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Research Article

Phytoplankton functional groups response to environmental parameters in Muling River basin of northeast China

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Abstract – The present study was carried out in the biggest tributary of Ussuri River of boundary between China and Russia. The Muling River basin has undergone a long-term dredging works, and waterbody became seriously turbid. The succession of phytoplankton functional groups succession and environmental factors in the river were sampled in 2015. We totally identified 83 species, belonging to 17 functional groups which 5 were predominant, including group F, M, MP, P and Y. The seasonal succession of phytoplankton functional groups was M/P-F/MP/P-MP/P. Results of Spearman correlation analysis and canonical correspondence analysis (CCA) revealed that phytoplankton functional groups were mainly influenced by nutrient concentrations and light availability including total nitrogen (TN), ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), N:P ratio (N:P), water depth (D) and transparency (SD) in the basin.

Keywords: Phytoplankton / functional groups / environmental parameters / canonical correspondence analysis

1 Introduction

Globally, most of aquatic ecosystems including lakes, rivers, reservoirs and freshwater and marine wetlands have undergone changes as a result of human-induced disturbances from land-use activities centered on human settlements, agriculture and industrial activities (Tockner *et al.*, 2010; Shen *et al.*, 2014). For example, in 1981 Tuanjie reservoir was built in Muling River with the purposes of controlling flood, generating electricity and irrigation activities. However, with the increasing population and demand of water for human consumption and hydro power, another reservoir was constructed in 2016 along the Muling River. The damming has been associated with degradation of water resources and reduction of diversity of aquatic communities and change phytoplankton species assemblages substantially (Burford and O'Donohue, 2006). Phytoplankton is among the most important primary producer that forms the vital energy source at the first trophic tier. As they also serve as food to many aquatic animals, they also have an important role in the material circulation in aquatic ecosystems by controlling the growth, reproductive capacity and population characteristics of aquatic biota. Moreover, phytoplankton communities are essential monitoring tool in water resources management because their spatiotemporal variations are closely related to water physicochemical factors (Wu *et al.*, 2013). Changes in physicochemical variables in the aquatic system can directly affect phytoplankton community structure (Ptacnik *et al.*, 2008; Zhu *et al.*, 2013). As a result, scientist have used phytoplankton community composition to study and understand aquatic ecosystems (Becker *et al.*, 2009).

Conventionally, plankton composition has been studied considering changes in biomass of major taxonomic classes. However according to (Reynolds 1998), this traditional classification does not to reflect the actual ecological functioning of an ecosystem since one taxonomic class represented by species with different structural and functional characteristics on life strategies is not sufficient to describe ecological functions. Phytoplankton classification system

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based on its morphological, physiological, and ecological characteristics and has attracted increasing attention in the recent years (Xiao *et al.*, 2011; Zhu *et al.*, 2013). Currently, 39 phytoplankton functional groups have been identified (Reynolds *et al.*, 2002; Padisák *et al.*, 2009). The concept of using phytoplankton functional groups has proved to be reliable approach to analyze spatial and temporal variations in phytoplankton community composition and understanding environmental condition of a given ecosystem (Cao *et al.* 2018). Recently, Ma *et al.* (2019) used phytoplankton functional groups in the assessment of environmental status of the lake in reveal the importance of physical–chemical variables in the spatial and temporal gradient. Varol (2019) studied trophic state of Batman Dam Reservoir by phytoplankton functional groups habitat preferences and diversity indices.

This work aimed to reveal the relationship between phytoplankton functional groups and physicochemical variables in Muling River basin using Spearman correlation analysis and canonical correspondence analysis.

2 Materials and methods

2.1 Study area

Muling River is the fifth-largest river in Heilongjiang Province, and it is also the main feeding river to the Ussuri River which is the boundary river of China and Russia (Fig. 1). It covers a surface of 18,427 km^2, with a length of 834 km, and its velocity ranges from 0.2 to 4360 m^3/s. S1–S19 located in the upstream, S20–S23 located in the midstream and S24–S28 located in the downstream.

2.2 Sampling procedure and laboratory analysis

We collected all samples in spring (May), summer (July) and autumn (September) periods from 28 sampling sites in 2015 (Fig. 1). Water transparency (SD) and water depth (D) were measured in the field using a Secchi disk and graduated portable staff gauge, respectively. Electric conductivity (EC), dissolved oxygen (DO), pH and water temperature (T) also measured in the field using a portable multi-probe (YSI 6600, YSI Inc., USA). We used the Chinese standard methods proposed by Ministry of Environmental Protection of People's Republic of China (MEP, 2002) to determine the concentration of total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), chemical oxygen demand (COD_{Mn}).

Three randomly replicate 1L phytoplankton samples were collected by using plankton net of 64 µm mesh from subsurface (0.5 m depth), and then fixed in the labelled bottle with Lugol's solution immediately. In the laboratory, the phytoplankton samples have undergone sedimentation during 48 hours and concentrated to 30 mL, and identified and counted them using an inverted microscope at 400× magnification in referring the identification key of Hu and Wei (2006). The species were classified to functional groups (FGs) according to Reynolds *et al.* (2002), and Padisák *et al.* (2009). All the phytoplankton taxa were framed as much as possible. Bio volume (mm^3/L) was estimated according to the solid geometric shape, and cell volumes of at least 40 algal units were estimated by approximation to the nearest solid geometric solid. Conversion of bio volume into biomass was done as $1mm^3/L=1mg/L$ (Ma *et al.*, 2019).

2.3 Statistical analysis

Statistical analysis was carried out using the SPSS 19.0 software and data were transformed to manage variance heterogeneity. Variation of environmental parameters and biomass of functional groups were analyzed using One-way ANOVA, followed by Tukey's honesty significant difference (HSD) test and Spearman correlation analysis was carried out to confirm the significances. We considered significant difference at $p < 0.05$ to high significant difference at $p < 0.01$ level. Relationships between functional groups and physicochemical parameters were analyzed using the detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) in CANOCO 4.5 software (Microcomputer Power). Monte Carlo simulations with 499 permutations were used to test the significance of the physicochemical variables in explaining the biomass of phytoplankton FG's data in the CCA.

3 Results

3.1 Environmental factors characteristics

The mean values of environmental factors are presented in Table 1, and most of the factors varied significantly. SD was remarkable higher in September and observed the highest mean values at S4 and S5 (Tab. 1; Fig. 2). Although relatively high values of D were observed in July, analysis of variances revealed no significant difference ($p > 0.05$) (Tab. 1). As one of the important physicochemical variable, EC differed during the study periods with significant value in September. In the most of the sites, dissolved oxygen (DO) differed significantly ($p < 0.05$). In July recorded the highest DO values followed by September. The results of pH values showed that the water of Muling River basin is alkaline in nature. Water temperature depicted a significant temporal variation with higher significant mean value reported in summer. The mean values of TP were the highest in July followed by May and September (Fig. 2g).

The N:P ratio presented a widely fluctuation from 0.44 to 23.95, and the seasonal mean values was increased more than 5 times. Tukey HSD test revealed that the mean N:P ratio in July was differences (Tab. 1). The concentration of ammonium nitrogen (NH_4^+–N) and nitrate nitrogen (NO_3^-–N) show both highly significant differences ($p < 0.01$). The top NH_4^+–N value was observed in July (Tab. 1).

3.2 Phytoplankton functional groups characteristics

A total of phytoplankton species were identified belonging to 43 genera and 7 phylum/class including Bacillariophyta (53.01%), Cryptophyta (2.41%), Chlorophyta (27.71%), Cyanophyceae (8.43%), Chrysophyceae (1.2%), Euglenophyceae (6.02%) and Miozoa (1.2%). And then classified into 17 functional groups namely: C, D, F, H1, J, L0, M, MP, N, P, S1,

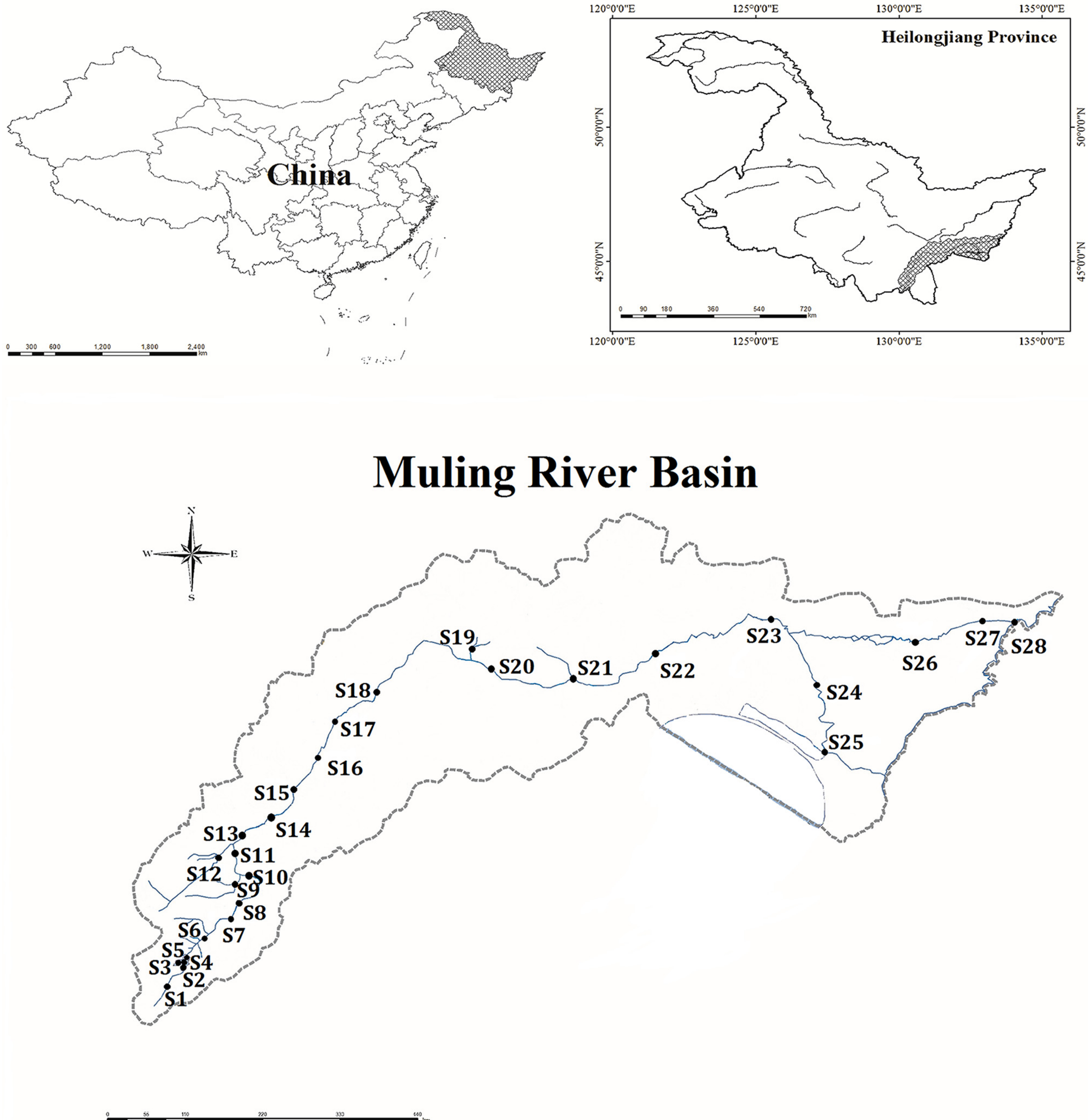

Fig. 1. Sampling sites locations in Muling River basin. S1~S19 located in the upstream, S20~S23 located in the midstream and S24~S28 located in the downstream.

W1, W2, X1, X2, X3, Y (Tab. 2). The predominant functional groups with more than 10% of total biomass were M, P, MP, Y and F which were the main contributors of biomass (Zhu *et al.*, 2013).

The biomass of phytoplankton functional groups was significantly differences among sampling sites and between seasons ($p < 0.01$) (Tab. 1). The dominant seasonal succession of functional groups was M/P-F/MP/P-MP/P. In spring, group P mainly composed by *Fragilaria breuisriata* Grunow, *Fragilaria virescen* Ralfs, *Fragilaria capucina* Desmaziéres, *Fragilaria capucina var. mesolepta* Rabenhorst, *Melosira granulate* Ralfs, *Melosira granulata var. angustissima* O. Müller and group M presented by *Microcystis robusta* Nygaard, *Microcystis incerta* Lemm were the important contributors (85.65% of total biomass). In summer, group F (*Westella botryoides* Wildeman, *Westellopsis linearis* Jao), MP (*Cymbella ventricosa* Kützing, *Cymbella perpusilla* Cleve, *Gomphonema constrictum* Ehrenberg) and P (*Fragilaria breuisriata* Grunow, *Fragilaria virescen* Ralfs, *Fragilaria capucina* Desmaziéres) were the important contributors with

Table 1. The seasonal variations of environmental factors and phytoplankton functional groups abundance and biomass. p values were from One-way ANOVA test. Differences between the seasons were tested by Tukey HSD ANOVA. All data were mean ± SE ($n=83$). Water transparency (SD), water depth (D), electric conductivity (EC), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N) and chemical oxygen demand (COD_{Mn}).

	May (Spring)	July (Summer)	September (Autumn)	p
SD (m)	0.35 ± 0.04^{a}	$0.32 \pm 0.07^{a,b}$	$0.48 \pm 0.07^{b,c}$	0.004^{**}
D (m)	1.86 ± 0.81^{a}	2.27 ± 1.09^{a}	2.17 ± 0.99^{a}	0.544
EC (ms/cm)	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.21 ± 0.02^{b}	0.008^{**}
DO (mg/L)	7.45 ± 0.29^{a}	$8.73 \pm 0.29^{a,b}$	$7.49 \pm 0.56^{b,c}$	0.024^{*}
pH	7.42 ± 0.12^{a}	7.03 ± 0.26^{b}	7.99 ± 0.06^{c}	0.001^{**}
T (°C)	14.81 ± 0.47^{a}	22.26 ± 0.55^{b}	6.89 ± 0.43^{c}	0.000^{**}
TN (mg/L)	1.73 ± 0.14^{a}	1.99 ± 0.21^{a}	1.62 ± 0.16^{a}	0.300
TP (mg/L)	0.6 ± 0.05^{a}	0.69 ± 0.04^{a}	0.36 ± 0.03^{b}	0.000^{**}
N:P	3.86 ± 0.56^{a}	$3.13 \pm 0.38^{a,b}$	$6.56 \pm 1.60^{a,c}$	0.019^{*}
NH_4^+–N (mg/L)	0.22 ± 0.01^{a}	0.35 ± 0.04^{b}	0.13 ± 0.01^{c}	0.000^{**}
NO_3^-–N (mg/L)	0.58 ± 0.07^{a}	1.52 ± 0.50^{a}	0.28 ± 0.03^{b}	0.000^{**}
COD_{Mn} (mg/L)	3.8 ± 0.13^{a}	3.98 ± 0.10^{a}	4.06 ± 0.12^{a}	0.281
Abundance ($^{*}10^{6}$cell/L)	2.34 ± 0.54^{a}	1.21 ± 0.37^{a}	1.29 ± 0.23^{a}	0.016^{*}
Biomass (mg/L)	3.83 ± 1.09^{a}	0.83 ± 0.23^{a}	1.29 ± 0.26^{a}	0.001^{**}

$^{*}p < 0.05$.
$^{**}p < 0.01$.

about 71.83% of total biomass. While in autumn (September) groups MP and P mainly composed by *Navicula radiosa* Kützing, *Meridion cirsulare* Agardh; *Fragilaria capucina* Desmaziéres, *Melosira granulata* Ralfs contributed about 71.65% of the total biomass (Fig. 3).

3.3 Spearman correlation analysis

Table 3 presents the results of correlation analysis between predominant biomass of phytoplankton functional groups and environmental factors. In 2015, the biomass of functional groups F was positively correlated with SD ($p < 0.01$), D ($p < 0.01$) and T ($p < 0.01$) and negatively related with pH ($p < 0.01$). Group M was negatively correlated with EC, pH, TN, N:P and NO_3^-–N, while positively with SD ($p < 0.01$) and D ($p < 0.01$). It was observed that, group M was positively correlated with SD and D ($p < 0.01$). Group MP composed by *Cymbella* sp., *Gomphonema* sp., *Navivuls* sp. and *Attheya* sp. was positively related with pH and TN. Group P was only positively correlated with SD ($p < 0.01$) and negatively related with EC ($p < 0.01$), TN ($p < 0.01$) and NH_4^+-N. Group Y was positively correlated with SD ($p < 0.01$), D ($p < 0.01$) and N:P and negatively related with NO_3^-–N.

3.4 Canonical correspondence analysis

Detrended correspondence analysis (DCA) results showed that the maximum gradient length was 3.234. Figure 4 showing the CCA results relationship between biomass of phytoplankton functional groups and environmental parameters. The results of Monte Carlo test revealed that the first canonical axis and all canonical axes were significantly different ($F=12.763$, $p=0.004$; $F=3.029$, $p=0.002$). The first two axes account for 63.1% of relation of functional groups-environment variables (axis 1: 45.0%, axis 2: 18.1%) and 24.8% of the functional groups variables (axis 1: 15.2%, axis 2: 9.6%). Axis 1 was mainly positive correlated with pH ($r=0.550$) and negatively correlated with SD ($r=-0.692$), D ($r=-0.675$) and T ($r=-0.611$). Axis 2 was positively correlated with TN ($r=0.422$), NH_4^+–N ($r=0.410$) and negatively correlated with D ($r=-0.455$), pH ($r=-0.323$).

4 Discussion

4.1 Seasonal succession of phytoplankton functional groups

Previous studies, the use of biomass of phytoplankton functional groups has proved to be suitable method to explain the dynamic of the phytoplankton community structure in aquatic systems (Li *et al.*, 2018). In this current study, the biomass total of phytoplankton functional groups varied significantly among sites and seasons. The seasonal and spatial variation of phytoplankton functional groups reflected changes of physicochemical factors and the succession of the different functional groups, which determined phytoplankton community structure. During the study period, the biomass of the functional groups was mainly dominated by group M and P accounting about 42.23% and 31.79% of the total biomass respectively.

Sampling sites (S1, S2, S3, S4 and S5) were located in the Tuanjie reservoir which is the headwater areas of Muling River. In this reservoir, three parts areas can be distinguished including riverine, transitional and lacustrine (Kimmel and Groeger, 1984). In spring, group M and P were dominant while in summer group C was dominant. In addition, we observed that the algae bloom which dominate by group M and P (in spring) and group C (in summer) respectively, including

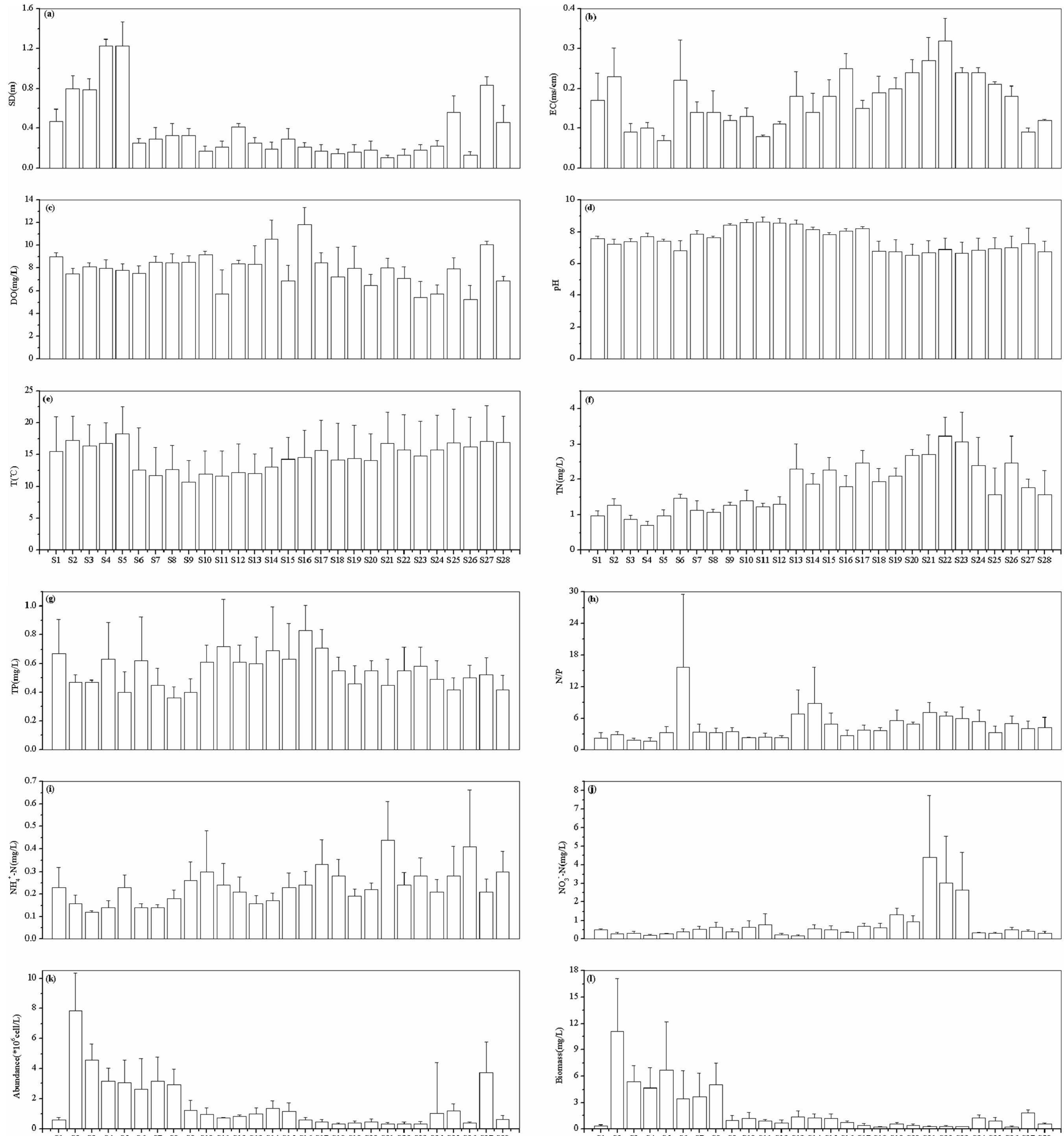

Fig. 2. The seasonal variations of environmental factors and phytoplankton functional groups abundance and biomass among sampling sites. Water transparency (SD), water depth (D), electric conductivity (EC), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^-–N) and chemical oxygen demand (COD_{Mn}).

Glenodinium (GALD < 50 µm) and *Cyclotella* (GALD < 10 µm). Similar results were reported in Liuxihe reservoir by Xiao *et al.* (2011). This proves that in Tuanjie reservoir environmental factors are more vital to phytoplankton multiply.

4.2 Driving factors of phytoplankton functional groups

In order to cope with environmental change, phytoplankton has undergone morphology adaptations as a survival strategy

Table 2. Phytoplankton functional groups (FGs) and relative biomass (%) in Muling River basin.

FGs	Genera	Biomass (%)
C	*Asterionella, Cyclotella*	3.72
D	*Synedra*	3.99
F	*Quadrigula, Westella, Westellopsis, Selenastrum, Closterium*	2.01
H1	*Anabaena*	1.05
J	*Chodatella, Scenedesmus, Crucigenia, Tetraedron, Pediastrum, Tetrastrum*	0.59
L0	*Chroococcus, Amphora, Gyrosigma*	0.23
M	*Microcystis*	7.65
MP	*Cymbella, Gomphonema, Navicula, Attheya, Diatoma, Surirella, Meridion, Cocconeis, Ulothrix*	9.67
N	*Cosmarium*	0.01
P	*Fragilaria, Melosira, Stauroneis, Tabellaria*	64.92
S1	*Phormidium*	0.02
W1	*Euglena, Eutreptia, Phacus*	0.01
W2	*Trachelomonas*	0.10
X1	*Ankistrodesmus*	0.13
X2	*Chlamydomonas*	1.45
X3	*Chromulina*	0.02
Y	*Cryptomonas, Glenodinium*	4.44

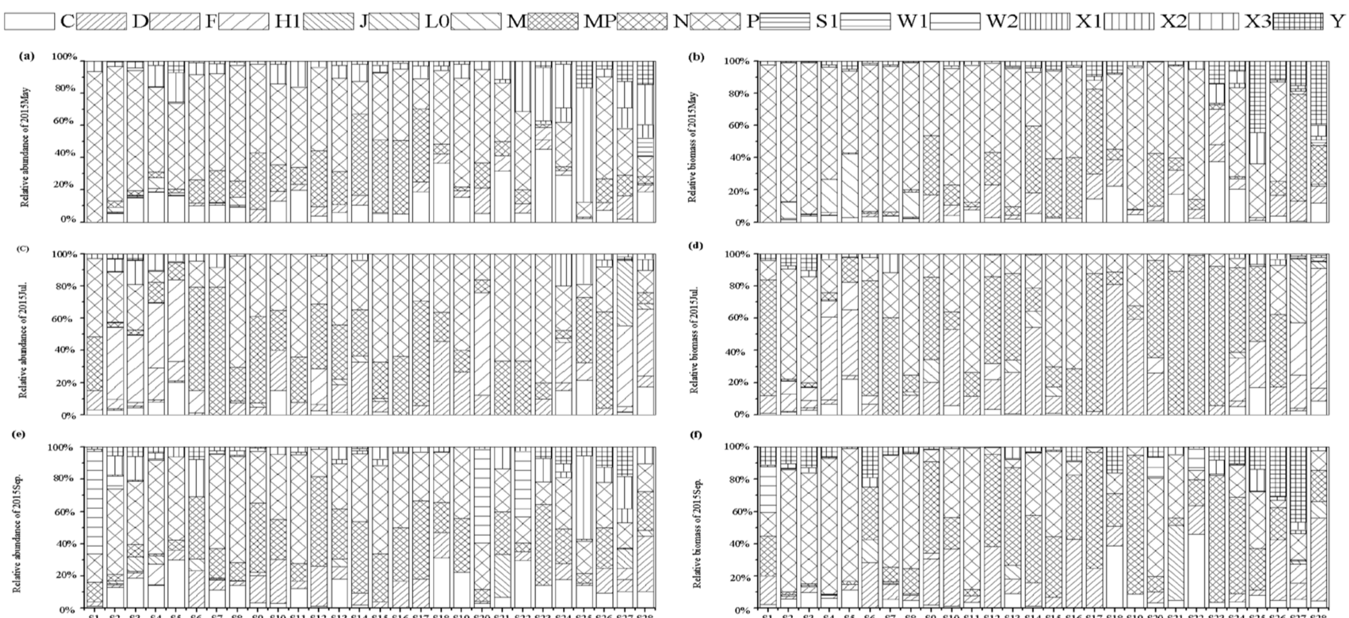

Fig. 3. Relative abundance and biomass of 17 Phytoplankton functional groups among sampling sites of three seasons. Upper letters of C, D, F, H1, J, L0, M, MP, N, P, S1, W1, W2, X1, X2, X3 and Y meaning 17 phytoplankton functional groups respectively. S1~S28 meaning sampling sites in the Muling River basin.

(Reynolds, 2007; Irwin *et al.*, 2006). According to Tilman *et al.* (1982), the main driving factors of dynamic phytoplankton community structure are light and nutrient. Geographically, the Muling River is located in northeast China and the light intensity varies with seasons (Yu *et al.*, 2012). We investigated 17 phytoplankton functional groups according to Padisák *et al.* (2009), and the number was more than that collected in Mudanjiang River (11 FGs) which located in the same province (Yu *et al.*, 2012). Yu *et al.* (2012) also reported that, the distribution of phytoplankton functional groups was significantly affected by tourism. The finding of Tian *et al.* (2015) revealed that phytoplankton community structure was influenced by hydrologic regime, and the water flushed bare riparian zone constantly in Hongze Lake, China. Hindering the light availability could be one of the main factors influencing growth of phytoplankton (Reynolds, 1998). Furthermore, the nutrient could affect the phytoplankton functional groups in the Muling River basin. We observed that nutrient

Table 3. Spearman correlations of predominant phytoplankton functional groups and environmental parameters. Water transparency (SD), water depth (D), electric conductivity (EC), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH_4^+–N) and nitrate nitrogen (NO_3^-–N).

	F	M	MP	P	Y
SD	0.409**	0.242*		0.294**	0.481**
D	0.497**	0.302**			0.345**
EC				−0.281**	
pH	−0.316**		0.223*		
T	0.306**				0.238*
TN				−0.418**	−0.327**
TP			0.226*		
N:P		−0.294**			0.232*
NH_4^+–N				−0.216*	
NO_3^-–N					−0.244*

**$P < 0.01$.
**$P < 0.05$.

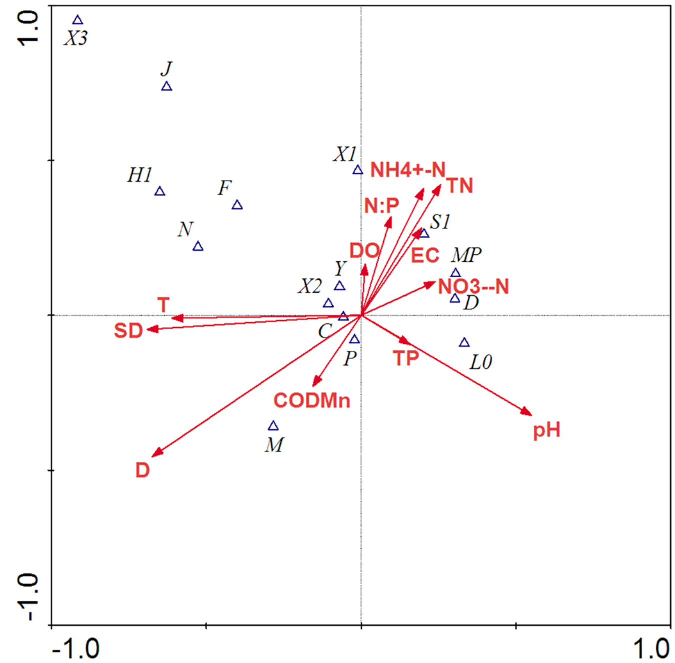

Fig. 4. CCA plot of relationships between biomass of phytoplankton functional groups and environment parameters. Water transparency (SD), water depth (D), electric conductivity (EC), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^-–N) and chemical oxygen demand (COD_{Mn}).

concentration were higher than before (Fig. 2f, i and j). That could be the precipitation will bring external nutrients from the surrounding croplands into the river via the surface runoff lead to higher concentration. The same situations were also noticed in Yangtze River basin in the middle of China (Wang *et al.*, 2011; Zhu *et al.*, 2013). N:P ratio is also a crucial factor influencing phytoplankton growth and distribution (Lagus *et al.*, 2004). Group H1 was sampled in all seasons except in May. Actually, group L0 is sensitive to prolonged or deep mixing (Reynolds *et al.*, 2002). We observed that group M was only observed in spring.

The CCA plots showed that group W1 was correlated with NO_3^-–N and NH_4^+–N, and these results indicated that this group adapt to rich organic matter habitat (Reynolds *et al.*, 2002; Padisák *et al.*, 2009). Group Y can use flagella to arrest suitable light and nutrient for growth, reproduction and avoid predators so that can appeared in all seasons (Bovo-Scomparin and Train, 2008), such as *Cryptomonas* and *Glenodinium*. The Spearman correlation results show that they are relatively correlated with water depth, transparency and total nitrogen (Tab. 3). Consistent with the correlation, the CCA plot results revealed that they were well adapted to high water depth and nitrogen and low SD. Besides, the whole Muling River basin impacted by the dredging resulted in turbidity and high nutrient conditions, which leading to a high biomass of phytoplankton and dominance of diatoms, such as group MP.

5 Conclusion

In this current study, we identified a total of 83 phytoplankton species belonging to 17 functional groups, including groups C, D, F, H1, J, L0, M, MP, N, P, S1, W1, W2, X1, X2, X3 and Y. The predominant functional groups were M, P, MP, Y and F. The seasonal succession of phytoplankton functional groups was followed as M/P-F/MP/P-MP/P from spring to summer and autumn. The biomass of phytoplankton functional groups was significantly higher in spring and lower in summer. Water depth, transparency, pH and COD_{Mn} were identified as the major factors influencing phytoplankton functional groups in Muling River basin. Water depth and COD_{Mn} were positively related with the predominant functional group M and P, while negatively with predominant group MP. The pH negatively related with predominant group Y and F.

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