Temporal variation revealed the composition change of phytoplankton assemblage responds to varied environmental indicators within an artificial freshwater engineered ecosystem

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Abstract

Artificial freshwater engineering ecosystems (AFwEEs) have attracted more and more attention. Phytoplankton is critical to the fluctuation of water quality in the AFwEEs. However, there is still a major knowledge gap regarding the ecology, composition, and temporal dynamics of phytoplankton assemblage composition in AFwEEs. Hence, an AFwEEs designed base on the submerged macrophytes (i.e., Vallisneria natans, Najas marina, and Potamogeton crispus) and fishes (i.e., Hypophthalmichthys molitrix, Hypophthalmichthys nobilis, and Ctenopharyngodon idellus) was established. The initial purpose for this survey was to conduct almost entire year (April/2019 – December/2019) sampling investigation to explain the interactions and relationship between multifarious environmental indicators and phytoplankton, also for the different phenomenon explanation of composition change in phytoplankton assemblage between AFwEEs and natural water environments. Consequences exhibited the assemblage dynamics of the phytoplankton were significant varied with seasonal succession, in which Cyanophyta dominated the phytoplankton assemblage while the mean relative abundance accounted for 66.4%, and then was Chlorophyta, for 18.8%. Principal component analysis (PCA) demonstrated temporal variation was significant impacted on phytoplankton assemblages. The dominant species, different assemblages, and distribution of phytoplankton were closely related to the dynamic changes of seasonal succession, which might change the composition of phytoplankton assemblage in the FwAEEs. Results of redundancy analysis (RDA) revealed the water temperature, nutrient concentration, DO concentration, and pH value were the main abiotic environmental indicators affecting phytoplankton assemblage. The trend of the four diversity indices indicated the phytoplankton assemblage became more stable in winter while the change extent of that was more significant in summer.

1. Introduction

Over the past two decades, environmental pollution specifically water pollution is alarming both in the developed and developing countries. Anthropogenic activities generate inputs via point or non-point sources, from which it may degrade waters and impair its use (i.e., potable supply, industry, agriculture, recreation, and etc.) (Jůza et al., 2019). The artificial freshwater engineering ecosystems (AFwEEs) are widely used to reverse the water environmental degradation caused by human activities (Benayas et al., 2009). AFwEEs exhibit good ecological benefits for its pollution control is carried out in an ecological way (Kagle et al., 2009). It not only plays an important role in the removal of pollutants in the water medium, but also greatly improves the ecological diversity of water bodies (Mohan et al., 2010; Bonnail et al., 2019). For its cost-effective and eco-friendly, more and more people have reached a consensus that water environmental ecological treatment has become the global focus (Chiranjeevi et al., 2013). Many researchers found that AFwEEs could effectively and sustainably restore the polluted water without introducing new chemical pollutants (Rezania et al., 2016; Everard et al., 2018).

Most theories and practices showed what matters for AFwEEs is multifarious planktonic microorganism, particularly phytoplankton (Guo et al., 2019). Peterson & Teal (1996) proposed that phytoplankton is one of ecological unit elements, which includes all the micro plants living in the water, usually for planktonic algae, not bacteria and other plants. Actually, phytoplankton exhibited a certain seasonal change with the interplay of various environmental indicators (i.e., nutrient concentration, temperature, and hydrological conditions, etc.). Its unique biological characteristics (i.e., wide geographical distribution, short reproductive cycle, and occupying a central position in the aquatic food webs) determine their importance in AFwEEs (Bondarenko et al., 2019). Jyothibabu et al. (2014) found that phytoplankton is very sensitive to the change of habitat environment. Namely, on the one hand, the change of its assemblage can not only reflect the change of water environment, but also predict the development and evolution trend of water in AFwEEs. On the other hands, for the distribution characteristics of phytoplankton are closely related
to water environment indicators, the changes of its cell density and assemblage composition could clearly and intuitively reflect the water quality and nutrition level of the water body. A research from Dalpadado et al. (2020) also exhibited that structural changes in the primary producer community (phytoplankton) are associated with changes in nutrient loading. In additional, phytoplankton could supply the herbivore with oxygen and nutrients (Srivastava et al., 2017), regulate the growth of aquatic animal, form an integrated ecosystem (Rodrigo et al., 2013). Therefore say up from this angle, the importance of phytoplankton to AFwEEs and ultimately to the planet itself has been highly recognized.

However, the changes of environmental indicators (i.e., climate change, physical indicators, and chemical indicators) have different effects on phytoplankton in AFwEEs. At the same time, under the frame of global warming and the impact of human activities on nature, some unforeseen changes are gradually emerging. Furthermore, the effectiveness of the AFwEEs in increasing provision of both biodiversity and phytoplankton diversity has not been evaluated systematically, especially in northern China. This is an urgent and necessary demand, to evaluate the temporal variation of relationships and interplaying between phytoplankton assemblage composition and environmental indicators, which is of vital importance to maintain the process and further assess the mechanism of AFwEEs.

In this study, an AFwEE designed base on submerged macrophytes (i.e., Vallisneria natans (V. natans), Najas marina (N. marina), and Potamogeton crispus (P. crispus)) and fishes (i.e., Silver carp (Hypophthalmichthys molitrix), bighead carp (Hypophthalmichthys nobilis), and grass carp (Ctenopharyngodon idellus)) is established in order to simulate the artificial ecological restoration project. Based on the other study on the AFwEE, the work aims to the variation of phytoplankton in the AFwEE. Furthermore it focuses on the relationship of phytoplankton data as well as 10 environmental indicators.

2. Material And Methods

2.1. Establishment of the AFwEE

AFwEE was built nearby Tangshan Qinglong River, and the illustration of its structure is shown in Fig. 1. The building structure of AFwEE was made of brick walls, which were coated with impermeable layers. The length, width, and height of the AFwEE were built as 7 m, 5 m, and 1.5 m, respectively. The river water was directly used as the original inflow of the AFwEE, which was extracted by the submersible pump (100 WQ90-26-11, Shandong Dong Beng Pump Co., Ltd, China). The water inlet pipe was divided into three branches to ensure uniform water distribution. Each water inlet was equipped with a flowmeter (LUGB-MIK-A, Asmik Sensor Co., Ltd, China) to measure the water inlet flow. The experimental effluent was collected uniformly and flows into the downstream of the river via the water pipes.

2.2. Cultivation of submerged macrophytes and fishes

In order to highly restore natural rivers, submerged macrophytes and fishes commonly found in northern China were introduced in the experiment. Submerged macrophytes and fishes were all purchased in Changlong Agricultural Products Co., Ltd (Beijing, China). The entire experiment durations were from March 2019 to December 2019. Among this period, pot experiments (with experiment water) of submerged macrophytes were carried out from March to April, which is to ensure the healthy growth of submerged macrophytes. After that the selected macrophytes will be transplanted into AFwEE in early April. Of note, the selected experimental season owns enough solar energy, which could be effectively utilized by photosynthesis of submerged macrophytes. Most experiments showed that this is the best growth period of submerged macrophytes (Lu et al., 2018; Yu et al., 2019). All macrophytes were cleaned with deionized water and uniformly sized (Initial length trim to 20 ± 2 cm). In order to reduce the impact of environmental
factors, a pot experiment was carried out with quartz sand fixation (average particle size 4–7 mm) to make macrophytes adapt to the water environment in advance. After adaptation of one month period, complete and healthy macrophytes were selected for subsequent experiments. Because of the tillering effect of new buds, planting density cannot be effectively controlled. At the same time, it is also considered to reduce human interference, so three selected submerged macrophytes are planted according to the density of 1:1:1.

When the growth of submerged macrophytes is stable, fishes will be placed in AFwEE (around June 15th). A certain number of fishes were selected and placed in AFwEE after one month of environmental adaptation (with experimental water). The breeding proportion of Silver carp, Bighead carp, and Grass carp are 7:5:6, respectively (Sin et al., 1987). Of note, only healthy fishes could be selected for experiments. For more detailed information about submerged macrophytes and fishes in Supplementary information (SI). Of note, all the biological experiments involved are non-invasive.

2.3. Determination of water quality parameters

Various chemical indicators (i.e., Chemical Oxygen Demand (COD), Total phosphorus (TP), Ammonia nitrogen (NH$_3$-N), Nitrate nitrogen (NO$_3$-N), and Total nitrogen (TN)) of water body were measured by the Palin-test Photometer Tube Tests (Palin-test Ltd., England) weekly. All kinds of reagents were purchased from Sigma-Aldrich Chemical Co., Ltd (Germany). Dissolved oxygen (DO), pH value, conductivity, water temperature (Temp.), and Chlorophyll a (Chl. a) were measured weekly by using the portable instruments (YSI-EXO1, WEISS instrument, the United States of America). Transparency (SD) was measured weekly with the help of Secchi disc, the measurement please refers to Effler (1988).

2.4. Determination of water and phytoplankton sampling

Water samples were collected with a Grasp (CG-001L) water-sampler once a week and 1 L of water was collected each time. Measurements were made before with the help of the 0.22 µmol syringe membrane filters. For detailed measurements please refers to Xu et al. (2020). All phytoplankton samplings are collected on a weekly basis. The determination of phytoplankton sampling was referred to Qian et al. (2016). Phytoplankton samples were collected 1 L for each time from the AFwEE also by the Grasp water-sampler and preserved in 4% neutralizing formaldehyde solution left 48 h for setting. And then a small siphon (inner diameter 3 mm) was used to absorb the supernatant. The remaining precipitation liquid (about 30 mL ~ 50 mL) was poured into a quantitative flask. Species identification of phytoplankton was conducted by 400X magnification of microscope and with the help of a 0.1 mL microscopic counting frame to detect the density. For details of species identification, please refer to Sun et al. (2017).

2.5. Calculation of diversity indices

The following equations are of the four representative diversity indices, including the Shannon–Wiener diversity index (Shannon and Weaver, 1949; Pielou, 1966a), the Margalef (1967) richness, the Pielou (1966b) evenness index, and the Simpson diversity index (Simpson, 1949; Pielou, 1969). Among them, they are listed as Eq. (1), Eq. (2), Eq. (3), and Eq. (4), respectively.
(1) Shannon Wiener diversity index (H)
\[ H = - \sum_{i=1}^{S} \left( \frac{n_i}{N} \right) \ln \left( \frac{n_i}{N} \right) \]  \hspace{1cm} (1)

(2) Margalef richness Index (M)
\[ M = \frac{S - 1}{\ln N} \]  \hspace{1cm} (2)

(3) Pielou evenness index (P)
\[ P = \frac{H}{\ln S} \]  \hspace{1cm} (3)

(4) Simpson diversity index (D)
\[ D = 1 - \sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^2 \]  \hspace{1cm} (4)

Where: \( S \) is the number of species within the tested phytoplankton sample, \( N \) is the total number of individuals in all species within the tested phytoplankton sample, and \( n_i \) is the number of individuals species \( i \) within a tested sample. Based on the original data of phytoplankton species, four indexes of phytoplankton diversity were calculated and used to analyze the dominant species of phytoplankton.

2.6. Data processing and statistical analysis

The original data is counted and calculated with Microsoft office, shown below are the average values calculated from \( N \) replicate measurements \( \pm \) S.D. For the phytoplankton assemblage, one-way variance analysis (ANOVA) was used to evaluate the variability of parameters between different months. Principal component analysis (PCA) was used to evaluate the difference of the biomass \( (10^{-3} \text{ mg/L}) \) of phytoplankton dominant species in different months. Redundancy analysis (RDA) was used to determine the impact of multifarious environmental indicators on the abundance of phytoplankton dominant species in different months. The dominant species and environmental indicators entering PCA and RDA were normalized by a logarithmic transformation \((\log_{10} (n+1))\). Of note, a large number of phytoplankton species occur only randomly and occasionally, and all of them do not provide any ecological information useful. Hence, only dominant species (For relative abundance are top 25) were selected for sequencing analysis. Graph-Pad Prism 8.2.1 and Origin 2019b are used for data calculation and graphic visualization.

3. Results And Discussion

3.1. Evaluation of temporal dynamics on physical and chemical indicators of water quality in AFwEE
In order to research the temporal dynamics of environment in AFwEE, we analyzed the variation in the abiotic environment indicators to accurately and effectively detect the seasonal change of the environment during the whole year. The physical-chemical characteristics are exhibited in Fig. 2. Clearly, water temperature varied significantly with months (ANOVA, \( p < .05 \)). The highest water temperature (28.5°C) was recorded in September while the lowest (4.8°C) in December (Fig. 2f). The average water temperature rises in spring and reaches the highest value in summer. The DO concentration ranged from 4.33 mg/L (December 2019) to 16.65 mg/L (August 2019) (Fig. 2g). In addition, due to the photosynthesis of aquatic plants, it is easy to observe that the DO concentration in June, July, and August increased rapidly. The pH range of water samples is not wide, it ranged from 6.88 to 8.95 (Fig. 2h). And it did not differ significantly according to different months (ANOVA, \( p = .22 \)).

3.2. Evaluation of phytoplankton assemblage composition in AFwEE

3.2.1. Species of phytoplankton assemblage

The entire species collected belong to 5 phylums (i.e., Cyanophyta, Bacillariophyta, Cryptophyta, Euglenophyta, and Chlorophyta) and 69 species. Among them, there are 9 species in Cyanophyta, 23 species in Bacillariophyta, 35 species in Chlorophyta and single species in Cryptophyta and Euglenophyta, respectively. The detailed summary is shown in Table 1.
| Phylum          | Species                  | Code | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|----------------|--------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cyanophyta     | Synechocystis sp.        |      | +   | +   | +   | +   | +   |     |     |     |     |
|                | Aphanocapsa sp.          |      | +   | +   | +   |     |     |     |     |     |     |
|                | Eucapsis sp.             | Bac 01 | + | + | + | + | + | + | + | + | + |
|                | Woronichinia sp.         |      | +   |     |     |     |     |     | + | + | + |
|                | Microcystis weisenbergii | Bac 02 | + | + | + | + | + | + | + | + | + |
|                | Anabaena flos-aquae      | Bac 03 | + | + | + | + | + | + | + | + | + |
|                | Pseudoanabaena sp.       | Bac 04 | + | + | + | + | + | + | + | + | + |
|                | Arthrospira sp.          | Bac 05 | + | + | + | + | + | + | + | + | + |
|                | Anabaena eucompaeta      | Bac 06 | + | + | + | + | + | + | + | + | + |
| Bacillariophyta| Merismopedia tenuissima  | Bac 07 | + | + | + | + | + | + | + | + | + |
|                | Merismopedia sp.         | Bac 08 | +   |     |     |     |     |     |     |     |     |
|                | Cyclotella sp.1          | Bac 09 | + | + |     | + |     |     |     |     |     |
|                | Cyclotella sp.2          | Bac 10 | + | + | + |     | + |     |     |     |     |
|                | Cyclotella sp.3          | Bac 11 | + | + | + | + |     |     |     |     |     |
|                | Cyclotella sp.4          | Bac 12 | + | + | + | + | + |     |     |     |     |
|                | Synedra sp.1             |      | + |     | + |     |     |     |     |     |     |
|                | Synedra acus             |      | + | + |     | + |     |     |     |     |     |
|                | Synedra ulna             |      | + | + |     | + |     |     |     |     |     |
|                | Navicula dicephala       |      | + | + |     |     | + |     |     |     |     |
|                | Navicula graciloides     |      | + | + | + |     | + |     |     |     |     |
|                | Navicula laevissima      |      | + |     |     |     |     |     |     |     |     |
|                | Amphora sp.              |      |     |     |     |     |     |     |     |     |     |
| Phylum      | Species                  | Code | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|------------|--------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|            | *Cymbella turgidula*     |      | +   |     |     |     |     |     |     |     |     |
|            | *Cymbella ventricosa*    |      | +   |     |     |     |     |     |     |     |     |
|            | *Gomphonema parvulum*    |      |     |     | +   |     |     |     |     |     |     |
|            | *Gomphonema simus*       |      |     |     |     |     |     |     |     | +   |     |
|            | *Coccineis placentalula* |      |     | +   | +   |     |     |     |     |     |     |
|            | *Achnanthes exigua*      |      |     |     |     |     |     |     | +   | +   |     |
|            | *Hantzschia amphioxys*   |      |     | +   |     |     |     |     |     |     |     |
|            | *Nitzschia frustulum*    |      |     | +   | +   |     |     |     |     |     |     |
|            | *Nitzschia linearis*     |      |     |     |     |     |     |     |     |     |     |
|            | *Nitzschia sublinearis*  |      |     |     |     |     |     | +   | +   | +   |     |
| Cryptophyta| *Cryptomonas erosa*      |      |     |     |     |     | +   |     |     |     |     |
| Euglenophyta| *Euglena spp.1*       |      |     |     |     |     |     | +   |     |     |     |
| Chlorophyta| *Chlamydomonas sp.*     |      |     |     |     |     |     |     |     |     | +   |
|            | *Chlamydomonas globosa*  |      |     |     |     | +   |     |     |     |     |     |
|            | *Pandorina morum*        |      |     |     |     |     |     |     |     |     | +   |
|            | *Schroederia spiralis*   |      |     |     |     |     |     |     |     |     | +   |
|            | *Schroederia nitzschioides* |   |     |     |     |     |     |     |     |     | +   |
|            | *Chlorella pyrenoidosa*  |      |     |     |     |     |     | +   | +   |     |     |
|            | *Chodatella wratislaviensis* | |     |     |     |     |     | +   |     |     |     |
|            | *Tetraedron minimum*     |      |     |     |     |     |     |     |     |     | +   |
|            | *Tetraedron trilobulatum*|      |     |     |     |     |     |     |     |     | +   |
| Phylum            | Species                | Code | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|-------------------|------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                   | Ankistrodesmus angustus| +    | +   | +   | +   |     |     |     |     |     |     |
|                   | Ankistrodesmus falcatus| +    | +   |     |     |     |     |     |     |     |     |
|                   | Selenastrum bibraianum | +    |     |     |     |     |     |     |     |     |     |
|                   | Selenastrum minutum    | +    |     |     |     |     |     |     |     |     |     |
|                   | Kirchneriella contorta | +    |     |     |     |     |     |     |     |     |     |
|                   | Oocystis borgei        | +    |     |     |     |     |     |     |     |     |     |
|                   | Nephrocytium agardhianum| +   |     |     |     |     |     |     |     |     |     |
|                   | Gloeocystis ampla      | +    |     |     |     |     |     |     |     |     |     |
|                   | Dictyosphaerium ehrenbergianum| + |     |     |     |     |     |     |     |     |     |
|                   | Pediastrum duplex      | +    |     |     |     |     |     |     |     |     |     |
|                   | Pediastrum simplex     | +    |     |     |     |     |     |     |     |     |     |
|                   | Pediastrum simplex var. duodenarium | + |     |     |     |     |     |     |     |     |     |
|                   | Scenedesmus bicaudatus | Bac 13 | + | + | + | + | + | + | + |
|                   | Scenedesmus quadricauda| Bac 14 | + | + | + | + |     |     |     |     |     |
|                   | Scenedesmus quadricauda var.parvus | Bac 15 | + | + | + | + |     |     |     |     |     |
|                   | Scenedesmus dimorphus  | Bac 16 | + | + | + | + | + |     |     |     |     |
|                   | Scenedesmus opoliensis | Bac 17 | + | + | + | + | + |     |     |     |     |
|                   | Scenedesmus abundans   | Bac 18 | + | + | + | + | + |     |     |     |     |
|                   | Scenedesmus armatus    | Bac 19 | + | + | + | + | + |     |     |     |     |
|                   | Pediastrum simplex     | Bac 20 | + | + | + | + | + |     |     |     |     |
### 3.2.2. Temporal variation of abundance and biomass of dominant phytoplankton

Consequences of this research showed that Cyanophyta dominated the phytoplankton assemblage in AFwEE almost during all experimental periods (Fig. 3a). According to cell density, it average accounts for more than 76% of phytoplankton abundance. It can be seen in Table 1, from April to September the detection rate of Bacillariophyta gradual declined. However, its relative abundance was remarkably larger in from April to June than other months, account for 14.6%, 11%, and 9%, respectively. In addition, most of the Bacillariophyta collected are pollution resistant assemblage, which also shows that the quality of river water environment is not good enough. And it also can be seen clearly that the relative abundance of Cryptophyta accounts for a large cent in April, for almost 15%. However, the Cryptophyta assemblage is less and less after June (Fig. 2b). It is worth mentioning that, from April to September, the relative abundance of Chlorophyta accounts for a large proportion, average for about 22%. Previous studies have confirmed that this is related to photosynthesis of aquatic plants (Liu et al., 2018). It is due to the initial stage of the experiment, the growth of aquatic plants has not been stable in AFwEE till May (Supplementary information, SI). However, from May to July, submerged macrophytes began to grow healthily and rapidly while the relative abundance of Chlorophyta increased significantly. It seems to be a common phenomenon. With the photosynthesis of submerged macrophytes, the content of Chl. \( a \) will significant increase (Fig. 4). And then the abundance and biomass of Chlorophyta in the vegetation zone are always very high (Zhu et al., 2010).

To further assess dominant phytoplankton species in AFwEE, a circos map (Cell density) and a heat map (Biomass) of phytoplankton at Species level in different months is given in Fig. 3b and Fig. 3c. As mentioned above, the dominant assemblage is Cyanophyta. Among that, the change of Cyanophyta is mainly due to *Eucapsis sp.*, *Microcystis wesenbergii*, *Anabaena flos-aquae*, and *Merismopedia tenuissima*. As time goes on, almost all of them show an obvious upward trend. However, dominance of *Eucapsis sp.*, *Microcystis wesenbergii*, and *Anabaena flos-aquae* might result in a decrease in numbers of others (Fares et al., 2020). Because these species in Cyanophyta assemblage has an effect on filtration, they are hard to be digested and also for the reason of that they release toxins, leading to changes in biodiversity (Yang et al., 2017; Li et al., 2020). After that, there are some detailed species of Bacillariophyta assemblage. The most significant changed species are *Cyclotella sp.1*, *Cyclotella sp.2*, *Cyclotella sp.3*, and *Cyclotella sp.4*, respectively. Shen et al. (2020) have labeled that the reduction of these algae is significantly related to the water temperature and nutrient concentration (i.e., NO\(_3\)-N and TP) changing. The rest are some phytoplankton assemblage related to Cryptophyta and Chlorophyta, among them the cell density of *Scenedesmus*...
*bicaudatus*, *Scenedesmus quadricauda*, and *Scenedesmus dimorphus* exhibit a clear decreasing trend with temporal variation.

### 3.2.3. Relationships between dominant phytoplankton species and temporal variations

Multivariate statistical analysis, especially principal component analysis (PCA), has been widely used to study the patterns and relationships of large-scale ecological data sets. PCA has been proved useful for qualitative analysis of the interactions between abiotic indicators affecting phytoplankton communities (El-Naggar et al., 2019). Hence, the PCA was carried out to evaluate the relationship between dominant phytoplankton species in AFwEE and temporal variations. According to the consequences of PCA, it is found that the temporal variations of phytoplankton species are very different. Conclusion of Fig. 5 exhibited that there was no significant difference in phytoplankton species between summer and autumn. This is because the summer and autumn season temperature (15–27°C) is more suitable for the growth and reproduction of most phytoplankton (Kumar et al., 2020). Hence, the phytoplankton assemblages are not limited by water temperature in these two seasons. However, the appropriate temperature will make Cyanophyta multiply in large quantities, and it is very easy to burst Cyanophyta blooms (Jia et al., 2018).

Regarding the effect of spring on phytoplankton species, the results showed that some assemblages (*i.e.*, Chlorophyta) could also reproduce in large quantities in spring. It is mentioned this is due to global warming, which promotes the reproduction of some phytoplankton assemblages in spring, while the maximum biomass and individual size of phytoplankton are decreasing gradually (Hoffmann et al., 2013). When in winter, low temperature environment and high nitrogen-phosphorus ratio will play a certain inhibitory role on the growth and reproduction of phytoplankton, especially for Cyanophyta. The phytoplankton composition in winter is relatively independent, which indicated that the phytoplankton assemblage composition is unique while Bacillariophyta is the main affected assemblage in AFwEE.

### 3.3. Relationships and interplay between dominant species of phytoplankton and environmental indicators.

Redundancy analysis (RDA) is an effective method to determine the main environmental indicators on a spatial scale via dimensionality reduction (Znachor et al., 2019). Most experiments and projects use RDA to obtain the sequencing analysis of aquatic ecology research (Waller et al., 2016). RDA provides further insight into the relationship between phytoplankton assemblage and environmental indicators. Hence, in order to further assess the relationship between phytoplankton and environmental indicators in AFwEE, RDA was performed with phytoplankton dominant species (25 species) and 10 environmental abiotic variables (*i.e.*, COD, TP, NH$_3$-N, NO$_3$-N, TN, Temp., DO, pH values, Transparency, and Chl. a).

The results indicated a strong correlation between abiotic variables and species distribution. The two axes explained 63.6% and 14.4% of the cumulative variance of the relationship between dominant species and environmental abiotic variables, and the eigenvalues were 0.183 and 0.067, respectively. As showed in Fig. 6, some species (*i.e.*, *Merismopedia tenuissima*, *Cyclotella sp.2*) in Bacillariophyta were related to the change of water temperature, phosphorus, and nitrogen. A study specifically targeting the Bacillariophyta revealed that three kinds (*i.e.*, motile ecological guild, low profile guild, and high profile guild) of Bacillariophyta exhibited different sensitivities to environmental indicators (Stenger-Kovács et al., 2013). For motile ecological guild is more sensitive to temperature while low profile guild and high profile guild was more sensitive to TP and other nutrients. Because phosphorus is a key element in biogeochemical cycle, high phosphorus load leads to high phytoplankton biomass (Lin et al., 2020). Overloading is the main reason led to water eutrophication in China. Namely, controlling nutrient concentration to a certain extent could not only avoid large-scale propagation of Bacillariophyta, but also effectively alleviate water eutrophication. And the dominant species in Cyanophyta also showed strong correction with water temperature, transparency, and pH values. Nowadays, water temperature is thought to be the most important environmental factor.
influencing the growth of phytoplankton assemblages. Previous literature reported that Cyanophyta grows and reproduces in large quantities and rapidly when the temperature of water body is high while it accesses a dormant phase when the environment temperature is not suitable for growing (Zhang et al., 2018). Most species of Cyanophyta were affected by the water temperature or trophic state. Especially for *Anabaena flos-aquae*, high temperature and high nutrient salt can effectively promote its growth and reproduction (Tian et al., 2012). Namely, temperature was the most important indictor affecting Cyanophyta abundance. The effect of other indicators on Cyanophyta abundance was more notable in warm periods than in periods with low temperature. Chlorophyta is relatively concentrated, and the changes of their assemblages are also affected by the changes of nutrients. Among them, TN and NH$_3$-N shows the greatest relevance. Meanwhile, the changes of DS concentration and transparency (negative correlation) also have great significant relationship with Chlorophyta. In fact, the aggregation of Chlorophyta is often affected by plant photosynthesis (Reid & Mosley, 2016). The strong light source in summer can promote the photosynthesis of aquatic plants, at which time the massive propagation of the Chlorophyta will often make the transparency of the water environment significantly decreased (Ying et al., 2020). On the whole, relative to natural water body, the phytoplankton assemblage composition in AFwEE exhibited a trend that warm-water species increase and cold-water species decrease on temperate. As for nutrient physiology, it could be found that favored nitrogen species are increase gradually.

3.4. Evaluation of temporal dynamic on four diversity indices
Phytoplankton assemblage diversity index reflects the relationship between phytoplankton assemblage and species density, as well as the disturbance degree of assemblage to environmental indicators and water quality (Gao & Song, 2005). In order to comprehensively evaluate the temporal variation of diversity of phytoplankton in AFwEE, four kinds indices were used.

The calculation results of phytoplankton are shown in Fig. 7. In general, the four indices have great changes in different months. The overall temporal variation of four indices of phytoplankton showed a trend of increasing first and then decreasing. In July, the Shannon Wiener diversity index and the Margalef richness index of phytoplankton got the highest value of 3.06 ± 0.25 and 2.72 ± 0.19, respectively, which explains that the biodiversity of AFwEE was the most complete and the ecological advantage was the largest in this period (Kadam et al., 2020). The Pielou evenness index was 0.725 ± 0.17 while the Simpson diversity index was 0.85 ± 0.16. Clearly, the richness of phytoplankton in summer was significantly different from that in other seasons (ANOVA, $p < .05$). However, as time goes on, the water temperature decreases while the four indices all decreased in different degrees, which indicated positive correlation with the water temperature. In December, the Shannon Wiener diversity index and the Margalef richness index got the minimum value of 2.02 ± 0.10 and 1.98 ± 0.08, respectively. And the Pielou evenness index was 0.41 ± 0.02 while the Simpson diversity index was 0.52 ± 0.07. Namely, the phytoplankton diversity of AFwEE follows certain seasonal rules. The abundance of phytoplankton in AFwEE reached the highest in summer, but there were only a few cold resistant assemblages in winter.

4. Conclusions
This paper analyzed the effects of temporal variation of various abiotic indicators on phytoplankton assemblage composition in AFwEE. The AFwEE was found to have a high and complete phytoplankton species diversity, with a total of 70 species from 5 phylums recorded. During the experiment, Cyanophyta was the dominant assemblages with an entire year average relative abundance of 66.4%, and then was Chlorophyta, for about 18.8%. PCA demonstrated that seasonal succession has a significant impact on phytoplankton assemblages. Among them, summer and autumn are more suitable for the reproduction of most phytoplankton assemblage. RDA demonstrated
that interactions between phytoplankton dominant species and abiotic environmental indicators, which were strongly related to water temperature, nutrient concentration, pH, DO. The temporal variations of four planktonic diversity indices reached a maximum at July. With the decrease of temperature, it gradually presents a downward trend. Phytoplankton assemblages tend to be stable till winter. After all, revealing the relationship between phytoplankton assemblage composition and water environmental indicators in AFwEE can provide more detailed and scientific theoretical consults and the method reference for construction of freshwater ecological engineering. In additional, this part of knowledge could be of interest in the development of management and strategy for plankton in aquaculture systems.

Declarations

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Author contributions

D. X. and H. W. conceptualized and designed the experiment. D. X. and AT. C. performed the experiments. D. X., RU. X., and YX. N. analyzed the data and made figures. D. X. and H. W. wrote the paper. All the authors read and contributed to the submitted version of the manuscript. D. X. and H. W. acquired the funding and were responsible for resources.

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Availability of data and materials

All relevant data within the manuscript and available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures on aquatic organism were performed according to the protocols approved by the Scientific Committee of Animal Experimentation of Water Science and Technology Hebei Institute (GB-593-1997).

Consent to participate

All authors were participated in this work.

Consent to publish

All authors agree to publish.

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Figure 1

Equipment sketch of the AFwEE
Figure 2

Temporal dynamics of COD (a), TP (b), NH₃-N (c), NO₃-N (d), TN concentration (e), Temperature (f), DO concentration (i), pH value (g), and transparency (h) of water in the AFwEE (Shown are the mean value of multiple sampling deviations)
Figure 3

Temporal variations of relative abundance of phytoplankton at Phylum level (a), circos map based on most abundant (top 25 Species) phytoplankton in density (104 Cells/L) (b), and heat map based on most abundant (top 25 Species) phytoplankton in biomass (10-3 mg/L) (c) within the AFwEE (Shown are the average of multiple sampling deviations)
Figure 4

Temporal variations of content of Chl. a within the AFwEE

Figure 5

PCA of temporal variations of phytoplankton dominant species (Cyanophyta, Bacillariophyta, and Chlorophyta) in the AFwEE
Figure 6

Relationship among phytoplankton dominant species (Cyanophyta, Bacillariophyta, and Chlorophyta) and environmental indicators in the AFwEE based on RDA.

Figure 7

Analysis of temporal variations of phytoplankton diversity indices in the AFwEE (Shown are the average of multiple sampling deviations).

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