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Title: Microbiomes respond predictably to built habitats on the seafloor

Running Title: Microbiomes respond to built habitats

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Abstract

The seafloor contains complex ecosystems where habitat heterogeneity influences biodiversity. Natural biological and geological features including vents, seeps and reefs create habitats that select for distinct populations of micro- and macrofauna. While largely studied for macrobiological diversity, built habitats may also select distinct microbiomes. Built habitat density on the seafloor is increasing with ocean sprawl expanding in the continental shelf and slope, potentially having widespread effects on benthic communities. This study addresses one type of built habitat, shipwrecks, on microbiomes in surrounding sediment. Using deep-sea sediment samples (762 total) from the Gulf of Mexico, we report elevated diversity and a predictable core microbiome around 9 shipwrecks. We show the sphere of influence of built habitats extends up to 300 m onto the seafloor. Supervised learning made predictions of sample proximity to structures based on frequency of taxa. Strongest predictions occurred in sediments nearest and furthest from sites for archaea and mid-transect for bacteria. The response of archaea to built habitats was consistent across sites, while bacteria showed greater between site variability. The archaeal core shipwreck microbiome was enriched in taxa (e.g. Bathyarchaeia, Lokiarchaeia, Thermoplasmata) not present in the surrounding seafloor. Shipwrecks shaped microbiomes in expected ways, providing insight on how built habitats impact microbiome biodiversity in the Anthropocene.

Introduction

The seafloor of the ocean is the largest continuous ecosystem on Earth and the most uncharted one, with less than 20% of seabed mapped (Levin et al., 2019). The seafloor is also a complex ecosystem where habitat heterogeneity is thought to be a major factor

influencing biodiversity (Zeppilli et al., 2016). Habitat heterogeneity allows for more species to co-exist in a specific area thus contributing to species diversity, richness, and a greater number of ecological niches (Zeppilli et al., 2019). Biodiversity in the deep sea is positively linked with ecosystem functioning, suggesting that heterogeneity influences ecosystem processes (Zeppilli et al., 2019). Contemporary advances in high-resolution bathymetry report an abundance of hydrothermal vents, cold seeps, gas hydrates, seamounts, canyons, and smaller habitat features contributing to a heterogeneous landscape (Danovaro et al., 2014). Some of these features, specifically, hydrothermal vents and seeps, create environments that are physically and chemically dissimilar from the surrounding seabed where poly-extremes of temperature and chemical gradients select for specialized populations of micro and macrofauna (Orcutt et al., 2011). Widespread hydrothermal vents and methane seeps are considered hotspots of biodiversity that harbor genetically distinct and biogeochemically relevant microbiomes (Dick et al., 2019; Dombrowski., 2018; Ruff et al., 2015;). Organic falls, like whale carcasses and wood falls, are also habitat features that create atypical environments attracting diverse microbial life (Bienhold et al., 2013; Goffredi & Orphan, 2010). Recent study suggests that even abyssal plains, once considered uniform in composition, are composed of heterogeneous rock patches that harbor nuclei of biodiversity (Riehl et al., 2020). These natural features have received substantial study for their importance as habitats that maintain deep sea diversity and function.

The mosaic of modern and historic built habitats (human made structures) on the ocean floor may play substantial roles in shaping and maintaining biodiversity, composition,

and function. These environments differ from the surrounding seabed, provide hard substrate for colonization, have high relief, increasing benthic and pelagic connectivity, and transform into artificial reefs. For decades, artificial reefs have been considered a beneficial surrogate to natural habitats, especially in mesophotic areas lacking hard bottom (Firth et al., 2016; Sammarco et al., 2004). Decommissioned oil rigs and platforms have been resourced as artificial reefs through government programs including the “Rigs to Reefs” in the United States (Macreadie et al., 2011). Biofouling and reef development on artificial hard surfaces is reported to have positive effects on biodiversity of regional fish stocks and can pose as physical barriers to illegal trawling practices (Firth et al., 2016; Walker et al., 2007). However, there is also debate regarding habitat value vs. habitat harm to marine macrofauna, especially where invasive species are concerned (Sammarco et al., 2004) and whether artificial reefs are “stepping-stones” for native or invasive species (Schulze et al., 2020). Artificial reefs could also promote high densities of native but habitat-limited species like benthic toxin producing dinoflagellates.

Shipwrecks transform into artificial reefs and have been long regarded as a positive influence on macrobiological biodiversity (Walker et al., 2007). While not as numerous as the built features associated with energy infrastructure, in the Gulf of Mexico and Baltic Sea, for example, there are over 2,000 and 100,000 known shipwrecks respectively (Björndal, 2012; Damour et al., 2015). The United Nations Educational, Scientific, and Cultural Organization (UNESCO) estimates over three million shipwrecks on the global sea floor (Koutsis and Stratigea, 2021; UNESCO, 2008). Along with

providing hard substrate, it is important to note some shipwrecks pose acute environmental hazards when they contain sunken munition, chemical agents, toxic chemicals and fuel that could leak into the surrounding seabed and water column (Czub et al., 2018).

While artificial reefs have been studied for their influence on macrobiological communities, they have largely been overlooked in microbial ecological studies. Two recent works highlighted a positive correlation between microbiome diversity and proximity to historic shipwrecks (Hamdan et al., 2018, 2021). A case study of a historic metal-hulled shipwreck revealed shipwrecks function as island-like systems for bacterial microbiomes (Hamdan et al., 2021). These works indicate shipwrecks shape microbial biogeography with their influence radiating from structure into the surrounding seafloor. The implication of these works is that built features function as catalysts of change for invisible diversity.

The pace of change for seafloor biodiversity may accelerate with the rising number of built features introduced into the seafloor as a consequence of the “ocean sprawl”, that is, the progressive addition of human made structures in the marine environment (Bishop et al., 2017; Duarte et al., 2013). Built features associated with fossil and renewable energy infrastructure are ubiquitous in some areas. For example, 60% of the world’s active offshore energy platforms are currently located in the Gulf of Mexico (Firth et al., 2016). By 2028, the amount of energy and aquaculture infrastructure on the ocean seafloor is expected to increase 50-70% (Bugnot et al., 2021). In addition to

energy infrastructure, shipwrecks, airplane wrecks, and space debris may also alter benthic communities in terms of composition, diversity and organismal abundance. This is the case from nearshore environments to the deepest areas on the global seabed (Amon et al., 2020).

Given the disconnect between the number of built habitats on the seafloor and biodiversity studies of the seabed surrounding them, the sphere of influence of built features on ecosystem dynamics is poorly understood. The response of microbial communities to built habitats is of particular interest, since microorganisms are the primary colonizers of introduced substrates, enabling them to become artificial reefs, and drive biogeochemical cycling (i.e., carbon, nitrogen, silica, trace metals) in all marine habitats (Svane & Petersen, 2001). Thus, the goals of this study were to address how historic deep-sea shipwrecks of different size, age and hull type (wood and metal) 1) shape microbiome composition in the surrounding seabed, and 2) determine if microbiomes respond to built features in stochastic or predictable ways to provide context for their potential as drivers of seabed biodiversity.

Materials and Methods

Sediment cores were collected during eight offshore expeditions spanning 2014–2019 aboard research vessels *Point Sur* (PS) and *Pelican* (PE) (Table S1). Samples from cruises PE15-22 (March 2014), PE16-23 (May 2016), PS17-26 (June 2017), and PS18-27 (June 2018) were collected using a MC800 deep-sea multi-corer (Ocean Instruments) fitted with a Tracklink Ultra-short baseline (USBL) transponder to provide positional information relative to shipwrecks. Samples from cruises PE14-15 (March

2014), PE15-02 (July 2014), PS19-06 (September 2018), and PS19-24 (June 2019) were collected using ROV *Global Explorer* (Oceaneering) and *Odysseus* (Pelagic Research Services) with Jason-style push cores. The shipwrecks in this study range in depth between 144 m (Halo) and 1950 m (Alcoa Puritan; Table S1; Fig.1).

Samples were collected and analyzed as previously described (Hamdan et al., 2018, 2021). Briefly, genomic DNA from sediment was extracted with the FastDNA SPIN kit (MP Biomedical Inc.). PCR gene amplification and sequencing was performed at the Integrated Microbiome Resource (IMR) facility at Dalhousie University (Halifax, Nova Scotia, Canada). Specifically, samples were analyzed on a MiSeq platform (Illumina) generating 300 bp paired-end sequences. The sequencing protocol involved PCR controls (4 per 380 samples), although the extraction protocol did not include kit blanks. Sequencing blanks were included for each individual Miseq run to help address background contamination. The primer sets B969F/BA1406R and A956F/A1401R were used respectively to target V6–V8 variable regions of the bacterial and archaeal 16S rRNA gene (Comeau et al., 2011).

Analysis of 16S rRNA amplicon sequences for archaea and bacteria was conducted using Quantitative Insights into Microbial Ecology 2 (QIIME2; Bolyen et al., 2019). Sequences were trimmed, denoised, and quality controlled with DADA2 (Callahan et al., 2016) for paired-end sequences at default settings with the exception of max number of expected errors set to 4. Taxonomic assignment on produced amplicon sequence variants (ASVs) was done using the trained classifier (q2-feature-classifier; Bokulich et al., 2018) with extracted reads from SILVA 132 reference database (Quast et al., 2013)

and Vsearch classification (Rognes et al., 2016) at 99%. Alpha diversity metrics were determined using the core-metrics plugin. Core microbiome at each shipwreck and each distance group as well as all shipwrecks and distance groups were determined with the feature-table core-features plugin.

PRIMER (v. 7) with PERMANOVA+ was used for statistical analysis of microbiome composition. Bray–Curtis dissimilarities were calculated from archaeal and bacterial ASV abundance tables. A permutational analysis of variance (PERMANOVA) was used to rule out sampling date and sampling device as confounders in this dataset (not shown). Subsequently PERMANOVA was used to identify differences in community composition with distance, sediment depth, water depth, and hull type. PERMANOVA used Type III (sequential) sum of squares and 9999 permutations. Diversity–extinction plots were created from ASV tables.

Distance decay of community similarity relationships were visualized through distance decay curves (DDC) to display how community similarity varies with geographic distance between samples (Zinger et al., 2014). DDCs were constructed for the bacterial and archaeal datasets separately in RStudio (Version 2022.02.1) using the methods described in Zinger et al. (2014) with the R packages *vegan* (Version 2.5-7; Oksanen et al., 2017), *ggplot2* (Version 3.3.5; Wickham, 2016), and *ggpubr* (Version 0.4.0; Kassambara, 2018). Bray-Curtis dissimilarity matrices were constructed for whole community ASV tables. In the matrices, 0.01 was added to each value to avoid calculating infinity values during log transformation. The Euclidean distance matrices

were constructed on distance of samples from shipwrecks (m). Both distance matrices were log transformed using natural log. A linear regression of log-transformed Bray-Curtis dissimilarities against the log-transformed Euclidean distance matrix was performed.

The QIIME2 q2-sample-classifier was used to make machine learning predictions of sample metadata variables from ASV tables. This analysis supported the goal of determining if microbiomes respond predictably to built features. The supervised learning tool splits input into training and test data sets. The fraction of input samples to include in the test set was set 0.2. The test set was excluded from training, but used in model validation (Bokulich et al., 2018). The training set trained and tested the estimator using a stratified k-fold cross-validation scheme. The random forest regressor predicted numerical variables including distance, water depth, and sediment depth from ASV feature tables with p-optimize-feature-selection parameter. The regressor model generated model summary and accuracy scatterplot showing predicted vs. true values for test sample. The random forest classifier was used to predict proximity of samples to shipwrecks from ASV tables. Receiver operating characteristic curves, area under the curve (AUC) analysis (calculated using scikit-learn) and confusion matrices summarizing the performance of the algorithm were generated as part of the pipeline. Abundance heatmaps of ASVs driving predictions in distance groups were generated with the qiime sample-classifier heatmap function. Data were visualized using ggplot2 (Version 3.3.3; Wickham, 2016) in RStudio (Version 1.4.1106) and Surfer (version 21, Golden Software) was used to create depth–distance diversity plots.

Sequences for *Anona* (2015–2018) and *Alcoa Puritan* are provided under NCBI Bioproject Number PRJNA612314. Sequences for *Halo*, *Viosca Knoll*, *Ewing Bank*, *Mica*, *Mardi Gras*, and 2014 samples for *Anona* are found under NCBI Bioproject Number PRJNA401282. Sequences for 15711 and 15470 shipwrecks are available under NCBI Bioproject Number PRJNA599410.

Results

Analysis of bacterial and archaeal 16S rRNA genes was conducted on sediments collected on single or multiple radial transects surrounding 9 shipwrecks in the Gulf of Mexico (Fig 1). The details of each shipwreck and the sampling design are described in Table S1. Field work generated sediment samples from distances of 2 – 1000 m away from shipwrecks, and from depths of 0 – 20 cm below the seafloor (cmbfs). This yielded extracted DNA from 666 and 788 archaeal and bacterial samples respectively, and produced ~27 million sequences for each domain. The smaller archaeal dataset is due to fewer analyses at one site, *Anona*, and no archaeal samples at *Alcoa Puritan*. Post quality control reads resulted in 41,752 amplicon sequence variants (ASVs) for archaea and 191,338 ASVs for bacteria. Low frequency reads were filtered and 650 samples for archaeal analysis and 762 for bacteria remained.

PERMANOVA revealed microbiome structure for both domains was primarily shaped by water depth (Table S2). Distance from the 9 shipwrecks for both archaea (Pseudo-F 12.4; $p < 0.001$) and bacteria (Pseudo-F 3.05; $p < 0.001$) (Table S2) significantly influenced microbiome composition, along with sediment depth, and hull type.

Alpha diversity of archaea and bacteria was significantly influenced by distance from the shipwrecks (Kruskal-Wallis; $p < 0.001$). Regression analysis of archaeal Shannon Index (Shannon, 1948) against distance showed a significant positive relationship between diversity and shipwreck proximity (Fig. 2a; $R^2 = 0.42$; $p < 0.001$). Bacterial diversity also increased with proximity to shipwrecks ($p < 0.0001$) although the relationship was not as strong (Fig. 2b; $R^2 = 0.13$; $p < 0.001$). A spatial analysis was generated to visualize the changes in alpha diversity with distance and sediment depth using data from all sites combined (Fig. 2c) and at each site individually (Fig. S1). Archaeal Shannon Index for all sites was highest near the shipwrecks and elevated in all depths. An area of elevated diversity extended up to 200 m away from all shipwrecks (Fig. 2c). This trend was observed at each site individually (Fig. S1) and the highest overall diversity was observed at the shipwreck *Halo*, a 436' long, steel-hulled wreck that rests in ~144m water depth, and at two 19th-century, wooden-hulled shipwrecks known as sites 15711 and 15470 resting in 525 m and 1800 m water depth respectively. Notably, the transects at sites 15711 and 15470 extended only 60 m into the surrounding seabed. Spatial analysis for bacterial Shannon Index showed similar results, with elevated diversity extending ~250 m from the shipwrecks at all sediment depths (Fig. 2c). At individual sites, the diversity – distance trend was not as consistent for bacteria as for archaea. For example, diversity at Ewing Bank increased throughout the entire transect (Fig S2). Similarly to alpha diversity, community similarity also decreased with increasing geographic distance. The DDC for bacteria showed slight decrease in microbial community similarity with increasing geographic distance, and the slope of the regression was significant ($p < 0.05$; Fig S3a). The DDC for archaea also showed

decreasing microbial community similarity with increasing geographic distance and the slope of the regression was more significant than for bacteria ($p < 0.001$; Fig S3b).

Core microbiome analysis is a tool to identify taxa consistently observed in certain habitats (Shade & Handelsman, 2012). Members of the core microbiome are often considered critical to habitat function. Membership in the core microbiome for archaea and bacteria was based on presence in 80% of samples in groups binned by distance range from the shipwrecks. The analysis on archaeal ASVs revealed changes in core community composition with distance from the shipwrecks (Fig 3a). The number of members of the core community was higher within 150 m from the shipwrecks ($n = 13$), and a wide range of phylotypes were observed. Crenarchaeota class Bathyarchaeia, Asgardaeota, Lokiarchaeia, and various members of Euryarchaeota Thermoplasmata were all detected in the archaeal core microbiome up to 150 m and disappeared at greater distances. Beyond 200 m the only core members belonged to Thaumarchaeota ($n = 2$). These patterns were evident when each shipwreck was analyzed individually (Fig. S4).

The core microbiome for bacteria also changed as a function of distance (Fig 3b). The diversity of bacterial core members was greatest at 2-9m from the sites with highest number of core members ($n=26$) and decreased beyond 200 m. At 500 and 1000 m the bacterial core consisted of only 7 taxa. Certain core phylotypes from the Acidobacteria, Bacteroidetes, Dependetiae, Latescibacteria Marinimicrobia, Nitrospirae, and Zixibacteria were present only in samples in nearest proximity to the shipwrecks. The

core also included cosmopolitan bacteria present at all distances affiliated with the Alpha-, Delta-, and Gammaproteobacteria (Fig 3b). The analysis for each shipwreck revealed a decline in core membership with distance from the sites, cosmopolitan members, and unlike the archaea, members specific to individual sites (Fig S5).

Machine learning can aid in studying microbial communities and their relationship to the surrounding environment. Here, supervised learning predicted numerical and categorical sample metadata values as a function of microbiome composition. The random forest regressor tool predicted sample distance from the shipwrecks, sediment depth and water depth based on frequency of ASVs. The most accurate predictions for archaea and bacteria were water depth and sediment depth (Table S3). Archaeal ASVs predicted proximity to shipwrecks more strongly ($r^2 = 0.29$, $p < 0.001$) than bacterial ASVs ($r^2 = 0.05$, $p = 0.007$; Table S3). To explore which ASVs were driving distance predictions in each distance group, we used the random forest classifier. The classifier indicates high predictive accuracy for archaea (macro-average area under the curve (AUC) = 0.85) and bacteria (macro-average AUC = 0.82) (Figs S6 and S7). The distance groups that were best predicted from archaeal ASVs were closest to (2-9m; AUC = 0.95 and the furthest from (1000m; AUC = 0.94; Fig S6a) the sites. For bacteria, 300 m (AUC = 0.91) and 1000 m group (AUC = 0.90) was best predicted from bacterial ASVs (Fig. S7a) while the closest distance group had a moderately high prediction (AUC = 0.79). Lastly, both archaeal and bacterial ASVs showed a strong prediction for

hull type (construction material) in with macro-average AUC = 0.98 for archaea (Fig. S6b) and macro-average AUC = 0.99 for bacteria (Fig. S7b).

Candidatus Nitrosopumilus was a strong predictor for every distance group (Fig 4a). Closest to shipwrecks (2-9m group) ASVs from uncultured Bathyarchaeia and Lokiarchaeia were the most important features driving the prediction. From 2 to 200 m, ASVs from Asgardaeota, Cranarchaeota, and Euryarchaeota were all considered important features in this model. We observed ASVs that were only considered strong predictors at distances from the shipwrecks within 200-300 m. These included Nanoarchaeota; Woesarchaeia and DSEG, Euryarchaeota; Methanomicrobia and Thermoplasmata; *Candidatus Nitrososphaera* from Thaumarchaeota, Nanoarchaeota; Nanohaloarchaeia, Hadesarchaeota, and Hydrothermaeota (Fig. 4a). The observed enrichment of important ASVs predicting proximity to shipwrecks between 2 and 200 m is consistent with the results obtained from the core microbiome analysis.

Bacterial ASVs strongly predicting close proximity to shipwrecks included those affiliated with Alphaproteobacteria; Rhodovibrionales; Kloniellaceae, Gammaproteobacteria; Sterodobacterales; Woeseiaceae, Bacteroidetes; Cytophagales, and Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae. ASVs from the Deltaproteobacteria, including NB1-j taxa were important features in all distance groups. This result was consistent with the core microbiome analysis. Like archaea, there were more important bacterial ASVs predicting proximity to shipwrecks in distance groups spanning 2 to 150 m away from the sites. The observed importance of Chlamydiae,

Chloroflexi; Dehalococcoidia, Dependuntiae; Babeliae, and Bacteroidetes; Ignavibacteria decreased after 150 m.

Discussion

The goal of this study was to understand how built features shape biodiversity in the surrounding environment and determine if benthic microbiomes respond to built habitats in predictable ways. Predictable, in this study means alpha diversity declines as a function of distance from the sites, as was observed in a prior case study for bacteria (Hamdan et al., 2021) and that ASVs are routinely associated with features. This information can inform on the impacts of 'ocean sprawl' (Bugnot et al., 2021; Duarte et al., 2013) in shelf and slope environments, a topic of particular importance in the Gulf of Mexico, given the abundance of operational built habitats and extant shipwrecks and decommissioned oil rigs on the shelf and slope. Using shipwrecks as model island-like systems (Hamdan et al., 2021), we show deep-sea shipwrecks modify archaeal and bacterial sediment microbiomes in a uniform manner. This was revealed by elevated microbial diversity in proximity to the sites and a distinct bacterial and archaeal core microbiome.

Unlike our earlier effort (Hamdan et al., 2021) which focused only on one shipwreck, *Anona* (included in this study), one domain, the current study includes 1432 samples from 9 shipwrecks of different hull types, sizes, at various water depths (144m to 1950m). In the previous study, we observed a significant relationship between bacterial alpha diversity and proximity to the shipwreck *Anona*. A significant relationship was

observed here, even when samples were derived from different water depths, a significant structuring factor on microbiomes. Hull material (steel vs. wood) was included in PERMANOVA analyses and was a structuring factor for bacteria and archaea (Table S2) suggesting the physical or chemical composition of the shipwrecks may select for different taxa.

The relationship between proximity and alpha diversity in sediments surrounding the shipwrecks was more pronounced for archaea than bacteria (Fig. 2). Archaeal communities formed a highly uniform core microbiome at all locations, regardless of environmental context (depth and hull type). Nearest to the shipwrecks, the core archaeal microbiome was more diverse and composed of heterotrophic taxa including Crenarchaeota; Bathyarchaeia and Asgardaeota; Lokiarchaeia and Euryarchaeota. The high contribution to the core microbiome of these taxa extended 200 m onto the seafloor, but declined at greater distances, replaced by seemingly ubiquitous Thaumarchaeota. This suggests a potential metabolic shift in communities surrounding the built habitat from dominant heterotrophy to chemolithoautotrophy. Bacterial core microbiome analysis also reveals increased core member diversity within 200 m of the sites. For bacteria, however, there was more variability in core microbiome membership among sites. Some ASVs, such as Bacteroidetes; Cytophagales were found in sediments closest to the shipwrecks at all sites, but with greater stochasticity near and distant from the wrecks, potentially due to local environmental conditions. The delivery of organic matter from riverine sources in particular may be a key determinant on what bacteria are found in near vs. offshore locations (Hempel et al., 2022).

Supervised learning was used to predict proximity to built features based on microbiome composition with strongest predictions based on archaeal ASVs at transect end members. These results suggest archaeal microbiomes surrounding built environments are distinguishable from background, respond to built habitats in a uniform, predictable way, and potentially could be a tool in detecting the influence of built habitats on seafloor ecosystems. Moreover, these data present a case that regionally dispersed core prokaryotic taxa characterize shipwreck influenced sediments differently than the surrounding seabed. The prediction of hull type was more accurate for bacteria than archaea potentially indicating built structure heterogeneity (age, size, hull material) may be more selective for bacteria than archaea. Likewise, as noted above, organic matter supply may differentially select core taxa from different domains. In deep sea, shipwrecks and associated biota may be the source of organic matter to the surrounding sediment core microbiome and sustain the microbial diversity (Hampel et al., 2022). Exploring differences between the domains may be critical to evaluating how built features interact with seafloor microbial biogeography.

The processes that formed the seabed carved canyons and escarpments provided the physical and chemical setting for hydrocarbon seeps, vents, and brine pools to form. These geological features are joined by wood and other organic falls, coral reefs, and built structures. This has resulted in establishment of a heterogeneous seafloor containing features that offer specialized habitats. Habitat heterogeneity leads to specialized niches or island-like systems (Ramette & Tiedje, 2007). Natural habitat

features have been a focus of marine microbial biogeography studies of the shelf and slope for decades. The appearance of routinely associated taxa that differ from the surrounding sediment (Dick, 2019; Inagaki et al., 2006; Ruff et al., 2015) with specific functional attributes has been observed in hydrothermal vent and seep settings. In the Guaymas Basin, a core microbiome was attributed with the presence of cold seeps and hydrothermally vented sediments, resulting in highly specific, adapted and predictable communities (Cruaud et al., 2017). Similarly, globally dispersed methane seeps contain similar key taxa again pointing to a predictable composition of natural seabed habitats (Hamdan et al., 2013; Ruff et al., 2015). In another example, an analysis of deep-sea *Lopheila pertusa* reefs in the western Atlantic revealed highly conserved core microbiomes across regions, with geographically conserved symbiotic taxa observed (Kellogg et al., 2017).

It is accepted that natural features form islands of biodiversity on the expansive seabed and attract genetically specialized populations (Schulze et al., 2020). It is also known that evolutionary processes, histories, and selective pressures drive diversification between site (Dombrowski et al., 2018; Moulana et al., 2020), and support biodiversity. Host association is also a key element differentiating microbiomes in habitats (Thompson et al., 2017). Based on current knowledge, natural features shape seafloor microbiomes in expected ways. The biogeographic patterns observed in aquatic and terrestrial environments are driven by a combination of physical, chemical and biological attributes (Hanson et al., 2019). Large scale meta-analyses show that natural systems exhibit predictability such that sample provenance can be attributed based on the

microbiome (Thompson et al., 2017). Our study is the first to show similar selective process occur at built habitat features. Unlike natural features, however, that have been present for decadal to geological time scales, built marine habitats are emerging at rapid rates, potentially altering dispersal between habitats. While the remains of historic shipwrecks can span the last 500 years, the majority of historic ones are 100 – 200 years old (Damour et al., 2016), along with an unknown number of modern wrecks. It is important to consider these in context with other infrastructure. Since the 1980's oil and gas infrastructure on the U.S. continental shelf and slope has expanded logarithmically (Bugnot et al., 2021). While the shipwrecks under study here have similar effects on microbial diversity and community structure as natural features, the progressive addition of potentially 8000 more energy platforms in the Gulf of Mexico by 2028 (Bugnot et al., 2021) could be impactful to the whole region. Ecological connectivity and built features have great potential to collide. It is unclear if the progressive addition of features will facilitate or impede movement of organisms, materials and energy across the seafloor (Bishop et al., 2017). Also, the connectivity of the seabed, if natural habitats are observed agnostic to the presence of built habitats, will remain unconstrained in the Anthropocene. This may represent an astonishing knowledge gap that impacts baseline studies across the shelf and slope. With the new knowledge from this study, that built features select specific microbiome members predictably, similar to natural analogs, we can begin to ask if they are bridges or barriers to genetic diversity, now and in the future when artificial features meet or exceed the number of natural habitats.

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Competing Interests

The authors declare no competing financial interests in relation to this work.

Data accessibility: Sequences for *Anona* (2015–2018) and *Alcoa Puritan* are provided under NCBI Bioproject Number PRJNA612314. Sequences for *Halo*, *Viosca Knoll*, *Ewing Bank*, *Mica*, *Mardi Gras*, and 2014 samples for *Anona* are found under NCBI Bioproject Number PRJNA401282. Sequences for 15711 and 15470 shipwrecks are available under NCBI Bioproject Number PRJNA599410.

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Reference:

- Amon, D. J., Kennedy, B. R. C., Cantwell, K., Suhre, K., Glickson, D., Shank, T. M., & Rotjan, R. D. (2020). Deep-Sea Debris in the Central and Western Pacific Ocean. *Frontiers in Marine Science*, 7(May), 1–15. <https://doi.org/10.3389/fmars.2020.00369>
- Bienhold, C., Pop Ristova, P., Wenzhöfer, F., Dittmar, T., & Boetius, A. (2013). How Deep-Sea Wood Falls Sustain Chemosynthetic Life. *PLoS ONE*, 8(1), 10–15. <https://doi.org/10.1371/journal.pone.0053590>
- Bishop, M. J., Mayer-Pinto, M., Airoidi, L., Firth, L. B., Morris, R. L., Loke, L. H. L., Hawkins, S. J., Naylor, L. A., Coleman, R. A., Chee, S. Y., & Dafforn, K. A. (2017). Effects of ocean sprawl on ecological connectivity: impacts and solutions. *Journal of Experimental Marine Biology and Ecology*, 492, 7–30. <https://doi.org/10.1016/j.jembe.2017.01.021>
- Björdal, C. G. (2012). Evaluation of microbial degradation of shipwrecks in the Baltic Sea. *International Biodeterioration and Biodegradation*, 70, 126–140. <https://doi.org/10.1016/j.ibiod.2012.01.012>
- Bokulich, N., Dillon, M., Bolyen, E., Kaehler, B., Huttley, G., & Caporaso, J. (2018). q2-sample-classifier: machine-learning tools for microbiome classification and regression. *Journal of Open Source Software*, 3(30), 934. <https://doi.org/10.21105/joss.00934>
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Bugnot, A. B., Mayer-Pinto, M., Airoidi, L., Heery, E. C., Johnston, E. L., Critchley, L. P., Strain, E. M. A., Morris, R. L., Loke, L. H. L., Bishop, M. J., Sheehan, E. V., Coleman, R. A., & Dafforn, K. A. (2021). Current and projected global extent of marine built structures. *Nature Sustainability*, 4(1), 33–41. <https://doi.org/10.1038/s41893-020-00595-1>
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>
- Comeau, A. M., Li, W. K. W., Tremblay, J. É., Carmack, E. C., & Lovejoy, C. (2011). Arctic ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE*, 6(11). <https://doi.org/10.1371/journal.pone.0027492>
- Cruaud, P., Vigneron, A., Pignet, P., Caprais, J. C., Lesongeur, F., Toffin, L., Godfroy, A., & Cambon-Bonavita, M. A. (2017). Comparative study of Guaymas Basin microbiomes: Cold seeps vs. hydrothermal vents sediments. *Frontiers in Marine Science*, 4(DEC), 1–15. <https://doi.org/10.3389/fmars.2017.00417>
- Czub, M., Kotwicki, L., Lang, T., Sanderson, H., Klusek, Z., Grabowski, M., Szubska, M., Jakacki, J., Andrzejewski, J., Rak, D., & Bełdowski, J. (2018). Deep sea habitats in the chemical warfare dumping areas of the Baltic Sea. *Science of the Total Environment*, 616–617, 1485–1497.

- <https://doi.org/10.1016/j.scitotenv.2017.10.165>
- Damour, M., Church, R., Warren, D., Horrell, C., & Hamdan, L. (2015). Gulf of Mexico Shipwreck Corrosion , Hydrocarbon Exposure , Microbiology , and Archaeology (GOM-SCHEMA) Project: Studying the Effects of a Major Oil Spill on Submerged Cultural Resources. *Underwater Archaeology Proceedings of the 2015 Society for Historical Archaeology Conference*, 49, 6–11.
- Danovaro, R., Snelgrove, P. V. R., & Tyler, P. (2014). Challenging the paradigms of deep-sea ecology. *Trends in Ecology and Evolution*, 29(8), 465–475. <https://doi.org/10.1016/j.tree.2014.06.002>
- Dick, G. J. (2019). The microbiomes of deep-sea hydrothermal vents: distributed globally, shaped locally. *Nature Reviews Microbiology*, 17(5), 271–283. <https://doi.org/10.1038/s41579-019-0160-2>
- Dombrowski, N., Teske, A. P., & Baker, B. J. (2018). Expansive microbial metabolic versatility and biodiversity in dynamic Guaymas Basin hydrothermal sediments. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-07418-0>
- Duarte, C. M., Pitt, K. A., Lucas, C. H., Purcell, J. E., Uye, S., Robinson, K., Brotz, L., Decker, M. B., Sutherland, K. R., Malej, A., Madin, L., Mianzan, H., Gili, J.-M., Fuentes, V., Atienza, D., Pagés, F., Breitburg, D., Malek, J., Graham, W. M., & Condon, R. H. (2013). Is global ocean sprawl a cause of jellyfish blooms? *Frontiers in Ecology and the Environment*, 11(2), 91–97. <https://doi.org/10.1890/110246>
- Firth, L. B., Knights, A. M., Bridger, D., Evans, A. J., Mieszkowska, N., Moore, P. J., O’connor, N. E., Sheehan, E. V., Thompson, R. C., & Hawkins, S. J. (2016). Ocean sprawl: Challenges and opportunities for biodiversity management in a changing world. *Oceanography and Marine Biology: An Annual Review*, 54(January 2017), 193–269. <https://doi.org/10.1201/9781315368597>
- Goffredi, S. K., & Orphan, V. J. (2010). Bacterial community shifts in taxa and diversity in response to localized organic loading in the deep sea. *Environmental Microbiology*, 12(2), 344–363. <https://doi.org/10.1111/j.1462-2920.2009.02072.x>
- Hamdan, L. J., Coffin, R. B., Sikaroodi, M., Greinert, J., Treude, T., & Gillevet, P. M. (2013). Ocean currents shape the microbiome of Arctic marine sediments. *The ISME Journal*, 7(4), 685–696. <https://doi.org/10.1038/ismej.2012.143>
- Hamdan, L. J., Hampel, J. J., Moseley, R. D., Mugge, R. L., Ray, A., Salerno, J. L., & Damour, M. (2021). Deep-sea shipwrecks represent island-like ecosystems for marine microbiomes. *The ISME Journal*. 15, 2883–2891 <https://doi.org/10.1038/s41396-021-00978-y>
- Hamdan, L. J., Salerno, J. L., Reed, A., Joye, S. B., & Damour, M. (2018). The impact of the Deepwater Horizon blowout on historic shipwreck-associated sediment microbiomes in the northern Gulf of Mexico. *Scientific Reports*, 8(1), 1–14. <https://doi.org/10.1038/s41598-018-27350-z>
- Hampel, J. J., Moseley, R. D., Mugge, R. L., Ray, A., Jones, D., Damour, M., Hamdan, L. J. Deep-sea wooden shipwrecks influence sediment microbiome diversity. *Limnology and Oceanography*, 67(2), 482–497. <https://doi.org/10.1002/lno.12008>
- Hanson, C. A., Müller, A. L., Loy, A., Dona, C., Appel, R., Jørgensen, B. B., & Hubert, C. R. J. (2019). Historical factors associated with past environments influence the biogeography of thermophilic endospores in arctic marine sediments. *Frontiers in Microbiology*, 10(FEB), 1–14. <https://doi.org/10.3389/fmicb.2019.00245>

- Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., Nealson, K. H., Horikoshi, K., D'Hondt, S., & Jørgensen, B. B. (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proceedings of the National Academy of Sciences of the United States of America*, 103(8), 2815–2820. <https://doi.org/10.1073/pnas.0511033103>
- Kassambara, A. (2018) ggpubr:"ggplot2" Based Publication Ready Plots. <https://CRAN.R-project.org/package=ggpubr>
- Kellogg, C. A., Goldsmith, D. B., & Gray, M. A. (2017). Biogeographic comparison of Lophelia-associated bacterial communities in the western atlantic reveals conserved core microbiome. *Frontiers in Microbiology*, 8, 1–15. <https://doi.org/10.3389/fmicb.2017.00796>
- Koutsi, D. and Stratigea, A. (2021). Shipwrecks' Underwater Mysteries—Identifying Commonalities Out of Globally-Distributed Knowledge. *Heritage*, 4, 3949-3969.
- Levin, L. A., Bett, B. J., Gates, A. R., Heimbach, P., Howe, B. M., Janssen, F., McCurdy, A., Ruhl, H. A., Snelgrove, P., Stocks, K. I., Bailey, D., Baumann-Pickering, S., Beaverson, C., Benfield, M. C., Booth, D. J., Carreiro-Silva, M., Colaço, A., Eblé, M. C., Fowler, A. M., ... Weller, R. A. (2019). Global Observing Needs in the Deep Ocean. *Frontiers in Marine Science*, 6(May), 1–32. <https://doi.org/10.3389/fmars.2019.00241>
- Macreadie, P. I., Fowler, A. M., & Booth, D. J. (2011). Rigs-to-reefs: Will the deep sea benefit from artificial habitat? *Frontiers in Ecology and the Environment*, 9(8), 455–461. <https://doi.org/10.1890/100112>
- Moulana, A., Anderson, R. E., Fortunato, C. S., & Huber, J. A. (2020). Selection is a significant driver of gene gain and loss in the pangenome of the bacterial genus *Sulfurovum* in geographically distinct deep-sea hydrothermal vents. *MSystems*, 5(2), 1–18.
- Oksanen, F.J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et. al. (2017) Vegan: Community Ecology Package. R package Version 2.4-3. <https://CRAN.R-project.org/package=vegan>
- Orcutt, B.N., Sylvan, J.B., Knab, N.J. & Edwards, K.J. (2011). Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiology and Molecular Biology Reviews*, 75, 361-422.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41, D590-D596.
- Ramette, A., & Tiedje, J. M. (2007). Biogeography: An emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microbial Ecology*, 53(2), 197–207. <https://doi.org/10.1007/s00248-005-5010-2>
- Riehl, T., Wöflf, A. C., Augustin, N., Devey, C. W., & Brandt, A. (2020). Discovery of widely available abyssal rock patches reveals overlooked habitat type and prompts rethinking deep-sea biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, 117(27), 15450–15459. <https://doi.org/10.1073/pnas.1920706117>
- Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584.

- Ruff, S. E., Biddle, J. F., Tesked, A. P., Knittel, K., Boetius, A., Ramette, A., Teske, A. P., Knittel, K., Boetius, A., & Ramette, A. (2015). Global dispersion and local diversification of the methane seep microbiome. *Proceedings of the National Academy of Sciences*, 112(13), 4015–4020. <https://doi.org/10.1073/pnas.1421865112>
- Sammarco, P. W., Atchison, A. D., & Boland, G. S. (2004). Expansion of coral communities within the Northern Gulf of Mexico via offshore oil and gas platforms. *Marine Ecology Progress Series*, 280, 129–143. <https://doi.org/10.3354/meps280129>
- Schulze, A., Erdner, D. L., Grimes, C. J., Holstein, D. M., & Miglietta, M. P. (2020). Artificial Reefs in the Northern Gulf of Mexico: Community Ecology Amid the “Ocean Sprawl.” *Frontiers in Marine Science*, 7, 447. <https://doi.org/10.3389/fmars.2020.00447>
- Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: The hunt for a core microbiome. *Environmental Microbiology*, 14(1), 4–12. <https://doi.org/10.1111/j.1462-2920.2011.02585.x>
- Shannon, CE (1948) The mathematical theory of communication. *Bell System Technical Journal* 27, 379-423
- Svane, I., & Petersen, J. K. (2001). On the problems of epibioses, fouling and artificial reefs, a review. *Marine Ecology*, 22(3), 169–188. <https://doi.org/10.1046/j.1439-0485.2001.01729.x>
- Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Xu, Z. Z., Jiang, L., ... Zhao, H. (2017). A communal catalogue reveals Earth’s multiscale microbial diversity. *Nature*, 551(7681), 457–463. <https://doi.org/10.1038/nature24621>
- UNESCO. (2008). Convention on the Protection of the Underwater Cultural Heritage will enter into force in January 2009. <http://www.unesco.org/new/en/culture/themes/underwater-cultural-heritage/underwater-cultural-heritage/wrecks/>
- Walker, S. J., Schlacher, T. A., & Schlacher-Hoenlinger, M. A. (2007). Spatial heterogeneity of epibenthos on artificial reefs: Fouling communities in the early stages of colonization on an East Australian shipwreck. *Marine Ecology*, 28(4), 435–445. <https://doi.org/10.1111/j.1439-0485.2007.00193.x>
- Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag
- Zeppilli, D., Pusceddu, A., Trincardi, F., & Danovaro, R. (2016). Seafloor heterogeneity influences the biodiversity-ecosystem functioning relationships in the deep sea. *Scientific Reports*, 6(April), 1–12. <https://doi.org/10.1038/srep26352>
- Zinger, L., Boetius, A., Ramette, A. (2014). Bacterial taxa-area and distance-decay relationships in marine environments. *Molecular Ecology*, 23, 954-964. <https://doi.org/10.1111/mec.12640>

Figures and Tables

Figure 1.

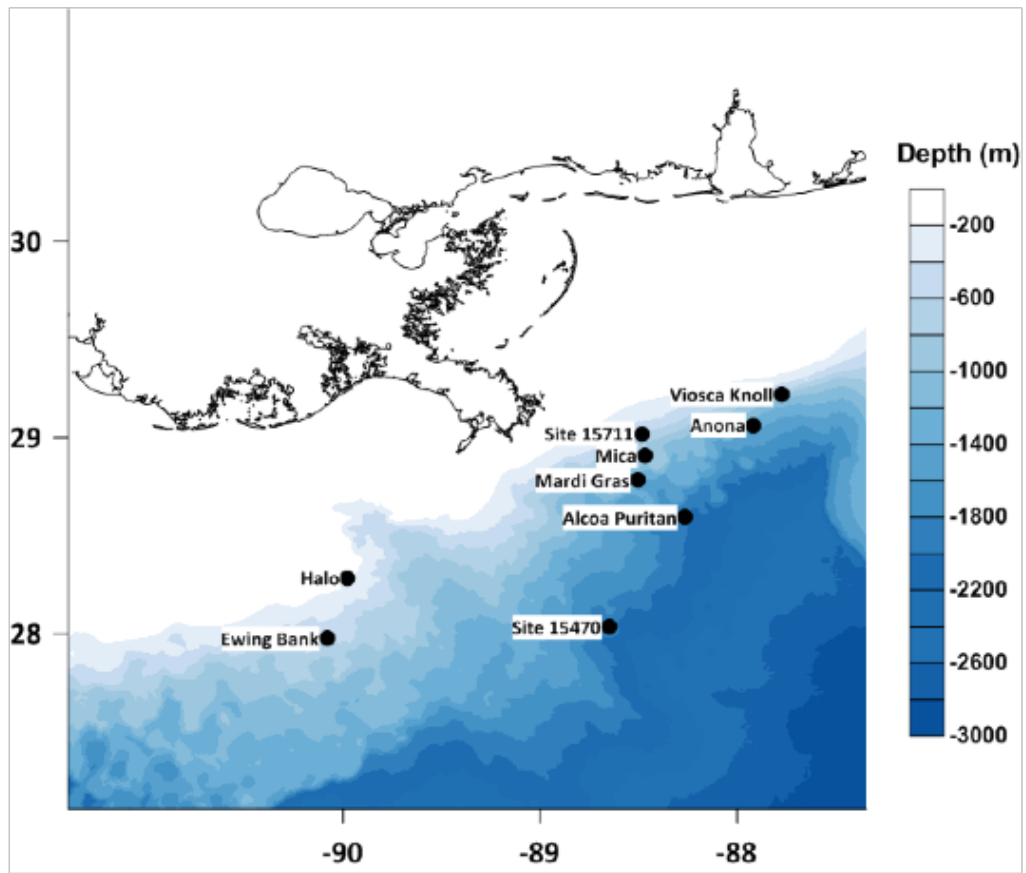


Figure 1. Map of historic shipwrecks sampled in the northern Gulf of Mexico

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Figure 2.

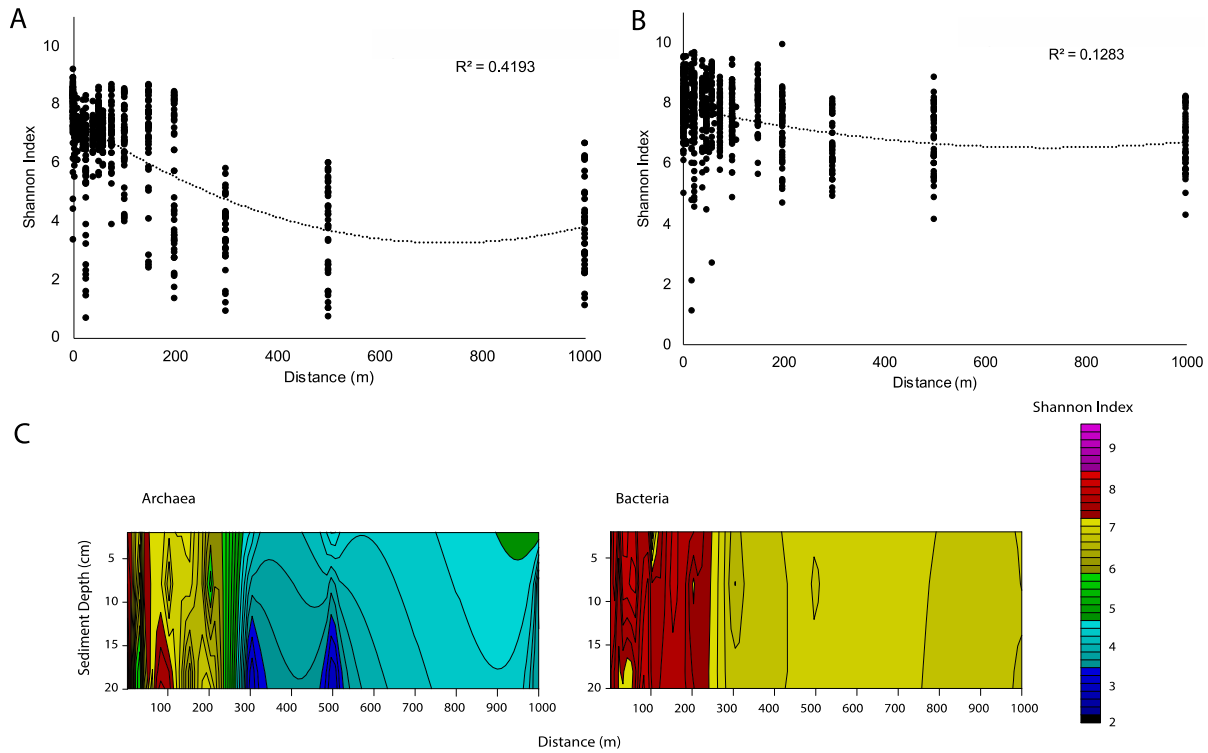


Figure 2. Shannon Index diversity–distance plots for archaea (A) and bacteria (B) with polynomial regression. Spatial analysis of archaeal ($n = 650$) and bacterial ($n = 762$) diversity with distance and sediment depth for all shipwreck samples (C). Contours constructed from data from four transects aggregated on one axis. Grid created with the Kriging method applied to observed data

Figure 3.

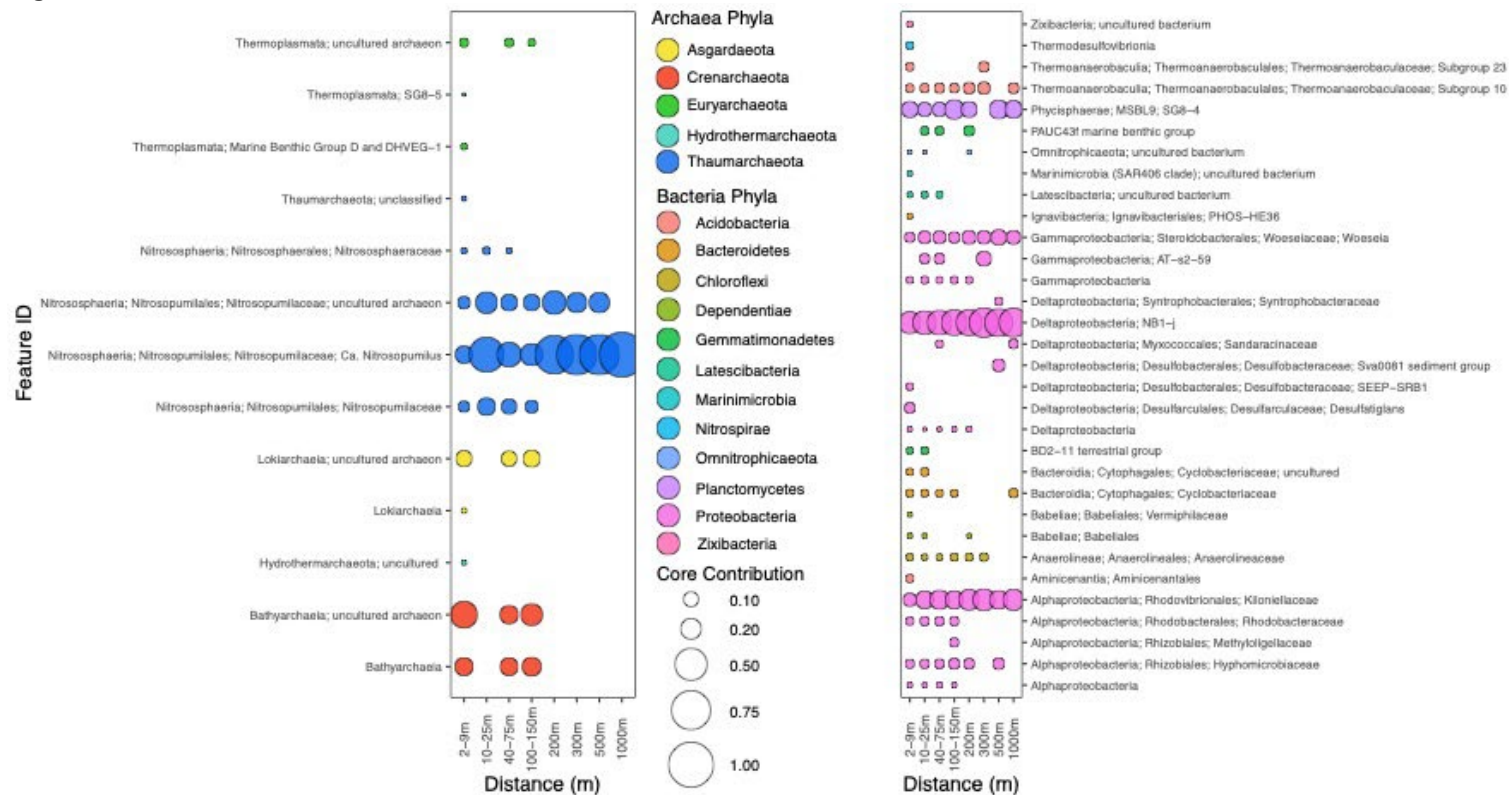


Figure 3. Core microbiome in sediments surrounding shipwrecks for archaea (left) and bacteria (right). Membership to the core was determined by presence in 80% of the samples.

Figure 4.

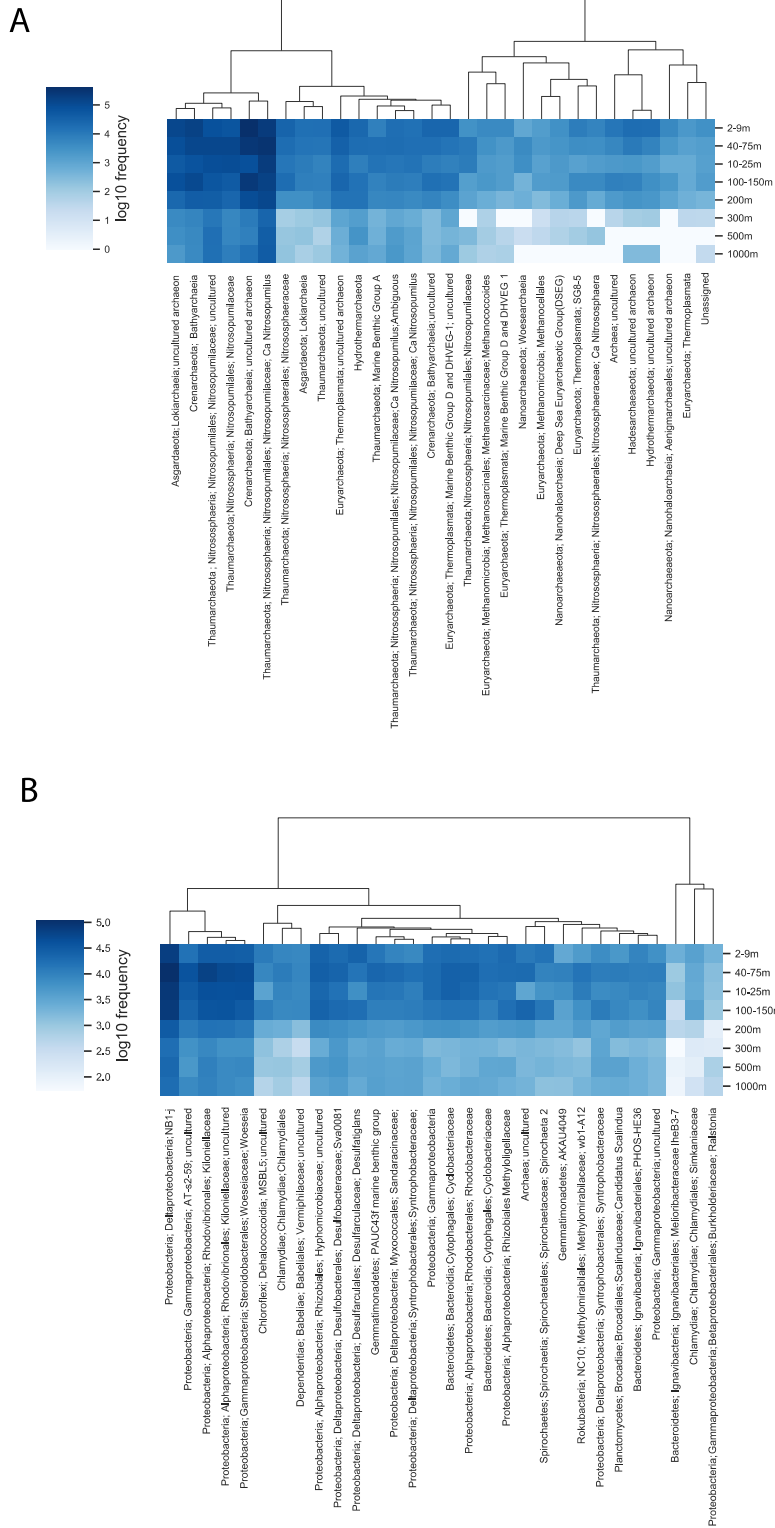
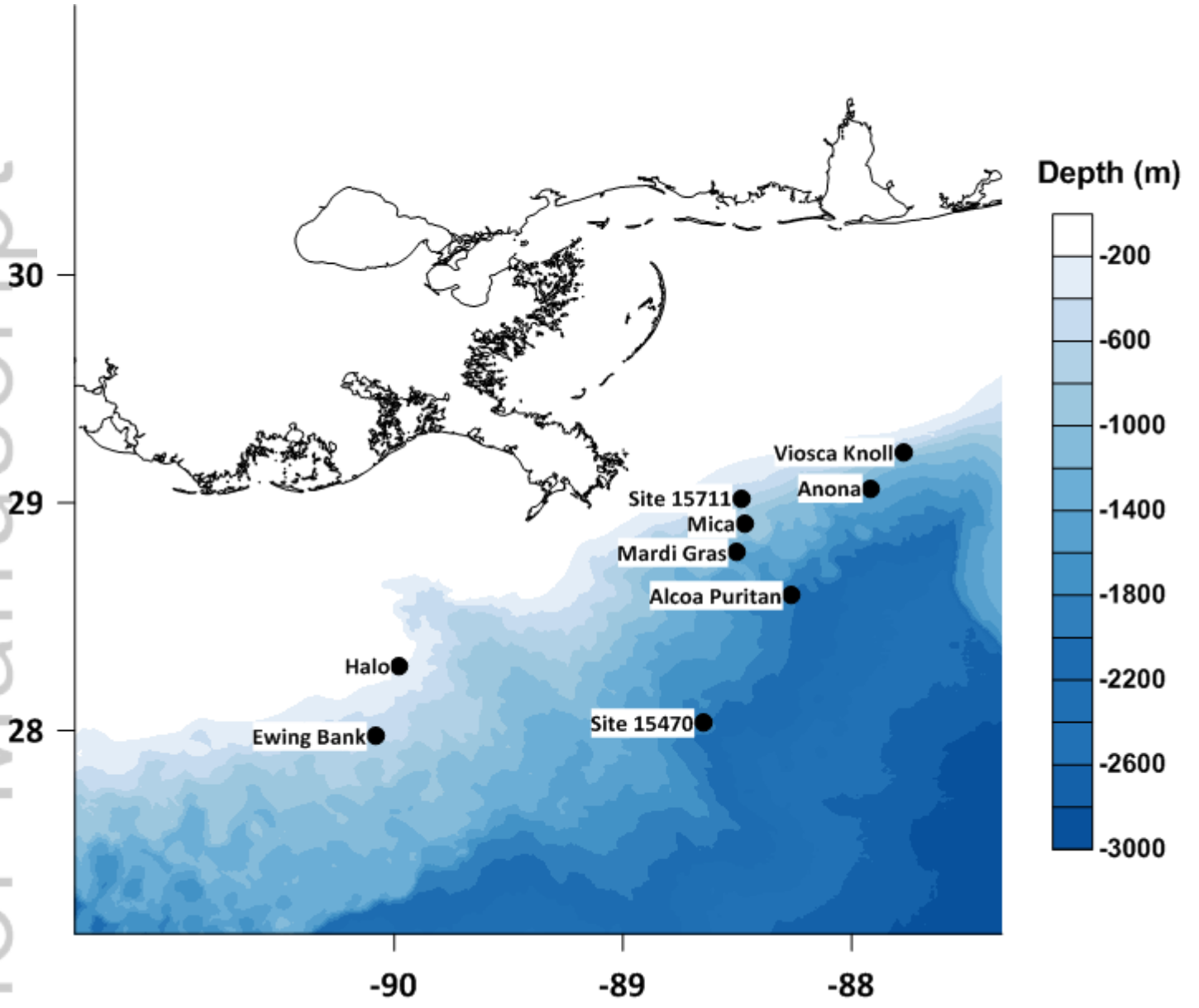
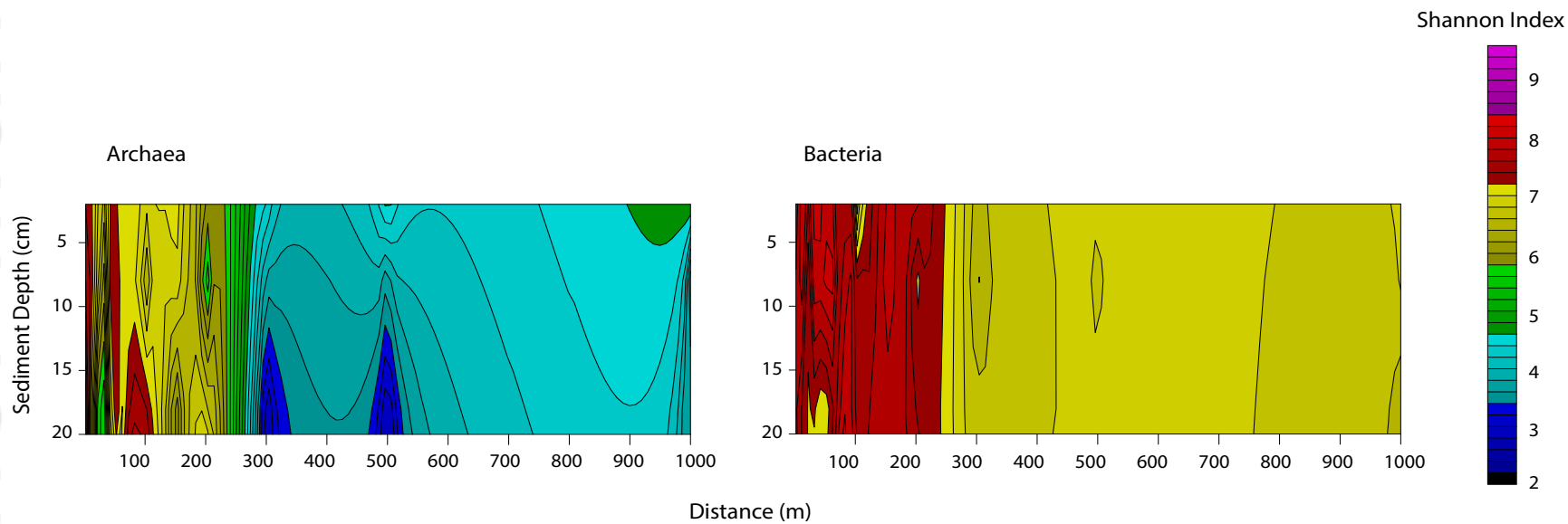
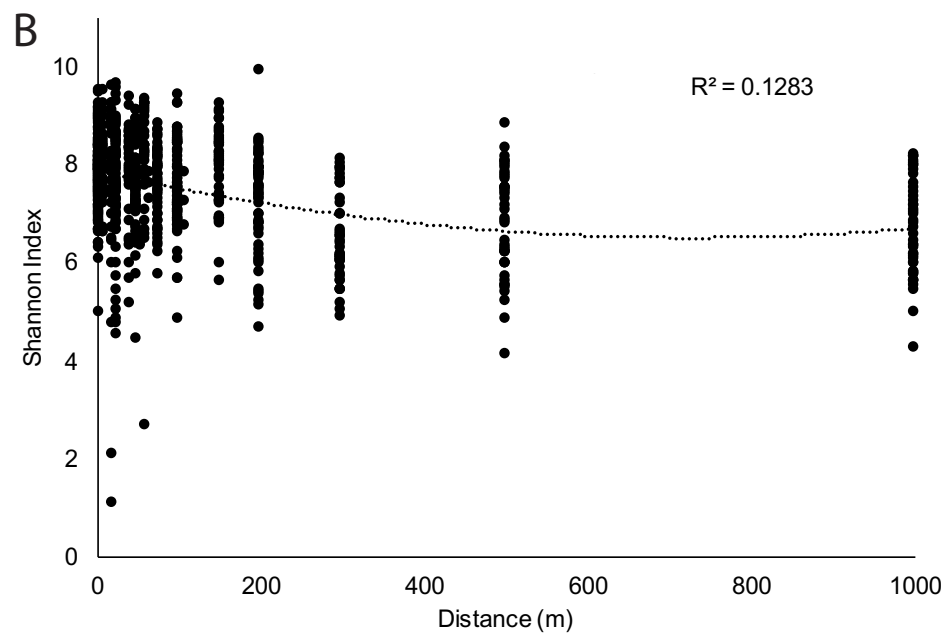
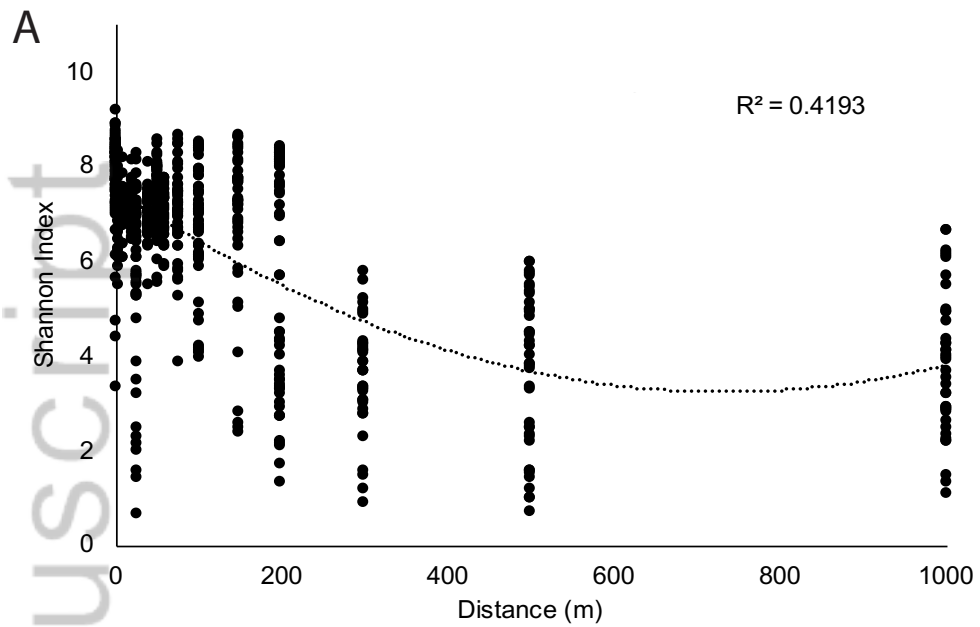
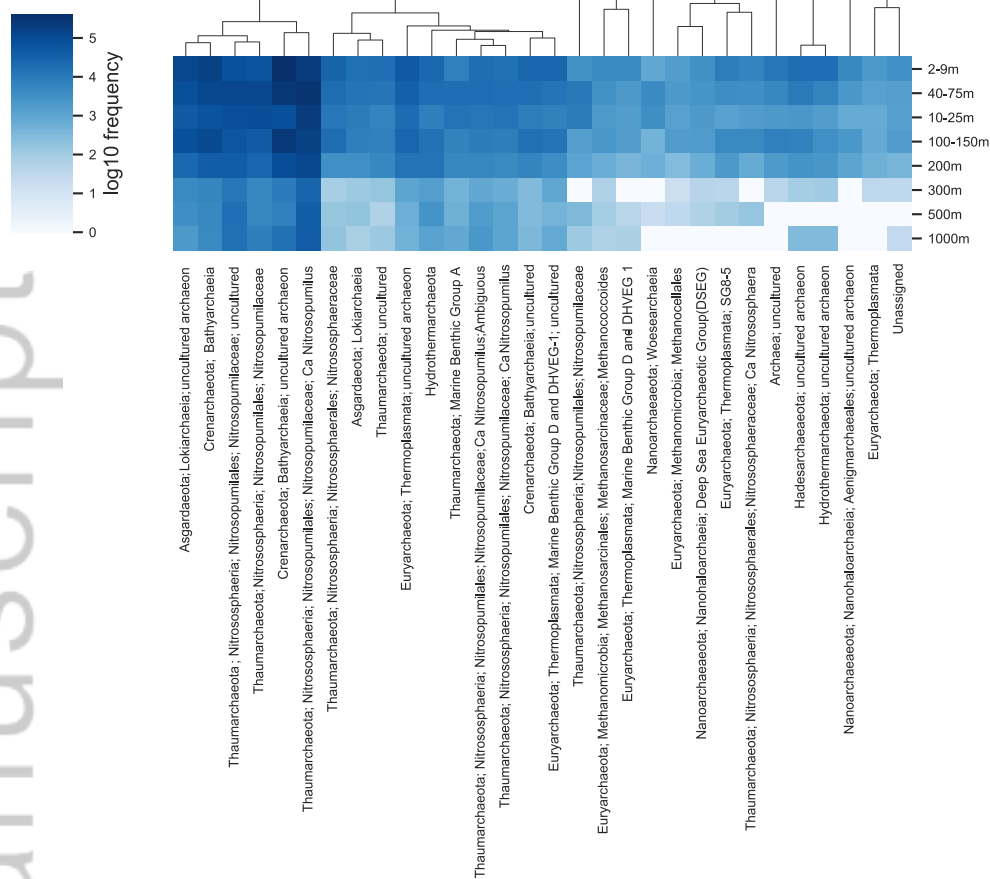


Figure 4. Abundance heatmap of the most important taxa in each distance group derived from sample-classifier Random Forest model for archaea (A) and bacteria (B). Distance groups are out of order as a result of the predicted output from the model.





A



B

