Metabolomics of Deep Sea Methane Seeps

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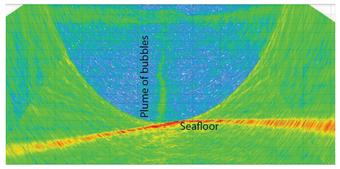
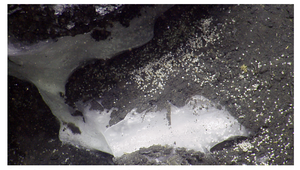
ABSTRACT

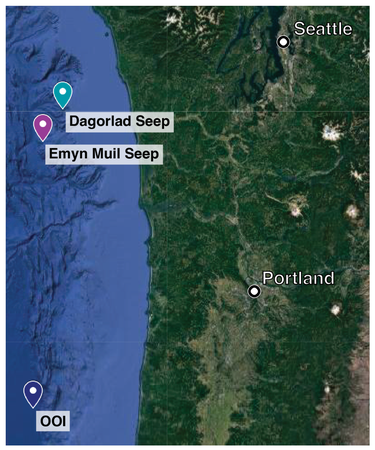
Deep sea methane seeps are home to a high diversity of microbial life. The unique metabolism of these microbes, which consume methane, makes them of interest due to potential applications for human and environmental health and well-being[1]. In this study, sediment cores were collected from an old and a new methane seep, both at approximately 1000 m depth, as well as a non-seep site (OOI). The Global Natural Product Social Molecular Networking (GNPS) platform was used to create a molecular network from MS2 data[2]. Compound classifications were predicted based on the fragmentation spectra using SIRIUS and CANOPUS[3-4]. The statistical package PRIMER v7 was used to compare and correlate the chemical and microbial profiles, and ultimately identify drivers of differences between the sites and depths. Interdisciplinary research to understand microbial and chemical diversity is essential for understanding the processes and role of ubiquitous methane seeps in global systems[1].



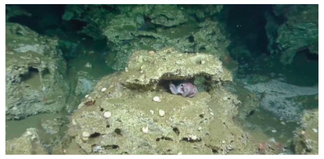
Methane bubbles from a seep as seen by multibeam sonar.



Methane hydrate at a seep

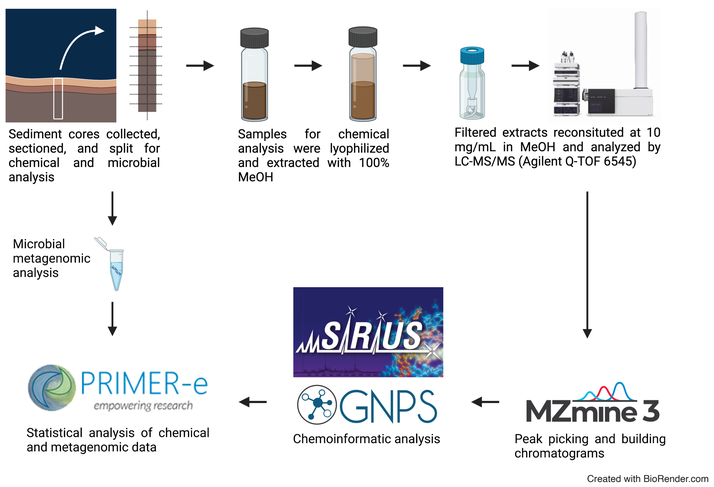


Dagorlad Seep (Young Seep)
Young methane seep
Soft sediment and microbial mats

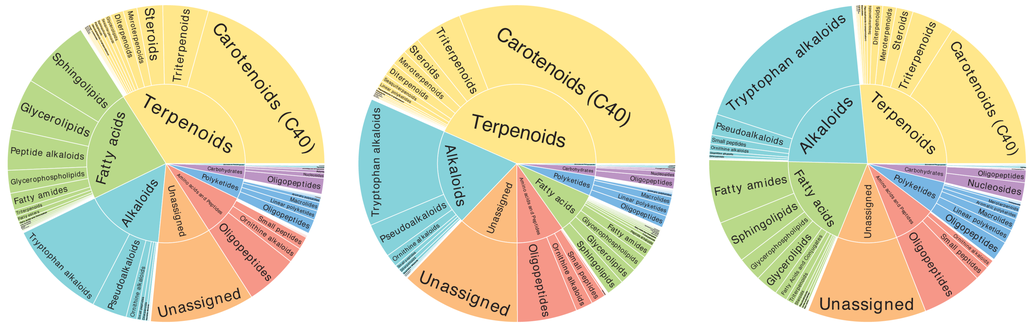


Emyn Muil Seep (Old Seep)
Old methane seep
Hard carbonate substrate

METHODS



STRUCTURAL CLASSIFICATION OF LCMS/MS FEATURES



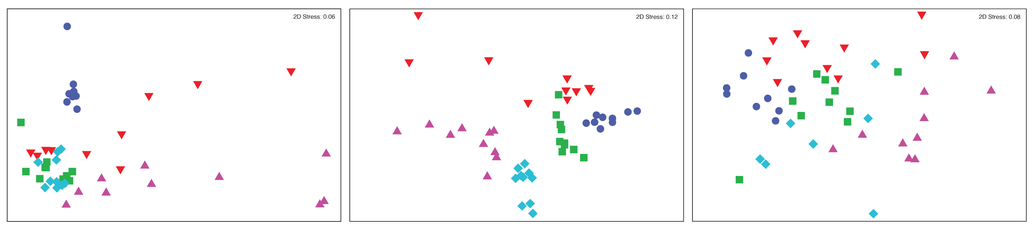
Emyn Muil Seep (Old)

Dagorlad Seep (Young)

OOI Slope Base

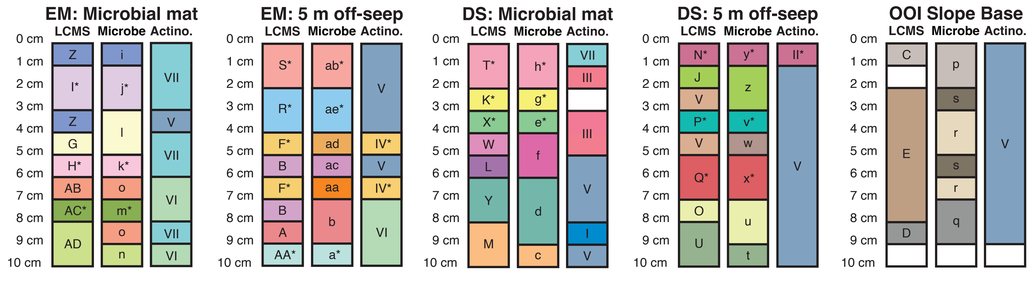
Sunburst plots of NP Classifier Pathways (inner rings) and Superclasses (outer rings) as assigned by CANOPUS[3,4]. Plots show proportions based on peak areas summed by site. The chemistry of the old seep with hard carbonate substrate is distinct from the young, soft sediment seep, with a relatively high proportion of fatty acids versus alkaloids. The chemical profiles of both methane seep sites display a higher proportion of terpenoids than the non-methane seep OOI site.

NMDS ANALYSES OF PUSH CORE CHEMICAL AND MICROBIAL DATA



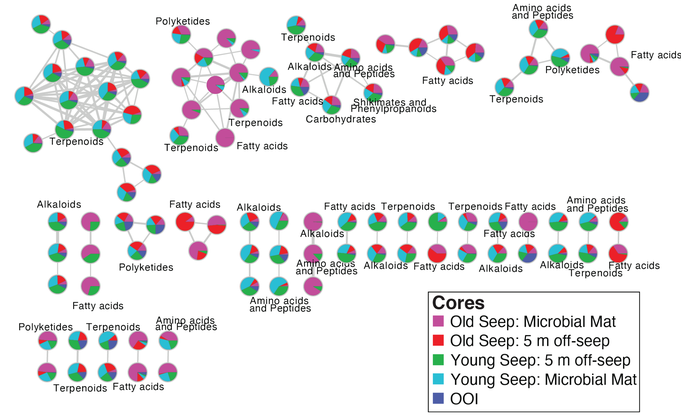
Non-metric multidimensional scaling (nMDS) plots of each dataset (chemical, microbial, and actinobacterial) colored by core. S17 Bray-Curtis nMDS plots were constructed in PRIMER v7 and a LogX1 transformation was used for all data prior to analysis. Sediment push cores from the old seep (hard carbonate) display much more variation in chemical profiles than the new seep (soft sediment) or the OOI non-seep site.

COMPARISON OF SEDIMENT PUSH CORE SLICES



Schematics of the five cores colored by the similarities between sections based on abundance of mass features, Microbial ASVs, and Actinobacterial ASVs. Groupings were determined using the SIMPROF routine in PRIMER v7. Sections within the same dataset that are labeled and colored the same are grouped together and different from the other samples within that data set (ex. Chemistry group Z at 0 and 3 cm in EM: Microbial Mat). Groupings that are consistent across multiple data sets are indicated with an asterix.

GNPS MOLECULAR NETWORKING OF LCMS/MS DATA



GNPS molecular network with singletons excluded[2]. Nodes are labeled with NP Classifier pathways assigned by CANOPUS[3,4]. GNPS molecular networking supports an abundance of terpenoids and fatty acids at the old carbonate seep and more alkaloids in the soft sediments of the young seep.

FUTURE WORK

- Determine correlations between the metabolites and microbes
- Optimization of environmental sampling methods for evaulation of *in situ* metabolome.
- Increase concentrations of metabolites retrieved using HP20 resin passive samplers in parallel with sediment cores.
- Antimicrobial testing of environmental samples and correlation of antimicrobial activity with components of crude samples.
- Test structure-function hypotheses using orthogonal approaches.
- Further sampling of additional shallow and deep methane seeps.

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