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

-PhD thesis-

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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 in their water, 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 natural 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, which resulted in species loss as well (Boros et al., 2013; Felföldi, 2020; 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.

There are previous studies that investigated the seasonality of the planktonic communities of soda lakes of the Carpathian Basin, for example in spring of 2013-2014, Szabó et al., (2020) observed sudden seasonal changes in the prokaryotic community composition. Pálffy et al. (2014) and Somogyi et al. (2009) found that in the summer, picocyanobacteria can dominate the water, while below 15 °C picoeukaryotic algae can cause mass production. 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 microbial communities and to what degree is seasonality determined by environmental variables and what extent desiccation influences the community composition.

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 micoruekaryotes and prokaryotes of soda pans.

2. Main objectives

To date, a comprehensive study focusing on the planktonic microeukaryotes and prokaryotes of shallow soda lakes has not been carried out, which examines both the taxonomic composition, core microbiome (taxa shared among all sites), and diversity along environmental gradients as well as delving into their seasonal dynamics and ecological interactions. However, soda pans, since 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. Seasonal changes of the microeukaryotic and bacterial communities

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. Temporal dynamics of microbial diversity along environmental gradients

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

3. Materials and methods

3.1. Seasonal changes of the microeukaryotic and bacterial communities

- Biweekly sampling of five soda pans of Kiskunság 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).
- Determination of environmental variables (water temperature, pH, conductivity, dissolved O₂, total nitrogen, total phosphorus, total suspended solids, chlorophyll a, dissolved organic carbon) by field and laboratory measurements.
- Identification of abundant zooplankton species by microscopy.
- Determination of bacterial cell number by fluorescent microscopy.
- Filtration of water samples, DNA extraction, PCR amplification of 18S and 16S rRNA gene.
- Determination of the microeukaryotic and bacterial communities by Illumina amplicon sequencing.
- Bioinformatic analysis of the sequences with the mothur program.
- Data and statistical analysis using R.
- Networks were created using the Extended Local Similarity Analysis (eLSA) tool.
- Visualization of networks by the Cytoscape program.
- Identification of keystone OTUs by the weighted topological importance (WI) index.

3.2. Temporal dynamics of microbial diversity along environmental gradients

- Sampling of twenty-six soda pans of Seewinkel in two very contrasting spring season, 2017 (dry), 2018 (wet).
- Determination of environmental variables (water depth, Secchi depth, pH, conductivity, total nitrogen, total phosphorus, total suspended solids) by field and laboratory measurements.

- Filtration of water samples, DNA extraction, PCR amplification of 18S and 16S rRNA gene.
- Determination of the microeukaryotic and bacterial communities by Illumina amplicon sequencing.
- Bioinformatic analysis of the sequences with the mothur program.
- Creation of six larger groups: ciliates, fungi, eukaryotic phytoplankton, heterotrophic flagellates and nanoflagellates, cyanobacteria and bacteria (non-cyanobacteria).
- Data and statistical analysis using R.

4. Results and Discussion

4.1. Seasonal changes of the microeukaryotic and bacterial communities

Seasonality had a great impact on the microeukaryotic and bacterial communities of all pans. Various common seasonal dynamics of the microbial succession were observed, 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). Based on our results, the microeukaryotic community was more sensitive to these changes, while the bacterial community remained more stable.

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.

Spring had similar 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 (richly connected with positive associations to others). Positive keystone eOTUs abundant in spring belonged to flagellates like Colpodellida (Mylnikov, 2009) or parasitic taxa such as Cryptomycotina (Letcher et al., 2017) and Perkinsozoa (Mangot et al., 2011). Positive actinobacterial keystone OTUs were assigned to lineages like Lunal-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 relative abundance of non-core4 after desiccation events, supporting the hypothesis that non-core OTUs are more important in the response to sudden environmental events.

All 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.

Overall, our results showed the studied soda pans despite drastic environmental changes and following community shifts are primarily populated by a common core microbiome and as a result of the identical climatic and meteorological conditions they have similar seasonal trends. However, the shared microbiome differed among pans of different habitat subtype (i.e., colored or turbid pan) and common seasonal trends were modified by local environmental events like desiccation. Adaptation to uniform seasonal changes was through species recruitment from the core community, while for microeukaryotes non-core members of the microbiome were involved in the response to sudden environmental events. Bacterial communities were resistant to stochastic events and adapted to the local stressors with species sorting from the core community and competitive exclusion.

4.2. Temporal dynamics of microbial diversity along environmental gradients

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. Based on our findings, conductivity was the most influential environmental variable shaping the 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 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., 2022). 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.

Our study found that HF-HNF richness and phylogenetic diversity increased exponentially with salinity, which is a pattern not previously reported from other saline systems, while 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).

In conclusion, 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. In addition, the response of prokaryotes (Bacteria and Cyanobacteria) to the environmental gradients generally differed from the microeukaryotic groups.

5. Thesis

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.) Does 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.

6. References

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7. 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 microeukaryotes differently in temporary saline waters. - *Scientific Reports*, 13:3243, <https://doi.org/10.1038/s41598-023-30385-6>

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8. Other papers

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