

Temporal variation in the prokaryotic community of a nearshore marine environment

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Abstract

Prokaryotic communities inhabiting surface waters of temperate areas exhibit patterns of seasonal succession. Studies describing these temporal changes are usually not performed at stations located in the proximity to the coast. The temporal variation of these communities was determined in the surface waters at two stations located in the close proximity to the eastern shore in the northern Adriatic Sea. Sequencing of the V4 region of the 16S rRNA gene identified highest community richness in December with distinct shifts in the community structure between spring, summer and autumn/winter. Temperature was shown to be the main environmental force explaining community temporal variation. *Synechococcus*, SAR86 clade, NS5 marine group and *Cryomorphaceae* were detected throughout the seasonal cycle. In contrast, the spring community was characterized by the NS4 marine group, *Formosa* and *Rhodobacteraceae*, the summer community by SAR11 subclades II and III, HIMB11, AEGEAN-169 marine group, OM60 (NOR5) clade and *Litoricola* and the autumn/winter community by SAR11 subclade Ia and *Archaea*. Taken together, prokaryotic communities inhabiting nearshore surface waters exhibit a general pattern in community composition similar to other surface associated assemblages. However, certain characteristic season-specific community structures and temporal patterns of specific taxonomic groups differ from other coastal areas.

18 Introduction

19 Prokaryotic picoplankton communities inhabiting marine surface waters exhibit a seasonal
20 succession. These temporal community changes were described for surface waters of polar,
21 temperate and (sub)tropical regions (Bunse and Pinhassi, 2017). In temperate regions changes were
22 mainly associated with summer water column stratification, winter mixing and spring phytoplankton
23 blooms (Teeling et al., 2012; Bunse and Pinhassi, 2017; Mestre et al., 2020). Although general
24 successional patterns in these waters have been reported, some local differences were also observed.
25 While some studies have reported the exchange of multiple community states during the year
26 (Gilbert et al., 2009; Sintes et al., 2013; El-Swaiss et al., 2015; Lindh et al., 2015), others have
27 observed a community separation in only two major groups, specifically seasons (Mestre et al.,
28 2020), indicating that beside global patterns local environmental conditions may influence seasonal
29 community change.

30 Seasonal community variation in temperate waters usually starts with assemblages
31 characteristic for spring phytoplankton blooms. The successional pattern of different microbial
32 groups during the pre-bloom, bloom and bloom-decay periods have been described in detail
33 (Teeling et al., 2012, 2016; Sintes et al., 2013). The pre-bloom community is generally dominated
34 by members of the alphaproteobacterial SAR11 clade, during the bloom *Bacteroidota* taxa such as
35 *Formosa*, *Polaribacter*, *Ulvibacter* and the VIS6 clade become abundant while the decay period is
36 characterized by *Gammaproteobacteria*, i.e. the SAR92 clade (Teeling et al., 2012, 2016; Sintes
37 et al., 2013). Beside taxa co-occurring with phytoplankton blooms, communities specific to
38 summer water stratification and winter mixing were also described (Mestre et al., 2020). Usually,
39 *Cyanobacteria* are enriched during the summer, while some sub-clades of SAR11 are characteristic
40 for summer and some for winter months (Salter et al., 2015; Mestre et al., 2020).

41 The majority of studies describing temporal changes in temperate areas were performed at
42 long-term time series stations, such as the L4 sampling site of the Western Channel Observatory

(Gilbert et al., 2009, 2012), Blanes Bay Microbial Observatory (BBMO) (Alonso-Sáez et al., 2007; Mestre et al., 2020), Linnaeus Microbial Observatory (Lindh et al., 2015), station Kabeltonne in the German Bight (Teeling et al., 2012, 2016) and station E2 of the RADIALES time-series project (Alonso-Sáez et al., 2015). Data obtained from such time-series studies have found that a set of abiotic and biotic factors drive the temporal community variation (Bunse and Pinhassi, 2017). It was suggested that biological interactions primarily affect microbial dynamics over time periods of days to weeks, while physicochemical parameters are mainly responsible for observed seasonal successional patterns (Gilbert et al., 2009; Fuhrman et al., 2015; Needham and Fuhrman, 2016; Bunse and Pinhassi, 2017; Mestre et al., 2020). In addition, several studies indicate that phytoplankton derived dissolved organic matter (DOM) drives community dynamics (Teeling et al., 2012, 2016; Lindh et al., 2015; Needham and Fuhrman, 2016; Bunse and Pinhassi, 2017). It is therefore worth investigating whether such general interactions also apply to nearshore microbial communities.

To describe the temporal variation of microbial communities located in the proximity of the shore and to disentangle the environmental variables responsible for their temporal change it is important to apply a high-frequency sampling approach. Monthly sampling of surface waters at two stations along the eastern coast of the northern Adriatic Sea was performed to determine the temporal variation of prokaryotic picoplankton communities in these habitats. In addition, to assess the main environmental parameters associated with community changes, compositional data were linked to a set of previously reported environmental parameters measured at the same time (Najdek et al., 2020a, 2020b).

Materials and methods

Sampling

Surface seawater from the northern Adriatic Sea was collected in the proximity of the shore (25 – 50 m distance) in two bays ~7 km apart from each other, Saline (45°7'5" N, 13°37'20" E) and Funtana (45°10'39" N, 13°35'42" E), by diving (depth, ~1.5 m). Samples were collected in 10 l containers and transported to the laboratory where 10 – 20 l were filtered through a 20 µm mesh. The filtrate was further sequentially filtered using a peristaltic pump through 3 µm and 0.2 µm polycarbonate membrane filters (Whatman, United Kingdom). Filters were dried briefly at room temperature and stored at –80 °C. Samples were collected monthly from July 2017 to October 2018. Concurrently of sampling for picoplankton community structure assessment, additional samples were collected to determine environmental parameters (temperature, salinity, orthophosphate, ammonium, nitrite, nitrate, orthosilicate, particulate matter, chlorophyll *a* and prokaryotic abundance) as reported previously (Najdek et al., 2020a, 2020b).

DNA isolation

Picoplankton DNA was isolated from 0.2 µm polycarbonate filters according to Massana et al. (1997) with slight modifications. Following phenol-chloroform extractions, 1/10 of 3 M chilled sodium acetate (pH 5.2) was added. DNA was precipitated by the addition of 1 volume of chilled isopropanol, incubating the mixtures overnight at –20 °C and centrifuging at 20,000 × *g* and 4 °C for 21 min. Pellets were washed twice with 500 µl of 70 % chilled ethanol and centrifuged after each washing step at 20,000 × *g* and 4 °C for 5 min. Air-dried pellets were re-suspended in 50 µl of deionized water.

Illumina 16S rRNA sequencing

The V4 region of the gene for 16S rRNA was sequenced using the Illumina MiSeq platform as described previously (Korlević et al., submitted). A two-step PCR procedure was applied to amplify the target region. In the first PCR, primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') from the Earth Microbiome Project (<https://earthmicrobiome.org/protocols-and-standards/16s/>) were used (Caporaso et al., 2012; Apprill et al., 2015; Parada et al., 2016). Tagged sequences were added to these primers on their 5' end. PCR products were purified and sent for Illumina MiSeq sequencing at IMG/M Laboratories, Martinsried, Germany. Prior to sequencing at IMG/M, adapter and sample-specific index sequences were incorporated during the second PCR amplification of the two-step PCR procedure using primers targeting the tagged region. Beside samples, a positive and a negative control were included in each sequencing batch. For the positive control a mock community consisting of evenly mixed DNA material originating from 20 bacterial strains (ATCC MSA-1002, ATCC, USA) was used, while the negative control comprised PCR reactions without DNA template. Reads obtained in this study (Bay of Saline) were combined with reads previously reported in a study describing temporal dynamics of surface associated microbial communities (Bay of Funtana) (Korlević et al., submitted) and analysed together. Sequences processed in this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession numbers SAMEA6648771 – SAMEA6648788, SAMEA6648824, SAMEA6648825, SAMEA8117500 – SAMEA8117516.

Sequence analysis

Sequences obtained in the present study were analysed using mothur (version 1.43.0) (Schloss et al., 2009) according to the MiSeq Standard Operating Procedure (MiSeq SOP; https://mothur.org/wiki/MiSeq_SOP) (Kozich et al., 2013) and recommendations given by the Riffomonas project to enhance data reproducibility (<http://www.riffomonas.org/>). Sequences were clustered into

operational taxonomic units (OTUs) at a similarity level of 97 % as suggested by the MiSeq SOP. Computing was performed on the computer cluster Isabella (University Computing Center, University of Zagreb). Alignment and classification was performed using the SILVA SSU Ref NR 99 database (release 138; <http://www.arb-silva.de>) (Quast et al., 2013; Yilmaz et al., 2014). Pipeline data processing and visualisation was done using R (version 3.6.0) (R Core Team, 2019) in combination with packages vegan (version 2.5.6) (Oksanen et al., 2019), tidyverse (version 1.2.1) (Wickham, 2017; Wickham et al., 2019) and multiple other packages (Neuwirth, 2014; Xie, 2014, 2015, 2019a, 2019b, 2019c; Xie et al., 2018; Allaire et al., 2019; McKinnon Edwards, 2019; Wilke, 2019; Zhu, 2019). The detailed analysis procedure including the RMarkdown file are available in the GitHub repository (https://github.com/MicrobesRovinj/Korlevic_SeawaterDynamics_x_2021). The average sequencing error rate of 0.01 % was calculated based on the ATCC MSA-1002 mock community included in each sequencing batch, which is in line with previously reported values for next-generation 16S rRNA amplicon sequencing (Kozich et al., 2013; Schloss et al., 2016). Also, negative controls processed together with the samples yielded on average only 2 sequences after quality curation.

Results

Sequencing of 17 samples from the Bay of Saline and 18 samples from the Bay of Funtana (one of the samples was a sequencing replicate) yielded 1.5 million reads after quality curation and exclusion of sequences without known relatives (no relative sequences), eukaryotic, chloroplast and mitochondrial sequences (Table S1). The number of reads per sample ranged from 25,360 to 77,466 (Fig. S1 and Table S1). Reads were clustered into 16,629 different OTUs at a similarity level of 97 %. To account for different sequencing depth reads were normalized to the minimum number of sequences per sample (25,360, Table S1) that resulted in 13,440 different OTUs ranging from 608 to 1,790 OTUs per sample (Fig. S2).

Temporal variations in richness and diversity were determined by calculating the observed number of OTUs, Chao1, ACE, Exponential Shannon and Inverse Simpson index (Jost, 2006). Similar trends in richness and diversity were observed at both stations (Fig. S2) characterized by a maximum richness in both, the Saline (Number of OTUs, 1,790 OTUs) and Funtana (Number of OTUs, 1,786 OTUs) Bay in December 2017. In contrast, the Inverse Simpson index did not show an elevated value in December 2017 indicating that rare OTUs contributed substantially to the observed richness maxima. To determine temporal changes in the proportion of shared OTUs and in communities the Jaccard's and Bray-Curtis similarity coefficients were calculated between consecutive sampling points (Fig. S3). Similar trends were observed at both stations with higher stability of shared bacterial and archaeal OTUs (Jaccard's similarity coefficient) than community similarity (Bray-Curtis similarity coefficient). A substantial decline in community similarity between March and April 2018 was observed at both stations indicating a pronounced community shift in this period (Fig. S3). Analysis of this time series data showed that only 0.3 % of OTUs were present throughout the study period while these persistent OTUs contributed to 62.0 % of sequences.

To evaluate the temporal variation of bacterial and archaeal communities Principal Coordinate

Analysis (PCoA) of Bray-Curtis distances was applied to the OTU community data (Fig. 1A). Communities specific to summer, autumn/winter and spring could be identified regardless of the station sampled. This separation in three specific communities was further supported by ANOSIM ($R = 0.72$, $p < 0.01$). To assess which environmental parameter mainly contributes to the observed temporal community variation, the community data were constrained by a set of previously reported environmental variables (Najdek et al., 2020a, 2020b) using Distance-Based Redundancy Analysis (db-RDA) (Fig. 1B). Nearly half ($R_a^2 = 45.5\%$) of the observed community variation could be explained by the variables. Separation between summer and autumn/winter communities was mainly explained by temperature, prokaryotic abundance, salinity and nitrite. In contrast, neither variable could strongly explain the spring community composition.

The classification of reads showed that the prokaryotic community was dominated by bacterial ($98.3 \pm 3.5\%$) over archaeal sequences ($1.7 \pm 3.5\%$) (Fig. 2). A higher relative contribution of archaeal reads was observed only in November 2017 ($7.5 \pm 2.2\%$), December 2017 ($13.2 \pm 1.4\%$) and February 2018 ($3.7 \pm 0.9\%$). The main taxonomic groups contributing to the higher relative abundance of *Archaea* in this period were the *Crenarchaeota* “*Candidatus Nitrosopumilus*” and the *Thermoplasmatota* Marine group II. The bacterial community was comprised of well-known seawater groups such as the *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*, *Marinimicrobia*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota* (Fig. 2). Generally, similar temporal patterns were observed at both stations.

Cyanobacteria comprised on average $5.1 \pm 2.8\%$ of the prokaryotic community. The highest relative contribution was recorded in winter ($8.2 \pm 5.6\%$), mainly caused by the high proportion in March 2018 ($13.0 \pm 1.6\%$) (Fig. 2). The cyanobacterial community was largely dominated by *Synechococcus*, especially during periods of higher cyanobacterial presence (Fig. 3). *Bacteroidota* comprised on average $21.8 \pm 6.2\%$ of the community. Slightly higher values were found in spring and summer ($23.9 \pm 4.6\%$) than in autumn and winter ($18.0 \pm 7.1\%$) (Fig. 2). Although *Bacteroidota* showed only slight temporal variations, taxa within this group exhibited strong seasonal

patterns (Fig. 4). Groups such as the NS5 marine group and uncultured *Cryomorphaceae* were present throughout the year, while sequences classified as *Balneola*, uncultured *Balneolaceae* and the NS11-12 marine group were more pronounced from May to October. In addition, *Formosa* and the NS4 marine group could be detected throughout the study period but specifically contributed to the *Bacteroidota* community in March and April 2018, respectively. Higher values of chloroplast sequences were also recorded at this time (Fig. S4). In addition, while uncultured *Saprospiraceae* from the Saline Bay samples contributed substantially to the *Bacteroidota* community in June and July 2018, they were not abundant in Funtana Bay (Fig. 4).

Sequences classified as *Alphaproteobacteria* showed the highest relative abundance and comprised on average 38.3 ± 8.0 % of the prokaryotic community (Fig. 2). The relative contribution of *Alphaproteobacteria* was higher in summer (41.3 ± 6.3 %) and winter (44.0 ± 2.2 %) than in autumn (34.9 ± 4.9 %) and spring (31.1 ± 11.5 %). The temporal variation of taxa within this class showed a more complex pattern (Fig. 5). The most pronounced community shift was observed in April 2018 when reads of the usually prominent SAR11 clade were scarce and *Stappiaceae*, *Asciadiaceihabitans* and no relative *Rhodobacteraceae* dominated the alphaproteobacterial community. Subclades within SAR11 also showed temporal patterns. Subclades II and III characterized the SAR11 community in summer from June to September, while from November to March reads classified as subclade Ia comprised the majority of SAR11-specific sequences. Except of the SAR11 subclades II and III, summer months were also characterized by two other alphaproteobacterial groups, the HIMB11 and the AEGEAN-169 marine group (Fig. 5).

Reads classified as *Gammaproteobacteria* comprised on average 21.6 ± 6.6 % of the prokaryotic community (Fig. 2). The season with the the highest relative contribution of gammaproteobacterial sequences was spring (29.9 ± 12.3 %), while in other seasons values ranged from 18.9 ± 2.6 to 22.3 ± 2.5 %. Within the *Gammaproteobacteria*, the SAR86 clade was present throughout the year while other taxa were season-specific, such as *Litoricola*, the OM60 (NOR5) clade and the SUP05 cluster (Fig. 6). *Litoricola* and the OM60 (NOR5) clade characterized

201 the gammaproteobacterial community from April to August, while sequences specific for the
202 SUP05 cluster were detected from November to March. Taxonomic groups of high-level bacterial
203 taxa that comprised a lower proportion of prokaryotic sequences such as the *Actinobacteriota*
204 and *Verrucomicrobiota* also showed temporal variations. During the period of higher relative
205 sequence abundance of *Actinobacteriota*, between September and December, the actinobacterial
206 community was comprised mainly of “*Candidatus Actinomarina*”. Similarly, in April and May
207 when *Verrucomicrobiota* specific reads exhibited the highest relative abundance, *Lentimonas* and
208 *Coralimargarita* were the main constituent of the *Verrucomicrobiota*.

Discussion

Prokaryotic communities inhabiting surface waters of temperate and (sub)polar regions exhibit patterns of seasonal succession (Bunse and Pinhassi, 2017). These temporal variations were mainly studied at long-term time series sites usually encompassing only one sampling station located further away from the coast (Gilbert et al., 2009; Mestre et al., 2020). In the present study the temporal variation of surface prokaryotic communities was determined at two sites in the close proximity of the shore. Similar patterns were observed at both sites indicating that the observed pattern might be representative for surface waters of a wider area.

Temporal changes in richness were considerable as indicated by the low proportion of OTUs present at each sampling date (0.3 %). This low number of persistent OTUs, however, comprised a high proportion of reads (62.0 %). Similar proportions of persistent core OTUs and their contribution to the total number of sequences were also reported in other time series studies (Gilbert et al., 2009, 2012). Analysis of the temporal variations in alpha-diversity showed maximal richness in December (Fig. S2). This observation is in agreement with previously reported richness maxima in other regions during colder months (Gilbert et al., 2012; Ladau et al., 2013; El-Swaiss et al., 2015; Mestre et al., 2020). It has been suggested that late autumn/winter overturn is responsible for this phenomenon by simply mixing populations from deeper parts of the water column with existing ones (García et al., 2015; Salter et al., 2015; Bunse and Pinhassi, 2017). However, a similar richness pattern was also observed in regional seas where seasonal overturning of the water column does not play a role, such as in the shallow North Sea where also a higher richness was observed in winter (Reinthal et al., 2005). Although the samples in this study were retrieved at very shallow locations, water column mixing taking place at deeper areas could bring additional taxa to these locations causing the observed increase in alpha-diversity.

The majority of studies analysing temporal community variation usually identified an exchange of multiple community states during the year (El-Swaiss et al., 2015; Lindh et al., 2015). In contrast,

some studies described only a switch between winter- and summer-specific assemblages (Ward et al., 2017; Mestre et al., 2020). These differences might be a consequence of local conditions. Indeed, some studies attributed the observed lower number of assemblages to the absence of large spring and fall phytoplankton blooms in some areas (Ward et al., 2017). We identified three distinct microbial assemblages characteristic for spring, summer and autumn/winter (Fig. 1A). This is in agreement with studies describing the exchange of multiple communities over an annual cycle with a distinct spring community assemblage (El-Swais et al., 2015; Lindh et al., 2015). The distinct spring community we detected is likely a response to a phytoplankton bloom that can occur in this region (Mozetič et al., 2010; Manna et al., 2021). Temperature and prokaryotic abundance were identified as main factors influencing the exchange of communities between the summer and autumn/winter period (Fig. 1B). It is not surprising that temperature and prokaryotic abundance are equally explaining this shift as higher prokaryotic abundances were reported in this area during summer months (Ivančić et al., 2010). The identification of temperature as the single most important driver of community change is in line with previously reported data (El-Swais et al., 2015; Ward et al., 2017; Mestre et al., 2020). It was proposed that temperature indirectly influences community change through phytoplankton nutrient limitation during water column stratification and nutrient input in times of water column mixing (Bunse and Pinhassi, 2017). Factors explaining the onset of a separate spring community were not identified. We hypothesise that based on a slightly higher value of chloroplast specific reads in these samples and the presence of taxa specific for phytoplankton blooms this community was a late prokaryotic response to a phytoplankton bloom even though the concentration of chlorophyll *a* could not explain it.

Differences between communities specific for spring, summer and autumn/winter observed at the level of OTUs could also be seen in the taxonomic composition (Figs. 2 – 6). The identified spring-specific community contained taxa previously associated with phytoplankton blooms (Figs. 4 and 5) (Teeling et al., 2012, 2016; Sintes et al., 2013). *Formosa* and members within the *Rhodobacteraceae* were associated with phytoplankton blooms in the North Sea (Teeling et al., 2012, 2016), while the NS4 marine group was found in previous studies describing bacterial communities

in different environments of the Adriatic Sea with no clear association with increased autotrophic biomass (Korlević et al., 2015, 2016). Observed variations in spring communities between different areas could be explained by differences in structure and supply of phototroph-derived organic matter. The summer community was characterized by the family *Balneolaceae* and the NS11-12 marine group from the *Bacteroidota*, the SAR11 subclades II and III, HIMB11 and the AEGEAN-169 marine group from the *Alphaproteobacteria* and from the *Gammaproteobacteria* the OM60 (NOR5) clade and *Litoricola* (Figs. 4 – 6). In contrast, the winter community was characterized by the archaeal “*Candidatus Nitrosopumilus*” and Marine group II, the alphaproteobacterial SAR11 subclade Ia and the gammaproteobacterial SUP05 cluster (Figs. 2, 5 and 6). Temporal and depth-related variation of different SAR11 subclades was also reported previously although we observed a different pattern in comparison to other surface associated SAR11 communities (Carlson et al., 2009; Vergin et al., 2013; Salter et al., 2015). In contrast to these former studies, we observed a higher contribution of the SAR11 subclade Ia to the community in the winter. A higher contribution of members in the summer community such as the HIMB11, the OM60 (NOR5) clade and *Litoricola* could result from their adaptation to more oligotrophic conditions during water column stratification in summer through the ability to use alternative pathways of energy supply (i.e. bacteriochlorophyll and proteorhodopsin) (Huggett and Rappe, 2012; Spring and Riedel, 2013; Durham et al., 2014). A study describing a strong co-dominance of “*Candidatus Nitrosopumilus*” and Marine group II suggests that nitrification by ammonia-oxidising archaea is coupled with ammonification performed by the members of the Marine group II (Kim et al., 2019). In addition, the presence of “*Candidatus Nitrosopumilus*” reads in our samples is not surprising as recently two new strains of ammonia-oxidising archaea within the genus *Nitrosopumilus* have been isolated from northern Adriatic coastal waters (Bayer et al., 2019).

Beside groups exhibiting specificity to one of the identified temporal communities, ubiquitous taxa were detected, such as *Synechococcus*, the flavobacterial NS5 marine group and *Cryomorphaceae* and the gammaproteobacterial SAR86 clade (Figs. 3, 4 and 6). The dominance of *Synechococcus* over other cyanobacterial groups in this coastal area was reported previously

(Šilović et al., 2012; Tinta et al., 2015). The known genome versatility of *Synechococcus* could explain the high contribution of this genus to the cyanobacterial community in fluctuating coastal environments (Palenik et al., 2003). The *Cryomorphaceae* are associated with organic matter remineralisation processes (Bowman, 2014), while a single-cell genome analysis of the NS5 marine group revealed the ability to degrade marine polysaccharides (Ngugi and Stingl, 2018). In addition, the NS5 marine group was previously detected in different seasons and environments of the Adriatic Sea (Korlević et al., 2015, 2016). These two groups are part of a basic remineralisation community present at this location throughout the year. The gammaproteobacterial SAR86 clade, previously reported in different environments of the Adriatic Sea (Korlević et al., 2015, 2016; Tinta et al., 2015), was also detected throughout the year. Recent analysis of metagenomic data suggests the existence of different functional and ecological ecotypes of this ubiquitous clade (Hoarfrost et al., 2020). It is possible that different ecotypes are also characteristic for different seasons.

In conclusion, prokaryotic communities inhabiting the proximity of the shore exhibit temporal variations similar to surface water assemblages in other temperate regions. As in other regions, a richness maximum was recorded in the colder period of the year and seasonal community shifts were observed. Temperature was identified as the main force driving seasonal community change. Besides these similarities, temporal analysis of taxonomic data identified a season-specific community structure and groups exhibiting temporal patterns different from other coastal areas indicating that beside global driving factors local conditions also influence the coastal prokaryotic community.

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Figure legends

Fig. 1. (A) Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities sampled in Saline and Funtana Bay. The proportion of explained variation by each axis is shown on the corresponding axis in parentheses. (B) Distance-Based Redundancy Analysis (db-RDA) of Bray-Curtis distances based on the same community data sampled at the same locations and constrained by a set of environmental parameters (T – temperature, S – salinity, PO_4^{3-} – orthophosphate, NH_4^+ – ammonium, NO_2^- – nitrite, NO_3^- – nitrate, SiO_4^{4-} – orthosilicate, PM – particulate matter, Chl *a* – chlorophyll *a* and PA – prokaryotic abundance). Scaling type 1 was selected for the biplot. The proportion of community data variation explained by environmental variables (R_a^2) is stated on the biplot, while the proportion of community data variation explained by each canonical axis is shown on the corresponding axis in parentheses. Samples in both plots originating from the same station or same season are labeled in different shape and color.

Fig. 2. Taxonomic classification and relative contribution of the most abundant ($\geq 1\%$) bacterial and archaeal sequences in communities sampled in Saline and Funtana Bay. No Relative – sequences without known relatives

Fig. 3. Taxonomic classification and relative contribution of the most abundant ($\geq 1\%$) cyanobacterial sequences in communities sampled in Saline and Funtana Bay. The proportion of cyanobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar.

Fig. 4. Taxonomic classification and relative contribution of the most abundant ($\geq 1\%$) sequences within the *Bacteroidota* in communities sampled in Saline and Funtana Bay. The proportion of sequences classified as *Bacteroidota* in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative (sequences without known relatives within the corresponding group)

Fig. 5. Taxonomic classification and relative contribution of the most abundant ($\geq 2\%$) alphaproteobacterial sequences in communities sampled in Saline and Funtana Bay. The proportion of alphaproteobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative (sequences without known relatives within the corresponding group)

Fig. 6. Taxonomic classification and relative contribution of the most abundant ($\geq 1\%$) gammaproteobacterial sequences in communities sampled in Saline and Funtana Bay. The proportion of gammaproteobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative (sequences without known relatives within the corresponding group)

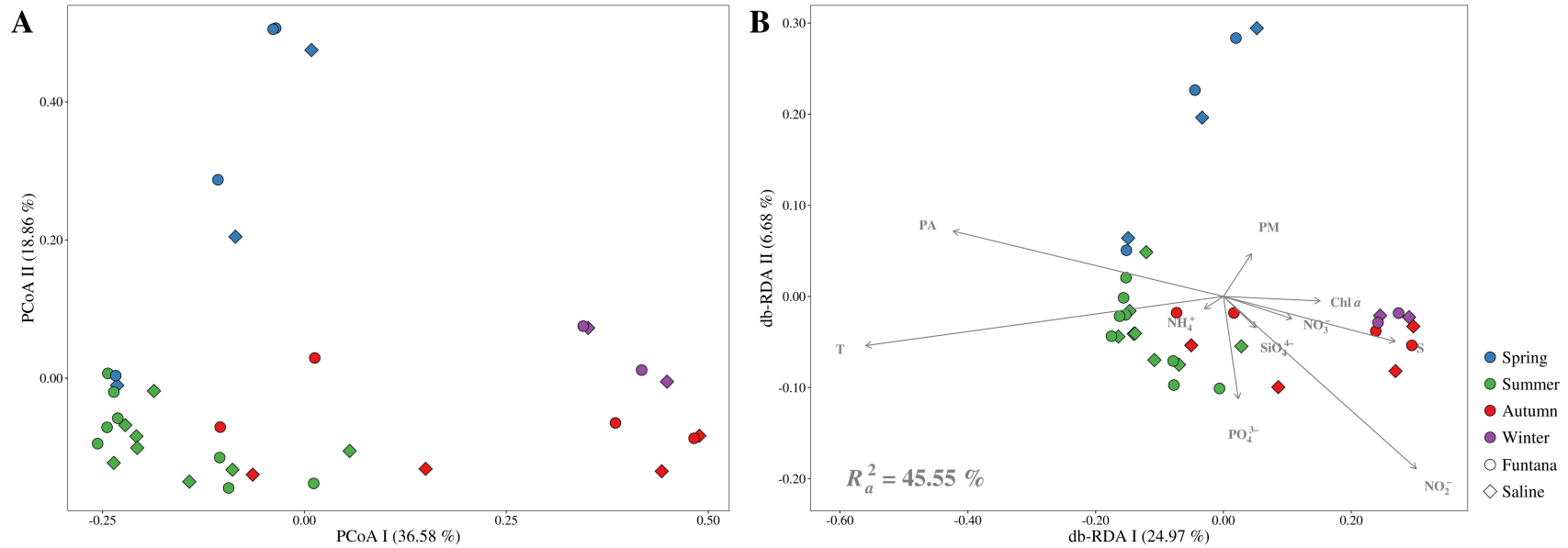


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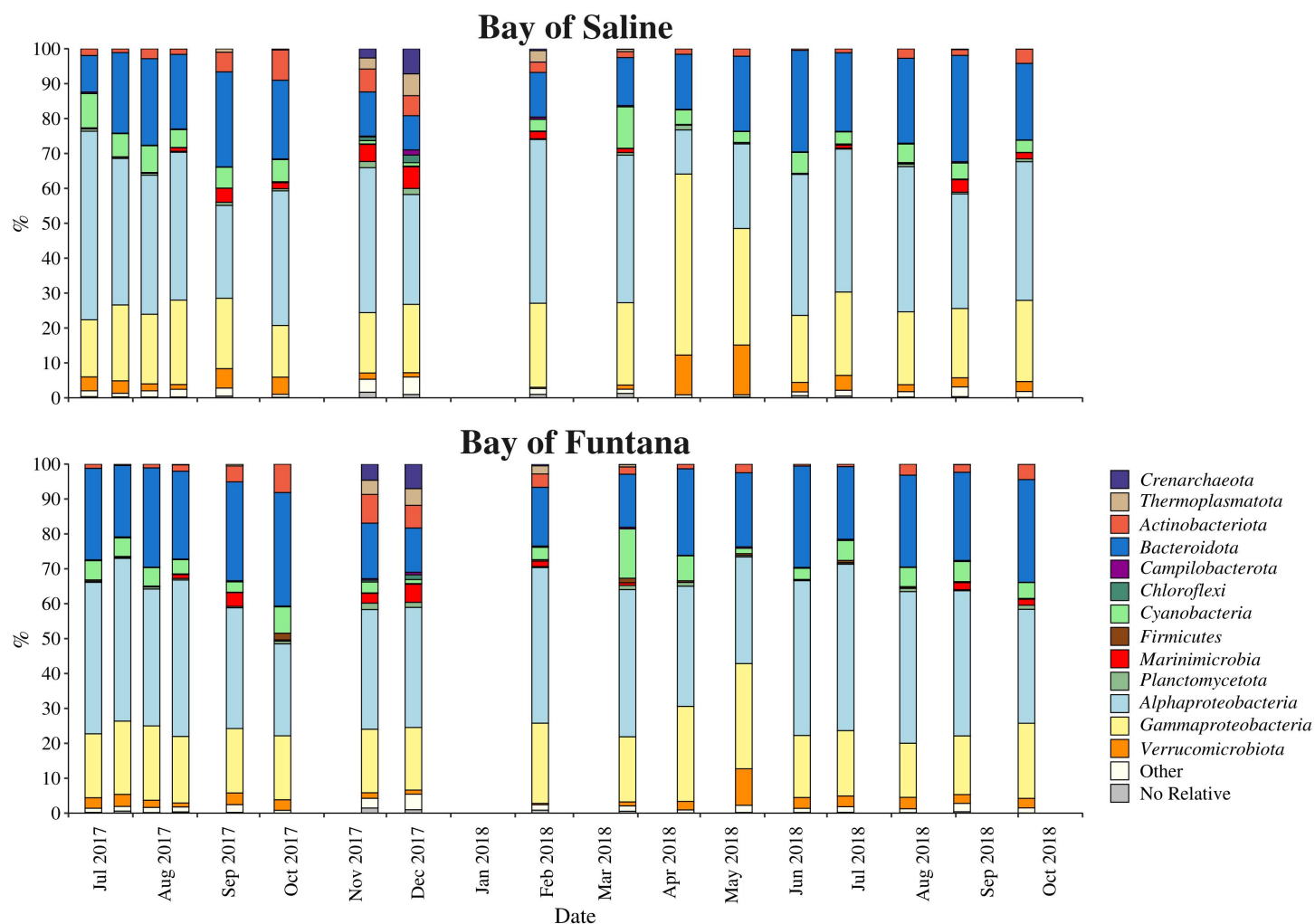


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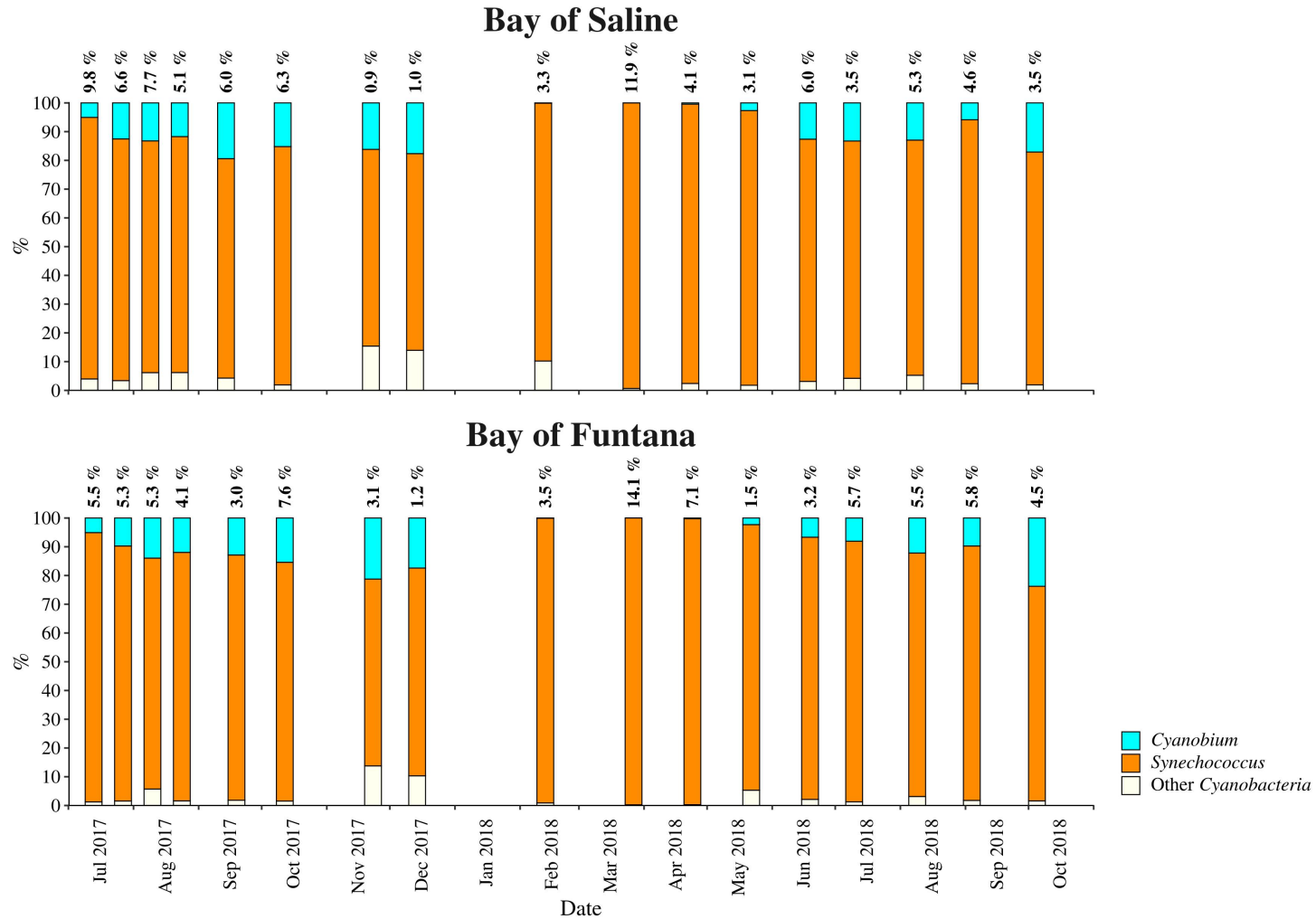


Fig. 3. Taxonomic classification and relative contribution of the most abundant (≥ 1 %) cyanobacterial sequences in communities sampled in Saline and Funtana Bay. The proportion of cyanobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar.

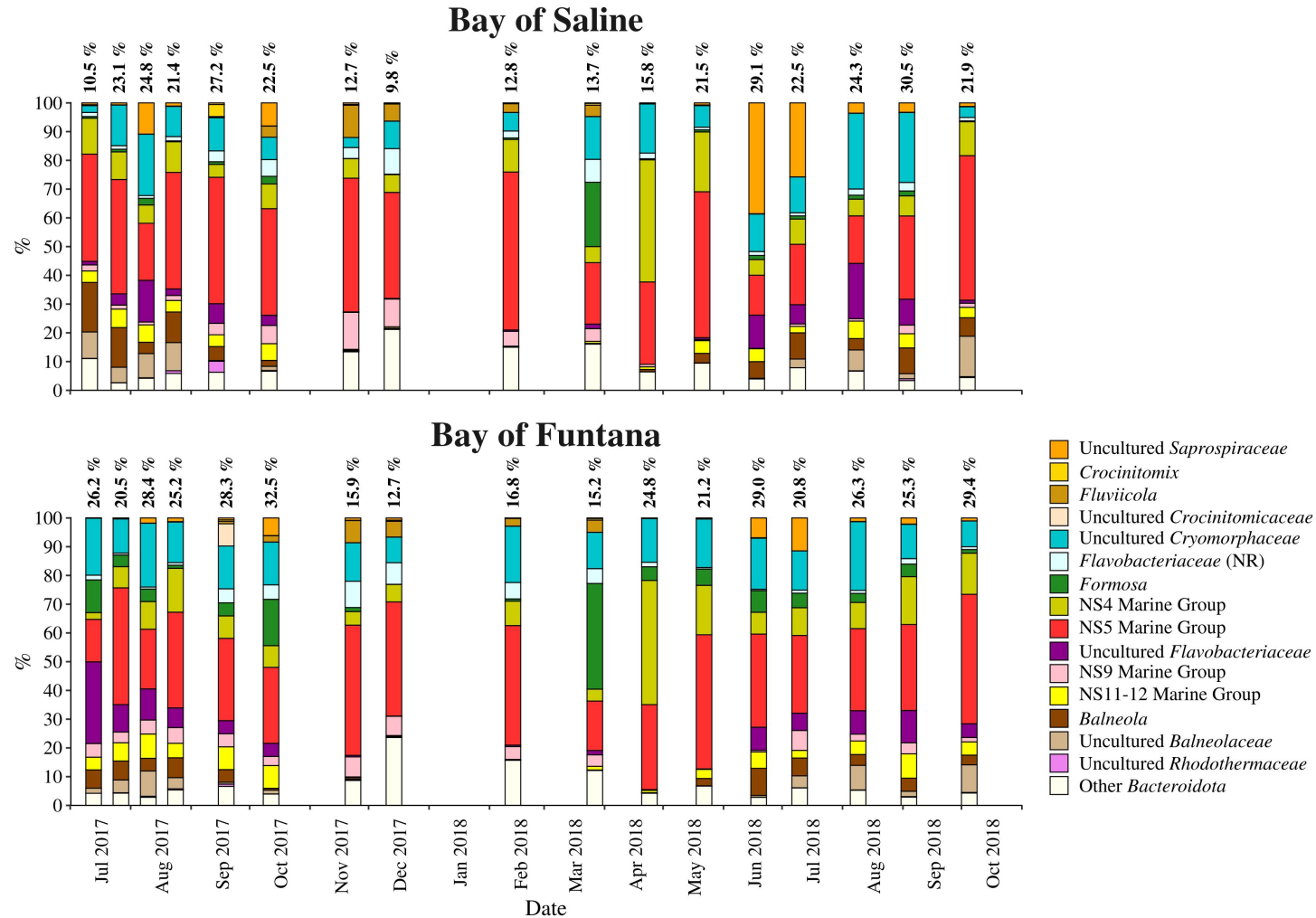


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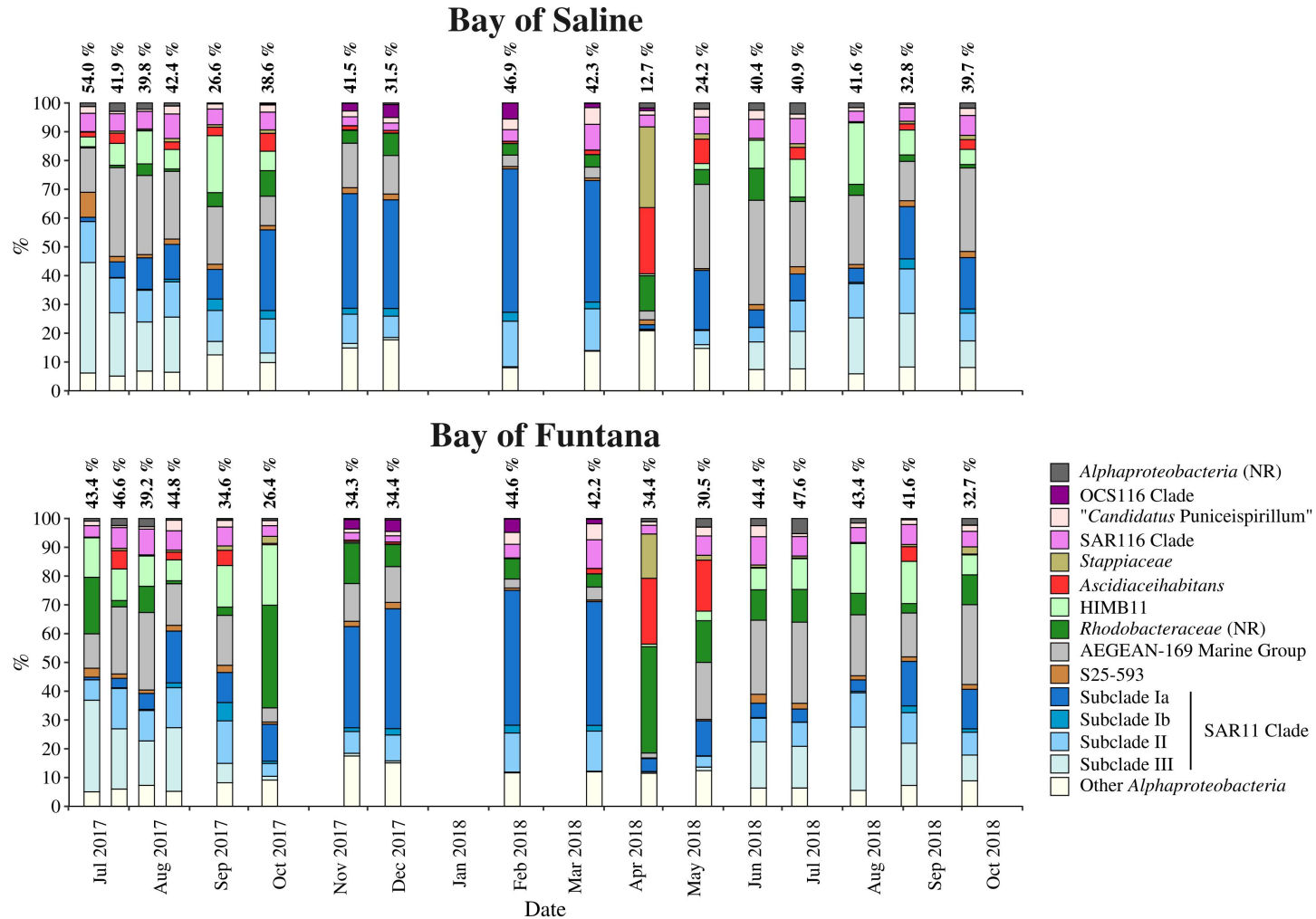


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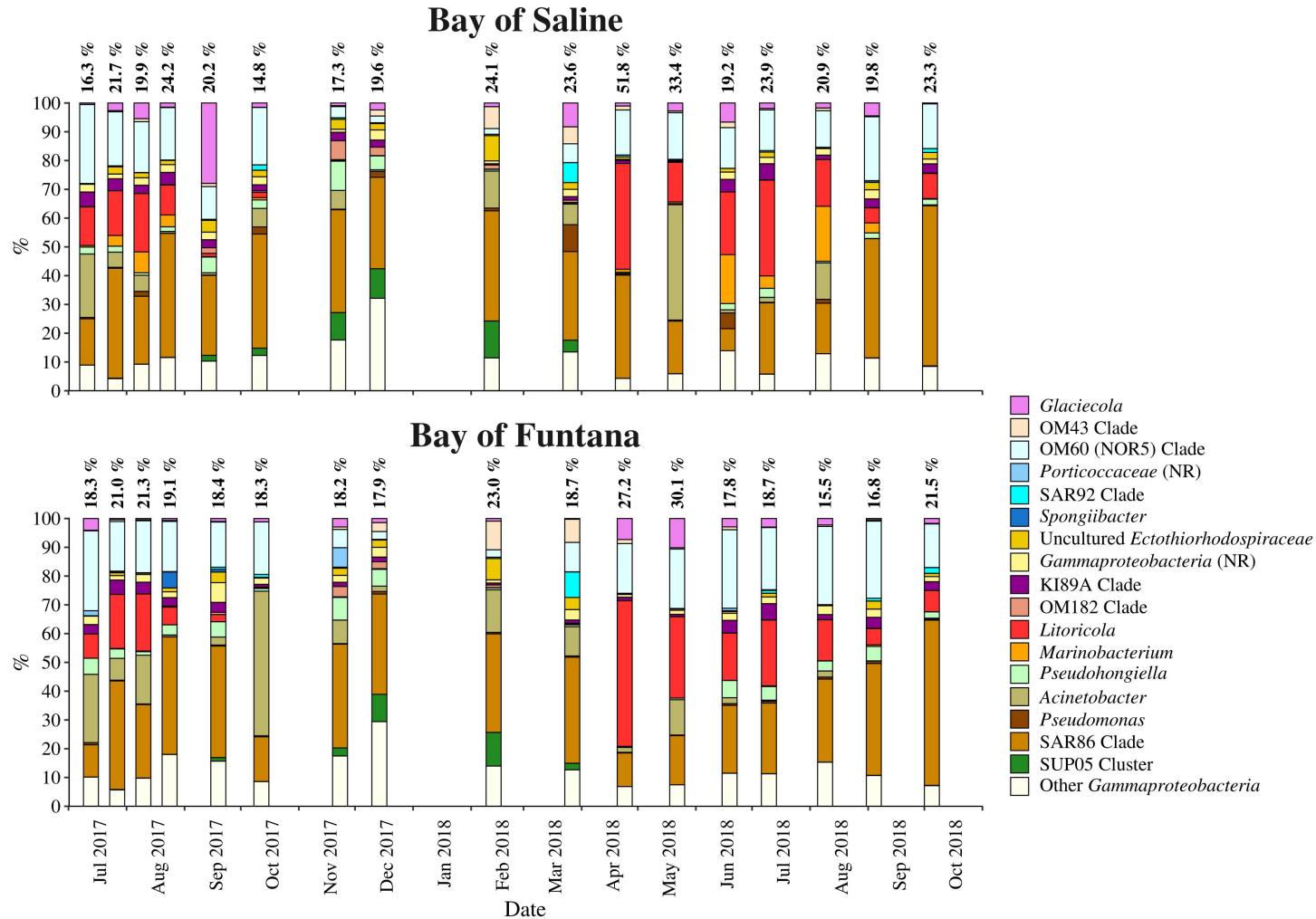


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