Kelp Derived DOC and Nutrients Influence Bacterial Growth and Biodiversity in Santa Barbara Channel

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Abstract

Previous research in the Santa Barbara Channel has shown that kelp releases dissolved organic carbon (DOC) and that there are microbial communities that are supported by organic matter. However, there has yet to be a link between these two processes in which kelp-derived DOC influences bacterial communities. In this study, we analyzed how carbon and nutrient inputs into seawater from the Santa Barbara Channel affect the microbial response including: growth rate, bacterial growth efficiency (BGE), and biodiversity. The treatment with glucose, nitrate, and phosphate additions was found to have the highest microbial growth response, and the treatment with kelp exudate, nitrate, and phosphate was the second highest. However, bacteria within treatments with kelp derived DOC resulted in greater BGE values. Additionally, different carbon and nutrient inputs caused microbial diversity to differ among treatments.

Introduction

Although they are microscopic, microorganisms are extremely abundant in all ecosystems on Earth and they are important organisms that are involved in global processes. They are drivers of a variety of biogeochemical processes such as nutrient cycling. Heterotrophic microbes are responsible for remineralization which is the process in which organic compounds are converted back into inorganic compounds (Burkhardt et al. 2014, Pomeroy et al. 2007). During this process, organic carbon is taken up by these heterotrophs and is used as a source of carbon for their metabolic processes (Lefévre et al. 1996). Through this process, microbes link dissolved organic carbon (DOC) to higher trophic levels in a process that is known as the microbial loop (Azam et al. 1983, Pomeroy et al. 2007).

Microbes are prevalent everywhere and extremely important in driving biological processes in all parts of the ocean.

Multiple sources of DOC exist in the environment, and different microbes can use distinct sources of DOC, which allows microbial communities to shift depending on the availability and type of DOC (Lozada et al. 2021, Manikandan et al. 2021, Nelson et al. 2013). For example, in tropical ecosystems, different types of algal DOC exudate can cause shifts in the microbial community composition (Manikandan et al. 2021, Nelson et al. 2013). Changing microbial community structures can completely change the metabolic function of these communities and have effects that extend to ecosystem scales.

Macroalgae is important in producing DOC (Khailov and Burlakova 1969, Wada et al. 2008), and supported by additional findings, kelp is known to enhance the concentrations of DOC in surrounding seawater (Pfister et al. 2019). The Santa Barbara Channel is a very productive ecosystem due to upwelling that occurs on the California coast (Brzenzinski and Washburn 2011), bringing up nutrients and also supporting large kelp forests. Kelp organic carbon exudate supports microbial communities, and it has been found that microbial communities of kelp beds have more diversity than those outside of kelp beds (Pfister et al. 2019).

There have been previous studies of the Santa Barbara Channel that have assessed DOC released by kelp (Reed et al. 2015) and looked at microbial communities supported by organic matter (Halewood et al. 2012). However, there has yet to be a study that analyzes how kelp organic matter exudates directly influences microbial community diversity and growth.

This experiment assessed how different carbon inputs influence the microbial composition from the coastal waters of the Santa Barbara Channel. Inoculum from coastal Santa Barbara was incubated in different treatments using kelp DOC exudate, glucose, and nutrients (phosphorus and nitrogen). We aimed to study how these different carbon sources and nutrients not only alter microbial community composition, but also the impacts on growth rate, abundance, and bacterial growth efficiency (BGE). We hypothesized that each treatment will have differing community compositions, and growth will be higher in the treatments which were enriched with nutrients and organic carbon.

Materials and Methods

Collection of Kelp Exudate

In this experiment, we aimed to add different sources of dissolved organic matter (DOM) to foster microbial communities. A source of DOM that we prepared was DOC exudate from the kelp species *Macrocystis pyrifera*. Pieces of the kelp were broken off where the nematocyst meets the stipe, and they were rinsed off with sea water. Each kelp piece was placed into a separate bag which was filled with water that contained nitrogen and phosphorus. They were sealed and incubated for five days, and the DOC was collected by filtering the water through a 0.2 μm filter.

Incubation

Surface seawater from Goleta pier was collected into an acid-washed polycarbonate bottle and partitioned into inoculum and media. The inoculum was seawater filtered through a 142 mm 1.2-μm filter, and the media was filtered through a 142 mm 0.2-μm filter.

With this media and inoculum, we prepared one control and three treatment carboys. The three treatments consisted of the addition of kelp exudate (K), kelp exudate and nutrients (KNP), and glucose and nutrients (GNP). For the control and GNP treatment, five liters of media were added and two liters of inoculum was added to acid-washed polycarbonate bottles, and for the kelp exudate treatments, two liters of inoculum, 10 μM kelp DOC exudate, and 4.5 liters of media was added to acid-washed polycarbonate bottles. In the treatments with nutrient amendments we added 1 μM NH_4^+ and 0.1 μM PO_4^{2-}. The GNP treatment also received 10 μM glucose.

Each treatment bottle was partitioned into two replicate five liter polycarbonate carboys, and each carboy was fitted with a positive pressure displacement cap to minimize handling and contamination. Each incubation carboy was sampled for cell abundance, DNA, and total organic carbon (TOC) three times at two day intervals.

Total Organic Carbon

At time point zero, twelve vials were filled three-quarters full with the water from each treatment bottle. At each sampling time point - including time point zero - three of the twelve vials were fixed with 60 μL 4N HCl. At the end of the sampling period, Shimadzu High Temperature Combustion systems were used to determine the amount of TOC at each time point, following the procedures from Hansell and Carlson 1998, Hansell 2005, Carlson et al. 2010, and

chapter 16 of the BATS method manual. DOC was calculated by subtracting the bacterial carbon by TOC:

$$DOC = TOC - Bacterial\,Carbon.$$

Cell Abundance and Bacterial Carbon

For microscopy sample collection, 9 mL of water was collected from each carboy into a 15 mL falcon tube with 1 mL of formalin at each time point. Cellulose backing filters and polycarbonate filters that were stained with Irgalan Black for at least one hour were placed on a filter base which was attached to a vacuum. A filter tower was attached to this apparatus, and the water/formalin samples were filtered through followed by 500 µL of DAPI. The filters were used to prepare microscope slides and were visualized using an epifluorescence microscope at 100x magnification following the procedure of Porter and Feig 1980, which allowed us to determine cell abundance and biovolume.

Bacterial carbon was calculated by multiplying the cell abundance by the biovolume derived carbon conversion factor for bacteria in the Santa Barbara Channel which is:

$$fg\,C\,cell^{-1} = 91.7(um^3)^{0.686} * \ 2.72.$$

The BGE per treatment and change in DOC were calculated using biovolume derived data. To calculate BGE, the change in cell carbon was divided by the change in DOC:

$$BGE = \frac{\Delta Cell\,Carbon}{\Delta DOC}$$

Exponential growth was identified as the growth occurring between day zero and day two, and the specific growth rate (µ) was calculated using the following equation:

$$\mu = \frac{ln(abundance)_{final} - ln(abundance)_{initial}}{T_{final} - T_{initial}}.$$

Doubling time was determined by

$$t_{doubling} = \frac{ln(2)}{\mu}.$$

DNA collection and sequencing

To collect DNA from each treatment, 250 mL of water from each polycarbonate carboy was filtered through a 0.2-µm Sterivex filter. We added 1 mL of sucrose lysis buffer to each filter and used parafilm to seal the openings. The DNA was extracted from the filters using methods from Fuhrman et al. 1988 and Massana et al. 1997, then quantified using a Qubit. They were then amplified using PCR and were prepared and sent to the UC Davis DNA Technologies Core for sequencing. The primers used were V4 designed for PE250. The methods we used for DNA preparation and sequencing are outlined in Kozich et al. 2013 and Parada et al. 2016. Unfortunately, during sequencing, there were issues with demultiplexing due to unknown errors. This

caused the GNP treatment to not have any data so the data from the 2018 class was used for this treatment.

Data Analysis

When the sequencing results were received, the data was run through DADA2 (Callahan et al. 2016) in RStudio to remove errors. The sequenced reads were trimmed at the length where quality decreases for both forward and reverse reads, which was at 240 base pairs for the forward reads and 150 for the reverse reads. The reads that contained unknown nucleotides were removed. The sequences were dereplicated and then variants with high error rates were removed. Bimeras were removed, and the sequences were compared to the Silva database to assign taxonomy.

The phyloseq package (McMurdie and Holmes 2013) in R was used to analyze the taxonomic data. We used Chao1 and Shannon to look at alpha diversity and non-metric dimensional scaling (NMDS) for beta diversity. We then visualized the biodiversity from each treatment with a stacked barplot.

Results

Microbial Abundance and Growth

The microscopy results showed that the patterns of cell abundance for each experiment are similar. They all increased by day two, and it appeared that growth slowed from day two to day four for K, KNP, and the control. However, GNP decreased in abundance from day two to day four (figure 1).

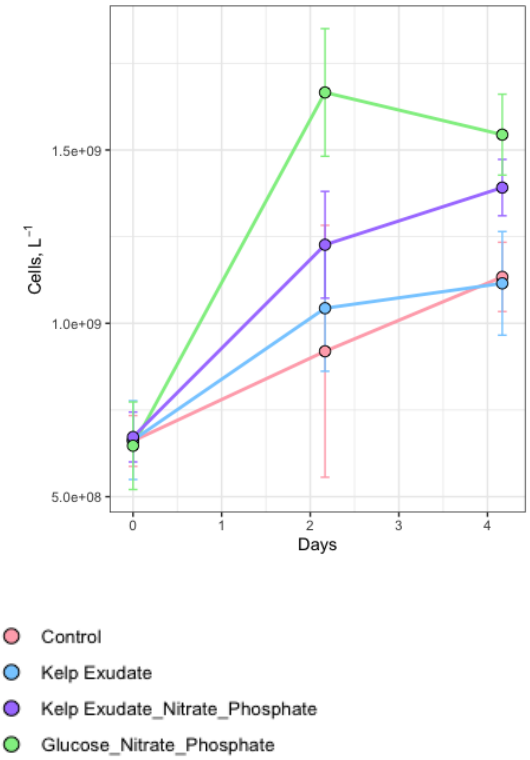

Figure 1. The amount of cells per liter over the three sampling time points.

GNP had the greatest μ and change in cell abundance with the lowest doubling time, followed by KNP, then K, and finally the control (table 1). Although GNP had the highest μ and cell abundance at day two, KNP had a higher biovolume per cell (figure 2).

Treatment	μ	Δ cell L^{-1}	Doubling time (d^{-1})
Control	0.15	2.59×10^8	4.54
K	0.21	3.81×10^8	3.31
KNP	0.28	5.54×10^8	2.50
GNP	0.44	1.02×10^9	1.59

Table 1. The values of specific growth rate, change in cell abundance per liter, and doubling time per treatment.

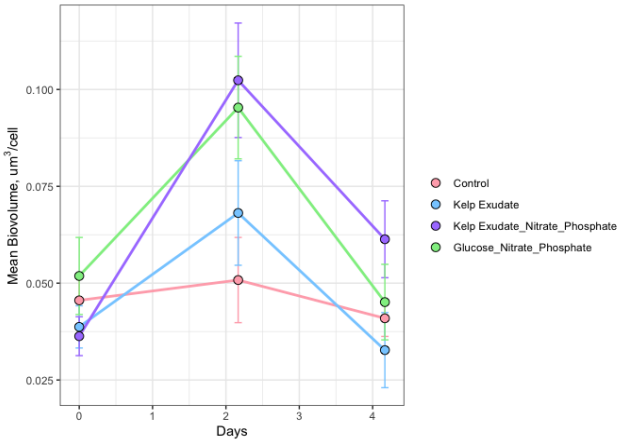

Figure 2. The mean biovolume per cell per treatment at each time point.

Microbial Carbon Consumption and BGE

The GNP treatment had an 18 μM change in DOC throughout the experiment while the control had a 3 μM change in DOC. The K and KNP treatments had a very similar change in DOC, with both treatments having an approximately 8 μM change in DOC (figure 4a). Although the bacteria in the GNP treatment had the highest uptake of DOC, the BGE of the bacteria from the KNP treatment was higher than the BGE of bacteria from the GNP treatment (figure 4b).

It is important to note that we aimed to add the same amount of carbon to each treatment bottle (10 μM), but we failed to attain this goal - as shown in figure 3. The total amount of carbon added to the GNP treatment was higher than the other treatments, and the initial organic carbon amounts for GNP, KNP, K, and the control were approximately 103 μM, 94 μM, 95

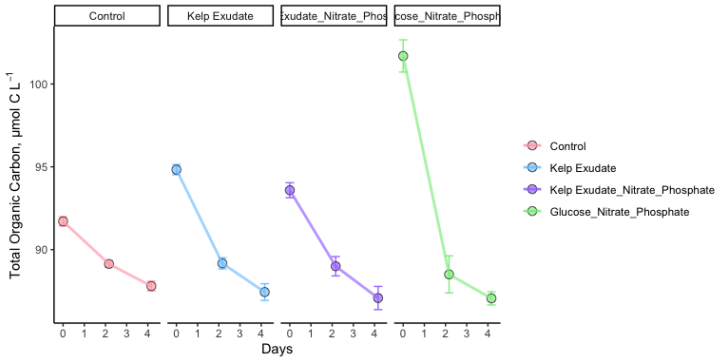

Figure 3. The GNP treatment had a larger amount of initial carbon input than the other treatments

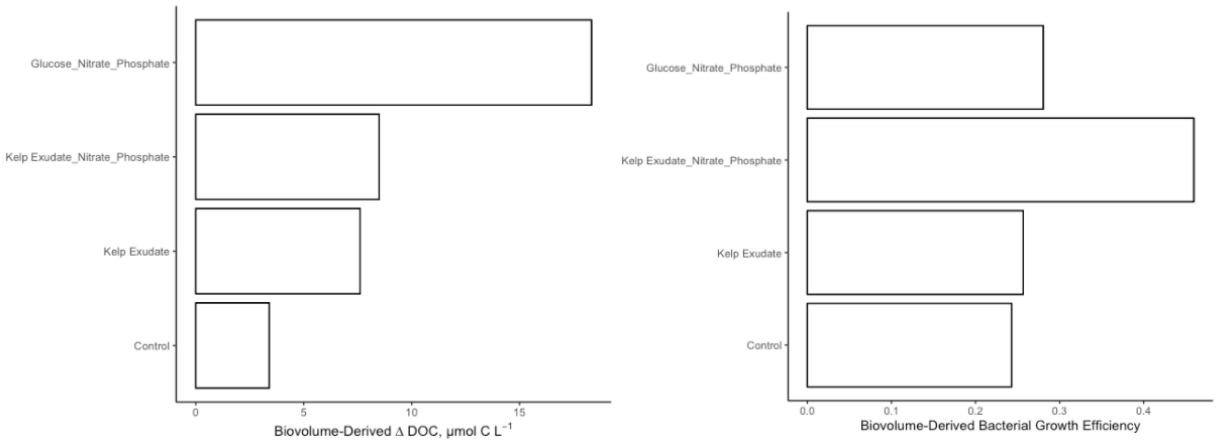

Figure 4. a) The change in DOC from day zero to day two per treatment. **b.)** Bacterial Growth Efficiency per treatment.

µM, and 92 µ M respectively. By day four of the experiment, all treatments had approximately 94 µM of organic carbon.

Microbial Biodiversity

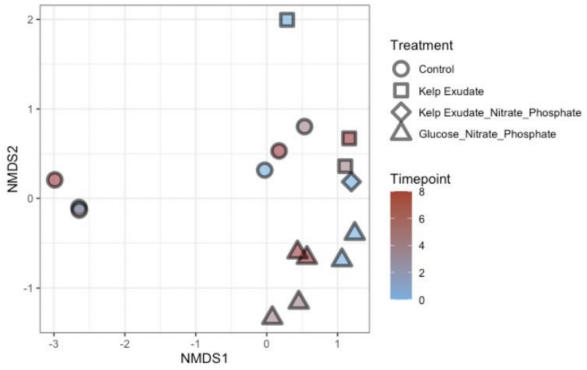

Figure 5. NMDS plot displays beta diversity. The closer together two points are, the community compositions are more similar.

Treatment K had a change in diversity between the different time points while GNP did not have as much of a difference (figure 5). KNP did not have data other than time point 0, so we cannot determine the difference in diversity between the different time points. The control treatment bottles had a large difference in biodiversity between the same time points, so it is difficult to conclusively analyze patterns.

Discussion

This study analyzes how the input of kelp derived organic carbon along with nutrients influences microbial response. Previous studies have looked at DOC release from kelp (Reed et al. 2015) and microbial communities (Halewood et al. 2012) in the Santa Barbara Channel, but there have yet to

be studies about the direct influence of kelp derived DOC on microbial community response. Our results show that different organic carbon inputs as well as nutrients influence microbial growth response and community composition.

Compared to the control, all treatments in which organic carbon and nutrients were added had an increase in μ, which is consistent with findings from Rivkin and Anderson 1997, which also found that additions of organic carbon and nutrients (although to different extents per environment) increase μ. The uptake of DOC in each treatment was also higher than the control treatment, and GNP bacteria had the highest uptake of DOC. However, KNP had the highest BGE.

Figure 2 displays the biovolume per cell at each time point per treatment, and the biovolume for KNP bacteria was higher than GNP bacteria. This shows us that even though microbes from GNP utilized more DOC than KNP microbes, the bacteria from KNP were using more of the organic carbon additions to build biomass, which is consistent with a higher BGE. Although GNP had a higher initial input of organic carbon, it is likely that it does not cause a large difference in BGE patterns.

BGE decreases when the carbon to nutrient ratio is higher than the optimal conditions (Polimene et al. 2006), which is 45C: 9N: 1P for bacteria (Goldman et al. 1987). GNP had an initial ratio of 105C: 6N: 1.7P while KNP had an initial ratio of 95C: 6N: 1.7P, and K's initial concentration was 95C: 0.1N: 0.15P. All of these ratios are much higher than Goldman et al. 1987's definition of bacterial ratio, but the K treatment had a much higher C:N and C:P ratio than GNP, so theoretically, K bacteria should have a much lower BGE. However, bacteria from K had a similar BGE as those from GNP. We can conclude that kelp derived carbon exudate is more efficiently taken up and converted into biomass than glucose. Even if all treatments received the same concentrations of carbon amendments at the beginning of the experiment, we would still expect glucose to be a less efficient source of carbon than kelp DOC.

In addition to differing microbial responses, the addition of different carbon and nutrient inputs impacts the diversity of microbes as well. Figure 5 shows that per time point, each treatment has differing microbial communities. However, because we used 2018 taxonomic data from a different experiment for our GNP treatment, we cannot make any definitive conclusions for comparisons of biodiversity data.

Additionally, the sequencing error resulted in only one replicate of the K treatment data and one time point for KNP data. Because of this, we cannot make a deeper analysis of how kelp DOC exudate influences the microbial community diversity over time; we only know that the different inputs appear to foster different microbial communities.

Conclusion

In conclusion, the addition of Santa Barbara Channel kelp derived organic carbon from as well as glucose and nutrients causes an increased μ, and kelp DOC is converted to biomass more efficiently than glucose. Because this experiment only used 16s rRNA to examine microbes, we only understand kelp exudate impacts on taxonomic diversity. Using proteomics or transcriptomics can answer questions about how kelp derived DOC from the Santa Barbara Channel changes metabolic function within a microbial community and examine the underlying mechanisms of bacteria efficiently utilizing kelp DOC exudate as a carbon source. No statistical tests were run to examine the data, so we cannot conclude that any of the results are significantly different from the control.

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