

Seasonal Dynamics of Epiphytic Microbial Communities on Marine Macrophyte Surfaces

Marino Korlević^{1*}, Marsej Markovski¹, Zihao Zhao², Gerhard J. Herndl^{2,3}, Mirjana Najdek¹

*To whom correspondence should be addressed: marino.korlevic@irb.hr

1. Center for Marine Research, Ruđer Bošković Institute, Croatia
2. Department of Functional and Evolutionary Ecology, University of Vienna, Austria
3. Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research, Utrecht University, The Netherlands

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 and comprising most of the
18 sequences could be identified together with low proportion taxa showing seasonal patterns
19 connected to the macrophyte growth cycle. Taken together, epiphytic microbial communities
20 of the seagrass *Cymodocea nodosa* and the macroalgae *Caulerpa cylindracea* appear to be
21 host-specific and 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 is 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 low
64 temporal frequency and/or methodologies that do not allow for 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 8,408 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 28,736 different OTUs at a similarity level of 97 %. Reads numbers were normalized to the
84 minimum number of sequences, 8,408 (Table S1), through rarefaction resulting in 17,114 different
85 OTUs that ranged from 379 to 2,016 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* (invaded [Number of OTUs, $1,676.9 \pm 140.3$ OTUs] and noninvaded
90 [Number of OTUs, $1,745.9 \pm 156.3$ OTUs]), middle values for *C. nodosa* (Number of OTUs,
91 $1,059.5 \pm 212.3$ OTUs) and lower values for picoplankton communities in the ambient seawater
92 (Number of OTUs, 530.8 ± 143.1 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* (invaded and noninvaded) 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 invaded and noninvaded
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 made also a high proportion of total sequences (40.3 – 52.4
110 %). To further disentangle the environmental and seasonal community dissimilarity a Principal
111 Coordinates Analysis (PCoA) based on Bray-Curtis distances and OTU abundances was applied.
112 It showed a clear separation between planktonic and surface associated communities (Figure 3). In
113 addition, a separation of epiphytic bacterial and archaeal communities based on host species was
114 determined. This separation was further supported by ANOSIM ($R = 0.96, p < 0.001$). Seasonal
115 changes of *C. nodosa* associated communities indicated a separation between Spring, Summer and
116 Autumn/Winter samples (ANOSIM, $R = 0.55, p < 0.001$), while communities from the surfaces
117 of *C. cylindracea* indicated a non so strongly supported, as in previous cases, separation between
118 Summer and Autumn/Winter/Spring samples (ANOSIM, $R = 0.31, p < 0.01$) (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* were consisted of same groups with the addition of *Planctomycetota* whose
128 contribution was higher in summer 2018. In addition, communities from invaded and noninvaded
129 *C. 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 (invaded [$(7.7 \pm 3.9\%)$] and noninvaded [$(7.8 \pm 2.4\%)$]) associated communities in autumn and
135 for seawater communities in winter ($8.8 \pm 7.5\%$). 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 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 *Pseudoaehrenia*, 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 invaded and noninvaded
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 characterisitic 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
¹⁸¹ *C. cylindracea*. On average they were comprising 11.2 ± 13.3 % of all sequences. While
¹⁸² seawater and *C. nodosa* communities were consisted of only 0.1 ± 0.08 % and 1.0 ± 0.7
¹⁸³ % *Desulfobacterota* sequences, respectively, in the invaded and noninvaded *C. cylindracea*
¹⁸⁴ communities their proportion was 25.7 ± 11.2 % and 24.0 ± 4.3 %, respectively (Figure 9). The
¹⁸⁵ community was mainly consisted of no relative *Desulfobacteraceae*, *Desulfatitalea*, no relative
¹⁸⁶ *Desulfobulbaceae*, *Desulfobulbus*, no relative *Desulfocapsaceae*, *Desulfopila*, *Desulforhopalus*,
¹⁸⁷ *Desulfotalea*, SEEP-SRB4 and uncultured *Desulfocapsaceae* (Figure 9).

188 **Discussion**

189 Surfaces of marine macrophytes are harboring biofilms consisted of diverse microbial taxa
190 (Egan *et al.*, 2013; Tarquinio *et al.*, 2019). No standard protocol has been developed to study
191 these macophyte associated microbes (Ugarelli *et al.*, 2019). Different procedures of microbial
192 cells removal 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* (invaded and
202 noninvaded), 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 probably a consequence
206 of part of epiphytic OTUs that are in contact with the sediment. *C. cylindracea* stolon is attached
207 to the sediment surface with rhizoids, so the stolon and rhizoids are in a direct contact with the
208 sediment. Part of the surface attached *Caulerpa cylindracea* community is therefore comprised of
209 OTUs that are in contact with a different environment and that could cause the observed increase
210 in richness. In addition, seasonal richness differences observed for surface attached communities
211 showed slightly higher values in spring and summer. This pattern could be explained by a higher
212 macrophyte growth in these seasons (M. Najdek, personal communication; Zavodnik *et al.*,
213 1998; Ruitton *et al.*, 2005). During active periods macrophytes exhibit a more dynamic chemical

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

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

238 Beside differences between seawater and surface associated communities, there were
239 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 *C. nodosa* and *C. cylindracea* was *Desulfobacterota*, whose sequences were more abundant in the *C. 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 find at every sampling time ($\leq 1.0\%$). Interestingly, these OTUs were accounting for a high proportion of sequences ($\leq 52.4\%$). 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 *C. nodosa* and *C. cylindracea* associated communities were identified that could be linked to the growth cycle of the seagrass and macroalgae (M. Najdek, personal communication). *C. nodosa* was characterized by a Spring community during maximum seagrass proliferation, a Summer community during a biomass maximum and a Autumn/Winter community during a biomass

267 decay. In contrast, *C. cylindracea* started to proliferate in late Spring and was characterized only
268 by a Summer community during maximal biomass increase and by a Autumn/Winter/Spring
269 community when the biomass were at the peak and the settlement started to subsequently decay.
270 Similar seasonal changes in the epiphytic community was described also for other macroalgae
271 (Tujula *et al.*, 2010; Lachnit *et al.*, 2011). Higher temporal stability of *C. cylindracea* surface
272 communities in comparison to *C. nodosa* were also observed in the higher proportion of shared
273 communities between two consecutive sampling points.

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

macrophytes and it is suggested that are involved in the hydrolysis and utilization of complex carbon sources (Burke *et al.*, 2011b; McIlroy and Nielsen, 2014; Crump *et al.*, 2018). On the other hand, families *Flavobacteriaceae* and *Rhodothermaceae* showed seasonal patterns, with *Flavobacteriaceae* being more pronounced from November to June and *Rhodothermaceae* from June to October. Within *Alphaproteobacteria* the family *Rhodobacteraceae* was comprising the majority of sequences thought the year. This metabolically versatile family is often associated with macrophyte surfaces and usually is one of the most abundant groups (Burke *et al.*, 2011b; Michelou *et al.*, 2013; Pujalte *et al.*, 2014). In addition, *Hyphomonadaceae* were found in all samples. Interestingly, some of the species within this group contain stalks on their cells which can be used to attach to the macrophyte surface (Weidner *et al.*, 2000; Abraham and Rohde, 2014). Within the *Gammaproteobacteria*, sequences without known representatives were the most pronounced group present throughout the year. In addition, *Granulosicoccus* was also found in almost all samples. *Gammaproteobacteria* are often a major constituent of macrophyte epiphytic communities (Burke *et al.*, 2011b; Michelou *et al.*, 2013; Crump *et al.*, 2018). Beside these two groups other less pronounced taxa showed seasonal and host-specific patterns. In example, *C. cylindracea* was characterized by *Thioploca*, a known sulfur sediment bacteria and *Cellvibrionaceae*, a family whose cultured members are known polysaccharides degraders (Jørgensen and Gallardo, 1999; Xie *et al.*, 2017). *Desulfobacterota* were found only associated with *C. cylindracea* and no group within this phylum showed seasonal patterns. The presence of this phylum only on *C. cylindracea* is to be expected as part of the epiphytic community is in a direct contact with the sediment. The *Desulfobacterota* community was dominated by *Desulfatitalea* and no relative *Desulfocapsaceae*, known sulfate sediment groups (Kuever, 2014; Higashioka *et al.*, 2015).

In temperate zones marine macrophytes are exhibiting growth cycles, so it is not surprising that the associated epiphytic microbial community is undergoing partial seasonal changes. In the present study, we could, in every analyzed high rank taxa, identify phylogenetic groups that were present throughout the year and that were comprising most of the sequences and lower

321 proportion taxa showing seasonal patterns connected to the macrophyte growth cycle. Studies
322 focusing on functional comparisons between communities associated with different hosts showed
323 that the majority of functions could be found in every community, indicating functional redundancy
324 (Roth-Schulze *et al.*, 2016). This difference between taxonomic and functional discrepancy was
325 explained by the lottery hypothesis that hypothesize an initial random colonization step performed
326 from a set of functionally equivalent taxonomic groups (Burke *et al.*, 2011a; Roth-Schulze *et al.*,
327 2016). It is possible that functional redundancy is a characteristic of high abundance taxa detected
328 to be present throughout the year, while seasonal and/or host-specific functions are an attribute
329 of taxa displaying successional patterns. Further studies connecting taxonomy with functional
330 properties will be required to elucidate the degree of functional redundancy or specificity in
331 epiphytic microbial communities.

332 **Experimental Procedures**

333 **Sampling**

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

348 **DNA Isolation**

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

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

361 **Illumina 16S rRNA Sequencing**

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

378 **Sequence Analysis**

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

396 **Acknowledgments**

397 This work was founded by the Croatian Science Foundation through the MICRO-SEAGRASS
398 project (IP-2016-06-7118). We would like to thank Margareta Buterer for technical support, Paolo
399 Paliaga for help during sampling and the University Computing Center of the University of Zagreb
400 for access to the cluster Isabella.

401 **References**

- 402 Abraham, W.R. and Rohde, M. (2014) The Family Hyphomonadaceae. In, *The prokaryotes: Alphaproteobacteria and betaproteobacteria*. Springer-Verlag Berlin Heidelberg, pp. 283–299.
- 403
- 404 Allaire, J.J., Xie, Y., McPherson, J., Luraschi, J., Ushey, K., Atkins, A., et al. (2019) rmarkdown: Dynamic Documents for R.
- 405
- 406 Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015) Minor revision to V4 region
- 407 SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic*
- 408 *Microbial Ecology* **75**: 129–137.
- 409
- 410 Armstrong, E., Rogerson, A., and Leftley, J. (2000) The Abundance of Heterotrophic Protists Associated with Intertidal Seaweeds. *Estuarine, Coastal and Shelf Science* **50**: 415–424.
- 411
- 412 Bengtsson, M.M., Bühler, A., Brauer, A., Dahlke, S., Schubert, H., and Blindow, I. (2017) Eelgrass Leaf Surface Microbiomes Are Locally Variable and Highly Correlated with Epibiotic
- 413 Eukaryotes. *Frontiers in Microbiology* **8**: 1312.
- 414
- 415 Bengtsson, M., Sjøtun, K., and Øvreås, L. (2010) Seasonal dynamics of bacterial biofilms on the kelp *Laminaria hyperborea*. *Aquatic Microbial Ecology* **60**: 71–83.
- 416
- 417 Borges, A.V. and Champenois, W. (2015) Seasonal and spatial variability of dimethylsulfoniopropionate (DMSP) in the Mediterranean seagrass *Posidonia oceanica*. *Aquatic Botany* **125**: 72–79.
- 418
- 419 Burke, C., Kjelleberg, S., and Thomas, T. (2009) Selective extraction of bacterial DNA from the surfaces of macroalgae. *Applied and environmental microbiology* **75**: 252–256.
- 420
- 421 Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011a) Bacterial community assembly based on functional genes rather than species. *Proceedings of the National*
- 422 *Academy of Sciences of the United States of America* **108**: 14288–14293.

- 423 Burke, C., Thomas, T., Lewis, M., Steinberg, P., and Kjelleberg, S. (2011b) Composition,
424 uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*.
425 *The ISME journal* **5**: 590–600.
- 426 Burns, B.P., Goh, F., Allen, M., and Neilan, B.A. (2004) Microbial diversity of extant
427 stromatolites in the hypersaline marine environment of Shark Bay, Australia. *Environmental*
428 *Microbiology* **6**: 1096–1101.
- 429 Cai, X., Gao, G., Yang, J., Tang, X., Dai, J., Chen, D., and Song, Y. (2014) An ultrasonic
430 method for separation of epiphytic microbes from freshwater submerged macrophytes. *Journal of*
431 *basic microbiology* **54**: 758–761.
- 432 Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al.
433 (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq
434 platforms. *The ISME Journal* **6**: 1621–1624.
- 435 Cardoso, D.C., Cretoiu, M.S., Stal, L.J., and Bolhuis, H. (2019) Seasonal development of a
436 coastal microbial mat. *Scientific Reports* **9**: 1–14.
- 437 Crump, B.C. and Koch, E.W. (2008) Attached bacterial populations shared by four species of
438 aquatic angiosperms. *Applied and environmental microbiology* **74**: 5948–5957.
- 439 Crump, B.C., Wojahn, J.M., Tomas, F., and Mueller, R.S. (2018) Metatranscriptomics and
440 amplicon sequencing reveal mutualisms in seagrass microbiomes. *Frontiers in Microbiology* **9**:
- 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* **37**:
443 462–476.
- 444 Fahimipour, A.K., Kardish, M.R., Lang, J.M., Green, J.L., Eisen, J.A., and Stachowicz, J.J.
445 (2017) Globalscale structure of the eelgrass microbiome. *Applied and Environmental Microbiology*

446 83:

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 *Desulfatitalea tepidiphila* S28bFT. *Genome Announcements* **3**:

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. **113**: 363–375.

457 Jørgensen, B.B. and Gallardo, V.A. (1999) *Thioploca* spp.: filamentous sulfur bacteria with
458 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: Deltaproteobacteria*
469 and *epsilonproteobacteria*. 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 Margulis, L. (1991) Symbiogenesis and symbioticism. In, Margulis,L. and Fester,R. (eds),
479 *Symbiosis as a source of evolutionary innovation*. Cambridge, Massachusetts: The MIT Press, pp.
480 1–14.
- 481 Massana, R., Murray, A.E., Preston, C.M., and DeLong, E.F. (1997) Vertical distribution and
482 phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Applied*
483 *and environmental microbiology* **63**: 50–56.
- 484 McIlroy, S.J. and Nielsen, P.H. (2014) The Family Saprospiraceae. In, *The prokaryotes: Other*
485 *major lineages of bacteria and the archaea*. Springer-Verlag Berlin Heidelberg, pp. 863–889.
- 486 Michelou, V.K., Caporaso, J.G., Knight, R., and Palumbi, S.R. (2013) The Ecology of
487 Microbial Communities Associated with *Macrocystis pyrifera*. *PLoS ONE* **8**: e67480.
- 488 Miranda, L.N., Hutchison, K., Grossman, A.R., and Brawley, S.H. (2013) Diversity and
489 Abundance of the Bacterial Community of the Red Macroalga *Porphyra umbilicalis*: Did Bacterial
490 Farmers Produce Macroalgae? *PLoS ONE* **8**: e58269.

- 491 Mobberley, J.M., Ortega, M.C., and Foster, J.S. (2012) Comparative microbial diversity
492 analyses of modern marine thrombolitic mats by barcoded pyrosequencing. *Environmental*
493 *Microbiology* **14**: 82–100.
- 494 Morrissey, K.L., Çavas, L., Willems, A., and De Clerck, O. (2019) Disentangling the influence
495 of environment, host specificity and thallus differentiation on bacterial communities in siphonous
496 green seaweeds. *Frontiers in Microbiology* **10**:
- 497 Neuwirth, E. (2014) RColorBrewer: ColorBrewer Palettes.
- 498 Nõges, T., Luup, H., and Feldmann, T. (2010) Primary production of aquatic macrophytes
499 and their epiphytes in two shallow lakes (Peipsi and Võrtsjärv) in Estonia. *Aquatic Ecology* **44**:
500 83–92.
- 501 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019)
502 vegan: Community Ecology Package.
- 503 Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016) Every base matters: assessing small
504 subunit rRNA primers for marine microbiomes with mock communities, time series and global
505 field samples. *Environmental Microbiology* **18**: 1403–1414.
- 506 Pujalte, M.J., Lucena, T., Ruvira, M.A., Arahal, D.R., and Macián, M.C. (2014) The
507 family Rhodobacteraceae. In, *The prokaryotes: Alphaproteobacteria and betaproteobacteria*.
508 Springer-Verlag Berlin Heidelberg, pp. 439–512.
- 509 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA
510 ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic*
511 *acids research* **41**: D590–6.
- 512 R Core Team (2019) R: A Language and Environment for Statistical Computing, Vienna,
513 Austria: R Foundation for Statistical Computing.

- 514 Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D.K.A., Thums, M., et al. (2014)
- 515 Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. *PLoS*
- 516 **one** **9**: e100289.
- 517 Reyes, J. and Sansón, M. (2001) Biomass and production of the epiphytes on the leaves of
- 518 *Cymodocea nodosa* in the Canary Islands. *Botanica Marina* **44**: 307–313.
- 519 Rickert, E., Wahl, M., Link, H., Richter, H., and Pohnert, G. (2016) Seasonal variations in
- 520 surface metabolite composition of *fucus vesiculosus* and *fucus serratus* from the Baltic Sea. *PLoS*
- 521 **ONE** **11**:
- 522 Roth-Schulze, A.J., Zozaya-Valdés, E., Steinberg, P.D., and Thomas, T. (2016) Partitioning of
- 523 functional and taxonomic diversity in surface-associated microbial communities. *Environmental*
- 524 *microbiology* **18**: 4391–4402.
- 525 Ruitton, S., Verlaque, M., and Boudouresque, C.F. (2005) Seasonal changes of the introduced
- 526 *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its
- 527 Mediterranean range. *Aquatic Botany* **82**: 55–70.
- 528 Salaün, S., Barre, S. la, Santos-Goncalvez, M.D., Potin, P., Haras, D., and Bazire, A. (2012)
- 529 Influence of Exudates of the Kelp *Laminaria Digitata* on Biofilm Formation of Associated and
- 530 Exogenous Bacterial Epiphytes. *Microbial Ecology* **64**: 359–369.
- 531 Schloss, P.D., Jenior, M.L., Koumpouras, C.C., Westcott, S.L., and Highlander, S.K. (2016)
- 532 Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. *PeerJ*
- 533 **4**: e1869.
- 534 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al.
- 535 (2009) Introducing mothur: open-source, platform-independent, community-supported software
- 536 for describing and comparing microbial communities. *Applied and environmental microbiology*
- 537 **75**: 7537–7541.

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

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

544 Ugarelli, K., Laas, P., and Stingl, U. (2019) The microbial communities of leaves and roots
545 associated with turtle grass (*Thalassia testudinum*) and manatee grass (*syringodium filliforme*) are
546 distinct from seawater and sediment communities, but are similar between species and sampling
547 sites. *Microorganisms* **7**:

548 Uku, J., Björk, M., Bergman, B., and Díez, B. (2007) Characterization and comparison of
549 prokaryotic epiphytes associated with three East African seagrasses. *Journal of Phycology* **43**:
550 768–779.

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

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

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

558 Xie, Y. (2014) knitr: A Comprehensive Tool for Reproducible Research in {R}. In,
559 Stodden, V., Leisch, F., and Peng, R.D. (eds), *Implementing reproducible computational research*.
560 Chapman; Hall/CRC.

561 Xie, Y. (2019a) knitr: A General-Purpose Package for Dynamic Report Generation in R.

562 Xie, Y. (2019b) TinyTeX: A lightweight, cross-platform, and easy-to-maintain LaTeX
563 distribution based on TeX Live. *TUGboat* 30–32.

564 Xie, Y. (2020) tinytex: Helper Functions to Install and Maintain 'TeX Live', and Compile
565 'LaTeX' Documents.

566 Xie, Y., Allaire, J.J., and Grolemund, G. (2018) R Markdown: The Definitive Guide, Boca
567 Raton, Florida: Chapman; Hall/CRC.

568 Xie, Z., Lin, W., and Luo, J. (2017) Comparative Phenotype and Genome Analysis of
569 Cellvibrio sp. PR1, a Xylanolytic and Agarolytic Bacterium from the Pearl River. *BioMed*
570 *research international* **2017**: 6304248.

571 Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al. (2014) The SILVA
572 and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic acids research* **42**:
573 D643–8.

574 Zavodnik, N., Travizi, A., and De Rosa, S. (1998) Seasonal variations in the rate of
575 photosynthetic activity and chemical composition of the seagrass *Cymodocea nodosa* (Ucr.) Asch.
576 *Scientia Marina* **62**: 301–309.

577 Zhu, H. (2019) kableExtra: Construct Complex Table with 'kable' and Pipe Syntax.

578 **Figure Captions**

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

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

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

592 **Figure 4.** Taxonomic classification and relative contribution of the most abundant (> 1 %) bacterial
593 and archaeal sequences on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea*
594 [Invaded and Noninvaded]) and in the ambient seawater.

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

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

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

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

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

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

620 **Figures**

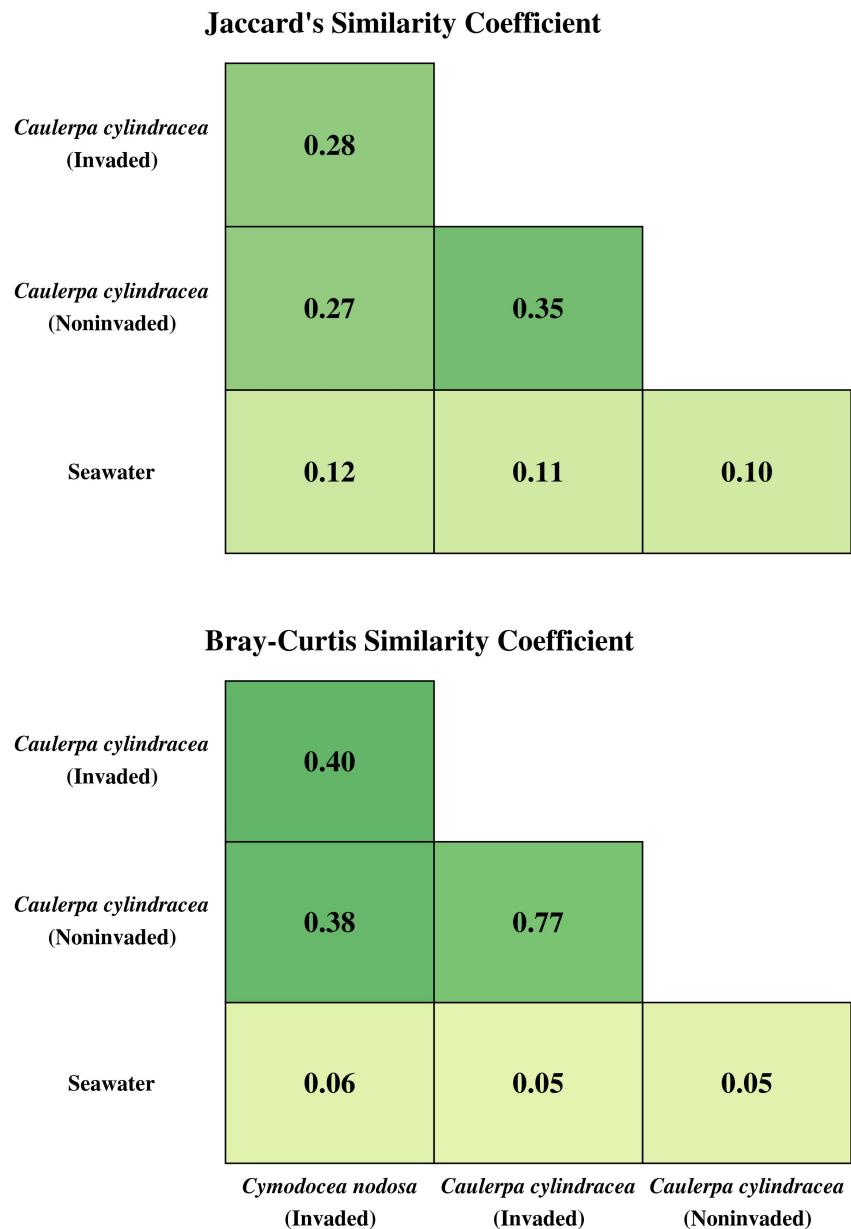


Figure 1. Proportion of shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) and shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) between communities associated with the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded])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

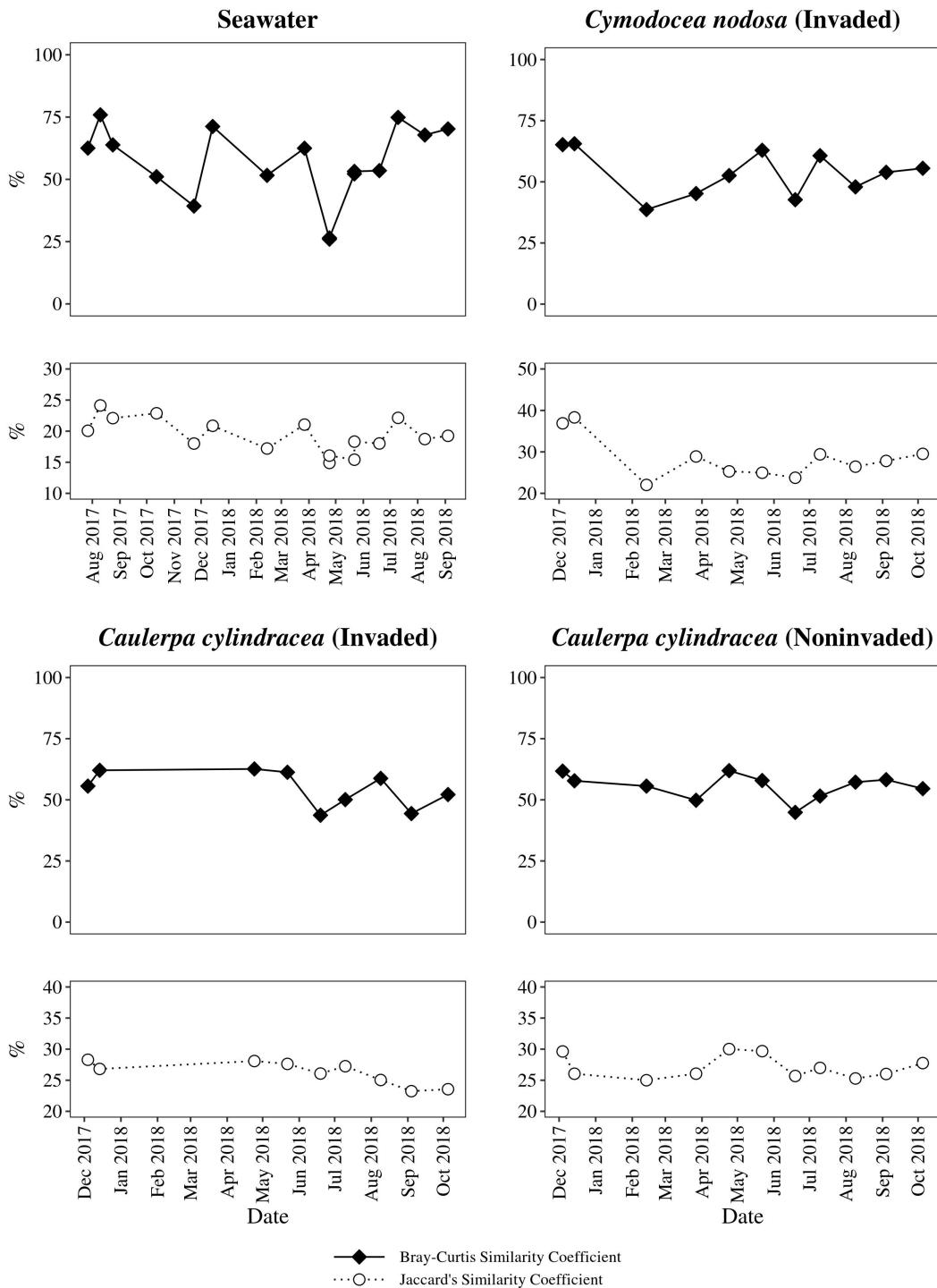


Figure 2. Proportion of shared bacterial and archaeal communities (Bray-Curtis Similarity Coefficient) and shared bacterial and archaeal OTUs (Jaccard's Similarity Coefficient) between consecutive sampling points and from the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded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

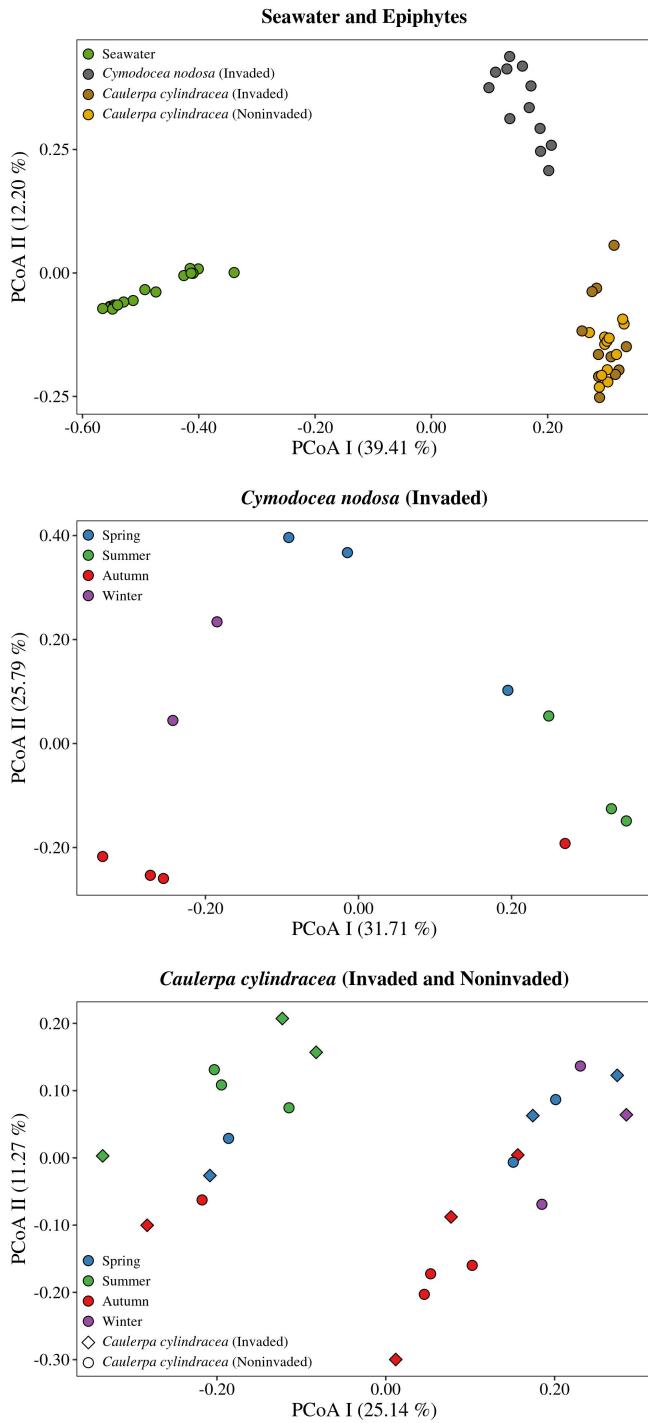


Figure 3. Principal Coordinates Analysis (PCoA) of Bray-Curtis distances based on OTU abundances of bacterial and archaeal communities from the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. Samples from the same environment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

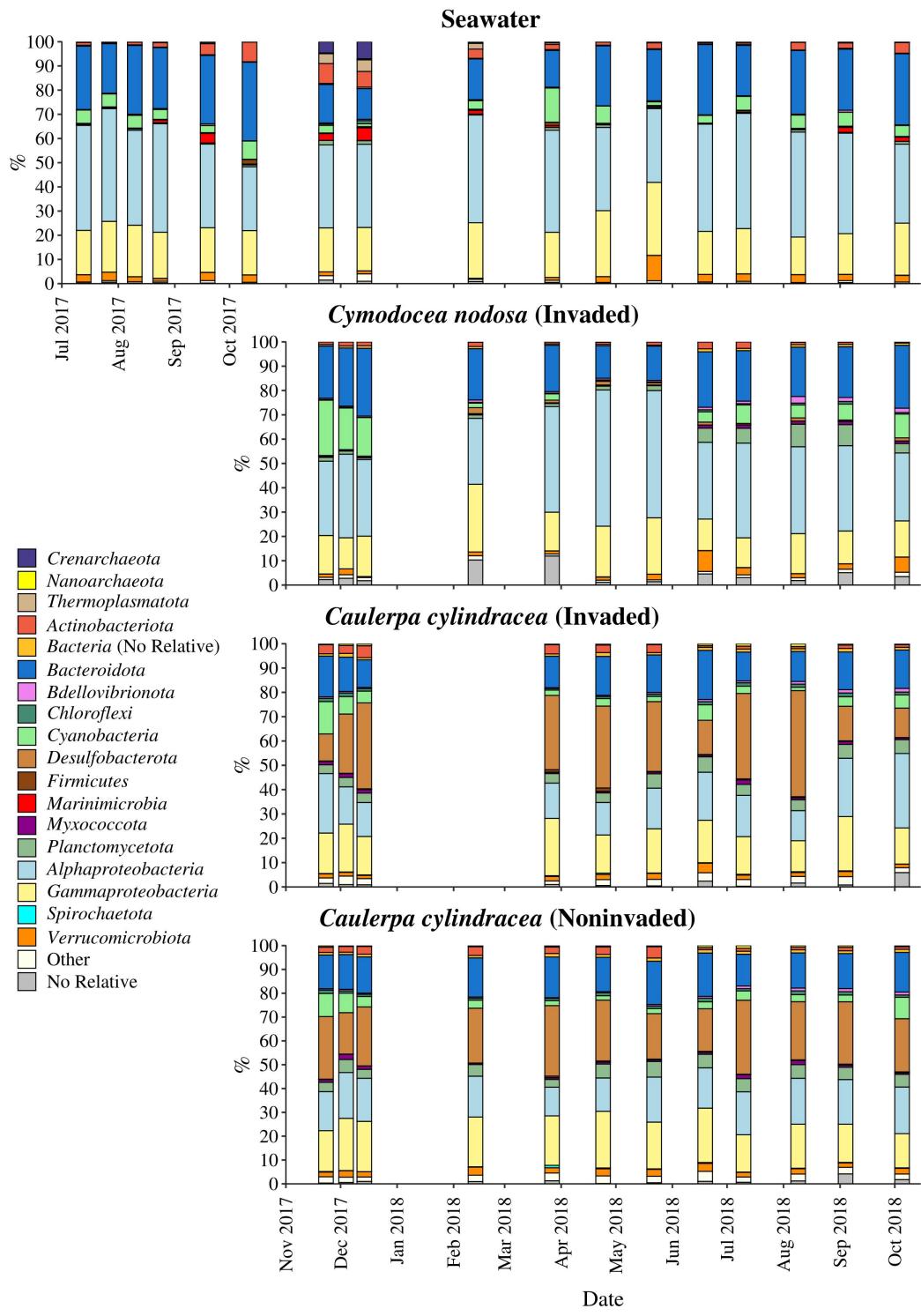


Figure 4. Taxonomic classification and relative contribution of the most abundant (> 1 %) bacterial and archaeal sequences on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater.

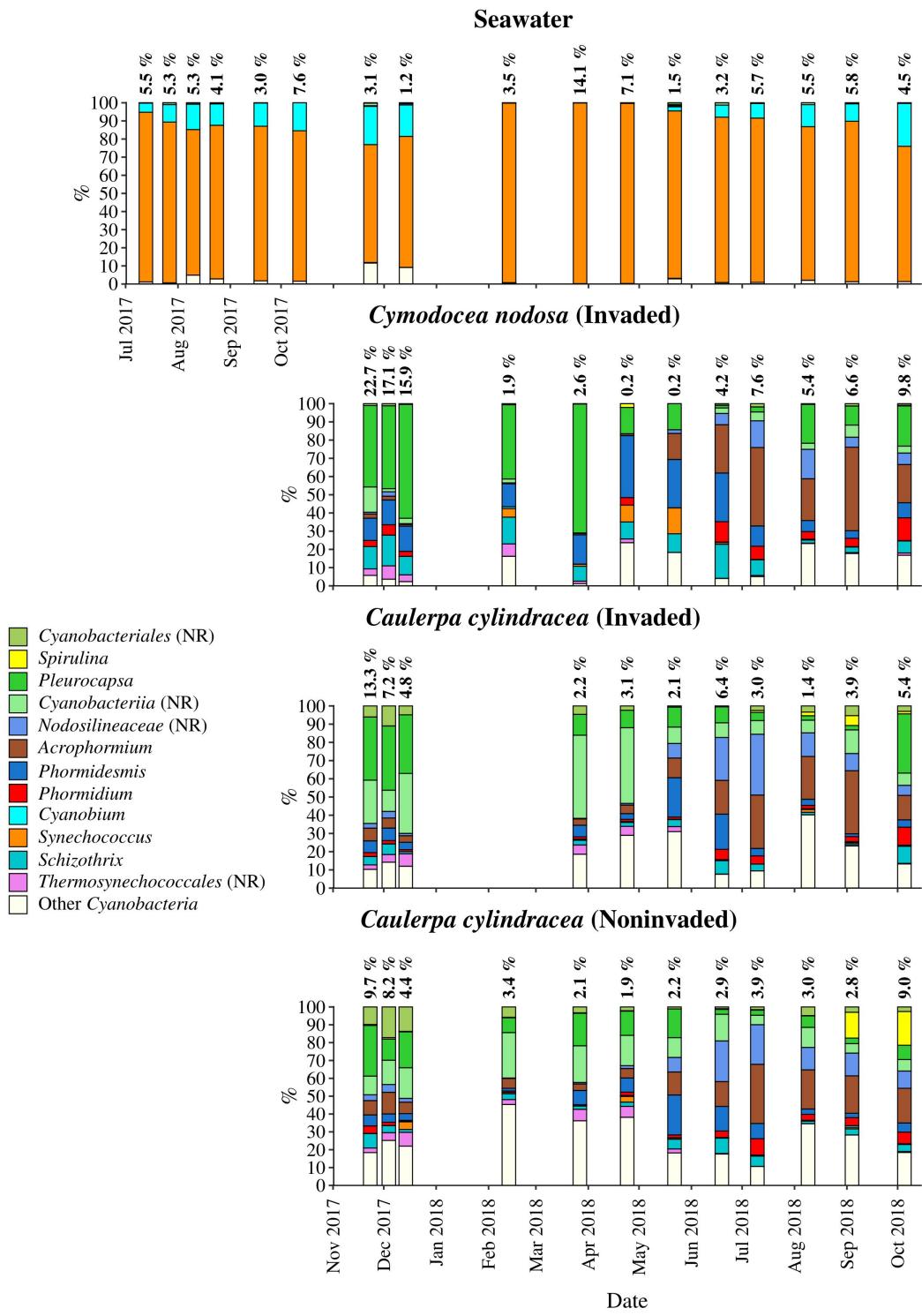


Figure 5. Taxonomic classification and relative contribution of the most abundant (> 1 %) cyanobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. The proportion of cyanobacterial sequences in the total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

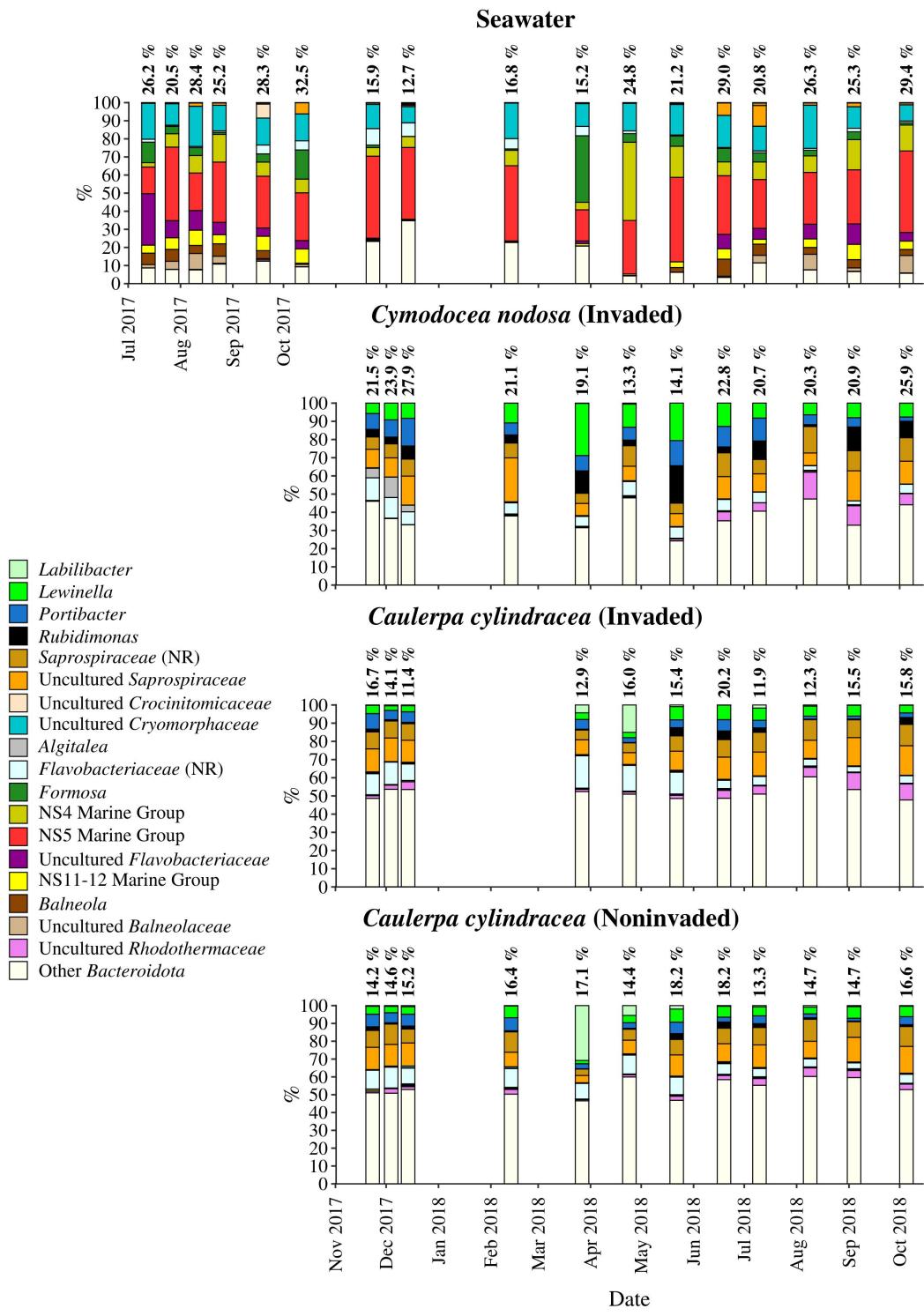


Figure 6. Taxonomic classification and relative contribution of the most abundant (> 2 %) sequences within the *Bacteroidota* on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. The proportion of sequences classified as *Bacteroidota*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

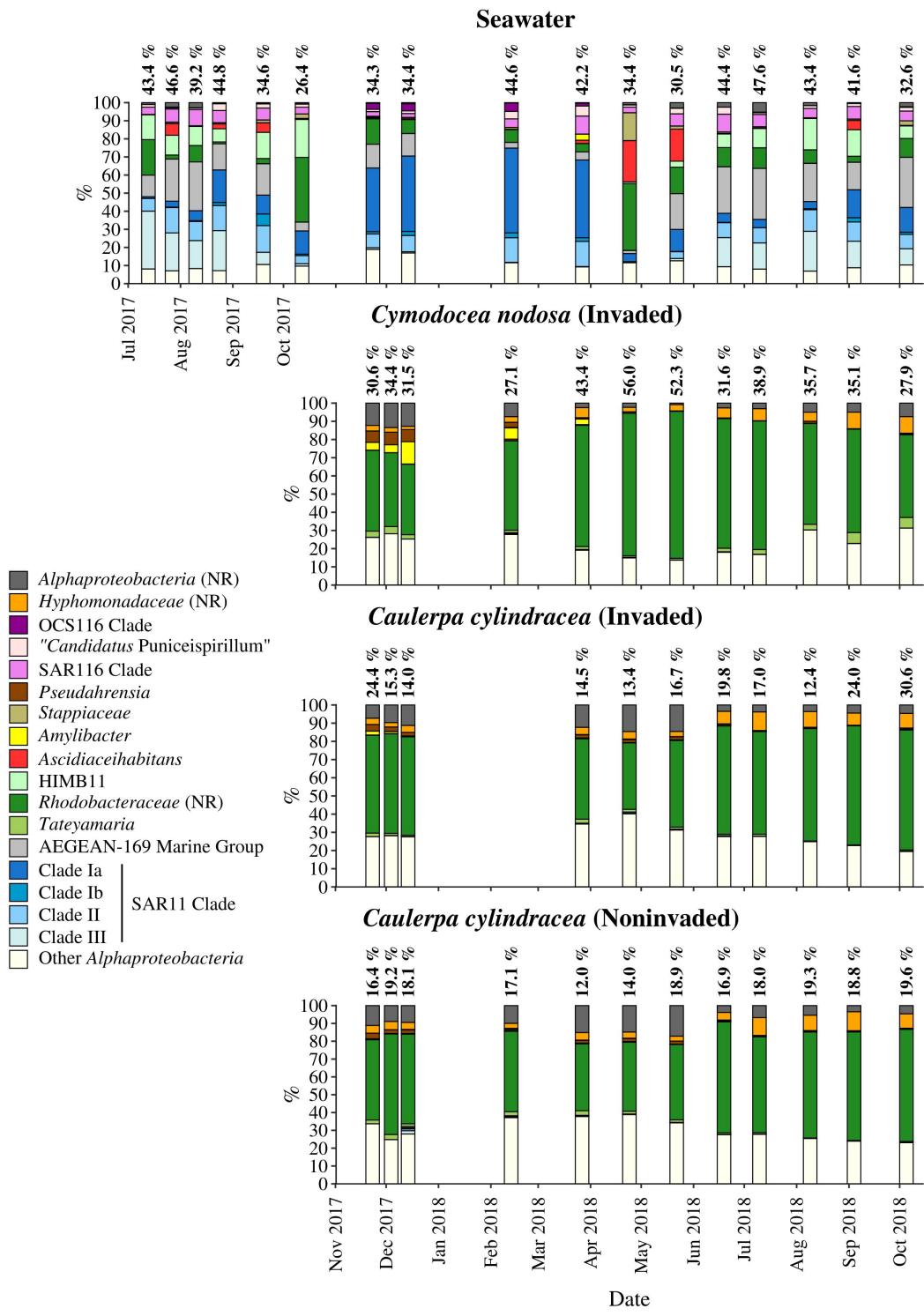


Figure 7. Taxonomic classification and relative contribution of the most abundant (> 2 %) alphaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. The proportion of alphaproteobacterial sequences in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

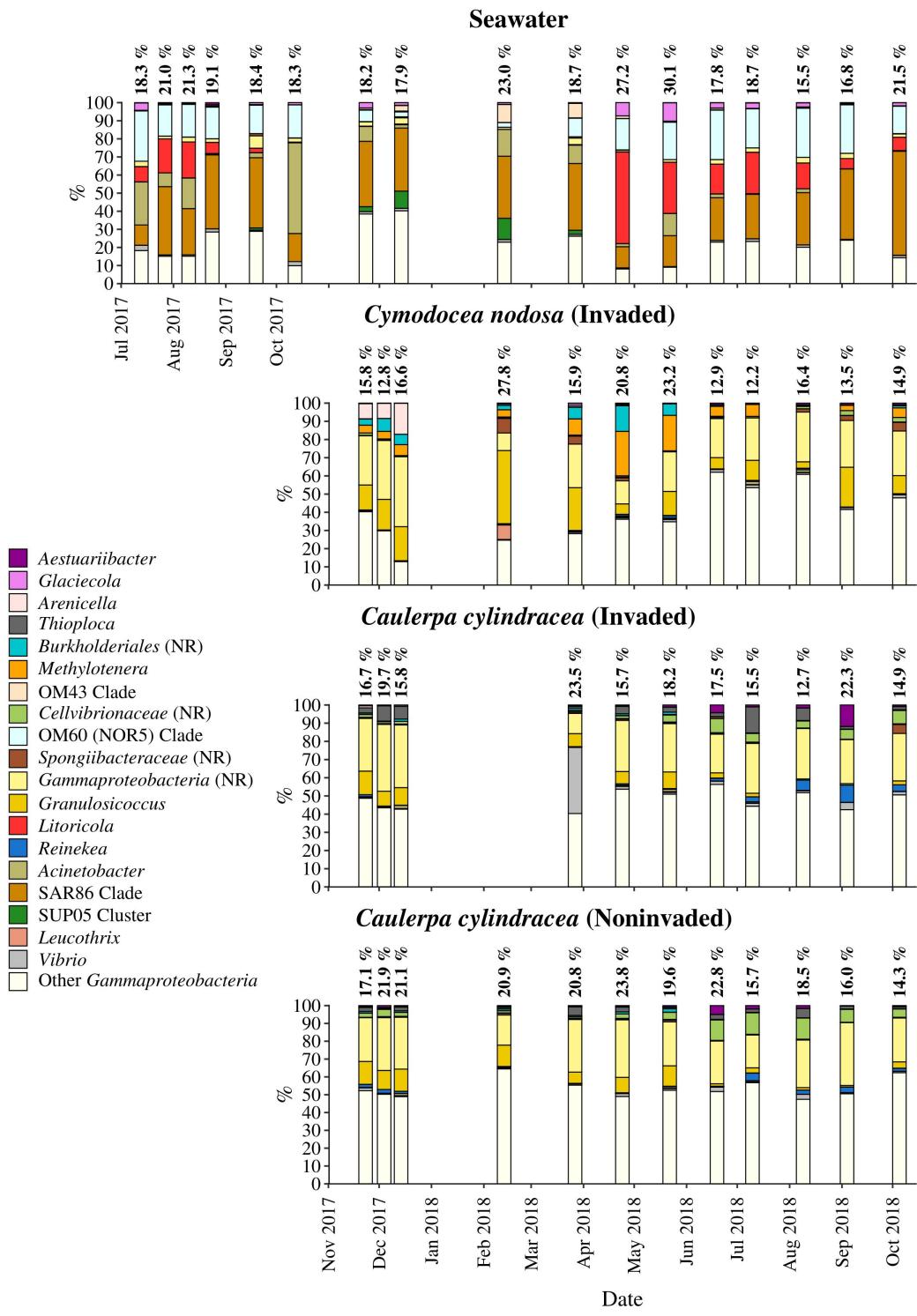


Figure 8. Taxonomic classification and relative contribution of the most abundant (> 2 %) gammaproteobacterial sequences on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. The proportion of gammaproteobacterial sequences in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

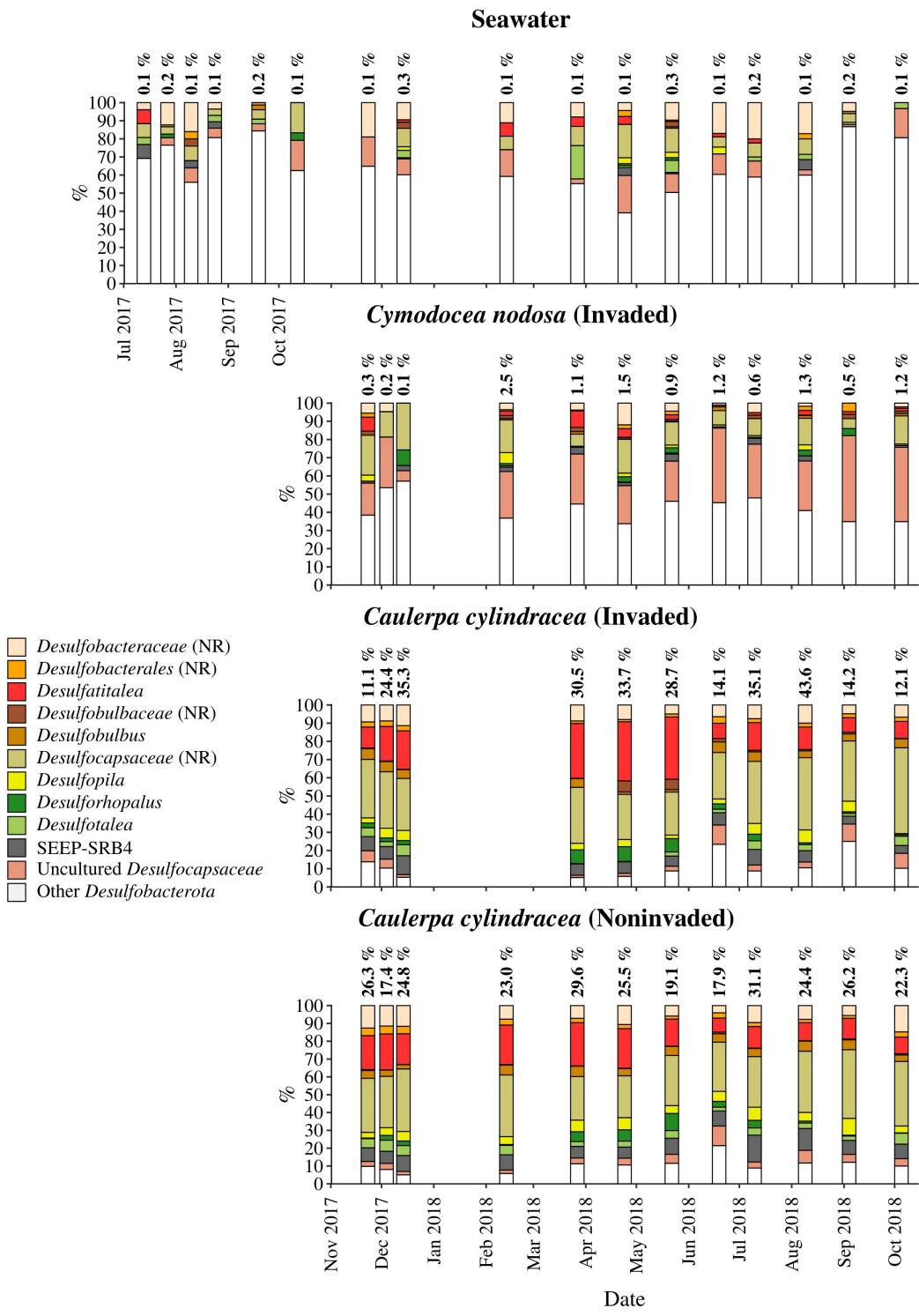


Figure 9. Taxonomic classification and relative contribution of the most abundant (> 1 %) sequences within the *Desulfobacterota* on the surfaces of macrophytes (*C. nodosa* [Invaded] and *C. cylindracea* [Invaded and Noninvaded]) and in the ambient seawater. The proportion of sequences classified as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