

# **Seasonal Dynamics of Epiphytic Microbial Communities on Marine Macrophyte Surfaces**

Marino Korlević<sup>1\*</sup>, Marsej Markovski<sup>1</sup>, Zihao Zhao<sup>2</sup>, Gerhard J. Herndl<sup>2,3</sup>, Mirjana Najdek<sup>1</sup>

1. Center for Marine Research, Ruđer Bošković Institute, Croatia

2. Department of Functional and Evolutionary Ecology, University of Vienna, Austria

3. Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea  
Research (NIOZ), Utrecht University, The Netherlands

\*To whom correspondence should be addressed:

Marino Korlević

G. Paliaga 5, 52210 Rovinj, Croatia

Tel.: +385 52 804 768

Fax: +385 52 804 780

e-mail: marino.korlevic@irb.hr

Running title: Seasonal dynamics of epiphytic communities

## 1 Abstract

2 Surfaces of marine macrophytes are inhabited by diverse microbial communities. Most  
3 studies focusing on epiphytic communities of macrophytes did not take into account temporal  
4 changes or applied low sampling frequency approaches. The seasonal dynamics of epiphytic  
5 microbial communities was determined in a meadow of *Cymodocea nodosa* invaded by *Caulerpa*  
6 *cylindracea* and in a monospecific settlement of *Caulerpa cylindracea* at monthly intervals. For  
7 comparison the ambient prokaryotic picoplankton community was also characterized. At the  
8 OTU level, the microbial community composition differed between the ambient water and the  
9 epiphytic communities exhibiting host-specificity. Also, successional changes were observed  
10 connected to the macrophyte growth cycle. Taxonomic analysis, however, showed similar high rank  
11 taxa (phyla and classes) in the ambient water and the epiphytic communities, with the exception  
12 of *Desulfobacterota*, which were only found on *Caulerpa cylindracea*. *Cyanobacteria* showed  
13 seasonal changes while other high rank taxa were present throughout the year. In months of high  
14 *Cyanobacteria* presence the majority of cyanobacterial sequences were classified as *Pleurocapsa*.  
15 Phylogenetic groups present throughout the year (e.g. *Saprosiraceae*, *Rhodobacteraceae*, members  
16 without known relatives within *Gammaproteobacteria*, *Desulfatitalea* and members without known  
17 relatives within *Desulfocapsaceae*) constituted most of the sequences, while less abundant taxa  
18 showed seasonal patterns connected to the macrophyte growth cycle. Taken together, epiphytic  
19 microbial communities of the seagrass *Cymodocea nodosa* and the macroalga *Caulerpa cylindracea*  
20 appear to be host-specific and contain taxa that undergo successional changes.

21 **Introduction**

22 Marine macrophytes (seagrasses and macroalgae) are important ecosystem engineers forming  
23 close associations with microorganisms belonging to all three domains of life (Egan et al., 2013;  
24 Tarquinio et al., 2019). Microbes can live within macrophyte tissue as endophytes or form epiphytic  
25 communities on surfaces of leaves, roots, rhizomes and thalli (Egan et al., 2013; Hollants et al.,  
26 2013; Aires et al., 2015; Tarquinio et al., 2019). Epiphytic and endophytic microbial communities  
27 exhibit a close functional relationship with the macrophyte host. It has been proposed that this  
28 close relationship constitutes a holobiont, an integrated community where the macrophyte organism  
29 and its symbiotic partners support each other (Margulis, 1991). In addition, as suggested by the  
30 hologenome theory endophytic microbes play a critical role in the adaptation and evolution of the  
31 host species (Aires et al., 2015).

32 Biofilms of microbial epiphytes can contain diverse taxonomic groups and harbour cell  
33 abundances from  $10^2$  to  $10^7$  cells  $\text{cm}^{-2}$  (Armstrong et al., 2000; Bengtsson et al., 2010; Burke  
34 et al., 2011b). In such an environment a number of positive and negative interactions between the  
35 macrophyte and the colonizing microorganisms have been described (Egan et al., 2013; Hollants  
36 et al., 2013; Tarquinio et al., 2019). Macrophytes can promote growth of associated microbes  
37 by nutrient exudation (Wood and Hayasaka, 1981), while in return microorganisms may support  
38 macrophyte performance through improved nutrient availability (Nielsen et al., 2001; de Oliveira  
39 et al., 2012), phytohormone production (Matsuo et al., 2003; Celdrán et al., 2012) and protection  
40 from toxic compounds (Küsel et al., 2006), oxidative stress (Sanchez-Amat et al., 2010), biofouling  
41 organisms (Dobretsov and Qian, 2002) and pathogens (Penesyan et al., 2009). Besides these positive  
42 interactions, macrophytes can negatively impact the associated microbes by producing reactive  
43 oxygen species (Weinberger, 2007) and secondary metabolites (Saha et al., 2011).

44 All these ecological roles are carried out by a taxonomically diverse community of  
45 microorganisms. At higher taxonomic ranks (phyla and classes) microbial taxa, such as

46 *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidota* and *Cyanobacteria*, have been  
47 associated with surfaces of seagrass leaves and macroalgal thalli (Crump and Koch, 2008; Tujula et  
48 al., 2010; Lachnit et al., 2011; Egan et al., 2013; Tarquinio et al., 2019; Ugarelli et al., 2019). While  
49 similar high rank taxa have been found on surfaces of different macrophyte species, in order to  
50 describe new ecological patterns it is also necessary to focus on lower taxonomic ranks (genus and  
51 OTUs) which tend to be host-specific (Lachnit et al., 2011; Hollants et al., 2013; Roth-Schulze et al.,  
52 2016). While the microbial community composition can vary between host species, metagenomic  
53 analyses revealed that the majority of microbial functions are conserved, showing that different  
54 epiphytic microbial species could be functionally similar (Burke et al., 2011a; Roth-Schulze et  
55 al., 2016; Cúcio et al., 2018). This discrepancy between the microbial taxonomic and functional  
56 composition might be explained by the lottery hypothesis (Sale, 1976). It postulates that an initial  
57 random colonization step takes place from a set of functionally equivalent taxonomic groups  
58 resulting in taxonomically different epiphytic communities sharing a core set of functional genes  
59 (Burke et al., 2011a; Stratil et al., 2013; Schmidt et al., 2015; Roth-Schulze et al., 2016).

60 Seagrasses are known to form close relationships with microbial communities associated with  
61 the surfaces of leaves, roots and rhizomes (Cúcio et al., 2016; Crump et al., 2018; Ugarelli et  
62 al., 2019; Ettinger and Eisen, 2020; Wang et al., 2020). For different seagrass species a distinct  
63 microbial community from ambient seawater or bulk sediment has been reported, however no  
64 species specific communities have been found (Cúcio et al., 2016; Crump et al., 2018; Ugarelli et al.,  
65 2019). It seems that seagrasses are selecting the associated microbial community but these microbes  
66 have not coevolved with their seagrass plant host. Similar to seagrasses, siphonous macroalgae  
67 of the genus *Caulerpa* are also closely associated with their microbial communities (Aires et al.,  
68 2013, 2015; Rizzo et al., 2016b; Stabili et al., 2017; Morrissey et al., 2019). While some studies  
69 have found similar culturable bacterial groups associated with the surface of a *Caulerpa* species  
70 from different geographic locations (Stabili et al., 2017), others have reported large compositional  
71 differences that were mainly attributed to different host species of this genus, biogeography and  
72 nutrient levels (Morrissey et al., 2019) again raising the question to which extent are associated

73 communities host-specific.

74 Since marine macrophytes in temperate zones are exhibiting seasonal changes in growth and  
75 physiology (Agostini et al., 2003; Najdek et al., 2020) it is important to verify if and how surface  
76 associated microbial communities are affected by these changes. The majority of studies describing  
77 macrophyte epiphytic microbial communities have not included possible seasonal changes (Crump  
78 and Koch, 2008; Lachnit et al., 2009; Burke et al., 2011b; Roth-Schulze et al., 2016; Ugarelli et  
79 al., 2019). If seasonal changes have been taken into account, low temporal frequency, applied  
80 methodologies and/or limited number of analysed host species did not allow for a detailed taxonomic  
81 analysis (Tujula et al., 2010; Lachnit et al., 2011; Bengtsson et al., 2012; Michelou et al., 2013;  
82 Miranda et al., 2013; Mancuso et al., 2016). In the present study we performed a descriptive analysis  
83 of seasonal bacterial and archaeal community dynamics on the surfaces of the seagrass *Cymodocea*  
84 *nodososa*, an abundant seagrass species in the Mediterranean (Short et al., 2001), and siphonous  
85 macroalga *Caulerpa cylindracea*, one of the most invasive macroalgal species (Klein and Verlaque,  
86 2008; Boudouresque et al., 2009). Bacterial and archaeal epiphytes were sampled in a meadow of  
87 *C. nodosa* invaded by the invasive *C. cylindracea* and in a locality of only *C. cylindracea* located in  
88 the proximity of the seagrass meadow. For comparison, the microbial community of the ambient  
89 seawater was also characterized. The presence of both macrophytes in the same area enabled (i)  
90 the assessment of differences in the bacterial and archaeal communities between host species and  
91 settlements of *C. cylindracea* and (ii) the evaluation of differences between surface associated and  
92 free living (ambient seawater) communities. In addition, these differences were evaluated on a  
93 monthly scale providing insight into seasonal changes (iii).

94 **Materials and methods**

95 **Sampling**

96 Sampling was performed in the Bay of Funtana, northern Adriatic Sea ( $45^{\circ}10'39''$  N,  $13^{\circ}35'42''$  E). The sea floor in the bay is partly covered by the invasive macroalga *C. cylindracea* that can be found in a monospecific settlement or mixed with the seagrass *C. nodosa* (Figure 1). *C. nodosa* leaves were retrieved from a meadow of *C. nodosa* invaded by the invasive *C. cylindracea* (mixed settlement; depth, 2 – 2.5 m), while *C. cylindracea* thalli were sampled in the same invaded meadow (mixed settlement; depth, 2 – 2.5 m) and in a monospecific settlement (depth, 1 – 1.5 m) of *C. cylindracea* located in the proximity (20 – 50 m) of the invaded meadow at approximately monthly intervals from November 2017 to October 2018 (Table S1). Leaves and thalli were collected by diving and transported to the laboratory in containers placed on ice and filled with seawater collected at the sampling site. Upon arrival to the laboratory, *C. nodosa* leaves were cut into sections of 1 – 2 cm, while *C. cylindracea* thalli were cut into 5 – 8 cm long sections. Leaves and thalli were washed three times with sterile artificial seawater (ASW) to remove loosely attached microbial cells. Ambient seawater was collected in 10 l containers by diving and transported to the laboratory where 10 – 20 l were filtered through a 20  $\mu\text{m}$  net. The filtrate was further sequentially filtered through 3  $\mu\text{m}$  and 0.2  $\mu\text{m}$  polycarbonate membrane filters (Whatman, United Kingdom) using a peristaltic pump. Filters were briefly dried at room temperature and stored at  $-80^{\circ}\text{C}$ . Seawater samples were also collected approximately monthly from July 2017 to October 2018.

113 **DNA isolation**

114 DNA from surfaces of *C. nodosa* and *C. cylindracea* was isolated from a pool of leaves (1 g  
115 wet weight) or thalli (2 g wet weight) on the sampling day using a previously modified and adapted  
116 protocol that allows for a selective epiphytic DNA isolation (Massana et al., 1997; Korlević et al.,

117 2021). Briefly, leaves and thalli were incubated in a lysis buffer and treated with lysozyme and  
118 proteinase K. Following the incubations, the mixture containing lysed epiphytic cells was separated  
119 from the leaves and thalli and extracted using phenol-chloroform. Finally, the extracted DNA  
120 was precipitated using isopropanol. DNA from seawater picoplankton was extracted from 0.2 µm  
121 polycarbonate filters according to Massana et al. (1997) with a slight modification. Following  
122 the phenol-chloroform extraction, 1/10 of 3 M sodium acetate (pH 5.2) was added. DNA was  
123 precipitated by adding 1 volume of chilled isopropanol, incubating the mixtures overnight at -20  
124 °C and centrifuging at 20,000 × g and 4 °C for 20 min. The pellet was washed twice with 500 µl  
125 of chilled 70 % ethanol and centrifuged after each washing step at 20,000 × g and 4 °C for 5 min.  
126 Dried pellets were re-suspended in 50 – 100 µl of deionized water. One DNA sample originating  
127 from seawater picoplankton was obtained per each sampling point.

128 **Illumina 16S rRNA sequencing**

129 Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene was performed as described  
130 previously (Korlević et al., 2021). The V4 region of the 16S rRNA gene was amplified using a  
131 two-step PCR procedure. In the first PCR, the 515F (5'-GTGYCAGCMGCCGCGTAA-3') and  
132 806R (5'-GGACTACNVGGGTWTCTAAT-3') primers from the Earth Microbiome Project (<https://earthmicrobiome.org/protocols-and-standards/16s/>) were used (Caporaso et al., 2012; Apprill et al.,  
133 2015; Parada et al., 2016). These primers contained on their 5' end a tagged sequence. Purified PCR  
134 products were sent for Illumina MiSeq sequencing at IMGM Laboratories, Martinsried, Germany.  
135 Prior to sequencing at IMGM, the second PCR amplification of the two-step PCR procedure was  
136 performed using primers targeting the tagged region incorporated in the first PCR. In addition,  
137 these primers contained adapter and sample-specific index sequences. Beside samples, a positive  
138 and negative control for each sequencing batch was sequenced. The negative control comprised  
139 PCR reactions without DNA template, while for a positive control a mock community composed  
140 of evenly mixed DNA material originating from 20 bacterial strains (ATCC MSA-1002, ATCC,  
141

142 USA) was used. Sequences obtained in this study have been deposited in the European Nucleotide  
143 Archive (ENA) at EMBL-EBI under the accession number PRJEB37267 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB37267>).  
144

## 145 Sequence and data analysis

146 Obtained sequences were analysed on the computer cluster Isabella (University Computing  
147 Center, University of Zagreb) using mothur (version 1.43.0) (Schloss et al., 2009) according to the  
148 MiSeq Standard Operating Procedure (MiSeq SOP; [https://mothur.org/wiki/MiSeq\\_SOP](https://mothur.org/wiki/MiSeq_SOP)) (Kozich et  
149 al., 2013) and recommendations provided by the Riffomonas project to enhance data reproducibility  
150 (<http://www.riffomonas.org/>). For alignment and classification of sequences the SILVA SSU Ref  
151 NR 99 database (release 138; <http://www.arb-silva.de>) was used (Quast et al., 2013; Yilmaz et al.,  
152 2014). Sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97  
153 %.

154 Pipeline data processing and visualization was done using R (version 3.6.0) (R Core Team,  
155 2019) combined with packages vegan (version 2.5-6) (Oksanen et al., 2019), tidyverse (version  
156 1.3.0) (Wickham, 2017; Wickham et al., 2019) and multiple other packages (Neuwirth, 2014;  
157 Xie, 2014, 2015, 2019a, 2019b, 2019c; Xie et al., 2018; Allaire et al., 2019; Wilke, 2019; Zhu,  
158 2019). Observed number of OTUs, Chao1, ACE, exponential of the Shannon diversity index and  
159 Inverse Simpson index were calculated after normalization to the minimum number of reads per  
160 sample using vegan's function `rrarefy` to account for different sequencing depths (Oksanen et  
161 al., 2019). Chao1 and ACE estimators were calculated using vegan's function `estimateR`, while  
162 Shannon and Inverse Simpson diversity indices were retrieved using vegan's function `diversity`  
163 (Oksanen et al., 2019). To express both diversity indices in terms of effective number of OTUs the  
164 exponential of the Shannon diversity index was calculated (Jost, 2006). The proportions of shared  
165 OTUs and communities between samples and community types (seawater, *C. nodosa* [mixed],  
166 *C. cylindracea* [mixed] and *C. cylindracea* [monospecific]) were expressed as the Jaccard's (on

presence/absence data) and Bray-Curtis similarity coefficient, respectively. The coefficients were calculated on the OTU data table using vegan's function `vegdist` and converted from dissimilarities to similarities (Borcard et al., 2011; Legendre and Legendre, 2012; Oksanen et al., 2019). The Principal Coordinates Analysis (PCoA) was performed on Bray-Curtis dissimilarities based on OTU abundances using the function `cmdscale` (Borcard et al., 2011; Legendre and Legendre, 2012). Differences between communities were tested by performing the Analysis of Similarities (ANOSIM) using the vegan's function `anosim` and 1000 permutations (Oksanen et al., 2019), while differences in relative contributions or proportions of shared OTUs and communities were tested by applying the Mann–Whitney  $U$  test using the function `wilcox.test`. In addition, differences between community type estimators or indices were tested by performing the Kruskal-Wallis  $H$  test (function `kruskal.test`) followed by a pairwise comparison using the Mann-Whitney  $U$  test (function `pairwise.wilcox`). Bonferroni correction was used to address the problem of multiple comparisons.

A total of 1.7 million sequences after quality curation and exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences were obtained (Table S1). The number of reads per sample ranged between 8,408 and 77,463 sequences (Table S1). Even when the highest sequencing effort was applied the rarefaction curves did not level off as commonly observed in high-throughput 16S rRNA amplicon sequencing approaches (Figure S1). Following quality curation and exclusion of sequences as mentioned above reads were clustered into 28,750 different OTUs. Read numbers were normalized to the minimum number of sequences (8,408, Table S1) using previously mentioned vegan's function `rrarefy` resulting in 17,201 different OTUs ranging from 352 to 2,062 OTUs per sample (Figure S2). Based on the ATCC MSA-1002 mock community included in the analysis an average sequencing error rate of 0.01 % was determined, which is in line with previously reported values for next-generation sequencing data (Kozich et al., 2013; Schloss et al., 2016). In addition, the negative controls processed together with the samples yielded only 2 sequences after sequence quality curation. The detailed analysis procedure including the R Markdown file is available as a GitHub repository

194 ([https://github.com/MicrobesRovinj/Korlevic\\_EpiphyticDynamics\\_FrontMicrobiol\\_2021](https://github.com/MicrobesRovinj/Korlevic_EpiphyticDynamics_FrontMicrobiol_2021)).

195 **Results**

196 A total of 35 samples originating from epiphytic archaeal and bacterial communities associated  
197 with surfaces of the seagrass *C. nodosa* and the macroalga *C. cylindracea* were analysed. In addition,  
198 18 samples (one of the samples was sequenced twice) originating from the ambient seawater were  
199 also processed for comparison. Generally, richness estimators and diversity indices showed similar  
200 trends. On average, higher values were found for *C. cylindracea* (mixed [Number of OTUs, 1,688.4  
201  $\pm$  136.6 OTUs] and monospecific [Number of OTUs, 1,750.4  $\pm$  165.7 OTUs]) than for *C. nodosa*  
202 (Number of OTUs, 1,063.7  $\pm$  210.6 OTUs) and lowest values were obtained for the microbial  
203 community of the ambient seawater (Number of OTUs, 531.0  $\pm$  143.9 OTUs) (Kruskal-Wallis,  $p <$   
204 0.0001) (Figure S2 and Tables S2 and S3). Temporal changes did not reveal such large dissimilarities.  
205 *C. nodosa* communities showed a slow increase in all calculated richness estimators towards the  
206 end of the study, while *C. cylindracea* (mixed and monospecific) communities were characterized  
207 by slightly higher values in spring and summer than in autumn and winter (Figure S2).

208 A clear separation between ambient seawater and surface associated communities was found  
209 (Figure 2). In addition, a separation of epiphytic bacterial and archaeal communities based on  
210 host species was detected. This separation was further supported by ANOSIM ( $R = 0.96$ ,  $p$   
211  $< 0.001$ ). The highest proportion of shared OTUs and community was found between mixed  
212 and monospecific *C. cylindracea* (Jaccard, 0.35; Bray-Curtis, 0.77), while lower shared values  
213 were calculated between ambient seawater and epiphytic communities (Jaccard, 0.10 – 0.11;  
214 Bray-Curtis, 0.05 – 0.06). Shared proportions of OTUs and communities between *C. nodosa* and  
215 *C. cylindracea* (either mixed or monospecific) were approximately in-between the values obtained  
216 for the comparison of ambient seawater with all other communities and for the comparison of the  
217 mixed and monospecific *C. cylindracea* associated community. Seasonal changes of *C. nodosa*

218 associated communities indicated a separation between spring, summer and autumn/winter samples  
219 (ANOSIM,  $R = 0.56$ ,  $p < 0.01$ ). For *C. cylindracea* associated communities a separation between  
220 summer and autumn/winter/spring samples was observed that was, however, not as strong as for *C.*  
221 *nodosa* associated communities (ANOSIM,  $R = 0.30$ ,  $p < 0.05$ ) (Figure 2). Shared proportions of  
222 OTUs between consecutive sampling points were lower for ambient seawater ( $19.6 \pm 2.5 \%$ ) than  
223 for *C. nodosa* ( $28.3 \pm 5.2 \%$ ) and *C. cylindracea* (mixed [ $26.3 \pm 2.1 \%$ ] and monospecific [ $27.2$   
224  $\pm 2.0 \%$ ]) associated communities ( $p < 0.0001$ ), while mean proportions of shared communities  
225 between consecutive sampling points did not show such a difference (seawater,  $57.4 \pm 14.7 \%$ ; *C.*  
226 *nodosa*,  $53.4 \pm 9.3 \%$ ; *C. cylindracea* [mixed],  $55.0 \pm 7.0 \%$ ; *C. cylindracea* [monospecific],  $55.1 \pm$   
227  $5.2 \%$ ) ( $p = 0.1$ ), although in ambient seawater higher fluctuations could be observed (Figure 3). In  
228 addition, only 0.4 – 1.0 % of OTUs from each surface associated community were present at all  
229 seasons. These persistent OTUs constituted a high proportion of total sequences (40.2 – 53.2 %)  
230 and were mainly contributing to abundant phylogenetic groups present throughout the year, e.g. the  
231 no relative *Rhodobacteraceae* in the case of *C. nodosa* or taxa within *Desulfobacterota* in the case  
232 of *C. cylindracea* (see below) (Table S4).

233 The taxonomic composition of both, macrophyte associated and ambient seawater community,  
234 was dominated by bacterial ( $99.1 \pm 2.1 \%$ ) over archaeal sequences ( $0.9 \pm 2.1 \%$ ) (Figure 4). Higher  
235 relative abundances of chloroplast related sequences were only observed in surface associated  
236 communities, with higher values in autumn/winter ( $37.2 \pm 11.2 \%$ ) than in spring/summer ( $20.9 \pm$   
237  $9.7 \%$ ) ( $p < 0.0001$ ) (Figure S3). Generally, at higher taxonomic ranks (phylum-class), epiphytic  
238 and ambient seawater microbial communities were composed of similar bacterial taxa. Ambient  
239 seawater communities were mainly comprised of *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*,  
240 *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota*. Communities associated with  
241 *C. nodosa* consisted additionally of *Planctomycetota* contributing more in summer 2018 than in  
242 other seasons. In addition, communities from mixed and monospecific *C. cylindracea* were similar  
243 and characterized by the same groups as ambient seawater and *C. nodosa* communities with the  
244 addition of *Desulfobacterota* (Figure 4). Larger differences between environments and host species

245 were observed at lower taxonomic ranks (Figures 5 – 9).

246 *Cyanobacteria* related sequences comprised, on average,  $5.5 \pm 4.4$  % of total sequences  
247 (Figure 5). Higher proportions were found for *C. nodosa* ( $16.4 \pm 5.3$  %) and *C. cylindracea* mixed  
248 ( $7.7 \pm 3.9$  %) and monospecific ( $7.8 \pm 2.4$  %) associated communities in autumn ( $p < 0.0001$ )  
249 and for ambient seawater communities in winter ( $8.8 \pm 7.5$  %). Large taxonomic differences  
250 between surface associated and ambient seawater cyanobacterial communities were observed.  
251 Ambient seawater communities were mainly comprised of *Cyanobium* and *Synechococcus*, while  
252 surface associated communities were comprised of *Pleurocapsa* and sequences within the class  
253 *Cyanobacteriia* that could not be further classified (no relative *Cyanobacteriia*) (Figure 5). In  
254 addition, seasonal changes in surface associated cyanobacterial communities were observed with  
255 *Pleurocapsa* and no relative *Cyanobacteriia* comprising larger proportions of *Cyanobacteria* in  
256 autumn and winter and *Acrophormium*, *Phormidesmis* and sequences without known relatives  
257 within the *Nodosilineaceae* (no relative *Nodosilineaceae*) in spring and summer (Figure 5).

258 Sequences classified as *Bacteroidota* comprised, on average,  $19.2 \pm 5.5$  % of all sequences  
259 (Figure 6). Similar to *Cyanobacteria*, large differences in the taxonomic composition between  
260 ambient seawater and surface associated communities were found (Figure 6). The ambient seawater  
261 community was characterized by the NS4 and NS5 marine groups, uncultured *Cryomorphaceae*,  
262 uncultured *Flavobacteriaceae*, NS11-12 marine group, *Balneola*, uncultured *Balneolaceae* and  
263 *Formosa*. In contrast, in surface associated communities *Lewinella*, *Portibacter*, *Rubidimonas*,  
264 sequences without known relatives within the *Saprospiraceae* (no relative *Saprospiraceae*),  
265 uncultured *Saprospiraceae*, sequences without known relatives within the *Flavobacteriaceae*  
266 (no relative *Flavobacteriaceae*) and uncultured *Rhodothermaceae* were found. Some groups  
267 showed minor seasonal changes such as no relative *Flavobacteriaceae* whose sequences were  
268 more abundant from November 2017 until June 2018. In contrast, uncultured *Rhodothermaceae*  
269 showed higher proportions from June 2018 until the end of the study period. Surface associated  
270 *Bacteroidota* communities were very diverse as observed in the high proportion of taxa clustering

271 as other *Bacteroidota* (Figure 6).

272 On average, *Alphaproteobacteria* were in comparison to the other high rank taxa the largest  
273 taxonomic group, comprising  $29.2 \pm 12.0$  % of all sequences (Figure 7). In accordance to  
274 the above described taxa, large differences between ambient seawater and surface associated  
275 communities were observed. Ambient seawater communities were composed mainly of the SAR11  
276 clade, AEGEAN-169 marine group, SAR116 clade, sequences without known relatives within  
277 the *Rhodobacteraceae* (no relative *Rhodobacteraceae*), HIMB11 and the OCS116 clade, while  
278 surface associated communities were composed mainly of no relative *Rhodobacteraceae* and to  
279 a lesser degree of *Pseudoahrensia*, *Amylibacter* and sequences without known relatives within  
280 the *Alphaproteobacteria* (no relative *Alphaproteobacteria*) and *Hyphomonadaceae* (no relative  
281 *Hyphomonadaceae*). Representatives of no relative *Rhodobacteraceae* comprised on average 54.7  
282  $\pm 11.5$  % of all alphaproteobacterial sequences in the epiphytic community (Figure 7). In addition,  
283 *Amylibacter* was detected mainly in *C. nodosa* from November 2017 until March 2018.

284 Sequences related to *Gammaproteobacteria* comprised on average  $18.6 \pm 3.9$  % of all  
285 sequences (Figure 8). Similar to above mentioned taxa, large taxonomic differences between  
286 ambient seawater and surface associated communities were found. Ambient seawater communities  
287 were mainly comprised of the OM60 (NOR5) clade, *Litoricola*, *Acinetobacter* and the SAR86  
288 clade, while epiphytic communities were mainly composed of sequences without known relatives  
289 within the *Gammaproteobacteria* (no relative *Gammaproteobacteria*) and *Granulosicoccus*. Beside  
290 these two groups specific to all three epiphytic communities, *C. nodosa* was characterized by  
291 *Arenicella*, *Methylotenera* and sequences without known relatives within the *Burkholderiales* (no  
292 relative *Burkholderiales*), while *Thioploca*, *Reinekea* and sequences without known relatives within  
293 *Cellvibrionaceae* (no relative *Cellvibrionaceae*) were more specific to both mixed and monospecific  
294 *C. cylindracea*. In addition, *Arenicella* was more pronounced in November and December 2017,  
295 while no relative *Burkholderiales* and *Methylotenera* were characteristic for the period from March  
296 until May 2018. For the *C. cylindracea* specific taxa no relative *Cellvibrionaceae* and *Reinekea*

297 showed seasonality and were characteristic for samples originating from June to October 2018.  
298 In addition, similar to *Bacteroidota*, a large proportion of the surface associated community was  
299 grouped as other *Gammaproteobacteria* indicating high diversity within this group (Figure 8).

300 *Desulfobacterota* were specific for *C. cylindracea*. In the mixed and monospecific *C.*  
301 *cylindracea* communities the proportion of *Desulfobacterota* was  $25.7 \pm 11.2\%$  and  $24.0 \pm$   
302  $4.3\%$ , respectively (Figure 9). In contrast, in ambient seawater and *C. nodosa* communities  
303 the contribution of *Desulfobacterota* was only  $0.1 \pm 0.08\%$  and  $1.0 \pm 0.7\%$ , respectively. In  
304 *C. cylindracea* the community consisted mainly of *Desulfatitalea*, *Desulfobulbus*, *Desulfopila*,  
305 *Desulforhopalus*, *Desulfotalea*, SEEP-SRB4, uncultured *Desulfocapsaceae* and sequences without  
306 known relatives within the *Desulfobacteraceae* (no relative *Desulfobacteraceae*), *Desulfobulbaceae*  
307 (no relative *Desulfobulbaceae*) and *Desulfocapsaceae* (no relative *Desulfocapsaceae*) (Figure 9).

308 **Discussion**

309 In the present study, we applied a selective epiphytic DNA isolation procedure based on  
310 direct cellular lysis (Korlević et al., 2021) coupled with a monthly sampling and Illumina amplicon  
311 sequencing to describe in detail the bacterial and archaeal communities associated with the surfaces  
312 of two marine macrophytes, *C. nodosa* and *C. cylindracea*. Highest richness was observed for *C.*  
313 *cylindracea* (mixed and monospecific) followed by *C. nodosa* and lowest richness was found in  
314 ambient seawater microbial communities. Higher richness of microbial communities associated with  
315 macrophytes than in ambient seawater has been described earlier (Mancuso et al., 2016; Ugarelli et  
316 al., 2019) and could be attributed to a larger set of inhabitable microniches existing on macrophyte  
317 surfaces than in the ambient seawater. The highest richness observed for *C. cylindracea* might be  
318 partly due to its contact with the sediment. The stolon of *C. cylindracea* is attached to the sediment  
319 surface with rhizoids and thus, the stolon and rhizoids are in direct contact with the sediment.  
320 Also, studies have shown that the presence of *C. cylindracea* can alter the content and biochemical  
321 composition of sedimentary organic matter (Pusceddu et al., 2016; Rizzo et al., 2017, 2020) possibly  
322 further expanding the number of inhabitable microniches and thus causing the observed increase in  
323 richness. Seasonal differences in richness observed for surface attached communities indicated a  
324 slightly higher richness in spring and summer. This pattern could be explained by a more intense  
325 macrophyte growth in these two seasons than in autumn and winter (Zavodnik et al., 1998; Ruitton et  
326 al., 2005; Najdek et al., 2020). During their main growth season in spring and summer macrophytes  
327 exhibit a more dynamic chemical interaction with the surface community probably causing an  
328 increase in the number of inhabitable microniches (Borges and Champenois, 2015; Rickert et al.,  
329 2016). Proportions of shared epiphytic OTUs between consecutive sampling points were low also  
330 indicated by the proportion of OTUs ( $\leq 1.0\%$ ) present at every sampling date (Figure 3). These  
331 persistent OTUs, however, accounted for a high proportion of sequences ( $\geq 40.2\%$ ), as is often  
332 the case with similar high-frequency sampling studies (Gilbert et al., 2009; Gilbert et al., 2012).  
333 In comparison to the seawater community, higher values of shared OTUs between consecutive

334 sampling points were observed for the macrophyte surface associated communities. It appears that  
335 macrophyte surfaces are providing more stable conditions than the ambient seawater.

336 We observed a strong differentiation between the surface attached and ambient seawater  
337 communities at the level of OTUs which is in agreement with most published studies (Burke et  
338 al., 2011b; Michelou et al., 2013; Mancuso et al., 2016; Roth-Schulze et al., 2016; Crump et al.,  
339 2018; Ugarelli et al., 2019; Sanders-Smith et al., 2020). This indicates that marine macrophytes  
340 are selecting microorganisms from the pool of microbial taxa present in the ambient seawater,  
341 modifying the microbial community once the macrophyte associated microbial biofilm develops  
342 (Salaün et al., 2012; Michelou et al., 2013). In addition, similar to the study of Roth-Schulze  
343 et al. (2016) seagrass and macroalgae specific microbial communities were identified, while no  
344 difference between *C. cylindracea* settlements was observed indicating that seagrass and macroalgae  
345 specific metabolism is involved in the selection and development of the associated biofilm. At the  
346 level of OTUs seasonal changes of *C. nodosa* and *C. cylindracea* associated communities were  
347 identified that could be linked to the growth cycle of the seagrass and macroalgae (Agostini et al.,  
348 2003; Najdek et al., 2020). *C. nodosa* was characterized by a spring community during maximum  
349 seagrass proliferation, a summer community during the highest standing stock of *C. nodosa* and an  
350 autumn/winter community during the decay of seagrass biomass. In contrast, *C. cylindracea* started  
351 to proliferate in late spring and was characterized only by a summer community during high growth  
352 rates and by an autumn/winter/spring community when the biomass was at the peak and decaying  
353 thereafter. Similar seasonal changes in the epiphytic community have also been described for other  
354 macroalgae (Tujula et al., 2010; Lachnit et al., 2011).

355 The taxonomic analysis showed higher chloroplast sequence abundances in autumn/winter  
356 than in spring/summer. This pattern is not surprising as seagrasses harbour more algal epiphytes  
357 during autumn/winter than in spring/summer (Reyes and Sansón, 2001). Furthermore, we used  
358 an adapted DNA isolation protocol that is known to partially co-extract DNA from planktonic  
359 eukaryotes (Korlević et al., 2015). In general, the taxonomic analysis identified epiphytic

360 phylogenetic groups present throughout the year comprising most of the reads, and taxa present  
361 in lower proportions showing seasonal patterns. The first group was comprised of members  
362 of the *Bacteroidota* family *Saprospiraceae*, the alphaproteobacterial *Rhodobacteraceae* and  
363 *Hyphomonadaceae*, the gammaproteobacterial genus *Granulosicoccus*, sequences without known  
364 relatives within *Gammaproteobacteria* and various taxa within *Desulfobacterota* (Figures 6 – 9).  
365 All these groups were found on all host species, with the exception of *Desulfobacterota* that  
366 was characteristic for *C. cylindracea*. In addition, the persistence of *Rhodobacteraceae* in the  
367 case of *C. nodosa* and *Desulfobacterota* in the case of *C. cylindracea* could be observed in the  
368 taxonomic classification of OTUs present at every sampling date. Within the *Bacteroidota* different  
369 groups within *Saprospiraceae* (e.g. *Lewinella*, *Portibacter* and *Rubidimonas*) were identified to be  
370 persistent. It has been suggested that members of this family are important in the hydrolysis and  
371 utilization of complex organic sources (McIlroy and Nielsen, 2014). Surface attached life style  
372 would be beneficial to these microbes as they could thrive on products of host cellular breakdown  
373 or by-products of host metabolism, so it not surprising that they are often found associated with  
374 macrophyte surfaces (Burke et al., 2011b; McIlroy and Nielsen, 2014; Crump et al., 2018).  
375 *Rhodobacteraceae* are often detected on macrophyte surfaces and usually are one of the most  
376 abundant groups (Burke et al., 2011b; Michelou et al., 2013; Mancuso et al., 2016). The functional  
377 association between macrophytes and members of this groups is difficult to assess based on 16S  
378 rRNA analysis as this family is phenotypically, metabolically, and ecologically very diverse (Pujalte  
379 et al., 2014). However, some interesting metabolic capacities linked to this group were described.  
380 Genomic analysis of *Rhodobacteraceae* strains and metatranscriptomic sequencing of seagrass  
381 microbiomes revealed the potential for biosynthesis of indole-3-acetic acid (IAA), a plant hormone  
382 (Simon et al., 2017), indicating a possible intake by seagrasses. However, another study found no  
383 effect of IAA on *C. nodosa* growth showing the complexity of macrophyte–microbes interactions  
384 (Muñoz, 1995). Another persistent alphaproteobacterial family was the *Hyphomonadaceae*, a group  
385 that contain species with stalks used to attach cells to different surfaces (Abraham and Rohde,  
386 2014). This group has been previously associated with seagrass surfaces (Weidner et al., 2000)

387 and it is believed that possessing stalks could be an advantage to keep the cells in the proximity of  
388 exudate excreted by the host (Weidner et al., 2000; Abraham and Rohde, 2014).

389 Within the *Gammaproteobacteria*, sequences without known representatives were the most  
390 pronounced group present throughout the year. *Gammaproteobacteria* are often a major constituent  
391 of macrophyte epiphytic communities (Burke et al., 2011b; Michelou et al., 2013; Crump et al.,  
392 2018). A study has attributed the expression of enzymes for the degradation of galactose-based  
393 algal polymers to this class indicating their possible involvement into epibiotic algal biofilm control  
394 (Crump et al., 2018). In addition, *Granulosicoccus* was also found in almost all samples. A species  
395 of this genus has been isolated from the leaf surface of the seagrass *Zostera marina* (Kurilenko et al.,  
396 2010), while sequences related to this genus have been found on the surfaces of macroalgae (Lachnit  
397 et al., 2011; Bengtsson et al., 2012), including *C. cylindracea* (Rizzo et al., 2016a), indicating this  
398 group preference for macrophyte surfaces. It is possible that bacteria of this genus can thrive on  
399 exudates of different macrophytes as it is known from cultivated members that they can utilise  
400 various sugars and amino acids (Ivanova and Webb, 2014). The presence of *Desulfobacterota* only  
401 on *C. cylindracea* is to be expected as part of the epiphytic community is in direct contact with the  
402 sediment. The *Desulfobacterota* community was comprised of known sulphate sediment groups  
403 such as the *Desulfatitalea* and no relative *Desulfocapsaceae* (Kuever, 2014; Higashioka et al., 2015).  
404 Sequences related to sulphur cycling bacterial groups have been found in *Caulerpa* endophytic  
405 and epiphytic communities (Aires et al., 2013). It is possible that these groups are involved into  
406 enhanced sulphate reduction rates observed in sediments underlying *Caulerpa* settlements causing  
407 unsuitable conditions to sulphide-sensitive seagrasses (Holmer et al., 2009).

408 The only high rank taxonomic group showing strong seasonal fluctuations was *Cyanobacteria*.  
409 Cyanobacterial sequences were more pronounced in November and December than in spring and  
410 summer. In the months of high cyanobacterial sequence abundances the majority of sequences from  
411 this group were classified as *Pleurocapsa*, a group known to colonize different living and non-living  
412 surfaces (Burns et al., 2004; Longford et al., 2007; Mobberley et al., 2012; Reisser et al., 2014;

413 Kolda et al., 2020). While we observed a strong temporal pattern for this group, a study of surface  
414 sediment cyanobacterial communities did not find any seasonal dynamics for *Pleurocapsa* (Kolda  
415 et al., 2020), indicating a possibility that there is a reduced selection of the epiphytic community  
416 by the seagrass during periods of low photosynthetic activity (Zavodnik et al., 1998), causing  
417 leaves to become a suitable surface for non-specific colonizers. Beside all these groups comprising  
418 most of the sequences, a set of taxa present in lower proportions and showing seasonal patterns  
419 was identified. This group was comprised of e.g. *Bacteroidota* sequences without known relatives  
420 within *Flavobacteriaceae* and *Rhodothermaceae*, the alphaproteobacterial *Amylibacter* and the  
421 gammaproteobacterial *Methylotenera*, *Reinekea* and sequences without known relatives within  
422 *Cellvibrionaceae* (Figures 6 and 8).

423 It is possible that *Flavobacteriaceae* and *Rhodothermaceae* are occupying similar niches  
424 with *Rhodothermaceae* being more adapted to higher temperatures as it is known that culturable  
425 members of this family exhibit mesophilic and thermophilic characteristics (Park et al., 2014).  
426 This would explain why we observed a higher presence of *Rhodothermaceae* in the warmer period  
427 of the year. A strain belonging to the *Rhodobacteraceae* genus *Amylibacter* has been isolated  
428 from the surface of a green macroalga indicating that members of this group can exhibit surface  
429 attached life style (Nedashkovskaya et al., 2016). In addition, since this is a relatively novel genus  
430 it is possible that novel taxa within *Rhodobacteraceae* will be described in the future elucidating  
431 the taxonomy of the currently high proportion of *Rhodobacteraceae* sequences without known  
432 relatives. The genus *Methylotenera* belongs to the methylotrophic family *Methylophilaceae*, a group  
433 capable of oxidising non-methane single-carbon compounds such as methanol and methylamine  
434 (Chistoserdova and Kalyuzhnaya, 2018). Interestingly, angiosperms produce methanol during  
435 cell-wall synthesis (Nemecek-Marshall et al., 1995; Dorokhov et al., 2018), so it is not surprising  
436 that we found members of this genus only on *C. nodosa* and in spring, during a period of maximum  
437 seagrass proliferation. Other studies have also found *Methylotenera*-specific sequences associated  
438 with seagrass roots and leaves indicating that this group members are important constituents of the  
439 seagrass microbiome (Crump et al., 2018; Sanders-Smith et al., 2020). Genomic and physiological

440 analyses of cultivated *Cellvibrionaceae* and *Reinekea* members showed the capabilities to use  
441 important algal polysaccharides (Avci et al., 2017; Xie et al., 2017) indicating their possible  
442 involvement into the degradation of *C. cylindracea* polysaccharides and/or the control of its algal  
443 epiphytes.

444 The epiphytic microbial community associated with marine macrophytes is undergoing  
445 seasonal changes that can be attributed to the fluctuations of environmental conditions, the growth  
446 cycle of macrophytes inhabiting temperate zones or the combined effect of both. In the present  
447 study, we could identify in analysed high rank taxa phylogenetic groups present throughout the  
448 year, comprising most of the sequences and a lower proportion of taxa showing seasonal patterns  
449 connected to the macrophyte growth cycle (Figures 4 and 9). Studies focusing on functional  
450 comparisons between communities associated with different hosts showed that the majority of  
451 functions could be found in every community, indicating functional redundancy (Roth-Schulze  
452 et al., 2016). This difference between phylogenetic variability and functional stability has been  
453 explained by the lottery hypothesis assuming an initial random colonization step performed by a  
454 set of functionally equivalent taxonomic groups (Burke et al., 2011a; Roth-Schulze et al., 2016).  
455 It is possible that functional redundancy is a characteristic of high abundance taxa detected to be  
456 present throughout the year, while seasonal and/or host-specific functions are an attribute of taxa  
457 displaying successional patterns. Further studies connecting taxonomy with functional properties  
458 will be required to elucidate the degree of functional redundancy or specificity in epiphytic microbial  
459 communities.

460 **Acknowledgments**

461 This work was funded by the Croatian Science Foundation through the MICRO-SEAGRASS  
462 project (project number IP-2016-06-7118). ZZ and GJH were supported by the Austrian Science  
463 Fund (FWF) project ARTEMIS (project number P28781-B21). We would like to thank Margareta  
464 Buterer for technical support, Paolo Paliaga for help during sampling and the University Computing  
465 Center of the University of Zagreb for access to the computer cluster Isabella.

466 **References**

- 467 Abraham, W. R., and Rohde, M. (2014). “The family *Hyphomonadaceae*,” in *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*, eds. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (Berlin, Heidelberg: Springer-Verlag), 283–299. doi:10.1007/978-3-642-30197-1\_260.
- 471 Agostini, S., Pergent, G., and Marchand, B. (2003). Growth and primary production of *Cymodocea nodosa* in a coastal lagoon. *Aquat. Bot.* 76, 185–193. doi:10.1016/S0304-3770(03)00049-4.
- 473 Aires, T., Moalic, Y., Serrao, E. A., and Arnaud-Haond, S. (2015). Hologenome theory supported by 474 cooccurrence networks of species-specific bacterial communities in siphonous algae (*Caulerpa*). *FEMS Microbiol. Ecol.* 91, fiv067. doi:10.1093/femsec/fiv067.
- 476 Aires, T., Serrão, E. A., Kendrick, G., Duarte, C. M., and Arnaud-Haond, S. (2013). Invasion 477 is a community affair: Clandestine followers in the bacterial community associated 478 to green algae, *Caulerpa racemosa*, track the invasion source. *PLoS One* 8, e68429. 479 doi:10.1371/journal.pone.0068429.
- 480 Allaire, J. J., Xie, Y., McPherson, J., Luraschi, J., Ushey, K., Atkins, A., et al. (2019). *rmardown: 481 Dynamic documents for R*.
- 482 Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 483 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 484 75, 129–137. doi:10.3354/ame01753.
- 485 Armstrong, E., Rogerson, A., and Leftley, J. W. (2000). The abundance of heterotrophic 486 protists associated with intertidal seaweeds. *Estuar. Coast. Shelf Sci.* 50, 415–424. 487 doi:10.1006/ECSS.1999.0577.

- 488 Avci, B., Hahnke, R. L., Chafee, M., Fischer, T., Gruber-Vodicka, H., Tegetmeyer, H. E., et  
489 al. (2017). Genomic and physiological analyses of ‘*Reinekea forsetii*’ reveal a versatile  
490 opportunistic lifestyle during spring algae blooms. *Environ. Microbiol.* 19, 1209–1221.  
491 doi:10.1111/1462-2920.13646.
- 492 Bengtsson, M. M., Sjøtun, K., Lanzén, A., and Øvreås, L. (2012). Bacterial diversity in relation to  
493 secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. *ISME J.* 6,  
494 2188–2198. doi:10.1038/ismej.2012.67.
- 495 Bengtsson, M., Sjøtun, K., and Øvreås, L. (2010). Seasonal dynamics of bacterial biofilms on the  
496 kelp *Laminaria hyperborea*. *Aquat. Microb. Ecol.* 60, 71–83. doi:10.3354/ame01409.
- 497 Borcard, D., Gillet, F., and Legendre, P. (2011). *Numerical ecology with R*. 1st ed. New York:  
498 Springer-Verlag doi:10.1007/978-1-4419-7976-6.
- 499 Borges, A. V., and Champenois, W. (2015). Seasonal and spatial variability of dimethylsulfoniopropionate  
500 (DMSP) in the Mediterranean seagrass *Posidonia oceanica*. *Aquat. Bot.* 125, 72–79.  
501 doi:10.1016/j.aquabot.2015.05.008.
- 502 Boudouresque, C. F., Bernard, G., Pergent, G., Shili, A., and Verlaque, M. (2009). Regression  
503 of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and  
504 stress: A critical review. *Botanica Marina* 52, 395–418. doi:10.1515/BOT.2009.057.
- 505 Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011a). Bacterial community  
506 assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci. U. S. A.* 108,  
507 14288–14293. doi:10.1073/pnas.1101591108.
- 508 Burke, C., Thomas, T., Lewis, M., Steinberg, P., and Kjelleberg, S. (2011b). Composition,  
509 uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*.  
510 *ISME J.* 5, 590–600. doi:10.1038/ismej.2010.164.

- 511 Burns, B. P., Goh, F., Allen, M., and Neilan, B. A. (2004). Microbial diversity of extant stromatolites  
512 in the hypersaline marine environment of Shark Bay, Australia. *Environ. Microbiol.* 6,  
513 1096–1101. doi:10.1111/j.1462-2920.2004.00651.x.
- 514 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al.  
515 (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq  
516 platforms. *ISME J.* 6, 1621–1624. doi:10.1038/ismej.2012.8.
- 517 Celdrán, D., Espinosa, E., Sánchez-Amat, A., and Marín, A. (2012). Effects of epibiotic bacteria  
518 on leaf growth and epiphytes of the seagrass *Posidonia oceanica*. *Mar. Ecol. Prog. Ser.* 456,  
519 21–27. doi:10.3354/meps09672.
- 520 Chistoserdova, L., and Kalyuzhnaya, M. G. (2018). Current trends in methylotrophy. *Trends  
521 Microbiol.* 26, 703–714. doi:10.1016/j.tim.2018.01.011.
- 522 Crump, B. C., and Koch, E. W. (2008). Attached bacterial populations shared by four species of  
523 aquatic angiosperms. *Appl. Environ. Microbiol.* 74, 5948–5957. doi:10.1128/AEM.00952-08.
- 524 Crump, B. C., Wojahn, J. M., Tomas, F., and Mueller, R. S. (2018). Metatranscriptomics and  
525 amplicon sequencing reveal mutualisms in seagrass microbiomes. *Front. Microbiol.* 9, 388.  
526 doi:10.3389/fmicb.2018.00388.
- 527 Cúcio, C., Engelen, A. H., Costa, R., and Muyzer, G. (2016). Rhizosphere microbiomes of european  
528 seagrasses are selected by the plant, but are not species specific. *Front. Microbiol.* 7, 440.  
529 doi:10.3389/fmicb.2016.00440.
- 530 Cúcio, C., Overmars, L., Engelen, A. H., and Muyzer, G. (2018). Metagenomic analysis shows  
531 the presence of bacteria related to free-living forms of sulfur-oxidizing chemolithoautotrophic  
532 symbionts in the rhizosphere of the seagrass *Zostera marina*. *Front. Mar. Sci.* 5, 171.  
533 doi:10.3389/fmars.2018.00171.

- 534 de Oliveira, L. S., Gregoracci, G. B., Silva, G. G. Z., Salgado, L. T., Filho, G. A.,  
535 Alves-Ferreira, M., et al. (2012). Transcriptomic analysis of the red seaweed *Laurencia*  
536 *dendroidea* (Florideophyceae, Rhodophyta) and its microbiome. *BMC Genomics* 13, 487.  
537 doi:10.1186/1471-2164-13-487.
- 538 Dobretsov, S. V., and Qian, P.-Y. (2002). Effect of bacteria associated with the green  
539 alga *Ulva reticulata* on marine micro- and macrofouling. *Biofouling* 18, 217–228.  
540 doi:10.1080/08927010290013026.
- 541 Dorokhov, Y. L., Sheshukova, E. V., and Komarova, T. V. (2018). Methanol in plant life. *Front.*  
542 *Plant Sci.* 9, 1623. doi:10.3389/fpls.2018.01623.
- 543 Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., and Thomas, T. (2013). The seaweed  
544 holobiont: Understanding seaweed-bacteria interactions. *FEMS Microbiol. Rev.* 37, 462–476.  
545 doi:10.1111/1574-6976.12011.
- 546 Ettinger, C. L., and Eisen, J. A. (2020). Fungi, bacteria and oomycota opportunistically isolated  
547 from the seagrass, *Zostera marina*. *PLoS One* 15, e0236135. doi:10.1371/journal.pone.0236135.
- 548 Gilbert, J. A., Field, D., Swift, P., Newbold, L., Oliver, A., Smyth, T., et al. (2009). The seasonal  
549 structure of microbial communities in the Western English Channel. *Environ. Microbiol.* 11,  
550 3132–3139. doi:10.1111/j.1462-2920.2009.02017.x.
- 551 Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., et al.  
552 (2012). Defining seasonal marine microbial community dynamics. *ISME J.* 6, 298–308.  
553 doi:10.1038/ismej.2011.107.
- 554 Higashioka, Y., Kojima, H., Watanabe, T., and Fukui, M. (2015). Draft genome  
555 sequence of *Desulfatitalea tepidiphila* S28bF<sup>T</sup>. *Genome Announc.* 3, e01326–15.  
556 doi:10.1128/genomeA.01326-15.

- 557 Hollants, J., Leliaert, F., De Clerck, O., and Willems, A. (2013). What we can learn from  
558 sushi: A review on seaweed-bacterial associations. *FEMS Microbiol. Ecol.* 83, 1–16.  
559 doi:10.1111/j.1574-6941.2012.01446.x.
- 560 Holmer, M., Marbà, N., Lamote, M., and Duarte, C. M. (2009). Deterioration of sediment quality  
561 in seagrass meadows (*Posidonia oceanica*) invaded by macroalgae (*Caulerpa* sp.). *Estuaries*  
562 *Coast* 32, 456–466. doi:10.1007/s12237-009-9133-4.
- 563 Ivanova, E. P., and Webb, H. K. (2014). “The family *Granulosicoccaceae*,” in *The*  
564 *Prokaryotes: Gammaproteobacteria*, eds. E. Rosenberg, E. F. DeLong, S. Lory,  
565 E. Stackebrandt, and F. Thompson (Berlin, Heidelberg: Springer-Verlag), 315–317.  
566 doi:10.1007/978-3-642-38922-1\_247.
- 567 Jost, L. (2006). Entropy and diversity. *Oikos* 113, 363–375. doi:10.1111/j.2006.0030-1299.14714.x.
- 568 Klein, J., and Verlaque, M. (2008). The *Caulerpa racemosa* invasion: A critical review. *Mar. Pollut.*  
569 *Bull.* 56, 205–225. doi:10.1016/j.marpolbul.2007.09.043.
- 570 Kolda, A., Ljubešić, Z., Gavrilović, A., Jug-Dujaković, J., Pikelj, K., and Kapetanović, D. (2020).  
571 Metabarcoding *Cyanobacteria* in coastal waters and sediment in central and southern Adriatic  
572 Sea. *Acta Bot. Croat.* 79, 157–169. doi:10.37427/botcro-2020-021.
- 573 Korlević, M., Markovski, M., Zhao, Z., Herndl, G. J., and Najdek, M. (2021). Selective DNA  
574 and protein isolation from marine macrophyte surfaces. *Front. Microbiol.* 12, 665999.  
575 doi:10.3389/fmicb.2021.665999.
- 576 Korlević, M., Pop Ristova, P., Garić, R., Amann, R., and Orlić, S. (2015). Bacterial diversity in the  
577 South Adriatic Sea during a strong, deep winter convection year. *Appl. Environ. Microbiol.* 81,  
578 1715–1726. doi:10.1128/AEM.03410-14.

- 579 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013).  
580 Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon  
581 sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79,  
582 5112–5120. doi:10.1128/AEM.01043-13.
- 583 Kuever, J. (2014). “The family *Desulfobulbaceae*,” in *The Prokaryotes: Deltaproteobacteria and*  
584 *Epsilonproteobacteria*, eds. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F.  
585 Thompson (Berlin, Heidelberg: Springer-Verlag), 75–86. doi:10.1007/978-3-642-39044-9\_267.
- 586 Kurilenko, V. V., Christen, R., Zhukova, N. V., Kalinovskaya, N. I., Mikhailov, V. V., Crawford, R.  
587 J., et al. (2010). *Granulosicoccus coccooides* sp. nov., isolated from leaves of seagrass (*Zostera*  
588 *marina*). *Int. J. Syst. Evol. Microbiol.* 60, 972–976. doi:10.1099/ijss.0.013516-0.
- 589 Küsel, K., Trinkwalter, T., Drake, H. L., and Devereux, R. (2006). Comparative evaluation  
590 of anaerobic bacterial communities associated with roots of submerged macrophytes  
591 growing in marine or brackish water sediments. *J. Exp. Mar. Biol. Ecol.* 337, 49–58.  
592 doi:10.1016/j.jembe.2006.06.004.
- 593 Lachnit, T., Blümel, M., Imhoff, J. F., and Wahl, M. (2009). Specific epibacterial communities  
594 on macroalgae: Phylogeny matters more than habitat. *Aquat. Biol.* 5, 181–186.  
595 doi:10.3354/ab00149.
- 596 Lachnit, T., Meske, D., Wahl, M., Harder, T., and Schmitz, R. (2011). Epibacterial community  
597 patterns on marine macroalgae are host-specific but temporally variable. *Environ. Microbiol.*  
598 13, 655–665. doi:10.1111/j.1462-2920.2010.02371.x.
- 599 Legendre, P., and Legendre, L. (2012). *Numerical ecology*. 3rd ed. Amsterdam: Elsevier.
- 600 Longford, S., Tujula, N., Crocetti, G., Holmes, A., Holmström, C., Kjelleberg, S., et al. (2007).  
601 Comparisons of diversity of bacterial communities associated with three sessile marine  
602 eukaryotes. *Aquat. Microb. Ecol.* 48, 217–229. doi:10.3354/ame048217.

- 603 Mancuso, F. P., D'Hondt, S., Willems, A., Airoldi, L., and De Clerck, O. (2016). Diversity and  
604 temporal dynamics of the epiphytic bacterial communities associated with the canopy-forming  
605 seaweed *Cystoseira compressa* (Esper) Gerloff and Nizamuddin. *Front. Microbiol.* 7, 476.  
606 doi:10.3389/fmicb.2016.00476.
- 607 Margulis, L. (1991). "Symbiogenesis and symbioticism," in *Symbiosis as a Source of Evolutionary  
608 Innovation: Speciation and Morphogenesis*, eds. L. Margulis and R. Fester (Cambridge,  
609 Massachusetts: The MIT Press), 1–14.
- 610 Massana, R., Murray, A. E., Preston, C. M., and DeLong, E. F. (1997). Vertical distribution and  
611 phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. *Appl.  
612 Environ. Microbiol.* 63, 50–56.
- 613 Matsuo, Y., Suzuki, M., Kasai, H., Shizuri, Y., and Harayama, S. (2003). Isolation and phylogenetic  
614 characterization of bacteria capable of inducing differentiation in the green alga *Monostroma  
615 oxyspermum*. *Environ. Microbiol.* 5, 25–35. doi:10.1046/j.1462-2920.2003.00382.x.
- 616 McIlroy, S. J., and Nielsen, P. H. (2014). "The family *Saprosiraceae*," in *The Prokaryotes: Other Major Lineages of Bacteria and the Archaea*, eds. E. Rosenberg, E. F. DeLong, S.  
617 Lory, E. Stackebrandt, and F. Thompson (Berlin, Heidelberg: Springer-Verlag), 863–889.  
618 doi:10.1007/978-3-642-38954-2\_138.
- 620 Michelou, V. K., Caporaso, J. G., Knight, R., and Palumbi, S. R. (2013). The ecology  
621 of microbial communities associated with *Macrocystis pyrifera*. *PLoS One* 8, e67480.  
622 doi:10.1371/journal.pone.0067480.
- 623 Miranda, L. N., Hutchison, K., Grossman, A. R., and Brawley, S. H. (2013). Diversity and  
624 abundance of the bacterial community of the red macroalga *Porphyra umbilicalis*: Did bacterial  
625 farmers produce macroalgae? *PLoS One* 8, e58269. doi:10.1371/journal.pone.0058269.

- 626 Mobberley, J. M., Ortega, M. C., and Foster, J. S. (2012). Comparative microbial diversity analyses  
627 of modern marine thrombolitic mats by barcoded pyrosequencing. *Environ. Microbiol.* 14,  
628 82–100. doi:10.1111/j.1462-2920.2011.02509.x.
- 629 Morrissey, K. L., Çavas, L., Willems, A., and De Clerck, O. (2019). Disentangling the influence of  
630 environment, host specificity and thallus differentiation on bacterial communities in siphonous  
631 green seaweeds. *Front. Microbiol.* 10, 717. doi:10.3389/fmicb.2019.00717.
- 632 Muñoz, J. T. (1995). Effects of some plant growth regulators on the growth of  
633 the seagrass *Cymodocea nodosa* (Ucria) Ascherson. *Aquat. Bot.* 51, 311–318.  
634 doi:10.1016/0304-3770(95)00481-E.
- 635 Najdek, M., Korlević, M., Paliaga, P., Markovski, M., Ivančić, I., Iveša, L., et al. (2020). Effects of  
636 the invasion of *Caulerpa cylindracea* in a *Cymodocea nodosa* meadow in the Northern Adriatic  
637 Sea. *Front. Mar. Sci.* 7, 602055. doi:10.3389/fmars.2020.602055.
- 638 Nedashkovskaya, O. I., Kukhlevskiy, A. D., Zhukova, N. V., and Kim, S. B. (2016). *Amylibacter*  
639 *ulvae* sp. nov., a new alphaproteobacterium isolated from the Pacific green alga *Ulva fenestrata*.  
640 *Arch. Microbiol.* 198, 251–256. doi:10.1007/s00203-015-1185-1.
- 641 Nemecek-Marshall, M., MacDonald, R. C., Franzen, J. J., Wojciechowski, C. L., and Fall, R. (1995).  
642 Methanol emission from leaves (enzymatic detection of gas-phase methanol and relation of  
643 methanol fluxes to stomatal conductance and leaf development). *Plant Physiol.* 108, 1359–1368.  
644 doi:10.1104/pp.108.4.1359.
- 645 Neuwirth, E. (2014). *RColorBrewer: ColorBrewer palettes*.
- 646 Nielsen, L. B., Finster, K., Welsh, D. T., Donelly, A., Herbert, R. A., de Wit, R., et al. (2001).  
647 Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments  
648 from *Zostera noltii* and *Spartina maritima* meadows. *Environ. Microbiol.* 3, 63–71.  
649 doi:doi.org/10.1046/j.1462-2920.2001.00160.x.

- 650 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019).  
651 *vegan: Community ecology package*.
- 652 Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: Assessing small  
653 subunit rRNA primers for marine microbiomes with mock communities, time series and global  
654 field samples. *Environ. Microbiol.* 18, 1403–1414. doi:10.1111/1462-2920.13023.
- 655 Park, S., Akira, Y., and Kogure, K. (2014). “The family *Rhodothermaceae*,” in *The Prokaryotes: Other Major Lineages of Bacteria and the Archaea*, eds. E. Rosenberg, E. F. DeLong, S.  
656 Lory, E. Stackebrandt, and F. Thompson (Berlin, Heidelberg: Springer-Verlag), 849–856.  
657 doi:10.1007/978-3-642-38954-2\_141.
- 658
- 659 Penesyan, A., Marshall-Jones, Z., Holmstrom, C., Kjelleberg, S., and Egan, S. (2009). Antimicrobial  
660 activity observed among cultured marine epiphytic bacteria reflects their potential as a source of  
661 new drugs. *FEMS Microbiol. Ecol.* 69, 113–124. doi:10.1111/j.1574-6941.2009.00688.x.
- 662 Pujalte, M. J., Lucena, T., Ruvira, M. A., Arahal, D. R., and Macián, M. C. (2014). “The family  
663 *Rhodobacteraceae*,” in *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*, eds.  
664 E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (Berlin, Heidelberg:  
665 Springer-Verlag), 439–512. doi:10.1007/978-3-642-30197-1\_377.
- 666 Pusceddu, A., Fraschetti, S., Scopa, M., Rizzo, L., and Danovaro, R. (2016). Meiofauna  
667 communities, nematode diversity and C degradation rates in seagrass (*Posidonia oceanica* L.)  
668 and unvegetated sediments invaded by the algae *Caulerpa cylindracea* (Sonder). *Mar. Environ.  
669 Res.* 119, 88–99. doi:10.1016/j.marenvres.2016.05.015.
- 670 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA  
671 ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic  
672 Acids Res.* 41, D590–D596. doi:10.1093/nar/gks1219.

- 673 R Core Team (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R  
674 Foundation for Statistical Computing.
- 675 Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D. K. A., Thums, M., et al. (2014).  
676 Millimeter-sized marine plastics: A new pelagic habitat for microorganisms and invertebrates.  
677 *PLoS One* 9, e100289. doi:10.1371/journal.pone.0100289.
- 678 Reyes, J., and Sansón, M. (2001). Biomass and production of the epiphytes on the  
679 leaves of *Cymodocea nodosa* in the Canary Islands. *Botanica Marina* 44, 307–313.  
680 doi:10.1515/BOT.2001.039.
- 681 Rickert, E., Wahl, M., Link, H., Richter, H., and Pohnert, G. (2016). Seasonal variations in surface  
682 metabolite composition of *Fucus vesiculosus* and *Fucus serratus* from the Baltic Sea. *PLoS*  
683 *One* 11, e0168196. doi:10.1371/journal.pone.0168196.
- 684 Rizzo, L., Fraschetti, S., Alifano, P., Pizzolante, G., and Stabili, L. (2016a). The alien species  
685 *Caulerpa cylindracea* and its associated bacteria in the Mediterranean Sea. *Mar. Biol.* 163, 4.  
686 doi:10.1007/s00227-015-2775-9.
- 687 Rizzo, L., Fraschetti, S., Alifano, P., Tredici, M. S., and Stabili, L. (2016b). Association of *Vibrio*  
688 community with the Atlantic Mediterranean invasive alga *Caulerpa cylindracea*. *J. Exp. Mar.*  
689 *Biol. Ecol.* 475, 129–136. doi:10.1016/j.jembe.2015.11.013.
- 690 Rizzo, L., Pusceddu, A., Bianchelli, S., and Fraschetti, S. (2020). Potentially combined  
691 effect of the invasive seaweed *Caulerpa cylindracea* (Sonder) and sediment deposition  
692 rates on organic matter and meiofaunal assemblages. *Mar. Environ. Res.* 159, 104966.  
693 doi:10.1016/j.marenvres.2020.104966.
- 694 Rizzo, L., Pusceddu, A., Stabili, L., Alifano, P., and Fraschetti, S. (2017). Potential effects of an  
695 invasive seaweed (*Caulerpa cylindracea*, Sonder) on sedimentary organic matter and microbial  
696 metabolic activities. *Sci. Rep.* 7, 12113. doi:10.1038/s41598-017-12556-4.

- 697 Roth-Schulze, A. J., Zozaya-Valdés, E., Steinberg, P. D., and Thomas, T. (2016). Partitioning  
698 of functional and taxonomic diversity in surface-associated microbial communities. *Environ.*  
699 *Microbiol.* 18, 4391–4402. doi:10.1111/1462-2920.13325.
- 700 Ruitton, S., Verlaque, M., and Boudouresque, C. F. (2005). Seasonal changes of the introduced  
701 *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its  
702 Mediterranean range. *Aquat. Bot.* 82, 55–70. doi:10.1016/j.aquabot.2005.02.008.
- 703 Saha, M., Rempt, M., Grosser, K., Pohnert, G., and Weinberger, F. (2011). Surface-associated  
704 fucoxanthin mediates settlement of bacterial epiphytes on the rockweed *Fucus vesiculosus*.  
705 *Biofouling* 27, 423–433. doi:10.1080/08927014.2011.580841.
- 706 Salaün, S., La Barre, S., Santos-Goncalvez, M. D., Potin, P., Haras, D., and Bazire, A. (2012).  
707 Influence of exudates of the kelp *Laminaria digitata* on biofilm formation of associated and  
708 exogenous bacterial epiphytes. *Microb. Ecol.* 64, 359–369. doi:10.1007/s00248-012-0048-4.
- 709 Sale, P. F. (1976). Reef fish lottery. *Nat. Hist.* 85, 60–65.
- 710 Sanchez-Amat, A., Solano, F., and Lucas-Elío, P. (2010). Finding new enzymes from bacterial  
711 physiology: A successful approach illustrated by the detection of novel oxidases in *Marinomonas*  
712 *mediterranea*. *Mar. Drugs* 8, 519–541. doi:10.3390/md8030519.
- 713 Sanders-Smith, R., Segovia, B. T., Forbes, C., Hessing-Lewis, M., Morien, E., Lemay, M. A., et al.  
714 (2020). Host-specificity and core taxa of seagrass leaf microbiome identified across tissue age  
715 and geographical regions. *Front. Ecol. Evol.* 8. doi:10.3389/fevo.2020.605304.
- 716 Schloss, P. D., Jenior, M. L., Koumpouras, C. C., Westcott, S. L., and Highlander, S. K. (2016).  
717 Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. *PeerJ*  
718 4, e1869. doi:10.7717/peerj.1869.

- 719 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009).
- 720 Introducing mothur: Open-source, platform-independent, community-supported software for  
721 describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- 722 doi:10.1128/AEM.01541-09.
- 723 Schmidt, V. T., Smith, K. F., Melvin, D. W., and Amaral-Zettler, L. A. (2015). Community assembly  
724 of a euryhaline fish microbiome during salinity acclimation. *Mol. Ecol.* 24, 2537–2550.
- 725 doi:10.1111/mec.13177.
- 726 Short, F. T., Coles, R. G., and Pergent-Martini, C. (2001). “Global seagrass distribution,” in *Global*  
727 *Seagrass Research Methods*, eds. F. T. Short and R. G. Coles (Amsterdam: Elsevier Science  
728 B.V.), 5–30.
- 729 Simon, M., Scheuner, C., Meier-Kolthoff, J. P., Brinkhoff, T., Wagner-Döbler, I., Ulbrich, M., et  
730 al. (2017). Phylogenomics of *Rhodobacteraceae* reveals evolutionary adaptation to marine and  
731 non-marine habitats. *ISME J.* 11, 1483–1499. doi:10.1038/ismej.2016.198.
- 732 Stabili, L., Rizzo, L., Pizzolante, G., Alifano, P., and Fraschetti, S. (2017). Spatial distribution of  
733 the culturable bacterial community associated with the invasive alga *Caulerpa cylindracea* in  
734 the Mediterranean Sea. *Mar. Environ. Res.* 125, 90–98. doi:10.1016/j.marenvres.2017.02.001.
- 735 Stratil, S. B., Neulinger, S. C., Knecht, H., Friedrichs, A. K., and Wahl, M. (2013).  
736 Temperature-driven shifts in the epibiotic bacterial community composition of the brown  
737 macroalga *Fucus vesiculosus*. *Microbiologyopen* 2, 338–349. doi:10.1002/mbo3.79.
- 738 Tarquinio, F., Hyndes, G. A., Laverock, B., Koenders, A., and Säwström, C. (2019). The seagrass  
739 holobiont: Understanding seagrass-bacteria interactions and their role in seagrass ecosystem  
740 functioning. *FEMS Microbiol. Lett.* 366, fnz057. doi:10.1093/femsle/fnz057.

- 741 Tujula, N. A., Crocetti, G. R., Burke, C., Thomas, T., Holmström, C., and Kjelleberg, S. (2010).  
742 Variability and abundance of the epiphytic bacterial community associated with a green marine  
743 *Ulvacean* alga. *ISME J.* 4, 301–311. doi:10.1038/ismej.2009.107.
- 744 Ugarelli, K., Laas, P., and Stingl, U. (2019). The microbial communities of leaves and roots  
745 associated with turtle grass (*Thalassia testudinum*) and manatee grass (*Syringodium filiforme*)  
746 are distinct from seawater and sediment communities, but are similar between species and  
747 sampling sites. *Microorganisms* 7, 4. doi:10.3390/microorganisms7010004.
- 748 Wang, L., Tomas, F., and Mueller, R. S. (2020). Nutrient enrichment increases size of *Zostera marina*  
749 shoots and enriches for sulfur and nitrogen cycling bacteria in root-associated microbiomes.  
750 *FEMS Microbiol. Ecol.* 96, fiaa129. doi:10.1093/femsec/fiaa129.
- 751 Weidner, S., Arnold, W., Stackebrandt, E., and Pühler, A. (2000). Phylogenetic analysis of  
752 bacterial communities associated with leaves of the seagrass *Halophila stipulacea* by  
753 a culture-independent small-subunit rRNA gene approach. *Microb. Ecol.* 39, 22–31.  
754 doi:doi.org/10.1007/s002489900194.
- 755 Weinberger, F. (2007). Pathogen-induced defense and innate immunity in macroalgae. *Biol. Bull.*  
756 213, 290–302. doi:10.2307/25066646.
- 757 Wickham, H. (2017). *tidyverse: Easily install and load the 'tidyverse'*.
- 758 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019).  
759 Welcome to the tidyverse. *J. Open Source Softw.* 4, 1686. doi:10.21105/joss.01686.
- 760 Wilke, C. O. (2019). *cowplot: Streamlined plot theme and plot annotations for 'ggplot2'*.
- 761 Wood, D. C., and Hayasaka, S. S. (1981). Chemotaxis of rhizoplane bacteria to amino acids  
762 comprising eelgrass (*Zostera marina* L.) root exudate. *J. Exp. Mar. Biol. Ecol.* 50, 153–161.  
763 doi:10.1016/0022-0981(81)90047-2.

- 764 Xie, Y. (2014). "knitr: A comprehensive tool for reproducible research in R," in *Implementing*  
765 *Reproducible Computational Research*, eds. V. Stodden, F. Leisch, and R. D. Peng (New York:  
766 Chapman and Hall/CRC), 3–32.
- 767 Xie, Y. (2015). *Dynamic Documents with R and knitr*. 2nd ed. Boca Raton, Florida: Chapman and  
768 Hall/CRC.
- 769 Xie, Y. (2019a). *knitr: A general-purpose package for dynamic report generation in R*.
- 770 Xie, Y. (2019b). TinyTeX: A lightweight, cross-platform, and easy-to-maintain LaTeX distribution  
771 based on TeX Live. *TUGboat* 40, 30–32.
- 772 Xie, Y. (2019c). *tinytex: Helper functions to install and maintain 'TeX Live', and compile 'LaTeX'*  
773 *documents*.
- 774 Xie, Y., Allaire, J. J., and Grolemund, G. (2018). *R Markdown: The Definitive Guide*. 1st ed. Boca  
775 Raton, Florida: Chapman and Hall/CRC.
- 776 Xie, Z., Lin, W., and Luo, J. (2017). Comparative phenotype and genome analysis of *Cellvibrio*  
777 sp. PR1, a xylanolytic and agarolytic bacterium from the Pearl River. *BioMed Res. Int.* 2017,  
778 6304248. doi:10.1155/2017/6304248.
- 779 Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al. (2014). The SILVA  
780 and "All-Species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res.* 42,  
781 D643–D648. doi:10.1093/nar/gkt1209.
- 782 Zavodnik, N., Travizi, A., and de Rosa, S. (1998). Seasonal variations in the rate of photosynthetic  
783 activity and chemical composition of the seagrass *Cymodocea nodosa* (Ucr.) Asch. *Sci. Mar.*  
784 62, 301–309. doi:10.3989/scimar.1998.62n4301.
- 785 Zhu, H. (2019). *kableExtra: Construct complex table with 'kable' and pipe syntax*.

786 **Figure legends**

787 **Figure 1.** Location of the mixed (*C. nodosa* and *C. cylindracea*) and monospecific (*C. cylindracea*)  
788 settlement in the Bay of Funtana, northern Adriatic Sea (© OpenStreetMap contributors,  
789 [www.openstreetmap.org/copyright](http://www.openstreetmap.org/copyright)).

790 **Figure 2.** Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU  
791 abundances of bacterial and archaeal communities from the surfaces of the macrophytes *C. nodosa*  
792 (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient  
793 seawater. Samples from different environments or seasons are labeled in different color and  
794 shape. The proportion of explained variation by each axis is shown on the corresponding axis in  
795 parentheses.

796 **Figure 3.** Proportion of shared bacterial and archaeal communities (Bray-Curtis similarity  
797 coefficient) and shared bacterial and archaeal OTUs (Jaccard's similarity coefficient) between  
798 consecutive sampling dates and from the surfaces of the macrophytes *C. nodosa* (mixed settlement)  
799 and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater.

800 **Figure 4.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) bacterial  
801 and archaeal sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C.*  
802 *cylindracea* (mixed and monospecific settlement) and in the ambient seawater. NR – No Relative  
803 (sequences without known relatives within the corresponding group)

804 **Figure 5.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ )  
805 cyanobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and  
806 *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion  
807 of cyanobacterial sequences in the total bacterial and archaeal community is given above the  
808 corresponding bar. NR – No Relative (sequences without known relatives within the corresponding  
809 group)

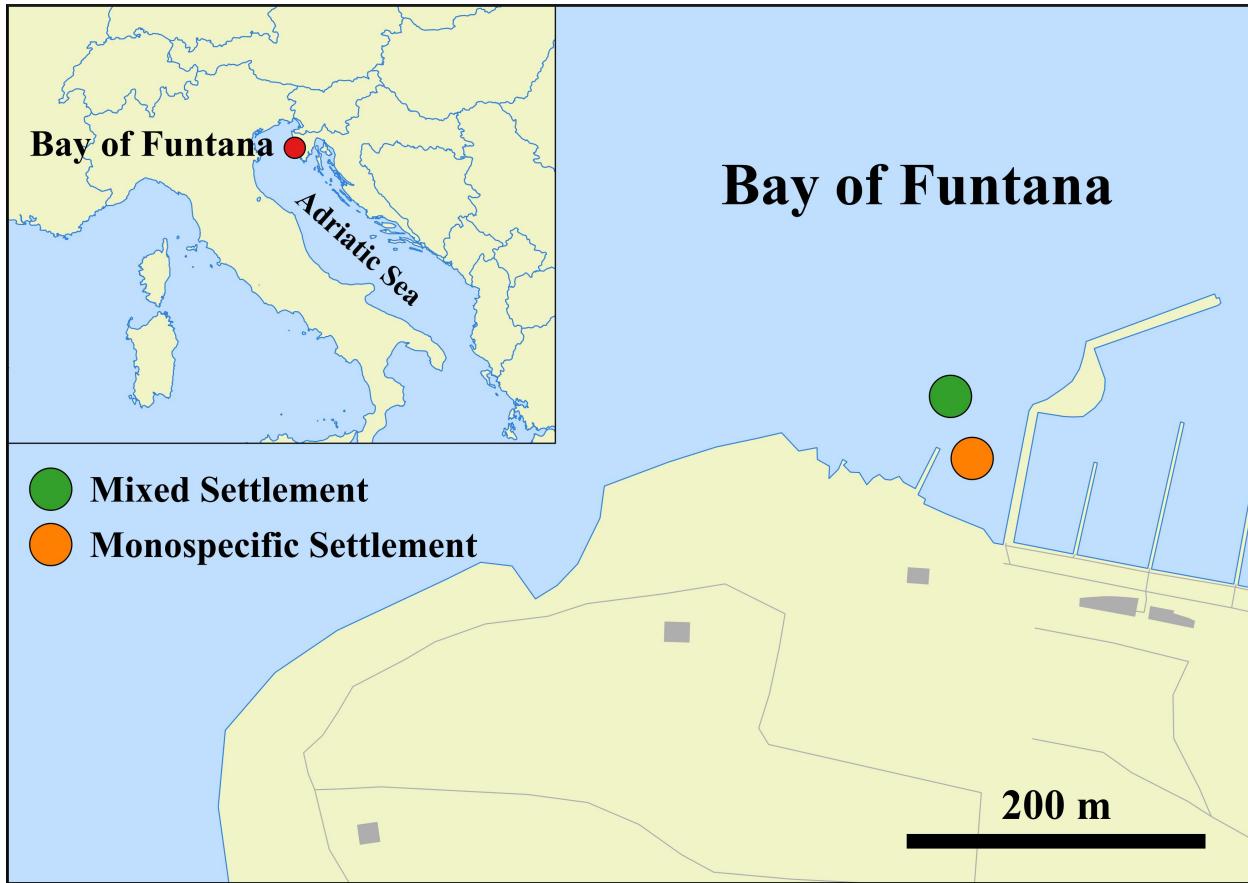
810 **Figure 6.** Taxonomic classification and relative contribution of the most abundant ( $\geq 2\%$ ) sequences  
811 within the *Bacteroidota* on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C.*  
812 *cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion of  
813 sequences classified as *Bacteroidota* in the total bacterial and archaeal community is given above the  
814 corresponding bar. NR – No Relative (sequences without known relatives within the corresponding  
815 group)

816 **Figure 7.** Taxonomic classification and relative contribution of the most abundant ( $\geq 2\%$ )  
817 alphaproteobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and  
818 *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion of  
819 alphaproteobacterial sequences in the total bacterial and archaeal community is given above the  
820 corresponding bar. NR – No Relative (sequences without known relatives within the corresponding  
821 group)

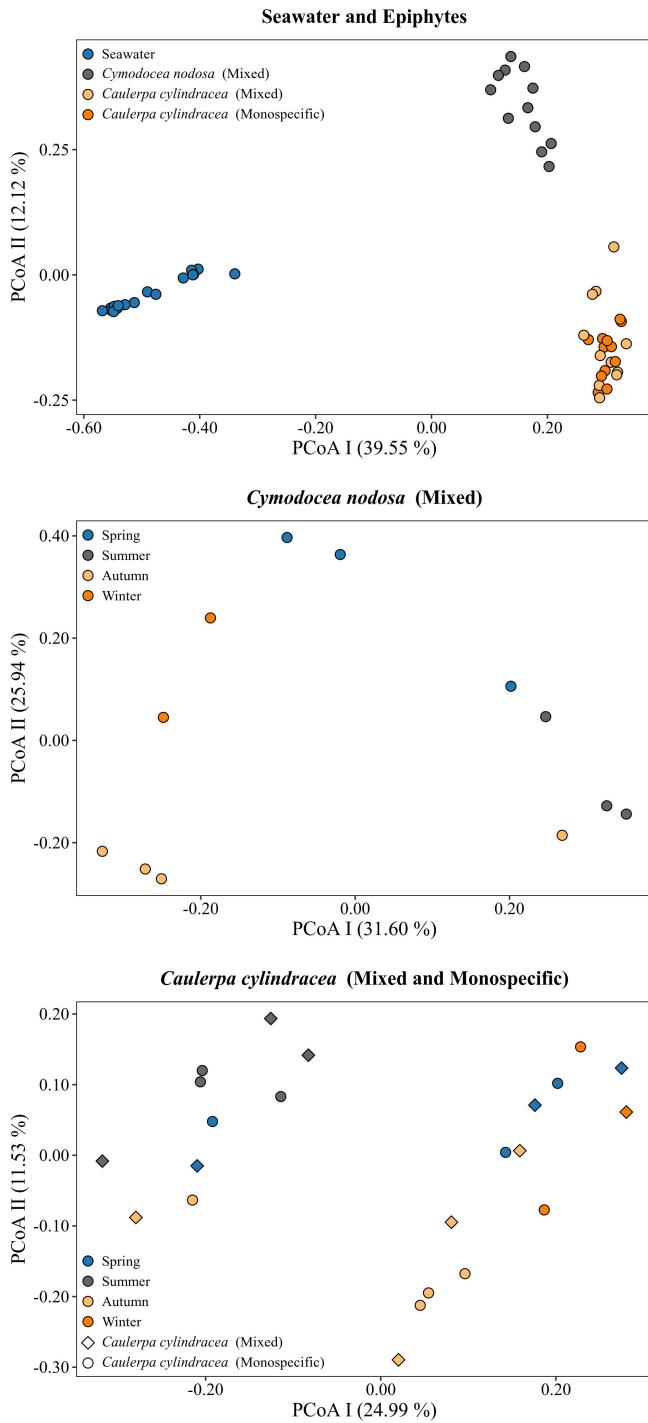
822 **Figure 8.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ )  
823 gammaproteobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement)  
824 and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion  
825 of gammaproteobacterial sequences in the total bacterial and archaeal community is given above the  
826 corresponding bar. NR – No Relative (sequences without known relatives within the corresponding  
827 group)

828 **Figure 9.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) sequences  
829 within the *Desulfobacterota* on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and  
830 *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion  
831 of sequences classified as *Desulfobacterota* in the total bacterial and archaeal community is given  
832 above the corresponding bar. NR – No Relative (sequences without known relatives within the  
833 corresponding group)

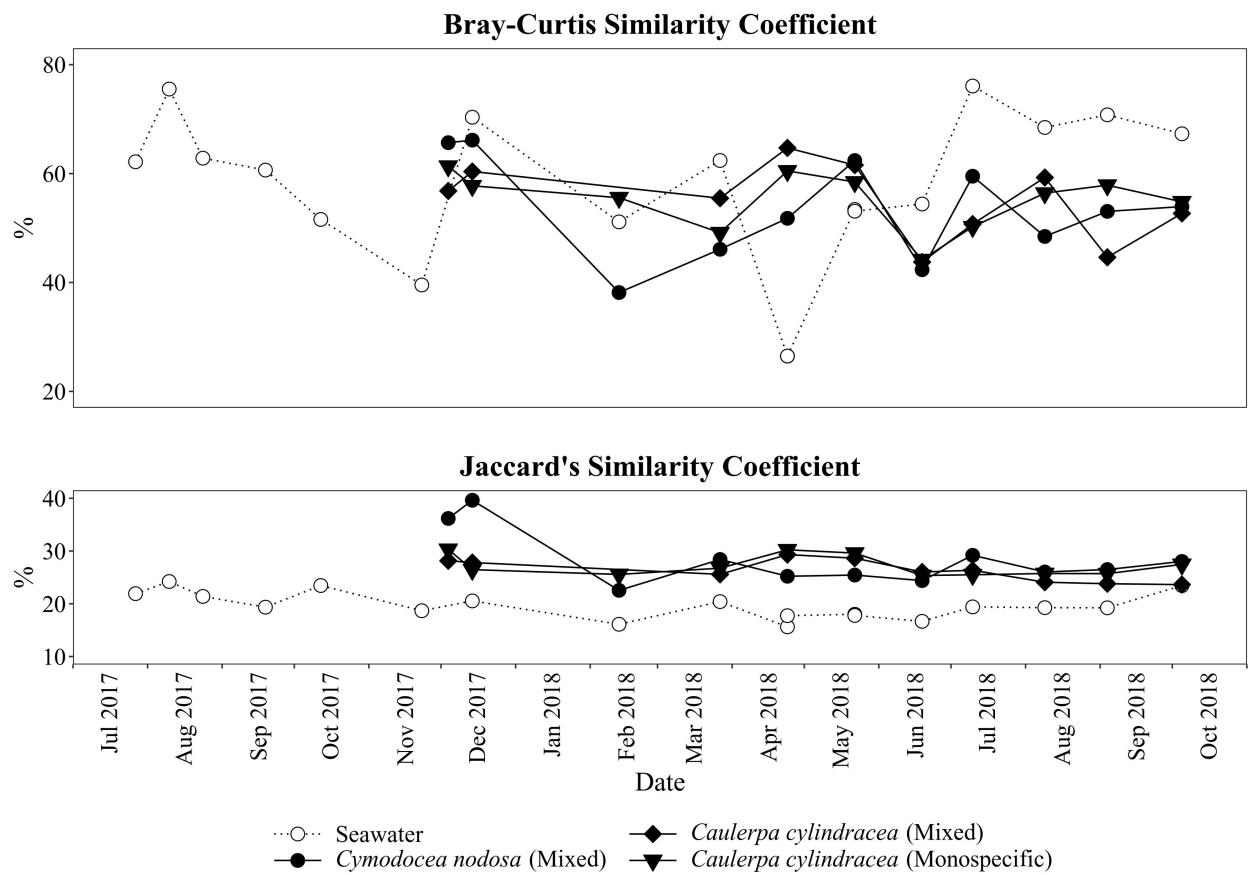
834 **Figures**



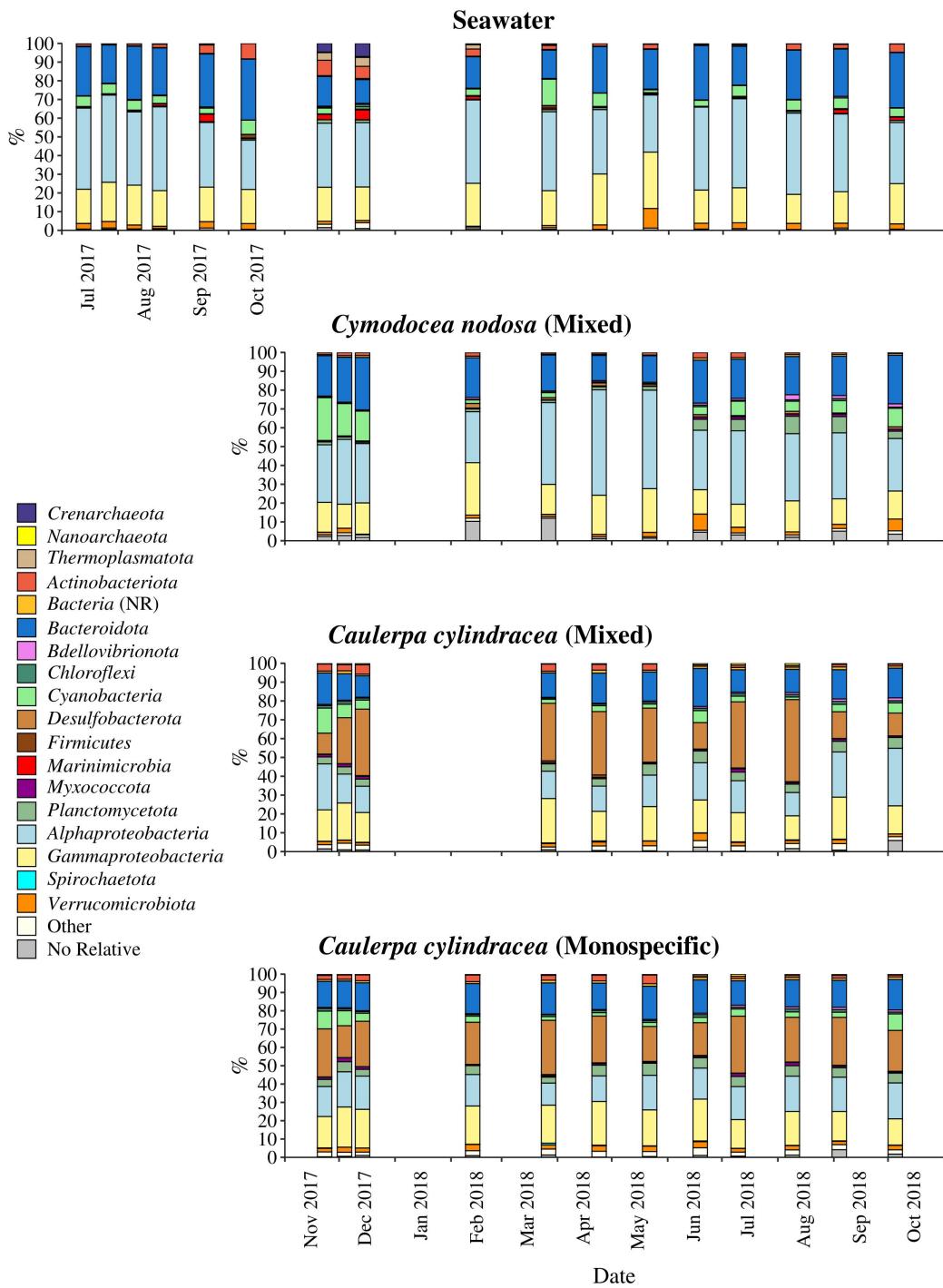
**Figure 1.** Location of the mixed (*C. nodosa* and *C. cylindracea*) and monospecific (*C. cylindracea*) settlement in the Bay of Funtana, northern Adriatic Sea (© OpenStreetMap contributors, [www.openstreetmap.org/copyright](http://www.openstreetmap.org/copyright)).



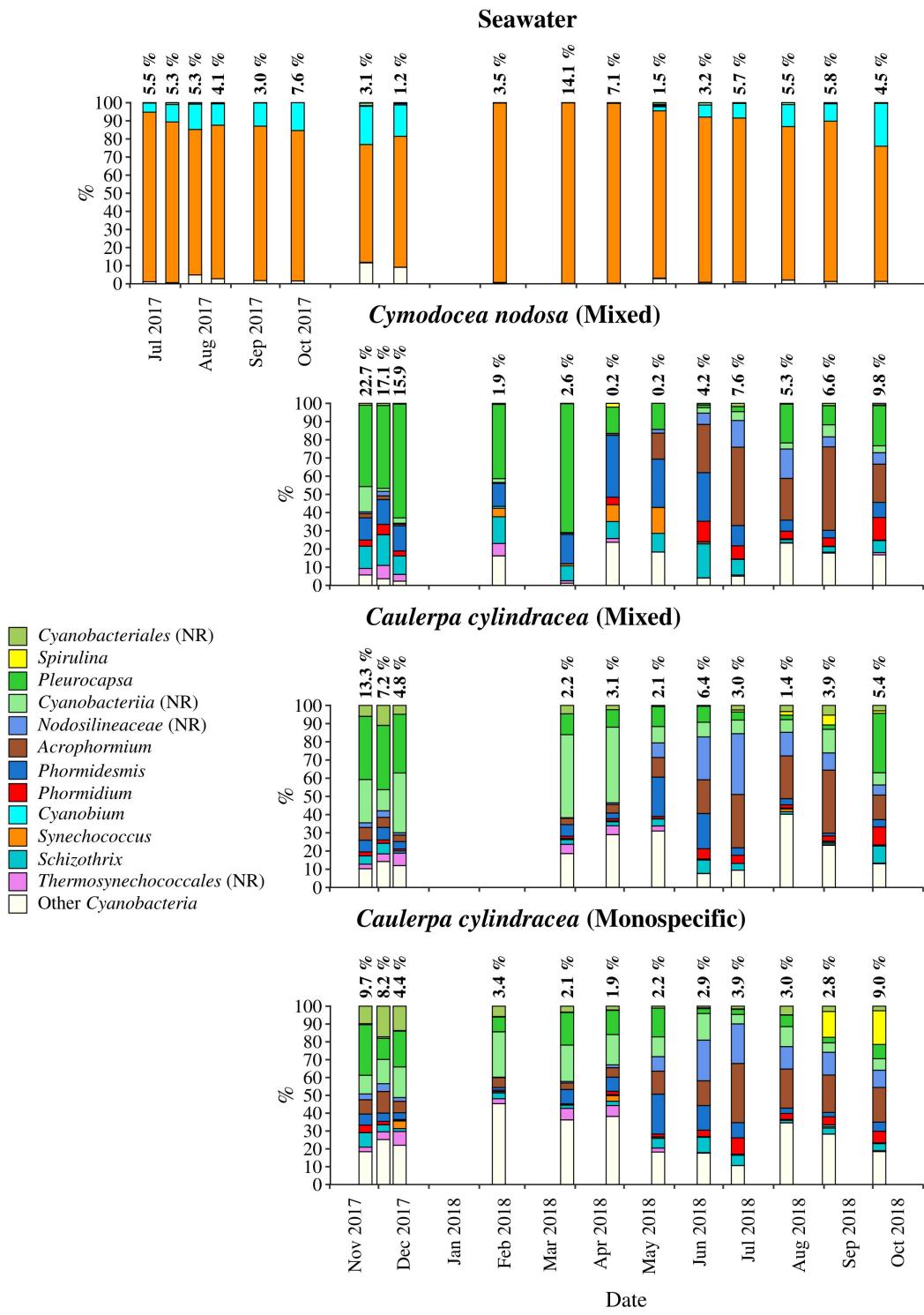
**Figure 2.** Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities from the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. Samples from different environments or seasons are labeled in different color and shape. The proportion of explained variation by each axis is shown on the corresponding axis in parentheses.



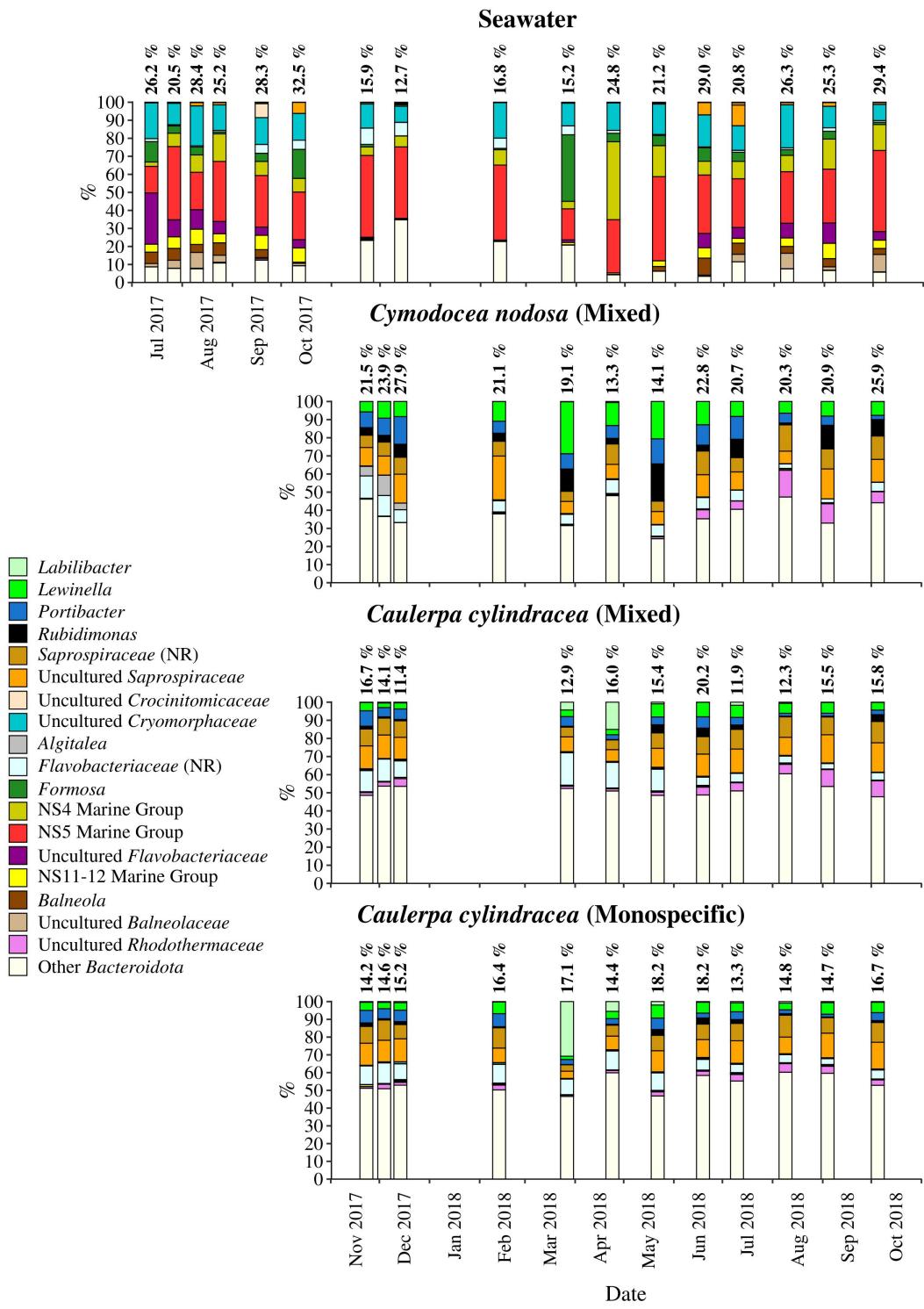
**Figure 3.** Proportion of shared bacterial and archaeal communities (Bray-Curtis similarity coefficient) and shared bacterial and archaeal OTUs (Jaccard's similarity coefficient) between consecutive sampling dates and from the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater.



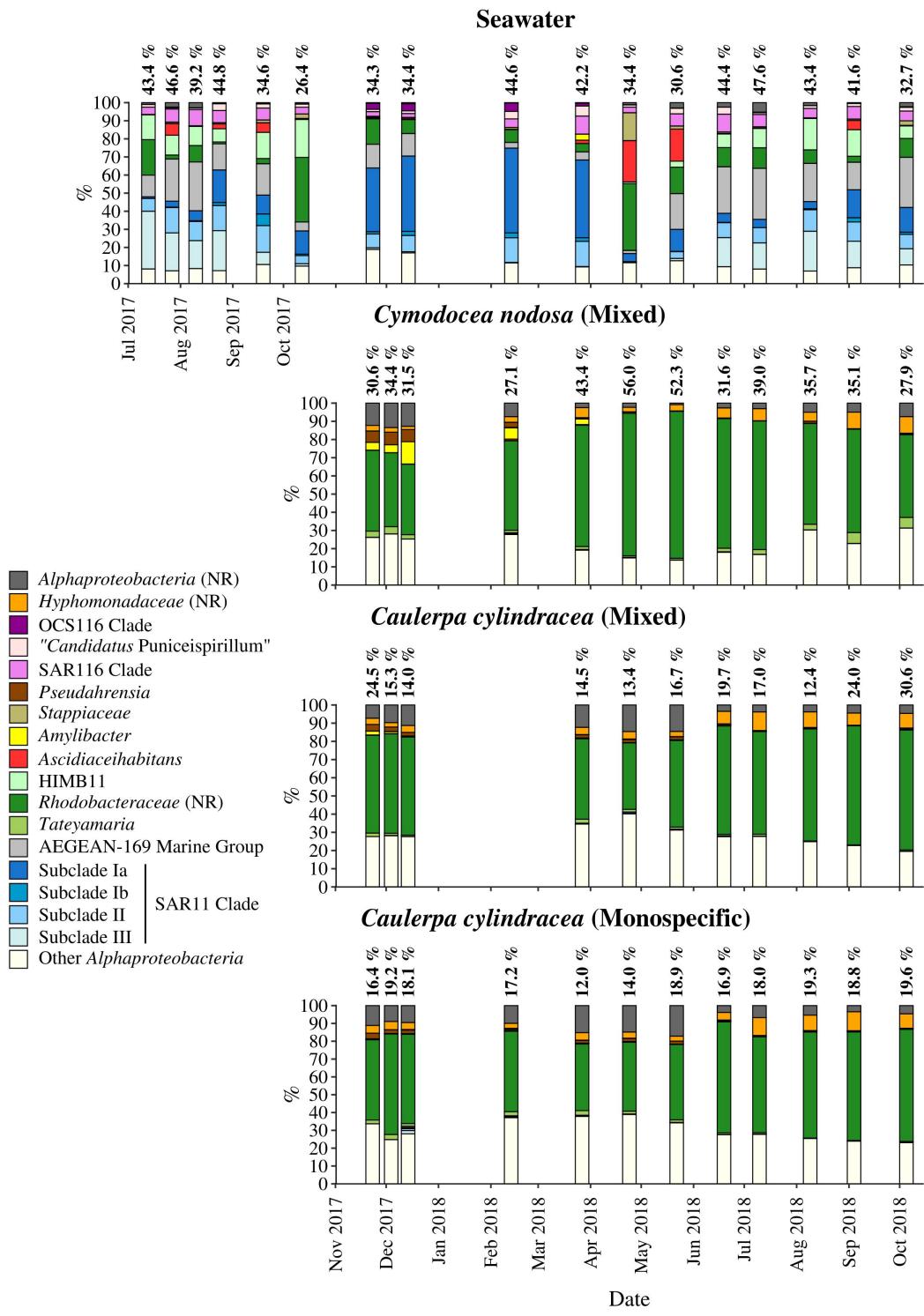
**Figure 4.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) bacterial and archaeal sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. NR – No Relative (sequences without known relatives within the corresponding group)



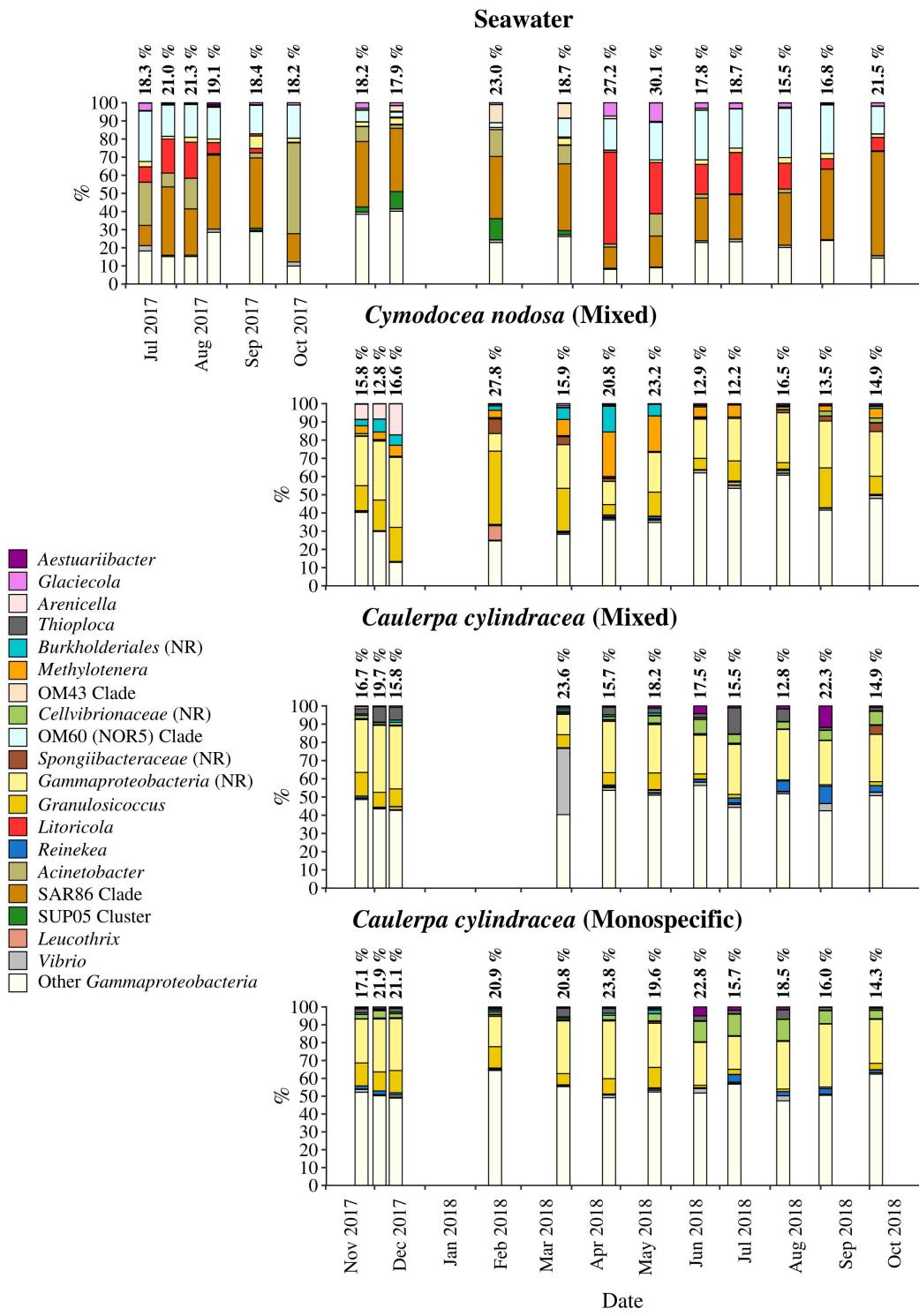
**Figure 5.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) cyanobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion of cyanobacterial 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)



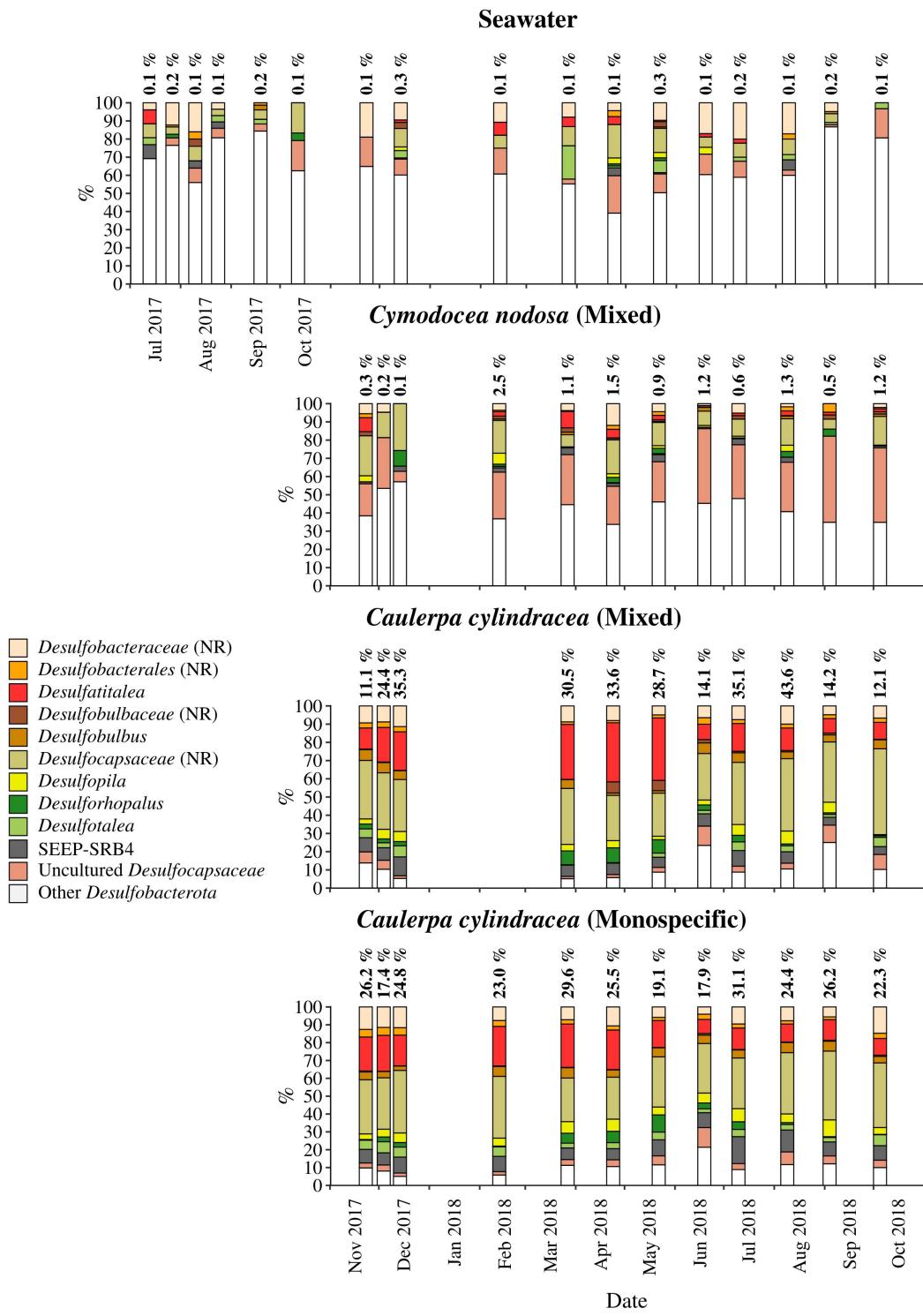
**Figure 6.** Taxonomic classification and relative contribution of the most abundant ( $\geq 2 \%$ ) sequences within the *Bacteroidota* on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion 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)



**Figure 7.** Taxonomic classification and relative contribution of the most abundant ( $\geq 2\%$ ) alphaproteobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. 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)



**Figure 8.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) gammaproteobacterial sequences on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. 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)



**Figure 9.** Taxonomic classification and relative contribution of the most abundant ( $\geq 1\%$ ) sequences within the *Desulfobacterota* on the surfaces of the macrophytes *C. nodosa* (mixed settlement) and *C. cylindracea* (mixed and monospecific settlement) and in the ambient seawater. The proportion of sequences classified as *Desulfobacterota* in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative (sequences without known relatives within the corresponding group)