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Presence of microplastic particles increased abundance of pathogens and antimicrobial resistance genes in microbial communities from the Oder river water and sediment

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High abundance of microplastic particles (MPs) in the water environment could be a factor in spreading of pathogens and antimicrobial resistance genes (AMR), especially antibiotic resistance genes (ARGs). The aim of our study was to assess changes in the microbial community developing on microplastic surfaces incubated in water from the Oder River—one of Central Europe's major rivers, flowing through three countries (Czechia, Germany, and Poland)—whose diverse, 20,000-km^2 catchment area (encompassing industrial, agricultural, and urban regions) ensures a relatively high abundance of microbial communities.Samples of water and sediment were taken from river in Wroclaw area. Then the water was poured into disinfected glass liquid containers and pre-drained sediment was added. Control samples of water and sediment were collected on day 0. Then microplastic particles were added (500 mg; ~1 mm). Subsequent sampling was performed after incubation on 7th and 14th day. From each group, samples of sediment and water were collected after the incubation period (n = 5/group), for extraction of microbial DNA and library preparation. Sequencing was performed, using MinION sequencer with 10.4.1 Flow cell. Galaxy Europe platform and R program (v 4.3.3), alpha diversity and PERMANOVA with Benjamini–Hochberg *p*-value correction for multiple comparisons were used. For identification of biomarker taxa being different between groups, ANCOMBC (Analysis of Compositions of Microbiomes with Bias Correction) was performed. Obtained results shown higher abundance of pathogenic bacteria such as *Aeromonas salmonicida Vibrio* spp., *Escherichia coli* or *Salmonella* after 7 days of incubation in water and sediment. Additionally, after 7 days of incubation numbers of ARGs was higher compared to control group.

Keywords Microplastic, Bacteria, Microbial community, Rivers, Antibiotic resistance genes, Pathogens

Since the technological advances and inventions allowing for commercial production of organic carbon-based polymers "plastics" in 1907, their presence and use in all aspects of our daily lives and many branches of industry increased multi-fold. Currently, more than half of plastics are being produced in Asia (mostly in China) at almost 30% of the global production. In 2020 around 367 million tons of plastics were produced, with a value of 16 billion EUR[1–3]. Such abundance of plastic materials in -environment and industrial processes leads to increasing presence of microplastic particles (MP, > 5 mm) and increased research interest due to their high potential for interaction with biota in the aquatic environment. Plastic, including MP, accounts for up to 95% of marine waste with further concern of very slow plastic material decomposition to constitutive elements, or not at all[4–6].

The primary source of MPs found in the environment, including surface waters other than marine environments, are anthropogenic in nature. MPs origin can be primary (produced as microplastic) and secondary (degraded to microplastic by intentional actions, or by environmental effects). Primary MPs therefore

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include the particles of plastic that are produced and released into the environment, such as personal care products containing microbeads, paint, washing wastewater, sewage sludge, plastic running tracks in schools, artificial turf, rubber road in cities, and vehicle tire wear. In contrast, secondary MPs are produced through the environmental degradation of larger pieces, such as plastic bags and bottles, fishing equipment waste (primarily from nets), or aquaculture pond liners[4,5,7,8].

It has been established that MPs have accumulative potential in trophic chains, which is one of the reasons for increased research interest in these particles. Recent studies reveal variations of plastic accumulation in living organisms, depending on presence of city agglomerations, sewage treatment plants, types of reservoirs, development of shores, and hydrodynamic[1–3]. In a study by Ferreira et al.[9] on the economically important estuarine apex predator *Cynoscion acoupa* (n = 552), it was shown that MPs were detected in more than half of the fish examined, accumulating more frequently in adults than juveniles.

Due to the physico-chemical properties of plastic particles, they can absorb other chemical agents (heavy metals, chemicals, polymeric or oligomeric substances) and release them after they are ingested or otherwise accumulated in the body. However, this secondary toxicity potential depends strongly on the phyco-chemical structures of MP and environmental conditions[10–12]. The phenomenon of MP surface adsorption is not limited to chemicals, and it has been observed that microbial communities can utilize MPs as a physical matrix for colonization or formation of biofilms, thus making MPs a novel ecological niche for various microorganisms, including pathogens. Bacterial communities will often organize in biofilms, in an effort to benefit from extracellular matrix protection as additional layer of defense for them[13,14]. Therefore, colonized MP's have potential to impact the microbial community content of the animal digestive systems.

MP can cause mechanical damage to the digestive system in animals, as well as disrupt the host's immune system and induce the onset of diseases (including chronic ones). This in turn can further promote infections and entry of opportunistic pathogens, interfering with genomic composition and expression in the intestinal microflora[15,16]. Kurchaba et al.[17] and Zhao et al.[18] showed that the addition of MP influenced the composition of the microbial community in *Danio rerio* larvae. Preliminary relationship was demonstrated between the microbiological composition of the shrimp (*Litopenaeus vannamei* digestive system and MP[19].

Kesy et al.[20], analyzed MP samples from the Baltic Sea to reveal the presence of pathogenic *Vibrio* spp. in addition to Alphaproteobacteria and Gammaproteobacteria. The authors also suggest that bacteria from genus *Vibrio* may act as early colonizers of MP surface in aquatic environments. Considering context of pathogens present on MPs, Zhang et al.[21] detected presence of 15 tetracycline resistance genes, three sulfonamide resistance genes, one quinolone resistance genes, two chloramphenicol resistance genes, four β-lactamase resistance genes, five aminoglycoside resistance genes, three macrolide resistance genes and 33 multidrug resistance genes on MP particle samples collected from aquaculture establishments. Indication of AMR (antimicrobial resistance genes) in the recent One Health operational framework, have been ranked as a priority, and global effort is ongoing in combating antimicrobial resistance with joined active roles of WHO, WOAH and FAO[22]. Antibiotics used more frequently in human medicine belong to β-lactams and macrolides and in veterinary medicine those are tetracyclines, sulphonamides, and trimethoprim[23,24].

Another case in which MP can potentially influence of the composition of water microbiota is its effect on phytoplankton, as already evidenced by studies of Hitchcock et al.[25] demonstrating the effect of MPs on Aphanocapsa, Pseudanabaena and in lesser degree on Crucigenia and Chlamydmonas taxa. Casabianca et al.[26], reviewed that MPs can affect photosynthesis, growth rate, colony size, and morphology of phytoplankton. Although this phenomenon cannot be held responsible for fish deaths related to toxins produced by algae like *Prymnesium parvum,* it may be a piece of a complex environmental puzzle.

Therefore, the aim of this study was to evaluate the influence of microplastic particles on the microbial community of water and sediment from Oder River, to detect and confirm the presence of *Prymnesium parvum* in the water samples of Oder River, and their growth dynamics in incubated water samples.

Materials and methods

Study design and sampling

Samples of water and sediment (fine-grained, mainly sand, with black mud developed in anaerobic conditions) were collected from the Oder right riverbed (51°05′45.7"N 17°05′32.1"E) on 20.03.2022. Oder river has been selected for this pre-eliminary study, as it is relatively large river at a sampling site whose upstream catchment spans approximately 20,000 km^2 and encompasses diverse land uses, including industrial zones, agricultural areas, and urban regions. This catchment houses nine cities with populations exceeding 100,000 (Ostrava, Częstochowa, Gliwice, Zabrze, Bytom, Ruda Śląska, Rybnik, Opole, and Liberec), as well as extensive industrial districts such as the Upper Silesian Industrial District (metallurgy, mining, energy) and the Ostrava-Karviná Coal District (heavy industry). Because of these varied anthropogenic influences, we anticipated that the river water would contain a broad spectrum of microorganisms. Sampling here thus provided an ideal opportunity to capture a highly diverse microbial community, influenced by multiple sources of pollutants and nutrients. Another key factor for this selection was, that in recent years, the microbial communities of the Oder were heavily impacted by the blooms of the *Prymnesium parvum*, and as such was suspected to be even more susceptible to the potential disbalance caused by the presence of MP particles. During the sampling water was characterized by following parameters: temperature (T): 7.7 °C, pH: 8.85, and conductivity: 1.1359 mS/cm.

The water was immediately poured into disinfected glass liquid containers (1 dm^3) in triplicate setup and 50 ml of pre-drained sediment was added. Control samples of water and sediment were collected on day 0 (C_WAT, n = 5; C_SED, n = 5). Microplastic particles were added (500 mg; ~ 1 mm in each dimension LxWxH). Subsequent sampling was performed on day 7 (I_INC_MP, n = 5; I_INC_SED, n = 5) and 14 (II_INC_MP, n = 5; II_INC_SED, n = 5) (Tab.1). Each biological replicate represented a mixture of samples from the triplicated containers.

Group name	Incubation time (days)	Type of sample
C_WAT	0 (control water)	Water
C_SED	0 (control sediment)	Sediment
I_INC_MP	7 (+ MPs)	Water
I_INC_SED	7 (+ MPs)	Sediment
II_INC_MP	14 (+ MPs)	Water
II_INC_SED	14 (+ MPs)	Sediment

Table. 1.. Study design. **SED – sediment/ WAT – water/ MP – microplastic/ INC—incubation.*

During the incubation water was constantly mixed and aerated. The following water quality parameters were monitored and were stable during incubation: temperature, pH, conductivity, and OD.

DNA extraction and library preparation

From each group, samples of sediment and water were collected after the incubation period (n = 5/group), for extraction of microbial DNA Genomic Bacteria AX Mini kit (A&A Biotechnology, Gdansk, Poland) was used (additionally with blank sample). DNA concentration and quality were measured with Qubit 4 Fluorometer using Qubit dsDNA HS Assay-Kit (Thermo Fisher Scientific Inc., US) and NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, US). Native barcoding V14 kit (Oxford Nanopore Technologies, UK) with Long Fragment Buffer (LFB) in accordance with the manufacturer's recommendations were used for the preparation of the libraries (Oxford Nanopore Technologies, UK).Sequencing was performed, using MinION sequencer (MIN-101B; Oxford Nanopore Technologies, UK) with 10.4.1 Flow cell (FLO-MIN114; Oxford Nanopore Technologies, UK). Three runs were performed – 10 samples/run, the amount data produces from each run was minimum 2 GB. The sequence data obtained in this study are deposited in the SRA of NCBI under accession number PRJNA1184693.

Sequencing and data analysis

Sequences were analyzed using the Galaxy Europe web platform [27] . The assessment of read quality before and after preprocessing was performed using FastQC, NanoPlot, and MultiQC[28] . Trimming and filtering of reads by length and quality were conducted using Porechop and Fastp[29] . Kraken2 and prebuilt RefSeq indexes (Standard Full) were then used for classification. For visualization of the results, the Krona chart and the R program (v4.2.3) with ggplot2 were used. For the detection of Antimicrobial Resistance Genes (AMR), the CARD database was queried using the ABRicate tool. A threshold of ≥ 80% (default value) was applied to the output files, and filtered data were used for further analysis. To create the ASV table, the Pavian web platform was used (https://fbreitwieser.shinyapps.io/pavian/). Downstream analyses were performed in R (v4.2.3). Alpha diversity was calculated using species richness based on ASV number, as well as Chao1, Shannon, and Simpson diversity indices. For statistical analysis, the Kruskal–Wallis test, Wilcoxon rank-sum test, and PERMANOVA with Benjamini–Hochberg p-value correction for multiple comparisons were applied. To identify biomarker taxa differing between groups, ANCOM-BC (Analysis of Compositions of Microbiomes with Bias Correction) was performed. Figures were prepared using the ggplot2, vegan, and phyloseq packages.

Results

General results of microbial composition

The minimum read length in tested samples was 200 bp, the average reads was 98 141, and the average bases per sample was 223 Mbp. Analysis of Alpha diversity (Fig. 1.) shown in the case of Shannon and Simpson differences in diversity in tested groups ($p = 0.03698$). The highest diversity was observed after 7th day of incubation in the case of both water and sediment ($p = 0.04456$) with subsequent decrease observed on the 14th day of incubation. The most abundant phyla in each group were Proteobacteria, followed by Actinobacteria (Fig. 2.). The highest level of Proteobacteria had been found in II_INC_MP group when compared to the other, and the highest level of Actinobacteria level had been in I_INC_SED. Additionally, group C_WAT was characterized by high level of Cyanobacteria. Other, phyla in tested samples were Bacteroidetes, Firmicutes and PVC (Planctomycetota, Verrucomicrobiota, and Chlamydiota) superphylum. The significant differences had been found between C_WAT and I_INC_MP ($p = 0.0087$), C_WAT and II_INC_MP ($p = 0.0033$) in Cyanobacteria level. Other differences were detected in Acidobacteria level between I_INC_SED and II_INC_SED ($p = 0.023$).

Sediment

In the control sediment samples the most prevalent class from Proteobacteria were Alphaproteobacteria (30%), Gammaproteobacteria (28%) and Betaproteobacteria (22%). In Alphaproteobacteria the most abundant bacteria families were *Brandyhizobacteriaceae, Rhizobiaceae* and *Phyllobacteriaceae* (Figs 2,3,4.). In Gammaproteobacteria most abundant were *Enterobacteriaceae, Xanthomonodaceae, Pseudomonadaceae, Aeromonadaceae,* and in case of Betaproteobacteria class – *Comamonadaceae* and *Burkholderiaceae*. At the genus level, fish and human pathogens such as *Aeromonas* (*Aeromonas salmonicida*), *Flavobacterium* (e.g. *Flavobacterium psychrophilum*), *Pseudomonas* (e. g. *Pseudomonas aeruginosa, Pseudomonas fluorescens*), *Eschericha coli* or *Klebsiella pneumoniae* were detected. The pathogen detection level was estimated to be 1 – 5% of the total bacteria abundance. The

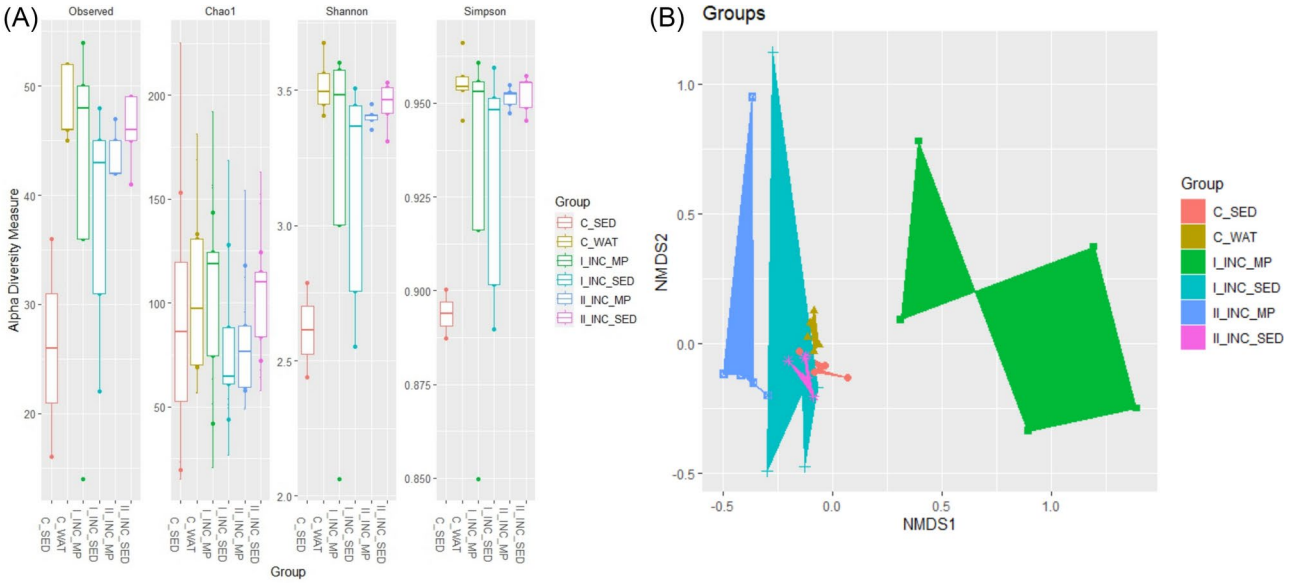

Fig. 1. Alpha (**A**) and Beta (**B**) diversity of tested samples. *C_SED – control sediment; C_WAT – control water; I_INC_MP – 7th day of water incubation with MPs; I_INC_SED—7th day of sediment incubation with MPs; II_INC_MP – 14th days of water incubation with MPs; II_INC_SED – 14th days of sediment incubation with MPs.*

Archaea constituted about 2% of whole microbial community, the most prevalent was phylum Euryarchaeota, with families Haloarculaceae and Halococcaceae (Fig 5.).

After 7 days of incubation with MPs, the microbial community changed significantly (Fig 5,6), and higher levels of Actinobacteria ($p = 0.01$) and lower levels of Proteobacteria compared to C_SED were detected. In Proteobacteria phyla, level of Enterobacteriaceae family increased from 35 to 44% and were dominated mostly by Escherichia coli (86%) and Salmonella enterica (4%), which was significant increase ($p = 0.0258$) compared to C_SED group. In case of Actinobacteria phyla, analysis have shown significant increase of Mycobacterium genus from 24% in C_SED group, up to 43% in I_INC_SED group, making them dominant in case of this phyla ($p = 0.00003$). Analysis have also evidenced significant increase of the Aeromonas ($p = 0.0048$) and Flavobacterium genera ($p = 0.009574$) in comparison to the C_SED group. Significant increase was also observed in Flavobacterium genus ($p = 0.0051$), from 45% up to 70% of Flavobacteriaceae family. However, larger number of pathogens were detected when compared to the previous group, such as: *Aeromonas hydrophila*, *Vibrio parahaemolyticus* or *Vibrio metschnikovii*.

On the other hand, in the case of Archaea (3%) after 7 days of incubation analysis has shown more genus-level diversity compared to C_SED group. In case of I_INC_SED group, samples were also characterized by additional phyla: Crenarchaeota with Sulfobaceae family. Previous class of the Archeons in this case, was characterized by presence of more families such as: Halorubraceae, Halobacteriaceae, Microbacteriaceae etc. (Fig. 7) when compared to C_SED samples.

After 14 days of incubation with MP, sediment samples were characterized with increasing similarity of microbial composition in treatment and control samples (C_SED) (Fig. 5,8). The abundance of Proteobacteria and Actinobacteria phyla were similar compared to C_SED group. In Proteoacteria phylum, most abundant classes were: Alphaproteobacteria (25%), Betaproteobacteria (22%) and Gammaproteobacteria (40%) (Fig. 5,9). The abundance of *Enetobacteriaceae* family (Gammaproteobacteria class) decreased from 44 to 24%, where *E. coli* constituted 96% and *Salmonella* genus was less than 0.5%, compared to I_INC_SED ($p < 0.0001$). However, the level of *Enterobacteriaceae* family, and the *Esherichia* genus were higher (62%) compared to C_SED (36%) ($p = 0.00003$).

Additionally, the level of *Aeromonadaceae* family was lower (1%; $p = 0.03954$) compared to the previous group (I_INC_SED). Samples were characterized by more *Aeromonas* species such as *A. salmonicida*, *A. hydrophila* and *A. veronii*. In this group, *A. salmonicida* were the most abundant species compared to other species (27%). Levels of *P. aeruginosa* and *P. fluorescens* were equal to previous group (about 3%). Abundance of *Mycobacterium*, *Vibrio* and *Flavobacterium* were similar when compared to I_INC_SED.

In the case of Archaea (Fig. 10), their abundance in the samples was similar to C_SED – about 2%. The genus diversity indicated in II_INC_SED decreased compared to the same group after 7 days of incubation with MPs. Order Natrialbales was characterized by increased genus numbers (*Halovarius*, *Natrialba, Natronobacterium* and *Salinarchaeum*), including Methanosarcinaceae family (*Methanohalophilus* and *Methanosarcina* genus) when compared to I_INC_SED.

In all groups, analysis revealed occurrences of antimicrobial resistance (AMR) genes (Table.2, Supplementary file Fig. 1). In control group, blaTEM-116 and erm(F) were detected, related to resistance to amoxicillin, ampicillin, erythromycin etc. In group I_INC_SED analysis showed more AMR genes, compared to control

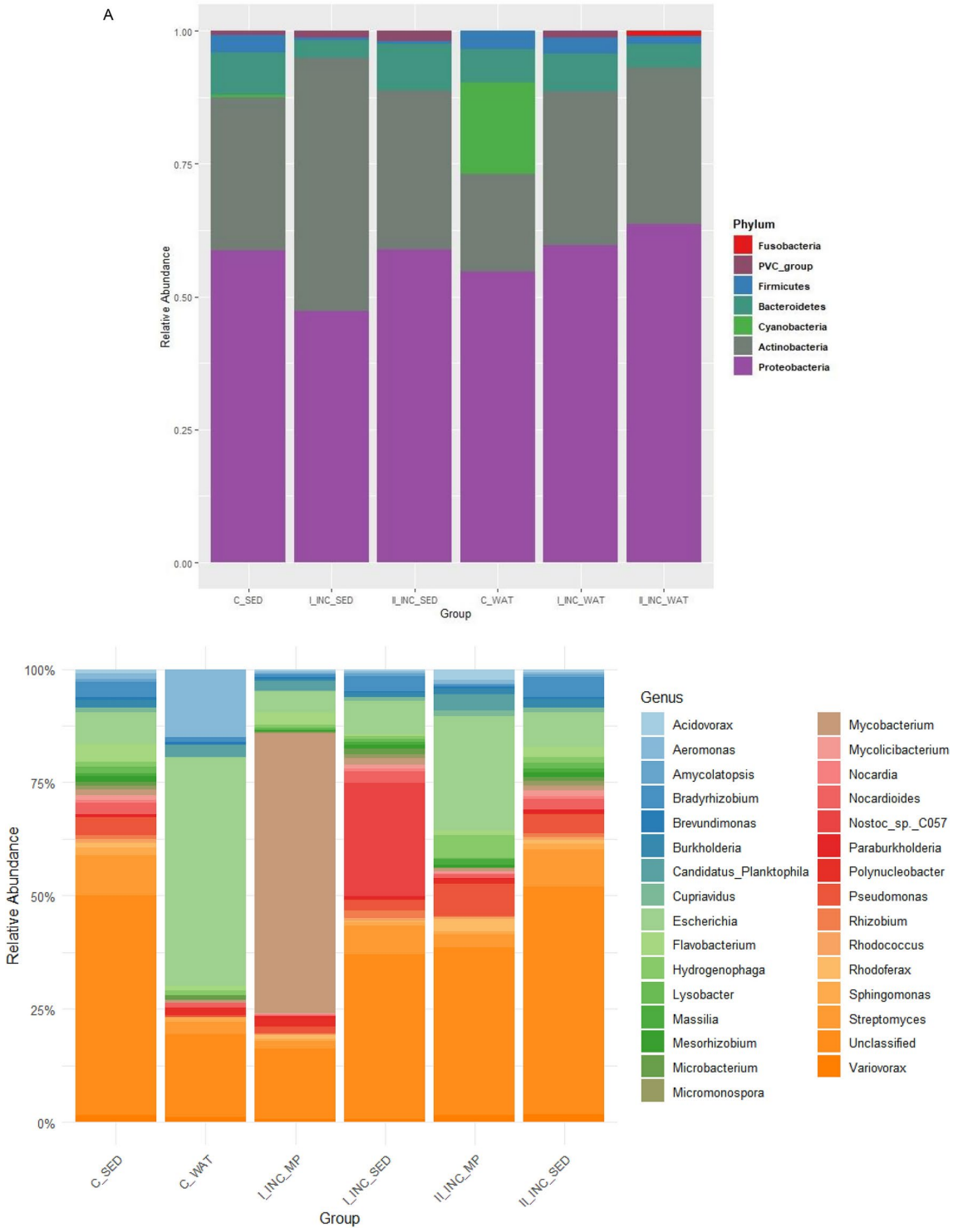

Fig. 2. Abundance (**A**) of bacterial phyla and genus in groups (< 3%) and heatmap (**B**) of bacteria in the tested groups (*C_SED – control sediment; C_WAT – control water; I_INC_MP – 7th day of water incubation with MPs; I_INC_SED—7th day of sediment incubation with MPs; II_INC_MP – 14th days of water incubation with MPs; II_INC_SED – 14th days of sediment incubation with MP)*.

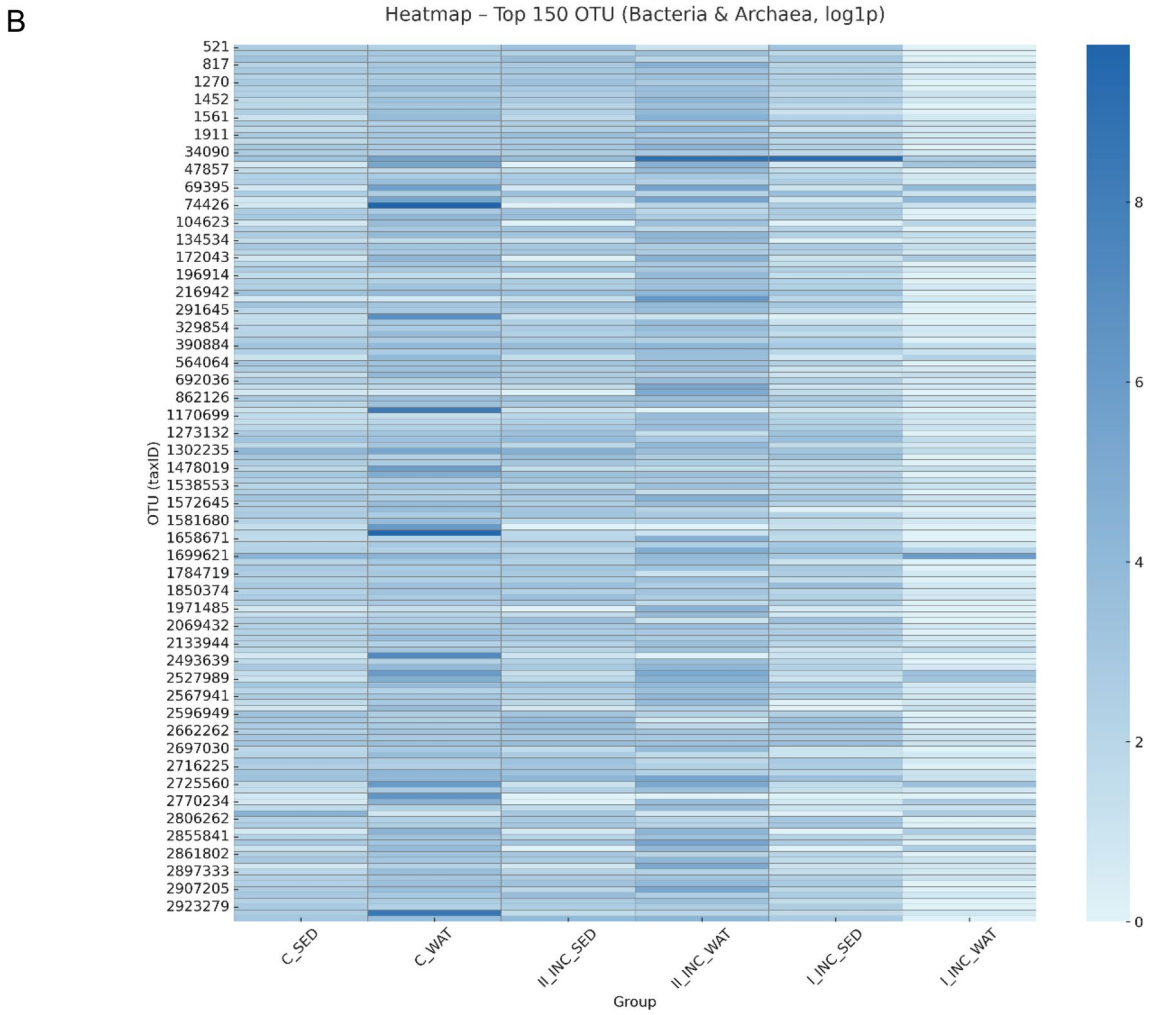

Figure 2. (continued)

group: blaTEM-116, otr(C), ole(C), and oqxB. Similar results were observed in the case of the II_INC_SED group, where analysis detected: dfrB3, tcr3, ole(C), and otr(A).

Water

Water samples in control group (C_WAT) were characterized by high abundance of Proteobacteria, Actinobacteria and Cyanobacteria phyla (Fig. 2A, 11). In the case of Proteobacteria phyla (Fig. 11,12), the most abundant class were Gammaproteobacteria (76%). In this class the most abundant were *Luteimonas*, *Klebsiella* and *Escherichia coli*. The level of *Aeromonas salmonicida* was 2%, and Pseudomonas was less than 2%. Most of the Cyanobacteria belonged to *Nostoc* sp. (97%). In Bacteroidetes phyla (3%), *Flavobacterium* genus abundance was 48%. In Actinobacteria phyla (13%), the *Mycobacterium* genus abundance was 16%.

Archaea accounted for less than 1% of the sequenced community (Fig. 13). The dominant phylum was Euryarchaeota, primarily represented by the *Halopiger* genus and several methanogenic taxa, including *Methanocaldococcus, Methanocella, Methanoregula, Methanosarcina*, and *Methanothrix*. The second most abundant group was Thaumarchaeota, featuring genera such as *Nitrosomarinus, Nitrosarchaeum, Nitrososcosmicus,* and *Nitrososphaera*. In the case of water samples after 7 days of incubation (I_INC_MPS) the microbial community (Fig. 11, 14) presented with changes in the level of Proteobacteria, Actinobacteria and Bacteroidetes ($p = 0.048$, $p = 0.039$, $p = 0.0236$) compared to the control group. The level of Gammaproteobacteria was higher (82%) compared to the control group. The *Aeromonas salmonicida* level was higher (10%) compared to control group ($p = 0.0002$), while the level of *Pseudomonas* was 1% and for *Vibrio* was about 0.3%. The other classes abundance was 8% for Betaproteobacteria and 4% for Alphaproteobacteria. On the other hand, the level of Cyanobacteria decreased significantly to 0.3% compared to the control group ($p = 0.0025$). The Bacteroidetes phylum abundance decreased to 2%, and *Flavobacterium* genus abundance was 24%, all lower when compared to the control group (C_WAT; $p = 0.0264$). In Actinobacteria phyla (9%), *Mycobacterium* genus abundance was significantly decreased to 4% ($p = 0.039$). In the I_INC_MPS group, the level of Archaea (Fig. 15) was 0.3%, and analysis has shown only Eurytarcheota phylum with *Haloferax*, *Natrinema* and *Mehtanococcoides* genus.

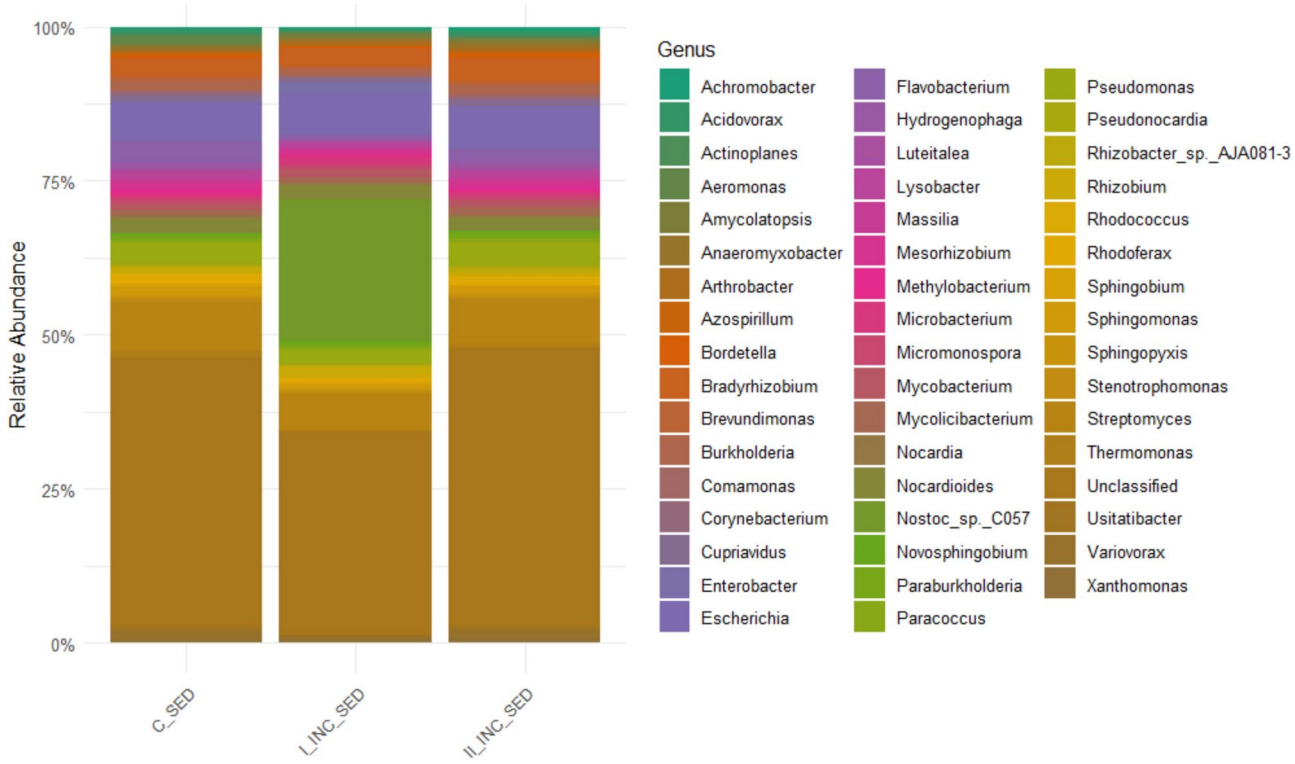

Fig. 3. Relative abundance of genus in groups in the tested groups (*C_SED – control sediment; I_INC_SED—7th day of sediment incubation with MPs; II_INC_SED – 14th days of sediment incubation with MP)*.

In the last group, after 14 days of incubation of the water with MPs (II_INC_MPS), the abundance of Proteobacteria was similar to the previous group (I_INC_MPS). In Proteobacteria phylum (Fig. 11,16) significant increase of Betaproteobacteria (42%), Alphaproteobacteria (19%) and decrease of Gammaproteobacteria (33%) ($p = 0.045$) classes was observed compared to I_INC_MPS group. In Gammaproteobacteria class, *E. coli* was significantly increased to 43% and *Pseudomonas* genus to 7% ($p = 0.039$), with *P. aeruginosa* and *P. fluorescens* abundance was highest when compared to the 7-day sample (3%), $p = 0.026$. In the case of Bacteroidetes, abundance has decreased significantly compared to 7-day sample (I_INC_MPS) ($p = 0.02995$). The Bacteroidetes phyla was on similar level to the control group (3%) and the prevalence of *Flavobacterium* genus has increased significantly to 37% ($p = 0.039$) when compared to the previous sampling (I_INC_MPS). The abundance of Actinobacteria phyla rose significantly to 36% ($p = 0.036$), and the same trend has been noticed for Mycobacterium genus (75%; $p = 0.008$) when compared to 7-day sample (I_INC_MPS). The Archaea in II_INC_MPS group (Fig. 17) was more diverse compared to the previous group – I_INC_MPS. After 14 days of incubation this domain was characterized by Halobacteria order represented by genus *Halomicrobium*, *Halobaculus*, *Halococcus*, Methanomicrobia order with *Methanofillis* genus, and Thermococci order with *Thermococcus* genus.

In the case of AMR genes (Table. 3, Supplementary file Fig. 1), in control water samples only one gene was indicated – vat(F), which is responsible for resistance to Dalfopristin, Pristinamycin, and Virginiamycin. In the case of water samples after 7th day of incubation with MPs, total of five AMR genes were indicated (dfrB3, mph(E), ole(C) tcr3 or otr(A)), dropping to three AMR genes at day 14 of incubation (qepA4, oqxB and ort(A)).

BLAST analysis in the tested samples suggested the presence of *Prymnesium parvum* in the tested water samples (Table 4), however, due to the small amount of genomic data, further analyzes are recommended.

Discussion

The presence of MPs in water environment and their bioavailability may contribute to, or alter the pattern of, the spreading of pathogens and AMR genes, especially in the context that environmental sources and human activities (wastewater discharge, plastic mulching, compost fertilization, sewage sludge amendment, and atmospheric rainfall), may all contribute to MPs pollution[13,30]. Recently, the MPs are described as a new ecological niche for bacteria, including animal and human pathogens e.g., *Vibrio* sp., *E. coli*, *Aeromonas, Salmonella, Klebsiella* or *Mycobacterium*[31–33].

We observed pathogens such as *Aeromonas salmonicida, Aeromonas hydrophila*, *Flavobacterium psychrophilum*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* or *Vibrio* sp. in sediment and water samples [Figs 2,3,4,5,6,7]. Significant increase of *E. coli*, *S. enterica*, *A. salmonicida* after 7 days of incubation with MPs were detected in sediments, and in water, increase of *A. salmonicida* and decrease of Cyanobacteria phyla were simultaneously observed [Fig. 4, 7b]. The changes continued, and after 14 days of incubation with MPs in sediment, microbial community level

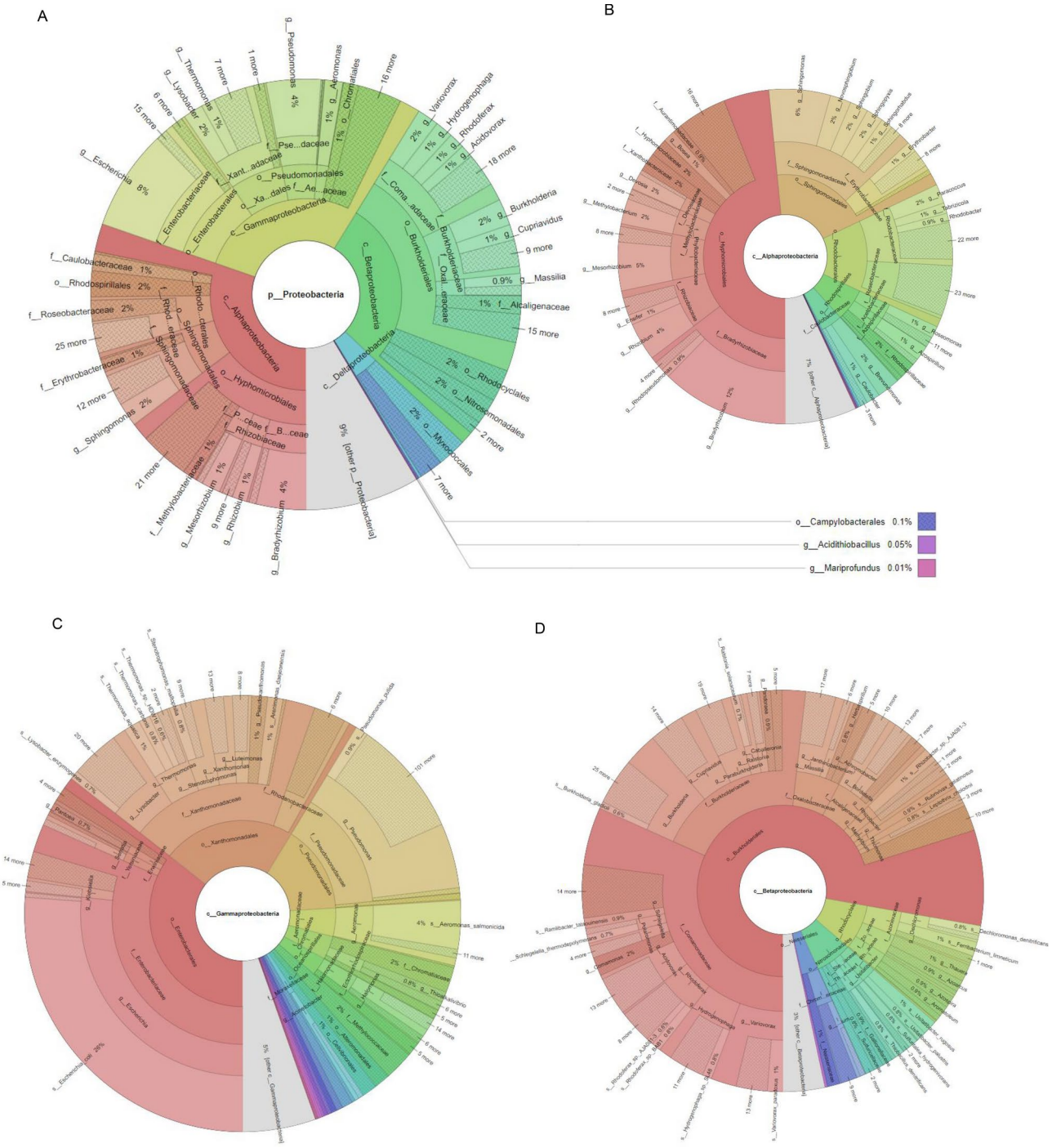

Fig. 4. Krona chart of most abundant classes from Proteobacteria phyla in C_SED group (**A** – Proteobacteria phyla; **B** – Alphaproteobacteria; **C** – Gammaproteobacteria; **D** – Betaproteobacteia).

of *Enterobacteriaceae* has significantly decreased, with *E. coli* becoming the dominant species, similar to the control group (and also with *Aeromonas* genus, similar changes were noted). However, other pathogens like *Pseudomonas*, *Mycobacterium*, *Vibrio* or *Flavobacterium* were similar at days 7 and 14 of incubation. In water samples, similar results were observed for *E. coli*, the *Pseudomonas*, *Flavobacterium* or *Mycobacterium* were significantly increased, and *A. salmonicida* decreased.

These results may be related to microbial competition and their use of MPs to create biofilms, making the MPs an environmentally favorable niche for some of the bacteria species. In studies performed by Delacuvellerie et al.[34], results of tested structure and color did not influence the microbial composition of plastisphere and the main factor influenced the microbial community of MPs was chemistry and geographical location of collected samples[34]. In study performed by Murphy et al.[35], MPs have influenced microbial community in water (River

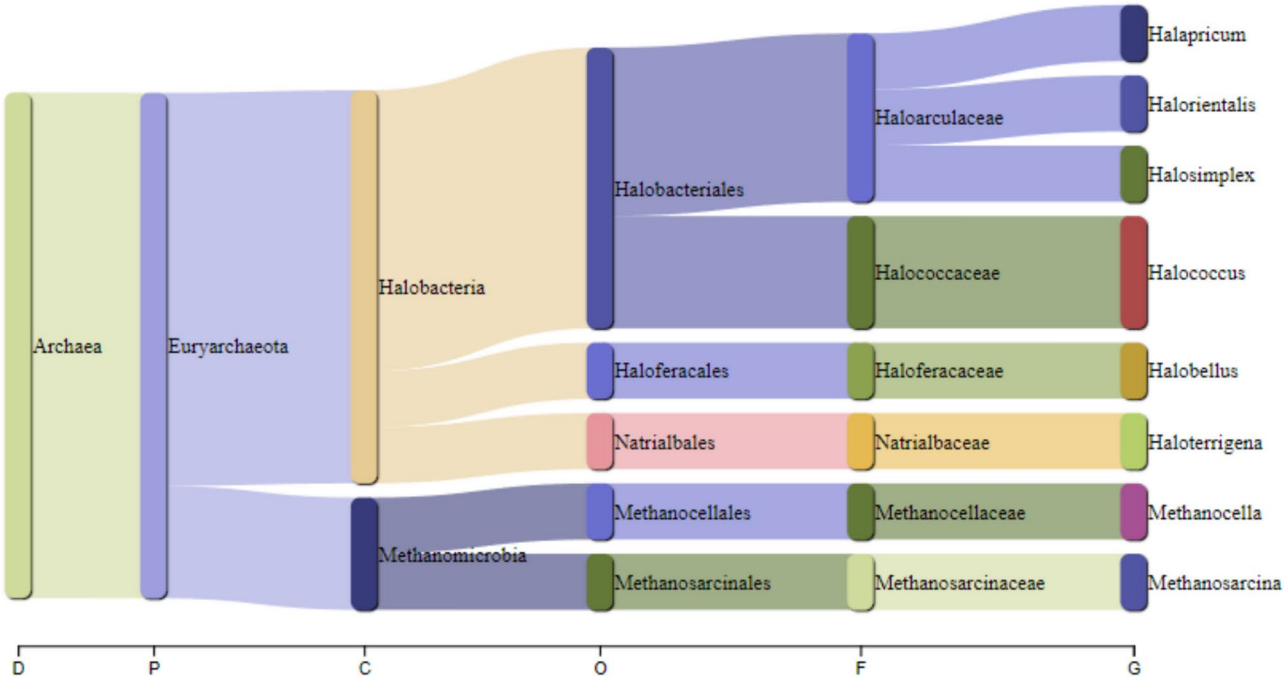

Fig. 5. Archaea in tested C_SED group.

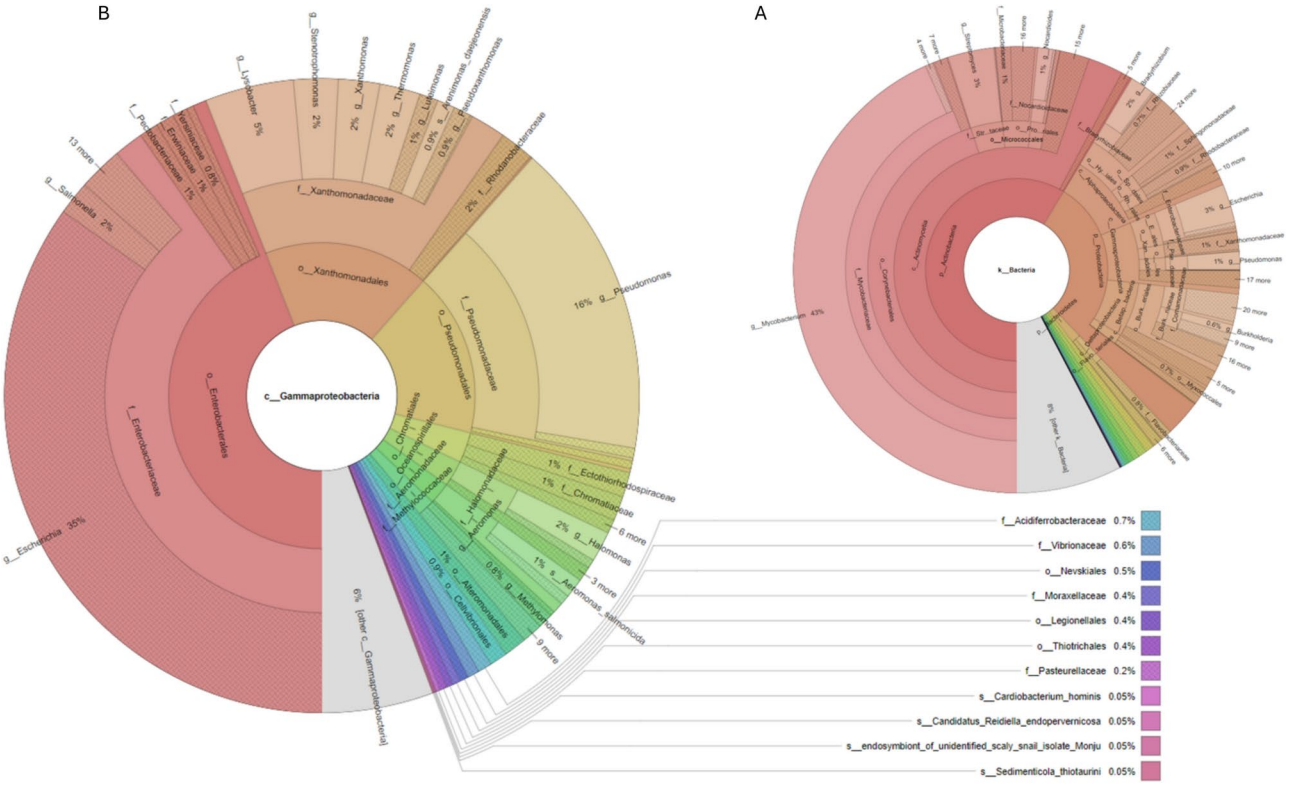

Fig. 6. Krona chart of I_INC_SED group (**A** – Bacteria domain; **B** – Gammproteobacteria).

Barrow, Carlow, Ireland) as another reservoir for pathogens. MPs acted as additional hard substrate favoring the attachment of autochthonous (and sometimes allochthonous) biofilm forming bacteria, likely influencing the microbial community and their physiology in sediments and water. For example, PET (Polyethylene terephthalate) particles can play a role as ecological refuge for allochthonous and rare potential pathogens,

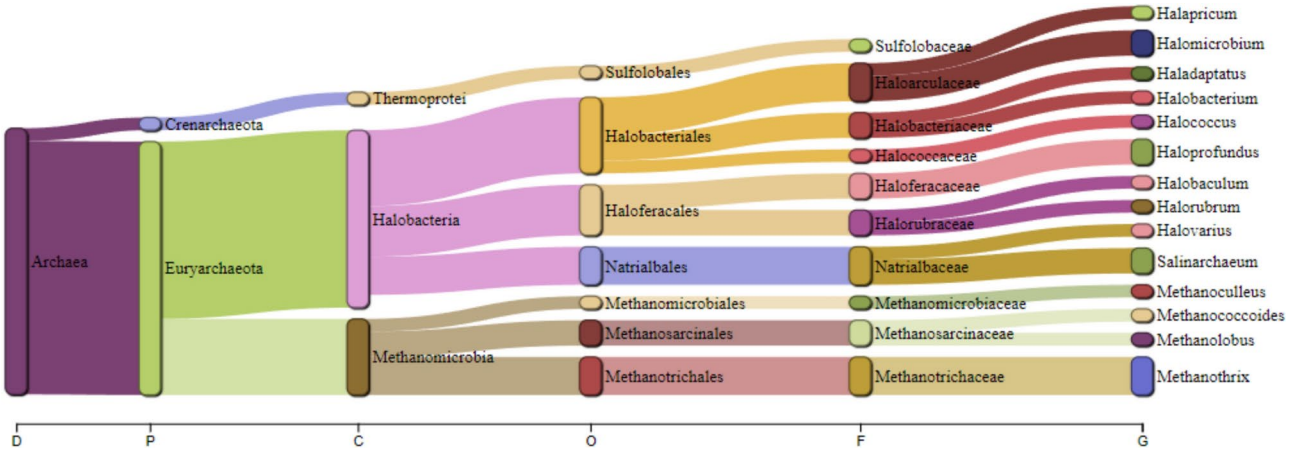

Fig. 7. Archaea indicated in I_INC_SED group.

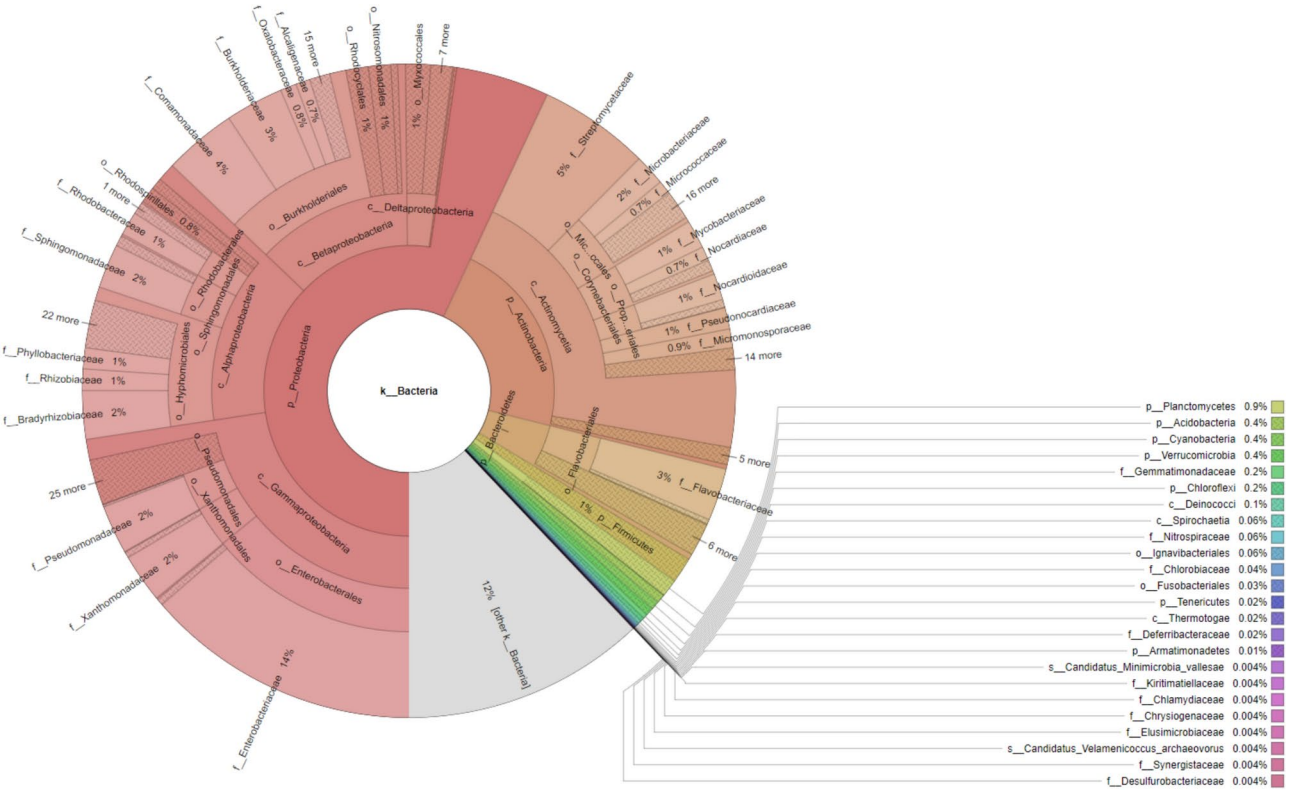

Fig. 8. Bacteria abundance in the sediment samples (II_INC_SED).

extending their presence in aquatic systems, and posing a direct threat to human health[36–38]. Based on recent studies, PET belongs to the one of the main type of MPs in the environment, alongside of polyethylene (PE), polypropylene (PP), polystyrene (PS) and Polyvinyl chloride (PVC)[36,39], indicating their collective availability as microbial colonization substrate.

The Archaea have complex interaction networks, high metabolic activities and different indicator species in soil environments compared to freshwater biofilms and estuarine waters. The most common representatives are Euryarchaeota and Crenarchaeota[40,41], and in our samples Euryarchaeota were dominant. Most of Archaea in freshwater environment have a significant impact on biogeochemical cycling. These microorganisms are using organic or inorganic electron donors and acceptors and thus, play crucial roles in global geochemical cycles including influencing greenhouse gas emissions[42]. Additionally, methanogenesis and anaerobic methane oxidation are important steps in the carbon cycle that are performed by anaerobic Archaea[41]. In our samples we can observe trends in diversity changes of Archaea in water samples depending on duration of incubation with MPs.

Fig. 9. Proteobacteria classes abundance in the sediment samples (II_INC_SED).

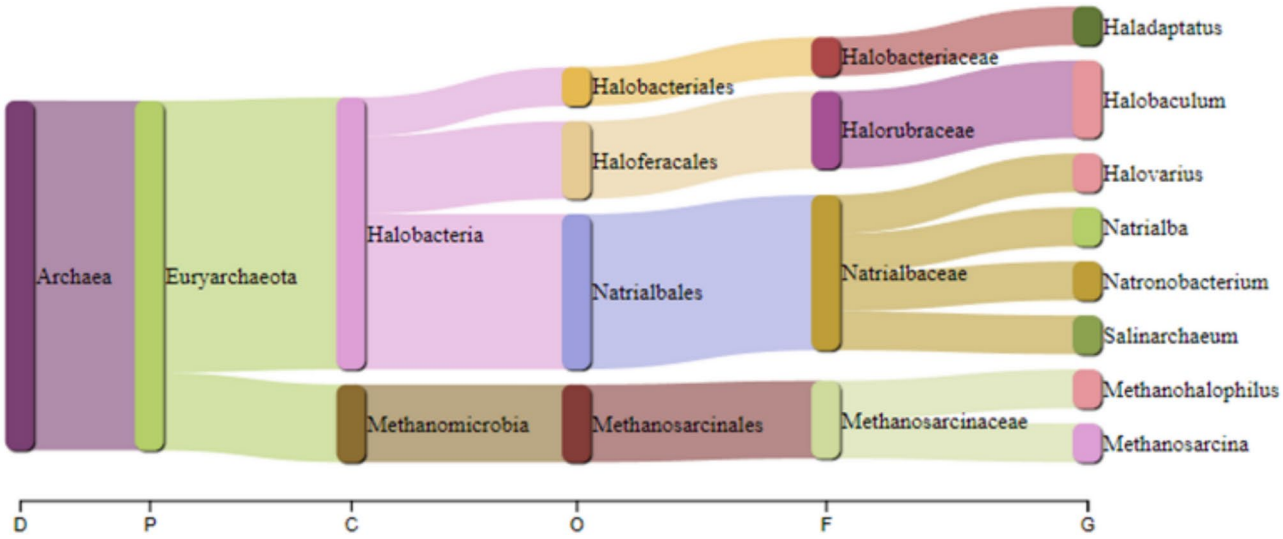

Fig.10. Archaea indicated in II_INC_SED group.

Antimicrobial resistance is another potential biological characteristic that can be affected by MPs, as bacteria colonized on MPs can carry, and therefore be involved in spreading of, antibiotic resistance genes (ARG) in the aquatic environments and sediment/water/biota interfaces. The ubiquitous use of antibiotics in human and animal medicine increased occurrence of ARG in environment. In the environment, common occurrence ARG are related to antibiotics such as beta – lactams, tetracycline, sulfonamides etc.[41,43]. MPs are also important adsorbents for various organic contaminants including antibiotics because of their specific structure and hydrophobic surfaces[39,41].

Sample	Gene	Resistance	Gene location	Database
C_SED	TEM -116	penicillin beta-lactam, cephalosporin, monobactam	Chromosome/Plasmid	CARD
	ErmF	streptogramin antibiotic, streptogramin B antibiotic, streptogramin A antibiotic, lincosamide antibiotic, macrolide antibiotic	Chromosome	CARD
I_INC_SED	otr(C)	tetracycline antibiotic	NA	CARD
	TEM -116	penicillin beta-lactam, cephalosporin, monobactam	Chromosome/Plasmid	CARD
	oleC	macrolide antibiotic	NA	CARD
	oqxB	tetracycline antibiotic, nitrofuran antibiotic, diaminopyrimidine antibiotic, glycylcycline, fluoroquinolone antibiotic	Chromosome/Plasmid	CARD
II_INC_SED	dfrB3	diaminopyrimidine antibiotic	NA	CARD
	oleC	macrolide antibiotic	NA	CARD
	otr(A)	tetracycline antibiotic	Chromosome	CARD

Table 2. Antimicrobial resistance genes in tested groups (*C_SED – control sediment; I_INC_SED—7th day of sediment incubation with MPs; II_INC_SED – 14th days of sediment incubation with MPs*).

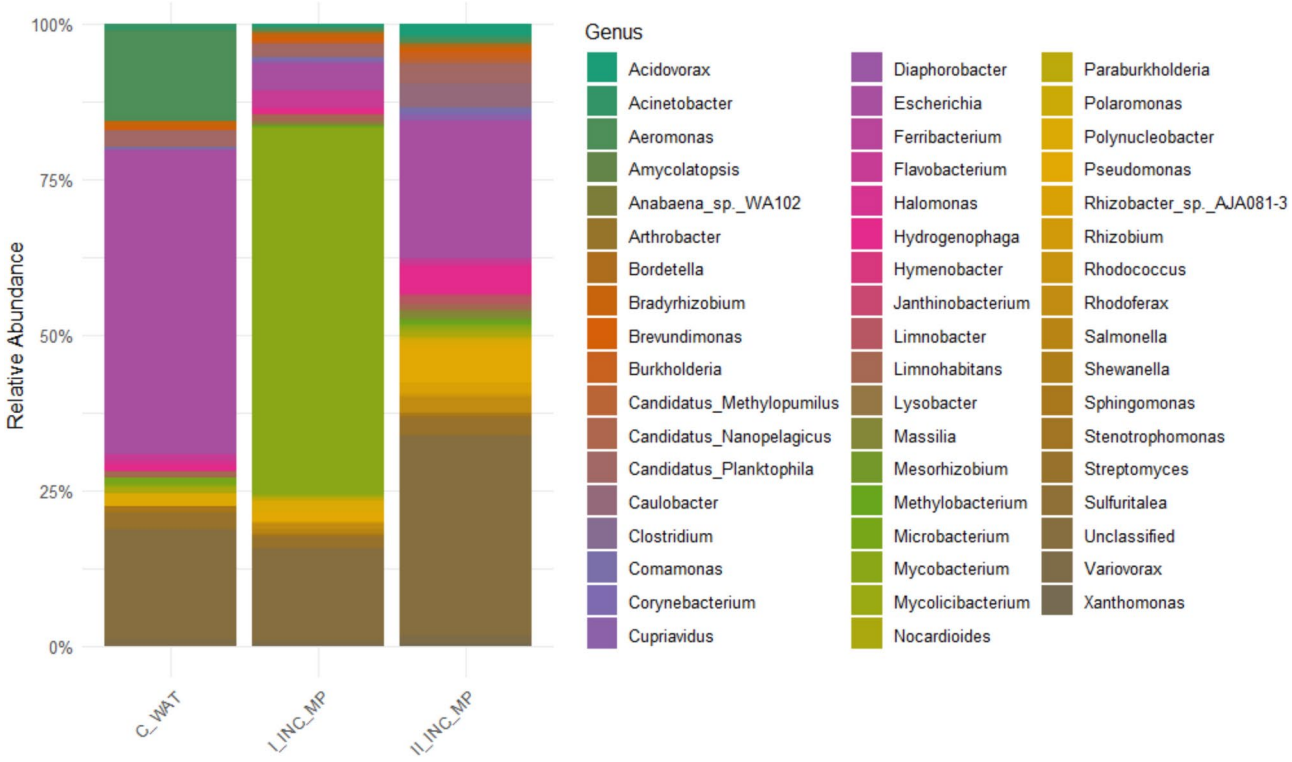

Fig.11. Relative abundance of genus in groups in the tested groups (*C_WAT – control water; I_INC_MP – 7th day of water incubation with MPs; II_INC_MP – 14th days of water incubation with MPs*).

We detected ARGs related to macrolide (erythromycin, lincomycin, oleandomycin etc.), tetracycline (doxycycline, minocycline etc.), fluoroquinolone/quinolone (nalidixic acid, ciprofloxacin), sulfonamide (trimethoprim) and β – lactams (amoxicillin, ampicillin, piperacillin etc.). Occurrence of ARGs such as drfB3 (trimethoprim), tcr3 (tetracycline, doxycycline), oleC (oleandomycin), otrA (tetracycline, doxycycline, minocycline) and oqxB (nalidixic acid, ciprofloxacin) was detected in both water and soil samples. In control group in both cases (C_SED, C_WAT) the numbers of ARG were 1 or 2, after 7th day of incubation the numbers of ARGs increased to 4 (C_SED) and 6 (C_WAT). ARG identification could be influenced by the season of sampling, which is related to changes occurring in the environment like water temperature, chemistry etc[44,45]. Additionally, MPs presence could increase gene exchange and the metabolites or degradation products of antibiotics that enter the aquatic environment. The gene exchange can occur through both vertical and horizontal gene transfer, which was tested on *E. coli* with ARG-coded plasmid performed by Li et al.[16]. As well aspathogenic bacteria developed on MPs could be a potential ARG host such as *Flavobacterium* [46] and in consequence, the antibiotic resistant pathogens or ARGs may be transferred from environment to human and animals[39]. The observed decrease in ARGs on day 14 compared to day 7 on the other hand may reflect the mechanisms of microbial succession within biofilm communities on microplastic surfaces. Early colonizers, often opportunistic

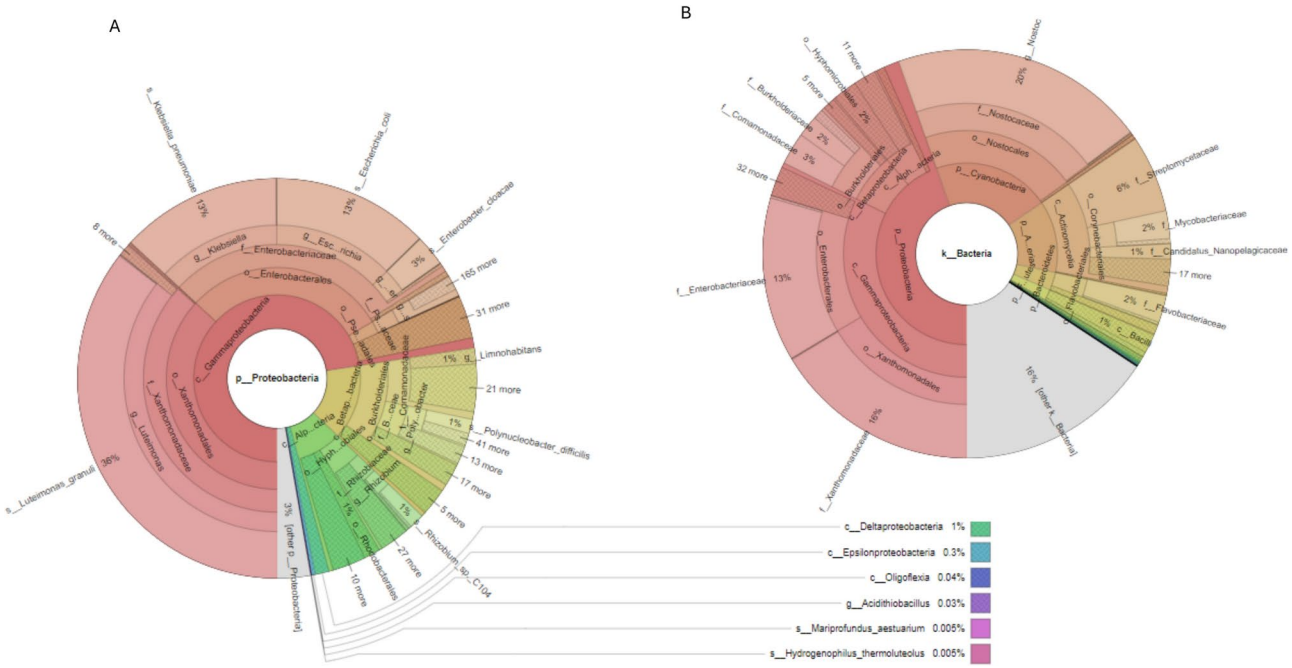

Fig.12. Proteobacteria (**A**) and bacteria domain (**B**) abundances in the water samples (C_WAT).

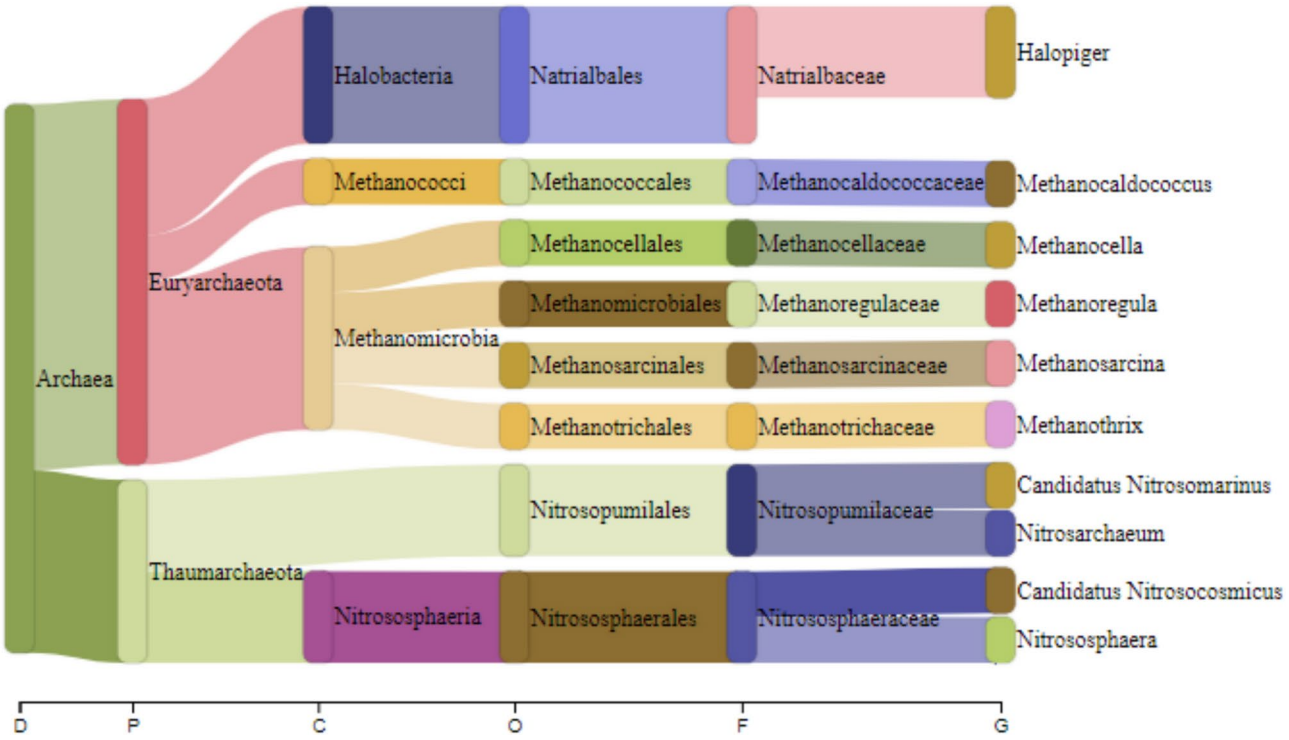

Fig. 13. Archaea indicated in water control group (C_WAT).

and ARG-enriched, may be outcompeted by slower-growing but more stable taxa over the time. Additionally, reduced HGT potential and environmental constraints such as nutrient depletion may have contributed to the observed decline in ARG abundance. This dynamic may be related to similar results presented in the previously cited studies of development of microbial communities on MPs of Delacuvellerie et al.,[34] and Liu et al.[47].

Despite the difference in the environment (soil) the studies performed by Gross et al.[48](2025) where *E.Coli* were exposed to various concentrations of the different MP types, including polyethylene, polystyrene, and

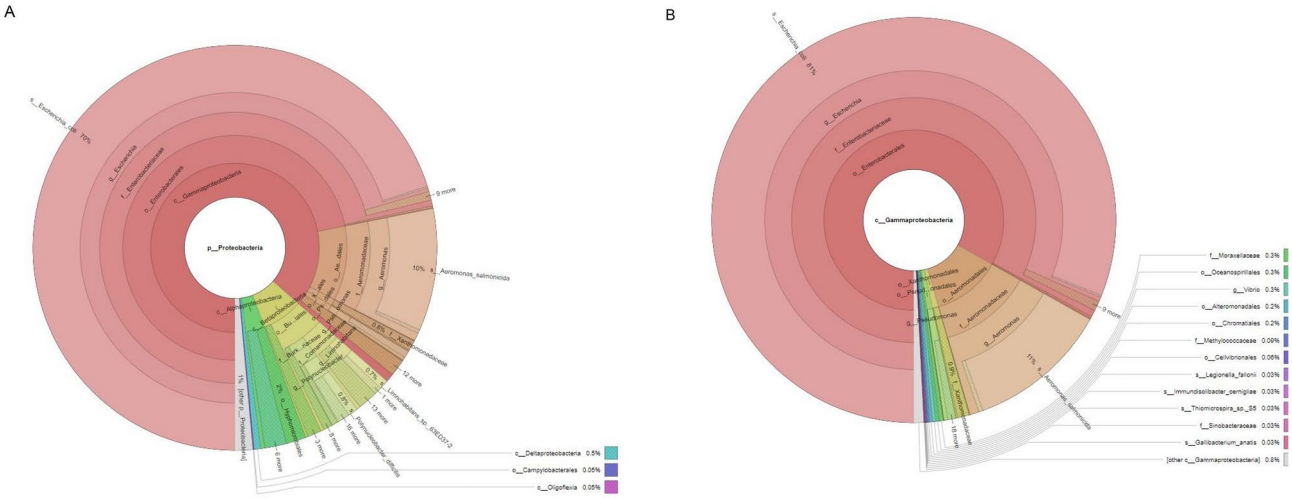

Fig.14. Proteobacteria (**A**) and Gammaproteobacteria (**B**) abundances in the water samples after 7th days of incubation with MPs (I_INC_WAT).

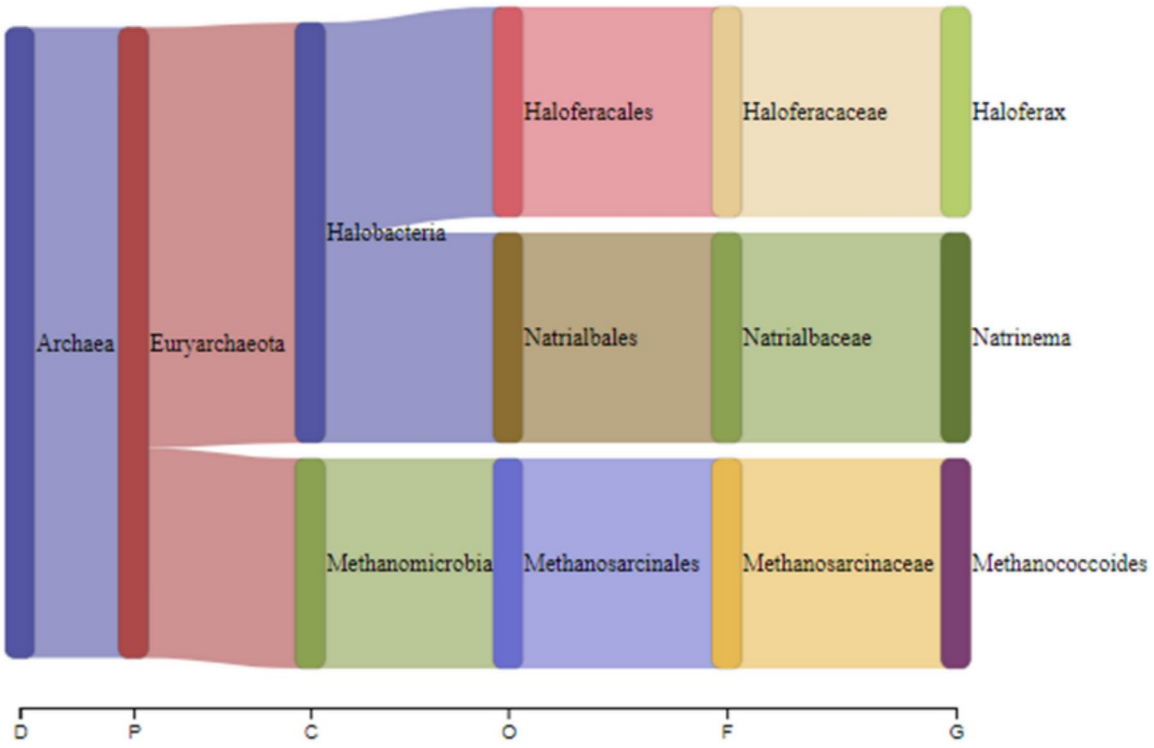

Fig.15. Archaea indicated in 7th days of water incubation with MPs (I_INC_MPS).

polypropylene, have exhibited elevated levels of multidrug resistant, and biofilm producing capabilities. This is especially alarming since the improved biofilm creation capabilities might even further impact not only the range in which those potentially pathogenic organisms will spread but also increase their potential for infecting the wildlife organisms. In studies by Liu et al.[47] performed on mariculture sediments, we can observe similar pattern, where PVC, and PS particles were not only constituting the perfect environment for the adhesion of the bacterial communities, but at the same time have significantly raised the HGT rates between bacteria forming it. Additionally, this have effected in increase of relative abundance of ARG's by up to 2,84 times. Another factor of risk associated with presence of the MP in the environment, have been reported by Wang et al.[49], in his microcosm studies, which have evidenced that not only amount of the and type of the MP can be factors attributing to danger related to their presence in the soil environment, but also MP diversity. In said studies, number of mobile genetic elements, virulence factor, and antibiotic resistance genes were correlated

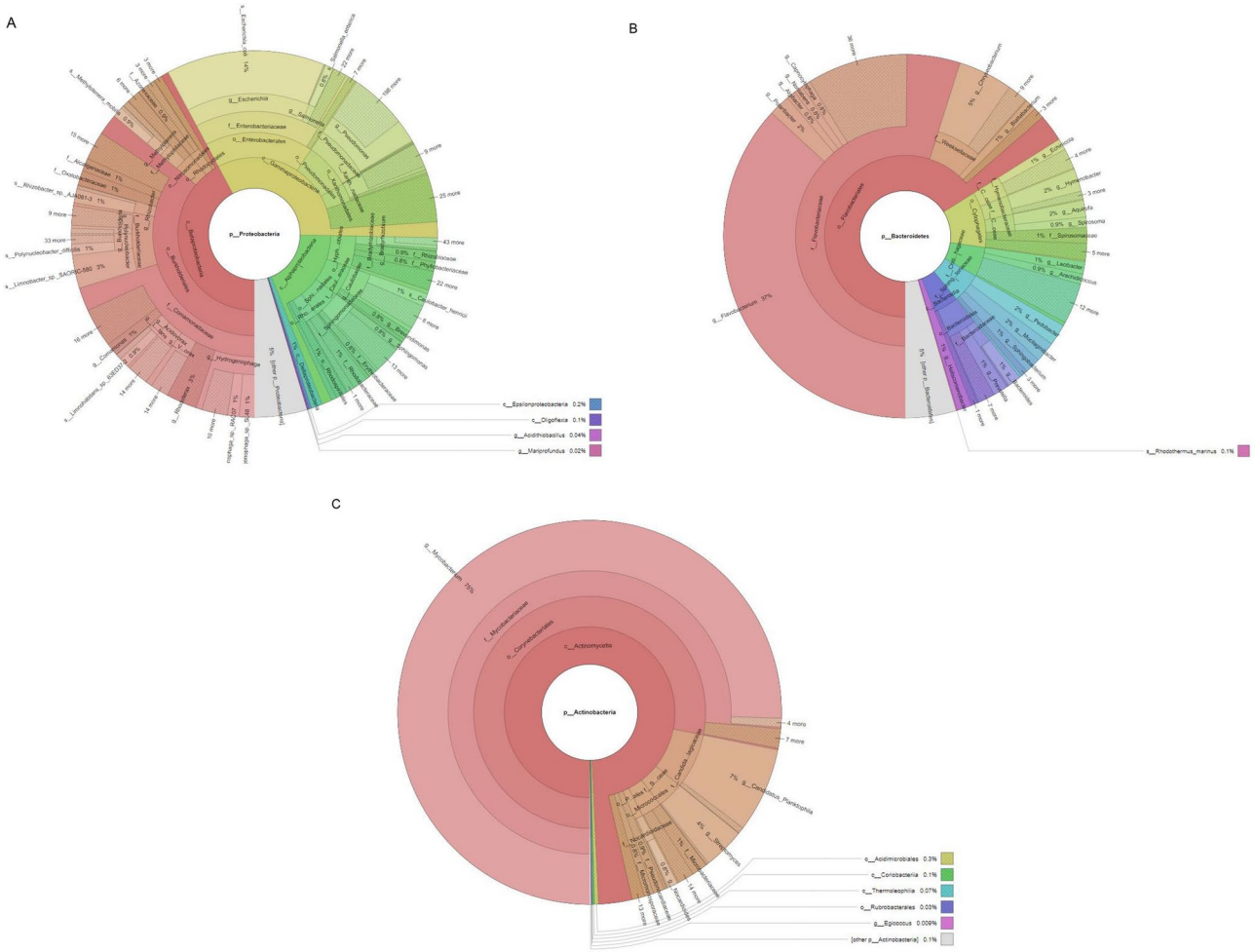

Fig.16. Proteobacteria (**A**), Bacteroidetes (**B**) and Actinobacteria (**C**) abundances in the water samples after 14th days of incubation with MPs (II_INC_WAT).

with diversity of the MP particles present in the soil. At the same time, authors have noticed that reduction of the plants diversity, and presence of the agriculture-derived fungicides were among other key factors attributing to this phenomenon.

Presented observations, might be even more alarming, if confirmed, in case of the water environment, due to the unique characteristics of the complex catchments like in case of the Oder river, which due to the presence of terrains associated with diverse purposes like agriculture, urban or industrial areas, might be particularly vulnerable to the effects associated with the impact of microplastic presence on microbial communities. At the same time blooms of the *Prymnesium parvum,* that have significantly impacted the ecological status of the Oder river, in recent years have greatly affected the microorganisms, plants and animals populations[50,51], potentially making it even more susceptible to the disbalance that might be caused by presence of various MP's particles. Presence of *Prymnesium parvum* has been confirmed in control group and incubated samples both, with the data showing that number of genes associated with that species have noticeably increased in case of the incubated water vs. the control water. This suggests that the amount of genetic material of the *Prymnesium parvum* in the incubated water samples was higher indicating a possibility that MPs supported growth of *Prymnesium parvum* in tested water samples, similarly to results from Hitchcock et al.[25]. Taken together, these findings underscore the need for more comprehensive investigations into the interplay between microplastics, toxic algal blooms such as *Prymnesium parvum*, and microbial communities—particularly in large and complex waterways like the Oder river. Future research efforts should adopt interdisciplinary approaches, combining microbiology, ecology, environmental chemistry, and public health perspectives to fully elucidate how MPs may amplify or otherwise alter the proliferation of harmful algae and the emergence of multi-drug resistant bacteria. Such insights will be pivotal for guiding conservation strategies, informing policy measures, and ultimately safeguarding both biodiversity and human populations that depend on these freshwater ecosystems.

Conclusions

Microplastic particles have the potential to significantly influence the composition of microbial communities in both riverine water and sediment environments. In this study, we observed trends suggesting that MPs may

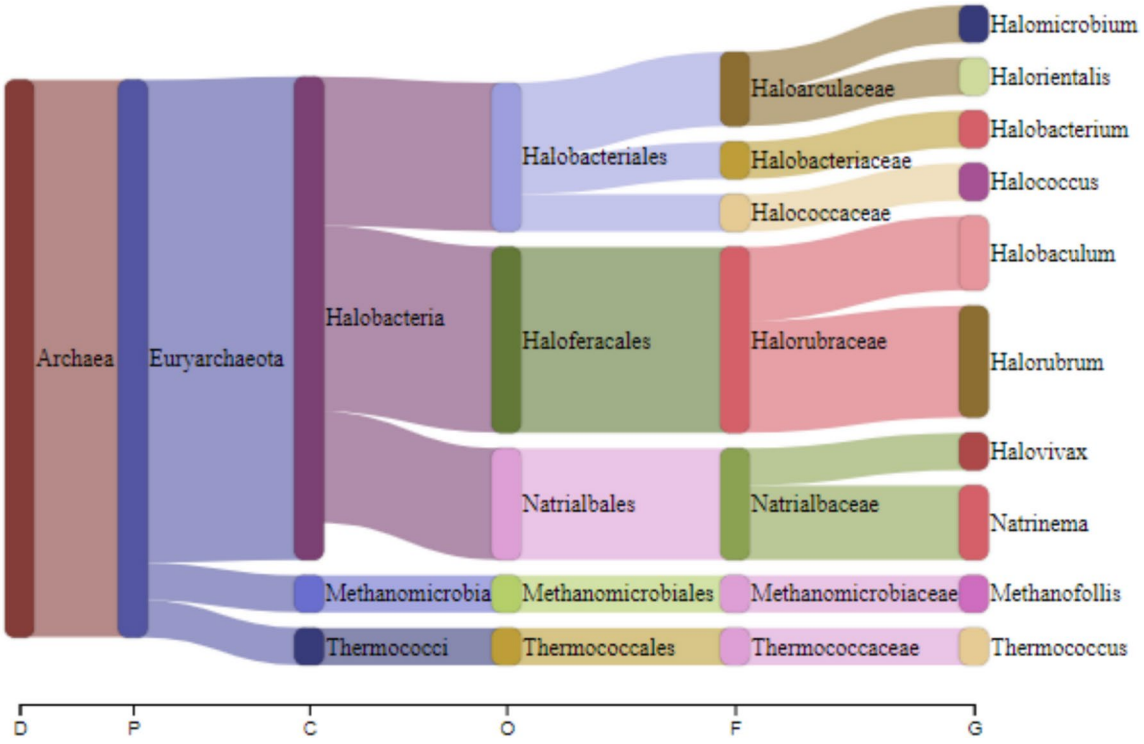

Fig.17. Archaea indicated in 14th days of water incubation with MPs (II_INC_MPS).

Sample	Gene	Resistance	Gene location	Database
C_WAT	vatF	streptogramin antibiotic, streptogramin A antibiotic	Chromosome	CARD
I_INC_MPS	dfrB3	diaminopyrimidine antibiotic	NA	CARD
	mphE	macrolide antibiotic	Chromosome/Plasmid	CARD
	oleC	macrolide antibiotic	NA	CARD
	tcr3	tetracycline antibiotic	NA	CARD
	otr(A)	tetracycline antibiotic	Chromosome	CARD
	srmB	macrolide antibiotic	NA	CARD
II_INC_MPS	QepA4	fluoroquinolone antibiotic	Plasmid	CARD
	oqxB	tetracycline antibiotic, nitrofuran antibiotic, diaminopyrimidine antibiotic, glycylcycline, fluoroquinolone antibiotic	Plasmid	CARD
	otr(A)	tetracycline antibiotic	Chromosome	CARD

Table 3. Antimicrobial resistance genes in tested groups (*C_WAT – control water; I_INC_MPS- 7th day of water incubation with MPs; II_INC_MPS – 14th days of water incubation with MPs*).

serve as surfaces for microbial colonization, potentially altering community structure over time. Furthermore, our findings, in combination with those reported in previous studies, point to the possibility that MPs can act as fomites—surfaces capable of harboring and transporting microorganisms—including potential pathogens and antimicrobial resistance (AMR) genes. While these observations raise important concerns, we recognize that the current study was limited in scope, both in terms of temporal and spatial sampling. As such, the findings should be interpreted with caution. Therefore, we acknowledge the presence of potential confounding factors that could have affected the results. Despite these limitations, the consistency of our findings with patterns observed in other aquatic and terrestrial systems lends preliminary support to the hypothesis that microplastics may play a role in the dissemination of pathogens and AMR elements in the environment. This represents an emerging public health concern, particularly in the context of the One Health framework, which emphasizes the interconnectedness of human, animal, and environmental health. Given the potential implications, we believe that further studies on this topic are necessary, especially in the case of rivers which are affected by anthropopressure as strongly as Oder river. Nevertheless, we strongly emphasize the need for more comprehensive studies—incorporating larger datasets, broader geographic coverage, and temporal replication—to robustly assess the ecological and public health risks associated with microplastic contamination.

Group	Sample	% of Identical matches
C_WAT	1	84.40
	2	79.871
I_MPS_WAT	1	83.333
	2	80.345
	3	83.951
	4	83.737
	5	78.723
II_MPS_WAT	1	82.531
	2	81.592
	3	83.495
	4	80.036
	5	81.336

Table 4. Results of BLAST analysis of water samples for detection of *Prymnesium parvum* (*C_WAT – control water; I_INC_MPS- 7th day of water incubation with MPs; II_INC_MPS – 14th days of water incubation with MPs*).

Data availability

The sequence data obtained in this study are deposited in the SRA of NCBI under accession number PRJNA1184693 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1184693).

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Author contributions

P.C.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. K.W.: Conceptualization, Data curation, Methodology, Investigation, Validation, Writing – review and editing. N.S.: Investigation, Data curation, Formal analysis; PP: Conceptualization, Resources, Validation. W.H.: Investigation, Formal analysis. Y.H.: Investigation, Formal analysis. W.D.: Writing – review and editing, Supervision. D.P.: Writing – review and editing, Supervision.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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