ABSTRACT

Phytoplankton is capable of responding to aquatic conditions and can therefore be used to monitor freshwater reservoir water quality. Numerous classification techniques, including morpho-functional approaches, have been developed. This study examined changes in phytoplankton assemblages and water quality, which were sampled quarterly from July 2018 to April 2019. The purpose was to contrast the applicability of three classification approaches (functional, morpho-functional and morphological-based functional groupings) for understanding the spatial and seasonal distribution of the biomass variance in phytoplankton functional groups and their driving environmental factors in the ecological zones of the Shanxi Reservoir through multivariate analysis. The results showed that the phytoplankton biomass was highest in the watercourse zone and lowest in the transition zone. Furthermore, the Shanxi Reservoir was characterized by several cyanobacteria (Microcystis spp.) and numerous bacillariophytes (Asterionella sp., Navicula spp. and Aulacoseira granulata). After evaluating the advantages and disadvantages of morpho-functional classifications, we determined that water temperature appeared to be an essential factor, and the morphology-based functional group approach provided the best results for demonstrating phytoplankton succession, despite having lower sensitivity than the others. Nevertheless, these approaches are all appropriate for identifying and monitoring phytoplankton community structure in aquatic systems of reservoirs with complex terrains.

Key words | environmental factors, morpho-functional classifications, phytoplankton assemblages, Shanxi Reservoir, spatial and seasonal distribution

HIGHLIGHTS

- Comparison of the MBFG, MFG, and FG approaches.
- Research in a river-type reservoir.
- Finding the environmental driving factors.
- Combining the concept of ecological zone.
- Evaluating the advantages and disadvantages of morpho-functional classifications.

INTRODUCTION

As a renewable but finite resource, freshwater needs to fulfill several changing needs, including the availability of, quality of and demand for water (de Freitas & Perry 2012). However, due to climate change and human activities, many natural freshwater resources have been polluted or damaged. As a result, many man-made lakes and reservoirs have been put into use. Freshwater reservoirs, as one of the primary water sources, offer crucial and diverse services, such as
providing flood control and drinking water, water for irrigation, transportation routes, and areas for the generation of hydropower and recreational activities (Markowska et al. 2020). However, many studies have argued that reservoirs represent highly dynamic aquatic environments, which profoundly impact riverine ecology and have complex biophysical effects (Naiman et al. 2000). The operation of dams alters both the community structure and abundance of aquatic organisms (Zhang et al. 2010; Li et al. 2013). For instance, rising water temperature, prolongs water stagnation time and nutrient enrichment, leading to an increase in phytoplankton (Nogueira et al. 2010).

Phytoplankton abundance and diversity can rapidly and sensitively respond to and reflect changes in the ecological features of aquatic environments (Padisák et al. 2005). Species that initially flourished in a certain set of living conditions may no longer be supported following environmental changes, while other species may be more adaptable (Zhou et al. 2019). For this reason, phytoplankton can be treated as a key control object for eutrophication prevention and used as an indicator of reservoir water quality. Therefore, it is necessary to enter phytoplankton monitoring as an improvement of water quality testing items of reservoirs (Lin et al. 2016). In addition, reservoirs have obvious hydrological changes during seasons, and terrain changes along the rivers (Wu et al. 2019). Consequently, it is essential to summarize the dynamic regularities of phytoplankton structures in reservoirs based on both seasonal and spatial variations. However, given the vast amount of data, illustrating how the phytoplankton community is related to environmental variables in reservoirs is still a considerable challenge. For many years, multivariate techniques have been widely utilized to examine these relationships (Jones & Francis 1982; Prézelin et al. 1991; Teissier et al. 2012). However, without a theoretical model, the randomness of the environmental variables has made it impossible to accurately assess phytoplankton and explain or predict dominant species (Reynolds et al. 2002).

According to some experimental studies, functional diversity may be more informative since taxonomic assemblages do not always accurately reflect ecological functions (Huston 1997; Tilman et al. 1997). Various methods of measuring functional diversity have been proposed to circumvent these issues (Petchey & Gaston 2006). Currently, the most commonly agreed upon approach involves aggregating species with similar appearances (ecological, physiological and morphological) into groups that reflect a specific habitat type and possibly an ecosystem function (Hadar et al. 1999; Salmaso & Padisák 2007). Conscious of the importance of functional properties, Reynolds (1980, 1984) created a phytoplankton-based classification system with 14 qualified associations containing species from a series of lakes in north-western England based on their responses to the surroundings. Then, the initial grouping was expanded to 31 functional groups (FGs) (Reynolds et al. 2002). A subsequent improvement of these methods was presented by Padisák et al. (2009), in the form of a more general approach that highlighted distinctive ecological features. Salmaso & Padisák (2007) evaluated 31 morpho-functional groups (MFG) based on the morphological and functional characteristics with a multivariate analysis technique and proposed expanding on the categories described by Tolotti et al. (2012). With the integration of machine learning techniques, Kruk et al. (2010) applied a new morphological-based functional groups (MBFG) approach, which has successfully been applied in different regions. To date, these three methods are the most effective solutions for identifying the connection between environmental variables and phytoplankton communities (Izaguirre et al. 2011; Hu et al. 2013; Bortolini et al. 2019); however, due to their different classification criteria, their abilities of performing the phytoplankton–environment relations are also diverse. Existing phytoplankton studies on lakes have shown that statistical analysis results presented using MBFG approach in describing the relation of phytoplankton and environmental factors were better than those with FG and MFG approaches (Izaguirre et al. 2012; Žutinić et al. 2014).

This study was conducted in a meso-eutrophic river-type reservoir located in a subtropical region with a mild climate in south-eastern China. There were several towns that focused on farming in the upper reaches of this reservoir which caused serious water pollution that continued for many years. Research on the physical and chemical factors of this reservoir has been carried out; however, the most recent thorough research on phytoplankton was carried out almost a decade ago (Zeng et al. 2011). Therefore, our research was conducive to updating the understanding of phytoplankton status and making the water quality testing items more comprehensive in this area. The Shanxi Reservoir has a winding watercourse through a canyon and considerable stratification during spring and summer, with relatively high nutrient concentrations. In light of these conditions, the study area was divided into the watercourse zone (Z1), riverine zone (Z2), transition zone (Z3) and lacustrine zone (Z4) based on the classification method provided by Wetzel (2001).

The emphasis of this study was to verify the applicability of three phytoplankton morpho-functional classification approaches and assess their performances in response to...
the dynamic changes in the phytoplankton community structure. The hypothesis of this research was that these three different phytoplankton morpho-functional classification approaches (FG, MFG, MBFG) can effectively detect changes in phytoplankton assemblages, that the variance of the biomass of phytoplankton functional groups (for each approach) can be explained by environmental factors, and that the MBFG approach was more applicable in representing phytoplankton succession in a large river-type reservoir.

**MATERIALS AND METHODS**

**Study site**

The Shanxi Reservoir (latitude 27°36′–27°50′N; longitude 119°47′–120°15′E) is located in the upstream region of the Feiyun River in southern Zhejiang Province, China (Figure 1). It was constructed in 2001 and has more than 14 main tributaries. As a vital large-scale drinking water resource, the reservoir supplies 1.2 × 10⁹ m³ of water annually to satisfy the demand of approximately 5 million residents in the surrounding counties. The total watershed area is 1,529 km², with a total volume of 1.82 × 10⁹ m³. The weather of the Shanxi watershed is regulated by a subtropical oceanic monsoon climate; the conditions are favourable, with a mean air temperature of approximately 18 °C and annual precipitation of 1,870 mm.

**Sampling**

Seven sampling sites were established in the Shanxi Reservoir, and the samples were collected at quarterly intervals from July 2018 to April 2019 (Figure 1). For each site, the samples for phytoplankton and water quality parameter measurements were taken simultaneously and respectively from the upper (1 m), middle (4–7 m) and lower (10–20 m) layers with a 5-L water collector. The main hydrometeorological characteristics of the four sampling periods are summarized in Table 1. Water temperature (Tem), pH, dissolved oxygen (DO), electrical conductivity (EC), turbidity (Tur), oxidation–reduction potential (ORP) and chlorophyll-a (Chl-a) were measured with a Hydrolab DS5X (Hach Corporation). The total phosphorus (TP) and permanganate index (IMn) were assessed in a laboratory using standard methods throughout the investigation period.

The volume of each phytoplankton sample (350 mL) was collected and fixed with 1% acidified Lugol’s iodine solution, and preserved in the dark at 4 °C for at least 24 h to allow sedimentation to occur. The phytoplankton identification and counting were performed with an Olympus BX51 (Olympus Corporation) microscope at ×400 magnification. The species contributing >5% to the total biomass were identified and classified into functional groups with the above-mentioned classification approaches. The biomasses of both the species and functional groups were estimated from the biological density, the species diversity variance approaches (FG, MFG, MBFG) can effectively detect changes in phytoplankton assemblages, that the variance of the biomass of phytoplankton functional groups (for each approach) can be explained by environmental factors, and that the MBFG approach was more applicable in representing phytoplankton succession in a large river-type reservoir.

**RESULTS**

**Environmental variables**

The different features of each zone are listed in Table 2. The highest Tur (mean value: 13.25) and EC (mean value: 45.29 mS cm⁻¹) were found in Z2 and Z1, respectively. The lowest Tur (mean value: 1.67) and EC (mean value: 35.68 mS cm⁻¹) were respectively observed in Z4 and Z3. Notably, apart from Z1, the maximum EC value in each zone appeared in summer, while the minimum Tur value appeared in spring. All the studied zones were acidic (pH ranged from 6.11 to 6.61) in spring, but alkali in other seasons. Reflecting the influence of the upstream farms, the water of Z1 contained high pollutants (mean...
IMn: 2.11 mg L$^{-1}$) and had a high alkalinity (mean pH 9.45) relative to that in the other zones.

Given that in the study area, the water temperature changes significantly (from 16.69 to 29.54 °C) with seasons, thermal stratification occurred in all the zones during the summer and spring periods. The average surface Tem of the Shanxi Reservoir in summer was 28.85 °C, and the difference between the upper and lower layers was 2.66 °C.
DO concentrations were relatively low in all the zones during every season, with the exception of summer (116.08 × 10^4 cells L^{-1} and 160.69 × 10^4 cells L^{-1}), respectively. Relative to the other zones, the biomass of Z3 was lower during most seasons (stratified averaged from 51.47 × 10^4 to 4.68 × 10^4 cells L^{-1}). Furthermore, Z4 also displayed lower phytoplankton biomass, except for a

**Table 1** | Annual precipitation, average water level and average volume of the Shanxi Reservoir

<table>
<thead>
<tr>
<th>Average precipitation (mm)</th>
<th>Average water level (m)</th>
<th>Average volume (10^6 m^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul. 2018</td>
<td>265.5</td>
<td>136.65</td>
</tr>
<tr>
<td>Oct. 2018</td>
<td>67.2</td>
<td>136.52</td>
</tr>
<tr>
<td>Jan. 2019</td>
<td>60.8</td>
<td>132.80</td>
</tr>
<tr>
<td>Apr. 2019</td>
<td>221.0</td>
<td>134.10</td>
</tr>
</tbody>
</table>

(Table S1). The stratification phenomenon was even more obvious in spring than in summer, with the Tem difference reaching 4.08 °C. Nevertheless, in winter and autumn, no apparent stratification was observed.

The variation in the ORP throughout the study area remained high over the sampling period (mean values from 273.60 to 307.50 mV), reaching the highest value in the lower layer of Z4 during spring while the minimum value of DO was also observed (2.38 mg L^{-1}). The lowest ORP occurred in the upper layer of Z1 during summer. The DO concentrations were relatively low in all the zones during every season, with the exception of summer when the maximum value was measured in Z2 (8.04 mg L^{-1}). The lowest and highest Chl-a values were detected in Z2 and Z4 during autumn and summer (mean values of 0.90 µg L^{-1} and 35.79 µg L^{-1}, respectively). Intermediate Chl-a values were recorded from Z1 and Z3 (mean values of 4.86 and 3.31 µg L^{-1}, respectively).

Every zone contained high concentrations of TP. For Z1, the highest TP concentration was recorded in the middle layer during summer (224.60 µg L^{-1}), while Z2, Z3 and Z4 exhibited the lowest TP during spring (Table S1). In Z2 and Z4, the highest TP values was recorded in winter, with the lowest values being 270.95 and 201.51 µg L^{-1}, respectively. In most of the seasons, the values of DO and TP both reached the maximums in the lower layer, while Chl-a showed the opposite trend in all zones except Z3 where TP and Chl-a decreased simultaneously.

**Phytoplankton community structures**

The four ecological zones showed substantial differences in total phytoplankton biomass, with significant seasonal variation (Figure 2). High biomasses were found in Z1 and Z2 as predicted, and the highest biomasses were found in spring and summer (116.08 × 10^4 cells L^{-1} and 160.69 × 10^4 cells L^{-1}), respectively. Relative to the other zones, the biomass of Z3 was lower during most seasons (stratified averaged from 51.47 × 10^4 to 4.68 × 10^4 cells L^{-1}). Furthermore, Z4 also displayed lower phytoplankton biomass, except for a

**Table 2** | Ranges of the main ecological features for the four zones studied in the Shanxi Reservoir

<table>
<thead>
<tr>
<th>Reservoir region</th>
<th>Watercourse zone</th>
<th>Riverine zone</th>
<th>Transition zone</th>
<th>Lacustrine zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO (mg L^{-1})</td>
<td>3.52–7.60</td>
<td>3.15–8.04</td>
<td>2.96–7.74</td>
<td>2.38–7.28</td>
</tr>
<tr>
<td>pH</td>
<td>6.61–11.67</td>
<td>6.11–11.70</td>
<td>6.61–11.53</td>
<td>6.11–11.27</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>168.00–420.00</td>
<td>171.50–431.00</td>
<td>207.50–448.00</td>
<td>216.00–432.00</td>
</tr>
<tr>
<td>Tur (NTU)</td>
<td>0.90–37.75</td>
<td>0.00–257.80</td>
<td>0.45–32.80</td>
<td>0.00–4.45</td>
</tr>
<tr>
<td>Chl-a (µg L^{-1})</td>
<td>0.87–20.48</td>
<td>0.64–8.99</td>
<td>0.90–10.00</td>
<td>1.27–39.56</td>
</tr>
<tr>
<td>EC (mS cm^{-1})</td>
<td>38.55–59.20</td>
<td>33.65–44.10</td>
<td>33.20–38.10</td>
<td>33.05–37.30</td>
</tr>
<tr>
<td>IMn (mg L^{-1})</td>
<td>0.02–18.50</td>
<td>0.91–2.13</td>
<td>0.72–2.09</td>
<td>1.07–2.07</td>
</tr>
<tr>
<td>TP (µg L^{-1})</td>
<td>16.24–224.60</td>
<td>4.08–270.95</td>
<td>1.95–175.57</td>
<td>12.18–201.51</td>
</tr>
</tbody>
</table>

Mean values and standard deviations (SD) are shown in the parentheses.
peak in winter \((9.46 \times 10^4 \text{ cells L}^{-1})\). All four zones generally exhibited a decrease throughout the year, with the highest value obtained from the middle layer of Z3 in spring and the lowest value obtained from the upper layer of Z2 in winter \((183.23 \times 10^4 \text{ and } 1.18 \times 10^4 \text{ cells L}^{-1}\), respectively). The seasonal variation in the Margalef index in each ecological zone was also evident. The phytoplankton diversity showed highly developed and complex structures in most of the zones from summer to autumn \((D > 6)\) but relatively simple structures from autumn to winter \((D < 6)\). The phytoplankton structure in Z3 was complex for most of the seasons; however, the index value was only 3.17 in winter. In contrast, Z4 mostly showed poor diversity, but the index value was 6.71 in autumn.

Altogether 72 species were identified and the differences in the proportions of the algal groups were also reflected among the zones (Figure 3). In Z2, Cyanophyta, Chlorophyta and Bacillariophyta were typical. The dominant groups of Z1 and Z3 were Cyanophyta and Bacillariophyta, while Chlorophyta and Bacillariophyta dominated Z4. Cyanophyta accounted for 41.01\% of the quarterly biomass.

Figure 2 | Seasonal variation in the total phytoplankton biomass (averaged for each stratification) and Margalef Index \((D)\) in each ecological zone during the study period.

Figure 3 | Seasonal variation in the algal groups (in relative biomass proportion) in the ecological zones during the study period: U (upper layer), M (middle layer), L (lower layer); Cyanophyta (Cyan), Chlorophyta (Chlo), Bacillariophyta (Baci), Euglenophyta (Eugl), Dinophyta (Dino); (a) summer, (b) autumn, (c) winter and (d) spring.
average in Z1. In contrast, the other zones were mainly dominated by Bacillariophyta, with a quarterly biomass average >50%.

A total of seven taxonomic divisions were registered during summer and autumn, decreasing to five in winter and increasing to six in spring. In Z1, cyanobacteria (Microcystis spp., Raphidiopsis sinensis) and several chlorophyceans (Chlamydomonas spp.), along with centric (Cyclorella sp.), planktonic (Asterionella sp.) and benthic diatoms (Achnanthes sp., Navicula sp.) were abundant throughout the year. In Z2, spring stratification was almost entirely dominated by the diatom Asterionella formosa. In the other seasons, cyanobacteria (Microcystis spp.), assorted diatoms (Achnanthes sp., Navicula sp. and Aulacoseira granulata) were codominant. In Z3, the community was dominated by Microcystis spp. (mainly Microcystis flos-aquae) and chlorophyceans (Chlamydomonas spp.), and although Euglena spp. were less numerous, they still contributed noticeably to the biomass. Z4 had a higher contribution of Microcystis spp., together with diatoms (mainly Rhizosolenia longiseta). Chlorophyceans (Treubaria triappendiculata and Scenedesmus spp.) were sporadically present in certain seasons (autumn and winter).

Altogether, 19 Reynolds FGs were recorded in the study area. Groups M, MP, P, C and T_B had the greatest effect on biomass in Z1. Group M (Microcystis spp.), was the most influential group in this zone throughout the year (Figure 4). Conversely, groups P (mainly Fragilaria spp., Closterium sp. and A. granulate) and T_B (Achnanthes sp., Nitzschia sp.) were only numerous during winter and spring, respectively. During summer and spring, Navicula spp. and Chlorococcum infusionum (group MP) dominated, with 15 and 20% of the biomass, respectively. Moreover, group C (Asterionella sp.) accounted for 15% of the biomass in autumn, increasing to 20% in winter. Z2 and Z3 were also characterized by group M during autumn stratification, with a mean proportion of approximately 50%; however, the biomass of these zones diminished in winter along with the diversity. This event was dominated by FG P and C, with the highest mean percentage (82.5%) of the phytoplankton population. During summer, groups S1 (Pseudanabaena sp.) and D (Synechla spp.) codominated Z3, although their amounts were lower in Z2. A similar phenomenon occurred in spring, when groups N (Tabellaria spp.) and B (small Cyclorella sp.) were abundant in Z3, while Z2 was controlled by groups MP and T_B. Group M served as a subdominant group in Z4, while the biomasses of groups N and C were relatively higher.

The phytoplankton species in the Shanxi Reservoir were grouped into 15 MFGs (Table 3 and Figure 4). In Z1, there were large vacuolated Chroococcales (group 5b) and pennates (group 6b), and the highest annual average of the total biomass (45%) was recorded. Unicellular chlorophytes (group 8a) were dominant throughout most of the seasons, except for spring, when small pennates (group 7b) became codominant with groups 5b and 6b. MFGs 5b, 6b and 7b were also observed in Z2 in spring, contributing 77% of the total biomass. However, the biomass of small and gelatinous colonies (groups 5d and 11b) in this zone was high during the summer period, which was not observed in the other zones. Thin filaments (mainly Oscillatoria sp.), which belong to group 5a, exhibited variance in Z3 in summer, contributing 26% of the total biomass. Group 6c (colonial pennates) showed the same phenomenon as group 5a and assembled in Z4, comprising 22% of the total biomass. As in the other zones, the biomass of large centrics (group 6a) was numerous in Z4 in winter. However, the unicellular chlorophytes (group 8a) declined in the other zones during spring stratification but increased to 33% in front of the dam.

The following MBFGs were found: I, III, IV, V, VI and VII (Table 3 and Figure 4). The most important contributors to the biomass were groups VI (Stephanodiscus sp., Asterionella sp. and Cyclotella sp.) and VII (Microcystis spp.). Group III represented by Raphidiopsis mediterranea contributed greatly to the total phytoplankton biomass in all seasons except for spring. The biomass of group I increased slightly in Z2 and Z4 during summer, mostly due to an increase in the biomass of chlorophyceans (Chlorella spp. and Chlorococcum sp.). Euglenophyceans, which are clustered into group V, dominated summer and spring in Z4, were rarely recorded in the other zones. In contrast, group IV (Pediastrum boryanum) was well represented in the other zones (except Z4) throughout the study period.

Redundancy and correlation analyses

All five dominant groups and eight environmental driving factors were subjected to RDA (Figure 5). From the species dataset, only the dominant groups: FG M, MP, P, C and N; MFG 5b, 6b, 7b, 6a and 8a; and MBFG III, IV, V, VI and VII were included in the analyses.

For the FGs, the RDA and Monte Carlo test showed that Tem (P = 0.002, F = 11.8), Chl-a (P = 0.006, F = 7.5), EC (P = 0.004, F = 3.8) and pH (P = 0.016, F = 3.8) contributed significantly to the phytoplankton distribution (Figure 5(a)). The first and second axes explained 55.6% of the total
variance, with eigenvalues of 0.4218 and 0.1022, respectively, and the Pearson correlation analysis showed a clear relationship between the environmental variables and species (0.8912 and 0.6413 for the first and second axes, respectively). The dominance of group N at every site could be explained by the occurrence of organic matter and high EC; however, this dominance was negatively associated with high phosphorus contents and pH values. Group MP was highly affected by temperature as well as group C, which also highly associated with Chl-a. Moreover,
Table 3 | Main MFGs, their biomass averaged over the stratifications (in relative proportion) and associated descriptors

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>FG</th>
<th>MFG</th>
<th>MBFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1 Summer</td>
<td>Microcystis spp./Chroococcos sp./Eunotia incisa</td>
<td>M/Lc/MP</td>
<td>5a/5b/6b</td>
</tr>
<tr>
<td>Autumn</td>
<td>Microcystis spp./Asterionella sp.</td>
<td>M/C</td>
<td>5b/6b/8a</td>
</tr>
<tr>
<td>Winter</td>
<td>Microcystis spp./Aulacoseira granulata/Asterionella sp.</td>
<td>M/P/C</td>
<td>5b/6a/8a</td>
</tr>
<tr>
<td>Spring</td>
<td>Microcystis spp./Navicula spp./Achnanthes spp.</td>
<td>M/MP/TP</td>
<td>5b/6b/7b</td>
</tr>
<tr>
<td>Z2 Summer</td>
<td>Microcystis spp./Staurodesmus spp.</td>
<td>M/N</td>
<td>5d/7b/11b</td>
</tr>
<tr>
<td>Autumn</td>
<td>Microcystis spp./Asterionella sp.</td>
<td>M/C</td>
<td>5b/6a/6b</td>
</tr>
<tr>
<td>Winter</td>
<td>Aulacoseira granulata/Asterionella sp.</td>
<td>P/C</td>
<td>5b/6a/8a</td>
</tr>
<tr>
<td>Spring</td>
<td>Microcystis spp./Navicula spp./Achnanthes spp.</td>
<td>M/MP/TP</td>
<td>5b/6b/7b</td>
</tr>
<tr>
<td>Z3 Summer</td>
<td>Navicula spp./Pseudanabaena sp./Synedra spp.</td>
<td>MP/S1/D</td>
<td>5a/6b/6c</td>
</tr>
<tr>
<td>Autumn</td>
<td>Microcystis spp./Aulacoseira granulata/Asterionella sp.</td>
<td>M/P/C</td>
<td>5b/6b/8a</td>
</tr>
<tr>
<td>Winter</td>
<td>Aulacoseira granulata/Asterionella sp.</td>
<td>P/C</td>
<td>6a</td>
</tr>
<tr>
<td>Spring</td>
<td>Microcystis spp./Cosmarium spp./Cyclotella spp.</td>
<td>M/N/B</td>
<td>5b/6a/8a</td>
</tr>
<tr>
<td>Z4 Summer</td>
<td>Cocconeis sp./Synedra spp.</td>
<td>MP/D</td>
<td>5b/6c/7b</td>
</tr>
<tr>
<td>Autumn</td>
<td>Microcystis spp./Aulacoseira granulata/Cymbella spp.</td>
<td>M/P/MP</td>
<td>6a/6b/8a</td>
</tr>
<tr>
<td>Winter</td>
<td>Aulacoseira granulata/Asterionella sp.</td>
<td>P/C</td>
<td>6a</td>
</tr>
<tr>
<td>Spring</td>
<td>Microcystis spp./Cosmarium spp./Cyclotella spp.</td>
<td>M/N/B</td>
<td>5b/6a/8a</td>
</tr>
</tbody>
</table>

Figure 5 | Biplot of the RDA based on the Shanxi Reservoir MFG biomasses as determined by methods proposed by (a) Reynolds et al. (2002), (b) Salmaso & Padsák (2007) and (c) Kruk et al. (2010).
Groups M and P were negatively associated with TP and pH. However, the influence of \( I_{\text{Mo}} \) and Tur was less evident.

Regarding the MFGs, Tem \((P = 0.002, F = 8.6)\), pH \((P = 0.004, F = 7.4)\), EC \((P = 0.002, F = 7.1)\), TP \((P = 0.002, F = 6.6)\), Chl-\(a\) \((P = 0.004, F = 5.5)\) and ORP \((P = 0.014, F = 4.2)\) contributed the most to biomass (Figure 5(b)). In this case, 56.5% of the total variance was explained by the first and second axes, with eigenvalues of 0.3970 and 0.1212, respectively, and the Pearson correlation analysis showed a clear relationship between the environmental variables and species (0.8282 and 0.7311 for the first and second axes, respectively). High DO and organic compounds inhibited the growth of group 6a. The dominance of groups 7b, 5b, 6b and 8a was positively associated with EC, Chl-\(a\) and Tem, but negatively correlated with TP and pH.

According to the RDA carried out with the significant MBFGs and environmental factors (Figure 5(c)), Tem \((P = 0.002, F = 16.4)\), pH \((P = 0.004, F = 5.6)\) and EC \((P = 0.036, F = 3.4)\) contributed the most. Altogether, 59.9% of the total variance was explained by the first and second axes, with eigenvalues of 0.5176 and 0.0567, respectively. The correlation analysis between the matrix of environmental factors and MBFG (0.8961 and 0.5778, respectively) was also significant. Group V was positively associated with Tem but negatively affected by pH and TP. EC contributed the most to groups VII and VI, while the effect of Tem was less significant but also considerable. In contrast, groups IV and III were closely correlated with Chl-\(a\), while they were less affected by temperature and EC and had a low tolerance to alkalinity.

**DISCUSSION**

Due to high water level fluctuations, truncated water flow, mixing regimes and other distinctions, the aquatic ecosystems of reservoirs are different from those of natural water bodies (Becker et al. 2009; Yang et al. 2018), and therefore are meaningful models for investigating plankton communities. There are several examples using the functional group approaches to find the influential environmental factors in reservoir ecosystems. For example, a comparison of taxonomic and Reynolds functional groups conducted in the Paso de las Piedras Reservoir in Argentina showed that the latter approach produced more specific results, and certain FGs have their own optima growth temperature and phosphorus uptake (Di Maggio et al. 2016). The community in a pennate diatom-dominated freshwater reservoir in the Czech Republic (Znachor et al. 2020) is generally controlled by nutrient input and water regime according to the MFG approach. Varol (2019) stated that in a monomictic reservoir, Reynolds functional groups F and T had narrower tolerance ranges for water temperature, pH and silicon dioxide, while groups G and T had narrower tolerance ranges for TP and nitrate nitrogen. For reservoirs situated in different regions, the effects of environmental driving factors on phytoplankton community structures are diverse when different approaches are applied. In this sense, the aim of our research was to evaluate the strength of different morphological and functional classification approaches to discriminate the effects of environmental driving factors on phytoplankton in a reservoir ecosystem.

In the Shaxi Reservoir, the most prevalent dominant taxonomic groups are cyanophytes and bacillariophytes (Figure 3). *Microcystis* spp., which are tolerant to relatively low nutrient availability (Reynolds 1995), were abundant throughout most of the year (except in winter). The composition of these species varied with depth, with large colonies dominating the water surface (Zhu et al. 2015). Meanwhile, another cyanobacterium, *M. flos-aquae*, has been frequently discovered in eutrophic to hypertrophic waterbodies (Padišák et al. 2009). Based on former studies, the determining factors of *M. flos-aquae* succession are temperature (Imai et al. 2008), N:P ratio and relatively high iron concentrations (Nalewajko & Murphy 2001), while our findings in the Shaxi Reservoir confirmed that water temperature and electric conductivity have strong effects on the abundance of *M. flos-aquae*. The cyanotoxin produced by *Microcystis* spp. may result in the oppression of growth and photosynthesis of some phytoplankton species (Perron et al. 2012) and diminish the diversity of diatom communities (Aboal et al. 2002, 2005). As a general indicator of eutrophic conditions (Reynolds 1998), *Asterionella* sp. was also affected considerably by some abiotic factors, such as water temperature and phosphorus content (Reynolds 1984). After the collapse of the *Microcystis* spp. bloom from autumn to winter, *Asterionella* sp., along with the large, unicellular *A. granulata*, became codominant, accounting for 70% of the total biomass in autumn, when an increase in ORP (from 218.19 to 235.39 mV) and inconspicuous stratification were recorded. *A. granulata* was shown to be associated with well-mixed and moderately eutrophic waters and has often been recorded in large, turbid water bodies; thus, it is an indicator of a mixed regime (O’Farrell et al. 2001; Goldenberg & Lehman 2012; Whitmore et al. 2018). The thermal stratification phenomenon appeared again in spring, promoting a diatom-dominated assemblage (Fedotov et al. 2012) consisting of *Navicula* spp. concomitant with
Cylcotella spp. and Achnanthes spp. Navicula spp. are typically found in inorganically turbid shallow lakes (Padisák et al. 2009) and can use low phosphate concentrations for growth as efficiently as other planktonic diatoms (Admiraal 1977). Cylcotella spp., the CR strategist species characteristic of mesotrophic water columns (Reynolds et al. 2002; Padisák et al. 2009), is sensitive to the onset of stratification, which fits the features of spring in the Shanxi Reservoir.

The spatial changes in phytoplankton community structure are also very obvious, and these changes are reflected in both the vertical and longitudinal directions. As one of the dominant groups during the spring period, Cosmarium spp. showed different patterns in different ecological zones. The layer-averaged biomasses of Cosmarium spp. in the transition and lacustrine zones were significantly higher than those in the other zones. In addition, the majority of the Cosmarium cells were found in the upper section (1–7 m) which is subject to numerous exchanges of substances and energy from the atmosphere and solar radiation (Agha et al. 2016). Concurrently, Achnanthes spp. showed a pattern similar to that of Cosmarium spp. in the vertical direction. However, Achnanthes spp. are mainly present in highly lotic environments (Padisák et al. 2009), which explained the fact that they appeared in large numbers in the channel area but were rarely found after the tributaries converged. The zone which is characterized by continuous or semi-continuous mixing (Z3) favoured A. granulata based on our investigation. Due to its unique cellular structure, A. granulata can easily sink to the lower water layer; however, it is well adapted for growth in rapidly flowing and turbid channels (O’Farrell 1994; Guenther & Bozelli 2004; Reid & Ogden 2009).

Throughout the year, the main FG of the Shanxi Reservoir was group M (Table 3; Figure 4). However, the dominance of codon M in different ecological zones varies with seasonal successions. The species of group M (Microcystis spp.) were often recorded in the watercourse and riverine zones. Microcystis spp., which were characterized by their preference for stable water bodies, would theoretically not follow the pattern found in the Shanxi Reservoir (Chu et al. 2007; Padisák et al. 2009). This difference might be caused by the large amount of organic matter in the upper reaches, which promoted the growth and reproduction of the cyanobacteria, while the buoyant cyanobacteria drifted along the flow, with their population gradually decreasing in the process (Moreno-Ostos et al. 2008). The species of group P were found to be subdominant in most of the zones in winter, which was confirmed by the fact that these species had a negative correlation with water temperature (Varol 2019). However, as one of the major species of group P, A. granulata was also found in the transition and lacustrine zones during autumn when the mixing criterion was satisfied and the surface water temperature was close to its optimum growth temperature of 25 °C (Tsuchida et al. 2006). The centric diatom Asterionella sp., which is representative of group C, also accounts for a large proportion of the total phytoplankton abundance in autumn and winter. However, during autumn, the dominance of group C was less apparent and limited to the lower latitudes. In contrast, two groups were found in the stratification periods (spring and summer): group MP, which includes littoral diatoms (Navicula spp.) that drift as plankton (Padisák et al. 2006) as a consequence of a high flushing rate in the epilimnion, and group N (Cosmarium spp.), which is usually related to mesotrophic epilimnia (Reynolds et al. 2002) and was present in the lower layer of the Z4 but had no advantage in the channel.

The MFG classification proposed by Salmoso & Padisák (2007) offered another approach (Table 3; Figure 4). In this case, the standards selected for distinguishing the groups include the traits proposed by Weithoff (2003), which can be estimated from descriptions of phytoplankton functional behaviours. The most significant groups were 5b, 6b, 7b, 6a and 8a. However, the temporal differences in the dominant groups were significant among all the associations. Group 5a (thin filaments) was abundant during all the seasons except winter, while group 6a (large centric diatoms) showed an overwhelming advantage in winter but declined in spring and was rarely found in summer. Groups 6b (large pennates) and 7b (small pennates) showed synchronized appearances at the time of water temperature stratification. However, in autumn, although the species from group 6b were abundant, those from group 7b were rarely recorded. The spatial differences in the morpho-functional approach were apparent. The lacustrine zone contained fewer algae from associations 5b (large vacuolated Chroococcales) and 7b (small pennates), mainly in the lower latitudes. In contrast, the algae from association 6a (large centric diatoms) were mostly recorded in the lacustrine zone from winter to spring. Association 8a was found to be a dominant group in the transition and lacustrine zones during autumn and spring but was often detected in the watercourse zone in winter. The results of the statistical analyses indicated that four prevalent groups were associated with relatively high EC values. Only group 6a showed a positive correlation with relatively high ORP, but a significant negative correlation with DO. The quantity of all the groups appeared highly correlated with pH and TP and...
slightly correlated with Tem and Chl-α, with four of the groups having positive correlations and group 6a having a negative correlation.

Because of the lower total variation in the data set, the percentage of explained variance obtained using the MBFG approach was higher than for the others. This phenomenon was reflected in many former studies, causing this approach to be considered as low-sensitive and inappropriate for detecting certain phytoplankton functional aspects. Nevertheless, this classification is still considered to be useful for ecosystem monitoring because of its objectivity, less relation to taxonomy, and easy identification (Izaguirre et al. 2012). In our study, the MBFGs also showed prominent seasonal characteristics of the phytoplankton community, especially in winter when the single population structure was more evident (Table 3; Figure 4). Furthermore, group VI was the most representative MBFG from winter to spring, and this trend was reflected in all the zones. However, although the abundance of group VII (large mucilaginous phytoplankton with low surface/volume colonies) was extraordinarily high in autumn, it declined in winter along with group V (medium to large size flagellates). The spatial differences are as follows: from summer to autumn, group III was not dominant in the riverine zone, group VII mainly dominated the watercourse and riverine zones in winter, and group I showed dominance during the summer period but was only abundant in the upper latitudes. Similar to that observed with the MFG classification, in the statistical analysis based on MBFG (Kruk et al. 2010), the forward selection procedure also indicated that Tem, pH and EC were significant in the ordering obtained successively; however, using the FG classification, EC was more important than pH. The statistical analysis also showed separations among the groups. Groups VII and VI were positively and negatively highly related to EC and pH, respectively. However, group V showed a highly positive relationship with water temperature but was less associated with EC and pH. Group III was highly related to Chl-α and slightly associated with EC and pH.

**CONCLUSION**

In the Shanxi Reservoir, the phenomenon of thermal stratification was more apparent in spring and summer and less evident in the other seasons based on our research. However, water temperature still appeared to be an essential driving factor according to the multivariate analyses. The vertical distribution of physiochemical indicators, such as TP, EC and Chl-α, as well as the phytoplankton structure showed different trends among the ecological zones of the Shanxi Reservoir.

In general, the FG, MFG and MBFG classifications constitute good approaches for analysing the differences between the ecological zones of the Shanxi Reservoir, and they can be valuable in environmental biomonitoring. The FG classification can reflect the relationship between environmental factors and phytoplankton structures; however, it requires comprehensive knowledge of phytoplankton identification. The MFG classification is helpful for explaining the phytoplankton–environment relationship and identifying driving factors, but the classification criteria for individuals of extreme sizes are not clear enough. The MBFG classification is the simplest and most efficient method and was found to be applicable by multivariate analyses in our study. It makes up for the complex, time-consuming and challenging disadvantages of the FG approach, and it showed a higher sensitivity to water temperature, which is useful for identifying seasonal variances. Although the MBFG classification does not explain the environment sufficiently, it still exhibited objectivity and efficiency, and is therefore suitable for long-term monitoring of aquatic ecosystems in reservoirs.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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