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Temporal and Vertical Distribution of Bacterioplankton at the Gray's Reef National Marine Sanctuary

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Running Head**:** Temporal variability of a coastal bacterioplankton population

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Abstract

Large spatial scales and long term shifts of bacterial community composition (BCC) in the open ocean can often be reliably predicted based on the dynamics of physical-chemical variables. The power of abiotic factors in shaping BCC at shorter time scales in shallow estuarine mixing zones is less clear. We examined diurnal variation of BCC at different water depths in spring and fall of 2011 at a station in the Gray's Reef National Marine Sanctuary (GRNMS). This site is located in the transition zone between the estuarine plume and continental shelf waters of the South Atlantic Bight. A total of 234,516 pyrotag sequences of bacterial 16S rRNA genes were recovered; they were were taxonomically affiliated with over 200 families of 23 bacterial phyla. Non-metric multidimensional scaling analysis revealed significant differences of BCCs between spring and fall samples, likely due to seasonality in the concentrations of dissolved organic carbon and nitrate+nitrite. Within each diurnal sampling, BCC changed significantly by depth only for spring samples, and between day and night only for fall samples. The former variations largely tracked changes of light availability, while the latter was most correlated with concentrations of polyamines and chlorophyll *a*. Our results suggest that at the GRNMS, a coastal mixing zone, diurnal variation of BCCs is attributable to mixing of local and imported bacterioplankton rather than to bacterial growth in response to environmental changes. Our results also indicated that like roseobacters, SAR11 bacteria may play an important role in processing dissolved organic material in coastal oceans.

Keywords: Bacterial community composition; Coastal seawater; Gray's Reef National Marine Sanctuary; Pyrosequencing

Introduction

Spatial variability of microbes, i.e., the occurrence of distinct patterns of bacterial community composition (BCC) among geographically isolated habitats or different depth zones has been well established in marine environments (1−5). Annually recurring patterns and pronounced seasonal variability in BCC have also been studied in a number of marine environments (6−9).

However, BCC variations at shorter time scales, such as diurnal cycles, are relatively understudied and available reports yield conflicting results. For example, BCC showed little diurnal change in the Western English Channel (10) but varied significantly in coastal California (11) and in the upper mixed layer of the Ligurian Sea (northwest of the Mediterranean Sea) (12). Moreover, some BCC studies are based solely on molecular fingerprinting methods, such as automated ribosomal intergenic spacer analysis (7) and terminal restriction fragment length polymorphism (13), therefore, lacking taxonomic resolution. Sequence-based studies are available but are often reported at very broad taxonomic levels (such as phylum/class) (8, 11) or focused only on a few specific taxa (14). Nonetheless, despite variations in sampling dimensions (spatial or vertical) and analytical methods, microbial ecologists have reached a general consensus that long-term dynamics of open ocean BCC are regulated by physical variables, most notably temperature (7, 8). However, whether these environmental factors apply similar impacts on BCC in estuarine mixing zones and at short time scales remain unclear.

This study investigated depth and diurnal dynamics of BCCs (i.e., relative abundance of bacterioplankton taxa) at the Gray's Reef National Marine Sanctuary (GRNMS) in two consecutive seasons and examined the potential correlations between BCCs and environmental factors. GRNMS is located c.a. 32 km offshore of the coast of Georgia (USA) in the transition zone between the nearshore estuarine plume and continental shelf waters in

the South Atlantic Bight. This coastal site has a long-term data set of physical and water quality variables as a result of the GRNMS monitoring program and the efforts of other researchers (15). Water chemistry at the reef is subjected to complex seasonal changes and exhibits intense short-term dynamics due to strong tidal mixing and wind-driven advection (15−17). Past GRNMS resource assessment efforts have documented community structures of algae, coral, sponges and fish (18), leaving the bacterioplankton unexplored.

Materials and methods

Sample collection and processing

Two sets of water samples were collected on two cruises of the R/V Savannah to the GRNMS (31° 24.04′ N, 80° 51.51′ W), one in spring (April 20-21, 2011) and the second in fall (October 5-6, 2011). Water samples were collected every 3 h during a 24-hour period (8 or 9 casts in total) using Niskin bottles mounted on a rosette sampling system (Sea-Bird Electronics, Bellevue, WA, USA). Samples taken after sunrise and before the following sunset were labeled as day samples, while samples taken after sunset and before the following sunrise were labeled as night samples. Depth profiles of environmental variables including temperature (T), salinity (S), and photosynthetically active radiation (PAR) were measured *in situ* with a conductivity-temperature-depth (CTD) water column profiler (Sea-Bird Electronics, Bellevue, WA, USA) that was mounted on the rosette sampler system. Water samples were collected at three different depths (~2, 4, and 17 m) in spring, when a pycnocline was present, and at two depths (~2 and 17 m) in fall, when the pycnocline was absent (Fig. S1). The tidal stage at time of sampling was quantified by transforming the data into tidal angles (19). Briefly, a conversion factor was first calculated by dividing the time interval between two successive high tides by 360°. Tidal angles were then calculated by multiplying this conversion factor by the time interval between a sampling and its previous

high tide. As a result, tidal angles of 0° and 180° were corresponding to high and low tides, respectively.

Immediately after collection, 1 liter of water samples were filtered sequentially through 3 µm and 0.2 µm pore size membrane filters (Pall Life Sciences, Ann Arbor, MI, USA). The filtrates were collected in 50 ml sterile conical centrifuge tubes and stored at −20 °C until analyses of organic carbon (DOC), dissolved nitrogen (DN), nitrate+nitrite (NO_x^-), and soluble reactive phosphorus (SRP). Filtrates were also collected in 5 ml amber glass vials and stored at −80°C for analyses of two labile dissolved organic nitrogen groups: dissolved free amino acids (DFAAs) and polyamines (PAs). Cells that were collected on 0.2 µm pore size membrane filters were frozen immediately in liquid nitrogen onboard and stored at −80 °C in lab until DNA extraction. Particulate material in 500 ml whole water samples was collected onto glass fiber filters (GF/F; Whatman International Ltd, Maidstone, England) for chlorophyll *a* (Chl *a*) measurement, and stored in the dark at −20 °C before analysis.

Part of the water (2 ml) that passed through the 3 µm pore size filters was preserved in 1% (final concentration) freshly prepared paraformaldehyde and stored at 4 °C until cells were enumerated by epifluorescence microscopy.

All glass ware and GF/F filters were combusted at 500 °C for 4 h before use. Triplicate samples were taken for analyses of nutrients and cell counts.

Nutrient analysis

Nutrient measurements were performed following standard procedures (20). Briefly, DOC and DN concentrations were determined with a TOC/TN analyzer (TOC-V_{CPN}; Shimadzu Corp., Tokyo, Japan) based on combustion oxidation/infrared detection and combustion/chemilluminescence detection methods, respectively. Concentrations of NO_x^- and SRP were determined based on the cadmium reduction method and the molybdenum blue

method, respectively, using flow injection protocols (Lachat QuikChem FIA+ 8000Series, Loveland, CO, USA).

Concentrations of DFAAs and PAs were measured using a Prominence 20A high-performance liquid chromatography system (Shimadzu Corp., Tokyo, Japan) equipped with a 250 × 4.6 mm i.d. 5 µm particle size, Phenomenex Gemini-NX C_{18} column (Phenomenex, Torrance, CA, USA) following a protocol developed specifically for seawater samples (21). Chl *a* was extracted from the GF/F filters with 90% acetone and measured by spectrophotometry (22).

DNA extraction, PCR, and Pyrotag sequencing

DNA was extracted from 0.2 µm pore size membrane filters using PowerSoil DNA extraction kits (MoBio Laboratory Inc., Carlsbad, CA, USA). The V4-V6 regions of 16S rRNA genes were PCR amplified using universal bacterial primers B530F (5′-GT GCC AGC MGC NGG GG-3′) (23) and B1100R (5′-GGG TTN CGN TCG TTG-3′) (24). The forward primers were constructed with an adaptor sequence (CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG) (23) and a 10 bp barcode tag, while the reverse primers were constructed with an adaptor sequence (CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG) only. The PCR program included an initial denaturation at 95 °C for 3 min, then 30 cycles of 95 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min, followed by a final elongation step at 72 °C for 5 min. For each sample, 5 separate PCR reactions (25 µl each) were performed, the resulting 125 µl of PCR amplicons were pooled and examined by gel electrophoresis (1% agarose) to verify amplicon length. The amplicons were excised from the gels and purified first with the QIAquick gel extraction kit (Qiagen, Chatsworth, CA, USA) and then with the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA). The quantity of purified PCR amplicons was determined using the Quant-iT PicoGreen ds DNA Assay Kit (Life technologies, Carlsbad, NY, USA). Equimolar amounts of PCR amplicons from 13 samples

(randomly assigned) were combined and sequenced in one run using a 454 GS Junior System (Roche 454 Life Sciences, Branford, CT, USA) with unidirectional Lib-L chemistry. Three pyrosequencing runs were performed to sequence a total of 39 samples collected in this study.

Taxonomic annotation of 16S rRNA gene pyrotag sequences

Bacterial 16S rRNA gene pyrotag sequences were assigned to their sample of origin based on barcodes. Primer and barcode sequences were then removed from each sequence. Sequences that had wrong base calls in the primer regions, that were shorter than 65 bp, or that contained chimeras (UCHIME) (25) were removed from further analysis. The remaining sequences were clustered into operational taxonomic units (OTUs) using CD-HIT (26) at the 97% identity cutoff level. Singleton OTUs were excluded from the OTU list to prevent potential overestimation of bacterial diversity (27). The longest sequence within each OTU group was selected as a representative and blasted against the SILVA SSU database for taxonomic annotation (28). Most sequences were summarized at the family level, except for those that were affiliated with marine bacterial groups that have no official taxonomic standings. The latter sequences were summarized at the clade level, such as SAR11 and SAR116. This family/clade level organization of sequences was referred to as at the family level hereafter for simplicity. Since the focus of the study was on bacterioplankton, sequences annotated as chloroplasts were excluded from further analyses.

Diversity and statistical analysis

Diversity calculations and statistical analyses were performed using PRIMER v5 (Plymouth Marine Laboratory, Plymouth, UK; 29) unless mentioned otherwise. Family-level diversity indices were calculated using the Shannon index. Family-level rarefaction curves were constructed to infer the library coverage (30) using the vegan package in R (31).

The similarity of BCC, i.e., relative abundance of bacterial taxa, among samples was examined using non-metric multidimensional scaling (NMDS) analysis, based on a Bray-Curtis matrix that was calculated using normalized and square-root transformed relative abundance of major bacterial families among samples (29). A hierarchical agglomerative clustering analysis was also performed based on the same matrix using group-average linkage. The robustness of NDMS grouping patterns was assessed by analysis of similarity (ANOSIM), an analogue of the standard univariate analysis of variance (ANOVA). The ANOSIM index r_{ANOSIM} was reported on a scale of 0 to 1. When $P < 0.05$, the sample groups were reported as well-separated with $r_{ANOSIM} > 0.75$, clearly different but overlapping with $0.5 < r_{ANOSIM} \leq 0.75$, separated but strongly overlapping with $0.25 < r_{ANOSIM} \leq 0.5$, or not separable with $r_{ANOSIM} < 0.25$ (29). Similarity percentage (SIMPER) analysis was conducted to identify the contribution of each bacterial family to the observed difference between sample groups.

Redundancy analysis (RDA; 32) was used to explore correlations between changes in BCC and environmental factors, including T, S, PAR, tidal stage (Tide), DOC, DN, NO_X^-, SRP, DFAAs, polyamines and Chl *a*, using the vegan package in the R software package (31). The significance of the correlation values was assessed statistically by a Monte Carlo analysis using 1000 permutations.

The significance of differences between depth and diurnal (day-night) variations in bacterial diversity and composition was tested using Student's *t* test (for paired samples), or one-way ANOVA (for multiple samples) within the R software package (33). Differences were deemed significant when $P < 0.05$. Potential correlations between the relative abundance of individual bacterial taxa and environmental variables were examined by calculating Pearson's product momentum correlation coefficients (*r*) using the R software

package (33). Significant correlations were reported when $P < 0.05$. Bonferroni corrections of P values were used for multiple tests.

Nucleotide Sequence accession number:

The 16S rRNA partial sequences were deposited in the NCBI Sequence Read Archive (SRA) under the project accession numbers: SRR1556928, SRR1602557 and SRR1602558.

Results

General statistics of 16S rRNA gene pyrotag sequences and taxonomic composition of bacterioplankton

A total of 234,516 partial sequences of bacterial 16S rRNA genes with an average read length of 561 bp passed quality control steps. The number of sequences per sample ranged from 1,516 to 17,689 in spring and from 1,260 to 12,862 in fall, which corresponded to 170 to 2,859 and 292 to 1,662 OTUs (roughly at the species level), respectively (Table S1). These OTUs were widely distributed across the Domain Bacteria with over 200 unique families from 23 phyla in total. Rarefaction curves of sequence libraries based on family-level annotations all reached plateau (Fig. S2), despite differences in library size (either by sequence or OTU number). Therefore, recovered pyrotag sequences were sufficient to represent bacterioplankton diversity in our samples at the family level.

Over 85% of recovered bacterial sequences were affiliated with only 12 families of 5 phyla, namely the *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Deferribacteres*, and *Bacteroidetes*. Each of these 12 families accounted for 2% or more of the total sequences (all spring and fall libraries together) (Fig. 1; Table 1) and was designated as major taxa. Among them, SAR11 (*Alphaproteobacteria*) and Family I *Cyanobacteria* (93.2% were *Synechococcus*) were the most abundant, accounting for 30.7% and 13.6% of sequences on average, respectively. Cluster analysis revealed that, in both seasons, patterns of SAR11 distribution differed significantly from Family I *Cyanobacteria* and other major taxa (Fig. 1).

Seasonal variations of BCC and influential environmental factors

Family-level Shannon index (H) values showed that spring communities were more diverse than those in fall (Table S1; *t* test, $P < 0.05$).

NMDS ordination analysis displayed a clear separation between spring and fall BCCs (Fig. 2). ANOSIM ($r_{ANOSIM} = 0.99$; $P < 0.05$) analyses further confirmed the robustness of this separation (Table S2). The SAR11 clade and Family I *Cyanobacteria* were the 2 most abundant taxa in both seasons, with the former over-represented in spring (average 37.6% of the sequences) relative to fall (21.5%) and the latter overrepresented in fall (20.1%) relative to spring (9.6%) (Table 1; *t* test, $P < 0.05$). SIMPER analysis further revealed that these two taxa contributed the most to the observed seasonal BCC dissimilarities (24.0% and 16.5% of dissimilarity, respectively), followed by *Vibrionaceae* (11.0% of dissimilarity) and *Pseudoalteromonadaceae* (9.7% of dissimilarity) (Table 1).

RDA was used to examine the potential correlation between environmental variables and the seasonality of BCC at the GRNMS (Fig. 3A). The two RDA axes, i.e., RDA1 and RDA2, each captured 60.7% and 4.3% of the total variance in BCC, respectively. Spring and fall samples were largely separated along RDA1, which broadly extracted the gradients of all measured environmental variables ($P < 0.05$) except PAR and Tide, with NO_x^-, DOC, and T contributing the most.

Depth profiles of BCC and influential environmental factors

Bacterial diversity (H values) showed no significant difference between depths in fall and overall samples ($P > 0.05$). However, bacterial diversity in spring was greater in surface than in either mid-depth or bottom samples (Table S1; *t* test, $P < 0.05$).

Analysis using NMDS followed by ANOSIM revealed significant BCC differences with depth (surface vs. mid-depth or bottom) only in spring ($r_{ANOSIM} = 0.52$, $r_{ANOSIM} = 0.77$, respectively; $P < 0.05$), but not in fall ($r_{ANOSIM} = 0.03$, $P > 0.05$; Figs. 1 and 2; Table S2).

SIMPER analysis showed that nearly 60% of the difference in spring BCC was due to differences in the abundances of SAR11, *Rhodobacteraceae,* Family I *Cyanobacteria* and Cytophaga Family Incertae Sedis (Table 1). Among these taxa, SAR11 and *Cytophaga* Family Incertae Sedis had higher relative abundance in mid-depth (average 42.1% and 6.5% of the sequences, respectively) and bottom (39.5% and 8.4%, respectively) samples than in surface samples (31.6% and 5.3%, respectively; ANOVA, $P < 0.05$). The distribution pattern was opposite for *Rhodobacteraceae* and Family I *Cyanobacteria*, whose relative abundances were the greatest in surface water (ANOVA, $P < 0.05$).

Consistently, RDA ordination also revealed a clear separation of spring BCCs based on sampling depth (Fig. 3B). This separation was mostly along the $RDA1_{spring}$ axis, which explained 78.2% of the total variance and was significantly correlated with PAR ($P < 0.05$).

Diurnal dynamics of BCC and influential environmental factors

Values of the Shannon index (H) revealed that, in either season, bacterioplankton diversity of the day samples was not different from that of the night samples (Table S1; *t* test, $P > 0.05$).

Analyses of BCC using NMDS and ANOSIM, on the other hand, identified significant differences between day and night samples in fall ($r_{ANOSIM} = 0.50$, $P < 0.05$), but not in spring ($r_{ANOSIM} = 0.00$, $P > 0.05$; Figures 1 and 2; Table S2). SIMPER analysis revealed that over 40% of the variance between day and night BCCs in fall was contributed by shifts in the relative abundances of SAR11, Family I *Cyanobacteria* (Table 1). SAR11 was more abundant during the day (28.9% averaged across all fall samples) than night (15.5%) (*t* test, $P < 0.05$), while, in contrast, Family I *Cyanobacteria* were more abundant at night (23.8%) than during the day (15.4%) (*t* test, $P < 0.05$). SIMPER analysis also identified *Pseudoalteromonadaceae* and *Vibrionaceae* as important contributors (each accounted for ~11% of the difference) to the identified day-night BCC shifts in fall. However, this was not

supported by the *t* test, which found that the relative abundances of these taxa in day samples were not significantly different from those in night samples in fall (*t* test; $P > 0.05$).

RDA analysis consistently separated the day and night BCCs in fall, mostly along the $RDA1_{fall}$ axis (47.9% of the total variance; Fig. 3C). This separation was significantly correlated with variance in PAR and concentrations of polyamines and Chl *a* ($P < 0.05$).

Correlation between major bacterial taxa and environmental factors

As mentioned above, SIMPER analysis followed by ANOVA or *t* tests revealed that the variation of BCC in time and depth was largely due to changes in the relative abundance of six taxa, including SAR11, Family I *Cyanobacteria*, *Cytophaga* Family Incertae Sedis, *Vibrionaceae*, *Pseudoalteromonadaceae* and *Rhodobacteraceae* (Tables 1). Pearson product-moment correlation coefficients revealed that these changes were correlated significantly with variations in environmental conditions between spring and fall (Table 2; $P < 0.05$ with Bonferroni correction). The relative abundances of SAR11 and *Cytophaga* Family Incertae Sedis were positively correlated with DOC ($r = 0.75$ and 0.76, respectively) and NO_x^- ($r = 0.52$ and 0.74, respectively), while negatively correlated with T, S, and SRP ($r \leq -0.58$). The pattern was the opposite for Family I *Cyanobacteria*, *Vibrionaceae*, and *Pseudoalteromonadaceae*, whose relative abundances were correlated positively with T, S, and SRP ($r \geq 0.52$), but negatively with DOC and NO_x^- ($r \leq -0.50$). The relative abundance of *Rhodobacteraceae* was not significantly correlated with any measured environmental variable.

Discussion

GRNMS is a live-bottom reef ecosystem located in the transition zone between the coastal/estuarine plume and continental shelf waters in the South Atlantic Bight. In accordance with the complex local hydrology and chemistry, bacterioplankton communities

in GRNMS water were highly diverse and showed significant temporal and depth variations in BCCs.

Despite sample variability, the two most abundant taxa were consistently SAR11 (*Alphaproteobacteria*) and Family I *Cyanobacteria* (93.2% were *Synechococcus*). These two taxa represent numerically and ecologically significant groups of marine heterotrophic and autotrophic bacterioplankton, respectively (34−38). *Rhodobacteraceae* was also identified as a major taxon at the GRNMS, but their relative abundance (7.5 ± 0.4% in average) was much lower than SAR11 (21.5 ± 1.7%). The *Rhodobacteraceae* family contains several genera that are collectively known as roseobacters. The relative abundance of roseobacters often exceeds that of SAR11 in nutrient-rich coastal regions, likely a result of roseobacters' stronger ability in processing plankton-derived DOC (39, 40). The observed predominance of SAR11 over roseobacters at the GRNMS has also been found in coastal sites of the *Sorcerer II* Global Ocean Sampling (41) and in Norwegian coastal waters of the North Sea (42). Moreover, we found a significant correlation between nutrients (both DOC and nitrate concentrations) and the relative abundance of SAR11 at the GRNMS (Table S3). These suggest that SAR11 clade may play a more important role in organic substrate turnover in coastal seawaters than previously thought.

Physical-chemical characteristics of seawater at the GRNMS, including T, S, turbidity and nutrient concentrations, typically show strong temporal and depth variabilities (17, this study). These factors have been repeatedly suggested as important regulators of BCC in many other marine ecosystems (3, 6, 8, 9). Our data further indicates that the observed temporal (both diurnal and seasonal) and depth variations of BCC at the GRNMS were correlated with mixes of different environmental variables. This suggests that the impact of individual environmental factors on BCCs vary among temporal and spatial scales.

316 BCC seasonality overwhelmed variations of BCC at either diurnal or depth scales
317 (Figs. 2 and 3). This pattern may be ascribed to the overriding influences of environmental
318 variables on defining the seasonal or long-term BCC dynamics at the GRNMS, as found in
319 other coastal sites (7, 9). Furthermore, we found that variables (i.e., NO_x^-, DOC, and T)
320 explaining the seasonality of BCC were not correlated with changes in BCC at shorter time
321 scales (day vs. night within 24 h) or among depths. Located in a coastal mixing zone,
322 seawater at the GRNMS actively exchanges with ambient environments through tidal
323 advection and turbulent mixing on the daily bases (15). The residence time of GRNMS water
324 has been estimated between 0.2-1.2 days (15), which may be too short to allow a detectable
325 growth of bacterial taxa. Therefore, the identified diurnal variation among our fall samples
326 (October 2011), when the water column is well mixed, may be more due to the mixing of
327 "new" bacterioplankton assemblages with local taxa than from differential growth of local
328 bacterial populations in response to changes of environmental conditions.
329 BCC variation with depth was found in spring (April 2011) (Fig. 1) when the water
330 column was stratified (Fig. S1). This indicates that the density difference between waters
331 above and below the pycnocline has created a barrier to prevent vertical mixing, which
332 allowed BCC of different depths to diverge along the PAR gradient. GRNMS is known for its
333 productive benthic community and substantial benthic nutrient fluxes (15, 16). However,
334 consistent with a previous study (15), significant vertical gradients of nutrient concentrations
335 were not observed by this study, likely resulting from the relatively large standing stock of
336 nutrients in the water column (15, Table S3). Therefore, the observed depth variations of
337 BCCs are more likely due to the difference between the content of dissolved organic and
338 inorganic nutrients across the pycnocline. Further studies on the correlation between nutrient
339 content and BCCs in GRNMS are needed to test this hypothesis.
340 **Conclusion**

Bacterioplankton community at the GRNMS consisted of a diverse group of taxa that are typically found in coastal marine environments, including *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Deferribacteres*, and *Bacteroidetes.* BCC at the GRNMS showed strong seasonal variations and was correlated with environmental factors that regulate the growth of bacterioplankton, such as T, S, and nutrient concentrations. Diurnal BCC variations at the GRNMS, on the other hand, are suggested to be more impacted by water mixing events (tides and vertical advection). The depth variations of BCCs are likely a result of poor vertical mixing and different content of nutrients between the surface and bottom waters.

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Figure legends

Fig. 1. Heatmap and accompanying cluster analysis of the relative abundance (%) of major GRNMS bacterioplankton at the family level in (A) spring (April, 2011) and (B) fall (October, 2011) samples. Sample identifiers are based on sampling season (sp, spring; fa, fall), depth (s, surface; m, mid-depth; b, bottom) and time (in 24-hour format). Regular and bold font styles are used to denote day and night samples, respectively.

Fig. 2. The non-metric multidimensional scaling ordination based on the relative abundance (%) of major GRNMS bacterioplankton at the family level in spring and fall samples. Sample identifiers are the same as in Fig.1. Symbols are used to denote different sampling depths in spring (black triangle: surface; gray triangle: mid-depth; white triangle: bottom) and in fall (black square: surface; white square: bottom).

Fig. 3. Bioplot diagrams of the RDA analysis on the relative abundance (%) of BCC by environmental variables at the GRNMS for (A) overall, (B) spring and (C) fall samples. Sample identifiers are the same as in Fig. 1. Environmental variables are in black boxes with white font. Light and dark gray shadings are used to indicate significantly different BCC sample clusters identified by ANOSIM analysis.

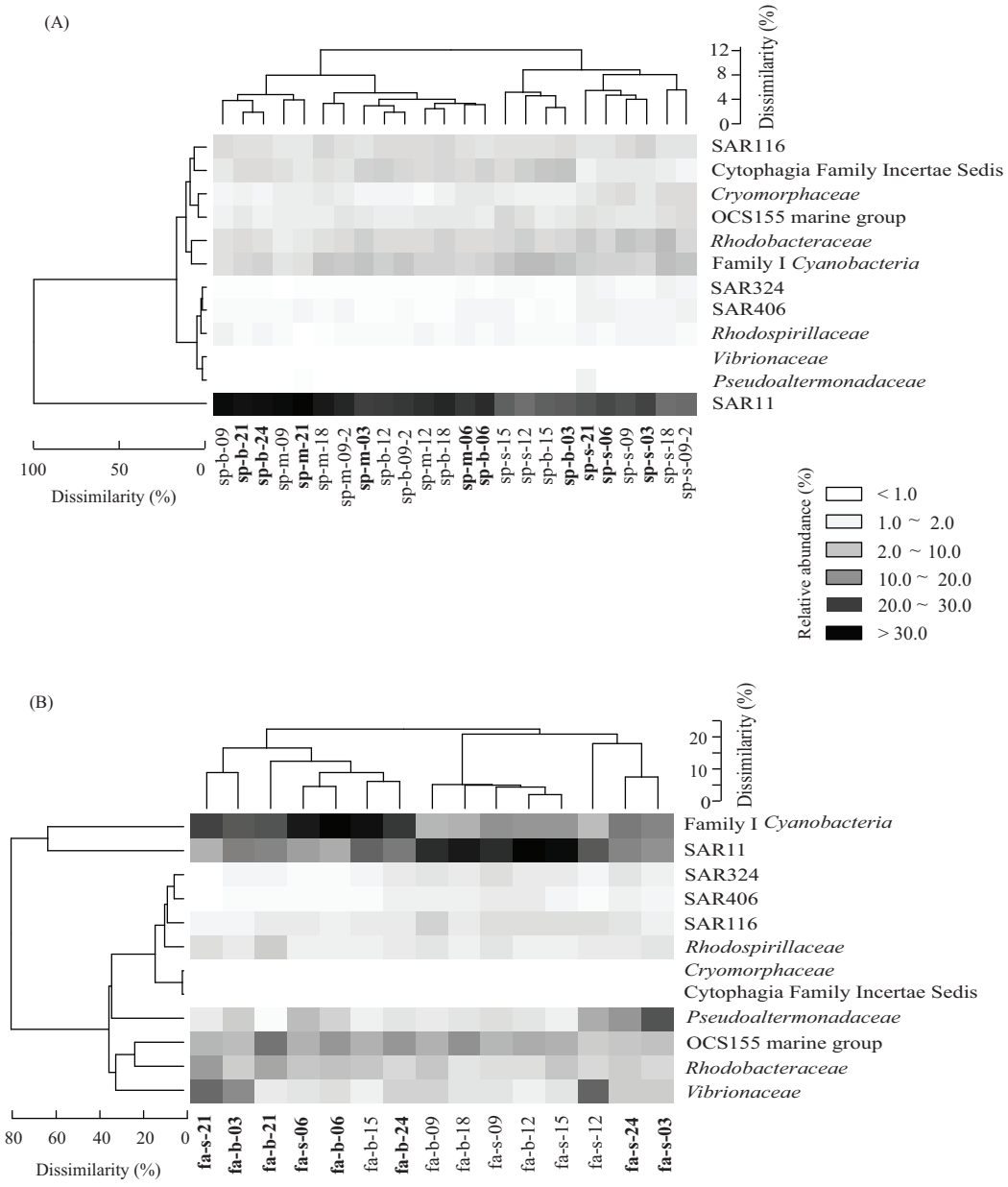

FIG 1 Heatmap and accompanying cluster analysis of the relative abundance (%) of major GRNMS bacterioplankton at the family level in (A) spring (April, 2011) and (B) fall (October, 2011) samples. Sample identifiers are based on sampling season (sp, spring; fa, fall), depth (s, surface; m, mid-depth; b, bottom), and time (in 24-hour format). Regular and bold font styles are used to denote day and night samples, respectively.

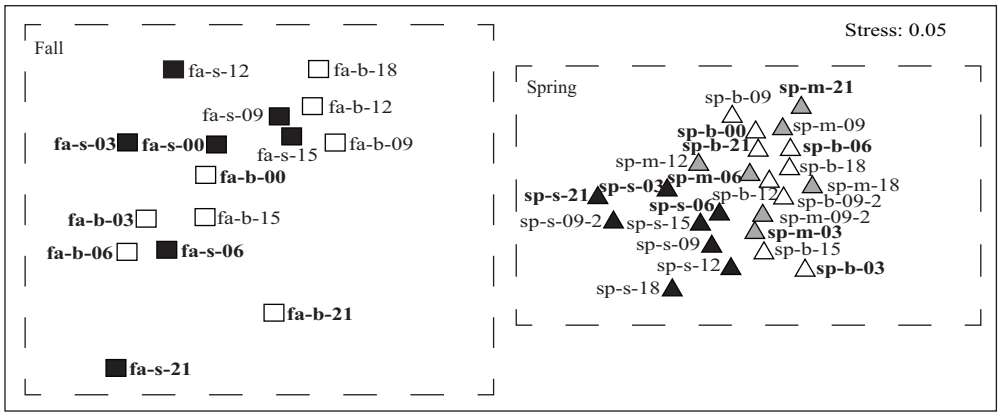

FIG 2 The non-metric multidimensional scaling ordination based on the relative abundance (%) of major GRNMS bacterioplankton at the family level in spring and fall samples. Sample identifiers are the same as in Fig. 1. Symbols are used to denote different sampling depths in spring (black triangle: surface; gray triangle: mid-depth; white triangle: bottom) and in fall (black square: surface; white square: bottom).

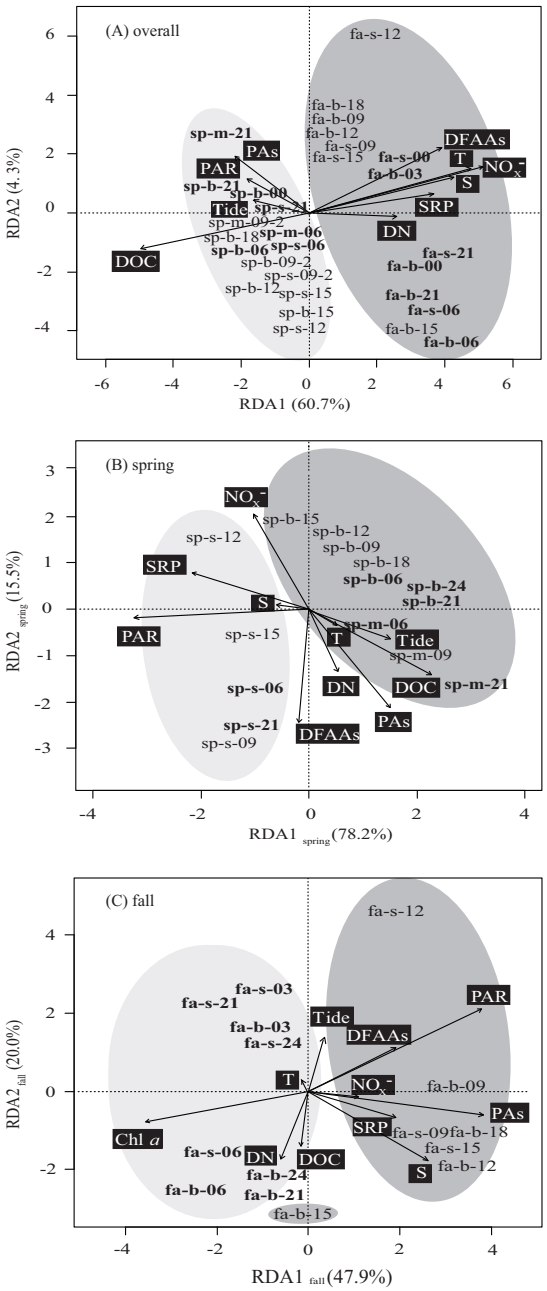

FIG 3 Bioplot diagrams of the RDA analysis on the relative abundance (%) of BCC by environmental variables at the GRNMS for (A) overall, (B) spring, and (C) fall samples. Sample identifiers are the same as in Fig. 1. Environmental variables are in the black boxes with white font. Light and dark gray shadings are used to indicate significantly different BCC sample clusters identified by ANOSIM analysis.

Table 1. The average abundance and relative contribution of major taxa to the observed BCC differences among samples of the GRNMS.

Bacterial taxa	over-all	Spring vs. Fall		Spring: Surface vs. mid-depth		Spring: Surface vs. bottom		Fall: day vs. night	
	Avg[a]	Avg sp/fa	δ_i	Avg s/m	δ_i	Avg s/b	δ_i	Avg D/N	δ_i
SAR11	30.7	37.6/21.5	24.0	31.6/42.1	29.2	31.6/39.5	29.2	28.9/15.5	22.4
Family I *Cyanobacteria*	13.6	9.6/20.1	16.5	10.2/8.8	8.6	10.2/9.6	8.7	15.4/23.8	18.1
Rhodobacteraceae	7.7	7.9/7.5	3.7	9.8/6.5	11.2	9.8/7.2	8.9	6.2/8.7	5.2
OCS155 marine group	7.5	4.9/11.5	9.6	6.1/4.6	5.2	6.1/4.2	6.3	11.0/11.5	5.4
SAR116	5.5	6.6/3.8	4.1	6.5/6.3	2.6	6.5/6.9	2.8	4.7/3.0	3.0
Cytophaga Family Incertae Sedis	4.3	6.8/0.3	9.6	5.3/6.5	7.4	5.3/8.4	12.0	1.7/0.9	1.8
Vibrionaceae	3.1	0.1/7.7	11.0	0.0/0.1	0.2	0.0/0.3	0.8	6.5/9.4	10.9
Pseudo-alteromonadaceae	2.9	0.4/6.9	9.7	0.7/0.3	1.9	0.7/0.1	1.8	4.7/9.2	11.0
Cryomorphaceae	2.7	4.3/0.2	5.9	5.8/3.8	7.0	5.8/3.2	7.8	0.1/0.2	0.2
Rhodospirillaceae	2.7	2.1/3.6	2.2	2.3/1.8	2.1	2.2/1.9	1.4	3.2/4.0	2.2
SAR324	2.0	1.5/2.7	2.2	2.3/1.1	4.0	2.3/1.1	4.0	3.2/2.4	2.5
SAR406	2.0	2.1/1.9	1.4	2.6/2.0	2.9	2.6/1.6	3.8	2.4/1.6	2.2

[a] Avg, average relative abundance (in percentage); δi, estimated individual contributions (in percentage) of bacteria taxa to the observed between-sample differences. Shaded values are to label top four contributors for each comparison group. Sp, spring; fa, fall; s, surface water; b, bottom water; D, day; N, night.

Table 2. Correlations between major bacterial taxa and environmental variables based on Pearson's product momentum correlation coefficient. Variables with significant ($P < 0.05$ with Bonferroni correction) correlations are in bold font.

Taxa	T	S	PAR	DOC	DN	NO_x^-	SRP	DFAAs	PAs
SAR11	**−0.70**	**−0.58**	−0.07	**0.75**	−0.27	**0.52**	**−0.72**	−0.34	0.14
Family I *Cyanobacteria*	**0.64**	**0.56**	−0.18	**−0.64**	0.30	**−0.50**	**0.64**	0.26	−0.15
Cytophaga Family Incertae Sedis	**−0.86**	**−0.66**	0.07	**0.76**	0.35	**0.74**	**−0.79**	**−0.61**	0.17
Vibrionaceae	**0.64**	**0.55**	0.09	**−0.61**	0.12	**−0.60**	**0.53**	0.40	−0.02
Pseudoalteromonadaceae	**0.60**	**0.52**	−0.02	**−0.56**	0.28	**−0.60**	**0.61**	0.48	0.01
Rhodobacteraceae	−0.02	−0.22	−0.09	−0.10	−0.24	0.10	0.01	−0.39	−0.36