

The effect of carbon subsidies on planktonic niche partitioning and recruitment of bacteria to marine biofilms

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Introduction

Biofilms are diverse and complex microbial habitats that, in many environments, are the rule rather than the exception for microbial lifestyle. Large and small scale architectural features of biofilms play an important role in their ecology and roles in biogeochemical cycles (Battin et al. 2007). While fluid mechanics have been shown to be important drivers of biofilm structure, it is less clear how resources and other abiotic factors affect biofilm structure. In addition how composition of the microbial community interacts with structure is also poorly understood.

At the grossest level planktonic microbial communities can be broken into two key groups; phototrophic Eukaryotes (hereafter algae) and heterotrophic Bacteria and Archaea. This dichotomy, while somewhat artificial (i.e. cyanobacteria are often the primary producer of organic matter in ocean ecosystems) has been shown to be a powerful paradigm for understanding community shifts and ecosystem function across lakes of varying trophic state (Cotner and Biddanda 2002). Heterotrophic bacteria derive some to all of their organic carbon requirements from the algae while simultaneously competing with them for limiting nutrients such as phosphorus. The presence of external carbon inputs, such as terrigenous carbon leaching from the watershed, can release the bacterioplankton from their reliance on algae for carbon shifting the relationship to strictly competitive. Increased carbon supply increases the resource space available to the bacteria while increased competition for P decreases the P available for algal biosynthesis (Figure 1). Along a gradient of allochthonous carbon subsidies the shifts in this relationship alters the pool size of each group and their associated resource space upon which a myriad of other factors that structure communities can play out.

While these gross level dynamics have been discussed conceptually (Cotner and Biddanda 2002) and demonstrated empirically many times, the affect that these shifts in size of the standing stock has on community composition and diversity has not been clearly evaluated in the plankton. In addition, how these putative shifts in planktonic community structure propagate to affect biofilm community structure is not well understood. Shifts in planktonic community composition should alter the available pool that can be recruited into a biofilm. Therefore as bacterial abundance increases the number of possible species that can be recruited to the biofilm should potentially increase alpha diversity within the biofilm. Alternatively carbon in excess of resource requirements may increase the production of extra cellular polysaccharides (EPS) and increase the probability that planktonic cells are incorporated into biofilm due to increased adhesion (i.e. stickiness). Thus through either mechanism I would expect increased carbon subsidies to result in increased alpha diversity in both the plankton and the biofilm.

I used an experimental mesocosm system to address these questions related to the structure and community composition of microbial biofilms. First, I asked if carbon subsidies shifted the balance between autotrophs and heterotrophs within the biofilm or its seed pool (plankton) and how this shift in pool size affected membership of the associated microbial communities. Second, I asked if carbon subsidies would affect who was recruited from the seed pool to the biofilm. For the second question I proposed two possible mechanisms for shifts in recruitment to the plankton. First, shifts in pool size of the plankton community would result in shifts in community composition and thus change the supply pool of available species that could be incorporated into the biofilm. Second, increased production of EPS due to excess resource carbon by planktonic bacteria would lead to an increased probability

of biofilm formation (e.g. stickiness), thus increasing the proportion of the plankton in the associated biofilm for any given treatment.

Material and Methods

I placed large test tube racks in 3 large (370L) mesocosms and three smaller racks in one smaller (185 L) mesocosm. Each mesocosm had an inflow and outflow with a flow rate adjusted so that the residence time of each tank was approximately 12 hours. Irregular variation in flow rate meant that flow rate varied around that target, however the residence time of each system never exceeded 12 h. Coverslips were attached to glass slides using nail polish and the slides attached to the test tube racks using binder clips. During the course of the experiment the metal portion of the binder clips oxidized and resulted in a gradient of Fe - oxide deposition across the surface of the coverslip. Coverslips were harvested and analyzed for community composition, Chlorophyll a, biomass, extracellular polysaccharide (EPS) and structure using epifluorescence and confocal laser microscopy.

Planktonic chlorophyll was collected on GF/F filters (Whatman) and extracted in 90-95% acetone for ~ 32 hours at -20°C before analysis on a Turner 10-AU fluorometer. For biofilm Chla analysis biomass was scrapped from the complete area of each coverslip and placed in a glass screw top beaker before acetone extraction and fluorometric analysis.

Bacterial abundance of the planktonic samples were analyzed using Dapi staining and direct visualization on a Zeiss Axio epifluorescence microscope after the methods of Porter and Feig (1980). Briefly, 1-3 mL of water was filtered through a 0.1 µm polycarbonate membrane filter and post stained with a combination of Dapi and Citifluor mountant media (Ted Pella Redding, Ca) to a final concentration of 1µg ml⁻¹. Confocal laser microscope images were produced by direct staining of the EPS with either Dapi or Concavallin A Texas Red or a combination of both.

Samples for dissolved organic carbon (DOC) analysis were collected in acid washed 50 mL falcon tubes after filtration through a 0.2 polycarbonate membrane filter attached to a 60 mL syringe. Syringes and filters were first flushed with the control sample to prevent leaching of carbon from the syringe or the filter into the sample. Samples were then frozen and analyzed for organic carbon content with a Shimadzu 500 TOC analyzer.

The composition of each plankton and biofilm community was analyzed by creating clone libraries for each sample. For plankton, cells were collected by filtering between 20 – 30 mL of water onto a 0.1 µm pore-size polycarbonate filter. For biofilm communities, biomass from the entire coverslip area of three separate slides were collected and combined in an eppendorf tube by gentle scrapping the slip surface with an ethanol rinsed and flamed razor blade. DNA was extracted using a Mobio Power Soil DNA isolation kit. 16s gene was amplified using generic eubacterial PCR primers 8F-1492R and cleaned by gel purification. PCR product was cloned into competent cells using a Topo TA cloning kit with PCR 4 sequencing vector (Invitrogen) and plated onto LB agar plates containing ampicillin to a final concentration of 50 µg per mL. After ~14 h at 35°C colonies were picked and grown in 96 well plates containing 1.2 mL of a super broth before extraction and sequencing using a Applied Biosystems Capillary sequencer model 3730.

Sequences were analyzed in Arb, Mothur and Unifrac. Rarefaction plots and Venn diagrams were composed in Mothur by grouping OTUs at a 97% sequence similarity using furthest neighbor cluster analysis. Community similarity indices and cluster analyses were conducted in Unifrac. All other statistical analyses were conducted in JMP.

Results

Additions of glucose to mesocosms resulted in increased dissolved organic carbon in the water column in the high carbon (C:P=500) treatment (Figure 2). Planktonic Chl a decreased with increasing carbon subsidies in each mesocosm (Figure 3a) but there was no significant difference in biofilm Chl a (Figure 3b). In addition after five days of treatment application the high carbon treatment had 4-fold higher planktonic bacterial abundance, as determined by DAPI counts, than the control and the 10 μ M carbon treatment (Figure 4). The treatments also resulted in significantly higher biomass of the biofilms in the high carbon treatment compared to the other treatments (Figure 5).

We then estimated both alpha (variation within a community) and beta (differences between communities) diversity in the high carbon (C:P = 500) and low carbon (C:P = 10) treatment mesocosms for both the plankton and the biofilms. Rarefaction plots composed by grouping OTUs that were 97% similar showed no differences between the treatments for either the plankton or biofilm communities (Figure 6). However, each plankton community was less rich in membership than each biofilm community. Further analyses revealed each community shared relatively few members within each other (Figure 7). A cluster analysis produced using Unifrac revealed that the planktonic groups grouped together while the low carbon biofilm grouped separately, but closer to the planktonic communities than to the high carbon biofilm (Figure 8).

Discussion

The goal of this study was to evaluate the effect of carbon subsidies on the alpha and beta diversity on planktonic and biofilm forming marine bacteria. I found no clear affect of resource treatment on alpha diversity in either the plankton or the biofilm between the high and low carbon treatments. The rarefaction curves for each group (plankton vs. biofilm) were nearly identical with the biofilm consistently having greater alpha diversity than the plankton. This is seemingly counterintuitive since the ultimate source of diversity in the biofilm must be the plankton. However, the dynamic spatial heterogeneity within the biofilm increases niche space and may lead to high levels of physiological heterogeneity. In addition, this result suggests that the planktonic community composition may vary temporally and have higher turnover than the biofilm community thus increasing biofilm diversity over time.

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Figure 1 – A conceptual diagram showing how allochthonous carbon subsidies could decouple the dependency of heterotrophic bacteria on (phototrophic eukaryotes) algae and lead to increased competition for phosphorus. Decreased phosphorus availability due to increased competition would decrease the resource space (niche) for the algae and lead to a decrease in pool size. This may or may not result in shifts in algal community diversity.

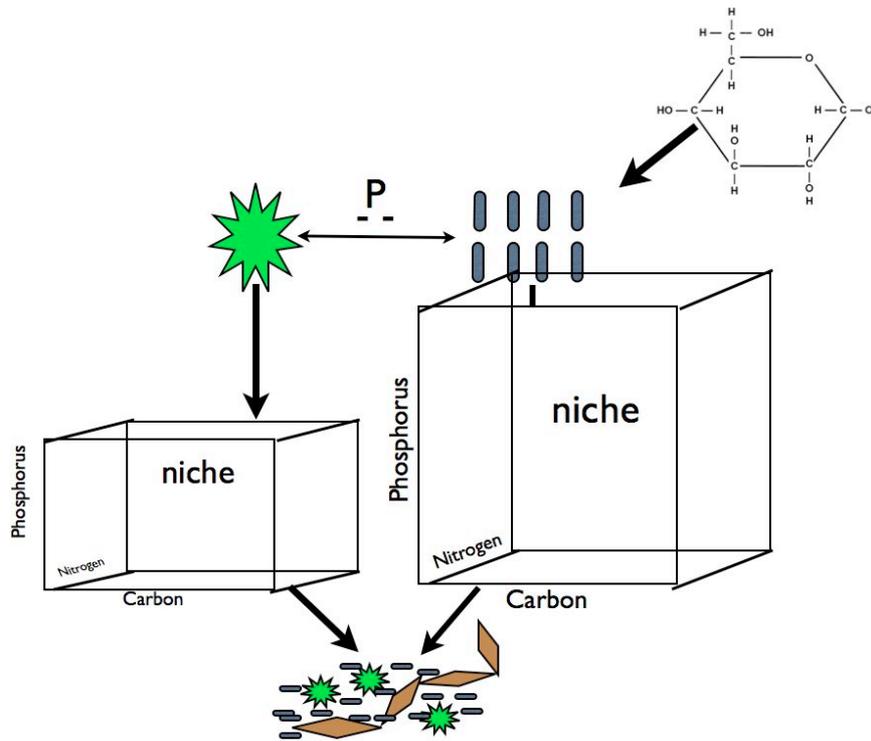


Figure 2 – Changes in dissolved organic carbon DOC with time during the course of the experiment. Values are plotted independently for treatments: control, C:P=10 and C:P = 100 (panel A) and C:P = 500 (panel B).

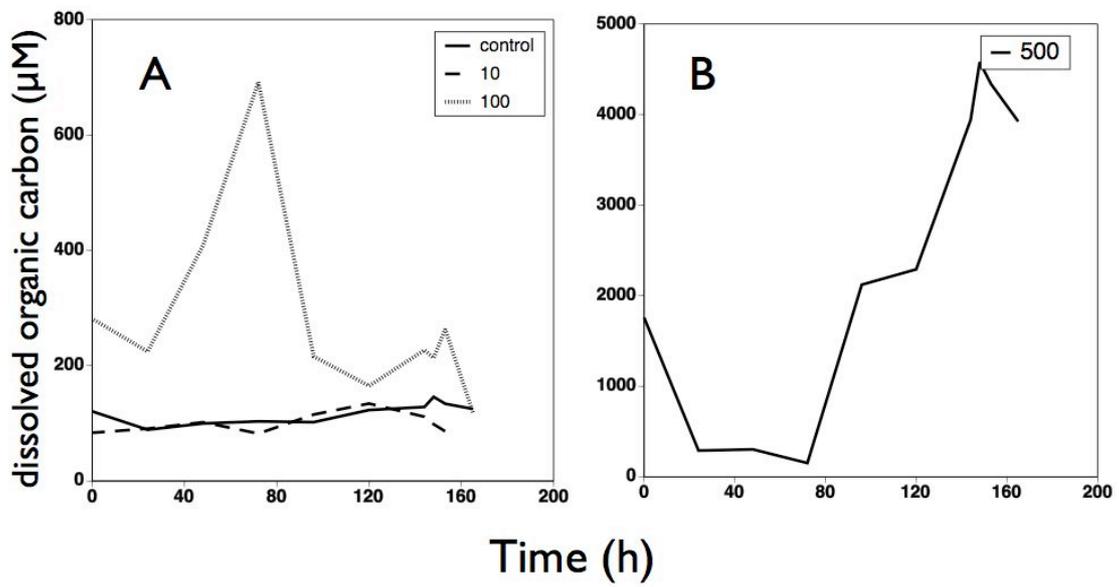


Figure 3 – Shows A) the amount of Chlorophyll A (Chla) for each treatment in the plankton (A) and the biofilm (B). Plankton Chla was measured on a single filter while biofilm Chla was measured individually for each of three slides.

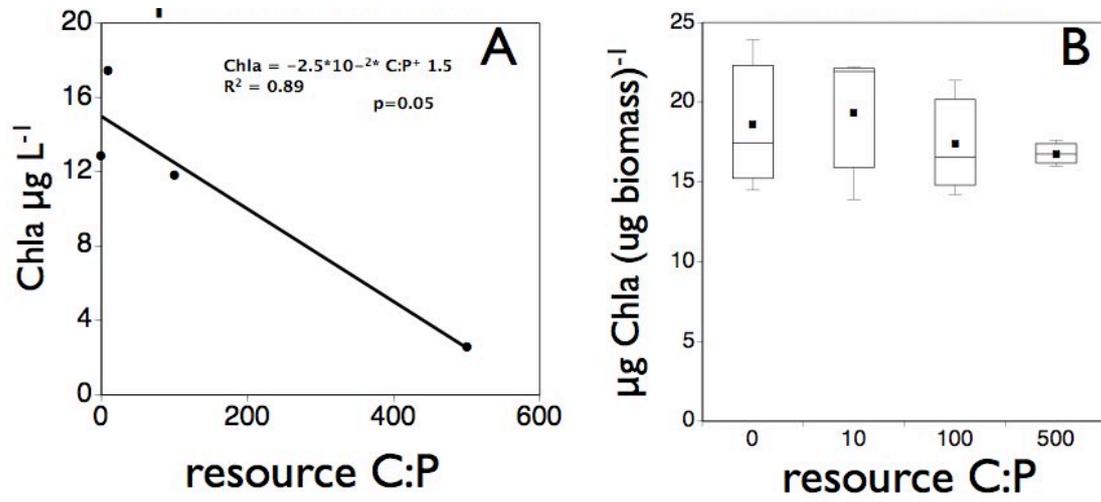


Figure 4 – Bacterial abundance between the high, low and control resource treatments measured on the 5th day of the experiment.

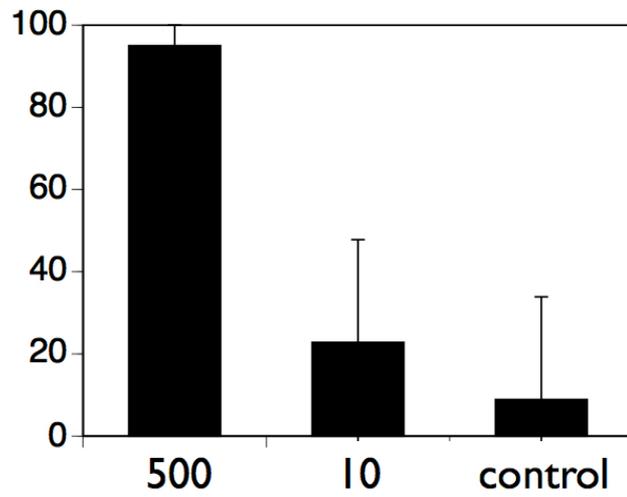


Figure 5 – Plot of biofilm biomass recovered from each cover slip. For each treatment biomass from three slides were weighed individually.

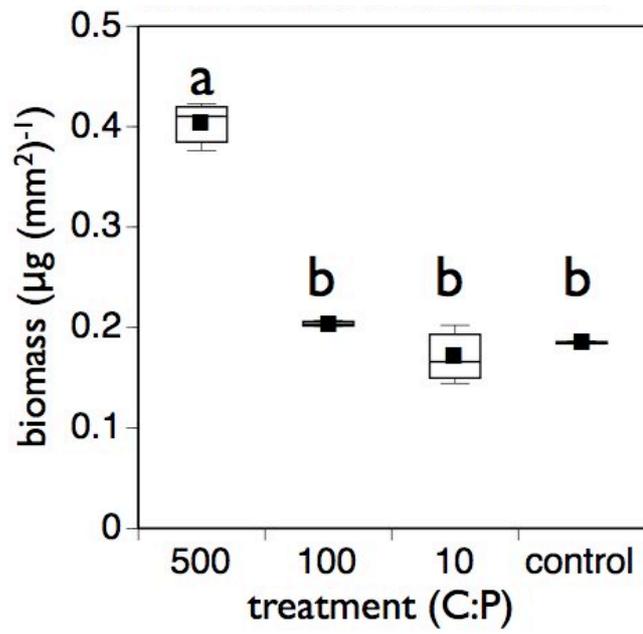


Figure 6 – A rarefaction plot of each of the four clone libraries. Labels are B = Biofilm , P = plankton, Treatment 1: CP=500, Treatment 3: CP=10. OTUs were grouped at 97 % similarity.

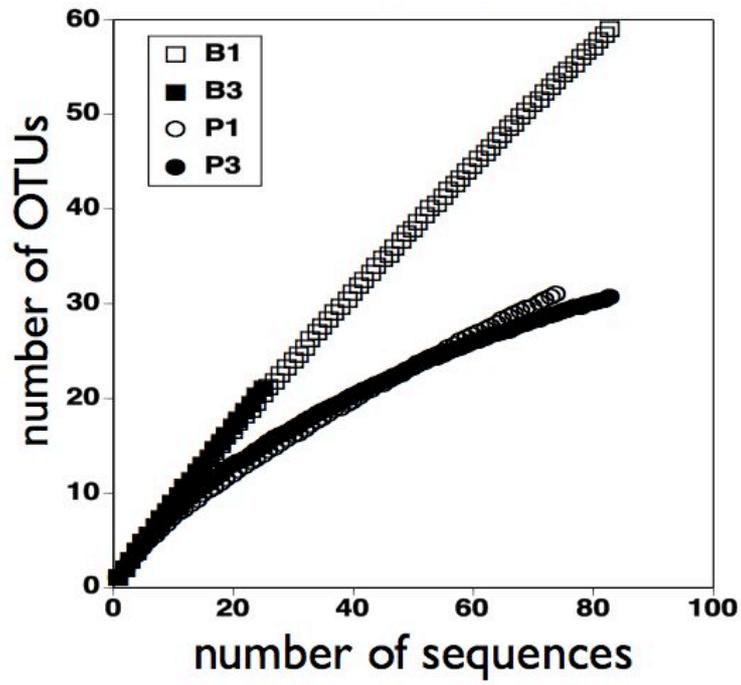


Figure 7 – A Venn diagram showing shared OTUs between clone libraries at 97% (A) and 90% (B) sequence similarity OUT cut-offs. Generated in Mothur.

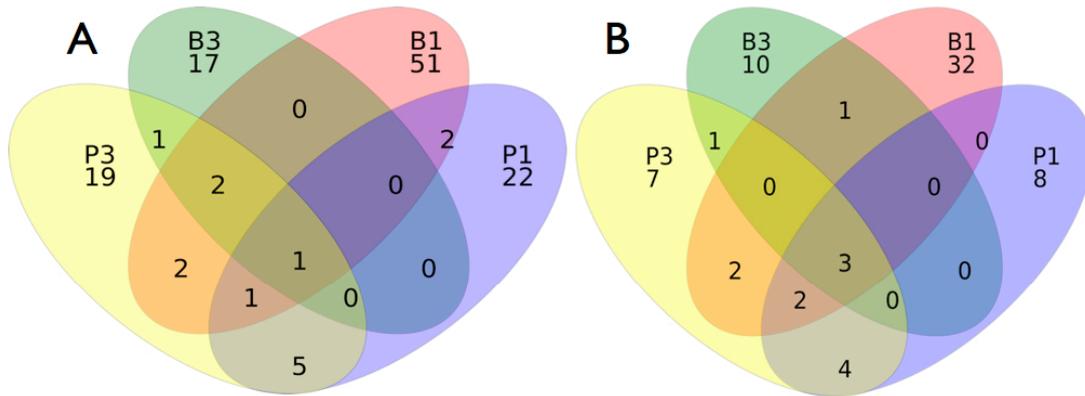


Figure 8 – Cluster analysis of each clone library generated in unifrac. Communities are denoted as defined in Figure X.

