Microbial ecology might serve as new indicator for the influence of green tide on the coastal water quality: Assessment the bioturbation of Ulva prolifera outbreak on bacterial community in coastal waters

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**ABSTRACT**

The massive outbreak of *Ulva prolifera* had brought significant ecological impacts on marine coastal areas. In order to clarify the influence of outbreak of *U. prolifera* on marine microbial ecosystem, the microbial communities in different stages of blooming were analyzed by high-throughput sequencing. The sequences were clustered into 2979 operational taxonomic units (OTUs), and Shannon estimator varied from 3.241 to 4.892. The OTUs number and Shannon estimator demonstrated that both parameters were higher in samples HY_2, QD_2, RZ_2 than in HY_1, QD_1, RZ_1, indicating that the massive outbreak of *U. prolifera* caused the increase of microbial community diversity in coastal waters. The outbreak of *U. prolifera* led to increase in the abundance of some bacteria. According to the network analysis, the differences in hot bacteria in different stages indicated that the outbreak of *U. prolifera* caused the variation of microbial community. Based on Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway analysis, the abundance of 41 metabolic pathways had changed due to the outbreak of *U. prolifera*. The microbial community could recover gradually after the disappearance of *U. prolifera*. The relative abundance of dominant genera *Methylophaga*, *Polaribacter*, *Marinobacterium*, *Marivita*, *Tenacibaculum*, *Vibrio*, *Planktomarina*, *Crocinitomix*, *Draconibacterium*, and *norank_f_Alteromonadaceae* in stage 6 (after the outbreak of *U. prolifera*) kept the same level as stage 1 (before the outbreak of *U. prolifera*). These findings suggested that microbial ecology might serve as new indicator for the influence of green tide on the coastal water quality since marine microbial ecosystem could be temporarily bioturbated during the outbreak of the green tide.

**ARTICLE INFO**

**Keywords:**
- *Ulva prolifera*
- Green tide
- Microbial communities
- Bioturbation
- Ecological impacts
- Coastal water

**1. Introduction**

In recent years, increased anthropogenic activities and climate change have resulted in the formation of massive outbreak of macroalgae, which has been a widespread environmental problem in coastal marine areas (Choi et al., 2006; Conover et al., 2016; Guidone and Thornber, 2013; Perrot et al., 2014; Smetacek and Zingone, 2013; Wei et al., 2018). The massive blooming of macroalgae is associated with intense eutrophication, and have been frequently happened in North America (Vadas and Beal, 1987), Europe (Lappalainen and Ponni, 2000), and the Asia-Pacific region (Morand and Briand, 1996; Ye et al., 2011). The macroalgae blooming caused by *Ulva prolifera* in the Southern Yellow Sea of China since 2007 was the largest green tide in the world (Liu et al., 2010; Liu et al., 2016).

The large-scale outbreak of *U. prolifera* can bring significant adverse ecological and economic impacts on marine coastal areas (Wang et al., 2009; Xing et al., 2015; Liu et al., 2016; Li et al., 2018; Xing et al., 2018), such as species invasion (Zhao et al., 2018; Zhang et al., 2019a, b), aquaculture and tourism (Zhang et al., 2019a, b). The massive outbreak of *U. prolifera* has changed marine community structures and functions, and they have had negative impacts on coastal marine environment by shading, biomass decomposition, and anoxia (Nelson et al., 2008). The *U. prolifera* outbreak have made negative influences on other benthic communities (Den Hartog, 1994), microbenthic...
communities (Sundbäck et al., 1990), macrofauna (Norkko and Bonsdorff, 1996), and microbial community (Lín et al., 2017). Nevertheless, the effect of the massive outbreak of *U. prolifera* on microbial ecology in coastal marine has not been reported.

The aim of the present study was to analyze bacterial communities in coastal environments where the blooming of *U. prolifera* happened in summer, using Illumina MiSeq sequencing method. The results in this study would provide initial information for evaluating the impact of the outbreak of *U. prolifera* on the coastal microbial ecology and searching for new indicators for the influence of green tide on the coastal water quality.

2. Materials and methods

2.1. Study areas and sampling

The water samples were collected from the coast of Haiyang, Qingdao, Rushan, Rizhao, and Yantai City, in China. During sampling, outbreaks of *U. prolifera* were observed in Haiyang, Qingdao, Rushan, Rizhao. Yantai was chosen as a negative control for no blooming of *U. prolifera*. The samples were collected from different periods of outbreak of *U. prolifera*: HY_1, QD_1, RS_1, RZ_1 (Stage 1, Group 1) were collected from one month before the blooming (*C₅* = 0 *g/m²*), HY_2, QD_2, RS_2, RZ_2 (Stage 2, Group 2) were collected from the beginning of the blooming (a small amount of *U. prolifera* drifted into the sampled coastal marine, *C₅* = 5–15 *g/m²*), HY_3, QD_3, RS_3, RZ_3 (Stage 3, Group 3) were collected from the massive blooming (*C₅* = 70–100 *g/m²*), HY_4, QD_4, RS_4, RZ_4 (Stage 4, Group 4) were collected from the end of the blooming (*U. prolifera* was collected or faded in the sampled coastal marine, *C₅* = 1–10 *g/m²*), HY_5, QD_5, RS_5, RZ_5 (Stage 5, Group 5) were collected from one week after the blooming (*C₅* = 0 *g/m²*), and HY_6, QD_6, RS_6, RZ_6 (Stage 6, Group 6) were collected from one month after the blooming (*C₅* = 0 *g/m²*). The samples YT_1, YT_2, YT_3, YT_4, YT_5, and YT_6 (Group Con) were collected from the end of the blooming (*C₅* = 0 *g/m²), HY_6, QD_6, RS_6, RZ_6 (Stage 6, Group 6) were collected from one month after the blooming (*C₅* = 0 *g/m²).

2.2. DNA extraction and PCR amplification

Microbial DNA was extracted from the samples using the E.Z.N.A.* soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V4-V5 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 515F (5′- GTG CCT ACC CTA ATG GTT GAC C-3′) and 907R (5′-CGT ATCT TCC TGT TAT TGA TCI-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μl mixture containing 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™ -ST (Promega, USA) according to the manufacturer’s protocol.

2.3. Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: SRP223104).

2.4. Data analysis

Raw fastq files were demultiplexed, quality-filtered by Trimomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotides mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

3. Results

3.1. Overview of diversity of bacteria in the samples collected before and after the outbreak of *U. prolifera*

A total of 1,144,934 reads with an average length of 395 bp were obtained from all the 30 samples. These sequences clustered into 2,979 OTUs at a 97% similarity level. The Good’s coverages of all samples were greater than 0.98, suggesting that the sequencing depth of all samples were sufficient to represent the bacterial community (Table 1). Shannon diversity estimator (Chao and Bunge, 2002; Magurran, 1988) showed variations of 3.241–4.892 in these samples (Table 1). OTUs number and Shannon estimator (Table 1) demonstrated that both parameters were higher in samples HY_2–4, QD_2–4, RZ_2–4 than in HY_1, QD_1, RZ_1, indicating that the mass outbreak of *U. prolifera* caused the increase of microbial community diversity in coastal zone of Haiyang, Qingdao, and Rizhao.

3.2. Taxonomic composition changes caused by the outbreak of *U. prolifera*

All of the sequences were classified from phylum to genus level, and 2979 OTUs were identified in all of these samples. The Venn diagram (Fig. 1) was used to show the shared and specific OTUs in different periods of the *U. prolifera* blooming. As shown in Fig. 1, the numbers of OTUs shared by Groups 1&2, 2&3, 3&4, 4&5, 5&6 were 1140, 1177, 1013, 1077, and 1065. It indicated that the microbial community of the coastal seawater changed greater with the outbreak of *U. prolifera*.
coverage is used to evaluate the depth of sequencing. Shannon index is used to calculate the diversity of bacteria community. The for city of Rushan, RZ is for city of Rizhao, and YT is for city of Yantai. The in the sampling period. HY is for city of Haiyang, QD is for city of Qingdao, RS is in the blooms, HY_4, QD_4, RS_4, RZ_4 (Group 4) were collected from the massive blooms, HY_3, QD_3, RS_3, RZ_3 (Group 3) were collected from the beginning of the blooms (a small amount of U. prolifera led to increase or decrease in the abundance of some bacteria (Fig. 2). However, comparing samples collected in stages one month before and after the outbreak of U. prolifera (Stages 1 and 6) in Haiyang, Qingdao, Ruzhan and Rizhao, the relative abundance of most genera Methylophaga, Polaribacter, Marinobacterium, Marinivita, Tenacibaculum, Vibrio, Planctomarina, Crocinotomix, Draconibacterium, norank_f_Altersonadaceae in stage 6 kept the same level as stage 1. The same variation trends were observed in Alteromonas, Planctomyces and Marinomonas detected in samples collected from Haiyang and Rushan, Nautilia and Ruederia detected in samples collected from Qingdao and Rushan, norank_f_Rhodobacteraceae and Thalassospira detected in samples collected from Haiyang, Rushan and Rizhao, unclassified_f_Rhodobacteraceae and norank_c_Cyanobacteria detected in samples collected from Rushan, Pelagibacter found in samples collected from Haiyang. The relative abundance of the most of bacteria such as norank_f_Rhodobacteraceae, unclassified_f_Rhodobacteraceae, Methylophaga, Polaribacter, norank_c_Cyanobacteria, Bacteroidia, Planctomyces, Tenacibaculum, Vibrio, Marinomonas, Thalassospira, and Draconibacterium did not change significantly in the samples (collected from Yantai). It indicated that the outbreak of U. prolifera had made some influence on the microbial community while the microbial

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shannon$^{(1)}$</th>
<th>Coverage$^{(1)}$</th>
<th>OTUs</th>
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<td>843</td>
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<td>HY_6</td>
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<td>YT_6</td>
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</table>

Fig. 1. The shared and specific OTUs in different groups collected from different periods of the U. prolifera blooms display by Venn graph. Each triangle in the figure represents a group. The numeral in the overlapped part shows the OTUs number shared. The number in the unoverlapped parts shows the specific OTUs number in each group. The groups 1, 2, 3, 4, 5, 6 refer to the six stages in U. prolifera blooms: Group 1 is for the stage one month before the blooms, Group 2 is for the stage of the beginning of the blooms, Group 3 is for the stage of the massive blooms, Group 4 is for the stage of the end of the blooms, Group 5 is for the stage of one week after the blooms, Group 6 is for the stage of one month after the blooms.

3.3. Differential species found in different stages of the U. prolifera blooming

LEfSe was used to find the potential discriminating taxa between different groups. The results showed that there were 27 bacterial taxa distinguishing groups 1–6 with LDA value greater than 4.0 (Fig. 3). One genus Methylophaga was enriched in group 1, 1 class (Bacteroides), 1 family (Draconibacteraceae), 1 order (Bacteroidia_Incertae_Sedis) and 1 genera (Scionambaeicola) were enriched in group 2, 1 phylum (Bacteroidetes), 2 order (Oceanspirales and Sphingobacteriales), 4 genera (Marinobacterium, Polaribacter, Litoricola and norank_c_Ycytrophaceae), 1 class (Sphingobacteria) and 2 family (Saprospiraceae and Litoricolaceae) were enriched in group 3, 1 genera (norank_f_Rhodobacteraceae), 1 family (Cymobacteriaceae), 1 phylum (Actinobacteria) and 1 class (Actinobacteria) were enriched in group 4, 1 order (Vibrionales), 1 family (Vibrionaceae) and 1 genera (Vibrio) were enriched in group 5, 1 phylum (Proteobacteria), 2 genera (Nautilia and Erythrobacter), 1 order (Sphingomonadales) and 1 family (Erythrobacteraceae) were enriched in group 6.

3.4. PCoA analysis

To further analyze the effect of U. prolifera on bacterial community composition, we summarized the binary chisq distances among the samples via PCoA. Samples were clustered according to the microbial community on genus level (Fig. 4). The results in Fig. 4 showed that group 1, 4, 5, 6 and control clustered well to PC1, and group 2 and 3 clustered well to PC2. The first two axes (PC1 and PC2) explained 20.18% of the taxonomic information. In addition to the blooming, the clustering results indicated that geographical and other environmental factors also might be important factors influencing the microbial communities of the coastal marine.
3.5. Correlation analysis of environmental factors

The influence of environmental factors on the microbial communities of each sample was quite different. Redundancy analysis (RDA) was performed to identify correlations between taxonomic composition on genus level and environmental factors, such as phosphate (PO$_4^{3-}$), total phosphorus (TP), ammonium nitrogen (NH$_4^+$), nitrate nitrogen (NO$_3^-$), nitrite nitrogen (NO$_2^-$), total nitrogen (TN), silicate (SiO$_3^{2-}$), inorganic carbon (IC), total organic carbon (TOC), total carbon (TC), sulphide (S) and pH (Fig. 5). The first two axes explained 23.35% of the taxonomic information. It was indicated that the microbial community structure of the samples was affected by many environmental factors.

Correlation heatmap in Fig. 6 showed the relationship between the top 50 bacteria (in genus level) and environmental factors. As shown in Fig. 6, bacteria Flavobacterium, norank_f_Rhodobacteraceae, Fluvicola, Marinobacterium, norank_f_Cryomorphaceae, Marivita, Polaribacter, Hyunsooolella, norank_f_Saprospiraceae, unclassified_o_Bacteroidales, Marinibacter, Draconibacterium and Seonamhaeicola were positively correlated with PO$_4^{3-}$, pH, TN, SiO$_3^{2-}$, TC, TOC, NO$_2^-$ and IC. The results indicated that PO$_4^{3-}$, pH, TN, SiO$_3^{2-}$, TC and TOC were important environmental factors that affected the microbial community structure.

3.6. Network analysis on genus level

To reveal the influence of green tide on the essential or hot bacteria in water, the network analysis was used to investigate the co-occurrence patterns between different bacteria on genus level in different stages of $U$. prolifera blooming. The relationships among the top 50 genera in stage 1 (Fig. 7a), stages 2, 3 and 4 (Fig. 7b), stages 5 and 6 (Fig. 7c) were shown in Fig. 7. The hot bacteria (in genus level) in the $U$. prolifera outbreak stages (Fig. 7b) were Acidimicrobiaceae, unclassified, OM190, unclassified, OM43, clade and Methylotenera. The relationships among the top 50 genera were negatively correlated with PO$_4^{3-}$, pH, TN, SiO$_3^{2-}$, TC, TOC, NO$_2^-$ and IC. The results indicated that PO$_4^{3-}$, pH, TN, SiO$_3^{2-}$, TC and TOC were important environmental factors that affected the microbial community structure.
However, there were 17 genera related with more than 6 genera in the green tide of 2014 in Yellow Sea (Zhao et al., 2018). Bio-invasion was the most important problem of the blooming, and it might be a great propulsive pressure to the local ecosystem. The relative abundances of the top 21 bacteria changed with the stages of the blooming. LEfSe and LDA analysis demonstrated that there were 27 different bacterial taxa in different stages of the blooming. All of the results indicated that the massive outbreak of U. prolifera had influence on the microbial community of the coastal waters. Lin et al. (2017) found that massive blooming of U. prolifera had serious effects on the bacterial community structures in aquaculture environment. The pond water with U. prolifera was dominated by Actinomycetales, and the pond water without U. prolifera was dominated by Rhodobacterales. The other studies showed that fish community (Le Luherne et al., 2016; Paumier et al., 2018) and macrozoobenthos like invertebrates, bivalve drifters (Quillien et al., 2015) also were affected by the green tides in coastal areas.

Although the outbreak of U. prolifera could affect the microbiome structure in coastal marine, the impacts were not everlasting. On the one hand, the microbiome in the natural environment could be influenced by many environmental factors except for the U. prolifera blooming. Compared with other environmental factors, the period of U. prolifera blooming was very short and limited. The PCoA clustering and RDA analysis confirmed that in addition to the blooming, other environmental factors also might be important factors influencing the microbial communities of the coastal marine. On the other hand, the natural seawater bodies had a strong self-purification ability, which could effectively deal with the impact of U. prolifera blooming, so that the microbial community structure of seawater would recover after the disappearance of the U. prolifera. In fact, environmental factors and the blooming of U. prolifera were correlated with each other. The environmental variation induced the blooming of U. prolifera, and then

![Fig. 3.](image-url)

(a) The cladograms of bacterial lineages with significant difference in different stages. The groups 1, 2, 3, 4, 5, 6 refer to the six stages in U. prolifera blooming: 1 is for the stage one month before the blooms, 2 is for the stage of the beginning of the blooms, 3 is for the stage of the massive blooms, 4 is for the stage of the end of the blooms, 5 is for the stage of one week after the blooms, 6 is for the stage of one month after the blooms. The bacterial groups from phylum to species level are listed from center to outside. Each circle is for the stage of one month after the blooms. The bacterial groups from stage of the end of the blooms, 5 is for the stage of one week after the blooms, 6 beginning of the blooms, 3 is for the stage of the massive blooms, 4 is for the stage of the blooming. The microorganisms became more active while the relationship between bacteria became closer under the conditions of U. prolifera blooming.

3.7. Functional prediction analysis based on Kyoto Encyclopedia of genes and Genomes (KEGG) pathways

Functional profile of microbial community was predicted based on the KEGG database, and most of the functions were shared by all of the samples. Comparative analysis was made on the abundance of the level 2 KEGG metabolic pathways. The abundance of 41 metabolic pathways had changed due to the outbreak of U. prolifera. The detailed level 2 KEGG metabolic pathways variations in different stages in U. prolifera blooming were shown in Fig. 8. The relative abundance of the 41 metabolic pathways shown in Fig. 8 decreased when the U. prolifera blooming broke out (in group 2 and 3), and then increased to as high as before (the Group 1 and Con). The expression level of pathways which had changed greatly were pathways correlated to amino acid metabolism, carbohydrate metabolism, membrane transport, energy metabolism, replication and repair, cell motility, cellular processes and signaling, and xenobiotics biodegradation and metabolism. The results indicated that the outbreak of U. prolifera affected the expression of many functions in the KEGG metabolic pathways of the bacteria, and these functions will recover after the blooming.

4. Discussion

The massive outbreak of U. prolifera had serious impacts on the microbial community of the coastal waters. In this study, the Venn diagram showed that there were new species produced in each stage of U. prolifera blooming. The Venn chart showed that there were 2979 OTUs found in these 6 groups, and 457 OTUs shared by all of the groups. It indicated that U. prolifera blooming brought in new microbial species to the coastal area. Macrocystis invasion was also found in the green tide of 2014 in Yellow Sea (Zhao et al., 2018). Bio-invasion was the most important problem of the blooming, and it might be a great propulsive pressure to the local ecosystem. The relative abundances of the top 21 bacteria changed with the stages of the blooming. LEfSe and LDA analysis demonstrated that there were 27 different bacterial taxa in different stages of the blooming. All of the results indicated that the massive outbreak of U. prolifera had influence on the microbial community of the coastal waters. Lin et al. (2017) found that massive blooming of U. prolifera had serious effects on the bacterial community structures in aquaculture environment. The pond water with U. prolifera was dominated by Actinomycetales, and the pond water without U. prolifera was dominated by Rhodobacterales. The other studies showed that fish community (Le Luherne et al., 2016; Paumier et al., 2018) and macrozoobenthos like invertebrates, bivalve drifters (Quillien et al., 2015) also were affected by the green tides in coastal areas.

![Fig. 7b](image-url)

(Stages 2, 3 and 4, when the U. prolifera outbroke), and 14 genera related with more than 6 genera in Fig. 7c (Stages 5 and 6, after the blooming). The microorganisms became more active while the relationship between bacteria became closer under the conditions of U. prolifera blooming.
Fig. 4. Principal coordinates analysis (PCoA) based on genus level. The numbers 1, 2, 3, 4, 5, 6 refer to the six stages in *U. prolifera* blooms: 1 is for the stage one month before the blooms, 2 is for the stage of the beginning of the blooms, 3 is for the stage of the massive blooms, 4 is for the stage of the end of the blooms, 5 is for the stage of one week after the blooms, 6 is for the stage of one month after the blooms. The Con refers to the control groups collected from Yantai, where *U. prolifera* has not outbroken.

Fig. 5. Redundancy analysis shows the relationships between environmental variables and each sample based on OTU level. Abbreviations: phosphate (PO$_4^{3-}$), total phosphorus (TP), ammonium nitrogen (NH$_4^+$), nitrate nitrogen (NO$_3^-$), nitrite nitrogen (NO$_2^-$), total nitrogen (TN), silicate (SiO$_3^{2-}$), inorganic carbon (IC), total organic carbon (TOC), total carbon (TC), sulphide (S) and pH. The numbers 1, 2, 3, 4, 5, 6 refer to the six stages in *U. prolifera* blooms: 1 is for the stage one month before the blooms, 2 is for the stage of the beginning of the blooms, 3 is for the stage of the massive blooms, 4 is for the stage of the end of the blooms, 5 is for the stage of one week after the blooms, 6 is for the stage of one month after the blooms. The Con refers to the control groups collected from Yantai, where *U. prolifera* has not outbroken.
environmental factors would change with the outbreak of U. prolifera (Li et al., 2015; Li et al., 2019; Liu et al., 2013). Multiple geographical, aquacultural and other biological factors were found correlated with the blooming of U. prolifera (Liu et al., 2013). The concentrations of dissolved organic carbon (DOC) and fluorescent dissolved organic matter (FDOM) were found increased significantly in the green tide areas (Li et al., 2019). Previous investigation showed that blooming might aid seagrasses to alleviate toxic effects of ammonium under eutrophic conditions and ecosystem to resistant to anthropogenic disturbance (Moreno-Marín et al., 2016). All of these studies suggested that the blooming of U. prolifera would induce the variation of a series of environmental factors, including the microbial community of the coastal seawater. The marine self-regulation could lead to the recovery of the microbial community shift after the disappearance of the blooming.

Functional prediction analysis was made based on KEGG database to explore the reasons for these variations of microbiome structure. The level 2 KEGG metabolic functions changed in different stages in U.

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**Fig. 6.** Correlation heatmap between the top 50 bacteria (in genus level) and environmental factors. The "***" refers to the level of the correlation, and *: 0.01 < P ≤ 0.05, **: 0.001 < P ≤ 0.01, ***: P ≤ 0.001. Abbreviations: phosphate (PO4\(^{3-}\)), total phosphorus (TP), ammonium nitrogen (NH4\(^+\)), nitrate nitrogen (NO3\(^-\)), nitrite nitrogen (NO2\(^-\)), total nitrogen (TN), silicate (SiO3\(^{2-}\)), inorganic carbon (IC), total organic carbon (TOC), total carbon (TC), sulphide (S) and pH.
Fig. 7. Network analysis of the top 50 species on genus level in (a) stage 1, (b) stages 2, 3 and 4, (c) stages 5 and 6 samples. The different colors of nodes represent different species, and the color of the line indicates the positive and negative correlation, red indicates the negative Pearson's correlation, and green indicates the positive Pearson's correlation. The more lines there are, the stronger the link between the species and other species.
blooming. The expression level of pathways, such as amino acid metabolism, carbohydrate metabolism, membrane transport, energy metabolism, replication and repair, cell motility, cellular processes and signaling decreased when the *U. prolifera* blooming broke out (in group 2 and 3), and then increased to as high as before (the Group 1 and Con). It was indicated that these pathways was repressed by the outbreak of *U. prolifera*, and then the expression levels recovered as control. The massive outbreak of *U. prolifera* might block the light invasion to the marine, and it might cause the amino acid metabolism, carbohydrate metabolism, replication and repair and other pathways repressed. Then the blooming might introduce the increase of organic chemicals such as nitrogen and phosphorus, and it might cause the expression level of the amino acid metabolism, carbohydrate metabolism or other pathways would be increased gradually. When *U. prolifera* disappeared entirely, the expression level of these pathways would recover as usual. Other studies reported also confirmed these results. The influence of *U. prolifera* species blooming on chlorophyll-a concentration in the Southern Yellow Sea was investigated previously (Sun et al., 2018). It was found that the concentration of chlorophyll-a increased with the growth of *U. prolifera* from April to mid-May, and then

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**Fig. 8.** The level 2 KEGG metabolic functions changed in different stages in *U. prolifera* blooms. The numbers 1, 2, 3, 4, 5, 6 refer to the six stages in *U. prolifera* blooms: 1 is for the stage one month before the blooms, 2 is for the stage of the beginning of the blooms, 3 is for the stage of the massive blooms, 4 is for the stage of the end of the blooms, 5 is for the stage of one week after the blooms, 6 is for the stage of one month after the blooms.
decreased sharply with the dramatically increased coverage of *U. prolifera* in June, and in the end slowly recovered and finally stabilized as *U. prolifera* decreased in July. Compared with other *Pseudomonas stutzeri* strains, the Clusters of Orthologous Groups (COGs) analysis revealed that the strain *Pseudomonas* sp. SI-3 isolated from *U. prolifera* had a higher expression of genes assigned to transcriptional regulation and signal transduction, and significant gene loss in several aromatic compounds degradation pathways (Fu et al., 2018). The morphology and photosynthetic performances of the green tide forming alga *U. prolifera* also changed in the blooming process (Zhang et al., 2013; Xu et al., 2012). All of these investigations indicated that the metabolic pathways would be altered by the blooming of *U. prolifera*, and the expression level of these pathways would recover after the disappearance of blooming.

5. Conclusions

The investigation demonstrated the temporary bioturbation of the massive outbreak of *U. prolifera* on the microbiome structure of the coastal waters. The microbial community structure would recover gradually after the disappearance of *U. prolifera*. The coastal marine environment changed with the outbreak of *U. prolifera*, and the self-purification capacity of the marine required the change of the functions of the bacteria. The expression level of the 41 metabolic pathways decreased when the *U. prolifera* blooming broke out, and then increased to as high as usual. The change of the function genes further determined the variation of the microbiome structure, which is a reflection of the self-purification capacity of the coastal marine environment. Since green tide could lead to temporary bioturbation on the marine micro-bial ecosystem, microbial ecology might serve as new indicator for the influence of green tide on the coastal water quality.

CRediT authorship contribution statement

Jiahua Wang: Writing - original draft, Investigation, Methodology, Software. Jian Lu: Conceptualization, Methodology, Supervision, Writing - review & editing. Yuxuan Zhang: Investigation, Data curation, Validation. Jun Wu: Validation, Methodology, Writing - review & editing.

Acknowledgements

This work was supported by National Natural Science Foundation of China (41671319, 41907152), Taishan Scholar Program of Shandong Province (No. tspn201812116), One Hundred Talents Program of the Chinese Academy of Sciences (KJF-STS-QYXZ-114), and Two-Hundred Talents Plan of Yantai (Y739011021).

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