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Vertical Distribution of Wetland Plant Roots and their Associated Bacteria in Groundwater-Fed Wetlands

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Vertical Distribution of Wetland Plant Roots and Their Associated Bacteria in Groundwater-fed Wetlands.

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Jennifer Diane Bailey ENTITLED Vertical Distribution of Wetland Plant Roots and Their Associated Bacteria in Groundwater-fed Wetlands BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Abstract

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As part of the effort to understand the community structure of bacteria and archaea in groundwater fed wetlands, the vertical distribution of plant root density and species presence was studied in correlation with the change in associated bacterial and archaeal communities. Three sampling sites at a local groundwater fed wetland were selected based on surface plant community structure: Site 1 was dominated by *Carex stricta*, site 2 was dominated by *Eleocharis erythropoda*, and site 3 was dominated by a 50/50 mix of *C. stricta* and *E. erythropoda*. Core samples at 4 depths down to 1.2 m were taken to collect data on soil moisture, root density, organic matter content, plant species presence, bacteria taxa presence, and quantification of bacteria taxa. DNA sequences were used to identify plants, bacteria, and archaea. Between 13,000 – 25,000 bacterial and archaeal species are estimated to be present at each depth, with the greatest diversity found in the upper layers. Species estimates were positively correlated with soil moisture, organic content, and root mass. In total, 242 microbial classes were found with a Simpson's dominance index $\leq$ 0.09, Shannon's diversity index $\geq$ 3.13, and Shannon's equitability $\geq$ 0.60, indicating a high diversity and somewhat even abundance. Compared to other wetlands, diversity indices are highest in wetlands with hydrology similar to fens (sub-surface vertically-flowing water). All soil parameters significantly ($p < 0.05$) influenced overall microbial community composition; most significantly the presence of dechlorinators, ammonia oxidizers, and methane oxidizers. Sub-surface samples revealed a greater species richness of plants than was expected from surface sampling; 22 species

were found through surface survey and 25 species were found through DNA analysis. *E. erythropoda* ($p \leq 0.05$) significantly influenced overall changes in microbial communities. Of the 25 plants, 12 plants were correlated with specific functional groups, with at least one plant strongly ($p \leq 0.10$) correlated with each functional group.

Key Words: Chlorinated ethenes, community structure, microbial ecology, soil microbes, wetlands, fens

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1. Introduction

Fens are rare in nature (Amon *et al.* 2002; Bedford & Godwin 2003), with surface emerging groundwater as their primary source of water; other wetlands have formed along streams, by flooding or pooling by lakes, or have rainfall as their primary source of water. Fens have upward-flowing, surface-directed water that provides a constant low level supply of nutrients and flushes away products of metabolism or degradation at a pace close to or equal to input from the aquifer supplying the water. Due to this, fens have two sources of nutrients available to their plant and microbial communities; one source from surface flowing water such as rain and the second source being surfaced directed groundwater. Soils in fens are populated by highly diverse communities of plants that bring oxygen to roots in what would be otherwise anaerobic zones. Jackson *et al.* (1996) found that on average over 90% of roots across nearly all biomes are found in the top 1m of soil. In fens, plant with roots reaching down 1m or more have an enormous surface area that brings them into contact with emerging groundwater as it flows freely through peaty soil (Amon *et al.* 2007). Leakage of oxygen from those roots, presumably at all depths, has the potential to create root to soil oxygen micro gradients (Colmer 2003). These gradients can produce a spectrum of redox conditions that should support a wide variety of microbial species and their wide range of biogeochemical activities.

Until recently, the structure of microbial communities in soils has been poorly studied, hampered by the inability to culture a majority of microbes present in laboratory conditions and the high cost and time required for DNA analysis. Between 1-10% of bacteria present in soil can be cultured using traditional petri dish and enrichment culture methods (Lutton et al. 2013). Enrichment (growing bacterial cultures on a medium with

specific qualities that favors a particular bacterium) has been useful in showing some functions of some bacteria, but it can miss the majority of microbes incapable of sustained growth in the enrichment medium. Previous attempts to describe microbial community composition by DNA analysis required the use of colony picking (Genhe *et al.* 2014; Moez *et al.* 2014). Colony picking is a method used to isolate pure DNA from a mixed sample to be sequenced via Sanger sequencing. Colony picking involves transforming a DNA area of interest into bacteria, plating and growing bacteria, picking a single colony, extracting then amplifying DNA of interest using PCR, and sequencing. For large samples, this method can be very time consuming, costly, and drastically limits the potential to find all relevant species. When species numbers are in the thousands, colony picking requires too much time and money to be a realistic approach to quantifying community structure.

New methods to identify the presence of bacteria, such as through analysis of key segments of their genomes (Bates *et al.* 2011), have enabled researchers to find and describe nearly every living thing in soils. By combining these sequence analyses with data from traditional isolation and enrichment methods, large databases of the genetic character of many bacterial taxa have been built. With the newest methods of identification, there is the potential to describe the presence of bacteria in a multidimensional habitat such as depth, soil quality, temperature, inorganic and organic composition and association with other things living within that matrix.

Progress has been made toward characterizing the microbial communities found in wetlands. The phylum proteobacteria is dominant across many wetland types (Genhe *et al.* 2014; Moez *et al.* 2014; Arroyo *et al.* 2015), followed by verrucomicrobia (Genhe

et al. 2014; Arroyo *et al.* 2015) and bacteroidetes (Genhe *et al.* 2014; Moez *et al.* 2014). Within proteobacteria, the beta-, gamma-, and deltaproteobacteria classes are found to occur most frequently. However, studies frequently show only the most dominant phyla or show the results of colony picking, so they characterize only the most common phyla instead of the whole community. Environmental parameters, such as available nitrogen and plant biomass (Arroyo *et al.* 2015; Chen *et al.* 2015), influence bacterial community structure. However, presence of specific functional groups within total community structure is still poorly understood and understanding could give insight on how microbes influence the availability of nutrients and behave as biochemical changers in their environment.

The structure of microbial communities may influence and, in turn, be influenced by the plant and animal communities in soil and on the soil surface. One hypothesis of this study is that the presence of certain microbes may be dependent on the plants growing in the soil. Both plants and microbes have the capacity to modify the soil and the various chemical compounds present there. For example, some widely distributed manmade compounds, such as trichloroethene (TCE) and perchloroethene (PCE), may contaminate groundwater or aquifers and are solvents known to be carcinogenic at levels as small as 5 ppb (ATSDR 1997). Several bacteria classes, including the well-researched Dehalococcoidetes, contain species capable of dechlorinating compounds such as TCE into nontoxic end products (MaymoGatell *et al.* 1997, Freeborn *et al.* 2005). Bacteria associated with dechlorination have been detected in both PCE/TCE contaminated wetlands and in more pristine environments, where these compounds are not present (Amon *et al.* 2007; Gruner 2008). Prior research has shown that specific plant roots may

be associated with dechlorination (Powell *et al*. 2011; Menon *et al.* 2013). The relationships between plants and bacteria are known to some extent through agricultural studies and the use of constructed waste treatment facilities. One such study used a constructed fen to maximize removal of TCE and PCE from contaminated groundwater moving upwardly through the soil and the roots of common wetland plants study (Amon *et al.* 2007). Other studies have also shown the ability of wetlands to remove chlorinated ethenes (Kadlec *et al.* 2012), as well as nitrogen and phosphorus (Moez *et al.* 2012; Sun *et al.* 2013) from regulated (ex. factories) and non-point (ex. roadway runoff) sources.

The nitrogen cycle is another example of plants and microbes modifying the chemical compounds in soil. The nitrogen cycle involves nitrogen-fixing bacteria reducing atmospheric nitrogen to ammonia, then nitrifying bacteria converting the ammonia into nitrates. From here, the nitrates can be taken up by plants as a nitrogen source to be eventually released as ammonia as dead plant matter decays, or denitrifiying bacteria can convert nitrates back into atmospheric nitrogen. In some situations, nitrogen availability can be a limiting nutrient for plants (Elser *et al.* 2007), notably in agriculture. Many crops are fertilized with anhydrous ammonia as a nitrogen source. The fertilizer can be washed into nearby streams by rain or irrigation and cause harmful algae blooms, loss of oxygen, and fish kills (Carpenter *et al.* 1998). However, wetlands are capable of being a “nutrient sink” by absorbing excess nitrogen from water that passes through them (Domingos *et al.* 2011; Genhe *et al.* 2014) which indicates that wetlands are a viable treatment option for non-point excess nitrogen sources and can help reduce the impact fertilizer runoff has on waterways.

Since few bacteria in soil can be detected using culture methods, studying plant and bacteria interactions requires the use of genetic sequencing. To identify plant and bacterial taxa that are found in wetlands Next-generation Genetic Sequencing (NGS) is used in this thesis, e.g. the IonTorrent PGM™ (current state of the art method). NGS systems offer many advantages for this project; they allow for sequencing of combined samples (multiplexing), accurate detection of taxonomically significant DNA sequences, quantification of the number of times a specific sequence was sequenced (copy numbers), avoidance of time consuming colony picking, and performance of a single PCR step with clean up (library preparation) is needed. NGS multiplexing can sequence the initial mixed sample, as well as a mix of many additional unique samples simultaneously, thereby reducing the cost and time of sequencing by reducing the runs required. Unique samples are tagged by an identifying set of base pairs, or "barcode", added to each strand of DNA, so the samples can be sorted based on their sample after sequencing. Single-nucleotide polymorphism detection allows for the identification of putative species when compared to known databases such as GenBank (Linder et al. 2000). Quantification of copy numbers allows for the specific proportional composition of bacterial communities to be elucidated (Kembel et al. 2012). Library preparation consists of adding IonTorrent PGM™ adaptor regions to the primers of the desired region to be sequenced. The adaptors bind to the sequencing wells of the IonTorrent PGM™ and are required for the DNA to be sequenced. Such adaptors can be added during PCR using the fusion primer method of including adaptors with study specific primers. The IonTorrent PGM™ can have different read lengths (the maximum length of base pairs that can be sequenced contiguously) available based on reagents that are used and the desired read length for a

run. The read length used needs to be greater than or equal to the length of the amplicon to be sequenced. A read length of at least 400 bp (Baldwin *et al.* 1995) is sufficient for identifing most species via sequencing the plant specific region and at least 291 bp (Bates *et al.* 2011) is required for bacteria.

For plant species identification, a portion of the internal transcribed spacer (ITS) region of the 18s-26s nuclear ribosomal is sequenced since this is a highly conserved gene but has hypervariable regions that can be used for species identification (Linder *et al.* 2000). The ITS region has many properties that lend itself to genetic study. First, the ITS region is frequently repeated in the genome making amplification and sequencing favorable. Second, the ITS region is highly conserved within taxa, making it useful for phylogenetic studies (Baldwin 1992; Baldwin *et al.* 1995) and species identification (Linder *et al.* 2000). Third, well tested, universal primers exist for the ITS region (White *et al.* 1990). Fourth, many ITS regions have already been sequenced and are available in the GenBank database for comparison to what is found in samples. As of September 2015, over 125,000 ITS region entries for "plants" were in GenBank.

For bacterial taxa identification, a portion of the 16S ribosomal DNA region can be sequenced. Similar to the ITS region, the 16S region is highly conserved within taxa of bacteria and archaea (Coenye *et al.* 2003). Several universal primers exist for the hypervariable regions within the 16s gene (Greisen et al. 1994). The primers 515F and 806R allow a large portion of the third and fourth variable region to be sequenced, which allows for detection to level of bacterial and archaeal species (Bates *et al.* 2011).

2. Goals and Objectives

The goal of this study is to characterize the distribution of various taxa of bacteria and archaea within the root zone of wetland plants in a fen, determine how that distribution may affect some of their functions, and quantify how plants influence or correlate with the distribution of the bacteria and archaea and whether subsurface plant presence relates to surface plant community.

Objective 1: Determine biomass of roots, water content, and organic matter content of soil at four depths for later correlation with individual plant root species and bacterial and archaeal taxa distribution.

Objective 2: Quantify and identify the presence of plants at four depths below the wetland surface by comparing DNA sequences to the GenBank database.

Objective 3: Quantify and identify the presence of known bacterial and archaeal classes at four depths below the wetland surface by comparing DNA sequences to the QIIME database. Determine number of bacterial and archaeal classes at each depth and show dominance if any.

Objective 4: Determine distribution of bacterial and archaeal genes known to be associated with various functional groups such as dechlorinators, methane oxidizers, methanogens, ammonia oxidizers, nitrite oxidizers, denitrifyers, and nitrogen fixers.

Objective 5: Compare vertical distribution and correlation of bacterial and archaeal functional groups to presence of species of plant roots.

Objective 6: Determine whether diversity and richness of bacteria and archaea can be correlated with plants or any other parameters investigated.

Objective 7: Provide data for use by others to further investigate microbial function in wetlands.

3. Methods

3.1 Site and Sampling Selection

Valle Greene is a local fen wetland (39°46'26.37"N, 84° 0'28.30"W) largely driven by ground-water emergence at the surface and part of large 1000+ hectare corridor dominated by groundwater discharge. Its soil structure and hydrologic function were used as a model for a wetland built at Wright Patterson Air Force Base, OH for bioremediation of chlorinated ethenes (Amon *et al.* 2007). Three sites at Valle Green were selected based on the plant communities found. Plant communities dominated by *Carex stricta* or *Eleocharis erythropoda* were selected for study since they are common wetland plants throughout North America and near monocultures could be found at Valle Greene. Site edges were at least 2 meters apart and surface dominance was determined by visual inspection of cover. Site 1 was dominated by *C. stricta* (80% of surface). Site 2 was dominated by *E. erythropoda* (90%). Site 3 was equally dominated by *C. stricta* (40% of surface) and *E. erythropoda* (40% of surface), or a 50/50 mix (80% of surface). At all three sites the plant communities covered at least a 3m radius circle. Samples were equally spaced (Figure 1) 1m from the circle's center, allowing a 1.5m or greater

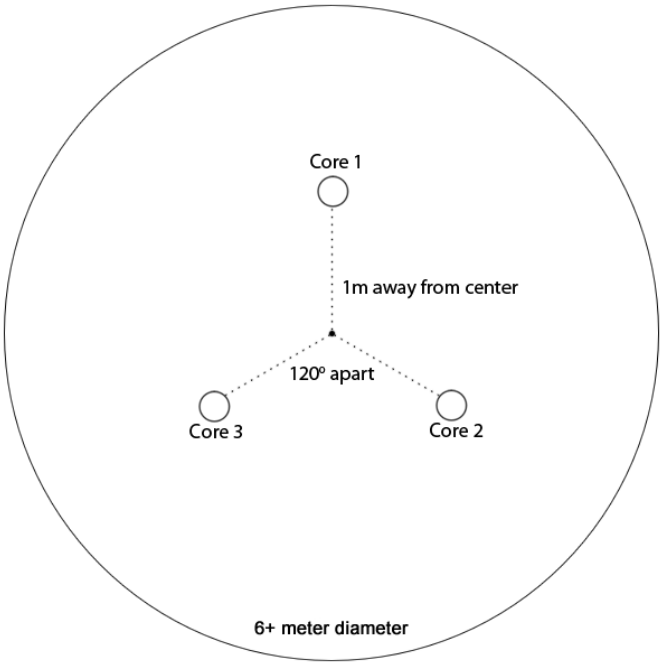

Figure 1 Diagram demonstrating the layout of a site.

separation between samples and the edge of the sampling space to try to reduce outside plant species influence by horizontal root growth. Samples were taken during July and August 2013 when plant growth was at its peak.

3.2 Sample Collection

At each site, 3 cores were collected using a 7.6 cm diameter aluminum piston corer (Amon *et al.* 2007). The soil is hemist with some clay and calcium carbonate sediment and in most cases rich with living roots. Cores were limited to a depth of 1.2 m due to a loose gravel layer found at that depth that could not be retained in the piston corer. The gravel was likely the top of the aquifer made of glacial outwash gravel.

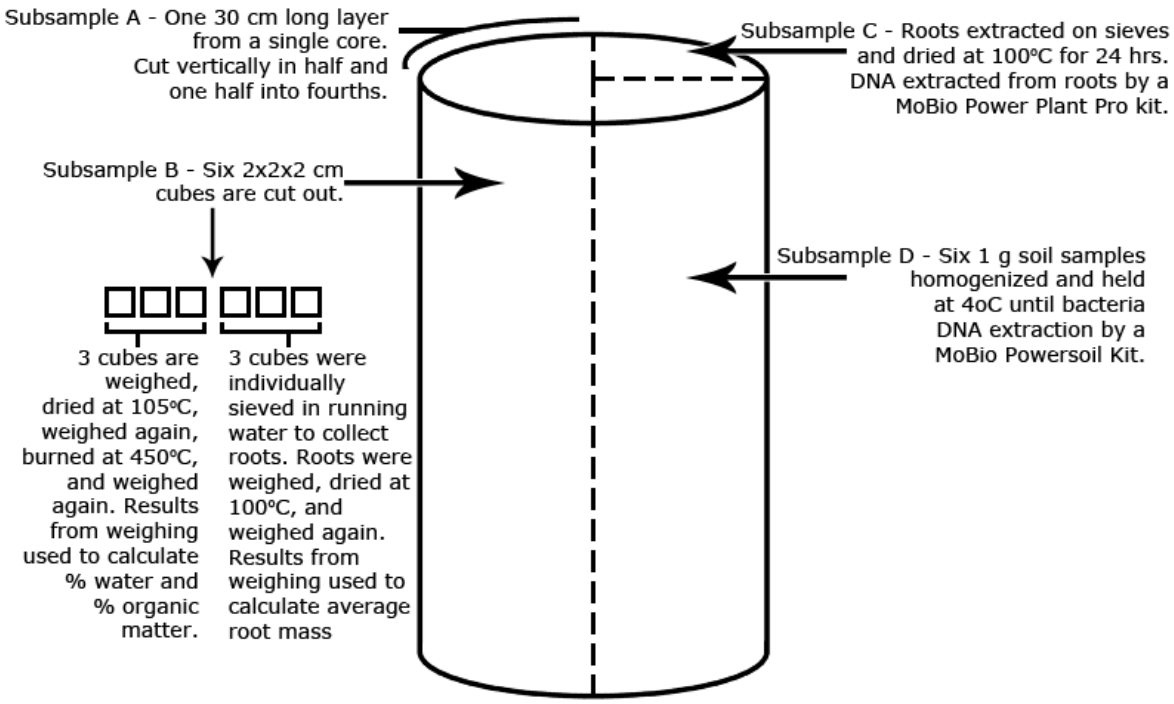

Figure 2. Example of core processing. Illustration represents one depth.

Following the general procedure in Amon et al. (2007), each core was divided into four 30 cm long sub-samples. Each sub-sample was cut in half vertically. From one

half, six 2x2x2 cm cubes were cut out; two from near the top, two from near the middle, and two from near the bottom taking care to avoid cross contamination from adjacent areas within the core and compression (Figure 2). For each sub-sample, three of the six cubes were weighed, then dried at 105 C and weighed for dry weight and then burned at 450 C, and reweighed to obtain information on soil moisture and organic matter content. Percent soil moisture is calculated by dividing dry weight by wet weight then subtracting from 100%. Organic matter content is calculated by dividing dry weight by burned weight then subtracting from 100%. Roots from the other three cubes from each sample were separated from soil by washing in running tap water on graded sieves (2.0, 0.85 and 0.088 mm mesh), rinsed, dried at 105 C for 24 hours and weighed to calculate root mass. Estimating root biomass by organic content negated small, but variable, amounts of fine soil retained in roots after thorough washing (Amon *et al.* 2007). The other half of each sub-sample was divided in half vertically, giving two vertical quarters. From the first quarter, all roots were extracted using the same method and dried at 100 C for 24 hours. The roots were then stored at -20 C until used for DNA extraction, which was no more than 24 hours later. From the second quarter, six 1g soil samples were randomly taken from the sub-sample for bacterial DNA extraction, homogenized in a tube and stored at 4 C, for no more than 24 hours.

3.3 DNA Extraction & Sequencing

To gather information on presence of **plant species**, the dried roots from a full quarter of a sub-sample were ground in a mortar and pestle and DNA was isolated using a Mo-Bio PowerPlant Pro® DNA Isolation kit. Due to roots generating “dirty” samples, an

additional cleanup step was performed using a Mo-Bio PowerClean® Pro DNA Clean-Up Kit. Extracted samples were stored at -80 C until ready for sequencing.

To gather information on **bacterial and archaeal community** composition, a Mo-Bio PowerSoil® DNA Isolation kit was used to isolate DNA from the homogenized 1g soil samples. Extracted samples were stored at -80 C until ready for sequencing.

To sequence plant DNA, universal primers 'ITS3' and 'ITS4' (White *et al.* 1990) were used to amplify DNA within the ITS2 region. To sequence bacterial and archaeal DNA, universal primers '515F' and '806R' (Bates *et al.* 2011) were used.

All samples were, under contract, sequenced on a Life Technology IonTorrent PGM™ at Wright State University's Center for Genomics Research, which provided files containing sequencing data and barcode assignment that were used in data analysis.

3.5 Community and Multivariate Analysis

For plant, bacteria, and archaea DNA sequence data, short (<150 bp) and low quality (average phred score <20) reads derived from Ion Torrent sequencing were removed using the program QIIME (Caporaso *et al.* 2010). This removes sequences too short to use in the study and sequences with less than 99% sequencing accuracy. Singletons (sequences appearing only onced) were also removed. Groups of DNA sequences were assigned to operational taxonomic units (OTUs) defined at a genetic distance of 3% by QIIME, hereafter called “species” for ease of understanding and relatability to other biological disciplines. Taxonomic assignment of bacteria and archaea from sequence reads was obtained using QIIME’s native database (Caporaso *et al.* 2010). Taxonomic assignment of plant sequence reads was obtained by pairing a custom ITS-2

database with QIIME. The custom database was created by combining ITS-2 sequence data obtained from GenBank (Benson *et al.* 2009) and taxonomic classification obtained from the USDA PLANTS Database (USDA 2015). QIIME provides data on kingdom, phylum, class, order, family, and genus in a spreadsheet that denotes percent of total sequences for that sample. Since bacteria and archaea are taxonomically separated most often by functional aspects, an examination of many sources can provide putative function(s). Through an extensive taxonomy literature review (Appendix A.1), each bacterial and archaeal class was assigned specific potential function(s) of wetland soils; aerobic, anaerobic, nitrogen fixing, denitrifying, nitrite oxidizing, ammonia oxidizing, methane generating, methane oxidizing and dechlorinating. Potential function was determined by a class having demonstrated function in pure culture or by having known functional gene sequences.

α-diversity analysis through Chao1 (estimates the total microbial species richness) and rarefaction curves and β-diversity analysis through weighted UniFrac principal coordinates plots (PCoA) were conducted using QIIME (Caporaso *et al.* 2010). Weighted UniFrac calculates values based on differences between microbial communities based on phylogenetic distances between observed organisms. These calculations can generate a PCoA plot where the principal coordinates represent the differences in phylogenetic values between communities. Multivariate analysis of community structure and patterns were conducted using the Vegan package (Oksanen *et al.* 2015) in R software (R core team 2015). The Adonis function, a multivariate analysis of variance (MANOVA) analog, was used to determine if a metric had significant influence on microbial community structure. Linear multiple regression analysis was used to determine the

strength and kind of the relationship between metrics and functional groups. While the small sample size of this study will prevent in-depth models from being created, a preliminary look into possible relationships can be gained. Significance for all tests was defined at $p \leq 0.10$ (strong trend) and $p \leq 0.05$ (significant).

Simpson's dominance, Shannon's diversity, and Shannon's equitability indices were calculated using R's diversity function. Simpson's dominance index calculates the probability that two individuals randomly selected from a sample will belong to the same taxa. The index ranges from 0 to 1, with 0 meaning all taxa are equally present and 1 meaning one taxon completely dominates the community. Shannon's diversity index takes into account the number of taxa and their evenness (Cain 2014). A low number indicates a lower amount of taxa with an uneven abundance and a higher number indicates a greater number of taxa with an even abundance. Typical values for natural communities fall between 1.5 and 3.5 (MacDonald 2003). Shannon's equitability is a measure of how similar the abundances of species are, with 0 indicating there is not a similar proportion of taxa and 1 indicating an even proportion of taxa (Cain 2014).

4. Results

4.1 Soil Parameters

Percent organic content and percent water content (Table 1) were highest at soil surface and decreased with depth at each site. Root density and percent water content (Table 1) were highest in the top layer at Site 2 – *E. erythropoda* when compared to the other two sites, but became similar to the other sites with increased depth. At all sites, root density generally decreased with depth, but increased in Layer 4. Layer 4 included the beginning of a loose gravel layer that groundwater flows through.

Table 2 – Organic content, water content and root mass by site and by depth. Values are avg ± one SD. All root density SDs are < 0.00001 and not shown. Numbers in () are percent of total for that site.

	Depth below surface			
	Layer 1 (0-30 cm)	Layer 2 (30-60 cm)	Layer 3 (60-90 cm)	Layer 4 (90-120 cm)
Avg % organic matter				
Site 1 – *C. stricta*	14.38 ± 8.19 (44)	9.01 ± 2.27 (28)	5.40 ± 1.45 (16)	3.93 ± 1.11 (12)
Site 2 – *E. erythropoda*	35.72 ± 12.49 (45)	23.04 ± 8.63 (29)	12.80 ± 5.11 (16)	7.26 ± 2.52 (10)
Site 3 – 50/50 Mix	23.26 ± 12.54 (48)	11.02 ± 3.95 (23)	9.81 ± 5.04 (20)	4.14 ± 1.11 (9)
Avg % water content				
Site 1 – *C. stricta*	41.23 ± 13.28 (33)	31.34 ± 3.33 (25)	31.34 ± 3.49 (25)	21.01 ± 7.23 (17)
Site 2 – *E. erythropoda*	65.31 ± 12.51 (40)	44.09 ± 9.08 (27)	34.69 ± 7.91 (21)	19.70 ± 2.70 (12)
Site 3 – 50/50 Mix	49.65 ± 11.45 (37)	34.76 ± 10.00 (26)	32.10 ± 9.92 (24)	18.54 ± 4.51 (13)
Avg mg/cm^3 root mass				
Site 1 – *C. stricta*	28.34 (56)	6.49 (13)	6.00 (12)	9.44 (19)
Site 2 – *E. erythropoda*	68.57 (77)	7.58 (9)	4.88 (5)	7.82 (9)
Site 3 – 50/50 Mix	52.30 (72)	6.81 (9)	5.74 (8)	8.23 (11)

4.2 Bacterial Community

4.2.1 Richness

The highest species richness of all sites was found in layer 1 (Table 2), steadily decreasing through layer 4 (Table 2). Site 3 had the highest estimate of 24,263 species for

layer 1, site 2 had the highest estimate of 23,270 and 18,903 species for layers 2 and 3 respectively, and site 1 had the highest estimate of 16,881 species for layer 4. Unlike site 1 and 2, site 3 showed a decrease in estimated microbial species, then an increase in layer 3, ending with a decrease in layer 4.

Table 2 – Chao1 estimates for bacterial species richness. Values are Avg ± SD

	Site			
Depth	Site 1 *C. stricta*	Site 2 *E. erythropoda*	Site 3 50/50 Mix	Overall Avg ± SD by layer
Layer 1 (0-30 cm)	21,039 ± 1,518	23,531 ± 266	24,263 ± 403	22,944 ± 1690
Layer 2 (30-60 cm)	20,797 ± 1,407	23,270 ± 4,166	14,080 ± 4,124	19,382 ± 4755
Layer 3 (60-90 cm)	17,797 ± 8,060	18,903 ± 4,495	15,297 ± 6,223	17,333 ± 1847
Layer 4 (90-120 cm)	16,881 ± 2,651	10,644 ± 1,1342	13,200 ± 3,617	13,575 ± 3135

Using rarefaction analysis, it is clear that while all sites have similar richness in my sampling (Figure 3), none of the sites were adequately sampled to give a true direct measure of richness. Chao 1 allows for extrapolation to obtain an estimated species number or richness. Similar species richness by α diversity can be seen in rarefaction curves (Figure 3) across sampling sites. Chao 1 estimates come from the extrapolation of the plateau point of these curves. Upper layers had higher species richness, while lower layers had lower species richness.

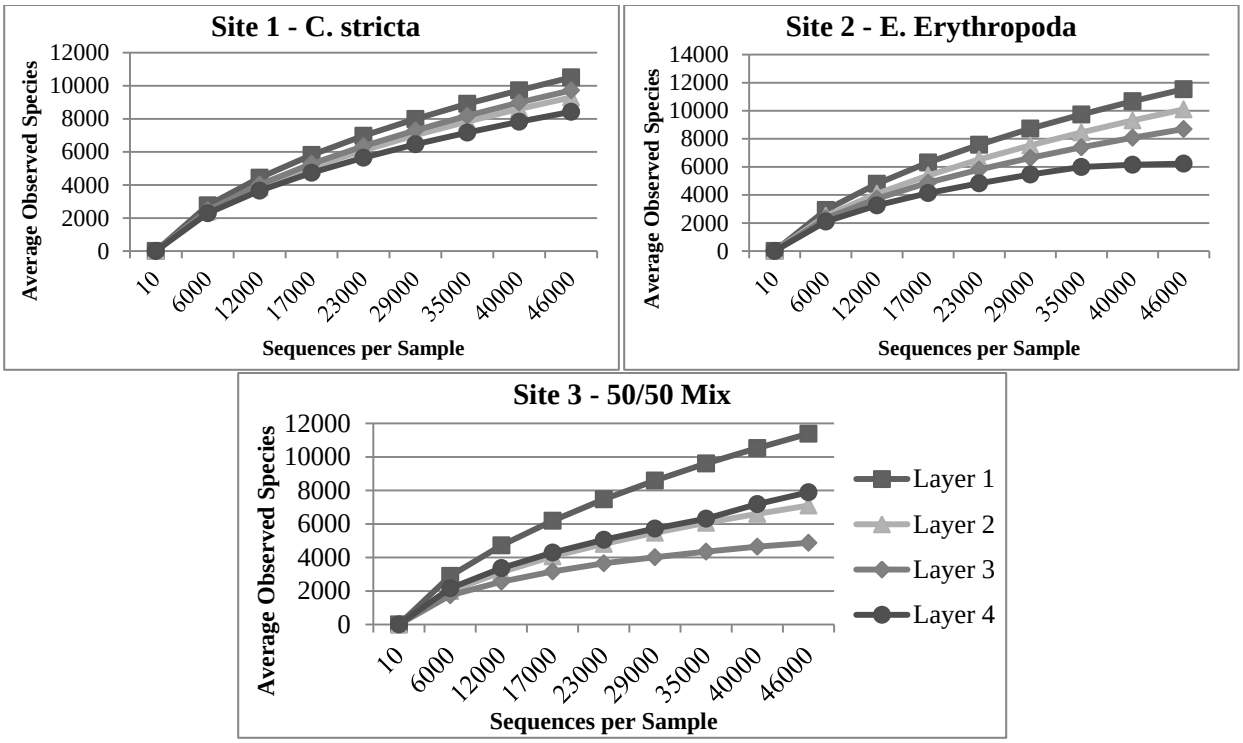

Figure 3. Alpha rarefaction curves for bacterial community species richness. Species were separated by 3% genetic difference. Each curve is generated from an average of three samples. Each layer is 30cm long, with Layer 1 at the top and Layer 4 at the bottom.

4.2.2 Similarity of Layers to One Another

β diversity through principal coordinate analysis (PCoA) showed grouping of samples when categorized by layer along PC1 (Figure 4). PC2 did not correlate with significant grouping. In this analysis, PC1 accounted for 15% of the variation. Across all sites and samples, bacteria communities were more similar when grouped by layer. Layers 2 and 3 showed some overlap, while layers 1 and 4 were distinctly separate.

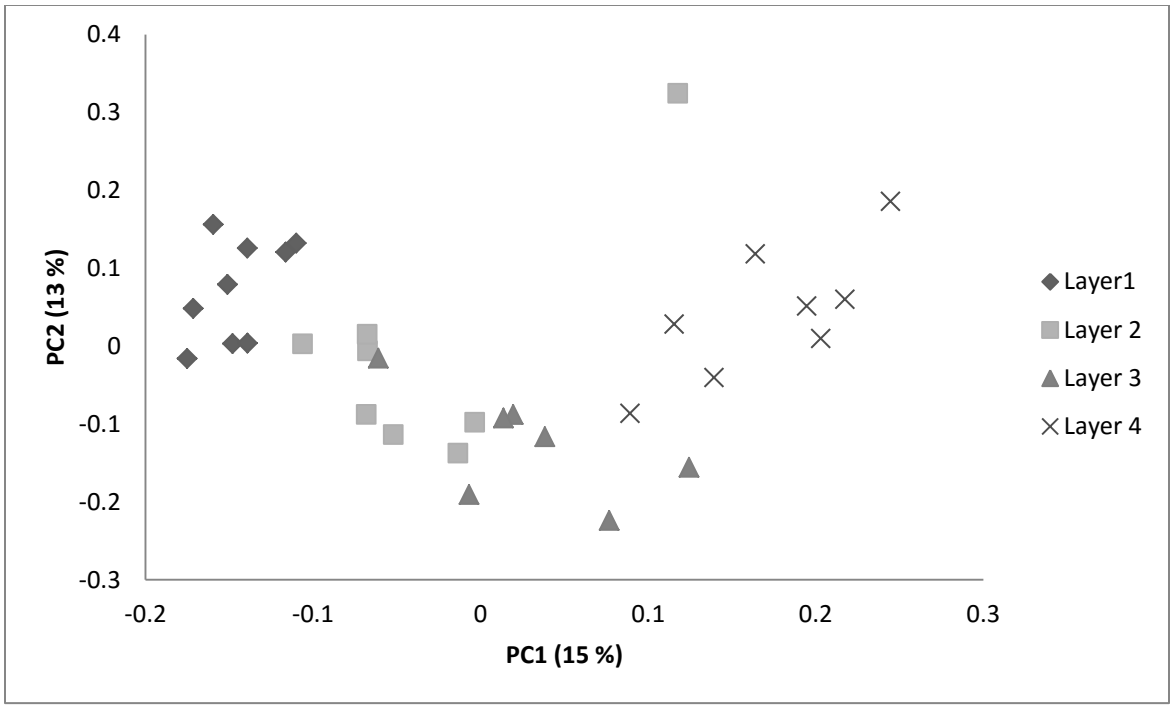

Figure 4. Bacteria community beta analysis through weighted UniFrac principal coordinate analysis. Numbers in parenthesis are the percentage of phylogenetic variance associated with each principal component. Clumping occurs when communities are grouped by layer.

4.2.3 Microbial Community Composition

In total, 242 classes were discovered by their sequence. Deltaproteobacteria were the dominant class across all sites (Table 3). On average, deltaproteobacteria were twice as abundant as next most common class. Betaproteobacteria and Nitrospira were the next two most abundant classes, with betaproteobacteria generally more dominant in the top and bottom layers and Nitrospira more dominant in the middle layers (Table 3). Beyond the top 3 classes, order of dominance changed among layers (Table 3). The top 25 classes make up 83.25% of the total layer community on average and each class outside the top 25 made up less than 1% of the community. The top 5 classes make up 50.11% of the total layer community on average; therefore 2% of total classes make

Table 3 – Average bacterial community composition by top 25 **Classes** in order of overall dominance. Values shown are percent of total community by site for each layer. Classes in square brackets are QIIME proposed taxonomies. K represents Kingdom with A and B representing archaea and bacteria respectively. A total of 242 classes were found.

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	**Class**	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Proteobacteria	Deltaproteobacteria	21.62	21.69	21.96	23.11	16.26	18.02	17.68	19.82	17.14	21.25	13.82	19.03
B	Proteobacteria	Betaproteobacteria	10.98	7.73	10.76	10.58	11.85	9.92	10.35	8.66	7.01	7.89	3.41	8.91
B	Nitrospirae	Nitrospira	5.76	10.54	10.84	8.84	5.44	10.67	10.95	7.72	6.40	11.88	5.66	8.61
B	Proteobacteria	Alphaproteobacteria	7.05	4.97	5.64	6.66	6.56	6.28	6.45	4.34	7.00	5.44	9.57	13.18
B	Chloroflexi	Anaerolineae	6.02	6.68	6.40	6.88	6.16	5.68	6.45	4.36	7.51	5.23	6.86	7.20
		Subtotal – top 5	51.43	51.61	55.6	56.07	46.27	50.57	51.88	44.9	45.06	51.69	39.32	56.93
B	Actinobacteria	Thermoleophilia	2.50	2.65	1.08	0.62	5.69	6.24	3.87	1.63	5.33	4.61	6.96	0.76
B	NC10	12-24*	1.87	5.23	4.83	3.66	1.46	3.99	4.52	0.43	1.37	5.93	1.70	2.18
B	Chloroflexi	S085*	0.74	2.44	3.16	4.20	0.49	1.67	3.54	2.50	0.54	2.73	6.64	5.70
B	Proteobacteria	Gammaproteobacteria	4.80	1.36	1.63	2.21	4.94	2.48	1.70	2.71	3.39	1.91	4.27	1.75
B	Gemmatimonadetes	Gemm-1	2.77	4.21	4.10	2.90	1.31	2.78	5.56	1.15	1.39	3.53	1.08	2.21
B	Acidobacteria	Acidobacteria-6	3.68	2.09	2.53	2.20	1.32	2.02	2.42	2.22	1.61	2.06	1.46	2.22
B	Planctomycetes	Phycisphaerae	1.94	1.64	1.62	1.81	1.89	1.65	1.46	4.93	2.60	1.24	1.45	1.56
B	Bacteroidetes	Bacteroidia	2.88	1.55	0.99	0.72	5.90	1.85	0.76	1.00	5.43	0.69	1.29	0.47
B	WS3	PRR-12*	2.04	1.76	1.78	1.70	1.30	1.38	2.62	0.54	1.27	1.38	0.77	1.03
B	Verrucomicrobia	[Pedosphaerae]	2.73	2.04	1.74	0.98	1.50	1.43	1.14	0.21	2.05	1.57	0.98	0.53
B	Acidobacteria	iii1-8*	1.57	1.04	0.77	0.37	1.37	2.11	0.83	0.09	2.36	2.40	1.41	0.24
B	Chlorobi	BSV26*	0.74	0.54	0.93	1.72	1.19	0.92	1.01	3.29	0.92	0.35	0.54	1.82
B	Gemmatimonadetes	Gemmatimonadetes	1.08	1.21	1.13	0.98	0.48	1.62	1.47	0.92	0.98	2.02	0.75	0.66
B	Acidobacteria	[Chloracidobacteria]	1.44	1.86	0.73	0.35	0.50	1.29	0.67	0.03	1.26	1.93	0.93	0.17
B	Actinobacteria	MB-A2-108*	0.41	0.56	0.39	0.24	1.15	1.39	1.05	0.10	0.75	1.49	2.92	0.36
B	Firmicutes	Clostridia	0.20	0.35	0.43	0.32	0.39	0.18	0.11	0.11	0.52	0.12	6.96	0.36
B	Acidobacteria	Solibacteres	1.11	1.14	0.75	0.84	0.71	0.73	0.69	0.45	1.26	0.57	0.72	0.51
A	Crenarchaeota	MCG*	0.28	0.23	0.52	0.98	0.73	0.63	0.42	3.35	0.83	0.31	1.28	1.29
B	Actinobacteria	Acidimicrobiia	0.76	0.47	0.29	0.29	1.15	1.12	0.75	0.18	1.28	0.92	1.17	0.42
B	Chloroflexi	Ellin6529	0.41	0.41	0.46	0.55	0.52	0.52	0.73	0.33	0.75	0.64	1.18	0.88
		Subtotal – top 25	85.38	84.39	85.46	83.71	80.26	86.57	87.2	71.07	80.95	88.09	83.78	82.05

*Some of the classes have not yet been given a formal name, but are recognized as unique and different from other classes.

up majority of the community. On average, archaea made up 1.93% of the microbial community in any layer. Only one archaea class was found in the top 25 classes and represented most of the archaea presence. A complete list of all 242 classes can be found in the Appendix – A.2.

Select genera were studied due to their close association with specific functional groups. *Dehalogenimonas sp.* and *Geobacter sp.* are known genera that contain dechlorinating genes. *Nitrospria sp.* are known to be nitrite oxidizers. *Geobacter sp.* was more abundant in Site 1 compared to Site 2 and 3 and was more abundant in upper layers. *Nitrospira sp.* are more abundant in the middle layers.

Table 4 – Average presence of select genera in order of class then genus. Values shown are percent of total community by site for each layer.

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
Phylum	**Class**	**Genus**	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
Actinobacteria	Actinobacteria	*Mycobacterium*	0.10	0.10	0.17	0.03	0.17	0.13	0.10	0.00	0.13	0.10	0.10	0.07
Chloroflexi	Dehalococcoidetes	*Dehalogenimonas*	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.01
Chloroflexi	Dehalococcoidetes	*Other*	0.10	0.27	0.29	0.31	0.48	0.24	0.42	0.15	0.58	0.30	0.18	0.56
Proteobacteria	Deltaproteobacteria	*Geobacter*	4.07	3.07	1.53	1.77	0.70	0.63	0.40	0.75	0.93	1.10	0.65	0.90
Nitrospirae	Nitrospira	*Nitrospira*	0.80	1.37	1.17	0.93	0.50	1.13	1.00	0.25	0.65	1.10	0.50	0.53

Several biodiversity indices were calculated by site by layer for the microbial phyla, classes and genera found (Table 5). For sites 1 & 2, all indices generally decreased with depth. For site 3, indices were highest in layer 1 and lowest in layer 2. For phyla, across all sites and layers, Simpson's index was below 0.23 indicating that all phyla were generally equally present. Shannon's index was above 2.11, indicating an even distribution in phyla and Shannon's equitability was above 0.61, indicating that the abundance of phyla was somewhat even. For classes, across all sites and layers, Simpson's index was below 0.09, indicating that all phyla were equally present. Shannon's index was above 3.13, indicating an even distribution in classes and Shannon's equitability was above 0.60 indicating that the abundance of classes was somewhat even. For genera, across all sites and layers, Simpson's index was below 0.03, indicating that all phyla were equally present. Shannon's index was above 4.11, indicating a very even distribution in genera, and Shannon's equitability was above 0.80 indicating that the abundance of classes was relatively even.

Table 5 – Biodiversity indices for each layer of each site. All calculations use taxa level indicated and are calculated by site for each layer. The summary column is the average across all sites and layers ± SD.

	Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix				
Phylum Indices	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Summary
Phylum Richness	26	28	36	33	40	37	32	39	34	27	41	40	34.4 ± 5.3
Simpson	0.0002	0.001	0.001	0.001	0.21	0.18	0.18	0.17	0.17	0.18	0.16	0.23	0.12 ± 0.09
Shannon	2.74	2.73	3.04	2.82	2.25	2.28	2.25	2.34	2.37	2.17	2.32	2.11	2.45 ± 0.30
Shannon's Equitability	0.84	0.82	0.85	0.81	0.61	0.63	0.64	0.63	0.67	0.68	0.63	0.57	0.70 ± 0.10
Class Indices													
Class Richness	200	198	189	198	213	202	188	167	211	158	182	185	190.9 ± 16.5
Simpson	0.08	0.08	0.09	0.09	0.06	0.07	0.07	0.08	0.06	0.08	0.06	0.08	0.08 ± 0.01
Shannon	3.24	3.24	3.16	3.14	3.39	3.27	3.20	3.17	3.44	3.13	3.37	3.14	3.24 ± 0.11
Shannon's Equitability	0.61	0.61	0.60	0.60	0.63	0.62	0.61	0.62	0.64	0.62	0.65	0.60	0.62 ± 0.02
Genus Indices													
Genus Richness	234	223	222	216	268	207	214	175	243	169	194	201	213.8 ± 27.8
Simpson	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.01	0.03	0.03	0.03	0.02 ± 0.01
Shannon	4.59	4.44	4.38	4.37	4.77	4.43	4.36	4.11	4.69	4.18	4.11	4.30	4.39 ± 0.21
Shannon's Equitability	0.84	0.82	0.81	0.81	0.85	0.83	0.81	0.80	0.85	0.83	0.80	0.81	0.82 ± 0.02

4.2.4 Distribution of Presumed Function

Since microbial taxonomy has attempted to classify bacteria and archaea by their functional features, it is possible to assign the bacteria found in this study to putative functional categories. Through literature review (Appendix 1), bacterial classes have been assigned to several functional groups (Table 6). While many classes and species may be capable of functions not yet described for them, these estimates represent the most likely *potential* microbial function. Across all sites, aerobic bacteria tended to decrease with depth. Presence of nitrogen fixing bacteria initially decreased with depth but then increased through layers 3 and 4. Ammonia-oxidizing bacteria were present at all depths but abundance varied. The highest concentration of nitrite oxidizers was found at site 2 and 3 with presence varying by depth. Denitrifying bacteria were present at all depths but abundance varied. Dechlorinating bacteria presence initially decreased with depth but then increased through layers 3 and 4. Across all sites and layers, on average, site 1 had the highest presence of dechlorinators and nitrite oxidizers and the lowest presence of methane oxidizers and nitrogen fixers. Site 2 had the highest presence of ammonia oxidizers and methane oxidizers and the lowest presence of nitrite oxidizers. Site 3 had the lowest presence of ammonia oxidizers and dechlorinators. Denitrifiers were evenly distributed across all the sites.

Table 6 – Potential microbial functional groups as average percent of community by site for each layer. Column totals >100% indicate a microbe can have more than one functional group. Functional groups were assigned by class. "Facultative" aerobes and anaerobes were included in both groups.

	Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
Functional Group	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
Aerobic	69.73	65.61	61.69	57.93	65.40	67.48	62.58	50.37	68.81	69.33	61.66	57.15
Anaerobic	62.38	56.78	60.48	63.36	58.76	55.27	58.73	54.72	56.78	55.80	54.63	65.05
Ammonia Oxidizing	16.38	9.84	13.13	13.21	17.19	12.84	12.13	11.43	11.29	10.11	8.03	10.82
Dechlorinating	38.18	31.72	35.52	37.21	35.14	31.50	30.58	33.36	29.46	31.66	29.75	31.55
DeNitrifying	24.45	23.95	27.81	26.31	24.47	27.39	28.32	20.84	21.28	26.02	19.83	30.98
Methane Oxidizing	22.82	14.06	18.03	19.45	23.35	18.68	18.49	15.71	17.40	15.24	17.24	23.84
Methanogens	0.34	0.40	0.15	0.13	0.46	0.09	0.07	0.13	0.66	0.11	0.61	0.10
Nitrogen Fixing	19.98	14.40	18.43	20.08	21.03	18.16	18.42	19.85	16.54	14.34	20.93	25.04
Nitrite Oxidizers	39.23	38.56	40.07	40.82	33.20	37.45	36.78	34.60	33.93	40.48	33.32	42.58

Denitrifiers, nitrogen fixers, and nitrifiers (ammonia oxidizers and nitrite oxidizers) are all part of the nitrogen cycle. Small differences exist between site and layer, but were all within 1 standard deviation indicating that the difference may not be significant. Averages of all sites by layer can be seen in Figure 5. The presence of nitrifiers was significantly higher than that of nitrogen fixers and denitrifiers across all layers.

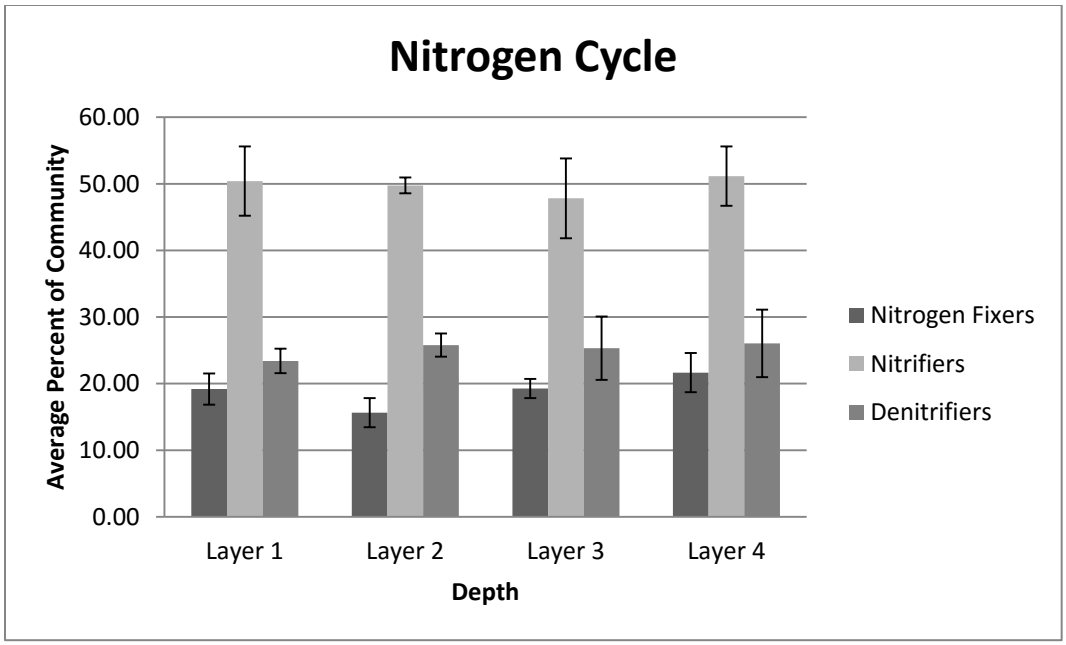

Figure 5. Average percent of community across all sites by layer for nitrogen fixers, nitrifiers (ammonia oxidizers and nitrite oxidizers), and denitrifiers. Error bars represent one standard deviation.

4.3 Plant Community

4.3.1 Surface Plant Community

Surface site surveys provided plant community composition (Table 7). Site 1 was covered by approximately 80% *C. stricta*, 10% *Typha sp.*, and 10% other. Eleven species were found at site 1. Site 2 was covered by approximately 90% *E. erythropoda* and 10% other. Six species were found at site 2. Site 3 was covered by approximately 40% *C. stricta*, 40% *E. erythropoda*, and 20% other. Fourteen species were found at site 3. In total, 22 species were found by surface survey.

Table 7 – Surface plant community composition in order of scientific name. An x under site indicates presence at site. Common names provided by the USDA Plants Database. (PLANTS)

Common Name	Scientific Name	Site 1	Site 2	Site 3
American Water Plantain	*Alisma subcordatum* Raf.			x
Indianhemp (Dogbane)	*Apocynum cannabinum* L.	x		
Nodding Beggartick	*Bidens cernua* L.		x	x
Longhair Sedge	*Carex comosa* Boott			x
Bottlebrush Sedge	*Carex hystericina* Muhl. Ex Willd.		x	x
Upright Sedge	*Carex stricta* Lam.	x		x
Swamp Thistle	*Cirsium muticum* Michx.	x		
Bald Spikerush	*Eleocharis erythropoda* Steud.		x	x
Common Boneset	*Eupatorium perfoliatum* L.	x		x
Common Marsh Bedstraw	*Galium palustre* L.			x
Manyflower Marshpennywort	*Hydrocotyle umbellata* L.		x	
Jewelweed	*Impatiens capensis* Meerb.	x		
Watercress	*Nasturtium officinale* W.T. Aiton		x	
Virginia Creeper	*Parthenocissus quinquefolia* (L.) Planch.	x		
Canadian Clearweed	*Pilea pumila* (L.) A. Gray			x
Swamp Rose	*Rosa palustris* Marshall	x		x
Dock	*Rumex sp.* L.			x
Sandbar Willow	*Salix interior* Rowlee			x
Green Bullrush	*Scirpus atrovirens* Willd.	x		
Wrinkleleaf Goldenrod	*Solidago rugosa* Mill.	x		
Aster	*Symphotrichum sp.* Nees	x		x
Cattail	*Typha sp.*	x	x	x

4.3.2 Sub-surface plant community

DNA sequencing of roots provided the sub-surface plant community composition (Table 8). Seven species were found at Site 1, 13 at site 2, and 17 at site 3. Site 1 is dominated by *Salix interior*. While there was no surface indication of *S. interior* inside the sampling plot, there was a stand about 2 meters outside the sample plot. This indicates the roots are spreading horizontally by at least 5m to be detected in the site. The next most dominant plant in site 1 was *C. stricta*, which was the most dominant by surface community estimation (Table 8). Site 2 was dominated on the surface by *E. erythropoda.* While *E. erythropoda* was consistently a large portion of the plant community by layer, the dominant species changed with depth. *Coreopsis sp*. dominated layer 1, *Nasturtium officinale* dominated layer 2 and *Geum canadense* dominated layer 3

(Table 8). Site 3 was dominated by *E. erythropoda*, except in layer 2 where *S. interior* dominated (Table 8). Twenty-five plants were discovered through DNA analysis with 5 of the 25 plants being annuals. On average, annuals made up 6.56 % (± 10.15) of the plant community and perennials made up 90.75% (± 11.53) of the plant community. A total of thirty plant species were seen by combining the surface visual survey, and DNA analysis of the subsurface.

Table 8 – Sub-surface plant community composition as, determined by DNA sequencing, in order of species name. Values shown are **percent of total plant community** by site by layer. P/A represents perennial or annual respectively. DNA from layer 4 of site 2 failed to amplify and therefore had no data.

		Site 1 – *C. stricta*				Site 2 – *E. erythropoda*			Site 3 – 50/50 Mix			
P/A	Plant species	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 1	Layer 2	Layer 3	Layer 4
P	*Alisma subcordatum* Raf.	0	0	0	0	0	0	0	0.10	0	0	0
P	*Allium cepa* L.	0	0	0	0	0	0	0	0.10	0	0	0
A	*Bidens cernua* L.	0	0	0	0	10.73	0	0.17	0.25	0	0	0.50
P	*Carex hystericina* Muhl. Ex Willd.	0	0	0	0	0	0.40	11.53	0.45	0.20	0.90	0
P	***Carex stricta*** Lam.	14.33	24.07	33.53	21.40	1.27	0.07	0.40	8.00	7.90	0.70	0.40
P	*Coreopsis sp.*	0	0	0	0	46.50	0.07	0.63	1.95	0	0	1.90
P	***Eleocharis erythropoda*** Steud.	0	0	0	0	40.33	36.43	23.07	79.90	22.30	39.10	66.40
P	*Eleocharis obtusa* (Willd.) Schult.	0	0	0	0	0	0.07	0.07	0.10	0.20	0	0
P	*Eupatorium perfoliatum* L.	13.73	0	0	0	0	0	0	0	1.00	0	0
P	*Geum canadense* Jacq.	0	0	0	0	0	0.03	29.97	0	0	0	0
A	*Helianthus annuus* L.	0	0	0	13.53	0	0	0	0	0.20	0	0
A	*Helianthus sp.*	0	0	0	2.37	0	0	0	0	0	0	0
P	*Hydrocotyle umbellata* L.	0	0	0	0	0.10	0.03	0	0	0	0	0
A	*Impatiens capensis* Meerb.	0	0	0	0	0	0	0	0	0.70	0	0
P	*Nasturtium officinale*	0	0	0	0	0.03	60.87	28.50	0	0	0	0
A	*Pilea pumila* (L.) A. Gray	0	0	0	0	0	0	0	0	13.00	30.70	0
P	*Polygonum sp.*	0.03	0	0	0	0	0	0	0	0	0	0
P	*Potentilla norvegica* L.	0	0.27	0	0	0	0	0	0	0	0	0
P	*Rosa palustris* Marshall	0	0	0	0	0	0	0	0	0.30	0	0
P	*Salix amygdaloides* Andersson	0	0	0	0	0	0.03	0	0	0	0	0
P	*Salix interior* Rowlee	70.40	75.57	65.40	61.23	0	1.50	2.60	2.45	37.70	21.30	30.40
P	*Scirpus atrovirens* Willd.	0	0	0	0	0	0.03	0	0	0.20	0	0
P	*Symphyotrichum boreale* (Torr. & A. Gray) Á. Löve & D. Löve	0.03	0	0	0	0	0	0	0.30	2.30	6.30	0.20
P	*Symphyotrichum sp.*	0	0	0	0	0	0	0	0	0.10	0	0
P	*Typha sp.*	0	0	0	0.07	0	0.03	0.47	0	0	0	0
	Other	1.40	0.13	1.07	1.37	1.00	0.47	2.53	6.50	13.80	0.90	0.20

In soil, Chao1 estimated 32 plant species found among the three sites. On average, site 1 had less plant species diversity than sites 2 and 3 (Table 9). Overall averages show a higher plant species diversity in upper layers and lower diversity in lower layers; starting at 10.35 and decreasing to 8.50 (Table 9).

Table 9 – Chao1 estimates for total plant species. DNA from layer 4 of site 2 failed to amplify and therefore had no data.

	Site			
Depth	Site 1 *C. stricta*	Site 2 *E. erythropoda*	Site 3 50/50 Mix	Avg (st. dev.)
Layer 1	5.67	13.33	12.04	10.35 ± 4.10
Layer 2	4.66	18.00	13.00	11.89 ± 6.74
Layer 3	3.00	12.67	7.00	7.56 ± 4.86
Layer 4	10.00	-	7.00	8.50 ± 2.12

Beta diversity through weighted UniFrac PCoA shows clumping of samples when categorized by site along PC1 and PC2 (Figure 6). Site 1 and site 2 were distinctly different groups while site 3 crossed the gap between the two, which is in agreement with surface surveys. In this analysis, PC1 accounted for 43.3% of the variation and PC2 accounted for 24.4% of the variation.

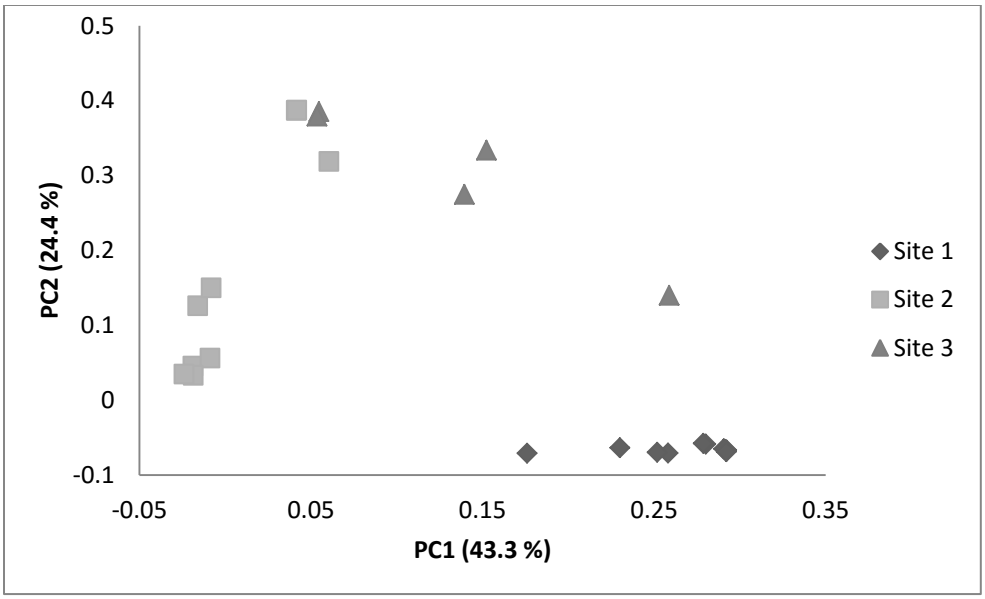

Figure 6. Sub-surface plant community analysis through weighted UniFrac principal coordinate analysis. Numbers in parenthesis are the percentage of phylogenetic variance associated with each principal coordinate. Clumping occurs when grouped by site.

4.4 Multivariate analysis of bacterial community structure and function

All soil parameters had a significant ($p < 0.05$) correlation to bacterial community structure (Table 10). Soil moisture had a strong ($p < 0.10$) correlation with the presence of dechlorinating classes. Soil moisture and organic content had significant (both $p < 0.05$) correlation to presence of ammonia oxidizing classes (Table 10). Abundance of ammonia oxidizing bacteria decreased with both soil moisture and organic content. Soil moisture, organic content, and root density all had a significant correlation with the presence of methane oxidizing classes (all $p < 0.05$). Methane oxidizers initially decreased with depth, along with soil moisture organic content and root density (Table 1), then increased in layer 4 along with root density. All soil measures ($p < 0.05$) had a significant correlation to methanogens.

Table 10 – Analysis of soil parameters using F model of Adonis (Vegan) function, an analog to MANOVA (multivariate analysis of variance). Values shown are p-values. Only plant species with at least one $p < 0.10$ are shown. Overall represents the complete community structure rather than a specific functional group.

	Functional Group							
Metric	Overall	Dechlorinating	Denitrifying	Nitrogen Fixing	Ammonia Oxidizing	Nitrite Oxidizing	Methane Oxidizing	Methane Generating
Depth	0.015**	0.395	0.493	0.973	0.177	0.591	0.238	0.186
Soil Moisture	0.019**	0.083*	0.74	0.386	0.027**	0.53	0.034**	0.214
Organic Content	0.039**	0.103	0.833	0.42	0.028**	0.623	0.05**	0.235
Root Density	0.013**	0.18	0.453	0.302	0.66	0.342	0.026**	0.104
Bidens cernua	0.15	0.55	0.82	0.31	0.16	0.008**	0.129	0.584
Carex hystericina	0.889	0.526	0.379	0.76	0.931	0.879	0.879	0.062*
Carex stricta	0.10*	0.22	0.58	0.36	0.97	0.084*	0.47	0.623
Coreopsis sp.	0.14	0.63	0.83	0.36	0.20	0.017**	0.127	0.613
Eleocharis erythropoda	0.04**	0.078*	0.22	0.86	0.65	0.02**	0.856	0.341
Eleocharis obtusa	0.72	0.25	0.76	0.08*	0.54	0.49	0.301	0.223
Eupatorium perfoliatum	0.24	0.065*	0.91	0.53	0.27	0.53	0.315	0.958
Geum canadense	0.824	0.586	0.252	0.87	0.933	0.939	0.802	0.001**
Hydrocotyle umbellata	0.24	0.46	0.83	0.27	0.063*	0.21	0.089*	0.89
Nasturtium officinale	0.876	0.585	0.14	0.873	0.715	0.929	0.794	0.022**
Pilea pumila	0.092*	0.35	0.069*	0.73	0.044**	0.57	0.445	0.689
Polygonium sp.	0.22	0.001**	0.79	0.39	0.19	0.56	0.336	0.999
Salix interior	0.12	0.17	0.84	0.44	0.87	0.043**	0.483	0.529
Scirpus atrovirens	0.66	0.82	0.61	0.047**	0.61	0.22	0.41	0.25
Symphyotrichum boreale	0.061*	0.25	0.078*	0.60	0.033**	0.49	0.455	0.647
Typha sp.	0.814	0.603	0.177	0.798	0.934	0.961	0.789	0.001**

* $p \leq 0.1$
** $p \leq 0.05$

Some plants are shown to have an influence on the presence of specific microbial functional groups (Table 10). Changes in presence of *C. stricta* ($p < 0.10$), *E. erythropoda* ($p < 0.05$), *P. pumila* ($p < 0.10$), and *Symphyotrichum boreale* ($p < 0.10$) had a strong correlation with the overall microbial community structure (Table 10). Changes in presence of *E. erythropoda* ($p < 0.10$), *E. perfoliatum* ($p < 0.10$), and *Polygonium* sp. ($p < 0.05$) had a strong correlation with the abundance of dechlorinators; as *E. erythropoda* presence increased (Table 8), the abundance of dechlorinating bacteria decreased (Table 6), and as *E. perfoliatum* and *Polygonium* sp. presence increased, the abundance of dechlorinating bacteria increased. Dechlorinating bacteria occurred most frequently where *E. perfoliatum* and *Polygonium* sp. were most abundant. Changes in presence of *P. pumila* and *S. boreale* had a strong (both $p < 0.10$) correlation with the

presence of denitrifying classes; the highest presence of *P. pumila* and *S. boreale* (Table 8) was associated with the lowest abundance of denitrifying bacteria (Table 6). Changes in presence of *E. obtusa* ($p < 0.05$) and *S. atrovirens* ($p < 0.05$) had a significant correlation with the presence of nitrogen fixing classes; as *E. obtusa* and *S. atrovirens* presence increased (Table 8), the abundance of nitrogen-fixing bacteria decreased (Table 6). Nitrogen-fixing bacteria occurred the least where *E. obtusa* and *S. atrovirens* occurred together. Changes in presence of *H. umbellate* ($p < 0.10$), *P. pumila* ($p < .05$), and *S. boreale* ($p < 0.05$) had a strong correlation with the presence of ammonia oxidizing classes; as *P. pumila* and *S. boreale* presence increased (Table 6), the abundance of ammonia oxidizing bacteria decreased (Table 8), and as *H. umbellata* presence increased, the abundance of ammonia oxidizing bacteria increased. The highest presence of *H. umbellata* was associated with the highest abundance of ammonia oxidizing bacteria. The highest presence of *P. pumila* and *S. boreale* were associated with the lowest abundance of ammonia oxidizing bacteria. Changes in presence of *H. umbellata* ($p < 0.10$) had a strong correlation with the presence of methane oxidizers; the highest presence of *H. umbellata* was associated with the highest abundance of methane oxidizers. Changes in presence of *B. cernua* ($p < 0.05$), *C. stricta* ($p < 0.10$), *Coreopsis sp.* ($p < 0.05$), *E. erythropoda* ($p < 0.05$), and *S. interior* ($p < 0.05$) had a significant correlation with the presence of nitrite oxidizers; as *B. cernua*, *Coreopsis* sp., *E. erythropoda*, and *S. interior* presence increased (Table 8), the abundance of nitrite oxidizers decreased (Table 6). The highest presence of *B. cernua* and *Coreopsis sp.* was associated with the lowest abundance of nitrite oxidizers bacteria. In site 1, as the presence of *C. stricta* increased (Table 8), the abundance of nitrite oxidizers bacteria increased (Table 6).

4.5 Regression Analysis

Regression models were created for each functional group and diversity measure based on what were found to be significant factors through MANOVA (Table 10) to further determine strength and type of relationship. The multiple regression model used layer, organic content, soil moisture, and root mass as independent variables to estimate potential species richness (Figure 7). The Chao1 based model accounted for 54% of overall variation in bacterial species richness and was a significant ($p<0.05$) predictor for estimates of species richness. While not a good model, it can still be used to infer type of relationship. Layer had a strong negative relationship with species richness. Organic content and soil moisture had a positive relationship with species richness.

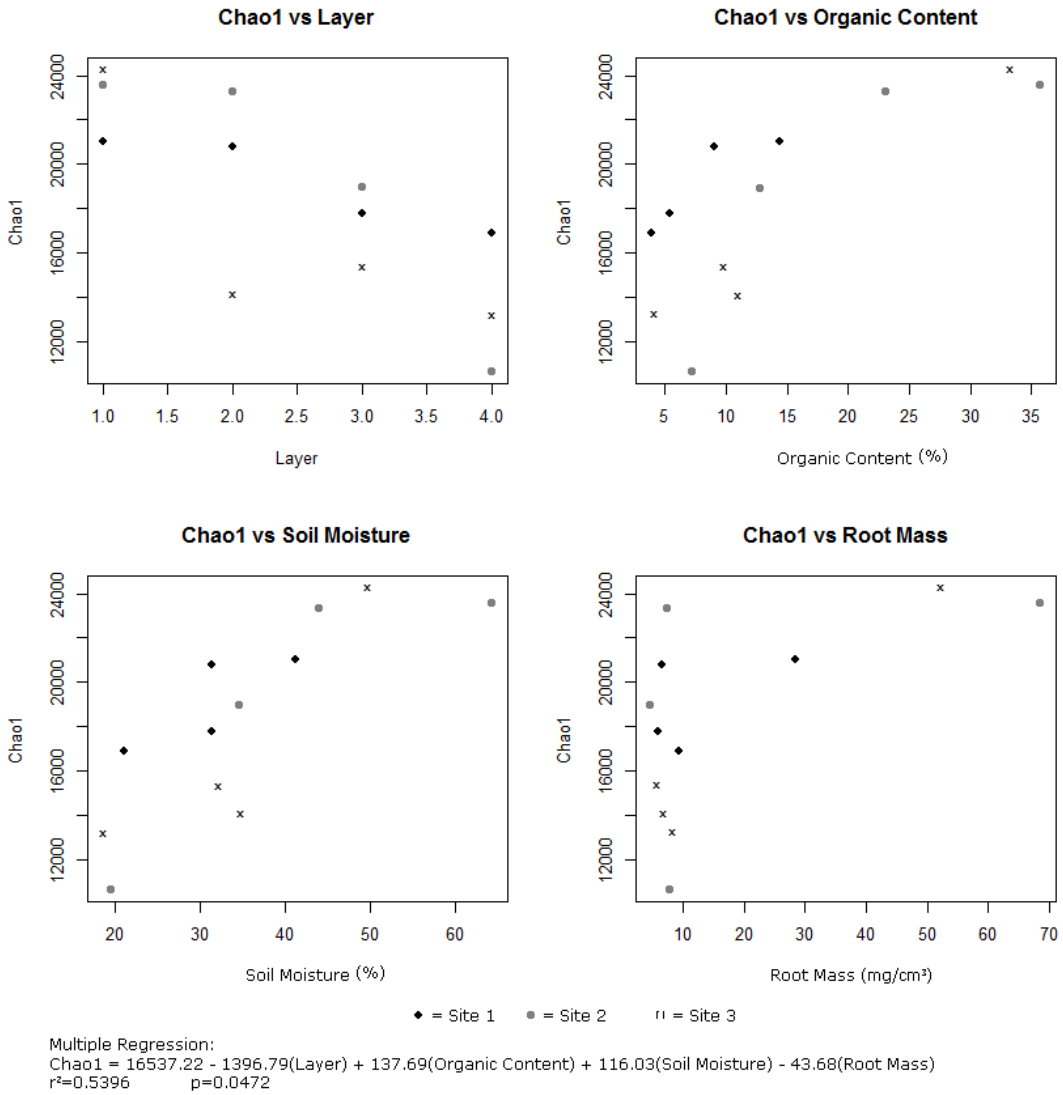

Figure 7. Scatter plots for individual variables used in multiple regression to predict Chao1 estimates. The multiple regression model accounted for 53.96% of variation seen in and was a significant ($p < 0.05$) predictor of Chao1 estimates. Depth and root mass had a negative impact on Chao1 species estimates while organic content and soil moisture had a positive impact.

The multiple regression model used organic content, *C. stricta* presence, and *E. erythropoda* presence as independent variables to estimate Simpson's index of classes (Figure 8). The model accounted for 50% of overall variation in Simpson's index and was a significant ($p<0.05$) predictor for Simpson's index of classes. While not a good model, it can still be used to infer type of relationship. Organic content and *E. erythropoda*

presence had a negative relationship with Simpson's index. *C. stricta* presence had a positive relationship with Simpson's index.

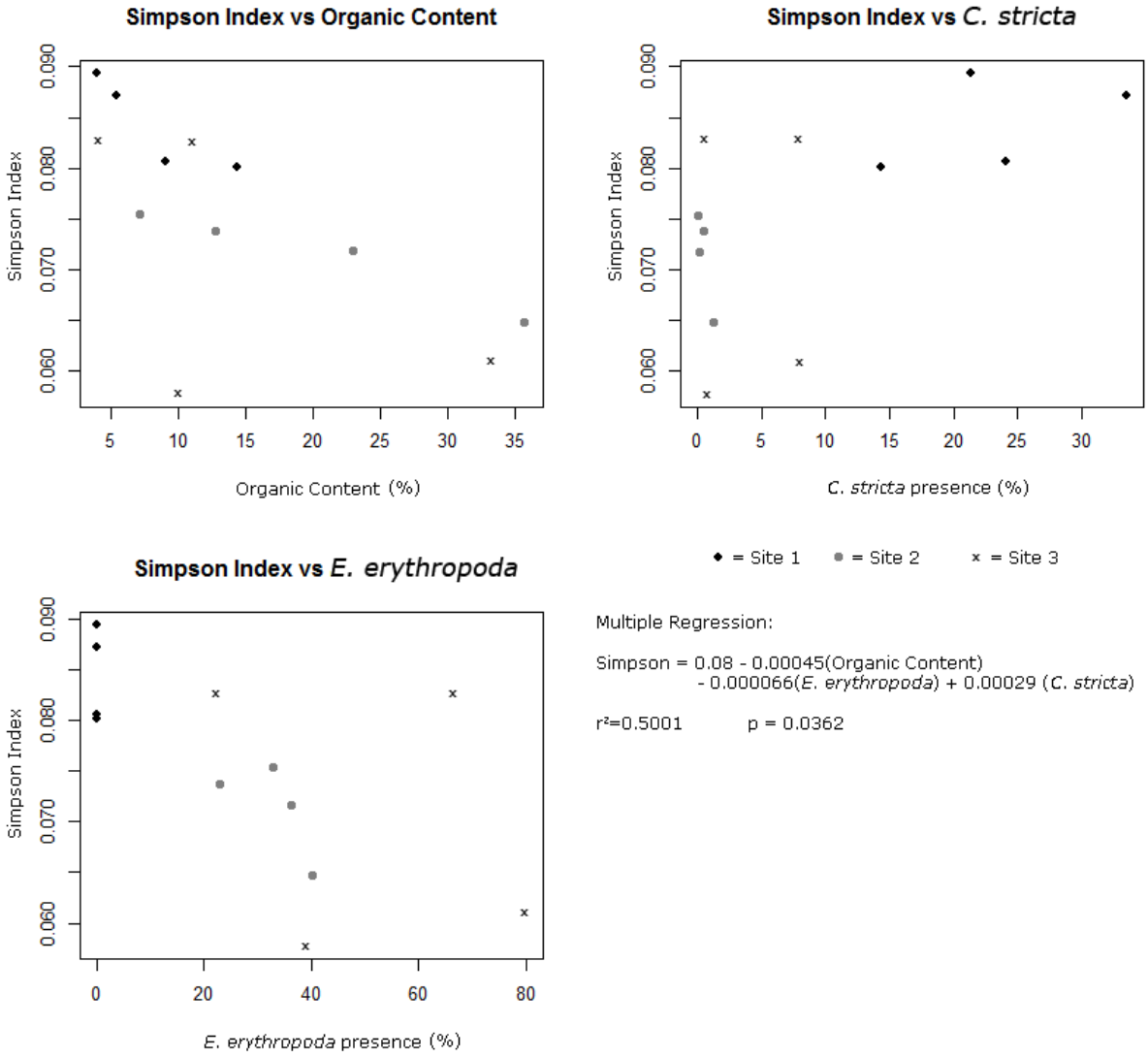

Figure 8. Scatter plots for individual variables used in multiple regression to predict Simpson's index for classes. The multiple regression model accounts for 50.01% of variation seen in and was significant ($p < 0.05$) predictor of Simpson's indices. Organic content and *E. erythropoda* presence had a negative impact on Simpson's index while *C. stricta* presence had a positive impact.

The multiple regression model used organic content, soil moisture, and root mass as independent variables to estimate Shannon's index for classes (Figure 9). The model accounts for 52% of variation in Shannon's index and was a significant ($p<0.05$) predictor

for Shannon's index for classes. While not a good model, it can still be used to infer type of relationship. Soil moisture had a negative relationship with Shannon's index. Organic content and root mass had a positive relationship with Shannon's index.

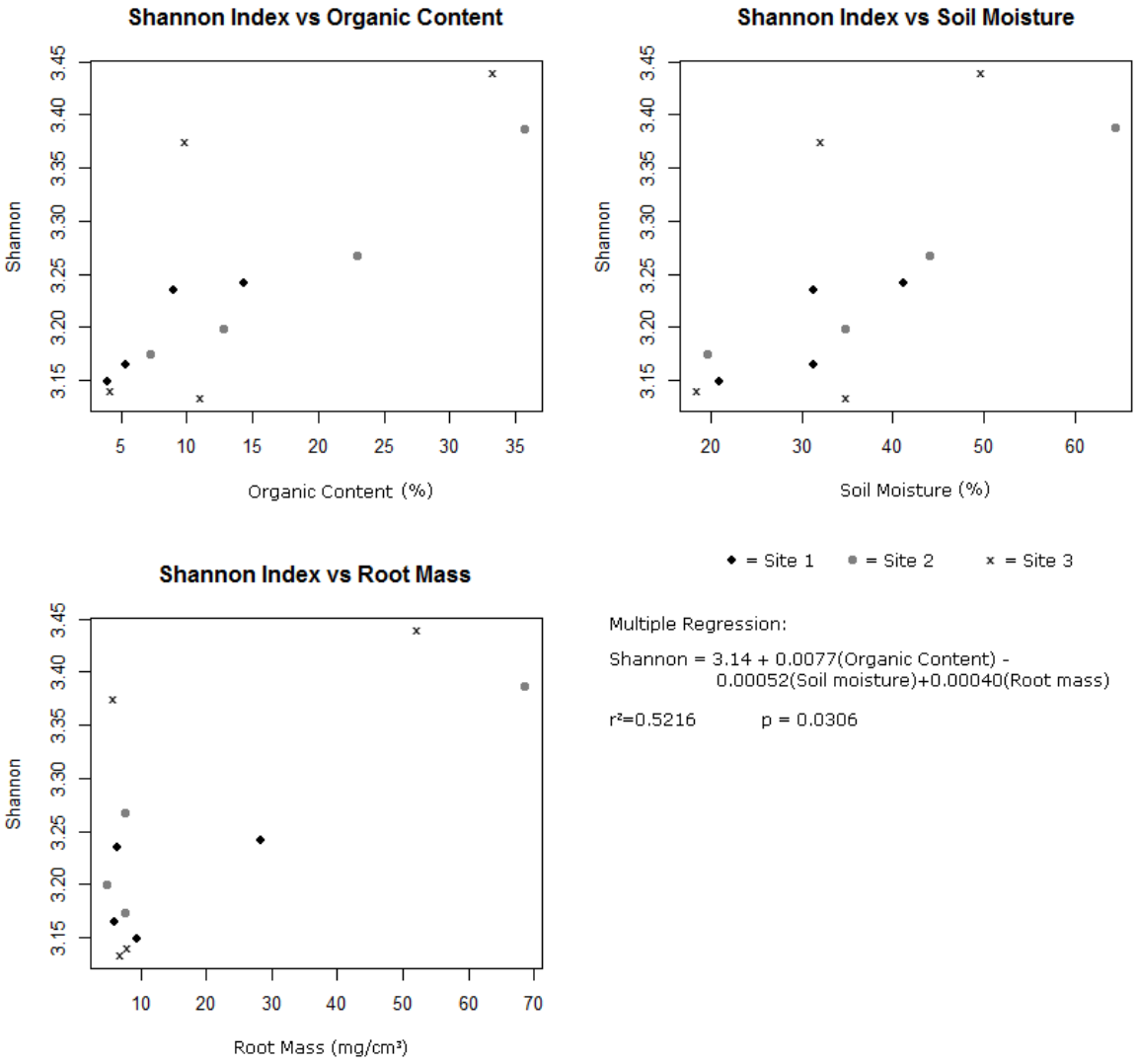

Figure 9. Scatter plots for individual variables used in multiple regression to predict Shannon's diversity index for classes. The multiple regression model accounted for 50.01% of variation seen in and was significant ($p < 0.05$) predictor of Shannon's indices. Organic content and *E. erythropoda* presence had a negative impact on Shannon's index while *C. stricta* presence had a positive impact.

The multiple regression model used site, soil moisture, and *E. erythropoda* presence as independent variables for the estimate of presence of dechlorinators (Figure 10). The

model accounts for 39% of variation and was a strong ($p < 0.10$) predictor for presence of dechlorinators. While a bad model, it can still be used to infer type of relationship. Site and presence had a negative relationship with dechlorinator presence. Soil moisture had a positive relationship with dechlorinator presence.

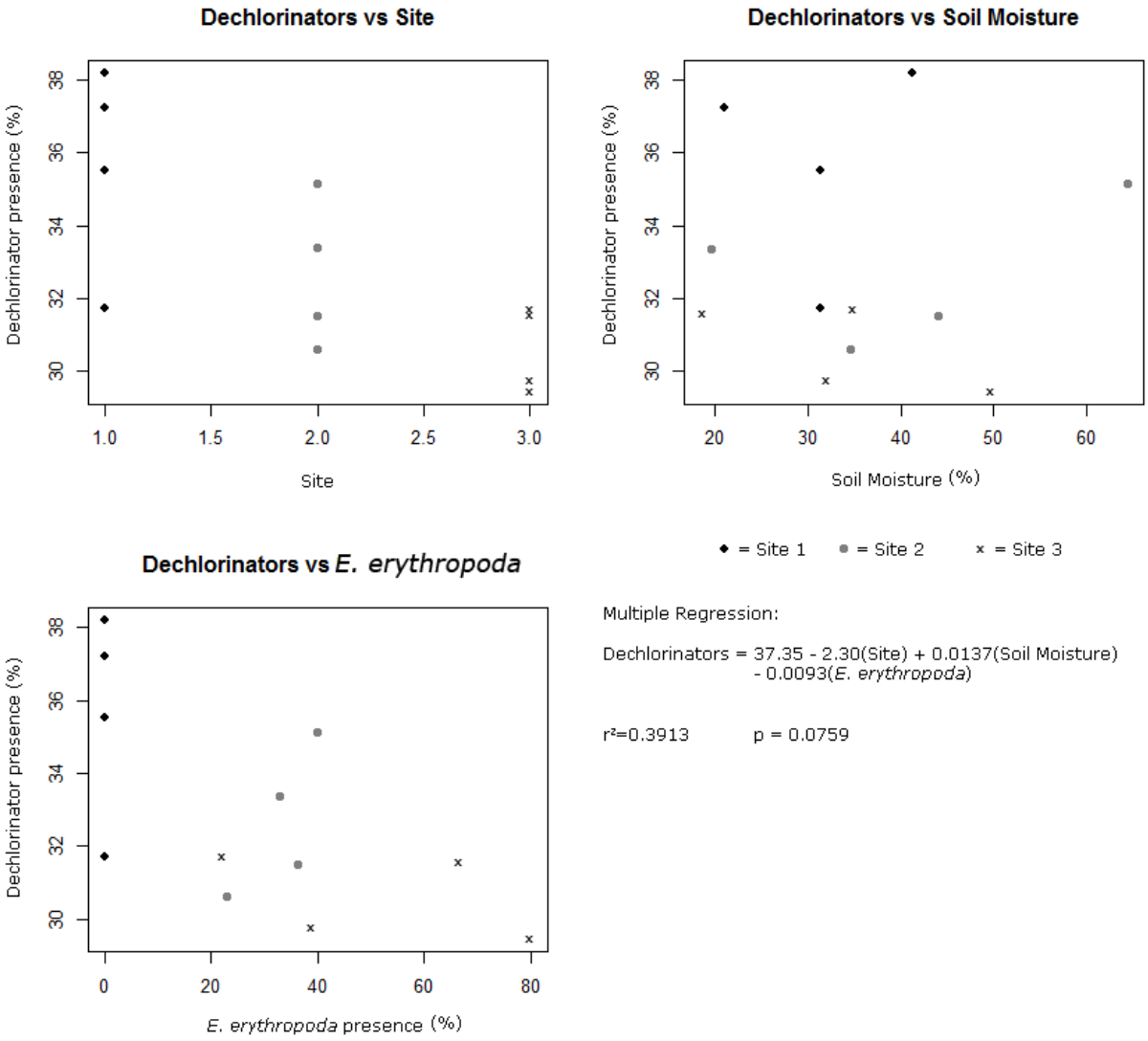

Figure 10. Scatter plots for individual variables used in multiple regression to predict the presence of dechlorinators. The multiple regression model accounts for 39.13% of variation seen in and was strong ($p < 0.10$) predictor of dechlorinator presence. Site and *E. erythropoda* presence had a negative impact on dechlorinator presence while soil moisture had a positive impact.

The multiple regression model used *B. cernua*, *Coreopsis sp.*, *E. erythropoda*, and *S. interior* presence as independent variables to estimate the presence of nitrite oxidizer presence (Figure 11). The model accounted for 47% of variation in nitrite oxidizers and was a strong ($p<0.10$) predictor for presence of nitrifiers. While not a good model, it can still be used to infer type of relationship. *E. erythropoda*, and *S. interior* presence had a positive relationship with nitrite oxidizer presence. *B. cernua* and *Coreopsis sp.* presence had a negative relationship with nitrite oxidizer presence. However, the significance of *B. cernua* and *Coreopsis sp.* presence is due to one outlying point and could be consider erroneous (Figure 11)..

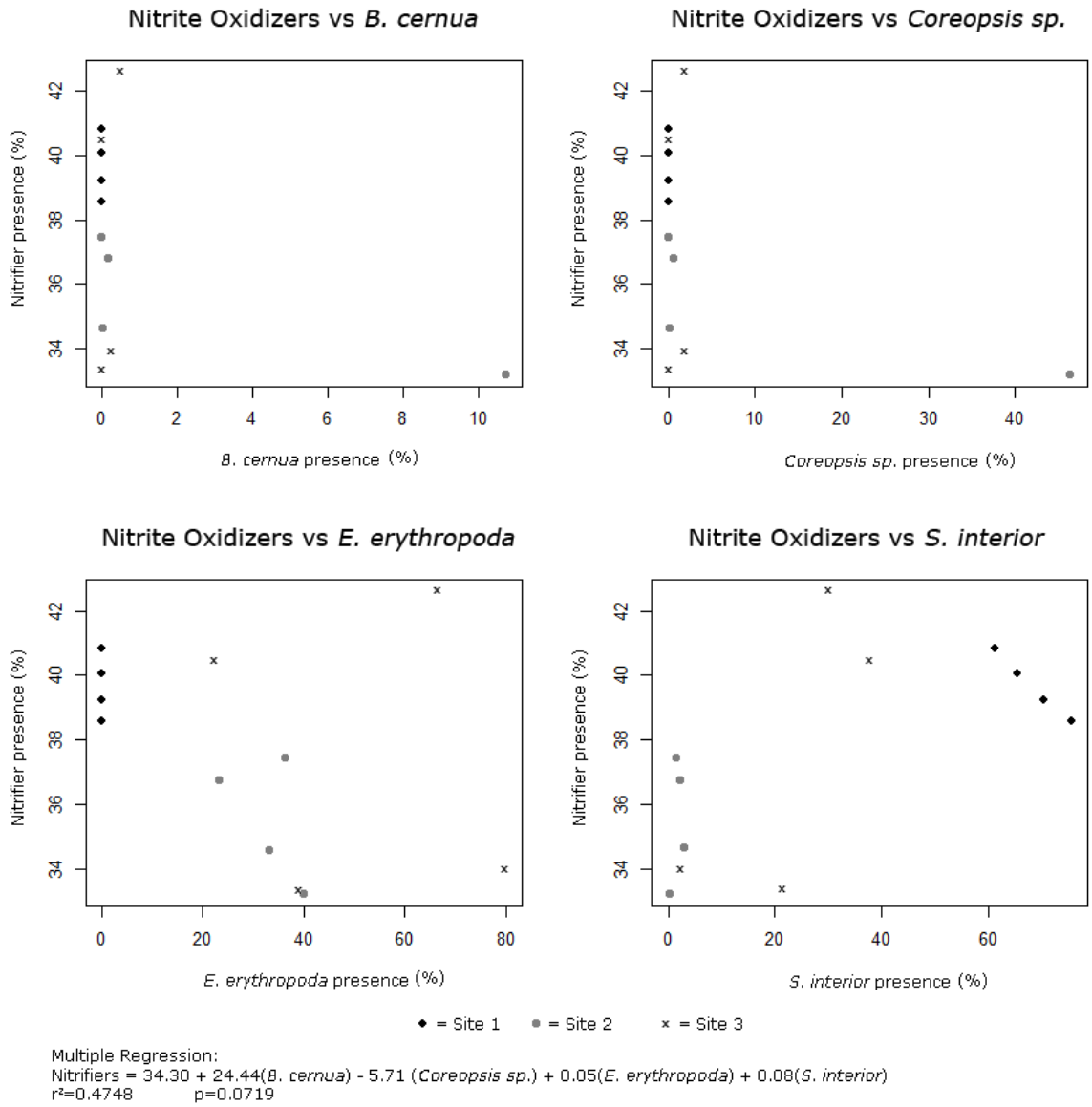

Figure 11. Scatter plots for individual variables used in multiple regression to predict the presence of nitrite oxidizers. The multiple regression model accounted for 47.48% of variation seen in and is a significant ($p < 0.10$) predictor of nitrite oxidizer presence. *Coreopsis sp.* presence had a negative impact on nitrifier presence while *E. erythropoda* and *S. interior* presence had a positive impact.

The multiple regression model used organic content, soil moisture, and *S. boreale* presence as independent variables to estimate the presence of ammonia oxidizer presence (Figure 11). The model accounted for 55% of variation in ammonia oxidizers and was a significant ($p<0.05$) predictor for presence of ammonia oxidizers. While not a good

model, it can still be used to infer type of relationship. Organic content and *S. boreale* had a negative relationship with ammonia oxidizer presence. Soil moisture had a positive relationship with ammonia oxidizer presence.

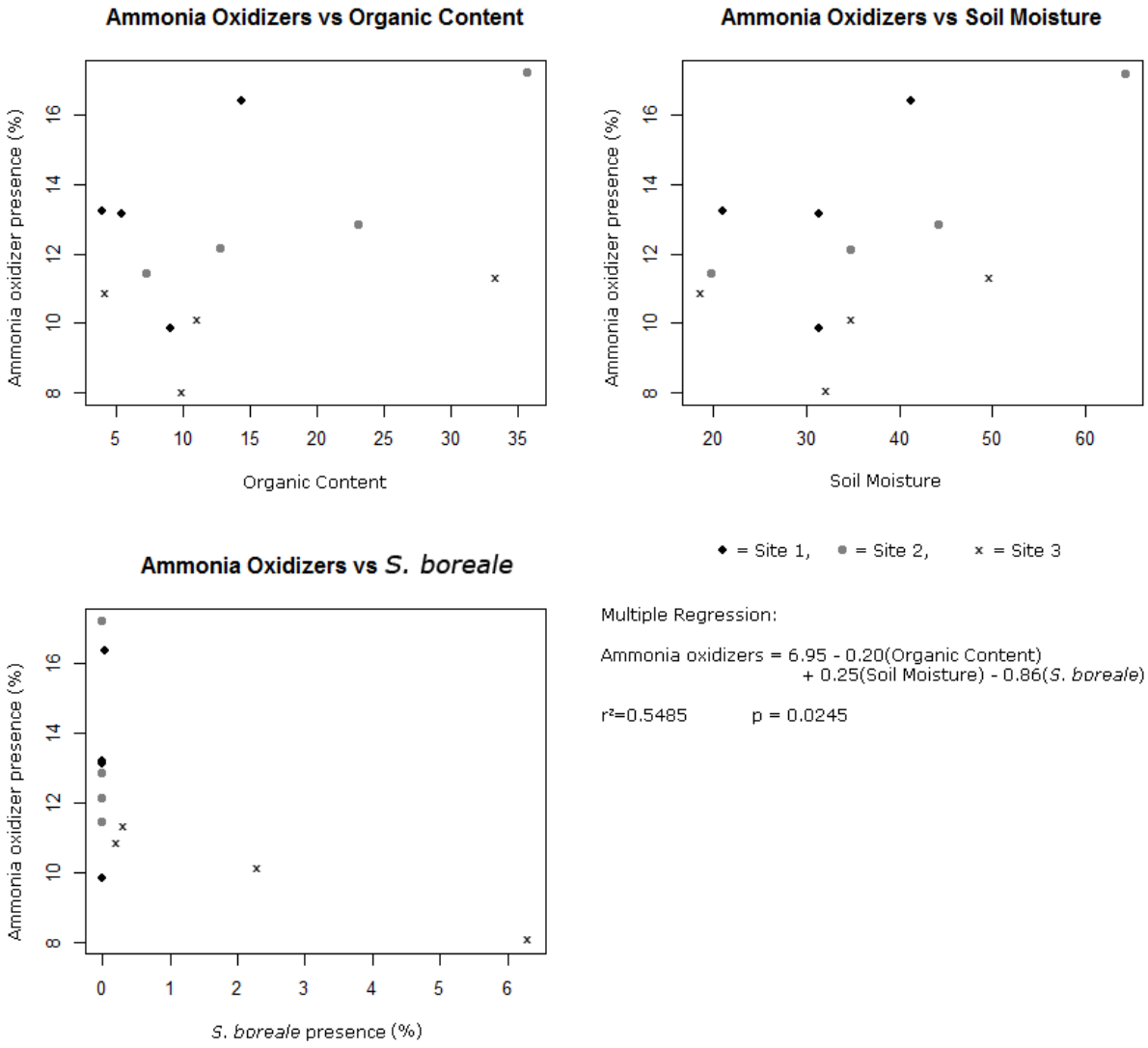

Figure 12. Scatter plots for individual variables used in multiple regression to predict the presence of ammonia oxidizers. The multiple regression model accounted for 54.85% of variation seen in and was a significant ($p < 0.05$) predictor of ammonia oxidizer presence. *S. boreale* presence and organic content had a negative impact on ammonia oxidizer presence while soil moisture had a positive impact.

Linear multiple regression failed to create significant models for the presence of denitrifiers, nitrogen fixers, methane oxidizers, and methanogens. Their relationships may be non-linear or require more data points to understand, both of which are beyond the scope of this study.

5. Discussion

The results of this study provides a new look at fens. Much of the information about how fens differ from other wetlands is summarized in an article by Amon and others (2002). The major feature that separates fens from all other wetlands is that they are driven by water that starts as groundwater, often deep below the surface, and moves through the soil and roots emerging at the surface. In most wetlands recognized as fens this is a continuous, year-round process. The water, constantly on the move, brings with it minerals, including nutrients, dissolved gases and the chemical species that are created by the plants, animals and microbes that live in the peat soil. Because the water comes from far below the surface it tends to be constant in temperature. Because the groundwater has flowed through the inorganic gravel or hydrologically conductive rock, the aquifer contains dissolved inorganic species characteristic of the parent material. In many fens, like the one in this report, the underlying is glacially derived and primarily limestone. The calcium and magnesium rich carbonates from limestone, once dissolved, create a carbonate buffer system that tends to keep the water in both the aquifer and the peat at a pH of near 7. Because the water flowing through the system often has a long flow-path its residence time underground has depleted nitrogen and phosphorous making it a poor source of these important nutrients and is generally not supportive of plants that grow rapidly. Plants at the surface may grow slowly, and leaves, stems and roots of these primarily perennial species tend to decay slowly, accumulating peat. In most fens, sedges make up a significant portion of the plant community. Because of the mineral rich content of the groundwater, these fens often accumulate calcium carbonate precipitates (marl) formed as the chemical conditions change when water approaches the surface.

Some fen plants are tolerant of or may need this unusual component of the soil and are found in “calcareous” fens and rarely elsewhere. All of these combined factors make fens a unique, but often species rich, habitat. This study investigates the relationship of the microbes present in fen soil and how they interact with the plants that make up the surface community. The following discussion is dependent on the above characteristics.

Biomass of roots, water content, and organic matter content were studied at each depth for correlation with individual plant root species and microbial taxa distribution. All soil parameters tested showed a strong influence on overall microbial community structure ($p < 0.10$, Table 10). Soil moisture, organic content and root density strongly influenced some of the selected microbial functional groups. Root mass generally decreased with depth, but increased in the bottom layer (Table 1). Nutrient-containing ground-water can travel through the gravel layer until it flows up and out the wetland. Plants may be stimulated by this source of nutrients and, once reached, grow more roots in this zone to uptake more nutrients before other organisms can. This pattern had also been seen in a sub-surface flow constructed wetland (Amon *et al.* 2007). Within the functional groups studied, root density had a significant ($p < 0.05$, Table 10) relationship with methane oxidizers. At sites 1 and 3, methane oxidizer presence followed the same non-linear pattern of first decreasing with depth then increasing in the final layer. This may indicate that not only are methane oxidizers present, but that they have an active relationship with plants found at each depth.

Arroyo *et al.* (2015) reported that organic content had a significant influence on bacterial community structure in natural and constructed wetlands. Their study did not include soil moisture; however, soil moisture and organic content are closely related

concepts as it is the organic content that retains the most water. Within the functional groups studied, organic content and soil moisture had a significant ($p < 0.05$, Table 10) relationship with ammonia oxidizers and methane oxidizers. Decomposing organic matter can be a source of ammonia and methane (Babson *et al.* 2013) for their respective oxidizers, and the soil moisture could be the means for products of degradation to flow from one microbe to another. Ammonia oxidizers and methane oxidizers occurred most frequently in the surface layer and the deepest layer. Both layers could have sufficient access to oxygen required for oxidation to occur. The surface layer has access to oxygen from the air and the deepest layer first has access to dissolved oxygen carried in groundwater. Ground water can have around 4 mg/L of dissolved oxygen (Suthersan 1996), which is high enough to support aerobic respiration (Stolper *et. al* 2010). This could create a more aerobic top and bottom layer and more anaerobic middle. Oxygen loss from roots could also provide the oxygen required for oxidation to occur (Colmer 2003; Smith *et al.* 2013).

In order to quantify and identify the presence of plants below the wetland surface, the ITS-2 region of their DNA was sequenced and identified. Plants employ many different rooting strategies, such as having a single large tap root or many small roots, or spreading roots along the surface or sending out deep roots. *S. interior* demonstrated far-reaching horizontal root spread through all layers at sites 1 and 3. Based on the size of the sample site and location of the closest stand, *S. interior* roots can reach at least 10m away. This indicates that horizontal root growth can account for up to 75% of roots found in an area. There was also a large patch of *B. cernua* on the surface between sites 2 and 3, with the edges of the patch in each site. *B. cernua* demonstrated shallow surface

horizontal rooting with 94% of its total presence across all sites and layers in layer 1 or 94% of its total presence was found in the top 30cm of soil. The root growth from *B. cernua* (an annual) can reach at least 2m away. These horizontal rooting patterns may indicate that future samples should be taken further apart or have a larger monoculture to sample from. *C. stricta, E. erythropoda,* and *S. boreale* were found at all depths. This indicates that plant size at surface may not predict rooting depth and spread, as *E. erythropoda* is a short plant (about 20-30 cm) that can root to a depth of at least 1.2m. *E. perfoliatum* (about 150 cm) was found only in the top 2 layers, with 93% of all growth found in the top layer. *A. subcordatum* and *A. cepa* were found only in layer 1 (Table 8), indicating a shallow rooting depth. *E. perfoliatum* and *A. subcordatum* are perennials that can grow to over a meter tall but are supported by roots that may only go down to 30 cm.

Some plants were present in layers 3-4 but not layer 1, including *P. pumila, P.norvegica, R. palustris, H. annuus, I. capensis,* and *Typha sp.* Many things could cause this pattern to occur. One is that the presence of the plants is due to horizontal root growth. Subsurface horizontal root growth is most likely for *P. pumila, I. capensis, Typha sp., and R. palustris* since they are present in the surface survey (Table 7). These plants appear to have roots that spread out with depth rather than at the surface. Another cause for presence at depth could be due to sequencing DNA from dead or decaying plant matter. While the sampling method thoroughly washed and separated roots to reduce influence of dead or decaying plant matter like pollen or leaves, the method would have allowed dead roots to be sequenced. Different plants also have different growing seasons and may have been dormant on the surface during the surface survey.

In order to quantify and identify the presence of known bacterial classes at 4 depths below the wetland surface, a portion of the 16s region was sequenced and compared to the QIIME database. Across all sites and layers, proteobacteria was the most frequently occurring phylum. Arroyo *et al.* (2015), Genhe *et al.* (2014), and Moez *et al.* (2014) found similar results in their studies, which included both natural and constructed wetlands that were around lagoons, had horizontal subsurface flow, or had vertical sub surface flow. In their studies, the proteobacteria, beta- and gammaproteobacteria were shown to be the most dominant. However, in this study delta- and betaproteobacteria were the most dominant (Table 3). Thus, wetlands (fens specifically in this study) may have changes in microbial community dominance due to geographic region or hydrology. In this study, depth does not appear to be a factor influencing presence of Deltaproteobacteria suggesting there may be a proportional change in presence of aerobic and anaerobic individuals with changes in oxygen availability since deltaproteobacteria contains both aerobes and anaerobes.

Betaproteobacteria and Nitrospira were the next most frequently occurring classes. Betaproteobacteria are a dominant class in other wetlands (Arroyo *et al.* 2015; Genhe *et al.* 2014; Moez *et al.* 2014), but Nitrospira may be uniquely dominant in Valle Greene (Table 3) or, perhaps, in fens. Compared to other wetland types, only fens have a constant supply of groundwater which could act as a second nutrition source for bacterial communities. This difference in hydrology could be an important part in determining dominant microbial classes.

When constructing treatment wetlands, this could be taken into consideration when designing water flow as Nitrospira are nitrite oxidizers. Since The Nitrospira

generally occur most frequently in layer 2 and 3, a wetland depth of 1.2m with proper hydrology may be required to support the formation of this community. The surface directed water flow of a fen may ease the movement of nutrients and create nutrient gradients across the depths and could drive the development of community structure. Nitrospira, an aerobic class, are more common in layer 2 and 3 while Betaproteobacteria, including a mix of facultative anaerobes and aerobes, are more common in layer 1 and 4. This indicates that plants may have anaerobic zones around the rhizosphere as oxygen is consumed for respiration. Respiration due to microbial decay of plant matter could also consume oxygen. A higher root density could also create larger zones with more dissolved oxygen due to radial oxygen loss (ROL) from roots. In wetland plants, ROL is highest at the root apex (Colmer 2003) and can reach cover up to 56 cm^2 horizontally (Smith & Luna 2013). This means that growing roots may release oxygen from the root apex as they grow down from the surface towards nutrient-containing groundwater. As the source of groundwater is reached, it appears that roots grow horizontally, increasing root mass (Table 1), maximizing nutrient uptake before stagnating and ceasing to grow. The low root mass in mid layers suggests that the zone would be less aerobic and this would better support anaerobic species, both facultative and obligate. A greater presence of Betaproteobacteria is, however, not seen and the strong representation of Nitrospira suggests a source of oxygen. It is possible that water flowing from the lowest layer where root mass is great may be laden with sufficient oxygen to support some of the oxidative steps required by the Nitrospira in the middle layers.

This study investigated the potential presence of six functional groups through genetic identification of bacterial classes. The use of DNA rather than mRNA based

methods means the presence of active functional genes cannot be certain. Some functional groups also overlap in classes but examination of the literature on known microbial functions can show their potential function. For example, Betaproteobacteria contains denitrifiers and ammonia oxidizers. Denitrifiers and various oxidizers were found at all depths indicating aerobic zones are available at all depths. Dissolved oxygen needed for oxidizing pathways can come from the constant supply of ground water or from plant roots due to ROL. Alternative compounds could also present to complete the pathway. For example, denitrifiers use nitrate as an electron acceptor to complete their metabolic pathway instead of dissolved oxygen. Likewise the presence of obligate anaerobes throughout the cores (Table 6, ex methanogens and denitrifiers) indicates the potential presence and possible activity of obligate anaerobes.

Site 1 and 2 were considered near monocultures of plants through a surface survey and each had the highest presence of a unique functional group. Both *C. stricta* and *E. erythropoda* also had a significant impact on overall microbial community structure (Table 10). Site 1 was dominated by *C. stricta* and had the highest overall presence of dechlorinators and nitrite oxidizers (Table 6). Site 2 was dominated by *E. erythropoda* and had the highest overall presence of ammonia oxidizers and methane oxidizers (Table 6). Site 3 was a 50/50 mix of the two and did not have the highest presence of any functional group. Sub-surface species presence and diversity (Table 8) reflected the surface diversity (Table 7) and indicates that monocultures may be used to increase or decrease the presence of specific functional groups.

Dechlorinating bacteria were the only bacteria studied that showed a significant difference by site (Figure 10). At the genera level, there was more *Geobacter sp.* found in

site 1 compared to site 2 and 3 (Table 4). Current data (Amon lab, present) demonstrates that the genes are not only present but active in *Geobacter sp.* at sites like Valle Greene. The main difference between sites was the plant community (Table 8), with *E. erythropoda* presence being a strong ($p < 0.10$) source of the difference between plant communities and changes in dechlorinator presence. This suggests that plant communities can be used to influence specific functional group presence in constructed wetlands.

While many studies report the ability of wetlands to remove excess nitrogen from waterways (Domingos et al. 2011, Sun *et al.* 2013; Genhe *et al.* 2014) and quantify influences on the microbial community (Arroyo et al. 2015), few have characterized the community itself. The functional groups required to complete the nitrogen cycle are nitrogen fixers, nitrifiers (ammonia oxidizers and nitrite oxidizers), and denirifiers. These functional groups are present across all sites and layers, with nitrogen fixers comprising 14.34 - 25.04% of the community, nitrite oxidizers part of 33.20 - 42.58% of the community, and denitrifiers part of 19.83 - 30.98% of the community (Table 6). At the genera level *Nitrospira sp.* presence also follows this relatively even trend (Table 4). None of the soil parameters studied had a significant correlation with the abundance of any specific functional groups (Table 10) (Figure 5). However, 9 of the 25 plants significantly influenced these functional groups (Table 10). Nitrifiers were significantly more abundant than nitrogen fixers and denitrifiers (Figure 5) and had the strongest correlation with plant type (Table 10). The difference in nitrifiers could be due to many factors. Plants can uptake nitrates, the end product of nitrification, and could support these microbes in order to uptake more nitrogen where nitrogen is a limiting nutrient.

However, four of the five plants associated with nitrifiers have a negative correlation. This negative correlation could indicate an excess of nitrates found in this area of the environment. If the plant community in an area of a fen has a negative impact on nitrifiers, but they still thrive, then there may be an excess of ammonium coming into or from the environment or plant generated oxygen may be insufficient. Many wetland plants continuously shed dead leaves and some roots as part of their life cycle, and this source of decaying matter could provide a supply of ammonium (from deamination of protein) to nitrifiers. While this trend could cause an overall decrease in ammonia nitrogen, oxidation will increase in nitrates specifically. This decrease in total nitrogen but increase in nitrates has been seen in other studies (Domingos *et al.* 2011; Genhe *et al.* 2014). The multidimensional aspects of the fen can allow nitrates could also be transported by the moving groundwater into areas of the fen that have a different parts of the bacterial and plant community. That community could uptake nitrates or denitrify to gaseous N which would cause a decrease in total nitrogen as water flows through the entire fen, not just one area but on the surface and in the soil.

Functional group variation with depth may also indicate more than is apparent from raw data. Microbes may move around or even change their metabolism as they exchange electrons as part of geochemical cycles. Some microbes, such as *Escherichia coli*, are "facultative" aerobes, meaning they can switch from an anaerobic to aerobic metabolism under the right conditions (Madigan *et al.* 2015). This shift in metabolism could also change their function in response to environmental conditions. Regardless of the source of variation, all functional groups studied are present at all depths (Table 6) indicating that complete nutrient cycles are available at least 1.2m deep at Valle Green.

Therefore, bioremediation using fen-like systems has the potential to treat a variety of groundwater contaminants as complete nutrient cycles are present and can break down a number of compounds including chlorinated ethenes (Amon *et al.* 2007). Fen-like systems also have the potential to create microenvironments throughout the root zone that support anaerobic and aerobic metabolisms at depth (Table 6).

The vertical distribution and correlation of functional groups to presence of species of plant roots present was investigated. Dechlorinators are influenced by *E. erythropoda*, *E. perfoliatum*, and *Polygonium sp*. (Table 10). The relation to *E. perfoliatum* and *Polygonium sp*. is unclear and may be because that those plants are only found in 1-2 layers in total and those layers have the highest amount of dechlorinators. When used in regression models, *E. perfoliatum* and *Polygonium sp*. provided large, seemingly erroneous coefficients since they provided an over 100% change in dechlorinator presence with 1% change in plant species presence and were not significant ($p > .50$). Since they were not significant and were a source of error, *E. perfoliatum* and *Polygonium sp*. were removed from the regression model. After they were removed a significant regression model could be created (Figure 10). This indicates that the relationship was likely due to outliers and not truely significant. *E. erythropoda* has a strong ($p < 0.10$, Table 10) and negative (Figure 10) association with dechlorinators, which may not make it a good plant to use in constructed wetlands treating chlorinated ethenes. However, this correlation is weak, as dechlorinator presence only decreases by 0.009% per percent increase in *E. erythropoda* presence.

Nitrite oxidizers appear to be influenced by *B. cernua, C. stricta, Coreopsis sp.*, *E. erythropoda*, and *S. interior* (Table 10). *B. cernua*, *E. erythropoda*, and *S. interior* had

a significant (all $p < 0.05$, Table 10) and positive association with the presence of nitrifiers (Figure 11). *Coreopsis sp.* had a significant ($p < 0.05$, Table 10) and negative association with the presence of nitrifiers (Figure 11). *H. umbellata* was correlated with ammonia oxidizers and methane oxidizers but not nitrite oxidizers (Table 10). This demonstrates that plants do seem to associate with specific microbial functional groups. More so, it demonstrates that the unique structure of a microbial community has both a negative and a positive association with plants that are present.

Of the functional groups studied, potential presence of nitrogen oxidizers was influenced by the greatest number of plants. The five associated plants are: *B. cernua, C. stricta, Coreopsis sp., E. erythropoda,* and *S. interior*. All of these plants generally have a negative influence on nitrogen oxidizer presence (Table 10). Inorganic nitrogen may not be a limiting nutrient for these plants in a fen, so the plants may favor other bacteria over the nitrifiers or they may be using ammonia as their preferred N source. Arroyo et al. (2014) showed in their study that the available total Kjeldahl nitrogen and ammonia nitrogen significantly influence bacterial community structure. Since some plants directly influence the functional groups of bacteria involved in the nitrogen cycle (Table 10), plants may, in turn, affect nutrient availability to the plant. By supporting or inhibiting microbial functional groups that alter nitrogen-containing compounds, the plant-microbial consortium may provide useable forms of N for other plants and microbes downstream in the surface directed flow of water. This plant-derived support could be in the form of CO_2, O_2, sugars, or amino acids or micronutrients in root exudates.

The overall bacterial community is influenced by *C. stricta, E. erythropoda, P. pumila, and S. boreale* (Table 10). Since these four plants seem to cause the overall

bacterial community composition to change, they may be good candidates to study in the future. A positive or negative relationship may indicate that a plant species either inhibits the growth of the bacteria in a functional group, or they more favorably interact with bacteria of other functional groups. Since these plants may influence one or more of the selected functional groups, they may be good candidates for future study on how they specifically influence bacteria communities and if it is consistent across geographic areas.

Diversity and richness were studied in order to determine if it can be correlated with plants or any other parameters investigated. This study estimated combined microbial species richness across three sites for a groundwater fed wetland at 13,000-24,000 species. In the study by Arroyo et al. (2015), four natural wetlands had between 18,000-26,000 species in their top 5 cm of soil. Bates *et al.* (2011) and Roesch *et al.* (2007) estimate bacterial species richness between 5,000-12,000 species for upland sites. However, these studies only have a sampling depth within 20 cm of the soil surface, which is comparable to Layer 1 of this study. Layer 1 contains the highest estimated richness at 21,000-24,000 species indicating that the estimated bacterial species richness for a wetland may be 10,000 higher than for an upland ecosystem. This may be due to the unique properties of a groundwater fed wetland and may not be true for other types of wetlands. One of those properties of a fen is its constant supply of nutrient rich (rich on an annualized basis) groundwater. Another study has shown the temperature of groundwater to be consistently between 12-15^{o}C (Amon et al. 2007). The flowing groundwater would create a stable temperature environment for bacteria to grow in that is well within the growth range of many soil bacteria (Atlas and Bartha 1998). This stable environment may allow less cold tolerant bacterial species to grow in the winter and less

heat tolerant bacterial species to grow in the summer. Water in most mid-western fens is also rich in dissolved minerals that providing carbonate buffer system which creates a system with a stable pH at, or slightly above, neutral (Amon 2002). Repeating this study across different wetland hydrology types could give more insight to how surface directed hydrologic flow influences bacterial communities.

Both Chao1 estimates (Table 2) and alpha diversity analysis through rarefaction (Figure 3) indicated higher bacterial species richness in upper layers. This could be caused by the increased availability of organic matter in the upper layers (Table 1) that is likely due to plant matter decaying. Organic matter and soil moisture also had a significant ($p < 0.05$, Table 10) and positive relation with Chao1 estimates (Figure 7), supporting the idea that increased organic matter availability can increase species diversity. Depth had a significant ($p < 0.05$, Table 10) and negative (Figure 7) relation with Chao1 estimates. Organic matter decreased with depth (Table 1), which further stresses the importance of available organic matter in determining species richness. Alpha diversity analysis also indicated that sampling coverage could be improved in future work. One way to increase sampling coverage may be to decrease the length of each layer. This would also allow a finer resolution of changes in bacterial community composition and directly increase the number of samples taken which should improve sample coverage.

Simpson and Shannon indices across all sites, layers, and taxa indicated that the microbial community had a high level of diversity and a relatively even distribution of taxa (Table 5). Between 26 and 41 phyla were identified, and it is not likely that a single phylum is dominant since low Simpson's values were calculated. In community ecology,

typical values for Shannon's diversity fall between 1.5 - 3.5. The phyla indices fall between 2.11 and 3.04, which indicates that the phyla distribution is relatively even. In a vertical flow constructed wetland for wastewater treatment, Sklarz *et al.* (2011) calculated similar a similar Shannon index for depths comparable to layer 1 and layer 2. Their estimates were 2.47 and 2.56 and mine were 2.45 and 2.39 for layer 1 and 2 respectively. In non-vertical flowing wetlands, the Shannon index is lower. Arroyo *et al.* (2015) had an average Shannon index of 1.62 for lagoons, and Ahn *et al.* (2011) had an average Shannon index of 2.25 for marshes. While, these studies only sampled to a depth comparable to layer 1, these differences may indicate that hydrology plays an important role in determining species evenness. Of the variables studied, organic content, *C. stricta* presence, and *E. erythropoda* presence influenced the Simpson index (Figure 8). Organic content, soil moisture, and root mass influenced the Shannon index (Figure 9). For both indices, organic content had the largest coefficient in regression, which indicates that organic matter availability plays an important role in species evenness. Chao1 estimates, or species richness, also had an association with organic matter availability (Figure 7). Stated before, common wetland plants (ex. sedges and rushes) continuously shed leaves throughout their lifecycle. This is what forms the peaty soil layer in wetlands and provides a continuous source of organic matter. While roots can provide direct nutrient exchange, this indirect organic matter source for bacteria may be just as important to study.

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7. Appendix

A.1 – List of sources used to assign functional group to bacterial and archaeal classes.
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Wikipedia articles on functional groups and dominate classes were used as a jumping point to get to some journal articles. For a functional group to be assigned, it had to have function referenced in at least two sources – only the first/main source is listed above.

A.2 – Complete table of bacterial classes. Classes in order of overall dominance. Values shown are percent of total community by site by, layer. Classes in square brackets are QIIME proposed taxonomies. K represents Kingdom with A and B representing archaea and bacteria respectively.

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Proteobacteria	Deltaproteobacteria	21.62	21.69	21.96	23.11	16.26	18.02	17.68	19.82	17.14	21.25	13.82	19.03
B	Proteobacteria	Betaproteobacteria	10.98	7.73	10.76	10.58	11.85	9.92	10.35	8.66	7.01	7.89	3.41	8.91
B	Nitrospirae	Nitrospira	5.76	10.54	10.84	8.84	5.44	10.67	10.95	7.72	6.40	11.88	5.66	8.61
B	Proteobacteria	Alphaproteobacteria	7.05	4.97	5.64	6.66	6.56	6.28	6.45	4.34	7.00	5.44	9.57	13.18
B	Chloroflexi	Anaerolineae	6.02	6.68	6.40	6.88	6.16	5.68	6.45	4.36	7.51	5.23	6.86	7.20
B	Actinobacteria	Thermoleophilia	2.50	2.65	1.08	0.62	5.69	6.24	3.87	1.63	5.33	4.61	6.96	0.76
B	NC10	12-24	1.87	5.23	4.83	3.66	1.46	3.99	4.52	0.43	1.37	5.93	1.70	2.18
B	Chloroflexi	S085	0.74	2.44	3.16	4.20	0.49	1.67	3.54	2.50	0.54	2.73	6.64	5.70
B	Proteobacteria	Gammaproteobacteria	4.80	1.36	1.63	2.21	4.94	2.48	1.70	2.71	3.39	1.91	4.27	1.75
B	Gemmatimonadetes	Gemm-1	2.77	4.21	4.10	2.90	1.31	2.78	5.56	1.15	1.39	3.53	1.08	2.21
B	Acidobacteria	Acidobacteria-6	3.68	2.09	2.53	2.20	1.32	2.02	2.42	2.22	1.61	2.06	1.46	2.22
B	Planctomycetes	Phycisphaerae	1.94	1.64	1.62	1.81	1.89	1.65	1.46	4.93	2.60	1.24	1.45	1.56
B	Bacteroidetes	Bacteroidia	2.88	1.55	0.99	0.72	5.90	1.85	0.76	1.00	5.43	0.69	1.29	0.47
B	WS3	PRR-12	2.04	1.76	1.78	1.70	1.30	1.38	2.62	0.54	1.27	1.38	0.77	1.03
B	Verrucomicrobia	[Pedosphaerae]	2.73	2.04	1.74	0.98	1.50	1.43	1.14	0.21	2.05	1.57	0.98	0.53
B	Acidobacteria	iii1-8	1.57	1.04	0.77	0.37	1.37	2.11	0.83	0.09	2.36	2.40	1.41	0.24
B	Chlorobi	BSV26	0.74	0.54	0.93	1.72	1.19	0.92	1.01	3.29	0.92	0.35	0.54	1.82
B	Gemmatimonadetes	Gemmatimonadetes	1.08	1.21	1.13	0.98	0.48	1.62	1.47	0.92	0.98	2.02	0.75	0.66
B	Acidobacteria	[Chloracidobacteria]	1.44	1.86	0.73	0.35	0.50	1.29	0.67	0.03	1.26	1.93	0.93	0.17
A	Crenarchaeota	MCG	0.28	0.23	0.52	0.98	0.73	0.63	0.42	3.35	0.83	0.31	1.28	1.29
B	Actinobacteria	MB-A2-108	0.41	0.56	0.39	0.24	1.15	1.39	1.05	0.10	0.75	1.49	2.92	0.36
B	Firmicutes	Clostridia	0.20	0.35	0.43	0.32	0.39	0.18	0.11	0.11	0.52	0.12	6.96	0.36
B	Acidobacteria	Solibacteres	1.11	1.14	0.75	0.84	0.71	0.73	0.69	0.45	1.26	0.57	0.72	0.51
B	Actinobacteria	Acidimicrobiia	0.76	0.47	0.29	0.29	1.15	1.12	0.75	0.18	1.28	0.92	1.17	0.42
B	Chloroflexi	Ellin6529	0.41	0.41	0.46	0.55	0.52	0.52	0.73	0.33	0.75	0.64	1.18	0.88
B	Firmicutes	Bacilli	0.33	0.73	0.41	0.12	0.33	0.56	0.47	0.06	1.52	0.58	1.91	0.16
B	Actinobacteria	Actinobacteria	0.66	0.70	0.56	0.23	0.62	0.51	0.58	0.12	0.86	0.81	1.20	0.27
B	Acidobacteria	BPC102	0.71	0.45	0.43	0.42	1.10	0.79	0.42	0.34	1.19	0.42	0.49	0.28
B	Chloroflexi	Dehalococcoidetes	0.12	0.13	0.29	0.62	0.65	0.41	0.30	1.57	0.55	0.19	0.83	1.13
B	Chlorobi	Ignavibacteria	0.45	0.22	0.17	0.30	0.61	0.35	0.13	3.21	0.56	0.08	0.14	0.26
B	Proteobacteria	Other	0.37	0.48	0.32	1.21	0.14	0.19	0.40	1.62	0.11	0.02	0.17	1.25
B	Spirochaetes	Spirochaetes	0.47	0.46	0.45	0.38	1.04	0.50	0.43	0.49	0.85	0.30	0.45	0.37
B	Planctomycetes	Planctomycetia	0.79	0.52	0.35	0.48	0.47	0.38	0.35	0.22	0.52	0.36	0.91	0.65
B	GN04	MSB-5A5	0.02	0.24	0.69	1.43	0.01	0.09	0.51	1.92	0.01	0.10	0.09	0.86
B	Acidobacteria	DA052	0.11	0.73	0.80	0.85	0.07	0.22	0.52	0.35	0.04	1.09	0.08	0.36
B	Bacteroidetes	[Saprospirae]	1.01	0.63	0.30	0.15	0.53	0.43	0.18	0.19	0.67	0.40	0.23	0.31

A.2 – Continued

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
A	Crenarchaeota	Thaumarchaeota	0.61	0.75	0.51	0.43	0.40	0.44	0.08	0.06	0.89	0.31	0.35	0.16
B	OP8	OP8_1	0.11	0.12	0.27	0.25	0.34	0.20	0.36	2.13	0.32	0.06	0.44	0.35
B	Chlorobi	SJA-28	0.32	0.50	0.41	0.43	0.30	0.45	0.35	0.21	0.44	0.43	0.22	0.47
A	Euryarchaeota	Thermoplasmata	0.02	0.05	0.07	0.14	0.22	0.09	0.22	1.78	0.16	0.04	0.08	0.37
B	Chloroflexi	Chloroflexi	0.23	0.52	0.25	0.12	0.10	0.26	0.21	0.00	0.15	0.96	0.27	0.08
B	Proteobacteria	Epsilonproteobacteria	0.01	0.01	0.00	0.01	2.29	0.02	0.00	0.00	0.56	0.00	0.01	0.01
B	Gemmatimonadetes	Gemm-5	0.41	0.43	0.29	0.13	0.12	0.43	0.32	0.00	0.18	0.32	0.10	0.07
A	Euryarchaeota	Methanomicrobia	0.31	0.39	0.15	0.11	0.34	0.08	0.07	0.11	0.53	0.11	0.54	0.08
B	GAL15	Other	0.02	0.12	0.22	0.42	0.08	0.10	0.17	0.24	0.03	0.07	0.18	0.90
B	Acidobacteria	Other	0.28	0.21	0.21	0.27	0.11	0.20	0.39	0.18	0.13	0.23	0.10	0.16
B	GN04	Other	0.12	0.16	0.16	0.34	0.16	0.13	0.27	0.42	0.14	0.08	0.09	0.29
B	Verrucomicrobia	[Spartobacteria]	0.42	0.27	0.11	0.02	0.30	0.26	0.09	0.00	0.44	0.18	0.20	0.01
B	Armatimonadetes	SJA-176	0.16	0.13	0.16	0.09	0.18	0.18	0.09	0.12	0.25	0.10	0.30	0.11
B	Chloroflexi	TK17	0.10	0.08	0.20	0.29	0.06	0.07	0.18	0.19	0.07	0.11	0.13	0.36
B	Acidobacteria	TM1	0.07	0.12	0.21	0.36	0.05	0.14	0.19	0.24	0.04	0.09	0.03	0.27
B	SBR1093	Other	0.03	0.25	0.36	0.17	0.03	0.24	0.20	0.00	0.05	0.20	0.07	0.14
B	GOUTA4	Other	0.20	0.15	0.10	0.11	0.21	0.14	0.13	0.10	0.22	0.15	0.15	0.04
B	Bacteroidetes	Flavobacteriia	0.07	0.04	0.01	0.00	0.91	0.21	0.10	0.00	0.09	0.01	0.23	0.00
B	Acidobacteria	OS-K	0.02	0.04	0.09	0.31	0.16	0.07	0.08	0.32	0.09	0.04	0.04	0.42
B	Chloroflexi	TK10	0.13	0.24	0.17	0.13	0.04	0.14	0.13	0.02	0.08	0.21	0.09	0.12
B	Acidobacteria	Sva0725	0.16	0.11	0.14	0.12	0.08	0.05	0.07	0.10	0.09	0.06	0.06	0.45
B	Verrucomicrobia	Opitutae	0.20	0.08	0.07	0.06	0.21	0.12	0.11	0.01	0.25	0.10	0.05	0.05
B	OP3	koll11	0.03	0.05	0.06	0.08	0.22	0.10	0.07	0.19	0.14	0.04	0.06	0.18
B	Bacteroidetes	Cytophagia	0.32	0.04	0.04	0.04	0.27	0.04	0.07	0.07	0.22	0.01	0.04	0.04
B	Actinobacteria	Rubrobacteria	0.12	0.20	0.07	0.01	0.10	0.14	0.06	0.00	0.13	0.17	0.16	0.00
B	SC4	Other	0.08	0.17	0.10	0.06	0.07	0.13	0.09	0.07	0.11	0.12	0.09	0.05
B	Planctomycetes	Pla3	0.19	0.10	0.16	0.14	0.05	0.06	0.10	0.04	0.10	0.06	0.03	0.09
B	Gemmatimonadetes	Gemm-2	0.19	0.08	0.08	0.14	0.04	0.10	0.15	0.08	0.04	0.06	0.03	0.07
B	NKB19	Other	0.09	0.06	0.07	0.12	0.13	0.09	0.06	0.15	0.10	0.04	0.04	0.08
B	Spirochaetes	[Leptospirae]	0.13	0.08	0.10	0.06	0.22	0.07	0.02	0.04	0.17	0.04	0.07	0.03
B	Acidobacteria	RB25	0.20	0.08	0.13	0.07	0.09	0.06	0.09	0.01	0.13	0.07	0.05	0.02
B	Acidobacteria	Acidobacteriia	0.06	0.11	0.08	0.02	0.06	0.16	0.12	0.00	0.17	0.14	0.06	0.02
B	Planctomycetes	OM190	0.30	0.08	0.10	0.07	0.10	0.05	0.07	0.01	0.11	0.04	0.02	0.02
B	BRC1	PRR-11	0.06	0.06	0.07	0.07	0.07	0.14	0.15	0.06	0.07	0.05	0.03	0.07
B	Bacteroidetes	Sphingobacteriia	0.11	0.04	0.05	0.04	0.35	0.08	0.03	0.01	0.08	0.01	0.04	0.06
B	MVS-104	Other	0.02	0.06	0.08	0.09	0.02	0.03	0.05	0.25	0.04	0.04	0.05	0.11
B	BRC1	Other	0.00	0.01	0.07	0.05	0.01	0.04	0.05	0.36	0.01	0.00	0.01	0.22

A.2 – Continued (2)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	TM6	SJA-4	0.07	0.04	0.04	0.05	0.08	0.05	0.03	0.10	0.06	0.01	0.06	0.20
B	Chloroflexi	Thermomicrobia	0.04	0.06	0.09	0.06	0.03	0.08	0.06	0.01	0.04	0.11	0.13	0.06
B	Verrucomicrobia	[Methylacidiphilae]	0.08	0.07	0.10	0.08	0.10	0.04	0.08	0.02	0.06	0.03	0.02	0.05
B	Fibrobacteres	TG3	0.04	0.01	0.03	0.03	0.17	0.05	0.02	0.03	0.16	0.01	0.11	0.04
B	Actinobacteria	OPB41	0.08	0.04	0.02	0.02	0.04	0.02	0.01	0.05	0.05	0.02	0.27	0.06
B	NC10	wb1-A12	0.01	0.37	0.21	0.02	0.00	0.03	0.02	0.00	0.00	0.02	0.00	0.00
B	GN04	GN15	0.04	0.04	0.07	0.09	0.09	0.04	0.06	0.12	0.04	0.02	0.01	0.05
B	Acidobacteria	PAUC37f	0.06	0.08	0.05	0.02	0.04	0.12	0.05	0.00	0.08	0.06	0.04	0.01
B	Elusimicrobia	Other	0.03	0.05	0.04	0.07	0.04	0.09	0.04	0.04	0.06	0.06	0.04	0.06
B	Planctomycetes	ODP123	0.06	0.07	0.07	0.03	0.05	0.04	0.04	0.04	0.07	0.06	0.03	0.05
B	Acidobacteria	S035	0.05	0.07	0.03	0.05	0.03	0.08	0.05	0.01	0.04	0.10	0.03	0.06
A	[Parvarchaeota]	Parvarchaea	0.06	0.02	0.06	0.06	0.10	0.03	0.02	0.08	0.10	0.00	0.03	0.04
B	Elusimicrobia	Elusimicrobia	0.07	0.03	0.04	0.03	0.10	0.05	0.03	0.02	0.10	0.02	0.04	0.03
B	Armatimonadetes	0319-6E2	0.04	0.07	0.05	0.06	0.01	0.03	0.04	0.02	0.03	0.04	0.03	0.07
B	OP3	BD4-9	0.03	0.03	0.02	0.04	0.07	0.03	0.01	0.04	0.08	0.03	0.01	0.07
B	Chloroflexi	Gitt-GS-136	0.11	0.04	0.03	0.02	0.05	0.06	0.04	0.00	0.06	0.05	0.02	0.00
A	Euryarchaeota	Methanobacteria	0.03	0.01	0.01	0.01	0.13	0.01	0.00	0.03	0.12	0.01	0.07	0.02
B	Gemmatimonadetes	JL-ETNP-Z39	0.01	0.00	0.02	0.08	0.02	0.02	0.02	0.08	0.01	0.02	0.04	0.07
B	AC1	HDBW-WB69	0.00	0.01	0.02	0.05	0.01	0.02	0.02	0.14	0.03	0.01	0.01	0.07
B	LCP-89	SAW1_B44	0.00	0.01	0.01	0.01	0.11	0.03	0.01	0.01	0.12	0.02	0.03	0.01
B	AC1	SHA-114	0.01	0.01	0.02	0.01	0.06	0.06	0.04	0.02	0.06	0.04	0.02	0.01
B	Planctomycetes	Other	0.06	0.04	0.03	0.04	0.03	0.02	0.03	0.01	0.04	0.02	0.01	0.02
B	Other	Other	0.03	0.03	0.02	0.03	0.05	0.03	0.01	0.05	0.03	0.03	0.01	0.04
B	WS2	Kazan-3B-09	0.01	0.01	0.04	0.02	0.03	0.05	0.03	0.07	0.03	0.00	0.01	0.05
B	Chloroflexi	Other	0.00	0.06	0.08	0.02	0.00	0.02	0.02	0.00	0.01	0.09	0.01	0.01
B	Acidobacteria	Holophagae	0.02	0.02	0.06	0.02	0.05	0.02	0.02	0.01	0.04	0.02	0.01	0.02
B	Armatimonadetes	[Fimbriimonadia]	0.08	0.02	0.03	0.04	0.02	0.01	0.02	0.03	0.02	0.01	0.01	0.04
B	Cyanobacteria	Chloroplast	0.11	0.05	0.03	0.03	0.01	0.00	0.00	0.00	0.01	0.00	0.04	0.02
B	LD1	Other	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.14	0.01	0.00	0.10	0.04
B	TM7	TM7-1	0.03	0.01	0.03	0.01	0.08	0.01	0.02	0.02	0.02	0.01	0.03	0.04
B	Verrucomicrobia	Verruco-5	0.01	0.01	0.03	0.01	0.08	0.02	0.02	0.01	0.06	0.01	0.01	0.01
B	Armatimonadetes	Chthonomonadetes	0.03	0.06	0.04	0.02	0.02	0.03	0.02	0.00	0.02	0.03	0.02	0.01
B	Chloroflexi	Ktedonobacteria	0.03	0.04	0.10	0.03	0.01	0.01	0.00	0.00	0.01	0.01	0.03	0.00
B	Chloroflexi	SAR202	0.00	0.02	0.06	0.06	0.00	0.01	0.02	0.01	0.01	0.03	0.01	0.05
B	OP8	OP8_2	0.00	0.03	0.00	0.00	0.13	0.01	0.02	0.00	0.05	0.01	0.01	0.00
B	Chlorobi	OPB56	0.07	0.01	0.01	0.01	0.04	0.01	0.01	0.02	0.03	0.01	0.00	0.01
B	GN02	BB34	0.01	0.01	0.03	0.02	0.03	0.04	0.02	0.02	0.03	0.00	0.02	0.02

A.2 – Continued (3)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Chlorobi	Other	0.05	0.01	0.02	0.02	0.03	0.02	0.02	0.00	0.03	0.01	0.01	0.01
B	Actinobacteria	Other	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.17	0.01
B	OP11	OP11-3	0.03	0.02	0.02	0.01	0.01	0.00	0.00	0.04	0.02	0.01	0.04	0.02
B	SBR1093	VHS-B5-50	0.01	0.00	0.01	0.06	0.00	0.01	0.02	0.04	0.00	0.00	0.02	0.02
B	Planctomycetes	[Brocadiae]	0.00	0.00	0.01	0.12	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.01
B	WS4	Other	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.02	0.10	0.01
B	Planctomycetes	BD7-11	0.01	0.01	0.01	0.02	0.00	0.00	0.01	0.00	0.03	0.00	0.08	0.00
B	TPD-58	Other	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.06	0.01
B	Caldithrix	Caldithrixae	0.00	0.00	0.04	0.03	0.01	0.01	0.01	0.03	0.00	0.00	0.03	0.01
B	FCPU426	Other	0.04	0.01	0.01	0.01	0.04	0.01	0.01	0.00	0.03	0.01	0.01	0.01
B	TM6	SBRH58	0.02	0.01	0.00	0.01	0.03	0.01	0.00	0.01	0.03	0.01	0.02	0.02
B	OD1	Other	0.01	0.01	0.00	0.04	0.00	0.00	0.00	0.04	0.01	0.00	0.02	0.03
B	GN02	3BR-5F	0.02	0.00	0.01	0.01	0.01	0.00	0.01	0.06	0.01	0.01	0.00	0.02
B	Chloroflexi	C0119	0.03	0.04	0.01	0.00	0.01	0.01	0.01	0.00	0.02	0.03	0.01	0.00
B	TM7	Other	0.02	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Spirochaetes	[Brevinematae]	0.01	0.00	0.00	0.00	0.03	0.01	0.00	0.01	0.04	0.01	0.02	0.01
B	Lentisphaerae	[Lentisphaeria]	0.02	0.02	0.01	0.01	0.05	0.01	0.00	0.00	0.03	0.00	0.01	0.00
A	Crenarchaeota	MBGB	0.01	0.01	0.01	0.00	0.03	0.01	0.00	0.01	0.03	0.01	0.02	0.00
B	Elusimicrobia	Endomicrobia	0.01	0.00	0.01	0.01	0.02	0.01	0.00	0.01	0.02	0.00	0.04	0.01
B	WS1	Other	0.01	0.01	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
B	OP11	Other	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.04	0.03
B	Acidobacteria	EC1113	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.00	0.01	0.01	0.02
B	Fibrobacteres	Other	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.03	0.01	0.01	0.00
B	WS2	SHA-109	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.03	0.00	0.01	0.00
B	BRC1	NPL-UPA2	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.01
B	Chloroflexi	SHA-26	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.02	0.01	0.01	0.01
B	AC1	B04R032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.04	0.00
B	KSB3	Other	0.01	0.02	0.01	0.01	0.04	0.01	0.00	0.00	0.01	0.00	0.01	0.00
B	Verrucomicrobia	Verrucomicrobiae	0.03	0.01	0.00	0.00	0.03	0.00	0.01	0.00	0.02	0.00	0.01	0.00
B	Spirochaetes	[Brachyspirae]	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.01	0.00	0.02	0.02
B	Planctomycetes	C6	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.00	0.00	0.00
B	PAUC34f	Other	0.01	0.00	0.01	0.01	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.01
B	Armatimonadetes	MD2902-B50	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.02	0.01
B	OP3	PBS-25	0.02	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00
B	Cyanobacteria	Other	0.01	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.01	0.01
A	Crenarchaeota	MBGA	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01
B	Gemmatimonadetes	Other	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.00

A.2 – Continued (4)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Tenericutes	Mollicutes	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.02	0.01	0.00	0.00
B	AC1	Other	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.01
B	Poribacteria	Other	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.01	0.02	0.00	0.01	0.01
B	NKB19	TSBW08	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00
B	Fibrobacteres	Fibrobacteria	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.00	0.00
B	Acidobacteria	Acidobacteria-5	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00
B	Fusobacteria	Fusobacteriia	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.03	0.00
B	Planctomycetes	028H05-P-BN-P5	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00
B	TM7	TM7-3	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.02	0.00
B	Elusimicrobia	OP2	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.01	0.01
B	[Caldithrix]	KSB1	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00
B	Planctomycetes	vadinHA49	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.01	0.00	0.00
B	Proteobacteria	Zetaproteobacteria	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00
B	NC10	Other	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00
B	Acidobacteria	GAL08	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01
B	OD1	Mb-NB09	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00
B	OP3	Other	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01
B	OP1	[Acetothermia]	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
B	GN02	GKS2-174	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
B	Chlamydiae	Chlamydiia	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00
B	Hyd24-12	WM88	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.00	0.00
A	Euryarchaeota	DSEG	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	OD1	ABY1	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
B	Cyanobacteria	4C0d-2	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	NC10	Other	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Bacteroidetes	At12OctB3	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	TA06	Other	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
B	Fibrobacteres	SBZC_2415	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00
B	TM7	SC3	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	WPS-2	Other	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	VHS-B3-43	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00
B	Fibrobacteres	B5-096	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00
B	OP8	SAW1_B6	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	OP9	JS1	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00
B	Spirochaetes	GN05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	GN04	5bav_B12	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
B	OP1	MSBL6	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00

A.2 – Continued (5)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Cyanobacteria	Nostocophycideae	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	ZB3	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	[Thermi]	Deinococci	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
B	Bacteroidetes	VC2_1_Bac22	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Firmicutes	Erysipelotrichi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
B	Proteobacteria	TA18	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	Gemmatimonadetes	Gemm-3	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Chloroflexi	Other	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Chloroflexi	P2-11E	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	OD1	SM2F11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	Cyanobacteria	ML635J-21	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	WS5	Other	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Cyanobacteria	Oscillatoriophycideae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
B	OP11	OP11-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
B	GN02	GN10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	WWE1	[Cloacamonae]	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	Fibrobacteres	Other	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
B	Actinobacteria	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
B	WS2	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
B	Acidobacteria	AT-s54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	SR1	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Acidobacteria	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
B	Armatimonadetes	SHA-37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A	[Parvarchaeota]	[Micrarchaea]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	TM7	MJK10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Planctomycetes	Pla4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	OP11	WCHB1-64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	OC31	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Verrucomicrobia	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
B	Bacteroidetes	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Caldiserica	OP5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Chloroflexi	[Thermobacula]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	OD1	ZB2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	KSB3	MAT-CR-H3-D11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Bacteroidetes	[Rhodothermi]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	BHI80-139	Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	Spirochaetes	MVP-15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A.3 – Complete table of bacterial classes. Classes in order by kingdom, by phylum, by class. Values shown are percent of total community by site by, layer. Classes in square brackets are QIIME proposed taxonomies. K represents Kingdom with A and B representing archaea and bacteria respectively.

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
A	[Parvarchaeota]	[Micrarchaea]	0	0	0	0	0	0	0	0	0	0	0	0
A	[Parvarchaeota]	Parvarchaea	0.06	0.02	0.06	0.06	0.1	0.03	0.02	0.08	0.1	0	0.03	0.04
A	Crenarchaeota	MBGA	0	0.01	0.01	0.02	0	0	0.01	0.01	0	0.01	0.01	0.01
A	Crenarchaeota	MBGB	0.01	0.01	0.01	0	0.03	0.01	0	0.01	0.03	0.01	0.02	0
A	Crenarchaeota	MCG	0.28	0.23	0.52	0.98	0.73	0.63	0.42	3.35	0.83	0.31	1.28	1.29
A	Crenarchaeota	Thaumarchaeota	0.61	0.75	0.51	0.43	0.4	0.44	0.08	0.06	0.89	0.31	0.35	0.16
A	Euryarchaeota	DSEG	0	0	0	0	0.01	0	0	0	0.01	0	0	0
A	Euryarchaeota	Methanobacteria	0.03	0.01	0.01	0.01	0.13	0.01	0	0.03	0.12	0.01	0.07	0.02
A	Euryarchaeota	Methanomicrobia	0.31	0.39	0.15	0.11	0.34	0.08	0.07	0.11	0.53	0.11	0.54	0.08
A	Euryarchaeota	Thermoplasmata	0.02	0.05	0.07	0.14	0.22	0.09	0.22	1.78	0.16	0.04	0.08	0.37
B	[Caldithrix]	KSB1	0	0	0	0	0.02	0	0	0	0.02	0	0	0
B	[Thermi]	Deinococci	0	0	0.02	0	0	0	0.01	0	0	0	0	0
B	AC1	B04R032	0	0	0	0	0	0	0	0.07	0	0	0.04	0
B	AC1	HDBW-WB69	0	0.01	0.02	0.05	0.01	0.02	0.02	0.14	0.03	0.01	0.01	0.07
B	AC1	Other	0	0	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0	0	0.01
B	AC1	SHA-114	0.01	0.01	0.02	0.01	0.06	0.06	0.04	0.02	0.06	0.04	0.02	0.01
B	Acidobacteria	[Chloracidobacteria]	1.44	1.86	0.73	0.35	0.5	1.29	0.67	0.03	1.26	1.93	0.93	0.17
B	Acidobacteria	Acidobacteria-5	0.02	0.01	0.01	0	0	0.01	0.01	0	0.01	0	0	0
B	Acidobacteria	Acidobacteria-6	3.68	2.09	2.53	2.2	1.32	2.02	2.42	2.22	1.61	2.06	1.46	2.22
B	Acidobacteria	Acidobacteriia	0.06	0.11	0.08	0.02	0.06	0.16	0.12	0	0.17	0.14	0.06	0.02
B	Acidobacteria	AT-s54	0	0	0	0	0	0	0	0	0	0	0	0
B	Acidobacteria	BPC102	0.71	0.45	0.43	0.42	1.1	0.79	0.42	0.34	1.19	0.42	0.49	0.28
B	Acidobacteria	DA052	0.11	0.73	0.8	0.85	0.07	0.22	0.52	0.35	0.04	1.09	0.08	0.36
B	Acidobacteria	EC1113	0	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0	0.01	0.01	0.02
B	Acidobacteria	GAL08	0	0	0	0.01	0.01	0	0	0.01	0	0	0	0.01
B	Acidobacteria	Holophagae	0.02	0.02	0.06	0.02	0.05	0.02	0.02	0.01	0.04	0.02	0.01	0.02
B	Acidobacteria	iii1-8	1.57	1.04	0.77	0.37	1.37	2.11	0.83	0.09	2.36	2.4	1.41	0.24
B	Acidobacteria	OS-K	0.02	0.04	0.09	0.31	0.16	0.07	0.08	0.32	0.09	0.04	0.04	0.42
B	Acidobacteria	Other	0.28	0.21	0.21	0.27	0.11	0.2	0.39	0.18	0.13	0.23	0.1	0.16
B	Acidobacteria	Other	0	0	0	0	0	0	0	0	0	0	0	0.01
B	Acidobacteria	PAUC37f	0.06	0.08	0.05	0.02	0.04	0.12	0.05	0	0.08	0.06	0.04	0.01
B	Acidobacteria	RB25	0.2	0.08	0.13	0.07	0.09	0.06	0.09	0.01	0.13	0.07	0.05	0.02
B	Acidobacteria	S035	0.05	0.07	0.03	0.05	0.03	0.08	0.05	0.01	0.04	0.1	0.03	0.06
B	Acidobacteria	Solibacteres	1.11	1.14	0.75	0.84	0.71	0.73	0.69	0.45	1.26	0.57	0.72	0.51
B	Acidobacteria	Sva0725	0.16	0.11	0.14	0.12	0.08	0.05	0.07	0.1	0.09	0.06	0.06	0.45
B	Acidobacteria	TM1	0.07	0.12	0.21	0.36	0.05	0.14	0.19	0.24	0.04	0.09	0.03	0.27

A.3 – Continued

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Actinobacteria	Acidimicrobiia	0.76	0.47	0.29	0.29	1.15	1.12	0.75	0.18	1.28	0.92	1.17	0.42
B	Actinobacteria	Actinobacteria	0.66	0.7	0.56	0.23	0.62	0.51	0.58	0.12	0.86	0.81	1.2	0.27
B	Actinobacteria	MB-A2-108	0.41	0.56	0.39	0.24	1.15	1.39	1.05	0.1	0.75	1.49	2.92	0.36
B	Actinobacteria	OPB41	0.08	0.04	0.02	0.02	0.04	0.02	0.01	0.05	0.05	0.02	0.27	0.06
B	Actinobacteria	Other	0	0	0	0.01	0	0.01	0	0	0.01	0.01	0.17	0.01
B	Actinobacteria	Other	0	0	0	0	0	0	0	0	0	0.01	0	0
B	Actinobacteria	Rubrobacteria	0.12	0.2	0.07	0.01	0.1	0.14	0.06	0	0.13	0.17	0.16	0
B	Actinobacteria	Thermoleophilia	2.5	2.65	1.08	0.62	5.69	6.24	3.87	1.63	5.33	4.61	6.96	0.76
B	Armatimonadetes	[Fimbriimonadia]	0.08	0.02	0.03	0.04	0.02	0.01	0.02	0.03	0.02	0.01	0.01	0.04
B	Armatimonadetes	0319-6E2	0.04	0.07	0.05	0.06	0.01	0.03	0.04	0.02	0.03	0.04	0.03	0.07
B	Armatimonadetes	Chthonomonadetes	0.03	0.06	0.04	0.02	0.02	0.03	0.02	0	0.02	0.03	0.02	0.01
B	Armatimonadetes	MD2902-B50	0	0	0	0	0	0	0.01	0.03	0	0	0.02	0.01
B	Armatimonadetes	SHA-37	0	0	0	0	0	0	0	0	0	0	0	0
B	Armatimonadetes	SJA-176	0.16	0.13	0.16	0.09	0.18	0.18	0.09	0.12	0.25	0.1	0.3	0.11
B	Bacteroidetes	[Rhodothermi]	0	0	0	0	0	0	0	0	0	0	0	0
B	Bacteroidetes	[Saprospirae]	1.01	0.63	0.3	0.15	0.53	0.43	0.18	0.19	0.67	0.4	0.23	0.31
B	Bacteroidetes	At12OctB3	0	0	0	0	0.01	0	0	0	0.01	0	0	0
B	Bacteroidetes	Bacteroidia	2.88	1.55	0.99	0.72	5.9	1.85	0.76	1	5.43	0.69	1.29	0.47
B	Bacteroidetes	Cytophagia	0.32	0.04	0.04	0.04	0.27	0.04	0.07	0.07	0.22	0.01	0.04	0.04
B	Bacteroidetes	Flavobacteriia	0.07	0.04	0.01	0	0.91	0.21	0.1	0	0.09	0.01	0.23	0
B	Bacteroidetes	Other	0	0	0	0	0	0	0	0	0	0	0	0
B	Bacteroidetes	Sphingobacteriia	0.11	0.04	0.05	0.04	0.35	0.08	0.03	0.01	0.08	0.01	0.04	0.06
B	Bacteroidetes	VC2_1_Bac22	0.02	0	0	0	0	0	0	0	0	0	0	0
B	BHI80-139	Other	0	0	0	0	0	0	0	0	0	0	0	0
B	BRC1	NPL-UPA2	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0	0	0.01
B	BRC1	Other	0	0.01	0.07	0.05	0.01	0.04	0.05	0.36	0.01	0	0.01	0.22
B	BRC1	PRR-11	0.06	0.06	0.07	0.07	0.07	0.14	0.15	0.06	0.07	0.05	0.03	0.07
B	Caldiserica	OP5	0	0	0	0	0	0	0	0	0	0	0	0
B	Caldithrix	Caldithrixae	0	0	0.04	0.03	0.01	0.01	0.01	0.03	0	0	0.03	0.01
B	Chlamydiae	Chlamydiia	0	0.01	0	0.02	0	0	0	0	0.01	0	0.01	0
B	Chlorobi	BSV26	0.74	0.54	0.93	1.72	1.19	0.92	1.01	3.29	0.92	0.35	0.54	1.82
B	Chlorobi	Ignavibacteria	0.45	0.22	0.17	0.3	0.61	0.35	0.13	3.21	0.56	0.08	0.14	0.26
B	Chlorobi	OPB56	0.07	0.01	0.01	0.01	0.04	0.01	0.01	0.02	0.03	0.01	0	0.01
B	Chlorobi	Other	0.05	0.01	0.02	0.02	0.03	0.02	0.02	0	0.03	0.01	0.01	0.01
B	Chlorobi	SJA-28	0.32	0.5	0.41	0.43	0.3	0.45	0.35	0.21	0.44	0.43	0.22	0.47
B	Chloroflexi	[Thermobacula]	0	0	0	0	0	0	0	0	0	0	0	0
B	Chloroflexi	Anaerolineae	6.02	6.68	6.4	6.88	6.16	5.68	6.45	4.36	7.51	5.23	6.86	7.2

A.3 – Continued (2)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Chloroflexi	C0119	0.03	0.04	0.01	0	0.01	0.01	0.01	0	0.02	0.03	0.01	0
B	Chloroflexi	Chloroflexi	0.23	0.52	0.25	0.12	0.1	0.26	0.21	0	0.15	0.96	0.27	0.08
B	Chloroflexi	Dehalococcoidetes	0.12	0.13	0.29	0.62	0.65	0.41	0.3	1.57	0.55	0.19	0.83	1.13
B	Chloroflexi	Ellin6529	0.41	0.41	0.46	0.55	0.52	0.52	0.73	0.33	0.75	0.64	1.18	0.88
B	Chloroflexi	Gitt-GS-136	0.11	0.04	0.03	0.02	0.05	0.06	0.04	0	0.06	0.05	0.02	0
B	Chloroflexi	Ktedonobacteria	0.03	0.04	0.1	0.03	0.01	0.01	0	0	0.01	0.01	0.03	0
B	Chloroflexi	Other	0	0.06	0.08	0.02	0	0.02	0.02	0	0.01	0.09	0.01	0.01
B	Chloroflexi	Other	0	0	0	0.01	0	0	0	0	0	0	0	0
B	Chloroflexi	P2-11E	0	0.01	0	0	0	0	0	0	0	0	0	0
B	Chloroflexi	S085	0.74	2.44	3.16	4.2	0.49	1.67	3.54	2.5	0.54	2.73	6.64	5.7
B	Chloroflexi	SAR202	0	0.02	0.06	0.06	0	0.01	0.02	0.01	0.01	0.03	0.01	0.05
B	Chloroflexi	SHA-26	0.01	0.02	0.01	0.01	0.01	0.01	0	0.01	0.02	0.01	0.01	0.01
B	Chloroflexi	Thermomicrobia	0.04	0.06	0.09	0.06	0.03	0.08	0.06	0.01	0.04	0.11	0.13	0.06
B	Chloroflexi	TK10	0.13	0.24	0.17	0.13	0.04	0.14	0.13	0.02	0.08	0.21	0.09	0.12
B	Chloroflexi	TK17	0.1	0.08	0.2	0.29	0.06	0.07	0.18	0.19	0.07	0.11	0.13	0.36
B	Cyanobacteria	4C0d-2	0	0	0	0	0.01	0	0	0	0	0	0.01	0
B	Cyanobacteria	Chloroplast	0.11	0.05	0.03	0.03	0.01	0	0	0	0.01	0	0.04	0.02
B	Cyanobacteria	ML635J-21	0	0	0	0	0.01	0	0	0	0	0	0	0
B	Cyanobacteria	Nostocophycideae	0	0	0	0	0.01	0	0	0	0	0	0.01	0
B	Cyanobacteria	Oscillatoriophycideae	0	0	0	0	0	0	0	0	0	0	0.01	0
B	Cyanobacteria	Other	0.01	0	0	0	0.02	0.03	0	0	0	0	0.01	0.01
B	Elusimicrobia	Elusimicrobia	0.07	0.03	0.04	0.03	0.1	0.05	0.03	0.02	0.1	0.02	0.04	0.03
B	Elusimicrobia	Endomicrobia	0.01	0	0.01	0.01	0.02	0.01	0	0.01	0.02	0	0.04	0.01
B	Elusimicrobia	OP2	0	0	0	0	0.01	0.01	0	0	0.02	0	0.01	0.01
B	Elusimicrobia	Other	0.03	0.05	0.04	0.07	0.04	0.09	0.04	0.04	0.06	0.06	0.04	0.06
B	FCPU426	Other	0.04	0.01	0.01	0.01	0.04	0.01	0.01	0	0.03	0.01	0.01	0.01
B	Fibrobacteres	B5-096	0	0	0	0	0	0	0	0	0.01	0.01	0	0
B	Fibrobacteres	Fibrobacteria	0.02	0	0	0	0.02	0	0	0	0.01	0.01	0	0
B	Fibrobacteres	Other	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0	0.03	0.01	0.01	0
B	Fibrobacteres	Other	0	0	0	0	0.01	0	0	0	0.01	0	0	0
B	Fibrobacteres	SBZC_2415	0	0	0	0	0.01	0	0	0	0.01	0	0.01	0
B	Fibrobacteres	TG3	0.04	0.01	0.03	0.03	0.17	0.05	0.02	0.03	0.16	0.01	0.11	0.04
B	Firmicutes	Bacilli	0.33	0.73	0.41	0.12	0.33	0.56	0.47	0.06	1.52	0.58	1.91	0.16
B	Firmicutes	Clostridia	0.2	0.35	0.43	0.32	0.39	0.18	0.11	0.11	0.52	0.12	6.96	0.36
B	Firmicutes	Erysipelotrichi	0	0	0	0	0	0	0	0	0	0	0.02	0
B	Fusobacteria	Fusobacteriia	0	0.01	0	0.01	0.01	0	0	0	0	0.01	0.03	0
B	GAL15	Other	0.02	0.12	0.22	0.42	0.08	0.1	0.17	0.24	0.03	0.07	0.18	0.9

A.3 – Continued (3)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	Gemmatimonadetes	Gemm-1	2.77	4.21	4.1	2.9	1.31	2.78	5.56	1.15	1.39	3.53	1.08	2.21
B	Gemmatimonadetes	Gemm-2	0.19	0.08	0.08	0.14	0.04	0.1	0.15	0.08	0.04	0.06	0.03	0.07
B	Gemmatimonadetes	Gemm-3	0.01	0	0	0	0	0	0	0	0	0	0	0
B	Gemmatimonadetes	Gemm-5	0.41	0.43	0.29	0.13	0.12	0.43	0.32	0	0.18	0.32	0.1	0.07
B	Gemmatimonadetes	Gemmatimonadetes	1.08	1.21	1.13	0.98	0.48	1.62	1.47	0.92	0.98	2.02	0.75	0.66
B	Gemmatimonadetes	JL-ETNP-Z39	0.01	0	0.02	0.08	0.02	0.02	0.02	0.08	0.01	0.02	0.04	0.07
B	Gemmatimonadetes	Other	0.01	0.01	0	0	0.01	0.01	0.01	0	0.01	0	0.01	0
B	GN02	3BR-5F	0.02	0	0.01	0.01	0.01	0	0.01	0.06	0.01	0.01	0	0.02
B	GN02	BB34	0.01	0.01	0.03	0.02	0.03	0.04	0.02	0.02	0.03	0	0.02	0.02
B	GN02	GKS2-174	0	0	0	0	0	0	0.01	0.01	0	0	0	0
B	GN02	GN10	0	0	0	0	0	0	0	0	0	0	0	0
B	GN04	5bav_B12	0	0	0	0.01	0	0	0	0	0	0	0	0.02
B	GN04	GN15	0.04	0.04	0.07	0.09	0.09	0.04	0.06	0.12	0.04	0.02	0.01	0.05
B	GN04	MSB-5A5	0.02	0.24	0.69	1.43	0.01	0.09	0.51	1.92	0.01	0.1	0.09	0.86
B	GN04	Other	0.12	0.16	0.16	0.34	0.16	0.13	0.27	0.42	0.14	0.08	0.09	0.29
B	GOUTA4	Other	0.2	0.15	0.1	0.11	0.21	0.14	0.13	0.1	0.22	0.15	0.15	0.04
B	Hyd24-12	WM88	0	0	0	0	0.02	0	0.01	0	0.01	0	0	0
B	KSB3	MAT-CR-H3-D11	0	0	0	0	0	0	0	0	0	0	0	0
B	KSB3	Other	0.01	0.02	0.01	0.01	0.04	0.01	0	0	0.01	0	0.01	0
B	LCP-89	SAW1_B44	0	0.01	0.01	0.01	0.11	0.03	0.01	0.01	0.12	0.02	0.03	0.01
B	LD1	Other	0	0	0	0	0.01	0	0	0.14	0.01	0	0.1	0.04
B	Lentisphaerae	[Lentisphaeria]	0.02	0.02	0.01	0.01	0.05	0.01	0	0	0.03	0	0.01	0
B	MVS-104	Other	0.02	0.06	0.08	0.09	0.02	0.03	0.05	0.25	0.04	0.04	0.05	0.11
B	NC10	24-Dec	1.87	5.23	4.83	3.66	1.46	3.99	4.52	0.43	1.37	5.93	1.7	2.18
B	NC10	Other	0	0	0.01	0	0.01	0	0	0.01	0.01	0	0	0
B	NC10	Other	0	0.01	0.01	0	0	0	0	0	0	0	0	0
B	NC10	wb1-A12	0.01	0.37	0.21	0.02	0	0.03	0.02	0	0	0.02	0	0
B	Nitrospirae	Nitrospira	5.76	10.54	10.84	8.84	5.44	10.67	10.95	7.72	6.4	11.88	5.66	8.61
B	NKB19	Other	0.09	0.06	0.07	0.12	0.13	0.09	0.06	0.15	0.1	0.04	0.04	0.08
B	NKB19	TSBW08	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0	0	0
B	OC31	Other	0	0	0	0	0	0	0	0	0	0	0	0
B	OD1	ABY1	0	0	0	0.01	0	0	0	0.01	0.01	0	0	0
B	OD1	Mb-NB09	0	0	0	0.01	0	0	0	0.01	0.01	0	0.01	0
B	OD1	Other	0.01	0.01	0	0.04	0	0	0	0.04	0.01	0	0.02	0.03
B	OD1	SM2F11	0	0	0	0	0	0	0	0	0	0	0.01	0
B	OD1	ZB2	0	0	0	0	0	0	0	0	0	0	0	0
B	OP1	[Acetothermia]	0	0	0	0.02	0	0	0	0	0	0	0	0.01

A.3 – Continued (4)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	OP1	MSBL6	0	0	0	0	0.01	0	0	0.01	0	0	0	0
B	OP11	OP11-3	0.03	0.02	0.02	0.01	0.01	0	0	0.04	0.02	0.01	0.04	0.02
B	OP11	OP11-4	0	0	0	0	0	0	0	0.01	0	0	0	0
B	OP11	Other	0.01	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0	0.04	0.03
B	OP11	WCHB1-64	0	0	0	0	0	0	0	0	0	0	0	0
B	OP3	BD4-9	0.03	0.03	0.02	0.04	0.07	0.03	0.01	0.04	0.08	0.03	0.01	0.07
B	OP3	koll11	0.03	0.05	0.06	0.08	0.22	0.1	0.07	0.19	0.14	0.04	0.06	0.18
B	OP3	Other	0	0	0	0	0.01	0	0	0	0.01	0	0	0.01
B	OP3	PBS-25	0.02	0	0	0.01	0.02	0	0	0	0.02	0	0	0
B	OP8	OP8_1	0.11	0.12	0.27	0.25	0.34	0.2	0.36	2.13	0.32	0.06	0.44	0.35
B	OP8	OP8_2	0	0.03	0	0	0.13	0.01	0.02	0	0.05	0.01	0.01	0
B	OP8	SAW1_B6	0	0	0	0	0.02	0	0	0	0	0	0	0
B	OP9	JS1	0	0	0	0	0.01	0	0	0.01	0.01	0	0	0
B	Other	Other	0.03	0.03	0.02	0.03	0.05	0.03	0.01	0.05	0.03	0.03	0.01	0.04
B	PAUC34f	Other	0.01	0	0.01	0.01	0.02	0	0	0	0.01	0	0.01	0.01
B	Planctomycetes	[Brocadiae]	0	0	0.01	0.12	0	0	0	0.04	0	0	0	0.01
B	Planctomycetes	028H05-P-BN-P5	0.01	0	0.01	0	0	0	0	0	0.01	0.01	0	0
B	Planctomycetes	BD7-11	0.01	0.01	0.01	0.02	0	0	0.01	0	0.03	0	0.08	0
B	Planctomycetes	C6	0.02	0.01	0.01	0.01	0.01	0	0.01	0	0.02	0	0	0
B	Planctomycetes	ODP123	0.06	0.07	0.07	0.03	0.05	0.04	0.04	0.04	0.07	0.06	0.03	0.05
B	Planctomycetes	OM190	0.3	0.08	0.1	0.07	0.1	0.05	0.07	0.01	0.11	0.04	0.02	0.02
B	Planctomycetes	Other	0.06	0.04	0.03	0.04	0.03	0.02	0.03	0.01	0.04	0.02	0.01	0.02
B	Planctomycetes	Phycisphaerae	1.94	1.64	1.62	1.81	1.89	1.65	1.46	4.93	2.6	1.24	1.45	1.56
B	Planctomycetes	Pla3	0.19	0.1	0.16	0.14	0.05	0.06	0.1	0.04	0.1	0.06	0.03	0.09
B	Planctomycetes	Pla4	0	0	0	0	0	0	0	0	0	0	0	0
B	Planctomycetes	Planctomycetia	0.79	0.52	0.35	0.48	0.47	0.38	0.35	0.22	0.52	0.36	0.91	0.65
B	Planctomycetes	vadinHA49	0	0	0	0	0.01	0	0	0	0.02	0.01	0	0
B	Poribacteria	Other	0	0	0	0	0.02	0.01	0	0.01	0.02	0	0.01	0.01
B	Proteobacteria	Alphaproteobacteria	7.05	4.97	5.64	6.66	6.56	6.28	6.45	4.34	7	5.44	9.57	13.18
B	Proteobacteria	Betaproteobacteria	10.98	7.73	10.76	10.58	11.85	9.92	10.35	8.66	7.01	7.89	3.41	8.91
B	Proteobacteria	Deltaproteobacteria	21.62	21.69	21.96	23.11	16.26	18.02	17.68	19.82	17.14	21.25	13.82	19.03
B	Proteobacteria	Epsilonproteobacteria	0.01	0.01	0	0.01	2.29	0.02	0	0	0.56	0	0.01	0.01
B	Proteobacteria	Gammaproteobacteria	4.8	1.36	1.63	2.21	4.94	2.48	1.7	2.71	3.39	1.91	4.27	1.75
B	Proteobacteria	Other	0.37	0.48	0.32	1.21	0.14	0.19	0.4	1.62	0.11	0.02	0.17	1.25
B	Proteobacteria	TA18	0	0	0	0	0.01	0	0	0	0.01	0	0	0
B	Proteobacteria	Zetaproteobacteria	0	0	0	0	0.01	0	0	0	0.01	0	0.02	0
B	SBR1093	Other	0.03	0.25	0.36	0.17	0.03	0.24	0.2	0	0.05	0.2	0.07	0.14

A.3 – Continued (5)

			Site 1 – *C. stricta*				Site 2 – *E. erythropoda*				Site 3 – 50/50 Mix			
K	Phylum	Class	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
B	SBR1093	VHS-B5-50	0.01	0	0.01	0.06	0	0.01	0.02	0.04	0	0	0.02	0.02
B	SC4	Other	0.08	0.17	0.1	0.06	0.07	0.13	0.09	0.07	0.11	0.12	0.09	0.05
B	Spirochaetes	[Brachyspirae]	0	0	0	0.02	0	0	0	0.02	0.01	0	0.02	0.02
B	Spirochaetes	[Brevinematae]	0.01	0	0	0	0.03	0.01	0	0.01	0.04	0.01	0.02	0.01
B	Spirochaetes	[Leptospirae]	0.13	0.08	0.1	0.06	0.22	0.07	0.02	0.04	0.17	0.04	0.07	0.03
B	Spirochaetes	GN05	0	0	0	0	0	0	0	0	0	0	0.01	0
B	Spirochaetes	MVP-15	0	0	0	0	0	0	0	0	0	0	0	0
B	Spirochaetes	Spirochaetes	0.47	0.46	0.45	0.38	1.04	0.5	0.43	0.49	0.85	0.3	0.45	0.37
B	SR1	Other	0	0	0	0	0	0	0	0	0	0	0	0
B	TA06	Other	0	0	0.01	0	0	0	0.01	0	0	0	0	0
B	Tenericutes	Mollicutes	0	0	0	0	0.06	0	0	0	0.02	0.01	0	0
B	TM6	SBRH58	0.02	0.01	0	0.01	0.03	0.01	0	0.01	0.03	0.01	0.02	0.02
B	TM6	SJA-4	0.07	0.04	0.04	0.05	0.08	0.05	0.03	0.1	0.06	0.01	0.06	0.2
B	TM7	MJK10	0	0	0	0	0	0	0	0	0	0	0	0
B	TM7	Other	0.02	0	0	0	0.13	0	0	0	0	0	0	0
B	TM7	SC3	0.01	0	0	0	0.02	0	0	0	0	0	0	0
B	TM7	TM7-1	0.03	0.01	0.03	0.01	0.08	0.01	0.02	0.02	0.02	0.01	0.03	0.04
B	TM7	TM7-3	0.01	0	0	0	0.02	0	0	0	0.01	0	0.02	0
B	TPD-58	Other	0	0	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.06	0.01
B	Verrucomicrobia	[Methylacidiphilae]	0.08	0.07	0.1	0.08	0.1	0.04	0.08	0.02	0.06	0.03	0.02	0.05
B	Verrucomicrobia	[Pedosphaerae]	2.73	2.04	1.74	0.98	1.5	1.43	1.14	0.21	2.05	1.57	0.98	0.53
B	Verrucomicrobia	[Spartobacteria]	0.42	0.27	0.11	0.02	0.3	0.26	0.09	0	0.44	0.18	0.2	0.01
B	Verrucomicrobia	Opitutae	0.2	0.08	0.07	0.06	0.21	0.12	0.11	0.01	0.25	0.1	0.05	0.05
B	Verrucomicrobia	Other	0	0	0	0	0	0	0	0	0	0	0	0.01
B	Verrucomicrobia	Verruco-5	0.01	0.01	0.03	0.01	0.08	0.02	0.02	0.01	0.06	0.01	0.01	0.01
B	Verrucomicrobia	Verrucomicrobiae	0.03	0.01	0	0	0.03	0	0.01	0	0.02	0	0.01	0
B	VHS-B3-43	Other	0	0	0	0	0	0	0	0.01	0	0	0.02	0
B	WPS-2	Other	0	0	0	0	0.01	0	0	0	0	0	0.01	0
B	WS1	Other	0.01	0.01	0	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
B	WS2	Kazan-3B-09	0.01	0.01	0.04	0.02	0.03	0.05	0.03	0.07	0.03	0	0.01	0.05
B	WS2	Other	0	0	0	0	0	0	0	0	0	0	0	0.01
B	WS2	SHA-109	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0	0.03	0	0.01	0
B	WS3	PRR-12	2.04	1.76	1.78	1.7	1.3	1.38	2.62	0.54	1.27	1.38	0.77	1.03
B	WS4	Other	0.01	0.02	0	0	0.01	0.01	0.01	0	0.01	0.02	0.1	0.01
B	WS5	Other	0.01	0	0	0	0	0	0	0	0	0	0	0
B	WWE1	[Cloacamonae]	0	0	0	0	0.01	0	0	0	0.01	0	0	0
B	ZB3	Other	0	0	0	0	0	0	0	0	0.01	0	0	0