# IN-SITU ACTIVITY OF THE HETEROTROPHIC MICROBIAL COMMUNITY IN SEEP SEDIMENTS FROM THE SANTA MONICA BASIN

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## Introduction

The role of microbes in marine sediments is of great importance for the understanding of processes and elemental cycles in the ocean. However, many microbes inhabiting the seafloor remain unculturable because the in-situ conditions are difficult to mimic in a laboratory setting. Incubations of sediment slurries are likewise limited as the retrieval of sample material from the seafloor is associated with depressurization and disruption of the sedimentary matrix, with still unknown effects on the microbial community and its activity.
To overcome some limitations of in vitro studies, we used an in-situ injector coring system deployed in the deep sea by a remotely operated vehicle. Specially designed push cores enable the injection of different substrates, including stable isotope labeled compounds, into the sediment, allowing an incubation directly in the deep-sea environment with minimal disturbance of the microbial community. For this study, an injector core and a reference neighboring push core without injected fluid were inserted in the center of an orange-colored sulfur-oxidizing microbial mat at an active methane seep in the Santa Monica Basin at 800 m water depth (Fig. 1a, b). $^{13}$C-labeled glucose, deuterated water and homopropargylglycine (HPG), an alkyne modified methionine analog, were injected through multiple needles aligned along the vertical core axis into the sediment. The core was incubated directly at the seafloor with the goal of better understanding the activity and diversity of heterotrophic microorganisms within this seep setting under in situ conditions. A similar injector core approach with $^{13}$C-labeled glucose (Takano et al., 2010) demonstrated archaeal activity after 9 days through the labeling of the glycerol backbone of archaeal membrane lipids. Here, we expand the scope of injected stable isotope labels and combine them with single cell resolved biorthogonal non-canonical amino acid tagging (BONCAT; Hatzenpichler et al., 2016) and fluorescence in situ hybridization microscopy (FISH) over a relatively short incubation period.

## Results

The injector core system was deployed during a RV *Western Flyer* expedition through the Monterey Bay Aquarium Research Institute (MBARI) in February 2020 (Fig. 1a+b). After a 48-hour incubation at the seafloor, sediment cores were recovered and processed for geochemistry, molecular and microscopy analysis. Sedimentary fatty acids were analyzed for their stable carbon and hydrogen isotopic composition via gas chromatography coupled to isotope ratio mass spectrometry back in the laboratory (c.f. Wegener et al., 2012).
We observed incorporation of both $^{13}$C from glucose and $^{2}$H from deuterated water for fatty acids with 14 to 18 carbon atoms, above the isotopic signatures measured from fatty acids of the background core collected from the same mat, being indicative of active biosynthesis during the seafloor incubation. For carbon isotope enrichment in fatty acids, the highest deviation from the control core was observed in the uppermost sediment horizons with a decreasing trend down to 6 cm, where both the injector core and background sediments show similar $^{13}$C values (Fig. 1c).

Alongside the observation of $^{13}C$ labeled fatty acids, we observed enrichment of $^{13}C$ in the porewater dissolved inorganic carbon (DIC) in the injector core relative to the background control, showing that a large fraction of glucose was used for respiration (Fig. 1d). Interestingly, the highest $^{13}C$ enrichment in the pools of both DIC and fatty acids are observed for the upper 0-3 cm horizon, with an offset from the observed depth of the highest concentration of injected fluid, assessed through the passive injection tracer bromide (Fig. 1e). This data supports that the highest activity of heterotrophic microbes is located just below the sediment-water interface. Preliminary results of selected sediment horizons from single cell BONCAT-FISH microscopy show also cells that are translational active.

These in-situ seafloor experiments demonstrated our ability to use independent molecular and isotopic methods for detecting microbial activity in deep-sea methane seep sediments over relatively short incubation periods. Both anabolic as well as catabolic microbial processes were detected through the incorporation of HPG and $^{13}C$ enrichment in pools of DIC and bacterial derived fatty acids. We show that these in-situ injector cores are useful tools for studying in-situ activity of marine microbes at both the community and single cell level.

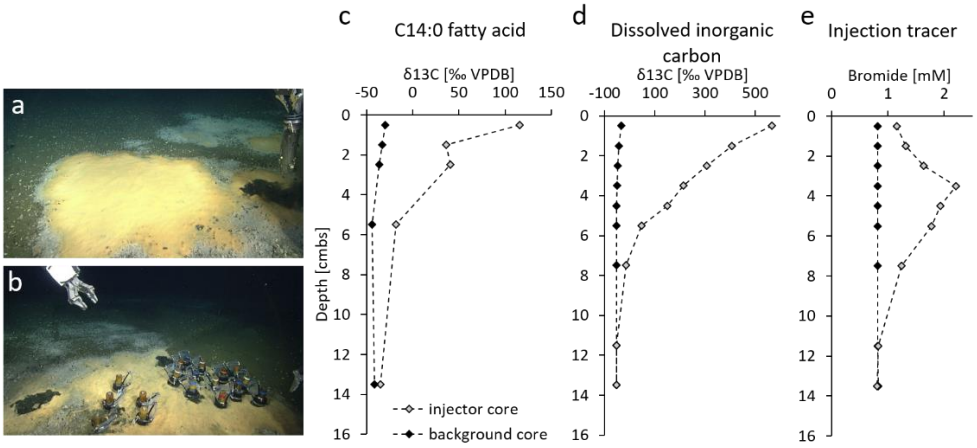


***Figure 1a)*** *Methane seep associated with an orange sulfur oxidizing microbial mat in the Santa Monica Basin (800 m, scientific party WF02-20),* ***b)*** *Deployed array of injector cores in the same mat (scientific party WF02-20),* ***c)*** *Tetradecanoic acid (C14:0) shows elevated $^{13}C$ values in the sediment core injected with $^{13}C$-glucose after 48-hours of incubation compared to the unamended background core,* ***d)*** *Active metabolism of $^{13}C$-labeled glucose is demonstrated from $^{13}C$ enrichment of pore water DIC,* ***e)*** *The passive injection tracer bromide demonstrates the depth of the injection front at the end of the incubation period.*

## References


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## Acknowledgements


We thank Sebastian Sudek (MBARI) for assistance with injector core maintenance.