SHENZHEN MSU-BIT UNIVERSITY

FACULTY OF BIOLOGY

CAO LIXI

曹李溪

Determinants of bacterial community composition in a subtropical endorheic urban pond

Master's thesis

Supervisor:

Dr. Pascaline Nyirabuhoro

Co-supervisor:

Dr. Jean Claude Ndayishimiye

This work is allowed to be defended

SHENZHEN–2023

Abstract

The dynamics of bacterioplankton communities in aquatic environments are of great importance as they provide valuable insights into the microbial diversity, ecological processes, and ecosystem functioning that are specific to these environments. However, there is still a lack of knowledge regarding the factors that determine variations in the bacterioplankton community in unique aquatic habitats such as endorheic ponds in urban environments. We conducted high-frequency sampling of bacterioplankton using high-throughput sequencing technology over a one-month period (August, 2022; n = 30) in an endorheic urban pond located in the subtropical monsoon climate zone of China. Our primary objective was to gain a comprehensive understanding of the factors that influence the dynamics of the bacterioplankton community in such a unique freshwater ecosystem. We classified the bacterioplankton community into different subcommunities: abundant taxa (AT), consisting of 117 operational taxonomic units (OTUs), conditionally rare taxa (CRT), consisting of 6,774 OTUs, and rare taxa (RT), consisting of 5,012 OTUs. The temporal dynamics of the bacterioplankton community exhibited substantial changes, largely driven by fluctuations in the dominant Actinobacteria and Proteobacteria phyla, as well as the Synechococcus genus, which is a group of cyanobacteria. The community compositions within subcommunities, particularly in CRT, exhibited significant differences. Water physicochemistry emerged as the most influential environmental condition, explaining pure variances of 0.8%, 0.01%, and 0.02% in AT, CRT, and RT subcommunities, respectively. Co-occurrence networks revealed significant species associations within the bacterioplankton community, emphasizing the key role of stochastic

processes in shaping the community structure, especially within CRT and RT subcommunities, which accounted for 40% and 18.1% of the explained community variance, respectively. The results suggest that the environment plays a crucial role as a determinant in shaping the composition of the bacterioplankton community in a subtropical endorheic urban pond.

Key words: Environmental conditions, bacterial community, community composition, community assembly mechanisms, endorheic pond, subtropical urban environment

Table of contents

1. Introduction

Bacterioplankton, which refer to the bacterial component of the plankton that drifts in the water column, play a central role in aquatic environments, including urban ponds (Sigee, 2005). They are involved in the breakdown and removal of contaminants, including organic matter and heavy metals, and in nutrient cycling in the ecosystem, and they can play a role in the food webs of urban ponds as they serve as a food source for higher trophic levels (Sigee, 2005; Sandrin et al., 2009). Several studies have shown that the bacterial community in urban ponds is different from that in natural water bodies (Karim et al., 2012; Hanashiro et al., 2019; Isabwe et al., 2022). The bacterial community in urban ponds is often dominated by opportunistic bacteria that can survive in the presence of anthropogenic contaminants (Karim et al., 2012; Hanashiro et al., 2019). Moreover, urban ponds have higher bacterial diversity compared to natural aquatic systems (Sigee, 2005). Factors that may affect the bacterial community structure in urban ponds include the physicochemical characteristics of the water, land use, and the presence of anthropogenic contaminants (Karim et al., 2012; Hanashiro et al., 2019).

Despite the significant research that has been carried out on the bacterial community in urban ponds, there are still substantial knowledge gaps (Hassall, 2014; Hill et al., 2017). One of the major gaps is the lack of comprehensive studies that examine the bacterial community structure in urban ponds over time (Karim et al., 2012). Most studies have been conducted on a single occasion, making it difficult to assess the seasonal variation in bacterial community structure (Sigee, 2005). Moreover, there is a lack of information on the effects of specific contaminants on the bacterial community structure in urban ponds. There is also a need for a better understanding of the factors

impacting the abundance and distribution of specific bacterial groups in urban ponds.

Studying bacterial communities in urban environments is important, as this can provide insight into the impact of human activities on microbial diversity and its subsequent effect on human health (Hassall, 2014; Hill et al., 2017). Urban environments are characterized by high levels of anthropogenic pollution, which can alter microbial diversity and abundance (Sigee, 2005). As bacterioplankton play a crucial role in nutrient cycling, food chain dynamics, and ecosystem processes, any ecological disturbance to microbial communities can have serious ecological consequences (Sandrin et al., 2009). Additionally, bacterioplankton in urban environments has been linked to several environmental health hazards, such as air and water pollution, soil contamination, and the spread of infectious diseases (Bahcall, 2015). Therefore, a better understanding of the spatio-temporal patterns of bacterial communities in urban environments is essential for designing effective monitoring and management strategies to mitigate these environmental health hazards (Hassall, 2014; Hill et al., 2017).

The novelty of this research lies in the fact that urban areas are constantly changing due to human activities, leading to opportunistic bacterial communities that differ from those in natural environments. This presents an opportunity to understand the mechanisms that drive the adaptation of these microbial communities to urban environments, including their resistance to contaminants and their ability to produce harmful substances on urban surfaces. Moreover, research on bacterial communities in urban environments is a relatively new field of environmental microbiology that has gained attention over the past decade (Sigee, 2005; Bahcall, 2015). Hence, there is a need for further research to fully understand the impact of urbanization on

microbial communities and associated ecological consequences (Bahcall, 2015) and the development of effective management strategies (Hassall, 2014; Hill et al., 2017; 2021).

This research aims to address the knowledge gap in previous research on bacterioplankton in urban ponds. It investigates the temporal variation in bacterioplankton community structure in urban ponds and factors influencing the abundance and distribution of specific bacterial groups. It also examines the impact of specific contaminants on the bacterial community structure in urban ponds. Based on previous research on the bacterioplankton in urban ponds, we developed the following research questions: (1) How does the diversity of freshwater bacteria change temporally and spatially in a subtropical endorheic urban pond? (2) How does the freshwater bacterial community respond to local environmental conditions in a subtropical endorheic urban pond? (3) In what ratios do stochastic processes control the bacterial community in a subtropical endorheic urban pond? We hypothesized that: (1) the upstream, midstream, and downstream portions of the Shenzhen MSU-BIT University (SMBU) pond may exhibit distinct bacterial diversity over a short period of time; (2) the bacterial community may respond differently to environmental conditions in the upstream, midstream, and downstream of SMBU pond; and (3) despite being in an urban area, the bacterial community in SMBU pond can be regulated by stochastic processes. The expected results provide insights into the factors that regulate bacterial communities in urban pond systems and can be valuable for developing effective strategies for managing and monitoring these environments.

2. Literature review

2.1. Pond ecology

Globally, ponds are recognized as one of the most ecologically important and biodiverse freshwater habitats, offering a unique opportunity to counteract the negative impacts of anthropogenic pressures and reverse the declining trend of aquatic biodiversity (Hill et al., 2021). In addition to their ecological significance, ponds also provide vital contributions to society through the provision of various ecosystem services (Milstein, 2012). Despite their importance, freshwater research, policy, and conservation have traditionally prioritized larger water bodies such as reservoirs and lakes, leading to significant knowledge gaps in our understanding and conservation of pond ecosystems (Sigee, 2005; Hill et al., 2021).

A lentic system (Fig. 1) is an ecological term used to describe "standing water" ecosystems, which include ponds, lakes, and reservoirs. These lentic systems can range from small, shallow ponds to large, deep lakes, and each has unique physical, chemical, and biological characteristics that contribute to its ecological functioning (Sigee, 2005). The ecologists conceptualize a lentic system as a heterogeneous mass of fresh or salt water, with characteristics varying physically (e.g., transparency and temperature), chemically (e.g., nutrients and contaminants), and biologically (e.g., growth rate and biomass of microbes) (O'Sullivan and Reynolds, 2004). The characteristics of a typical lentic system can vary spatially and temporally (e.g., on the scale of diel, season, year, or geological time) (Last and Smol, 2002). The nearshore (littoral zone) allows the penetration of sunlight all the way to the sediment. It can serve as a substrate for algae, invertebrates, plants, and habitat for fish (Moss, 2017). The open water (limnetic zone), in contrast,

does not allow penetration of the sunlight all the way to the bottom of the lake. The bottom sediment (benthic zone), which is the lowest level, has a close relationship with the substrate and has a surface layer abundant with organisms (O'Sullivan and Reynolds, 2004; Smol, 2008). The benthic zone is greatly influenced by the overlying water and biological activity taking place in a limnetic zone; hence, most of organisms in a benthic zone are lastingly attached to the constituents of benthic layers (e.g., soil, mud, sand, and rocky outcrops) (Smol, 2008). The benthic zone may also contain a broad range of chemical compounds (e.g., molecules and irons) delivered from the watershed and atmosphere; thus, the water and sediment chemistry are placed in the central function of climate that can affect a watershed, hydrology as well as aquatic organisms (O'Sullivan and Reynolds, 2004; Smol, 2008).

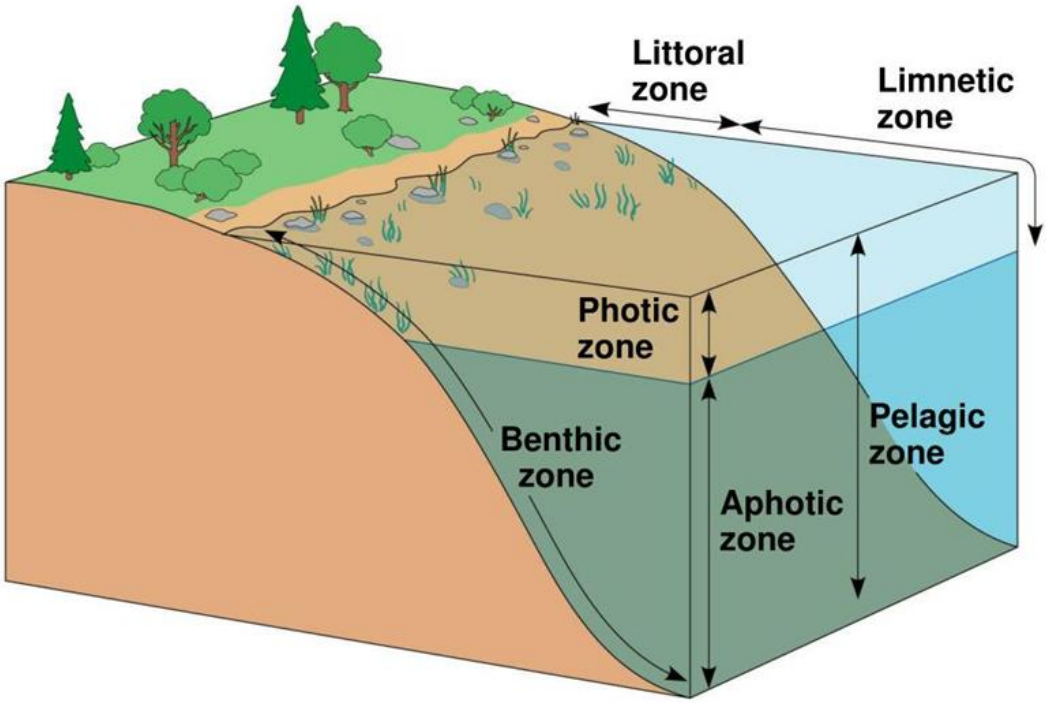

Fig. 1. Zonation of a lentic system (Source: Pearson Education, Inc. Publishing as Pearson Benjamin Cummings, 2005).

2.2. Endorheic pond system

Closed basin ponds, also known as endorheic ponds, are a unique type of body of water that can be found in many parts of the world, particularly in arid or semi-arid regions (Ordónez et al., 1994; Martin-Rosales and Leduc, 2003; Seeboonruang, 2014; Bellia and Lanfranco, 2020). Unlike other bodies of water, endorheic ponds do not have an outflow, can range in size from small pools to large lakes, and are formed by precipitation or runoff (Hawes et al., 2021). Due to the fact that the water in endorheic ponds cannot drain into a river or ocean, it can only leave the pond through evaporation or seepage into the ground (Sigee, 2005).

The unique characteristics of endorheic ponds make them vital habitats for a variety of flora and fauna, including many species of birds, insects, and fish (Ordónez et al., 1994; Martin-Rosales and Leduc, 2003; Seeboonruang, 2014; Bellia and Lanfranco, 2020). For the reason that the water in endorheic ponds is typically saltier than other bodies of water, it can support species that are adapted to high-salinity environments (Sigee, 2005). For example, the Great Salt Lake is home to several unique species, including brine shrimp and brine flies, that are found nowhere else in the world (Post, 1977; Adams et al., 2015). In addition to supporting flora and fauna, endorheic ponds serve as important resources for local communities. In many arid or semi-arid regions, these ponds are used for irrigation, livestock watering, and even as a source of drinking water (Sigee, 2005). However, the water in endorheic ponds is not regularly replenished by rivers or other sources of freshwater; consequently, they can be vulnerable to contamination and other forms of environmental degradation (Adams et al., 2015).

The most important threats to endorheic ponds are overuse and climate change (Ordónez et al., 1994; Martin-Rosales and Leduc, 2003; Seeboonruang, 2014; Bellia and Lanfranco, 2020). Endorheic ponds cannot be replenished by an outflow and are vulnerable to depletion if they are overused. In some arid or semi-arid regions, these ponds are also used for mining and industrial purposes, which can introduce contaminants into the water and degrade its quality. As temperatures rise and precipitation patterns shift, many of these ponds become more vulnerable to drought and other forms of water scarcity. This could have serious consequences for the flora and fauna that depend on these ponds for their survival, as well as for the communities that rely on them as a resource (Sigee, 2005).

2.3. Background on bacterioplankton

Scientists developed the energy pyramid, which is a graphical representation of the feeding levels in an ecosystem, to predict the effects of changes in one part of the ecosystem on others and to develop strategies for managing and conserving natural resources (Fig. 2). Bacterioplankton occupy a central position in the energy pyramid as they are the primary decomposers of organic matter in aquatic ecosystems (Prasad, 2022). They break down complex organic molecules into simpler compounds, making the nutrients available to other aquatic organisms. As such, bacterioplankton are at the base of the food web and support the survival of other organisms, such as phytoplankton and zooplankton, which in turn support higher trophic levels (Sigee, 2005).

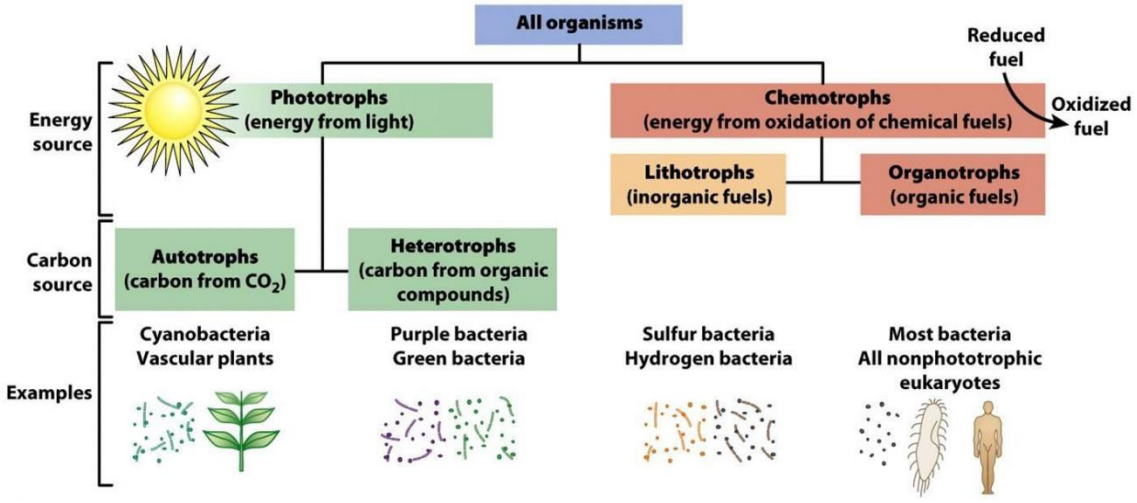

Fig. 2. Classifications of organisms based on source of energy (Source: www.biology-forums.com).

Bacterioplankton can live in aerobic or anaerobic conditions and are produced by mitosis or asexual reproduction. They are very small in size but very abundant (Hobbie et al., 1977). Bacterioplankton are divided into the following groups: femtoplankton (< 0.2 μm); picoplankton (0.2–2.0 μm); nanoplankton (2.0–20.0 μm); microplankton (20–200 μm); mesoplankton (0.2–2.0 mm); macroplankton (> 2 mm) (Sieburth et al., 1978). However, a strict classification of the functional groups is sometimes difficult; as many bacterioplankton have a combination of the above functions (Kirchman, 2008). Cyanobacteria group such as *Prochlorococcus* and *Synechococcus* are recognized on top rank of phototrophic bacteria in aquatic ecosystems because of their important role in food chain as drivers of light for primary production (Lindholm and Weppling, 1987; Vicente and Miracle, 1988; Vila et al., 1996).

Cyanobacteria provided the first largescale biotic source of oxygen on early earth and are capable of oxygenic photosynthesis (Hamilton et al., 2015) and fixing nitrogen (Caldwell and Tiedje, 1975; Jurtshuk, 1996; Petrash et al., 2018). Gammaproteobacteria are new types of phototrophs because of their

proteorhodopsin proteins, which are retinal-based photoreceptors enabling phototrophic function in water (Sabehi et al., 2005). Chemotrophic bacteria require carbon to survive and are classified into two important categories (Pjevac et al., 2015). Chemoautotrophs are capable to synthesize their own source of energy from oxidation of reduced inorganic compounds such as iron, sulfur, sulphide, and magnesium. Contrary to popular belief, chemoheterotrophs are unable to synthesize their own food, so they get their energy from the oxidation of inorganic minerals in their environment (Jaspers et al., 2001). This makes them major decomposers of organic matter and allows them to mineralize a high proportion of the daily primary production (Sigee, 2005).

Bacterioplankton community composition, production, and abundance change seasonally in most aquatic systems, and these changes appear to be linked to climatic variations that affect several biogeochemical processes (Hobbie et al., 1977; Jurtshuk, 1996; Nyirabuhoro et al., 2020, 2021). Because of their quick turnover rates and relationship with numerous environmental variables, bacterioplankton are sensitive to environmental alteration such as climate change, increases in nutrient concentrations or pollution by a wide array of human-made chemicals (Hobbie et al., 1977; Jurtshuk, 1996; Jiao et al., 2018). As an example, in subtropical reservoirs, cyanobacteria are able to produce blooms during eutrophication and warm weather (Yang et al., 2008, 2012). These cyanobacterial blooms can result in the death of organisms such as fish and other aquatic fauna (Dokulil and Teubner, 2000). Even though their importance in aquatic ecosystems is obvious, bacterioplankton taxa in various subtropical reservoirs are less described.

2.4. Rare bacteria and their importance

The rare biosphere consists of microbes that exist in low abundance within the community (Pedrós-Alió, 2012; Lynch and Neufeld, 2015; Nyirabuhoro et al., 2020). However, when conditions become favorable, the rare biosphere microbes can disproportionately affect ecosystem function (Shade et al., 2014). The exploration of this rare biosphere has numerous points of interest in ecology (Pedrós-Alió, 2012). First, it can show a reasonable estimate of the total number of bacterial taxa in the environment; right now, we do not even know the accurate order of magnitude. Second, it is able to answer the problem of whether "everything is everywhere." Third, it allows the investigation of ecological mechanisms that let existence of many species in low numbers. Fourth, it opens an opportunity for research into the huge reserve of genes with potential applications hidden in the rare biosphere. More importantly, rare microbes possess an extraordinarily diverse set of enzymes, some of which can prove greatly relevant to industrial processes, such as thermophilic cellulases for biofuels applications (Lynch and Neufeld, 2015). Isolation in pure culture was the only way to detect some rare bacteria, but current culturing techniques are incapable of isolating most of the bacteria in nature. The current development of fast and cheap high-throughput sequencing and imaging techniques, including flow cytometry, permits access to rare species. The use of these tools is starting to reveal functional relationships.

One of the most familiar forms in biodiversity investigation is that only a few species are common, whereas most species are rare (Lennon and Jones, 2011; Shade et al., 2014). This phenomenon can be demonstrated using a rank abundance curve (Fig. 3), which shows the total number of species and their

relative abundances in a sample or community. Theory predicts that rare species may be at risk of extinction even though they represent a reservoir of genetic diversity that is capable of responding speedily to environmental change (Lennon and Jones, 2011). For example, it is likely that rare taxa are disproportionately active relative to common taxa because rare taxa consist of populations that lack the ability to enter and exit dormancy (Jones and Lennon, 2010). Alternatively, microbial rank abundance curves may be more dynamic and possibly characterized by transitions between active and dormant states that can eventually affect the relative abundance of microbial taxa. In either case, the rare biosphere seems to be metabolically active and potentially important when attempting to make links between the structure and function of microbial communities (Lennon and Jones, 2011; Shade et al., 2014).

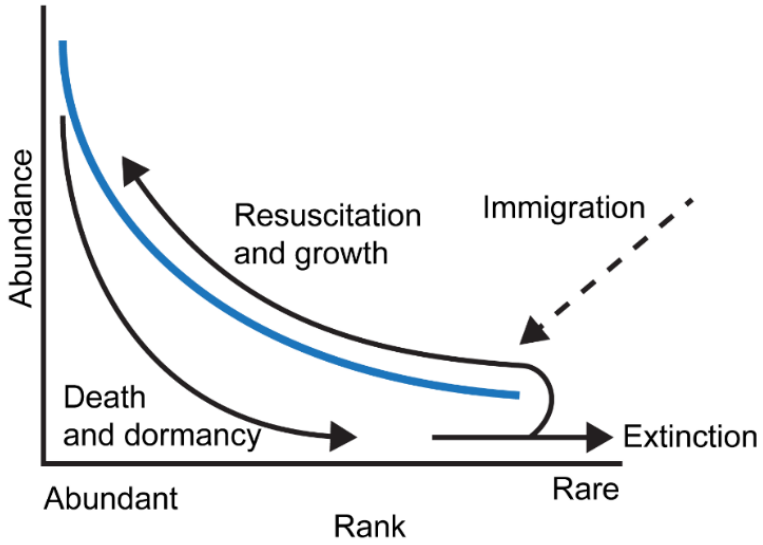

Fig. 3. A dynamic rank abundance curve for a microbial community that is influenced by dormancy. In most ecosystems, a small number of microbial species are dominant (abundant), and the remaining species are rare. The relationship between species abundance and the rank order of species in a community is shown by the blue line. Abundance can change over time owing to various factors (black lines). For example, species abundance can decrease

because of predation and resource limitation, as well as dormancy, which prevents microorganisms from replicating. However, dormancy can also reduce the likelihood of extinction; after resuscitation, a population may return to a dominant position in the rank abundance curve. In the absence of dormancy, the persistence of a given species is more dependent on immigration (dashed arrow) and the species is more likely to be lost from the local community (Source: Lennon and Jones, 2011).

2.5. Criteria for defining rare taxa from abundant ones

Several high-throughput sequencing methods that were formerly developed for small subunit ribosomal ribonucleic acid gene sequencing have shown an enormous complement of low-abundance microbial taxa (Liu et al., 2015). Defining the rare biosphere has been arbitrary and the methods mostly employed include relative abundance cut-offs sequence counts in generated data sets (e.g., two sequences per sample) and empirical thresholds. The choice of thresholds can depend on the technology used to find out species (basic unit of classification and a taxonomic rank of an organism, as well as a unit of biodiversity) or the sake of the study (Reid and Buckley, 2011). Some researchers used a cutoff of 0.1% local relative abundance for rare taxa definition (Fuhrman, 2009; Vergin et al., 2013); others used 0.01% (Galand et al., 2009; Liu et al., 2015; Logares et al., 2015; Nyirabuhoro et al., 2020). Further, a threshold of 1% of relative abundance has been extensively utilized in numerous studies to define abundant species (Pedrós-Alió, 2012; Vergin et al., 2013; Logares et al., 2014). Practically, it is widespread to remove low-abundance sequences from analyses at a specific ecological threshold, such as the contribution to community dissimilarity (Lynch and Neufeld, 2015). Despite the threshold employed for defining the rare biosphere, microbial

community abundance distributions based on marker gene surveys typically reveal a long tail of low-relative-abundance operational taxonomic units. The length and shape of this tail differ depending on the diversity of the sampled community and on the underlying species-abundance distribution (Fig. 4), which is inadequately understood for most microbial communities (Lynch and Neufeld, 2015). The microbes that are periodically recruited from the rare biosphere can switch between abundant and rare, depending on periodic environmental conditions such as temperature, nutrient and seasonality (Aanderud et al., 2016; Nyirabuhoro et al., 2020, 2021). The microbes that are occasionally recruited from the rare biosphere persist with relatively rare abundances, responding to occasional episodic cues such as precipitation and pressure (Lynch and Neufeld, 2015). Microbial taxa that demonstrate periodic increases in abundance but are permanently rare are adapted to live at low relative abundance, constantly avoiding predation. This lifestyle is associated with increased susceptibility to starvation. Permanently rare taxa, which include potential keystone species, demonstrate persistent low-abundance distributions and an increased susceptibility to starvation. Transiently rare taxa are occasionally rare due to immigration. Their persistence depends on appropriate conditions for survival and reproduction. Taxa showing periodic or occasional recruitment from the rare biosphere can be considered to be conditionally rare taxa (CRT).

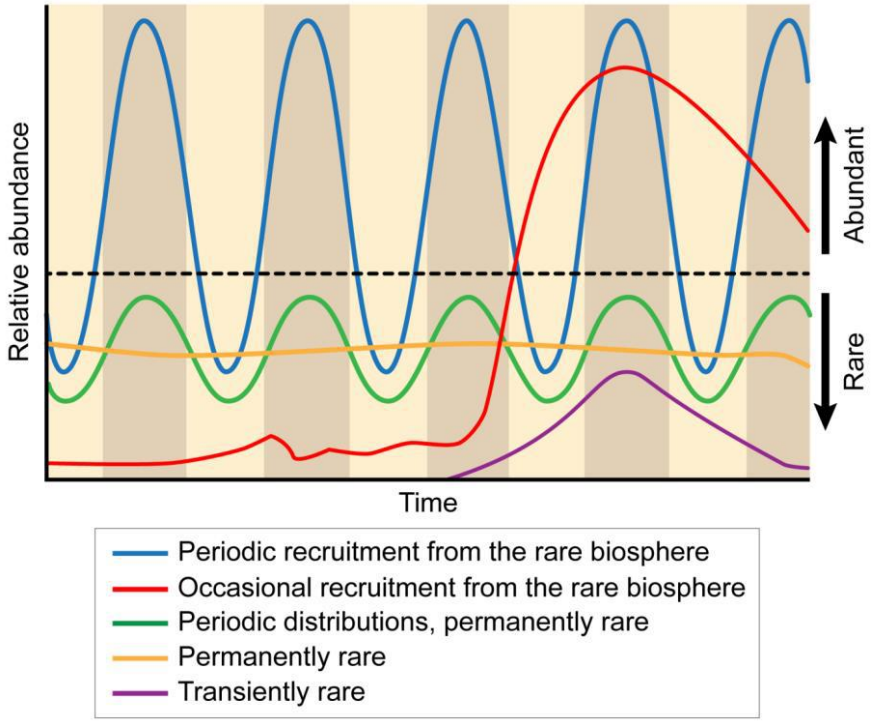

Fig. 4. Hypothetical temporal abundance profiles for rare-biosphere microorganisms (Source: Lynch and Neufeld, 2017). Abundance refers to the numerical count or relative contribution of a taxon to a community observation. Abundance includes a continuum from most to least abundant of dominant, prevalent, and rare taxa; Relative abundance refers to the evenness of distribution of individuals among species in a community.

2.6. Importance of conditionally rare and abundant taxa

Previous studies acknowledged microbes that are rare at definite points in time and space and shift to abundance at other points (conditionally rare taxa) as major contributors to community dynamics in different ecosystems (Campbell et al., 2011; Shade et al., 2014). Within an observation of a microbial community, conditionally rare taxa may be prevalent and remain important for ecosystem function (Shade et al., 2014). They are important in maintaining the function and stability of ecosystems; hence, they are

considered the seed bank of the community, which is a reservoir of inactive individuals that can potentially be resuscitated in the future under a different set of environmental conditions (Lennon and Jones, 2011). Despite the importance of the seed bank, conditionally rare taxa can be active with potential ecological roles: First, they are potentially responsible for changes in the community structure and composition over space and time (Campbell et al., 2011; Hugoni et al., 2013). This may be due to the ease of their reproduction by binary fission, so that species can grow rapidly under suitable conditions in nature (Campbell et al., 2011). Second, some nutrient cycling processes offer illustrative examples of the effects of conditionally rare taxa. Conditionally rare bacteria are highly active foundation species in freshwater and important for nitrogen and carbon uptake as "keystone species" (species with key roles in community structure and/or ecosystem functioning). As an example, a decrease of 75% of the measured species richness may reduce soil-denitrifying activity by a factor of 4-5-fold, suggesting that dominant species cannot perform this process alone (Jousset et al., 2017). Third, conditionally rare bacteria play a key role in the degradation of organic compounds, including pollutants. As an example, the removal of rare microbes in activated sludge and freshwater can seriously diminish the capacity to degrade pollutants and toxins (Fuentes et al., 2014). Fourth, conditionally rare bacteria also revealed their importance in medicine. In the human lung, a high diversity of low-abundance bacteria is likely to be linked with a reduced severity of bacterial infection in individuals with cystic fibrosis. On the other hand, rare species may contribute to pathogenesis when the conditions become favorable to them (e.g., oral microflora can lead to periodontal disease) (Jousset et al., 2017).

2.7. Traditional and new methods in water sampling strategy

Water is an essential liquid that is vital for all life on earth (Sigee, 2005). In environmental microbiology, water is a crucial factor, as it is often used as a sample matrix for the detection and quantification of microorganisms (Maier et al., 2009). The quality of water is a key factor in determining the health of an ecosystem, and monitoring aquatic environments is an essential part of environmental management (Sigee, 2005; Maier et al., 2009). Microbes, such as bacteria, viruses, and fungi, are abundant in water, and their presence or absence provides valuable information about the health of ecosystems (Varnam and Evans, 2000; Sigee, 2005; Maier et al., 2009). For example, the presence of certain bacteria in water indicates contamination of the water with fecal matter or other pollutants (Sigee, 2005).

Water can be collected from various sources, including rural and urban ponds, rivers, lakes, oceans, groundwater, wastewater, and even drinking water (Varnam and Evans, 2000; Sigee, 2005; Maier et al., 2009). Sampling is defined as the process of selecting a portion of material (i.e., small enough in volume) to be transported suitably and handled in the laboratory while still accurately representing the part of the environment that was sampled (Madrid and Zayas, 2007). The main difficulties in sampling the environment are representativeness and integrity (Sigee, 2005; Madrid and Zayas, 2007). This is because it is important to obtain a sample that accurately reflects the characteristics of the environment being studied (Madrid and Zayas, 2007). A representative water sample is one that precisely represents the larger populations of diverse microorganisms, while integrity refers to the preservation of the physical, chemical, and biological characteristics of water during collection, transportation, and analysis (Sigee, 2005). If a water sample

is not representative, it may not provide accurate information about the environment, including microbial communities (Madrid and Zayas, 2007). On the contrary, if water is collected from a more contaminated environment, such as stormwater from urban areas, industrial wastewater, or radioactively contaminated cooling water from nuclear power plants, but the sample is not representative of the larger body of water, the results of the analysis may not accurately reflect the level of contamination (Varnam and Evans, 2000; Sigee, 2005; Maier et al., 2009). Similarly, if samples are not handled with integrity, water may become contaminated and altered, which can affect the accuracy and reliability of the results (Madrid and Zayas, 2007). Therefore, ensuring representativeness and integrity is important in obtaining reliable data from a water sample (Sigee, 2005; Madrid and Zayas, 2007). This can be achieved through careful planning of the sampling, the use of appropriate sampling and sample preservation techniques, and the careful handling and transport of the sample to the laboratory (Fig. 5).

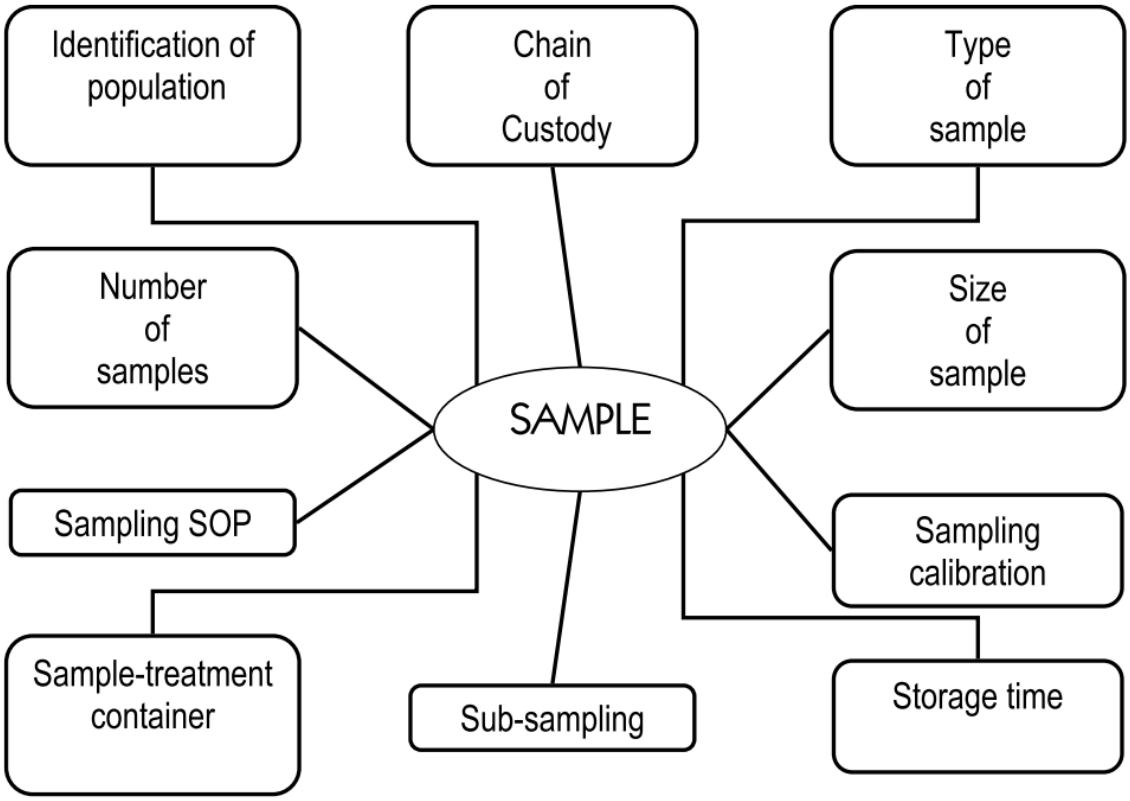

Fig. 5. Items to be taken into account while developing a sampling plan (Source: Madrid and Zayas, 2007).

Traditional methods of water sampling involve physically collecting water from a specific location and transporting it to a laboratory for analysis (Madrid and Zayas, 2007). These approaches typically involve using a sampling bottle, which is typically made of glass or plastic material, to collect the water sample (Sigee, 2005). In general, the bottle is filled with water by submerging it to the desired depth, and then the bottle is sealed to prevent contamination or loss of sample integrity during transportation. Other traditional water sampling methods may involve using automated samplers such as submersible utility pumps to collect water samples at a specific time interval or flow rate (Madrid and Zayas, 2007). Moreover, traditional water sampling methods typically involve measuring physical characteristics of

water, such as depth, transparency, temperature, electrical conductivity, total dissolved solids, and turbidity, as well as chemical properties like pH, dissolved oxygen, nutrients, and heavy metals, and biological characteristics, such as microbial content (Sigee, 2005). Although traditional water sampling methods have been widely used for many years, they do have limitations, such as the potential for sample contamination, inconsistency in sample collection techniques, and the need for extensive laboratory analysis (Madrid and Zayas, 2007). As a result, more advanced practices have been developed to provide more accurate and efficient water sampling and analysis (Varnam and Evans, 2000; Sigee, 2005; Maier et al., 2009).

Advanced water sampling methods refer to new and innovative techniques which address the limitations of traditional water sampling methods. Some examples of advanced water sampling methods include real-time water quality monitoring, autonomous underwater vehicles (AUVs), optical sensors, passive sampling, and DNA-based methods (Varnam and Evans, 2000; Sigee, 2005; Maier et al., 2009). Real-time water quality monitoring involves using automated sensors measuring various water quality parameters in real-time. This technique provides continuous monitoring and data collection, which identify changes in water quality and detect contamination events more quickly (Barabde and Danve, 2015). Autonomous underwater vehicles (AUVs) are unmanned vehicles that are programmed to collect water samples and data at specific locations and depths. AUVs are mostly equipped with several sensors measuring water quality parameters. AUVs are particularly advantageous for sampling deep water and large water bodies, as they can be operated autonomously or controlled remotely from a base station (Griffiths, 2002). Optical sensors employ light-based technology to quantify various water quality parameters, such as dissolved organic matter,

chlorophyll-*a*, and turbidity. Like AUVs, optical sensors also provide fast, accurate, and non-invasive measurement of water quality parameters (Sigee, 2005). Passive sampling involves using specialized supplies, such as resins or membranes, to collect water over a period of time. This technique provides a more representative water sample over time to help identify trends and changes in aquatic environments (Vrana et al., 2005). DNA-based methods use genetic sequencing technology to quantify microbial communities in water. This technique provides a more accurate and detailed analysis of microbes present in water, allowing for the detection of potential health risks and contamination events more quickly (Sigee, 2005; Blancher et al., 2022).

2.8. DNA-based monitoring of environmental microbial community

DNA-based methods provide a comprehensive understanding of microbial community composition and functional potential (Bruce et al., 2021; Blancher et al., 2022). The procedure for DNA-based monitoring of environmental microbial communities involves several steps, including sample collection, DNA extraction, amplification, and analysis of sequencing data (Fig. 6). Several techniques (e.g., polymerase chain reaction (PCR) based techniques, metagenomics, microarrays, quantitative PCR (qPCR), and DNA barcoding) have been developed for DNA-based biomonitoring. Each technique has its own strengths, limitations, and specific applications. Hence, researchers must cautiously consider which technique to use depending on their research question, available resources, and the specific characteristics of the sample being analyzed. For example, water may require different techniques than soil or air samples due to differences in the physicochemical properties of these matrices (Sigee, 2005).

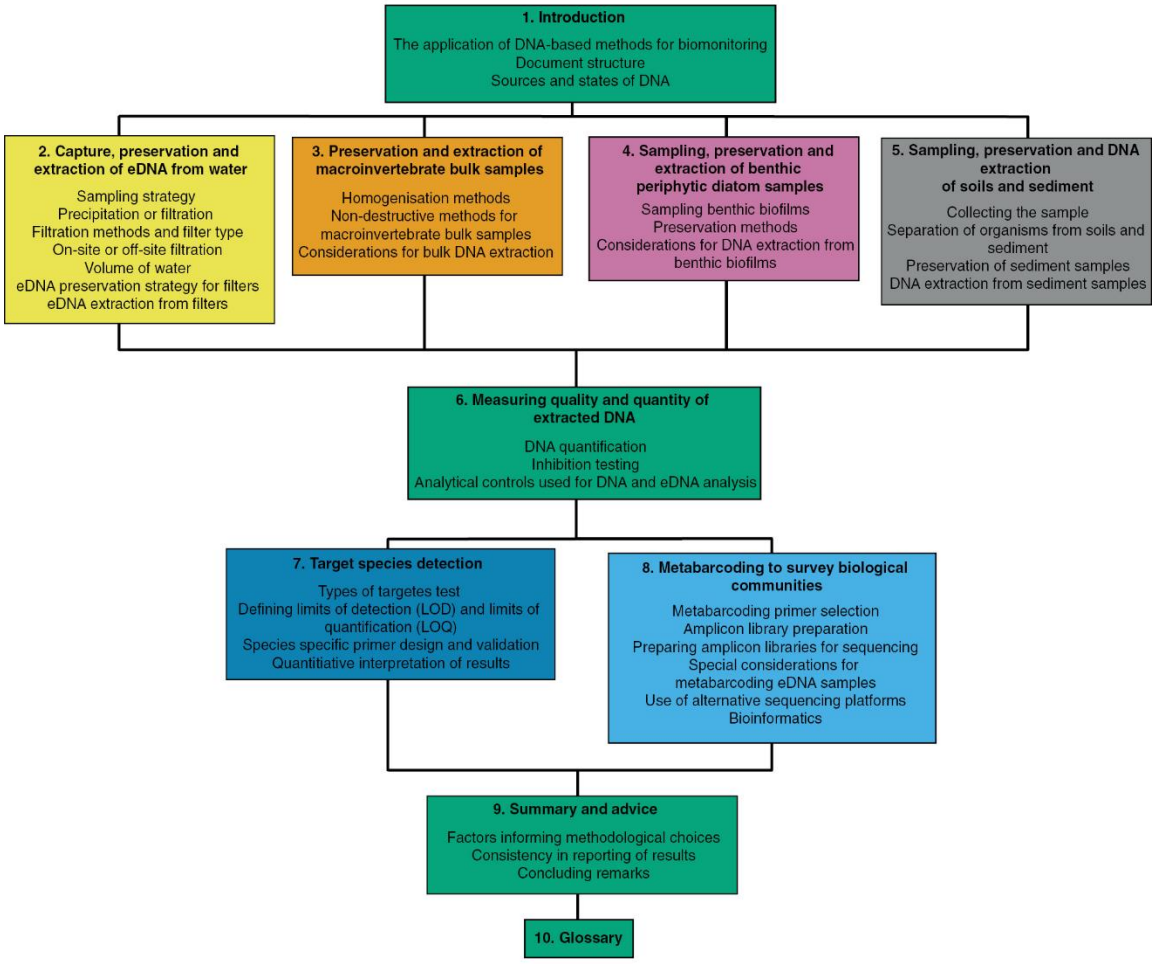

Fig. 6. Decision-making process in selecting a methodology for DNA-based biomonitoring together with practical recommendations to guide this process (Source: Bruce et al., 2021).

Amplicon sequencing involves amplifying a specific DNA region, such as the 16S rRNA gene for bacteria or the internal transcribed spacer (ITS) region for fungi, using polymerase chain reaction (PCR). The amplified DNA fragments are then sequenced using high-throughput sequencing technologies (Lundberg et al., 2013). Amplicon sequencing is relatively inexpensive and has several advantages, including high throughput, sensitivity, and specificity. It can be used to analyze multiple samples instantly for microbial community composition and diversity. However, the technique has some limitations,

including potential biases in amplification and the inability to identify novel microbes (Sigee, 2005).

16S rRNA amplicon sequencing is a widely used technique for freshwater bacterial community analysis (Nyirabuhoro et al., 2020, 2021). The technique involves several steps from sampling to OTU table generation (Fig. 7), which can be summarized as follows: sample collection, DNA extraction, amplification of the 16S rRNA gene, sequencing, quality control and filtering, operational taxonomic unit (OTU) clustering, taxonomic assignment, statistical analysis, and OTU table generation. The first step is to collect the environmental sample, such as water, that contains the bacterial community of interest. The bacterial DNA is extracted from the sample using a commercial kit or a laboratory protocol. This step requires that the DNA extraction method be optimised to obtain high-quality DNA that is appropriate for downstream processing. The bacterial 16S rRNA gene is amplified using PCR with universal primers that target conserved regions of the gene. The PCR conditions should be optimized to ensure that the amplification is specific, efficient, and reproducible. The amplified DNA is sequenced using a high-throughput sequencing platform, such as Illumina or PacBio. The sequencing depth should be appropriate to ensure that the diversity and abundance of the bacterial community are accurately represented. The raw sequence data are subjected to quality control and filtering to eliminate low-quality reads, adapter sequences, and chimeric sequences that may affect downstream analysis. The sequence data are then clustered into operational taxonomic units (OTUs) based on a defined similarity threshold, typically 97%. These OTUs serve as proxies for bacterial species or phylotypes. The OTUs are assigned to taxonomic categories based on reference databases, such as Greengenes or SILVA. The taxonomic assignment provides evidence

of the identity and abundance of the bacterial community. The OTU table is analyzed using different statistical methods to compare the bacterial community structure and composition between samples, identify biomarkers, and evaluate the effect of environmental factors on the community. The final output of 16S rRNA amplicon sequencing is an OTU table, which lists the abundance of each OTU in each environmental sample. The OTU table is a valuable resource for further downstream analysis, such as functional profiling and network analysis (Nyirabuhoro et al., 2020, 2021; Al et al., 2022).

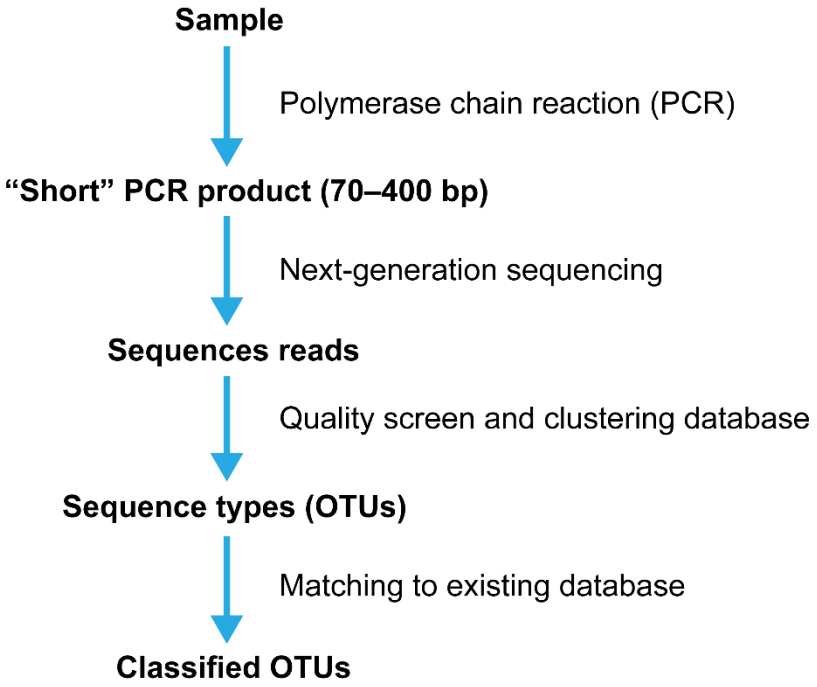

Fig. 7. The process of generating an OTU (operational taxonomic unit) table from 16S rRNA amplicon sequencing involves multiple steps.

Metagenomic sequencing is a more comprehensive technique for DNA-based biomonitoring (Ni et al., 2013). It involves sequencing all the DNA in a sample, including the DNA of microorganisms as well as that of other organisms present in the sample. It has several advantages, including the

capability to identify novel microorganisms and functional genes. However, the technique has limitations such as high cost, potential biases in DNA extraction and sequencing, and greater computational requirements (Sigee, 2005).

Shotgun metagenomic sequencing involves sequencing all the DNA in a sample without prior amplification or enrichment (Quince et al., 2017). The technique has several advantages, including the ability to identify novel microorganisms and functional genes, as well as the ability to determine the genomic content of individual microorganisms. However, the technique has limitations such as high cost, potential biases in DNA extraction and sequencing, and greater computational requirements (Sigee, 2005; Sharpton, 2014).

2.9. Numerical and statistical techniques

Numerical and statistical methods play an important role in analyzing biodiversity and microbial community data (Young et al., 1998; Legendre and Legendre, 2012). These methods aid researchers to estimate and understand the diversity and complexity of microbial communities and their relationships with environment (Sigee, 2005). The choice of statistical method depends on the research objectives and the characteristics of the microbial community being studied (Ilstrup, 1990; Young et al., 1998).

Some of the commonly used statistical methods for analyzing biodiversity and bacterial communities are diversity indices, analysis of similarities (ANOSIM), non-metric multidimensional scaling (NMDS), redundancy analysis (RDA) or canonical correspondence analysis (CCA), and variance partitioning analysis (VPA) (Burlage et al., 1998; Sigee, 2005). Diversity indices are mathematical measures used to calculate the diversity

and evenness of species in a community. These indices are commonly used to compare the diversity of microbial communities under different environmental conditions (Peet, 1974). Examples of diversity indices include the Shannon-Wiener diversity index, Simpson diversity index, and the species richness index (Sigee, 2005). ANOSIM is used to compare the similarity of microbial communities in different environments. ANOSIM calculates a similarity index (R) between microbial communities and tests whether there is a significant difference between the communities (Anderson and Walsh, 2013). Non-metric multidimensional scaling (NMDS) is used to visualize and compare the similarity of microbial communities based on their taxonomic composition. NMDS can help to identify patterns in microbial community data that may be associated with environmental factors (Zuur et al., 2007). RDA and CCA are multivariate statistical methods that are employed to analyze the relationships between multiple environmental variables and the distribution of species in a community. These methods allow researchers to identify which environmental factors are most strongly associated with changes in species composition. The difference between RDA and CCA is that RDA assumes linear relationships between species and environmental variables, whereas CCA can model non-linear relationships (ter Braak and Šmilauer, 2012). Variance Partitioning Analysis (VPA) is used to partition the variation in species composition into components associated with different environmental factors. VPA is based on the idea that multiple environmental factors can contribute to the variation in species composition, and VPA provides a way to quantify the relative contributions of these factors to the variation observed in the community (Zhou and Ning, 2017).

2.10. Network analysis

A network is a system composed of interconnected elements, referred to as nodes or vertices, and the relationships between them, represented as edges or links (Brinkmeier and Schank, 2005). Network analysis is a field that applies mathematical and statistical techniques to model networks in various domains, including ecology (Fath et al., 2007; Wulff et al., 2012), providing a framework to understand the complex patterns of interactions, dependencies, and flows within a system. Key concepts in network analysis include nodes, edges, degree, centrality, clustering, network density, modularity, small-world effect, and network visualization (Brinkmeier and Schank, 2005).

Nodes, also referred to as vertices, are the fundamental components of a network, representing distinct entities such as genes in a biological network. Edges, also known as links, represent the connections between nodes, illustrating how nodes are interrelated. The degree of a node in a network denotes the count of edges connected to that specific node, serving as a metric for its connectivity and significance within the network. Centrality measures assess the importance of a node within a network, with various types of centrality measures, including degree centrality, betweenness centrality, and eigenvector centrality, capturing different aspects of node significance. Clustering pertains to the inclination of nodes in a network to assemble into tightly interconnected clusters or groups, estimating the degree to which nodes within a cluster exhibit stronger connections to one another compared to nodes in different clusters. Network density, on the other hand, quantifies the level of interconnectivity among nodes by measuring the proportion of actual edges present in relation to all possible edges within a network. Modularity serves as a metric that gauges the existence of modules or communities within a

network, assessing the extent to which nodes within a module form denser connections amongst themselves compared to nodes in other modules. The small-world effect characterizes the phenomenon observed in numerous real-world networks, where nodes tend to form clusters or communities, while still maintaining a relatively short average path length between any two nodes. This property indicates that the network demonstrates both local clustering and global connectivity. Network visualization encompasses the graphical depiction of a network, enabling visual exploration and analysis of its structure, relationships, and patterns. Various layout algorithms and visual encoding techniques are employed to represent nodes, edges, and their attributes in a meaningful and informative manner (Brinkmeier and Schank, 2005).

2.11. Community assembly models

Understanding the ecological processes governing freshwater bacterial community biogeography, diversity, functions, and succession is a central but poorly understood topic in ecology (Nyirabuhoro et al., 2020, 2021). One of the most central questions in ecology is how microbial diversity is produced and maintained (Nemergut et al., 2013; Zhou and Ning ,2017). The processes shaping the diversity among species are largely considered to be ecological processes and are grouped into four central ecological concepts: ecological dispersal, diversification, drift, and selection (Vellend, 2010; Nemergut et al., 2013; Zhou and Ning, 2017).

Dispersal is referred to as the process of continually moving from one place to another and the successful establishment of organisms across space (Nemergut et al., 2013; Zhou and Ning, 2017). Many factors (e.g., environmental filtering and biotic interactions) influence the movement of

organisms; thus, dispersal can be ambiguously treated as being deterministic or stochastic (Nyirabuhoro et al., 2020, 2021). Diversification is an evolutionary process of producing new genetic variation and is situated between speciation and extinction (Nemergut et al., 2013). The importance of diversification is largely ignored in community ecology research because it involves long-term evolutionary processes spanning millions of years for many microbes (Zhou and Ning, 2017). At present, no specific method is available to examine the relative importance of diversification in shaping microbial community structure (Nemergut et al., 2013; Dini-Andreote et al., 2015; Zhou and Ning, 2017). Drift is referred to as stochastic variation with respect to species characteristics in the relative abundances of different species within a community over time due to inborn random processes such as birth, death, and reproduction (Zhou and Ning, 2017). Drift is important when selection is weak and the local community size is small (Evans et al., 2017). Ecological drift is unambiguously stochastic and difficult to test empirically because no species in nature are precisely demographically similar (Zhou and Ning, 2017). Selection is referred to as the ecological forces that change community structure due to fitness differences for example in survival, growth and reproduction among different organisms (Nemergut et al., 2013; Zhou and Ning, 2017). Ecological selection can be generated by deterministic variables (e.g., moisture, pH and temperature) at both local and regional scales and synergistic effects of biotic interactions such as competition, mutualism and predation (Dini-Andreote et al., 2015). Ecological selection can be divided into two main categories: homogeneous and heterogeneous selections. For homogeneous selection, environmental conditions are not changing and little variation in community structure could exist. For heterogeneous selection, environmental conditions change across

space and time, and high variation in community structure is often expected (Zhou and Ning, 2017). Ecological selection is unequivocally not stochastic (Dini-Andreote et al., 2015).

Numerous statistical approaches have been developed to assess the relative importance of environmental influence and dispersal limitation (Zhou and Ning, 2017). Three important types of multivariate statistical approaches are often employed to compare community structure differences between and within treatments: permutational multivariate analysis of variance, analysis of similarities, permutational analysis of multivariate dispersions and ordination methods (e.g., principal-coordinates analysis, nonmetric multidimensional scaling, principal-component analysis and detrended correspondence analysis); correlation-type analyses between community structure and environment variables (e.g., Mantel test, multiple regression on (dis) similarity matrices, redundancy analysis and canonical correspondence analysis); variation partitioning analysis. Multivariate analysis approaches are often compounded by the issue of unmeasured environmental variables because it is very difficult to measure all environmental variables in practice. In variation partitioning analysis, great caution is necessary to partition community variation, and it should be utilized as an exploratory tool together with other techniques such as neutral theory-based models and null model analysis (Zhou and Ning, 2017).

Neutral theory-based process models are one of the major approaches used to infer processes from diversity patterns (Fig. 8). There are over ten different neutral models, each with slightly different predictions for different factors (Nemergut et al., 2013; Zhou and Ning, 2017). The most important one is Hubbell's neutral model, which has only three parameters: population size of the local community; rate of immigration; and fundamental diversity

number. All of these parameters are estimated theoretically and directly from ecological data, but in practice, it is a challenge to do so because quantifying the population size of a metacommunity is problematic (Zhou and Ning, 2017). Further, the rates of migration and speciation cannot be estimated directly; thus, the parameters can only be indirectly quantified by fitting a neutral model to the observed community structure data (Sloan et al., 2006).

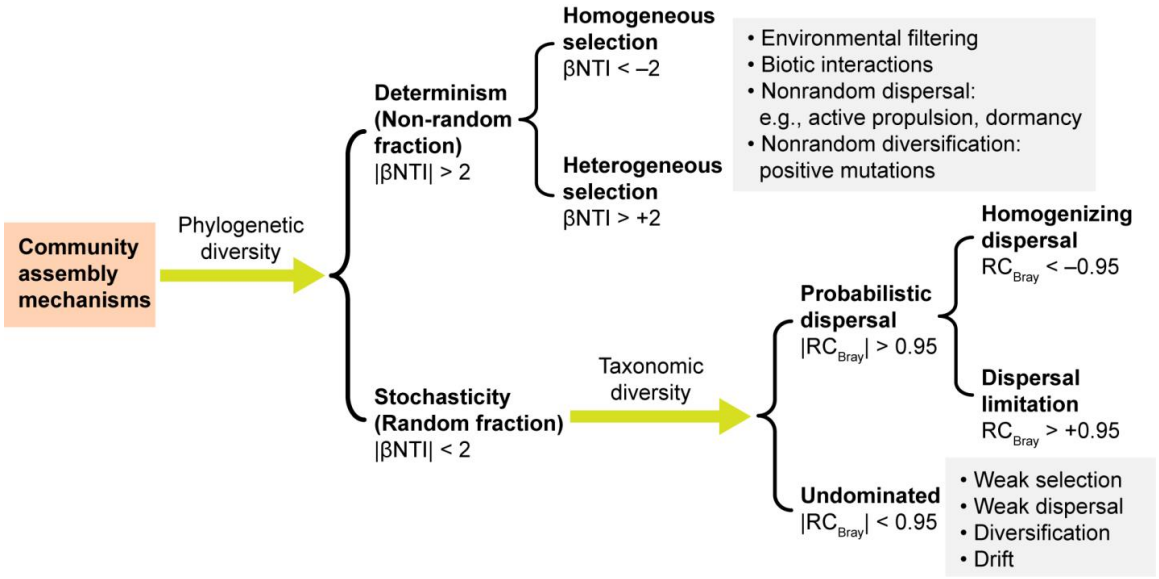

Fig. 8. A diagram showing ecological processes influencing microbial community within the context of the determinism and stochasticity dualism and various steps in partitioning different ecological processes on the basis of both phylogenetic and taxonomic diversity. βNTI, β nearest-taxon index based on a null model test of the phylogenetic β-diversity index β mean nearest-taxon distance; RC_{Bray}, modified Raup-Crick index based on a null model test of the Bray-Curtis taxonomic β-diversity index (Source: Zhou and Ning, 2017).

2.12. Research methodology

The methodology for a research project on DNA-based biomonitoring of microbial communities depends on the specific research questions and study

area but should follow a similar general framework of study area selection, sampling design, sample collection and processing, data analysis, interpretation and discussion, and conclusions and recommendations (Sigee, 2005).

Study area selection involves selecting a specific region or site, such as urban park ponds, where the presence of bacterioplankton will be assessed. Sampling design involves developing a sampling design that is appropriate for the study area and research questions. This could involve selecting a specific sampling method and determining the number and location of sampling sites. Sample collection and processing involve the collection of environmental samples from the selected sites using the chosen sampling method, processing the samples in the laboratory by filtering, washing, or centrifuging to separate the bacterioplankton from other organisms and debris, and using 16S rRNA amplicon sequencing to identify bacterioplankton. Data analysis requires analyzing the OUTs using appropriate statistical methods, such as ordination techniques, and assessing the diversity, abundance, and distribution of the bacterioplankton in relation to environmental variables such as water quality, weather, and air pollution. Interpretation and discussion involve interpreting the results of the data analysis in the context of the research questions and existing literature on the bacterial community and discussing the potential implications of the results for understanding of the determinants of bacterial community in urban park ponds. Conclusions and recommendations involve summarizing the main findings of the study, drawing conclusions about the determinants of bacterial community in urban park ponds, and making recommendations for future research or management actions that could help protect and conserve these organisms and the ecosystems they inhabit.

3. Materials and methods

3.1. Study area description and sampling

This study was conducted in three adjacent ponds located at the main campus of Shenzhen MSU-BIT University (SMBU) in Shenzhen, China (Fig. 9). SMBU pond is built within a wetland that has been transformed into a garden-forest landscape. They are situated on the east bank of the Pearl River estuary in Shenzhen, a sub-provincial city in the south eastern province of Guangdong, China. SMBU pond is located within an urban area covering 1,748 km2, with elevations ranging from 0 to 944 m above sea level. In addition, a geographic area is characterized by a warm, monsoon-influenced, humid subtropical climate, with mild and relatively dry winters attributed, in part, to the South China Sea. SMBU pond experiences very humid and hot weather conditions during the summer, when the monsoon reaches its peak intensity. The average annual temperature and precipitation in the area are 22.4 °C and 1,948 mm, respectively (Zhong et al., 2022).

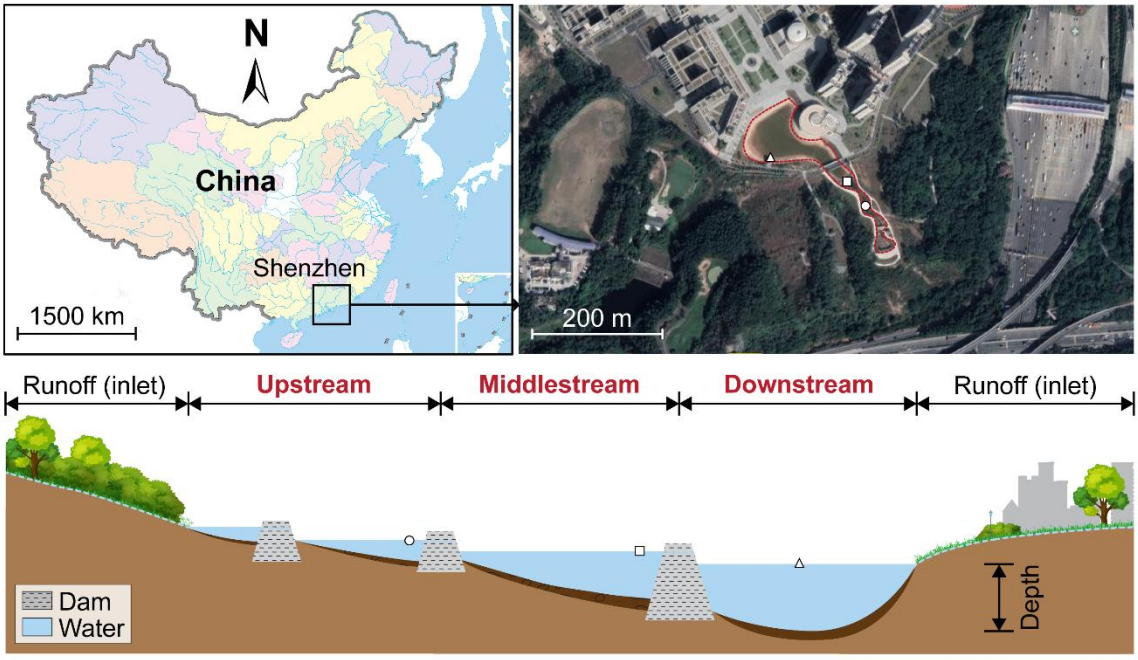

Fig. 9. The study site map (first row, left) showing the locations of the Shenzhen MSU-BIT University (SMBU) pond (first row, right) in Shenzhen, Guangdong, southeast China. The cross-section (second row) of three studied portions of SMBU pond (upstream, midstream, and downstream). The three sampling stations are shown using different symbols. The map was created by QGIS version 3.24.1 (QGIS Development Team (2022).

A total of 30 water samples were collected from three stations in SMBU pond, which are the upstream, midstream, and upstream, in August 2022 at approximately 9:00 a.m. The water samples were first pre-filtered using a 200 μm pore-sized sieve to remove large particles; then, a volume of 600 mL was filtered through a 0.22 μm polycarbonate membrane (47 mm diameter, Millipore, Billerica, MA) using a vacuum filtration system (filtering time: 30–60 min). The filter membranes with microbial plankton were then packed into sterilized tubes and preserved at 80 ◦C until DNA extraction.

Environmental variables that were measured for each pond are typically meteorological variables, air quality parameters, and the physicochemical properties of water. Air temperature and humidity were measured using Live Thermometer version 1.1. Light intensity, wind speed, and atmospheric pressure were measured using Smart Luxmeter version 1.0.7, ZephyrFree WindMeter version 3.1.2, and GPS Essentials version 4.4.64, respectively. Carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide (NO_2), ozone (O_3), sulphur dioxide (SO_2), ammonia (NH_3), particulates PM2.5, and particulates PM10 were measured using Live Thermometer version 1.1. Water depth was estimated using a sounding cable: a plumb bob was attached to a rope, released slowly into the water, and when the plumb bob just touched the rock bottom of the pond, a mark was formed on the rope exactly at the water

level, then the depth was measured by tape. Transparency was measured using a Secchi disc. Water temperature, turbidity, total dissolved solids, electrical conductivity, pH, oxydo-reduction potential, dissolved oxygen saturation, dissolved oxygen concentration, resistivity, and salinity were measured using a multiparameter device (HI 9829, HANNA Instrument Inc., Woonsocket, RI, USA), and the mean-values were computed for further analysis.

3.2. DNA extraction, Illumina sequencing and bioinformatics

Total DNA of bacterioplankton was extracted directly from the membrane using the FastDNA SPIN Kit and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The DNA quality and concentration were tested using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After checking for quality, 20-µl DNA sample was amplified using universal bacterial primers (341F-806R) and subjected to sequencing of the V3-V4 hypervariable region of the bacterial 16S rRNA gene in two library batches on Illumina MiSeq and HiSeq platforms at the Novogene sequencing facility (Novogene Technology Co. Ltd, Beijing, China). Each DNA sample was individually PCR-amplified in triplicated 25 µl reactions included an initial denaturation at 94 °C for 5 min, followed by 25 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C. At the end of the amplification, the amplicons were subjected to final 7 min extension at 72 °C. Each reaction contained 1 × PCR buffer, 2.5 mM dNTPs, 0.625 U of Taq DNA polymerase, 10 µM of each primer, and 20 ng of target DNA. The triplicate PCR products were pooled together, and sequencing was performed on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) using 2 × 250 bp paired-end sequencing

approach. The removal of barcode and primer sequence was carried out in QIIME 1.9.1 (Caporaso et al., 2010).

Bioinformatic analyses of 16S rRNA gene sequences were conveyed using VSEARCH (Rognes et al., 2016). Chimeras were discarded using default settings in VSEARCH from a set of unique sequences to construct biologically corrected sequences. Quality-filtered sequences were assigned to OTUs at a 97% sequence similarity threshold. The OTU taxonomies were assigned using the sintax algorithm on query sequences mapped against the Greengenes database (DeSantis et al., 2006). Unknown OTUs were removed before the downstream analyses. The resulting OTU tables were subjected to subsequent processing for singleton, archaea, chloroplast, and mitochondrial sequence removal. Finally, the bacterial sequences were normalized to the same number of sequences (48378 per sample), and 11903 OTUs at a 97% sequence similarity level were obtained. In addition, the bacterioplankton community was categorized into three categories: abundant taxa (AT), conditionally rare taxa (CRT), and rare taxa (RT) (Nyirabuhoro et al., 2020).

3.3. Definition of abundant and rare taxa

The differentiation of the rare from the abundant biosphere is not based on a specific or fixed threshold of relative abundance; thus, the cutoff point is artificial (Pedrós-Alió, 2012; Lynch and Neufeld, 2015). The selection of thresholds may vary depending on the methodology used to identify species or the purpose of the research (Reid and Buckley, 2011). Some studies have defined rare taxa using a cutoff of 0.1% as a local relative abundance (Fuhrman, 2009; Vergin et al., 2013), while others have used a threshold of 0.01% (Mangot et al., 2013; Logares et al., 2014, 2015; Liu et al., 2015). Moreover, a relative abundance threshold of 1% has frequently been utilized

to define abundant species in numerous investigations (Vergin et al., 2013; Logares et al., 2014; Liu et al., 2015). For this study, the bacterial community was divided into different categories of taxa by setting a local relative abundance threshold of 1% for abundant taxa and 0.01% for rare taxa. Our community was artificially classified into five categories (Dai et al., 2016; Chen et al., 2017; Xue et al., 2018; Liu et al., 2019): (i) always abundant taxa (AAT), OTUs with a relative abundance ≥ 1% in all samples; (ii) conditionally abundant taxa (CAT), OTUs with relative abundance ≥ 1% in some samples and ≥ 0.01% in other samples, but never being rare; (iii) conditionally abundant or rare taxa (CRAT), OTUs with relative abundance from rare < 0.01% to abundant ≥1% in samples; (iv) conditionally rare taxa (CRT), OTUs with < 0.01% local relative abundance in some samples and ≥ 0.01% in others but never ≥ 1%; (v) rare taxa (RT), OTUs with relative abundance < 0.01% in all samples (Table 1). To simplify the analysis, OTUs with relative abundance > 1% at least once in a sample (i.e., AAT, CAT, and CRAT) were combined together as abundant taxa (AT) in this study **(**Nyirabuhoro et al., 2020**).**

Table 1. Criteria used for categorizing the different taxa within bacterioplankton, and number of total OTUs and associated sequences represented by each taxon.

Taxa	Selection Criteria
Abundant taxa (AT)	
–	≥ 1% local relative abundance at least once
Always abundant taxa	≥ 1% local relative abundance always
Conditionally abundant taxa	

	≥ 1% local relative abundance in some samples and > 0.01% in all samples
Conditionally rare and abundant taxa	≥ 1% local relative abundance in some samples and < 0.01% in others
Moderate taxa (MT)	
–	< 1% and > 0.01% local relative abundance in all samples
Conditionally rare taxa (CRT)	
–	< 1% and < 0.01% local relative abundance in samples
Rare taxa (RT)	
–	< 0.01% local relative abundance always
All taxa	–

The selected taxa categories for this study are in bold.

3.4. Statistical analyses

The bacterioplankton diversity was assessed using the Shannon-Wiener index, a widely used metric in ecological studies due to its sensitivity to differences between sites (Magurran, 1988). To compare the Shannon-Wiener index across the three stations, we used the Mann-Whitney U test with a statistical significance level of $P < 0.05$. NMDS based on Bray-Curtis was applied to investigate differences in bacterioplankton communities among three stations. The degree of separation of bacterial community composition

across the seasons was tested with ANOSIM. The R-value is supposed to vary between 0 and 1. Values of R closer to 1 indicate complete separation of sample groups, while values near 0 indicate no separation between groups. Both NMDS and ANOSIM were performed in PRIMER 6.0 software. The bacterial community dynamics at phylum and genus levels of taxonomic resolution were quantified using relative abundance data in R version 4.3.0 (R Core Team, 2023).

To examine the correlation between bacterioplankton (sequence data) and environmental variables, Pearson correlation coefficients were calculated with a significance level set at $P < 0.05$. In addition, RDA and VPA in R version 4.3.0 (R Core Team, 2023) were used to quantify the bacterial community response to environmental conditions. VPA was implemented by removing collinearity shown by the variance inflation factor (> 10) and using sequence data of bacterioplankton at the subcommunity level, significant environmental variables ($P < 0.05$) in RDA, and three groups of environmental variables: weather, physical, and chemical parameters.

The importance of stochastic and deterministic processes in shaping bacterial communities was assessed using the neutral community model (Sloan et al., 2006) and the null model (Gotelli and Mccabe, 2002), respectively. For the Sloan neutral community model, the parameters *N*, *m*, and *Nm* describe the metacommunity size, immigration rate, and dispersal between communities, respectively. The least-square method was employed to determine the best-fit distribution curve of the neutral model in the R environment (R Core Team, 2023). To calculate the difference between observed and simulated communities and the standardized effect size in the null model, we utilized sequence data, the C-score metric, 5000 random

matrices, SIM9 algorithm, and EcoSim Professional version 1.0 (Entsminger, 2014).

To analyze the co-occurrence patterns in different bacterioplankton subcommunities, we performed the network analysis in the R environment (R Core Team, 2023). The relationships between OTUs were calculated using Spearman's rank correlations, and only strong and statistically significant correlations ($|r| > 0.6$, $P < 0.01$) were integrated into the networks, which were visualized and analyzed using Gephi version 0.9.2 (Bastian et al., 2009). To characterize the network topology, we calculated the degree, betweenness, and closeness across different bacterioplankton subcommunities using the Wilcoxon rank-sum test with a significant difference of $P < 0.05$ (Xue et al., 2018).

4. Results

4.1. Temporal dynamics of environmental conditions

Table 2, which displays the mean and standard errors of 25 measured environmental variables at each station (n = 10) and across the three stations in SMBU pond (n = 30), provides a more comprehensive overview of different environmental conditions in a subtropical endorheic urban pond. At all stations, the meteorological variables indicate relatively warm conditions, which are characteristic of a humid subtropical climate. The variation in air temperature, light intensity, wind speed, humidity, and air pressure across the three stations ranged from 27 to 29 °C, 26336.5 to 51835.6 lx, 1.42 to 1.78 m s^{-1}, 76.8 to 80.1%, and 999.905 to 999.946 kPa, respectively. The concentrations of carbon monoxide, nitrogen monoxide, nitrogen dioxide, ozone, sulphur dioxide, ammonia, particulates PM10, and particulates PM2.5 varied across the three stations, with ranges of 525.448 to 624.181, 9.815 to 21.805, 22.647 to 31.642, and 37.561 to 58.746, 47.659 to 73.313, 0.068 to 0.229, 57.795 to 90.22, and 48.969 to 75.765 μg m^3, respectively, indicating the air quality differences among the sampling sites. The pond was generally shallow, with warm water temperatures ranging from 27.993 to 30.399 °C. It was also transparent, well-oxygenated, and had variable pH levels.

Table 2. The mean values plus standard errors of twenty-five measured environmental variables in the garden of Shenzhen MSU-BIT University.

Parameter	Upstream	Middle stream	Downstream
Air temperature (°C)	29.02 ± 0.72	28.2 ± 0.749	27.15 ± 0.744

Light intensity (lx)	51835.6	26336.5	29286.4
	± 15435.64	± 9897.895	± 11038
Wind speed (m/s)	1.78	1.42	1.49
	± 0.269	± 0.179	± 0.282
Humidity (%)	76.8	78.4	80.1
	± 2.867	± 2.941	± 2.233
Pressure (hPa)	999.905	999.912	999.946
	± 0.577	± 0.574	± 0.574
Carbon monoxide (μg m^3)	525.448	624.181	602.151
	± 60.324	± 78.504	± 94.941
Nitrogen monoxide (μg m^3)	9.815	20.51	21.805
	± 8.358	± 9.816	± 11.657
Nitrogen dioxide (μg m^3)	26.175	31.642	22.647
	± 4.784	± 5.636	± 4.403
Ozone O_3 (μg m^3)	58.746	37.561	38.141
	± 21.465	± 17.848	± 19.426
Sulfur dioxide (μg m^3)	56.743	73.313	47.659
	± 16.954	± 17.683	± 14.823
Ammonia (μg m^3)	0.112	0.068	0.229
	± 0.05	± 0.03	± 0.066
Particulates PM10 (μg m^3)	57.795	74.674	90.22
	± 16.928	± 20.51	± 28.419
Particulates PM2.5 (μg m^3)	48.969	62.873	75.765
	± 15.828	± 18.144	± 26.076
Water depth (m)	0.469	0.49	1.187
	± 0.016	± 0.012	± 0.075

Transparency (m)	0.396 ± 0.031	0.42 ± 0.031	0.898 ± 0.098
pH	6.644 ± 0.062	7.047 ± 0.07	8.048 ± 0.086
Oxydo-reduction potential (mV)	173.09 ± 10.727	169.17 ± 10.553	133.02 ± 10.753
Dissolved oxygen saturation (%)	73.82 ± 12.258	96.43 ± 11.586	198.73 ± 18.793
Dissolved oxygen concentration (ppm)	5.663 ± 1.017	7.03 ± 0.736	13.672 ± 0.981
Electrical conductivity (µs cm^{-1})	407.7 ± 31.186	370.1 ± 38.367	192.6 ± 11.025
Resistivity (MΩcm)	0.003 ± 0	0.003 ± 0	0.005 ± 0
Total dissolved solids (ppm)	205 ± 15.496	185.2 ± 19.163	96.6 ± 5.518
Salinity (PSU)	0.196 ± 0.015	0.175 ± 0.019	0.087 ± 0.005
Turbidity (FNU)	8.710 ± 3.076	8.56 ± 2.304	3.58 ± 0.728
Water temperature (°C)	27.993 ± 0.402	29.097 ± 0.425	30.399 ± 0.421

The averaging time for each air pollutant (i.e., carbon monoxide, nitrogen monoxide, nitrogen dioxide, ozone, sulphur dioxide, ammonia, particulate matter 10, or particulate matter 2.5) is one hour; however, the results are interpreted using the annual limits for environmental air pollutants set by the World Health Organization in its guidelines for global air quality (World

Health Organization, 2021) and the China National Ambient Air Quality Standard (GB3095-2012).

4.2. Temporal dynamics of bacterioplankton community

The rarefaction curves generated for similarity-based OTUs with a sequence similarity level of 97% showed the adequacy of the sampling effort and compared the microbial community diversity across the samples (Fig. 10).

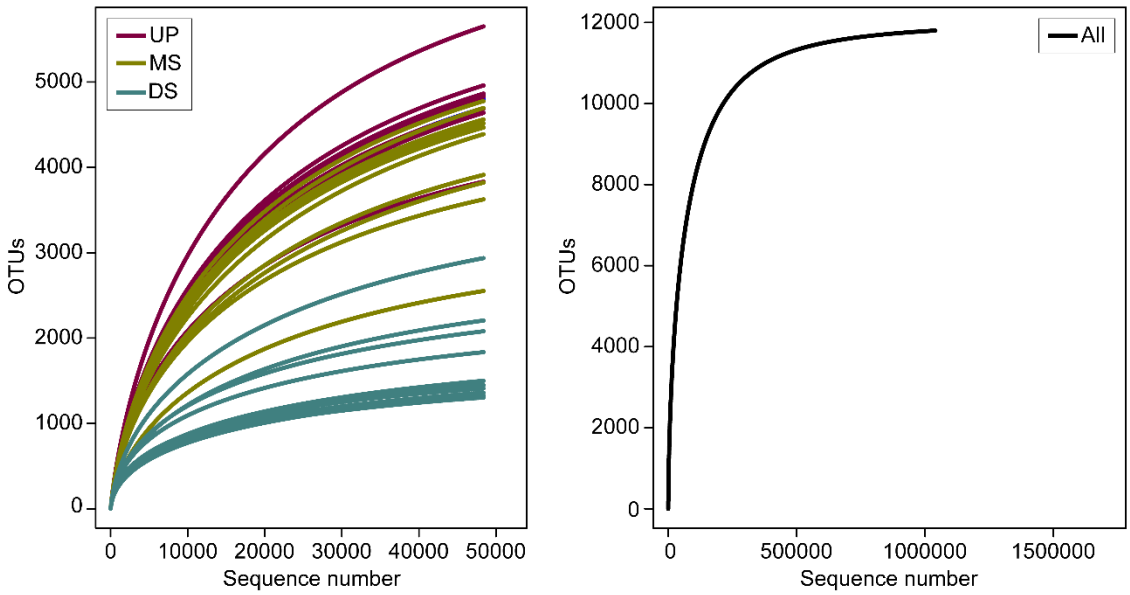

Fig. 10. Rarefaction curves of similarity-based operational taxonomic units (OTUs) at 97% sequence similarity level. Left - the individual samples, right – the combined set of 30 samples. Sampling stations: US, upstream; MS, midstream; DS, downstream.

A total of 11,903 OTUs were obtained from 30 samples collected at three stations. Among them, AT, CRT, and RT subcommunities accounted for 117, 6,774, and 5,012 OTUs, respectively (Fig. 11a). Shannon-Wiener index exhibited a substantial change over time, particularly in CRT and RT subcommunities, indicating a significant difference among the three stations ($P < 0.01$). In CRT, the mean plus standard error values for the Shannon-

Wiener index were 7.20 ± 0.04, 6.96 ± 0.07, and 6.01 ± 0.09 at the upstream, midstream, and downstream locations, respectively. For RT, the corresponding values were 6.94 ± 0.05, 6.74 ± 0.09, and 5.40 ± 0.15 (Fig. 11b). The bacterioplankton community showed substantial dissimilarity within three taxa categories, with a significant difference among groups of samples (AT: Global R = 0.693, *P* = 0.001; CRT: Global R = 0.741, *P* = 0.001; RT: Global R = 0.599, *P* = 0.001) (Fig. 11c).

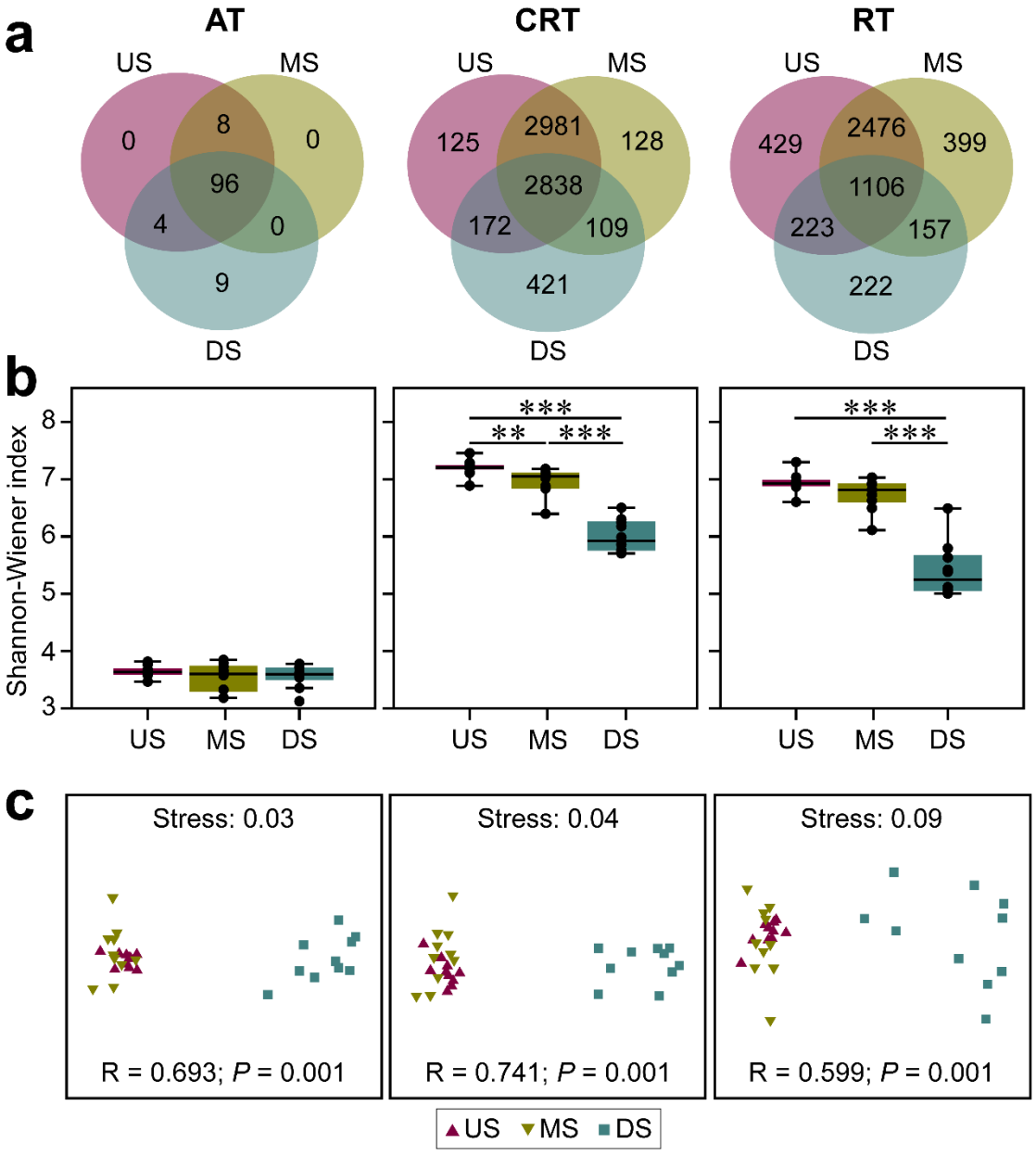

Fig. 11. Community structuring of bacterioplankton. (a) Venn diagram showing the numbers of unique and shared OTUs between three different groups of samples. (b) Shannon-Wiener index across three different groups of samples. Stars indicate a significant difference at $^{**}P<0.01$ $^{***}P<0.001$ according to Tukey's post-hoc test. (c) Non-metric multidimensional scaling (NMDS) plots based on the Bray-Curtis distance of taxonomic composition of bacterial communities. Sampling stations: US, upstream; MS, midstream; DS, downstream. Taxa categories: AT, abundant taxa; CRT, conditionally rare taxa; RT, rare taxa.

The bacterioplankton community exhibited significant temporal changes across all taxa categories (Fig. 12). The dominant phyla, Actinobacteria and Proteobacteria, showed slight temporal variation, with Actinobacteria ranging between 13.72% and 52.85% and Proteobacteria ranging between 12.65% and 52.28%. Cyanobacteria, on the other hand, displayed substantial fluctuations over time, with a high relative abundance of 51.53% on the 223rd Julian day of 2022 and a low relative abundance of 3.06% on the 234th Julian day of 2022. The temporal change at the genus level was remarkable for *Synechococcus*, which peaked on the 223rd Julian day of 2022 with a relative abundance of 35.22% (Fig. 12).

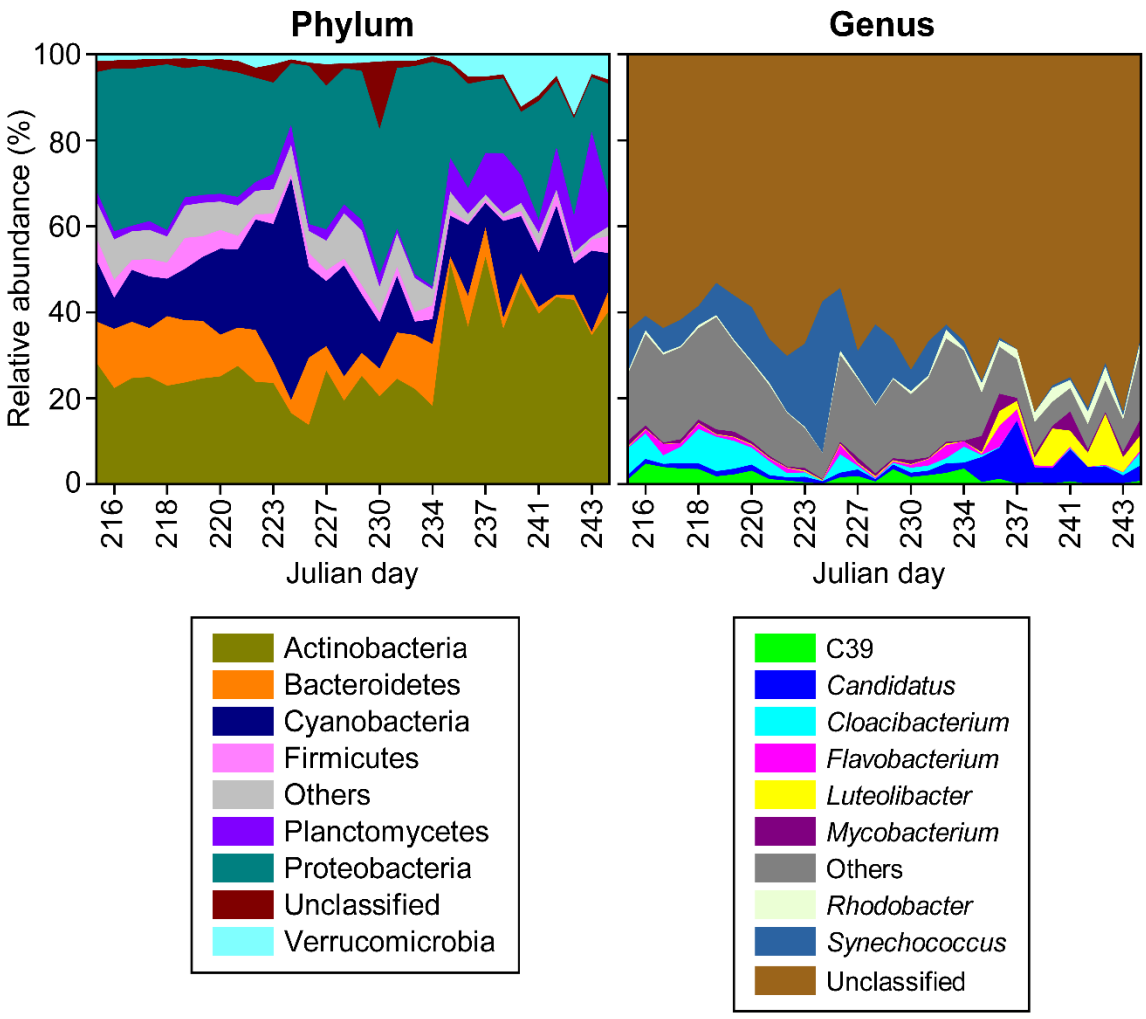

Fig. 12. Temporal variation of the bacterial community in SMBU pond in August 2022. The dynamics of the community are shown at the phylum and genus levels of taxonomic resolution.

4.3. Relationships between bacterioplankton and the environment

The bacterial abundance was strongly correlated with water physicochemical parameters, especially water depth, transparency, pH, dissolved oxygen, electrical conductivity, salinity, and water temperature, as well as a few meteorological and air quality parameters (Fig. 13). Actinobacteria showed significant positive correlations with ammonia, water depth, transparency, pH, and dissolved oxygen ($P < 0.05$), and significant

negative correlations with electrical conductivity and salinity ($P < 0.01$). Proteobacteria exhibited significant positive correlations with oxydo-reduction potential, electrical conductivity, and salinity ($P < 0.05$), and significant negative correlations with water depth, pH, and dissolved oxygen ($P < 0.01$). There was no significant correlation found between cyanobacteria and any environmental variable. Moreover, *Synechococcus* showed only a negative correlation with NH_4, water depth, transparency, and pH.

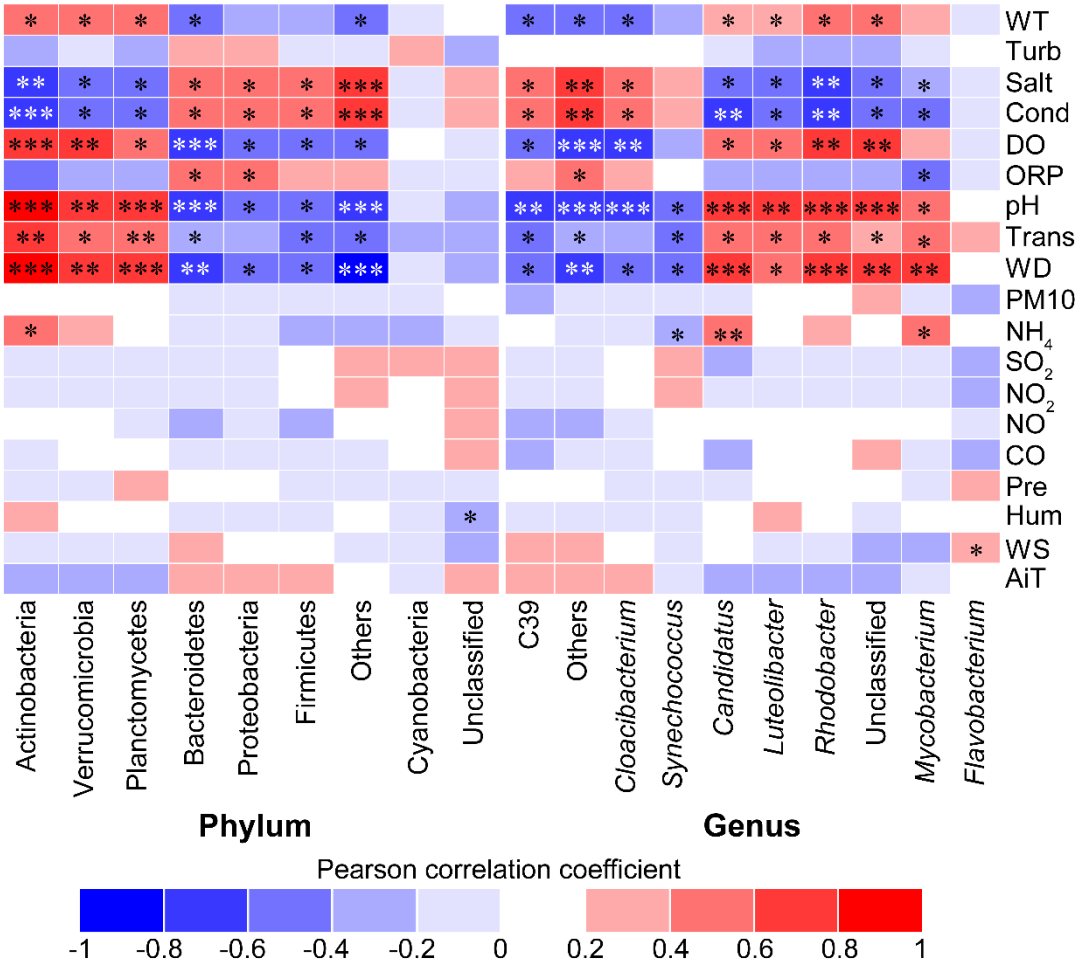

Fig. 13. Heat maps showing Spearman correlation coefficients between environmental variables and bacterial relative abundance (sequence data) at phylum and genus levels, respectively. Significant levels: *P<0.05; **P<0.01; ***P<0.001. AiT, air temperature; WS, wind speed; Hum, humidity; Pre,

pressure; CO, carbon monoxide; NO, nitrogen monoxide; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; NH_4, ammonia; PM10, particulates PM10; WD, water depth; Trans, transparency; ORP, oxydo-reduction potential; DO, dissolved oxygen concentration; Cond, electrical conductivity; Sal, salinity; Tur, turbidity; WT, water temperature.

Water physicochemistry and weather were responsible for explaining significant changes in the composition of the bacterioplankton community across the three sites (Fig. 14). Water physicochemistry accounted for the highest proportion of community variation in both AT, CRT, and RT subcommunities, with proportions of 0.8%, 0.01%, and 0.02% based on pure variances, respectively. The weather accounted for 0.2%, 0.6%, and 0.3% of the pure variances in the AT, CRT, and RT subcommunities, respectively.

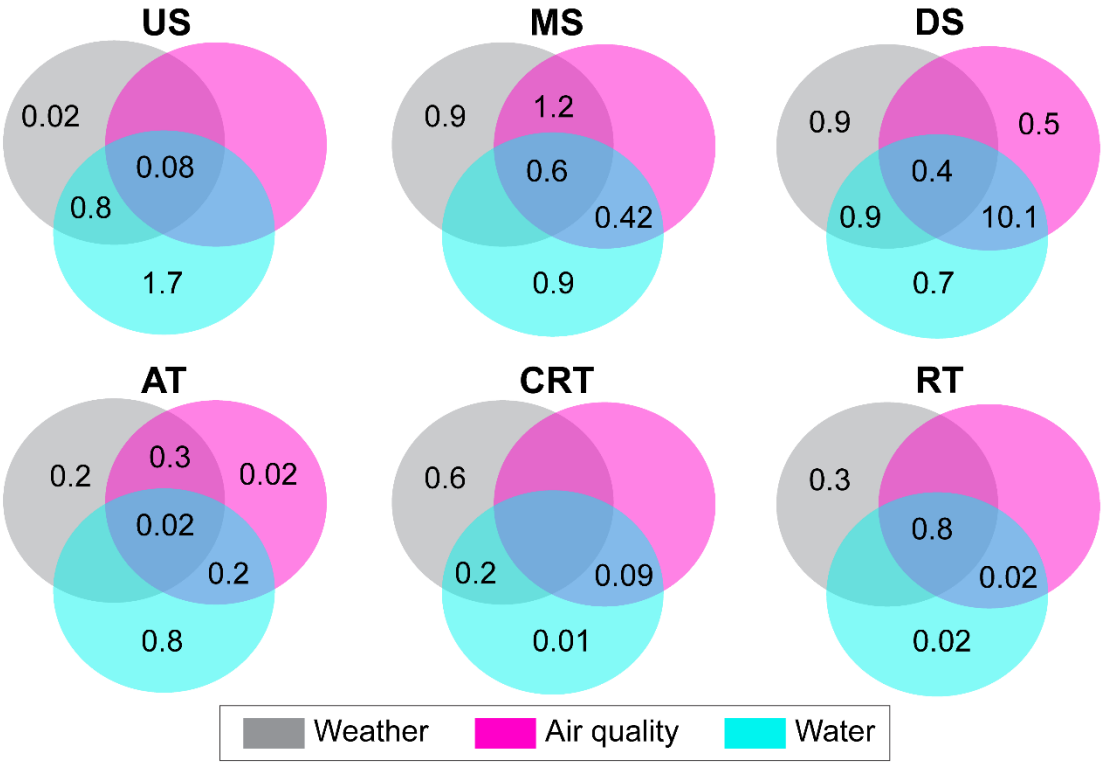

Fig. 14. The Venn diagrams showing the results of variation partitioning analysis (VPA) based on the bacterioplankton community in the pond and in

the three subcommunities, and three groups of environmental variables (weather, air quality, and water physiochemistry). Note that the variances < 0.01 and residuals are not displayed for simplicity. Sampling stations: US, upstream; MS, midstream; DS, downstream. Taxa categories: AT, abundant taxa; CRT, conditionally rare taxa; RT, rare taxa.

4.4. Co-occurrence patterns and community assembly mechanisms

The co-occurrence patterns among OTUs showed potential connections between bacterioplankton, indicating complex ecological networks of interacting species that change over time (Fig. 15). The networks had a total of 9,080 nodes and 78,340 edges (n = 30). Furthermore, six major selected modules contributed a total of 78.04% of nodes (i.e., OTUs), with each module's contribution varying between 6.65% and 23.98%. The values for betweenness centrality varied across the bacterioplankton subcommunities, with 19,482 ± 4,187 for AT, 24,562 ± 800 for CRT, and 27,304 ± 1,108 for RT. Similarly, the values for closeness centrality were 0.021 ± 0.016 for AT, 0.047 ± 0.003 for CRT, and 0.065 ± 0.004 for RT. In terms of degree centrality, the highest value was observed in AT, with a value of 79.8 ± 8.5 for AT, 23.3 ± 0.6 for CRT, and 7.9 ± 0.1 for RT.

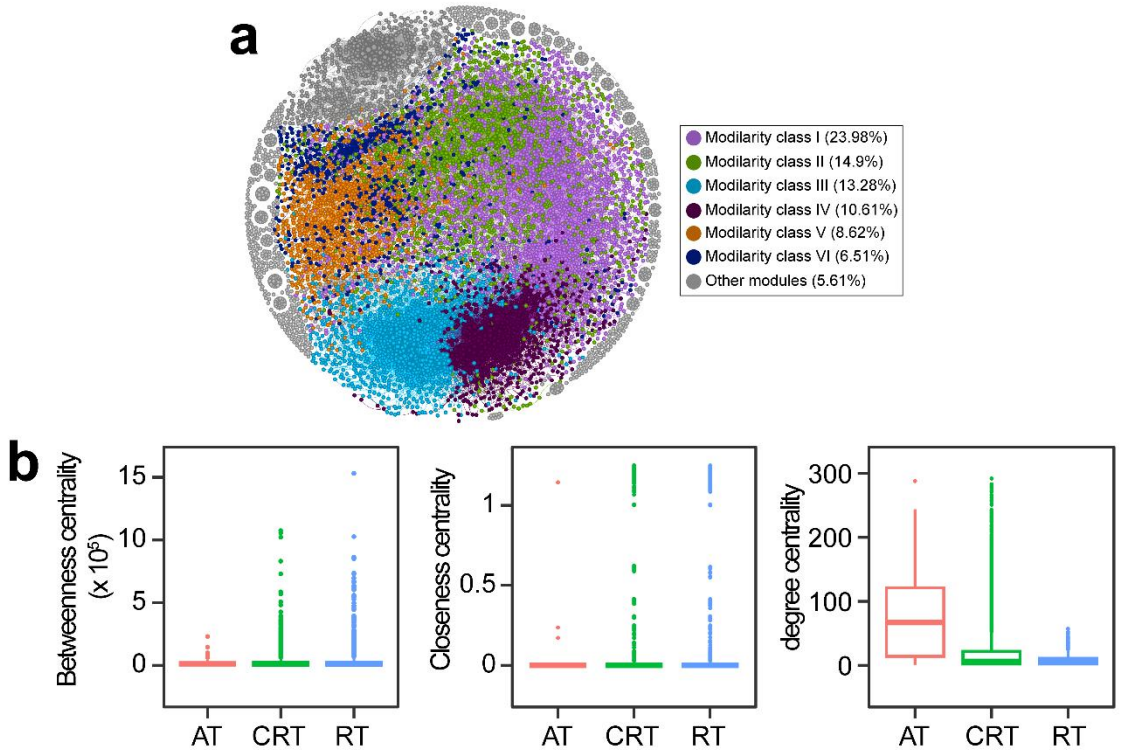

Fig. 15. The co-occurrence patterns among OTUs revealed by network analysis. Properties of the correlation-based network. (a) The nodes were colored according to different types of modularity classes. A connection stands for a strong (Spearman's $r > 0.8$ or $r < -0.8$) and significant (P-value < 0.01) correlation. The size of each node is proportional to the number of connections (i.e., degrees). (b) Comparison of node-level topological features among four different subcommunities. Taxa categories: AT, abundant taxa; CRT, conditionally rare taxa; RT, rare taxa.

The neutral community model, which investigates the degree to which bacterioplankton communities are influenced by stochastic processes, revealed a high degree of fit across the three sites, with values ranging between 72.1% and 77.5% of the explained community variance (Fig. 16). At the subcommunity level, the fit was relatively low. Specifically, in AT, the explained community variance was less than zero, indicating that there was

no fit to the neutral model. In CRT and RT, the values were 40% and 18.1%, respectively.

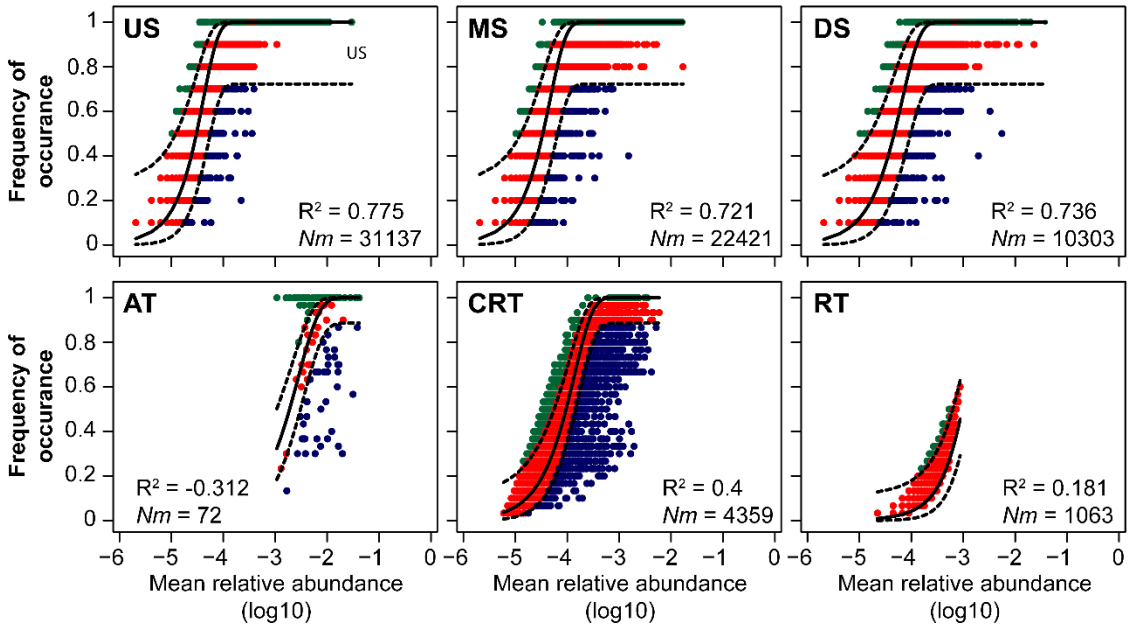

Fig. 16. The frequency of occurrence in the bacterial community OTUs as a function of mean relative abundance in the pond and across different categories of taxa. Lines indicate the best fit for the neutral community model. *Nm* indicates the size of the metacommunity multiplied by the immigration rate. R^2 indicates the fit to the neutral model, and negative R^2 values indicate no fit to this model. The X axis was log-transformed. Sampling stations: US, upstream; MS, midstream; DS, downstream. Taxa categories: AT, abundant taxa; CRT, conditionally rare taxa; RT, rare taxa.

5. Discussion

5.1. Dynamics of rare and abundant bacterioplankton subcommunities

Bacterioplanktonic communities, consisting of a few abundant taxa (AT) and many CRT and RT, are a central component of aquatic environments (Liu et al., 2015; Wang et al., 2020). However, understanding the processes of abundant and rare bacterioplankton dynamics dictates the use of high-frequency measurements (Nyirabuhoro et al., 2021). The high-frequency sampling, conducted twice weekly, facilitated the identification of diverse temporal variations in the AT, CRT, and RT sub-communities in the SMBU pond (Fig. 12). These findings suggest that high-frequency sampling is crucial to capturing the build-up and breakdown of episodic shifts in bacterioplanktonic communities (Nyirabuhoro et al., 2020, 2021). Our observations align with the findings of Avila et al. (2017), who demonstrated that fundamental patterns of seasonal changes and successions of bacterioplankton communities are typically reflected only in data obtained through low-frequency sampling. Indeed, our observations are consistent with the findings of Lindh et al. (2015), who also emphasized that these patterns may not accurately capture sharp transitions in certain microbial taxa. This is due to the rapid responses exhibited by microbial communities in response to environmental variations, with such interactions occurring at short temporal scales, ranging from hours to days.

Our chosen sampling strategy (i.e., twice a week) further emphasizes the significance of employing high-resolution time series analysis in microbial community studies (Sigee, 2005). This approach enables the detection of rapid changes resulting from species interactions as well as prolonged dynamics attributed to shifts in overall ecological conditions (Mo et al., 2021).

Moreover, compared to previous strategies, our sampling strategy, which involves regular sampling intervals and frequent sampling of bacterial communities, enhances the characterization of AT, CRT, and RT subcommunity dynamics (Liu et al., 2015; Jiao et al., 2018; Nyirabuhoro et al., 2020).

All taxonomic groups, including phyla and genera, exhibited significant daily fluctuations, and these dynamics remained consistent over a one-month period (Fig. 12). This suggests that the bacterial community in the endorheic pond can undergo temporal dynamics over the short term, similar to reservoirs or lakes (Sigee, 2005). In comparison to previous research, our findings contribute to a deeper understanding of the temporal patterns exhibited by bacterial communities across different timescales. Specifically, our results suggest the presence of a potential seasonal cycle pattern, particularly during warm and cold conditions in the subtropics (Mo et al., 2021; Nyirabuhoro et al., 2021).

The temporal dynamics of the bacterioplankton community, as revealed by the Shannon-Wiener diversity index (Fig. 11b), indicate that CRT and RT subcommunities made significant contributions. Additionally, it suggests that rare taxa have the ability to recover and thrive in response to favorable environmental conditions (Fig. 13), leading to an increase in their relative abundance (Sigee, 2005). Liu et al. (2015), Wang et al. (2020), and Nyirabuhoro (2021) propose that this phenomenon is more likely to occur in subtropical regions, particularly under warm and eutrophic conditions. They suggest that certain rare taxa have adaptations to low-nutrient environments, while others may prefer high temperatures or low pH levels. These adaptations and preferences increase their likelihood of thriving and contributing to the community dynamics in such subtropical regions. Our

findings are also consistent with previous studies that underscore the importance of rare taxa in community dynamics and their ability to enter a state of dormancy when faced with unfavorable conditions (Lennon and Jones, 2011; Shade et al., 2012; Wang et al., 2020).

Indeed, competition among microbial species for limited resources can be a potential explanation for the observed temporal dynamics in the bacterioplankton community (Nyirabuhoro, 2021). In a competitive environment, certain microbial species may outcompete others for resources, leading to changes in relative abundances and community composition (Nemergut et al., 2013; Dini-Andreote et al., 2015; Zhou and Ning, 2017). This competitive interaction can drive shifts in the dominant taxa and contribute to the temporal patterns observed in the bacterioplankton community (Nyirabuhoro, 2020, 2021). Additionally, competition can induce alterations in community compositions over time, with certain species potentially becoming more dominant while others experience a decrease in abundance or complete disappearance (Wikner and Hagström, 1999; Joint et al., 2002).

The significant variation in Bray-Curtis dissimilarity observed among numerous samples of AT, CRT, and RT subcommunities (Fig. 11c) obviously indicates the high responsiveness of each subcommunity to changes in various environmental conditions at a fine temporal scale (Jiao et al., 2017; Xue et al., 2018). This finding further emphasizes the sensitivity and adaptability of bacterioplankton to their immediate surroundings, highlighting their remarkable capability to respond and adjust to even subtle changes in environmental conditions (Lennon and Jones, 2011; Shade et al., 2012; Wang et al., 2020). Furthermore, our observations are consistent with the findings of Lennon and Jones (2011), which suggest that the adaptability of different

bacterioplankton subcommunities is attributed to their distinct physiological and metabolic characteristics exhibited by each phylo-type. As a result, the unique physiology and metabolic functions of these bacterioplankton groups enable them to respond and adjust to variations in their environment, ultimately contributing to the overall dynamics and resilience of the microbial community.

5.2. Bacterial community response to environmental variation

The dominance of Proteobacteria and Actinobacteria in a microbial community, as indicated in Fig. 12, can be attributed to various factors. These include adaptation to environmental conditions, metabolic versatility, and ecological interactions. These bacterial groups have evolved mechanisms to effectively respond to variations in temperature, pH, salinity, and other physicochemical parameters that are characteristic of subtropical environments (Mo et al., 2018; Nyirabuhoro, 2020, 2021). Furthermore, Proteobacteria and Actinobacteria may engage in mutualistic relationships, where they derive benefits from associations with other microbes, or competitive interactions, where they outcompete other bacterial groups for resources. These interactions contribute to their ability to establish dominance and shape the bacterioplankton community composition in subtropical aquatic environments (Liu et al., 2015; Mo et al., 2018; Nyirabuhoro, 2020, 2021; Wang et al., 2020). The dominance of certain genera, such as *Synechococcus*, during specific times can be attributed to the availability of nutrients in the environment. These nutrients facilitate the abundant reproduction of rare taxa under favorable environmental conditions, leading to the dominance of specific genera at those times (Ruiz-González et al., 2015).

Previous research on rare and abundant bacterioplankton communities primarily focused on the biogeography across different spatial scales and along various environmental gradients (Gilbert et al., 2012; Liu et al., 2015; Logares et al., 2013; Mo et al., 2018; Wang et al., 2020). However, in specific geographic locations like the subtropics, the extent to which temporal changes in bacterial communities are impacted by shifts in environmental conditions across space may raise questions (Liu et al., 2015; Wang et al., 2020). Hence, the varying environmental conditions across the three stations in SMBU pond (Table 2), together with the correlations between several physicochemical variables and bacterioplankton subcommunities (Fig. 13), provide insights into how spatial variation can potentially influence the composition of the bacterioplankton community in an endorheic pond.

The pure variance, accounting for 0.8% in AT subcommunity (Fig. 14), underscores the significance of water physicochemistry as a crucial factor in shaping the bacterioplankton community in endorheic ponds. Similarly, the pure variance of 0.6% and 0.2% for CRT and RT, respectively (Fig. 14), highlights the importance of weather in shaping bacterioplankton subcommunities in endorheic ponds. These findings suggest that both water physicochemistry and weather conditions can have a significant impact on driving the dynamics of the bacterioplankton community in endorheic ponds, similar to what is observed in urban reservoirs (Nyirabuhoro, 2021). On the other hand, the low proportion of explained variation (Fig. 14) suggests that there are other factors beyond the measured environmental conditions that contribute to the complexity of bacterioplankton community dynamics (Mo et al., 2018). Firstly, there are measured environmental variables that were not included in the analysis. For example, certain environmental variables have been excluded from RDA and VPA due to issues of multicollinearity. These

omitted variables could potentially have a significant influence on the dynamics and composition of the bacterioplankton community. Additionally, the influence of species interactions within the bacterioplankton community cannot be fully quantified using methods like RDA and VPA. This is because bacterioplankton species can engage in various complex interactions, such as competition, predation, and cooperation, which can have significant effects on the dynamics of the community. These interactions may not be fully captured by the measured environmental variables alone, underscoring the importance of considering species interactions when interpreting the dynamics of the bacterioplankton community (Zhou and Ning, 2017).

In general, variations in the physicochemical characteristics of water and meteorological variables explain the observed variations in AT, CRT, and RT subcommunities in endorheic ponds through various mechanisms. Firstly, water quality parameters such as temperature, pH, dissolved oxygen, and nutrient concentrations have a direct impact on the growth and metabolism of bacterioplankton. So, any changes in these physicochemical parameters can result in a shift in the composition of the bacterial community, leading to changes in the relative abundance of different subcommunities (Sigee, 2005). Secondly, weather conditions such as temperature, precipitation, and wind can also have an indirect effect on the bacterial community through their impact on water quality parameters. For example, rainfall can increase the amount of nutrients and organic matter entering the aquatic environment, which can stimulate the growth of certain bacterioplankton groups (Mo et al., 2018; 2021). Lastly, high temperatures can increase the metabolic rates of some bacterioplankton, leading to changes in community composition (Nyirabuhoro et al., 2021).

5.3. Assembly mechanisms underlying occurrence of rare and abundant bacterioplankton

The integration of network analyses and relative importance of ecological processes allows for a more comprehensive understanding of how these processes shape microbial communities (Konopka et al., 2015). Furthermore, the occurrence of rare and abundant bacterioplankton in the environment is typically influenced by community assembly processes, which play a crucial role in determining the composition of microbial communities (Mo et al., 2021; Nyirabuhoro et al., 2021). These community assembly processes play a crucial role in determining which species become rare and which become abundant within a given community (Zhou and Ning, 2017). The co-occurrence patterns among OTUs in the bacterioplankton communities of SMBU pond clearly indicate the presence of complex ecological networks where different bacterioplankton species interact with each other (Fig. 15). These co-occurrence patterns further provide evidence of interactions that are likely to be shaped by stochastic processes, similar to other freshwater systems in urban environments (Isabwe et al., 2018; Mo et al., 2021; Nyirabuhoro et al., 2021).

The use of a Sloan neutral community model resulted in higher R^2-values (Fig. 16), suggesting that a substantial portion of the bacterioplankton community variation across three stations and within CRT and RT subcommunities could be explained by neutral processes (Isabwe et al., 2018). This further suggests that factors such as migration, births, and deaths within the population play a crucial role in shaping the dynamics of the bacterioplankton community (Zhou and Ning, 2017). While the importance of stochastic processes in shaping microbial community assembly in freshwater is often found to be low in numerous studies (Chisholm and Pacala, 2010;

Roguet et al., 2015; Isabwe et al., 2018), it is important to acknowledge that stochastic processes can still have an impact on the community dynamics, especially during times of environmental change, such as natural disasters and extreme weather events (Zhou and Ning, 2017).

5.4. Ecological implication and recommendations for future research

Endorheic ponds are important ecosystems with their own unique characteristics and ecological significance (Ordónez et al., 1994; Martin-Rosales and Leduc, 2003; Seeboonruang, 2014). The dynamics of the bacterioplankton community in these ponds are influenced by changes in environmental conditions that are specific to this type of ecosystem. Fluctuations in water levels, salinity, temperature, and nutrient availability, among other factors, were found to be important in studied subtropical urban endorheic pond. These environmental changes revealed a significant impact on the composition, abundance, and diversity of the bacterioplankton community in water. Changes in the structure and diversity of the bacterioplankton community can serve as indicators of environmental health and ecosystem functioning (Sigee, 2005). By studying the dynamics of various bacterioplankton subcommunities, such as AT, CRT, and RT, researchers can gain insights into the specific ecological patterns that characterize urban ponds and assess the impacts of environmental changes on the microbial community.

The limited study period and the use of only a few sampling stations can pose limitations when trying to demonstrate the dynamics of different bacterioplankton subcommunities (Liu et al., 2015; Wang et al., 2020). In the case of SMBU pond, the findings indicated the presence of bacterioplankton community dynamics (Fig. 12). However, it is important to note that our study

was conducted over a one-month period, which may not capture the full range of variations and long-term trends. Therefore, conducting a more extensive and prolonged investigation that encompasses multiple months would be valuable in order to evaluate the influence of environmental factors on the bacterioplankton community across different timescales. For future studies, it is recommended to prioritize conducting research on a larger spatial scale by including more endorheic ponds from various urban environments and geographic regions. Additionally, considering a wide range of climate zones would provide insights into how microbial communities in endorheic ponds respond to varying climatic conditions. By expanding the scope of the study, it would be possible to generalize the conclusions and findings to a broader context, increasing the robustness and applicability of the research outcomes.

6. Conclusion

This study presents the temporal dynamics of bacterioplankton in the surface waters of an endorheic pond located at Shenzhen MSU-BIT University in Shenzhen, southeast China. We conducted high-frequency sampling over a one-month period to gain a better understanding of the factors that drive the dynamics of the bacterioplankton community in such a unique freshwater ecosystem. The temporal dynamics of the bacterioplankton community were primarily associated with variations in the Actinobacteria and Proteobacteria phyla, as well as the presence of the *Synechococcus* genus. The community compositions within the three studied subcommunities, specifically in CRT, showed notable differences. Water physicochemistry played a more significant role in shaping the bacterioplankton community composition within AT subcommunity, whereas weather conditions had a greater impact on CRT and RT subcommunities. Species associations within the bacterioplankton community, uncovered through co-occurrence networks, highlighted the prominent influence of stochastic processes in shaping the community structure, particularly within CRT and RT subcommunities. These findings highlight the importance of conducting high-frequency sampling studies on microbial communities to enhance our understanding, modelling, and prediction of microbial responses to environmental change in the future.

Acknowledgement

First, I would like to express my sincere gratitude to my supervisor, Dr. Nyirabuhoro Pascaline and co-supervisor, Dr. Jean Claude Ndayishimiye, for all their help and private support in both theoretical and experimental work. From the time he acknowledged my application for my postgraduate studies, they took the greatest care to guarantee the best conditions possible for the continuation of my research training, and he had regular work discussions in providing advice.

Second, I feel great happiness in expressing my heartfelt thanks to my current colleagues at the Faculty of Biology at Shenzhen MSU-BIT University (SMBU). There are no words that can restrict the great help, support, and care of each one of you, Cheng Xinyun, and Zhao Qing. I really want to express my thanks for your special role in the smooth running of my laboratory work. I heartily appreciate your help; without such a lifetime full of happiness as a team, close teamwork, and being supportive in both social life and research work, I could not have reached this far.

Third, I recognize and thank Prof. Yuri Mazei and Prof. Damir Saldaev for their insightful thoughts and discussions by correspondence.

Fourth, I would like to acknowledge with gratitude, the unwavering support of my parents, who have been my pillars of strength throughout my academic journey. Their love, encouragement, and unwavering support have been instrumental in helping me achieve my goals. I am also thankful to my relatives and parents-in-law, who have supported me and stood by me through thick and thin. Their encouragement and support have been a source of motivation for me and have helped me reach where I am today.

This work was supported by the Shenzhen Natural Science Foundation (20200828181231001) and the Russian Science Foundation (19–14-00102). This research was performed within the framework of the development program of the Interdisciplinary Scientific and Educational School of Lomonosov Moscow State University, “The Future of the Planet and Global Environmental Change.”

References

1. Aanderud, Z. T., Vert, J. C., Lennon, J. T., Magnusson, T. W., Breakwell, D. P., & Harker, A. R. (2016). Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. Frontiers in Microbiology, 7, 853.
2. Adams, W. J., DeForest, D. K., Tear, L. M., Payne, K., & Brix, K. V. (2015). Long-term monitoring of arsenic, copper, selenium, and other elements in Great Salt Lake (Utah, USA) surface water, brine shrimp, and brine flies. Environmental monitoring and assessment, 187, 1-13.
3. Al, M. A., Xue, Y., Xiao, P., Xu, J., Chen, H., Mo, Y., ... & Yang, J. (2022). Community assembly of microbial habitat generalists and specialists in urban aquatic ecosystems explained more by habitat type than pollution gradient. Water Research, 220, 118693.
4. Anderson, M. J., & Walsh, D. C. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing?. Ecological monographs, 83(4), 557-574.
5. Bahcall, O. G. (2015). Urban microbiome. Nature Reviews Genetics, 16(4), 194-195.
6. Barabde, M., & Danve, S. (2015). Real time water quality monitoring system. International Journal of Innovative Research in Computer and Communication Engineering, 3(6), 5064-5069.
7. Bastian, M., Heymann, S., & Jacomy, M. (2009) .Gephi: an open source software for exploring and manipulating networks. International AAAI Conference on Weblogs and Social Media.
8. Baxter, B. K. (2018). Great Salt Lake microbiology: a historical perspective. International Microbiology, 21(3), 79-95.

9. Bellia, A. F., & Lanfranco, S. (2020). Evaluation of a dinoflagellate bloom in a perennial endorheic Mediterranean pond. Biodiversity Journal, 2020,11 (4): 961–968.
10. Blancher, P., Lefrançois, E., Rimet, F., Vasselon, V., Argillier, C., Arle, J., ... & Bouchez, A. (2022). A strategy for successful integration of DNA-based methods in aquatic monitoring. Metabarcoding and Metagenomics 6:e85652.
11. Brinkmeier, M., & Schank, T. (2005). Network statistics. Network analysis: methodological foundations, 293-317.
12. Bruce, K., Blackman, R. C., Bourlat, S. J., Hellström, M., Bakker, J., Bista, I., ... & Deiner, K. (2021). A practical guide to DNA-based methods for biodiversity assessment. Pensoft Advanced Books.
13. Burlage, R. S., Atlas, R., Stahl, D., Sayler, G., & Geesey, G. (Eds.). (1998). Techniques in microbial ecology. Oxford University Press on Demand.
14. Caldwell, D. E., & Tiedje, J. M. (1975). The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. Canadian Journal of Microbiology, 21(3), 377-385.
15. Campbell, B. J., Yu, L., Heidelberg, J. F., & Kirchman, D. L. (2011). Activity of abundant and rare bacteria in a coastal ocean. Proceedings of the National Academy of Sciences, 108(31), 12776-12781.
16. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature methods, 7(5), 335-336.
17. Chen, W., Pan, Y., Yu, L., Yang, J., & Zhang, W. (2017). Patterns and processes in marine microeukaryotic community biogeography from

Xiamen coastal waters and intertidal sediments, southeast China. Frontiers in microbiology, 8, 1912.

18. Chisholm, R. A., & Pacala, S. W. (2010). Niche and neutral models predict asymptotically equivalent species abundance distributions in high-diversity ecological communities. Proceedings of the National Academy of Sciences, 107(36), 15821-15825.
19. Dai, T., Zhang, Y., Tang, Y., Bai, Y., Tao, Y., Huang, B., & Wen, D. (2016). Identifying the key taxonomic categories that characterize microbial community diversity using full-scale classification: a case study of microbial communities in the sediments of Hangzhou Bay. FEMS microbiology ecology, 92(10), fiw150.
20. Dini-Andreote, F., Stegen, J. C., Van Elsas, J. D., & Salles, J. F. (2015). Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proceedings of the National Academy of Sciences, 112(11), E1326-E1332.
21. Dokulil, M. T., & Teubner, K. (2000). Cyanobacterial dominance in lakes. Hydrobiologia, 438, 1-12.
22. Entsminger, G. L. (2014). EcoSim Professional: Null Modeling Software for Ecologists, Version 1 Acquired Intelligence Inc. Kesey-Bear and Pinyon Publishing, Montrose, CO, USA.
23. Evans, S., Martiny, J. B., & Allison, S. D. (2017). Effects of dispersal and selection on stochastic assembly in microbial communities. The ISME journal, 11(1), 176-185.
24. Fath, B. D., Scharler, U. M., Ulanowicz, R. E., & Hannon, B. (2007). Ecological network analysis: network construction. Ecological modelling, 208(1), 49-55.

25. Fuentes, S., Méndez, V., Aguila, P., & Seeger, M. (2014). Bioremediation of petroleum hydrocarbons: catabolic genes, microbial communities, and applications. Applied microbiology and biotechnology, 98, 4781-4794.
26. Fuhrman, J. A. (2009). Microbial community structure and its functional implications. Nature, 459(7244), 193-199.
27. Galand, P. E., Casamayor, E. O., Kirchman, D. L., & Lovejoy, C. (2009). Ecology of the rare microbial biosphere of the Arctic Ocean. Proceedings of the National Academy of Sciences, 106(52), 22427-22432.
28. Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., ... & Field, D. (2012). Defining seasonal marine microbial community dynamics. The ISME journal, 6(2), 298-308.
29. Gotelli, N. J., & McCabe, D. J. (2002). Species co-occurrence: a meta-analysis of JM Diamond's assembly rules model. Ecology, 83(8), 2091-2096.
30. Griffiths, G. (Ed.). (2002). Technology and applications of autonomous underwater vehicles (Vol. 2). CRC Press.
31. Hanashiro, F. T. T., Mukherjee, S., Souffreau, C., Engelen, J., Brans, K. I., Busschaert, P., & De Meester, L. (2019). Freshwater bacterioplankton metacommunity structure along urbanization gradients in Belgium. Frontiers in microbiology, 10, 743.
32. Hassall, C. (2014). The ecology and biodiversity of urban ponds. Wiley Interdisciplinary Reviews: Water, 1(2), 187-206.
33. Hawes, I., Howard-Williams, C., Gilbert, N., & Joy, K. (2021). Towards an environmental classification of lentic aquatic ecosystems in the McMurdo Dry Valleys, Antarctica. Environmental Management, 67, 600-622.

34. Hill, M. J., Biggs, J., Thornhill, I., Briers, R. A., Gledhill, D. G., White, J. C., ... & Hassall, C. (2017). Urban ponds as an aquatic biodiversity resource in modified landscapes. Global change biology, 23(3), 986-999.
35. Hill, M. J., Greaves, H. M., Sayer, C. D., Hassall, C., Milin, M., Milner, V. S., ... & Wood, P. J. (2021). Pond ecology and conservation: research priorities and knowledge gaps. Ecosphere, 12(12), e03853.
36. Hobbie, J. E., Daley, R. J., & Jasper, S. (1977). Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and environmental microbiology, 33(5), 1225-1228.
37. Ilstrup, D. M. (1990). Statistical methods in microbiology. Clinical Microbiology Reviews, 3(3), 219-226.
38. Isabwe, A., Yang, J. R., Wang, Y., Liu, L., Chen, H., & Yang, J. (2018). Community assembly processes underlying phytoplankton and bacterioplankton across a hydrologic change in a human-impacted river. Science of the Total Environment, 630, 658-667.
39. Isabwe, A., Yang, J. R., Wang, Y., Wilkinson, D. M., Graham, E. B., Chen, H., & Yang, J. (2022). Riverine bacterioplankton and phytoplankton assembly along an environmental gradient induced by urbanization. Limnology and Oceanography, 67(9), 1943-1958.
40. Jaspers, E., Nauhaus, K., Cypionka, H., & Overmann, J. (2001). Multitude and temporal variability of ecological niches as indicated by the diversity of cultivated bacterioplankton. FEMS Microbiology Ecology, 36(2-3), 153-164.
41. Jiao, C., Zhao, D., Huang, R., Cao, X., Zeng, J., Lin, Y., & Zhao, W. (2018). Abundant and rare bacterioplankton in freshwater lakes subjected to different levels of tourism disturbances. Water, 10(8), 1075.

42. Joint, I., Henriksen, P., Fonnes, G. A., Bourne, D., Thingstad, T. F., & Riemann, B. (2002). Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquatic Microbial Ecology, 29(2), 145-159.
43. Jones, S. E., & Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences, 107(13), 5881-5886.
44. Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., ... & Hol, W. H. (2017). Where less may be more: how the rare biosphere pulls ecosystems strings. The ISME journal, 11(4), 853-862.
45. Jurtshuk, P.J. Bacterial Metabolism. In Medical Microbiology, 4th ed.; Baron, S., Ed.; University of Texas Medical Branch at Galveston: Galveston, TX, USA, 1996; Chapter 4.
46. Karim, M. A., Begum, Z. T., & Islam, M. S. (2012). Bacterial Load in Relation to Physicochemical parameters and phytoplankton abundance of an urban pond. Bangladesh Journal of Botany, 41(1), 15-20.
47. Konopka, A., Lindemann, S., & Fredrickson, J. (2015). Dynamics in microbial communities: unraveling mechanisms to identify principles. The ISME journal, 9(7), 1488-1495.
48. Last, W. M., & Smol, J. P. (2002). Tracking Environmental Change using Lake Sediments. Volume 1: Basin Analysis, Coring, and Chronological Techniques. New York, USA: Kluwer Academic Publishers.
49. Legendre, P., & Legendre, L. (2012). Numerical ecology. Elsevier.
50. Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature reviews microbiology, 9(2), 119-130.

51. Lindh, M. V., Sjöstedt, J., Andersson, A. F., Baltar, F., Hugerth, L. W., Lundin, D., ... & Pinhassi, J. (2015). Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. Environmental microbiology, 17(7), 2459-2476.
52. Lindholm, T., & Weppling, K. (1987). Blooms of phototrophic bacteria and phytoplankton in a small brackish lake on Åland, SW Finland. Acta Acad. Aboensis, 47, 45-53.
53. Liu, L., Chen, H., Liu, M., Yang, J. R., Xiao, P., Wilkinson, D. M., & Yang, J. (2019). Response of the eukaryotic plankton community to the cyanobacterial biomass cycle over 6 years in two subtropical reservoirs. The ISME journal, 13(9), 2196-2208.
54. Liu, L., Yang, J., Yu, Z., & Wilkinson, D. M. (2015). The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. The ISME journal, 9(9), 2068-2077.
55. Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., ... & Massana, R. (2014). Patterns of rare and abundant marine microbial eukaryotes. Current Biology, 24(8), 813-821.
56. Logares, R., Lindström, E. S., Langenheder, S., Logue, J. B., Paterson, H., Laybourn-Parry, J., ... & Bertilsson, S. (2013). Biogeography of bacterial communities exposed to progressive long-term environmental change. The ISME journal, 7(5), 937-948.
57. Logares, R., Mangot, J. F., & Massana, R. (2015). Rarity in aquatic microbes: placing protists on the map. Research in microbiology, 166(10), 831-841.
58. Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. Nature methods, 10(10), 999-1002.

59. Lynch, M. D., & Neufeld, J. D. (2015). Ecology and exploration of the rare biosphere. Nature Reviews Microbiology, 13(4), 217-229.
60. Madrid, Y., & Zayas, Z. P. (2007). Water sampling: Traditional methods and new approaches in water sampling strategy. TrAC Trends in Analytical Chemistry, 26(4), 293-299.
61. Magurran, A. E. (1988). Ecological Diversity and Its Measurement. Princeton University Press, Princeton, NJ, USA.
62. Maier, R. M., Pepper, I. L., Gerba, C. P., & Gentry, T. J. (Eds.) (2009). Environmental microbiology. Elsevier, CA, USA.
63. Mangot, J. F., Domaizon, I., Taib, N., Marouni, N., Duffaud, E., Bronner, G., & Debroas, D. (2013). Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. Environmental Microbiology, 15(6), 1745-1758.
64. Martin-Rosales, W., & Leduc, C. (2003). Variability of the dynamics of temporary ponds in a semi-arid endorheic system (southwest Niger). In Hydrology in Mediterranean and semiarid regions: international conference, Montpellier, France, 1-4 April 2003 (pp. 174-178). IAHS Press.
65. Milstein, A. (2012). Pond ecology. Aquaculture Pond Fertilization: Impacts of Nutrient Input on Production, 23-32.
66. Mo, Y., Peng, F., Gao, X., Xiao, P., Logares, R., Jeppesen, E., ... & Yang, J. (2021). Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. Microbiome, 9(1), 1-17.
67. Mo, Y., Zhang, W., Yang, J., Lin, Y., Yu, Z., & Lin, S. (2018). Biogeographic patterns of abundant and rare bacterioplankton in three

subtropical bays resulting from selective and neutral processes. The ISME journal, 12(9), 2198-2210.

68. Moss, B. (2017). Ponds and Small Lakes: Microorganisms and Freshwater Ecology. Exter, UK: Pelagic Publishing Ltd.
69. Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F., ... & Ferrenberg, S. (2013). Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews, 77(3), 342-356.
70. Ni, J., Yan, Q., & Yu, Y. (2013). How much metagenomic sequencing is enough to achieve a given goal?. Scientific reports, 3(1), 1-7.
71. Nyirabuhoro, P., Gao, X., Ndayishimiye, J. C., Xiao, P., Mo, Y., Ganjidoust, H., & Yang, J. (2021). Responses of abundant and rare bacterioplankton to temporal change in a subtropical urban reservoir. FEMS Microbiology Ecology, 97(4), fiab036.
72. Nyirabuhoro, P., Liu, M., Xiao, P., Liu, L., Yu, Z., Wang, L., & Yang, J. (2020). Seasonal variability of conditionally rare taxa in the water column bacterioplankton community of subtropical reservoirs in China. Microbial Ecology, 80, 14-26.
73. O'Sullivan, P., & Reynolds, C. S. (2004). The Lakes Handbook: Limnology and Limnetic Ecology (Vol. 1) [EB]. Oxford, UK: Blackwell Science Ltd.
74. Odum, E. P. (1968). Energy flow in ecosystems: a historical review. American Zoologist, 8(1), 11-18.
75. Ordónez, S., Moral, S. S., Del, M. D. L. A. G., & Badiola, E. R. (1994). Precipitation of salts from Mg^{2+}-(Na^{+})-SO_4^{2-}-Cl-Playa-Lake Brines: the endorheic saline ponds of La Mancha, Central Spain.

76. Pedrós-Alió, C. (2012). The rare bacterial biosphere. Annual review of marine science, 4, 449-466.
77. Peet, R. K. (1974). The measurement of species diversity. Annual review of ecology and systematics, 5(1), 285-307.
78. Petrash, D. A., Jan, J., Sirová, D., Osafo, N. O. A., & Borovec, J. (2018). Iron and nitrogen cycling, bacterioplankton community composition and mineral transformations involving phosphorus stabilisation in the ferruginous hypolimnion of a post-mining lake. Environmental Science: Processes & Impacts, 20(10), 1414-1426.
79. Pjevac, P., Korlević, M., Berg, J. S., Bura-Nakić, E., Ciglenečki, I., Amann, R., & Orlić, S. (2015). Community shift from phototrophic to chemotrophic sulfide oxidation following anoxic holomixis in a stratified seawater lake. Applied and Environmental Microbiology, 81(1), 298-308.
80. Post, F. J. (1977). The microbial ecology of the Great Salt Lake. Microbial ecology, 3, 143-165.
81. Prasad, K. H. (2022). Ecosystem Ecology. In Insect Ecology: Concepts to Management (pp. 189-207). Singapore: Springer Nature Singapore.
82. QGIS Development Team (2022). QGIS Geographic Information System. Open Source Geospatial Foundation, Gossau, Switzerland.
83. Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature biotechnology, 35(9), 833-844.
84. R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
85. Reid, A., Buckley, M., & Mcfall, M. (2011). The rare biosphere: a report from the American Academy of Microbiology. Washington, DC: American Academy of Microbiology, 356.

86. Roguet, A., Laigle, G. S., Therial, C., Bressy, A., Soulignac, F., Catherine, A., ... & Lucas, F. S. (2015). Neutral community model explains the bacterial community assembly in freshwater lakes. FEMS Microbiology Ecology, 91(11).
87. Ruiz-González, C., Niño-García, J. P., & Del Giorgio, P. A. (2015). Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecology letters, 18(11), 1198-1206.
88. Sabehi, G., Loy, A., Jung, K. H., Partha, R., Spudich, J. L., Isaacson, T., ... & Béjà, O. (2005). New insights into metabolic properties of marine bacteria encoding proteorhodopsins. PLoS biology, 3(8), e273.
89. Sandrin, T.R.; Dowd, S.E.; Herman, D.C.; Maier, R.M. Aquatic Environments. In Environmental Microbiology; Elsevier: Amsterdam, The Netherlands, 2009; pp. 103–122.
90. Seeboonruang, U. (2014). Physico-chemical characterization of saline subsurface system near small endorheic ponds in Thailand. Environmental earth sciences, 71, 3273-3286.
91. Shade, A., Jones, S. E., Caporaso, J. G., Handelsman, J., Knight, R., Fierer, N., & Gilbert, J. A. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. MBio, 5(4), e01371-14.
92. Shade, A., Read, J. S., Youngblut, N. D., Fierer, N., Knight, R., Kratz, T. K., ... & McMahon, K. D. (2012). Lake microbial communities are resilient after a whole-ecosystem disturbance. The ISME journal, 6(12), 2153-2167.
93. Sharpton, T. J. (2014). An introduction to the analysis of shotgun metagenomic data. Frontiers in plant science, 5, 209.

94. Sieburth, J. M., Smetacek, V., & Lenz, J. (1978). Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions 1. Limnology and oceanography, 23(6), 1256-1263.
95. Sigee, D. C. (2005). Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. John Wiley & Sons.
96. Sloan, W. T., Lunn, M., Woodcock, S., Head, I. M., Nee, S., & Curtis, T. P. (2006). Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environmental microbiology, 8(4), 732-740.
97. Smol, J P. (2008). Pollution of lakes and rivers: a paleoenvironmental perspective. Oxford, UK: Blackwell Science Ltd.
98. ter Braak, C. J. F., & Šmilauer, P. (2012). CANOCO Reference Manual and User's Guide: Software for Ordination (version 5.0). Microcomputer Power, Ithaca.
99. Varnam, A., & Evans, M. (2000). Environmental microbiology. Tylor and Francis Group, FL, USA.
100. Vellend, M. (2010). Conceptual synthesis in community ecology. The Quarterly review of biology, 85(2), 183-206.
101. Vergin, K. L., Done, B., Carlson, C. A., & Giovannoni, S. J. (2013). Spatiotemporal distributions of rare bacterioplankton populations indicate adaptive strategies in the oligotrophic ocean. Aquatic microbial ecology, 71(1), 1-13.
102. Vicente, E., & Miracle, M. R. (1988). Physicochemical and microbial stratification in a meromictic karstic lake of Spain: With 6 figures and 2

tables in the text. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 23(1), 522-529.

103. Vila, X., Dokulil, M., Garcia-Gil, L. J., Abella, C. A., Borrego, C. M., & Bañeras, L. (1996). Composition and distribution of phototrophic bacterioplankton in the deep communities of several central European lakes: the role of light quality. Ergebnisse der Limnologie, (48), 183-196.
104. Vrana, B., Allan, I. J., Greenwood, R., Mills, G. A., Dominiak, E., Svensson, K., ... & Morrison, G. (2005). Passive sampling techniques for monitoring pollutants in water. TrAC Trends in Analytical Chemistry, 24(10), 845-868.
105. Wang, Y., Ye, F., Wu, S., Wu, J., Yan, J., Xu, K., & Hong, Y. (2020). Biogeographic pattern of bacterioplanktonic community and potential function in the Yangtze River: roles of abundant and rare taxa. Science of the Total Environment, 747, 141335.
106. Wikner, J., & Hagström, Å. (1999). Bacterioplankton intra-annual variability: importance of hydrography and competition. Aquatic Microbial Ecology, 20(3), 245-260.
107. World Health Organization (2021). WHO global air quality guidelines: particulate matter (PM2. 5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide: executive summary.
108. Wulff, F., Field, J. G., & Mann, K. H. (Eds.). (2012). Network analysis in marine ecology: methods and applications (Vol. 32). Springer Science & Business Media.
109. Xue, Y., Chen, H., Yang, J. R., Liu, M., Huang, B., & Yang, J. (2018). Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. The ISME Journal, 12(9), 2263-2277.

110. Yang, J., Yu, X., Liu, L., Zhang, W., & Guo, P. (2012). Algae community and trophic state of subtropical reservoirs in southeast Fujian, China. Environmental Science and Pollution Research, 19, 1432-1442.
111. Yang, X. E., Wu, X., Hao, H. L., & He, Z. L. (2008). Mechanisms and assessment of water eutrophication. Journal of Zhejiang University Science B 9: 197–209.
112. Young, L. L., Young, L. J., & Young, J. (1998). Statistical ecology. Springer Science & Business Media.
113. Zhou, J., & Ning, D. (2017). Stochastic community assembly: does it matter in microbial ecology?. Microbiology and Molecular Biology Reviews, 81(4), e00002-17.
114. Zuur, A. F., Ieno, E. N., & Smith, G. M. (2007). Principal coordinate analysis and non-metric multidimensional scaling. Analysing ecological data, 259-264.