

## Bacterial anomalies indicative of deep-sea sediment hydrocarbon seeps

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### Summary

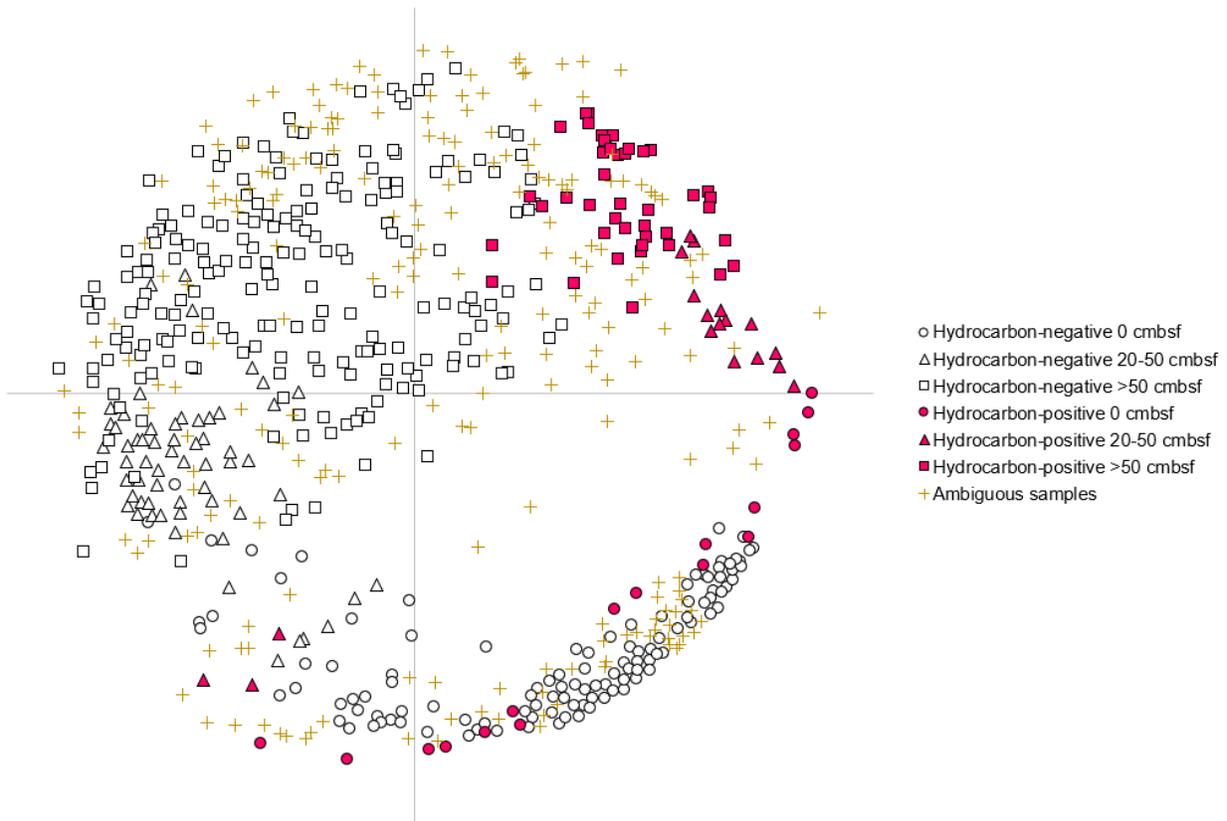
Deep sea oil and gas exploration relies predominantly on conventional geochemical analyses of subsurface marine sediments to identify the presence of a working petroleum system and de-risk exploratory drilling. In this study, molecular microbiological methods were investigated as a complementary tool for hydrocarbon seep detection by uncovering biodiversity anomalies in seabed samples. Piston coring was performed in >2500 m water depth at 43 geographic locations off the coast of Nova Scotia resulting in samples from the upper 10 metres below seafloor (mbsf). Sediments were assessed for hydrocarbon liquids and gases that typically signify seepage in seabed samples. In addition, over 650 bacterial 16S rRNA gene amplicon libraries allowed microbial communities to be compared along vertical depth profiles (0, 20-50, and >50 cmbsf) in these 43 locations. Select sites showed strong evidence of both biogenic and thermogenic hydrocarbons. NMDS, ANOSIM, and IndicSpecies analyses revealed significantly different microbial communities in the presence of hydrocarbons, characterized by select bacterial indicator taxa associated with specific sediment depths. Caldatribacteriota and Campilobacterota groups were observed in high relative sequence abundance in hydrocarbon-positive sediments, notably in the 20-50 cmbsf shallow intervals. Correlation of these microbial groups with geochemical parameters suggests that specific taxa within Caldatribacteriota and Campilobacterota lineages could signal the presence of hydrocarbons, and furthermore differentiate between hydrocarbons of thermogenic- and biogenic-origin. These patterns suggest that microbial DNA sequencing has the potential to play a key role in offshore exploration.

### Theory / Method / Workflow

Marine sediments down to 1000 cmbsf were collected by piston coring at 43 deep sea locations in the Atlantic Ocean where water depths exceeded 2500 m. Geochemical analysis of headspace gases and sediment extracts revealed the presence and type of hydrocarbons such that sites were ranked as positive, negative, or ambiguous (i.e., weak geochemical signals). Microbial DNA sequencing of the V3-V4 region of bacterial 16S rRNA genes on an in-house Illumina MiSeq allowed biodiversity assessments of operational taxonomic units (OTUs) sharing >97% sequence identity. Microbial communities in surface sediments (0 cmbsf and 20-50 cmbsf) and subsurface sediments (50-1000 cmbsf) were compared through NMDS, ANOSIM, and IndicSpecies analyses using R.

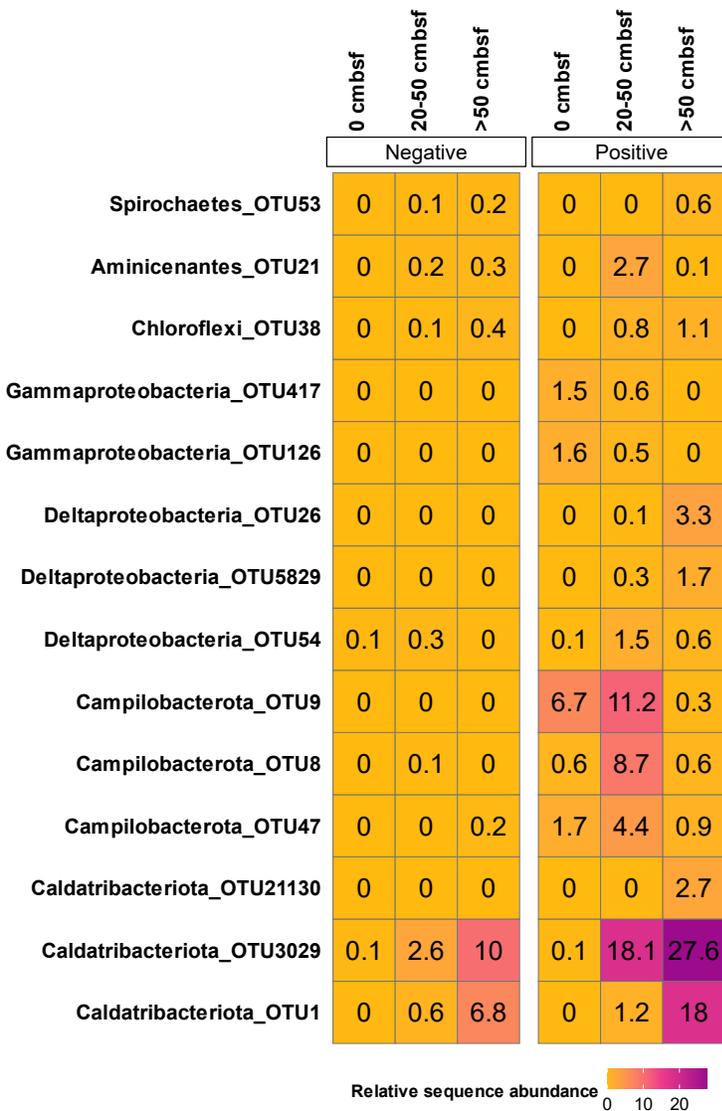
## Results, Observations, Conclusions

NMDS analysis of 668 bacterial 16S rRNA gene amplicon libraries suggests bacterial communities in hydrocarbon-positive sediments are distinct from hydrocarbon-negative sediments, particularly at depths of 20-50 cmbsf and >50 cmbsf (Figure 1). Bacterial communities in hydrocarbon-ambiguous sediments resembled both hydrocarbon-negative and hydrocarbon-positive sediments, potentially enabling re-consideration of ambiguous sites with microbial profiles like those in hydrocarbon-positive samples. ANOSIM revealed that three out of nine hydrocarbon-ambiguous sites were significantly different from hydrocarbon-negative sites and these were investigated further (Figure 3).



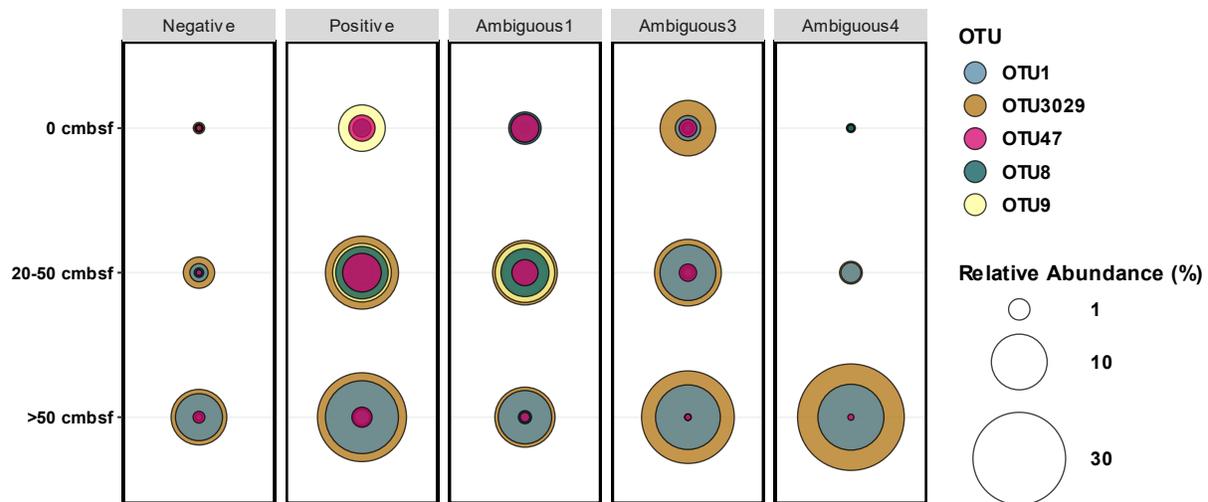
**Figure 1.** Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of 668 sediment samples from 0 cmbsf, 20-50 cmbsf, and >50 cmbsf depths. Ordination is based on Bray-Curtis dissimilarities among samples. Points represent samples, shapes represent depths, colours represent hydrocarbon-status.

Fourteen bacterial OTUs were identified as indicator species for hydrocarbon-positive samples (Figure 2). Average relative sequence abundances for these OTUs varied in 0 cmbsf, 20-50 cmbsf, and >50 cmbsf sediments. Indicator OTU 3029 (phylum Caldatribacteriota) and OTUs 8, 9, and 47 (phylum Campilobacterota) were detected in high relative sequence abundance in 20-50 cmbsf sediment from hydrocarbon-positive sites. This suggests that their use as bioindicators for hydrocarbon seeps can be applied to relatively shallow surface sediments, whereas OTUs 1 and 3029 were detected in high relative abundance in deeper sediments (>50 cmbsf) that are typically sampled in piston-coring geochemical surveys.



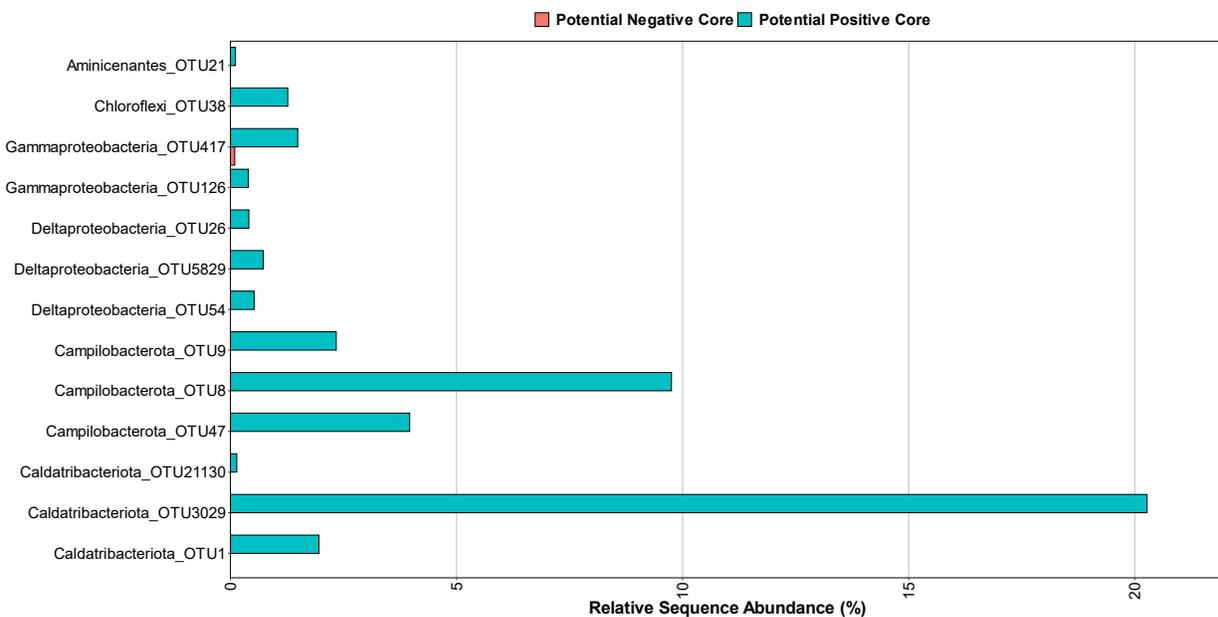
**Figure 2.** Heatmap of relative 16S rRNA gene sequence abundances of 14 indicator OTUs at 0 cmbsf, 20-50 cmbsf, and >50 cmbsf depths compared between hydrocarbon-negative and hydrocarbon-positive samples.

Depth distribution patterns of Caldatribacteriota and Campilobacterota indicator OTUs in 0 cmbsf, 20-50 cmbsf, and >50 cmbsf sediments from three hydrocarbon-ambiguous sites were compared with a hydrocarbon-positive and a hydrocarbon-negative site (Figure 3). Depth distribution patterns of these OTUs in site 'Ambiguous1' most resemble those in hydrocarbon-positive sites, where geochemical signals of both biogenic and thermogenic hydrocarbons were detected. Thermogenic, but not biogenic, hydrocarbon signals were detected in site 'Ambiguous4', where there is a marked absence of Campilobacterota OTUs 8, 9, and 47 at 0-50 cmbsf, suggesting that Caldatribacteriota OTUs may be indicators for the presence of thermogenic hydrocarbons and Campilobacterota OTUs may be indicators for biogenic hydrocarbons.



**Figure 3.** Bubble plot showing the relative abundance (depicted by size) of indicator OTUs (depicted by colour) 1 and 3029 (Phylum Caldatribacteriota) and OTUs 8, 9, and 47 (Phylum Campilobacterota) in hydrocarbon-negative and hydrocarbon-positive samples and samples from three hydrocarbon-ambiguous sites.

Bacterial indicator OTUs for hydrocarbon seepage identified in this study were tested in the field by performing DNA sequencing on the RV Atlantic Condor on deep sea hydrocarbon seep samples obtained with a remotely operated vehicle (Figure 4). Positive and negative seabed samples, supported by visual (video) evidence were sampled by push coring and compared. Oxford Nanopore sequencing of amplified bacterial 16S rRNA genes confirmed that the bacterial indicator OTUs identified in this study (Figs. 2-3) were detected in the positive core and were almost entirely absent in the negative core. Specifically, Caldatribacteriota OTU 3029 and Campilobacterota OTU 8 comprised ~10-20% of the total sequences in the positive core.



**Figure 4.** Negative (A) and positive (B) seabed sediments collected on board the RV Atlantic Condor ranged in appearance and texture. (C) DNA sequencing was performed at sea using the Oxford Nanopore MinION sequencing device. (D) Bar chart graph comparing relative sequence abundances of indicator OTUs between a negative and a positive seabed sample.

### Novel/Additive Information

A bioassay was developed for hydrocarbon exploration in the deep-sea using a DNA-based assessment of *in situ* bacterial species in shallow and deep marine sediments. Bacterial OTUs affiliated with Caldatribacteriota and Campilobacterota are indicative of thermogenic and biogenic hydrocarbons. These OTUs were readily detected in shallow <50 cmbsf sediments in hydrocarbon-positive sites. Preliminary testing of application of this bioassay at sea with visual (ROV) control at seabed seepage sites corroborates these findings. Anomalies in seabed sediment microbial diversity patterns can be used as bioindicators for hydrocarbon seeps in the deep sea and provide a complementary tool for de-risking offshore oil exploration.

## **Acknowledgements**

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## **References**

Dong X, Kleiner M, Sharp CE, Thorson E, Li C, Liu D and Strous M (2017) Fast and Simple Analysis of MiSeq Amplicon Sequencing Data with MetaAmp. *Front. Microbiol.* 8:1461. doi: 10.3389/fmicb.2017.01461