina libraries were prepared with about 100 ng of the DNA pools using the Ovation Rapid DR Multiplex System 1-96 (NuGEN Technologies, Inc., CA, USA). Furthermore, the libraries were pooled and size-selected by preparative gel electrophoresis and the amplicon sequencing was performed on an Illumina MiSeq platform by LGC Genomics (Berlin, Germany) (Szabó et al., 2022).

Bioinformatic analysis was performed the same way as we describe in chapter 3.3.3. with the modification that we used mothur v.1.43 and the lowest read count was 8620 reads per sample for the 16S rRNA dataset and 2432 reads per sample for the 18S rRNA dataset.

The rarefied microeukaryotic and prokaryotic data were further split into six larger groups based on taxonomy (according to higher categories) and function and later on referred to as groups. This included four groups of microeukaryotes, namely (1) ciliates, (2) fungi, (3) eukaryotic phytoplankton (referred later simply as phytoplankton), and (4) heterotrophic flagellates and nanoflagellates (HF-HNF) and two groups of prokaryotes, namely (1) autotrophic cyanobacteria and (2) non cyanobacteria (referred to as bacteria). The groups were created with the subset_taxa function in the “phyloseq” package (McMurdie and Holmes, 2013).

Data are openly available in the NCBI SRA database as part of the BioProject accession PRJNA748202.

5.3.4 Statistical analysis

We used a set of environmental variables as reference data from the 2009 summer and 2010 spring for 16 pans, which were also sampled in our study period (2017 and 2018). 2009 and 2010 were characterized by higher mean annual precipitation and lower annual mean temperature in the region so we could use them as references for our study (Figure 21.). We applied PCA to assess the overall environmental variability across four years (2009, 2010, 2017 and 2018). Prior to all analyses, we normalized the distribution of all environmental parameters, log transformation was used for conductivity, TP, TN, and TSS, and square root transformation was used for water depth data. All data were standardized to unit variance and then we calculated an Euclidean distance matrix from all normalized environmental data using the "vegan" package (Oksanen, 2017). Pairwise comparisons with a PERMANOVA was applied to test the differences between the four years using the "pairwiseAdonis" R package by adjusting for multiple comparisons, using the same Euclidean distance matrix (Martinez, 2020).

Paired t-tests were used to test the differences between the two years' environmental variables which were sampled in both years, with the "ggpubr" package (Kassambra, 2022).

Richness (OTU richness) and Pielou's evenness (from now on referred to as evenness) were calculated using the "phyloseq" package (McMurdie and Holmes, 2013). Phylogenetic trees were created with the FastTree software (Qiime2 plugin) (Burian et al., 2022). Phylogenetic diversity (PD) was calculated by Rao's quadratic entropy with the function "raoD" from the R package "picante" (Kembel et al., 2010). The three measures of diversity were compared in the two sampling years with paired t-tests on a subset of samples that were sampled in both sampling years.

Non-metric multidimensional scaling (NMDS) was used to visualize microeukaryotic and prokaryotic plankton communities based on OTU composition using Bray-Curtis distance using the "vegan" package (Oksanen, 2017).

Canonical correspondence analysis (CCA) was performed to examine the relationship between environmental variables and the community composition of prokaryotes, microeukaryotes (based on phyla), and the six major groups (Ciliates, Fungi, HF-HNF, Phytoplankton, Bacteria,

Cyanobacteria) (based on relative abundances of OTUs) separately (using the package "vegan", (Oksanen, 2017)). We log(x+1) transformed the relative abundance data of prokaryotes and microeukaryotes prior to analysis. To reduce the complexity of the datasets, only those prokaryotic and microeukaryotic phyla were included in the analysis that had more than three occurrences and more than five percent maximum relative abundance each year. In the case of the six major groups OTU tables were used, but OTUs that have less than four occurrences were excluded from the analysis. Permutation tests (n = 199) with backward and forward stepwise model selection were used to identify significant environmental variables. Total variation explained by significant variables was determined by partial CCAs, where only significant variables were kept as constraining variables and all of the others were partialled out as conditioning variables. The explained variation was constrained by the ratio of constrained and total inertia.

We compared the fit of multiple linear regression models (ML, with the "MASS" package of R) (Venables and Ripley, 2002) and generalized additive models with smooth terms (GAM, using the "mgcv" package of R, Wood, 2017) to determine which environmental variables had the strongest effect on diversity (richness, evenness, and phylogenetic diversity/PD). We selected the best fitted models based on Akaike's Information Criteria (AIC). We fitted separate models for the diversity indices of prokaryotic and microeukaryotic groups and the three time periods: the two sampling years separately or pooled together. We created a summary table of the best fitted models using (ML or GAM) the "ComplexHeatmap" package (Gu, 2022). For each group and time period (2017, 2018 separately and 2017-2018 together), we used GAM model predictions to identify the direction of responses of richness, evenness, and PD to environmental variables (using the "mgcv" package of R, Wood, 2017). We only used richness, evenness, and PD if their relationship was significant in at least one of the three time periods.

We applied multiple linear regression models with the same selection method to test the changes in local richness (subtracting 2018 values from 2017 values per habitat) and changes in each environmental variable between 2017 and 2018 to link differences in local richness to potential environmental drivers.

Also, we used generalized additive models (GAM) with smooth terms (using the "mgcv" package of R, Wood, 2017) to analyze the temporal trends of the annual mean temperature and

annual precipitation data from 2009 till 2020. We created six models for the temperature and precipitation data with different numbers of knots (2-7). The same model selection was used as before, while the final model was checked with the "gam_check" function of the "mgcv" package of R.

5.4 Results

We found significantly lower water levels in 2017, which was coupled with significantly higher values of conductivity, TN, TP, TSS and pH (Figure 22., Table S7.).

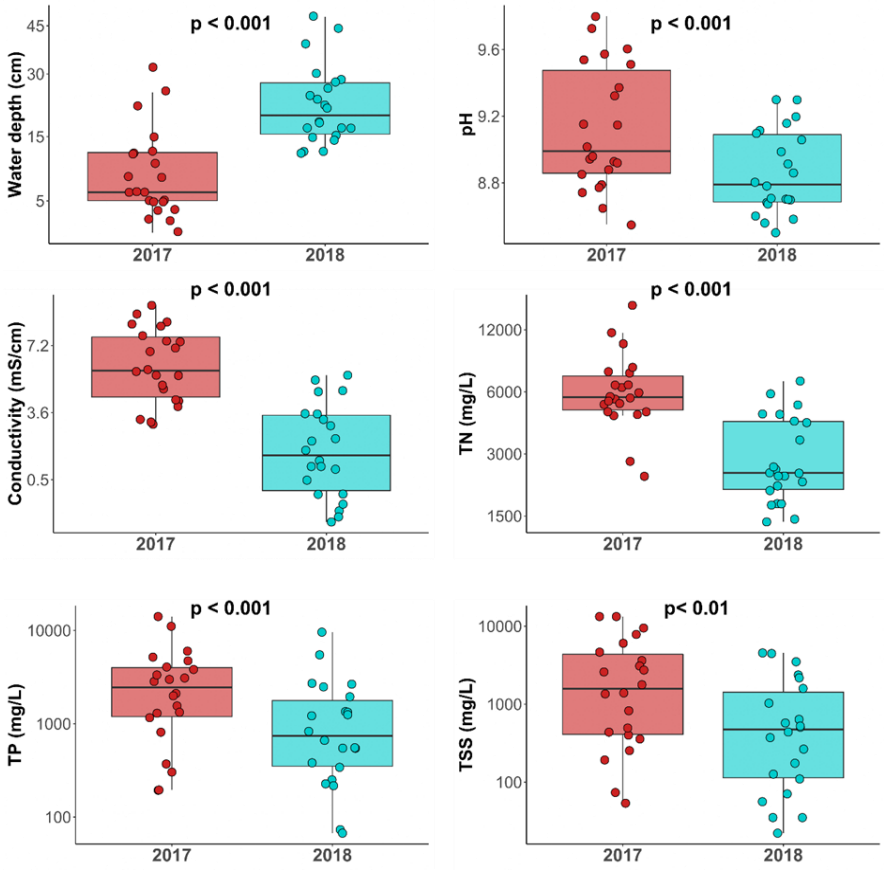

Figure 22. Differences in the environmental variables between the two sampling years. P values indicate significant differences between the two years based on paired t-tests.

According to the pairwise PERMANOVA and PCA, the spring of 2018 (wetter) was similar to the spring conditions of 2010, while it was significantly different from the dry spring (2017)

and the reference summer conditions (2009). Furthermore, the dry spring conditions (2017) statistically did not differ from the reference summer conditions (2009) (Table 6., Figure 23.).

Table 6. Results of the pairwise comparisons of the reference data (2009 summer, 2010 spring) and our data (2017 spring, 2018 spring data and 2009 summer, 2010 spring) (p values are adjusted for multiple comparisons based on Bonferroni method, $p < 0.05$, bold: significant, Df = Degree of freedom, SS = Sum of square, F =F value)

Pairs		Df	SS	F	R^2	p adjusted
2009 summer	2010 spring	1	3.036	0.998	0.0322	1.000
2009 summer	2017 spring	1	4.964	1.853	0.0582	1.000
2009 summer	**2018 spring**	**1**	**24.136**	**9.776**	**0.246**	**0.018**
2010 spring	2017 spring	1	9.718	4.185	0.122	0.153
2010 spring	2018 spring	1	10.750	5.089	0.145	0.051
2017 spring	**2018 spring**	**1**	**30.977**	**17.697**	**0.371**	**0.003**

The evenness of the Ciliates was higher in 2017. Phytoplankton showed different patterns, higher evenness, PD and lower richness in 2017 (Figure 24.). Diversity indices of the rest of the groups (Ciliate richness and PD, and all of the indices of Fungi and HF-HNF) did not show

a difference between the two years. Richness, evenness and PD of Bacteria was higher in 2017, while Cyanobacteria only had higher richness and PD values in 2017.

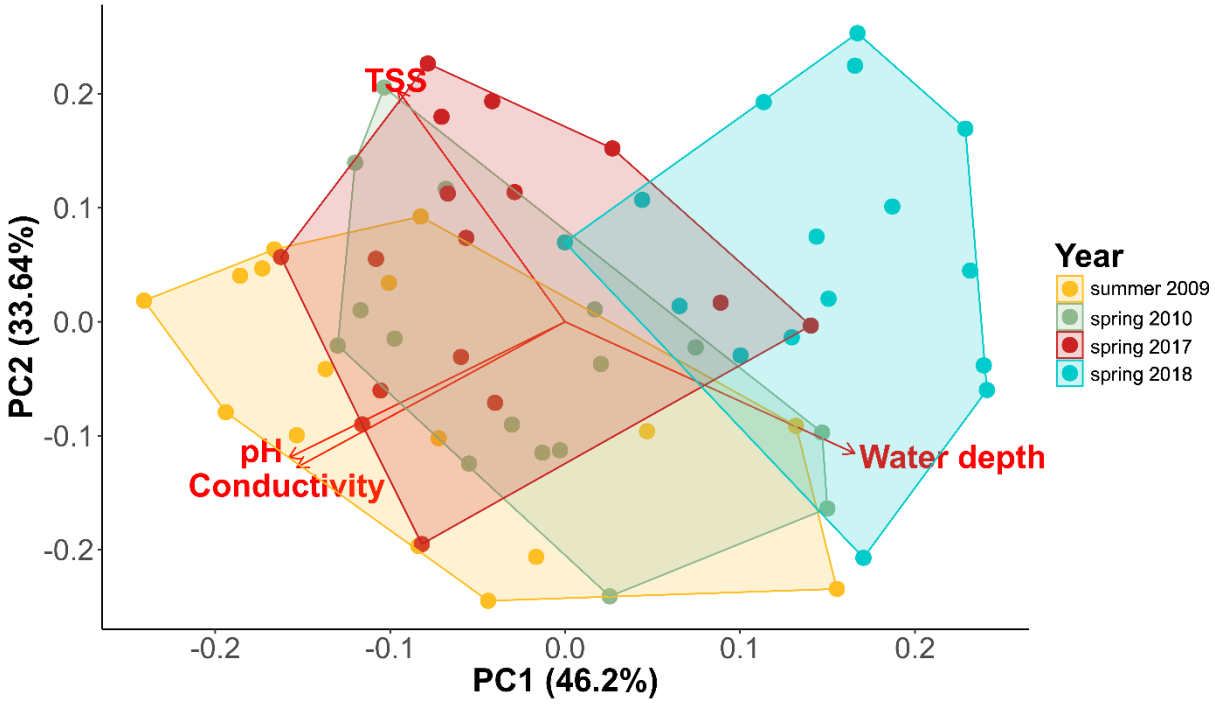

Figure 23. Separation of samples (each soda pan is represented by one filled circle per year) based on the local environmental variables in the Seewinkel regional data (2017: dry spring, 2018: wetter spring) and the earlier reference data (2009: summer, 2010: spring)

Microeukaryoitc and prokaryotic communities showed clear separtaion based on the two sampling years (Figure S1.)

For microeukaryotic phyla, conductivity was a significant predictor in both years according to the CCAs (Figure 25. a), together with either TP (2017) or pH (2018) (Table S8.). A gradient was visible along the conductivity axis in both years, with mixotrophic (Dinoflagellata and/or Cryptophyta) and heterotrophic groups (Stramenopiles, Centroheliozoa, Alveolata_unclassified, Ciliophora) being more abundant in less saline waters, while Haptophyta and Discoba had increasing relative abundance along the conductivity gradient (Figure 25. a, Table S9.).

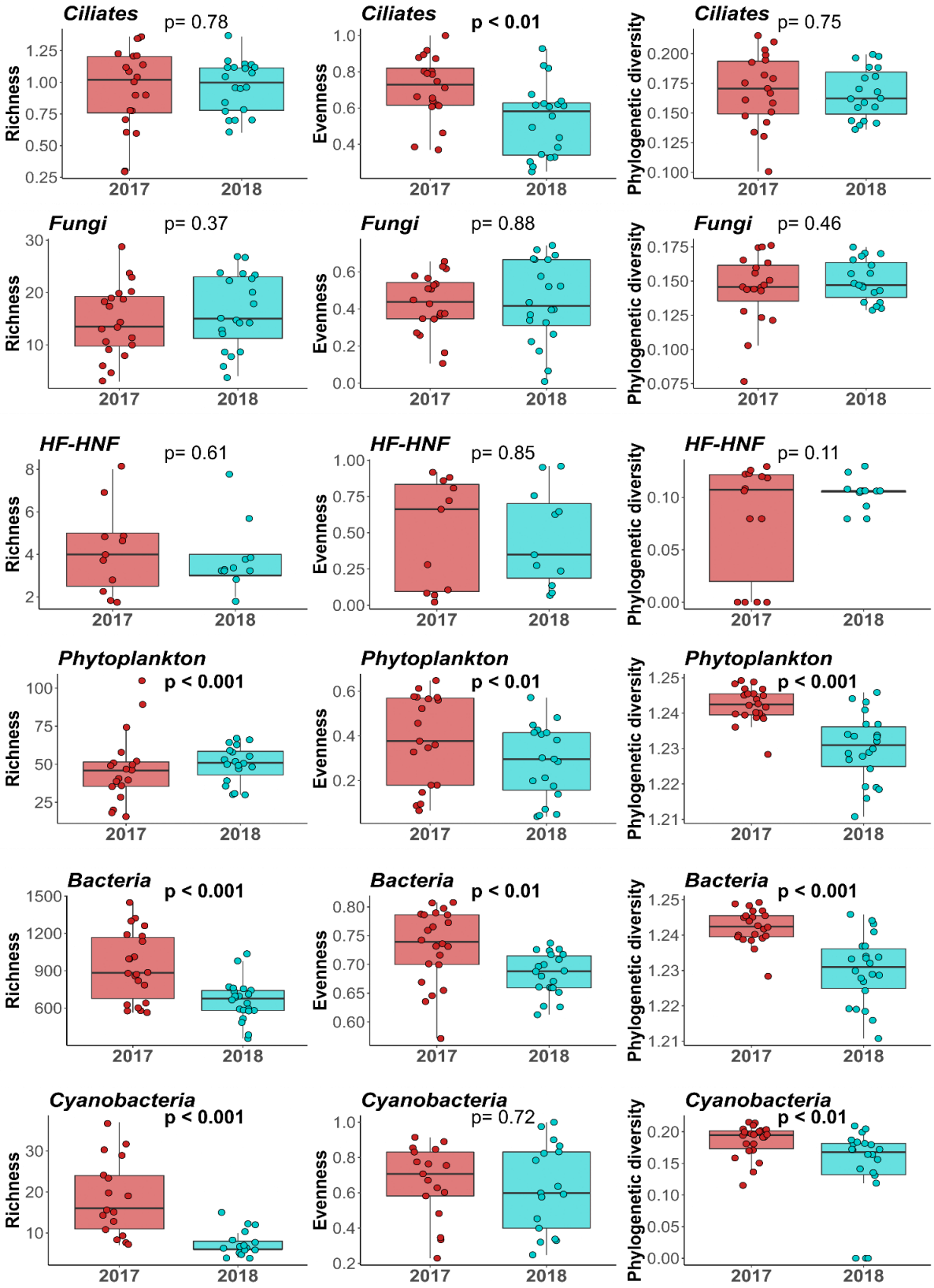

Figure 24. Richness, evenness and phylogenetic diversity of the microeukaryotic and prokaryotic groups in the two sampling years. Significance values are based on paired t-tests (bold: significant)

Prokaryotic phylum composition was significantly influenced by water depth and TP in both years, with conductivity being additionally significant in 2018 (Figure 25. b, Table S10.). While the significant environmental predictors were similar in the two years, the relative position of the major taxonomic groups and response to the environmental predictors varied in the multivariate space. Cyanobacteria was more abundant in more saline and deeper pans which was more pronounced in 2018 (Figure 25. b, Table S11.).

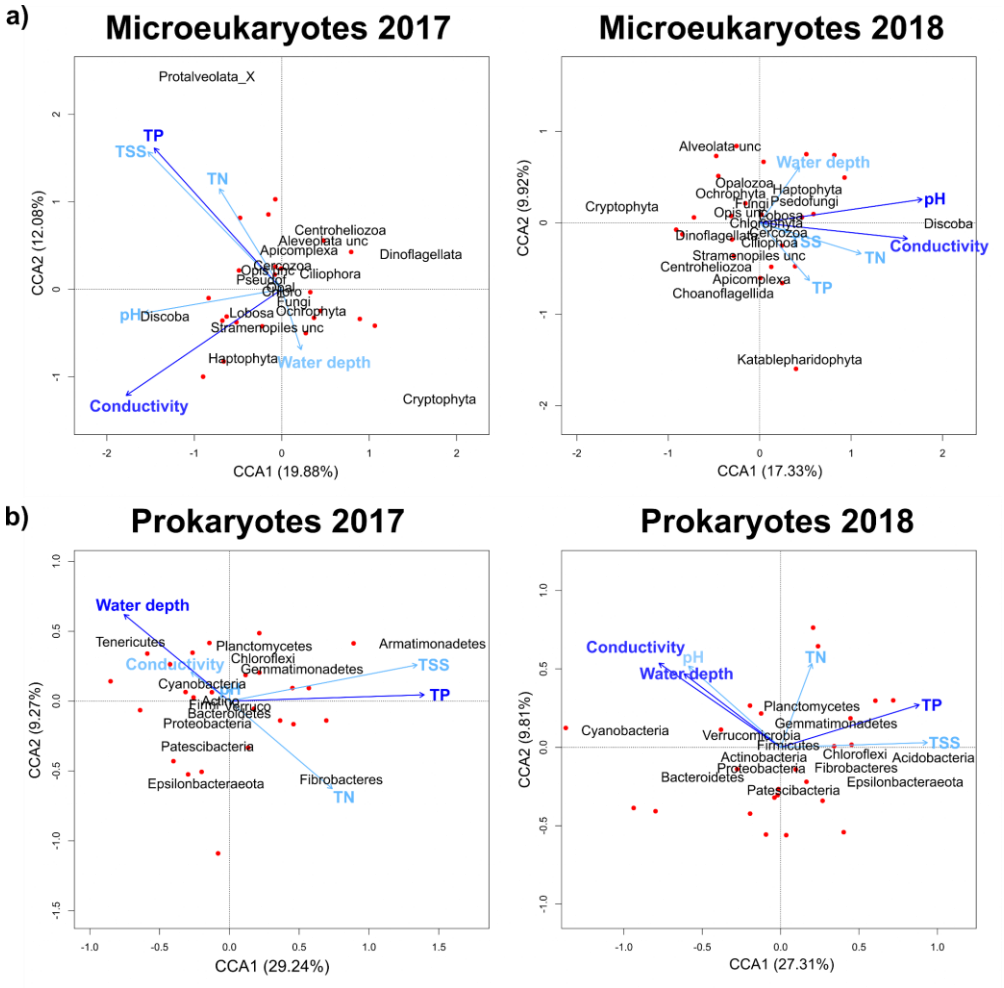

Figure 25. Canonical correspondence analysis (CCA) biplots based on the phylum composition of microeukaryotes (a) and prokaryotes (b) in the two years (red points: habitat scores, dark blue arrows: significant environmental variables, light blue arrows: all the other environmental variables). Abbreviation of the phyla names: unc = unclassified, Opis unc = Opisthokonta unclassified, Firmi= Firmicutes, Verruco = Verrucomicrobia, Actino = Actinobacteria, Chloro = Chlorphyta, Psedof = Pseudofungi, Ochro = Ochrophyta, Opal = Opalozoa

Water depth, conductivity and TP were similarly important predictors of OTU composition in the major groups separate CCAs (Table 7.). A trend was visible among the groups that the environmental variables explained a higher variation in OTU composition in 2017 than in 2018. Conductivity was the only environmental variable that was significant in almost all models.

Table 7. Significant environmental variables ($p<0.05$) of the OTU composition of prokaryotic and microeukaryotic groups based on separate CCAs (presented in the supplementary material, Figure S2.-S3.)

		Water depth	pH	Conductivity	TN	TP	TSS	Explained variation
2017								
Microeukaryotes	**Ciliates**					●	●	0.16
	Fungi	●		●		●		0.14
	HF-HNF			●				0.12
	Phytoplankton	●		●	●	●		0.25
Prokaryotes	**Bacteria**	●		●		●		0.16
	Cyanobacteria	●		●		●		0.15
2018								
Microeukaryotes	**Ciliates**			●				0.06
	Fungi			●				0.04
	HF-HNF			●				0.07
	Phytoplankton	●		●				0.12
Prokaryotes	**Bacteria**	●		●		●		0.15
	Cyanobacteria							NA

Overall, linear responses were more frequent in the multiple regression models for environmental factors and the local richness indices in the six major groups (Bacteria, Cyanobacteria, Ciliates, Fungi, HF-HNF, Phytoplankton), but there were also several cases where GAM predictions provided a better fit (e.g. for five out of the six evenness models in 2018; Figure 26.). U-shaped patterns were observed in the case of Bacteria (PD with TSS) (Figure S4.), ciliates (richness with TSS, evennes with water depth) (Figure S6.), and phytoplankton (PD with conductivity and pH) (Figure S9.).

Prokaryote richness (Bacteria and Cyanobacteria) was in general poorly explained by the environmental data (R^2 of the ML Bacteria was 0.175 in 2017 and 0.189 in 2018, while R^2 of LM Cyanobacteria was 0.141 in 2017 and merely 0.05 in 2018), and no significant predictor was found for either group in the 2018 data. In the other four microeukaryotic groups (and in

Cyanobacteria), a general trend was visible that the environment was a stronger predictor of richness in 2017 than in 2018 according to R^2 values of the final models (Figure 26).

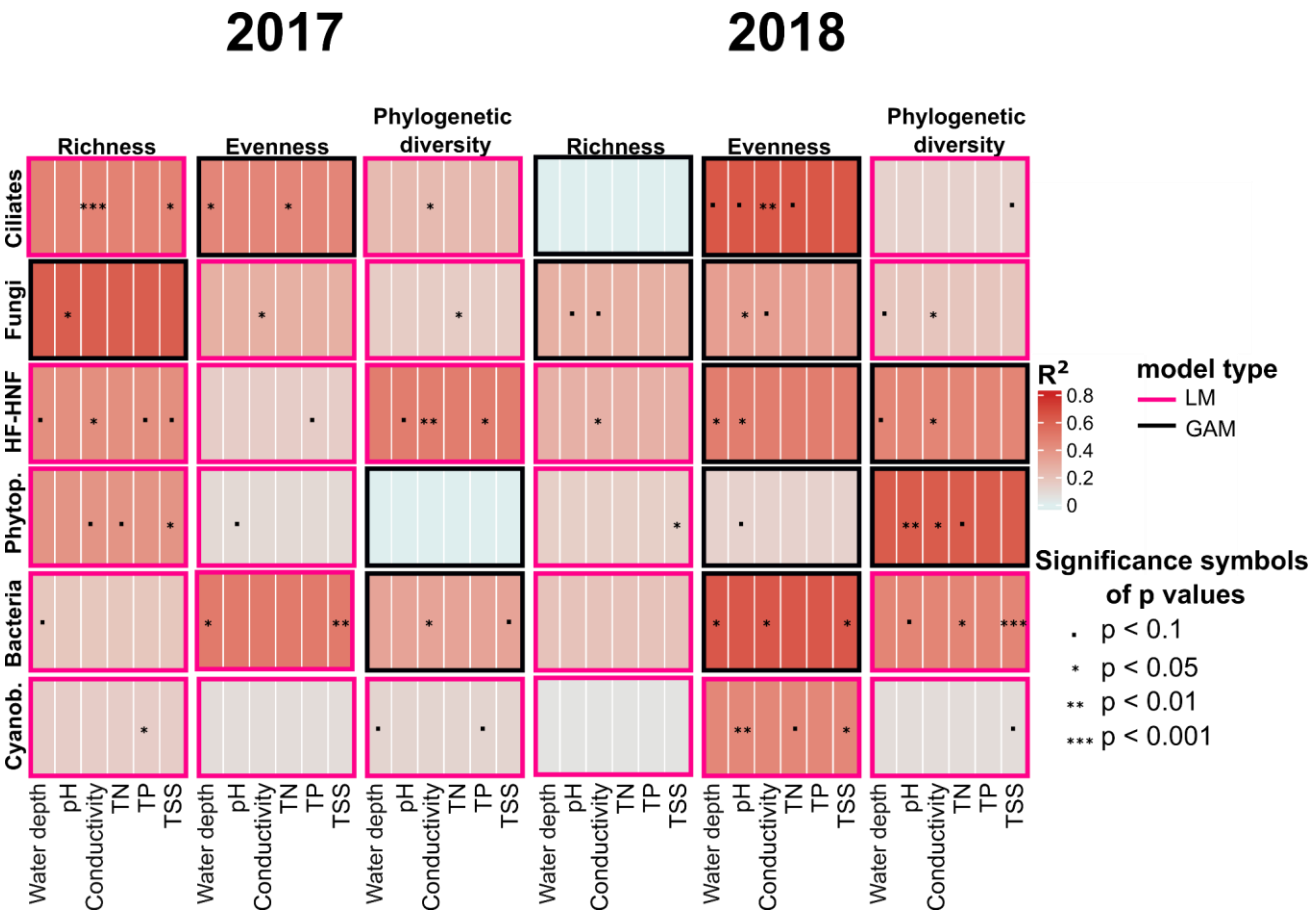

Figure 26. Heatmap of the best-fit models with the significant environmental variables for the six major taxonomic groups (in rows) and their local diversity indices (richness, evenness, phylogenetic diversity) in the two sampling years (in columns). One model is represented by one box, where the shading indicates the overall R^2 of each multiple regression model (either linear or GAM-based, indicated by the colour of the frame of each box), while the significance symbols belong to individual predictors in the final model. Abbreviation of the group names: Cyanob. = Cyanobacteria, Phytop. = Phytoplankton, HF-HNF = Heterotrophic flagellates and nanoflagellates

Conductivity and TSS were found to be the most frequent significant predictors for richness, both being at least marginally significant in four of the 12 models (six groups in two years; Figure 26.). Conductivity was at least marginally significant for three groups in 2017 (showing

a negative relationship with ciliates; Figure S6. and phytoplankton; Figure S9., and positive with HF-HNF; Figure S8. and marginally significant for Fungi in 2018 (with a humped-shaped relationship, Figure S7.). TSS was similarly a significant predictor in four cases, for three groups in 2017 (humped-shaped relationship with HF-HNF; Figure S8. and U-shaped relationships with a longer negative trend in the cases of ciliates; Figure S6. and phytoplankton; Figure S9.) and for a single group in 2018 (negative relationship with phytoplankton; Figure S9.).

The most frequent predictor of evenness was pH (Figure 26.), showing a negative relationship with phytoplankton evenness in 2017 and having a significant effect on all groups except for Bacteria, in 2018 (negative on HF-HNF; Figure S8. and Cyanobacteria; Figure S5., positive on Ciliata; Figure S6., humped-shaped on phytoplankton; Figure S9. and Fungi; Figure S7.). This was followed by the importance of water depth and conductivity. Water depth was a positive predictor of ciliate and bacterial evenness in 2017, while these relationships changed in 2018, to a negative relationship for bacteria and a u-shaped relationship for ciliates (Figure S3., Figure S6.), in addition to a further positive relationship with HF-HNF (Figure S9.). Conductivity was only significant for Fungi in 2017 (positive), while it had a significant effect on the evenness values of Fungi, Ciliata, and Bacteria in 2018 (all showing a humped-shaped relationship with conductivity; Figure S7., Figure S6., Figure S4.).

Similarly to the richness, PD was also mostly explained by conductivity (in six) and TSS (in four models). Conductivity was a significant positive linear predictor of HF-HNF in both years (Figure S8.), was a negative predictor of Ciliata PD in 2017 (Figure S6.), showed a humped-shaped relationship with Bacteria in 2017 (Figure S4.), with Fungi in 2018 and a U-shaped relationship with phytoplankton in 2018 (Figure S7., Figure S9.). TSS showed a marginally significant U-shaped relationship with Bacteria PD in 2017 when Bacteria PD was overall much higher than in 2018 (Figure S4.), while this relationship, together with Cyanobacteria, was positive in 2018. In 2018, Ciliata was the third group with TSS among the selected predictors, but here the relationship was negative and only marginally significant.

In three of the six groups, the relationship between changes in richness and environmental variables was significant between the two years (Table 8.). Richness changes of Bacteria and ciliates were significant with changes in conductivity. Changes in fungi richness corresponded to the change in pH between the two sampling years. All of the significant responses were

negative, which indicates a decrease in richness with increasing conductivity and pH (Figure 27.).

Table 8. Summary statistics of the multiple linear regression models testing the relationships between planktonic microbial richness change and environmental variables change from 2017 to 2018

(*= $p < 0.01$, NS = not significant)

Group	AIC	R^2	p (model)	Significance	p	Direction of relationship
Ciliates	Conductivity	0.249	0.064	*	0.027	-
	TP	0.249	0.064	NS	0.108	
	TSS	0.249	0.064	NS	0.190	
Fungi	pH	0.274	0.013	*	0.013	-
HF-HNF	Water depth	0.351	0.265	NS	0.104	
	pH	0.351	0.265	NS	0.382	
	TP	0.351	0.265	NS	0.319	
	TN	0.351	0.265	NS	0.105	
	TSS	0.351	0.265	NS	0.119	
Phytoplankton	Water depth	-0.349	0.962	NS	0.493	
	Conductivity	-0.349	0.962	NS	0.531	
	pH	-0.349	0.962	NS	0.916	
	TP	-0.349	0.962	NS	0.996	
	TN	-0.349	0.962	NS	0.586	
	TSS	-0.349	0.962	NS	0.802	
Bacteria	Conductivity	0.153	0.045	*	0.045	-
Cyanobacteria	Conductivity	0.147	0.129	NS	0.111	
	TP	0.147	0.129	NS	0.177	

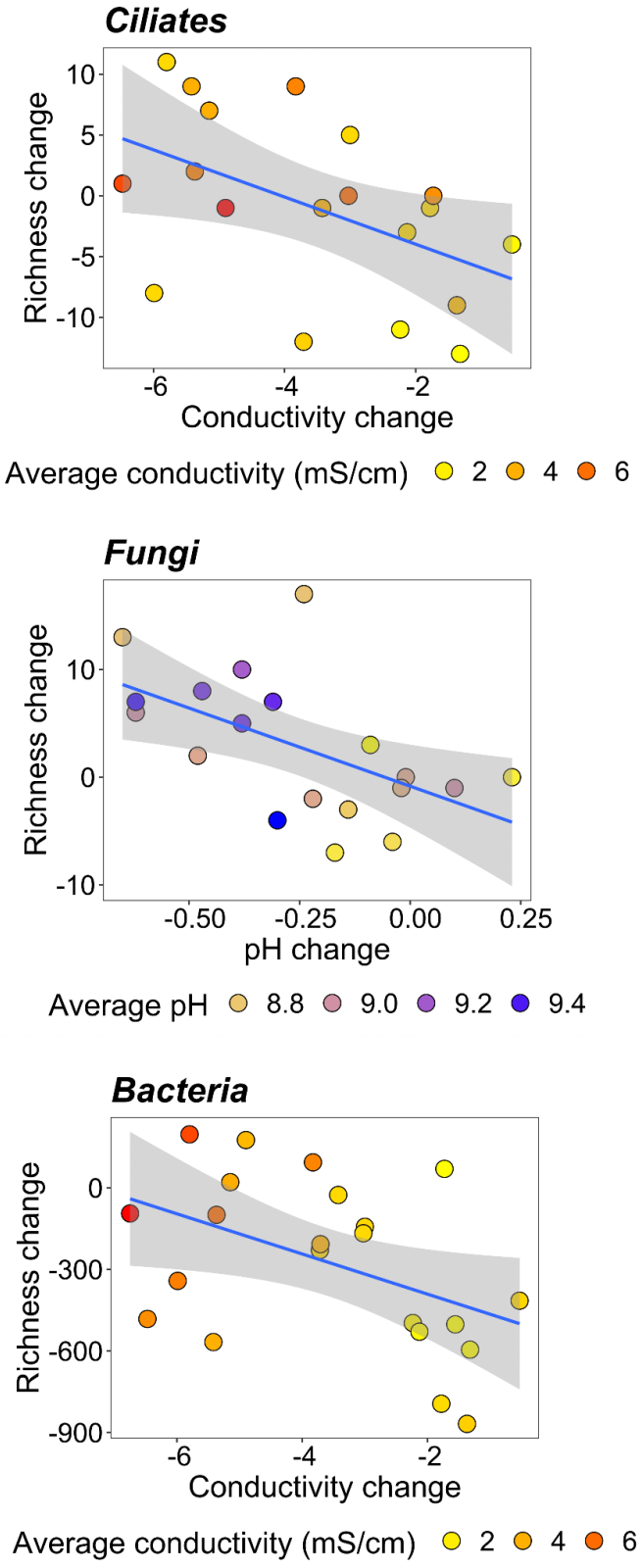

Figure 27. The relationship between planktonic microbial richness changes and significant environmental variable changes ($p < 0.01$)

5.5 Discussion

In this study, we investigated the diversity patterns of microbial communities across environmental gradients in two consecutive spring seasons with different weather and hydrological conditions. We observed that the dry spring of 2017 had more similar conditions to the reference summer data, while the wetter spring of 2018 was comparable to the reference spring data from a decade ago, indicating a seasonal shift in the aquatic environment. We found that the environmental variables had a stronger effect on community composition in the dry spring, and conductivity, TSS, and TP were the most important environmental variables affecting the diversity and community patterns across six major groups of microbes. Additionally, we noted that the response of prokaryotes (Bacteria and Cyanobacteria) to the environmental gradients generally differed from the microeukaryotic groups. We suggest that climate change may drive shifts in the community composition of microbial communities in soda pans, where microeukaryotic plankton may be more adversely affected by the increasing environmental stress associated with drier weather conditions and stronger environmental stress gradients.

Based on our findings, conductivity was the most influential environmental factor that shaped microbial diversity patterns. Although the salinity gradient in the pans we studied was relatively narrow (0.6 to 11 mS/cm conductivity, equivalent to 0.4-8.8 g/L salinity, as per the conversion factor of Boros et al., 2014). Previous research suggested that significant changes in saline lake communities occur at lower salinity levels, particularly in the range of 3-10 g/L (Hammer, 1990; Schallenberg et al., 2003; Williams, 1987). A previous study that covered all soda pans in the Pannonian ecoregion identified a salinity threshold of 3.9 g/L, beyond which there was a marked decrease in zooplankton species richness (Horváth et al., 2013). Our results were largely in line with this threshold for most microbial groups, as we observed negative trends in richness for five out of six groups above 3.2 mS/cm conductivity (2.6 g/L salinity), except for HF-HNF, which displayed a contrasting pattern of increased richness with conductivity. As most conductivity values in 2017 were above 3 mS/cm, this may also account for the relatively lower biodiversity values observed for most groups in 2017 compared to 2018. Previous research on ephemeral ponds showed that prokaryotic richness was higher along the salinity gradient than eukaryotic richness, which was in line with the results of our study as well. Fungi was found to be more abundant at lower salinity values and were less dominant as salinity increased in

ephemeral ponds (Menéndez-Serra et al., 2021). Our results showed a similar trend, richness of Fungi decreased from 3.6 mS/cm conductivity which is equivalent to 2.88 g/L salinity. Furthermore, according to Csitári et al., 2022 not only the levels of salinity but the anion type of the salt also impacts the growth and survival of the microbes. They found that carbonate and bicarbonate anions were a stronger selective force for the bacteria than chloride or sulfate.

Previous studies on soda pans in Hungary have shown that pico-sized algae dominate the phytoplankton communities (Felföldi et al., 2009; Pálffy et al., 2014; Somogyi et al., 2022b, 2009). However, the algae's small size and the high inorganic turbidity of the pans have made it difficult to identify them through traditional microscopic techniques. This challenge may explain the lack of community-level studies on phytoplankton in these systems. A previous review of microscopic data available from soda pans concluded that the number of eukaryotic phytoplankton species rarely exceeds 10 (Padisák and Naselli-Flores, 2021). In contrast, our study found an average eukaryotic phytoplankton OTU richness of 47 in 2017 and 49 in 2018, with a range of 16 (Runde Lacke in 2017) to 105 (Obere Höllacke in 2017). This result shows that these habitats have a much higher diversity of phytoplankton than previously assumed, consistent with molecular results on the picocyanobacteria diversity of soda pans in Hungary and Austria (Felföldi et al., 2009; Somogyi et al., 2022b).

Only phytoplankton exhibited a U-shaped pattern in phylogenetic diversity across the salinity gradient in 2018, with the lowest diversity occurring at intermediate salinity (2.9 g/L), beyond which diversity increased again. Comparable U-shaped patterns of species richness in phytoplankton along salinity gradients have been observed from transitional gradients, ranging from freshwater to brackish to marine environments (Olli et al., 2022, 2019). However, in soda pans, the increase in phylogenetic diversity was not accompanied by a parallel increase in OTU richness, indicating that the pattern was most likely due to the disappearance of closely related taxa rather than the emergence of salt-tolerant OTUs. A previous review from the Carpathian Basin found that the salt tolerance of picoeukaryotic strains from freshwater, soda, and hypersaline lakes differed. Strains isolated from freshwater and soda pans were only able to grow between 0-4.4 g/L salinity, while isolates from hypersaline environments were able to grow from 2.3 to 16.1 g/L salinity range (Somogyi et al., 2022b).

HF-HNF richness and phylogenetic diversity showed a clear increasing trend with salinity. Heterotrophic nanoflagellates (HF-HNF) are the main protozoan grazers of bacterio- and picophytoplankton (Tikhonenkov et al., 2015). However, classical microscopic techniques for their identification and enumeration can be challenging, especially in turbid habitats such as soda pans, which are characterized by high levels of suspended sediment (Arndt et al., 2000). Previous research showed that heterotrophic flagellate diversity is primarily influenced by salinity and temperature (Azovsky et al., 2016; Je Lee and Patterson, 1998) and follows the "rule of critical salinity" meaning that species richness has a minimum in brackish waters (~5-8 g/L of salinity) (Tikhonenkov et al., 2006). Our study found that HF-HNF richness and phylogenetic diversity increased exponentially with salinity, which can be due to the appearance of salt-tolerant species. This is was a pattern not previously reported from other saline systems. Our results highlight the importance of using molecular techniques for reliable identification and enumeration of phytoplankton and heterotrophic flagellates and nanoflagellates in soda pans.

Although not all taxa were equally constrained by the environmental variables in the microbial communities, our results indicate that environmental factors played a more significant role in shaping community variation in the dry spring of 2017. This is consistent with the concept of species sorting becoming more influential in harsher environmental conditions (Datry et al., 2016; Van der Gucht et al., 2007; Vanschoenwinkel et al., 2010). The response of biodiversity indices to changes in environmental conditions between the two years indicated that elevated levels of salinity and other parameters can be associated with lower levels of biodiversity for most of the microbial groups. We promote longer term monitoring studies to track changes in these systems over time and to establish direct links between changing climatic conditions and their biota.

6. Conclusions

We gained a comprehensive insight into the seasonal dynamics and the impact of contrasting hydrological conditions of shallow soda pans by incorporating network analysis, the identification of keystone species, and the separate consideration of the microeukaryotic and prokaryotic communities. The identified microbial community composition is unique in the presence of certain dominant species (*Choricystis*, *Chloroparva*, acIII-A1, *Algoriphagus*, *Nitriliruptor*), although it showed similarities to other soda lakes (Afonina and Tashlykova, 2020; Dimitriu et al., 2008; Schagerl, 2016). In case of our two study sites, we found that the phytoplankton community was similar, genera *Choricystis*, unclassified Chlorophyceae, unclassified Chlorellales were the most abundant primary producers, while genus *Chloroparva*, one of the most dominant green algae of the five soda pans of Kiskunság was not detected in the Seewinkel microeukaryotic dataset. Genus *Nitzschia* is a dominant diatom species from soda pans in the region (Ács et al., 2017; Stenger-Kovács and Lengyel, 2015) which was also abundant in both of our study sites. Heterotrophic flagellates and nanoflagellates are really important bacterivores in the microbial food web (Kellogg et al., 2019). Genera *Spumella* and *Paraphysomonas* were present in both microeukaryotic dataset. Despite the importance of parasitism in biodiversity, ecological studies that include parasites are still relatively rare (Beng et al., 2021). Representatives of parasitic fungi have been identified from both study sites, like class Chytridiomycota, genera *Pythium* and *Pirsonia*. Genus *Pythium* was only abundant in Sós-ér in spring (the only colored soda pan in Kiskunság) and Westliche Wörthenlacke in the dry spring, 2017 (colored Seewinkel soda pan). The infection of *Pythium* can be delayed due to high salinity (Herrero et al., 2020), so that both of the soda pans had relatively lower salinity in the spring (Sós-ér ~ 4.9 g/L, Westliche Wörthenlacke ~ 2.9 g/L) was in line with the ideal infection conditions. Although, there was no significant relationship between green algae and genus *Pythium* abundance at either site. Genus *Pirsonia*, parasitoid nanoflagellates on diatom species, was also present at both study sites. *Pirsonia* was observed with high relative abundance in all of the Kiskunság soda pans in spring and autumn, while it was only abundant in a couple of Seewinkel soda pans, like Oberer-Stinkersee and Albersee. So the Seewinkel and Kiskunság soda pans had a similar microeukaryotic community composition with the same abundant microeukaryotic taxons. Due to the lack of information on microeukaryotes of soda pans in general and especially on fungi, parasites, and heterotrophic nanoflagellates, the comparison with different aquatic environments is still challenging.

The results obtained from the models confirmed the observation that the microbial communities of continental waters are influenced by the salinity of the water (Newton et al., 2011). However, a similar conductivity range was measured in other saline lakes for example Kazakhstan or Transylvania but using the same method, their microbial community composition was different from the investigated soda lakes. This implied that other environmental factors also influence the community composition which was in line with our findings. Next to salinity, water depth and TP were the most important environmental variables of the diversity and community structure of the microbes. Water depth clearly separated the colored soda pans from the turbid ones and also higher water levels in the spring season resulted in different microbial community structure, than the more shallow periods. Deeper water depth coupled with higher TP levels as well.

Although the two studies were conducted at different time and spatial scales, both studies emphasized the sensitivity of these habitats to a changing climate. Based on the biweekly sampling (through three seasons) of five soda pans, we gained more detailed knowledge of the community composition, the core microbiome, and their seasonal patterns, while the comparison of two very contrasting years of twenty-six soda pans showed that the increasing annual mean temperature and the decreasing annual precipitation can have a negative impact on the diversity of multiple microbial groups. The limitation of both studies is their sampling effort. The results of the first study implicated a very quickly changing microeukaryotic community and the second study revealed that the biodiversity of multiple microbial groups can be negatively impacted by climate change. We only focused on five soda pans with biweekly sampling through three seasons and we only compared two years and twenty-six soda pans. So we promote more intense (weekly or even daily samplings) and long-term sampling with the inclusion of more soda pans.

Considering the results of both studies, future research should focus on long-term biodiversity studies to better understand the functional roles of the most sensitive microbial groups in these ecosystems. Also, it is crucial to examine the impacts of changing environmental conditions (the more frequent desiccation and refillment periods) on microbial communities. Furthermore, integrating network analyses, keystone species and the separation of microeukaryotic and prokaryotic groups into the data analysis can provide a more detailed insight into the microbial dynamics of soda pans. These types of research will enhance our understanding of the ecological responses of microbial communities to environmental changes and help the

development of effective conservation and management strategies to preserve these vulnerable aquatic ecosystems.

7. Thesis points

I.) How similar are the seasonal changes of planktonic microeukaryotic and prokaryotic communities in nearby soda pans?

The similarities of planktonic microbial communities from spring to autumn are altered by local effects (like occasional desiccation periods), which drives the community composition and structure by shifting the dominance and relationships of taxa. Therefore, the similar communities in the beginning of the year (spring) become more distinct when local effects are more pivotal (during summer and autumn).

II.) How core and non-core microbial taxa contribute to the adaptation of the microbial communities, and how does this contribution vary between microeukaryotic and bacterial communities?

Adaptation to seasonal changes differed between bacterial and microeukaryotic communities. For microeukaryotic communities non-core members of the microbiome were involved in the response to sudden environmental events, while bacterial communities adapted to extreme conditions through species sorting from the core community.

III.) Do the identity and strength of the main environmental drivers change between subsequent years?

The environmental variables had a stronger effect on microbial community composition in the dry spring, than in the wet spring. The identity of the main environmental drivers remained the same in the two spring seasons and conductivity had the strongest effect in both years.

8. Summary

Soda lakes possess an alkaline pH, distinctive ion composition, and high salinity. In addition to being a unique habitat, they given home to distinct biota due to their turbidity, humic, and nutrient content. Most of the world's soda lakes are difficult to access, so their microbial communities and their structure in response to seasonal changes and environmental gradients have not been thoroughly investigated.

Our research focused on the changes in the planktonic microeukaryotic and prokaryotic communities of soda pans through time and space. For our first study, the sampling was carried out biweekly from April 12 to November 14 in 2017, throughout three seasons (spring, summer, and autumn), while for the second study water samples were collected from twenty-six soda pans in two consecutive spring seasons, 2017 (dry) and 2018 (wet) with very different hydrological conditions. The community composition of the microeukaryotic and prokaryotic communities was determined based on the 18S rRNA and the 16S rRNA genes by Illumina amplicon sequencing. To reveal local and time shifted correlations between microeukaryotic and prokaryotic communities we created networks using the extended local similarity analysis (eLSA) based on the parallel time-series data of the five soda pans. Keystone OTUs of the networks were identified by weighted topological importance (WI). For the second study, we created six functional groups according to taxonomy and function (ciliates, heterotrophic flagellates and nanoflagellates, fungi, phytoplankton and bacteria, cyanobacteria). The effect of environmental variables on the diversity of microeukaryotic and prokaryotic functional groups were tested within and across the two years.

Our results showed that the microbial communities inhabiting soda pans are diverse and soda pans are vulnerable to the effects of climate change. Through the use of network analyses and identification of keystone taxa, we expanded our understanding of the community composition in extreme aquatic habitats. Our findings demonstrate that the studied soda pans despite the drastic environmental changes and subsequent community composition shifts are mainly populated by a common core microbiome and due to the identical climatic and meteorological conditions they share similar seasonal dynamics. However, the shared microbiome is limited among pans of different habitat subtypes, and common seasonal trends were modified by local stressors such as desiccation and refillment. Conductivity, total suspended solids (TSS), total phosphorus (TP) and water depth were the most important environmental variables affecting

the microbial diveristy and community structure. Also environmental variables had a stronger effect on microbial community composition in the dry period than the wet.

Our study revealed that despite the high variability of soda pans, they follow a similar seasonal patterns, but environmental changes (like desiccation) can disrupt this trend. Also microeukaryotic communities responded differently to local stressors and environmental gradients than prokaryotes.

9. Összefoglaló

A szikes tavak lúgos pH-val, jellegzetes ionösszetétellel és az édesvizeknél magasabb sótartalommal rendelkeznek. A fentiek miatt élővilágúk is különleges, továbbá zavarosságúk, humusz- és tápanyagtartalmuk miatt is egyedülálló élőhelynek minősülnek. A világban található szikes tavak többsége nehezen megközelíthető, ebből kifolyólag eddig nem készült olyan átfogó tanulmány, amely a planktonikus mikroeukarióta és bakteriális közösség szerkezetének és kapcsolatainak változására fókuszált volna különféle tér és időbeni skálák függvényében.

Az első projekt keretében kétheti rendszerességgel végeztünk mintavételt (2017. április 12 - november 14) három évszakon át (tavasz, nyár, ősz). A második projekt során huszonhat szikes tavat vizsgáltunk meg egymást követő két nagyon eltérő hidrológiai viszonyokkal rendelkező év tavaszán, 2017 (száraz) és 2018 (nedves). A mikroeukarióta és prokarióta közösségek összetételét 18S rRNS és a 16S rRNS gének alapján azonosítottuk amplikon szekvenálással. A mikroeukarióta és prokarióta közösségek lokális és időben eltolt összefüggéseit "kiterjesztett lokális hasonlóság analízis" (extended local similarity analysis, eLSA) segítségével vizsgáltuk meg, amely során kapcsolati hálózatokat hoztunk létre az öt szikes tó párhuzamos adatai alapján. A kapcsolati hálózatok kulcs OTU-it súlyozott topológiai fontosságuk alapján határoztuk meg. A második projekthez hat csoportot hoztunk létre taxonómia és funkció szerint (ciliáták, heterotróf flagellláták és nanoflagellláták, gombák, fitoplankton és baktériumok, cianobaktériumok). A két évben és a két év között is megvizsgáltuk a környezeti változók mikroeukarióta és a prokarióta funkcionális csoportok diverzitására gyakorolt hatását.

A kapott eredmények alapján a szikes tavakat benépesítő mikrobiális közösségek változatosak, és a szikes tavak érzékenyek a klímaváltozás hatásaira. Kapcsolati hálózatok és kulcsfajok azonosítása révén bővítettük a szélsőséges vízi élőhelyek közösségi összetételének megértését. Eredményeink azt mutatják, hogy a vizsgált szikes tavak a drasztikus környezeti változások és az ezt követő közösség összetételbeli eltolódások ellenére főként egy közös "mag mikrobiom" (core microbiome) népesíti be. Az azonos éghajlati és meteorológiai viszonyok miatt hasonló szezonális dinamikát figyeltünk meg a közösségekben. Noha a közös "mag mikrobiom" (core microbiome) korlátozott a különböző élőhely altípusokba tartózó szikes tavak között. A közös szezonális tendenciákat helyi stresszorok, például a kiszáradás és feltöltődés módosították. A vezetőképesség, az összes lebegőanyag tartalom (TSS), az összes foszfor (TP) és a vízmélység voltak a legfontosabb környezeti változók, amelyek befolyásolták a mikrobiális diverzitást és a

közösség szerkeztét. A környezeti változók erősebb hatást gyakoroltak a mikrobiális közösség összetételére száraz, alacsonyabb vízállású időszakban, mint esősebb és mélyebb vízmélységű periódusban.

A szikes tavak nagy változékonysága ellenére hasonló szezonális mintázatot követnek, amit környezeti változások (például a kiszáradás) módosíthatnak. A mikroeukarióta közösségek helyi stresszorokra és környezeti gradiensekre adott válasza különbözött a prokariótákétól.

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11. Contribution to the research

Species identification of zooplankton samples, bacterial cell number enumeration, DNA extraction of the soda pans of Neusiedlersee-Seewinkel National Park, all of the statistical analyses (ordinations, network analysis, PERMANOVA, Mantel test, multiple linear regression and generalized additive models etc.) and data visualization were my own work. Extended Local Similarity Analysis (eLSA) and bioinformatic analysis were run by me with the help of Attila Szabó. I participated in the samplings conducted in the Kiskunság National Park and in the Neusiedlersee-Seewinkel National Park. I took part in the on-site measurements of environmental variables and sample processings.

The sampling of soda pans of the Kiskunság National Park was conducted by Bianka Csitári, Dr. Tamás Felföldi, and Dr. Attila Szabó with the help of Dr. Boros Emil, Nóra Tugyi, Balázs Németh, Tímea Szabó, and Tamás Sápi. Samples from the Neusiedlersee-Seewinkel National Park were collected by Dunja Lukic, Dr. Zsófia Horváth, Dr. Csaba F. Vad, and Robert Ptacnik.

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Christoffer Bergvall (Uppsala University, Sweden) measured the TP, TN, and DOC concentrations of the Hungarian soda pans.

DNA extraction and PCR of the soda pans of the Kiskunság National Park were done by Bianka Csitári, under the supervision of Dr. Anna J. Székely at Uppsala University, Uppsala, Sweden.

Weighted topological importance (WI) was calculated by András Hidas and Dr. Ferenc Jordán.

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13. List of publications

13.1. Articles related to the dissertation

Zsuzsanna Márton, Bianka Csitári, Tamás Felföldi, Ferenc Jordán, András Hidas, Attila Szabó, Anna J Székely. (2023) Contrasting Response of Microeukaryotic and Bacterial Communities to the Interplay of Seasonality and Stochastic Events in Shallow Soda Lakes. - FEMS Microbiology Ecology, Accepted for publication

Zsuzsanna Márton, Beáta Szabó, Csaba F. Vad, Károly Pálffy, Zsófia Horváth. (2023) Environmental changes associated with drying climate are expected to affect functional groups of pro- and microuekaryotes differently in temporary saline waters. - Scientific Reports, 13:3243, https://doi.org/10.1038/s41598-023-30385-6

Beáta Szabó, Attila Szabó, Csaba F Vad, Emil Boros, Dunja Lukić, Robert Ptacnik, **Zsuzsanna Márton**, Zsófia Horváth. (2022) Microbial stowaways: Waterbirds as dispersal vectors of aquatic pro-and microeukaryotic communities. - Journal of Biogeography, 49:1286-1298, https://doi.org/10.1111/jbi.14381

13.2 Other papers

Kristóf Korponai, Sára Szuróczki, **Zsuzsanna Márton**, Attila Szabó, Paula V. Morais, Diogo Neves Proença, Erika Tóth, Emil Boros, Károly Márialigeti, Tamas Felfoldi. (2023) Habitat distribution of the Belliella genus in continental waters and the description of Belliella alkalica sp. nov., Belliella calami sp. nov. and Belliella filtrata sp. nov. - International Journal of Systematic and Evolutionary Microbiology, 73., https://doi.org/10.1099/ijsem.0.005928

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14. Appendix

Table S1. Measured environmental variables of the Kiskunság soda pans

Sample Code	Pan name	Sampling date	Water depth (cm)	pH	Water temperature (°C)	DO (%)	Salinity (g/L)	TN (µg/L)	TP (µg/L)	SRP (µg/L)	DOC (mg/L)	Bacterial cell number	Chlorphyll a (µg/L)
S01	**Sós-ér**	2017.04.12	44.0	8.53	15.9	66.8	4.136	13287.46	1326.0	440	83.63846	5.82E+06	268.40
Z01	**Zab-szék**	2017.04.12	9.8	9.24	15.4	117.1	3.408	9772.38	4650.0	2567	23.71777	8.21E+06	319.50
V01	**Pan no. 60**	2017.04.12	27.5	9.32	13.7	82.5	4.048	11584.84	4726.6	3350	32.06806	1.48E+08	15.40
K01	**Kelemen-szék**	2017.04.12	14.5	8.9	14.1	82.9	2.560	7740.23	3860.0	1953	20.88538	1.48E+07	247.20
B01	**Böddi-szék**	2017.04.12	9.3	9.08	17.9	115.4	5.136	10266.69	3516.6	1326.6	34.96339	8.55E+06	319.50
S02	**Sós-ér**	2017.04.26	40.0	8.89	16.2	71.0	4.120	10651.15	536.6	323.3	87.89753	3.90E+06	264.20
Z02	**Zab-szék**	2017.04.26	26.0	9.56	15.3	93.1	3.440	8454.23	4030.0	2566	27.13761	5.51E+06	323.80
V02	**Pan no. 60**	2017.04.26	24.5	9.37	15.2	102.0	3.952	9387.92	4830.0	3453	31.04001	1.75E+08	2.90
K02	**Kelemen-szék**	2017.04.26	13.5	9.16	16.6	105.9	2.440	6586.85	3450.0	1800	19.87831	8.93E+08	251.40
B02	**Böddi-szék**	2017.04.26	14.0	9.43	15.7	92.0	5.040	8454.23	2693.0	1250	28.24959	4.03E+06	374.90
S03	**Sós-ér**	2017.05.17	42.0	8.71	24.0	47.0	4.096	8783.77	523.0	440	83.82729	9.01E+06	117.13
Z03	**Zab-szék**	2017.05.17	15.0	9.35	23.7	116	3.376	6916.39	3353.0	1087	26.00466	6.92E+06	204.48
V03	**Pan no. 60**	2017.05.17	27.0	9.37	21.2	91.1	4.00	8783.77	4826.6	1480	34.85849	1.75E+08	1.83
K03	**Kelemen-szék**	2017.05.17	17.0	9.2	22.5	104.0	2.448	6312.24	2510.0	1283	20.17204	3.95E+06	541.69
B03	**Böddi-szék**	2017.05.17	9.0	9.23	26.3	122.6	5.272	7905.00	1596.6	1067	29.84411	5.85E+06	238.48
S04	**Sós-ér**	2017.05.29	37.0	8.74	24.6	52.0	4.904	11013.80	1053.3	853	107.49340	4.33E+06	78.79
Z04	**Zab-szék**	2017.05.29	20.0	9.52	24.2	115.2	4.112	4313.70	3526.0	1806	28.54331	7.98E+07	8.52
V04	**Pan no. 60**	2017.05.29	23.0	9.19	23.9	45.4	4.928	13991.56	4003.3	1233	46.71254	9.88E+07	1.92
K04	**Kelemen-szék**	2017.05.29	13.0	9.05	27.8	91.9	3.208	6249.28	3466.0	1567	32.25689	4.71E+06	89.46
B04	**Böddi-szék**	2017.05.29	4.5	9.45	25.2	104.3	6.488	7043.36	2416.6	1506	37.04047	7.22E+06	105.05
S05	**Sós-ér**	2017.06.15	25.0	9.22	25.9	46.6	7.280	15480.46	1130.0	880	162.35780	8.74E+06	49.22

Sample Code	Pan name	Sampling date	Water depth (cm)	pH	Water temperature (°C)	DO (%)	Salinity (g/L)	TN (µg/L)	TP (µg/L)	SRP (µg/L)	DOC (mg/L)	Bacterial cell number	Chlorphyll a (µg/L)
Z05	**Zab-szék**	2017.06.15	15.0	9.49	30.9	139.1	5.840	7589.29	4746.6	3393	45.89430	8.74E+07	93.74
V05	**Pan no. 60**	2017.06.15	12.0	9.46	29.4	153.6	8.00	16324.17	4350.0	1487	76.60995	2.09E+08	3.41
K05	**Kelemen-szék**	2017.06.15	4.0	9.42	30.3	112.6	5.600	6497.43	4343.3	2577	52.12555	6.84E+07	306.72
B05	**Böddi-szék**	2017.06.15	10.0	9.49	26.1	99.0	10.160	8184.85	3340.0	1643	61.79762	3.27E+06	107.91
S06	**Sós-ér**	2017.06.29	25.0	9.49	24.9	86.3	6.800	14822.40	1266.0	733	156.6721	8.48E+06	195.92
Z06	**Zab-szék**	2017.06.29	7.0	9.74	24	98.1	6.080	12779.60	7800.0	4386	45.07605	7.98E+07	255.6
V06	**Pan no. 60**	2017.06.29	10.0	9.65	22.1	114.1	5.376	17254.31	8513.3	5987	53.63615	1.44E+08	112.46
K06	**Kelemen-szék**	2017.06.29	2.0	9.81	24.8	79.7	2.0400	12974.16	7380.0	1056	10.08036	7.60E+07	362.18
B06	**Böddi-szék**	2017.06.29	12.0	9.78	21.4	86.3	9.248	11612.30	4666.0	1537	51.74790	1.18E+08	204.48
S07	**Sós-ér**	2017.07.13	23.0	9.5	26.5	120.4	9.960	14530.58	1213.3	473	243.36400	4.75E+07	257.65
Z07	**Zab-szék**	2017.07.13	22.0	9.65	23.6	97.8	9.280	15989.72	9473.3	6787	326.02770	1.48E+07	136.29
B07	**Böddi-szék**	2017.07.13	7.0	9.58	24.1	102.2	15.280	15795.17	6406.6	2250	446.87620	1.18E+08	230.04
S08	**Sós-ér**	2017.07.27	46.0	9.67	20.3	109.6	0.8560	14822.40	1266.0	733	212.31270	1.49E+07	164.31
Z08	**Zab-szék**	2017.07.27	5.0	9.84	19.6	92.7	7.440	12779.60	7800.0	4386	54.85303	1.14E+08	598.61
V08	**Pan no. 60**	2017.07.27	15.0	9.57	21.2	127.4	8.00	21242.62	12813.0	7880	96.39469	9.12E+07	426.08
B08	**Böddi-szék**	2017.07.27	10.0	9.42	22.3	108.2	8.960				126.06130	9.12E+07	153.36
S09	**Sós-ér**	2017.08.14	8.0	9.8	25.5	179.0	8.352	18421.62	1926.6	126.6	217.97750	1.10E+08	183.26
Z09	**Zab-szék**	2017.08.14	8.0	9.78	27.2	113.2	10.304	34083.06	14733.0	6560	610.94480	3.61E+08	51.28
V09	**Pan no. 60**	2017.08.14	4.0	9.65	28.1	157.5	8.976	17059.76	10706.0	6866	367.14980	1.52E+08	672.64
K09	**Kelemen-szék**	2017.08.14	5.0	9.83	27.8	180.0	7.016	11028.64	4646.0	1753	69.93811	6.46E+07	498.42
B09	**Böddi-szék**	2017.08.14	5.0	9.77	24.4	135.4	12.424	8791.29	5480.0	1603	78.70801	7.60E+07	85.36
S10	**Sós-ér**	2017.08.29	7.0	9.72	27.0	157.3	12.640	42156.96	1253.0	93.3	359.38690	6.08E+07	106.46
Z10	**Zab-szék**	2017.08.29	1.5	10.01	24.9	150.2	27.760	40989.65	22983.0	9846	3341.15500	3.80E+08	588.72
V10	**Pan no. 60**	2017.08.29	2.0	9.93	25.9	62.5	13.088				1281.69600	4.37E+08	

Sample Code	Pan name	Sampling date	Water depth (cm)	pH	Water temperature (°C)	DO (%)	Salinity (g/L)	TN (µg/L)	TP (µg/L)	SRP (µg/L)	DOC (mg/L)	Bacterial cell number	Chlorphyll a (µg/L)
B10	**Böddi-szék**	2017.08.29	5.0	9.69	25.7	120.6	23.280	19005.28	8193.0	4817	1266.38000	1.44E+08	13.18
S11	**Sós-ér**	2017.09.11	10.0	9.52	25.4	191.0	15.080	33827.90	1273.0	93	352.88290	1.14E+07	52.29
V11	**Pan no. 60**	2017.09.11	12.0	9.63	25.2	129.1	11.440	19811.60	25013.0	9046	820.96090	3.52E+08	696.71
B11	**Böddi-szék**	2017.09.11	3.5	9.73	24.8	198.0	18.800	15244.9	4360.0	2883	545.27540	2.47E+08	264.16
S12	**Sós-ér**	2017.09.27	15.0	9.39	19.9	42.9	5.512	11291.10	796.0	380	161.41370	1.89E+07	80.96
Z12	**Zab-szék**	2017.09.27	4.0	9.65	21.1	139.6	5.096	6892.40	3960.0	2950	35.92850	6.84E+07	12.78
V12	**Pan no. 60**	2017.09.27	11.0	9.66	18.9	110.3	5.464	12427.80	7480.0	5403	49.41905	1.75E+08	28.57
K12	**Kelemen-szék**	2017.09.27	2.0	9.37	19.4	107.5	4.096	13070.30	4560.0	2906	33.66259	6.84E+07	267.09
B12	**Böddi-szék**	2017.09.27	11.5	9.76	18.2	92.0	5.816	18605.70	2173.0	1023	29.02587	2.21E+07	51.12
S13	**Sós-ér**	2017.10.16	15.0	9.16	18.8	71.3	6.912	207157.50	580.0	390	800.5000	3.61E+08	57.94
V13	**Pan no. 60**	2017.10.16	5.5	9.56	19.5	108.6	7.144	174046.20	11373.0	3807	710.9000	2.66E+08	26.86
B13	**Böddi-szék**	2017.10.16	8.5	9.6	17.4	130	8.624	78957.50	4300.0	1663	598.6000	1.14E+08	255.68
S14	**Sós-ér**	2017.11.14	20.0	9.25	6.9	82.6	3.760	6245.49	766.6	283	377.7000	2.00E+08	NA
Z14	**Zab-szék**	2017.11.14	6.0	9.61	5.9	88.7	2.848	4841.37	1900.0	1013	208.8000	1.82E+08	NA
V14	**Pan no. 60**	2017.11.14	15.6	9.69	6.7	99.1	4.456	6439.17	3826.6	2350	496.1000	2.57E+08	NA
K14	**Kelemen-szék**	2017.11.14	11.0	9.39	7.1	89.5	2.64	3824.59	2346.6	1137	306.3000	1.44E+08	NA
B14	**Böddi-szék**	2017.11.14	9.0	9.55	6.0	92.0	4.784	2614.15	1453.3	1147	428.9000	1.71E+08	NA

Table S2. Zooplankton community data of the Kiskunság soda pans

Sample Code	Pan name	Sampling date	*D.magna* (individual/L)	*D. atkinsoni* (individual/L)	*M. brachiata* (individual/L)	*A. spinosus* (individual/L)	*A. bacilifer* (individual/L)	*M. viridis* (individual/L)	*Cd. reticulata* (individual/L)	Copepoda (individual/L)	Cladocera (individual/L)
S01	**Sós-ér**	2017.04.12	68.0	2.0			198.0	2.0		200.0	70.0
Z01	**Zab-szék**	2017.04.12	11.0	5.0	1.0	130.0				131.00	17.0
V01	**Pan no. 60**	2017.04.12	0.2			527.0				527.0	0.2
K01	**Kelemen-szék**	2017.04.12	1.5	3.0	4.5	369.5				369.5	9.0
B01	**Böddi-szék**	2017.04.12	10.0	3.5	5.0	74.5				74.5	18.5
S02	**Sós-ér**	2017.04.26	9.9				24.5			24.5	9.9
Z02	**Zab-szék**	2017.04.26	2.0			87.0				87.0	2.0
V02	**Pan no. 60**	2017.04.26		0.5		244.5				244.5	0.5
K02	**Kelemen-szék**	2017.04.26		12.0	1.0	544.5				544.5	13.0
B02	**Böddi-szék**	2017.04.26	2.0		1.0	173.5				173.5	3.0
S03	**Sós-ér**	2017.05.17	56.0	48.0		2.5	40.0		0.15	42.5	104.2
Z03	**Zab-szék**	2017.05.17	12.5		2.5	202.0		1.0		202.0	14.5
V03	**Pan no. 60**	2017.05.17	4.0	2.0	3.0	171.5				171.5	9.0
K03	**Kelemen-szék**	2017.05.17		12.0	11.0	267.5				267.5	13.0
B03	**Böddi-szék**	2017.05.17	5.5		28.0	225.0	18.0			243.0	33.5
S04	**Sós-ér**	2017.05.29	6.2				13.5			13.5	6.2
Z04	**Zab-szék**	2017.05.29	3.5		2.0	1006.5				1006.5	5.5
V04	**Pan no. 60**	2017.05.29	111.4		285.7	65.7				65.7	397.2
K04	**Kelemen-szék**	2017.05.29	40.0		161.5	78.0				78.0	201.5
B04	**Böddi-szék**	2017.05.29	1.0		10.0	92.5	8.5			101.0	11.0
S05	**Sós-ér**	2017.06.15	3.5		0.1	1.5	20.1			21.6	3.5
Z05	**Zab-szék**	2017.06.15	0.4		5.2	47.0				47.0	5.5
V05	**Pan no. 60**	2017.06.15			40.0	950.0				950.0	40.0
K05	**Kelemen-szék**	2017.06.15	9.0		6.0	1263.0				1263.0	15.0

Sample Code	Pan name	Sampling date	*D.magna* (individual/L)	*D. atkinsoni* (individual/L)	*M. brachiata* (individual/L)	*A. spinosus* (individual/L)	*A. bacilifer* (individual/L)	*M. viridis* (individual/L)	*Cd. reticulata* (individual/L)	Copepoda (individual/L)	Cladocera (individual/L)
B05	**Böddi-szék**	2017.06.15	10.0		50.0	622.5		15.0		637.5	60.0
S06	**Sós-ér**	2017.06.29			3.0		50.0			3.0	50.0
Z06	**Zab-szék**	2017.06.29			4.5	194.5				194.5	4.5
V06	**Pan no. 60**	2017.06.29				273.5				273.5	0.0
K06	**Kelemen-szék**	2017.06.29	5.0		20.0	25.0				25.0	25.0
B06	**Böddi-szék**	2017.06.29			6.0	158.5				158.5	6.0
S07	**Sós-ér**	2017.07.13			1.0		2034.0	0.1		2034.1	1.0
Z07	**Zab-szék**	2017.07.13			2140.0	1190.0				1190.0	2140.0
V07	**Pan no. 60**	2017.07.13			5.0	1195.0					
B07	**Böddi-szék**	2017.07.27			8.0	592.0	153.0			745.0	8.0
S08	**Sós-ér**	2017.07.27					0.1	0.1		0.2	0.0
Z08	**Zab-szék**	2017.07.27			30.0	845.0				845.0	30.0
V08	**Pan no. 60**	2017.07.27				820.0				820.0	0.0
B08	**Böddi-szék**	2017.08.14				55.5				55.5	0.0
S09	**Sós-ér**	2017.08.14			2.0					0.0	2.0
Z09	**Zab-szék**	2017.08.14			150.0	960.0				960.0	150.0
V09	**Pan no. 60**	2017.08.14				92.0				92.0	0.0
K09	**Kelemen-szék**	2017.08.14	1.0		6.0	2.0				2.0	7.0
B09	**Böddi-szék**	2017.08.29			1.4	175.0				175.0	1.4
S10	**Sós-ér**	2017.08.29			11.9	0.9				0.9	11.9
Z10	**Zab-szék**	2017.08.29				40.0				40.0	0.0
V10	**Pan no. 60**	2017.08.29				203.0				203.0	0.0
B10	**Böddi-szék**	2017.09.11				240.0	10.0			250.0	0.0
S11	**Sós-ér**	2017.09.11		0.1	0.1	0.4	0.1			0.5	0.2
V11	**Pan no. 60**	2017.09.11				1490.0				1490.0	0.0
B11	**Böddi-szék**	2017.09.27			1730.0	243.0				243.0	1730.0

Sample Code	Pan name	Sampling date	*D.magna* (individual/L)	*D. atkinsoni* (individual/L)	*M. brachiata* (individual/L)	*A. spinosus* (individual/L)	*A. bacilifer* (individual/L)	*M. viridis* (individual/L)	*Cd. reticulata* (individual/L)	Copepoda (individual/L)	Cladocera (individual/L)
S12	**Sós-ér**	2017.09.27	50.0		375.0		130.0			130.0	425.0
Z12	**Zab-szék**	2017.09.27			2.0	44.0				44.0	2.0
V12	**Pan no. 60**	2017.09.27			0.3	80.7				80.7	0.3
K12	**Kelemen-szék**	2017.09.27	10.0		171.0		1.0			1.0	181.0
B12	**Böddi-szék**	2017.09.27			70.0	47.5				47.5	70.0
S13	**Sós-ér**	2017.10.16	6.7	0.2	7.0		32.5			32.5	13.2
V13	**Pan no. 60**	2017.10.16			103.5	146.5				146.5	103.5
B13	**Böddi-szék**	2017.10.16			386.0	91.0				91.0	386.0
S14	**Sós-ér**	2017.11.14	64.5		8.0	7.0	36.5			43.5	72.5
Z14	**Zab-szék**	2017.11.14			12.0	49.0				49.0	12.0
V14	**Pan no. 60**	2017.11.14			300.0	1450.0	150.0			1600.0	300.0
K14	**Kelemen-szék**	2017.11.14			86.5	32.5	10.5			43.0	86.5
B14	**Böddi-szék**	2017.11.14			8.65	15.9				15.ö	8.7

Table S3. Relative abundance of the dominant microuekaryotic genera and bacterial clades and their abbreviated names

	Abbreviation	Genus/clade	Böddi-szék		Pan no. 60		Kelemen-szék		Zab-szék		Sós-ér	
			Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max
Microeukaryotes	Ad	Andalucia			0.012	0-0.158						
	An	Anomoeoneis					0.011	0-0.090	0.011	0-0.099	0.021	0-0.164
	Ca	Chlamydomonadales_X_unclassified							0.021	0-0.241		
	Cd	Chrysophyceae_Clade-D_X									0.011	0-0.128
	Ce	Cercozoa_unclassified	0.005	0-0.056					0.025	0-0.134		
	Cf	Chrysophyceae_Clade-F_X									0.013	0-0.126
	Ch	Choricystis	0.211	0.002-0.575	0.115	0.003-0.624	0.363	0-0.825	0.304	0.007-0.864	0.051	0-0.225
	Cl	Chloroparva	0.041	0.005-0.195	0.192	0.018-0.472			0.024	0.004-0.101	0.013	0-0.097
	Cr	Chlorophyta_unclassified	0.013	0-0.137					0.011	0-0.062	0.026	0-0.231
	Ct	Chytridiomycetes_unclassified							0.007	0-0.093		
	Cu	Chlorellales_X_unclassified	0.091	0.003-0.307	0.163	0.009-0.419	0.035	0-0.296	0.126	0-0.651	0.138	0-0.538
	Cy	Chrysophyceae_X_unclassified							0.034	0-0.230		
	Di	Diacronema							0.008	0-0.099	0.033	0-0.231
	Fu	Fungi_unclassified	0.005	0-0.051	0.013	0-0.141					0.009	0-0.098
	Ha	Halocafeteria							0.011	0-0.059		
	Hl	Halteria					0.069	0-0.592			0.007	0-0.051
	Hn	Hanusia									0.009	0-0.067
	Hp	Hoplorhynchus									0.007	0-0.094
	Ht	Halteriidae_X									0.021	0-0.167
	Ko	Komma									0.051	0-0.537
	Ma	Marvania	0.012	0-0.073			0.048	0-0.375				

Na	Nannochloris	0.046	0-0.439	0.019	0-0.107						
Ni	Nitzschia	0.039	0-0.300			0.051	0-0.292	0.031	0-0.275		
Nn	Nannochloropsis			0.067	0-0.626					0.005	0-0.053
No	Novel-clade-2_X							0.032	0-0.325		
Nv	Novel-Gran-6_X			0.011	0-0.103						
Oc	Ochrophyta_unclassified			0.005	0-0.064					0.029	0-0.067
Op	Opisthokonta_unclassified			0.019	0-0.241					0.017	0-0.227
Pa	Paraphysomonas			0.009	0-0.122					0.037	0-0.268
Pe	Perkinsida_XXX			0.034	0-0.417						
Pi	Pirsonia_unclassified	0.006	0-0.085	0.008	0-0.101	0.014	0-0.129				
Pl	Platyophryida_unclassified							0.005	0-0.061		
Pn	Pirsonia									0.019	0-0.259
Pr	Prorocentrum					0.032	0-0.167			0.011	0-0.094
Ps	Pseudodendromonadales_XX									0.006	0-0.69
Py	Pythium									0.012	0-0.103
Ra	Raphid-pennate_unclassified							0.005	0-0.059		
Se	Sessilida_unclassified					0.006	0-0.052				
So	Sordariomycetes_unclassified									0.049	0-0.557
Sp	Spumella	0.037	0-0.408	0.063	0-0.793			0.013	0-0.153		
St	Stramenopiles_unclassified			0.007	0-0.097			0.026	0-0.306		
Su	Surirella	0.005	0-0.057								
Te	Tetracystis									0.007	0-0.091
Tr	Tremula			0.005	0-0.074						
Vo	Vorticella									0.008	0-0.116

	Wi	Wislouchiella					0.027	0-0.203				
	Ab	Absconditabacteriales_SR1										
	Ai	Acidithiobacillaceae							0.007	0-0.089	0.007	0-0.101
	ac	acIII-A	0.026	0.032-0.059	0.034	0.017-0.095	0.064	0.018-0.232	0.043	0.013-0.157	0.025	0.002-0.086
	aV	acIV-C	0.011	0-0.054								
	Ac	Actinobacteria									0.013	0-0.056
	Al	Algoriphagus									0.012	0-0.061
	ba	bacII-A									0.037	0-0.216
	bc	bacV									0.015	0-0.064
	Ba	Balneolaceae	0.016	0.002-0.068	0.008	0-0.059						
	Bu	Burkholderiaceae					0.026	0-0.143			0.042	0-0.165
Bacteria	Cc	Candidatus_Campbellbacteria					0.008	0-0.051				
	Ce	Cecembia	0.005	0-0.066								
	Cr	Cryomorphaceae	0.009	0-0.071								
	Cy	Cyanobium_PCC-6307			0.119	0.002-0.281			0.021	0-0.102	0.008	0-0.067
	Cl	Cyclobacteriaceae	0.005	0-0.066							0.011	0-0.058
	Er	Erysipelotrichaceae_UCG-004									0.093	0-0.318
	Fl	Flavobacteriaceae									0.042	0-0.328
	Ge	Gemmatimonadetes			0.038	0-0.224						
	Hy	Hydrogenophaga									0.027	0-0.093
	Iu	Ilumatobacter			0.025	0.001-0.087						
	Il	Ilumatobacteraceae	0.036	0.011-0.086	0.021	0.002-0.054	0.029	0.006-0.106	0.034	0-0.168		
	Iz	Izimaplasmatales	0.011	0.001-0.052								
	Ki	Kiritimatiellaeota			0.053	0-0.224					0.006	0-0.067

Lu	Luna1-A	0.059	0.009-0.195	0.014	0-0.059	0.075	0.017-0.186			0.071	0-0.319
Mt	Methylophilaceae	0.024	0.007-0.063							0.035	0-0.067
Me	Methylotenera					0.024	0.005-0.067				
Mi	Microscillaceae					0.012	0-0.061				
Ni	Nitriliruptoraceae	0.092	0.032-0.201	0.085	0.020-0.292	0.019	0.002-0.063	0.083	0.018-0.324	0.055	0-0.295
No	Nodularia_PCC-9350									0.058	0-0.404
Ox	Oxyphotobacteria	0.013	0-0.079			0.035	0-0.199	0.017	0-0.051		
Pa	Parcubacteria							0.037	0-0.134	0.008	0-0.114
Pi	Phycisphaeraceae			0.024	0-0.130	0.014	0-0.115			0.006	0-0.066
Pl	Planktosalinus	0.044	0-0.152	0.019	0-0.141			0.018	0-0.056	0.004	0-0.057
Rh	Rhodobaca	0.024	0-0.082					0.016	0-0.058		
Ru	Ruminiclostridium_1	0.011	0-0.088					0.013	0-0.069		
Sa	Saprospiraceae	0.014	0-0.064	0.023	0-0.113	0.008	0-0.073			0.005	0-0.052
Sp	Sphingobacteriales			0.006	0-0.063	0.009	0-0.052			0.012	0-0.085
Sp	Sporichthyaceae	0.019	0.003-0.053					0.028	0.006-0.052		
Sy	Synechococcus_MBIC10613	0.036	0-0.219	0.028	0.001-0.132						

Table S4. Mantel test results between the microeukaryotic and bacterial communities, and environmental variables and zooplankton species based on Spearman's rank correlation (rho = 1 "strong positive correlation", rho = -1 "strong negative correlation") (bold: significant)

Mantel test between		**Spearman's correlation coefficient (rho)**	**Significance of the test (p)**
Microeukaryotes	Water depth	-0.004	0.470
	Water temperature	-0.022	0.617
	Salinity	**0.190**	**0.005**
	DOC	**0.313**	**0.001**
	TP	**0.248**	**0.001**
	TN	0.188	0.007
	pH	0.066	0.138
	Chlorophyll-a	0.051	0.179
	Daphnia magna	-0.090	0.864
	Daphnia atkinsoni	-0.057	0.725
	Moina brachiata	0.003	0.446
	Arctodiaptomus spinosus	0.021	0.331
	Arctodiaptomus bacilifer	-0.108	0.910
	Megacyclops viridis	-0.112	0.895
	Copepoda	-0.019	0.571
	Cladocera	0.007	0.451
Bacteria	Water depth	0.101	0.068
	Water temperature	**0.121**	**0.046**
	Salinity	0.079	0.101
	DOC	**0.135**	**0.045**
	TP	**0.194**	**0.003**
	TN	0.176	0.012
	pH	0.075	0.129
	Chlorophyll-a	0.075	0.139
	Daphnia magna	-0.048	0.712
	Daphnia atkinsoni	0.017	0.395
	Moina brachiata	-0.056	0.759
	Arctodiaptomus spinosus	**0.129**	**0.044**
	Arctodiaptomus bacilifer	-0.123	0.943
	Megacyclops viridis	-0.064	0.743
	Copepoda	0.102	0.084
	Cladocera	-0.050	0.734

Table S5. Impact of pan identity and seasonality on the structure of microeukaryotic and bacterial communities based on two-way PERMANOVA analysis (number of * indicates the statistical significance with p, 0 '***, 0.001 '**', 0.01 '*', 0.05 '·', 0.1 ' ', 1)

			Pan identity (R^2)	Pan identity (p)	Seasonality (R^2)	Seasonality (p)	Pan identity* Seasonality (R^2)	Pan identity* Seasonality (p)
All pans	**Microeukaryotes**	All OTUs	0.186	0.001***	0.144	0.001***	0.162	0.001 ***
		Core5	0.178	0.001***	0.151	0.001***	0.147	0.001***
		Non-core5	0.153	0.001***	0.069	0.001***	0.173	0.001***
	Bacteria	All OTUs	0.255	0.001***	0.115	0.001***	0.163	0.001 ***
		Core5	0.188	0.001***	0.176	0.001***	0.127	0.007 **
		Non-core5	0.151	0.001***	0.125	0.001***	0.148	0.001***

Turbid pans	**Microeukaryotes**	All turbid pan OTUs	0.144	0.001***	0.206	0.001***	0.132	0.006 **
		Core4	0.145	0.001***	0.226	0.001***	0.124	0.015 *
		Non-core4	0.129	0.001***	0.084	0.001***	0.164	0.001***
	Bacteria	All turbid pan OTUs	0.177	0.001 ***	0.160	0.001***	0.137	0.003 **
		Core4	0.177	0.001***	0.171	0.001***	0.129	0.012 *
		Non-core4	0.167	0.001***	0.108	0.001***	0.159	0.001***

Table S6. GPS coordinates of the sampling sites in the Seewinkel region and the sampling years we collected samples from each site (black dots)

Name	Longitude	Latitude	2017	2018
Albersee	16.77011917	47.77513639	●	●
Apetloner Meierhoflacke	16.82404028	47.72157583		●
Auerlacke	16.88677778	47.78917083	●	●
Birnbaumlacke	16.86491056	47.81771528	●	●
Grosse Neubruchlacke	16.84219833	47.78612111	●	●
Herrnsee	16.7699880	47.7445640		●
Kirchsee	16.78548861	47.75867861		●
Krautingsee	16.78047972	47.75602861	●	●
Kühnbrunnlacke	16.87862583	47.79273556	●	●
Lange Lacke	16.87876472	47.75747528	●	●
Martenhofenlacke	16.8566950	47.75050167	●	●
Mittlerer Stinkersee	16.78752194	47.80675889	●	●
Neufeldlacke	16.84024833	47.76447667	●	●
Unnamed	16.8443250	47.7680360	●	
Obere Höllacke	16.80793222	47.82711944	●	●
Oberer Stinkersee	16.79251722	47.81376222	●	●
Ochsebrunnlacke	16.84461722	47.81073583	●	●
Östliche Wörthenlacke	16.88009389	47.77334361	●	●
Östliche Fuchslochlacke	16.86618583	47.79044083	●	●

Runde Lacke	16.79274361	47.78574472	●	●
Sechsmahdlacke	16.88411194	47.78378861	●	●
Stundlacke	16.87170639	47.79890389	●	●
Südlicher Silbersee	16.77973694	47.79113278	●	●
Westliche Wörthenlacke	16.87077667	47.77092167	●	●
Westliche Fuschslochlacke	16.85234944	47.79007667	●	●
Zicklacke	16.78475194	47.76696444	●	●

Table S7. Measured environmental variables and zooplankton abundance of the Seewinkel soda pans

Sample Code	Pan name	Sampling year	Water depth (cm)	Conductivity (mS/cm)	pH	TP (mg/L)	TN (mg/L)	TSS (mg/L)	Copepoda (ind/L)	Cladocera (ind/L)
Sp17_1	**Albersee**	2017	4	6.65	9.37	3093.261	7375.923	1393.333	333.0	64.0
Sp17_2	**Auerlacke**	2017	8	2.14	8.65	2112.152	5435.816	2590.000	0.0	0.0
Sp17_4	**Birnbaumlacke**	2017	5	2.21	8.79	11077.190	10061.590	13290.000	0.0	0.0
Sp17_7	**Grosse_Neubruchlacke**	2017	12	6.72	8.94	3817.773	5502.635	6040.000	0.0	0.0
Sp17_9	**Krautingsee**	2017	6	4.20	9.32	2987.603	15425.900	1353.333	0.0	0.0
Sp17_10	**Kühnbrunnlacke**	2017	2	4.54	8.93	4059.277	4520.273	3625.000	0.0	0.0
Sp17_11	**Lange_Lacke**	2017	32	3.48	9.15	2836.663	5174.400	1776.667	1460.0	38.0
Sp17_12	**Martenhofenlacke**	2017	3	2.95	9.15	3334.765	6186.069	13280.000	162.5	211.3
Sp17_13	**Mittlerer_Stinkersee**	2017	15	7.23	9.51	1553.674	5120.475	401.111	634.0	18.0
Sp17_14	**Unnamed**	2017	9	4.14	8.60	507.3363	8749.823	37.000	0.0	0.0
Sp17_16	**Obere_Höllacke**	2017	6	8.47	9.73	1327.264	4714.870	192.667	341.0	19.0
Sp17_17	**Oberer_Stinkersee**	2017	5	8.72	9.60	6006.402	7264.557	4650.000	827.7	33.8
Sp17_18	**Ochsenbrunnlacke**	2017	6	4.18	8.92	14094.200	5833.216	9470.000	0.0	0.0
Sp17_19	**Östliche_Fuchslochlacke**	2017	12	2.73	8.77	4723.413	6365.426	3090.000	0.0	0.0
Sp17_20	**Östliche_Wörthenlacke**	2017	22	30.00	8.55	193.623	2301.166	73.750	0.0	0.0
Sp17_21	**Runde_Lacke**	2017	8	9.73	9.57	812.035	6350.187	437.778	610.0	6.1
Sp17_22	**Sechsmahdlacke**	2017	5	8.23	8.88	1991.400	5588.211	2736.667	0.0	0.0
Sp17_23	**Standlacke**	2017	4	2.29	8.85	1297.076	7741.671	823.333	0.0	0.0
Sp17_24	**Südlicher_Silbersee**	2017	12	10.95	9.80	303.196	4719.559	53.750	608.0	100.0
Sp17_27	**Westliche_Fuschslochlacke**	2017	3	6.10	9.02	5176.232	4575.370	7840.000	0.0	0.0
Sp17_28	**Westliche_Wörthenlacke**	2017	25	3.66	8.74	195.124	2717.322	254.000	0.0	0.0

Sp17_29	**Zicklacke**	2017	10	5.81	9.54	370.741	5312.728	495.000	351.3	20.7
Sp17_Neu2	**Neufeldlacke**	2017	5	4.42	8.96	1161.230	11376.880	358.000	0.0	0.0
Sp18_1	**Albersee**	2018	18	1.50	8.99	250.814	2067.481	126.667	35.5	3.5
Sp18_1_d	**Auerlacke**	2018	23	0.819	8.56	664.364	2487.675	110.000	3.0	30.5
Sp18_4	**Birnbaumlacke**	2018	15	0.653	8.68	1349.167	2382.278	372.857	34.5	111.5
Sp18_7	**Grosse_Neubruchlacke**	2018	22	1.30	8.70	1326.933	2163.492	506.667	3400.0	305.0
Sp18_8	**Kirchsee**	2018	21	0.70	8.80	993.425	2237.309	745.000	92.0	234.5
Sp18_9	**Krautingsee**	2018	28	1.20	8.70	547.265	4182.024	175.000	283.0	16.0
Sp18_10	**Kühnbrunnlacke**	2018	12	0.821	8.58	2712.844	3447.667	1590.000	145.0	28.5
Sp18_11	**Lange_Lacke**	2018	28	1.70	8.67	545.783	1697.004	35.143	234.0	4.5
Sp18_12	**Martenhofenlacke**	2018	17	0.715	8.50	828.894	1693.326	440.000	21.5	59.5
Sp18_13	**Mittlerer_Stinkersee**	2018	24	3.40	9.20	1946.516	5763.620	1030.000	2330.0	490.0
Sp18_16	**Obere_Höllacke**	2018	15	2.48	9.11	2484.576	5092.678	2370.000	3.5	49.5
Sp18_17	**Oberer Stinkersee**	2018	14	3.35	9.3	2656.518	2307.066	2175.000	368.0	6.5
Sp18_18	**Ochsenbrunnlacke**	2018	17	1.16	8.91	9587.555	4585.35	4540.000	2435.0	145.0
Sp18_19	**Östliche_Fuchslochlacke**	2018	17	0.60	8.60	1249.855	2306.686	638.000	16.0	126.0
Sp18_20	**Östliche_Wörthenlacke**	2018	44	2.47	8.78	73.295	1428.247	22.200	123.0	38.5
Sp18_21	**Runde_Lacke**	2018	30	3.93	9.10	553.194	4605.643	524.000	4085.0	75.0
Sp18_22	**Sechsmahdlacke**	2018	21	1.76	8.86	1217.246	1963.351	574.000	3.5	42.0
Sp18_23	**Stundlacke**	2018	12	0.56	8.71	381.252	1670.877	3.400	133.0	9.0
Sp18_24	**Südlicher_Silbersee**	2018	48	4.20	9.30	216.722	4254.319	35.200	244.0	2.5
Sp18_27	**Westliche_Fuschslochlacke**	2018	18	1.20	8.80	5480.219	6642.566	4460.000	65.0	6.5
Sp18_28	**Westliche_Wörthenlacke**	2018	39	2.29	8.70	67.208	1385.758	70.857	26.5	150.0
Sp18_29	**Zicklacke**	2018	26	2.10	9.16	341.232	2554.642	265.333	25.0	38.0

Sp18_30	**Apetloner_Meierhoflacke**	2018	35	4.06	9.10	677.704	4478.811	125.652	59.5	36.0
Sp18_31	**Herrnsee**	2018	31	9.14	9.15	265.636	5081.263	64.857	795.0	240.0
Sp18_Neu2	**Neufeldlacke**	2018	12	0.99	9.06	227.098	2392.044	56.296	53.5	31.0

Table S8. Interset correlations (Pearson's correlation coefficients) between the environmental variables and the canonical axes of the CCA of microeukaryotic communities (bold: significant environmental variable for the community composition)

Environmental variables	CCA1	CCA2
2017		
Water depth	0.09	-0.31
pH	-0.62	-0.12
Conductivity	**-0.70**	**-0.54**
TP	**-0.58**	**0.72**
TN	-0.28	0.52
TSS	-0.60	0.70
2018		
Water depth	0.22	0.35
pH	**0.88**	**0.15**
Conductivity	**0.80**	**-0.10**
TP	0.27	-0.36
TN	0.55	-0.19
TSS	0.15	-0.06

Table S9. Scores of microeukaryotic phyla based on CCA of the microeukaryotic communities

Groups	CCA1	CCA2
2017		
Alveolata_unclassified	0.23	0.17
Apicomplexa	0.05	0.16
Centroheliozoa	0.22	0.21
Cercozoa	-0.10	0.04
Chlorophyta	-0.09	0.00
Ciliophora	0.13	0.03
Cryptophyta	0.78	-0.49
Dinoflagellata	0.62	0.21
Discoba	-0.65	-0.12
Fungi	-0.03	-0.03
Haptophyta	-0.16	-0.28
Lobosa	-0.18	-0.14
Ochrophyta	0.02	-0.05
Opalozoa	-0.07	0.00
Opisthokonta_unclassified	-0.02	0.06
Protalveolata_X	-0.41	1.02
Pseudofungi	-0.08	0.02
Stramenopiles_unclassified	-0.13	-0.19
2018		
Alveolata_unclassified	-0.17	0.31

Apicomplexa	-0.10	-0.23
Centroheliozoa	-0.25	-0.21
Cercozoa	0.09	-0.04
Chlorophyta	0.06	0.01
Choanoflagellida	-0.34	-0.31
Ciliophora	-0.03	-0.06
Cryptophyta	-0.68	0.08
Dinoflagellata	-0.17	-0.08
Discoba	0.93	-0.01
Fungi	0.00	0.08
Haptophyta	0.19	0.11
Katablepharidophyta	0.17	-0.58
Lobosa	0.07	0.04
Ochrophyta	-0.06	0.08
Opalozoa	-0.02	0.10
Opisthokonta_unclassified	0.10	0.03
Pseudofungi	0.17	0.09
Stramenopiles_unclassified	0.00	-0.18

Table S10. Interset correlations (Pearson's correlation coefficients) between the environmental variables and the canonical axes of the CCA of prokaryotic communities (bold: significant environmental variable for the community composition)

Environmental variables	CCA1	CCA2
2017		
Water depth	**-0.52**	**0.57**
pH	0.00	0.08
Conductivity	-0.19	0.19
TP	**0.96**	**0.04**
TN	0.51	-0.58
TSS	0.93	0.24
2018		
Water depth	**-0.52**	**0.51**
pH	-0.50	0.57
Conductivity	**-0.66**	**0.59**
TP	**0.75**	**0.30**
TN	0.17	0.59
TSS	0.79	0.03

Table S11. Scores of prokaryotic phyla based on the CCA of the prokaryotic communities

Groups	CCA1	CCA2
2017		
Actinobacteria	-0.03	-0.01
Armatimonadetes	0.57	0.12
Bacteroidetes	-0.01	-0.02
Chloroflexi	0.03	0.09
Cyanobacteria	-0.08	0.02
Epsilonbacteraeota	-0.08	-0.15
Fibrobacteres	0.26	-0.16
Firmicutes	-0.04	-0.01
Gemmatimonadetes	0.13	0.07
Patescibacteria	-0.07	-0.06
Planctomycetes	0.03	0.10
Proteobacteria	-0.02	-0.03
Tenericutes	-0.30	0.12
Verrucomicrobia	0.02	-0.01
2018		
Acidobacteria	0.30	-0.03
Actinobacteria	0.00	-0.01
Bacteroidetes	-0.07	-0.03
Chloroflexi	0.13	0.00
Cyanobacteria	-0.41	0.02

Epsilonbacteraeota	0.25	-0.06
Fibrobacteres	0.16	-0.03
Firmicutes	0.04	0.01
Gemmatimonadetes	0.09	0.04
Patescibacteria	-0.02	-0.09
Planctomycetes	0.05	0.06
Proteobacteria	-0.08	-0.03
Tenericutes	0.09	0.62
Verrucomicrobia	-0.05	0.03

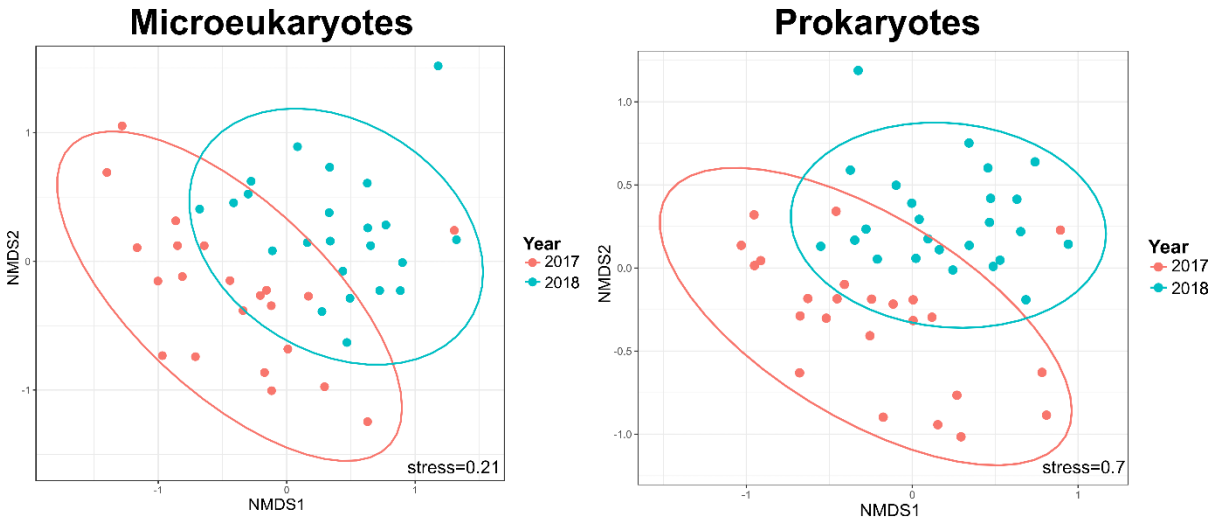

Figure S1. Non-metric multidimensional scaling (NMDS) ordination of planktonic microeukaryotic and prokaryotic communities of the soda pans

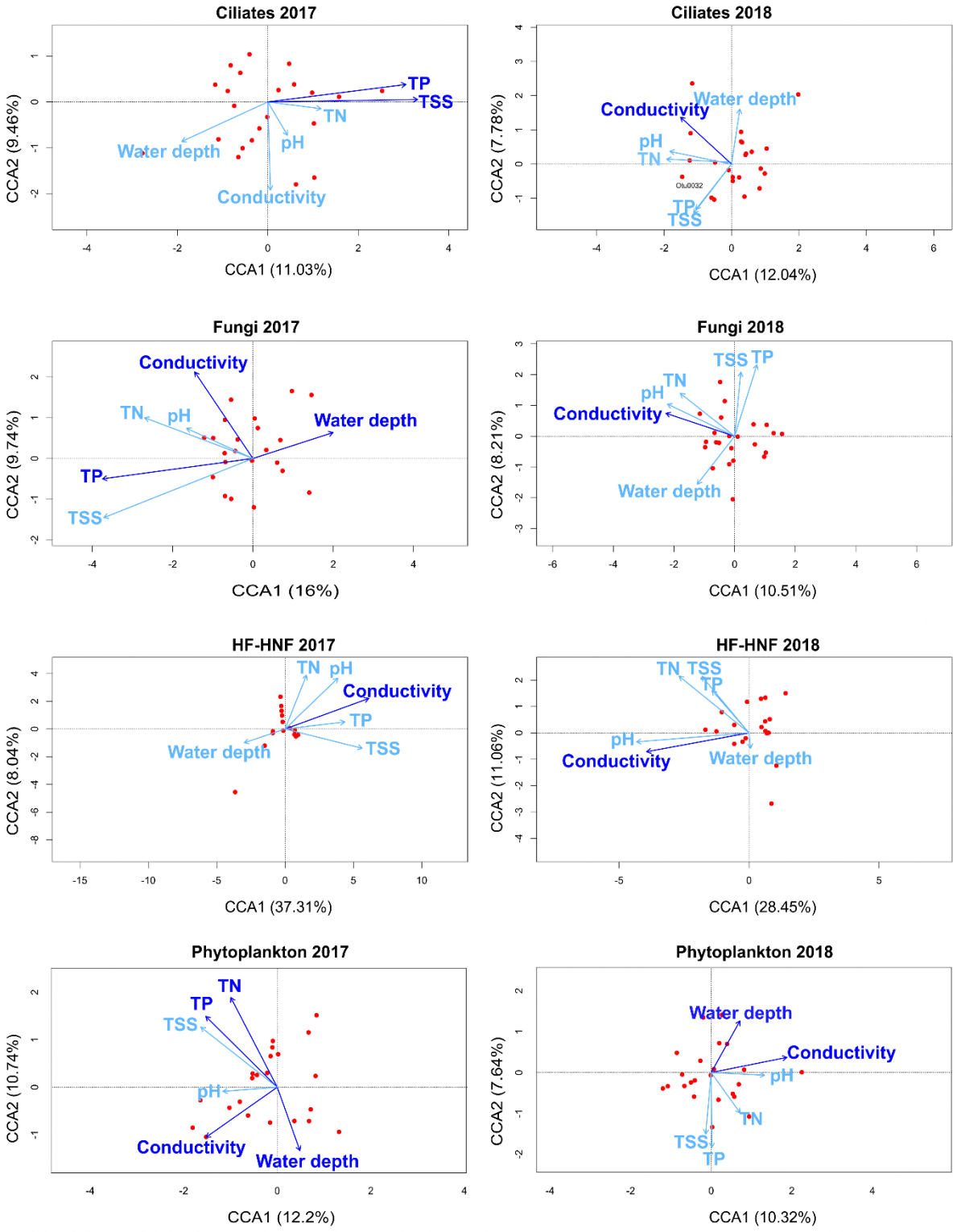

Figure S2. CCAs based on the OTU composition of the microeukaryotes (red points: habitat scores, dark blue arrows: significant environmental variables, light blue arrows: all other environmental variables)

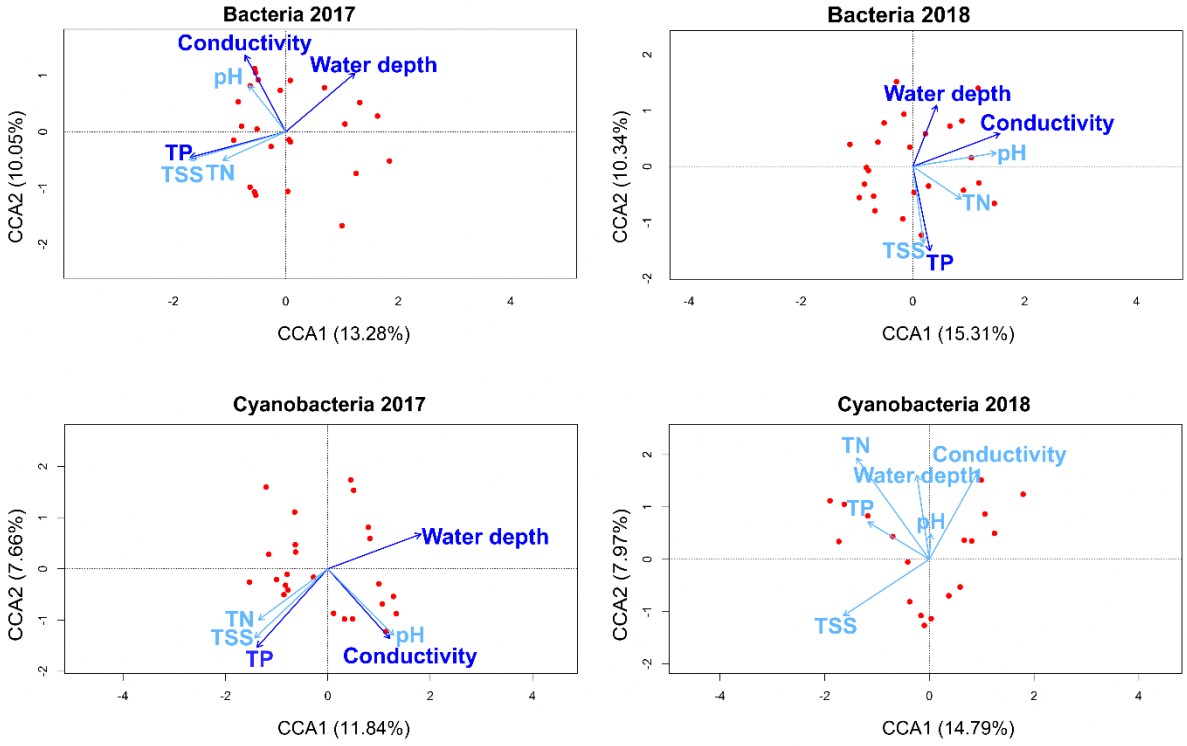

Figure S3. CCAs based on OTU composition of the prokaryotes (red points: habitat scores, dark blue arrows: significant environmental variables, light blue arrows: all other environmental variables)

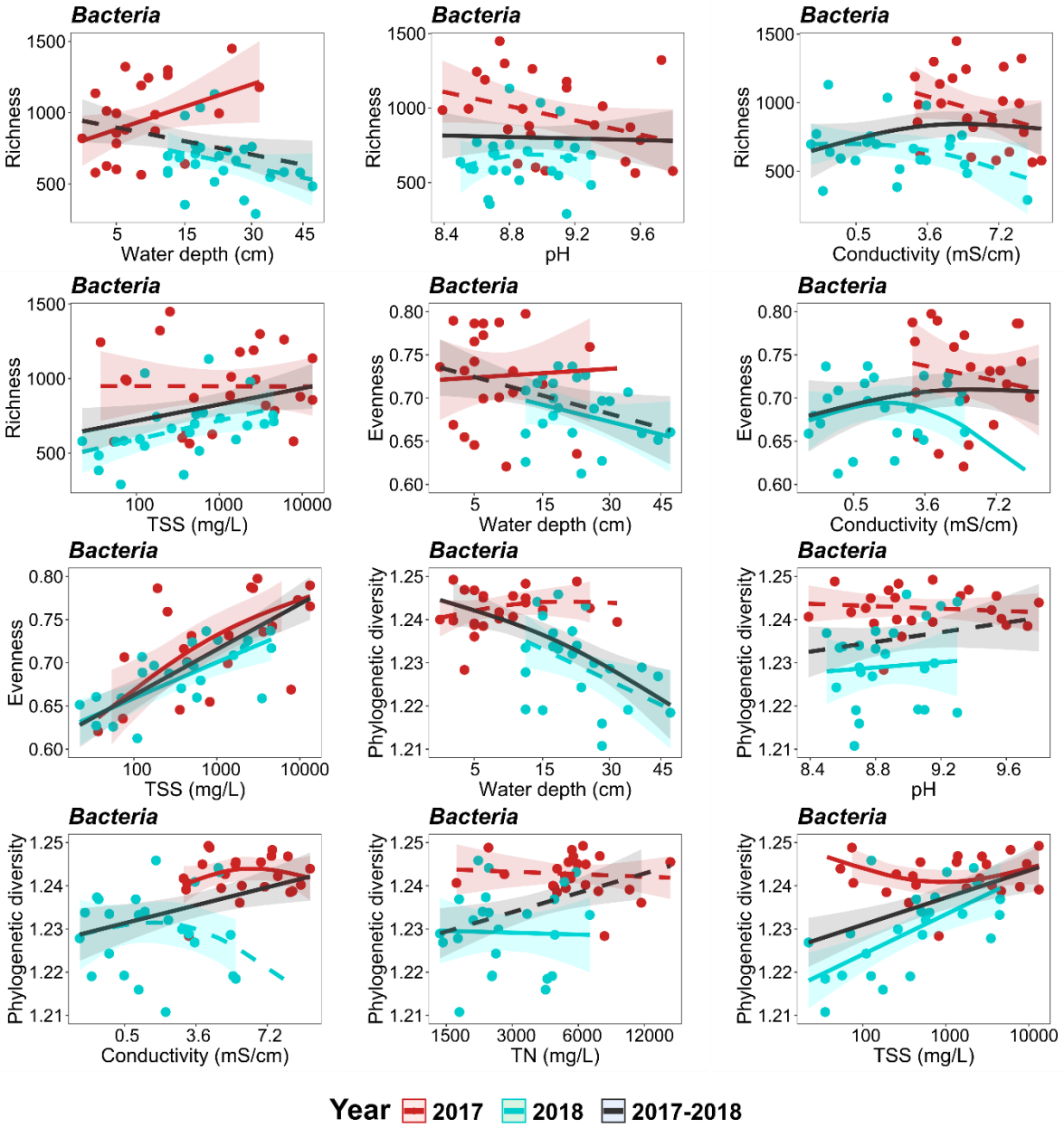

Figure S4. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)

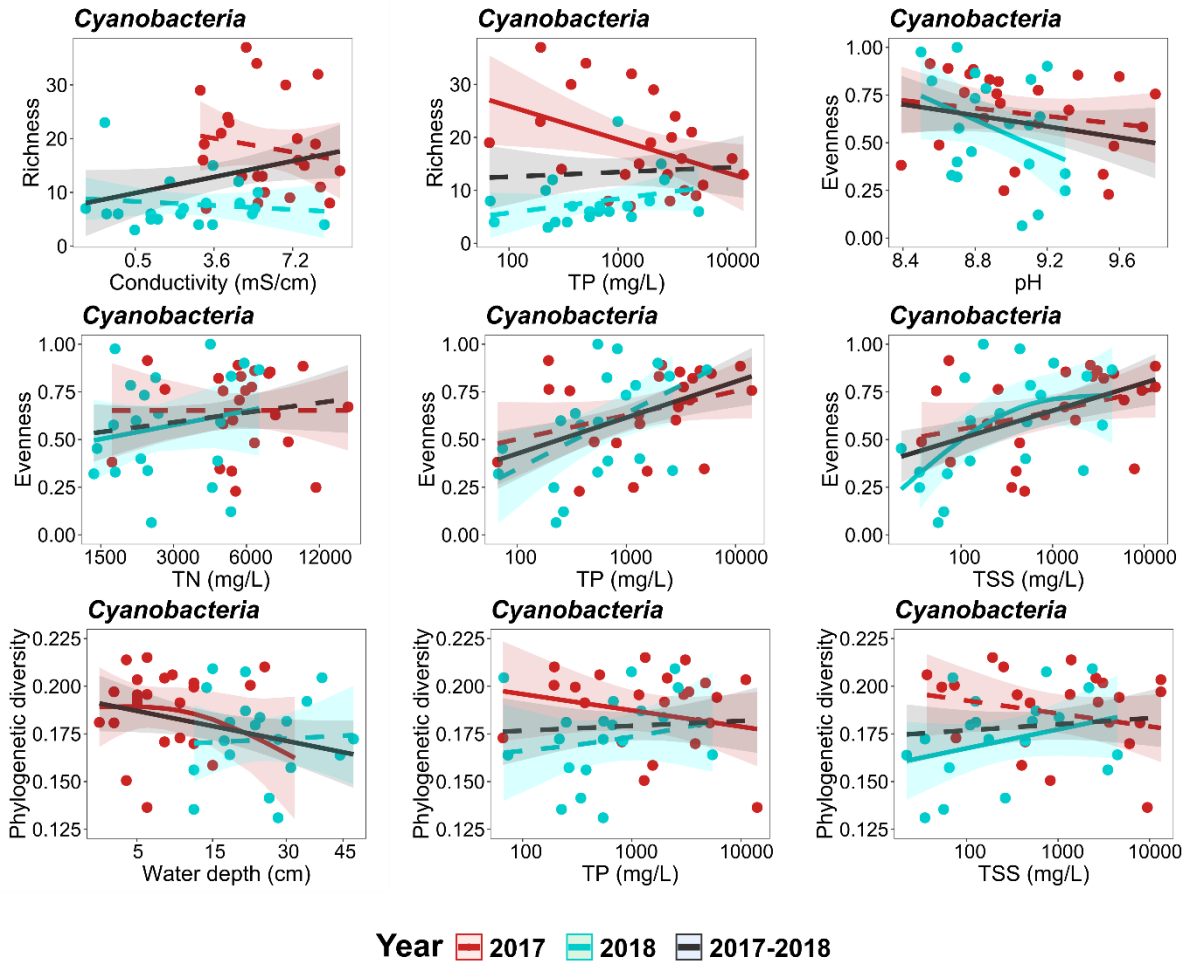

Figure S5. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)

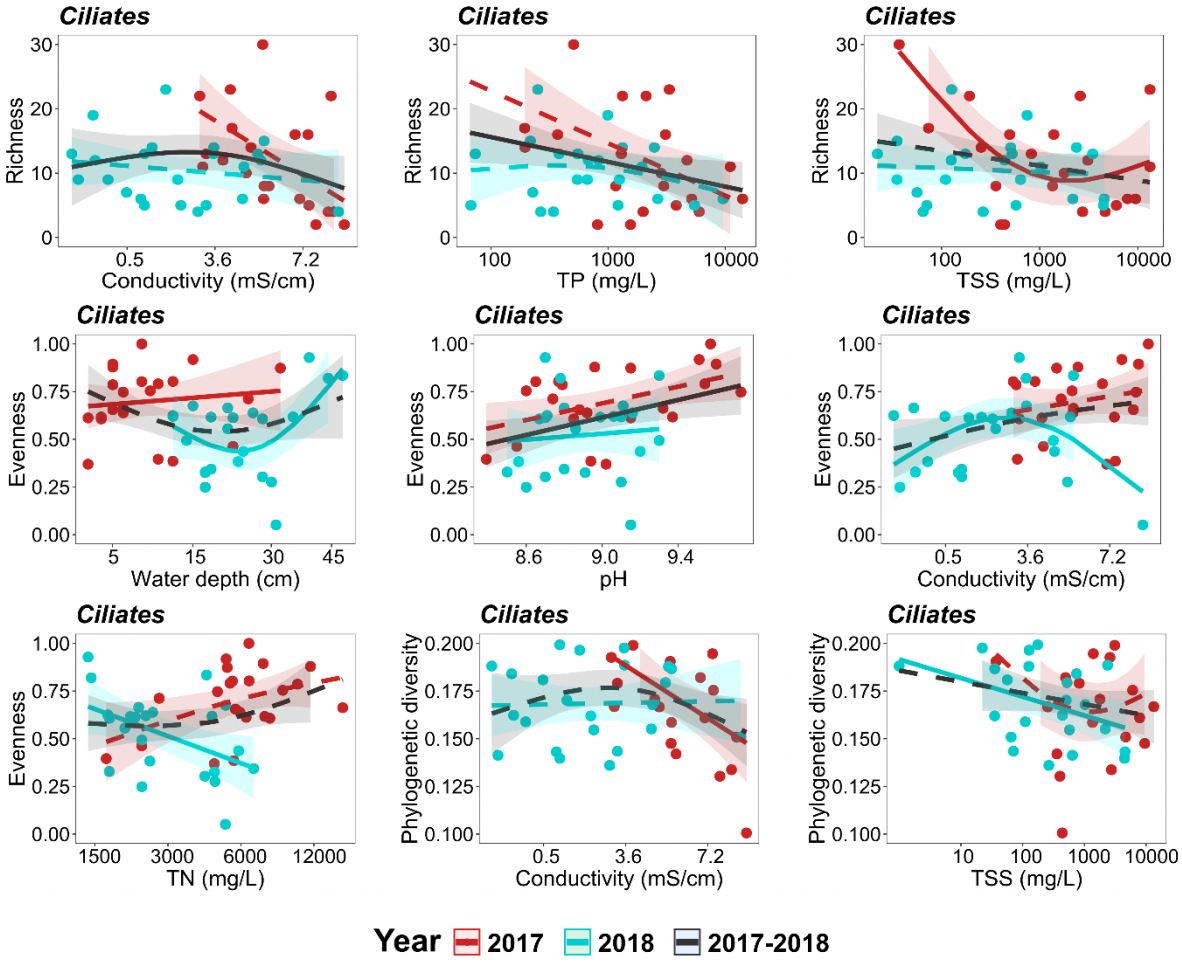

Figure S6. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)

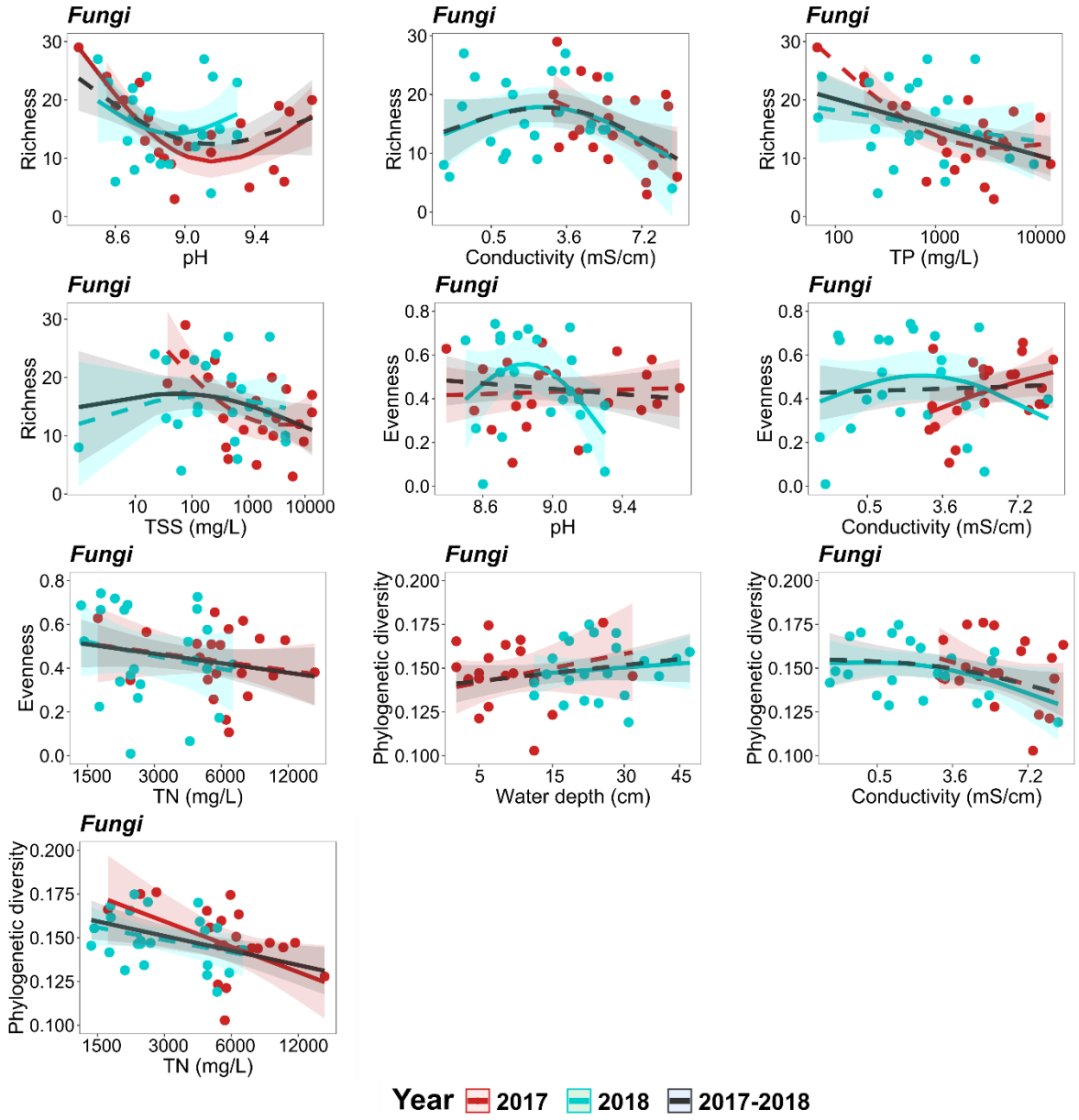

Figure S7. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)

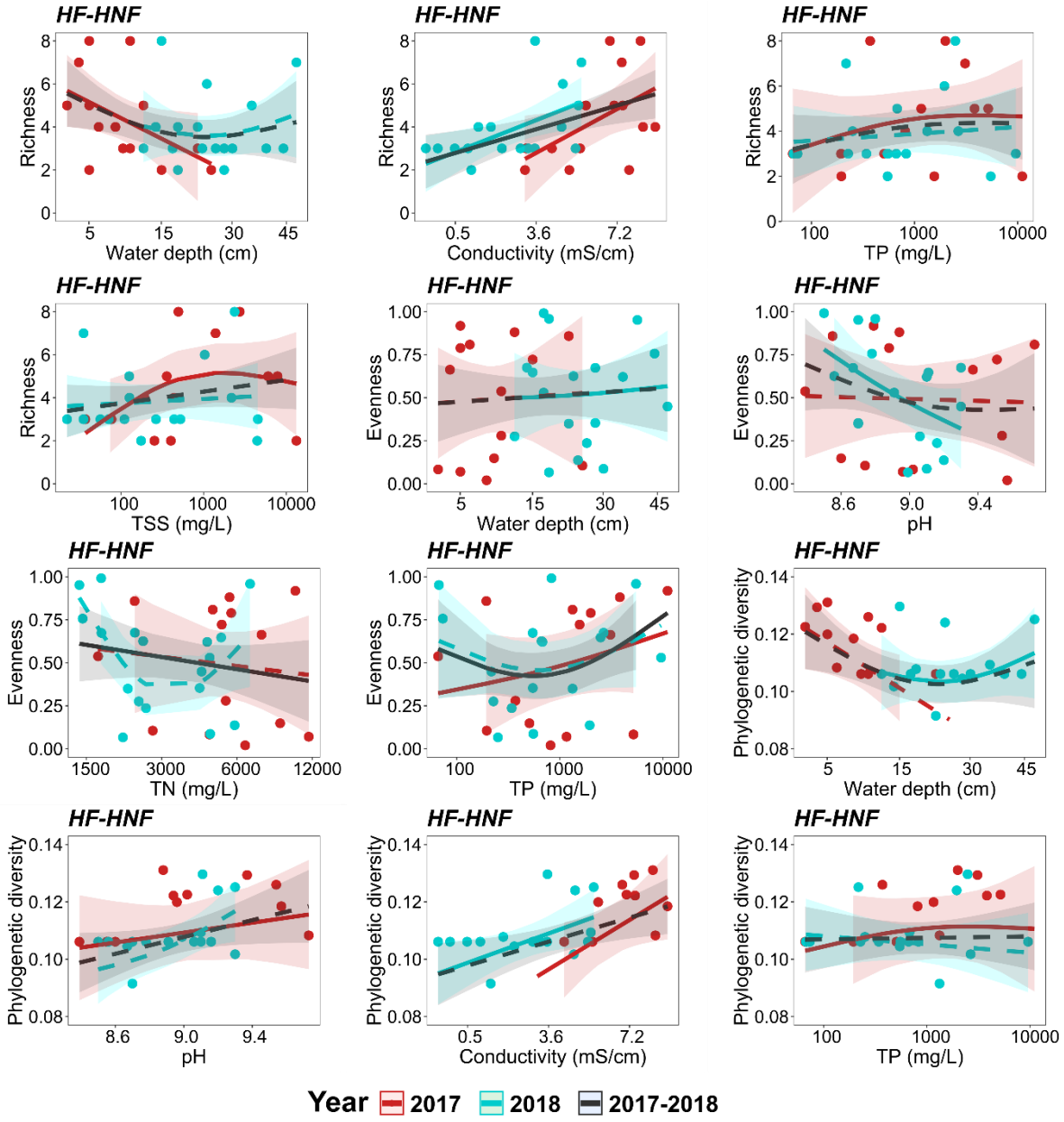

Figure S8. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)

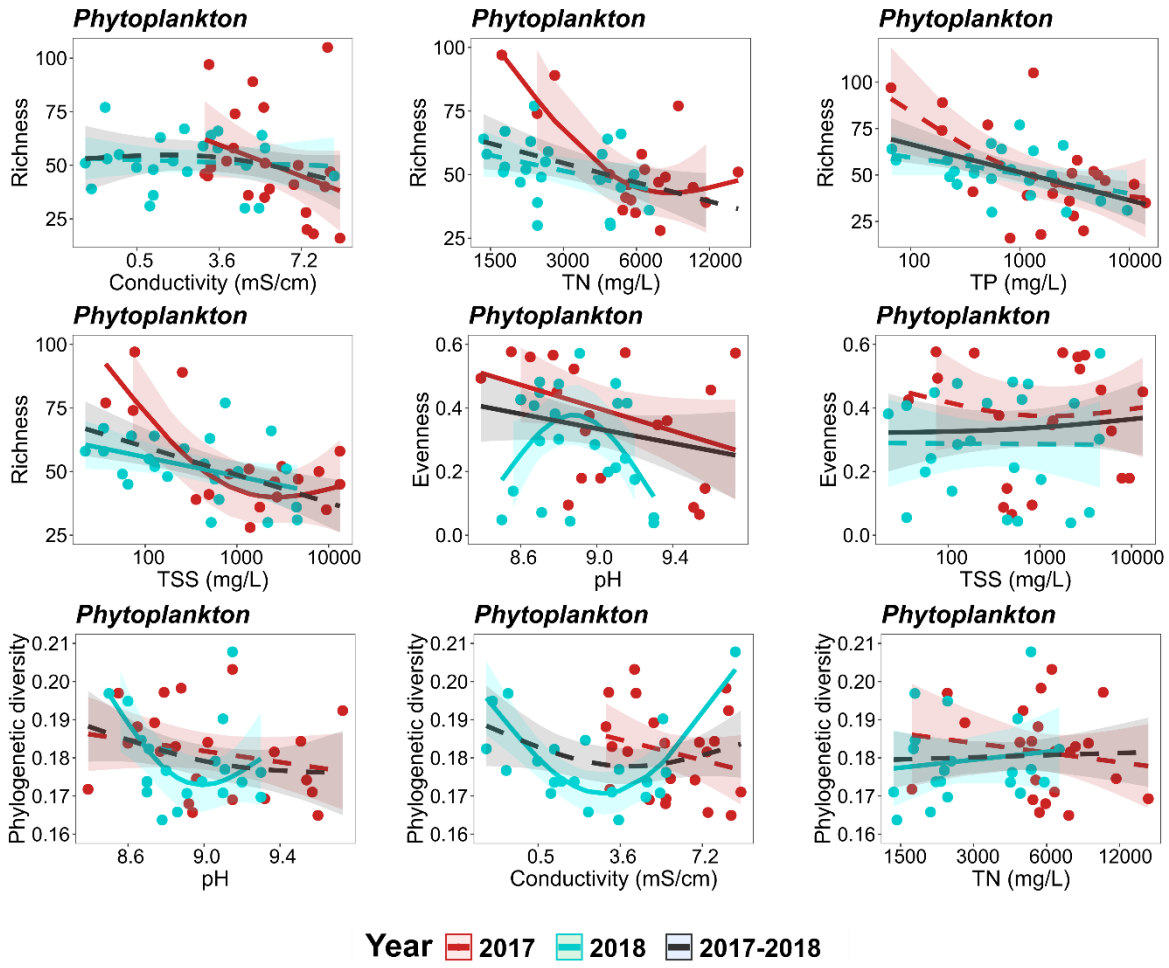

Figure S9. Relationship of richness, evenness, and phylogenetic diversity with the significant environmental variables. Fitted trend lines are based on predicted GAM models in 2017, 2018, and for the pooled dataset. Solid lines: significant relationships, dashed lines: not significant ($p < 0.05$)