Elucidating microbial species-specific effects on organic matter transformation in marine sediments

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November 22, 2022

Abstract

Microbial transformation and decomposition of organic matter in sediments constitutes one of the largest fluxes of carbon in marine environments. Mineralization of sedimentary organic matter by microorganisms results in selective degradation such that bioavailable or accessible compounds are rapidly metabolized while more recalcitrant, complex compounds are preserved and buried in sediment (Mahmoudi et al., 2017). Recent studies have found that the ability to use different carbon sources appears to vary among microorganisms, suggesting that the availability of certain pools of carbon can be specific to the taxa that utilize the pool. This implies that organic matter mineralization in marine environments may depend on the metabolic potential of the microbial populations that are present and active. The goal of our study was to investigate the extent to which organic matter availability and transformation may be species-specific using sediment from Guaymas Basin (Gulf of California). We carried out time-series incubations using bacterial isolates and sterilized sediment in the IsoCaRB system (Beaupre et al., 2016) which allowed us to measure the production rates and natural isotopic signatures (δ13C and Δ14C) of microbially-respired CO2. Separate incubations using two different marine bacterial isolates (Vibrio sp. and Pseudoalteromonas sp.) and sterilized Guaymas Basin sediment under oxic conditions showed that the rate and total quantity of organic matter metabolized by these two species differs. Approximately twice as much CO2 was collected during the Vibrio sp. incubation compared to the Pseudoalteromonas sp. incubation. Moreover, the rate at which organic matter was metabolized by the Vibrio sp. was much higher than the Pseudoalteromonas sp. indicating the intrinsic availability of organic matter in sediments may depend on the species that is present and active. Isotopic analyses of microbially respired CO2 will be used to constrain the type and age of organic matter that is accessible to each species. Moreover, molecular analysis of subsamples collected from each incubation will link carbon utilization with the underlying gene expression. Our study sheds light on the degree to which the metabolic capacities of microorganisms affect carbon transformation in sedimentary environments.

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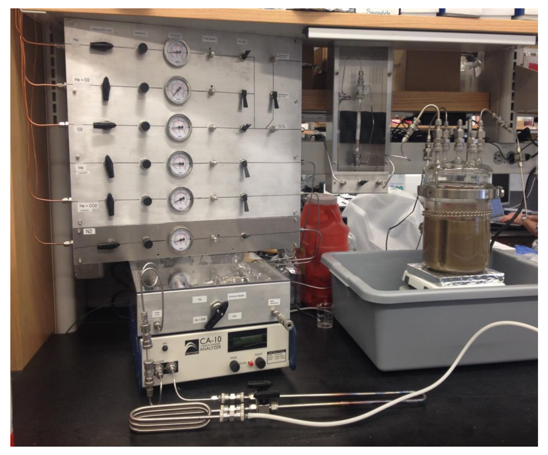
Abstract

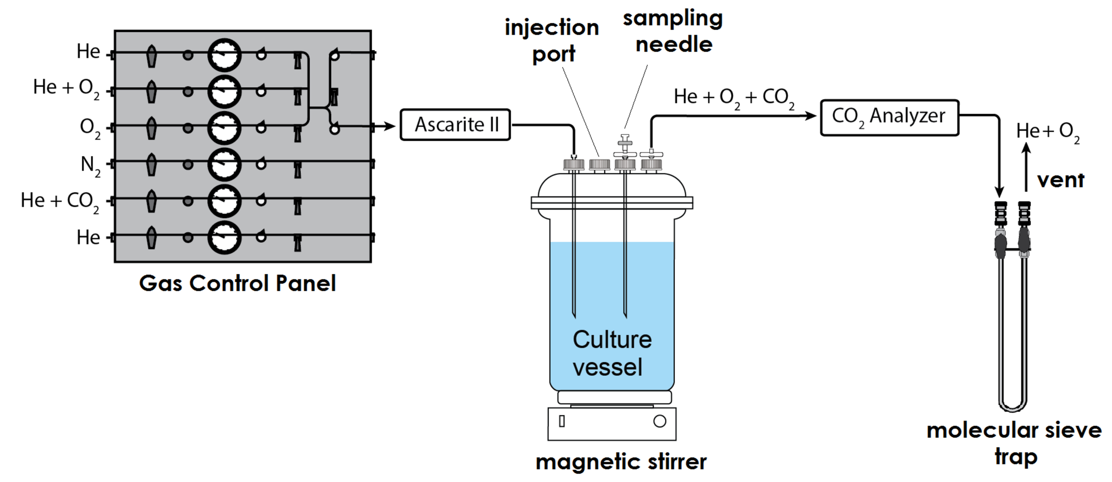
Microbial transformation and decomposition of organic matter in sediments constitutes one of the largest fluxes of carbon in marine environments. Recent studies have found that the ability to use different carbon sources appears to vary among microorganisms[1-3], suggesting that the availability of certain pools of carbon can be specific to the taxa that utilize the pool. This implies that organic matter mineralization in marine environments may depend on the metabolic potential of the microbial populations that are present and active. The goal of our study was to investigate the extent to which organic matter availability and transformation may be species-specific using sediment from Guaymas Basin (Gulf of California). We carried out time-series incubations using bacterial isolates and sterilized sediment in the IsoCaRB system which allowed us to measure the production rates and natural isotopic signatures of microbially-respired CO_2. Separate incubations using two different marine bacterial isolates (*Vibrio* sp. and *Pseudoalteromonas* sp.) and sterilized Guaymas Basin sediment under oxic conditions showed that the rate and total quantity of organic matter metabolized by these two species differs. Isotopic analyses of microbially respired CO_2 will be used to constrain the type and age of organic matter that is accessible to each species. Moreover, molecular analysis of subsamples collected from each incubation will link carbon utilization with the underlying gene expression. Our study sheds light on the degree to which the metabolic capacities of microorganisms affect carbon transformation in sedimentary environments.

The IsoCaRB System

The Isotopic Carbon Respirometer-Bioreactor (IsoCaRB) system allows us to probe the time-dependent relationships between microbial metabolic activity, respiration (*via* CO_2 flux) and the associated reactivity (or accessibility) of natural organic matter. This system continuously monitors respiratory CO_2 production and collects it quantitatively for natural abundance ^{14}C and ^{13}C analyses allowing us to decipher the age and source of organic matter being utilized, as well as its rate of remineralization[4,5].



Beaupre, Mahmoudi & Pearson (2016)



Simplified schematic of the IsoCaRB system

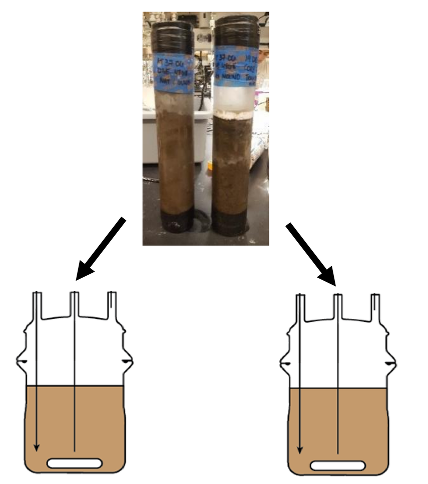
The system permits sampling of the growth media for downstream molecular analyses in order to explore relationships between the utilization of organic matter and underlying gene expression and enzymatic activity.

Experimental Approach

Do specific taxa degrade distinct pools of organic matter such that the composition of the microbial community will affect the rate and type of organic matter that is degraded?

Incubate sterilized sediment with model organisms in the IsoCaRB system to evaluate the potential species-specific effect on organic carbon degradation.

sterilized sediment



Species A Species B

- ***Microbially-respired CO_2*** was continuously monitored for quantification of total CO_2 as well as the rate of CO_2 production for each incubation.
- ***Natural abundance ^{13}C and ^{14}C analysis of CO_2*** was used to determine the source and age of the organic carbon utilized by each species.
- ***Cell counts*** were carried out in triplicate on daily subsamples to observe changes in cell density over the course of the incubation.
- ***Transcriptomics*** will be carried out on daily subsamples to observe changes gene expression.

Guaymas Basin is an an ideal study site – it contains wide range of potential microbial carbon sources with a large spectrum in radiocarbon ages[6].

Guaymas Basin is a young, active spreading center, with water depth of ~2000 m. It is characterized by hydrocarbon seeps and hydrothermal plumes and near-surface sediments are rich in organic matter (TOC = 3-12%)[7].

Sediment was decarbonated and sterilized using gamma-irradiation (total dose ~45 kGy).

Various marine isolates were incubated with sterilized sediment to identify organisms that could potentially degrade sedimentary organic matter.

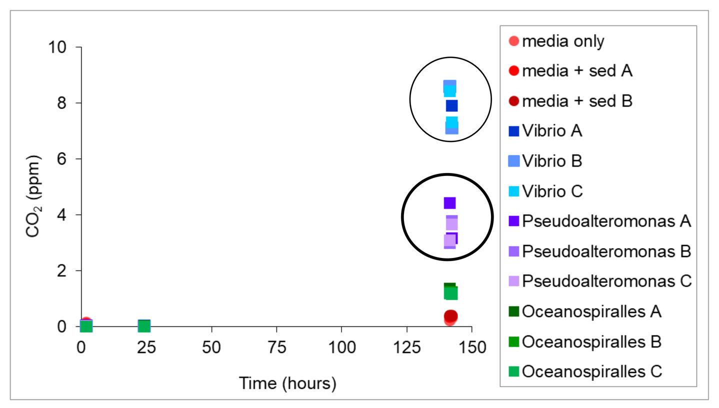


Figure 1. Results of benchtop tests for three candidate species incubated in gas-tight serum bottles with 50mL of carbon-free seawater media and 0.6g of sterilized Guaymas Basin sediment. CO_2 headspace measurements were carried out at day 0 and day 5 to assess whether species could metabolize sedimentary organic matter as their sole carbon and energy source.

Two different bacterial isolates were selected (*Vibrio splendidus* and *Pseudoalteromonas* sp. *3D05)* and subsequently used for IsoCaRB incubations.

Incubation in the IsoCaRB system consisted of:
(1)20-22g of decarbonated sterilized sediment;
(2) 2L of minimal media;
(3) 50mL log phase cells washed with carbon free media (cell density = 5 x 10^8 cells/mL).

Results

The rate and total quantity of organic matter metabolized by these two species differs, indicating the intrinsic availability of organic matter in sediments may depend on the species that are present and active.

Approximately 4.6 mg of carbon was collected during the *Pseudoalteromonas* sp. incubation and 2.3 mg was collected from the *Vibrio* sp. incubation.

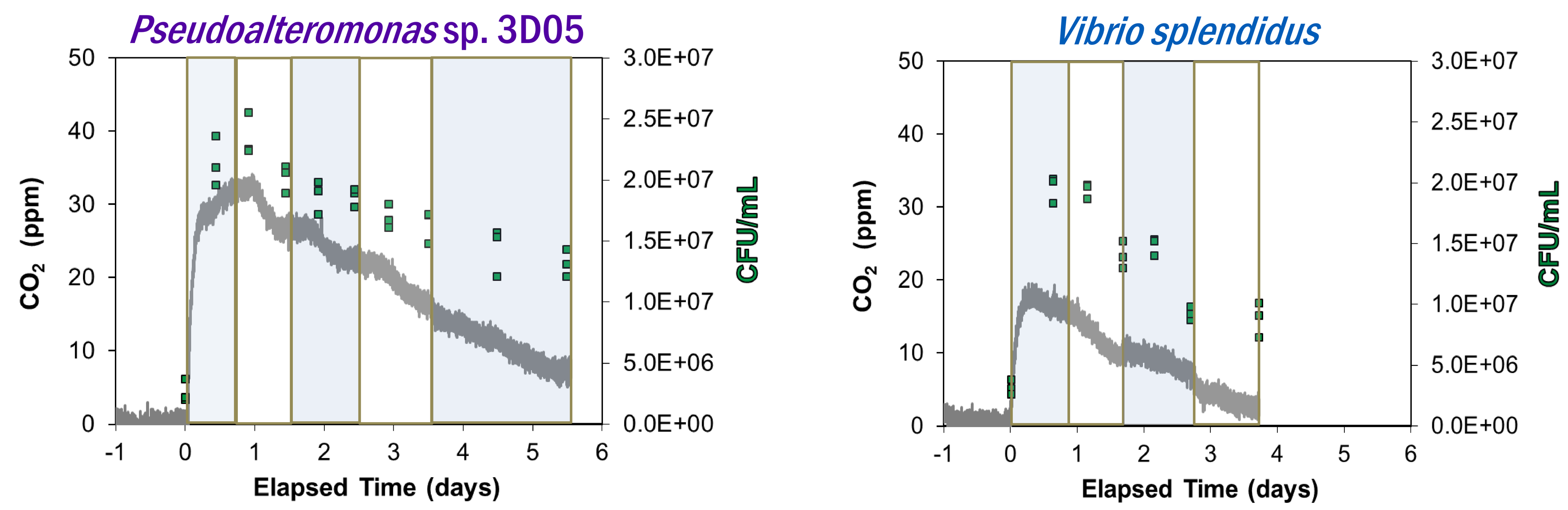


Figure 2. Microbial CO_2 production rates (gray line) of respired CO_2 released during incubation of 22g of Guaymas Basin sediment (0-9 cm; cores 7871-6, 7810-10,) by (A)*Pseudoalteromonas* sp. and (b)*Vibrio* sp. The width of each box spans the time interval during which each CO_2 fraction was collected for isotopic analysis to constrain the type and age of organic matter that is accessible to each species. For cell densities (green squares), subsamples were serially diluted in minimal media and plated on marine broth plates for CFU counts.

Although organic matter transformation appears to be species-specific, intrinsic sediment properties are still an important control on organic matter degradation.

When incubated with a different series of cores, an opposite pattern was observed such that *Vibrio* sp. metabolized substantially more organic matter than *Pseudoalteromonas* sp.

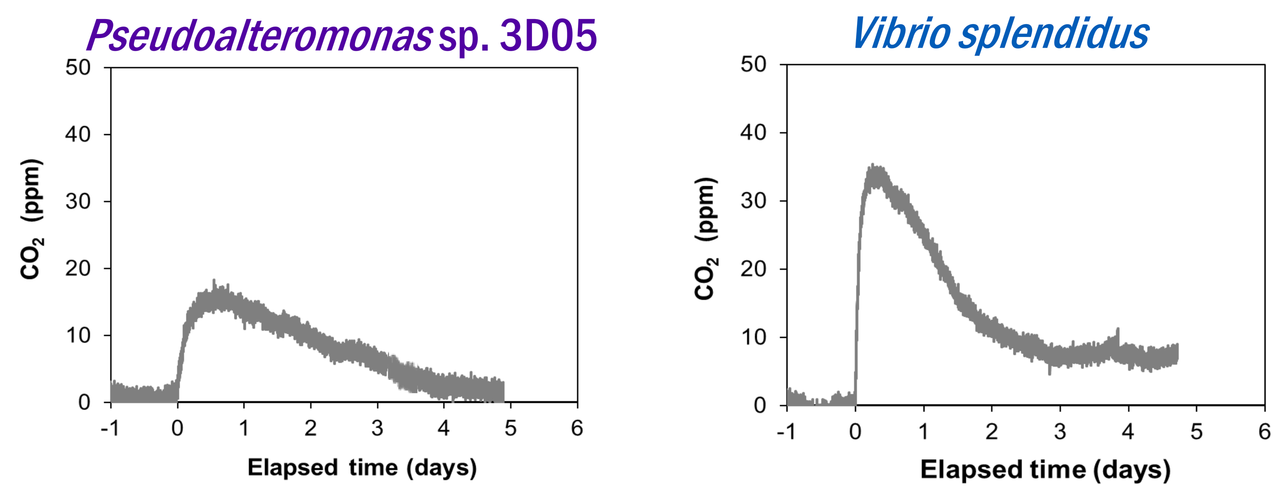


Figure 3. Microbial CO_2 production rates (gray line) of respired CO_2 released during incubation of 20g of Guaymas Basin sediment (0-8 cm; cores 4490-25, 4490-26) by (A) *Vibrio* sp. and (b) *Pseudoalteromonas* sp. Approximately 2.1 mg of carbon was collected during the *Pseudoalteromonas* sp. incubation and 3.6 mg was collected during the *Vibrio* sp. incubation.

Significantly less organic matter was metabolized by *Vibrio* sp. when incubated with sediment from deeper depths.

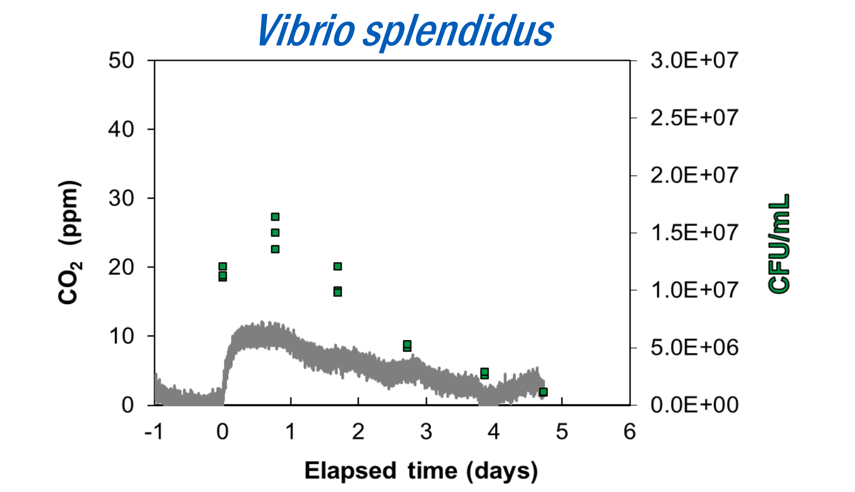


Figure 4. Microbial CO_2 production rates (gray line) of respired CO_2 released during incubation of 20g of Guaymas Basin sediment (0-14 cm; cores 4490-25, 4490-26) with *Vibrio* sp. Approximately 0.9 mg of carbon was collected during the incubation.

References

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