Review article

Spatial and temporal characteristics of microbial communities in the Seine river in the greater Paris area under anthropogenic perturbation

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ABSTRACT

Microorganisms play an important role in maintaining the proper functioning of river ecosystems and are promising candidates for environmental indicators. They are also highly sensitive to environmental changes. It is necessary to have basic knowledge about them in order to know the ecological status of river ecosystem. To our knowglege, there is very little information on the status of microorganisms in surface water of the Seine River, although the Seine River is one of the rivers that suffers the greatest impact from humain activities in the world due to a weak dilution effect. It is therefore necessary to carry out a microbial analysis to assess the ecological status of the Seine River and to use it as a reference to compare with the future state when, for instance, new disinfection technologies of wastewater are implemented.

To this end, the microbial communities of the Seine surface water were analyzed, taking into account the spatial effect, including the tributaries, and from upstream to downstream of the Paris conurbation and the temporal aspect, with a monitoring over 4 seasons. The results showed that the microbiome of the water is highly diverse and involved a variety of functions. The main phyla making up the surface water microbiome were *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, while other minor phyla were *Deinococcota*, *Patescibacteria*, *Gemmatimonadota*, *Cyanobacteria*, *Bdellovibrionota*, *Acidobacteriota*, *Campilobacterota*, *Myxococcota*, and *Desulfobacterota*. Overall, the microbial community did not change spatially (with the exception of some minor differences between upstream and downstream), but did vary seasonally. The main factors influencing this microbiome were temperature, nitrate and orthophosphate concentrations. The main predicted functions were related to cell metabolism, in particular carbohydrates, amino acids, lipids, energy, vitamins and cofactors, and cell mobility. The microbial compositions showed a strong balance between microbial groups and were involved in the degradation of recalcitrant compounds.

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1. Introduction

The Seine River is the most important river in France and the second longest, with a total length of 776 km, crossing the Parisian Basin before reaching Normandy. Its catchment area covers 78,650 km^2 (14 % of the surface of France) and it is mostly located in the Parisian Basin [1]. It cannot be denied that the Seine River is an important part of the life of the people of Paris in particular and the people of France in general. Moreover, the water of the Seine River is used as a source of drinking water, for irrigation, as industrial water, and even for swimming [2]. The Seine River is also important as a habitat for algae, rooted plants, benthic animals, fish, and other wildlife [3,4]. With water scarcity, population growth and global warming, rivers are increasingly used for human needs. Given their importance, it is essential to maintain their ecological status so that they can function properly. To achieve this, a good knowledge of their water quality is essential.

Due to the rapid urbanization and increased impervious surface, the Seine River is frequently contaminated by various anthropogenic pollutants originating from domestic sewage, industrial wastewater, and combined sewer overflows. As noted in [5], urban rivers like the Seine River face significant environmental stress primarily related to water quality, rather than natural stressors such as hydrological factors. Water quality assessment is therefore essential to gain an overall understanding of environmental and ecosystemic quality. Several assessment methods are employed to assess the ecological status of river ecosystems, with a focus on water quality indicators such as PO_4^{3-} levels [6] and aquatic organisms including phytoplankton, and macroinvertebrates ([7]; L. [8]). Among aquatic organisms, microorganisms stand out due to short life cycle and high metabolic rate, and their community structure and functional genes can rapidly reflect the resilience of river ecosystems to pollution inputs [5].

Microorganisms are considered the most diverse and abundant biological group in the world, and are parts of the composition of the river biocenosis. They play a very important role in maintaining the good functioning of river ecosystems [9]. As the microbial community in river ecosystems, the bacterial community plays an important role in various biogeochemical processes and maintains important services to river ecosystems, including the degradation of pollutants and organic matter [10]. In the food web, they are the primary producers, carrying out photosynthesis or other autotrophic processes, but also the primary decomposers, breaking down pollutants and other materials to recycle their constituents as nutrients. Therefore, the presence and characteristics of microorganisms in a particular environment can directly influence the presence and abundance of other organisms. Bacterial populations tend to
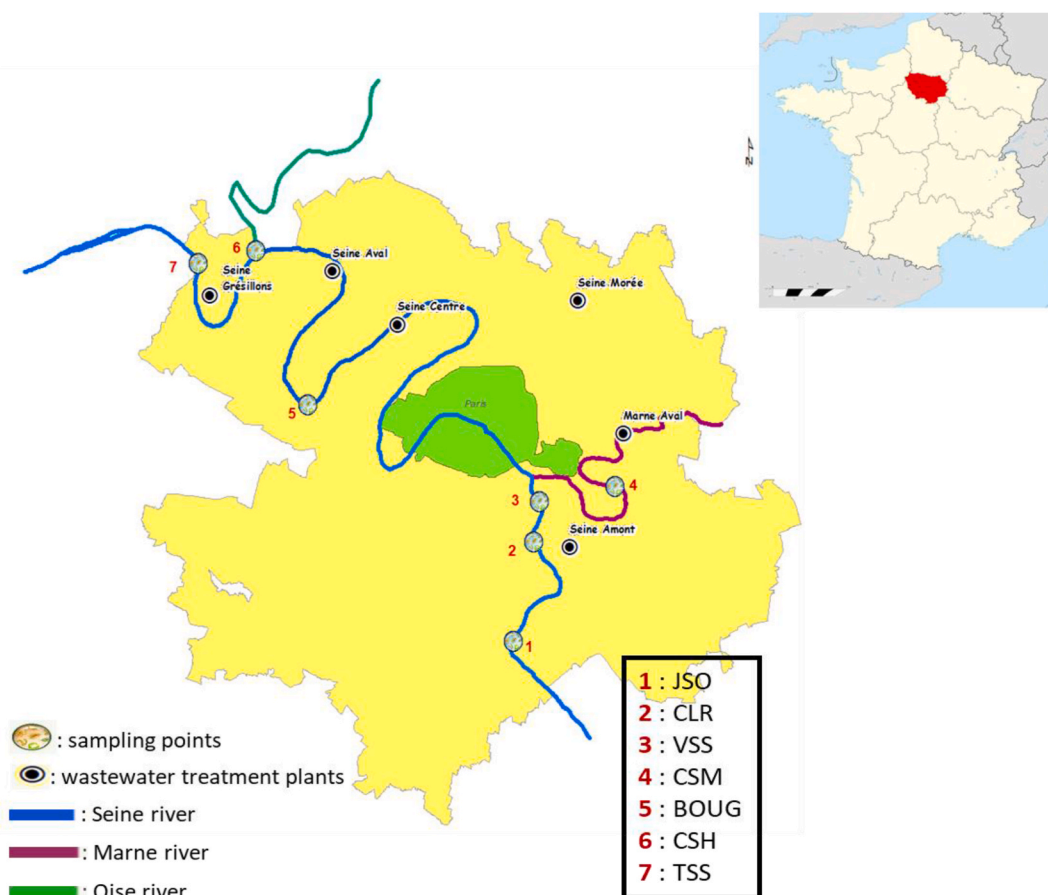
Fig. 1. Location of sampling sites (Juvisy-sur-Orge (JSO), Choisy-le-Roi (CLR), Vitry-sur-Seine (VSS), Bougival (Boug), Triel-sur-Seine (TSS), Champigny-sur-Marne (CSM), and Conflans-Sainte-Honorine (CSH)).

respond rapidly to spatiotemporal environmental variation [11–13]. They are highly sensitive to changes in environmental parameters such as pH, temperature, nutrient levels, and especially the presence of chemical contaminants [14–16]. It is also known that the diversity of the bacterial community can reflect its ecological status and the functionning of river ecosystems. Therefore, microbial communities are a good promising candidate for environmental indicators [17,18].

Regular monitoring of bacterial abundance, composition and diversity in the river is an important step towards a better understanding of the influences of anthropogenic activities to help for the conservation and sustainable development of the Seine River ecosystem. To date, the information on the variations of the bacterial community in the surface water of the Seine River at the scale of Parisian conurbation in response to treat wastewater discharges from wastewater treatment plants (WWTP) remains less known. In the activity report (phase 6) of the Piren-Seine programme in 2011, the bacterial diversity in the surface water column exhibited a great diversity with a dominance for the following phyla: *Actinobacteria*, *Proteobacteia*, *Betaproteobacteria*, and *Bacteroidetes* at three sites Marnay-sur-Seine, Bougival and Triel-sur-Seine which are located from upstream to downstream of the Seine River, respectively [19]. This is the only reference study on the microbial diversity of the Seine River. Nevertheless, the Seine River is one of the rivers in France under the strongest anthropic pressure, especially at the scale of the Paris conurbation, due to the discharge of wastewater from various sources such as domestic, industrial, agricultural ones during dry weather and of combined sewer overflows during wet weather [1]. Along the Seine, from downstream to upstream, in the Ile-de-France region, there are seven WWTPs managed by Siaap (the Greater Paris sanitation authority). The daily volume of wastewater treated by these WWTPs is about 2.5 million m^3/d during dry weather and up to 5 million m^3/d (mixture of wastewater and stormwater) during wet weather [2]. The Seine River also receives water from its main tributaries including the Orge, the Marne, and the Oise Rivers, which can also be sources of pollution. These contributions are likely to significantly influence the microbial community both in terms of metabolism function and diversity [20,21].

The Bathing plan, launched in 2018 in anticipation of the Olympic and Paralympic Games, calls for the use of numerous cutting-edge technologies to treat urban wastewater in WWTP before it is discharged into the Seine River, and to clean up the Seine River in the Paris capital. It is therefore necessary to take stock of the ecological status of the Seine River prior to these applications. In this work, we will study the bacterial community (diversity, abundance) in order to be able to compare it before and after any changes that may occur in the future such as droughts, the implementation of new WWTP effluent disinfection techniques, like the use of performic acid use. To this end, we characterized the microbial abundance using the cytometry (BactoSense, bNovate, Swiss) and the microbial diversity using NGS technology along the Seine River, from upstream to downstream, in five locations and at two sampling points located just before the confluence with two tributaries: the Oise and Marne Rivers, as shown in Fig. 1S. These sampling points were selected in order to understand (i) the influence of treated wastewater discharges from upstream to downstream and (ii) the effect of two tributaries (one upstream and one downstream) on the microbial communities in the surface water of the Seine River, and (iii) also to apprehend spatial and temporal variability.

2. Materials and methods

2.1. Study sites and sampling campagns

This study is carried out on the Seine River, and on the Marne and Oise Rivers, two of its main tributaries. The Seine, Marne and Oise Rivers are 777, 525 and 341 km long respectively. The Seine River has an oceanic rainfall regime, flowing from the southeast to the northwest. The study area is limited to the Paris conurbation on a stretch of the Seine River about 110 km long. It has a temperate climate, half continental and half oceanic. The average annual temperature is between 11 and 13 °C and the average annual rainfall is about 820 mm/year. The Seine River flows through Paris conurbation, which is highly urbanised with a high population density. Five sampling sites were selected on the Seine River: (i) three upstream of Paris, namely Juvisy-sur-Orge (JSO), Choisy-le-Roi (CLR) and Vitry-sur-Seine (VSS) and (ii) two downstream, namely Bougival (BOU) and Triel-sur-Seine (TSS) (Fig. 1). One sampling point, Champigny-sur-Marne (CSM), was selected on the Marne River and another point, Conflans-Sainte-Honorine (CSH), on the Oise River, before the confluence with the Seine River. Four sampling campaigns were carried out during four consecutive seasons: spring (April 2021), summer (July 2021), autumn (November 2021) and winter (January 2022).

Water samples were collected in triplicates at a depth of approximately 50 cm, mixed and homogenized to form a single sample. The collected water was then transferred to sterile bottles pre-rinsed with the sampled water. The samples were stored at 4 °C before being transported to the laboratory for the various analyses.

2.2. Physicochemical and hydrometeorological parameters

Parameters including temperature, pH and electrical conductivity (EC) were measured on site after sampling collection using a HACH multi-parameter probe (sensION+, Hach Company, Germany). The physico-chemical parameters were measured by the internal Siaap laboratory, which is under European accreditation, according to usual international AFNOR standards [22]. The 5-day biochemical oxygen demand (BOD5), which measures the amount of dissolved oxygen consumed by microorganisms and the chemical oxygen demand (COD), which measures the amount of oxygen required to chemically oxidize organic and inorganic compounds, were measured according to the NF EN 1899-2 and NF T 90–101, standards, respectively. Dissolved organic carbon (DOC), representing the fraction of dissolved organic carbon compounds serving as substrates for microbial metabolisms, was measured according to NF EN 1484 standard. Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were quantified according to NF EN ISO 11732 standard through flow analysis and spectrometric dectection, NF EN ISO 10304-1 standard by ion chromatography, respectively. Orthophosphates (PO_4^{3-}) were quantified according to NF EN ISO 15681-2 by spectrometric analysis with ammonium

molybdate. Total suspended solids (TSS) were measured by gravimetric analysis analysis (NF EN 872 standard). All measured parameters are ordinary water quality parameters.

The hydrometeorological parameters taken into account include the flow of the Seine River from the records of the Vigicrues monitoring stations in France (https://www.vigicrues.gouv.fr/), the ambient air temperature and relative humidity from Infoclimat stations (https://www.infoclimat.fr/).

2.3. Bacterial abundance

The number of bacterial cells in water samples was determined using an automated flow cytometer (FCM), BactoSense (bNovate, Swiss), according to the provided recommendations. Homogenized water samples (2 ml) were transferred to a screw-capped plastic microtube (bNovate, Swiss) for use in the cytometer. An aliquot (260 μl) of the sample was automatically aspirated by the instrument. 90 μl was used for the analysis and 150 μl for instrument clean. A cleaning step was performed before and after each measurement. Each sample was measured in three replicates. Mineral bottled water (Vittel, France) was used as a standard to check the status of the BactoSense for every five measurements. The collected aliquot is labelled with SYBR Green I and propidium iodide (PI) and incubated at 37 °C for 10 min before counting. The automation of the process ensures reproducible measurements for all samples.

The raw FCM data files were then analyzed using custom software that allowed batch processing of the large data sets generated in this study. In brief, FCM gates were constructed to separate signals from labelled cells (total cell concentration, TCC) from signals from the background and to distinguish between intact cells and cells with altered membranes using two fluorochromes SYBR Green 1 and PI. SYBR Green 1 (FL1 channel at 525 nm) is a marker that can penetrate all cells regardless of the integrity of the cell membrane; whereas PI (FL3 channel at 721 nm) can only penetrate cells with damaged membranes [23].

2.4. Bacterial diversity and composition

2.4.1. DNA Extraction, amplification, and Illumina sequencing

To collect the microorganisms, water samples (500 ml) were filtered through a vacuum pump system with 0.22 μm nitrate-cellulose Whatman filters (Sartorius, France) in a microbiological safety cabinet (BIOII, ADS Laminaire, France). The vacuum pump system and the filters were sterillized at 121 °C for 21 min. After filtration, the filters containing the microorganisms were placed in labelled sterile Petri dishes under the flow hood for 30 min to dry. DNA was extracted directly from these filters using the DNeasy PowerWater Kit (QIAGEN) according to the manufacturer's instructions. The amount of genomic DNA was assessed on a 1 % agarose gel and quantified with Quant-iTTM PicoGreen ds DNA assay kit according to the manufacturer's instructions and rapidly stored at −20 °C for future analysis.

Bacterial 16S rRNA gene libraries were constructed using the "16S metagenomic sequencing library preparation" protocol provided by Illumina for the Illumina MiSeq system. The V3–V4 variable regions of the 16S rRNA gene of the bacterial communities were amplified using the forward primer (5′-TAC GGG AGG CAG CAG-3′) [24] and the reverse primer (5′-CCA GGG TAT CTA ATC C-3′). Each primer set contains the Illumina adapter sequence: forward primer target sequence (5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′) and reverse primer target sequence (5′-GACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′). PCR conditions were as follows: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 45 s, and finally 72 °C for 10 min. DNA amplification was verified by agarose gel electrophoresis (1.5 %). DNA purity and concentration were assessed using Nanodrop ND-1000 (Thermo-Fisher Scientific). Amplified DNA was purified using the QIAquick96 PCR Purification Kit (Qiagen, CA) according to the manufacturer's instructions. Purified amplicons were normalized to 120 ng/μl in a new low-retention microtube prior to sequencing. The amplified DNA was sequenced on a 2x250 paired-end Illumina MiSeq platform by Eurofins Genomics (Germany) on an Illumina MiSeq plateform according to standard protocols.

2.4.2. Data processing and analysis

The raw sequences (.fastq) of the samples sequenced by Eurofins (Germany) were imported into the FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a galaxy instance (v.2.3.0) (h ttp://sigenae-workbench.toulouse.inra.fr/galaxy/). Pair-end reads were merged using the software FLASH (v1.2.6) with an average merging rate of 98 % [25]. Barcode and primer sequences were removed using cutadapt [26]. Sequences were then clustered into operational taxonomic units (OTUs) using SWARM [27], which generates the OTU abundance table, where an OTU is defined at the 97 % sequence similarity level. Chimeras were removed using the VSEARCH tool [28]. The taxonomic assignation of each OTU was performed using the BLAST tool against with the database SILVA-132 16S rRNA [29]. The Shannon index and the Chao1 index were calculated to estimate alphadiversity. The Shannon index was used to estimate species richness and evenness, while the Chao1 index provides an estimate of species richness based on abundance.

From the data obtained by 16S RNA sequencing, it is possible to predict the functions of the different identified bacteria. Gene prediction is performed using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to estimate the gene families contributing to the bacterial metagenome. PICRUSt will correlate phylogeny with function. The abundance of the different genes identified will be analyzed using KEGG (Kyoto Encyclopedia of Genes and Genomes). A metabolic pathway is a set of chemical reactions that lead either to the synthesis of a molecule (known as anabolism) or to its degradation (catabolism). The enzymes involved in each metabolic pathway are grouped together in the KEGG pathway tool. These pathways are organized into six major groups (known as level 1): metabolism (of sugars, lipids, carbohydrates, etc.), processing of genetic information (transcription, translation, DNA repair).

2.5. Statistical analysis

Before carrying out the various analyses to test for significant differences between treatments, the normality of the data matrices was tested using the *Shapiro-Wilk* test implemented in the R-3.6.1 software [30].

In order to evaluate the surface water quality of the Seine River and its tributaries, the Marne and the Oise River, over time, we compared our physico-chemical dataset with the data collected over the last 10 years (from 2012 to 2022) by a permanent measuring system installed onsite by the Siaap as part of the MeSeine measuring network.

Differences between the physical and chemical parameters, the relative abundance of microbial cells, and the alphadiversity index were analyzed by one-way ANOVA (Fisher's post hoc test) with a 95 % confidence interval. Two-way ANOVA was also performed to investigate the effect of the two independent variables sampling sites and seasons on the physic-chemical factors for 95 % confidence interval, with Tukey's post hoc test.

Further analyses, such as Principal Component Analysis (PCA), Redundancy Analysis (RDA), and the correlation tests, were conducted using R software [30]. To visualize the spatio-temporal effect on physico-chemical characteristics, a PCA was performed by with the *ade4* package [31]. A permutation test was used to determine whether the differences observed between the modality were large enough to reject the null hypothesis. Values were considered different if the probability of a null hypothesis was less than 0.05. Redundancy analysis (RDA) were performed using the "*vegan*" package in R [32]. For the correlation test, depending on the results of the normality test, we selected either the Pearson or Spearman test. If both variables exhibited a normal distribution, Pearson's test was chosen; otherwise, Spearman's test was used.

3. Results

3.1. Physical–chemical properties of surface water

The values of the physico-chemical parameters are shown in Table 1S. The mean surface water temperature was 5.2 °C in winter and 21.1 °C in summer, and two-way ANOVA analysis showed significant differences in water temperature between seasons, but not between sites (Table 1). pH values varied slightly between 7.7 and 8.3, with the highest value measured at JSO in autumn, and no significant spatio-temporal differences were found. Conductivity values ranged from 472 to 696 µS/cm with high values at CSH, located on the Oise River, a tributary of the Seine, and showed a significant difference between sites. Nitrogen concentrations in the water varied between 0.03 and 0.33 mg/l for NH_4^+, 0.03 and 0.54 mg/l for NO_2^- and 16.1 and 29.3 mg/l for NO_3^-. The highest nitrogen concentrations were measured at TSS downstream of the Paris conurbation and there were no significant spatial nor temporal variability. However, the concentration of NO_3^- was significantly higher in the Seine River than in its tributaries. The concentration of PO_4^{3-} varied between 0.01 and 0.4 mg/l, with low values in spring and high values in summer. COD, which ranged from 2 to 15.1 mg/l, was only significantly different between seasons, with the lowest values measured in autumn, while BOD, which ranged from 0.9 to 5.1 mg/l, showed no spatial nor temporal variability. The TSS content ranged from 2 to 23 mg/l and varied significantly between seasons, with a significant increase in winter.

To assess water quanlity, we compared our physico-chemical dataset with data collected over the last 10 years (from 2012 to 2022) by a permanent measurement system installed on site (Table S3). The comparison is made site-by-site and season-by-season. The results for the Bougival site, used as a model, are shown in Fig. 2. For all sites, the results obtained for these 4 seasons were representative and consistent with the results obtained over the long-term monitoring period by a permanent measuring system installed on site making our dataset representative.

A principal component analysis (PCA) of these physico-chemical parameters was conducted for all sites across four seasons. Axes 1 and 2 of the PCA explained 35.89 % and 19.81 % of the variance, respectively (Fig. 3). Analysis of data from rivers, including the Seine River and its tributaries, and sampling sites revealed no significant differences between rivers and sampling sites, as confirmed by discriminant analysis performed on 10,000 permuations (p > 0.05) (Fig. 3A&B&C). However, seasonal variations were evident. Summer samples, positioned above axis 2 in Fig. 3A, were separated from other seasons, likely due to higher values of temperature and dissolved organic carbon (DOC).

When comparing the upstream samples (average of JSO, VSS, and CLR sites) with the downstream samples (average of BOUG and TSS sites), significant differences were found for NH_4^+, NO_2^-, DOC, PO_4^{3-}, and EC, with higher values in the downstream sites than in the upstream ones. Furthermore, to understand the seasonal effect, the analysis of variance was performed on (i) our data and (ii) the data obtained during the long-term monitoring. The results showed that in both cases PO_4^{3-} and NO_3^- concentrations varied significantly

Table 1

Effects of rivers and seasons on physical and chemical properties of surface water were tested by a two-way ANOVA analysis. F values and significance level: non significatif ^{ns}P > 0.05, *0.01 < P ≤ 0.05, **0.001 < P ≤ 0.01, ***P ≤ 0.001 (N = 7 for Rivers, N = 4 for seasons).

	pH	NO_2^-	NO_3^-	NH_4^+	DOC	PO_4^{3+}	BDO	COD	T°C	EC	TSS
Rivers	0.9^{ns}	0.65^{ns}	6E-10***	0.76^{ns}	0.0006***	0.9^{ns}	0.6^{ns}	0.6^{ns}	0.9^{ns}	0.02*	0.5^{ns}
Seasons	0.9^{ns}	0.5^{ns}	6E-5***	0.6^{ns}	0.06^{ns}	0.003**	0.4^{ns}	4E-5***	3E-25***	0.4^{ns}	0.006**
Rivers*Seasons	0.9^{ns}	0.9^{ns}	8E-7***	0.9^{ns}	0.03*	0.2^{ns}	0.9^{ns}	0.009**	3E-17***	0.2^{ns}	0.04*

DOC, dissolved organic carbon; BOD, biochemical oxygen demand; COD, chemical oxygen demand; EC, electrical conductivity; TSS, total suspended solids.
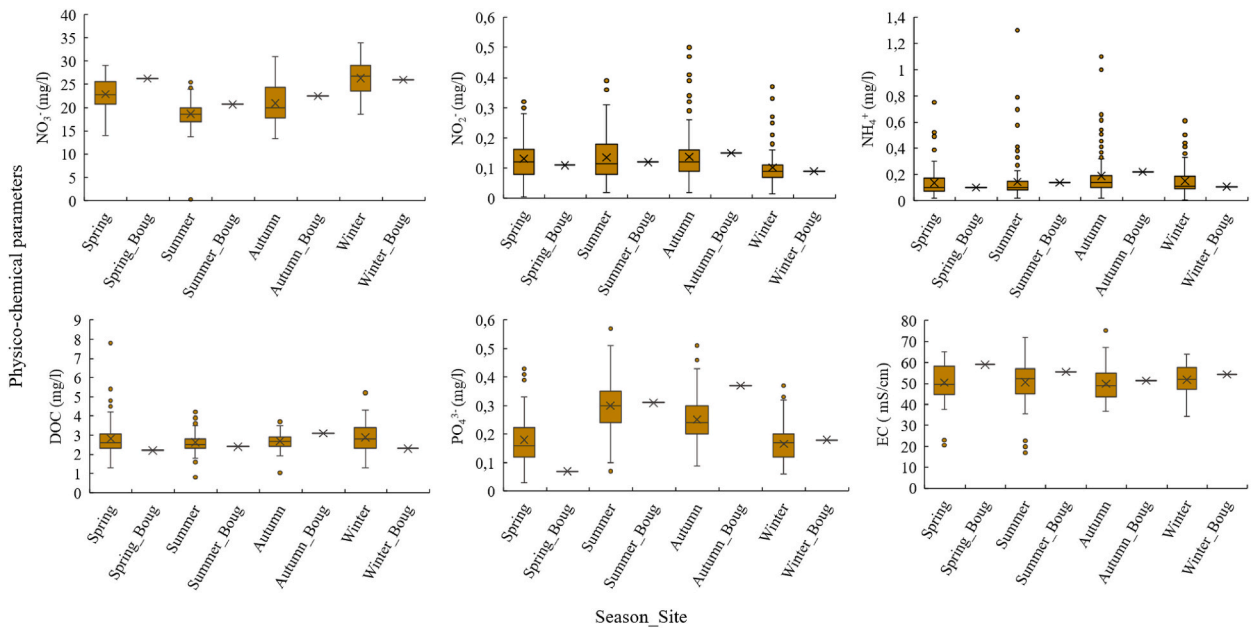
Fig. 2. Comparison of the physico-chemical properties of Seine water in Bougival site to the average values obtained from 2012 to 2022 by a permanent measuring system installed on-site. NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; PO_4^{3-}, orthophosphates; DOC, dissolved organic carbon; EC, electrical conductivity.
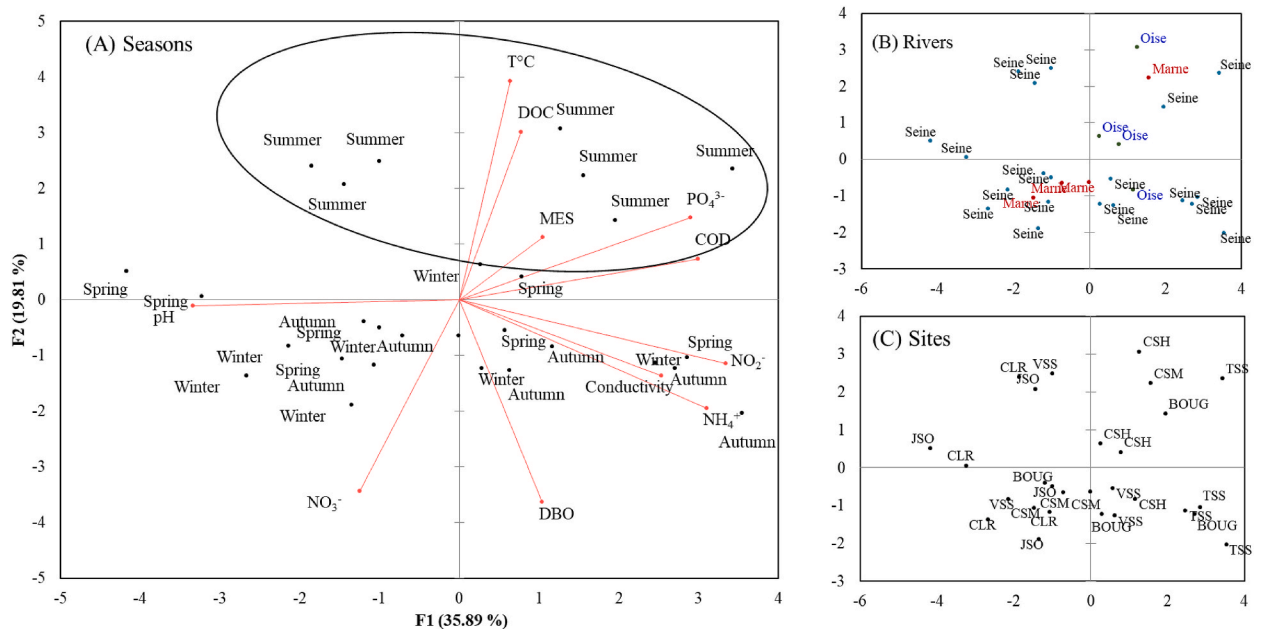
Fig. 3. PCA analysis of physico-chemical parameters of the surface water of the Seine river and its tributaries (Marne and Oise Rivers) for 4 seasons (spring, summer, autumn, and winter) (A) differences between the seasons, (B) differences between the rivers and (C) difference between the sites, Juvisy-sur-Orge (JSO), Choisy-le-Roi (CLR), Vitry-sur-Seine (VSS), Bougival (Boug), Triel-sur-Seine (TSS), Champigny-sur-Marne (CSM), and Conflans-Sainte-Honorine (CSH).

between the seasons.

3.2. Microbial abondance

Total microbial cells (TTC) and damaged microbial cells (DMC) were quantified in the water samples and shown in Fig. 4A for TTC
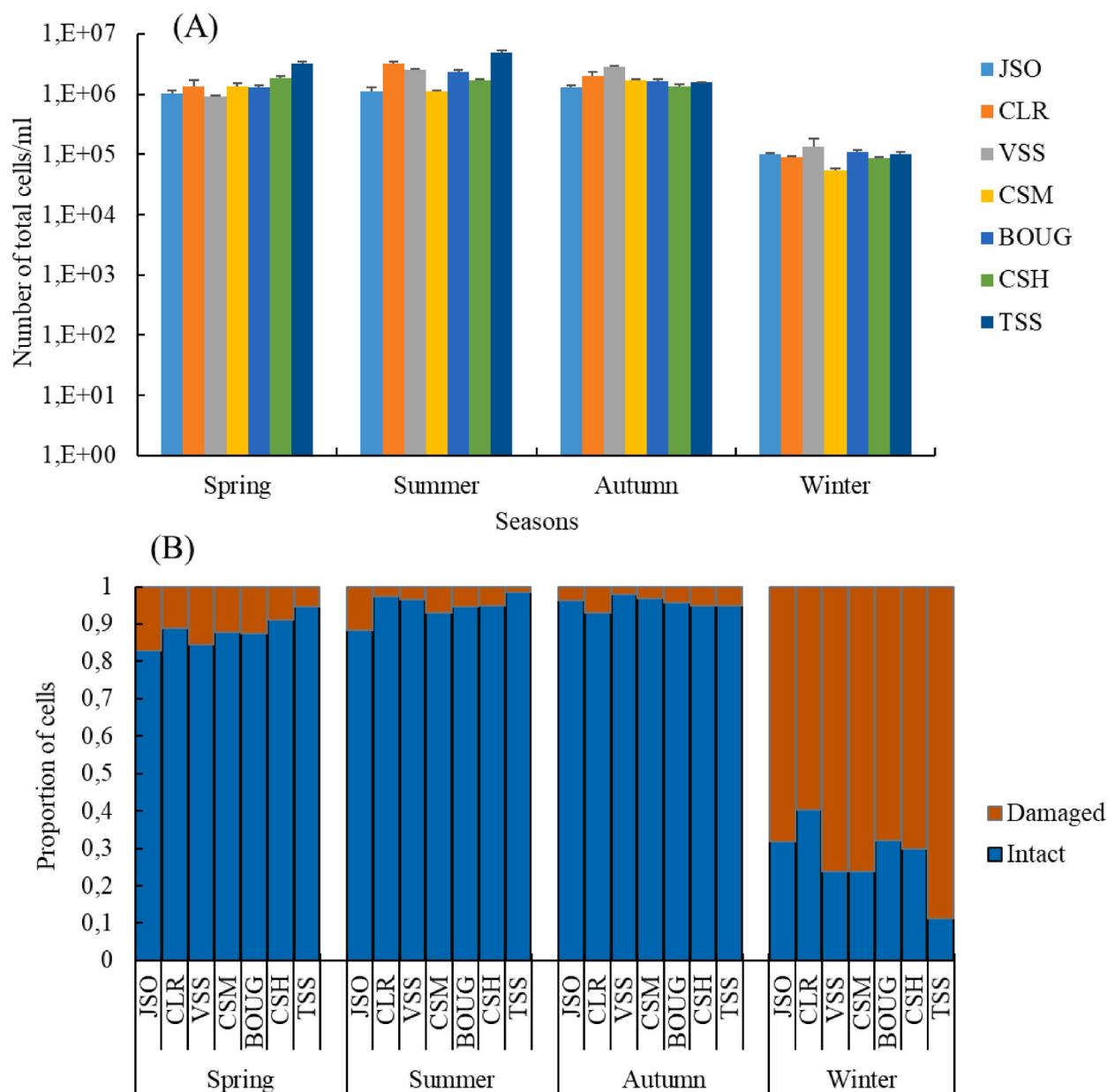
Fig. 4. Total bacterial cells (A) and proportion of intact/damaged bacterial cells (B) of water samples for 4 seasons at 7 sampling sites. N = 12 for each site and each season. JSO, Juvisy-sur-Orge; CLR, Choisy-le-Roi; VSS, Vitry-sur-Seine; CSM, Champigny-sur-Marne; BOUG, Bougival; CSH, Conflans-Sainte-Honorine; TSS, Triel-sur-Seine.

and Fig. 4B for DMC. Total microbial cell counts ranged from 9.0×10^4 to 4.9×10^6 cells/ml. Total microbial cells in winter samples were significantly lower than those in other seasons (Fig. 4A). The ANOVA analysis to evaluate the spatio-temporal variability showed that the variability between the Seine River and its tributaries was not significant. However, significant differences were observed between seasons. The number of damaged cells represented on average 15 % of the total microbial cells in spring, summer and autumn, but this value was very high (>70 %) in winter (Fig. 4B).

3.3. Diversity and richness analysis of bacterial communities

To study the bacterial communities of 28 samples (7 sites x 4 seasons) from the Seine River and its tributaries, Illumina MiSeq sequencing was used to sequence the bacterial 16S rRNA gene V3–V4 regions. After removing low quality sequences and chimeras, an average of 6×10^6 high quality 16S rRNA sequences were obtained. The OTUs were determined to calculate the richness (Chao1), the

Shannon's diversity of the microbial communities.

The mean values of Chao1 and Shannon (H) indices ranged from 570 to 3549 and from 3.7 to 5.8, respectively (Fig. 5). When comparing spatial diversity between rivers and between sites, the Chao1 index was similar for all rivers and did not vary between upstream and downstream of the Seine River in the study area (Fig. 5A). Nevertheless, the analysis of variance showed a significant variability of the richness according to the seasons, with higher richness in winter and lower richeness in spring (Fig. 5B). The Shannon index, like the Chao1 index, showed only seasonal significant variability, ranging from H_{spring} (4.15) < H_{summer} (5) < H_{autumn} (5.4) < H_{winter} (5.6).

3.4. Composition of bacterial communities

A total of 35 phyla were identified in surface water samples collected at 7 sites along the Seine River and its tributaries. Based on relative abundances, the dominant groups (greater than 1 % abundance) of each sample are shown in Fig. 6. As shown, among them, *Proteobacteria* (62.2 %), *Actinobacteriota* (16.5 %), *Bacteroidota* (17 %) were the most frequently detected phyla in all samples and together accounted for 90–95 % of the relative abundance. The phyla *Firmicutes* (2.2 %), *Cyanobacteria* (0.2 %), *Bdellovibrionota* (0.4 %), and *Campilobacterota* (0.5 %) constituted between 5 and 10 % of the abundance.

In terms of spatial variability, there were no significant differences in the relative abundance of these phyla between rivers nor between upstream (JSO, CLR and VSS) and downstream (BOUG and TSS) sites of the Seine River. In particular, the relative abundance of *Firmicutes* was significantly higher in downstream samples than in upstream samples.

In terms of temporal variability, the main phyla were similar at all sites. In spring, the most dominant phyla were *Proteobacteria* (55.7 %), *Actinobacteriota* (42.4 %) and *Firmicutes* (1.2 %), while in summer *Proteobacteria* (75.9 %), *Actinobacteriota* (8 %) and *Bacteroidota* (13.4 %) were dominant. As in summer, the most abundant bacterial phyla in autumn are *Proteobacteria* (76.0 %), *Actinobacteriota* (7.1 %) and *Bacteroidota* (15.1 %). In winter, *Proteobacteria* (41.9 %), *Actinobacteriota* (11.5 %) and *Bacteroidota* (37 %) were the most abundant phyla. The bacterial composition in winter was characterized by an increase in the abundance of *Bacteroidota* from 15.1 to 37.0 % and the presence of *Campylobacterota* (1.6 %). An analysis of variance showed a significant variation between the different seasons, but a similarity in abundance between autumn and summer. A decrease in the abundance of *Proteobacteria* was observed in winter along with an increase in *Bacteroidota*. On the other hand, a higher abundance of *Firmicutes* and *Campylobacterota* was observed. Spring is characterised by an abundance of *Actinobacteriota*. The variations in the abundance of the bacterial phyla were more pronounced from one season to another than from one site to another.

To better explain the structure of the microbial communities in different sites and seasons, the relative abundance and classification of OTUs were analyzed at the class and genus level. At the class level, the dominant classes identified were *Alphaproteobacteria* and *Gammaproteobacteria* belonging to *Proteobacteria*, *Actinobacteria* belonging to *Actinobacteriota*, *Bacteroidia* of the *Bacteroidota*, the *Bacili* and *Clostridia* belonging to the *Firmicutes* and the *Campylobacteria* affiliated with *Campylobacterota* (Fig. 7A). No significant spatial variability was observed between upstream and downstream of Paris, nor between the Seine River and its tributaries. However, the abundance of these bacterial classes varied according to the season. In spring, the dominant classes were *Alphaproteobacteria* (55.0 %) and *Actinobacteria* (41.4 %) with a very low abundance of *Gammaproteobacteria* (0.2 %). The most dominant classes in summer and autumn were similar and consisted of *Gammaproteobacteria* while in winter it was the *Bacteroidia* (37 %). Higher abundances of *Bacili*, *Clostridia* and *Campylobacteria* were observed in winter.

At the level of the bacterial genera, *Brevundimonas*, *Sphingomonas*, *Flavobacterium*, *Methylotenera limnohabitans* were the most dominant (Fig. 7B). The abundance of these dominant phyla did not show any spatial variability between the Seine River and its tributaries, nor between upstream and downstream samples. However, some genera such as *Zoogloea*, *Methylotenera* and *Comamonas* were more abundant at sites downstream than upstream of the Paris conurbation. Significant seasonal variability was observed. The dominant genera identified were *Brevundimonas*, *Sphingomonas*, *Nocardioides*, *Novosphingobium* in spring, and *Brevundimonas*, *Sphingorhabdus*, *Flavobacterium*, and *Methylotenera* in summer. *Novosphingobium*, *Flavobacterium*, *Methylotenera*, *Pseudomonas*, *Limnohabitans*,
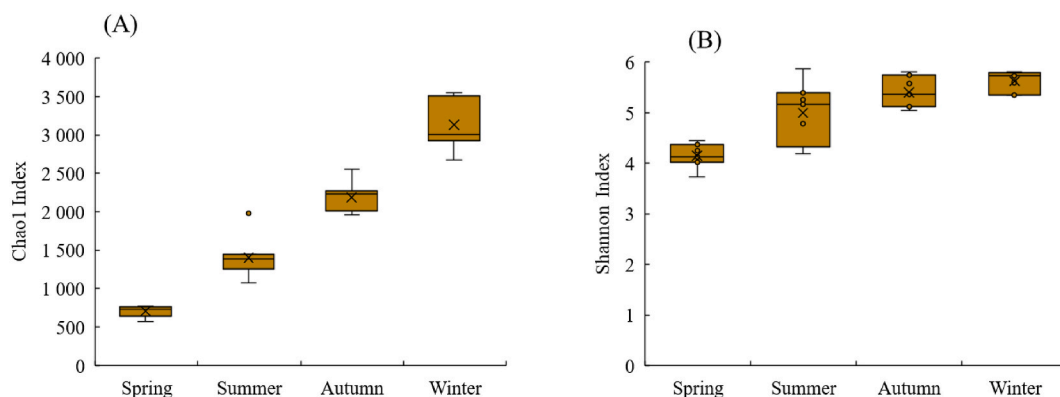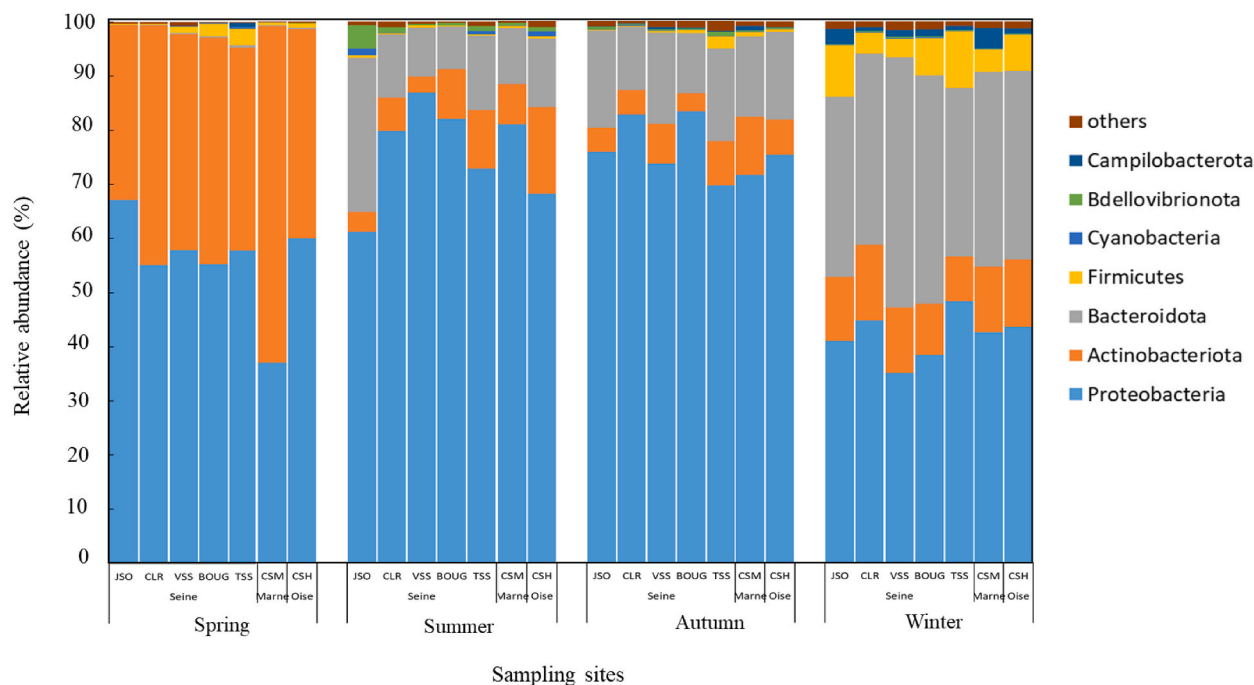

Fig. 5. Diversity index of Chao1 (A) and Shannon (B) of bacterial communities in water samples of the Seine River and its tributaries (Marne and Oise Rivers) for 4 seasons.

Fig. 6. Relative abundance (%) of the dominant phyla (>1 %) of the Seine water in 4 seasons of 2021 at 7 sampling sites JSO, Juvisy-sur-Orge; CLR, Choisy-le-Roi; VSS, Vitry-sur-Seine; CSM, Champigny-sur-Marne; BOUG, Bougival; CSH, Conflans-Sainte-Honorine; TSS, Triel-sur-Seine.

OM43 clade dominated in autumn; while *Flavobacterium*, *Rhodoferax*, *Limnohabitans*, *Pseudarcicella*, and *Fluviicola* dominated in winter.

### 3.5. Functional predictions of bacterial communities

PICRUSt analysis indicated that the major functional gene families were related to metabolism (~80 %), genetic information processing (11 %), cellular processes (4 %) and computational processing for all sites, regardless of season (Fig. 8). At level 2 of the KEGG pathways, metabolic functions included carbohydrates, amino acids, cofactors and vitamins, terpenoids and polyketides, lipids, energy, xenobiotic metabolism and degradation; membrane transport and cell motility, growth and death (Fig. 8). The relative abundance of genes related to these functions did not show significant variability between upstream and downstream sites on the Seine River, nor between rivers, but did differ significantly between seasons ($p < 0.001$) in the Seine River. Furthermore, 0.2 % of the bacterial genes were associated with human infectious diseases of the parasitic and bacterial type, with 0.11 % of parasitic infections in summer and 0.08 % in the other seasons.

### 3.6. Correlations between microbial community properties and environmental factors

RDA analysis was performed to determine the potential relationships between the bacterial community and physicochemical properties both at the phyla (Fig. 9A) and genus levels (Fig. 9B). At the phylum level, the results showed that air temperature, COD, and TSS were the factors that significantly influenced community composition. *Proteobacteria*, *Cyanobacteria* and *Bdellovibrionota* were characterised by temperature and COD, while *Firmicutes* and *Campilobacterota* were correlated with NO_3^- concentration. At the genus level, temperature, PO_4^{3-}, TSS, NO_3^-, and DOC were the main factors that significantly influenced the community composition.

Correlation test was further conducted to examine the correlations between the physicochemical properties, and microbial community characteristics, including microbial abundance, alpha diversity indices and relative abundance of dominant phyla and class had close relationships (Table 2). Microbial abundance was positively correlated with DOC and air temperature and negatively correlated with NO_3^- concentration. In the relationship between physicochemical properties and alpha diversity indices, air temperature showed a negative correlation with Chao1 and Shannon, while TSS concentration showed a negative correlation with air temperature. At the phyla level, the relative abundance of *Proteobacteria* was positively correlated with temperature, PO_4^{3-} concentration and DOC, but negatively correlated with NO_3^-. The relative abundance of predicted genes involved in amino acid, carbohydrate and terpenoids/polyketides metabolism was positively correlated with NO_3^- and negatively correlated with PO_4^{3-} and dissolved organic carbon. We also observed that the relative abundance of genes identified for infectious diseases of bacterial origin was positively correlated with BOD and negatively correlated with water temperature.

(A)
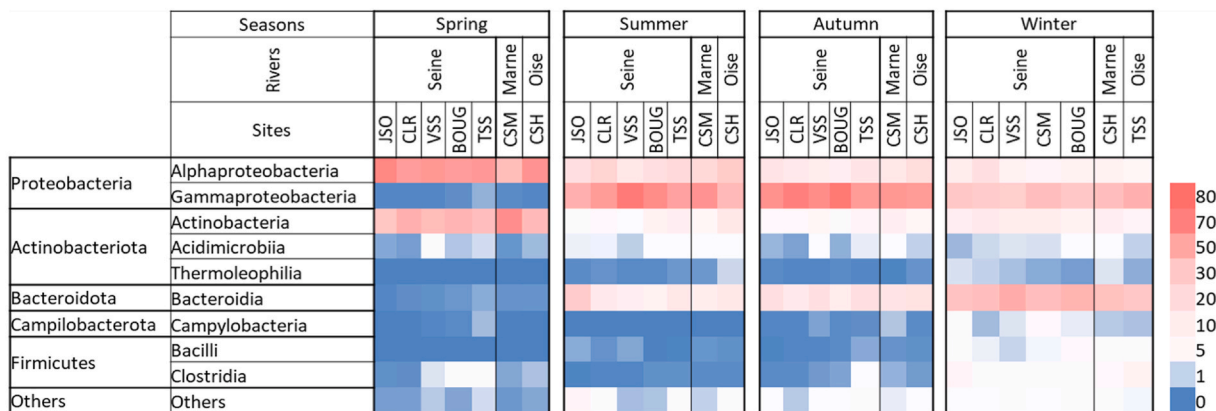
(B)
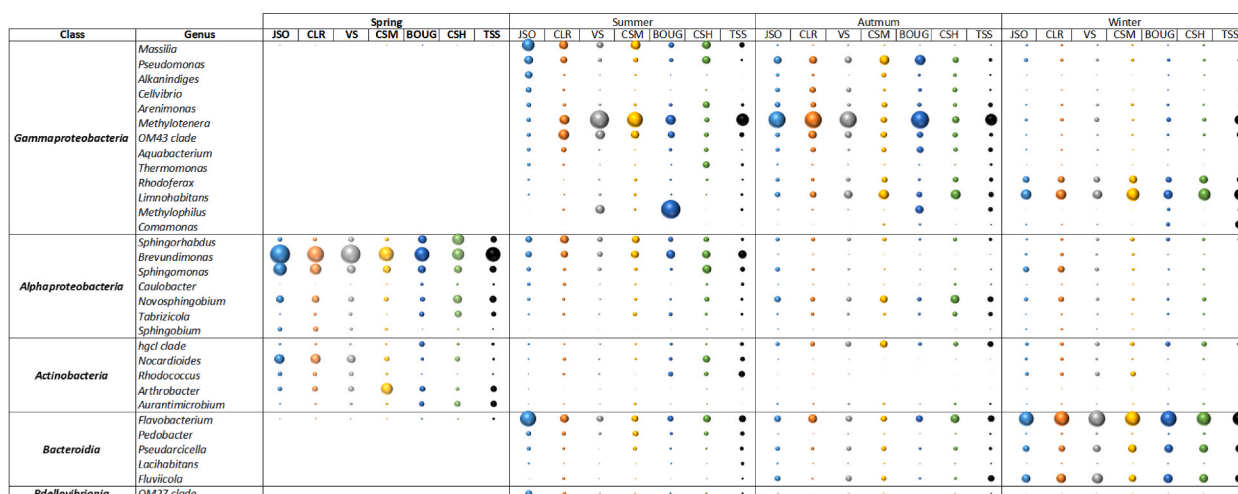
Fig. 7. Relative abundance (%) of the dominant classes (A) and genus (B) of the Seine water for 4 seasons of 2021 at 7 sampling sites. JSO, Juvisy-sur-Orge; CLR, Choisy-le-Roi; VSS, Vitry-sur-Seine; CSM, Champigny-sur-Marne; BOUG, Bougival; CSH, Conflans-Sainte-Honorine; TSS, Triel-sur-Seine..

3.7. Correlations between the relative abundance of microbial community at the phyla level and the relative abundance of functional genes

The correlation test was performed to examine the relationships between the relative abundance of microbial composition at the phyla level and the relative abundance of functional genes predicted in the KEGG pathway analysis (Table 3). A positive correlation was observed between the relative abundance of genes identified for infectious diseases of bacterial origin and the abundance of *Campilobacterota*, *Firmicutes*, and *Bacteroidota*. Furthermore, the abundance of *Actinobacteria* was positively correlated with amino acid, carbohydrate, polyketide and terpenoid metabolism, xenobiotic biodegradation, but negatively correlated with vitamin and cofactor metabolism. An inverse relationship was observed between these metabolites and the abundance of *Proteobacteria*. Bacteridota was positively correlated with glycan biosynthesis and metabolism.

4. Discussion

The Seine River in France, nestled in the Paris conurbation with its 10 million inhabitants (2020), is facing strong anthropogenic pressures [1]. Continuous monitoring of Seine physico-chemical profile remains imperative. Notably, findings suggest a decade-long stability, consistent with global riverine patterns. Seine water pH (~8.0) aligns with counterparts observed in the Qing and Yongding Rivers, China [33,34]. Nitrate-nitrogen levels (22 mg/l) are comparable to those recorded in the Yellow River Delta [35]. N–NH$_4^+$ and P-PO$_4^{3-}$ concentration mirror those of the Maozhou River, China. Seine water's NH$_4^+$ variability, notably lower than that of the Maozhou River, suggests potentially superior effluent management pratices [36]. Upstream Seine River exhibits elevated of N–NH$_4^+$, N–NO$_2$, and
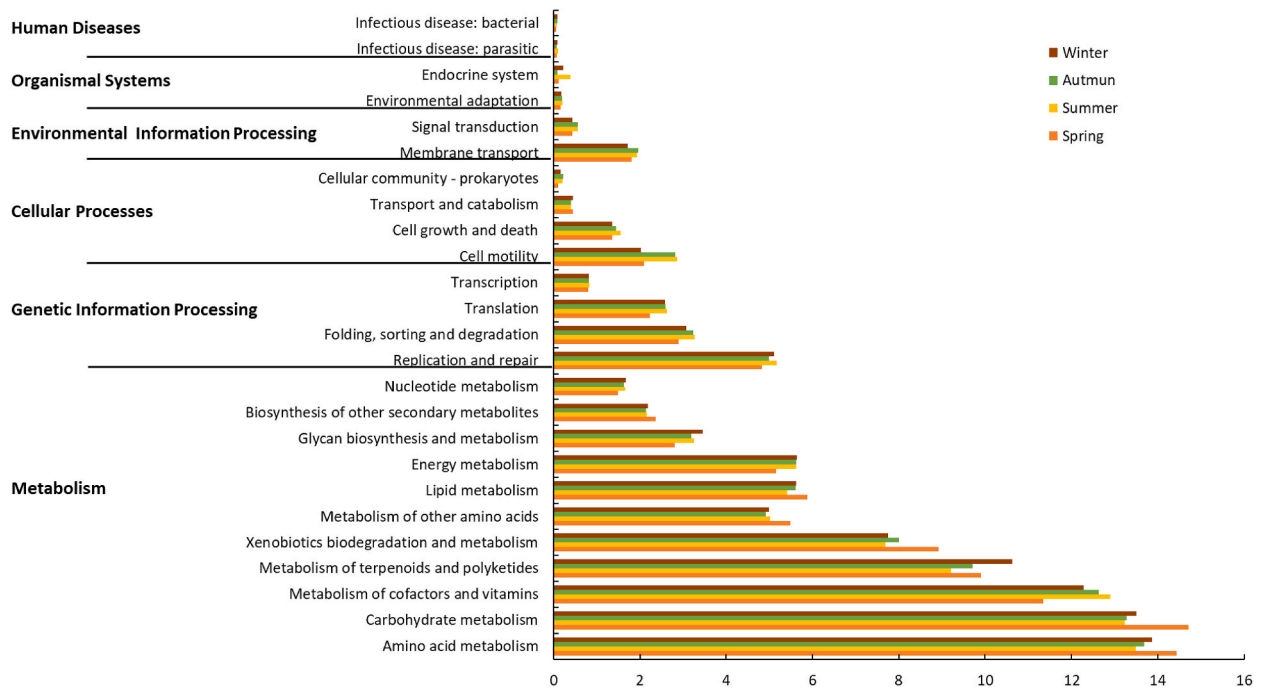
Fig. 8. Predicted function of operational taxonomic units based on KEGG modules level 2 of bacterial communities of the Seine water.
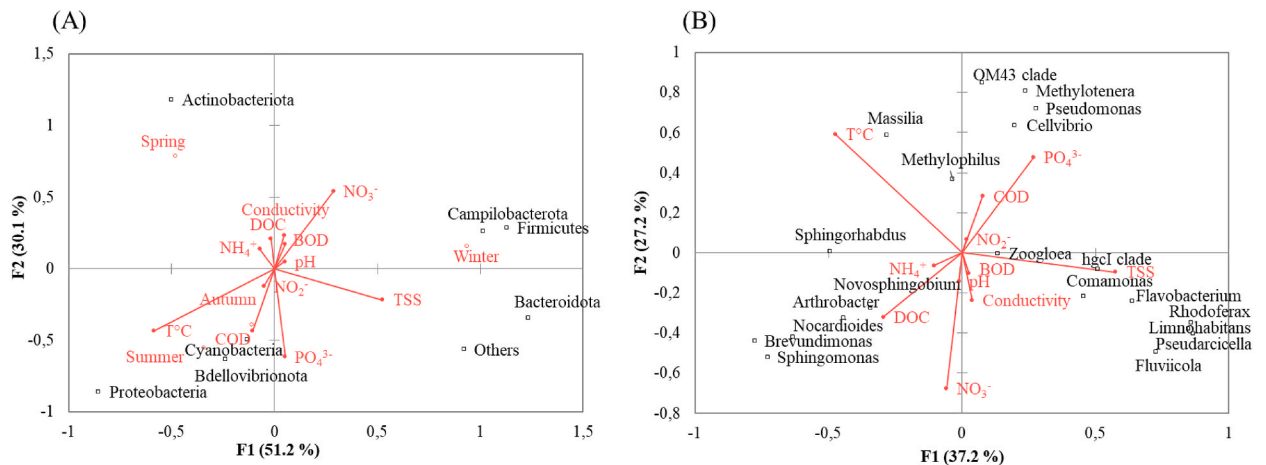

Fig. 9. Redundancy analysis (RDA) of the Seine water samples based on the bacterial communities at the phyla level (A) and the genus level (B) and environmental variables.

P-PO$_4^{3-}$ concentrations due to accumulation from upstream to downstream. This phenomenon was also observed in the case of the Maozhou River [36]. Regarding seasonal variability, fluctuations in water/air temperature and water flow significantly influence parameters like N–NO$_3^-$, N–NH$_4^+$ and TSS.

Water microbes play a vital role in maintaining the ecological balance of river ecosystems, with microbial diversity serving as an indicator of ecosystem health [37]. Changes in environmental parameters can strongly influence microbial abundance and composition/diversity. Here, we investigated surface water microbial communities at five sites along the Seine River at the scale of the Paris conurbation and two other sites on two tributary, namely the Marne and Oise Rivers, during four seasons in 2021. The total cell count in the Seine water was approximately 10^6 cells/ml, similar to previous findings from the PIREN-Seine program [19] and comparable to microbial abundance in the Wensum River in England and the Lancang River, China [38]. As far as microbial diversity is concerned, to our knowledge, this is one of the first studies to characterize the microbial community of the Seine River using the NGS technique. The dominant phyla were *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, *Cyanobacteria*, *Bdellovibrionota*, and *Campilobacterota*, mirroring patterns seen in other urban river ecosystems [12,36,39–42]. These results showed that the surface water of the Seine River

Table 2
Correlation matix between microbial community parameters including bacterial abundance (TCC = total cells, ICC = intact cells and DCC = damaged cells), diversity indices, relative abundance of phyla and genera with physicochemical parameters. DOC, dissolved organic carbon; BOD, biochemical oxygen demand; COD, chemical oxygen demand; EC, electrical conductivity; TSS, total suspended solids (N = 28).

	Variables	pH	NO_2^-	NO_3^-	NH_4^+	DOC	PO_4^{3+}	BOD	COD	T°C	EC	TSS
Microbial abundance (ml)	TCC	−0.33	0.33	*-0.44	0.14	*0.43	0.24	0.01	0.05	**0.57	0.08	−0.25
	ICC	−0.29	0.27	*-0.46	0.11	*0.42	0.22	0.01	0.02	**0.56	0.011	−0.24
	DCC	0.01	0.01	0.25	−0.20	−0.14	−0.35	−0.12	0.37	*0.45	0.038	*-0.42
Diversity indices	Chao1	0.18	−0.18	0.02	0.03	0.08	0.19	0.26	−0.31	***-0.73	−0.082	**0.54
	Shannon	0.12	−0.17	−0.09	0.05	0.20	0.30	0.14	−0.21	*-0.46	0.028	0.31
Relative abundance (%) of the dominant phyla	Proteobacteria	−0.16	0.19	**-0.58	0.10	*0.44	*0.47	−0.08	−0.18	**0.53	−0.16	−0.06
	Actinobacteriota	0.07	−0.03	**0.49	−0.08	*-0.40	**-0.52	−0.23	0.35	−0.05	0.32	−0.30
	Bacteroidota	0.03	−0.14	−0.02	0.14	0.09	0.23	0.24	−0.18	**-0.65	−0.06	**0.53
	Firmicutes	−0.19	0.14	0.33	0.34	−0.06	0.00	0.27	0.06	**-0.62	0.34	0.28
	Cyanobacteria	−0.10	0.11	−0.25	0.18	0.33	0.19	0.14	0.10	−0.22	0.13	0.35
	Bdellovibrionota	−0.16	0.05	**-0.61	0.02	*0.45	***0.66	−0.10	−0.02	0.31	−0.17	0.33
	Campilobacterota	0.01	0.09	0.37	0.36	−0.12	−0.07	*0.41	−0.34	***-0.84	0.25	0.25
Relative abundance (%) of the dominant genus	Brevundimonas	0.06	−0.17	0.18	−0.28	*-0.41	*-0.38	*-0.47	**0.54	**0.56	−0.14	−0.33
	Sphingomonas	0.24	*-0.38	0.33	*-0.39	*-0.39	**-0.53	*-0.46	0.41	0.34	−0.19	−0.34
	Nocardioides	0.09	−0.31	0.24	*-0.38	*-0.38	−0.35	**-0.50	**0.60	*0.43	−0.12	−0.23
	Novosphingobium	0.18	−0.03	0.27	−0.04	0.08	*-0.43	0.11	−0.26	−0.09	0.27	*-0.44
	Arthrobacter	0.11	−0.24	0.34	−0.33	*-0.37	**-0.55	−0.32	*0.48	0.23	−0.07	−0.25
	Sphingorhabdus	0.09	−0.07	−0.27	−0.29	−0.17	−0.23	*-0.42	0.36	***0.60	−0.01	−0.29
	hgcI clade	0.01	0.36	0.21	*0.45	0.17	0.02	*0.38	*-0.42	**-0.62	0.31	0.06
	Flavobacterium	0.10	−0.24	−0.13	−0.03	0.10	0.21	0.18	−0.08	**-0.49	−0.15	**0.59
	Massilia	0.05	−0.30	**-0.59	−0.22	0.08	*0.39	−0.34	0.13	0.18	*-0.40	*0.39
	Methylotenera	−0.19	0.16	**-0.49	0.16	*0.46	***0.65	0.08	−0.34	0.12	−0.22	0.24
	Pseudomonas	0.12	−0.08	**-0.59	0.00	0.32	*0.43	0.09	*-0.42	0.01	−0.22	0.12
	Rhodoferax	0.07	−0.11	−0.05	0.16	0.07	0.20	0.22	−0.35	***-0.75	0.041	*0.45
	Zoogloea	−0.01	0.11	0.18	0.27	0.28	0.15	**0.59	*-0.47	***-0.76	0.148	0.31
	Limnohabitans	0.05	−0.05	−0.09	0.21	0.08	0.22	0.27	−0.37	***-0.75	0.033	**0.51
	Pseudarcicella	0.07	−0.08	−0.09	0.15	0.05	0.21	0.23	−0.21	***-0.68	−0.069	**0.57
	Methylophilus	−0.32	0.32	*-0.39	0.29	0.34	***0.72	0.16	−0.21	0.09	−0.111	0.32
	OM43 clade	−0.12	0.11	***-0.69	0.08	*0.39	***0.63	0.01	−0.21	0.21	−0.199	0.25
	Fluviicola	0.03	−0.11	0.05	0.13	0.07	0.23	0.28	−0.29	***-0.71	−0.048	**0.49
	Comamonas	0.03	0.08	−0.04	0.26	0.17	0.34	*0.40	*-0.39	***-0.64	0.101	*0.43
	Cellvibrio	0.15	−0.12	*-0.41	−0.04	0.35	0.34	0.26	**-0.55	−0.19	−0.225	0.12

Table 3

Correlation matrix between the relative abundance of the microbial community at the phyla level and the relative abundance of functional genes (N = 28).

Variables	Proteobacteria	Actinobacteriota	Bacteroidota	Firmicutes	Cyanobacteria	Bdellovibrionota	Campilobacterota
Amino acid metabolism	***-0.64	***0.79	−0.27	0.18	−0.14	***-0.69	0.14
Carbohydrate metabolism	***-0.66	***0.87	−0.31	0.32	−0.27	***-0.81	0.23
Metabolism of cofactors and vitamins	***0.64	***-0.83	0.29	−0.28	0.13	***0.72	−0.16
Metabolism of terpenoids and polyketides	***-0.80	*0.43	*0.41	***0.73	0.26	**-0.49	***0.78
Xenobiotics biodegradation and metabolism	−0.21	**0.61	*-0.41	−0.063	−0.20	*-0.48	−0.12
Metabolism of other amino acids	*-0.43	***0.74	*-0.46	−0.05	−0.11	**-0.5	−0.22
Lipid metabolism	*-0.43	***0.67	−0.22	0.16	−0.15	**-0.57	0.21
Energy metabolism	0.32	***-0.75	**0.53	0.09	0.11	**0.53	0.26
Folding, sorting and degradation	***0.64	***-0.79	0.27	−0.25	0.06	***0.66	−0.05
Cell motility	***0.82	***-0.65	−0.11	***-0.63	0.02	***0.65	**-0.53
Translation	**0.51	***-0.77	*0.37	−0.11	0.07	**0.58	0.06
Glycan biosynthesis and metabolism	−0.227	*-0.42	***0.64	*0.44	0.34	0.25	*0.40
Biosynthesis of other secondary metabolites	−0.299	*0.47	−0.22	0.14	−0.01	−0.32	0.09
Membrane transport	***0.93	***-0.66	−0.30	**-0.52	−0.19	*0.46	*-0.41
Cell growth and death	***0.74	**-0.60	−0.17	**-0.62	−0.14	**0.56	*-0.56
Nucleotide metabolism	0.174	***-0.62	**0.56	0.19	0.20	*0.46	0.30
Transcription	*0.47	*-0.46	−0.03	*-0.47	−0.07	*0.39	−0.28
Signal transduction	***0.87	***-0.78	−0.05	**-0.52	−0.01	***0.64	−0.41
Transport and catabolism	***-0.83	**0.58	0.21	*0.42	0.17	*-0.37	0.29
Environmental adaptation	***0.76	***-0.87	0.17	−0.29	0.01	***0.75	−0.27
Endocrine system	0.186	0.017	−0.14	−0.22	−0.05	0.31	**-0.54
Cellular community - prokaryotes	***0.71	***-0.88	0.26	−0.28	0.155	***0.76	−0.16
Infectious disease: parasitic	0.01	−0.18	0.19	0.19	*0.38	0.340	−0.15
Infectious disease: bacterial	−0.082	*-0.37	**0.52	*0.44	0.10	0.169	**0.53

presented a typical profile of high bacterial rank, commonly found in other river ecosystems. This seems logical, since each phylum plays a different role in maintaining the proper functioning of the river ecosystem. Among them, *Proteobacteria*, constituting over 60 % of the community, are known for their role in organic matter degradation, and have a rapid growth in nutrient-rich environments [43–46], while *Actinobacteriota* contribute to the carbon cycle in aquatic ecosystems [47]. However, the relative abundance of these phyla may vary depending on environmental conditions in space and time.

4.1. Spatial variation

A notable similarity in microbial abundance, diversity indices (Chao1 and Shannon) and taxonomic characteristics was observed across various sampling sites along a 110 km stretch of the Seine River, and its tributaries. This suggested that WWTP effluents have minimal impact on the Seine's microbial community, probably due to effective control of physico-chemical parameters prior to discharge. However, downstream samples exhibited significantly higher relative abundance of *Firmicutes*, known for their capability to degrade recalcitrant organic compounds [48]. The physico-chemical data revealed higher content of NH_4^+, NO_2^-, PO_4^{3-}, and DOC in downstream samples, attributed to accumulation from upstream – a phenomenon observed in a variety of ecosystems [49–52]. Furthermore, the positive correlation between *Firmicutes* abundance at phyla and genus levels and nutrient concentrations such as NO_3^-, PO_4^{3-}, TSS, NO_3^-, and DOC (Fig. 9A and B) confirms their role as copiotrophs or fast-growing organisms. Regarding *Flaviobacterium*, a genus within the *Bacteroidota* phylum, it is a common heterotrophic organism found in surface waters, playing a crucial role in organic matter degradation [42]. At the Juvisy-sur-Orge site, in summer 2021, its relative abundance reached up to 20 %, significantly higher than at the other sites. This higher abundance is likely due to the untreated water or poorly treated effluent reaching the JSO site upstream of the Paris conurbation, resulting in elevated organic matter levels that favor *Flaviobacterium* growth. The positive correlation between *Flaviobacterium* abundance and organic matter concentration further supports this observation. Although comprehensive sampling strategies were implemented along a 110 km stretch of the Seine River, extending both before and after WWTP discharges as well as upstream and downstream of the Paris conurbation, using NGS technology with high sensitivity for detecting microbial composition, no significant differences were observed between the sampling sites. This suggests either a persistent ecosystem over time or the establishment of microbial communities long ago. To further elucidate this, comparative analysis with sites located both well downstream and upstream of the Paris conurbation is necessary. For instance, sites such as Vernon or Porcheville, located at distances of 140 km and 100 km from Paris respectively, can provide insight into downstream conditions. Similarly, upstream sites like Marnay-sur-Seine, situated approximately 120 km from Paris, can offer valuable comparative data. Furthermore,

while NGS technology serves as an effective method for identifying microbial diversity in terms of taxonomic resolution, the data provide a broad overview of microbial composition, but may overlook finer-scale functional community dynamics. Therefore, it is imperative to analyze the functions of microorganisms involved in various biogeochemical cycles, such as those related to nutrient cycles, to gain more comprehensive insights.

4.2. Temporal variation

The seasonal variation significantly influences river microbial communities, as observed in various studies where its effects often surpass those of location [53,54]. Temperature changes, particularly, exert a significant influence on the microbial composition as demonstrated for the Ganjiang River [55]. The same is true in our case. The microbial community in the Seine River appeared to be very similar between sampling sites, but varied from season to season.

Seasonal variability in diversity indices was observed, with the Shannon and Chao1 indices higher in winter than in to other seasons. This was in good agreement with finding in the Fuhe and Pearl urban rivers [12,42], where temperature emerged as a key factor influencing bacterial composition [44,56]. The significant rise in temperature over the last could have repercussions on microbial communities, as microorganisms tend to thrive at low temperatures.

The *Proteobacteria* group, dominant in all samples, exhibits a varying relative abundance with seasons. Particularly, we observed an increase in their abundance from spring to summer, followed by a stability phase in autumn, and a decrease in winter. This trend is in line with studies showing that *Proteobacteria*, known to be heterotrophic bacteria thriving in high temperatures and nitrate-rich environments, prefer for warmer seasons (Y.-S. [8,57]). The correlation test confirms a significant correlation between their abundance, temperature and the concentration of N–NO$_3^-$, and COD (Fig. 9). At the class level, *Proteobacteria* were predominantly divided into *Gammaproteobacteria* and *Alphaproteobacteria*, with no *Betaproteabacteria* detected. This contrasts with findings in the Donjiang River, where *Betaproteobacteria* were dominant [44,58]. Environmental parameters and selection pressures likely influence this selection. For example, *Alphaproteobacteria* and *Gammaproteobacteria* prefer salted water, which is not the case for *Betaproteobacteria* [44,59]. As noted in [60], the Seine estuary is characterized by marine dominance, likely due to its relatively short convergence length compared to its width at the mouth. Consequently, the water in the estuary likely exhibits salinity characteristics. *Betaproteobacteria* are fast growing, nutrient-hungry and highly vulnerable to predation pressure, while *Alphaproteobacteria* are known to be predation-resistant, competitive in low-nutrient conditions and able to use recalcitrant organic compounds such as humic substances [44]. Since the Seine water is low in nutrients, and probably degrades the recalcitrant organic compounds remaining in WWTP effluent, *Alphaproteobacteria* were able to resist and proliferate. The presence of specific genera like *Novosphingobium*, *Sphingobium* and *Sphingomonas*, members of *Alphaproteobacteria*, which are known to degrade a variety of slowly degradable complex organic compounds such as polycyclic aromatic hydrocarbons (PAHs), phthalate esters, aromatic hydrocarbons and dibenzofurans [61–63], supports this hypothesis.

Seasonal shifts in the abundance between *Alphaproteobacteria* and *Gammaproteobacteria* vary, with *Alphaproteobacteria* dominating in spring and *Gammaproteobacteria* in the other seasons. This changes in abundance differed depending on the season and river. For instance, while *Alphaproteobacteria* were more abundant than *Gammaproteobacteria* in both dry and wet seasons in the Dongjiang River [44], they were found in equal proportions in another study in the same river but not in the same study year [58]. The higher abundance of *Alphaproteobacteria* in spring could be due to their ability to form filaments and aggregates during winter [59], and then thriving from spring onwards. This increase was mainly attributed to *Brevundimonas*, whose abundance decreased in summer and again in autumn and winter, possibly due to the proliferation of cyanobacteria. *Brevundimonas* is known to interact strongly with cyanobacterial blooms that developed well during the summer [64]. In our sample, cyanobacteria were detected during the summer at relative low abundance. This may be due to the sampling method. *Cyanobacteria* tend to live on the surface, whereas the Seine water samples were taken at greater depths. While many *Gammaproteobacteria* members originate from anthropogenic or zoonotic sources and may be transient passengers [44,59], they were not detected in our samples, indicating a good water quality for the Seine River. This absence includes members of the order *Enterobacteriales* (family *Enterobacteriaceae*, class *Gammaproteobacteria*), often used as bioindicators for monitoring pollutants in surface waters [65].

The *Actinobacteriota*, primarily comprising the *Actinobacteria* group, exhibited a peak in abundance during spring, declining steadily from summer to winter. This group is known for its ability to mobilize nitrogen, notably through pathways involving the incorporation of nucleic and amino acids such as arginine, leucine, and thymidine [59]. Our study revealed positive correlations between *Actinobacteriota* abundance and genes associated with amine acid metabolism, carbohydrate metabolism, polyketides and terpenoids metabolism and membrane transport (Table 3). These findings are consistent with the results reported by [66]), highlighting the role of *Actinobacteriota* in synthesizing polyketides and terpenoids. Additionally, the correlation test demonstrated changes in *Actinobacteriota* abundance linked to nutrient elements, revealing a positive correlation between with N–NO$_3^-$ and negative correlations with PO$_4^{3-}$ and DOC. This is in line with results from other studies [36,42]. Furthermore, the decrease in *Actinobacteria* abundance in summer may be attributed to elevated nutrient levels or competition from rapidly growing phyla, such as the *Proteobacteria* group, during this season.

The *Bacteroidota* phylum, primarily comprising the class *Bacteroidia*, plays an important role in degrading complex biopolymer loads and dissolved organic carbon inputs from cyanobacterial blooms [59]. Consequently, they are often used to understand top-down control in freshwater environments [67]. In general, *Bacteroidota* abundance is highly dependent on cyanobacterial blooms and organic matter, with peak abundance in summer when cyanobacterial blooms dominate. However, our study noted a lower level of *Bacteroidota* during summer and autumn compared to winter, with no presence in spring. We hypothesize that natural mechanisms (*i.e.,* top-down control) regulate the presence of different microorganisms to maintain system equilibrium.

Similar trends were observed for the phylum *Firmicutes*, with its abundant being higher in winter (>7 %) compared to the other

seasons (<1 %). This findings is in agreement with Wu et al. [68], suggesting that the increased abundance of *Firmicutes* in winter could be attributed to their ability to adapt to low temperatures. Given that many members of this group are heat-sensitive bacteria capable of surviving at low temperatures, the strong negative correlation between *Firmicutes* abundance and temperature supports this observation.

4.3. Functional predictions of bacterial communities

Bacterial gene functions in the Seine surface water were predicted using the PICRUSt algorithm and annotated from the KEGG databases at level 2 (Fig. 8). Amino acid metabolism, carbohydrate metabolism and vitamin and cofactor metabolism were the top three functions of the microbial community in the Seine surface water, similar to the results obtained by [42] for the Fuhe River in Jiangxi province, China. They also processed the ability to perform polyketide and terpenoid metabolism, xenobiotic biodegradation and metabolism, glycan biosynthesis, energy metabolism, metabolism of other amino acid as well as nucleotide metabolism. The results of the spatial-temporal variability using two-ways ANOVA analysis showed significant seasonal variability in the abundance of these genes whose functions are predicted. In spring and summer, the relative abundance of genes related to cofactor and vitamin metabolism, infectious diseases of both bacterial and parasitic origins, and cell mobility, growth and mortality were higher than in autumn and winter. There was also an enrichment of genes related to carbohydrates, energy metabolism, amino acids, terpenoids and polyketides in winter compared to summer, and a decrease in membrane transport and cell mobility. These observations are consistent with those of [42] and contrary to those obtained by [12] for microbial communities in the urban Pearl River in China. This could be explained by the stress caused by variation in environmental conditions, which creates competition among the microbial communities present [35]. A small abundance of genes related to infectious diseases of both bacterial and parasitic origins was found, and its was positively correlated with the abundance of *Campilobacterota, Firmicutes,* and *Bacteroidota*. These microbial groups were composed of bacteria that can be pathogenic.

5. Conclusions

Our study utilized NGS technology to enhance our understanding of microbial community dynamics in the Seine River. In particular, this study represents the first comprehensive characterization of microbial composition and diversity in the Seine surface water, taking into account both spatial and temporal dimensions. Additionally, physico-chemical parameters of the Seine water were analyzed and compared with values from the past 10 years in order to assess water quality. The findings indicate that these parameters have remained stable over time. In terms of microbial community diversity, the results revealed a highly diverse microbiome that participates in a variety of ecological functions. The main phyla making up the surface water microbiome were *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, and other minor phyla. Spatial variabilities in microbial composition were minor, with the exception of *Firmicutes*, whose abundance differed between upstream and downstream locations. This change can be explained by the phenomena of organic matter accumulation from upstream to downstream, as the *Firmicutes* group tends to thrive in nutrient-rich environments. However, seasonal fluctuations were nevertheless evident, primarily influenced by temperature, nitrate and ortho-phosphate concentrations. It is interesting to note that the *Proteobacteria* group consists of heterotrophic microorganisms that thrive at high temperatures, resulting in higher abundance during summer. Conversely, the *Firmicutes* group is known for its ability to adapt to low temperature, with higher abundance during winter, as confirmed by a strong negative correlation between the abundance of this phylum and temperature. For the future, the results of this study will provide a baseline of the microbial diversity in the Seine River, once new technologies to disinfect the WWTP effluent, such as performic acid disinfection in 2023, have been implemented. This will enable us to assess the effects on the Seine microbial communities of the discharge of wastewater treated with performic acid.

Data availability statement

The data associated with this study have been deposited with the NCBI Sequence Read Archive under BioProject access number PRJNA996616.

CRediT authorship contribution statement

Sadia Bagagnan: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Sabrina Guérin-Rechdaoui:** Visualization, Supervision, Project administration. **Vincent Rocher:** Supervision, Project administration, Investigation. **Vanessa Alphonse:** Methodology, Formal analysis. **Régis Moilleron:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **My Dung Jusselme:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30614.

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