



Investigation of water quality and aquatic ecological succession of a newly constructed river replenished by reclaimed water in Beijing

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ABSTRACT

The potential to create new ecosystems in rivers is possible through the use of reclaimed water as a replenishment source, although the long-term effects of this method are unknown. In this study, the water quality and aquatic ecological evolution of a newly constructed river replenished by reclaimed water in Beijing (the Jing River) were investigated, and the conventional water quality, phytoplankton indicators, and submerged plant growth conditions from October 2018 to December 2020 were analyzed. Spearman's correlation and redundancy analysis between possible influential environmental factors and algal indicators were conducted. The results show that the major water quality indicators could meet the water quality standards for landscape water. There were seven phyla present, including 322 species of phytoplankton. The phytoplankton density increased, followed by a decreasing trend. Phytoplankton densities at each monitoring site reached 10×10^6 to 25×10^6 cells/L in 2019 before decreasing in 2020, then ranging from 8×10^6 to 20×10^6 cells/L. Phytoplankton growth was influenced by changing water quality and ecosystems. Consequently, the submerged plant coverage rate gradually increased from 2018 (0%) to 2020 (26.27%–37.06%), as did biodiversity. Through the implementation of ecological restoration measures in the Jing River, the reclaimed water environment evolved into a more natural water environment, which could provide some reference for similar areas to use reclaimed water as a water replenishment source.

1. Introduction

With the development of the social economy, water shortages are becoming increasingly serious in China [1]. Simultaneously, water pollution and ecological degradation have become predominant issues requiring urgent attention. Efficient utilization of

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reclaimed water following wastewater treatment is one of the important steps in solving these problems. Reclaimed water could replace conventional water resources in areas such as industrial production [2], municipal miscellaneous use [3], irrigation [4], groundwater recharge [5,6], and ecological water replenishment [7,8], but these processes require good water quality and ecological stability [9].

There are numerous advantages to using reclaimed water. The investment in reclaimed water preparation is inexpensive compared with long-distance freshwater diversion (such as China's South-North Water Diversion Project). Reclaimed water, referred to as the second water source, has a wide range of applications. The reuse of reclaimed water could alleviate the pressure of freshwater and make the best use of available resources [3,10]. The use of reclaimed water for environmental purposes has significant social and non-market benefits as well [11]. Reclaimed water can build regional landscapes and improve local climates by recharging environmental water bodies [12]. Reclaimed water is widely used in developed countries such as the United States, Japan, Israel, Singapore, and Australia, where the utilization rates of reclaimed water are over 70% [10]. Most of China's reclaimed water has been used to meet landscape environmental requirements that have little contact with humans and pose low environmental risks. Many Chinese cities have used reclaimed water to create man-made lakes, streams, and rivers, as well as to maintain environmental water for wetlands [13]. Though research into reclaimed water utilization in China is still novel, the efficient use of reclaimed water in Beijing has developed rapidly. The successful implementation of reclaimed water projects depends heavily on public awareness and a positive public attitude toward reclaimed water. Beijing is a typical northern megacity in China, facing serious water resource pressure. According to the Beijing Water Resources Bulletin, the total water supply in Beijing was $4.06 \times 10^9 \text{ m}^3$ in 2020, which included $1.70 \times 10^9 \text{ m}^3$ for domestic use, $1.74 \times 10^9 \text{ m}^3$ for environmental use, $0.30 \times 10^9 \text{ m}^3$ for industrial use, and $0.32 \times 10^9 \text{ m}^3$ for agricultural use, of which the environmental sources were comprised mainly of reclaimed water [14]. China's reclaimed water utilization is rapidly developing, owing to advances in wastewater treatment technology, increased capital investment, and strong policy support, and reclaimed water is expected to play an important role in addressing China's serious water resource crisis.

Although the efficient use of reclaimed water has a series of advantages, it also poses certain hazards. As reclaimed water use increased, its safety attracted mounting attention. Chemical and biological contaminants have been found in reclaimed water due to various sources and treatment methods, such as persistent organic pollutants (POPs), endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs), heavy metals, endotoxins, pathogens, antibiotics, and disinfection by-products [3]. Trace amounts of these substances are often present in the environment. And there are biological risks, such as, POPs, which are bio-accumulative and persistent in the natural environment [15], EDCs, which have endocrine disrupting potential [16], and PPCPs, which are designed for inducing changes in specific organs/receptors of humans and animals, although unknown effects may occur in other organs/receptors [17].

It is even more remarkable that nitrogen and phosphorus nutrients in reclaimed water were higher than in natural water [18]. Therefore, its use could lead to the eutrophication of rivers and lakes replenished by reclaimed water and result in the excessive growth of harmful phytoplankton in the ecosystem [19]. Harmful algae typically grow in large numbers under suitable environmental factors, and this would harm fish or other organisms in the water, which could affect the stability of the water ecosystem.

It is important to analyze water quality and ecological changes following the recharge of reclaimed water into rivers and lakes. Most current research focuses on the change process of natural river ecosystems after reclaimed water replenishment [20], such as aesthetic characteristics and algal bloom control, but most natural river ecosystems have been largely established over many years of succession, and there are complex environmental factors (e.g., complex surrounding pollution sources, diverse ecosystem species, etc.) in the influences on river ecosystems [21]. Many studies are analyzing water quality improvement by ecological restoration measures as well [18], and some studies are analyzing the effects of pollutants in the water on aquatic organisms [22]. Relatively few studies have been conducted on newly constructed water projects that are directly replenished with reclaimed water, and the effects of reclaimed water on river ecology have not been specifically addressed. An investigation of the water quality and aquatic ecological development of a newly constructed river replenished by reclaimed water could better explain the impact of reclaimed water on the water environment in the early stages of aquatic ecological succession. This process can bridge the gap between the artificial river and the natural steady-state river, thus optimizing the ecological restoration process of the river to reach the natural stable state faster. Continuous monitoring and investigation of water quality and ecological changes in the newly constructed river after replenishment of reclaimed water and analysis of its impact on the initial construction of the ecosystem could better guide the ecological restoration process, influencing the ecosystem toward health and stability.

In this study, a newly constructed river in Beijing only replenished with reclaimed water was selected, and water quality and ecological succession were analyzed; no point source pollution exists in the surrounding area. The results of this research may provide suggestions for the ecological reuse of reclaimed water in similar rivers and provide a technical case for alleviating environmental water shortages in water-scarce areas.

2. Material and methods

2.1. Research area overview

There is a new reclaimed water recharge river in Beijing's urban sub-center, the Jing River, formerly the Fengzi Ditch, a north-south drainage trench in the Tongzhou District prior to 2018. The main function of this trench was the domestic sewage discharge of the surrounding villages. With the construction of Beijing's urban sub-center, a new Beijing administrative office area was nearby.

The Jing River was completed in October 2018, and the recharge water source was reclaimed water (approximately $10,000 \text{ m}^3$ per day) from the East River Wastewater Treatment Plant in Tongzhou District, Beijing. The production process of reclaimed water is the

A2/O + MBR process, and reclaimed water is piped to point S1 (Fig. 1) for recharge. The Jing River is 2.4 km long, with a water surface area of approximately 160,000 m² and a storage capacity of approximately 250,000 m³. The normal water level of the river is 18 m at the elevation of the Yellow Sea. The Jing River cross-section is trapezoid, with a 5 m wide shallow area on each bank with a depth of 0.5 m and a gentle slope (ratio of 1:2.5) from the shallow area to the river bottom on both banks. In the center of the river, the depth is 1.8 m. The soil of the river bottom is clay, and the measured water flow after the completion of the river is 0.20 ± 0.05 m/s. The Jing River, which is upstream of the Jian River and downstream of the North Canal, currently regulates water flow through a pumping station. The Jing River has been the subject of ecological restoration, wherein approximately 20,000 m² of submerged plants were planted in April 2019, primarily *Vallisneria natans* (Lour.) H. Hara in the shallow part of the river. The planting density was 25–30 clusters/m² and 3–5 plants/cluster. *Vallisneria natans* (Lour.) H. Hara was purchased from Baiyang Lake, Hebei Province.

In this study, the river water was sampled and measured along the flow direction. Four sampling sections were selected from north to south: S1, S2, S3, and S4 (Fig. 1).

2.2. Sampling and survey

The water sampling was conducted in accordance with the standard method issued by the China State Environmental Protection Administration [23]. 2.5 L of water was collected from 0.5 m below the surface at each sampling site monthly from October 2018 to December 2020. The samples were then transported to the laboratory for testing. Phytoplankton samples (1 L) were fixed with Lugol's iodine solution (1% v/v) and allowed to settle for 48 h prior to counting using a microscope (Model CX31, Olympus Inc., Tokyo, Japan) [24]. The areas of submerged plants were determined by GPS, and plant coverage was calculated. Additionally, submerged plants were surveyed around each sampling section (a 100 m × 20 m plot with the long axis parallel to the river). In each plot, five quadrats (1 m × 1 m) were randomly selected to identify species and test the photosynthetic efficiency of each submerged plant. Simultaneously, to analyze the causes of water quality changes, the microbial community changes in the early stages of ecosystem development were also sampled and analyzed.

2.3. Measurement and analysis

Water temperature (WT), pH, and dissolved oxygen (DO) of the river water were determined in situ using a portable multi-parameter water quality analyzer (Proplus, YSI, USA). Chemical oxygen demand (COD), ammonia-nitrogen (NH₃-N), total nitrogen (TN), and total phosphorus (TP) were measured in accordance with standard methods [23]. The COD was determined using the potassium dichromate method; the NH₃-N was determined using Nessler's reagent colorimetry; the TN was determined using potassium persulfate oxidation-UV spectrophotometry; and the TP was determined using the ammonium molybdate spectrophotometric method (potassium persulfate was used to digest the samples).

Phytoplankton density and species were determined using a microscope (Model CX31, Olympus Inc., Tokyo, Japan). Phytoplankton samples were identified to the species level according to 'Chinese Freshwater Algae-System, Classification, and Ecology' [25]. All phytoplankton cells were counted in a 0.1 mL fixed sample that was concentrated from 1 L to 30 mL to account for all species. Algal biovolumes were calculated from cell numbers and cell size measurements. The biomass conversion assumes that 1 mm³ of volume is equivalent to 1 mg of fresh-weight biomass [26]. The phytoplankton samples were collected monthly, and the results were averaged by year and quarter. For each year, the mean value from March to May was designated as spring data, that from June to August as summer data, that from September to November as autumn data, and that from December to February as winter data.

Plant coverage was calculated by dividing the area of submerged plant growth by the area of the sample plot. The survey time was

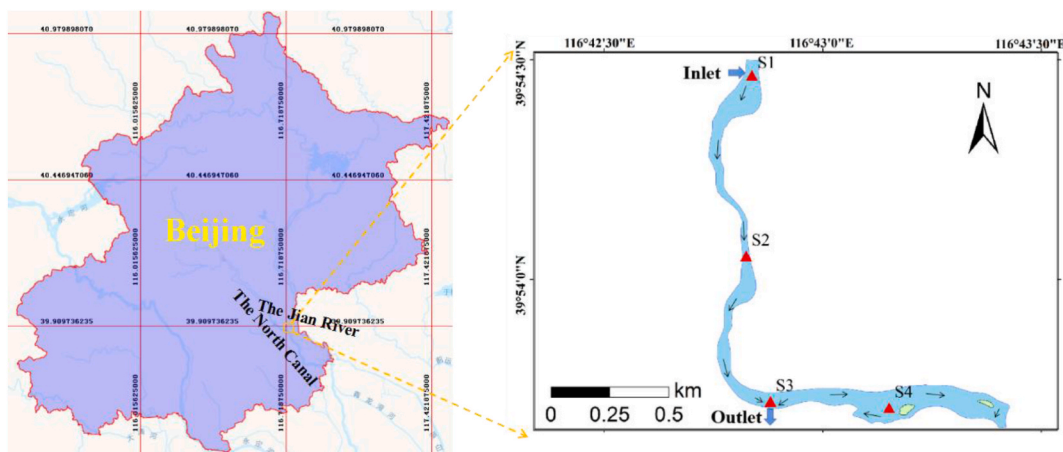


Fig. 1. Study areas and sampling sections (The positions S1, S2, S3, and S4 were chosen along the Jing River from north to south, with S1 being the reclaimed water inlet position and S3 being the reclaimed water outlet position.).

the growth period of submerged plants. Because some areas were frozen in winter (December–February), the survey of submerged plant coverage was not conducted for that period, and the survey of submerged plant coverage was conducted monthly at other times. A schematic diagram of annual changes in submerged plant growth coverage was created using ArcGIS software (using the Inverse Distance Weight). Pulse amplitude modulation (PAM) fluorometry (DIVING-PAM-II, Heinz Walz GmbH, Germany) was used to determine the effective and maximum quantum yield of photosystem II within the submerged plant in situ. Within the plant survey, three submerged plants of different species were randomly selected, and the photosynthetic parameters of the plants were measured after dark adaptation of the plant leaves for 20 min. The maximum light energy conversion efficiency (F_v/F_m) of each submerged plant was calculated [27,28], which indicated the photosynthetic efficiency and represented the health status of the submerged plants. The test was performed four times in July 2019, September 2019, November 2019, and July 2020. To better analyze the initial aquatic ecological succession of the Jing River, the changes in plants during the same season were selected for comparison (July 2019 and July 2020).

The composition of soil microbial communities was analyzed by gene sequencing in March and July 2019. DNA was extracted from each sample using the PowerSoil DNA Isolation Kit in accordance with the manufacturer's protocol. The quality and concentration of DNA extracted from soil samples were detected by 1% agarose gel electrophoresis and spectrophotometry. The 16S rRNA gene was amplified from the genomic DNA using primers 338F (5'-ACTCCTACGGGAGCAGCAG-3') and 806R (5'-GGACTACNNGGTAT-3') targeting the V3–V4 region [29]. PCR products were detected by 1% agarose gel electrophoresis and purified with the Agencourt AMPure XP nucleic acid purification kit. PCR products were used to construct a microbial diversity sequencing library, and the Illumina Miseq PE300 high-throughput sequencing platform was used for paired-end sequencing.

Statistical analyses were conducted using Origin software (OriginLab, USA) for Spearman's correlation and redundancy analysis between possible influential factors and algal indicators. The phytoplankton community structure was affected by various abiotic factors such as nutrients (N, P), temperature, and pH, with phytoplankton photosynthesis having an impact on the pH and dissolved oxygen [30]. Spearman's correlation analysis was conducted between the density of different phytoplankton species and environmental factors. Redundancy analysis was conducted for the relationship between spatial and temporal algal growth density (seasonal-regional) and environmental factors. A total of five environmental variables were selected, TN, TP, WT, pH, and DO. The changes in sediment microbial communities before and after submerged plant remediation at the phylum and genus taxonomic levels were analyzed by R version 4.2.0 using the circlize package [31].

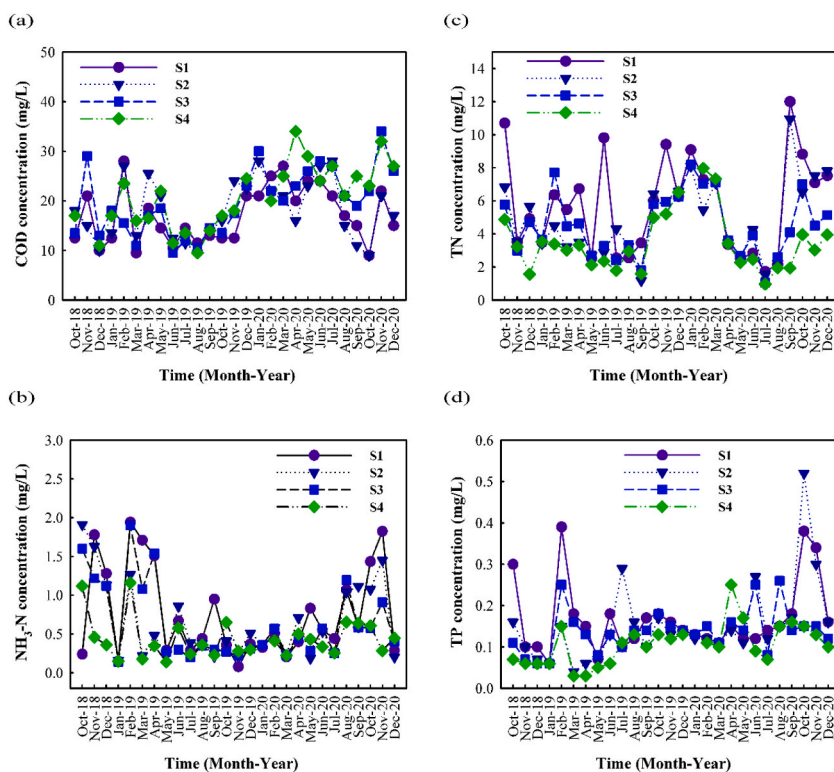


Fig. 2. Monthly dynamics of water parameters in the Jing River: (a) COD, (b) $\text{NH}_3\text{-N}$, (c) TN, and (d) TP.

3. Results

3.1. Temporal variation of water quality

Monthly data from four sampling sites was collected from October 2018 to December 2020. The results of the main environmental variables such as COD, $\text{NH}_3\text{-N}$, TN, and TP are shown in Fig. 2. The COD, $\text{NH}_3\text{-N}$, and TP were generally lower than 30 mg/L, 1.5 mg/L, and 0.3 mg/L, respectively, and TN concentrations were generally below 15 mg/L. The trend of the COD (Fig. 2[a]) indicator over time increased along the flow direction of reclaimed water, except for the second half of 2019, when COD was more stable. Nutrient concentrations ($\text{NH}_3\text{-N}$, TN, and TP) (Fig. 2[b–d]) at the S1 sampling point were higher than those at other points, and they showed a decreasing trend with the direction of water flow.

3.2. Changes in phytoplankton

The growth trends of phytoplankton in the Jing River were analyzed. Microscopy analyses from October 2018 to December 2020 revealed seven phyla, including 322 species of phytoplankton. The annual variation of phytoplankton density in the Jing River was evident, with phytoplankton density increasing from 2018 to 2019, then decreasing from 2019 to 2020 (Fig. 3[a]). Phytoplankton growth was low, and phytoplankton densities detected at the four monitoring sites in 2018 were all below 5×10^6 cells/L. The phytoplankton detected in the Jing River in 2018 were mainly Bacillariophyta. Phytoplankton densities increased rapidly in 2019, reaching 10×10^6 to 25×10^6 cells/L in each monitoring site, with higher densities of Cyanophyta, Bacillariophyta, and Chlorophyta than other algae. Phytoplankton densities decreased in 2020, ranging from 8×10^6 to 20×10^6 cells/L. Except for the high density of Cyanophyta at S3, the sites were dominated by Bacillariophyta and Chlorophyta in 2020. The phytoplankton biomass trend differed from the density trend, which showed an increase (Fig. 3 [b]). From 2019 to 2020, although phytoplankton density decreased in the Jing River, phytoplankton biomass increased. The highest phytoplankton biomass was over 25 mg/L at the S3 sampling point in 2020.

3.3. Response of phytoplankton to environmental factors

Spearman's correlation was measured for different phytoplankton microscopy results and corresponding environmental states to analyze the relationship between phytoplankton growth and environmental factors (Fig. 4[a]). The response to environmental factors varied among phytoplankton species, and there was a correlation between different phytoplankton phyla. The results suggest that Chrysophyta is negatively related to TP concentration ($r = -0.60$, $p \leq 0.05$), and that Pyrrophyta is strongly negatively related to TN concentration ($r = -0.68$, $p \leq 0.01$). The correlation between phytoplankton growth and water temperature, pH, and dissolved oxygen concentration was more significant. For example, Chlorophyta showed a positive correlation with water temperature ($r = 0.54$, $p \leq 0.05$); pH value ($r = 0.61$, $p \leq 0.05$); and DO concentration ($r = 0.73$, $p \leq 0.01$).

Redundancy analysis was conducted (Fig. 4[b]) to further understand the relationship between spatial and temporal changes of phytoplankton (Fig. S5) in the Jing River and environmental factors (Fig. 2[c–d] and Figs. S2–S4). The results show that the phytoplankton densities at each monitoring site were more closely related to environmental factors such as WT, pH, and DO concentration in spring and autumn (except for Spring-S4 and Autumn-S2), and more dependent on nutrients such as TN and TP in summer and winter (except for Summer-S3 and Winter-S4).

3.4. Growth conditions of submerged plants

Changes in plant coverage (Fig. 5[a] and Fig. S6) were analyzed through on-site surveys. In 2018, the Jing River had recently been filled with reclaimed water. The ecosystem had not yet been established, and there were no submerged plants. Therefore, the submerged plant coverage rate of the entire river was 0%. The Jing River's submerged plant coverage rates increased in 2019, and the annual mean values of submerged plant coverage in different areas of the river ranged from 11.62% to 13.25%. The results of the 2020

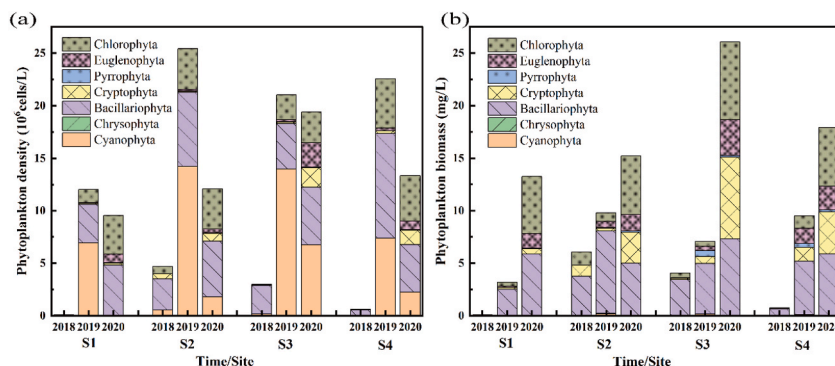


Fig. 3. Changes reported in phytoplankton densities in the Jing River: (a) phytoplankton density, and (b) phytoplankton biomass.

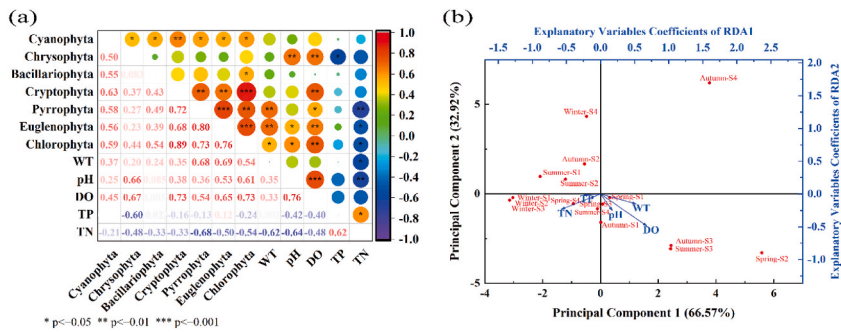


Fig. 4. Response of phytoplankton to environmental factors: (a) Spearman's correlation between possible influential factors and the densities of different phytoplankton species, and (b) redundancy analysis between spatial and temporal phytoplankton growth density (seasonal-regional) and environmental factors.

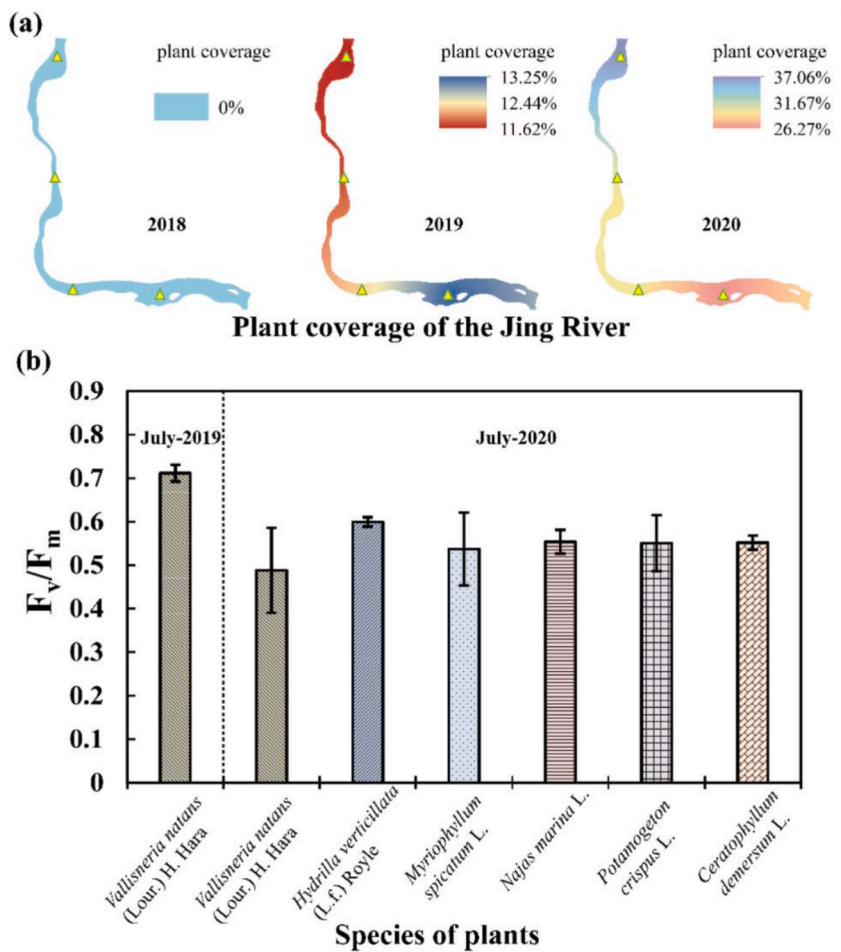


Fig. 5. Growth conditions of submerged plants: (a) changes in submerged plant coverage (using the Inverse Distance Weight), and (b) maximum light energy conversion efficiency (F_v/F_m) of each submerged plant in the Jing River.

submerged plant survey show that the annual mean values of submerged plant coverage in different areas of the Jing River varied between 26.27% and 37.06%.

Approximately 20,000 m² of submerged plants (mainly *Vallisneria natans* [Lour.] H. Hara) were planted in the Jing River in April 2019, along with the introduction of other aquatic species. As a result, the process of restoration and improvement of the Jing River ecosystem was accelerated. In the second half of 2019, *Hydrilla verticillata* (L.f.) Royle, *Myriophyllum spicatum* L., *Najas marina* L., and

Potamogeton crispus L. were successively found. In 2020, a submerged plant species, *Ceratophyllum demersum* L., was also found.

Although the diversity of submerged plant species in the Jing River had increased, the growth status of submerged plants in the reclaimed water environment also required attention. The growth status of different submerged plants was monitored as well. The health status of submerged plants was analyzed by comparing changes in the maximum light energy conversion efficiency (F_v/F_m) of submerged plants (Fig. 5[b]). The test results from July 2019 show that the F_v/F_m values of *Vallisneria natans* (Lour.) H. Hara were approximately 0.7, and in July 2020, the F_v/F_m values of different submerged plant species were approximately 0.6, an overall downward trend from July 2019 to July 2020.

3.5. The changes in sedimentary microbial communities

At the initial stage of aquatic ecological succession, Proteobacteria, Bacillariophyta, and Bacteroidetes were the top three dominant microorganisms at the phylum level in Jing River soil in March 2019, and after planting the submerged plants, in July 2019, Proteobacteria, Bacteroidetes, and Firmicutes had become the top three (Fig. 6 [a] and [c]). The top 10 genera were *Fistulifera*, *Flavobacterium*, *Dechloromonas*, *Gemmatimonas*, *Geobacter*, *Sphingomonas*, *Rhodofexax*, *Roseisolibacter*, *Arenimonas*, and *Luteolibacter* in Jing River soil in March 2019. After planting the submerged plants, *Povalibacter*, *Vicinamibacter*, *Tabrizicola*, *Ilumatobacter*, *Fistulifera*, *Geobacter*, *Dechloromonas*, *Zhizhongheella*, *Chromatocurvus*, and *Lentimicrobium* had become the top 10 (Fig. 6 [b] and [d]).

4. Discussion

4.1. Causes of changes in water quality and current status evaluation

In this study, we investigated the water quality and aquatic ecological succession of a newly constructed river replenished by reclaimed water in Beijing, China (Jing River). Most of the water quality indicators met the requirements of relevant water quality standards (concentrations of COD, NH_3-N , and TP generally met the Chinese national standards for surface water in Class IV [GB3838-2002] (Table S1), and the concentration of TN generally met the Chinese national standards for reclaimed water reuse in scenic

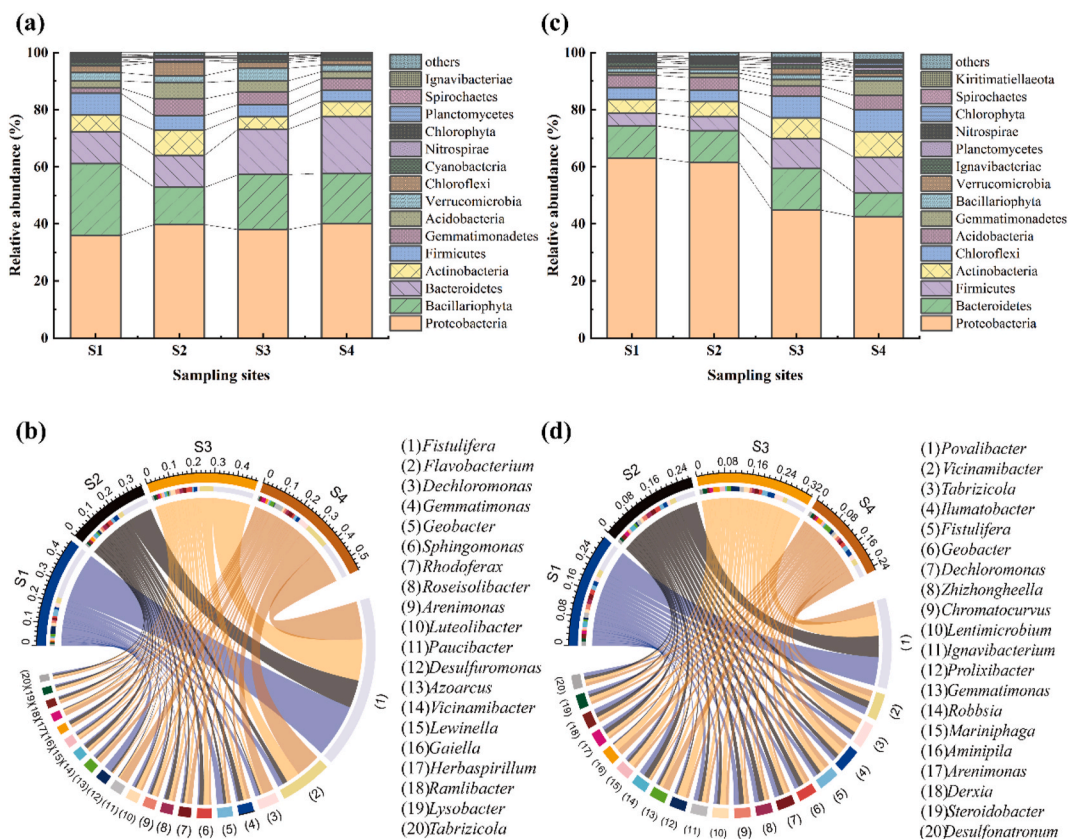


Fig. 6. Changes in microbial communities: (a) relative abundance of soil microbial populations at the phylum level in March 2019, (b) circos diagram visualizing the linkage between soil samples and microbial taxa at the genus level in March 2019 (top 20 genera), (c) relative abundance of soil microbial populations at the phylum level in July 2019, and (d) circos diagram visualizing the linkage between soil samples and microbial taxa at the genus level in July 2019 (top 20 genera).

environments [GB/T 18921-2019] (Table S1)). But these water quality indicators of reclaimed water were higher than those of natural rivers (the COD, NH₃-N, TN, and TP in natural rivers of Beijing areas are usually lower than 15 mg/L, 0.5 mg/L, 1.5 mg/L, and 0.1 mg/L respectively). The COD, NH₃-N, TN, and TP in the Jing River change with time (Fig. 2), influenced primarily by the quality of incoming reclaimed water and the development of the ecosystem. Studies have shown that water quality indicators changed after reclaimed water replenished the river, and some pollution intolerant species disappeared [21]. After the reclaimed water is replenished in the natural river, it does affect the water quality and has an impact on aquatic organisms. However, in this study, due to the shortage of natural water sources, the Jing River uses only reclaimed water for recharge; therefore, the water quality of the river is greatly influenced by the quality of the incoming reclaimed water (Table S2). Additionally, submerged plants were introduced into the Jing River, and the related ecosystem changes will also influence the water quality.

The trend of the COD indicator was irregular (Fig. 2[a]), generally lower than 30 mg/L, and the reason for this trend may be related to the alteration of the Jing River water ecosystem. In the first year of reclaimed water replenishment, the COD concentration of the four sampling points had little difference, but in the second year of reclaimed water replenishment, the COD concentration at S4 point was significantly higher than that at S1 point (when the influent COD concentration of S1 was about 20 mg/L, the COD concentration at S4 point was about 30 mg/L). With the development of and changes to the ecosystem, the phytoplankton biomass increased, which resulted in an increase in COD detection results [32]. Because the S3 sampling point is the outlet position, and the water around the S4 sampling point is relatively static, these environmental conditions are more suitable for phytoplankton biomass accumulation.

Some of the higher values of the monitoring results were due to higher concentrations in the influent water (Fig. 2[b-d]). The nutrient concentrations at the S1 sampling point were relatively higher than at other points, and the nutrient concentrations showed a decreasing trend with the direction of water flow. However, the changing pattern of nutrient concentrations in the Jing River with time was not evident. The higher values of the monitoring results were due to higher concentrations in the influent water at this monitoring time. The nutrient concentrations in the Jing River were also influenced by the different growth statuses of aquatic plants, phytoplankton, and microbial communities in different seasons [33]. Moreover, sediment has an impact on water quality, and plants absorb nutrients from the water. In the subsequent long-term monitoring process, these factors should also be considered to make the monitoring analysis more comprehensive.

4.2. Causes of phytoplankton change and current status evaluation

Phytoplankton densities of the Jing River increased rapidly in 2019 probably owing to sufficient nutrients and decreased in 2020 probably owing to the improving ecosystem (Fig. 3[a]). The Jing River had just been replenished with water (October 2018), and the temperature in Beijing dropped rapidly; the phytoplankton densities detected at the four monitoring sites in 2018 were below 5×10^6 cells/L. The phytoplankton detected in the Jing River in 2018 were mainly Bacillariophyta (Fig. 3[a]). The reason for this is that Bacillariophyta tend to be the dominant algal species in late autumn and early winter as they are prevalent at lower temperatures (approximately 10 °C) [34]. Improved biodiversity makes the ecosystem more stable and less susceptible to the overgrowth of a single species, thus inhibiting the occurrence of water blooms [35].

From 2019 to 2020, phytoplankton biomass increased in the Jing River, indicating that larger algae, such as Bacillariophyta, Cryptophyta, and Euglenophyta, became more abundant [25,36]. With the increase in the proportion of larger algae, the phytoplankton biomass showed an increasing trend when the phytoplankton density decreased. Chlorophyta also contributed to the increase in biomass, and the dominant species were *Scenedesmus quadricauda*, *Nephrocytium agardhianum*, *Chlorella vulgaris*, etc. Nutrients and light are the main environmental factors, affecting the growth and spatial distribution of phytoplankton [34]. The reason for this was that the Jing River had replenished the reclaimed water with sufficient nutrient salts rich in nitrogen and phosphorus, while the shallow water depth (0.5 m) and suitable light provided good conditions for the growth of these algae.

The contradicting results of phytoplankton density and biomass could be analyzed from two perspectives: the phytoplankton density had decreased, which probably meant the risk of water bloom was reduced; and the increase in phytoplankton biomass could make the primary productivity higher, which could provide a richer food source for aquatic animals in the water ecosystem such as fish. During the study, after planting submerged plants in April 2019, an increasing number of fish species were found in the Jing River. Before the restoration of water ecology, only *Carassius auratus* was found. With the improvement of the ecosystem, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Ctenopharyngodon idella*, *Parabramis pekinensis*, *Rhinogobius giurinus*, *Misgurnus anguillicaudatus*, and *Channa argus* were gradually found (Fig. S1). Filter feeding, herbivorous, omnivorous, and carnivorous fish were captured gradually during field investigation, and the increased biodiversity made the ecosystem more stable. This was an indication that the water ecosystem of the Jing River was progressively improving.

Considering the high risk of phytoplankton bloom caused by the utilization of reclaimed water in the Jing River (some harmful algae were detected, such as *Microcystis* sp. [the highest density was 2.4×10^6 cells/L at S4 in September 2019], Table S3), the response of phytoplankton to environmental factors was analyzed. Analysis of the response between phytoplankton and environmental factors in the Jing River showed that the factors previously thought to mainly affect phytoplankton growth (such as nitrogen and phosphorus nutrient concentrations) [33,37] did not show positive correlations with phytoplankton growth. This could be due to the high nitrogen and phosphorus concentrations in reclaimed water, which far exceed the limit of algae growth. The high concentration of nitrogen and phosphorus makes it not a limiting factor for algae growth. Some studies have analyzed the threshold of algae growth in reclaimed water [1], the nitrogen threshold values of different algae range from 0.17 to 0.298 mg/L and the phosphorus threshold values of different algae range from 0.0096 to 0.017 mg/L. Although various nitrogen and phosphorus removal technical processes had been applied in the wastewater treatment plant, the nitrogen and phosphorus concentrations in reclaimed water were still very high; the effluent standards being 15 mg/L and 0.3 mg/L, respectively. The concentration of nitrogen and phosphorus in the Jing River was

much higher than reported threshold values.

The nitrogen and phosphorus in reclaimed water were readily absorbed by phytoplankton, while other environmental factors, such as light conditions and water temperatures, also affected the growth of phytoplankton. The high concentration of nitrogen and phosphorus in the inlet water of the Jing River helped meet the growth demand of phytoplankton. Therefore, the nutrient concentration was not a limiting factor. However, the light conditions are also sufficient in the Jing River (there are a lot of shallow water areas, the depths of which are approximately 0.5 m).

Temperature has a direct impact on plant metabolism, which includes both photosynthetic and respiratory activities [30]. So that under suitable water temperature, phytoplankton of the Jing River may grow in large quantities. Meanwhile, phytoplankton photosynthesis may have an impact on pH and dissolved oxygen [38,39]. The growth of phytoplankton could change the DO and pH of the Jing River. Therefore, environmental indicators such as DO and pH are correlated with phytoplankton in the Jing River.

There was also a correlation between different phytoplankton phyla, and this was because some phytoplankton phyla have similar suitable growth conditions. The ranges of temperatures at which various types of algae can maintain healthy growth differ widely (the thermal niche), and some phytoplankton phyla have similar suitable temperature optimums. Therefore, these phytoplankton often occur together [40].

For the redundancy analysis, each sampling point in different seasons was a ranked sample, which was projected vertically on the vector of explanatory variables (environmental factors) or their extensions, and the longer the projection distance of the intersection point in the positive direction of the vector, the greater the influence of the variable in the corresponding sample. Because the rapid change in spring and autumn temperatures most likely influenced phytoplankton growth, while summer and winter temperatures were more extreme (higher or lower), the effect of nitrogen and phosphorus nutrients on phytoplankton growth became apparent. Simultaneously, because the ecosystem was still in its early stages, some ecological indicators were not stable, resulting in some abnormal positions.

4.3. Effects of submerged plant cultivation

Planting submerged plants in the Jing River was the main ecological restoration method implemented. Therefore, the changes in initial water quality and ecology of the Jing River were closely related to the growth status of aquatic plants. In shallow freshwater ecosystems, submerged plant communities promote physical and chemical changes [41]. An increase in the growth cover of submerged plants could improve the ecosystem's ability to maintain water quality, and although it cannot effectively improve water quality, it could inhibit the excessive growth of algae and provide baiting and spawning grounds for fish and other aquatic animals. This conclusion has also been verified in existing studies, including a 4-year study that encompassed a change from phytoplankton to plant dominance; there was a large decline in phytoplankton biomass and production and an increase in plant biomass [42]. Once a submerged vegetation stand has been established, water transparency is increased through a variety of positive feedback mechanisms, which stimulate plant growth. The mechanisms implemented by submerged plants to stabilize the clear state are as follows: protection for grazers on algae as large-bodied zooplankton, attenuation of water currents and sediment stabilization, support for epiphyton that sequester nutrients, reduction of inorganic macronutrients by uptake from the water, and production of allelopathic compounds. Although confirmation of allelopathy at the ecosystem level is still speculative, submerged plants are known to prevent excessive phytoplankton growth, causing the clear state to be self-stabilizing [41,43].

The health status of submerged plants was also analyzed. In general, when the F_v/F_m value is approximately 0.8, the growth of submerged plants is relatively healthy [44], while lower values indicate plant stress, which proves that submerged plants were somewhat inhibited in this environment [45]. The F_v/F_m values of different submerged plant species were approximately 0.6, with an overall downward trend from July 2019 to July 2020 (Fig. 5[b]). In the case of *Vallisneria natans* (Lour.) H. Hara, the F_v/F_m value was 0.7 in July 2019, but in July 2020, the F_v/F_m value had dropped to 0.5 (Fig. 5[b]). The reason is that in the early stages of aquatic ecological succession, submerged plant and algae growth were still in competition, and reclaimed water replenishment resulted in higher algae densities in the Jing River, which affected the transparency of the water. The sunlight entering the water was reduced, which in turn inhibited the growth of submerged plants (Fig. 5[b]). However, environmental factors such as temperature in different seasons also affect the growth of aquatic plants, among which *Potamogeton crispus* L. had an F_v/F_m value of 0.7 in November 2019, and decreased to below 0.6 in July 2020 (Fig. 5[b]), mainly because *Potamogeton crispus* L. is a cold-season plant that can grow normally in low temperatures. The high temperatures in the summer were not its normal growth period; therefore, the growth state was inhibited. The light and temperature affect the growth of aquatic plants, but temperature is a natural evolution process and has little relationship with reclaimed water. Light condition of water is indirectly affected by reclaimed water replenishment. Therefore, the influence of reclaimed water replenishment on the growth of aquatic plants is mainly achieved by affecting the density of algae and thus the water light conditions.

The changes in the microbial community before and after ecological restoration were compared. The results show that the abundance of each bacterial phylum was slightly different at each sampling point, but the overall composition was similar (Fig. 6). The gradual stability of the microbial community was a good phenomenon, and the emergence of some microorganisms had the effect of improving water quality. Intracellular storage of carbon and energy, as well as other nutrients, has long been documented among fungi and bacteria. It has been suggested that Proteobacteria and Bacteroidetes have storage traits [46]. Therefore, the increase in the overall share of Proteobacteria and Bacteroidetes could reduce the concentration of carbon and other nutrients in water. After the submerged plants were planted (in July 2019), the distribution of microbial species at the genus level was more balanced (Fig. 6[d]). This may be caused by the change in temperature and other environmental factors when microbial samples were collected in different seasons. However, submerged plants may change the habitat of surrounding organisms after planting. Before there were submerged plants,

microbial survival was mainly affected by sediments. After submerged plant introduction, they provided a habitat for microorganisms [47]. Three genera were always in the top 10, *Fistulifera*, *Geobacter*, and *Dechloromonas*, with the others varying (Fig. 6[b, d]). The genera observed suggest the presence of a diverse bacterial community able to catalyze a wide range of biogeochemical cycles of C, N, P, and sulfur, water purification, or primary production in aquatic environments [29,48]. The results also show the presence of harmful bacteria such as *Legionella*, which were not listed because of their low percentages (it ranks 242nd in percentage). *Legionella*, a high-epidemic pathogen, is generally encountered in reclaimed water [3]. In future studies, surveillance data regarding *Legionella* should be increased. The abundance of each bacterial phylum was slightly different at each sampling point, but the overall composition was similar, and the genera observed suggest the presence of a diverse bacterial community, which could guide the aquatic ecological succession in a good direction.

4.4. Analysis of the aquatic ecosystem in the Jing River

After the Jing River was replenished with reclaimed water, the quality of the water met the relevant landscape environmental water quality standards, but there were differences between reclaimed water and natural water. The eutrophication of reclaimed water led to the rapid proliferation of phytoplankton. Through the introduction of submerged plants (Fig. S6), the biological habitat of the Jing River was optimized. Plant coverage increased naturally, and the number of submerged plants increased as well, preventing excessive phytoplankton density growth. Although the biomass of phytoplankton was still increasing, the proportion of large phytoplankton increased, leading to an increase in primary productivity and providing a good bait environment for fish.

The health of the river environment requires the functional positioning of the river. The Jing River is positioned as a drainage and landscape river. Plant coverage and diversity of aquatic species are not as high as those of a natural river (plant coverage was more than 80% and the fish species were 24 in a natural river of Beijing) [49], although the phytoplankton grew in large numbers and the growth state of submerged plants was inhibited after the Jing River replenishment with reclaimed water. However, its water quality was up to standards, and after planting submerged plants, the density of phytoplankton gradually decreased and the biodiversity of fish increased. In these respects, the Jing River is still improving in a good direction.

5. Conclusions

By analyzing the process of water quality and aquatic ecological development after the Jing River was replenished with reclaimed water, the results show that the major water quality indicators could meet the water quality standards of landscape water. Nevertheless, the phytoplankton density rapidly increased. The coverage of submerged plants increased, but the growth rate of submerged plants was inhibited. After the introduction of submerged plants of Jing River in April 2019, the density of phytoplankton gradually decreased and the biodiversity of fish and microorganisms increased, which accelerated the stability of the ecosystem.

Although some ecological problems appeared after the replenishment of reclaimed water, the ecological system gradually stabilized and developed toward the direction of biological species diversification through artificial control measures such as submerged plants cultivation. In future research, the implementation of ecological restoration measures for reclaimed water should focus more on aquatic ecological succession (such as regulatory biological chain), rather than water quality purification, by accelerating the naturalization of the aquatic ecological succession, which in turn leads to the overall improvement of the water's environmental quality. Simultaneously, trace pollutants, harmful algae and potential pathogenic bacteria in the reclaimed water should also be paid more attention.

Author contribution statement

Zhaoxin Li: Conceived and designed the experiments, Analyzed and interpreted the data, Wrote the paper.

Zhiyan Sun: Analyzed and interpreted the data, Wrote the paper.

Lei Zhang: Performed the experiments, materials, analysis tools or data.

Nan Zhan: Contributed reagents, materials, analysis tools or data.

Chunhua Lou: Contributed reagents, materials, analysis tools or data.

Jijian Lian: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e17045>.

References

- [1] K. Song, Y. Lu, G. Dao, Z. Chen, Y. Wu, S. Wang, J. Liu, H.-Y. Hu, Reclaimed water for landscape water replenishment: threshold nitrogen and phosphorus concentrations values for bloom control, *Algal Res.* 62 (2022), 102608.
- [2] C. Cherchi, M. Kesaano, M. Badruzzaman, K. Schwab, J.G. Jacangelo, Municipal reclaimed water for multi-purpose applications in the power sector: a review, *J. Environ. Manag.* 236 (2019) 561–570.
- [3] S. Deng, X. Yan, Q. Zhu, C. Liao, The utilization of reclaimed water: possible risks arising from waterborne contaminants, *Environ. Pollut.* 254 (2019), 113020.
- [4] S.R. Yanala, K.R. Pagilla, Use of biochar to produce reclaimed water for irrigation use, *Chemosphere* 251 (2020), 126403.
- [5] G. Ding, G. Chen, Y. Liu, M. Li, X. Liu, Occurrence and risk assessment of fluoroquinolone antibiotics in reclaimed water and receiving groundwater with different replenishment pathways, *Sci. Total Environ.* 738 (2020), 139802.
- [6] W. Wu, R. Liao, Y. Hu, H. Wang, H. Liu, S. Yin, Quantitative assessment of groundwater pollution risk in reclaimed water irrigation areas of northern China, *Environ. Pollut.* 261 (2020), 114173.
- [7] D. Ao, L. Luo, M. Dzakpasu, R. Chen, T. Xue, X.C. Wang, Replenishment of landscape water with reclaimed water: optimization of supply scheme using transparency as an indicator, *Ecol. Indic.* 88 (2018) 503–511.
- [8] X.Y. Ma, Q. Li, X.C. Wang, Y. Wang, D. Wang, H.H. Ngo, Micropollutants removal and health risk reduction in a water reclamation and ecological reuse system, *Water Res.* 138 (2018) 272–281.
- [9] Y. Meng, W. Liu, H. Fiedler, J. Zhang, X. Wei, X. Liu, M. Peng, T. Zhang, Fate and risk assessment of emerging contaminants in reclaimed water production processes, *Front. Environ. Sci. Eng.* 15 (2021) 1–16.
- [10] Q. Li, W. Wang, X. Jiang, D. Lu, Y. Zhang, J. Li, Analysis of the potential of reclaimed water utilization in typical inland cities in northwest China via system dynamics, *J. Environ. Manag.* 270 (2020), 110878.
- [11] F. Alcon, J. Martin-Ortega, J. Berbel, M.D. De Miguel, Environmental benefits of reclaimed water: an economic assessment in the context of the Water Framework Directive, *Water Pol.* 14 (1) (2012) 148–159.
- [12] H.J. Zhao, Y. Wang, L.L. Yang, L.W. Yuan, D.C. Peng, Relationship between phytoplankton and environmental factors in landscape water supplemented with reclaimed water, *Environ. Int.* 58 (2015) 113–121.
- [13] N. Xiang, F. Xu, M.J. Shi, D.Y. Zhou, Assessing the potential of using water reclamation to improve the water environment and economy: scenario analysis of Tianjin, China, *Water Pol.* 17 (3) (2015) 391–408.
- [14] J. Dou, Z. Zhu, Current status of reclaimed water in China: an overview, *J. Water Reuse Desalin.* 8 (2018) 293–307.
- [15] J. Aravind kumar, T. Krithiga, S. Sathish, A.A. Renita, D. Prabu, S. Lokesh, R. Geetha, S.K.R. Namasivayam, M. Sillanpaa, Persistent organic pollutants in water resources: fate, occurrence, characterization and risk analysis, *Sci. Total Environ.* 831 (2022), 154808.
- [16] H. Cho, C.S. Ryu, S.A. Lee, Z. Adeli, B.T. Meupea, Y. Kim, Y.J. Kim, Endocrine-disrupting potential and toxicological effect of para-phenylphenol on *Daphnia magna*, *Ecotoxicol. Environ. Saf.* 243 (2022), 113965.
- [17] A. Sengar, A. Vijayanandan, Human health and ecological risk assessment of 98 pharmaceuticals and personal care products (PPCPs) detected in Indian surface and wastewaters, *Sci. Total Environ.* 807 (2022), 150677.
- [18] W.H. Wang, Y. Wang, Z. Li, C.Z. Wei, J.C. Zhao, L.Q. Sun, Effect of a strengthened ecological floating bed on the purification of urban landscape water supplied with reclaimed water, *Sci. Total Environ.* 622–623 (2018) 1630–1639.
- [19] X. Lv, J. Zhang, P. Liang, X. Zhang, K. Yang, X. Huang, Phytoplankton in an urban river replenished by reclaimed water: features, influential factors and simulation, *Environ. Int.* 112 (2020), 106090.
- [20] J. Zhang, Z. Wei, H. Jia, X. Huang, Factors influencing water quality indices in a typical urban river originated with reclaimed water, *Front. Environ. Sci. Eng.* 11 (4) (2017) 1–10.
- [21] N. Prat, M. Rieradevall, C. Barata, A. Munné, The combined use of metrics of biological quality and biomarkers to detect the effects of reclaimed water on macroinvertebrate assemblages in the lower part of a polluted Mediterranean river (Llobregat River, NE Spain), *Ecol. Indic.* 24 (2013) 167–176.
- [22] R.S.A. Kitamura, J.C.M. Brito, H.C. Silva de Assis, M.P. Gomes, Physiological responses and phytoremediation capacity of floating and submerged aquatic macrophytes exposed to ciprofloxacin, *Environ. Sci. Pollut. Res.* (2022) 1–18.
- [23] EPAC, The Methods for the Examination of Water and Wastewater, fourth ed., China Environmental Science Press, Beijing, 2002 (in Chinese).
- [24] Z. Zhang, J. Gao, Y. Cai, The effects of environmental factors and geographic distance on species turnover in an agriculturally dominated river network, *Environ. Monit. Assess.* 191 (4) (2019) 1–17.
- [25] H.J. Hu, Y.X. Wei, The Freshwater Algae of China-Systematics, Taxonomy and Ecology, Science press, Beijing, 2006 (in Chinese).
- [26] Z. Wu, Y. Cai, X. Liu, C.P. Xu, Y. Chen, L. Zhang, Temporal and spatial variability of phytoplankton in Lake Poyang: the largest freshwater lake in China, *J. Great Lake Res.* 39 (2013) 476–483.
- [27] H.K. Lichtenthaler, C. Buschmann, M. Knapp, How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio Rfd of leaves with the PAM fluorometer, *Photosynthetica* 43 (3) (2005) 379–393.
- [28] D. Xu, Y. Xia, Z. Li, Y. Gu, C. Lou, H. Wang, J. Han, The influence of flow rates and water depth gradients on the growth process of submerged macrophytes and the biomass composition of the phytoplankton assemblage in eutrophic water: an analysis based on submerged macrophytes photosynthesis parameters, *Environ. Sci. Pollut. Res.* 27 (2020) 31477–31488.
- [29] W. Xiu, J. Lloyd, H. Guo, W. Dai, S. Nixon, N.M. Bassil, C. Ren, C. Zhang, T. Ke, D. Polya, Linking microbial community composition to hydrogeochemistry in the western Hetao Basin: potential importance of ammonium as an electron donor during arsenic mobilization, *Environ. Int.* 136 (2020), 105489.
- [30] X. Liu, L. Chen, G. Zhang, J. Zhang, Y. Wu, H. Ju, Spatiotemporal dynamics of succession and growth limitation of phytoplankton for nutrients and light in a large shallow lake, *Water Res.* 194 (2021), 116910.
- [31] Z. Gu, L. Gu, R. Eils, M. Schlesner, B. Brors, Circlize implements and enhances circular visualization in R, *Bioinformatics* 30 (19) (2014) 2811–2812.
- [32] W. Wang, P. Yang, J. Xia, S. Zhang, S. Hu, Changes in the water environment and its major driving factors in Poyang Lake from 2016 to 2019, China, *Environ. Sci. Pollut. Res.* (2022) 1–15.
- [33] D. Xu, H. Bai, Z. Li, S. Lu, Z. Cheng, X. Shen, H. Wang, Acclimation of phytoplankton to diverse environmental variables at the initial operation stage of a free water surface constructed wetland, *J. Clean. Prod.* 317 (2021), 128442.

- [34] J. Wei, M. Wang, C. Chen, H. Wu, L. Lin, M. Li, Seasonal succession of phytoplankton in two temperate artificial lakes with different water sources, *Environ. Sci. Pollut. Res.* 27 (2020) 42324–42334.
- [35] H.S. Ayele, M. Atlabachew, Review of characterization, factors, impacts, and solutions of Lake eutrophication: lesson for lake Tana, Ethiopia, *Environ. Sci. Pollut. Res.* 28 (12) (2021) 14233–14252.
- [36] T.K. Marella, I.Y. López-Pacheco, R. Parra-Saldívar, S. Dixit, A. Tiwari, Wealth from waste: diatoms as tools for phycoremediation of wastewater and for obtaining value from the biomass, *Sci. Total Environ.* 724 (2020), 137960.
- [37] Y. Yang, H. Chen, M. Abdullah Al, J.C. Ndayishimiye, J.R. Yang, A. Isabwe, A. Luo, J. Yang, Urbanization reduces resource use efficiency of phytoplankton community by altering the environment and decreasing biodiversity, *J. Environ. Sci.* 112 (2022) 140–151.
- [38] G. Minelgaite, P.C. Frost, M.A. Xenopoulos, D.A. Stephansen, M.L. Fejerskov, J. Vollertsen, Planktonic algae abundance and diversity are similar in urban stormwater ponds of different geographic locations and natural shallow lakes, *Urban Ecosyst.* 23 (2020) 841–850.
- [39] N. Wang, J. Xiong, X.C. Wang, Y. Zhang, H. Liu, B. Zhou, P. Pan, Y. Liu, F. Ding, Relationship between phytoplankton community and environmental factors in landscape water with high salinity in a coastal city of China, *Environ. Sci. Pollut. Res.* 25 (2018) 28460–28470.
- [40] J.O. Nalley, D.R. O'Donnell, E. Litchman, Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria, *Algal Res.* 35 (2018) 500–507.
- [41] T.F. Ferreira, L.O. Crossetti, D.M.M. Marques, L. Cardoso, C.R. Fragozo Jr., E.H. van Nes, The structuring role of submerged macrophytes in a large subtropical shallow lake: clear effects on water chemistry and phytoplankton structure community along a vegetated-pelagic gradient, *Limnologia* 69 (2018) 142–154.
- [42] S.F. Mitchell, Primary production in a shallow eutrophic lake dominated alternately by phytoplankton and by submerged macrophytes, *Aquat. Bot.* 33 (1–2) (1989) 101–110.
- [43] K. Li, W. He, Q. Hu, S. Gao, Ecological restoration of reclaimed wastewater lakes using submerged plants and zooplankton, *Water Environ. J.* 28 (3) (2014) 323–328.
- [44] H.S. Jiang, Y. Zhang, L. Yin, W. Li, Q. Jin, W. Fu, T. Zhang, W. Huang, Diurnal changes in photosynthesis by six submerged macrophytes measured using fluorescence, *Aquat. Bot.* 149 (2018) 33–39.
- [45] L.M. Rasmusson, A. Nualla-ong, T. Wutiruk, M. Björk, M. Gullström, P. Buapet, Sensitivity of photosynthesis to warming in two similar species of the aquatic angiosperm ruppia from tropical and temperate habitats, *Sustainability* 13 (2021) 9433.
- [46] K. Mason-Jones, S.L. Robinson, G. F Veen, S. Manzoni, W.H. van der Putten, Microbial storage and its implications for soil ecology, *ISME J.* 16 (2021) 617–629.
- [47] M. Hempel, M. Blume, I. Blindow, E.M. Gross, Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater, *BMC Microbiol.* 8 (2008) 58.
- [48] A. Barthès, L. Ten-Hage, A. Lamy, J.L. Rols, J. Leflaive, Resilience of aggregated microbial communities subjected to drought—small-scale studies, *Microb. Ecol.* 70 (2014) 9–20.
- [49] Q. Kong, Z. Xin, Y. Zhao, L. Ran, X. Xia, Health assessment for mountainous rivers based on dominant functions in the huajiu river, Beijing, China, *Environ. Manag.* (2022) 1–14.