The effect of carbon subsidies on marine planktonic niche partitioning and recruitment during biofilm assembly

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2 ABSTRACT

The influence of resource availability on planktonic and biofilm microbial community membership is poorly understood. Heterotrophic bacteria derive some to all of their organic carbon (C) from photoautotrophs while simultaneously competing with photoautotrophs for inorganic nutrients such as phosphorus (P) or nitrogen (N). Therefore, C inputs have the potential to shift the competitive balance of aquatic microbial communities by increasing the resource space available to heterotrophs (more C) while decreasing the resource space available to photoautotrophs (less mineral nutrients due to increased competition from heterotrophs). To test how resource dynamics affect membership of planktonic communities and assembly of biofilm communities we amended a series of flow-through mesocosms with C to alter the availability of C among treatments. Each mesocosm was fed with unfiltered seawater and incubated with sterilized microscope slides as surfaces for biofilm formation. The highest C treatment had the highest planktonic heterotroph abundance, lowest planktonic photoautotroph abundance, and highest biofilm biomass. We surveyed bacterial 16S rRNA genes and plastid 23S rRNA genes to characterize biofilm and planktonic community membership and structure. Regardless of resource additions, biofilm communities had higher alpha diversity than planktonic communities in all mesocosms. Heterotrophic plankton communities were distinct from heterotrophic biofilm communities in all but the highest C treatment where heterotrophic plankton and biofilm communities resembled each other after 17 days. Unlike the heterotrophs, photoautotrophic plankton communities were different than photoautotrophic biofilm communities in composition in all treatments including the highest C treatment. Our results suggest that although resource amendments affect community membership and structure, microbial lifestyle (biofilm versus planktonic) has a stronger influence on community composition.

Keywords: microbial ecology, 16S, 23S, planktonic, biofilm, carbon subsidies, resource stoichiometry

1 INTRODUCTION

Biofilms are diverse and complex microbial consortia, and, the biofilm lifestyle is the rule rather than the exception for microbes in many environments. Large and small-scale biofilm architectural features play an important role in their ecology and influence their role in biogeochemical cycles (**Battin et al.**, 2007).

30 Fluid mechanics impact biofilm structure and assembly (**Hodl et al.**, 2011; **Besemer et al.**, 2009; **Battin**
31 **et al.**, 2003), but it is less clear how other abiotic factors such as resource availability affect biofilm
32 assembly. Aquatic biofilms initiate with seed propagules from the planktonic community (**Hodl et al.**,
33 2011; **McDougald et al.**, 2011). Thus, resource amendments that influence planktonic communities may
34 also influence the recruitment of microbial populations during biofilm community assembly.

35   In a crude sense, biofilm and planktonic microbial communities divide into two key groups: oxygenic
36 phototrophs including eukaryotes and cyanobacteria (hereafter "photoautotrophs"), and heterotrophic
37 bacteria and archaea. This dichotomy, admittedly an abstraction (e.g. non-phototrophs can also be
38 autotrophs), can be a powerful paradigm for understanding community shifts across ecosystems of varying
39 trophic state (**Cotner and Biddanda**, 2002). Heterotrophs meet some to all of their organic carbon (C)
40 requirements from photoautotroph produced C while simultaneously competing with photoautotrophs for
41 limiting nutrients such as phosphorous (P) (**Bratbak and Thingstad**, 1985). The presence of external C
42 inputs, such as terrigenous C leaching from the watershed (**Jansson et al.**, 2008; **Karlsson et al.**, 2012)
43 or C exudates derived from macrophytes (**Stets and Cotner**, 2008a,b), can alleviate heterotroph reliance
44 on photoautotroph derived C and shift the heterotroph-photoautotroph relationship from commensal and
45 competitive to strictly competitive (see **Stets and Cotner**, 2008a, Figure 1). Therefore, increased C supply
46 should increase the resource space available to heterotrophs and increase competition for mineral nutrients
47 decreasing nutrients available for photoautotrophs (assuming that heterotrophs are superior competitors
48 for limiting nutrients as has been observed (see **Cotner and Wetzel**, 1992, Figure 1)). These dynamics
49 should result in the increase in heterotroph biomass relative to the photoautotroph biomass along a
50 gradient of increasing labile C inputs. We refer to this differential allocation of limiting resources among
51 components of the microbial community as niche partitioning.

52   While these gross level dynamics have been discussed conceptually (**Cotner and Biddanda**, 2002) and
53 to some extent demonstrated empirically (**Stets and Cotner**, 2008a), the effects of biomass dynamics on
54 photoautotroph and heterotroph membership and structure has not been directly evaluated in plankton or
55 biofilms. In addition, how changes in planktonic communities propagate to biofilms during community
56 assembly is not well understood. We designed this study to test if C subsidies shift the biomass balance
57 between autotrophs and heterotrophs within the biofilm or its seed pool (i.e. the plankton), and, to measure
58 how changes in biomass pool size alter composition of the plankton and biofilm communities. Specifically,
59 we amended marine mesocosms with varying levels of labile C input and evaluated differences in
60 photoautotroph and heterotrophic bacterial biomass in plankton and biofilm samples along the C gradient.
61 In each treatment we characterized plankton and biofilm community composition by PCR amplifying and
62 DNA sequencing 16S rRNA genes and plastid 23S rRNA genes.

2   MATERIALS AND METHODS

63 *2.0.1 Experimental Design*   Test tube racks were placed in one smaller (185 L, control) and 3 larger
64 (370 L) flow-through mesocosms. All mesocosms were fed directly with marine water from an inflow
65 source in Great Bay, Woods Hole, MA, approximately 200 m from the shore. Each mesocosm had
66 an adjustable flow rate that resulted in a residence time of approximately 12 h. Irregular variation in
67 inflow rate caused the actual mesocosm flow rate to vary around the targeted flow rate throughout the day.
68 However, regular monitoring ensured that the entire volume of each system was flushed approximately
69 two times per day (i.e. maintained a residence time of ∼12 h). To provide a surface for biofilm formation,
70 we attached coverslips to glass slides using nail polish and then attached each slide to the test tube racks
71 using office-style binder clips. Twice daily 10 mL of 37 mM KPO_4 and 1, 5 and 50 mL of 3.7 M glucose
72 were added to each of 3 mesocosms to achieve target C:P resource amendments of 10, 100 and 500. The
73 goal of the resource amendments was to create a gradient of labile carbon. The same amount of P was
74 added to each treated mesocosm to ensure that response to additions of C were not inhibited by extreme
75 P limitation. The control mesocosm did not receive any C or P amendments.

76 *2.0.2 DOC and Chlorophyll Measurements* To assess the efficacy of the C additions, we sampled
77 each mesocosm twice daily during the first week of the experiment to evaluate dissolved organic C
78 (DOC) content. After the initiation of the experiment we collected plankton on filters regularly to evaluate
79 planktonic Chl *a* and bacterial abundance. Once it was clear that pool size of each community had been
80 altered (day 8) we filtered plankton onto 0.2 μm filters and harvested coverslips to assess bacterial and
81 algal biofilm community composition (16S and plastid 23S rDNA). In addition, all mesocosms were
82 analyzed for community composition a second time (day 17) to assess how community composition of
83 both the plankton and biofilm communities had changed over time. Control samples were only analyzed
84 for community composition on day 17.

85 Samples for dissolved organic C (DOC) analysis were collected in acid washed 50 mL falcon tubes
86 after filtration through a 0.2 μm polycarbonate membrane filter (Millipore GTTP GTTP02500, Sigma
87 Aldrich P9199) attached to a 60 mL syringe. Syringes and filters were first flushed multiple times with
88 the control sample to prevent leaching of C from the syringe or the filter into the sample. Samples were
89 then frozen and analyzed for organic C content with a Shimadzu 500 TOC analyzer (**Wetzel and Likens**,
90 2000). Biomass of all biofilm samples were measured by difference in pre-(without biofilm) and post-
91 (with biofilm) weighed GF/F filters after oven drying overnight at 60 $^{\circ}$C.

92 For Chl *a* analysis, we collected plankton on GF/F filters (Whatman, Sigma Aldrich Cat. # Z242489) by
93 filtering between 500 mL and 1L from the water column of each mesocosm for each treatment. For biofilm
94 samples, all biofilm was gently removed from the complete area of each coverslip (3 coverslips for each
95 treatment per sampling event) before being placed in a test tube for extraction with 90-95% acetone for
96 ~32 hours at -20 $^{\circ}$C and analyzed immediately using a Turner 10-AU fluorometer (**Wetzel and Likens**,
97 2000).

98 Bacterial abundance of the planktonic samples was analyzed using DAPI staining and direct
99 visualization on a Zeis Axio epifluorescence microscope after the methods of **Porter and Feig** (1980).
100 Briefly, 1-3 mL of water was filtered from three separate water column samples through a 0.2 μm black
101 polycarbonate membrane filter and post stained with a combination of DAPI and Citifluor mountant media
102 (Ted Pella Redding, Ca) to a final concentration of 1 μL mL^{-1}.

103 *2.0.3 DNA extraction* For plankton, cells were collected by filtering between 20 – 30 mL of water
104 onto a 0.2 μm pore-size polycarbonate filter (Whatman Nucleopore 28417598, Sigma-Aldrich cat#
105 WHA110656). For biofilm communities, biomass from the entire coverslip area of three separate slides
106 was collected and combined in an Eppendorf tube by gently scraping the slip surface with an ethanol
107 rinsed and flamed razor blade. DNA from both the filter and the biofilm was extracted using a Mobio
108 Power Soil DNA isolation kit (MoBio Cat. # 12888).

109 *2.0.4 PCR* Samples were amplified for 454 sequencing using a forward and reverse fusion primer. The
110 forward primer was constructed with (5'-3') the Roche A linker, an 8-10 bp barcode, and the forward gene
111 specific primer sequence. The reverse fusion primer was constructed with (5'-3') a biotin molecule, the
112 Roche B linker and the reverse gene specific primer sequence. The gene specific primer pair for bacterial
113 SSU rRNA genes was 27F/519R (**Lane**, 1991). The primer pair p23SrV_f1/p23SrV_r1 was used to target
114 23S rRNA genes on plastid genomes (**Sherwood and Presting**, 2007). Amplifications were performed in
115 25 μL reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 μL of each
116 5 uM primer, and 1 μL of template. Reactions were performed on ABI Veriti thermocyclers (Applied
117 Biosytems, Carlsbad, California) under the following thermal profile: 95 $^{\circ}$C for 5 min, then 35 cycles
118 of 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min, followed by one cycle of 72°C for 10 min and
119 4 $^{\circ}$C hold. Amplification products were visualized with eGels (Life Technologies, Grand Island, New
120 York). Products were then pooled equimolar and each pool was cleaned with Diffinity RapidTip (Diffinity
121 Genomics, West Henrietta, New York), and size selected using Agencourt AMPure XP (BeckmanCoulter,
122 Indianapolis, Indiana) following Roche 454 protocols (454 Life Sciences, Branford, Connecticut). Size
123 selected pools were then quantified and 150 ng of DNA was hybridized to Dynabeads M-270 (Life

124 Technologies) to create single stranded DNA following Roche 454 protocols (454 Life Sciences). Single
125 stranded DNA was diluted and used in emPCR reactions, which were performed and subsequently
126 enriched. Sequencing followed established manufacture protocols (454 Life Sciences).

127 *2.0.5   Sequence quality control*   The 16S rRNA gene and plastid 23S rRNA gene sequence collections
128 were demultiplexed and sequences with sample barcodes not matching expected barcodes were discarded.
129 We used the maximum expected error metric (**Edgar**, 2013) calculated from sequence quality scores to
130 cull poor quality sequences from the dataset. Specifically, we discarded any sequence with a maximum
131 expected error count greater than 1 after truncating the sequence to 175 nt. The forward primer and
132 barcode was trimmed from the remaining reads. We checked that all primer trimmed, error screened,
133 and truncated sequences were derived from the targeted region of the LSU or SSU rRNA gene (23S
134 and 16S sequences, respectively) by aligning the reads to Silva LSU or SSU rRNA gene alignment
135 ("Ref" collection, release 115) with the Mothur (**Schloss et al.**, 2009) NAST-algorithm (**DeSantis et al.**,
136 2006) aligner and inspecting the alignment coordinates. Reads falling outside the expected alignment
137 coordinates were culled from the dataset. Remaining reads were trimmed to consistent alignment
138 coordinates such that all reads began and ended at the same position in the SSU rRNA gene and
139 screened for chimeras with UChime in "denovo" mode (**Edgar et al.**, 2011) via the Mothur UChime
140 wrapper. 19,978 of 56,322 16S rRNA gene sequencing reads and 44,719 or 78,695 plastid 23S rRNA
141 gene sequencing reads passed quality control.

142 *2.0.6   Taxonomic annotations*   Sequences were taxonomically classified using the UClust (**Edgar**,
143 2010) based classifier in the QIIME package (**Caporaso et al.**, 2010) with the Greengenes database and
144 taxonomic nomenclature (version "gg_13_5" provided by QIIME developers, 97% OTU representative
145 sequences and corresponding taxonomic annotations, (**McDonald et al.**, 2012)) for 16S reads or the Silva
146 LSU database ("Ref" set, version 115, EMBL taxonomic annotations, (**Quast et al.**, 2013)) for the 23S
147 reads as reference. We used the default parameters for the algorithm (i.e. minimum consensus of 51% at
148 any rank, minimum sequence identity for hits at 90% and the maximum accepted hits value was set to 3).

149 *2.0.7   Sequence clustering*   Reads were clustered into OTUs following the UParse pipeline. Specifically
150 USearch (version 7.0.1001) was used to establish cluster centroids at a 97% sequence identity level from
151 the quality controlled data and to map quality controlled reads to the centroids. The initial centroid
152 establishment algorithm incorporates a quality control step wherein potentially chimeric reads are not
153 allowed to become cluster seeds. Additionally, we discarded singleton reads. Eighty-eight and 98% of
154 quality controlled reads could be mapped back to our cluster seeds at a 97% identity cutoff for the 16S
155 and 23S sequences, respectively.

156 *2.0.8   Alpha and Beta diversity analyses*   Alpha diversity calculations were made using PyCogent
157 Python bioinformatics modules (**Knight et al.**, 2007). Rarefaction curves show average OTU counts
158 from 25 re-samplings at intervals of 10 sequences for each sample. Beta diversity analyses were made
159 using Phyloseq (**McMurdie and Holmes**, 2014) and its dependencies (**Oksanen et al.**, 2013). A sparsity
160 threshold of 25% was used for ordination of both plastid 23S and bacterial 16S libraries. Additionally, we
161 discarded any OTUs from the plastid 23S rRNA gene data that could not be annotated as belonging in the
162 Eukaryota or cyanobacteria for differential abundance, ordination and Adonis analyses. Cyanobacterial
163 DNA sequences were removed from 16S rRNA gene sequence collections for ordination, Adonis and
164 differential abundance analyses. We operated under the assumption that non-cyanobacterial bacteria
165 are predominantly heterotrophs in our mesocosm setup and refer to non-cyanobacterial bacteria as
166 "heterotrophs" in the manuscript (this abstraction is useful however there are likely exceptions – i.e.
167 autotrophs among the non-cyanobacterial bacteria). All DNA sequence based results were visualized
168 using GGPlot2 (**Wickham**, 2009). Adonis tests and principal coordinate ordinations were performed using
169 the Bray-Curtis similarity measure for pairwise library comparisons. Adonis tests employed the default
170 value for number of permutations (999) ("adonis" function in Vegan R package, **Oksanen et al.** (2013)).

171 Principal coordinates of OTUs were found by averaging site principal coordinate values for each OTU
172 with OTU relative abundance values (within sites) as weights. The principal coordinate OTU weighted
173 averages were then expanded to match the site-wise variances (**Oksanen et al.**, 2013).

174 *2.0.9   Identifying enriched OTUs*   We used an RNA-Seq differential expression statistical framework to
175 find OTUs enriched in the given sample classes (R package DESeq2 developed by **Love et al.** (2014))
176 (for review of RNA-Seq differential expression statistics applied to microbiome OTU count data see
177 **McMurdie and Holmes** (2014)). We use the term differential abundance coined by **McMurdie and**
178 **Holmes** (2014) to denote OTUs that have different relative abundance across sample classes. We were
179 particularly interested in two sample classes: 1) lifestyle (biofilm or planktonic) and, 2) high C (C:P
180 = 500) versus not high C (C:P = 10, C:P = 100 and C:P = control). A differentially abundant OTU is
181 enriched on one side of a sample class (e.g. enriched in the biofilm versus the plankton). Differential
182 abundance could mark an enrichment of the OTU towards either side of the sample class and the direction
183 of the enrichment is apparent in the sign (positive or negative) of the enrichment. Differential abundance
184 was moderated (see **Love et al.** (2014)) such that the fold change OTU of an OTU across two categories
185 of a sample class can be used to rank the enrichment of OTUs that span a wide range of base abundance.
186 The DESeq2 RNA-Seq statistical framework has been shown to improve power and specificity when
187 identifying differentially abundant OTUs across sample classes in microbiome experiments **McMurdie**
188 **and Holmes** (2014).

189 The specific DESeq2 (**Love et al.**, 2014) parameters we used were as follows: All dispersion estimates
190 from DESeq2 were calculated using a local fit for mean-dispersion. Native DESeq2 independent filtering
191 was disabled in favor of explicit independent filtering by sparsity. The sparsity thresholds that produced
192 the maximum number of OTUs with adjusted p-values for differential abundance below a false discovery
193 rate of 10% were selected. Cook's distance filtering was also disabled when calculating p-values with
194 DESeq2. We used the Benjamini-Hochberg method to adjust p-values for multiple testing (**Benjamini**
195 **and Hochberg**, 1995). Identical DESeq2 methods were used to assess enriched OTUs from relative
196 abundances grouped into high C (C:P = 500) or low C (C:P < 500 and control) categories.

197 IPython Notebooks with computational methods used to create all figures and tables as well as taking
198 raw sequences through quality control preprocessing are provided at the following URL:

199 `https://github.com/chuckpr/BvP_manuscript_figures.`

200 Version information for all R libraries is provided at the end of each IPython Notebook.

3   RESULTS

3.1   BULK COMMUNITY CHARACTERISTICS

201 We first assessed the effect of the resource treatments on the dissolved chemistry and bulk community
202 characteristics of the plankton and the biofilms. In the control treatment the mean DOC level was 0.12
203 $+/-$ 0.02 μmoles L^{-1}. The lower C treatment (C:P = 10) was statistically indistinguishable from the
204 control at 0.10 $+/-$ 0.02 μmoles C L^{-1}. The intermediate treatment (C:P = 100) increased in DOC to
205 0.70 μmoles C L^{-1} before decreasing to 0.12 μmoles C L^{-1} at the end of the experiment, with a mean of
206 0.28 $+/-$ 0.16 μmoles C L^{-1} over the course of the experiment. Only the highest C treatment (C:P = 500)
207 had DOC levels that were significantly higher (2.53 $+/-$ 1.6 μmoles C L^{-1}) than the control treatment,
208 over the course of the experiment. The high DOC levels in the highest C treatment were consistent with
209 C being supplied in excess of the metabolic requirements of the community (i.e. C saturation), but not
210 higher than what has been observed in coastal marine ecosystems.

211 This increase in DOC in the higher C treatments was associated with decreases in planktonic Chl *a* in
212 each treatment (Figure 2A), however there was no significant difference in biofilm Chl *a* among treatments

213 (Figure 2B). In combination with the decrease in planktonic Chl *a* on the 6th day of the experiment the
214 highest C treatment had approximately 4-fold higher planktonic heterotroph abundance than the control
215 and the 10 μM C treatment (Figure 2D). Similarly, biofilms had significantly higher total biomass in the
216 high C treatment compared to the other treatments (Figure 2D). Thus the shift in resource C:P altered the
217 pool size of both the photoautotroph and heterotroph communities. Clear differences in heterotroph and
218 photoautotroph pool size among treatments allowed us to address how shifts in pool sizes were related to
219 community membership and structure within and among plankton and biofilm communities.

3.2 PLANKTONIC AND BIOFILM COMMUNITY STRUCTURE

220 *3.2.1 Alpha diversity* We used rarefaction curves to evaluate alpha diversity in all treatments for both
221 the plankton and the biofilm communities. Rarefaction curves showed heterotroph and photoautotroph
222 OTU richness was consistently higher in the biofilm compared to the planktonic communities (Figure 3).
223 For both the photoautotroph and heterotroph sequence datasets the biofilm and plankton communities
224 had the fewest OTUs in the highest C treatment (C:P = 500) (Figure 3). When planktonic rRNA gene
225 sequences from all planktonic samples were pooled, individual biofilm heterotroph community richness
226 still generally exceeded the pooled planktonic heterotroph richness. For photoautotrophs, the individual
227 biofilm richness was similar to the pooled photoautotroph planktonic richness (Figure 4).

228 *3.2.2 Community membership biofilm versus plankton* Heterotroph community membership between
229 the plankton and biofilm communities was notably different for all treatments except for the highest C
230 treatment where the plankton and biofilm communities during the second sampling event (day 17) were
231 more similar to each other than any other community (Figure 5). Photoautotroph plankton and biofilm
232 communities were also different in OTU composition, however, the similarity among photoautotroph
233 plankton and biofilm communities in the highest C treatment was not observed as it was for the heterotroph
234 communities (Figure 5).

235 In heterotroph libraries, 19,978 sequences were distributed into 636 OTUs and 58% of quality controlled
236 sequences fell into the top 25 OTUs in order of decreasing sum of relative abundance across all samples.
237 In photoautotroph libraries 44,719 23S plastid rRNA gene sequences were distributed into 359 OTUs and
238 71% of sequences fell into the top 25 OTUs sorted by mean relative abundance across all samples.

239 To investigate differences in community structure and membership between the heterotroph biofilm
240 and overlying planktonic communities we identified the most enriched OTUs in biofilm compared to the
241 planktonic communities and vice versa. The most enriched OTUs were enriched in planktonic samples
242 (with respect to biofilm) as opposed to biofilm samples (Figure 6). This is consistent with the higher alpha
243 diversity in biofilm communities compared to planktonic communities and evidence that sequence counts
244 were spread across a greater diversity of taxa in the biofilm libraries compared to the planktonic libraries
245 (i.e. biofilm communities had higher evenness than planktonic communities). Of the top 5 enriched
246 heterotroph OTUs between the two lifestyles (biofilm or plankton), 1 is annotated as *Bacteroidetes*, 1
247 *Gammaproteobacteria*, 1 *Betaproteobacteria*, 1 *Alphaproteobacteria* and 1 *Actinobacteria* and all 5 were
248 enriched in the planktonic libraries relative to biofilm (Table 1). Of the 25 most enriched OTUs among
249 lifestyles only 2 heterotroph OTU centroid DNA sequences shared high sequence identity ($>= 97\%$) with
250 cultured isolates ("OTU.32" and "OTU.48", Table 1).

251 We similarly assessed membership among biofilm and plankton in the photoautotroph communities.
252 Photoautotroph 23S plastid rRNA gene sequence libraries also clustered strongly by lifestyle (Figure 5).
253 Biofilm libraries were predominantly enriched in *Stramenopile* OTUs whereas planktonic libraries were
254 enriched in *Haptophyceae*, *Cryptophyta* and *Viridiplantae* OTUs based on OTU positions in sample
255 ordination space (Figure 5, see Ordination Methods). When photoautotroph OTUs were ordered by
256 differential abundance between lifestyles (see Figure 6), 16 of the 25 most enriched OTUs were enriched
257 in the biofilm and 9 were enriched in the planktonic samples. Fourteen of these 16 biofilm enriched
258 OTUs were *Stramenopiles* of class *Bacillarophyta*, the remaining OTUs were classified as members of

259 the *Chlorophyta* and *Dinophyceae*. The 9 planktonic enriched OTUs (above) were distributed into the
260 *Viridiplantae* (5 OTUs), *Cryptophyta* (1 OTUs), *Haptophyceae* (1 OTU), *Stramenopiles* (1 OTU) and
261 cyanobacteria (1 OTU). The 10 most enriched photoautotroph OTUs between lifestyles were evenly split
262 between planktonic and biofilm enriched OTUs. As with the heterotrophs, photoautotroph OTU fold
263 change between lifestyles are qualitatively consistent with OTU positions in sample ordination space (see
264 Figures 6 and 5).

265   In both the heterotroph and photoautotroph communities low abundance members of the planktonic
266 communities became highly abundant members of the biofilm (Figure 7). The separation in community
267 membership among biofilm and planktonic communities is supported statistically by the Adonis test
268 (**Anderson**, 2001) for both the heterotroph and photoautotroph libraries (p-value 0.003 and 0.002,
269 respectively). The lifestyle category represents 18% and 45% for pairwise sample distance variance
270 in heterotroph and photoautotroph libraries, respectively. The Adonis test result is also consistent with
271 lifestyle (biofilm versus planktonic) clustering along the first principal component for the photoautotroph
272 libraries but not for the heterotroph libraries (Figure 5).

273 *3.2.3 Heterotroph community membership changes with C amendments* Although community
274 membership was predominately driven by lifestyle, we also investigated how resource amendments
275 affected community membership and structure. Because the abiotic (e.g. DOC) and all biomass indicators
276 (e.g. heterotroph abundance, Chl *a*) were only significantly different in the highest resource C:P treatment
277 we compared resource C:P = 500 (high C) to all other mesocosms (i.e. control, C:P = 10, and C:P
278 = 100 – low C). The high and low carbon amended mesocosms had statistically different heterotroph
279 communities (Adonis p-value 0.018) but not photoautotroph communities (Adonis p-value 0.59). Nine
280 heterotroph OTUs were enriched in the high C treatment relative to low C. Four of the 9 high C enriched
281 OTUs were annotated as *Alteromonadales*, 3 as *Campylobacterales* and 1 each into *Vibrionales* and
282 *Pseudomonadales*. The most enriched OTU in low C mesocosms was annotated as belonging to the
283 "HTCC2188" candidate order and shared 99% identity with a 16S sequence annotated as "marine gamma
284 proteobacterium HTCC2089" (accession AY386332). We only observed differences at the community
285 level between high and low C amendments in the heterotroph communities and therefore did not assess
286 differential abundance of OTUs between high and low C treatments in photoautotroph communities.

4 DISCUSSION

4.1 BIOMASS POOL SIZE

287 The goal of this study was to evaluate how changes in available C affected the biomass pool size and
288 composition of planktonic and biofilm communities. Our results suggest that C subsidies increased
289 heterotroph biomass in both plankton and biofilm communities. C amendments also resulted in decreased
290 photoautotroph biomass in the plankton community, but there was no significant change in biofilm
291 photoautotroph biomass between resource treatments. Although the DOC concentration in the highest
292 C treatment was significantly higher than the other treatments, the concentrations we measured were in
293 the range of those reported in natural marine ecosystems (**Mopper et al.**, 1980) and it is has been noted
294 that glucose concentrations in coastal marine ecosystems may fluctuate over several orders of magnitude
295 (**Alonso and Pernthaler**, 2006). The changes in the biomass pool size that did occur were consistent
296 with changing relationships (commensal to competitive) between the autotrophic and heterotrophic
297 components of the plankton communities but not necessarily of the biofilm communities. While we
298 recognize that other mechanisms may drive the shift in biomass pool size of these two components
299 of the microbial community (e.g. increased grazing pressure on the photoautotrophs with C additions,
300 or production of secondary metabolites by the heterotrophs that inhibit algal growth) previous studies
301 **Stets and Cotner** (2008a); **Cotner and Biddanda** (2002) and the data reported here suggest that altered
302 nutrient competition among heterotrophic and photoautotrophic members of the plankton is the most
303 parsimonious explanation for the observed shift in biomass pool size.

4.2 BIOFILM AND PLANKTON ALPHA AND BETA DIVERSITY

304 Beyond changes in the biomass pool size of each community, we explored how shifts in resource C
305 affected the membership and structure of each community, and the recruitment of plankton during biofilm
306 community assembly. Intuitively, shifts in planktonic community composition should alter the available
307 pool of microorganisms that can be recruited into a biofilm. For example, if planktonic diversity increases,
308 the number of potential taxa that can be recruited to the biofilm should also increase, potentially increasing
309 diversity within the biofilm. Similarly, a decrease in mineral nutrients available to photoautotrophs
310 should decrease photoautotroph pool size, potentially decreasing photoautotroph diversity and therefore
311 candidate photoautotroph taxa that are available for biofilm formation. In addition, C in excess of resource
312 requirements may increase the production of extracellular polysaccharides (EPS) by planktonic cells thus
313 increasing the probability that planktonic cells are incorporated into a biofilm by adhesion. Each of these
314 mechanisms suggest that an increase in labile C to the system should result in increased alpha diversity
315 in heterotrophic plankton and heterotrophic biofilm communities while decreasing alpha diversity within
316 both planktonic and biofilm photoautotroph communities.

317 We highlight three key results that we find important for understanding aquatic biofilm assembly.
318 First, biofilm community richness exceeded planktonic community richness (Figure 3) in all mesocosms.
319 Second, for the control, C:P = 10 and C:P = 100 resource treatments the membership and structure of
320 the heterotroph biofilm and plankton communities were more similar within a lifestyle (plankton versus
321 biofilm) than within a resource treatment. However, for the bacteria in the highest C treatment (C:P =
322 500) both membership and structure of biofilm and planktonic communities at day 17 were more similar
323 to each other than to communities from other treatments (Figure 5). Third, C subsides acted differently
324 on the photoautotroph and heterotroph communities. Specifically, while the highest level of C subsides
325 (C:P = 500) resulted in a merging of membership in the heterotroph plankton and heterotroph biofilm
326 communities the same merging of membership was not observed for the photoautotroph biofilm and
327 plankton communities which had distinct membership in all treatments.

328 We propose two potential mechanisms for the increased richness of the biofilm communities relative
329 to the planktonic community richness. First, it is possible that the planktonic community composition of
330 our flow through incubators was dynamic in time. In this case the biofilm community would represent
331 a temporally integrated sample of the planktonic organisms moving through the reactor resulting in
332 higher apparent alpha diversity (i.e. mass effects would be the dominant assembly mechanism). Second,
333 the biofilm environment may disproportionately enrich the low abundance members of the planktonic
334 community. In this case it is probable that the biofilm would incorporate the most abundant members
335 from the planktonic community (i.e. mass effects) but also select and enrich (i.e. species sorting) the
336 least abundant members of the planktonic community resulting in a higher level of detectable alpha
337 diversity. The second mechanism would result if the biofilm environment represented a more diverse
338 microhabitat including sharply delineated oxygen, nutrient and pH gradients that are not present in the
339 planktonic environment. In this case the more diverse microhabitat would be able to support a more diverse
340 community due to an abundance of additional environmental habitats (i.e. niches).

341 We evaluated the first mechanism by comparing membership among the plankton samples taken 9
342 days apart (day 8 and day 17). While heterotrophic plankton communities were not identical between
343 the time points (Figure 5), communities within a treatment were more similar to each other than other
344 heterotroph plankton communities regardless of time. In addition, the control and two lowest C treatments
345 (C:P = 10 and C:P = 100) separated completely from biofilm communities in principle coordinate space
346 (Bray-Curtis distance metric). This suggests that the biofilm community was not integrating variable
347 bacterioplankton community membership, but rather was at least in part selecting for a community that
348 was composed of distinct populations when compared to the most abundant members of the plankton
349 community. As noted above, in the highest C treatment (C:P = 500) the heterotroph biofilm and plankton
350 community membership had significant overlap at the final timepoint (Figure 5). However, heterotrophic
351 plankton community composition for the highest C treatment among timepoints (8 and 17 days) were
352 also qualitatively as similar to each other as any other community. Thus, variable planktonic community

353 composition among timepoints would not explain the higher diversity observed in the biofilm compared
354 to the planktonic community. Rather, two results point to enrichment of planktonic community members
355 within the biofilm as the mechanism for higher diversity in the biofilm compared to the plankton. First, the
356 increasing similarity between the plankton and the biofilm communities between each time point in the
357 highest resource C treatment suggests that *in situ* resource conditions were sufficient to alter the relative
358 abundance of the populations within each community. Second, an analysis of the OTU relative abundance
359 in biofilm and planktonic libraries where OTUs are sorted by planktonic sample rank (Figure 7) shows
360 that the least abundant members of the plankton community were routinely highly abundant within the
361 biofilm community. This was true for both photoautotroph and heterotroph communities, at all treatment
362 levels and both timepoints. While we did not (could not) specifically measure niche diversity within
363 the biofilm communities our results suggest that the biofilm habitat selected for unique members of the
364 photoautotroph and heterotrophic community that were in low abundance in the planktonic habitat but
365 readily became major constituents of the biofilm community.

366 Few studies have simultaneously evaluated the relationship among membership and/or diversity of the
367 plankton and the biofilm community from complex environmental microbial communities. One notable
368 study looked at planktonic community composition and biofilm formation on glass beads placed for three
369 weeks in three boreal freshwater streams (**Besemer et al.**, 2012). While that study system is markedly
370 different than our study, the analyses and questions addressed in each study were sufficiently similar to
371 merit comparison. **Besemer et al.** (2012) concluded that the biofilm community membership was most
372 likely driven by species sorting over mass effects. This is consistent with what we report here. However,
373 in the **Besemer et al.** (2012) study the authors reported that planktonic diversity was significantly higher
374 relative to biofilm diversity (the opposite of what we found in our study). Given the differences in
375 the source of the planktonic community among studies, this result is not surprising. While biofilm
376 communities were established on glass beads in **Besemer et al.** (2012) and glass slides (this study) over a
377 similar time period (21 days, **Besemer et al.** (2012) and 17 days this study) the origin of the planktonic
378 community in each study was different. The **Besemer et al.** (2012) study was conducted in three boreal
379 streams during snow melt when connectivity between the terrestrial and aquatic habitats was high and
380 potentially highly variable depending on how hydrologic pathways differed among precipitation events.
381 In this study the source community was a marine intake located approximately 200 m from the shore
382 during July when communities are more stable over the 17 day period of the incubation. A separate study
383 conducted in alpine and sub-alpine streams clearly showed that stream plankton communities reflected
384 localized precipitation events and could be traced largely to soil source communities from drainages
385 within the watershed (**Portillo et al.**, 2012). While planktonic communities in lake ecosystems can be
386 linked to soil communities in the watershed, as residence time of the system slows the relative influence
387 of species sorting increases. Thus, in headwater ecosystems stream plankton communities can often be
388 composed primarily of soil organisms (**Crump et al.**, 2012). In addition to the diverse source communities
389 the **Besemer et al.** (2012) study sampled the plankton community at multiple timepoints and integrated
390 the samples before sequencing further increasing community richness as compared to the current study
391 where the plankton community was sampled and analyzed only at two independent timepoints. Indeed,
392 when we pool OTU counts from all planktonic libraries and compare the rarefaction curve of the pooled
393 planktonic libraries (photoautotrophs and heterotrophs) against sample-wise biofilm libraries, we found
394 more total heterotroph and photoautotroph planktonic OTUs than in any given single biofilm sample.
395 It appears, however, that sample-wise heterotroph biofilm rarefaction curves may exceed the integrated
396 planktonic curve upon extrapolation and most exceed the integrated planktonic curve at sampling depths
397 where data is present for the biofilm and pooled planktonic library (Figure 4). This result is consistent
398 with our conclusion that temporal heterogeneity in the plankton was not sufficient to explain the higher
399 diversity in the biofilm sample but would explain the relative differences between planktonic and biofilm
400 diversity found in **Besemer et al.** (2012) compared to this study.

401 In addition, for this study, it is important to note that biofilm community richness peaked at the
402 intermediate treatment (C:P = 100) and appeared to decrease between each time point although with
403 only two time points it was unclear how pronounced the temporal effect was nor is it possible assess
404 the statistical significance of this effect (Figure 3). Since biomass of the plankton and the biofilm

405 increased with increasing C subsidies the intermediate peak in OTU richness is consistent with a classic
406 productivity-diversity relationship that has been shown for many ecosystems and communities both
407 microbial and otherwise. However, as with other experiments our experimental design did not allow
408 us to tell whether resources drove productivity that subsequently drove changes in diversity or whether
409 resources drove diversity which altered productivity. Rather, we note that as diversity decreased in the
410 highest C treatment, heterotrophic plankton and biofilm membership became increasingly similar. This
411 suggests that environments that contained high amounts of labile C selected for fewer dominant taxa,
412 overwhelming the lifestyle species sorting mechanisms that appeared to dominate biofilm community
413 assembly in all other treatments. Similarly, while we did not measure extracellular polymeric substances
414 (EPS), direct microscopy showed that planktonic cells in the highest C treatment (C:P = 500) were
415 surrounded by what appeared to be EPS. Because biofilm EPS also appeared to increase from the low
416 to high C treatments it is possible that abundant planktonic cells were more readily incorporated into
417 biofilms due both to increased "stickiness" of the planktonic cells as well as the biofilm itself. While we
418 did not observe flocculating DOC which has been shown to dominate high DOC environments in nature,
419 we did measure a substantial increase in DOC in the C:P = 500 treatment which was more than 2-fold
420 higher than any of the other treatments. Thus additional adhesion of the plankton and the biofilm may also
421 explain the merging of the planktonic and biofilm heterotroph membership in the highest C treatment.

4.3 LIFESTYLE (BIOFILM OR PLANKTONIC) ENRICHED OTUS

422 There are only a few studies that attempt to compare biofilm community composition and the overlying
423 planktonic community (**Besemer et al.**, 2007, 2012; **Jackson et al.**, 2001; **Lyautey et al.**, 2005). Those
424 studies illustrate community composition among the two habitats are unique, with few taxa found in
425 both. This is consistent with our findings in this experimental system with a natural marine planktonic
426 source community. In addition, our study also evaluated photoautotroph community composition which
427 showed a similar result suggesting that both the photoautotroph and heterotroph biofilm communities
428 are comprised of phylogenetically distinct organisms that exist in low abundance in the surrounding
429 habitat (i.e. the plankton) but are readily enriched in the biofilm lifestyle. Most of the biofilm enriched
430 photoautotroph OTUs were *Bacillariophyta* although there were also many *Bacillariophya* OTUs enriched
431 in the planktonic libraries. We also found more *Cryptophyta* and *Viridiplantae* were enriched in the
432 planktonic photoautotroph libraries. It appears that these broad taxonomic groups were selected against in
433 biofilms under our experimental conditions. Heterotroph OTUs enriched in planktonic samples displayed
434 more dramatic differential abundance patterns than heterotroph OTUs enriched in biofilm samples, but,
435 biofilm enriched heterotroph OTUs were spread across a greater phylogenetic breadth (Figure 6). This
436 is also consistent with the idea of greater niche diversity in the biofilm environment as opposed to the
437 plankton. Greater niche diversity should select for a more diverse set of taxa but individual taxa would not
438 be as numerically dominant in a more uniform environment like the planktonic environment. At the Order
439 level, enriched heterotroph OTUs tended to have members that were enriched in both the plankton and the
440 biofilm suggesting the phylogenetic coherence of lifestyle is not captured at the level of Order. It should be
441 noted, however, that taxonomic annotations in reference databases and therefore environmental sequence
442 collections show little equivalency in phylogenetic breadth between groups at the same taxonomic rank
443 (**Schloss and Westcott**, 2011). Unfortunately, at higher taxonomic resolution (e.g. Genus-level), groups
444 did not possess a sufficient numbers of OTUs to evaluate coherence between taxonomic annotation and
445 lifestyle. Carbon amendments did not affect photoautotroph library membership and structure to the same
446 degree as it affected heterotroph library composition. As expected, heterotroph OTUs enriched in the high
447 C amended mesocosm (C:P = 500) include OTUs in classic copiotroph families such as *Altermonodales*
448 and *Pseudomonadaceae*. Interestingly, the most depleted OTU in the high C treatments is annotated
449 as being in the HTCC2188 order of the *Gammaproteobacteria* and shares 99% sequence identity with
450 another "HTCC" strain (accession AY386332). HTCC stands for 'high throughput culture collection' and
451 is a prefix for strains cultured under low nutrient conditions (**Cho and Giovannoni**, 2004; **Connon and**
452 **Giovannoni**, 2002).

4.4 CONCLUSION

453 In summary this study shows that changes in low resolution community level dynamics are concurrent
454 with changes in the underlying constituent populations that compose them. We found that autotrophic
455 pools and heterotrophic pools responded differently to amendments of labile C as hypothesized. Notably
456 while C amendments altered both pool size and membership of the heterotroph communities we did not
457 see similar dynamics within the photoautotroph communities. Planktonic photoautotrophs decreased in
458 response to C amendments presumably in response to increased competition for mineral nutrients from
459 a larger heterotroph community, however there was not a similar decrease in biofilm photoautotroph
460 community. In addition membership of the photoautotroph communities between the plankton and biofilm
461 lifestyles did not become more similar in the photoautotrophs as it did for the bacterial heterotrophs
462 in the highest C treatment. Consistent with a growing body of work our results suggest that complex
463 environmental biofilms are a unique microbial community that form from taxa that are found in low
464 abundance in the neighboring communities. This membership was affected by C amendments for
465 heterotrophic but not photoautotrophic microbes and then only in the most extreme resource environment.
466 This suggests that lifestyle is a major division among environmental microorganisms and although biofilm
467 forming microbes must travel in planktonic form at some point, reproductive success and metabolic
468 contributions to biogeochemical processes comes from those taxa primarily if not exclusively while they
469 are part of a biofilm. Our results point to lifestyle (planktonic or biofilm) as an important trait that explains
470 a portion of the exceptional diversity found in snapshots used to characterize environmental microbial
471 communities in space and time.

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REFERENCES

477 Alonso, C. and Pernthaler, J. (2006), Roseobacter and SAR11 dominate microbial glucose uptake in
478 coastal north sea waters, *Environ. Microbiol.*, 8, 11, 2022–2030, doi:10.1111/j.1462-2920.2006.01082.
479 x
480 Anderson, M. J. (2001), A new method for non-parametric multivariate analysis of variance, *Austral
481 Ecology*, 26, 1, 32–46, doi:10.1111/j.1442-9993.2001.01070.pp.x
482 Battin, T. J., Kaplan, L. A., Denis, N. J., and Hansen, C. M. (2003), Contributions of microbial biofilms
483 to ecosystem processes in stream mesocosms., *Nature*, 426, 439–42
484 Battin, T. J., Sloan, W. T., Kjelleberg, S., Daims, H., Head, I. M., Curtis, T. P., et al. (2007), Microbial
485 landscapes: new paths to biofilm research., *Nat. Rev. Microbiol.*, 5, 76–81
486 Benjamini, Y. and Hochberg, Y. (1995), r, *J. R. Stat. Soc.*, 57, 1, 289–300, doi:10.2307/2346101
487 Besemer, K., Hödl, I., Singer, G., and Battin, T. J. (2009), Architectural differentiation reflects bacterial
488 community structure in stream biofilms., *ISME J*, 3, 1318–24
489 Besemer, K., Peter, H., Logue, J. B., Langenheder, S., Lindstrm, E. S., Tranvik, L. J., et al. (2012),
490 Unraveling assembly of stream biofilm communities., *ISME J*, 6, 1459–68
491 Besemer, K., Singer, G., Limberger, R., Chlup, A. K., Hochedlinger, G., Hödl, I., et al. (2007),
492 Biophysical controls on community succession in stream biofilms, *Appl. Environ. Microbiol.*, 73, 15,
493 4966–4974, doi:10.1128/aem.00588-07

494 Bratbak, G. and Thingstad, T. F. (1985), Phytoplankton-bacteria interactions: an apparent paradox?
495   Analysis of a model system with both competition and commensalism, *Marine Ecology - Progress*
496   *Series*, 25, 23–30
497 Caporaso, J. G., Kuczynski, J., Stombaugh, J., K., B., Bushman, F. D., Costello, E. K., et al. (2010),
498   QIIME allows analysis of high-throughput community sequencing data., *Nat. Methods*, 7, 335–6
499 Cho, J. C. and Giovannoni, S. J. (2004), Cultivation and growth characteristics of a diverse group of
500   oligotrophic marine gammaproteobacteria, *Appl. Environ. Microbiol.*, 70, 1, 432–440, doi:10.1128/
501   aem.70.1.432-440.2004
502 Connon, S. A. and Giovannoni, S. J. (2002), High-throughput methods for culturing microorganisms in
503   very-low-nutrient media yield diverse new marine isolates, *Appl. Environ. Microbiol.*, 68, 8, 3878–
504   3885, doi:10.1128/aem.68.8.3878-3885.2002
505 Cotner, J. B. and Biddanda, B. A. (2002), Small players large role: microbial influence on biogeochemical
506   processes in pelagic aquatic ecosystems, *Ecosystems*, 5, 2, 105–121, doi:10.1007/s10021-001-0059-3
507 Cotner, J. B. and Wetzel, R. G. (1992), Uptake of dissolved inorganic and organic bphosphorus
508   compounds by phytoplankton and bacterioplankton, *Limnol. Oceangr.*, 37, 2, 232–243, doi:10.4319/lo.
509   1992.37.2.0232
510 Crump, B. C., Amaral-Zettler, L. A., and Kling, G. W. (2012), Microbial diversity in arctic freshwaters is
511   structured by inoculation of microbes from soils., *ISME J*, 6, 1629–39
512 DeSantis, T. Z. J., Hugenholtz, P., Keller, K., L., B. E., Larsen, N., Piceno, Y. M., et al. (2006), NAST: a
513   multiple sequence alignment server for comparative analysis of 16S rRNA genes., *Nucleic Acids Res.*,
514   34, W394–9
515 Edgar, R. C. (2010), Search and clustering orders of magnitude faster than BLAST., *Bioinformatics*, 26,
516   2460–1
517 Edgar, R. C. (2013), UPARSE: highly accurate OTU sequences from microbial amplicon reads., *Nat.*
518   *Methods*, 10, 996–8
519 Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. (2011), UCHIME improves
520   sensitivity and speed of chimera detection., *Bioinformatics*, 27, 2194–200
521 Hodl, I., Hodl, J., Worman, A., Singer, G., K., B., and Battin, T. J. (2011), Voronoi tessellation captures
522   very early clustering of single primary cells as induced by interactions in nascent biofilms, *PLoS ONE*,
523   6, 10, e26368, doi:10.1371/journal.pone.0026368
524 Jackson, C. R., Churchill, P. F., and Roden, E. E. (2001), Successional changes in bacterial assemblage
525   structure during epilithic biofilm development, *Ecology*, 82, 2, 555–566, doi:10.1890/0012-9658(2001)
526   082[0555:scibas]2.0.co;2
527 Jansson, M., Hickler, T., Jonsson, A., and Karlsson, J. (2008), Links between terrestrial primary
528   production and bacterial production and respiration in lakes in a climate gradient in subarctic sweden,
529   *Ecosystems*, 11, 3, 367–376, doi:10.1007/s10021-008-9127-2
530 Karlsson, J., Berggren, M., Ask, J., Byström, P., A., J., Laudon, H., et al. (2012), Terrestrial organic matter
531   support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers,
532   *Limnol. Oceangr.*, 57, 4, 1042–1048, doi:10.4319/lo.2012.57.4.1042
533 Knight, R., Maxwell, P., Birmingham, A., Carnes, J., Caporaso, J. G., Easton, B. C., et al. (2007),
534   PyCogent: a toolkit for making sense from sequence., *Genome Biol.*, 8, R171
535 Lane, D. J. (1991), 16S/23S rRNA sequencing, *Nucleic Acid Techniques in Bacterial Systematics*, 125–
536   175
537 Love, M. I., Huber, W., and Anders, S. (2014), Moderated estimation of fold change and dispersion for
538   RNA-Seq data with DESeq2, doi:10.1101/002832
539 Lyautey, E., Jackson, C. R., Cayrou, J., Rols, J., and F., G. (2005), Bacterial community succession in
540   natural river biofilm assemblages, *Microb. Ecol.*, 50, 4, 589–601, doi:10.1007/s00248-005-5032-9
541 McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., et al. (2012), An
542   improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria
543   and archaea., *ISME J*, 6, 610–8
544 McDougald, D., Rice, S. A., Barraud, N., Steinberg, P. D., and Kjelleberg, S. (2011), Should we stay or
545   should we go: mechanisms and ecological consequences for biofilm dispersal., *Nat. Rev. Microbiol.*,
546   10, 39–50

547 McMurdie, P. J. and Holmes, S. (2014), Waste not, want not: why rarefying microbiome data is
548   inadmissible., *PLoS Comput. Biol.*, 10, e1003531
549 Mopper, K., Dawson, R., Liebezeit, G., and Ittekkot, V. (1980), The monosaccharide spectra of natural
550   waters, *Marine Chemistry*, 10, 1, 55–66, doi:10.1016/0304-4203(80)90058-4
551 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2013), vegan:
552   Community Ecology Package, R package version 2.0-10
553 Porter, K. G. and Feig, Y. S. (1980), The use of DAPI for identifying and counting aquatic microflora,
554   *Limnol. Oceangr.*, 25, 5, 943–948, doi:10.4319/lo.1980.25.5.0943
555 Portillo, M. C., Anderson, S. P., and Fierer, N. (2012), Temporal variability in the diversity and
556   composition of stream bacterioplankton communities., *Environ. Microbiol.*, 14, 2417–28
557 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., T., S., Yarza, P., et al. (2013), The SILVA ribosomal RNA
558   gene database project: improved data processing and web-based tools., *Nucleic Acids Res.*, 41, D590–6
559 Schloss, P. D. and Westcott, S. L. (2011), Assessing and improving methods used in operational taxonomic
560   unit-based approaches for 16s rrna gene sequence analysis, *Appl. Environ. Microbiol.*, 77, 10, 3219–
561   3226, doi:10.1128/aem.02810-10
562 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009),
563   Introducing mothur: open-source, platform-independent, community-supported software for describing
564   and comparing microbial communities., *Appl. Environ. Microbiol.*, 75, 7537–41
565 Sherwood, A. R. and Presting, G. G. (2007), Universal primers amplify a 23s rdna plastid marker in
566   eukaryotic algae and cyanobacteria, *Journal of Phycology*, 43, 3, 605–608, doi:10.1111/j.1529-8817.
567   2007.00341.x
568 Stets, E. G. and Cotner, J. B. (2008a), Littoral zones as sources of biodegradable dissolved organic carbon
569   in lakes, *Can. J. Fish. Aquat. Sci.*, 65, 11, 2454–2460, doi:10.1139/f08-142
570 Stets, E. G. and Cotner, J. B. (2008b), The influence of dissolved organic carbon on bacterial phosphorus
571   uptake and bacteria-phytoplankton dynamics in two Minnesota lakes, *Limnol. Oceangr.*, 53, 1, 137–
572   147, doi:10.4319/lo.2008.53.1.0137
573 Wetzel, R. G. and Likens, G. E. (2000), Limnological Analyses (Springer New York), doi:10.1007/
574   978-1-4757-3250-4
575 Wickham, H. (2009), ggplot2 (Springer New York), doi:10.1007/978-0-387-98141-3

FIGURES

Table 1. Results for BLAST search against Living Tree Project (top 25 lifestyle enriched bacterial OTUs (Operational Taxonomic Unit)

| OTU	Phylum	$log_2(plankton:biofilm)$	Species Name	%Identity	Accession
OTU.103	Bacteroidetes	7.78	*Zunongwangia profunda*	89.66	DQ855467
OTU.105	Proteobacteria	8.09	*Microbulbifer yueqingensis*	90.14	GQ262813
OTU.11	Proteobacteria	9.59	*Methylobacillus glycogenes*	93.96	FR733701
OTU.123	Proteobacteria	8.96	*Flexibacter roseolus* *Flexibacter elegans*	83.46 83.46	AB078062 AB078048
OTU.165	Proteobacteria	-7.05	*Kangiella spongicola* *Kangiella marina*	92.05 92.05	GU339304 JN559388
OTU.166	Proteobacteria	-7.52	*Halomonas halocynthiae*	92.62	AJ417388
OTU.19	Proteobacteria	9.31	*Neptuniibacter caesariensis*	90.07	AY136116
OTU.195	Proteobacteria	7.17	*Methylobacillus glycogenes*	94.63	FR733701
OTU.20	Proteobacteria	9.07	*Ruegeria halocynthiae* *Phaeobacter daeponensis*	96.15 95.49	HQ852038 DQ981486
OTU.207	Proteobacteria	9.30	*Methylobacillus glycogenes*	91.28	FR733701
OTU.223	Proteobacteria	7.94	*Methyloferula stellata* *Methylocapsa aurea* *Beijerinckia indica subsp. lacticogenes* *Beijerinckia indica subsp. indica* *Beijerinckia derxii subsp. venezuelae*	87.02 87.02 87.02 87.02 87.02	FR686343 FN433469 AJ563931 CP001016 AJ563934
OTU.26	Actinobacteria	8.58	*Corallomonas stylophorae*	88.17	GU569894
OTU.31	Bacteroidetes	9.63	*Sediminitomix flava* *Kordia algicida*	91.33 91.33	AB255370 AY195836
OTU.32	Bacteroidetes	8.90	*Bizionia echini*	97.32	FJ716799
OTU.36	Actinobacteria	9.55	*Pseudoclavibacter soli*	95.95	AB329630
OTU.369	Actinobacteria	7.93	*Agrococcus terreus*	96.0	FJ423764
OTU.40	Bacteroidetes	7.68	*Aureitalea marina*	91.33	AB602429
OTU.44	Proteobacteria	8.92	*Glaciecola mesophila* *Aestuariibacter salexigens* *Aestuariibacter halophilus*	92.62 92.67 92.67	AJ488501 AY207502 AY207503
OTU.48	Actinobacteria	7.28	*Microterricola viridarii* *Leifsonia pindariensis*	97.33 97.33	AB282862 AM900767
OTU.62	Proteobacteria	8.75	*Haliea rubra* *Congregibacter litoralis* *Chromatocurvus halotolerans*	91.55 91.55 91.55	EU161717 AAOA01000004 AM691086
OTU.69	Proteobacteria	9.34	*Sneathiella glossodoripedis*	87.94	AB289439
OTU.71	Bacteroidetes	7.40	*Aequorivita sublithincola*	95.77	AF170749
OTU.83	Actinobacteria	7.27	*Microbacterium invictum*	92.47	AM949677
OTU.84	Proteobacteria	-7.44	*Alcanivorax dieselolei* *Alcanivorax balearicus*	93.29 93.29	AY683537 AY686709
OTU.89	Actinobacteria	7.17	*Corallomonas stylophorae*	87.91	GU569894
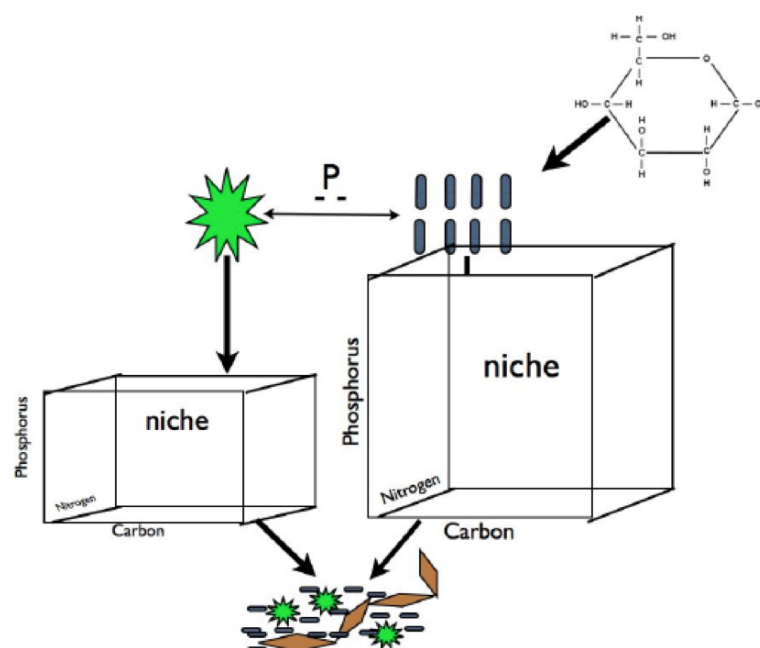
Figure 1. Carbon subsidies in the form of glucose alleviate the dependence of heterotrophic bacteria on photoautotroph derived C exudates. This should result in an increase in resource space and biomass for heterotrophs and a decrease in resource space and biomass for photoautotrophs due to increased competition for mineral nutrients (for simplicity we illustrate competition for P but this is equally applicable other elements that may limit primary production). We hypothesized that this predicted change in biomass pool size of these two groups will result in changes in the plankton community composition of both groups that will propagate to to the composition of biofilm communities for both groups. We refer to shifts in the demand and availability of resources among components of the microbial community as 'partitioning. Blue rods represent heterotrophs, green stars represent photoautotrophs, brown diamonds represent EPS or other cohesive components of a biofilm.
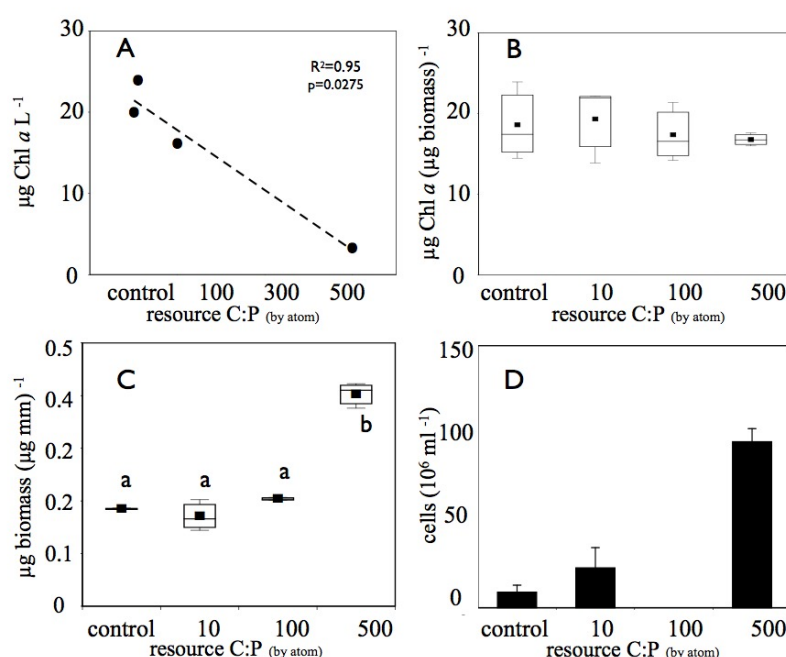
Figure 2. Increased C amendments diminished A) planktonic photoautotroph biomass (estimated as Chl *a*) but B) not biofilm photoautotroph biomass. In contrast, both C) biofilm total biomass and D) number of planktonic bacterial cells increased with increasing C subsidies. Only the highest C treatment produced biomass that was significantly greater than ($p < 0.05$) the other treatments (significant differences among treatments are denoted by different letters). The bacterial abundance sample for the C:P = 100 treatment was lost before analysis and is therefore not reported in panel D.
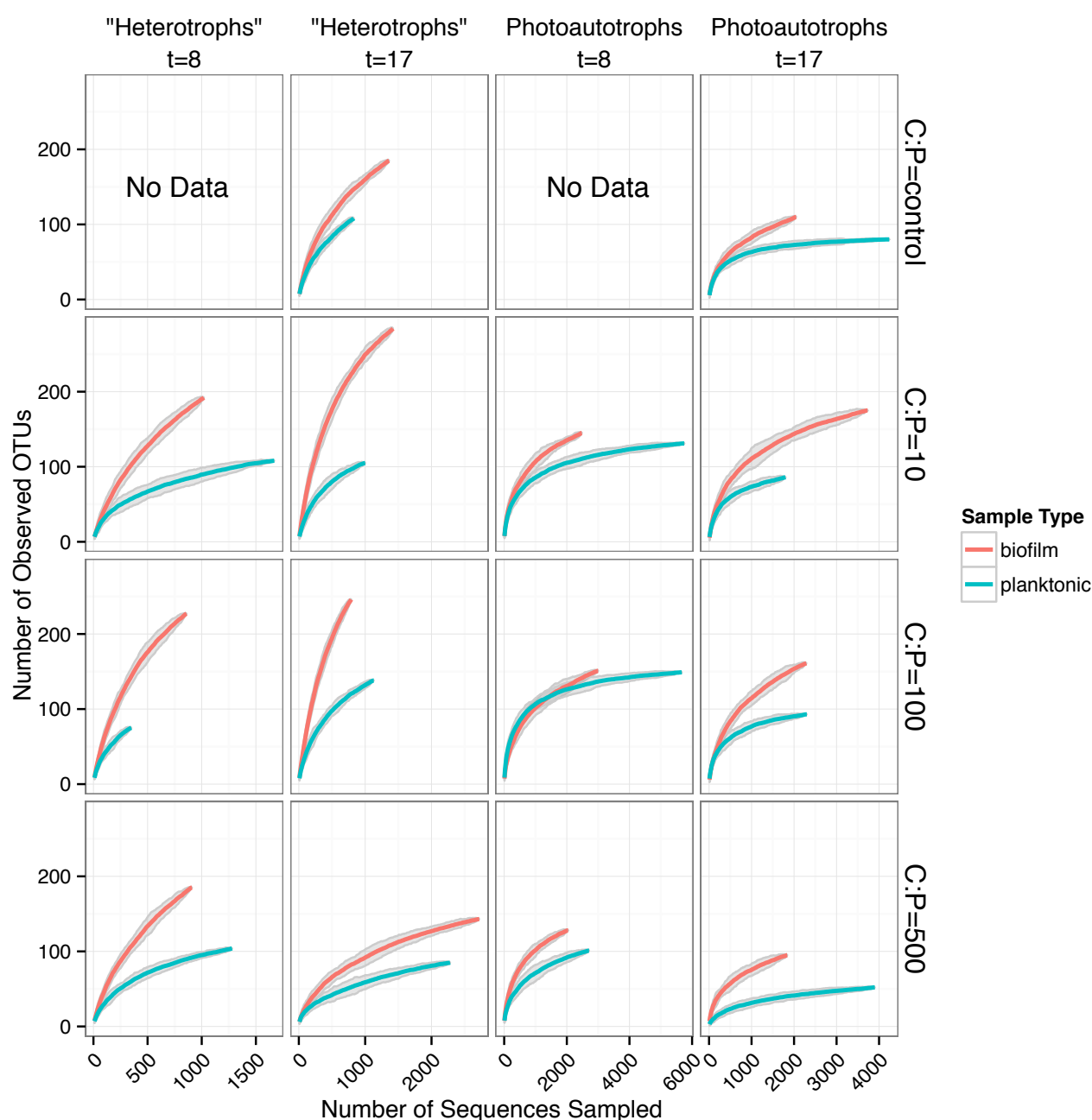
Figure 3. Rarefaction curves for all biofilm versus plankton libraries. Each panel represents a single C:P treatment and time point. Richness is greater for all biofilm communities when compared to corresponding planktonic communities. Gray ribbons are 99% confidence intervals around each rarefaction point based on variance from 25 re-samplings.
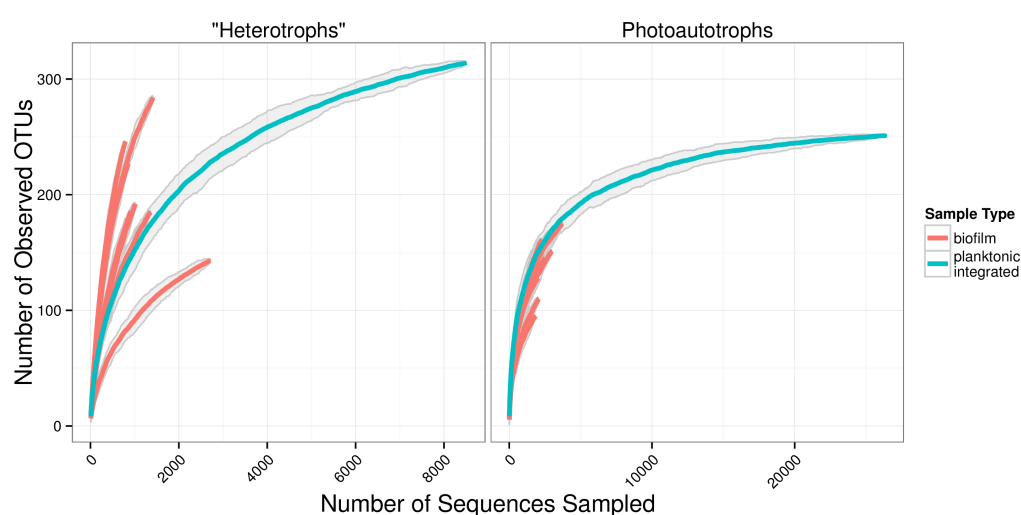
Figure 4. Rarefaction plots for all samples. Planktonic libraries have been integrated such that the count for each OTU is the sum of counts across all samples. Gray ribbons are 99% confidence intervals around each rarefaction point based on variance from 25 re-samplings.
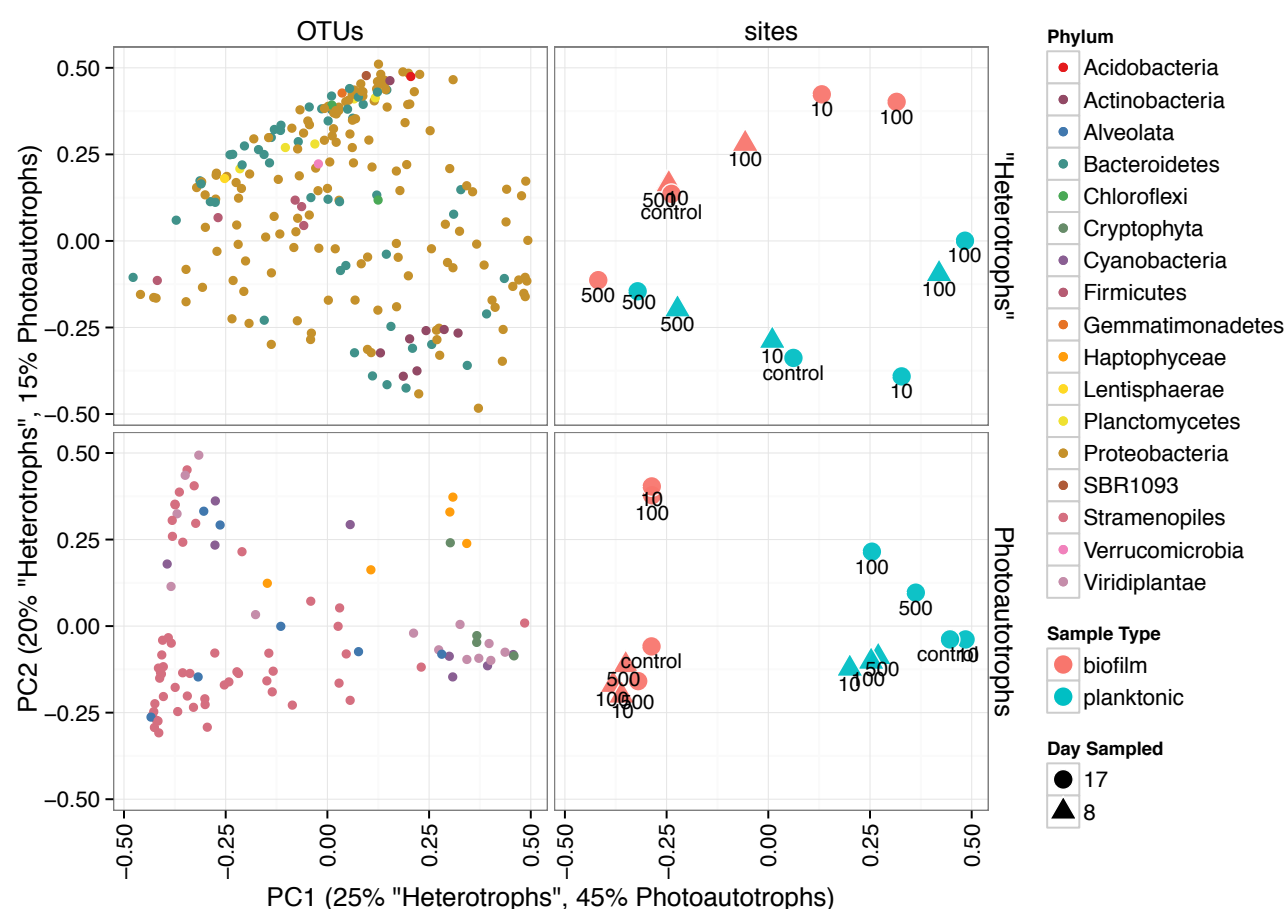
Figure 5. Principal coordinates ordination of bray-curtis distances for 23S rRNA plastid libraries and 16S rRNA gene libraries. OTU points are weighted principal coordinate averages (weights are relative abundance values in each sample) and the variance along each principal axis is expanded to match the site variance. Point annotations denote the amended C:P ratio for the mesocosm from which each sample was derived.
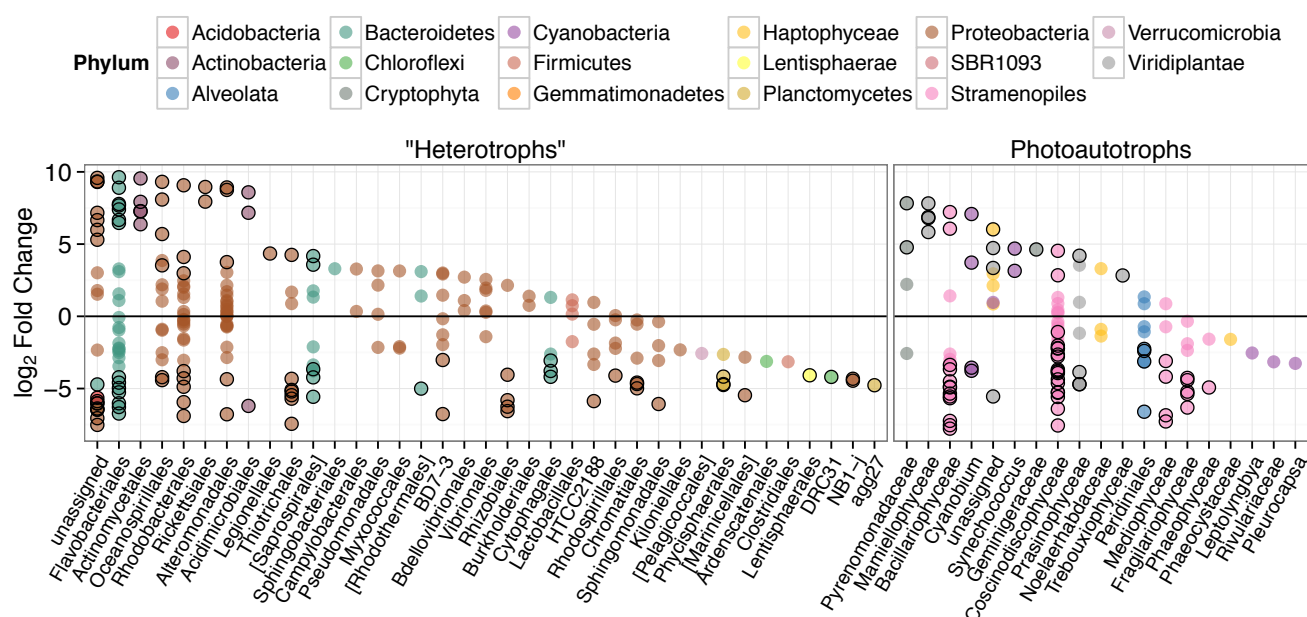
Figure 6. log_2 of lifestyle OTU abundance fold change between biofilm and plankton communities. Each point represents one OTU and points are grouped along the x-axis by Order. Outlined points have adjusted p-values below a false discovery rate of 0.10. Positive fold change values represents enrichment in planktonic samples.
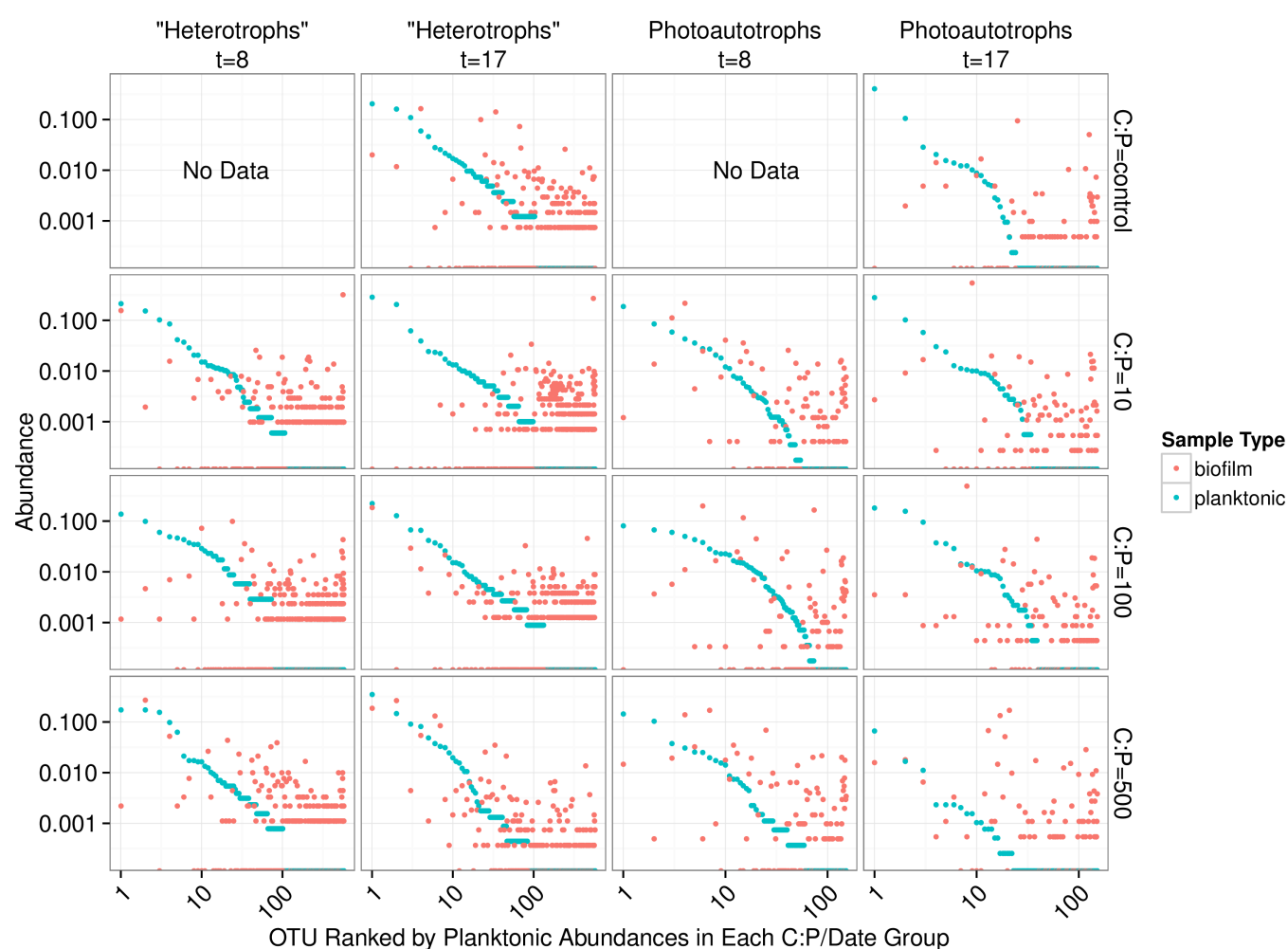
Figure 7. Rank abundance plots. Each panel represents a single time point and C:P. The "rank" of each OTU is based on planktonic sample relative abundance. Each position along the x-axis represents a single OTU. Both the x and y axes are scaled logarithmically.