

**Environmental Controls on the Diversity and Distribution of Endosymbionts Associated
with *Phacoides pectinatus* (Bivalvia: Lucinidae) from Shallow Mangrove and Seagrass
Sediments, St. Lucie County, Florida**

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Abstract

Lucinid bivalves are capable of colonizing traditionally inhospitable shallow marine sediments due to the chemosynthetic metabolic functions of bacterial endosymbionts located within their gills. Because lucinids can often be the dominant sediment infauna species, defining their abundances and roles in sediment and pore fluid biogeochemical cycling is necessary to understand coastal biological diversity and the sensitivity of threatened coastal ecosystems over time. However, there has been limited research done to evaluate the diversity and distribution of many lucinid chemosymbiotic systems. The goals of this study were to assess the distribution of the lucinid *Phacoides pectinatus* and its endosymbiont communities from Ft. Pierce, St. Lucie County, Florida, and to define the environmental controls on potential free-living *P. pectinatus* endosymbionts to understand symbiont distribution patterns and host acquisition. Nearly all of the encountered *P. pectinatus* hosts were confined to within one meter of the mangrove canopy. *P. pectinatus* distribution correlated to concentrations of organic carbon in the sediments, but not to total dissolved sulfide concentrations or sea grass vegetation densities. Sequencing of bacterial 16S rRNA genes from sediment and pore fluids from the *P. pectinatus* habitat revealed that Proteobacteria dominated the communities, including Alpha-, Delta-, and Gammaproteobacteria classes. Other major phyla included the Bacteroidetes, Chloroflexi, Planctomycetes, and Spirochaetae. 16S rRNA genes obtained from *P. pectinatus* gills and feet were closely related to novel bacterial communities comprised of *Sedimenticola*, *Kistimonas*, *Methylomarinum*, and *Spirochaeta* spp., unclassified Rickettsiales (Alphaproteobacteria) and unclassified Lentisphaerae. This level of potential endosymbiont diversity has not been previously reported for lucinids. Moreover, potential endosymbiont populations differed by lucinid location, as gills

containing higher Rickettsiales abundances occurred in areas with the lowest clam density and hosts from deeper sediments contained a higher proportion of *Methylomarinum*. Despite broad sediment and pore water bacterial diversity, no genetically similar sequences that matched those from *P. pectinatus* hosts were retrieved from the environment. These results, while strengthening our understanding of a lucinid-symbiont system, still highlight how limited our knowledge is about these systems. These results provide new directions for future research and can be used to help understand how sensitive these systems are to environmental change.

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CHAPTER ONE: INTRODUCTION

Coastal ecosystems are important to global geochemical cycles (Jickells, 1998; Scavia *et al.*, 2002; Bianchi, 2006) particularly seagrass beds and mangrove coastlines that are at risk for being diminished or lost (Valiela & Cole, 2002; Fourqurean *et al.*, 2012). However, microbial taxonomic and functional diversity largely responsible for sulfur, nitrogen, and carbon cycling in shallow coastal marine sediments associated with seagrass and mangroves still remain poorly understood. Some microbes colonizing these sediments are free-living cells that can also live in chemosymbiotic associations with lucinid bivalves (clams). Lucinid-bacteria chemosymbiotic systems represent one of the only associations where the host uptakes symbionts through environmental acquisition (Gros *et al.*, 1996). It is unclear how habitat loss or changes to the environment, such as sea-level-rise, will affect the microbial communities in marine sediments and influence the transmission of potential bacterial symbionts for lucinids. As lucinids often are the sole burrowing infauna species in their sediment habitats, a reduction in bacterial communities capable of being endosymbionts may have unknown consequences to overall coastal communities and ecosystems. Before addressing how natural or anthropogenic environmental changes will, or already have, affected lucinid populations, comprehensive investigations are needed to understand each lucinid chemosymbiotic system, how lucinids are distributed throughout the environment, what endosymbiont diversity exists, and if regionalized observations of bacterial diversity distribution patterns for potential lucinid endosymbionts in a habitat are mirrored within the hosts. The goals of this thesis research were to evaluate the distribution of *Phacoides pectinatus* and its endosymbiont communities from Ft. Pierce, St.

Lucie County, Florida, and to define the environmental controls on potential free-living *P. pectinatus* endosymbionts to understand symbiont distribution patterns and host acquisition.

Background

Discoveries of chemosymbiosis between marine invertebrates and microbes occurred originally at hydrothermal vents (Cavanaugh *et al.*, 1981; Felbeck, 1981; Jannasch, 1984). This work led to the recognition of chemosymbiotic associations in numerous other marine environments (Glover *et al.*, 2004; Dubilier *et al.*, 2008; Glover *et al.*, 2008; Brissac *et al.*, 2011; van der Heide *et al.*, 2012), although, research interests have largely focused on associations from hydrothermal vents and cold seeps (Cavanaugh *et al.*, 2006; Dubilier *et al.*, 2008). Chemosymbiotic relationships have also been identified in underexplored, yet far more accessible, shallow marine habitats (Dubilier *et al.*, 2008).

The evolutionary success of bacterial chemosymbiosis is evident from the known presence of bacteria within hosts belonging to seven animal phyla (Stewart *et al.*, 2005; Dubilier *et al.*, 2008). Throughout the Bivalvia, symbiotic relationships are common in the Solemyidae, Lucinidae, Vesicomyidae, Thyasiridae, Mytilidae, Manzanellidae, and Teredinidae (Von Cosel & Olu, 2009; Anderson, 2014). The evolutionary emergence of chemosymbiosis in Bivalvia appears to have had multiple origins that lead to different Bivalvia-Bacteria associations (Anderson, 2014). Although fossil Solemyidae reveal the emergence of chemosymbiosis as early the Ordovician (Taviani *et al.*, 2011), lucinid fossil records extend to the Silurian (Liljedahl, 1991; Taylor & Glover, 2006). Lucinids have the greatest taxonomic diversity among all the Bivalvia families and also have more chemosymbiotic associations than other bivalves, which makes the Lucinidae a good evolutionary system to study (Taylor *et al.*, 2011). Fossil

lucinids can be used as paleoindicators of ancient marine habitats, although there still needs to be a better understanding of extant lucinid ecology in order to interpret paleoecological information from lucinid shells and assemblages (Anderson, 2014).

Understanding modern lucinid ecology is potentially challenging because they occupy a wide range of habitats, including fjords, mangrove swamps, seagrass beds, deep-sea cold seeps, and hydrothermal vents (Glover *et al.*, 2004; Dubilier *et al.*, 2008; Glover *et al.*, 2008; Brissac *et al.*, 2011; van der Heide *et al.*, 2012). Lucinids also live in strong redox gradients that become established between a consistent supply of hydrogen sulfide and oxygen in shallow, near-shore environments, usually at sediment depths of 20 cm or greater (Gros *et al.*, 1996; Stewart *et al.*, 2005; van der Heide *et al.*, 2012). Hydrogen sulfide is primarily produced by free-living sulfate-reducing microorganisms in shallow marine sediments where there is a constant flux of organic material. Lucinids burrow across the redox interface with their foot to increase substrate uptake (Dufour & Felbeck, 2003; Stewart, 2011) and to overcome potential thermodynamic issues related to the rates of abiotic oxidation of hydrogen sulfide being more favorable at higher oxygen concentrations (Stewart, 2011). The sulfide-oxygen redox gradient is also important because all lucinids studied to date have endosymbionts from the class Gammaproteobacteria that are thought to oxidize reduced sulfur compounds, like hydrogen sulfide. However, knowledge about the potential metabolic versatility of lucinid endosymbionts is still relatively poor, such as whether symbionts can utilize other reduced compounds like hydrogen or methane.

Additionally, the degree to which lucinids impact their environment has been understudied, as well as how changes in near-shore habitats may impact the lucinid system. Lucinid burrowing and active metabolism can affect sediment pore water geochemistry, nutrient

suspension, and vegetation density (Arndt *et al.*, 2001; Reynolds *et al.*, 2007). Although vegetation provides organic matter and oxygen to the sediment of a lucinid habitat, and dense seagrass root systems have the potential to encapsulate finer sediment and maintain anoxic conditions, plant productivity depends on the capacity of lucinids to remove hydrogen sulfide, a known plant toxin (Reynolds *et al.*, 2007; Stanley, 2014). Chemosymbiotic lucinids are thought to moderate hydrogen sulfide levels for seagrass and play an important role in the overall health of nearshore marine ecosystems (Bagarinao, 1992; Borum *et al.*, 2005; van der Heide *et al.*, 2012). In turn, seagrass beds offer lucinids a sanctuary from predation and possibly a nutrient reservoir (Bologna & Heck, 2002; Boström *et al.*, 2006).

Lucinid Symbiont Acquisition, Identity, and Ecology

Vertical transmission is the most common mechanism for symbiont acquisition among the Bivalvia, whereby juveniles inherit endosymbionts from the previous generation (Decker *et al.*, 2013). In contrast, the primary mechanism for lucinids to acquire their endosymbionts is through horizontal transmission directly from the environment, without host displacement of bacteria (Gros *et al.*, 2003a; Gros *et al.*, 2003b; Caro *et al.*, 2007; Brissac *et al.*, 2009; Gros *et al.*, 2012). Evidence that lucinids continuously obtain endosymbionts from their surroundings comes from repeated starvation experiments (Caro *et al.*, 2009) and from the inability of endosymbionts to reproduce while isolated within the bacteriocyte (Caro *et al.*, 2009). Nonetheless, bacterial release by a lucinid host has yet to be witnessed (Brissac *et al.*, 2009). Consequently, if bacteria are acquired from the habitat and bacteria are not expelled from hosts, then there is potential that the bacterial composition within a host may change several times

within one lifespan (Reynolds *et al.*, 2007; Gros *et al.*, 2012). Repeated environmental acquisition throughout a host's lifespan may reflect the geospatial distribution of the host or of the free-living bacteria (that can become endosymbionts) through time. Symbiont acquisition from the environment, after the host larval stage, also means that bacterial symbionts must be free-living within a lucinid habitat and must also survive in the environment without a host. Bacteria closely related, if not genetically identical, to lucinid endosymbionts have been found from marine sediments where lucinids have been retrieved (Gros *et al.*, 2003b; Green-García & Engel, 2012). But, sulfur-oxidizing symbiont populations in that habitats are usually rare compared to the overall bacterial diversity (Green-García & Engel, 2012). In general, there is insufficient information about the distribution and genetic and functional diversity of bacteria within shallow marine sediments where lucinids live, mostly because only a few taxa have been investigated previously (Green-García & Engel, 2012). Horizontal symbiont acquisition is unique to lucinids, and bacteria associated with other chemosymbiotic non-lucinid bivalves (i.e. mussels) have yet to be discovered within the environment.

Regardless of the habitat, lucinid bacterial endosymbionts are considered to be strictly monophyletic and sulfur-oxidizers from the Gammaproteobacteria class (Durand & Gros, 1996; Durand *et al.*, 1996). All known (i.e. published) descriptions of the lucinid endosymbionts include that they oxidize reduced sulfur, specifically hydrogen sulfide, and fix carbon dioxide into consumable organic carbon that is used by the host (Distel *et al.*, 1988). A recent metagenomics study (A.S. Engel, unpublished) of the lucinid species *Phacoides pectinatus* from St. Lucie County, Florida, provides evidence that there is greater genetic diversity among lucinid endosymbionts than previously known, and that metabolism beyond sulfur cycling is possible,

specifically for nitrogen and methane. To date, no lucinid symbionts have been shown to utilize methane, which could potentially mean that *P. pectinatus* is the first lucinid to have a dual symbiotic system. This has significant implications for how we view extant and extinct lucinid chemosymbiosis. Several other chemosymbiotic bivalves, such as the mytilid mussels, also have sulfur-oxidizing and methane-oxidizing dual symbionts, although these discoveries have almost exclusively been limited to deep marine habitats (Fisher *et al.*, 1993; Distel *et al.*, 1995; Duperron *et al.*, 2005).

The sensitivity of the lucinid-bacteria chemosymbiotic system in shallow marine ecosystems is unknown. The distribution and diversity of free-living bacteria in lucinid habitats, that may become lucinid endosymbionts, could be impacted by natural and anthropogenic perturbations, such as sea-level rise due to climate change, or urban development and ecotourism (Borum *et al.*, 2005; Jackson, 2008). Hypothetically, sea-level changes affect the depth of sediment redox gradients, with deepening water columns causing lucinids to burrow at shallow rather than deeper depths in sediment. This may put lucinids at risk for predation, but also may affect vegetation productivity if sulfide is not removed sufficiently. Changes in water depth may also affect the type of redox metabolism of endosymbionts, such as transitioning from sulfur oxidation to methane oxidation because of changes in reduced gases availability in the sediments (Schmaljohann, 1996). To our knowledge, there has been limited research to understand the biogeochemistry and ecology of free-living sulfur-oxidizing symbionts within marine sediments, and to our knowledge there has been almost no research to evaluate the role of other potential free-living symbionts, like methane-oxidizers from sediments associated with lucinids.

Phacoides pectinatus

Phacoides pectinatus, formerly *Lucinia pectinata* (Gmelin, 1791), is a large thick-shelled clam species that thrives in the reducing sulfidic mud of mangrove swamps throughout the western Atlantic Ocean basin (Narchi & Farani Assis, 1980; Frenkiel *et al.*, 1996). In addition to potentially having novel endosymbiont communities (Engel, unpublished), *P. pectinatus* contains other unusual features not observed in lucinids, such as a line of discrete mantle gills around the anterior ventral mantle, intracellular sulfur bodies, large lysosomes, and high hemoglobin concentrations within the gills (Narchi & Farani Assis, 1980; Frenkiel *et al.*, 1996; Liberge *et al.*, 2001). Unlike the seasonal reproductive capacity expressed by several other bivalves and some lucinids, a large proportion of a *P. pectinatus* population can be mature all the year round (Frenkiel *et al.*, 1997). As a protandric species, up to 90% of a population may be males in a young community, but the proportion of females rises to 50% once the community reaches an average size of 50 mm (Frenkiel *et al.*, 1997). Reproduction occurs by gelatinous egg mass and intracapsular development, which have only been reported for five other bivalve species (Collin & Giribet, 2010).

Objectives and Hypothesis

For my thesis, I wanted to determine sediment and pore water bacterial diversity from a *P. pectinatus* habitat and to evaluate how bacterial diversity may change as a function of geochemical conditions. The following research objectives were to:

- 1) Describe the physical lucinid sediment habitat, including pore water organic and inorganic aqueous and gas-phase geochemistry, sediment properties, flora, and fauna;

- 2) Characterize the abundance and diversity of free-living microbial communities from the environment, and compare to lucinid endosymbionts, by using 16S rRNA gene sequences;
- 3) Evaluate geochemical and biological controls on *P. pectinatus* and endosymbiont bacterial diversity associated with lucinids.

These objectives allowed me to test the following hypotheses:

- 1) Free-living symbiont abundances correspond to fluctuations in chemical gradients and are taxonomically more similar to each other when from similar habitats.
- 2) Lucinid endosymbionts are not species-specific to their host, but species-specificity does correspond to environmental conditions and especially variations due to vegetation type and density.
- 3) The symbiont population within *P. pectinatus* hosts and environment will express metabolic pathways capable of carbon and nitrogen cycling that are novel to lucinid chemosymbiosis due to their unique evolutionary lineage, and the extent of this function will correlate to geographic location.

CHAPTER TWO: MATERIALS AND METHODS

Study Site and Sample Collection

Wildcat Cove is a state land and aquatic preserve located on the east coast of Florida, northeast of the city of Fort Pierce, St. Lucie County (Figure 1). Much of the area surrounding Wildcat Cove is part of the Fort Pierce Inlet State Park. The cove is connected to the Indian River Lagoon, one of most biologically diverse estuaries in the United States (Young, 1974). Recent canal development, urban development, and pollution and pesticide loading have caused water quality to degrade within the lagoon (Division of Recreation and Parks, 2006). The area is underlain by organic rich sediments and soils that drain poorly, which cover the Anastasia Formation and Pamlico Sand geologic formation (Division of Recreation and Parks, 2006). At Wildcat Cove, natural soil and nutrient migration from the land to the water are disrupted by a mosquito impoundment between the cove and marsh interior, and dikes are positioned along impoundment. Healthy red mangrove forests of *Rhizophora mangle* grow along the coastline. Abundant *P. pectinatus* populations were located at this site during previous sampling trips in 2006, 2011, and 2013. Sampling focused on areas where the backwater swamp drains from canals through the mangroves into the cove and inlet. The shallow water in the cove (<2 meters) has sparse to moderate seagrass beds that include several of the species observed throughout the greater Indian River Lagoon, including *Halodule wrightii*, *Syringodium filiforme*, *Thalassia testudinum*, *Halophila johnsonii*, *Halophila engelmannii*, *Halophila decipiens*, and *Ruppia maritime* (Rey & Rutledge, 2010).

Sampling at Wildcat Cove was done during July 2014, as permitted by a Florida Fish and Wildlife Conservation Commission Special Activity License (SAL-14-1599-SR). Two different types of ecological sampling methods were used to characterize the lucinid sediment habitat ecologically and biologically and to determine lucinid abundances.

The first method employed 1 m² quadrats over two partial 10 x 10 m sampling grids (Figure 2). The quadrats provided a framework to characterize the geochemistry, vegetation, lucinid density (e.g., live and articulated dead shells), and other epi- and infauna, particularly molluscs. Articulated shells of dead lucinids represented recently deceased specimens that had undergone limited to no relocation. When comparing the articulated shells to living lucinids, shifts in the community structure, abundances, or shell morphology could indicate change in the environment (Green *et al.*, 1989; Korpanty *et al.*, 2014). Per the permit limitations, mangrove roots could not be disturbed. Sediment was excavated from up to 30 cm depth, starting from the edge of the mangrove vegetation canopy and progressing towards the open water (i.e. the cove interior) (Figure 2, blue boxes). From one grid, comprised of seven quadrats, no *P. pectinatus* specimens were encountered. Another grid was established 10 meters to the south-southwest, and closer to mangrove canopy, and 19 quadrats were sampled. Collectively the two grids resulted in the examination of 26 m² surface area and 7.8 m³ of sediment.

The other sampling method involved two 50-m long transects that paralleled the shoreline, approximately 1-3 m from the edge of the mangrove canopy, starting from quadrats 1-1, and the 1-10 from the second grid and continuing north and south away from the grid. Stations were set up every 5 m along each transect and vegetative cover at the surface was evaluated from a 70 cm² area and sediment up to 20 cm deep was sampled amounting to approximately 0.0014

m³ of material. The number of live lucinids and articulated dead shells were counted and collected from the sediment.

To correspond with the second grid, raw and filtered ocean water, and raw and filtered pore waters were collected and analyzed at six points (Figure 2, red stars). Pore water was pumped from approximately 25 cm deep in the sediments using a stainless steel piezometer and Geotech peristaltic pump (series II) with Geotech silicone tubing. Standard electrode methods were used (Eaton *et al.*, 2005). All analytical geochemistry methods were done as previously described in Green-García & Engel (2012). Briefly, pore water and ocean water pH and temperature were measured with a Fisher Scientific Accumet AP115 portable probe, and conductivity was measured with a Fisher Scientific Accumet AP75 probe. Temperature, pH, and conductivity were monitored continuously to verify ocean water was not being pumped into the piezometer sample hole. Dissolved oxygen was measured using an YSI Pro2030 dissolved oxygen and conductivity probe. Dissolved oxygen and sulfide concentrations from pore water were determined using CHEMetrics methods, specifically the Indigo Carmine and Methylene Blue methods, respectively, with a V-2000 Multi-Analyte Photometer.

Ocean and pore waters were filtered through 0.22-µm Millipore Express™ Sterivex, PES (polyethersulfone) membrane filter cartridges that were used for DNA extractions. Filtered water was collected in separate, prepared HDPE bottles for anions and cations, alkalinity, and fluorescence spectroscopy, and baked glass vials were used for total organic and dissolved inorganic carbon. Cations were preserved with trace metal grade nitric acid in the field. Alkalinity was determined after manual titration using 0.1 N sulfuric acid to an endpoint of pH 4.3. Major anions and cations were measured with Dionex (ThermoFisher) ICS2000 ion

chromatographs with standards checks accurate within two standard deviations. Concentrations of total inorganic and dissolved organic carbon (TIC and DOC, respectively) were analyzed from filtered water with a Shimadzu Model TOC- V Total Carbon Analyzer. DOC is the difference between dissolved nonpurgeable organic carbon (NPOC) and TIC. The standard used for minimum detection limit was $C_8H_5KO_4$, and the precision between replicate sample injections was 2% of the relative percent difference (RPD) for DOC >4 mg/L and 5% RPD for DOC <4 mg/L. Total nitrogen (TN) was measured by high temperature catalytic oxidation with chemiluminescence minimum detection level of 0.01 mg/L.

Pore water dissolved gases were measured from serum bottles by headspace gas chromatography and the bubble-strip method using a SRI Instruments 8610C gas chromatograph with a flame ionization detector, flame photometric detector, and a thermal conductivity detector with a methanizer. Gases of interest included CO_2 , CH_4 , H_2S , and atmospheric gases such as O_2 and N_2 .

Fluorescence data from the dissolved organic matter (DOM) of filtered water was obtained using methods of Birdwell & Engel (2010) on a Horiba Scientific Fluoromax4 spectrofluorometer with a Xenon lamp, analyzed by using a 1-cm quartz cuvette at room temperature. Excitation emission matrices (EEM) were created by collecting a series of emission scans ranging from 250-550 nm in 2.5-nm steps and excitation scans across 240-550 nm in 5-nm steps. Primary and secondary inner filter effects were corrected with absorbance (200-700 nm wavelength range) made by a Thermo Scientific Evolution 200 series spectrophotometer on deionized water. EEM spectra were corrected for Raman scattering by subtracting a DI water blank EEM spectrum Fluorescence index or FI (McKnight *et al.*, 2001),

biological/autochthonous index or BIX (Huguet *et al.*, 2009), and Humification index or HIX (Zsolnay *et al.*, 1999) were calculated with the corrected EEMs.

Sediment samples from within the quadrats were collected by push core to a depth of 5 to 10 cm, depending on the depth that oyster shell beds were encountered and could not be penetrated. Cores were sectioned immediately in the field every 1 cm. At every 5 m along the two 50-m transects, a 500 g sample of homogenized sediment was also collected. Subsamples of sediment from the quadrat cores and from the transects were analyzed using methods described in Fitch & Crowe (2010) and Green-García & Engel (2012) to determine water and organic carbon content using the loss-on-ignition (LOI) methodology.

Oceanographic and Meteorological Data

Historic meteorological data for Wildcat Cove were gathered from an open database curated by the National Oceanic & Atmospheric Administration (NOAA) (<http://www.ncdc.noaa.gov/cdo-web/>). Station FORT PIERCE 3.4 NNE, FL US GHCND:US1FLSL0015 was approximately 1 km from sampling area (Lat: 27.487° N Lon: - 80.304° W). This information provided a proxy for the hydrological stability of Wildcat Cove. In addition, local tide data were obtained from NOAA's Center for Operational Oceanographic Products and Services (<http://tidesandcurrents.noaa.gov/>) using station ID 8722212 for Fort Pierce, South Jetty, Florida.

DNA Extraction, Amplicon Pyrosequencing, and Analysis

Total nucleic acids were extracted from 0.25 g of sediment in triplicate using a MoBio PowerSoil DNA isolation kit, following manufacturer instructions. For samples with noticeably greater water content, samples were centrifuged to obtain more sediment. Ocean and pore water total nucleic acids were collected using the MoBio PowerWater DNA isolation kit, following manufacturer instructions. The volume of water filtered through each Sterivex filter was recorded and at least two filters were used at each sample location.

DNA was obtained from the gills and feet of representative *P. pectinatus* hosts from quadrats by using Qiagen DNeasy Blood & Tissue kits, following manufacturer instructions. Unused tissues and shells of individuals are vouchered as cataloged specimens in the South Dakota School of Mines and Technology, Museum of Geology, under the accession number SDSM 2014-003. Feet samples were also used for DNA extraction, based on the assumption that DNA from clams would be obtained by that no bacterial DNA would be recovered from the foot unless a host was infected; in essence, the feet served as extraction controls (Green-García & Engel, 2012). For quadrats 1-2, 1-4, 1-8, and 1-10, gills from two specimens were used from the top 10 cm and two specimens were used from the 10-30 cm sediment depth. For quadrats 1-6 and 3-10, fewer individuals were used due to lack of recovery in the top 10 cm of the sediment.

Extracted DNA quality and quantity were checked on a NanoDrop UV/Vis spectrophotometer and by running TBE electrophoresis gels. Positive PCR amplification of bacterial 16S rRNA genes was done by amplifying genes using protocols described in Green-García & Engel (2012) and examining amplified products on TBE gels with ethidium bromide staining after gel electrophoresis.

Confirmed extractions of water, sediment, gill, and feet samples were sent to the Molecular Research LP commercial facility in Shallowater, Texas, for sample purification and amplicon tag pyrosequencing on a 454 GS FLX Titanium instrument. 16S rRNA genes were amplified with primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 534r (5'-ATTACCGCGGCTGCTGG-3') to obtain reads for the V1-V3 region of the gene on a 454 GS FLX Titanium platform (Dowd *et al.*, 2008).

Resulting amplicons were screened and processed in the computer program MOTHUR (version 1.35.1) (Schloss *et al.*, 2011). Primers and barcodes were trimmed from the raw sequence files for each of the sample libraries. In addition, sequence reads that did not achieve a quality score of 35, contained an ambiguous base call, or comprised nine or more homopolymers were omitted from downstream analysis. After screening, sequences were aligned to the Silva rRNA gene database (release 119) (Quast *et al.*, 2013). Screening and filtering steps were used to ensure that sequences had the same start and end region of the gene. Prior to taxonomic classification with previously described reference files, chimera were removed using the algorithm chimera.uchime (Edgar *et al.*, 2011). Sequences that affiliated to mitochondria, chloroplasts, and Archaea were also removed from the sample datasets. Phylip-formatted, lower-triangle distance matrices were generated (Felsenstein, 2005). Diversity indices were calculated with MOTHUR for each individual library. Sequences were assigned to operational taxonomic units (OTUs) using average neighbor clustering based on 97% sequence identities. OTUs comprised of shared reads from across different samples were generated from merged and reprocessed files. When calculating OTUs, the unique command in MOTHUR was used to adjust for computational limitations due to the merged files sizes.

All raw sequence fastq and qual for each sample was submitted to the NCBI Sequence Read Archive (SRA) for the BioProject PRJNA300417, <http://www.ncbi.nlm.nih.gov/bioproject/PRJNA300417>).

Statistical Analyses

The degree of significance that quadrat location and depth played on organic carbon content (measured by LOI) to explain the distribution of bacterial communities was measured through analysis of variance (ANOVA) using the computer program SAS 9.4. Normality of each variable was tested using the procedure *proc univariate* and subsequent Shapiro-Wilks test. ANOVAs were performed using *proc glm*.

Bray-Curtis dissimilarity matrices were created for sediment cores, water samples, and gill and foot tissues communities. The indices were run stepwise with analysis of molecular variance (AMOVA) to determine if sub-population pools were different significantly between each other. For all analysis, an *F*-test value was reported with the *p*-value of < 0.05 representing significance. To determine the geochemical controls on water communities, non-metric multidimensional scaling (NMDS) was performed using Bray-Curtis dissimilarity matrices on water sample's OTUs (in 1,000 iterations) with the lowest recorded stress (<0.2) and R^2 values being reported. Water chemistries were subsequently added to the NMDS using the R package *vegan* and *envfit*, which fits environmental vectors onto the NMDS ordination plot to create projection points with corresponding maximum correlations (Oksanen et al., 2015). No geochemical variable were found to significantly explain the function, but NMDS stress was <0.2 and permutational multivariate analysis of variance (PERMANOVA) was calculating using

the adonis function in the vegan package for R to determine the significance of each individual environment parameter that could affect the means of bacterial community ordinations.

PERMANOVA did not identify significance among the geochemical variables, but the NMDS stress for each analysis was <0.2 and therefore reported.

CHAPTER THREE: RESULTS

Aqueous and Sediment Geochemistry

Sampling pore water chemistry along either side of the grid occurred during transitions from high to low tide over three days (Figure 3). During sampling, the sediment surface of the entire grid was always inundated. The water depth ranged across the grid from a maximum of 80 cm (high tide) to a minimum of 37 cm (low tide). As such, although positions located in row 10 were further from the coastline, water depths at time of sampling were shallower than at other the locations due to tidal fluctuations. According to NOAA's database, precipitation was measured in 12 of 20 days leading up to the sample period, with a moderate event (1.47 cm) occurring the day prior to sampling. The Fort Pierce region did not experience a drought throughout 2014.

The basic physicochemical parameters, major ion compositions, and dissolved gases for the pore waters were different than the ocean water (Tables 1 through 3). Measured pH values in the pore waters ranged from 7.05 to 7.50, and were consistently lower than the overlying ocean and adjacent marsh and canal water. Conductivity was higher in the pore waters compared to ocean water, which coincided with higher cation and anion concentrations for the pore waters than the ocean waters (Table 2). Total organic carbon content ranged from 77.35 to 26.99 mg/L, and was higher in the pore waters then ocean or marsh samples. DOC strongly correlated with alkalinity ($R^2 = 0.984$). TN ranged from 0.30 to 1.53 mg/L, with no trend with distance away from the shoreline (Table 2). Fluorescence indices FI and BIX for pore waters indicated DOC input from terrigenous sources, compared to stronger proteinaceous or microbial sources (i.e. likely from marine phytoplankton) for ocean and marsh waters (Table 3). Oxygen concentrations

in the pore waters varied from 78.0 $\mu\text{mol/L}$ to 181.9 $\mu\text{mol/L}$ based on colorimetric or electrode measurements, with generally higher values towards the cove. Sulfide concentrations ranged from 11.7 $\mu\text{mol/L}$ to 56.1 $\mu\text{mol/L}$ and there was no apparent trend with distance from the shoreline (Table 1). There was no detectable sulfide in the overlying ocean waters. The concentration of dissolved methane (CH_4) and carbon dioxide (CO_2) varied between the pore waters and ocean water (Table 3). Dissolved CO_2 concentrations for pore waters were an order of magnitude higher than ocean water, which was approximately in equilibrium with the partial pressure of CO_2 in the atmosphere (Table 3).

Sediment cores varied in length from 4 to 9 cm deep, and their depths were dictated by the position of a continuous oyster shell layer. Organic carbon content quantified from LOI measurements significantly differed when clustered by row within the sampling grid (ANOVA F-value = 6.47 and P-value = 0.0042), but not when measured by column within the grid (ANOVA F-value = 1.74, P-value = 0.1965), thereby indicating that organic carbon content in the sediments was higher in closer proximity to the mangrove canopy (Table 4).

Fauna and Vegetation of Wildcat Cove

A total of 190 *P. pectinatus* live specimens were collected from Wildcat Cove, with 140 individuals being from within the second 10 x 10 m sampling grid (Figure 4) and 50 were from the two transects parallel to the mangroves. Only nine of the 190 *P. pectinatus* were found more than one meter from the mangrove canopy. *P. pectinatus* were found at all sediment depths between 0 and 30 cm. The average shell size of live *P. pectinatus* was 2.06 cm, although the size distribution was bimodal, with peaks at 1.8 cm and 4.6 cm (Figure 5). Lucinid abundances

correlated to sediment organic carbon content (measured by LOI) in the grid ($r^2 = 0.595$) and for one of the transects ($r^2 = 0.776$). The greatest *P. pectinatus* abundances from the grid row 1, strongly correlated to sediment organic carbon content ($r^2 = 0.961$) (Table 4).

Dead but articulated *P. pectinatus* were also collected (Figure 6), with articulated dead shells being recovered from eight quadrats where no living specimens were collected. No other lucinid species or other chemosymbiotic bivalves were discovered at Wildcat Cove. However, several living non-chemosymbiotic infaunal bivalves, including *Abra* (46 individuals), *Anadara* (1), *Anomalocardia* (2), *Caryocorbula* (6), *Chione* (32), *Geukensia* (11), *Isognomon alatus* (5), *Laveicardium* (1), *Mulinia* (1), *Tagelus* (2), and four unknown taxa, were found in the grid. Articulated dead shells of *Amygdalum*, *Eurytellina*, *Grippina*, *Macoma*, *Mercenaria*, *Semele*, and *Tellinidae* were also retrieved.

The seagrass *H. wrightii* was present in the sampling grid, with coverage ranging from 0 to 60% across the second sampled grid. Column 10, closest to the marsh inlet, had the greatest seagrass coverage, and columns 2 and 4 (farthest from the inlet) had for the lowest coverage. *R. mangle* mangroves were present along the shoreline, and mangrove leaf litter and propagules were found in large quantities throughout the upper layers of the sediment across the sampling grid. Trumpet petticoat algae (*Padina pavonica*) was also identified in 14 of the 25 quadrats and represented the dominant alga encountered. Sponges were observed throughout the grid, including several fire sponge (*Tedania ignis*) specimens.

Lucinid Gill and Foot Bacterial Diversity

The taxonomic affiliations of bacterial communities retrieved from 21 *P. pectinatus* gills were evaluated from 358,707 16S rRNA gene sequence amplicons. Most of the sequence reads were affiliated with the Proteobacteria (99.70% of all the reads), as well as the Spirochaetes (0.28%) and Lentisphaerae (0.03%) phyla. Within the Proteobacteria, Gammaproteobacteria dominated (93.67% of all the proteobacterial reads), and 20 of the 21 gills analyzed were dominated by amplicons affiliated with the genus *Sedimenticola*, regardless of whether the hosts were collected from shallow sediment (<10 cm) or from deep sediment (10-30 cm), as noted on Figures 6 and 7 by “S” for shallow or “D” for deep. *Sedimenticola* spp. sequences from the lucinid tissues clustered into a total of 22 OTUs at the 97% sequence similarity level (Figure 8).

All of the *P. pectinatus* hosts analyzed shared at least one OTU with another host. A lucinid specimen from the upper 10 cm of quadrat 1-8 contained the highest OTU count (8) among the *Sedimenticola*-like sequences, whereas a specimen from quadrat 1-10 contained the lowest OTU abundance (1). *Sedimenticola* spp. belong to an unclassified clade of sulfur-oxidizers that includes other identified symbionts from marine invertebrates (Dubilier *et al.*, 2008; Carlström *et al.*, 2015), although this genus has not been previously associated with chemosymbiosis. It is unclear whether the endosymbionts from any lucinids, including *P. pectinatus*, actually belong to the *Sedimenticola* genus or whether the retrieved sequences were simply closely related to this genus. Additional research is needed to confirm this taxonomic assignment.

In addition to sequences affiliated with *Sedimenticola*, other bacterial sequences were retrieved from *P. pectinatus* hosts. Some were also affiliated with the Gammaproteobacteria,

including the genera *Kistimonas* and *Methylomarinum*. *Kistimonas* belongs to the order Oceanospirillales and represented the second highest taxonomic group for 17 of the 21 hosts examined. Hosts from quadrat 1-2 had the highest numbers of *Kistimonas* spp. reads, and a total of 68 OTUs were identified to *Kistimonas* at the 97% sequence similarity level. Clustering of the gill and foot sequences revealed that *Kistimonas*-like sequences has the largest taxonomic diversity of any bacterial group retrieved from 18 *P. pectinatus*. *Kistimonas* spp. have been observed from bivalves previously; however, the role of *Kistimonas* spp. within its host is often unclear (Jensen *et al.*, 2010). *Methylomarinum* was identified in all of the tissue samples from Wildcat Cove, was the second largest group retrieved from 17 of 21 lucinid gills, and clustered into 156 OTUs at the 97% sequence similarity level (Figure 8). Relative abundances ranged from 0.19% from hosts in the quadrat 1-6 at shallow depths to 3.46% from hosts at depth in quadrat 1-8. Overall, *Methylomarinum*-like sequence reads were more abundant from lucinid hosts collected from deeper sediment (10-30 cm) compared to shallower depths (0-10 cm) in four of the five quadrats (recall, no shallow lucinids were collected from quadrat 1-10). *Methylomarinum* spp. belong to a clade of methanotrophic bacteria that also comprises symbionts of marine invertebrates, primarily mussels (Hirayama *et al.*, 2013). No literature reports show that *Methylomarinum* spp. are associated with lucinids.

Spirochaeta is a genus of bacteria within the phylum Spirochaetes. Sequence reads affiliated with this genus were present in low relative abundances (<2% sequence reads per sample), but had higher relative abundances from individuals within quadrat 1-8. Although comparatively minor for all gill communities, *Spirochaeta* spp. have been reported previously as a potential symbiont for deep-sea (from 507 to 1691 m water depth) lucinid clams (Duperron *et*

al., 2007). Like *Sedimenticola* spp., *Spirochaeta* spp. had limited taxonomic diversity from the gills and all of the reads clustered into four groups (97% sequence similarity), with one OTU representing 92.5% of the sequences retrieved from all hosts.

16S rRNA gene amplicons retrieved from one deep lucinid host from quadrat 1-4 were predominately affiliated with an unclassified Rickettsiales, an order of the Alphaproteobacteria class. For 16 of the 21 *P. pectinatus* hosts examined, Rickettsiales made up an average of <1% of the gill communities, amounting for less than 10 sequence reads per specimen in 11 hosts (Figure 7). But, hosts from quadrat 1-4 and 1-6 contained higher abundances of Rickettsiales-like reads, with an average of 15% per host and as high 72% for a specimen from quadrat 1-4. Although these amplicons could not be assigned to a genus, the sequences fell into the LWSR-14 clade of Rickettsiales and clustered into 176 OTUs at the 97% sequence similarity level. No other reports in the literature indicate that lucinids or other chemosymbiotic bivalves, like mussels, could have Rickettsiales infections.

Sequence reads affiliated with an unclassified Lentisphaerae were retrieved as part of the gills communities, but there were generally few sequence reads and only greater than five reads for two hosts from quadrat 1-8.

In addition to the gills, one foot per lucinid host per quadrat resulted in 48,570 sequence reads combined (Figure 7). Proteobacteria dominated the sequence reads (67.67% of all the foot reads), followed by Lentisphaerae (31.80%) and Spirochaetes (0.05%). Every foot contained sequences assigned to each taxonomic group previously described from the gill tissue, but an NMDS ordination plot (Figure 9) and AMOVA tests based upon Bray-Curtis dissimilarity matrix indices based on the OTUs (Figure 10) indicated that gill and feet

taxonomic diversity was significantly different (F -value = 5.754, P -value = 0.00005).

Collectively, 31.8% of the sequences from the foot were affiliated to Lentisphaerae, but were the dominant taxonomic group in only one foot from quadrat 1-2. The most frequently encountered genus retrieved was *Sedimenticola* spp. from quadrats 1-6, 1-10, and 3-10 (56.78%, 55.77%, and 47.31%, respectively). Unclassified Rickettsiales were retrieved from quadrats 1-4 and 1-8 (74.38% and 53.77%, respectively). Several additional bacterial sequences were recovered from the feet but were not from the gills, including Fibrobacteria, Epsilonproteobacteria, Dehalococcoidia, and Deltaproteobacteria. To our knowledge, this is the first study to uncover these bacterial groups within the feet of lucinids. It is unclear whether they actively participate in a chemosymbiotic relationship, or if they indicate potential host infection or environmental contamination.

Bacterial Populations in Environmental Water and Sediment

Aqueous bacterial populations were examined from six pore water samples, one overlying ocean water sample, and two marsh water samples (collected from high and low tides). A total of 41,476 sequence reads were obtained from water. Based on 97% sequence similarity, pore water communities (679 to 1217 OTUs) were more diverse than either ocean (303 OTUs) or marsh waters (326 and 433 OTUs). Marsh and ocean waters were significantly different from pore water communities (ANOVA F = 1.598, p -value = 0.0018). An NMDS created from Bray-Curtis dissimilarity matrices is shown in Figure 11. Regardless of sample type, all aquatic communities analyzed were dominated by Proteobacteria, with Alpha- and Gammaproteobacteria representing the most abundant classes. The third most diverse class was

Dehalococcoidia from the Chloroflexi phylum. From the shore to the open water, the relative proportions of Alpha- and Deltaproteobacteria increased with decreasing abundances of Gammaproteobacteria. Alternatively, along column 10, Gammaproteobacteria increased with decreasing Alphaproteobacteria and variable Deltaproteobacteria abundances. Sequence reads affiliated with Dehalococcoidia were only retrieved in the pore waters, and relative abundances decreased from column 1 and increased along column 10 from the shore to the cove.

Dehalococcoidia, which are known organohalide-respiring anaerobic bacteria that prefer anoxic conditions (Wasmund *et al.*, 2014), were retrieved from areas with higher dissolved sulfide concentrations.

Rickettsiales were the most abundant group of bacteria retrieved from the water samples that were also identified in gill tissues. Rickettsiales had greater proportions in non-pore waters (i.e., in the ocean and marsh waters). Sequence reads affiliated with the *Sedimenticola* and *Spirochaeta* spp. were retrieved from all of the pore waters, but not in ocean or marsh waters. *Methylo Marinum* spp. were present in pore waters from quadrat 1-1 (corresponding to water sample LUC_14_004) and 10-1 (corresponding to water sample LUC_14_006). *Kistimonas* spp. were not present in any water communities. Lentisphaerae sequence reads were retrieved from water samples.

The bacterial communities from seven sediment cores are presented in Appendix B (Tables 7-11). Cores depth varied from 3 to 6 cm depending on the location of an oyster hash bed that was impenetrable with push core device. The largest portion of sequence reads from all the cores clustered into the Proteobacteria phylum, with Deltaproteobacteria, Gammaproteobacteria, and Alphaproteobacteria having the highest relative abundances among

the sediment OTUs. The relative abundances and diversity of Gammaproteobacteria decreased with depth for all sediment cores, whereas the relative abundances of Deltaproteobacteria increased with depth. Sequences similar to the diversity of *P. pectinatus* gills and feet, particularly *Sedimenticola*, *Methylo Marinum*, *Spirochaeta*, and unclassified Rickettsiales and Lentisphaerae, were retrieved in all of the sediment cores. *Kistimonas* spp. were only retrieved from quadrat 1-2 at a depth of 10-20 cm. *Sedimenticola* spp. sequences were retrieved from all sediment depth, but were in low abundance and ranged from 1 to 13 reads. Reads affiliated with *Spirochaeta* spp. were retrieved in greater relative abundances from quadrats 1-2 and 3-2, whereas reads affiliated with Rickettsiales represented a minor sediment population (<7 reads), although the highest abundances were from quadrat 1-10.

CHAPTER FOUR: DISCUSSION

Lucinid bivalves in coastal environments are beneficial to seagrass communities (Reynolds *et al.*, 2007; van der Heide *et al.*, 2007). However, limited research has been dedicated to uncovering how other organisms or processes influence lucinid populations and, alternatively, what environmental parameters might impact the lucinid-symbiont system. The lack of attention paid to studying lucinids from shallow coastal habitats may be attributed to the long-standing assumption that lucinid endosymbiont populations are thought to belong exclusively to a monophyletic group of Gammaproteobacteria (Gros *et al.*, 2003). However, greater metabolic and taxonomic symbiont diversity are possible (Green-García & Engel, 2012), particularly given the ecology and life style of lucinids, their ability to acquire symbionts from the environment, and their ability to acquire bacteria throughout their lifespan (Caro *et al.*, 2009). These characteristics have important implications for lucinid resiliency to environmental fluctuations and may provide benefits to their environment from nutrient cycling.

Therefore, the aim of this research was to describe the physical lucinid habitat, determine the degree of taxonomic specificity for free-living and endosymbionts, and to identify environmental trends underlying the distribution of the host and symbionts. To accomplish these goals, a systematic sampling scheme was completed at Wildcat Cove, a back-channel inlet of the Indian River Lagoon, where *P. pectinatus* have been previously studied since 2006. As a functional land and aquatic preserve, the area is likely removed from many of the anthropogenic pressures and offers a unique site for extended studies on a lucinid system. The available meteorological and water quality data suggest that the region is hydrologically stable. However, Wildcat Cove is also separated from an inland marsh due to the installation of a mosquito

impoundment along the coastline. These impoundments have been shown to negatively affect natural water and nutrient circulation (Division of Recreation and Parks, 2006). Reduced supply of nutrient and organic litter could explain the observance of lower seagrass densities in quadrats located further from the marsh connecting canal. The effect of this impoundment on the *P. pectinatus* system is unclear, as neither *P. pectinatus* nor their potential symbionts showed specific spatial distribution patterns associated with the canal or its inlet.

The geochemistry of Wildcat Cove is suitable for lucinid populations. All known and previously studied lucinid endosymbionts are assumed to be sulfide-oxidizing bacteria, which require a geochemical habitat that contains sulfide and oxygen (Stewart *et al.*, 2005). In Wildcat Cove, the highest dissolved oxygen concentrations measured (181.9 $\mu\text{mol/L}$) and most concentrations were below this value. The highest dissolved oxygen corresponded to the second highest sulfide concentration (51.8 $\mu\text{mol/L}$) measured, at grid quadrat 10-10. A sulfide-oxygen redox gradient is required by lucinid communities (van der Heide *et al.*, 2012), and needs to remain stable across changing water depths due to tidal cycles. The fact that lucinids were recovered from 0 to 30 cm depth indicate that they likely live at all redox gradients, with high oxygen and low sulfide and low to no oxygen and high sulfide. However, the success at each of these redox extremes may vary, which also likely relates to the diversity of bacteria across the gradients.

One stronger correlation of lucinid abundance to geochemical parameter was to sediment organic carbon content. The higher concentrations of organic content closer to the mangroves was also observed in the pore water where DOM fluorescence data showed FI, BIX, and HIX values (Table 3) transitioned from a terrestrial/plant to proteinaceous/microbial phytoplankton

signature. Organic carbon, sourced from mangrove litter likely fuels anaerobic chemoorganotrophic processes, such as sulfate reduction (Reynolds *et al.*, 2007) and methanogenesis, which produce electron donors useful for bacterial chemosymbionts for lucinids, specifically H₂S and CH₄, respectively. Several studies confirm that sulfide concentrations increase and oxygen concentrations decrease with increasing contributions of organic carbon sourced from marine vegetation (van der Heide *et al.*, 2012).

It is unclear from taxonomic information alone which bacteria identified are active symbionts within the lucinid gills, as some sequences could be attributed to environmental associations, deleterious infection, or contamination (i.e. located on the surface of gill or not encapsulated in the gill tissue). However, previously documented research suggest that the taxonomic identity of the groups discussed herein are likely involved in either a chemosymbiotic relationship or affiliated to some degree with the *P. pectinatus*.

Only recently have the predicted metabolic function of *Sedimenticola* spp. based upon phylogeny been observed, revealing that *Sedimenticola* spp. are capable of oxidizing thiosulfate, tetrathionate, elemental sulfur, and sulfide during autotrophic growth (Russ *et al.*, 2014; Carlström *et al.*, 2015; Flood *et al.*, 2015). *Sedimenticola* spp. may also have some capacity to utilize aromatic or other carbons sources in their metabolisms (Flood *et al.*, 2015). Even though *Sedimenticola* affiliates with other sulfur-oxidizing symbionts of bivalves and tube worms, many of the previous culture studies suggests that *Sedimenticola* spp. are strictly anaerobes that reduce selenate to selenite through the degradation of aromatic compound. (Knight *et al.*, 2002; Narasingarao & Häggblom, 2006). In addition to sulfur oxidizing, genes encoding for an almost complete denitrification pathway were described and may help account for the increase in total

nitrogen along column 10 (0.39 mg/L to 1.53 mg/L). The potential for this genus to different metabolic functions was suggested previously to give *Sedimenticola* spp. a competitive advantage in denitrifying conditions (Russ *et al.*, 2014). The capacity for *Sedimenticola* spp. to perform some of these functions might be highly dependent on oxygen concentrations (Flood *et al.*, 2015), which could also explain why the highest abundance of *P. pectinatus* occurred in quadrats associated with the lowest oxygen concentrations.

In 17 of the 21 gills analyzed, bacterial sequences that identified to *Kistimonas* represented the second most abundant taxonomic group. *Kistimonas* spp. belong to the Oceanospirillales order, whose role within animal hosts may range from being a beneficial symbiont (Goffredi *et al.*, 2005) to an infectious intranuclear parasite responsible for high host fatality rates (Kerk *et al.*, 1992). As *Kistimonas* spp. were found in all *P. pectinatus* specimens and their relative abundances did not vary with *P. pectinatus* populations, the potential relationships to the host are unlikely to be detrimental. However, Zielinski *et al.* (2009) show that *Kistimonas* spp. invade host nuclei cell lacking chemosymbiotic bacteria in bathymodiolus mussels and repopulated in the cell until the host cell ruptured. Such a process produces a genetically similar community; however, *Kistimonas* clustered in 68 OTUs at the 97% identity cutoff, suggesting the communities at Wildcat Cove are more genetically diverse. Additional research is needed before clarifying what type of relationship is being expressed between *Kistimonas* and *P. pectinatus*.

Like *Sedimenticola* spp. and *Kistimonas* spp., *Methylomarinum* spp. also belong to the class of Gammaproteobacteria. Found in all tissue samples, *Methylomarinum* spp. represent potential novel symbionts, as no published studies have shown lucinids with a methanotrophic

bacteria or dual symbiosis. *Methylo Marinum*, a member of Methylococcaceae family, only recently has been described as a gram negative mesophile that metabolizes methane and methanol, as well as utilizes ammonium, nitrate and formamide as their sole nitrogen source (Hirayama *et al.*, 2013). Although certain deep marine mussels have been shown to have dual symbiosis with methanotrophs (Duperron *et al.*, 2005), symbionts of bacterial lineages within the Methylococcaceae are rare. The higher proportion of *Methylo Marinum* in lucinid gills collected at greater depths suggest that environmental position may impact endosymbiont diversity, which supports my third hypothesis. As sediment becomes more anoxic with depth, methanotrophs may offer lucinids a more energetically preferable metabolic pathway compared to the more traditionally observed sulfide-oxidizing symbiont. Shifts in gill-related *Methylo Marinum* spp., relative abundances were also observed from quadrat to quadrat, and could possibly be explained by a changing redox gradient, as described previously. Differences in endosymbiont populations during a single sample period due to environmental position indicates that perturbations to the environment would likely effect the bacteria being incorporated into a lucinid's bacteriocytes over time.

Spirochaeta 16S rRNA gene reads were recovered for 19 *P. pectinatus* and they represented a minor phylotype as because relative abundances never surpassed 1.8% of the total gill bacterial communities. The two *P. pectinatus* containing no *Spirochaeta* were located in quadrats 1-4 and 1-6. Of the 989 sequence reads, *Spirochaeta* clustered into four OTUs, with one OTU representing 92.5 % of the sequences. This suggests narrow genetic diversity. Duperron *et al.* (2007) recovered a clone sequence from a cold-seep (507 to 1691 m) in the eastern Mediterranean from a lucinid clam and showed through phylogenetic analysis that there is a

clade containing several symbionts associated with other thyasirids and lucinids, including *P. pectinatus*. Limited additional research has expanded on *Spirochaeta* potential symbiotic association in lucinid clams.

An unclassified Rickettsiales was present in 16 of the 21 hosts analyzed, but represented less than one percent of the bacterial communities. High Rickettsiales relative abundances (72% and 15%) were observed in clams located in quadrats 1-4 and 1-6. Rickettsiales clustered into 176 OTUs, the highest number of any bacteria group associated with the gills. This suggested large taxonomic diversity. Several bacterial lineages of Rickettsiales have been related to disease and high mortality outbreaks for other marine bivalves (Carvalho *et al.*, 2013; Costa *et al.*, 2012; and Villalba *et al.*, 1999). Most *P. pectinatus* (16) contained Rickettsiales in low abundances and some of these were from high clam density areas. But, because of the available literature, and due to finding high Rickettsiales communities with low *P. pectinatus* abundances, this bacterial group likely indicates an infectious pathogen for *P. pectinatus*. These results are the first to suggest a potential pathogen for lucinids. The relationship needs further examination.

Lastly unclassified class of Lentisphaerae. Sequence counts in the gills were low, but were retrieved as high numbers from the feet. Lentisphaerae, a recently discovered phylum with relatively few culture studies (Cho *et al.*, 2004), making higher level taxonomic affiliations difficult. Lentisphaerae could be actively participating as a symbiont, although no published material has shown the harboring of Lentisphaerae or any other microbes in non-gill lucinid tissues making these results surprising. But, a report by Wentrup *et al* (2013) found the deep-sea mussel *Bathymodiolus* contains chemosynthetic bacteria throughout all the tissues during juvenile development. Because gills develop after the mantle and foot in the *Bathymodiolus*, the

authors speculated that incorporating symbionts in all tissues was a method for the host to obtain nutrients during early growth stages. *P. pectinatus* may have a similar infection strategy during their development which may mean they maintain bacterial communities in their non-gill tissues through adulthood. Moreover, ongoing transmission of endosymbionts into non-gill tissue could explain the greater taxonomic diversity identified from the host overall compared to previous studies. Future work should address whether bacterial communities do exist in the foot and possibly the mantle, using methods like fluorescence in situ hybridization (Caro *et al.*, 2009; Gros *et al.*, 2012).

Because all known lucinids obtain their symbionts from environmental transmission, similar symbionts should be found in close proximity to the clams in the habitats. Both sediment and water microbial communities contained 16S rRNA genes that were taxonomically affiliated with gill and feet symbionts, although the reads were usually less than 10. Only non-tissue sequences retrieved from the feet were shared at the 95% similarity level. Free-living symbionts for some lucinids have been found in sediments previously, but were estimated to be a small fraction of the total bacterial population (Gros *et al.*, 2003b; Green-García & Engel, 2012).

CHAPTER FIVE: CONCLUSION

Lucinid bivalves are in a chemosymbiotic relationship with bacteria obtained from the environment. Previous reports have suggested that lucinid endosymbiont populations belong to a monophyletic clade of sulfur oxidizing *Gammaproteobacteria* (Distel, 1998; Gros et al., 2003a) and that this association stabilizes sea grass beds through the removal of sulfide (Stanley, 2014; van der Heide *et al.*, 2012). A total of 190 *Phacoides pectinatus* were found at Wildcat Cove who harbored endosymbionts in both the foot and gills and represented novel bacterial communities including *Sedimenticola*, *Kistimonas*, *Methylomarinum*, and *Spirochaeta* spp., as well as unclassified Rickettsiales (Alphaproteobacteria) and unclassified Lentisphaerae. *Phacoides pectinatus* populations positioned closer to mangroves with higher organic carbon content. High Rickettsiales abundance associated with lower lucinid quantities and *Methylomarinum* were expressed in higher proportions from gills collected in deeper sediments.

These results suggest that the *P. pectinatus* contain the most taxonomically and metabolically diverse symbionts observed within any lucinid bivalves reported thus far, and the first to harbor bacteria within non-gill tissues. Further work, possibly microscopy based studies, should focus upon addressing these symbionts' locations in host tissue. Determining which symbiont populations are encapsulated in bacteriocytes, more accurately quantifying bacterial communities, and if these populations display an organizational hierarchy could indicate which types of relationships are being expressed. Identifying the role of the bacteria is especially true for foot tissue where no bacteriocytes have ever been described. Examining symbiont-*P. pectinatus* relationships would provide light on whether the greater diversity of the of *P. pectinatus* with

respect to other lucinids was in response to fluctuating environmental conditions or anthropogenic impacts. However, the evolutionary emergence of these symbionts is unknown.

As endosymbiont communities changed across the sampling grid possibly due to the positioning of *P. pectinatus*, brief temporal shifts in symbiont communities of a *P. pectinatus* population seems possible if not likely. Highly specific symbiont-host relationships expressed by other lucinid reports suggest that endosymbiont populations do not undergo changes rapidly. As the *P. pectinatus* is one of the few lucinids associated with mangroves (Frenkiel *et al.*, 1996) and its phylogenetic positioning is often long unstable branches within lucinids (Taylor *et al.*, 2011), a diverse and antiquated symbiont population or an uncharacteristically flexible transmission strategy could also be possible. Only limited number of lucinid taxa have had their symbiont populations adequately studied. A more comprehensive database would reveal whether the symbionts observed within *P. pectinatus* are representative of a unique system or are the identities and functions of lucinid endosymbionts as a whole vastly underexplored

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APPENDIX

Tables

Table 1 - Field physiochemical parameters from Wildcat Cove, St. Lucie County, Florida.

Sample Name	Grid Location	Water Type	Time	Tide	pH	Conductivity (mS)	Temperature (°C)	Sulfide (μmol/L)^a	Oxygen (μmol/L)^a
LUC_14_004	1_1	Pore	9:05 AM	High	7.07	53.1	28.3	56.1	78.0
LUC_14_005	5_1	Pore	11:04 AM	Receding	7.11	53.5	29.8	21.5	136.6
LUC_14_006	10_1	Pore	12:55 AM	Low	7.50	51.7	30.9	11.7	156.6
LUC_14_007	1_10	Pore	9:12 AM	High	7.18	53.3	29.6	17.6	124.5
LUC_14_008	5_10	Pore	12:34 PM	Receding	7.10	56.0	30.8	22.2	156.4
LUC_14_009	Ocean	Ocean	12:35 PM	Receding	8.03	51.0	31.6	NM	NM
LUC_14_010	10_10	Pore	1:44 PM	Low	7.25	54.0	32.2	51.8	181.9
LUC_14_011	Canal	Marsh	8:30 AM	High	7.71	49.8	30.0	NM	137.5*
LUC_14_012	Canal	Marsh	3:10 PM	Low	7.74	49.6	31.2	NM	156.3*

NM: Not Measured

^a Measured by using CHEMetrics methods, except where noted with an asterisk (*) to denote YSI probe measurements.

Table 2 - Cation and anion concentrations (mmol/L) at Wildcat Cove, St. Lucie County, Florida

Sample Name	Grid Location	Water Type	Alkalinity mg/L	DOC mg/L	TN mg/L	mmol/L							
						Na ⁺	NH ₄ ⁺	K ⁺	Mg ²⁺	Ca ²⁺	F ⁻	Cl ⁻	SO ₄ ²⁻
LUC_14_004	1_1	Pore	296.90	53.64	0.57	575.2	1.38	13.2	70.3	13.9	0.08	584.4	29.5
LUC_14_005	5_1	Pore	326.20	56.65	0.72	486.3	1.24	11.1	59.4	11.8	0.09	598.5	30.1
LUC_14_006	10_1	Pore	227.80	39.49	0.56	587.1	1.41	13.5	71.6	14.1	0.09	584.4	29.4
LUC_14_007	1_10	Pore	361.85	63.44	0.39	567.4	0.00	13.0	69.4	13.7	0.08	605.2	30.6
LUC_14_008	5_10	Pore	441.93	76.18	0.87	563.5	0.00	12.9	68.9	13.6	0.09	623.6	31.5
LUC_14_009	Ocean	Ocean	160.66	26.99	0.30	556.3	1.34	12.7	67.9	13.4	0.07	552.9	28.0
LUC_14_010	10_10	Pore	374.05	60.53	1.53	620.3	1.42	14.3	75.8	15.1	0.09	605.9	30.7
LUC_14_011	Canal	Marsh	147.23	30.15	0.40	559.2	1.19	12.8	68.3	13.5	0.12	568.0	28.6
LUC_14_012	Canal	Marsh	148.69	28.76	0.38	582.6	1.46	13.3	71.0	14.1	0.07	568.3	28.7

Table 3 - Dissolved gases measured by gas chromatography and fluorescence spectroscopy indices for water from Wildcat Cove, St. Lucie County, Florida.

Sample Name	Location	Water Type	Dissolved Gases		Fluorescence Indices		
			CH ₄ μmol/L	CO ₂ μmol/L	FI	HIX	BIX
LUC_14_004a	1_1	Pore	0.48	204.01	1.86	0.95	0.53
LUC_14_004b	1_1	Pore	0.35	212.35	1.86	0.95	0.54
LUC_14_005a	5_1	Pore	0.82	246.86	1.90	0.96	0.56
LUC_14_005b	5_1	Pore	0.60	228.28	-	-	-
LUC_14_006a	10_1	Pore	0.27	80.76	1.91	0.93	0.57
LUC_14_006b	10_1	Pore	0.59	95.32	-	-	-
LUC_14_007a	1_10	Pore	0.43	252.94	1.84	0.97	0.51
LUC_14_007b	1_10	Pore	ND	337.19	1.84	0.97	0.51
LUC_14_008a	5_10	Pore	0.31	14.94	1.87	0.96	0.54
LUC_14_008b	5_10	Pore	0.54	393.50	1.88	0.96	0.55
LUC_14_009a	Ocean	Ocean	0.44	19.07	1.96	0.92	0.69
LUC_14_009b	Ocean	Ocean	0.61	28.81	-	-	-
LUC_14_010a	10_10	Pore	0.30	298.99	1.91	0.94	0.56
LUC_14_010b	10_10	Pore	0.43	13.55	-	-	-
LUC_14_011a	Canal	Marsh	-	-	1.96	0.93	0.69
LUC_14_011b	Canal	Marsh	-	-	-	-	-
LUC_14_012a	Canal	Marsh	-	-	1.98	0.92	0.69
LUC_14_012b	Canal	Marsh	-	-	-	-	-

Table 4 - Average percent organic carbon based on loss-on-ignition (LOI) measurements and *P. pectinatus* abundances^a (in parentheses) from Wildcat Cove sediments.

Meters	Transect 4 ^c	Transect 5 ^c	Sampling Grid	Column 2	Column 4	Column 6	Column 8	Column 10
5	7.01 (2)	4.34 (1)	Row 1 ^b :	7.17 (43)	5.20 (18)	3.83 (3)	4.55 (19)	5.74 (47)
10	2.84 (3)	3.26 (0)	Row 3 ^b :	2.67 (0)	2.34 (0)	4.07 (0)	4.80 (1)	2.64 (6)
15	6.29 (6)	4.06 (0)	Row 5 ^b :	2.00 (0)	3.51 (1)	NM	NM	2.99 (0)
20	5.86 (5)	2.83 (0)	Row 7 ^b :	2.28 (0)	3.19 (0)	NM	NM	4.72 (1)
25	4.26 (3)	6.44 (1)	Row 9 ^b :	2.05 (0)	2.14 (0)	NM	NM	4.07 (0)
30	9.36 (3)	5.01 (1)		Mean % OC LOI	Standard Deviation	r ² ^d		
35	11.77 (3)	4.09 (1)	Grid	3.68	1.39	0.595		
40	9.57 (3)	8.31 (4)	Row 1	5.30	1.13	0.961		
45	2.66 (2)	3.90 (1)	Transect 4	6.58	2.81	0.001		
50	6.19 (5)	14.31 (4)	Transect 5	5.65	3.26	0.776		

NM: Not Measured

^a Lucinid abundances from the quadrats were taken from 0.3 m² of sediment and from the transects from 0.0014 m³ sediment

^b LOI performed on centimeter sections to a depth of 4 cm and averaged

^c Samples were taken as homogenized aliquots prior to LOI

^d Coefficient of Determination (r²) between percent organic carbon from LOI and *P. pectinatus* abundances

Table 5 - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
1-2_0-1cm	1.1103	2.9984	4.1070	2.8486	1.7383	41.993	2.7510	1.6407	5.615
1-2_1-2cm	1.1238	2.9987	4.1209	2.6820	1.5582	48.010	2.4678	1.3440	13.747
1-2_2-3cm	1.1233	3.0018	4.1228	3.2349	2.1116	29.602	3.1510	2.0277	3.973
1-2_3-4cm	1.1077	3.0007	4.1069	3.0051	1.8974	36.736	2.9036	1.7959	5.349
1-2_4-5cm	1.1153	3.0008	4.1148	3.1809	2.0656	31.135	3.0922	1.9769	4.294
1-2_5-6cm	1.1290	2.9984	4.1260	3.2222	2.0932	30.157	3.1317	2.0027	4.324
1-2_6-7cm	1.0956	2.9981	4.0918	3.0634	1.9678	34.323	2.9381	1.8425	6.368
1-2 ~10cm	1.1063	2.9986	4.1024	2.8222	1.7159	42.729	2.7645	1.6582	3.363
1-2 ~20cm	1.1092	2.9984	4.1057	2.8695	1.7603	41.255	2.8232	1.7140	2.630
1-4_0-1cm	1.1069	2.9979	4.1022	2.5450	1.4381	51.988	2.4609	1.3540	5.848
1-4_1-2cm	1.1065	2.9980	4.0976	2.8606	1.7541	41.356	2.7888	1.6823	4.093
1-4_2-3cm	1.0985	2.9986	4.0957	2.9388	1.8403	38.599	2.8315	1.7330	5.831
1-4_3-4cm	1.1077	3.0004	4.1059	2.9398	1.8321	38.893	2.8480	1.7403	5.011
1-4_Deep	1.1109	3.0009	4.1101	3.0758	1.9649	34.486	3.0052	1.8943	3.593
1-6_0-2cm	1.1056	2.9995	4.1030	2.6869	1.5813	47.244	2.6012	1.4956	5.420
1-6_2-4cm	1.0985	2.9980	4.0944	3.1605	2.0620	31.173	3.1142	2.0157	2.245
1-8_0-1cm	1.0978	2.9970	4.0847	2.7463	1.6485	44.809	2.6716	1.5738	4.531
1-8_1-2cm	1.1065	3.0281	4.1330	2.8420	1.7355	42.657	2.7685	1.6620	4.235
1-8_2-3cm	1.1024	3.0375	4.1379	3.0924	1.9900	34.442	2.9952	1.8928	4.884
1-8_3-4cm	1.0843	3.0487	4.1318	3.2424	2.1581	29.185	3.1443	2.0600	4.546
1-8_4-5cm	1.0973	3.0193	4.1144	3.1014	2.0041	33.575	2.9878	1.8905	5.668

Table 5 (Continued) - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
1-8_5-6cm	1.0930	3.0475	4.1399	3.0785	1.9855	34.835	2.9556	1.8626	6.190
1-8_Deep	1.1108	3.0609	4.1699	2.8460	1.7352	43.277	2.7556	1.6448	5.210
1-10_0-1cm	1.0997	3.0310	4.1285	2.3924	1.2927	57.320	2.3200	1.2203	5.601
1-10_1-2cm	1.1068	3.0362	4.1413	2.5988	1.4920	50.832	2.5116	1.4048	5.845
1-10_2-3cm	1.1252	3.0570	4.1806	3.1318	2.0066	34.326	3.0396	1.9144	4.595
1-10_3-4cm	1.0949	3.0502	4.1429	3.0093	1.9144	37.192	2.8772	1.7823	6.900
1-10_4-5cm	1.1119	3.0319	4.1423	3.2564	2.1445	29.234	3.1796	2.0677	3.581
1-10_Deep	1.1025	3.0265	4.1279	3.2440	2.1415	29.216	3.1840	2.0815	2.802
3-2_0-1cm	1.0981	3.0532	4.1519	2.9775	1.8794	38.457	2.9316	1.8335	2.442
3-2_1-2cm	1.1147	3.0200	4.1335	3.2570	2.1423	29.035	3.2059	2.0912	2.385
3-2_2-3cm	1.1046	3.0645	4.1677	3.2955	2.1909	28.474	3.2196	2.1150	3.464
3-2_3-4cm	1.1277	3.0283	4.1545	3.4098	2.2821	24.604	3.3556	2.2279	2.375
3-2_4-5cm	1.1018	3.0489	4.1484	3.4343	2.3325	23.439	3.3870	2.2852	2.028
3-2_Deep	1.1037	3.0557	4.1586	3.0159	1.9122	37.405	2.9222	1.8185	4.900
3-4_0-1cm	1.1054	3.0252	4.1292	3.1633	2.0579	31.943	3.0952	1.9898	3.309
3-4_1-2cm	1.1048	3.0518	4.1564	3.3574	2.2526	26.183	3.2994	2.1946	2.575
3-4_2-3cm	1.1179	3.0062	4.1239	3.4271	2.3092	23.180	3.3842	2.2663	1.858
3-4_3-4cm	1.0966	3.0460	4.1417	3.4519	2.3553	22.653	3.4134	2.3168	1.635
3-4_4-5cm	1.1013	3.0521	4.1527	3.4631	2.3618	22.599	3.4278	2.3265	1.495
3-6_0-1cm	1.0961	3.0573	4.1521	2.9813	1.8852	38.312	2.9368	1.8407	2.360
3-6_1-2cm	1.1035	3.0233	4.1267	2.6941	1.5906	47.387	2.6047	1.5012	5.621
3-6_2-3cm	1.1134	3.0325	4.1454	2.8508	1.7374	42.698	2.7668	1.6534	4.835

Table 5 (Continued) - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
3-6_3-4cm	1.0957	3.0544	4.1482	3.0679	1.9722	35.391	2.9995	1.9038	3.468
3-8_0-1cm	1.1138	3.0276	4.1396	2.1705	1.0567	65.077	2.0995	0.9857	6.719
3-8_1-2cm	1.1137	3.0454	4.1570	2.7732	1.6595	45.470	2.6783	1.5646	5.719
3-8_2-3cm	1.1173	3.0465	4.1619	2.9916	1.8743	38.439	2.9152	1.7979	4.076
3-8_3-4cm	1.1236	3.0573	4.1799	3.3091	2.1855	28.492	3.2508	2.1272	2.668
3-8_4-5cm	1.1114	3.0277	4.1375	3.1959	2.0845	31.116	3.1283	2.0169	3.243
3-8_5-6cm	1.1040	3.0478	4.1503	3.3074	2.2034	27.670	3.2430	2.1390	2.923
3-8_6-7cm	1.1216	3.0628	4.1837	3.3636	2.2420	26.782	3.2893	2.1677	3.314
3-8_7-8cm	1.1077	3.0284	4.1344	3.3564	2.2487	25.705	3.2980	2.1903	2.597
3-10_0-1cm	1.1026	3.0144	4.1167	3.3114	2.2088	26.718	3.2671	2.1645	2.006
3-10_1-2cm	1.1032	3.0602	4.1622	3.2833	2.1801	28.732	3.2260	2.1228	2.628
3-10_2-3cm	1.1213	3.0399	4.1612	3.3735	2.2522	25.912	3.3170	2.1957	2.509
3-10_3-4cm	1.1038	3.0230	4.1250	3.3057	2.2019	27.118	3.2302	2.1264	3.429
3-10_4-5cm	1.1092	3.0075	4.1153	3.2742	2.1650	27.980	3.2008	2.0916	3.390
3-10_5-6cm	1.1040	3.0394	4.1418	3.3820	2.2780	25.012	3.3273	2.2233	2.401
3-10_Deep	1.1136	3.0370	4.1500	3.2241	2.1105	30.493	3.1553	2.0417	3.260
5-2_0-1cm	1.0916	3.0518	4.1423	3.3662	2.2746	25.440	3.3253	2.2337	1.798
5-2_1-2cm	1.1161	3.0497	4.1648	3.3858	2.2697	25.552	3.3407	2.2246	1.987
5-2_2-3cm	1.0933	3.0547	4.1467	3.3372	2.2439	26.511	3.2873	2.1940	2.224
5-2_3-4cm	1.1009	3.0277	4.1278	3.0357	1.9348	36.080	3.2474	2.1465	-10.942
5-2_4-5cm	1.1009	3.0331	4.1332	3.3135	2.2126	27.032	3.2406	2.1397	3.295
5-4_0-1cm	1.1184	3.0468	4.1638	2.8505	1.7321	43.124	2.7831	1.6647	3.891

Table 5 (Continued) - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
5-4_1-2cm	1.1084	3.0421	4.1495	2.9022	1.7938	41.015	2.8382	1.7298	3.568
5-4_2-3cm	1.1094	3.0516	4.1595	3.1081	1.9987	34.471	3.0431	1.9337	3.252
5-4_3-4cm	1.1250	3.0190	4.1425	3.2072	2.0822	30.996	3.1383	2.0133	3.309
5-4_4-5cm	1.1284	3.0302	4.1576	3.1611	2.0327	32.896	3.0854	1.9570	3.724
5-10_0-1cm	1.0923	3.0554	4.1461	2.6487	1.5564	49.034	2.5899	1.4976	3.778
5-10_1-2cm	1.0908	3.0592	4.1490	3.2039	2.1131	30.904	3.1549	2.0641	2.319
5-10_2-3cm	1.1073	3.0475	4.1541	3.2327	2.1254	30.242	3.1685	2.0612	3.021
5-10_3-4cm	1.1136	3.0501	4.1622	3.3204	2.2068	27.613	3.2575	2.1439	2.850
5-10_4-5cm	1.1150	3.0458	4.1597	3.3627	2.2477	26.177	3.3011	2.1861	2.741
5-10_5-6cm	1.1036	3.0136	4.1160	3.3054	2.2018	26.909	3.2461	2.1425	2.693
7-2_0-1cm	1.1171	3.0352	4.1515	2.9656	1.8485	39.082	2.9202	1.8031	2.456
7-2_1-2cm	1.1004	3.0169	4.1162	3.3881	2.2877	24.143	3.3507	2.2503	1.635
7-2_2-3cm	1.0954	3.0327	4.1269	3.3415	2.2461	25.908	3.2825	2.1871	2.627
7-2_3-4cm	1.1007	3.0459	4.1452	3.4315	2.3308	23.442	3.3752	2.2745	2.415
7-2_4-5cm	1.1094	3.0472	4.1555	3.1633	2.0539	32.573	3.3985	2.2891	-11.451
7-2_5-6cm	1.1077	3.0505	4.1579	3.3161	2.2084	27.598	3.2460	2.1383	3.174
7-2_6-7cm	1.1106	3.0319	4.1410	3.3236	2.2130	26.973	3.2597	2.1491	2.887
7-4_0-1cm	1.1105	3.0354	4.1446	2.7605	1.6500	45.618	2.6947	1.5842	3.988
7-4_1-2cm	1.1238	3.0466	4.1687	3.1793	2.0555	32.494	3.1167	1.9929	3.045
7-4_2-3cm	1.1057	3.0587	4.1627	3.2465	2.1408	29.971	3.1862	2.0805	2.817
7-4_3-4cm	1.1112	3.0339	4.1437	3.2531	2.1419	29.369	3.1905	2.0793	2.923
7-4_4-5cm	1.1171	3.0436	4.1597	3.2551	2.1380	29.731	3.1825	2.0654	3.396

Table 5 (Continued) - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
7-10_0-1cm	1.1075	3.0522	4.1578	2.4335	1.3260	56.529	2.3691	1.2616	4.857
7-10_1-2cm	1.1090	3.0454	4.1535	2.8326	1.7236	43.386	2.7415	1.6325	5.285
7-10_2-3cm	1.1111	3.0122	4.1217	2.8675	1.7564	41.659	2.7805	1.6694	4.953
7-10_3-4cm	1.1046	3.0352	4.1384	3.1054	2.0008	34.050	3.0294	1.9248	3.798
7-10_4-5cm	1.1090	3.0565	4.1648	3.1480	2.0390	33.274	3.0656	1.9566	4.041
7-10_5-6cm	1.1044	3.0528	4.1558	3.2429	2.1385	29.917	3.1068	2.0024	6.364
7-10_6-7cm	1.1009	3.0507	4.1502	3.3288	2.2279	26.937	3.2612	2.1603	3.034
7-10_7-8cm	1.1070	3.0065	4.1127	3.3088	2.2018	26.746	3.2328	2.1258	3.452
7-10_8-9cm	1.1040	3.0389	4.1409	3.3766	2.2726	25.167	3.3136	2.2096	2.772
9-2_0-1cm	1.0805	3.0080	4.0872	3.0631	1.9826	34.061	3.0070	1.9265	2.830
9-2_1-2cm	1.1038	3.0445	4.1473	3.4197	2.3159	23.907	3.3810	2.2772	1.671
9-2_2-3cm	1.0902	3.0262	4.1158	3.3560	2.2658	25.112	3.3087	2.2185	2.088
9-2_3-4cm	1.1140	3.0502	4.1635	3.4662	2.3522	22.866	3.4284	2.3144	1.607
9-2_4-5cm	1.0879	3.0465	4.1337	3.4318	2.3439	23.045	3.3909	2.3030	1.745
9-4_0-1cm	1.1050	3.0099	4.1142	3.2871	2.1821	27.486	3.2392	2.1342	2.195
9-4_1-2cm	1.1056	3.0378	4.1398	3.3498	2.2442	26.037	3.3004	2.1948	2.201
9-4_2-3cm	1.1132	3.0470	4.1594	3.4482	2.3350	23.347	3.4010	2.2878	2.021
9-10_0-1cm	1.1077	3.0572	4.1633	2.9288	1.8211	40.401	2.8837	1.7760	2.477
9-10_1-2cm	1.0885	3.0394	4.1270	2.9418	1.8533	39.006	2.8370	1.7485	5.655
9-10_2-3cm	1.0895	3.0450	4.1335	3.0567	1.9672	35.375	2.9742	1.8847	4.194
9-10_3-4cm	1.0957	3.0024	4.0975	3.1110	2.0153	32.864	3.0315	1.9358	3.945
9-10_4-5cm	1.1114	3.0364	4.1470	3.1935	2.0821	31.411	3.0904	1.9790	4.952

Table 5 (Continued) - Organic carbon content measured by Loss on Ignition (LOI)

Sample Name (LUC_14_002)	W Al dish (g)	W wet sample (g)	W wet sample + dish (g)	W dry sample + dish (g)	W water loss (g)	% water loss	W muffled sample + dish (g)	Organic Carbon LOI (g)	% Organic Matter LOI
9-10_5-6cm	1.0965	3.0603	4.1558	3.1646	2.0681	32.400	3.0740	1.9775	4.381
9-10_6-7cm	1.1075	3.0518	4.1585	3.2602	2.1527	29.443	3.1853	2.0778	3.479
9-10_7-8cm	1.1031	3.0513	4.1535	3.2980	2.1949	28.046	3.2205	2.1174	3.531
9-10_8-9cm	1.1048	3.0194	4.1233	3.2214	2.1166	29.879	3.1418	2.0370	3.761

Table 6 - Summary of 454 tag pyrosequencing data for each sample. Operational Taxonomic Units (OTUs) and diversity indices were calculated at 97% level.

Sample Name	Number sequences (raw)	Number sequences after trimming	Mean length after trimming (base pairs)	Number Sequences. After screening, filtering, and chimera removal	No. OTUs (97% sequence identity)	Chao Diversity	Shannon Diversity (H') Index
LUC_002_12_Sc	26140	14191	366.1	14190	19	20.00	1.51
LUC_002_12_Se	24530	13324	366.8	13318	22	25.00	1.59
LUC_002_12_Dd	34592	12042	364.7	12037	23	24.66	1.66
LUC_002_12_De	20328	8759	356.0	8758	25	40.00	1.85
LUC_002_12_Ddf	17469	7843	327.3	7835	32	84.50	1.48
LUC_002_14_Sb	23714	13144	366.0	13138	15	15.60	1.31
LUC_002_14_Sc	25853	14827	368.1	14821	24	27.75	1.69
LUC_002_14_Da	34920	16826	324.9	16757	21	34.00	1.30
LUC_002_14_Dd	36286	20475	371.6	20472	24	33.00	1.23
LUC_002_14_Daf	14888	8315	310.1	8282	45	115.86	1.76
LUC_002_16_S	41318	23720	372.2	23715	17	24.20	0.74
LUC_002_16_Da	60623	36722	360.1	36687	35	51.50	2.25
LUC_002_16_Db	18920	9108	351.1	9103	28	47.50	1.48
LUC_002_16_Daf	18233	9042	349.0	9036	25	85.00	1.75
LUC_002_18_Sa	27514	15542	371.0	15539	20	22.50	1.19
LUC_002_18_Sb	14401	8251	375.0	8251	12	15.75	0.82
LUC_002_18_Db	32892	17692	366.5	17686	35	44.17	1.59
LUC_002_18_Dc	32917	18232	367.7	18223	24	39.00	1.06
LUC_002_18_Dbf	24906	12293	309.8	12229	77	362.17	2.18
LUC_002_110_Sb	44738	25581	373.6	25576	23	36.75	0.78
LUC_002_110_Sd	20454	11748	376.9	11746	10	10.75	0.61

Table 6 (Continued) - Summary of 454 tag pyrosequencing data for each sample. Operational Taxonomic Units (OTUs) and diversity indices were calculated at 97% level.

Sample Name	Number sequences (raw)	Number sequences after trimming	Mean length after trimming (base pairs)	Number Sequences. After screening, filtering, and chimera removal	No. OTUs (97% sequence identity)	Chao Diversity	Shannon Diversity (H') Index
LUC_002_110_Dc	8646	2728	370.7	2562	8	10.00	0.92
LUC_002_110_De	36458	20245	370.8	20238	26	27.25	1.31
LUC_002_110_Dcf	9209	3579	360.7	3564	100	567.63	3.21
LUC_002_310_Da	29937	16374	372.3	16369	22	49.50	1.11
LUC_002_310_Db	43213	24548	374.2	24546	21	23.14	0.90
LUC_002_310_Daf	16138	7860	345.9	7843	46	96.25	2.02
LUC14_002_12_01	27041	10496	310.9	9872	2304	3544.67	6.92
LUC14_002_12_12	20870	7879	305.2	7172	2022	4190.27	6.45
LUC14_002_12_23	14685	5926	304.1	5726	1505	2931.77	6.08
LUC14_002_12_34	19861	7941	302.7	7681	2049	4357.13	6.41
LUC14_002_12_45	25695	10298	304.4	9573	2443	4742.09	6.58
LUC14_002_12_56	22933	9155	301.7	8398	2491	5565.12	6.67
LUC14_002_12_67	21436	8848	305.6	8231	2804	5953.26	7.02
LUC14_002_12_D1	23747	10050	307.2	9420	3122	5967.52	7.25
LUC14_002_12_D2	27774	11502	300.5	11000	1046	1360.23	6.29
LUC14_002_14_01	27534	10729	308.6	10179	2096	4178.93	6.28
LUC14_002_14_12	25252	9648	310.1	9001	2213	4673.71	6.48
LUC14_002_14_23	25769	10456	312.8	9708	2583	5309.53	6.74
LUC14_002_14_34	24820	10233	309.3	9561	2617	4242.19	6.38
LUC14_002_14_D	21990	9558	305.2	9297	2828	6084.41	6.90
LUC14_002_16_02	25981	9409	301.1	8720	2221	4995.01	6.46
LUC14_002_16_24	28290	10867	304.6	10406	2424	4921.78	6.47

Table 6 (Continued) - Summary of 454 tag pyrosequencing data for each sample. Operational Taxonomic Units (OTUs) and diversity indices were calculated at 97% level.

Sample Name	Number sequences (raw)	Number sequences after trimming	Mean length after trimming (base pairs)	Number Sequences. After screening, filtering, and chimera removal	No. OTUs (97% sequence identity)	Chao Diversity	Shannon Diversity (H') Index
LUC14_002_18_01	10851	3664	287.7	3526	1013	2204.09	5.85
LUC14_002_18_12	10884	3818	289.5	3375	1179	2638.92	6.30
LUC14_002_18_23	12318	4082	289.6	3711	1395	3649.58	6.47
LUC14_002_18_34	12638	4230	281.7	4151	1441	3536.72	6.48
LUC14_002_18_45	10562	3726	282.3	3328	1283	2703.29	6.37
LUC14_002_18_56	8427	2877	286.1	2625	974	2378.10	6.06
LUC14_002_18_D	14973	5483	282.9	5020	1962	4718.13	6.69
LUC14_002_110_01	23124	8949	308.3	8489	2018	4045.77	6.25
LUC14_002_110_12	26838	10585	308.5	9660	2150	4337.70	6.17
LUC14_002_110_23	26338	10024	304.6	9650	2159	4543.57	6.19
LUC14_002_110_34	25131	9712	309.1	8966	2554	5781.72	6.94
LUC14_002_110_45	25737	10719	310.9	9859	2870	6545.86	6.82
LUC14_002_110_D	30483	12644	297.5	11893	3575	7305.70	7.20
LUC14_002_310_01	25405	10278	309.1	9905	2323	4776.93	6.37
LUC14_002_310_12	20671	8411	307.0	7876	2261	4872.07	6.55
LUC14_002_310_23	25007	10364	311.2	9524	2339	4949.35	6.42
LUC14_002_310_34	25934	10735	311.6	9911	2157	4462.72	6.10
LUC14_002_310_45	28321	11790	311.3	10777	3074	6596.78	6.86
LUC14_002_310_56	25409	10398	308.3	10256	2773	5903.13	6.80
LUC14_002_310_D	32353	14098	298.9	13331	3672	6988.22	7.16
LUC14_004pw	15170	5684	304.1	5197	1091	3068.99	4.96
LUC14_005pw	24419	8761	298.2	7922	1127	1524.01	5.45

Table 6 (Continued) - Summary of 454 tag pyrosequencing data for each sample. Operational Taxonomic Units (OTUs) and diversity indices were calculated at 97% level.

Sample Name	Number sequences (raw)	Number sequences after trimming	Mean length after trimming (base pairs)	Number Sequences. After screening, filtering, and chimera removal	No. OTUs (97% sequence identity)	Chao Diversity	Shannon Diversity (H') Index
LUC14_006pw	16510	6667	298.7	6410	978	2106.98	4.27
LUC14_007pw	15709	6353	300.0	5977	822	2054.02	3.94
LUC14_008pw	13367	4628	294.2	4219	1037	2556.56	5.14
LUC14_009ow	15890	5898	299.0	5449	382	989.95	3.43
LUC14_010pw	13076	4552	295.9	4137	1012	2434.78	5.36
LUC14_011mw	18468	7322	317.7	6460	388	588.00	2.96

Table 7 - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
		Total	9177	6906	5667	7338	9515	8296	8105	9341	10917
2	0.2.1	Acidobacteria	327	247	230	204	529	359	342	325	372
2	0.2.10	Candidate_division_BRC1	8	2	4	7	17	5	9	17	35
2	0.2.11	Candidate_division_JS1								1	15
2	0.2.12	Candidate_division_KB1	1	1		1	2		1	7	11
2	0.2.13	Candidate_division_OD1	16	13	6	4	9	19	17	19	42
2	0.2.14	Candidate_division_OP11	4		1	2	1	1	4	6	50
2	0.2.15	Candidate_division_OP3	7	3	2	6	6	7	9	4	5
2	0.2.16	Candidate_division_OP8	2	25	33	53	55	73	92	237	370
2	0.2.19	Candidate_division_TM7	12	8	5	9		2	7	4	
2	0.2.2	Actinobacteria	325	188	182	173	375	231	228	255	209
2	0.2.20	Candidate_division_WS3	289	149	134	147	262	274	235	311	461
2	0.2.22	Chlamydiae	1	2		6	3	1	5	3	16
2	0.2.23	Chlorobi	44	58	40	56	41	58	50	37	
2	0.2.24	Chloroflexi	470	417	446	472	942	976	952	2145	3721
2	0.2.25	Chrysiogenetes		4	9	2		1	2	8	
2	0.2.26	Cyanobacteria	921	461	201	523	198	218	227	152	104
2	0.2.27	Deferribacteres	129	124	98	106	93	129	111	183	185
2	0.2.28	Deinococcus-Thermus	2	1	2	2		2	1	3	3
2	0.2.29	Dictyoglomi		1							
2	0.2.30	Elusimicrobia	17	5	16	6	16	14	22	39	27
2	0.2.31	Fibrobacteres	34	18	18	36	17	23	25	35	36
2	0.2.32	Firmicutes	157	99	128	143	174	155	173	193	193
2	0.2.33	Fusobacteria	13	43	8	304	45	52	12	25	
2	0.2.34	GAL08						2			

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
2	0.2.35	GOUTA4	1	1					2	6	
2	0.2.36	Gemmatimonadetes	140	86	76	63	147	103	123	92	50
2	0.2.37	Hyd24-12	21	14	5	8	17	7	4	5	
2	0.2.38	JL-ETNP-Z39		3	6	4	2	2		1	2
2	0.2.39	LD1-PA38	2				1	3	2	9	10
2	0.2.4	Armatimonadetes		1						3	1
2	0.2.40	Lentisphaerae	83	33	21	65	63	48	26	53	45
2	0.2.41	NPL-UPA2	2	1		1	3		2	6	5
2	0.2.42	Nitrospirae	22	4	5	10	8	10	5	19	66
2	0.2.44	Planctomycetes	585	468	452	613	825	746	551	757	1014
2	0.2.45	Proteobacteria	4845	3583	2961	3907	4845	3985	3923	3706	3053
2	0.2.46	RsaHF231		2							
2	0.2.48	SHA-109	5	6	2	3	2	1	2	1	13
2	0.2.5	BD1-5	11	4	6	5	6	5	3	6	2
2	0.2.50	Spirochaetae	125	75	44	61	78	122	127	199	314
2	0.2.51	Synergistetes				1	2	1	2	1	
2	0.2.52	TA06	81	43	24	22	41	73	70	191	286
2	0.2.53	TM6	20	14	13	15	12	30	18	42	20
2	0.2.54	Tenericutes	13	4	5	6	13	5	3	11	
2	0.2.56	Thermotogae	3					2			
2	0.2.57	Verrucomicrobia	45	13	11	11	35	15	7	9	6
2	0.2.7	Bacteroidetes	1082	944	531	622	684	635	830	280	188
2	0.2.8	CKC4						1			
2	0.2.9	Caldiserica	3	3		1	2		2	1	1
3	0.2.1.1	Acidobacteria	100	79	56	76	177	134	157	172	240

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.1.2	Holophagae	132	108	125	86	255	143	110	72	64
3	0.2.1.3	Subgroup_22	92	57	45	41	90	79	74	80	68
3	0.2.1.4	Subgroup_26	3	3	4	1	7	3	1	1	
3	0.2.10.1	unclassified	8	2	4	7	17	5	9	17	35
3	0.2.11.1	unclassified								1	15
3	0.2.12.1	Unknown_Class		1		1			1	4	
3	0.2.12.2	unclassified	1				2			3	11
3	0.2.13.1	Unknown_Class								1	
3	0.2.13.2	unclassified	16	13	6	4	9	19	17	18	42
3	0.2.14.1	unclassified	4		1	2	1	1	4	6	50
3	0.2.15.1	unclassified	7	3	2	6	6	7	9	4	5
3	0.2.16.1	unclassified	2	25	33	53	55	73	92	237	370
3	0.2.19.2	unclassified	12	8	5	9		2	7	4	
3	0.2.2.1	Acidimicrobiia	256	153	124	115	284	135	144	90	11
3	0.2.2.11	Thermoleophilia	9	4	10	3	16	14	9	16	32
3	0.2.2.2	Actinobacteria	54	28	42	48	54	62	53	116	135
3	0.2.2.3	Coriobacteriia	5	1	4		11	12	17	24	17
3	0.2.2.4	FFCH16263		1		2	4	5	1	3	4
3	0.2.2.5	KIST-JJY010					1				
3	0.2.2.7	Nitriliruptoria					1				
3	0.2.2.8	OPB41	1	1	2	5	4	3	4	6	10
3	0.2.20.1	Unknown_Class	1	1					2	1	3
3	0.2.20.2	unclassified	288	148	134	147	262	274	233	310	458
3	0.2.22.1	Chlamydiae	1	2		6	3	1	5	3	16
3	0.2.23.1	Chlorobia	12	2	1	3	2	4		2	

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.23.2	Ignavibacteria	32	56	39	53	39	54	50	35	
3	0.2.24.1	Anaerolineae	313	224	267	253	451	481	521	487	773
3	0.2.24.11	KD4-96	54	39	56	40	173	65	36	38	
3	0.2.24.12	KZNMV-5-B42	2	1		2	4	7	1	11	10
3	0.2.24.13	Ktedonobacteria		1			1	6	2	8	37
3	0.2.24.14	MSB-5B2	16	3	1	4	24	14	28	329	668
3	0.2.24.16	S085						7		8	8
3	0.2.24.17	SAR202_clade		1			1			8	24
3	0.2.24.2	Ardenticatenia	47	63	54	71	130	90	70	63	37
3	0.2.24.20	TK10								1	2
3	0.2.24.3	Caldilineae	18	18	21	20	50	23	15	6	7
3	0.2.24.5	Chloroflexia	1								
3	0.2.24.6	Dehalococcoidia	19	63	47	82	108	283	278	1151	2024
3	0.2.24.9	JG30-KF-CM66		4					1	35	131
3	0.2.25.1	Chrysiogenetes		4	9	2		1	2	8	
3	0.2.26.2	Cyanobacteria	223	193	143	175	137	115	104	86	90
3	0.2.26.3	ML635J-21		1							
3	0.2.26.4	Melainabacteria	7	2		6	5	3	2		
3	0.2.27.1	Deferribacteres	129	124	98	106	93	129	111	183	185
3	0.2.28.1	Deinococci	2	1	2	2		2	1	3	3
3	0.2.29.1	Dictyoglomia		1							
3	0.2.30.1	Elusimicrobia	17	5	16	6	16	14	22	39	27
3	0.2.31.1	Fibrobacteria	34	18	18	36	17	23	25	35	36
3	0.2.32.1	Bacilli	56	20	25	21	33	31	50	80	72
3	0.2.32.2	Clostridia	80	65	87	95	111	108	109	96	102

Table 7 (Continued) - Core 1-2: 16S rRNA sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.32.3	Erysipelotrichia	7	3	10	1	8	1	8	2	5
3	0.2.32.4	Negativicutes	14	11	6	26	22	15	6	14	14
3	0.2.32.5	OPB54								1	
3	0.2.33.1	Fusobacteriia	13	43	8	304	45	52	12	25	
3	0.2.34.1	unclassified						2			
3	0.2.35.1	unclassified	1	1					2	6	
3	0.2.36.1	Gemmatimonadetes	140	86	76	63	147	103	123	92	50
3	0.2.37.1	unclassified	21	14	5	8	17	7	4	5	
3	0.2.38.1	unclassified		3	6	4	2	2		1	2
3	0.2.39.1	unclassified	2				1	3	2	9	10
3	0.2.4.2	Chthonomonadetes								1	
3	0.2.4.3	unclassified		1						2	1
3	0.2.40.1	113B434	2	2	2	7		1		2	
3	0.2.40.10	ML1228J-2	1								
3	0.2.40.11	ML635J-35	2		1			3		1	
3	0.2.40.12	MSBL3	3							5	
3	0.2.40.13	Oligosphaeria	21	2		11		2			
3	0.2.40.15	R76-B128	17	11	6	32	34	12	6	7	6
3	0.2.40.17	SBYG-2613	2	3		1			3	1	
3	0.2.40.18	SS1-B-03-39		1							
3	0.2.40.20	WCHB1-41	7		3		3	8	1	3	27
3	0.2.40.21	c5LKS8	4						1	1	
3	0.2.40.3	BS5		1							
3	0.2.40.5	LD1-PA20	3	3	1	2	4	4	8	7	3
3	0.2.40.6	LD1-PA26	12	5	7	10	15	11	7	21	9

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.40.7	LD1-PA34				2	1	4		2	
3	0.2.40.8	LD1-PB3	9	5	1		6	3		3	
3	0.2.41.1	unclassified	2	1		1	3		2	6	5
3	0.2.42.1	Nitrospira	22	4	5	10	8	10	5	19	66
3	0.2.44.1	028H05-P-BN-P5	5	5	3	3	1	5	3	15	4
3	0.2.44.10	SGST604	7	6	3		5	4	2	1	11
3	0.2.44.11	SPG12-401-411-B72	13	6	4	5	1	5	5	3	29
3	0.2.44.13	vadinHA49	5	3	3	7	10	8	1	10	13
3	0.2.44.2	BD7-11	7	3	1	3	2		1	2	
3	0.2.44.3	MBMPE71				1			1	9	49
3	0.2.44.4	MD2896-B258							1	4	
3	0.2.44.5	OM190	24	25	15	37	31	39	22	43	29
3	0.2.44.6	Phycisphaerae	292	151	127	251	214	313	214	393	555
3	0.2.44.7	Pla3_lineage	10	4	6	4	9	15	14	3	18
3	0.2.44.8	Pla4_lineage	3	6	4	7		3	3	8	28
3	0.2.44.9	Planctomycetacia	219	259	286	295	552	354	284	266	278
3	0.2.45.1	AEGEAN-245					1				
3	0.2.45.10	FGL7S									3
3	0.2.45.11	Gammaproteobacteria	1824	1395	803	1176	1208	980	966	882	481
3	0.2.45.12	JTB23		2	1						
3	0.2.45.13	MACA-EFT26				1				1	7
3	0.2.45.14	Milano-WF1B-44	13	17	7	20	18	10	14	7	
3	0.2.45.15	Proteobacteria_Incertae_Sedis	109	42	26	51	42	42	41	27	13
3	0.2.45.16	SC3-20	6	5	2	5	1	1	4	3	
3	0.2.45.17	SPOTSOCT00m83		2	1		1			4	

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.45.19	TA18	3	3	4		1		3	1	4
3	0.2.45.2	ARKDMS-49		5		4	4		3	11	
3	0.2.45.21	pItb-vmat-80	14	2		2	1	2	1	2	
3	0.2.45.3	ARKICE-90		1		1			1		
3	0.2.45.4	Alphaproteobacteria	604	349	291	295	515	329	390	158	111
3	0.2.45.5	BJGMM-U27	3			7	3	3		1	4
3	0.2.45.6	Betaproteobacteria	6			2	2	3	1	16	30
3	0.2.45.7	Deltaproteobacteria	2235	1748	1817	2331	3020	2595	2458	2573	2379
3	0.2.45.8	Elev-16S-509	1		3						
3	0.2.45.9	Epsilonproteobacteria	27	12	6	12	28	20	41	20	21
3	0.2.46.1	unclassified		2							
3	0.2.48.1	unclassified	5	6	2	3	2	1	2	1	13
3	0.2.5.1	unclassified	11	4	6	5	6	5	3	6	2
3	0.2.50.1	Spirochaetes	125	75	44	61	78	122	127	199	314
3	0.2.51.1	Synergistia				1	2	1	2	1	
3	0.2.52.1	unclassified	81	43	24	22	41	73	70	191	286
3	0.2.53.1	unclassified	20	14	13	15	12	30	18	42	20
3	0.2.54.1	Mollicutes	13	4	5	6	13	5	3	11	
3	0.2.56.1	Thermotogae	3					2			
3	0.2.57.2	OPB35_soil_group	8	1	1	1	8	1		1	5
3	0.2.57.3	Opitutae	14	11	7	6	12	5	4	6	
3	0.2.57.5	Spartobacteria				1		1			
3	0.2.57.6	UA11			1	1		1		1	
3	0.2.57.7	Verrucomicrobia_Incertae_Sedis						2			1
3	0.2.57.8	Verrucomicrobiae	23	1	2	2	15	5	3	1	

Table 7 (Continued) - Core 1-2: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	10-20 cm	20-30 cm
3	0.2.7.1	BD2-2	52	73	45	83	71	78	93	61	43
3	0.2.7.10	SB-5	5	8	6	2	3	18	23	19	65
3	0.2.7.12	Sphingobacteriia	154	80	30	59	50	48	81	19	18
3	0.2.7.13	VC2.1_Bac22	22	21	17	23	24	18	9	11	
3	0.2.7.16	vadinHA17								1	
3	0.2.7.3	Bacteroidetes_Incertae_Sedis				3	1	2	1	3	
3	0.2.7.4	Bacteroidia	9	7	6	11	6	5	2		1
3	0.2.7.5	Cytophagia	165	121	79	121	129	92	126	40	40
3	0.2.7.6	Flavobacteriia	665	626	347	316	394	373	492	126	21
3	0.2.7.7	Ika33		1							
3	0.2.7.8	ML602M-17	4								
3	0.2.7.9	SB-1	6	7	1	4	6	1	3		
3	0.2.8.1	unclassified						1			
3	0.2.9.1	Caldisericia	3	3		1	2		2	1	1

Table 8 - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
		Total	8819	8325	8925	9263	9254
2	0.2.1	Acidobacteria	215	209	260	338	406
2	0.2.10	Candidate_division_BRC1	3		5	5	11
2	0.2.12	Candidate_division_KB1	1	2	4	5	3
2	0.2.13	Candidate_division_OD1	11	11	11	6	26
2	0.2.14	Candidate_division_OP11					6
2	0.2.15	Candidate_division_OP3		2	4		18
2	0.2.16	Candidate_division_OP8	15	8	23	24	288
2	0.2.18	Candidate_division_SR1	2			2	
2	0.2.19	Candidate_division_TM7	21	10	9	4	
2	0.2.2	Actinobacteria	248	219	218	311	198
2	0.2.20	Candidate_division_WS3	104	120	145	162	206
2	0.2.22	Chlamydiae	1			3	2
2	0.2.23	Chlorobi	47	56	56	60	25
2	0.2.24	Chloroflexi	405	320	403	579	2065
2	0.2.25	Chrysiogenetes	4				7
2	0.2.26	Cyanobacteria	1724	931	1191	610	104
2	0.2.27	Deferribacteres	44	45	73	88	180
2	0.2.28	Deinococcus-Thermus	3			1	4
2	0.2.29	Dictyoglomi		3			
2	0.2.30	Elusimicrobia	6	1	6	5	34
2	0.2.31	Fibrobacteres	10	12	15	19	46
2	0.2.32	Firmicutes	118	104	151	202	213
2	0.2.33	Fusobacteria	69	60	70	60	10
2	0.2.36	Gemmatimonadetes	56	54	41	70	69
2	0.2.37	Hyd24-12	5	2	17	28	5
2	0.2.38	JL-ETNP-Z39	3				9
2	0.2.39	LD1-PA38			3	1	1
2	0.2.4	Armatimonadetes					3
2	0.2.40	Lentisphaerae	32	37	44	42	35
2	0.2.41	NPL-UPA2	1	2		2	
2	0.2.42	Nitrospirae	4	8	14	16	34
2	0.2.44	Planctomycetes	380	518	572	684	863
2	0.2.45	Proteobacteria	4738	4599	5095	5018	3809
2	0.2.46	RsaHF231	1				
2	0.2.47	SBYG-2791				1	1

Table 8 (Continued) - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
2	0.2.48	SHA-109	8	11	12	4	
2	0.2.5	BD1-5	6	4	5	9	4
2	0.2.50	Spirochaetae	39	58	64	83	173
2	0.2.51	Synergistetes				8	5
2	0.2.52	TA06	23	17	28	71	108
2	0.2.53	TM6	9	4	9	16	46
2	0.2.54	Tenericutes	5	2	5	15	28
2	0.2.55	Thermodesulfobacteria	1	1		1	
2	0.2.56	Thermotogae		2	2	2	
2	0.2.57	Verrucomicrobia	33	20	38	18	9
2	0.2.6	BHI80-139	1				
2	0.2.7	Bacteroidetes	1781	1540	1106	986	241
2	0.2.9	Caldiserica		8	6	2	
3	0.2.1.1	Acidobacteria	89	62	71	110	192
3	0.2.1.2	Holophagae	88	109	139	163	128
3	0.2.1.3	Subgroup_22	36	35	49	61	84
3	0.2.1.4	Subgroup_26	2	3	1	4	2
3	0.2.10.1	unclassified	3		5	5	11
3	0.2.12.1	Unknown_Class		2	3		3
3	0.2.12.2	unclassified	1		1	5	
3	0.2.13.1	Unknown_Class					3
3	0.2.13.2	unclassified	11	11	11	6	23
3	0.2.14.1	unclassified					6
3	0.2.15.1	unclassified		2	4		18
3	0.2.16.1	unclassified	15	8	23	24	288
3	0.2.18.1	unclassified	2			2	
3	0.2.19.2	unclassified	21	10	9	4	
3	0.2.2.1	Acidimicrobiia	177	180	141	227	59
3	0.2.2.11	Thermoleophilia	6	7	24	14	10
3	0.2.2.2	Actinobacteria	62	31	40	59	104
3	0.2.2.3	Coriobacteriia	2	1	9	5	14
3	0.2.2.4	FFCH16263			3	5	6
3	0.2.2.5	KIST-JJY010	1		1		1
3	0.2.2.8	OPB41				1	4
3	0.2.20.1	Unknown_Class				1	3

Table 8 (Continued) - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
3	0.2.20.2	unclassified	104	120	145	161	203
3	0.2.22.1	Chlamydiae	1			3	2
3	0.2.23.1	Chlorobia	8	5	8	2	3
3	0.2.23.2	Ignavibacteria	39	51	48	58	22
3	0.2.24.1	Anaerolineae	201	147	208	302	589
3	0.2.24.11	KD4-96	91	91	91	108	49
3	0.2.24.12	KZNMV-5-B42				2	8
3	0.2.24.13	Ktedonobacteria			4		8
3	0.2.24.14	MSB-5B2	11	8	9	15	135
3	0.2.24.16	S085	1				18
3	0.2.24.17	SAR202_clade					1
3	0.2.24.2	Ardenticatenia	39	51	49	77	52
3	0.2.24.3	Caldilineae	24	13	25	44	4
3	0.2.24.6	Dehalococcoidia	37	9	17	31	1179
3	0.2.24.7	Elev-1554					1
3	0.2.24.9	JG30-KF-CM66	1	1			21
3	0.2.25.1	Chrysiogenetes	4				7
3	0.2.26.2	Cyanobacteria	360	255	407	306	59
3	0.2.26.3	ML635J-21	1				
3	0.2.26.4	Melainabacteria	5	1	4	6	4
3	0.2.27.1	Deferribacteres	44	45	73	88	180
3	0.2.28.1	Deinococci	3			1	4
3	0.2.29.1	Dictyoglomia		3			
3	0.2.30.1	Elusimicrobia	6	1	6	5	34
3	0.2.31.1	Fibrobacteria	10	12	15	19	46
3	0.2.32.1	Bacilli	17	15	25	32	69
3	0.2.32.2	Clostridia	83	76	112	159	133
3	0.2.32.3	Erysipelotrichia	2	5	5	2	3
3	0.2.32.4	Negativicutes	16	8	9	9	8
3	0.2.33.1	Fusobacteriia	69	60	70	60	10
3	0.2.36.1	Gemmatimonadetes	56	54	41	70	69
3	0.2.37.1	unclassified	5	2	17	28	5
3	0.2.38.1	unclassified	3				9
3	0.2.39.1	unclassified			3	1	1
3	0.2.4.2	Chthonomonadetes					1

Table 8 (Continued) - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
3	0.2.4.3	unclassified					2
3	0.2.40.1	113B434			1	2	1
3	0.2.40.11	ML635J-35			1	1	
3	0.2.40.12	MSBL3		5		1	
3	0.2.40.13	Oligosphaeria	1	8	9	5	
3	0.2.40.15	R76-B128	17	14	15	13	14
3	0.2.40.17	SBYG-2613	1	1	3	1	
3	0.2.40.20	WCHB1-41	1		4	2	2
3	0.2.40.21	c5LKS8	5	1	3		
3	0.2.40.5	LD1-PA20		2	1	3	3
3	0.2.40.6	LD1-PA26		3	2	7	12
3	0.2.40.7	LD1-PA34	2	2	2	5	2
3	0.2.40.8	LD1-PB3	5	1	3	2	1
3	0.2.41.1	unclassified	1	2		2	
3	0.2.42.1	Nitrospira	4	8	14	16	34
3	0.2.44.1	028H05-P-BN-P5	3	3	1	12	2
3	0.2.44.10	SGST604	2	6	5	2	5
3	0.2.44.11	SPG12-401-411-B72	1	4	4	5	13
3	0.2.44.13	vadinHA49		1	2	3	4
3	0.2.44.2	BD7-11			2	1	5
3	0.2.44.3	MBMPE71	1				6
3	0.2.44.4	MD2896-B258					1
3	0.2.44.5	OM190	38	31	29	24	22
3	0.2.44.6	Phycisphaerae	66	86	116	132	564
3	0.2.44.7	Pla3_lineage	4	1	4	7	11
3	0.2.44.8	Pla4_lineage		4	5	7	15
3	0.2.44.9	Planctomycetacia	265	382	404	491	215
3	0.2.45.1	AEGEAN-245					2
3	0.2.45.11	Gammaproteobacteria	1301	1401	1604	1263	641
3	0.2.45.12	JTB23				1	
3	0.2.45.13	MACA-EFT26				3	
3	0.2.45.14	Milano-WF1B-44	14	29	21	19	13
3	0.2.45.15	Proteobacteria_Incertae_Sedis	91	60	90	51	22
3	0.2.45.16	SC3-20	7	3	6	6	
3	0.2.45.17	SPOTSOCT00m83	2	1		2	5

Table 8 (Continued) - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
3	0.2.45.19	TA18	7	1	4	3	
3	0.2.45.2	ARKDMS-49		1	3	2	6
3	0.2.45.21	pItb-vmat-80	5	3	4	2	4
3	0.2.45.3	ARKICE-90		2			
3	0.2.45.4	Alphaproteobacteria	1253	897	764	668	297
3	0.2.45.5	BJGMM-U27	1	1	1	3	
3	0.2.45.6	Betaproteobacteria	3	1	2	3	11
3	0.2.45.7	Deltaproteobacteria	2027	2178	2568	2967	2779
3	0.2.45.8	Elev-16S-509			2		1
3	0.2.45.9	Epsilonproteobacteria	27	21	26	25	28
3	0.2.47.1	unclassified				1	1
3	0.2.48.1	unclassified	8	11	12	4	
3	0.2.5.1	unclassified	6	4	5	9	4
3	0.2.50.1	Spirochaetes	39	58	64	83	173
3	0.2.51.1	Synergistia				8	5
3	0.2.52.1	unclassified	23	17	28	71	108
3	0.2.53.1	unclassified	9	4	9	16	46
3	0.2.54.1	Mollicutes	5	2	5	15	28
3	0.2.55.1	Thermodesulfobacteria	1	1		1	
3	0.2.56.1	Thermotogae		2	2	2	
3	0.2.57.2	OPB35_soil_group	1		5	3	2
3	0.2.57.3	Opitutae	20	15	19	10	6
3	0.2.57.4	S-BQ2-57_soil_group					1
3	0.2.57.6	UA11			2		
3	0.2.57.7	Verrucomicrobia_Incertae_Sedis	1				
3	0.2.57.8	Verrucomicrobiae	11	5	12	5	
3	0.2.6.1	unclassified	1				
3	0.2.7.1	BD2-2	61	72	63	71	53
3	0.2.7.10	SB-5	7	12	16	14	12
3	0.2.7.12	Sphingobacteriia	213	129	121	116	26
3	0.2.7.13	VC2.1_Bac22	17	25	21	31	
3	0.2.7.3	Bacteroidetes_Incertae_Sedis	1			1	5
3	0.2.7.4	Bacteroidia	14	10	12	10	8
3	0.2.7.5	Cytophagia	144	159	122	130	36
3	0.2.7.6	Flavobacteriia	1312	1123	735	609	98

Table 8 (Continued) - Core 1-4: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	10-30 cm
3	0.2.7.7	Ika33		2	1		
3	0.2.7.8	ML602M-17			1		
3	0.2.7.9	SB-1	12	8	14	4	3
3	0.2.9.1	Caldisericia		8	6	2	

Table 9 - Core 1-6: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-2 cm	2-4 cm
		Total	8057	9706
2	0.2.1	Acidobacteria	198	278
2	0.2.10	Candidate_division_BRC1		9
2	0.2.12	Candidate_division_KB1	1	3
2	0.2.13	Candidate_division_OD1	7	21
2	0.2.14	Candidate_division_OP11		1
2	0.2.15	Candidate_division_OP3	2	5
2	0.2.16	Candidate_division_OP8	8	19
2	0.2.18	Candidate_division_SR1		2
2	0.2.19	Candidate_division_TM7	9	14
2	0.2.2	Actinobacteria	208	265
2	0.2.20	Candidate_division_WS3	115	197
2	0.2.22	Chlamydiae	3	
2	0.2.23	Chlorobi	55	77
2	0.2.24	Chloroflexi	311	476
2	0.2.25	Chrysiogenetes	3	2
2	0.2.26	Cyanobacteria	907	1019
2	0.2.27	Deferribacteres	46	124
2	0.2.28	Deinococcus-Thermus	4	2
2	0.2.30	Elusimicrobia	1	2
2	0.2.31	Fibrobacteres	22	31
2	0.2.32	Firmicutes	87	134
2	0.2.33	Fusobacteria	240	143
2	0.2.35	GOUTA4		2
2	0.2.36	Gemmatimonadetes	58	86
2	0.2.37	Hyd24-12	9	19
2	0.2.38	JL-ETNP-Z39	1	3
2	0.2.39	LD1-PA38	3	
2	0.2.40	Lentisphaerae	35	88
2	0.2.41	NPL-UPA2	1	4
2	0.2.42	Nitrospirae	3	43
2	0.2.44	Planctomycetes	563	538
2	0.2.45	Proteobacteria	4210	5089
2	0.2.46	RsaHF231	1	
2	0.2.48	SHA-109	2	3

Table 9 (Continued) - Core 1-6: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-2 cm	2-4 cm
2	0.2.5	BD1-5	6	15
2	0.2.50	Spirochaetae	53	85
2	0.2.51	Synergistetes	1	4
2	0.2.52	TA06	22	54
2	0.2.53	TM6	11	12
2	0.2.54	Tenericutes	15	9
2	0.2.55	Thermodesulfobacteria		2
2	0.2.57	Verrucomicrobia	22	35
2	0.2.7	Bacteroidetes	1473	1488
2	0.2.9	Caldiserica	2	3
3	0.2.1.1	Acidobacteria	70	97
3	0.2.1.2	Holophagae	85	106
3	0.2.1.3	Subgroup_22	40	66
3	0.2.1.4	Subgroup_26	3	9
3	0.2.10.1	unclassified		9
3	0.2.12.1	Unknown_Class	1	2
3	0.2.12.2	unclassified		1
3	0.2.13.2	unclassified	7	21
3	0.2.14.1	unclassified		1
3	0.2.15.1	unclassified	2	5
3	0.2.16.1	unclassified	8	19
3	0.2.18.1	unclassified		2
3	0.2.19.2	unclassified	9	14
3	0.2.2.1	Acidimicrobiia	159	192
3	0.2.2.11	Thermoleophilia	17	4
3	0.2.2.2	Actinobacteria	29	63
3	0.2.2.3	Coriobacteriia	1	1
3	0.2.2.4	FFCH16263	2	4
3	0.2.20.1	Unknown_Class	2	
3	0.2.20.2	unclassified	113	197
3	0.2.22.1	Chlamydiae	3	
3	0.2.23.1	Chlorobia	7	11
3	0.2.23.2	Ignavibacteria	48	66
3	0.2.24.1	Anaerolineae	137	292
3	0.2.24.11	KD4-96	56	49

Table 9 (Continued) - Core 1-6: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

Taxa level	rank ID	taxon	0-2 cm	2-4 cm
3	0.2.24.12	KZNMV-5-B42		1
3	0.2.24.14	MSB-5B2	7	3
3	0.2.24.16	S085	3	
3	0.2.24.2	Ardenticatenia	66	73
3	0.2.24.3	Caldilineae	26	23
3	0.2.24.5	Chloroflexia		1
3	0.2.24.6	Dehalococcoidia	15	34
3	0.2.25.1	Chrysiogenetes	3	2
3	0.2.26.2	Cyanobacteria	242	320
3	0.2.26.3	ML635J-21	2	
3	0.2.26.4	Melainabacteria	2	2
3	0.2.27.1	Deferribacteres	46	124
3	0.2.28.1	Deinococci	4	2
3	0.2.30.1	Elusimicrobia	1	2
3	0.2.31.1	Fibrobacteria	22	31
3	0.2.32.1	Bacilli	11	18
3	0.2.32.2	Clostridia	65	74
3	0.2.32.3	Erysipelotrichia	6	2
3	0.2.32.4	Negativicutes	5	40
3	0.2.33.1	Fusobacteriia	240	143
3	0.2.35.1	unclassified		2
3	0.2.36.1	Gemmatimonadetes	58	86
3	0.2.37.1	unclassified	9	19
3	0.2.38.1	unclassified	1	3
3	0.2.39.1	unclassified	3	
3	0.2.40.1	113B434	1	5
3	0.2.40.11	ML635J-35	1	1
3	0.2.40.12	MSBL3	2	1
3	0.2.40.13	Oligosphaeria	5	3
3	0.2.40.15	R76-B128	19	26
3	0.2.40.17	SBYG-2613		6
3	0.2.40.20	WCHB1-41		16
3	0.2.40.21	c5LKS8		3
3	0.2.40.5	LD1-PA20		10
3	0.2.40.6	LD1-PA26	2	17

Table 9 (Continued) - Core 1-6: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-2 cm	2-4 cm
3	0.2.40.7	LD1-PA34	1	
3	0.2.40.8	LD1-PB3	3	
3	0.2.40.9	Lentisphaeria	1	
3	0.2.41.1	unclassified	1	4
3	0.2.42.1	Nitrospira	3	43
3	0.2.44.1	028H05-P-BN-P5	2	4
3	0.2.44.10	SGST604	1	3
3	0.2.44.11	SPG12-401-411-B72	10	4
3	0.2.44.13	vadinHA49	3	8
3	0.2.44.2	BD7-11		3
3	0.2.44.4	MD2896-B258	2	
3	0.2.44.5	OM190	24	48
3	0.2.44.6	Phycisphaerae	131	145
3	0.2.44.7	Pla3_lineage	5	8
3	0.2.44.8	Pla4_lineage	6	6
3	0.2.44.9	Planctomycetacia	379	309
3	0.2.45.1	AEGEAN-245		1
3	0.2.45.11	Gammaproteobacteria	1581	1652
3	0.2.45.12	JTB23	1	
3	0.2.45.14	Milano-WF1B-44	12	15
3	0.2.45.15	Proteobacteria_Incertae_Sedis	58	77
3	0.2.45.16	SC3-20	2	1
3	0.2.45.17	SPOTSOCT00m83	1	2
3	0.2.45.19	TA18	6	7
3	0.2.45.2	ARKDMS-49		1
3	0.2.45.21	pItb-vmat-80	8	3
3	0.2.45.3	ARKICE-90	2	3
3	0.2.45.4	Alphaproteobacteria	662	533
3	0.2.45.5	BJGMM-U27	1	2
3	0.2.45.6	Betaproteobacteria	7	6
3	0.2.45.7	Deltaproteobacteria	1851	2773
3	0.2.45.8	Elev-16S-509	1	
3	0.2.45.9	Epsilonproteobacteria	17	13
3	0.2.48.1	unclassified	2	3
3	0.2.5.1	unclassified	6	15

Table 9 (Continued) - Core 1-6: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-2 cm	2-4 cm
3	0.2.50.1	Spirochaetes	53	85
3	0.2.51.1	Synergistia	1	4
3	0.2.52.1	unclassified	22	54
3	0.2.53.1	unclassified	11	12
3	0.2.54.1	Mollicutes	15	9
3	0.2.55.1	Thermodesulfobacteria		2
3	0.2.57.2	OPB35_soil_group		8
3	0.2.57.3	Opitutae	20	19
3	0.2.57.6	UA11		2
3	0.2.57.8	Verrucomicrobiae	2	6
3	0.2.7.1	BD2-2	54	112
3	0.2.7.10	SB-5	8	15
3	0.2.7.12	Sphingobacteriia	175	161
3	0.2.7.13	VC2.1_Bac22	19	40
3	0.2.7.16	vadinHA17		1
3	0.2.7.3	Bacteroidetes_Incertae_Sedis	3	
3	0.2.7.4	Bacteroidia	25	7
3	0.2.7.5	Cytophagia	167	184
3	0.2.7.6	Flavobacteriia	1015	954
3	0.2.7.7	Ika33	1	
3	0.2.7.8	ML602M-17	3	
3	0.2.7.9	SB-1	3	14
3	0.2.9.1	Caldisericia	2	3

Table 10 - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
		Total	3198	3060	3616	3722	3283	2585	8873
1	0.2	Bacteria	3198	3060	3616	3722	3283	2585	8873
2	0.2.1	Acidobacteria	63	64	102	127	130	86	440
2	0.2.10	Candidate_div_BRC	4		5	1	3	2	26
2	0.2.12	Candidate_division_KB1		1				1	
2	0.2.13	Candidate_div_OD1	2	7	3	3	2	2	6
2	0.2.14	Candidate_division_OP11				1			
2	0.2.15	Candidate_division_OP3		1			4	2	22
2	0.2.16	Candidate_div_OP8	1	5	5	20	11	7	112
2	0.2.18	Candidate_division_SR1					1		
2	0.2.19	Candidate_div_TM7	9	6	3	4	1	4	
2	0.2.2	Actinobacteria	89	96	104	112	78	93	198
2	0.2.20	Candidate_div_WS3	41	49	78	72	56	36	212
2	0.2.22	Chlamydiae	1	1		1	2	1	10
2	0.2.23	Chlorobi	16	10	10	16	14	6	26
2	0.2.24	Chloroflexi	124	139	173	270	226	168	854
2	0.2.25	Chrysiogenetes	3				1		
2	0.2.26	Cyanobacteria	441	510	196	242	129	167	302
2	0.2.27	Deferribacteres	13	21	46	43	26	18	112
2	0.2.28	Deinococcus-Thermus			1	2	3		2
2	0.2.29	Dictyoglomi	2						
2	0.2.30	Elusimicrobia			2	2	4	2	22
2	0.2.31	Fibrobacteres	5	1	6	15	22	5	26
2	0.2.32	Firmicutes	40	45	69	79	69	48	226
2	0.2.33	Fusobacteria	114	117	47	38	70	6	14

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
2	0.2.36	Gemmatimonadetes	15	27	24	29	43	28	122
2	0.2.37	Hyd24-12	1	2	6	5	2	2	12
2	0.2.38	JL-ETNP-Z39							2
2	0.2.39	LD1-PA38		2					
2	0.2.4	Armatimonadetes			1				
2	0.2.40	Lentisphaerae	15	15	22	19	14	31	66
2	0.2.41	NPL-UPA2					1		4
2	0.2.42	Nitrospirae		4	4	7	7	2	16
2	0.2.44	Planctomycetes	171	201	276	348	294	227	794
2	0.2.45	Proteobacteria	1815	1625	2083	2072	1852	1417	5624
2	0.2.46	RsaHF231			1	1			
2	0.2.47	SBYG-2791	1	2	2		1		
2	0.2.48	SHA-109	3	3				1	3
2	0.2.5	BD1-5	1	4	7	7	7	3	16
2	0.2.50	Spirochaetae	26	15	29	30	20	13	67
2	0.2.51	Synergistetes		1		2		2	
2	0.2.52	TA06	3	9	16	19	12	2	43
2	0.2.53	TM6	2	2	3	7	11	4	27
2	0.2.54	Tenericutes		4	5	7		6	5
2	0.2.56	Thermotogae				1			
2	0.2.57	Verrucomicrobia	7	13	7	9	4	1	6
2	0.2.7	Bacteroidetes	498	372	374	224	207	230	456
2	0.2.9	Caldiserica		1	1	4	1		6
3	0.2.1.1	Acidobacteria	18	23	36	58	40	25	174
3	0.2.1.2	Holophagae	31	25	50	41	58	43	116

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.1.3	Subgroup_22	13	15	16	27	29	18	144
3	0.2.1.4	Subgroup_26	1	1		1	3		6
3	0.2.10.1	unclassified	4		5	1	3	2	26
3	0.2.12.1	Unknown_Class		1				1	
3	0.2.13.2	unclassified	2	7	3	3	2	2	6
3	0.2.14.1	unclassified				1			
3	0.2.15.1	unclassified		1			4	2	22
3	0.2.16.1	unclassified	1	5	5	20	11	7	112
3	0.2.18.1	unclassified					1		
3	0.2.19.2	unclassified	9	6	3	4	1	4	
3	0.2.2.1	Acidimicrobiia	59	67	84	79	50	65	96
3	0.2.2.11	Thermoleophilia	3	8	4	9	10	11	14
3	0.2.2.2	Actinobacteria	27	20	15	21	11	14	74
3	0.2.2.3	Coriobacteriia				3	5	2	6
3	0.2.2.4	FFCH16263		1	1			1	2
3	0.2.2.5	KIST-JJY010							4
3	0.2.2.8	OPB41					2		2
3	0.2.20.1	Unknown_Class				1		2	
3	0.2.20.2	unclassified	41	49	78	71	56	34	212
3	0.2.22.1	Chlamydiae	1	1		1	2	1	10
3	0.2.23.1	Chlorobia	1	2		3			
3	0.2.23.2	Ignavibacteria	15	8	10	13	14	6	26
3	0.2.24.11	KD4-96	34	38	31	56	43	38	44
3	0.2.24.12	KZNMV-5-B42				2			4
3	0.2.24.13	Ktedonobacteria	1		1	5			2

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.24.14	MSB-5B2		1	5	1	2	3	46
3	0.2.24.16	S085				1			
3	0.2.24.17	SAR202_clade							6
3	0.2.24.2	Ardenticatenia	12	22	26	23	32	17	40
3	0.2.24.3	Caldilineae	15	15	9	17	12	14	12
3	0.2.24.5	Chloroflexia			1				2
3	0.2.24.6	Dehalococcoidia	6	8	8	32	46	23	270
3	0.2.24.9	JG30-KF-CM66			1				4
3	0.2.25.1	Chrysiogenetes	3				1		
3	0.2.26.2	Cyanobacteria	112	195	99	125	79	128	214
3	0.2.26.4	Melainabacteria	1		2		5	1	4
3	0.2.27.1	Deferribacteres	13	21	46	43	26	18	112
3	0.2.28.1	Deinococci			1	2	3		2
3	0.2.29.1	Dictyoglomia	2						
3	0.2.30.1	Elusimicrobia			2	2	4	2	22
3	0.2.31.1	Fibrobacteria	5	1	6	15	22	5	26
3	0.2.32.1	Bacilli	10	1	14	12	7	10	38
3	0.2.32.2	Clostridia	22	33	47	56	57	35	148
3	0.2.32.3	Erysipelotrichia				3	2		6
3	0.2.32.4	Negativicutes	8	11	8	8	3	3	34
3	0.2.33.1	Fusobacteriia	114	117	47	38	70	6	14
3	0.2.36.1	Gemmatimonadetes	15	27	24	29	43	28	122
3	0.2.37.1	unclassified	1	2	6	5	2	2	12
3	0.2.38.1	unclassified							2
3	0.2.39.1	unclassified		2					

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.4.3	unclassified			1				
3	0.2.40.1	113B434		1		1			2
3	0.2.40.10	ML1228J-2				2			4
3	0.2.40.11	ML635J-35		1					2
3	0.2.40.12	MSBL3			1			1	8
3	0.2.40.13	Oligosphaeria	1	4		1			2
3	0.2.40.15	R76-B128	5	5	8	9	6	21	28
3	0.2.40.17	SBYG-2613	1	2	4	2			2
3	0.2.40.20	WCHB1-41	1	1	2		1		2
3	0.2.40.21	c5LKS8			1				
3	0.2.40.5	LD1-PA20	2	1	2	1	1	4	
3	0.2.40.6	LD1-PA26	2		1	2	1	2	10
3	0.2.40.7	LD1-PA34	1		1		4		
3	0.2.40.8	LD1-PB3	2		2			3	6
3	0.2.41.1	unclassified					1		4
3	0.2.42.1	Nitrospira		4	4	7	7	2	16
3	0.2.44.1	028H05-P-BN-P5	1					3	
3	0.2.44.10	SGST604		1	2	4	1	1	4
3	0.2.44.11	SPG12-401-411-B72		7	2	2	1		10
3	0.2.44.13	vadinHA49	3	1	2	5	3		10
3	0.2.44.2	BD7-11		1		2			
3	0.2.44.3	MBMPE71							2
3	0.2.44.5	OM190	9	8	16	24	11	7	48
3	0.2.44.6	Phycisphaerae	31	45	87	93	104	60	394
3	0.2.44.7	Pla3_lineage	2		4	2	5		10

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.44.8	Pla4_lineage	2	2		3	2		2
3	0.2.44.9	Planctomycetacia	123	136	163	213	167	156	314
3	0.2.45.1	AEGEAN-245	1						
3	0.2.45.10	FGL7S			1				
3	0.2.45.11	Gammaproteobacteria	697	645	915	733	532	421	1908
3	0.2.45.12	JTB23		1					1
3	0.2.45.13	MACA-EFT26					1		
3	0.2.45.14	Milano-WF1B-44	4	9	15	23	6	8	7
3	0.2.45.15	Proteobacteria_Incertae_S	19	26	41	41	25	10	16
3	0.2.45.16	SC3-20	1	1	3				2
3	0.2.45.17	SPOTSOCT00m83	1	5	2	1			3
3	0.2.45.19	TA18	1			2	2	1	
3	0.2.45.2	ARKDMS-49					2		2
3	0.2.45.21	pItb-vmat-80	5	5	10	4		2	
3	0.2.45.3	ARKICE-90	3						
3	0.2.45.4	Alphaproteobacteria	208	167	183	128	89	162	268
3	0.2.45.5	BJGMM-U27		2	1				10
3	0.2.45.6	Betaproteobacteria	1		2			1	2
3	0.2.45.7	Deltaproteobacteria	860	751	887	1127	1186	790	3356
3	0.2.45.8	Elev-16S-509						13	6
3	0.2.45.9	Epsilonproteobacteria	14	13	23	13	9	9	14
3	0.2.46.1	unclassified			1	1			
3	0.2.47.1	unclassified	1	2	2		1		
3	0.2.48.1	unclassified	3	3				1	3
3	0.2.5.1	unclassified	1	4	7	7	7	3	16

Table 10 (Continued) - Core 1-8: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.50.1	Spirochaetes	26	15	29	30	20	13	67
3	0.2.51.1	Synergistia		1		2		2	
3	0.2.52.1	unclassified	3	9	16	19	12	2	43
3	0.2.53.1	unclassified	2	2	3	7	11	4	27
3	0.2.54.1	Mollicutes		4	5	7		6	5
3	0.2.56.1	Thermotogae				1			
3	0.2.57.2	OPB35_soil_group	1	2		1	2		
3	0.2.57.3	Opitutae	4	10	2	7	2	1	5
3	0.2.57.7	Verrucomicrobia_Incertae_Sedis			1				
3	0.2.57.8	Verrucomicrobiae	2	1	4	1			1
3	0.2.7.1	BD2-2	18	13	27	17	19	11	34
3	0.2.7.10	SB-5			2	2	1	2	16
3	0.2.7.12	Sphingobacteriia	59	38	33	14	18	17	28
3	0.2.7.13	VC2.1_Bac22	8	15	8	7	6	8	22
3	0.2.7.3	Bacteroidetes_Incertae_Sedis	4						
3	0.2.7.4	Bacteroidia	6	7	17	5	7	6	16
3	0.2.7.5	Cytophagia	60	57	30	27	33	36	80
3	0.2.7.6	Flavobacteriia	341	239	253	151	121	148	248
3	0.2.7.7	Ika33		1				1	2
3	0.2.7.9	SB-1	2	2	4	1	2	1	10
3	0.2.9.1	Caldisericia		1	1	4	1		6

Table 11 - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
		Total	7967	8806	9292	8581	9591	11833
2	0.2.1	Acidobacteria	137	177	270	205	365	479
2	0.2.10	Candidate_div._BRC1	4	4	8	5	11	11
2	0.2.12	Candidate_division_KB1		2	2	1	5	6
2	0.2.13	Candidate_div._OD1	4	12	4	13	10	39
2	0.2.14	Candidate_division_OP11		4			4	12
2	0.2.15	Candidate_div._OP3	4	1	5	6	14	6
2	0.2.16	Candidate_div._OP8	9	14	17	23	42	371
2	0.2.18	Candidate_div._SR1	3	2	1	1	1	
2	0.2.19	Candidate_div._TM7	7	11	11	9	1	
2	0.2.2	Actinobacteria	164	157	266	171	258	282
2	0.2.20	Candidate_div._WS3	104	153	155	130	183	392
2	0.2.22	Chlamydiae	3	1		3	2	13
2	0.2.23	Chlorobi	39	57	32	31	40	32
2	0.2.24	Chloroflexi	319	372	540	327	719	3407
2	0.2.25	Chrysiogenetes	7	9	2	3		
2	0.2.26	Cyanobacteria	923	1183	679	729	561	149
2	0.2.27	Deferribacteres	72	62	62	134	114	210
2	0.2.28	Deinococcus-Thermus					1	9
2	0.2.29	Dictyoglomi	2					
2	0.2.30	Elusimicrobia	1	4	7	4	11	45
2	0.2.31	Fibrobacteres	8	23	21	13	28	43
2	0.2.32	Firmicutes	135	161	151	157	202	288
2	0.2.33	Fusobacteria	149	315	88	207	42	46
2	0.2.35	GOUTA4						7
2	0.2.36	Gemmatimonadetes	42	49	93	86	93	102
2	0.2.37	Hyd24-12	14	18	6	6	10	1
2	0.2.38	JL-ETNP-Z39			1			3
2	0.2.39	LD1-PA38			2	3	1	2
2	0.2.4	Armatimonadetes						2
2	0.2.40	Lentisphaerae	31	44	55	57	74	51
2	0.2.41	NPL-UPA2				2	2	2
2	0.2.42	Nitrospirae	9	9	4	8	14	20
2	0.2.44	Planctomycetes	310	347	759	458	712	972
2	0.2.45	Proteobacteria	4129	4321	5257	4605	5265	4068

Table 11 (Continued) - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
2	0.2.47	SBYG-2791				2		
2	0.2.48	SHA-109	3	9	5	9	3	5
2	0.2.5	BD1-5	7	6	1	9	9	6
2	0.2.50	Spirochaetae	59	115	58	96	101	207
2	0.2.51	Synergistetes	1	3	1			
2	0.2.52	TA06	29	29	16	23	43	256
2	0.2.53	TM6	2	6	5	11	24	70
2	0.2.54	Tenericutes	12	4	7	2	7	8
2	0.2.55	Thermodesulfobacteria					1	
2	0.2.56	Thermotogae	3			1	1	1
2	0.2.57	Verrucomicrobia	15	38	34	34	11	15
2	0.2.7	Bacteroidetes	1719	1932	1023	1377	868	230
2	0.2.8	CKC4						1
2	0.2.9	Caldiserica	1	3	2	2	2	3
3	0.2.1.1	Acidobacteria	51	53	89	82	145	261
3	0.2.1.2	Holophagae	67	96	130	86	156	89
3	0.2.1.3	Subgroup_22	18	21	50	37	60	121
3	0.2.1.4	Subgroup_26	1	7	1		4	8
3	0.2.10.1	unclassified	4	4	8	5	11	11
3	0.2.12.1	Unknown_Class		2	2	1	3	2
3	0.2.12.2	unclassified					2	4
3	0.2.13.1	Unknown_Class						2
3	0.2.13.2	unclassified	4	12	4	13	10	37
3	0.2.14.1	unclassified		4			4	12
3	0.2.15.1	unclassified	4	1	5	6	14	6
3	0.2.16.1	unclassified	9	14	17	23	42	371
3	0.2.18.1	unclassified	3	2	1	1	1	
3	0.2.19.2	unclassified	7	11	11	9	1	
3	0.2.2.1	Acidimicrobiia	118	108	185	118	170	109
3	0.2.2.11	Thermoleophilia	3	5	23	4	13	20
3	0.2.2.2	Actinobacteria	41	39	54	40	61	115
3	0.2.2.3	Coriobacteriia		2	1	6	5	19
3	0.2.2.4	FFCH16263		1	3	1	1	5
3	0.2.2.5	KIST-JJY010					1	
3	0.2.2.7	Nitriliruptoria		1		1		

Table 11 (Continued) - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
3	0.2.2.8	OPB41	2			1	7	14
3	0.2.20.1	Unknown_Class		1	1	1	3	7
3	0.2.20.2	unclassified	104	152	154	129	180	385
3	0.2.22.1	Chlamydiae	3	1		3	2	13
3	0.2.23.1	Chlorobia	5	9		3	2	
3	0.2.23.2	Ignavibacteria	34	48	32	28	38	32
3	0.2.24.1	Anaerolineae	179	194	242	190	360	914
3	0.2.24.11	KD4-96	39	36	112	9	67	56
3	0.2.24.12	KZNMV-5-B42	2	3	1		1	16
3	0.2.24.13	Ktedonobacteria					8	6
3	0.2.24.14	MSB-5B2		6	2	7	16	342
3	0.2.24.16	S085	1				1	35
3	0.2.24.17	SAR202_clade				2		2
3	0.2.24.2	Ardenticatenia	43	62	106	61	81	60
3	0.2.24.20	TK10						7
3	0.2.24.21	Thermomicrobia			1		1	
3	0.2.24.3	Caldilineae	30	25	38	17	16	11
3	0.2.24.5	Chloroflexia					2	
3	0.2.24.6	Dehalococcoidia	25	46	38	41	166	1914
3	0.2.24.7	Elev-1554						1
3	0.2.24.9	JG30-KF-CM66						43
3	0.2.25.1	Chrysiogenetes	7	9	2	3		
3	0.2.26.2	Cyanobacteria	401	328	327	345	292	109
3	0.2.26.4	Melainabacteria	4	8	5	4	7	3
3	0.2.27.1	Deferribacteres	72	62	62	134	114	210
3	0.2.28.1	Deinococci					1	9
3	0.2.29.1	Dictyoglomia	2					
3	0.2.30.1	Elusimicrobia	1	4	7	4	11	45
3	0.2.31.1	Fibrobacteria	8	23	21	13	28	43
3	0.2.32.1	Bacilli	4	14	13	17	37	80
3	0.2.32.2	Clostridia	84	134	99	122	136	184
3	0.2.32.3	Erysipelotrichia	4	5	3	5	13	6
3	0.2.32.4	Negativicutes	43	8	36	13	16	18
3	0.2.33.1	Fusobacteriia	149	315	88	207	42	46
3	0.2.35.1	unclassified						7

Table 11 (Continued) - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
3	0.2.36.1	Gemmatimonadetes	42	49	93	86	93	102
3	0.2.37.1	unclassified	14	18	6	6	10	1
3	0.2.38.1	unclassified			1			3
3	0.2.39.1	unclassified			2	3	1	2
3	0.2.4.3	unclassified						2
3	0.2.40.1	113B434		1	3	2	2	
3	0.2.40.10	ML1228J-2						1
3	0.2.40.11	ML635J-35			3		14	1
3	0.2.40.12	MSBL3	1			2		1
3	0.2.40.13	Oligosphaeria	3	2	8	10	6	3
3	0.2.40.14	PBS-III-20		2				
3	0.2.40.15	R76-B128	11	24	20	33	26	11
3	0.2.40.17	SBYG-2613	5	2	2	3	2	
3	0.2.40.18	SS1-B-03-39		1	2			
3	0.2.40.20	WCHB1-41		3	1		3	9
3	0.2.40.21	c5LKS8			1		1	
3	0.2.40.4	DEV055		1				
3	0.2.40.5	LD1-PA20	2	1	7	1	4	6
3	0.2.40.6	LD1-PA26	6	4	4	3	9	15
3	0.2.40.8	LD1-PB3	3	3	4	2	7	3
3	0.2.40.9	Lentisphaeria				1		1
3	0.2.41.1	unclassified				2	2	2
3	0.2.42.1	Nitrospira	9	9	4	8	14	20
3	0.2.44.1	028H05-P-BN-P5	2			1	5	1
3	0.2.44.10	SGST604			5	4	4	10
3	0.2.44.11	SPG12-401-411-B72	9	6	8	6	9	9
3	0.2.44.13	vadinHA49	6	5	3	6	8	6
3	0.2.44.2	BD7-11	1		3	2		1
3	0.2.44.3	MBMPE71						5
3	0.2.44.4	MD2896-B258						2
3	0.2.44.5	OM190	25	25	36	26	25	31
3	0.2.44.6	Phycisphaerae	66	91	154	174	247	564
3	0.2.44.7	Pla3_lineage	7	5	4	6	6	17
3	0.2.44.8	Pla4_lineage	5	3	6	8	2	12
3	0.2.44.9	Planctomycetacia	189	212	540	225	406	314

Table 11 (Continued) - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
3	0.2.45.10	FGL7S		1				
3	0.2.45.11	Gammaproteobacteria	1453	1430	1591	1665	1433	581
3	0.2.45.12	JTB23	1			1	1	1
3	0.2.45.13	MACA-EFT26		1	1			
3	0.2.45.14	Milano-WF1B-44	40	18	16	45	23	8
3	0.2.45.15	Proteobacteria_Incertae_Sedis	76	54	103	89	75	13
3	0.2.45.16	SC3-20	2	4	8	9	1	1
3	0.2.45.17	SPOTSOCT00m83	2		6	3		1
3	0.2.45.19	TA18	2	1	4	10	1	
3	0.2.45.2	ARKDMS-49			3	1	3	4
3	0.2.45.21	pItb-vmat-80	8	6	3	5		
3	0.2.45.3	ARKICE-90	1		2	1	1	
3	0.2.45.4	Alphaproteobacteria	734	798	569	545	620	293
3	0.2.45.5	BJGMM-U27	1			1	1	
3	0.2.45.6	Betaproteobacteria	3	1	4	7	1	26
3	0.2.45.7	Deltaproteobacteria	1784	1999	2902	2165	3066	3120
3	0.2.45.8	Elev-16S-509		1	1			
3	0.2.45.9	Epsilonproteobacteria	22	7	44	58	39	20
3	0.2.46.1	unclassified	5	3				
3	0.2.47.1	unclassified				2		
3	0.2.48.1	unclassified	3	9	5	9	3	5
3	0.2.5.1	unclassified	7	6	1	9	9	6
3	0.2.50.1	Spirochaetes	59	115	58	96	101	207
3	0.2.51.1	Synergistia	1	3	1			
3	0.2.52.1	unclassified	29	29	16	23	43	256
3	0.2.53.1	unclassified	2	6	5	11	24	70
3	0.2.54.1	Mollicutes	12	4	7	2	7	8
3	0.2.55.1	Thermodesulfobacteria					1	
3	0.2.56.1	Thermotogae	3			1	1	1
3	0.2.57.2	OPB35_soil_group	1	5	4	3	1	1
3	0.2.57.3	Opitutae	13	19	15	29	7	12
3	0.2.57.4	S-BQ2-57_soil_group		2				
3	0.2.57.5	Spartobacteria		1				
3	0.2.57.6	UA11	1	4	4			
3	0.2.7.1	BD2-2	76	125	46	84	96	50

Table 11 (Continued) - Core 1-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	10-30 cm
3	0.2.7.10	SB-5	8	22	9	13	11	40
3	0.2.7.12	Sphingobacteriia	207	175	99	123	59	8
3	0.2.7.13	VC2.1_Bac22	33	33	28	23	19	9
3	0.2.7.3	Bacteroidetes_Incertae_Sedis	5	1	5	1	2	1
3	0.2.7.4	Bacteroidia	28	12	11	22	29	4
3	0.2.7.5	Cytophagia	138	185	101	141	109	35
3	0.2.7.6	Flavobacteriia	1191	1363	705	958	532	82
3	0.2.7.7	Ika33			1	2		
3	0.2.7.8	ML602M-17	3			1		
3	0.2.7.9	SB-1	30	16	18	9	11	1
3	0.2.8.1	unclassified						1
3	0.2.9.1	Caldisericia	1	3	2	2	2	3

Table 12 - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
		Total	9362	7615	9180	9517	10630	9028	13279
2	0.2.1	Acidobacteria	206	196	305	266	515	326	372
2	0.2.10	Candidate_division_BRC1	19	12	6	8	18	10	8
2	0.2.12	Candidate_division_KB1		4	9	7	7	3	2
2	0.2.13	Candidate_division_OD1	2	8	20	12	11	17	40
2	0.2.14	Candidate_division_OP11	2				4	1	14
2	0.2.15	Candidate_division_OP3	6	10	4	2	12	8	15
2	0.2.16	Candidate_division_OP8	29	30	33	23	70	81	460
2	0.2.18	Candidate_division_SR1	1		1	3			
2	0.2.19	Candidate_division_TM7	16	6	2	9	5	4	
2	0.2.2	Actinobacteria	210	133	250	292	245	190	288
2	0.2.20	Candidate_division_WS3	201	199	187	162	271	214	514
2	0.2.22	Chlamydiae	1	7		2	4	4	8
2	0.2.23	Chlorobi	45	40	59	41	63	56	35
2	0.2.24	Chloroflexi	430	400	587	608	964	847	4437
2	0.2.25	Chrysiogenetes	9	1	10	1	2	8	7
2	0.2.26	Cyanobacteria	859	533	603	673	385	819	189
2	0.2.27	Deferribacteres	82	85	106	86	123	142	216
2	0.2.28	Deinococcus-Thermus	1	1	3		4	4	7
2	0.2.3	Aquificae				1		1	
2	0.2.30	Elusimicrobia	6	1	9	5	6	7	48
2	0.2.31	Fibrobacteres	22	54	22	35	17	33	42
2	0.2.32	Firmicutes	169	233	237	258	258	207	231
2	0.2.33	Fusobacteria	337	405	184	202	11	114	43
2	0.2.35	GOUTA4						1	2

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
2	0.2.36	Gemmatimonadetes	79	70	66	76	116	101	73
2	0.2.37	Hyd24-12	27	70	25	10	7	26	5
2	0.2.38	JL-ETNP-Z39	1						7
2	0.2.39	LD1-PA38					1	2	1
2	0.2.4	Armatimonadetes	2		1	1			2
2	0.2.40	Lentisphaerae	77	78	82	50	48	71	39
2	0.2.41	NPL-UPA2	3	2	2	2	3	6	5
2	0.2.42	Nitrospirae	5	2	8	8	19	11	15
2	0.2.44	Planctomycetes	471	406	660	607	1023	660	1115
2	0.2.45	Proteobacteria	4981	3834	4731	4677	5281	4508	4266
2	0.2.46	RsaHF231				1		1	
2	0.2.47	SBYG-2791	1				3	1	
2	0.2.48	SHA-109	10	5	9	3	7	2	4
2	0.2.5	BD1-5	6	9	12	3	18	20	10
2	0.2.50	Spirochaetae	100	126	99	96	88	105	167
2	0.2.51	Synergistetes	5	1	3	3	2	4	6
2	0.2.52	TA06	43	24	41	32	70	67	278
2	0.2.53	TM6	10	9	15	14	33	23	39
2	0.2.54	Tenericutes	6	11	6	9	14	17	25
2	0.2.56	Thermotogae			4	1	1	1	1
2	0.2.57	Verrucomicrobia	45	32	30	13	19	18	15
2	0.2.7	Bacteroidetes	1376	833	1088	1607	1020	833	267
2	0.2.8	CKC4					2	1	1
2	0.2.9	Caldiserica	2	6	1	2	3	2	2
3	0.2.1.1	Acidobacteria	70	43	113	76	202	119	218

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.1.2	Holophagae	91	103	124	120	217	128	70
3	0.2.1.3	Subgroup_22	44	49	62	65	87	77	84
3	0.2.1.4	Subgroup_26	1	1	6	5	9	2	
3	0.2.10.1	unclassified	19	12	6	8	18	10	8
3	0.2.12.1	Unknown_Class		3	1	4	6	2	
3	0.2.12.2	unclassified		1	8	3	1	1	2
3	0.2.13.2	unclassified	2	8	20	12	11	17	40
3	0.2.14.1	unclassified	2				4	1	14
3	0.2.15.1	unclassified	6	10	4	2	12	8	15
3	0.2.16.1	unclassified	29	30	33	23	70	81	460
3	0.2.18.1	unclassified	1		1	3			
3	0.2.19.2	unclassified	16	6	2	9	5	4	
3	0.2.2.1	Acidimicrobiia	112	90	167	182	138	111	97
3	0.2.2.11	Thermoleophilia	10	4	9	8	15	7	16
3	0.2.2.2	Actinobacteria	82	33	67	95	83	49	114
3	0.2.2.3	Coriobacteriia	1	3	2	1	3	11	39
3	0.2.2.4	FFCH16263	4	2	4	5	4	8	15
3	0.2.2.5	KIST-JJY010	1						
3	0.2.2.8	OPB41		1	1	1	2	4	7
3	0.2.20.1	Unknown_Class	1	2	1		1	2	12
3	0.2.20.2	unclassified	200	197	186	162	270	212	502
3	0.2.22.1	Chlamydiae	1	7		2	4	4	8
3	0.2.23.1	Chlorobia	7	4	1	5	6	4	1
3	0.2.23.2	Ignavibacteria	38	36	58	36	57	52	34
3	0.2.24.1	Anaerolineae	255	267	321	259	457	456	871

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.24.11	KD4-96	35	30	76	148	60	31	47
3	0.2.24.12	KZNMV-5-B42	1	3	8	2	4	6	30
3	0.2.24.13	Ktedonobacteria	5	1	6	1	4	2	12
3	0.2.24.14	MSB-5B2	2	1	6	3	22	18	587
3	0.2.24.16	S085						1	36
3	0.2.24.17	SAR202_clade				2		2	8
3	0.2.24.18	SHA-26							1
3	0.2.24.2	Ardenticatenia	65	60	71	62	93	49	79
3	0.2.24.20	TK10							4
3	0.2.24.21	Thermomicrobia						3	
3	0.2.24.3	Caldilineae	31	10	39	20	21	14	9
3	0.2.24.5	Chloroflexia	2		1			1	
3	0.2.24.6	Dehalococcoidia	34	28	59	111	302	264	2714
3	0.2.24.9	JG30-KF-CM66					1		39
3	0.2.25.1	Chrysiogenetes	9	1	10	1	2	8	7
3	0.2.26.2	Cyanobacteria	325	269	262	276	238	261	141
3	0.2.26.3	ML635J-21	1					1	
3	0.2.26.4	Melainabacteria	2	5	1	6	4	8	6
3	0.2.27.1	Deferribacteres	82	85	106	86	123	142	216
3	0.2.28.1	Deinococci	1	1	3		4	4	7
3	0.2.3.1	Aquificae				1		1	
3	0.2.30.1	Elusimicrobia	6	1	9	5	6	7	48
3	0.2.31.1	Fibrobacteria	22	54	22	35	17	33	42
3	0.2.32.1	Bacilli	21	15	14	15	68	44	68
3	0.2.32.2	Clostridia	97	185	167	221	170	138	123

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.32.4	Negativicutes	47	30	53	16	16	24	26
3	0.2.32.5	OPB54							5
3	0.2.33.1	Fusobacteriia	337	405	184	202	11	114	43
3	0.2.35.1	unclassified						1	2
3	0.2.36.1	Gemmatimonadetes	79	70	66	76	116	101	73
3	0.2.37.1	unclassified	27	70	25	10	7	26	5
3	0.2.38.1	unclassified	1						7
3	0.2.39.1	unclassified					1	2	1
3	0.2.4.3	unclassified	2		1	1			2
3	0.2.40.1	113B434	3		5	1	3	1	
3	0.2.40.10	ML1228J-2	1		1	3	1		1
3	0.2.40.11	ML635J-35		2	1	1			
3	0.2.40.12	MSBL3	1		2		1	2	2
3	0.2.40.13	Oligosphaeria	7	6	8	5	4	21	8
3	0.2.40.14	PBS-III-20					1		
3	0.2.40.15	R76-B128	25	32	32	20	14	25	6
3	0.2.40.17	SBYG-2613	6	13	6			2	
3	0.2.40.2	B01R017	1						
3	0.2.40.20	WCHB1-41	16	9	6	2	2	3	3
3	0.2.40.21	c5LKS8	4	1		1	2		
3	0.2.40.4	DEV055				1			
3	0.2.40.5	LD1-PA20	4	6	8	2	3	2	
3	0.2.40.6	LD1-PA26	4	4	3	8	9	8	16
3	0.2.40.7	LD1-PA34		1	1	3	4	1	
3	0.2.40.8	LD1-PB3	5	4	8	3	4	5	3

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.40.9	Lentisphaeria			1			1	
3	0.2.41.1	unclassified	3	2	2	2	3	6	5
3	0.2.42.1	Nitrospira	5	2	8	8	19	11	15
3	0.2.44.1	028H05-P-BN-P5	3	4		5	7	2	1
3	0.2.44.10	SGST604	7	3	3	5	6	2	4
3	0.2.44.11	SPG12-401-411-B72	4	4	10	7	9	11	20
3	0.2.44.13	vadinHA49	10	3	3	7	11	10	10
3	0.2.44.2	BD7-11	1	3	2	2	3	4	4
3	0.2.44.3	MBMPE71			3	2	1		13
3	0.2.44.5	OM190	35	28	49	46	72	28	26
3	0.2.44.6	Phycisphaerae	153	146	189	169	497	303	757
3	0.2.44.7	Pla3_lineage	7	6	5	8	12	15	12
3	0.2.44.8	Pla4_lineage	18	9	10	5	10	7	20
3	0.2.44.9	Planctomycetacia	233	200	386	351	395	278	248
3	0.2.45.1	AEGEAN-245	1						
3	0.2.45.10	FGL7S			1				
3	0.2.45.11	Gammaproteobacteria	1828	1127	1480	1734	1643	1276	525
3	0.2.45.12	JTB23							2
3	0.2.45.14	Milano-WF1B-44	23	7	7	33	24	14	1
3	0.2.45.15	Proteobacteria_Incertae_Sedis	92	47	61	65	95	64	32
3	0.2.45.16	SC3-20	4	2	2	4		2	3
3	0.2.45.17	SPOTSOCT00m83	6		2	1	1	2	
3	0.2.45.18	Skagenf62							1
3	0.2.45.19	TA18	1	3	5	3			
3	0.2.45.2	ARKDMS-49			4	1	3	1	11

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.45.21	pItb-vmat-80	3	4	2	1	1	7	
3	0.2.45.3	ARKICE-90	4			3		1	
3	0.2.45.4	Alphaproteobacteria	501	313	573	430	513	376	259
3	0.2.45.5	BJGMM-U27	3	1		2	3	1	10
3	0.2.45.6	Betaproteobacteria	6	1	5	2	2		31
3	0.2.45.7	Deltaproteobacteria	2501	2280	2572	2391	2952	2744	3364
3	0.2.45.8	Elev-16S-509	3		2			1	
3	0.2.45.9	Epsilonproteobacteria	5	49	15	7	44	19	27
3	0.2.46.1	unclassified				1		1	
3	0.2.47.1	unclassified	1				3	1	
3	0.2.48.1	unclassified	10	5	9	3	7	2	4
3	0.2.5.1	unclassified	6	9	12	3	18	20	10
3	0.2.50.1	Spirochaetes	100	126	99	96	88	105	167
3	0.2.51.1	Synergistia	5	1	3	3	2	4	6
3	0.2.52.1	unclassified	43	24	41	32	70	67	278
3	0.2.53.1	unclassified	10	9	15	14	33	23	39
3	0.2.54.1	Mollicutes	6	11	6	9	14	17	25
3	0.2.56.1	Thermotogae			4	1	1	1	1
3	0.2.57.2	OPB35_soil_group	5	6	9		1	1	3
3	0.2.57.3	Opitutae	20	14	11	8	16	16	6
3	0.2.57.6	UA11	5	1	1				
3	0.2.57.7	Verrucomicrobia_Incertae_Sedis	1					1	5
3	0.2.57.8	Verrucomicrobiae	14	11	9	5	2		1
3	0.2.7.1	BD2-2	92	91	115	106	98	100	45
3	0.2.7.10	SB-5	2	2	11	7	8	16	13

Table 12 (Continued) - Core 3-10: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	10-30 cm
3	0.2.7.12	Sphingobacteriia	148	68	136	106	75	63	21
3	0.2.7.13	VC2.1_Bac22	43	24	28	52	32	20	2
3	0.2.7.16	vadinHA17							1
3	0.2.7.3	Bacteroidetes_Incertae_Sedis	6		2	6	6	3	2
3	0.2.7.4	Bacteroidia	13	7	8	17	6	22	7
3	0.2.7.5	Cytophagia	136	82	104	179	115	68	27
3	0.2.7.6	Flavobacteriia	925	542	677	1124	673	533	141
3	0.2.7.7	Ika33		1				1	
3	0.2.7.9	SB-1	11	16	7	10	7	7	8
3	0.2.8.1	unclassified					2	1	1
3	0.2.9.1	Caldisericia	2	6	1	2	3	2	2

Table 13 - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
		Total	5171	7654	6357	5911	4113	6406
2	0.2.1	Acidobacteria	14	147	80	14	30	6
2	0.2.10	Candidate_div._BRC1	2	3		1	1	1
2	0.2.12	Candidate_div._KB1		3			2	
2	0.2.13	Candidate_div._OD1	12	37	6	6	5	
2	0.2.14	Candidate_div._OP11	2	1	1	2		
2	0.2.15	Candidate_div._OP3	29	40	11	4	12	
2	0.2.16	Candidate_div._OP8	15	94	16	2	2	2
2	0.2.19	Candidate_div._TM7		5	1		4	4
2	0.2.2	Actinobacteria	42	212	119	55	36	68
2	0.2.20	Candidate_div._WS3	23	63	34	11	34	7
2	0.2.22	Chlamydiae	2	2		2	3	
2	0.2.23	Chlorobi		16	5	6	3	2
2	0.2.24	Chloroflexi	292	473	163	32	210	20
2	0.2.25	Chrysiogenetes		2			1	
2	0.2.26	Cyanobacteria	154	656	302	468	227	272
2	0.2.27	Deferribacteres	14	28	14	9	11	
2	0.2.28	Deinococcus-Thermus		1		2	1	
2	0.2.30	Elusimicrobia	6	4		1	1	1
2	0.2.31	Fibrobacteres	1	8	5	2	4	
2	0.2.32	Firmicutes	39	116	76	36	29	14
2	0.2.33	Fusobacteria	30	30	15	8	19	
2	0.2.36	Gemmatimonadetes	11	31	11	6	22	1
2	0.2.37	Hyd24-12	1		1	1	1	
2	0.2.38	JL-ETNP-Z39						

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
2	0.2.39	LD1-PA38	3	4	1	1	3	
2	0.2.40	Lentisphaerae	18	77	8	6	15	6
2	0.2.41	NPL-UPA2	4		1		1	
2	0.2.42	Nitrospirae	3		1			
2	0.2.44	Planctomycetes	65	336	155	43	70	42
2	0.2.45	Proteobacteria	3925	4684	4930	4627	2925	5601
2	0.2.48	SHA-109					3	
2	0.2.5	BD1-5	2	30	8	7	2	4
2	0.2.50	Spirochaetae	10	27	2	3	14	1
2	0.2.51	Synergistetes		1				
2	0.2.52	TA06	24	69	16	13	10	
2	0.2.53	TM6	32	51	15	8	15	
2	0.2.54	Tenericutes	7	27	15	18	7	5
2	0.2.56	Thermotogae			1			
2	0.2.57	Verrucomicrobia	14	47	9	8	16	7
2	0.2.7	Bacteroidetes	398	588	388	574	459	365
2	0.2.9	Caldiserica			1			
3	0.2.1.1	Acidobacteria	8	74	26	8	9	2
3	0.2.1.2	Holophagae	2	50	33	4	13	2
3	0.2.1.3	Subgroup_22	4	15	18	2	7	2
3	0.2.1.4	Subgroup_26		8	3		1	
3	0.2.10.1	unclassified	2	3		1	1	1
3	0.2.12.1	Unknown_Class					1	
3	0.2.12.2	unclassified		3			1	
3	0.2.13.1	Unknown_Class	2					

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
3	0.2.13.2	unclassified	10	37	6	6	5	
3	0.2.14.1	unclassified	2	1	1	2		
3	0.2.15.1	unclassified	29	40	11	4	12	
3	0.2.16.1	unclassified	15	94	16	2	2	2
3	0.2.19.2	unclassified		5	1		4	4
3	0.2.2.1	Acidimicrobiia	16	91	61	24	13	26
3	0.2.2.11	Thermoleophilia		10	7		2	5
3	0.2.2.2	Actinobacteria	24	111	51	31	20	37
3	0.2.2.4	FFCH16263	1					
3	0.2.2.5	KIST-JJY010	1					
3	0.2.2.7	Nitriliruptoria					1	
3	0.2.20.1	Unknown_Class	8			1	6	
3	0.2.20.2	unclassified	15	63	34	10	28	7
3	0.2.22.1	Chlamydiae	2	2		2	3	
3	0.2.23.1	Chlorobia		2		2		2
3	0.2.23.2	Ignavibacteria		14	5	4	3	
3	0.2.24.1	Anaerolineae	21	56	55	9	25	10
3	0.2.24.11	KD4-96	8	90	18	1	5	2
3	0.2.24.12	KZNMV-5-B42					2	
3	0.2.24.13	Ktedonobacteria	1				1	
3	0.2.24.14	MSB-5B2	11	36		2	2	
3	0.2.24.17	SAR202_clade	5	1	2		3	
3	0.2.24.2	Ardenticatenia	2	20	20	1	8	8
3	0.2.24.20	TK10	1					
3	0.2.24.3	Caldilineae	3	6	11		2	

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
3	0.2.24.6	Dehalococcoidia	240	237	57	19	161	
3	0.2.24.9	JG30-KF-CM66		27			1	
3	0.2.25.1	Chrysiogenetes		2			1	
3	0.2.26.2	Cyanobacteria	125	382	246	402	142	248
3	0.2.26.4	Melainabacteria	6	15	4	1		1
3	0.2.27.1	Deferribacteres	14	28	14	9	11	
3	0.2.28.1	Deinococci		1		2	1	
3	0.2.30.1	Elusimicrobia	6	4		1	1	1
3	0.2.31.1	Fibrobacteria	1	8	5	2	4	
3	0.2.32.1	Bacilli	4	22	2	6	7	1
3	0.2.32.2	Clostridia	30	92	67	28	20	11
3	0.2.32.3	Erysipelotrichia	2		2	1		2
3	0.2.32.4	Negativicutes	2	2	4	1	1	
3	0.2.32.5	OPB54	1		1		1	
3	0.2.33.1	Fusobacteriia	30	30	15	8	19	
3	0.2.36.1	Gemmatimonadetes	11	31	11	6	22	1
3	0.2.37.1	unclassified	1		1	1	1	
3	0.2.38.1	unclassified						
3	0.2.39.1	unclassified	3	4	1	1	3	
3	0.2.40.11	ML635J-35	6	37		5	4	1
3	0.2.40.12	MSBL3	1	3			1	
3	0.2.40.13	Oligosphaeria	2	12	3		3	3
3	0.2.40.15	R76-B128	3	14	1		5	
3	0.2.40.17	SBYG-2613		5	2			
3	0.2.40.2	B01R017	2					

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
3	0.2.40.20	WCHB1-41		2		1		
3	0.2.40.21	c5LKS8	1	2				
3	0.2.40.6	LD1-PA26	3	2	2		2	
3	0.2.40.9	Lentisphaeria						2
3	0.2.41.1	unclassified	4		1		1	
3	0.2.42.1	Nitrospira	3		1			
3	0.2.44.1	028H05-P-BN-P5		8	2	1		
3	0.2.44.10	SGST604	3				2	
3	0.2.44.11	SPG12-401-411-B72	2	2	2	3	4	
3	0.2.44.13	vadinHA49	1	9			2	
3	0.2.44.2	BD7-11	1					
3	0.2.44.3	MBMPE71		1	1			
3	0.2.44.5	OM190	3	11	13	5	1	21
3	0.2.44.6	Phycisphaerae	30	144	62	23	43	5
3	0.2.44.7	Pla3_lineage		4				
3	0.2.44.8	Pla4_lineage			3			
3	0.2.44.9	Planctomycetacia	25	157	72	11	18	16
3	0.2.45.11	Gammaproteobacteria	2169	1551	852	1074	887	3738
3	0.2.45.12	JTB23			1	1	1	
3	0.2.45.14	Milano-WF1B-44	2	14	4	4	2	
3	0.2.45.15	Proteobacteria_Incertae Sedis	138	167	251	576	258	415
3	0.2.45.16	SC3-20	2	1		1	2	
3	0.2.45.19	TA18					1	
3	0.2.45.2	ARKDMS-49	1			1		

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
3	0.2.45.21	pItb-vmat-80	5	6	1	2	1	
3	0.2.45.4	Alphaproteobacteria	1265	2111	3133	2618	1292	1358
3	0.2.45.5	BJGMM-U27	1		1		1	
3	0.2.45.6	Betaproteobacteria	5	18	25	39	14	26
3	0.2.45.7	Deltaproteobacteria	289	729	647	225	448	48
3	0.2.45.9	Epsilonproteobacteria	48	87	15	86	18	16
3	0.2.48.1	unclassified					3	
3	0.2.5.1	unclassified	2	30	8	7	2	4
3	0.2.50.1	Spirochaetes	10	27	2	3	14	1
3	0.2.51.1	Synergistia		1				
3	0.2.52.1	unclassified	24	69	16	13	10	
3	0.2.53.1	unclassified	32	51	15	8	15	
3	0.2.54.1	Mollicutes	7	27	15	18	7	5
3	0.2.56.1	Thermotogae			1			
3	0.2.57.2	OPB35_soil_group		3			1	
3	0.2.57.3	Opitutae	12	30	7	8	8	3
3	0.2.57.6	UA11	1		1			1
3	0.2.57.7	Verrucomicrobia _Incertae_Sedis		10	1		1	
3	0.2.57.8	Verrucomicrobiae	1	4			6	3
3	0.2.7.1	BD2-2	5	1	4	6	4	
3	0.2.7.10	SB-5	2	3		1	2	
3	0.2.7.12	Sphingobacteriia	12	19	16	20	14	7
3	0.2.7.13	VC2.1_Bac22	1	14	4	5	5	
3	0.2.7.16	vadinHA17					1	

Table 13 (Continued) - Porewater communities: 16S rRNA gene sequence reads at phylum (2) and class (3) level

taxa level	Rank ID	taxon	LUC_14 004	LUC_14 005	LUC_14 006	LUC_14 007	LUC_14 008	LUC_14 010
3	0.2.7.3	Bacteroidetes_ Incertae_Sedis		1				
3	0.2.7.4	Bacteroidia	2	11		52	3	3
3	0.2.7.5	Cytophagia	19	128	17	31	46	15
3	0.2.7.6	Flavobacteriia	357	398	347	459	384	340
3	0.2.7.9	SB-1		13				
3	0.2.9.1	Caldisericia		1				

Table 14 - Lucinid tissue from quadrat 1-2 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	rank ID	taxon	1-2 Sc	1-2 Se	1-2 Dd	1-2 De	1-2 Ddf
		Total	14190	13318	12037	8758	7823
2	0.2.2	Actinobacteria					8
2	0.2.7	Bacteroidetes					4
2	0.2.24	Chloroflexi					6
2	0.2.31	Fibrobacteres					51
2	0.2.32	Firmicutes					6
2	0.2.40	Lentisphaerae	3	2	3		5461
2	0.2.42	Nitrospirae					3
2	0.2.26	Cyanobacteria	2			3	
2	0.2.45	Proteobacteria	14156	13303	12029	8748	2247
2	0.2.50	Spirochaetae	29	13	5	7	30
2	0.2.54	Tenericutes					7
3	0.2.2.2	Actinobacteria					8
3	0.2.7.12	Sphingobacteriia					4
3	0.2.24.1	Anaerolineae					2
3	0.2.24.6	Dehalococcoidia					4
3	0.2.31.1	Fibrobacteria					51
3	0.2.32.2	Clostridia					6
3	0.2.40.1	113B434					4
3	0.2.40.6	LD1-PA26	3	2	3		5457
3	0.2.42.1	Nitrospira					3
3	0.2.26.2	Cyanobacteria				3	
3	0.2.45.4	Alphaproteobacteria	30	23	5	1	635
3	0.2.45.7	Deltaproteobacteria					2
3	0.2.45.9	Epsilonproteobacteria					54
3	0.2.45.11	Gammaproteobacteria	14126	13280	12024	8747	1555
3	0.2.45.14	Milano-WF1B-44					1
3	0.2.50.1	Spirochaetes	29	13	5	7	30
3	0.2.54.1	Mollicutes					7

Table 15 - Lucinid tissue from quadrat 1-4 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-4 Sb	1-4 Sc	1-4 Da	1-4 Dd	1-4 Daf
		Total	13139	14821	16757	20470	8282
2	0.2.1	Acidobacteria					10
2	0.2.7	Bacteroidetes					4
2	0.2.26	Cyanobacteria					13
2	0.2.31	Fibrobacteres					7
2	0.2.40	Lentisphaerae				1	953
2	0.2.44	Planctomycetes					3
2	0.2.45	Proteobacteria	13091	14792	16756	20359	7282
2	0.2.50	Spirochaetae	48	29	1	110	1
2	0.2.54	Tenericutes					3
2	0.2.57	Verrucomicrobia					3
3	0.2.1.1	Acidobacteria					6
3	0.2.1.2	Holophagae					4
3	0.2.7.6	Flavobacteriia					1
3	0.2.7.12	Sphingobacteriia					3
3	0.2.26.2	Cyanobacteria					13
3	0.2.31.1	Fibrobacteria					7
3	0.2.40.1	113B434					135
3	0.2.40.6	LD1-PA26				1	818
3	0.2.44.6	Phycisphaerae					1
3	0.2.44.9	Planctomycetacia					2
3	0.2.45.4	Alphaproteobacteria	5	790	12145		6126
3	0.2.45.6	Betaproteobacteria					4
3	0.2.45.9	Epsilonproteobacteria					2
3	0.2.45.7	Deltaproteobacteria	6				
3	0.2.45.11	Gammaproteobacteria	13080	14002	4611	20359	1150
3	0.2.50.1	Spirochaetes	48	29	1	110	1
3	0.2.54.1	Mollicutes					3
3	0.2.57.3	Opitutae					3

Table 16 - Lucinid tissue from quadrat 1-6 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-6 S	1-6 Da	1-6 Db	1-6 Daf
		Total	23715	36680	24108	9036
2	0.2.2	Actinobacteria				
2	0.2.7	Bacteroidetes				
2	0.2.31	Fibrobacteres				
2	0.2.40	Lentisphaerae	1	1	7	1064
2	0.2.45	Proteobacteria	23712	36599	24093	7927
2	0.2.50	Spirochaetae		80	8	21
2	0.2.54	Tenericutes				
3	0.2.2.2	Actinobacteria				
3	0.2.7.5	Cytophagia				
3	0.2.7.6	Flavobacteriia				
3	0.2.31.1	Fibrobacteria				
3	0.2.26.2	Cyanobacteria		1		
3	0.2.40.1	113B434			7	1
3	0.2.40.6	LD1-PA26	1	1		1063
3	0.2.45.4	Alphaproteobacteria	1036	6439		466
3	0.2.45.7	Deltaproteobacteria				
3	0.2.45.11	Gammaproteobacteria	22676	30160	24103	7459
3	0.2.50.1	Spirochaetes		80	8	21
3	0.2.54.1	Mollicutes				

Table 17 - Lucinid tissue from quadrat 1-8 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-8 Sa	1-8 Sb	1-8 Db	1-8 Dc	1-8 Dbf
		Total	15539	8251	17682	18223	12208
2	0.2.2	Actinobacteria					5
2	0.2.7	Bacteroidetes					45
2	0.2.26	Cyanobacteria	1		4		176
2	0.2.27	Deferribacteres					2
2	0.2.31	Fibrobacteres					44
2	0.2.20	Candidate_division_WS3					
2	0.2.33	Fusobacteria	2		4		199
2	0.2.36	Gemmatimonadetes					1
2	0.2.1	Acidobacteria				1	
2	0.2.40	Lentisphaerae	4	10	29	40	4509
2	0.2.44	Planctomycetes					6
2	0.2.45	Proteobacteria	15521	8235	17511	17852	7174
2	0.2.50	Spirochaetae	11	6	134	330	36
2	0.2.53	TM6					2
2	0.2.54	Tenericutes					9
3	0.2.2.1	Acidimicrobiia					1
3	0.2.2.2	Actinobacteria					4
3	0.2.7.4	Bacteroidia					2
3	0.2.7.5	Cytophagia					2
3	0.2.7.6	Flavobacteriia					35
3	0.2.7.12	Sphingobacteriia					6
3	0.2.26.2	Cyanobacteria	1		4		176
3	0.2.27.1	Deferribacteres					2
3	0.2.31.1	Fibrobacteria					44
3	0.2.1.2	Holophagae					
3	0.2.20.2	unclassified					
3	0.2.33.1	Fusobacteriia	2		4		199
3	0.2.36.1	Gemmatimonadetes					1
3	0.2.1.1	Acidobacteria				1	
3	0.2.40.1	113B434		9	1		1
3	0.2.40.6	LD1-PA26	4	1	28	40	4506
3	0.2.40.12	MSBL3					2
3	0.2.44.5	OM190					2
3	0.2.44.9	Planctomycetacia					4
3	0.2.40.13	Oligosphaeria					

Table 17 (Continued) - Lucinid tissue from quadrat 1-8 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-8 Sa	1-8 Sb	1-8 Db	1-8 Dc	1-8 Dbf
3	0.2.45.4	Alphaproteobacteria	2		11	1189	6255
3	0.2.45.6	Betaproteobacteria				2	
3	0.2.45.7	Deltaproteobacteria				1	2
3	0.2.45.9	Epsilonproteobacteria					37
3	0.2.45.11	Gammaproteobacteria	15519	8235	17500	16660	880
3	0.2.50.1	Spirochaetes	11	6	134	330	36
3	0.2.53.1	unclassified					2
3	0.2.54.1	Mollicutes					9

Table 18 - Lucinid tissue from quadrat 1-10 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-10 Sb	1-10 Sd	1-10 Dc	1-10 De	1-10 Dcf
		Total	25576	11746	2562	20238	3564
2	0.2.1	Acidobacteria					5
2	0.2.2	Actinobacteria					4
2	0.2.5	BD1-5		5			
2	0.2.7	Bacteroidetes					11
2	0.2.13	Candidate_division_OD1					3
2	0.2.16	Candidate_division_OP8					4
2	0.2.20	Candidate_division_WS3					6
2	0.2.24	Chloroflexi					13
2	0.2.26	Cyanobacteria	1	1			17
2	0.2.27	Deferribacteres					5
2	0.2.31	Fibrobacteres					3
2	0.2.32	Firmicutes					4
2	0.2.33	Fusobacteria					13
2	0.2.36	Gemmatimonadetes					2
2	0.2.40	Lentisphaerae	1	3			902
2	0.2.44	Planctomycetes					19
2	0.2.45	Proteobacteria	25527	11691	2559	20107	2438
2	0.2.48	SHA-109					11
2	0.2.50	Spirochaetae	47	51	3	131	97
2	0.2.52	TA06					2
3	0.2.1.1	Acidobacteria					3
3	0.2.1.2	Holophagae					2
3	0.2.2.1	Acidimicrobiia					2
3	0.2.2.2	Actinobacteria					2
3	0.2.5.1	unclassified					5
3	0.2.7.6	Flavobacteriia					5
3	0.2.7.9	SB-1					1
3	0.2.7.12	Sphingobacteriia					5
3	0.2.13.2	unclassified					3
3	0.2.16.1	unclassified					4
3	0.2.20.2	unclassified					6
3	0.2.24.1	Anaerolineae					4
3	0.2.24.3	Caldilineae					5
3	0.2.24.6	Dehalococcoidia					4

Table 18 (Continued) - Lucinid tissue from quadrat 1-10 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at phylum (2) and class (3) levels

taxa level	Rank ID	taxon	1-10 Sb	1-10 Sd	1-10 Dc	1-10 De	1-10 Dcf
3	0.2.26.2	Cyanobacteria	1	1			17
3	0.2.27.1	Deferribacteres					5
3	0.2.31.1	Fibrobacteria					3
3	0.2.32.2	Clostridia					4
3	0.2.33.1	Fusobacteriia					13
3	0.2.36.1	Gemmatimonadetes					2
3	0.2.40.3	BS5					2
3	0.2.40.6	LD1-PA26	1	3			899
3	0.2.40.15	R76-B128					1
3	0.2.44.1	028H05-P-BN-P5					1
3	0.2.44.5	OM190					7
3	0.2.44.6	Phycisphaerae					5
3	0.2.44.9	Planctomycetacia					5
3	0.2.44.10	SGST604					1
3	0.2.45.4	Alphaproteobacteria	2		1		345
3	0.2.45.7	Deltaproteobacteria					27
3	0.2.45.9	Epsilonproteobacteria					12
3	0.2.45.11	Gammaproteobacteria	25525	11691	2558	20107	2050
3	0.2.45.14	Milano-WF1B-44					1
3	0.2.45.15	Proteobacteria_Incertae_Sedis					3
3	0.2.48.1	unclassified					11
3	0.2.50.1	Spirochaetes	47	51	3	131	97
3	0.2.52.1	unclassified					2

Table 19 - Lucinid tissue from quadrat 3-10 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at the phylum (2) and class (3) levels

taxa level	Rank ID	taxon	3-10 Da	3-10 Db	3-10 Daf
		Total	16369	24546	7840
2	0.2.1	Acidobacteria	1		
2	0.2.20	Candidate_division_WS3	2		
2	0.2.2	Actinobacteria			12
2	0.2.7	Bacteroidetes			8
2	0.2.16	Candidate_division_OP8			1
2	0.2.22	Chlamydiae			1
2	0.2.26	Cyanobacteria			3
2	0.2.31	Fibrobacteres			27
2	0.2.32	Firmicutes			25
2	0.2.33	Fusobacteria	1		8
2	0.2.40	Lentisphaerae	1	4	2581
2	0.2.42	Nitrospirae			4
2	0.2.44	Planctomycetes			2
2	0.2.45	Proteobacteria	16324	24526	5061
2	0.2.50	Spirochaetae	40	16	103
2	0.2.54	Tenericutes			4
3	0.2.1.2	Holophagae	1		
3	0.2.20.2	unclassified	2		
3	0.2.2.2	Actinobacteria			10
3	0.2.2.4	FFCH16263			2
3	0.2.7.5	Cytophagia			1
3	0.2.7.6	Flavobacteriia			2
3	0.2.7.12	Sphingobacteriia			5
3	0.2.16.1	unclassified			1
3	0.2.22.1	Chlamydiae			1
3	0.2.26.2	Cyanobacteria			2
3	0.2.31.1	Fibrobacteria			27
3	0.2.32.1	Bacilli			25
3	0.2.33.1	Fusobacteriia	1		8
3	0.2.40.13	Oligosphaeria	1		
3	0.2.40.1	113B434			15
3	0.2.40.6	LD1-PA26		4	2566
3	0.2.42.1	Nitrospira			4
3	0.2.44.9	Planctomycetacia			2
3	0.2.45.4	Alphaproteobacteria	1	4	893

Table 19 (Continued) - Lucinid tissue from quadrat 3-10 shallow (0-10 cm) and deep (10-30 cm) regions: 16S rRNA gene sequence reads at the phylum (2) and class (3) levels

taxa level	Rank ID	taxon	3-10 Da	3-10 Db	3-10 Daf
3	0.2.45.6	Betaproteobacteria			4
3	0.2.45.7	Deltaproteobacteria	2	6	16
3	0.2.45.9	Epsilonproteobacteria			143
3	0.2.45.11	Gammaproteobacteria	16321	24516	4005
3	0.2.50.1	Spirochaetes	40	16	103
3	0.2.54.1	Mollicutes			4

Table 20- Top operational taxonomic units (OTU) from sediments in Core 1-2

Class	0 cm	1 cm	2 cm	3 cm	4 cm	5 cm	6 cm	10-20 cm	20-30 cm
Deltaproteobacteria	441	400	254	407	372	381	384	455	152
Gammaproteobacteri	338	289	120	203	164	160	153	181	49
Dehalococcoidia	10	33	20	38	48	113	97	473	171
Phycisphaerae	108	81	38	83	70	118	85	192	82
Planctomycetacia	80	121	102	110	157	120	89	117	48
Anaerolineae	118	81	82	71	100	112	114	147	70
Alphaproteobacteria	150	109	67	64	72	84	89	57	22
unclassified	110	75	57	54	83	106	81	96	59
Spirochaetes	68	40	27	38	36	49	45	84	64
Actinobacteria	54	30	34	33	46	40	33	59	32

Table 21 - Top operational taxonomic units (OTU) from sediments in Core 3-2

	0	1	2	3	4	5
	cm	cm	cm	cm	cm	cm
Deltaproteobacteria	325	352	503	287	300	372
Gammaproteobacteria	399	287	298	269	197	194
Planctomycetacia	193	172	134	103	161	113
Alphaproteobacteria	218	138	150	108	119	66
Flavobacteriia	150	61	105	58	57	29
unclassified	68	57	69	60	56	89
Phycisphaerae	51	40	72	55	66	102
Anaerolineae	75	63	92	52	64	59
Sphingobacteriia	120	41	71	39	19	9
Holophagae	46	47	40	35	48	45

Table 22 - Top operational taxonomic units (OTU) from sediments in Core 1-4

	0	1	2	3	10-30
	cm	cm	cm	cm	cm
Deltaproteobacteria	402	357	462	422	501
Gammaproteobacteria	323	298	313	253	142
Alphaproteobacteria	299	180	165	119	59
Planctomycetacia	136	154	156	148	102
Dehalococcoidia	34	6	14	13	417
Phycisphaerae	42	52	52	63	249
Anaerolineae	92	65	91	94	168
unclassified	68	68	78	76	125
Flavobacteriia	159	92	66	58	13
Sphingobacteriia	111	45	45	42	3

Table 23 - Top operational taxonomic units (OTU) from sediments in Core 1-6

	0-2 cm	2-4 cm
Deltaproteobacteria	397	509
Gammaproteobacteria	362	265
Alphaproteobacteria	200	131
Planctomycetacia	140	132
Flavobacteriia	131	70
unclassified	71	109
Sphingobacteriia	107	62
Anaerolineae	76	116
Phycisphaerae	62	79
Cytophagia	71	56

Table 24 - Top operational taxonomic units (OTU) from sediments in Core 1-8

	0	1	2	3	4	5	10-30
	cm	cm	cm	cm	cm	cm	cm
Deltaproteobacteria	224	172	226	276	252	116	326
Gammaproteobacteria	201	180	191	164	101	67	161
Planctomycetacia	70	68	71	78	60	50	71
Alphaproteobacteria	85	58	44	50	32	49	49
Anaerolineae	39	31	50	66	36	31	76
Phycisphaerae	26	24	40	35	37	22	80
unclassified	18	28	44	37	28	21	63
Flavobacteriia	65	56	33	26	13	20	18
Spirochaetes	15	12	25	18	15	14	43
Dehalococcoidia	6	6	6	16	24	16	69

Table 25 - Top operational taxonomic units (OTU) from sediments in Core 1-10

	0	1	2	3	4	10-30
	cm	cm	cm	cm	cm	cm
Deltaproteobacteria	347	419	418	426	476	573
Gammaproteobacteria	335	269	255	282	215	124
Alphaproteobacteria	230	231	130	129	116	50
Dehalococcoidia	15	24	13	25	75	572
Planctomycetacia	105	93	179	87	133	126
Phycisphaerae	47	46	62	76	102	242
Anaerolineae	82	94	81	76	110	193
unclassified	74	73	65	46	90	177
Flavobacteriia	157	126	48	80	35	10
Spirochaetes	38	58	30	50	45	108

Table 26 - Top operational taxonomic units (OTU) from sediments in Core 3-10

	0	1	2	3	4	5	10-30
	cm	cm	cm	cm	cm	cm	cm
Deltaproteobacteria	485	423	425	341	431	476	532
Gammaproteobacteria	353	197	251	256	252	222	110
Dehalococcoidia	25	14	38	40	111	88	713
Phycisphaerae	80	59	81	62	140	124	252
Anaerolineae	122	109	93	84	112	117	196
Planctomycetacia	116	66	131	93	120	116	111
unclassified	96	76	83	68	108	84	168
Alphaproteobacteria	167	89	134	81	78	99	54
Spirochaetes	61	64	48	46	51	55	85
Actinobacteria	48	29	43	52	62	47	72

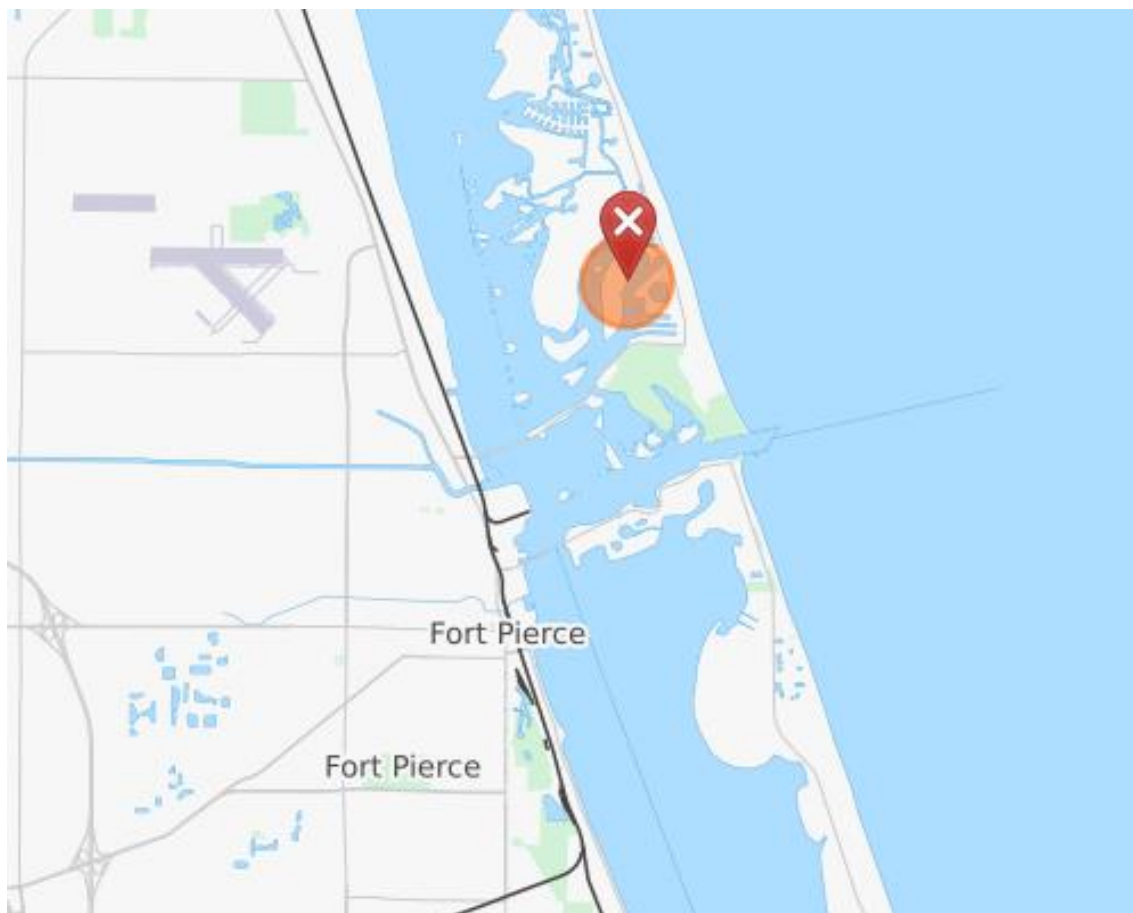
Table 27 - Living non-lucinid bivalves collected from Wildcat Cove, Florida

Quadrat (Depth)	Bivalve Taxa	Quantity
1-3S (Grid 1)	Chione	3
3-2S (Grid 1)	Chione	2
3-3S (Grid 1)	Chione	3
3-3S (Grid 1)	Mulinia	1
5-2S (Grid 1)	Chione	1
7-2S (Grid 1)	Cariacorbula	1
1-10D	Tagelus	1
1-10S	Abra	1
1-10S	Anadara	1
1-10S	Cariacorbula	1
1-10S	Chione	3
1-10S	Chione	1
1-2S	Abra	1
1-2S	Chione	2
1-6D	Chione	1
1-6S	Abra	6
1-6S	Anomalocardia	1
1-6S	Cariacorbula	1
1-6S	Chione	1
1-6S	Chione	1
1-8D	Chione	1
1-8S	Abra	1
1-8S	Abra	6
1-8S	Cariacorbula	1
1-8S	Chione	2
1-8S	Chione	1
3-10D	Chione	2
3-10S	Abra	9
3-10S	Cariacorbula	1
3-10S	Chione	1
3-2D	Live specimen	1
3-6D	Abra	4
3-6D	Chione	1
3-8S	Abra	2
5-10S	Abra	3

**Table 27 (Continued) - Living non-lucinid bivalves
collected from Wildcat Cove, Florida**

Quadrat (Depth)	Bivalve Taxa	Quantity
5-10S	Chione	1
5-4S	Chione	1
7-10S	Abra	8
9-10S	Cariacorbula	1
9-2S	Abra	1
9-4S	Chione	1
9-4S	Lavicardium	1
9-4S	Tagelus	1
Transect 4 (15 m)	Abra	1
Transect 4 (20 m)	Geukensia	1
Transect 4 (45 m)	Unidentified	2
Transect 4 (5 m)	Abra	1
Transect 5 (45 m)	Abra	1
Transect 5 (50 m)	Abra	1
Transect 5 (35 m)	Unidentified	1

Figures



**Figure 1 - Fort Pierce, St. Lucie County, Florida, showing location of Wildcat Cove.
From Openstreetmap.org**

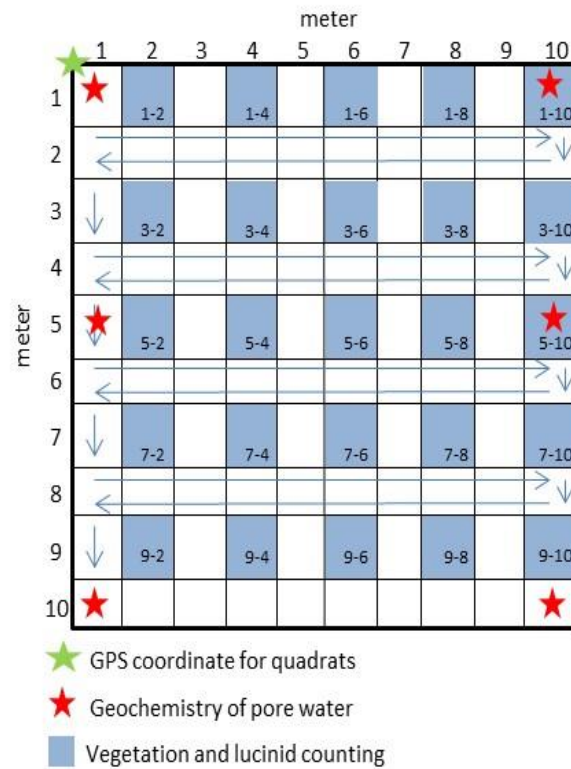


Figure 2 - Sampling grid used at Wildcat Cove, St. Lucie County, Florida. Blue colored quadrats were sampled (except for 5-6, 5-8, 7-6, 7-8, 9-6, and 9-8).

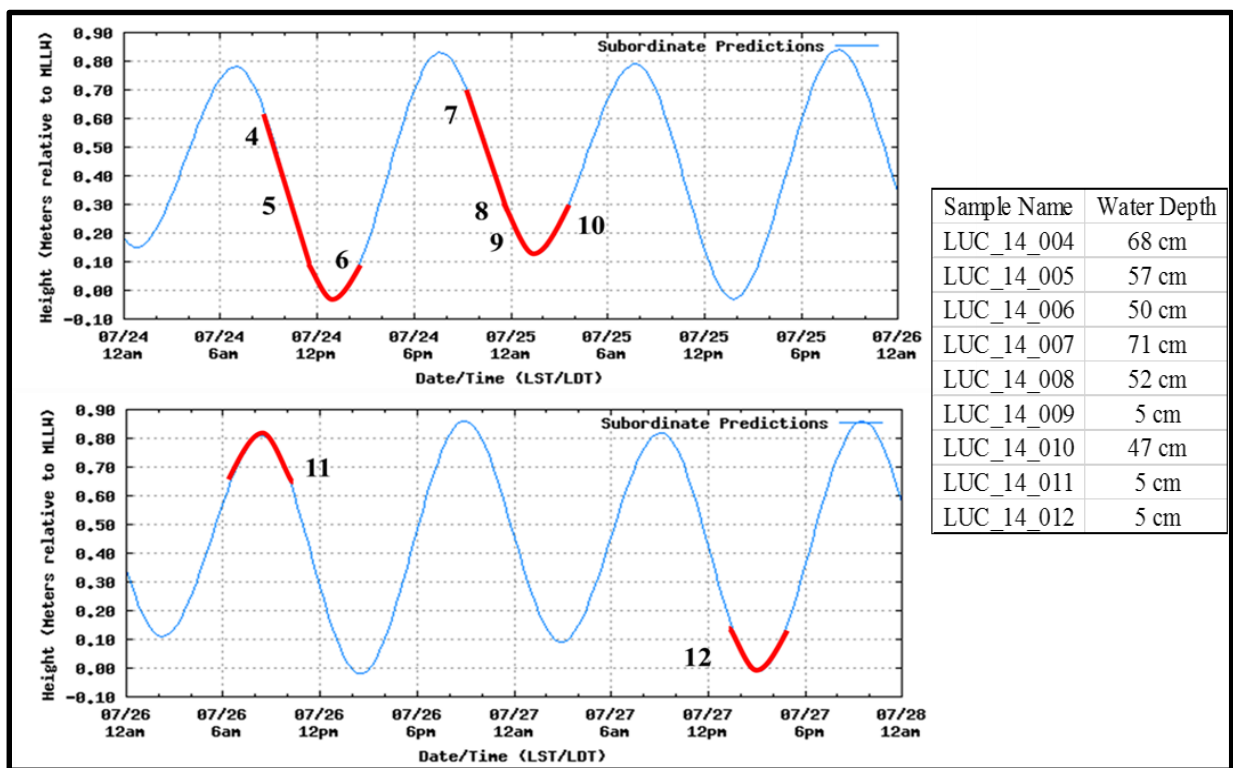


Figure 3 - Tidal cycles during sampling at Wildcat Cove, St. Lucie County, Florida. Red areas highlight sampling times during tidal cycle and corresponding numbers refer to water depth during sampling.

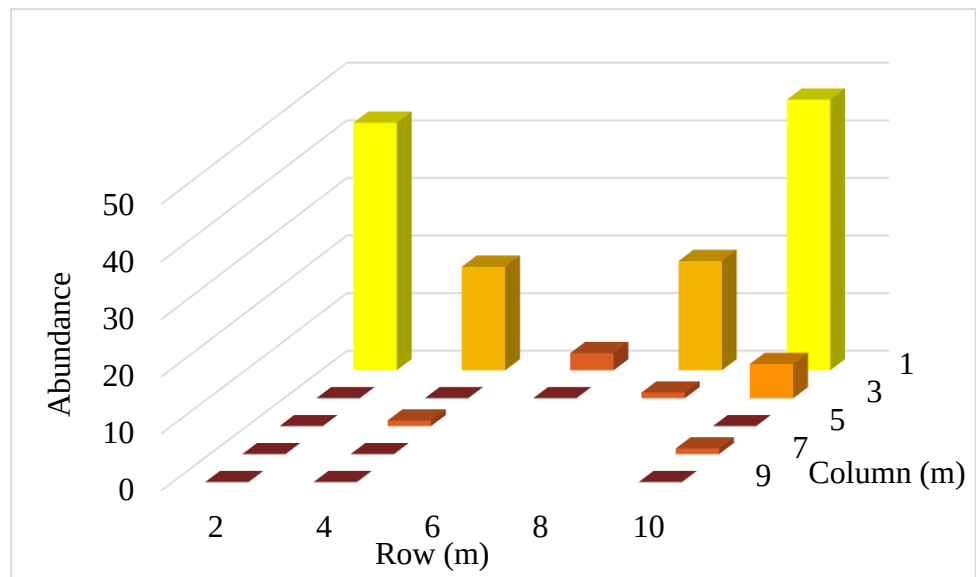


Figure 4 - Numbers of live *Phacoides pectinatus* abundances in the sampling grid at Wildcat Cove, St. Lucie County, Florida.

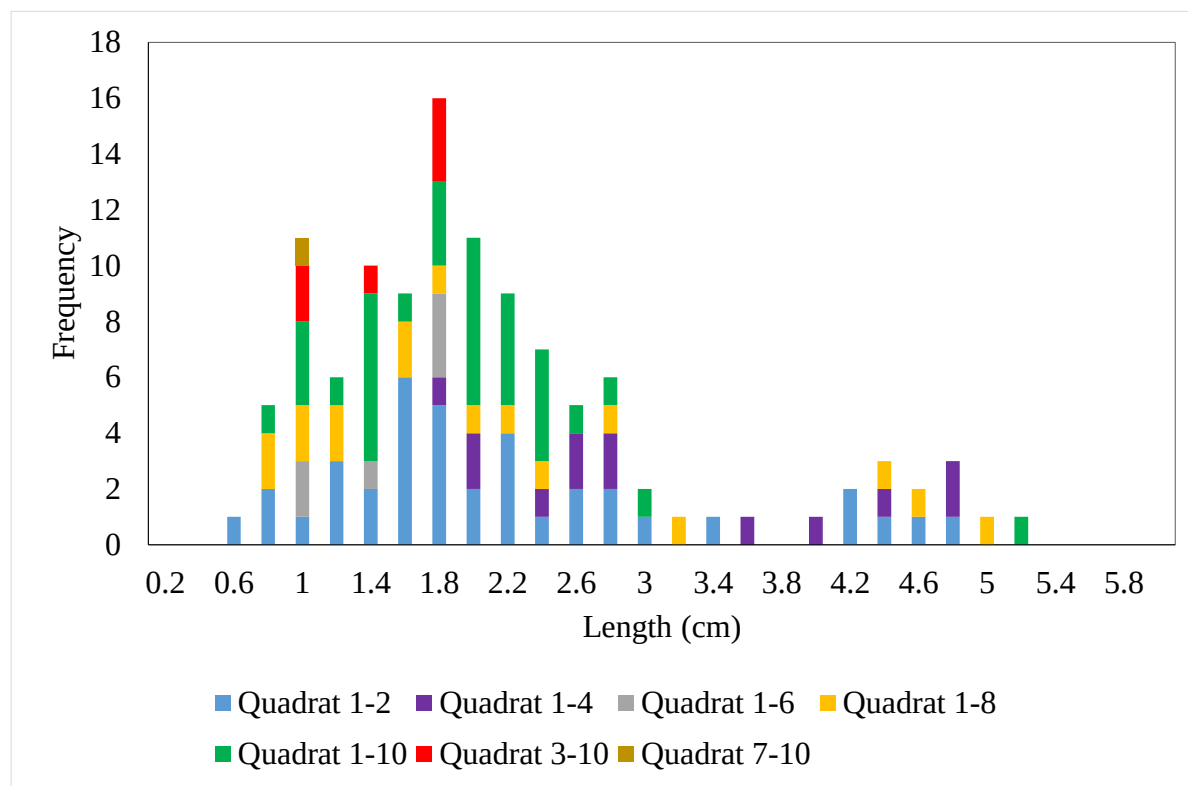


Figure 5 - Live *Phacoides pectinatus* shell lengths recovered per quadrat within the sampling grid at Wildcat Cove, St. Lucie County, Florida.

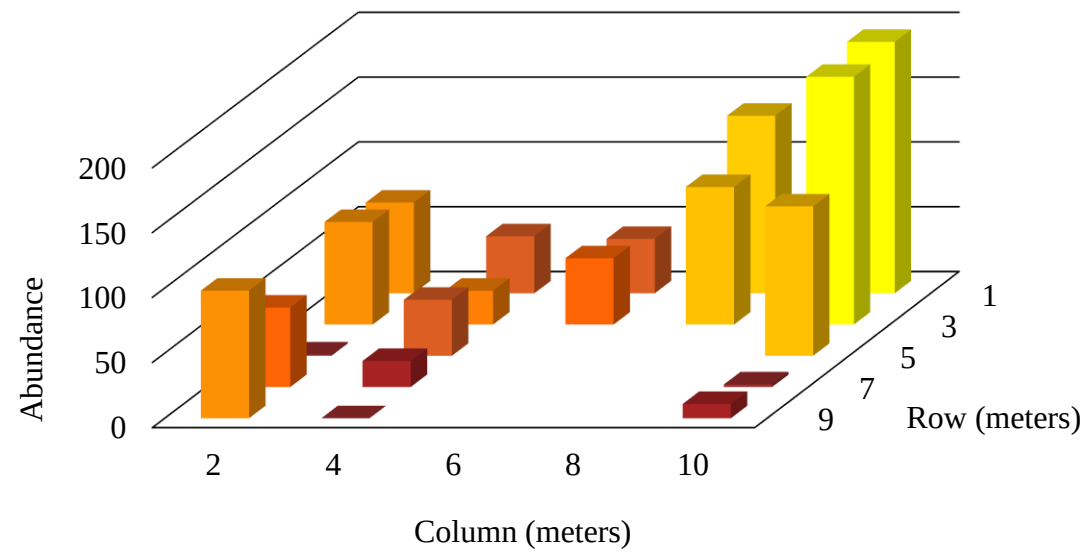


Figure 6 - Numbers of dead and articulated *Phacoides pectinatus* shells in the sampling grid at Wildcat Cove, St. Lucie County, Florida.

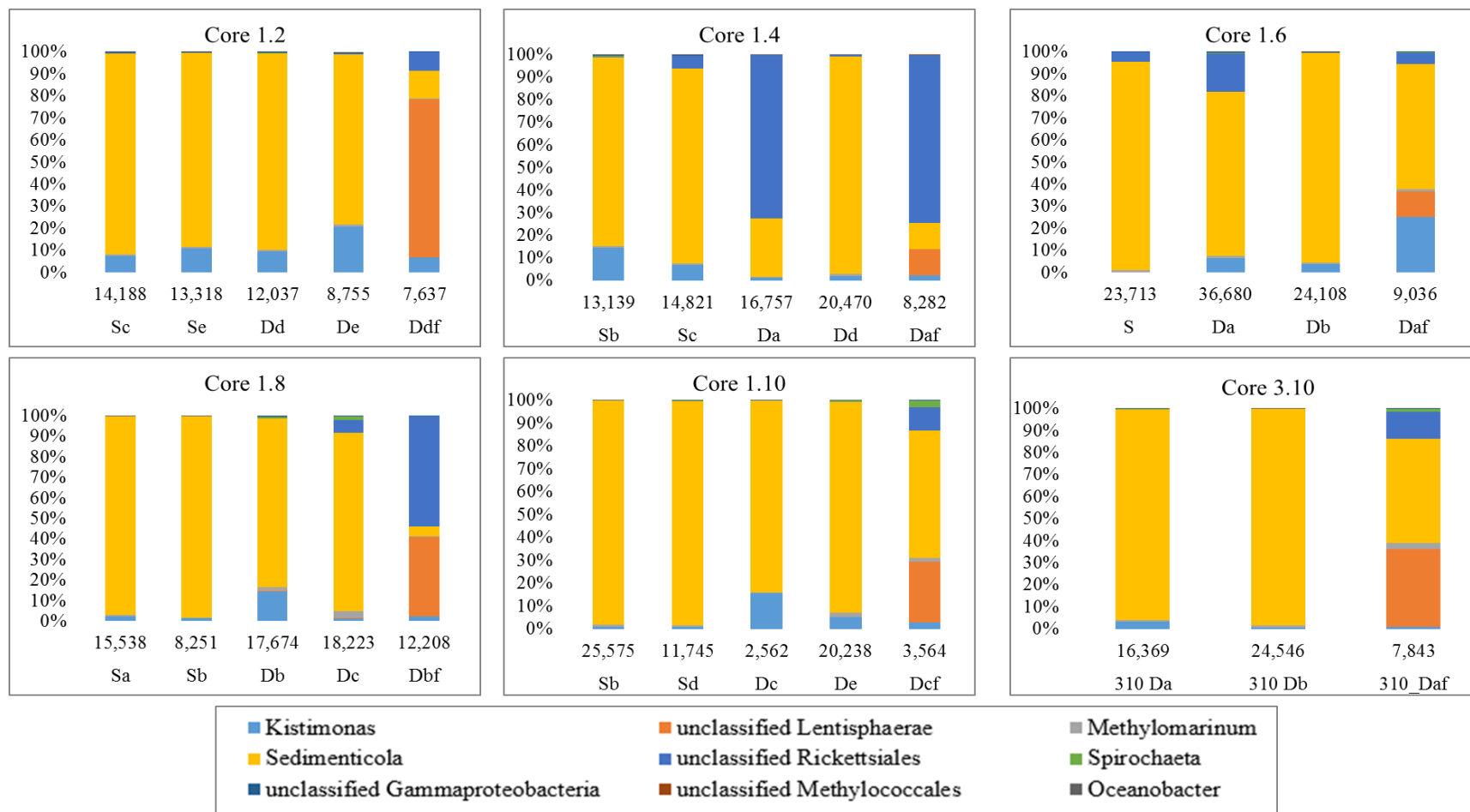


Figure 7 - Total taxonomic sequence identities from gill and foot tissue. The letter S refers to host *P. pectinatus* collected from 0-10 cm and letter D refers to *P. pectinatus* hosts collected from 10-30 cm depth. Sequence counts for each sample are below sample ID.

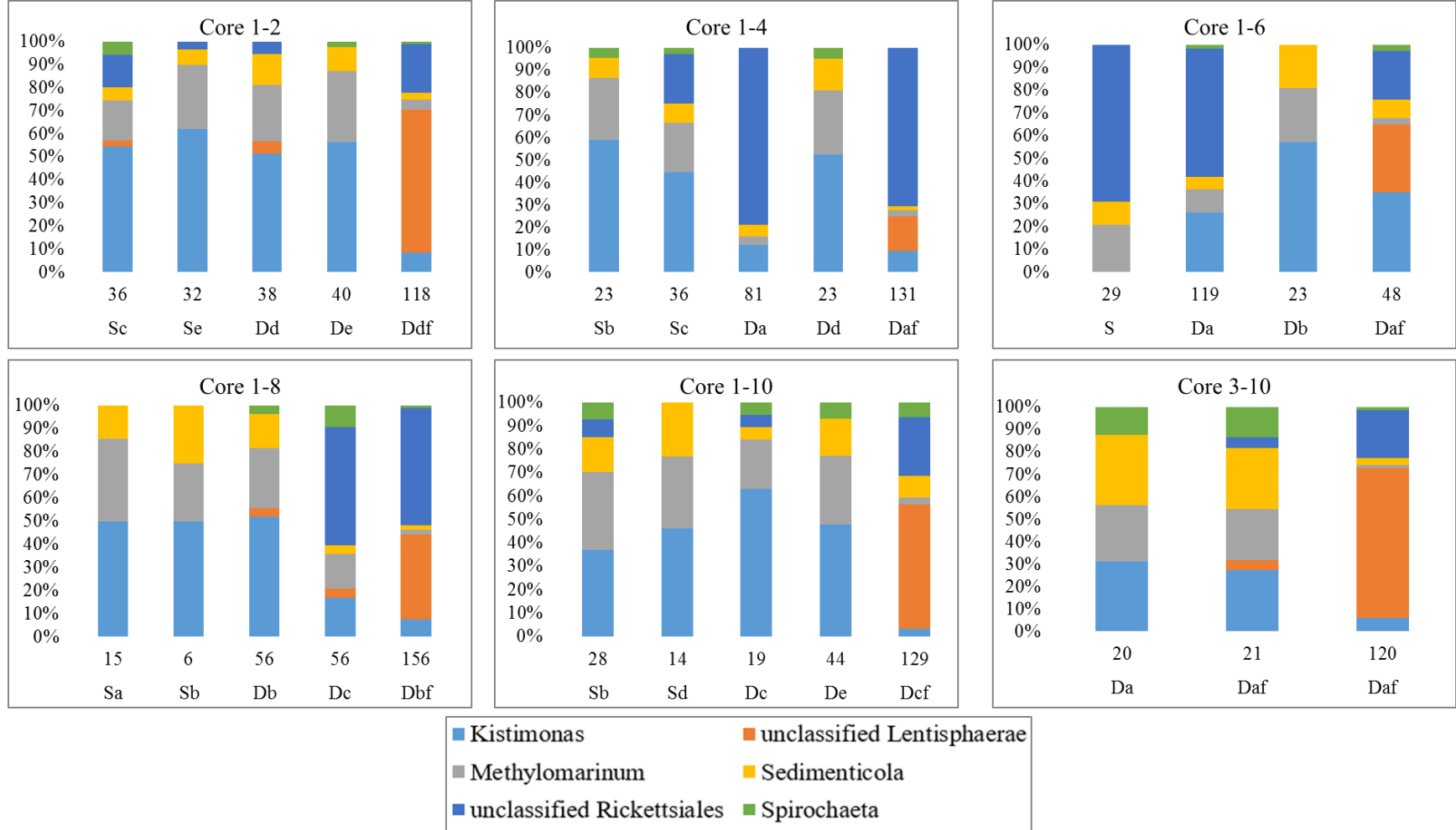


Figure 8 - Operational Taxonomic Units (OTUs) at the 97% level from gill and foot tissue. The letter S refers to 0-10 cm and letter D refers to 10-30 cm). Sequence counts are below sample ID.

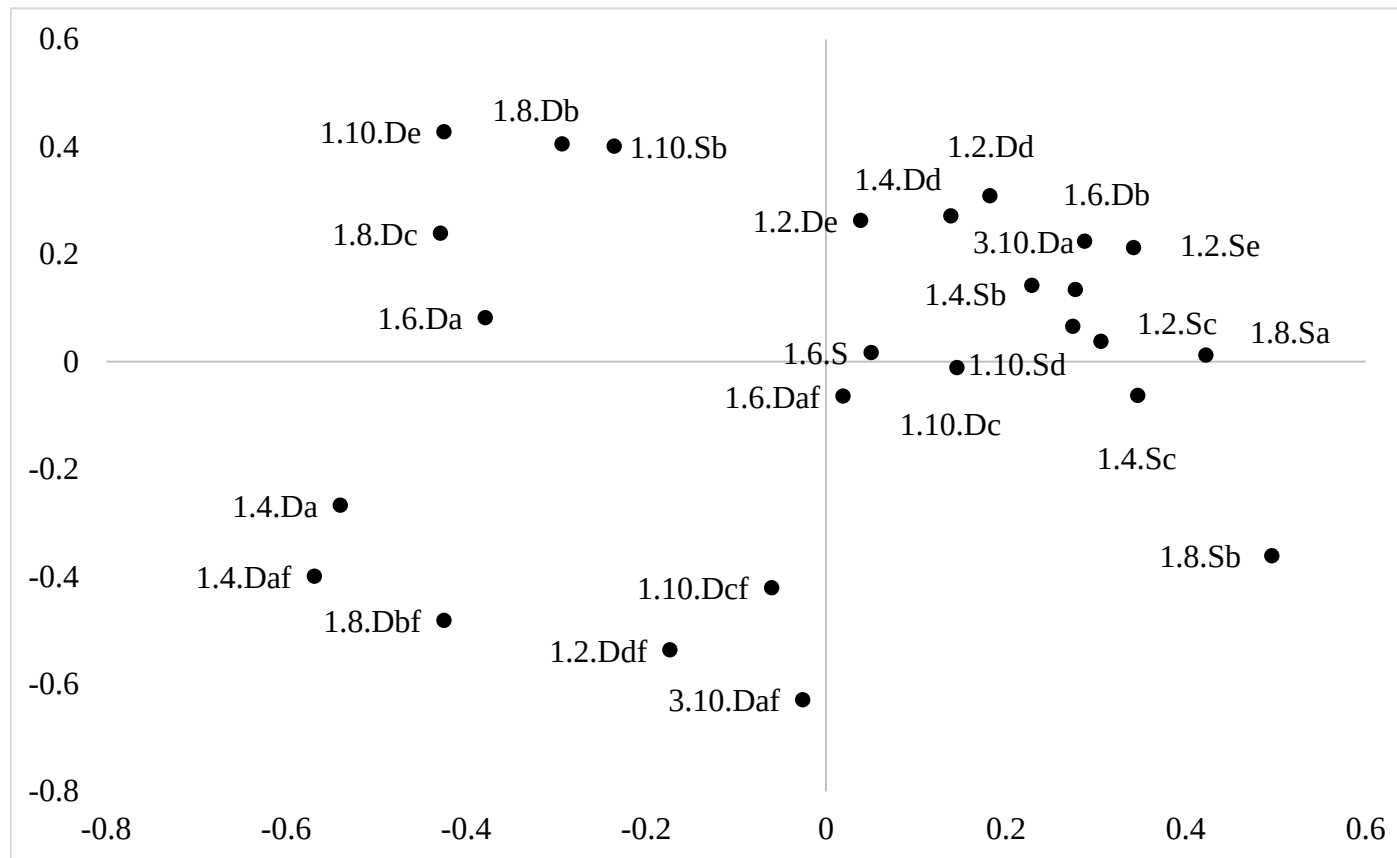


Figure 9 - Non-metric multidimensional scaling (NMDS) for tissue samples with stress value = 0.160 and $R^2 = 0.825$. The labels shown are describing the quadrat, depth, and specimen. The communities associated with the lucinid's foot (label with f) were significantly different (F-value = 5.75 and $p = 0.00005$)

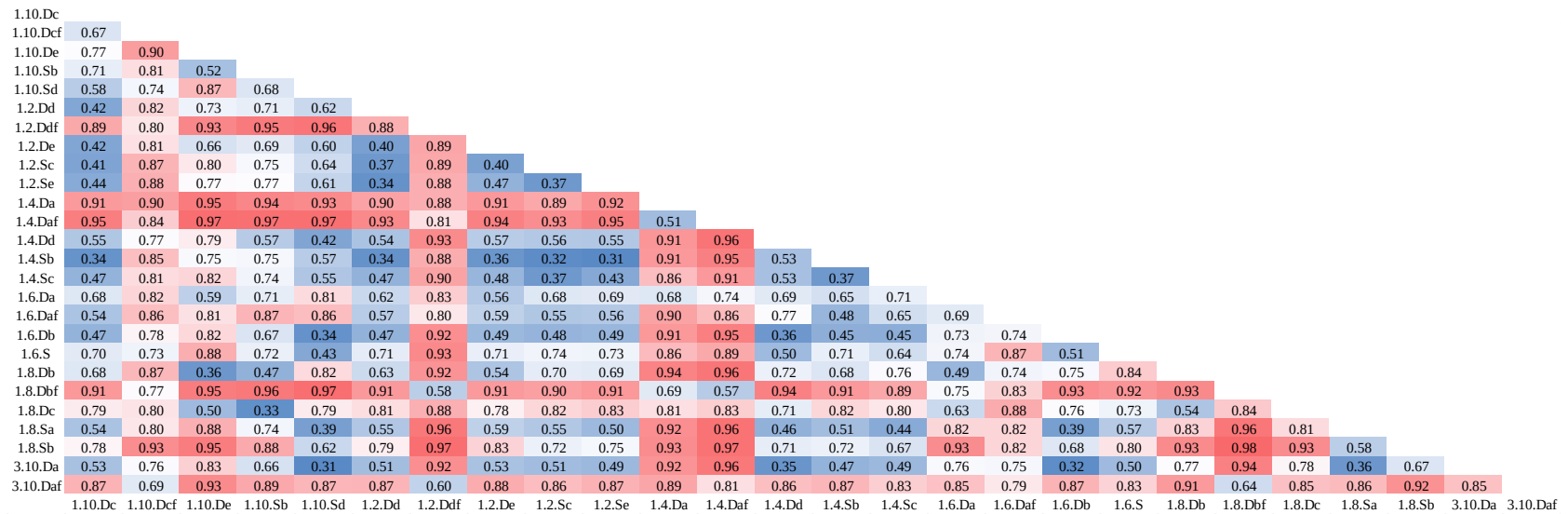


Figure 10 - Bray-Curtis similarity matrix indices for gills and foot at 97% similarity. Red means the diversity of two samples was highly similar and blue means that two samples are not similar.

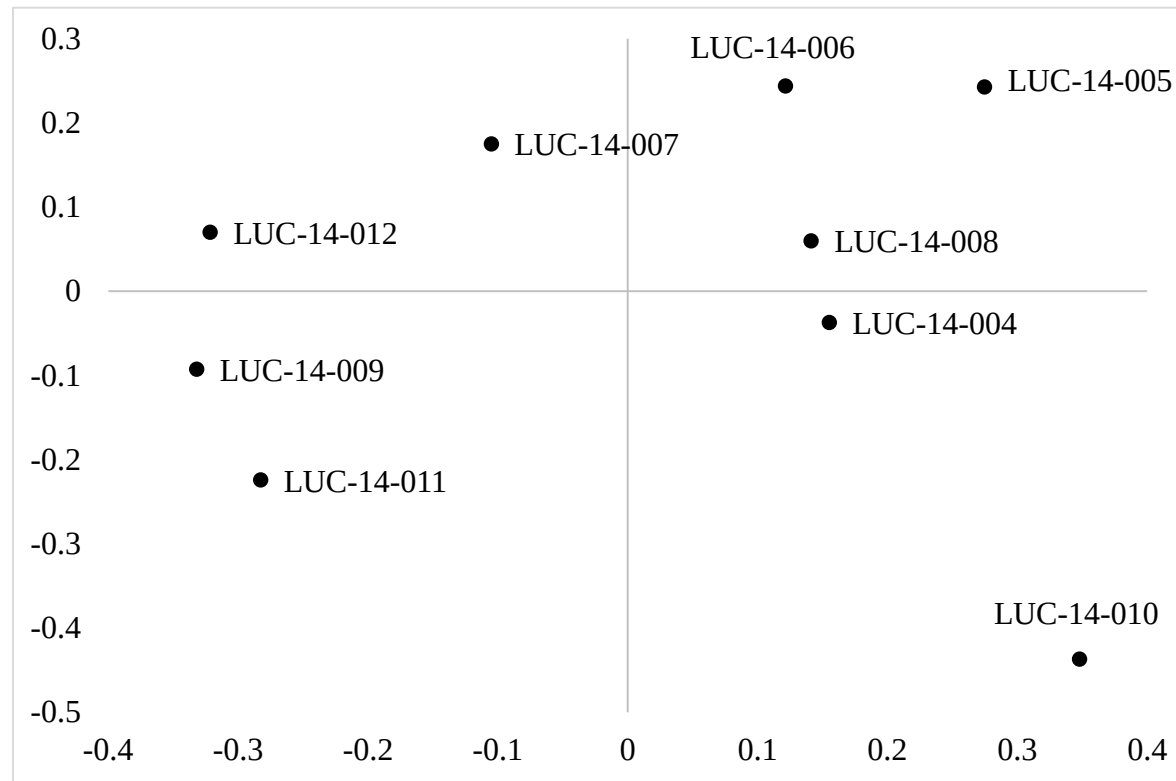


Figure 11 - Non-metric multidimensional scaling (NMDS) for water samples with stress value = 0.192 and $R^2 = 0.267$.

VITA

Walt Doty is a Knoxville native. Go Vols. He earned his Bachelors of Science in Chemistry from Sewanee: The University of the South in 2012. Under the guidance of his advisor, Dr. John Shibata, he investigated the persistence and fate of pesticides and herbicides applied to the University's campus. In 2013, Walt began working with Dr. Annette Engel at The University of Tennessee. His early work focused on determining the distribution of the *soxB* genes along a sulfur spring located in Oklahoma. In 2014, he officially started his graduate program in the Department of Earth and Planetary Sciences.