

# **Prokaryotic community temporal variation in a coastal marine environment**

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Running title: Temporal variation of a coastal prokaryotic community

## **1 Abstract**

2 Prokaryotic communities inhabiting surface waters of temperate areas exhibit patterns of  
3 seasonal succession. Studies describing these temporal changes were mainly not performed at  
4 stations located in the proximity of the coast. The temporal variation of these communities was  
5 determined in the northern Adriatic Sea surface waters sampled at two stations located in the close  
6 proximity of its western shore. Sequencing of the V4 region of the 16S rRNA gene identified a  
7 community richness maximum in December and a temporal exchange of a spring, summer and  
8 autumn/winter-specific community. Temperature was shown to be the main environmental force  
9 explaining community temporal variation. Taxonomic analysis determined low-level taxa present  
10 throughout the year and groups specific to each identified temporal community. The *Synechococcus*,  
11 SAR86 clade, NS5 marine group and *Cryomorphaceae* were detected through the year. In contrast,  
12 the spring community was characterized by the NS4 marine group, *Formosa* and *Rhodobacteraceae*,  
13 the summer community by SAR11 subclades II and III, HIMB11, AEGEAN-169 marine group,  
14 OM60 (NOR5) clade and *Litoricola* and the autumn/winter community by SAR11 subclade Ia  
15 and *Archaea*. Taken together, prokaryotic communities inhabiting coastal surface waters exhibit  
16 general phenomena similar to other surface associated assemblages, but are also characterized by  
17 season-specific community structures and temporal patterns of certain taxonomic groups that differ  
18 from other areas.

19 **Introduction**

20 Prokaryotic picoplankton communities inhabiting marine surface waters exhibit patterns of  
21 seasonal succession. These temporal community changes were described for surface waters of polar,  
22 temperate and (sub)tropical regions (Bunse and Pinhassi, 2017). In temperate regions changes were  
23 mainly associated with summer water column stratification, winter mixing and spring phytoplankton  
24 blooms (Teeling et al., 2012; Bunse and Pinhassi, 2017; Mestre et al., 2020). Although general  
25 successional patterns in these waters were reported, some local differences were also observed.  
26 While some studies have reported the exchange of multiple community states during the year  
27 (Gilbert et al., 2009; Sintes et al., 2013; El-Swais et al., 2015; Lindh et al., 2015), others have  
28 observed a community separation in only two major groups (Mestre et al., 2020), indicating that  
29 beside global patterns local environmental conditions may influence seasonal 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 summer periods, while the SAR11 clade exhibit an interesting  
40 trend with some sub-clades being characteristic for summer and some for winter months (Salter et  
41 al., 2015; Mestre et al., 2020).

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

44 (Gilbert et al., 2009, 2012), Blanes Bay Microbial Observatory (BBMO) (Alonso-Sáez et al., 2007;  
45 Mestre et al., 2020), Linnaeus Microbial Observatory (Lindh et al., 2015), station Kabeltonne in the  
46 German Bight (Teeling et al., 2012, 2016) and station E2 of the RADIALES time-series project  
47 (Alonso-Sáez et al., 2015). Data obtained from such time-series studies have found that a set of  
48 abiotic and biotic factors drive the temporal community variation (Bunse and Pinhassi, 2017). It  
49 was suggested that biological interactions primarily affect microbial dynamics over shorter time  
50 periods of days to weeks, while physicochemical parameters are mainly responsible for observed  
51 seasonal successional patterns (Gilbert et al., 2009; Fuhrman et al., 2015; Needham and Fuhrman,  
52 2016; Bunse and Pinhassi, 2017; Mestre et al., 2020). In addition, several studies indicate that  
53 the phytoplankton derived dissolved organic matter (DOM) indirectly drives community dynamics  
54 (Teeling et al., 2012, 2016; Lindh et al., 2015; Needham and Fuhrman, 2016; Bunse and Pinhassi,  
55 2017). It is therefore worth investigating if such general such general interactions also apply to  
56 coastal microbial communities inhabiting the proximity of the shore.

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

65 **Materials and methods**

66 **Sampling**

67 Surface seawater from the northern Adriatic Sea was collected in the proximity of the coast  
68 (25 – 50 m) in two closely located bays (~7 km apart), Saline (45°7'5" N, 13°37'20" E) and  
69 Funtana (45°10'39" N, 13°35'42" E), by diving (depth, ~1.5 m) in 10 l containers and transported  
70 to the laboratory where 10 – 20 l were filtered through a 20 µm mesh net. The filtrate was further  
71 sequentially filtered using a peristaltic pump through 3 µm and 0.2 µm polycarbonate membrane  
72 filters (Whatman, United Kingdom). Filters were dried briefly at room temperature and stored at  
73 –80 °C. Samples were collected monthly from July 2017 to October 2018. At the same time when  
74 samples for picoplankton community structure assessment were collected additional samples were  
75 retrieved to determined a set of environmental parameters as reported previously (Najdek et al.,  
76 2020a, 2020b).

77 **DNA isolation**

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

85 **Illumina 16S rRNA sequencing**

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

104 **Sequence analysis**

105 Sequences obtained in the present study were analysed using mothur (version 1.43.0) (Schloss  
106 et al., 2009) according to the MiSeq Standard Operating Procedure (MiSeq SOP; [https://mothur.org/wiki/MiSeq\\_SOP](https://mothur.org/wiki/MiSeq_SOP)) (Kozich et al., 2013) and recommendations given by the Riffomonas project  
107 to enhance data reproducibility (<http://www.riffomonas.org/>). Computing was performed on the  
108

109 computer cluster Isabella (University Computing Center, University of Zagreb). Alignment and  
110 classification was performed using the SILVA SSU Ref NR 99 database (release 138; [http://www.arb-  
111 silva.de](http://www.arb-silva.de)) (Quast et al., 2013; Yilmaz et al., 2014). Pipeline data processing and visualisation was  
112 done using R (version 3.6.0) (R Core Team, 2019) in combination with packages vegan (version  
113 2.5.6) (Oksanen et al., 2019), tidyverse (version 1.2.1) (Wickham, 2017; Wickham et al., 2019) and  
114 multiple other packages (Neuwirth, 2014; Xie, 2014, 2015, 2019a, 2019b, 2019c; Xie et al., 2018;  
115 Allaire et al., 2019; McKinnon Edwards, 2019; Wilke, 2019; Zhu, 2019). The detailed analysis  
116 procedure including the RMarkdown file are available in the GitHub repository (**TO BE ADDED**).  
117 The average sequencing error rate of 0.01 % was calculated based on the ATCC MSA-1002 mock  
118 community included in each sequencing batch, which is in line with previously reported values for  
119 next-generation 16S rRNA amplicon sequencing (Kozich et al., 2013; Schloss et al., 2016). Also,  
120 negative controls processed together with the samples yielded on average only 2 sequences after  
121 quality curation.

122 **Results**

123 Sequencing of 17 samples from the Bay of Saline and 18 samples from the Bay of Funtana  
124 (one of the samples was a sequencing replicate) yielded 1.6 million reads after quality curation and  
125 exclusion of sequences without known relatives (no relative sequences), eukaryotic, chloroplast and  
126 mitochondrial sequences (Table S1). The number of reads per sample ranged from 25,366 to 77,466  
127 (Fig. S1 and Table S1). Reads were clustered into 16,656 different OTUs at a similarity level of 97  
128 %. To account for different sequencing depth reads were normalized to the minimum number of  
129 sequences per sample (25,366, Table S1) that resulted in 13,501 different OTUs ranging from 598  
130 to 1,825 OTUs per sample (Fig. S2).

131 Temporal variations in richness and diversity were determined by calculating the observed  
132 number of OTUs, Chao1, ACE, Exponential Shannon and Inverse Simpson (Jost, 2006). Similar  
133 trends in richness and diversity were observed at both stations (Fig. S2) characterized by a December  
134 2017 richness maximum both in the Saline (Number of OTUs, 1,777 OTUs) and Funtana (Number  
135 of OTUs, 1,825 OTUs) Bay. Interestingly, the Inverse Simpson index did not show an elevated  
136 value in December 2017 as the Exponential Shannon index indicating that rare OTUs contributed  
137 substantially to the observed richness maxima. To determine temporal changes in the proportion of  
138 shared OTUs and communities the Jaccard's and Bray-Curtis similarity coefficients were calculated  
139 between consecutive sampling points (Fig. S3). Similar trends were observed at both stations  
140 with higher stability of shared bacterial and archaeal OTUs (Jaccard's similarity coefficient) than  
141 shared communities (Bray-Curtis similarity coefficient). In addition, a stronger drop in shared  
142 communities between March and April 2018 was observed at both stations indicating a more  
143 pronounced community shift in this period (Fig. S3). Analysis of this time series data showed  
144 that only 0.3 % of OTUs were present throughout the study period while these persistent OTUs  
145 contributed to 61.8 % of sequences.

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

<sup>147</sup> Analysis (PCoA) of Bray-Curtis distances was applied to the OTU community data (Fig. 1A).  
<sup>148</sup> Communities specific to summer, autumn/winter and spring could be identified regardless of  
<sup>149</sup> the station sampled. This separation in three specific communities was further supported by  
<sup>150</sup> ANOSIM ( $R = 0.72$ ,  $p < 0.01$ ). To assess which environmental parameter mainly contributes to the  
<sup>151</sup> observed temporal community variation the community data was constrained by a set of previously  
<sup>152</sup> reported environmental variables (Najdek et al., 2020a, 2020b) using Distance-Based Redundancy  
<sup>153</sup> Analysis (db-RDA) (Fig. 1B). Nearly half ( $R_a^2 = 45.7\%$ ) of the observed community variation  
<sup>154</sup> could be explained by the explanatory variables. Separation between summer and autumn/winter  
<sup>155</sup> communities could mainly be explained by temperature, prokaryotic abundance, salinity and nitrite.  
<sup>156</sup> In contrast, neither variable could strongly explain the separate spring community.

<sup>157</sup> The classification of reads showed that the prokaryotic community was dominated by bacterial  
<sup>158</sup> ( $98.3 \pm 3.5\%$ ) over archaeal sequences ( $1.7 \pm 3.5\%$ ) (Fig. 2). A higher relative contribution  
<sup>159</sup> of archaeal reads was observed only in November 2017 ( $7.5 \pm 2.2\%$ ), December 2017 ( $13.2 \pm$   
<sup>160</sup>  $1.4\%$ ) and February 2018 ( $3.7 \pm 0.9\%$ ). The main taxonomic group contributing to the higher  
<sup>161</sup> relative abundance of *Archaea* in this period was the *Crenarchaeota* “*Candidatus Nitrosopumilus*”  
<sup>162</sup> and the *Thermoplasmatota* Marine group II. The bacterial community was comprised of well  
<sup>163</sup> known seawater groups such as the *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*, *Marinimicrobia*,  
<sup>164</sup> *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota* (Fig. 2). In addition, similar  
<sup>165</sup> temporal patterns were observed at both stations.

<sup>166</sup> *Cyanobacteria* comprised on average  $5.1 \pm 2.8\%$  of the prokaryotic community. The  
<sup>167</sup> highest relative contribution was recorded in winter ( $8.2 \pm 5.6\%$ ), mainly caused by the high  
<sup>168</sup> proportion in the March 2018 sample ( $13.0 \pm 1.6\%$ ). The cyanobacterial community was largely  
<sup>169</sup> dominated by *Synechococcus*, especially during periods of higher cyanobacterial presence (Fig. 3).  
<sup>170</sup> *Bacteroidota* comprised on average  $21.8 \pm 6.2\%$  of the community. Slightly higher values were  
<sup>171</sup> characteristic for spring and summer ( $23.9 \pm 4.6\%$ ) in comparison to autumn and winter ( $18.0 \pm$   
<sup>172</sup>  $7.1\%$ ) (Fig. 2). Although *Bacteroidota* showed only slight temporal variations, taxa within

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

182 Sequences classified as *Alphaproteobacteria* showed the highest relative abundance and  
183 comprised on average  $38.3 \pm 8.0\%$  of the prokaryotic community (Fig. 2). Analysis of temporal  
184 alphaproteobacterial variation showed higher sequence contribution in summer ( $41.3 \pm 6.3\%$ )  
185 and winter ( $44.0 \pm 2.2\%$ ) in comparison to autumn ( $34.9 \pm 4.9\%$ ) and spring ( $31.1 \pm 11.5\%$ ).  
186 Temporal variation of taxa within this class showed different patterns (Fig. 5). The most pronounced  
187 community shift was observed in April 2018 when reads of the usually prominent SAR11 clade  
188 were scarce and *Stappiaceae*, *Ascidiaeihabitans* and no relative *Rhodobacteraceae* dominated  
189 the alphaproteobacterial community. Subclades within the SAR11 clade also showed different  
190 temporal patterns. Subclades II and III characterized the SAR11 community in summer from June  
191 to September, while from November to March reads classified as subclade Ia comprised the majority  
192 of SAR11 specific sequences. Except by the SAR11 subclades II and III, summer months were also  
193 characterized by two other alphaproteobacterial groups, the HIMB11 and the AEGEAN-169 marine  
194 group (Fig. 5).

195 Reads classified as *Gammaproteobacteria* comprised on average  $21.6 \pm 6.6\%$  of the  
196 prokaryotic community (Fig. 2). The season characterized by the highest relative contribution of  
197 gammaproteobacterial sequences was spring ( $29.9 \pm 12.3\%$ ), while in other periods values ranged  
198 from  $18.9 \pm 2.6$  to  $22.3 \pm 2.5\%$ . Temporal variation analysis of taxa within *Gammaproteobacteria*

199 showed groups present throughout the year, such as the SAR86 clade, but also season specific taxa,  
200 such as *Litoricola*, OM60 (NOR5) clade and SUP05 cluster (Fig. 6). *Litoricola* and the OM60  
201 (NOR5) clade characterized the gammaproteobacterial community from April to August, while  
202 sequences specific for the SUP05 cluster were detected from November to March. Taxonomic  
203 groups of high level bacterial taxa that comprised a lower proportion of prokaryotic sequences  
204 such as the *Actinobacteriota* and *Verrucomicrobiota* also showed temporal variations. During  
205 the period of higher relative sequence abundance of *Actinobacteriota*, in the time span between  
206 September and December, the actinobacterial community was comprised mainly of the “*Candidatus*  
207 *Actinomarina*”. Similarly, in April and May when *Verrucomicrobiota* specific reads were at their  
208 maximal relative abundance *Lentimonas* and *Coraliomargarita* were the main constituent of the  
209 *Verrucomicrobiota* specific community.

210 **Discussion**

211 Prokaryotic communities inhabiting surface waters of temporal areas exhibit patterns of  
212 seasonal succession (Bunse and Pinhassi, 2017). These temporal variations were mainly studied at  
213 long-term time series sites which usually encompass only one sampling station located further away  
214 from the coast (Gilbert et al., 2009; Mestre et al., 2020). In the present study temporal variation  
215 of surface prokaryotic communities was determined in the close proximity of the shore. Drawn  
216 conclusions were further strengthen by the analysis of time series data at two closely located stations.  
217 Similar patterns were observed at both sites indicating that determined phenomena could also be  
218 characteristic for prokaryotic communities inhabiting surface waters of a wider area.

219 Temporal changes in richness were substantial as indicated by the proportion of OTUs present  
220 at every sampling point (0.3 %). Sequences clustered into these persistent OTUs comprised a high  
221 proportion of reads (61.8 %). Similar proportions of persistent core OTUs and their contribution  
222 to the total number of sequences were also reported in other time series studies (Gilbert et al.,  
223 2009, 2012). Analysis of temporal variations in alpha diversity showed maximal richness values in  
224 December. This observed event is in line with previously reported richness maxima in other areas  
225 during colder months (Gilbert et al., 2012; Ladau et al., 2013; El-Swais et al., 2015; Mestre et al.,  
226 2020). It has been suggested that late autumn/winter overturn is responsible for this phenomenon  
227 by simply mixing population from deeper parts of the water column with existing ones (García et  
228 al., 2015; Salter et al., 2015; Bunse and Pinhassi, 2017). Although the samples in this study were  
229 retrieved at very shallow locations, water column mixing taking place at deeper areas could bring  
230 additional taxa to these locations causing the observed increase in alpha diversity.

231 The majority of studies analysing temporal community variation usually identified an exchange  
232 of multiple community states during the year (El-Swais et al., 2015; Lindh et al., 2015). In contrast,  
233 some studies described only a switch between winter and summer specific assemblages (Ward et al.,  
234 2017; Mestre et al., 2020). It is possible that these differences are a consequence of local conditions.

Indeed, some studies attributed the observed lower number of states to the absence of large spring and fall phytoplankton blooms in some areas (Ward et al., 2017). Analysis of OTU community data from our samples identified three separate microbial assemblages characteristic for spring, summer and autumn/winter. This is in line with studies describing the exchange of multiple states during the year with a separate spring community assemblage (El-Swais et al., 2015; Lindh et al., 2015). We hypothesize that the separate spring community is a late response to a phytoplankton bloom that can occur in this area (Mozetič et al., 2010; Manna et al., 2021). Temperature and prokaryotic abundance were identified as the main factors influencing the exchange of communities between the summer and autumn/winter period. It is not surprising that temperature and prokaryotic abundance are similar in 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 hypothesize 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. The identified spring-specific community contained taxa previously associated with phytoplankton blooms (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

262 explained by differences in structure and supply of phototroph-derived organic matter. The  
263 summer community was characterized by the family *Balneolaceae* and the NS11-12 marine group  
264 from the *Bacteroidota*, the SAR11 subclades II and III, HIMB11 and the AEGEAN-169 marine  
265 group from the *Alphaproteobacteria* and from the *Gammaproteobacteria* the OM60 (NOR5)  
266 clade and *Litoricola*. In contrast, the winter community was characterized by the archaeal  
267 “*Candidatus Nitrosopumilus*” and Marine group II, the alphaproteobacterial SAR11 subclade Ia  
268 and the gammaproteobacterial SUP05 cluster. Temporal and depth-related variation of different  
269 SAR11 subclades was also reported previously although in our data a different pattern could be  
270 observed in comparison to other surface associated SAR11 communities (Carlson et al., 2009;  
271 Vergin et al., 2013; Salter et al., 2015). In example, the higher contribution of the SAR11 subclade  
272 Ia to the community in winter. Higher contribution of members in the summer community such as  
273 the HIMB11, the OM60 (NOR5) clade and *Litoricola* could result from their adaptation to more  
274 oligotrophic conditions during water column stratification in summer through the ability to use  
275 alternative methods of energy supply (i.e. bacteriochlorophyll *a* and proteorhodopsin) (Huggett and  
276 Rappe, 2012; Spring and Riedel, 2013; Durham et al., 2014). Furthermore, higher contribution of  
277 the AEGEAN-169 marine group to the summer community in our samples could be explained by  
278 the hypothesised adaptation of this group to ultraoligotrophic waters and high solar irradiance  
279 (Reintjes et al., 2019). The co-occurrence of “*Candidatus Nitrosopumilus*” and the Marine  
280 group II in winter samples is interesting. A study describing a strong co-dominance of these two  
281 groups suggested that nitrification by ammonia-oxidising archaea is coupled with ammonification  
282 performed by the members of the Marine group II (Kim et al., 2019). In addition, the presence of  
283 “*Candidatus Nitrosopumilus*” reads in our samples is not surprising as recently two new strains  
284 of ammonia-oxidising archaea within the genus *Nitrosopumilus* have been isolated from northern  
285 Adriatic coastal waters (Bayer et al., 2019).

286 Beside these groups that showed specificity to one of the identified temporal communities  
287 taxonomic analysis revealed taxa present through the year, such as the cyanobacterial *Synechococcus*,  
288 the flavobacterial NS5 marine group and *Cryomorphaceae* and the gammaproteobacterial SAR86

289 clade. The dominance of *Synechococcus* over other cyanobacterial groups in this coastal area was  
290 reported previously, so it is not surprising that we also observed a strong dominance of *Synechococcus*  
291 related sequences (Šilović et al., 2012; Tinta et al., 2015). The known genome versatility of  
292 *Synechococcus* could explain the high contribution of this genus to the cyanobacterial community in  
293 fluctuating coastal environments (Palenik et al., 2003). The *Cryomorphaceae* were associated with  
294 organic matter remineralisation processes (Bowman, 2014), while a single-cell genome analysis  
295 of the NS5 marine group revealed the ability to degrade marine polysaccharides. In addition, the  
296 NS5 marine group was previously detected in different seasons and environments of the Adriatic  
297 Sea (Korlević et al., 2015, 2016). It seems that members of these two groups are part of a basic  
298 remineralisation community present at this location throughout the year. The gammaproteobacterial  
299 SAR86 clade, previously reported in different environments of the Adriatic Sea (Korlević et al., 2015,  
300 2016; Tinta et al., 2015), was also detected throughout the year. Recent analysis of metagenomic  
301 data suggested the existence of different functional and ecological ecotypes of this ubiquitous clade  
302 (Hoarfrost et al., 2020). It is possible that different ecotypes are also characteristic for different  
303 seasons.

304 In conclusion, prokaryotic communities inhabiting the proximity of the shore exhibit temporal  
305 variations similar to surface water assemblages in other temperate areas. As in other areas a richness  
306 maximum was recorded in the colder period of the year, the exchange of multiple community states  
307 during the year was observed and temperature was identified as the main force driving temporal  
308 community change (Gilbert et al., 2012; Ladau et al., 2013; El-Swais et al., 2015; Lindh et al., 2015;  
309 Ward et al., 2017; Mestre et al., 2020). Beside these similarities, temporal analysis of taxonomic data  
310 identified season-specific community structures and groups exhibiting temporal patterns different  
311 from other areas indicating that beside global driving factors local conditions also influence the  
312 coastal prokaryotic community.

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494 **Figure legends**

495 **Fig. 1.** (A) Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU  
496 abundances of bacterial and archaeal communities sampled in Saline and Funtana Bay. The  
497 proportion of explained variation by each axis is shown on the corresponding axis in parentheses.  
498 (B) Distance-Based Redundancy Analysis (db-RDA) of Bray-Curtis distances based on the same  
499 community data sampled at the same locations and constrained by a set of environmental parameters  
500 (T – temperature, S – salinity,  $\text{PO}_4^{3-}$  – orthophosphate,  $\text{NH}_4^+$  – ammonium,  $\text{NO}_2^-$  – nitrite,  $\text{NO}_3^-$  –  
501 nitrate,  $\text{SiO}_4^{4-}$  – orthosilicate, PM – particulate matter, Chl *a* – chlorophyll *a* and PA – prokaryotic  
502 abundance). Scaling type 1 was selected for the biplot. The proportion of community data variation  
503 explained by environmental variables ( $R_a^2$ ) is stated on the biplot, while the proportion of community  
504 data variation explained by each canonical axis is shown on the corresponding axis in parentheses.  
505 Samples in both plots originating from the same station or same season are labeled in different  
506 shape and color.

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

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

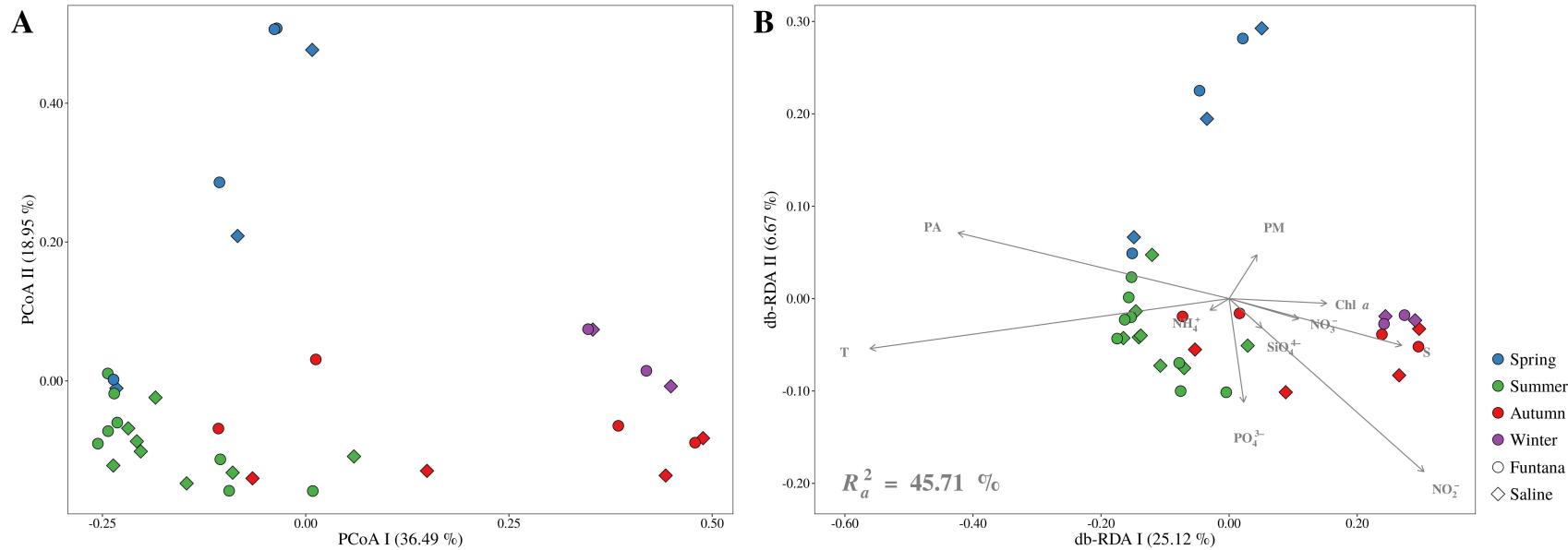
514 **Fig. 4.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) sequences  
515 within the *Bacteroidota* in communities sampled in Saline and Funtana Bay. The proportion of  
516 sequences classified as *Bacteroidota* in the total bacterial and archaeal community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

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

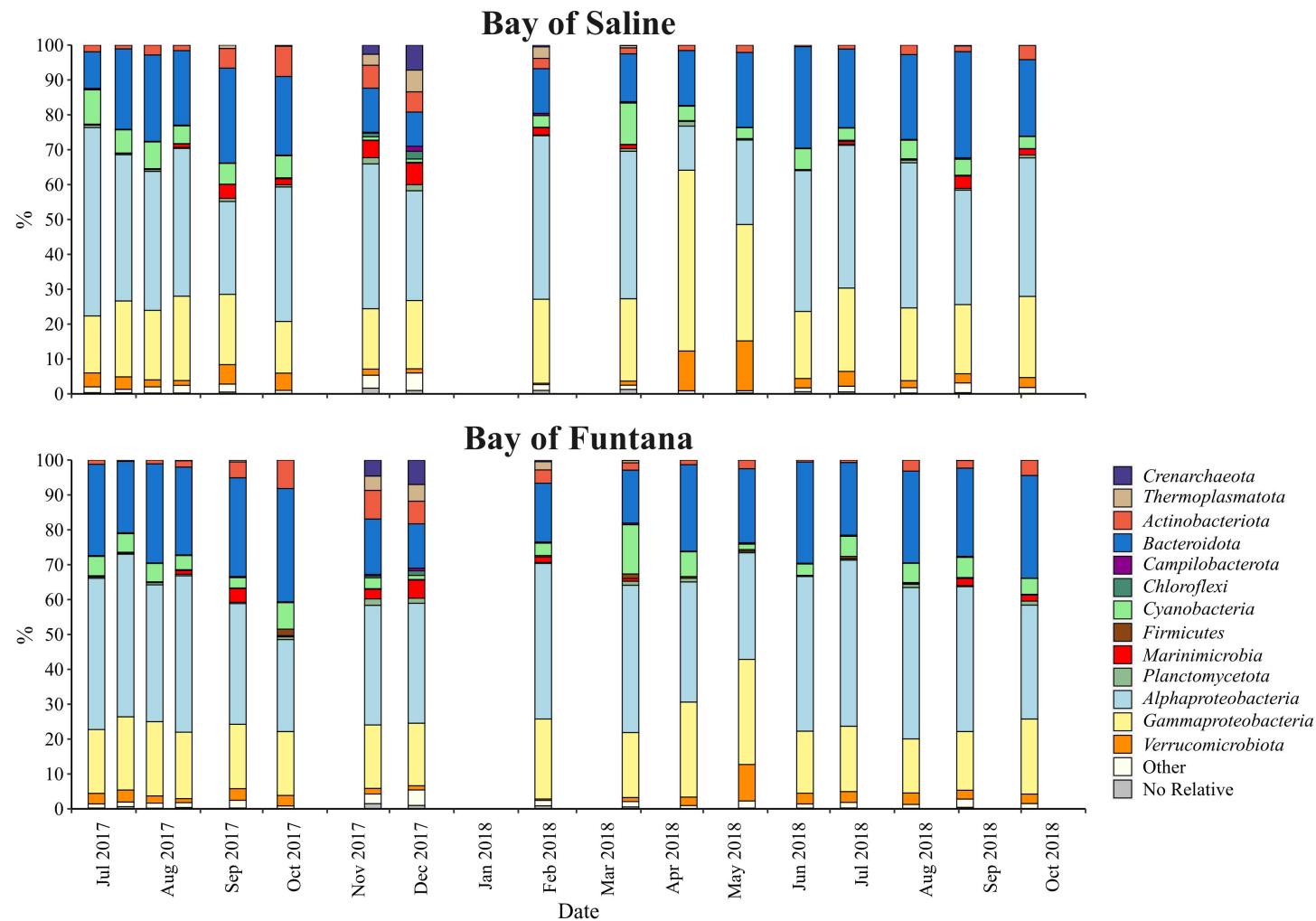
524 **Fig. 6.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ )  
525 gammaproteobacterial sequences in communities sampled in Saline and Funtana Bay. The  
526 proportion of gammaproteobacterial sequences in the total bacterial and archaeal community is  
527 given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

529 **Figures**

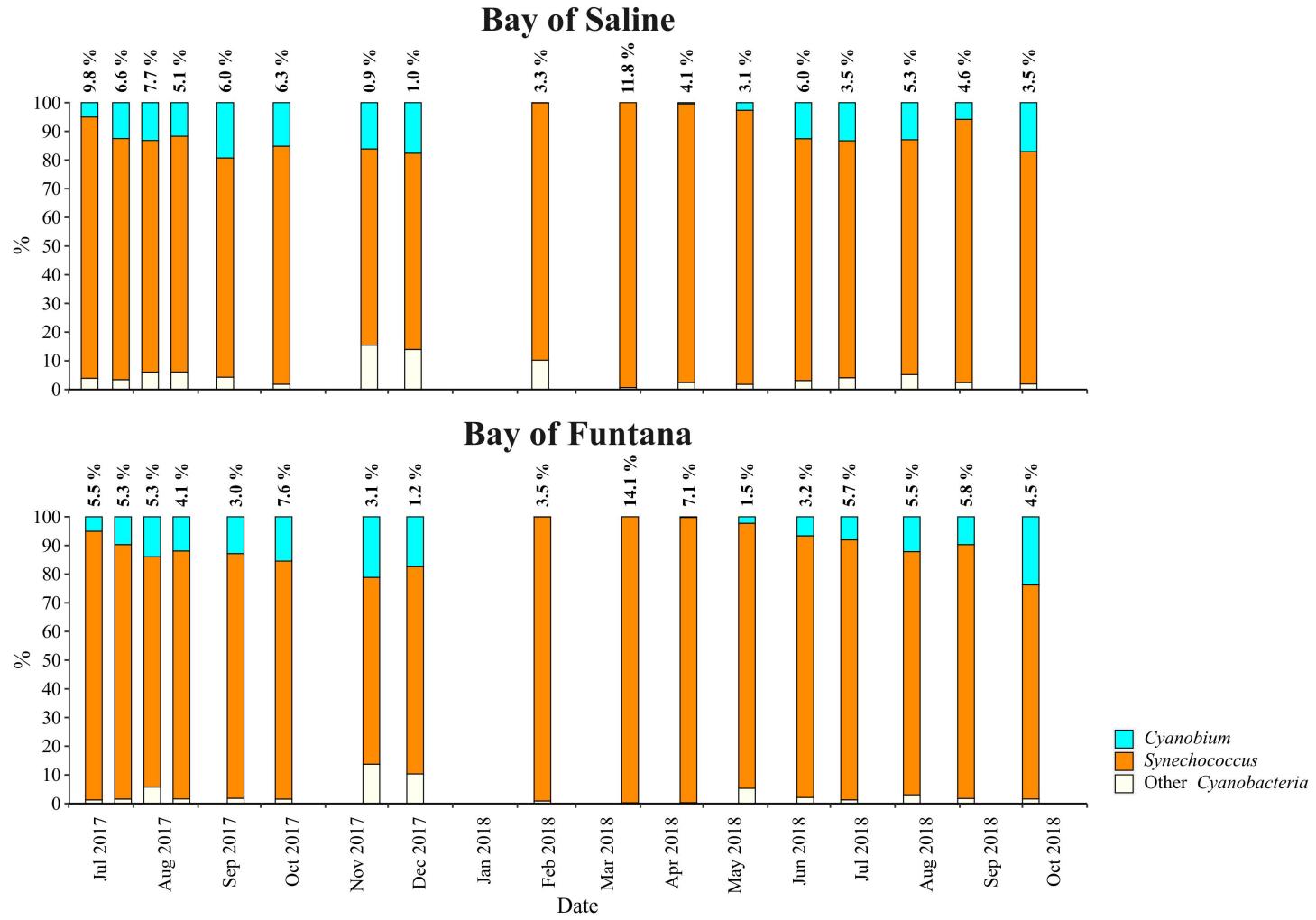
27



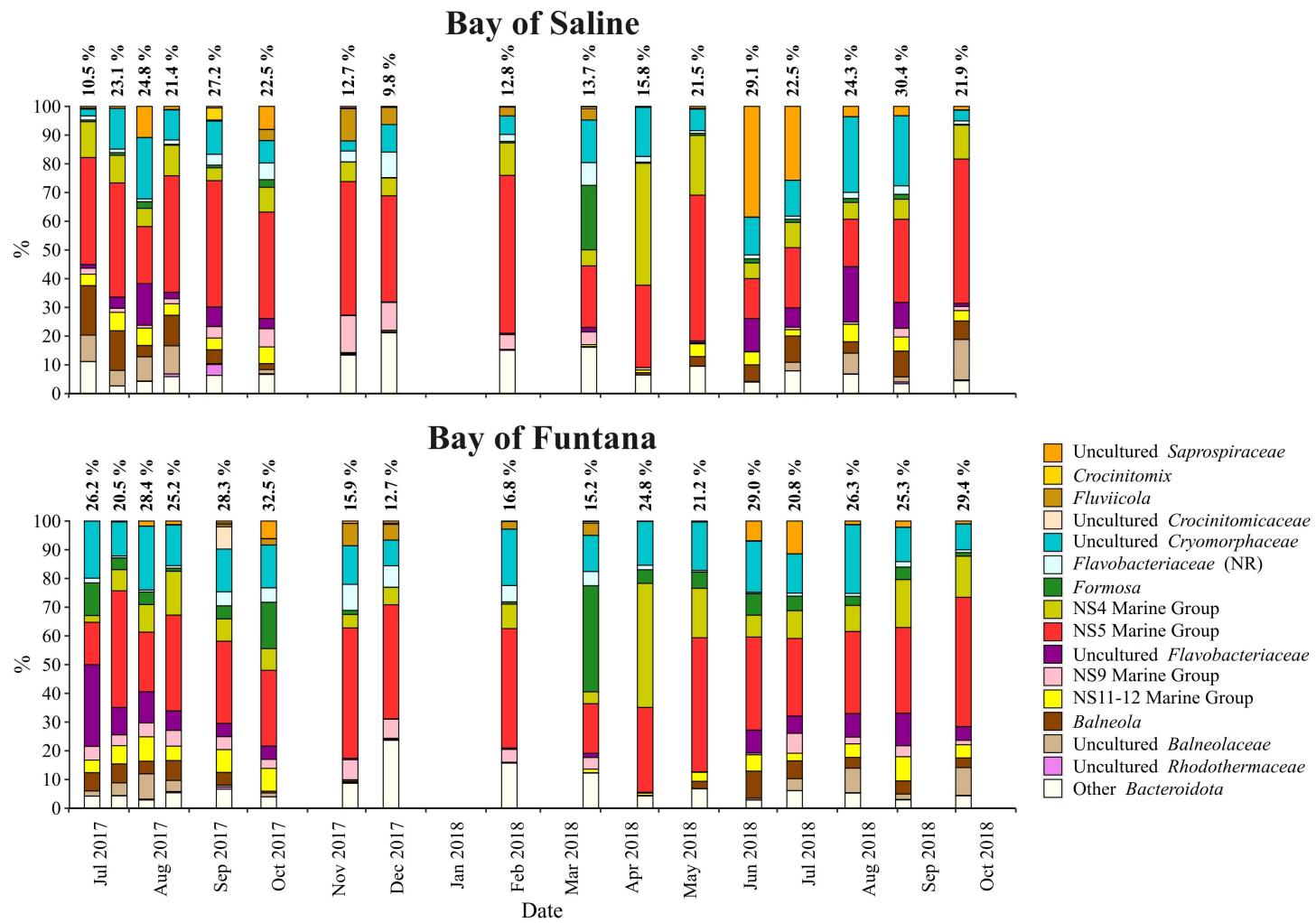
**Fig. 1.** (A) Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities sampled in Saline and Funtana Bay. The proportion of explained variation by each axis is shown on the corresponding axis in parentheses. (B) Distance-Based Redundancy 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,  $\text{PO}_4^{3-}$  – orthophosphate,  $\text{NH}_4^+$  – ammonium,  $\text{NO}_2^-$  – nitrite,  $\text{NO}_3^-$  – nitrate,  $\text{SiO}_4^{4-}$  – 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 ( $R^2_a$ ) 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.



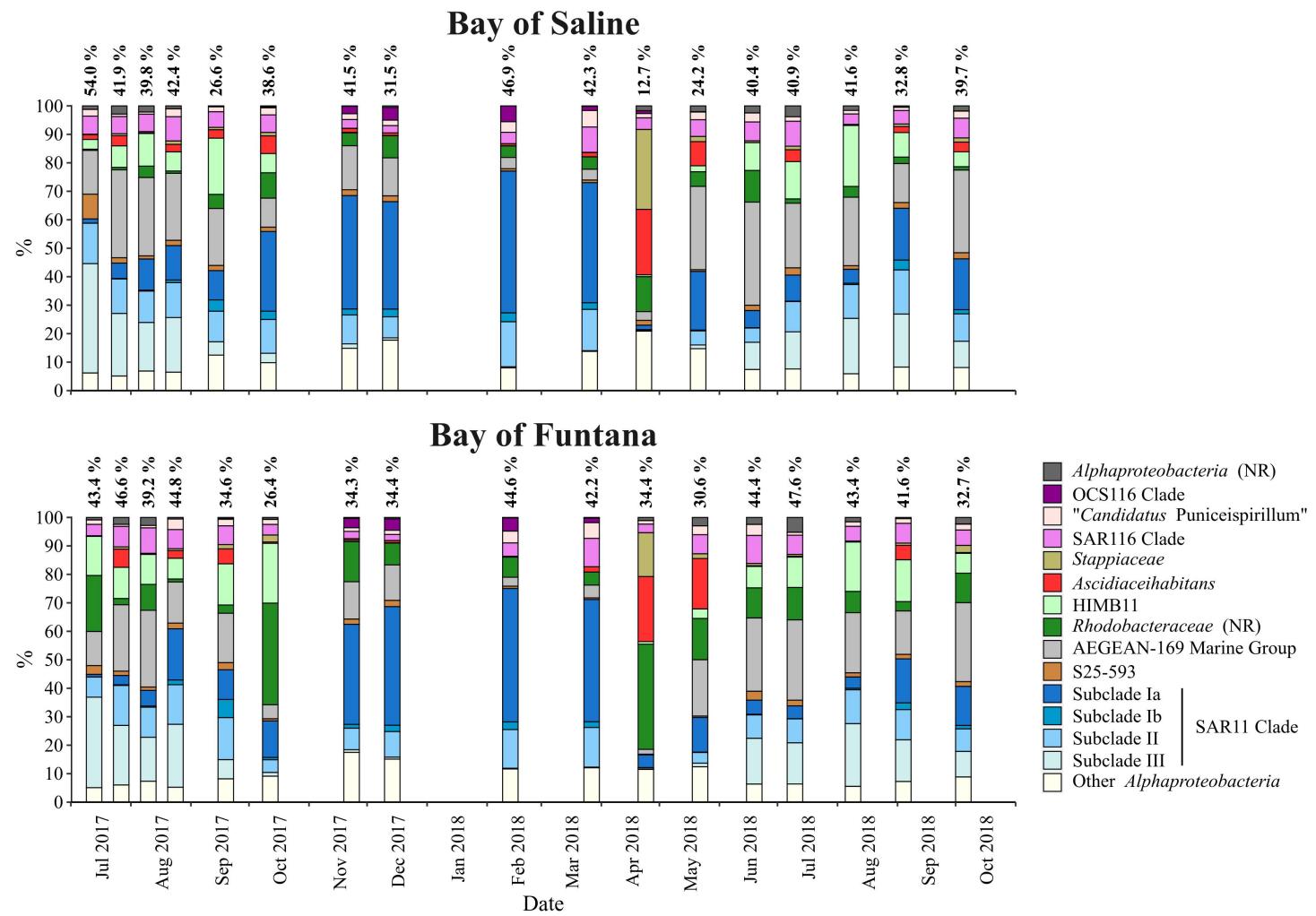
**Fig. 2.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) bacterial and archaeal sequences in communities sampled in Saline and Funtana Bay. No Relative – sequences without known relatives



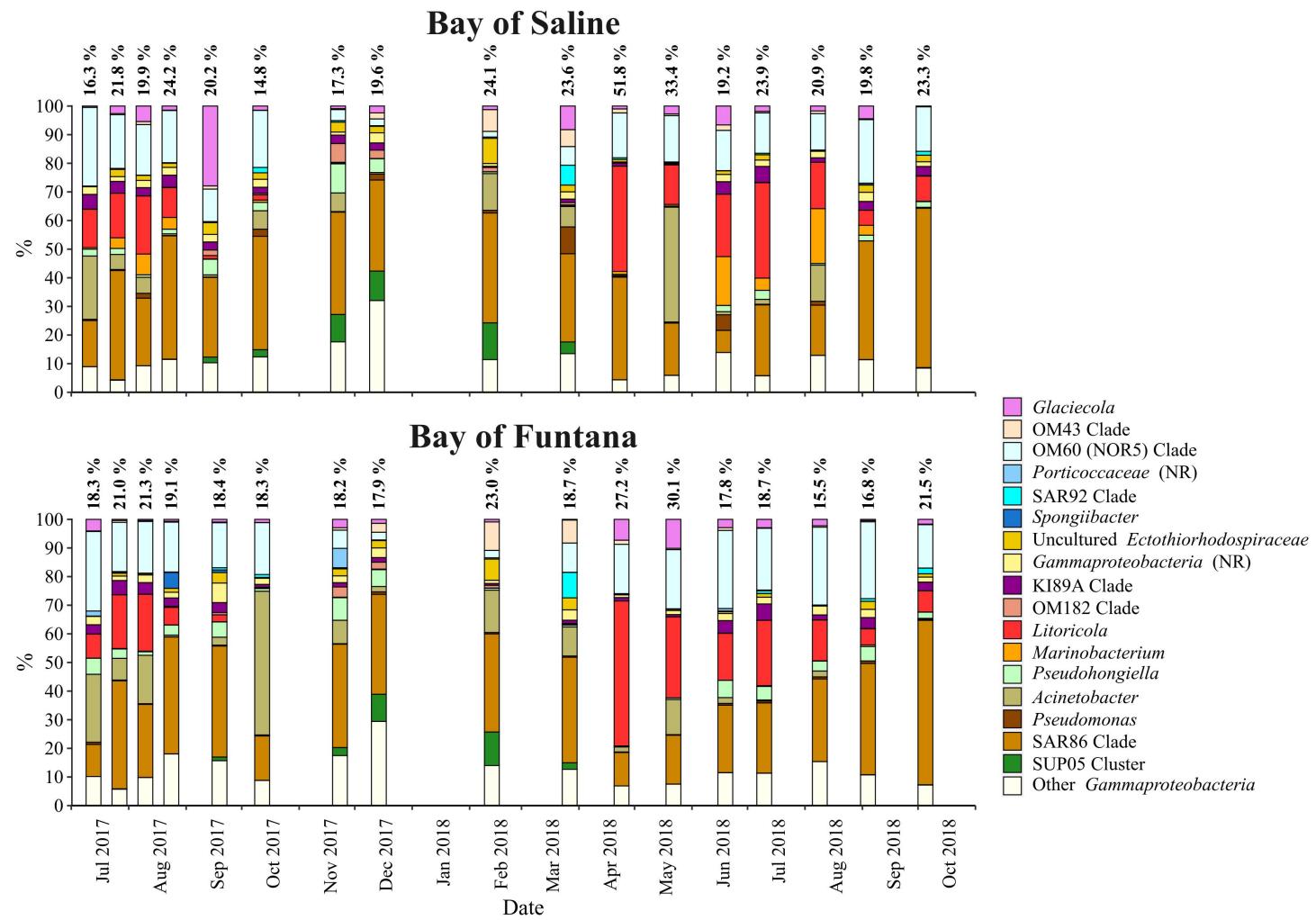
**Fig. 3.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) cyanobacterial sequences in communities sampled in Saline and Funtana Bay. The proportion of cyanobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar.



**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)