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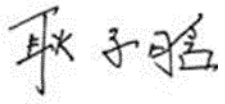

Dynamics and assembly mechanisms of microbial communities in subtropical urban ponds

Master's thesis

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Abstract

The dynamics of freshwater bacterial communities in aquatic environments are important as they provide valuable insights into the processes that drive ecosystem functioning and assess the overall health of these ecosystems. However, much remains unknown about the determinants of bacterial community dynamics in urban ponds, especially in the context of a warming climate. In this study, we conducted a whole-metagenomic shotgun sequencing analysis of bacterial communities in subtropical urban ponds located in Shenzhen, southern China, to understand the temporal dynamics and assembly mechanisms of bacterial communities in response to warm conditions (26.4–32.1 °C). Over a one-month period, 30 surface water samples collected from three adjacent ponds yielded a total of 381 bacterial species, corresponding to 21 phyla. Among these species, Proteobacteria were the most prevalent, with 40.42% of them classified as pathogenic bacteria affecting both humans and animals. The bacterial diversity and species relative abundance exhibited distinct patterns over a relatively short time interval of 2 to 3 days, underscoring the dynamic nature of bacterial communities and the unique characteristics of each pond as a potential habitat. The bacterial community tended to return to a community type resembling an earlier sampling period, demonstrating the potential for a seasonal cycle and ecological processes involving both random changes in population (stochastic processes) and non-random patterns of species (deterministic processes). Stochastic processes explained 72.1–77.5% of the community composition, representing a stronger influence of stochastic processes over deterministic processes in shaping the bacterial community. The results suggest that under warm conditions, the bacterial community is largely regulated by stochastic

processes, with temperature playing a vital role in increasing species richness and species relative abundance, including those of human and animal pathogenic bacteria.

Keywords: Bacterial community; high-frequency sampling; temporal dynamics; ecological processes; subtropical zone; urban ponds

1. Introduction

Urbanization, as one of the most significant global changes in the 21st century, is profoundly restructuring the patterns of biodiversity and ecosystem services (Díaz et al., 2019; Hobohm et al., 2021). In this process, urban ecosystems, especially urban water bodies such as ponds, are increasingly valued for their key role in urban biodiversity (Alikhani et al., 2021; Hill et al., 2021). Subtropical urban ponds, with their unique climatic conditions and rapid urbanization characteristics, serve as an ideal place for studying the impact of urbanization on aquatic ecosystems (Yao et al., 2023; Li et al., 2023; Ndayishimiye et al., 2023).

Microbial communities, as an essential component of aquatic ecosystems, play a crucial role in maintaining the biogeochemical cycles of water bodies, the degradation of pollutants, and the stability of ecosystems (Fuhrman, 2009; Pernthaler et al., 2013; Singh, 2015). However, current research on microbial communities in subtropical urban ponds is relatively scarce, particularly regarding how they respond to environmental changes brought about by rapid urbanization and the understanding of their assembly mechanisms and ecological functions under the influence of urbanization is still insufficient (Li et al., 2023). Over the past half-century, researchers have increasingly focused on the biodiversity of urban water bodies. For example, Fenchel and Jørgensen (1977) emphasized the core role of bacteria in maintaining ecosystem health. This foundational work was built upon by Trap et al. (2016), who underscored the importance of plant-bacterial interactions in promoting plant growth and adaptability. Additionally, Idi et al. (2015) and Mehrotra et al. (2021) demonstrated the significant role of bacteria in the bioremediation process, highlighting their contribution to the removal of

various pollutants. In terms of microbial diversity in urban ponds, Hassall (2014) and Hill et al. (2021) provided a comprehensive review of their current status by recommending a series of strategies to promote biodiversity. They highlighted the potential of urban ponds to support threatened species and suggested that, if managed properly, urban ponds could sustain more biodiversity than currently exists. Although Hassall (2014) has provided valuable insights into the ecological functions and biodiversity of urban ponds, there remains a need for a deeper understanding of the dynamics and assembly mechanisms of microbial communities in subtropical urban ponds. This includes investigating how various environmental factors and anthropogenic activities influence microbial diversity and interactions within these unique ecosystems (Li et al., 2023; Ndayishimiye et al., 2023).

This study intends to fill the existing knowledge gap by comprehensively applying whole-metagenomic sequencing technique, statistical methods, and ecological models to explore the dynamics and assembly mechanisms of microbial communities in response to warm conditions in subtropical urban ponds. The deliberate emphasis on warm conditions arises from the previous acknowledgment of the substantial impact of temperature on shaping bacterial community dynamics in a subtropical climate zone (Nyirabuhoro et al., 2020, 2021). The research questions are therefore: (1) How do the compositions of the bacterial community in subtropical urban ponds change in response to warm conditions? (2) To what extent are stochastic processes involved in shaping the assembly mechanisms of the bacterial community in warm conditions? The expected results of this research include shifts in bacterial compositions, which may reveal specific taxa becoming more dominant or declining in abundance in response to warm conditions, thereby indicating temperature-driven alterations in microbial community structure.

Additionally, the investigation into the role of stochastic processes in community assembly is expected to unveil the extent to which random events keep up bacterial communities under warmer environmental conditions.

2. Literature review

2.1. Urban pond: zonation patterns and their ecological significance

Urban ponds are an integral component of aquatic ecosystems, serving as focal points of biodiversity and ecological services in urban environments (Hassall, 2014). The spatial organization of a pond "zonation," is crucial for understanding the biodiversity distribution and conservation efforts (Li et al., 2023; Ndayishimiye et al., 2023). Urban ponds encompass a variety of distinct zones, including the riparian zone, emergent vegetation zone, littoral zone, open water zone, and submerged vegetation zone (Fig. 1). Each of these zones plays a unique and essential role in shaping the overall functioning and biodiversity of these aquatic environments (Li et al., 2023).

The riparian zone, covering the terrestrial habitat adjacent to the pond's edge, functions as a transitional area between aquatic and upland ecosystems. Vegetation such as trees, shrubs, and grasses along the pond shores offer shade, stabilize banks, and mitigate runoff, thus reducing erosion and nutrient input (Fig. 1). The riparian zone sustains a variety of wildlife, including mammals, birds, and reptiles, which rely on this habitat for foraging, nesting, and dispersal activities (Thomas et al., 1979; Birx-Raybuck et al., 2010). The zone of emergent vegetation, characterized by clusters of plants rooted in shallow water, acts as a dynamic boundary between land and water (Fig. 1). These plants fulfill multiple roles within urban pond ecosystems, serving as habitats, food sources, and agents for erosion control. The complex structure of

emergent vegetation offers shelter and nesting sites for avian species such as herons and marsh wrens, whereas also contributing to water quality improvement by filtering pollutants and stabilizing sediments (Shimoda, 2005). The littoral zone, typically found as a shallower area enclosing the pond's edge, serves as a vital interface between terrestrial and aquatic environments (Fig. 1). In this zone, emergent vegetation such as reeds, rushes, and cattails thrives, offering habitat, sustenance, and shelter for diverse organisms. Amphibians, including frogs and salamanders, employ the littoral zone for breeding and foraging, whereas insects such as dragonflies and water beetles rely on it for protection and reproduction. Additionally, the accumulation of litter and detritus in the littoral zone contributes to nutrient cycling and sustains microbial communities crucial for decomposing organic matter (Rannap et al., 2010). Unlike the littoral zone, the open water zone occupies the central area of the pond, featuring deeper water and sparse vegetation (Fig. 1). Although it may appear uniform, the open water zone hosts a wide variety of aquatic organisms adapted to pelagic environments. Fish species such as bass, carp, and bluegill may flourish in these deeper waters, feeding on smaller fish and invertebrates. Waterfowl such as ducks and geese rely on the open water zone for feeding and resting, contributing significantly to the avian diversity within urban pond ecosystems (Rannap et al., 2010; Sychra et al., 2010). Below the surface of urban ponds lies the submerged vegetation zone, where aquatic plants such as pondweeds, water milfoil, and hornwort thrive (Fig. 1). These submerged plants play a vital role in oxygenation of the water column, carbon sequestration, and providing habitats for diverse aquatic organisms. Fish species such as sunfish and bass find refuge among submerged vegetation, while invertebrates like snails and

freshwater mussels feed on algae and detritus attached to plant surfaces (Rannap et al., 2010).

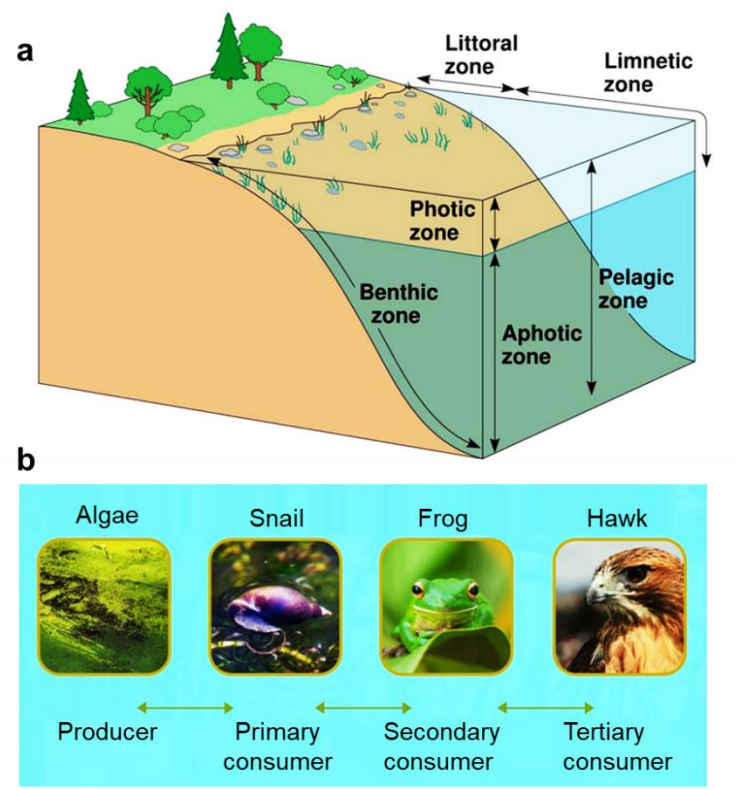

Fig. 1. Zonation patterns and ecological significance of a pond. (a) The body of water characterized by six distinct zones, each contributing uniquely to the pond's ecological dynamics (Source: Pearson Education, Inc. Publishing as Pearson Benjamin Cummings, 2005). (b) Pond ecosystem food chain representing the interdependencies among various aquatic and terrestrial species (Source: Earth Reminder, 2024).

2.2. Urban pond: categories and their societal significance

Urban ponds, typically found in urban environments, can be categorized based on their origin, design, and purpose. Each category brings its own unique contribution to urban life, collectively playing a significant role in enhancing environmental quality and supporting biodiversity. Urban ponds

are broadly categorized into ornamental ponds, stormwater ponds, and retention ponds (Hancock et al., 2010; Hassall and Anderson, 2015; Oertli et al., 2023). Ornamental ponds are largely designed for aesthetic purposes and are typically found in gardens, parks, and public spaces. Ornamental ponds display decorative elements such as fountains, sculptures, and carefully selected plantings, which create visually attractive landscapes that enhance the beauty of urban areas. These ponds serve as focal points for relaxation and social interaction (Oertli et al., 2023). Stormwater ponds are designed to manage urban runoff. They collect rainwater from impervious surfaces such as roads and rooftops, mitigating flooding risks and decreasing the burden on urban drainage systems. By filtering sediments and contaminants from stormwater, they improve water quality before it is released into natural water bodies, thus protecting aquatic ecosystems downstream (Hassall and Anderson, 2015). Retention ponds, often integrated into urban infrastructure, hold water for extended periods, facilitating control of water flow and preventing erosion. They are typically designed to manage large volumes of water, particularly during heavy rainfalls, and gradually release it to prevent downstream flooding. Additionally, retention ponds serve as sediment traps, improving water quality by allowing contaminants to settle before the water is discharged (Hancock et al., 2010).

Urban ponds provide numerous benefits that extend beyond their major functional categories, substantially contributing to societal well-being and urban sustainability (Hassall, 2014; Blicharska and Johansson, 2016; Hill et al., 2021). These water bodies contribute to the environmental health of cities by regulating temperature, enhancing air quality, and supporting urban biodiversity (Blicharska and Johansson, 2016). The vegetation around ponds acts as a carbon sink, sequestering carbon dioxide and releasing oxygen,

thereby mitigating the urban heat island effect. Additionally, these green spaces provide vital habitats for animals, promoting ecological balance in urban areas (Hassall, 2014; Hill et al., 2021). Urban ponds enhance the quality of life for city dwellers by offering recreational spaces for activities such as walking, bird watching, and fishing. The presence of water bodies within urban environments creates visually pleasing landscapes that offer psychological benefits, such as stress reduction and improved mental health. Ponds also serve as community gathering spots, fostering social cohesion and promoting outdoor activities (Blicharska and Johansson, 2016). In addition to their recreational roles, urban ponds serve as living laboratories for environmental education. They provide opportunities for schools and communities to engage in hands-on learning about ecosystems, water management, and conservation (Byrd et al., 2007). These water bodies also hold cultural significance, often being central to local traditions, festivals, and artistic expressions (Shukla and Shukla, 2018). By managing stormwater runoff and decreasing flood risks, urban ponds play a critical role in protecting urban infrastructure and communities. They help mitigate the impacts of extreme weather events, which are becoming increasingly common due to climate change (Hancock et al., 2010; Hassall and Anderson, 2015).

2.3. Proneness of urban ponds: pollution and climate change

Urban ponds are significantly threatened by environmental pollution and climate change, jeopardizing their health and the services they provide (Hassall, 2014; Hill et al., 2021). Pollution, one of the most pressing issues for urban ponds, arises from diverse sources, including runoff from roads, industrial discharges, and improper waste disposal. Urbanization and industrial activities introduce harmful substances such as heavy metals,

nutrients, and organic contaminants into the water, leading to several detrimental effects (Bhat et al., 2012). Excessive nutrients, originating from agricultural runoff, lawn fertilizers, and sewage discharges, promote rapid algae growth, leading to eutrophication. As algae decompose, they reduce oxygen levels in the water, causing hypoxic conditions that can result in fish kills and the loss of aquatic biodiversity. Moreover, algal blooms produce toxins harmful to both aquatic life and humans (Waajen et al., 2014). Urban ponds are also prone to contamination from pesticides, heavy metals, and industrial pollutants. These contaminants accumulate in the sediment and biota, posing long-term risks to the ecosystem health. For example, heavy metals are toxic to aquatic organisms, affecting their growth, reproduction, and survival (Allinson et al., 2015). Plastic pollution is another growing concern for urban ponds. Microplastics, originating from degraded plastic waste, are ingested by aquatic organisms, leading to physical and chemical harm. Plastics also serve as vectors for other pollutants, potentially intensifying the contamination of urban pond ecosystems (Brooks et al., 2023).

Climate change presents additional complexities to the challenges faced by urban ponds (Hassall, 2014; Hill et al., 2021). Rising temperatures, altered precipitation patterns, and an increased frequency of extreme weather events all significantly impact these delicate ecosystems. Higher temperatures intensify nutrient loading by promoting algal blooms, and warmer water holds less dissolved oxygen, further stressing aquatic life. Furthermore, temperature variations disrupt the breeding cycles of amphibians and other temperature-sensitive species, potentially leading to population declines. Climate change also affects the hydrology of urban ponds through changes in precipitation patterns. Increased rainfall results in more stormwater runoff, which carries

more contaminants into ponds. Prolonged droughts reduce water levels, concentrating pollutants and stressing aquatic organisms (Hassall, 2014).

2.3. Bacterioplankton: background information

Bacterioplankton are bacterial organisms residing within the water column of various aquatic environments, such as oceans, lakes, and rivers (Sigee, 2005). They are an integral component of the microbial community, pivotal in decomposing organic substances and facilitating nutrient recycling within the ecosystem (Hobbie et al., 1977). Bacterioplankton can thrive in both aerobic and anaerobic conditions and reproduce through mitosis or asexual reproduction (Sigee, 2005; Prasad, 2022). Despite their tiny size, they are highly abundant. Bacterioplankton are categorized into several groups based on size: 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); and macroplankton (>2 mm). Yet, strict classification of these functional groups is challenging, as many bacterioplankton exhibit overlapping functions (Sigee, 2005).

Bacterioplankton hold a central role in the energy pyramid, serving as the primary decomposers of organic matter in aquatic ecosystems (Prasad, 2022). They facilitate the breakdown of complex organic molecules into simpler compounds, thus rendering nutrients accessible to other aquatic organisms. As a result, bacterioplankton form the foundation of the food web, sustaining the survival of organisms such as phytoplankton and zooplankton, which, in turn, support higher trophic levels (Sigee, 2005; Prasad, 2022).

Cyanobacteria played a ground-breaking role as the first biotic source of oxygen on early Earth, indicating capabilities in oxygenic photosynthesis and nitrogen fixation (Jurtshuk, 1996; Petrash et al., 2018). Gammaproteobacteria

(synonym Proteobacteria) represent new types of phototrophs, distinguished by their possession of proteorhodopsin proteins, which serve as retinal-based photoreceptors enabling phototrophic function in water. Chemotrophic bacteria, relying on carbon for nourishment, are broadly categorized into two important groups. Chemoautotrophs possess the capacity to synthesize their own energy source through the oxidation of reduced inorganic compounds such as iron, sulfur, sulfide, and magnesium. In contrast, chemoheterotrophs lack the ability to synthesize their own food, thus deriving energy from the oxidation of inorganic minerals in their environment. This characteristic renders them pivotal decomposers of organic matter, enabling the mineralization of a substantial proportion of daily primary production (Sigee, 2005).

Seasonal variations in climatic conditions often induce changes in the composition, production, and abundance of bacterioplankton communities across aquatic systems (Nyirabuhoro et al., 2020, 2021). Due to their rapid turnover rates and complex relationships with environment, bacterioplankton exhibit sensitivity to environmental changes, including climate change, increased nutrients, and anthropogenic pollution (Hobbie et al., 1977; Jurtshuk, 1996). In warmer climate zones, cyanobacteria frequently form blooms in eutrophic lakes and reservoirs. These blooms often result in detrimental consequences, including the mortality of organisms such as fish and other aquatic fauna (Sigee, 2005).

2.4. Bacterial community analysis: sampling and analytical techniques

Bacterial community analysis is a comprehensive process that involves the detailed analysis of bacterial samples to understand the diversity, structure, and function of bacterial populations within ecosystems (Fig. 2). This process

is also crucial for scientists to discover how bacterial communities interact with each other and their environment, how they respond to environmental changes, and their roles in various ecological processes (Cloete and Muyima, 1997; Sigee, 2005).

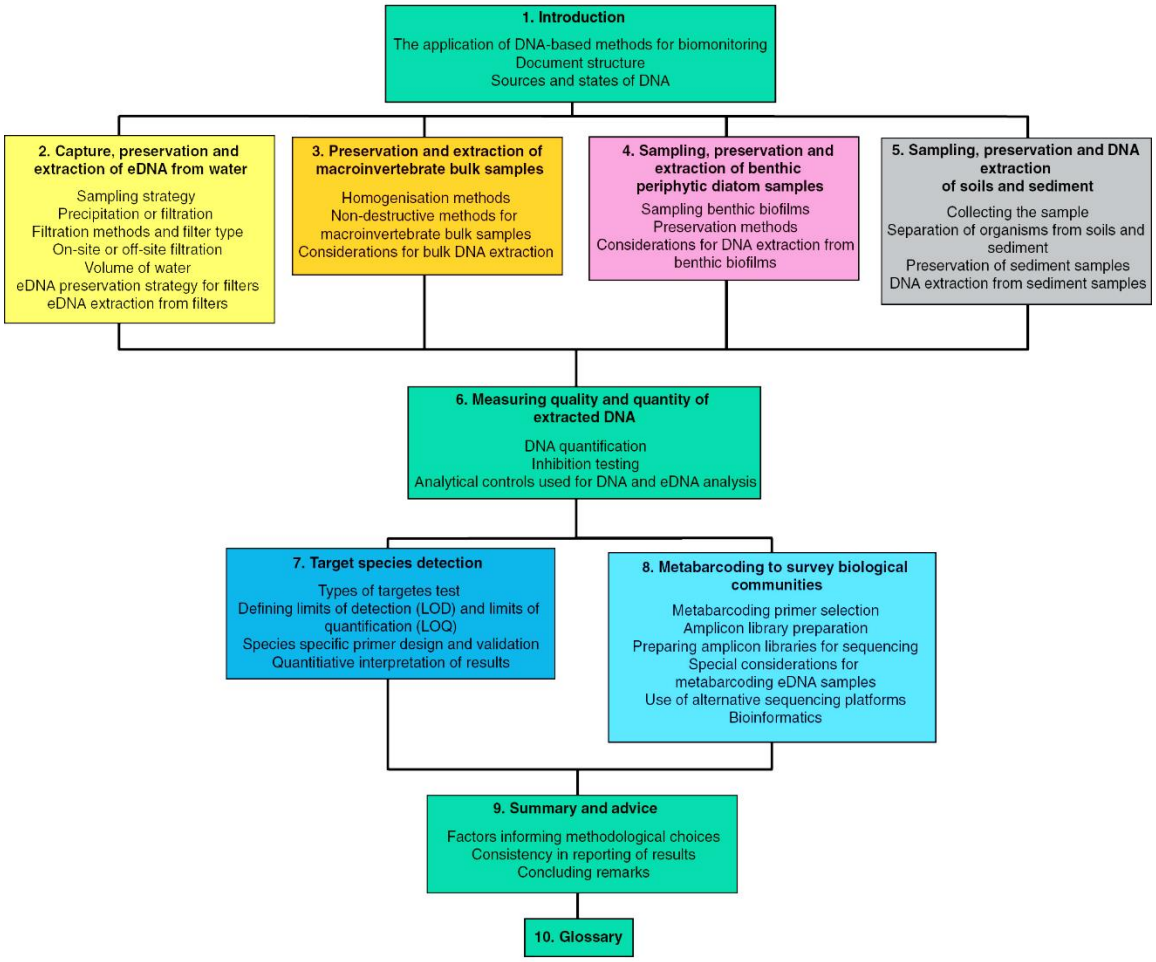

Fig. 2. The standardized practical procedure of DNA-based biomonitoring (Source: Bruce et al., 2021).

Sampling plan (Fig. 3) form the cornerstone of bacterial community analysis (Staley et al., 2015). In aquatic environments such as lakes, rivers, and oceans, sampling involves collecting water samples at various depths using devices such as Niskin bottles and grab samplers (Sigee, 2005). These

samples capture the microbial diversity present in different water layers, providing insights into vertical stratification and community dynamics (Nyirabuhoro et al., 2020). Depth-integrated sampling involves collecting water samples from multiple depths within a water column to assess vertical stratification and nutrient gradients (Sigee, 2005).

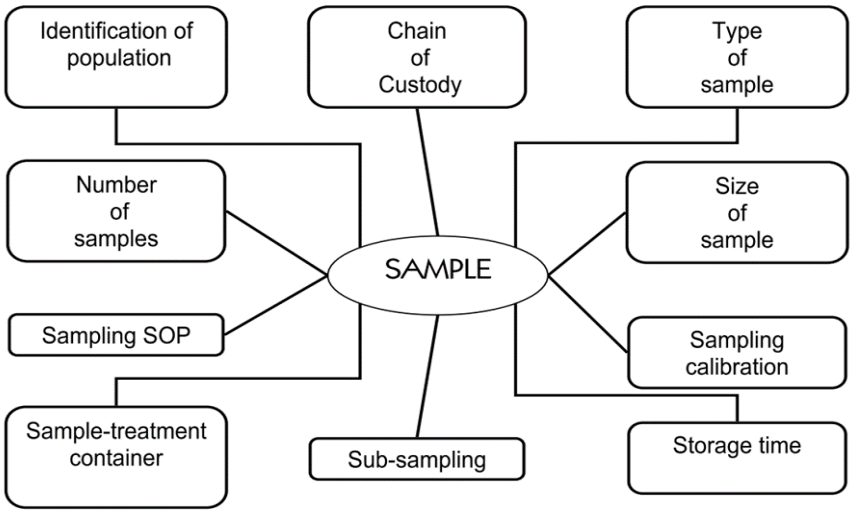

Fig. 3. Considerations for developing a successful sampling plan (Source: Madrid and Zayas, 2007).

The water sampler, water filtration system, and membrane filters for bacterial DNA extraction are crucial for bacterial community analysis (Nyirabuhoro et al., 2020, 2021). Water samples are typically collected from the target environment, which could be a lake, river, ocean, or drinking water supply, ensuring multiple samples are taken for representativeness (Sigee, 2005). These samples are largely collected using deep water sampler (Fig. 4a) The filtration system typically includes a vacuum pump and filtration unit (Fig. 4a) and membrane filters (Fig. 4b) with pore sizes capable of capturing bacteria (e.g., 0.2 to 0.45 µm). For large volumes, multiple filters may be required. Ideally, filters are processed immediately to prevent DNA

degradation. To avoid contamination, sterile, DNA-free containers and equipment are essential, and wearing gloves and other protective gear is necessary to maintain sample integrity. In case immediate extraction is not possible, filters are generally stored at -20°C or -80°C in sterile containers, and adding a DNA preservation reagent helps stabilize the DNA (Nyirabuhoro et al., 2020, 2021).

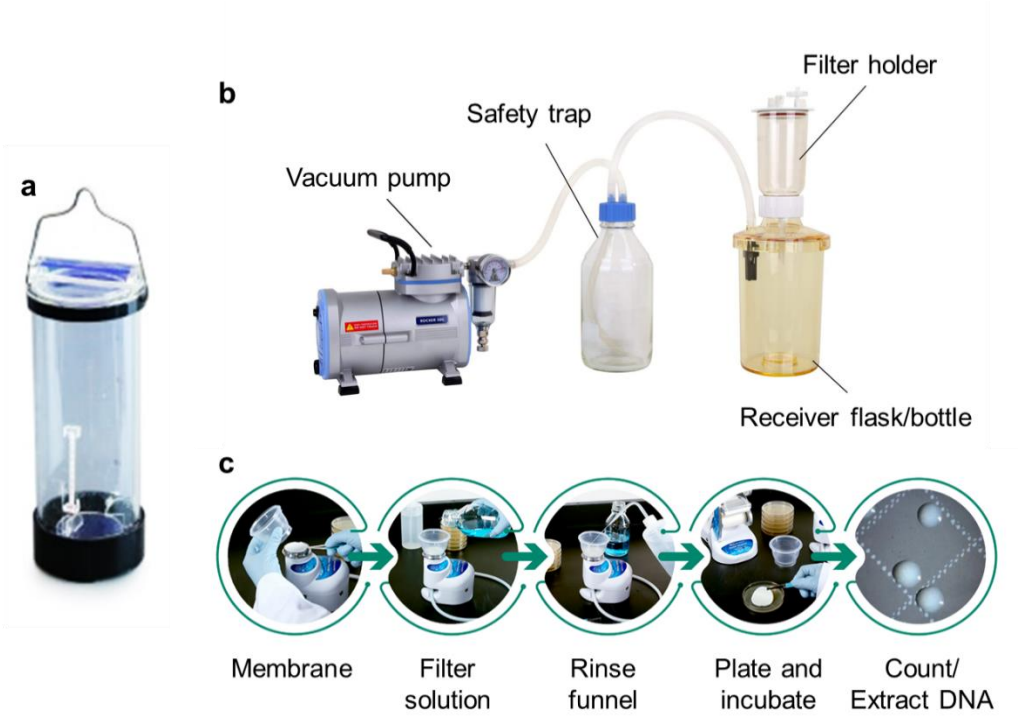

Fig. 4. Equipment for bacterial community sampling from water. (a) Deep water sampler. (b) Vacuum filtration system: comprising a vacuum pump, safety trap, filter holder, and receiver flask. This system, also known as negative pressure filtration or suction filtration, operates by creating a negative pressure in the container beneath the membrane. This negative pressure facilitates the passage of liquid through the membrane, effectively separating solids from the liquid phase (Source: Rocker Scientific, 2023). (c) Membrane filter technique. The technique was introduced in the late 1950s

and is widely recognized as the standard method for microbiological contamination in fluid samples (Source: Cytiva, 2024).

The top-secret for a successful DNA extraction (Fig. 5) lies in effectively breaking open bacterial cells to release their genetic material, a process known as “cell lysis.” This can be achieved through mechanical, chemical, or thermal methods, each with its advantages and chosen based on the specific requirements of analysis and the characteristics of target bacterial cells (Sigee, 2005). Mechanical lysis involves physically disrupting bacterial cells to release their DNA. The most commonly used method in mechanical lysis is bead beating, where samples are agitated with small beads. The physical force generated during this process disrupts the cell walls and membranes, effectively releasing the intracellular contents. This method is particularly effective for tough, resilient bacteria that may resist other lysis methods. However, it requires specialized equipment and can be labor-intensive (Di Carlo et al., 2003). Chemical lysis employs specific chemicals to break down bacterial cell walls and membranes. Lysis buffers typically contain detergents, such as sodium dodecyl sulfate and enzymes such as lysozymes. Detergents dissolve the lipid components of cell membranes, whereas enzymes target and break down peptidoglycan in bacterial cell walls. This method is widely utilized as it can be finely tuned to target specific cellular components and is generally effective across a broad range of bacterial species. Chemical lysis is also relatively straightforward to perform and is easily incorporated into automated DNA extraction workflows (Mehta et al., 2015). Thermal lysis relies on the application of heat to break open bacterial cells. Heat disrupts cell membranes and denatures proteins, leading to the release of DNA. This method is simple and cost-effective, often used as a preliminary step in

combination with other lysis techniques to ensure complete cell disruption. However, care must be taken to avoid overheating, which can damage the DNA and reduce the efficiency of subsequent analyses (Shetty et al., 2017).

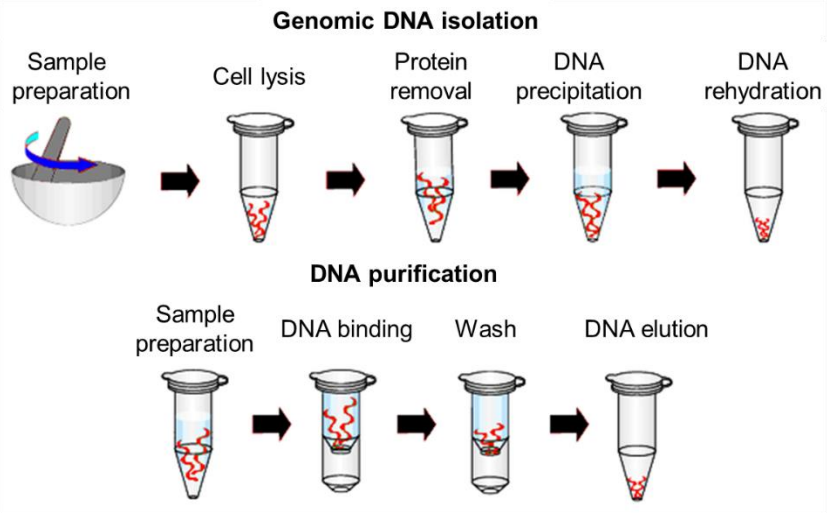

Fig. 5. The DNA extraction process, which typically covers both genomic DNA isolation and DNA purification. Genomic DNA isolation is the initial step, involving the extraction of DNA from a biological sample such as bacterial cells or tissue. This process involves breaking open the cells to release the genomic DNA and separating it from other cellular components such as proteins and lipids. DNA purification involves refining the isolated DNA further to eliminate any contaminants or impurities that could hinder downstream applications such as polymerase chain reaction and sequencing (Source: Mavink, 2024).

Shotgun metagenomics is a powerful molecular technique employed in microbial ecology and environmental microbiology to analyze the genetic material of complex microbial communities present in various environments (Sharpton, 2014; Quince et al., 2017; Tremblay et al., 2022). The technique involves the sequencing and analysis of DNA directly extracted from

environmental samples, bypassing the requirement for isolating individual microbial species through culturing. Unlike targeted approaches that focus on specific genes or regions of the genome, shotgun metagenomics provides a comprehensive view of the whole genetic content “metagenome,” of a microbial community (Quince et al., 2017).

The standard workflow (Fig. 6) for shotgun metagenomics starts with the collection and isolation of DNA from environmental samples. Subsequently, the extracted DNA undergoes fragmentation into smaller fragments, followed by sequencing using advanced next-generation sequencing technologies capable of high-throughput processing. The resultant sequence data are then subjected to bioinformatic analysis, training to reconstruct the genomes of individual organisms present in the sample, identify functional genes and pathways, and evaluate microbial diversity and community structure (Quince et al., 2017). A notable advantage of shotgun metagenomics lies in its capacity to unveil the functional potential of microbial communities (Sharpton, 2014; Quince et al., 2017; Tremblay et al., 2022). Through analysis of the collective gene repertoire encoded in the metagenome, scientists definitely infer the metabolic capabilities and ecological processes such as nutrient cycling, carbon metabolism, and symbiotic interactions in ecosystems (Sharpton, 2014; Tremblay et al., 2022).

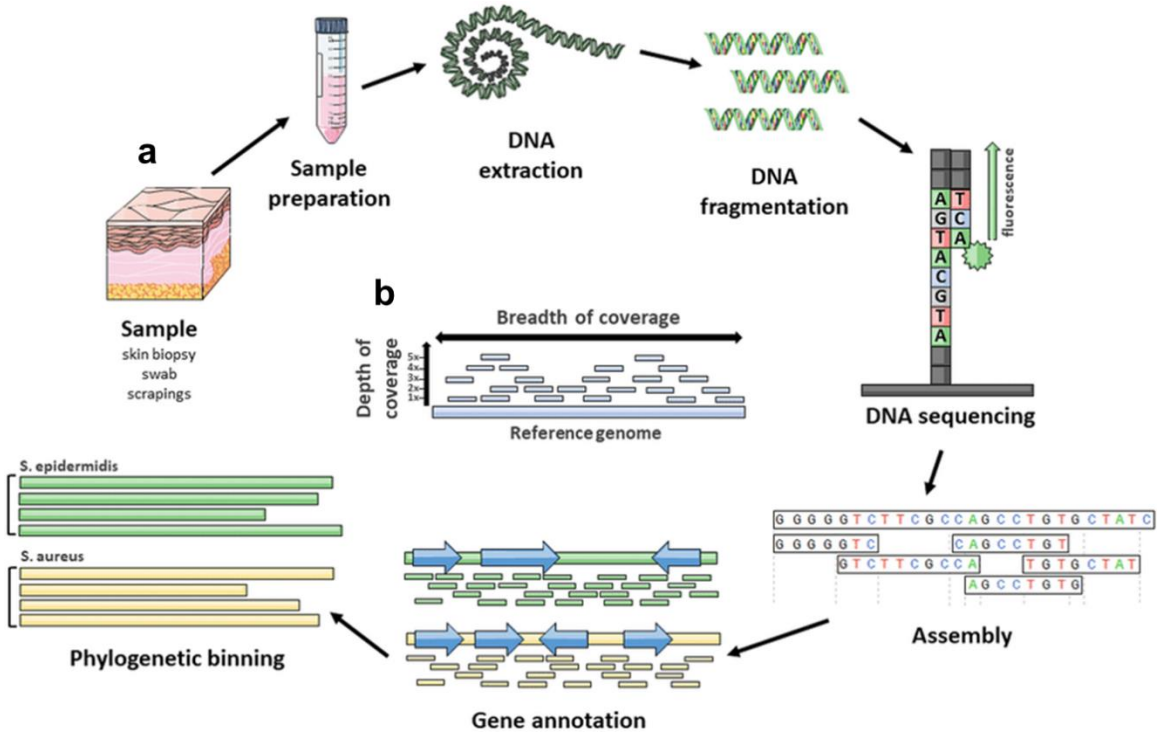

Fig. 6. The typical workflow of a whole shotgun metagenomic sequencing study. (a) After the collection of samples and subsequent DNA extraction, the genetic material undergoes fragmentation. These short DNA fragments are then sequenced concurrently, often employing methods such as sequencing by synthesis (e.g., Illumina sequencing). Subsequently, the resulting short reads are assembled in silico to produce sufficiently long segments suitable for gene annotation and whole-genome assembly. This assembly process relies on either a reference database genome or be performed de novo. Following assembly, a variety of procedures can be conducted, including phylogenetic binning of the resulting sequences. To ensure the success of the assembly, it is vital to achieve adequate representation of genomes, referred to as "depth of coverage," within the short fragment. (b) This aspect becomes particularly crucial in metagenomic analysis due to the high complexity of samples, emphasizing the importance of achieving comprehensive coverage across a breadth of genetic material (Godlewska et al., 2020).

Shotgun metagenomics finds diverse applications across multiple fields of biology (Sharpton, 2014; Guo et al., 2016; Xie et al., 2016; Dai et al., 2018; Godlewska et al., 2020; Zhang et al., 2021; Tremblay et al., 2022). In the field of environmental microbiology, it serves as a valuable tool for monitoring microbial communities inhabiting a wide range of habitats in soils, marine ecosystems, freshwater ecosystems, and extreme environments (Guo et al., 2016; Dai et al., 2018; Zhang et al., 2021). Shotgun metagenomics holds promise for unraveling the complexities of the microbiome and its implications for health and disease. Notably, it has been instrumental in characterizing the gut microbiota concerning conditions such as obesity, inflammatory bowel disease, and colorectal cancer (Xie et al., 2016). The effectiveness of shotgun metagenomics extends to expanding fields such as biotechnology, agriculture, and bioremediation. In biotechnology, it facilitates the discovery of novel enzymes, metabolic pathways, and natural products, thereby offering potential applications in both industry and medicine. In agriculture, shotgun metagenomics aids in the optimization of soil and plant microbiomes to bolster crop productivity and fortify resilience against environmental stressors. In bioremediation, it guides the development of microbial-based strategies for remediating contaminated sites by identifying microorganisms equipped to degrade pollutants (Sharpton, 2014; Guo et al., 2016; Tremblay et al., 2022). Despite its diverse advantages, shotgun metagenomics also presents several limitations. These include the substantial computational and bioinformatics demands associated with data analysis, susceptibility to sequencing and assembly inaccuracies, and the complexities inherent in accurately quantifying microbial abundance and activity solely from sequence data. Moreover, shotgun metagenomics

overlook rare microbial taxa and cannot capture certain types of genetic information, such as RNA transcripts or proteins (Quince et al., 2017).

2.5. Bacterial community analysis: statistical and model approaches

Numerical and statistical methodologies are essential tools in the analysis of microbial community data (Legendre and Legendre, 2012; ter Braak and Šmilauer, 2012). These methods aid scientists in quantifying the diversity and discovering complexity of microbial communities, along with their associations with the environment (Sigee, 2005). The selection of a statistical approach is basically determined by the research objectives and the characteristics inherent to the microbial community under investigation (Legendre and Legendre, 2012; ter Braak and Šmilauer, 2012).

The commonly employed statistical techniques for analyzing microbial community include diversity indices, analysis of similarities, non-metric multidimensional scaling, redundancy analysis, canonical correspondence analysis, and variance partitioning analysis (Magurran, 1988; Legendre and Legendre, 2012; ter Braak and Šmilauer, 2012; Paliy and Shankar, 2016). Diversity indices serve as mathematical measures to quantify the diversity and evenness of species within a community. Diversity indices such as the species richness index, Shannon-Wiener diversity index, and Simpson diversity index are frequently utilized to compare microbial community diversity across varying environmental conditions (Magurran, 1988). The analysis of similarities facilitates the comparison of microbial community similarity across diverse environments. It computes a similarity index between microbial communities and assesses whether significant differences exist among them (Anderson and Walsh, 2013). The non-metric multidimensional scaling aids in visualizing and contrasting the similarity of microbial

communities based on their taxonomic composition. This technique enables the identification of patterns within microbial community data that may correlate with environmental factors (Zuur et al., 2007). Redundancy analysis and canonical correspondence analysis represent multivariate statistical approaches utilized to analyze the relationships between multiple environmental variables and species distribution within a community. These methodologies enable scientists to determine the environmental factors most strongly linked to changes in species composition. Notably, redundancy analysis assumes linear relationships between species and environmental variables, whereas canonical correspondence analysis accommodates non-linear relationships (ter Braak and Šmilauer, 2012). The variance partitioning analysis partitions the variation in species composition according to distinct environmental factors. This method is grounded on the premise that various environmental factors contribute to the observed variation in species composition (Zhou and Ning, 2017).

The ecological processes shaping the diversity and compositional changes in microbial communities is inadequately understood topic in microbial ecology (Vellend, 2010; Nemergut et al., 2013; Zhou and Ning, 2017). The mechanisms underpinning species diversity are predominantly categorized into four fundamental ecological concepts: ecological dispersal, diversification, drift, and selection (Nemergut et al., 2013; Zhou and Ning, 2017). Dispersal denotes the continuous movement and successful colonization of organisms across spatial boundaries. Numerous factors, such as environmental filtering and biotic interactions, influence organismal movement, rendering dispersal a concept that can be perceived as either deterministic or stochastic (Zhou and Ning, 2017). Diversification represents an evolutionary mechanism that generates new genetic variation and lies

between speciation and extinction. Despite its significance, diversification is often overlooked in community ecology studies due to its association with long-term evolutionary processes spanning millions of years, particularly for microbes (Nemergut et al., 2013; Zhou and Ning, 2017). Currently, no specific approach exists to assess the relative importance of diversification in shaping microbial community structure (Zhou and Ning, 2017). Drift encompasses stochastic variations in the relative abundances of different species in a community over time, stemming from inherent random processes such as birth, death, and reproduction. Drift becomes prominent when selection pressure is weak, and the local community size is small. Ecological drift is inherently stochastic and challenging to empirically validate due to inherent demographic dissimilarities among species in nature (Zhou and Ning, 2017). Ecological selection refers to ecological forces that reshape community structure based on fitness disparities, such as differences in survival, growth, and reproduction among organisms (Nemergut et al., 2013; Zhou and Ning, 2017). Ecological selection arises from deterministic factors such as moisture, pH, and temperature, operating at both local and regional scales, as well as from synergistic effects of biotic interactions like competition, mutualism, and predation. Selection is categorized into two main types: homogeneous and heterogeneous selections. Homogeneous selection occurs when environmental conditions remain stable, resulting in minimal variation in community structure. In contrast, heterogeneous selection occurs when environmental conditions vary across space and time, leading to high variability in community composition. Ecological selection is unequivocally non-stochastic (Zhou and Ning, 2017).

Various statistical methods have been developed to evaluate the relative significance of environmental factors and dispersal constraints (Legendre and

Legendre, 2012; ter Braak and Šmilauer, 2012). Three main categories of multivariate statistical techniques are commonly employed to compare differences in community structure between and within treatments: permutational multivariate analysis of variance, analysis of similarities, and permutational analysis of multivariate dispersions, along with ordination methods such as principal-coordinates analysis, nonmetric multidimensional scaling, principal-component analysis, and detrended correspondence analysis (ter Braak and Šmilauer, 2012). Correlation-based analyses, including the Mantel test, multiple regression on dissimilarity matrices, redundancy analysis, and canonical correspondence analysis, are employed to assess relationships between community structure and environmental variables (Legendre and Legendre, 2012; ter Braak and Šmilauer, 2012). Additionally, variation partitioning analysis is employed to dissect community variation; however, caution must be exercised in its application, considering the challenge of unmeasured environmental variables (ter Braak and Šmilauer, 2012). Variation partitioning analysis is best utilized as an exploratory tool alongside other techniques such as neutral theory-based models and null model analysis (Zhou and Ning, 2017).

Neutral theory-based models represent an important approach for inferring processes from diversity patterns (Fig. 7). There are over ten distinct neutral models, each offering slightly different predictions concerning various factors. Among these, Hubbell's neutral model holds particular importance, featuring only three parameters: the population size of the local community, the rate of immigration, and the fundamental diversity number. Although these parameters are theoretically estimated and derived from ecological data, practical challenges arise in their quantification, particularly concerning the population size of a metacommunity, which proves problematic to quantify

(Nemergut et al., 2013; Zhou and Ning, 2017). Additionally, the rates of migration and speciation cannot be directly assessed, demanding the indirect quantification of parameters through the fitting of a neutral model to observed community structure data (Sloan et al., 2006).

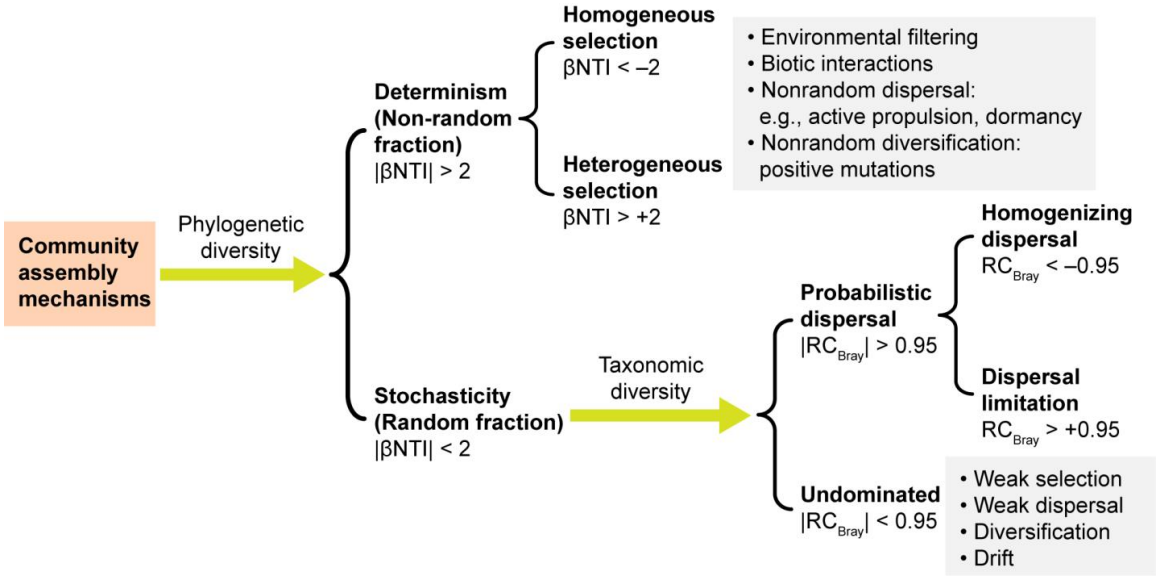

Fig. 7. The determinism and stochasticity in shaping microbial communities. The steps involved in partitioning these ecological processes are determined by both phylogenetic and taxonomic diversity metrics. Key indicators include βNTI, representing the β nearest-taxon index derived from a null model test of the phylogenetic β-diversity index, and RC_{Bray}, denoting the modified Raup-Crick index based on a null model test of the Bray-Curtis taxonomic β-diversity index (Source: Zhou and Ning, 2017).

3. Material and methods

3.1. Study site description and sampling

The Longcheng ponds are situated on the east bank of the Pearl River estuary in Shenzhen, a sub-provincial city in the southeastern province of Guangdong, China (Fig. 8). These urban ponds were created by transforming

a wetland into a garden-forest landscape. These ponds cover an area of 1.1 ha, with a maximum depth of 2 m and an altitude of 51.62 m. They are subject to a subtropical climate zone with clearly defined seasonal cycles annually. The average annual temperature and precipitation are 22.4 °C and 1,948 mm, respectively (Zhong et al., 2022). One notable characteristic of the Longcheng ponds is their isolated location, lacking connections to streams and rivers that can serve as inlets or outlets for water exchange (Fig. 8a). This isolation, coupled with adapted organisms to the specific environmental conditions, makes it a small endorheic urban system. Longcheng ponds serve as biotopes for various aquatic fauna, such as black swans, fish, frogs, dragonflies, and snails, as well as diverse flora, including lilies and free-floating algae in the water, along with reeds along the pond's edges (Fig. 8a–g).

The studied urban pond system comprises three adjacent ponds, each with distinct environmental characteristics (Supplementary Table 1). The replenishment of each pond is determined by a combination of natural processes, such as rainfall and terrestrial runoff, as well as artificial interventions such as municipal water supplies and inter-pond discharge. Pond 1 receives natural replenishment from rainwater and terrestrial runoff, supplemented by artificial inputs from municipal water supplies (upstream). Similarly, Pond 2 undergoes replenishment like Pond 1, with the additional contribution of discharge from Pond 1 (middlestream). In the case of Pond 3, it shares the replenishment characteristics of Pond 1, with the further inclusion of discharge from both pond 1 and 2 (downstream). Notably, Pond 3 lacks an outlet for water discharge (Fig. 8h).

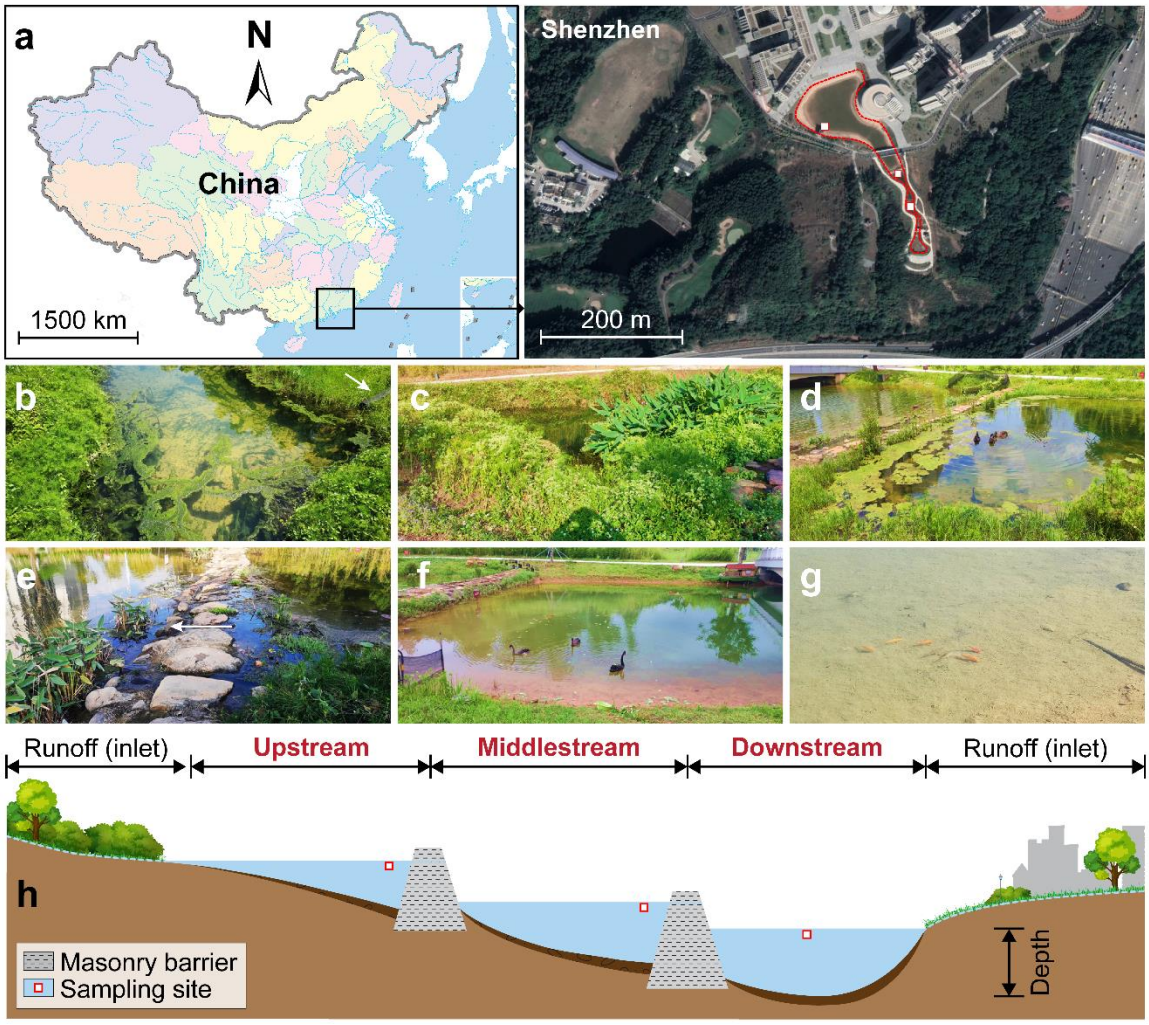

Fig. 8. Description of Longcheng Pond. (a) Geographical location of the ponds in Shenzhen, Guangdong, southeastern China. (b) The pond structure in its upstream section shows pond scum in both surface water and at the bottom, with marshland vegetation surrounding the body of water, and a drainage pipe (white arrow) serving as artificial replenishing facilities. (b–d) Diversity of marsh vegetation within and along the pond. (e–f) Structure of the masonry barriers splitting the pond into three sections, with connectivity of these sections (white arrow) mostly determined by an excess of water moving from the upstream section to other sections. (f) The bottom of the

pond in the downstream section, which holds a relatively lesser amount of pond scum, several black swans, and fishes.

Previous research on the dynamics of microbial communities in urban ponds, conducted at both local and regional levels, underscored the importance of recognizing ponds as distinct environments depending on their connectivity to adjacent environments (Burdíková et al., 2012; Ndayishimiye et al., 2023; Li et al., 2023). To ensure consistency with previous implications, we developed a sampling approach considering that each pond represents a unique habitat, and establishing one sampling station in the deepest zone adequately produces a representative sample. Sampling was conducted during warmer conditions, with water temperatures ranging from 26.4 to 32.1 °C and pH levels varying from 6.4 to 8.6 (Supplementary Table 1). Samples were collected from the surface water at a depth of 0 to 20 cm, twice a week in August and September 2022 (i.e., from the 216^{th} to 244^{th} days of 2022), around 9:00 a.m. A total of 30 water samples were obtained from three stations, comprising one upstream (n = 10), another midstream (n = 10), and one downstream (n = 10) (Fig. 8h). This high-frequency sampling approach is particularly beneficial when studying systems with rapid changes, variability, or short-term events, aiming to obtain a more comprehensive and detailed understanding of the biosphere, including various ecological phenomena (Nyirabuhoro et al., 2021). After the fieldwork, samples were immediately transported to the laboratory for further analysis.

3.2. DNA extraction, metagenomic sequencing, and bioinformatics

Initially, the water samples underwent pre-filtration using a 200 μm pore-sized sieve to eliminate large particles. Subsequently, 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 containing microbial plankton were then carefully packed into sterilized tubes and preserved at -80 °C until DNA extraction.

Total DNA of microplankton 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). DNA samples were then dry-frozen and shipped to Tianjin Novogene Bioinformatic Technology Co., Ltd. (Tianjin, China) for sequencing. A total amount of 0.2 μg DNA per sample was used as input material for the DNA library preparations. Sequencing library was generated using NEBNext® UltraTM DNA Library Prep Kit for Illumina (NEB, USA, #Catalog #: E7370L) following manufacturer's recommendations and index codes were added to each sample. Briefly, genomic DNA sample was fragmented by sonication to a size of 350 bp. Then DNA fragments were endpolished, A-tailed, and ligated with the full-length adapter for Illumina sequencing, followed by further PCR amplification. After PCR products were purified by AMPure XP system (Beverly, USA). Subsequently, library quality was assessed on the Agilent 5400 system (Agilent, USA) and quantified by QPCR (1.5 nM). The qualified libraries were pooled and sequenced on Illumina platforms with PE150 strategy in Novogene Bioinformatics Technology Co., Ltd (Beijing, China), according to effective library concentration and data amount required. The original fluorescence image files obtained from Illumina platform were transformed to short reads (Raw data) by base calling and these short reads

are recorded in FASTQ format (Cock et al., 2010), which contains sequence information and corresponding sequencing quality information. Sequence artifacts, including reads containing adapter contamination, low-quality nucleotides and unrecognizable nucleotide (N), undoubtedly set the barrier for the subsequent reliable bioinformatics analysis. Hence quality control was an essential step and was applied to guarantee the meaningful downstream analysis. we used Fastp version 0.23.1 (Chen et al., 2018) to perform basic statistics on the quality of the raw reads. The steps of data processing were as follows: discard a paired reads if either one read contains adapter contamination; discard a paired reads if more than 10% of bases are uncertain in either one read; and discard a paired reads if the proportion of low quality (Phred quality < 5) bases is over 50% in either one read.

3.3. Identification of pathogenic bacteria

Kraken2 was employed to annotate and classify all valid sequences from each sample, using the parameter "--confidence 0.2." Subsequently, the results were forwarded to Bracken with default parameters to annotate and estimate the bacterial diversity and composition of the samples at the lowest taxonomic level. The search for potential bacterial pathogens was conducted using ePATHogen. The risk assessment involved the classification of pathogenic bacteria into diverse risk groups (National Research Council, 2010) and monitoring temporal changes in taxonomic diversity and abundance. Risk group 1 includes bacteria that typically do not cause disease in healthy mature humans or animals. Risk group 2 involves bacteria associated with diseases that are rarely serious. Risk group 3 comprises bacteria linked to serious or lethal diseases. An unclassified group consists of all pathogenic bacteria that have not been considered within a utilized classification system until the end

of December 2023. The potential risk posed by bacterial pathogens in urban park ponds to both human (human risk group) and animal (animal risk group) populations was estimated by linking the diversity, characterized by species richness and species relative abundance, with each risk group. Venn diagrams, pie charts, and stacked charts in OriginLab Corporation version 9.90.225 were further employed to create graphic representations of the data.

3.4. Statistical analysis

To quantify the rate rates and patterns of community variation over time, we employed time-lag regression analysis, where delta time represents the interval between two sampling days (Collins et al., 2000). This method is a valuable tool for measuring temporal variation in bacterial communities, serving as an extension of auto-correlation analysis for short time series of community data (Nyirabuhoro et al., 2021). A linear regression line with a significant positive slope shows that the microbial community is undergoing directional change over time. If the linear regression line is not significant or the slope is not significantly different from zero, it indicates fluctuation or stochastic variation over time. A linear regression line with negative slope implies that community composition is becoming more similar to an earlier state in the time series (Collins et al., 2000).

The relative importance of stochastic and deterministic processes in shaping bacterial communities was quantified using the neutral community model (Sloan et al., 2006) and the null model (Gotelli and Mccabe, 2002), respectively. The neutral community model explains the patterns observed in bacterial communities, considering the typical scales at which they are found. A key aspect of the model hypothesizes that bacterial composition is governed by factors such as species diversity, species abundance, migration probability,

compositional drift, and extinction (Nyirabuhoro et al., 2021). In practice, *Nm* is an estimate of dispersal between communities, where *N* represents the metacommunity size, and *m* expresses the rate of individuals immigrating from the source community into the local community. The proportion of variability, R^2 , quantifies the degree to which the detection frequency fits the model: R^2 value approaching 1 indicates that the community composition follows a completely stochastic process; R^2 value of ≤0 suggests that the model does not match the community composition (Sloan et al., 2006). The neutral community model was computed using the packages "Hmisc," "minpack.lm," and "stats4" and R version 4.4.0 (R Core Team, 2024).

The checkerboard score, also known as the C-score (Stone et al., 1990), was employed to assess the non-random distribution of bacteria. This metric was selected due to its robustness against minor fluctuations in the data (Nyirabuhoro et al., 2021). Initially, the species table was transformed into a binary matrix indicating their presence or absence, which was then subjected to analysis for various combinations (Stone et al., 1990; Gotelli et al., 2002). To enable comparison of results across the sites, standardized effect sizes (SES) were computed for each matrix. SES were determined by calculating the difference between the observed index (I_{obs}) and the mean of the simulated index (I_{sim}) divided by the standard deviation of the simulated index (σ_{sim}) (Gotelli et al., 2002). SES for the C-score was computed using a burn-in of 30,000 simulations and the sequential swap randomization algorithm implemented using the package "EcoSimR" and R version 4.4.0 (R Core Team, 2024). The strength of deterministic processes on microbial communities was further reflected in the magnitude of SES for the C-Score. SES values between -2 and 2 suggest the dominance of stochastic processes

in shaping the microbial community. SES values less than -2 (i.e., aggregation) or greater than 2 (i.e., segregation) show that deterministic processes are more important in community assembly than stochastic processes (Mo et al., 2021; Lin et al., 2023).

4. Results

4.1. Taxonomic diversity and composition of the bacterial community

A taxonomic diversity of 381 bacterial species, or 21 phyla, was identified from Longcheng ponds (Fig. 9a). Pathogenic bacteria (Table 1) accounted for 40.42% of the total bacterial species, corresponding to 66.67% of the total phyla (Fig. 9a). The predominance of the phylum Proteobacteria in the bacterial community coincided with its high taxonomic diversity, comprising a total of 69 pathogenic bacteria, which accounted for 18.11% of the total bacterial species. Among these pathogenic Proteobacteria, Alphaproteobacteria accounted for 20 species (5.25% of the total bacterial species), Gammaproteobacteria for 18 (4.72%), Betaproteobacteria for 12 (3.15%), Deltaproteobacteria for 11 (2.89%), and Epsilonproteobacteria for 8 (2.10%).

The diversity patterns of the bacterial community exhibited substantial variations, especially among phyla with a higher prevalence of pathogens (Fig. 9b). The species richness of pathogenic bacteria ranged from 11 to 26 species at each station, resulting in a mean value of 20 ± 1 species for the three stations collectively. It decreased markedly on the 227th day, with 11 species midstream, and peaked on the 218th day, with 26 species both midstream and downstream. The prevalence of Proteobacteria markedly increased the species richness of pathogenic bacteria, with values ranging from 3 to 15 at each

station (or a mean value of 9 ± 1 species for the three stations collectively). These pathogenic Proteobacteria decreased markedly on the 227th day, with 5 species in the middlestream, and peaked on the 218th day, with a total of 15 species in both the upstream and middlestream.

The temporal patterns of the bacterial species relative abundance showed substantial variations, particularly among phyla with a higher prevalence of pathogens (Fig. 9c). The relative abundance of pathogenic bacteria ranged from 26.8% to 54.9%, resulting in a mean value of 39.3 ± 1.3% for the three stations collectively. It decreased markedly on the 223rd day, with 26.8% downstream, and peaked on 244th day, with 54.9% midstream. The prevalence of Proteobacteria distinctly increased the relative abundance of pathogenic bacteria, with values ranging from 8.8% to 35.2% (or a mean value of 20.0 ± 1.4% for the three stations collectively). These pathogenic Proteobacteria dropped markedly on the 216th day, with a relative abundance of 8.8% downstream, and peaked on 220th day, with 35.2% upstream.

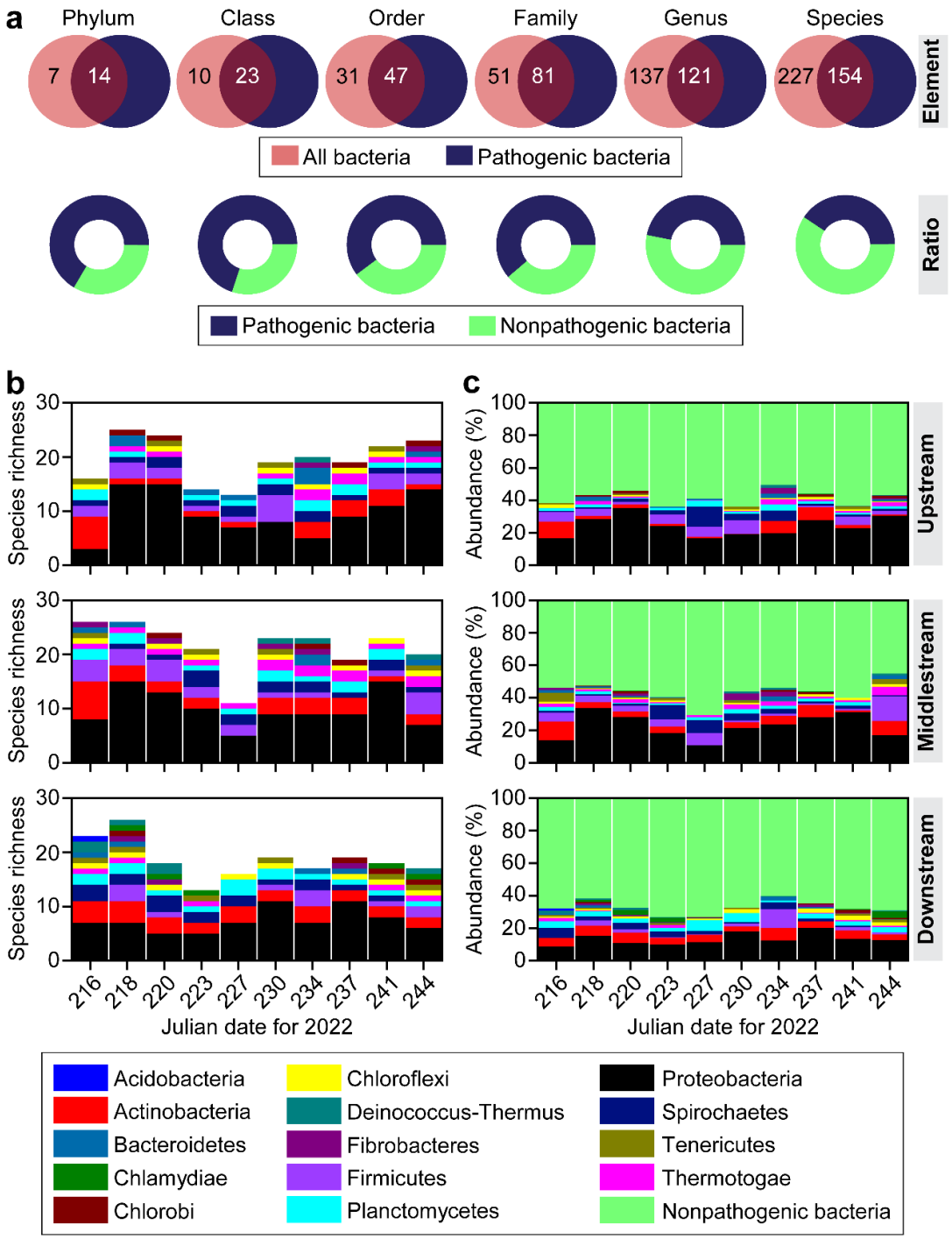

Fig. 9. Taxonomic diversity of pathogenic bacteria in Longcheng Pond between August 4 and September 1, 2022. (a) Taxonomic hierarchy of pathogenic bacteria, illustrating broader classifications to more specific ones. The quantity of pathogenic bacteria and their corresponding percentages in

the taxonomic hierarchy are represented using elements and ratios, respectively. (b) Temporal variations in species richness at the phylum level. (c) Temporal variations in species relative abundance at the phylum level.

Table 1. The taxonomic diversity of 154 bacterial species from Longcheng Pond organized into diverse risk groups for humans and animals.

Species name	Human pathogen	Animal pathogen
Acetohalobium arabaticum	Risk group 1	Unclassified
Acholeplasma laidlawii	Risk group 1	Risk group 2
Acidimicrobium ferrooxidans	Risk group 1	Risk group 1
Acidithiobacillus caldus	Risk group 1	Unclassified
Acidobacterium capsulatum	Risk group 1	Unclassified
Acidovorax avenae	Risk group 1	Risk group 1
Acidovorax citrulli	Risk group 1	Risk group 1
Acinetobacter baumannii	Risk group 2	Risk group 1
Actinobacillus pleuropneumoniae	Risk group 1	Risk group 2
Actinobacillus suis	Risk group 2	Risk group 2
Aeromonas veronii	Unclassified	Risk group 1
Alicyclobacillus acidocaldarius	Risk group 1	Risk group 1
Amphibacillus xylanus	Risk group 1	Unclassified
Anaerococcus prevotii	Risk group 2	Risk group 1
Anaeromyxobacter dehalogenans	Risk group 1	Risk group 1
Anaplasma centrale	Risk group 1	Risk group 3
Anaplasma marginale	Risk group 1	Risk group 3
Asticcacaulis excentricus	Risk group 1	Unclassified
Atopobium parvulum	Risk group 2	Risk group 1

Bacillus anthracis	Risk group 2	Risk group 2
Bacillus cereus	Risk group 3	Risk group 3
Bacillus clausii	Risk group 1	Risk group 1
Bacillus pumilus	Risk group 1	Risk group 1
Bdellovibrio bacteriovorus	Risk group 1	Unclassified
Beijerinckia indica	Risk group 1	Unclassified
Bifidobacterium animalis	Risk group 1	Risk group 1
Bifidobacterium asteroides	Risk group 1	Unclassified
Bordetella avium	Risk group 1	Risk group 2
Borrelia duttonii	Risk group 2	Risk group 1
Buchnera aphidicola	Risk group 1	Unclassified
Burkholderia cepacia	Risk group 2	Risk group 1
Burkholderia glumae	Risk group 1	Risk group 1
Campylobacter concisus	Risk group 2	Risk group 1
Campylobacter curvus	Risk group 2	Risk group 1
Caulobacter segnis	Risk group 1	Risk group 1
Cellulomonas fimi	Risk group 1	Unclassified
Chloroherpeton thalassium	Risk group 1	Unclassified
Clavibacter michiganensis	Risk group 1	Unclassified
Comamonas testosteroni	Risk group 1	Unclassified
Coriobacterium glomerans	Risk group 1	Risk group 1
Corynebacterium argentoratense	Risk group 2	Risk group 1
Corynebacterium callunae	Risk group 1	Risk group 1
Corynebacterium glutamicum	Risk group 1	Risk group 1
Cytophaga hutchinsonii	Risk group 1	Risk group 1
Delftia acidovorans	Risk group 2	Risk group 1

Desulfarculus baarsii	Risk group 1	Unclassified
Desulfobulbus propionicus	Risk group 1	Unclassified
Desulfomonile tiedjei	Risk group 1	Unclassified
Desulfovibrio aespoeensis	Risk group 1	Unclassified
Desulfovibrio africanus	Risk group 1	Unclassified
Desulfovibrio vulgaris	Risk group 1	Risk group 1
Erysipelothrix rhusiopathiae	Risk group 2	Risk group 2
Erythrobacter litoralis	Risk group 1	Unclassified
Eubacterium eligens	Risk group 1	Unclassified
Eubacterium rectale	Risk group 1	Risk group 1
Fibrobacter succinogenes	Risk group 1	Unclassified
Filifactor alocis	Risk group 1	Risk group 1
Finegoldia magna	Risk group 2	Risk group 1
Flavobacterium branchiophilum	Risk group 1	Risk group 1
Flavobacterium psychrophilum	Risk group 1	Unclassified
Flexibacter litoralis	Risk group 1	Risk group 1
Francisella novicida	Risk group 2	Risk group 2
Gardnerella vaginalis	Risk group 2	Risk group 1
Gluconacetobacter xylinus	Risk group 1	Risk group 1
Gordonia polyisoprenivorans	Risk group 1	Unclassified
Haemophilus influenzae	Risk group 2	Risk group 1
Haemophilus parainfluenzae	Risk group 2	Risk group 2
Halanaerobium praevalens	Risk group 1	Unclassified
Haliscomenobacter hydrossis	Risk group 1	Unclassified
Halobacteroides halobius	Risk group 1	Unclassified
Halothiobacillus neapolitanus	Risk group 1	Risk group 1

Helicobacter cinaedi	Risk group 2	Risk group 2
Helicobacter heilmannii	Risk group 2	Risk group 2
Helicobacter mustelae	Risk group 2	Risk group 2
Helicobacter pylori	Risk group 2	Risk group 2
Herpetosiphon aurantiacus	Risk group 1	Risk group 1
Hirschia baltica	Risk group 1	Unclassified
Isosphaera pallida	Risk group 1	Unclassified
Jonesia denitrificans	Risk group 2	Risk group 1
Kitasatospora setae	Risk group 1	Unclassified
Kocuria rhizophila	Risk group 1	Risk group 1
Lactobacillus buchneri	Risk group 1	Unclassified
Lactobacillus casei	Risk group 1	Risk group 1
Lactobacillus delbrueckii	Risk group 1	Risk group 1
Lactobacillus fermentum	Risk group 1	Risk group 1
Lactobacillus rhamnosus	Risk group 1	Risk group 1
Lactobacillus ruminis	Risk group 1	Unclassified
Lactobacillus sanfranciscensis	Risk group 1	Unclassified
Leptospira biflexa	Risk group 2	Risk group 1
Leptospira interrogans	Risk group 2	Risk group 2
Leptothrix cholodnii	Risk group 1	Unclassified
Leuconostoc gelidum	Risk group 1	Risk group 1
Magnetospirillum gryphiswaldense	Risk group 1	Risk group 1
Maricaulis maris	Risk group 1	Risk group 1
Meiothermus ruber	Risk group 1	Unclassified
Meiothermus silvanus	Risk group 1	Unclassified
Microbacterium testaceum	Risk group 1	Risk group 1

Mobiluncus curtisii	Risk group 2	Risk group 1
Moraxella catarrhalis	Risk group 2	Risk group 1
Mycobacterium leprae	Risk group 2	Risk group 1
Mycobacterium marinum	Risk group 2	Risk group 2
Mycoplasma hyopneumoniae	Risk group 1	Risk group 2
Myxococcus stipitatus	Risk group 1	Risk group 1
Nakamurella multipartita	Risk group 1	Unclassified
Nocardiopsis dassonvillei	Risk group 2	Risk group 1
Novosphingobium aromaticivorans	Risk group 1	Unclassified
Octadecabacter antarcticus	Risk group 1	Unclassified
Octadecabacter arcticus	Risk group 1	Unclassified
Olsenella uli	Risk group 1	Unclassified
Ornithobacterium rhinotracheale	Risk group 1	Risk group 2
Paracoccus aminophilus	Risk group 1	Unclassified
Pirellula staleyi	Risk group 1	Unclassified
Planctomyces limnophilus	Risk group 1	Unclassified
Polynucleobacter necessarius	Risk group 1	Unclassified
Prevotella intermedia	Risk group 2	Risk group 1
Propionibacterium avidum	Risk group 2	Risk group 1
Pseudomonas fluorescens	Risk group 1	Risk group 1
Pseudomonas putida	Risk group 1	Risk group 1
Pseudoxanthomonas spadix	Risk group 1	Risk group 1
Renibacterium salmoninarum	Risk group 1	Risk group 1
Rhodobacter sphaeroides	Risk group 1	Unclassified
Rhodococcus erythropolis	Risk group 1	Risk group 1
Rhodomicrobium vannielii	Risk group 1	Unclassified

Rhodospirillum photometricum	Risk group 1	Unclassified
Rhodospirillum rubrum	Risk group 1	Risk group 1
Roseobacter litoralis	Risk group 1	Unclassified
Rothia mucilaginosa	Risk group 2	Risk group 1
Rubrivivax gelatinosus	Risk group 1	Unclassified
Ruegeria pomeroyi	Risk group 1	Risk group 1
Saprospira grandis	Risk group 1	Risk group 2
Segniliparus rotundus	Risk group 1	Risk group 1
Simkania negevensis	Risk group 2	Risk group 2
Sorangium cellulosum	Risk group 1	Risk group 1
Spiroplasma diminutum	Risk group 1	Unclassified
Staphylococcus saprophyticus	Risk group 2	Risk group 1
Stenotrophomonas maltophilia	Risk group 2	Risk group 2
Stigmatella aurantiaca	Risk group 1	Risk group 1
Streptococcus suis	Risk group 2	Risk group 2
Sulfurimonas denitrificans	Risk group 1	Unclassified
Thermobacillus composti	Risk group 1	Unclassified
Thermotoga hypogea	Risk group 1	Unclassified
Thermotoga neapolitana	Risk group 1	Unclassified
Thermotoga thermarum	Risk group 1	Unclassified
Thermus thermophilus	Risk group 1	Unclassified
Thiocystis violascens	Risk group 1	Unclassified
Thiomonas intermedia	Risk group 1	Risk group 1
Treponema paraluiscuniculi	Risk group 2	Risk group 2
Tropheryma whipplei	Risk group 2	Risk group 1
Turneriella parva	Risk group 1	Risk group 1

Ureaplasma urealyticum	Risk group 2	Risk group 1
Variovorax paradoxus	Risk group 1	Unclassified
Wigglesworthia glossinidia	Risk group 1	Unclassified
Wolinella succinogenes	Risk group 1	Unclassified
Xylella fastidiosa	Risk group 1	Risk group 1

Risk group 1 includes bacteria that typically do not cause disease in healthy mature humans or animals. Risk group 2 involves bacteria associated with diseases that are rarely serious. Risk group 3 comprises bacteria linked to serious or lethal diseases. An unclassified group consists of all pathogenic bacteria that have not been considered within a utilized classification system until the end of December 2023.

4.2. Ecological processes shaping the bacterial community

The bacterial community was unstable, with the species composition of pathogenic bacteria over time converging towards a community-type characteristic of an earlier sampling period (Fig. 10). The species composition exhibited sensitivity to the sampling time span, displaying divergent trends of varying strengths and significance: $R^2 = 0.122$ ($P > 0.05$) upstream; $R^2 = 0.041$ ($P > 0.05$) middlestream; and $R^2 = 0.133$ ($P < 0.05$) downstream. The neutral community model, which estimates the influence of stochastic processes on the bacterial community, demonstrated a strong fit across the three stations. The explained community variation was 77.5% upstream, 72.1% middlestream, and 73.6% downstream. The null model, which evaluates the impact of deterministic processes and species segregation, demonstrated a higher I_{obs} compared to I_{sim} and SES for C-score falling between 9 and 21.5.

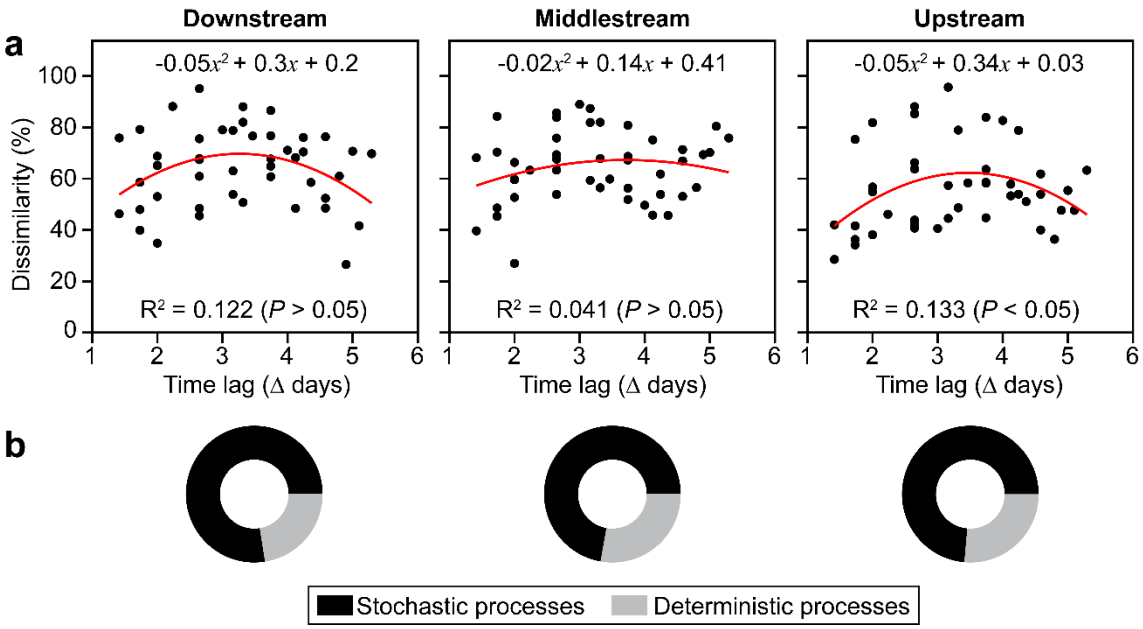

Fig. 10. Driving factors of bacterial community stability. (a) The convergent dynamic in bacterial community composition during short-term periods. The delta time (∆) describes the time elapsed between two sampling days. (b) The relative importance of stochastic and deterministic processes in shaping the bacterial community.

5. Discussion

5.1. Compositional changes of the bacterial community in response to warm conditions

Bacterial communities are integral component of aquatic environments (Sigee, 2005). Nevertheless, understanding their ecological dynamics especially in urban waters dictates the use of high-frequency measurements (Mo et al., 2021; Nyirabuhoro et al., 2021). In this study, high-frequency sampling, carried out twice a week from three adjacent urban ponds, enabled the analysis of the bacterial community change in response to warm conditions (Fig. 9). Compositional changes in response to warm conditions

obviously underscore the importance of high-frequency sampling in capturing the episodic shifts in bacterial communities (Nyirabuhoro et al., 2021). Our observations align with those of Avila et al. (2017), who showed that fundamental patterns of seasonal changes and successions in bacterial communities are typically evident only in data obtained through low-frequency sampling. They are also consistent with those of Lindh et al. (2015), who emphasized that such patterns may fail to capture sharp transitions in certain microbial taxa. This is because microbial communities exhibit rapid responses to environmental variations, with interactions occurring over short temporal scales, ranging from seconds to seasons (Nguyen et al., 2021).

The dominance of the Proteobacteria phylum in the bacterial community of Longcheng ponds, coupled with its extensive taxonomic diversity comprising 69 pathogenic bacterial species (Fig. 9), demonstrates the significance of the temporal changes in environmental conditions in shaping the bacterial community and competitive advantage of Proteobacteria over other bacterioplankton groups in degrading anthropogenic inputs (He et al., 2017; Vaz-Moreira et al., 2017). Chiriac et al. (2017) highlighted the significance of environmental dynamics, indicating that that in warmer waters (20 to 65°C), increasing temperatures lead to decreased Cyanobacteria abundance and increased Proteobacteria and Chloroflexi abundances. Cooper et al. (2015) underscore the competitive advantage of Proteobacteria, noting their involvement in mutualistic relationships and competitive interactions, which enable them to outcompete other bacterial groups for resources. The prevalence of Proteobacteria additionally indicates a potential microbial marker for disease, as this phylum ranks among the most abundant phyla, housing numerous known human pathogens particularly in warm conditions (Rizzatti et al., 2017).

5.2. Influence of stochastic processes in shaping bacterial community

Environmental dynamics are inherent natural phenomena, which can exert both stochastic (random) and deterministic (predictable) influences on microbial communities over time (Nemergut et al., 2013; Zhou and Ning, 2017). Our sampling strategy of collecting surface water twice a week, especially in subtropical warm conditions, effectively enables the detection of rapid changes resulting from species interactions as well as prolonged dynamics caused by shifting ecological conditions (Fig. 9b–c). In supporting the consistency and effectiveness of our strategy, Nyirabuhoro et al. (2021) showed that high-frequency sampling over 13 months in a subtropical urban reservoir better compares patterns and ecological processes at short (0–8 weeks), medium (9–24 weeks), and long (25–53 weeks) time intervals for bacterial subcommunities such as abundant taxa, conditionally rare taxa, and rare taxa. This method revealed directional changes in bacterial communities during short- and medium-time intervals and convergent dynamics due to seasonal cycles over longer periods. In our study, covering a one-month period (i.e., a short time period of delta time intervals in a range of 2 to 3 days), the results show convergent dynamics (Fig. 10a). Therefore, as demonstrated by Nyirabuhoro et al. (2021), the observed convergent dynamics may suggest that the species composition and abundances of sampled bacterial community are highly dynamic and responsive to environmental changes, especially those linked to seasonal variations. This is particularly relevant in subtropical China, where autumn, characterized by a significant decrease in both air and water temperatures, may begin as early as August.

The neutral community model is a powerful tool providing a better insight into the assembly and dynamics of bacterial community in urban

waters, especially the role of neutral processes such as migration, births, and deaths within the population (Nyirabuhoro et al., 2021; Mo et al., 2021; Li et al., 2023; Ndayishimiye et al., 2023). The Sloan neutral community model which show explained community variation between 72.1% and 77.5% (Fig. 10b), clearly indicates a substantial influence of stochastic processes in shaping the bacterial community over time (Nyirabuhoro et al., 2021). Higher explained community variation with values in the same range may further underscore the typical role of migration in the neutral processes as these three studied ponds are adjacent (Li et al., 2023). In supporting the consistency and significance of our findings, Nemergut et al. (2013) and Zhou and Ning (2017) underscore that even though stochastic processes are largely deemed less important in shaping microbial community assembly in aquatic ecosystems, they can drive microbial community dynamics, particularly during extreme weather events and natural disasters.

Employing the null model alongside the neutral community model offers insights into the competitive interactions among microorganisms and the importance of deterministic processes in shaping their microbial community assembly within urban waters (Nyirabuhoro et al., 2021; Li et al., 2023; Ndayishimiye et al., 2023). The competitive interactions among bacterial species for limited resources (Fig. 10) potentially explain observed $I_{obs} > I_{sim}$ and higher SES for C-score > 2 (Nyirabuhoro, 2021). Our findings align with observations from various environments reviewed by Nemergut et al. (2013), Dini-Andreote et al. (2015), and Zhou and Ning (2017), as certain microbial species outcompete others in highly dynamic environments, leading to shifts in relative abundances and community composition. These competitive

interactions drive changes in dominant taxa and contribute to temporal patterns within microbial communities. Additionally, competition induces alterations in community compositions over time, potentially resulting in the increased dominance of certain microorganisms whereas others decline or are completely eliminated.

5.3. Ecological implication and priorities for future research

Urban ponds represent significant ecosystems with distinct characteristics and ecological importance (Hassall, 2014; Hill et al., 2021). The dynamics of microbial communities in these ponds are predominantly driven by changes in specific environmental conditions unique to this ecosystem type (Hill et al., 2021). Variations in the structure and diversity of bacterial communities in the ponds act as indicators of ecosystem dynamics and health (Sigee, 2005). The analysis of dynamics and assembly mechanisms of bacterial communities in Longcheng ponds enables a better understanding of specific ecological patterns characterizing subtropical urban ponds and assessing the impacts of environmental changes on microbial communities.

Although our study revealed the dynamics of the bacterial community and community assembly mechanisms (Figs. 10–11), it is crucial to acknowledge limitations stemming from the short duration of the research and limited sampling stations. Conducting research over only a one-month period may fail to capture the full spectrum of variations and long-term trends, especially considering that climate change can disrupt seasonal cycles and inter-annual variations in microbial communities. Therefore, conducting more extensive and prolonged investigations across multiple warm months would be beneficial to comprehensively evaluate how environmental changes impact bacterial communities over various timescales.

For future research, it is advisable to prioritize larger spatial scales by including more urban ponds from various cities and geographic regions. Additionally, incorporating a diverse range of climate zones would offer insights into bacterial community dynamics in response to varying climatic conditions. Expanding the scope of study would further allow for generalizing conclusions to a broader context, enhancing the feasibility and applicability of research outcomes.

6. Conclusion

This study presents the temporal dynamics and assembly mechanisms of bacterial communities in response to warm conditions in three adjacent subtropical urban ponds in Shenzhen, southeast China. We conducted whole-metagenomic shotgun sequencing of 30 surface water samples over a one-month period to better understand the bacterial community compositional changes between 26.4 and 32.1°C and how stochastic processes contribute to the assembly mechanisms of these bacterial communities. A total of 381 bacterial species, representing 21 phyla, were identified, with Proteobacteria being the most prevalent and 40.42% of the species classified as pathogenic bacteria. The distinct patterns of diversity and species relative abundance in each studied pond over a relatively short time scale underscore the dynamic nature of bacterial community and uniqueness of each pond as a potential habitat. The bacterial community that tends to converge towards a community type resembling an earlier sampling period indicates the potential for a seasonal cycle, with ecological processes encompassing both random changes in population (stochastic processes) and non-random patterns of species (deterministic processes). Stochastic processes, explaining 72.1% and 77.5% of the community composition in the three studied ponds, underscores a

stronger effect of stochastic processes over deterministic processes in shaping the bacterial community.

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Supplementary information

Supplementary Table 1. The mean values plus standard errors of twenty-five measured environmental variables in Longcheng ponds, Shenzhen, southern China, between the 216th and 244th days of 2022.

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 ± 15435.64	26336.5 ± 9897.895	29286.4 ± 11038
Wind speed (m/s)	1.78 ± 0.269	1.42 ± 0.179	1.49 ± 0.282
Humidity (%)	76.8 ± 2.867	78.4 ± 2.941	80.1 ± 2.233
Pressure (hPa)	999.905 ± 0.577	999.912 ± 0.574	999.946 ± 0.574
Carbon monoxide (μg m^3)	525.448 ± 60.324	624.181 ± 78.504	602.151 ± 94.941
Nitrogen monoxide (μg m^3)	9.815 ± 8.358	20.51 ± 9.816	21.805 ± 11.657
Nitrogen dioxide (μg m^3)	26.175 ± 4.784	31.642 ± 5.636	22.647 ± 4.403
Ozone O_3 (μg m^3)	58.746 ± 21.465	37.561 ± 17.848	38.141 ± 19.426
Sulfur dioxide (μg m^3)	56.743 ± 16.954	73.313 ± 17.683	47.659 ± 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	0.42	0.898
	± 0.031	± 0.031	± 0.098
pH	6.644	7.047	8.048
	± 0.062	± 0.07	± 0.086
Oxydo-reduction potential	173.09	169.17	133.02
(mV)	± 10.727	± 10.553	± 10.753
Dissolved oxygen saturation	73.82	96.43	198.73
(%)	± 12.258	± 11.586	± 18.793
Dissolved oxygen	5.663	7.03	13.672
concentration (ppm)	± 1.017	± 0.736	± 0.981
Electrical conductivity (µs	407.7	370.1	192.6
cm^{-1})	± 31.186	± 38.367	± 11.025
Resistivity (MΩcm)	0.003	0.003	0.005
	± 0	± 0	± 0
Total dissolved solids (ppm)	205	185.2	96.6
	± 15.496	± 19.163	± 5.518
Salinity (PSU)	0.196	0.175	0.087
	± 0.015	± 0.019	± 0.005

Turbidity (FNU)	8.710	8.56	3.58
	± 3.076	± 2.304	± 0.728
Water temperature (℃)	27.993	29.097	30.399
	± 0.402	± 0.425	± 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).