

Seasonal Dynamics of Epiphytic Microbial Communities on Marine Macrophyte Surfaces

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1 Abstract

2 Surfaces of marine macrophytes (seagrasses and macroalgae) are inhabited by diverse
3 microbial communities. Most studies focusing on macrophyte epiphytic communities did not
4 take into account temporal changes or applied low sampling frequency approaches. Illumina
5 sequencing of the V4 16S rRNA region was performed to determine the seasonal dynamics
6 of epiphytic communities sampled from the surfaces of the seagrass *Cymodocea nodosa* and
7 invasive macroalga *Caulerpa cylindracea*. Leaves and thalli were sampled in a meadow of
8 *Cymodocea nodosa* invaded by the invasive *Caulerpa cylindracea* and in a monospecific
9 settlement of *Caulerpa cylindracea* located in the proximity of the meadow at monthly intervals.
10 For comparison the ambient prokaryotic plankton community was also characterized. Sequencing
11 results at the OTU level showed a clear differentiation between ambient water and epiphytic
12 communities and a host-specific community assemblage. In addition, successional changes were
13 observed that could be connected to the macrophyte growth cycle. Taxonomic analysis showed
14 similar high rank groups in the ambient water and epiphytic communities, with the exception of
15 *Desulfobacterota* that were found only on *Caulerpa cylindracea*. Only *Cyanobacteria* showed
16 seasonal change, while other high rank taxa were present throughout the year. In every analyzed
17 high rank taxa, phylogenetic groups present throughout the year comprised most of the sequences
18 and could be identified together with low proportion taxa showing seasonal patterns connected to
19 the macrophyte growth cycle. Taken together, epiphytic microbial communities of the seagrass
20 *Cymodocea nodosa* and the macroalgae *Caulerpa cylindracea* appear to be host-specific and
21 contain taxa that undergo successional changes.

22 **Introduction**

23 Marine macrophytes (seagrasses and macroalgae) are important ecosystem engineers that
24 form close associations with microorganism belonging to all three domains of life (Egan *et al.*,
25 2013; Tarquinio *et al.*, 2019). Microbes can live within macrophyte tissue as endophytes or can
26 form epiphytic communities on surfaces of leaves and thalli (Egan *et al.*, 2013; Hollants *et al.*,
27 2013; Tarquinio *et al.*, 2019). Epiphytic and endophytic microbial communities form a close
28 functional relationship with the macrophyte host. It was proposed that this close relationship
29 constitutes a holobiont, an integrated community where the macrophyte organism and its symbiotic
30 partners support each other (Margulis, 1991; Egan *et al.*, 2013; Tarquinio *et al.*, 2019).

31 Biofilms formed from microbial epiphytes can contain diverse taxonomic groups and harbor
32 cell densities from 10^2 to 10^7 cells cm^{-2} (Armstrong *et al.*, 2000; Bengtsson *et al.*, 2010; Burke *et*
33 *al.*, 2011b). In such an environment a number of positive and negative interactions between the
34 macrophyte and colonizing microorganisms have been described (Egan *et al.*, 2013; Hollants *et*
35 *al.*, 2013; Tarquinio *et al.*, 2019). Macrophytes can promote growth of associated microbes by
36 nutrient exudation, while in return microorganisms may support macrophyte performance through
37 improved nutrient availability, phytohormone production and protection form toxic compounds,
38 oxidative stress, biofouling organisms and pathogens (Egan *et al.*, 2013; Hollants *et al.*, 2013;
39 Tarquinio *et al.*, 2019). Beside this positive interactions, macrophytes can negatively impact
40 the associated microbes such as pathogenic bacteria by producing reactive oxygen species and
41 secondary metabolites (Egan *et al.*, 2013; Hollants *et al.*, 2013; Tarquinio *et al.*, 2019).

42 All these ecological roles are carried out by a taxonomically diverse community of
43 microorganisms. At higher taxonomic ranks a core set of macrophyte epiphytic taxa was
44 described consisting mainly of *Alphaproteobacteria*, *Gammaproteobacteria*, *Desulfobacterota*,
45 *Bacteroidota*, *Cyanobacteria*, *Actinobacteriota*, *Firmicutes*, *Planctomycetota*, *Chloroflexi* and
46 *Verrucomicrobiota* (Crump and Koch, 2008; Tujula *et al.*, 2010; Lachnit *et al.*, 2011). In contrast,

47 at lower taxonomic ranks host specific microbial communities were described (Lachnit *et al.*,
48 2011; Roth-Schulze *et al.*, 2016). Recently, it was shown that even different morphological niches
49 within the same alga had a higher influence on bacterial community variation than biogeography
50 or environmental factors (Morrissey *et al.*, 2019). While there is high community variation
51 between host species it was observed that the majority of metagenome determined functions were
52 conserved both between host species and individuals (Burke *et al.*, 2011a; Roth-Schulze *et al.*,
53 2016). This discrepancy between taxonomic and functional composition could be explained by
54 the lottery hypothesis. It postulates that an initial random colonization step is performed from
55 a set of functionally equivalent taxonomic groups resulting in taxonomically different epiphytic
56 communities sharing a core set of functional genes (Burke *et al.*, 2011a; Roth-Schulze *et al.*,
57 2016). In addition, some of the variation in the observed data could be attributed to different
58 techniques used in various studies, such as different protocols for epiphytic cell detachment and/or
59 DNA isolation, as no standard protocol to study epiphytic communities was established (Ugarelli
60 *et al.*, 2019; Korlević *et al.*, submitted).

61 The majority of studies describing macrophyte epiphytic communities did not encompass
62 seasonal changes (Crump and Koch, 2008; Lachnit *et al.*, 2009; Burke *et al.*, 2011b; Roth-Schulze
63 *et al.*, 2016; Ugarelli *et al.*, 2019). In addition, if seasonal changes were taken into account
64 low temporal frequency and/or methodologies that do not allow high taxonomic resolution were
65 used (Tujula *et al.*, 2010; Lachnit *et al.*, 2011; Miranda *et al.*, 2013; Michelou *et al.*, 2013). In
66 the present study we describe the seasonal dynamics of bacterial and archaeal communities on
67 the surfaces of the seagrass *Cymodocea nodosa* and siphonous macroalgae *Caulerpa cylindracea*
68 determined on a mostly monthly scale. Bacterial and archaeal epiphytes were sampled in a meadow
69 of *C. nodosa* invaded by the invasive *C. cylindracea* and in a locality of only *C. cylindracea*
70 located in the proximity of the meadow. In addition, for comparison, the community of the ambient
71 seawater was characterized.

72 **Results**

73 Sequencing of the 16S rRNA V4 region yielded a total of 1.8 million sequences after
74 quality curation and exclusion of eukaryotic, chloroplast, mitochondrial and no relative sequences
75 (Table S1). A total of 35 samples originating from epiphytic archaeal and bacterial communities
76 associated with surfaces of the seagrass *C. nodosa* and macroalga *C. cylindracea* were analyzed. In
77 addition, 18 samples (one of the samples was sequenced two times) originating from picoplankton
78 archaeal and bacterial communities in the ambient seawater were also processed for comparison.
79 The number of reads per sample ranged between 8,409 and 77,465 sequences (Table S1). Even
80 when the highest sequencing effort was applied the rarefaction curves did not level off that is a
81 common observation in high-throughput 16S rRNA amplicon sequencing approaches (Figure S1).
82 Following quality curation and exclusion of sequences mentioned before reads were clustered
83 into 28,691 different OTUs at a similarity level of 97 %. Read numbers were normalized to the
84 minimum number of sequences, 8,409 (Table S1), through rarefaction resulting in 17,071 different
85 OTUs that ranged from 386 to 2,009 OTUs per sample (Figure S2). To determine seasonal changes
86 of richness and diversity the Observed Number of OTUs, Chao1, ACE, Exponential Shannon (Jost,
87 2006) and Inverse Simpson were calculated after normalization through rarefaction. Generally,
88 richness estimators and diversity indices showed similar trends. On average, higher values were
89 found for *C. cylindracea* (mixed [Number of OTUs, $1,679.5 \pm 127.4$ OTUs] and monospecific
90 [Number of OTUs, $1,736.3 \pm 156.5$ OTUs]), middle values for *C. nodosa* (Number of OTUs,
91 $1,059 \pm 216.2$ OTUs) and lower values for picoplankton communities in the ambient seawater
92 (Number of OTUs, 528.2 ± 135.4 OTUs) (Figure S2). Seasonal changes did not show such large
93 dissimilarities. *C. nodosa* communities showed a slow increase towards the end of the study,
94 while *C. cylindracea* (mixed and monospecific) communities were characterized by slightly larger
95 values in Spring and Summer in comparison to Autumn and Winter (Figure S2).

96 To determine the proportion of shared archaeal and bacterial OTUs and communities sampled
97 in different environments the Jaccard's Similarity Coefficient on presence-absence data and

98 Bray-Curtis Similarity Coefficient were, respectively, calculated. Coefficients were determined
99 after normalization through rarefaction and binning of samples from a particular environment. The
100 highest proportion of shared OTUs and community was found between mixed and monospecific
101 *C. cylindracea* (Jaccard, 0.35; Bray-Curtis, 0.77), while lower shared values were calculated
102 between seawater and epiphytic communities (Figure 1). Shared proportion between *C. nodosa*
103 and *C. cylindracea* were approximately in the middle between these two extremes. To assess
104 seasonal changes in the proportion of shared OTUs and communities the Jaccard's and Bray-Curtis
105 Similarity Coefficients were calculated between consecutive sampling points (Figure 2). Both
106 coefficients showed similar trends. Temporal proportional changes were more pronounced for
107 seawater in comparison to *C. nodosa* and especially *C. cylindracea* associated communities
108 (Figure 2). In addition, only 0.4 – 1.0 % of OTUs from each surface associated community were
109 found at every time point. These OTUs also made a high proportion of total sequences (39.9
110 – 51.8 %). To further disentangle the environmental and seasonal community dissimilarity a
111 Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances and OTU abundances was
112 applied. It showed a clear separation between planktonic and surface associated communities
113 (Figure 3). In addition, a separation of epiphytic bacterial and archaeal communities based on
114 host species was determined. This separation was further supported by ANOSIM ($R = 0.96, p <$
115 0.001). Seasonal changes of *C. nodosa* associated communities indicated a separation between
116 Spring, Summer and Autumn/Winter samples (ANOSIM, $R = 0.53, p < 0.01$). For *C. cylindracea*
117 associated communities a separation between Summer and Autumn/Winter/Spring samples was
118 observed that was not so strongly supported (ANOSIM, $R = 0.30, p < 0.05$) (Figure 3).

119 The taxonomic composition of both, macrophyte associated and seawater communities,
120 was dominated by bacterial ($99.1 \pm 2.1 \%$) over archaeal sequences ($0.9 \pm 2.1 \%$) (Figure 4).
121 Higher relative abundances of chloroplast related sequences were only observed in surface
122 associated communities, with higher values in Autumn/Winter ($37.2 \pm 11.2 \%$) in comparison to
123 Spring/Summer ($20.9 \pm 9.7 \%$) (Figure S3). Generally, at higher taxonomic ranks (phylum-class)
124 epiphytic and seawater microbial communities were composed of similar bacterial taxa.

125 Seawater communities were mainly comprised of *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*,
126 *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota*. Communities associated
127 with *C. nodosa* consisted of same groups with the addition of *Planctomycetota* whose contribution
128 was higher in summer 2018. In addition, communities from mixed and monospecific *C.*
129 *cylindracea* were similar and characterized by same groups as seawater and *C. nodosa*
130 communities with the addition of *Desulfobacterota* (Figure 4). Larger differences between
131 environments and host species could be observed at lower taxonomic ranks (Figure 5 – 9).

132 *Cyanobacteria* related sequences were comprising, on average, $5.5 \pm 4.4\%$ of total sequences
133 (Figure 5). Higher proportions were found for *C. nodosa* ($16.4 \pm 5.3\%$) and *C. cylindracea*
134 (mixed [$(7.7 \pm 3.9\%)$] and monospecific [$(7.8 \pm 2.4\%)$]) associated communities in autumn and
135 for seawater communities in winter ($8.8 \pm 7.4\%$). Large taxonomic differences between surface
136 associated and seawater cyanobacterial communities were observed. Seawater communities
137 were mainly comprised of *Cyanobium* and *Synechococcus*, while surface associated communities
138 were consisted of *Pleurocapsa* and sequences without known relatives within *Cyanobacteriia*
139 (Figure 5). In addition, seasonal changes in surface associated communities were observed
140 with *Pleurocapsa* and no relative *Cyanobacteriia* comprising larger proportions in autumn and
141 winter and *Acrophormium*, *Phormidesmis* and no relative *Nodosilineaceae* in spring and summer
142 (Figure 5).

143 Sequences classified as *Bacteroidota* were comprising, on average, $19.2 \pm 5.5\%$ of all
144 sequences (Figure 6). Similarly to *Cyanobacteria*, large differences in the taxonomic composition
145 between seawater and surface associated communities were found (Figure 6). The seawater
146 community was characterized by the NS4 and NS5 marine groups, uncultured *Cryomorphaceae*,
147 uncultured *Flavobacteriaceae*, NS11-12 marine group, *Balneola*, uncultured *Balneolaceae* and
148 *Formosa*. In contrast, in surface associated communities *Lewinella*, *Portibacter*, *Rubidimonas*, no
149 relative *Sapspiraceae*, uncultured *Sapspiraceae*, no relative *Flavobacteriaceae* and uncultured
150 *Rhodothermaceae* were found. Some groups showed slight seasonal changes such as no relative

151 *Flavobacteriaceae* that were more pronounced from November 2017 until June 2018. In contrast,
152 uncultured *Rhodothermaceae* showed higher proportions from June 2018 until the end of the study
153 period. Surface associated *Bacteroidota* communities were very diverse as could be observed in
154 the high proportion of taxa that grouped as other *Bacteroidota* (Figure 6).

155 On average, *Alphaproteobacteria* were in comparison to other high rank taxa the largest
156 taxonomic group, comprising 29.2 ± 12.0 % of all sequences (Figure 7). In accordance to previous
157 taxa, high differences between seawater and surface associated communities were observed.
158 Picoplankton communities were composed mainly of the SAR11 clade, AEGEAN-169 marine
159 group, SAR116 clade, no relative *Rhodobacteraceae*, HIMB11 and OCS116 clade, while surface
160 associated communities were composed in high proportion of no relative *Rhodobacteraceae* and to
161 a lesser degree of *Pseudoahrensia*, no relative *Alphaproteobacteria*, no relative *Hyphomonadaceae*
162 and *Amylibacter*. Representatives of no relative *Rhodobacteraceae* were comprising on average
163 40.6 ± 23.2 % of all alphaproteobacterial sequences from the epiphytic community (Figure 7). In
164 addition, *Amylibacter* was detected mainly in *C. nodosa* from November 2017 until March 2018.

165 Sequences related to *Gammaproteobacteria* were comprising, on average, 18.6 ± 3.9 %
166 of all sequences (Figure 8). Similarly to previous taxa, large taxonomic differences between
167 seawater and surface associated communities were found. Seawater communities were mainly
168 comprised of the OM60 (NOR5) clade, *Litoricola*, *Acinetobacter* and the SAR86 clade,
169 while epiphytic communities were mainly composed of no relative *Gammaproteobacteria* and
170 *Granulosicoccus*. Beside these two groups specific to all three epiphytic communities, *C. nodosa*
171 was characterized by *Arenicella*, no relative *Burkholderiales* and *Methylotenera*, while *Thioploca*,
172 no relative *Cellvibrionaceae* and *Reinekea* were more specific to both mixed and monospecific
173 *C. cylindracea*. In addition, *Arenicella* was more pronounced in November and December 2017,
174 while no relative *Burkholderiales* and *Methylotenera* were more characteristic for the period from
175 March until May 2018. For the *C. cylindracea* specific taxa no relative *Cellvibrionaceae* and
176 *Reinekea* showed some seasonality and were characteristic for samples originating from June to

¹⁷⁷ October 2018. In addition, similarly to *Bacteroidota*, a large proportion of the surface associated
¹⁷⁸ community was grouped as other *Gammaproteobacteria* indicating high diversity within this
¹⁷⁹ group (Figure 8).

¹⁸⁰ In contrast to previously described high rank taxa, *Desulfobacterota* were specific to *C.*
¹⁸¹ *cylindracea*. On average they comprised 11.2 ± 13.3 % of all sequences. Seawater and *C.*
¹⁸² *nodosa* communities consisted of only 0.1 ± 0.08 % and 1.0 ± 0.7 % *Desulfobacterota* sequences,
¹⁸³ respectively. In the mixed and monospecific *C. cylindracea* communities their proportion was
¹⁸⁴ 25.7 ± 11.2 % and 24.0 ± 4.3 %, respectively (Figure 9). The community consisted mainly of
¹⁸⁵ no relative *Desulfobacteraceae*, *Desulfatitalea*, no relative *Desulfobulbaceae*, *Desulfobulbus*,
¹⁸⁶ no relative *Desulfocapsaceae*, *Desulfopila*, *Desulforhopalus*, *Desulfotalea*, SEEP-SRB4 and
¹⁸⁷ uncultured *Desulfocapsaceae* (Figure 9).

188 **Discussion**

189 Surfaces of marine macrophytes harbor biofilms consisting of diverse microbial taxa (Egan
190 *et al.*, 2013; Tarquinio *et al.*, 2019). No standard protocol has been developed to study these
191 macrophyte associated microbes (Ugarelli *et al.*, 2019). Different procedures for removal of
192 microbial cells from host surfaces were described, such as host tissue shaking (Nõges *et al.*, 2010),
193 scraping (Uku *et al.*, 2007) and ultrasonication (Cai *et al.*, 2014). All these methods showed
194 different removal efficiencies but none was enabling a complete removal of attached microbial
195 cells. In the present study, we applied an earlier developed removal protocol (Korlević *et al.*,
196 submitted), based on a previous idea of direct cellular lysis (Burke *et al.*, 2009), to ensure an
197 almost complete cell detachment. The application of a direct lysis procedure coupled with a high
198 frequency sampling protocol and Illumina high resolution amplicon sequencing has enabled us to
199 make a detailed description of bacterial and archaeal communities associated with the surfaces of
200 two marine macrophytes, *C. nodosa* and *C. cylindracea*.

201 In the present study, highest richness values were observed for *C. cylindracea* (mixed and
202 monospecific), middle for *C. nodosa* and lowest for seawater derived communities. Higher values
203 for seagrass associated communities in comparison to seawater were described earlier and could
204 be attributed to a larger set of inhabitable microniches existing on macrophyte surfaces (Ugarelli
205 *et al.*, 2019). In addition, highest values observed for *C. cylindracea* are partly due to its contact
206 with the sediment. *C. cylindracea* stolon is attached to the sediment surface with rhizoids, so
207 the stolon and rhizoids are in a direct contact with the sediment. In addition, seasonal richness
208 differences observed for surface attached communities showed slightly higher values in spring
209 and summer. This pattern could be explained by a higher macrophyte growth in these seasons
210 (M. Najdek, personal communication; Zavodnik *et al.*, 1998; Ruitton *et al.*, 2005). During active
211 periods macrophytes exhibit a more dynamic chemical interaction with the surface community
212 probably causing an increase in the number of inhabitable microniches (Borges and Champenois,
213 2015; Rickert *et al.*, 2016).

214 Since the colonization of macrophyte surfaces is performed from a pool of prokaryotic cells
215 from the ambient seawater, it was interesting to see to which extent these two communities differ.
216 We observed a strong differentiation between the surface attached and seawater communities
217 at the level of OTUs that is in agreement with most published studies (Burke *et al.*, 2011b;
218 Michelou *et al.*, 2013; Roth-Schulze *et al.*, 2016; Crump *et al.*, 2018; Ugarelli *et al.*, 2019).
219 These data indicate that marine macrophytes are selecting, from a pool of seawater microbial taxa,
220 the one that can colonize and proliferate on their surfaces (Salaün *et al.*, 2012; Michelou *et al.*,
221 2013). In contrast to these findings Fahimipour *et al.* (2017) found, in a global study of *Zostera*
222 *marina*, similarities between leaves and seawater samples. Discrepancies between our data and this
223 study could be explained by differences in studied seagrass species, methodological variations or
224 biogeographic trends as Fahimipour *et al.* (2017) were analyzing samples from different locations
225 throughout the northern hemisphere. It is possible that ambient seawater and leaves communities
226 from the same location are differing but are still more similar to each other when compared to
227 other sampling locations. Indeed, it was found that prokaryotic communities vary substantially
228 between different sampling sites (Bengtsson *et al.*, 2017). When the taxonomic composition at
229 high ranks was analyzed no such strong differentiation was noticed. Phyla and classes such as:
230 *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and
231 *Verrucomicrobiota* were described that is in agreement with previously reported data (Burke *et al.*,
232 2011b; Egan *et al.*, 2013; Michelou *et al.*, 2013). In contrast, when low taxonomic ranks were
233 analyzed (i.g. family and genus) a strong differentiation was observed. A similar differentiation
234 at lower taxonomic ranks was described for other species of macrophytes (Egan *et al.*, 2013;
235 Michelou *et al.*, 2013; Ugarelli *et al.*, 2019).

236 Beside differences between seawater and surface associated communities, there were
237 discussions if the prokaryotic epiphytic community is host-specific or there are generalists taxa
238 characteristic to all or many macrophytes (Egan *et al.*, 2013). Similarly to previously described
239 differences between seawater and surface attached communities, at high taxonomic ranks no
240 strong differentiation between communities associated with different host was observed. The only

241 high rank phylum that was differing between *C. nodosa* and *C. cylindracea* was *Desulfobacterota*,
242 whose sequences were more abundant in the *C. cylindracea* associated community. As already
243 mentioned, the rhizoids and part of the stolon are in contact with the sediment, so *Desulfobacterota*
244 are probably a part of the epiphytic community that was in contact with the sediment. Similar high
245 rank taxa found in this study were described to be specific for other species of macrophytes (Burke
246 *et al.*, 2011b; Lachnit *et al.*, 2011; Bengtsson *et al.*, 2017). In contrast to high taxonomic ranks,
247 a substantial differentiation between host specific communities was found, which supports the
248 host-specific hypothesis. Similar host-specificity was observed for different species of macroalgae
249 and seagrasses (Lachnit *et al.*, 2011; Roth-Schulze *et al.*, 2016; Ugarelli *et al.*, 2019; Morrissey *et*
250 *al.*, 2019). Taken together, at high taxonomic ranks a core set of taxa could be described that is
251 characteristic for all or many macrophytes, while at low taxonomic ranks a community specific to
252 host species could be identified (Egan *et al.*, 2013).

253 Seasonal richness changes in the epiphytic community were substantial as could be observed
254 in the proportion of OTUs that could be found at every sampling time ($\leq 1.0\%$). Interestingly,
255 these OTUs were accounting for a high proportion of sequences ($\leq 51.8\%$). A very similar
256 proportion of persistent OTUs and their sequence contribution was reported in high frequency
257 studies describing seasonal picoplankton changes (Gilbert *et al.*, 2009, 2012). In comparison to
258 the seawater community, a lower degree of seasonal shifts was observed for the surface associated
259 communities. It seems, microniches on the surfaces of macrophytes are providing more stable
260 conditions in comparison to the seawater. At the level of OTUs seasonal changes of *C. nodosa* and
261 *C. cylindracea* associated communities were identified that could be linked to the growth cycle of
262 the seagrass and macroalgae (M. Najdek, personal communication). *C. nodosa* was characterized
263 by a Spring community during maximum seagrass proliferation, a Summer community during a
264 biomass maximum and a Autumn/Winter community during a biomass senescence. In contrast,
265 *C. cylindracea* started to proliferate in late Spring and was characterized only by a Summer
266 community during maximal biomass increase and by a Autumn/Winter/Spring community when
267 the biomass was at the peak and the settlement started to subsequently decay. Similar seasonal

268 changes in the epiphytic community was also described for other macroalgae (Tujula *et al.*,
269 2010; Lachnit *et al.*, 2011). Higher temporal stability of *C. cylindracea* surface communities
270 in comparison to *C. nodosa* were also observed in the higher proportion of shared communities
271 between two consecutive sampling points.

272 Analysis of seasonal chloroplast sequence abundances showed higher values in Autumn/Winter
273 in comparison to Spring/Summer. This pattern is not surprising as seagrasses are known to harbor
274 more epiphytes during Autumn/Winter (Reyes and Sansón, 2001). Furthermore, we used an
275 adapted DNA isolation protocol that is known to partially coextract DNA from planktonic
276 eukaryotes (Korlević *et al.*, 2015). Strong seasonal fluctuations of high rank epiphytic taxa
277 were not observed, with the exception of *Cyanobacteria*. Cyanobacterial sequences were more
278 pronounced in November and December in comparison to Spring and Summer. Interestingly,
279 in these high proportion months the majority of cyanobacterial sequences were classified as
280 *Pleurocapsa*, a group known to colonized different living and nonliving surfaces (Burns *et*
281 *al.*, 2004; Longford *et al.*, 2007; Mobberley *et al.*, 2012; Reisser *et al.*, 2014). It is possible
282 that during periods of low metabolic activity there is a reduced interaction and selection of
283 the epiphytic community by the seagrass, causing leaves to become a suitable surface for
284 nonspecific colonizers (Zavodnik *et al.*, 1998). *Pleurocapsa* was replaced in Spring and
285 Summer by *Acrophormium*, *Phormidesmis* and no relative *Nodosilineaceae*. A study of coastal
286 microbial mats found also higher proportion of *Nodosilineaceae* sequences in Summer, while
287 *Phormidesmis* sequences were at their peak in Autumn (Cardoso *et al.*, 2019). Other high rank
288 taxa did not show strong successional patterns. In every analyzed group, with the exception of
289 *Desulfobacterota*, taxa present throughout the year in similar proportions and season specific
290 taxa could be identified. Within *Bacteroidota* different groups withing the family *Saprospiraceae*
291 (i.g. *Lewinella*, *Portibacter* and *Rubidimonas*) were detected through the year. Members of
292 this family are often found in association with macrophytes and it is suggested that they are
293 involved in the hydrolysis and utilization of complex carbon sources (Burke *et al.*, 2011b; McIlroy
294 and Nielsen, 2014; Crump *et al.*, 2018). On the other hand, families *Flavobacteriaceae* and

295 *Rhodothermaceae* showed seasonal patterns, with *Flavobacteriaceae* being more pronounced
296 from November to June and *Rhodothermaceae* from June to October. Within *Alphaproteobacteria*
297 the family *Rhodobacteraceae* was comprising the majority of sequences throughout the year. This
298 metabolically versatile family is often associated with macrophyte surfaces and usually is one of
299 the most abundant groups (Burke *et al.*, 2011b; Michelou *et al.*, 2013; Pujalte *et al.*, 2014). In
300 addition, *Hyphomonadaceae* were found in all samples. Interestingly, some of the species within
301 this group contain stalks on their cells which can be used to attach to the macrophyte surface
302 (Weidner *et al.*, 2000; Abraham and Rohde, 2014). Within the *Gammaproteobacteria*, sequences
303 without known representatives were the most pronounced group present throughout the year. In
304 addition, *Granulosicoccus* was also found in almost all samples. *Gammaproteobacteria* are often
305 a major constituent of macrophyte epiphytic communities (Burke *et al.*, 2011b; Michelou *et al.*,
306 2013; Crump *et al.*, 2018). Beside these two groups other less pronounced taxa showed seasonal
307 and host-specific patterns. For example, *C. cylindracea* was characterized by *Thioploca*, a known
308 sulfur sediment bacteria and *Cellvibrionaceae*, a family whose cultured members are known
309 polysaccharide degraders (Jørgensen and Gallardo, 1999; Xie *et al.*, 2017). *Desulfobacterota*
310 were found only associated with *C. cylindracea* and no group within this phylum showed seasonal
311 patterns. The presence of this phylum only on *C. cylindracea* is to be expected as part of the
312 epiphytic community is directly in contact with the sediment. The *Desulfobacterota* community
313 was dominated by *Desulfatitalea* and no relative *Desulfocapsaceae*, known sulfate sediment
314 groups (Kuever, 2014; Higashioka *et al.*, 2015).

315 In temperate zones marine macrophytes are exhibiting growth cycles, so it is not surprising
316 that the associated epiphytic microbial community is undergoing partial seasonal changes. In the
317 present study, we could, in every analyzed high rank taxa, identify phylogenetic groups that were
318 present throughout the year and that were comprising most of the sequences and lower proportion
319 taxa showing seasonal patterns connected to the macrophyte growth cycle. Studies focusing
320 on functional comparisons between communities associated with different hosts showed that
321 the majority of functions could be found in every community, indicating functional redundancy

322 (Roth-Schulze *et al.*, 2016). This difference between taxonomic and functional discrepancy was
323 explained by the lottery hypothesis that hypothesizes an initial random colonization step performed
324 from a set of functionally equivalent taxonomic groups (Burke *et al.*, 2011a; Roth-Schulze *et al.*,
325 2016). It is possible that functional redundancy is a characteristic of high abundance taxa detected
326 to be present throughout the year, while seasonal and/or host-specific functions are an attribute
327 of taxa displaying successional patterns. Further studies connecting taxonomy with functional
328 properties will be required to elucidate the degree of functional redundancy or specificity in
329 epiphytic microbial communities.

330 **Experimental Procedures**

331 **Sampling**

332 Sampling was performed in the Bay of Funtana, northern Adriatic Sea (45°10'39" N,
333 13°35'42" E). Leaves and thalli were sampled in a meadow of *Cymodocea nodosa* invaded by the
334 invasive *Caulerpa cylindracea* (mixed settlement) and in a monospecific settlement of *Caulerpa*
335 *cylindracea* located in the proximity of the meadow at approximately monthly intervals from
336 December 2017 to October 2018 (Table S1). Leaves and thalli were collected by diving and
337 transported to the laboratory in containers placed on ice and filled with site seawater. Upon arrival
338 to the laboratory, *C. nodosa* leaves were cut into sections of 1 – 2 cm, while *C. cylindracea* thalli
339 were cut into 5 – 8 cm long sections. Leaves and thalli were washed three times with sterile
340 artificial seawater (ASW) to remove loosely attached microbial cells. Ambient seawater was
341 collected in 10 l containers by diving and transported to the laboratory where the whole container
342 volume was filtered through a 20 µm net. The filtrate was further sequentially filtered through 3
343 µm and 0.2 µm polycarbonate membrane filters (Whatman, United Kingdom) using a peristaltic
344 pump. Filters were briefly dried at room temperature and stored at –80 °C. Seawater samples
345 were also collected approximately monthly from July 2017 to October 2018.

346 **DNA Isolation**

347 DNA from surfaces of *C. nodosa* and *C. cylindracea* was isolated using a previously modified
348 and adapted protocol that allows for a selective epiphytic DNA isolation (Massana *et al.*, 1997;
349 Korlević *et al.*, submitted). Briefly, leaves and thalli are incubated in a lysis buffer and treated with
350 lysozyme and proteinase K. Following the incubations, the mixture containing lysed epiphytic cells
351 is separated from leaves and thalli and extracted using a phenol-chloroform procedure. Finally, the
352 extracted DNA is precipitated using isopropanol. DNA from seawater picoplankton was isolated

353 from 0.2 µm polycarbonate filters according to Massana *et al.* (1997) with a slight modification.
354 Following the phenol-chloroform extraction steps 1/10 of chilled 3 M sodium acetate (pH 5.2) was
355 added. DNA was precipitated by adding 1 volume of chilled isopropanol, incubating the mixtures
356 overnight at -20 °C and centrifuging at 20,000 × g and 4 °C for 21 min. The pellet was washed
357 twice with 500 µl of chilled 70 % ethanol and centrifuged after each washing step at 20,000 × g
358 and 4 °C for 5 min. Dried pellets were resuspended in 50 – 100 µl of deionized water.

359 **Illumina 16S rRNA Sequencing**

360 Illumina MiSeq sequencing of the V4 16S rRNA region was performed as described
361 previously (Korlević *et al.*, submitted). The V4 region of the 16S rRNA gene was amplified using
362 a two-step PCR procedure. In the first PCR the 515F (5'-GTGYCAGCMGCCGCGTAA-3')
363 and 806R (5'-GGACTACNVGGGTWTCTAAT-3') primers from the Earth Microbiome Project
364 (<http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/>) were used (Caporaso
365 *et al.*, 2012; Apprill *et al.*, 2015; Parada *et al.*, 2016). These primers contained on their 5' end
366 a tagged sequence. Purified PCR products were sent for Illumina MiSeq sequencing at IMGM
367 Laboratories, Martinsried, Germany. Before sequencing at IMGM, the second PCR amplification
368 of the two-step PCR procedure was performed using primers targeting the tagged region
369 incorporated in the first PCR. In addition, these primers contained adapter and sample-specific
370 index sequences. Beside samples, a positive and negative control for each sequencing batch was
371 sequenced. Negative control was comprised of PCR reactions without DNA template, while for
372 a positive control a mock community composed of evenly mixed DNA material originating from
373 20 bacterial strains (ATCC MSA-1002, ATCC, USA) was used. Sequences obtained in this study
374 have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession
375 number PRJEB37267.

376 **Sequence Analysis**

377 Obtained sequences were analyzed on the computer cluster Isabella (University Computing
378 Center, University of Zagreb) using mothur (version 1.43.0) (Schloss *et al.*, 2009) according
379 to the MiSeq Standard Operating Procedure (MiSeq SOP; https://mothur.org/wiki/MiSeq_SOP)
380 (Kozich *et al.*, 2013) and recommendations given from the Riffomonas project to enhance data
381 reproducibility (<http://www.riffomonas.org/>). For alignment and classification of sequences the
382 SILVA SSU Ref NR 99 database (release 138; <http://www.arb-silva.de>) was used (Quast *et*
383 *al.*, 2013; Yilmaz *et al.*, 2014). Pipeline data processing and visualization was done using R
384 (version 3.6.0) (R Core Team, 2019), packages vegan (version 2.5-6) (Oksanen *et al.*, 2019), and
385 tidyverse (version 1.3.0) (Wickham *et al.*, 2019) and multiple other packages (Xie, 2014, 2015,
386 2019a, 2019b, 2020; Neuwirth, 2014; Xie *et al.*, 2018; Allaire *et al.*, 2019; Zhu, 2019). The
387 detailed analysis procedure including the R Markdown file for this paper are available as a GitHub
388 repository (https://github.com/mkorlevic/Korlevic_EpiphyticDynamics_EnvironMicrobiol_2020).
389 Based on the ATCC MSA-1002 mock community included in the analysis an average sequencing
390 error rate of 0.01 % was determined, which is in line with previously reported values for
391 next-generation sequencing data (Kozich *et al.*, 2013; Schloss *et al.*, 2016). In addition, the
392 negative controls processed together with the samples yielded on average only 2 sequences after
393 sequence quality curation.

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399 **References**

- 400 Abraham, W.R. and Rohde, M. (2014) The Family *< i>Hypomonadaceae< /i>*. In, *The*
401 *prokaryotes: < i>Alphaproteobacteria< /i> and < i>Betaproteobacteria< /i>*. Springer-Verlag
402 Berlin Heidelberg, pp. 283–299.
- 403 Allaire, J.J., Xie, Y., McPherson, J., Luraschi, J., Ushey, K., Atkins, A., et al. (2019)
404 rmarkdown: Dynamic Documents for R.
- 405 Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015) Minor revision to V4 region
406 SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic*
407 *Microbial Ecology* **75**: 129–137.
- 408 Armstrong, E., Rogerson, A., and Leftley, J. (2000) The Abundance of Heterotrophic Protists
409 Associated with Intertidal Seaweeds. *Estuarine, Coastal and Shelf Science* **50**: 415–424.
- 410 Bengtsson, M.M., Bühler, A., Brauer, A., Dahlke, S., Schubert, H., and Blindow, I. (2017)
411 Eelgrass Leaf Surface Microbiomes Are Locally Variable and Highly Correlated with Epibiotic
412 Eukaryotes. *Frontiers in Microbiology* **8**: 1312.
- 413 Bengtsson, M., Sjøtun, K., and Øvreås, L. (2010) Seasonal dynamics of bacterial biofilms on
414 the kelp *< i>Laminaria hyperborea< /i>*. *Aquatic Microbial Ecology* **60**: 71–83.
- 415 Borges, A.V. and Champenois, W. (2015) Seasonal and spatial variability of dimethylsulfoniopropionate
416 (DMSP) in the Mediterranean seagrass *< i>Posidonia oceanica< /i>*. *Aquatic Botany* **125**: 72–79.
- 417 Burke, C., Kjelleberg, S., and Thomas, T. (2009) Selective Extraction of Bacterial DNA from
418 the Surfaces of Macroalgae. *Applied and Environmental Microbiology* **75**: 252–256.
- 419 Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011a) Bacterial
420 community assembly based on functional genes rather than species. *Proceedings of the National*

- 421 *Academy of Sciences of the United States of America* **108**: 14288–14293.
- 422 Burke, C., Thomas, T., Lewis, M., Steinberg, P., and Kjelleberg, S. (2011b) Composition,
423 uniqueness and variability of the epiphytic bacterial community of the green alga *< i>Ulva*
424 *australis*< /i>. *The ISME Journal* **5**: 590–600.
- 425 Burns, B.P., Goh, F., Allen, M., and Neilan, B.A. (2004) Microbial diversity of extant
426 stromatolites in the hypersaline marine environment of Shark Bay, Australia. *Environmental*
427 *Microbiology* **6**: 1096–1101.
- 428 Cai, X., Gao, G., Yang, J., Tang, X., Dai, J., Chen, D., and Song, Y. (2014) An ultrasonic
429 method for separation of epiphytic microbes from freshwater submerged macrophytes. *Journal of*
430 *Basic Microbiology* **54**: 758–761.
- 431 Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al.
432 (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq
433 platforms. *The ISME Journal* **6**: 1621–1624.
- 434 Cardoso, D.C., Cretoiu, M.S., Stal, L.J., and Bolhuis, H. (2019) Seasonal development of a
435 coastal microbial mat. *Scientific Reports* **9**: 9035.
- 436 Crump, B.C. and Koch, E.W. (2008) Attached Bacterial Populations Shared by Four Species
437 of Aquatic Angiosperms. *Applied and Environmental Microbiology* **74**: 5948–5957.
- 438 Crump, B.C., Wojahn, J.M., Tomas, F., and Mueller, R.S. (2018) Metatranscriptomics and
439 Amplicon Sequencing Reveal Mutualisms in Seagrass Microbiomes. *Frontiers in Microbiology* **9**:
440 388.
- 441 Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., and Thomas, T. (2013) The
442 seaweed holobiont: understanding seaweed-bacteria interactions. *FEMS Microbiology Reviews*
443 **37**: 462–476.

- 444 Fahimipour, A.K., Kardish, M.R., Lang, J.M., Green, J.L., Eisen, J.A., and Stachowicz,
445 J.J. (2017) Global-Scale Structure of the Eelgrass Microbiome. *Applied and Environmental*
446 *Microbiology* **83**: e03391–16.
- 447 Gilbert, J.A., Field, D., Swift, P., Newbold, L., Oliver, A., Smyth, T., et al. (2009) The
448 seasonal structure of microbial communities in the Western English Channel. *Environmental*
449 *Microbiology* **11**: 3132–3139.
- 450 Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbrück, L., Reeder, J., Temperton, B., et al.
451 (2012) Defining seasonal marine microbial community dynamics. *The ISME Journal* **6**: 298–308.
- 452 Higashioka, Y., Kojima, H., Watanabe, T., and Fukui, M. (2015) Draft genome sequence of
453 *< i>Desulfatitalea tepidiphila</i> S28bF^T*. *Genome Announcements* **3**: e01326–15.
- 454 Hollants, J., Leliaert, F., De Clerck, O., and Willems, A. (2013) What we can learn from
455 sushi: a review on seaweed-bacterial associations. **83**: 1–16.
- 456 Jost, L. (2006) Entropy and diversity. *Oikos* **113**: 363–375.
- 457 Jørgensen, B.B. and Gallardo, V.A. (1999) *< i>Thioploca</i>* spp.: filamentous sulfur
458 bacteria with nitrate vacuoles. *FEMS Microbiology Ecology* **28**: 301–313.
- 459 Korlević, M., Markovski, M., Zhao, Z., Herndl, G.J., and Najdek, M. Selective DNA and
460 Protein Isolation from Marine Macrophyte Surfaces.
- 461 Korlević, M., Pop Ristova, P., Garić, R., Amann, R., and Orlić, S. (2015) Bacterial Diversity in
462 the South Adriatic Sea during a Strong, Deep Winter Convection Year. *Applied and Environmental*
463 *Microbiology* **81**: 1715–1726.
- 464 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013)
465 Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon
466 Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and environmental*

- 467 *microbiology* **79**: 5112–5120.
- 468 Kuever, J. (2014) The Family Desulfobulbaceae. In, *The prokaryotes: <i>Deltaproteobacteria</i> and <i>Epsilonproteobacteria</i>*. Springer-Verlag Berlin Heidelberg, pp. 75–86.
- 470 Lachnit, T., Blümel, M., Imhoff, J.F., and Wahl, M. (2009) Specific epibacterial communities
471 on macroalgae: phylogeny matters more than habitat. *Aquatic Biology* **5**: 181–186.
- 472 Lachnit, T., Meske, D., Wahl, M., Harder, T., and Schmitz, R. (2011) Epibacterial community
473 patterns on marine macroalgae are host-specific but temporally variable. *Environmental
474 Microbiology* **13**: 655–665.
- 475 Longford, S., Tujula, N., Crocetti, G., Holmes, A., Holmström, C., Kjelleberg, S., et al. (2007)
476 Comparisons of diversity of bacterial communities associated with three sessile marine eukaryotes.
477 *Aquatic Microbial Ecology* **48**: 217–229.
- 478 Margulies, L. (1991) Symbiogenesis and Symbiontism. In, Margulies, L. and Fester, R. (eds),
479 *Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis*. Cambridge,
480 Massachusetts: The MIT Press, pp. 1–14.
- 481 Massana, R., Murray, A.E., Preston, C.M., and DeLong, E.F. (1997) Vertical Distribution
482 and Phylogenetic Characterization of Marine Planktonic *Archaea* in the Santa Barbara
483 Channel. *Applied and environmental microbiology* **63**: 50–56.
- 484 McIlroy, S.J. and Nielsen, P.H. (2014) The Family *Saprospiraceae*. In, *The
485 prokaryotes: Other major lineages of <i>Bacteria</i> and the <i>Archaea</i>*. Springer-Verlag
486 Berlin Heidelberg, pp. 863–889.
- 487 Michelou, V.K., Caporaso, J.G., Knight, R., and Palumbi, S.R. (2013) The Ecology of
488 Microbial Communities Associated with *Macrocystis pyrifera*. *PloS one* **8**: e67480.
- 489 Miranda, L.N., Hutchison, K., Grossman, A.R., and Brawley, S.H. (2013) Diversity and

490 Abundance of the Bacterial Community of the Red Macroalga *Porphyra umbilicalis*: Did Bacterial
491 Farmers Produce Macroalgae? *PloS one* **8**: e58269.

492 Mobberley, J.M., Ortega, M.C., and Foster, J.S. (2012) Comparative microbial diversity
493 analyses of modern marine thrombolic mats by barcoded pyrosequencing. *Environmental*
494 *Microbiology* **14**: 82–100.

495 Morrissey, K.L., Çavas, L., Willems, A., and De Clerck, O. (2019) Disentangling the
496 Influence of Environment, Host Specificity and Thallus Differentiation on Bacterial Communities
497 in Siphonous Green Seaweeds. *Frontiers in Microbiology* **10**: 717.

498 Neuwirth, E. (2014) RColorBrewer: ColorBrewer Palettes.

499 Nõges, T., Luup, H., and Feldmann, T. (2010) Primary production of aquatic macrophytes
500 and their epiphytes in two shallow lakes (Peipsi and Võrtsjärv) in Estonia. *Aquatic Ecology* **44**:
501 83–92.

502 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019)
503 vegan: Community Ecology Package.

504 Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016) Every base matters: assessing small
505 subunit rRNA primers for marine microbiomes with mock communities, time series and global
506 field samples. *Environmental Microbiology* **18**: 1403–1414.

507 Pujalte, M.J., Lucena, T., Ruvira, M.A., Arahal, D.R., and Macián, M.C. (2014)
508 The Family Rhodobacteraceae. In, *The prokaryotes: <i>Alphaproteobacteria</i> and*
509 *<i>Betaproteobacteria</i>*. Springer-Verlag Berlin Heidelberg, pp. 439–512.

510 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA
511 ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic*
512 *Acids Research* **41**: D590–D596.

- 513 R Core Team (2019) R: A Language and Environment for Statistical Computing, Vienna,
514 Austria: R Foundation for Statistical Computing.
- 515 Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D.K.A., Thums, M., et al. (2014)
516 Millimeter-Sized Marine Plastics: A New Pelagic Habitat for Microorganisms and Invertebrates.
517 *PloS one* **9**: e100289.
- 518 Reyes, J. and Sansón, M. (2001) Biomass and Production of the Epiphytes on the Leaves of
519 *Cymodocea nodosa* in the Canary Islands. *Botanica Marina* **44**: 307–313.
- 520 Rickert, E., Wahl, M., Link, H., Richter, H., and Pohnert, G. (2016) Seasonal Variations in
521 Surface Metabolite Composition of *Fucus vesiculosus* and *Fucus serratus* from the Baltic Sea. *PloS*
522 *one* **11**: e0168196.
- 523 Roth-Schulze, A.J., Zozaya-Valdés, E., Steinberg, P.D., and Thomas, T. (2016) Partitioning of
524 functional and taxonomic diversity in surface-associated microbial communities. *Environmental*
525 *microbiology* **18**: 4391–4402.
- 526 Ruitton, S., Verlaque, M., and Boudouresque, C.F. (2005) Seasonal changes of the introduced
527 *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its
528 Mediterranean range. *Aquatic Botany* **82**: 55–70.
- 529 Salaün, S., Barre, S. la, Santos-Goncalvez, M.D., Potin, P., Haras, D., and Bazire, A. (2012)
530 Influence of Exudates of the Kelp *Laminaria Digitata* on Biofilm Formation of Associated and
531 Exogenous Bacterial Epiphytes. *Microbial Ecology* **64**: 359–369.
- 532 Schloss, P.D., Jenior, M.L., Koumpouras, C.C., Westcott, S.L., and Highlander, S.K. (2016)
533 Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. *PeerJ*
534 **4**: e1869.
- 535 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al.

536 (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software
537 for Describing and Comparing Microbial Communities. *Applied and environmental microbiology*
538 **75**: 7537–7541.

539 Tarquinio, F., Hyndes, G.A., Laverock, B., Koenders, A., and Säwström, C. (2019) The
540 seagrass holobiont: understanding seagrass-bacteria interactions and their role in seagrass
541 ecosystem functioning. *FEMS Microbiology Letters* **366**: fnz057.

542 Tujula, N.A., Crocetti, G.R., Burke, C., Thomas, T., Holmström, C., and Kjelleberg, S. (2010)
543 Variability and abundance of the epiphytic bacterial community associated with a green marine
544 *< i>Ulvacean</i>* alga. *The ISME Journal* **4**: 301–311.

545 Ugarelli, K., Laas, P., and Stingl, U. (2019) The Microbial Communities of Leaves and Roots
546 Associated with Turtle Grass (*< i>Thalassia testudinum</i>*) and Manatee Grass (*< i>Syringodium
547 filliforme</i>*) are Distinct from Seawater and Sediment Communities, but Are Similar between
548 Species and Sampling Si. *Microorganisms* **7**: 4.

549 Uku, J., Björk, M., Bergman, B., and Díez, B. (2007) CHARACTERIZATION AND
550 COMPARISON OF PROKARYOTIC EPIPHYTES ASSOCIATED WITH THREE EAST
551 AFRICAN SEAGRASSES. *Journal of Phycology* **43**: 768–779.

552 Weidner, Arnold, Stackebrandt, and Pühler (2000) Phylogenetic Analysis of Bacterial
553 Communities Associated with Leaves of the Seagrass *< i>Halophila stipulacea</i>* by a
554 Culture-Independent Small-Subunit rRNA Gene Approach. *Microbial Ecology* **39**: 22–31.

555 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., et al. (2019)
556 Welcome to the Tidyverse. *Journal of Open Source Software* **4**: 1686.

557 Xie, Y. (2015) Dynamic Documents with R and knitr, 2nd ed. Boca Raton, Florida: Chapman;
558 Hall/CRC.

- 559 Xie, Y. (2014) knitr: A Comprehensive Tool for Reproducible Research in R. In, Stodden,V.,
560 Leisch,F., and Peng,R.D. (eds), *Implementing reproducible computational research*. Chapman;
561 Hall/CRC.
- 562 Xie, Y. (2019a) knitr: A General-Purpose Package for Dynamic Report Generation in R.
- 563 Xie, Y. (2019b) TinyTeX: A lightweight, cross-platform, and easy-to-maintain LaTeX
564 distribution based on TeX Live. *TUGboat* 30–32.
- 565 Xie, Y. (2020) tinytex: Helper Functions to Install and Maintain 'TeX Live', and Compile
566 'LaTeX' Documents.
- 567 Xie, Y., Allaire, J.J., and Grolemund, G. (2018) R Markdown: The Definitive Guide, Boca
568 Raton, Florida: Chapman; Hall/CRC.
- 569 Xie, Z., Lin, W., and Luo, J. (2017) Comparative Phenotype and Genome Analysis of
570 *< i>Cellvibrio</i> sp. PR1*, a Xylanolytic and Agarolytic Bacterium from the Pearl River.
571 *BioMed Research International* **2017**: 6304248.
- 572 Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al. (2014) The SILVA
573 and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research* **42**:
574 D643–D648.
- 575 Zavodnik, N., Travizi, A., and De Rosa, S. (1998) Seasonal variations in the rate of
576 photosynthetic activity and chemical composition of the seagrass *< i>Cymodocea nodosa</i>*
577 (Ucr.) *Asch. Scientia Marina* **62**: 301–309.
- 578 Zhu, H. (2019) kableExtra: Construct Complex Table with 'kable' and Pipe Syntax.

579 **Figure Captions**

580 **Figure 1.** Proportion of shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient)
581 and shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) between
582 assemblages associated with the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea*
583 [Mixed and Monospecific]) and communities in the ambient seawater.

584 **Figure 2.** Proportion of shared bacterial and archaeal communities (Bray-Curtis Similarity
585 Coefficient) and shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) between
586 consecutive sampling points and from the surfaces of macrophytes (*C. nodosa* [Mixed] and *C.*
587 *cylindracea* [Mixed and Monospecific]) and in the ambient seawater.

588 **Figure 3.** Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU
589 abundances of bacterial and archaeal communities from the surfaces of macrophytes (*C. nodosa*
590 [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. Samples
591 from the same environment or same season are labeled in different colors. The proportion of
592 explained variation by each axis is shown on the corresponding axis in parentheses.

593 **Figure 4.** Taxonomic classification and relative contribution of the most abundant (> 1 %) bacterial
594 and archaeal sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea*
595 [Mixed and Monospecific]) and in the ambient seawater. NR – No Relative

596 **Figure 5.** Taxonomic classification and relative contribution of the most abundant (> 1 %)
597 cyanobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea*
598 [Mixed and Monospecific]) and in the ambient seawater. The proportion of cyanobacterial
599 sequences in the total bacterial and archaeal community is given above the corresponding bar. NR
600 – No Relative

601 **Figure 6.** Taxonomic classification and relative contribution of the most abundant (> 2 %)
602 sequences within the *Bacteroidota* on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C.*

603 *cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of sequences
604 classified as *Bacteroidota* in the total bacterial and archaeal community is given above the
605 corresponding bar. NR – No Relative

606 **Figure 7.** Taxonomic classification and relative contribution of the most abundant (> 2 %)
607 alphaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C.*
608 *cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of
609 alphaproteobacterial sequences in the total bacterial and archaeal community is given above the
610 corresponding bar. NR – No Relative

611 **Figure 8.** Taxonomic classification and relative contribution of the most abundant (> 2 %)
612 gammaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and
613 *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of
614 gammaproteobacterial sequences in the total bacterial and archaeal community is given above the
615 corresponding bar. NR – No Relative

616 **Figure 9.** Taxonomic classification and relative contribution of the most abundant (> 1 %)
617 sequences within the *Desulfobacterota* on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C.*
618 *cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of sequences
619 classified as *Desulfobacterota* in the total bacterial and archaeal community is given above the
620 corresponding bar. NR – No Relative

621 **Figures**

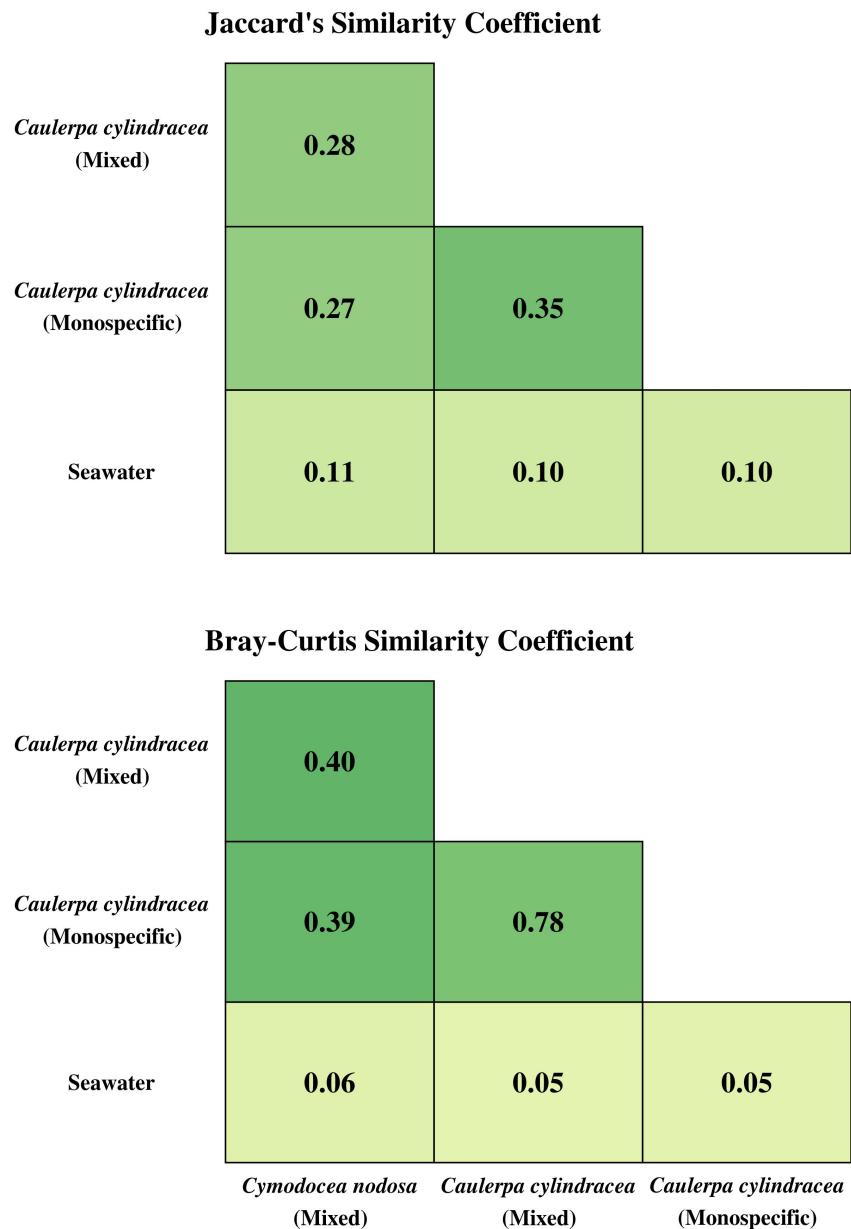


Figure 1. Proportion of shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) and shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) between assemblages associated with the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and communities in the ambient seawater.

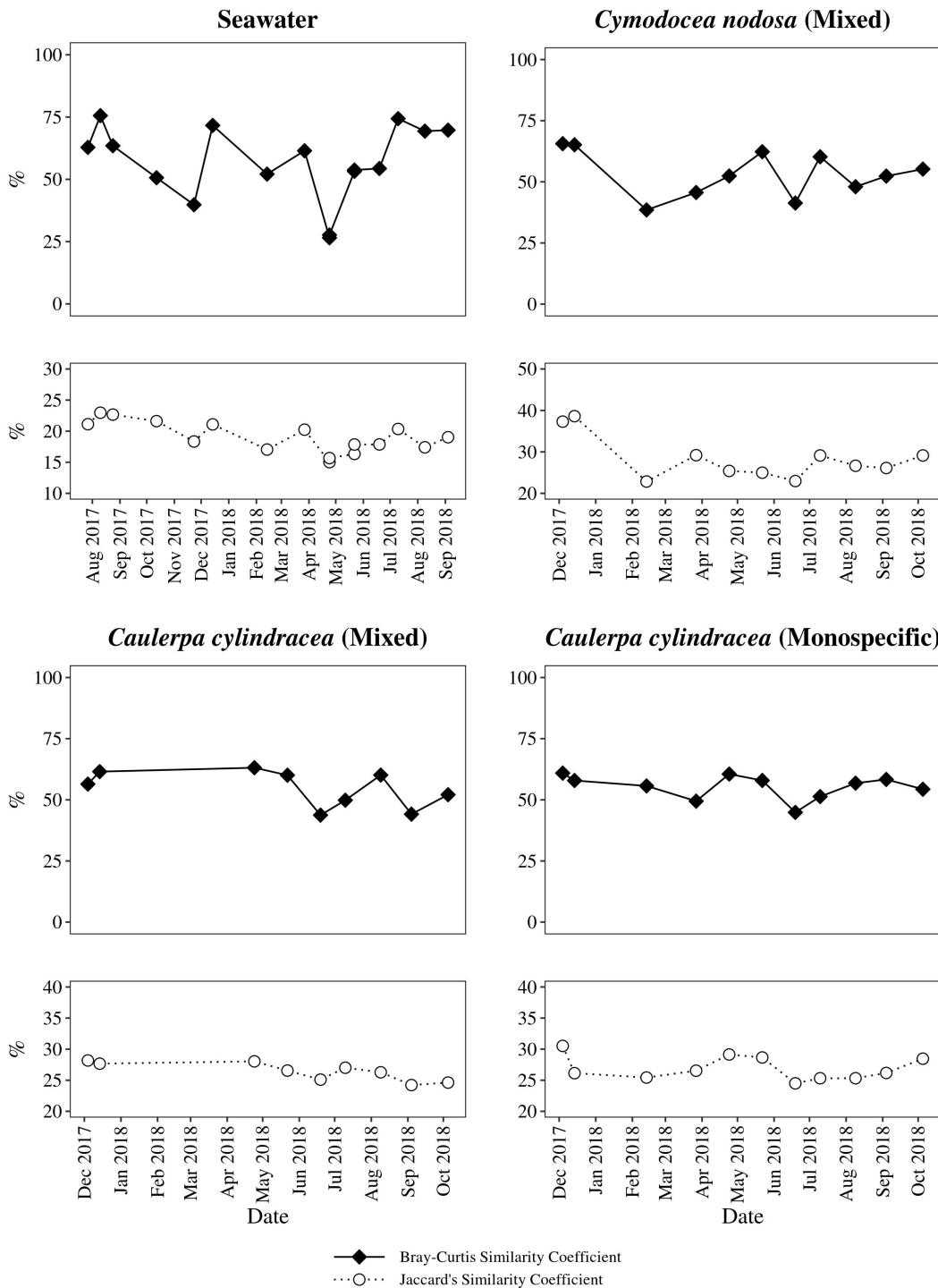


Figure 2. Proportion of shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) and shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) between consecutive sampling points and from the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater.

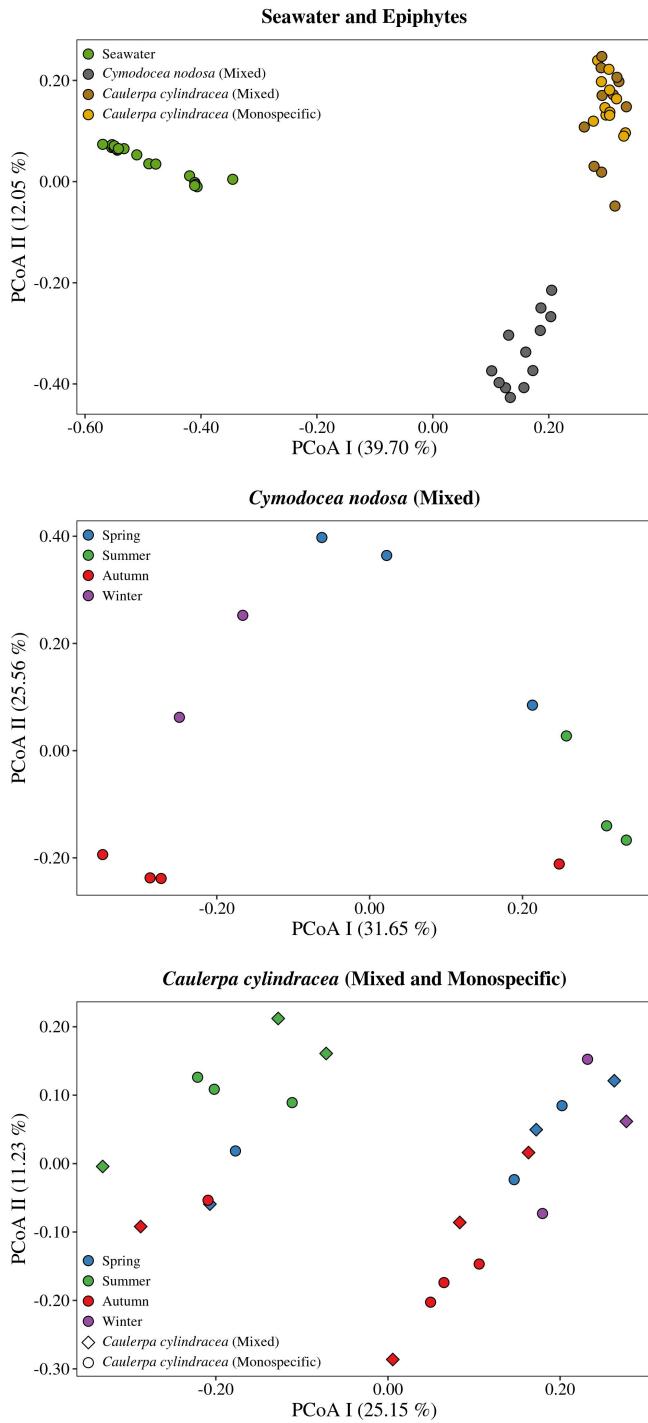


Figure 3. Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities from the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. Samples from the same environment or same season are labeled in different colors. The proportion of explained variation by each axis is shown on the corresponding axis in parentheses.

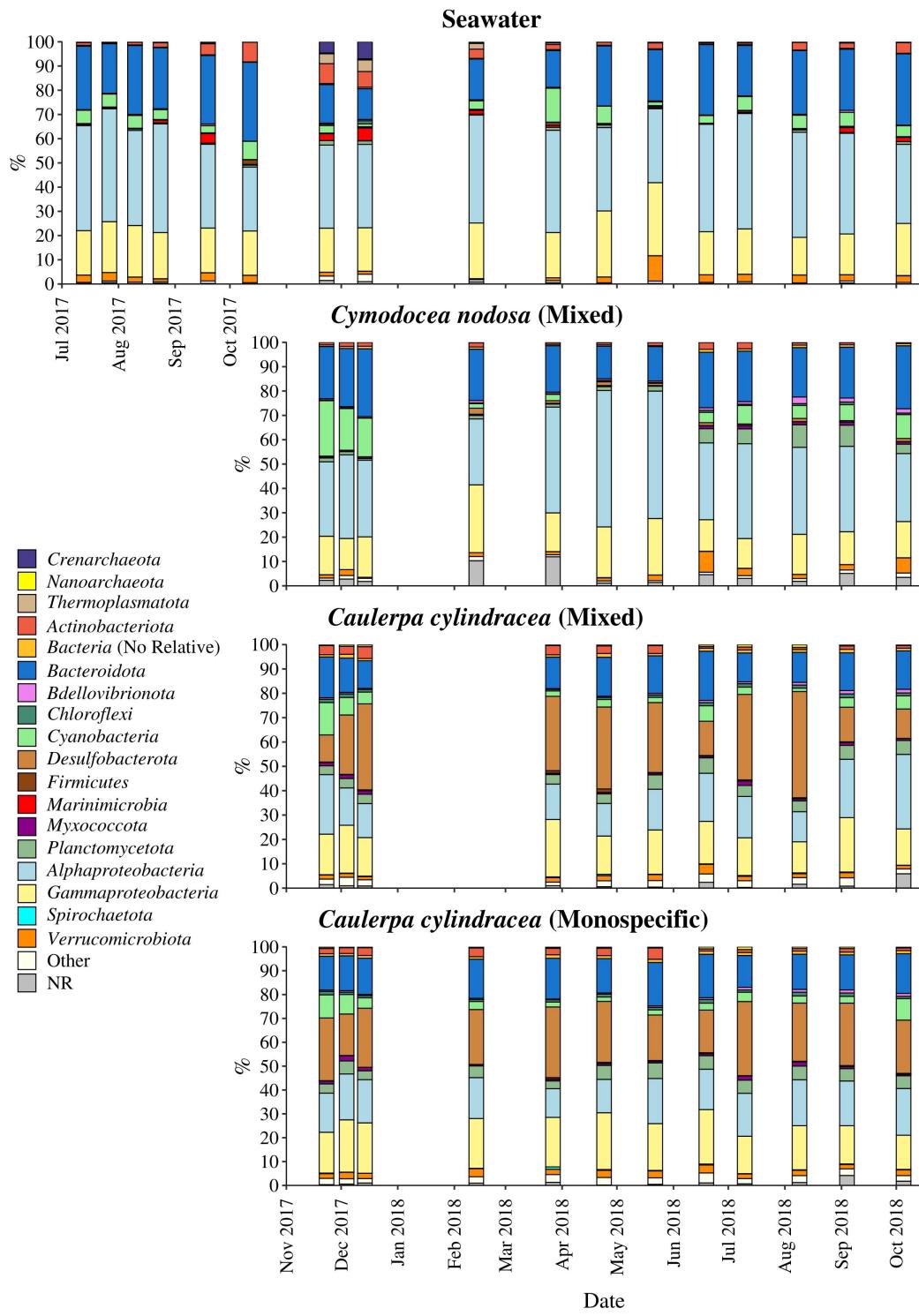


Figure 4. Taxonomic classification and relative contribution of the most abundant (> 1 %) bacterial and archaeal sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. NR – No Relative

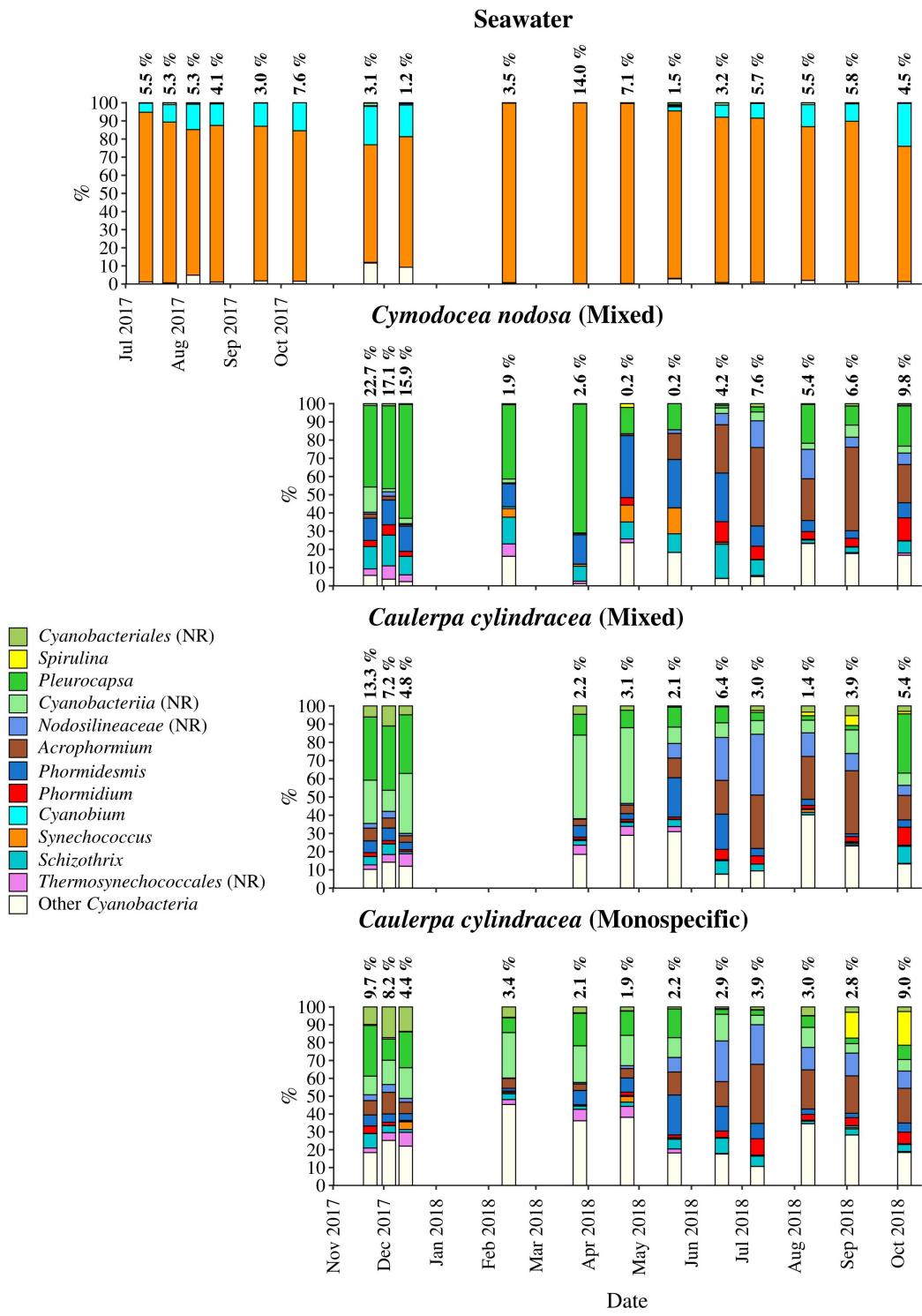


Figure 5. Taxonomic classification and relative contribution of the most abundant (> 1 %) cyanobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of cyanobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative

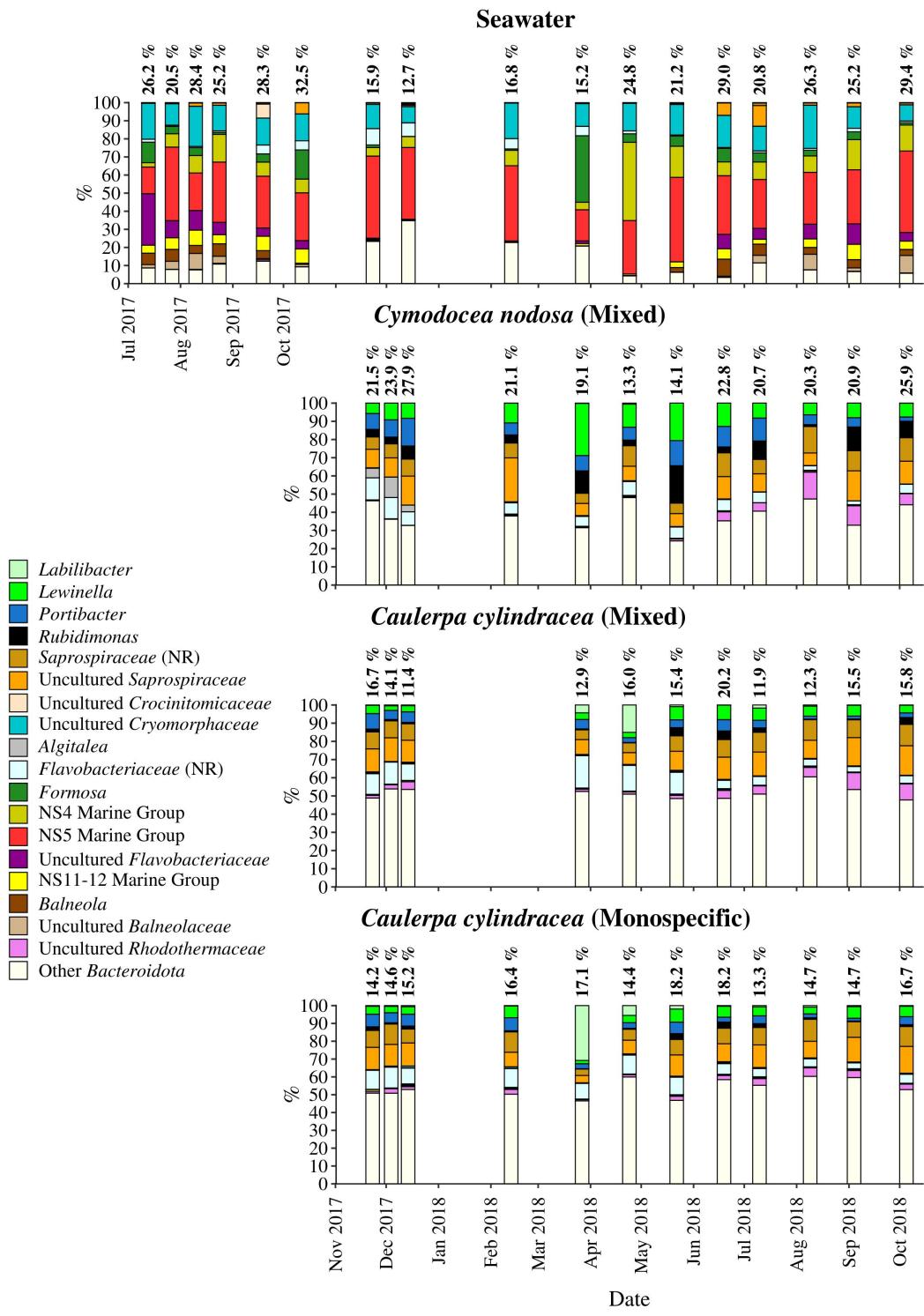


Figure 6. Taxonomic classification and relative contribution of the most abundant (> 2 %) sequences within the *Bacteroidota* on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of sequences classified as *Bacteroidota* in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative

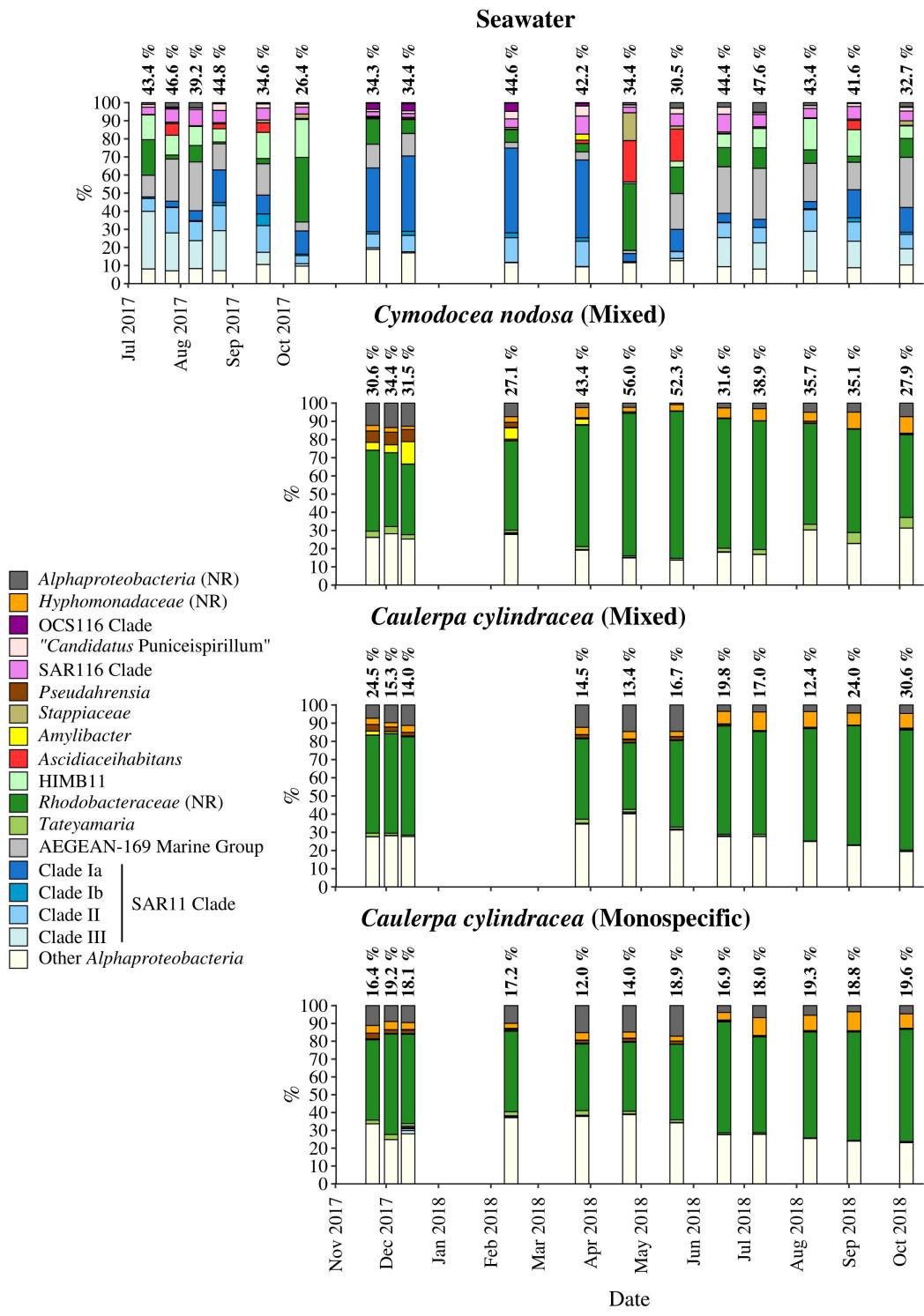


Figure 7. Taxonomic classification and relative contribution of the most abundant (> 2 %) alphaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of alphaproteobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative

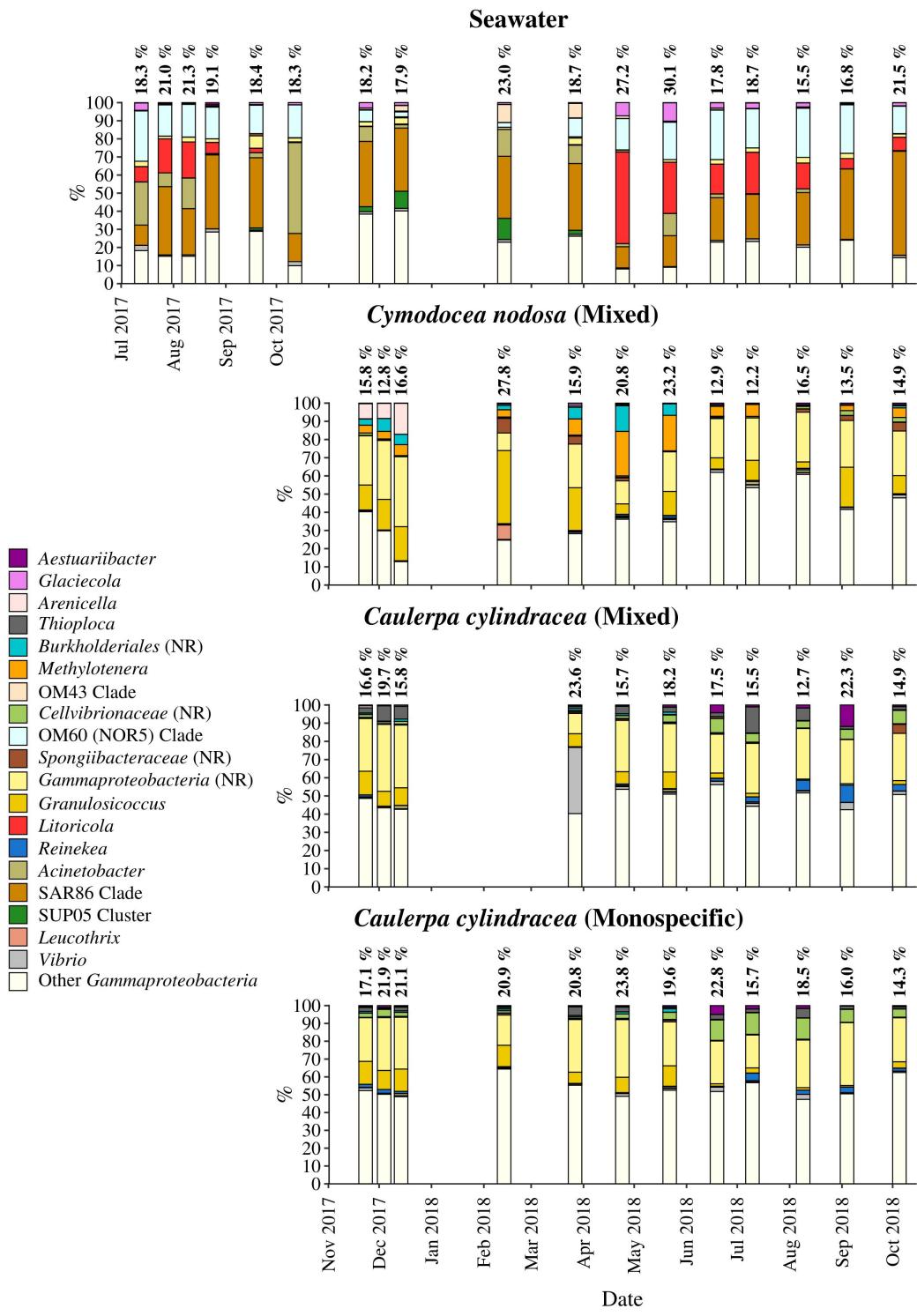


Figure 8. Taxonomic classification and relative contribution of the most abundant (> 2 %) gammaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of gammaproteobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative

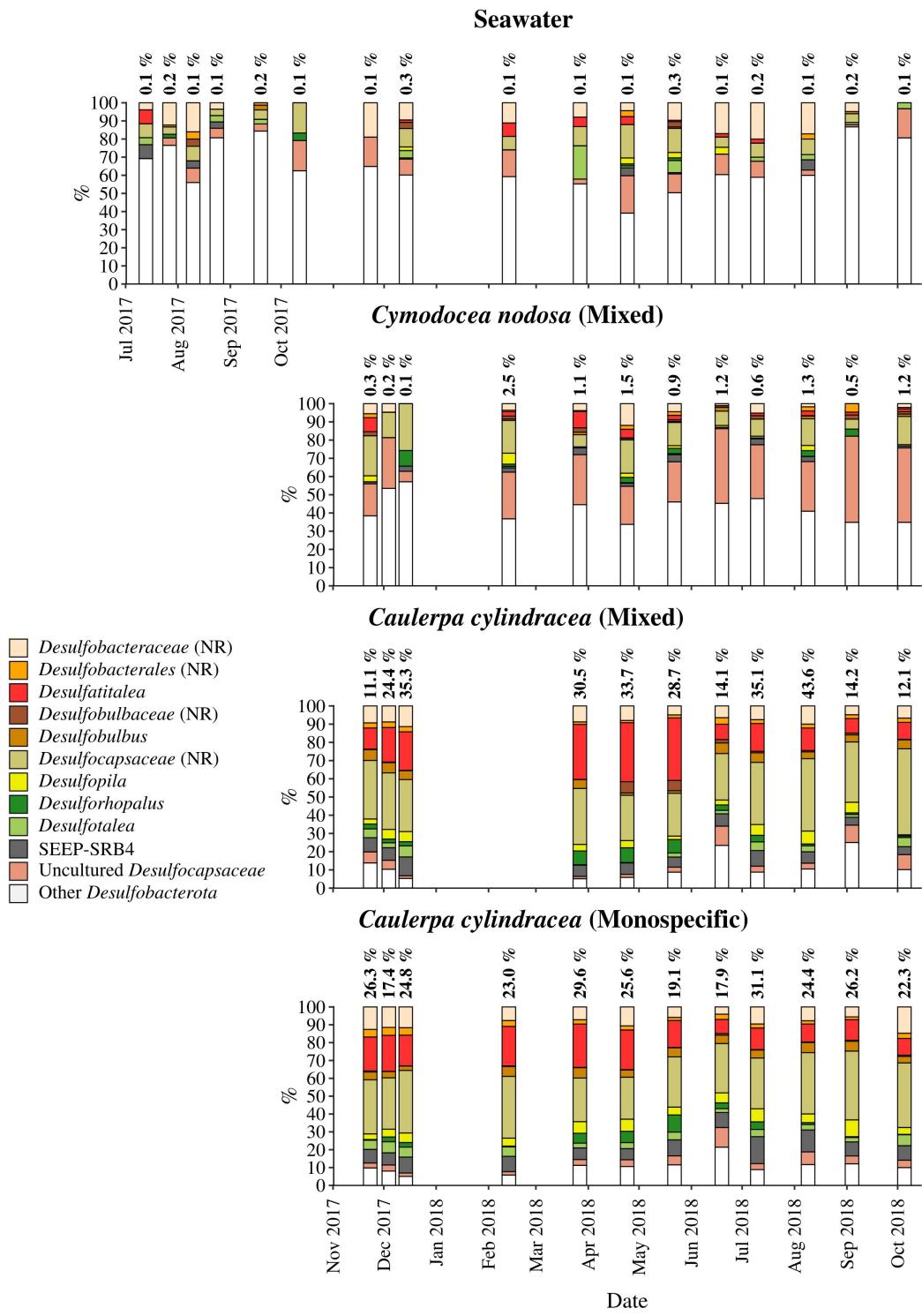


Figure 9. Taxonomic classification and relative contribution of the most abundant (> 1 %) sequences within the *Desulfobacterota* on the surfaces of macrophytes (*C. nodosa* [Mixed] and *C. cylindracea* [Mixed and Monospecific]) and in the ambient seawater. The proportion of sequences classified as *Desulfobacterota* in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative