Science of the Total Environment 949 (2024) 174845

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Organic matter composition fluctuations disrupt free-living bacterial communities more than particle-associated bacterial communities in coastal waters

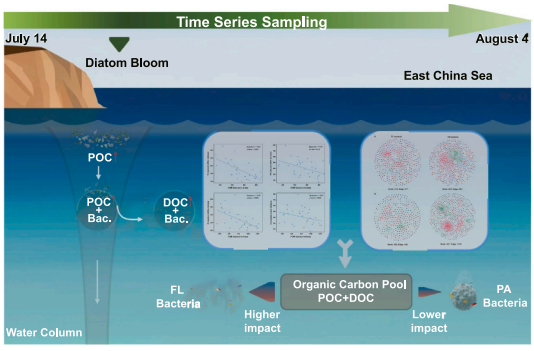
Shujing Liu, Quanrui Chen, Le Liu, Changjie Dong, Xuanyun Qiu, Kai Tang *

State Key Laboratory of Marine Environmental Science, Fujian Key Laboratory of Marine Carbon Sequestration, College of Ocean and Earth Sciences, Xiamen University, China

HIGHLIGHTS

- FL bacterial communities were significantly less stable than PA communities.
- Amino acids and peptides in DOM significantly increased during the diatom bloom.
- The relative intensities of major POM molecule classes remained stable.
- DOM and POM compositions influenced FL communities more than PA communities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Abasiofiok Mark Ibekwe

Keywords:
Bacteria
Organic matter
Particle
Metabolomics
Bloom

ABSTRACT

Marine organic matter fuels the growth of microbial communities, shaping the composition of bacteria that specialize in its breakdown. However, responses of free-living (FL) and particle-associated (PA) bacterial communities to the changing pools of dissolved organic matter (DOM) and particulate organic matter (POM) remained unclear. This study investigates the composition of size-fractionated bacterial communities, DOM and POM in coastal waters over a 22-day period that includes a diatom bloom. Co-occurrence analysis showed that the FL bacterial communities were significantly less stable than PA communities. During the diatom bloom, we observed a significant increase in DOM molecules, particularly those derived from amino acids and peptides. In contrast, the relative intensities of major POM molecule classes remained stable despite the algal bloom's influence. Our study revealed a strong negative correlation between bacterial alpha-diversity and the amount of molecules in the organic matter pool. Similarly, bacterial community beta-diversity was found to be related to the composition of organic matter pool. However, the composition of organic matter was more strongly related to the composition of FL bacterial communities compared to PA communities. This suggests that FL bacteria exhibit greater variations in temporal dynamics and higher sensitivity to the specific structure of organic matter molecules.

* Corresponding author.
E-mail address: tangkai@xmu.edu.cn (K. Tang).

https://doi.org/10.1016/j.scitotenv.2024.174845
Received 21 May 2024; Received in revised form 13 July 2024; Accepted 14 July 2024
Available online 23 July 2024
0048-9697/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

1. Introduction

The ocean contributes half of global primary production, where the biological transformation of inorganic substrates to organic carbon drives biogeochemical cycles (Field et al., 1998; Falkowski, 1998). This organic matter supports marine food web, acting as a crucial links between organisms and nonliving organic carbon pool (Romankevich et al., 2023). Marine organic matter is one of the most diverse and complex mixtures of compounds on our planet, consisting of millions of different molecules (Verdugo et al., 2004; Azam and Malfatti, 2007). It is typically categorized into two major pools: DOM and POM. DOM consists of smaller, soluble molecules that pass through filters with a pore size of around 0.7 μm (Meinhard et al., 2002), while POM includes larger, insoluble particles living biomass and detrital organic along with inorganic matter. DOM and POM are stock carbon reservoir in the oceans, which are estimated at 662 Gt and 30 Gt, respectively (Hansell et al., 2009; Post et al., 1990).

Microbial communities play a critical role in regulating the composition and turnover of both DOM and POM, influencing the overall efficiency of the coastal carbon cycle (Azam and Malfatti, 2007; Jiao et al., 2010). This distinction is particularly relevant when considering the impact of organic matter on bacterial lifestyles. Marine bacteria exist as either free-living or particle-associated communities (Grossart, 2010). POM, densely colonized by various microbes with high hydrolytic enzyme activities, plays a key role in the formation and decomposition of detritus-dominated micropatches (Zhao et al., 2020; Grossart et al., 2007). These bacteria break down the aggregate organic matter, converting it into dissolved ones and forming DOM plumes in the ocean (Stocker, 2012). Over the past few decades, there has been extensive research on the compositional differences between FL and PA bacteria, along with their responses to environmental variations in marine ecosystems (Post et al., 1990; Jiao et al., 2010). Deterministic and stochastic processes significantly influenced the assembly of FL and PA bacterial communities in coastal waters (Wang et al., 2020), while the quantitative importance of assembly pattern was different across the different lifestyles (Shi et al., 2022; Yuan et al., 2021). PA bacterial genomes are generally larger and more variable, encoding for a wider range of metabolic and regulatory capabilities compared to FL bacterial genomes (Smith et al., 2013). FL bacterial genomes are typically characterized by have smaller genomes and exhibiting fewer metabolic genes and high-affinity transporters for nutrient uptake, enabling them thrive in nutrient-deficient seawater (Luo and Moran, 2015). Genes involved in denitrification, N_2-fixing, and polysaccharide utilization, are significantly richer in the PA fraction (Farnelid et al., 2019; Boeuf et al., 2019), whereas dimethylsulfoniopropionate was mainly utilized by FL bacteria (Farnelid et al., 2019). These findings suggest distinct preferences for organic matters utilization by the FL and PA communities. Therefore, understanding how variations in organic matter composition influence both free-living and particle-associated bacteria is crucial for a comprehensive understanding of the interplay between organic matter and bacterial communities in the marine environment. However, such studies are lacking.

Untargeted liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been developed for detecting DOM composition (Petras et al., 2017). Various multivariate statistical methods, such as canonical correlation analysis and network analysis, were used to investigate the relationship between bacterial communities and organic matter signatures (Osterholz et al., 2016; Zhao et al., 2019). Previous study investigated the connections between bacterial diversity (alpha-diversity) and DOM characteristics using linear models (Muscarella et al., 2019). Additionally, multivariate models have been employed to explore the influence of DOM composition on bacterial community structure (beta-diversity) (Muscarella et al., 2019). Network analysis, in particular, provides graphical statistics to identify potential associations between bacterial populations and specific chemical compounds within the DOM pool (Zhao et al., 2019). For instance, a study analyzing DOM and bacterial communities along a latitudinal transect in the North Sea revealed a link between actively growing bacterial populations and labile DOM fractions, which was influenced by local phytoplankton blooms and storm (Osterholz et al., 2016). Similarly, a study conducted in the Delaware Bay during fall and winter, has shown that the composition of DOM molecules exhibits greater stability compared to bacterial communities along a salinity gradient (Osterholz et al., 2018). Furthermore, network analysis of incubations with added viral-lysed picocyanobacteria-derived DOM revealed changes in bacterial community composition in response to the chemical diversity of the DOM (Zhao et al., 2019).

Climate change and human activities have increased eutrophication in Chinese seas, including the East China Sea (Maúre et al., 2021). Receiving massive nutrients and organic matter inputs from the Changjiang River, the East China Sea exhibiting the largest eutrophic zone (Chang and Isobe, 2003). Eutrophication-induced blooms in surface water are frequently observed in the East China Sea (Wang et al., 2021). Phytoplankton blooms release substantial amounts of organic matter, creating favorable conditions for bacterial growth (Dai et al., 2023). The East China Sea is also influenced by complicated hydrodynamic processes such as river flux, tide event, and the Taiwan Warm Current, affecting the dynamics of organic matter (Chen et al., 1999). This dynamic organic matter pool in the East China Sea provides a natural laboratory to examine how organic matters influence bacterial communities. Here, we conducted a time-series sampling over 22-day period in this region to analyze the structure of bacterial communities, including FL and PA bacteria, and the molecular composition of DOM and POM. This study unveils novel insights into the interactions between marine bacteria and organic matter in dynamic environments.

2. Materials and methods

2.1. Sample collection

A summer cruise was conducted from July 11 to August 4, 2022. Samples were collected from two sites in the East China Sea: A1 (30.12079°N, 122.72947°E) and C4 (30.1282°N, 122.74599°E). Water samples were collected at eight time points from surface (A1: 3 m, C4: 5 m), middle (A1: 17 m, C4: 25 m), and bottom (A1: 40 m, C4: 60 m) layers. Depth, salinity, temperature, and dissolved oxygen concentration were obtained through Conductivity-Temperature-Depth (CTD) sensors. For microbial diversity analysis, approximately 1 L seawater was prefiltered through a 20 μm pore size filter to remove particulate impurities, and then through 3 μm and 0.2 μm pore size filters (47 mm diameter, Millipore, USA) to collect the corresponding size-fractionated biomass. For composition analysis of organic matters, approximately 1 L seawater was filtered through 0.7 μm pore size glass fiber membrane filters (47 mm diameter, Whatman, USA) to collect POM for subsequent analysis of POM composition. The PPL columns (200 mg, Bond Elut PPL, Agilent Technologies, USA) were used to enrich DOM in filtrate which was acidified to pH 2. All filters were rapidly frozen with liquid nitrogen and then stored at −80°C. The PPL columns were stored at −20°C. DOM concentration was analyzed by the Shimadzu TOC-VCPH analyzer. POM concentration was analyzed on a CHNS elemental analyzer (vario EL cube, Elementar, Germany).

2.2. DNA extraction and sequencing

Corresponding size-fractionated microbial genomic DNA was extracted from 3 μm and 0.2 μm membranes using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA). For picoeukaryotic communities, the primers “528F” and “706R” were applied to amplify V4 region of the 18S rRNA gene (Cheung et al., 2010). For bacterial communities, the universal primers “343F” and “798R” was used to amplified V3-V4 region of the 16S rRNA gene (Nossa et al., 2010). Amplicon sequencing was performed on the Illumina Miseq platform with the 2 × 250 bp

paired-end approach. All the raw sequences were analyzed using QIIME2 v2023.2 (Bolyen et al., 2019). Briefly, cutadapt v4.2 was used to removed primers. The DADA2 pipeline was then used to identify sequences and abundance table of amplicon sequence variants (ASVs). Representative sequences of ASVs were compared against the SILVA v138 database for taxonomic classification (Yilmaz et al., 2013).

2.3. LC–MS/MS sample preparation and analysis

POM compounds were extracted using an acetonitrile:methanol: water solution (2:2:1v/v/v, −20°C). Filter samples were homogenized using the FastPrep-24 Homogenizer (MP Biomedicals) and then incubated at −20°C for 2 h. After centrifugation, the supernatant was filtered through a 13 mm, 0.2 μm hydrophilic polyvinylidene fluoride membrane filter (JINTENG, Tianjin, China). DOM was eluted from the column using 100% methanol. POM and DOM samples were analyzed using a Q-Exactive high performance liquid chromatography system which was connected to an orbitrap mass spectrometer (Thermo Fisher Scientific) in data-dependent acquisition mode (DDA mode). Chromatographic separation was performed using a Waters ACQUITY UPLC BEH Amide (2.1 × 100 mm, 1.7 μm). The flow rate was set to 0.4 mL/min. The mobile phase was composed of a mixture of (A) acetonitrile and (B) 10 mM acetic acid. The chromatographic elution gradient was as follows: 0.00–1.00 min, isocratic 5% B; 1.00–10.00 min, gradient from 5% to 40% B; 10.00–15.00 min, isocratic 40% B; followed by equilibration 15.00–17.00 min, gradient from 40% to 5% B; 17.00–20.00 min, isocratic 5% B. Taurine-d4 was added in each sample as an isotopically labelled internal standard.

2.4. LC-MS/MS data processing

This analysis followed the standardized LC-MS/MS data processing protocol established by the Earth Microbiome Project (Shaffer et al., 2022). The raw mass spectrometry data (.raw) were transformed to open format (.mzML) using ProteoWizard v3.0.19 (Chambers et al., 2012). The mzML files were processed with MZmine v2.5.3 to obtain a features abundance table and their fragment information. These features were annotated systematically using Sirius v4.4.0 (Duhrkop et al., 2019). Molecular formula were computed with SIRIUS and refined with ZODIAC module (Ludwig et al., 2020). Structure annotation was done with CSI:FingerID module (Duhrkop et al., 2015). Chemical class annotations were conducted with CANOPUS module (Duhrkop et al., 2021) according to the NPClassifier ontology (Kim et al., 2021).

2.5. Quantification of community assembly mechanisms

Infer community assembly mechanisms by phylogenetic bin-based null model analysis (iCAMP) was used to quantify the relative importance of community assemble in FL and PA bacterial communities (Ning et al., 2020). Assembly processes were identified by the β-mean nearest taxon distance (β-NTI) and Bray-Curtis-based Raup-Crick (RC_{Bray}). The heterogeneous selections, homogeneous selections, dispersal limitation, homogenizing dispersal, and ecological drift were estimated by $\beta\text{-NTI} > 2$, $\beta\text{-NTI} < -2$, $|\beta\text{-NTI}| \leq 2$ but $RC_{Bray} > 0.95$, $|\beta\text{-NTI}| \leq 2$ but $RC_{Bray} < -0.95$, and $|\beta\text{-NTI}| \leq 2$ but $|RC_{Bray}| \leq 0.95$, respectively. The neutral community model (NCM) was used to calculate relative contribution of stochastic process in FL and PA bacterial communities (Sloan et al., 2006).

2.6. Statistical analysis

The relationship between bacterial communities and environmental factors was revealed by the Mantel test. The hypothesis that organic matter compositions affects bacterial community alpha-diversity and beta-diversity was tested according to previous research (Muscarella et al., 2019). Linear models were used to determine if richness of organic matters would affect the richness of bacterial communities. Distance-based redundancy analysis (dbRDA) was used to confirm relationships between organic matter composition and community composition (Legendre and Anderson, 1999). The co-occurrence network of bacterial ASVs and organic matter molecular features are shown based on a Spearmen's correlation coefficient of $|r| > 0.6$ and $p < 0.05$.

3. Results

3.1. Picoeukaryotic community composition and chlorophyll a concentration

Overall, 18S rRNA genes sequencing yielded 1,899 picoeukaryotic ASVs. The most abundant plankton ASVs belonged to the groups of Dinoflagellata and Diatomea (Fig. S1). In the surface layer of A1, the relative abundance of diatoms reached 83.0% on July 15 and 75.5% on July 23, 2022. At station C4, surface diatom abundance peaked at 38.0% on July 23, 2022. Chlorophyll *a* concentration at A1 and C4 stations peaked on July 23, with the values of 83.23 and 61.75 $\mu g/cm^3$, respectively (Fig. S2). These findings suggest that the samples of July 15 and July 23 were influenced by the diatom bloom. The concentration of dissolved and particulate organic carbon was fluctuating, ranging from 104.08 to 225.12 μM, 6.78 to 98.32 μM, respectively (Fig. S3).

3.2. Bacterial community composition

This study identified a total of 5,380 bacterial ASVs belong to 36 phyla. Proteobacteria (73.55%), Bacteroidia (14.29%), Actinobacteriota (7.37%), Cyanobacteria (1.02%) and Frimicutes (1.30%) dominated the overall bacterial communities (Fig. 1). Bacterial communities were separated based on time at station A1 and C4 based on principal coordinates analysis (PCoA) (adonis $R^2 = 0.0892$ for FL communities, $p < 0.001$; adonis $R^2 = 0.0074$ for PA communities, $p < 0.001$) (Fig. S4). Wilcoxon tests revealed significantly higher alpha-diversity values (observed species, ACE, Shannon, and Chao1) in the PA communities compared to the FL communities (Fig. S5). Neutral processes significantly influenced the temporal patterns observed in both FL and PA communities. NCM explained > 70% of the variation in bacterial structure for both free-living and particle-associated samples (Fig. 1C and D). Since this model does not account for the deterministic process, we used iCAMP for a more complete description of community assembly pattern. The iCAMP results suggested that drift (35%) and dispersal limitation (34%) dominated the assembly of PA communities, while drift (42%) followed by homogeneous selection (37%) primarily governed the assembly of FL communities (Fig. S6).

To explore co-occurrence patterns among the FL and PA bacterial communities (at the ASV level), network analysis were performed (Fig. 2). The FL network had 369 nodes and 4,110 edges (Fig. 2A), while the PA network had 419 nodes and 6,280 edges (Fig. 2B), indicating that a more complex metacommunity co-occurrence pattern in PA communities. Networks were divided into five major modules in FL bacterial network and six major modules in PA bacterial network. The closeness centrality of PA bacterial network was significantly higher than that of the FL network ($p < 0.001$) (Fig. 2C). In addition, the PA networks had higher degree, average degree and clustering coefficient compared to the FL networks (Table S1). Under random species loss, the co-occurrence network of PA bacterial community exhibited significantly higher robustness than that of FL community ($p < 0.05$) (Fig. 2D).

3.3. Molecular signature and spatiotemporal distribution of organic matter

Untargeted LC-MS/MS data generated for 90 DOM samples, resulting in 773 unique molecular structures across all samples (Table S2). These unique DOM molecular features were classified based on NPClassifier ontology, including alkaloids, fatty acids, terpenoids, shikimates and

phenylpropanoids, amino acids and peptides, polyketides, and carbohydrates. The most prevalent chemical pathway are alkaoids (23.7%) and fatty acids (24.0%) in non-bloom samples, while amino acids and peptides (36.5%) dominated in bloom-affected samples (Fig. 3A). At the superclass level, bloom-affected samples exhibited higher relative abundances of small peptides, oligopeptides, and fatty amides compared to other samples (Fig. 3B). Additionally, more DOM molecular features were identified in bloom-affected samples. Compared to the molecular composition of DOM, a greater number of molecular features (1,266) were detected in POM samples (Table S3). Alkaloids was the most prevalent chemical pathway of POM, accounting for 54.21%, followed by shikimates and phenylpropanoids (9.53%) and carbohydrates (9.33%). The chemical pathway of POM remained relatively stable across all sample (Fig. 4A), with the exception of a slight increase in saccharides at the superclass level in boom-affected samples (Fig. 4B). PCoA analysis of organic matter composition revealed a clear distinction between sampling times. Specifically, DOM composition exhibited a stronger distinction (adonis $R^2 = 0.1683$, $p = 0.002$) compared to POM composition (adonis $R^2 = 0.0996$, $p = 0.033$) (Fig. S4).

3.4. Correlation between organic matter and microbial diversity

Linear models were used to investigate the relationship between organic matter and bacterial alpha-diversity. Significant negative correlations between DOM richness and bacterial taxon richness across samples, within free-living samples ($r = -0.66$, $p < 0.0001$) and within particle-associated samples ($r = -0.41$, $p = 0.026$) (Fig. 5A). Alpha-diversity of POM molecular features also showed a significant correlation with that of FL bacterial samples ($r = -0.59$, $p = 0.0006$), but no significant correlation was found with PA bacterial samples ($r = -0.41$, $p > 0.05$) (Fig. 5B). Next, dbRDA was used to investigate the relationship between organic matter composition and bacterial beta-diversity. Based on the dbRDA models, DOM composition explained 23.3% and 16.0% of the variation in FL and PA bacterial community composition, respectively (Fig. 6A). And POM composition explained 23.0% and 15.1% of the variation in FL and PA bacterial community composition, respectively (Fig. 6B).

3.5. Co-occurrence network between the microbial community and organic matter molecules

Spearman's correlations was used to computed the relationships between bacterial ASVs and the identified molecular features, evaluating the connection between microbial populations and organic matter molecules. Spearman's correlation-based network for FL bacteria comprised 434 nodes and 271 edges for ASVs and DOM molecular features, while the PA bacteria network had 603 nodes and 884 edges (Fig. 7A). The network for FL and PA bacteria with POM features contained 393 nodes and 446 edges, and 657 nodes and 1,105 edges, respectively (Fig. 7B). Across all networks, the average *m/z* values of features in the positive correlations were: DOM and FL (298.4), DOM and PA (296.5), POM and FL (376.0), and POM and PA (379.5). However, features in the corresponding negative networks had higher average *m/z* values: DOM and FL (328.6), DOM and PA (332.3), POM and FL (641.3), and POM and PA (485.4). In all networks, Flavobacteriales were the most abundant taxa based on ASV nodes, followed by Rhodobacterales and SAR11 (Fig. 7). Fatty acids and alkaloids were the majority of organic matter features nodes, while positive network primarily contained amino acids and peptides, and carbohydrates associated nodes.

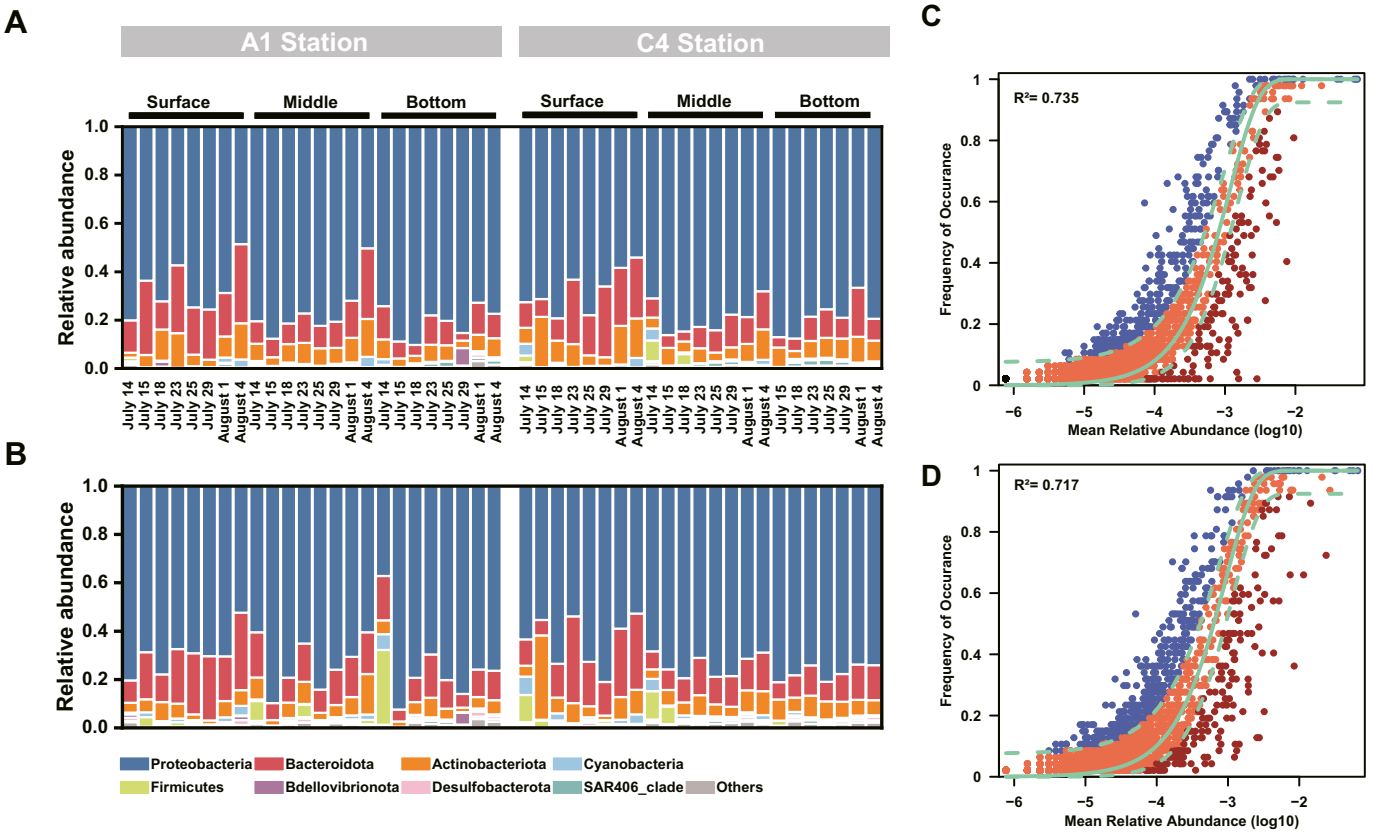


Fig. 1. Taxonomic composition of bacterial communities at the phylum level in the free-living fraction (A) and particle-associated fraction (B). Fit of Sloan's neutral community model for the free-living (C) and particle-associated (D) bacterial communities. Blue and red points indicate the ASVs that occur more and less frequently than predicted by the model. The dashed green lines represent 95% confidence intervals around the model prediction (solid green line). R^2 indicates the goodness-of-fit to the model.

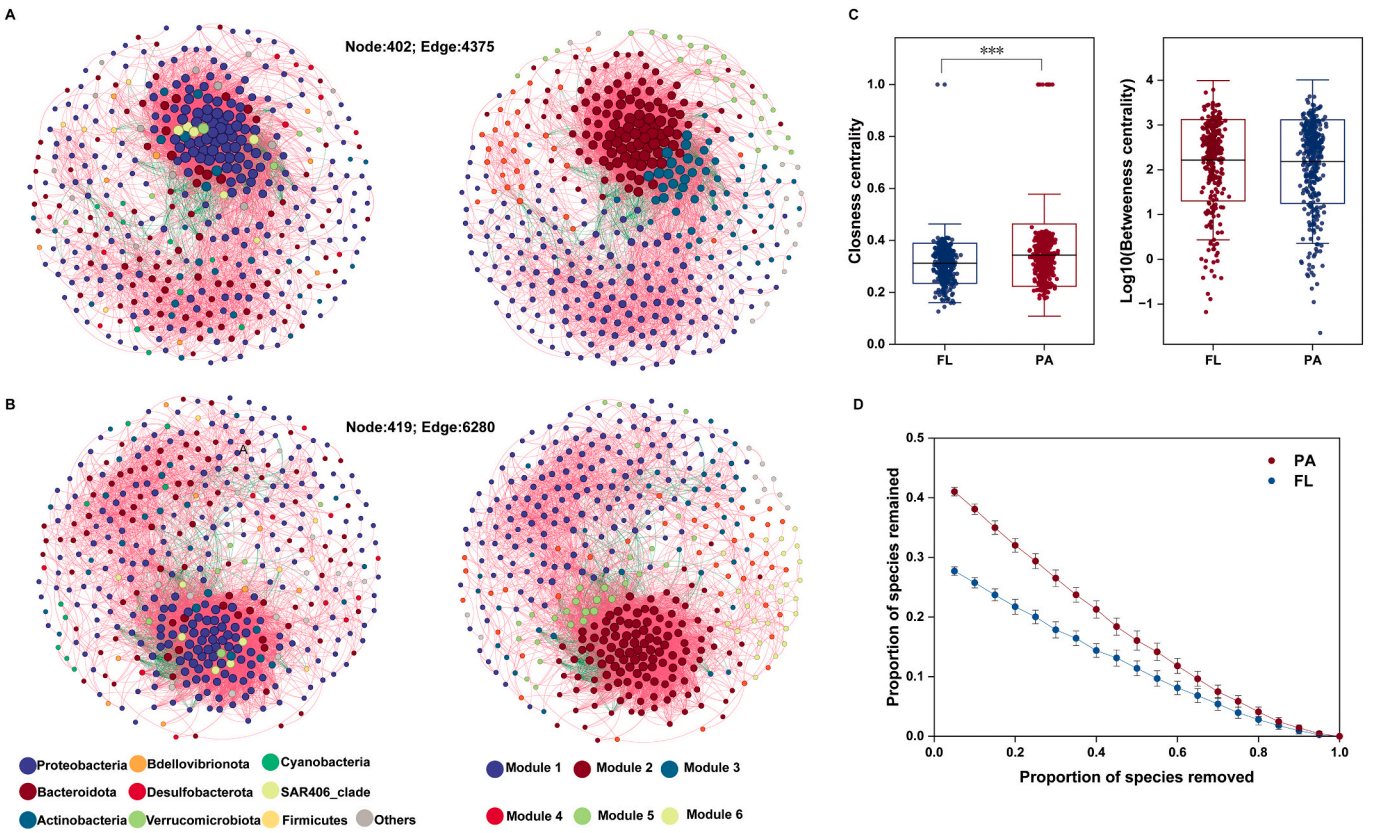


Fig. 2. Co-occurrence network of bacterial communities colored by corresponding phylum and modules in the free-living (A) and particle-associated (B) samples. Comparison of closeness centrality and betweenness centrality for the free-living and particle-associated bacterial network (C). Random removal robustness of the free-living and particle-associated bacterial networks (D).

4. Discussion

4.1. Bacterial community structure under bloom-induced disturbance

Over a 22-day period, PA bacterial communities had significantly higher richness compared to FL bacterial communities (Fig. S5). PA communities are generally more diverse than FL one in coastal seawater (Wang et al., 2020; Crump et al., 1999; Liu et al., 2020), whereas the reverse pattern is observed in oligotrophic seas (Roth Rosenberg et al., 2021; Zhang et al., 2020). Bloom can also stimulate a shift in bacterial lifestyle shift from FL to PA state by altering bacterial cell surfaces, promoting adhesion and particle formation (Liu et al., 2019; Rinta-Kanto et al., 2012). The most abundant bacterial taxa in both FL and PA communities were SAR11, Rhodobacterales, Flavobacteriales, and Alteromonadales (Fig. 1). SAR11 and Rhodobacterales consistently maintained high abundance across all samples, typically utilizing phytoplankton-derived metabolites (Alonso and Pernthaler, 2006). Flavobacteriales, the dominant lineage in the natural bacterial community associated with diatoms, often metabolize complex organic matter that require exoenzyme activity, especially during phytoplankton blooms (Pinhassi et al., 2004; Shibl et al., 2020). Alteromonadales genera, such as *Alteromonas* and *Pseudoalteromonas* usually, are typically copiotrophic opportunists (Pedler et al., 2014; Reintjes et al., 2019), and their relative abundance increases under the influence of blooms (Fig. 1). Co-occurrence network analysis suggested that the PA community network was more stable than the FL community network, evidenced by significantly higher robustness (Fig. 2). PA bacteria are generally colonizing the surface of organic particle, gaining access to abundant resource (Meinhard et al., 2002). Additionally, particles can provide a physical barrier, protecting them from predators and environmental pressures (Dang and Lovell, 2016). Consequently, PA bacteria are usually less susceptible to environmental disturbance such as dissolved oxygen, temperature, pH (Zhao et al., 2021; Tang et al., 2017).

Both FL and PA bacterial communities were strongly driven by stochastic processes, with NCM explaining 73.5% and 71.5% of the community variation, respectively (Fig. 1). The mantel test showed no significant relationship between measured environmental factor (including temperature, salinity, pH, dissolved oxygen, dissolved organic carbon concentration, total organic nitrogen concentration, particle organic nitrogen concentration) and FL and PA communities, while dissolved oxygen, particle organic carbon concentration were significantly related to PA bacterial communities (Table S4). The relative importance of stochastic processes in our samples was higher than communities in other coastal systems (Shi et al., 2022; Mo et al., 2018). Strong currents are present at our studied areas, likely increasing dispersal-related processes and ecological drift. However, the pattern of community assembly differed between the two lifestyles (Fig. S6). Homogeneous selection played a relatively greater role in structuring FL communities compared to PA communities (Fig. S6). This observation could be explained by the fact that FL bacteria exhibited similar response to environmental factors such as light, and predation, while particles protected PA bacteria from environmental influences (Langenheder and Lindström, 2019; Zhou and Ning, 2017). Dispersal limitation increased in PA communities compared to FL communities (Fig. S6). The attachable particles serve as a dispersal barrier between the PA bacterial community and surrounding seawater environment, whereas passive dispersal driven by water movement is less limited for FL bacteria (Villarino et al., 2018).

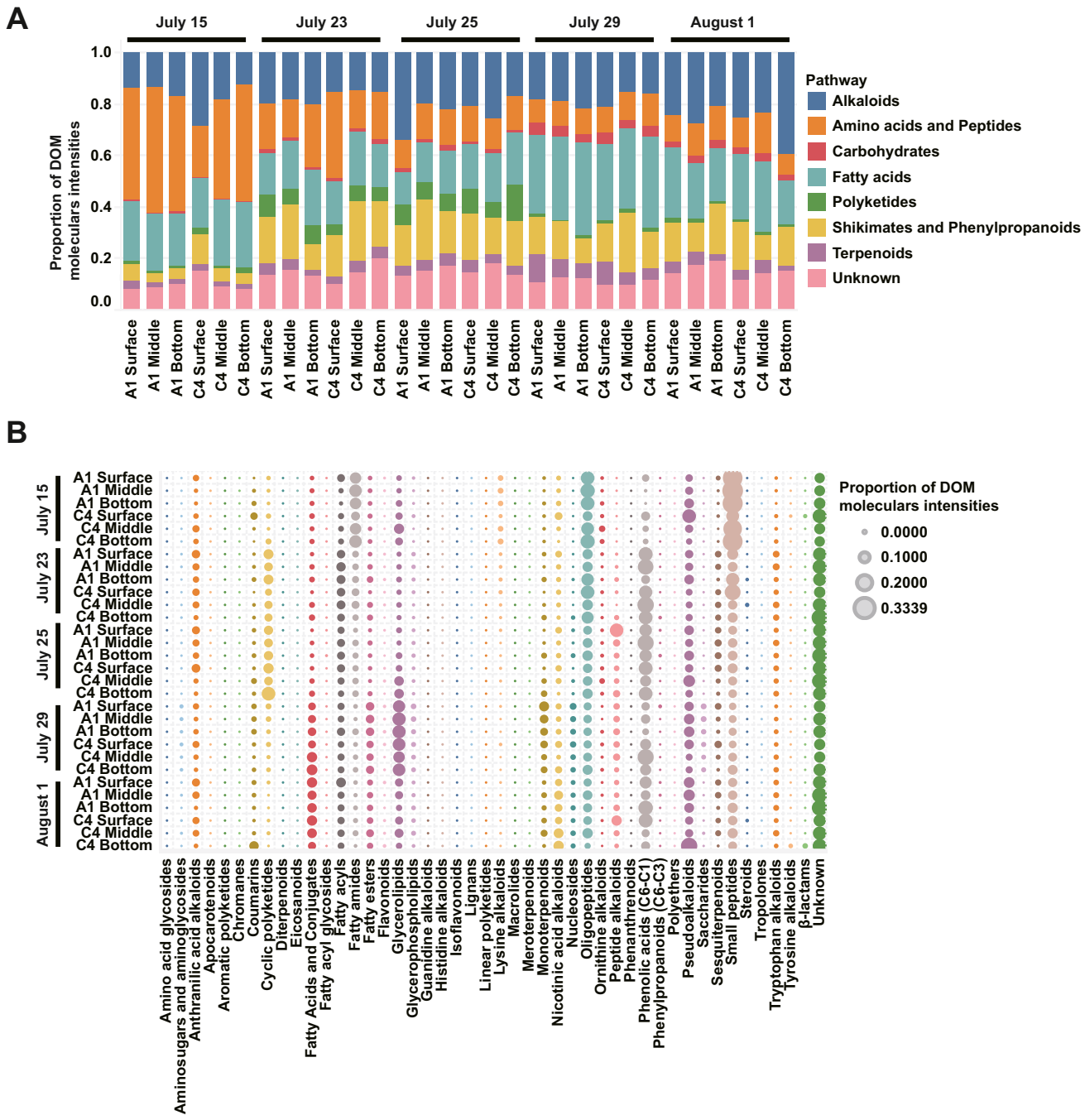


Fig. 3. Chemical pathway (A) and chemical superclass (B) annotations based on the NPClassifier ontology are shown according to the relative intensities of DOM molecular feature.

4.2. Spatiotemporal variation in the organic matter composition

The number of identified organic matter molecular features was significantly higher in bloom-affected samples than in non-bloom samples (Table S5), particularly for DOM. This is expected, as phytoplankton growth releases a significant amount of organic nutrients (Azam and Malfatti, 2007). Bloom-affected samples exhibited a shift in DOM composition, with a significant increase in the relative abundance of compounds belonging to amino acids and peptides (Fig. 3). Our previous research in Xiamen sea also found significant increases in amino acids, dipeptides and lipids molecules during bloom development (Han et al., 2021). While PCoA analysis of the POM molecular data revealed a clear distinction between sampling times (Fig. S4), while the overall chemical composition of POM molecules showed minimal variation across all samples (Fig. 4). The Changjiang Estuary carries a large number of land-based substances into the East China Sea, which makes the estuary and the adjacent sea areas rich in nutrition and vigorous production (Osterholz et al., 2018). The organic matter in our studied areas is a mixture of terrestrial and predominantly marine-derived components, with estimates suggesting a 50–80% marine contribution in the adjacent shelf sea (Liang et al., 2023). Marine-derived POM primarily originates from the biomass of autotrophic organisms, while DOM can also be derived from the release of organic matter by phytoplankton into the seawater (Eppley and Peterson, 1979). Terrestrial organic matter, dominated by resistant chemical composition such as cellulose, lignin, and cutin is relatively more aged and refractory than marine algal-

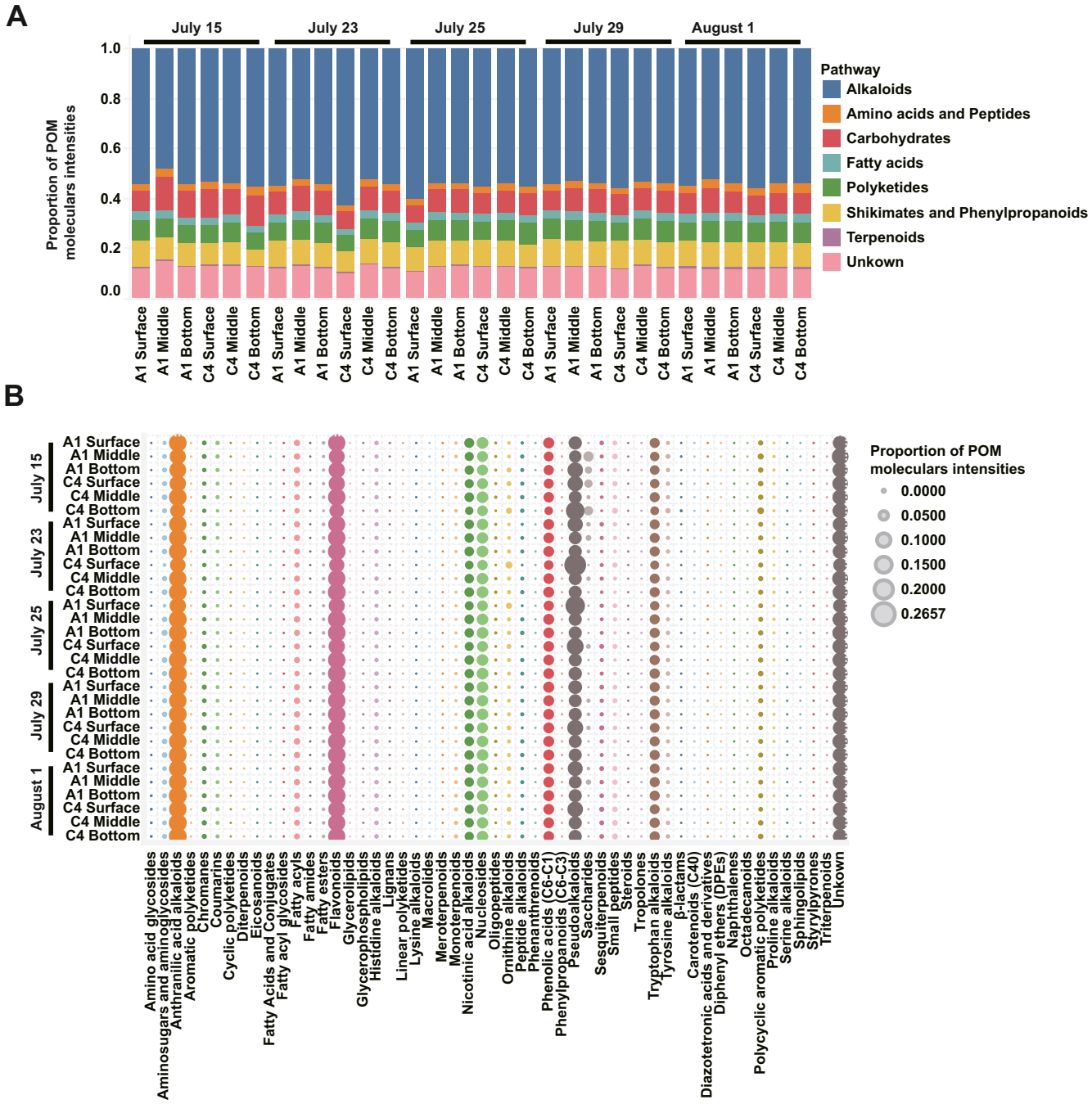


Fig. 4. Chemical pathway (A) and chemical superclass (B) annotations based on the NPClassifier ontology are shown according to the relative intensities of POM molecular features.

derived organic matter (Hedges et al., 2000). Pathways such as viral lysis of cells, grazing by heterotrophic organisms (resulting in the release of phytoplankton cell contents), and extracellular enzymatic hydrolysis of POM by microbial communities contribute to the increase of DOM in seawater (Jiao et al., 2010). Diatoms and dinoflagellates are the two dominant phytoplankton groups in the East China Sea (Fig. S1). The diatom bloom primarily increased the total concentration of POM (Fig. S3 and S6), while the relative abundances of major POM molecule classes remained stable, suggesting minimal alteration in the overall POM composition. The phytoplankton bloom introduced an increased quantity of organic matter, providing more resources for bacteria, which led to an augmentation in bacterial abundance (Table S5). Increased microbial growth, biomass, and turnover likely drove the release of more amino acids and peptides in DOM compositions (Fig. 3).

4.3. *The relationship between organic matter molecules and bacteria*

Substrate availability and interactions among microorganisms influence the spatiotemporal variation in microbial community composition (Fu et al., 2020; Seymour et al., 2017; Teeling et al., 2012). Our analysis revealed a strong negative correlation between bacterial alpha-diversity and the number of molecules within the DOM pool (Fig. 5A). Similarly, bacterial community beta-diversity was related to DOM composition (Fig. 6A). These findings suggest that the supply of DOM

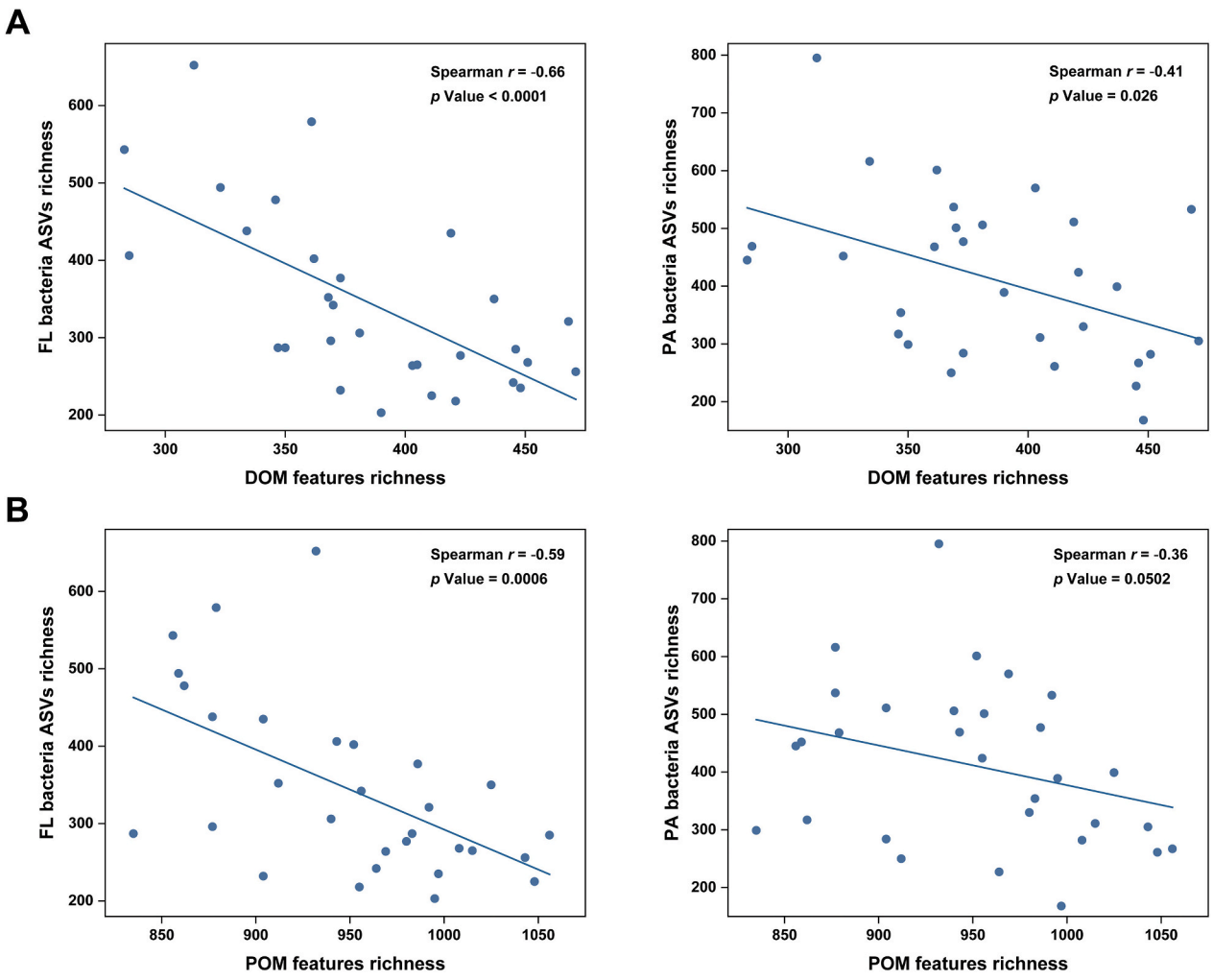


Fig. 5. Relationship between and bacterial community alpha diversity and DOM (A) and POM (B) components. The blue lines denote the ordinary least squares linear regression across all samples between organic matter features richness and bacterial ASV richness.

strongly influenced microbial populations. Previous addition incubation experiments also supported the molecular-level interactions between microbial population and DOM molecular changes (Wang et al., 2022). The negative correlation between bacterial richness and DOM molecular richness is inconsistent with previous observations that more diverse DOM sustains higher bacterial diversity (Chen et al., 2022). This discrepancy may be explained by the diatom bloom releasing a significant amount of organic matter, leading to an increase in the variety of organic matter molecules. However, opportunistic bacteria gain a competitive advantage, resulting in a decrease in microbial abundance in bloom-affected samples (Han et al., 2021). The stronger correlation between FL bacteria and DOM was expected, as FL bacteria are the primary degraders of DOM in costal ecosystems (Karner and Herndl, 1992). Higher covariance between DOM diversity and free-living bacteria diversity, compared to PA bacteria, has also been observed in lake ecosystems (Kajan et al., 2023).

An interesting finding is that the composition of organic matter was more strongly related to the composition of FL bacterial communities compared to PA communities. The alpha diversity of the FL community exhibited a stronger correlation with that of organic matter molecules including both DOM and POM compared to PA community (Fig. 5). Similarly, the beta diversity of both DOM and POM explained the beta diversity of FL bacteria to a greater extent than it did for PA bacteria (Fig. 6). Previous research reveals that compositions of POM, including stable isotope composition, C/N ratio, and chemical composition, is more strongly related to the FL community composition than to the PA community (Zhang et al., 2016). The particle microenvironment properties, rather than chemical composition, might have shaped the corresponding PA bacterial community structure (Hu et al., 2022). We also found that anaerobic bacteria such as Desulfobulbaceae, Desulfobacteraceae, Desulfocapsaceae, Phycisphaeraceae, Lachnospiraceae, Hyphomicrobiaceae and Magnetospiraceae were enriched in particle-associated communities (Fig. 1), suggesting the presence of low-oxygen microzones within these particles. This finding highlights the importance of particulate microniches, such as low-oxygen environments, in shaping the structure of particle-associated bacterial communities. This also supports the notion that PA bacterial communities are more resilient to bloom-induced disturbances, while FL bacteria exhibit higher variations in temporal dynamics and greater sensitivity to the structure composition of organic matter molecules.

Diatoms, as primary producers, significantly contribute to the formation of both particulate and dissolved organic matter through their photosynthesis and the release of extracellular polymeric substances and other organic compounds (Eppley and Peterson, 1979). Our statistical

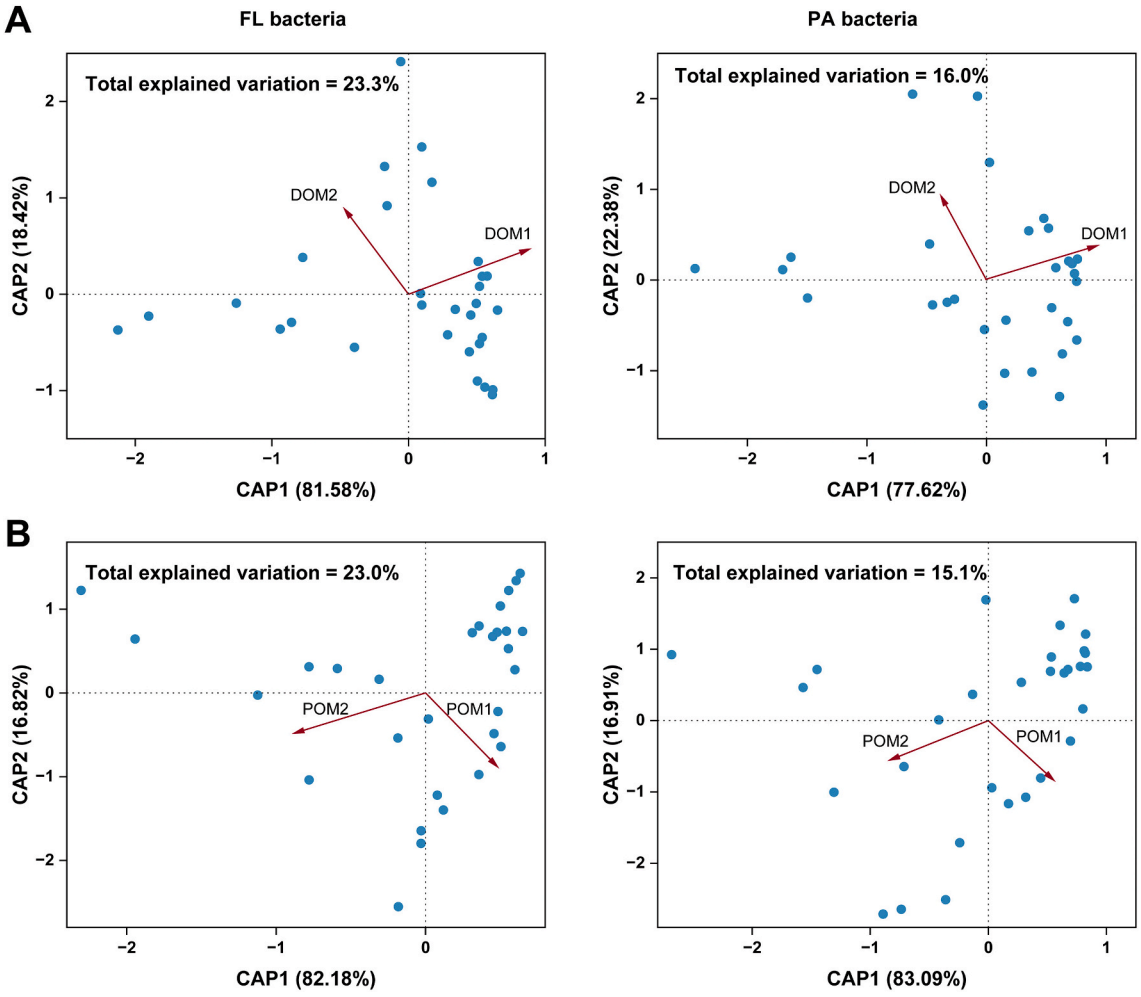


Fig. 6. Relationship between bacterial community beta-diversity and the beta-diversity of DOM (A) and POM (B). The vectors represent the correlation between bacterial community composition and the first and second axes of the organic matter PCoA.

analysis revealed an enrichment of specific bacterial taxa known to associate with diatoms were enriched in both PA and FL bacterial communities (Fig. 1). For example, members of the Rhodobacteraceae and Flavobacteriaceae families, commonly associated with diatom blooms, were prevalent in our samples. This suggests a strong influence of diatom-derived organic matter on these bacterial populations (Fig. 1). These findings highlight the crucial role of diatom-bacteria interactions in shaping the composition of organic matter and bacterial communities.

Strong positive correlations in co-occurrence networks are related to the production of new molecular features, while negative correlations may indicate degradation processes of these molecular features (Hu et al., 2022). In all networks of organic matter features and bacterial ASVs, features with higher *m/z* values were found in the negative network, while feature with lower *m/z* values were found in the negative network (Fig. 7). This suggests that bacteria likely degraded high *m/z* organic matters to low *m/z* organic matters. Phytoplankton can produce and release relative high molecular weight DOM into the environment, which can be degraded into low molecular weight DOM (Jiao et al., 2014). Our results are consistent with observed correspondence between DOM features and the microbial community in coastal estuaries (Chen et al., 2021). Additionally, laboratory incubation experiments supported this link, demonstrating that bacterial communities preferentially consume high molecular weight DOM fractions and transform them into lower molecular forms (Zheng et al., 2019). Flavobacteriales were identified as the major bacteria in the network of organic matter and bacterial communities (Fig. 7). The importance of Flavobacteriales in organic matter degradation is well-recognized, likely due to their ability to utilize a diverse sets of enzyme systems for both polysaccharides and proteins (Fernández-Gómez et al., 2013).

5. Conclusion

This study links the biochemical properties of size-fractioned organic matter to PA and FL bacterial communities, revealing how organic matter composition shapes bacterial community structure. Co-occurrence network analysis suggested that PA bacterial communities are more stable than FL bacterial communities during bloom disturbance. The relative proportion of amino acids and peptides in DOM were significantly increased during bloom-affected period, while the major classes of POM molecules exhibited relative stability across all samples. The richness of organic matter molecules showed a significant negative correlation with the bacterial community richness. Additionally, bacterial community beta-diversity was related to organic matter compositions. Interestingly, the composition of organic matter molecules, including DOM and POM, was more strongly correlated with FL bacterial communities than PA communities. This suggests that FL bacteria exhibited greater sensitivity to the structure of organic matter molecules compared to PA bacteria.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174845.

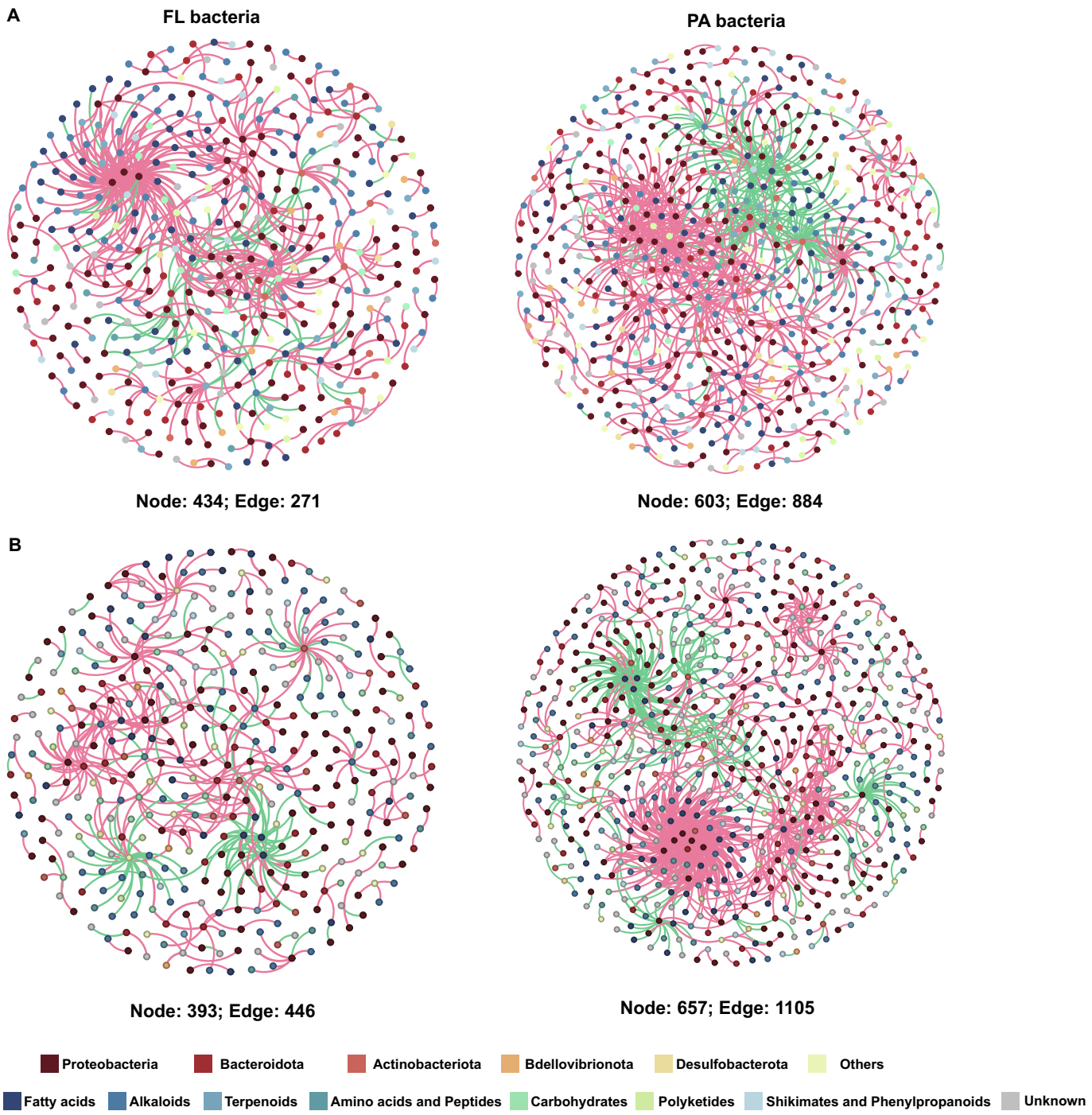


Fig. 7. Co-occurrence network of bacterial ASVs and DOM molecular features (A), as well as POM (B) molecular features. The node colors represent the microbial taxonomy and chemical pathway of organic matters features. Red and green lines represent significant positive and negative relationships ($|r| > 0.6$ and $p < 0.05$), respectively.

CRediT authorship contribution statement

Shujing Liu: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Quanrui Chen:** Writing – review & editing, Methodology, Investigation. **Le Liu:** Writing – review & editing, Methodology, Investigation. **Changjie Dong:** Writing – review & editing, Methodology, Formal analysis. **Xuanyun Qiu:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Kai Tang:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

The sequences of 16S rRNA and 18S rRNA, and LC-MS/MS raw data have been uploaded to the National Omics Data Encyclopedia (NODE, https://www.biosino.org/node/) database with the identifier

OEX024199, OEX024200, OEX027721, and OEX027720, respectively.

Acknowledgments

This study was supported by the National Natural Science Foundation of China project (92251306), the National Key Research and Development Program of China (2020YFA0608300), and the National Natural Science Foundation of China project (42188102, 42276120, 42076160).

References

Alonso, C., Pernthaler, J., 2006. Roseobacter and SAR11 dominate microbial glucose uptake in coastal North Sea waters. Environ. Microbiol. 8, 2022–2030.
Azam, F., Malfatti, F., 2007. Microbial structuring of marine ecosystems. Nat. Rev. Microbiol. 5, 782–791.
Boeuf, D., et al., 2019. Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. Proc. Natl. Acad. Sci. USA 116, 11824–11832.
Bolyen, E., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.
Chambers, M.C., et al., 2012. A cross-platform toolkit for mass spectrometry and proteomics. Nat. Biotechnol. 30, 918–920.
Chang, P.-H., Isobe, A., 2003. A numerical study on the Changjiang diluted water in the Yellow and East China seas. J. Geophys. Res. 108, 3299.
Chen, J., et al., 1999. The processes of dynamic sedimentation in the Changjiang estuary. J. Sea Res. 41, 129–140.
Chen, Q., et al., 2021. Correspondence between DOM molecules and microbial community in a subtropical coastal estuary on a spatiotemporal scale. Environ. Int. 154, 106558.
Chen, Q., et al., 2022. Increased microbial and substrate complexity result in higher molecular diversity of the dissolved organic matter pool. Limnol. Oceanogr. 67, 2360–2373.
Cheung, M.K., Au, C.H., Chu, K.H., Kwan, H.S., Wong, C.K., 2010. Composition and genetic diversity of picoeukaryotes in subtropical coastal waters as revealed by 454 pyrosequencing. ISME J. 4, 1053–1059.
Crump, B.C., Armbrust, E.V., Baross, J.A., 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. 65, 3192–3204.
Dai, Y., et al., 2023. Coastal phytoplankton blooms expand and intensify in the 21st century. Nature 615, 280–284.
Dang, H., Lovell, C.R., 2016. Microbial surface colonization and biofilm development in marine environments. Microbiol. Mol. Biol. Rev. 80, 91–138.
Duhrkop, K., Shen, H.B., Meusel, M., Rousu, J., Bocker, S., 2015. Searching molecular structure databases with tandem mass spectra using CSI:FingerID. Proc. Natl. Acad. Sci. USA 112, 12580–12585.
Duhrkop, K., et al., 2019. SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nat. Methods 16, 299–302.
Duhrkop, K., et al., 2021. Systematic classification of unknown metabolites using high-resolution fragmentation mass spectra. Nat. Biotechnol. 39, 462–471.
Eppley, R.W., Peterson, B.J., 1979. Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282, 677–680.
Falkowski, P.G., 1998. Biogeochemical controls and feedbacks on ocean primary production. Science 281, 200–206.
Farnelid, H., et al., 2019. Diverse diazotrophs are present on sinking particles in the North Pacific subtropical gyre. ISME J. 13, 170–182.
Fernández-Gómez, B., et al., 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. ISME J. 7, 1026–1037.
Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240.
Fu, H., Uchimiya, M., Gore, J., Moran, M.A., 2020. Ecological drivers of bacterial community assembly in synthetic phycospheres. Proc. Natl. Acad. Sci. USA 117, 3656–3662.
Grossart, H.-P., 2010. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. Environ. Microbiol. Rep. 2, 706–714.
Grossart, H.-P., Tang, K.W., Kiørboe, T., Ploug, H., 2007. Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. FEMS Microbiol. Lett. 266, 194–200.
Han, Y., et al., 2021. Opportunistic bacteria with reduced genomes are effective competitors for organic nitrogen compounds in coastal dinoflagellate blooms. Microbiome 9, 71.
Hansell, D., Carlson, C., Repeta, D., Schlitzer, R., 2009. Dissolved organic matter in the ocean: a controversy stimulates new insights. Oceanography 22, 202–211.
Hedges, J.I., et al., 2000. Organic matter in Bolivian tributaries of the Amazon River: a comparison to the lower mainstream. Limnol. Oceanogr. 45, 1449–1466.
Hu, A., et al., 2022. Ecological networks of dissolved organic matter and microorganisms under global change. Nat. Commun. 13, 3600.
Jiao, N., et al., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599.
Jiao, N., et al., 2014. Mechanisms of microbial carbon sequestration in the ocean – future research directions. Biogeosciences 11, 5285–5306.
Kajan, K., Osterholz, H., Stegen, J., Gligora Udovič, M., Orlić, S., 2023. Mechanisms shaping dissolved organic matter and microbial community in lake ecosystems. Water Res. 245, 120653.
Karner, M., Herndl, G.J., 1992. Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. Mar. Biol. 113, 341–347.
Kim, H.W., et al., 2021. NPClassifier: a deep neural network-based structural classification tool for natural products. J. Nat. Prod. 84, 2795–2807.
Langenheder, S., Lindström, E.S., 2019. Factors influencing aquatic and terrestrial bacterial community assembly. Environ. Microbiol. Rep. 11, 306–315.
Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24.
Liang, S., et al., 2023. Source, composition, and reactivity of particulate organic matter along the Changjiang estuary salinity gradient and adjacent sea. Mar. Chem. 252, 104245.
Liu, M., et al., 2019. Community dynamics of free-living and particle-attached bacteria following a reservoir Microcystis bloom. Sci. Total Environ. 660, 501–511.
Liu, Y., et al., 2020. Differences in metabolic potential between particle-associated and free-living bacteria along Pearl River estuary. Sci. Total Environ. 728, 138856.
Ludwig, M., et al., 2020. Database-independent molecular formula annotation using Gibbs sampling through ZODIAC. Nat. Mach. Intell. 2, 629–641.
Luo, H., Moran, M.A., 2015. How do divergent ecological strategies emerge among marine bacterioplankton lineages? Trends Microbiol. 23, 577–584.
Maúre, E.d.R., Terauchi, G., Ishizaka, J., Clinton, N., DeWitt, M., 2021. Globally consistent assessment of coastal eutrophication. Nat. Commun. 12, 6142.
Meinhard, S., Hans-Peter, G., Bernd, S., Helle, P., 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28, 175–211.
Mo, Y., et al., 2018. Biogeographic patterns of abundant and rare bacterioplankton in three subtropical bays resulting from selective and neutral processes. ISME J. 12, 2198–2210.
Muscarella, M.E., Boot, C.M., Broeckling, C.D., Lennon, J.T., 2019. Resource heterogeneity structures aquatic bacterial communities. ISME J. 13, 2183–2195.
Ning, D., et al., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nat. Commun. 11, 4717.
Nossa, C., et al., 2010. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J. Gastroenterol. 16, 4135–4144.
Osterholz, H., et al., 2016. Deciphering associations between dissolved organic molecules and bacterial communities in a pelagic marine system. ISME J. 10, 1717–1730.
Osterholz, H., Kirchman, D.L., Niggemann, J., Dittmar, T., 2018. Diversity of bacterial communities and dissolved organic matter in a temperate estuary. FEMS Microbiol. Ecol. 94, fiy119.
Pedler, B.E., Aluwihare, L.I., Azam, F., 2014. Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. Proc. Natl. Acad. Sci. USA 111, 7202–7207.
Petras, D., et al., 2017. High-resolution liquid chromatography tandem mass spectrometry enables large scale molecular characterization of dissolved organic matter. Front. Mar. Sci. 4, 405.
Pinhassi, J., et al., 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Appl. Environ. Microbiol. 70, 6753–6766.
Post, W.M., et al., 1990. The global carbon cycle. Am. Sci. 78, 310–326.
Reintjes, G., Arnosti, C., Fuchs, B., Amann, R., 2019. Selfish, sharing and scavenging bacteria in the Atlantic Ocean: a biogeographical study of bacterial substrate utilisation. ISME J. 13, 1119–1132.
Rinta-Kanto, J.M., Sun, S., Sharma, S., Kiene, R.P., Moran, M.A., 2012. Bacterial community transcription patterns during a marine phytoplankton bloom. Environ. Microbiol. 14, 228–239.
Romankevich, E.A., Vetrov, A.A., Ulyantsev, A.S., 2023. Organic matter in the ocean: indicators of biogeochemical processes. Oceanology 63, S131–S142.
Roth Rosenberg, D., et al., 2021. Particle-associated and free-living bacterial communities in an oligotrophic sea are affected by different environmental factors. Environ. Microbiol. 23, 4295–4308.
Seymour, J.R., Amin, S.A., Raina, J.-B., Stocker, R., 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. Nat. Microbiol. 2, 17065.
Shaffer, J.P., et al., 2022. Standardized multi-omics of Earth's microbiomes reveals microbial and metabolite diversity. Nat. Microbiol. 7, 2128–2150.
Shi, J., et al., 2022. Stochastic processes shape the aggregation of free-living and particle-attached bacterial communities in the Yangtze River Estuary, China. J. Basic Microbiol. 62, 1514–1525.
Shibl, A.A., et al., 2020. Diatom modulation of select bacteria through use of two unique secondary metabolites. Proc. Natl. Acad. Sci. USA 117, 27445–27455.
Sloan, W.T., et al., 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. 8, 732–740.
Smith, M.W., Zeigler Allen, L., Allen, A.E., Herfort, L., Simon, H.M., 2013. Contrasting genomic properties of free-living and particle-attached microbial assemblages within a coastal ecosystem. Front. Microbiol. 4.
Stocker, R., 2012. Marine microbes see a sea of gradients. Science 338, 628–633.
Tang, X., et al., 2017. Spatiotemporal dynamics of bacterial community composition in large shallow eutrophic Lake Taihu: high overlap between free-living and particle-attached assemblages. Limnol. Oceanogr. 62, 1366–1382.
Teeling, H., et al., 2012. Substrate-controlled succession of marine Bacterioplankton populations induced by a phytoplankton bloom. Science 336, 608–611.
Verdugo, P., et al., 2004. The oceanic gel phase: a bridge in the DOM–POM continuum. Mar. Chem. 92, 67–85.

Villarino, E., et al., 2018. Large-Scale Ocean connectivity and planktonic body size. Nat. Commun. 9, 142.
Wang, Y., et al., 2020. Patterns and processes of free-living and particle-associated bacterioplankton and archaeaplankton communities in a subtropical river-bay system in South China. Limnol. Oceanogr. 65, S161–S179.
Wang, Y., et al., 2021. Coastal eutrophication in China: trend, sources, and ecological effects. Harmful Algae 107, 102058.
Wang, Y., et al., 2022. Linking microbial population succession and DOM molecular changes in Synechococcus-derived organic matter addition incubation. Microbiol. Spectr. 2, e02308–e02321.
Yilmaz, P., et al., 2013. The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648.
Yuan, H., et al., 2021. Diversity distribution, driving factors and assembly mechanisms of free-living and particle-associated bacterial communities at a subtropical marginal sea. Microorganisms 9, 2445.
Zhang, Y., Xiao, W., Jiao, N., 2016. Linking biochemical properties of particles to particle-attached and free-living bacterial community structure along the particle density gradient from freshwater to open ocean. J. Geophys. Res. 121, 2261–2274.
Zhang, Y., Jing, H., Peng, X., 2020. Vertical shifts of particle-attached and free-living prokaryotes in the water column above the cold seeps of the South China Sea. Mar. Pollut. Bull. 156, 111230.
Zhao, Z., et al., 2019. Microbial transformation of virus-induced dissolved organic matter from picocyanobacteria: coupling of bacterial diversity and DOM chemodiversity. ISME J. 13, 2551–2565.
Zhao, Z., Baltar, F., Herndl, G.J., 2020. Linking extracellular enzymes to phylogeny indicates a predominantly particle-associated lifestyle of deep-sea prokaryotes. Sci. Adv. 6, eaaz4354.
Zhao, D., et al., 2021. Disproportionate responses between free-living and particle-attached bacteria during the transition to oxygen-deficient zones in the Bohai seawater. Sci. Total Environ. 791, 148097.
Zheng, Q., et al., 2019. Molecular characteristics of microbially mediated transformations of Synechococcus-derived dissolved organic matter as revealed by incubation experiments. Environ. Microbiol. 21, 2533–2543.
Zhou, J., Ning, D., 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81, e00002–e00017.