Impact of land use on microbial communities in the Great Bend Prairie Aquifer

by

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Abstract

The High Plains aquifer is a vital source of irrigation and drinking water in the central United States. Aquifer geochemistry and water quality can be significantly affected by microbially mediated redox reactions. Therefore, it is important to understand how environmental controls on aquifer microbial communities affect the health of the aquifer. This study examines the relationship between land use and aquifer microbiology in the Great Bend Prairie aquifer, a portion of the High Plains aquifer in south-central Kansas. In the summer of 2018, we collected samples from soils of different land use near groundwater monitoring wells that were sampled two years prior. The soil samples were collected in replicates of four at each well site, and the groundwater was sampled from wells screened in the shallow and deep aquifer at each site. Results demonstrate significant variation in nitrate levels with land use in both soils and groundwater. Soil extractable NO_3-N in irrigated crop soils is significantly higher (86.05 mg/kg) than in pasture (3.84 mg/kg) and non-irrigated crop (29.48 mg/kg) soils on average. Shallow wells below crop soils contain average NO_3-N concentrations three times higher than those of the shallow wells below pasture soils and of the deep wells, at 18 mg/L. Coupled with this impact, microbial community composition also varies significantly with land use in both soils and groundwater. Our statistical analysis shows that NO_3-N and soil microbial communities are significantly correlated, with land use as the best defining factor in soil community similarity. Groundwater microbial communities were significantly correlated with land use and geographic markers, with well depth as the best defining factor in groundwater microbial community. Specifically, the aquifer contains many genera capable of participating in the nitrification and denitrification processes, including genera within bacterial orders *Nitrospirales* (nitrification) and *Pseudomonadales* (denitrification), as well as archaeal phylum *Crenarchaeota* (nitrification and nitrite reduction). Shallow crop wells have the highest average relative abundances of *Pseudomonadales* (12.14%) and *Nitrospirales* (7.03%), compared to other wells. Deep crop wells have the highest average relative abundances of *Crenarchaeota* (3.25%). Taken together, our results show that land use not only affects the chemistry and microbiology of soils but also the underlying water table aquifer. These findings advance our understanding of environmental controls on aquifer microbiology.

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Dedication

This thesis would not have been possible without my Lord and Savior, Jesus Christ. I would never have considered pursuing degrees in Geology without the curiosity, passion, incredible mentors, support people, funding opportunities, and everything else that He has given me in my life journey thus far.

Chapter 1 - Introduction

Groundwater is a critical resource globally. It ranked in 2013 as the most extracted raw material in the world, with an estimated withdrawal rate of 982 km^3/year (2019a). According to 2010 estimates, India, China, and the United States were the top three extractors of groundwater at 231.00 km^3/year, 111.95 km^3/year, and 111.70 km^3/year, respectively (2019a). Around 70% of the groundwater extracted worldwide is used for agriculture purposes, with about 38% of irrigated lands in the world using groundwater as their water source (Siebert et al. 2010). In addition, an estimated 50% of drinking water worldwide is sourced from groundwater (2019a).

These statistics highlight the importance of understanding variables involved in groundwater quality to ensure the continued use of aquifers as water resources. Potential threats to groundwater quality include over-withdrawal, climate change, and contamination from agriculture, aquifer bedrock deposits, underground storage tanks, and hydraulic fracking (Böhlke 2002; Green et al. 2011; Warner et al. 2012; Kirk et al. 2016; Ham et al. 2017). Trace heavy metals (e.g. arsenic) are toxic in small concentrations, while other compounds like nitrate become dangerous with increased concentrations (Nolan 2005; J. O. Nriagu et al. 2007; Richard et al. 2014; Zheng and Ayotte 2015). Microbially-driven redox reactions in groundwater can increase or decrease proportions of mobile and bioavailable compounds like arsenic and nitrate (Khan and Hoque 2003; Weber et al. 2006; Wallenstein et al. 2006; Kirk et al. 2015; Whaley-Martin et al. 2016; Kumar et al. 2017). Thus, understanding controls on the composition and function of aquifer microbial communities is important because microorganisms can strongly influence the chemistry of aquifers.

In this study we consider the impact of land use on aquifer microbiology. Previous studies have shown that land use can significantly influence soil microbiology as well as soil chemical and physical properties (Huang et al. 2002; Jiang et al. 2011; Shi et al. 2019). For example, long-term soil tillage and crop cultivation has been found to decrease biomass and potential enzymatic activity of soil microbiology, decrease percentage of water-stable soil aggregates, increase erosion of topsoil, and increase soil NO_3-N and available soil P (Karlen et al. 1999; Huang et al. 2002; Murphy et al. 2006; Dorr de Quadros et al. 2012; Zuber and Villamil 2016).

Previous studies have also shown that land use can affect the chemistry of aquifers (Spalding 1984; Lasagna et al. 2016). Below well-drained cropland soils, high nitrate concentrations have been measured in the intermediate vadose zone and in shallow aquifer wells (Spalding 1984; Spalding and Kitchen 1988). Mobility of nitrate in soil, irrigation and N-fertilizer application rates, and soil texture contribute to the rapid movement of nitrate into and attenuation of nitrate within underlying water table aquifers (Spaldingb et al. 1978; Lasagna et al. 2016).

Whether land use also affects the microbiology of aquifers remains unclear. The goal of our study is to examine this issue. Specifically, we examine whether aquifer microbiology varies significantly with land use and consider the resemblance between aquifer and soil microbial communities.

We addressed these goals in the Great Bend Prairie aquifer, a portion of the High Plains aquifer located in south-central Kansas. This aquifer is an ideal location for our analysis because several variables make it sensitive to surface activities. In the field area, the water table is only about 20 meters below land surface, increasing the potential for soil microbial communities and geochemical environments to influence groundwater quality. In addition, most of the soils in our field area are sandy, providing permeable and porous media through which regular percolation of water containing nutrients and microbes can occur. The field area also contains different land uses to compare with each other, including non-irrigated cropland, irrigated cropland, and pasture. Finally, previous research on this portion of the aquifer was conducted by Don Whittemore in the 1970s and by Alexandria Richard from 2016 to 2018, providing comparative points in time to consider as well as a larger pool of data for statistical analysis (Whittemore 1993; Richard 2018).

Chapter 2 - Background

Great Bend Prairie Aquifer

The High Plains aquifer is one of the world's largest freshwater aquifers, stretching under 112 million acres from South Dakota to Texas (Lapham et al. 2005). The aquifer supplies water to 30 percent of all irrigation systems using groundwater in the U.S (Dennehy 2000). It also provides drinking water to 82 percent of the population living above the aquifer system (Dennehy 2000). The Great Bend Prairie aquifer is a portion of the High Plains aquifer in south-central Kansas. It largely coincides with the Groundwater Management District #5 (GMD 5) and lies under the counties of Pratt, Stafford, Barton, and Edwards, as well as parts of Kiowa, Pawnee, Reno and Rice (Figure 2-1).

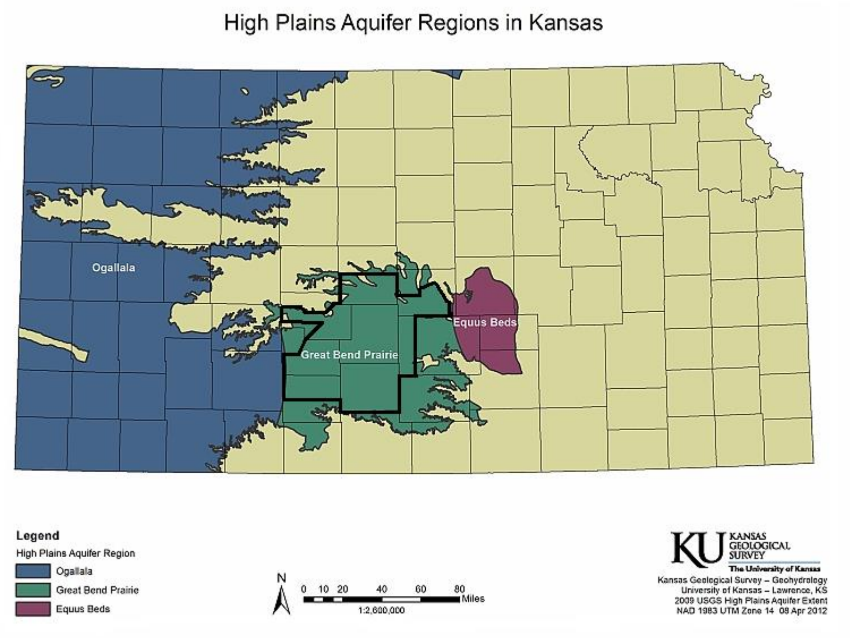

Figure 2-1: Placement of Great Bend Prairie aquifer and GMD 5 in Kansas, adapted from "High Plains Aquifer Regions in Kansas," 2012.

The Great Bend Prairie aquifer is an unconsolidated aquifer flowing eastward in Kansas through interbedded silts, sands, and clays deposited by the Arkansas River (Whittemore 1993). The bedrock of the aquifer consists of Cretaceous and Permian shales, sandstones, siltstones, and evaporites (Whittemore 1993). Because parts of the Permian bedrock contain salt deposits, salt intrusion of the lower aquifer waters has been found (Whittemore 1993) but does not seem to be spreading to upper aquifer waters as a result of agricultural overdraft of well water (Whittemore 1993; Richard 2018). The upper aquifer may be protected from salt intrusion by low permeability clays or by horizontal groundwater flow (Whittemore 1993).

Groundwater nitrate levels in the Great Bend Prairie aquifer were found to be higher in the shallow freshwater than in the more saline bedrock waters in the late 1970s and 1980s (Whittemore 1993). More recent examination shows increasing nitrate levels in twenty of twenty-four wells sampled in 2016, with more rapid increase of nitrate concentrations in shallow aquifer wells (Richard 2018), as shown in Figure 2-2. In the late 1970s and 1980s, nitrate levels exceeded the EPA safety limit of 10 mg/L for public drinking water in three of twenty-four wells (Whittemore 1993). By 2016, seven of twenty-four wells had nitrate concentrations above 10 mg/L (Richard 2018).

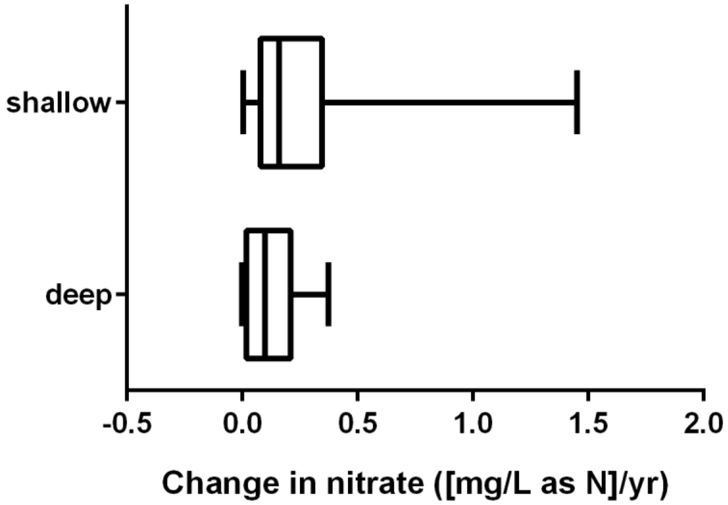

Figure 2-2: Rate of change in nitrate in shallow and deep aquifer wells between the 1970s and 2016. Made by Kirk from 2016 data (Richard 2018).

Indeed, rising groundwater nitrate levels have been correlated with land use in several other studies as well. Specifically, high nitrate concentrations have been measured in the vadose zone and in shallow aquifer wells below well-drained cropland soils (Spaldingb et al. 1978; Sophocleous 1990). Nitrate movement through soils into shallow aquifers has been shown to be directly correlated to precipitation, irrigation, and rate of N-fertilizer application. Sandy and loamy soils allow high leaching rates of nitrate, and fine-grained soils slow but do not stop vertical nitrate movement toward shallow aquifers (Spaldingb et al. 1978; Spalding 1984; Puckett et al. 2011) In addition, zones of nitrate concentration have been measured in groundwater, with higher concentrations in the upper shallow aquifer and decreasing concentrations in the lower shallow aquifer to deep aquifer (Spalding 1984). These zones can represent decades of nitrate accumulation in the groundwater (Puckett et al. 2011).

Rising nitrate concentrations in groundwater and the deleterious effects of water with high nitrate levels on humans have been increasingly documented in recent years (Spalding and Kitchen 1988; Nolan 2005; Rivett et al. 2008; Richard et al. 2014). However, some microbes can act as natural remediators by reducing nitrate into other forms of nitrogen (e.g. NO_2^-, NH_3, NH_4^+, NO, N_2O, N_2 gas) (Lasagna et al. 2016). Microbial respiration of nitrate to N_2 gas (denitrification) has long been considered the primary nitrate removal process from the biologically reactive pool (Lasagna et al. 2016). Growing evidence shows dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (Anammox) as important alternative nitrogen transformation pathways that are also microbially mediated (Burgin and Hamilton 2007; Nelson et al. 2016). Because microbial activities drive many redox reactions in aquifers, they can have a major influence on groundwater chemistry and quality (Puckett et al. 2011; Kirk et al. 2015; Ham et al. 2017). As a result, aquifer microbial communities are important to consider when seeking to understand the quality of groundwater.

The High Plains aquifer appears to have an abundant supply of dissolved oxygen, suggesting that aerobic respiration is the overall dominant process (Figure 2-3). However, the reduction of nitrate through denitrification, DNRA, or Anammox occurs in anoxic conditions, or environments with very little to no oxygen (McMahon and Chapelle 2008; Bethke et al. 2011; Madigan et al. 2018). The presence of oxygen in the aquifer implies a limited capacity for nitrate reduction. Nonetheless, some anaerobic reaction zones appear to be present even in the shallow aquifer and may have a significant impact in reducing ever-increasing levels of nitrate (McMahon and Chapelle 2008; Lasagna et al. 2016). Indeed, the aquifer’s interbedded clay layers would allow limited flow of water into and through them (Ochsner 2019), creating anoxic microenvironments in which anaerobic microbes would thrive. Similarly, anoxic microenvironments in oxic stream hyporheic zones have been shown to play an important role in denitrification (DeLong and Rosenberg 2013; Wondzell and Zarnetske 2014).

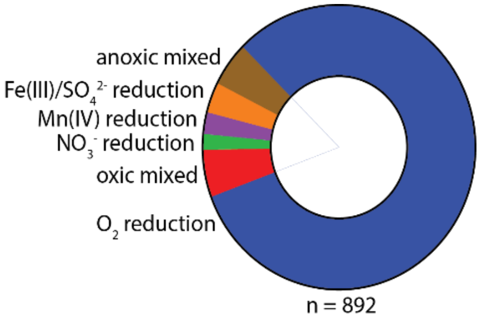

Figure 2-3: Redox processes occurring in the High Plains aquifer based on the characterization by Kirk of 892 groundwater samples taken from the USGS National Water Information System (McMahon and Chapelle 2008).

GMD 5 Soils

Pratt, Stafford, Barton, and Edwards are the four primary counties above the Great Bend Prairie aquifer in GMD 5. More detailed county-wide soil descriptions are in Appendix A, and all county soil descriptions are sourced from the Web Soil Survey of the United States Department of Agriculture's Natural Resources Conservation Service (2014a). In general, loamy soils are consistent throughout each of the four counties, with some more clayey soils and many sandy soils interbedded. The percent sand of GMD 5 soils tends to be higher compared to that of other Kansas soils (Figure 2-4). In general, the soils of all four counties drain well, with little ponding. As a result, these soils will likely tend to be oxic environments that encourage dominantly aerobic microbial communities (Koike and Hattori 1978; Hoffmann et al. 2007; Ochsner 2019).

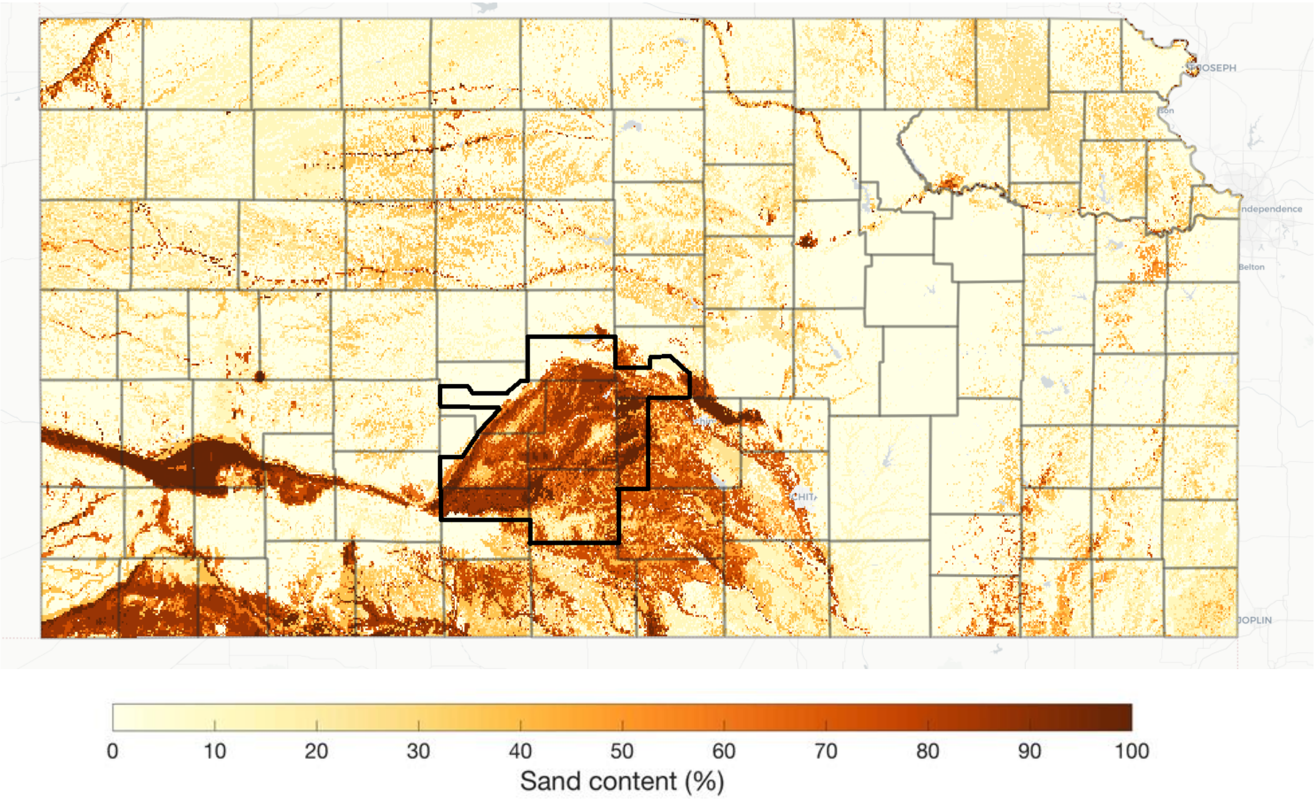

Figure 2-4: Percent sand in soils across Kansas at a resolution of 800 m and a depth of 20 cm, with the outline of GMD 5, modified from NCRS data compilation (Patrignani 2018).

The Carwile soil unit in the Stafford and Edwards counties is more of an outlier, however, having a mixture of clayey loam and sandy loam that drains somewhat poorly, with frequent ponding. As a result, depth to water table in the Carwile soil tends to be 0 inches, while other soil profiles in the four counties tend to have a depth to water table of over 80 inches. The frequent ponding and poor drainage of the Carwile soils would limit soil oxygen availability more than surrounding soils, increasing the probability of anoxic conditions conducive to higher rates of denitrification (Or et al. 2007; Madigan et al. 2018; Ochsner 2019).

The soils within GMD 5 are classified as a mixture of mollisols, alfisols, and entisols, compared to the general soil class of mollisols across the state of Kansas (1998). Most of the soils originated as alluvial deposits of the Arkansas River, with more recent overlying alluvial deposits from multiple area streams and with wind-reworked surface soils (Whittemore 1993; Evans 2015). Trends in soil type near our sampled wells seem to result more from geologic depositional history, rather than from the weathering of the soils through time (Figure 2-5).

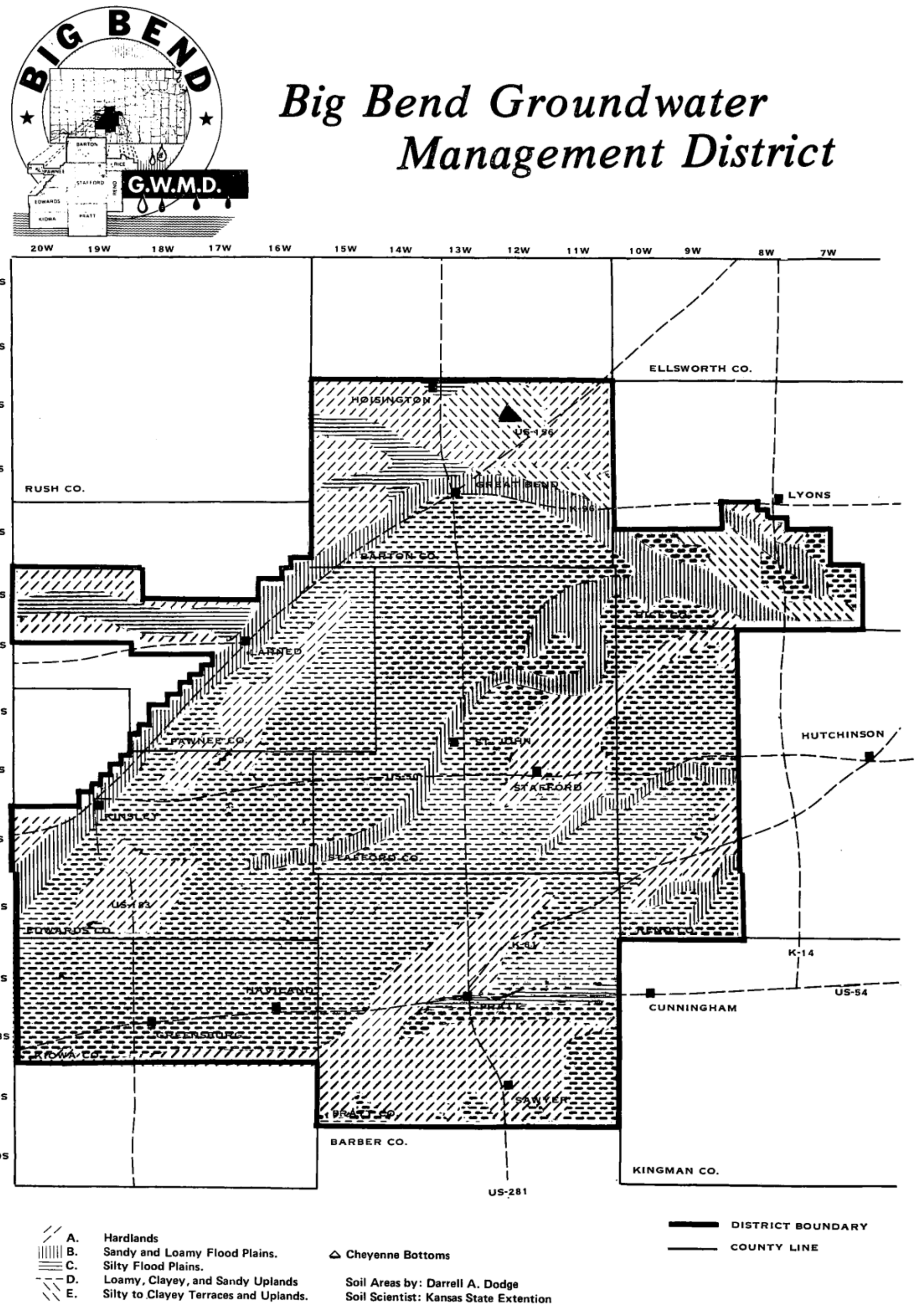

Figure 2-5: Map of area soils with geological history descriptors (1979).

Impacts of Land Use

Impact of Land Use on Soils

Land management practices affect soil in many ways, including pH, compaction, organic matter, microbial activity, and topsoil erosion across a range of soil types (Huang et al. 2002; Dorr de Quadros et al. 2012; Zuber and Villamil 2016). Long-term cultivation decreases soil quality by changing soil structure, decreasing soil organic matter and biological activity, altering soil pH, and increasing soil bulk density, erosion of topsoil by wind and runoff, and soil compaction (Huang et al. 2002; Jiang et al. 2011). Cultivated fields also tend to contain higher levels of soil NO_3-N, most likely as a result of N-fertilizer application (Karlen et al. 1999; Huang et al. 2002).

In pastures, grazing has been found to have a range of effects on soil, from being harmful to helping replenish nutrients, depending on the grazing methods employed, soil quality indicators measured, and other factors. For example, grazing decreases aboveground biomass and leaf surface, which can lead to decreased soil respiration under drought conditions (Shi et al. 2019). Trampling can decrease soil pore size and amount; however, it can also augment the physical breakdown of plant residues, which in turn, can increase litter decomposition rate under wet conditions (Shi et al. 2019). Cattle grazing and excreta inputs can increase soil available N and its spatial heterogeneity in areas with high and low plant diversity (Liu et al. 2016). In steppe topsoils, physical and chemical parameters deteriorated significantly after heavy grazing, while the parameters remained stable under reduced grazing management (Steffens et al. 2008).

Covering cultivated fields with grasses for 5-10 years (e.g. through the Conservation Reserve Program) can reverse some of these changes by increasing soil organic matter and microbial biomass carbon and decreasing erosion (Huang et al. 2002; Murphy et al. 2006).

Overall, higher levels of soil C, N, and organic matter, along with lower soil bulk density, have been found in soils of never cultivated fields (Murphy et al. 2006; Dorr de Quadros et al. 2012).

Soil Microbial Response to Land Use

Coupled with these impacts to soil quality, land use also affects biological activity and the composition of microbial communities (Huang et al. 2002; Dorr de Quadros et al. 2012). Microbial communities drive many of the reactions occurring in soils either directly or indirectly, including redox reactions, hydrolysis reactions, and acid-base reactions, among others (Treusch et al. 2005; Nunan et al. 2007; Strickland et al. 2009; Nelson et al. 2016; Jelen et al. 2016; Fierer 2017). Fungi are the primary decomposers of complex carbon molecules (e.g. lignin, cellulose), and specialized mycorrhizal fungi supply plant with important minerals and water (Madigan et al. 2018). Certain bacterial extracellular enzymes are also involved in the decomposition of organic carbon molecules; as a result, measured soil enzymatic activity ratios can indicate microbial nutrient limitation (Sinsabaugh and Follstad Shah 2012; Madigan et al. 2018). In addition, high enzymatic activity can indicate higher rates of bioavailable nutrient release, such as nitrogen, phosphorous, and organic compounds (Rousk et al. 2010; Hsiao et al. 2016).

Soils contain diverse microbial communities that can be affected by changes to the soil environment (Or et al. 2007). Tilling and compaction of soil changes soil structure, which in turn affects soil aerobic and anaerobic zones that result from oxygen availability and water percolation ability (Turlapati et al. 2015; Keiluweit et al. 2017). Coupled nitrification-denitrification, specifically, has been shown to occur in the oxic and anoxic zonation of aggregates larger than 0.5 cm and increased NH_4^+ availability, with denitrification taking over as the dominant N-process in aggregate sizes above 1.5 cm (Kremen et al. 2005; Hoffmann et al.

2007; Ayoubi et al. 2011). Addition of fertilizers affects soil water pH and soil nutrient availability, which are key factors in microbial community composition (Bru et al. 2011; Turlapati et al. 2015). In fact, the abundance of archaea involved in nitrification can increase with decreasing soil pH; the abundance of bacteria involved in nitrification, however, tends to increase with increasing pH (Nicol et al. 2008). Certain plants may even directly compete with microbes for NH_4^+ by exuding microbial activity inhibitors, such as phenolic acids or tannins (Lata et al. 2004). Geochemical, physical, and biological changes in the soil can influence the rate of bioavailable nutrient release and leaching into groundwater, depending on what microbes are actively providing enzymes involved in soil reactions (Jones and Olson-Rutz 2011; van der Perk et al. 2018). Because microbial communities are so involved in biogeochemical cycling and soil health, change in soil microbial community composition and diversity due to land use affects the health of soils (Murphy et al. 2006; Ayoubi et al. 2011).

Chapter 3 - Methods

Sampling in the Field

Soil Samples

In June and July of 2018, we sampled soil around ten monitoring wells in GMD 5. Soil sampling sites were chosen in different land use areas located near wells sampled in 2016 and 2017 (Richard 2018), as indicated in Figure 3-1. Selected sites were as follows: non-irrigated cropland near wells 6, 51, and 50B; irrigated cropland near wells 21, 50A, 42, and 3; and pasture near wells 52, 36, 34, and 10. The land use for soils near well 3 was converted from irrigated cropland to pasture between the summers of 2016 and 2018.

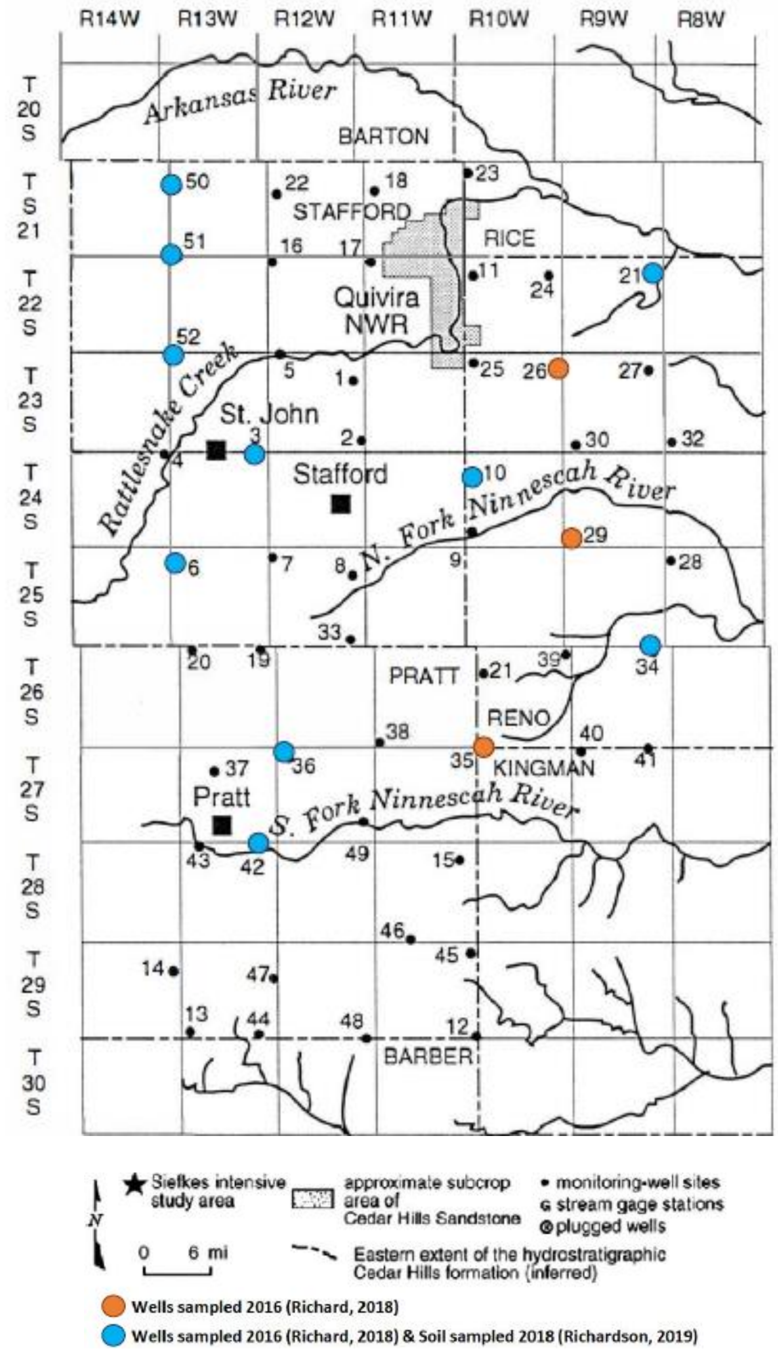

Figure 3-1: Map of all well sampling sites, modified from Whittemore 1993 and Richard 2018.

Samples were taken from four sites near each well, spaced 10-20 meters from each well and from each other (Figure 3-2). We attempted to keep similar spacing between well site and soil sampling sites at every well to be more consistent in our study. However, obstructions on the land surface such as drainage ditches, fences, and vegetation prevented identical spacing in some cases. At each of the four sites per well, we took soil from three depths, where possible: 0-5 cm, 5-15 cm, and 15-30 cm. We were able to obtain samples up to a depth of 30 cm at most of our sampling sites; however, factors such as soil dryness and compaction at Wells 6, 36, 34, 3, and 50 prevented us from taking 15-30 cm cores with a hand auger at some of our replicate sites (details in Appendix E). Soil microbial communities are known to be diverse and soil conditions are heterogeneous (Or et al. 2007; Fierer 2017). Therefore, 2-5 cores of soil approximately two centimeters apart were taken at each of the four sampling sites per well. These cores were then homogenized to mitigate large discrepancies resulting from sample heterogeneity and to ensure enough sample for all required analyses.

Figure 3-2: Example of approximate spacing of soil sampling sites.

Each core of soil was measured for correct depth while still in the hand auger, and soil was scooped out of the auger with a metal scoopula. We sterilized hand augers, scoopulas, and gloves with 95% lab-grade ethanol between samples to limit cross-contamination. We stored samples in Ziploc bags on ice in the field and at 4º C in the lab.

Groundwater Samples

In August 2018, we sampled the shallow and deep aquifer at well 50 to compare with the geochemical and microbial community data collected by Richard (2018) in the summers of 2016 and 2017. Well 50B is screened in the aquifer base with a total depth of 132.1 ft, and well 50C is screened in the upper aquifer with a total depth of 51.4 ft. We used a bladder pump (Geotech Geocontrol PRO) to collect groundwater samples. Well 50B was purged at an approximate rate of 0.49 ounces per second, and Well 50C was purged at an approximate rate of 0.53 ounces per second. After pH, temperature, and electrical conductivity measurements (Oakton PC-450 meter) had stabilized over five consecutive readings five minutes apart, we averaged four final measurements to obtain our reported results and then collected water samples.

We filtered groundwater samples for anion and cation analysis with a 0.45 micron filter and stored them in Nalgene bottles. Samples for cation analysis were preserved with 40 µL of trace metal grade nitric acid. Microorganisms were collected using autoclave-sterilized materials (120º C for 30 min). We used 60 mL sterile syringes to take up water and filtered each sample through mixed cellulose ester (MCE) membranes (0.22 microns) until the membranes clogged (Kirk et al. 2015). Filters used for Well 50B became clogged after 240 mL groundwater, and filters used for Well 50C became clogged after 720 mL groundwater. Filters were stored in individual bags and placed on ice with water samples. Upon return to the lab, we stored all samples at 4º C. Microbe samples were subsequently preserved with 0.2 mL of sucrose lysis buffer, plugged with a luer lok plug, and stored at -80º C (Kirk et al. 2015). A summary of methods used in 2016 groundwater sampling by Richard is given in Appendix G.

Geochemical Analysis

Soil Geochemistry

Soil environmental parameters were measured for all 112 soil samples. All samples were homogenized in the lab within 2 weeks of field work, using 95% ethanol to sterilize bowls and gloves between samples. Approximately 10-20 g of each sample was separated out for microbial DNA analysis and stored in -80º C.

Soil pH was measured on fresh, homogenized samples in the Geology Department's Geomicrobiology lab. Approximately 2 grams of soil were weighed out per flask. Two replicates per sample were measured, with the addition of 10mL 18 MΩ deionized water to one flask and 10 mL 0.01 M $CaCl_2$ to another flask. Flasks were then capped and shaken on a platform shaker at moderate levels (6 of 10) for twenty minutes. Finally, an Oakton General-Purpose pH probe (single junction) connected to an Oakton PC-450 meter was used to measure pH of the soil suspensions (see Appendix C).

Samples were prepared for soil particle size analysis (PSA) in the Geomicrobiology lab, using 30% hydrogen peroxide and a 12-hour 80º C water bath to remove organic material. PSA was completed with a laser diffraction particle size analyzer (Malvern Mastersizer 3000) in the Geography Department's Paleoenvironmental Lab. Data from PSA is reported as % by volume. Soil texture was evaluated according to USDA guidelines (Figure 3-3). Soil classification was identified by consulting Soil Web with Google Earth Pro (USDA-NCSS SSURGO and STATSGO Soil Survey Products).

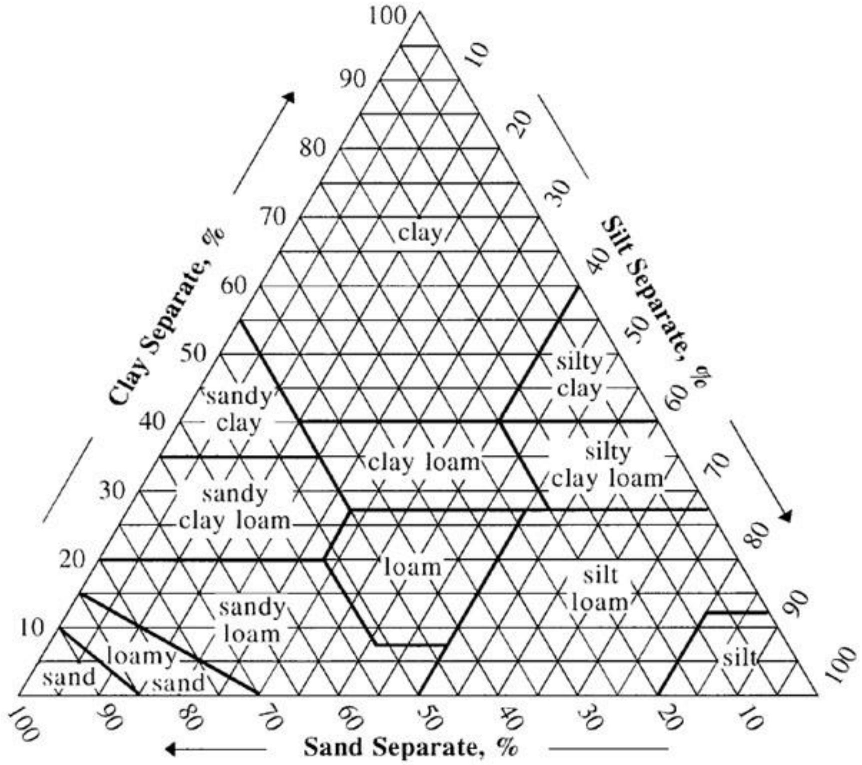

Figure 3-3: Soil texture for samples was evaluated with this USDA Texture Triangle diagram and other USDA guidelines (2019b).

Soil organic matter (SOM), NO_3-N and NH_4-N, and extractable element (Na^+, K^+, Ca^{2+}, Mg^{2+}, P) analyses were performed in the Kansas State University Soil Testing Lab. SOM was analyzed via loss on ignition. NO_3-N and NH_4-N were measured using 1N KCl extraction. Soil Na^+, K^+, Ca^{2+}, and Mg^{2+} were extracted with the ammonium acetate method. Soil phosphorous was extracted via Mehlich-3 method. Protocols and references for each analysis performed in the Soil Testing Lab are given in Appendix C.

Groundwater Geochemical Analysis

Groundwater environmental parameters of pH, temperature, and electrical conductivity were measured in the field for Wells 50B and 50C, as previously described. Geochemical analyses of our groundwater samples were performed in the Geomicrobiology lab of the Geology Department at KSU. We measured total alkalinity with burette titration of 10 mL sample, 0.02 N H_2SO_4 titrant, and USGS Gran alkalinity titration calculation (2012; Kirk et al. 2015). In addition, anion and cation concentrations were measured using a Thermo Fisher Scientific™ Dionex ICS-1100 ion chromatograph (IC). For each IC run, standards, quality control samples, and unknown samples were analyzed. Unknown samples with ion concentrations outside the standard range were diluted and measured again. A summary of 2016 groundwater geochemical analysis performed by Richard can be found in Appendix G.

Microbial Community Analysis

Soil and Aquifer Samples

Total microbial community DNA extraction and amplification analyses were performed in the Molecular Microbial Ecology in the Division of Biology at Kansas State University. Ninety-one samples were chosen for DNA analysis, comprising of 85 soil samples (all 0-5 cm and 5-15 cm depth samples) and six groundwater samples from Wells 50B and 50C (sampled in the summers of 2016, 2017, and 2018). Soil DNA samples were stored on ice in the field for nine hours or less and were then stored at -80º C in the lab until extraction analysis. Extraction of DNA was performed using the Qiagen DNeasy® PowerSoil® Kit protocol as modified by Dr. Lydia Zeglin (pers. comm.; see Appendix B for details).

Soil DNA extract solutions and control samples were diluted 1:10, with 5 µL sample and 45 µL 18 MΩ deionized water (sterilized via UV radiation), before being used for downstream applications. Groundwater DNA extract solutions, however, were not diluted because aquifer microbial communities are naturally less concentrated than those of soils. After DNA extraction and dilution of soil and control samples, all diluted and aquifer samples were quantified in a FilterMax F5 Multi-Mode Microplate Reader, following the protocol used in Dr. Zeglin's lab (pers. comm.; Appendix B). This quantification ensured that enough DNA was extracted per sample to be used in polymerase chain reaction (PCR) amplification.

Amplification of genomic DNA was conducted by the PCR methodology (Appendix B) modified by Dr. Zeglin from the Earth Microbiome Project (Caporaso et al. 2012). This methodology uses 515F and 806R modified universal primers to amplify and sequence the variable V4 region of the prokaryotic 16S rRNA gene. For most of the soil samples, the PCR runs were comprised of 25 cycles, using 1:10 diluted gDNA. A PCR run of 25 cycles has been found to reduce amplification bias, resulting in better data quality. However, some samples required more than 1 µL diluted gDNA to amplify. Additional cycles in a PCR run were only used when undiluted gDNA did not amplify in a 25-cycle PCR run. Amplification success was verified using electrophoresis in 1% agarose gel.

After three PCR replicates per sample were run and combined, an EXO-SAP reaction was used to remove from the samples any unused PCR primers and dNTPs (deoxyribonucleotide triphosphates which are monomer-like units of DNA that are the building materials for gene segments amplified in the PCR reaction (2017)). Individual samples were then quantified, and an equal amount of DNA per sample (100 ng) was added to a single tube to create the combined Illumina library. The combined library was gel purified and then quantified to obtain the precise library length needed for sequencing quality. Each of the protocols for these procedures is located in Appendix B. Finally, the combined and purified Illumina library (concentration of 6.411 ng/L and 24.284 nM) was sent to the KSU Department of Plant Pathology for Illumina MiSeq sequencing.

Taxonomic Analysis

Sequencing data results from our soil and groundwater samples were provided in the form of FastQ files by the sequencing lab. We processed these files in QIIME (Caporaso et al. 2010), using a protocol modified by Dr. Lydia Zeglin (for complete list of commands, see Appendix D). First, we joined the forward and reverse Illumina reads (script: join_paired_ends.py). We then validated the mapping file comprised of environmental metadata and the barcodes indicating which sequences belong to each sample (script: validate_mapping_file.py). Next, we split all sequences into their sample groups, using the barcodes specified in the mapping file (script: split_libraries_fastq.py). We defined OTUs (operational taxonomic unit) at 97% similarity using the Greengenes reference database (script: pick_open_reference_otus.py). Taxonomies were assigned using RDP's (Ribosomal Database Project) hierarchical assignment algorithm (script: parallel_assign_taxonomy_rdp.py). We cleaned up the OTU assignment output by adding the taxonomy assignment to the OTU matrix and filtering out OTUs that did not align with the reference 16S gene sequence from the final OTU matrix (scripts: biom add-metadata, filter_otus_from_otu_table.py). We summarized the OTU output into .txt table format (script: biom summarize-table). Finally, we checked for chimeras using the QIIME ChimeraSlayer (script: parallel_identify_chimeric_seqs.py) and removed identified chimeras from the data matrix (scripts: filter_otus_from_otu_table.py, filter_fasta.py). A summary of 2016 groundwater microbial analysis and data processing can be found in Appendix G.

Alpha Diversity

Alpha diversity tests were primarily completed in QIIME (Caporaso et al. 2010). We evaluated each sample for OTU richness and relative abundance (script: summarize_taxa_through_plots.py), which yielded relative abundance bar and area charts from phylum level to genus level, with data tables for each. Relative abundance of all genera in soil samples were averaged by land use: irrigated crop land, non-irrigated crop land, pasture, and irrigated-to-pasture land. Relative abundance of all genera in groundwater samples were averaged by depth and land use at well sites: irrigated-shallow, non-irrigated-crop-shallow, pasture-shallow, irrigated-deep, non-irrigated-crop-deep, and pasture-deep. The average of replicate 2016 samples from well 50 was used in calculation of total relative abundance averages for irrigated-shallow and irrigated-deep land uses. Well 50 samples from 2017 and 2018 were not included in the average relative abundance calculations in order to maintain consistency in the sampling year and analysis methodology used to obtain microbial community data, as well as to not bias averages toward well 50 microbial communities. In addition, phyla and order level relative abundance for groundwater samples were averaged by depth and land use at well sites as well, applying a broader definition for land use: shallow-crop, deep-crop, shallow-pasture, and deep-pasture. Finally, groundwater microbial community analysis was not done for Well 3 because microbial samples for this well were not collected in 2016. Well 3 was the site of land converted from irrigated crops to pasture between 2016 and 2018.

Beta Diversity

To consider the relationship of microbial communities with environmental characteristics, we performed several beta diversity tests (for list of commands, see Appendix D). Microbial community and geochemistry correlations were analyzed for statistical significance in RStudio v. 3.4.2 (R Core Team 2019), primarily using the packages Phyloseq (McMurdie and Holmes 2013, 2019) and Vegan (Oksanen; R Core Team 2019). After loading and merging the metadata, taxonomy, and OTU files, we created three data subsets for ease and accuracy of analysis (package: phyloseq, command: subset_samples). The three data subsets are comprised of control samples, soil samples, and aquifer samples.

Relationships between microbial communities and environmental data were examined with linear regression analyses. Pairwise dissimilarites were calculated via the Bray-Curtis method (Shade et al. 2013), and dissimilarities were visualized with non-metric dimensional scaling (NMDS) plots (script: ordinate("NMDS", "unifrac", weighted=TRUE), script: plot_ordination; (Oksanen)). In addition, we used PERMANOVA (PERmutational Multivariate ANalysis Of Variance; (Anderson 2017)) tests to evaluate whether influence of specific environmental characteristics on community composition was statistically significant (package: vegan, commands: vegdist(dist.matrix, method = "bray"), adonis). In PERMANOVA, F-tests are used to understand statistical significance between multiple variables, based on sequential sums of squares from permutations of the raw data (Anderson 2017). An F-value less than 0.05 is considered statistically significant.

Relating Nitrogen Cycle Processes with Specific OTUs

The metagenomes of several microbial OTUs have been found to contain genetic pathways encoding traits associated with N-cycling (Nelson et al. 2016). Sequences from our samples were classified as being in some of these potential N-cycling groups. Results from this analysis are generalized to N-oxidation, N-reduction, and N-fixation for simplicity. Appendix E, section titled “Microbial Communities: Relative Abundance Tables,” contains the specific genera and N-cycling pathways for each soil and groundwater-related land use category.

Several considerations are important to remember when discussing specific OTUs and their involvement in processes occurring within the soil and aquifer. First, we do not have an exhaustive list of all microbial groups capable of nitrogen cycling. The OTUs we have specified are those we know of (as of 2016) whose metagenomes have been recorded as having the genetic ability to code for traits associated with N-cycling processes (Nelson et al. 2016). Secondly, the fact that some members of a specific OTU have the genetic potential to be involved in a process does not mean that all do. Many genes that encode traits are passed horizontally (i.e. passing genes through any method other than vertically to progeny as humans do), like some associated with denitrification, often because they are useful for surviving or thriving in an environment (Fierer 2017; Madigan et al. 2018). This can complicate analysis of genomes and make it difficult to be accurate when assigning function based on taxonomy (Fierer 2017; Madigan et al. 2018). Thirdly, having the genetic potential to be involved in N-cycling processes does not mean that the OTUs are actually *performing* these processes (Myrold et al. 2014). Rather, they are *able* to participate in such processes if their living conditions require them to use their genetic potential to make the proteins necessary for these processes (Myrold et al. 2014). In addition, our method of understanding what microbes are in each sample only allows us to see the DNA of microbes present (living, dormant, dead). To know which microbes are *active* in each sample, we would need to measure the enzymatic activity in each environment (Myrold et al. 2014). Finally, the N-oxidation and N-reduction processes that we have summarized for simplicity are a combination of six N-cycle processes (Nelson et al. 2016). The nitrogen-oxidizing cycles include nitrification (ammonium to nitrite to nitrate), as well as general oxidation of ammonium to nitrite and oxidation of nitrite to nitrate. The nitrogen-reducing cycles include denitrification, dissimilatory nitrate to nitrite, dissimilatory nitrite to ammonium, assimilatory nitrate to nitrite, and assimilatory nitrite to ammonium. Dissimilatory nitrate reduction to nitrite and nitrite to ammonium is an N-reduction process that is not well-studied in soils. However, many genera contain the genetic pathways required for this process, and it could have a noticeable impact on nitrate reduction in different environments (Nelson et al. 2016). In summary, while relating specific functions to OTUs in our samples is not a certain measure of the ability of microbes in our samples to be involved in the nitrogen cycle, the results of this type of analysis are interesting and worth considering.

Chapter 4 - Results

Geochemistry

Soil Samples

Soils of recently converted land use (irrigated cropland to pasture) exhibited the most neutral and highest average pH at 6.40, measured with 0.01 M $CaCl_2$ (Figure 4-1). Irrigated crop soils were close behind with an average pH of 6.34. The lowest average pH at 5.20 was measured in non-irrigated crop soils, and pasture soils had an average pH of 5.64. All pH measurements with DI water were higher than those with 0.01 M $CaCl_2$, but trends in pH with land use were the same for results from each method (Table 4-1).

Some soil ions varied substantially between soils of different land use, while others were relatively similar (Table 4-1). Average soil sodium was lowest in pasture soils (5.48 mg/kg) and highest in irrigated crop soils (41.79 mg/kg). Phosphorous as phosphate average concentrations were lowest in pasture soils at 13.28 mg/kg and highest in the recently converted land use (irrigated cropland to pasture) soils at 178.53 mg/kg (Figure 4-1). Soil potassium was highest on average in non-irrigated crop soils (303.46 mg/kg) and lowest in pasture soils (178.04 mg/kg). The highest average values of soil calcium were 2429.60 mg/kg in the irrigated crop soils, with the irrigated to pasture soils containing the lowest average soil calcium (672.25 mg/kg). Average soil magnesium was lowest in the irrigated crop soils (116.35 mg/kg) and highest in the dry crop soils (211.15 mg/kg). Finally, average nitrogen as ammonium concentrations were highest in the non-irrigated crop soils (47.99 mg/kg) and lowest in the irrigated to pasture soils (4.36 mg/kg).

Nitrogen as nitrate concentrations were highest on average in irrigated crop soils at 86.05 mg/kg, with non-irrigated crop soils at 29.48 mg/kg as second highest (Figure 4-1). Concentrations of NO_3^- ranged from 7 to 333.8 mg/kg as N in irrigated crop soils and 1.2 to

330.6 mg/kg as N in non-irrigated crop soils. Nitrate concentrations ranged from 1 to 21.4 mg/kg as N in the converted irrigation to pasture soils, with an average of 6.20 mg/kg as N. In pasture soils, the NO_3^- concentrations ranged from 1 to 14.1 mg/kg as N and contained the lowest average of all four land use soils at 3.84 mg/kg as N.

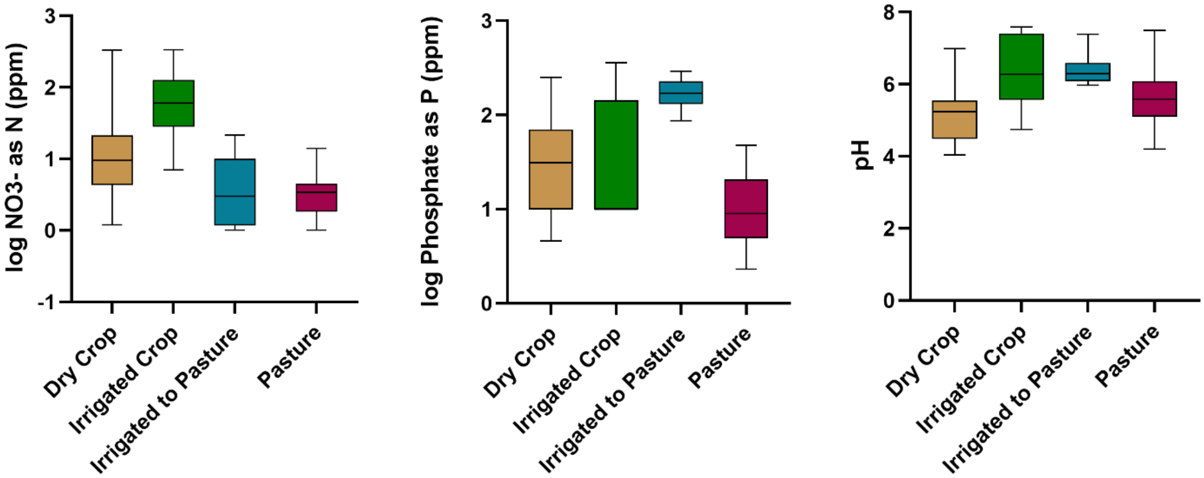

Figure 4-1: Soil extractable NO_3-N and PO_4-P, as well as soil pH, averaged by land use.

Table 4-1: Soil geochemistry averages by land use.

	Avg. Non-irrigated Crop n=27	Avg. Irrigated Crop n=34	Avg. Irrigated to Pasture n=10	Avg. Pasture n=41
OM LOI %	1.87	1.74	1.86	1.95
Ca^{2+} mg/kg	1314.95	2429.60	672.25	1159.04
Mg^{2+} mg/kg	211.15	116.35	122.77	146.02
Na^{+} mg/kg	31.80	41.79	6.17	5.48
K^{+} mg/kg	303.46	241.12	246.93	178.04
P-M* mg/kg	46.79	83.21	178.53	13.28
NO_3-N mg/kg	29.48	86.05	6.20	3.84
NH_4-N mg/kg	47.99	5.93	4.36	8.41
pH (DI)	5.93	6.89	7.10	6.34
pH (0.01 M $CaCl_2$)	5.20	6.34	6.40	5.64

*Phosphorous extracted by the Mehlich-3 method.

Soil organic matter content varied by sample, ranging from 0.40% to 5.20%; however, when averaged by land use, SOM percentages ranged from 1.741 to 1.95. Samples from the pasture sites had the highest average percentage of organic matter at 1.95. Average soil organic matter percentage measured as 1.87 and 1.86 for non-irrigated crop soils and converted land use soils, respectively. Irrigated crop soils had the lowest amount of organic matter at an average percentage of 1.74.

Particle size analysis demonstrates that the samples contain a high percentage of sand (Table 4-2). Most of the soils are composed of an average 70% or higher volume of sand, ranging from sand to sandy loam in soil texture. The soils near wells 51 and 42 are the exception, with an average of 58.8% and 51.4% volume sand respectively in the first five centimeters and decreasing sand content with increasing depth. All samples contained an average of less than 3% volume of clay, with the remaining percent volume as silt.

Table 4-2: Average particle size by percent volume in soils near each well, at sampling depths of 0-5 cm and 5-15 cm.

Sampled Near	**Land Use**	**Bottom Depth (cm)**	**Soil Type**	**Sand**	**Silt**	**Clay**	**Primary Soil Taxonomy**
				Average % Volume			
Well 50	Irrigated & Non-irrigated Crop	5.00	Loamy Sand	76.6	22.3	1.10	Mollisol
		15.00		76.1	22.9	0.99	
Well 51	Non-irrigated Crop	5.00	Sandy Loam	58.8	38.9	2.32	Alfisol/ Mollisol
		15.00		54.0	43.4	2.60	
Well 52	Pasture	5.00	Sand	89.6	10.1	0.31	Alfisol
		15.00		90.1	9.50	0.36	
Well 42	Irrigated Crop	5.00	Sandy/Silt Loam	51.4	48.6	0.00	Mollisol
		15.00		46.7	52.9	0.40	
Well 36	Pasture	5.00	Sandy Loam	70.1	28.9	0.96	Mollisol
		15.00		71.1	28.2	0.77	
Well 34	Pasture	5.00	Loamy Sand	77.1	22.2	0.65	Mollisol
		15.00		80.4	19.2	0.49	
Well 21	Irrigated Crop	5.00	Loamy Sand	82.4	17.0	0.58	Mollisol
		15.00		84.7	14.9	0.48	
Well 10	Pasture	5.00	Loamy Sand	77.5	21.5	0.95	Alfisol
		15.00		76.1	23.1	0.87	
Well 6	Non-irrigated Crop	5.00	Sand	90.6	8.97	0.41	Mollisol
		15.00		91.2	8.36	0.40	
Well 3	Irrigated to Pasture	5.00	Sand	91.3	8.40	0.29	Alfisol/ Entisol
		15.00		90.6	9.14	0.31	

Groundwater Samples

Groundwater samples collected in 2016 and 2018 from Well 50B and Well 50C varied in composition (Table 4-3). Shallow groundwater alkalinity (mg/L $CaCO_3$) increased by 26.8. We were unable to measure the alkalinity of the 2018 sample from the deep aquifer because the sample became acidic between collection and the alkalinity titration. Change in nitrate concentration between 2016 and 2018 is most notable, with NO_3-N increasing by 57.22 mg/L in the deep aquifer well and decreasing by 7.53 mg/L in the shallow aquifer well. In addition, chloride concentration increased slightly in the deep groundwater by 2.40 mg/L and decreased in the shallow groundwater by 36.11 mg/L. Groundwater NH_4-N, PO_4-P, and NO_2-N concentrations were below detection limits for most samples (see Appendix C for MCLs). A summary of 2016 groundwater chemistry results is given in Appendix G.

Table 4-3: Well 50B and 50C data results from summer 2016 and summer 2018.

	Well 50B (Deep Aquifer)		Well 50C (Shallow Aquifer)		Δ Well 50B	Δ Well 50C
Year	**2016**	**2018**	**2016**	**2018**		
Temperature (ºC)	18.20	24.70	15.90	21.90	6.50	6.00
Groundwater pH	7.34	7.27	7.02	6.74	-0.07	-0.28
C (µS/cm)	639.00	675.00	1095.00	996.10	36.00	-98.90
Alkalinity (mg/L $CaCO_3$)	176.30	Not measured	238.90	265.70	NA	26.80
F^- (mg/L)	0.46	0.38	0.26	0.27	-0.08	0.01
Cl^- (mg/L)	50.62	53.02	113.53	77.42	2.40	-36.11
Br^- (mg/L)	0.12	0.14	0.25	0.26	0.02	0.01
SO_4^{2-} (mg/L)	36.39	39.29	55.90	53.12	2.89	-2.78
Na^+ (mg/L)	56.99	53.26	48.47	47.89	-3.74	-0.58
K^+ (mg/L)	8.98	3.12	3.86	4.15	-5.86	0.29
Mg^{2+} (mg/L)	3.08	8.61	15.83	14.16	5.53	-1.66
Ca^{2+} (mg/L)	66.72	56.86	127.42	113.92	-9.86	-13.50
Sr^{2+} (mg/L)	bdl*	2.20	5.71	3.51	bdl	-2.20
NO_3-N (mg/L)	10.84	68.05	28.84	21.31	57.22	-7.53
PO_4-P (mg/L)	bdl	bdl	bdl	0.02	bdl	bdl
NH_4-N (mg/L)	bdl	0.08	bdl	bdl	bdl	bdl
NO_2-N (mg/L)	bdl	0.06	bdl	bdl	bdl	bdl

*Note: bdl means below detection limit (see Appendix C).

Microbial Communities

Taxonomy and Alpha Diversity

Quantification of the samples after DNA extraction yielded sufficient DNA for amplification, with groundwater samples containing the lowest concentration (Appendix E). In the end, however, two groundwater samples did not amplify well: from well 50B (sampled in 2017) and from well 50C (sampled in 2016). The amount of gDNA and the number of PCR cycles required to amplify each sample are specified in Appendix E.

Alpha diversity analysis in QIIME described microbial community diversity in each sample. Relative abundance bar and area charts from phylum level to genus level, with data tables for each, were made in QIIME to display alpha diversity results. The complete set of data tables from QIIME are available at 10.6084/m9.figshare.8091281. All relative abundance area and bar charts are located in Appendix F, along with the legend for the order level charts. Relative abundance charts at the phylum level were also made in GraphPad Prism (v. 8.1.1).

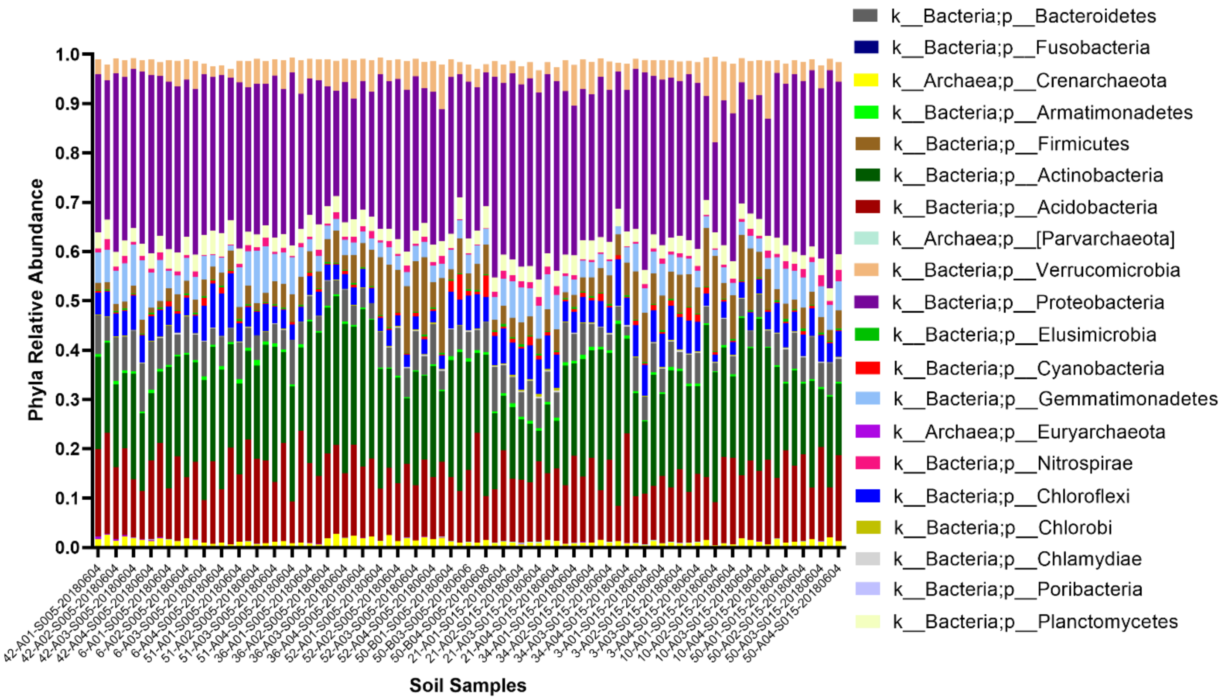

Figure 4-2: Bar chart of archaeal and bacterial phyla in soil samples.

Relative Abundance in Soil Samples

On average in the soil samples (Figure 4-2), the highest percentages of relative abundance belong to bacteria in phyla *Proteobacteria* (31. 29%), *Actinobacteria* (20.89%), *Acidobacteria* (14.47%), *Bacteroidetes* (5.47%), and *Firmicutes* (5.23%). Order *Actinomycetales* (phylum *Actinobacteria*, class *Actinobacteria*) is the bacterial order with the highest average relative abundance (10.59%), while the highest average percentage of an archaeal order in the soils belongs to *Nitrososphaerales* (p.*Crenarchaeota*, c. *Thaumarchaeota*) at 1.16%.

In irrigated crop soils, orders *Actinomycetales* (p. *Actinobacteria*, c. *Actinobacteria*) and *Rhizobiales* (p. *Proteobacteria*, c. *Alphaproteobacteria*) are the first and second highest percentages of average relative abundance at 8.78% and 5.99%. *Actinomycetales* is a diverse order of primarily aerobic bacteria that are commonly found in soils (Madigan et al. 2018). *Rhizobiales* is also a very diverse order of primarily aerobic bacteria, including phototrophs, chemoorganotrophs, chemolithotrophs, and symbionts (Madigan et al. 2018). Bacterial order *Nitrospirales* (p. *Nitrospirae*, c. *Nitrospira*) and archaeal order *Nitrososphaerales* (p.*Crenarchaeota*, c. *Thaumarchaeota*) contain members with the potential to be involved in the nitrification of NH_4^+ and urea (Madigan et al. 2018). *Nitrososphaerales* are common soil archaea that may directly compete with bacterial nitrifiers in soils with high NH_4^+ concentrations (Madigan et al. 2018). Orders *Nitrososphaerales* and *Nitrospirales* have average relative abundances of 1.37% and 1.29% in irrigated soils. Finally, order *Pseudomonadales* (p. *Proteobacteria*, c. *Gammaproteobacteria*) has members with the potential to be involved in denitrification of NO_3^-, and this order is at an average relative abundance of 0.66% in irrigated soil samples.

The non-irrigated crop soils also have orders *Actinomycetales* (12.30%) and *Rhizobiales* (5.99%) as the first and second highest percentages of average relative abundance. The potential nitrifiers, archaeal order *Nitrososphaerales* and bacterial order *Nitrospirales* are at average relative abundances of 1.10% and 0.62%. The order *Pseudomonadales,* with potential denitrifiers, has a 0.75% average relative abundance.

The orders with the highest average relative abundance in the irrigated to pasture soils are also *Actinomycetales* (13.26%) and *Rhizobiales* (6.87%). Archaeal order *Nitrososphaerales* (0.86%) and bacterial order *Nitrospirales* (0.71%) contain potential nitrifiers. Average relative abundance of the order *Pseudomonadales,* with potential denitrifiers, is 0.40% in irrigated to pasture soil samples.

Pasture soils contain *Actinomycetales* (11.22%), *Bacillales* of phylum *Firmicutes* (7.39%), and *Rhizobiales* (6.83%) as the orders with the highest percentages of average relative abundance. Members of *Bacillales* are commonly found in soils and tend to be aerobic chemoorganotrophs (Madigan et al. 2018). The potential nitrifiers, archaeal order *Nitrososphaerales* and bacterial order *Nitrospirales* are at average relative abundances of 1.42% and 0.74%. The order *Pseudomonadales,* with potential denitrifiers, has a 0.74% average relative abundance.

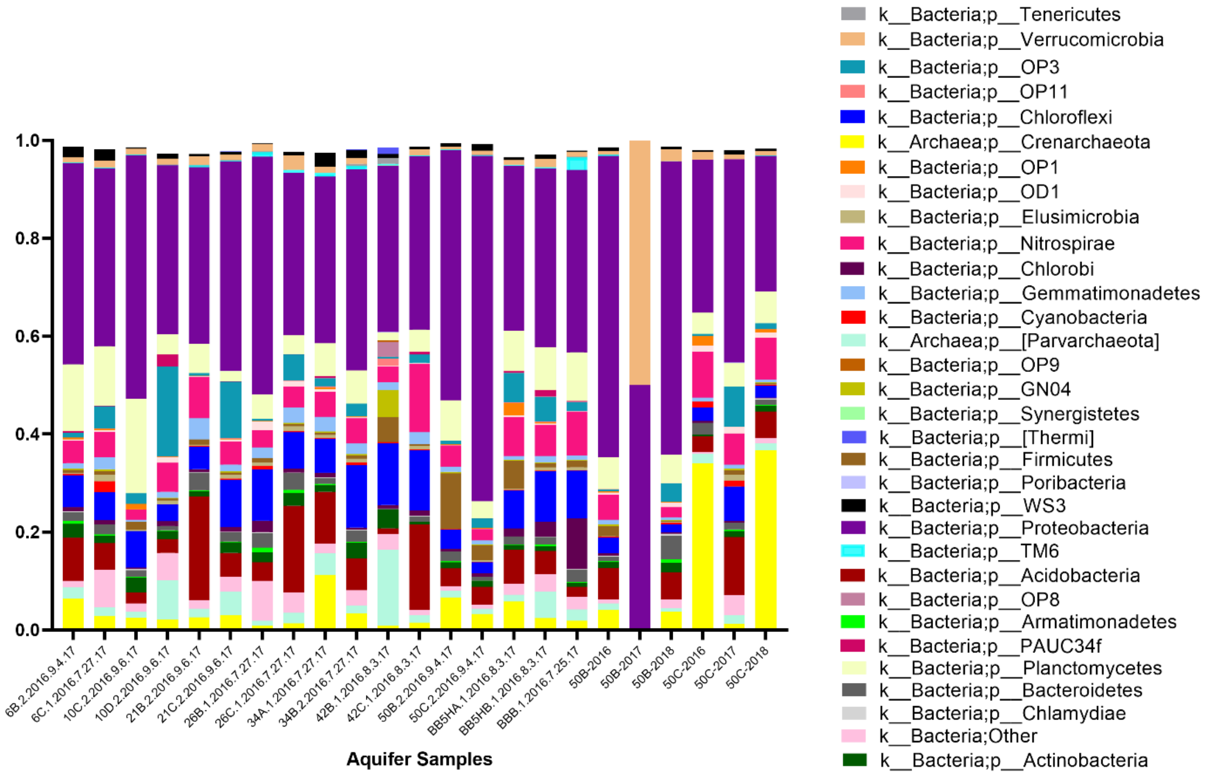

Figure 4-3: Bar chart of archaeal and bacterial phyla in groundwater samples.

Relative Abundance in Groundwater Samples

In the groundwater samples (Figure 4-3), the highest average relative abundance percentages belong to bacteria in phyla *Proteobacteria* (40.93%), *Chloroflexi* (7.89%), *Planctomycetes* (7.31%), *Acidobacteria* (7.28%), and *Nitrospirae* (5.64%). The bacterial order with the highest average relative abundance is *Pseudomonadales* (p. *Proteobacteria*, c. *Gammaproteobacteria*) at 7.46%. In addition, the archaeal order with the highest average relative abundance is *Cenarchaeales* (p. *Crenarchaeota*, c. *Thaumarchaeota*) at 3.13%.

For shallow and deep wells, *Pseudomonadales* is the order with the highest average relative abundances of 8.23% and 7.55%, respectively. As mentioned earlier, members of *Pseudomonadales* tend to be chemoorganotrophs, using oxygen (aerobic) and sometimes nitrate (facultatively aerobic) as electron acceptors (Madigan et al. 2018). Both well depths have similar average relative abundances of order *Nitrospirales* within phylum *Nitrospirae*, as second highest in the shallow wells (5.99%) and third highest in the deep wells (5.36%). Nitrite oxidizers, specifically of autotrophic genus *Nitrospira*, are in the order *Nitrospirales* and are common in many natural environments (Madigan et al. 2018). The order with second highest average relative abundance in the deep aquifer wells is *Rhodospirillales* of the class *Alphaproteobacteria* (5.47%). Order *Rhodospirillales* has a diverse array of microbes, including facultatively aerobic chemoorganotrophs (DeLong and Rosenberg 2014), photoautotrophs that can use H_2S and Fe^{2+} as electron donors (Madigan et al. 2018), and anaerobic respirers that use a variety of electron acceptors and donors (Madigan et al. 2018).

The shallow wells below soils used for crop cultivation have the orders *Pseudomonadales* (12.14%) and *Nitrospirales* (7.03%) as first and second highest in average relative abundance, both of which have members with the potential to be involved in the nitrogen cycle. The two highest average relative abundances of archaeal orders are 2.32% for order *Cenarchaeales* and 1.4% for order *YLA114* (p. *Parvarchaeota*, c. *Parvarchaea*). While phylum *Parvarchaeota* is a relatively unknown archaeal group, phylum *Crenarchaeota* tends to contain chemolithotrophic autotrophs that enjoy temperatures above boiling point (Madigan et al. 2018). However, growing evidence shows that *Crenarchaeota* is commonly found in a variety of environments and may play a role in ammonia oxidation and nitrite reduction (Treusch et al. 2005; Hansel et al. 2008; Timonen and Bomberg 2009).

In the deep wells below soils used for crop cultivation, orders *Pseudomonadales* and *Nitrospirales* again have the highest average relative abundance at 7.70% and 5.88%. The highest average relative abundances for archaeal groups in these deep crop wells are of order

WCHD3-30 from phylum *Parvarchaeota* (3.54%) and order *Cenarchaeales* from phylum *Crenarchaeota* (3.25%).

The order with the highest average relative abundance in shallow wells below soils used as pasture is *Nitrospirales* (5.42%), and the second highest is an unidentified order (4.90%) of phylum *Chloroflexi*. Members within *Nitrospirales* oxidize nitrite to nitrate, as mentioned above (Madigan et al. 2018). Phylum *Chloroflexi* seems to be widespread throughout nature but members are difficult to cultivate and are not as well known (Madigan et al. 2018). However, some genera within *Chloroflexi* have the potential to be involved in N-reduction processes, including denitrification (Nelson et al. 2016). The highest average relative abundances for archaeal groups in these shallow pasture wells are of order *YLA114* from phylum *Parvarchaeota* (2.94%) and order *Cenarchaeales* of phylum *Crenarchaeota* (2.15%).

The deep wells below soils used as pasture have the orders *Rhodospirillales* and *Pseudomonadales* as first and second highest in average relative abundance (8.07% and 7.31%, respectively). As previously mentioned, order *Pseudomonadales* contains some potential nitrate reducers (Madigan et al. 2018), and order *Rhodospirillales* contains a diverse array of microbes. Orders *Cenarchaeales* (2.72%) and *YLA114* (0.69%) are the archaeal orders with the highest average relative abundance percentages in these deep pasture wells.

Relating Nitrogen Cycle Processes with Specific OTUs

Specific microbial OTUs were classified as having the potential to be involved in N-cycling (Nelson et al. 2016). As mentioned previously, results from this analysis are generalized to N-oxidation, N-reduction, and N-fixation for simplicity (Table 4-4), and Appendix E contains the specific genera and N-cycling pathways for each soil and groundwater-related land use category. Considerations to be aware of when relating function with specific OTUs are described in detail in the methods section.

Compared to the soils of other land uses, the irrigated crop soil samples contain slightly higher average relative abundances of potential N-oxidizers (2.17%) and lower average relative abundances of potential N-reducers (7.69%). In addition, shallow aquifer wells below irrigated crop soils contain higher relative abundances of potential N-reducers (24.74%) compared to groundwater samples from other wells.

Table 4-4: Relative abundance of OTUs with the potential to be involved in different N-cycle processes. Average relative abundance of genera to the 0.05% are included in this analysis.

Category (Averaged)		Relative Abundance of Genera with N-Cycling Traits (all are in percentages of relative abundance)				
		# genera	Total	N-oxidation	N-reduction	N-fixation
Soil Samples	Irrigated Crop (n=24)	44	9.661	2.170	7.691 (5.73)	1.538
	Non-irrigated Crop (n=21)	45	11.947	1.470	10.61 (7.49)	2.168
	Pasture (n=32)	41	11.629	1.658	10.06 (7.76)	1.859
	Irrigated-to-Pasture (n=8)	47	12.865	1.743	11.51 (9.12)	1.797
Shallow Groundwater Samples	Irrigated Crop (n=3)	26	26.321	1.578	24.74 (6.91)	5.234
	Non-irrigated Crop (n=1)	19	9.768	3.613	6.06 (3.48)	2.436
	Pasture (n=4)	26	5.865	1.383	4.46 (2.83)	1.414
Deep Groundwater Samples	Irrigated Crop (n=3)	34	18.466	3.129	15.54 (4.10)	3.211
	Non-irrigated Crop (n=2)	22	11.077	4.890	6.40 (3.59)	3.022
	Pasture (n=3)	19	15.117	4.060	11.25 (3.42)	1.004

(Note: Numbers in red are the sum of genera with encoding ability for traits associated with denitrification.)

Beta Diversity: Soil Communities and Environmental Factors

Our overall NMDS results show soil microbial community clustering by land use, but not with the variables of soil type, soil taxonomy, or sampling site. The microbial communities from irrigated soils and pasture soils cluster in opposite corners, while the microbial communities from non-irrigated crop soils are spread between the other two major land use clusters (Figure 4-4). Interestingly, the microbial communities from the recently converted land (irrigated to pasture) still plot closer to the irrigated crop soil cluster than to the pasture soil cluster.

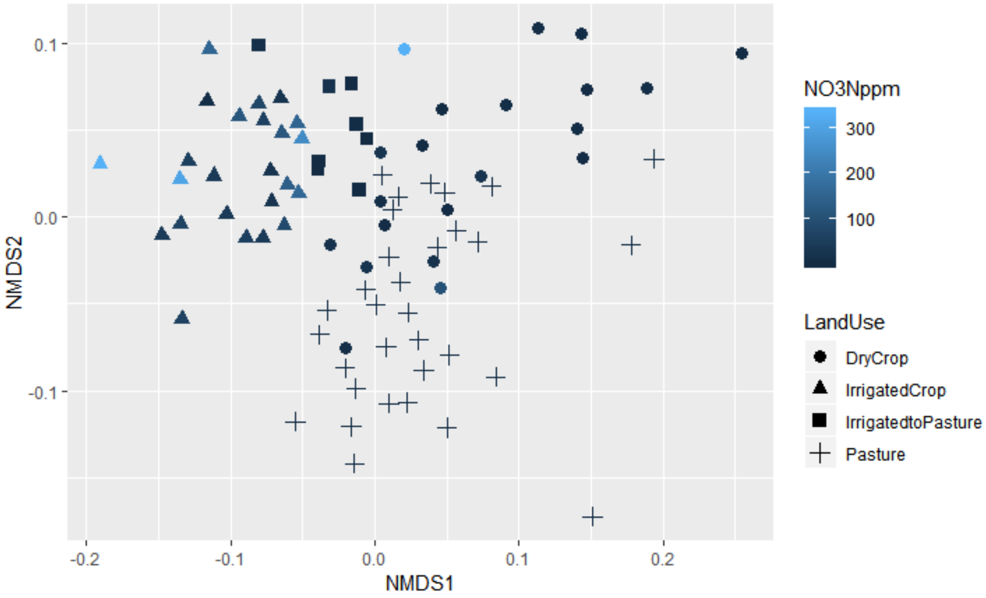

Figure 4-4: NMDS relationship of soil microbial communities, land use, and NO_3-N (mg/kg).

In general, no discernable pattern is visible on the NMDS plots between soil microbial communities and soil environmental characteristics like extractable elements, pH, and OM. Concentrations of extractable Ca^{2+} are the exception to this general observation. Some of the irrigated soil samples have the highest concentrations of Ca^{2+}, and those microbial communities are noticeably clustered (Figure 4-5). Other NMDS plots showing the relationship of soil microbial communities with numerical or categorical environmental factors are in Appendix F.

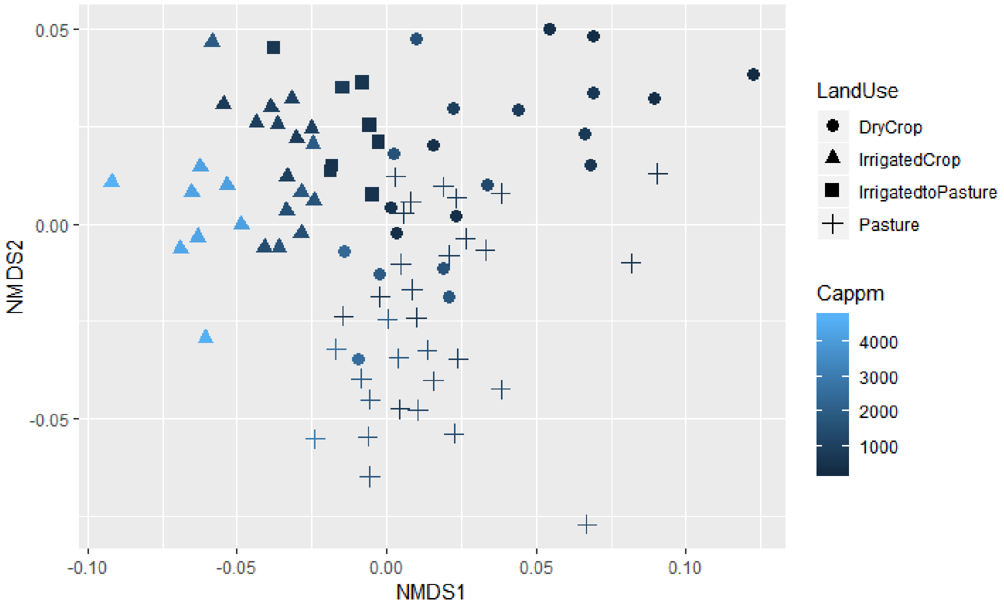

Figure 4-5: NMDS relationship of soil microbial communities, land use, and Ca^{2+} (mg/kg).

Bray-Curtis dissimilarity and PERMANOVA provide a further test of relationships between microbial community composition and environmental parameters. The soil microbial communities are significantly correlated with land use, NO_3-N concentration, pH, sampling site variables (site, longitude, latitude), soil type variables (soil type, soil taxonomy, percent volume sand, percent volume silt, percent volume clay), macronutrient concentrations (Ca^{2+}, Mg^{2+}, K^+, and P), and Na^+ concentration (Table 4-5). Soil microbial communities were not significantly correlated with NH4-N concentration or with percent organic matter (OM), having f-values of 0.214 and 0.13 respectively.

Beta Diversity: Groundwater Communities and Environmental Factors

We made several NMDS plots to consider the relationship of our groundwater microbial communities with groundwater environmental factors (Appendix F). The groundwater microbial community data clusters with well depth category (i.e. wells screened in the shallow, deep, and bedrock portions of the aquifer). However, these groundwater community clusters with well depth are not as defined as the soil community clusters with land use, and there are clear groundwater community outliers (Figure 4-6). None of the groundwater chemistry factors seem to contribute to the groundwater microbial community clustering patterns. The two grey community scatter data (Figure 4-6) are the samples for well 50B and well 50C that were collected in 2017 and were analyzed for microbial communities but not for water chemistry.

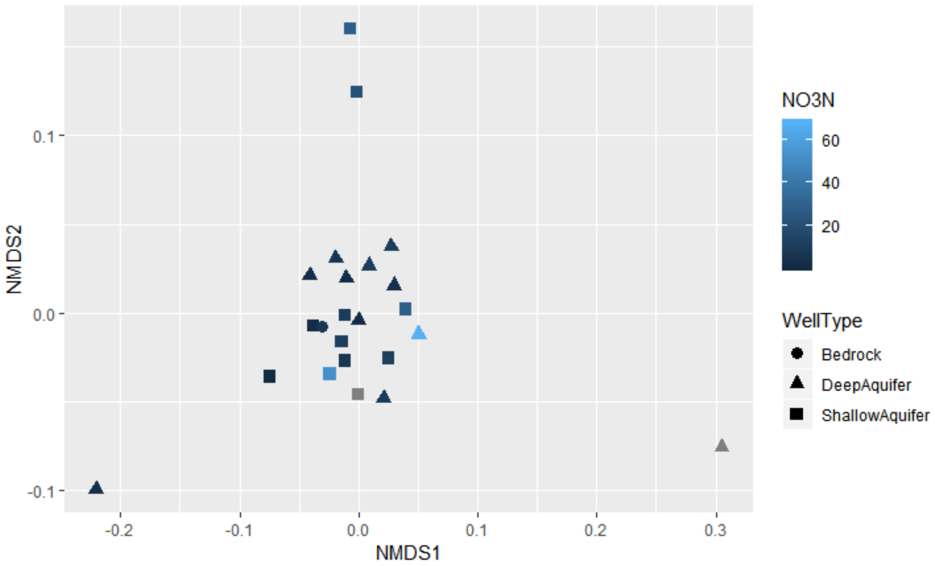

Figure 4-6: NDMS relationship of groundwater microbial communities, well depth, and NO_3-N (mg/L).

We also plotted the groundwater microbial communities with different combinations of categorical environmental factors, including well depth, land use, NO_3-N as a category, and Cl^- as a category (Appendix F). NO_3-N and Cl^- as categories together with groundwater microbial communities could not be plotted as NMDS relationships by the program. The Cl^- categories with well depth show that the very high concentrations of Cl^- are in the deep groundwater samples, while the very low Cl^- concentrations are in the bedrock well and one of the shallow aquifer wells (Figure 4-7).

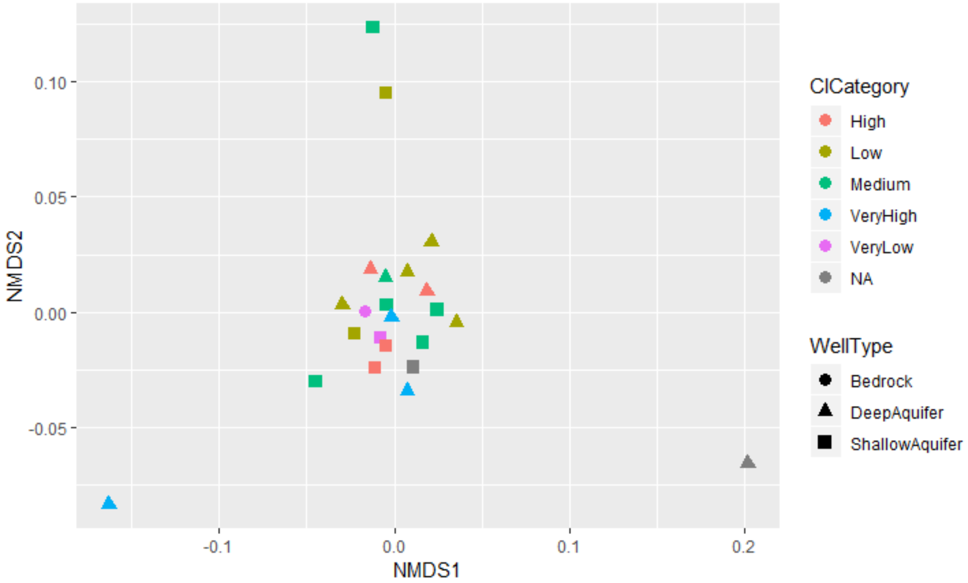

Figure 4-7: NMDS relationship of groundwater microbial communities, well depth, and Cl^- as a category (Very Low = 0-39.99 Low = 40-99.99, Medium = 100-299.99, High = 300-999.99, Very High = 1000-above).

The Bray-Curtis dissimilarity and PERMANOVA significance tests show that groundwater microbial communities are significantly correlated with sampling site (f-value = 0.001), latitude (f-value = 0.001), and longitude (f-value = 0.006). There is also a significant correlation between groundwater communities and overall land use (f-value = 0.036). Groundwater microbial communities were not significantly correlated with well depth, with an f-value of 0.235 (Table 4-5). In addition, groundwater communities and NO_3-N categories were non-conformable arrays, as were groundwater communities with NO_3-N concentrations, pH, and Cl^- concentrations.

Comparing Soil and Groundwater Microbial Communities

For most of our statistical analysis, we separated soil and groundwater microbial communities with their environmental metadata because these environments and their communities are fundamentally different. However, we did analyze all samples with general environmental factors as categories to see if any patterns were visible. We chose environmental factors related to our research goals that could be applied to both soil and groundwater samples including sampling site, overall land use, pH as categories, and NO_3-N as categories. Only a few of these tests revealed correlations within the data that could be plotted. The tests that did plot show microbial communities clustering primarily according to soil and groundwater sample type and not visibly related to environmental categories (Figure 4-8). The two grey community points (Figure 4-8) that also plot as "N/A" are the samples for Well 50B and Well 50C collected in 2017 and analyzed for microbial communities but not for water chemistry.

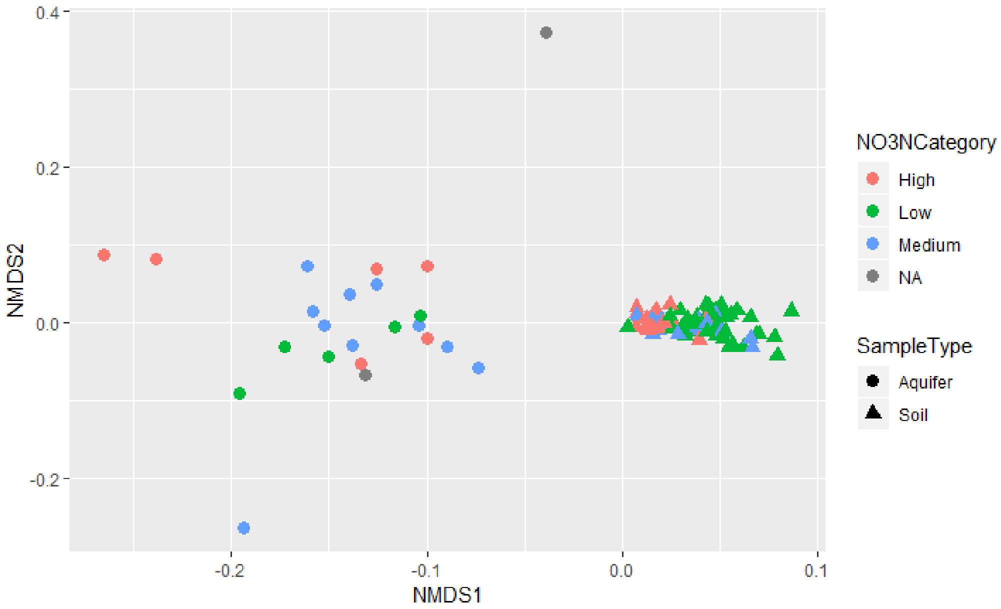

Figure 4-8: NMDS relationship of all microbial communities, sample type, and NO_3-N as a category (Low = 0-9.99 mg/kg, Medium = 10-49.99 mg/kg, High = 50 mg/kg-above).

Sampling site, overall land use, sample type, year sampled, and latitude were significantly correlated ($f \leq 0.002$) with all microbial communities (Table 4-5). Longitude is significantly correlated with all microbial communities as well (f-value = 0.039). Other variables were non-conformable with all microbial communities. Statistical analysis commands that compare microbial communities with environmental factors are located in Appendix D.

Table 4-5: Comparison of Bray-Curtis, PERMANOVA statistical significance results between three subsets of microbial communities (all samples, soil samples, and groundwater samples).

	Vs. All Microbial Communities	Vs. Soil Microbial Communities	Vs. Groundwater Microbial Communities
Bray-Curtis, Permanova	*F-value significance: ≤0.001, <0.01, <**0.05***		
Sampling Site	*0.001*	*0.001*	*0.001*
Overall Land Use	*0.001*	*0.001*	**0.036**
NO_3-N Category	n.c.*	*0.001*	n.c.
Sample Type	*0.001*	N/A*	N/A
Soil Land Use	n.c.	*0.001*	N/A
NH_4-N	n.c.	0.214	N/A
NO_3-N	n.c.	*0.001*	n.c.
Soil Type	n.c.	*0.001*	N/A
Well Depth	n.c.	N/A	0.235
Year Sampled	*0.001*	N/A	**0.026**
Latitude	0.002	*0.001*	*0.001*
Longitude	**0.039**	**0.012**	0.006

*Note: n.c. means that the arrays were non-conformable, and N/A refers to the inability to perform the test because the variables involved did not apply (e.g. well depth does not apply to soils).

Chapter 5 - Discussion

Land Use Impact on Soils

Many studies have found that land use significantly impacts soil chemistry and microbial communities (Huang et al. 2002; Murphy et al. 2006; Ayoubi et al. 2011; Dorr de Quadros et al. 2012; Keiluweit et al. 2017). Our results also show statistically significant effects of land on soil chemistry and microbial communities in our study area. Land use remains the best defining factor for the microbial community composition patterns in NMDS plots of our soil samples (Figure 4-4). Land use also correlated significantly with soil microbial community composition along with many environmental factors affected by land use, including NO_3-N concentrations and pH (Table 4-5).

One of the most considerable differences between our soils of different land uses is the soil NO_3-N concentrations. Average concentration of NO_3-N in the irrigated soils is significantly higher than in the other soils, and non-irrigated crop soils also have a high average compared to pasture soils (Table 5-1). Nitrification of ammonium-based fertilizers is a contributor to the higher levels of nitrate in irrigated and non-irrigated crop soils (Richard 2018), and in general, this area and similarly agriculturally dominated areas are experiencing a documented, overall increase in soil and groundwater nitrate concentrations over time (Spaldingb et al. 1978; Spalding and Kitchen 1988; Whittemore 1993; Richard 2018). The manner in which fertilizer is applied (e.g. application method or more recent application time) could artificially increase soil NH_4-N and NO_3-N concentrations for a short period of time (2014b). In addition, different amounts of ammonium-based fertilizers are applied to different types of crops (Leikam et al. 2003). For example, larger amounts of ammonium-based fertilizers are applied to corn than to wheat (Leikam et al. 2003). This is likely why our irrigated soils, growing corn exclusively, have significantly higher concentrations of nitrate even compared to the non-irrigated soils, which were primarily used for wheat production during our sampling time period. Another source of nitrate to our irrigated crop soils could be the considerably elevated levels of nitrate in the shallow groundwater used for irrigation (Table 5-1). In addition, the elevated nitrate concentrations in the irrigated soils could be partly influenced by low rates of denitrification. The high percent volume of sand found in our irrigated soils, except for the soils near well 42 (Table 4-2), would allow for more rapid leaching of nitrate, especially with constant or regular irrigation (Spaldingb et al. 1978; Spalding and Kitchen 1988). Larger pore sizes in sandy soils would allow for more oxygen input as well. Because denitrification occurs under anoxic conditions, soils with high amounts of sand are unlikely to promote high rates of denitrification (Koike and Hattori 1978; Hoffmann et al. 2007). The combination of oxic conditions, large pore sizes, and constant irrigation could considerably decrease rates of denitrification occurring in our irrigated soils. However, denitrification rates would need to be measured to know whether this is indeed a factor in the high nitrate concentrations.

While highly sandy soils do not encourage the formation of soil aggregates, ceasing tillage practices could still allow the accumulation of soil organic matter and root networks to anchor the sand in a way that could in turn enable more anaerobic microsite formation (Jiang et al. 2011; Bakker et al. 2014). This could, in fact, be true for the converted irrigated-to-pasture soils which have 90-91% average volume of sand, as well as the highest average of putative denitrifiers (Table 4-4). Converting irrigated crop soils to pasture soils is, by definition, the cessation of irrigation and fertilizer application. The combination of these factors could cause nitrate to leach through the soil less quickly and encourage the growth of OTUs with the genetic ability to code for traits associated with denitrification and other nitrate/nitrite reduction processes.

In general, the soil microbial communities are more similar to each other than to the groundwater microbial communities (Figure 4-2, Figure 4-3, Figure 4-8). All four land uses have *Actinomycetales* (phylum *Actinobacteria*) as the order with the highest average relative abundance. This order contains primarily aerobic bacteria that are common in soils (Madigan et al. 2018). The irrigated crop soils are somewhat set apart from the other soils, however, containing a relative abundance of 8.78% *Actinomycetales*, compared to the non-irrigated crop soils (12.30%), the irrigated to pasture soils (13.26%), and the pasture soils (11.22%). Order *Rhizobiales* (p. *Proteobacteria*) and order *Bacillales* (p. *Firmicutes*) are two other primarily aerobic bacterial groups that comprise a higher relative abundance percentage of the microbial community in our soil samples (Madigan et al. 2018).

Considering specific microbial groups in our soil samples with the potential to engage in the nitrogen cycle, the bacterial order *Nitrospirales* (p. *Nitrospirae*) and the archaeal order *Nitrososphaerales* (p.*Crenarchaeota*) contain putative nitrifiers (Madigan et al. 2018). The irrigated crop soils had a slightly higher total average of putative ammonium oxidizers and the highest averages of NO_3-N concentrations (Table 4-4, Table 5-1). Ammonium oxidizers are obligate chemolithotrophs or mixotrophs, which means that we can somewhat accurately connect their function with the presence of OTUs genetically capable of ammonium oxidation (Madigan et al. 2018). While all of our soils contain putative denitrifiers, including those in the order *Pseudomonadales* (p. *Proteobacteria*), the pasture and converted soils have the highest average relative abundance of these OTUs (Table 4-4), similar to results found in other studies (Philippot et al. 2009).

Besides soil NH_4-N and NO_3-N concentrations, another soil geochemistry measurement that stands out is the average calcium concentrations in irrigated soils (Table 4-1). Well 42 soils contain two to three times the concentration of calcium as other soils (Appendix E), which inflates average Ca^{2+} values for irrigated soils. The underlying soil type, Udic Calciustolls, is the most likely cause (2014a). We found gravel-sized pieces of rock in each soil sample we collected near well 42. After bringing the samples back to the lab, we confirmed that the rock was likely carbonate because each piece of rock effervesced profusely with hydrochloric acid. The microbial community in the soils near well 42 is somewhat different from those of the other soils (Figure 4-5). Phylum *Acidobacteria*, order *iii1-15*, has the highest relative abundance at 8.52%. Order *Actinomycetales* (p. *Actinobacteria*) follows with 8.00%, and order *Xanthomonadales* (c. *Gammaproteobacteria*) is third highest (4.90%).

Finally, average soil pH was more similar in irrigated crop and irrigated to pasture soils compared with non-irrigated and pasture soils (Figure 4-1). Average pH was significantly higher for irrigated crop soils compared to non-irrigated crop soils ($p < 0.0001$) and pasture soils ($p = 0.0009$). In irrigated to pasture soils, average soil pH was significantly higher than that of non-irrigated crop ($p = 0.0003$) and pasture soils ($p = 0.0321$). Similarities in pH could be a reason that the microbial communities from the recently converted (irrigated to pasture) soils plotted closer to the microbial community cluster of the irrigated crop soils than to that of the pasture soils (Figure 4-4). Altogether our results indicate that land use is the main contributor influencing field-scale microbial community patterns in our soils, with other factors in our dataset contributing to small-scale similarities and dissimilarities within more localized soil communities.

Table 5-1: Averages of NO_3-N concentrations in groundwater samples at different depths under different land uses, with averages of NO_3-N concentrations in soil samples of different land uses.

Groundwater: Average NO_3-N			Soil: Average NO_3-N		
Depth-Land Use	#of samples	Avg NO_3-N (mg/L)	Land Use	#of samples	Avg NO_3-N (mg/kg)
Shallow-crop	8	18	Non-irrigated crop	27	29.48
Shallow-pasture	4	3.8	Irrigated crop	34	86.05
Deep-crop	6	3.9	Irrigated to pasture	10	6.20
Deep-pasture	3	6.5	Pasture	41	3.84

Land Use Impact on Aquifer

Similar to the results of other studies (Spalding 1984; Whittemore 1993; Lasagna et al. 2016; Richard 2018), our research shows land use to have an effect on groundwater chemistry in our study area (Table 5-1). In addition, land use has a significant correlation with groundwater microbial community composition, as do sampling site, latitude, and longitude (Table 4-5). These results support our hypothesis that land use influences groundwater microbial communities. Well depth category (i.e. wells screened in the shallow, deep, and bedrock portions of the aquifer) is the best defining factor for groundwater community composition patterns in our NMDS plot results. However, neither well depth category nor chloride concentration is significantly correlated with groundwater microbiology. This result diverges from our sub-hypothesis, which states that more saline environments from salt deposit contamination in deep aquifer would have a stronger effect on groundwater microbial community composition than land use. One possible reason for this result is that we biased our study toward less saline environments by collecting groundwater samples that we knew had lower chlorine concentrations in order to decrease field sampling costs.

In order to consider how land use influences our aquifer microbial communities, we must consider how land use affects the chemistry of our groundwater samples. Groundwater NH_4-N concentrations are very low in all samples, with most samples being below detection limits. This result implies that nitrification of ammonium-based fertilizers is occurring in the overlying soil or vadose zone. Groundwater NO_3-N concentrations in 2016, on the other hand, were elevated in many samples and tended to be higher in shallow wells under soils used for growing crops than in other wells (Table 5-1). Isotopic analyses of the groundwater nitrate indicate that ammonia-based fertilizers are the primary source (Appendix G - 1), with potential sewage and manure contributions (Richard 2018). Some of the existing groundwater nitrate is possibly cycling directly back to the aquifer under irrigated crop soils, with the shallow groundwater as the main source of irrigation water (Whittemore 1993) and with high leaching rates through sandy soils (Spalding 1984; Spalding and Kitchen 1988). However, the overall increasing levels of groundwater nitrate, especially in the shallow aquifer (Whittemore 1993; Richard 2018), suggest continued nitrate leaching of newly oxidized ammonium-based fertilizers.

Concerning specific microbial groups in our groundwater samples with the potential to be involved in the nitrogen cycle (Figure 4-3, Table 4-4), bacterial order *Pseudomonadales* contains putative denitrifiers (Madigan et al. 2018). In addition, bacterial order *Nitrospirales* and archaeal phylum *Crenarchaeota* contain putative nitrifiers (Madigan et al. 2018). The shallow aquifer wells below soils used for crop cultivation have noticeably higher relative abundance averages of orders *Pseudomonadales* (12.14%) and *Nitrospirales* (7.03%), as well as a higher relative abundance average of genera that have the potential to be involved in N-reduction (Table 4-4). In comparison, lower relative abundances of orders *Pseudomonadales* and *Nitrospirales* are present in the deep aquifer wells below cultivated soils (7.70% and 5.88%), in the shallow wells below pasture soils (1.34% and 5.42%), and in the deep wells below pasture soils (7.31% and 4.48%). Average relative abundances of archaeal phylum *Crenarchaeota* are higher in the shallow crop wells at 6.52%, compared to the deep crop wells at 3.82%, the shallow pasture wells at 2.30%, and the deep pasture wells at 3.08%.

For the time-sequence study that we did at well 50, we noticed some differences and some similarities in the groundwater chemistry and microbiology. Between 2016 and 2018, NO_3-N concentration increased by 57.22 mg/L in the deep well 50B and decreased by 7.53 mg/L in the shallow well 50C (Table 4-3). This large increase of NO_3-N concentration in the deep aquifer could be due to vertical leakage from the surface through the well casing (Whittemore 1993). Another possibility is that sampling method differences between 2016 and 2018, such as differences in pumping rate or placement of pump hose within the well, could have contributed to different results. Factors affecting the decrease in shallow groundwater NO_3-N concentrations could be dispersion into the deep aquifer, temporal variation, high rates of irrigation using shallow groundwater, and nitrate reduction by anaerobic microbes.

The analysis of the microbial communities in well 50B and 50C yielded different results between samples collected in 2016, 2017, and 2018 (Figure 4-3). The samples collected in 2016 were amplified by MR. DNA labs, along with the other 2016 samples. Duplicate samples from well 50, collected in 2016, were analyzed in 2018. DNA sequencing results for these two analyses varied greatly in the first round of analysis, showing high relative abundance of the genus *Acinetobacter* (c. *Gammaproteobacteria*, o. *Pseudomonadales*) in well 50B (26.37%) and well 50C (50.28%). From the second round of analysis, well 50B contained 35.27% *Acinetobacter*, and well 50C contained 0.40% *Acinetobacter*. This genus is relatively common in soil and water, as well as on human skin (2010; Madigan et al. 2018). Relative abundance of the genus *Acinetobacter* from well 50B is surprisingly high compared to that of other genera; however, the similarity between the two replicates that were analyzed in different years by different people makes likelihood of contamination difficult to determine. On the other hand, the presence of *Acinetobacter* in our control samples, ranging from 0.01% in one negative control to 13.09% in another negative control, means that contamination during collection or analysis is possible. Other possible explanations include variation caused by different primer sets being used in 2016 and 2018 analysis, as well as sampling bias or amplification bias (Fierer 2017). In addition, the genus of highest relative abundance in well 50C of 2018 is *Nitrosopumilus* of phylum *Crenarchaeota* at a relative abundance of 35.42%, and the replicate Well 50C of 2016 has 33.60% of *Nitrosopumilus* (Figure 4-3). This genus contains aerobic, ammonia-oxidizing archaea that tend to require more saline environments for survival (Qin et al. 2016). While this genus is surprisingly high in relative abundance, the higher Cl^- concentration in well 50C (113.5 mg/L) compared to that of well 50B (50.6 mg/L) is a possible factor. In conclusion, the differences between microbial communities in well 50 samples (2016, 2017, and 2018) may be a result of environmental differences, sampling bias, amplification bias, or contamination or a combination thereof.

Links between Soil and Aquifer Microbiology

Microbial community composition is noticeably distinct between the soil and the groundwater (Figure 4-2, Figure 4-3). On average in the soil samples, the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes,* and *Firmicutes* have the higher percentages of relative abundance. In the groundwater, however, the higher average relative abundance percentages belong to bacteria in phyla *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, and *Nitrospirae*. In addition, the archaeal order of highest average relative abundance in the groundwater community belongs to *Cenarchaeales* (p. *Crenarchaeota*, c. *Thaumarchaeota*) at 3.13%; the highest average percentage of an archaeal order in the soils belongs to *Nitrososphaerales* (p.*Crenarchaeota*, c. *Thaumarchaeota*) at 1.16%.

Despite noticeable differences in average microbial community composition between the soil and groundwater, microbial communities correlated significantly with sampling site, as well as longitude and latitude. These statistically significant results suggest that soil and groundwater microbial community compositions at one sampling site may be related to one another in a way that is distinct from microbial communities at other sampling sites. Any similarity in soil and groundwater microbial community composition at one sampling site would most likely result from environmental commonalities.

The primary commonality that we see in our samples is the higher average concentrations of nitrate in irrigated crop soils and groundwater below crop soils compared to other soil and groundwater samples (Table 5-1). Average NO_3-N concentration in irrigated soils is significantly higher than in other soil averages (f = 0.001, Table 5-1). Average nitrate concentrations in shallow crop wells are three times higher than in other wells (Table 5-1), and isotopic analysis of the groundwater nitrate indicates ammonia-based fertilizers as the primary source (Appendix G - 1), with potential sewage and manure contributions (Richard 2018). Extrapolating this further, these higher concentrations of nitrate in soil and aquifer, most likely due to land use, would provide an increased consumable supply for microbes involved in N-reduction.

Another commonality between the aquifer and the soil at individual sampling sites could be abundance of anoxic microenvironments. As mentioned before, anoxic microenvironments in unsaturated soil profiles and in the saturated, unconsolidated aquifer create favorable conditions for higher rates of denitrification (Or et al. 2007; McMahon and Chapelle 2008; DeLong and Rosenberg 2013; Wondzell and Zarnetske 2014; Lasagna et al. 2016; Madigan et al. 2018). With most of the GMD 5 soils being sourced from alluvial deposits, clay lenses occur throughout the sandy soils (Whittemore 1993; Evans 2015). However, a few specific soil types in the study area (e.g. the Carwile soils (Typic Argiaquolls) near wells 50, 6 and 51) have moderate water storage in the soil profile, somewhat poor drainage, and some ponding of water in soils (2014a). These combined factors could lead to a decrease in the amount of available soil oxygen, while increasing relative proportion of anoxic microenvironments in the subsurface. In the shallow aquifer, clay layers interbedded with sand layers would create anoxic microenvironments that are conducive to higher rates of denitrification (Wondzell and Zarnetske 2014; Lasagna et al. 2016). As a result, the soil and groundwater microbial communities could look similar because of a common soil profile that contributes to more anoxic microenvironment formation.

Finally, we hypothesize that groundwater recharge through the overlying soil could physically transport cells into the shallow portion of the aquifer. Cell transport from soil to aquifer would require that microbes cover large distances relative to their size. However, the proximity of the aquifer to the surface, drainage rates due to irrigation at irrigated crop sites, and high drainage potential of the sandy soils in the study area make this scenario a possibility. In addition, aquifer microbes could be added to the soil microbial community as a result of the irrigation water being sourced from the aquifer. More data collection and analyses comparing specific sites need to be done to confirm whether the soil and groundwater microbial communities at one site are related because of cell transport via groundwater recharge and irrigation. However, our results do imply that the microbial communities in the soil and aquifer at one site are possibly related.

Concluding Remarks

Our results indicate that land use has an impact on aquifer microbial communities. With accumulation of nitrate in the shallow aquifer, populations of N-reducing microbes will be vital to maintaining the health of the aquifer. From current statistical analysis, our groundwater microbial communities do not seem to be more influenced by aquifer-specific geochemical factors (i.e. more saline water in the deep aquifer) than by land use factors. Groundwater NH_4-N concentrations consistently below detection limits, high groundwater NO_3-N concentrations in shallow wells below soils used for crop cultivation, and the elevated presence of putative nitrifiers in irrigated crop soils provide some evidence that more nitrification could be occurring in the irrigated soils than in the aquifer. Overall increasing levels of groundwater nitrate, higher average nitrate concentrations in irrigated crop soils and in the shallow aquifer below cultivated soils, and elevated average relative abundances of putative denitrifiers in the shallow aquifer below cultivated soils provide evidence of continued nitrate leaching from newly oxidized ammonium-based fertilizers.

Our results also suggest that groundwater and soil microbial communities at one site could be related to each other in a way that is distinct from communities at other sites. Site-specific environmental factors, like high concentrations of nitrate from land use and increased numbers of anoxic microenvironments from a common soil profile, could encourage site-specific similarities in soil and groundwater microbial communities. In addition, the aqueous transport of microbial cells between soil and aquifer could increase microbial community similarities at specific sites, especially at irrigated sites.

Additional statistical analysis, laboratory tests, sample collection, and county-specific records are needed to develop a more holistic picture of land use impact on soil and aquifer microbial communities. For example, collecting more samples at sites specific to different types of land use will provide more geochemical and microbial community data, which will allow for more reliable statistical analysis. More groundwater samples with microbial community analysis will enlarge our data pool and bring more accuracy to our understanding of the Great Bend Prairie aquifer's microbial community. To better quantify the interaction of aquifer microbial composition and geochemistry through time, groundwater samples could be collected over time. A similar study of soil microbial communities and geochemistry by land use in GMD 5 would better quantify changes in soil microbial community composition through growing seasons. In addition, analyzing enzymatic activity in soils and aquifer would show which microbes are actually active and provide a better understanding of what processes are taking place (Myrold et al. 2014).

Additional tests in situ or in the lab to find soil salinity, soil moisture, and soil mineral content would provide helpful information relating to possible anoxic environments, soil geochemistry, and other factors influencing microbial community composition. Measuring N_2 and other denitrification products, for example, could enhance accuracy of denitrification estimates within the aquifer (McMahon and Chapelle 2008). Calculation of soil water percolation rates and groundwater flow rates would provide a more accurate understanding of how quickly nitrate is leaching into the aquifer from soils and then diffusing through the aquifer.

More statistical analysis could be run on current data to examine whether soil geochemistry besides nitrate (e.g. Ca^{2+}, Na^{+}) and soil texture have a noticeable influence on soil microbial community composition. For example, other environmental variables could be defined as categories, which would allow for more combinations and variations of PERMANOVA tests and NMDS plots with Bray-Curtis dissimilarity distances. Examining statistical significance of microbial communities to environmental metadata by sampling site would provide a better understanding of how site differences in soil type, nutrient concentration, well type, and other factors correlate with microbial community composition.

Finally, records from the Kansas Department of Agriculture, counties, and local farmers could be consulted to obtain more information about types of fertilizers applied to wheat (non-irrigated crop) and corn (irrigated crop) grown in the area, season of application (initial to final), and application rates through a growing season. Quantification and statistical analysis of these additional environmental factors will provide a more holistic picture and provide more insight into how soil geochemistry and texture affect local soil microbial community composition.

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Appendix A - County-wide Soil Descriptions

Pratt county soils are comprised of different classes of loam, from silty loam to clayey loam to fine sandy loam to a combination of all three, coming mainly from Eolian deposits and clayey to loamy alluvium. These soils have no ponding, are mostly well-drained, moderate to high water storage in profile (7.7 to 10.5 inches), and are considered to be prime farmland or farmland of statewide importance. Langdon soil, which comprises 3.9% of soils in Pratt county, however, consists primarily of fine sand to fine sandy loam (parentage of Eolian deposits) and has somewhat excessive drainage, with low water storage in profile (about 6.0 inches). It is not considered to be prime farmland. Depth to water table in Pratt county soil profiles is over 80 inches, with soils classified as non-saline to very slightly saline (0.0 to 2.0 mmhos/cm).

Stafford county contains primarily loamy fine sands, with parent material of Eolian deposits and alluvium. These soils are mostly well-drained, with no ponding, and have moderate to high storage of water in profile. The Carwile soil (about 18.6% of Stafford county soils) is more of an outlier, having a mixture of clayey, sandy loam that drains somewhat poorly, with frequent ponding. Stafford county soils are classified as non-saline to very slightly saline (0.0 to 2.0 mmhos/cm) and are considered prime farmland or farmland of statewide importance. Depth to water table in most soil profiles is over 80 inches, with the exception of the Carwile soil having a depth to water table of 0 inches.

Barton county soils are comprised primarily of silty fine loams, clay silt loams, and fine sandy loams, with parent material of Eolian deposits, loess, and alluvium. These soils are typically well drained and have low to negligible drainoff, with low to very high water storage in profiles (6.0 to 12.8 inches). Most of the soil profiles are considered prime farmland or farmland of statewide importance. The main exception in these soils is the Nibson-Wakeen silt loams

(3.9% of Barton county soils), which are residuum soils from limestone and shale weathering. Although these are also silty clay loams, they exhibit high to very high drainoff, good to excessively high drainage, and low water storage in profiles (3.8 to 5.6 inches); they are not considered good farmland. Depth to water table in all the soils is over 80 inches, and all are classified as non-saline to very slightly saline (0.0 to 2.0 mmhos/cm).

Edwards county soil profiles are more heterogenous than Pratt, Stafford, and Barton soil profiles, comprised of loam (silty to fine sandy to clayey), clay, and sand layers. Parent material is primarily Eolian deposits and alluvium (loamy to clayey), with some loess and calcareous loess. The loamy soils are primarily well drained, with no ponding and low to high water storage in profile (about 6.0 to 11.7 inches). Carwile clay rich soils are somewhat poorly drained, with frequent ponding and moderate water storage in profile (about 8.2 inches). Tivoli fine sand soils are somewhat excessively drained, with low water storage in profile (about 3.4 inches). Depth to water table in all the soils is over 80 inches, with the exception of the Carwile soils (depth to water table is about 0 inches), and all are classified as non-saline to very slightly saline (0.0 to 2.0 mmhos/cm). The Edwards soil profiles are considered prime farmland or farmland of statewide importance, except for the Tivoli soils (not prime farmland).

Appendix B - DNA Analysis Protocols

DNA Extraction

Extraction of total microbial DNA from soil and groundwater followed the protocol dictated by the Qiagen DNeasy® PowerSoil® Kit and modified by Dr. Lydia Zeglin. The twenty-two step, modified protocol using the provided solutions (C1-C6) is as follows:

1. Add approximately 0.5 grams soil to each PowerBead Tube provided, and gently vortex tubes for 5 seconds.
2. Add Solution C1 (60 µL) to each tube, and vortex for 5 seconds.
3. Use MP Fast Prep®-24 to mix solution in tubes at 5.5 m/s for 30 seconds.
4. Centrifuge tubes at 10,000x g for 30 seconds.
5. Pipet supernatant into clean 2 mL collection tubes (approximately 500 µL supernatant per sample).
6. Add Solution C2 (250 µL) to each tube, vortex for 5 seconds, and incubate at 4º C for 5 minutes.
7. Centrifuge at 10,000x g for 1 minute.
8. Pipet up to 600 µL of supernatant into clean 2 mL collection tubes, using care to avoid settled pellets.
9. Add Solution C3 (200 µL) to each tube, vortex for 5 seconds, and incubate at 4º C for 5 minutes.
10. Centrifuge at 10,000x g for 1 minute.
11. Pipet up to 750 µL of supernatant into clean 2 mL collection tubes, using care to avoid settled pellets.

12. Mix Solution C4 by shaking before adding 1200 µL to the supernatant in each tube. Vortex for 5 seconds.
13. Pipet up to 675 µL per sample into MB Spin Columns and centrifuge at 10,000x g for 1 minute. Discard flow through.
14. Repeat step 13 until all liquid of each sample is processed through the MB Spin Columns.
15. Pipet Solution C5 (500 µL) into each tube and centrifuge at 10,000x g for 30 seconds.
16. Discard flow through and centrifuge again at 10,000x g for 1 minute.
17. Carefully place MB Spin Columns into clean 2 mL collection tubes, taking care to avoid getting Solution C5 onto the columns.
18. Pipet Solution C6 (50 µL) into center of white filter membrane in each MB Spin Column and incubate tubes at room temperature for 5 minutes.
19. Centrifuge tubes at room temperature for 30 seconds at 10,000x g.
20. Pipet flow through back into center of white filter membrane in each MB Spin Column and incubate tubes at room temperature for 5 minutes.
21. Centrifuge tubes at room temperature for 30 seconds at 10,000x g and discard MB Spin Columns.
22. Store extracted DNA in labelled 2 mL collection tubes at -20º C until used for downstream application.

For aquifer samples, the same protocol was followed; however, in place of soil in the first step, the filters used to collect groundwater microbes were cut into small pieces and placed into a provided PowerBead Tube. The three control samples were prepared with the same protocol, except that 500 µL 18MΩ deionized water (sterilized via UV radiation) was added to the initial PowerBead Tubes in place of soil or aquifer filter.

DNA Quantification

Quantify DNA samples using the following steps:

1. Take standards (broad range), dye, and buffer out of refrigerator before quantification. Put dye into a drawer to reduce exposure to light.
2. Put buffer and dye into 50 mL tube. Vortex and put back in drawer.
3. Vortex and centrifuge each standard and unknown sample before using.
4. Pipet 1 µL of each standard into 3 separate wells in plate.
5. Pipet 1 µL of each unknown sample into 2 separate wells in plate.
6. Cover with parafilm to keep samples from evaporating while pipetting into all wells.
7. Put buffer-dye solution in trough. Use multi-pipet to put buffer-dye solution in each well (200 µL).
8. Use multi-pipet to mix. Incubate 2 minutes in drawer.
9. Make sure FilterMax F5 Multi-Mode Microplate Reader is using the SoftMax Pro 6.5.1 program on the computer.
10. Set fluorescent wavelengths at 485 λ / 535 λ. Insert Filter 1 into instrument.
11. Place well plate in instrument and push “read.”

16S rRNA gene PCR

Polymerase chain reaction (PCR) amplification of the V4 region of the 16S rRNA gene followed the Earth Microbiome Project (EMP) approach, as specified in the protocol given by

Dr. Lydia Zeglin and taught to me by Janaye Hanschu, Dr. Zeglin's laboratory technician. I used the modified universal primers 515F and 806R. The reverse primer included 96 unique tags to indicate the 96 samples in my library, consisting of 85 soil samples, 6 aquifer samples, 3 control samples, and positive and negative controls. These universal primers for the V4 region have Illumina sequencing adapters attached, allowing for one PCR run (instead of two) to amplify and attach sequencing adapters to the target genes in preparation for sequencing.

Preparation before a PCR run is described in the steps below:

1. Sterilize all instruments and equipment used in UV radiation hood.
2. Calculate how much of each "ingredient" is needed for the template PCR reaction recipe, referred to from now on as Master Mix (MM). Unfreeze, vortex, and centrifuge all except GoTaq enzyme. MM template recipe is shown in this table:

PCR Reagent	**Volume for 1 reaction**
5x Promega master mix buffer (color free)	5 µL
515F primer (10 µM)	0.5 µL
806R primer (10 µM)	(0.5 µL)
dntps (10 mM)	0.5 µL
1% BSA	1 µL
GoTaq, 1.25 U	0.25 µL
genomic DNA	(1 µL)
PCR grade water	16.25 µL
TOTAL for 1 reaction	25 µL

3. Pipet all "ingredients" into a 2 mL collection tube, except GoTaq enzyme, 806R primer, and gDNA. Vortex and centrifuge.
4. Pipet GoTaq enzyme into 2 mL collection tube and mix by stirring with pipet tip.
5. Pipet 0.5 µL 806R primer, 1 µL gDNA, and 23.5 µL MM into an individual test tube per sample.

6. Centrifuge test tubes for 10 seconds
7. Place in thermocycler for 25-35 cycles (takes 2-3 hours per run). A PCR run of fewer cycles reduces amplification bias, increasing data quality, so it is best to start with 25 cycles and increase the number of cycles only if needed.

   The cycles of a PCR run in a thermocycler are described below:

   1) 94º C for 3 minutes (Hot Start)
   2) 94º C for 45 seconds (Dissociation)
   3) 55º C for 1 minute (Primer Annealing)
   4) 72º C for 1.5 minutes (Extension)
   5) Repeat steps 2-4 for 25 cycles
   6) 72º C for 10 minutes (Finishing)
   7) 4-10º C for infinity (Hold)
8. Run an electrophoresis gel to ascertain if PCR amplified gDNA. If so, store at 4º C until ready to combine and purify samples for sequencing.

   Steps for preparing gel are listed below:

   1) Add a 1/100 ratio of agarose and eluent water into a flask (e.g. 0.88 grams agarose to 88 mL eluent water). Eluent water added to agarose must be exactly the same as eluent water surrounding gel.
   2) Heat agarose and eluent water in microwave until mixture boils. Then, take out and swirl to mix, heat again till boiling, take out and swirl to mix, etc. until agarose is completely dissolved.
   3) Cool mixture until hot flask can be touched comfortably, but mixture has not yet solidified.

    4) Pour mixture into mold and let mixture solidify (15-25 minutes).
    5) Submerge gel in box with eluent water.
    6) Place 1 µL Promega loading dye combined with gel red into one dot per sample, with 2-4 dots of 1 µL dye for known DNA ladders, on parafilm.
    7) Pipet 5 µL gDNA into a gel dot for each sample, mix together in pipet tip, and place in a well in the gel.
    8) Pipet 5 µL 100 bp DNA ladder into 2-4 gel dots each (preferably ladders first and last in each row of DNA sample).
    9) Put cover onto box & push “on” and “run.” Run 30-40 minutes and check under UV light to see if gDNA amplified.

9. Run three replicate PCRs (that worked) per sample.

ILL Library Preparation

Sample Combination and EXO-SAP Reaction

Triple replicates of samples were combined into one well of amplicons per sample. EXO-SAP (exonuclease and shrimp alkaline phosphatase), which degrades short DNAs and dntps, was used to clean up the samples. The protocol is below:

1. Dilute EXO-SAP to a 1:10 ratio with water. (For example, I combined 20 µL EXO-SAP with 180 µL 18 MΩ water to equal 200 µL Master Mix.)
2. Add 2 µL of the EXO-SAP Master Mix to each combined PCR well.
3. Incubate plate in Thermocycler at 37º C for 30 minutes, then at 80º C for 20 minutes (to deactivate).

4. Quantitate amount of DNA remaining in each well with the PicoGreen (“Quan-it dsDNA”) assay, using quantification protocol.
5. Normalize samples to the same number of sequence reads per sample for unbiased comparison in sequencing analysis. This is the combined ILL library.

Gel Purification and ILL Library Submission

Gel purification makes sure the combined ILL library is a uniform size before submission. The steps are below:

1. Prepare a small, thick gel with 5 large wells.
2. Vortex combined DNA library and centrifuge for 25 seconds.
3. Put 30 µL dye in 5 pools on parafilm.
4. Pipet 120 µL gDNA into each of 4 pools of dye. Use pipet to mix gDNA and dye. Put into wells.
5. Pipet 100 bp ladder (120 µL) into fifth pool of dye and mix. Place into final well.
6. Run gel for 40-60 minutes and put under UV light.
7. Cut out the fat band at ~400 bp and place in a collection tube. (Use protective gear.)
8. Dissolve gel containing DNA before quantification.
    a. Weigh gel containing DNA. Add a 1:3 ratio of Buffer QG (e.g. 1770 µL DNA and 5310 µL Buffer QG).
    b. Wait 10-20 minutes for gel containing DNA to dissolve in Buffer QG (1:3 ratio), vortexing every 2 minutes.
    c. Add 50 µL of 3M sodium acetate.

   d. Add one volume of isopropanol to DNA (1* 1770 µL DNA = 1770 µL isopropanol)
   e. Add all volume from 15 mL tube into two filter tubes, gradually. Keep centrifuging for 1 min (at 13000 rpm). Discard flow through.
   f. Add 500 µL Buffer QG to tube. Centrifuge for 1 minute at 13000 rpm. Discard flow through.
   g. Add 750 µL Buffer PE to tube. Let stand for 5 minutes.
   h. Centrifuge for 1 minute at 13000 rpm. Discard flow through. Repeat to remove residue.
   i. Place filter tubes into clean collection tubes.

9. To elute the DNA from filter:
   a. Add 25 µL of Buffer EB to each filter.
   b. Incubate 5 minutes at room temperature. Centrifuge 1 minute at 13000 rpm.
   c. Pipet flow through back into filter.
   d. Incubate 5 minutes at room temperature. Centrifuge 1 minute at 13000 rpm.
   e. Combine the 25 µL and 25 µL from each tube to make 50 µL. Label tube.
10. Quantify the gel purified library concentration with PicoGreen, following quantification protocol.
11. Submit the combined and purified library at the Integrated Genomic Facility in the Department of Plant Pathology. Give the length of the library and the concentration of the library in both ng/µL and nM, using the following equation(s) to convert to nM:

$$(X\ \text{ng}\ \mu\text{L}^{-1}) * (1\ \text{nmol bp}\ 660\ \text{ng}^{-1}) * (400\ \text{bp}^{-1}) * (10^{6}\mu\text{L}\ \text{L}^{-1}) = Y\ \text{nM}$$

$$Y\ \text{nM} = X\ \text{ng}\ \mu\text{L}^{-1} * 1{,}000{,}000/(400 * 600)$$

Appendix C - Soil Analysis Protocols

Soil pH

Soil pH was measured on fresh samples in the Geomicrobiology lab of the KSU Department of Geology. Each sample was homogenized ~2 grams of soil was measured out per flask, with one flask for pH analysis with 10mL 18 MΩ deionized water added and with another flask for pH analysis with 10 mL 0.01 M $CaCl_2$ added. Flasks were then capped and shaken on a platform shaker at level 6 for twenty minutes. An Oakton PC-450 meter was used to measure pH of the soil suspensions. The protocol used for measuring soil pH is from the Billings lab (pers. comm.) in the Department of Ecology and Evolutionary Biology at the University of Kansas.

Particle Size Analysis

Samples for soil particle size analysis (PSA) were prepared in the Geology Department's Geochemistry Lab and analyzed in the Geography Department's Paleoenvironmental Lab. Approximately 0.5 grams of air-dried sample was measured into a 50 mL tube, with 1 mL 30% hydrogen peroxide and 5 mL 18 MΩ deionized water, to remove organic material. The mixture was allowed to react for 15-20 minutes until rapid effervescent stopped, after which 25-30 mL 18 MΩ deionized water was added. Samples were then placed in an 80º C water bath for approximately 12 hours until bubbling ceased. After being cooled to room temperature, samples were washed with 18 MΩ deionized water and centrifuged at 5000 rpm for 4 minutes. Supernatant was poured off, with care taken to avoid disturbing settled soil particles. The centrifuging and supernatant removal steps were repeated twice more, after which 18 MΩ deionized water was added to reach a final volume of ~30 mL per tube.

After preparation was completed, each sample was analyzed with a laser diffraction particle size analyzer (Malvern Mastersizer 3000). Test runs on the instrument were performed to find the most accurate program settings for the samples. The finalized program setting had a stirrer speed of ~2700 rpm and 9.5% - 13.5% obscuration. For each sample, we rinsed and filled a beaker with 18 MΩ deionized water, placed the beaker under the fan, and started the program. Each soil sample was shaken to loosen settled grains and homogenize the sample before mixing the sample and pipetting enough sample (~4-8 mL) to be within the specified obscuration range. After the sample was run through the instrument, the tubes were drained into a specimen cup and the instrument's fan and tubes were rinsed with 3 beakers of tap water. PSA data is reported as % by volume.

K-State Soil Testing Lab Analysis

All soil samples were prepared in the Kirk lab as described below before being sent to the K-State Soil Testing Lab for analysis. For all extractions, soil elemental concentrations are provided in mg/kg of soil. Soil organic matter is reported in percent weight after loss-on-ignition was performed on soil samples.

Sample preparation before analysis

1. On a paper plate, dry each sample at room temperature. Put a second paper plate on top to prevent contamination of sample.
2. Use rubber mallet or rolling pin to disaggregate samples.
3. Isolate fraction <2 mm by sieving samples.
4. Give >4 g of sample to K-State soil testing lab for analysis. Each extraction requires 2 g.

Mehlich-3 Phosphorous Extraction

1. For Mehlich-3, a solution of glacial acetic acid, ammonium nitrate, ammonium fluoride and nitric acid is used to extract the soil phosphorous. The extraction and colorimetric assay are described on pp 23-25 of Frank et al. (1998).
2. A Lachat Quickchem 8000 is used to perform the colorimetric assay for the extraction.

Ca, K, Mg, & Na Extraction

1. The ammonium acetate (1M, pH 7.0) method (Warncke and Brown, 1998) with a low-sodium filter paper was used to extract soil cations (Ca, K, Mg, & Na).
2. Analysis was done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer (manufactured by Varian Australia Pty Ltd, Mulgrave, Vic Australia), and a Model Analyst 200 (AA) Spectrometer (from Perkin Elmer Life and Analytical Sciences, Shelton, CT).

Nitrate, Nitrite & Ammonium Analysis

1. A 1M KCl extraction (2 g in 20 ml, 15 min.) and cadmium reduction/colorimetry for analysis are used for soil-extractable nitrate (Gelderman and Beegle, 1998).
2. Ammonia is extracted from soil by 1 M KCl (2 g in 20 ml, 30 min.) in a modification of the method described in Keeney and Nelson (1982). Ammonia in soil extracts was analyzed by an indophenol colorimetric reaction.

3. Analyses of both nitrate and ammonia are performed on a Rapid Flow Analyzer (Model RFA-300) from Alpkem Corporation, Clackamas, OR 97015.

LOI measurement of SOC

1. Loss on Ignition for Organic Matter content is a modified version of Combs and Nathan (1998). The modifications are that 1 g of soil is used, drying at 150º C for 2 hours and igniting at 400º C for 3 hours.

References

Alpkem Corporation. (1986). RFA Methodology No. A303-S170. (pp. 18-19). Nitrate/Nitrite Nitrogen Clackamas, OR.

Alpkem Corporation. (1986). RFA Methodology No. A303-S021. Ammonia Nitrogen. Clackamas, OR.

Combs, S.M and Nathan, M.V. (1998). Soil organic matter. Recommended Chemical Soil Test Procedures for the North Central Region. (pp. 53-58). Missouri Ag. Exp. Stn. SB 1001. Colombia, MO.

Frank, et al. (1998). Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Publication No. 221 (Revised). University of Missouri Agricultural Experiment Station, Columbia, MO.

Gelderman, R.H., and Beegle, D. (1998). Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Publication No. 221 (Revised). University of Missouri Agricultural Experiment Station, Columbia, MO.

Keeney, D.R., and Nelson, D.W. (1982). Nitrogen - Inorganic Forms in A.L. Page et al. (ed.) Methods of Soil Analysis, Part 2. Agronomy 9. (pp. 648-649). ASA and SSSA., Madison, WI.

Mehlich, A. (1984). Mehlich-3 soil test extractant - a modification of Mehlich-2 extractant. Communications in Soil Science and Plant Analysis, 15. (pp. 1409-1416).

Warncke, D.M. and Brown, J.R. (1998). Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Publication. (pp. 31-32). University of Missouri Agricultural Experiment Station, Columbia, MO.

IC Detection Limits

ICS-1100 Ion Chromatograph (IC) Detection Limits	**F^-**	**Cl^-**	**Br^-**	**NO_2^-**	**NO_3^-**	**PO_4^{3-}**	**SO_4^{2-}**	**Na^+**	**NH_4^+**	**K^+**	**Mg^{2+}**	**Ca^{2+}**	**Sr^{2+}**
MDL	0.06	1.54	0.08	0.13	0.016	0.48	0.71	0.22	0.13	0.16	0.19	0.50	0.17

All units are in mg/L.

Appendix D - Statistical Analysis Commands

QIIME: Illumina 16S Amplicon Data Analysis

The commands listed below were used to sort through and filter the raw sequencing data in QIIME, as well as perform some alpha diversity analysis on the filtered DNA data. These commands were adapted from the protocols written and used by Dr. Lydia Zeglin and Dr. Matthew Kirk.

1. Modify the MiSeqReporter config file (MiSeq Reporter.exe.config) to include this line below <configuration> and <appSettings>:

```
<add key="CreateFastqForIndexReads" value="1"/>
```

This file can be modified in any text editor. There should already be a list of “<add key=” strings to paste this one below. Save and run the MiSeq Reporter analysis.

2. Transfer fastq output files to the Mac that already has QIIME loaded. For instructions on loading QIIME, and on all QIIME commands, go to http://qiime.org/index.html. There should be three files, one ending in I1_001.fastq, one ending in R1_001.fastq and one ending in R2_001.fastq.

3. (a) Navigate to and set the working directory:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$
```

(b) Join the paired end reads for my data.

```
join_paired_ends.py -f $PWD/Undetermined_S0_L001_R1_001.fastq -r $PWD/Undetermined_S0_L001_R2_001.fastq -b $PWD/Undetermined_S0_L001_I1_001.fastq -o $PWD/fastq-join_joined
```

start 14:01, end 14:34

4. Check the mapping file (a .txt file, see http://qiime.org/index.html for instructions) to make sure it is set up correctly.

To validate my mapping file:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ validate_mapping_file.py -m 515F806Rprimers_CRR.txt
```

To validate Allie's mapping file:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ validate_mapping_file.py -m 092717MK519F-mapping2.txt
```

5. Demultiplex the library, using the sample names and associated index sequences noted in the mapping file.

Step 5a Command for my data:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ split_libraries_fastq.py -o slout -i fastq-join_joined/fastqjoin.join.fastq -b fastq-join_joined/fastqjoin.join_barcodes.fastq --rev_comp_mapping_barcodes -m 515F806Rprimers_CRR.txt
```

start 15:44, end 16:09

Step 5b Commands for Allie's Data: (Same as above)

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ cd Allie_sequence_data
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ split_libraries.py -f 092717MK519F-full.fasta -m 092717MK519F-mapping2.txt -q 092717MK519F-full.qual -b hamming_8 -o split_libraries_A_output
```

start 11:41, end 11:50

Step 5c Join my data files and Allie's data files:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ head Allie_sequence_data/split_libraries_A_output/seqs.fna
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ tail Allie_sequence_data/split_libraries_A_output/seqs.fna
```

Check the data file beginning and end:

```
head slout/seqs.fna
tail slout/seqs.fna
cat Allie_sequence_data/split_libraries_A_output/seqs.fna slout/seqs.fna > CombiChristinaAllie/slout_seqs.fna
```

6. OTUs are chosen in QIIME's workflows using different methods, depending on the type of amplicon data that is analyzed and the availability of databases for different gene amplicons. For bacterial 16S, this protocol uses the current "QIIME-developer-preferred" OTU picking workflow with the reference database file of greengenes 97% similar non-redundant sequence database (gg/13_8_otus/rep_set/97_otus.fasta). The tutorials at http://qiime.org/tutorials/otu_picking.html and http://qiime.org/tutorials/open_reference_illumina_processing.html explain what is being done to the data at this step. I used 18 processors to complete this step on the big Mac in the Zeglin lab.

Step 6 Command (for combined samples from here on):

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ cd CombiChristinaAllie
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ PWD
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ pick_open_reference_otus.py -i $PWD/slout_seqs.fna -o
$PWD/OpenRefOTUs -r $PWD/gg_13_8_otus/rep_set/97_otus.fasta -aO 18
```

start 13:01, end 16:54

7. Assign taxonomy using RDP. This protocol used the Ribosomal Database Project (RDP) taxonomy assigner, which uses a hierarchical assignment algorithm and uses more computing power. I used a command flag to increase the memory allocation for this RDP taxonomy matching step as well, in order to avoid command failure.

Step 7 Command:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ parallel_assign_taxonomy_rdp.py -i
$PWD/OpenRefOTUs/rep_set.fna -o $PWD/OpenRefOTUs/rdp_assigned_taxonomy -T --jobs_to_start 18 --
rdp_max_memory 20000
```

start 18:46, end 18:53

8. Manually complete OTU assignment workflow on RDP taxonomy data. In preparation for downstream analysis, this step cleans up the output of the OTU assignment workflow by appending the taxonomy assignment to the OTU matrix and then removing the OTUs that failed to align to the reference 16S gene sequence from the final OTU matrix.

Step 8 = 2 Commands:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ biom add-metadata -i
$PWD/OpenRefOTUs/otu_table_mc2.biom --observation-metadata-fp
$PWD/OpenRefOTUs/rdp_assigned_taxonomy/rep_set_tax_assignments.txt -o
$PWD/OpenRefOTUs/otu_table_mc2_w_rdp-tax.biom --sc-separated taxonomy --observation-header
OTUID,taxonomy
```

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ filter_otus_from_otu_table.py -i $PWD/OpenRefOTUs/otu_table_mc2_w_rdp-tax.biom -o $PWD/OpenRefOTUs/otu_table_mc2_w_rdp-tax_no_pynast_failures.biom -e $PWD/OpenRefOTUs/pynast_aligned_seqs/rep_set_failures.fasta
```

start 18:56, end 18:58

9. Summarize the OTU picking output to be sure the read/OTU/sample distribution makes sense. Note: I changed output file from second command in Step 8 from “otu_table_mc2_w_rdp-tax_no_pynast_failures.biom” to “ANALYSIS-otu-table.biom”

Step 9 Commands:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ biom summarize-table -i OpenRefOTUs/ANALYSIS-otu-table.biom -o OpenRefOTUs/ANALYSIS-otu-table_summary.txt
```

10. Check for chimeras using chimera slayer. The output .txt file lists all possible chimeric sequences. Not all sequences that Chimera Slayer identifies are actually chimeric, but it is most conservative with so much data to remove any suspicious reads. This step takes the most time (5-7 hours). Note: reference file “core_set_aligned.fasta.imputed” is from previous folder made by Dr. Zeglin (taken from another source) in 2014.

Step 10 Command:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ parallel_identify_chimeric_seqs.py -i $PWD/OpenRefOTUs/pynast_aligned_seqs/rep_set_aligned.fasta -r $PWD/core_set_aligned.fasta.imputed -o $PWD/chimera_slayer_chimeric_seqs.txt -O 18
```

start 19:16 (Friday), end 00:49 (Saturday)

11. The chimeric sequences need to be removed from the dataset, both from the main fasta file and from the biom table that goes into downstream analysis—the two appropriate commands are below. In addition, any other known contaminant sequences should also be removed from the final dataset and biom table (there were no known contaminant sequences in my samples at the time I did this analysis).

Step 11 = 2 Commands:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ filter_otus_from_otu_table.py -i
$PWD/OpenRefOTUs/ANALYSIS-otu-table.biom -o $PWD/OpenRefOTUs/ANALYSIS-otu-table-clean.biom -e
$PWD/chimera_slayer_chimeric_seqs.txt
```

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ filter_fasta.py -f
OpenRefOTUs/pynast_aligned_seqs/rep_set_aligned.fasta -o
OpenRefOTUs/pynast_aligned_seqs/non_chimeric_rep_set_aligned.fasta -s chimera_slayer_chimeric_seqs.txt -n
```

start 12:50 end 12:52

12. Alpha diversity analysis commands: I created a new folder to work in and moved the final clean OTU output matrix to the new folder, to stay more organized. I also moved the output rep_set.tre file to the new directory.

ALPHA DIVERSITY from Dr. Kirk’s protocol

(a) Normalize number of sequences in each sample:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ biom summarize-table -i $PWD/Combi_CR-
AR_Analysis/ANALYSIS-otu-table-clean.biom -o $PWD/Combi_CR-AR_Analysis/otu_table_filtered_summary.txt
```

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ single_rarefaction.py -i $PWD/Combi_CR-AR_Analysis/ANALYSIS-otu-table-clean.biom -o $PWD/Combi_CR-AR_Analysis/otu_table_filtered_normalized.biom -d 160
```

Note: 15009 is the number of sequences in Sample 93 (Samples 90,95,92,96,6, and 8 have a smaller number of sequences)

(b) Summarize the taxonomy of sequences in each sample

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$  validate_mapping_file.py -m $PWD/Combi_CR-AR_Analysis/515F806Rprimers_Mapping_CRR2.txt -B
```

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$  summarize_taxa_through_plots.py -i $PWD/AnalysisOTUs3/ANALYSIS-otu-table-clean.biom -o $PWD/AnalysisOTUs3/summarize_taxa_through_plots_output_corrected3 -m $PWD/AnalysisOTUs3/515F806Rprimers_Mapping_CRR2_corrected.txt -s
```

start 18:04, end 18:28

ALPHA DIVERSITY from Dr. Zeglin’s protocol (I decided to go with this one because I used her protocol for all of the other steps. Also, I had one sample with 2 sequences and, when I was trying to normalize the samples as required in Dr. Kirk’s protocol, I wasn’t sure how to do that without taking out the sample with only 2 sequences.)

Step 12 Commands:

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ alpha_rarefaction.py -i $PWD/AnalysisOTUs3/ANALYSIS-otu-table-clean.biom -m $PWD/AnalysisOTUs3/515F806Rprimers_Mapping_CRR2_corrected.txt -o AnalysisOTUs3/alpha_rarefaction_output3 -t $PWD/AnalysisOTUs3/rep_set.tre -aO 18
```

start 21:46, end 22:14

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ alpha_diversity.py -i $PWD/AnalysisOTUs3/ANALYSIS-otu-table-clean.biom -m chao1,PD_whole_tree,observed_otus  -t $PWD/AnalysisOTUs3/rep_set.tre -o AnalysisOTUs3/C_R_alpha_diversity.txt
```

start 22:16, end 22:18

BETA DIVERSITY (these commands did not work for me, no matter the changes I made to the mapping file and commands)

```
MacQIIME ZLab-Mac:Christina_Nov2018 lzeglin$ jackknifed_beta_diversity.py -i $PWD/AnalysisOTUs3/ANALYSIS-otu-table-clean.biom -m $PWD/AnalysisOTUs3/515F806Rprimers_Mapping_CRR2_corrected.txt -t $PWD/AnalysisOTUs3/rep_set.tre  -o $PWD/AnalysisOTUs3/BetaDiversity/jackknifed_beta_diversity -e 160
```

start 22:25 end 22:36 with errors

```
beta_diversity_through_plots.py -i $PWD/AnalysisOTUs3/ANALYSIS-otu-table-clean.biom -e 160 -o AnalysisOTUs3/BetaDiversity/PCoA160 -t $PWD/AnalysisOTUs3/rep_set.tre -m $PWD/AnalysisOTUs3/515F806Rprimers_Mapping_CRR2_corrected.txt -aO18
```

start 22:37 end 22:48 with errors

RStudio: Statistical Analysis Commands

The commands listed below were used to load different packages in RStudio, load and merge the DNA data and environmental metadata, and perform beta diversity analysis with graph and table outputs. These commands were adapted from some used and sent to me by Kent Connell.

Beginning Codes in RStudio: Load Packages, Load Data, & Merge Data Files

```
##Install phyloseq
```

```
source("http://bioconductor.org/biocLite.R")
biocLite("phyloseq")

source("http://bioconductor.org/biocLite.R")
biocLite("multtest")

source("http://bioconductor.org/biocLite.R")
biocLite("BiocUpgrade")

install.packages("stringi")

#To set a working directory
setwd("C:/Users/Christina/Documents/Articles and Reviews_MastersResearch/RStudio_Run1")
getwd()

## Loading libraries
library(phyloseq)
library(ggplot2)
library(scales)
library(grid)
library(tidyverse)
```

```
## Load mapping file
mapfile<-"Mapping_02232019.txt"
map<-import_qiime_sample_data(mapfile)

## Load tree file
tree<-read_tree("rep_set.tre")

## Load OTU table
otufile<-"ANALYSIS-otu-table-clean.biom"
biomfile<-import_biom(otufile,parseFunction=parse_taxonomy_default)

## Merge the three objects together in phyloseq
testdata<-merge_phyloseq(biomfile,tree,map)
print(testdata)
```

Example Codes for Subset Data Files & NMDS Plots

```
#create subsets of data
bacteria.blank<-subset_samples(testdata, Blank=="Y")
bacteria.blank

bacteria.noblank<-subset_samples(testdata, Blank=="N")
bacteria.noblank
```

```
bacteria.soil<-subset_samples(testdata, Soil=="Y")

bacteria.soil


bacteria.aquifer<-subset_samples(testdata, Aquifer=="Y")

bacteria.aquifer


# Turn taxa abundance into relative abundance

total <-median(sample_sums(bacteria.soil))

standf <- function(x, t=total) round(t * (x / sum(x)))

bacteria.soilready<-transform_sample_counts(bacteria.soil, standf)

bacteria.soilready


total <-median(sample_sums(bacteria.aquifer))

standf <- function(x, t=total) round(t * (x / sum(x)))

bacteria.aquiferready<-transform_sample_counts(bacteria.aquifer, standf)

bacteria.aquiferready


total <-median(sample_sums(bacteria.noblank))

standf <- function(x, t=total) round(t * (x / sum(x)))

bacteria.noblankready<-transform_sample_counts(bacteria.noblank, standf)

bacteria.noblankready


#ordination for NMDS- Considering NO3N
```

```
ordu <- ordinate(bacteria.soilready, "NMDS", "unifrac", weighted=TRUE)
alldata<-plot_ordination(bacteria.soilready, ordu, color="NO3Nmg/kg", shape="LandUse") + geom_point(size=3)
alldata

ordu <- ordinate(bacteria.aquiferready, "NMDS", "unifrac", weighted=TRUE)
alldata2<-plot_ordination(bacteria.aquiferready, ordu, color="NO3N", shape="WellType") + geom_point(size=3)
alldata2
```

Example Codes for Bray Tests

```
# make a data frame from the sample_data
sampledf <- data.frame(sample_data(bacteria.soilready))

#BraySignificance- LandUse,NO3N, NH4N
bacteria.perm<-adonis(bray.not.na ~ LandUse * NO3Nmg/kg * NH4Nmg/kg, data = sampledf)
bacteria.perm

#BraySignificance- LandUse, pH, NO3N
bacteria.perm1<-adonis(bray.not.na ~ LandUse * pHDI * NO3Nmg/kg, data = sampledf)
bacteria.perm1
```

```
#BraySignificance- LandUse, OM, NO3N

bacteria.perm2<-adonis(bray.not.na ~ LandUse * OMLOI * NO3Nmg/kg, data = sampledf)

bacteria.perm2
```

Appendix E - Data Tables

Soil Sampling Site: General Information

Sample Name	Type of Landuse	Latitude, Longitude	Top Depth (cm)	Bottom Depth (cm)	Depth Difference (cm)	Wet Weight (g)	Dry Weight (g)	Water Weight (g)	% Volume Clay	% Volume Silt	% Volume Sand	Soil Type
6-A01-S005-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	0.00	5.00	5.00	14.00	13.93	0.07	0.26	6.49	93.23	Sand/Loamy Sand
6-A01-S015-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	5.00	15.00	10.00	16.09	15.92	0.17	0.31	6.99	92.68	Sand/Loamy Sand
6-A01-S030-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	15.00	30.00	15.00	15.05	14.76	0.29	0.31	7.61	92.06	Sand/Loamy Sand
6-A02-S005-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	0.00	5.00	5.00	13.02	12.76	0.26	0.31	7.56	92.14	Sand/Loamy Sand
6-A02-S015-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	5.00	15.00	10.00	15.59	14.95	0.64	0.29	6.84	92.85	Sand/Loamy Sand
6-A02-S030-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	15.00	30.00	15.00	19.06	17.94	1.12	0.34	7.96	91.68	Sand/Loamy Sand
6-A03-S005-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	0.00	5.00	5.00	15.69	15.18	0.51	0.64	12.46	86.95	Sandy Loam

6-A03-S015-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	5.00	15.00	10.00	17.38	17.03	0.35	0.56	10.83	88.64	Sandy Loam
6-A04-S005-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	0.00	5.00	5.00	17.25	17.01	0.24	0.42	9.36	90.21	Sand/Loamy Sand
6-A04-S015-20180604	Non-irrigated crop	37°54'27"N, 98°48'10"W	5.00	15.00	10.00	16.63	16.39	0.24	0.42	8.78	90.78	Sand/Loamy Sand
52-A01-S005-20180604	Pasture	38°5'12"N, 98°48'9"W	0.00	5.00	5.00	16.62	16.38	0.24	0.26	7.91	91.84	Sand/Loamy Sand
52-A01-S015-20180604	Pasture	38°5'12"N, 98°48'9"W	5.00	15.00	10.00	16.36	15.93	0.43	0.30	7.94	91.76	Sand/Loamy Sand
52-A01-S030-20180604	Pasture	38°5'12"N, 98°48'9"W	15.00	30.00	15.00	16.11	15.50	0.61	0.36	8.90	90.72	Sand/Loamy Sand
52-A02-S005-20180604	Pasture	38°5'12"N, 98°48'9"W	0.00	5.00	5.00	16.25	15.83	0.42	0.20	7.61	92.15	Sand/Loamy Sand
52-A02-S015-20180604	Pasture	38°5'12"N, 98°48'9"W	5.00	15.00	10.00	17.59	17.26	0.33	0.29	6.73	93.00	Sand/Loamy Sand
52-A02-S030-20180604	Pasture	38°5'12"N, 98°48'9"W	15.00	30.00	15.00	17.82	16.84	0.98	0.46	11.15	88.40	Sandy Loam
52-A03-S005-20180604	Pasture	38°5'12"N, 98°48'9"W	0.00	5.00	5.00	16.67	16.47	0.20	0.32	10.45	89.23	Sandy Loam

52-A03-S015-20180604	Pasture	38°5'12"N, 98°48'9"W	5.00	15.00	10.00	16.42	15.52	0.90	0.34	9.53	90.09	Sand/Loamy Sand
52-A03-S030-20180604	Pasture	38°5'12"N, 98°48'9"W	15.00	30.00	15.00	16.55	15.96	0.59	0.40	11.27	88.37	Sandy Loam
52-A04-S005-20180604	Pasture	38°5'12"N, 98°48'9"W	0.00	5.00	5.00	16.59	15.97	0.62	0.46	14.38	85.16	Sandy Loam
52-A04-S015-20180604	Pasture	38°5'12"N, 98°48'9"W	5.00	15.00	10.00	18.66	17.72	0.94	0.52	13.80	85.69	Sandy Loam
52-A04-S030-20180604	Pasture	38°5'12"N, 98°48'9"W	15.00	30.00	15.00	17.31	16.81	0.50	0.49	13.74	85.80	Sandy Loam
36-A01-S005-20180604	Pasture	37°43'58"N, 98°40'33"W	0.00	5.00	5.00	16.32	15.88	0.44	0.49	30.17	69.32	Sandy Loam
36-A01-S015-20180604	Pasture	37°43'58"N, 98°40'33"W	5.00	15.00	10.00	15.54	14.98	0.56	0.30	30.70	69.00	Sandy Loam
36-A02-S005-20180604	Pasture	37°43'58"N, 98°40'33"W	0.00	5.00	5.00	16.61	16.49	0.12	1.06	29.42	69.50	Sandy Loam
36-A02-S015-20180604	Pasture	37°43'58"N, 98°40'33"W	5.00	15.00	10.00	19.71	19.32	0.39	0.70	25.49	73.83	Sandy Loam
36-A03-S005-20180604	Pasture	37°43'58"N, 98°40'33"W	0.00	5.00	5.00	15.49	15.16	0.33	1.17	29.80	68.99	Sandy Loam
36-A03-S015-20180604	Pasture	37°43'58"N, 98°40'33"W	5.00	15.00	10.00	16.63	16.15	0.48	0.93	27.23	71.78	Sandy Loam

36-A03-S030-20180604	Pasture	37°43'58"N, 98°40'33"W	15.00	30.00	15.00	18.87	18.23	0.64	0.94	28.95	70.12	Sandy Loam
36-A04-S005-20180604	Pasture	37°43'58"N, 98°40'33"W	0.00	5.00	5.00	15.13	15.00	0.13	1.10	26.30	72.61	Sandy Loam
36-A04-S015-20180604	Pasture	37°43'58"N, 98°40'33"W	5.00	15.00	10.00	16.02	15.56	0.46	1.13	29.25	69.62	Sandy Loam
51-A01-S005-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	0.00	5.00	5.00	18.74	16.30	2.44	2.34	41.75	55.93	Loam
51-A01-S015-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	5.00	15.00	10.00	24.09	21.41	2.68	2.95	50.82	46.26	Loam
51-A01-S030-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	15.00	30.00	15.00	21.59	18.93	2.66	5.25	78.1	16.7	Silt loam
51-A02-S005-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	0.00	5.00	5.00	20.95	18.69	2.26	3.46	54.65	41.94	Silt loam/Loam
51-A02-S015-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	5.00	15.00	10.00	24.19	20.95	3.24	2.55	46.77	50.67	Loam
51-A02-S030-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	15.00	30.00	15.00	23.69	20.69	3.00	4.25	81.7	14.05	Silt Loam
51-A03-S005-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	0.00	5.00	5.00	21.98	19.68	2.30	2.18	35.73	62.08	Sandy Loam

51-A03-S015-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	5.00	15.00	10.00	23.72	20.62	3.10	2.48	39.13	58.38	Loam
51-A03-S030-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	15.00	30.00	15.00	23.77	20.61	3.16	0.89	20.62	78.48	Sandy Loam
51-A04-S005-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	0.00	5.00	5.00	20.49	18.20	2.29	1.31	23.57	75.11	Sandy Loam
51-A04-S015-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	5.00	15.00	10.00	25.60	22.50	3.10	2.41	36.73	60.84	Sandy Loam
51-A04-S030-20180604	Non-irrigated crop	38°10'30"N, 98°48'11"W	15.00	30.00	15.00	23.46	20.09	3.37	3.08	45.35	51.55	Loam
34-A01-S005-20180604	Pasture	37°49'30"N, 98°15'21"W	0.00	5.00	5.00	15.93	15.74	0.19	1.07	32.41	66.50	Sandy Loam
34-A01-S015-20180604	Pasture	37°49'30"N, 98°15'21"W	5.00	15.00	10.00	17.27	16.75	0.52	0.73	26.89	72.37	Sandy Loam
34-A02-S005-20180604	Pasture	37°49'30"N, 98°15'21"W	0.00	5.00	5.00	15.84	15.80	0.04	0.66	24.64	74.65	Sandy Loam
34-A02-S015-20180604	Pasture	37°49'30"N, 98°15'21"W	5.00	15.00	10.00	18.52	18.27	0.25	0.52	19.08	80.41	Sandy Loam
34-A03-S005-20180604	Pasture	37°49'30"N, 98°15'21"W	0.00	5.00	5.00	23.01	22.96	0.05	0.42	15.57	84.02	Sandy Loam

34-A03-S015-20180604	Pasture	37°49'30"N, 98°15'21"W	5.00	15.00	10.00	23.09	22.96	0.13	0.31	14.09	85.61	Sandy Loam
34-A04-S005-20180604	Pasture	37°49'30"N, 98°15'21"W	0.00	5.00	5.00	19.55	19.54	0.01	0.46	16.25	83.24	Sandy Loam
34-A04-S015-20180604	Pasture	37°49'30"N, 98°15'21"W	5.00	15.00	10.00	21.17	20.72	0.45	0.40	16.55	83.06	Sandy Loam
10-A01-S005-20180604	Pasture	37°59'5"N, 98°27'47"W	0.00	5.00	5.00	21.75	21.36	0.39	0.81	18.23	80.95	Sandy Loam
10-A01-S015-20180604	Pasture	37°59'5"N, 98°27'47"W	5.00	15.00	10.00	26.00	25.34	0.66	0.87	21.07	78.06	Sandy Loam
10-A01-S030-20180604	Pasture	37°59'5"N, 98°27'47"W	15.00	30.00	15.00	24.46	23.38	1.08	0.74	28.76	70.51	Sandy Loam
10-A02-S005-20180604	Pasture	37°59'5"N, 98°27'47"W	0.00	5.00	5.00	22.35	21.77	0.58	0.63	14.67	84.71	Sandy Loam
10-A02-S015-20180604	Pasture	37°59'5"N, 98°27'47"W	5.00	15.00	10.00	20.39	19.84	0.55	0.74	19.43	79.80	Sandy Loam
10-A02-S030-20180604	Pasture	37°59'5"N, 98°27'47"W	15.00	30.00	15.00	22.28	21.50	0.78	0.54	14.94	84.48	Sandy Loam
10-A03-S005-20180604	Pasture	37°59'5"N, 98°27'47"W	0.00	5.00	5.00	22.06	21.43	0.63	1.25	27.60	71.15	Sandy Loam
10-A03-S015-20180604	Pasture	37°59'5"N, 98°27'47"W	5.00	15.00	10.00	21.90	21.22	0.68	0.84	25.83	73.35	Sandy Loam

10-A03-S030-20180604	Pasture	37°59'5"N, 98°27'47"W	15.00	30.00	15.00	34.71	32.47	2.24	1.62	46.47	51.91	Loam
10-A04-S005-20180604	Pasture	37°59'5"N, 98°27'47"W	0.00	5.00	5.00	23.15	21.97	1.18	1.11	25.68	73.19	Sandy Loam
10-A04-S015-20180604	Pasture	37°59'5"N, 98°27'47"W	5.00	15.00	10.00	21.22	20.32	0.90	1.02	25.96	73.06	Sandy Loam
10-A04-S030-20180604	Pasture	37°59'5"N, 98°27'47"W	15.00	30.00	15.00	33.16	31.36	1.80	0.94	40.49	58.55	Loam
3-A01-S005-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	0.00	5.00	5.00	20.54	19.32	1.22	0.33	9.19	90.46	Sand/Loamy Sand
3-A01-S015-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	5.00	15.00	10.00	22.03	21.10	0.93	0.32	8.82	90.84	Sand/Loamy Sand
3-A01-S030-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	15.00	30.00	15.00	26.07	24.82	1.25	0.35	9.09	90.57	Sand/Loamy Sand
3-A02-S005-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	0.00	5.00	5.00	21.24	21.06	0.18	0.20	7.44	92.39	Sand/Loamy Sand
3-A02-S015-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	5.00	15.00	10.00	22.64	21.74	0.90	0.41	11.51	88.09	Loamy Sand
3-A02-S030-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	15.00	30.00	15.00	20.69	19.29	1.40	2.10	41.55	56.34	Loam

3-A03-S005-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	0.00	5.00	5.00	22.94	22.88	0.06	0.32	7.99	91.70	Sand/Loamy Sand
3-A03-S015-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	5.00	15.00	10.00	23.92	23.64	0.28	0.26	7.49	92.27	Sand/Loamy Sand
3-A04-S005-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	0.00	5.00	5.00	20.29	20.24	0.05	0.32	8.99	90.70	Sand/Loamy Sand
3-A04-S015-20180604	Irrigated to Pasture	37°59'59"N, 98°42'4"W	5.00	15.00	10.00	20.26	20.08	0.18	0.24	8.73	91.01	Sand/Loamy Sand
21-A01-S005-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	0.00	5.00	5.00	20.38	18.55	1.83	0.74	17.56	81.71	Sandy Loam
21-A01-S015-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	5.00	15.00	10.00	22.69	20.20	2.49	0.57	14.74	84.70	Sandy Loam
21-A01-S030-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	15.00	30.00	15.00	23.71	20.17	3.54	0.64	20.74	78.59	Sandy Loam
21-A02-S005-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	0.00	5.00	5.00	21.22	19.78	1.44	0.71	23.00	76.25	Sandy Loam
21-A02-S015-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	5.00	15.00	10.00	21.25	19.40	1.85	0.59	17.92	81.50	Sandy Loam
21-A02-S030-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	15.00	30.00	15.00	22.87	21.01	1.86	0.4	15.71	83.87	Sandy Loam

21-A03-S005-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	0.00	5.00	5.00	21.18	19.42	1.76	0.40	12.91	86.67	Sandy Loam
21-A03-S015-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	5.00	15.00	10.00	21.46	19.39	2.07	0.37	12.96	86.67	Sandy Loam
21-A03-S030-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	15.00	30.00	15.00	27.69	25.77	1.92	0.51	15.45	84.05	Sandy Loam
21-A04-S005-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	0.00	5.00	5.00	20.15	19.18	0.97	0.46	14.55	84.99	Sandy Loam
21-A04-S015-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	5.00	15.00	10.00	26.29	24.43	1.86	0.40	13.81	85.76	Sandy Loam
21-A04-S030-20180604	Irrigated crop	37°48'27"N, 98°27'56"W	15.00	30.00	15.00	23.28	21.92	1.36	0.42	14.11	85.46	Sandy Loam
50-A01-S005-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	0.00	5.00	5.00	21.38	19.81	1.57	1.12	24.82	74.05	Sandy Loam
50-A01-S015-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	5.00	15.00	10.00	25.06	22.12	2.94	1.21	24.18	74.59	Sandy Loam
50-A02-S005-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	0.00	5.00	5.00	28.13	24.12	4.01	1	24	75	Sandy Loam
50-A02-S015-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	5.00	15.00	10.00	27.08	24.33	2.75	0.97	22.39	76.66	Sandy Loam
50-A02-S030-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	15.00	30.00	15.00	29.10	26.38	2.72	0.66	17.97	81.38	Sandy Loam

50-A03-S005-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	0.00	5.00	5.00	19.75	16.69	3.06	0.99	23.53	75.51	Sandy Loam
50-A03-S015-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	5.00	15.00	10.00	25.44	22.41	3.03	1.06	25.33	73.6	Sandy Loam
50-A03-S030-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	15.00	30.00	15.00	29.12	25.88	3.24	0.48	12.17	87.34	Sandy Loam
50-A04-S005-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	0.00	5.00	5.00	20.74	18.00	2.74	0.87	19.47	79.64	Sandy Loam
50-A04-S015-20180604	Irrigated crop	38°15'15"N, 98°48'10"W	5.00	15.00	10.00	24.84	23.28	1.56	0.57	17.39	82.06	Sandy Loam
50-B01-S005-20180604	Non-irrigated crop	38°15'18"N, 98°48'05"W	0.00	5.00	5.00	20.78	20.65	0.13	0.91	18.74	80.34	Sandy Loam
50-B02-S005-20180604	Non-irrigated crop	38°15'18"N, 98°48'05"W	0.00	5.00	5.00	21.08	20.94	0.14	0.87	18.24	80.91	Sandy Loam
50-B03-S005-20180604	Non-irrigated crop	38°15'18"N, 98°48'05"W	0.00	5.00	5.00	21.23	20.34	0.89	1	19.15	79.84	Sandy Loam
50-B03-S015-20180604	Non-irrigated crop	38°15'18"N, 98°48'05"W	5.00	15.00	10.00	20.77	19.38	1.39	1.12	25.36	73.5	Sandy Loam
50-B04-S005-20180604	Non-irrigated crop	38°15'18"N, 98°48'05"W	0.00	5.00	5.00	22.85	22.68	0.17	1.16	20.78	78.07	Sandy Loam

42-A01-S005-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	0.00	5.00	5.00	24.14	22.74	1.40	0	54.97	45.04	Silt Loam/Loam
42-A01-S015-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	5.00	15.00	10.00	26.97	23.97	3.00	0.79	59.52	39.67	Silt Loam
42-A01-S030-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	15.00	30.00	15.00	25.24	23.35	1.89	0	68.92	31.07	Silt Loam
42-A02-S005-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	0.00	5.00	5.00	23.09	21.82	1.27	0	48.12	51.9	Loam
42-A02-S015-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	5.00	15.00	10.00	24.71	22.82	1.89	0.79	54.65	44.54	Silt Loam/Loam
42-A02-S030-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	15.00	30.00	15.00	26.50	24.68	1.82	0	65.58	34.4	Silt Loam
42-A03-S005-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	0.00	5.00	5.00	24.52	23.03	1.49	0	46.29	53.73	Loam
42-A03-S015-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	5.00	15.00	10.00	25.88	23.40	2.48	0	41.38	58.61	Loam
42-A03-S030-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	15.00	30.00	15.00	28.69	26.16	2.53	0	67.89	32.11	Silt Loam
42-A04-S005-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	0.00	5.00	5.00	19.69	18.07	1.62	0	45.08	54.93	Loam
42-A04-S015-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	5.00	15.00	10.00	25.87	23.28	2.59	0	56.19	43.83	Silt Loam/Loam

42-A04-S030-20180604	Irrigated crop	37°38'16"N, 98°41'54"W	15.00	30.00	15.00	31.18	27.95	3.23	0	62.45	37.54	Silt Loam

Soil Geochemistry

Sample Name	OM LOI %	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	P-M mg/kg	NO3-N mg/kg	NH4-N mg/kg	pH (DI)	pH (0.01 M CaCl2)
6-A01-S005-20180604	1.3	440.0	82.1	2.2	119.2	41.4	2.7	5.6	5.92	5.61
6-A01-S015-20180604	0.9	377.7	56.5	6.0	72.9	30.3	2.2	6.8	5.79	5.19
6-A01-S030-20180604	0.6	380.6	52.9	7.5	87.0	28.5	1.2	7.4	6.02	5.24
6-A02-S005-20180604	1.3	459.7	79.2	5.5	153.8	26.9	11.3	15.4	6.61	5.75
6-A02-S015-20180604	0.9	378.8	63.5	6.9	85.7	20.8	4.2	6.7	6.4	5.54
6-A02-S030-20180604	0.4	294.5	51.3	5.2	82.6	17.2	1.5	5.5	6.62	5.53
6-A03-S005-20180604	1.9	385.8	50.7	4.3	161.9	86.9	9.6	7.2	5.24	4.41
6-A03-S015-20180604	1.4	374.6	54.0	8.2	114.2	74.2	10.8	6.9	5.11	4.7
6-A04-S005-20180604	1.5	306.7	46.9	6.2	116.4	80.1	6.4	6.5	5.11	4.23
6-A04-S015-20180604	1.4	225.4	35.7	7.9	77.7	85.3	6.1	7.2	4.84	4.04
52-A01-S005-20180604	2.5	643.4	112.7	9.7	161.1	15.2	7.1	6.9	6.55	6.01
52-A01-S015-20180604	1.6	487.1	71.2	2.5	107.7	6.4	4.4	5.8	6.42	5.88
52-A01-S030-20180604	0.8	480.4	65.9	0.3	89.8	5.2	3.4	5.3	6.32	5.6
52-A02-S005-20180604	0.7	720.7	102.1	3.5	157.5	13.4	10.5	6.0	6.65	6.12
52-A02-S015-20180604	2.0	404.3	62.7	5.5	119.9	7.0	4.6	5.6	6.75	6.04

52-A02-S030-20180604	0.7	462.8	73.8	3.1	117.9	4.5	2.1	5.2	6.95	6.32
52-A03-S005-20180604	0.6	730.5	107.4	3.2	98.6	9.9	6.0	8.9	6.39	5.65
52-A03-S015-20180604	2.1	574.4	88.3	2.5	87.9	3.9	4.0	6.6	6.34	5.61
52-A03-S030-20180604	1.1	526.3	89.6	1.8	93.2	2.4	2.3	6.3	6.47	5.69
52-A04-S005-20180604	0.7	851.1	126.7	2.7	161.3	10.3	6.8	7.8	6.5	5.8
52-A04-S015-20180604	3.2	585.3	84.8	2.1	123.0	3.1	4.8	6.2	6.46	5.58
52-A04-S030-20180604	1.2	595.6	95.3	1.7	141.1	2.7	1.7	6.8	6.3	5.51
36-A01-S005-20180604	0.8	2,709.9	89.5	9.9	253.4	12.6	3.0	7.7	8	7.11
36-A01-S015-20180604	2.7	3,019.3	63.7	8.6	198.5	5.9	1.8	8.8	8.27	7.49
36-A02-S005-20180604	2.9	1,846.6	153.8	9.5	246.5	12.2	2.1	8.0	6.65	5.97
36-A02-S015-20180604	1.6	1,767.7	101.4	10.4	170.0	5.4	2.8	7.7	6.85	6.34
36-A03-S005-20180604	3.2	2,131.0	157.6	11.6	253.2	22.8	1.9	8.2	7.41	6.84
36-A03-S015-20180604	2.1	1,886.5	159.8	15.3	198.9	14.6	3.4	9.0	7.44	6.68
36-A03-S030-20180604	2.7	1,807.0	137.4	8.3	170.8	10.6	2.2	6.8	7.58	6.94
36-A04-S005-20180604	2.1	1,964.8	188.6	4.9	294.1	26.4	3.8	11.2	6.61	6.1

36-A04-S015-20180604	1.5	1,716.1	151.2	9.7	258.8	7.5	3.5	11.0	6.76	6.17
51-A01-S005-20180604	3.3	1,652.9	236.8	19.0	717.2	88.6	28.0	8.8	6.21	5.26
51-A01-S015-20180604	2.3	1,975.0	267.0	30.5	426.4	55.3	25.4	9.9	6.1	5.24
51-A01-S030-20180604	3.0	3,715.5	660.6	190.1	366.5	10.1	10.3	6.9	7.21	6.39
51-A02-S005-20180604	2.6	2,407.8	351.8	21.8	549.4	69.5	21.1	7.0	6.26	5.53
51-A02-S015-20180604	1.5	2,578.0	358.1	49.9	404.4	52.7	12.2	7.1	6.59	5.81
51-A02-S030-20180604	1.8	4,001.9	659.1	205.6	446.5	4.6	6.2	4.2	7.73	6.99
51-A03-S005-20180604	2.7	1,336.2	215.0	15.1	541.2	53.8	26.2	8.6	5.31	4.48
51-A03-S015-20180604	2.2	1,656.8	251.0	15.4	362.6	31.3	20.2	6.2	5.73	4.92
51-A03-S030-20180604	1.4	2,202.7	357.6	49.1	277.6	14.2	15.4	5.8	6.22	5.35
51-A04-S005-20180604	2.2	1,613.6	305.2	20.2	599.7	252.0	330.6	804.9	6.62	6.11
51-A04-S015-20180604	2.0	1,781.3	302.8	24.3	364.0	54.3	105.7	193.1	5.58	5.06
51-A04-S030-20180604	1.9	3,073.7	580.0	108.0	403.8	35.3	108.9	135.5	5.93	5.51
34-A01-S005-20180604	2.6	1,136.9	172.7	6.0	293.3	47.8	5.0	11.9	5.91	5.03
34-A01-S015-20180604	2.2	919.5	92.1	4.0	132.2	28.3	3.7	6.7	5.67	4.77

34-A02-S005-20180604	2.1	902.9	109.2	5.0	195.0	35.2	1.5	7.4	5.63	5.02
34-A02-S015-20180604	3.7	745.3	85.5	2.9	104.2	24.5	1.8	7.2	5.72	4.86
34-A03-S005-20180604	2.8	504.6	63.9	2.2	116.0	33.7	2.4	6.5	5.4	4.63
34-A03-S015-20180604	2.1	547.5	62.0	4.1	89.4	29.9	2.8	4.4	5.55	4.81
34-A04-S005-20180604	2.6	422.3	63.0	4.7	121.2	30.9	9.8	10.1	4.82	4.2
34-A04-S015-20180604	1.9	499.5	67.5	5.6	79.1	18.1	14.1	6.2	4.95	4.27
10-A01-S005-20180604	1.9	1,050.1	189.7	7.7	184.5	23.5	1.8	42.0	5.36	4.99
10-A01-S015-20180604	1.4	1,114.0	224.8	10.8	167.0	10.3	1.0	21.3	5.44	4.91
10-A01-S030-20180604	1.3	1,601.6	346.9	11.8	203.6	8.2	1.5	13.1	5.83	5.2
10-A02-S005-20180604	1.1	991.6	175.5	8.6	198.9	9.0	2.6	5.5	6.19	5.53
10-A02-S015-20180604	2.9	873.1	155.6	2.2	144.7	4.7	3.5	6.6	5.96	5.29
10-A02-S030-20180604	1.9	780.1	154.1	1.5	118.3	8.7	1.0	5.2	5.99	5.25
10-A03-S005-20180604	1.4	1,419.0	213.6	0.4	232.4	7.8	4.3	6.2	6.19	5.43
10-A03-S015-20180604	2.8	1,227.6	198.4	0.5	195.6	3.5	3.8	5.8	6.3	5.51
10-A03-S030-20180604	1.6	2,137.8	437.7	9.7	364.5	2.3	1.7	4.0	6.59	5.8

10-A04-S005-20180604	1.7	1,708.7	281.7	5.6	318.7	8.6	7.4	8.5	6.29	5.43
10-A04-S015-20180604	1.1	1,418.2	268.8	1.7	259.9	3.4	4.0	5.8	6.26	5.32
10-A04-S030-20180604	4.2	1,609.4	340.7	2.7	278.0	4.0	1.6	4.5	6.41	5.56
3-A01-S005-20180604	1.9	816.9	126.5	0.8	229.8	292.0	21.4	6.7	6.78	6.08
3-A01-S015-20180604	1.2	649.6	107.3	1.1	182.3	249.0	16.2	5.0	6.98	6.29
3-A01-S030-20180604	5.2	310.2	101.5	1.2	205.6	134.0	3.5	3.5	7.29	6.63
3-A02-S005-20180604	1.7	502.2	83.6	2.4	180.9	162.0	7.8	5.4	6.85	6.1
3-A02-S015-20180604	1.2	487.7	89.7	0.6	222.1	179.0	5.5	3.9	7.05	6.29
3-A02-S030-20180604	1.8	1,791.9	312.0	49.6	967.7	87.3	2.5	4.1	8.1	7.38
3-A03-S005-20180604	1.3	488.9	88.9	1.1	126.3	137.0	1.1	4.1	6.68	5.97
3-A03-S015-20180604	0.5	472.7	91.8	0.5	139.3	129.0	1.8	3.4	6.81	6.16
3-A04-S005-20180604	2.7	602.3	112.1	2.0	107.9	199.0	1.0	4.2	7.19	6.51
3-A04-S015-20180604	1.1	600.1	114.3	2.4	107.4	217.0	1.2	3.3	7.26	6.56
21-A01-S005-20180604	1.0	1,048.8	96.8	37.2	317.2	135.0	28.9	5.2	6.78	6.36
21-A01-S015-20180604	0.6	1,007.7	82.0	30.1	209.1	134.0	27.8	5.5	6.7	6.14
21-A01-S030-20180604	2.7	1,139.1	78.1	54.6	145.0	252.0	13.5	4.4	7.16	6.47
21-A02-S005-20180604	1.1	1,022.0	111.6	74.6	225.7	173.0	155.6	13.5	5.16	4.75
21-A02-S015-20180604	1.5	1,077.7	89.0	115.4	179.5	190.0	62.8	5.8	6.09	5.59

21-A02-S030-20180604	1.1	931.9	75.7	112.3	174.5	210.0	13.5	3.2	6.93	6.28
21-A03-S005-20180604	2.3	955.9	85.1	45.4	203.5	123.0	76.8	5.7	6.05	5.68
21-A03-S015-20180604	1.9	820.7	61.2	40.0	107.7	137.0	9.0	3.8	6.87	6.28
21-A03-S030-20180604	1.3	774.7	57.1	50.9	87.4	115.0	7.0	3.4	6.96	6.4
21-A04-S005-20180604	2.1	864.1	91.3	38.4	174.9	140.0	118.7	16.4	5.47	4.77
21-A04-S015-20180604	1.9	959.1	77.4	55.1	151.9	148.0	119.4	4.7	5.49	5.17
21-A04-S030-20180604	0.8	716.1	54.0	39.7	118.0	130.0	22.2	2.6	6.47	5.99
50-A01-S005-20180604	1.9	1,854.8	202.2	23.6	552.7	357.0	137.8	8.2	6.31	5.93
50-A01-S015-20180604	1.1	1,513.3	163.6	15.1	390.2	210.0	27.2	4.4	6.74	6.21
50-A02-S005-20180604	0.6	1,715.6	142.7	27.3	356.9	185.0	161.1	14.0	5.6	5.16
50-A02-S015-20180604	1.8	1,536.9	146.5	36.6	429.4	10.0	84.7	5.1	5.86	5.4
50-A02-S030-20180604	1.3	1,645.4	191.0	38.6	444.6	10.0	55.3	4.4	5.76	5.23
50-A03-S005-20180604	0.6	1,974.6	161.6	52.3	336.7	10.0	247.1	12.3	5.47	5.11
50-A03-S015-20180604	3.7	1,450.3	116.6	35.4	206.5	10.0	57.7	5.6	6.28	5.86
50-A03-S030-20180604	2.3	1,412.6	161.8	48.0	218.2	10.0	14.8	3.8	6.25	5.67

50-A04-S005-20180604	3.0	1,939.9	165.7	40.2	377.2	10.0	157.1	10.2	6.04	5.56
50-A04-S015-20180604	1.9	1,706.9	148.7	31.8	249.8	10.0	41.6	4.6	6.71	6.3
50-B01-S005-20180604	1.3	937.2	134.8	10.0	394.9	10.0	3.6	4.6	6.12	5.4
50-B02-S005-20180604	3.8	677.3	99.6	10.1	355.2	10.0	4.4	3.6	5.21	4.5
50-B03-S005-20180604	1.8	814.1	122.4	10.7	340.1	10.0	9.0	4.7	5.44	4.72
50-B03-S015-20180604	1.3	699.7	110.1	12.5	221.8	10.0	4.7	4.3	5.07	4.33
50-B04-S005-20180604	3.9	756.1	116.4	6.3	350.7	10.0	8.1	5.4	5.2	4.49
42-A01-S005-20180604	2.1	4,160.6	136.7	26.6	308.4	10.0	44.9	4.7	8.09	7.38
42-A01-S015-20180604	2.3	4,629.9	97.8	34.4	157.4	10.0	64.1	3.8	8.13	7.5
42-A01-S030-20180604	1.5	4,827.7	78.8	32.3	130.0	10.0	41.6	3.3	8.21	7.59
42-A02-S005-20180604	1.9	4,168.1	133.2	18.7	310.5	10.0	43.4	4.8	7.88	7.22
42-A02-S015-20180604	1.3	4,299.0	118.8	15.0	220.6	10.0	66.8	4.9	8.23	7.43
42-A02-S030-20180604	1.7	4,948.9	91.0	16.3	146.4	10.0	83.1	3.2	8.18	7.53
42-A03-S005-20180604	2.5	4,246.2	146.9	28.6	332.9	10.0	303.5	5.6	7.64	7.25
42-A03-S015-20180604	1.8	4,689.6	133.1	28.2	198.4	10.0	333.8	6.6	7.83	7.38

42-A03-S030-20180604	1.4	4,947.5	117.2	35.4	136.3	10.0	138.8	4.2	8.08	7.56
42-A04-S005-20180604	2.4	4,216.3	133.0	44.2	279.9	10.0	59.3	5.5	8.21	7.47
42-A04-S015-20180604	2.1	4,363.5	119.1	44.9	188.6	10.0	44.6	4.7	8.22	7.48
42-A04-S030-20180604	1.7	5,041.1	90.6	53.6	132.1	10.0	62.1	3.6	8.24	7.54

Aquifer Geochemistry: Well 50 Time Sequence

Well ID	Latitude, Longitude	Well type	Year Sampled	T °C (field)	Aquifer pH (field)	C (µS/cm)	Alkalinity (meq/L)	Alkalinity (mg/L as CaCO3)	F- mg/L	Cl- mg/L	NO2- mg/L	Br- mg/L	NO3- mg/L	PO4 3- mg/L
50B-2016	38°15'18"N, 98°48'05"W	Deep Aquifer	2016	18.2	7.34	639	3.52	176.3	0.462	50.616	n.a.	0.1233	48.003	n.a.
50B-2018	38°15'18"N, 98°48'05"W	Deep Aquifer	2018	24.7	7.27	675	n.a.	n.a.	0.379	53.019	0.182	0.1353	301.39	n.a.
50B Averaged	n.a.	n.a.	n.a.	21.45	7.305	657	3.52	176.3	0.42	51.817	0.182	0.1293	174.69	n.a.
Δ 50B	n.a.	n.a.	2	-6.5	0.07	-36	n.a.	n.a.	0.083	-2.403	0.182	-0.012	-253.4	n.a.
50C-2016	38°15'18"N, 98°48'05"W	Shallow Aquifer	2016	15.9	7.02	1095	4.77	238.9	0.257	113.53	n.a.	0.248	127.72	n.a.
50C-2018	38°15'18"N, 98°48'05"W	Shallow Aquifer	2018	21.9	6.74	996.1	n.a.	265.7	0.267	77.417	n.a.	0.2641	94.354	0.0617
50C Averaged	n.a.	n.a.	n.a.	18.9	6.88	1045.55	4.77	252.3	0.262	95.471	n.a.	0.2561	111.04	0.0617
Δ 50C	n.a.	n.a.	2	-6	0.28	98.9	n.a.	-26.8	-0.01	36.108	n.a.	-0.016	33.366	0.0617

Well ID	SO4 2- mg/L	Na+ mg/L	NH4+ mg/L	K+ mg/L	Mg2+ mg/L	Ca2+ mg/L	Sr2+ mg/L	SiO2 mg/L	HCO3 mg/L	NO3-N mg/L	PO4-P mg/L	NH4-N mg/L	NO2-N mg/L	Water type
50B-2016	36.394	56.99	n.a.	8.9806	3.0765	66.721	n.a.	n.a.	214.76	10.839	n.a.	n.a.	n.a.	Ca-HCO3
50B-2018	39.289	53.26	0.1066	3.1225	8.6062	56.86	2.1992	n.a.	n.a.	68.055	n.a.	0.0829	0.0554	Ca-HCO3
50B Averaged	37.841	55.13	0.1066	6.0516	5.8414	61.791	2.1992	n.a.	214.76	39.447	n.a.	0.0829	0.0554	n.a.
Δ 50B	-2.895	3.735	0.1066	5.8581	-5.53	9.861	2.1992	n.a.	n.a.	-57.22	n.a.	0.0829	0.0554	n.a.
50C-2016	55.905	48.47	n.a.	3.8631	15.827	127.42	5.7108	n.a.	291.02	28.84	n.a.	n.a.	n.a.	Ca-HCO3
50C-2018	53.123	47.89	n.a.	4.1492	14.163	113.92	3.5126	265.7	n.a.	21.306	0.0201	n.a.	n.a.	Ca-HCO3
50C Averaged	54.514	48.18	n.a.	4.0062	14.995	120.67	4.6117	265.7	291.02	25.073	0.0201	n.a.	n.a.	n.a.
Δ 50C	2.7815	0.58	n.a.	-0.286	1.6642	13.498	2.1982	n.a.	n.a.	7.534	0.0201	n.a.	n.a.	n.a.

Microbial Communities: Sequencing Tables

Sequencing Data Table: 2018 Samples

Soil Sample ID	Sample Nickname	Land Use	Pre-PCR (ng/L)	Post-PCR (ng/L)	Normalised (uL)	Barcode Sequence	Linker Primer Sequence	Primer Name
42-A01-S005-20180604	42-1-05	Irrigated Crop	5.8015	4.6427	21.5392	ACGAGACTGATT	GTGCCAGCMGCCGCGGTAA	806rcbc1
42-A01-S015-20180604	42-1-15	Irrigated Crop	3.1506	6.1005	16.3920	GCTGTACGGATT	GTGCCAGCMGCCGCGGTAA	806rcbc2
42-A02-S005-20180604	42-2-05	Irrigated Crop	5.8177	4.5330	22.0606	ATCACCAGGTGT	GTGCCAGCMGCCGCGGTAA	806rcbc3
42-A02-S015-20180604	42-2-15	Irrigated Crop	4.6331	7.0659	14.1524	TGGTCAACGATA	GTGCCAGCMGCCGCGGTAA	806rcbc4
42-A03-S005-20180604	42-3-05	Irrigated Crop	6.1801	13.820	7.2355	ATCGCACAGTAA	GTGCCAGCMGCCGCGGTAA	806rcbc5
42-A03-S015-20180604	42-3-15	Irrigated Crop	3.6286	4.8911	20.4451	TACAGCGCATAC	GTGCCAGCMGCCGCGGTAA	806rcbc9
42-A04-S005-20180604	42-4-05	Irrigated Crop	6.4830	6.7981	14.7099	ACCGGTATGTAC	GTGCCAGCMGCCGCGGTAA	806rcbc10
42-A04-S015-20180604	42-4-15	Irrigated Crop	3.9679	2.7286	36.6487	AATTGTGTCGGA	GTGCCAGCMGCCGCGGTAA	806rcbc11
6-A01-S005-20180604	6-1-05	Non-irrigated Crop	8.1598	4.5345	22.0530	TGCATACACTGG	GTGCCAGCMGCCGCGGTAA	806rcbc12
6-A01-S015-20180604	6-1-15	Non-irrigated Crop	4.9884	8.8705	11.2734	AGTCGAACGAGG	GTGCCAGCMGCCGCGGTAA	806rcbc13
6-A02-S005-20180604	6-2-05	Non-irrigated Crop	8.3132	8.5660	11.6741	ACCAGTGACTCA	GTGCCAGCMGCCGCGGTAA	806rcbc14

6-A02-S015-20180604	6-2-15	Non-irrigated Crop	4.8602	5.0738	19.7089	GAATACCAAGTC	GTGCCAGCMGCCGCGGTAA	806rcbc15
6-A03-S005-20180604	6-3-05	Non-irrigated Crop	4.8041	9.1810	10.8921	GTAGATCGTGTA	GTGCCAGCMGCCGCGGTAA	806rcbc16
6-A03-S015-20180604	6-3-15	Non-irrigated Crop	3.9982	12.7440	7.8468	TAACGTGTGTGC	GTGCCAGCMGCCGCGGTAA	806rcbc17
6-A04-S005-20180604	6-4-05	Non-irrigated Crop	4.1478	6.3510	15.7456	CATTATGGCGTG	GTGCCAGCMGCCGCGGTAA	806rcbc18
6-A04-S015-20180604	6-4-15	Non-irrigated Crop	3.6160	14.8429	6.7372	CCAATACGCCTG	GTGCCAGCMGCCGCGGTAA	806rcbc19
51-A01-S005-20180604	51-1-05	Non-irrigated Crop	9.3534	10.2750	9.7323	GATCTGCGATCC	GTGCCAGCMGCCGCGGTAA	806rcbc20
51-A01-S015-20180604	51-1-15	Non-irrigated Crop	4.2060	6.5733	15.2130	CAGCTCATCAGC	GTGCCAGCMGCCGCGGTAA	806rcbc21
51-A02-S005-20180604	51-2-05	Non-irrigated Crop	1.4896	8.9625	11.1575	AGGCTTACGTGT	GTGCCAGCMGCCGCGGTAA	806rcbc63
51-A02-S015-20180604	51-2-15	Non-irrigated Crop	0.7494	4.8691	20.5377	TCTCTACCACTC	GTGCCAGCMGCCGCGGTAA	806rcbc64
51-A03-S005-20180604	51-3-05	Non-irrigated Crop	5.4997	6.4532	15.4962	CAAACAACAGCT	GTGCCAGCMGCCGCGGTAA	806rcbc22
51-A03-S015-20180604	51-3-15	Non-irrigated Crop	3.5289	7.3394	13.6251	GCAACACCATCC	GTGCCAGCMGCCGCGGTAA	806rcbc23
51-A04-S005-20180604	51-4-05	Non-irrigated Crop	3.8560	6.4846	15.4212	GCGATATATCGC	GTGCCAGCMGCCGCGGTAA	806rcbc24
51-A04-S015-20180604	51-4-15	Non-irrigated Crop	2.5000	2.3260	42.9916	CGAGCAATCCTA	GTGCCAGCMGCCGCGGTAA	806rcbc25
36-A01-S005-20180604	36-1-05	Pasture	20.5824	12.0039	8.3306	AGTCGTGCACAT	GTGCCAGCMGCCGCGGTAA	806rcbc26
36-A01-S015-20180604	36-1-15	Pasture	11.3922	5.7091	17.5158	GTATCTGCGCGT	GTGCCAGCMGCCGCGGTAA	806rcbc27
36-A02-S005-20180604	36-2-05	Pasture	21.6212	9.6168	10.3984	CGAGGGAAAGTC	GTGCCAGCMGCCGCGGTAA	806rcbc28

36-A02-S015-20180604	36-2-15	Pasture	8.1491	9.1653	10.9107	CAAATTCGGGAT	GTGCCAGCMGCCGCGGTAA	806rcbc29
36-A03-S005-20180604	36-3-05	Pasture	19.5798	10.7701	9.2850	AGATTGACCAAC	GTGCCAGCMGCCGCGGTAA	806rcbc30
36-A03-S015-20180604	36-3-15	Pasture	13.4243	7.0997	14.0851	AGTTACGAGCTA	GTGCCAGCMGCCGCGGTAA	806rcbc31
36-A04-S005-20180604	36-4-05	Pasture	18.3357	17.9597	5.5680	GCATATGCACTG	GTGCCAGCMGCCGCGGTAA	806rcbc32
36-A04-S015-20180604	36-4-15	Pasture	14.5867	5.4207	18.4477	CAACTCCCGTGA	GTGCCAGCMGCCGCGGTAA	806rcbc33
52-A01-S005-20180604	52-1-05	Pasture	12.8323	3.6493	27.4028	TTGCGTTAGCAG	GTGCCAGCMGCCGCGGTAA	806rcbc34
52-A01-S015-20180604	52-1-15	Pasture	6.6976	4.8273	20.7157	TACGAGCCCTAA	GTGCCAGCMGCCGCGGTAA	806rcbc35
52-A02-S005-20180604	52-2-05	Pasture	0.1910	5.4989	18.1856	CACTACGCTAGA	GTGCCAGCMGCCGCGGTAA	806rcbc36
52-A02-S015-20180604	52-2-15	Pasture	0.4260	1.9255	51.9348	TGCAGTCCTCGA	GTGCCAGCMGCCGCGGTAA	806rcbc37
52-A03-S005-20180604	52-3-05	Pasture	0.6595	4.0654	24.5976	ACCATAGCTCCG	GTGCCAGCMGCCGCGGTAA	806rcbc38
52-A03-S015-20180604	52-3-15	Pasture	0.1773	3.8230	26.1573	TCGACATCTCTT	GTGCCAGCMGCCGCGGTAA	806rcbc39
52-A04-S005-20180604	52-4-05	Pasture	0.9482	2.0011	49.9716	GAACACTTTGGA	GTGCCAGCMGCCGCGGTAA	806rcbc40
52-A04-S015-20180604	52-4-15	Pasture	0.5717	1.5961	62.6540	GAGCCATCTGTA	GTGCCAGCMGCCGCGGTAA	806rcbc41
21-A01-S005-20180604	21-1-05	Irrigated Crop	10.3582	11.9694	8.3547	CATTCGTGGCGT	GTGCCAGCMGCCGCGGTAA	806rcbc47
21-A01-S015-20180604	21-1-15	Irrigated Crop	10.9720	15.8705	6.3010	TACTACGTGGCC	GTGCCAGCMGCCGCGGTAA	806rcbc48
21-A02-S005-20180604	21-2-05	Irrigated Crop	3.6656	8.1702	12.2395	GGCCAGTTCCTA	GTGCCAGCMGCCGCGGTAA	806rcbc49

21-A02-S015-20180604	21-2-15	Irrigated Crop	4.0971	4.7032	21.2623	GATGTTCGCTAG	GTGCCAGCMGCCGCGGTAA	806rcbc50
21-A03-S005-20180604	21-3-05	Irrigated Crop	7.8799	7.1965	13.8957	CTATCTCCTGTC	GTGCCAGCMGCCGCGGTAA	806rcbc51
21-A03-S015-20180604	21-3-15	Irrigated Crop	4.0623	3.3348	29.9866	ACTCACAGGAAT	GTGCCAGCMGCCGCGGTAA	806rcbc52
21-A04-S005-20180604	21-4-05	Irrigated Crop	4.7631	2.7728	36.0650	ATGATGAGCCTC	GTGCCAGCMGCCGCGGTAA	806rcbc53
21-A04-S015-20180604	21-4-15	Irrigated Crop	4.8418	5.2611	19.0073	GTCGACAGAGGA	GTGCCAGCMGCCGCGGTAA	806rcbc54
34-A01-S005-20180604	34-1-05	Pasture	11.3708	18.9253	5.2839	TGTCGCAAATAG	GTGCCAGCMGCCGCGGTAA	806rcbc55
34-A01-S015-20180604	34-1-15	Pasture	6.4008	6.0754	16.4599	CATCCCTCTACT	GTGCCAGCMGCCGCGGTAA	806rcbc56
34-A02-S005-20180604	34-2-05	Pasture	8.7164	5.9787	16.7261	TATACCGCTGCG	GTGCCAGCMGCCGCGGTAA	806rcbc57
34-A02-S015-20180604	34-2-15	Pasture	8.3561	4.9925	20.0301	AGTTGAGGCATT	GTGCCAGCMGCCGCGGTAA	806rcbc58
34-A03-S005-20180604	34-3-05	Pasture	5.6784	5.1779	19.3129	ACAATAGACACC	GTGCCAGCMGCCGCGGTAA	806rcbc59
34-A03-S015-20180604	34-3-15	Pasture	5.2559	5.1064	19.5833	CGGTCAATTGAC	GTGCCAGCMGCCGCGGTAA	806rcbc60
34-A04-S005-20180604	34-4-05	Pasture	2.3991	3.7203	26.8794	GTGGAGTCTCAT	GTGCCAGCMGCCGCGGTAA	806rcbc61
34-A04-S015-20180604	34-4-15	Pasture	3.1677	3.2584	30.6899	GCTCGAAGATTC	GTGCCAGCMGCCGCGGTAA	806rcbc62
3-A01-S005-20180604	3-1-05	Irrigated to Pasture	4.1570	3.8373	26.0601	ACTTCCAACTTC	GTGCCAGCMGCCGCGGTAA	806rcbc65
3-A01-S015-20180604	3-1-15	Irrigated to Pasture	6.1740	1.9723	50.7027	CTCACCTAGGAA	GTGCCAGCMGCCGCGGTAA	806rcbc66

3-A02-S005-20180604	3-2-05	Irrigated to Pasture	4.7157	1.8663	53.5819	GTGTTGTCGTGC	GTGCCAGCMGCCGCGGTAA	806rcbc67
3-A02-S015-20180604	3-2-15	Irrigated to Pasture	3.1234	7.1696	13.9477	CCACAGATCGAT	GTGCCAGCMGCCGCGGTAA	806rcbc68
3-A03-S005-20180604	3-3-05	Irrigated to Pasture	4.6663	3.7033	27.0031	TATCGACACAAG	GTGCCAGCMGCCGCGGTAA	806rcbc69
3-A03-S015-20180604	3-3-15	Irrigated to Pasture	6.3280	6.5841	15.1881	GATTCCGGCTCA	GTGCCAGCMGCCGCGGTAA	806rcbc70
3-A04-S005-20180604	3-4-05	Irrigated to Pasture	6.9681	4.2672	23.4346	CGTAATTGCCGC	GTGCCAGCMGCCGCGGTAA	806rcbc71
3-A04-S015-20180604	3-4-15	Irrigated to Pasture	4.6602	2.7056	36.9598	GGTGACTAGTTC	GTGCCAGCMGCCGCGGTAA	806rcbc72
10-A01-S005-20180604	10-1-05	Pasture	4.6400	1.9474	51.3508	ATGGGTTCCGTC	GTGCCAGCMGCCGCGGTAA	806rcbc73
10-A01-S015-20180604	10-1-15	Pasture	1.6635	3.8094	26.2510	TAGGCATGCTTG	GTGCCAGCMGCCGCGGTAA	806rcbc74
10-A02-S005-20180604	10-2-05	Pasture	11.7901	7.6069	13.1460	AACTAGTTCAGG	GTGCCAGCMGCCGCGGTAA	806rcbc75
10-A02-S015-20180604	10-2-15	Pasture	7.6675	2.0307	49.2431	ATTCTGCCGAAG	GTGCCAGCMGCCGCGGTAA	806rcbc76
10-A03-S005-20180604	10-3-05	Pasture	26.5048	8.6988	11.4958	AGCATGTCCCGT	GTGCCAGCMGCCGCGGTAA	806rcbc77
10-A03-S015-20180604	10-3-15	Pasture	9.0514	4.8281	20.7119	GTACGATATGAC	GTGCCAGCMGCCGCGGTAA	806rcbc78
10-A04-S005-20180604	10-4-05	Pasture	26.2826	6.0831	16.4390	GTGGTGGTTTCC	GTGCCAGCMGCCGCGGTAA	806rcbc79
10-A04-S015-20180604	10-4-15	Pasture	6.8102	2.8658	34.8947	TAGTATGCGCAA	GTGCCAGCMGCCGCGGTAA	806rcbc80
50A-A01-S005-20180604	50A-1-05	Irrigated Crop	11.6026	13.1069	7.6296	TGCGCTGAATGT	GTGCCAGCMGCCGCGGTAA	806rcbc81

50A-A01-S015-20180604	50A-1-15	Irrigated Crop	4.7270	8.0432	12.4329	ATGGCTGTCAGT	GTGCCAGCMGCCGCGGTAA	806rcbc82
50A-A02-S005-20180604	50A-2-05	Irrigated Crop	6.1933	2.1361	46.8146	GTTCTCTTCTCG	GTGCCAGCMGCCGCGGTAA	806rcbc83
50A-A02-S015-20180604	50A-2-15	Irrigated Crop	5.2641	2.0450	48.9008	CGTAAGATGCCT	GTGCCAGCMGCCGCGGTAA	806rcbc84
50A-A03-S005-20180604	50A-3-05	Irrigated Crop	7.6675	2.5480	39.2460	GCGTTCTAGCTG	GTGCCAGCMGCCGCGGTAA	806rcbc85
50A-A03-S015-20180604	50A-3-15	Irrigated Crop	4.3500	1.5507	64.4856	GTTGTTCTGGGA	GTGCCAGCMGCCGCGGTAA	806rcbc86
50A-A04-S005-20180604	50A-4-05	Irrigated Crop	10.7074	3.2043	31.2081	GGACTTCCAGCT	GTGCCAGCMGCCGCGGTAA	806rcbc87
50A-A04-S015-20180604	50A-4-15	Irrigated Crop	7.4392	2.5661	38.9690	CTCACAACCGTG	GTGCCAGCMGCCGCGGTAA	806rcbc88
50B-A01-S005-20180604	50B-1-05	Non-irrigated Crop	9.3340	3.2362	30.9001	TTGGGTACACGT	GTGCCAGCMGCCGCGGTAA	806rcbc42
50B-A02-S005-20180605	50B-2-05	Non-irrigated Crop	2.9898	4.0965	24.4110	AAGGCGCTCCTT	GTGCCAGCMGCCGCGGTAA	806rcbc43
50B-A03-S005-20180606	50B-3-05	Non-irrigated Crop	6.6948	4.5338	22.0566	TAATACGGATCG	GTGCCAGCMGCCGCGGTAA	806rcbc44
50B-A03-S015-20180607	50B-3-15	Non-irrigated Crop	3.6504	4.1787	23.9312	TCGGAATTAGAC	GTGCCAGCMGCCGCGGTAA	806rcbc45
50B-A04-S005-20180608	50B-4-05	Non-irrigated Crop	2.8686	3.1955	31.2935	TGTGAATTCGGA	GTGCCAGCMGCCGCGGTAA	806rcbc46

Aquifer Sample ID	**Sample Nickname**	**Well Type**	**Pre-PCR (ng/L)**	**Post-PCR (ng/L)**	**Normalised (uL)**	**Barcode Sequence**	**Linker Primer Sequence**	**Primer Name**
50B-2016	50B-2016	Deep Aquifer	0.2472	4.6911	21.3171	CTGCTATTCCTC	GTGCCAGCMGCCGCGGTAA	806rcbc89
50B-2017	50B-2017	Deep Aquifer	0.1812	n.a.	n.a.	ATGTCACCGCTG	GTGCCAGCMGCCGCGGTAA	806rcbc90
50B-2018	50B-2018	Deep Aquifer	0.4318	2.5239	39.6213	TGTAACGCCGAT	GTGCCAGCMGCCGCGGTAA	806rcbc91

50C-2016	50C-2016	Shallow Aquifer	0.2208	n.a.	n.a.	AGCAGAACATCT	GTGCCAGCMGCCGCGGTAA	806rcbc92
50C-2017	50C-2017	Shallow Aquifer	0.1939	1.4962	66.8339	TGGAGTAGGTGG	GTGCCAGCMGCCGCGGTAA	806rcbc93
50C-2018	50C-2018	Shallow Aquifer	0.4261	2.5366	39.4226	TTGGCTCTATTC	GTGCCAGCMGCCGCGGTAA	806rcbc94
Control Sample ID	**Sample Nickname**	**Lab Method**	**Pre-PCR (ng/L)**	**Post-PCR (ng/L)**	**Normalised (uL)**	**Barcode Sequence**	**Linker Primer Sequence**	**Primer Name**
Blank_3	Blank 3	DNA Extraction	0.0987	2.2111	45.2263	GTCGTGTAGCCT	GTGCCAGCMGCCGCGGTAA	806rcbc6
Blank_4	Blank 4	DNA Extraction	0.1722	1.4452	69.1960	GATCCCACGTAC	GTGCCAGCMGCCGCGGTAA	806rcbc95
Blank_5	Blank 5	DNA Extraction	0.1658	1.3455	74.3214	TACCGCTTCTTC	GTGCCAGCMGCCGCGGTAA	806rcbc96
Positive_control	E Coli	PCR	n.a.	44.2883	2.2579	AGCGGAGGTTAG	GTGCCAGCMGCCGCGGTAA	806rcbc7
Negative_control	Water	PCR	n.a.	1.9250	51.9472	ATCCTTTGGTTC	GTGCCAGCMGCCGCGGTAA	806rcbc8

Sequencing Data Table: 2016 Samples

#SampleID	Location	County	Well Type	Barcode Sequence	Linker Primer Sequence	Barcode Name	Project Name
10C.2.2016.9.6.17	SEC6 T24S R10W	Reno	Deep Aquifer	TGAGAGAC	CAGCMGCCGCGGTAA	ill519Fmodbar3	092717MK519F
10D.2.2016.9.6.17	SEC6 T24S R10W	Reno	Shallow Aquifer	TGAGAGGT	CAGCMGCCGCGGTAA	ill519Fmodbar4	092717MK519F
21B.2.2016.9.6.17	SEC1 T26S R11W	Pratt	Deep Aquifer	TGAGATTT	CAGCMGCCGCGGTAA	ill519Fmodbar5	092717MK519F
21C.2.2016.9.6.17	SEC1 T26S R11W	Pratt	Shallow Aquifer	TGAGCAAG	CAGCMGCCGCGGTAA	ill519Fmodbar6	092717MK519F
26B.1.2016.7.27.17	SEC1 T23S R10W	Reno	Deep Aquifer	TGAGCACA	CAGCMGCCGCGGTAA	ill519Fmodbar7	092717MK519F
26C.1.2016.7.27.17	SEC1 T23S R10W	Reno	Shallow Aquifer	TGAGCACT	CAGCMGCCGCGGTAA	ill519Fmodbar8	092717MK519F
34A.1.2016.7.27.17	SEC36 T25S R9W	Reno	Bedrock	TGAGCAGT	CAGCMGCCGCGGTAA	ill519Fmodbar9	092717MK519F
34B.2.2016.7.27.17	SEC36 T25S R9W	Reno	Shallow Aquifer	TGAGCCAA	CAGCMGCCGCGGTAA	ill519Fmodbar10	092717MK519F
42B.1.2016.8.3.17	SEC1 T28S R13W	Pratt	Deep Aquifer	TGAGCCTC	CAGCMGCCGCGGTAA	ill519Fmodbar11	092717MK519F
42C.1.2016.8.3.17	SEC1 T28S R13W	Pratt	Shallow Aquifer	TGAGCGAT	CAGCMGCCGCGGTAA	ill519Fmodbar12	092717MK519F
50B.2.2016.9.4.17	SEC6 T21S R13W	Stafford	Deep Aquifer	TGAGCGGT	CAGCMGCCGCGGTAA	ill519Fmodbar13	092717MK519F
50C.2.2016.9.4.17	SEC6 T21S R13W	Stafford	Shallow Aquifer	TGAGCGTT	CAGCMGCCGCGGTAA	ill519Fmodbar14	092717MK519F
6B.2.2016.9.4.17	SEC6 T25S R13W	Stafford	Deep Aquifer	TGAGACGA	CAGCMGCCGCGGTAA	ill519Fmodbar1	092717MK519F
6C.1.2016.7.27.17	SEC6 T25S R13W	Stafford	Shallow Aquifer	TGAGACTA	CAGCMGCCGCGGTAA	ill519Fmodbar2	092717MK519F
BB5HA.1.2016.8.3.17			Deep Aquifer	TGAGCTTA	CAGCMGCCGCGGTAA	ill519Fmodbar15	092717MK519F
BB5HB.1.2016.8.3.17			Shallow Aquifer	TCTAGCAT	CAGCMGCCGCGGTAA	arch2A519Fbar16	092717MK519F
BBB.1.2016.7.25.17			Deep Aquifer	TCTAGCCA	CAGCMGCCGCGGTAA	arch2A519Fbar17	092717MK519F

Microbial Communities: Relative Abundance Tables

Nitrogen Cyclers: Relative Abundance in Irrigated Crop Soils

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Irrigated Soils %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	1.38306	Ammonia Oxidizer				
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.62946	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.6254	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.58652	Oxidizes Nitrite to Nitrate				
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.48406	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.42649	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	0.4104	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		

k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.40499	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.39894	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter	0.33725	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.30382	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.25105	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.23735	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus	0.21991	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.18133	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.1583	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	Denitrification
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Micromonospora	0.15623	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0.15261	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.149	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	0.14615	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.14479	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.14403	Dissimilatory Nitrite to Ammonium	Denitrification			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.14206	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.13783	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/Nitrification
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Geodermatophilus	0.12036	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	0.10792	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	0.10612	Assimilatory Nitrate to Ammonium				
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.09349	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__Sorangium	0.08801	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.08658	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.07914	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.07618	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	0.06771	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.06445	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.06363	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas	0.06358	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter	0.06247	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrifcation	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrate to Nitrite/Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.05744	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella	0.05602	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.05466	Dissimilatory Nitrate to Nitrite	Denitrification			

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Pantoea	0.05297	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	0.0506	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Cellvibrio	0.05002	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas	0.04817	Denitrification	Assimilatory Nitrite to Ammonium		
Total Relative Abundance in Irrigated Crop Soils (%)	9.66055				

Nitrogen Cyclers: Relative Abundance in Non-irrigated Crop Soils

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Non-irrigated Soils %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	1.1063	Ammonia Oxidizer				
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	1.0507	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite			
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.7973	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	0.7649	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.7460	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.6713	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.6413	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.5656	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia	0.5424	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.3061	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.2628	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.2606	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter	0.2412	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.2299	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.2281	Oxidizes Nitrite to Nitrate			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Geodermatophilus	0.2150	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	0.2116	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Amycolatopsis	0.2026	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0.2017	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite

k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus	0.1968	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Micromonospora	0.1938	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.1610	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.1549	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.1521	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.1503	Dissimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.1442	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella	0.1363	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.1203	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	Denitrification
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.1182	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__Conexibacter	0.1168	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	

k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.1053	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.0988	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.0778	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	0.0765	Assimilatory Nitrate to Ammonium				
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.0750	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia	0.0745	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter	0.0682	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrifcation	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrate to Nitrite/ Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	0.0681	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.0676	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/ Nitrification
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.0676	Dissimilatory Nitrate to Nitrite	Denitrification			

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.0646	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	0.0610	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.0537	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Serratia	0.0521	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas	0.0458	Dissimilatory Nitrate to Nitrite	Denitrification		
Total Relative Abundance in Non-irrigated Crop Soils (%)	11.95				

Nitrogen Cyclers: Relative Abundance in Pasture Soils

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Non-irrigated Soils %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	1.481	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium	
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	1.392	Ammonia Oxidizer				
k__Bacteria;p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter	0.777	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.769	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.75	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	0.681	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.575	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.476	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.376	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.375	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.239	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.202	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia	0.197	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus	0.195	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.19	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.19	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	0.184	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.179	Oxidizes Nitrite to Nitrate			

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Amycolatopsis	0.174	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.165	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	Denitrification
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella	0.157	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Micromonospora	0.154	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.148	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0.144	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.137	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.135	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.133	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	0.105	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.105	Dissimilatory Nitrite to Ammonium	Denitrification		

k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	0.093	Assimilatory Nitrate to Ammonium				
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.089	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.087	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/ Nitrification
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Geodermatophilus	0.08	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.078	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae];g__Exiguobacterium	0.078	Dissimilatory Nitrate to Nitrite				
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	0.075	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__Conexibacter	0.067	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.051	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite	

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.05	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptosporangiaceae;g__Streptosporangium	0.048	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Serratia	0.047	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
Total Relative Abundance in Pasture Soils (%)	11.63			

Nitrogen Cyclers: Relative Abundance in Irrigated to Pasture Soils

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Non-irrigated Soils %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	1.4171	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	1.3622	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	1.184	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	0.8524	Ammonia Oxidizer				
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.5878	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.5216	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.5053	Oxidizes Nitrite to Nitrate				
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.499	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		

k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus	0.4761	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.422	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.2974	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.2856	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0.2658	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.26	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.2274	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Geodermatophilus	0.2254	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.2101	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.199	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/ Nitrification
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.1987	Dissimilatory Nitrite to Ammonium	Denitrification			

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.1954	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter	0.1868	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrifcation	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrate to Nitrite/Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	0.1827	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.1771	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.1687	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.1574	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation		
k__Bacteria;p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter	0.1327	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Micromonospora	0.1303	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.1187	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae;g__Anaerolinea	0.1139	Assimilatory Nitrite to Ammonium	Denitrification			

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.1093	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia	0.109	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.0975	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	0.0949	Assimilatory Nitrate to Ammonium				
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__Conexibacter	0.0844	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae];g__Exiguobacterium	0.0813	Dissimilatory Nitrate to Nitrite				
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	0.0773	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.0745	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__Sorangium	0.0689	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.0665	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.0654	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			

k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas	0.0567	Denitrification	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	0.0559	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	0.0554	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Pantoea	0.0547	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.0544	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia	0.0521	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.0466	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
Total Relative Abundance in Irrigated-to-Pasture Soils (%)	12.865				

Nitrogen Cyclers: Relative Abundance in Deep Wells under Irrigated Crop Land

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	IRRIGATED %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	9.1590648	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	1.2753528	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	1.2377103	Ammonia Oxidizer				
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	1.1670743	Oxidizes Nitrite to Nitrate				
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae];g__Exiguobacterium	0.9923509	Dissimilatory Nitrate to Nitrite				
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.9521914	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma	0.401389	Nitrogen Fixation				
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae;g__Desulfarculus	0.3725621	Nitrification	Assimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Xanthomonas	0.3383828	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		

k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.2303481	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Pantoea	0.2271771	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter	0.1711154	Nitrification	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia	0.1491641	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.1365377	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.1328673	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.1284405	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Marinobacter	0.1210548	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	0.1170354	Ammonia Oxidizer			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	0.115145	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.1015213	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Gallionellales;f__Gallionellaceae;g__Gallionella	0.0939542	Denitrification				
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.0888823	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.0884805	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.0804624	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.0665119	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/Nitrification
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	0.0652574	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.0649709	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter	0.0631981	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrifcation	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrate to Nitrite/Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.0622843	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__Desulfotalea	0.0592439	Dissimilatory Nitrite to Ammonium				

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	0.0544993	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__Spirochaeta	0.0539651	Nitrogen Fixation	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.049653	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	0.0481366	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
Total Relative Abundance in Deep Irrigated Wells %	**18.465985**				

Nitrogen Cyclers: Relative Abundance in Deep Wells under Non-irrigated Crop Land

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	**Non-irrigated %**	**Primary N-Cycling**	**Secondary N-Cycling**	**Tertiary N-Cycling**	**Quaternary N-Cycling**
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	2.182	Ammonia Oxidizer			
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	2.11	Oxidizes nitrite to nitrate			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	1.546	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	1.322	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.907	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	0.555	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.471	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.407	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	0.389	Ammonia Oxidizer			

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	0.276	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter	0.21	Nitrification	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.153	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.096	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.075	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Gallionellales;f__Gallionellaceae;g__Gallionella	0.073	Denitrification			
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.073	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.064	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.057	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	0.056	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium	0.056	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	

k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.049	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.048	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation
Total Relative Abundance in Deep Non-irrigated Crop Wells (%)	11.08				

Nitrogen Cyclers: Relative Abundance in Deep Wells under Pasture

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Pasture %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	6.863222	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	2.544985	Oxidizes Nitrite to Nitrate			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Psychromonadaceae;g__Psychromonas	1.595394	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	1.044288	Ammonia Oxidizer			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.918749	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.357554	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	0.279419	Ammonia Oxidizer			
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.21343	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Sulfurimonas	0.166065	Denitrification	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.158006	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.153988	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae;g__Pelobacter	0.133236	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.123661	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	0.116612	Nitrogen Fixation	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.113515	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter	0.102621	Nitrification	Assimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.096552	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae;g__Desulfarculus	0.089186	Nitrification	Assimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium	0.046839	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	
Total Relative Abundance in Deep Pasture Wells (%)	15.11732				

Nitrogen Cyclers: Relative Abundance in Shallow Wells under Irrigated Crop Land

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Irrigated %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	17.151191	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	1.5436992	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	1.5340018	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.8540796	Oxidizes Nitrite to Nitrate			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	0.8272195	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrite to Ammonium	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Caulobacter	0.707862	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia	0.5333843	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite	
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	0.472756	Ammonia Oxidizer			
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.4588794	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.3165596	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation

k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera	0.2511133	Ammonia Oxidizer			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.2377676	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.2290207	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.1987872	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Polaromonas	0.1284349	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.1214974	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.1175059	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.1155349	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.0861443	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.0861045	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Dissimilatory Nitrite to Ammonium
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.0805409	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification	

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__Methylotenera	0.0569196	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.0551989	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.0536343	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.052234	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella	0.0506832	Dissimilatory Nitrate to Nitrite	Denitrification		
Total Relative Abundance in Shallow Irrigated Wells %	26.320754				

Nitrogen Cyclers: Relative Abundance in Shallow Wells under Non-irrigated Crop Land

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Non-irrigated %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	2.139113	Oxidizes Nitrite to Nitrate			
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	1.473758	Ammonia Oxidizer			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	1.422938	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	1.003209	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.957095	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.627712	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	0.586303	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.322796	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium		
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Cellvibrio	0.239039	Assimilatory Nitrite to Ammonium			

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.230569	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification	
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.219276	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.106344	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.09411	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.082817	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Azoarcus	0.063995	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Nitrogen Fixation	Denitrification
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.054584	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma	0.049878	Nitrogen Fixation			
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	0.047055	Not specified			
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Coxiella	0.047055	Dissimilatory Nitrite to Ammonium			
Total Relative Abundance in Shallow Non-irrigated Crop Wells %	9.767643				

Nitrogen Cyclers: Relative Abundance in Shallow Wells under Pasture

OTU ID: Genetic Potential for Involvement in Nitrogen Cycle	Pasture %	Primary N-Cycling	Secondary N-Cycling	Tertiary N-Cycling	Quaternary N-Cycling	Fifth/Sixth N-Cycling
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.907106	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0.89838	Oxidizes Nitrite to Nitrate				
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.690627	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.486547	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus	0.392079	Ammonia Oxidizer				
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.383748	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.316535	Assimilatory Nitrite to Ammonium				
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.305877	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Denitrification		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.21353	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	

k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	0.175712	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium			
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma	0.113877	Nitrogen Fixation				
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Gallionellales;f__Gallionellaceae;g__Gallionella	0.099667	Denitrification				
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.092641	Dissimilatory Nitrate to Nitrite	Dissimilatory Nitrite to Ammonium	Denitrification	Nitrogen Fixation	Assimilatory Nitrate to Nitrite/ Nitrification
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.08729	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.085261	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite			
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia	0.082548	Assimilatory Nitrite to Ammonium	Denitrification	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.080609	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	0.074884	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	Nitrogen Fixation	
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.062104	Assimilatory Nitrite to Ammonium	Assimilatory Nitrate to Nitrite			
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.060565	Dissimilatory Nitrate to Nitrite	Assimilatory Nitrite to Ammonium	Nitrogen Fixation	Assimilatory Nitrate to Nitrite	

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.060297	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.055073	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	0.047764	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification	
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella	0.047107	Dissimilatory Nitrate to Nitrite	Denitrification		
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0.045646	Dissimilatory Nitrite to Ammonium	Assimilatory Nitrite to Ammonium	Dissimilatory Nitrate to Nitrite	Denitrification
Total Relative Abundance in Shallow Pasture Wells %	5.865472				

Amount of gDNA and Number of PCR Cycles Required for Amplification

Sample Number	Sample ID	Type	Primer Number	PCR A Results	Redo Results	Notes	PCR B Results	Redo Results	Notes	PCR C Results	Redo Results	Notes
1	42-1-05	Soil	1	Y		Key: Red lettering = samples to redo	Y			Y		
2	42-1-15	Soil	2	Y			Y			Y		
3	42-2-05	Soil	3	Y			Y			Y		
4	42-2-15	Soil	4	Y			Y			Y		
5	42-3-05	Soil	5	Y			Y			Y		
6	Blank 3	Water through extraction	6	N/A			N/A			N/A		
7	Positive	E Coli	7	N/A		If (+) control did not amplify, I redid the whole batch	N/A			N/A		
8	Negative	PCR water	8	N/A			N/A			N/A		
9	42-3-15	Soil	9	Y			Y			Y		
10	42-4-05	Soil	10	Y			Y			Y		
11	42-4-15	Soil	11	Y			Y			N	Y	1 uL diluted (1:10)
12	6-1-05	Soil	12	Y			Y			Y		
13	6-1-15	Soil	13	Y			Y			Y		
14	6-2-05	Soil	14	Y			Y			Y		
15	6-2-15	Soil	15	Y			N	Y	1 uL diluted (1:10)	Y		
16	6-3-05	Soil	16	Y			Y			Y		
17	6-3-15	Soil	17	Y			Y			Y		
18	6-4-05	Soil	18	Y			Y			Y		
19	6-4-15	Soil	19	Y			Y			Y		
20	51-1-05	Soil	20	Y			Y			Y		
21	51-1-15	Soil	21	Y			N	Y	1 uL diluted (1:10)	Y		
22	51-3-05	Soil	22	Y			Y			Y		
23	51-3-15	Soil	23	Y			Y			Y		

24	51-4-05	Soil	24	Y			Y		Y		
25	51-4-15	Soil	25	Y			Y		Y		
26	36-1-05	Soil	26	Y			Y		Y		
27	36-1-15	Soil	27	Y			Y		Y		
28	36-2-05	Soil	28	Y			Y		Y		
29	36-2-15	Soil	29	Y			Y		Y		
30	36-3-05	Soil	30	Y			Y		Y		
31	36-3-15	Soil	31	Y			Y		Y		
32	36-4-05	Soil	32	Y			Y		Y		
33	36-4-15	Soil	33	Y			Y		Y		
34	52-1-05	Soil	34	Y			Y		Y		
35	52-1-15	Soil	35	Y			Y		Y		
36	52-2-05	Soil	36	N	Y	1 uL undiluted	Y	1 uL undiluted	Y		1 uL undiluted
37	52-2-15	Soil	37	Y			Y		N	Y	1 uL diluted (1:10)
38	52-3-05	Soil	38	N	Y	1 uL undiluted	Y	1 uL undiluted	Y		1 uL undiluted
39	52-3-15	Soil	39	N	Y	1 uL undiluted	Y	1 uL undiluted	Y		1 uL undiluted
40	52-4-05	Soil	40	Y			Y		Y		
41	52-4-15	Soil	41	Y			Y		Y		
42	50B-1-05	Soil	42	Y			Y		Y		
43	50B-2-05	Soil	43	Y			Y		Y		
44	50B-3-05	Soil	44	Y			Y		Y		
45	50B-3-15	Soil	45	Y			Y		Y		
46	50B-4-05	Soil	46	Y			Y		Y		
47	21-1-05	Soil	47	Y			Y		Y		
48	21-1-15	Soil	48	Y			Y		Y		
49	21-2-05	Soil	49	N	Y	1 uL undiluted	Y	1 uL undiluted	Y		1 uL undiluted
50	21-2-15	Soil	50	N	Y	1 uL undiluted	Y	1 uL undiluted	Y		1 uL undiluted
51	21-3-05	Soil	51	Y			Y		Y		
52	21-3-15	Soil	52	Y			Y		Y		

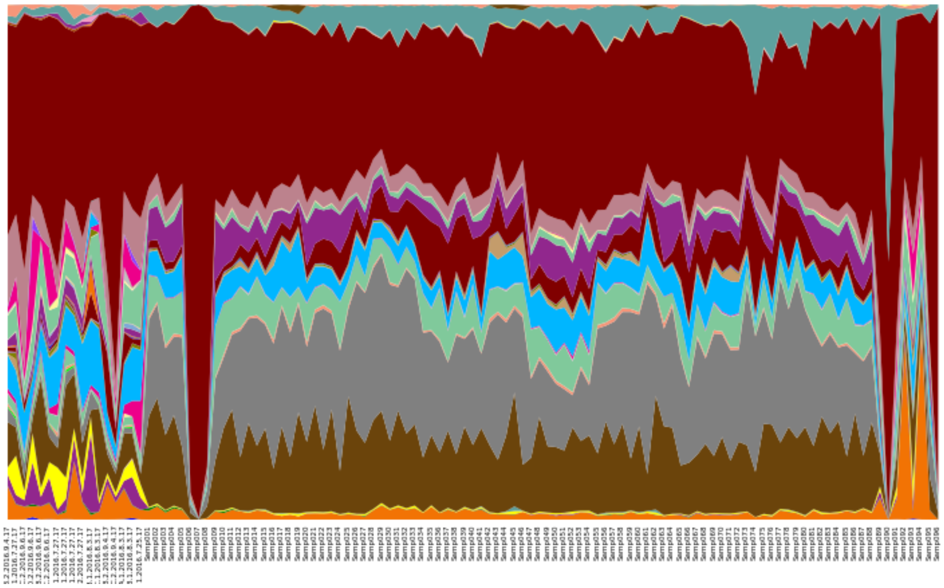
53	21-4-05	Soil	53		Y				Y				Y		
54	21-4-15	Soil	54		Y				Y				Y		
55	34-1-05	Soil	55		Y				Y				Y		
56	34-1-15	Soil	56		Y				Y				Y		
57	34-2-05	Soil	57		Y				Y				Y		
58	34-2-15	Soil	58		Y				Y				Y		
59	34-3-05	Soil	59		Y				Y				Y		
60	34-3-15	Soil	60		Y				Y				Y		
61	34-4-05	Soil	61		Y				Y				Y		
62	34-4-15	Soil	62		Y				Y				Y		
63	51-2-05	Soil	63	N		Y	1 uL undiluted		Y		1 uL undiluted		Y		1 uL undiluted
64	51-2-15	Soil	64	N		Y	1 uL undiluted		Y		1 uL undiluted		Y		1 uL undiluted
65	3-1-05	Soil	65		Y				Y				Y		
66	3-1-15	Soil	66		Y			N		Y	1 uL diluted (1:10)		Y		
67	3-2-05	Soil	67		Y				Y				Y		
68	3-2-15	Soil	68		Y			N		Y	1 uL undiluted	N		Y	1 uL undiluted
69	3-3-05	Soil	69		Y				Y				Y		
70	3-3-15	Soil	70	N		Y	1 uL undiluted		Y		1 uL undiluted		Y		1 uL undiluted
71	3-4-05	Soil	71		Y				Y				Y		
72	3-4-15	Soil	72		Y				Y				Y		
73	10-1-05	Soil	73		Y				Y				Y		
74	10-1-15	Soil	74	N			3 uL undiluted				3 uL undiluted				3 uL undiluted
75	10-2-05	Soil	75		Y			N		Y	1 uL undiluted	N		Y	1 uL undiluted
76	10-2-15	Soil	76		Y			N		Y	1 uL diluted (1:10)		Y		
77	10-3-05	Soil	77	N		Y	1 uL undiluted				1 uL undiluted				1 uL undiluted
78	10-3-15	Soil	78		Y				Y				Y		
79	10-4-05	Soil	79		Y				Y				Y		
80	10-4-15	Soil	80		Y				Y				Y		
81	50A-1-05	Soil	81	N		Y	3 uL undiluted		Y		3 uL undiluted		Y		3 uL undiluted
82	50A-1-15	Soil	82	N		Y	3 uL undiluted, 28 cycles		Y		3 uL undiluted, 28 cycles		Y		3 uL undiluted, 28 cycles

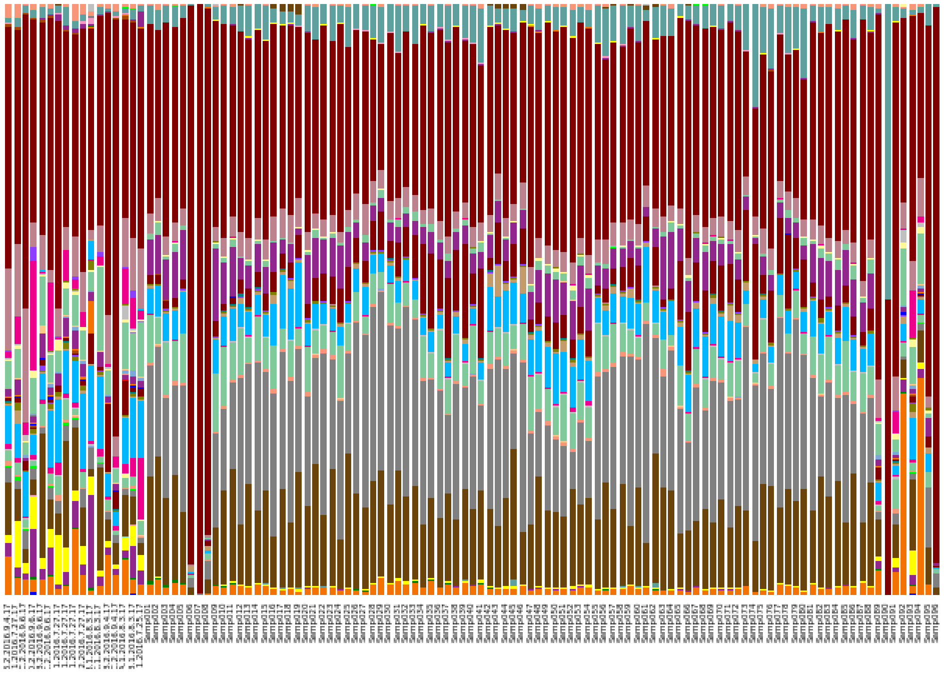
83	50A-2-05	Soil	83	Y			Y		Y	
84	50A-2-15	Soil	84	N	Y		Y		Y	
85	50A-3-05	Soil	85	N	Y		Y		Y	
86	50A-3-15	Soil	86	N	Y		Y		Y	
87	50A-4-05	Soil	87	Y			Y		Y	
88	50A-4-15	Soil	88	N	Y		Y		Y	
89	50B-2016	Soil	89	N	Y	5 uL undiluted, 28 cycles	Y	5 uL undiluted, 28 cycles	Y	5 uL undiluted, 28 cycles
90	50B-2017	Soil	90	N			N		N	
91	50B-2018	Soil	91	N			N		N	
92	50C-2016	Soil	92	N			N		N	
93	50C-2017	Soil	93	N			N		N	
94	50C-2018	Soil	94	N	Y	5 uL undiluted, 28 cycles	N		N	
95	Blank 4	Water through extraction	95	N/A			N/A		N/A	
96	Blank 5	Water through extraction	96	N/A			N/A		N/A	

Appendix F - Microbial Community Graphs

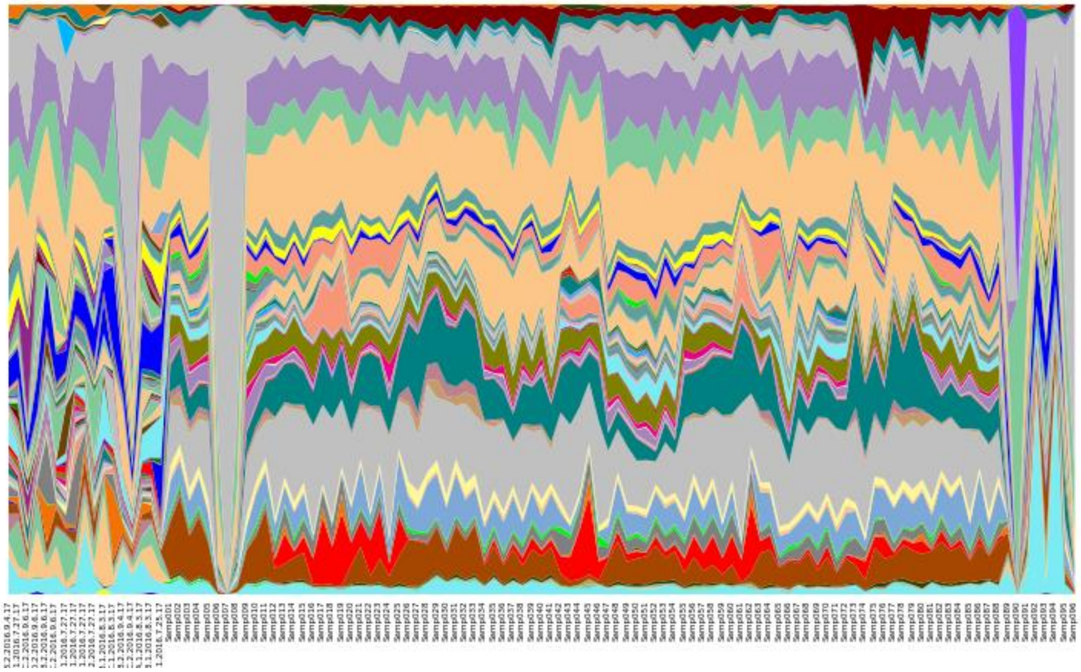
Alpha Diversity Results

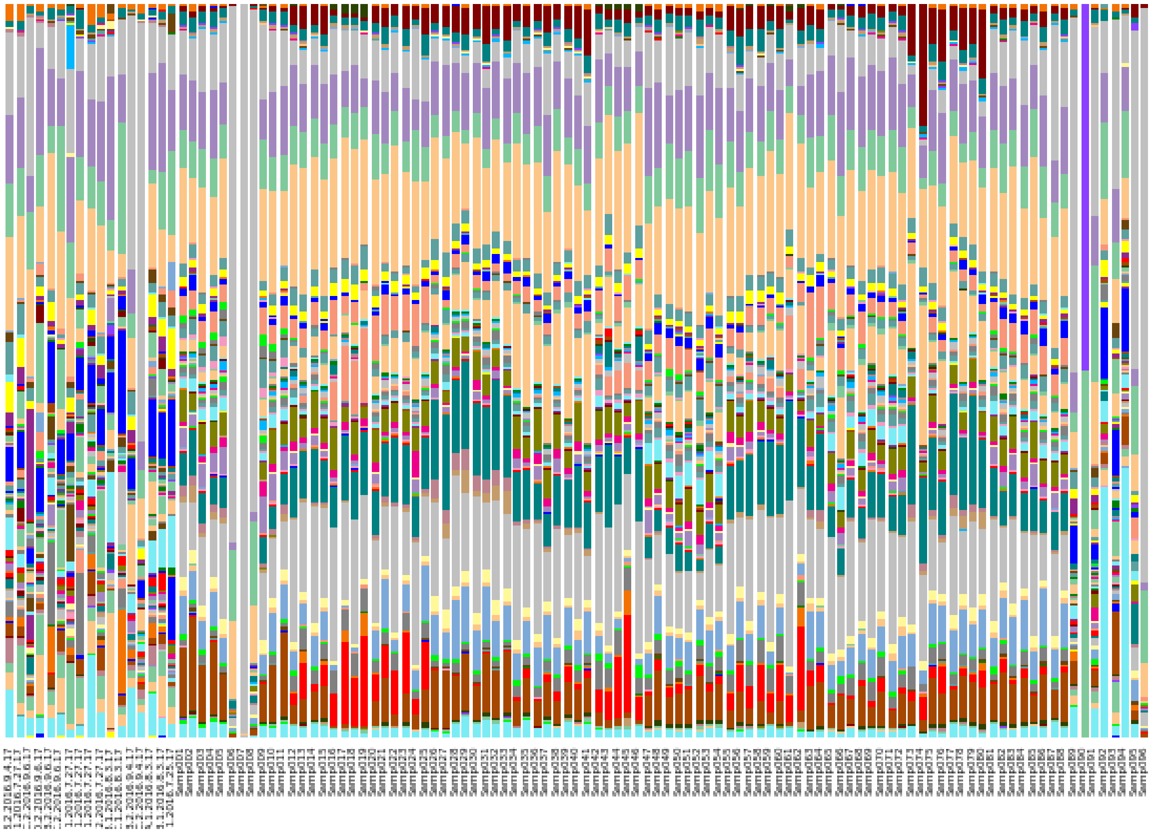
Phyla Level Area and Bar Charts of Archaeal and Bacterial Taxonomies



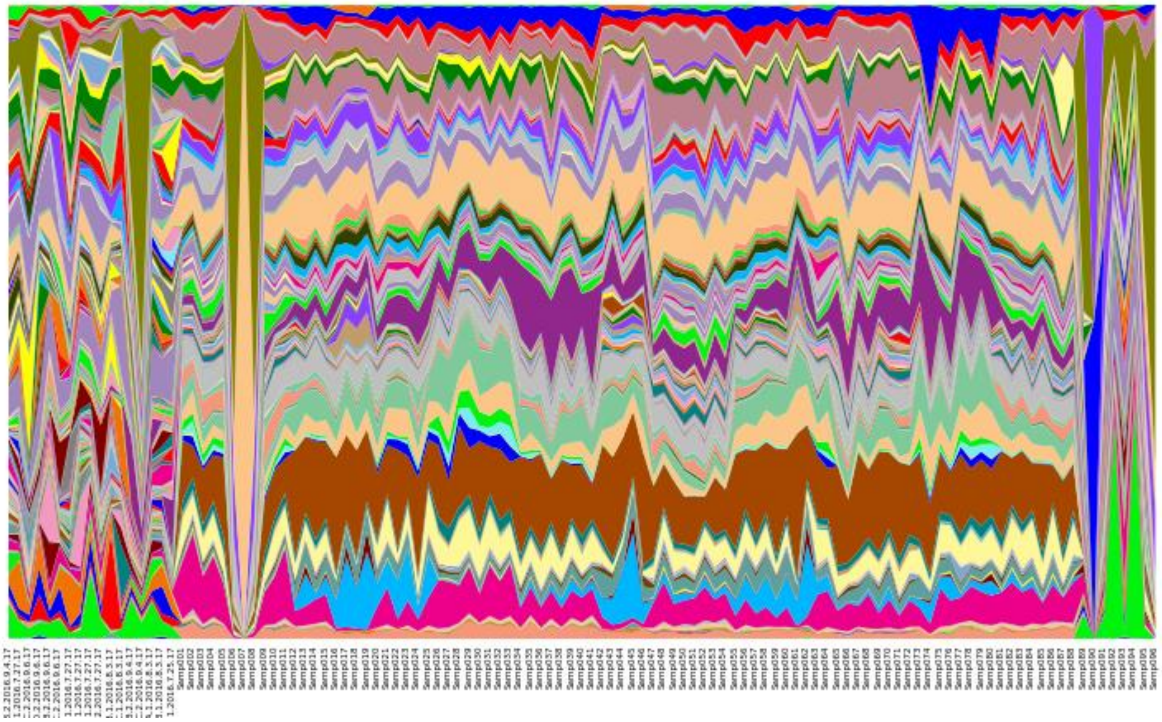


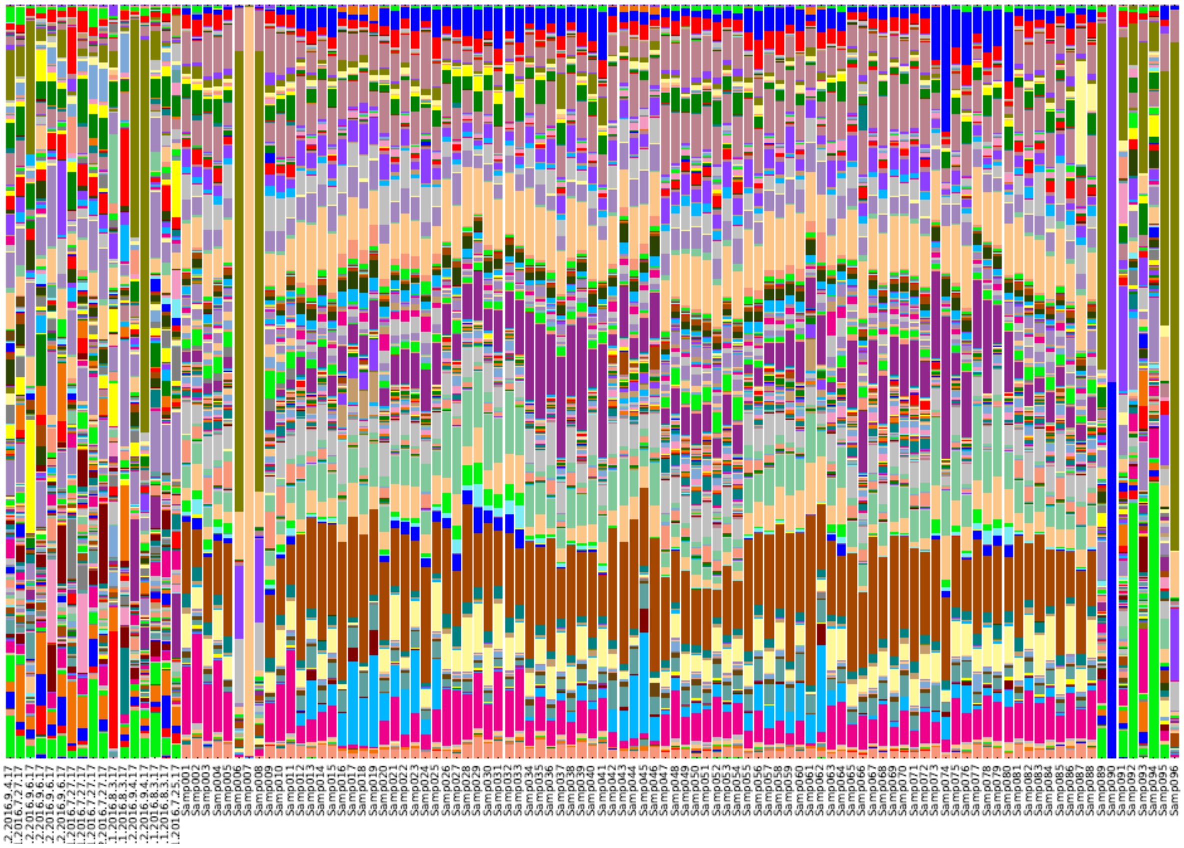
Class Level Area and Bar Charts of Archaeal and Bacterial Taxonomies





Order Level Area and Bar Charts of Archaeal and Bacterial Taxonomies





Legend for Relative Abundance Area and Bar Charts of All Samples through Order Level

- 🟥 Unclassified;Other;Other;Other
- 🟦 k__Archaea;Other;Other;Other
- 🟧 k__Archaea;p__Crenarchaeota;Other;Other
- 🟩 k__Archaea;p__Crenarchaeota;c__MBGA;o__
- 🟪 k__Archaea;p__Crenarchaeota;c__MBGA;o__NRP-J
- 🟨 k__Archaea;p__Crenarchaeota;c__MBGB;o__
- 🟦 k__Archaea;p__Crenarchaeota;c__MCG;o__
- 🟪 k__Archaea;p__Crenarchaeota;c__MCG;o__B10
- 🟩 k__Archaea;p__Crenarchaeota;c__MCG;o__pGrfC26
- 🟫 k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;Other
- ⬜ k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__AK31
- 🟩 k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales
- 🟧 k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales
- 🟦 k__Archaea;p__Euryarchaeota;Other;Other
- 🟧 k__Archaea;p__Euryarchaeota;c__DSEG;Other
- 🟩 k__Archaea;p__Euryarchaeota;c__DSEG;o__ArcA07
- 🟪 k__Archaea;p__Euryarchaeota;c__DSEG;o__DHVE3
- 🟨 k__Archaea;p__Euryarchaeota;c__DSEG;o__HydGC-84-221A
- ⬜ k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales
- 🟪 k__Archaea;p__Euryarchaeota;c__Methanomicrobia;Other
- 🟦 k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__
- 🟫 k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanocellales
- 🟩 k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales
- 🟪 k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales
- 🟫 k__Archaea;p__Euryarchaeota;c__Thermoplasmata;Other
- 🟩 k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2
- 🟥 k__Archaea;p__[Parvarchaeota];Other;Other
- 🟩 k__Archaea;p__[Parvarchaeota];c__[Micrarchaea];o__[Micrarchaeles]
- 🟫 k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];Other
- 🟥 k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];o__WCHD3-30
- 🟦 k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];o__YLA114
- 🟧 k__Bacteria;Other;Other;Other
- 🟩 k__Bacteria;p__;c__;o__
- 🟪 k__Bacteria;p__AC1;c__;o__
- 🟨 k__Bacteria;p__AC1;c__HDBW-WB69;o__
- 🟦 k__Bacteria;p__AC1;c__SHA-114;o__
- 🟪 k__Bacteria;p__AD3;Other;Other
- 🟩 k__Bacteria;p__AD3;c__ABS-6;o__
- 🟫 k__Bacteria;p__AD3;c__JG37-AG-4;o__
- ⬜ k__Bacteria;p__Acidobacteria;Other;Other
- 🟩 k__Bacteria;p__Acidobacteria;c__;o__
- 🟧 k__Bacteria;p__Acidobacteria;c__AT-s2-57;o__
- 🟦 k__Bacteria;p__Acidobacteria;c__AT-s54;o__
- 🟧 k__Bacteria;p__Acidobacteria;c__Acidobacteria-5;o__
- 🟩 k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;Other
- 🟪 k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__
- 🟨 k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__BPC015
- ⬜ k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__CCU21
- 🟪 k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15
- 🟦 k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales
- 🟫 k__Bacteria;p__Acidobacteria;c__BPC102;o__
- 🟩 k__Bacteria;p__Acidobacteria;c__BPC102;o__B110
- 🟪 k__Bacteria;p__Acidobacteria;c__BPC102;o__MVS-40
- 🟫 k__Bacteria;p__Acidobacteria;c__DA052;Other
- 🟩 k__Bacteria;p__Acidobacteria;c__DA052;o__E29
- 🟥 k__Bacteria;p__Acidobacteria;c__DA052;o__Ellin6513
- 🟩 k__Bacteria;p__Acidobacteria;c__DA052;o__HDB_SIOH1004
- 🟫 k__Bacteria;p__Acidobacteria;c__EC1113;o__
- 🟥 k__Bacteria;p__Acidobacteria;c__GAL08;o__
- 🟦 k__Bacteria;p__Acidobacteria;c__Holophagae;o__Holophagales
- 🟧 k__Bacteria;p__Acidobacteria;c__OS-K;o__
- 🟩 k__Bacteria;p__Acidobacteria;c__PAUC37f;o__
- 🟪 k__Bacteria;p__Acidobacteria;c__RB25;o__
- 🟨 k__Bacteria;p__Acidobacteria;c__S035;o__
- 🟦 k__Bacteria;p__Acidobacteria;c__Solibacteres;Other
- 🟪 k__Bacteria;p__Acidobacteria;c__Solibacteres;o__JH-WHS99
- 🟩 k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales
- 🟫 k__Bacteria;p__Acidobacteria;c__Sva0725;o__Sva0725
- ⬜ k__Bacteria;p__Acidobacteria;c__TM1;o__
- 🟩 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];Other
- 🟧 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__
- 🟦 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__11-24
- 🟧 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__DS-100
- 🟩 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__Ellin7246
- 🟪 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__PK29
- 🟨 k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41
- ⬜ k__Bacteria;p__Acidobacteria;c__iii1-8;Other
- 🟪 k__Bacteria;p__Acidobacteria;c__iii1-8;o__
- 🟦 k__Bacteria;p__Acidobacteria;c__iii1-8;o__32-20
- 🟫 k__Bacteria;p__Acidobacteria;c__iii1-8;o__DS-18
- 🟩 k__Bacteria;p__Acidobacteria;c__iii1-8;o__SJA-36
- 🟪 k__Bacteria;p__Actinobacteria;Other;Other
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- 🟩 k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales
- 🟥 k__Bacteria;p__Actinobacteria;c__Actinobacteria;Other
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- 🟫 k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales
- 🟥 k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales
- 🟦 k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Micrococcales
- 🟧 k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__WCHB1-81
- 🟩 k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales
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- 🟨 k__Bacteria;p__Actinobacteria;c__MB-A2-108;o__
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- 🟪 k__Bacteria;p__Actinobacteria;c__Nitriliruptoria;Other
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- 🟧 k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales
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🟪 k__Bacteria;p__Armatimonadetes;c__;o__
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🟦 k__Bacteria;p__Armatimonadetes;c__Armatimonadia;o__Armatimonadales
🟫 k__Bacteria;p__Armatimonadetes;c__Armatimonadia;o__FW68
🟩 k__Bacteria;p__Armatimonadetes;c__Chthonomonadetes;o__Chthonomonadales
🟪 k__Bacteria;p__Armatimonadetes;c__Chthonomonadetes;o__SJA-22
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🟫 k__Bacteria;p__Armatimonadetes;c__SJA-176;o__GAB-B06
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🟨 k__Bacteria;p__BRC1;c__;o__
🟦 k__Bacteria;p__BRC1;c__NPL-UPA2;o__
🟥 k__Bacteria;p__BRC1;c__PRR-11;o__
🟦 k__Bacteria;p__Bacteroidetes;Other;Other
🟫 k__Bacteria;p__Bacteroidetes;c__At12OctB3;o__
⬛ k__Bacteria;p__Bacteroidetes;c__BME43;o__
🟩 k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales
🟧 k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales
🟦 k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales
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🟩 k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales
🟪 k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22;o__
🟨 k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales]
⬜ k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales]
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🟫 k__Bacteria;p__Caldithrix;c__Caldithrixae;o__Caldithrixales
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🟫 k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales
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🟥 k__Bacteria;p__Chlorobi;c__;o__
🟩 k__Bacteria;p__Chlorobi;c__BSV26;Other
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🟦 k__Bacteria;p__Chlorobi;c__BSV26;o__C20
🟧 k__Bacteria;p__Chlorobi;c__BSV26;o__PK329
🟩 k__Bacteria;p__Chlorobi;c__BSV26;o__VC38
🟪 k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales
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🟩 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__
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🟪 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales
🟨 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__DRC31
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🟦 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__MSB-1E9
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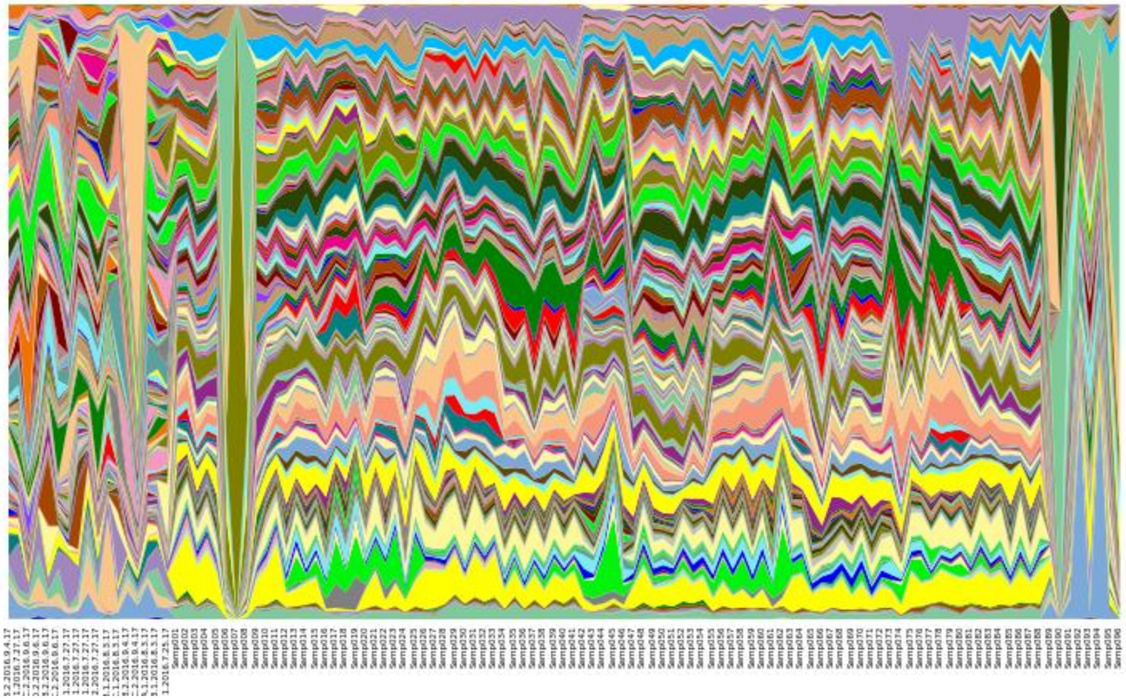
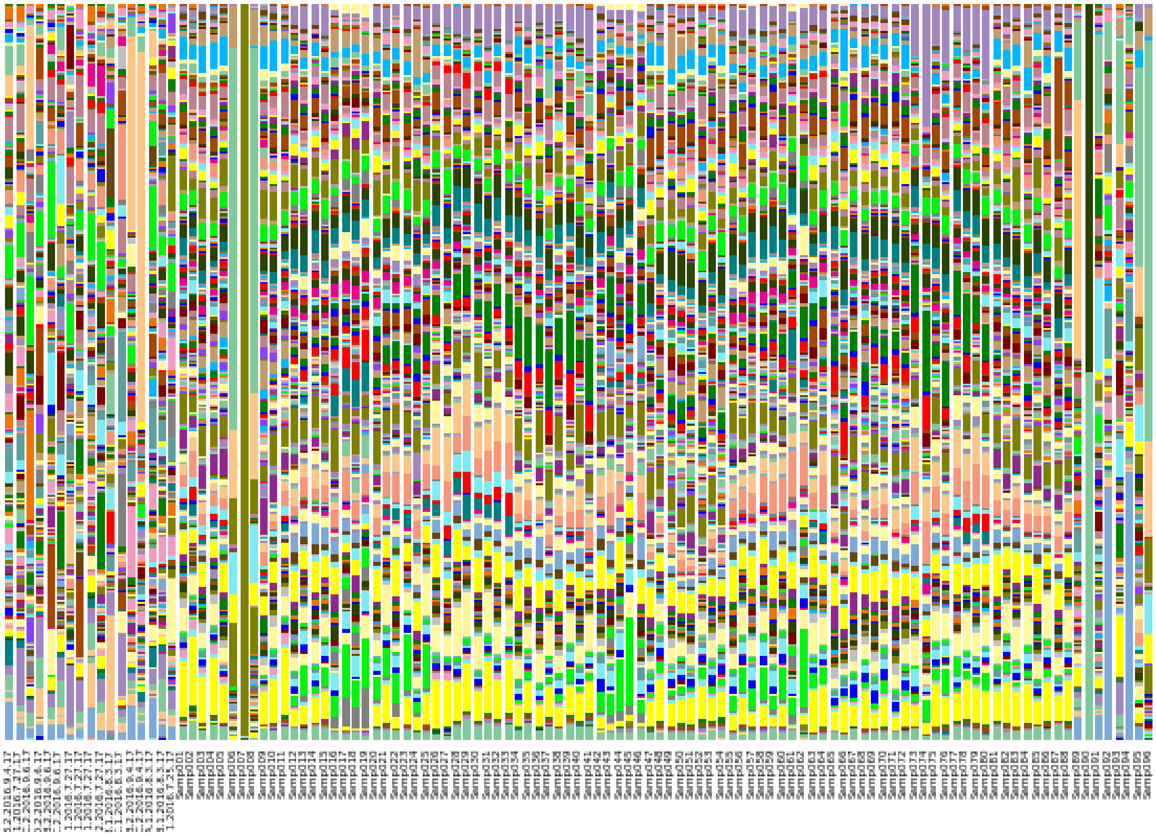
🟫 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SB-34
🟦 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031
🟥 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SHA-20
🟩 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SJA-15
🟫 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__WCHB1-50
🟥 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__envOPS12
🟦 k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__pLW-97
🟧 k__Bacteria;p__Chloroflexi;c__C0119;o__
🟩 k__Bacteria;p__Chloroflexi;c__Chloroflexi;Other
🟪 k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__AKIW781
🟨 k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__Chloroflexales
🟦 k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__Herpetosiphonales
🟥 k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__[Roseiflexales]
🟦 k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;Other
🟫 k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__
⬛ k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__Dehalococcoidales
🟩 k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__FS117-23B-02
🟧 k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__GIF9
🟦 k__Bacteria;p__Chloroflexi;c__Ellin6529;o__
🟧 k__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__
🟩 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;Other
🟪 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__
🟨 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__B10-SB3A
⬜ k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__B12-WMSP1
🟪 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__Elev-1554
🟦 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__JG30-KF-AS9
🟫 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__Ktedonobacterales
🟩 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__TK10
🟪 k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales
🟫 k__Bacteria;p__Chloroflexi;c__P2-11E;o__
🟦 k__Bacteria;p__Chloroflexi;c__S085;o__
🟥 k__Bacteria;p__Chloroflexi;c__SAR202;o__
🟩 k__Bacteria;p__Chloroflexi;c__SHA-26;o__
🟫 k__Bacteria;p__Chloroflexi;c__SL56;o__
🟥 k__Bacteria;p__Chloroflexi;c__TK10;Other
🟦 k__Bacteria;p__Chloroflexi;c__TK10;o__
🟧 k__Bacteria;p__Chloroflexi;c__TK10;o__AKYG885
🟩 k__Bacteria;p__Chloroflexi;c__TK10;o__B07_WMSP1
🟪 k__Bacteria;p__Chloroflexi;c__TK17;Other
🟨 k__Bacteria;p__Chloroflexi;c__TK17;o__
🟦 k__Bacteria;p__Chloroflexi;c__TK17;o__TK18
🟥 k__Bacteria;p__Chloroflexi;c__TK17;o__mle1-48
🟦 k__Bacteria;p__Chloroflexi;c__Thermomicrobia;Other
🟫 k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__
⬛ k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__AKYG1722
🟩 k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__Ellin6537
🟧 k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45
🟦 k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__Sphaerobacterales
🟧 k__Bacteria;p__Chloroflexi;c__[Thermobacula];o__[Thermobaculales]
🟩 k__Bacteria;p__Cyanobacteria;Other;Other
🟫 k__Bacteria;p__Cyanobacteria;c__;o__
🟪 k__Bacteria;p__Cyanobacteria;c__4C0d-2;Other
🟨 k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__MLE1-12
⬜ k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__SM1D11
🟪 k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__SM2F09
🟦 k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2
🟩 k__Bacteria;p__Cyanobacteria;c__Chloroplast;Other
🟪 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__
🟫 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta
🟦 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Cryptophyta
🟥 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Euglenozoa
🟩 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Haptophyceae
🟫 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Stramenopiles
🟥 k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta
🟦 k__Bacteria;p__Cyanobacteria;c__ML635J-21;o__
🟧 k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales
🟩 k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;Other
🟪 k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales
🟨 k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Oscillatoriales
🟦 k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;Other
🟥 k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales
🟦 k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Synechococcales
🟫 k__Bacteria;p__Elusimicrobia;Other;Other
⬛ k__Bacteria;p__Elusimicrobia;c__;o__
🟩 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;Other
🟧 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__
🟦 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales
🟧 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__FAC88
🟩 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__IIb
🟪 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__MD2894-B20
🟨 k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__MVP-88
⬜ k__Bacteria;p__Elusimicrobia;c__Endomicrobia;o__
🟪 k__Bacteria;p__Elusimicrobia;c__OP2;o__
🟦 k__Bacteria;p__FBP;c__;o__
🟫 k__Bacteria;p__FCPU426;c__;o__
🟩 k__Bacteria;p__Fibrobacteres;Other;Other
🟪 k__Bacteria;p__Fibrobacteres;c__B5-096;o__
🟫 k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;Other
🟦 k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__258ds10
🟥 k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__Fibrobacterales
🟩 k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__Ucp1540
🟫 k__Bacteria;p__Fibrobacteres;c__SBZC_2415;o__
🟥 k__Bacteria;p__Fibrobacteres;c__TG3;o__
🟦 k__Bacteria;p__Fibrobacteres;c__TG3;o__TG3-1
🟧 k__Bacteria;p__Firmicutes;Other;Other
🟩 k__Bacteria;p__Firmicutes;c__Bacilli;Other
🟪 k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales
🟨 k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales
🟦 k__Bacteria;p__Firmicutes;c__Bacilli;o__Haloplasmatales
🟥 k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales
🟦 k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales
🟫 k__Bacteria;p__Firmicutes;c__Clostridia;Other
⬛ k__Bacteria;p__Firmicutes;c__Clostridia;o__
🟩 k__Bacteria;p__Firmicutes;c__Clostridia;o__BSA2B-08
🟧 k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales
🟦 k__Bacteria;p__Firmicutes;c__Clostridia;o__Halanaerobiales
🟧 k__Bacteria;p__Firmicutes;c__Clostridia;o__MBA08
🟩 k__Bacteria;p__Firmicutes;c__Clostridia;o__Natranaerobiales
🟪 k__Bacteria;p__Firmicutes;c__Clostridia;o__OPB54
🟨 k__Bacteria;p__Firmicutes;c__Clostridia;o__SHA-98
⬜ k__Bacteria;p__Firmicutes;c__Clostridia;o__Thermoanaerobacterales
🟪 k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales
🟦 k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales
🟫 k__Bacteria;p__GAL15;c__;o__
🟩 k__Bacteria;p__GN01;c__;o__
🟪 k__Bacteria;p__GN02;Other;Other
🟦 k__Bacteria;p__GN02;c__;o__
🟫 k__Bacteria;p__GN02;c__3BR-5F;o__
🟥 k__Bacteria;p__GN02;c__BB34;o__
🟩 k__Bacteria;p__GN02;c__BD1-5;o__
🟫 k__Bacteria;p__GN02;c__GKS2-174;o__
🟥 k__Bacteria;p__GN02;c__GN07;o__
🟦 k__Bacteria;p__GN02;c__GN10;o__
🟧 k__Bacteria;p__GN02;c__IIB17;o__
🟩 k__Bacteria;p__GN04;Other;Other
🟨 k__Bacteria;p__GN04;c__;o__
🟪 k__Bacteria;p__GN04;c__5bav_B12;o__
🟦 k__Bacteria;p__GN04;c__GN15;o__
🟥 k__Bacteria;p__GN04;c__MSB-5A5;o__
🟦 k__Bacteria;p__GOUTA4;c__;o__
🟫 k__Bacteria;p__Gemmatimonadetes;Other;Other
⬛ k__Bacteria;p__Gemmatimonadetes;c__;o__
🟩 k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__
🟧 k__Bacteria;p__Gemmatimonadetes;c__Gemm-2;o__
🟦 k__Bacteria;p__Gemmatimonadetes;c__Gemm-3;o__
🟧 k__Bacteria;p__Gemmatimonadetes;c__Gemm-5;o__
🟩 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;Other
🟪 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__
🟨 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__C114
⬜ k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290
🟪 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonada
🟦 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__KD8-87
🟫 k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__N1423WL
🟩 k__Bacteria;p__Gemmatimonadetes;c__JL-ETNP-Z39;o__

■ k__Bacteria;p__H-178;c__;o__
■ k__Bacteria;p__KSB3;c__;o__
■ k__Bacteria;p__Kazan-3B-28;c__;o__
■ k__Bacteria;p__LCP-89;c__;o__
■ k__Bacteria;p__LCP-89;c__SAW1_B44;o__
■ k__Bacteria;p__LD1;c__;o__
■ k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales
■ k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales
■ k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Z20
■ k__Bacteria;p__MVP-21;c__;o__
■ k__Bacteria;p__MVS-104;c__;o__
■ k__Bacteria;p__NC10;Other;Other
■ k__Bacteria;p__NC10;c__;o__
■ k__Bacteria;p__NC10;c__12-24;Other
■ k__Bacteria;p__NC10;c__12-24;o__
■ k__Bacteria;p__NC10;c__12-24;o__JH-WHS47
■ k__Bacteria;p__NC10;c__12-24;o__MIZ17
■ k__Bacteria;p__NC10;c__12-24;o__Methylomirabiliales
■ k__Bacteria;p__NC10;c__wb1-A12;o__
■ k__Bacteria;p__NKB19;Other;Other
■ k__Bacteria;p__NKB19;c__;o__
■ k__Bacteria;p__NKB19;c__TSBW08;o__
■ k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales
■ k__Bacteria;p__OC31;c__;o__
■ k__Bacteria;p__OD1;Other;Other
■ k__Bacteria;p__OD1;c__;o__
■ k__Bacteria;p__OD1;c__ABY1;o__
■ k__Bacteria;p__OD1;c__Mb-NB09;o__
■ k__Bacteria;p__OD1;c__SM2F11;o__
■ k__Bacteria;p__OD1;c__ZB2;o__
■ k__Bacteria;p__OP1;c__MSBL6;o__
■ k__Bacteria;p__OP1;c__[Acetothermia];o__[Acetothermales]
■ k__Bacteria;p__OP11;Other;Other
■ k__Bacteria;p__OP11;c__;o__
■ k__Bacteria;p__OP11;c__OP11-1;o__
■ k__Bacteria;p__OP11;c__OP11-2;o__
■ k__Bacteria;p__OP11;c__OP11-3;o__
■ k__Bacteria;p__OP11;c__OP11-4;o__
■ k__Bacteria;p__OP11;c__WCHB1-64;Other
■ k__Bacteria;p__OP11;c__WCHB1-64;o__
■ k__Bacteria;p__OP11;c__WCHB1-64;o__K2-4-19
■ k__Bacteria;p__OP11;c__WCHB1-64;o__d153
■ k__Bacteria;p__OP3;Other;Other
■ k__Bacteria;p__OP3;c__;o__
■ k__Bacteria;p__OP3;c__BD4-9;o__
■ k__Bacteria;p__OP3;c__PBS-25;o__
■ k__Bacteria;p__OP3;c__koll11;Other
■ k__Bacteria;p__OP3;c__koll11;o__
■ k__Bacteria;p__OP3;c__koll11;o__GIF10
■ k__Bacteria;p__OP8;c__OP8_1;Other
■ k__Bacteria;p__OP8;c__OP8_1;o__
■ k__Bacteria;p__OP8;c__OP8_1;o__HMMVPog-54
■ k__Bacteria;p__OP8;c__OP8_1;o__SHA-124
■ k__Bacteria;p__OP8;c__OP8_2;o__
■ k__Bacteria;p__OP9;c__JS1;o__SB-45
■ k__Bacteria;p__PAUC34f;c__;o__
■ k__Bacteria;p__Planctomycetes;Other;Other
■ k__Bacteria;p__Planctomycetes;c__;o__
■ k__Bacteria;p__Planctomycetes;c__028H05-P-BN-P5;o__
■ k__Bacteria;p__Planctomycetes;c__BD7-11;o__
■ k__Bacteria;p__Planctomycetes;c__C6;o__MVS-107
■ k__Bacteria;p__Planctomycetes;c__C6;o__Ucm1571
■ k__Bacteria;p__Planctomycetes;c__C6;o__d113
■ k__Bacteria;p__Planctomycetes;c__ODP123;Other
■ k__Bacteria;p__Planctomycetes;c__ODP123;o__
■ k__Bacteria;p__Planctomycetes;c__ODP123;o__T8-B82
■ k__Bacteria;p__Planctomycetes;c__OM190;Other
■ k__Bacteria;p__Planctomycetes;c__OM190;o__
■ k__Bacteria;p__Planctomycetes;c__OM190;o__CL500-15
■ k__Bacteria;p__Planctomycetes;c__OM190;o__agg27
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;Other
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__AKAU3564
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__C86
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__CCM11a
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__CPla-3
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__MBMPE71
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__MSBL9
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__ODP1230B3009
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__Phycisphaerales
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__Pla1
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__S-70
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__SHA-43
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__SHUX583
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101
■ k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__mle1-8
■ k__Bacteria;p__Planctomycetes;c__Pla3;o__
■ k__Bacteria;p__Planctomycetes;c__Pla4;o__
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;Other
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__B97
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales
■ k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales
■ k__Bacteria;p__Planctomycetes;c__[Brocadiae];o__Brocadiales
■ k__Bacteria;p__Planctomycetes;c__vadinHA49;o__DH61
■ k__Bacteria;p__Planctomycetes;c__vadinHA49;o__PHOS-HE93
■ k__Bacteria;p__Planctomycetes;c__vadinHA49;o__p04_C01

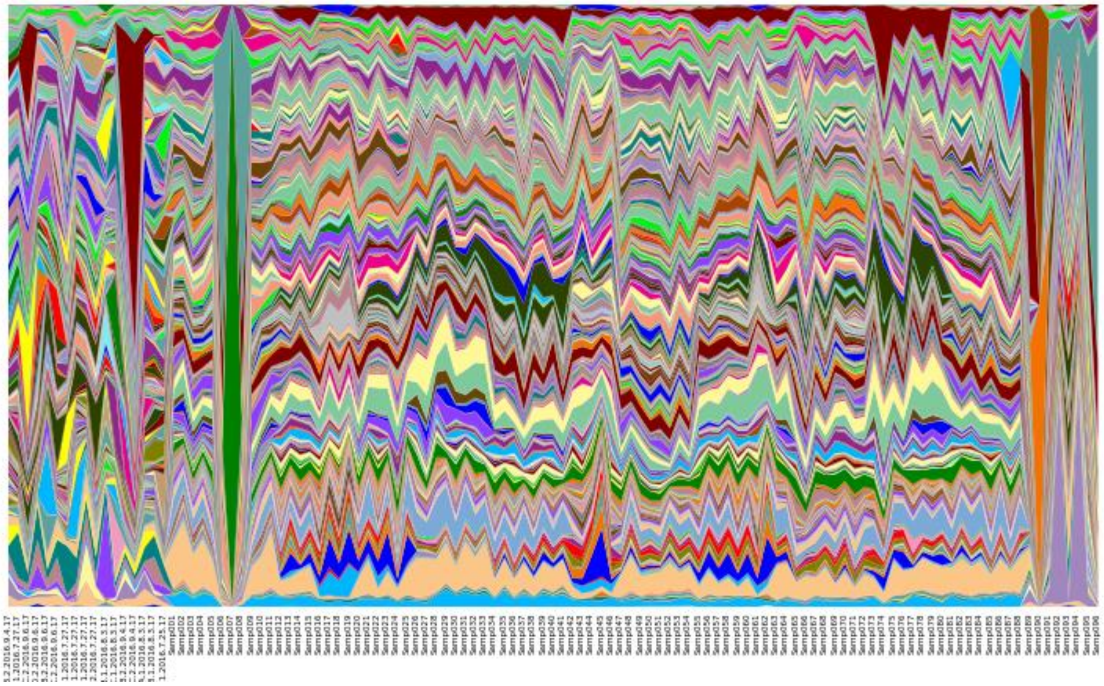
■ k__Bacteria;p__Poribacteria;c__;o__
■ k__Bacteria;p__Proteobacteria;Other;Other
■ k__Bacteria;p__Proteobacteria;c__;o__
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__BD7-3
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales
■ k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__A21b
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__ASSO-13
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Gallionellales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__IS-44
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MKC10
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Procabacteriales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__SBla14
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__SC-I-84
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Thiobacterales
■ k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Tremblayales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;Other
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__BPC076
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__DTB120
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__FAC87
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__GMD14H09
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__GW-28
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MBNT15
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NKB15
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__PB19
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__SAR324
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Spirobacillales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Sva0485
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Sva0853
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Thermodesulfobacteria
■ k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales]
■ k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__34P16
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Acidithiobacillales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Chromatiales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__HOC36
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__HTCC2188
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PYR10d3
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales
■ k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales]
■ k__Bacteria;p__Proteobacteria;c__TA18;Other
■ k__Bacteria;p__Proteobacteria;c__TA18;o__CV90
■ k__Bacteria;p__Proteobacteria;c__TA18;o__PHOS-HD29
■ k__Bacteria;p__Proteobacteria;c__Zetaproteobacteria;o__Mariprofundales
■ k__Bacteria;p__SAR406;c__AB16;o__noFP_H7
■ k__Bacteria;p__SBR1093;Other;Other
■ k__Bacteria;p__SBR1093;c__;o__
■ k__Bacteria;p__SBR1093;c__VHS-B5-50;o__
■ k__Bacteria;p__SC4;c__;o__
■ k__Bacteria;p__SR1;c__;o__
■ k__Bacteria;p__Spirochaetes;c__GN05;o__
■ k__Bacteria;p__Spirochaetes;c__GN05;o__LG048
■ k__Bacteria;p__Spirochaetes;c__MVP-15;o__PL-11B10
■ k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__M2PT2-76
■ k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales
■ k__Bacteria;p__Spirochaetes;c__[Brachyspirae];o__[Brachyspirales]
■ k__Bacteria;p__Spirochaetes;c__[Brevinematae];o__[Brevinematales]
■ k__Bacteria;p__Spirochaetes;c__[Leptospirae];o__[Leptospirales]
■ k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales
■ k__Bacteria;p__TM6;Other;Other
■ k__Bacteria;p__TM6;c__;o__
■ k__Bacteria;p__TM6;c__F38;o__
■ k__Bacteria;p__TM6;c__SBRH58;o__
■ k__Bacteria;p__TM6;c__SJA-4;Other
■ k__Bacteria;p__TM6;c__SJA-4;o__
■ k__Bacteria;p__TM6;c__SJA-4;o__S1198
■ k__Bacteria;p__TM6;c__SJA-4;o__YJF2-48
■ k__Bacteria;p__TM7;Other;Other
■ k__Bacteria;p__TM7;c__;o__
■ k__Bacteria;p__TM7;c__MJK10;o__
■ k__Bacteria;p__TM7;c__SC3;o__
■ k__Bacteria;p__TM7;c__TM7-1;o__
■ k__Bacteria;p__TM7;c__TM7-3;Other
■ k__Bacteria;p__TM7;c__TM7-3;o__
■ k__Bacteria;p__TM7;c__TM7-3;o__EW055
■ k__Bacteria;p__TM7;c__TM7-3;o__I025
■ k__Bacteria;p__TPD-58;c__;o__
■ k__Bacteria;p__Tenericutes;c__Mollicutes;Other
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__Acholeplasmatales
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__Entomoplasmatales
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales
■ k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39
■ k__Bacteria;p__Tenericutes;c__RF3;o__ML615J-28
■ k__Bacteria;p__Verrucomicrobia;Other;Other
■ k__Bacteria;p__Verrucomicrobia;c__;o__
■ k__Bacteria;p__Verrucomicrobia;c__Opitutae;Other
■ k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__
■ k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__HA64
■ k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales
■ k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__[Cerasicoccales]
■ k__Bacteria;p__Verrucomicrobia;c__Verruco-5;Other
■ k__Bacteria;p__Verrucomicrobia;c__Verruco-5;o__LD1-PB3
■ k__Bacteria;p__Verrucomicrobia;c__Verruco-5;o__WCHB1-41
■ k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales
■ k__Bacteria;p__Verrucomicrobia;c__[Methylacidiphilae];Other
■ k__Bacteria;p__Verrucomicrobia;c__[Methylacidiphilae];o__Methylacidiphilales
■ k__Bacteria;p__Verrucomicrobia;c__[Methylacidiphilae];o__S-BQ2-57
■ k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];Other
■ k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__
■ k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales]
■ k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales]
■ k__Bacteria;p__WPS-2;c__;o__
■ k__Bacteria;p__WS1;c__;o__
■ k__Bacteria;p__WS2;c__Kazan-3B-09;o__
■ k__Bacteria;p__WS2;c__SHA-109;o__
■ k__Bacteria;p__WS3;c__PRR-12;o__
■ k__Bacteria;p__WS3;c__PRR-12;o__GN03
■ k__Bacteria;p__WS3;c__PRR-12;o__LD1-PA13
■ k__Bacteria;p__WS3;c__PRR-12;o__PBS-III-9
■ k__Bacteria;p__WS3;c__PRR-12;o__SSS58A
■ k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1
■ k__Bacteria;p__WS3;c__PRR-12;o__wb1_H11
■ k__Bacteria;p__WS4;c__;o__
■ k__Bacteria;p__WS5;c__;o__
■ k__Bacteria;p__WS6;c__B142;o__
■ k__Bacteria;p__ZB3;c__;o__
■ k__Bacteria;p__ZB3;c__BS119;o__
■ k__Bacteria;p__[Caldithrix];c__KSB1;Other
■ k__Bacteria;p__[Caldithrix];c__KSB1;o__
■ k__Bacteria;p__[Caldithrix];c__KSB1;o__GW-22
■ k__Bacteria;p__[Caldithrix];c__KSB1;o__MSB-5B5
■ k__Bacteria;p__[Caldithrix];c__KSB1;o__Ucn15732
■ k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales
■ k__Bacteria;p__[Thermi];c__Deinococci;o__Thermales

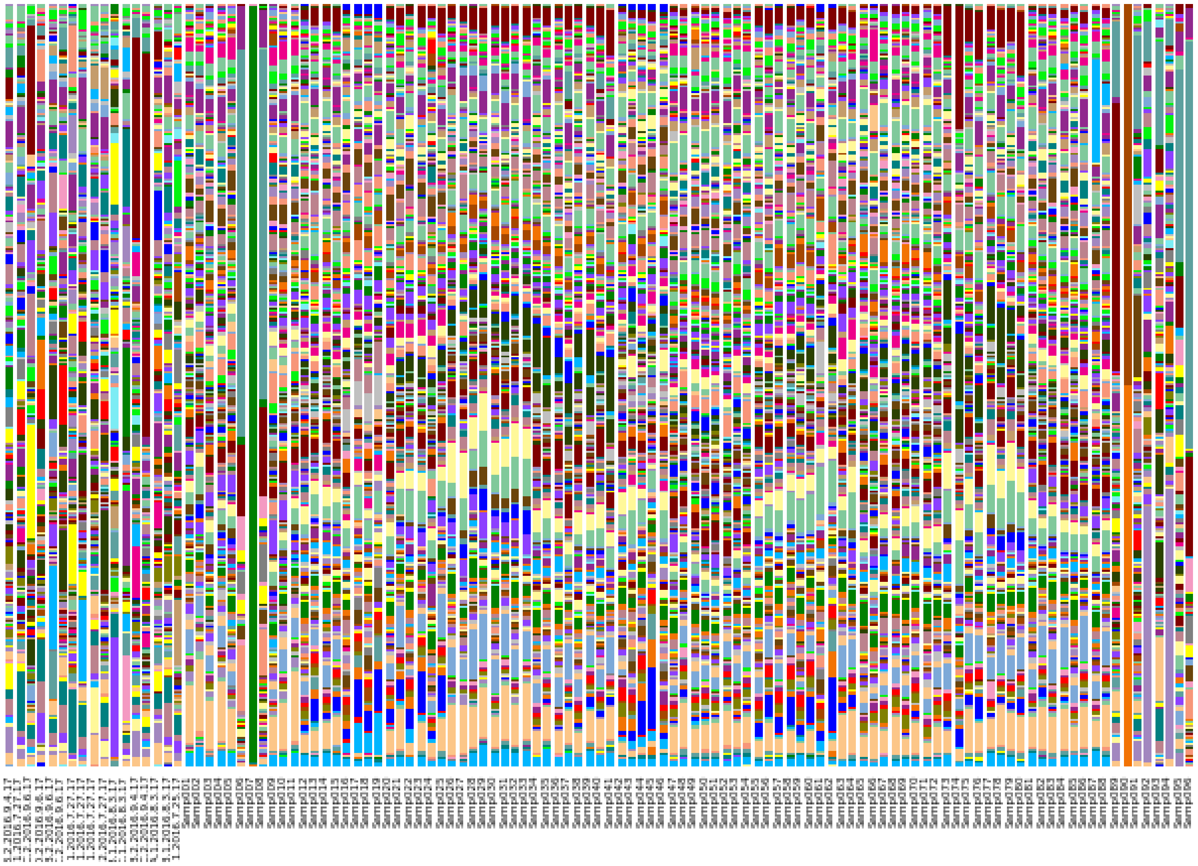
Family Level Area and Bar Charts of Archaeal and Bacterial Taxonomies





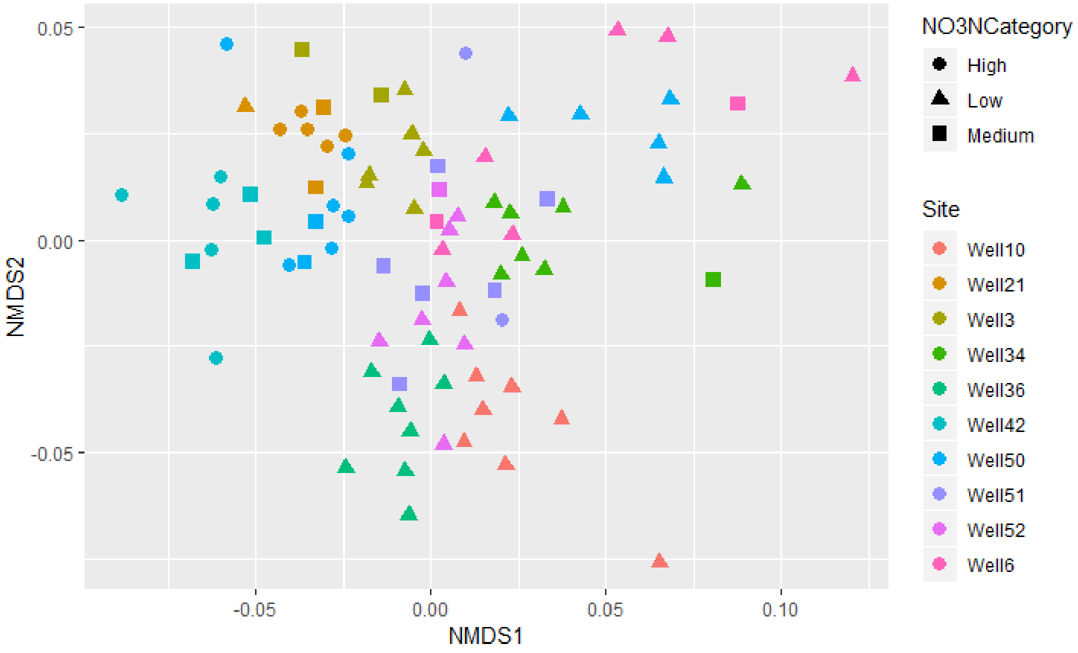
Genus Level Area and Bar Charts of Archaeal and Bacterial Taxonomies

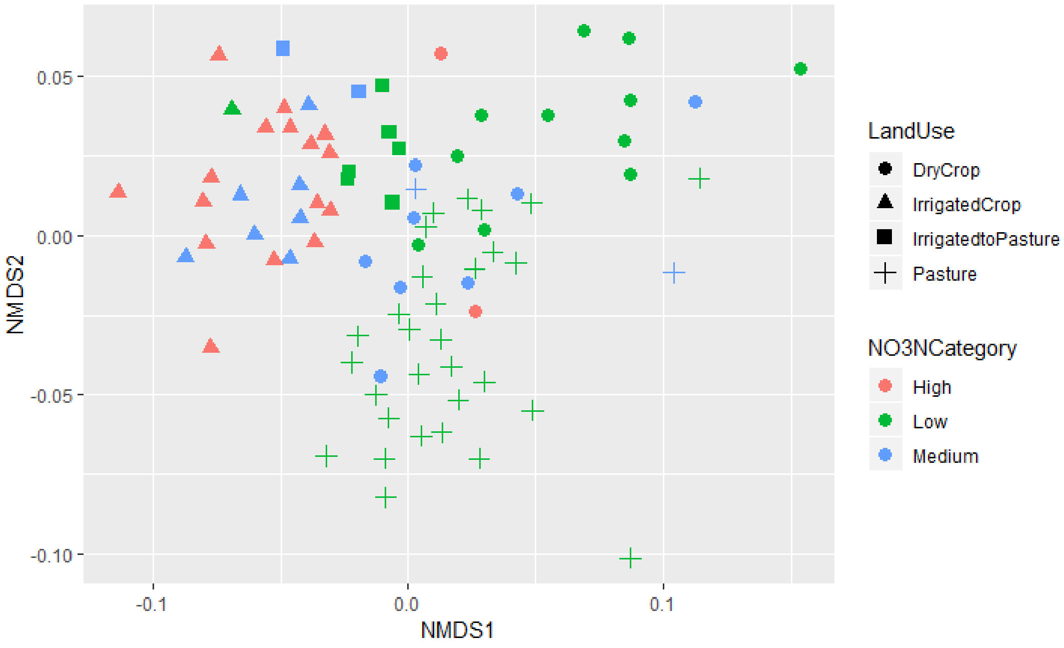




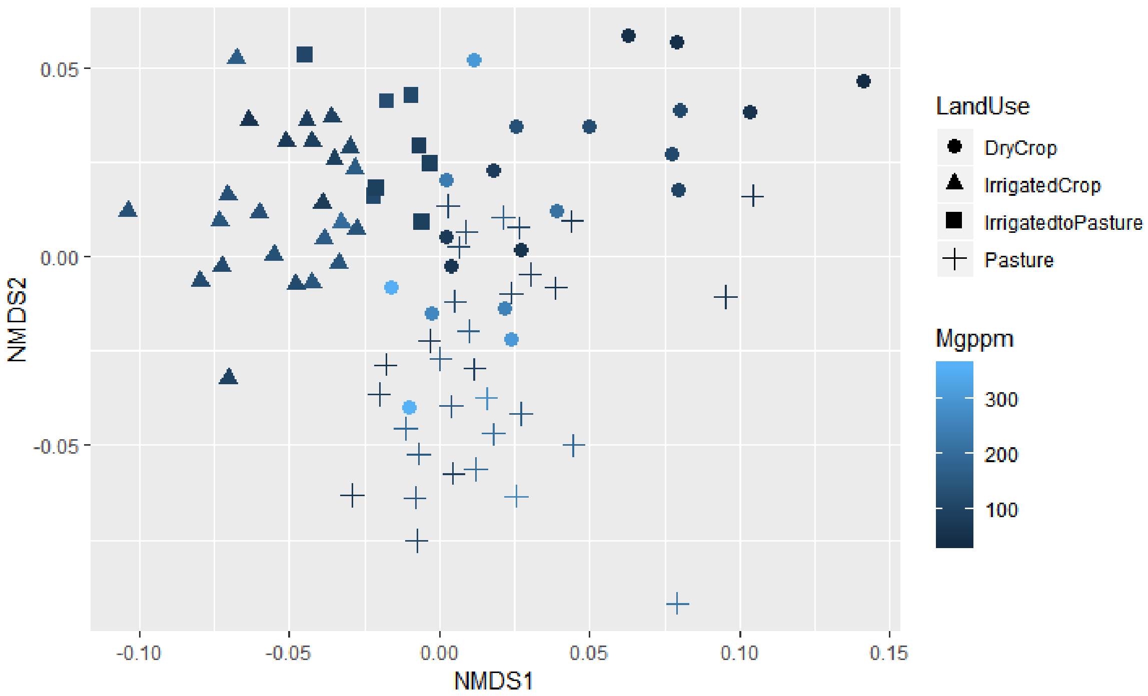
Beta Diversity Results

Soil NMDS Plots: Microbial Communities and Categories

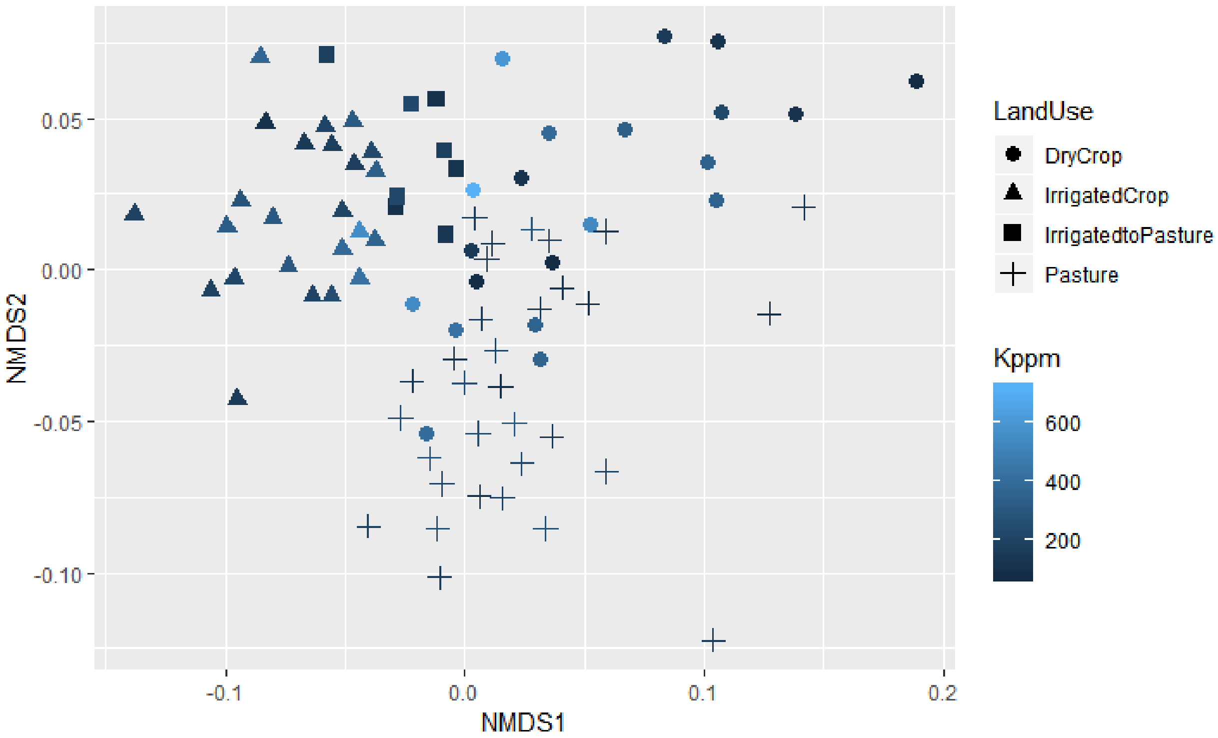




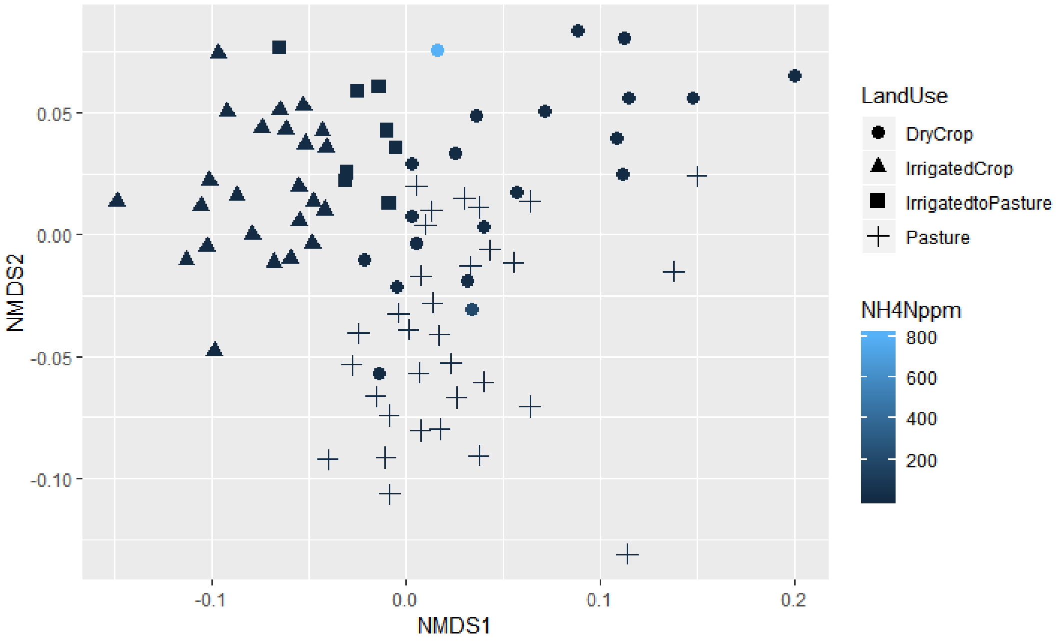
Soil NMDS Plots: Microbial Communities and Land Use with Other Factors



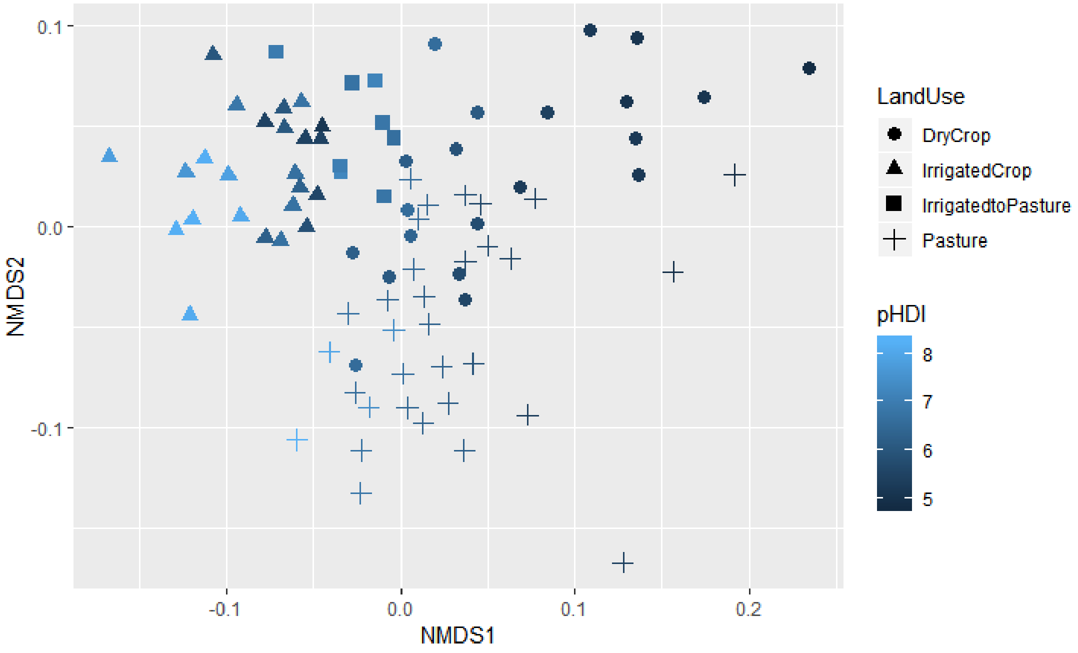
NMDS2
NMDS1
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
OMLOI
3
2
1



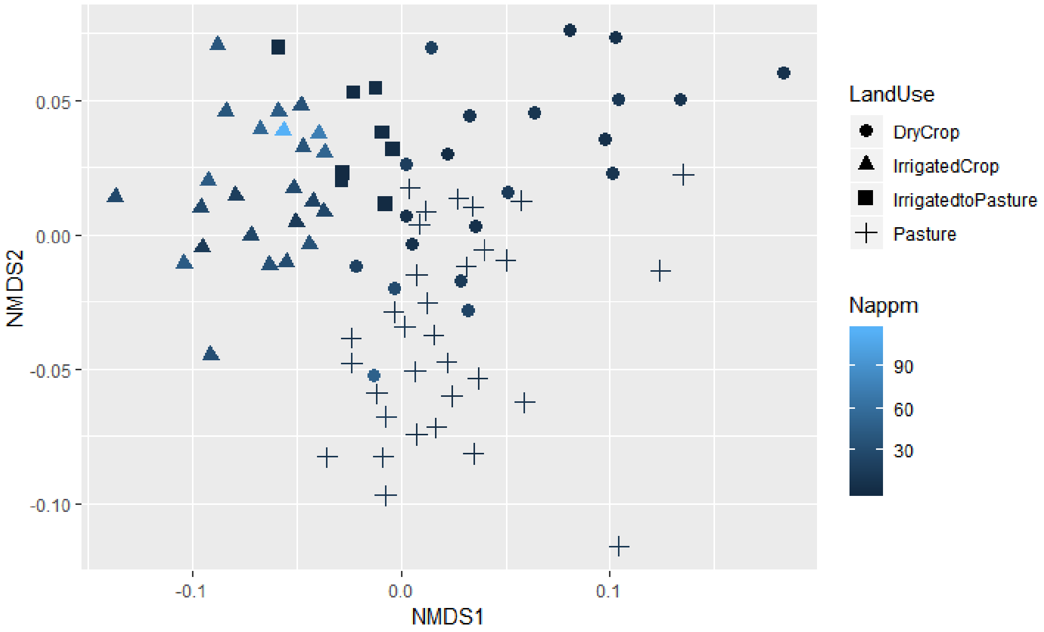
NMDS2
NMDS1
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
0.2
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
Kppm
600
400
200

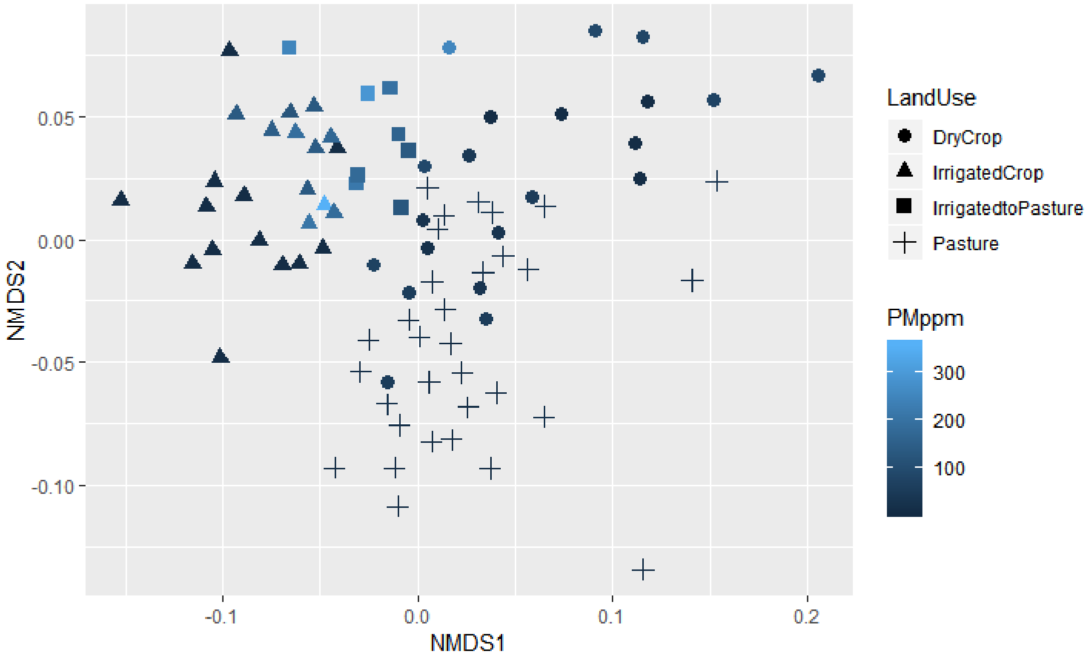


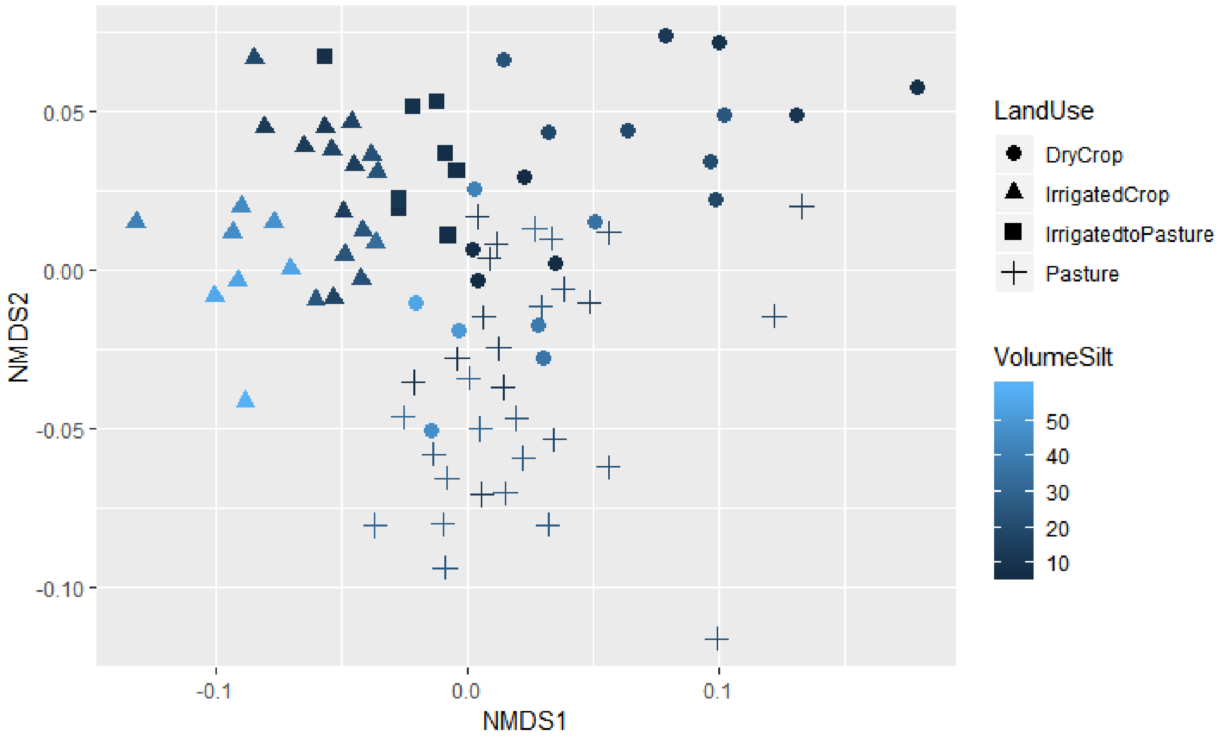
0.05
0.00
-0.05
-0.10
NMDS2
-0.1
0.0
0.1
0.2
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
NH4Nppm
800
600
400
200

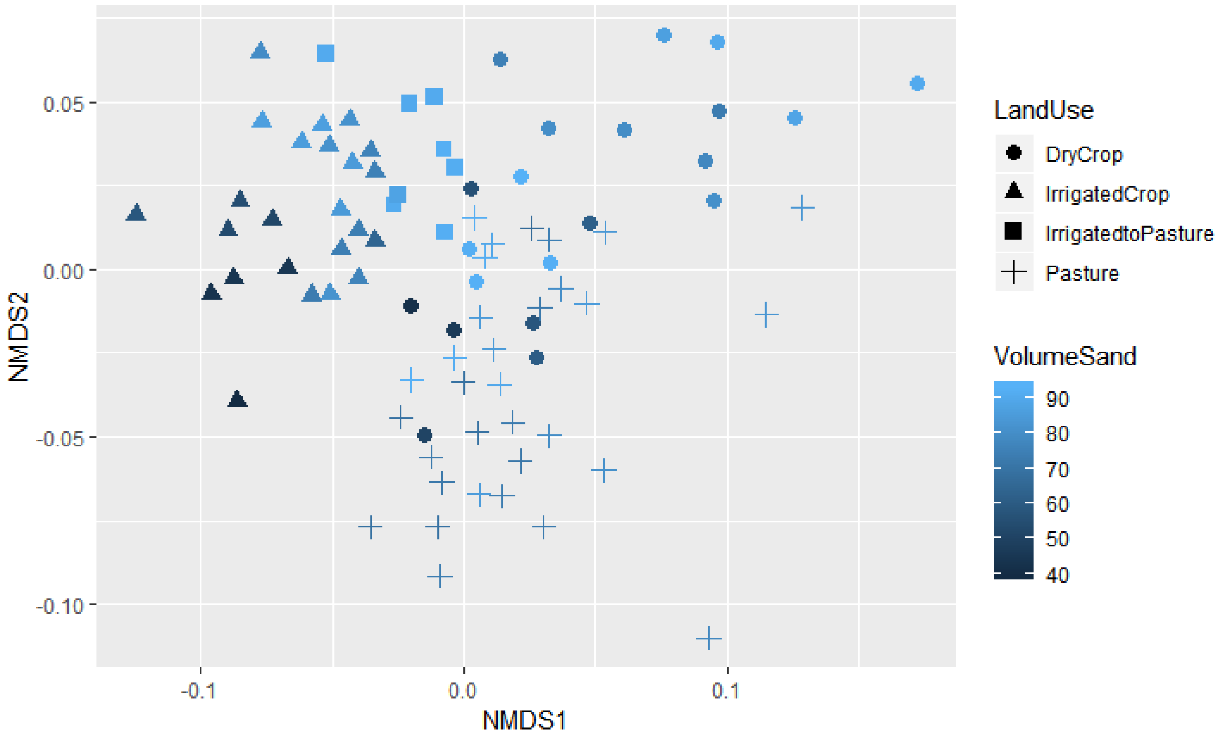


0.1
0.0
-0.1
NMDS2
-0.1
0.0
0.1
0.2
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
pHDI
8
7
6
5

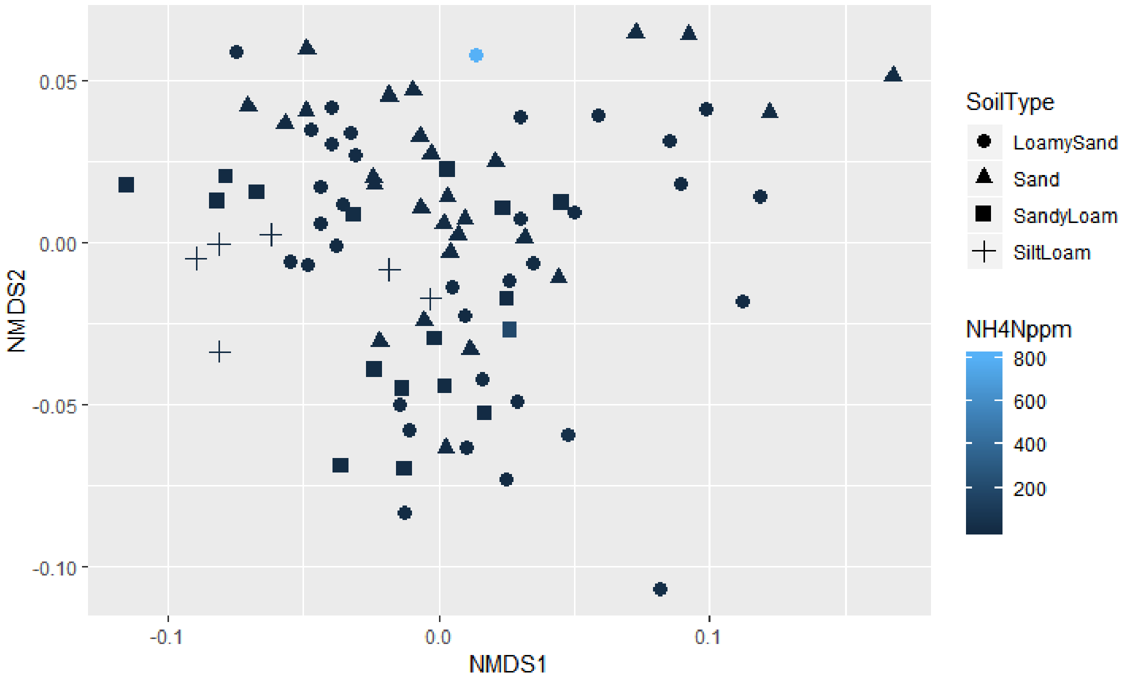

NMDS2
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
Nappm
90
60
30

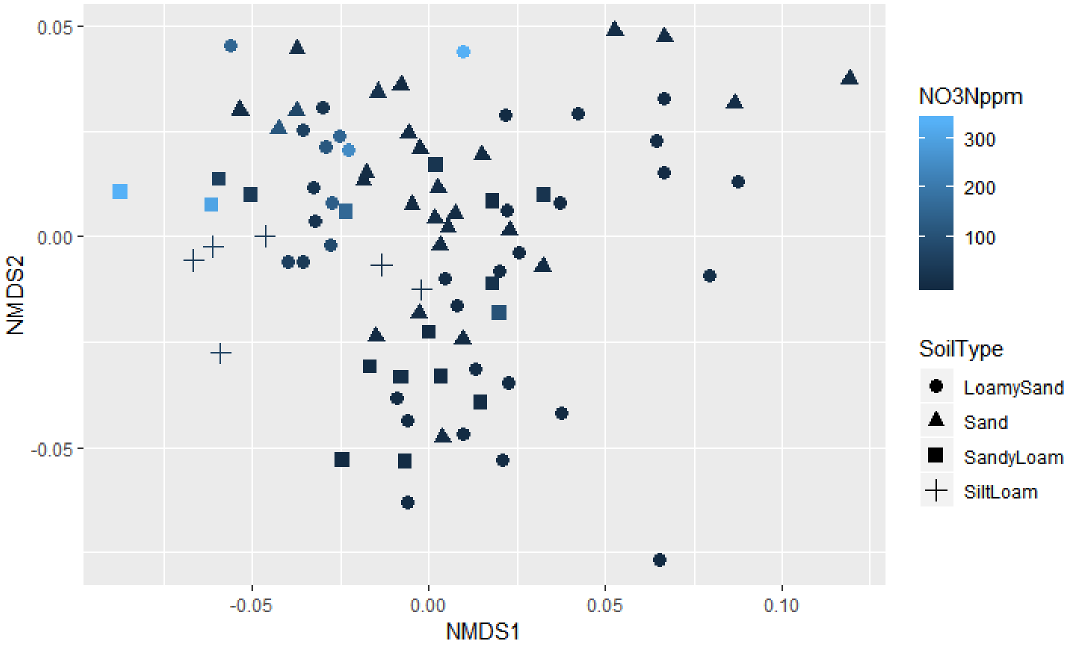

NMDS2
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
0.2
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
PMppm
300
200
100

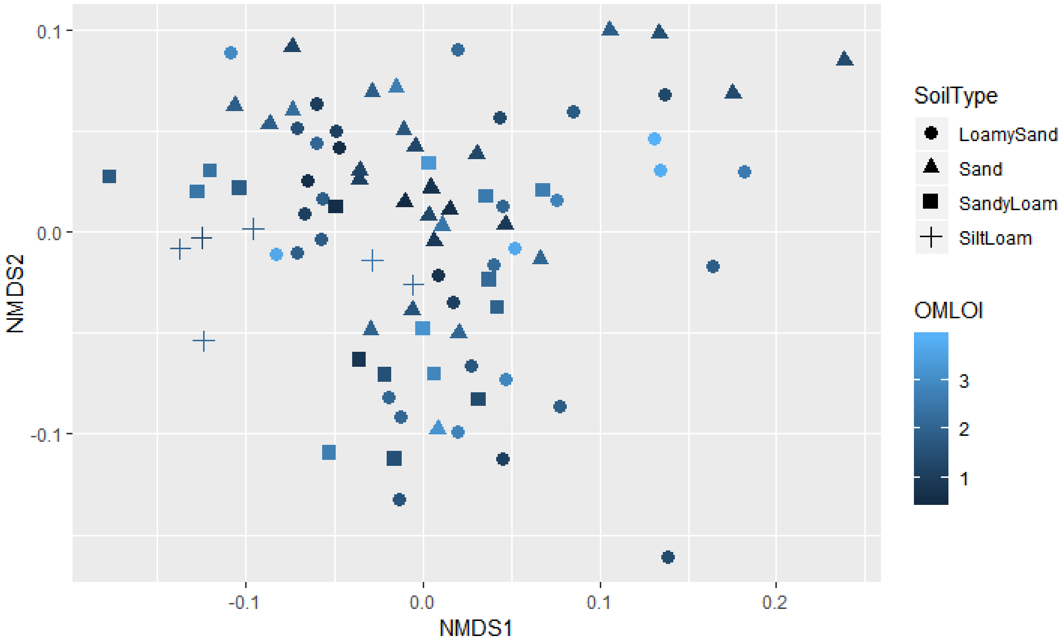

NMDS2
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
VolumeSilt
50
40
30
20
10

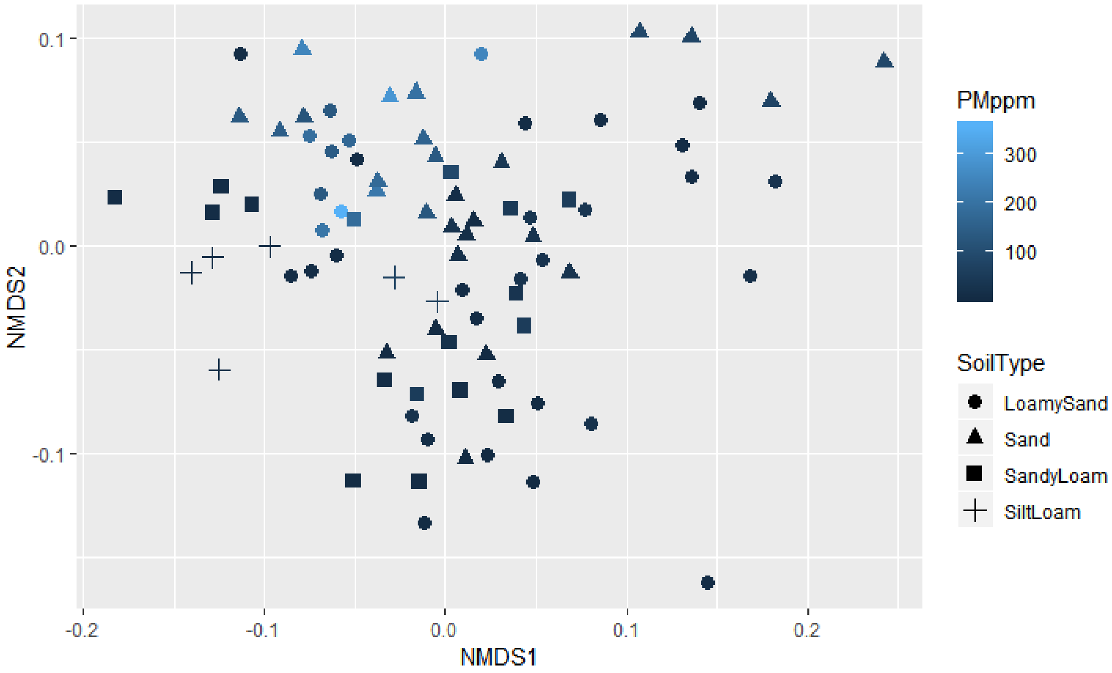

NMDS2
0.05
0.00
-0.05
-0.10
-0.1
0.0
0.1
NMDS1
LandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
VolumeSand
90
80
70
60
50
40

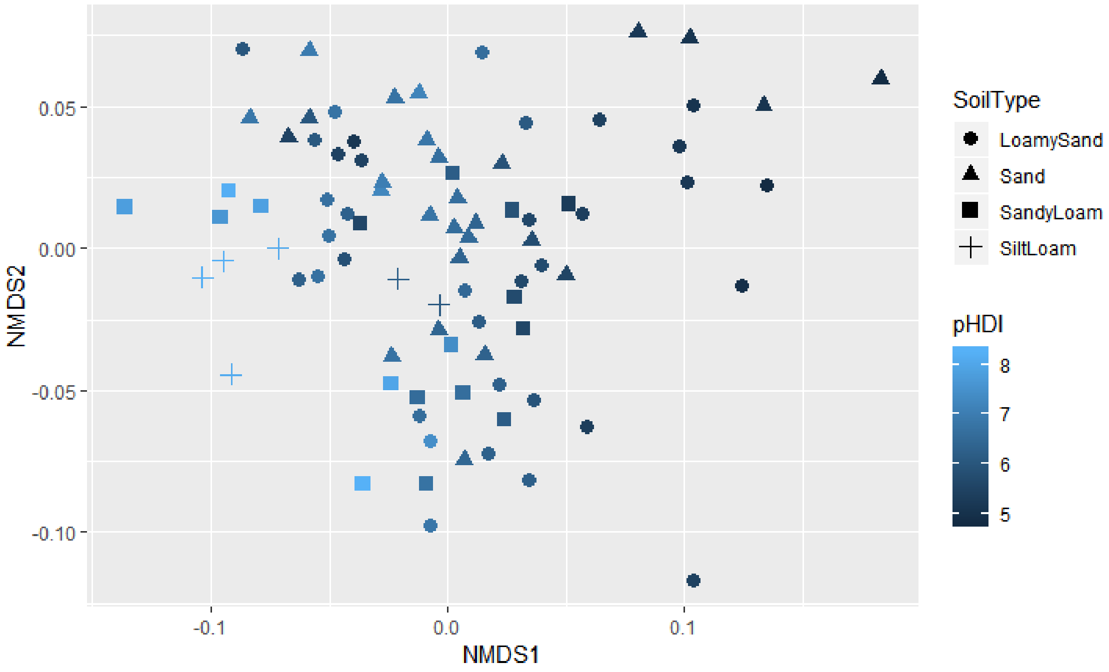
Soil NMDS Plots: Microbial Communities and Soil Type with Other Factors



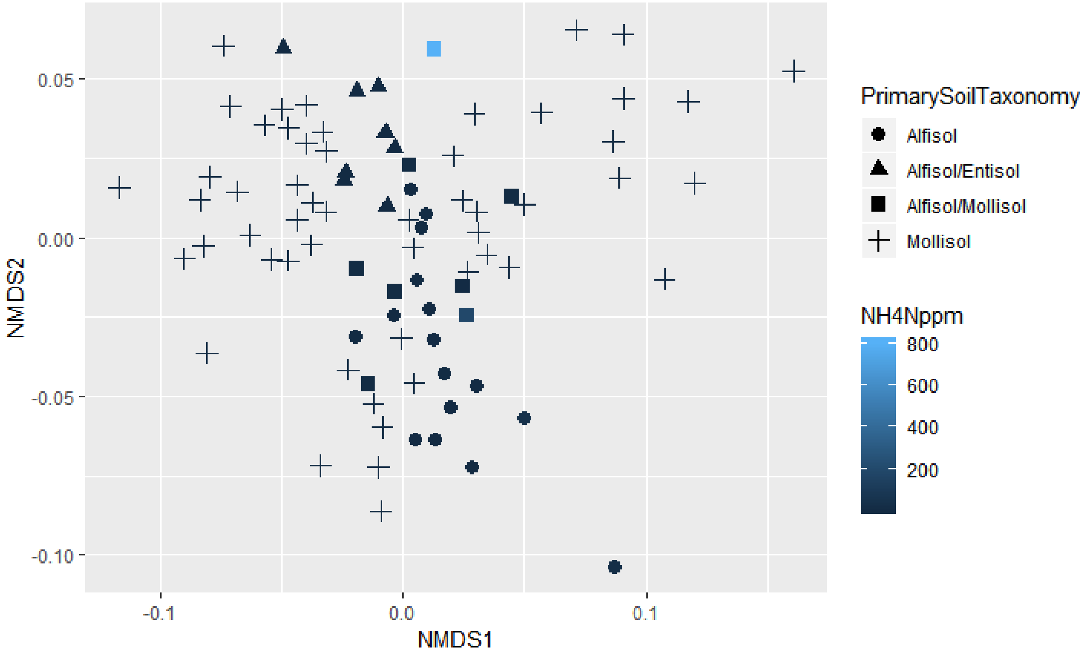


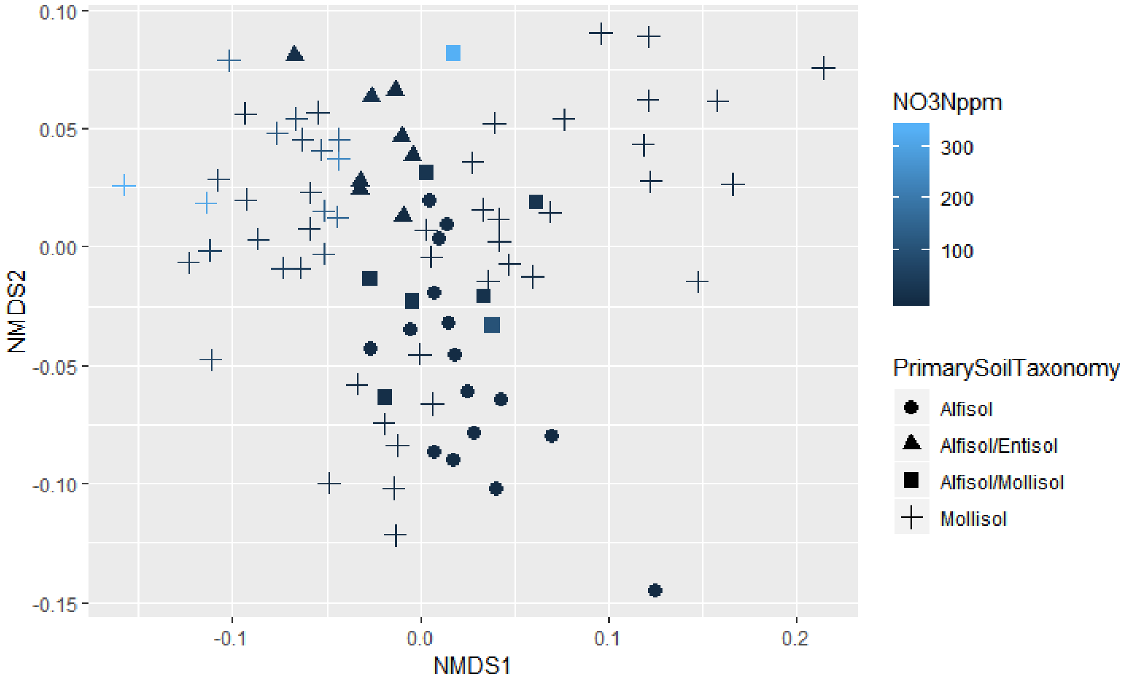

0.1
0.0
-0.1
NMDS2
-0.1
0.0
0.1
0.2
NMDS1
SoilType
LoamySand
Sand
SandyLoam
SiltLoam
OMLOI
3
2
1

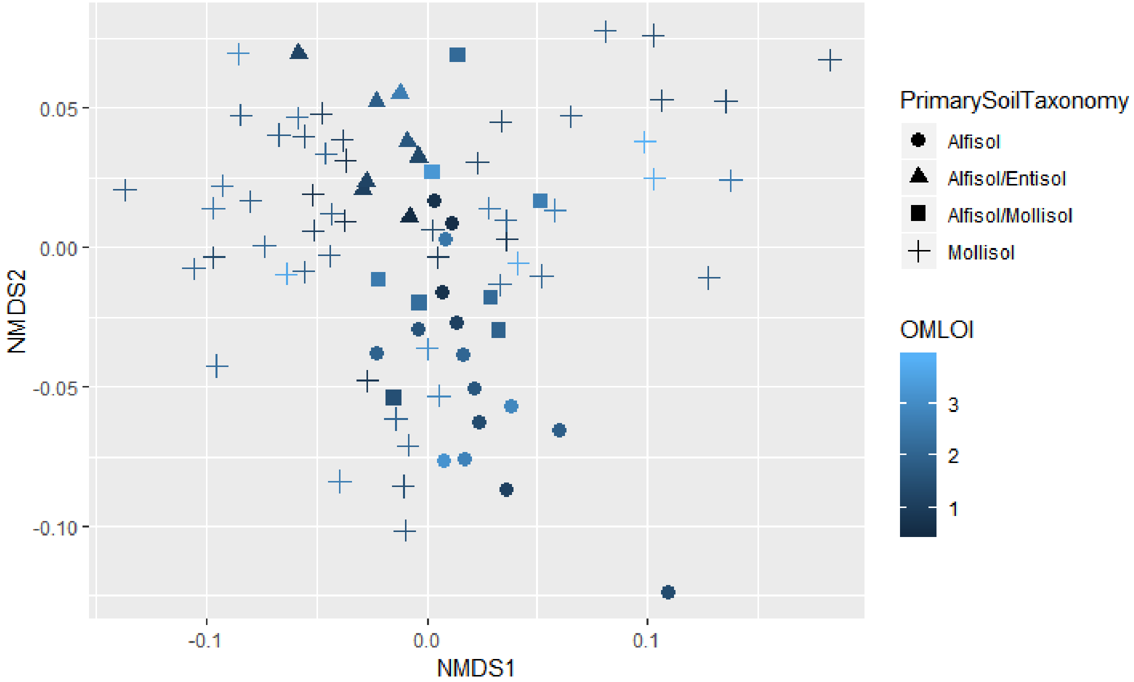

0.1
0.0
-0.1
NMDS2
-0.2
-0.1
0.0
0.1
0.2
NMDS1
PMppm
300
200
100
SoilType
LoamySand
Sand
SandyLoam
SiltLoam

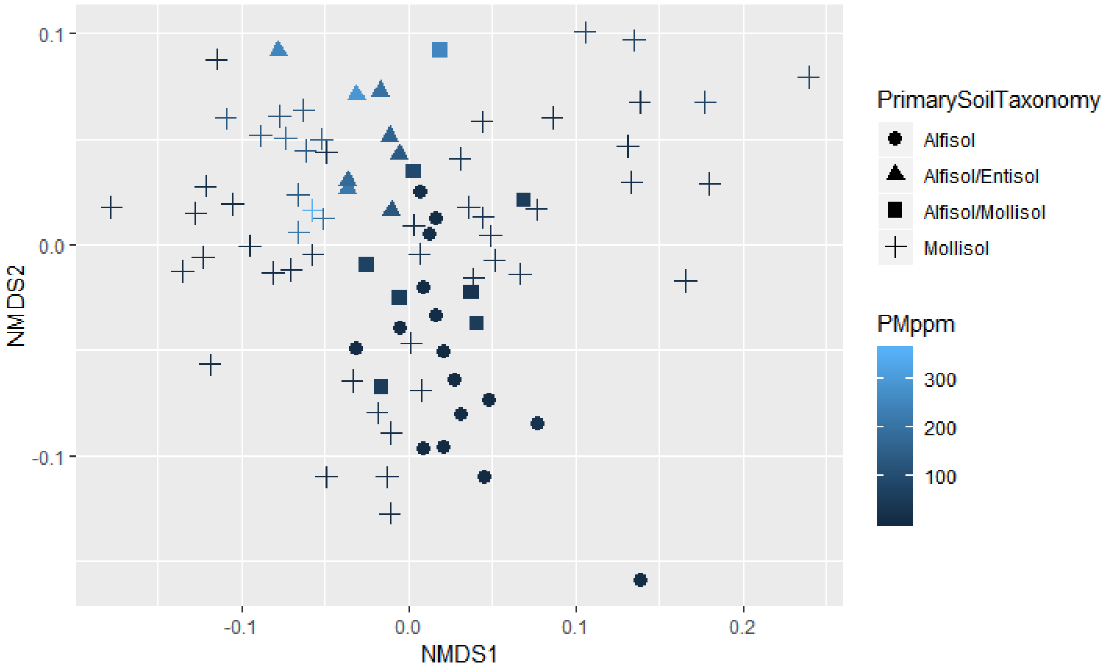


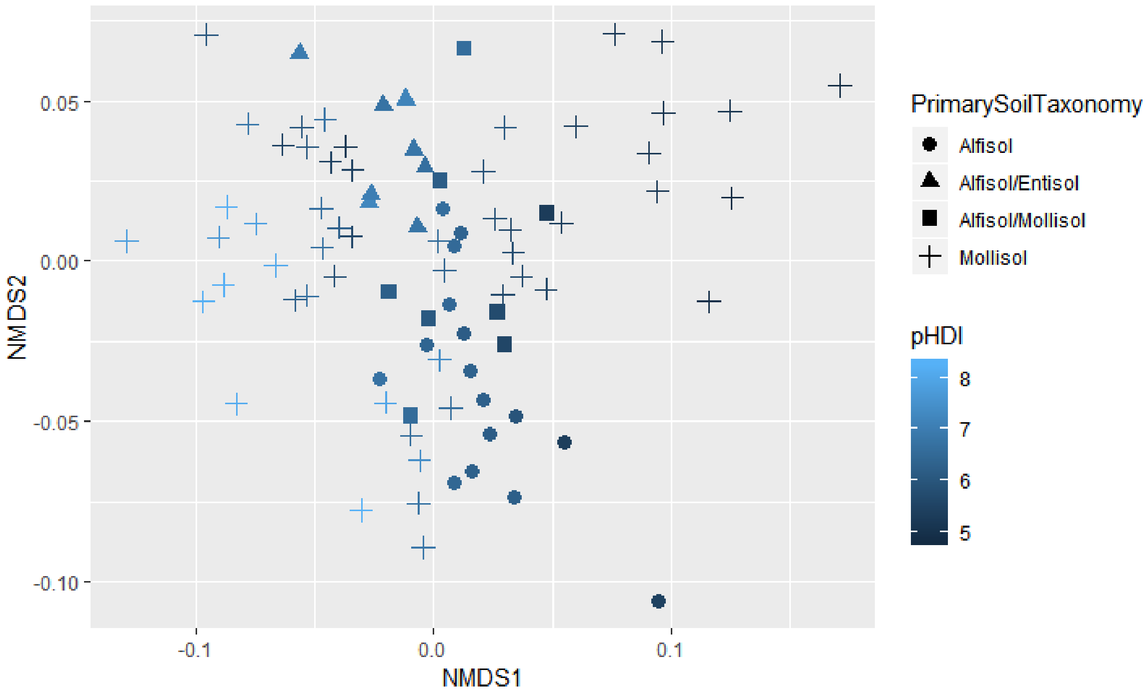
Soil NMDS Plots: Microbial Communities and Soil Taxonomy with Other Factors



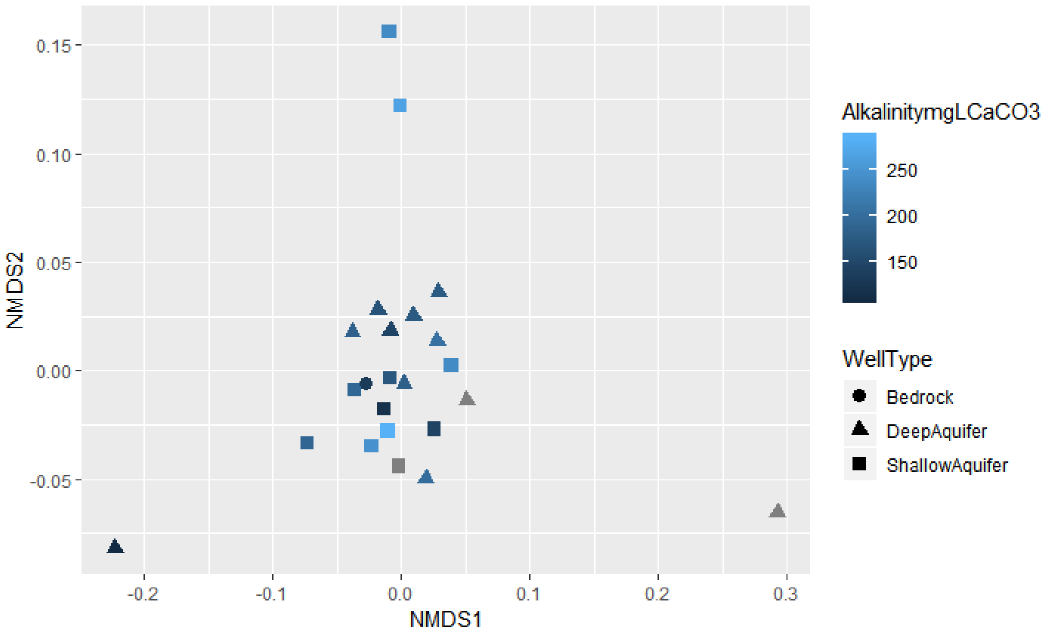

NO3Nppm
300
200
100
PrimarySoilTaxonomy
● Alfisol
▲ Alfisol/Entisol
■ Alfisol/Mollisol
+ Mollisol
NMDS2
0.10
0.05
0.00
-0.05
-0.10
-0.15
NMDS1
-0.1
0.0
0.1
0.2

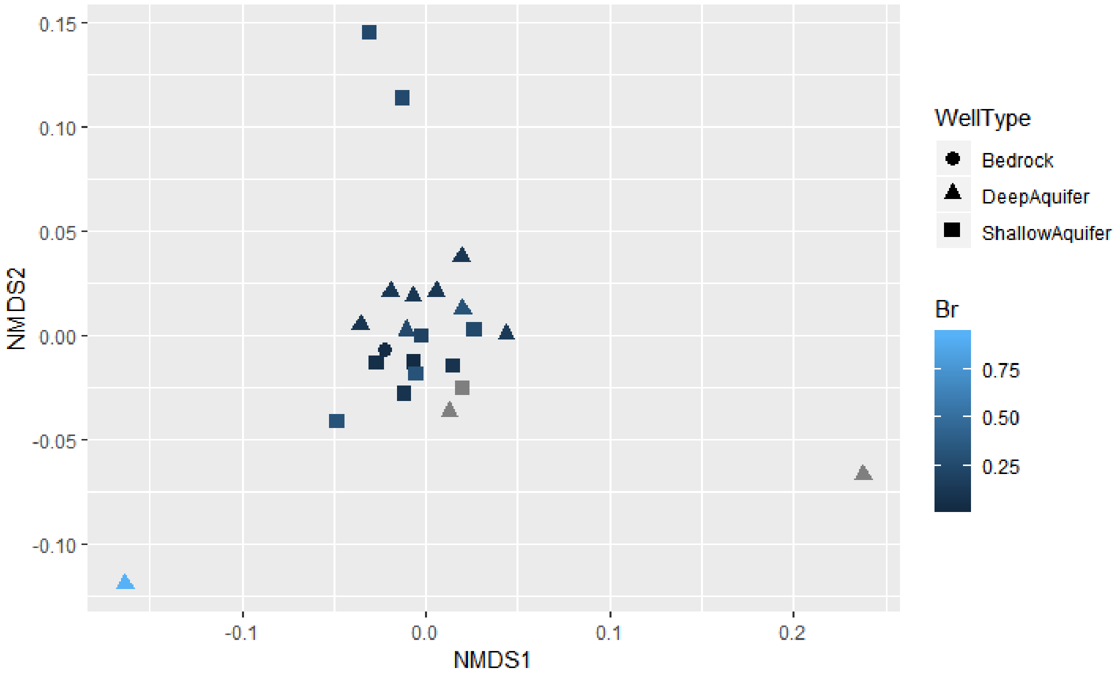

PrimarySoilTaxonomy
● Alfisol
▲ Alfisol/Entisol
■ Alfisol/Mollisol
+ Mollisol
OMLOI
3
2
1
NMDS2
0.05
0.00
-0.05
-0.10
NMDS1
-0.1
0.0
0.1

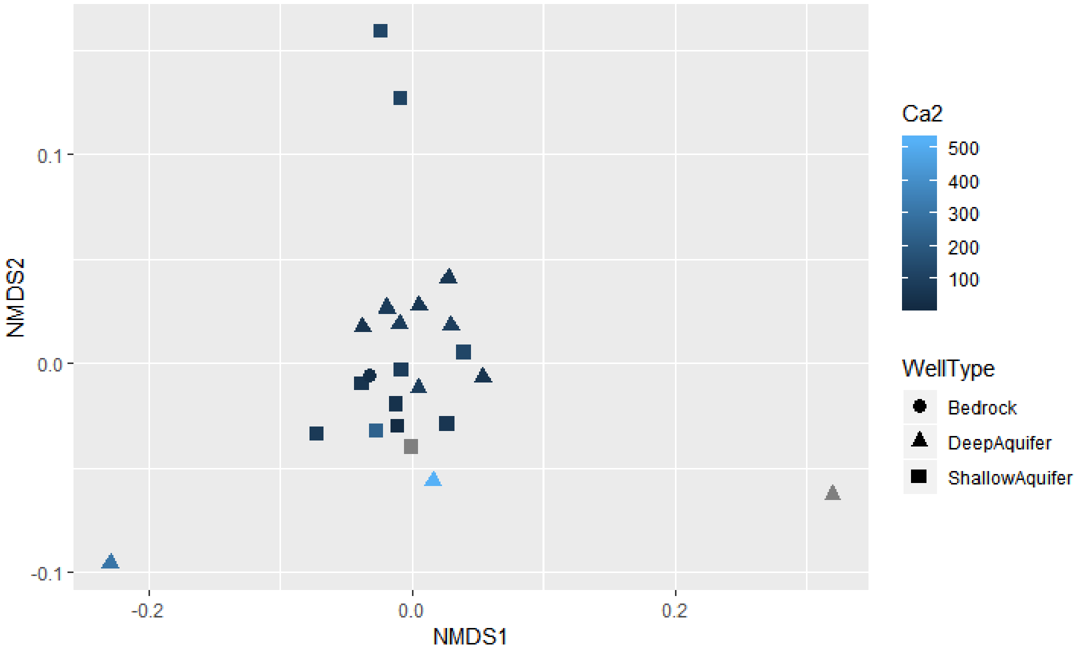

0.1
0.0
-0.1
NMDS2
-0.1
0.0
0.1
0.2
NMDS1
PrimarySoilTaxonomy
Alfisol
Alfisol/Entisol
Alfisol/Mollisol
Mollisol
PMppm
300
200
100

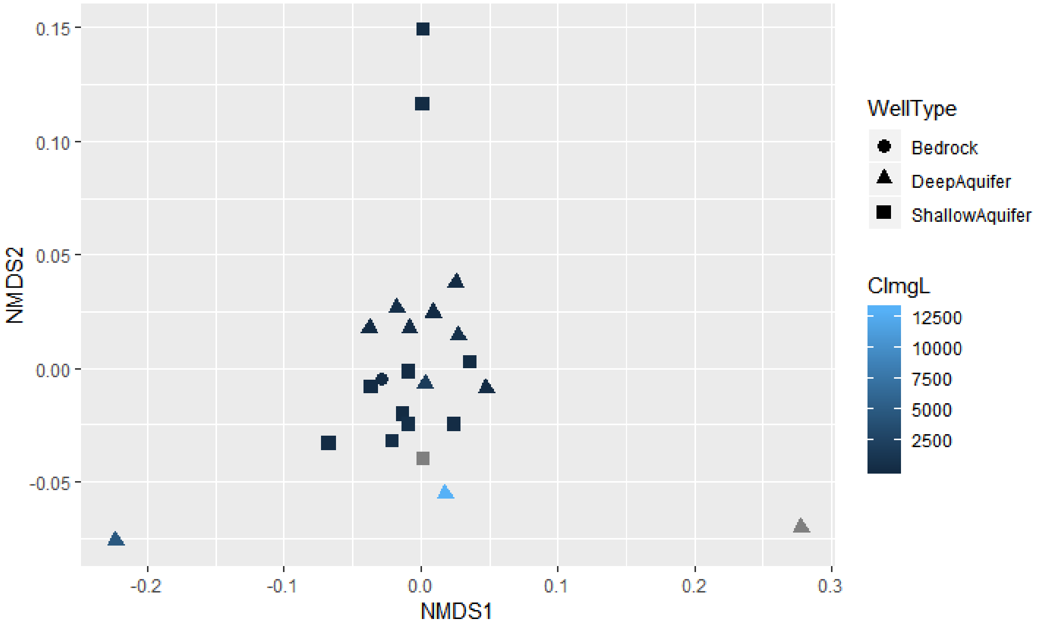

0.05
0.00
-0.05
-0.10
NMDS2
-0.1
0.0
0.1
NMDS1
PrimarySoilTaxonomy
Alfisol
Alfisol/Entisol
Alfisol/Mollisol
Mollisol
pHDI
8
7
6
5

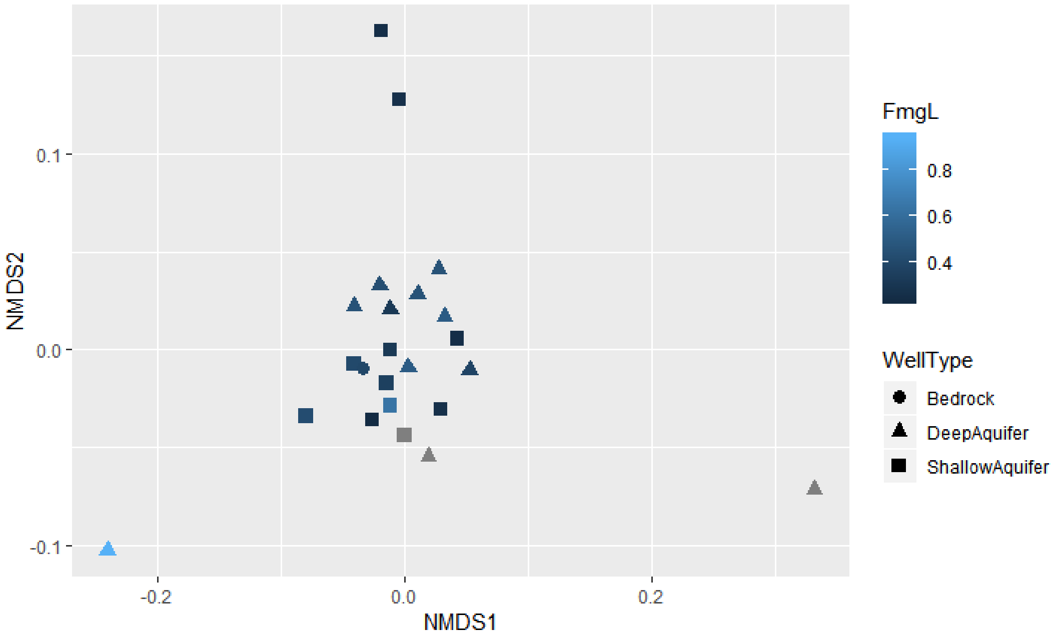
Groundwater NMDS Plots: Microbial Communities, Well Depth, & Other Factors

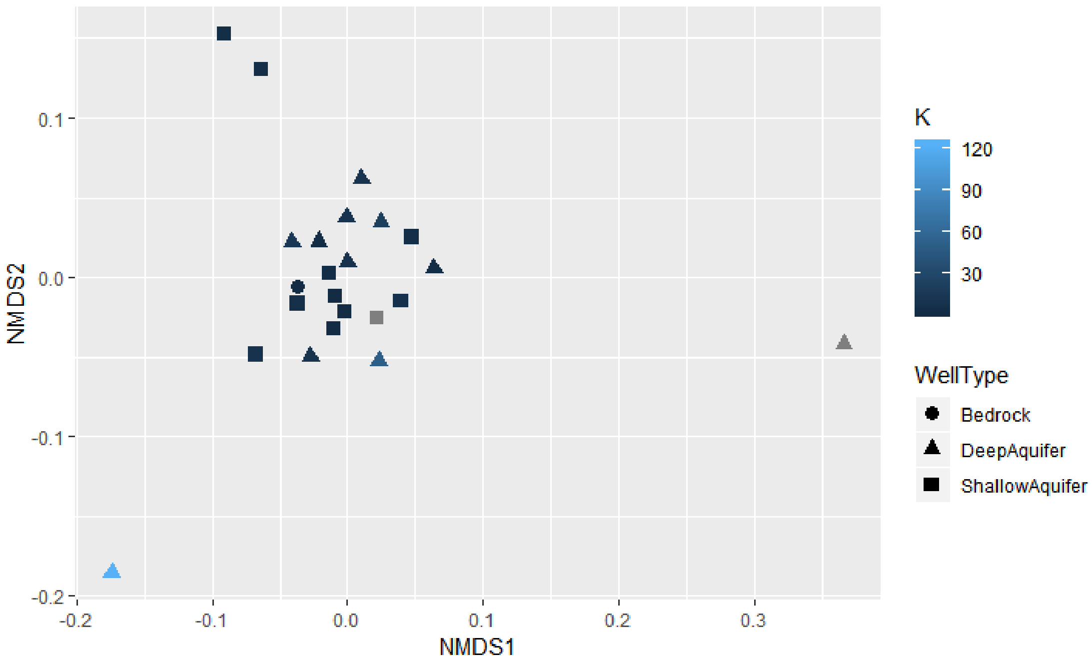


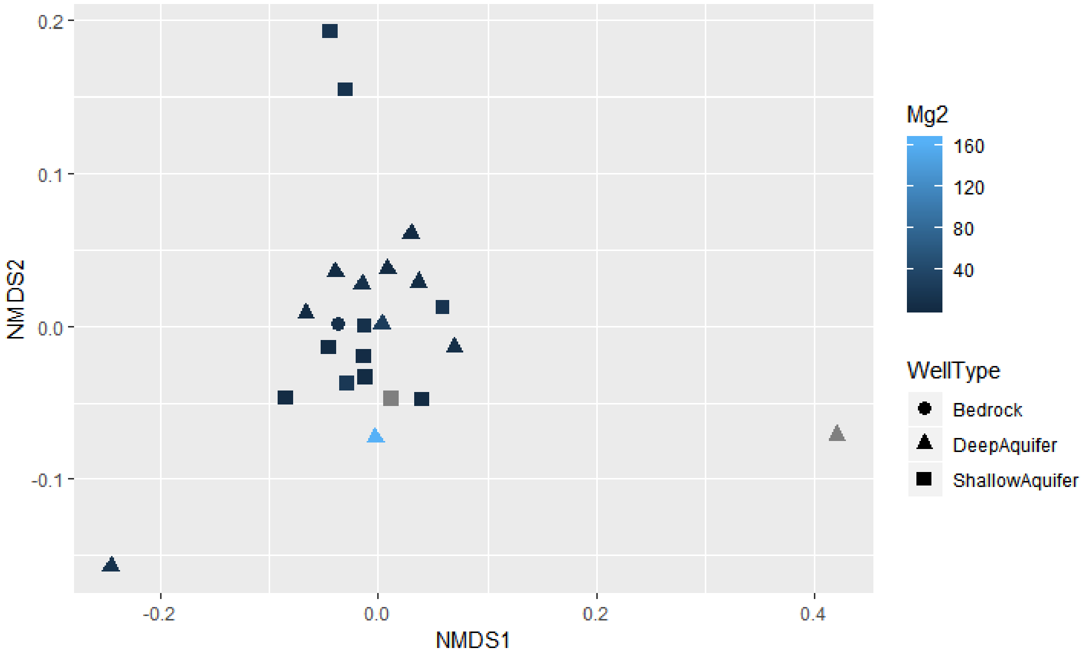



NMDS2
0.1
0.0
-0.1
-0.2
0.0
0.2
NMDS1
Ca2
500
400
300
200
100
WellType
● Bedrock
▲ DeepAquifer
■ ShallowAquifer

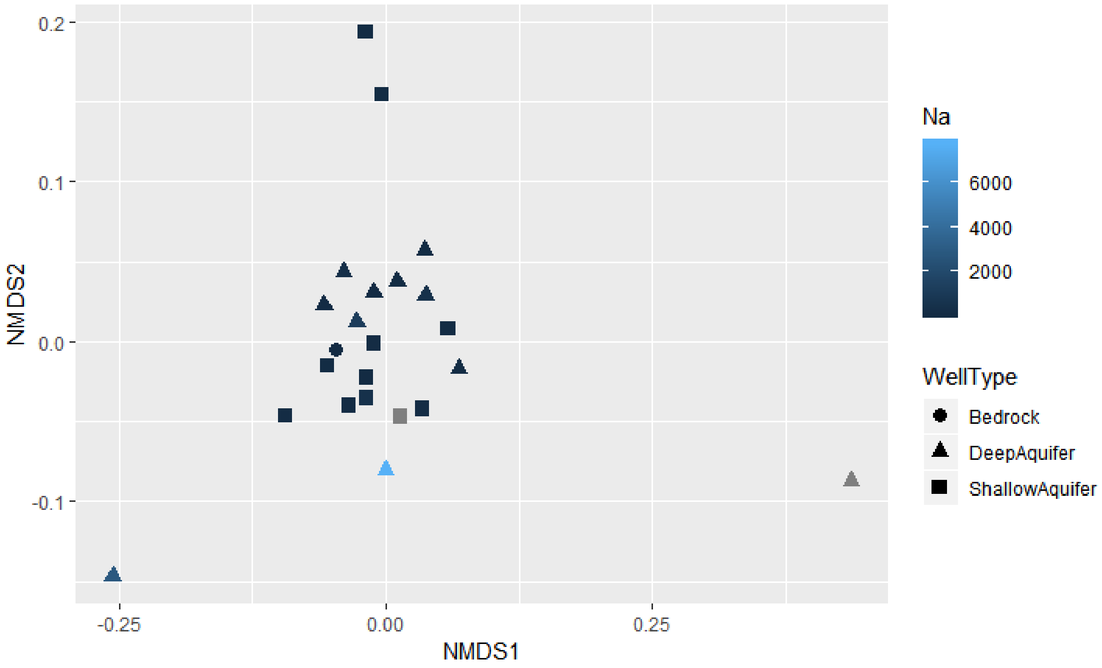

NMDS2
0.15
0.10
0.05
0.00
-0.05
-0.2
-0.1
0.0
0.1
0.2
0.3
NMDS1
WellType
● Bedrock
▲ DeepAquifer
■ ShallowAquifer
ClmgL
12500
10000
7500
5000
2500


NMDS2
0.1
0.0
-0.1
-0.2
0.0
0.2
NMDS1
FmgL
0.8
0.6
0.4
WellType
● Bedrock
▲ DeepAquifer
■ ShallowAquifer

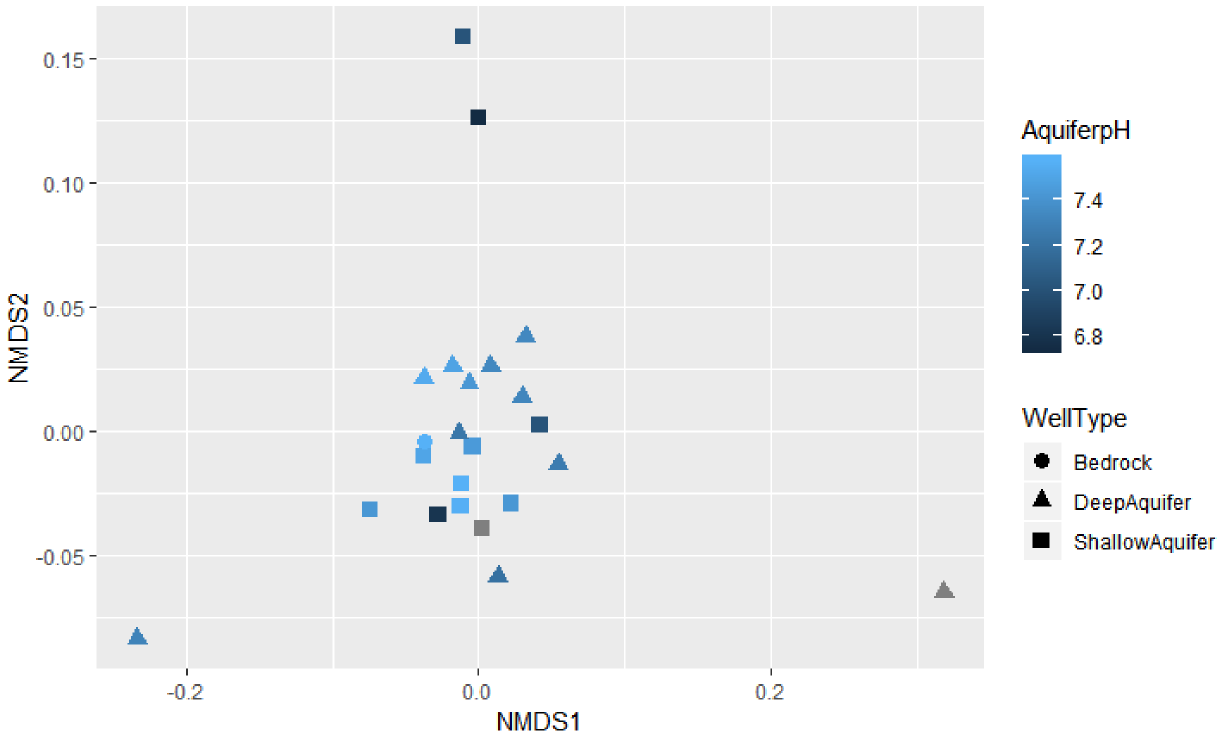

NMDS2
0.1
0.0
-0.1
-0.2
-0.2
-0.1
0.0
0.1
0.2
0.3
NMDS1
K
120
90
60
30
WellType
● Bedrock
▲ DeepAquifer
■ ShallowAquifer



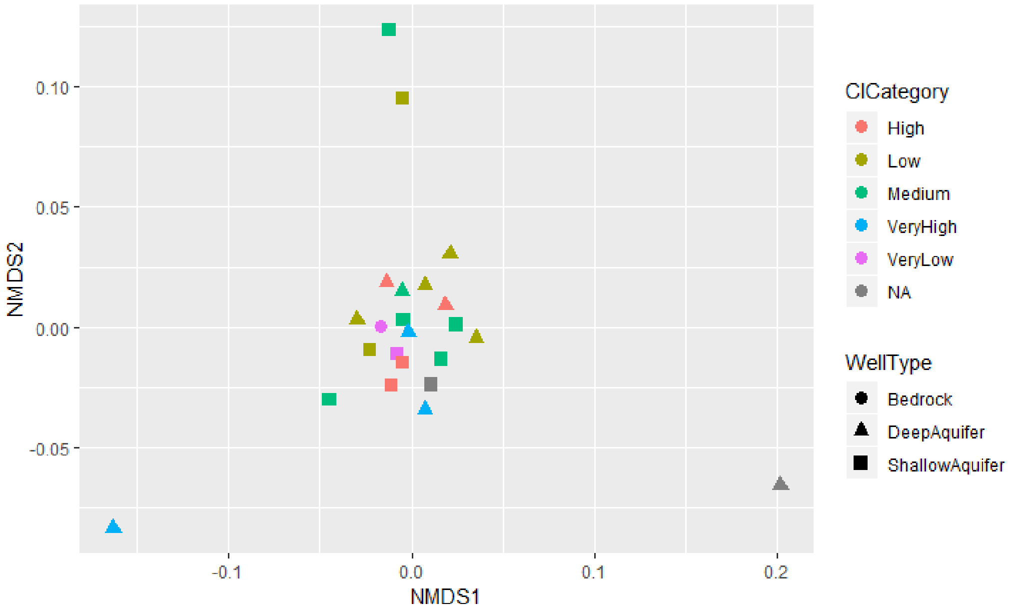
0.2
0.1
0.0
-0.1
NMDS2
-0.2
0.0
0.2
0.4
NMDS1
Mg2
160
120
80
40
WellType
Bedrock
DeepAquifer
ShallowAquifer

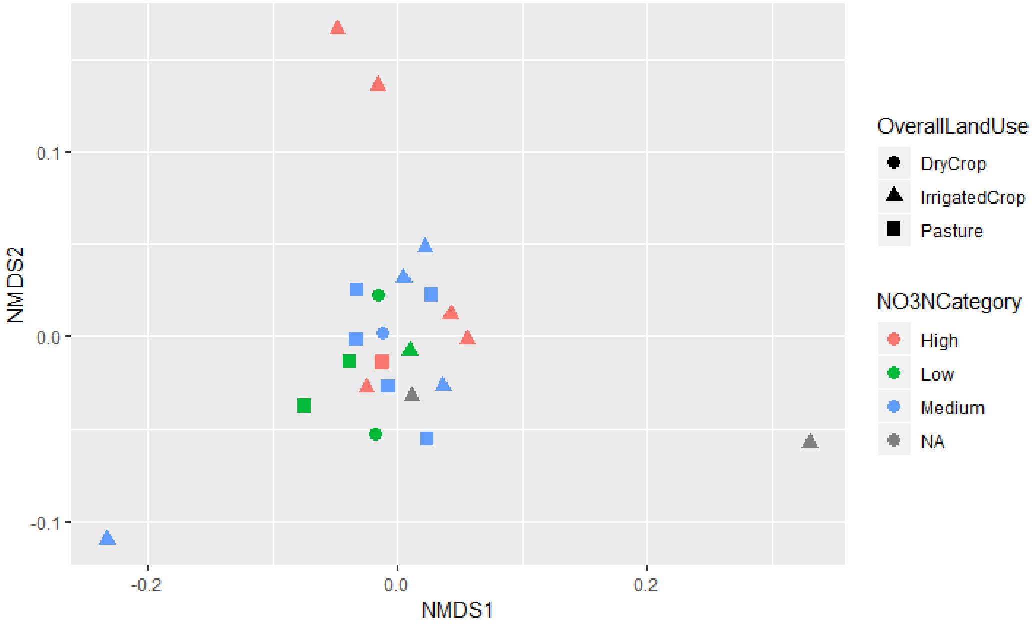


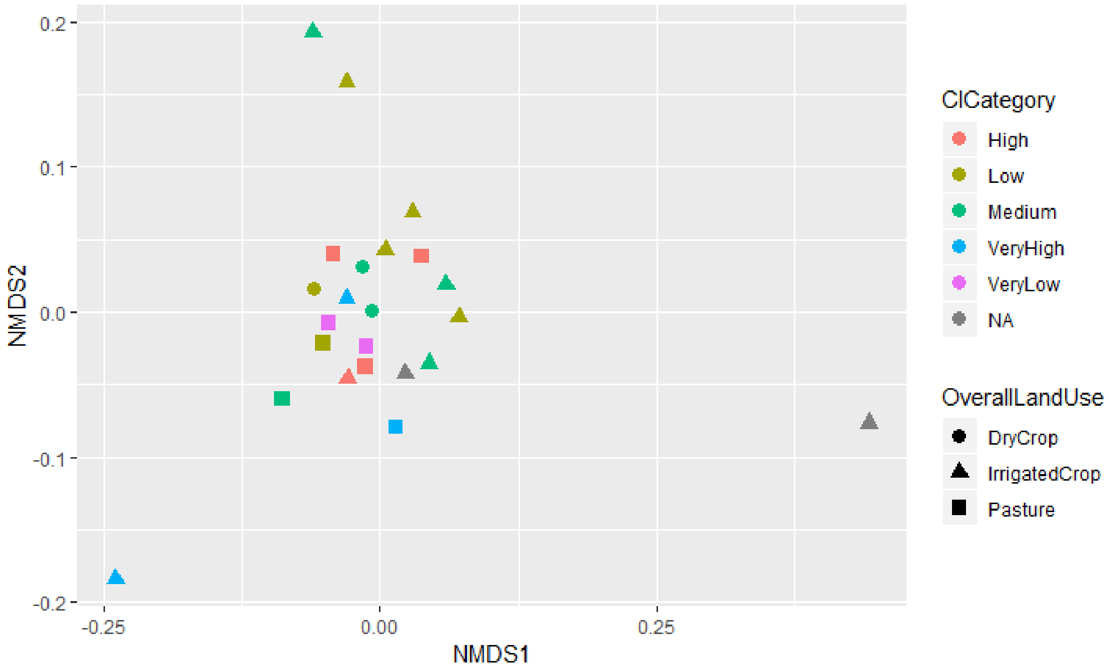
0.2
0.1
0.0
-0.1
NMDS2
-0.25
0.00
0.25
NMDS1
Na
6000
4000
2000
WellType
Bedrock
DeepAquifer
ShallowAquifer



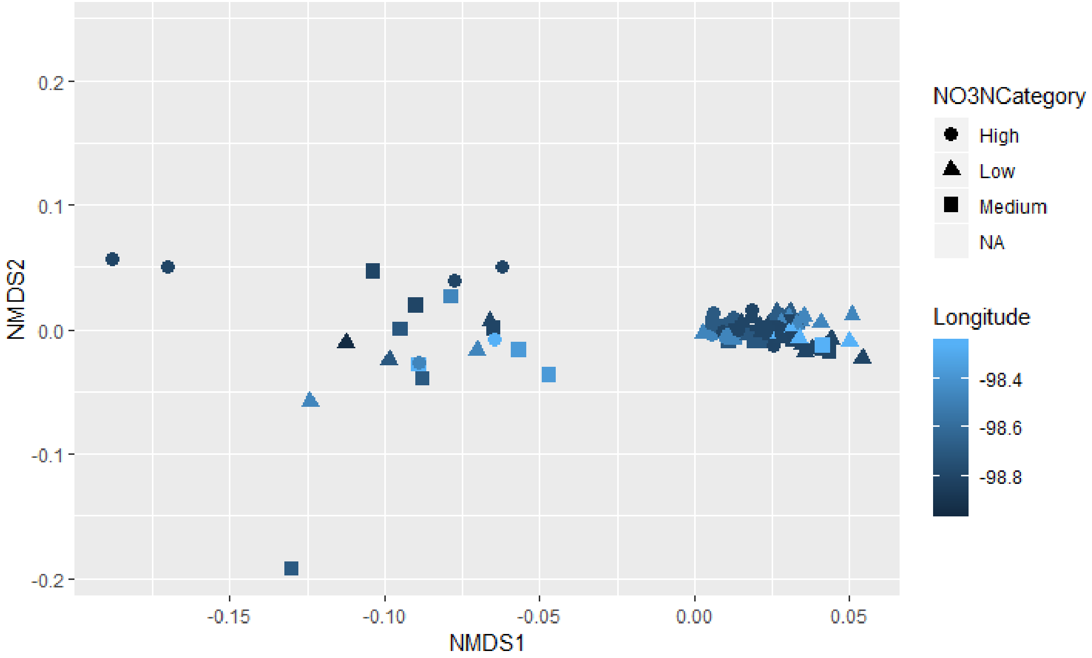
Groundwater NMDS Plots: Microbial Communities with Categories

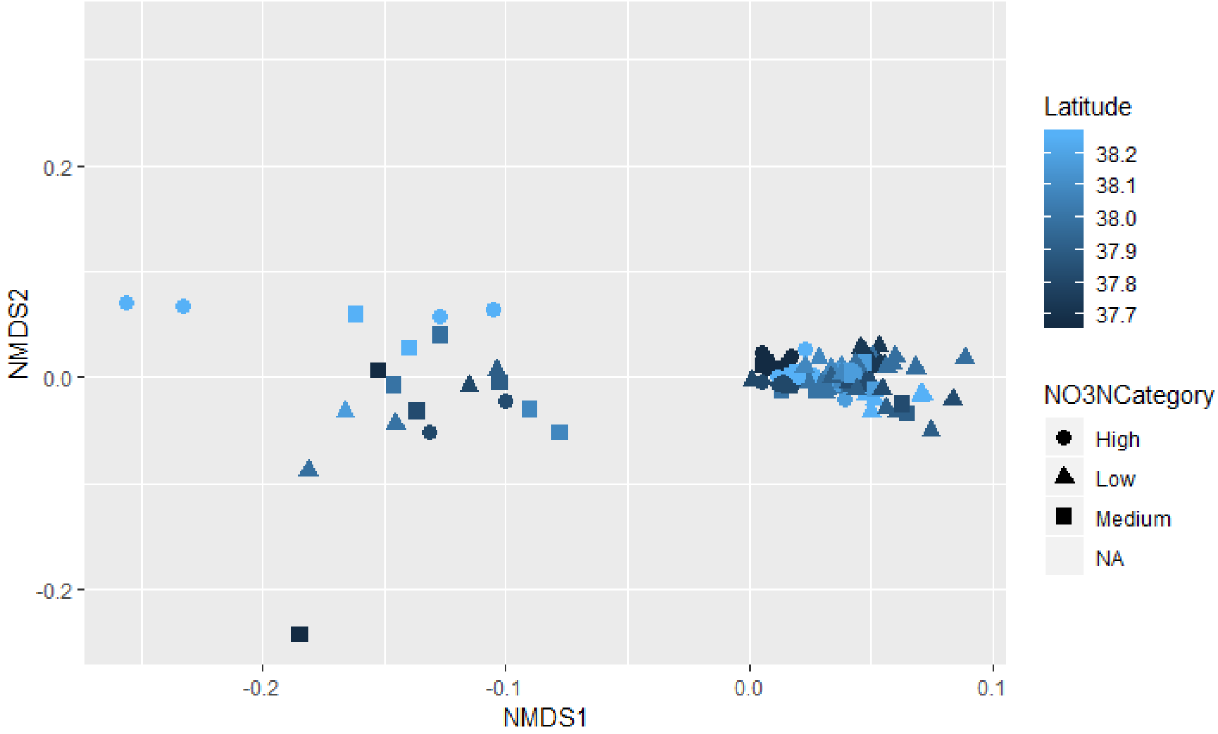


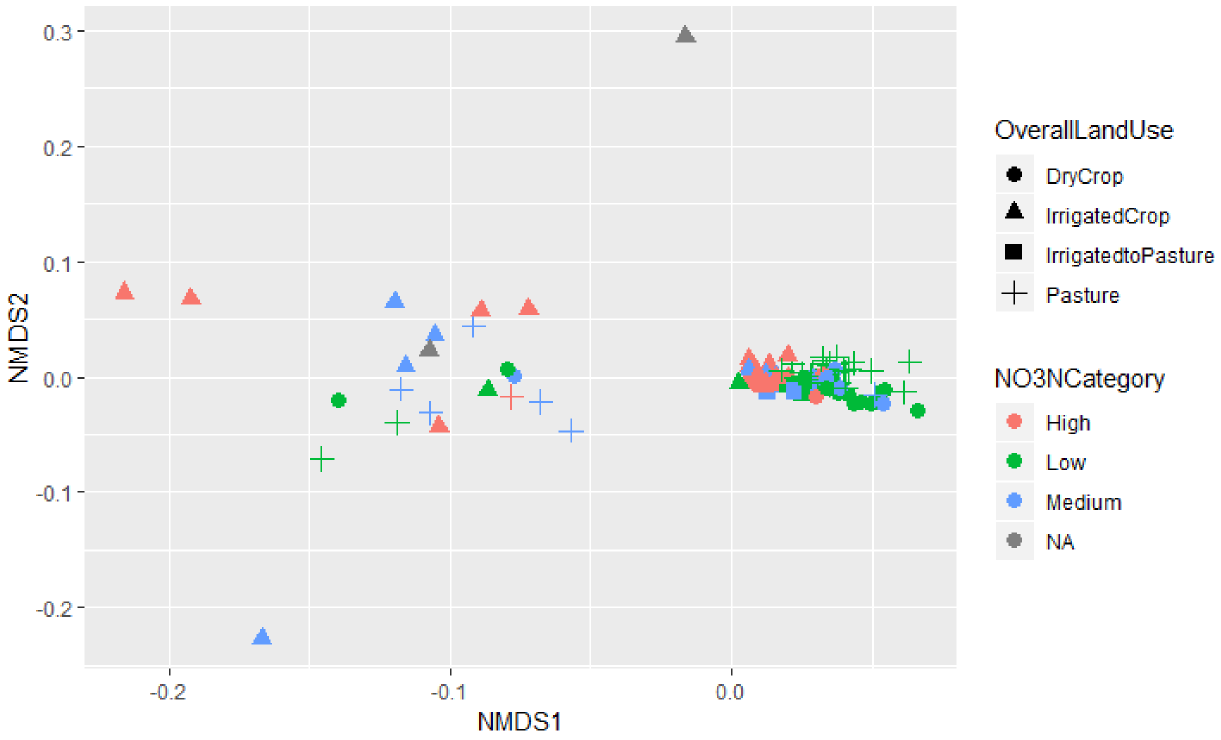

NMDS2
0.1
0.0
-0.1
-0.2
0.0
0.2
NMDS1
OverallLandUse
DryCrop
IrrigatedCrop
Pasture
NO3NCategory
High
Low
Medium
NA


NMDS2
0.2
0.1
0.0
-0.1
-0.2
-0.25
0.00
0.25
NMDS1
ClCategory
High
Low
Medium
VeryHigh
VeryLow
NA
OverallLandUse
DryCrop
IrrigatedCrop
Pasture

All Microbial Community NMDS Plots



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0.1
0.0
-0.1
-0.2
NMDS2
-0.2
-0.1
0.0
NMDS1
OverallLandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
NO3NCategory
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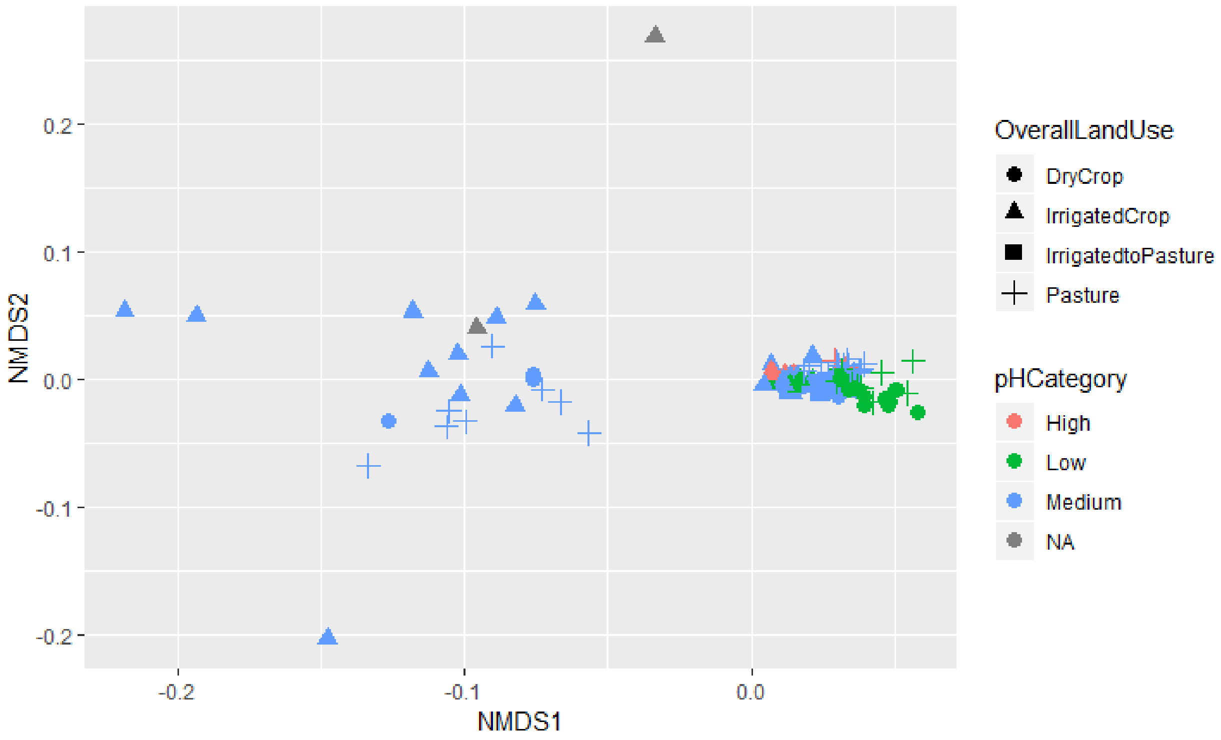
Low
Medium
NA


0.2
0.1
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-0.1
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NMDS2
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0.0
NMDS1
OverallLandUse
DryCrop
IrrigatedCrop
IrrigatedtoPasture
Pasture
pHCategory
High
Low
Medium
NA

Appendix G - Summary of Pilot Study in 2016

Methods

Groundwater Sampling

Groundwater samples from monitoring wells spread throughout GMD 5 were collected by Richard in the summer of 2016 (Richard 2018). The twenty-four wells are located at the following thirteen sites: 3, 6, 10, 21, 29, 34, 35, 36, 42, 50, 51, 52, and BB8 (Figure 3-1). Thirteen of the sampled wells, with an average depth of 72.2 ft, are screened in the upper aquifer; ten wells are screened in the aquifer base, with an average depth of 141.1 ft; and the final well, screened in the underlying bedrock at a depth of 54.1 ft, was thought to be an upper aquifer well. Sample collection methods were similar to those used in this study, with two main exceptions: a single stage submersible DC pump (Geotech SS Geosub) was used to extract groundwater and dissolved oxygen (DO) levels were measured in the field with a dissolved oxygen test kit (LaMotte).

Laboratory Analysis

Groundwater samples collected in 2016 were analyzed similarly to the 2018 samples, with some additional tests. Groundwater pH, temperature, and electrical conductivity were measured slightly differently in the field, in that 2016 data was recorded after three stabilized measurements compared to five in 2018. Alkalinity and concentration of anions and cations for both sets of samples were measured with the same protocols and instruments. Samples from 2016 were also analyzed for trace elements by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in the Department of Biochemistry's Redox Biology Center at the University of

Nebraska-Lincoln. Finally, stable nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios of nitrate were measured for all 2016 samples by the Environmental Isotope Laboratory in the Department of Earth and Environmental Sciences at the University of Waterloo on a Trace Gas-GVI IsoPrime-Isoptope Ratio Mass Spectrometer (TG-IRMS).

Groundwater geochemistry and microbial samples collected in summer 2016 (Richard 2018) allowed for a statistically viable data pool in our study. DNA extraction was performed by Richard in the Geomicrobiology lab, following the Qiagen DNeasy® PowerSoil® Kit protocol, and samples were then sent to MR. DNA lab for amplification and sequencing. The sequencing results were provided by MR. DNA lab in the form of FastA, quality, and mapping files. We processed these samples in QIIME (Caporaso et al. 2010) with most of the same steps described above. The primary modification in the protocol was that we did not have to join the forward and reverse Illumina reads, as they were already joined in the raw data files provided. The other slight modification because of the type of data files provided was a variation of the command to split sequences into their sample groups based on the barcodes in the mapping file (script: split_libraries.py).

Results

Groundwater Geochemistry

In 2016, samples from the deeper portion of the aquifer had the higher temperature and pH, averaged at 17.1º C and 7.42 respectively. Shallow groundwater samples, in contrast, had the lower average values of temperature (16. 4º C) and pH (7.30). Average Total Dissolved Solids (TDS) content was calculated as the sum of major ion concentrations. Differences in TDS content are primarily a result of variation in chloride and sodium concentrations. TDS levels averaged 509 ± 299 mg/L and 4,155 ± 7,138 mg/L in the shallow and deep groundwater samples, respectively. Comparatively, the sample from the bedrock portion of the aquifer had higher temperature (22. 4º C) and pH (7.58) values and lower TDS content (222 mg/L) than the other samples.

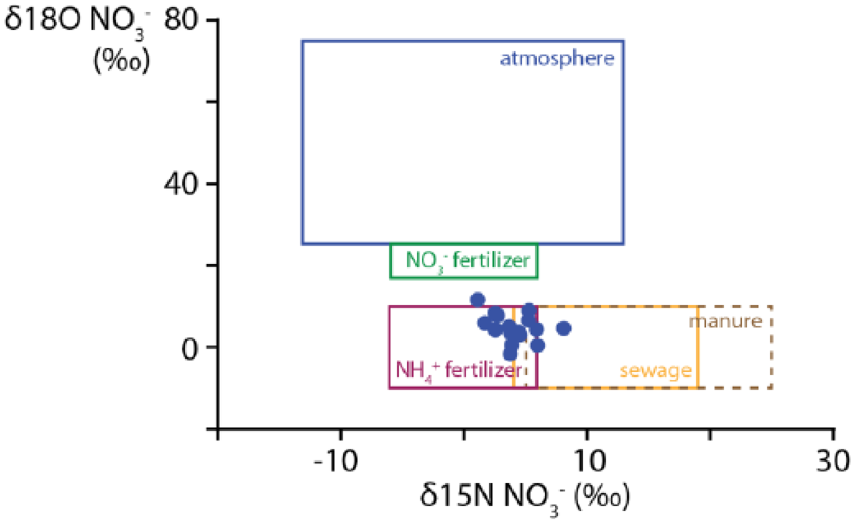
Dissolved constituent concentrations (mg/L) including chloride, sodium, bromide, sulfate, magnesium, and calcium were highest in the deep groundwater samples on average. In the shallow groundwater samples, chloride concentrations ranged from 5.1 to 427 mg/L and sodium concentrations ranged from 11.7 to 410.7 mg/L. Chloride and sodium concentrations in the deep portion of the aquifer ranged from 35.5 to 12,958 mg/L and from 40 to 7,816 mg/L, respectively.

Ammonium and nitrite concentrations were below detection limits (see Appendix C) for most samples. However, nitrate concentrations ranged from 0.5 to 52.3 mg/L as N in the shallow groundwater and 1.6 to 10.8 mg/L as N in the deep groundwater. Twenty of twenty-four samples increased in nitrate concentrations between Whittemore's 1970-1980 measurements and Richard's 2016 measurements. Nitrate concentrations surpassed the EPA standard for public drinking water (10 mg/L as N) in seven samples, of which six were collected from the shallow aquifer.

Most of the collected samples contained trace element concentrations below EPA drinking water standards. Lead concentrations were the exception, with 21 of 24 samples exceeding the EPA action level of 15 µg/L. The well 10D sample had a uranium concentration of 61.16 µg/L, above the EPA specified Maximum Contaminate Level (MCL) of 30 µg/L. The sample collected from 21C had a barium concentration of 2.46 mg/L (MCL = 2 mg/L). Manganese concentrations in samples from 10C, 26B, 42B, 50B, and BB5HA were above the

EPA Secondary MCL (SMCL) of 0.05 mg/L. Iron in the 42C sample exceeded the SMCL of 0.3 mg/L.

Nitrate sources, such as atmospheric nitrogen, chemical fertilizers, and manure, vary in their nitrogen isotopic compositions. As a result, stable oxygen ($^{18}O/^{16}O$) and nitrogen ($^{15}N/^{14}N$) isotope ratios of nitrate can be used to identify sources of nitrate in groundwater (Xue et al. 2009). Most of the samples fell into the $\delta^{15}N$ range of 0 to +10‰ and the $\delta^{18}O$ range of -10 to +20‰ (Appendix G - 1). Nitrate oxidized from ammonium-based fertilizers, with sewage and manure contributions, typically falls within these ranges.



Appendix G - 1: Nitrate isotopes for 2016 groundwater samples from Richard, 2018.

Groundwater Microbiology

Groundwater samples were also analyzed for microbial communities. On average, the phylum *Proteobacteria* (39.9%) had the highest relative abundance for all wells. Many genera within *Proteobacteria* have the potential to be involved in nitrification, denitrification, iron oxidation, and sulfur reduction (Madigan et al. 2018). Other observed phyla containing genera capable of involvement in the nitrogen cycle were *Chloroflexi* (7.45%), *Planctomycetes* (6.98%), *Nitrospirae* (5.67%), and *Crenarchaeota* (3.4%). Some species of *Chloroflexi* have the potential to be involved in denitrification (Kawaichi et al. 2013). *Planctomycetes* contain genera with the potential to be involved in anaerobic ammonium oxidation (Falkiewicz-Dulik et al. 2014). *Nitrospirae* genera are known nitrifiers and some are capable of denitrification coupled with iron oxidation (Madigan et al. 2018). Finally, phylum *Crenarchaeota* contains species that are autotrophic ammonia oxidizing chemolithotrophs (Madigan et al. 2018).