Can functional units of protozoan periphytons be used to evaluate ecological quality status under harmful algal blooms in marine ecosystems?

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Research Article

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Abstract

Based on biological traits the ecological quality status (EQS) under the pressure of two harmful algal bloom (HAB) species was evaluated using functional units (FUs) of protozoan periphytons. Five treatments with different concentrations of *Alexandrium tamarense* and *Gymnodinium catenatum* were designed as10^0, 10^2, 10^3, 10^4 and 10^5 cell ml^{-1}. A total of 21 FUs were identified from 25 test protozoan species. Vagile algivores with large sizes showed a decreasing trend towards high concentrations, while the vagile bacterivores and non-selectives with small sizes were dominating in concentrations (10^4 cell ml^{-1}) of both algal species. Ellipse test on pair-wise functional distinctness indices revealed a significant departure of test protozoan samples from an expected pattern when algal concentrations were over 10^4 cell ml^{-1}. Thus, it is suggested that FUs of protozoan periphyton assemblages may be used as a useful tool for evaluating the effects of HAB on ecological quality status in marine ecosystems.

Introduction

Harmful algal bloom (HAB) is a range of different types of blooms, and cause both serious damages to the marine ecosystem and deleterious effects on other animals and humans (Sonak et al., 2018). The HAB species *Alexandrium tamarense* have a great ecological impact due to its toxins formation which leads to paralytic shell fish poisoning, and thus it is supposed to be a threat to shell fish consumers as well as to marine aquaculture (Hallegraeff, 1993). The other HAB species *Gymnodinum catenatum* is a chain-forming dinoflagellate species and also associated with shellfish poisoning (Hallegraef and Fraga 1998).

As primary consumers and facilitators, protozoa play a vital role in maintaining ecological balance of microbial food webs by mediating the flux of energy and materials from low trophic levels to the high (Norf et al., 2009; Xu et al., 2011; Bai et al., 2019; Gui et al., 2021). Protozoan periphytons have proved to be useful bioindicator for bioassessment of water quality status in marine ecosystems because of their ease of collection, short life span and delicate pellicle which make them more sensitive to environmental fluctuations than other metazoans (Xu et al., 2002, 2005; Ismael and Dorgham, 2003; Jiang et al., 2007; Shi et al., 2012). However, previous studies have demonstrated that the traditional strategy for bioassessment surveys using protozoa was commonly at taxon-abundance/biomass resolution, and thus was subject to strong disturbance of high “signal to noise” ratios due to functional redundancy in a protozoan community (Xu et al., 2011; Bai et al., 2019).

In this study, the effects of two HAB species *Alexandrium tamarense* and *Gymnodinium catenatum* on FUs of test protozoan periphytons were investigated. The objectives of this study were (1) to observe the effects on functional pattern of test protozoan assemblages to different algal cell concentrations, (2) to demonstrate the variations in functional distinctness of test protozoan FUs, and (3) to confirm the feasibility of protozoan FUs in indication of the HAB effects on environmental quality status in marine ecosystems.

Materials And Methods

2.1. Study site and dataset collection

The test protozoan periphytons were collected in coastal waters of the Yellow Sea, near the mouth of Jiao Zhou Bay, Qingdao, northern China (Fig. 1). This was a typical coastal area with a depth of 9 m and an average tidal interval of 3m. Diaphaneity was measured as 2.5 during sampling period.

Samples were collected using glass slides as an artificial substratum following the sampling procedure described by Xu et al. (2011). In brief, 40 glass slides (2.5 × 7.5 cm) were fixed to polyvinyl chloride frames (5×2.5×7.5 cm). Four PVC frames were immersed at 1 m below the water surface, and left to allow biofilm dwelling ciliates to colonize the slides for periods of 14 days. The slide samples were transported to laboratory in *in situ* water and stored in a cooling box (Xu et al., 2011). After the domestication period of 3 days under laboratory conditions in an illumination culture cabinet (temperature 21.6°C, illumination 3960 1x) 30 glass slides with ciliate communities were used as test communities. The experiment was conducted during the summer season.

Identification and enumeration were 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 followed was Xu et al., 2014. WTW Multi 3500i sensor was used to measure the water temperature (T), pH, salinity (Sal) 3 and dissolved oxygen (DO in marine environment according to the “Standard Protocols for the Examination of Water and Wastewater” (APHA, 1992).

2.2 Experimental designation

Two harmful algal species *Alexandrium tamarense* and *Gymnodinum catenatum* were used as test species, obtained from Laboratory of Applied Microalgae Biology, Ocean University of China. All bioassay experiments were conducted in petri dishes during a period of 14 days. For each test microalgae five treatments with same gradients of concentrations were prepared as by Wang et al., (2017) including 10^0 (treatment 1 as control), 10^2 (treatment 2), 10^3 (treatment 3), 10^4 (treatment 4) and 10^5 (treatment 5) cells ml^{-1}, respectively. One glass slide with ciliate communities was transferred to the petri dish filled with 20 ml filtered sea water without and with test micro-algae for both control and other 8 treatments, 2 replicates were used as parallel tests for each treatment (Wang et al., 2017). During the experimental time period, the species composition, and individual abundances of the ciliates in control and all treatments were recorded.

2.3. Functional unit grouping

Combining the four ecological traits: (1) feeding type: bacterivores (B), algivores (A), raptors (R) and non-selectives (N) (Fenchel, 1969; Pratt and Cairns, 1985; Fernandez-Leborans and Fernandez-Fernandez, 2002; Abdullah Al et al., 2018; Wang and Xu, 2015; Sikder et al., 2019b); (2) source of food supply: inside/outside the biofilm (Zhong et al., 2017; Guo et al., 2019); (3) body size, (S1–S8) (Kerr and Deckie, 2001; Jiang et al., 2012; Wang et al., 2016a, b; Xu et al., 2016a, b; Xu and Xu, 2016; Zhao et al., 2016); and

(4) modes of locomotion: planktonic (P), sessile (S), and vagile (V), the functional units (FUs) were proposed for the test protozoan periphytons.

2.4. Data analysis

Four functional distinctness measures were proposed based on the four biological traits (size, locomotion, type and source of food supply): trophic-functional diversity (Δ_φ), trophic-functional distinctness (Δ^*_φ), average trophic-functional distinctness (Δ_φ^+), and variation in trophic-functional distinctness (Λ_φ^+). The calculations were carried out logistically according to taxonomic distinctness indices following the equations (1)–(4):

$$\Delta_\varphi = [\Sigma\Sigma_{i<j}\, \omega_{ij}\, x_i\, x_j] / [N(N-1)/2] \quad (1)$$

$$\Delta^*_\varphi = [\Sigma\Sigma_{i<j}\, \omega_{ij}\, x_i\, x_j] / [\Sigma\Sigma_{i<j}\, x_i\, x_j] \quad (2)$$

$$\Delta_\varphi^+ = [\Sigma\Sigma_{i<j}\, \omega_{ij}] / [S(S-1)/2] \quad (3)$$

$$\Lambda_\varphi^+ = [\Sigma\Sigma_{i<j}\, (\omega_{ij} - \Delta^+)] / [S(S-1)/2] \quad (4)$$

where x_i ($i = 1, 2, \ldots, S$) is the abundance of the ith species; N represents total number of individuals in the sample; ω_{ij} is the "distinctness weight" given to the path length linking species i and j (with $i < j$); S is the number of species (Xu et al., 2016).

All four trophic-functional distinctness measures were computed using the 'DIVERSE' routine in PRIMER v7.0.17 based on a trait-resemblance matrix. Shade plot analysis was used to summarize the distribution of functional units at each of the algal cell concentrations (Anderson et al., 2008). Metric multidimensional scaling (mMDS) ordination based on bootstrapped average analysis was used to demonstrate the variations in functional units pattern of the ciliate communities among the different algal cell concentrations of *Alexandrium tamarense* and *Gymnodinium catenatum* (Clarke and Gorley, 2015). Ellipse tests for pair-wised indices (Δ_φ^+, Λ_φ^+) were conducted to signify the departure of samples from an expected functional pattern using the routine TAXDTEST of the program PRIMER (Clarke and Gorley, 2015).

Results

3.1. Distribution of functional units

The observed species list of functional units and abundance at five different algal cell concentrations have been described in Table S1. Total number of test samples ciliates species were classified into 21 (FUs) showed in Fig. 2.

Clustering analysis classified 21 FUs into three groups (I-III) Fig. 2. Group I and II showed an increasing trend from 10^0 to 10^5 algal concentration while same result is shown by group I (Fig. 2b). However, FUs included in group II and III represented a decreasing trend from control to those with high algal concentrations.

3.2. Spatial variation in FUs pattern

The bootstrapped-average-analysis-based mMDS revealed different patterns of the test ciliates communities for both algal cell gradients (Fig. 3). For example, the functional patterns in control (10^0) demonstrate a significant difference from those of the treatments with concentrations (10^2–10^5) of both *A. tamarense* and *G. catenatum* (Fig. 3).

Coordinate-based routine CAP demonstrate that in test protozoan samples algivores with medium and large size with both inside and outside food supply and vagile (AIS4v, AOS6v) are strictly and typically found at control group in both algal species Fig. 4. In addition, it is clearly found that small to large size and vagile algivorous are significantly found at control and 10^2 cell ml^{-1} while bacterivores and non-selectives with large size inside food supply and vagile are found dominating at high concentrations (10^4 and 10^5) of both test algal species *A. tamarense* and *G. catenatum*.

3.3. Variations in trophic functional distinctness

The ellipse tests on pair-wise trophic-functional distinctness measures (Δ_φ^+ and Λ_φ^+) shows that the number of ciliated protozoan samples with significant departure from the expected trophic-functional pattern generally increased from the relatively lower to higher algal gradients of both algal species. For an instance, a sublist of (10 and 20) sizes of test protozoans were applied for each algal concentration and only one sample is found inside 20 contour at control group while the rest of samples showed a clear departure from the expected pattern (Fig. 5).

Discussion

Previous studies have demonstrated that ciliated protozoa play an important role in controlling HABs flux by feeding on them (Strom and Morello, 1998; Yih and Coats, 2000; Lawrence et al., 2001). A pelagic ciliate *Favella ehrenbergii* is recognized and highly credited for grazing on HABs specie *Alexandrium tamarense* (Needler (1949). However, a number of investigations have revealed the significant loss of micro-zooplankton (including ciliates) communities feeding on high concentrations of HABs (Watras et al., 1985; Sellner and Brownlee, 1990; Jeong and Latz, 1994). Several authors also demonstrated that feeding on harmful algal blooms ciliates growth and reproduction is highly stimulated (Gifford, 1985; Stoecker et al., 1986; Kamiyama, 1997; Jeong et al., 1999).

Protozoan periphytons have been considered as primary contributors in functional process of microbial food webs in aquatic ecosystems and a slight change in functional community pattern may lead to a significant change and an imbalance ecosystem (e.g., Xu et al., 2011, 2014; Zhong et al., 2017; Bai et al.,

2019). Some previous studies also demonstrates that food supply should be a driven factor in ecosystem functioning of different environmental conditions (Xu et al., 2016; Abdullah Al et al., 2018, 2019; Sikder et al., 2019a, b). In present study we combine four biological traits (feeding type, resource of food supply, body-size rank and movement type) in form of functional units (FUs) to identify how different concentrations of algae (*A. tamarense* and *G. catenatum*) can affect the functional distribution of protozoan communities.

In this study, a significant variation in functional unit distribution was found among different algal cell concentrations. Bacterivores with food supply inside and outside of almost all sizes (e.g., BOS6s, BIS2v, and BIS4v) showed a clear increasing trend from control to high algal concentrations of both species. While the same result was found in large size nonselective with planktonic food supply (e.g., NIS4v and NIS5v). Similarly, algivores with small and large size (e.g., AIS2v, AIS3v, AIS4v, AIS5v, AOS6v) with both types of food supply were dominant at control group while showed a decreasing pattern towards high algal concentrations. Metric multidimensional scaling also supported the results and have a clear distinction among control and other algal cell concentrations. Furthermore, the ellipse tests revealed that the pair-wise indices (Δ_{φ}^{+} and Λ_{φ}^{+}) may identify the departure of the protozoan samples from a set of expected trophic-functional patterns along the algal gradients.

Summary

In summary, the vagile algivores with large sizes showed decreasing trend towards high concentrations, while the vagile bacterivores and non-selective with small sizes dominated samples with high concentrations (10^4 cell ml^{-1}) of both algal species. Ellipse test on pair-wise functional distinctness indices revealed a significant departure of test protozoan samples from an expected pattern when the algal concentrations were over 10^4 cell ml^{-1}. It is suggested that the FUs of protozoan periphyton assemblages may be used as a useful tool for evaluating the effects of HAB on ecological 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

Uroosa: Writing Original Draft, Study Designing, Revision Editing; **Syed Shabi Ul Hassan Kazmi:** Revision Editing; **Mohammad Shafiqur Rahman:** 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

The data will be available on request.

Ethical Approval

Not Applicable.

Publication/Participation consent

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

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Figures

Figure 1

Sampling station, located in coastal waters of the Yellow Sea, northern China. 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.

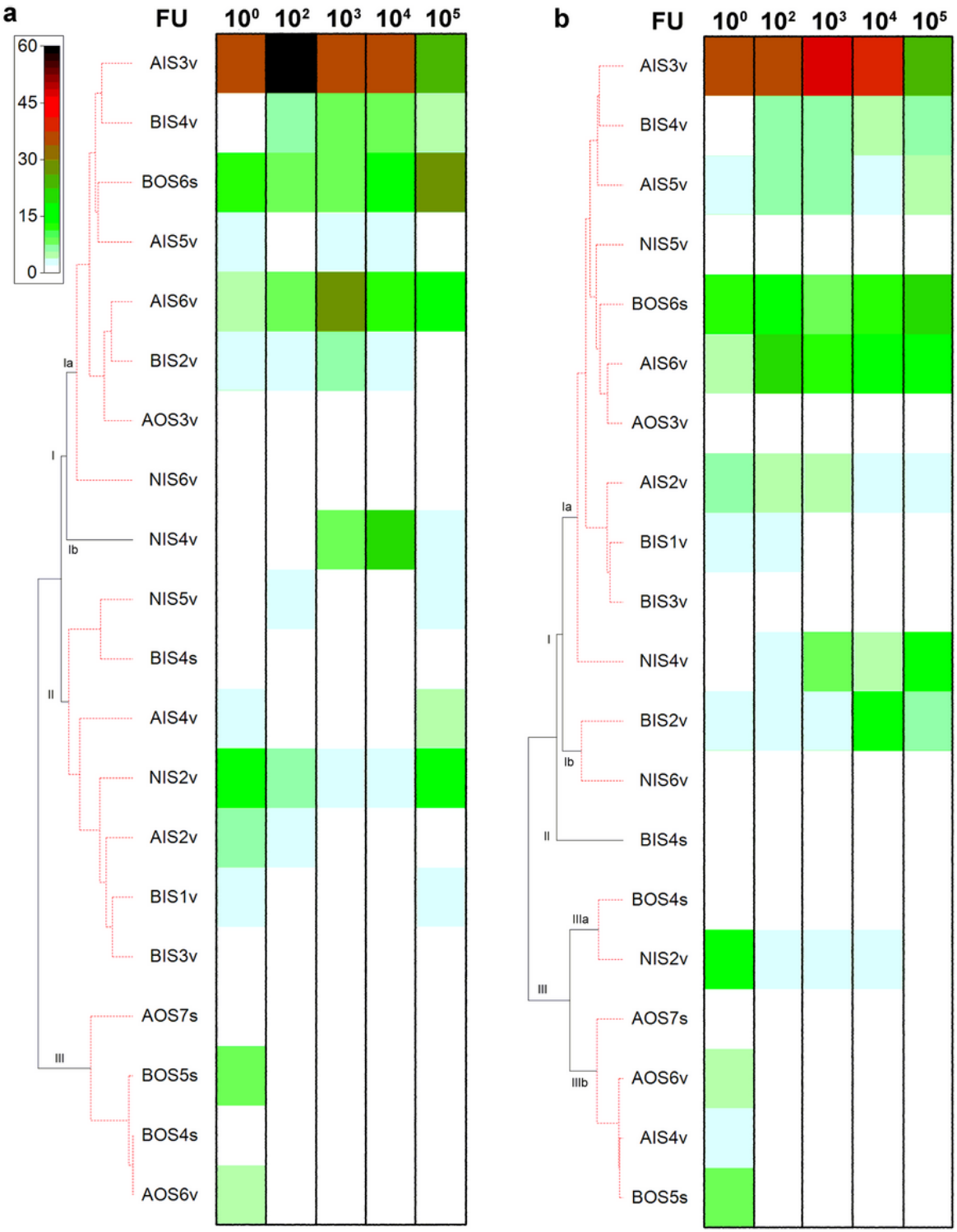

Figure 2

Shade plotting analysis, showing special distribution of functional units (FUs) at five test algal treatments 100, 102, 103, 104 and 105 cell ml-1 of A. tamarense (a) and G. catenatum (b).

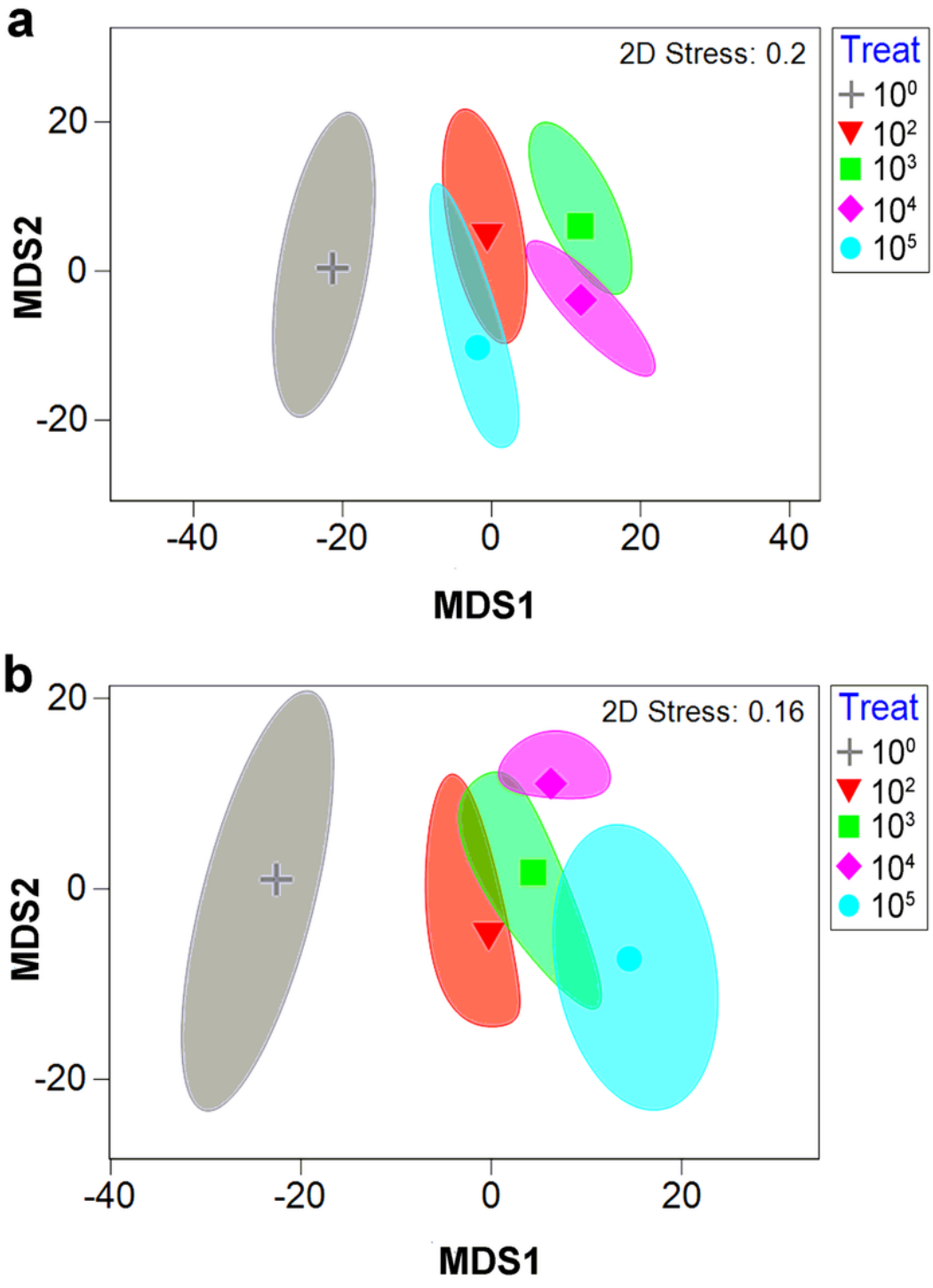

Figure 3

Metric multi-dimensional scaling based on Bootstrapped average analysis- ordinations, showing the functional patterns at five treatments 100, 102, 103, 104 and 105 cell ml-1 of A. tamarense (a) and G. catenatum (b).

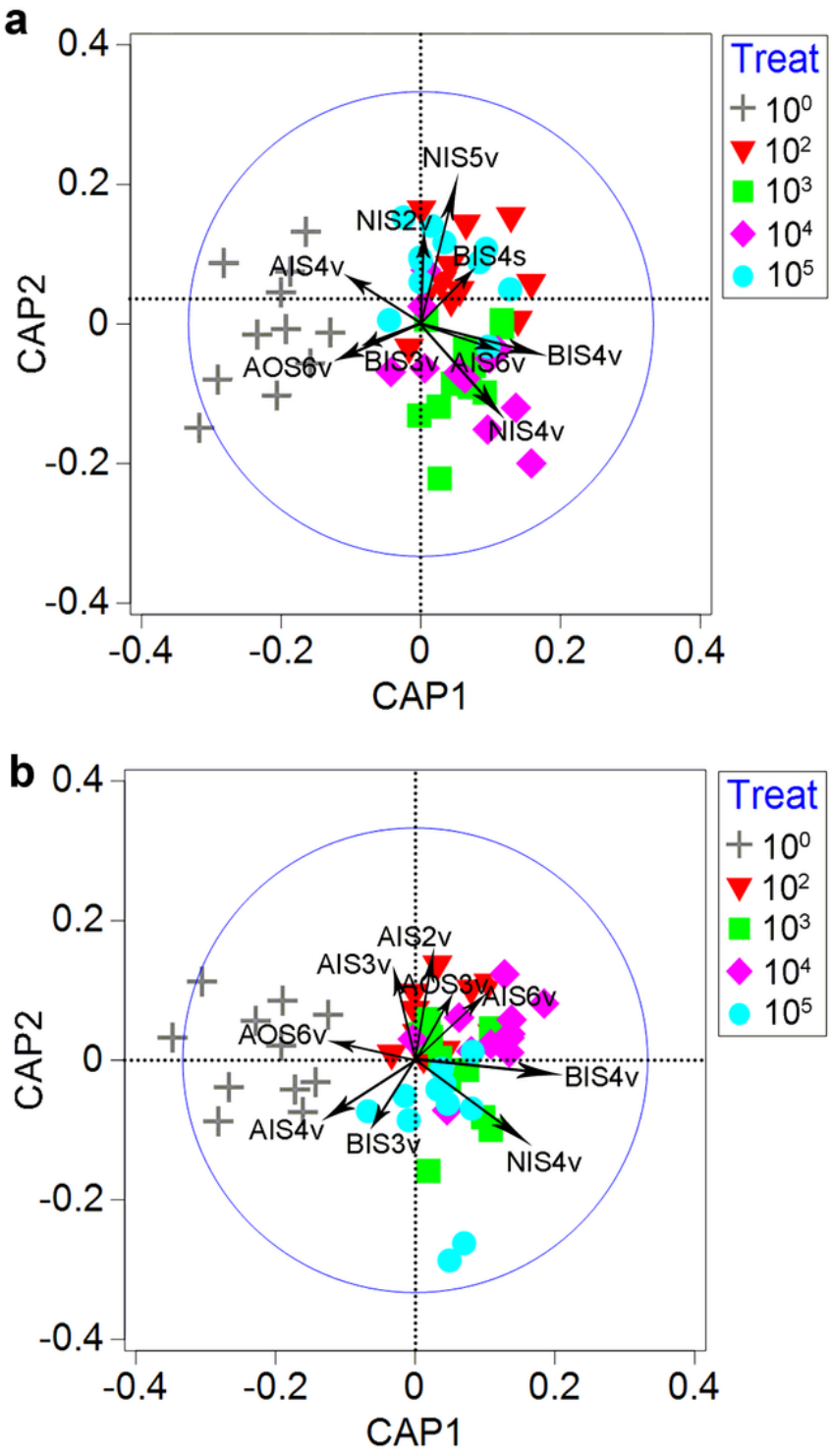

Figure 4

Canonical analyses of principle coordinates CAP, showing the vectors of FUs to the samples at five treatments of A. tamarense (a) and G. catenatum (b).

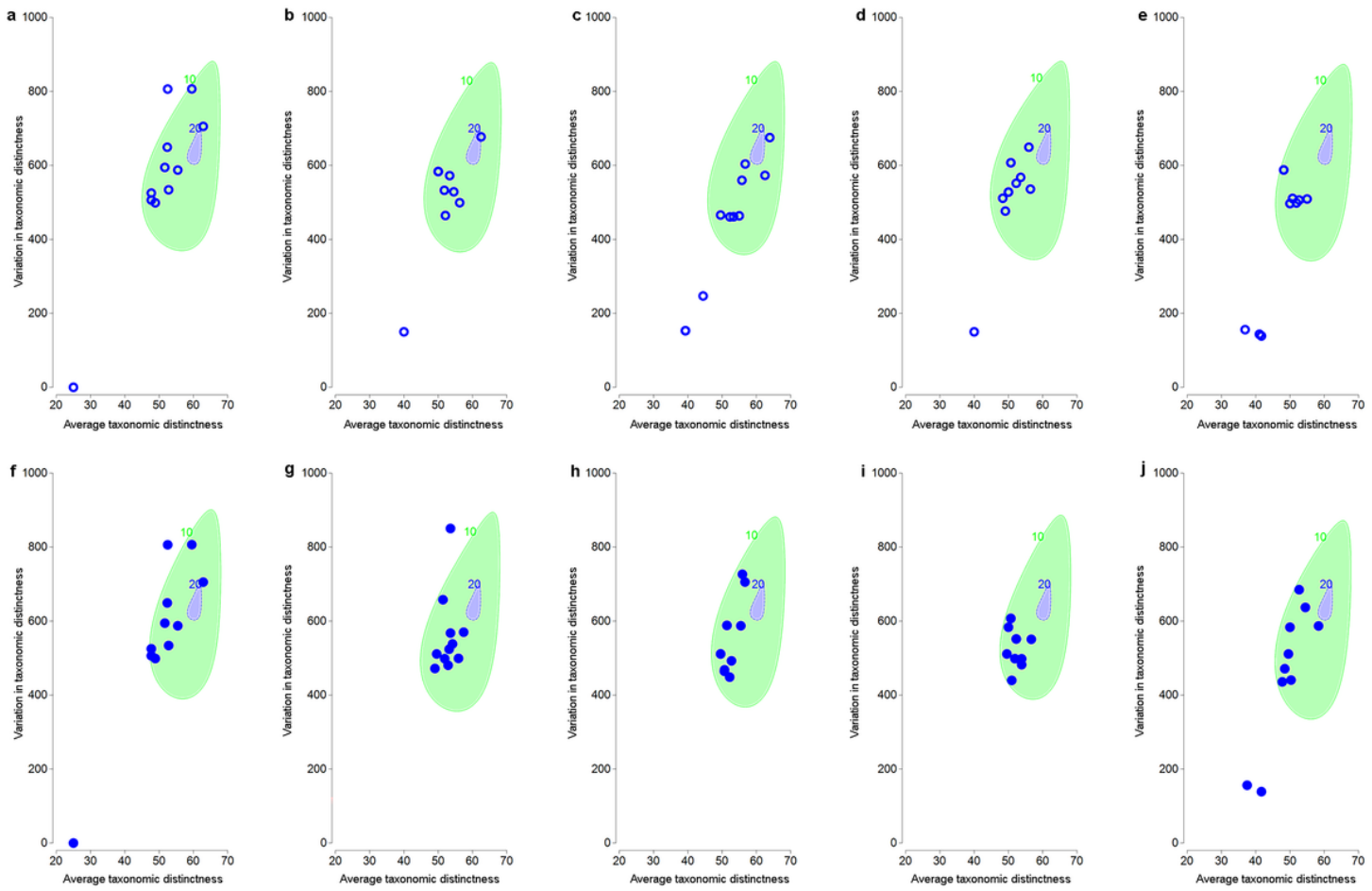

Figure 5

Ellipse tests, showing the departure of the test protozoan samples from an expected functional pattern at five treatments 100, 102, 103, 104 and 105 cell ml-1 of A. tamarense and G. catenatum.

Supplementary Files

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