

# Insights into bioassessment of water quality status using functional units of protozoan periphytons in marine ecosystems

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## Research Article

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# Abstract

Protozoan periphytons with their ecological features are considered as a robust bioindicator for bioassessment of both environmental stress and anthropogenic impacts in aquatic ecosystems. Keeping in view the ability of protozoan fauna for discriminating water quality status, a 1-year baseline survey was conducted using glass slides as an artificial substratum in coastal waters of Yellow sea, northern China. Four sampling sites (A–D) were selected from a clean area to a polluted station, and samples were collected monthly at a depth of 1 m. Environmental variables such as salinity, chemical oxygen demand (COD), dissolved oxygen (DO), soluble reactive phosphates (SRP), ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) were measured synchronically to compare with biotic factors. From a total of 144 identified protozoan species, 53 functional units (FUs) were proposed based on four biological traits: feeding type, resource of food supply, body size and movement type. These FUs represented a clear variability in spatial distribution among four study sites. The relative abundances of the sessile colonial bacterivores (e.g., BOS5s) showed an increasing trend from sites A to D. Multivariate analysis revealed that the patterns of the protozoan FUs showed a significant variation among four sampling sites, and were driven by the increasing levels of nutrients (e.g.,  $\text{NH}_4\text{-N}$ ) and decreasing ranks of DO ( $P < 0.05$ ). The bacterivorous FUs (e.g., BOS5s and BIS3v) were significantly positively related to  $\text{NH}_4\text{-N}$ , while the vagile algivorous FUs (e.g., AIS8v, AOS3v, AOS6v) and sessile predators (e.g., RIS4s) were significantly positively correlated with transparency. These findings suggested that FUs of protozoan periphytons may be used as a useful bioindicators of water quality status in marine ecosystems.

## Introduction

Protozoa are the primary contributors in microbial food webs, and play a significant role to arbitrating the mutability of energy and matter from lower to higher trophic levels (Finlay and Esteban 1998; Eisenmann et al. 2001; Fischer et al., 2002; Weitere et al., 2003; Kathol et al., 2009; Xu et al., 2009a; Guo et al., 2019; Kazmi et al., 2020a). Otherwise, they have been engaged as an important sweeper to remove the organic pollutants and other harmful contaminants to improve the water quality status in aquatic ecosystems (Cairns et al., 1972; Cairns and Henebry, 1982; Corliss, 2002; Weitere et al., 2003; Xu et al., 2005; Jiang et al., 2007; Norf et al., 2007, 2009; Shi et al., 2012). Furthermore, the protozoan periphytons have widely been used as robust bioindicators for assessing environmental stress and anthropogenic impact on water quality status due to their rapid response towards environmental fluctuations, ease of sampling and observation (Coppellotti and Matarazzo, 2000; Ismael and Dorgham, 2003; Xu et al., 2014a; Risse-Buhl and Kusel, 2009; Xu et al., 2009a).

According to the specific feeding types protozoa have been classified into the four trophic-functional groups like; bacterivores (B), algivores (A), raptors (R) and non-selectives (N), respectively (Fenchel 1969; Pratt and Cairns 1985; Fernandez-Leborans and Fernandez-Fernandez, 2002; Parry, 2004; Jiang et al., 2013a; Wang and Xu, 2015; Abdullah Al et al., 2018; Sikder et al., 2019b). Similarly, body size spectrum as an internal function, have been proved a useful operative tool to summarize the functional dynamics of a

community (Sheldon et al., 1972; San martin et al., 2006; Kamenir et al., 2010; Jiang et al., 2012; Xu and Xu, 2016). Traditionally seven body size ranks; S1, 2–17  $\mu\text{m}$ ; S2, 22–27  $\mu\text{m}$ ; S3, 29–36  $\mu\text{m}$ ; S4, 37–49  $\mu\text{m}$ ; S5, 53–71  $\mu\text{m}$ ; S6, 84–92  $\mu\text{m}$ ; S7, 127–153  $\mu\text{m}$  have been used as robust bio indicator to summarize the environmental changes (Kerr and Deckie, 2001; Jiang et al., 2012; Wang et al., 2016a, 2016b; Xu et al., 2016a, 2016b; Xu and Xu, 2016; Zhao et al., 2016; Uroosa et al., 2020). Although, variety of approaches have been used previously based on the collecting and analyzing the protozoan communities (Railkin, 1998; Struder-Kypke, 1999; Kralj et al., 2006; Norf et al., 2007; Xu et al., 2009a). Whereas, recent investigations demonstrated the biodiversity measures more feasible and effective bio indicator in functional space compared with taxonomic resolutions due to low “signal to noise” ratios from functional redundancy (Zhong et al., 2017; Xu et al., 2018). However, there is no information available based on use of “Functional Units” of protozoan assemblages for the assessment of water quality status. Therefore, in this study by combining the previous discussed traditional traits following source of food supply (inside/outside the biofilm) (Jiang et al., 2013b; Zhong et al., 2017; Guo et al., 2019) modes of locomotion, planktonic (P), sessile (S) and vagile (V) a combine (collective of four traits) “Functional Units (FUs)” of protozoan periphytons were proposed to be a possible bioindicators of water quality status in marine environment.

For this purpose, a 1-year cycle baseline study was conducted in coastal waters of Yellow Sea, Northern China with following aims and objectives: (1) to examine the response of functional units patterns in different water quality status, (2) to demonstrate the effectiveness of the functional units for indication of water quality status, using biofilm-dwelling protozoa in marine ecosystem.

## Methods

### Study area and dataset collection

The study area was located in coastal waters of the Yellow Sea, northern China, four study sites were selected according to the gradient of pollution (Fig.1, A–D). Site A, a relatively clean area, situated in the Olympic Sailing Center area; site B, a slightly polluted area located near the mouth of Jiaozhou Bay; site C, a moderately polluted area with minor discharge from rivers in Jiaozhou Bay; and site D, a heavily polluted area in Jiaozhou Bay stressed mainly by organic pollutants and nutrients from the discharge of domestic sewage and industrial discharge via several rivers (Fig. 1).

Protozoan samples were collected every month over a 1-year cycle from August 2011 to July 2012, using microscopic glass slides as an artificial substratum at a depth of 1 m. A total of 960 glass slides (2.5×7.5cm) were used to collect the protozoan fauna at a depth of 1m below the water surface. Two PVC frames with 20 slides for each sampling site were collected at the exposure time of 14 days. Samples were transferred into Petri dishes containing *in situ* water and were processed within 2 hrs according to the methods by Xu et al. (2014a).

Identification and enumeration was performed under an inverted microscope with magnification of 10–400X. The references key for species identification was followed according to Song et al. (2009). The

enumeration strategy was followed Xu et al., 2014b.

WTW Multi 3500i sensor was used to measure the water temperature (T), pH, salinity (Sal) 3 and dissolved oxygen (DO), the concentration of ammonium nitrogen (NH<sub>4</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), nitrite nitrogen (NO<sub>2</sub>-N), and soluble reactive phosphate (SRP) in marine environment according to the "Standard Protocols for the Examination of Water and Wastewater" (APHA, 1992).

#### Functional Unit (FU) formulation

Combining the four ecological traits; i) feeding type bacterivores (B), algivores (A), raptors (R) and non-selectives (N) (Fenchel, 1969; Pratt and Cairns, 1985; Fernandez-Leborans and Fernandez-Fernandez, 2002; Parry, 2004; Abdullah Al et al., 2018; Jiang et al., 2013a; Wang and Xu, 2015; Sikder et al., 2019b), ii) source of food supply (inside/outside the biofilm) (Jiang et al., 2013b; Zhong et al., 2017; Guo et al., 2019), iii) body size, (S1-S8) (Kerr and Deckie, 2001; Jiang et al., 2012; Wang et al., 2016a, 2016b; Xu et al., 2016a, 2016b; Xu and Xu, 2016; Zhao et al., 2016), iv) modes of locomotion, planktonic (P), sessile (S), and vagile (V) a collective/composite 53 "Functional Units (FU)" were proposed as possible bioindicators of water quality status in marine ecosystems.

#### Data analyses

The functional unit diversity ( $H_{FU}$ ), evenness ( $J_{FU}$ ) and the richness ( $D_{FU}$ ) were calculated as follows:

$$H_{FU} = - \sum_{i=1}^s P_i (\ln P_i)$$

$$J_{FU} = H' / \ln S_{FU}$$

$$D_{FU} = (S - 1) / \ln N_{FU}$$

where  $H_{FU}$  = observed diversity index;  $P_i$  = proportion of the total count arising from the  $i^{\text{th}}$  species;  $S_{FU}$  = total number of FUs; and  $N_{FU}$  = total abundance of FUs.

All multivariate analyses of spatial variations in the biofilm-dwelling protozoans were analyzed using the PRIMER v7.1.17 package (Clarke and Gorley, 2006) and the PERMANOVA+ v1.0.7 for PRIMER (Anderson et al., 2008). The shade plot with clustering analysis was used to summarize the functional unit distribution of the protozoan periphytons among four sampling sites (Anderson et al., 2008). Using the submodule CAP (canonical analysis of principal coordinates) of PERMANOVA+ on Bray–Curtis similarities from the transformed species-abundance, the spatial differences in functional units among the four sampling sites were summarized while, the environmental status of the four sampling sites was summarized based on Euclidean distances from the log-transformed/normalized abiotic data (Anderson et al., 2008). The metric multidimensional scaling (mMDS) ordination based on the bootstrapped average analysis were used to show the variations in the functional unit patterns of the protozoan periphytons

and environmental variables among four sampling sites (Clarke and Gorley, 2015). PERMANOVA test was used to signify the differences in the functional unit patterns among four sampling sites (Anderson et al., 2008). The significance level of biota-environment correlations was tested using the routine RELATE, following the submodule BIOENV (biota-environment correlation analysis) to explore the potential environmental drivers for changes of the protozoan functional units along the pollution gradient (Clarke and Gorley, 2006; Xu et al., 2008; Jiang et al., 2011, 2013b; Xu et al., 2014a).

Correlation analyses were performed using statistical program SPSS v16.0 to observe the Pearson relationships between FUs/diversity and environmental variables (Xu et al., 2008; Xu et al., 2014a).

## Results

### Physico-chemical/environmental variables

The observed values of physico-chemical factors at four sampling sites (A–D) during the study period are summarized in Table S1. Clear variations have been observed in abiotic factors along the pollution incline from site A to D, respectively. Worth mentioning, site D (heavily polluted area) showed the lowest salinity and pH values whereas, DO and transparency were gradually decreased from sites A to D. Meanwhile,  $\text{NH}_4\text{-N}$  was observed to be increased from A to D sites, however lower values of SRP and  $\text{NO}_3\text{-N}$  were recorded at sites A and B (clean/slightly polluted area) as compared to the sites C and D (moderately/heavily polluted area) (Table S1).

### Spatial distribution of functional units

The observed species list with functional units (FUs) and abundance at four sampling sites during the study period have been showed in Table. S2. A total of 144 protozoan species have been identified, which were assigned into 53 FUs (Table S2).

The spatial distribution of the total 53 FUs among four sampling sites were summarized in Fig. 2. Based on the index of association matrix from standardized FU-abundance data, the clustering analysis divided 53 FUs into four groups (I–IV) (Fig. 2a). Groups I and II showed a decreasing trend from clean area (site A) to heavily polluted area (site D), whereas groups III and IV generally represented a revert gradient, in particular group IIIb (Fig. 2a).

### Spatial variations in functional unit patterns

The protozoan samples showed clear spatial variations in functional unit patterns from site A to D (Fig. 3). In clean area, the bacterivores and raptors with inside and outside feeding source were predominant during the period of June to October, and were primary contributors during the July to October at site B (Fig. 3a, b). At site C the dominant FUs were (raptors feeder with inside food supply) during Jan to June (Fig. 3c). However, it should be noted that the sessile bacterivores with outside food

source (e.g., BOS5s) generally dominated the samples during an increasing period from clean area (site A) to heavily polluted area (site D), while the other FUs showed an invert trend (Fig. 3).

The CAP ordination demonstrated that the pattern of FUs at three sites was discriminated from those in all polluted areas (site B–C) by CAP 1 (Fig. 4a). It should be addressed that, of the vectors with correlation  $> 0.25$ , the FU BOS2s (i.e., small bacterivores with outside resource of food supply) were pointing towards samples at site A, while the FU BOS5s (i.e., large bacterivores with outside resource of food supply) pointed towards the samples in heavily polluted area (site D) (Fig. 4a).

Furthermore, the PERMANOVA test revealed that there was a significant difference in the protozoan FUs pattern among four studied areas (pseudo- $F = 2.1829$ ,  $P < 0.05$ ), and patterns of all polluted samples were significantly different from those in clean area ( $t = 1.7–1.8$ ,  $P < 0.05$ ).

### Linkage between biota and abiota

The CAP ordinations on abiotic data revealed a similar pattern to the biotic data (Fig. 4b). It is clear that the vectors  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  directly pointed towards sample in heavily polluted area (site D), while the transparency was towards samples in clean area (site A) (Fig. 4b).

Based on bootstrapped average analyses on both biotic and abiotic matrices, mMDS ordinations revealed a similar pattern between both datasets (Fig. 5). The RELATE analysis revealed the significant relationship between the FU patterns and changes in environmental variables ( $\rho = 0.467$ ,  $P < 0.05$ ).

Based on the multivariate biota-environment (BIOENV) analysis, top 10 matches of environmental factors with the protozoan FU pattern were mainly nutrients  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  with the combination of DO (Table 1).

Pearson correlation analysis showed that 14 FUs were significantly correlated with environmental variables (Table 2). For example, BOS5s, AOS2p&v, AOS7s and BIS3v were significantly positively correlated with  $\text{NH}_4\text{-N}$ , while AIS8v, AOS3v, AOS6v, BOS5v and RIS4s were significantly positively related to transparency ( $P < 0.05$ ) (Table 2). The numbers of FUs showed a significantly positive relationship to  $\text{NH}_4\text{-N}$ , but a significantly negative correlation with DO and transparency ( $P < 0.05$ ), whereas the abundances of FUs represented a revert case (Table 3). Of three biodiversity indices at FU resolution, the FU richness ( $D_{FU}$ ) was significantly positively correlated with  $\text{NH}_4\text{-N}$ , while the other indices ( $J_{FU}$  and  $H_{FU}$ ) showed a significantly negative relationship to DO ( $P < 0.01$ ) (Table 3).

## Discussion

Recent studies have reported that there is a strong “signal to noise” ratio due to the functional redundancy at species-abundance resolution, when using the communities of organisms for the assessment of environmental/ecological quality status (Ellis, 1995; Heino and Soininen, 2007; Van der Linden et al., 2012; Zhang et al., 2012, 2014; Heymans et al., 2014; Jiang et al., 2014a; Zhong et al., 2014;

Hunter Jr. et al., 2016). Contrary to this, the community-level bioindicators based on a small taxonomic or functional species pools are considered as more effective towards environmental heterogeneity (Jiang et al., 2014a; Xu et al., 2014b; Zhong et al., 2014; Xu and Xu, 2017). In this study, we proposed 53 FUs based on four biological traits (feeding type, resource of food supply, body-size rank and movement type) of the protozoan periphytons, for making sure about the hypothesis that the FUs of the fauna may be used as a potential bioindicators of water quality status in marine ecosystems.

In this study, the functional unit pattern of the protozoan fauna showed a significant variation among four sampling sites. In particular, the large sessile bacterivores feeding on food supply from plankton (e.g., BOS5s) occurred in high abundance at moderately or heavily polluted areas, while the other FUs (e.g., BIS1v) showed decreasing trend from clean site to the polluted ones.

Furthermore, multivariate correlation/matching analyses demonstrated the functional unit patterns of the protozoan fauna were significantly associated with the changes of environmental variables, in particular the nutrients  $\text{NH}_4\text{-N}$  and/or  $\text{NO}_3\text{-N}$  in combination with DO. The Pearson correlation analysis revealed FUs BOS5s and BIS3v were significantly positively related to  $\text{NH}_4\text{-N}$ , while AIS8v, AOS3v, AOS6v and RIS4s were significantly positively correlated with transparency, with NIS4v and RIS4s positively associating DO ( $P < 0.05$ ). These findings suggested that the FUs of the protozoan periphytons may reflect the water quality status, and these FUs might be used as a useful bioindicator of water quality status in marine ecosystems.

Biodiversity parameters such as species number, richness, evenness, and diversity have widely been used as a traditional bioindicator to discriminate environmental/ecological quality status (Huston, 1979; Magurran, 1991; Ismael and Dorgham, 2003; Tan et al., 2010; Jiang et al., 2011; Shi et al., 2012; Xu et al., 2012a, 2012b; Zhang et al., 2012). Generally, higher values of these indices indicate better water quality status, with the possible exception of low levels of organic pollution (Ismael and Dorgham, 2003). In our study, however, all these parameters at FU resolution had higher values in samples at less-stressed areas than in those at more polluted sites. For example, the FU number and richness index were significantly positively correlated with  $\text{NH}_4\text{-N}$ . These findings suggest that these traditional parameters have a weakness in bioassessment of water quality.

## Conclusion

In conclusion, the FUs of the protozoan periphytons represented a significant variation in spatial distribution, and were driven by the increasing levels of nutrients (e.g.,  $\text{NH}_4\text{-N}$ ) and decreasing ranks of DO among four sampling sites. The bacterivorous FUs (e.g., BOS5s and BIS3v) were significantly positively related to  $\text{NH}_4\text{-N}$ , while the vagile algivorous FUs (e.g., AIS8v, AOS3v, AOS6v) and sessile predators (e.g., RIS4s) were significantly positively correlated with transparency. Therefore, it is suggested that FUs of protozoan periphytons may be used as a useful bioindicators of water quality status in marine ecosystems.

# Declarations

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## Declaration of Interest

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

## Credit authorship contribution statement

**Syed Shabi Ul Hassan Kazmi:** Writing Original Draft, Study Designing, Revision Editing; **Uroosa:** Proof reading; **Henglong Xu:** Supervision, Conceptualization, and Revision.

## Publication/Participation consent

All authors have participated and approved the final version of manuscript.

## Data availability Statement

Beside the supplementary material, the data will be available on request.

## Ethical Approval

Not Applicable.

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## Tables

**Table 1** Summary of results from biota-environment (BIOENV) analysis showing the 10 best matches of environmental variables with spatial variations in the FU abundance of the ciliates at four sampling sites in coastal waters of the Yellow Sea, near Qingdao, northern China during the study period.

Ranks	Environmental variables	$\rho$ value	$P$ value
1	DO	0.420	<0.05
2	DO, NH <sub>4</sub> -N	0.340	<0.05
3	DO, NO <sub>3</sub> -N	0.339	<0.05
4	S, DO	0.319	<0.05
5	DO, SRP	0.293	<0.05
6	DO, NO <sub>3</sub> -N, NH <sub>4</sub> -N	0.292	<0.05
7	S, DO, NO <sub>3</sub> -N	0.278	<0.05
8	DO, NO <sub>2</sub> -N	0.278	<0.05
9	DO, NO <sub>3</sub> -N, SRP	0.273	<0.05
10	S, DO, NH <sub>4</sub> -N	0.261	<0.05

$\rho$  value, Spearman correlation coefficient;  $P$  value, statistical significance level. Sal, salinity; DO, dissolve oxygen; NH<sub>4</sub>-N, ammonium nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; NO<sub>2</sub>-N, nitrite nitrogen; SRP, soluble reactive phosphate; COD, chemical oxygen demand.

**Table 2** Pearson correlations between average values of FU-abundance and average of environmental variables at four study sites in coastal waters of the Yellow Sea, near Qingdao, northern China during the study period.

FU	Sal	pH	DO	Tra	NO <sub>2</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	SRP	COD
AIS8v	-0.286	0.355	0.936	<b>0.970*</b>	0.163	-0.300	-0.624	-0.083	0.440
AOS2p&v	-0.060	-0.596	-0.818	0.806	0.583	0.883	<b>0.971*</b>	0.772	0.248
AOS3v	-0.303	0.343	0.934	<b>0.972*</b>	0.142	-0.304	-0.628	-0.087	0.418
AOS5p&v	-0.362	0.278	0.907	<b>0.957*</b>	0.166	-0.242	0.577	-0.022	0.423
AOS6v	-0.311	0.366	-0.934	<b>0.974*</b>	0.131	-0.306	-0.630	-0.089	0.406
AOS7s	-0.106	-0.622	-0.902	-0.781	0.596	0.906	<b>0.977*</b>	0.803	0.260
BIS3v	-0.452	-0.830	-0.712	-0.620	0.526	<b>0.990*</b>	<b>0.971*</b>	0.935	0.192
BIS4v	0.728	0.842	0.391	0.249	-0.530	-0.946	-0.785	<b>-0.982*</b>	-0.262
BOS5s	-0.330	-0.850	-0.930	-0.847	0.172	0.831	<b>0.954*</b>	0.700	-0.206
BOS5v	-0.311	0.336	0.934	<b>0.974*</b>	0.131	0.306	-0.629	-0.088	0.406
BOS8s	-0.311	0.336	0.934	<b>0.974*</b>	0.131	-0.306	-0.629	-0.088	0.406
NIS4v	0.092	0.687	<b>0.991*</b>	0.948	0.095	-0.574	-0.812	-0.390	0.444
RIS4s	-0.244	0.421	<b>0.968*</b>	<b>0.999**</b>	-0.047	-0.474	-0.760	-0.268	0.262
RIS6v	0.300	0.665	0.618	0.565	-0.720	<b>-0.977*</b>	-0.937	-0.937	-0.415

\*\* Correlation is significant at the 0.01 level; \* Correlation is significant at the 0.05 level. Sal, salinity; DO, dissolve oxygen; Tra, transparency; NH<sub>4</sub>-N, ammonium nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; NO<sub>2</sub>-N, nitrite nitrogen; SRP, soluble reactive phosphate; COD, chemical oxygen demand.

**Table 3** Pearson correlations between the average number ( $S_{FU}$ ), abundance ( $N_{FU}$ ), richness ( $D_{FU}$ ), evenness ( $J_{FU}$ ) and the diversity ( $H_{FU}$ ) of the protozoan functional units and average of environmental variables at four study sites in coastal waters of the Yellow Sea, near Qingdao, northern China during the study period.

Parameter	Sal	pH	DO	Tra	NO <sub>2</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	SRP	COD
$S_{FU}$	-0.149	-0.735	<b>-0.955*</b>	<b>-0.921*</b>	0.226	0.792	<b>0.956*</b>	0.639	-0.145
$N_{FU}$	-0.066	0.547	<b>0.973*</b>	<b>0.960*</b>	0.198	-0.415	-0.703	-0.212	0.513
$D_{FU}$	-0.530	<b>-0.945*</b>	-0.839	-0.716	0.124	0.852	<b>0.915*</b>	0.756	-0.244
$J_{FU}$	-0.180	-0.699	<b>-0.925*</b>	-0.858	-0.331	0.432	0.674	0.254	-0.651
$H_{FU}$	-0.127	-0.670	<b>-0.941*</b>	-0.886	-0.306	0.430	0.684	0.245	-0.626

\*\* Correlation is significant at the 0.01 level; \* Correlation is significant at the 0.05 level. Sal, salinity; DO, dissolve oxygen; Tra, transparency; NH<sub>4</sub>-N, ammonium nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; NO<sub>2</sub>-N, nitrite nitrogen; SRP, soluble reactive phosphate; COD, chemical oxygen demand.

## Figures

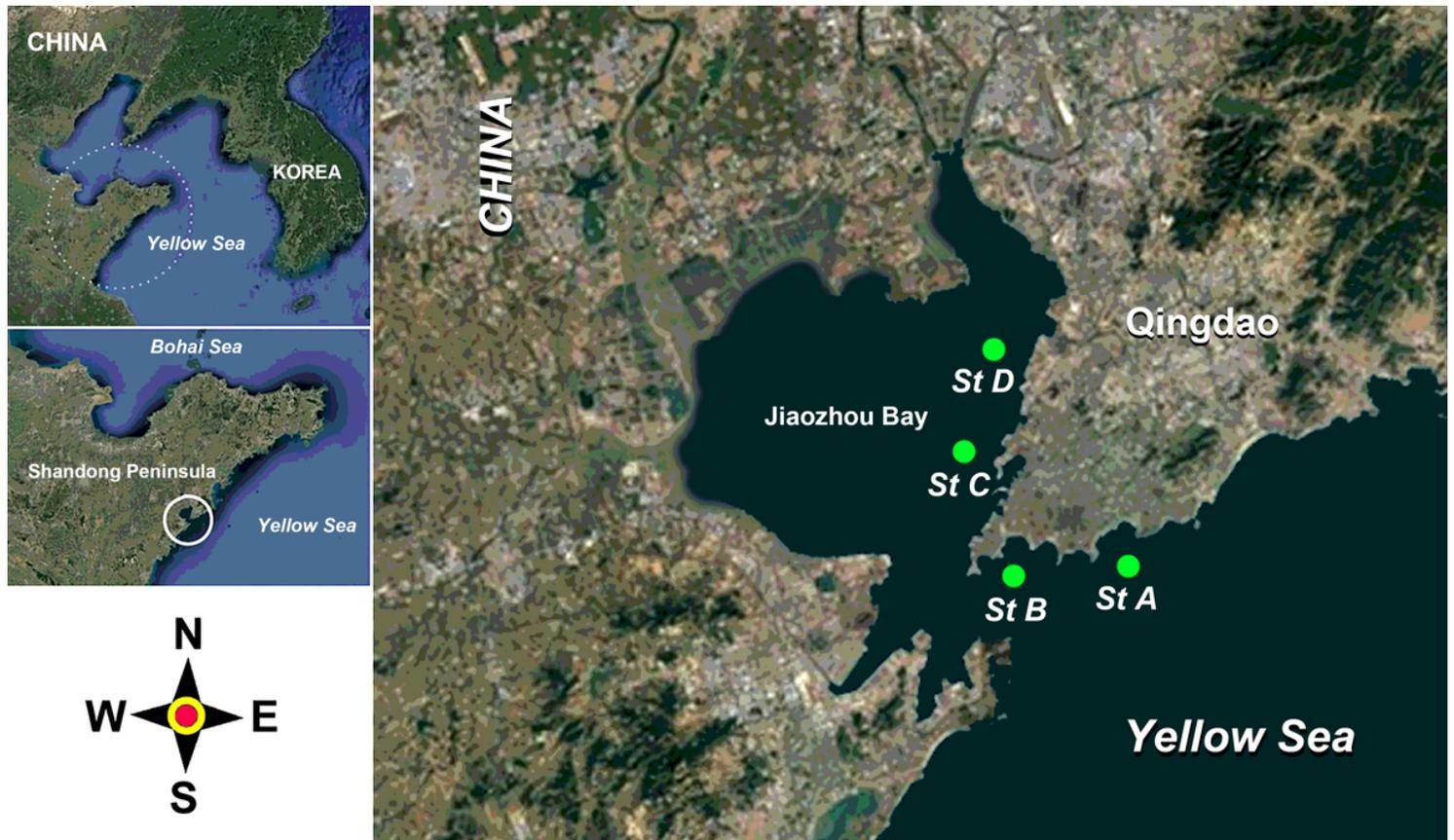
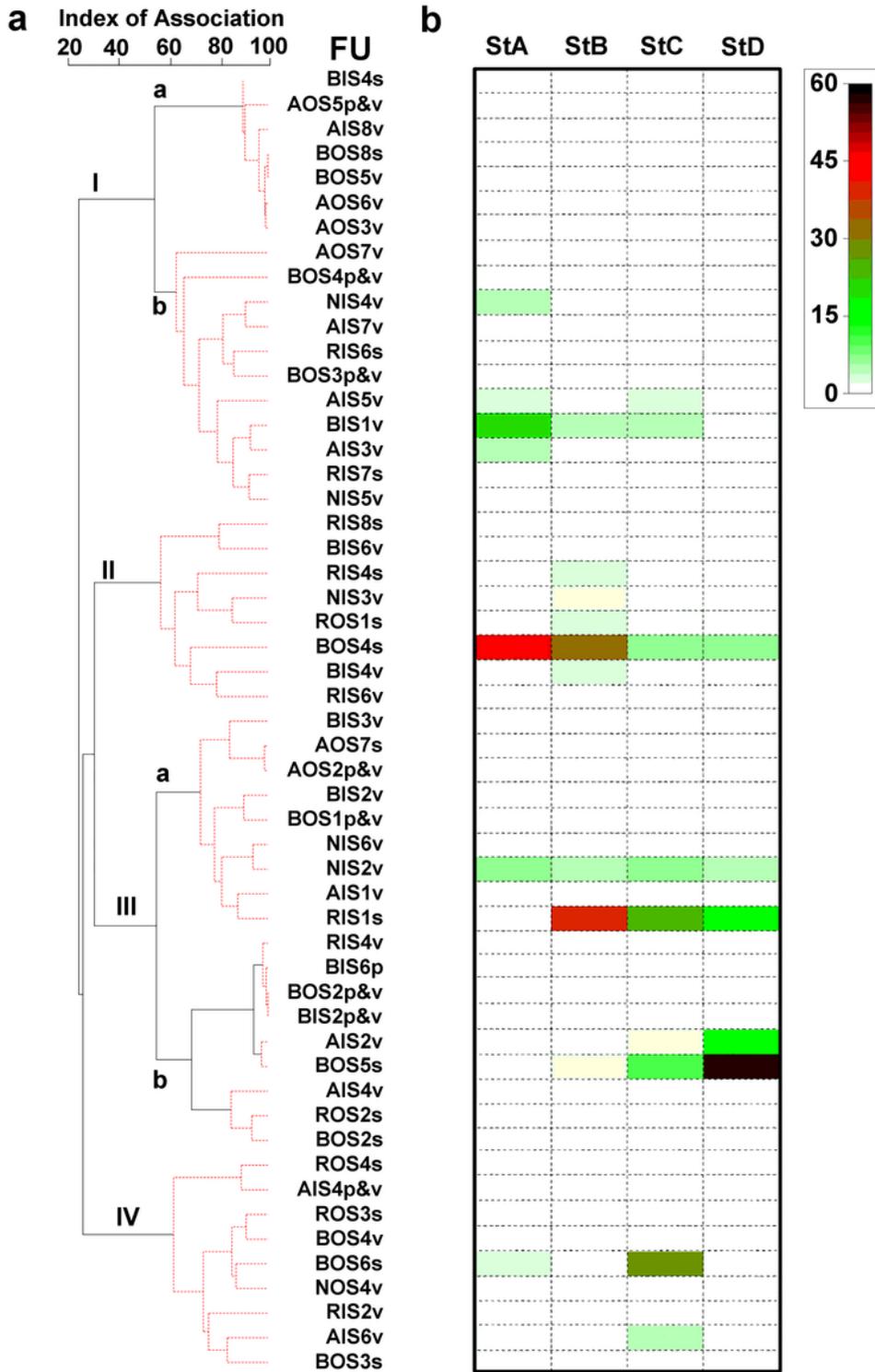


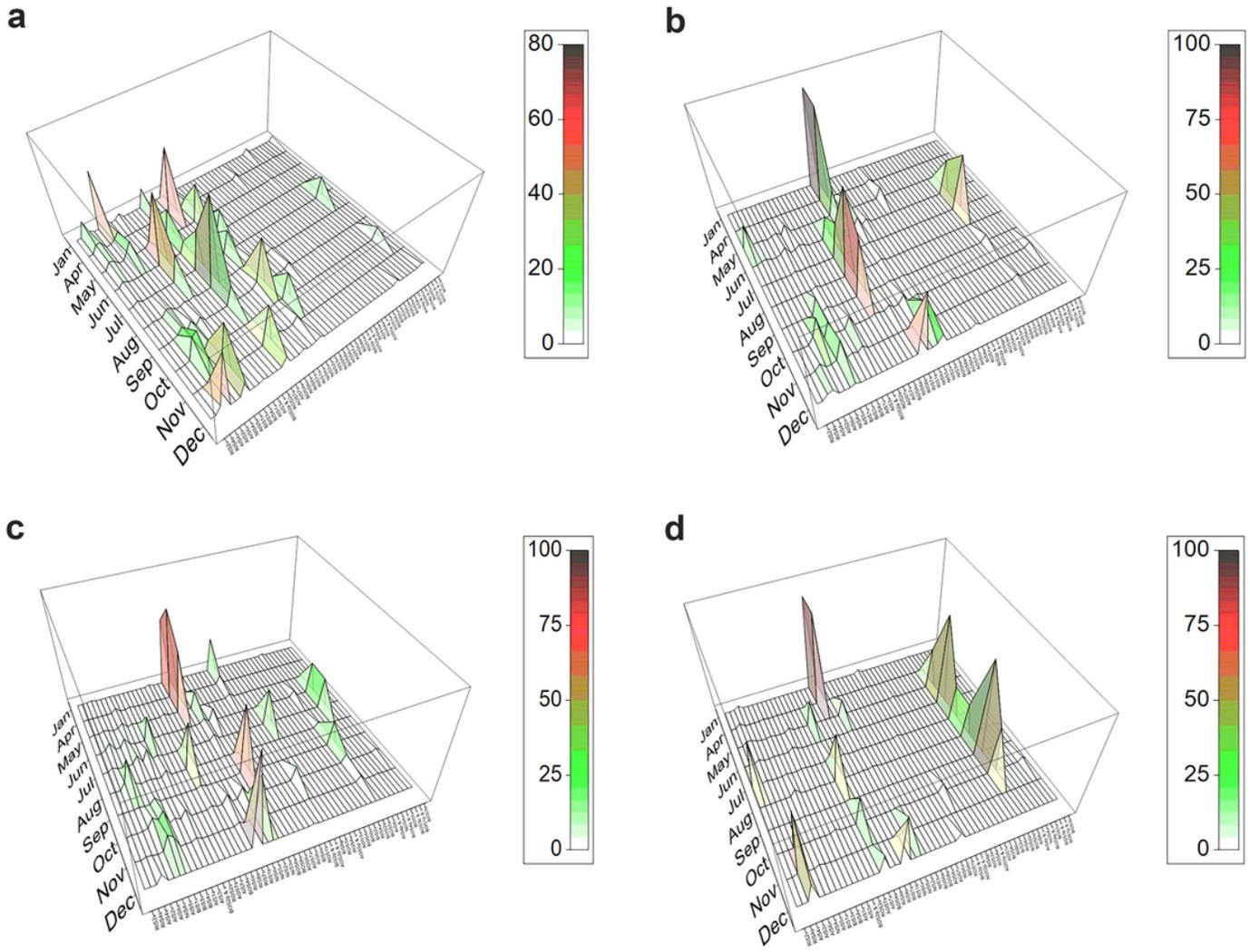
Figure 1

Sampling sites (A–D) in coastal waters of the Yellow Sea, near Qingdao, northern China. Site A, relatively clean area; site B, slightly polluted area near the mouth of Jiaozhou Bay, relatively far from the rivers that discharges sewage; site C, moderately polluted area with minor discharge from rivers in Jiaozhou Bay; and site D, heavily polluted area in Jiaozhou Bay mainly in the form of organic pollutants and nutrients. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



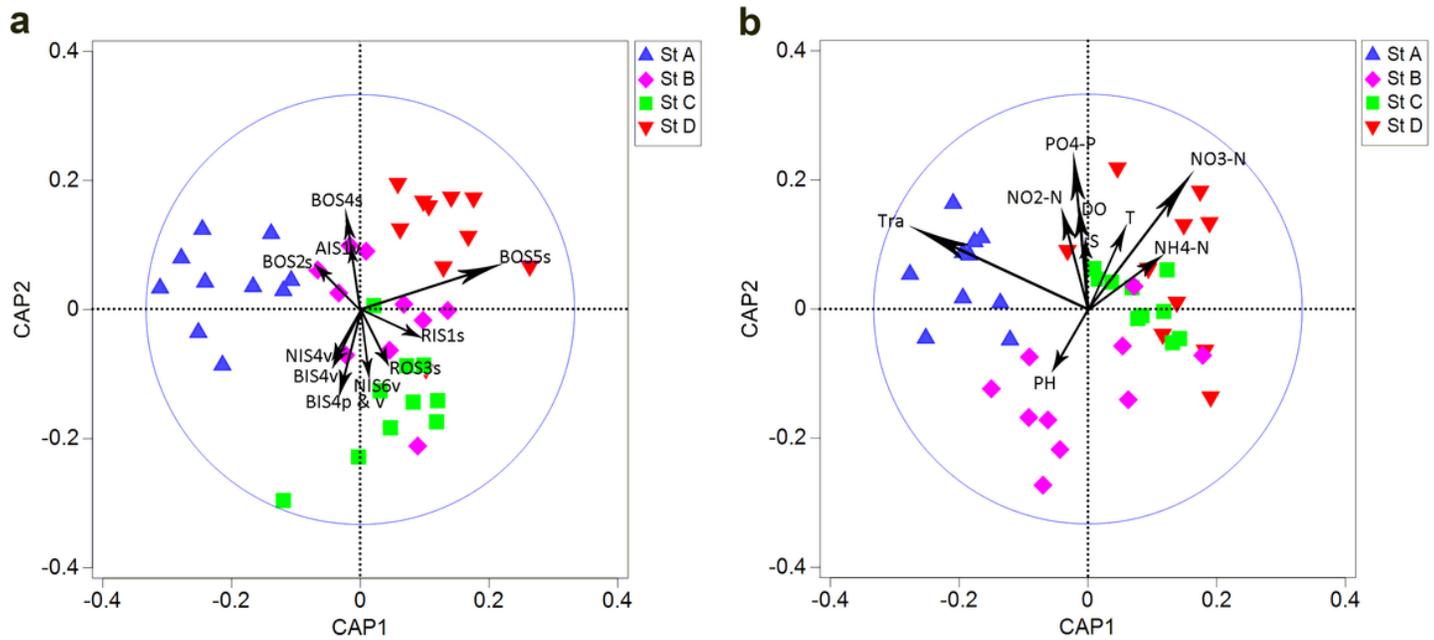
**Figure 2**

Shade plot with cluster analysis on index of association, showing the spatial distribution of the protozoan functional units among four study sites (A–D) in coastal waters of Yellow sea, northern China.



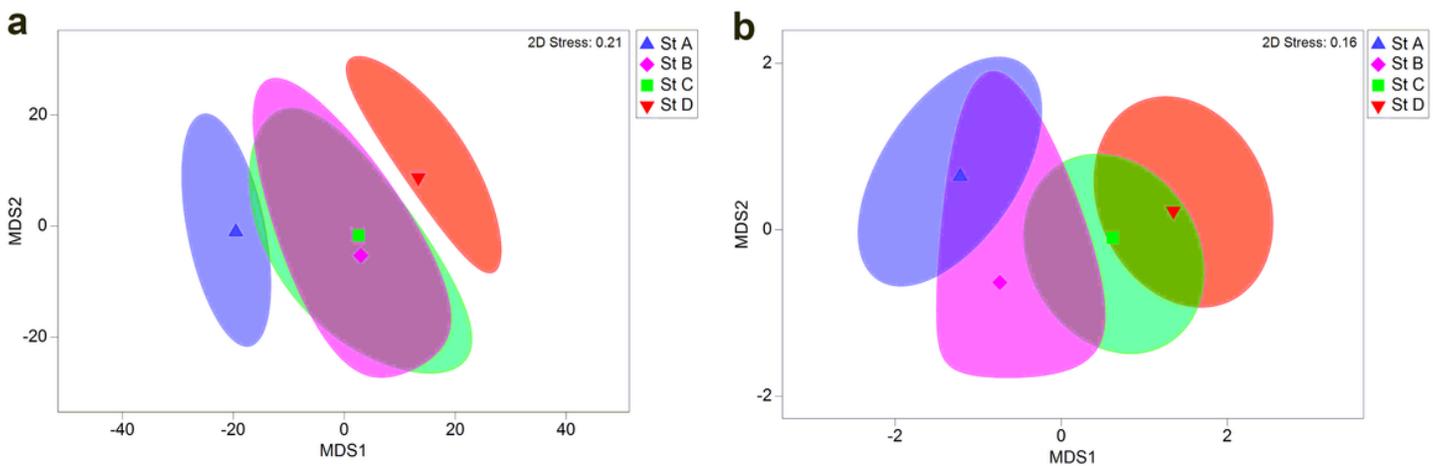
**Figure 3**

Surface plot of functional units (FUs), showing the temporal distribution of the protozoan functional units during 1-year cycle at four study sites (A–D) in coastal waters of Yellow sea, northern China.



**Figure 4**

Canonical analyses of principle coordinates (CAP) for functional units (FUs) of the protozoan periphyton fauna (a) with vectors of correlations > 0.25, and for environmental variables (b) with vectors of nine variables, showing spatial variations in both biotic and abiotic data among four study sites (A–D) in coastal waters of Yellow sea, northern China.



**Figure 5**

Metric multidimensional scaling ordinations (mMDS) based on bootstrapped average analyses, matching the spatial variations in the protozoan functional units (FUs) (a) and environmental variables (b) at four study sites (A–D) in coastal waters of Yellow sea, northern China.

## Supplementary Files

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