GREAT AUSTRALIAN BIGHT
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Spatial distribution and diversity in hydrocarbon degrading microbes in the Great Australian Bight II: Analysis of bacterial and archaeal community structure

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GREAT AUSTRALIAN BIGHT RESEARCH PROGRAM

The Great Australian Bight Research Program is a collaboration between BP, CSIRO, the South Australian Research and Development Institute (SARDI), the University of Adelaide, and Flinders University. The Program aims to provide a whole-of-system understanding of the environmental, economic and social values of the region; providing an information source for all to use.

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Acknowledgments

Funding for this study was provided by the Great Australian Bight Research Program - a collaboration between BP, CSIRO, the South Australian Research and Development Institute (SARDI), the University of Adelaide, and Flinders University. The Program aims to provide a whole-of-system understanding of the environmental, economic and social values of the region; providing an information source for all to use. The crew and scientists, especially Mark Green, Mark Lewis, Matt Sherlock, Jeff Cordell and Michael Watson on board the *RV Southern Surveyor* are thanked for obtaining samples. Franzis Althaus is thanked for her assistance with figures.

Executive summary

Following an oil spill, most of the oil released into the environment is metabolised by bacteria and other microorganisms. Knowing the relationship of indigenous bacteria within the Great Australian Bight to those known to degrade hydrocarbons elsewhere will aid in the assessment of, and response to, an unplanned release of hydrocarbons in the environment. To this end, we analysed the community composition of Bacteria and Archaea in sediment samples collected from sites within the Great Australian Bight. We found a diverse community of bacteria in all samples. These included some bacteria related to those with a known capacity to degrade hydrocarbons and that have been shown to increase in abundance in response to previous spills in other locations. By contrast, the Archaea were less diverse, and seem to be dominated by fewer operational taxonomic units. There was a very strong relationship between the structure of the bacterial assemblage present in a sample and that of the archaeal assemblage. The sediment and water samples had different microbial communities, and the sediment samples were clustered more tightly, indicating a higher degree of relatedness. The microbial communities in sediment samples collected from the continental shelf (200 and 400 m) differed from those collected from the continental slope (depths greater than 1000 m). For the benthic samples, diversity and species evenness decreased with depth. In general, community composition related best to depth and sediment grain size in benthic samples and to depth and nutrients in pelagic samples. This report provides important information about the baseline indigenous microbial communities present in areas of the Great Australian Bight without a hydrocarbon source.

1 Introduction

Most oil that is released into the environment is ultimately degraded by microorganisms (Head et al. 2006). For example, following the recent *Deepwater Horizon* oil spill, marine bacteria were found to respond very quickly to the spill, with known hydrocarbon degrading bacteria significantly more abundant inside the plume than outside it (Hazen et al. 2010). It is estimated that approximately half of the oil that was released during the spill was metabolised by microorganisms (Joye 2015). The Gulf of Mexico has high natural inputs of petroleum hydrocarbons from seeps, and as a consequence, a high abundance of hydrocarbon degrading bacteria (Kappell et al. 2014). However, hydrocarbon degrading microorganisms are widespread in the environment, normally making up 1% of the microbial population in pristine environments, increasing to 10% in environments with a petroleum hydrocarbon source (Atlas 1995).

Since oil is a complex mixture of more than 10,000 different chemical compounds, different microorganisms have developed the ability to degrade different constituents of oil over evolutionary time (Head et al. 2006). Numerous bacteria can degrade hydrocarbons – oil degrading bacteria have been isolated from the α, β, δ, and γ Proteobacteria and the Firmicutes (Head et al. 2006). Alkanes, or linear arrangements of carbon and hydrogen, are the most labile fraction of oil. Alkane degraders, such as *Alcanivorax,* Oceanospirillaceae*, Pseudomonas* and *Marinobacter*, are typically the fastest responding microorganisms to a new source of oil. Polycyclic aromatic hydrocarbons (PAH) are also readily degraded by microorganisms, and bacteria such as *Colwellia* and *Cycloclasticus* are typically abundant in areas with aromatic hydrocarbons present (Dubinsky et al. 2013, Kleindienst et al. 2015). Gas is also associated with the breakdown of some petroleum products and may be discharged from petroleum wells. Microorganisms such as *Methylomonas* oxidise methane to carbon dioxide in oxic environments. Following the *Deepwater Horizon* wellhead blowout, a "phylochip" array was used to measure changes in microbial species abundance. Although all taxa were active in the hydrocarbon enriched plume throughout the response, changes in relative abundance matched patterns that would be predicted by changes in the hydrocarbon chemistry (Dubinsky et al. 2013).

Since the existing microbial community structure likely controls the rate at which an ecosystem can respond to an oil spill (Kostka et al. 2014) our overall goal is to be able to predict the capacity of indigenous organisms in the Great Australian Bight (GAB) to degrade oil – both as discharged as part of routine operations, and in case of an oil spill. The presence of a high abundance and diversity of microbial oil degraders may also point to the natural presence of oil in the system, and provide pointers to the regions prospectivity. To that end, we characterized the prokaryotic diversity using next generation 16S rDNA gene sequencing. We used the 16S gene sequences to identify the proportion of the community that were known or expected oil degraders, and how they are distributed in both the sediment and the water column prior to the commencement of drilling by BP. Differences in the community structure are related to available environmental parameters, such as depth, sediment grain size and temperature. In a complementary report, we examine the presence of specific gene sequences encoding enzymes that are involved in key steps in conserved pathways for the degradation of different fractions of oil from the same samples (Hook et al. 2016).

2 Materials and Methods

2.1 Sample collection

Sediment samples were collected from the surface of cores collected at the stations shown in Figure 1 below, taken during the *Southern Surveyor* cruise, 3-22 April 2013, and a second cruise from 30 November to 21 December 2015 conducted on the *RV Investigator.* A 6-core multicorer from KC (Denmark) was incorporated into an instrumented coring platform (ICP) that could be controlled from the vessel and allowed reliable collection of sediment samples at depths between 200 and 3000 m (Sherlock et al. 2014). Triplicate sediment cores from each deployment were subsampled for microbial analysis using 30mm diameter minicores. The top 2 cm of each minicore was extruded, placed into a DNA free tube, and frozen until they were transferred to CSIRO laboratories for DNA extraction. A fourth core from each deployment was sampled for sediment grain size and carbon content. Water samples were collected at maximum depth at each Station using a Niskin bottle on the ICP (located ~ 1m off bottom). Microbial cells were collected by filtration of 2 L seawater through a 0.22 µm pore Sterivex™ GP filter (Millipore®, Massachusetts. Cat. # SVGPL10RC), using a 6 channel peristaltic pump. Pump tubing was rinsed with ~200ml seawater from the appropriate depth prior to cell collection. Pumping continued for 1 min after the sample had cleared the filter to dry. Both ends of the filter were capped, placed in individual snap-lock bags and stored at -80**°.**

Near bottom nutrients (nitrate, nitrite, ammonia, phosphate and silicate), as well as temperature, salinity and oxygen levels, were obtained from a separate CTD cast taken at each station.

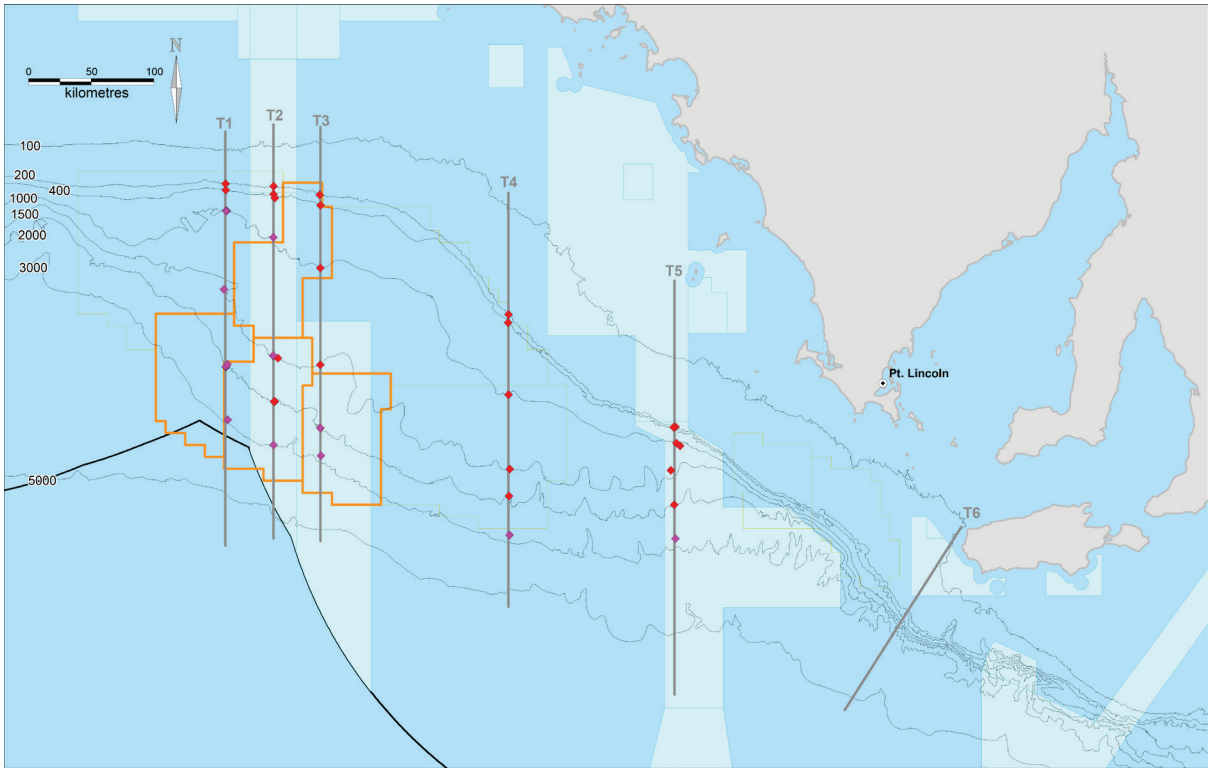


Figure 1. Transects and sampling locations used in the 2013 and 2015 cruises. Sampling locations used for development of the functional assays are shown in red. BP's lease blocks are shown in orange, and the light blue shading indicates Commonwealth marine reserves.

2.2 DNA extractions

Sediment

Ten grams of sediment were used for each DNA extraction. DNA was extracted using the PowerMax® Soil DNA Isolation Kit (Mo Bio Laboratories Inc, USA), modified as follows: 10 minutes incubation at 70 °C after adding the lysis solution (C1), and extending the incubation times to 30 minutes. Once DNA was eluted, the sample was concentrated to dry pellets in a "speed vac" vacuum concentrator, and then washed in 100% ethanol to remove excess salts.

Water samples

DNA was extracted from sterivex filters following a modified version of the PowerWater® Sterivex™ DNA Isolation Kit (Mo Bio Laboratories Inc, USA) (Appleyard et al. 2011). Filters were removed from -80 °C and brought to room temperature before adding 1875 µL lysis buffer (200 mM sodium phosphate buffer, pH 7.0 containing: 1% CTAB, 2% PVP K30, 0.3 M NaCl and lysozyme at a final concentration of 5 mg mL^{-1}) and 125 µL MT Buffer (MP Biomedicals, LLC, USA) via the inlet valve. Sterivex filters were recapped and attached to a horizontal vortexer (Vortex-Genie 2, Mo Bio Laboratories Inc, USA) and vortexed at speed setting 6 for 1 h. A 3 mL syringe was attached to the inlet of the sterivex and using back pressure from the syringe the contents of the sterivex was removed and split into 2 x 2.0 mL microfuge tubes (approximately 1 mL per tube). 900 µL of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added to each tube and mixed by inverting several times. After centrifuging at 13 000 rpm x 10 min, the supernatant was removed and combined in a new 2.0 mL microfuge. Proteinase K was added (20 µL of 20mg/mL stock) followed by incubation at 60 °C for 2 h. 500 µL of Chloroform:Isoamyl Alcohol (CI) (24:1) was added and mixed by inverting several times. After centrifuging at 13 000 rpm x 10 min, the supernatant was removed to a new 2.0 mL microfuge tube and the CI extraction step was repeated. Following centrifugation the supernatant was removed to a 5.0 mL tube and 3 mL of ST4 Buffer (prewarmed to 65 °C) was added. The barrel of a 20 mL syringe was attached to a filter column that was then attached to a vacuum manifold (Vac-Man® Laboratory Vacuum Manifold, Promega Corp, USA). The contents of the 5.0 mL tube was poured into the syringe barrel and pulled through the filter column using the vacuum. Once the entire volume had been pulled through the column the 20 mL syringe barrel was removed. With the vacuum still flowing the filter was washed with 500 µL ST5 followed by 500 µL ST6 and dried by continuing vacuum flow for a further 2 min after the ST6 was entirely pulled through. The vacuum was turned off and the filter transferred to a new 2.0 mL collection tube and allowed to air dry on the bench for 10 min. To elute DNA, the filter was incubated with 80 µL of 0.1 x TE at 37 °C for 45 min, followed by a final spin at 13 000 rpm x 2 min.

The quality and quantity of all DNA was checked using a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™). DNA was aliquoted into multiple plates, vacuum dried and stored at -20 °C.

2.3 PCR and Sequencing

PCR and sequencing of extracted DNA was carried out at the Ramaciotti Center for Genomics (Sydney, Australia). Bacterial and archaeal assemblages were surveyed using small-subunit ribosomal RNA gene amplicon sequencing methods. Amplicons for the V1 to V3 regions of the 16S rRNA gene were prepared using bacterial primers 27F – 519R (Lane 1991, Lane et al. 1985) and archaeal primer A2F – 519R (DeLong 1992, Lane et al. 1985) and sequenced at the same facility using the Illumina MiSeq platform (Illumina, Inc., USA), with 300 bp paired reads, according to the manufacturer's directions. Details of the methodology can be found at (http://support.illumina.com/sequencing/documentation.html).

2.4 Bioinformatics

Amplicons were analysed in a strictly standardized fashion following the bioinformatics workflow established for the Bioplatforms Australia (BPA) Biome of Australian Soils (BASE) project (Bisset et al. in revision) and adopted by other Australian microbial biodiversity initiatives. This allows the microbial diversity detected in the GAB to be placed within the broader context of microbial diversity in other Australian environments. Full details of the bioinformatics workflow can be found here: (https://ccgapps.com.au/bpa-metadata/base/information).

2.5 Phylogeny

Preliminary classification was performed using MOTHUR's (Schloss et al 2009) implementation of the Wang classifier (Wang et al 2007) at 60 % sequence similarity cut-off with the Green Genes database and taxonomy files (DeSantis et al 2006: http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). OTU sequences that did not classify to the correct lineage (i.e. bacteria or archaea) were discarded from further analysis.

2.6 Data Analysis and Statistics

Matrices containing relative abundance data for all OTUs across all samples were generated using the python command uc2otutab.py provided on the drive5 Bioinformatics and software services site (http://www.drive5.com/) to assist in processing OTU data. Rarefaction curves were generated using the rarefaction.single command in MOTHUR (Schloss et al 2009). Preliminary community composition analysis was performed using the Primer-E Multivariate Statistics for Ecologists software package (Version 7; Clarke and Gorley 2015). For community composition analysis, the relative abundance data was fourth root transformed and a Bray-Curtis dissimilarity matrix was constructed after removing taxa that accounted for <0.001% of total reads. Spatial patterns in assemblage composition for bacteria and archaea separately were examined using permutational multivariate analysis of variance (PERMANOVA) and visualised using principal co-ordinates analysis (PCO), and the Relate procedure in Primer was used to examine the concordance in patterns between the two groups, and between benthic and pelagic samples. Environmental variables were correlated to community composition data using distance-based linear modelling (DISTLM) with distance-based redundancy analysis (dbRDA), and Pearson correlations with environmental variables were also overlaid on the principal co-ordinates plots. For the DISTLM, the overall best model was selected from all environmental procedures using the BEST procedure and the small sample size variation of AIC (AIC_C) with 9999 permutations. All analyses involving environmental variables were conducted at the station level, as environmental data corresponding to individual cores were not collected.

3 Results

3.1 Sequencing output and quality assurance

The output from the sequencing runs, as partitioned by dataset and environment, is summarised in Table 1 below. After QC and checks for chimeras, we were able to identify 2,414,482 16S rRNA sequences from Bacteria from benthic samples (Figure 2) that were grouped at 97% sequence similarity into 7993 Operational Taxonomic Units (OTU's). From the pelagic samples, 2,306,778 16s rRNA sequences could be grouped into 4522 OTU's (Figure 3). For the Archaea, 4,920,756 16S rRNA sequences could be grouped into 496 OTU's from benthic samples (Figure 4) and 2,335,864 16s rRNA sequences could be grouped into 290 OTU's from pelagic samples (Figure 5). OTU's defined at 97% rRNA sequence similarity are practically equivalent to species level taxonomy and the standard way of looking at 16S rRNA based genomic surveys. Our results indicate that we had sufficient sequencing depth (i.e. number of reads) to discern trends, as shown in Figures 2-5 below. In all samples, the curve is either beginning to plateau or has neared plateauing, suggesting that additional read depth would not have substantially increased the number of taxa identified.

Table 1. The number of reads and OTU's derived from the sequencing results, divided into data sets and partitioned by environment, where 16S are bacterial sequences, and A16S are archaeal sequences.

Dataset	***Post OTU clustering (97%)***		***Partitioned by environment***			***Removing OTUs < 0.001%***	
	# Reads	# OTUs		# Reads	# OTUs	# Reads	# OTUs
16S	4,771,424	10,236	Benthic	2,414,482	7 993	2,392,189	4,838
			Pelagic	2,306,778	4 522	2,212,238	2,122
A16S	7,256,651	604	Benthic	4,920,756	496	4,918,504	328
			Pelagic	2,335,864	290	2,335,253	180

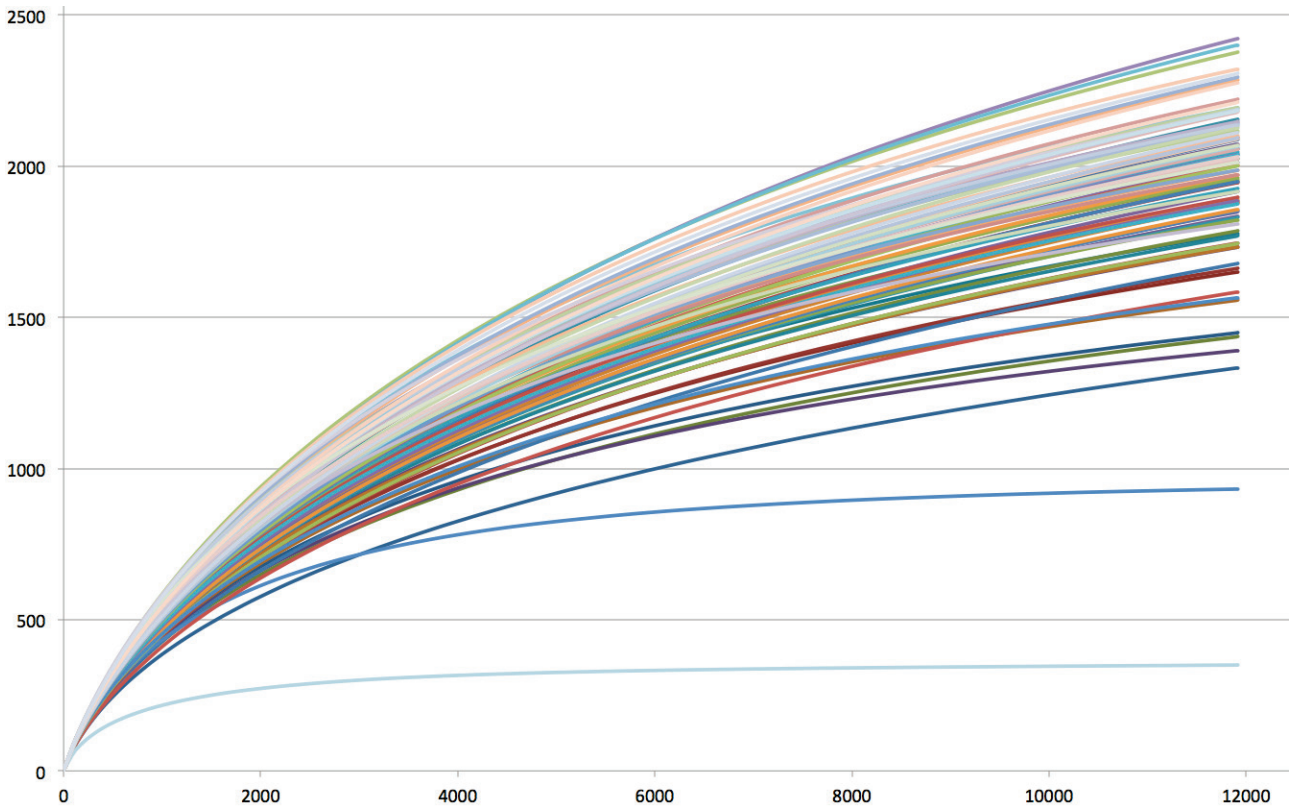


Figure 2. Rarefaction curve of bacterial benthic samples, showing the number of new bacterial taxa identified (y-axis) relative to the number of sequences analysed (x-axis) for each station.

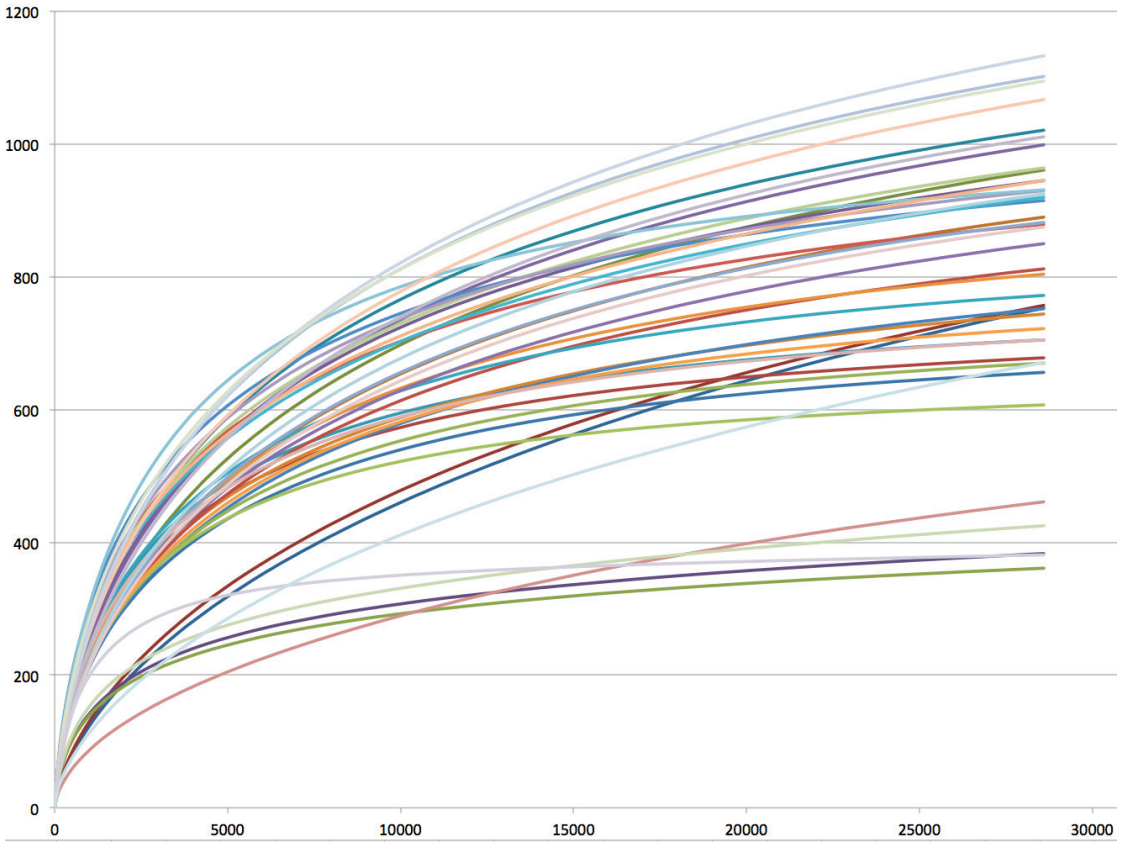


Figure 3. Rarefaction curve of bacterial pelagic samples, showing the number of new bacterial taxa identified (y-axis) relative to the number of sequences analysed (x-axis) for each station.

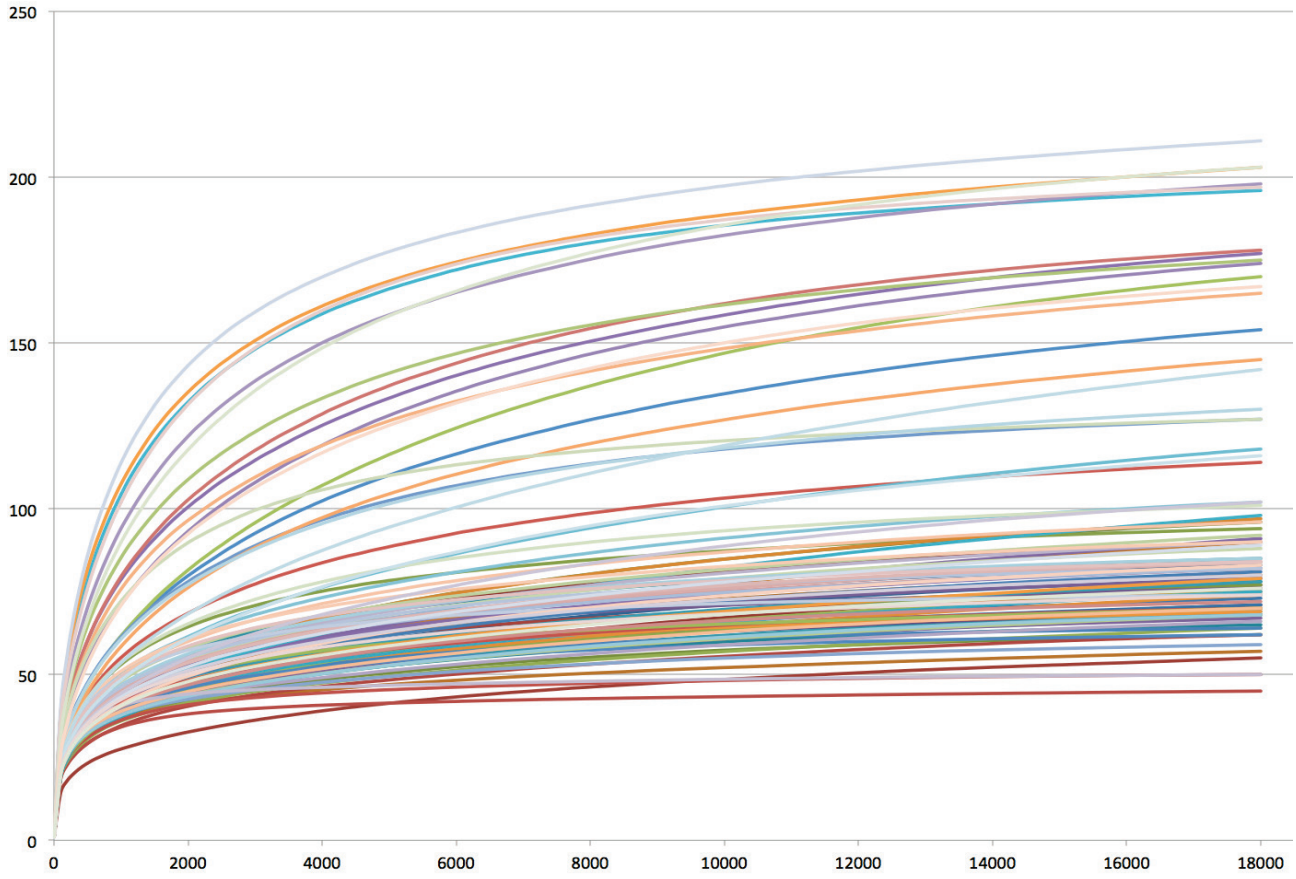


Figure 4. Rarefaction curve, showing the number of new archaeal taxa identified in benthic samples (y-axis) relative to the number of sequences analysed (x-axis) for each station.

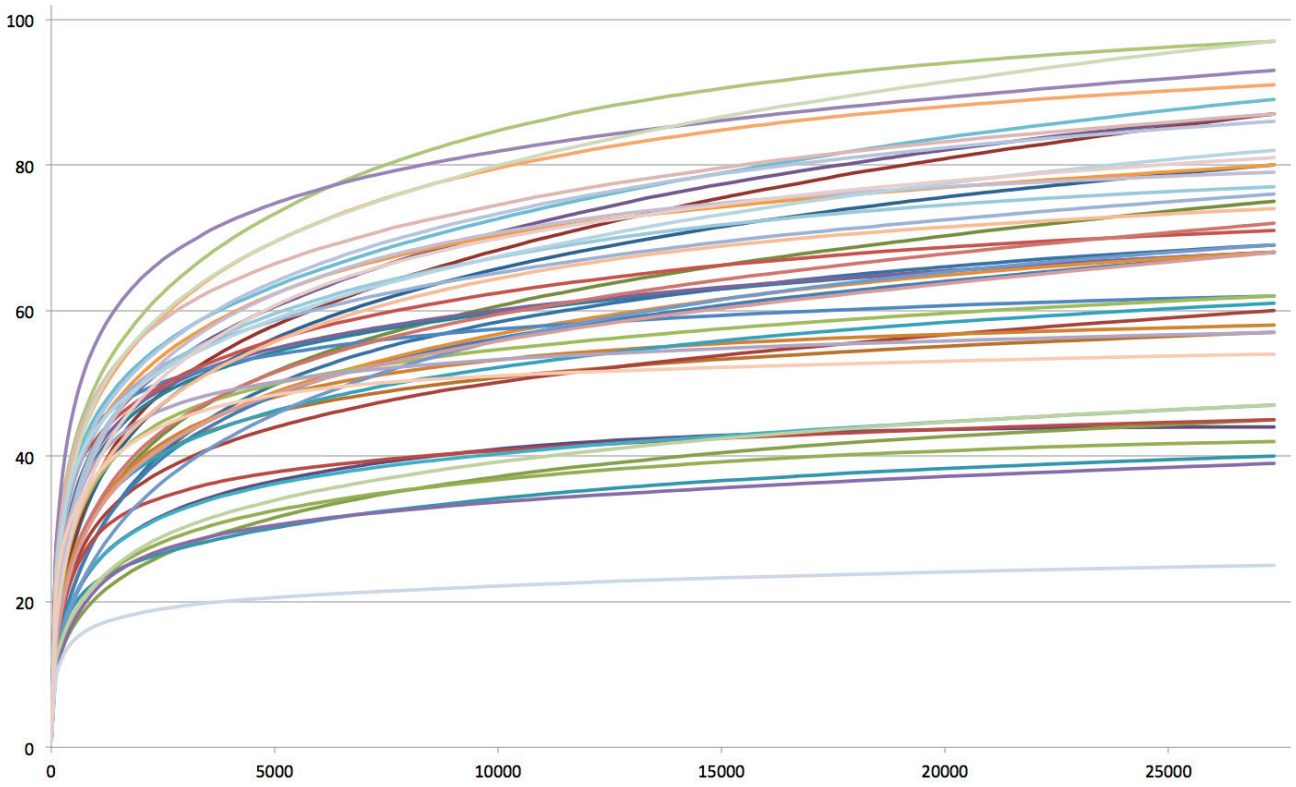


Figure 5. Rarefaction curve, showing the number of new archaeal taxa identified in pelagic samples (y-axis) relative to the number of sequences analysed (x-axis) for each station.

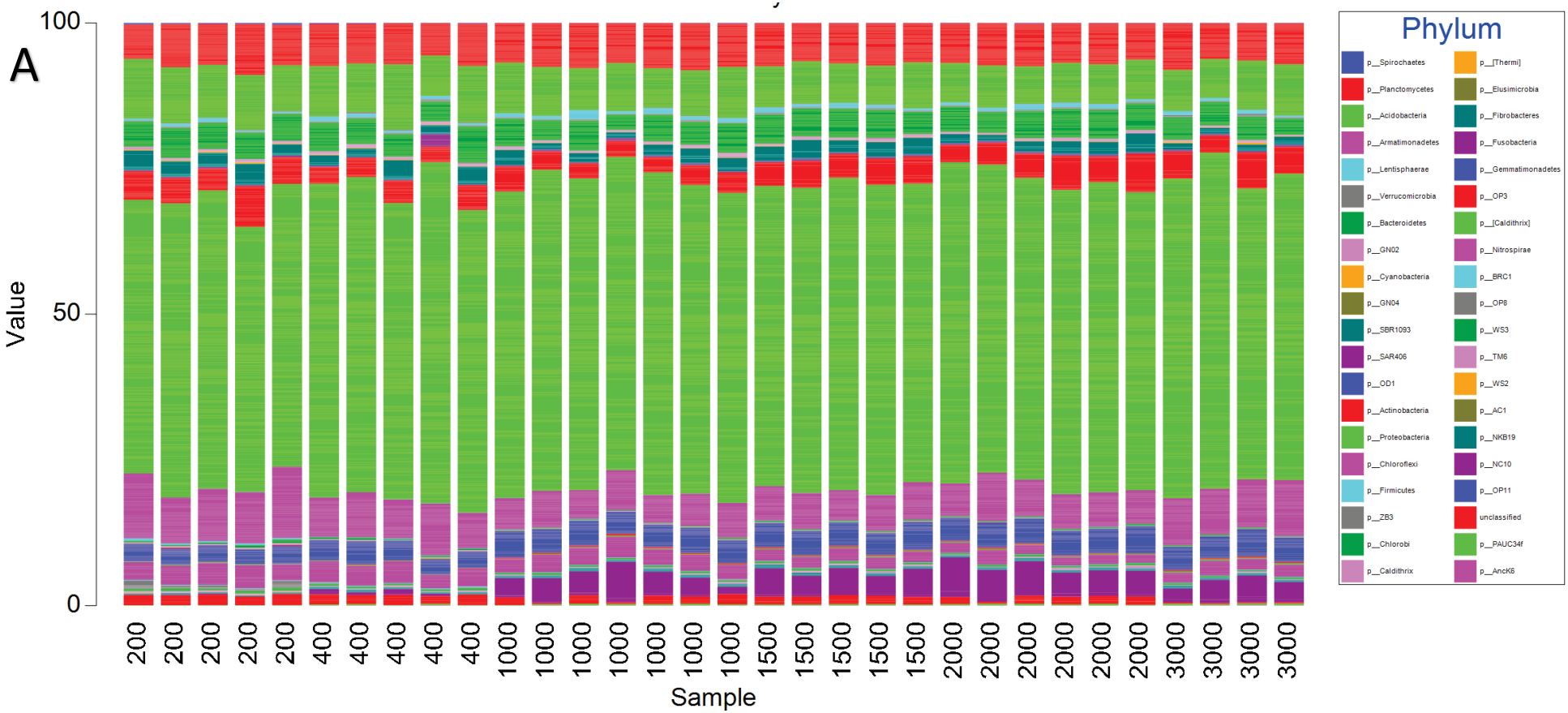
The rarefaction curves in Figures 4 and 5 demonstrate that we have much lower diversity for Archaea than for bacteria, even though we had equivalent or greater depth of coverage (Table 1). In most stations, the diversity begins to plateau at 500 OTUs. By contrast, the bacteria are much more diverse, and typically contain more than 5000 OTU's per station. For all taxa, our sequencing depth greatly exceeds what would be required to describe the diversity of the dominant Archaea present in the regions of the Great Australian Bight included in the survey.

3.2 Community composition

3.2.1 Bacteria

As shown in Figure 6, bacteria in benthic samples were detected from a wide variety of taxonomic groups. There are few obvious visual differences in the community composition with transect or depth, with samples taken in the same location having very similar composition. When these data are examined at the phylum level (Figure 6A), Proteobacteria (bright green) are the most abundant taxon, making up approximately 50% of analysed sequences in all samples. When examined at the class level (Figure 6B), the most abundant taxa are the gamma proteobacteria (shown in red), the delta proteobacteria (shown in aqua), and the alpha proteobacteria (shown in khaki). Many of the OTU's could not be classified at the order level (Figure 6C, shown in grey), indicating that many of the bacteria found in the GAB are novel and have not been described before. The most abundant orders were: *Rhodospirillales* (in fuschia), *Thiotrichales* (in pink), *Rhizobiales* (in turquoise), *Acidimicrobiales* (in turquoise), the NB1-j clade and the *Nitrospirales* (also shown in pink).

OTU's related to bacteria with a known capacity to degrade hydrocarbons and which were enriched in petroleum containing samples collected during the response to the *DeepWater Horizon* wellhead blowout were identified, as described in Table 2. These taxa were not abundant, as would be expected without a measurable hydrocarbon source (Ahmed et al., 2015). Even though these taxa are rare, they were evenly distributed across all samples and consistently present (Figure 7). There may be a slight increase in the abundance of families containing known hydrocarbon degrading organisms with depth (figure 7B), but without further functional analysis, these relationships are difficult to interpret on the basis of phylogeny alone.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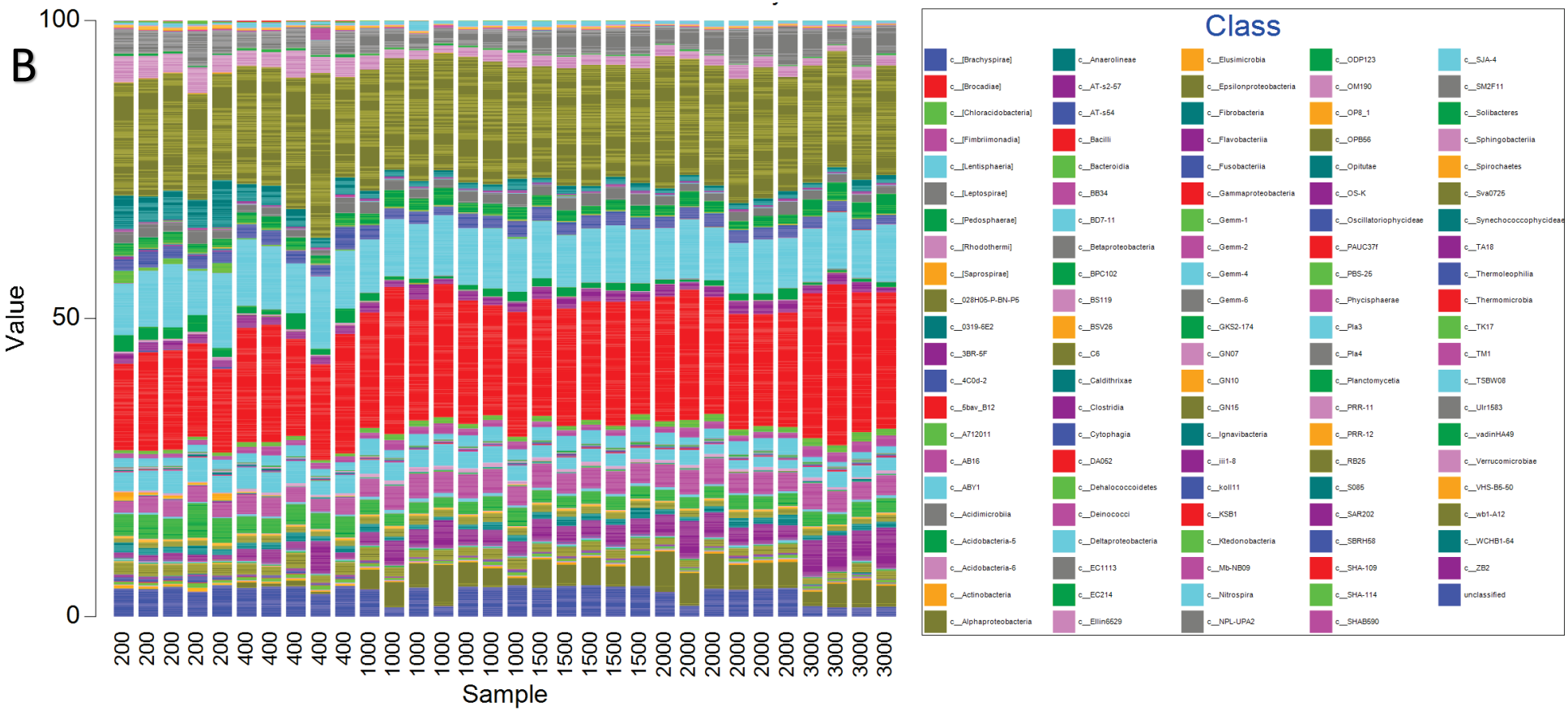
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c__GN15
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c__iii1-8
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c__Mb-NB09
c__Nitrospira
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c__Pla4
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c__Thermoleophilia
c__Thermomicrobia
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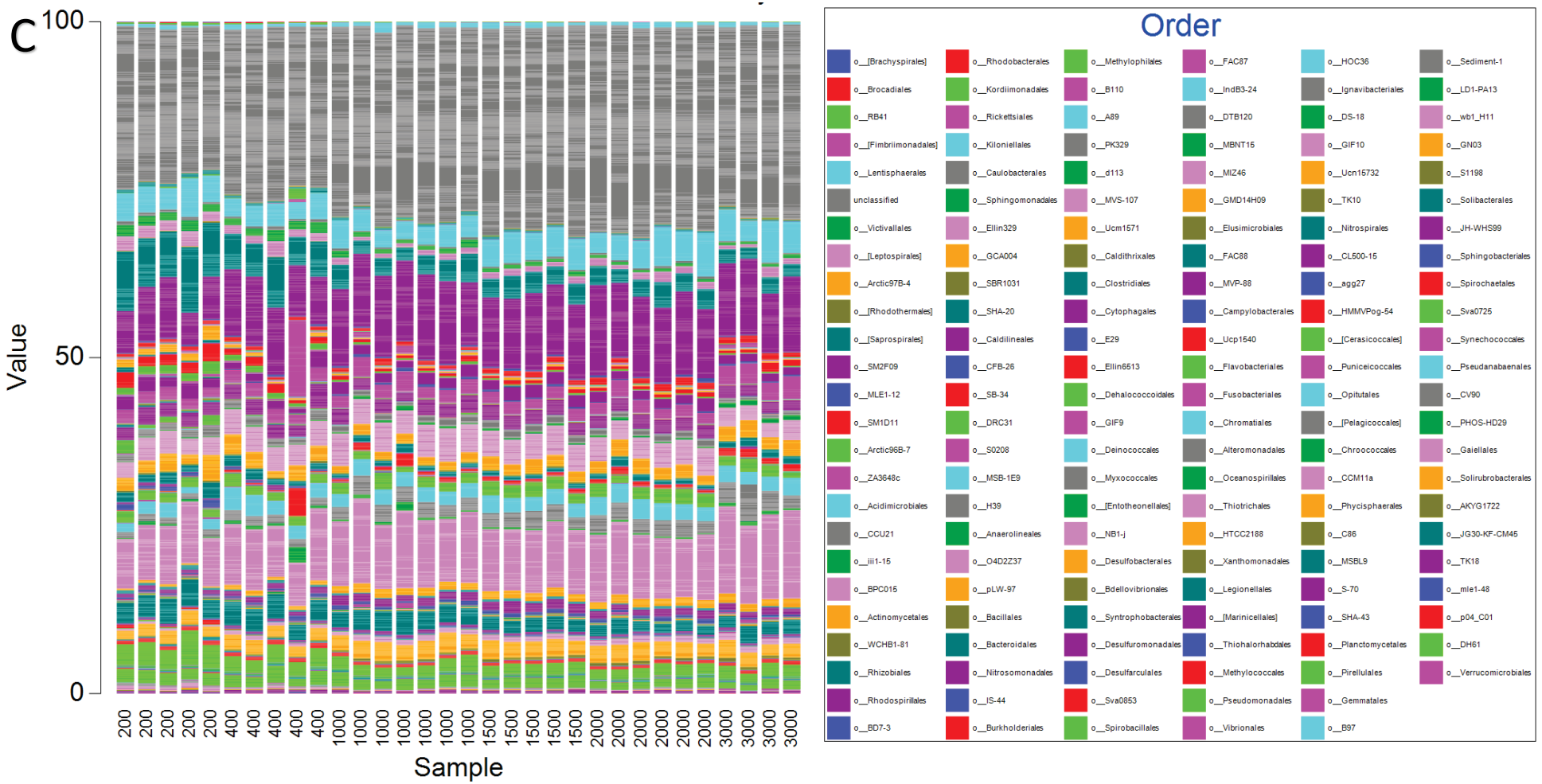


Figure 6. The representation of different taxonomic groups of bacteria in benthic samples collected at each station. Depth and transect are on the x axis, and % contribution of each taxonomic group to the overall composition of the sample are on the y axis. Only taxonomic groups that make up more than 0.001% of the community are shown, chloroplast and mitochondrial rRNA have been removed. Each vertical line represents a different sample, but for clarity, only depths are indicated in the x-axis labels. The taxonomic resolution in Figure 6A is to phylum, panel B shows class, panel C shows order.

Table 2. A comparison of the taxonomic identification of microbes identified in the Great Australian Bight to those enriched in samples originating from the response to the *DeepWater Horizon* wellhead blowout. Closed symbols denote families, open symbols genera.

Taxa found enriched in oil containing samples from the GOM	Reference	Taxa found in the GAB	Number of OTU's from this taxonomic group found in the GAB	Number of reads aligning to this group	Proportion of reads (of 2.3 M) aligning to this group
Pseudomonadales	1,4	Pseudomonadales (order)	3	96	$4.1*10^{-5}$
Oceanospirillales	1, 3, 4	Oceanospirillales (order)	91	23328	0.01
		• Oceanospirillaceae (family)	• 26	• 8408	• 0.004
		○ Marinomonas (genus)	○ 1	○ 266	○ $1.1*10^{-4}$
		• Oleiphilaceae (family)	• 2	• 105	• $4.6*10^{-5}$
		• Halomonadaceae (family)	• 1	• 239	• $1*10^{-4}$
		• Alcanivoracaceae (family)	• 9	• 1516	• $6.6*10^{-5}$
		○ Alicanivorax (genus)	○ 2	○ 473	○ $2.1*10^{-4}$
Methylococcales	1	Methylococcales (order)	2	242	$1.1*10^{-4}$
Alteromonadales	1,3,4	Alteromonadales (order)	139	45096	0.02
		• Colwelliaceae (family)	• 10	• 1301	• $5.5*10^{-4}$
		• Moritellaceae (family)	• 6	• 1778	• $7.7*10^{-4}$
		• Shewanellaceae (family)	• 4	• 323	• $1.4*10^{-4}$
		• Alteromonadaceae (family)	• 67	• 36158	• 0.015
		○ Marinobacter (genus)	○ 4	○ 3821	○ $1.6*10^{-3}$
Flavobacteriales	1	Flavobacteriales (order)	152	38994	0.015
Sphingobacteriales	1	Sphingobacteriales (order)	79	11084	0.005
		• Saprospiraceae (family)	• 43	• 3157	• 0.0013
Desulfuromonadales	2	Desulfuromonadales (order)	20	3725	0.0016
Desulfobacterales	2	Desulfobacterales (order)	79	30977	0.013

1. Dubinsky et al., 2013, enriched in the plume; anomaly; 2. Kimes et al., 2013; 3. Chakraborty et al., 2012; 4. Hazen et al., 2010

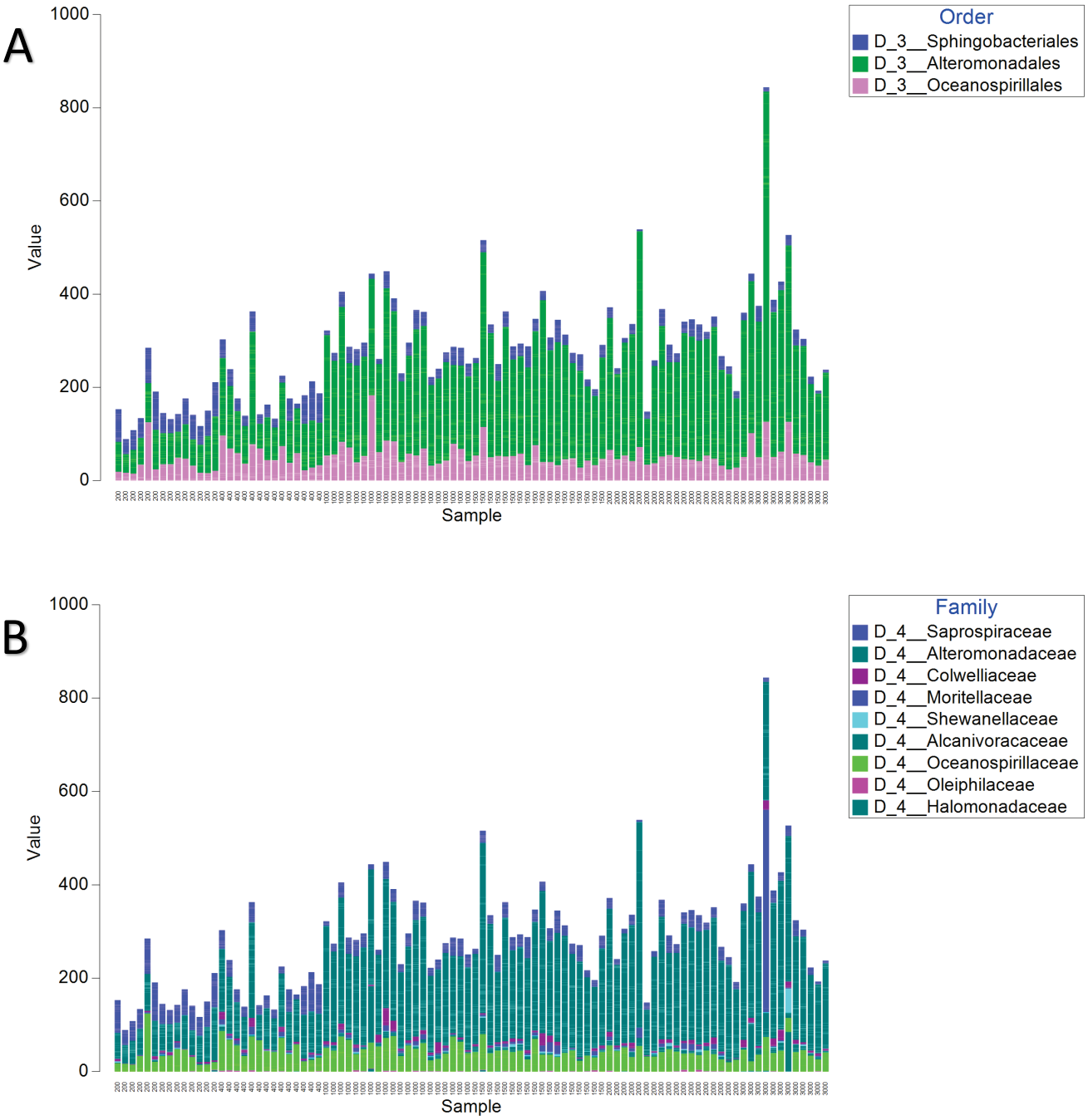
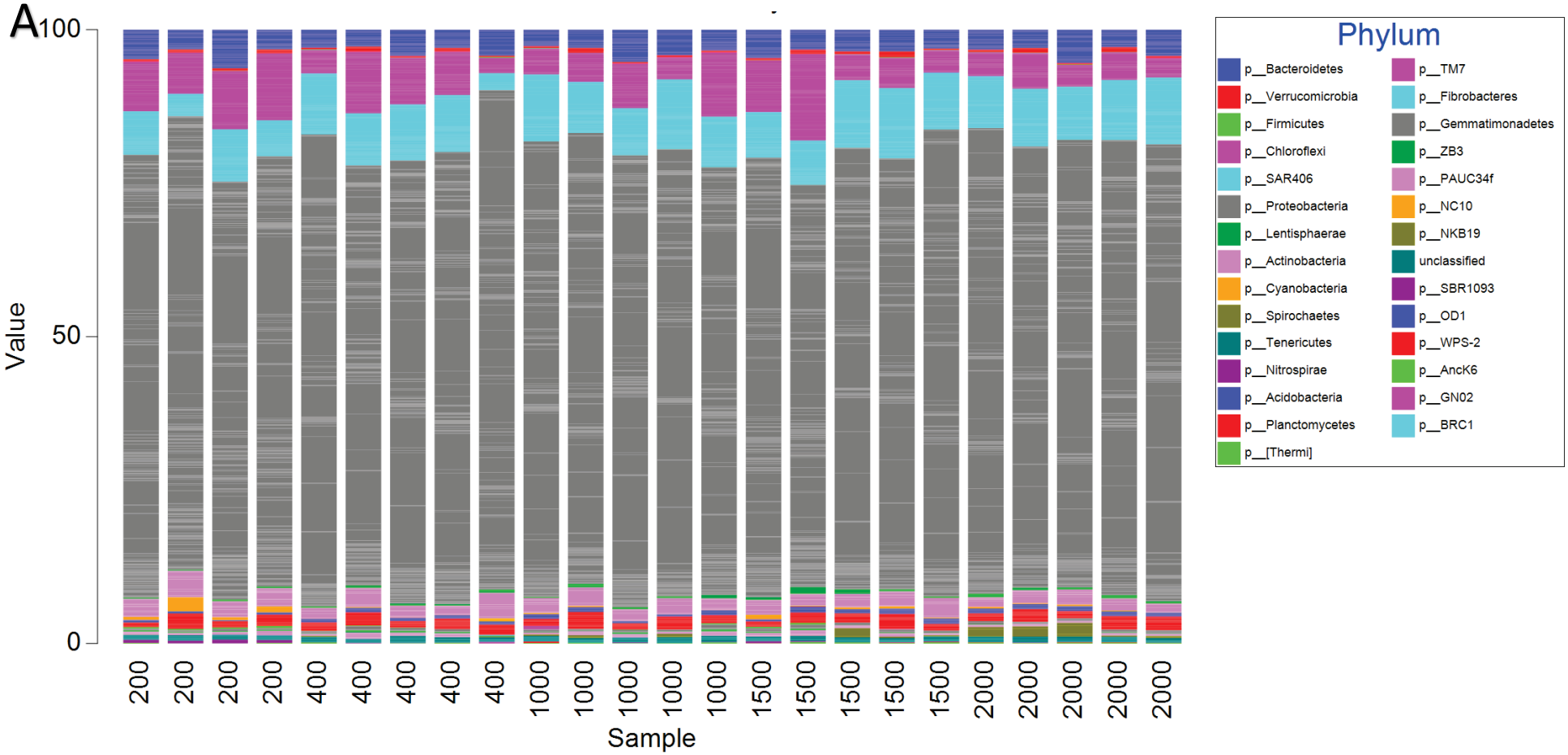


Figure 7. The relative abundance of bacteria in orders (panel A) or families (panel B) known to contain hydrocarbon degrading organisms. The station (in order of transect) and depth are shown on the x axis, and the abundance on the y axis. For clarity, only depth is labelled.

When bacteria found in pelagic samples are examined, a somewhat different taxonomic distribution is apparent (Figure 8). Like the benthic samples, few differences in composition are apparent with either transect or depth. In both the benthic and pelagic samples, the dominant phylum is the proteobacteria (Figure 8A). Again, at the level of class (Figure 8B), the alpha proteobacteria (shown in bright green), the gamma proteobacteria (shown in orange) and the delta proteobacteria (shown in purple), make up the most abundant taxa. At the taxonomic level of order (Figure 8C), about 15% of the OTU's (shown in red) are unclassified, again indicating that many of the bacteria found in the GAB are novel and have not been described before. The *Rickettsiales* (shown in lilac), the *Altermonadales* (shown in fuchsia) and the *Acidomicrobiales* (shown in aqua) are among the most abundant orders in the pelagic samples.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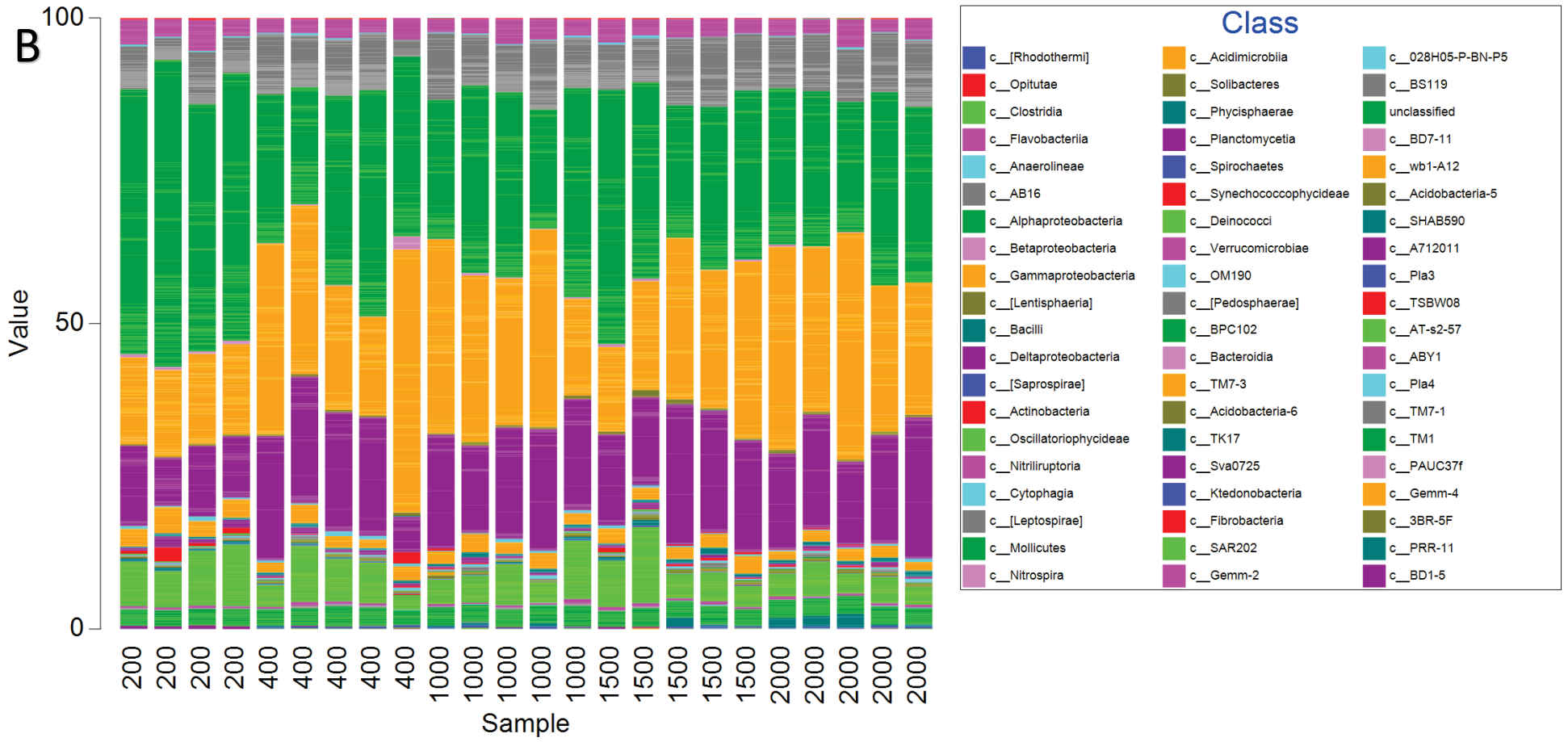
Sample
Phylum
p__Bacteroidetes
p__Verrucomicrobia
p__Firmicutes
p__Chloroflexi
p__SAR406
p__Proteobacteria
p__Lentisphaerae
p__Actinobacteria
p__Cyanobacteria
p__Spirochaetes
p__Tenericutes
p__Nitrospirae
p__Acidobacteria
p__Planctomycetes
p__[Thermi]
p__TM7
p__Fibrobacteres
p__Gemmatimonadetes
p__ZB3
p__PAUC34f
p__NC10
p__NKB19
unclassified
p__SBR1093
p__OD1
p__WPS-2
p__AncK6
p__GN02
p__BRC1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Sample
Class
c__[Rhodothermi]
c__Opitutae
c__Clostridia
c__Flavobacteriia
c__Anaerolineae
c__AB16
c__Alphaproteobacteria
c__Betaproteobacteria
c__Gammaproteobacteria
c__[Lentisphaeria]
c__Bacilli
c__Deltaproteobacteria
c__[Saprospirae]
c__Actinobacteria
c__Oscillatoriophycideae
c__Nitriliruptoria
c__Cytophagia
c__[Leptospirae]
c__Mollicutes
c__Nitrospira
c__Acidimicrobiia
c__Solibacteres
c__Phycisphaerae
c__Planctomycetia
c__Spirochaetes
c__Synechococcophycideae
c__Deinococci
c__Verrucomicrobiae
c__OM190
c__[Pedosphaerae]
c__BPC102
c__Bacteroidia
c__TM7-3
c__Acidobacteria-6
c__TK17
c__Sva0725
c__Ktedonobacteria
c__Fibrobacteria
c__SAR202
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c__028H05-P-BN-P5
c__BS119
unclassified
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c__wb1-A12
c__Acidobacteria-5
c__SHAB590
c__A712011
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c__TM1
c__PAUC37f
c__Gemm-4
c__3BR-5F
c__PRR-11
c__BD1-5

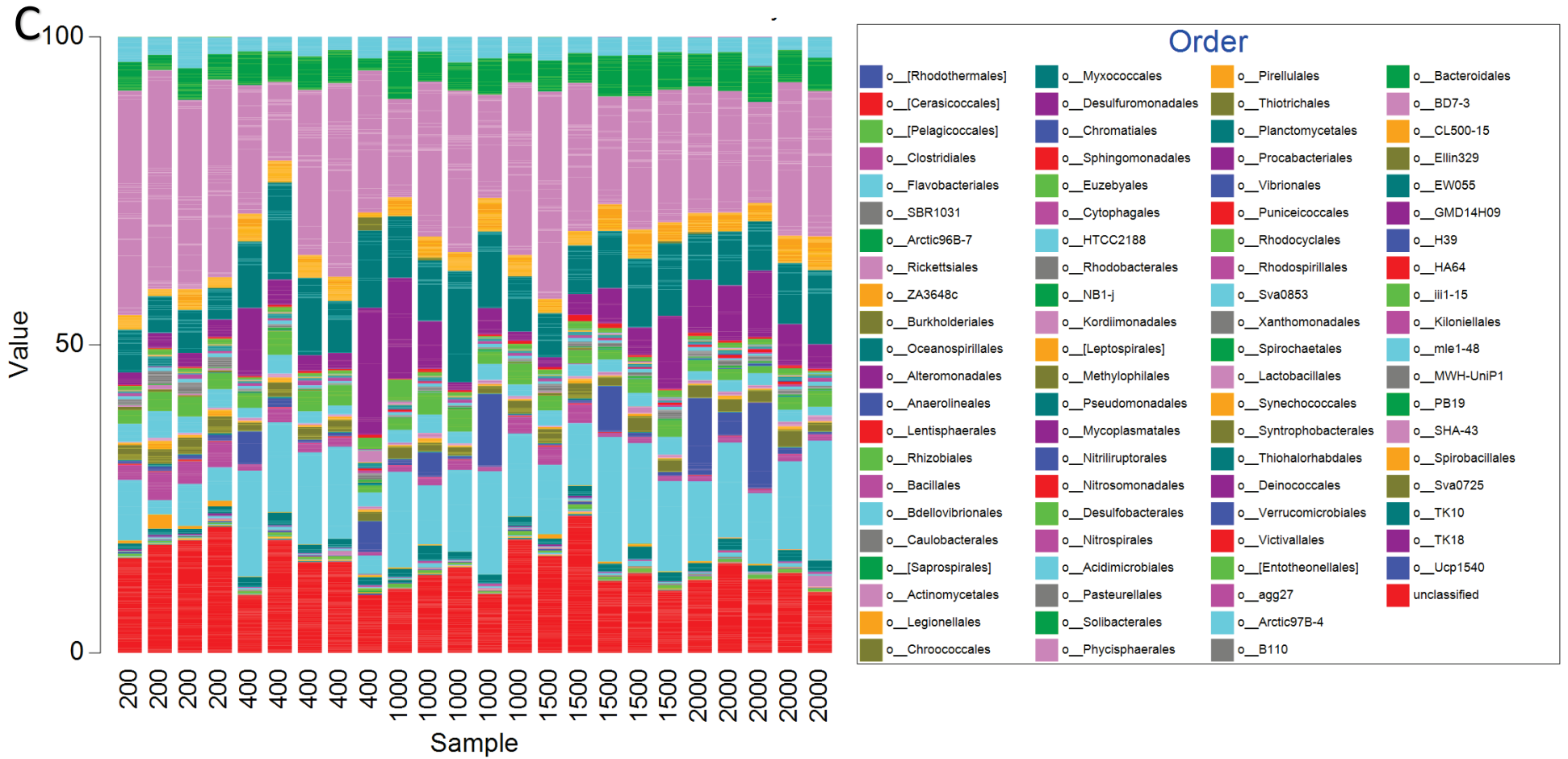
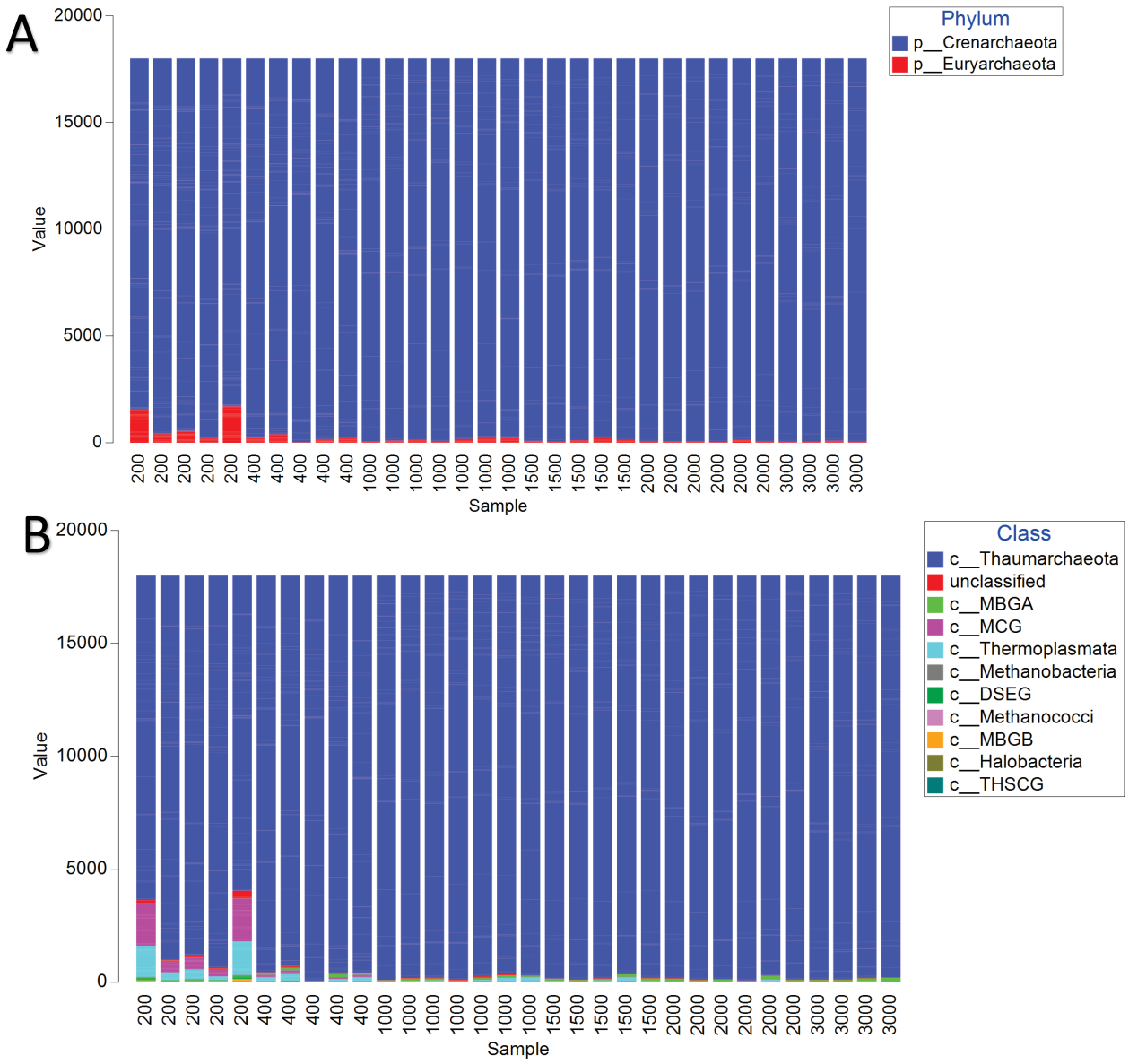


Figure 8. The representation of different taxonomic groups of bacteria in pelagic samples collected at each station. Depth and transect are on the x axis, and % contribution of each taxonomic group to the overall composition of the sample is on the y axis. Only taxonomic groups that make up more than 0.001% of the community are shown, chloroplast and mitochondrial rRNA have been removed. Each vertical line represents a different sample, but for clarity, only depths are labelled. The taxonomic resolution in Figure 8A is to phylum, panel B shows class, panel C shows order.

3.2.2 Archaea

The comparative abundances of different taxonomic groups of Archaea in benthic samples are shown in Figure 9. Both Archaeal phyla are present at all depths, however, the Euryarchaeota are more abundant at the 200 m depths than in the remaining samples (Figure 9A). This increased diversity at the shallower depths is apparent at the class and family level as well (Figure 9B and 9C), although in all samples, a single genus of ammonia oxidising archaea, the genus *Nitrosopumilus* (phylum Crenarchaeota, class Thaumarchaeaota, order Cenarchaeales, all three shown in blue) was most abundant. This genus contributed over two thirds of the total observed sequences across the whole dataset.



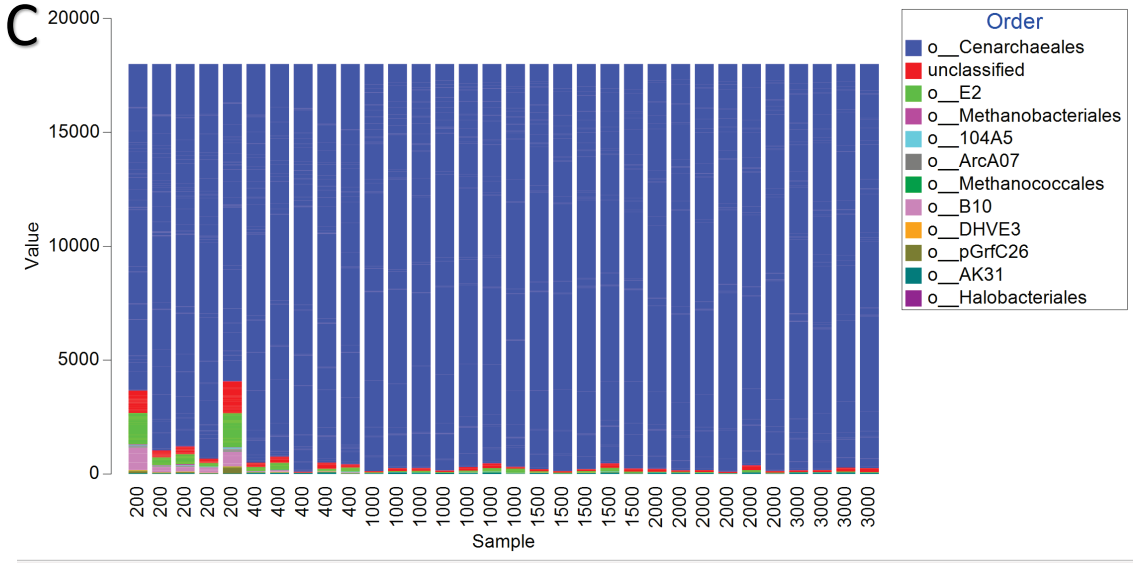
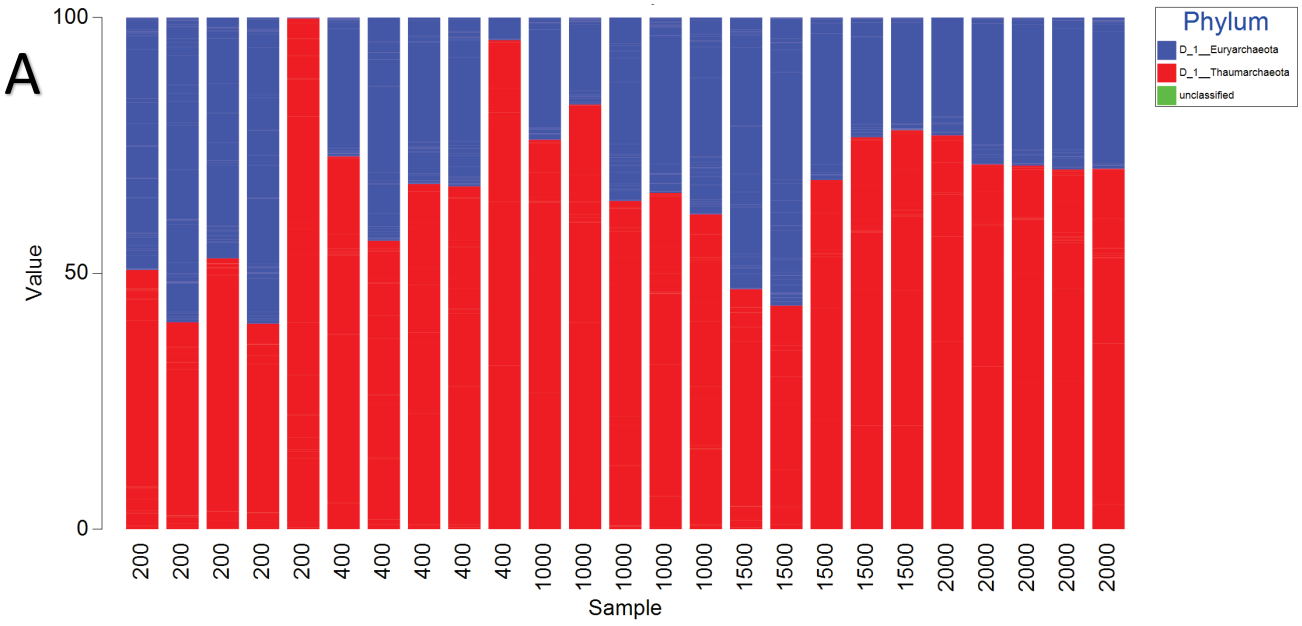


Figure 9. Comparative abundances of different groups of *Archaea* collected from benthic samples at the different depths. Depth and transect are on the x axis, and % contribution of each taxonomic group to the overall composition of the sample are on the y axis. Only taxonomic groups that make up more than 0.001% of the community are shown. For clarity, station numbers are not shown. Figure 9A is taxonomic resolution to phylum, panel B shows class, panel C shows order.

The comparative abundances of different orders of *Archaea* from pelagic samples are shown in Figure 10. There was greater variability between different samples in pelagic Archaeal composition relative to the benthic or bacterial samples, with the shallow water depths from transect 5 being particularly different from the rest (i.e. Figure 10A). In most of these samples, the distribution of phyla is more even between the *Euryarchaeaota* (shown in red) and the *Crenarchaeota* (shown in blue) (Figure 10A). There is a greater proportion of members of the class *Thermoplasmata* (shown in green) in pelagic samples relative to benthic, although the *Thaumarchaetota* (shown in blue) are more than 50% of many of the pelagic samples (Figure 10B). Of the sequences that can be classified to order, the *Cenarchaeles* are most abundant (Figure 10C). Again, similar to the benthic samples, over two thirds of the archaea detected in the pelagic samples belong to the genus *Nitrosopumilus.*



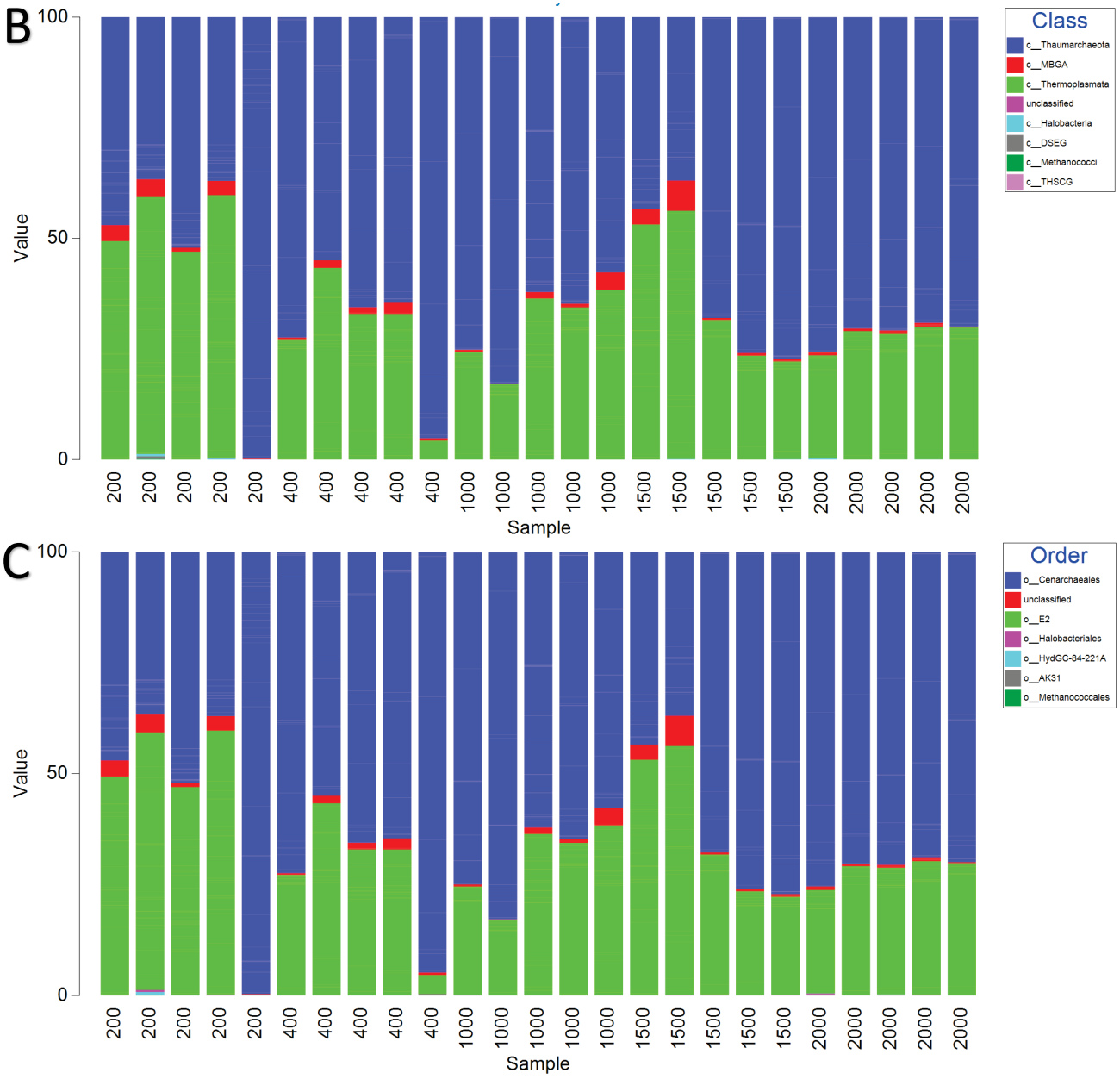


Figure 10. Comparative abundances of different groups of *Archaea* collected from pelagic samples at the different depths. Depth and transect are on the x axis, and % contribution of each taxonomic group to the overall composition of the sample are on the y axis. Only taxonomic groups that make up more than 0.001% of the community are shown. For clarity, station numbers are not shown. Figure 10A is taxonomic resolution to phylum, panel B shows class, panel C shows order.

3.2.3 Diversity Indices

Trends in the biodiversity of microbial communities were explored further using Shannon and Simpson indexes of biodiversity, and by exploring the dominance and evenness of the distribution of different OTU's. As shown in Figure 11 below, there is a general trend towards less bacterial diversity in the sediment with depth (panel 11A). This is reflected in a greater degree of dominance of some OTU's in the samples collected at deeper stations (panel 11B). In bacterial communities from pelagic samples, however, there is more variability in both diversity (Figure 12A) and evenness (Figure 12B), obscuring any trends in ecological parameters with depth.

For the Archaeal communities, the decrease in diversity and species evenness with depth is even more pronounced, as depicted in Figure 13. The differences between the 200m stations and the deeper communities are most noticeable. However, as shown in Figure 14, variability between different stations obscures any differences in either diversity or dominance with depth in pelagic samples.

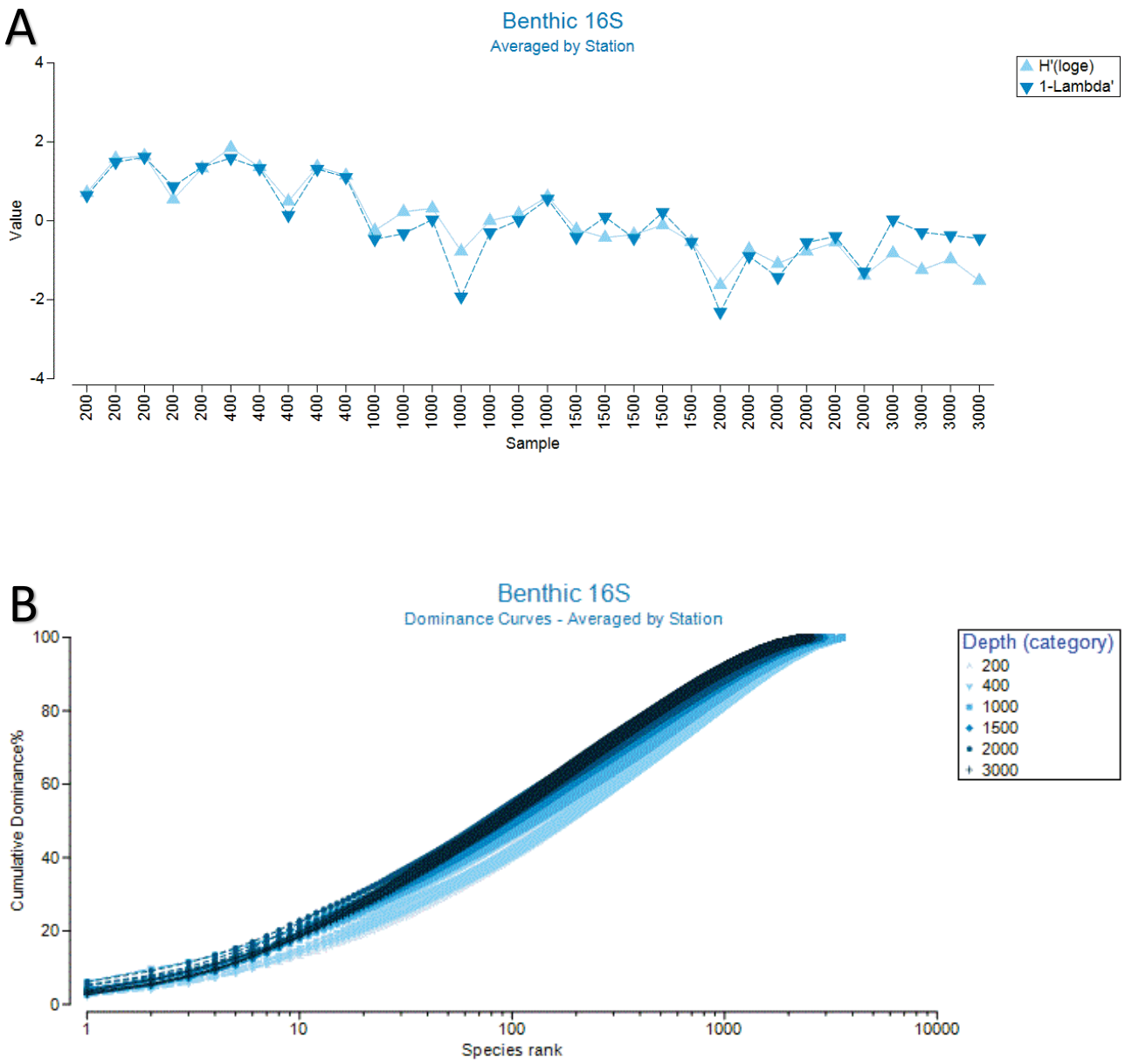


Figure 11. Biodiversity metrics in the benthic bacterial samples. Panel A shows diversity as determined by either the Shannon (H) or Simpson (1-lambda) metrics; Panel B shows evenness for each station.

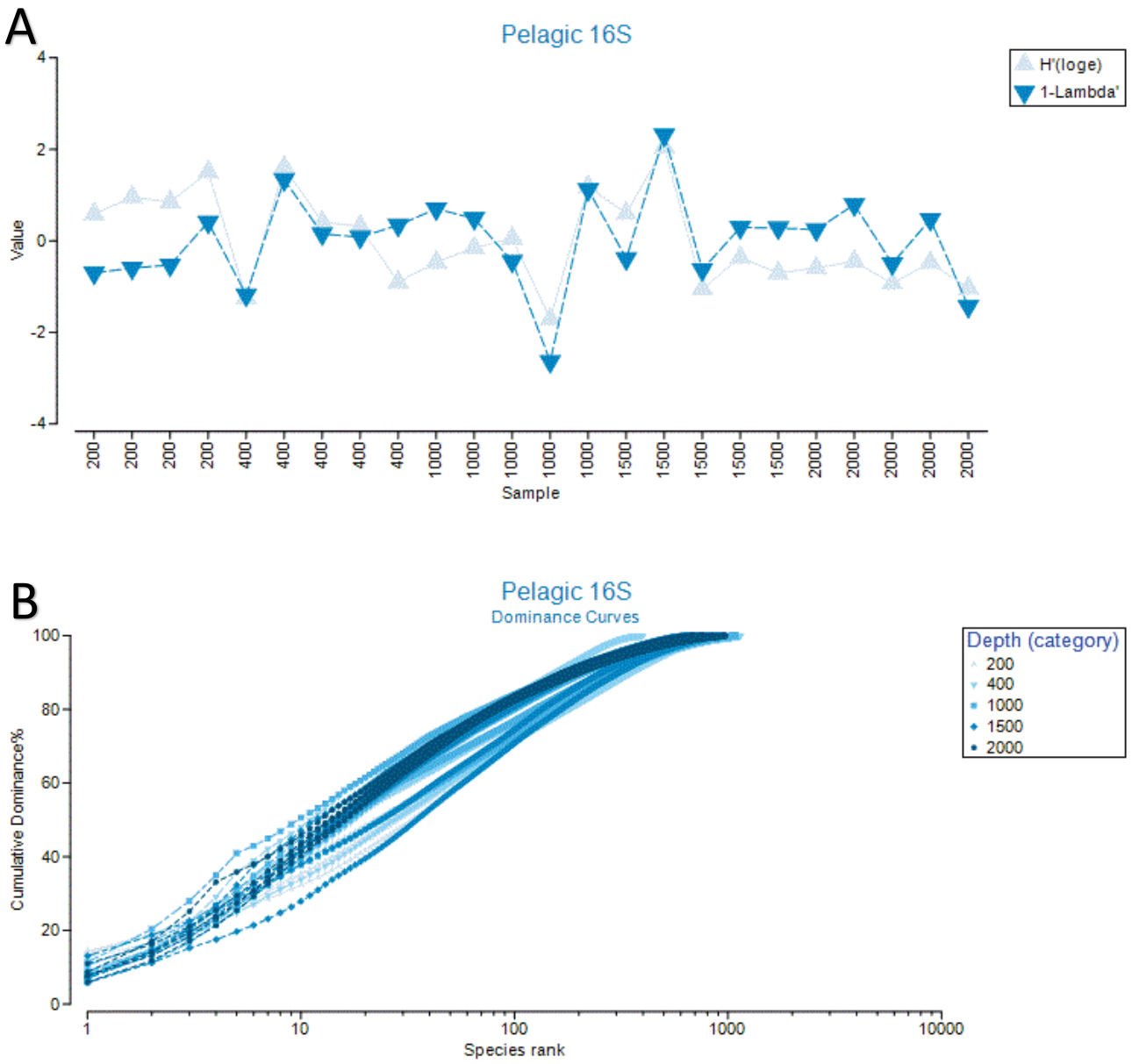


Figure 12. Biodiversity metrics in the pelagic bacterial samples. Panel A shows diversity as determined by either the Shannon (H) or Simpson (1-lambda) metrics; Panel B shows evenness for each station.

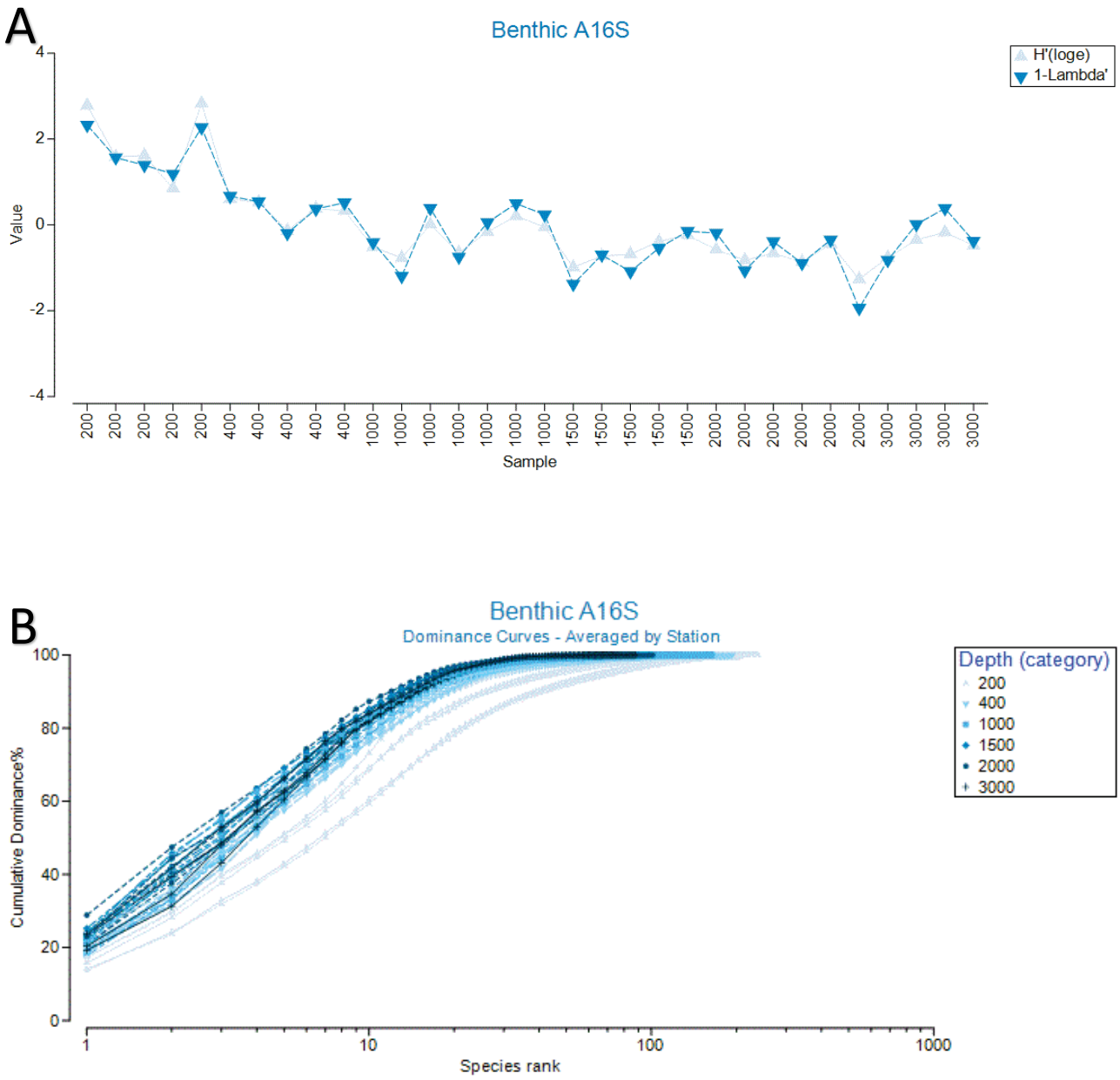


Figure 13. Biodiversity metrics in the benthic archaeal samples. Panel A shows diversity as determined by either the Shannon (H) or Simpson (1-lambda) metrics; Panel B shows evenness for each station.

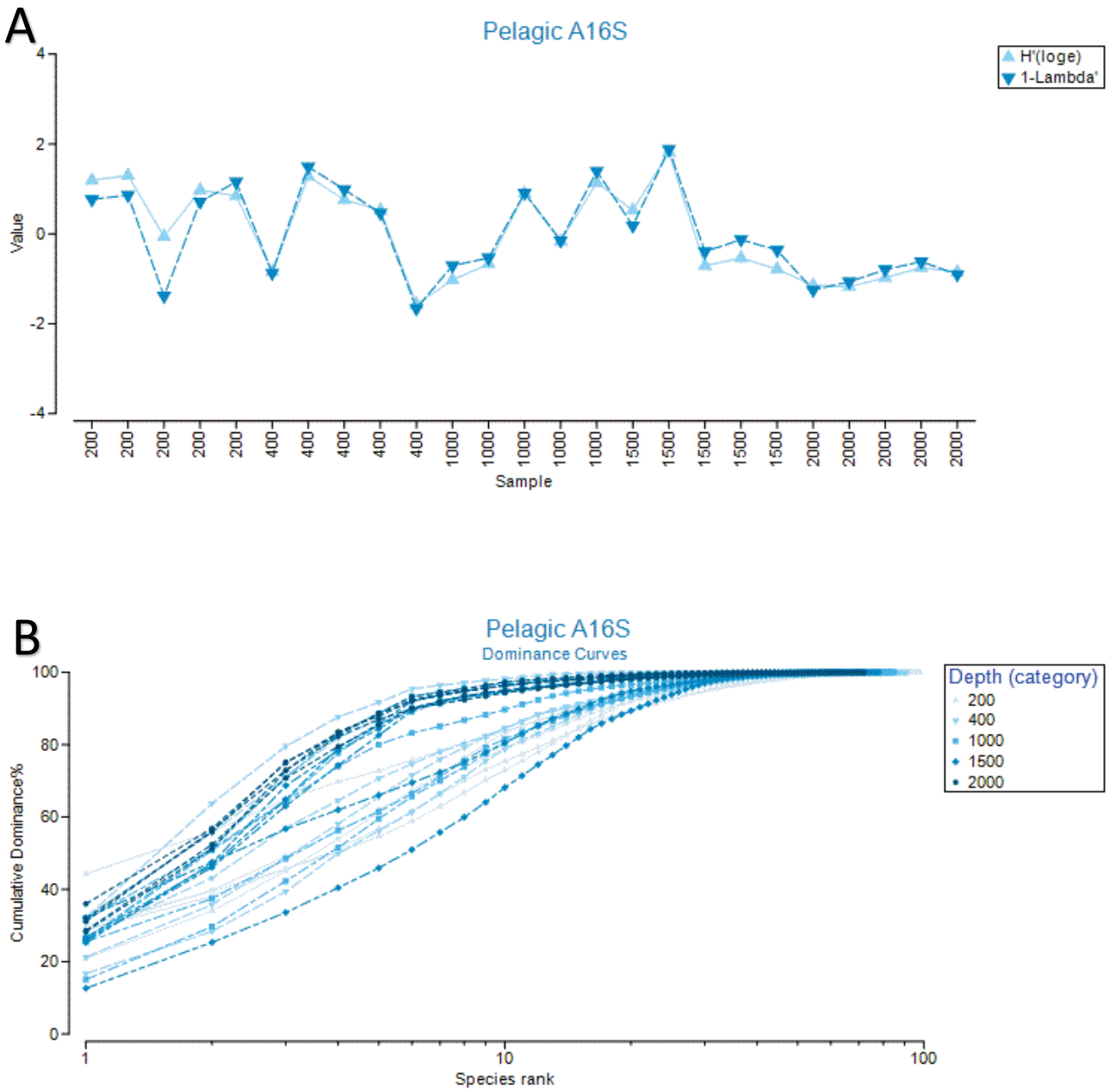


Figure 14. Biodiversity metrics in the pelagic archaeal samples. Panel A shows diversity as determined by either the Shannon (H) or Simpson (1-lambda) metrics; Panel B shows evenness for each station.

3.3 Relationships between community composition and environmental variables

The relationships between the taxonomic compositions of different samples were visualised using principle co-ordinates analysis. As shown in Figure 15, the benthic bacterial samples cluster tightly with depth, with little variation either between different replicates in the same transect or different transects, although there was an interaction between depth and transect (PERMANOVA: $F_{19,67}$=1.36, p<0.001). Pairwise tests indicated that Transects 1 and 2 both differed to transect 5 at 1000m only. Samples from 200m were consistently different to all other depths, except on transects 3 and 4, where they were the same as the 400m samples. Similarly, 400m samples differed to all deeper samples except for the 1000m samples on transect 3. Samples from 1000m consistently differed from those from 3000m, and also the 2000m samples on transects 1 and 2, and 1500m samples differed from 3000m on transects 1 and 4. When only those taxonomic families known

to contain hydrocarbon degrading taxa are analysed (Figure 16), the same trends are apparent both spatially and with regards to the clustering of depths. This suggests that the differences in abundance of the taxa related to known hydrocarbon degraders are determined by the same environmental factors influencing the overall bacterial community. Pelagic bacterial assemblages also separated out by depth (PERMANOVA: $F_{4,15}$=2.99, p=0.011), but not transect (PERMANOVA: $F_{4,15}$=0.60, p=0.87), although there was higher variation between samples at each depth (Figure 17). Pairwise tests indicated that 200m samples differed to all other depths except for 1500m, but all other depth pairs were equal. These differences could reflect that the deep waters are a more variable environment than the deep sediment. Although there was a significant relationship between the benthic and pelagic bacterial assemblages at the station level, the correlation was only weak (RELATE: ρ=0.352, p=0.009).

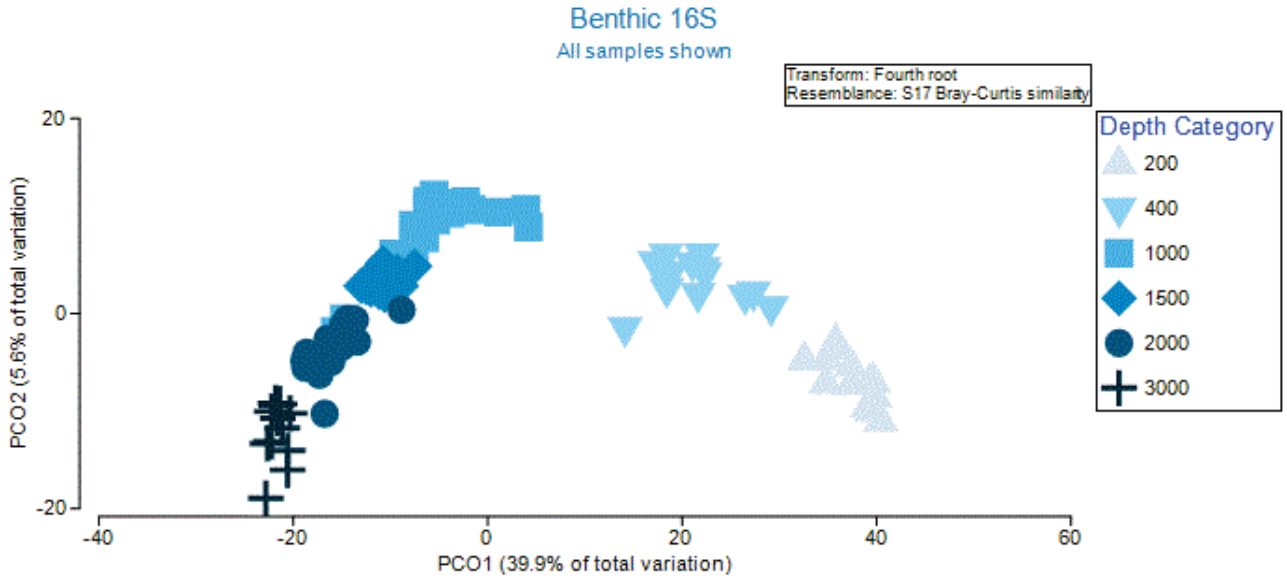


Figure 15. Relationships between bacterial benthic samples as visualised via principle co-ordinates analysis.

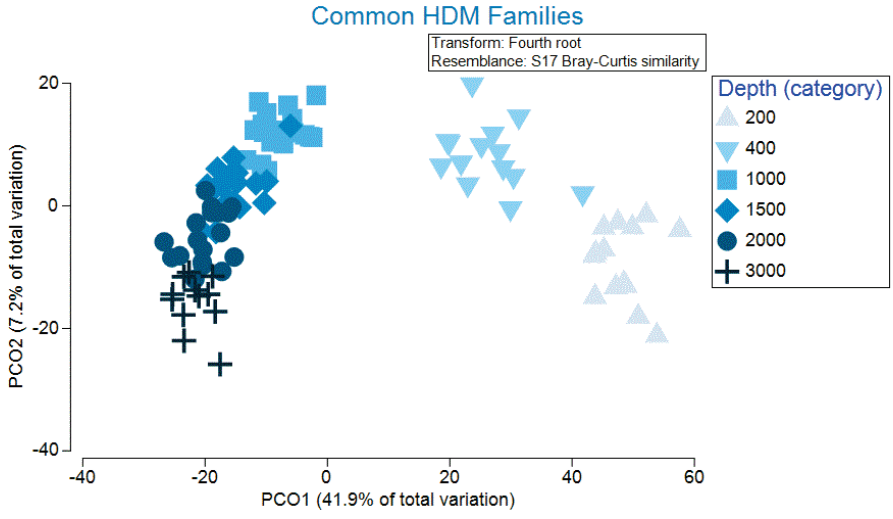


Figure 16. Relationships between families containing known hydrocarbon degrading taxa in benthic samples as visualised via principle co-ordinates analysis.

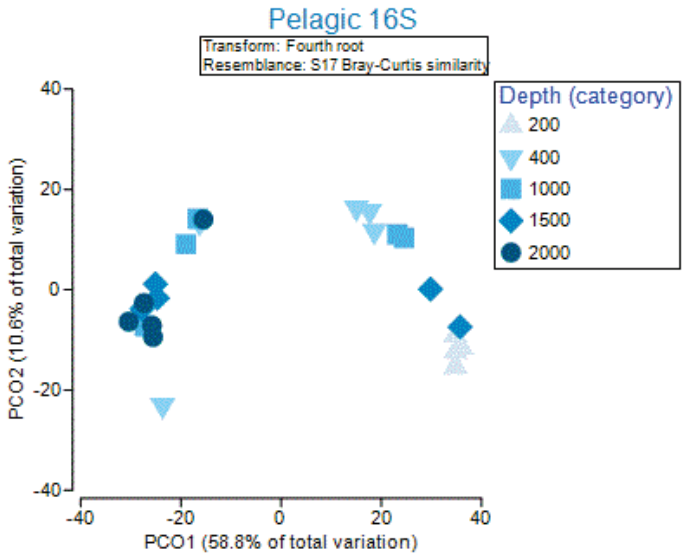


Figure 17. Relationships between the bacterial composition of different pelagic samples as visualised via principle co-ordinates analysis.

The relationships amongst the Archaeal communities in benthic samples are shown in Figure 18. Although the depth clusters are not as tight as observed in the benthic bacterial samples, close relationships amongst the same depth and among shelf (200-400m) or slope (>1000m) samples are apparent. There is less spatial resolution between the samples collected from depths greater than 1000m, as shown in Figure 18, but this may be a result of fewer OTU's being present in the sample. Again, there is an interaction between depth and transect (PERMANOVA: $F_{19,70}$=2.20, p<0.001). Pairwise tests indicated that at shallower depths (200-1000 m) transect 5 is generally different to the other transects, at 1500 m, transect 1 is different, and at 2000 and 3000 m there are no differences between transects. The composition of the archaeal assemblage is highly correlated with that of the bacterial assemblage (RELATE: ρ=0.895, p<0.001).

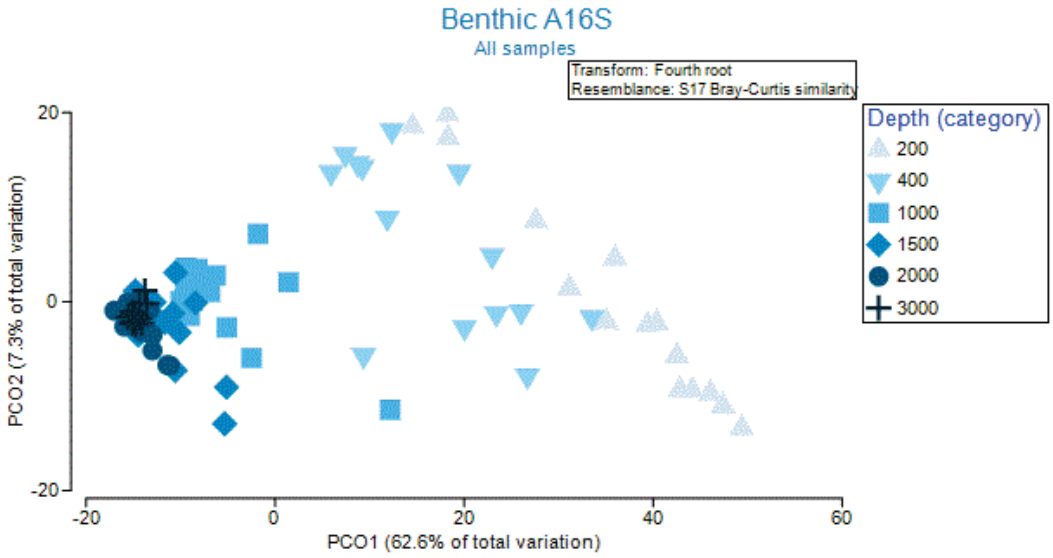


Figure 18. Relationships between the archaeal composition of different benthic samples as visualised via principle co-ordinates analysis.

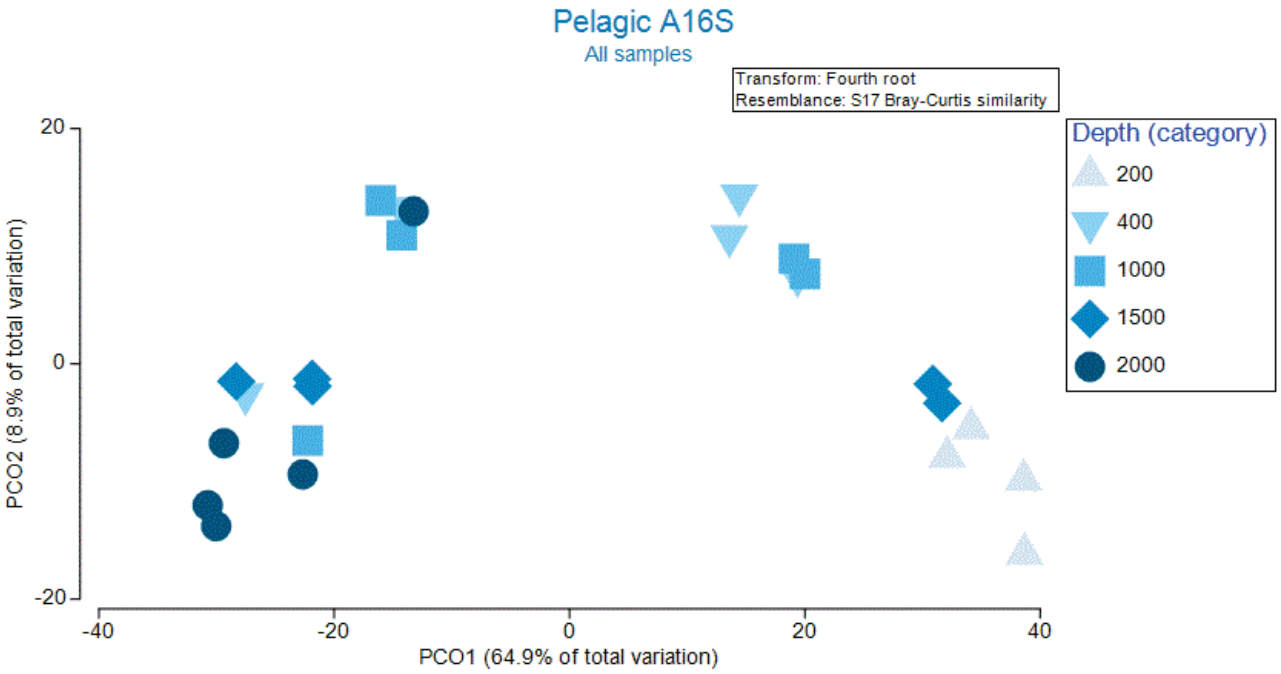


Figure 19. Relationships between the archaeal composition of different pelagic samples as visualised via principle co-ordinates analysis.

The relationships amongst the Archaeal communities in pelagic samples are shown in Figure 19. Again, assemblages separated out by depth (PERMANOVA: $F_{4,16}$=3.44, p=0.005), but not transect (PERMANOVA: $F_{4,16}$=0.673, p=0.70), and there was higher variation between samples at each depth than for benthic assemblages, suggesting that there may be greater environmental variability in oceanic waters than in sediment. The composition of the archaeal assemblage is very highly correlated with that of the bacterial assemblage (RELATE: ρ=0.965, p<0.001). Again, while the relationship between benthic and pelagic assemblages was significant, it was only weak (RELATE: ρ=0.419, p=0.005).

The relationship between community composition and environmental variables was determined via distance-based linear modelling. For benthic bacteria, the final selected model (r^2=0.60) only contained temperature and depth, with none of the sediment variables being important (Table 3). For benthic archaea, the best model also included coarse sand (r^2=0.73). Much less of the variation in pelagic microbial assemblages was explained by the measured environmental variables, with the best model for bacteria containing temperature and oxygen (r^2=0.31), and for archaea temperature only (r^2=0.21). Transect never had a significant relationship with community composition (F < 1; P > 0.312).

Table 3. Relationships between community composition and environmental parameters as determined by distance-based linear modelling. Only variables contributing to the final selected model are included. Values for Pseudo-F and P represent values for marginal tests for each variable individually.

Variable	Pseudo-F	P value
16S Benthic		
Temperature	35.27	< 0.001
Depth	23.22	< 0.001
A16S Benthic		
Temperature	58.52	< 0.001
Depth	27.42	< 0.001
Coarse sand (0.5-1 mm)	21.783	< 0.001
16S Pelagic		
Temperature	6.46	0.003
Oxygen	4.16	0.015
A16S Pelagic		
Temperature	6.24	0.002

To further explore these relationships, they were visualised with both principle co-ordinates analysis and distance based redundancy analysis. Both approaches showed similar relationships between environmental variable towards and community composition. For instance, for benthic bacterial samples (Figure 20), both analyses indicated that depth was the major environmental variable separating stations from 1000-3000m, while temperature and sediment grain size composition played a greater role in separating out 200-1000m stations. For reference, the sediment grain size distributions are provided in Table 4. There are slight differences in the influence of clay between the two analyses. There are also strong relationships between the pelagic bacteria assemblage and environmental variables (Figure 21), particularly nutrient concentrations and depth. The environmental factors that are important are similar in both analyses, but the relationships between them are different depending on the analysis type.

Similar relationships are seen between archaeal community composition and environmental variables. For instance, as shown in Figure 22, depth, temperature, and sediment grain size are the primary determinants of archaeal community composition, although the relationships between these variable differ depending on the analysis tool used. For pelagic samples, depth, nutrient levels and temperature are primarily influencing differences in community composition, as shown in Figure 23. Again, while both types of analysis identify the same environmental variables as being important influencers of Archaeal biodiversity, the relationships between these parameters differs depending on the analysis type used.

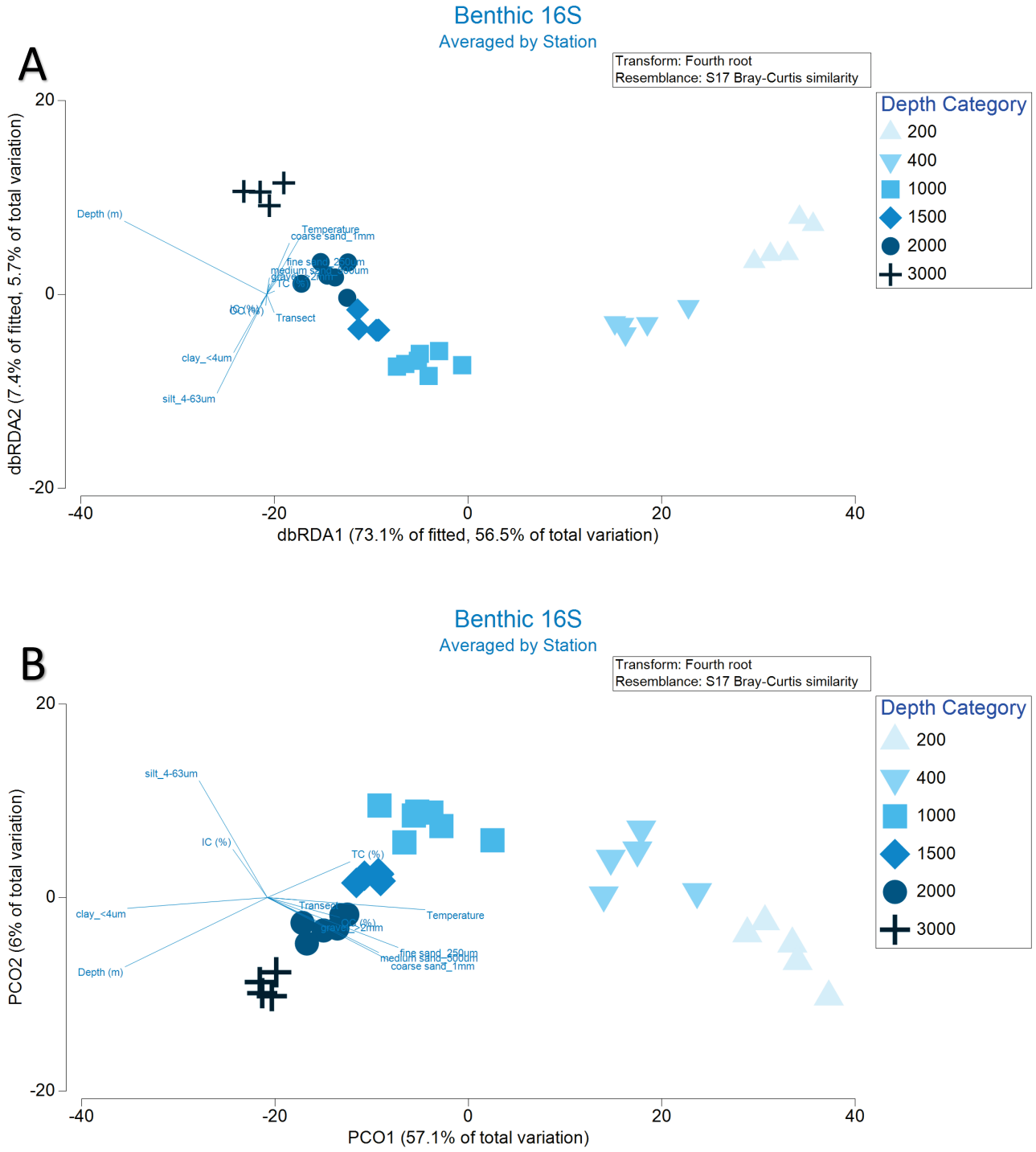


Figure 20. Relationships between community composition in benthic bacterial samples and environmental variables as determined via distance-based redundancy analysis (panel A) or principle co-ordinates analysis (panel B).

Table 4. Sediment grain size distribution (%) with depth for each of the sampling transects.

Transect	Depth (m)	Clay <4µm	Silt 4-63µm	Very fine sand 63-125µm	Fine sand 125-250µm	Medium sand 250-500µm	Coarse sand 0.5-1mm	Very coarse sand 1-2mm	Gravel >2mm
1	200	13.86	0.16	25.42	22.43	24.47	17.02	10.51	0.74
2	200	14.5	0.16	25.84	19.41	20.35	16.67	17.56	2.48
3	200	15.22	0.13	23.83	18.84	23.19	20.26	13.76	4.52
4	200	14.55	0.15	16.64	14.18	23.11	23.42	22.49	9.72
5	200	13.23	0.15	21.88	16.16	16.02	16	29.8	17.59
1	400	10.5	19.27	41.94	21.22	13.52	3.56	0.49	0.21
2	400	10.84	0.75	56.26	24.51	12.98	4.26	1.25	3.5
3	400	11.48	15.13	43.39	23.34	13.6	4.05	0.49	0.74
4	400	9.8	0.64	44.59	23.05	19.08	8.8	3.85	0.38
5	400	11.08	0.61	55.03	23.96	11.98	5.47	2.94	0.51
1	1000	4.31	30.02	53.14	9.02	5.01	2.68	0.14	3.49
2	1000	4.36	27.32	56.37	7	5.29	3.74	0.29	1.2
3	1000	4.07	32.08	44.48	7.78	8.59	6.63	0.44	12.39
4	1000	4.13	36.82	52.72	6.07	2.97	1.4	0.02	6.93
5	1000	4.83	25.54	47.05	16.6	8.24	2.09	0.48	0.21
1	1500	2.71	37.53	47.11	5.55	5.29	4.12	0.4	2.96
2	1500	2.69	2.4	61.69	10.7	12.19	11.49	1.52	1.99
3	1500	2.73	33.24	45.06	8.17	7.61	5.35	0.57	1.34
4	1500	2.77	40.77	40.64	5.55	5.55	6.23	1.26	7
5	1500	2.74	31.6	47.13	7.99	6.52	6.08	0.67	6.55
1	2000	2.28	34.16	45.15	6.4	6.94	6.55	0.8	11.84
2	2000	2.31	38.45	39.42	6.82	7.28	6.9	1.13	0.73
3	2000	2.3	31.34	30.66	8.36	12.91	14.5	2.23	0.07
4	2000	2.38	37.61	42.96	5.62	6.09	6.54	1.16	12.56
5	2000	2.34	45.84	42.66	4.68	3.5	2.96	0.36	6.75
1	3000	1.78	53.43	35.63	6.95	3.21	0.77	0	0
2	3000	1.89	51.81	32.3	8.33	5.64	1.89	0.04	0
3	3000	1.89	28.61	36.51	20.55	13.35	0.98	0	0
4	3000	1.81	36.85	39.29	11.81	9.73	2.33	0	0

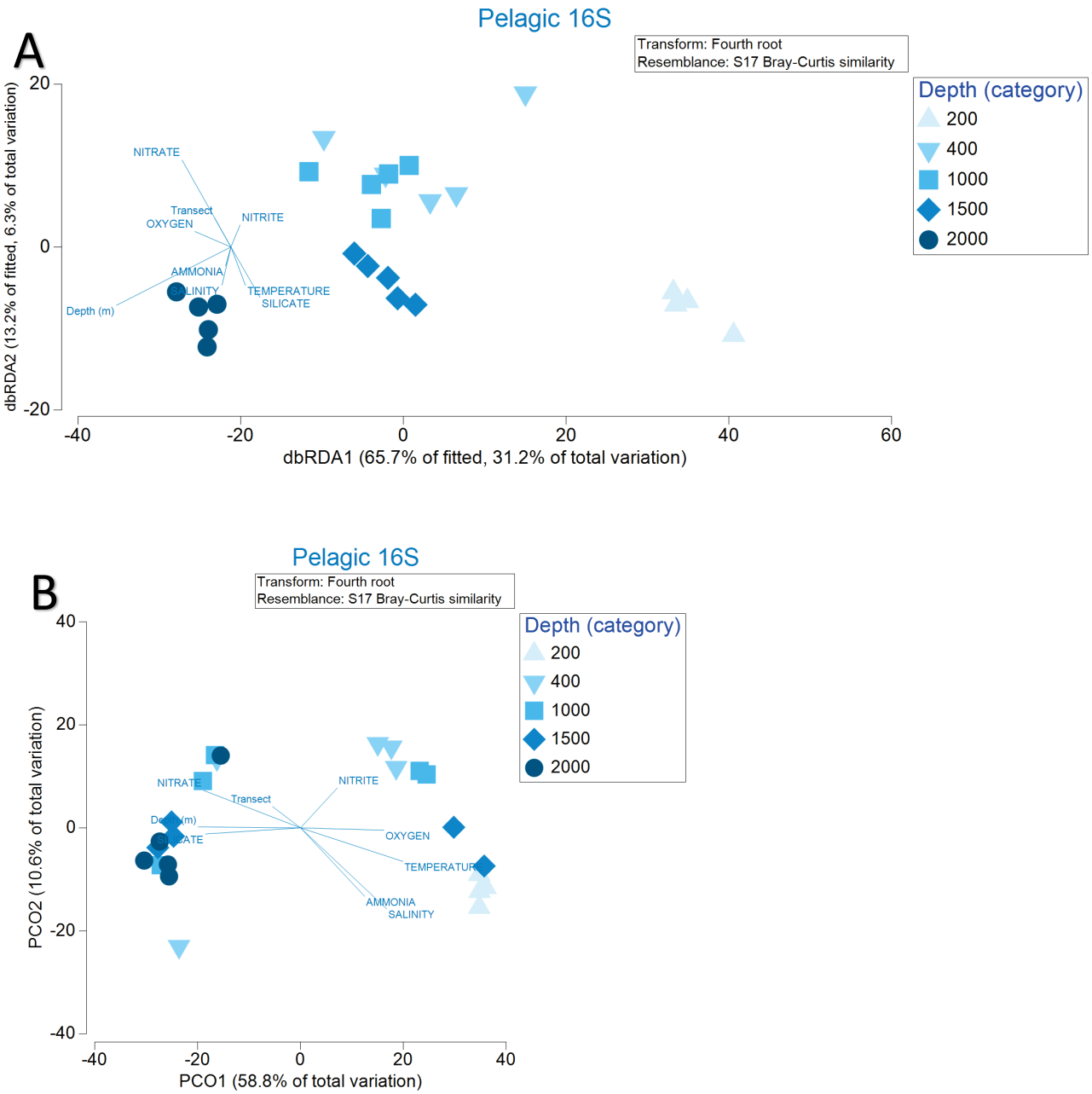


Figure 21. Relationships between community composition in pelagic bacterial samples and environmental variables as determined via distance based redundancy analysis (panel A) or principle co-ordinates analysis (panel B).

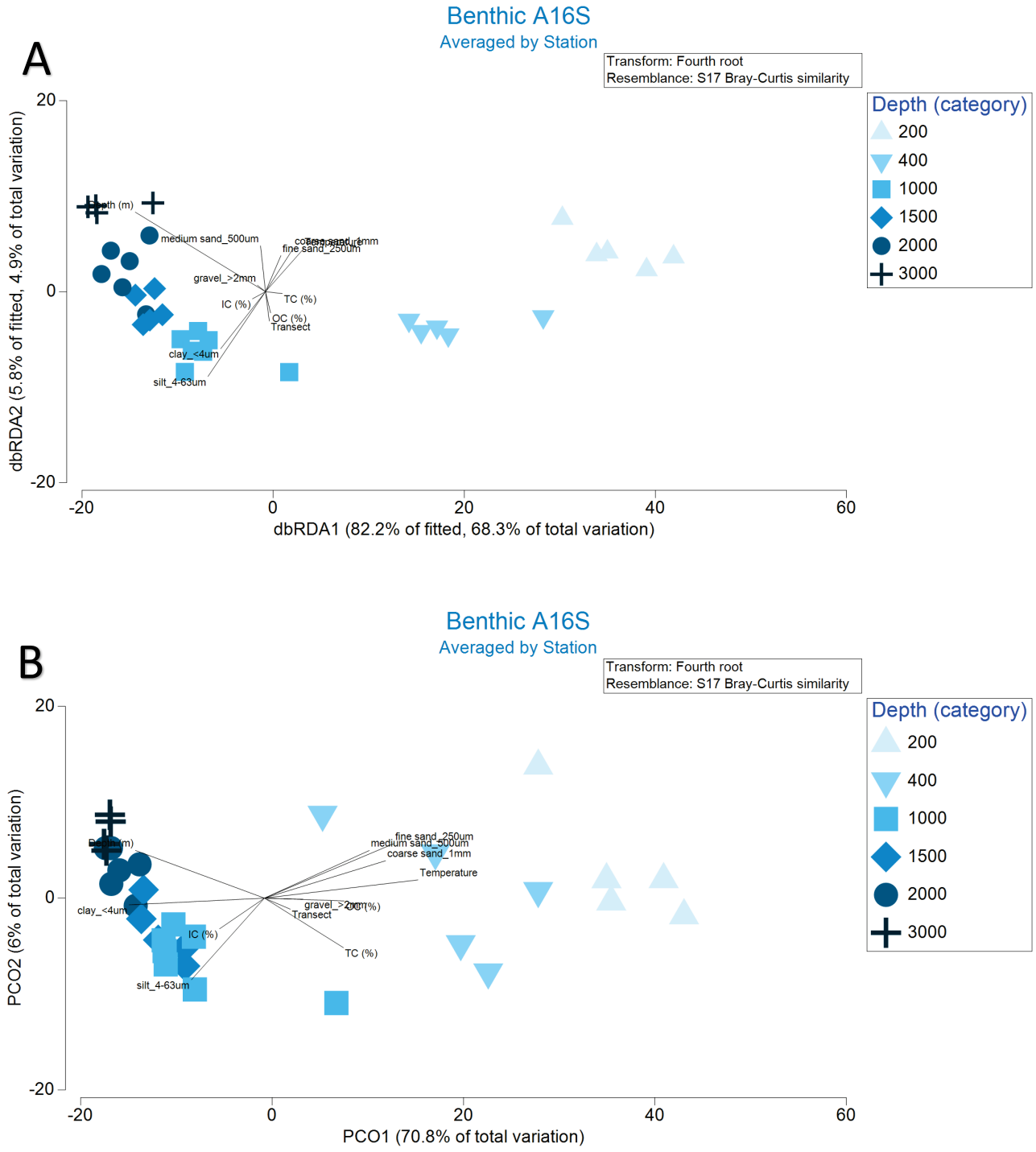


Figure 22. Relationships between community composition in benthic archaeal samples and environmental variables as determined via distance based redundancy analysis (panel A) or principle co-ordinates analysis (panel B).

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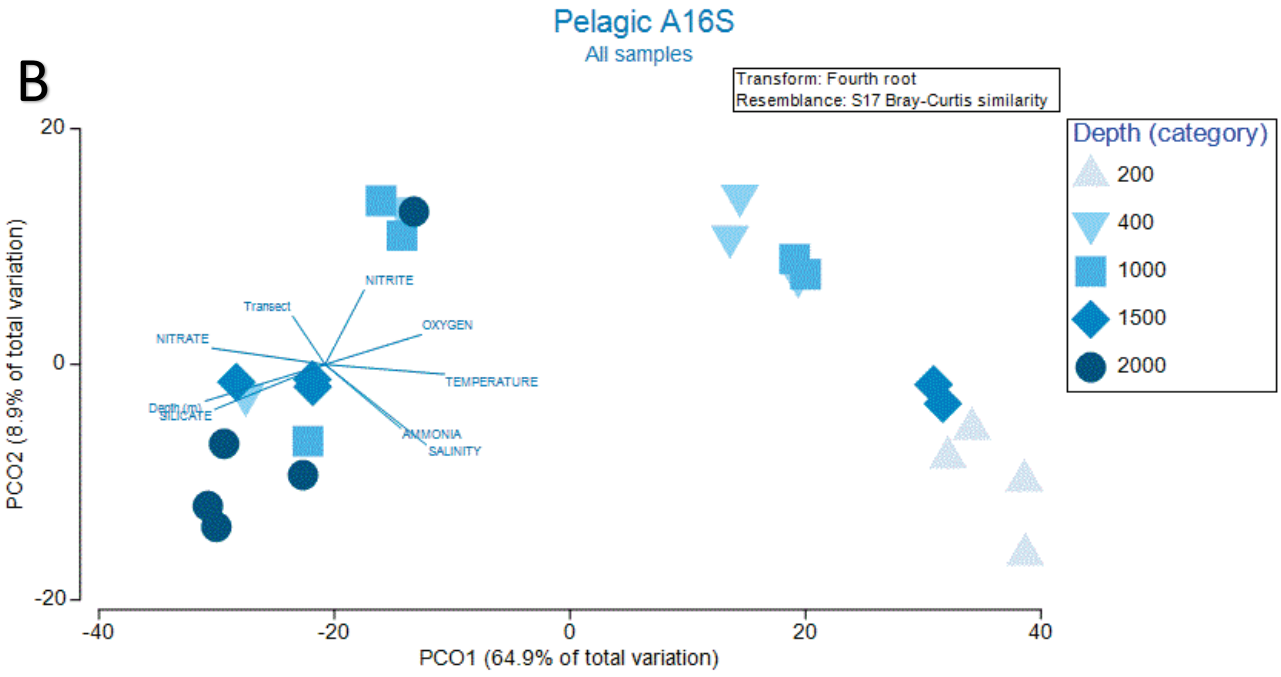

Figure 23. Relationships between community composition in pelagic archaeal samples and environmental variables as determined via distance based redundancy analysis (panel A) or principle co-ordinates analysis (panel B).

4 Discussion

The study presented in this report provides important baseline information about the indigenous microorganisms in the Great Australian Bight. At the stations surveyed, there is a greater diversity in the bacterial community than in the archaeal community. The major taxonomic groups present in both benthic and pelagic samples were identified. The composition of the benthic samples was more homogenous in samples collected along different transects but at the same depths than were the pelagic samples, as indicated by the relatedness in the PCO plots. There was an overall decrease in species diversity and evenness with depth in the benthic samples, but not the pelagic samples, likely due to the increased inter-station variability. Community composition of both Bacteria and Archaea differs between the shelf and the slope sites. For benthic samples, differences in community composition were best predicted by depth and temperature, although there were also strong relationships to sediment grain size, although these variables were poorly represented in the final models as they were highly correlated to depth and/or temperature. For the pelagic samples, temperature and oxygen level were the best predictor variables, although nutrient concentrations and depth were highly correlated to these, and also good predictors in their own right. At all stations surveyed, bacteria related to known hydrocarbon degrading taxa were identified. However, bacteria related to hydrocarbon degrading taxa likely do not all have the capacity to degrade hydrocarbons, and may instead fill other ecological roles. The distribution of microorganisms with the capacity to degrade petroleum will be best understood using a weight of evidence approach – incorporating both the community structure discussed in this report as well as the functional genomics discussed in the companion report (Hook et al., 2016). The GAB is relatively pristine, consequently about 1% of taxa would be expected to have the ability to degrade oil (Atlas 1995).

The rate at which these indigenous bacteria would respond to a hydrocarbon source such as an oil spill is not known. Since the GAB has few known sources of hydrocarbons (either as seeps or from anthropogenic contamination), the indigenous microbes may not respond as quickly to an oil spill as was seen in the GOM (e.g. Hazen et al. 2010) or as would be expected in other, more contaminated environments (Bargiela et al. 2015). Bacteria in environments with continuous inputs of crude oil are expected to respond more quickly to pollution events (Bargiela et al. 2015). Mesocosm studies or controlled releases may be the best means of determining the rate of response of the indigenous microbial community within the GAB.

Numerous studies have shown that known hydrocarbon degrading bacteria, including members of the ɣ-Proteobacteria, were more abundant in sediment and water samples impacted by the *Deepwater Horizon* wellhead blowout (Hazen et al. 2010, Kostka et al. 2011, Kimes et al. 2013). In addition, following the *Deepwater Horizon* oil spill, there was a change in the abundance of hydrocarbon degrading bacteria that corresponded with the predominant petroleum hydrocarbons in the water column (Chakraborty et al. 2012, Dubinsky et al. 2013). Although multiple modes of hydrocarbon metabolism were occurring simultaneously, the relative dominance of each, inferred from taxonomic composition as measured using the Phylochip, changed as the oil was captured and the well shut in. When oil was freely flowing, *Oceanospirillaceae* and *Pseudomonas* were abundant in the water column, but their abundance declined after the wellhead was shut (Dubinsky et al. 2013). The PAH degrading bacterial groups including *Colwellia, Cycloclasticus,* and *Pseudoalteromonas,* as well as the methanotroph *Methylomonas,* were abundant for a longer time period. Distance from the wellhead, temperature and nutrient availability did not seem to change the bacterial composition within the plume – only the chemical composition of the oil itself did (Chakraborty et al. 2012, Dubinsky et al. 2013).

Bacteria from related taxonomic groups to those found after the *Deepwater Horizon* spill have been identified in our initial analysis of samples from the GAB. Potential hydrocarbon degraders in the α, δ, and ɣ Proteobacteria were all identified, as were methane degrading bacteria. This suggests that the microorganisms present within the GAB have at least some indigenous metabolic capacity to oxidise hydrocarbons. This could be important, as previous studies have shown that the microbial community structure likely controls the potential of an ecosystem to degrade oil (Kostka et al. 2014). However,

measuring relatedness to hydrocarbon degrading bacteria alone may not predict all bacteria with the capacity to degrade hydrocarbons, as some studies have shown that the capacity to degrade PAHs in particular is encoded on a plasmid and may be conferred by horizontal gene transfer (Louvado et al. 2015).

Determining which bacteria will respond to a rapid influx of oil, as occurs in an oil spill, will be more challenging. Some reports from the *Deepwater Horizon* oil spill indicate that the microbial community was dominated temporarily by a few, rare, specialist oil degrading taxa (Yang et al. 2016). The bacteria that responded had been rare before the spill (Kleindienst et al. 2015) and were rare once increased concentrations of hydrocarbons could no longer be detected in the water column. Known hydrocarbon degrading bacteria, including *Oceanospirillales, Alcanivorax, Marinobacter*, as well as *Methylococcaceae* and *Methylocystaceae,* were detected but were not differentially abundant between sites near the well head and reference sites one year after the *Deepwater Horizon* wellhead blowout (Yergeau et al. 2015). How the microbial assemblage present in the GAB will actually respond to the presence of oil can only be determined by measuring changes in their abundance following an input of oil.

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