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# The effect of urbanization on planktonic and biofilm bacterial communities in different water bodies of the Danube River in Hungary

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**Freshwaters play an essential role in providing ecosystem services worldwide, however, the water quality of different water bodies is strongly influenced by human activities such as urbanization, industry and agriculture. In this study, water and biofilm samples were collected from the main channel of the Danube River upstream and downstream of a metropolitan, from a regulated side arm within an urbanized area, and from two differently separated oxbow lakes located in nature conservation areas. The taxonomic diversity of bacterial communities was revealed by 16S rRNA gene-based amplicon sequencing using Illumina MiSeq platform. The results showed that all samples were dominated by phyla Pseudomonadota, Actinobacteriota and Bacteroidota. The bacterial community structures, however, clearly differentiated according to planktonic and epilithic or epiphytic habitats, as well as by riverine body types (main channel, side arm, oxbow lakes). The taxonomic diversity of biofilm communities was higher than that of planktonic ones in all studied habitats. Human impacts were mainly reflected in the slowly changing biofilm composition compared to the planktonic ones. Genera with pollution tolerance and/or degradation potential, such as *Acinetobacter*, *Pseudomonas* and *Shewanella* were mainly detected in biofilm communities of the highly urbanized section of the river side arm.**

**Keywords** Water quality, Epilithic, Epiphytic, Planktonic, Microbial communities, Metropolitan, Danube River

In the 21st century, the uneven distribution and limited availability of freshwater resources have led to severe water scarcity and water quality problems due to the increased water use associated with human population growth as well as the effects of climate change[1]. Water resources are highly sensitive; thus, they can be easily degraded[2]. Due to the multiple benefits of densely populated areas, rivers worldwide have been heavily impacted by urbanization[3]. The main anthropogenic factors include industry, municipal water use, agriculture, and construction or alteration of water bodies, furthermore the usage of the river for transportation[1]. The impact of urbanization and anthropogenic factors on the chemical and microbiological properties of rivers has become the focus of research nowadays[4–8].

Human activities, such as pollution of rivers and construction of dams, can lead to deterioration in water quality and to a decrease in water quantity. Thus, the water cannot be used in industrial processes or agricultural activities and cannot participate in the drinking water supply[6]. In addition, contamination of river waters can significantly affect human health[5,9]. At a certain pollution level, water bodies are capable of self-purification, whereby physical, chemical and (micro)biological processes occur in complementary and parallel ways[1,10]. Bacterial communities inhabiting freshwaters play a key role in biogeochemical cycles, by transforming and/or

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| Sampling sites | Temperature (°C) | Conductivity (mS/cm) | pH | Dissolved oxygen (mg/L) | TOC (mg/L) | $NO_3^-$ (mg/L) | $PO_4^{3-}$ (µg/L) |
|---|---|---|---|---|---|---|---|
| Upstream Budapest (DNW) | 13.57 ± 0.46 a | 0.29 ± 0.01 a | 7.54 ± 0.17 a | 9.74 ± 0.51 a | 3.35 ± 1.52 a | 6.23 ± 0.9 ab | 105.83 ± 56.8 a |
| Downstream Budapest (DSW) | 13.06 ± 1.14 a | 0.30 ± 0.01 ab | 7.81 ± 0.20 ab | 10.02 ± 0.78 a | 2.67 ± 0.99 a | 6.24 ± 0.86 a | 110.83 ± 74.16 a |
| Soroksári-Danube (SW) | 13.32 ± 0.24 a | 0.37 ± 0.02 bd | 8.07 ± 0.13 bc | 9.69 ± 0.63 a | 2.88 ± 0.59 a | 7.17 ± 0.98 a | 53.69 ± 7.07 a |
| Nyéki- Danube (NW) | 17.35 ± 0.46 b | 0.37 ± 0.00 cd | 7.82 ± 0.07 ab | 7.46 ± 0.59 b | 8.52 ± 0.51 b | 2.15 ± 0.23 b | 45.09 ± 14.00 ab |
| Riha Lake (RW) | 17.63 ± 0.23 b | 0.64 ± 0.00 c | 8.27 ± 0.04 c | 7.94 ± 0.34 b | 19.12 ± 0.41 b | 4.96 ± 0.24 b | 5.44 ± 2.25 b |

**Table 1**. Physical and chemical variables of the water samples at the different sampling sites (average ± SD; $n = 12$ per sample) (notes: means followed by different letters in the same row differ significantly in Dunn's post hoc test at 95% confidence interval. Different letters (a–d) in the same line indicate significant statistical difference ($p < .05$, Dunn's post-hoc)).

| Sampling sites | Community | SEQ. No. | Coverage | Chao | ACE | Sobs | Inverse Simpson |
|---|---|---|---|---|---|---|---|
| Upstream Budapest (DNW) | Planktonic | 3311 | 0.98 ± 0.00 | 283.17 ± 23.02 | 291.49 ± 26.59 | 208.95 ± 5.29 | 23.06 ± 0.58 |
| Downstream Budapest (DSW) | Planktonic | 3311 | 0.98 ± 0.00 | 285.94 ± 22.26 | 302.53 ± 30.34 | 207.00 ± 5.02 | 21.43 ± 0.49 |
| Soroksári-Danube (SW) | Planktonic | 3311 | 0.98 ± 0.00 | 292.25 ± 26.25 | 313.86 ± 40.76 | 212.78 ± 6.73 | 21.99 ± 0.68 |
| Nyéki- Danube (NW) | Planktonic | 3311 | 0.99 ± 0.00 | 187.54 ± 25.13 | 217.04 ± 39.80 | 129.49 ± 5.53 | 17.14 ± 0.43 |
| Riha Lake (RW) | Planktonic | 3311 | 0.99 ± 0.00 | 196.58 ± 21.40 | 206.26 ± 28.78 | 146.03 ± 5.37 | 14.24 ± 0.50 |
| Upstream Budapest (DNB) | Biofilm | 22689 | 1.00 ± 0.00 | 403.04 ± 13.28 | 396.03 ± 8.14 | 351.02 ± 3.46 | 13.16 ± 0.07 |
| Downstream Budapest (DSB) | Biofilm | 22689 | 1.00 ± 0.00 | 368.62 ± 11.83 | 364.04 ± 7.50 | 318.06 ± 2.90 | 15.04 ± 0.09 |
| Soroksári-Danube (SB) | Biofilm | 22689 | 1.00 ± 0.00 | 329.57 ± 5.17 | 329.69 ± 3.54 | 313.17 ± 1.81 | 16.07 ± 0.09 |
| Nyéki- Danube (NB) | Biofilm | 22689 | 1.00 ± 0.00 | 349.18 ± 11.33 | 348.63 ± 8.25 | 321.83 ± 4.08 | 13.33 ± 0.16 |
| Riha Lake (RB) | Biofilm | 22689 | 1.00 ± 0.00 | 362.84 ± 9.70 | 360.84 ± 6.68 | 339.83 ± 3.50 | 13.85 ± 0.16 |

**Table 2**. Observed (Sobs) and estimated (Chao1 and ACE) bacterial species richness, and diversity indices (Inverse Simpson) calculated from the 16S rRNA gene-based amplicon sequencing data of the planktonic and biofilm bacterial communities (sequence numbers were subsampled to the read number of the sample having the lowest sequence count).

degrading various organic materials and toxic compounds[11,12]. Aquatic environments provide diverse habitats for different microorganisms. They can populate water bodies as planktonic communities and form biofilms on all underwater biotic and abiotic surfaces. In littoral zones, where the flow rate is low, submerged plant surfaces can be an appropriate habitat for bacterial biofilm formation[13]. These complex plant-microbial consortia allow highly efficient self-purification of water bodies through the ability of macrophytes and biofilm-forming bacteria to degrade, assimilate and remove contaminants. In heavily polluted water bodies, however, this self-purification is insufficient to restore good water quality[1]. Most of research so far has focused on planktonic communities, therefore, our knowledge of riverine biofilm-forming (epiphytic or epilithic) communities is rather limited[12,14–17].

The Danube River is the second longest river in Europe. Hungary is in the center of the Danube Basin where the river crosses the capital in a north-south direction. In the Budapest metropolitan area, the Danube River is exposed to significant anthropogenic effects. The Danube, due to its outstanding importance and potential or actual environmental pollution events, has long been the subject of various physical-chemical and microbiological studies, along its entire length, in different sections, and in relation to its various water bodies[14,18–21]. These studies, however, have not addressed the impact of different human activities on the overall bacterial communities by examining various riverine water bodies and habitat types in parallel.

The aim of this study, therefore, was to compare the planktonic, epilithic, and epiphytic bacterial communities of three different riverine water bodies (main river channel, side arm and oxbow lakes) of the Danube River, and to assess the potential anthropogenic effects of the metropolitan area on these bacterial communities.

## Results

### The physical and chemical characteristics of the water samples

The mean values and standard deviations of the measured water physical-chemical characteristics according to the sampling sites are presented in the Table 1. Neither the upstream and downstream main river channel sites nor the side arm and the downstream main river channel sampling sites differed significantly for most of the environmental parameters. However, in the case of the electric conductivity and pH values a significant difference was detected between the upstream main river channel and the side arm. The two oxbows differed significantly from the main river channel sampling sites. The pH of the side arm and the oxbow lakes was comparable. The Nyéki-Danube oxbow and the side arm did not differ based on the electric conductivity and phosphate content of the water. The Nyéki-Danube and the Riha lake oxbows clearly separated based on the pH values, although further significant differences were not detected (Supplementary Table 1).

The Principal Component Analysis confirmed the findings discussed above. The environmental variables contributed differently to the separation of the sampling sites (Fig. 1). Water temperature, electrical conductivity,

TOC values were higher at the oxbow sampling sites compared to the main river channel and the side arm. Conversely, dissolved oxygen and nitrate concentrations were the highest in the main river channel and the side arm of the river.

The results of the Spearman's Rank-Order Correlation analysis (Supplementary Table 2.) indicated negative correlations between the dissolved oxygen (DO) concentration in the main river channel and nitrate, phosphate, and total organic carbon (TOC) contents. In the case of the downstream sample a stronger negative correlation was detected in all the three parameters than in the upstream sample. Furthermore, TOC, nitrate and phosphate contents were strongly positively correlated at the two main river channel sampling sites.

## The diversity of the planktonic and biofilm bacterial communities

In total 2 755 240 sequences were assigned to 552 OTUs (Operational Taxonomic Unit). Good's coverage indicated high sequencing depth across all sampling sites and habitats (Table 2). Diversity analysis revealed higher values in the case of the epilithic and epiphytic communities compared to the planktonic ones, based on the predicted number of OTUs (Sobs) and the estimated richness (ACE, Chao1) and diversity (Inverse Simpson) indices. The diversity of the planktonic communities was the highest in the side arm, comparing the five sampling sites. The main river channel sampling sites had a higher diversity compared to the oxbows (Table 2). In contrast, the epiphytic communities of the side arm had the lowest diversity among the biofilm samples.

## The taxonomic composition of the planktonic bacterial communities

The dissimilarity of the sampling locations is shown in the Fig. 2A based on the relative abundance of the dominant planktonic bacterial phyla. Among the identified bacterial taxa, the phyla Pseudomonadota, Actinobacteriota and Bacteroidota were the predominant across all sampling sites with relative abundance values above 10%. The relative abundance values of the phyla Pseudomonadota and Actinobacteriota showed opposite trends. The Pseudomonadota had the lowest, and the Actinobacteriota had the highest relative abundance in the separated Riha lake oxbow. The phylum Verrucomicrobiota exhibited the highest abundance (> 1%) in the at the upstream and downstream of the main river channel sampling sites, while the lowest values in the Nyéki-Danube oxbow lake.

The separation of sampling sites based on planktonic bacterial orders is shown in Fig. 3A. The water samples from the main river channel and the Soroksári-Danube side arm formed an overlapping subgroup, while the two oxbow lakes were clearly separated. The orders Burkholderiales (Pseudomonadota), Frankiales (Actinobacteriota) and Flavobacteriales (Bacteroidota) were predominant within the planktonic bacterial communities at each sampling site. Furthermore, within the phylum Bacteroidota, the orders Chitinophagales, Cytophagales, Kapabacteriales and Sphingobacteriales all had a relative abundance higher than 1% at least one sampling site. Other abundant orders were identified as Chthoniobacterales (Verrucomicrobiota), Microtrichales, and Corynebacteriales (Actinobacteriota) and SAR11_clade (Pseudomonadota) (Supplementary Table 3).

Across all sampling sites, several bacterial genera were abundant, including *Flavobacterium* (Flavobacteriales), *Fluviicola* (Flavobacteriales), *hgcI_clade* (Frankiales), *NS11-12_marine_group* (Sphingobacteriales), *Limnohabitans* (Burkholderiales) and *Polynucleobacter* (Burkholderiales). Notably, genera *Limnohabitans*

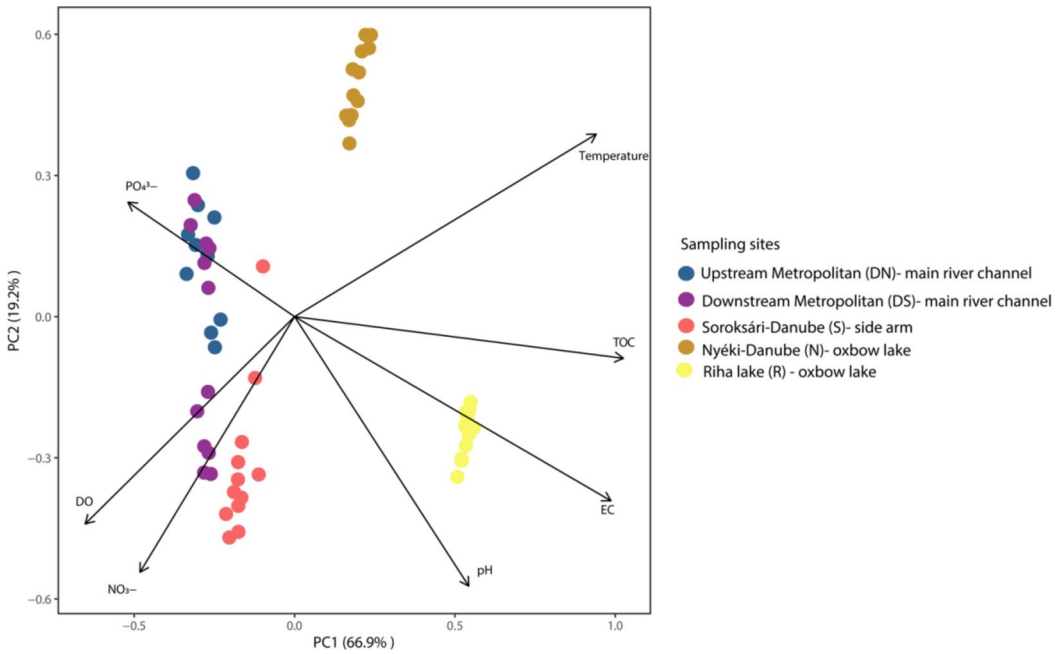


**Fig. 1**. The principal component analysis (PCA) biplot based on the water physical-chemical variables of the different sampling sites.

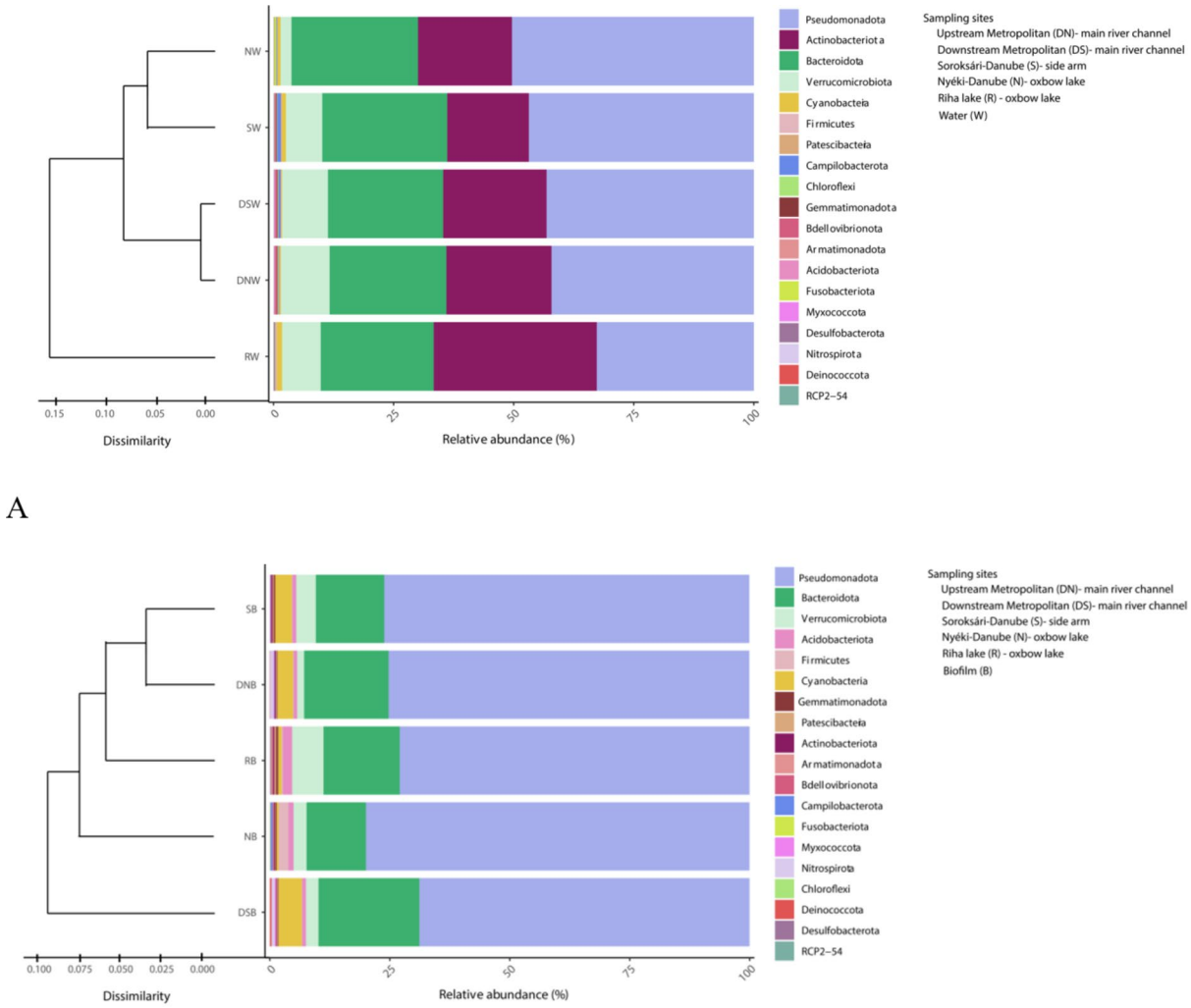


**Fig. 2**. Percentage distribution of 16S rRNA gene amplicon sequences among the phyla in the planktonic (**A**) and biofilm (**B**) bacterial communities together with the results of Bray-Curtis similarity index-based cluster analysis.

(Burkholderiales) and *Polynucleobacer* (Burkholderiales) exceeded 10% relative abundance at least one sampling site (Fig. 4A, Supplementary Table 3).

In addition, genera representative of the planktonic community of the sampling sites were *Terrimicrobium* (Chthoniobacterales) for the main river channel, *Dinghuibacter* (Chitinophagales), *Kapabacteriales* (Kapabacteriales) and the *Clade_III* (SAR11_clade) for the Riha oxbow lake, and *Mycobacterium* (Corynebacteriales) and *Pseudarcicella* (Cytophagales) for the Nyéki-Danube oxbow lake (Supplementary Table 3).

## The taxonomic composition of the biofilm bacterial communities

The dissimilarity of the sampling sites is represented in the Fig. 2B based on the relative abundance of the dominant biofilm bacterial phyla. The relative abundance of phyla Pseudomonadota, Bacteroidota, Verrucomicrobiota, Cyanobacteria, Firmicutes, Acidobacteriota and Nitrospirota were above 1% at least one biofilm sample. The phyla Pseudomonadota, Bacteroidota and Verrucomicrobiota were the most abundant at each sampling site. The phylum Cyanobacteria was characteristic of the main river channels and the side arm. The phylum Firmicutes was the most abundant in the Nyéki-Danube oxbow lake, while the representatives of the phylum Acidobacteriota showed the highest abundance in the oxbow lakes. The relative abundance of the phylum Nitrospirota reached 1% only in the main river channel samples.

The sampling sites also had a clear separation according to the bacterial orders of the biofilms (Fig. 3B). The two epilithic samples from the main river channel and the two epiphytic samples from the oxbow lakes formed separated subgroups. The side arm epiphytic samples clearly separated from both subgroups. They were closer to the main river channel epilithic than the oxbow epiphytic samples.

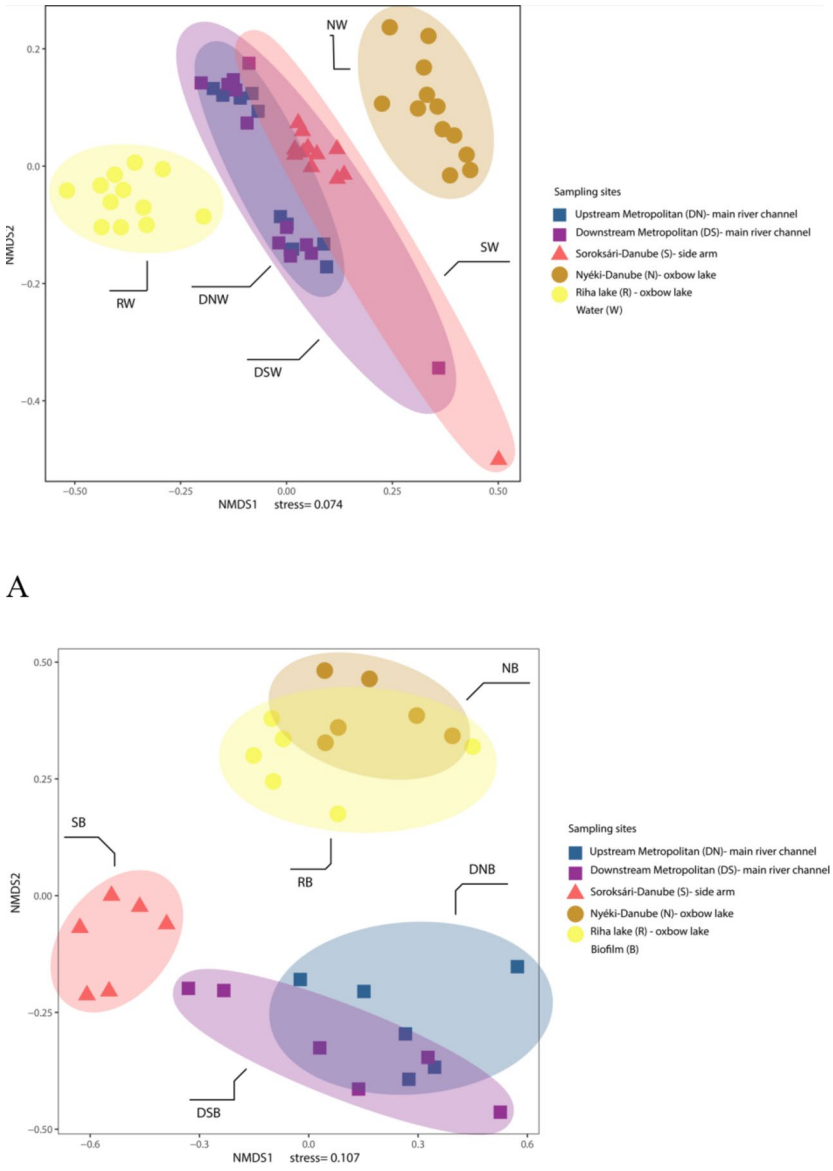


**Fig. 3**. The non-metric multidimensional scaling (NMDS) ordination of planktonic (**A**) and biofilm (**B**) bacterial communities based on the relative abundance of orders above 1%.

The orders Aeromonadales, Enterobacterales and Pseudomonadales (Pseudomonadota), and Flavobacteriales (Bacteroidota) had a relative abundance above 10% at least one sampling site (Supplementary Table 3). The order Enterobacterales (Pseudomonadota) was characteristic of the oxbow lakes (Supplementary Table 3).

At genus level, various taxa were detected above 2% relative abundance at least at one sampling site (Supplementary Table 3). Within the identified genera (Fig. 4B), *Pseudomonas* (Pseudomonadales) was predominant in the epilithic communities of the main river channel. Other genera typical of this habitat were *Acinetobacter* (Pseudomonadales), *Shewanella* (Alteromonadales), *Deefgea* (Burkholderiales) and *Flavobacterium* (Flavobacteriales). The genera *Rhodobacter* (Rhodobacterales) and *Methylotenera* (Methylophilales) were present in the highest relative abundance in the epiphytic biofilm of the side arm compared to the other samples. The genera *Aeromonas* (Aeromonadales), *Rahnella* (Enterobacterales) and *Rhodoferax* (Burkholderiales) were characteristic of the epiphytic communities in the oxbow lakes.

## Discussion

This study focused on a detailed comparative analysis of planktonic and biofilm (both epilithic and epiphytic) samples, from different sections of the same river system, affected to varying degrees by human activity.

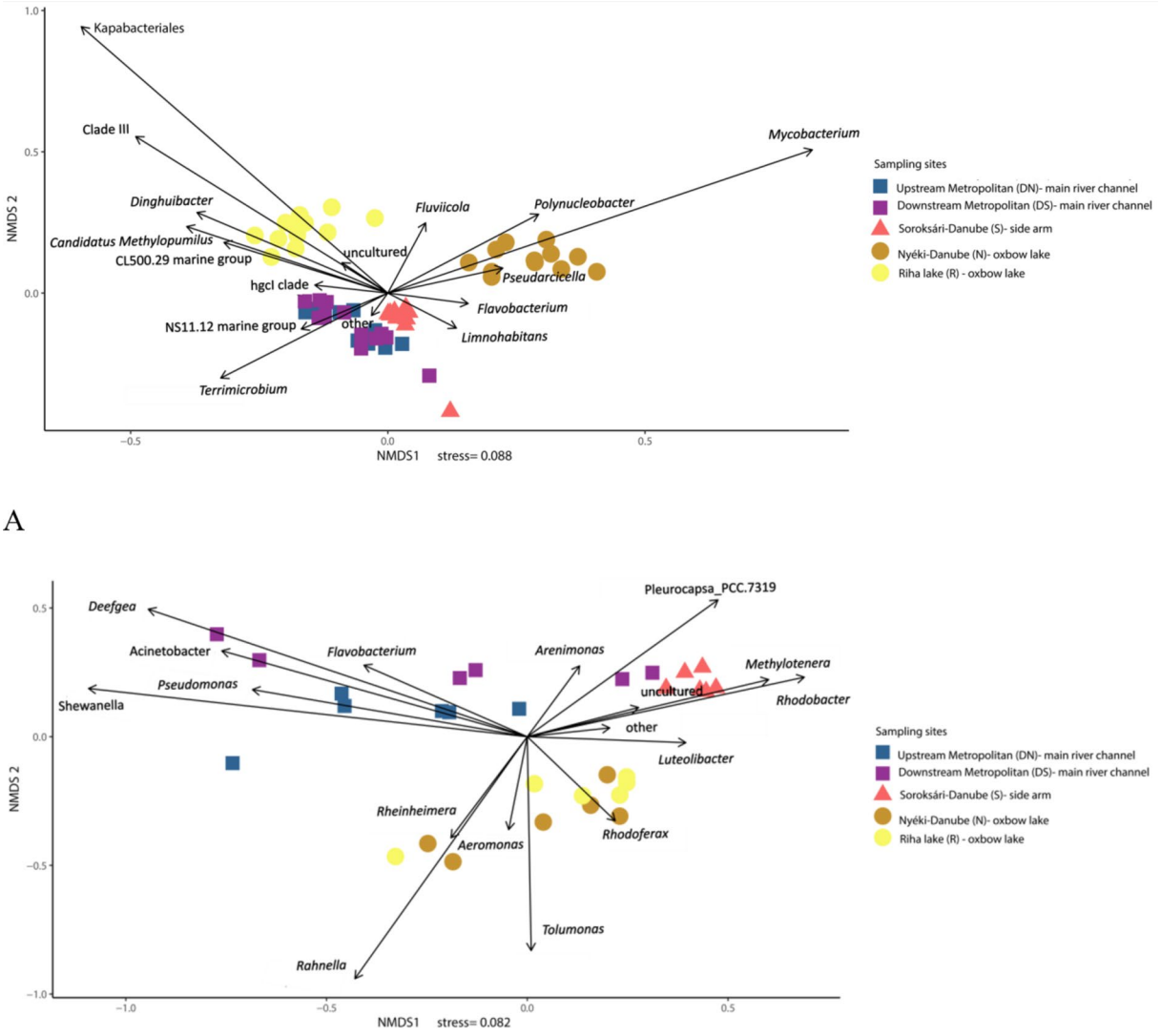


**Fig. 4**. Non-metric MultiDimensional Scaling (NMDS) ordination of the planktonic (**A**) and biofilm (**B**) bacterial communities based on the relative abundance of genera above 2%.

The 16S rRNA gene-based amplicon sequencing data indicated significant differences in the composition of planktonic and biofilm bacterial communities across all taxonomic levels (from phylum to genus) and habitat types (main river channel, side arm and oxbow). Although all sample types were dominated by the phylum Pseudomonadota (previously Proteobacteria), like in other freshwater habitats[22–26], there was a twofold difference in its relative abundance in favor of biofilm samples compared to planktonic ones. The relative abundance of the phylum Actinobacteriota (previously Actinobacteria), however, was high only in the planktonic communities. Previous studies also reported high relative abundance of the phylum Actinobacteria in freshwater planktonic communities[22–26]. Among the dominant phyla, the relative abundance of Bacteroidota (previously Bacteroidetes) was higher in the planktonic bacterial communities compared to biofilms. The presence of this phylum could be a possible indication of faecal contamination[18,27]. In 2007, the Soroksári-Danube side arm was found to be a faecal contamination hot spot, however, for 2013 the extent of the pollution decreased[18].

At lower taxonomic levels, hitherto uncultivated, typical freshwater bacteria dominated the planktonic communities e.g. hgcI clade (Actinobacteriota) and NS11.12 marine group (Bacteroidota). Previously, the hgcI clade was a dominant community member, e.g. in pelagic freshwater bacterial communities. Additionally, they play an integral role in the nutrient cycle by fixing carbon dioxide and taking up nitrogen-rich and phosphate containing compounds. Their genome encodes a high variety of degrading enzymes e.g. lysozymes, chitinase[28]. The occurrence of the NS11-12 marine group was reported from coastal, urbanized and/or polluted environments[29–31]. Despite its typical association with marine and brackish environments, the NS11-12 marine group has been increasingly detected in freshwater systems, such as Lake Balaton, Zala River, and Lake Fertő in Hungary[29,32]. This unexpected presence suggests a broader ecological tolerance and potential role in diverse

aquatic habitats. While its detection in freshwater is less common, it may be indicative of higher concentrations of organic matter from algal blooms or other external sources[29,33]. The genus *Limnohabitans* (Pseudomonadota) was abundant in all studied planktonic communities. It is known as a ubiquitous member of neutral and alkaline planktonic communities, by degrading autochthonous organic matter from algae (e.g. monosaccharides and some amino acids)[34–36]. The growth rate might be positively influenced by the high nutrient content of the water[37,38]. In the Nyéki Danube oxbow lake water, the genera *Mycobacterium* (Actinobacteriota*)* and *Pseudarcicella* (Bacteroidota) were abundant. Members of the genus *Mycobacterium* are highly resistant and could live in many different environments, due to their high variation in degrading enzymes and the ability to metabolize many different compounds. Fast growing, non-tuberculotic mycobacteria were previously frequently isolated from various environmental (e.g. soil and sediment) samples[39]. Growth of mycobacteria is possible even at low nutrient levels; therefore, these bacteria can survive in oligotrophic environments e.g. in the biofilms of drinking water systems[40–42]. A recently published metagenome-based correlation network analysis pointed to the possibility of *Pseudarcicella* being an indicator bacterium of good water quality in freshwater lakes[43]. In both oxbow lakes, other typical freshwater bacterial genera, e.g. *Fluviicola* (Bacteroidota)[15,44] and *Polynucleobacter* (Pseudomonadota)[45] were also detected. The *Polynucleobacter* genus inhabits environments with a high variation in physical-chemical parameters, e.g. pH[46,47]. The genus *Candidatus Methylopumilus* (Pseudomonadota) is commonly found in freshwaters, especially where the water body has a connection with plants or is surrounded by different plants[48], similarly to the Riha-lake sampling site.

The biofilm bacterial communities, both epilithic and epiphytic ones, showed higher taxonomic diversity than the planktonic ones in all Danube riverine water bodies. Similar results were also found previously[49–53]. Both species richness and taxonomic diversity were the lowest in the biofilm of the Soroksári-Duna side arm compared to the other sampling sites. Of the sampling sites, this side arm is the most affected by the negative impact of urbanization. In the Danube metropolitan area, the sampling sites separated according to the differentiation of the phosphate concentration in the main river channel and the nitrate concentration in the regulated side arm. Both the nitrogen and phosphorus compounds are plant nutrients known as the main non-point pollutants in rivers[53,54]. The water quality and the contamination of the side arm have already caused serious problems in the past decades. Several studies have reported the eutrophication of the water body and the increased nutrient content (mainly N and P) since the 1990s[55–57]. In this densely populated region, several diffuse pollutants of anthropogenic origin, such as nitrogen, phosphorus, sulphate, chloride, potassium, and vanadium, were also identified through a detailed physical-chemical analysis of the Danube water[20]. The measured physical-chemical parameters can also be related to differences observed in the sequence data.

Bacterial genera (e.g. *Acinetobacter*, *Pseudomonas* and *Shewanella*) capable of degrading pollutants and toxic compounds[58–60] were found with higher relative abundance in the epilithic than planktonic communities in the main river channel. Members of all three genera isolated from a wastewater treatment system showed high adhesion during biofilm formation[61]. Recently, *Acinetobacter* and *Pseudomonas* was found to be the most abundant genera in microplastic-associated biofilms in the Pearl River Delta (China), as well[62]. In the epiphytic biofilm of the Soroksári-Danube side arm, the genera *Methylotenera* and *Rhodobacter* (Pseudomonadota) was abundant. Members of the *Methylotenera* genus can play a role in the nitrogen reduction. Furthermore, the genus was identified as a major oil degrading group in Nigeria[63]. The *Rhodobacter* species can be used for bioremediation and wastewater pollutant removal due to their metabolic versatility[64–66]. Members of the genus is highly abundant in eutrophic freshwaters near the shore; they are not common in a low nutrient content environment[37]. Bacterial genera, e.g. *Rhodobacter*, *Acinetobacter* and *Pseudomonas* are known for their storage and production capacity of polyhydroxy-alkanoates (PHA)[58,67]. The PHA molecule is used for energy storing; however, it can also improve the stress resistance of the microbes.

In the epiphytic biofilms of the oxbow lakes, the high relative abundance of genera *Aeromonas* and *Rheinheimera* (Pseudomonadota) could be a good water quality indicator[43]. Representatives of both genera are frequently isolated from reed periphyton in freshwater environment[32,68,69]. The genus *Rahnella* (Pseudomonadota), a naturally occurring biofilm forming member of Enterobacteriales[70], was also characteristic of epiphytic communities in oxbow lakes. The whole genome sequencing of *Rahnella aquatilis* strain ZF7 revealed its potential for plant growth promotion, biocontrol and stress tolerance[71], which may have implications for water quality.

## Conclusions

In this study, differently regulated river sections of the Danube River were analyzed to explore the impact of anthropogenic exposure of different water bodies on the composition of planktonic and biofilm bacterial communities using many replicates. The results showed that different abundances of genera *Pseudomonas*, *Acinetobacter*, *Shewanella*, *Aeromonas* and *Rheinheimera* in both the planktonic and biofilm bacterial communities may be indicative of human impacts. The negative effects of anthropogenic activities, however, were more evident in the more stable biofilm communities compared to the rapidly changing planktonic ones. The differences in the relative abundances of *Rhodobacter* genus by water type and habitat may reflect this effect. The taxonomic composition of biofilm bacterial communities can therefore be useful indicators of long-term changes in water quality.

## Methods

*Description of the sampling locations*

Five sampling locations were selected along the Hungarian section of the Danube River with various types and degrees of human influence (Fig. 5).

Two sampling sites were designated in the main river channel, upstream (between 1678 and 1674 river km) and downstream (between 1607 and 1604 river km) from the Hungarian capital[14,15,21,72]. Here, the river supplies water to about 2.5 million people in the metropolis and its agglomeration area.

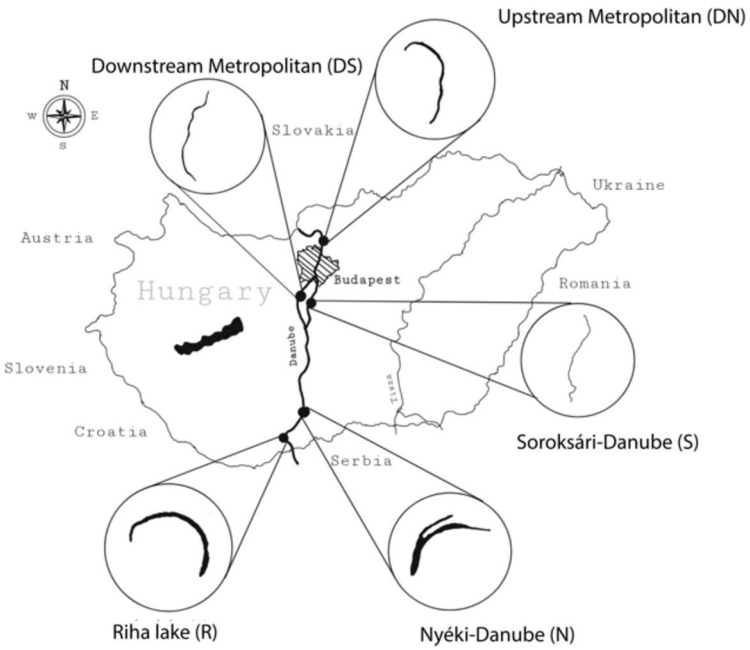


**Fig. 5**. Sampling locations of the different riverine water bodies along the Hungarian section of river Danube (Abbreviations: upstream (north) from Budapest, DN; downstream (south) from Budapest, DS; Soroksári-Danube, S; Nyéki-Danube, N; Riha lake, R).

Three additional sampling sites were designated in a side arm of the Danube and two backwaters. The Soroksári-Danube (between 1642 and 1586 river km) is the second largest side arm of the Danube in Hungary (Fig. 5). It maintains a continuous connection with the main river channel; although, a sluice system regulates its flow rate and water level. Due to the Kvassay and the Tass sluices, the flow rate of the side arm decreased drastically compared to the main river channel. The riverbed of the Soroksári-Danube becomes wider and deeper downstream, and the riparian reed zone becomes more extensive[73]. The side arm is heavily exposed to human activities. Surrounded by a densely populated agglomeration area with significant industrial activities, it also offers various recreational activities (e.g. relaxation, water sports, and fishing). Due to the water regulation and the resulting decrease in flow rate, sedimentation and pollution have increased in the side arm compared to the main river channel[72,74].

The Nyéki-Danube (at the 1479 river km) is situated in the Gemenc floodplain area of the Duna-Dráva National Park (Fig. 5). This former river sidearm naturally became an oxbow lake more than 200 years ago. Although, it is in contact with the main river channel during floods. Its riverine body is surrounded by a wide reed belt[75]. Due to the floodings, its water level fluctuates greatly. Based on the properties of the water body, the water renewal is slow, so the high sedimentation results in periodically repeated drying out[72,74,76].

Riha lake (at 1447 river km) is a separated oxbow lake (Fig. 5). This water body is also located within the area of the Duna-Dráva National Park, but there is a livestock farm in its vicinity. The oxbow lake has completely lost its connection with the main river channel and is fed only by groundwater and rainwater. The water body is surrounded by a reed belt, the lakebed is uneven and very shallow in some places[72,74].

## Sample collection and in situ physical-chemical measurements

In the main river channel of the Danube, three transects perpendicular to the shore have been designated upstream (between 1678 and 1674 river km) and downstream (between 1607 and 1604 river km) from the Hungarian capital as described by[21]. At the Soroksári-Danube side arm (between 1642 and 1586 river km), Nyéki-Danube (at the 1479 river km) and Riha lake (at 1447 river km), 300–500 m long sections parallel to the shore were selected as described by[72]. Water and biofilm samples were collected in May at the beginning of the vegetation period.

For microbiological analysis of planktonic bacterial communities and ex situ chemical measurements, water samples were collected with twelve replicates from a depth of 50 cm at each of the five sampling sites. The samples were collected into sterile 1 l glass bottles and stored at 4–6 °C until laboratory processing within 24 h.

Biofilms were collected from different substrates with 6 replicates per sampling sites. In the main river channel, where reeds are absent, biofilm communities develop on the pebble surfaces. Therefore, in the upstream and downstream sampling sites, epilithic biofilm samples were taken via from 1, 2, and 5 m water depths at the transects using benthic dredging. Samples from different depths were combined as composite samples per transect. A At the Soroksári-Danube side arm, the Nyéki-Danube and the Riha lake, a reed belt lines the shores and plays a key role in the water self-purification processes. These water bodies have muddy sediment without a gravel bed. Consequently, reed stems were sampled every 25–35 m along the open water edge of the reed stands. The biofilm samples were collected into disposable plastic bags and stored at 6–8 °C until laboratory processing

within 24 h. A total of 90 samples (12 replicates of water samples and 6 replicates of biofilm samples from all 5 sampling sites) were used for DNA isolation, sequencing and statistical analysis.

In situ measurements were conducted at each sampling site. Temperature (°C), pH, electrical conductivity (S $cm^{-1}$), dissolved oxygen (mg $L^{-1}$), nitrate-N (mg $L^{-1}$) were analysed by YSI EXO2 Multi-Parameter Water Quality Sonde in situ at the sampling sites. Orthophosphate (mg $L^{-1}$) was determined by Spekord 210 Plus spectrophotometre (Analytik Jena, Germany), following Eaton et al. (2005)[77]. Total organic carbon (TOC, mg $L^{-1}$) was determined by a Multi N/C 2100 S TC-TN analyser (Analytik Jena, Germany) equipped with a nondispersive infrared detector and a chemiluminescent detector, in accordance with the corresponding international standards (MSZ EN 1484:1998).

### DNA extraction, Illumina MiSeq amplicon sequencing and bioinformatic analysis

For environmental DNA extraction 500 ml of water samples were concentrated by filtration using 0.22 µm pore-sized polycarbonate filters (Millipore, Billerica, MA, USA). Biofilm from the reed stems and pebbles was washed with saline solution using a sterile paintbrush. The samples were centrifugated (10000 rpm for 2 min). For DNA extraction, biomass from the water on the filter surface, and approximately 100 mg of biofilm compacted by centrifuge were used. DNA extraction was performed using the DNeasy Power Soil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration of the samples was measured using Qubit 4 fluorometer (Thermo Fisher Scientific, USA).

The V3-V4 region of the 16S rRNA gene was amplified by PCR using the primers Bact 341F (5'-CCT ACG GGN GGC WGC AG-3') and Bact 805R (5'-GAC TAC NVG GGT ATC TAA TCC-3') designed for the study of Bacteria and Archaea during next-generation sequencing[78]. Before sequencing, the amplicon library was assessed using Agilent 2100 Bioanalyzer System (Agilent Technologies, Inc., USA). For sequencing, the Illumina MiSeq platform (Illumina, San Diego, California, USA) pair-end, dual-index sequencing technique was used, via the MiSeq Reagent Kit v3 providing 300 base long reads. The sequences in fastq format are deposited in NCBI as BioProjects PRJNA 838445 and 1119742.

The sequences were analysed using Qiime2 software[79]. The 500-base long aplicon sequences were achieved via the joining of the read pairs using vsearch module of the software. Using the same module, the quality check of the read was conducted. For the OTU (Operational Taxonomic Unit) picking a 97% identity threshold was used and the OTUs with coverage under 0.005% were filtered out in the vsearch module[80,81]. The taxonomical classification was conducted using SILVA SSU v.138 database (https://www.arb-silva.de/).

### Statistical analysis

Prior to the statistical analysis, subsampling was performed using the Mothur v. 1.48.0 software[82]. For subsampling, the lowest sequencing number was selected based on the two sample types (water, biofilm). In case of water samples, the lowest read number was 3311 with 498 OTUs, 22,689 read number with 532 OTUs was selected for the biofilm samples. For the bacterial community analyses, the relative abundance of the OTUs was calculated. The Shannon, the Inverse Simpson diversity indexes, the Chao1 species richness and the Good's coverage were also calculated by Mothur v. 1.48.0 software[82]. The R 4.2.3 software[83] was used for statistical analysis and data visualisation. Shapiro-Wilk test was selected to examine the normality of the data, while the Bartlett test was used to examine the homogeneity of the data. Kruskal-Wallis and Dunn's post-hoc tests with Bonferroni correction were performed to analyse significant differences between the water samples. principal component analysis (PCA), non-metric multidimensional scaling (NMDS), and UPGMA (unweighted pair group method using arithmetic averages) analysis were performed. The hierarchical cluster analysis and the non-metric multidimensional scaling were based on Bray-Curtis distances, and Euclidean distance was used in the principal component analysis. In the statistical test, the 0.05 p-value was considered significant. The correlation between the environmental parameters and the bacterial taxa was evaluated in R 4.2.3 software[83] using envfit function within the vegan package.

## Data availability

The sequences in fastq format are deposited in NCBI as BioProjects PRJNA 838445 and 1119742.

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## Author contributions
K.J.L. and A.K.B. as first authors contributed equally to this work. A.I.E. and A.K.B. contributed to the conceptualization and design the study. D.A., G.K. and A.I.E. participated in the sampling. D.A. and G.K. performed the sample preparation for DNA sequencing. P.B.K. performed NGS data analyses. K.J.L. made statistical analyses with the instruction of A.K.B and A.I.E. K.J.L. and A.K.B. wrote the main part of the manuscript. All authors contributed, revised and approved the final version of the manuscript.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Additional information
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