

Variation Characteristics of the Ciliate Protozoan Community in the Intertidal Zone by the Outbreak of *Enteromorpha Prolifera*

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Abstract. Ciliates, a representative group of unicellular eukaryotes, play multidimensional and crucial roles within environmental ecosystems. They constitute a vital component of intertidal ecosystems and are significant drivers for ecosystems' material cycling, functional regulation, and energy flow. Given that their community structure is susceptible to external environmental variations, ciliates can be utilized as biological indicator species, thereby playing a pivotal role in biological monitoring and environmental conservation research. This study focuses on ciliates to investigate the influence of *Enteromorpha prolifera* outbreaks on the ciliate protozoan community in the intertidal ecological setting. The findings indicate that during *Enteromorpha prolifera* outbreaks, the ciliate community structure generally transitions from simplicity to complexity. Specifically, species diversity and the relationships of interaction among species continuously increase. Additionally, within the ciliate community, the succession of dominant species occurs due to certain morphological and functional disparities. Furthermore, the alterations in environmental factors induced by *Enteromorpha prolifera* outbreaks have a significant impact on the composition of the ciliate community. These results all suggest the potential for ciliates to serve as a biological indicator group for the occurrence of *E. prolifera* green tides.

Keywords: Ciliate; *Enteromorpha Prolifera*; Community Structure; Intertidal Zone; Ecological Effect.

1. Introduction

In recent years, with the rapid development of the social economy, an increasing amount of environmental pollution has been discharged into the oceans and rivers due to human activities. This has continuously pressured the quality of the ecological environment in coastal and estuarine regions [1]. Researchers generally regard continuous coastal ecosystem degradation as a major factor contributing to the increasing frequency and scale of outbreaks of marine organisms, such as algae [2-4]. Recently, the global occurrence of large-scale algal blooms has been rising [5]. The green tide, which is caused by large attached green algae, such as *Enteromorpha prolifera*, detaching from their substrates and is influenced by multiple factors, including light, temperature, and seawater eutrophication, is a prime example [6-7]. *Enteromorpha prolifera* belongs to the family *Ulvaceae* and the genus *Enteromorpha*. The thallus of this alga is bright green, composed of a single layer of cells, and forms a tubular shape or adheres to form a ribbon-like structure [8]. Single-celled algae are characterized by their small size and large surface area, enabling them to absorb nutrients relatively quickly [9].

Additionally, the propagules of *Enteromorpha prolifera* can survive under adverse conditions such as low light and temperature [10]. It is a eurythermal, euryhaline, acid-tolerant, and slightly alkaliphilic seaweed with strong environmental adaptability and rapid reproductive capacity [11]. Consequently, it is prone to large-scale reproduction and outbreaks once the conditions are right. Although *Enteromorpha prolifera* is non-toxic, its large-scale proliferation can have impacts such as blocking sunlight and competing with other marine organisms for oxygen [12]. Consequently, it changes the community structure of the marine ecosystem [13]. Some studies have also indicated that certain chemical substances it secretes may adversely affect other marine organisms [14]. During the outbreak of *Enteromorpha prolifera*, a portion of it drifts to the intertidal zone along with ocean

currents, attaches to the area, and spreads rampantly, causing extremely detrimental effects on the ecological environment [15].

The intertidal zone is the coastal area between the average high and low tide levels. [16]. This zone typically undergoes drastic environmental fluctuations and harbors a unique ecological setting [17]. Moreover, it ranks among the most vulnerable ecosystems [18]. The biological communities within the intertidal zone are intricate and display a high level of biodiversity. In addition to common arthropods and mollusks, many protozoans are also present, including ciliates [19].

Ciliates are classified under the phylum *Ciliophora*. They are a group of single-celled microeukaryotes with diverse body forms. Among protists, ciliates represent the most intricately differentiated population [20]. Ciliates act as a connection between small plankton and ultra-micro plankton [21]. They are an essential component in the material cycling of the marine micro-food web [22], making a significant contribution to the energy flow within marine ecosystems [23]. Ciliates have a short individual life cycle. Their species composition and community structure are susceptible to changes in the external environment [24]. Consequently, they find extensive applications in research related to biological monitoring and environmental protection [25]. Therefore, when exploring the impact of the *Enteromorpha prolifera* outbreak on the ecological environment of the intertidal zone, it is of great significance to study and analyze the changing characteristics of ciliate protozoan communities. Additionally, calculating the intertidal zone microbial ecosystem and analyzing the interactions therein to construct co-occurrence networks is conducive to understanding and interpreting the complexity and robustness of the ecosystem network [26].

However, currently, the majority of research on ciliates focuses on the model organism *Tetrahymena thermophila* [27]. These model organisms feature easy culturing and convenient genetic manipulation. Even research on environmental ecological groups mainly focuses on various planktonic and benthic ciliates [28-29]. Research on ciliate communities in the intertidal zone remains relatively scarce. There are still specific gaps in our understanding of the succession of dominant ciliate species and the spatiotemporal variations of ciliate communities before and after the outbreak of *Enteromorpha prolifera*.

This study selected ciliates as the research subject to explore the assembly patterns of ciliate communities and their responses to the *Enteromorpha prolifera* outbreak within the ecological context of the intertidal zone. We postulate that the outbreak of *Enteromorpha prolifera* influences the ciliate community by modifying the interactions of environmental driving factors and the food web. Thus, by determining environmental factors and analyzing the succession of dominant orders within the ciliate community, we can clarify the position and function of intertidal ciliates during changes in the micro-food web influenced by *Enteromorpha prolifera*.

2. Materials and Methods

2.1 Sample Collection

From March to April 2025, sandy samples were collected from the intertidal zone of Qingdao Zhanqiao for preliminary experiments. Sampling was conducted at appropriate tidal times, on average, once every two weeks. The collected sandy samples were subjected to ice-driven filtration. The filtrate was then collected and examined under a microscope to identify the ciliate species present. Simultaneously, the ice-driven filtration experiment established a volume gradient of the sandy samples to determine the optimal volume for ice-driven filtration.

The formal experimental sample collection was carried out from May to July 2025. The samples were collected from three coastal areas in Qingdao: the intertidal zone of Qingdao Sculpture Park (120.456°E, 36.079°N), Qingdao Taipingjiao Park (120.358°E, 36.051°N), and Qingdao Zhanqiao (120.320°E, 36.062°N). Sampling was conducted at approximately two-week intervals. Both the collection and preservation of samples adhered strictly to the "Specifications for Oceanographic Survey" (GB/T 12763.6) [30]. In light of on-site hydrological observations, during sampling, sandy samples were excavated from the mid-tidal zone of each area using a shovel and placed into plastic

buckets. Each time, samples were collected to nearly fill the buckets, with a sampling depth of 30 cm. Subsequently, a certain amount of seawater was added to the surface of the samples. These samples were then properly preserved and transported back to the laboratory for further analysis.

During each sampling event, the YSI series water quality analyzer was first employed to measure the characteristic parameters of nearshore seawater. These parameters included dissolved oxygen (DO), pH value, temperature, salinity, atmospheric pressure, and electrical conductivity. Simultaneously, 50 mL of seawater from each sampling site was collected into centrifuge tubes. The seawater samples were then filtered through 0.22- μm filter membranes and stored for subsequent analysis of nutrients and salts. This allowed for the determination of the levels of nitrate, phosphate, silicate, nitrite, and ammonium in the seawater.

2.2 Sample Processing

The collected samples were subjected to ice-driven filtration treatment. Place the lids of two culture dishes on the bottom of one, and put the sand gap ciliate separator on top. The bottoms of the two culture dishes are used alternately to collect the filtrate. Three parallel filtrations were performed for each sand sample collected at a sampling point, resulting in 9 filtration systems. The collected filtrate was first examined under a microscope to determine the quantity and types of ciliates. Subsequently, the filtrate was transferred to wide-mouth bottles, with approximately 20 mL of filtrate collected in each bottle.

All the samples' filtrates were centrifuged and enriched using an oil centrifuge. The enriched centrifugates were transferred to 15 mL centrifuge tubes, and 9 tubes of enriched centrifugates were collected. Approximately 200 μL of Lugol's iodine solution was added to each tube of enriched liquid to fix the ciliates, and they were stored at a low temperature for subsequent analysis.

Subsequently, gene sequencing was performed. RNA was extracted from the ciliated protozoa samples enriched and fixed at three sampling points using the E.Z.N.A. Soil RNA Kit (Omega), with three parallel extractions for each sampling point. The extracted RNA samples were purified using RNA Clean Beads (Nanjing Vazyme Biotechnology Co., Ltd.). The gDNA in the purified RNA samples was first removed using the Evo M-MLV Reverse Transcription Premix Kit (Axygen Biotechnology). Then the RNA was reverse transcribed into cDNA and stored at $-80\text{ }^{\circ}\text{C}$. The samples were then sent to LC Sciences for sequencing.

2.3 Statistical Analysis

On the QIIME2 platform, the raw amplicon sequences obtained from sequencing were converted into phylogenetic trees and Amplicon Sequence Variant (ASV) tables with species annotations. Statistical analyses and result visualizations were accomplished using R version 4.3.3. α -diversity analysis was employed to investigate differences in species richness and composition. Based on β -diversity analysis, Canonical Correspondence Analysis (CCA) was performed on the acquired environmental factor data. This was done to clarify whether the microbial community assembly process leaned towards determinism or randomness.

The ciliate community's co-occurrence network was constructed on the MENAP website (<http://ieg4.rccc.ou.edu/mena/>). Gephi version 0.9.7 was employed to visualize the co-occurrence network. This enabled the analysis of network stability, complexity, and resistance to interference within microbial communities. Additionally, it enabled the exploration of the succession of dominant species within the ciliate population and the changes in community structure that occurred over time.

3. Result

3.1 α -diversity Analysis

This study conducted α -diversity analysis on the species composition at three sampling points before, during, and after the outbreak of *Enteromorpha prolifera* (as shown in Figure 1). This was done to identify the differences in species richness and species composition. Among the metrics of

Shannon index, Simpson index, Richness index, and Pielou evenness index, a higher value indicates a greater within-species diversity. The lowercase letters (a, b, c) above the box plots in the figure denote the results of one-way analysis of variance (ANOVA).

The intertidal zone of the Zhanqiao Pier exhibits a notable superiority over the intertidal zones of the Sculpture Garden and Taipingjiao Point in terms of the Shannon index, Simpson index, species richness, and evenness index. Moreover, this superiority remains consistent across the three phases of the *Enteromorpha prolifera* outbreak.

The α -diversity indices of the three regions all generally and significantly decreased during the green tide outbreak (i.e., Phase 2), indicating that the ecological environment in these regions underwent significant changes during the *Enteromorpha prolifera* outbreak, resulting in a general decline in biodiversity. However, they gradually recovered after the green tide receded.

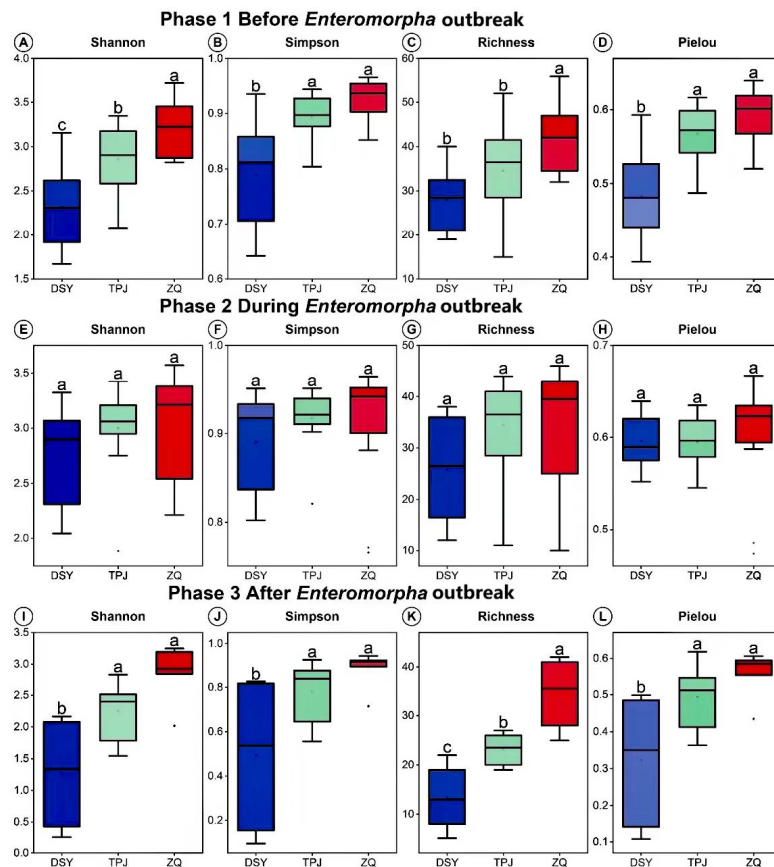


Figure 1. presents the α -diversity indices of the three sampling sites in this study during different phases of the *Enteromorpha prolifera* outbreak. DSY refers to the intertidal zone of Qingdao Sculpture Garden, TPJ to the intertidal zone near Qingdao Taipingjiao Park, and ZQ to the intertidal zone near Qingdao Zhanqiao Pier. The abbreviations in the figures and tables hereinafter follow this convention and will not be elaborated on individually.

3.2 Succession of Dominant Orders of Ciliates

Figure 2 shows, from left to right, the compositional structures of ciliate communities at three sampling sites, respectively, before the outbreak of *Enteromorpha*, during the outbreak, and after its recession. Evidently, the *Enteromorpha* outbreak has significantly influenced the ciliates' population structure. This finding confirms the assembly pattern of the ciliate community and its responses to environmental changes and the outbreak of *Enteromorpha prolifera*.

Analyzed from the perspective of dominant orders, the dominant taxon in the intertidal zone of the Zhanqiao Pier has consistently been Karyorelictea, showing relatively insignificant changes. Conversely, the dominant taxon in the Sculpture Garden's intertidal zone has transitioned from CONtreeP to Litostomatea. In the intertidal zone of Taiping Bridge, before the outbreak of

Enteromorpha, although the overall proportion of the dominant order Litostomatea did not exhibit marked alterations, the compositions of two taxa, CONthreeP and Karyorelictea, underwent substantial changes successively, thereby resulting in the succession of dominant species. This suggests that the ciliate community in the intertidal zone of Zhanqiao Pier is relatively stable in terms of community structure, particularly in the dominant orders, indicating a high level of ecological stability and resilience.

In terms of the structural changes of the ciliate community by the outbreak of *Enteromorpha prolifera*, although the proportion of the Karyorelictea taxon in the intertidal zone of the Sculpture Garden continued to increase, it generally exhibited a trend of first decreasing and then increasing. The Litostomatea taxon generally showed an upward trend. The Spirotrichea taxon generally presented a pattern of increasing and then decreasing proportionally. Moreover, the Heterotrichea taxon showed a slight overall increase during the outbreak of *Enteromorpha prolifera* and a downward trend towards the end of the recession.

From this, we can gain insights into some of the responses of the ciliate protozoan community structure to the outbreak of *Enteromorpha prolifera*. The Karyorelictea taxa are typically benthic, relying on a stable sediment environment and being sensitive to oxygen concentration [31]. Therefore, the massive outbreak of *E. prolifera* competes for oxygen, which inhibits the growth of this taxon. However, their population gradually recovers after the *E. prolifera* subsides. The Litostomatea taxa, with strong motility and feeding on bacteria or small protists [32], may utilize the bacterial community released during the decomposition of *E. prolifera* as a food source. This could contribute to an increase in their numbers in the later stages, thus showing an overall upward trend. The Spirotrichea taxa, as ciliates proficient in filter-feeding on algae or suspended particles [33], can directly consume other small algae or the suspended particles generated by *E. prolifera*, rapidly increasing their numbers.

Nevertheless, the excessive proliferation of algae increases the water body's viscosity, restricting their motility. Consequently, they exhibit an overall trend of increasing first and then decreasing. Regarding the Heterotrichea taxa, due to their relatively large size and dominance in low-oxygen environments [34], although they account for a small proportion, they can experience a notable increase during the *E. prolifera* outbreak. Although their numbers may decline again after the *E. prolifera* retreats, they may become a dominant taxon in the later stage, similar to the intertidal zone of the trestle. Instead, their numbers may increase significantly again and take a position in the community composition.

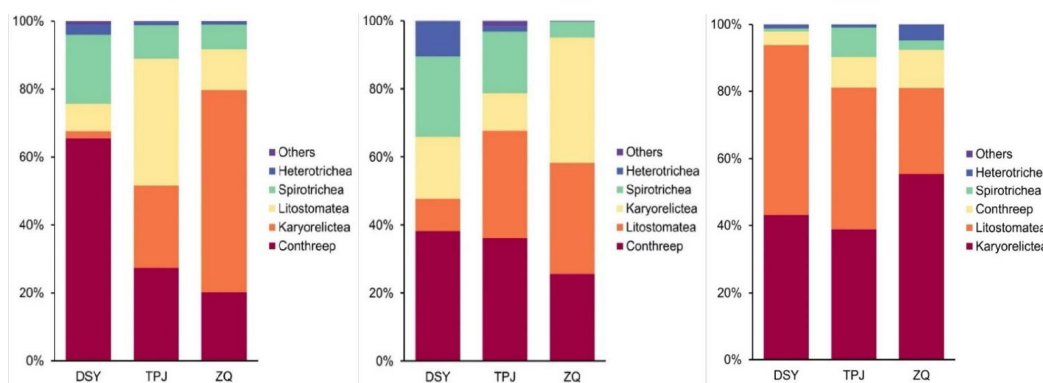


Figure 2. Ciliate community compositions at three sampling sites before, during, and following the outbreak of *E. prolifera*

3.3 Co-occurrence Network Graph

The ciliate community's co-occurrence network was visualized using Gephi (0.9.7) (Figure 3). Topological feature data were then extracted and tabulated (Table 1) to facilitate more accurate analysis of the data occurrence network graph.

As can be discerned from the co-occurrence network graphs and the table of topological features, with the outbreak of *Enteromorpha prolifera*, the ciliate community structures in the three regions all exhibit a trend from simplicity to complexity, accompanied by a continuous increase in species diversity and interactions. This suggests that the changes in environmental conditions triggered by the outbreak of *Enteromorpha prolifera* have compelled the ciliate community to undergo adaptive adjustments to maintain ecological balance.

By constructing the visualization of the co-occurrence network, one can intuitively observe the succession of dominant species within the ciliate population and the alterations in community structure. The topological analysis table can render the conclusions derived from the visual representation more precise. For instance, in the co-occurrence network diagram of the ciliate community under study, without the aid of the topological feature table, it would be difficult to discern that, before the outbreak of *Enteromorpha prolifera*, the co-occurrence network in the intertidal zone of the pier had a low network diameter but a high average clustering coefficient. Moreover, it would be impossible to conclude that it exhibited high compactness. Evidently, the topological analysis table significantly facilitates the interpretation of visual data. It facilitates a deeper understanding of the ciliate community's response to the *Enteromorpha prolifera* outbreak.

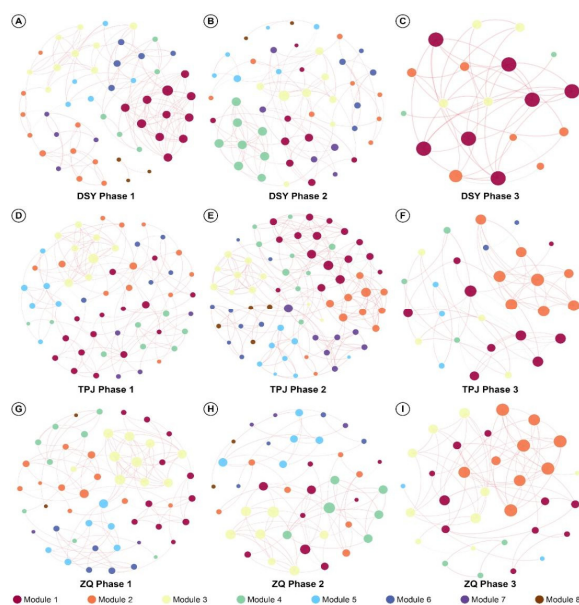


Figure 3. Co-occurrence network diagrams of ciliate communities at three sampling sites during different periods

Table 1. Topological Characteristics of Ciliate Co-occurrence Networks

Sample	Node	Positive Edge	Negative Edge	Average Degree	Average Clustering Coefficient	Network Diameter	Modularity
DSY Phase 1	52	142	5	5.654	0.749	9	0.675
TPJ Phase 1	51	98	5	4.039	0.661	12	0.710
ZQ Phase 1	19	35	2	3.895	0.965	2	0.592
DSY Phase 2	58	137	6	4.931	0.671	8	0.674
TPJ Phase 2	73	214	14	6.247	0.709	9	0.708
ZQ Phase 2	29	53	9	4.276	0.832	5	0.649
DSY Phase 3	55	124	4	4.655	0.742	13	0.744
TPJ Phase 3	43	72	5	3.581	0.680	11	0.738
ZQ Phase 3	33	71	1	4.364	0.860	4	0.670

3.4 Canonical Correspondence Analysis (CCA)

A CCA was performed on the characteristic indicators of nearshore seawater measured by the YSI series water quality detector and nutrient salt assays. The results are presented in Figure 4. From the perspective of the CCA results, before the outbreak of *Enteromorpha prolifera* (phase 1), among the

environmental factors, pH and silicate had the most substantial impact on the samples. During the outbreak of *Enteromorpha prolifera* (phase 2), temperature and ammonium emerged as the two environmental factors with the most prominent influence. Subsequently, dissolved oxygen, salinity, pH, and other factors played secondary roles.

Finally, in the aftermath of the retreat of *E. prolifera* (Phase 3), temperature remains the environmental factor with the highest weight. Next is dissolved oxygen, followed by air pressure. The degrees of influence of salinity and electrical conductivity on ciliates are not substantially different. Despite numerous attempts, other indicators did not appear in the visualization of the canonical analysis. This implies that only the aforementioned five indicators played a crucial role in the environmental factors following the retreat of *E. prolifera*, while the others can be deemed negligible. This clearly demonstrates that the outbreak of *E. prolifera* has significantly impacted the environmental factors affecting the ciliate community. Notably, from the pre-outbreak to the outbreak phase of *E. prolifera*, the influence of ammonium on the ciliate community has increased significantly, supplanting the role of silicate before the outbreak. Before the outbreak, ammonium had one of the lowest levels of influence on ciliates.

This is likely attributable to the fact that the outbreak of *E. prolifera* accelerates the cycling of nitrogen and phosphorus, thereby altering the competitive relationships through nutrient restructuring [35]. During the absorption and decomposition processes associated with the outbreak of *E. prolifera*, the release of high concentrations of ammonia-nitrogen, phosphate, and organic carbon significantly accelerates the cycling of nitrogen and phosphorus [36]. As a result, the eutrophication characteristics of the water body shift from silicon limitation to nitrogen-phosphorus limitation. At this juncture, the dynamics of the ciliate community are more strongly driven by the nitrogen-phosphorus ratio rather than the silicate concentration. Consequently, the influence weight of silicate in the environmental factor analysis is substantially reduced, while that of ammonium is significantly elevated. This phenomenon can also account for the substantial increase in phosphate's influence. Therefore, it is highly probable that the outbreak of *E. prolifera* accelerates nitrogen-phosphorus cycling, thus altering competitive relationships and resulting in nutrient restructuring.

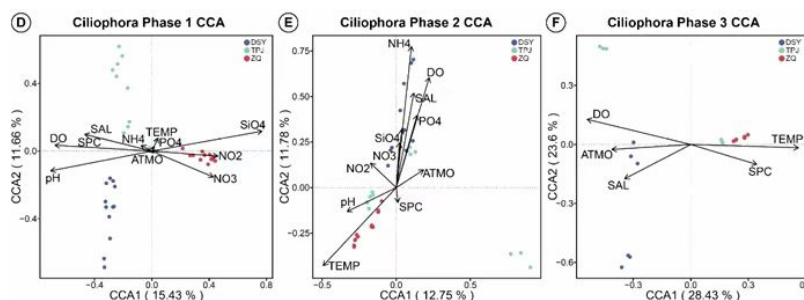


Figure 4. Canonical Correspondence Analysis of Seawater Characteristics Influenced by the Outbreak of *Enteromorpha prolifera*. TEMP: Temperature; ATMO: Atmospheric Pressure; DO: Dissolved Oxygen; SPC: Specific Conductance; SAL: Salinity; pH: pH Value; NH_4^+ : Ammonium Ion; NO_3^- : Nitrate Ion; NO_2^- : Nitrite Ion; PO_4^{3-} : Phosphate Ion; SiO_4^{4-} : Silicate Ion

4. Discussion

4.1 The Impacts of Environmental Factors

The influence of the *Enteromorpha prolifera* outbreak on the canonical correspondence analysis of ciliates' environment is primarily manifested in the significant regulatory effects of the resulting environmental factor alterations on ciliates' community structure and distribution patterns. For example, consider the dynamic imbalance of dissolved oxygen. During the outbreak of *E. prolifera*, the algal biomass releases oxygen during the day through photosynthesis and consumes oxygen at night. This characteristic exacerbates the fluctuations in dissolved oxygen levels within the water body. During the daytime, the surface water may become supersaturated with oxygen. Depletion

occurs at night, due to the algae's respiratory activities, and in some cases, anoxic zones may even form [37]. As aerobic or facultative anaerobic protozoa, Ciliates exhibit a high correlation between their population abundance, dominant species succession, and the dissolved oxygen gradient. In CCA, dissolved oxygen often represents the most prominent ordination axis. Consequently, the oxygen deficiency resulting from the *E. prolifera* outbreak may lead to a decline in the population of aerobic ciliates. In contrast, hypoxia-tolerant or anaerobic ciliate taxa may assume a dominant position within the community.

Simultaneously, the eutrophication of seawater, particularly the elevation of nitrogen and phosphorus levels, is a crucial factor contributing to the outbreak of *E. prolifera* [38]. The excessive input of nutrients can also stimulate the reproduction of certain ciliates, thereby further modifying their community structure.

Furthermore, the light shading caused by the massive outbreak of *E. prolifera* can also induce a photosynthetic inhibition effect. The floating layer formed by *E. prolifera* can block more than 90% of the effective light radiation underwater, suppressing the photosynthesis of benthic algae and disrupting the original producer community [39]. For example, if the photosynthesis of diatoms is inhibited, certain ciliate groups that symbiotically associate with diatoms may decline due to the food chain disruption. Conversely, ciliates that rely on heterotrophy or saprotrophy may proliferate as the amount of organic detritus increases. In CCA, both the depth of light penetration and the concentration of organic detritus have the potential to emerge as new dominant environmental variables.

In addition to the above, the outbreak of *E. prolifera* can also give rise to certain pH fluctuations, thereby influencing the community structure of ciliates. During the period of the outbreak, the absorption of CO₂ through photosynthesis may result in a local increase in pH values (>8.5), while the respiratory processes at night cause the pH to decline (<7.5) [39]. These substantial pH fluctuations are likely to impact the calcification processes and enzyme activities of some ciliates. Consequently, this further affects the community structure of ciliates [40].

4.2 Characteristics and Ecological Functions of Ciliates

There may be a replacement of dominant species among ciliate communities due to the differences in morphology and function during the outbreak of *E. prolifera*. For example, populations sensitive to oxygen concentration may decrease in hypoxic environments. Populations with specialized predatory structures such as cirri, which possess strong motility and feed on bacteria or small protozoa, may experience an increase in numbers during the mid- to late-stage of decomposition, as a result of the bacterial communities released after the decay of seaweeds. Populations proficient in filter-feeding on algae or suspended particles may proliferate due to increased food resources. Populations with a relatively high tolerance to anaerobic or low-oxygen environments may be able to participate in the sulfur cycle, thereby adapting to hypoxic conditions and emerging as dominant groups in the later stages. Alternatively, during the decay stage of *E. prolifera*, ciliate groups capable of participating in the decomposition process may also assume a dominant position.

Therefore, due to the morphological and functional differences, ciliate populations that can adapt to the environment during the outbreak of *E. prolifera* may indeed become dominant populations under natural selection during the outbreak and participate in the restructuring of the community. In the long run, if *E. prolifera* disasters occur frequently, it may drive the succession of ciliate communities towards tolerance species that can withstand low-oxygen and polluted conditions.

Additionally, several limitations persist in this study. Primarily, regarding the temporal scale, this research solely collected ciliate samples before and after the *Ulva prolifera* bloom from May to July 2025. This approach is inherently restricted and subject to contingency. Furthermore, the sampling sites were confined to the intertidal zones of only three areas in Qingdao. As such, the conclusions reached cannot be guaranteed to be universally applicable to all intertidal regions. Consequently, only the feasibility of using ciliates as a monitoring indicator for the *Ulva prolifera* green tide outbreak can be tentatively established. Additional relevant investigations are essential to validate these findings.

5. Conclusion

This study isolated ciliates in the intertidal zone and identified them using IC-driven filtration, centrifugal enrichment, and staining fixation. The extracted RNA was successfully sequenced by reverse transcribing it into cDNA. The α -diversity analysis was performed on the collected ciliate samples, revealing differences in species richness and composition. Subsequently, the dominant taxa within the ciliate population and a CCA of environmental factors were further analyzed. A co-occurrence network diagram of the ciliate community was constructed through computational analysis to gain insight into the composition and assembly mechanisms of ciliates in the intertidal zone environment. It was observed that the ciliate communities in the three sampling regions had transformed. Before the *E. proliferata* outbreak, the community structure was relatively loose. During the outbreak, the community composition gradually became more complex, with more connections forming among species. Finally, after the *E. proliferata* subsided, a highly complex species structure emerged, characterized by persistent interactions among species. These findings suggest that, as *E. proliferata* influences the intertidal zone habitat, the structure of the ciliate community exhibits a trend from simplicity to complexity, accompanied by a continuous increase in species diversity and the number of interspecific interactions. This research preliminarily reveals the response of the ciliate community assembly pattern to the *E. proliferata* outbreak.

Regarding the species succession within the ciliate community and its spatiotemporal variations, a detailed analysis of the population composition within the ciliate community was conducted before and after the *E. proliferata* outbreak. It was discovered that the outbreak can lead to the succession of dominant species among ciliates due to specific morphological and functional differences. For instance, during the outbreak of *E. proliferata*, populations that are sensitive to oxygen concentration tend to decline in number. At the same time, ciliate groups involved in decomposition processes predominate in the later stages of the outbreak. Additionally, the alterations in environmental factors triggered by the outbreak significantly influence the changes in the ciliate community structure. These observations highlight the interactive relationship between the intertidal protist community and its environment, shedding light on the role and significance of intertidal ciliates within the context of microbial network dynamics influenced by *E. proliferata*. Moreover, they suggest that ciliates may serve as a biological indicator group for the occurrence of *E. proliferata* green tides.

Acknowledgments

The Biology Laboratory of Ocean University of China supported this work. We also thank LC Sciences for their assistance with gene sequencing. The authors are grateful to all those who assisted with the fieldwork during this study. At the same time, we extend our sincere thanks and best wishes to the teachers who assisted in the preparation of this thesis. Their expertise and advice have been invaluable in shaping this work.

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