Comparison of Potential Bioavailable Organic Carbon and Microbial Characterization of Two Carbon Amended Sites

Marian Georgette Alicea

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Mark A. Widdowson, chair

Andrea M. Dietrich

Amy J. Pruden-Bagchi

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Abstract

Enhanced Reductive Bioremediation (ERB) is a sustainable remediation technology for the in situ treatment of chlorinated solvent contamination in aquifers. However, monitoring efforts employed to measure performance metrics rely on inferences of the subsurface environment from water samples collected at monitoring wells, ignoring the microbial activity that occurs at the granular level of aquifer sediment. This study compared potential bioavailable organic carbon (PBOC) and microbial diversity of two ERB sites. A two-sample t-test and a one-way ANOVA test with Tukey's HSD were performed to show differences between ERB and non-ERB samples and their degree of variability at selected geospatial locations downgradient of ERB treatment. Non-parametric multidimensional scaling (MDS) with similarity analysis was performed along with other data visualization plots to show microbial diversity. At Tinker AFB, results from the t-test showed that the PBOC concentrations from the ERB samples were statistically significantly greater than the samples without treatment (95% confidence; p-value = 0.018). For Dover AFB, results from the ANOVA with Tukey's HSD showed that there is a significant difference between the samples (DV3) collected in the ERB treatment zone to all other samples upgradient and downgradient of the ERB treatment. MDS and similarity analysis performed on relative abundance results from the Illumina MiSeq Sequencing of 16S rRNA genes showed large similarities among the samples within each site and the only observed differences occurred when comparing any sample to DV3, nearest to treatment.

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General Audience Abstract

Enhanced Reductive Bioremediation (ERB) is a sustainable remediation technology for the treatment of chlorinated solvent contamination in groundwater within the subsurface. ERB acts as a stimulant of microbial communities to accelerate remediation. Current ways to measure the success of remediation technologies use water from monitoring wells and they ignore the microbial activity that occurs within the subsurface sediment, where the water is stored. This study compared potential bioavailable organic carbon (PBOC) and microbial diversity of two ERB sites. PBOC is the amount of carbon in sediment that is consumable by bacteria. Microbial diversity are the various communities of microscopic organisms present in a sediment sample. A two-sample t-test and a one-way ANOVA test with Tukey's HSD were performed to show differences between ERB and non-ERB samples and their degree of variability at selected geospatial locations downgradient of ERB treatment. A two-sample t-test determined if there is a statistical difference between two values. A one-way ANOVA test compared multiple values to each other and all their possible combinations. The Tukey's HSD showed how different those values were from each other, from the ANOVA test results. Non-parametric multidimensional scaling (MDS) with similarity analysis was performed along with other data visualization plots to show microbial diversity. These visualization techniques helped determine similarities and demostrate microbial diversity. At Tinker AFB, results from the t-test showed that the PBOC concentrations from the ERB samples were statistically significantly greater than the samples without treatment (95% confidence; p-value = 0.018). For Dover AFB, results from the ANOVA with Tukey's HSD showed that there is a significant difference between the samples (DV3) collected in the ERB treatment zone to all other samples upgradient and downgradient of the ERB treatment. MDS and similarity analysis performed on relative abundance results from the Illumina MiSeq Sequencing of 16S rRNA genes showed large similarities among the samples within each site and the only observed differences occurred when comparing any sample to DV3, nearest to treatment.

Dedication

For my dearly departed and always in my heart mamá, Maria Georgina Pérez Dávila. All am and all I'll be; you are the air I breathe.

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Chapter 1. Introduction

Groundwater is a renewable resource, but chemical contamination can render an aquifer unusable for human activities and consumption without costly treatment. Chemical contaminants can potentially percolate into an aquifer via storm water runoff, irrigation, or improper disposal and dumping, among others (Fitts 2002). While soil can serve as a natural filter and can keep an aquifer from becoming contaminated, there are numerous instances in which chemicals bypass this natural filtration process and accumulate in groundwater resources and aquifers. One source of these contaminants are dense non-aqueous phase liquids (DNAPLs). DNAPLs are characterized as immiscible fluids in water, yet the solubility of individual components of DNAPLs are typically higher than that allowed by drinking water standards (Fitts 2002).

With densities greater than that of water, DNAPLs can move vertically though the subsurface and penetrate deeply into an aquifer. When introduced into the subsurface environment, these DNAPLs descend through the subsurface following the path of least resistance until they reach an impervious layer, at which point they will accumulate and pool (Berkowitz et al. 2014). Figure 1-1 illustrates what happens when a DNAPL is improperly disposed of or a leak occurs. The immiscible nature of DNAPLs allows unimpeded travel directly into aquifers with minimal disruption from sorption during transport (Stroo and Ward 2010). This migration of DNAPLs continues until the substance reaches an impervious layer. Over time, DNAPLs settle at the bottom of aquifers where they remain as permanent contaminants of groundwater (Lee et al. 2007).

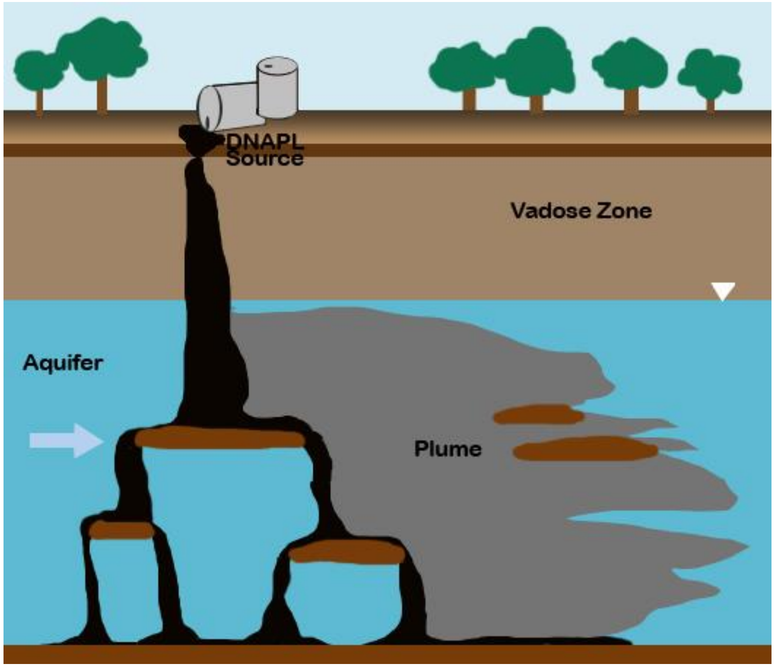

Figure 1-1. Likely DNAPL behavior in an aquifer following a spill, after Stroo and Ward 2010.

1.1 Chlorinated Ethenes

Chlorinated ethenes are typically derived from DNAPLs, and their fate and transport are largely dictated by their physical and chemical properties. Table 1-1 lists the main physical and chemical properties of chlorinated ethenes of interest.

Table 1-1. Physical and chemical properties of chlorinated ethenes of interest and their daughter products at 25°C and 1 atm (Stroo and Ward 2010; USEPA 2003).

Chlorinated Compound	Density (g/cm^3)	K_H (atm/M)	Water solubility (mg/L)	Octanol Partition Coefficient (log K_{ow})	MCL (mg/L)
Tetrachloroethene (PCE)	1.62	27	150	3.4	0.005
Trichloroethene (TCE)	1.46	12	1,100	2.42	0.005
cis-dichloroethene (*cis*-DCE)	1.28	7.4	3,500	1.85	0.07
trans-dichloroethene (*trans*-DCE)	1.26	6.7	6,300	2.09	0.1
Vinyl Chloride (VC)	0.91	22	2,700	1.62	0.002

Chlorinated ethenes are characterized by having a double-bonded carbon center with at most two hydrogen substituents per carbon as seen in Figure 1-2 (Stroo and Ward 2010). Chlorinated ethenes have been widely used as solvents for many industrial purposes since the early 20th century, for activities such as dry cleaning, paint-stripping, and metal degreasing (Lee et al. 2007). Dry cleaners have been the major culprit of contamination of chlorinated ethenes in the United States (Ruder et al. 2001). The low flammability of chlorinated solvents allowed dry cleaning locations to become wide-spread in towns and cities (Sinsheimer et al. 2002). Furthermore, improper disposal of these solvents has caused widespread chlorinated ethene contamination in aquifers (Bradley 2000). The aerospace industry and electronics manufacturing are also industries that rely heavily on chlorinated solvents for degreasing (Jendrzejewski et al. 2001). Over time, degreasing activities became a major source of groundwater contamination from chlorinated ethenes since disposing of degreasing sludge often consisted of pouring it onto dry wells and allowing it to evaporate, but most of it ended up leaching into groundwater resources (Jackson and Dwarakanath 1999).

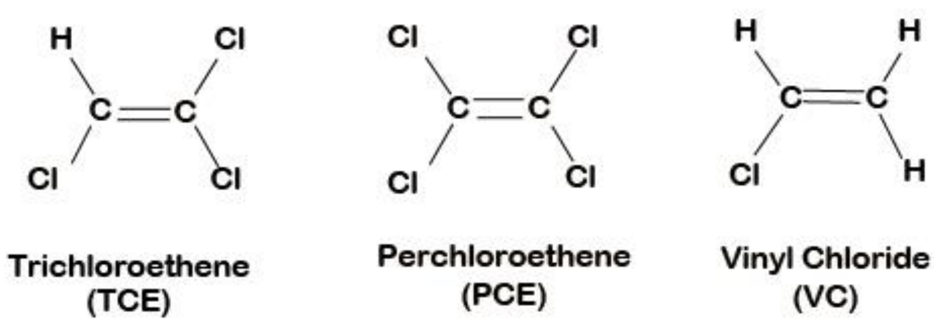

Figure 1-2. Chemical structures of chlorinated ethenes of interest.

PCE and TCE are known to cause severe negative human health and environmental impacts. Chlorinated ethenes have been identified as central nervous system disruptors and have been found to induce cancer in the liver and kidneys (Seo et al. 2011). The International Agency for Research on Cancer has classified PCE as a Group 2A Carcinogen (Ruder et al. 2001). Recent studies have found that both PCE and TCE are suspected human carcinogens (Christensen et al. 2013). High cancer mortality rates among dry clean workers has been documented, among numerous other health complications directly attributed to long-term exposure of chlorinated ethenes (Ruder et al. 2001).

PCE and TCE are difficult to remediate in the subsurface due to their recalcitrant nature and origin in DNAPL sources. As shown in Table 1-1, the solubility of PCE and TCE is much greater than the maximum contaminant levels (MCL) permitted in drinking water (USEPA 2003). These values will become more relevant when characterizing study sites in the methods section.

1.2 Bioremediation

Bioremediation is an engineered approach for cleaning up contaminated groundwater sites, including chlorinated ethenes. Bioremediation is the process where microorganisms consume compounds as a substrate and subsequently convert contaminants into innocuous byproducts (Stroo and Ward 2010). This natural attenuation of chlorinated ethenes is catalyzed by microorganisms under strongly reducing environmental conditions given an abundant source of electron donors (Bedard 2003). Several reaction pathways involving different bacteria genera are capable of reductively dechlorinating chlorinated ethenes, but these all depend on subsurface redox reactions (Kotik et al. 2013). In strict anaerobic environments, or in the absence of dissolved oxygen (DO), certain microorganisms utilize chlorinated ethenes as terminal electron acceptors in a process called chlororespiration. Strongly-reducing redox conditions highly favor reductive dechlorination (Maymó-Gatell et al. 2001). Reductive dechlorinating pathways are illustrated in Figure 1-3. This stepwise process replaces one chlorine atom at a time with a hydrogen atom as shown in Figure 1-3 (Griffin et al. 2004). At present, *Dehalococcoides spp.* are the only microbes found to degrade PCE, TCE, and *cis*-DCE into non-toxic ethene under strongly-reducing anaerobic conditions (Maymó-Gatell et al. 2001). Other bacteria populations have been found to degrade PCE or TCE through chlororespiration, but only to cis-DCE as the end-product (Griffin et al. 2004).

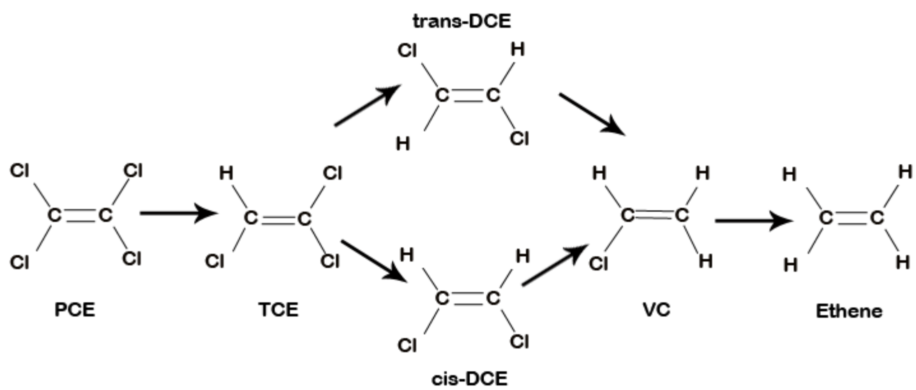

Figure 1-3. Reductive dechlorination pathways by *Dehalococcoides spp.*, (e.g., Loffler and Edwards 2006 and others).

Table 1-2 summarizes these known microorganisms. However, incomplete reductive dechlorination leaves toxic chlorinated substrates in the subsurface. Furthermore, the presence of *Dehalococcoides spp.* alone does not guarantee bioremediation since PCE and TCE are stable in anaerobic conditions. An electron donor is often needed to begin and maintain the reductive dechlorinating bacterial process. In general, complete reductive dechlorination of chlorinated ethenes can be attributed to a collective of microbial communities rather than just one species. Table 1-2 summarized microbes of interest and what is known about reductive dechlorinators (Griffin et al. 2004). Aulenta *et al*. and Lien *et al*. (Aulenta et al. 2006; Lien et al. 2016) provide additional information on species rank for reductive dechlorinators.

Table 1-2. Known bacteria genera responsible for reductive dechlorination and their end-products (Bedard 2003; Griffin et al. 2004; Sharma and McCarty 1996; Sung et al. 2003; Ye et al. 2010).

Contaminant	**End-Product**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**e^- donor**
PCE	TCE	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfitobacterium	Hydrogen
PCE/TCE	Ethene	Chloroflexi	Dehalococcoidetes	Dehalococcoidales	Dehalococcoidaceae	Dehalococcoides	Hydrogen
PCE/TCE	cis-DCE	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Dehalobacter	Hydrogen
PCE/TCE	cis-DCE	Proteo-bacteria	Delta-proteobacteria	Desulfuromonadales	Desulfuromonadaceae	Desulfuromonas	Acetate
PCE/TCE	cis-DCE	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Hydrogen, Lactate, and Pyruvate
PCE/TCE	cis-DCE	Proteo-bacteria	Gamma-proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Acetate and yeast extract
PCE/TCE	cis-DCE	Proteo-bacteria	Epsilon-proteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	Pyruvate, Formate, and Methyl Viologen hydrate

During reductive dechlorination, chlorinated ethenes are utilized as electron acceptors during anaerobic chlororespiration (Häggblom and Bossert 2003). An anaerobic environment is the first requirement in degrading chlorinated ethenes. Under these conditions, fermenting bacteria utilize readily available organic compounds and can produce acetate, lactate, carbon dioxide, and dissolved hydrogen gas as a by-product (Borden and Rodriguez 2006). These by-products then become electron donors for dechlorinating bacteria as seen on Table 1-2. Given that ideal dehalogenators (Table 1-2) utilize dissolved hydrogen as their electron donor, the universal electron donor, they compete to some degree in anaerobic conditions with other hydrogenotrophs (Ferguson and Pietari 2000). In groundwater systems, dehalogenators must compete with nitrate-reducers, manganese(IV)-reducers, Fe(III)-reducers, sulfate reducers, autotrophic methanogens, and homoacetogens (Chapelle 2001). Studies have shown that chlororespitators more favorably uptake hydrogen and can outcompete methanogens under highly reducing conditions (Aulenta et al. 2006; Löffler et al. 1999). Strongly reducing conditions, a fermentable carbon source, and the right dehalogenating microbial communities must all be present for the success of complete anaerobic reductive dechlorination through natural attenuation.

1.3 Enhanced Reductive Bioremediation (ERB)

Biostimulation is a key component to achieving *in situ* anaerobic bioremediation of chlorinated ethenes. Biostimulation of chlorinated ethenes involves the addition of an electron donor into the subsurface to stimulate bioremediation (Stroo and Ward 2010). In the case of chlorinated ethenes, enhanced reductive bioremediation (ERB) is the delivery of a fermentable organic carbon substrate that would lead to the production of dissolved hydrogen, the electron donor required by *Dehalococcoides spp.* for complete reductive dechlorination. Any organic compound that can be fermented into hydrogen and acetate can potentially be used as a substrate for stimulating reductive dechlorination (Borden and Rodriguez 2006). Furthermore, the fermentation process itself can further consume DO, thus optimizing reducing condition for reductive dechlorinators.

Carbon amendment injections have become a common practice at chlorinated sites where natural sources of bioavailable organic carbon are insufficient for effective and sustained rates of reductive dechlorination. Ideal carbon amendments should be easily fermentable and these can be alcohols, low-molecular-weight fatty acids, carbohydrates, vegetable oils, and plant debris (Stroo and Ward 2010). Patil *et al.* demonstrated complete reductive dechlorination of PCE to ethene in their study utilizing acetate as a carbon amendment (Patil et al. 2014). In another study, Pérez-de-Mora *et al.* assessed changes in dechlorinating and non-dechlorinating bacteria biostimulated with ethanol. The results showed decreased concentration of chlorinated ethenes and only a moderate increase in ethene (Pérez-de-Mora et al. 2014). The addition of acetate in an RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) contaminated site showed reduction of contaminants and increased reducing conditions at monitoring wells downgradient of treatment (Livermore et al. 2013). Overall, it has been found that the success of complete TCE dechlorination can be critically dependent on biostimulant selection (McLean et al. 2015).

In spite of groundwater flow, bacteria do not travel very far because they have an affinity to adhere to mineral surfaces and this can be further augmented by cell-to-cell adhesion (Stroo and Ward 2010). As such, stimulating bacterial growth through ERB can create biofilms at or near the carbon injection sites causing clogs in the delivery system, and even slowing down the travel distance of the added carbon treatment (Stroo and Ward 2010). The increase in microbial density that occurs can create more competition for fermenting bacteria and as a result more competition for dechlorinators. While reductive dechlorinators can outcompete other microbial groups in strongly reducing conditions, they are outperformed for H_2 before those conditions are achieved (Aulenta et al. 2006). One way to combat competition is to utilize slow-fermenting sources of H_2 such as edible oils, decaying biomass, peat, mulch, etc. After DO depletion, these slow fermenting carbon sources will still remain in the subsurface and will continue to support reductive dechlorination (Aulenta et al. 2006).

Identification of optimal slow-fermenting H_2 sources to induce and support reductive dechlorination has been ongoing. A study evaluating different “food-grade organic substrates” was conducted where hydrogen and acetate biogas production were used as indicators for substrate fermentation, thus producing ideal reductive dechlorinating conditions (Borden and Rodriguez 2006). In that same study, emulsified vegetable oil (EVO) was found to support continued biogas production for a period of over 14 months (Borden and Rodriguez 2006). EVO, with its smaller droplets dissolved in water, can be readily distributed throughout an aquifer and various porous media (Borden 2007). Studies show that EVO injections into the subsurface have resulted in accelerated detoxification of TCE and PCE into ethene (Borden 2007; Long and Borden 2006). Because of its relative insolubility, injection of EVO into the groundwater results in a significant, slow-release organic carbon source that stimulates PCE and TCE degradation. The EVO injection technique is preferred over other carbon substrates due to its relatively immobile and slow biodegradation characteristics (Borden and Rodriguez 2006). A pilot study using direct-push EVO injections over a 2-day period was monitored for DO depletion and the increased presence of chlorinated ethenes daughter products not from source contamination. Results showed strongly reducing conditions two years following the treatment, supporting EVO as an ideal substrate for reductive dechlorination (Borden 2007).

While ERB has become routine, one significant limitation has been observed at chlorinated sites. According to Tillotson and Borden, organic carbon does not travel more than 10 meters downgradient, and at sites with more permeable media, total organic carbon (TOC) was found up to 25 meters downgradient (Tillotson and Borden 2015). Whether reductive dechlorination through biostimulation is employed as a primary remediation strategy or considered as a transition technology prior to employing a more passive approach (e.g., Monitored Natural Attenuation, MNA), decision making is hindered by data limitations to determine when such transitions can take place.

1.4 Metrics for Enhanced Reductive Bioremediation (ERB)

Short-term sustainability of ERB has been demonstrated by evaluating groundwater constituents collected from monitoring wells. Currently, TOC and DO concentrations (elevated and depleted levels, respectively) along with the increased presence of chlorinated ethene-daughter products not from source contamination are the most utilized metrics to assess the effectiveness of ERB (Bradley and Chapelle 2007). Monitoring wells are often installed to evaluate the extent of contamination through source characterization and to assess the long-term effectiveness of a treatment (Stroo and Ward 2010). However, measuring daughter products of chlorinated ethenes only describes the chemical quality of groundwater and measuring DO only gives a suggestive presence of an organic carbon source that is promoting reducing conditions (Borden 2007; Jendrzejewski et al. 2001). These parameters alone are not indicative of the consumption and availability of carbon for microbial processes (Bradley and Chapelle 2007; Chapelle et al. 2012).

Using groundwater TOC as a measurement is indicative of substrate availability for reductive dechlorination. However, TOC only provides a measure of dissolved organic carbon and does not provide any information about dissolved bioavailable organic carbon, nor does it account for any organic carbon in the soil, biodegradable or otherwise (Chapelle et al. 2009). Natural organic carbon can contain a varying multitude of complex chemical structures and a wide range of uptake rates by microorganisms, or bioavailability (Chapelle et al. 2009). Furthermore, studies have shown that the products of biomass decay are efficient in contributing to reductive dechlorination beyond that of carbon amendment treatments (Chapelle et al. 2007; Yang and McCarty 2000). In attempts to provide information regarding the bioavailability of organic carbon present in groundwater, a study utilizing total hydrolysable neutral sugars (THNS) and total hydrolysable amino acids (THAA) as biochemical indicators confirmed that bioavailable organic carbon cannot be determined by TOC measures alone (Chapelle et al. 2009).

1.5 Potentially Bioavailable Organic Carbon (PBOC)

Quantifying organic carbon in sediment requires combustion related processes or measurement of organic carbon in chemical extractions. However, combustion techniques do not distinguish among carbon fractions without pre-treatment. Chemical methods using mild extractants can remove organic carbon from mineral surfaces. Potentially bioavailable organic carbon (PBOC) is a 5-step chemical extraction process that measures the sorbed organic carbon utilized as an electron donor for reductive dechlorination (Rectanus et al. 2007). Rectanus *et al.* demonstrated a method for evaluating carbon present in aquifer sediment readily available to support reductive dechlorination (Rectanus et al. 2007). Thomas *et al.* further evaluated the relationship between PBOC and hydrolysable amino acids (HAA) and demonstrated a positive relationship between the two in aquifer sediment. Because bioavailable carbon is defined as more hydrolysable form of carbohydrates and amino acids, microorganisms have shown to more easily uptake these forms of carbon over bulk organic carbons (Thomas et al. 2012). Chapelle *et al*. further demonstrated the positive correlation between PBOC and reductive dechlorination on 15 different sites contaminated with chlorinated ethenes not treated with an anthropogenic carbon source. Using PBOC, TOC, and water chemistry data, the study approximated that a 200 mg/kg concentration of PBOC is required to initiate and sustain reductive dechlorination (Chapelle et al. 2012). PBOC was utilized at another study to support the hypothesis of bioavailable organic carbon depletion at chloroethene-contaminated sites without anthropogenic carbon sources where monitored natural attenuation is the primary remediation strategy (Thomas et al. 2013). These findings suggest that PBOC is a promising and low cost metric for evaluating short-term and long-term sustainability of ERB.

1.6 Microbial Molecular Tools

Combining microbial molecular tools and analytical approaches can help in the improvement of ERB strategies. These microbial molecular tools utilize the 16s ribosomal RNA genes as a fingerprint of microbial types. Denaturing Gradient Gel Electrophoresis (DGGE) and Real-Time Quantitative Polymerase Chain Reaction (qPCR) can both aid in the monitoring of specific organisms (Adetutu et al. 2015). DGGE and qPCR allow identification and quantification of specific organisms such as known dechlorinators (Griffin et al. 2004; Hunkeler et al. 2011; Mattes et al. 2015). *Dehalococcoides spp.* can be used as a biomarker to evaluate the efficacy of reductive dechlorination (Lien et al. 2016). DGGE requires a database of known genes and provides a closest relative species. However, DGGE has limitations for reproducibility when more than 10 microbe species are of interest (Patil et al. 2014). Another microbial molecular tool, qPCR, requires that target microbial species be identified (Cupples 2008). The qPCR method demands time consuming calibration curves to quantify specific species and this process can be very costly (Adetutu et al. 2015).

More promising are Next Generation Sequencing tools which can be used as exploratory tools, to identify relative abundance of microbial taxa, and for more in-depth taxonomic classification of thousands and even millions of organisms simultaneously depending on the method; these can also identify even low-frequency variants (Caporaso et al. 2011). 454-pyrosequencing has been applied to determine the microbial composition of groundwater at chloroethene contaminated sites (Kotik et al. 2013). The Illumina GAIIx platform has demonstrated superiority in the next generation sequencing tools with the ability to run millions of sequences per run as opposed to PhyloChip and 454 pyrosequencing that can run thousands of sequences (Caporaso et al. 2011).

ERB is not a targeted approach and adding carbon to the subsurface environment stimulates more than just reductive dechlorination. ERB studies have reported greater species richness and diversity in carbon amended samples than those without. DNA concentrations of microbial communities are consistently higher in samples with carbon addition, and studies reported increase in targeted microbial communities as well as an increase in microbial communities as a whole (David et al. 2015; Jayamani and Cupples 2015). Studies have identified amplified microbial communities in samples with carbon addition and have also reported the abundance of different microbial species not present in samples without carbon amendments (David et al. 2015; Pérez-de-Mora et al. 2014; Singleton et al. 2013). A study comparing different types of carbon amendments and the microbial response at chlorinated sites found more variations in diversity of microbial communities among the different carbon substrate samples than those without any carbon addition (David et al. 2015). Studies comparing microbial communities over time also reported changing community structures even within the same sampling location (Lee et al. 2011; Livermore et al. 2013). More specifically, using 454 pyrosequencing, Livermore et al. (2013) reported a shift in dominant microbial communities over time from Betaproteobacteria to Deltaproteobacteria and Bacteroidetes taxa at a site a biostimulated site contaminated with explosives. By applying PhyloChip, Lee et al. (2011) identified abundance of Proteobacteria, Bacteroidetes, Firmicutes, and Chloroflexi phyla at an ERB treated chloroethene contaminated site. David et al. (2015) tested lab generated microcosms utilizing 454 pyrosequencing to demonstrate differences in microbial communities in reaction to varying organic substrates. They identified an increase in communities that produce electron donors for reductive dechlorination as well as an increase in microorganisms that compete with dechlorinators for electron donors (David et al. 2015). Any time a substrate is introduced into the subsurface to elicit a microbial response, utilizing whole genome based next generation microbial sequencing tools, along with other analytical tools, will only help to generate a better understanding of changes to aquifer microbial communities and dominant species.

1.7 Objectives & Hypotheses

The overall goal of this study is to advance methods to quantitatively assess the long-term sustainability of added organic carbon into aquifers to stimulate reductive dechlorination at chlorinated sites by addressing important knowledge gaps related to organic carbon availability and impacts to microbial communities. The hypotheses of this study are:

1. Subsurface injections of organic carbon (ERB treatment) result in elevated levels of PBOC in aquifer sediment samples collected in the immediate vicinity of injection points.

2. There is no statistically significant increase of PBOC in aquifer sediment beyond 10 to 25 meters downgradient of ERB treatment.
3. Differences in microbial communities are expected near carbon amendments relative to non-ERB treated aquifer sediment samples; however, impacts to microbial communities will not be significantly different downgradient of ERB treatment.

To test these hypotheses, aquifer sediment samples from two ERB sites contaminated with chlorinated solvents where evaluated using the PBOC extraction method. Illumina MiSeq, an exploratory microbial tool, was utilized to identify communities of microbes and their relative abundance at each site. Results were compared within each site, between ERB treatment and no treatment, and also between ERB treated samples and their geospatial location in relation to the treatment location.

Chapter 2. Materials and Methods

2.1 Site Background and Sample Collection

Aquifer sediment samples were collected from two study sites; Tinker Air Force Base (AFB) and Dover AFB. Both sites are characterized by having large chlorinated ethenes contaminated plumes. Both sites were also treated with an anthropogenic organic carbon substrate for ERB. For this study, samples from both sites include samples treated with organic carbon and not treated with organic carbon. Samples from Dover AFB were collected at different distances along the groundwater flow path in relation to the organic carbon treatment injections.

Tinker Air Force Base Site (Oklahoma City, OK)

Aquifer sediment was derived from soil core samples collected from Tinker AFB in Oklahoma by GSI Environmental Inc. (Houston, TX) in November 2014. Figure 2-1 shows the location of Tinker AFB. Chlorinated volatile organic compounds (CVOCs) are all contaminants of concern at Tinker AFB (EPA 2015). The circle in the picture within Figure 2-1 depicts the extent of the TCE contaminant plume, after the EPA Region 6 current status report (EPA 2015). Tinker AFB sits on a portion of the Central Oklahoma aquifer, a major aquifer that supplies drinking water to portions of the state of Oklahoma (Parsons 1998). Remediation strategies began in 1985 and have been ongoing since (Parsons 1998).

The precise location where the Tinker AFB samples were collected is unknown. The Tinker AFB samples are mostly fine-grained silty clay and clayey silt, suggesting relatively low permeability. The water table was found at 3-5 meters below ground surface (bgs.) and the samples were collected between 8 and 12 meters bgs. (GSI-Environmental 2012).

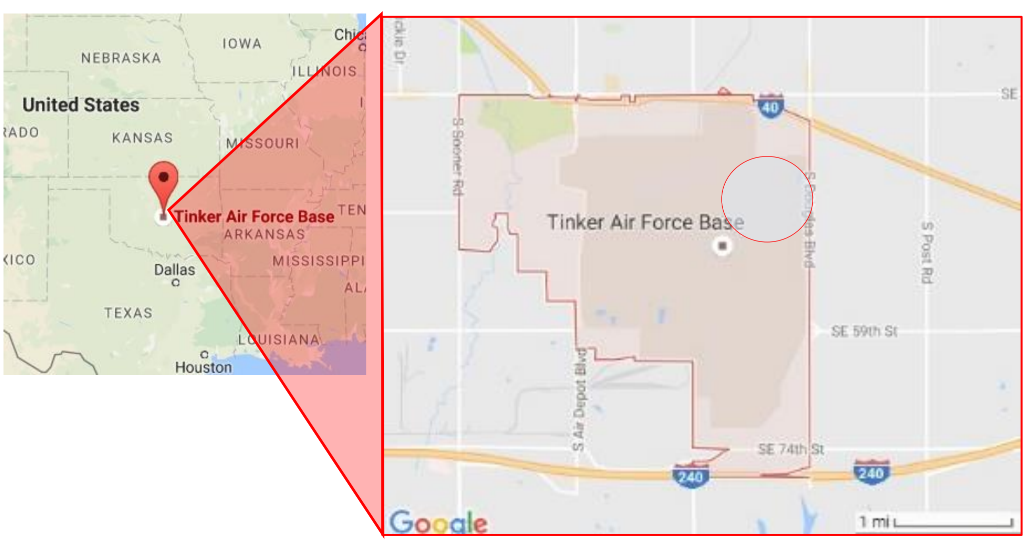

Figure 2-1. Aerial map of Tinker AFB location and approximate location of TCE contaminant plume in reference to property boundaries, represented by red circle (Google Maps & after EPA Current Status Report 2015).

Core samples were retrieved using a direct-push device from two boreholes, SBGSI-1 and SBGSI-4, at Tinker AFB. Samples were retrieved with a plastic liner; cores were cut into smaller segments still in the sleeves for a total of six segments at each borehole. Individual samples were quickly transferred into single clean half-liter glass jars. Sample jars were typically filled to capacity and capped without headspace. If groundwater was available from an adjacent well or during sample collection, groundwater was used to fill the void space/top off the sample jars. The soil/water ratio was at least 2:3 soil. All samples were shipped overnight and packed in ice. Sample jars were stored at 4°C without light immediately upon arrival to Durham Hall (Blacksburg, VA). Table 2-1 lists the samples from these two bore holes, as well as depth bgs., contaminants at site, and treatment. Core samples from SBGSI-1 are from an area treated with EVO as a carbon amendment and core samples from SBGSI-4 did not receive treatment. Treatment administration, duration, and date are unknown.

Table 2-1. Tinker AFB samples and known information provided by GSI Environmental.

Sample ID	**Sample Name**	**CVOC Exposure**	**EVO Addition**	**Depth (m bgs)**	**Average Depth (m bgs)**
TK131	SBGSI-1	Yes	Yes	9.5-9.6	9.55
TK133	SBGSI-1	Yes	Yes	9.6-10	9.8
TK135	SBGSI-1	Yes	Yes	10-10.7	10.35
TK136	SBGSI-1	Yes	Yes	10.7-11	10.85
TK136.5	SBGSI-1	Yes	Yes	11-11.6	11.3
TK138	SBGSI-1	Yes	Yes	11.6-12.2	11.9
TK426	SBGSI-4	Yes	No	8-8.5	8.25
TK428	SBGSI-4	Yes	No	8.5-9.1	8.8
TK430	SBGSI-4	Yes	No	9.1-9.6	9.35
TK431	SBGSI-4	Yes	No	9.6-10	9.8
TK433	SBGSI-4	Yes	No	10-10.7	10.35
TK435	SBGSI-4	Yes	No	10.7-11	10.85

Dover Air Force Base Site (Dover, DE)

Aquifer sediment was obtained from soil core samples that were collected from Dover Air Force AFB in Delaware by Solutions-IES (Raleigh, NC) in August 2014. Figure 2-2 shows the location of Dover AFB. CVOCs are all contaminants of concern at this site (Bloom 2006). Dover AFB sits on the Columbia Formation and is composed of silts and clay for the first 3 m. bgs. and becomes sandier with depth, it is 5.1-5.6 meters thick, with a clay and silt formation underneath acting as an aquitard (Bloom 2006). The water table can be found at 3-5 meter bgs. (Bloom 2006). Solutions-IES described the samples collected as originating from a shallow, surficial aquifer.

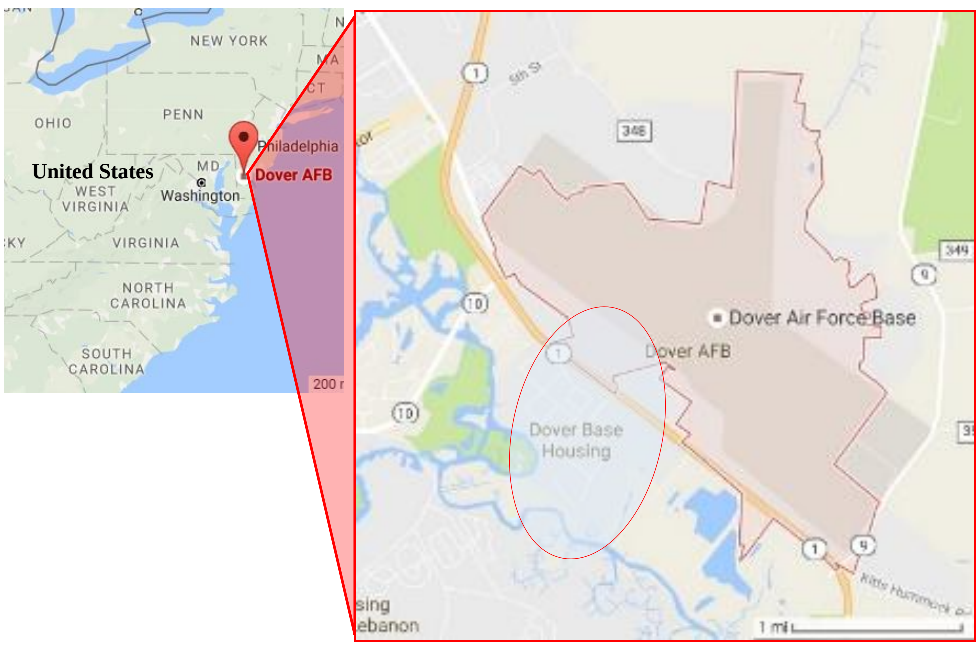

Figure 2-2. Aerial map of Dover AFB location and approximate location of TCE contaminant plume in reference to property boundaries, represented by red circle (Google Maps & after Bloom 2015).

The largest chlorinated ethene plume at Dover AFB was designated as Area 6 and is estimated at having dimensions of 700 meters wide and 1,830 meters long (Bloom 2015). Due to source migration, there is a heavily contaminated plume core within Area 6 with dimension of 290 meters wide and 1,219 meters long, and chlorinated ethene concentrations greater than 1,000 μg/L (Bloom 2015). The circle in the picture on the right within Figure 2-2 approximates the contaminant plume named Area 6 (Bloom 2015). The samples for this study were collected from within this more heavily contaminated plume core as well as outside of the Area 6 plume.

ERB was administered by installing rows of permanent wells perpendicular to groundwater flow, approximately 3 meters apart called plume injection/circulation transect (PICT), that alternate between injection wells, monitoring wells, and extraction wells (Bloom 2006; Bloom 2015). A combination of soluble sodium lactate and EVO substrates were added through a push and pull method, in which groundwater was pumped out of the aquifer, mixed with the substrate in a processing trailer above ground, and pumped right back into the aquifer (Bloom 2015). The treatment source for this study is known as PICT-3. PICT-3 is comprised of a linear system of 10-cm diameter wells permanently installed perpendicular to groundwater flow and it is part of a larger ERB remediation plan at Area 6 in Dover AFB. All geospatial references of samples for this study listed on Table 2-2 are show in reference to PICT-3. Four injection events were completed between 2006 and 2011 at PICT 3 prior to sample collection for this study (ORNL and URS 2011). The first injection event circulated groundwater for approximately 100 hours and each subsequent injection event circulated groundwater with the organic carbon substrate in the above ground processing trailer for 230 to 310 hours (ORNL and URS 2011).

Six core samples were retrieved using a direct-push device from Dover AFB at predetermined distances from PICT-3. One sample was collected upgradient of PICT-3, before treatment, and four samples were collected downgradient of PICT-3 along the contaminated plume at recorded distances listed on Table 2-2. One sample was collected from outside the contaminated plume at an undetermined location. All six samples were collected at an average of 11.4 meters bgs. Individual samples were quickly transferred into single half-liter polyethylene jars. Sample jars were typically filled 2/3 full. All samples were shipped overnight and packed in ice. Sample jars were stored at 4°C without light immediately upon arrival. Refer to Table 2-2 for samples, contaminant at site, treatment, distance from treatment, and time it took for the organic carbon to reach each sample location.

Table 2-2. Dover AFB samples and known information provided by Solutions IES.

Sample ID	**Contaminant**	**Distance from EVO Injection (m)**	**Upgradient or Downgradient**	**Travel Time** (yr.)**	**Organic Carbon Contact at sample collection?**
DV1	-	Background	Upgradient	-	-
DV2	CVOC	-47	Upgradient	-	-
DV3	CVOC	1	Downgradient	0	Yes
DV4	CVOC	10	Downgradient	0.22	Yes
DV5	CVOC	25	Downgradient	0.56	Yes
DV6	CVOC	50	Downgradient	1.12	Yes

*Groundwater velocity = 44.5 m/y (ORNL and URS 2011)
**Travel time = distance (m)/groundwater velocity (m/yr.)

The upgradient/downgradient values in Table 2-2 were assigned based on whether a sample was collected from a location before or after treatment at PICT-3, and in reference to known direction of groundwater flow as see on Figure 2-3 (ORNL and URS 2011). Travel time was calculated using the given distance from PICT-3 (m) and dividing by the groundwater velocity reported in the Monitoring Report for Area 6 (ORNL and URS 2011). Two groundwater velocities were referenced in the report, a shallow and a deep velocity. Since the collection depth of the sample was given as an average of 11.4 meters bgs., and since this value falls within the range of deep wells as listed on the report, the groundwater velocity for deep wells of 44.5 meters per year was used in this calculation (ORNL and URS 2011). Note that this groundwater velocity does not take into account the retardation factor from the added anthropogenic organic carbon. The last column on the right of Figure 2-2 answers the question of whether the aquifer sediment samples retrieved at each of these locations would have received the organic carbon addition at the time of sample collection (approximately 3 years after the last ERB treatment event). Since all travel times downgradient of PICT-3 are less than 3 years, they would have all had contact with the anthropogenic carbon source.

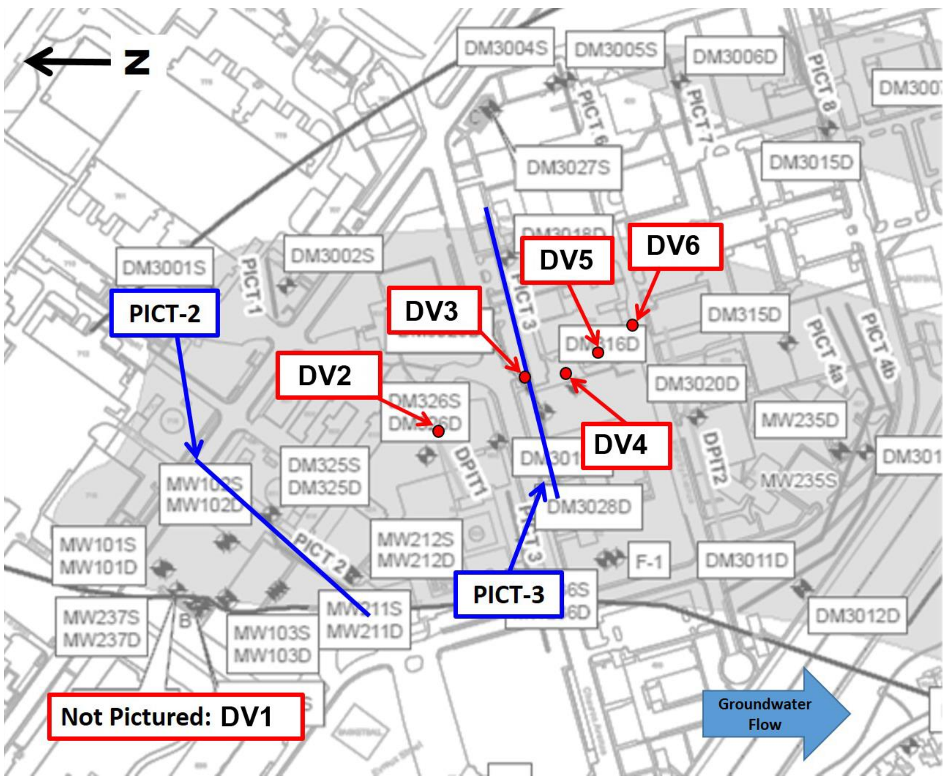

Figure 2-3. Map of samples from Dover AFB in reference to ERB treatment, PICT-3 (ORNL and URS 2011).

Figure 2-3 shows an approximate schematic of the DOVER AFB sample collection locations in reference to PICT-3. A site map of Area 6 including PICTs and monitoring wells can be found in Appendix L. Appendices M and N contain biological parameters and chlorinated ethene concentrations over time (2006-2011) in reference to the carbon injection events. Appendix M contains information on well DM3029D, one of the monitoring wells in PICT-3 and Appendix N has data on DM316D, a monitoring well located downgradient of PICT-3.

2.2 Experimental Methods

PBOC Extraction Method

The PBOC extraction method by Rectanus *et al*. (2007), was adapted to quantify the mass of extracted carbon per mass of aquifer sediment. This is a five-step chemical extraction process that measures the amount of sorbed organic carbon. Three technical replicates of each 5-step PBOC extraction were performed. An amount of 10 g of soil from each sample was dried at 70°C in a 24-hour period and sieved through 2mm openings to exclude any soil classified as gravel. Dried soil was combined with 20mL of the extractant in a 50mL sterile centrifuge tube. Two extraction solutions were used as part of the PBOC extraction method: 0.1% sodium pyrophosphate (pH 8.5, Crystalline/Certified ACS; Fisher Scientific, Pittsburgh, Pennsylvania) and 0.5 N sodium hydroxide (pH 13, Pellets/Certified ACS; Fisher Scientific, Pittsburgh, Pennsylvania). Both extractants were diluted with Nanopure (Barnstead, Dubuque, Iowa) water (pH 5) to achieve the desired concentrations as stated above. The five extractions were as follows:

- Extraction 1: 20mL of 0.1% sodium pyrophosphate dilution
- Extraction 2: 20mL of 0.1% sodium pyrophosphate dilution
- Extraction 3: 20mL of 0.1% sodium pyrophosphate dilution
- Extraction 4: 20mL of 0.5N sodium hydroxide dilution
- Extraction 5: 20mL of 0.1% sodium pyrophosphate dilution

Each extraction was followed by a 24-hour cycle in a rotary tumbler. Each sample was then centrifuged for 10-60 minutes at 2,000-6,000 rpm for solid separation. Time and speed on the centrifuge depended on the composition of the soil samples. Sandy soils required less time than samples with higher silt/clay compositions. The supernatant was decanted and stored at 4°C in carbon-free (Fisherbrand Sparkleen washed, acid washed, and baked at 400°C for 1 hour) 25mL glass vials per extraction for later analysis of total aqueous organic carbon (C_{TOC}).

Concentration of C_{TOC} were measured through combustion catalytic oxidation (Shimadzu TOC-VCSN, Columbia, Maryland), with a detection limit of 0.05 mg/L of carbon. The complete standard operating procedure (SOP) for the PBOC extraction method and the TOC analyzer Shimadzu TOC-VCSN is found in Appendices A and B, respectfully. This method shows how PBOC is a measurable solution for determining the amount of bioavailable organic carbon utilized as an electron donor source in microbial reductive dechlorination.

DNA Extraction, PCR Amplification, and Illumina MiSeq Sequencing

FastDNA Spin Kit for soil (MP Biomedicals in Solon, OH) was used to extract total genomic DNA as per the manufacturer's protocol (www.mpbio.com). The complete MPBio FastDNA SPIN Kit for Soil Protocol is found in Appendix C. To analyze the taxonomic composition of the aquifer sediment samples, the 16S rRNA genes were amplified for the three technical replicates from all DNA extractions with the universal barcoded primers 515F/806R31. Barcode primers can be found in Appendix Q. The well thermocycler conditions were as follows: initial denaturation at 94°C for 3 minutes, 35 cycles of 94 °C for 45 seconds, 50 °C for 60 seconds, 72° for 90 seconds, with a final extension at 72 °C for 10 minutes (Caporaso et al. 2011). Amplification protocol and primer sequences used are listed in Appendices D and E, respectfully.

Individual PCR product concentrations were measured using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Life Technologies, USA) as per manufacturer protocol (www.thermofisher.com). Complete Qubit dsDNA HS Assay Kit SOP can be found in Appendix F. PCR products were pooled and mixed proportionally with each other according to mass. Pooled PCR product was purified using QIAquick PCR purification kit (QIAGEN, GmbH, Germany) according to manufacturer's instructions (www.qiagen.com). SOP for the QIAquick PCR purification kit can be found in Appendix G. PCR pooled product was visualized on an agarose gel and was determined that another purification was warranted. A second purification was performed on the pooled PCR product using the Agencourt AMPure XP PCR purification (Beckman Coulter, USA) as per the manufacturer's instructions (www.beckmancoulter.com). This additional purification was performed by the Biocomplexity Institute of Virginia Tech (BI) (Blacksburg, VA, USA) (www.bi.vt.edu). Sequencing was done on an Illumina MiSeq benchtop sequencer using pair-end 250bp kits and was performed by BI.

2.3 Methods of Data Analyses

PBOC Concentrations

Due to instrument detection limits, TOC concentrations for PBOC concentration calculations were multiplied by the dilution factors used for the TOC analyzer before data processing. The mass of TOC (M_i) for each of the five extractions was found using the C_{TOCi} concentration measured in milligrams per liter (mg/L) of each extraction and the volume (V_i) of extractant added to each extraction measured in liters (L). PBOC was calculated using the sum of TOC mass for all five extractions and the original mass (M_S) of each soil sample added measured in kilograms (kg). The units of PBOC are in milligrams per kilograms (mg/kg). Equations 1 and 2 below summarize the PBOC equation (Thomas et al. 2013; Thomas et al. 2012).

$$M_i = C_{TOCi} \times V \quad (1)$$

$$PBOC\ \left({}^{mg}/_{kg}\right) = \sum M_i \times M_s \quad (2)$$

Appendix I contains all recorded experimental values and TOC concentrations for all five extractions per technical replicate of each sample. This information was used to apply the PBOC concentration equations listed above to find individual PBOC concentration per replicate of each sample.

Illumina Sequencing

Pan Ji, a doctoral student under Dr. Pruden, performed the initial data processing from the BI output. From personal communication with Pan Ji, PANDAseq (ref) was used for joining paired-end sequence reads. Stitched reads were pre-filtered based on length (252-255bp) and threshold score (0.90) before feeding into the Quantitative Insights into Microbial Ecology (QIIME) version 1.8.0 (ref), following an open source online protocol (http://qiime.org/tutorials/tutorial.html). Denovo OTU picking method was applied (pick_de_novo_otus.py script was used to conduct). Singletons (defined as an OTU represented by 1 sequence, and appears only once in the whole OTU table) were removed from the OTU table prior to downstream analysis. A total of 4,377,675 reads were achieved from 75 samples, with a median value of 63539. Operational taxonomic unit (OTU) outputs were generated using average-neighbor clustering and no cutoff value.

2.4 Data Treatment and Analyses

PBOC Concentrations

Once the PBOC values were calculated for all three technical replicates from each sample for both Tinker AFB and Dover AFB samples, descriptive statistics were generated. Arithmetic mean, standard deviation, standard error of the mean, maximum and minimum values were calculated for all samples. Appendix I contains all tabulated descriptive statistic results. Box-and-whisker plots were generated to show variability among samples within each site using Tableau (Seattle, Washington), a data visualization software. Appendix J contains the metadata table used for R and Tableau analyses. Further site-specific data treatment was performed to tests the hypotheses of this study. Appendix K contains the R scripts for all site-specific hypothesis testing.

Tinker AFB—Specific Data Treatment

To compare PBOC result for the Tinker AFB samples, a t-tests was performed using R-Studio (Boston, MA). A two sample t-test was selected to test the first hypothesis that, in general, carbon amended samples have a greater PBOC concentration than non-treated samples. To justify the two-sample t-test, it was assumed that the samples were independent simple random samples with a population following a normal distribution (Daniel and Cross 2013). Because the sampling size is small, a t-test was further justified. A sample size is considered small if it is less than or equal to 30 (Daniel and Cross 2013).

Dover AFB—Specific Data Treatment

A one-way analysis of variance (ANOVA) test with a Tukey's honest significant difference (HSD) were performed on the PBOC data from Dover AFB. A one-way ANOVA investigates one source of variation, in this case organic carbon amendments, of a completely randomized experiment design (Daniel and Cross 2013). A one-way ANOVA test will be used to test any variability in the means of the Dover AFB samples. However, a one-way ANOVA only tests variability without identifying the source of the variability nor the level of its significance.

To identify which sample means are different and to what extent, the Tukey's HSD was performed. The Tukey's HSD utilizes a single value, the HSD, to compare against all means and compute a difference (Daniel and Cross 2013). With the Tukey's HSD, all possible differences between pairs of means are measured, and any value greater than the HSD is therefore significant (Daniel and Cross 2013). The following comparisons are of particular importance to test the second hypothesis of this study, stating that added organic carbon does not significantly affect PBOC concentrations further than 10-25 meters downgradient of injection location:

1. Downgradient samples (DV4, DV5, and DV6) to the treatment sample (DV3).
2. Upgradient sample (DV2) to treatment (DV3).
3. Upgradient (DV2) to downgradient (DV4, DV5, DV6)
4. Variability among individual samples downgradient of treatment (DV4 to DV5, DV4 to DV6, and DV5 to DV6).

Instead of performing individual t-tests between each pair of sample means listed above, the ANOVA with Tukey's HSD test evaluated all samples simultaneously.

Illumina Sequencing

The data provided by Pan Ji was the relative abundance of microbial communities present within each sample replicate per taxonomic rank. I combined the three technical replicates using the geometric mean of the three values. Any zero values were assigned a value of one before taking the geometric mean, then one was subtracted from each of the resultant value. This was done to include all microbial variability within each sample. Then the values for each sample were normalized to meet the relative abundance criteria that all values per sample must sum up to 1. Appendix H shows all the resulting relative abundance data per sample at phyla, class, and order ranks.

To down-weigh high abundance of species, an overall Log(x+1) transformation was performed. To identify resemblance among microbial communities of the samples, a Bra−Curtis similarity resemblance measure was done to create a lower triangular resemblance matrix. Non-Parametric Multi-Dimensional Scaling (MDS) using a Kruskal stress formula followed. This provided a dimensionless plot of the samples. For an MDS plot, the closer the samples are to each other, the larger the similarities among microbial communities. Conversely, the further away, the less similar their microbial communities are between samples. To quantify these similarities, a Hierarchical Cluster Analysis was overlaid on the MDS plot. The above described procedures were all completed using Primer-E 6.0 (Plymouth, United Kingdom).

Relative abundance values along with site and sample attributes were used to generate relative abundance pie charts and heat maps for side-by-side comparison for each site. These were all created using Tableau. Note that DV1 (Dover AFB), the background sample from an unknown location, will be excluded from any microbial diversity comparisons where a geospatial location is required for hypothesis testing.

Tinker AFB Sample Selection

Due to extraneous limitations, not all Tinker AFB samples were sequenced. Only four samples were able to be sequences and a selection decision needed to be made. The decision was based on Tinker AFB mean PBOC concentrations listed on

Table 3-1, the table of tabulated mean PBOC concentration results for Tinker AFB samples that will be introduced and explained in the results and discussion section. From the samples with ERB treatment (SBGSI-1), the sample with the highest and lowest PBOC measured concentrations were selected, TK138 and TK131. This selection was made to determine if differences in mean PBOC concentration would have an effect on microbial communities for SBGSI-1. From the samples without ERB treatment (SBGSI-4), the two lowest mean PBOC concentrations were selected, TK430 and TK431. In this case, the lowest two were selected based on the premise that SBGSI-4 did not receive the ERB treatment.

Chapter 3. Results and Discussion

3.1 PBOC Comparison

Tinker AFB

To support the first hypothesis which states that subsurface carbon amendments will result in elevated levels of PBOC in aquifer sediment samples collected at the injection points, PBOC of the Tinker AFB samples was measured. Table 3-1 lists results of the PBOC concentrations per sample in reference to depth bgs at each borehole. It can be observed from

Table 3-1 that the PBOC concentrations at SBGSI-1, the samples treated with an anthropogenic carbon, are generally greater than the PBOC concentrations at SBGSI-4, the samples without an addition of carbon.

Table 3-1: Mean PBOC concentrations for Tinker AFB samples in reference to ERB treatment and depth bgs.

Sample ID	Sample	PBOC* (mg/kg)	Depth (m)	Depth Average (m)	Treatment
TK131	SBGSI-1	147.92 ± 20.58	9.5-9.6	9.55	Yes
TK133	SBGSI-1	166.41 ± 16.98	9.6-10	9.8	Yes
TK135	SBGSI-1	216.74 ± 8.22	10-10.7	10.35	Yes
TK136	SBGSI-1	201.74 ± 11.01	10.7-11	10.85	Yes
TK136.5	SBGSI-1	168.02 ± 45.70	11-11.6	11.3	Yes
TK138	SBGSI-1	222.98 ± 23.94	11.6-12.2	11.9	Yes
TK426	SBGSI-4	161.37 ± 31.63	8-8.5	8.25	No
TK428	SBGSI-4	143.93 ± 12.97	8.5-9.1	8.8	No
TK430	SBGSI-4	125.61 ± 4.99	9.1-9.6	9.35	No
TK431	SBGSI-4	121.04 ± 12.37	9.6-10	9.8	No
TK433	SBGSI-4	186.29 ± 17.81	10-10.7	10.35	No
TK435	SBGSI-4	143.33 ± 36.96	10.7-11	10.85	No

* Arithmetic mean of each set of three technical replicates ± standard deviation

Figure 3-1 shows the measured PBOC concentrations per sample in a box-and-whisker plot, inclusive of technical triplicates. Result of samples with and without EVO treatment are shown side-by-side with the 200 mg/kg PBOC concentration needed for reductive dechlorination clearly marked. As seen in Figure 3-1, four out of the six samples treated with EVO have values above the 200 mg/kg threshold (Chapelle et al. 2012), whereas all samples from SBGSI-4, the untreated samples, are well below 200 mg/kg of PBOC. From Figure 3-1, one can speculate that the ERB treatment was applied at depths below 10 meters by observing the increase in PBOC concentrations at SBGSI-1 with relation to depth below ground surface. To demonstrate the degree in difference between the two groups, SBGSI-1 and SBGSI-4, a two-sample t-test was performed on the mean PBOC concentrations.

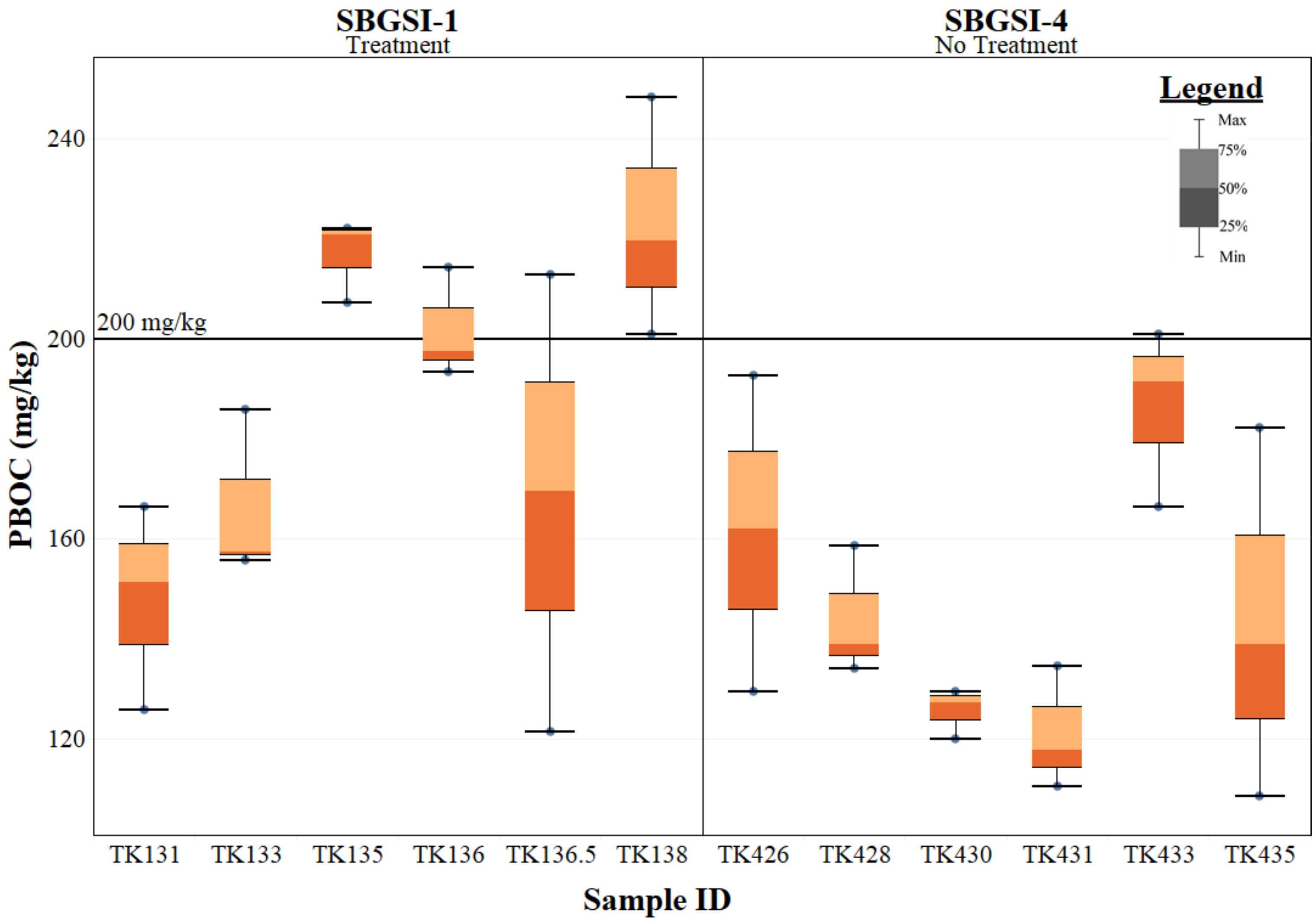

Figure 3-1. Box-and-Whiskers plot of PBOC concentrations for Tinker AFB grouped in reference to ERB treatment. The 200mg/kg PBOC concentration line is clearly marked as this is the threshold found to support reductive dechlorination (Chapelle et al. 2012).

Statistical Results and Analysis

The first hypothesis of this study states that samples from carbon amended sites will have significantly higher PBOC concentrations than those not treated with organic carbon. To test the first hypothesis, the 10-step hypothesis testing procedure is as follows:

1. **Data.** The arithmetic mean from the Tinker PBOC concentrations of the technical replicates was used for this test. This results in 6 samples from each population (SBSI-1 and SBGSI-4).
2. **Assumptions.** The data consist of two independent simple random samples of PBOC concentrations, one sample from a population of no treatment and the other sample from a population treated with a carbon amendment. Normal distribution of both populations was assumed. Furthermore, the population variances are unknown, but are assumed to be equal, have a small sampling size, and at a level of significance of $\alpha=0.05$.
3. **Hypothesis.** H_0: $\mu_1-\mu_2\geq0$, H_A: $\mu_1-\mu_2<0$, where μ_1 and μ_2 are the population means of SBGSI-4 and SBGSI-1 respectively.
4. **Test statistic.** $t=\frac{(\overline{x_1}-\overline{x_2})-(\mu_1-\mu_2)_0}{\sqrt{\frac{s_p^2}{n_1}+\frac{s_p^2}{n_2}}}$, where $\overline{x_1}\ and\ \overline{x_2}$ are the sample mean of SBGSI-4 and SBGSI-1 respectively, s_p^2 is the pooled sample variance, and n_1 and n_2 are the number of samples for SBGSI-4 and SBGSI-1 respectively.
5. **Distribution of test statistic.** Degrees of freedom$=n_1+n_2-2=12-2=10$. When the null hypothesis is true, the test statistic follows a t-distribution with 10 degrees of freedom.
6. **Decision rule.** Let $\alpha=0.05$. The critical value of t using R Studio is -1.81. Reject H_0 if $t_{computed}<-1.81$.

7. **Calculation of test statistic.** Using R Studio, t was calculated to be -2.421.
8. **Statistical Decision.** Because -2.421 is less than -1.81, reject H_0!
9. **Conclusion.** It can be concluded that the mean PBOC concentration of samples treated with carbon amendments is greater than those without treatment.
10. **P-value.** For this test, using R-studio, p-value= 0.018.

The results showed that there is a true difference in means between the SBGSI-1 and the SBGSI-4 samples given that the critical t value is less than -1.81. This means that the amount of PBOC among the samples within SBGSI-1 (EVO injection) is statistically significantly greater than the amount of PBOC in the sample group from SBGSI-4 (no EVO injection). The p-value of 0.018 is significant at a 0.05 level. This supports the first hypothesis that PBOC concentrations will be greater from samples treated with carbon than those without treatment. Complete results for the two-sample t-test from R can be found in Appendix P.

Dover AFB

To further support the first hypothesis which states that subsurface injections of a carbon amendment will have higher PBOC concentrations than those not treated with a carbon addition, PBOC concentrations between the Dover AFB samples were compared. The PBOC concentrations of the Dover AFB samples were measured in reference to each sample's geospatial location to the carbon treatment at PICT-3. Table 3-2 shows the mean PBOC concentrations of Dover AFB samples in reference to their distance from PICT-3 (see Figure 2-3).

As seen in Table 3-2, mean PBOC concentrations is greatest at the treatment area (DV3), and are not elevated at sample sites downgradient from the treatment. All other samples, including the downgradient samples, are well below the 200 mg/kg threshold. From Table 3-2, it can also be observed that the mean PBOC concentration of DV1 is much greater than the mean PBOC concentration of DV2. This difference may be due to natural variability of soil in the region. It can also be observed in Table 3-2 that the mean PBOC values for the upgradient (DV2) and the downgradient (DV4, DV5, and DV6) samples are within range of each other's standard deviation.

Table 3-2. Mean PBOC concentrations for Dover AFB samples in reference to distance to ERB treatment.

Sample ID	PBOC* (mg/kg)	Distance from PICT-3 (m)	Upgradient or Downgradient	Travel Time (yr.)	Organic Carbon Contact at sample collection?
DV1	109.54 ± 3.85	Background	Upgradient	-	-
DV2	69.52 ± 15.25	-47	Upgradient	-	-
DV3	326.78 ± 28.68	1	Downgradient	0	Yes
DV4	71.86 ± 8.40	10	Downgradient	0.22	Yes
DV5	59.37 ± 11.10	25	Downgradient	0.56	Yes
DV6	59.22 ± 5.31	50	Downgradient	1.12	Yes

*Arithmetic mean of experimental triplicates ± standard deviation

Figure 3-2 shows the measured PBOC concentrations per sample in a box-and-whisker plot, inclusive of technical triplicate values. Samples are aligned corresponding to their distance from PICT-3. The 200 mg/kg PBOC threshold for reductive dechlorination is clearly marked. All values above this threshold will theoretically support reductive dechlorination and all those below will not (Chapelle et al. 2012). Figure 3-2 shows that only the sample adjacent to the carbon substrate addition (DV3) contains enough PBOC to support reductive dechlorination.

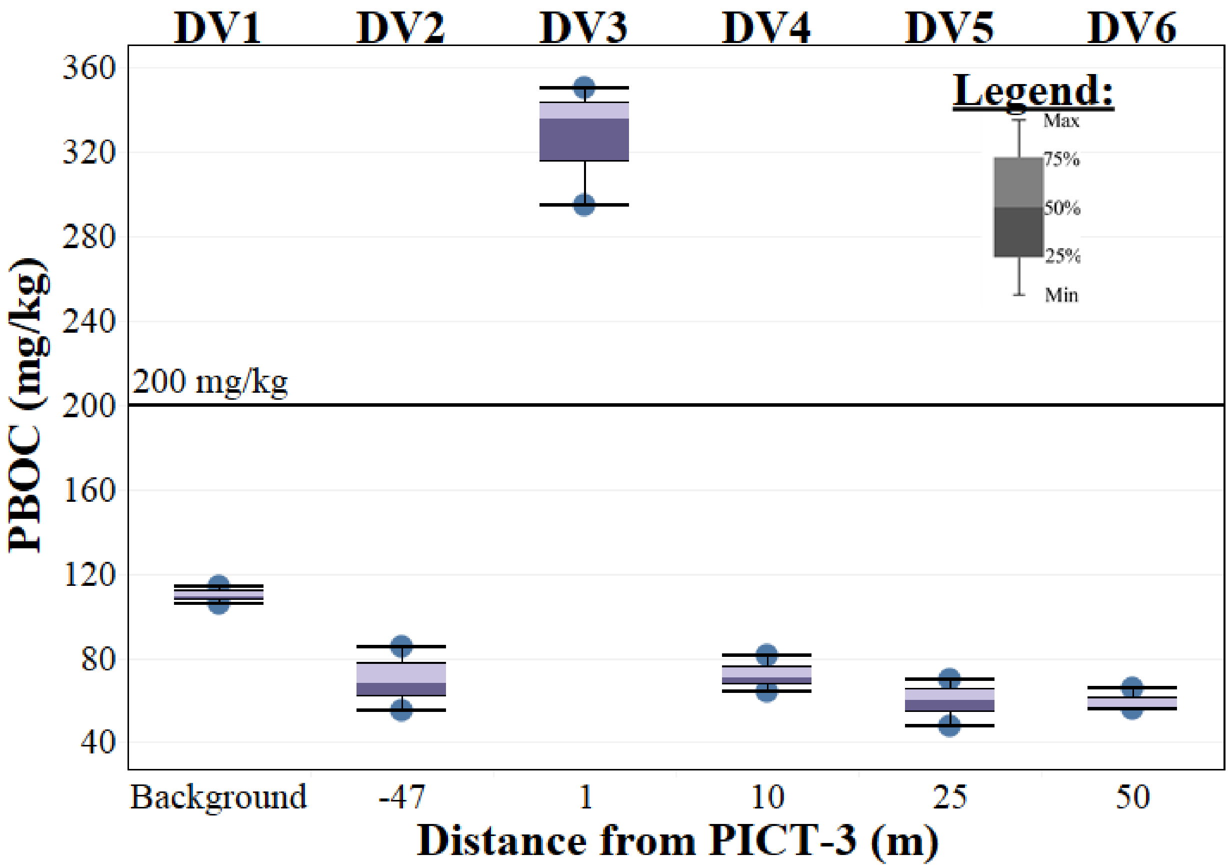

Figure 3-2. Box-and-Whiskers plot of PBOC concentrations from Dover AFB samples, arranged in reference to ERB treatment at PICT-3. The 200mg/kg PBOC concentration line is clearly marked as this is the threshold found to support reductive dechlorination (Chapelle et al. 2012).

To test the first hypothesis and to demonstrate whether there is a statistically significant difference in PBOC among the samples, a one-way analysis of variance (ANOVA) was performed on the Dover AFB PBOC concentrations.

Statistical Results and Analysis

To test the first hypotheses using the Dover AFB PBOC data, the 10-step hypothesis testing procedure for a one-way ANOVA is as follows:

1. **Data.** PBOC concentrations of the technical replicates was used for this test.

2. **Assumptions.** Normal distribution of population was assumed. Furthermore, the population variance is unknown, but are assumed to be equal, and have a small sampling size.
3. **Hypothesis.** H_0: $\mu_1=\mu_2= \mu_3=\mu_4=\mu_5= \mu_6$, H_A: at least one is different, where μ_n are the population means of the Dover AFB samples.
4. **Test statistic.** Variance Ratio (V.R.)= $\frac{among\ groups\ means\ square\ (MSA)}{within\ groups\ means\ square\ (MSW)}$.
5. **Distribution of test statistic.** F-distribution with 5 degrees of freedom for the numerator and 12 degrees of freedom for the denominator.
6. **Decision rule.** Let α=0.05. Using R Studio, V.R.=3.11. Reject H_0 if $V.R._{computed}$>3.11.
7. **Calculation of test statistic.** Using R Studio, V.R.=153.3.
8. **Statistical Decision.** Because 153.3 is greater than 3.11, reject H_0!
9. **Conclusion.** It can be concluded that not all PBOC concentration are equal.
10. **P-value.** For this test, using R-studio, p-value= 1.93×10^{-10}.

Results from the one-way ANOVA show that not all mean PBOC concentrations of the six Dover samples are equal. With such a small p-value of 1.93×10^{-10}, this difference is extremely significant. However, this result does not specify which mean is different, and therefore is not a definitive supporting test for the first hypothesis.

To find out which mean or means are statistically different and to what degree, a Tukey's HSD tests was performed. Complete results from the one-way ANOVA and Tukey's HSD test can be found in Appendix O. Results for Tukey's HSD were group per hypothesis. Table 3-3 shows the comparison of means relevant to the first hypothesis which states that ERB treated samples will have greater PBOC concentrations than those without the treatment. Table 3-3 and Table 3-4 both list which pairs are being compared, their difference, 95% confidence interval, p-value in reference to each pair, and whether the result is significant within a 95% confidence. Note that p-values of 0 mean that the p-value is infinitesimally small and cannot be calculated. Results from Table 3-3 show that the sample (DV3) closest to the treatment, PICT-3, having the largest mean PBOC concentration is in fact significantly different than all other samples. These results support the first hypothesis showing a significant difference of PBOC between the sample closest to PICT-3 than the samples upgradient, downgradient, and the background sample from unknown location.

Table 3-3. ANOVA with Tukey HSD for Dover AFB to compare the effects of ERB treatment to all other samples.

Comparison	**Difference**	**Confidence Interval**		**P-value Adjusted**	**Significant at α=0.05?**
		Lower Bound	**Upper Bound**		
DV3-DV1	217.2400	177.0021	257.4779	0.0000	Yes
DV3-DV2	257.2567	217.0187	297.4946	0.0000	Yes
DV4-DV3	-254.9267	-295.1646	-214.6887	0.0000	Yes
DV5-DV3	-267.4067	-307.6446	-227.1687	0.0000	Yes
DV6-DV3	-267.5600	-307.7979	-227.3221	0.0000	Yes

The results in Table 3-3 also support the second hypothesis which states that there will be no statistically significant increase of PBOC in aquifer sediment beyond 10 to 25 meters downgradient of ERB treatment. If PBOC concentrations downgradient from the treatment were consistent downgradient, the means between DV3 and DV4-DV6 would be similar and there would not a statistical significance at α=0.05. This shows that there is no statistically significant increase of PBOC in aquifer sediment beyond 10 to 25 meters downgradient of ERB treatment.

To further support the second hypothesis of this study states that there will not be significant variability of PBOC concentrations beyond 10-25 meters downgradient from the location of carbon addition into the subsurface, in this case PICT-3. Table 3-4 shows paired comparisons for the PBOC concentrations pertaining to samples upgradient and downgradient of PICT-3.

Table 3-4. ANOVA with Tukey HSD for Dover AFB to compare upgradient samples to downgradient samples from treatment as well as samples within the downgradient group.

Comparison	**Difference**	**Confidence Interval**		**P-value Adjusted**	**Significant at α=0.05?**
		Lower Bound	**Upper Bound**		
DV4-DV2	2.3300	-37.9079	42.5679	0.9999	No
DV5-DV2	-10.1500	-50.3879	30.0879	0.9521	No
DV6-DV2	-10.3033	-50.5413	29.9346	0.9492	No
DV5-DV4	-12.4800	-52.7179	27.7579	0.8947	No
DV6-DV4	-12.6333	-52.8713	27.6046	0.8899	No
DV6-DV5	-0.1533	-40.3913	40.0846	1.0000	No

From Table 3-4, comparing upgradient samples to downgradient samples and within the downgradient group yields the same results of no significant variability at a 95% confidence. In this case, it can be concluded that the PBOC concentrations are not significantly different from each other between the upgradient and downgradient groups, and the samples within the downgradient group. The result from both Table 3-3 and Table 3-4 support the second hypothesis by showing a statistically significant difference between any sample compared to DV3 and by showing no significant difference between the samples further away than 10-25 meters from the treatment at PICT-3, regardless of direction of groundwater flow.

3.2 Microbial Diversity

Tinker AFB

Similarities and Multidimensional Scaling (MDS)

To test the third hypothesis of this study which states that an increase in microbial community diversity is expected at aquifer sediment samples with ERB treatment (i.e., differences relative to non-treated areas), a MDS analysis with cluster similarities overlay was performed. Figure 3-3 shows the MDS plot of Tinker AFB at the class rank of taxonomy with cluster of similarities overlay. Figure 3-3 shows that all samples share a 50% similarity in microbial communities at class rank. Interestingly, out of the four samples displayed, the three selected specifically for having the lowest PBOC concentrations regardless of ERB, all share a 60% similarity. This small margin of difference among similarity clusters may be due to natural variability of soil in the region. These results did not show a clear distinction of microbial communities between the samples treated with EVO and those without treatment. With similarities of 50% or greater among all samples sequences, this test does not support the third hypothesis that impacts to microbial communities will be significantly different between ERB treated and non-treated samples.

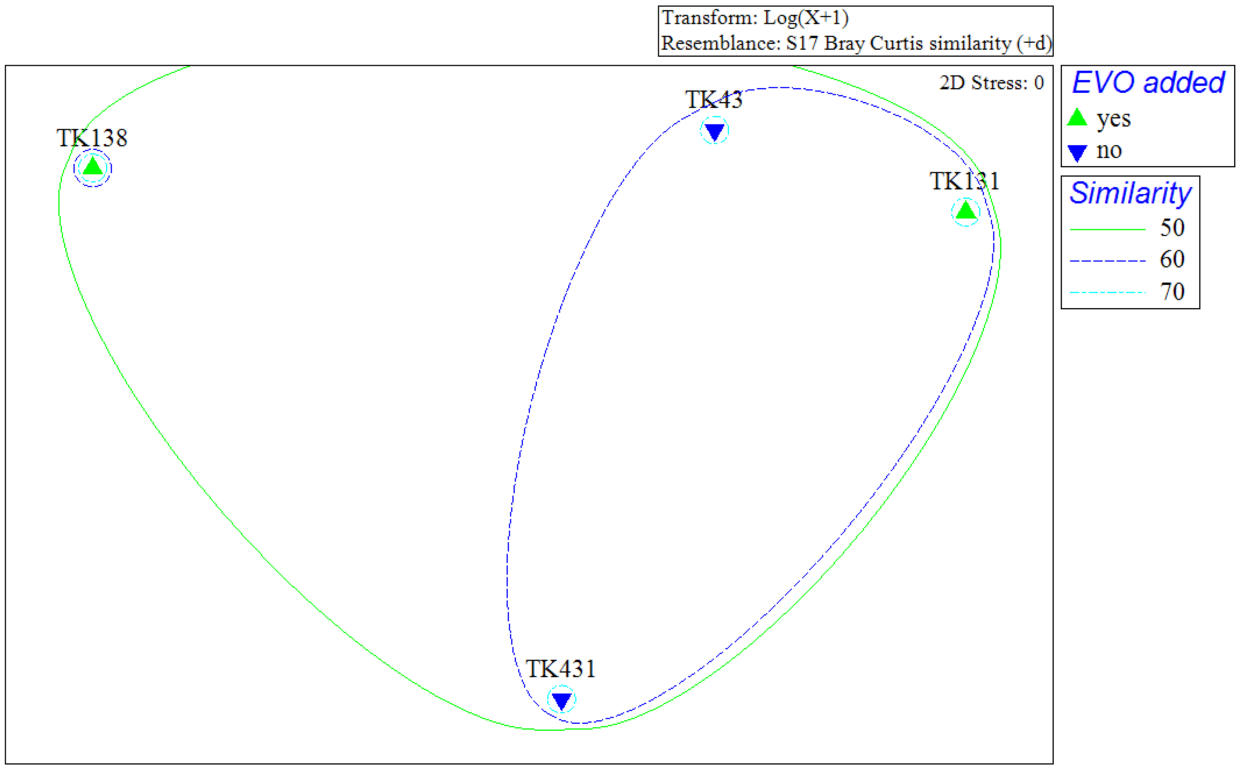

Figure 3-3. MDS plot of similarities at class rank for Tinker AFB. Overlaid cluster analysis represents samples with similarities at 50% (solid green) and 60% (dark blue dash).

Microbial communities profiled from Illumina Sequencing of 16S rRNA genes

To further test the third hypothesis of this study, Figure 3-4 shows a pie chart matrix of relative abundance per sample at Tinker AFB for all phyla present and only class rank of proteobacteria present since this was the most abundanct group. These were grouped by either ERB treatment or no treatment. This was constructed to show side-by-side comparison of microbial communities and their abundance in reference to treatment. Phyla results are relatively similar across all samples in Figure 3-4, with the most dominant phyla being Proteobacteria. Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes. These were present in all samples regardless of treatment and in great abundance. These were all present in abundance in other similar studies (David et al. 2015;

Lee et al. 2011). Other notable phyla present in all samples, but in smaller abundance were Chloroflexi, Chlorobi, Verrucomicrobia, and WS3. Presence of these were also noted in similar studies (Adetutu et al. 2015; David et al. 2015; Lee et al. 2011). Firmicutes, Chloroflexi, and Proteobacteria are all phyla from known dechlorinating bacteria as listed in Table 1-2. Known bacteria genera responsible for reductive dechlorination and their end-products (Bedard 2003; Griffin et al. 2004; Sharma and McCarty 1996; Sung et al. 2003; Ye et al. 2010). Class rank of proteobacteria shows a similar consistency of types of proteobacteria present across all samples. These findings are consistent with the MDS plot with cluster similarity overlay of Figure 3-3. These results do not support the third hypothesis that impacts to microbial communities will be significantly different between ERB treated and non-treated samples.

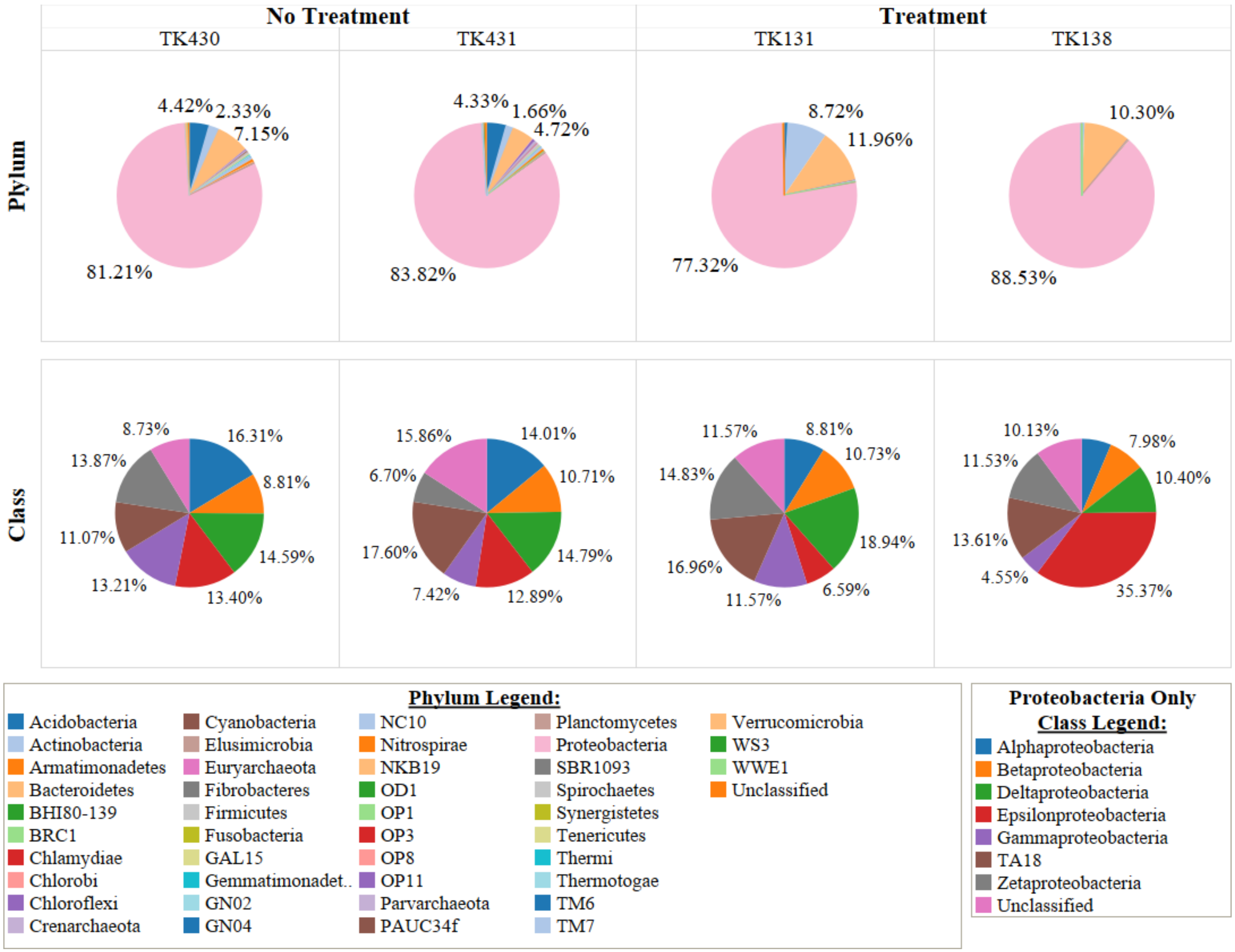

Figure 3-4. Pie chart matrix of relative abundance per sample at Tinker AFB for all phyla present, with only class for Proteobacteria, the most abundant phyla present.

Effects of carbon amendments on microbial abundance

To further explore similarities and the effects of ERB at Tinker AFB, and to continue testing the third hypothesis, a heat map of the Tinker AFB was generated. Figure 3-5 shows a heat map of the Tinker AFB samples at the class rank with relative abundances of 1.5% or greater. Relative abundance values for samples are grouped based on ERB treatment. Relative abundance is represented by an array of colors with white corresponding to a value of zero to dark blue for the most relative abundance present. Overall, from Figure 3-5, there are no clear distinctions of microbial communities between those samples that received treatment and those samples that did not receive carbon addition. This lack of microbial diversity does not support the third hypothesis that there will be a significant differences of microbial diversity between ERB treated and non-treated samples.

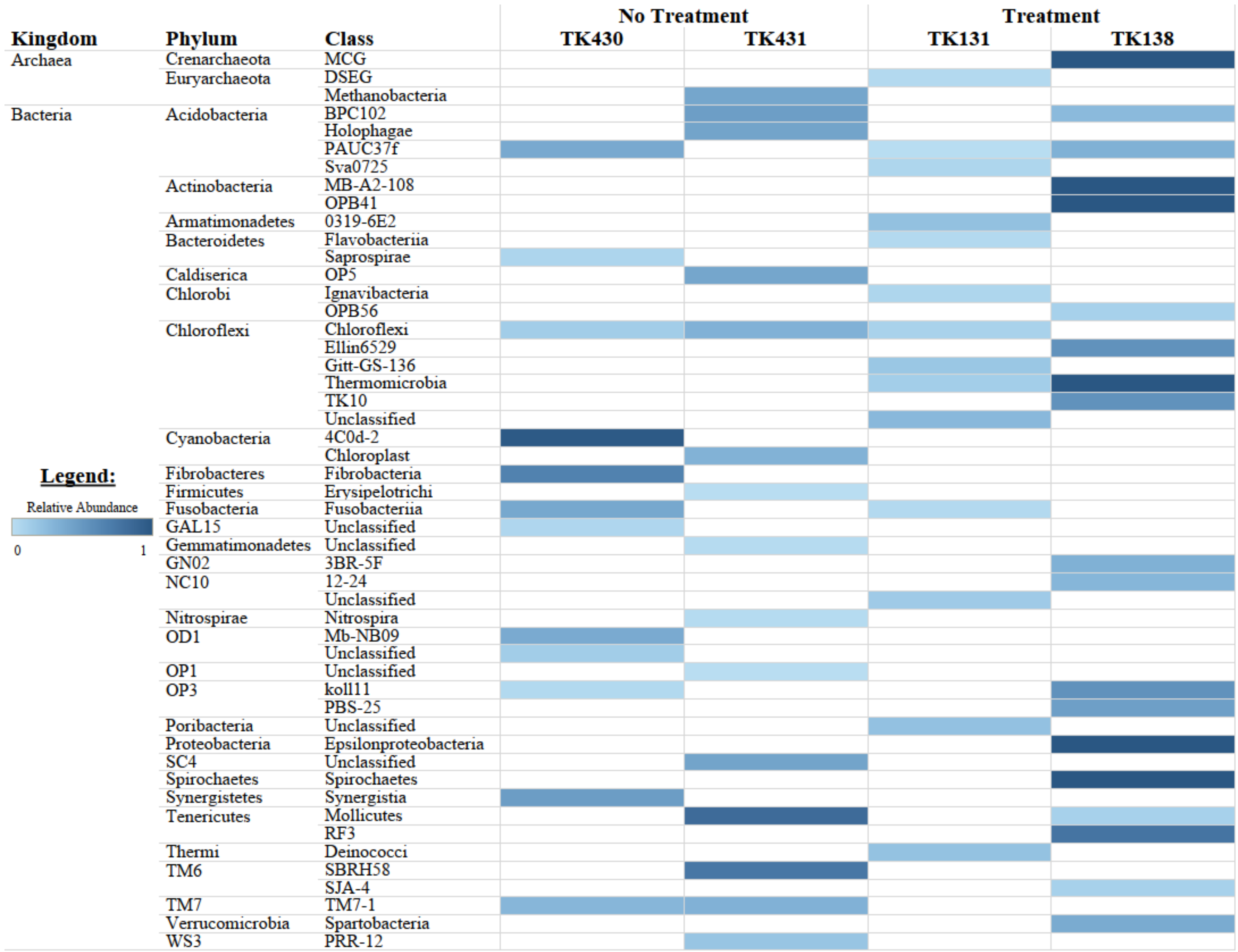

Figure 3-5. Heat map of relative abundances greater than 1.5% at class rank in reference to treatment for Tinker AFB Samples.

Dover AFB

Similarities and Multidimensional Scaling (MDS)

To test the third hypothesis of this study which states that impacts to microbial communities from carbon amendments will not be significantly different downgradient of ERB treatment, MDS analysis with cluster similarities overlay was performed. Figure 3-6 shows the MDS plot of Dover AFB at the class rank of taxonomy with cluster of similarities overlay. Figure 3-6 shows a 40% similarity among all samples downgradient of PICT-3. The strongest similarities are between samples DV4 and DV5, which are closer together in distance, with a 60% similarity. All samples share at least a 20% similarity in microbial communities at the class rank. Samples DV2 and DV3 show relatively more independent microbial community composition in comparison to the other samples. Overall, microbial community similarities seem to be distinctively divided by upgradient, treatment, and downgradient groups. At only a 20% similarity, these results support the third hypothesis that impacts to microbial communities will not be significantly different downgradient of ERB treatment.

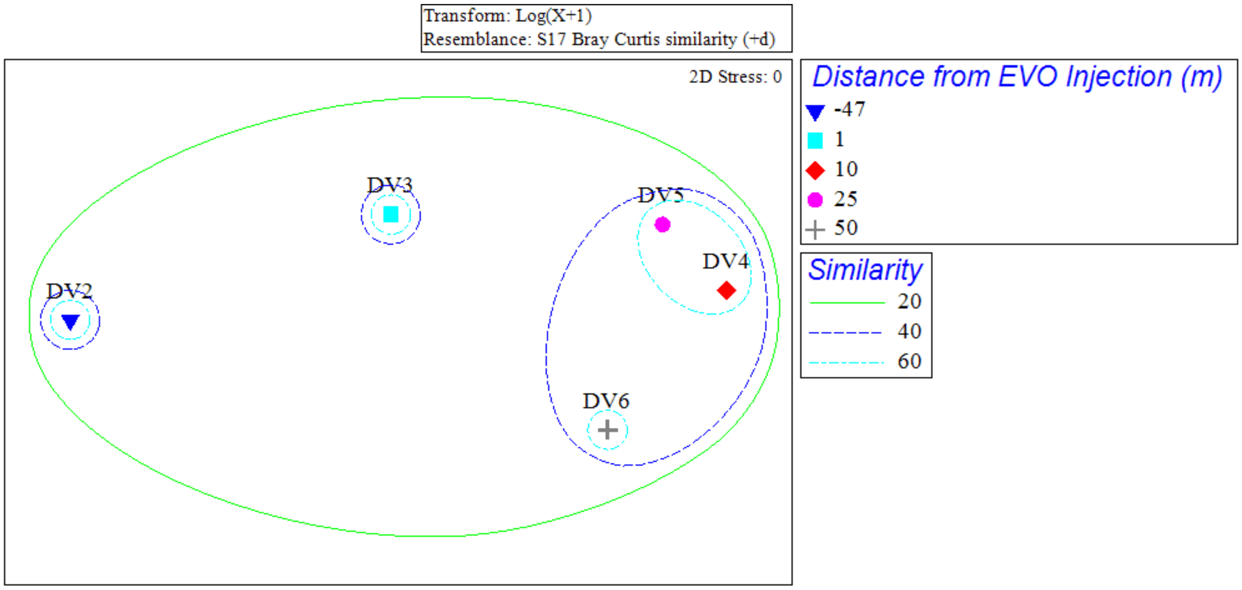

Figure 3-6. MDS plot of similarities at class rank for Dover AFB. Overlaid cluster analysis represents samples with similarities of 60% (light blue dash), 40% (dark blue dash), and 20% (green solid).

Microbial communities profiled from Illumina Sequencing of 16S rRNA genes

Figure 3-7 shows a pie chart matrix of relative abundance per sample at Dover AFB. The first row is that of all phyla present, and the second row focuses in on the class within Proteobacteria, the most abundant phyla present. The samples are arranged in reference to upgradient (DV2), at treatment (DV3) and downgradient (D4, DV5, DV6) of treatment. This was constructed to show side-by-side comparison of microbial communities and their abundance in reference to treatment. Similarly, to the Tinker samples, Proteobacteria has the greatest abundance of all phyla present in all Dover samples. Also present in large abundances are Actinobacteria, Bacteroidetes, and Firmicutes. These were all phyla present in other similar studies (David et al. 2015; Lee et al. 2011). Of interest regarding dechlorinators, the phyla Chloroflexi was found in samples DV3 through DV6, the sample closest to treatment and all downgradient samples. When looking at class rank, there is more variability in microbial communities, with the largest group being unclassified.

Results in Figure 3-7 show that more similarity in microbial communities exist between the downgradient samples than all other samples. It is at the class rank for proteobacteria that similarities among the downgradient samples can be observed. These results from the phyla rank further support the third hypothesis that impacts to microbial communities will be most significant at or near the treatment, but not much more significantly different downgradient of ERB treatment.

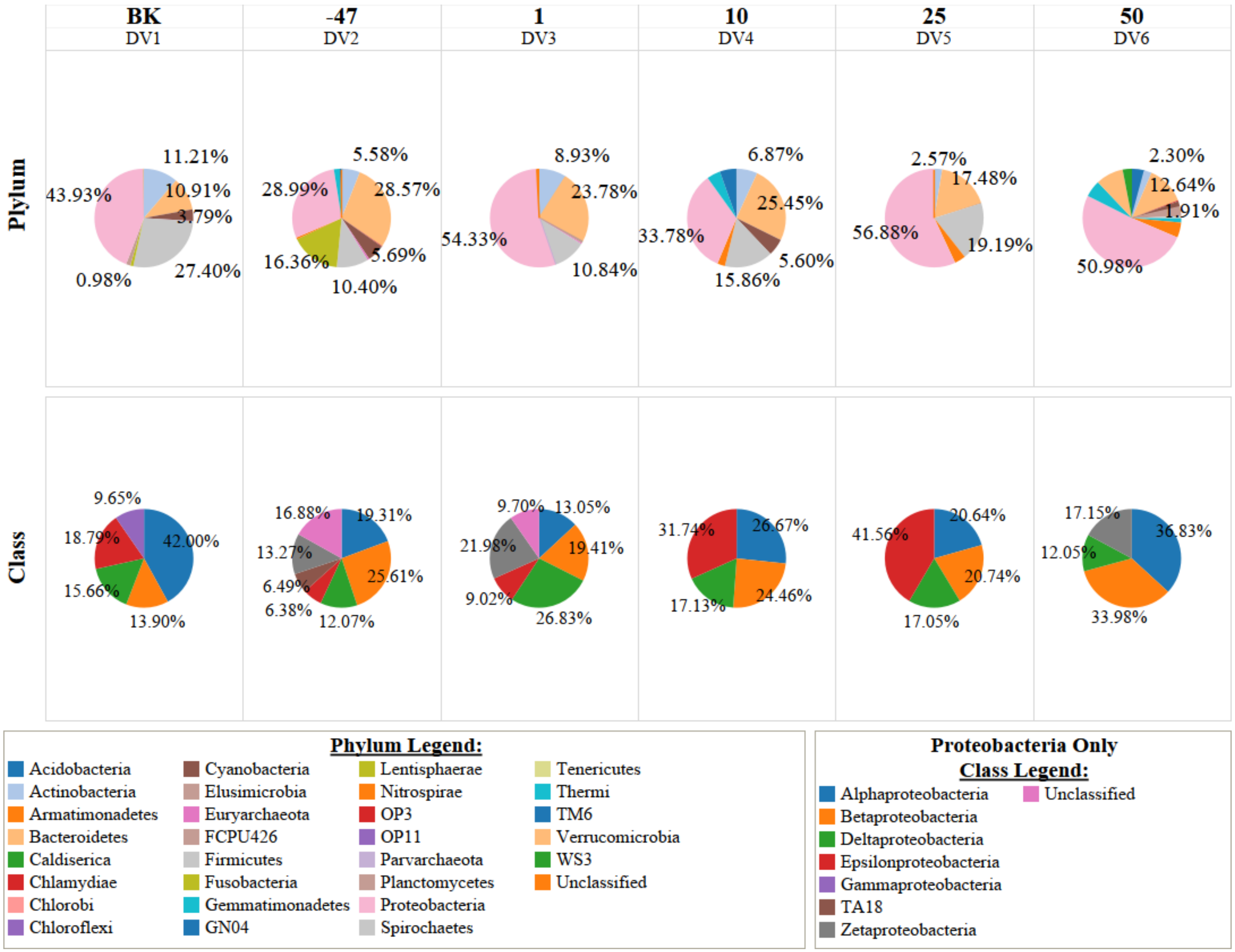

Figure 3-7. Pie chart matrix of relative abundance per sample at Dover AFB for all phyla present, with only class for Proteobacteria, the most abundant phyla present.

Effects of carbon amendments on microbial abundance

To further explore similarities and the effects of the introduction of ERB into the subsurface environment at Dover AFB and to support the third hypothesis, a heat map was generated. Figure 3-8 shows a heat map of the Dover AFB samples at class rank with relative abundances of 1.5% or greater. Relative abundance values for samples are grouped based on ERB treatment. Relative abundance is represented by an array of colors with white corresponding to a value of zero to dark blue for the most relative abundance present. Relative abundance values for samples are arranged in reference to distance from PICT-3. Most notable is the sample DV3, located one meter from the EVO injection, having a blue hue in almost every cell. These findings support the third hypothesis that microbial diversity is not significantly increased downgradient from treatment and that the only major increase of diversity is closest to the treatment injection.

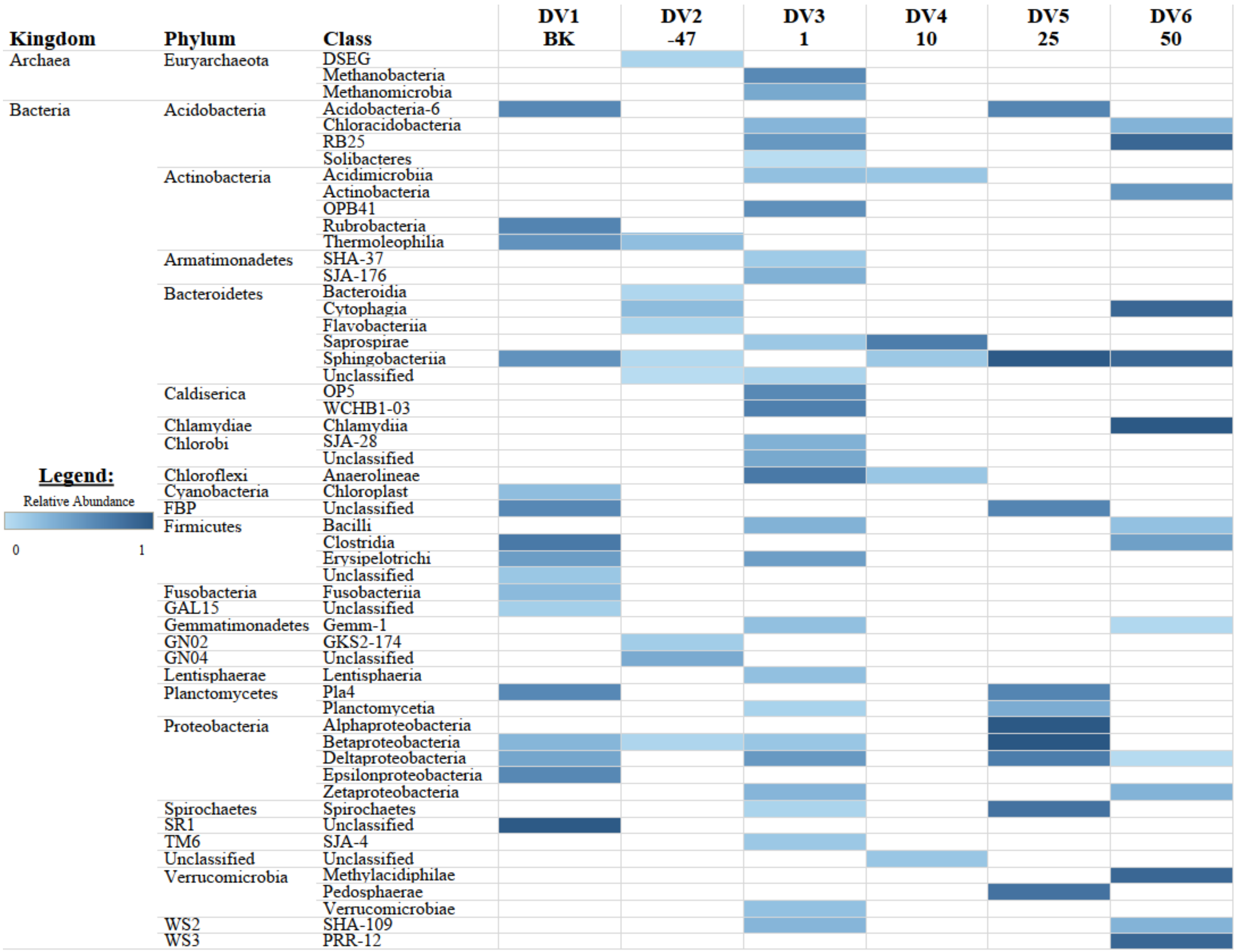

Figure 3-8. Heat map of relative abundance greater than 1.5% at class rank in reference to distance from treatment for Dover AFB in meters.

Chapter 4. Conclusions

4.1 Summary of Findings

The purpose of this study was to test two ERB sites using the PBOC extraction method and Illumina MiSeq Sequencing, by comparing PBOC concentrations and microbial community findings, to measure the effectiveness of ERB and identify some of its limitations. To achieve this, three hypotheses were tested. The first hypothesis was that ERB treatment results in an increase of PBOC concentration at and near the subsurface injection points. The second hypothesis was that no statistically significant increase of PBOC concentration would be observed in aquifer sediment beyond 10-25 meters downgradient of ERB treatment. The third hypothesis was that an increase in microbial community diversity would be observed at or near the ERB treatment, but not much further downgradient.

To validate these hypotheses, aquifer sediment from two ERB treated sites was collected. The PBOC extraction method was applied to the aquifer sediment samples PBOC concentrations were measured. DNA extraction, PCR amplification, and Illumina MiSeq Sequencing were also performed on the aquifer sediment samples to identify microbial communities and their relative abundance. Resultant data from these experimental methods was treated for further statistical analysis and data visualization.

To test the first hypothesis, a two-sample t-test was performed using the Tinker AFB PBOC concentrations. Results from the two-sample t-test performed on the Tinker AFB samples showed that the mean PBOC concentration of ERB samples is greater than samples without treatment (95% confidence; p-value = 0.018). Results from the one-way ANOVA test with Tukey's HSD of Dover

AFB PBOC concentrations showed that these is a significant difference between DV3 (the sample closest to PICT-3) to the samples upgradient, downgradient, and the background sample from unknown location (95% confidence, p-value=0.0000). Results of PBOC analysis at both the Tinker and Dover sites support the first hypothesis, showing that ERB treated aquifer sediment samples will have greater PBOC concentrations than those not treated with a carbon amendment.

To test the second hypothesis, a one-way ANOVA test with Tukey's HSD was done using the Dover AFB PBOC concentrations. Since no geospatial information was provided for the Tinker AFB samples, the second hypothesis could not be tested on the Tinker site. The results from the one-way ANOVA test with Tukey's HSD performed on the Dover AFB samples also showed that PBOC concentrations are not significantly different between the upgradient sample (DV2) to the downgradient samples (DV4, DV5, and DV6). There is also no significant difference when comparing the downgradient sample to one another. The only statistically significant difference occurred when any sample was compared to the sample closest to PICT-3 (DV3). These results support the second hypothesis by showing no significant difference in PBOC concentrations between the samples further away than 10-25 meters from the ERB treatment.

To test the third hypothesis, non-parametric MDS with a hierarchical similarity analysis was performed on both Tinker and Dover sites. The non-parametric MDS and cluster analysis performed on relative abundance results from the Illumina MiSeq Sequencing of 16S rRNA genes showed large similarities among the samples from Tinker AFB (50% similarities), regardless of ERB treatment or distance from treatment. This test on the Tinker samples does not support the third hypothesis that impacts to microbial communities will be significantly different between ERB treated and non-treated samples. The same MDS with cluster analysis performed on the Dover samples showed great similarities between the downgradient samples than all other samples

(40% similarities). All of the Dover samples shared at least some similarities (20% similarities). The results from the Dover samples support the third hypothesis that impacts to microbial communities will not be significantly different downgradient of ERB treatment.

To further explore the microbial community composition and to test the third hypothesis, relative abundance pie charts and heat maps were generated for data visualization of both Tinker and Dover sites. The pie charts and heatmaps for Tinker AFB samples supported the MDS analysis, by all samples sharing similar compositions of phyla and class taxa. These findings did not support the third hypothesis that there will be more variety on microbial composition in ERB treated aquifer sediment samples. For the Dover AFB samples, the pie charts and heatmaps supported the MDS analysis as well, showing similar compositions of phyla and class taxa in the samples upgradient and downgradient of the ERB treatment, and a much different composition for DV3, the sample closest to the treatment. This singular difference between the microbial communities present at DV3, and the lack of variability among the samples downgradient of DV3 confirm the third hypothesis which states that there will be greater microbial diversity at ERB application, but not much microbial diversity downgradient of the treatment. It can be speculated that the differences in microbial communities between the Tinker and Dover samples may be due to differences in soil composition, variations in hydraulic conductivity of the two sites, or even differences in the type and frequency of the ERB treatment application. Furthermore, the high PBOC concentrations at DV3 could be related to the high microbial diversity in the same sample. Conversely, the lack in microbial diversity at Tinker ABF could also be related to the minor PBOC concentration elevations for the same samples. This study supports previous works that have demonstrated more abundant DNA concentrations of microbial species in samples with carbon addition, and studies that have reported increased microbial communities as a whole when carbon has been added into the subsurface.

4.2 Concluding—Impacts of Results

This study seeks to support PBOC as a low cost metric for evaluating the sustainability of ERB by providing an additional metric along with groundwater constituents. The first hypothesis shows that PBOC concentrations will be greater in aquifer sediment samples from ERB treated sites than those without. The second hypothesis states that PBOC concentrations measured at or further away than 10 to 25 meters from carbon amendments will be significantly less than PBOC concentrations measured closer to the ERB treatment. Lastly, the third hypothesis stating that microbial diversity will only be observed at and near the ERB treatment and no other variability of samples was observed. These results show an important consideration when designing and implementing ERB treatments. Since carbon amendments do not travel very far from where they are introduced into the subsurface, traditional installation plans for injection wells of ERB treatment and frequency of treatments should be reconsidered.

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List of Appendices

Appendix A. PBOC Standard Operating Procedure

Potentially Bioavailable Organic Carbon (PBOC) Extraction Method for Aquifer Sediment

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) established the chemical extraction procedure for quantifying potentially bioavailable organic carbon present in aquifer sediments.

1.2 General Procedure

The Potentially Bioavailable Organic Carbon (PBOC) Extraction Method consist of a five step chemical extraction process where three sequential 24-hour extractions with 0.1% pyrophosphate are followed with a 24-hour 0.5 N NaOH extraction and a final 24-hour 0.1% pyrophosphate extraction. The first sequential 0.1% pyrophosphate extractions (extractions 1-3) represent the loosely-extractable organic carbon associated with the sediment. And, the final to extraction (extractions 4-5), where a 0.5 N NaOH solution is followed by 0.1% pyrophosphate solution, represent the more strongly-associated organic carbon associated with the sediment.

2.0 REQUIRED SUPPLIES AND EQUIPMENT

This SOP requires all of the following supplies and equipment for the PBOC chemical extraction process.

- Aquifer sediment (dried at 70°C for 24-hour and sieved through 2-mm openings)
- 50mL Polypropylene centrifuge tubes with centristar cap (www.fishersci.com)
- 25mL glass vials with caps for sample storage
- Analytical Balance

- Stir plate and stir bar
- 20 mL graduated cylinder or 10mL pipette
- 0.1% sodium pyrophosphate (pH 8.5, crystalline/certified ACS; www.fishersci.com)
- 0.5 N NaOH (pH 13, pellets/certified ACS; www.fishersci.com)
- Rotary Tumbler (Dayton 3m137B Motor or equivalent) to ensure proper mixing of exposed aquifer sediments and extracting solution
- Centrifuge (Model Eppendorf Centrifuge 5430) required for solid separation
- Shimadzu Total Organic Carbon Analyzer (TOC-VCSN or equivalent; www.shimadzu.com) to measure extracted bioavailable organic carbon

3.0 GLASSWARE CLEANING

3.1 Glassware needed (for 2 samples, with three technical replicates)

2	60mL beakers
2	400mL beakers
2	1000mL volumetric flasks with lids
2	1000mL Pyrex glass bottles with lids and plastic rims
30	25mL glass vials with lids
6	Centrifuge tubes (not to be washed, set aside)

3.2 General Procedure

Always wear appropriate protective equipment when handling sharps and chemicals: safety glasses and nitrile gloves.

- Utilize Sparkleen or available detergent and follow manufacturer's instructions for cleaning the glassware.

- Soak glassware in acid bath for at least two hours at a minimum or soak overnight soak, but do not leave in longer than a day (or weekend at most)

Always wear appropriate protective equipment when utilizing the acid bath: lab coat, safety goggles (not glasses), acid bath approved gloves, and closed toe shoes.

- Wrap in aluminum foil and bake in furnace oven for 1 hour at 300°C, let oven cool down to room temperature (25°C) for at least 20 minutes before taking out of the oven.

Always wear appropriate protective equipment when working with extreme temperatures. Make sure to always use insulated gloves when removing glassware from furnace oven.

4.0 SOLUTIONS FOR PBOC EXTRACTIONS

4.1 0.1% Sodium Pyrophosphate Solution

- Weigh 1g of Sodium Pyrophosphate powder
- Measure 400mL of nanopure water using a beaker
- Combine powder into beaker and mx using a stir bar and a stir plate
- Pour solution into a 1000mL volumetric flask and fill with nanopure water to the 1000mL line. Place cap on flask invert several times making sure to hold the cap in place.
- Transfer the solution to 1000mL Pyrex glass bottles and seal tightly. Immediately store below bench.

Always wear appropriate protective equipment when handling sharps and chemicals: safety glasses and nitrile gloves.

4.2 0.5N NaOH Solution

- Weigh 20g of NaOH pellets
- Measure 400mL of nanopure water using a beaker
- Combine powder into beaker and mx using a stir bar and a stir plate
- Pour solution into a 1000mL volumetric flask and fill with nanopure water to the 1000mL line. Place cap on flask and invert several times making sure to hold the cap in place.
- Transfer the solution to 1000mL Pyrex glass bottles and seal tightly. Immediately store below bench.

Always wear appropriate protective equipment when handling caustic chemicals: lab coat, safety goggles (not glasses), acid bath approved gloves, and closed toe shoes.

5.0 SAMPLE PREPARATION

- Dry aquifer sediment sample in oven at 70°C for 24 hours. Dry enough soil taking into account wet vs. dry weights
- Process through #8 sieve (~2mm) by hand to remove gravel and break up samples. May use pestle and mortar to break down larger pieces before sieving if necessary.
- Store in clean vials with labels. Include sample name and date on label. Store in 4°C walk-in fridge.

6.0 PROCEDURE FOR PBOC EXTRACTION METHOD

Always wear appropriate protective equipment when handling sharps and chemicals: safety glasses and nitrile gloves.

6.1 Prepare samples

- Record weight of each centrifuge tube (tube and cap)
- Weigh 10 grams of aquifer sediment in polypropylene centrifuged tube. Record weight.
- Label tubes and caps appropriately.

6.2 Extraction cycles 1-3

- Transfer 0.1% Sodium Pyrophosphate into a beaker from the stock solution.
- Accurately measure 20mL of 0.1% Sodium Pyrophosphate using a pipette and add solution into each centrifuge tube with sample.
- Manually shake tubes to ensure sample is suspended in the solution.
- Record total weigh on the cap of the centrifuge tube
- Tightly pack tubes into large plastic jars and place on rotary tumbler for 24 hours
- After removing from tumbler, place tubes in centrifuge making sure the centrifuge remains balanced.
- Centrifuge at 2000-5000rpms for 10-30 minutes depending on soil type (soil with predominately clays and silts take longer to separate than sands), repeat until solid separation.
- Decant supernatant using a new filtered tip on the pipette per each sample, place in empty 25mL glass vial and store at 4°C until analyzed for extracted bioavailable organic carbon.
- Repeat for extractions 2-3.

6.3 Extraction cycle 4

- Transfer 0.5N NaOH into a beaker from the stock solution.
- Accurately measure 20mL of 0.5N NaOH using a pipette and add solution into each centrifuge tube with sample and close lid tightly.
- Manually shake tubes to ensure sample is suspended in the solution.
- Record total weigh on the cap of the centrifuge tube.
- Tightly pack tubes into large plastic jars and place on rotary tumbler for 24 hours
- After removing from tumbler, place tubes in centrifuge making sure the centrifuge remains balanced.
- Centrifuge at 2000rpms for 5-10 minutes depending on soil type (soil with predominately clays and silts take longer to separate than sands), repeat until solid separation.
- Decant supernatant using a new filtered tip on the pipette per each sample, place in empty 25mL glass vial and store at 4°C until analyzed for extracted bioavailable organic carbon.

6.4 Extraction cycle 5

- Transfer 0.1% Sodium Pyrophosphate into a beaker from the stock solution.
- Accurately measure 20mL of 0.1% Sodium Pyrophosphate using a pipette and add solution into each centrifuge tube with sample.
- Manually shake tubes to ensure sample is suspended in the solution.
- Record total weigh on the cap of the centrifuge tube
- Tightly pack tubes into large plastic jars and place on rotary tumbler for 24 hours

- After removing from tumbler, place tubes in centrifuge making sure the centrifuge remains balanced.
- Centrifuge at 2000-3000rpms for 5-15 minutes depending on soil type (soil with predominately clays and silts take longer to separate than sands), repeat until solid separation.
- Decant supernatant using a new filtered tip on the pipette per each sample, place in empty 25mL glass vial and store at 4°C until analyzed for extracted bioavailable organic carbon.

7.0 TOC Analysis

7.1 Materials:

- Auto sample vials for TOC analyzer – follow glassware cleaning procedures
- Shimadzu Total Organic Carbon Analyzer (TOC)-VCSN

7.2 Analysis of TOC concentrations: see Standard Methods and Appendix C

Always wear appropriate protective equipment when handling sharps and chemicals: safety glasses and nitrile gloves. Use fume hood when handling acids.

7.3 Discard any remaining TOC samples after analysis into correspondingly labeled waste container, do not pour into drain. See Julie for guidance.

Appendix B. Shimadzu TOC-VCSN Standard Operating Procedure

To turn on machine, press power button on the lower right side.

On the computer, double click on the TOC-Control V icon.

- Select the Sample Table Editor
- Select OK at the next screen

Using the left window, scroll down to TOC-VCSN:

- Right click and select Connect
    - Use settings on PC
    - Next screen shows initializing system. Nothing to click.
- Right click on TOC-VCSN and select Background Monitor
    - Place window in upper right corner of screen
    - Instrument is ready when all circles are green.

Check the following items:

- Zero Air has greater than 500 psi left in the cylinder. This is indicated on the gauge closest to the cylinder. If it is below 500 psi tell Jody.
- Rinse water for the auto-sampler-refill if it has not been changed for a week or is below 200mls. It is the container behind the auto-sampler. If you have a large run, make sure you fill this. It rinses in between each sample.
- Offline water-empty and refill daily
- Dilution water-refill daily.
- Acid (2M HCl, used during catalyst regeneration)-tell Jody if this is below 50mls

- Inside machine:
    - Humidifier (front tube), add water if level is below “Low” mark. Level should be between the “High and Low” markings.
    - Drain Vessel, add water into the plastic tube up front until water flows through the drain line on the right hand side of the instrument. The drain vessel is the back plastic bottle.
    - Pressure gauge is at 200 kPa
- Zero Air, make sure ball float top is at 150. This is located on the front of the instrument. Check this after you have connected the instrument and the computer.
- Right click on TOC-VCSN and select Maintenance
    - Select Washing. This is very important step if you change the Rinse, Dilution and Offline waters.
    - Select Flow Lines, click in each box
    - Wait until machine stops washing. Close window.
    - This makes sure fresh water is in the lines.

Allow at least 20 minutes for warm up or until all items in Background Monitor window are checked green. If the system takes longer than 30 minutes to be ready (all circles are green) re-generate catalyst. See page 4

METHOD DETECTION LIMITS:

Using a calibration curve-highest standard:	1 ppm = 0.036 mg/l
	2 ppm = 0.069 mg/l
	10 ppm = 0.125 mg/l

100 ppm= 0.175 mg/l

Setting up Samples to run

1) To start from scratch (no previous file), Begin with the Calibration Curve Wizard.

a) Click on File then new and double clicking on Calibration Curve icon
b) This gets you into the Calibration Curve Wizard
c) Page 1 is system information. You should not have to change anything on this page. Click NEXT
d) Page 2 is Calibration Curve Type. Select "Edit calibration points manually". Under Calibration using automatic dilution select "Dilution from Standard Solution. Click NEXT
e) Page 3 is Analysis information. Use the pull down menu beside Analysis to select "NPOC". Type in your sample name and sample ID, for example Cal and 1. You can change these at a later time. Calibration Method should be "Linear Regression" and deselect Zero Shift. Check (select) "Multiple Injections". Click the browse button next to the file name. This will take you to the directory where calibration curves are saved. Type in the name you wish to give the calibration curve, for example 10ppm 09_13_06. Click NEXT
f) Page 4 is Calibration Measurement Parameters. On this page you select the units of your standards, the number of injections per standard, the number of washes (usually use 0), purge time (usually 1.0 or 1:30min), acid addition is always 0. Click NEXT
g) Page 5 is Calibration Points List. At the top of page type in an injection volume of 150 μL. To add a calibration point, click on the desired number and then click on "Add". This will take to you to the edit screen. Enter the concentration of your standard solution and the auto dilution needed to obtain your desired concentration. For example, if your standard solution was 10 ppm and you wanted a calibration standard of 1 ppm the auto dilution value

you would enter is 10. The first standard you should enter is the Blank. The concentration will be 0 and the dilution value will be 1. Continue entering your standards. Before you click "Add" make sure the injection volume is 150 μL. When you have entered all your standards (before you click "Next") enter 150 μL for the injection volume. Now click NEXT. If you use 100 ppm stock use 100 μL injection vol.

h) Page 6 is Error Checking. If you check this, you should choose stop unless you have enough standard solution for the instrument to try another calibration run. If it fails twice I would select "Stop". Enter a lower limit of 0.995.

i) Page 7 is Peak Time Parameters. Use default settings.

j) Page 8 is History. DO NOT enable history log. Click on FINISH.

2) Now you need to generate your sample table.

a) Click on File then new and double clicking on Sample Run icon

b) Make no changes to window that automatically opens. Click OK

c) Enter the name you name you want the table to save as.

d) Click on Insert then choose Sample.

e) Page 1 is Parameter Source. Click on Calibration Curve and then the Browse button. Choose the calibration curve you generated above. Click NEXT

f) Page 2 is Analysis Information. You can enter a sample name and a sample ID. Dilution should be set at 1.00 and number of determinations is 1. Click NEXT

g) Page 3 is Calibration Curves. You are already doing a calibration curve so do not check anything on this page. Click NEXT

h) Page 4 is Injection parameters. Do not need to change anything on this page. Should be the same as your calibration curve parameters. Click NEXT

i) Page 5 is Peak Time Parameters. Check Use default settings. Click NEXT

j) Page 6 is USP/EP. Do not need to change anything on this page. Click Finish

k) Choose the vial position. It should be Off Line. (Position 0)

l) Click on Insert then choose Calibration Curve. Click on Calibration Curve and then the Browse button. Choose the calibration curve you generated above. Click NEXT

m) Choose the vial positions for your standards. The first standard, conc. 0.000, is your blank and is nanopure water only. I usually have nanopure in the vial in position 1. The rest of your standards will have the position of the stock solution. For me this is usually position 2.

n) Click on Insert then choose Auto Generate

o) Page 1 is Sample Source. Click on Calibration Curve and then the Browse button. Choose the calibration curve you generated above. Click NEXT

p) Page 2 is Sample Parameters. Enter the number of samples you have and the position number of your first sample vial in the auto-sampler. You can enter a sample name and a sample ID. If you click Index start each sample description will receive an increasing counting number. For example, if you put “A” for the sample ID and check indexing and enter “1” the first ID will be A1 the next one A2 and so on. Click NEXT

q) Page 3 is Calibration Curves. You are already doing a calibration curve so do not check anything on this page. Click NEXT

r) Page 4 is Calibration Curve Check. Do not check anything on this page. Click NEXT

s) Page 5 is Controls. Do not check anything on this page. Click Finish

t) The vial positions should be already in place. Make sure they are correct.

u) Save the changes you made to the sample table.

3) To setup a sample table using an existing file:
   a) Click on File Then Open, select the sample table file you wish to use
   b) Click on File Then Save As. Give it a new file name.
      i) To make sure you don't accidentally destroy the file you copied, close the sample table window. Click on File Then Open and open the file you created in 3.b.
   c) You now need to delete the old data in the file.
      i) Click on the 7^{th} icon from the right. (Delete all data)
      ii) The samples designation will change from Completed to Defined.
   d) Make the needed corrections to your sample ID's
   e) Save your changes using either Save or Save As.

To run your Samples:

1) You must connect your Sample Table. Click on the yellow lightning bolt icon
2) Click on the Stop light icon.
3) This takes you to the Standby Window. Choose either Keep running or Shutdown instrument. By choosing Shutdown the instrument will automatically turn itself off 30 minutes after your samples have run. Click Standby
4) Sparing/Acid Addition window. Make sure your samples are in correct positions in the auto-sampler. Click OK. Normally for your standards it will have position 1 for the first one (conc. 0.000) and then position 2 for the rest. That is if the vial in position 1 is nanopure water.
5) Last window, deselect External Acid addition. Click Start.

To Export Data:

1) Click on File, then ASCII Export.
2) Choose Export results by analysis type. This will create a text file that can be opened in Excel

Items to do at least weekly:

- Re-generate catalyst (May not need to do weekly)
    - Right click on TOC-VCSN and select Maintenance
    - Select re-generate TC catalyst
    - Select start
- Replace rinse, offline, dilution waters

Wash flow lines after replacing water

Appendix C. MPBIO FastDNA SPIN Kit for Soil Standard operating Procedure

(http://www.mgp.cz/files/kity/FastDNA_Spin_Kit_for%20soil.pdf)

1. Add up to 500 mg of soil sample to a Lysing Matrix E tube.
2. Add 978 µl Sodium Phosphate Buffer to sample in Lysing Matrix E tube.
3. Add 122 µl MT Buffer.
4. Homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0.
5. Centrifuge at 14,000rcf for 15 minutes to eliminate excessive debris from large samples, or from cells with complex cell walls.
6. Transfer supernatant to a clean 2.0 ml microcentrifuge tube. Add 250 µl PPS (Protein Precipitation Solution) and mix by shaking the tube by hand 10 times.
7. Centrifuge at 14,000rcf for 5 minutes to pellet precipitate. Transfer supernatant to a clean 15 ml tube.
8. Resuspend Binding Matrix suspension and add 1.0 ml to supernatant in 15 ml tube.
9. Place on rotator or invert by hand for 2 minutes to allow binding of DNA. Place tube in a rack for 3 minutes to allow settling of silica matrix.
10. Remove and discard 500 µl of supernatant being careful to avoid settled Binding Matrix.
11. Resuspend Binding Matrix in the remaining amount of supernatant. Transfer approximately 600 µl of the mixture to a SPIN™ Filter and centrifuge at 14,000rcf for 1 minute. Empty the catch tube and add the remaining mixture to the SPIN™ Filter and centrifuge as before. Empty the catch tube again.
12. Add 500 µl prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip. Ensure that 100mL of 100% ethanol has been added to the Concentrated SEWS-M.
13. Centrifuge at 14,000rcf for 1 minute. Empty the catch tube and replace.
14. Without any addition of liquid, centrifuge a second time at 14,000rcf for 2 minutes to “dry” the matrix of residual wash solution. Discard the catch tube and replace with a new, clean catch tube.

15. Air dry the SPIN™ Filter for 5 minutes at room temperature, by leaving the tube caps open.
16. Gently resuspend Binding Matrix (above the SPIN filter) in 50 µl of DES (DNase/ Pyrogen-Free Water).

Centrifuge at 14,000rcf for 1 minute to bring eluted DNA into the clean catch tube. Discard the SPIN filter. DNA is now ready for PCR and other downstream applications. Store at -20°C for extended periods or 4°C until use.

Appendix D. 16S rRNA Amplification Protocol, version 4.3

Primers for paired-end 16s community sequencing on the Illumina HiSeq platform using bacteria/archaeal primers 515F/806R. Please see this article:

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J.

For running these libraries in the MiSeq and HiSeq please make sure you read the supplementary methods of the above manuscript very well – you will need to make your sample more complex by adding 30-50% PhiX to your run.

Primer Constructs designed by Greg Caporaso

The primer sequences in this protocol are always listed in the 5' -> 3' orientation. This is the orientation that should be used for ordering. See primer tips and getting started for information on ordering, concentration, and resuspension. (http://www.earthmicrobiome.org/files/2013/04/EMP_primer_ordering_and_resuspension.doc)

515f PCR Primer Sequence – Forward primer

Field description (space-delimited):

5′ Illumina adapter

Golay barcode

Forward primer pad

Forward primer linker

Forward primer (515f)

AATGATACGGCGACCACCGAGATCTACAC XXXXXXXXXXXX TATGGTAATT GT GTGYCAGCMGCCGCGGTAA

806r PCR primer sequence – Reverse primer

Field description (space-delimited):

Reverse complement of 3′ Illumina adapter

Reverse primer pad

Reverse primer linker

Reverse primer (806r)

CAAGCAGAAGACGGCATACGAGAT AGTCAGTCAG CC GGACTACNVGGGTWTCTAAT

Illumina PCR Conditions: 515f-806r region of the 16S rRNA gene (Caporaso et al PNAS 2010):

Complete reagent recipe (master mix) for 1X PCR reaction

PCR Grade H2O (note 1, below) 13.0 µL

5 Primer Hot MM (note 2, below) 10.0 µL

Forward primer (10µM) 0.5 µL

Reverse primer (10µM) 0.5 µL

Template DNA 1.0 µL

Total reaction volume 25.0 µL

Notes:

PCR grade water was purchased from MoBio Laboratories (MoBio Labs: Item#17000-11)

Five Prime Hot Master Mix (5 prime: Item# 2200410)

Final primer concentration of master mix: 0.2 µM

Thermocycler Conditions for 96 well thermocycler:

94°C 3 minutes

94°C 45 seconds

50°C 60 seconds

72°C 90 seconds

Repeat steps 2-4 35 times

72°C 10 minutes

4°C HOLD

Thermocycler Conditions for 384 well thermocycler:

94°C 3 minutes

94°C 60 seconds

50°C 60 seconds

72°C 105 seconds

Repeat steps 2-4 35 times

72°C 10 minutes

4°C HOLD

Protocol:

Amplify samples in each of the three technical replicates, meaning each sample will be amplified in 3 replicate 25 µL PCR reactions.

Combine the three technical replicate PCR reactions for each sample into a single volume. Combination will result in a total of 75 µL of amplicon for each sample. Do NOT combine amplicons from different samples at this point.

Run amplicons for each sample on an agarose gel. Expected band size for 515f/806r is roughly 300 – 350 bp.

Quantify amplicons with Picogreen (see manufacturers protocol; Invitrogen Item #P11496).

Combine an equal amount of amplicon from each sample into a single, sterile tube. Generally, 240 ng of DNA per sample are pooled. However, higher amounts can be used if the final pool will be gel isolated or when working with low biomass samples. (Note: When working with multiple plates of samples, it is typical to produce a single tube of amplicons for each plate of samples.)

Clean Amplicon pool using MoBio UltraClean PCR Clean-Up Kit #12500 according to the manufacturer's instructions. If working with more than 96 samples, the pool may need to be split evenly for cleaning and then recombined. (Optional: if spurious bands were present on gel (in step 3), ½ of the final pool can be run on a gel and then gel extracted to select only the target bands.)

Measure concentration and 260/280 of final pool that has been cleaned. For best results the 260/280 should be between 1.8-2.0.

Send an aliquot for sequencing along with sequencing primers listed below.

IMPORTANT: Sequencing requires use of 16S and Index sequencing primers, constructs below

Appendix E. Primer sequences used for PCR amplification

Read 1 sequencing primer:

Field description (space-delimited):

Forward primer pad

Forward primer linker

Forward primer

TATGGTAATT GT GTGYCAGCMGCCGCGGTAA

Read 2 sequencing primer:

Field description (space-delimited):

Reverse primer pad

Reverse primer linker

Reverse primer

AGTCAGTCAG CC GGACTACNVGGGTWTCTAAT

Index sequence primer:

Field description (space-delimited):

Reverse complement of reverse primer

Reverse complement of reverse primer linker

Reverse complement of reverse primer pad

ATTAGAWACCCBDGTAGTCC GG CTGACTGACT

Appendix F. Qubit dsDNA HS Assay Kit Standard Operating Procedure

Qubit® dsDNA HS Assay Kit

1. Prepare samples and standards

   This protocol assumes that you are preparing standards for calibrating the Qubit® Fluorometer. If you plan to use the last calibration performed on the instrument (see “Qubit® Fluorometer calibration” on page 2), you need fewer tubes (step 1.1) and less working solution (step 1.3).

1.1. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® dsDNA HS Assay requires 2 standards.

   Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856) or Axygen® PCR-05-C tubes (part no. 10011-830).

1.2. Label the tube lids. Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit® Fluorometer requires the standards to be inserted into the instrument in the right order.

1.3. Prepare the Qubit® working solution by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. **Do not mix the working solution in a glass container.**

   Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit® working solution, and each sample tube requires anywhere from 180–199 µL. Prepare sufficient Qubit® working solution to accommodate all standards and samples.

   For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit® reagent plus 1990 µL of Qubit® buffer).

1.4. Add 190 µL of Qubit® working solution to each of the tubes used for standards.

1.5. Add 10 µL of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit® standard is added to 190 µL of Qubit® working solution.

1.6. Add Qubit® working solution to individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere from 1–20 µL. Add a corresponding volume of Qubit® working solution to each assay tube: anywhere from 180–199 µL.

1.7. Add each sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8. Allow all tubes to incubate at room temperature for 2 minutes. Proceed to "Reading standards and samples"; follow the procedure appropriate for your instrument:

- "Qubit® 3.0 Fluorometer" on page 4
- "Qubit® 2.0 Fluorometer" on page 5

2. Read standards and samples Qubit® 3.0 Fluorometer

2.1. On the Home screen of the Qubit® 3.0 Fluorometer, press **DNA**, then select **dsDNA High Sensitivity** as the assay type. The "Read standards" screen is displayed. Press **Read Standards** to proceed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 2.4. Otherwise, continue with step 2.2.

2.2. Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

2.3. Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard #2.

The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the Qubit® 3.0 Fluorometer User Guide.

2.4. Press **Run samples**.

2.5. On the assay screen, select the sample volume and units:

a) Press the + or – buttons on the wheel to select the sample volume added to the assay tube (from 1–20 µL).

b) From the dropdown menu, select the units for the output sample concentration.

2.6. Insert a sample tube into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove the sample tube.

The instrument displays the results on the assay screen. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit® 3.0 Fluorometer User Guide.*

2.7. Repeat step 2.6 until all samples have been read.

3. Qubit® 2.0 Fluorometer

3.1. On the Home screen of the Qubit® 2.0 Fluorometer, press **DNA**, then select **dsDNA High Sensitivity** as the assay type. The Standards screen is displayed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press **No** and skip to step 3.5. Otherwise, continue with step 3.2.

3.2. On the Standards screen, press **Yes** to read the standards.

3.3. Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read**. When the reading is complete (~3 seconds), remove Standard #1.

3.4. Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read**. When the reading is complete, remove Standard #2.

When the calibration is complete, the instrument displays the Sample screen.

3.5. Insert a sample tube into the sample chamber, close the lid, then press **Read.** When the reading is complete (~3 seconds), remove the sample tube.

The instrument displays the results on the Sample screen. The value displayed corresponds to the concentration after your sample was diluted into the assay tube. To find the concentration of your original sample, you can record this value and perform the calculation yourself (see "Calculating the sample concentration" below) or the instrument can perform this calculation for you (see "Dilution Calculator" on page 6).

3.6. Repeat step 3.5 until all samples have been read.

Calculate the sample concentration – Qubit® 2.0 Fluorometer

Note: The Qubit® 3.0 Fluorometer performs this calculation automatically.

The Qubit® 2.0 Fluorometer gives values for the Qubit® dsDNA HS Assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

Concentration of your sample = QF value × 200/ x

where QF value = the value given by the Qubit® 2.0 Fluorometer

x = the number of microliters of sample added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer. For example, if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation is in ng/mL.

4. Dilution Calculator– Qubit® 2.0 Fluorometer

The Dilution Calculator feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instructions below.

4.1. After the sample measurement is complete, press **Calculate Stock Conc**. The Dilution Calculator screen is displayed.

4.2. Using the volume roller wheel, select the volume of your original sample that you added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

4.3. To change the units in which the original sample concentration is displayed:

a) Press **ng/mL**.

b) On the unit selection pop-up window, select a unit for your original sample concentration.

c) Touch anywhere on the screen to close the pop-up window. The Qubit® 2.0 Fluorometer automatically converts the units to your selection.

Note: The unit button next to your sample concentration reflects the change in units. For example, if you changed the unit to pg/µL, the button displays pg/µL.

4.4. To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement is saved in the *.csv file and tagged with a time and date stamp.

4.5. To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or press **Read Next Sample.**

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and units on the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix G. QIAquick PCR purification kit Protocol

QIAquick® PCR Purification Kit Protocol

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10 μl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
2. Place a QIAquick column in a provided 2 ml collection tube or into a vacuum manifold. For details on how to set up a vacuum manifold, refer to the QIAquick Spin Handbook.
3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s or apply vacuum to the manifold until all the samples have passed through the column. Discard flow-through and place the QIAquick column back in the same tube.
4. To wash, add 0.75 ml Buffer PE to the QIAquick column centrifuge for 30–60 s or apply vacuum. Discard flow-through and place the QIAquick column back in the same tube.
5. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
6. Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 50 μl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 μl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.
8. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Appendix H. Illumina Sequencing Results—Normalized

Phylum rank

Site	Sample ID	Kingdom	Phylum	Phylum Relative Abundance
Dover	DV1	Archaea	Euryarchaeota	0.001104978
Dover	DV1	Bacteria	Other	0.00071359
Dover	DV1	Bacteria	Acidobacteria	0.000336037
Dover	DV1	Bacteria	Actinobacteria	0.112065515
Dover	DV1	Bacteria	Bacteroidetes	0.10908657
Dover	DV1	Bacteria	Cyanobacteria	0.03793317
Dover	DV1	Bacteria	Firmicutes	0.274044795
Dover	DV1	Bacteria	Fusobacteria	0.009753495
Dover	DV1	Bacteria	GN04	2.41021E-05
Dover	DV1	Bacteria	Gemmatimonadetes	2.41021E-05
Dover	DV1	Bacteria	Planctomycetes	0.014143715
Dover	DV1	Bacteria	Proteobacteria	0.439251497
Dover	DV1	Bacteria	Spirochaetes	0.000602553
Dover	DV1	Bacteria	Tenericutes	0.000891779
Dover	DV1	Bacteria	Verrucomicrobia	2.41021E-05
Dover	DV2	Archaea	Euryarchaeota	0.0063376980
Dover	DV2	Bacteria	Other	0.0002274583
Dover	DV2	Bacteria	Acidobacteria	0.0045690381
Dover	DV2	Bacteria	Actinobacteria	0.0558298851
Dover	DV2	Bacteria	Bacteroidetes	0.2857383125
Dover	DV2	Bacteria	Chlamydiae	0.0023582132
Dover	DV2	Bacteria	Cyanobacteria	0.0568772717
Dover	DV2	Bacteria	Firmicutes	0.1039916058
Dover	DV2	Bacteria	Fusobacteria	0.1636348582
Dover	DV2	Bacteria	Nitrospirae	0.0060429214
Dover	DV2	Bacteria	Proteobacteria	0.2899262754
Dover	DV2	Bacteria	TM6	0.0051585914
Dover	DV2	Bacteria	Verrucomicrobia	0.0004421650
Dover	DV2	Bacteria	Thermi	0.0188657058
Dover	DV3	Unclassified	Other	1.5776E-05
Dover	DV3	Archaea	Euryarchaeota	0.003705162
Dover	DV3	Archaea	Parvarchaeota	1.04995E-05
Dover	DV3	Bacteria	Other	0.007920434

Dover	DV3	Bacteria	Acidobacteria	0.000154864
Dover	DV3	Bacteria	Actinobacteria	0.089283977
Dover	DV3	Bacteria	Armatimonadetes	0.000771258
Dover	DV3	Bacteria	Bacteroidetes	0.237834926
Dover	DV3	Bacteria	Caldiserica	0.000481143
Dover	DV3	Bacteria	Chlorobi	0.000111459
Dover	DV3	Bacteria	Chloroflexi	0.000521394
Dover	DV3	Bacteria	Cyanobacteria	7.39332E-05
Dover	DV3	Bacteria	Elusimicrobia	0.005408248
Dover	DV3	Bacteria	FCPU426	1.04995E-05
Dover	DV3	Bacteria	Firmicutes	0.108392019
Dover	DV3	Bacteria	Gemmatimonadetes	3.15519E-05
Dover	DV3	Bacteria	Lentisphaerae	3.14986E-05
Dover	DV3	Bacteria	OP11	0.000102324
Dover	DV3	Bacteria	OP3	0.000158108
Dover	DV3	Bacteria	Planctomycetes	0.000268191
Dover	DV3	Bacteria	Proteobacteria	0.54325891
Dover	DV3	Bacteria	Spirochaetes	0.001228405
Dover	DV3	Bacteria	TM6	2.0999E-05
Dover	DV3	Bacteria	Verrucomicrobia	0.000204421
Dover	DV4	Bacteria	Other	0.000128028
Dover	DV4	Bacteria	Actinobacteria	0.07186938
Dover	DV4	Bacteria	Bacteroidetes	0.266434363
Dover	DV4	Bacteria	Chloroflexi	0.000128028
Dover	DV4	Bacteria	Cyanobacteria	0.05862308
Dover	DV4	Bacteria	Firmicutes	0.165976306
Dover	DV4	Bacteria	Nitrospirae	0.026470567
Dover	DV4	Bacteria	Proteobacteria	0.353606651
Dover	DV4	Bacteria	TM6	0.056763598
Dover	DV4	Bacteria	Thermi	0.046729629
Dover	DV5	Bacteria	Other	0.000261707
Dover	DV5	Bacteria	Acidobacteria	0.000190682
Dover	DV5	Bacteria	Actinobacteria	0.025735199
Dover	DV5	Bacteria	Bacteroidetes	0.174883951
Dover	DV5	Bacteria	Cyanobacteria	0.001125717
Dover	DV5	Bacteria	Firmicutes	0.191907365
Dover	DV5	Bacteria	Nitrospirae	0.036625876
Dover	DV5	Bacteria	Planctomycetes	0.000147685
Dover	DV5	Bacteria	Proteobacteria	0.568909652
Dover	DV5	Bacteria	Spirochaetes	0.000212167
Dover	DV5	Bacteria	Verrucomicrobia	0.000212167

Dover	DV6	Bacteria	Acidobacteria	0.041834873
Dover	DV6	Bacteria	Actinobacteria	0.023017012
Dover	DV6	Bacteria	Bacteroidetes	0.126410802
Dover	DV6	Bacteria	Chlamydiae	0.003233302
Dover	DV6	Bacteria	Chloroflexi	6.4666E-05
Dover	DV6	Bacteria	Cyanobacteria	0.019050163
Dover	DV6	Bacteria	Elusimicrobia	0.030172284
Dover	DV6	Bacteria	Firmicutes	0.007226249
Dover	DV6	Bacteria	Gemmatimonadetes	0.013773867
Dover	DV6	Bacteria	Nitrospirae	0.049582396
Dover	DV6	Bacteria	Proteobacteria	0.509849783
Dover	DV6	Bacteria	Verrucomicrobia	0.090516851
Dover	DV6	Bacteria	WS3	0.030172284
Dover	DV6	Bacteria	Thermi	0.055095469
Tinker	TK131	Unclassified	Other	2.48534E-05
Tinker	TK131	Archaea	Other	5.4396E-05
Tinker	TK131	Archaea	Crenarchaeota	0.000420465
Tinker	TK131	Archaea	Euryarchaeota	0.000112432
Tinker	TK131	Archaea	Parvarchaeota	0.00013951
Tinker	TK131	Bacteria	Other	0.002254567
Tinker	TK131	Bacteria	Acidobacteria	0.007995989
Tinker	TK131	Bacteria	Actinobacteria	0.087175194
Tinker	TK131	Bacteria	Armatimonadetes	1.22562E-05
Tinker	TK131	Bacteria	BRC1	1.93962E-05
Tinker	TK131	Bacteria	Bacteroidetes	0.119579985
Tinker	TK131	Bacteria	Chlamydiae	6.07684E-05
Tinker	TK131	Bacteria	Chlorobi	9.92412E-05
Tinker	TK131	Bacteria	Chloroflexi	0.000980612
Tinker	TK131	Bacteria	Cyanobacteria	0.000335975
Tinker	TK131	Bacteria	Elusimicrobia	7.04108E-05
Tinker	TK131	Bacteria	FBP	6.62758E-05
Tinker	TK131	Bacteria	Fibrobacteres	5.80961E-05
Tinker	TK131	Bacteria	Firmicutes	0.001185682
Tinker	TK131	Bacteria	Fusobacteria	7.2528E-05
Tinker	TK131	Bacteria	GAL15	3.89779E-05
Tinker	TK131	Bacteria	GN02	5.4396E-05
Tinker	TK131	Bacteria	GN04	0.000131248
Tinker	TK131	Bacteria	Gemmatimonadetes	0.000331979
Tinker	TK131	Bacteria	NC10	0.000482801
Tinker	TK131	Bacteria	Nitrospirae	0.000883396
Tinker	TK131	Bacteria	OD1	8.96267E-05

Tinker	TK131	Bacteria	OP1	2.7198E-05
Tinker	TK131	Bacteria	OP3	9.01666E-05
Tinker	TK131	Bacteria	Planctomycetes	0.001609143
Tinker	TK131	Bacteria	Poribacteria	8.66643E-06
Tinker	TK131	Bacteria	Proteobacteria	0.773152232
Tinker	TK131	Bacteria	SBR1093	3.00214E-05
Tinker	TK131	Bacteria	Synergistetes	1.8132E-05
Tinker	TK131	Bacteria	TM6	2.46404E-05
Tinker	TK131	Bacteria	Tenericutes	1.65989E-05
Tinker	TK131	Bacteria	Verrucomicrobia	0.002248558
Tinker	TK131	Bacteria	WS3	3.45216E-05
Tinker	TK131	Bacteria	Thermi	9.066E-06
Tinker	TK138	Archaea	Crenarchaeota	0.000214161
Tinker	TK138	Archaea	Euryarchaeota	3.71933E-05
Tinker	TK138	Archaea	Parvarchaeota	1.00957E-05
Tinker	TK138	Bacteria	Other	0.000157795
Tinker	TK138	Bacteria	Acidobacteria	0.001631671
Tinker	TK138	Bacteria	Actinobacteria	0.00452504
Tinker	TK138	Bacteria	Armatimonadetes	1.88519E-05
Tinker	TK138	Bacteria	Bacteroidetes	0.102966442
Tinker	TK138	Bacteria	Chlamydiae	2.32531E-05
Tinker	TK138	Bacteria	Chlorobi	7.78198E-05
Tinker	TK138	Bacteria	Chloroflexi	0.000381544
Tinker	TK138	Bacteria	Cyanobacteria	0.000176524
Tinker	TK138	Bacteria	Elusimicrobia	9.42596E-06
Tinker	TK138	Bacteria	Firmicutes	0.001514943
Tinker	TK138	Bacteria	GAL15	5.97383E-05
Tinker	TK138	Bacteria	GN02	6.59817E-05
Tinker	TK138	Bacteria	Gemmatimonadetes	7.05268E-05
Tinker	TK138	Bacteria	NC10	0.000232095
Tinker	TK138	Bacteria	Nitrospirae	0.000486532
Tinker	TK138	Bacteria	OP3	6.77242E-05
Tinker	TK138	Bacteria	Planctomycetes	0.000164633
Tinker	TK138	Bacteria	Proteobacteria	0.885268308
Tinker	TK138	Bacteria	SBR1093	9.42596E-05
Tinker	TK138	Bacteria	Spirochaetes	0.000113112
Tinker	TK138	Bacteria	TM6	5.65558E-05
Tinker	TK138	Bacteria	TM7	2.82779E-05
Tinker	TK138	Bacteria	Tenericutes	0.000141389
Tinker	TK138	Bacteria	Verrucomicrobia	0.001310052
Tinker	TK138	Bacteria	WS3	3.77038E-05

Tinker	TK138	Bacteria	WWE1	1.1138E-05
Tinker	TK138	Bacteria	Thermi	4.7213E-05
Tinker	TK430	Archaea	Crenarchaeota	0.001475843
Tinker	TK430	Archaea	Euryarchaeota	0.000322353
Tinker	TK430	Archaea	Parvarchaeota	0.000432034
Tinker	TK430	Bacteria	Other	0.003108525
Tinker	TK430	Bacteria	Acidobacteria	0.044152221
Tinker	TK430	Bacteria	Actinobacteria	0.023256876
Tinker	TK430	Bacteria	Armatimonadetes	0.000105799
Tinker	TK430	Bacteria	BHI80-139	4.65632E-05
Tinker	TK430	Bacteria	BRC1	0.000197894
Tinker	TK430	Bacteria	Bacteroidetes	0.071446491
Tinker	TK430	Bacteria	Chlamydiae	9.56988E-05
Tinker	TK430	Bacteria	Chlorobi	0.000397024
Tinker	TK430	Bacteria	Chloroflexi	0.003452607
Tinker	TK430	Bacteria	Cyanobacteria	0.003196479
Tinker	TK430	Bacteria	Elusimicrobia	0.000875291
Tinker	TK430	Bacteria	FBP	0.001047672
Tinker	TK430	Bacteria	Fibrobacteres	0.000803215
Tinker	TK430	Bacteria	Firmicutes	0.003930018
Tinker	TK430	Bacteria	Fusobacteria	0.000218693
Tinker	TK430	Bacteria	GAL15	0.00055442
Tinker	TK430	Bacteria	GN02	0.001583149
Tinker	TK430	Bacteria	GN04	0.00177162
Tinker	TK430	Bacteria	Gemmatimonadetes	0.002976464
Tinker	TK430	Bacteria	NC10	0.005245207
Tinker	TK430	Bacteria	NKB19	0.001117517
Tinker	TK430	Bacteria	Nitrospirae	0.0070222
Tinker	TK430	Bacteria	OD1	0.000219467
Tinker	TK430	Bacteria	OP11	2.91384E-05
Tinker	TK430	Bacteria	OP3	0.000654162
Tinker	TK430	Bacteria	OP8	1.45692E-05
Tinker	TK430	Bacteria	PAUC34f	0.000153342
Tinker	TK430	Bacteria	Planctomycetes	0.002304947
Tinker	TK430	Bacteria	Proteobacteria	0.811211093
Tinker	TK430	Bacteria	SBR1093	0.000162971
Tinker	TK430	Bacteria	Spirochaetes	1.45692E-05
Tinker	TK430	Bacteria	Synergistetes	7.2846E-05
Tinker	TK430	Bacteria	TM6	3.06685E-05
Tinker	TK430	Bacteria	TM7	6.1337E-05
Tinker	TK430	Bacteria	Verrucomicrobia	0.005414128

Tinker	TK430	Bacteria	WS3	0.000824886
Tinker	TK431	Archaea	Crenarchaeota	0.005449749
Tinker	TK431	Archaea	Euryarchaeota	0.002173654
Tinker	TK431	Archaea	Parvarchaeota	0.000857938
Tinker	TK431	Bacteria	Other	0.005488059
Tinker	TK431	Bacteria	Acidobacteria	0.043302267
Tinker	TK431	Bacteria	Actinobacteria	0.016570886
Tinker	TK431	Bacteria	Armatimonadetes	0.000252581
Tinker	TK431	Bacteria	Bacteroidetes	0.047209882
Tinker	TK431	Bacteria	Chlorobi	0.00101373
Tinker	TK431	Bacteria	Chloroflexi	0.007165261
Tinker	TK431	Bacteria	Cyanobacteria	0.00069733
Tinker	TK431	Bacteria	Elusimicrobia	6.96407E-05
Tinker	TK431	Bacteria	FBP	0.000285832
Tinker	TK431	Bacteria	Firmicutes	0.005796374
Tinker	TK431	Bacteria	Fusobacteria	0.001370123
Tinker	TK431	Bacteria	GAL15	0.000977739
Tinker	TK431	Bacteria	GN02	0.000232053
Tinker	TK431	Bacteria	GN04	0.001682041
Tinker	TK431	Bacteria	Gemmatimonadetes	0.00047913
Tinker	TK431	Bacteria	NC10	0.004576465
Tinker	TK431	Bacteria	Nitrospirae	0.006232967
Tinker	TK431	Bacteria	OD1	0.000694709
Tinker	TK431	Bacteria	OP1	6.15563E-05
Tinker	TK431	Bacteria	OP3	0.000397714
Tinker	TK431	Bacteria	Planctomycetes	0.00384153
Tinker	TK431	Bacteria	Proteobacteria	0.838006147
Tinker	TK431	Bacteria	SBR1093	0.000101921
Tinker	TK431	Bacteria	Spirochaetes	1.09935E-05
Tinker	TK431	Bacteria	TM6	0.000980347
Tinker	TK431	Bacteria	TM7	7.69547E-05
Tinker	TK431	Bacteria	Tenericutes	0.000945443
Tinker	TK431	Bacteria	Thermotogae	3.29806E-05
Tinker	TK431	Bacteria	Verrucomicrobia	0.001276974
Tinker	TK431	Bacteria	WS3	0.001469158
Tinker	TK431	Bacteria	Thermi	0.000219871

Class rank

Site	Sample ID	Kingdom	Phylum	Class	Class Relative Abundance
Dover	DV1	Archaea	Euryarchaeota	Methanomicrobia	0.068497139
Dover	DV1	Bacteria	Acidobacteria	Acidobacteria-6	0.02904093
Dover	DV1	Bacteria	Acidobacteria	Acidobacteriia	0.014939865
Dover	DV1	Bacteria	Actinobacteria	Actinobacteria	0.059917466
Dover	DV1	Bacteria	Actinobacteria	MB-A2-108	0.008101202
Dover	DV1	Bacteria	Actinobacteria	Rubrobacteria	0.029879768
Dover	DV1	Bacteria	Actinobacteria	Thermoleophilia	0.027397277
Dover	DV1	Bacteria	Bacteroidetes	Bacteroidia	0.011101307
Dover	DV1	Bacteria	Bacteroidetes	Cytophagia	0.012430381
Dover	DV1	Bacteria	Bacteroidetes	Flavobacteriia	0.009727346
Dover	DV1	Bacteria	Bacteroidetes	Saprospirae	0.010575282
Dover	DV1	Bacteria	Bacteroidetes	SM1A07	0.059610063
Dover	DV1	Bacteria	Bacteroidetes	Sphingobacteriia	0.027397277
Dover	DV1	Bacteria	Chlorobi	Unclassified	0.068497139
Dover	DV1	Bacteria	Cyanobacteria	Chloroplast	0.020318285
Dover	DV1	Bacteria	Cyanobacteria	ML635J-21	0.010039589
Dover	DV1	Bacteria	FBP	Unclassified	0.02904093
Dover	DV1	Bacteria	FCPU426	Unclassified	0.014939865
Dover	DV1	Bacteria	Firmicutes	Bacilli	0.013188984
Dover	DV1	Bacteria	Firmicutes	Clostridia	0.031695959
Dover	DV1	Bacteria	Firmicutes	Erysipelotrichi	0.025638168
Dover	DV1	Bacteria	Firmicutes	Unclassified	0.018824235
Dover	DV1	Bacteria	Fusobacteria	Fusobacteriia	0.020821799
Dover	DV1	Bacteria	GAL15	Unclassified	0.017554342
Dover	DV1	Bacteria	Gemmatimonadetes	Gemm-3	0.014939865
Dover	DV1	Bacteria	GN04	Unclassified	0.014939865
Dover	DV1	Bacteria	PAUC34f	Unclassified	0.014939865
Dover	DV1	Bacteria	Planctomycetes	Pla4	0.02904093
Dover	DV1	Bacteria	Planctomycetes	Planctomycetia	0.011448612
Dover	DV1	Bacteria	Proteobacteria	Alphaproteobacteria	0.065039848
Dover	DV1	Bacteria	Proteobacteria	Betaproteobacteria	0.021521803
Dover	DV1	Bacteria	Proteobacteria	Deltaproteobacteria	0.024254429
Dover	DV1	Bacteria	Proteobacteria	Epsilonproteobacteria	0.029097472
Dover	DV1	Bacteria	Proteobacteria	Gammaproteobacteria	0.014939865

Dover	DV1	Bacteria	SR1	Unclassified	0.037349663
Dover	DV1	Bacteria	Tenericutes	RF3	0.055277502
Dover	DV1	Bacteria	Unclassified	Unclassified	0.013095816
Dover	DV1	Bacteria	Verrucomicrobia	Spartobacteria	0.014939865
Dover	DV2	Archaea	Crenarchaeota	Thaumarchaeota	0.010919623
Dover	DV2	Archaea	Euryarchaeota	DSEG	0.016692565
Dover	DV2	Archaea	Euryarchaeota	Unclassified	0.003538689
Dover	DV2	Archaea	Parvarchaeota	Parvarchaea	0.011658191
Dover	DV2	Archaea	Unclassified	Unclassified	0.005732526
Dover	DV2	Bacteria	Acidobacteria	Acidobacteria-5	0.014159421
Dover	DV2	Bacteria	Acidobacteria	Acidobacteria-6	0.009725033
Dover	DV2	Bacteria	Acidobacteria	BPC102	0.014988096
Dover	DV2	Bacteria	Acidobacteria	Chloracidobacteria	0.008512187
Dover	DV2	Bacteria	Acidobacteria	EC1113	0.011988118
Dover	DV2	Bacteria	Acidobacteria	GAL08	0.010422255
Dover	DV2	Bacteria	Acidobacteria	iii1-8	0.006183355
Dover	DV2	Bacteria	Acidobacteria	PAUC37f	0.004221638
Dover	DV2	Bacteria	Acidobacteria	S035	0.008135004
Dover	DV2	Bacteria	Acidobacteria	Solibacteres	0.005614256
Dover	DV2	Bacteria	Acidobacteria	TM1	0.012876616
Dover	DV2	Bacteria	Acidobacteria	Unclassified	0.00378807
Dover	DV2	Bacteria	Actinobacteria	Acidimicrobiia	0.014861981
Dover	DV2	Bacteria	Actinobacteria	Actinobacteria	0.008821063
Dover	DV2	Bacteria	Actinobacteria	Rubrobacteria	0.010668137
Dover	DV2	Bacteria	Actinobacteria	Thermoleophilia	0.020194168
Dover	DV2	Bacteria	Actinobacteria	Unclassified	0.009970419
Dover	DV2	Bacteria	AD3	ABS-6	0.005654271
Dover	DV2	Bacteria	AD3	JG37-AG-4	0.008089022
Dover	DV2	Bacteria	AD3	Unclassified	0.004691848
Dover	DV2	Bacteria	Armatimonadetes	0319-6E2	0.004714701
Dover	DV2	Bacteria	Armatimonadetes	Armatimonadia	0.010919623
Dover	DV2	Bacteria	Armatimonadetes	Chthonomonadetes	0.008220206
Dover	DV2	Bacteria	Armatimonadetes	Fimbriimonadia	0.008640774
Dover	DV2	Bacteria	Bacteroidetes	Bacteroidia	0.016264468
Dover	DV2	Bacteria	Bacteroidetes	Cytophagia	0.020600005
Dover	DV2	Bacteria	Bacteroidetes	Flavobacteriia	0.016692565
Dover	DV2	Bacteria	Bacteroidetes	Saprospirae	0.009372244
Dover	DV2	Bacteria	Bacteroidetes	Sphingobacteriia	0.015747673
Dover	DV2	Bacteria	Bacteroidetes	Unclassified	0.015208772

Dover	DV2	Bacteria	BRC1	PRR-11	0.00327845
Dover	DV2	Bacteria	Chlamydiae	Chlamydiia	0.006458548
Dover	DV2	Bacteria	Chlorobi	BSV26	0.006645477
Dover	DV2	Bacteria	Chloroflexi	Anaerolineae	0.00760216
Dover	DV2	Bacteria	Chloroflexi	C0119	0.009320205
Dover	DV2	Bacteria	Chloroflexi	Dehalococcoidetes	0.003709457
Dover	DV2	Bacteria	Chloroflexi	Ellin6529	0.011368558
Dover	DV2	Bacteria	Chloroflexi	Ktedonobacteria	0.01314089
Dover	DV2	Bacteria	Chloroflexi	P2-11E	0.006949736
Dover	DV2	Bacteria	Chloroflexi	S085	0.003538689
Dover	DV2	Bacteria	Chloroflexi	SAR202	0.010209773
Dover	DV2	Bacteria	Chloroflexi	Thermomicrobia	0.003709457
Dover	DV2	Bacteria	Chloroflexi	TK10	0.006794482
Dover	DV2	Bacteria	Chloroflexi	TK17	0.012550846
Dover	DV2	Bacteria	Chloroflexi	Unclassified	0.012786184
Dover	DV2	Bacteria	Cyanobacteria	4C0d-2	0.006153329
Dover	DV2	Bacteria	Cyanobacteria	Oscillatoriophycideae	0.005732526
Dover	DV2	Bacteria	Cyanobacteria	Unclassified	0.011658191
Dover	DV2	Bacteria	Elusimicrobia	Unclassified	0.014159421
Dover	DV2	Bacteria	FBP	Unclassified	0.009725033
Dover	DV2	Bacteria	FCPU426	Unclassified	0.005732526
Dover	DV2	Bacteria	Fibrobacteres	Fibrobacteria	0.013172921
Dover	DV2	Bacteria	Firmicutes	Bacilli	0.011200438
Dover	DV2	Bacteria	Firmicutes	Clostridia	0.005881581
Dover	DV2	Bacteria	GAL15	Unclassified	0.005473545
Dover	DV2	Bacteria	Gemmatimonadetes	Gemm-1	0.012095749
Dover	DV2	Bacteria	Gemmatimonadetes	Gemm-2	0.006316718
Dover	DV2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	0.003317364
Dover	DV2	Bacteria	GN02	3BR-5F	0.007047968
Dover	DV2	Bacteria	GN02	GKS2-174	0.01782826
Dover	DV2	Bacteria	GN04	MSB-5A5	0.009577618
Dover	DV2	Bacteria	GN04	Unclassified	0.011658191
Dover	DV2	Bacteria	GN04	Unclassified	0.023417504
Dover	DV2	Bacteria	NC10	12-24	0.003709457
Dover	DV2	Bacteria	Nitrospirae	Nitrospira	0.009818138
Dover	DV2	Bacteria	OD1	ABY1	0.011318181
Dover	DV2	Bacteria	OD1	Mb-NB09	0.007507984
Dover	DV2	Bacteria	OD1	Unclassified	0.004278782
Dover	DV2	Bacteria	OD1	ZB2	0.009365295

Dover	DV2	Bacteria	OP3	koll11	0.007246145
Dover	DV2	Bacteria	OP3	PBS-25	0.00773784
Dover	DV2	Bacteria	PAUC34f	Unclassified	0.014159421
Dover	DV2	Bacteria	Planctomycetes	028H05-P-BN-P5	0.009835356
Dover	DV2	Bacteria	Planctomycetes	BD7-11	0.004783037
Dover	DV2	Bacteria	Planctomycetes	C6	0.008305807
Dover	DV2	Bacteria	Planctomycetes	OM190	0.013048285
Dover	DV2	Bacteria	Planctomycetes	Phycisphaerae	0.008001059
Dover	DV2	Bacteria	Planctomycetes	Pla3	0.006783424
Dover	DV2	Bacteria	Planctomycetes	Pla4	0.009725033
Dover	DV2	Bacteria	Planctomycetes	Planctomycetia	0.004177863
Dover	DV2	Bacteria	Planctomycetes	Unclassified	0.00950125
Dover	DV2	Bacteria	Planctomycetes	vadinHA49	0.008953144
Dover	DV2	Bacteria	Proteobacteria	Alphaproteobacteria	0.012383867
Dover	DV2	Bacteria	Proteobacteria	Betaproteobacteria	0.016425964
Dover	DV2	Bacteria	Proteobacteria	Deltaproteobacteria	0.007741128
Dover	DV2	Bacteria	Proteobacteria	Epsilonproteobacteria	0.004090171
Dover	DV2	Bacteria	Proteobacteria	TA18	0.004159984
Dover	DV2	Bacteria	Proteobacteria	Unclassified	0.010828112
Dover	DV2	Bacteria	Proteobacteria	Zetaproteobacteria	0.008512187
Dover	DV2	Bacteria	SBR1093	Unclassified	0.011988118
Dover	DV2	Bacteria	SBR1093	VHS-B5-50	0.010422255
Dover	DV2	Bacteria	Spirochaetes	Brevinematae	0.006183355
Dover	DV2	Bacteria	TM6	SBRH58	0.00752933
Dover	DV2	Bacteria	TM6	SJA-4	0.007869435
Dover	DV2	Bacteria	TM6	Unclassified	0.004159984
Dover	DV2	Bacteria	Unclassified	Unclassified	0.00877397
Dover	DV2	Bacteria	Verrucomicrobia	Methylacidiphilae	0.006524754
Dover	DV2	Bacteria	Verrucomicrobia	Opitutae	0.010828112
Dover	DV2	Bacteria	Verrucomicrobia	Pedosphaerae	0.006819505
Dover	DV2	Bacteria	Verrucomicrobia	Spartobacteria	0.007103615
Dover	DV2	Bacteria	Verrucomicrobia	Unclassified	0.004691848
Dover	DV2	Bacteria	WS2	SHA-109	0.008512187
Dover	DV2	Bacteria	WS3	PRR-12	0.00949724
Dover	DV3	Archaea	Euryarchaeota	Methanobacteria	0.02888448
Dover	DV3	Archaea	Euryarchaeota	Methanomicrobia	0.023759168
Dover	DV3	Archaea	Parvarchaeota	Parvarchaea	0.009331471
Dover	DV3	Bacteria	Acidobacteria	Acidobacteria-6	0.013634001
Dover	DV3	Bacteria	Acidobacteria	BPC102	0.009507118

Dover	DV3	Bacteria	Acidobacteria	Chloracidobacteria	0.021557249
Dover	DV3	Bacteria	Acidobacteria	Holophagae	0.009331471
Dover	DV3	Bacteria	Acidobacteria	RB25	0.026515511
Dover	DV3	Bacteria	Acidobacteria	Solibacteres	0.015006757
Dover	DV3	Bacteria	Actinobacteria	Acidimicrobiia	0.019920335
Dover	DV3	Bacteria	Actinobacteria	Actinobacteria	0.053348623
Dover	DV3	Bacteria	Actinobacteria	OPB41	0.027709032
Dover	DV3	Bacteria	Actinobacteria	Thermoleophilia	0.009183705
Dover	DV3	Bacteria	Actinobacteria	Unclassified	0.009960165
Dover	DV3	Bacteria	Armatimonadetes	0319-6E2	0.009331471
Dover	DV3	Bacteria	Armatimonadetes	SHA-37	0.018169304
Dover	DV3	Bacteria	Armatimonadetes	SJA-176	0.022272505
Dover	DV3	Bacteria	Bacteroidetes	Bacteroidia	0.014934082
Dover	DV3	Bacteria	Bacteroidetes	Saprospirae	0.018662942
Dover	DV3	Bacteria	Bacteroidetes	Unclassified	0.016679224
Dover	DV3	Bacteria	Caldiserica	OP5	0.02888448
Dover	DV3	Bacteria	Caldiserica	WCHB1-03	0.030426909
Dover	DV3	Bacteria	Chlorobi	SJA-28	0.022284756
Dover	DV3	Bacteria	Chlorobi	Unclassified	0.023759168
Dover	DV3	Bacteria	Chloroflexi	Anaerolineae	0.031758195
Dover	DV3	Bacteria	Chloroflexi	Unclassified	0.009074678
Dover	DV3	Bacteria	Cyanobacteria	4C0d-2	0.014760948
Dover	DV3	Bacteria	Cyanobacteria	Unclassified	0.009331471
Dover	DV3	Bacteria	FBP	Unclassified	0.013634001
Dover	DV3	Bacteria	Firmicutes	Bacilli	0.022089219
Dover	DV3	Bacteria	Firmicutes	Clostridia	0.009365514
Dover	DV3	Bacteria	Firmicutes	Erysipelotrichi	0.025496569
Dover	DV3	Bacteria	Gemmatimonadetes	Gemm-1	0.019920335
Dover	DV3	Bacteria	GN04	Unclassified	0.009331471
Dover	DV3	Bacteria	Lentisphaerae	Lentisphaeria	0.019886633
Dover	DV3	Bacteria	OP1	OP11-2	0.013935214
Dover	DV3	Bacteria	OP3	koll11	0.009982697
Dover	DV3	Bacteria	Planctomycetes	Pla4	0.013634001
Dover	DV3	Bacteria	Planctomycetes	Planctomycetia	0.016932281
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	0.012801185
Dover	DV3	Bacteria	Proteobacteria	Betaproteobacteria	0.019030926
Dover	DV3	Bacteria	Proteobacteria	Deltaproteobacteria	0.026312767
Dover	DV3	Bacteria	Proteobacteria	Epsilonproteobacteria	0.008847093
Dover	DV3	Bacteria	Proteobacteria	Unclassified	0.009507118

Dover	DV3	Bacteria	Proteobacteria	Zetaproteobacteria	0.021557249
Dover	DV3	Bacteria	SC4	Unclassified	0.009331471
Dover	DV3	Bacteria	Spirochaetes	Spirochaetes	0.01670881
Dover	DV3	Bacteria	TM6	SJA-4	0.018662942
Dover	DV3	Bacteria	Unclassified	Unclassified	0.050005898
Dover	DV3	Bacteria	Verrucomicrobia	Opitutae	0.009507118
Dover	DV3	Bacteria	Verrucomicrobia	Pedosphaerae	0.014279884
Dover	DV3	Bacteria	Verrucomicrobia	Spartobacteria	0.009960165
Dover	DV3	Bacteria	Verrucomicrobia	Verrucomicrobiae	0.019782803
Dover	DV3	Bacteria	WS2	SHA-109	0.021557249
Dover	DV3	Unclassified	Unclassified	Unclassified	0.009960165
Dover	DV4	Bacteria	Actinobacteria	Acidimicrobiia	0.019017766
Dover	DV4	Bacteria	Actinobacteria	Actinobacteria	0.049479746
Dover	DV4	Bacteria	Bacteroidetes	Bacteroidia	0.067755052
Dover	DV4	Bacteria	Bacteroidetes	Saprospirae	0.031106777
Dover	DV4	Bacteria	Bacteroidetes	Sphingobacteriia	0.018600372
Dover	DV4	Bacteria	Chloroflexi	Anaerolineae	0.019017766
Dover	DV4	Bacteria	Cyanobacteria	4C0d-2	0.046492732
Dover	DV4	Bacteria	Cyanobacteria	Chloroplast	0.163100033
Dover	DV4	Bacteria	Firmicutes	Bacilli	0.041195749
Dover	DV4	Bacteria	Firmicutes	Clostridia	0.064429275
Dover	DV4	Bacteria	Nitrospirae	Nitrospira	0.040750867
Dover	DV4	Bacteria	Proteobacteria	Alphaproteobacteria	0.071028948
Dover	DV4	Bacteria	Proteobacteria	Betaproteobacteria	0.065128371
Dover	DV4	Bacteria	Proteobacteria	Deltaproteobacteria	0.04561696
Dover	DV4	Bacteria	Proteobacteria	Epsilonproteobacteria	0.084527046
Dover	DV4	Bacteria	Thermi	Deinococci	0.069416762
Dover	DV4	Bacteria	TM6	SJA-4	0.084318011
Dover	DV4	Bacteria	Unclassified	Unclassified	0.019017766
Dover	DV5	Bacteria	Acidobacteria	Acidobacteria-6	0.029629178
Dover	DV5	Bacteria	Actinobacteria	Actinobacteria	0.06374795
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Dover	DV5	Bacteria	Bacteroidetes	Bacteroidia	0.051445332
Dover	DV5	Bacteria	Bacteroidetes	Saprospirae	0.116247741
Dover	DV5	Bacteria	Bacteroidetes	Sphingobacteriia	0.037253476
Dover	DV5	Bacteria	Cyanobacteria	Chloroplast	0.055314543
Dover	DV5	Bacteria	FBP	Unclassified	0.029629178
Dover	DV5	Bacteria	Firmicutes	Bacilli	0.053124922
Dover	DV5	Bacteria	Firmicutes	Clostridia	0.043703746

Dover	DV5	Bacteria	Nitrospirae	Nitrospira	0.056911261
Dover	DV5	Bacteria	Planctomycetes	Pla4	0.029629178
Dover	DV5	Bacteria	Planctomycetes	Planctomycetia	0.023072871
Dover	DV5	Bacteria	Proteobacteria	Alphaproteobacteria	0.037582338
Dover	DV5	Bacteria	Proteobacteria	Betaproteobacteria	0.037769325
Dover	DV5	Bacteria	Proteobacteria	Deltaproteobacteria	0.031048162
Dover	DV5	Bacteria	Proteobacteria	Epsilonproteobacteria	0.075665022
Dover	DV5	Bacteria	Spirochaetes	Spirochaetes	0.032967677
Dover	DV5	Bacteria	Unclassified	Unclassified	0.040665501
Dover	DV5	Bacteria	Verrucomicrobia	Pedosphaerae	0.032967677
Dover	DV6	Bacteria	Acidobacteria	Chloracidobacteria	0.021898653
Dover	DV6	Bacteria	Acidobacteria	RB25	0.034979716
Dover	DV6	Bacteria	Acidobacteria	Solibacteres	0.042845136
Dover	DV6	Bacteria	Actinobacteria	Actinobacteria	0.026684484
Dover	DV6	Bacteria	Bacteroidetes	Bacteroidia	0.072440394
Dover	DV6	Bacteria	Bacteroidetes	Cytophagia	0.034979716
Dover	DV6	Bacteria	Bacteroidetes	Saprospirae	0.01316854
Dover	DV6	Bacteria	Bacteroidetes	Sphingobacteriia	0.034979716
Dover	DV6	Bacteria	Chlamydiae	Chlamydiia	0.037484731
Dover	DV6	Bacteria	Chloroflexi	Anaerolineae	0.075009337
Dover	DV6	Bacteria	Cyanobacteria	4C0d-2	0.087714272
Dover	DV6	Bacteria	Cyanobacteria	Chloroplast	0.046118867
Dover	DV6	Bacteria	Firmicutes	Bacilli	0.01971784
Dover	DV6	Bacteria	Firmicutes	Clostridia	0.025163171
Dover	DV6	Bacteria	Gemmatimonadetes	Gemm-1	0.015968498
Dover	DV6	Bacteria	Nitrospirae	Nitrospira	0.057482495
Dover	DV6	Bacteria	Proteobacteria	Alphaproteobacteria	0.047017163
Dover	DV6	Bacteria	Proteobacteria	Betaproteobacteria	0.043377004
Dover	DV6	Bacteria	Proteobacteria	Deltaproteobacteria	0.015380112
Dover	DV6	Bacteria	Proteobacteria	Zetaproteobacteria	0.021898653
Dover	DV6	Bacteria	Thermi	Deinococci	0.063873981
Dover	DV6	Bacteria	Verrucomicrobia	Methylacidiphilae	0.034979716
Dover	DV6	Bacteria	Verrucomicrobia	Pedosphaerae	0.069959433
Dover	DV6	Bacteria	WS2	SHA-109	0.021898653
Dover	DV6	Bacteria	WS3	PRR-12	0.034979716
Tinker	TK131	Archaea	Crenarchaeota	MBGA	0.005730981
Tinker	TK131	Archaea	Crenarchaeota	Thaumarchaeota	0.00708032
Tinker	TK131	Archaea	Euryarchaeota	DSEG	0.015760921
Tinker	TK131	Archaea	Parvarchaeota	Parvarchaea	0.01274975

Tinker	TK131	Archaea	Unclassified	Unclassified	0.010710143
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-5	0.008508528
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-6	0.005123383
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteriia	0.005730981
Tinker	TK131	Bacteria	Acidobacteria	BPC102	0.014132428
Tinker	TK131	Bacteria	Acidobacteria	Chloracidobacteria	0.007347129
Tinker	TK131	Bacteria	Acidobacteria	EC1113	0.003946348
Tinker	TK131	Bacteria	Acidobacteria	Holophagae	0.008925119
Tinker	TK131	Bacteria	Acidobacteria	iii1-8	0.004914293
Tinker	TK131	Bacteria	Acidobacteria	PAUC37f	0.015431044
Tinker	TK131	Bacteria	Acidobacteria	RB25	0.009797471
Tinker	TK131	Bacteria	Acidobacteria	S035	0.007706446
Tinker	TK131	Bacteria	Acidobacteria	Solibacteres	0.009900086
Tinker	TK131	Bacteria	Acidobacteria	Sva0725	0.016065214
Tinker	TK131	Bacteria	Acidobacteria	TM1	0.008306791
Tinker	TK131	Bacteria	Acidobacteria	Unclassified	0.005154186
Tinker	TK131	Bacteria	Actinobacteria	Acidimicrobiia	0.007305619
Tinker	TK131	Bacteria	Actinobacteria	Actinobacteria	0.006763318
Tinker	TK131	Bacteria	Actinobacteria	OPB41	0.005355071
Tinker	TK131	Bacteria	Actinobacteria	Rubrobacteria	0.009222675
Tinker	TK131	Bacteria	Actinobacteria	Thermoleophilia	0.004552735
Tinker	TK131	Bacteria	Actinobacteria	Unclassified	0.008378383
Tinker	TK131	Bacteria	Armatimonadetes	0319-6E2	0.017925147
Tinker	TK131	Bacteria	Armatimonadetes	Armatimonadia	0.00708032
Tinker	TK131	Bacteria	Armatimonadetes	Fimbriimonadia	0.003577256
Tinker	TK131	Bacteria	Bacteroidetes	Bacteroidia	0.004550163
Tinker	TK131	Bacteria	Bacteroidetes	Cytophagia	0.013113636
Tinker	TK131	Bacteria	Bacteroidetes	Flavobacteriia	0.015760921
Tinker	TK131	Bacteria	Bacteroidetes	Rhodothermi	0.003262297
Tinker	TK131	Bacteria	Bacteroidetes	Saprospirae	0.013317327
Tinker	TK131	Bacteria	Bacteroidetes	Sphingobacteriia	0.007801982
Tinker	TK131	Bacteria	Bacteroidetes	Unclassified	0.005507338
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Tinker	TK131	Bacteria	Chlamydiae	Chlamydiia	0.011964811
Tinker	TK131	Bacteria	Chlorobi	BSV26	0.00743549
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Tinker	TK131	Bacteria	Chlorobi	SJA-28	0.006536384
Tinker	TK131	Bacteria	Chlorobi	Unclassified	0.007154512
Tinker	TK131	Bacteria	Chloroflexi	Anaerolineae	0.01343631

Tinker	TK131	Bacteria	Chloroflexi	Chloroflexi	0.016405182
Tinker	TK131	Bacteria	Chloroflexi	Ellin6529	0.005276552
Tinker	TK131	Bacteria	Chloroflexi	Gitt-GS-136	0.017384856
Tinker	TK131	Bacteria	Chloroflexi	Ktedonobacteria	0.005667607
Tinker	TK131	Bacteria	Chloroflexi	P2-11E	0.007384
Tinker	TK131	Bacteria	Chloroflexi	SAR202	0.006150263
Tinker	TK131	Bacteria	Chloroflexi	Thermomicrobia	0.016779413
Tinker	TK131	Bacteria	Chloroflexi	TK10	0.011324561
Tinker	TK131	Bacteria	Chloroflexi	Unclassified	0.018641611
Tinker	TK131	Bacteria	Cyanobacteria	4C0d-2	0.006838468
Tinker	TK131	Bacteria	Cyanobacteria	Chloroplast	0.009846851
Tinker	TK131	Bacteria	Cyanobacteria	ML635J-21	0.00903828
Tinker	TK131	Bacteria	Cyanobacteria	Oscillatoriophycideae	0.010710143
Tinker	TK131	Bacteria	Cyanobacteria	Unclassified	0.01274975
Tinker	TK131	Bacteria	Elusimicrobia	Unclassified	0.008508528
Tinker	TK131	Bacteria	FBP	Unclassified	0.005123383
Tinker	TK131	Bacteria	FCPU426	Unclassified	0.010710143
Tinker	TK131	Bacteria	Fibrobacteres	Fibrobacteria	0.011438672
Tinker	TK131	Bacteria	Firmicutes	Bacilli	0.006850208
Tinker	TK131	Bacteria	Firmicutes	Clostridia	0.00334013
Tinker	TK131	Bacteria	Fusobacteria	Fusobacteriia	0.015760921
Tinker	TK131	Bacteria	GAL15	Unclassified	0.00792995
Tinker	TK131	Bacteria	Gemmatimonadetes	Gemm-1	0.004619132
Tinker	TK131	Bacteria	Gemmatimonadetes	Gemm-3	0.008359423
Tinker	TK131	Bacteria	Gemmatimonadetes	Gemm-5	0.006524594
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Tinker	TK131	Bacteria	GN02	BB34	0.005355071
Tinker	TK131	Bacteria	GN02	GN07	0.005355071
Tinker	TK131	Bacteria	GN04	GN15	0.004179693
Tinker	TK131	Bacteria	GN04	MSB-5A5	0.010966553
Tinker	TK131	Bacteria	GN04	Unclassified	0.01274975
Tinker	TK131	Bacteria	NC10	12-24	0.00405266
Tinker	TK131	Bacteria	NC10	Unclassified	0.017112463
Tinker	TK131	Bacteria	NC10	wb1-A12	0.00943487
Tinker	TK131	Bacteria	Nitrospirae	Nitrospira	0.008075509
Tinker	TK131	Bacteria	OD1	ABY1	0.004007604
Tinker	TK131	Bacteria	OD1	Mb-NB09	0.005656187
Tinker	TK131	Bacteria	OD1	Unclassified	0.007154512
Tinker	TK131	Bacteria	OD1	ZB2	0.002283608

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Tinker	TK131	Bacteria	OP3	koll11	0.003237576
Tinker	TK131	Bacteria	OP3	PBS-25	0.00794728
Tinker	TK131	Bacteria	OP3	Unclassified	0.006524594
Tinker	TK131	Bacteria	PAUC34f	Unclassified	0.008508528
Tinker	TK131	Bacteria	Planctomycetes	BD7-11	0.00799906
Tinker	TK131	Bacteria	Planctomycetes	OM190	0.007154512
Tinker	TK131	Bacteria	Planctomycetes	Phycisphaerae	0.004432867
Tinker	TK131	Bacteria	Planctomycetes	Pla3	0.004938036
Tinker	TK131	Bacteria	Planctomycetes	Pla4	0.005123383
Tinker	TK131	Bacteria	Planctomycetes	Planctomycetia	0.011930199
Tinker	TK131	Bacteria	Planctomycetes	Unclassified	0.008175569
Tinker	TK131	Bacteria	Poribacteria	Unclassified	0.017887653
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	0.004365618
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	0.005314477
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	0.009380181
Tinker	TK131	Bacteria	Proteobacteria	Epsilonproteobacteria	0.003265151
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Tinker	TK131	Bacteria	Proteobacteria	TA18	0.008402185
Tinker	TK131	Bacteria	Proteobacteria	Unclassified	0.005730243
Tinker	TK131	Bacteria	Proteobacteria	Zetaproteobacteria	0.007347129
Tinker	TK131	Bacteria	SBR1093	Unclassified	0.003946348
Tinker	TK131	Bacteria	SC4	Unclassified	0.008925119
Tinker	TK131	Bacteria	Spirochaetes	Brevinematae	0.004914293
Tinker	TK131	Bacteria	Synergistetes	Synergistia	0.003577256
Tinker	TK131	Bacteria	Tenericutes	Mollicutes	0.003282122
Tinker	TK131	Bacteria	Thermi	Deinococci	0.017850238
Tinker	TK131	Bacteria	TM6	SJA-4	0.004854757
Tinker	TK131	Bacteria	TM6	Unclassified	0.008402185
Tinker	TK131	Bacteria	Unclassified	Unclassified	0.004439064
Tinker	TK131	Bacteria	Verrucomicrobia	Opitutae	0.005730243
Tinker	TK131	Bacteria	Verrucomicrobia	Pedosphaerae	0.009518382
Tinker	TK131	Bacteria	Verrucomicrobia	Spartobacteria	0.006834275
Tinker	TK131	Bacteria	Verrucomicrobia	Unclassified	0.003262297
Tinker	TK131	Bacteria	Verrucomicrobia	Verrucomicrobiae	0.004898736
Tinker	TK131	Bacteria	WS2	SHA-109	0.007347129
Tinker	TK131	Bacteria	WS3	PRR-12	0.007014167
Tinker	TK131	Unclassified	Unclassified	Unclassified	0.004893446
Tinker	TK138	Archaea	Crenarchaeota	MBGA	0.003541631

Tinker	TK138	Archaea	Crenarchaeota	MCG	0.027528633
Tinker	TK138	Archaea	Crenarchaeota	Thaumarchaeota	0.012756892
Tinker	TK138	Archaea	Euryarchaeota	Unclassified	0.005505728
Tinker	TK138	Archaea	Parvarchaeota	Parvarchaea	0.011586503
Tinker	TK138	Bacteria	Acidobacteria	Acidobacteria-5	0.006331586
Tinker	TK138	Bacteria	Acidobacteria	Acidobacteria-6	0.005990236
Tinker	TK138	Bacteria	Acidobacteria	Acidobacteriia	0.003541631
Tinker	TK138	Bacteria	Acidobacteria	BPC102	0.018472313
Tinker	TK138	Bacteria	Acidobacteria	Chloracidobacteria	0.008974867
Tinker	TK138	Bacteria	Acidobacteria	EC1113	0.010409077
Tinker	TK138	Bacteria	Acidobacteria	iii1-8	0.004084809
Tinker	TK138	Bacteria	Acidobacteria	PAUC37f	0.019270043
Tinker	TK138	Bacteria	Acidobacteria	RB25	0.004876634
Tinker	TK138	Bacteria	Acidobacteria	Solibacteres	0.008523582
Tinker	TK138	Bacteria	Acidobacteria	Sva0725	0.011011453
Tinker	TK138	Bacteria	Acidobacteria	TM1	0.01142075
Tinker	TK138	Bacteria	Acidobacteria	Unclassified	0.005617747
Tinker	TK138	Bacteria	Actinobacteria	Acidimicrobiia	0.005243739
Tinker	TK138	Bacteria	Actinobacteria	Actinobacteria	0.008763827
Tinker	TK138	Bacteria	Actinobacteria	MB-A2-108	0.027528633
Tinker	TK138	Bacteria	Actinobacteria	OPB41	0.027528633
Tinker	TK138	Bacteria	Actinobacteria	Rubrobacteria	0.013185949
Tinker	TK138	Bacteria	Actinobacteria	Thermoleophilia	0.011699972
Tinker	TK138	Bacteria	Armatimonadetes	Armatimonadia	0.012756892
Tinker	TK138	Bacteria	Armatimonadetes	Fimbriimonadia	0.005505728
Tinker	TK138	Bacteria	Bacteroidetes	Bacteroidia	0.013413903
Tinker	TK138	Bacteria	Bacteroidetes	Cytophagia	0.009018692
Tinker	TK138	Bacteria	Bacteroidetes	Saprospirae	0.011996978
Tinker	TK138	Bacteria	Bacteroidetes	Sphingobacteriia	0.007155202
Tinker	TK138	Bacteria	Chlamydiae	Chlamydiia	0.006791098
Tinker	TK138	Bacteria	Chlorobi	BSV26	0.006925295
Tinker	TK138	Bacteria	Chlorobi	OPB56	0.01651718
Tinker	TK138	Bacteria	Chloroflexi	Anaerolineae	0.01066085
Tinker	TK138	Bacteria	Chloroflexi	Ellin6529	0.022022906
Tinker	TK138	Bacteria	Chloroflexi	P2-11E	0.008875882
Tinker	TK138	Bacteria	Chloroflexi	S085	0.005505728
Tinker	TK138	Bacteria	Chloroflexi	SAR202	0.004119487
Tinker	TK138	Bacteria	Chloroflexi	Thermomicrobia	0.027528633
Tinker	TK138	Bacteria	Chloroflexi	TK10	0.022022906

Tinker	TK138	Bacteria	Cyanobacteria	4C0d-2	0.007327948
Tinker	TK138	Bacteria	Cyanobacteria	Chloroplast	0.008865652
Tinker	TK138	Bacteria	Cyanobacteria	ML635J-21	0.00523044
Tinker	TK138	Bacteria	Cyanobacteria	Unclassified	0.011586503
Tinker	TK138	Bacteria	Elusimicrobia	Unclassified	0.006331586
Tinker	TK138	Bacteria	FBP	Unclassified	0.005990236
Tinker	TK138	Bacteria	Firmicutes	Bacilli	0.007821386
Tinker	TK138	Bacteria	Firmicutes	Clostridia	0.00750634
Tinker	TK138	Bacteria	GAL15	Unclassified	0.006147068
Tinker	TK138	Bacteria	Gemmatimonadetes	Gemm-1	0.006074143
Tinker	TK138	Bacteria	Gemmatimonadetes	Gemm-3	0.009476208
Tinker	TK138	Bacteria	Gemmatimonadetes	Gemmatimonadetes	0.007622988
Tinker	TK138	Bacteria	GN02	3BR-5F	0.019270043
Tinker	TK138	Bacteria	GN04	Unclassified	0.011586503
Tinker	TK138	Bacteria	NC10	12-24	0.018838009
Tinker	TK138	Bacteria	NC10	wb1-A12	0.006103053
Tinker	TK138	Bacteria	Nitrospirae	Nitrospira	0.0142142
Tinker	TK138	Bacteria	OP3	koll11	0.022022906
Tinker	TK138	Bacteria	OP3	PBS-25	0.020848989
Tinker	TK138	Bacteria	PAUC34f	Unclassified	0.006331586
Tinker	TK138	Bacteria	Planctomycetes	028H05-P-BN-P5	0.011011453
Tinker	TK138	Bacteria	Planctomycetes	BD7-11	0.009604063
Tinker	TK138	Bacteria	Planctomycetes	Phycisphaerae	0.012313242
Tinker	TK138	Bacteria	Planctomycetes	Pla4	0.005990236
Tinker	TK138	Bacteria	Planctomycetes	Planctomycetia	0.003541631
Tinker	TK138	Bacteria	Planctomycetes	Unclassified	0.005505728
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	0.004998659
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	0.006213268
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	0.008092055
Tinker	TK138	Bacteria	Proteobacteria	Epsilonproteobacteria	0.027528633
Tinker	TK138	Bacteria	Proteobacteria	Gammaproteobacteria	0.003541631
Tinker	TK138	Bacteria	Proteobacteria	TA18	0.010592018
Tinker	TK138	Bacteria	Proteobacteria	Unclassified	0.007880295
Tinker	TK138	Bacteria	Proteobacteria	Zetaproteobacteria	0.008974867
Tinker	TK138	Bacteria	SBR1093	Unclassified	0.010409077
Tinker	TK138	Bacteria	Spirochaetes	Brevinematae	0.004084809
Tinker	TK138	Bacteria	Spirochaetes	Spirochaetes	0.027528633
Tinker	TK138	Bacteria	Tenericutes	Mollicutes	0.01651718
Tinker	TK138	Bacteria	Tenericutes	RF3	0.024787211

Tinker	TK138	Bacteria	Thermi	Deinococci	0.013788602
Tinker	TK138	Bacteria	TM6	SJA-4	0.01651718
Tinker	TK138	Bacteria	TM6	Unclassified	0.010592018
Tinker	TK138	Bacteria	TM7	SC3	0.00825859
Tinker	TK138	Bacteria	Unclassified	Unclassified	0.009928549
Tinker	TK138	Bacteria	Verrucomicrobia	Opitutae	0.007880295
Tinker	TK138	Bacteria	Verrucomicrobia	Pedosphaerae	0.00665389
Tinker	TK138	Bacteria	Verrucomicrobia	Spartobacteria	0.019829604
Tinker	TK138	Bacteria	WS2	SHA-109	0.008974867
Tinker	TK138	Bacteria	WS3	PRR-12	0.011011453
Tinker	TK138	Bacteria	WWE1	Cloacamonae	0.004042246
Tinker	TK430	Archaea	Crenarchaeota	MBGA	0.007994083
Tinker	TK430	Archaea	Crenarchaeota	MCG	0.011102472
Tinker	TK430	Archaea	Crenarchaeota	Thaumarchaeota	0.009050622
Tinker	TK430	Archaea	Parvarchaeota	Parvarchaea	0.012495358
Tinker	TK430	Bacteria	Acidobacteria	Acidobacteria-5	0.00777745
Tinker	TK430	Bacteria	Acidobacteria	Acidobacteria-6	0.005870078
Tinker	TK430	Bacteria	Acidobacteria	Acidobacteriia	0.007994083
Tinker	TK430	Bacteria	Acidobacteria	BPC102	0.011978653
Tinker	TK430	Bacteria	Acidobacteria	Chloracidobacteria	0.008393253
Tinker	TK430	Bacteria	Acidobacteria	EC1113	0.011251426
Tinker	TK430	Bacteria	Acidobacteria	iii1-8	0.01178187
Tinker	TK430	Bacteria	Acidobacteria	PAUC37f	0.01995864
Tinker	TK430	Bacteria	Acidobacteria	Solibacteres	0.010342714
Tinker	TK430	Bacteria	Acidobacteria	Sva0725	0.00336671
Tinker	TK430	Bacteria	Acidobacteria	TM1	0.012561994
Tinker	TK430	Bacteria	Acidobacteria	Unclassified	0.008263511
Tinker	TK430	Bacteria	Actinobacteria	Acidimicrobiia	0.007085799
Tinker	TK430	Bacteria	Actinobacteria	Actinobacteria	0.004540701
Tinker	TK430	Bacteria	Actinobacteria	MB-A2-108	0.011102472
Tinker	TK430	Bacteria	Actinobacteria	Rubrobacteria	0.01085346
Tinker	TK430	Bacteria	Actinobacteria	Thermoleophilia	0.012794008
Tinker	TK430	Bacteria	Armatimonadetes	0319-6E2	0.008261967
Tinker	TK430	Bacteria	Armatimonadetes	Armatimonadia	0.009050622
Tinker	TK430	Bacteria	Armatimonadetes	Chthonomonadetes	0.007745669
Tinker	TK430	Bacteria	Bacteroidetes	Bacteroidia	0.007803008
Tinker	TK430	Bacteria	Bacteroidetes	Cytophagia	0.010788026
Tinker	TK430	Bacteria	Bacteroidetes	Saprospirae	0.016132154
Tinker	TK430	Bacteria	Bacteroidetes	Sphingobacteriia	0.005868765

Tinker	TK430	Bacteria	Bacteroidetes	Unclassified	0.004354428
Tinker	TK430	Bacteria	BHI80-139	Unclassified	0.013466839
Tinker	TK430	Bacteria	BRC1	PRR-11	0.005723407
Tinker	TK430	Bacteria	Chlamydiae	Chlamydiia	0.008752437
Tinker	TK430	Bacteria	Chlorobi	BSV26	0.008260744
Tinker	TK430	Bacteria	Chlorobi	SJA-28	0.009387914
Tinker	TK430	Bacteria	Chlorobi	Unclassified	0.011378917
Tinker	TK430	Bacteria	Chloroflexi	Anaerolineae	0.005835958
Tinker	TK430	Bacteria	Chloroflexi	C0119	0.006733419
Tinker	TK430	Bacteria	Chloroflexi	Chloroflexi	0.01685271
Tinker	TK430	Bacteria	Chloroflexi	Ellin6529	0.005663589
Tinker	TK430	Bacteria	Chloroflexi	Ktedonobacteria	0.011446813
Tinker	TK430	Bacteria	Chloroflexi	P2-11E	0.009705063
Tinker	TK430	Bacteria	Chloroflexi	SAR202	0.008251877
Tinker	TK430	Bacteria	Chloroflexi	Thermomicrobia	0.006733419
Tinker	TK430	Bacteria	Chloroflexi	TK10	0.006523682
Tinker	TK430	Bacteria	Chloroflexi	TK17	0.006733419
Tinker	TK430	Bacteria	Chloroflexi	Unclassified	0.007947682
Tinker	TK430	Bacteria	Cyanobacteria	4C0d-2	0.02727035
Tinker	TK430	Bacteria	Cyanobacteria	Chloroplast	0.010496663
Tinker	TK430	Bacteria	Cyanobacteria	Unclassified	0.012495358
Tinker	TK430	Bacteria	Elusimicrobia	Unclassified	0.00777745
Tinker	TK430	Bacteria	FBP	Unclassified	0.005870078
Tinker	TK430	Bacteria	Fibrobacteres	Fibrobacteria	0.023455194
Tinker	TK430	Bacteria	Firmicutes	Bacilli	0.01006007
Tinker	TK430	Bacteria	Firmicutes	Clostridia	0.00965463
Tinker	TK430	Bacteria	Fusobacteria	Fusobacteriia	0.020072626
Tinker	TK430	Bacteria	GAL15	Unclassified	0.016034743
Tinker	TK430	Bacteria	Gemmatimonadetes	Gemm-1	0.006297462
Tinker	TK430	Bacteria	Gemmatimonadetes	Gemm-5	0.010258621
Tinker	TK430	Bacteria	Gemmatimonadetes	Gemmatimonadetes	0.007372519
Tinker	TK430	Bacteria	Gemmatimonadetes	Unclassified	0.006733419
Tinker	TK430	Bacteria	GN02	GN07	0.004578725
Tinker	TK430	Bacteria	GN04	MSB-5A5	0.01107599
Tinker	TK430	Bacteria	GN04	Unclassified	0.012495358
Tinker	TK430	Bacteria	NC10	12-24	0.010901741
Tinker	TK430	Bacteria	NC10	wb1-A12	0.009252433
Tinker	TK430	Bacteria	Nitrospirae	Nitrospira	0.009555967
Tinker	TK430	Bacteria	NKB19	Unclassified	0.003319034

Tinker	TK430	Bacteria	OD1	ABY1	0.006733419
Tinker	TK430	Bacteria	OD1	Mb-NB09	0.019863587
Tinker	TK430	Bacteria	OD1	Unclassified	0.01685271
Tinker	TK430	Bacteria	OP3	koll11	0.015820228
Tinker	TK430	Bacteria	OP3	Unclassified	0.011590807
Tinker	TK430	Bacteria	OP8	OP8_1	0.004213651
Tinker	TK430	Bacteria	PAUC34f	Unclassified	0.00777745
Tinker	TK430	Bacteria	Planctomycetes	BD7-11	0.011590807
Tinker	TK430	Bacteria	Planctomycetes	Brocadiae	0.008069319
Tinker	TK430	Bacteria	Planctomycetes	OM190	0.009317712
Tinker	TK430	Bacteria	Planctomycetes	Phycisphaerae	0.010437891
Tinker	TK430	Bacteria	Planctomycetes	Pla4	0.005870078
Tinker	TK430	Bacteria	Planctomycetes	Planctomycetia	0.010353611
Tinker	TK430	Bacteria	Planctomycetes	Unclassified	0.003723458
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	0.009868724
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	0.00532889
Tinker	TK430	Bacteria	Proteobacteria	Deltaproteobacteria	0.008826415
Tinker	TK430	Bacteria	Proteobacteria	Epsilonproteobacteria	0.008106458
Tinker	TK430	Bacteria	Proteobacteria	Gammaproteobacteria	0.007994083
Tinker	TK430	Bacteria	Proteobacteria	TA18	0.006697122
Tinker	TK430	Bacteria	Proteobacteria	Unclassified	0.005281531
Tinker	TK430	Bacteria	Proteobacteria	Zetaproteobacteria	0.008393253
Tinker	TK430	Bacteria	SBR1093	Unclassified	0.011251426
Tinker	TK430	Bacteria	Spirochaetes	Brevinematae	0.01178187
Tinker	TK430	Bacteria	Spirochaetes	Spirochaetes	0.004213651
Tinker	TK430	Bacteria	Synergistetes	Synergistia	0.021068257
Tinker	TK430	Bacteria	TM6	SJA-4	0.009387914
Tinker	TK430	Bacteria	TM6	Unclassified	0.006697122
Tinker	TK430	Bacteria	TM7	TM7-1	0.018775827
Tinker	TK430	Bacteria	Unclassified	Unclassified	0.008991738
Tinker	TK430	Bacteria	Verrucomicrobia	Methylacidiphilae	0.006289467
Tinker	TK430	Bacteria	Verrucomicrobia	Opitutae	0.005281531
Tinker	TK430	Bacteria	Verrucomicrobia	Pedosphaerae	0.009049932
Tinker	TK430	Bacteria	Verrucomicrobia	Spartobacteria	0.009318064
Tinker	TK430	Bacteria	WS2	SHA-109	0.008393253
Tinker	TK430	Bacteria	WS3	PRR-12	0.005798429
Tinker	TK431	Archaea	Crenarchaeota	MBGA	0.006161858
Tinker	TK431	Archaea	Crenarchaeota	MCG	0.007456282
Tinker	TK431	Archaea	Crenarchaeota	Thaumarchaeota	0.01086429

Tinker	TK431	Archaea	Euryarchaeota	Methanobacteria	0.020237914
Tinker	TK431	Archaea	Euryarchaeota	Methanomicrobia	0.005484564
Tinker	TK431	Archaea	Parvarchaeota	Parvarchaea	0.004649768
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-5	0.006318232
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-6	0.00756618
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteriia	0.006161858
Tinker	TK431	Bacteria	Acidobacteria	AT-s54	0.006855705
Tinker	TK431	Bacteria	Acidobacteria	BPC102	0.020922448
Tinker	TK431	Bacteria	Acidobacteria	Chloracidobacteria	0.005565763
Tinker	TK431	Bacteria	Acidobacteria	EC1113	0.013445937
Tinker	TK431	Bacteria	Acidobacteria	Holophagae	0.020473659
Tinker	TK431	Bacteria	Acidobacteria	iii1-8	0.003281489
Tinker	TK431	Bacteria	Acidobacteria	RB25	0.004066393
Tinker	TK431	Bacteria	Acidobacteria	S035	0.006582869
Tinker	TK431	Bacteria	Acidobacteria	Solibacteres	0.008705213
Tinker	TK431	Bacteria	Acidobacteria	Sva0725	0.004900989
Tinker	TK431	Bacteria	Acidobacteria	TM1	0.006379178
Tinker	TK431	Bacteria	Acidobacteria	Unclassified	0.005641184
Tinker	TK431	Bacteria	Actinobacteria	Acidimicrobiia	0.014038826
Tinker	TK431	Bacteria	Actinobacteria	Actinobacteria	0.014025884
Tinker	TK431	Bacteria	Actinobacteria	MB-A2-108	0.007456282
Tinker	TK431	Bacteria	Actinobacteria	Rubrobacteria	0.008245986
Tinker	TK431	Bacteria	Actinobacteria	Thermoleophilia	0.006453069
Tinker	TK431	Bacteria	Armatimonadetes	0319-6E2	0.004562135
Tinker	TK431	Bacteria	Armatimonadetes	Armatimonadia	0.01086429
Tinker	TK431	Bacteria	Armatimonadetes	Fimbriimonadia	0.005888108
Tinker	TK431	Bacteria	Armatimonadetes	Unclassified	0.004851306
Tinker	TK431	Bacteria	Bacteroidetes	Bacteroidia	0.009008187
Tinker	TK431	Bacteria	Bacteroidetes	Cytophagia	0.004970284
Tinker	TK431	Bacteria	Bacteroidetes	RhodUnclassifiedmi	0.006316473
Tinker	TK431	Bacteria	Bacteroidetes	Saprospirae	0.007348087
Tinker	TK431	Bacteria	Bacteroidetes	Sphingobacteriia	0.00414059
Tinker	TK431	Bacteria	Bacteroidetes	Unclassified	0.004066393
Tinker	TK431	Bacteria	Caldiserica	OP5	0.020237914
Tinker	TK431	Bacteria	Chlorobi	BSV26	0.011737226
Tinker	TK431	Bacteria	Chlorobi	Unclassified	0.005484564
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	0.014568014
Tinker	TK431	Bacteria	Chloroflexi	Chloroflexi	0.019195986
Tinker	TK431	Bacteria	Chloroflexi	Ellin6529	0.007492967

Tinker	TK431	Bacteria	Chloroflexi	Gitt-GS-136	0.002946432
Tinker	TK431	Bacteria	Chloroflexi	P2-11E	0.014940521
Tinker	TK431	Bacteria	Chloroflexi	SAR202	0.006113394
Tinker	TK431	Bacteria	Chloroflexi	Thermomicrobia	0.004994305
Tinker	TK431	Bacteria	Chloroflexi	TK10	0.006481857
Tinker	TK431	Bacteria	Chloroflexi	Unclassified	0.013282569
Tinker	TK431	Bacteria	Cyanobacteria	4C0d-2	0.006453677
Tinker	TK431	Bacteria	Cyanobacteria	Chloroplast	0.019148945
Tinker	TK431	Bacteria	Cyanobacteria	Unclassified	0.004649768
Tinker	TK431	Bacteria	Elusimicrobia	Unclassified	0.006318232
Tinker	TK431	Bacteria	FBP	Unclassified	0.00756618
Tinker	TK431	Bacteria	Firmicutes	Bacilli	0.011847332
Tinker	TK431	Bacteria	Firmicutes	Clostridia	0.003924759
Tinker	TK431	Bacteria	Firmicutes	Erysipelotrichi	0.015404598
Tinker	TK431	Bacteria	Fusobacteria	Fusobacteriia	0.003417706
Tinker	TK431	Bacteria	GAL15	Unclassified	0.005254498
Tinker	TK431	Bacteria	Gemmatimonadetes	Gemm-1	0.014669563
Tinker	TK431	Bacteria	Gemmatimonadetes	Gemm-5	0.009551591
Tinker	TK431	Bacteria	Gemmatimonadetes	Gemmatimonadetes	0.012139864
Tinker	TK431	Bacteria	Gemmatimonadetes	Unclassified	0.015570443
Tinker	TK431	Bacteria	GN02	GKS2-174	0.012471086
Tinker	TK431	Bacteria	GN04	MSB-5A5	0.008724747
Tinker	TK431	Bacteria	GN04	Unclassified	0.010858839
Tinker	TK431	Bacteria	NC10	12-24	0.009800525
Tinker	TK431	Bacteria	NC10	wb1-A12	0.007338088
Tinker	TK431	Bacteria	Nitrospirae	Nitrospira	0.015619132
Tinker	TK431	Bacteria	OD1	ABY1	0.0071327
Tinker	TK431	Bacteria	OD1	Mb-NB09	0.010626648
Tinker	TK431	Bacteria	OP1	Unclassified	0.015354908
Tinker	TK431	Bacteria	OP3	koll11	0.009342273
Tinker	TK431	Bacteria	OP3	Unclassified	0.004679118
Tinker	TK431	Bacteria	PAUC34f	Unclassified	0.006318232
Tinker	TK431	Bacteria	Planctomycetes	BD7-11	0.01023683
Tinker	TK431	Bacteria	Planctomycetes	Brocadiae	0.005624418
Tinker	TK431	Bacteria	Planctomycetes	Phycisphaerae	0.008126501
Tinker	TK431	Bacteria	Planctomycetes	Pla3	0.003378361
Tinker	TK431	Bacteria	Planctomycetes	Pla4	0.00756618
Tinker	TK431	Bacteria	Planctomycetes	Planctomycetia	0.012192165
Tinker	TK431	Bacteria	Planctomycetes	Unclassified	0.005593715

Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	0.011634662
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	0.008894579
Tinker	TK431	Bacteria	Proteobacteria	Deltaproteobacteria	0.012279785
Tinker	TK431	Bacteria	Proteobacteria	Epsilonproteobacteria	0.010704031
Tinker	TK431	Bacteria	Proteobacteria	Gammaproteobacteria	0.006161858
Tinker	TK431	Bacteria	Proteobacteria	TA18	0.014608833
Tinker	TK431	Bacteria	Proteobacteria	Unclassified	0.01316932
Tinker	TK431	Bacteria	Proteobacteria	Zetaproteobacteria	0.005565763
Tinker	TK431	Bacteria	SBR1093	Unclassified	0.013445937
Tinker	TK431	Bacteria	SC4	Unclassified	0.020473659
Tinker	TK431	Bacteria	Spirochaetes	Brevinematae	0.003281489
Tinker	TK431	Bacteria	Spirochaetes	Leptospirae	0.006855705
Tinker	TK431	Bacteria	Tenericutes	Mollicutes	0.025628408
Tinker	TK431	Bacteria	Thermi	Deinococci	0.005484564
Tinker	TK431	Bacteria	Thermotogae	Thermotogae	0.008245986
Tinker	TK431	Bacteria	TM6	SBRH58	0.024455156
Tinker	TK431	Bacteria	TM6	Unclassified	0.014608833
Tinker	TK431	Bacteria	TM7	TM7-1	0.019195986
Tinker	TK431	Bacteria	Unclassified	Unclassified	0.007024116
Tinker	TK431	Bacteria	Verrucomicrobia	Opitutae	0.006313615
Tinker	TK431	Bacteria	Verrucomicrobia	Pedosphaerae	0.003793631
Tinker	TK431	Bacteria	Verrucomicrobia	Spartobacteria	0.00673384
Tinker	TK431	Bacteria	WS2	SHA-109	0.005565763
Tinker	TK431	Bacteria	WS3	PRR-12	0.017539444

Order rank

Site	Sample ID	Kingdom	Phylum	Class	Order	Order Relative Abundance
Dover	DV1	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.182140607
Dover	DV1	Bacteria	Firmicutes	Bacilli	Bacillales	0.149717674
Dover	DV1	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.034090359
Dover	DV1	Bacteria	Firmicutes	Clostridia	Clostridiales	0.234309046
Dover	DV1	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.129639303
Dover	DV1	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.039239416
Dover	DV1	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.056383472
Dover	DV1	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	0.025300929
Dover	DV1	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.142355113
Dover	DV1	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.006824081
Dover	DV2	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.172547506
Dover	DV2	Bacteria	Firmicutes	Clostridia	Clostridiales	0.049539837
Dover	DV2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.165951662
Dover	DV2	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.191200819
Dover	DV2	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.420760176
Dover	DV3	Archaea	Euryarchaeota	Methanobacteria	Methanomicrobiales	0.003060489
Dover	DV3	Archaea	Euryarchaeota	Methanobacteria	Methanosarcinales	0.000470919
Dover	DV3	Bacteria	Other	Other	Other	0.008044892
Dover	DV3	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.085739892
Dover	DV3	Bacteria	Actinobacteria	OPB41	Other	0.004457814
Dover	DV3	Bacteria	Armatimonadetes	SJA-176	RB046	0.000759189
Dover	DV3	Bacteria	Bacteroidetes	Other	Other	0.000268329
Dover	DV3	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.240256038

Dover	DV3	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	0.000293673
Dover	DV3	Bacteria	Caldiserica	WCHB1-03	Other	0.000488704
Dover	DV3	Bacteria	Chloroflexi	Anaerolineae	SJA-15	0.000213485
Dover	DV3	Bacteria	Elusimicrobia	Endomicrobia	Other	0.005489476
Dover	DV3	Bacteria	Firmicutes	Other	Other	8.70057E-05
Dover	DV3	Bacteria	Firmicutes	Bacilli	Bacillales	0.002146627
Dover	DV3	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.000396433
Dover	DV3	Bacteria	Firmicutes	Clostridia	Other	0.000199193
Dover	DV3	Bacteria	Firmicutes	Clostridia	Clostridiales	0.105015633
Dover	DV3	Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	0.000155667
Dover	DV3	Bacteria	Proteobacteria	Other	Other	5.30962E-05
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Other	0.000142342
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	0.077165701
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.12419809
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	4.39379E-05
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	0.000905404
Dover	DV3	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.001726465
Dover	DV3	Bacteria	Proteobacteria	Betaproteobacteria	Other	0.000162375
Dover	DV3	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.006437103
Dover	DV3	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	0.023735509
Dover	DV3	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	0.00011515
Dover	DV3	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	0.090523343
Dover	DV3	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	0.000103932
Dover	DV3	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	0.131135479
Dover	DV3	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.084654274
Dover	DV3	Bacteria	Synergistetes	Spirochaetes	Spirochaetales	0.001247708
Dover	DV3	Bacteria	Verrucomicrobia	Pedosphaerae	Pedosphaerales	0.000106633
Dover	DV4	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.145593295

Dover	DV4	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.019935984
Dover	DV4	Bacteria	Bacteroidetes	Saprospirae	Saprospirales	0.424798721
Dover	DV4	Bacteria	Firmicutes	Bacilli	Bacillales	0.228154204
Dover	DV4	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.025863669
Dover	DV4	Bacteria	Firmicutes	Clostridia	Clostridiales	0.018198035
Dover	DV4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.031212677
Dover	DV4	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	0.013393727
Dover	DV4	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	0.002487112
Dover	DV4	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	0.019519408
Dover	DV4	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.053597591
Dover	DV4	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.017245579
Dover	DV5	Bacteria	Other	Other	Other	0.001151586
Dover	DV5	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.078963177
Dover	DV5	Bacteria	Firmicutes	Clostridia	Clostridiales	0.023113011
Dover	DV5	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.265808145
Dover	DV5	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.073333072
Dover	DV5	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	0.008776516
Dover	DV5	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	0.099456397
Dover	DV5	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	0.086965383
Dover	DV5	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.339724102
Dover	DV5	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.02270861
Dover	DV6	Bacteria	Firmicutes	Clostridia	Clostridiales	0.025597467
Dover	DV6	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.974402533
Tinker	TK131	Archaea	Crenarchaeota	MBGA	Other	6.7421E-05
Tinker	TK131	Archaea	Crenarchaeota	Thaumarchaeota	AK31	0.000146534
Tinker	TK131	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	0.000120607
Tinker	TK131	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	0.000143291
Tinker	TK131	Archaea	Euryarchaeota	Thermoplasmata	E2	0.000138488

Tinker	TK131	Archaea	Parvarchaeota	Parvarchaea	WCHD3-30	9.43181E-05
Tinker	TK131	Archaea	Parvarchaeota	Parvarchaea	YLA114	8.11266E-05
Tinker	TK131	Bacteria	Other	Other	Other	0.002925641
Tinker	TK131	Bacteria	Acidobacteria	Other	Other	0.00038684
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-5	Other	0.000120576
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-6	Other	6.52546E-05
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-6	CCU21	6.86953E-05
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	0.00131256
Tinker	TK131	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	2.53783E-05
Tinker	TK131	Bacteria	Acidobacteria	BPC102	B110	9.43181E-05
Tinker	TK131	Bacteria	Acidobacteria	DA052	E29	0.000211156
Tinker	TK131	Bacteria	Acidobacteria	DA052	ELLIN6513	0.000255568
Tinker	TK131	Bacteria	Acidobacteria	DA052	HDB-SIOH1004	0.000270803
Tinker	TK131	Bacteria	Acidobacteria	EC1113	Other	0.000557773
Tinker	TK131	Bacteria	Acidobacteria	PAUC37f	Other	2.12048E-05
Tinker	TK131	Bacteria	Acidobacteria	S035	Other	5.07565E-05
Tinker	TK131	Bacteria	Acidobacteria	Solibacteres	JH-WHS99	5.96728E-05
Tinker	TK131	Bacteria	Acidobacteria	Solibacteres	Solibacterales	0.000566532
Tinker	TK131	Bacteria	Acidobacteria	TM1	Other	5.46758E-05
Tinker	TK131	Bacteria	Acidobacteria	Chloracidobacteria	RB41	0.002196563
Tinker	TK131	Bacteria	Acidobacteria	iii1-8	32-20	4.61153E-05
Tinker	TK131	Bacteria	Acidobacteria	iii1-8	DS-18	9.22307E-05
Tinker	TK131	Bacteria	Actinobacteria	Other	Other	5.34327E-05
Tinker	TK131	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	0.000464334
Tinker	TK131	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.037437719
Tinker	TK131	Bacteria	Actinobacteria	MB-A2-108	0319-7L14	0.000205636
Tinker	TK131	Bacteria	Actinobacteria	Rubrobacteria	Rubrobacterales	2.73379E-05
Tinker	TK131	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	0.000749068

Tinker	TK131	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	0.028192037
Tinker	TK131	Bacteria	Bacteroidetes	Other	Other	0.000147709
Tinker	TK131	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.000139195
Tinker	TK131	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	0.040104386
Tinker	TK131	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	0.004341048
Tinker	TK131	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	0.011078166
Tinker	TK131	Bacteria	Bacteroidetes	Saprospirae	Saprospirales	0.084622872
Tinker	TK131	Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	6.65098E-05
Tinker	TK131	Bacteria	Chlorobi	BSV26	C20	7.82676E-05
Tinker	TK131	Bacteria	Chloroflexi	Other	Other	6.04282E-05
Tinker	TK131	Bacteria	Chloroflexi	Anaerolineae	Other	0.00012233
Tinker	TK131	Bacteria	Chloroflexi	Anaerolineae	H39	2.21699E-05
Tinker	TK131	Bacteria	Chloroflexi	Anaerolineae	SB-34	8.14016E-05
Tinker	TK131	Bacteria	Chloroflexi	Gitt-GS-136	Other	2.46528E-05
Tinker	TK131	Bacteria	Chloroflexi	P2-11E	Other	0.000101601
Tinker	TK131	Bacteria	Chloroflexi	S085	Other	0.000304265
Tinker	TK131	Bacteria	Chloroflexi	SAR202	Other	8.71525E-05
Tinker	TK131	Bacteria	Chloroflexi	Thermomicrobia	JG30-KF-CM45	2.30577E-05
Tinker	TK131	Bacteria	Cyanobacteria	Chloroplast	Stramenopiles	0.000141952
Tinker	TK131	Bacteria	Cyanobacteria	Chloroplast	Streptophyta	0.000122057
Tinker	TK131	Bacteria	Firmicutes	Bacilli	Bacillales	0.00065628
Tinker	TK131	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.000117533
Tinker	TK131	Bacteria	Firmicutes	Clostridia	Clostridiales	0.000336493
Tinker	TK131	Bacteria	GAL15	Other	Other	5.05797E-05
Tinker	TK131	Bacteria	GN04	MSB-5A5	Other	0.000155716
Tinker	TK131	Bacteria	Gemmatimonadetes	Gemm-1	Other	0.000303832
Tinker	TK131	Bacteria	Gemmatimonadetes	Gemm-3	Other	2.38819E-05
Tinker	TK131	Bacteria	NC10	12-14	Other	2.21699E-05

Tinker	TK131	Bacteria	NC10	12-14	MIZ17	0.00032433
Tinker	TK131	Bacteria	NC10	12-14	Methylomirabiliales	0.000180535
Tinker	TK131	Bacteria	NC10	wb1-A12	Other	2.79324E-05
Tinker	TK131	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	0.00114634
Tinker	TK131	Bacteria	OP3	PBS-25	Other	5.07565E-05
Tinker	TK131	Bacteria	Planctomycetes	Phycisphaerae	CCM11a	2.53783E-05
Tinker	TK131	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	5.07565E-05
Tinker	TK131	Bacteria	Planctomycetes	Phycisphaerae	WD2101	2.90508E-05
Tinker	TK131	Bacteria	Planctomycetes	Pla3	Other	7.00167E-05
Tinker	TK131	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	0.000408091
Tinker	TK131	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	0.000394228
Tinker	TK131	Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	0.000341152
Tinker	TK131	Bacteria	Proteobacteria	Other	Other	0.008519335
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Other	0.002676074
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	0.019603091
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Ellin329	7.88561E-05
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.027768282
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	0.012867082
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	0.005123227
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	0.00176157
Tinker	TK131	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.129631174
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	Other	0.006255425
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.137548229
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	Ellin6067	0.001158843
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	MND1	0.000256046
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	0.005907403
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	0.001307137
Tinker	TK131	Bacteria	Proteobacteria	Betaproteobacteria	SC-I-84	6.04282E-05

Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Other	0.000890827
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	4.18986E-05
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	1.39662E-05
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	9.60224E-05
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	MIZ46	4.02855E-05
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	0.127598297
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Sva0853	3.66018E-05
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	0.001613671
Tinker	TK131	Bacteria	Proteobacteria	Deltaproteobacteria	Entotheonellales	2.01427E-05
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Other	0.004123263
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	0.007572115
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	0.000290412
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	0.000106865
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.163049041
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	6.21211E-05
Tinker	TK131	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.107956876
Tinker	TK131	Bacteria	Verrucomicrobia	Opitutae	Opitutales	0.00063807
Tinker	TK131	Bacteria	Verrucomicrobia	Pedosphaerae	Pedosphaerales	0.00062609
Tinker	TK131	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	0.0009685
Tinker	TK131	Bacteria	WS3	PRR-12	Sediment-1	4.4797E-05
Tinker	TK138	Archaea	Crenarchaeota	Thaumarchaeota	AK31	7.18028E-05
Tinker	TK138	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	0.000156289
Tinker	TK138	Archaea	Euryarchaeota	Thermoplasmata	E2	4.36719E-05
Tinker	TK138	Bacteria	Other	Other	Other	0.000198098
Tinker	TK138	Bacteria	Acidobacteria	Other	Other	0.000113861
Tinker	TK138	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	8.85405E-05
Tinker	TK138	Bacteria	Acidobacteria	BPC102	B110	5.5777E-05
Tinker	TK138	Bacteria	Acidobacteria	DA052	E29	0.000130474

Tinker	TK138	Bacteria	Acidobacteria	DA052	ELLIN6513	0.000146537
Tinker	TK138	Bacteria	Acidobacteria	DA052	HDB-SIOH1004	4.99919E-05
Tinker	TK138	Bacteria	Acidobacteria	EC1113	Other	0.000194251
Tinker	TK138	Bacteria	Acidobacteria	Solibacteres	JH-WHS99	3.02804E-05
Tinker	TK138	Bacteria	Acidobacteria	Solibacteres	Solibacterales	0.000744582
Tinker	TK138	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	0.000104625
Tinker	TK138	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.003749518
Tinker	TK138	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	0.000115849
Tinker	TK138	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	0.000797636
Tinker	TK138	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.000123291
Tinker	TK138	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	0.00375463
Tinker	TK138	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	0.000764288
Tinker	TK138	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	0.066264939
Tinker	TK138	Bacteria	Bacteroidetes	Saprospirae	Saprospirales	0.051569228
Tinker	TK138	Bacteria	Chloroflexi	P2-11E	Other	8.21937E-05
Tinker	TK138	Bacteria	Chloroflexi	S085	Other	0.000188698
Tinker	TK138	Bacteria	Chloroflexi	SAR202	Other	3.81509E-05
Tinker	TK138	Bacteria	Cyanobacteria	Chloroplast	Streptophyta	8.89322E-05
Tinker	TK138	Bacteria	Firmicutes	Bacilli	Bacillales	0.001229413
Tinker	TK138	Bacteria	Firmicutes	Clostridia	Clostridiales	0.000301791
Tinker	TK138	Bacteria	NC10	12-14	MIZ17	0.000173312
Tinker	TK138	Bacteria	NC10	wb1-A12	Other	5.6511E-05
Tinker	TK138	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	0.0006108
Tinker	TK138	Bacteria	Proteobacteria	Other	Other	0.004049442
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Other	0.008897703
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	0.043791474
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Ellin329	0.000205958
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.018800925

Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	0.002896993
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	0.001570575
Tinker	TK138	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.081494198
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	Other	0.024315165
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.155018623
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	MND1	0.000290967
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	0.00602075
Tinker	TK138	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	0.00781693
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	Other	0.000241293
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	0.015145715
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	0.019036378
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	0.000884017
Tinker	TK138	Bacteria	Proteobacteria	Deltaproteobacteria	Entotheonellales	2.09953E-05
Tinker	TK138	Bacteria	Proteobacteria	Gammaproteobacteria	Other	0.001466045
Tinker	TK138	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	0.011479291
Tinker	TK138	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.459369044
Tinker	TK138	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.003746078
Tinker	TK138	Bacteria	Verrucomicrobia	Opitutae	Opitutales	0.001365325
Tinker	TK138	Bacteria	Thermi	Deinococci	Deinococcales	3.81509E-05
Tinker	TK430	Archaea	Crenarchaeota	MBGA	Other	0.000147264
Tinker	TK430	Archaea	Crenarchaeota	Thaumarchaeota	AK31	0.000688386
Tinker	TK430	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	0.000437543
Tinker	TK430	Archaea	Euryarchaeota	Thermoplasmata	E2	0.000418799
Tinker	TK430	Archaea	Parvarchaeota	Parvarchaea	WCHD3-30	0.000218294
Tinker	TK430	Bacteria	Other	Other	Other	0.004038569
Tinker	TK430	Bacteria	Acidobacteria	Other	Other	0.00419804
Tinker	TK430	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	0.004251026
Tinker	TK430	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	0.001349336

Tinker	TK430	Bacteria	Acidobacteria	DA052	E29	0.005988306
Tinker	TK430	Bacteria	Acidobacteria	DA052	ELLIN6513	0.00966551
Tinker	TK430	Bacteria	Acidobacteria	DA052	HDB-SIOH1004	0.003722794
Tinker	TK430	Bacteria	Acidobacteria	EC1113	Other	0.005050607
Tinker	TK430	Bacteria	Acidobacteria	Solibacteres	JH-WHS99	0.000475906
Tinker	TK430	Bacteria	Acidobacteria	Solibacteres	Solibacterales	0.003868621
Tinker	TK430	Bacteria	Acidobacteria	TM1	Other	0.001215745
Tinker	TK430	Bacteria	Acidobacteria	Chloracidobacteria	11-24	0.00021571
Tinker	TK430	Bacteria	Acidobacteria	Chloracidobacteria	RB41	0.002912391
Tinker	TK430	Bacteria	Acidobacteria	iii1-8	32-20	0.00019297
Tinker	TK430	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	0.001360667
Tinker	TK430	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.020126454
Tinker	TK430	Bacteria	Actinobacteria	MB-A2-108	0319-7L14	0.000335131
Tinker	TK430	Bacteria	Actinobacteria	Rubrobacteria	Rubrobacterales	0.00010307
Tinker	TK430	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	0.004140467
Tinker	TK430	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	0.001452826
Tinker	TK430	Bacteria	Bacteroidetes	Other	Other	0.000421384
Tinker	TK430	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	0.0048296
Tinker	TK430	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	0.00960879
Tinker	TK430	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	0.002615627
Tinker	TK430	Bacteria	Bacteroidetes	Saprospirae	Saprospirales	0.072081778
Tinker	TK430	Bacteria	Chloroflexi	Other	Other	0.000146026
Tinker	TK430	Bacteria	Chloroflexi	Anaerolineae	Other	0.000441792
Tinker	TK430	Bacteria	Chloroflexi	S085	Other	0.000909024
Tinker	TK430	Bacteria	Firmicutes	Bacilli	Bacillales	0.002687066
Tinker	TK430	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.001691192
Tinker	TK430	Bacteria	Firmicutes	Clostridia	Clostridiales	0.000357027
Tinker	TK430	Bacteria	Firmicutes	Clostridia	OPB54	7.36319E-05

Tinker	TK430	Bacteria	GN04	MSB-5A5	Other	0.002301673
Tinker	TK430	Bacteria	Gemmatimonadetes	Gemm-1	Other	0.002738023
Tinker	TK430	Bacteria	Gemmatimonadetes	Gemm-5	Other	0.000460828
Tinker	TK430	Bacteria	NC10	12-14	MIZ17	0.003149953
Tinker	TK430	Bacteria	NC10	12-14	Methylomirabiliales	0.000778336
Tinker	TK430	Bacteria	NC10	wb1-A12	Other	0.000878662
Tinker	TK430	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	0.009123181
Tinker	TK430	Bacteria	OP3	koll11	Other	0.000710638
Tinker	TK430	Bacteria	OP8	OP8-1	Other	0.000710638
Tinker	TK430	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	0.000875246
Tinker	TK430	Bacteria	Proteobacteria	Other	Other	0.003064553
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Other	0.002909912
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	0.033429945
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.033865676
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	0.00081451
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	0.004754149
Tinker	TK430	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.012761414
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Other	0.009439796
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	A21b	0.000699417
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.18999925
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Ellin6067	0.019169052
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	MND1	0.000454236
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	0.000104482
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	0.00113349
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	0.001182276
Tinker	TK430	Bacteria	Proteobacteria	Betaproteobacteria	SC-I-84	0.00045829
Tinker	TK430	Bacteria	Proteobacteria	Deltaproteobacteria	Other	0.00109055
Tinker	TK430	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	0.000109147

Tinker	TK430	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	0.150643945
Tinker	TK430	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	0.024946034
Tinker	TK430	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	0.000165856
Tinker	TK430	Bacteria	Proteobacteria	Gammaproteobacteria	Other	0.000793333
Tinker	TK430	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	0.000615817
Tinker	TK430	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.262898966
Tinker	TK430	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.043143668
Tinker	TK430	Bacteria	Verrucomicrobia	Opitutae	Opitutales	0.000420698
Tinker	TK430	Bacteria	Verrucomicrobia	Pedosphaerae	Pedosphaerales	0.004065329
Tinker	TK430	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	0.001732593
Tinker	TK430	Bacteria	WS3	PRR-12	Sediment-1	0.000969064
Tinker	TK431	Archaea	Crenarchaeota	MBGA	Other	0.001172171
Tinker	TK431	Archaea	Crenarchaeota	MCG	Other	3.26345E-05
Tinker	TK431	Archaea	Crenarchaeota	Thaumarchaeota	AK31	0.002028294
Tinker	TK431	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	0.002176299
Tinker	TK431	Archaea	Euryarchaeota	Thermoplasmata	E2	0.002183172
Tinker	TK431	Archaea	Parvarchaeota	Parvarchaea	WCHD3-30	0.000258709
Tinker	TK431	Archaea	Parvarchaeota	Parvarchaea	YLA114	0.000632707
Tinker	TK431	Bacteria	Other	Other	Other	0.005996512
Tinker	TK431	Bacteria	Acidobacteria	Other	Other	0.002185854
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-5	Other	0.000596232
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-6	Other	0.000419338
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-6	CCU21	9.08855E-05
Tinker	TK431	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	0.002760881
Tinker	TK431	Bacteria	Acidobacteria	BPC102	B110	0.001824782
Tinker	TK431	Bacteria	Acidobacteria	BPC102	MVS-40	0.00054447
Tinker	TK431	Bacteria	Acidobacteria	DA052	Other	2.50081E-05
Tinker	TK431	Bacteria	Acidobacteria	DA052	E29	0.007669422

Tinker	TK431	Bacteria	Acidobacteria	DA052	ELLIN6513	0.016761272
Tinker	TK431	Bacteria	Acidobacteria	DA052	HDB-SIOH1004	0.002948466
Tinker	TK431	Bacteria	Acidobacteria	EC1113	Other	0.00507217
Tinker	TK431	Bacteria	Acidobacteria	Solibacteres	JH-WHS99	0.000557855
Tinker	TK431	Bacteria	Acidobacteria	Solibacteres	Solibacterales	0.001124575
Tinker	TK431	Bacteria	Acidobacteria	TM1	Other	0.001257969
Tinker	TK431	Bacteria	Acidobacteria	Chloracidobacteria	RB41	0.000408409
Tinker	TK431	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	0.000579984
Tinker	TK431	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	0.012692671
Tinker	TK431	Bacteria	Actinobacteria	MB-A2-108	0319-7L14	4.90723E-05
Tinker	TK431	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	0.002250034
Tinker	TK431	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	0.000445851
Tinker	TK431	Bacteria	Armatimonadetes	Other	Other	0.000212477
Tinker	TK431	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	0.000847926
Tinker	TK431	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	0.000468266
Tinker	TK431	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	0.009851174
Tinker	TK431	Bacteria	Bacteroidetes	Saprospirae	Saprospirales	0.032186852
Tinker	TK431	Bacteria	Chlorobi	BSV26	A89	0.000603686
Tinker	TK431	Bacteria	Chloroflexi	Other	Other	0.000561197
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	Other	0.00093438
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	H39	0.000114509
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	SB-34	0.000970926
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	SHA-20	0.000115211
Tinker	TK431	Bacteria	Chloroflexi	Anaerolineae	pLW-97	0.000535252
Tinker	TK431	Bacteria	Chloroflexi	P2-11E	Other	0.001409691
Tinker	TK431	Bacteria	Chloroflexi	S085	Other	0.001970241
Tinker	TK431	Bacteria	Chloroflexi	SAR202	Other	0.000124295
Tinker	TK431	Bacteria	Firmicutes	Bacilli	Bacillales	0.001920353

Tinker	TK431	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.002402149
Tinker	TK431	Bacteria	Firmicutes	Clostridia	Clostridiales	0.000725542
Tinker	TK431	Bacteria	Firmicutes	Clostridia	OPB54	1.51476E-05
Tinker	TK431	Bacteria	GAL15	Other	Other	0.001068323
Tinker	TK431	Bacteria	GN02	GKS2-174	Other	0.000253552
Tinker	TK431	Bacteria	GN04	MSB-5A5	Other	0.001772071
Tinker	TK431	Bacteria	Gemmatimonadetes	Gemm-1	Other	0.000298142
Tinker	TK431	Bacteria	NC10	12-14	MIZ17	0.003703337
Tinker	TK431	Bacteria	NC10	12-14	Methylomirabiliales	0.000554786
Tinker	TK431	Bacteria	NC10	wb1-A12	Other	0.00057987
Tinker	TK431	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	0.006810433
Tinker	TK431	Bacteria	OD1	ABY1	Other	0.000145019
Tinker	TK431	Bacteria	OD1	Mb-NB09	Other	0.0004649
Tinker	TK431	Bacteria	Planctomycetes	Other	Other	0.000245005
Tinker	TK431	Bacteria	Planctomycetes	Phycisphaerae	CCM11a	0.001059547
Tinker	TK431	Bacteria	Planctomycetes	Pla3	Other	0.000318819
Tinker	TK431	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	0.000376495
Tinker	TK431	Bacteria	Planctomycetes	Brocadiae	Brocadiales	0.000245937
Tinker	TK431	Bacteria	Proteobacteria	Other	Other	0.003868966
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Other	0.001025691
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	0.01793745
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	0.014893103
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	0.001128431
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	0.002414143
Tinker	TK431	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	0.010867418
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Other	0.004530109
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	0.150511243
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Ellin6067	0.01081653

Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	0.008603773
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	0.000253047
Tinker	TK431	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	0.000837806
Tinker	TK431	Bacteria	Proteobacteria	Deltaproteobacteria	Other	0.00145537
Tinker	TK431	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	0.069127075
Tinker	TK431	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	0.028283737
Tinker	TK431	Bacteria	Proteobacteria	Deltaproteobacteria	Entotheonellales	6.63391E-05
Tinker	TK431	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	1.90848E-05
Tinker	TK431	Bacteria	Proteobacteria	Gammaproteobacteria	Other	0.00054606
Tinker	TK431	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	0.516024356
Tinker	TK431	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	0.005803994
Tinker	TK431	Bacteria	Verrucomicrobia	Pedosphaerae	Pedosphaerales	0.000769797
Tinker	TK431	Bacteria	WS3	PRR-12	Sediment-1	0.001605271

Appendix I. Complete PBOC data with all extraction values and descriptive statistics

Sample	Ext.	C_{TOC} (mg/L) Dilute	C_{TOC} (mg/L)	V (L)	M_i (mg)	ΣM (mg)	M_S (g)	PBOC (mg/kg)	Mean	Std. Div.	Std. Error of mean	max	min
DOVER 6-SS1-D	1	3.868	19.34	0.02	0.3868								
DOVER 6-SS1-D	2	1.898	9.49	0.02	0.1898								
DOVER 6-SS1-D	3	1.455	7.275	0.02	0.1455	1.0612	10.00	106.12					
DOVER 6-SS1-D	4	1.804	9.02	0.02	0.1804								
DOVER 6-SS1-D	5	1.587	7.935	0.02	0.1587								
DOVER 6-SS1-E	1	3.582	17.91	0.02	0.3582								
DOVER 6-SS1-E	2	1.520	7.6	0.02	0.152								
DOVER 6-SS1-E	3	2.180	10.9	0.02	0.218	1.1383	10.01	113.716	109.539	3.854	2.23	114	106
DOVER 6-SS1-E	4	2.257	11.285	0.02	0.2257								
DOVER 6-SS1-E	5	1.844	9.22	0.02	0.1844								
DOVER 6-SS1-F	1	3.448	17.24	0.02	0.3448								
DOVER 6-SS1-F	2	2.210	11.05	0.02	0.221								
DOVER 6-SS1-F	3	1.559	7.795	0.02	0.1559	1.0889	10.01	108.781					
DOVER 6-SS1-F	4	1.998	9.99	0.02	0.1998								
DOVER 6-SS1-F	5	1.674	8.37	0.02	0.1674								
DOVER 6-SS2-D	1	2.165	10.825	0.02	0.2165								
DOVER 6-SS2-D	2	1.282	6.41	0.02	0.1282								
DOVER 6-SS2-D	3	0.9353	4.6765	0.02	0.09353	0.68271	10.00	68.271					
DOVER 6-SS2-D	4	0.9458	4.729	0.02	0.09458								
DOVER 6-SS2-D	5	1.499	7.495	0.02	0.1499				69.523	15.25	8.8	85.4	54.9
DOVER 6-SS2-E	1	2.682	13.41	0.02	0.2682								
DOVER 6-SS2-E	2	1.429	7.145	0.02	0.1429	0.8536	10.00	85.36					
DOVER 6-SS2-E	3	1.576	7.88	0.02	0.1576								
DOVER 6-SS2-E	4	1.573	7.865	0.02	0.1573								

DOVER 6-SS2-E	5	1.276	6.38	0.02	0.1276								
DOVER 6-SS2-F	1	2.174	10.87	0.02	0.2174								
DOVER 6-SS2-F	2	0.9229	4.6145	0.02	0.09229								
DOVER 6-SS2-F	3	1.181	5.905	0.02	0.1181	0.54938	10.00	54.938					
DOVER 6-SS2-F	4	0.7416	3.708	0.02	0.07416								
DOVER 6-SS2-F	5	0.4743	2.3715	0.02	0.04743								
DOVER 6-SS3-D	1	15.80	79	0.02	1.58								
DOVER 6-SS3-D	2	5.162	25.81	0.02	0.5162								
DOVER 6-SS3-D	3	3.184	15.92	0.02	0.3184	3.5009	10.00	350.09					
DOVER 6-SS3-D	4	6.984	34.92	0.02	0.6984								
DOVER 6-SS3-D	5	3.879	19.395	0.02	0.3879								
DOVER 6-SS3-E	1	17.09	85.45	0.02	1.709								
DOVER 6-SS3-E	2	4.920	24.6	0.02	0.492								
DOVER 6-SS3-E	3	2.518	12.59	0.02	0.2518	3.3549	10.00	335.49	326.778	28.68	16.6	350	295
DOVER 6-SS3-E	4	5.974	29.87	0.02	0.5974								
DOVER 6-SS3-E	5	3.047	15.235	0.02	0.3047								
DOVER 6-SS3-F	1	14.97	74.85	0.02	1.497								
DOVER 6-SS3-F	2	5.147	25.735	0.02	0.5147								
DOVER 6-SS3-F	3	3.176	15.88	0.02	0.3176	2.9505	10.01	294.755					
DOVER 6-SS3-F	4	3.596	17.98	0.02	0.3596								
DOVER 6-SS3-F	5	2.616	13.08	0.02	0.2616								
DOVER 6SS4G	1	2.641	13.205	0.02	0.2641								
DOVER 6SS4G	2	2.636	13.18	0.02	0.2636								
DOVER 6SS4G	3	0.9928	4.964	0.02	0.09928	0.80945	10.00	80.918					
DOVER 6SS4G	4	1.109	5.545	0.02	0.1109				71.855	8.395	4.85	80.9	64.3
DOVER 6SS4G	5	0.7157	3.5785	0.02	0.07157								
DOVER 6SS4H	1	2.934	14.67	0.02	0.2934	0.70327	10.00	70.3036					
DOVER 6SS4H	2	1.368	6.84	0.02	0.1368								

DOVER 6SS4H	3	0.9312	4.656	0.02	0.09312								
DOVER 6SS4H	4	1.081	5.405	0.02	0.1081								
DOVER 6SS4H	5	0.7185	3.5925	0.02	0.07185								
DOVER 6SS4I	1	2.584	12.92	0.02	0.2584								
DOVER 6SS4I	2	1.070	5.35	0.02	0.107								
DOVER 6SS4I	3	0.7834	3.917	0.02	0.07834	0.64365	10.00	64.3436					
DOVER 6SS4I	4	1.352	6.76	0.02	0.1352								
DOVER 6SS4I	5	0.6471	3.2355	0.02	0.06471								
DOVER 6SS5G	1	2.304	11.52	0.02	0.2304								
DOVER 6SS5G	2	0.8631	4.3155	0.02	0.08631								
DOVER 6SS5G	3	0.6505	3.2525	0.02	0.06505	0.60388	10.00	60.3679					
DOVER 6SS5G	4	1.668	8.34	0.02	0.1668								
DOVER 6SS5G	5	0.5532	2.766	0.02	0.05532								
DOVER 6SS5H	1	1.832	9.16	0.02	0.1832								
DOVER 6SS5H	2	0.8189	4.0945	0.02	0.08189								
DOVER 6SS5H	3	0.4340	2.17	0.02	0.0434	0.47822	10.00	47.8061	59.3729	11.1	6.41	69.9	47.8
DOVER 6SS5H	4	0.8465	4.2325	0.02	0.08465								
DOVER 6SS5H	5	0.8508	4.254	0.02	0.08508								
DOVER 6SS5I	1	2.262	11.31	0.02	0.2262								
DOVER 6SS5I	2	0.9685	4.8425	0.02	0.09685								
DOVER 6SS5I	3	1.826	9.13	0.02	0.1826	0.69968	10.00	69.9447					
DOVER 6SS5I	4	1.187	5.935	0.02	0.1187								
DOVER 6SS5I	5	0.7533	3.7665	0.02	0.07533								
DOVER 6SS6G	1	1.661	8.305	0.02	0.1661								
DOVER 6SS6G	2	0.9360	4.68	0.02	0.0936								
DOVER 6SS6G	3	0.7911	3.9555	0.02	0.07911	0.56511	10.00	56.4922	59.2209	5.31	3.07	65.3	55.8
DOVER 6SS6G	4	1.199	5.995	0.02	0.1199								
DOVER 6SS6G	5	1.064	5.32	0.02	0.1064								

DOVER 6SS6H	1	1.934	9.67	0.02	0.1934								
DOVER 6SS6H	2	0.9253	4.6265	0.02	0.09253								
DOVER 6SS6H	3	0.8736	4.368	0.02	0.08736	0.55849	10.00	55.8304					
DOVER 6SS6H	4	1.157	5.785	0.02	0.1157								
DOVER 6SS6H	5	0.6950	3.475	0.02	0.0695								
DOVER 6SS6I	1	2.299	11.495	0.02	0.2299								
DOVER 6SS6I	2	0.9616	4.808	0.02	0.09616								
DOVER 6SS6I	3	0.6896	3.448	0.02	0.06896	0.65362	10.00	65.3402					
DOVER 6SS6I	4	1.477	7.385	0.02	0.1477								
DOVER 6SS6I	5	1.109	5.545	0.02	0.1109								
Tinker SBGSI1 31-31.5G	1	4.828	24.14	0.02	0.4828								
Tinker SBGSI1 31-31.5G	2	2.317	11.585	0.02	0.2317								
Tinker SBGSI1 31-31.5G	3	2.973	14.865	0.02	0.2973	1.25795	10	125.795					
Tinker SBGSI1 31-31.5G	4	0.8755	4.3775	0.02	0.08755								
Tinker SBGSI1 31-31.5G	5	1.586	7.93	0.02	0.1586								
Tinker SBGSI1 31-31.5H	1	5.834	29.17	0.02	0.5834								
Tinker SBGSI1 31-31.5H	2	2.272	11.36	0.02	0.2272								
Tinker SBGSI1 31-31.5H	3	1.842	9.21	0.02	0.1842	1.6648	10	166.48	147.922	20.58	11.9	166	126
Tinker SBGSI1 31-31.5H	4	1.756	8.78	0.02	0.1756								
Tinker SBGSI1 31-31.5H	5	4.944	24.72	0.02	0.4944								
Tinker SBGSI1 31-31.5I	1	1.871	9.355	0.02	0.1871								
Tinker SBGSI1 31-31.5I	2	3.780	18.9	0.02	0.378								
Tinker SBGSI1 31-31.5I	3	1.307	6.535	0.02	0.1307	1.5149	10	151.49					
Tinker SBGSI1 31-31.5I	4	2.259	11.295	0.02	0.2259								
Tinker SBGSI1 31-31.5I	5	5.932	29.66	0.02	0.5932								
Tinker SBGSI1 31.5-33G	1	1.396	6.98	0.02	0.1396								
Tinker SBGSI1 31.5-33G	2	4.618	23.09	0.02	0.4618	1.5742	10	157.42	166.407	16.98	9.8	186	156
Tinker SBGSI1 31.5-33G	3	2.038	10.19	0.02	0.2038								

Tinker SBGSI1 31.5-33G	4	2.086	10.43	0.02	0.2086									
Tinker SBGSI1 31.5-33G	5	5.604	28.02	0.02	0.5604									
Tinker SBGSI1 31.5-33G	1	2.718	13.59	0.02	0.2718									
Tinker SBGSI1 31.5-33H	2	2.992	14.96	0.02	0.2992									
Tinker SBGSI1 31.5-33H	3	6.407	32.035	0.02	0.6407	1.8599	10	185.99						
Tinker SBGSI1 31.5-33H	4	2.292	11.46	0.02	0.2292									
Tinker SBGSI1 31.5-33H	5	4.190	20.95	0.02	0.419									
Tinker SBGSI1 31.5-33I	1	4.135	20.675	0.02	0.4135									
Tinker SBGSI1 31.5-33I	2	2.979	14.895	0.02	0.2979									
Tinker SBGSI1 31.5-33I	3	2.799	13.995	0.02	0.2799	1.5581	10	155.81						
Tinker SBGSI1 31.5-33I	4	2.574	12.87	0.02	0.2574									
Tinker SBGSI1 31.5-33I	5	3.094	15.47	0.02	0.3094									
Tinker SBGSI1 33-35G	1	6.108	30.54	0.02	0.6108									
Tinker SBGSI1 33-35G	2	3.969	19.845	0.02	0.3969									
Tinker SBGSI1 33-35G	3	5.076	25.38	0.02	0.5076	2.2083	10	220.83						
Tinker SBGSI1 33-35G	4	3.095	15.475	0.02	0.3095									
Tinker SBGSI1 33-35G	5	3.835	19.175	0.02	0.3835									
Tinker SBGSI1 33-35H	1	5.468	27.34	0.02	0.5468									
Tinker SBGSI1 33-35H	2	4.495	22.475	0.02	0.4495									
Tinker SBGSI1 33-35H	3	3.612	18.06	0.02	0.3612	2.0728	10	207.28	216.74	8.218	4.74	222	207	
Tinker SBGSI1 33-35H	4	3.348	16.74	0.02	0.3348									
Tinker SBGSI1 33-35H	5	3.805	19.025	0.02	0.3805									
Tinker SBGSI1 33-35I	1	5.534	27.67	0.02	0.5534									
Tinker SBGSI1 33-35I	2	3.966	19.83	0.02	0.3966									
Tinker SBGSI1 33-35I	3	6.185	30.925	0.02	0.6185	2.2211	10	222.11						
Tinker SBGSI1 33-35I	4	3.108	15.54	0.02	0.3108									
Tinker SBGSI1 33-35I	5	3.418	17.09	0.02	0.3418									
Tinker SBGSI1 35-36G	1	5.370	26.85	0.02	0.537	1.9764	10	197.64	201.743	11.01	6.36	214	193	

Tinker SBGSI1 35-36G	2	3.678	18.39	0.02	0.3678									
Tinker SBGSI1 35-36G	3	3.929	19.645	0.02	0.3929									
Tinker SBGSI1 35-36G	4	2.996	14.98	0.02	0.2996									
Tinker SBGSI1 35-36G	5	3.791	18.955	0.02	0.3791									
Tinker SBGSI1 35-36H	1	5.121	25.605	0.02	0.5121									
Tinker SBGSI1 35-36H	2	4.097	20.485	0.02	0.4097									
Tinker SBGSI1 35-36H	3	5.612	28.06	0.02	0.5612	2.1422	10	214.22						
Tinker SBGSI1 35-36H	4	2.859	14.295	0.02	0.2859									
Tinker SBGSI1 35-36H	5	3.733	18.665	0.02	0.3733									
Tinker SBGSI1 35-36I	1	4.987	24.935	0.02	0.4987									
Tinker SBGSI1 35-36I	2	3.739	18.695	0.02	0.3739									
Tinker SBGSI1 35-36I	3	3.589	17.945	0.02	0.3589	1.9337	10	193.37						
Tinker SBGSI1 35-36I	4	3.524	17.62	0.02	0.3524									
Tinker SBGSI1 35-36I	5	3.498	17.49	0.02	0.3498									
Tinker SBGSI1 36.5-38G	1	4.461	22.305	0.02	0.4461									
Tinker SBGSI1 36.5-38G	2	3.804	19.02	0.02	0.3804									
Tinker SBGSI1 36.5-38G	3	6.649	33.245	0.02	0.6649	2.1289	10	212.89						
Tinker SBGSI1 36.5-38G	4	2.907	14.535	0.02	0.2907									
Tinker SBGSI1 36.5-38G	5	3.468	17.34	0.02	0.3468									
TINKER SBGSI136-38H	1	2.898	14.49	0.02	0.2898									
TINKER SBGSI136-38H	2	2.598	12.99	0.02	0.2598				168.023	45.7	26.4	213	122	
TINKER SBGSI136-38H	3	2.125	10.625	0.02	0.2125	1.2153	10	121.53						
TINKER SBGSI136-38H	4	2.251	11.255	0.02	0.2251									
TINKER SBGSI136-38H	5	2.281	11.405	0.02	0.2281									
TINKER SBGSI1 36-38I	1	5.982	29.91	0.02	0.5982									
TINKER SBGSI1 36-38I	2	3.136	15.68	0.02	0.3136	1.6965	10	169.65						
TINKER SBGSI1 36-38I	3	2.835	14.175	0.02	0.2835									
TINKER SBGSI1 36-38I	4	2.371	11.855	0.02	0.2371									

TINKER SBGSI1 36-38I	5	2.641	13.205	0.02	0.2641								
TINKER SBGSI1 38-40G	1	6.006	30.03	0.02	0.6006	2.0091	10	200.91	222.977	23.94	13.8	248	201
TINKER SBGSI1 38-40G	2	2.993	14.965	0.02	0.2993								
TINKER SBGSI1 38-40G	3	4.869	24.345	0.02	0.4869								
TINKER SBGSI1 38-40G	4	3.116	15.58	0.02	0.3116								
TINKER SBGSI1 38-40G	5	3.107	15.535	0.02	0.3107								
TINKER SBGSI1 38-40H	1	5.121	25.605	0.02	0.5121	2.1959	10	219.59					
TINKER SBGSI1 38-40H	2	4.607	23.035	0.02	0.4607								
TINKER SBGSI1 38-40H	3	5.148	25.74	0.02	0.5148								
TINKER SBGSI1 38-40H	4	2.766	13.83	0.02	0.2766								
TINKER SBGSI1 38-40H	5	4.317	21.585	0.02	0.4317								
TINKER SBGSI1 38-40I	1	5.215	26.075	0.02	0.5215	2.4843	10	248.43					
TINKER SBGSI1 38-40I	2	5.700	28.5	0.02	0.57								
TINKER SBGSI1 38-40I	3	6.598	32.99	0.02	0.6598								
TINKER SBGSI1 38-40I	4	3.259	16.295	0.02	0.3259								
TINKER SBGSI1 38-40I	5	4.071	20.355	0.02	0.4071								
TINKER SBGSI4 26-28A	1	3.756	18.78	0.02	0.3756	1.2944	10	129.44	161.37	31.63	18.3	193	129
TINKER SBGSI4 26-28A	2	2.135	10.675	0.02	0.2135								
TINKER SBGSI4 26-28A	3	4.504	22.52	0.02	0.4504								
TINKER SBGSI4 26-28A	4	1.406	7.03	0.02	0.1406								
TINKER SBGSI4 26-28A	5	1.143	5.715	0.02	0.1143								
TINKER SBGSI4 26-28B	1	3.456	17.28	0.02	0.3456	1.927	10	192.7					
TINKER SBGSI4 26-28B	2	5.574	27.87	0.02	0.5574								
TINKER SBGSI4 26-28B	3	4.133	20.665	0.02	0.4133								
TINKER SBGSI4 26-28B	4	3.148	15.74	0.02	0.3148								
TINKER SBGSI4 26-28B	5	2.959	14.795	0.02	0.2959								
TINKER SBGSI4 26-28C	1	3.167	15.835	0.02	0.3167	1.6197	10	161.97					
TINKER SBGSI4 26-28C	2	3.272	16.36	0.02	0.3272								

TINKER SBGSI4 26-28C	3	5.241	26.205	0.02	0.5241								
TINKER SBGSI4 26-28C	4	2.499	12.495	0.02	0.2499								
TINKER SBGSI4 26-28C	5	2.018	10.09	0.02	0.2018								
TINKER SBGSI4 28-30A	1	3.431	17.155	0.02	0.3431	1.5863	10	158.63	143.933	12.97	7.49	159	134
TINKER SBGSI4 28-30A	2	3.056	15.28	0.02	0.3056								
TINKER SBGSI4 28-30A	3	4.995	24.975	0.02	0.4995								
TINKER SBGSI4 28-30A	4	2.325	11.625	0.02	0.2325								
TINKER SBGSI4 28-30A	5	2.056	10.28	0.02	0.2056								
TINKER SBGSI4 28-30B	1	2.439	12.195	0.02	0.2439	1.3906	10	139.06					
TINKER SBGSI4 28-30B	2	3.145	15.725	0.02	0.3145								
TINKER SBGSI4 28-30B	3	4.342	21.71	0.02	0.4342								
TINKER SBGSI4 28-30B	4	1.729	8.645	0.02	0.1729								
TINKER SBGSI4 28-30B	5	2.251	11.255	0.02	0.2251								
TINKER SBGSI4 28-30C	1	3.879	19.395	0.02	0.3879	1.3411	10	134.11					
TINKER SBGSI4 28-30C	2	1.735	8.675	0.02	0.1735								
TINKER SBGSI4 28-30C	3	3.431	17.155	0.02	0.3431								
TINKER SBGSI4 28-30C	4	2.471	12.355	0.02	0.2471								
TINKER SBGSI4 28-30C	5	1.895	9.475	0.02	0.1895								
TINKER SBGSI4 30-31.5A	1	3.450	17.25	0.02	0.345	1.2957	10	129.57	125.61	4.994	2.88	130	120
TINKER SBGSI4 30-31.5A	2	1.649	8.245	0.02	0.1649								
TINKER SBGSI4 30-31.5A	3	4.160	20.8	0.02	0.416								
TINKER SBGSI4 30-31.5A	4	1.864	9.32	0.02	0.1864								
TINKER SBGSI4 30-31.5A	5	1.834	9.17	0.02	0.1834								
TINKER SBGSI4 30-31.5B	1	3.664	18.32	0.02	0.3664	1.2726	10	127.26					
TINKER SBGSI4 30-31.5B	2	2.855	14.275	0.02	0.2855								
TINKER SBGSI4 30-31.5B	3	2.953	14.765	0.02	0.2953								
TINKER SBGSI4 30-31.5B	4	1.526	7.63	0.02	0.1526								
TINKER SBGSI4 30-31.5B	5	1.728	8.64	0.02	0.1728								

TINKER SBGSI4 30-31.5C	1	2.601	13.005	0.02	0.2601	1.2	10	120					
TINKER SBGSI4 30-31.5C	2	3.322	16.61	0.02	0.3322								
TINKER SBGSI4 30-31.5C	3	2.275	11.375	0.02	0.2275								
TINKER SBGSI4 30-31.5C	4	2.022	10.11	0.02	0.2022								
TINKER SBGSI4 30-31.5C	5	1.780	8.9	0.02	0.178								
TINKER SBGSI4 31.5-33A	1	3.431	17.155	0.02	0.3431	1.17838	10	117.838	121.038	12.37	7.14	135	111
TINKER SBGSI4 31.5-33A	2	3.608	18.04	0.02	0.3608								
TINKER SBGSI4 31.5-33A	3	2.369	11.845	0.02	0.2369								
TINKER SBGSI4 31.5-33A	4	1.407	7.035	0.02	0.1407								
TINKER SBGSI4 31.5-33A	5	0.9688	4.844	0.02	0.09688								
TINKER SBGSI4 31.5-33B	1	4.736	23.68	0.02	0.4736	1.3469	10	134.69					
TINKER SBGSI4 31.5-33B	2	3.537	17.685	0.02	0.3537								
TINKER SBGSI4 31.5-33B	3	2.514	12.57	0.02	0.2514								
TINKER SBGSI4 31.5-33B	4	1.585	7.925	0.02	0.1585								
TINKER SBGSI4 31.5-33B	5	1.097	5.485	0.02	0.1097								
TINKER SBGSI4 31.5-33C	1	2.687	13.435	0.02	0.2687	1.10585	10	110.585					
TINKER SBGSI4 31.5-33C	2	3.596	17.98	0.02	0.3596								
TINKER SBGSI4 31.5-33C	3	2.556	12.78	0.02	0.2556								
TINKER SBGSI4 31.5-33C	4	1.339	6.695	0.02	0.1339								
TINKER SBGSI4 31.5-33C	5	0.8805	4.4025	0.02	0.08805								
TINKER SBGSI4 33-35A	1	4.384	21.92	0.02	0.4384	2.0097	10	200.97	186.287	17.81	10.3	201	166
TINKER SBGSI4 33-35A	2	4.406	22.03	0.02	0.4406								
TINKER SBGSI4 33-35A	3	5.510	27.55	0.02	0.551								
TINKER SBGSI4 33-35A	4	3.431	17.155	0.02	0.3431								
TINKER SBGSI4 33-35A	5	2.366	11.83	0.02	0.2366								
TINKER SBGSI4 33-35B	1	3.984	19.92	0.02	0.3984	1.9142	10	191.42					
TINKER SBGSI4 33-35B	2	5.179	25.895	0.02	0.5179								
TINKER SBGSI4 33-35B	3	3.833	19.165	0.02	0.3833								

TINKER SBGSI4 33-35B	4	3.015	15.075	0.02	0.3015								
TINKER SBGSI4 33-35B	5	3.131	15.655	0.02	0.3131								
TINKER SBGSI4 33-35C	1	3.909	19.545	0.02	0.3909								
TINKER SBGSI4 33-35C	2	4.712	23.56	0.02	0.4712								
TINKER SBGSI4 33-35C	3	3.128	15.64	0.02	0.3128	1.6647	10	166.47					
TINKER SBGSI4 33-35C	4	2.159	10.795	0.02	0.2159								
TINKER SBGSI4 33-35C	5	2.739	13.695	0.02	0.2739								
TINKER SBGSI4 35-36A	1	3.397	16.985	0.02	0.3397								
TINKER SBGSI4 35-36A	2	1.072	5.36	0.02	0.1072								
TINKER SBGSI4 35-36A	3	2.521	12.605	0.02	0.2521	1.8226	10	182.26					
TINKER SBGSI4 35-36A	4	2.116	10.58	0.02	0.2116								
TINKER SBGSI4 35-36A	5	9.120	45.6	0.02	0.912								
TINKER SBGSI4 35-36B	1	3.075	15.375	0.02	0.3075								
TINKER SBGSI4 35-36B	2	3.129	15.645	0.02	0.3129								
TINKER SBGSI4 35-36B	3	2.831	14.155	0.02	0.2831	1.39	10	139	143.33	36.96	21.3	182	109
TINKER SBGSI4 35-36B	4	2.309	11.545	0.02	0.2309								
TINKER SBGSI4 35-36B	5	2.556	12.78	0.02	0.2556								
TINKER SBGSI4 35-36C	1	2.802	14.01	0.02	0.2802								
TINKER SBGSI4 35-36C	2	2.593	12.965	0.02	0.2593								
TINKER SBGSI4 35-36C	3	1.975	9.875	0.02	0.1975	1.0873	10	108.73					
TINKER SBGSI4 35-36C	4	1.638	8.19	0.02	0.1638								
TINKER SBGSI4 35-36C	5	1.865	9.325	0.02	0.1865								

Appendix J. Metadata Table

Site	Sample ID	Sample	PBOC (mg/kg)	Depth (m)	Depth Average (m)	Distance from PICT-3 (m)	Treatment
Dover	DV1	6-SS1	106.12			Background	No
Dover	DV1	6-SS1	113.72			Background	No
Dover	DV1	6-SS1	108.78			Background	No
Dover	DV2	6-SS2	68.27			-47	No
Dover	DV2	6-SS2	85.36			-47	No
Dover	DV2	6-SS2	54.94			-47	No
Dover	DV3	6-SS3	350.09			1	Yes
Dover	DV3	6-SS3	335.49			1	Yes
Dover	DV3	6-SS3	294.76			1	Yes
Dover	DV4	6-SS4	80.92			10	Yes
Dover	DV4	6-SS4	70.30			10	Yes
Dover	DV4	6-SS4	64.34			10	Yes
Dover	DV5	6-SS5	60.37			25	Yes
Dover	DV5	6-SS5	47.81			25	Yes
Dover	DV5	6-SS5	69.94			25	Yes
Dover	DV6	6-SS6	56.49			50	No
Dover	DV6	6-SS6	55.83			50	No
Dover	DV6	6-SS6	65.34			50	No
Tinker	TK131	SBGSI-1	125.80	9.5-9.6	9.55		Yes
Tinker	TK131	SBGSI-1	166.48	9.5-9.6	9.55		Yes
Tinker	TK131	SBGSI-1	151.49	9.5-9.6	9.55		Yes
Tinker	TK133	SBGSI-1	157.42	9.6-10	9.8		Yes
Tinker	TK133	SBGSI-1	185.99	9.6-10	9.8		Yes
Tinker	TK133	SBGSI-1	155.81	9.6-10	9.8		Yes
Tinker	TK135	SBGSI-1	220.83	10-10.7	10.35		Yes
Tinker	TK135	SBGSI-1	207.28	10-10.7	10.35		Yes
Tinker	TK135	SBGSI-1	222.11	10-10.7	10.35		Yes
Tinker	TK136	SBGSI-1	197.64	10.7-11	10.85		Yes
Tinker	TK136	SBGSI-1	214.22	10.7-11	10.85		Yes
Tinker	TK136	SBGSI-1	193.37	10.7-11	10.85		Yes
Tinker	TK136.5	SBGSI-1	212.89	11-11.6	11.3		Yes
Tinker	TK136.5	SBGSI-1	121.53	11-11.6	11.3		Yes

Tinker	TK136.5	SBGSI-1	169.65	11-11.6	11.3		Yes
Tinker	TK138	SBGSI-1	200.91	11.6-12.2	11.9		Yes
Tinker	TK138	SBGSI-1	219.59	11.6-12.2	11.9		Yes
Tinker	TK138	SBGSI-1	248.43	11.6-12.2	11.9		Yes
Tinker	TK426	SBGSI-4	129.44	8-8.5	8.25		No
Tinker	TK426	SBGSI-4	192.7	8-8.5	8.25		No
Tinker	TK426	SBGSI-4	161.97	8-8.5	8.25		No
Tinker	TK428	SBGSI-4	158.63	8.5-9.1	8.8		No
Tinker	TK428	SBGSI-4	139.06	8.5-9.1	8.8		No
Tinker	TK428	SBGSI-4	134.11	8.5-9.1	8.8		No
Tinker	TK430	SBGSI-4	129.57	9.1-9.6	9.35		No
Tinker	TK430	SBGSI-4	127.26	9.1-9.6	9.35		No
Tinker	TK430	SBGSI-4	120	9.1-9.6	9.35		No
Tinker	TK431	SBGSI-4	117.838	9.6-10	9.8		No
Tinker	TK431	SBGSI-4	134.69	9.6-10	9.8		No
Tinker	TK431	SBGSI-4	110.585	9.6-10	9.8		No
Tinker	TK433	SBGSI-4	200.97	10-10.7	10.35		No
Tinker	TK433	SBGSI-4	191.42	10-10.7	10.35		No
Tinker	TK433	SBGSI-4	166.47	10-10.7	10.35		No
Tinker	TK435	SBGSI-4	182.26	10.7-11	10.85		No
Tinker	TK435	SBGSI-4	139	10.7-11	10.85		No
Tinker	TK435	SBGSI-4	108.73	10.7-11	10.85		No

Appendix K. R script for PBOC Analysis

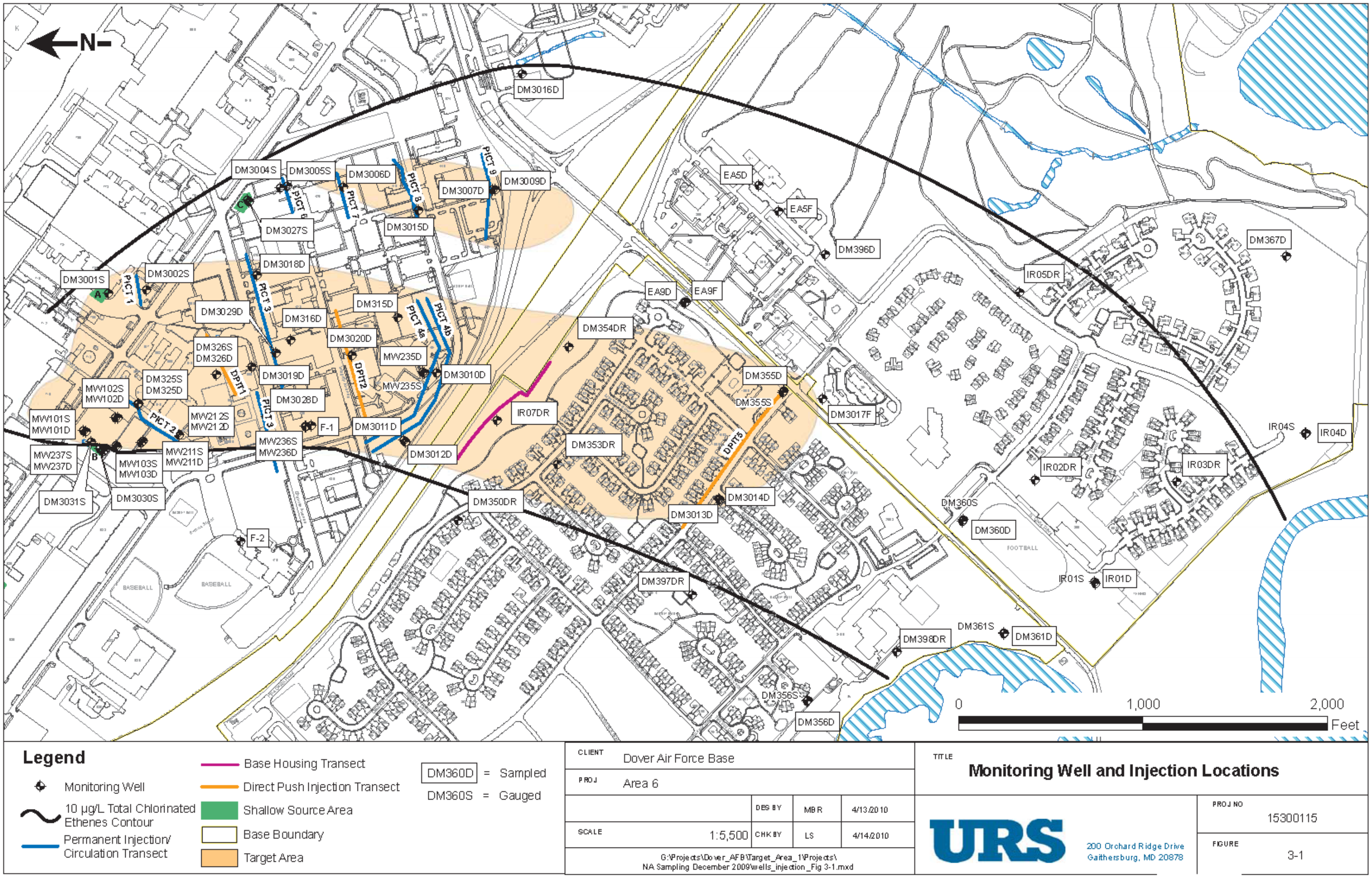
For two-sample t-test—Tinker AFB

```
qt(0.05,10)

data = read.table("tinkerforR.csv", header=T, sep=',')

t.test(data$SBGSI4,data$SBGSI1,mu=-1.812461,alternative ="less", var.equal=TRUE)
```

For one-way ANOVA and Tukey HSD—Dover AFB

```
qf(0.95,5,12)

data = read.table("DoverforR.csv", header=T, sep=',')

data$SampleID = as.factor(data$SampleID)

fit = aov(data$PBOC~data$SampleID)

summary(fit)

TukeyHSD(fit)
```

Appendix L. Dover AFB site map of Area 6 including PICT and monitoring wells (ORNL and URS 2011)



N
DM3016D
DM3004S
DM3005S
DM3006D
DM3007D
DM3009D
DM3015D
DM3027S
DM3018D
DM3001S
DM3002S
DM3029D
DM316D
DM315D
DM3020D
MW235D
DM3010D
DM326S
DM326D
DM325S
DM325D
DM3019D
DM3028D
MW235S
MW102S
MW102D
MW101S
MW101D
MW212S
MW212D
F-1
DM3011D
MW236S
MW236D
MW237S
MW237D
MW103S
MW103D
MW211S
MW211D
DM3012D
DM3031S
DM3030S
F-2
PICT 1
PICT 2
PICT 3
PICT 4a
PICT 4b
PICT 6
PICT 7
PICT 8
PICT 9
DPIT1
DPIT2
DPIT5
IR07DR
DM354DR
DM353DR
DM350DR
EA5D
EA5F
EA9D
EA9F
DM396D
DM355D
DM355S
DM3017F
DM3014D
DM3013D
DM397DR
DM356S
DM356D
DM398DR
DM361S
DM361D
DM360S
DM360D
IR05DR
DM367D
IR04S
IR04D
IR02DR
IR03DR
IR01S
IR01D
BASEBALL
FOOTBALL
0
1,000
2,000
Feet
Legend
Monitoring Well
10 µg/L Total Chlorinated Ethenes Contour
Permanent Injection/ Circulation Transect
Base Housing Transect
Direct Push Injection Transect
Shallow Source Area
Base Boundary
Target Area
DM360D = Sampled
DM360S = Gauged
CLIENT Dover Air Force Base
PROJ Area 6
DES BY MBR 4/13/2010
CHK BY LS 4/14/2010
SCALE 1:5,500
G:\Projects\Dover_AFB\Target_Area_1\Projects\NA Sampling December 2009\wells_injection_Fig 3-1.mxd
TITLE Monitoring Well and Injection Locations
URS
200 Orchard Ridge Drive
Gaithersburg, MD 20878
PROJ NO 15300115
FIGURE 3-1

3-3

Appendix M. Dover AFB, well DM3029D biological parameters and chloroethene concentrations over time in reference to carbon injection events (ORNL and URS 2011)

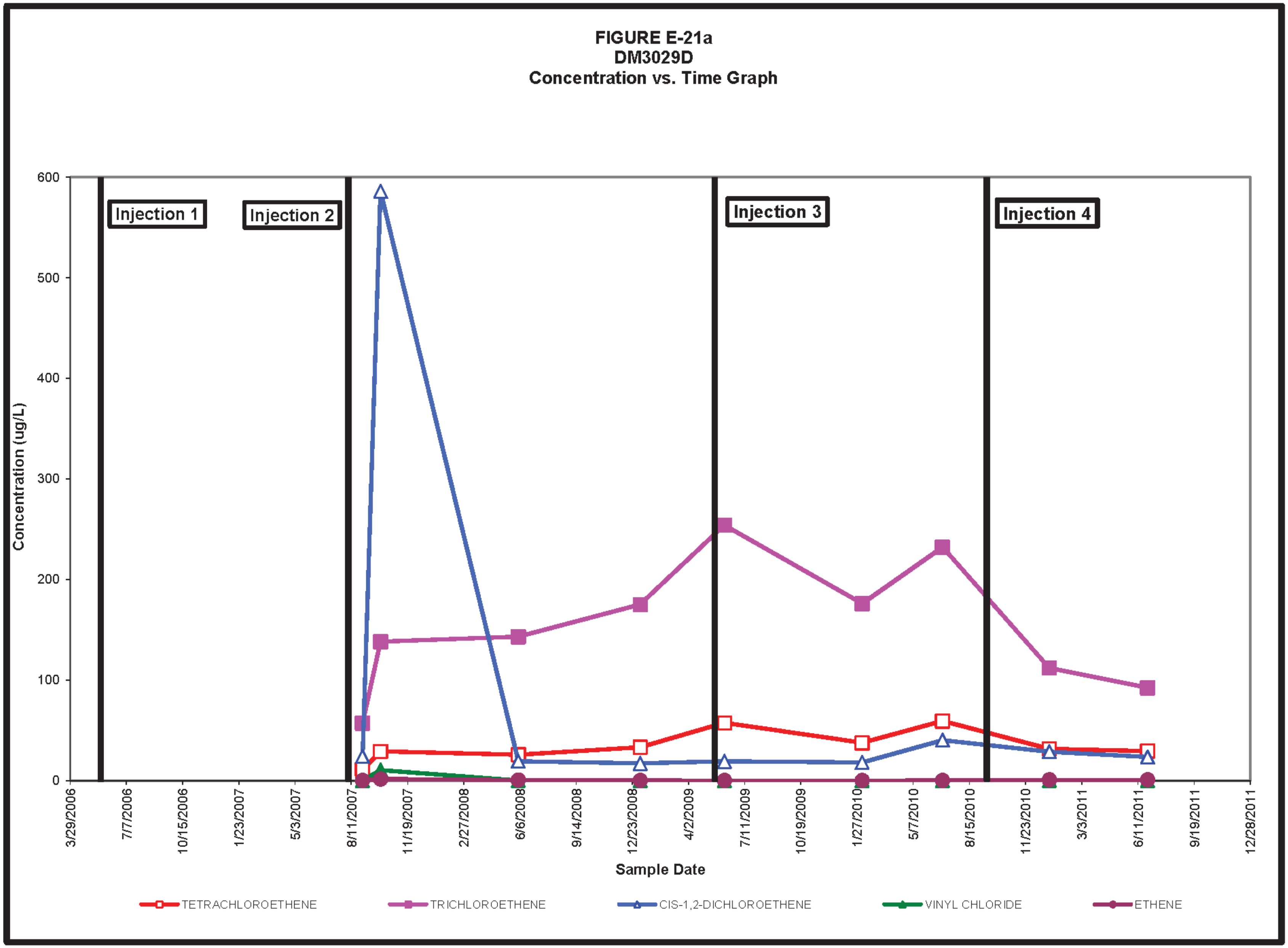
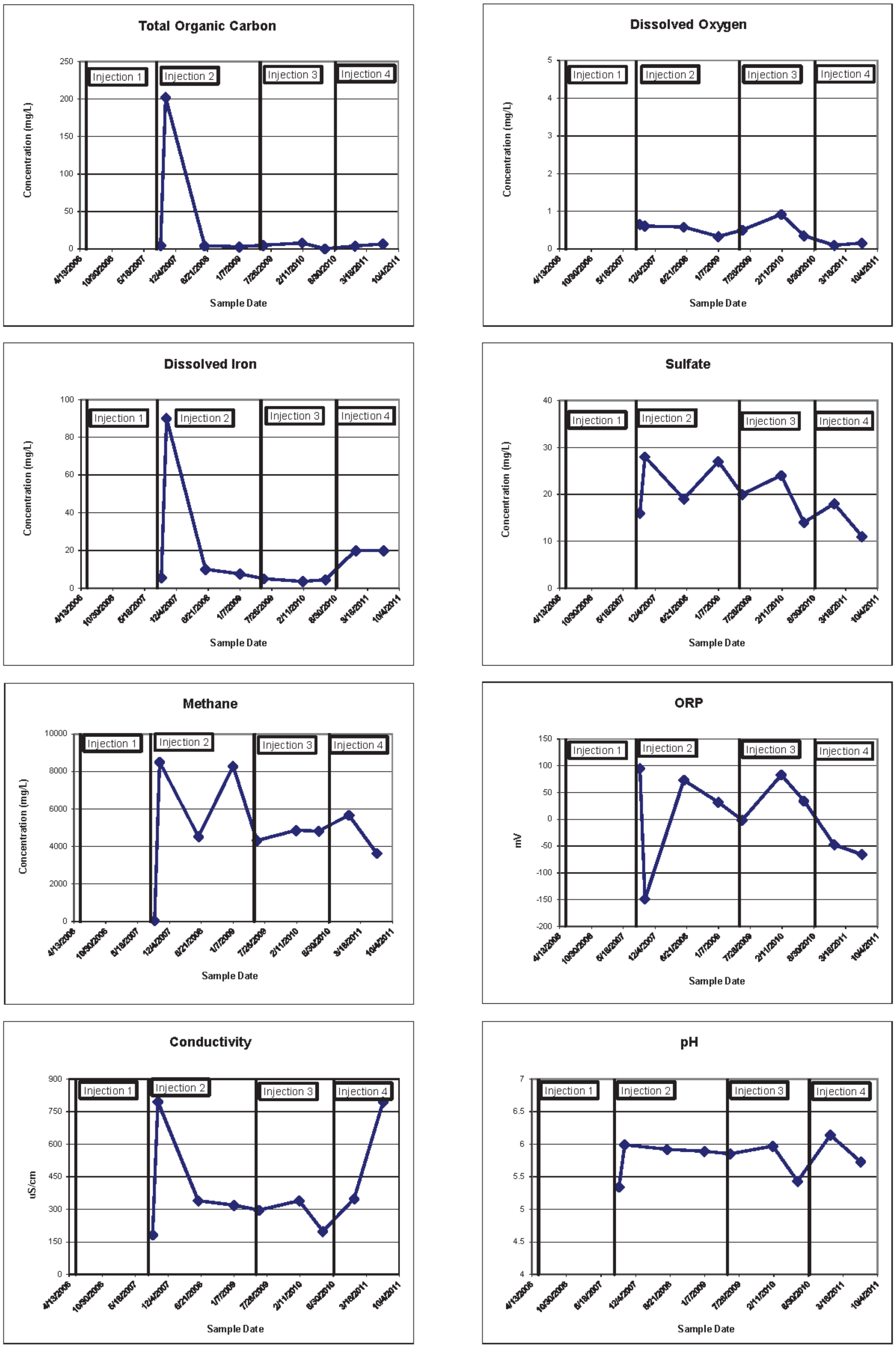


FIGURE E-21a
DM3029D
Concentration vs. Time Graph
Injection 1
Injection 2
Injection 3
Injection 4
Concentration (ug/L)
600
500
400
300
200
100
0
3/29/2006
7/7/2006
10/15/2006
1/23/2007
5/3/2007
8/11/2007
11/19/2007
2/27/2008
6/6/2008
9/14/2008
12/23/2008
4/2/2009
7/11/2009
10/19/2009
1/27/2010
5/7/2010
8/15/2010
11/23/2010
3/3/2011
6/11/2011
9/19/2011
12/28/2011
Sample Date
TETRACHLOROETHENE
TRICHLOROETHENE
CIS-1,2-DICHLOROETHENE
VINYL CHLORIDE
ETHENE

DM3029D - PICT 3
Indicators Over Time



Appendix N. Dover AFB, well DM316D biological parameters and chloroethene concentrations over time in reference to carbon injection events (ORNL and URS 2011)

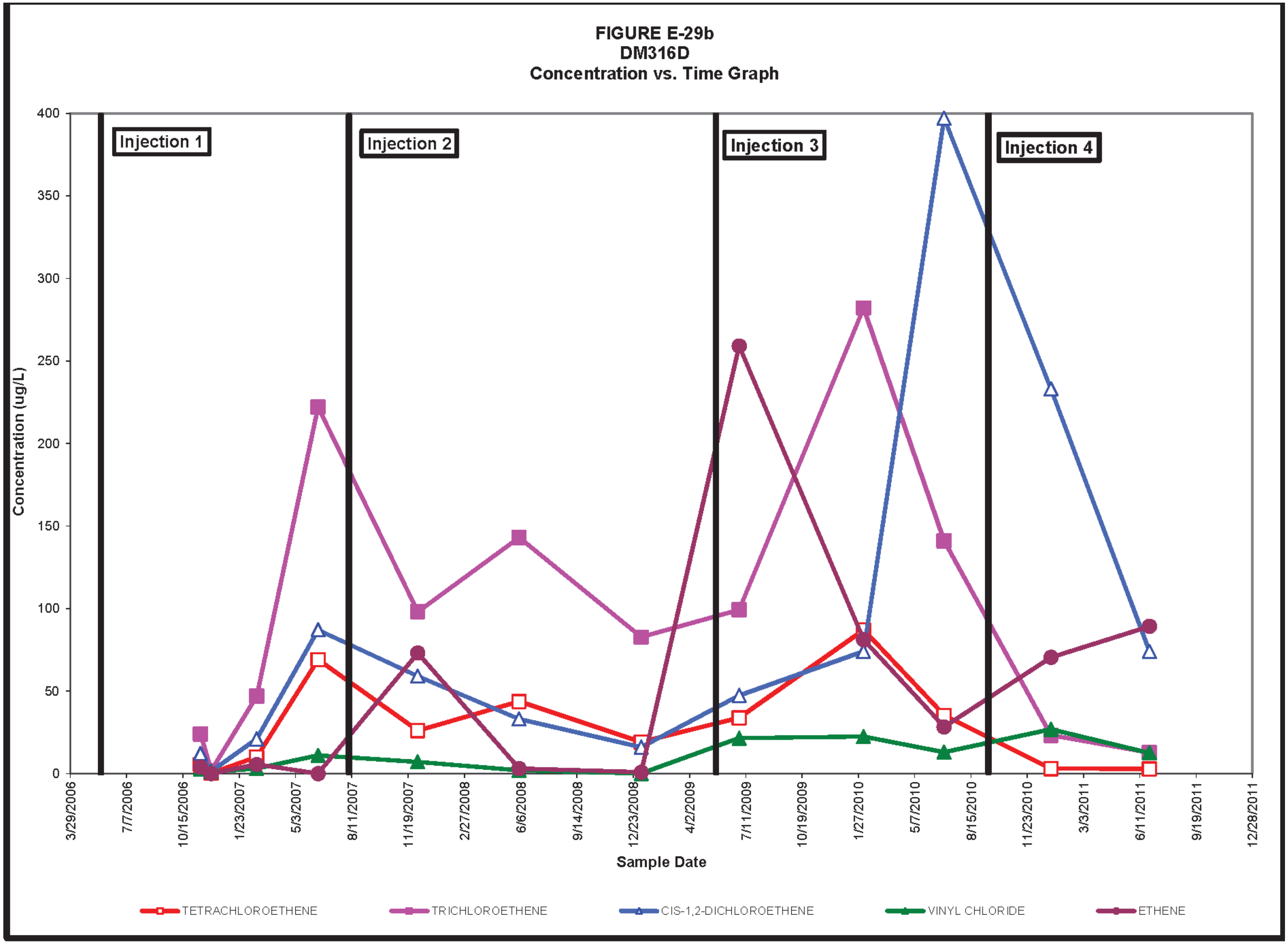
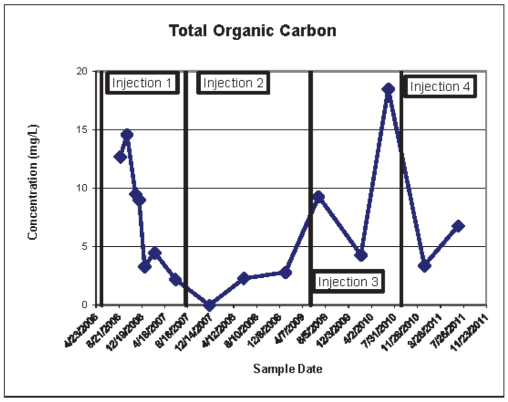
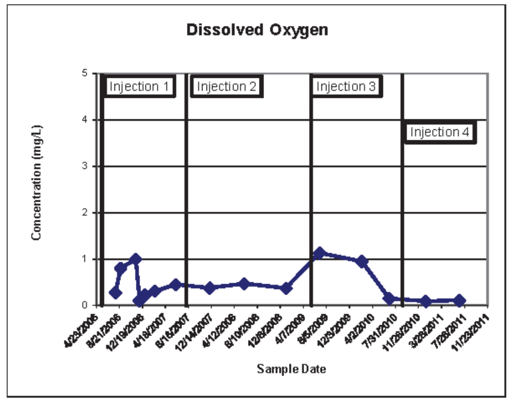
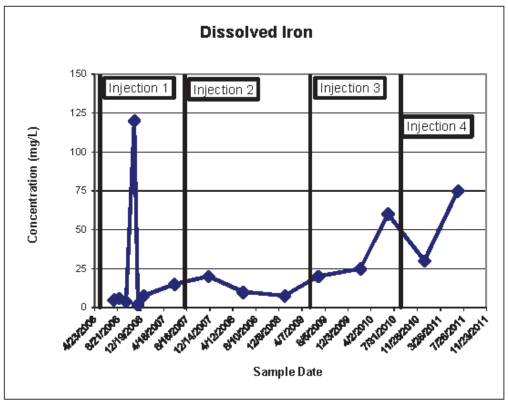


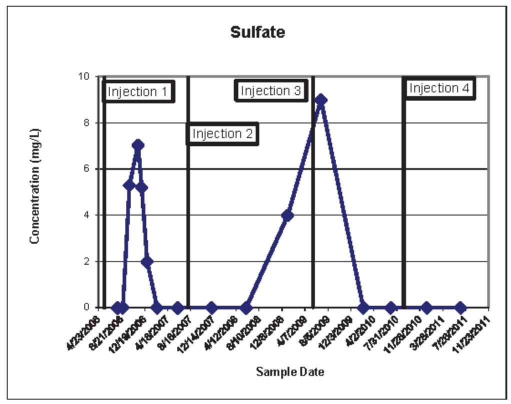
FIGURE E-29b
DM316D
Concentration vs. Time Graph
Injection 1
Injection 2
Injection 3
Injection 4
Concentration (ug/L)
400
350
300
250
200
150
100
50
0
3/29/2006
7/7/2006
10/15/2006
1/23/2007
5/3/2007
8/11/2007
11/19/2007
2/27/2008
6/6/2008
9/14/2008
12/23/2008
4/2/2009
7/11/2009
10/19/2009
1/27/2010
5/7/2010
8/15/2010
11/23/2010
3/3/2011
6/11/2011
9/19/2011
12/28/2011
Sample Date
TETRACHLOROETHENE
TRICHLOROETHENE
CIS-1,2-DICHLOROETHENE
VINYL CHLORIDE
ETHENE

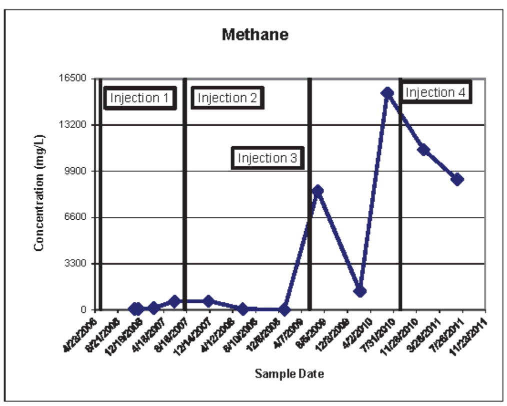
DM316D - PICT 3
Indicators Over Time

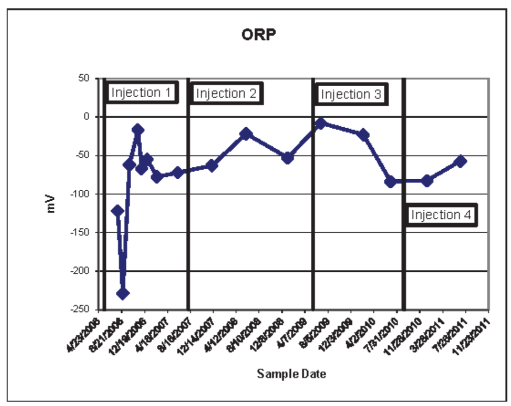


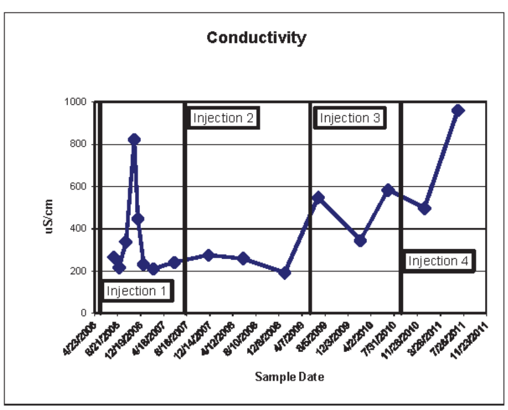


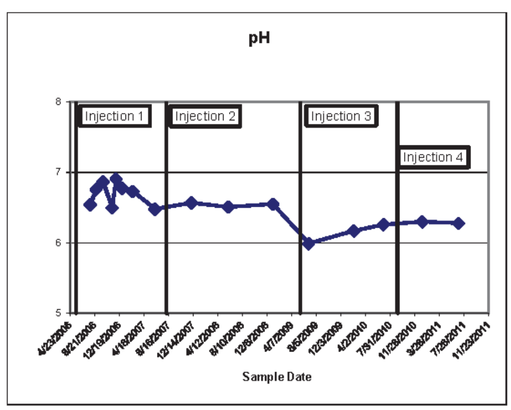












Appendix O. Complete results for One-way ANOVA and Tukey's HSD tests

Summary of results for the one-way ANOVA test.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P-value
Treatment	5	165028	33006	153.3	1.93E-10
Residuals (error)	12	2583	215		

Tabulated results for the Tukey HSD test with all possible sample comparisons, 95% confidence interval, adjusted p-value, and their significance at α=0.05.

Comparison	Difference	Confidence Interval		P-value Adjusted	Significant at α=0.05?
		Lower Bound	Upper Bound		
DV2-DV1	-40.0167	-80.2546	0.2213	0.0516	No
DV3-DV1	217.2400	177.0021	257.4779	0.0000	Yes
DV4-DV1	-37.6867	-77.9246	2.5513	0.0713	No
DV5-DV1	-50.1667	-90.4046	-9.9287	0.0124	Yes
DV6-DV1	-50.3200	-90.5579	-10.0821	0.0121	Yes
DV3-DV2	257.2567	217.0187	297.4946	0.0000	Yes
DV4-DV2	2.3300	-37.9079	42.5679	0.9999	No
DV5-DV2	-10.1500	-50.3879	30.0879	0.9521	No
DV6-DV2	-10.3033	-50.5413	29.9346	0.9492	No
DV4-DV3	-254.9267	-295.1646	-214.6887	0.0000	Yes
DV5-DV3	-267.4067	-307.6446	-227.1687	0.0000	Yes
DV6-DV3	-267.5600	-307.7979	-227.3221	0.0000	Yes
DV5-DV4	-12.4800	-52.7179	27.7579	0.8947	No
DV6-DV4	-12.6333	-52.8713	27.6046	0.8899	No
DV6-DV5	-0.1533	-40.3913	40.0846	1.0000	No

Appendix P. Complete results from the two sample t-test in R

Two Sample t-test

data:
data$SBGSI4 and data$SBGSI1

t-value	degrees of freedom	p-value
-2.421	10	0.018

alternative hypothesis:
true difference in means is less than -1.812461

95 percent confidence interval:

Lower bound	Upper bound
-infinity	-11.50519

sample estimates:

mean of x	mean of y
146.9279	187.3022

Appendix Q. Primer Barcode used for Illumina MiSeq Sequencing

Sample name	Primer Number	Reverse Complement of Golay barcode	Reverse Complement, broken up by portion marked					
DV1B	806rcbc29	ATCCCGAATTTG	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ATCCCGAATTTG	ATCTCGTATGCCGTCTTCTGCTTG
DV2B	806rcbc30	GTTGGTCAATCT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GTTGGTCAATCT	ATCTCGTATGCCGTCTTCTGCTTG
DV1C	806rcbc31	TAGCTCGTAACT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TAGCTCGTAACT	ATCTCGTATGCCGTCTTCTGCTTG
DV2C	806rcbc32	CAGTGCATATGC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	CAGTGCATATGC	ATCTCGTATGCCGTCTTCTGCTTG
DV6C	806rcbc45	GTCTAATTCCGA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GTCTAATTCCGA	ATCTCGTATGCCGTCTTCTGCTTG
DV6B	806rcbc46	TCCGAATTCACA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TCCGAATTCACA	ATCTCGTATGCCGTCTTCTGCTTG
DV5C	806rcbc48	GGCCACGTAGTA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GGCCACGTAGTA	ATCTCGTATGCCGTCTTCTGCTTG
DV5B	806rcbc49	TAGGAACTGGCC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TAGGAACTGGCC	ATCTCGTATGCCGTCTTCTGCTTG
DV5A	806rcbc50	CTAGCGAACATC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	CTAGCGAACATC	ATCTCGTATGCCGTCTTCTGCTTG
DV4C	806rcbc51	GACAGGAGATAG	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GACAGGAGATAG	ATCTCGTATGCCGTCTTCTGCTTG
DV4B	806rcbc52	ATTCCTGTGAGT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ATTCCTGTGAGT	ATCTCGTATGCCGTCTTCTGCTTG
DV4A	806rcbc53	GAGGCTCATCAT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GAGGCTCATCAT	ATCTCGTATGCCGTCTTCTGCTTG
DV3C	806rcbc54	TCCTCTGTCGAC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TCCTCTGTCGAC	ATCTCGTATGCCGTCTTCTGCTTG
DV3B	806rcbc55	CTATTTGCGACA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	CTATTTGCGACA	ATCTCGTATGCCGTCTTCTGCTTG
TK430C	806rcbc60	GTCAATTGACCG	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GTCAATTGACCG	ATCTCGTATGCCGTCTTCTGCTTG
TK430B	806rcbc61	ATGAGACTCCAC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ATGAGACTCCAC	ATCTCGTATGCCGTCTTCTGCTTG
TK431C	806rcbc62	GAATCTTCGAGC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GAATCTTCGAGC	ATCTCGTATGCCGTCTTCTGCTTG
TK431B	806rcbc63	ACACGTAAGCCT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ACACGTAAGCCT	ATCTCGTATGCCGTCTTCTGCTTG

TK431A	806rcbc64	GAGTGGTAGAGA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GAGTGGTAGAGA	ATCTCGTATGCCGTCTTCTGCTTG
TK138C	806rcbc65	GAAGTTGGAAGT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GAAGTTGGAAGT	ATCTCGTATGCCGTCTTCTGCTTG
TK138B	806rcbc66	TTCCTAGGTGAG	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TTCCTAGGTGAG	ATCTCGTATGCCGTCTTCTGCTTG
TK138A	806rcbc67	GCACGACAACAC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GCACGACAACAC	ATCTCGTATGCCGTCTTCTGCTTG
TK131C	806rcbc68	ATCGATCTGTGG	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ATCGATCTGTGG	ATCTCGTATGCCGTCTTCTGCTTG
TK131B	806rcbc69	CTTGTGTCGATA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	CTTGTGTCGATA	ATCTCGTATGCCGTCTTCTGCTTG
DV6A	806rcbc72	GAACTAGTCACC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	GAACTAGTCACC	ATCTCGTATGCCGTCTTCTGCTTG
DV1A	806rcbc101	AACACAAGGAGT	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	AACACAAGGAGT	ATCTCGTATGCCGTCTTCTGCTTG
TK131A	806rcbc102	AATGTCCGTGAC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	AATGTCCGTGAC	ATCTCGTATGCCGTCTTCTGCTTG
TK430A	806rcbc103	TACTTCGCTCGC	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TACTTCGCTCGC	ATCTCGTATGCCGTCTTCTGCTTG
DV2A	806rcbc106	TGACCTCCAAGA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	TGACCTCCAAGA	ATCTCGTATGCCGTCTTCTGCTTG
DV3A	806rcbc107	ACAAGGAGGTGA	ATTAGAWACCCVHGTAGTCC	GG	CTGACTGACT	ACAAGGAGGTGA	ATCTCGTATGCCGTCTTCTGCTTG