

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 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 ambient seawater were also
59 processed for comparison. The number of reads per sample ranged between 8,407 and 77,465
60 sequences (Table S1). Even when the highest sequencing effort was applied the rarefaction curves
61 did 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.
69 On average, higher values were found for *Caulerpa cylindracea* (invaded [Number of OTUs,
70 $1,688.5 \pm 125.6$ OTUs] and noninvaded [Number of OTUs, $1,744.8 \pm 150.8$ OTUs]), middle
71 values for *Cymodocea nodosa* (Number of OTUs, $1,062.8 \pm 209.6$ OTUs) and lower values for
72 picoplankton communities in the ambient seawater (Number of OTUs, 528.1 ± 135.8 OTUs)
73 (Figure S2). Seasonal changes did not show such large dissimilarities. *Cymodocea nodosa*
74 communities showed a slow increase towards the end of the study, while *Caulerpa cylindracea*
75 (invaded and noninvaded) communities were characterized by slightly larger values in Spring and
76 Summer in comparison to Autumn and Winter (Figure S2).

77 To determine the proportion of shared archaeal and bacterial OTUs and communities sampled

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

101 The taxonomic composition of both, macrophyte associated and seawater communities,
102 was dominated by bacterial ($99.1 \pm 2.1 \%$) over archaeal sequences ($0.9 \pm 2.1 \%$) (Figure 4).
103 Higher relative abundances of chloroplast related sequences were only observed in surface
104 associated communities, with higher values in Autumn/Winter ($37.2 \pm 11.2 \%$) in comparison to

105 Spring/Summer ($20.9 \pm 9.7\%$) (Figure S3). Generally, at higher taxonomic ranks (phylum-class)
106 epiphytic and seawater microbial communities were composed of similar bacterial taxa.
107 Seawater communities were mainly comprised of *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*,
108 *Alphaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobiota*. Communities associated
109 with *Cymodocea nodosa* were consisted of same groups with the addition of *Planctomycetota*
110 whose contribution was higher in summer 2018. In addition, communities from invaded and
111 noninvaded *Caulerpa cylindracea* were similar and characterized by same groups as seawater
112 and *Cymodocea nodosa* communities with the addition of *Desulfobacterota* (Figure 4). Larger
113 differences between environments and host species could be observed at lower taxonomic ranks
114 (Figure 5 – 9).

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

126 Sequences classified as *Bacteroidota* were comprising, on average, $19.2 \pm 5.5\%$ of all
127 sequences (Figure 6). Similarly to *Cyanobacteria*, large differences in the taxonomic composition
128 between seawater and surface associated communities were found (Figure 6). The seawater
129 community was characterized by the NS4 and NS5 marine groups, uncultured *Cryomorphaceae*,
130 uncultured *Flavobacteriaceae*, NS11-12 marine group, *Balneola*, uncultured *Balneolaceae* and

131 *Formosa*. In contrast, in surface associated communities *Lewinella*, *Portibacter*, *Rubidimonas*, no
132 relative *Saprospiraceae*, uncultured *Saprospiraceae*, no relative *Flavobacteriaceae* and uncultured
133 *Rhodothermaceae* were found. Some groups showed slight seasonal changes such as no relative
134 *Flavobacteriaceae* that were more pronounced from November 2017 until June 2018. In contrast,
135 uncultured *Rhodothermaceae* showed higher proportions from June 2018 until the end of the study
136 period. Surface associated *Bacteroidota* communities were very diverse as could be observed in
137 the the high proportion of taxa that grouped as other *Bacteroidota* (Figure 6).

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

149 Sequences related to *Gammaproteobacteria* were comprising, on average, 18.7 ± 3.9 % of all
150 sequences (Figure 8). Similarly to previous taxa, large taxonomic differences between seawater
151 and surface associated communities were found. Seawater communities were mainly comprised
152 of the OM60 (NOR5) clade, *Litoricola*, *Acinetobacter* and the SAR86 clade, while epiphytic
153 communities were mainly composed of no relative *Gammaproteobacteria* and *Granulosicoccus*.
154 Beside these two groups specific to all three epiphytic communities, *Cymodocea nodosa* was
155 characterized by *Arenicella*, no relative *Burkholderiales* and *Methylotenera*, while *Thioploca*,
156 no relative *Cellvibrionaceae* and *Reinekea* were more specific to both invaded and noninvaded

¹⁵⁷ *Caulerpa cylindracea*. In addition, *Arenicella* was more pronounced in November and December
¹⁵⁸ 2017, while no relative *Burkholderiales* and *Methylotenera* were more characteristic for the
¹⁵⁹ period from March until May 2018. For the *Caulerpa cylindracea* specific taxa no relative
¹⁶⁰ *Cellvibrionaceae* and *Reinekea* showed some seasonality and were characteristic for samples
¹⁶¹ originating from June to October 2018. In addition, similarly to *Bacteroidota*, a large proportion
¹⁶² of the surface associated community was grouped as other *Gammaproteobacteria* indicating high
¹⁶³ diversity within this group (Figure 8).

¹⁶⁴ In contrast to previously described high rank taxa, *Desulfobacterota* were specific to *Caulerpa*
¹⁶⁵ *cylindracea*. On average they were comprising $11.2 \pm 13.3\%$ of all sequences. While seawater
¹⁶⁶ and *Cymodocea nodosa* communities were consisted of only $0.1 \pm 0.08\%$ and $1.0 \pm 0.7\%$
¹⁶⁷ *Desulfobacterota* sequences, respectively, in the invaded and noninvaded *Caulerpa cylindracea*
¹⁶⁸ communities their proportion was $25.7 \pm 11.3\%$ and $24.0 \pm 4.3\%$, respectively (Figure 9). The
¹⁶⁹ community was mainly consisted of no relative *Desulfobacteraceae*, *Desulfatitalea*, no relative
¹⁷⁰ *Desulfobulbaceae*, *Desulfobulbus*, no relative *Desulfocapsaceae*, *Desulfopila*, *Desulforhopalus*,
¹⁷¹ *Desulfotalea*, SEEP-SRB4 and uncultured *Desulfocapsaceae* (Figure 9).

172 **Discussion**

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

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

198 dynamic chemical interaction with the surface community probably causing an increase in the
199 number of inhabitable microniches (Borges and Champenois, 2015; Rickert *et al.*, 2016).

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

222 Beside differences between seawater and surface associated communities, there were
223 discussions if the prokaryotic epiphytic community is host-specific or there are generalists taxa

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

Seasonal richness changes in the epiphytic community were substantial as could be observed in the proportion of OTUs that could be found at every sampling time ($\leq 1.0\%$). Interestingly, these OTUs were accounting for a high proportion of sequences ($\leq 51.3\%$). A very similar proportion of persistent OTUs and their sequence contribution was reported in high frequency studies describing seasonal picoplankton changes (Gilbert *et al.*, 2009, 2012). In comparison to the seawater community, a lower degree of seasonal shifts was observed for the surface associated communities. It seems, that microniches on the surfaces of macrophytes are providing more stable conditions in comparison to the seawater. At the level of OTUs seasonal changes of *Cymodocea nodosa* and *Caulerpa cylindracea* associated communities were identified that could be linked to the growth cycle of the seagrass and macroalgae (M. Najdek, personal communication). *Cymodocea nodosa* was characterized by a Spring community during maximum seagrass proliferation, a Summer community during a biomass maximum and a Autumn/Winter

251 community during a biomass decay. In contrast, *Caulerpa cylindracea* started to proliferate in late
252 Spring and was characterized only by a Summer community during maximal biomass increase
253 and by a Autumn/Winter/Spring community when the biomass were at the peak and the settlement
254 started to subsequently decay. Similar seasonal changes in the epiphytic community was described
255 also for other macroalgae (Tujula *et al.*, 2010; Lachnit *et al.*, 2011). Higher temporal stability
256 of *Caulerpa cylindracea* surface communities in comparison to *Cymodocea nodosa* were also
257 observed in the higher proportion of shared communities between two consecutive sampling
258 points.

259 Seasonal patterns of high rank epiphytic taxa did not show strong changes. Only
260 *Cyanobacteria* were characterized by strong seasonal differences. Cyanobacterial sequences were
261 more pronounced in the November and December 2017 in comparison to Spring and Summer.
262 Interestingly, in these high proportion months the majority of cyanobacterial sequences were
263 classified as *Pleurocapsa*, a group known to colonized different living and nonliving surfaces
264 (Burns *et al.*, 2004; Longford *et al.*, 2007; Mobberley *et al.*, 2012; Reisser *et al.*, 2014). It is
265 possible than during periods of low metabolic activity of the i.g. seagrass there is no so active
266 interaction and selection of the epiphytic community, so the seagrass leaves are becoming a
267 suitable surface for nonspecific colonizers (Zavodnik *et al.*, 1998). *Pleurocapsa* was replaced in
268 Spring ans Summer by *Acrophormium*, *Phormidesmis* and no relative *Nodosilineaceae*. A study
269 of coastal microbial mats found also higher proportion of *Nodosilineaceae* sequences in Summer,
270 while *Phormidesmis* sequences were at their peak in Autumn (Cardoso *et al.*, 2019). Other high
271 rank taxa did not showed strong successional patterns. In each analyzed group low rank taxa

272 **Experimental Procedures**

273 **Sampling**

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

289 **DNA Isolation**

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

295 a phenol-chloroform procedure. Finally, the extracted DNA is precipitated using isopropanol.
296 DNA from seawater picoplankton was isolated from 0.2 μ m polycarbonate filters according to
297 (Massana *et al.*, 1997) with a slight modification. Following the phenol-chloroform extraction
298 steps 1/10 of chilled 3 M sodium acetate (pH 5.2) was added. DNA was precipitated by adding 1
299 volume of chilled isopropanol, incubating the mixtures overnight at -20 °C and centrifuging at
300 20,000 \times g and 4 °C for 21 min. The pellet was washed twice with 500 μ l of chilled 70 % ethanol
301 and centrifuged after each washing step at 20,000 \times g and 4 °C for 5 min. Dried pellets were
302 resuspended in 50 – 100 μ l of deionized water.

303 **Illumina 16S rRNA Sequencing**

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

320 **Sequence Analysis**

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

337 **Acknowledgments**

338 **References**

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490 **Figure Captions**

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

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

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

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

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

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

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

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

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

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

Jaccard's Similarity Coefficient

<i>Caulerpa cylindracea</i> (Invaded)	0.28		
<i>Caulerpa cylindracea</i> (Noninvaded)	0.27	0.35	
Seawater	0.12	0.10	0.10

Bray-Curtis Similarity Coefficient

<i>Caulerpa cylindracea</i> (Invaded)	0.39		
<i>Caulerpa cylindracea</i> (Noninvaded)	0.38	0.77	
Seawater	0.06	0.05	0.05
<i>Cymodocea nodosa</i>	(Invaded)	<i>Caulerpa cylindracea</i> (Invaded)	<i>Caulerpa cylindracea</i> (Noninvaded)

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

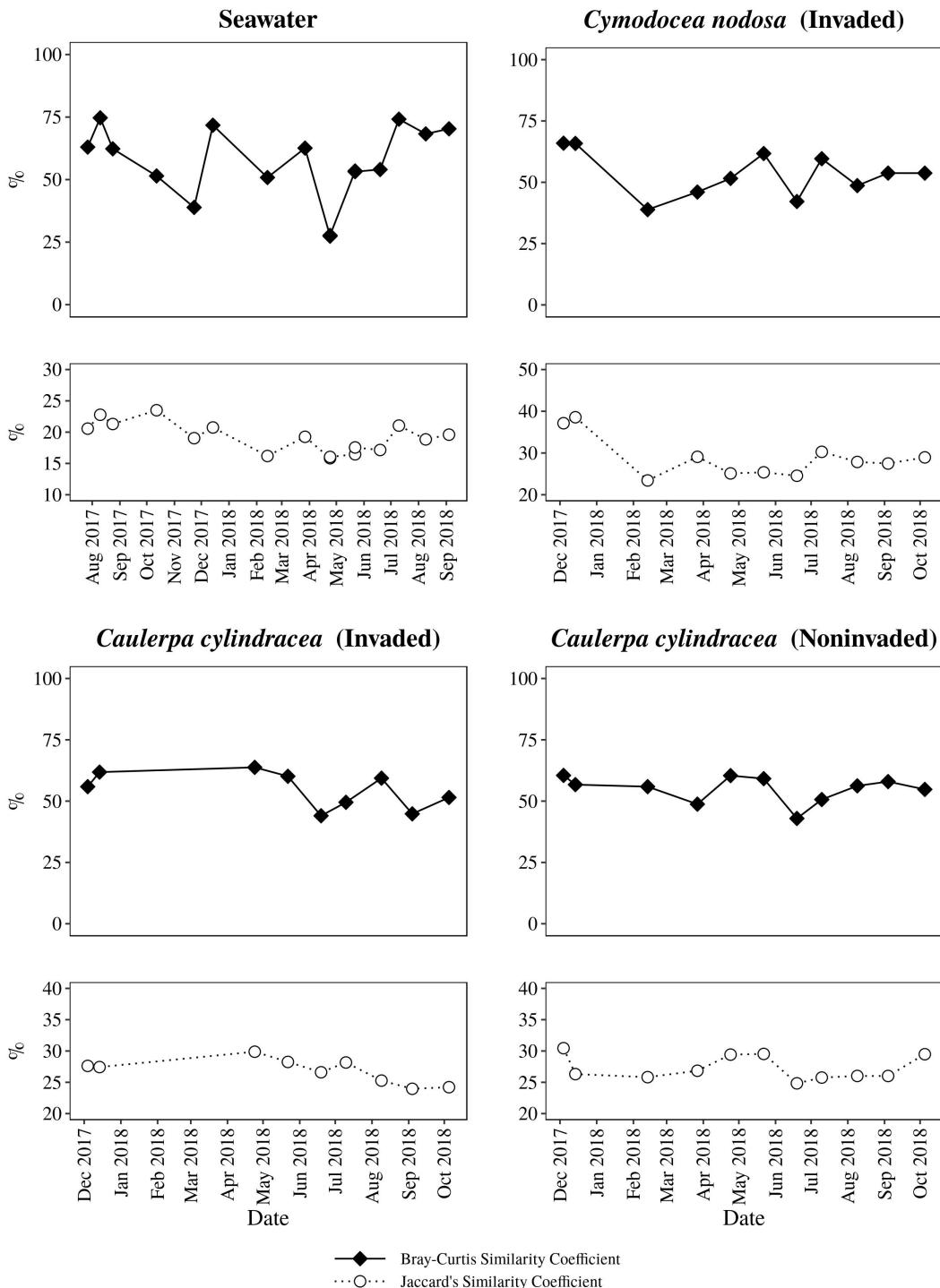


Figure 2. Proportion of shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) and shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) between consecutive sampling points and from the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea*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

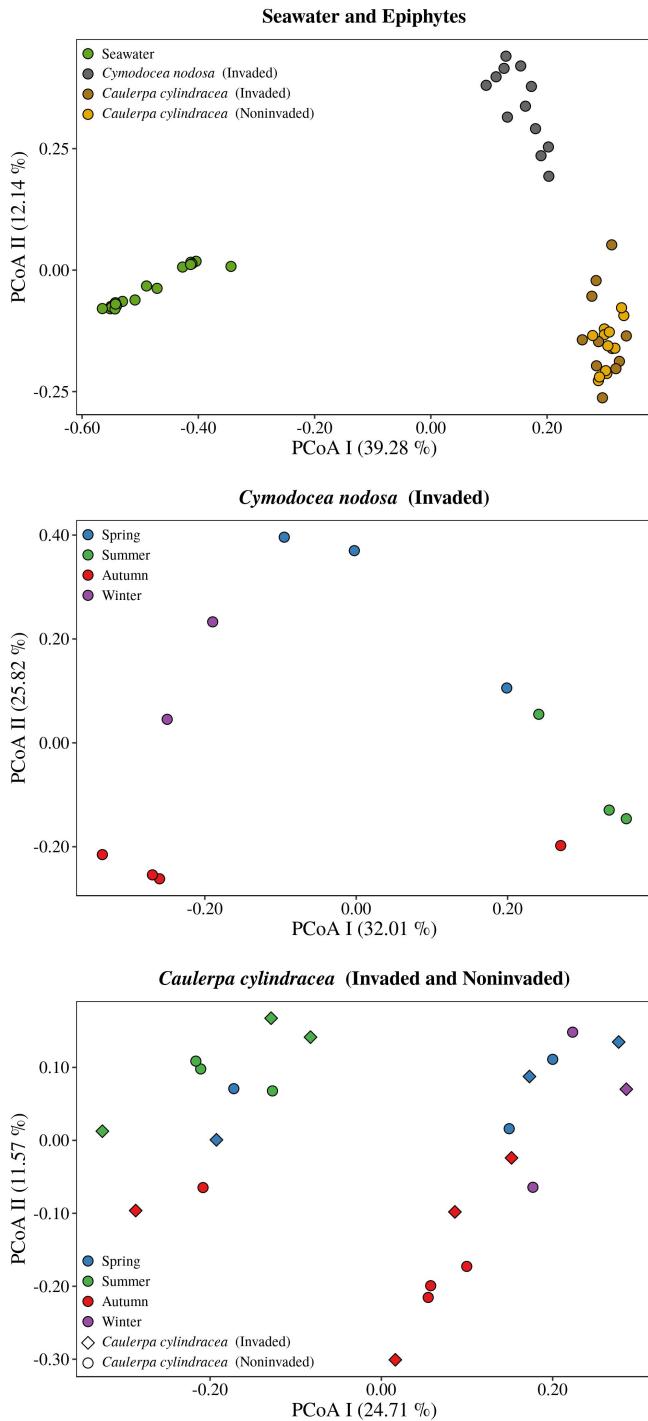


Figure 3. Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities from the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. Samples from the 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.

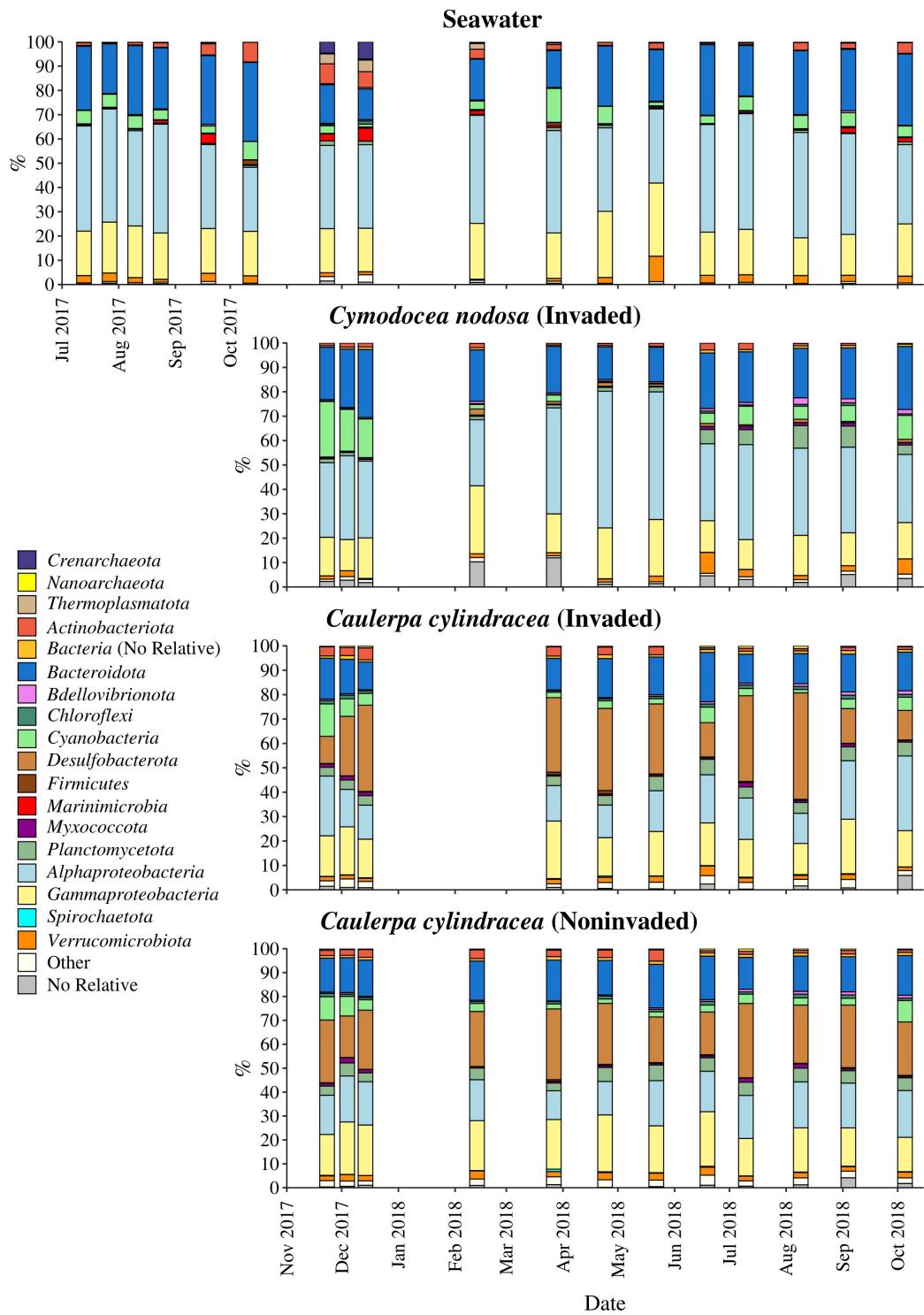


Figure 4. Taxonomic classification and relative contribution of the most abundant bacterial and archaeal sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater.

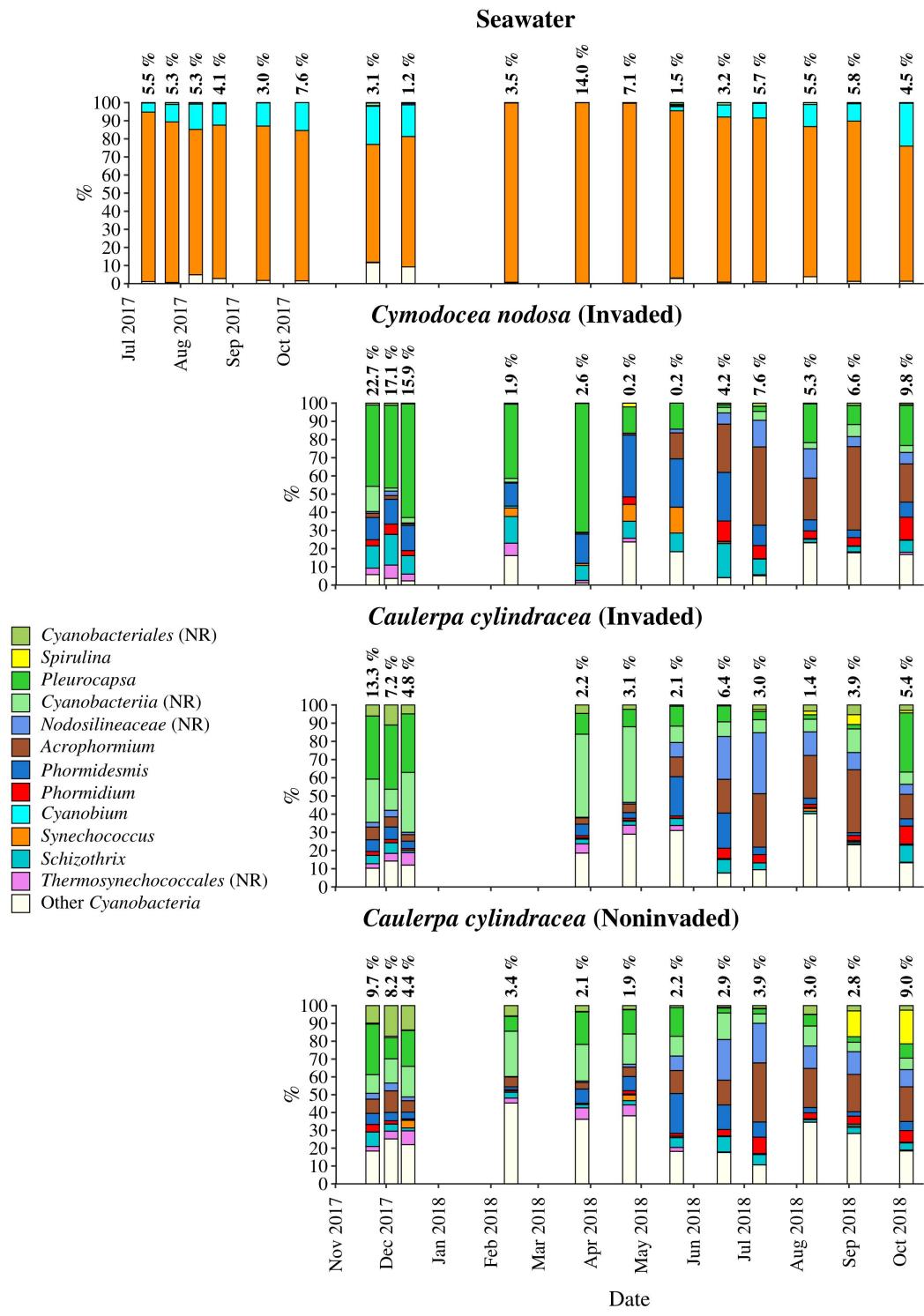


Figure 5. Taxonomic classification and relative contribution of the most abundant cyanobacterial sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of cyanobacterial sequences in the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

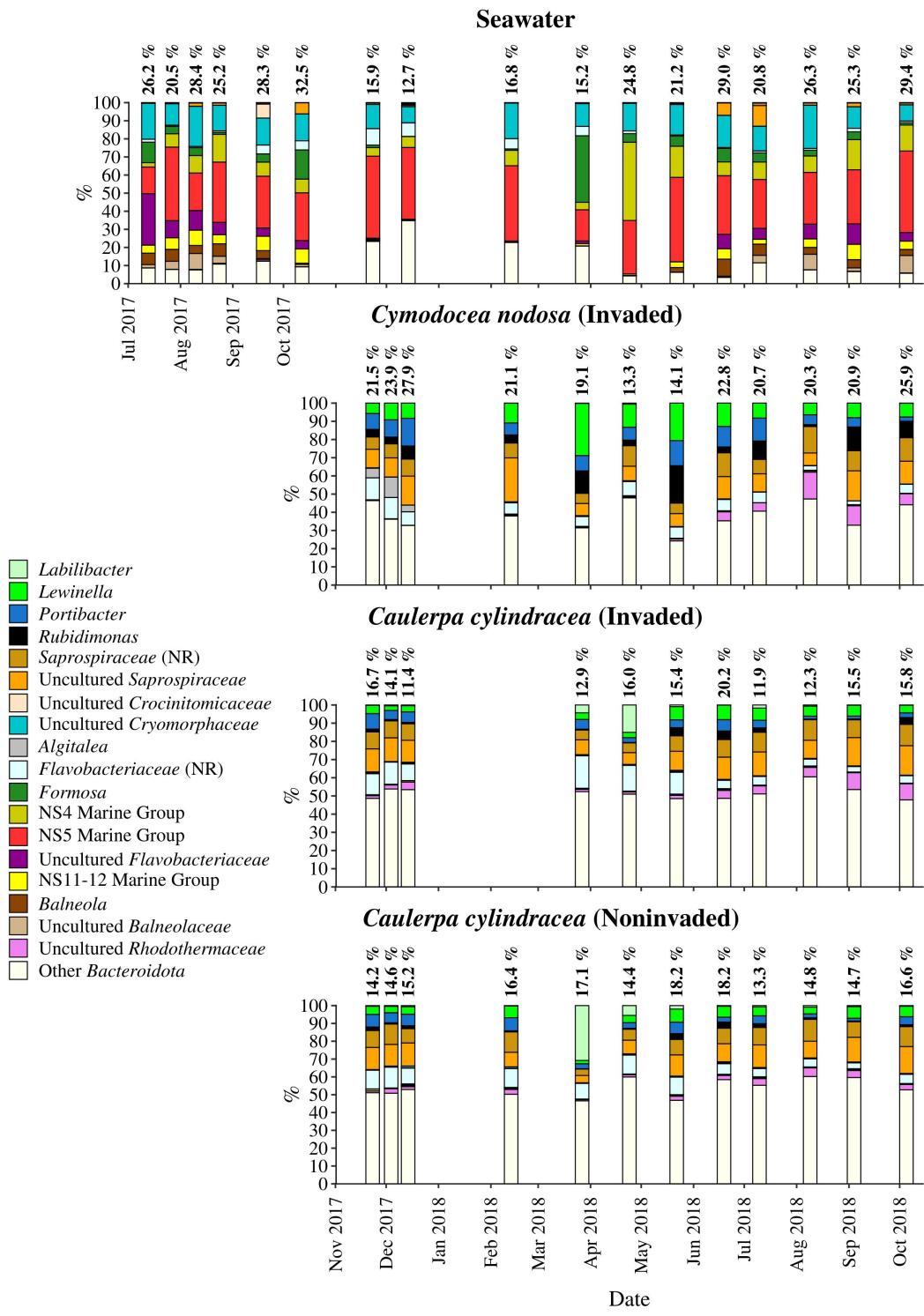


Figure 6. Taxonomic classification and relative contribution of the most abundant sequences within the *Bacteroidota* on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of sequences classified as *Bacteroidota* in the total bacterial and archaeal community is given above the corresponding bar. NR – No Relative

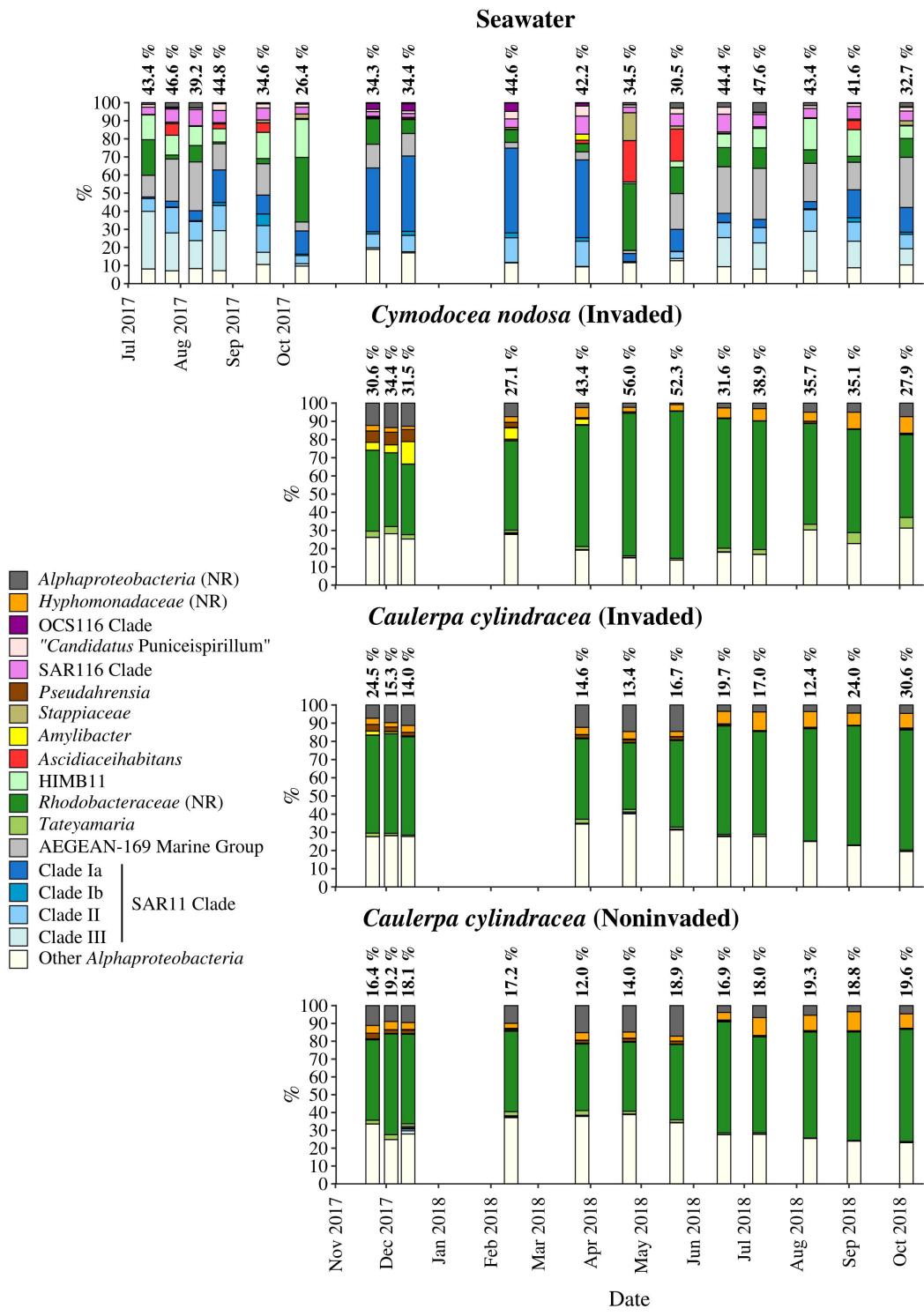


Figure 7. Taxonomic classification and relative contribution of the most abundant alphaproteobacterial sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of alphaproteobacterial sequences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

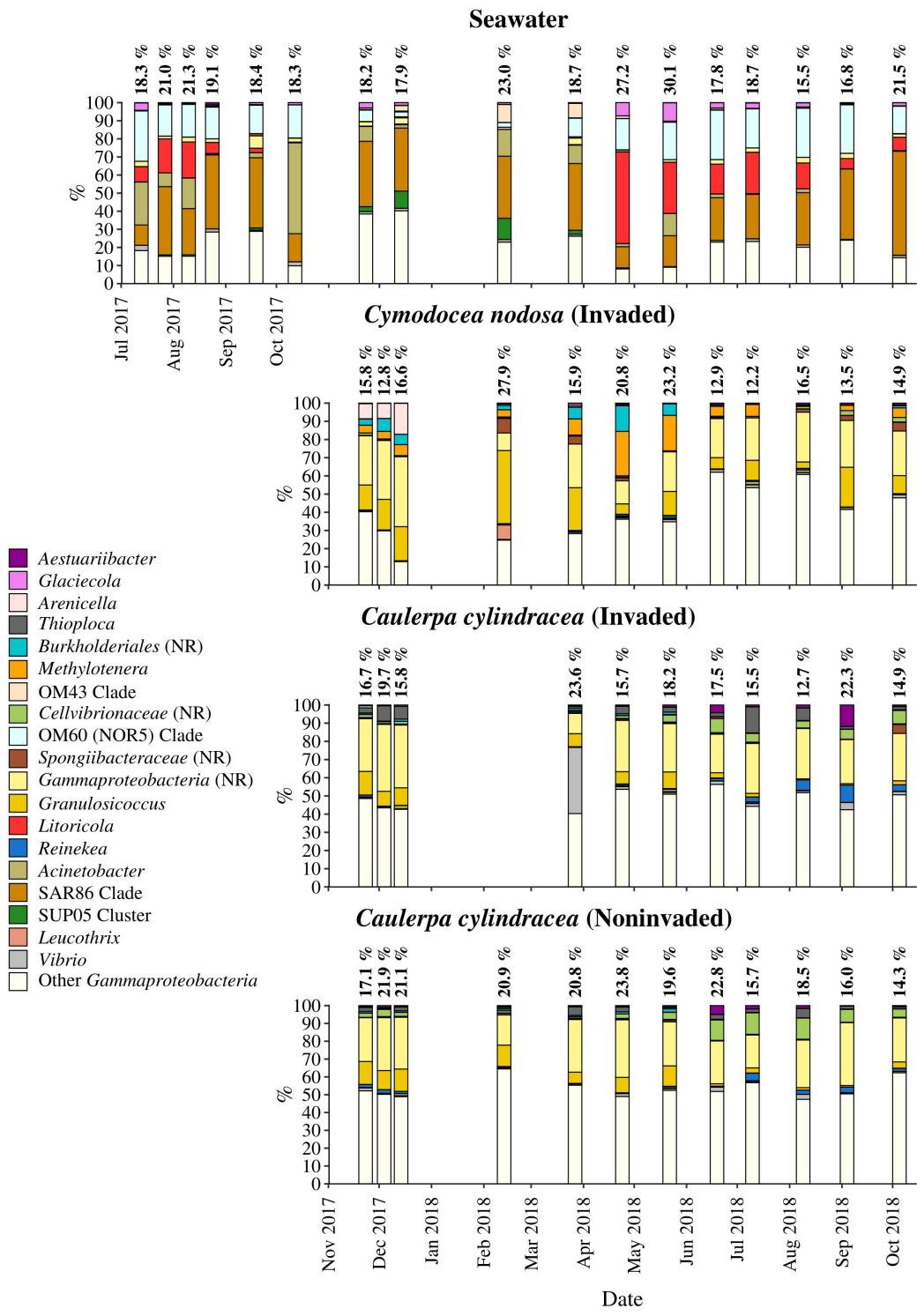


Figure 8. Taxonomic classification and relative contribution of the most abundant gammaproteobacterial sequences on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of gammaproteobacterial sequences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

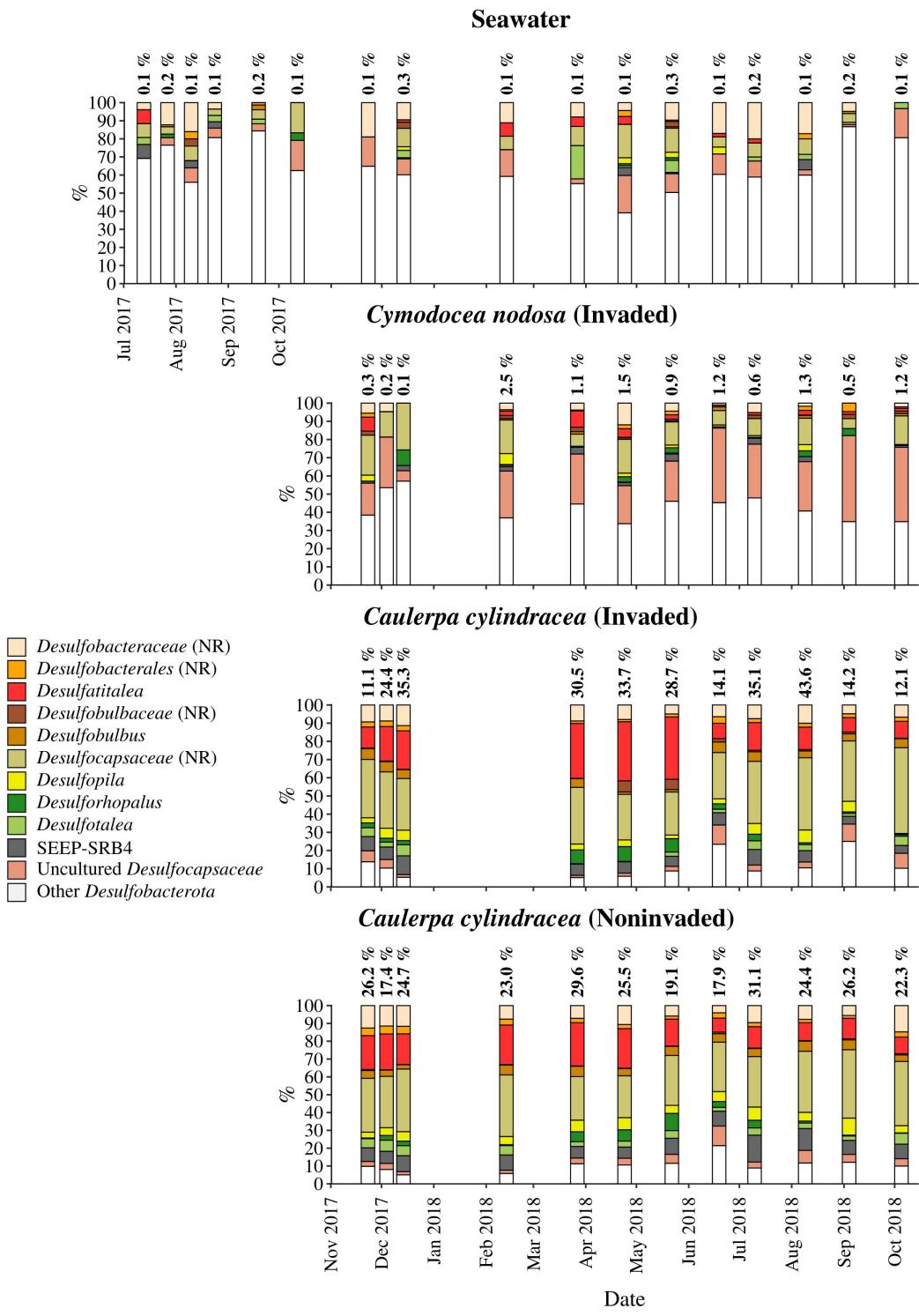


Figure 9. Taxonomic classification and relative contribution of the most abundant sequences within the *Desulfobacterota* on the surfaces of macrophytes (*Cymodocea nodosa* [Invaded] and *Caulerpa cylindracea* [Invaded and Noninvaded]) and in the surrounding seawater. The proportion of sequences classified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