

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

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## 1 **Abstract**

## 2 Introduction

3 Marine macrophytes (seagrasses and macroalgae) are important ecosystem engineers that  
4 form close associations with microorganism belonging to all three domains of life (Egan *et al.*,  
5 2013; Tarquinio *et al.*, 2019). Microbes can live within macrophyte tissue as endophytes or can  
6 form epiphytic communities on surfaces of leaves and thalli (Egan *et al.*, 2013; Hollants *et al.*,  
7 2013; Tarquinio *et al.*, 2019). Epiphytic and endophytic microbial communities form a close  
8 functional relationship with the macrophyte host. It was proposed that this close relationship  
9 constitutes a holobiont, an integrated community where the macrophyte organism and its symbiotic  
10 partners support each other (Margulis, 1991; Egan *et al.*, 2013; Tarquinio *et al.*, 2019).

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

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

27 at lower taxonomic ranks host specific microbial communities were described (Lachnit *et al.*,  
28 2011; Roth-Schulze *et al.*, 2016). Recently, it was shown that even different morphological niches  
29 within the same alga had a higher influence on bacterial community variation than biogeography  
30 or environmental factors (Morrissey *et al.*, 2019). While there is high community variation  
31 between host species is was observed that the majority of metagenome determined functions were  
32 conserved both between host species and individuals (Burke *et al.*, 2011a; Roth-Schulze *et al.*,  
33 2016). This discrepancy between taxonomic and functional composition could be explained by  
34 the lottery hypothesis. It postulates that an initial random colonization step is performed from  
35 a set of functionally equivalent taxonomic groups resulting in taxonomically different epiphytic  
36 communities sharing a core set of functional genes (Burke *et al.*, 2011a; Roth-Schulze *et al.*,  
37 2016). In addition, some of the variation in the observed data could be attributed to different  
38 techniques used in various studies, such as different protocols for epiphytic cell detachment and/or  
39 DNA isolation, as no standard protocol to study epiphytic communities was established (Ugarelli  
40 *et al.*, 2019; Korlević *et al.*, submitted).

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

52 **Results**

53 Sequencing of the 16S rRNA V4 region yielded a total of 1.8 million sequences after  
54 quality curation and exclusion of eukaryotic, chloroplast, mitochondrial and no relative sequences  
55 (Table S1). A total of 35 samples originating from epiphytic archaeal and bacterial communities  
56 associated with surfaces of the seagrass *Cymodocea nodosa* and macroalga *Caulerpa cylindracea*  
57 were analyzed. In addition, 18 samples (one of the samples was sequenced two times) originating  
58 from picoplankton archaeal and bacterial communities in the surrounding seawater were also  
59 processed. The number of reads per sample ranged between 8,407 and 77,465 sequences  
60 (Table S1). Even when the highest sequencing effort was applied the rarefaction curves did  
61 not level off that is a common observation in high-throughput 16S rRNA amplicon sequencing  
62 approaches (Figure S1). Following quality curation and exclusion of sequences mentioned before  
63 reads were clustered into 28,726 different OTUs at a similarity level of 97 %. Reads numbers  
64 were normalized to the minimum number of sequences, 8,407 (Table S1), through rarefaction  
65 resulting in 17,007 different OTUs that ranged from 366 to 1,998 OTUs per sample (Figure S2).  
66 To determine seasonal changes of richness and diversity the Observed Number of OTUs, Chao1,  
67 ACE, Exponential Shannon (Jost, 2006) and Inverse Simpson were calculated after normalization  
68 through rarefaction. Generally, richness estimators and diversity indices showed similar trends. On  
69 average, higher values were found for *Caulerpa cylindracea* (invaded [Number of OTUs, 1,688.5  
70  $\pm$  125.6 OTUs] and noninvaded [Number of OTUs, 1,744.8  $\pm$  150.8 OTUs]), middle values for  
71 *Cymodocea nodosa* (Number of OTUs, 1,062.8  $\pm$  209.6 OTUs) and lower values for picoplankton  
72 communities in the surrounding seawater (Number of OTUs, 528.1  $\pm$  135.8 OTUs) (Figure S2).  
73 Seasonal changes did not show such large dissimilarities. Seawater communities richness was  
74 stable during the studied period with the exception of one sampling point in December 2017  
75 when larger values were observed. *Cymodocea nodosa* communities showed a slow increase  
76 towards the end of the study, while *Caulerpa cylindracea* (invaded and noninvaded) communities  
77 were characterized by slightly larger values in Spring and Summer in comparison to Autumn and

78 Winter (Figure S2).

79 To determine the proportion of shared archaeal and bacterial OTUs and communities sampled  
80 in different environments the Jaccard's Similarity Coefficient on presence-absence data and  
81 Bray-Curtis Similarity Coefficient were, respectively, calculated. Coefficients were determined  
82 after normalization through rarefaction and binning of samples from a particular environment. The  
83 highest proportion of shared OTUs and community was found between invaded and noninvaded  
84 *Caulerpa cylindracea* (Jaccard, 0.35; Bray-Curtis, 0.77), while lower shared values were  
85 calculated between seawater and epiphytic communities (Figure 1). Shared proportion between  
86 *Cymodocea nodosa* and *Caulerpa cylindracea* were approximately in the middle between these  
87 two extremes. To assess seasonal changes in the proportion of shared OTUs and communities the  
88 Jaccard's and Bray-Curtis Similarity Coefficients were calculated between consecutive sampling  
89 points (Figure 2). Both coefficients showed similar trends. Temporal proportional changes were  
90 more pronounced for seawater in comparison to *Cymodocea nodosa* and especially *Caulerpa*  
91 *cylindracea* associated communities (Figure 2). In addition, only 0.4 – 1.0 % of OTUs from  
92 each surface associated community were found at every time point. These OTUs made also a  
93 high proportion of total sequences (39.1 – 51.3 %). To further disentangle the environmental and  
94 seasonal community dissimilarity a Principal Coordinates Analysis (PCoA) based on Bray-Curtis  
95 distances and OTU abundances was applied. It showed a clear separation between planktonic  
96 and surface associated communities (Figure 3). In addition, a separation of epiphytic bacterial  
97 and archaeal communities based on host species was determined. This separation was further  
98 supported by ANOSIM ( $R = 0.96, p < 0.001$ ). Seasonal changes of seawater communities  
99 indicated a separation between Spring, Summer and Autumn/Winter samples (ANOSIM,  $R =$   
100 0.64,  $p < 0.001$ ). Epiphytic microbial communities associated with *Cymodocea nodosa* showed a  
101 similar pattern (ANOSIM,  $R = 0.56, p < 0.01$ ), while communities from the surfaces of *Caulerpa*  
102 *cylindracea* indicated a non so strongly supported, as in previous cases, separation between  
103 Summer and Autumn/Winter/Spring samples (ANOSIM,  $R = 0.31, p < 0.05$ ) (Figure 3).

104 The taxonomic composition of both, macrophyte associated and seawater communities,  
105 was dominated by bacterial ( $99.1 \pm 2.1 \%$ ) over archaeal sequences ( $0.9 \pm 2.1 \%$ ) (Figure 4).  
106 Higher relative abundances of chloroplast related sequences were only observed in surface  
107 associated communities, with higher values in Autumn/Winter ( $37.2 \pm 11.2 \%$ ) in comparison to  
108 Spring/Summer ( $20.9 \pm 9.7 \%$ ) (Figure S3). Generally, at higher taxonomic ranks (phylum-class)  
109 epiphytic and seawater microbial communities were composed of similar bacterial taxa.  
110 Seawater communities were mainly comprised of *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*,  
111 *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota*. Communities associated  
112 with *Cymodocea nodosa* were consisted of same groups with the addition of *Planctomycetota*  
113 whose contribution was higher in summer 2018. In addition, communities from invaded and  
114 noninvaded *Caulerpa cylindracea* were similar and characterized by same groups as seawater  
115 and *Cymodocea nodosa* communities with the addition of *Desulfobacterota* (Figure 4). Larger  
116 differences between environments and host species could be observed at lower taxonomic ranks  
117 (Figure 5 – 9).

118 *Cyanobacteria* related sequences were comprising, on average,  $5.5 \pm 4.4 \%$  of total sequences  
119 (Figure 5). Higher proportions were found for *Cymodocea nodosa* ( $16.4 \pm 5.3 \%$ ) and *Caulerpa*  
120 *cylindracea* (invaded [ $7.7 \pm 3.9 \%$ ] and noninvaded [ $7.8 \pm 2.4 \%$ ]) associated communities  
121 in autumn and for seawater communities in winter ( $8.8 \pm 7.4 \%$ ). Large taxonomic differences  
122 between surface associated and seawater cyanobacterial communities were observed. Seawater  
123 communities were mainly comprised of *Cyanobium* and *Synechococcus*, while surface associated  
124 communities were consisted of *Pleurocapsa* and sequences without known relatives within  
125 *Cyanobacteriia* (Figure 5). In addition, seasonal changes in surface associated communities  
126 were observed with *Pleurocapsa* and no relative *Cyanobacteriia* comprising larger proportions in  
127 autumn and winter and *Acrophormium*, *Phormidesmis* and no relative *Nodosilineaceae* in spring  
128 and summer (Figure 5).

129 Sequences classified as *Bacteroidota* were comprising, on average,  $19.2 \pm 5.5 \%$  of all

130 sequences (Figure 6). Similarly to *Cyanobacteria*, large differences in the taxonomic composition  
131 between seawater and surface associated communities were found (Figure 6). The seawater  
132 community was characterized by the NS4 and NS5 marine groups, uncultured *Cryomorphaceae*,  
133 uncultured *Flavobacteriaceae*, NS11-12 marine group, *Balneola*, uncultured *Balneolaceae* and  
134 *Formosa*. In contrast, in surface associated communities *Lewinella*, *Portibacter*, *Rubidimonas*, no  
135 relative *Saprosiraceae*, uncultured *Saprosiraceae*, no relative *Flavobacteriaceae* and uncultured  
136 *Rhodothermaceae* were found. Some groups showed slight seasonal changes such as no relative  
137 *Flavobacteriaceae* that were more pronounced from November 2017 until June 2018. In contrast,  
138 uncultured *Rhodothermaceae* showed higher proportions from June 2018 until the end of the study  
139 period. Surface associated *Bacteroidota* communities were very diverse as could be observed in  
140 the the high proportion of taxa that grouped as other *Bacteroidota* (Figure 6).

141 On average, *Alphaproteobacteria* were in comparison to other high rank taxa the largest  
142 taxonomic group, comprising  $29.2 \pm 12.0$  % of all sequences (Figure 7). In accordance to previous  
143 taxa, high differences between seawater and surface associated communities were observed.  
144 Picoplankton communities were composed mainly of the SAR11 clade, AEGEAN-169 marine  
145 group, SAR116 clade, no relative *Rhodobacteraceae*, HIMB11 and OCS116 clade, while surface  
146 associated communities were composed in high proportion of no relative *Rhodobacteraceae* and to  
147 a lesser degree of *Pseudoahrensia*, no relative *Alphaproteobacteria*, no relative *Hyphomonadaceae*  
148 and *Amylibacter*. Representatives of no relative *Rhodobacteraceae* were comprising on average  
149  $40.6 \pm 23.2$  % of all alphaproteobacterial sequences from the epiphytic community (Figure 7).  
150 In addition, *Amylibacter* was detected mainly in *Cymodocea nodosa* from November 2017 until  
151 March 2018.

152 Sequences related to *Gammaproteobacteria* were comprising, on average,  $18.7 \pm 3.9$  % of all  
153 sequences (Figure 8). Similarly to previous taxa, large taxonomic differences between seawater  
154 and surface associated communities were found. Seawater communities were mainly comprised  
155 of the OM60 (NOR5) clade, *Litoricola*, *Acinetobacter* and the SAR86 clade, while epiphytic

156 communities were mainly composed of no relative *Gammaproteobacteria* and *Granulosicoccus*.  
157 Beside these two groups specific to all three epiphytic communities, *Cymodocea nodosa* was  
158 characterized by *Arenicella*, no relative *Burkholderiales* and *Methylotenera*, while *Thioploca*,  
159 no relative *Cellvibrionaceae* and *Reinekea* were more specific to both invaded and noninvaded  
160 *Caulerpa cylindracea*. In addition, *Arenicella* was more pronounced in November and December  
161 2017, while no relative *Burkholderiales* and *Methylotenera* were more characteristic for the  
162 period form March until May 2018. For the *Caulerpa cylindracea* specific taxa no relative  
163 *Cellvibrionaceae* and *Reinekea* showed some seasonality and were characterisitic for samples  
164 originating from June to October 2018. In addition, similarly to *Bacteroidota*, a large proportion  
165 of the surface associated community was grouped as other *Gammaproteobacteria* indicating high  
166 diversity within this group (Figure 8).

167 In contrast to previously described high rank taxa, *Desulfobacterota* were specific to  
168 *Caulerpa cylindracea*. On average they were comprising  $11.2 \pm 13.3$  % of all sequences. While  
169 seawater and *Cymodocea nodosa* communities were consisted of only  $0.1 \pm 0.08$  % and  $1.0 \pm 0.7$  % *Desulfobacterota* sequences, respectively, in the invaded and noninvaded *Caulerpa cylindracea* communities their proportion was  $25.7 \pm 11.3$  % and  $24.0 \pm 4.3$  %, respectively  
170 (Figure 9). In addition, *Caulerpa cylindracea* associated communities were characterized by  
171 higer proportions in Winter and Summer (invaded,  $30.9 \pm 12.4$  %; noninvaded,  $26.9 \pm 3.4$  %)  
172 in comparison to Autumn and Spring (invaded,  $22.8 \pm 10.3$  %; noninvaded,  $21.9 \pm 3.8$  %). The  
173 community was mainly consisted of no relative *Desulfobacteraceae*, *Desulfatitalea*, no relative  
174 *Desulfobulbaceae*, *Desulfobulbus*, no relative *Desulfocapsaceae*, *Desulfopila*, *Desulforhopalus*,  
175 *Desulfotalea*, SEEP-SRB4 and uncultured *Desulfocapsaceae* (Figure 9).

178 **Discussion**

179 Surfaces of marine macrophytes are harboring biofilms consisted of diverse microbial taxa  
180 (Egan *et al.*, 2013; Tarquinio *et al.*, 2019). No standard protocol has been developed to study  
181 these macophyte associated microbes (Ugarelli *et al.*, 2019). Different procedures of microbial  
182 cells removal from host surfaces were described, such as host tissue shaking (Nõges *et al.*, 2010),  
183 scraping (Uku *et al.*, 2007) and ultrasonication (Cai *et al.*, 2014). All these methods showed  
184 different removal efficiencies but none was enabling a complete removal of attached microbial  
185 cells. In the present study, we applied an earlier developed removal protocol (Korlević *et al.*,  
186 submitted), based on a previous idea of direct cellular lysis (Burke *et al.*, 2009), to ensure an  
187 almost complete cell detachment. The application of a direct lysis procedure coupled with a high  
188 frequency sampling protocol and Illumina high resolution amplicon sequencing has enabled us to  
189 make a detailed description of bacterial and archaeal communities associated with the surfaces of  
190 two marine macrophytes, *Cymodocea nodosa* and *Caulerpa cylindracea*.

191 In the present study, highest richness values were observed for *Caulerpa cylindracea* (invaded  
192 and noninvaded), middle for *Cymodocea nodosa* and lowest for seawater derived communities.  
193 Higher values for seagrass associated communities in comparison to seawater were described  
194 earlier and could be attributed to a larger set of inhabitable microniches existing on macrophyte  
195 surfaces (Ugarelli *et al.*, 2019). In addition, highest values observed for *Caulerpa cylindracea*  
196 are probably a consequence of part of epiphytic OTUs that are in contact with the sediment.  
197 *Caulerpa cylindracea* stolon is attached to the sediment surface with rhizoids, so the stolon and  
198 rhizoids are in a direct contact with the sediment. Part of the surface attached *Caulerpa cylindracea*  
199 community is therefore comprised of OTUs that are in contact with a different environment and that  
200 could cause the observed increase in richness. In addition, seasonal richness differences observed  
201 for surface attached communities showed slightly higher values in spring and summer. This  
202 pattern could be explained by a higher macrophyte growth in these seasons (M. Najdek, personal  
203 communication; Zavodnik *et al.*, 1998; Ruitton *et al.*, 2005). During active periods macrophytes

204 exhibit a more dynamic chemical interaction with the surface community probably causing an  
205 increase in the number of inhabitable microniches (Borges and Champenois, 2015; Rickert *et al.*,  
206 2016).

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

229 Beside differences between seawater and surface associated communities, there were

230 discussions if the prokaryotic epiphytic community is host-specific or there are generalists taxa  
231 characteristic to all or many macrophytes (Egan *et al.*, 2013). Similarly to previously described  
232 differences between seawater and surface attached communities, at high taxonomic ranks no  
233 strong differentiation between communities associated with different host was observed. The only  
234 high rank phylum that was differing between *Cymodocea nodosa* and *Caulerpa cylindracea* was  
235 *Desulfobacterota*, whose sequences were more abundant in the *Caulerpa cylindracea* associated  
236 community. As already mentioned, the rhizoids and part of the stolon are in contact with the  
237 sediment, so *Desulfobacterota* are probably a part of the epiphytic community that is in contact  
238 with the sediment. Similar high rank taxa found in this study were described to be specific for  
239 other species of macrophytes (Burke *et al.*, 2011b; Lachnit *et al.*, 2011; Bengtsson *et al.*, 2017). In  
240 contrast to high taxonomic ranks, a substantial differentiation between host specific communities  
241 was found, which supports the host-specific hypothesis. Similar host-specificity was observed for  
242 different species of macroalgae and seagrasses (Lachnit *et al.*, 2011; Roth-Schulze *et al.*, 2016;  
243 Ugarelli *et al.*, 2019; Morrissey *et al.*, 2019). Taken together, at high taxonomic ranks a core set of  
244 taxa could be described that is characteristic for all or many macrophytes, while at low taxonomic  
245 ranks a community specific to host species could be identified (Egan *et al.*, 2013).

246 Few studies describing microbial epiphytic communities were encompassing seasonal shifts  
247 (Tujula *et al.*, 2010; Lachnit *et al.*, 2011; Michelou *et al.*, 2013). Seasonal changes in the  
248 epiphytic community were substantial as could be observed in the proportion of OTUs that could  
249 be find at every sampling time ( $\leq 1.0\%$ ). Interestingly, these OTUs were accounting for a high  
250 proportion of sequences ( $\leq 51.3\%$ ). A very similar proportion of persistent OTUs and their  
251 sequence contribution was reported in high frequency studies describing seasonal picoplankton  
252 changes (Gilbert *et al.*, 2009, 2012). In comparison to the seawater community, a lower degree of  
253 seasonal shifts was observed for the surface associated communities. It seems, that microniches  
254 on the surfaces of macrophytes are providing more stable conditions in comparison to the  
255 seawater. At the level of OTUs seasonal changes of *Cymodocea nodosa* and *Caulerpa cylindracea*  
256 associated communities were identified that could be linked to the growth cycle of the seagrass

257 and macroalgae (M. Najdek, personal communication). *Cymodocea nodosa* was characterized  
258 by a Spring community during maximum seagrass proliferation, a Summer community during  
259 a biomass maximum and a Autumn/Winter community during a biomass decay. In contrast,  
260 *Caulerpa cylindracea* started to proliferate in late Spring and was characterized only by a Summer  
261 community during maximal biomass increase and by a Autumn/Winter/Spring community when  
262 the biomass were at the peak and the settlement started to subsequently decay. Similar seasonal  
263 changes in the epiphytic community was described also for other macroalgae (Tujula *et al.*, 2010;  
264 Lachnit *et al.*, 2011). The higher temporal stability of *Caulerpa cylindracea* surface communities  
265 in comparison to *Cymodocea nodosa* could be also observed in the higher proportion of shared  
266 communities between two consecutive sampling points.

267 **Experimental Procedures**

268 **Sampling**

269 Leaves of *Cymodocea nodosa* were sampled in a *Cymodocea nodosa* meadow located in the  
270 proximity of the village of Funtana (45°10'39" N, 13°35'42" E). Thalli of *Caulerpa cylindracea*  
271 were sampled in the same *Cymodocea nodosa* invaded meadow in Funtana and on a locality of  
272 only *Caulerpa cylindracea* located close to the invaded meadow. Sampling of leaves and thalli  
273 was performed approximately monthly from December 2017 to October 2018 (Table S1). Leaves  
274 and thalli were collected by diving and transported to the laboratory in containers placed on ice  
275 and filled with site seawater. Upon arrival to the laboratory, *Cymodocea nodosa* leaves were cut  
276 into sections of 1 – 2 cm, while *Caulerpa cylindracea* thalli were cut into 5 – 8 cm long sections.  
277 Leaves and thalli were washed three times with sterile artificial seawater (ASW) to remove loosely  
278 attached microbial cells. Surrounding seawater was collected in 10 l containers by diving and  
279 transported to the laboratory where the whole container volume was filtered through a 20 µm net.  
280 The filtrate was further sequentially filtered through 3 µm and 0.2 µm polycarbonate membrane  
281 filters (Whatman, United Kingdom) using a peristaltic pump. Filters were briefly dried at room  
282 temperature and stored at –80 °C. Seawater samples were also collected approximately monthly  
283 from July 2017 to October 2018.

284 **DNA Isolation**

285 DNA from surfaces of *Cymodocea nodosa* and *Caulerpa cylindracea* was isolated using  
286 a previously modified and adapted protocol that allows for a selective epiphytic DNA isolation  
287 (Massana *et al.*, 1997; Korlević *et al.*, submitted). Briefly, leaves and thalli are incubated  
288 in a lysis buffer and treated with lysozyme and proteinase K. Following the incubations, the  
289 mixture containing lysed epiphytic cells is separated from leaves and thalli and extracted using

290 a phenol-chloroform procedure. Finally, the extracted DNA is precipitated using isopropanol.  
291 DNA from seawater picoplankton was isolated from 0.2 µm polycarbonate filters according to  
292 (Massana *et al.*, 1997) with a slight modification. Following the phenol-chloroform extraction  
293 steps 1/10 of chilled 3 M sodium acetate (pH 5.2) was added. DNA was precipitated by adding  
294 1 volume of chilled isopropanol, incubating the mixtures overnight at -20 °C and centrifuging at  
295 20,000 × g and 4 °C for 21 min. The pellet was washed twice with 500 µl of chilled 70 % ethanol  
296 and centrifuged after each washing step at 20,000 × g and 4 °C for 5 min. Dried pellets were  
297 resuspended in 50 – 100 µl of deionized water.

298 **Illumina 16S rRNA Sequencing**

299 Illumina MiSeq sequencing of the V4 16S rRNA region was performed as described  
300 previously (Korlević *et al.*, submitted). The V4 region of the 16S rRNA gene was amplified using  
301 a two-step PCR procedure. In the first PCR the 515F (5'-GTGYCAGCMGCCGCGTAA-3')  
302 and 806R (5'-GGACTACNVGGGTWTCTAAT-3') primers from the Earth Microbiome Project  
303 (<http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/>) were used (Caporaso  
304 *et al.*, 2012; Apprill *et al.*, 2015; Parada *et al.*, 2016). These primers contained on their 5' end  
305 a tagged sequence. Purified PCR products were sent for Illumina MiSeq sequencing at IMGM  
306 Laboratories, Martinsried, Germany. Before sequencing at IMGM, the second PCR amplification  
307 of the two-step PCR procedure was performed using primers targeting the tagged region  
308 incorporated in the first PCR. In addition, these primers contained adapter and sample-specific  
309 index sequences. Beside samples, a positive and negative control for each sequencing batch was  
310 sequenced. Negative control was comprised of PCR reactions without DNA template, while for a  
311 positive control a mock community composed of evenly mixed DNA material originating from 20  
312 bacterial strains (ATCC MSA-1002, ATCC, USA) was used. The sequences obtained in this study  
313 have been submitted to the European Nucleotide Archive (ENA) under accession numbers **TO BE**  
314 **ADDED LATER!**

315 **Sequence Analysis**

316       Obtained sequences were analyzed on the computer cluster Isabella (University Computing  
317   Center, University of Zagreb) using mothur (version 1.43.0) (Schloss *et al.*, 2009) according  
318   to the MiSeq Standard Operating Procedure (MiSeq SOP; [https://mothur.org/wiki/MiSeq\\_SOP](https://mothur.org/wiki/MiSeq_SOP))  
319   (Kozich *et al.*, 2013) and recommendations given from the Riffomonas project to enhance data  
320   reproducibility (<http://www.riffomonas.org/>). For alignment and classification of sequences  
321   the SILVA SSU Ref NR 99 database (release 138; <http://www.arb-silva.de>) was used (Quast *et*  
322   *al.*, 2013; Yilmaz *et al.*, 2014). Pipeline data processing and visualization was done using R  
323   (version 3.6.0) (R Core Team, 2019), packages vegan (version 2.5-6) (Oksanen *et al.*, 2019), and  
324   tidyverse (version 1.3.0) (Wickham *et al.*, 2019) and multiple other packages (Xie, 2014, 2015,  
325   2019a, 2019b, 2020; Neuwirth, 2014; Xie *et al.*, 2018; Allaire *et al.*, 2019; Zhu, 2019). The  
326   detailed analysis procedure including the R Markdown file for this paper are available as a GitHub  
327   repository (**TO BE ADDED LATER!**). Based on the ATCC MSA-1002 mock community  
328   included in the analysis an average sequencing error rate of 0.01 % was determined, which is in  
329   line with previously reported values for next-generation sequencing data (Kozich *et al.*, 2013;  
330   Schloss *et al.*, 2016). In addition, the negative controls processed together with the samples  
331   yielded on average only 2 sequences after sequence quality curation.

332 **Acknowledgments**

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469 **Figure Captions**

470 **Figure 1.** Proportion of shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient)  
471 and shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) between  
472 communities associated with the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and  
473 *Caulerpa cylindracea* [Invaded and Noninvaded]) and coomunities in the surrounding seawater.

474 **Figure 2.** Proportion of shared bacterial and archaeal communities (Bray-Curtis Similarity  
475 Coefficient) and shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) between  
476 consecutive sampling points and from the surfaces of macrophytes (*Cymodocea nodosa* [Invaded]  
477 and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater.

478 **Figure 3.** Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU  
479 abundances of bacterial and archaeal communities from the surfaces of macrophytes (*Cymodocea*  
480 *nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding  
481 seawater. Samples from the same environment or same season are labeld in different colors. The  
482 proportion of explained variation by each axis is shown on the corresponding axis in parentheses.

483 **Figure 4.** Taxonomic classification and relative contribution of the most abundant bacterial and  
484 archaeal sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa*  
485 *cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater.

486 **Figure 5.** Taxonomic classification and relative contribution of the most abundant cyanobacterial  
487 sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa*  
488 *cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of  
489 cyanobacterial sequences in the total bacterial and archaeal community is given above the  
490 corresponding bar. NR – No Relative

491 **Figure 6.** Taxonomic classification and relative contribution of the most abundant sequences  
492 within the *Bacteroidota* on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and

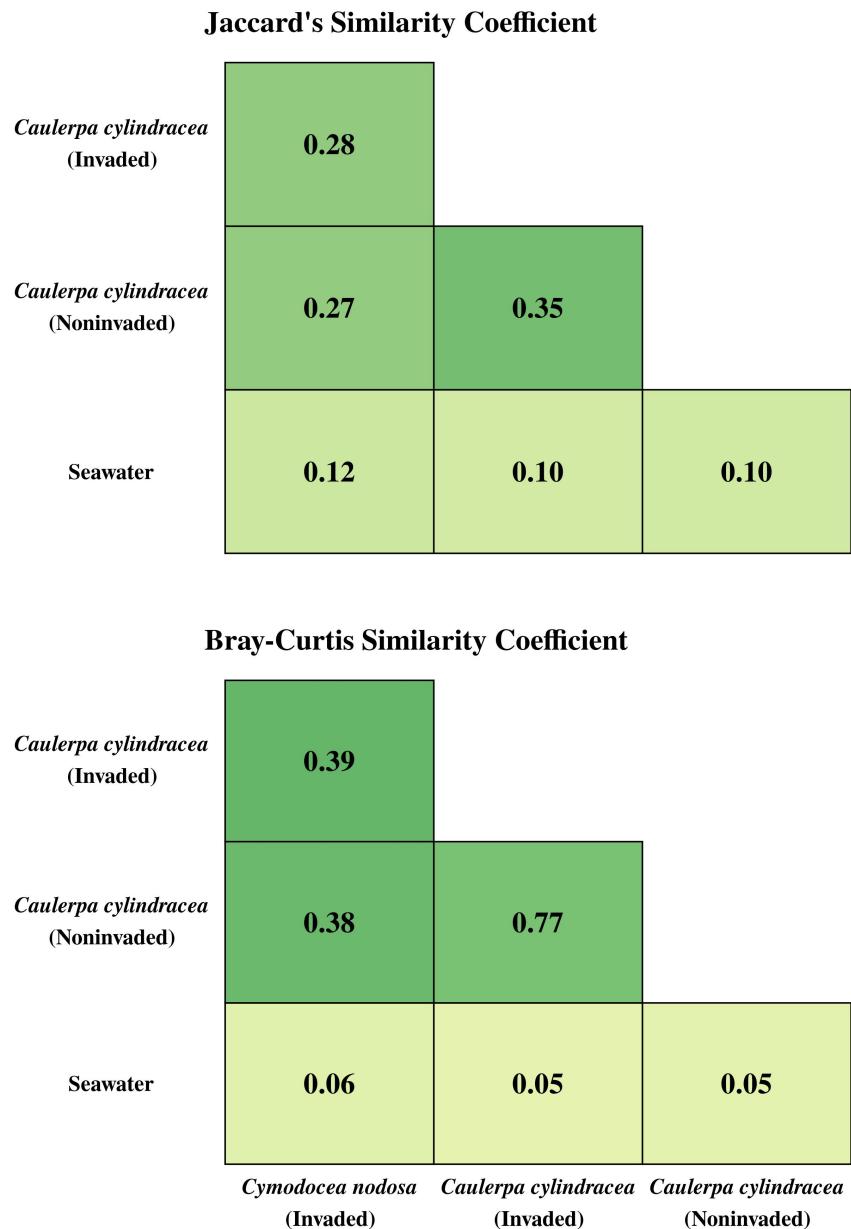
493 *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion  
494 of sequences classified as *Bacteroidota* in the total bacterial and archaeal community is given  
495 above the corresponding bar. NR – No Relative

496 **Figure 7.** Taxonomic classification and relative contribution of the most abundant alphaproteobacterial  
497 sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa*  
498 *cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of  
499 alphaproteobacterial sequences in the total bacterial and archaeal community is given above the  
500 corresponding bar. NR – No Relative

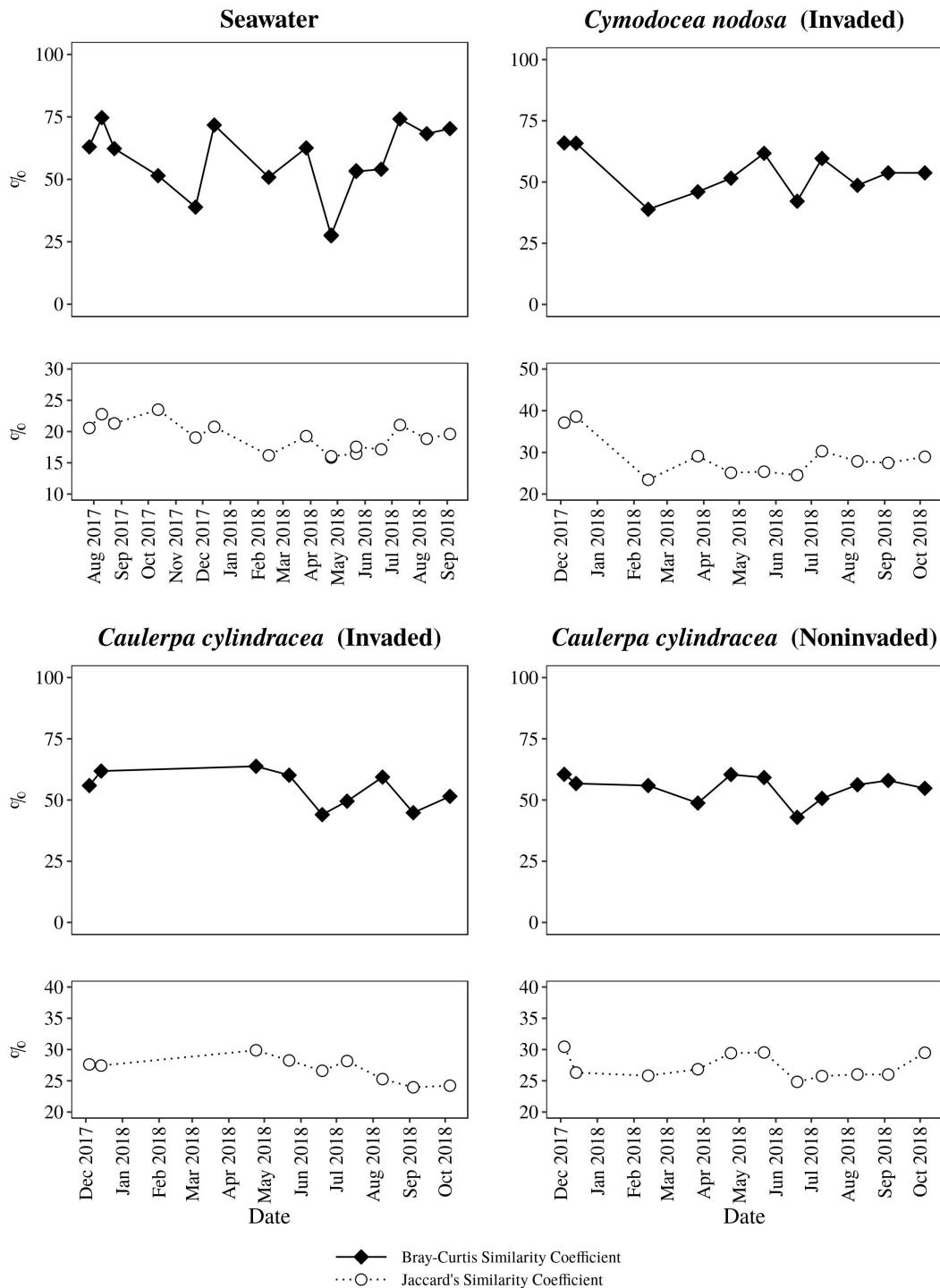
501 **Figure 8.** Taxonomic classification and relative contribution of the most abundant gammaproteobacterial  
502 sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa*  
503 *cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of  
504 gammaproteobacterial sequences in the total bacterial and archaeal community is given above the  
505 corresponding bar. NR – No Relative

506 **Figure 9.** Taxonomic classification and relative contribution of the most abundant sequences  
507 within the *Desulfobacterota* on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and  
508 *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion  
509 of sequences classified as *Desulfobacterota* in the total bacterial and archaeal community is given  
510 above the corresponding bar. NR – No Relative

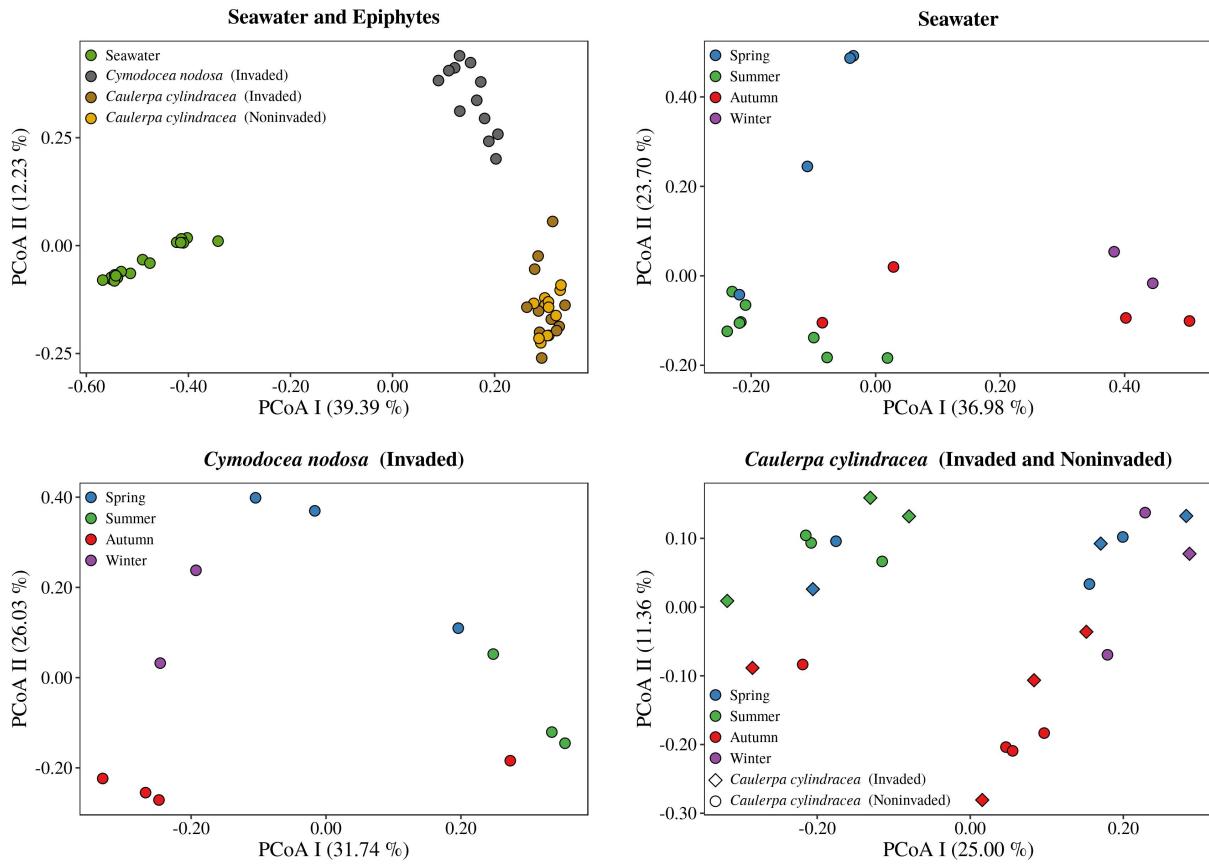
511 **Figures**



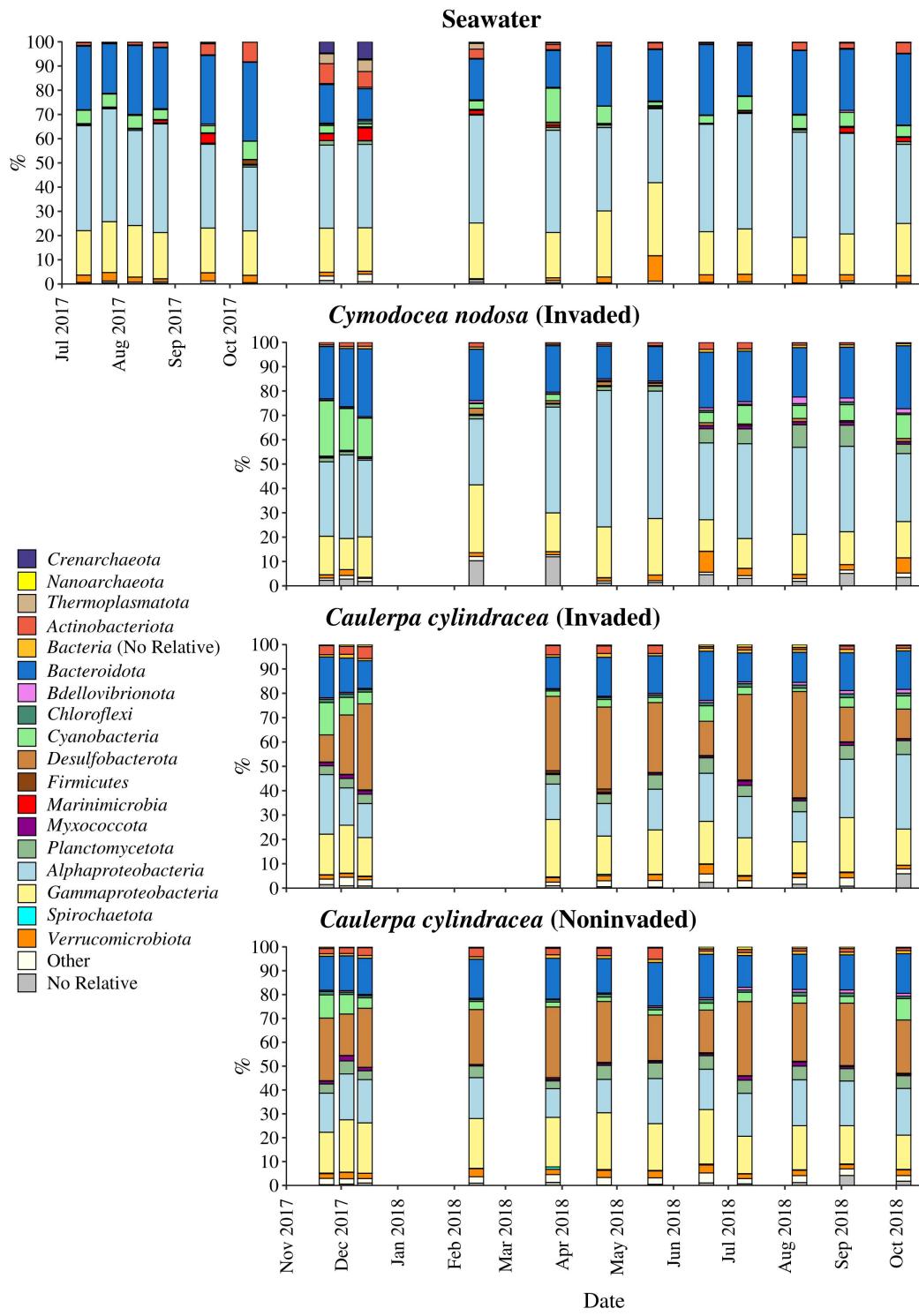
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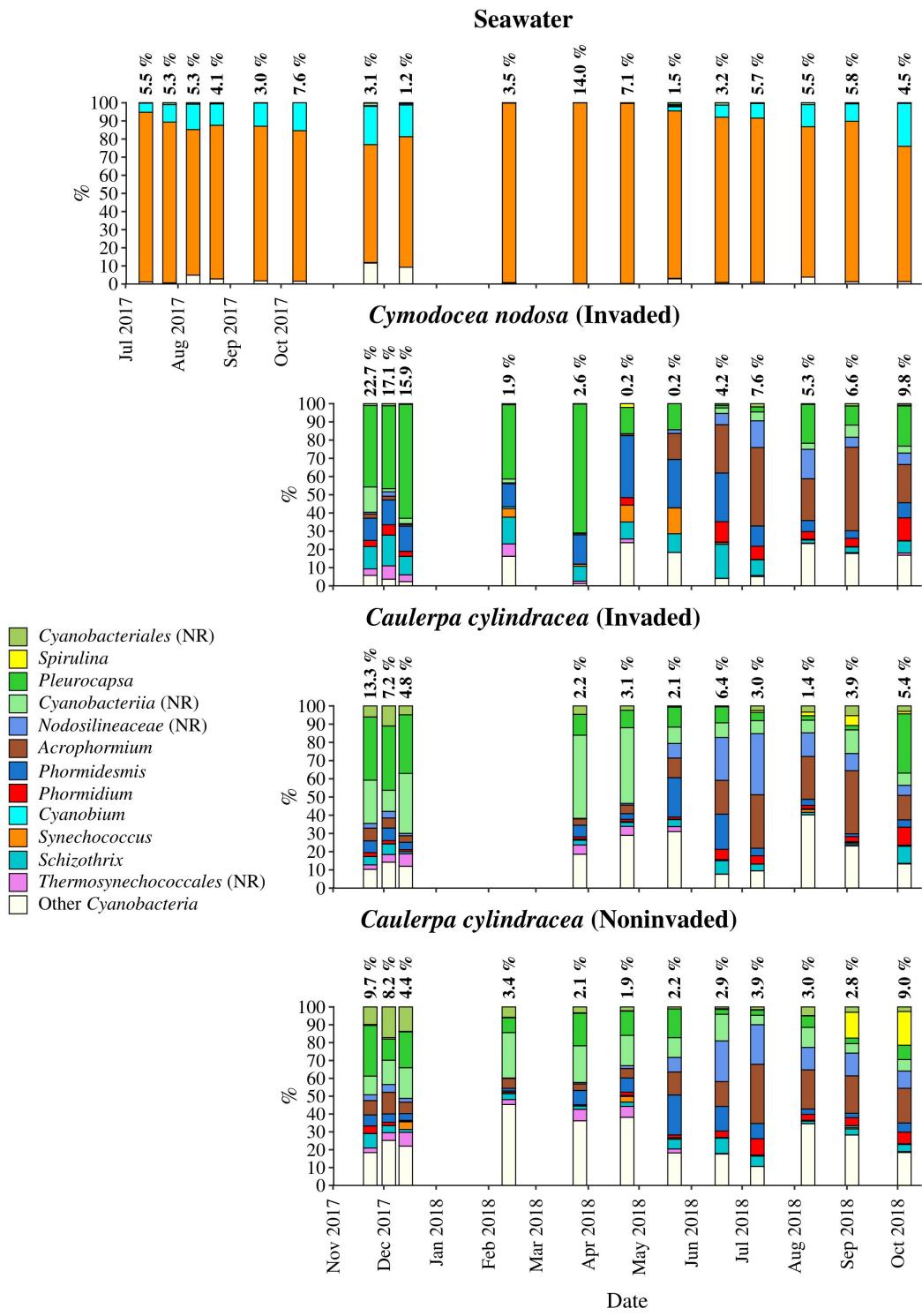
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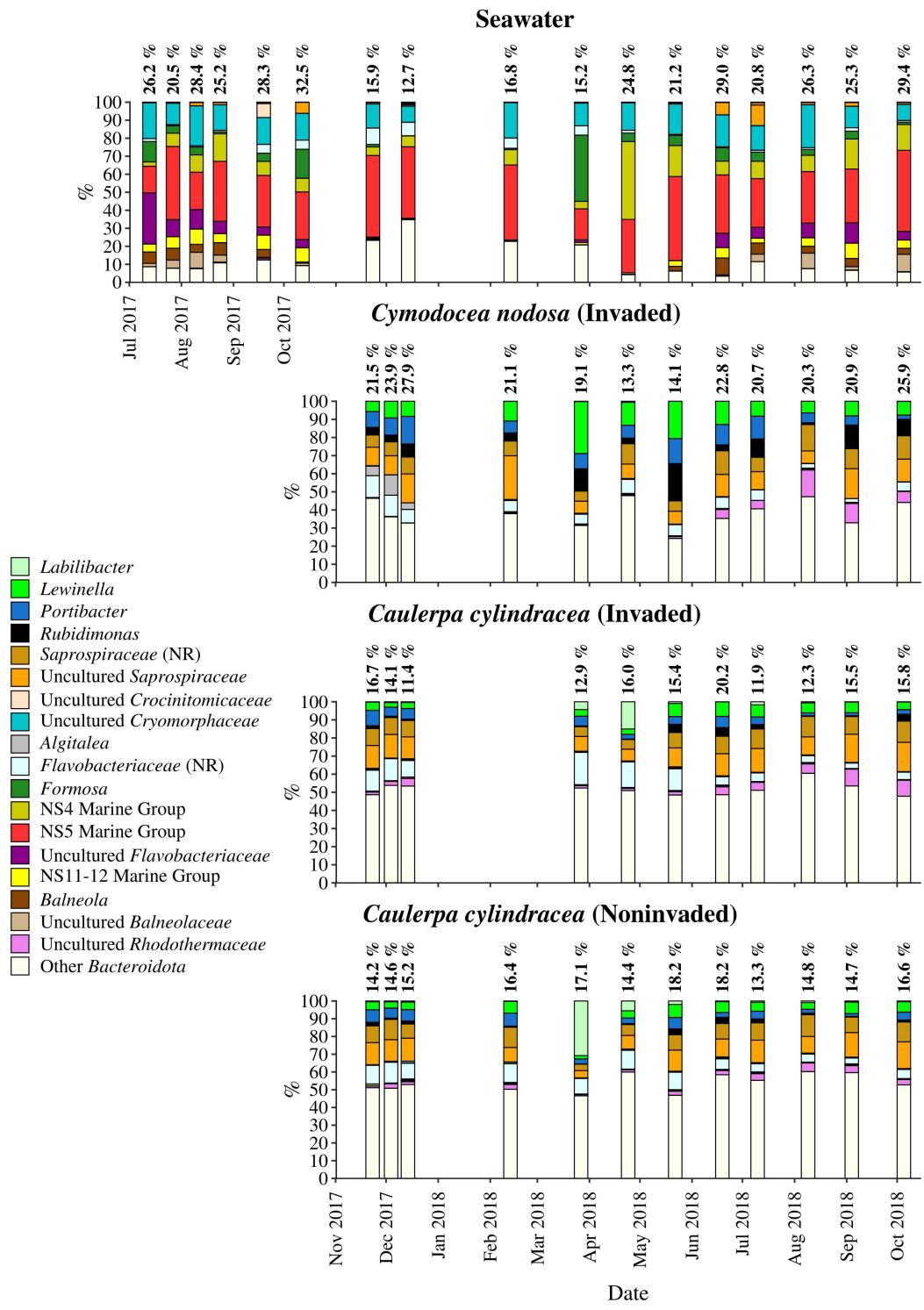
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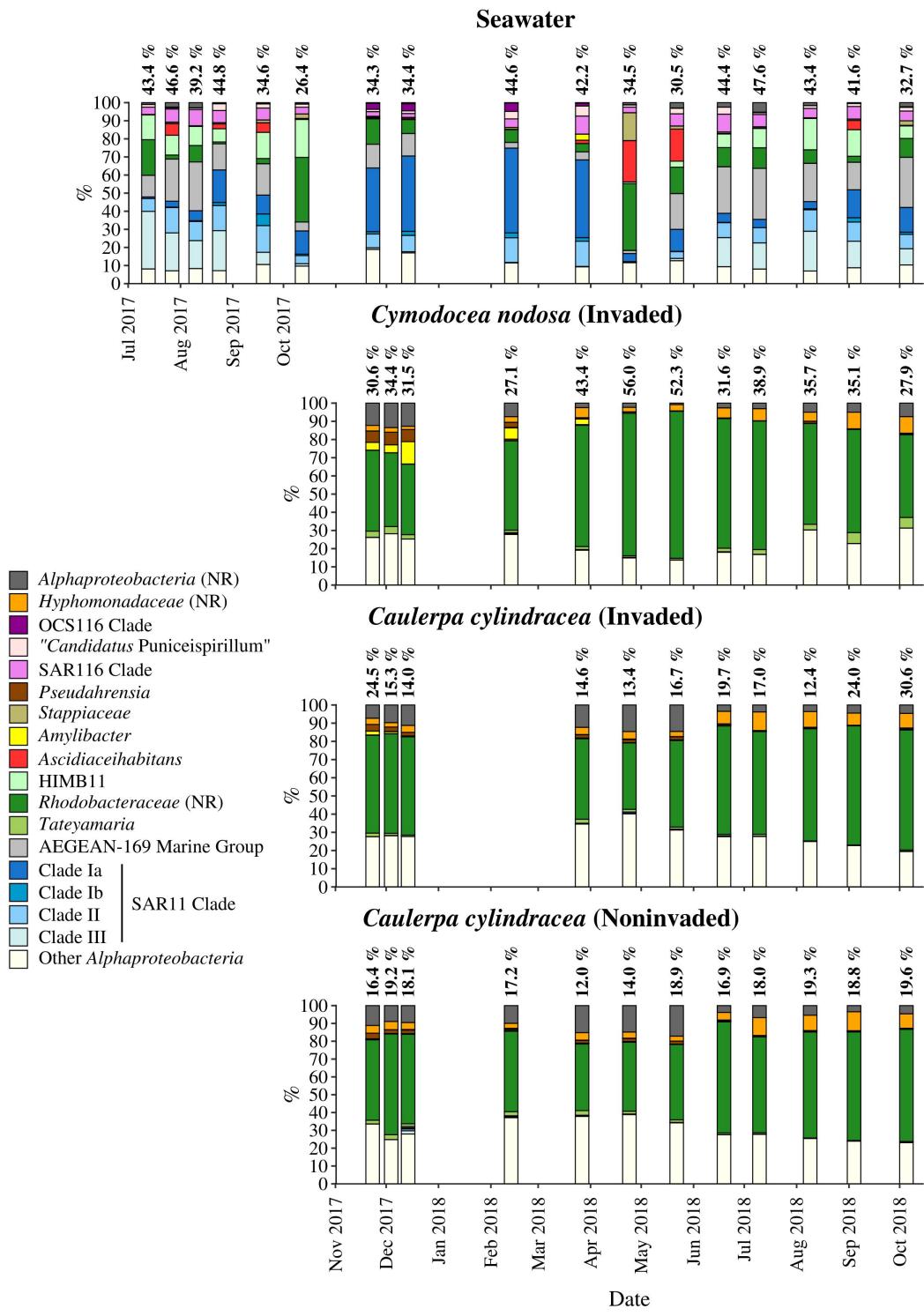
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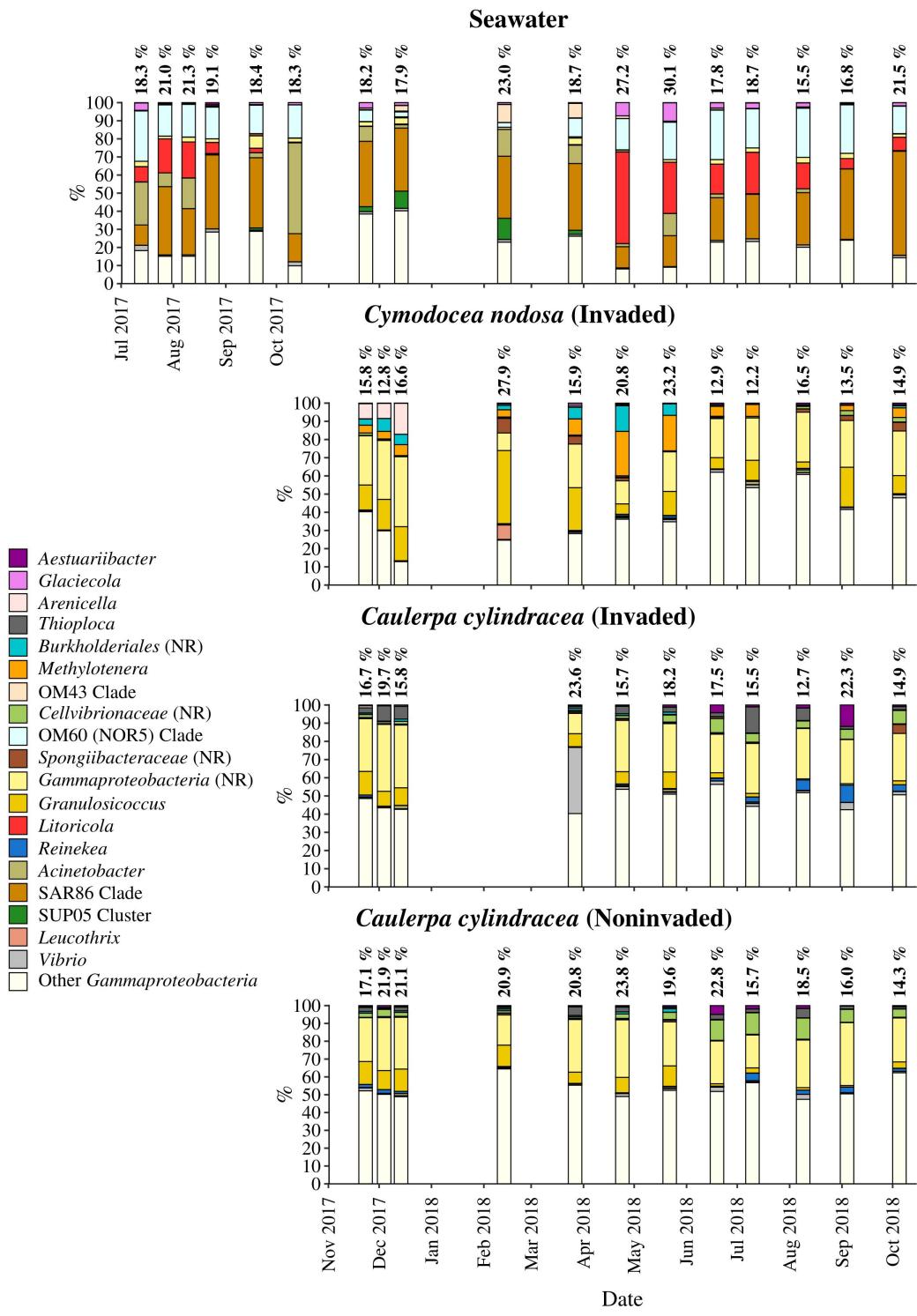
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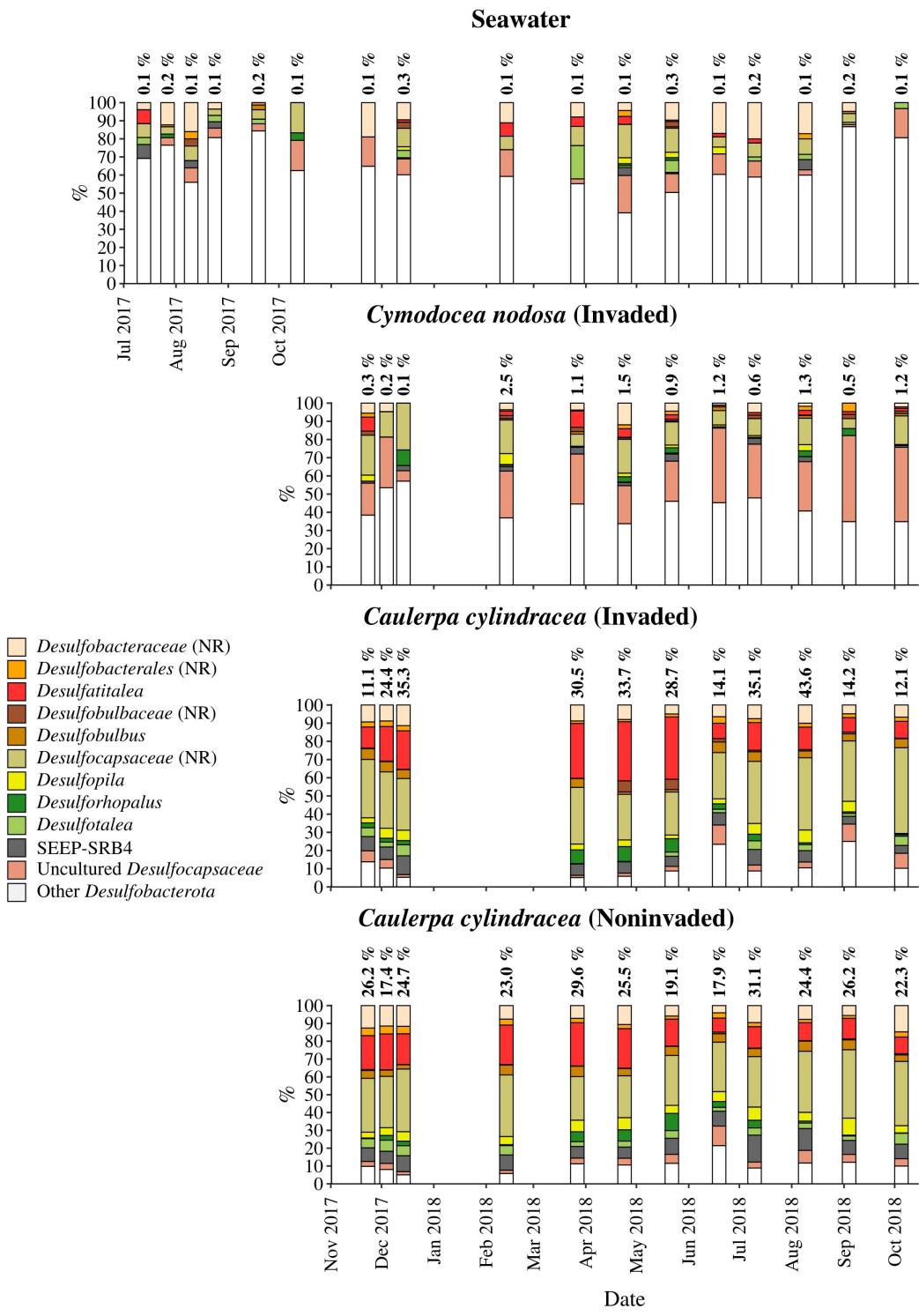
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**Figure 7.** Taxonomic classification and relative contribution of the most abundant alphaproteobacterial sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding 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 gammaproteobacterial sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of gammaproteobacterial sequences in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative



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