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Effects of green tide on microbial communities in waters of the Jiangsu coastal area, China

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Abstract

Recently, green tide outbreaks have resulted in severe coastal ecology and economic effects in China. Jiangsu coastal areas are usually the site of early green tide outbreaks. To clarify the effects of green tide outbreaks in Jiangsu coastal areas, this study analyzed microbial communities during green tide-free and green tide outbreak periods (May and July, respectively) through 16S rDNA sequencing. Sequences were clustered into 4117 operational taxonomic units (OTUs), 1044 and 3834 of which were obtained from the May and July groups, respectively. Redundancy analysis indicated that green tide occurrence was closely associated with the temperature, pH, and concentrations of various nutrients. Diversity analysis revealed that the July group had a richer microbial community than the May group, in agreement with the results of propagule culture. Moreover, comparative analysis revealed that samples in the May and July groups clustered together. According to Megan analysis, the May group had much more *Psychrobacter*, *Sulfitobacter*, and *Marinomonas* than the July group, whereas the other genera were predominantly found in July, such as *Ascidiacerhabitans*, *Synechococcus Hydrotalea*, and *Burkholderia-Paraburkholderia*. These findings suggest that green tide outbreaks affect marine microbial communities, and detecting the changes in the identified genera during green tide outbreaks may contribute to green tide forecasting.

Practitioner Points

- Jiangsu coastal areas are usually the site of early green tide outbreaks.
- Green tide occurrence was related to the concentrations of various nutrients.
- Microbial species and community structure significantly changed after green tide outbreak.

Yiming Yuan and Biyun Luo have contributed equally to this work and share first authorship.

KEYWORDS
16S rDNA, green tide, macroalgae, microbial community, Southern Yellow Sea

INTRODUCTION

Green tides are harmful algal blooms caused by the explosive reproduction of green macroalgae (Fu et al., 2022; J. L. Liu et al., 2021) During green tide blooms, macroalgae reproduce rapidly and drift with the wind and sea surface circulation (Jin et al., 2018; Son et al., 2015). The large-scale green tide blooms alter the survival and reproduction of other marine organisms, and can even affect human activities and health; moreover, decaying algal masses wash ashore and cause substantial ecological and economic losses (Crispim et al., 2003; Jin et al., 2018; Paul et al., 2007; Steinberg & de Nys, 2002; C. Zhang et al., 2019). In recent years, green tides have frequently occurred in coastal marine areas (Landsberg, 2002), such as those in Europe, North America and the Asia-Pacific region (Lappalainen & Ponni, 2000; Yabe et al., 2009). In China, since 2007, the macroalgae blooms have continually occurred in the Southern Yellow Sea for 15 years, usually from May to August each year (W. Guo et al., 2016; Y. Wang et al., 2019). Direct losses caused by macroalgal blooms from 2008 to 2012 amounted to approximately two billion RMB (X. Q. Liu et al., 2016). The green tide in China is usually caused by *Ulva prolifera*. Affected by climatic, thermal, nutritional, biological and other factors, *U. prolifera* easily blooms in shallow sea and offshore coastal areas, then erupts into a green tide (X. Q. Liu et al., 2016; Mayali & Azam, 2004).

In general, the eutrophication of seawater environments is regarded as the cause of most green tides (Gladyshev & Gubelit, 2019). In the case of China, some researchers have suggested that coastal aquaculture ponds have resulted in outbreaks of green tides. Some believe that the cause of green tide outbreaks might be *Porphyra* aquaculture, because the macroalga *U. prolifera* has been found to be the dominant fouling species growing on *Neopyropia yezoensis* rafts (D. Y. Liu et al., 2009). Marine ecosystems are enormous and complex, and marine microbial communities contribute to productivity, nutrient cycling, biological decomposition, and marine ecosystem resilience (Smriga et al., 2010). In addition, macroalgae have complex relationships with bacteria.

Currently, increasing numbers of signal transduction molecules among microorganisms are being identified, including defensins, pheromones, attractants, and other signaling molecules (Crispim et al., 2003; Lubarsky et al., 2010; Paul et al., 2007; Steinberg & de Nys, 2002) Some of these chemical signals are universal, whereas others are species specific (J. Q. Li et al., 2011). The organic compounds released by macroalgae can induce the growth of bacteria; subsequently, macroalgae release secondary metabolites that inhibit bacterial attachment (Maximilien et al., 1998). Meanwhile, the attached bacteria inhibit other organisms' attachment (Rao et al., 2006). Some bacteria even degrade the cell components of other organisms (Weinberger et al., 1999).

Although the direct cause of green tide is controversial, green tides in the Southern Yellow Sea often originate from the coastal waters of Jiangsu Province (Xu et al., 2009). To verify the correlations of marine microbial communities with periods before and after green tide outbreaks, we collected water samples from the coastal waters of Jiangsu Province before and after green tide outbreaks. We then performed 16S rDNA sequencing to analyze the differences in microbial communities during the green tide outbreaks. Our results provide information that may aid in understanding and predicting green tide outbreaks.

MATERIALS AND METHODS

Sample collection

Samples were collected from the coastal waters of four cities in Jiangsu, China, during May (9th and 10th) and July (2nd and 3rd) of 2019. Samples were collected in Lianyungang (L), Sheyang (S), Dafeng (D), and Rudong (R) from north to south, and the numbers of sample sites were determined on the basis of the different coastline lengths (Figure 1a). The northernmost site, Lianyungang, had the longest coastline, containing five sampling sites (M/J-L1, 119°30′17.16″E, 34°45′50.10″N; M/J-L 2, 119°42′05.76″E, 34°45′50.10″N; M/J-L 3, 119°53′56.22″E, 34°45′50.10″N; M/J-L 4, 120°13′05.94″E, 34°45′50.10″N; and M/J-L 5, 120°31′16.02″E, 34°45′50.10″N), and each sample from five sampling sites was denoted M-L and J-L according to the sampling times of May and July, respectively. Sheyang, samples were collected from three sampling sites denoted M/J-S1 (120°44′24.96″E), M/J-S2 (33°45′51.00″N, 120°56′46.56″E, 33°45′51.00″N), and M/J-S3 (121°16′37.20″E, 34°45′50.10″N). Next to Sheyang, two sampling sites in Dafeng were located at 121°34′38.58″E, 33°13′41.10″N (M/J-D1) and

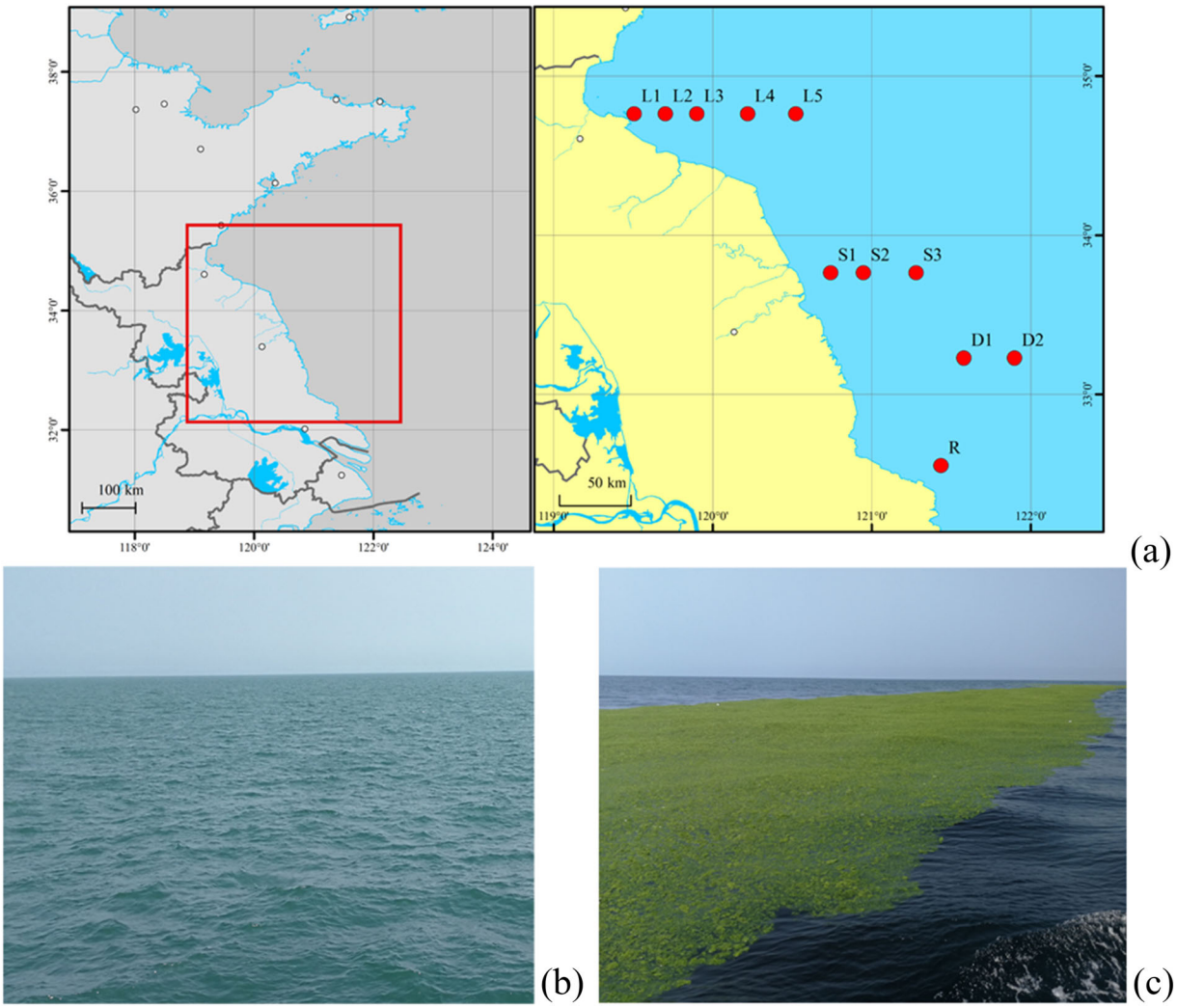

FIGURE 1 Sampling sites in coastal areas of Jiangsu Province, China. Samples were collected in Lianyungang (L), Sheyang (S), Dafeng (D), and Rudong (R) from north to south. Abbreviations: L1, Lianyungang Site 1; L2, Lianyungang Site 2; L3, Lianyungang Site 3; L4, Lianyungang Site 4; L5, Lianyungang Site 5; S1, Sheyang Site 1; S2, Sheyang Site 2; S3, Sheyang Site 3; D1, Dafeng Site 1; D2, Dafeng Site 2; R, Rudong site

121°53′48.30″E, 33°13′41.10″N (M/J-D2). The southernmost sample site in Rudong was located at 121°26′03.48″E, 32°33′06.66″N, denoted by M-R and J-R. For each site, water samples were collected approximately 15 m below the water surface with 0.5 m sampling depth.

For sequencing, because the large-scale green tide did not bloom in early May, the samples collected in May were pooled into one sample for each sampling site, and every sample was repeatedly sampled three times to eliminate error. A 500 ml volume of subseawater (0.5–1 m) for each sampling was collected with a water collector (GB 17378. 7–2007, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China) and stored in an ice bucket until filtration through 0.22 μm membranes (60 mm, Jinjing, CHN). The filter paper was a cellulose acetate microporous filter membrane with a diameter of 60 mm. All samples were stored at −80°C (Thermo, USA). Additionally, the water samples from each sample sites were cultured for propagule analysis.

To analyze the presence of green tide outbreaks, visual inspection is the method of choice. Green tide was clearly not observed in May (Figure 1b) but was observed in July (Figure 1c). In addition, in this study, the propagules of each sample were cultured. The 1 L water samples were filtered with 100-mesh cloth, and the filters were transported in the dark at low temperature. In the laboratory, samples were cultured under a temperature of 16°C to 18°C, light intensity of 80–100 μmol/(m^2·s), and light–dark cycle of 12 h. Culture media were

changed every 5 days. The total algal seeding was counted 20 days later. Sediment samples were resuspended in sterile seawater (salinity: 2.5% to 3.0%), the supernatant was removed as a control, and the sediment was cultured in seawater (with 500 μmol/L dissolved inorganic nitrogen [DIN] and 30 μmol/L PO_4-P). Culturing was performed as described above. Additionally, dry air was bubbled into the culture beakers 7 days later, and nutrients were supplied every 7 days. The algal seeding, including spores, gametes, zygotes, and micropropagules, was counted after 3–4 weeks of culture. Moreover, the *Ulva* species were identified by molecular identification. After DNA extraction and PCR reaction, the amplified products were sequenced to identify species (Akcali & Kucuksezgin, 2011).

Analysis of environmental factors

Environmental factors, such as pH, dissolved oxygen (DO), NO_2-N and PO_4-P, were determined with Chinese standard methods (HJ506-2009, Ministry of Environmental Protection of the People's Republic of China; HY/T 147.1-2013, State Oceanic Administration of the People's Republic of China). Briefly, pH was measured through potentiometry measurement; DO was determined through the iodimetry method; NO_2-N was determined through the diazo-azo method; NO_3-N was determined through the zinc cadmium reduction method; NH_4-N was determined through the sodium hypobromite oxidation method; PO_4-P was determined through ascorbic acid reduced phosphomolybdate blue spectrophotometry; and SiO_3-Si was determined through silicon molybdenum blue spectrophotometry. Spectrophotometry was used to detect the visible light.

DNA extraction and high-throughput sequencing

Genomic DNA was extracted from all samples with a DNeasy plant mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The quantity of isolated genomic DNA was verified with agarose gel electrophoresis and with a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). Specific primers with barcodes were used for amplification of the V3–V4 region, which is the best choice for marine bacterial detection, because it can successfully detect all clones at taxonomic resolution (Wear et al., 2018). Every sample was amplified with three replications, and PCR amplification products were quantified with a QuantiFluor™-ST Handheld Fluorometer with UV/Blue Channels (Promega, USA). For library construction, approximately 580 bp DNA fragments were prepared and sequenced on an Illumina PE250 (Illumina, USA) instrument.

Bioinformatics analysis

The sequences with low quality (>20), lengths less than 50 bp, and presence of N bases were removed. On the basis of the overlap of reads, paired reads were merged. Chimeras were removed with Usearch software version 7.1 (Edgar, 2010) (http://drive5.com/uparse/) and the gold database. Obtained reads were assembled, and then, the clean reads were created. Clean reads were clustered into operational taxonomic units (OTUs) at 97% similarity, and chimeras were removed again with Usearch software. The obtained OTUs were BLAST searched against SILVA, dataset covering the three domains of Archaea, Bacteria and Eukaryota, which updates data while preserving the original data (Quast et al., 2013) (Release119, http://www.arb-silva.de). Taxonomic analysis of each sample was performed with the RDP classifier algorithm (Q. Wang et al., 2007).

To confirm the microbial diversity, the Shannon index (http://www.mothur.org/wiki/Shannon) was calculated. Differences between samples were analyzed with *t* tests in STAMP version 2.1.3 (Parks et al., 2014) (https://beikolab.cs.dal.ca/software/STAMP). Principal coordinate analysis (PCoA) was performed in R3.4.3 with the vegan 2.4-6 package (Dixon, 2003). Correlation analysis of environmental factors was performed with vegan (Dixon, 2003).

RESULTS

Propagule culture

After propagule culture shown (Table 1), *Ulva flexuosa* and *U. prolifera* were found at almost all sites in the May group, whereas only *U. prolifera* was found at the M-L site. In July, with the green tide bloom, *U. flexuosa* was still found at almost sites; moreover, *Ulva compressa* and *Ulva linza* appeared at these sites. Among the sampling sites, J-L3 had the most species in propagule cultures, whereas only *U. flexuosa* was found at J-D1, J-S3 and J-L2, and some sites did not yield propagule cultures. In a comparison of the propagules in the May and July groups, *U. linza* and *U. compressa* were found in only the July groups, thus indicating that these two species may be indicators of early green tide blooming. Meanwhile, the July groups showed more species, thereby indicating that the increasing species may forecast green tide

TABLE 1 Summary of propagule culture

Month	Sites	Species	Count
May	M-R	*Ulva flexuosa*, *Ulva prolifera*	2
	M-D	*U. flexuosa*, *U. prolifera*	2
	M-S	*U. flexuosa*, *U. prolifera*	2
	M-L	*U. flexuosa*	1
July	J-D1	*U. flexuosa*	1
	J-D2	*U. prolifera*, *Ulva linza*	2
	J-S1	*U. flexuosa*, *U. prolifera*	2
	J-S2	*U. flexuosa*, *Ulva compressa*	2
	J-S3	*U. flexuosa*	1
	J-L1	*U. flexuosa*, *U. compressa*, *U. prolifera*	3
	J-L2	*U. flexuosa*	1
	J-L3	*U. flexuosa*, *U. prolifera*, *U. compressa*, *U. linza*	4
	J-L4	*U. flexuosa*, *U. prolifera*, *U. compressa*	3
	J-L5	*U. flexuosa*, *U. compressa*, *U. linza*	3

blooming. In brief, the changes in propagule species may indicate blooming of the green tide.

Correlation analysis of environmental factors

In July, the temperature clearly increased, whereas the suspended solids, DO, NO_3-N, PO_4-P, and SiO_3-Si all decreased to varying degrees (Table 2). The nutrient salts almost completely disappeared after the green tide bloomed: the more severe the green tide outbreak, the clearer the decrease in environmental factors. The optimum growth temperature of *U. prolifera* has been reported to be 18°C to 23°C (Kim et al., 2011). The temperature of seawater monitored this time was below 18°C in May and rose to 21.82°C to 28°C in July, thereby confirming the aforementioned relationship. DO ranged from 6.52 to 10.2 mg/L, with an average of 8.97 mg/L in May and a decrease to 6.95 mg/L in July. The inorganic nitrogen in May ranged from 0.2036 to 0.3871 mg/L, thus confirming water quality class II to class III (GB 3097-1997, Ministry of Environmental Protection of the People's Republic of China). The range of inorganic nitrogen in July was 0.0034–0.3059 mg/L. J-R was consistent with class III water quality, whereas J-S1 and J-S2 were consistent with class II water quality, and other sites were consistent with class I water quality. The range of active phosphate was 0.0047–0.0188 mg/L; except for M-S and J-R, all other sites were consistent with class I water quality.

In addition, redundancy analysis (Figure 2) was performed to identify correlations between taxonomic composition at the phylum level and the environmental factors. As shown in Figure 2, the other factors were close to the abscissa except for NH_4-N, thus indicating that the microbial community structure of each sample was affected by the environmental factors. Salinity had the greatest effects on microbial community structure, and nutrients had greater effects than temperature. Moreover, DO, NO_3-N, PO_4-P, and SiO_3-Si showed negative correlations with the microbial community structure, whereas temperature and NO_2-N were positively correlated with the microbial community structure.

OTU cluster analysis and annotation

High-throughput sequencing yielded a total of 1,035,171 high-quality sequences, of which 252,368 were obtained from the samples collected in May (before green tide bloom), and 782,803 were obtained from the samples in July (during green tide bloom). The average length of these sequences was approximately 418 bp. After clustering, 4117 OTUs were obtained, comprising 1044 and 3834 from the samples collected in May and July, respectively. After annotation, all OTUs were classified into 47 phyla, 113 classes, 227 orders, 438 families, 868 genera, and 1620 species. Of these, 30 phyla, 72 classes, 149 orders, 266 families, 481 genera, and 738 species were found in the May group, whereas 47 phyla, 115 classes, 225 orders, 426 families, 807 genera, and 1491 species were found in the July group.

Alpha-diversity analysis

The alpha-diversity analysis of samples was performed on the basis of the Shannon index and rank abundance

TABLE 2 Environmental factors of sample sites

Site	Date	Depth (m)	Sampling depth	Temp. (°C)	S. S. (mg/dm^3)	pH	Salinity	DO (mg/dm^3)	NO_2-N (mg/dm^3)	NO_3-N (mg/dm^3)	NH_4-N (mg/dm^3)	PO_4-P (mg/dm^3)	SiO_3-Si (mg/dm^3)	N/P
M-R	2019-05-09	22.4	0.5	17.1	83.2	8.17	29.432	8.57	0.0044	0.349	0.0337	0.0069	0.205	56
M-D	2019-05-09	13.2	0.5	16.7	78.0	8.11	31.674	8.765	0.0027	0.1855	0.0154	0.0148	0.230	14
M-S	2019-05-09	16.1	0.5	16.7	115.3	8.08	29.226	8.33	0.0021	0.360	0.0156	0.0186	0.902	20
M-L	2019-05-10	16.48	0.5	16.9	20.5	8.16	30.537	10.20	0.0071	0.211	0.133	0.0047	0.536	75
J-R	2019-08-21	16	0.5	28.00	10.6	8.24	27.844	6.26	0.0039	0.302	*	0.0188	0.467	16
J-D1	2019-08-22	14	0.5	27.82	74.8	8.08	29.533	6.50	0.0041	0.190	*	0.0081	0.182	24
J-D2	2019-08-21	13	0.5	27.16	45.4	8.19	30.387	6.51	0.0044	0.148	*	0.0086	0.192	18
J-S1	2019-07-03	17.5	0.5	24.44	99.8	8.07	29.399	6.84	0.0030	0.270	*	0.0085	0.411	32
J-S2	2019-07-03	17.5	0.5	24.33	97.7	8.02	30.536	7.01	0.0034	0.281	0.0023	0.0120	0.581	24
J-S3	2019-07-02	17	0.5	24.33	10.1	8.17	30.535	7.01	0.0095	0.0945	*	*	0.147	213
J-L1	2019-07-03	8	0.5	25.68	14.3	7.90	29.850	6.73	0.0135	0.103	0.0065	*	0.201	246
J-L2	2019-07-03	15	0.5	23.95	8.4	8.18	30.360	7.12	0.0025	0.0009	*	*	0.166	12
J-L3	2019-07-03	18	0.5	23.18	10.7	8.16	30.200	7.24	0.0120	0.102	*	*	0.215	233
J-L4	2019-07-03	19	0.5	21.82	11.2	8.04	30.710	7.51	0.0077	0.0836	0.0098	*	0.0980	202
J-L5	2019-07-03	24	0.5	25.29	8.5	8.34	30.010	7.74	0.0051	0.0647	0.0014	*	0.0470	142

Abbreviations: DO, dissolved oxygen; M/J-D1, Dafeng Site 1 in May or July; M/J-D2, Dafeng Site 2 in May or July; M/J-L1, Lianyungang Site 1 in May or July; M/J-L2, Lianyungang Site 2 in May or July; M/J-L3, Lianyungang Site 3 in May or July; M/J-L4, Lianyungang Site 4 in May or July; M/J-L5, Lianyungang Site 5 in May or July; M/J-R, Rudong site in May or July; M/J-S1, Sheyang Site 1 in May or July; M/J-S2, Sheyang Site 2 in May or July; M/J-S3, Sheyang Site 3 in May or July; S, surface; S. S., suspended solids; Temp., water temperature.

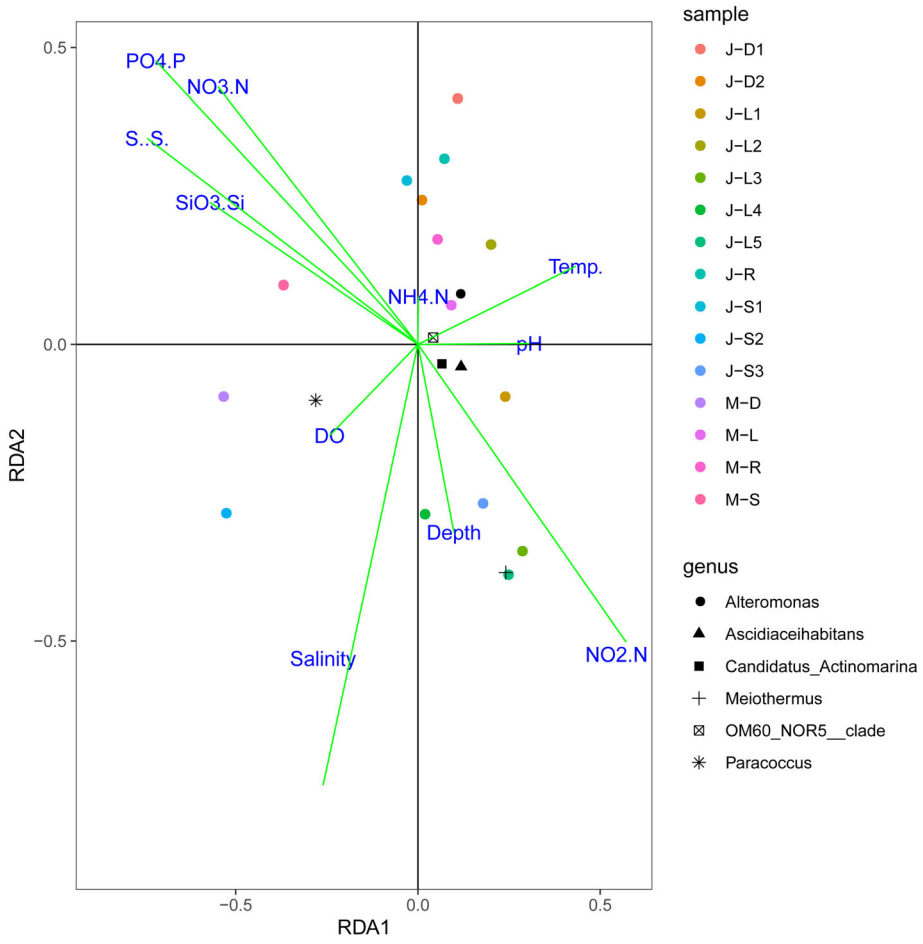

FIGURE 2 Redundancy analysis showing the relationships between environmental factors and green tide samples. Abbreviations: Tep., water temperature; DO, dissolved oxygen; L1, Lianyungang Site 1; L2, Lianyungang Site 2; L3, Lianyungang Site 3; L4, Lianyungang Site 4; L5, Lianyungang Site 5; S1, Sheyang Site 1; S2, Sheyang Site 2; S3, Sheyang Site 3; D1, Dafeng Site 1; D2, Dafeng Site 2; R, Rudong site

(Figure 3). As shown in Figure 3a, the J-R group had the highest average Shannon index, whereas the M-R group had the lowest. In the J-D2 group, the average Shannon index exceeded 5, thus indicating rich diversity. Most July groups had higher average Shannon index values than the May groups, thus suggesting that the July groups had richer diversity than the May groups. Among the July groups, the diversity of J-S3 was lowest, in agreement with the finding of fewer propagules. In addition, the gentle curve of the Shannon index reflected the reliability and sufficiency of the sequencing data. The rank abundance indicates the diversity and uniformity of sequencing data. As shown in Figure 3b, J-R had the richest diversity (more than 2500 OTUs), followed by J-D2 (more than 2000 OTUs), and the July groups (J-L2, J-L1, J-D1 etc.) had markedly higher diversity than the May groups.

Comparative analysis between the May and July groups

As shown in Figure 4, the samples in May and July clustered together. M-S, M-R, and M-D clearly clustered together, and M-L was distant from them. In the July groups, J-D1, J-D2, and J-R clustered together, thus indicating similar community composition. The second cluster was composed of J-L1, J-L2, J-L3, J-L4, J-L5, and J-S3, and the last cluster comprised J-S1 and J-S2, thus implying their similar community composition. Because the samples were distributed essentially according to their geographic locations in the PCoA plot, the types or stages of the green tide in different locations might have varied.

Taxonomic composition

The top three most abundant phyla in May and July were Proteobacteria, Deinococcus-Thermus, and Bacteroidetes. At the genus level, the top five genera in May were *Rhodobacteraceae*, *Vibrio*, *Pseudoalteromonas*, *Sulfitobacter*, and *Thermus*, four of which (those besides *Thermus*) belong to the Proteobacteria (Figure 5). After the green tide bloom, the relative abundance of Deinococcus-Thermus clearly increased. As shown in Figure 5, the top five genera in July were *Thermus*, *Pseudoalteromonas*, *Meiothermus*, *Pseudomons*, and *Alteromonas*, and the top genera belonged to Deinococcus-Thermus (more than 10%).

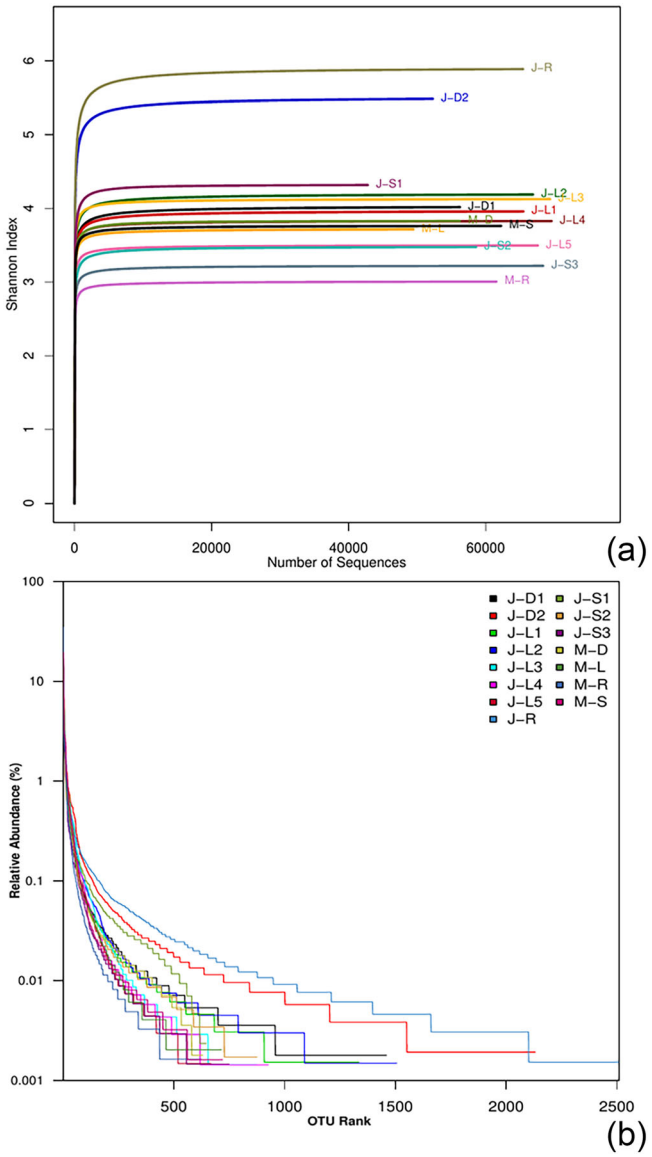

FIGURE 3 Alpha-diversity analysis of samples in May and July. (a) Shannon index analysis of samples and (b) rank abundance analysis of samples. Abbreviations: M/J-L1, Lianyungang Site 1 in May or July; M/J-L2, Lianyungang Site 2 in May or July; M/J-L3, Lianyungang Site 3 in May or July; M/J-L4, Lianyungang Site 4 in May or July; M/J-L5, Lianyungang Site 5 in May or July; M/J-S1, Sheyang Site 1 in May or July; M/J-S2, Sheyang Site 2 in May or July; M/J-S3, Sheyang Site 3 in May or July; M/J-D1, Dafeng Site 1 in May or July; M/J-D2, Dafeng Site 2 in May or July; M/J-R, Rudong site in May or July

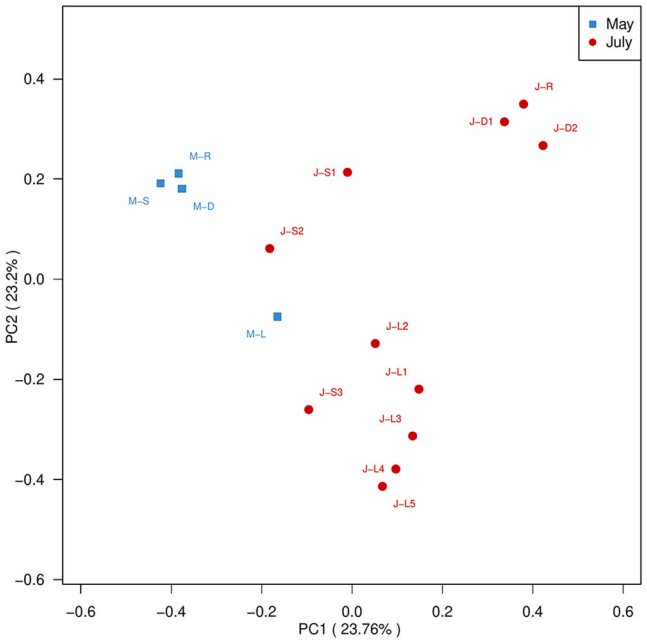

FIGURE 4 Principal coordinate analysis of samples in May and July. Abbreviations: M/J-L1, Lianyungang Site 1 in May or July; M/J-L2, Lianyungang Site 2 in May or July; M/J-L3, Lianyungang Site 3 in May or July; M/J-L4, Lianyungang Site 4 in May or July; M/J-L5, Lianyungang Site 5 in May or July; M/J-S1, Sheyang Site 1 in May or July; M/J-S2, Sheyang Site 2 in May or July; M/J-S3, Sheyang Site 3 in May or July; M/J-D1, Dafeng Site 1 in May or July; M/J-D2, Dafeng Site 2 in May or July; M/J-R, Rudong site in May or July

To further determine the taxonomic differences during the green tide bloom, we assessed the taxonomic composition in the May and July groups with Megan analysis (Figure 6). At the bacteria level, the July group clearly had higher species abundance (73.3%). In addition, every phylum had higher abundance in the July group; only the ratio of Proteobacteria in the May group was higher than that in the July group. At the class level, Alphaproteobacteria and Gammaproteobacteria were present in the same proportion as the Proteobacteria. Notably, among Gammaproteobacteria, the May group had more Pseudonadales and Vibrionales than the July group and also had more Moraxellaceae, Pseudomonadaceae, and Vibrionaceae. At the genus level, for these families, the May group had more abundant *Pseudomonas* (54.55%), *Vibrio* (72.73%), and *Psychrobacter* (nearly 100%) species than the July group. Similarly, most *Sulfitobacter* and *Marinomonas* were found in the May group. These results suggested that a significant decrease in *Psychrobacter*, *Sulfitobacter*, and *Marinomonas* may directly indicate the occurrence of green tide. Accordingly, some genera were predominantly found in July group, such as *Alteromonas* (97.14%), *Ascidiacerhabitans* (96.30%), *Synechococcus* (nearly 100%), *OM60_NOR5_clade* (nearly 100%), *Hydrotalea* (nearly 100%), and *Burkholderia-Paraburkholderia* (nearly 100%). Significant increases in these genera also imply green tide occurrence.

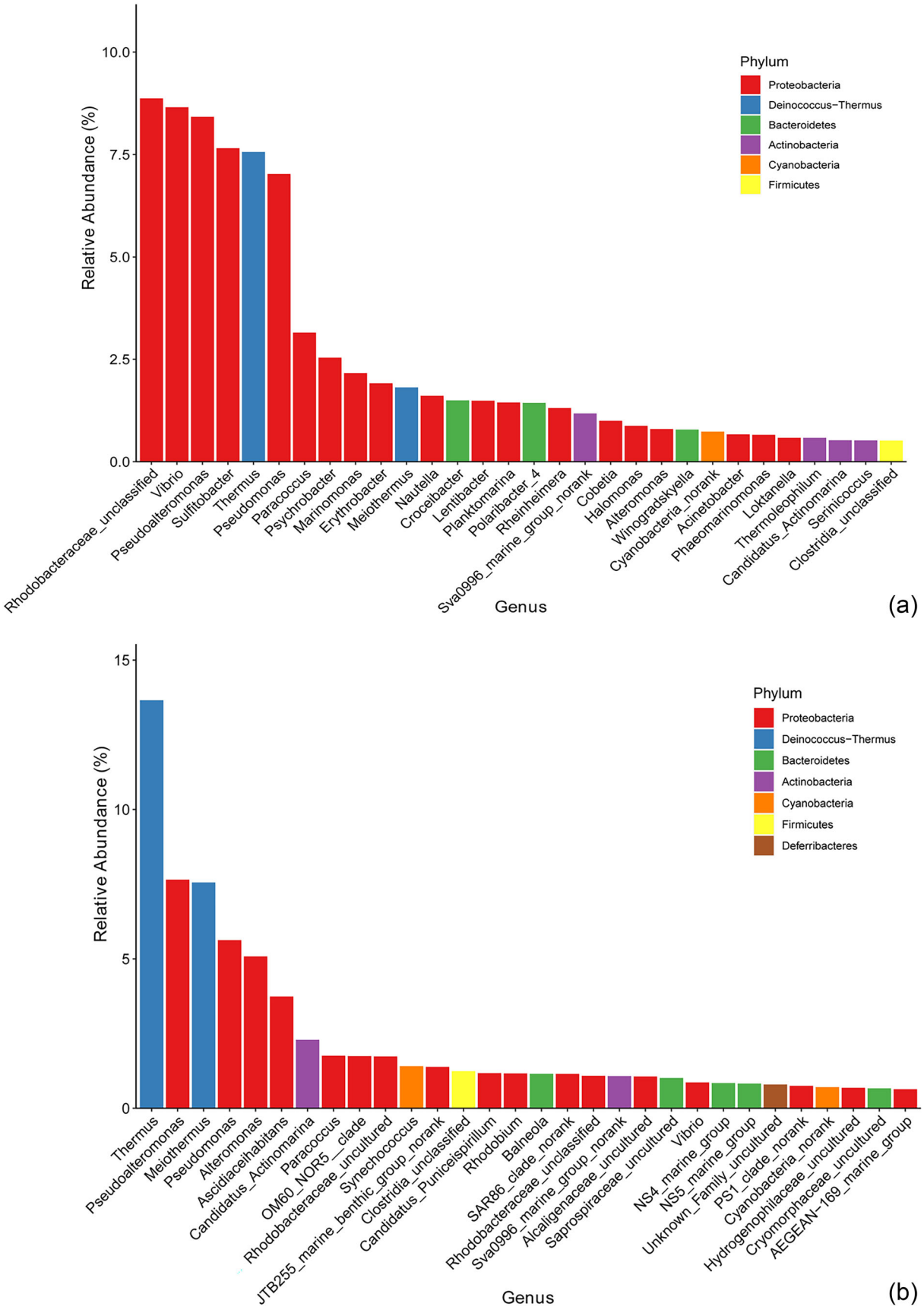

FIGURE 5 Dominant microbial genera in green tide samples. (a) Dominant microbial genera in the May group and (b) dominant microbial genera in the July group

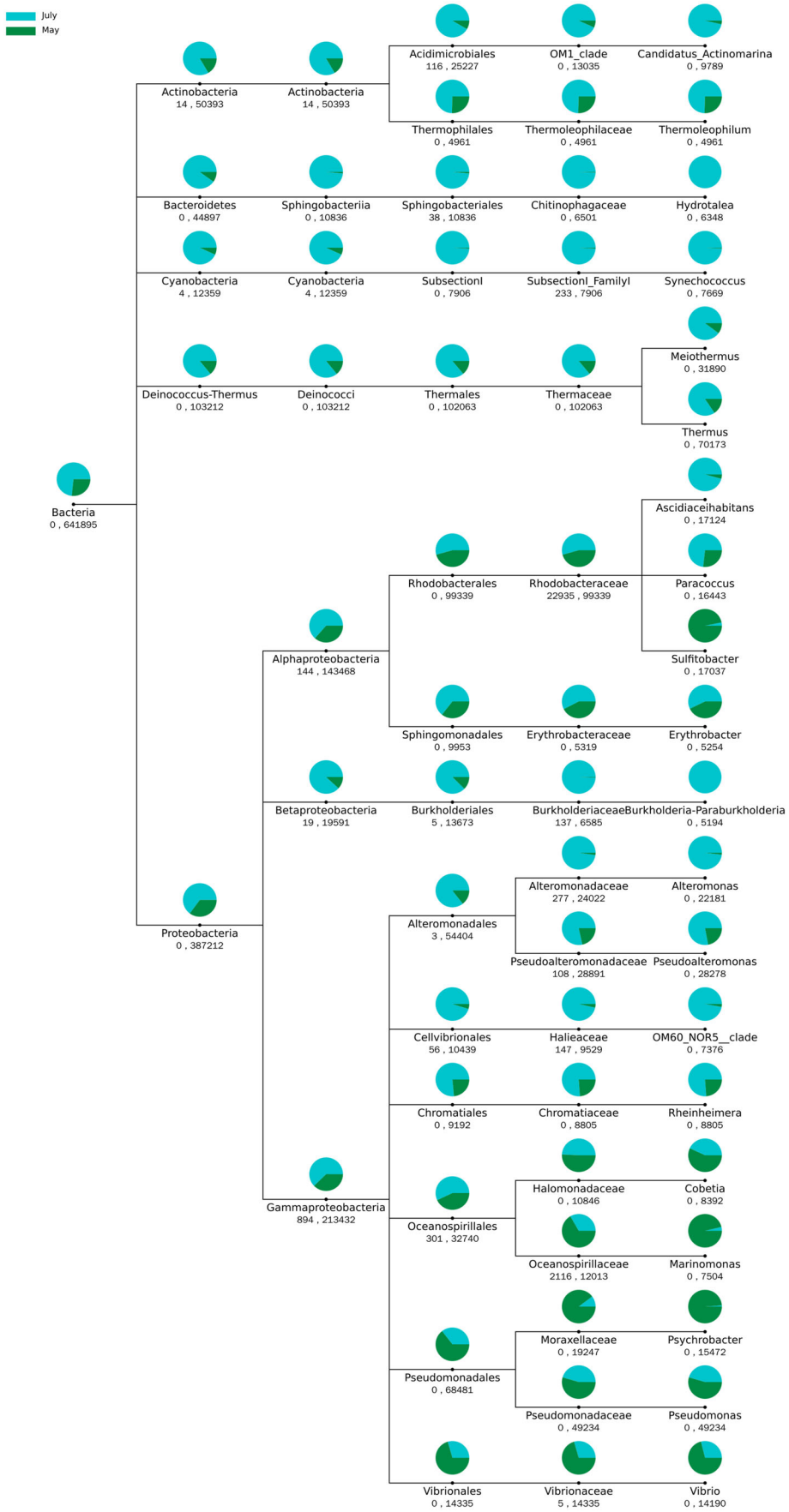

FIGURE 6 Megan analysis of samples in May and July

DISCUSSION

To explore the changes in microbial diversity during green tide blooms, we compared water samples before and after green tide blooming in coastal areas of Jiangsu Province, China. On the basis of 16S rDNA sequencing, the microbial community abundance and structure were systematically investigated. A previous study has indicated that the abundance of some bacteria increases with green tide outbreaks, then gradually reverts to normal with green tide decay, in multiple locations in Shandong, China (J. H. Wang et al., 2020). In contrast, during green tide blooming in the Qingdao offshore region, a decrease in the abundance and diversity of the surface and sediment bacterial community has been observed (Qu et al., 2020). Overall, this finding is consistent with our results, in which most samples in the July groups had higher microbial community diversity than the samples in the May groups. Marine investigations have indicated that the blooming of red tides usually causes uneven distribution of organic matter, thus resulting in marked increases in several bacteria in a certain water layers (C. Zhang, 2010). This finding is consistent with the increase in microbial diversity observed after the green tide outbreak in this study. Studies have detected large numbers of propagules in the Southern Yellow Sea during green tide outbreaks, and their spatial and temporal distribution is consistent with the outbreak process and drift path of green tide (Huo et al., 2014; Taylor et al., 2001; J. H. Zhang et al., 2013). In this study, more species of propagules were observed in the July group than the May group, in agreement with the higher microbial diversity in July than May, thus indicating that the change in propagules may forecast the outbreak of green tide.

The bacterial community ratios in the May and July groups clearly changed during the green tide bloom (Figure 6). Among the species flourishing more in May than July, *Sulfitobacter* is known to promote diatom cell division via secretion of the hormone indole-3-acetic acid, thus affecting the global distribution of diatoms (Amin et al., 2015). Hence, diatoms are expected to be strongly influenced by decreased *Sulfitobacter* in green tide blooming. Beyond *Sulfitobacter*, the other species in the order Rhodobacterales increased during the green tide outbreak. These bacteria have been verified to be associated with green tide and have been reported to produce algicidal substances that directly or indirectly inhibit algal growth (Mayali & Azam, 2004). In contrast, with the outbreak of macroalgae, photosynthesis is suppressed, thus resulting in generation and accumulation of large amounts of hydrogen sulfide (Nedergaard et al., 2003). Rhodobacterales are dominant in eutrophic environments, because they have versatile metabolic characteristics; for example, they function in decreasing the concentration of hydrogen sulfide in water environments (Brinkhoff et al., 2008; Lenk et al., 2012). Moreover, bacteria that have high resistance and sulfate-reducing characteristics gradually proliferate. Deinococcus-Thermus comprises two orders, both with exceptional resistance to environmental stresses (Ho et al., 2016). Because of the sensitivity of these bacteria to the environment, their changes might be key indicators of green tide outbreaks.

In the spring of 2012, the concentration of DIN in Jiangsu coastal water increased to three times that in 1985, and this increase has become more pronounced in recent years. DIN is positively correlated with the biomass of green macroalgae (Shi et al., 2014). Moreover, during the green tide outbreak, massive macroalgal reproduction consumes organic matter and inorganic nutrients in marine environments. From 2008 to 2012, the nutrients in the green tide areas of the Southern Yellow Sea were found to be high in the coastal area and low in the far sea area (Shi et al., 2014). In this study, the nutrients at each site presented similar characteristics overall. Additionally, the concentrations of nutrients all decreased after the green tide outbreak (Table 2). Previous studies have demonstrated that environmental factors (i.e., dissolved organic carbon, fluorescent dissolved organic matter and DO) change with the outbreak of green tides (H. M. Li et al., 2016; X. Z. Li et al., 2019; F. Liu et al., 2013). Furthermore, microbial communities are altered by green tide blooms and the decreased inorganic nutrients. In some aquaculture ponds, bacterial communities with *U. prolifera* have been found to have higher diversity than those in *U. prolifera*-free ponds (Lin et al., 2017), and green tide blooms have been found to increase microbial community diversity in the coastal waters of Shandong, China (J. H. Wang et al., 2020). Moreover, among the microorganisms cultured, bacterial communities have been found to exhibit relatively high diversity in low-nutrient conditions (Connon & Giovannoni, 2002). A similar result was found in our study: The diversity of the microbial community was lower in the May group than the July group (Figure 3). In summary, the diversity of microbial communities increased under the low-nutrient concentrations during green tide outbreaks. Beyond limiting nutrients being key to algal blooms, Redfield (1960) has proposed that a ratio of nutrient salt concentrations of Si, N, and P is 16:16:1 is conductive to the growth and reproduction of phytoplankton, whereas other ratios cause changes in plankton population structure. Moreover, much higher N/P promote *U. prolifera* growth; that is, N has a greater influence on *U. prolifera* than P (Yang et al., 2020). In this

study, apart from the M-D and J-L2 sites, which showed <16:1 N/P, other sites all showed N/P > 16:1, indicating a suitable environment for *U. prolifera*.

Specific microbial groups show direct associations with physical and chemical factors in the environment (Takai et al., 2001). For example, the temperature index can robustly distinguish winter and summer–autumn communities (Kan & Sun, 2011). In China, the biomass of bacteria in autumn is more than three times that in spring in the fresh water flushed area of the Yangtze River Estuary (Liu et al., 2001). The green tide outbreaks always occur in summer, thus indicating that temperature plays an essential role in green tide blooming (Fan et al., 2018; Kim et al., 2011). In our study, redundancy analysis showed a clear positive correlation between temperature and bacterial communities during green tide blooming. As previous studies have reported, *U. prolifera* spore production is elevated at temperatures ranging from 18°C to 25°C (Kim et al., 2011). An appropriate temperature leads to thriving of green tides and increases the diversity of the microbial community.

In general, climate change and human disturbance are external factors involved in the occurrence of green tides in the Yellow Sea, and the occurrence of El Niño or La Niña may affect the duration of green tide outbreaks in the Yellow Sea (J. Yang et al., 2017). Meanwhile, foggy days and lower sea surface temperatures indirectly prolong the extinction cycle of green tide (L. N. Guo et al., 2015). The necessary conditions for the occurrence of large-scale green tide in the Yellow Sea can be explained by four aspects: specific provenances in the shoal, attachment and amplification of aquaculture rafts, expansion of high nutrients in the sea area, and hydrodynamic transport. Floating ecotype *U. prolifera* is the main organism causing green tides, and the micropropagules serve as seed banks in green tides.

Amounts of studies reported that when the green tide outbroken, the organic released by phytoplankton increases rapidly, which can stimulate the growth of specific bacterial groups, thus causing changes in bacterial community structure (Gonzalez et al., 2000; M. Liu et al., 2011). Because marine heterotrophic bacteria rely mainly on phytoplankton and dissolved organic matter released by zooplankton during predation, a clear correlation exists between the standing stocks of heterotrophic bacteria and phytoplankton. Simultaneously, the growth of marine heterotrophic bacteria also depends on the concentrations and properties of inorganic nutrients. Heterotrophic bacteria can use DIN and dissolved inorganic phosphorus as nitrogen and phosphorus sources (G. Z. Zhang et al., 2022). Only when heterotrophic planktonic bacteria in the water are not limited by dissolved organic matter do inorganic nutrients have an important effect on their growth (G. Z. Zhang et al., 2022). Meanwhile, the change of marine bacteria also affects the green tide outbreak and environmental factors. For example, during the outbreak of green tide, increased *Rhodobacterales* inhibited algal growth and decreased the concentration of hydrogen sulfide. Furthermore, the seasonal change would also affects the environmental factors, and the seasonal environmental changes would directly/indirectly effects on the green tide outbreak and microbial community structure (Bunse & Pinhassi, 2017; G. Z. Zhang et al., 2022). These results indicated that the relationships among the changes in microbial community structure, environmental factors, and algal growth were not unidirectional and unique but were complex. However, to explore the multiple relationship between the three, even seasonal change related effects, requires prolonged data collection and accumulation, further research, and discussion.

CONCLUSION

In this study, we investigated the changes in the diversity and structures of microbial communities during green tide outbreaks in the coastal area of Jiangsu Province in China. As shown by the Shannon index and rank abundance analyses, the microbial community increased after the green tide bloomed. *Psychrobacter*, *Sulfitobacter*, and *Marinomonas* dominated in the May group, whereas *Ascidiacerhabitans*, *S. Hydrotalea*, and *Burkholderia-Paraburkholderia* dominated in the July group. These findings indicate that the green tide significantly influences the microbial community, and the changes in some key microbial communities might serve as a new indicator of green tide outbreaks.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in metagenome or environmental sample from seawater metagenome at https://www.ncbi.nlm.nih.gov/biosample/SAMN18011962/.

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