Testing environmental controls on methane generation during microbial degradation of coal and oil from the Cherokee basin, Kansas

by

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B.S., Kansas State University, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Geology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Approved by:

Major Professor
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Abstract

Biodegradation of crude oil to methane has long been known to exist in shallow petroleum reservoirs. It is only in the past decade, however, in which the concept of in-reservoir petroleum biodegradation has changed from a model emphasizing aerobic crude-oil degradation (with oxygen delivered down from meteoric waters) to a more recent model in which crude-oil degradation is driven by anaerobic processes (methanogenic microorganisms). In this study, we examine controls on microbial conversion of crude oil and coal into methane in middle-Pennsylvanian strata in the Cherokee Basin, Kansas, USA and how access to oil or coal influence microbial communities. Specifically, we considered the following hypotheses: 1) microorganisms in the basin are capable of generating methane by degrading crude oil or coal and 2) potential controls on the rate of methane formation include microbial diversity, formation water chemistry, nutrient abundance, and carbon dioxide abundance. To test these hypotheses, we used three sets of laboratory experiments constructed of materials from the Cherokee basin, Kansas. One set tested environmental controls on methane generation from oil, another from coal, and a third was a control experiment that utilized methanogenic substrates rather than oil or coal. In the experiments with oil and coal, environmental factors tested ammonium/phosphate availability, feedlot wastewater injection, and carbon dioxide abundance. Our experiments also tested the influence of salinity, by including materials from a well producing water with relatively low salinity and a well producing water with relatively high salinity. The cultures were allowed to incubate from approximately 75 to 170 days, during which headspace of oil and coal bioreactors were sampled periodically and analyzed for methane concentrations. Post incubation analyses included microbial DNA sequencing. We determined that a higher concertation of methanogens existed in the lower salinity well, which has higher potential for practical stimulatory injection. Of methane produced, the only significant (Mann Whitney) treatment had access to oil in lower salinity formation water. Access to coal resulted in no significant results. Microbial diversity, in the form of methanogenic archaea abundance, formation water chemistry (salinity), and wastewater nutrient often correlated with increased, yet insignificant, rates of methane production, while carbon dioxide abundance showed no benefit. Of methanogenic substrates consumed, we determined that most Cherokee basin methanogens preferred methanol over hydrogen and acetate.

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Acknowledgements

The completion of this thesis could not have been completed without the support of current and former members of the Geomicrobiology research group including Kyle Marquart, Ben Haller, Janet Paper, and Dr. Kirk - the captain himself. Janet spent many late hours troubleshooting the gas chromatograph on my behalf, and many more hours training me how to use QIIME (microbial analysis software). Ben assisted with field sampling, and provided general laboratory assistance more times than could be recollected. Kyle showed me the ins-and-outs of using the ion chromatograph, microbial DNA extraction, and insight into microbial interpretation. Dr. Kirk went above and beyond as a research advisor, providing me the stepping stones I needed, when I needed them, to navigate through this research project. To all professors, relatives, friends, lab-mates, and others who in one way or another shared support, either financially, morally, or physically: Thank you.

This research project was made possible due to generous grant scholarships from both *American Association of Petroleum Geologists* and *Geological Society of America.* Additional thanks is given to the folks at *Quest Cherokee L.L.C.,* formerly known as *PostRock Energy Corp.,* in Chanute, KS. They not only provided professional insight into the basin, but more importantly time out of their busy schedule to grant us access to various wells. Lastly, credit is given to the department head, Dr. Pamela Kempton, and fellow K-State Geology Dept. faculty who granted me three semesters of a vital GTA position which allowed myself to teach classes in exchange for coursework and living expenses while pursuing research.

Chapter 1 - Introduction

In a world of increasing population and demand for energy, the importance of microbial methanogenesis is often overlooked (Gieg et al. 2008). Methanogenic archaea are capable of producing methane, the primary component of natural gas, and have the potential to re-supply our world's currently depleting gas reservoirs if properly stimulated. By learning about the structure and environmental controls of these complex anaerobic organisms, direct nutrient-stimulation approaches may be developed to uncap the methanogenic potential of these living assets and harness their power as an energy source (Figure 2).

Biogenic production of methane from complex organic matter, such as oil and coal, is done through the combined effort of fermentative bacteria that are syntrophically associated with methanogenic archaea (Strąpoć et al. 2011). Specifically, microbial conversion of hydrocarbons into methane consists of two phases: (1) Bacterial fermentation of polymers and monomers from the organic matter into fatty acids, organic acids, alcohol, hydrogen, and carbon dioxide, and (2) subsequent reduction of those substrates follows via secondary fermentative bacteria (syntrophs), homoacetogenic bacteria, and acetoclastic, methylotrophic, and hydrogenotrophic methanogenic archaea (Schink 2006).

Temperature, formation water salinity, microbial identity, and nutrient availability are some of the more common controls on methanogenesis (Wilhelms et al. 2001, Strąpoć et al. 2011). Physical properties of coal and oil may further control the rate of degradation through hindrance of fermentation bacteria (Jones et al. 2008). Other components such as livestock wastewater and CO_2

gas may yield potential as well-injectable nutrient sources to stimulate methane production, even though they are unconventional (waste) resources. For more details on environmental controls, refer to subsection, titled *Environmental Controls on Methanogenesis*.

The extent is not known to which waste nutrients such as these may be utilized for gas production in subsurface reservoirs. Furthermore, because biostimulation of coalbed methane reservoirs is a relatively new concept (Jones et al. 2008), the extent is not known to which environmental controls, such as salinity or availability of oil and coal, serve upon methanogenic stimulation. It is possible that stimulation of a high salinity well would be vastly different than a low salinity well. The same question about stimulation strategy could be posed with regard to the availability of oil or coal within a well.

In response to these questions, we prepared three laboratory experiments constructed of materials from the Cherokee basin, Kansas. One set tested environmental controls on methane generation from oil. A parallel set focused on coal. The third set was a control experiment that utilized methanogenic substrates rather than coal or oil. In the first two experiments, environmental factors tested ammonium/phosphate availability, feedlot wastewater injection, salinity, and CO_2 abundance. The Cherokee basin is a good fit for study because it is likely that biogenic gas may be forming in the Cherokee basin subsurface. Additionally, the basin stratigraphy contains black shale (oil) and coal, which may provide further insight into methanogenic influence held by fossil fuels.

Environmental controls on methanogenesis

When fermenting bacteria have access to coal, molecules may be more resistant to biodegradation with increasing thermal maturity and/or depth of the coal under study (Jones et al. 2008). Microbial methane generation was observed from subbituminous coals in Alaska, Texas, and the Powder River basin, not seen in higher-maturity coals from Pennsylvania (Strąpoć et al. 2011, Jones et al. 2008). The Cherokee basin, for comparison, is partially-mature in rank (less mature than Pennsylvania fields, and more mature than the Powder River basin). When fermenting bacteria have access to oil, they tend to break up the lighter, easier-to-degrade components of crude oil, while avoiding the heavier crude components (Shelton et al. 2016). Just like humans, bacteria prefer to use less energy when possible, particularly bacteria faced with the task of oil degradation. For most hydrocarbon-digestive bacterial groups, presence of heavier oil limits substrate production more-so than lighter oil. Because of this, the lighter or heavier a crude is, in terms of API gravity, may indicate heavy to minimal biodegradation, respectively. For reference (Meyer et al. 1984), the majority of oil recovered within the Cherokee basin is "light" in classification (APIgrav = > 25°). Cherokee basin reserve estimates from 1984 recorded recovery of 66 million barrels of "light" crude oil, 4 million barrels of "medium" crude (APIgrav = 20 to 25°), and 0 barrels of "heavy" or "extremely heavy" crude (APIgrav = < 20°).

Temperature is a fundamental control on methane generation in the subsurface. Microorganisms, particularly those that produce methane, are able to survive across a wide spectrum of temperatures. However, the upper temperature limit for microbial gas generation in subsurface hydrocarbon reservoirs appears to be about 80 to 90°C (Wilhelms et al. 2001). Previous work in the Cherokee basin determined that temperature correlates significantly with microbial diversity, particularly methanogens such as *Methanomicrobiales, Methanobacteriales,* and *Methanococcus*

(Kirk et al. 2015). Separately, in an anaerobic scenario that explored the influence of temperature, with respect to pH on biogas accumulation through methanogenic digestion of pig manure with straw, it was found that temperature was a more prominent factor affecting the community of methanogens than pH (Song et al. 2016). In another study, in which biological production of CH_4 was evaluated as a function of incubation temperature, cultures grown at 30 and 38°C accumulated 118% greater CH_4 than of that accumulated in 22°C tubes (Green et al. 2008).

The chemistry of formation water (FW), including TDS (total dissolved solids), pH, and ion chemistry, greatly varies within the Cherokee basin. Previous work determined TDS to range from 34.9 to 91.3 g L^{-1}. There is an indication of a link between TDS, well depth, and longitude (Figure 1). Previous data also suggests that composition of the archaeal community is most closely related to variation in FW salinity (Kirk et al. 2015) which incorporates the assumption that with brinier FW, it is some degree harder for microbes to survive or effectively function. A summary of the overall Cherokee basin geochemistry may be seen in Table 1.

Methanogens represent a diverse array of microorganism groups whose nutritional requirements vary considerably (Balch et al. 1979). For example, *Methanobacterium* species have minimal nutrient requirements and grow autotrophically on H_2-CO_2 with sulfide and ammonium as the sole sources of sulfur and nitrogen (Bhatnagar et al. 1984, Bryant et al. 1971). For this reason, we will be supplying some cultures with ammonium. Nitrogen is an essential component of DNA, RNA, and proteins, and is also needed to synthesize amino acids (Bryant et al. 1971). Ammonium chloride (NH_4Cl) was also incorporated as a nitrogen source (Bhatnagar et al. 1984). Separately, previous studies have shown supplementation of phosphate into wells to correspond to higher rates

of methanogenesis (Pfeiffer et al. 2010). *In situ* microbially enhanced CBM stimulation performed in the Powder River Basin showed an increase in methane production (relative to the curve of expected production decline) after injection of a phosphate treatment (Strąpoć et al. 2011 and Pfeiffer et al. 2010), so for this reason, monopotassium phosphate $KH_2(PO_4)_4$ is incorporated as a phosphate source.

Several authors have attempted nutrient bio-stimulation of methanogenesis from coal (Green et al. 2008). Nutrients that have been tested and showed some degree of success include: ammonia, phosphate, yeast extract, milk, urea, agar, trace metals, and various vitamins (Strąpoć et al. 2011). To our knowledge, nobody has previously tested the impact of livestock wastewater injection on the rate of gas formation from coal and oil, particularly in the Cherokee basin. In multiple basins, however, biogenic methane was initiated or stimulated after the introduction of meteoric (precipitation-influx based) water into the system (Bachu et al. 2003). High availability of water, vitamins, and other nutrients such as urea (the main nitrogen-containing substance in the urine of mammals, $CO(NH_2)_2$) are saturated within sterilized livestock wastewater. However, waste nutrients such as these are often lacking in CBM reservoirs such as the Cherokee basin. Because of this, sterilized livestock wastewater is to be tried –at the laboratory level- as a stimulant.

Carbon dioxide (CO_2) is often released as a byproduct waste gas which, when released into the atmosphere, can trap global heat as a greenhouse gas. This induces stress on the environment through unnecessary warming and ocean acidification. With many new technological advancements in CO_2 sequestration/monitoring (i.e. the injection of CO_2 into the subsurface as a means for underground storage and eventual mineralization), there is also hinted potential for CO_2

to be used as a methanogenic stimulant in subsurface natural gas reservoirs. Most known methanogenic archaea possess the ability to reduce CO_2 to CH_4 (Mayumi et al. 2013). Mayumi determined that in a microcosm mimicking high-T oil reservoir conditions, methanogenesis occurs in both high and low CO_2 conditions, but that an increase in CO_2 pressure into the system accelerates the rate of methanogenesis more than twice that under low CO_2 conditions. This is distinct from our own study focusing on relatively low temperature/low pressure coalbeds. Therefore, the effects of injection of CO_2 upon low temperature environments, as seen within the Cherokee basin, must be better understood in the laboratory if there is any future possibility to practically inject CO_2 into shallow commercial gas wells.

There are an assortment of other factors which may influence methanogenic processes in the subsurface. Pore size distribution and surface area of the coal particles play an important factor into the degree of biological interaction that the coal may endure, in terms of bacterial penetration, reproduction, and habitable substrate availability within the coal (Budwill, 2003). In general, a greater surface area available for microbe-interaction is associated with a higher rate of methanogenesis (Green et al. 2008). Ensuring the storage of laboratory cultures and materials in a dark environment is also important to prevent the rise of phototrophic groups which would not exist *in-situ.*

The Cherokee basin

The Cherokee basin of southeastern Kansas is located across Wilson, Neosho, Montgomery, and Labette counties (Figure 3). Cherokee basin coal beds are a good potential target for future stimulation, and therefore a good locality to study Coalbed Methane (CBM) processes. We know that the Cherokee basin is shallow, and consists of low ranking, partially mature coals.

Furthermore, we have conclusive isotopic and microbial evidence that biogenic gas is being commercially pumped from the wells (Kirk et al. 2015). From this information, it is likely that biogenic gas is concurrently forming in the Cherokee basin subsurface. Previous microbial community analysis of FW indicated that the local archaeal community consisted almost entirely of methanogens, with an assortment of various bacterial groups also thriving in the FW. Isotopic signatures of methane from the basin cluster within the "biogenic gas" margin as determined (Figure 4) through their molecular composition, and abundance of CH_4 relative to higher hydrocarbons (Faiz et al. 2006). Generally, CBM forms either through thermal maturation of organic matter at temperatures above 70°C or through microbiological degradation of organic matter (Faiz et al. 2006). Of these two formation pathways, only microbial methane continues to form in many coal seams today because they have been uplifted and cooled (Faiz et al. 2006), as is the likely case of the Cherokee basin.

The geologic group of interest for this study is the Cherokee Group of Pennsylvanian Age. There are a number of different coals 400' to 1200' deep (Figure 5) within the Cherokee group that differ in thickness, maturity and lateral extent (Newell et al. 1987). Most coal beds are approximately 1-5 feet thick. There are two economically important coals within the basin: The Weir-Pittsburg coal, and the Riverton coal (Woody 1985). Within the Pennsylvanian group, vitrinite reflectance is between 0.5 -0.7% which shows that the group is partially mature (Newell 2010). The Mulky formation, underlying the Fort Scott limestone, is similarly ranking (mature) and Pennsylvanian aged. Samples of this coal layer were collected from a highway outcrop, crushed, and used as a source of coal in Experiment 2.

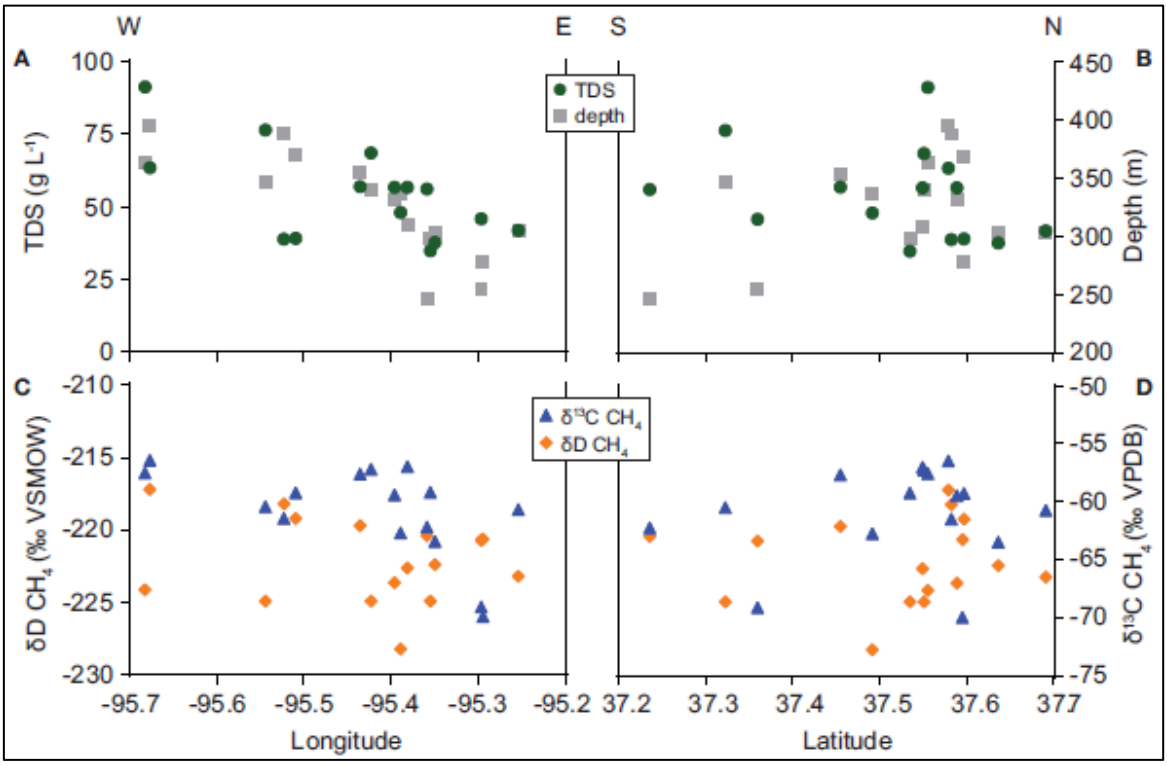


Figure 1 – Well longitude, depth, and TDS

(Kirk et al. 2015): Spatial variation in (A,B) TDS concentration and total well depth and (C,D) carbon and hydrogen isotope ratios of methane from 16 well samples collected across the Cherokee Basin. Average TDS increased proportionally with well depth, and increased with longitude.

Table 1 - Cherokee basin water chemistry

(Kirk. et al. 2015) Summary of geochemistry collected from produced water in the Cherokee Basin in 2015 previous study.

	Min	Max	Avg	*SD*
PRODUCED WATER				
pH	6.6	7.6	7.0	0.3
T (°C)	15.2	28.2	19.0	3.7
Cond. (mS cm^{-1})	46.4	79.6	60.9	10.2
Alk.(meq L^{-1})	3.3	8.5	4.9	1.5
Cl^- (M)	0.6	1.6	1.0	0.3
Br^- (mM)	0.9	3.7	1.8	0.8
SO_4^{2-} (μM)	7	112	50	22
Na^+ (M)	0.5	1.2	0.8	0.2
K^+ (mM)	1.7	4.7	2.6	0.9
Mg^{2+} (mM)	14.2	78.7	36.0	19.0
Ca^{2+} (mM)	11.7	58.5	31.8	12.5
Fe^{2+} (μM)	2	1502	372	438
NPOC (μM)	109	2018	295	483
TN (mM)	0.8	2.1	1.3	0.4

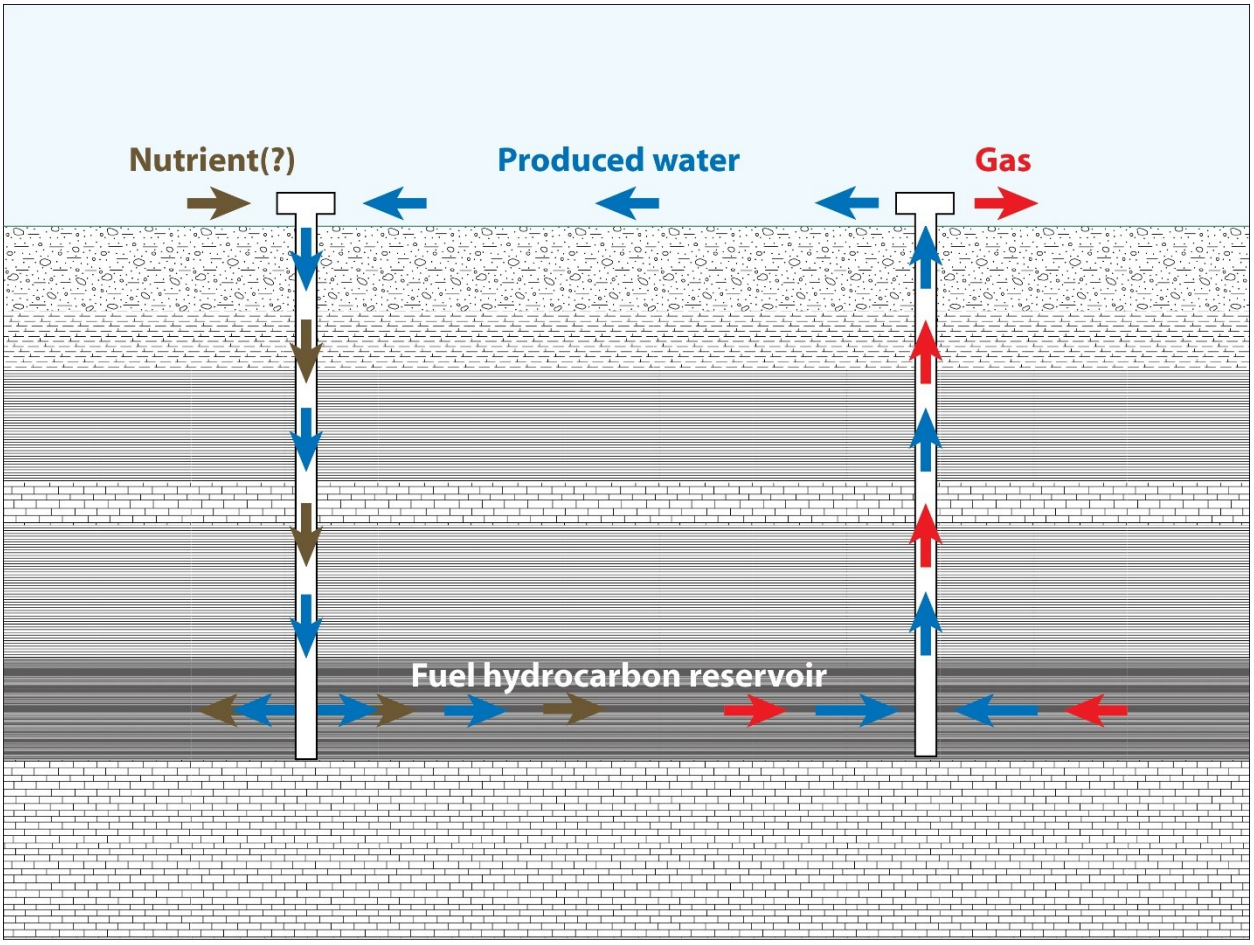


Figure 2 – Biostimulation diagram

Methane may be stimulated biogenically through nutrient injection into the subsurface, where coal and nutrients (brown) are directly consumed microbially *in situ.* Methane (red) is then recovered, along with produced water (blue), where the methane is sold and the water is re-stocked with nutrients and flushed back into the subsurface. Theoretically, this cycle could continue as long as there are nutrients being injected, methanogens present, and organic matter available to degrade.

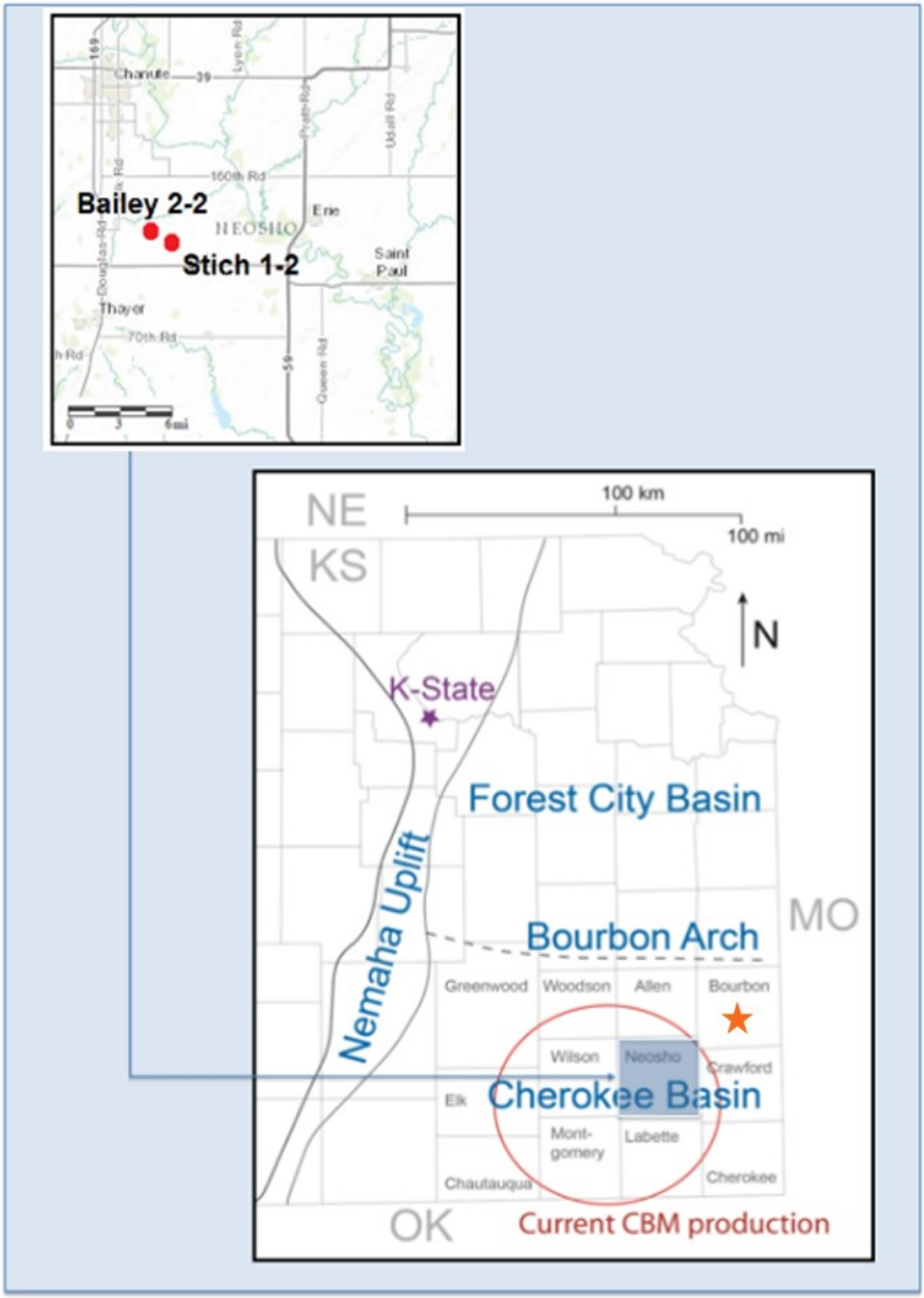


Figure 3 - Map of SE Kansas

The Cherokee Basin is a natural gas coalbed methane field in Southeastern Kansas. Working with energy company personnel, we collected oil, water, and microbe samples from two commercial gas wells in the Cherokee Basin during February 2015. The lease name of the southern well is Stich 1-2, while the lease name of the northern well is Bailey 2-2. Southeast of Chanute, both wells are located in Neosho County and are separated by approximately 1.5 miles of lateral (W-E) difference and .5 miles vertically (N-S). Coal was collected in Bourbon County.

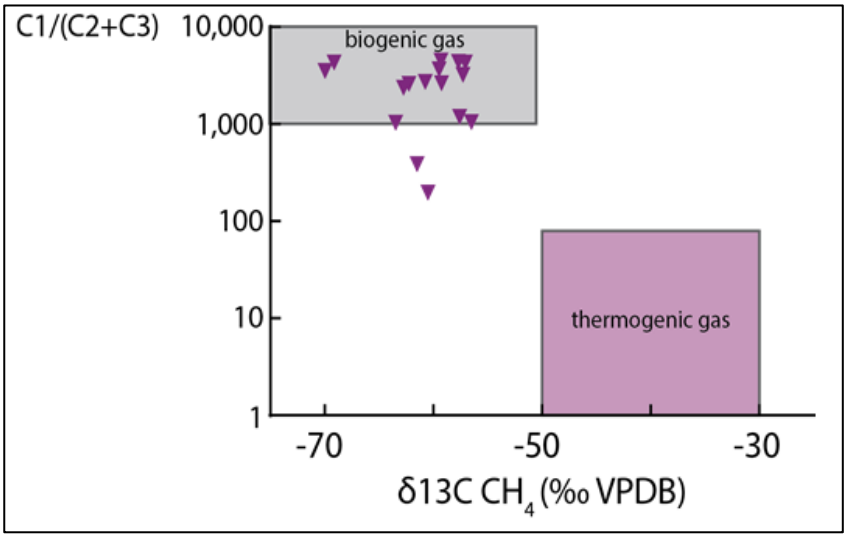


Figure 4 - Cherokee basin isotopes

(Kirk et al. 2015) Bernard plot showing y axis (abundance of methane relative to ethane and propane) and x axis ($\delta^{13}C$ isotopic composition standard to VPDB). Biogenic methane has a $\delta^{13}C$ signature of -60 parts per thousand (per mil, ‰) whereas thermogenic methane has a value around -40. The majority of wells sampled in the Cherokee basin indicate largely biogenic gas production. The reference standard established for this carbon-13 work was the VPDB (Vienna Pee Dee Belemnite).

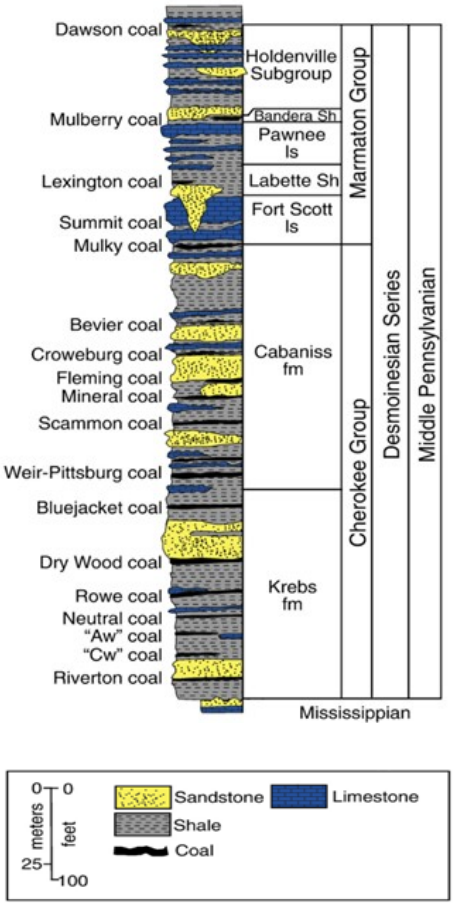


Figure 5 - Cherokee basin stratigraphy

(Woody, 1985): There are a number of different coals 400' to 1200' deep within the Cherokee group that differ in thickness, maturity and lateral extent. The Weir-Pittsburg coal and the Riverton coal are the two most economically important beds.

Chapter 2 - Methods

Field sampling

In February 2015 we collected formation water (FW) from two wells: Stich 1-2 and Bailey 2-2 (Figure 3) in SE Kansas. These two wells were chosen because they were noted, according to PostRock Energy Corp., to produce oil within the FW. Preparation for this sampling phase included flushing 8, 125 mL glass serum bottles with nitrogen gas (to disallow oxidation of the FW to-be-filled), and sterilization of these and other equipment (glass jars, DNA filters, sampling hoses, valve adapters, etc.) using an autoclave. On the premises, standard water-collection protocol were followed using previously mentioned sterile equipment. Produced oil was sampled directly from the wellhead faucet and collected into a sterile 1000 mL glass jar. This sampling was for oil only, which would later settle out above the FW. After running the wellhead faucet for a few minutes to flush the system, oil-free/oxygen-free FW was sequentially transferred directly using a rubber hose that connected the well (via valve adapter) to each serum bottle (via sterile needle). In order to maintain equilibrium and anaerobic conditions, another sterile needle perforated the serum bottle cap for outflow pressure –only during the duration of this transfer. 0.25 μM filters were utilized to collect microbes from within the FW and folded, using sterilized (ethanol-dipped and blazed) prongs and tweezers, into 10mL plastic vials. One drop (200 μL) of a sucrose lysis buffer was immediately added into the filter vials to preserve the collected cells and prepare for future extraction. All FW collected was immediately taken back to Kansas State University and stored in a dark cabinet at lab room temperature 22°C.

Next, we sampled livestock wastewater as an experimental nutrient for methanogenesis. This was collected from a waste-drainage ditch towards the south end of the KSU cattle feedlot in

Manhattan, KS. To the best of our ability, wastewater was collected from the top of the shallow water column into fresh 50 mL plastic tubes. Next, the unfiltered wastewater was taken back to the laboratory and immediately autoclave-sterilized three cumulative times -over the course of six days- in order to sterilize and eliminate any pathogenic microorganisms that may have been present. An attempt was made to filter the wastewater with 45μM nylon filters, however due to rapid cloggage, this proved ineffective/costly so we decided to use sterile, but unfiltered, wastewater as the experimental reagent instead.

To collect a Cherokee basin coal source for exp. 2, we traveled to a highway outcrop 10 miles south of the town of Fort Scott in Bourbon County, KS. Here, Pennsylvanian-aged coal deposits from the Mulky formation (Figure 5) were obtained. Digging roughly one foot into the outcrop to obtain less weathered material, we wrapped up the coal in aluminum foil and brought it back to Kansas State University. Then it was crushed using a simple mortar and pestle and sieved down to particles <49μM.

Laboratory experiments

Experiment 1: Setup and treatment design (oil availability)

The first experiment was designed to see if the microbes could make methane from oil and test environmental factors that may affect rate. On March 16, 2015 a total of 48 anaerobic bioreactors were prepared. The bioreactors contained 10mL of FW from wells Stich 1-2 and Bailey 2-2, and treatments incorporated 0.04 grams of the associated oil collected (Figure 6). To account for any accidental oxygen contamination into our anaerobic cultures, we added 100 μM $FeCl_2$ to each culture -including controls- to react with any encountered O_2 to produce iron (III) oxide-hydroxide

($Fe(OH)_3$. Our aim was to keep the oxygen from interfering with the anaerobic methanogens, but we also developed a visible way of recognizing error via mineralization. Listed below is the balanced chemical reaction for the formation of iron oxide-hydroxide precipitate.

$$4Fe^{2+}_{(aq)} + O_{2(g)} + 10H_2O_{(l)} \rightarrow 4Fe(OH)_{3(s)} + 8H^{+}_{(aq)}$$

Four treatments were tested for each of the wells: (1) *No amendment control*: These controls were unaffected aside from $FeCl_2$, (2) *phosphate + ammonium amendment:* The addition of 0.10 mL of 1mM KH_2PO4_4 and 0.02 mL of 50mM NH_4Cl, (3) *wastewater amendment:* The addition of 0.10 mL of unfiltered, sterile wastewater, and (4) *high-CO_2 amendment*: The injection of 100% CO_2 gas during both initial headspace preparation and periodic gas exchanges. Headspace gas of all cultures (except CO_2 based) were created with 98% N_2, 2% CO_2. Similar concentrations were used for routine gas exchanges as required for gas chromatography (see *gas chromatography* method). During the duration of this experiment, all bioreactors were stored in a dark cabinet at lab room temperature 22°C. Table 2 shows the configurational matrix of Experiment 1. Samples were designed in triplicate.

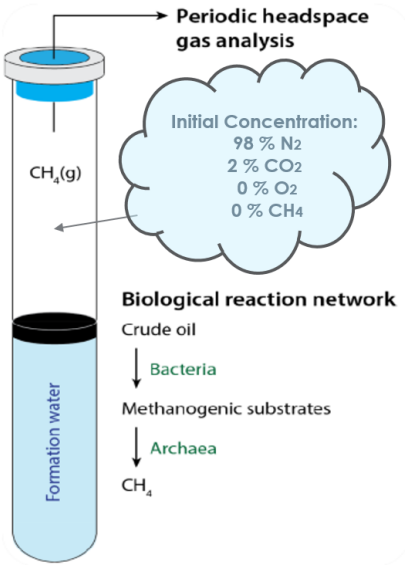


Figure 6 - Bioreactor configuration

This is a diagram showing the standard configuration of a Bioreactor treatment for Experiment 1. (1) Amendment controls were unaffected, (2) PO_4/NH_4 amendments added .1 mL of 1mM KH_2PO4_4 and .02 mL of 50mM NH_4Cl, (3) sterile livestock wastewater amendments added .1 mL of sterile wastewater, and (4) the CO_2 amendment applied 100 % CO_2 gas during initial preparation and gas exchanges. The only difference between Experiments 1 & 2 is that Experiment 2 replaced oil with coal. The 2-part microbial process of methanogenesis includes the breakdown of crude oil into substrates (acetate, CO_2, methanol) by bacteria, and then the utilization of substrates by archaea.

Treatment and ID	**Well A (Stich 1-2)**			**Well B (Bailey 2-2)**		
	1	**2**	**3**	**4**	**5**	**6**
FW + OIL **OA**	OA1	OA2	OA3	OA4	OA5	OA6
FW control **OB**	OB1	OB2	OB3	OB4	OB5	OB6
FW + OIL + PO_4/NH_4 **OC**	OC1	OC2	OC3	OC4	OC5	OC6
FW + PO_4/NH_4 control **OD**	OD1	OD2	OD3	OD4	OD5	OD6
FW + OIL + CO_2 **OE**	OE1	OE2	OE3	OE4	OE5	OE6
FW + CO_2 control **OF**	OF1	OF2	OF3	OF4	OF5	OF6
FW + OIL + Sterile Wastewater **OG**	OG1	OG2	OG3	OG4	OG5	OG6
FW + Sterile Wastewater control **OH**	OH1	OH2	OH3	OH4	OH5	OH6

Table 2 - Matrix of Experiment 1

Configurational matrix of Experiment 1: Sample ID's consist of O (due to the fact that this is the *Oil* experiment), the letter A-H (based upon *specific treatment)*, and a number 1-6 *(corresponding to triplicate number).* B treatments are *formation water controls*, whereas D, F, and H are *treatment controls.*

Experiment 2: Setup and treatment design (coal availability)

The second experiment was designed to see if the microbes could make methane from coal and also test environmental factors that may affect rate. On Oct. 10, 2015 a total of 48 anaerobic bioreactors were prepared. The bioreactors contained 10mL of FW from wells Stich 1-2 and Bailey 2-2 and 0.08 grams of the Mulky Coal collected from an outcrop near Fort Scott, KS (see field methods).

To account for any accidental oxygen contamination into our anaerobic cultures, we added 100 μM $FeCl_2$ to each culture. Four treatments were tested for each of the wells: (1) *No amendment control:* These controls were unaffected aside from $FeCl_2$, (2) *phosphate + ammonium amendment:* The addition of 0.10 mL of 1mM KH_2PO4_4 and 0.02 mL of 50mM NH_4Cl, (3) *wastewater amendment:* The addition of 0.1ml of sterile, unfiltered wastewater, (4) *high-CO_2 amendment*. This amendment was the injection of 100 % CO_2 gas during both initial headspace preparation and periodic gas exchanges. Headspace gas of all cultures (except CO_2 based) were created with 98% N_2, 2% CO_2. Similar concentrations were used for routine gas exchanges as required for gas chromatography. During the duration of this experiment, all bioreactors were stored in a dark cabinet at lab room temperature 22°C. Table 3 shows the configuration of experiment 2. Samples were designed in triplicate.

Treatment and ID	**Well A (Stich 1-2)**			**Well B (Bailey 2-2)**		
	1	**2**	**3**	**4**	**5**	**6**
FW + COAL **CA**	CA1	CA2	CA3	CA4	CA5	CA6
FW control **CB**	CB1	CB2	CB3	CB4	CB5	CB6
FW + COAL + PO_4/NH_4 **CC**	CC1	CC2	CC3	CC4	CC5	CC6
FW + PO_4/NH_4 control **CD**	CD1	CD2	CD3	CD4	CD5	CD6
FW + COAL + CO_2 **CE**	CE1	CE2	CE3	CE4	CE5	CE6
FW + CO_2 control **CF**	CF1	CF2	CF3	CF4	CF5	CF6
FW + COAL + Sterile Wastewater **CG**	CG1	CG2	CG3	CG4	CG5	CG6
FW + Sterile Wastewater control **CH**	CH1	CH2	CH3	CH4	CH5	CH6

Table 3 - Matrix of Experiment 2

Configurational matrix of Experiment 2: Sample ID's consist of C (if the sample has coal), one letter A-H (based upon *specific treatment)*, and a number 1-6 *(corresponding to triplicate number).* B treatments are *formation water controls*, whereas D, F, and H are *treatment controls.*

Experiment 3: Setup and treatment design (substrate consumption)

The third experiment was designed evaluate which methanogenic substrates were most consumed by Cherokee basin methanogens. In addition to Stich 1-2 and Bailey 2-2, water from 13 other

Cherokee basin wells were used for this experiment. On Dec 18, 2015, 60 anaerobic cultures were prepared using 10mL FW from 15 various wells. The same four non-duplicated treatments were used on each well (A-O). (1) *PO_4 + NH_4 control*: This control contained 0.10 mL of 1mM $KH_2(PO_4)_4$ and 0.02 mL of 50mM NH_4Cl. (2) *Hydrogen treatment*: This treatment's initial headspace contained 55% Nitrogen, 5% CO_2, and 40% H_2 in addition to ammonium and phosphate nutrients. (3) *Acetate treatment*: This treatment contained 6.3 mM acetate in addition to ammonium and phosphate nutrients. (4) *Methanol treatment:* This treatment contained 8.4mM methanol in addition to ammonium and phosphate nutrients. Once again, 100 μM $FeCl_2$ was added to each culture (including controls) to react with any encountered O_2 and produce iron (III) oxide hydroxide. Headspace gas of all cultures (except H_2 based) were created with 95% N_2, 5% CO_2 . Unlike Experiments 1 and 2, which had routine gas exchanges, Experiment 3 only underwent one round of GC gas measurement (74 days after the bioreactors were prepared). During the duration of this experiment, all bioreactors were stored at room temperature 22°C in a dark, dry cabinet. Table 4 shows the configuration of Experiment 3.

Treatment And ID	Well														
	SA	**SB**	**SC**	**SD**	**SE**	**SF**	**SG**	**SH**	**SI**	**SJ**	**SK**	**SL**	**SM**	**SN**	**SO**
FW + PO_4/NH_4 Control 1	SA1	SB1	SC1	SD1	SE1	SC1	SG1	SH1	SI1	SJ1	SK1	SL1	SM1	SN1	SO1
FW + PO_4/NH_4 + Acetate 2	SA2	SB2	SC2	SD2	SE2	SC2	SG2	SH2	SI2	SJ2	SK2	SL2	SM2	SN2	SO2
FW + PO_4/NH_4 + Methanol 3	SA3	SB3	SC3	SD3	SE3	SC3	SG3	SH3	SI3	SJ3	SK3	SL3	SM3	SN3	SO3
FW + PO_4/NH_4 + Hydrogen 4	SA4	SB4	SC4	SD4	SE4	SC4	SG4	SH4	SI4	SJ4	SK4	SL4	SM4	SN4	SO4

Table 4 - Matrix of Experiment 3

Configurational matrix of Experiment 3: Sample ID's consist of S (to indicate that this is the methanogenic *substrate* experiment), one letter A-O (based upon well name*)*, and a number 1-4 *(corresponding substrate treatment).* Due to the large number of wells tested, treatment types were not reproduced (i.e. no duplicates or triplicates).

Chemical analyses

A dual column GOWMAC 580 series gas chromatograph was periodically used for the purpose of headspace gas analysis. This machine was designed to take a gas compound and separate the gas components between H_2, O_2, N_2, CH_4, and CO_2. Then, through use of a helium carrier gas and the sorbent material within the gas column, the gas components separate and the quantity of those components are recorded via a peak. For example, H_2 flows through the machine faster than CH_4, because the column sorbent has a higher affinity for CH_4 than H_2. The machine also has a thermal conductivity detector which exposes a filament that varies in electrical resistivity, depending on the temperature of the filament (this fluctuates with respect to the specific gas compounds detected). These changes allow *CLARITY* software to record gas peaks, which when compared to a calibrated curve, provide insight into the quantity of the gasses present.

Because any thermal changes in the system may yield entirely different results, it is important to keep the settings consistent for each periodic use. The following settings were applied: Regulator outlet (helium) pressure was set to 40psi. Ensured carrier gas for each line (both A and B) was 30 mL/min. After gas was flowing for 5 minutes, the machine was turned on, and the following knob adjustments were made: (a) detector current: 120mA, (b) column temperature: 80°C, (c) detector temperature: (100°C), and (d) injector temperature: 100°C. The column heater and fan were turned on, after which 45 minutes were allotted for settings to apply. Once *CLARITY* software was turned on, and calibration settings applied, then 0.50 mL gas sample were injected via syringe/needle into the gas chromatograph (port A).

When a culture tube needed to be sampled more than once (days or weeks apart), pressure still needed to remain in equilibrium over time. As such, a pre-determined concentration of gas was exchanged into the culture tube first, before GC out-sampling, to dilute the headspace gas and neutralize pressure. Concentrations of 98% N, and 2% CO_2 were used for Experiments 1 and 2, with the exception of CO_2-based cultures where 100% CO_2 gas was exchanged. Data was adjusted in the following manner: Bioreactor headspace was diluted and exchanged with 0.50 mL non-methane gas each sampling period (required to retain pressure equilibrium).. Knowing that each bioreactor had 10mL FW and 18mL headspace (a 30mL culture tube was used, with 2mL taken up by rubber seal), $1/36^{th}$ of the "raw" methane value collected for each sampling period was added to following sampling periods in sequence.

All FW analyzed during this project by myself, as well as our lab's previous study (Kirk et al. 2015) was done so in the Kansas State University Geomicrobiology lab. The analysis machine, a Dionex ion chromatography system, is capable of running both cation and anion analyses. All procedures were followed to protocol with application of the following settings: (a) system A: rate 1.2 mL/min, current 31mA, and (b) system B: rate 1.0mL/min, current 65mA. These settings were allowed 10 minutes to adjust, and depending on if the run was for anion or cation analyses, the ion eluent often needed to be prepared and filled. System A –anion system- required a carbonate eluent, while System C –cation system – required a sulfuric acid eluent. Prior to analysis, all formation samples underwent concentration dilution between 50 & 500 fold so that data fell within acceptable guidelines. From there, samples were loaded into corresponding slots into the auto-sampler, which allowed *Chromelion,* the IC chemical data collection software, to sequence the samples in order.

Rate calculation

The ideal gas law (PV=nRT) was used with GC-obtained data to calculate daily methane accumulation (μmolCH_4/day). Solving for n (number of moles) = PV/RT. Assumptions include: Lab room temperature (T) = 295.15, pressure (P) = 1atm, gas constant (R) = 0.0821, and volume (V) was calculated by multiplying in culture headspace (0.018) by % CH_4 headspace (varied by treatment). Final value was "total moles" accumulated, so it was then divided by the number of days incubated (for example, 165 days in Experiment 1) and converted (from moles to micromoles) by multiplying 1,000,000.

Extraction and sequencing of DNA

DNA extraction was performed in the Kansas State University Geomicrobiology lab using the UltraClean Soil DNA Isolation Kit (*MO BIO).* The protocol consisted of 22 steps designed to extract the DNA using six solutions (C1-C6), with intermittent use of hot-water baths, cold-water baths, vortexing, and centrifuging. The detailed extraction procedure, for one given microbe filter (which was performed 22 times – one for each DNA sample) is as follows: (1) The microbe filter was initially cut and shredded on a sterile petri dish using a sterilized exacto knife and material was subsequently placed into a PowerBead tube. (2) The tube was vortexed for 10 seconds. (3) Solution 1 (a detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms – required to complete cell lysis) was heated up to 60 °C and any preexisting precipate was dissolved. (4) 60μL of Solution C1 was added into the PowerBead tube and vortexed for 2 minutes. (5) Tube was heated to 70 °C for 10 minutes, then vortexed 2 min, re-heated 10 minutes, then vortexed for another 2 minutes. (6) Tube was centrifuged for 30 seconds at 10,000g at room temperature. (7) Supernatant was transferred to a clean 2 mL collection tube. (8) 250 μL

of Solution C2 (a reagent to precipitate non-DNA organic and inorganic matter such as humic substances, cell debris, and proteins) was added to the tube, vortexed for 5 seconds, and incubated at 4 °C for 5 minutes. (9) The tube was centrifuged for 1 minute at 10,000g. (10) 600 µL of supernatant was transferred to a clean 2mL collection tube. (11) 200 µL of solution C3 (another reagent to precipitate additional non-DNA organic and inorganic matter) was added to the 2mL collection tube, vortexed briefly, and incubated at 4 °C for 5 minutes. (12) The tube was centrifuged for 1 minute at 10,000g. (13) 750 mL of supernatant was transferred to a clean 2mL collection tube. (14) Solution C4 (a salt solution designed to allow DNA to bind to spin filters) was shaken and 1.2 mL added to the supernatant and vortexed for 5 seconds. (15) 675 µL of solution was added into a tube with a spin filter and centrifuged at 10,000g for 1 minute at room temperature. The flow-through was discarded, and then an additional 675 µL of solution was added, centrifuged, and discarded. This process was repeated another time, so that there were a total of three loads for each sample performed. This step binds the DNA to the filter membrane. (16) 500 µL of Solution C5 (an ethanol-based wash solution used to further clean the DNA of residual salt, humic acid, and other contaminants) was added to the spin-filter tube, centrifuged at 30 seconds at 10,000g. (17) The flow through was discarded. (18) The spin filter was centrifuged for 1 minute at 10,000g to ensure removal of Solution C5. (19) The spin filter was placed into a clean 2mL collection tube. (20) 50 µL of Solution C6 (a sterile elution buffer designed to ensure release of DNA from the spin filter) was placed into the center of the white filter membrane. (21) The spin filter tube was centrifuged at room temperature for 30 seconds at 10,000g. (22) The spin filter was discarded, and the DNA in the tube was ready for sequencing.

Next we measured the concentrations of DNA using the NanoDrop ND-1000 Spectrophotometer in the integrated genomics facility at Kansas State University. We initialized the NanoDrop equipment was for Nucleic Acids analyses, and used purified water and KIM-wipes for routine wiping of the measurement pedestal. Data was collected (absorbance numbers) and stored within the program, and all data was printed off as hard-copy at the end of the session. The DNA "concentration" data may be found in Appendix 7.

Next, we contacted MR DNA laboratory to amplify and sequence 16S rRNA genes within the DNA sample collected. 16S rRNA gene V4 variable region PCR primers 519Fmod (CAGCMGCCGCGGTAA) and 806Rmod (GGACTACHVGGGTWTCTAAT) were used in a 28 cycle PCR (Polymerase Chain Reaction). These set of primers (519F-806R) were utilized for amplification of both bacteria and archaea DNA (similar to Wutcher et al. 2013). These prokaryotic primers allowed sequencing for 88.1% of bacterial and 90.5% of archaeal 16S rDNA sequences available in the *greengenes* database (DeSantis et al. 2006). Using the HotStarTaq Plus Master Mix Kit (Qiagen, USA), DNA was amplified under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a 5 minute final elongation step was performed at 72°C. The laboratory products were then checked in 2% agarose gel to determine amplification success using electrophoresis, a technique that applies –to protiens- a negative charge, causing them to shift oppositely (towards positive). Multiple samples were pooled together into proportions based on molecular weight and DNA concentrations. Samples were purified using Ampure XP beads, and the final products were used to prepare the Illumina DNA library. Sequencing was performed at MR. DNA in Shallowater, TX using an Illumina MiSeq System following manufacturing protocol.

Microbial community analyses

Sequencing data was processed using the most recent version of QIIME software (v.1.9.1) QIIME software commands were input using python script (.py). In order to process and analyze data, we used eight essential functions: (1) *Demultiplexing.* The split_libraries.py command used data in the .qual file to identify low quality sequences and extracts out only the samples present in the mapping file. (2) *Operational Taxonomic Units (OTU) Picking.* The pick_de_novo_otus.py command aligned sequences and binned them into OTUs. This also created a phylogenic tree, and assigned a consensus taxonomy to each OTU. (3) *Filtering OTUs*. The filter_otus_from_otu_table.py script removed OTUs with only one representative sequence, and created a new, filtered OTU table. (4) *Summarize Taxonomy*. The summarize_taxa_through_plots.py script output HTML-formatted charts that displayed the taxonomy of each sample (phylum, class, order, family, and genus). (5) *Normalization.* The single_rarefaction.py script normalized the number of sequences in each sample, in order to compare diversity between samples (beta diversity). We had to adjust the counts/sample of all samples to that of the lowest. My sample with the lowest counts was B2 (28,280 counts / sample). As such, this value was used for normalization. (6) *Rarefied OTU tables for alpha diversity*. The alpha_rarefaction.py script generated OTU tables for alpha diversity, and created associated alpha rarefaction plots. (7) *Compute Alpha Diversity*. The Alpha_diversity.py script calculated alpha diversity using an OTU table. (8) *Compute beta Diversity and create principal component (PCoA) plots*. The beta_diversity_through_plots.py script compared sample communities to one another, and output a scaleable, vector-based PCoA plot. Plots and charts were generated using QIIME software.

Chapter 3 - Results

Formation water chemistry (Bailey 2-2 and Stich 1-2)

Formation water chemistry (IC data) of the two sampled wells may be seen in Table 5. All ion concentrations, particularly salinity, fall within the guidelines previously set by other wells in the basin (appendix 6). Stich 1-2 holds higher Na, Cl concentrations than Bailey 2-2.

Table 5 - Well Water Chemistry

			Temp	Conductivity	Alk (CaCO3)	HCO_3	Cl	Br	SO_4	Mg	Ca	K	Na	Fe
	sample data	pH	°C	(mS)	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Marion Bailey 2-2	2/12/2015	6.67	15.5	51.2	133.2	162.4	33058	108.16	1.5	1283	1415.9	190	19898	n/a
William Stich 1-2	2/5/2015	7.45	16.9	59.4	248.1	302.4	43785	129.41	1.2	850	1008	297	25837	n/a

Rate variation from raw formation water

The rates of methanogenesis vary on a day to day basis, depending upon numerous environmental and catabolic controls involved. There are periods of rapid methanogenesis which may be observed through temporal headspace variation (Figure 8). However, applying the law of average in conjunction with ideal gas law variables allowed daily rates of methanogenesis to be calculated from headspace gas abundance into units of $\mu molCH_4$/day (see rate calculation method for more details). These rates may provide insight into relative bio-stimulation strategies among both treatments and FW of varying salinity. Upon incubation termination of Experiment 1, Bailey 2-2's FW controls had a higher percentage of headspace CH_4 than in Stich 1-2's. This was consistently the case throughout Experiments 1 and 2. (In Experiment 1, Bailey's FW control average was 1.496 $\mu molCH_4$ /day higher than Stich's counterpart. In Experiment 2, Bailey's FW control average was 2.09 $\mu molCH_4$ /day higher than Stich's.)

Rate variation from coal and oil

Bailey's FW microbes were significantly (Mann Whitney) capable of using oil (0.04 grams) to produce methane in Experiment 1. *FW + oil* treatments showed a rate of 2.92 and 1.33 μmolCH_4/day for Bailey and Stich respectively, which corresponds to a net increase of +0.18 and +0.09 μmolCH_4/day compared to their FW controls. Treatments with coal (0.08 grams) did not yield significant methane accumulation relative to controls, and this was the case in both Bailey and Stich bioreactors. At the end of Experiment 2's incubation, treatments averaged +0.73 and +0.60 μmolCH_4/day relative to control in the wells, respectively. Standard deviation for both treatments and cultures involved with Experiment 2 were higher than so in Experiment 1, which attributes to the non-significance of methane observed from coal (Figure 7).

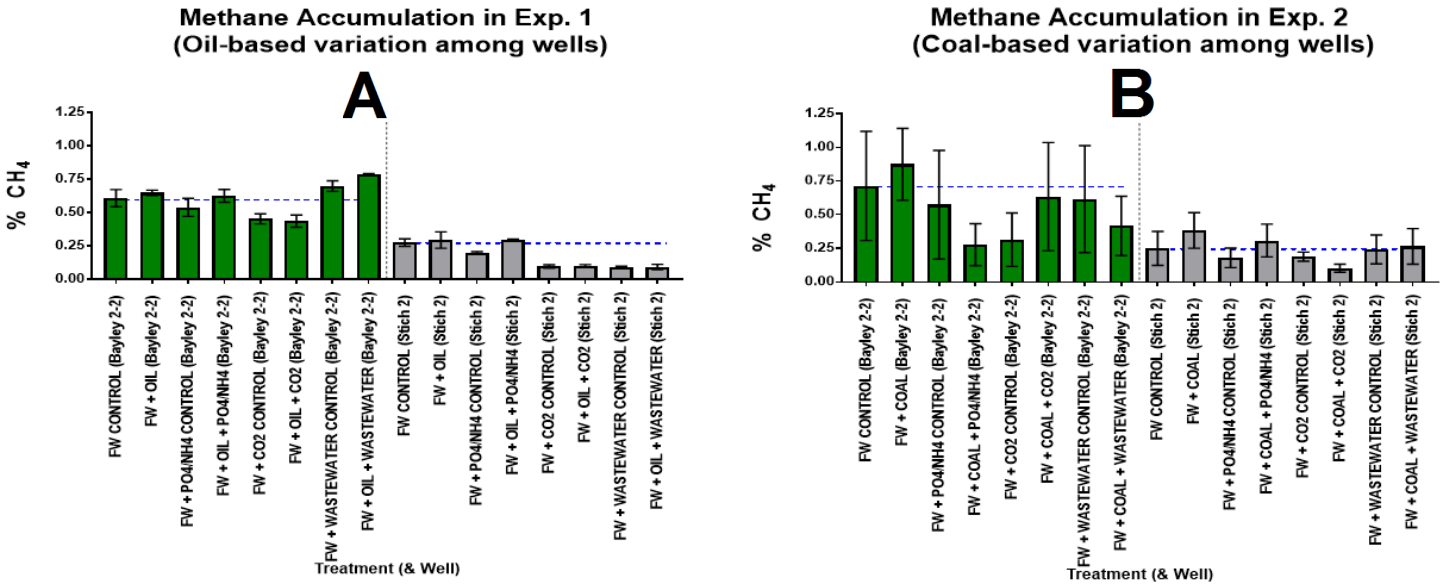


Figure 7 - Average methane accumulation (Exp. 1 & 2)

Abundance of methane in the headspace of cultures at the end of the incubation for (A) Experiment 1 and (B) Experiment 2. For both charts, the dashed blue line indicates the abundance of methane in the FW control, samples inoculated with FW from Bailey are shaded green (i.e., bars on the left), and samples inoculated with FW from Stich are shaded gray (i.e., bars on the right).

Rate variation from waste

Methanogenic response to wastewater varied greatly with respect to the specific FW used in conjunction. As expected, Bailey's methanogens benefited from waste availability, producing CH_4 at net daily rates of +0.80 and +0.41 μmol/day, respectively, for both the FW+oil+wastewater and FW+wastewater treatments. Contrarily, Stich's methanogens suffered from waste exposure, exhibiting net productivity loss of -0.83 and -0.82 μmolCH_4 /day, respectively, in both FW+oil+wastewater and FW+wastewater treatments. Bailey's waste treatment accumulations were noted as being close to significant ($P < 0.08$), but not actually significant ($P < 0.05$) from controls.

Rate variation from non-significant majority

The remainder of the experimental treatments were not significant in any way. Methane abundance in FW+oil+CO_2 were not significantly different from the control cultures for Bailey or Stich. Methane abundance in FW+oil+NH_4/CH_4 were not significantly different from the control cultures for Bailey or Stich. Methane abundance in oil+wastewater were not significantly different from the control cultures for Bailey or Stich. In Experiment 1, there was no indication of a link between CO_2 or nutrient treatment abundance and increased methanogenic activity. Of Bailey's low salinity bioreactors, the FW+oil+wastewater treatment had the highest average rate of methane accumulation (3.54 μmol/day), followed by the FW+wastewater treatment (3.15 μmol/day), followed by the FW+oil treatment (2.92 μmol/day). Of Stich's high salinity bioreactors, only two treatment rates were higher than the controls. Coal was present in both of these cultures (FW+oil and FW+oil+NH_4/PO_4). In Experiment 2, there is also no indication of a link between CO_2 or nutrient treatment abundance and increased methanogenic activity, and there were no significant

results associated with this experiment. Of Bailey's low salinity bioreactors, the FW+coal treatment had the highest average rate of methane accumulation (3.938 µmol/day), followed by the FW control (3.213 µmol/day). Of Stich's high salinity bioreactors, only three treatment rates were higher than the controls. Coal was present in all three of these cultures (FW+coal, FW+coal+PH4/NH4, and FW+coal+wastewater). Daily methanogenic rates for all treatments are listed in Table 6.

A	FW + OIL (Bailey 2-2)	FW + OIL (Stich 1-2)	FW CONTROL (Bailey 2-2)	FW CONTROL (Stich 1-2)	FW + OIL + PO4/NH4 (Bailey 2-2)	FW + OIL + PO4/NH4 (Stich 1-2)	FW + PO4/NH4 CONTROL (Bailey 2-2)	FW + PO4/NH4 CONTROL (Stich 1-2)	FW + OIL + CO2 (Bailey 2-2)	FW + OIL + CO2 (Stich 1-2)	FW + CO2 CONTROL (Bailey 2-2)	FW + CO2 CONTROL (Stich 1-2)	FW + OIL + WASTEWATER (Bailey 2-2)	FW + OIL + WASTEWATER (Stich 1-2)	FW + WASTEWATER CONTROL (Bailey 2-2)	FW + WASTEWATER CONTROL (Stich 1-2)
µmolCH4 / day	2.918	1.326	2.737	1.241	2.817	1.329	2.425	0.897	1.962	0.456	2.043	0.435	3.538	0.416	3.147	0.420
(+/-) NET µmolCH4 / day (relative to FWcontrol)	0.181	0.084	0.000	0.000	0.079	0.088	-0.312	-0.344	-0.775	-0.785	-0.695	-0.807	0.800	-0.826	0.410	-0.821
(+/-) NET µmolCH4 / day (relative to treatment control)	0.181	0.084	0.000	0.000	0.391	0.432	0.000	0.000	-0.081	0.022	0.000	0.000	0.391	-0.005	0.000	0.000

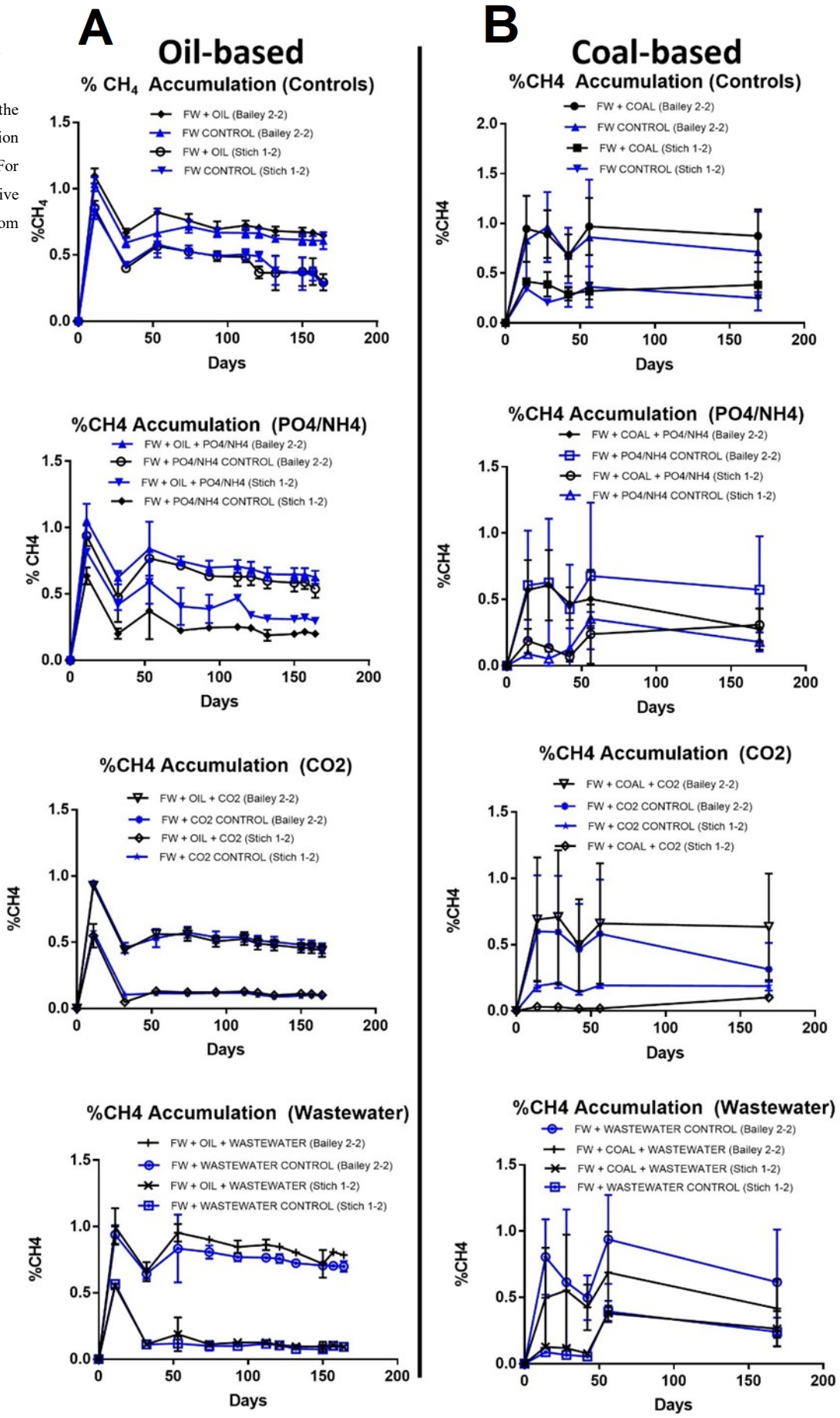
Table 6 – Daily methane rate (average) (Exp. 1, 2)

The average rates of methanogenesis for Experiment 1 (**A**) and Experiment 2 (**B**) are listed in by total µmolCH_4 / day, average µmol/CH_4 / day relative to FW control, and average net µmolCH_4 / day relative to treatment control.

B	**FW + COAL (Bailey 2-2)**	**FW + COAL (Stich 1-2)**	**FW CONTROL (Bailey 2-2)**	**FW CONTROL (Stich 1-2)**	**FW + COAL + PO4/NH4 (Bailey 2-2)**	**FW + COAL + PO4/NH4 (Stich 1-2)**	**FW + PO4/NH4 CONTROL (Bailey 2-2)**	**FW + PO4/NH4 CONTROL (Stich 1-2)**	**FW + COAL + CO2 (Bailey 2-2)**	**FW + COAL + CO2 (Stich 1-2)**	**FW + CO2 CONTROL (Bailey 2-2)**	**FW + CO2 CONTROL (Stich 1-2)**	**FW + COAL + WASTEWATER (Bailey 2-2)**	**FW + COAL + WASTEWATER (Stich 1-2)**	**FW + WASTEWATER CONTROL (Bailey 2-2)**	**FW + WASTEWATER CONTROL (Stich 1-2)**
µmolCH4 / day	3.938	1.722	3.213	1.123	1.247	1.388	2.582	0.807	2.855	0.460	1.414	0.842	1.876	1.189	2.770	1.085
(+/-) NET µmolCH4 / day (relative to FWcontrol)	0.725	0.599	0.000	0.000	-1.966	0.265	-0.631	-0.316	-0.358	-0.663	-1.799	-0.280	-1.337	0.066	-0.443	-0.038
(+/-) NET µmolCH4 / day (relative to treatment control)	0.725	0.599	0.000	0.000	-1.335	0.581	0.000	0.000	1.441	-0.382	0.000	0.000	-0.893	0.104	0.000	0.000

Figure 8 - Temporal variation of headspace CH_4 (Exp. 1, 2)

Corrected temporal variation of methane in the headspace of cultures at the end of the incubation for Experiment 1 (A) and Experiment 2 (B). For both charts, the blue line indicates the relative treatment or FW control. Standard deviation from mean is plotted.



Microbial Community Composition

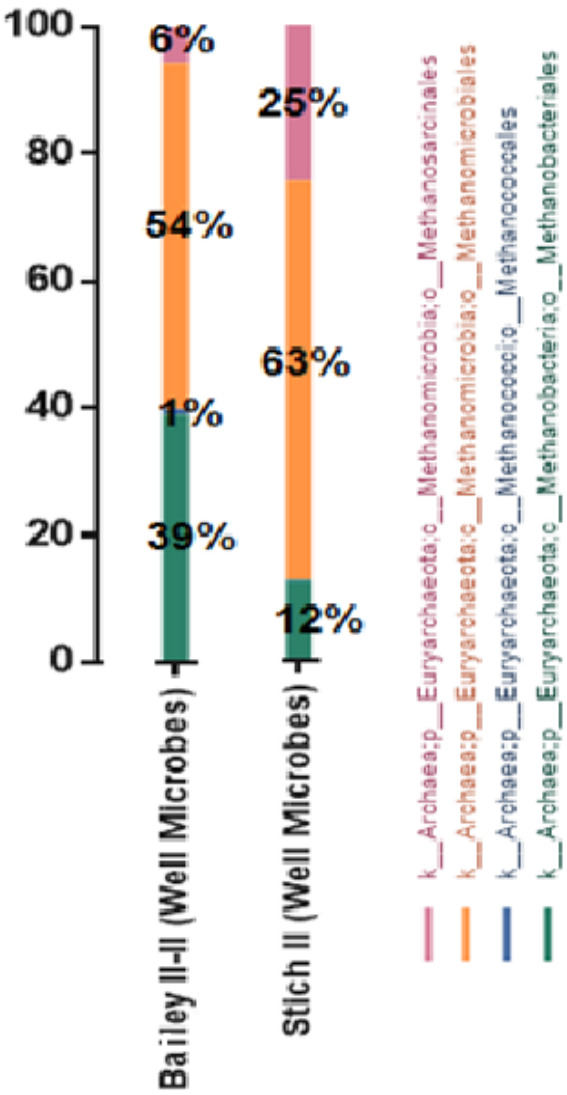


Figure 9 – Archaeal abundance of raw formation water (bar graph)

Relative abundance of archaea in wells Bailey 2-2 and Stich 1-2. All identified archaea are known methanogens.

Relative abundance of archaea from two natural gas wells

DNA concentrations extracted from two natural gas wells (Bailey 2-2 and Stich 1-2) each measured approximately 44 ng/µl (Appendix 7). Once processed, archaeal sequences represented 3.303% and 1.677% of the total Illumina reads, respectively, for the wells. Archaeal sequences all belong to the phylum *Euryarchaeota*, and consist of almost entirely (>99%) methanogens. For Bailey, these methanogens were most notably (listed in descending quantity of % abundance within archaeal order) *Methanomicrobiales* (54.3%) *Methanobacteriales* (38.9%), *Methanosarcinales* (5.9%), *and Methanococcales* (0.9%). For Stich, these methanogens were most notably (listed in descending quantity of % abundance within archaeal order) *Methanomicrobiales* (62.7%), *Methanosarcinales* (24.7%), *Methanobacteriales* (12.4%), *and Methanococcales* (0.2%). From this data, it's clear that Bailey accommodated 8.4% less *Methanomicrobiales,* 18.8% less *Methanosarcinales,* and 26.5% more *Methanobacteriales*. A

higher percentage of *Methanosarcinales* is within Stich. Relative abundances of wellsite archaea are visualized in bar and pie graph formats (Figures 9 and 10).

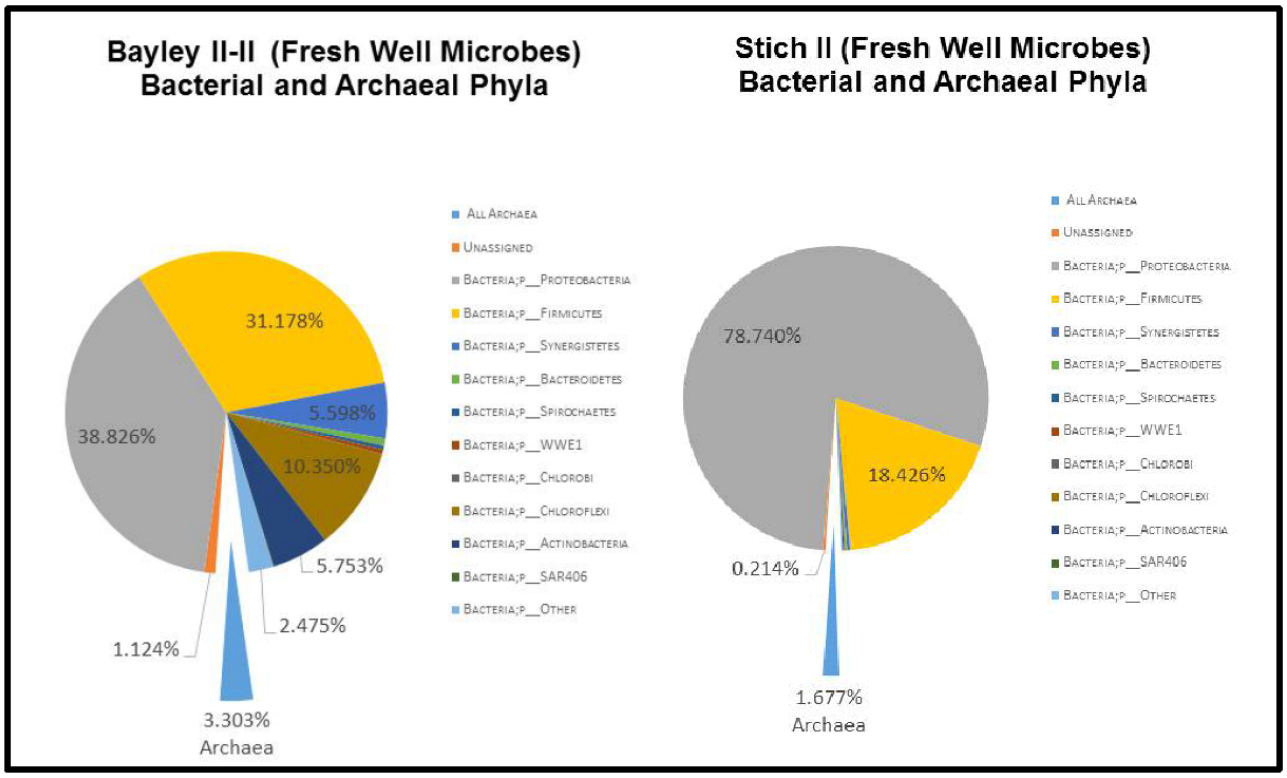


Figure 10 – Microbial abundance of raw formation water (pie graph)

Initial well composition of total microbial groups, archaea and bacteria. Bailey 2-2 encompasses nearly twice the amount of archaea (3.303% of total llumina reads) than that of Stich 1-2 (1.677%).

Relative abundance of bacteria from two natural gas wells

Illumina reads from the combined phyla *Firmicutes* and *Proteobacteria* covered 73.2% (Bailey 2-2) and 98.5% (Stich 1-2) of the overall bacterial groups identified in each well. *Proteobacteria* dominated in both FW samples. *Proteobacteria* could be separated by class into *Alpha* (α), *Beta* (β), *Delta* (δ), *Epsilon* (ε), *or Gamma* (γ) *Proteobacteria.* For Bailey, these respective class abundances of total *Proteobacteria* were (α) 20.5%, (β) 4.6%, (δ) 36.4%, (ε) 8.1%, and (γ) 30.4%. For Stich, these respective class abundances of *Proteobacteria* were (α) 1.3%, (β) 0.3%, (δ) 92%,

(ε) 6.0%, and (γ) 0.4%. δ-*Proteobacteri*a dominated within Stich FW, representing 72.5% of the wells' total Illumina reads, whereas δ-*Proteobacteria* also dominated in Bailey FW, but only represented 14.1% of the wells' total Illumina reads. Stich appears much less bacterially diverse in other spectra. Relative to Stich, Bailey 2-2 had higher abundances of *Chloriflexi* (10.8%), *Actinobacteria* (6.0%), *Synergistetes* (5.9%) and an assortium of other bacteria (2.92%). All of these combined phyla encompass < 2% of total Illumina reads within Stich. Visual bar graphs of the relative abundances of bacterial phyla may be seen below (Figure 11), along with a breakdown of *Firmicutes* and *Proteobacteria* groups.

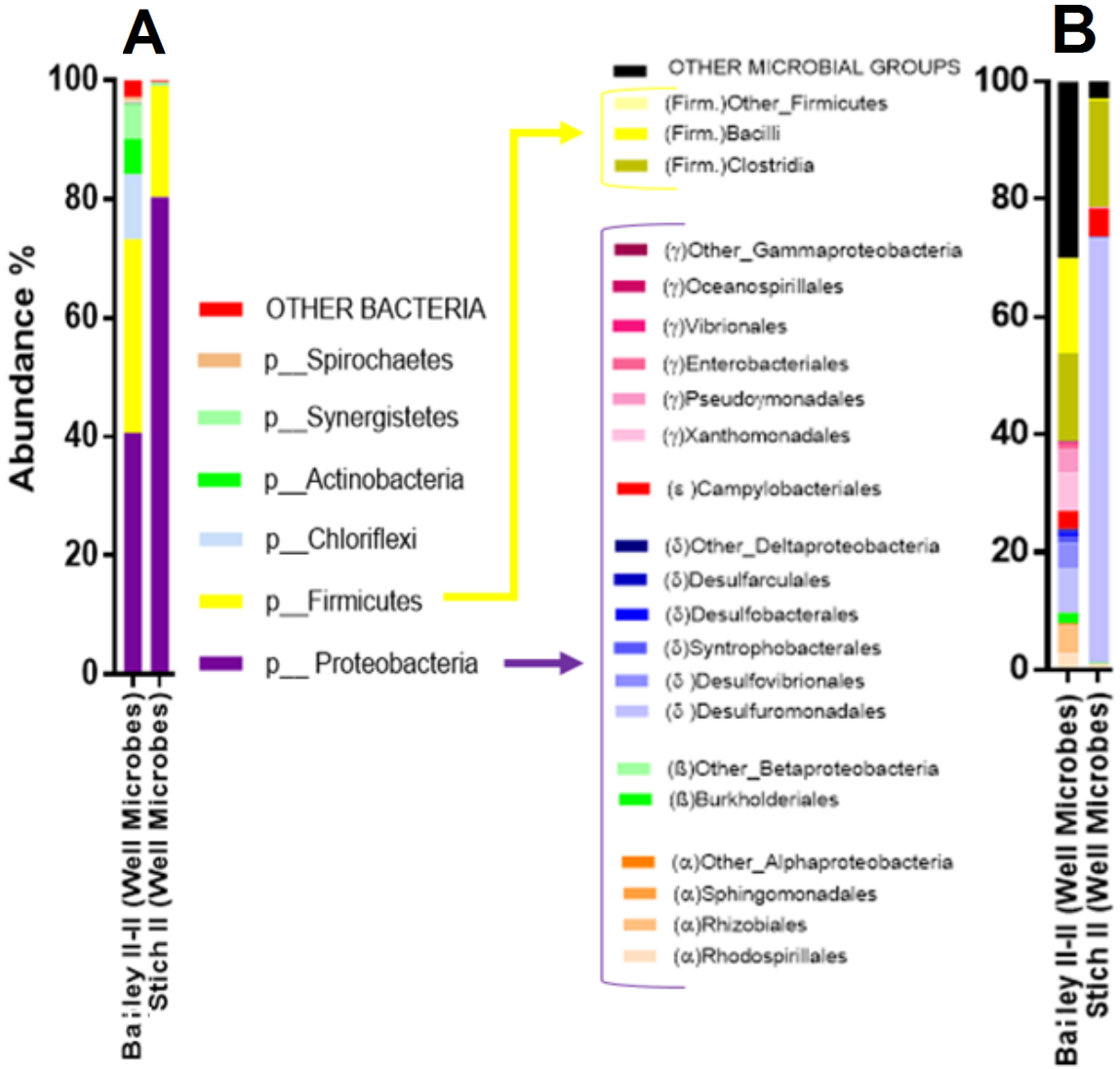


Figure 11 – Bacterial abundance of raw formation water

Identified bacterial assortium of wells Stich 1-2 and Bailey 2-2 characterized from Illumina reads: (A) range of bacterial phylum, and relative abundance of *Firmicutes* and *Proteobacteria* phylum. (B) classes of *Firmicutes and Proteobacteria.*in greater detail.

Relative abundance of archaea between treatments

In Experiment 1 (oil), most archaeal OTU's found in the oil incubation experiments were also present in the initial well community. In cultures with Bailey's FW, access to oil may have contributed to shrinking (relative to initial FW community) the archaeal proportions of *Methanomicrobiales* (from 54.4 to 36.6%). Access to oil may also have increased proportions of *Methanosaetaceae* (from 5.4 to 9.8%). Relative abundance of *Methanobacteriaceae* increased (from 30.2 to 48.7%), however a higher shift increase was seen relative to the FW control (from 30.2 to 56%). Access to waste induced proportionally higher abundancies of *Methanobacteriaceae* (from 30.2 to 63.8%) and *Methanosaetaceae* (from 5.4 to 22.4%), and proportionally lower abundancies of *Methanomicrobiales* (from 54.4 to 12.1%). In cultures with Stich's FW, access to oil may have contributed towards shrinking proportions of *Methanomicrobiales* (from 60.7 to 11.8%), simultaneously allowing growth of *Methanobacteriaceae* (from 12.4 to 76.5%). In the FW control, *Methanomicrobiales* ended up dominating everything (from 60.7 to 100%). Access to waste didn't have nearly the microbially diversifying effect that oil did, but mirrored an effect that the Stich FW control had; where *Methanomicrobiales* ended up dominating all other microbial groups (from 60.7 to 97.4%).

In Experiment 2 (coal), most archaeal OTU's found in the coal incubation experiments were also present in the initial well FW**.** In cultures with Bailey's FW, access to coal may increase (relative to initial FW community) abundancies of *Methanobacteriaceae* (from 30.2 to 61.8%) and *Methanosaetaceae* (from 5.4 to 25%), while decreasing abundance of *Methanomicrobiales* (from 54.4 to 13.2%). Cultures with waste access were marked with an increase in *Methanosaetaceae* (from 5.4 to 86.6%). In cultures with Stich's FW, access to coal increased proportional

abundancies of *Methanobacteriaceae* (from 12.4 to 45.5%), while shrinking abundancies of *Methanomicrobiales* (from 60.7 to 38.6). Access to waste seemed to increase the population of *Methanosaetaceae* (from 0.0 to 19.4%) and *Methanobacteriaceae* (from 12.4 to 61.3%), while decreasing the abundancy of *Methanomicrobiales* (from 60.7 to 9.7%). Visual relative abundance of archaea by treatment type is depicted below (Figure 12).

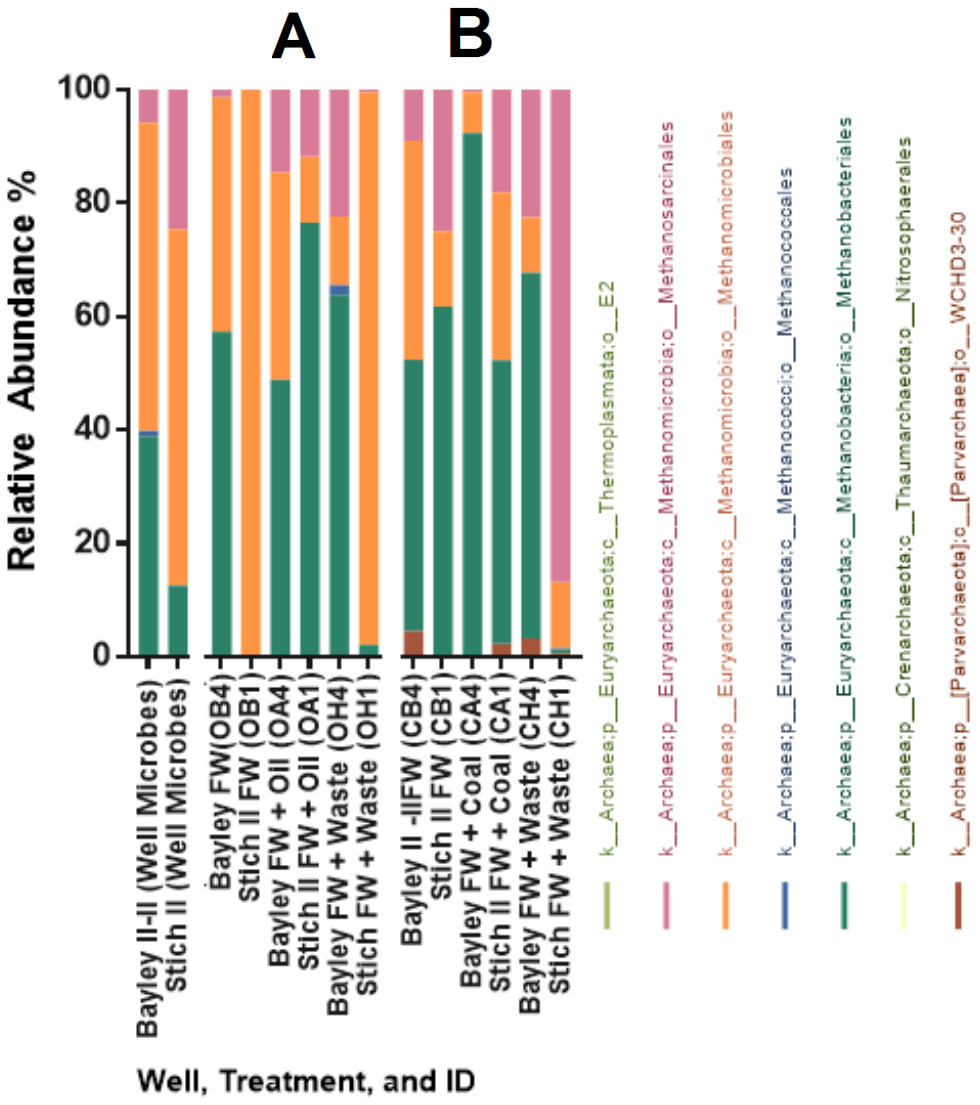


Figure 12 - Archaeal variation from treatment (exp. 1&2)

Left bars indicate initial well communities (Stich 1-2 and Bailey 2-2). Middle bars (A) indicate Experiment 1 treatments of oil, waste, and controls. Right bars (B) indicate Experiment 2 treatments of oil, waste, and controls.

Relative abundance of bacteria between treatments

Visual relative abundance of bacteria by treatment type is depicted below (Figure 13). In both Experiments 1 (oil) and 2 (coal), all incubations resulted in massive growth of *Campylobacteriales*. Even the treatment with the smallest proportion (Bailey's *FW + waste*) resulted in an overall Illumina identity abundance of 33.5%. Also, much of the diversity characterized within Bailey's FW is not seen in other treatment incubations, or even in the control. There are four main bacterial orders (3 *Proteobacteria* & 1 *Firmicute*) that frequently dominate. These are *Desulfuromonadales*, *Campylobacteriales, Rhodospirillales* (mainly in the case of Bailey's FW) or the *Firmicute* class, *Clostridia*. Another observation of Experiment 2 is that there is a higher proportion of *Desulfobacterales* associated with Bailey's FW, as compared with Stich's FW. Stich's FW (experiment 2 control) was marked by an unexpected abundance of *Rhodospirillales* (this group was almost exclusively seen in Bailey's Experiment 1 treatments prior).

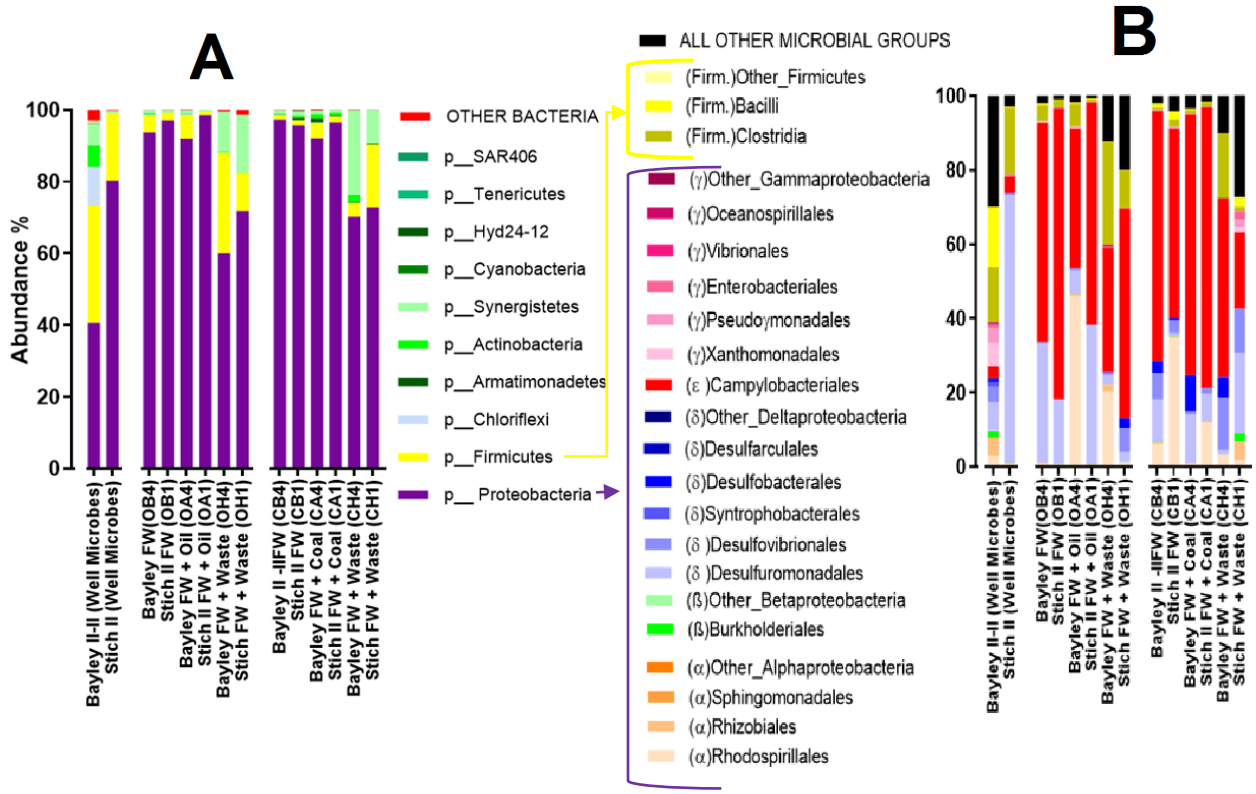


Figure 13 - Bacterial variation from treatment (exp. 1 &2)

Each bar set (A & B) is broken down into three segments: Stich1-2 / Bailey2-2 microbes –a basis for comparison-(left), Exp. 1 treatments (middle), and Exp. 2 treatments (right): (A) Overall bacterial community, categorized by Phylum. (B) *Firmicutes* (order), and *Proteobacteria (*classes) in greater detail.

Rate variation of substrate consumption reactors

Table 7 shows rates of methane growth (µmol/day) during substrate catabolism. On average, Methanol served as the most utilized substrate, followed hydrogen, followed by acetate. Bailey and Stich controls produced more methane than the controls for any other well, at rates of 7.760 and 6.565 µmolCH_4/day, respectively. Middleton 9-1 was shown to have the highest methane production from both hydrogen and methanol substrates. For this reason, we decided to sequence Middleton's treatments, rather than Bailey's. It was noticed that the newly sampled wells (Stich and Bailey) corresponded to higher methanogenic rates than all others (sampled in 2013) which is possibly an effect of nutrient depletion in laboratory storage over time.

Table 7 - Substrate-consumed methanogenesis (Exp. 3)

Methane accumulation with respect to substrate availability (acetate, methanol, and hydrogen) and control. Middle columns show average µmolCH_4 /day. Right column shows net growth relative to control. (green values are positive, red are negative). Stich was shown to have high consumption of acetate, whereas Middleton was shown to have the highest methane production from methanol and hydrogen substrates.

Well Name	Well ID	NH4/PO4 control	NH4/PO4 + Acetate	NH4/PO4 + Methanol	NH4/PO4 + H2	NH4/PO4 control	NH4/PO4 + Acetate	NH4/PO4 + Methanol	NH4/PO4 + H2
RWJ FARMS 29-2	SA	2.008	0.201	2.429	0.231	0.000	-1.807	0.422	-1.777
Middleton 9-1	SB	2.750	3.373	56.334	89.069	0.000	0.622	53.584	86.318
Jerry Brant 3-4	SC	3.564	4.638	0.000	37.262	0.000	1.074	-3.564	33.698
Theodore Housel 28-1	SD	0.994	2.781	40.996	1.596	0.000	1.787	40.002	0.602
Baugher Trust 1-11	SE	0.944	1.706	40.996	21.773	0.000	0.763	40.052	20.829
Cheyney Land 24-4	SF	2.078	2.329	55.190	44.489	0.000	0.251	53.112	42.411
Nunnenkamp 5-3	SG	0.211	2.811	54.949	63.512	0.000	2.600	54.738	63.301
Joseph Stich 1-1	SH	1.516	1.616	16.081	1.255	0.000	0.100	14.565	-0.261
MIH Alexander 18-3	SI	2.068	2.700	24.292	26.310	0.000	0.632	22.225	24.242
Lester Arthur 26-2	SJ	3.192	3.363	30.115	23.469	0.000	0.171	26.922	20.277
Triplett 25-2	SK	1.817	3.624	36.780	28.027	0.000	1.807	34.963	26.210
Kepley RA 23-1	SL	1.616	9.717	40.374	22.506	0.000	8.101	38.757	20.889
Ben Hinkle 30-1	SM	5.551	4.096	25.316	84.481	0.000	-1.456	19.765	78.930
Stich 2	SN	6.565	8.522	6.685	6.936	0.000	1.957	0.120	0.371
Bayley 2-2	SO	7.760	3.935	3.473	4.527	0.000	-3.825	-4.286	-3.232
		Avg µmolCH4 / day				Avg µmolCH4 / Day relative to contro			

Relative archaeal abundance of substrate consumption reactors

Visual relative abundance of archaea by substrate type is depicted below (Figure 14). Middleton 9-1's initial archaeal identity was 0.63%, whereas Stich 1-2's was 1.01%. Relative abundance of archaea in cultures with acetate was 9.69% in Middleton and 0.55% in Stich. Relative abundance of archaea in cultures with methanol was 18.53% in Middleton, and 0.03% in Stich. Relative abundance of archaea in cultures with H_2 was 3.56% in Middleton, and 0.27% in stich. The archaeal groups are almost entirely methanogens (> 99%). Archaea groups present include *Methanomicrobiales, Methanobacteriales, Methanococcales, Methanobacteriales,* and *Methanococcales.*

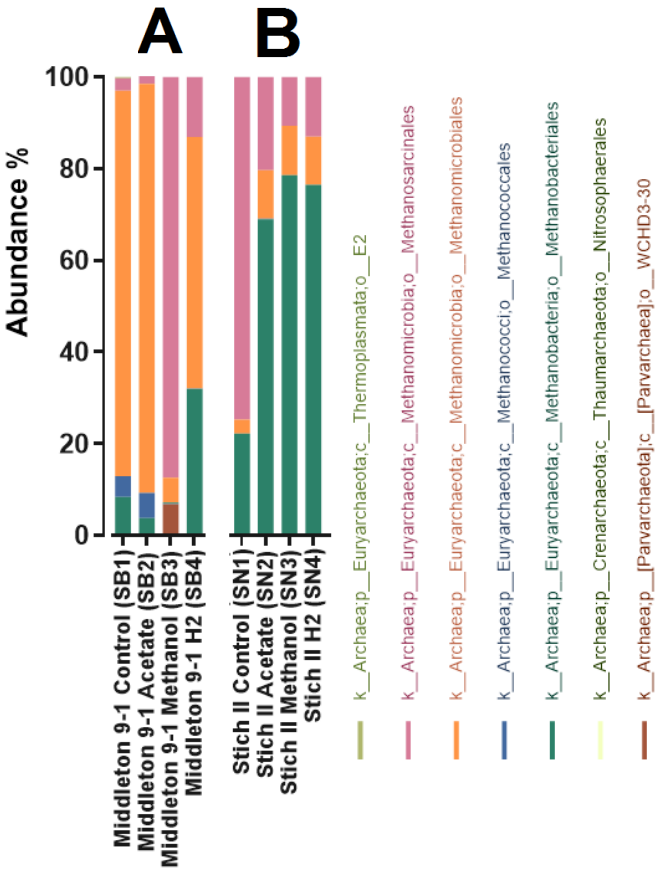


Figure 14 - Relative archaea abundance from substrate consumption (Exp. 3)

Resulting archaeal community from wells' Middleton 9-1 (A) and Stich 1-2 (B) substrate treatments (acetate, methanol, hydrogen) and control.

Relative bacterial abundance of substrate consumption reactors

The key difference between Middleton 9-1's and Stich 1-2's bacterial groups is that *Proteobacteria* have preference within Stich's FW, while bacterial phyla *Firmicutes, Armatimonadetes, WWE1, Spriochaetes,* and *Chlorobi* take preference within Middleton. These microbes (aside from *Firmicutes*) have not been abundantly present within Stich or Bailey's FW, and is likely related to

Middleton's unknown formation water chemistry. Visual relative abundance of bacteria by substrate type is depicted below (Figure 15).

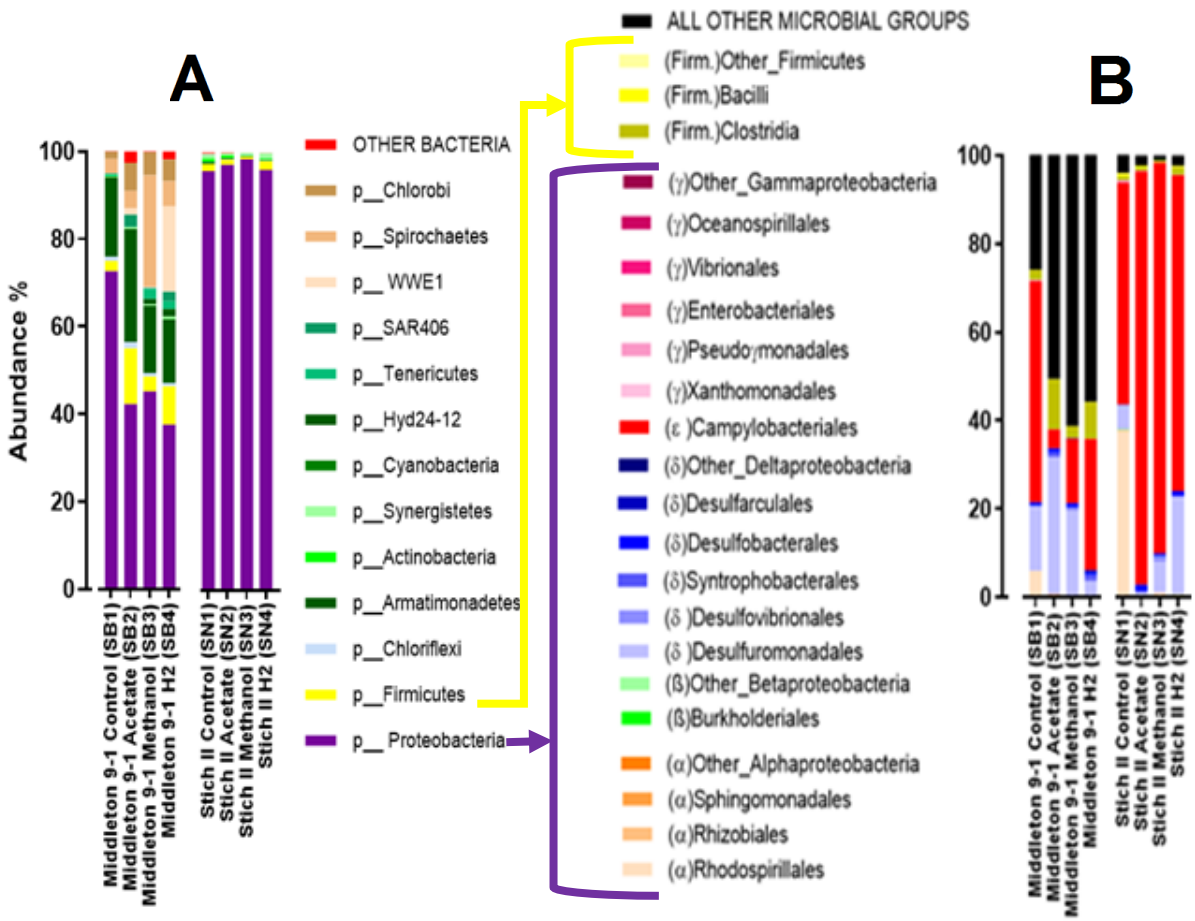


Figure 15 – Relative bacterial abundance from substrate consumption (Exp. 3)
Each of the two bar graph sets (A,B) are split in half (by wells Middleton 9-1 and Stich 1-2): (A) overall bacterial community categorized by Phylum, and (B) *Firmicutes* (order) / *Proteobacteria* (class) in greater detail.

Chapter 4 - Discussion

A comparison with previous Cherokee basin data

Chemical data from wells' Bailey 2-2 and Stich 1-2 plot within the range previously established in the Cherokee basin (Appendix 6). SO_4 concentrations of both wells were 1.50 and 1.20 mg/L respectively, falling within the range observed previously (0.67 – 10.72 mg/L). Salinity and dissolved ion concentrations also fell within the range of previous work. To our knowledge, the geochemistry of produced water from the wells has changed little since the initial study. A once-proposed weak correlation (between increased TDS with longitude or longitude) was not supported through the addition of the two newly-sampled wells Stich 1-2 and Bailey 2-2 (R^2 value calculated from Cherokee basin samples decreased from .4375 to .3773, with respect to longitude, and.0766 to .0647). Microbial groups closely matched those seen in the basin previously.

The dominant groups of microbes: *Firmicutes, Proteobacteria,* and *Methanogens*

Firmicutes and *Proteobacteria* were the dominant groups of bacteria among wells Stich 1-2 and Bailey 2-2. The majority of *Firmicutes* among the bacterial sequences lie within the *Clostridia* and *Bacilli* classes. The *Clostridia* class exist only in anaerobic conditions (i.e. they are obligate anaerobes, meaning that oxygen is toxic to them). Various *Firmicute* and *Proteobacteria* groups are regarded as facultative aerobes, meaning they prefer to produce ATP through oxygen as an energy source if available, but may rely upon fermentative breakdown of other substrates if necessary. Alternatively facultative anaerobes- in which fermentation of non-oxygen sources are preferred- have the capacity to utilize oxygen if needed (Hogg, 2005). The archaeal groups

identified were all anaerobic, methanogen groups and made up of approximately 1.6 and 3.2 % of the total identified microbes in Stich 1-2, and Bailey 2-2, respectively.

Methanogens as picky eaters

As my data methanogenic abundance and substrate-consumption assay supports, Mouser (2016) implies that methanogens are generally in low abundance (< 2% of total community) even in methanogenic ecosystems, such as the Cherokee basin, which have economic accumulations of methane. It is suggested that archaeal sequence identity in shale gas wells lies around 1-2%, and that methanogenic diversity is usually low; usually constricted to methylotrophic *Methanosarcinales*, *Methanomicrobiales*, or *Methanobacteriales* orders (these were the exact methanogens collected from the Cherokee basin). Each of these microorganisms may be distinguished by their catabolic preference of particular methanogenic substrates. The most abundant methane-producing groups from the Cherokee Basin (all within the kingdom of archaea) were classified into the methanogenic orders *Methanobacteriales, Methanomicrobiales,* and *Methanosarcinales.*

Methanobacteriales are distinguished by using catabolic substrates (generally H_2) to reduce CO_2 to methane (Bonin, 2006). Alternatively, various genera of the group do have the capacity of utilizing secondary alcohols or formate as electron donors to reduce CO_2 to methane. One member of the group, *Methanosphaera,* even uses H_2 to reduce methanol to methane (Bonin, 2006). *Methanomicrobiales* may be categorized by the fact that all genera within the order can utilize H_2 and CO_2 as a substrate for methanogenesis, *most* genera can utilize formate, and *many* species utilize alcohols. *Methanosarcinales* are the only known microorganisms capable of splitting

acetate to methane and CO_2, and this how they typically function in most anaerobic environments that lack light and alternative electron acceptors other than CO_2. Additionally, various genera within the family of *Methanosarcina* can dismute methyl compounds to make methane (Kendal et al. 2006). The reactions of these methanogenic pathways may be seen below:

Acetate fermentation: $CH_3COO^- + H^+ \rightarrow CO_2 + CH_4$

Methylated compounds: $4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2H_2O$

CO_2 reduction: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

Relative to Stich 2-2's archaeal community, Bailey 2-2's community consisted of a small amount (0.86%) of the methanogenic order *Methanococcales.* This order was not identified in FW from Stich 1-2. This group is characterized by the use of CO_2 as the electron acceptor, with hydrogen or formate as the electron donors (Whitman et al. 2006).

Geo-environmental factors as a control on microbial habitability

Relative to Stich 1-2's bacterial community, Bailey 2-2's community was much more diverse. There was a higher abundance of aerobic bacterial groups, such as *Firmicute* groups *Sporosarcina* and *Jeotgalicocus.* While these may have stemmed from laboratory error (accidental air exposure), growth might also have occurred if there were high levels of oxygen in the water to begin with (possibly due to meteoric water input). In addition, there were lower relative abundances of microbes suited for more saline conditions, such as *Halanaerobium*, that correspond to the lower concentrations of Na^+ and Cl^- seen in the formation water. Relative to Bailey 2-2's bacterial community, Stich 1-2's community was less diverse. There was a lower abundance of the same aerobic bacterial groups, such as *Sporosarcina* and *Jeotgalicocus,* suggesting that the formation

water within the well had less access to oxygen, and was more consistent with expected anaerobic conditions. In addition, there were higher relative abundances of microbes suited for brinier conditions, such as *Halanaerobium*, which correspond to the higher concentrations of Na^+ and Cl^- seen in the FW.

Initial *Dethiosulfovibrio* well concentrations of Stich 1-2 and Bailey 2-2 were 0.15% and 1.01% respectively. Stich cultures with oil yielded the same concentrations of *Dethiosulfovibrio* as seen in the control, whereas abundance decreased down to 0.27% within Bailey 2-2. Additionally, access to coal yielded an increased abundance of 0.77% (in Stich) and a decreased abundance to 0.87% (in Bailey). I presume these shifts may be partially due to *Dethiosulfovibro's* affinity for salt. *Dethiosulfovibrio* are *Synergistic* bacteria that have the ability to biodegrade protein extracts, producing organic acids such as acetic, isobutyric, isovaleric, and 2-methylbutyric acids. These microbes have the ability to reduce thiosulfate and prefer living where salt contents are up to 3% (Wolicka et al. 2012). This, however, is just one example of a fermenting bacterial group which is directly affected by salinity. Several groups of sulfate-reducing *Deltaproteobacteria*, such as *Desulfovibronales* and *Syntrophobacterales,* are present in the FW of both wells, which partially explain the low SO_4 concentrations seen within the formation water chemistry.

Impact of oil and coal availability on microbial community composition

In cultures with Bailey's FW, access to oil resulted in a decrease (relative to initial FW community) of the archaeal proportions of *Methanomicrobiales* (from 54.4 to 36.6%) even more so than the FW control microbes alone (from 54.4 to 41.3%). Access to oil also increased proportions of *Methanosaetaceae* (from 5.4 to 9.8%), even more so than the cultured FW control alone, which

decreased (from 5.4 to 1.3%). In cultures with Stich's FW, access to oil resulted in decreased proportions of *Methanomicrobiales* (from 60.7 to 11.8%), simultaneously allowing growth of *Methanobacteriaceae* (from 12.4 to 76.5%). In the FW control, *Methanomicrobiale*s ended up dominating everything (from 60.7 to 100%). *Methanosarcinales* populations are often found when the environmental availability of oil is present, whereas overall archaeal diversity is often higher in coal environments (Meslé et. al. 2013).

In cultures with Bailey's FW, access to coal seemed to increase (relative to initial FW community) abundancies of *Methanobacteriaceae* (from 30.2 to 61.8%) and *Methanosaetaceae* (from 5.4 to 25%), while decreasing abundance of *Methanomicrobiales* (from 54.4 to 13.2%). In cultures with Stich's FW, access to coal increased proportional abundancies of *Methanobacteriaceae* (from 12.4 to 45.5%), while reducing abundancies of *Methanomicrobiales* (from 60.7 to 38.6%). The absence of *Methanococcus* methanogens being found within cultures may be due to the fact that most of these archaea are thermophilic and would become out populated by the more mesophilic groups, especially incubating in the room-temperature setting of a 22ºC laboratory (Meslé et al. 2013).

Impact of waste availability on microbial community composition

A previous study analyzed the impact of the installation of a Waste-Water Treatment Plant (W.W.T.P.) upon the benthic-zone microbial community of an attached river (Atashgahi et al. 2015). They found that post W.W.T.P. samples of the river yielded lower relative abundances of *Lactobacillales* (*Bacilli* class), *Clostridales* (*Clostridia* class), *Rhodocyclclales* (*β–Proteobacteria* class), and *Desulfobacterales* (*δ-Proteobacteria* class), while increasing relative abundances of *Burkholderiales* (*β–Proteobacteria* class), and *Xanthomonadales* (*γ–Proteoboacteria* class). A

primary factor influencing the microbial shifts in this W.W.T.P. study (other than implied trace amounts of waste) is chemical byproduct vinyl chloride (C_2H_3Cl). Because this compound does not form naturally, it is most likely not present in unfiltered livestock wastewater. However, all of these same bacterial groups did exist (if at very least a small percentage) and an attempt was made to compare the bacterial shifts within Atashgahi et al.'s (2015) experiment with the relative shifts within mine.

The results of both wastewater-introduction scenarios (i.e. my study vs Atashgahi et al. (2015)) have similar growth/decline patterns between some shared microbial groups (e.g. *Lactobacillales)* and different growth/decline patterns in others (e.g. *Clostridales)*. Once again, this may be a function of salinity or higher levels of bacterial diversity in the FW. With respect to the initial FW communities, the introduction of waste of both scenarios caused (at the order level) *Lactobacillales* to decrease from an initial 7.27% in Bailey FW and 0.17% in Stich down to 0.05% in Bailey and 0.09% in Stich. *Clostridales* decreased in Atashgahi et al.'s study, increased in Bailey, and decreased in Stich. Similar inconsistencies occur within many randomly chosen bacterial groups, so no correlations could be determined between the two studies. When comparing methanogenic groups, Atashgahi et al. noticed an overall reduction in the methanogen-to-bacteria ratio after the introduction of the W.W.T.P. Though it does not imply causation by any means, introduction of waste (as well as coal and oil treatments) into Bailey 2-2's and Stich 1-2's FW cultures also showed reduction in the methanogen-to-bacteria ratio (e.g. % archaeal identity).

Access to waste increased populations of the family *Methanosaetaceae* in both Stich and Bailey Experiment 1 cultures, and was confirmed through Experiment 2 replication. Because this strain is an obligate acetoclastic methanogen, livestock waste injection seemed to serve as a good source of acetate. Many incubations were characterized by relatively high abundances of

Campylobacteriales. Often, this particular order is mistaken for its pathogenic family, *Campylobacter.* However, this species is just one of three families under *Campylobacteraceae Campylobacteriales*. The other two members, *Sulfospirillum* and *Arcobacter*, are non-pathogenic, free-living, environmental organisms that have been isolated from a wide range of sulfur-rich marine or terrestrial environments, and grow where oxygen levels are less than 4% (Vandamme et. al. 2015). These (*Sulfospirillum* and *Arcobacter*) are the specific strains which were found within my cultures, so it's important to note that pathogens were not being incubated. However, if the concept of injecting unfiltered wastewater into a real well is ever to be tested, such livestock wastewater should need to be thoroughly sterilized (as was done prior to my incubations), and assurances should be made to distance the injection from potable aquifer sources. Otherwise, there's the looming potential of unintentionally injecting pathogens into a drinking water supply which would be unideal (and quite possibly, a legal mess).

Visualization of beta diversity clustering via principle coordinate analysis (PCoA)

PCoA data points plot within three cluster sets; the top left quadrant, top right quadrant, or bottom right quadrant. As a whole, 1/3 of total variation (22.77% + 10.66%) within relative microbial communities is represented via PCoA (Figure 16).

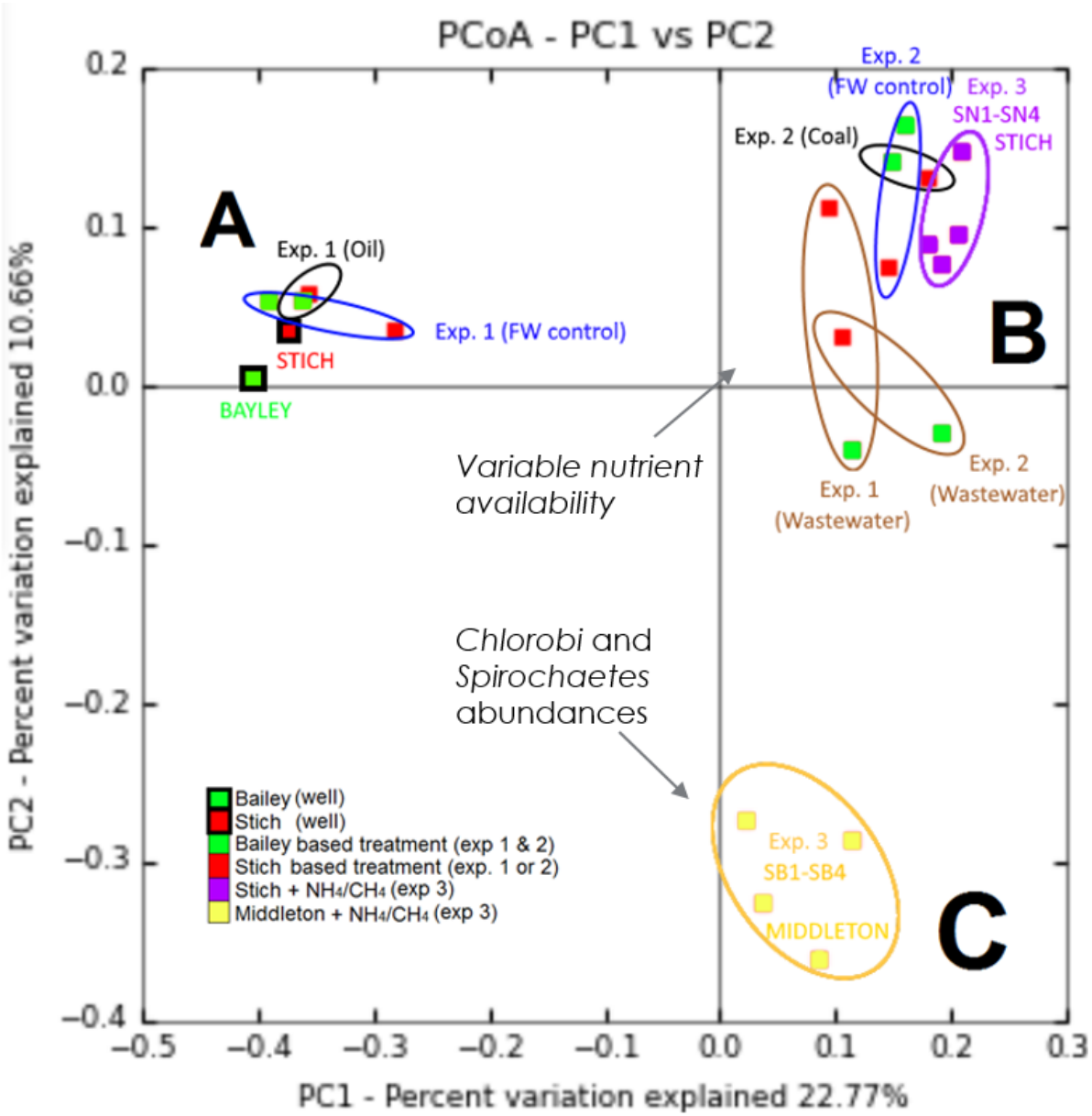


Figure 16 - Principal coordinate analysis (PCoA)

Initial wellsite sequences of Bailey and Stich plotted close together in the left cluster (A), along with Exp. 1's oil treatment and control. Plotting in the top right quadrant (B) sits Exp. 2's coal treatment and control, along with Exp. 1 & 2's wastewater amendments, and the Stich cluster from Exp. 3. In the bottom right quadrant (C) sits Middleton 9-1's Exp. 3 cultures. Because Exp. 3 cultures (substrate assays) were prepared with ammonium and phosphate amendments, they plotted together relatively precisely (as seen in both Stich and Middleton ellipses), though very far away from the well's original microbial coordinate (as is the case for Stich). Regardless, this nutrient induced higher levels of the phyla *Chlorobi* and *Spirochaetes* in Middleton 9-1's control, yet produced extremely high levels of *Proteobacteria* in Stich 1-2's control (> 95% well identity).

It is difficult to determine the influence of the different substrates (i.e. H_2, acetate, and methanol) and the resulting overall abundance of methanogens (relative to bacteria). Strangely, the substrate treatments affected Middleton very differently than they did Stich. Middleton's end control culture yielded a methanogen abundance –relative to total well identity- of 0.104 %. Comparatively, Middleton's acetate substrate allowed for net archaeal growth up to 1.129 %, Middleton's H_2 substrate allowed growth up to 1.608 %, and Middleton's methanol substrate allowed for net archaeal growth up to 17.554 %. Stich's end control culture yielded a methanogen abundance of 0.985%. Comparatively, Stich's acetate substrate allowed for a net archaeal loss down to 0.489%, Stich's methanol substrate allowed for a net archaeal loss down to 0.031%, and Stich's H_2 substrate allowed for a net archaeal loss down to 0.239%. All of the substrates increased the % archaeal community in Middleton FW relative to controls, but declined the % archaeal community in Stich FW relative to controls. The substrates had an opposite effect in each well:

Relative level of Middleton's substrate benefit (highest to lowest): Methanol > H_2 > Acetate > Control

Relative level of Stich' substrate benefit (highest to lowest): Control > Acetate > H_2 > Methanol

The scenario makes a considerably higher amount of sense when understanding that Middleton's methanol and acetate-dismantling group *Methanosarcinales* (though low in abundance at the start) was able to seize the methanol substrate conditions and then out-compete all other methanogens. These conditions occurred oppositely in Stich, which had an initially high concentration of *Methanosarcinales* but could not out-compete *Methanobacteriales,* even after methanol substrate became available. This is just one microbial implication that underlines the observed methanogenesis (or lack thereof). Most likely, the chemical conditions of the FW itself (again,

possibly due to higher saline conditions in Stich) was more suitable for *Methanobacteriales* which, as an unfortunate result, hindered methanogenesis.

Oppositions to the overarching methanogenic conceptual model

Since the start of this research, there have been exciting new discoveries in the field of microbial methanogenesis. Though it steers away from our conceptual "2-step" methanogenic process, a research team at the *National Institute of Advanced Industrial Science and Technology in Japan* has identified two strains of *Methermicoccus Shengliensis* which, acting alone, have proved to degrade coal directly into methane without the use of fermentative bacteria and their byproducts such as methoxylated aromatic compounds (Mayumi et al. 2016). These particular strains were not identified in my experiments. Nevertheless, these newly identified bacterial strains yield remarkable potential with respect to applied energy recovery from abandoned coal mines or natural gas fields, and make us wonder just what else microbes –both discovered and undiscovered- are capable of.

Significance summarized

In terms of total methane (µmol) accumulated by the final incubation sampling, standard deviation of triplicates was much lower in Experiment 1 (SD 3.8 – 50.2) than in Experiment 2 (SD 21.9 - 301.3). Because of this, Experiment 1 has a relative higher significance than Experiment 2 However, all treatments (from both Stich and Bailey based FW) with oil or coal were calculated for using the Mann-Whitney unpaired, non-parametric test for variation from treatment controls. We chose the unpaired, non parametric U test instead of a T test for the following reasons: In the case of my study, each methane dataset had random chance of error, but we decided not to assume

normal distribution because we had a low number of data points (only three per treatment). Additionally, there was no way of ensuring microbial population (and methanogenic potential) equality among treatments with their specific controls. In doing this, mean value ranks were calculated from triplicate treatments, and a P value was assigned. None of the Stich-based treatments in Experiment 1 had any significance from their controls ($P > 0.99$). However, Bailey's *FW + oil* treatment did show significance from controls ($P < 0.0291$). Being the only statistically significant result of the entire thesis, this treatment satisfied one of the goals of the overlying hypothesis; to observe methanogenesis from oil. All remaining others (treatments) failed the Mann Whitney test of significance.

	Mann Whitney Signifigance (P Values) (Exp. 1, Oil)									Mann Whitney Signifigance (P Values) (Exp. 2, Coal)							
Controls (below) vs Treatments (right)	FW+ oil (Bailey)	FW + oil (Stich)	FW + oil + PO4/NH4 (Bailey)	FW + oil + PO4/NH4 (Stich)	FW + oil + CO2 (Bailey)	FW + oil + CO2 (Stich)	FW + oil + Waste (Bailey)	FW + oil + Waste (Stich)		FW+ oil (Bailey)	FW + oil (Stich)	FW + oil + PO4/NH4 (Bailey)	FW + oil + PO4/NH4 (Stich)	FW + oil + CO2 (Bailey)	FW + oil + CO2 (Stich)	FW + oil + Waste (Bailey)	FW + oil + Waste (Stich)
FW Control (Bailey)	0.029									0.999							
FW Control (Stich)		0.999									0.999						
PO4/NH4 Control (Bailey)			0.116									0.999					
PO4/NH4 Control (Stich)				0.999									0.999				
CO2 Control (Bailey)					0.562									0.999			
CO2 Control (Stich)						0.999									>0.999		
Waste Control (Bailey)							0.079									>0.999	
Waste Control (Stich)								0.999									0.999

Table 8 - Mann Whitney significance (Exp. 1, 2, & 3)

P values were calculated from an un-paired, non parametric analyses. Values greater than 0.05 are not significant. Bailey's *FW + oil* treatment was only the significant result (as compared with control treatment) Bailey's *FW + waste* treatment was relatively close towards significance.

Potential sources of error

There are several sources of error associated with the execution of Experiments 1-3. For example, a higher level of quality control in culture tubes / blue rubber caps during culture preparation in Experiment 2 would have prevented potentially avoidable oxygen contamination, which eventually became apparent in the form of orange-red iron oxide precipitate at the bottom of some cultures. This would have resulted in a lower SD to be able to better effectively judge the influence of coal availability upon methanogenesis. Additionally, the laboratory gas chromatograph had been having some issues when we first started the experiment, but it took months of trial and error and column swaps before getting it right. Looking back at Experiment 1, there is no way to know the exact accuracy of the gas chromatograph at the beginning of the experiment, particularly because the first two samplings consisted of an older, fallible GC-rod (which was later found out to be plugged by a piece of septum). With regards to shortfalls of Exp. 3, it would have been very hard to produce the amount of triplicates with the amount of FW we still had available in the lab. However, there may have been enough to at least produce duplicate controls, and this would have provided us with a degree of precision associated with the experiment. Additionally, it may have been noticed that the Cherokee basin water chemistry (Appendix 6) does not have iron concentrations listed for the two new wells (Bailey 2-2 and Stich 1-2). This is because iron spectrophotometer data was unintentionally lost. Had it been recovered, this data could have provided valuable insight into iron reduction processes.

Lastly, it is important to know that there is the chance we didn't consider other variables which may have induced stress upon the microbial populations. Examples of these variables include: shifts in temperature and/or pressure of the FW from the subsurface layers to within the well itself

– this may have resulted in higher groups of mesophilic bacteria over initially thermophilic groups, and/or reduced populations of high-pressure tolerant bacterial groups, such as the *Firmicute Carnobacterium.* It is difficult to determine what may be the limiting factor that slowed methanogenic rate within the cultures. It very well could be the lack of produced substrates, such as methylbutyric acids produced via bacterial fermentation. Or it, instead, could be low concentrations of the methanogenic archaea or fermentation bacteria themselves. Alternatively, it might be the unhealthy biologic conditions such as salinity in which the various microorganisms reside. Most likely, however, there are a combination of all of the above components which have resulted in low methanogenic significance.

Conclusion and future work

From this work, we have determined that: a) microbes from the Cherokee basin are capable of producing methane from oil in laboratory conditions, and b) a higher concentration of methanogens existed in lower-salinity conditions. This suggests higher potential for practical stimulatory injection. With respect to the initial hypotheses tested, we determined that the only methanogenically significant treatment had access to oil in lower salinity formation water, and access to coal resulted in no significant results (likely laboratory error and small-scale uncertainty). Methanogenic archaeal abundance, formation water chemistry (salinity), and wastewater nutrients often produced higher (yet insignificant) rates of methane production. And though other studies show carbon dioxide to stimulate methanogenesis in higher T / P conditions (Mayumi et al. 2013), this was not observed in any manner within our low T, low P incubations. Of methanogenic substrates consumed, we determined that most Cherokee basin methanogens preferred methanol over hydrogen and acetate.

I would propose future work to include experiment replication but in larger batches. This would help remove some of the uncertainty associated with such a small scale, and hopefully yield more significant standings of various treatments. More work should be performed in the laboratory to assist with nutrient development, particularly to stimulate methane production from Cherokee basin microbes in set conditions of varying salinity. Additionally, information such as coal surface area and coal-seam extent should be further studied. By normalizing the rate results (e.g., wastewater enhanced methane formation rate by X μmol/day/m^2 relative to the control) to the coal surface area available in a given cultures, we may then multiply that value by an estimate of the surface area of the seam itself. However, because most laboratory methanogenesis experiments are conducted with crushed coal having a large net surface area, one would expect higher reaction rates than if solid coal was used (Budwill 2013). This information, weighed against the cost of implementing the stimulation strategy, will yield insight into if an energy company could ultimately make a profit from stimulating a coalbed.

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Appendix 1 – Raw GC data (Experiment 1)

				3/16/2015			3/27/2015			4/17/2015			5/8/2015			5/29/2015			6/17/2015			7/6/2015			7/15/2015			7/26/2015			8/13/2015			8/20/2015			8/27/2015
	GC0	GC0	GC0	GC1	GC1	GC1	GC2	GC2	GC2	GC3	GC3	GC3	GC4	GC4	GC4	GC5	GC5	GC5	GC6	GC6	GC6	GC7	GC7	GC7	GC8	GC8	GC8	GC9	GC9	GC9	GC10	GC10	GC10	GC11	GC11	GC11
(Day)	0	0	0	11	11	11	32	32	32	53	53	53	74	74	74	93	93	93	112	112	112	121	121	121	132	132	132	150	150	150	157	157	157	164	164	164
	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4
A1	98.00	0.00	0.00	97.31	0.16	1.11	71.24	0.04	0.59	N/A	N/A	N/A	100.76	0.06	0.67	95.16	0.04	0.55	99.43	0.07	0.59	101.05	0.06	0.57	98.21	0.06	0.53	99.45	0.07	0.51	100.13	0.06	0.48	100.56	0.06	0.46
A2	98.00	0.00	0.00	84.96	0.08	1.09	71.45	0.11	0.65	94.07	0.03	0.75	97.97	0.08	0.73	94.90	0.23	0.64	97.67	0.09	0.63	98.86	0.11	0.60	97.71	0.07	0.56	98.60	0.10	0.54	99.55	0.08	0.51	95.96	0.06	0.47
A3	98.00	0.00	0.00	74.48	0.09	1.00	70.55	0.12	0.62	N/A	N/A	N/A	97.43	0.03	0.66	94.29	0.14	0.63	100.20	0.08	0.63	98.84	0.07	0.58	97.24	0.06	0.54	99.17	0.08	0.52	100.68	0.07	0.52	98.65	0.06	0.48
A1-3 AVG	98.00	0.00	0.00	85.58	0.11	1.07	71.08	0.09	0.62	94.07	0.03	0.75	98.72	0.06	0.69	94.78	0.14	0.61	99.10	0.08	0.62	99.58	0.08	0.58	97.72	0.06	0.54	99.07	0.08	0.52	100.12	0.07	0.50	98.39	0.06	0.47
A4	98.00	0.00	0.00	84.02	0.16	0.89	N/A	N/A	N/A	93.52	0.03	0.54	100.00	0.04	0.50	95.85	0.15	0.47	97.89	0.07	0.45	100.16	0.08	0.33	98.80	0.07	0.41	99.11	0.11	0.39	99.98	0.08	0.37	100.56	0.09	0.24
A5	98.00	0.00	0.00	85.14	0.21	0.81	69.94	0.04	0.37	94.17	0.07	0.50	101.04	0.08	0.44	95.25	0.09	0.39	101.61	0.07	0.39	100.38	0.16	0.25	98.90	0.17	0.18	97.94	0.07	0.21	101.77	0.07	0.21	99.00	0.10	0.14
A6	98.00	0.00	0.00	71.71	0.22	0.79	N/A	N A	N/A	94.26	0.08	0.54	98.50	0.08	0.47	93.75	0.09	0.43	97.95	0.09	0.38	99.80	0.10	0.27	96.24	0.12	0.22	99.58	0.10	0.22	99.65	0.05	0.23	100.33	0.14	0.17
A4-6 AVG	98.00	0.00	0.00	80.29	0.20	0.83	69.94	0.04	0.37	93.98	0.06	0.53	99.85	0.07	0.47	94.95	0.11	0.43	99.15	0.08	0.41	100.11	0.11	0.28	97.98	0.12	0.27	98.88	0.09	0.27	100.47	0.07	0.27	99.96	0.11	0.18
B1	98.00	0.00	0.00	82.79	0.20	0.95	71.05	0.21	0.53	76.92	0.05	0.49	99.40	0.09	0.62	96.26	0.08	0.54	98.74	0.13	0.52	99.58	0.07	0.51	96.99	0.07	0.47	98.70	0.06	0.43	101.60	0.07	0.42	93.88	0.08	0.37
B2	98.00	0.00	0.00	80.94	0.04	1.02	70.03	0.11	0.58	90.36	0.04	0.81	98.95	0.04	0.60	95.47	0.08	0.59	101.29	0.07	0.58	99.51	0.08	0.54	98.85	0.07	0.50	98.71	0.17	0.48	100.66	0.06	0.47	98.38	0.07	0.47
B3	98.00	0.00	0.00	87.25	0.63	1.05	21.94	0.01	0.15	72.03	0.05	0.53	100.39	0.09	0.71	95.16	0.08	0.61	97.57	0.08	0.59	99.94	0.07	0.58	92.16	0.07	0.50	98.18	0.06	0.50	94.93	0.06	0.47	97.47	0.07	0.47
B1-3 AVG	98.00	0.00	0.00	83.66	0.29	1.01	54.34	0.11	0.42	79.77	0.05	0.61	99.58	0.07	0.64	95.63	0.08	0.58	99.20	0.09	0.56	99.67	0.07	0.54	96.00	0.07	0.49	98.53	0.10	0.47	99.06	0.06	0.45	96.58	0.07	0.44
B4	98.00	0.00	0.00	88.67	0.23	0.83	71.12	0.06	0.37	91.43	0.09	0.47	98.54	0.09	0.45	97.25	0.11	0.40	100.27	0.08	0.39	101.34	0.09	0.37	98.15	0.07	0.20	99.25	0.25	0.21	99.00	0.07	0.21	99.07	0.10	0.18
B5	98.00	0.00	0.00	62.02	0.20	0.74	71.41	0.08	0.39	91.93	0.10	0.52	99.81	0.05	0.43	97.64	0.08	0.42	102.26	0.08	0.42	98.82	0.11	0.38	98.86	0.17	0.25	96.59	0.11	0.16	101.14	0.07	0.23	99.76	0.16	0.13
B6	98.00	0.00	0.00	71.77	0.18	0.83	70.52	0.05	0.42	95.24	0.08	0.60	99.48	0.09	0.51	92.87	0.08	0.45	101.81	0.07	0.46	99.51	0.19	0.43	97.59	0.16	0.39	99.58	0.15	0.37	100.22	0.06	0.28	101.90	0.24	0.16
B4-6 AVG	98.00	0.00	0.00	74.16	0.20	0.80	71.02	0.06	0.39	92.86	0.09	0.53	99.28	0.08	0.46	95.92	0.09	0.42	101.45	0.07	0.42	99.89	0.13	0.39	98.20	0.13	0.28	98.47	0.17	0.25	100.12	0.07	0.24	100.24	0.17	0.16
C1	98.00	0.00	0.00	65.98	0.07	0.89	69.70	0.03	0.53	80.96	0.04	0.54	99.83	0.08	0.63	94.01	0.08	0.55	99.47	0.07	0.55	99.08	0.06	0.52	96.52	0.07	0.48	97.47	0.07	0.47	99.93	0.10	0.45	95.20	0.06	0.41
C2	98.00	0.00	0.00	89.94	0.11	1.09	70.80	0.54	0.59	N/A	N/A	N/A	97.80	0.20	0.70	94.57	0.10	0.63	100.02	0.09	0.62	95.54	0.10	0.57	96.10	0.16	0.50	95.87	0.10	0.48	99.48	0.07	0.47	100.46	0.08	0.46
C3	98.00	0.00	0.00	87.14	0.12	1.11	70.64	0.12	0.62	93.15	0.02	0.91	97.10	0.04	0.68	93.89	0.08	0.63	98.29	0.07	0.62	99.85	0.07	0.60	96.81	0.06	0.55	99.90	0.17	0.53	100.87	0.08	0.51	98.54	0.07	0.47
C1-3 AVG	98.00	0.00	0.00	81.02	0.10	1.03	70.38	0.23	0.58	87.05	0.03	0.73	98.24	0.11	0.67	94.16	0.08	0.60	99.26	0.08	0.60	98.16	0.08	0.56	96.48	0.10	0.51	97.75	0.11	0.49	100.09	0.08	0.48	98.06	0.07	0.45

C4	98.00	0.00	0.00	82.19	0.19	0.81	72.63	0.57	0.41	93.67	0.09	0.65	98.01	0.10	0.25	96.11	1.46	0.24	99.83	0.80	0.39	100.92	0.85	0.27	97.60	0.53	0.23	100.22	0.49	0.23	99.17	0.31	0.21	87.86	0.27	0.18
C5	98.00	0.00	0.00	74.67	0.15	0.78	69.88	0.03	0.36	82.62	0.04	0.43	99.48	0.09	0.45	96.29	0.20	0.40	100.27	0.07	0.39	98.23	0.13	0.24	96.68	0.16	0.22	96.50	0.09	0.20	101.88	0.06	0.23	100.39	0.23	0.19
C4-5 AVG	98.00	0.00	0.00	78.43	0.17	0.80	71.25	0.30	0.39	88.14	0.06	0.54	98.74	0.09	0.35	96.20	0.83	0.32	100.05	0.43	0.39	99.58	0.49	0.25	97.14	0.35	0.22	98.36	0.29	0.21	100.52	0.18	0.22	94.12	0.25	0.19
C6 (not graphed)	98.00	0.00	0.00	40.84	0.05	1.33	69.90	0.08	1.56	94.19	0.08	1.88	98.08	0.10	1.57	93.65	0.20	1.31	100.01	0.09	1.20	99.47	0.11	1.04	94.66	0.09	0.88	98.12	0.13	0.78	100.10	0.11	0.70	99.26	0.21	0.69
D1	98.00	0.00	0.00	75.16	0.08	0.91	29.37	0.23	0.24	87.47	0.36	0.72	101.72	0.12	0.63	96.51	0.15	0.54	98.25	0.09	0.48	99.11	0.08	0.48	96.96	0.08	0.43	97.76	0.09	0.40	100.68	0.08	0.40	91.89	0.11	0.34
D2	98.00	0.00	0.00	57.65	0.05	0.84	72.52	0.10	0.60	93.63	0.05	0.72	99.66	0.07	0.66	97.53	0.10	0.57	99.82	0.07	0.60	100.57	0.08	0.58	97.21	0.06	0.52	98.19	0.08	0.50	99.72	0.11	0.48	95.99	0.06	0.44
D3	98.00	0.00	0.00	84.26	0.08	0.99	61.96	0.09	0.47	92.65	0.04	0.70	99.85	0.06	0.63	95.36	0.08	0.53	100.07	0.08	0.49	100.49	0.08	0.48	98.70	0.05	0.44	98.39	0.08	0.41	99.88	0.08	0.42	96.19	0.06	0.34
D1-3 AVG	98.00	0.00	0.00	72.36	0.07	0.91	54.62	0.14	0.44	91.25	0.15	0.71	100.41	0.08	0.64	96.47	0.11	0.54	99.38	0.08	0.52	100.06	0.08	0.51	97.62	0.06	0.47	98.11	0.08	0.44	100.09	0.09	0.43	94.69	0.08	0.37
D4	98.00	0.00	0.00	78.89	0.27	0.56	70.31	0.04	0.20	91.10	0.04	0.49	99.53	0.06	0.19	95.45	0.19	0.18	100.91	0.07	0.22	100.83	0.07	0.19	97.73	0.09	0.08	97.70	0.22	0.15	100.36	0.12	0.16	99.35	0.19	0.14
D5	98.00	0.00	0.00	61.28	0.20	0.61	41.32	0.03	0.14	63.15	0.05	0.19	27.92	0.01	0.06	97.54	0.08	0.24	99.29	0.07	0.20	98.70	0.03	0.19	97.36	0.12	0.16	97.52	0.13	0.15	100.41	0.06	0.15	96.45	0.16	0.14
D6	98.00	0.00	0.00	68.25	0.23	0.69	70.89	0.03	0.20	N / A	N/A	N / A	99.78	0.09	0.19	93.52	0.16	0.21	99.16	0.06	0.21	101.26	0.17	0.20	94.56	0.15	0.17	97.38	0.20	0.12	98.67	0.06	0.16	100.55	0.12	0.13
D4-6 AVG	98.00	0.00	0.00	69.48	0.24	0.62	60.84	0.04	0.18	77.12	0.04	0.34	75.74	0.05	0.15	95.50	0.14	0.21	99.79	0.06	0.21	100.26	0.09	0.19	96.55	0.12	0.14	97.53	0.18	0.14	99.81	0.08	0.15	98.78	0.16	0.13
E1	0.00	0.00	0.00	2.96	1.06	0.89	14.13	3.48	0.41	17.62	4.20	0.49	19.65	5.00	0.50	19.91	5.06	0.43	19.00	4.58	0.44	19.30	4.63	0.39	17.37	4.01	0.37	18.93	4.35	0.34	17.69	3.95	0.34	16.94	3.73	0.30
E2	0.00	0.00	0.00	3.59	1.23	0.91	14.23	3.48	0.40	19.00	4.41	0.53	21.63	5.23	0.54	22.02	5.21	0.47	20.74	4.56	0.48	20.79	4.57	0.43	18.77	3.96	0.40	20.39	4.31	0.37	18.82	3.85	0.36	19.02	3.88	0.33
E3	0.00	0.00	0.00	2.81	1.02	0.90	N / A	N/A	N / A	N / A	N/A	N / A	20.67	4.77	0.47	21.62	4.97	0.42	19.66	4.26	0.42	19.74	4.31	0.37	18.16	3.85	0.36	18.67	3.96	0.33	18.07	3.79	0.32	17.04	3.52	0.28
E1-3 AVG	0.00	0.00	0.00	3.12	1.10	0.90	14.18	3.48	0.40	18.31	4.30	0.51	20.65	5.00	0.50	21.18	5.08	0.44	19.80	4.47	0.45	19.94	4.50	0.40	18.10	3.94	0.38	19.33	4.21	0.35	18.19	3.86	0.34	17.67	3.71	0.31
E4	0.00	0.00	0.00	4.46	1.40	0.44	4.94	1.18	0.03	19.17	4.42	0.10	21.71	5.42	0.09	22.36	5.60	0.08	20.19	4.86	0.09	20.51	4.92	0.08	18.31	4.25	0.06	19.55	4.45	0.07	19.08	4.28	0.07	17.98	3.96	0.06
E5	0.00	0.00	0.00	3.87	1.25	0.58	8.03	1.94	0.05	20.69	4.71	0.11	22.21	5.34	0.10	23.68	5.65	0.10	21.20	4.73	0.10	21.81	4.83	0.09	19.87	4.22	0.07	21.02	4.41	0.08	19.67	4.02	0.08	17.93	3.60	0.07
E6	0.00	0.00	0.00	3.89	1.28	0.59	4.92	1.21	0.03	19.65	4.52	0.12	22.31	5.48	0.11	23.17	5.71	0.11	27.82	5.05	0.11	32.35	4.71	0.10	26.18	3.46	0.08	27.97	3.96	0.09	24.01	3.21	0.08	24.70	3.58	0.07
E4-6 AVG	0.00	0.00	0.00	4.07	1.31	0.53	5.96	1.44	0.03	19.84	4.55	0.11	22.07	5.42	0.10	23.07	5.65	0.10	23.07	4.88	0.10	24.89	4.82	0.09	21.45	3.98	0.07	22.85	4.27	0.08	20.92	3.84	0.07	20.20	3.71	0.06
F1	0.00	0.00	0.00	2.94	1.09	0.91	15.41	3.86	0.46	18.33	4.49	0.55	20.84	5.57	0.55	21.74	5.80	0.51	19.62	5.19	0.49	20.15	5.35	0.45	18.20	4.79	0.43	19.44	5.13	0.40	18.48	4.87	0.38	17.71	4.67	0.34
F2	0.00	0.00	0.00	N / A	N/A	N / A	14.81	3.69	0.42	18.19	4.44	0.51	20.28	5.41	0.51	20.74	5.50	0.45	19.07	5.04	0.45	19.46	5.16	0.41	17.48	4.60	0.39	18.70	4.94	0.36	17.11	4.47	0.34	16.72	4.41	0.30
F3	0.00	0.00	0.00	2.49	0.95	0.93	15.06	3.77	0.41	15.02	3.64	0.41	20.74	5.17	0.49	21.80	5.33	0.44	19.45	4.48	0.43	20.29	4.68	0.40	18.22	4.06	0.38	19.42	4.39	0.35	18.85	4.23	0.34	17.69	3.96	0.31
F1-3 AVG	0.00	0.00	0.00	2.71	1.02	0.92	15.09	3.77	0.43	17.18	4.19	0.49	20.62	5.38	0.52	21.43	5.54	0.47	19.38	4.90	0.46	19.97	5.06	0.42	17.97	4.48	0.40	19.19	4.82	0.37	18.15	4.52	0.35	17.37	4.34	0.32

F4	0.00	0.00	0.00	3.05	1.06	0.56	15.40	3.75	0.08	18.87	4.46	0.10	20.88	5.40	0.09	21.40	5.51	0.09	19.29	4.89	0.09	20.10	5.12	0.07	17.71	4.46	0.06	19.52	4.96	0.06	19.30	4.88	0.07	17.36	4.38	0.06
F5	0.00	0.00	0.00	N/A	N/A	N/A	15.59	3.76	0.09	19.44	4.45	0.11	21.36	5.32	0.09	21.72	5.42	0.09	20.11	4.93	0.09	20.50	5.08	0.08	18.32	4.47	0.06	20.27	5.03	0.07	19.39	4.78	0.07	17.59	4.34	0.06
F6	0.00	0.00	0.00	6.51	1.95	0.57	15.44	3.69	0.10	N/A	N/A	N/A	20.58	5.15	0.11	21.80	5.46	0.11	19.36	4.79	0.11	19.67	4.91	0.09	17.54	4.32	0.08	19.65	4.91	0.07	18.62	4.65	0.08	17.26	4.28	0.07
F4-6 AVG	0.00	0.00	0.00	4.78	1.50	0.57	15.48	3.73	0.09	19.16	4.45	0.10	20.94	5.29	0.10	21.64	5.46	0.10	19.59	4.87	0.10	20.09	5.04	0.08	17.85	4.42	0.06	19.81	4.96	0.07	19.10	4.77	0.07	17.40	4.33	0.06
G1	98.00	0.00	0.00	88.95	0.24	1.10	55.60	0.04	0.53	89.88	0.03	0.93	100.68	0.03	0.80	99.15	0.12	0.79	101.16	0.07	0.76	100.78	0.10	0.72	97.66	0.11	0.65	77.72	0.11	0.42	101.24	0.07	0.61	99.49	0.06	0.56
G2	98.00	0.00	0.00	54.53	0.27	0.83	70.51	0.52	0.67	86.76	0.15	0.84	100.31	0.11	0.83	93.66	0.21	0.72	100.93	0.10	0.75	97.83	0.13	0.70	97.16	0.22	0.65	95.79	0.11	0.62	100.01	0.08	0.60	99.88	0.09	0.57
G3	98.00	0.00	0.00	66.85	0.36	0.99	70.89	0.03	0.65	N/A	N A	N A	100.80	0.09	0.82	94.87	0.14	0.73	98.47	0.07	0.71	100.54	0.12	0.69	95.59	0.15	0.63	98.83	0.28	0.60	97.69	0.06	0.63	103.72	0.08	0.60
G1-3 AVG	98.00	0.00	0.00	70.11	0.29	0.97	65.66	0.20	0.62	88.32	0.09	0.88	100.60	0.08	0.82	95.89	0.16	0.74	100.19	0.08	0.74	99.72	0.12	0.70	96.80	0.16	0.64	90.78	0.17	0.54	99.65	0.07	0.61	101.03	0.07	0.58
G4	98.00	0.00	0.00	35.17	0.08	0.55	70.78	0.51	0.09	N/A	N/A	N/A	102.48	0.11	0.08	99.77	0.32	0.11	98.91	0.09	0.10	100.70	0.12	0.10	98.62	0.13	0.05	96.39	0.08	0.08	100.09	0.07	0.08	99.89	0.15	0.06
G5	98.00	0.00	0.00	23.24	0.06	0.53	71.99	0.04	0.11	95.06	0.04	0.25	100.46	0.08	0.12	97.64	0.16	0.12	100.49	0.07	0.12	98.68	0.15	0.06	97.72	0.19	0.08	99.01	0.20	0.08	100.58	0.06	0.07	101.72	0.18	0.07
G6	98.00	0.00	0.00	N/A	N/A	N/A	71.46	0.04	0.10	69.76	0.04	0.09	100.58	0.15	0.08	94.59	0.20	0.09	98.72	0.07	0.09	100.45	0.12	0.09	96.66	0.14	0.07	97.57	0.24	0.03	99.08	0.06	0.07	101.55	0.06	0.05
G4-6 AVG	98.00	0.00	0.00	29.21	0.07	0.54	71.41	0.20	0.10	82.41	0.04	0.17	101.17	0.11	0.10	97.33	0.23	0.10	99.37	0.08	0.10	99.94	0.13	0.08	97.67	0.15	0.07	97.66	0.17	0.06	99.92	0.06	0.08	101.05	0.13	0.06
H1	98.00	0.00	0.00	64.06	0.18	0.87	71.40	0.06	0.58	58.18	0.04	0.49	100.19	0.05	0.69	97.34	0.31	0.66	100.81	0.07	0.66	100.68	0.15	0.62	98.69	0.16	0.57	98.58	0.07	0.54	101.93	0.07	0.53	100.26	0.06	0.50
H2	98.00	0.00	0.00	81.96	0.06	0.99	70.33	0.50	0.58	98.21	0.12	0.90	101.20	0.10	0.71	97.23	0.11	0.65	98.87	0.09	0.61	97.55	0.13	0.59	98.12	0.20	0.56	97.44	0.11	0.52	100.99	0.07	0.50	97.16	0.09	0.47
H3	98.00	0.00	0.00	59.38	0.24	0.88	72.57	0.54	0.65	92.73	0.07	0.93	99.98	0.11	0.77	96.71	0.22	0.70	98.21	0.09	0.67	100.21	0.08	0.65	93.94	0.08	0.59	97.80	0.12	0.56	97.91	0.08	0.53	100.43	0.08	0.54
H1-3 AVG	98.00	0.00	0.00	68.47	0.16	0.91	71.43	0.37	0.60	83.04	0.08	0.77	100.46	0.09	0.72	97.09	0.21	0.67	99.30	0.08	0.65	99.48	0.12	0.62	96.92	0.15	0.57	97.94	0.10	0.54	100.28	0.07	0.52	99.28	0.08	0.50
H4	98.00	0.00	0.00	84.63	0.28	0.57	65.09	0.55	0.08	94.89	0.18	0.10	100.08	0.14	0.10	99.66	0.31	0.08	88.12	0.10	0.08	101.63	0.18	0.07	98.87	0.21	0.02	100.45	0.13	0.03	102.61	0.09	0.06	98.48	0.08	0.05
H5	98.00	0.00	0.00	80.61	0.20	0.56	71.00	0.51	0.09	96.05	0.10	0.09	100.06	0.17	0.10	98.04	0.32	0.07	97.66	0.09	0.09	98.91	0.13	0.07	98.60	0.21	0.05	97.47	0.16	0.05	96.88	0.07	0.08	101.88	0.11	0.06
H6	98.00	0.00	0.00	47.33	0.08	0.53	71.63	0.04	0.11	94.34	0.16	0.10	47.89	0.05	0.04	95.75	0.20	0.07	100.01	0.07	0.09	101.03	0.10	0.09	96.21	0.15	0.07	97.99	0.33	0.05	98.69	0.05	0.07	100.82	0.07	0.06
H4-6 AVG	98.00	0.00	0.00	70.86	0.19	0.55	69.24	0.37	0.09	95.09	0.14	0.10	82.68	0.12	0.08	97.82	0.28	0.08	95.26	0.09	0.09	100.52	0.14	0.08	97.89	0.19	0.05	98.63	0.21	0.04	99.39	0.07	0.07	100.39	0.09	0.06

Appendix 2 – Raw GC data (Experiment 2)

			10/8/2015			10/22/2015			11/5/2015			11/19/2015			12/3/2015			3/25/2016
	GC0	GC0	GC0	GC1	GC1	GC1	GC2	GC2	GC2	GC3	GC3	GC3	GC4	GC4	GC4	GC5	GC5	GC5
(Day)	0	0	0	14	14	14	28	28	28	42	42	42	56	56	56	169	169	169
	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	%O2	%CH4	%N2	O%	%CH4
A1	98.00	0.00	0.00	88.69	0.07	1.15	82.30	0.37	0.95	69.32	0.07	0.82	84.92	0.07	0.97	86.67	0.10	0.93
A2	98.00	0.00	0.00	88.28	0.08	1.06	92.08	0.07	1.00	69.59	0.06	0.59	103.47	0.07	1.09	87.27	0.09	0.86
A3	98.00	0.00	0.00	87.99	0.10	0.55	95.82	0.08	0.58	68.69	0.06	0.43	101.25	0.07	0.58	88.78	0.51	0.50
A1-3 AVG	98.00	0.00	0.00	88.32	0.08	0.92	90.06	0.17	0.84	69.20	0.06	0.61	96.55	0.07	0.88	87.57	0.23	0.76
A4	98.00	0.00	0.00	91.35	0.06	0.42	95.81	0.08	0.46	68.80	0.04	0.19	102.75	0.08	0.24	85.42	2.83	0.33
A5	98.00	0.00	0.00	92.34	0.09	0.40	91.36	0.09	0.23	71.81	0.13	0.29	100.36	0.47	0.38	48.01	0.80	0.21
A6	98.00	0.00	0.00	91.21	0.06	0.40	98.26	0.06	0.40	69.71	0.05	0.30	104.45	0.06	0.23	89.91	0.06	0.47
A4-6 AVG	98.00	0.00	0.00	91.63	0.07	0.41	95.14	0.07	0.37	70.11	0.07	0.26	102.52	0.20	0.28	74.45	1.23	0.34
B1	98.00	0.00	0.00	89.93	0.05	1.23	97.79	0.06	1.25	69.05	0.04	0.83	102.38	0.05	1.15	88.22	0.03	0.89
B2	98.00	0.00	0.00	82.82	0.06	0.83	93.68	0.08	0.91	69.41	0.05	0.68	106.88	0.07	1.01	93.71	0.07	0.73
B3	98.00	0.00	0.00	88.88	0.85	0.36	137.03	2.26	0.59	65.78	2.53	0.34	105.53	3.25	0.16	8.78	83.00	0.21
B1-3 AVG	98.00	0.00	0.00	87.21	0.32	0.81	109.50	0.80	0.92	68.08	0.87	0.61	104.93	1.12	0.77	63.57	27.70	0.61
B4	98.00	0.00	0.00	90.79	0.05	0.36	98.03	0.06	0.22	71.75	0.05	0.33	106.30	0.16	0.45	94.17	0.84	0.35
B5	98.00	0.00	0.00	91.20	0.47	0.35	97.80	0.11	0.20	71.03	0.20	0.26	105.78	0.12	0.44	77.29	15.52	0.15
B6	98.00	0.00	0.00	89.44	1.18	0.30	95.44	1.27	0.15	75.46	0.24	0.14	103.53	2.32	0.11	80.83	14.57	0.14
B4-6 AVG	98.00	0.00	0.00	90.48	0.57	0.34	97.09	0.48	0.19	72.75	0.16	0.24	105.20	0.87	0.33	84.09	10.31	0.21
C1	98.00	0.00	0.00	91.03	0.05	0.33	84.20	0.05	0.31	71.24	0.04	0.35	104.66	0.05	0.44	94.81	0.09	0.40
C2	98.00	0.00	0.00	80.35	1.97	0.57	92.18	4.23	0.61	65.24	5.44	0.38	99.20	8.84	0.24	75.18	14.39	0.09
C3	98.00	0.00	0.00	88.19	1.39	0.77	94.89	2.66	0.81	67.34	3.12	0.55	99.35	5.29	0.66	76.82	15.23	0.16
C1-3 AVG	98.00	0.00	0.00	86.52	1.14	0.56	90.42	2.31	0.58	67.94	2.87	0.42	101.07	4.72	0.45	82.27	9.90	0.22

C4	98.00	0.00	0.00	91.59	0.06	0.29	97.45	0.07	0.12	46.37	0.63	0.07	105.23	0.66	0.47	93.57	0.11	0.39
C5	98.00	0.00	0.00	92.51	0.05	0.14	91.89	0.06	0.13	69.01	0.04	0.09	103.99	0.07	0.10	81.09	8.60	0.16
C6	98.00	0.00	0.00	88.36	0.05	0.12	98.62	0.05	0.12	73.69	0.05	0.02	104.87	0.05	0.10	89.45	0.14	0.30
C4-6 AVG	98.00	0.00	0.00	90.82	0.06	0.18	95.99	0.06	0.13	63.02	0.24	0.06	104.69	0.26	0.22	88.04	2.95	0.28
D1	98.00	0.00	0.00	88.89	0.06	1.00	97.17	0.06	1.07	66.37	0.05	0.70	106.64	0.06	1.13	90.90	0.12	0.91
D2	98.00	0.00	0.00	90.93	0.81	0.58	95.75	1.87	0.56	59.78	1.13	0.35	101.94	1.55	0.60	79.70	11.37	0.25
D3	98.00	0.00	0.00	92.66	0.06	0.20	93.00	0.05	0.16	56.31	0.03	0.10	106.17	0.25	0.12	88.82	0.21	0.34
D1-3 AVG	98.00	0.00	0.00	90.83	0.31	0.59	95.31	0.66	0.60	60.82	0.40	0.38	104.92	0.62	0.62	86.47	3.90	0.50
D4	98.00	0.00	0.00	91.51	0.05	0.11	96.49	0.05	0.10	69.37	0.04	0.29	106.46	0.61	0.30	83.37	12.21	0.13
D5	98.00	0.00	0.00	92.56	0.05	0.09	104.04	0.06	0.00	40.70	0.03	0.04	109.54	0.06	0.40	40.37	3.11	0.11
D6	98.00	0.00	0.00	89.40	0.06	0.06	97.23	0.07	0.05	55.23	0.03	0.03	108.73	0.06	0.31	90.83	0.06	0.24
D4-6 AVG	98.00	0.00	0.00	91.15	0.05	0.09	99.25	0.06	0.05	55.10	0.03	0.12	108.24	0.25	0.34	71.52	5.13	0.16
E1	0.00	0.00	0.00	15.32	3.75	0.19	16.88	3.93	0.16	17.32	2.71	0.11	23.30	3.54	0.14	25.12	3.65	0.18
E2	0.00	0.00	0.00	12.68	3.23	1.09	11.82	2.82	1.11	8.67	2.06	0.72	16.84	4.04	0.93	29.73	7.14	0.87
E3	0.00	0.00	0.00	16.38	4.36	0.74	14.96	3.64	0.74	10.88	2.54	0.52	19.39	4.43	0.70	30.15	6.67	0.60
E1-3 AVG	0.00	0.00	0.00	14.79	3.78	0.67	14.55	3.46	0.67	12.29	2.44	0.45	19.85	4.00	0.59	28.33	5.82	0.55
E4	0.00	0.00	0.00	18.67	4.57	0.03	18.19	4.12	0.03	10.32	2.44	0.01	26.50	5.85	0.01	67.40	15.36	0.13
E5	0.00	0.00	0.00	18.33	4.79	0.03	17.00	4.11	0.03	6.71	1.60	0.01	21.73	4.93	0.02	33.07	7.35	0.10
E6	0.00	0.00	0.00	18.58	4.77	0.03	18.02	4.48	0.03	13.50	3.34	0.02	21.57	5.05	0.02	32.43	7.16	0.07
E4-6 AVG	0.00	0.00	0.00	18.53	4.71	0.03	17.74	4.24	0.03	10.18	2.46	0.02	23.27	5.28	0.02	44.30	9.95	0.10
F1	0.00	0.00	0.00	3.23	0.71	0.13	2.86	0.61	0.12	1.87	0.41	0.08	4.37	1.04	0.11	19.96	5.33	0.08
F2	0.00	0.00	0.00	15.20	4.09	0.92	14.29	3.80	0.90	11.80	3.18	0.70	16.50	4.33	0.83	40.75	10.79	0.27
F3	0.00	0.00	0.00	18.71	4.99	0.70	16.99	4.47	0.67	12.67	3.36	0.48	22.45	5.75	0.63	40.11	10.04	0.40
F1-3 AVG	0.00	0.00	0.00	12.38	3.26	0.58	11.38	2.96	0.56	8.78	2.31	0.42	14.44	3.71	0.52	33.61	8.72	0.25
F4	0.00	0.00	0.00	18.38	4.67	0.21	18.03	4.50	0.22	11.55	2.92	0.13	19.16	4.72	0.18	26.90	6.71	0.16

F5	0.00	0.00	0.00	19.31	4.96	0.20	18.08	4.58	0.20	12.09	3.07	0.13	21.96	5.41	0.19	23.38	5.87	0.13
F6	0.00	0.00	0.00	14.52	3.86	0.14	17.38	4.51	0.19	11.57	2.91	0.11	20.28	5.11	0.16	30.98	7.82	0.20
F4-6 AVG	0.00	0.00	0.00	17.40	4.50	0.18	17.83	4.53	0.20	11.74	2.97	0.12	20.47	5.08	0.17	27.08	6.80	0.16
G1	98.00	0.00	0.00	92.99	0.05	0.07	90.94	0.06	0.06	70.89	0.06	0.22	110.14	0.06	0.33	91.76	0.12	0.24
G2	98.00	0.00	0.00	93.70	0.06	0.75	97.93	0.06	0.79	63.29	0.05	0.47	108.90	0.06	0.87	88.41	0.08	0.57
G3	98.00	0.00	0.00	0.46	0.06	0.64	4.95	0.08	0.72	71.52	0.04	0.48	105.00	0.29	0.70	87.33	11.16	0.24
G1-3 AVG	98.00	0.00	0.00	62.38	0.06	0.49	64.61	0.07	0.53	68.57	0.05	0.39	108.01	0.14	0.63	89.17	3.79	0.35
G4	98.00	0.00	0.00	90.48	0.07	0.15	98.00	0.10	0.14	69.71	0.29	0.08	104.79	1.05	0.36	82.59	13.34	0.11
G5	98.00	0.00	0.00	91.01	0.19	0.12	96.80	0.07	0.10	68.88	0.94	0.06	105.16	1.39	0.42	90.96	2.36	0.23
G6	98.00	0.00	0.00	91.43	0.39	0.11	98.57	0.07	0.10	71.08	0.05	0.07	106.83	0.09	0.31	91.42	0.18	0.38
G4-6 AVG	98.00	0.00	0.00	90.97	0.22	0.12	97.79	0.08	0.11	69.89	0.42	0.07	105.59	0.84	0.36	88.33	5.29	0.24
H1	98.00	0.00	0.00	89.14	0.06	1.05	96.66	0.08	1.14	55.78	0.06	0.57	107.89	0.06	1.16	100.35	0.24	0.93
H2	98.00	0.00	0.00	94.57	0.07	0.80	98.95	0.06	0.08	63.65	0.04	0.50	107.60	0.08	0.89	60.02	0.06	0.39
H3	98.00	0.00	0.00	96.42	0.06	0.50	99.40	0.06	0.51	65.08	0.68	0.28	108.23	1.45	0.54	82.74	6.71	0.26
H1-3 AVG	98.00	0.00	0.00	93.37	0.06	0.78	98.34	0.07	0.58	61.50	0.26	0.45	107.91	0.53	0.86	81.03	2.34	0.53
H4	98.00	0.00	0.00	94.78	0.06	0.10	98.73	0.06	0.06	64.15	0.06	0.06	107.87	0.05	0.30	97.14	0.07	0.33
H5	98.00	0.00	0.00	.93.268	0.05	0.09	97.83	0.08	0.07	65.01	0.05	0.05	108.77	0.06	0.39	88.68	7.95	0.12
H6	98.00	0.00	0.00	94.20	0.06	0.07	99.18	0.06	0.06	60.27	0.03	0.04	108.87	0.07	0.45	92.51	0.43	0.20
H4-6 AVG	98.00	0.00	0.00	94.49	0.06	0.09	98.58	0.07	0.06	63.15	0.05	0.05	108.50	0.06	0.38	92.78	2.81	0.22

Appendix 3 – Raw GC data (Experiment 3)

				12/18/2015			2/29/2016
		GC0	GC0	GC0	GC1	GC1	GC1
	(Day)	0	0	0	74	74	74
		%N2	%O2	%CH4	%N2	%O2	%CH4
RWJ FARMS 29-2	A						
1: NH4/PO4 Only	A1	95.00	0.00	0.00	99.70	0.00	0.20
2: NH4/PO4 / H2	A2	55.00	0.00	0.00	82.30	0.20	0.02
3: NH4/PO4 / Acetate	A3	95.00	0.00	0.00	78.67	0.03	0.02
4: NH4/PO4 / Methanol	A4	95.00	0.00	0.00	88.45	0.05	0.24
Middleton 9-1	B						
1: NH4/PO4 Only	B1	95.00	0.00	0.00	89.57	0.18	0.27
2: NH4/PO4 / H2	B2	55.00	0.00	0.00	75.74	3.22	8.87
3: NH4/PO4 / Acetate	B3	95.00	0.00	0.00	95.20	0.04	0.34
4: NH4/PO4 / Methanol	B4	95.00	0.00	0.00	76.95	0.04	5.61
Jerry Brant 3-4	C						
1: NH4/PO4 Only	C1	95.00	0.00	0.00	81.75	0.05	0.36
2: NH4/PO4 / H2	C2	55.00	0.00	0.00	71.06	3.31	3.71
3: NH4/PO4 / Acetate	C3	95.00	0.00	0.00	98.32	0.05	0.46
4: NH4/PO4 / Methanol	C4	95.00	0.00	0.00	79.87	4.01	0.00
Theodore Housel 28-1	D						
1: NH4/PO4 Only	D1	95.00	0.00	0.00	85.25	0.04	0.10
2: NH4/PO4 / H2	D2	55.00	0.00	0.00	42.43	0.04	0.16
3: NH4/PO4 / Acetate	D3	95.00	0.00	0.00	97.76	0.17	0.28
4: NH4/PO4 / Methanol	D4	95.00	0.00	0.00	90.53	0.05	4.08
Baugher Trust 1-11	E						
1: NH4/PO4 Only	E1	95.00	0.00	0.00	36.30	0.03	0.09
2: NH4/PO4 / H2	E2	55.00	0.00	0.00	74.16	2.85	2.17
3: NH4/PO4 / Acetate	E3	95.00	0.00	0.00	82.84	0.05	0.17
4: NH4/PO4 / Methanol	E4	95.00	0.00	0.00	81.61	0.05	4.08
Cheyney Land 24-4	F						
1: NH4/PO4 Only	F1	95.00	0.00	0.00	90.15	0.17	0.21
2: NH4/PO4 / H2	F2	55.00	0.00	0.00	65.54	2.25	4.43
3: NH4/PO4 / Acetate	F3	95.00	0.00	0.00	82.52	0.06	0.23
4: NH4/PO4 / Methanol	F4	95.00	0.00	0.00	83.85	0.05	5.50
Nunnenkamp 5-3	G						

1: NH4/PO4 Only	G1	95.00	0.00	0.00	55.14	0.03	0.02
2: NH4/PO4 / H2	G2	55.00	0.00	0.00	67.36	1.96	6.33
3: NH4/PO4 / Acetate	G3	95.00	0.00	0.00	83.15	0.04	0.28
4: NH4/PO4 / Methanol	G4	95.00	0.00	0.00	75.73	0.04	5.47
Joseph Stich 1-1	H						
1: NH4/PO4 Only	H1	95.00	0.00	0.00	83.43	0.02	0.15
2: NH4/PO4 / H2	H2	55.00	0.00	0.00	51.35	0.09	0.13
3: NH4/PO4 / Acetate	H3	95.00	0.00	0.00	84.00	0.06	0.16
4: NH4/PO4 / Methanol	H4	95.00	0.00	0.00	80.01	0.05	1.60
MIH Alexander 18-3	I						
1: NH4/PO4 Only	I1	95.00	0.00	0.00	85.03	0.06	0.21
2: NH4/PO4 / H2	I2	55.00	0.00	0.00	77.22	3.23	2.62
3: NH4/PO4 / Acetate	I3	95.00	0.00	0.00	66.18	0.05	0.27
4: NH4/PO4 / Methanol	I4	95.00	0.00	0.00	77.76	0.04	2.42
Lester Arthur 26-2	J						
1: NH4/PO4 Only	J1	95.00	0.00	0.00	85.18	0.08	0.32
2: NH4/PO4 / H2	J2	55.00	0.00	0.00	74.15	2.82	2.34
3: NH4/PO4 / Acetate	J3	95.00	0.00	0.00	84.53	0.05	0.34
4: NH4/PO4 / Methanol	J4	95.00	0.00	0.00	72.64	1.21	3.00
Triplett 25-2	K						
1: NH4/PO4 Only	K1	95.00	0.00	0.00	92.98	0.12	0.18
2: NH4/PO4 / H2	K2	55.00	0.00	0.00	79.04	0.82	2.79
3: NH4/PO4 / Acetate	K3	95.00	0.00	0.00	109.58	0.05	0.36
4: NH4/PO4 / Methanol	K4	95.00	0.00	0.00	75.24	0.04	3.66
Kepley RA 23-1	L						
1: NH4/PO4 Only	L1	95.00	0.00	0.00	94.61	0.05	0.16
2: NH4/PO4 / H2	L2	55.00	0.00	0.00	77.38	3.66	2.24
3: NH4/PO4 / Acetate	L3	95.00	0.00	0.00	133.90	0.05	0.97
4: NH4/PO4 / Methanol	L4	95.00	0.00	0.00	101.85	0.04	4.02
Ben Hinkle 30-1	M						
1: NH4/PO4 Only	M1	95.00	0.00	0.00	122.55	0.06	0.55
2: NH4/PO4 / H2	M2	55.00	0.00	0.00	73.11	1.88	8.42
3: NH4/PO4 / Acetate	M3	95.00	0.00	0.00	81.69	0.08	0.41
4: NH4/PO4 / Methanol	M4	95.00	0.00	0.00	83.35	1.86	2.52
Stich 2 (From my sampling)	N						
1: NH4/PO4 Only	N1	95.00	0.00	0.00	73.26	0.25	0.65
2: NH4/PO4 / H2	N2	55.00	0.00	0.00	54.26	0.15	0.69

3: NH4/PO4 / Acetate	N3	95.00	0.00	0.00	85.11	0.06	0.85
4: NH4/PO4 / Methanol	N4	95.00	0.00	0.00	83.68	0.06	0.67
Bailey 2-2 (From my sampling)	O						
1: NH4/PO4 Only	O1	95.00	0.00	0.00	86.77	0.05	0.77
2: NH4/PO4 / H2	O2	55.00	0.00	0.00	50.56	0.14	0.45
3: NH4/PO4 / Acetate	O3	95.00	0.00	0.00	77.48	0.05	0.39
4: NH4/PO4 / Methanol	O4	95.00	0.00	0.00	74.62	0.27	0.35

Appendix 4 – Microbial Beta Diversity (phylum - family)

			Experiment 1						Experiment 2						Experiment 3							
	Bailey Well	Stich Well	OB4	OB1	OA4	OA1	OH4	OH1	CA1	CA4	CB1	CB4	CH1	CH4	SB1	SB2	SB3	SB4	SN1	SN2	SN3	SN4
k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];o__WCHD3-30;f__	0.000%	0.000%	0.043%	0.002%	0.038%	0.003%	0.004%	2.204%	0.013%	0.007%	0.020%	0.009%	0.002%	0.019%	0.527%	8.559%	0.971%	1.952%	0.029%	0.057%	0.004%	0.028%
k__Archaea;p__Crenarchaeota;c__MCG;o__;f__	0.000%	0.000%	0.059%	0.000%	0.051%	0.022%	0.021%	0.045%	0.016%	0.031%	0.262%	0.015%	0.012%	0.001%	0.047%	0.017%	0.014%	1.108%	0.225%	0.374%	0.027%	0.204%
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.001%	0.000%	0.003%	0.334%	0.043%	0.021%	0.000%	0.000%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae	0.997%	0.209%	0.001%	0.000%	0.010%	0.002%	0.007%	0.001%	0.003%	0.013%	0.001%	0.004%	0.004%	0.152%	0.004%	0.137%	16.174%	0.321%	0.750%	0.109%	0.004%	0.035%
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__WSA2	0.286%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.029%	0.532%	0.005%	0.001%	0.000%	0.001%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanococci;o__Methanococcales;f__Methanococcaceae	0.028%	0.003%	0.000%	0.000%	0.005%	0.002%	0.000%	0.009%	0.000%	0.000%	0.000%	0.001%	0.001%	0.001%	0.013%	0.013%	0.012%	0.143%	0.008%	0.002%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__	1.796%	1.017%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.001%	0.001%	0.001%	0.001%	0.000%	0.000%	1.260%	0.000%	0.000%	0.001%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanomicrobiaceae	0.000%	0.034%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.016%	0.016%	0.010%	0.000%	0.000%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosaetaceae	0.177%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.003%	0.076%	0.028%	0.001%	0.002%	0.001%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosarcinaceae	0.018%	0.412%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.005%	0.003%	0.002%	0.000%	0.000%	0.000%	0.000%
k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2;f__[Methanomassiliicoccaceae]	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;Other;Other;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.010%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%
k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Deinococcaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.043%	0.010%	0.003%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.001%
k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Ellin6075	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%
k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__mb2424	0.004%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Acidobacteria;c__BPC102;o__B110;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Acidobacteria;c__Holophagae;o__Holophagales;f__Holophagaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.013%	0.000%	0.006%
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__;o__;f__	0.191%	0.019%	0.003%	0.000%	0.000%	0.007%	0.005%	0.012%	0.002%	0.001%	0.074%	0.007%	0.002%	0.002%	0.001%	0.000%	0.001%	0.002%	0.038%	0.187%	0.061%	0.069%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0.000%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.117%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Iamiaceae	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.001%	0.000%	0.046%	0.012%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.007%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Brevibacteriaceae	0.085%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0.233%	0.002%	0.011%	0.002%	0.005%	0.003%	0.001%	0.000%	0.000%	0.012%	0.000%	0.013%	0.000%	0.236%	0.001%	0.000%	0.000%	0.001%	0.001%	0.001%	0.000%	0.000%

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae	1.641%	0.031%	0.190%	0.045%	0.128%	0.028%	0.212%	0.059%	0.716%	0.962%	0.449%	0.143%	0.033%	1.197%	0.151%	0.057%	0.017%	0.047%	0.804%	0.398%	0.046%	0.176%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermabacteraceae	0.035%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.004%	0.000%	0.024%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dietziaceae	0.025%	0.000%	0.001%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae	0.021%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae	0.035%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0.067%	0.003%	0.006%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.007%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0.972%	0.014%	0.021%	0.012%	0.015%	0.012%	0.001%	0.001%	0.009%	0.005%	0.009%	0.012%	0.001%	0.184%	0.000%	0.001%	0.001%	0.001%	0.001%	0.002%	0.001%	0.001%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae	1.521%	0.036%	0.042%	0.011%	0.051%	0.005%	0.009%	0.003%	0.022%	0.151%	0.019%	0.026%	0.004%	0.001%	0.006%	0.004%	0.001%	0.001%	0.001%	0.001%	0.005%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae	0.004%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.026%	0.000%	0.003%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae	0.361%	0.007%	0.007%	0.002%	0.008%	0.007%	0.001%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0.032%	0.000%	0.007%	0.003%	0.000%	0.000%	0.001%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.001%	0.000%	0.000%	0.002%	0.001%	0.000%	0.000%	0.000%	0.005%	0.004%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0.007%	0.000%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.020%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae	0.014%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.011%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__WCHB1-81;f__At425_EubF1	0.067%	0.003%	0.004%	0.002%	0.000%	0.000%	0.003%	0.000%	0.000%	0.006%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.009%	0.001%	0.000%	0.009%	0.010%	0.000%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae	0.103%	0.000%	0.003%	0.000%	0.000%	0.000%	0.006%	0.018%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__OPB41;o__;f__	0.209%	0.019%	0.014%	0.003%	0.051%	0.007%	0.014%	0.006%	0.001%	0.004%	0.007%	0.025%	0.001%	0.221%	0.008%	0.009%	0.005%	0.004%	0.000%	0.032%	0.013%	0.031%
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__;f__	0.021%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	0.050%	0.007%	0.001%	0.003%	0.003%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__	0.021%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.024%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae	0.025%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Armatimonadetes;c__[Fimbriimonadia];o__[Fimbriimonadales];f__[Fimbriimonadaceae]	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0.014%	0.000%	0.000%	0.000%	0.000%	0.000%	0.004%	0.003%	0.000%	0.010%	0.008%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0.301%	0.104%	0.048%	0.000%	0.036%	0.015%	0.372%	0.405%	0.116%	1.036%	0.598%	0.110%	0.187%	0.213%	17.170%	21.592%	12.016%	12.362%	0.350%	0.207%	0.079%	0.128%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae]	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BA008	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.086%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.031%	0.000%	0.000%	0.000%	0.008%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.102%	0.023%	0.006%	0.000%	0.000%	0.000%	0.003%	0.003%	0.001%	0.000%	0.000%	0.000%	0.005%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__RF16	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7	0.099%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1	0.060%	0.000%	0.001%	0.000%	0.003%	0.000%	0.005%	0.005%	0.005%	0.016%	0.003%	0.008%	0.007%	0.004%	0.762%	1.571%	0.279%	1.489%	0.001%	0.011%	0.005%	0.018%
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0.018%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.022%	0.000%	0.031%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.043%	0.000%	0.000%	0.007%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	0.000%	0.005%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.004%	0.000%	0.004%	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae	0.212%	0.007%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__BRC1;c__NPL-UPA2;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.023%	0.014%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__BRC1;c__PRR-11;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.004%	0.025%	0.007%	0.033%	0.000%	0.001%	0.000%	0.000%
k__Bacteria;p__CD12;c__;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.009%	0.000%	0.000%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.010%	0.000%	0.017%	0.010%	0.008%	0.000%	0.000%	0.000%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__[Melioribacteraceae]	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.006%	0.001%	0.014%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__Ignavibacteriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.035%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__IheB3-7	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.005%	0.003%	0.002%	0.003%	0.003%	0.003%	0.001%	0.181%	1.158%	0.035%	0.612%	0.005%	0.004%	0.005%	0.001%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__OTUb7	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.024%	0.021%	0.012%	0.022%	0.012%	0.015%	0.015%	0.014%	1.433%	4.583%	4.157%	4.108%	0.121%	0.028%	0.028%	0.040%
k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.017%	0.003%	0.014%	0.001%	0.001%	0.000%	0.002%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae	10.255%	0.118%	0.297%	0.061%	0.353%	0.075%	0.030%	0.003%	0.004%	0.143%	0.017%	0.084%	0.005%	0.005%	0.574%	1.045%	0.540%	0.689%	0.003%	0.049%	0.006%	0.035%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.076%	0.012%	0.001%	0.008%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__CFB-26;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.001%	0.000%	0.001%	0.000%	0.285%	0.007%	0.001%	0.005%	0.000%	0.000%	0.004%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__envOPS12;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__GCA004;f__	0.000%	0.000%	0.003%	0.002%	0.005%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.022%	0.002%	0.021%	0.000%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__OPB11;f__	0.014%	0.000%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.274%	0.006%	0.001%	0.000%	0.004%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SHA-31	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SHA-20;f__	0.035%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Anaerolineae;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__Dehalococcoidales;f__Dehalococcoidaceae	0.014%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%
k__Bacteria;p__Chloroflexi;c__Dehalococcoidetes;o__GIF9;f__	0.018%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Ellin6529;o__;f__	0.007%	0.000%	0.006%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__MLE1-12;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.004%	0.000%
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__	0.053%	0.003%	0.006%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.057%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__	0.021%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.003%	0.066%	0.057%	0.089%	0.008%	0.003%	0.111%	0.003%	0.003%	0.001%	0.001%	0.000%	0.001%	0.000%	0.007%
k__Bacteria;p__Deferribacteres;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae	0.021%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__	0.004%	0.000%	0.001%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae]	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.006%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	0.311%	0.007%	0.010%	0.009%	0.003%	0.010%	0.006%	0.003%	0.005%	0.048%	0.013%	0.011%	0.001%	0.002%	0.006%	0.004%	0.001%	0.003%	0.129%	0.003%	0.000%	0.017%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Listeriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae	0.071%	0.005%	0.006%	0.002%	0.000%	0.005%	0.002%	0.003%	0.000%	0.025%	0.016%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.009%	0.000%	0.001%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae	4.540%	0.104%	0.077%	0.042%	0.061%	0.076%	0.002%	0.001%	0.000%	0.001%	0.000%	0.007%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.008%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae	3.953%	0.094%	0.161%	0.062%	0.141%	0.061%	0.065%	0.017%	0.574%	1.903%	0.398%	0.192%	0.030%	1.580%	0.093%	0.034%	0.016%	0.028%	0.354%	0.234%	0.035%	0.141%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.004%	0.001%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae	0.004%	0.000%	0.004%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.027%	0.001%	0.000%	0.000%	0.000%	0.002%	0.003%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__	0.011%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae	0.018%	0.000%	0.000%	0.000%	0.000%	0.000%	0.007%	0.035%	0.012%	0.000%	0.013%	0.001%	0.007%	0.001%	0.001%	0.001%	0.000%	0.001%	0.001%	0.000%	0.000%	0.010%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae	4.438%	0.101%	0.064%	0.042%	0.092%	0.058%	0.014%	0.009%	0.000%	0.020%	0.001%	0.031%	0.001%	0.435%	0.002%	0.003%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae	0.354%	0.007%	0.004%	0.002%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.003%	0.006%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.012%	0.002%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae	2.097%	0.048%	0.046%	0.017%	0.038%	0.028%	0.007%	0.001%	0.034%	0.068%	0.013%	0.016%	0.001%	0.356%	0.003%	0.001%	0.002%	0.001%	0.001%	0.022%	0.004%	0.012%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae	0.099%	0.000%	0.001%	0.002%	0.003%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.017%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae	0.244%	0.010%	0.021%	0.011%	0.013%	0.005%	0.022%	0.019%	0.369%	0.137%	0.062%	0.030%	0.004%	0.292%	0.015%	0.009%	0.003%	0.008%	0.068%	0.040%	0.015%	0.059%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;Other	0.011%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.010%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Bacilli;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__	0.244%	0.003%	0.003%	0.000%	0.003%	0.002%	0.037%	0.075%	0.000%	0.001%	0.001%	0.001%	0.003%	0.001%	0.015%	0.233%	0.003%	0.074%	0.000%	0.001%	0.001%	0.003%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae]	1.245%	0.072%	0.018%	0.016%	0.059%	0.018%	0.017%	0.014%	0.013%	0.009%	0.024%	0.012%	0.012%	0.007%	1.649%	5.245%	2.271%	1.108%	0.049%	0.033%	0.024%	0.021%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0.308%	0.003%	0.004%	0.003%	0.010%	0.002%	0.003%	0.025%	0.027%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.019%	0.001%	0.002%	0.001%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae]	5.934%	1.224%	0.140%	0.028%	0.161%	0.065%	0.030%	0.051%	0.145%	0.275%	0.530%	0.072%	0.027%	0.704%	0.112%	4.761%	0.109%	4.824%	0.398%	0.339%	0.072%	0.564%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	0.467%	0.024%	0.010%	0.009%	0.033%	0.008%	0.000%	0.000%	0.023%	0.019%	0.000%	0.006%	0.001%	0.000%	0.000%	0.000%	0.000%	0.019%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0.106%	0.005%	0.006%	0.003%	0.000%	0.000%	0.007%	0.009%	0.000%	0.000%	0.006%	0.004%	0.006%	0.001%	0.309%	0.043%	0.052%	0.009%	0.003%	0.004%	0.001%	0.017%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae	0.025%	0.005%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae	0.240%	0.135%	0.014%	0.002%	0.010%	0.010%	0.000%	0.002%	0.027%	0.000%	0.003%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.017%	0.010%	0.001%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__JTB215	0.018%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0.173%	0.009%	0.001%	0.002%	0.003%	0.003%	0.038%	0.149%	0.002%	0.001%	0.001%	0.001%	0.007%	0.001%	0.041%	0.022%	0.023%	0.031%	0.001%	0.001%	0.001%	0.070%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae	1.531%	0.029%	0.024%	0.017%	0.043%	0.020%	0.007%	0.006%	0.004%	0.004%	0.005%	0.005%	0.006%	0.004%	0.008%	0.065%	0.049%	2.074%	0.030%	0.014%	0.005%	0.008%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0.286%	0.015%	0.006%	0.005%	0.005%	0.002%	0.022%	0.078%	0.000%	0.013%	0.000%	0.006%	0.004%	0.000%	0.001%	0.001%	0.003%	0.000%	0.001%	0.000%	0.000%	0.118%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Sulfobacillaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Syntrophomonadaceae	0.053%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.052%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.002%	0.001%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.065%	0.004%	0.000%	0.001%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other	0.000%	0.002%	0.004%	0.000%	0.000%	0.002%	0.002%	0.001%	0.000%	0.008%	0.001%	0.003%	0.001%	0.001%	0.008%	0.862%	0.059%	0.106%	0.037%	0.008%	0.001%	0.004%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Halanaerobiales;f__syntriaceae	4.307%	16.519%	4.046%	1.830%	5.776%	0.571%	27.565%	9.796%	0.405%	1.652%	0.271%	0.777%	17.335%	0.381%	0.084%	0.092%	0.076%	0.095%	0.418%	0.444%	0.337%	0.921%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Halanaerobiales;f__Halobacteroidaceae	0.053%	0.000%	0.001%	0.000%	0.000%	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.020%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Thermoanaerobacterales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.001%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae	0.035%	0.002%	0.000%	0.003%	0.000%	0.000%	0.006%	0.009%	0.001%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%
k__Bacteria;p__Firmicutes;Other;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.029%	0.000%	0.000%	0.000%
k__Bacteria;p__OP9;c__JS1;o__BA021;f__	0.987%	0.012%	0.007%	0.009%	0.008%	0.012%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%
k__Bacteria;p__OP9;c__JS1;o__SB-45;f__	0.403%	0.015%	0.015%	0.003%	0.020%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.007%	0.000%	0.000%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae	0.255%	0.157%	0.003%	0.005%	0.005%	0.010%	0.001%	0.859%	0.002%	0.000%	0.091%	0.000%	0.031%	0.001%	0.001%	0.003%	0.003%	0.003%	0.003%	0.000%	0.000%	0.001%
k__Bacteria;p__OP3;c__koll11;o__;f__	0.085%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.003%	0.001%	0.002%	0.000%	0.002%	0.000%	0.000%

k__Bacteria;p__OP8;c__OP8_2;o__;f__	0.085%	0.002%	0.003%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Hyd24-12;c__WM88;o__;f__	0.046%	0.002%	0.001%	0.002%	0.000%	0.000%	0.005%	0.003%	0.004%	0.003%	0.004%	0.004%	0.003%	0.002%	0.083%	0.007%	1.005%	1.683%	0.078%	0.008%	0.001%	0.003%
k__Bacteria;p__Gemmatimonadetes;c__Gemm-3;o__;f__	0.028%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__GN02;c__GKS2-174;o__;f__	0.025%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae	0.018%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.032%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__OP8;c__OP8_1;o__;f__	0.011%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__MSBL9;f__	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.092%	0.000%	0.001%	0.000%	0.003%	0.000%	0.010%	0.000%	0.005%	0.030%	0.001%	0.019%	0.000%	0.001%	0.001%	0.000%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.021%	0.015%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.016%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__LCP-89;c__SAW1_B44;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__0319-6A21	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__OD1;c__;o__;f__	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__OP3;c__;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.002%	0.002%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__AKAU3564;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.009%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.019%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Planctomycetes;c__vadinHA49;o__PeHg47;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__;o__;f__	0.028%	0.002%	0.003%	0.000%	0.000%	0.000%	0.002%	0.000%	0.001%	0.001%	0.001%	0.001%	0.003%	0.001%	0.018%	0.092%	0.004%	0.010%	0.000%	0.001%	0.001%	0.002%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0.156%	0.010%	0.018%	0.006%	0.018%	0.003%	0.011%	0.003%	0.084%	0.054%	0.020%	0.017%	0.003%	0.001%	0.002%	0.000%	0.001%	0.001%	0.001%	0.013%	0.004%	0.027%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	0.350%	0.005%	0.003%	0.006%	0.020%	0.003%	0.001%	0.000%	0.001%	0.016%	0.000%	0.002%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.032%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Aurantimonadaceae	0.057%	0.005%	0.003%	0.000%	0.003%	0.005%	0.002%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae	0.007%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae	0.184%	0.003%	0.011%	0.000%	0.020%	0.005%	0.006%	0.003%	0.030%	0.013%	0.013%	0.001%	0.001%	0.001%	0.004%	0.000%	0.001%	0.001%	0.038%	0.018%	0.001%	0.002%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae	1.687%	0.056%	0.060%	0.036%	0.161%	0.033%	0.017%	0.002%	0.001%	0.010%	0.000%	0.089%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Cohaesibacteraceae	0.046%	0.007%	0.035%	0.003%	0.143%	0.012%	1.543%	0.007%	0.087%	0.049%	0.011%	0.011%	0.007%	4.927%	0.008%	0.006%	0.003%	0.008%	0.015%	0.009%	0.016%	0.007%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0.325%	0.003%	0.006%	0.005%	0.023%	0.003%	0.003%	0.001%	0.000%	0.000%	0.008%	0.007%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae	1.531%	0.063%	0.295%	0.026%	0.153%	0.046%	0.127%	0.003%	0.030%	0.043%	0.025%	0.008%	0.001%	0.217%	0.003%	0.006%	0.011%	0.002%	0.091%	0.017%	0.006%	0.002%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	0.053%	0.003%	0.000%	0.002%	0.000%	0.000%	0.002%	0.000%	0.000%	0.009%	0.003%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.012%

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0.407%	0.009%	0.015%	0.006%	0.028%	0.020%	0.000%	0.000%	0.000%	0.000%	0.000%	0.020%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.042%	0.000%	0.000%	0.001%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	0.011%	0.000%	0.000%	0.003%	0.005%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	0.067%	0.000%	0.003%	0.000%	0.005%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.004%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.001%	0.176%	0.001%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	0.039%	0.002%	0.001%	0.002%	0.003%	0.000%	0.000%	0.000%	0.010%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	2.765%	0.807%	0.769%	0.542%	45.957%	0.454%	20.263%	1.263%	6.242%	34.978%	0.851%	12.014%	3.398%	1.574%	5.898%	0.489%	0.181%	0.240%	37.555%	0.301%	1.040%	0.567%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__	0.025%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	0.219%	0.017%	0.007%	0.011%	0.036%	0.010%	0.002%	0.005%	0.043%	0.010%	0.048%	0.004%	0.000%	0.000%	0.001%	0.001%	0.001%	0.001%	0.020%	0.011%	0.000%	0.007%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	1.153%	0.217%	0.034%	0.025%	0.056%	0.045%	0.010%	0.007%	0.072%	0.081%	0.035%	0.013%	0.003%	1.152%	0.008%	0.007%	0.002%	0.006%	0.041%	0.016%	0.007%	0.056%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	0.293%	0.000%	0.007%	0.003%	0.013%	0.007%	0.005%	0.001%	0.045%	0.050%	0.024%	0.012%	0.003%	0.436%	0.001%	0.003%	0.000%	0.000%	0.001%	0.006%	0.002%	0.001%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae	0.248%	0.005%	0.003%	0.003%	0.005%	0.005%	0.001%	0.003%	0.004%	0.013%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.001%	0.003%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	0.032%	0.002%	0.001%	0.002%	0.000%	0.002%	0.002%	0.001%	0.027%	0.001%	0.005%	0.004%	0.001%	0.448%	0.002%	0.000%	0.000%	0.001%	0.061%	0.001%	0.000%	0.001%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae	0.028%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	0.007%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae	0.004%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.000%	0.000%	0.001%	0.002%	0.000%	0.000%	0.000%	0.021%	0.000%	0.000%	0.018%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__	0.000%	0.000%	0.004%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales;f__Hydrogenophilaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae	0.364%	0.012%	0.014%	0.011%	0.013%	0.017%	0.001%	0.001%	0.002%	0.001%	0.001%	0.001%	0.000%	0.002%	0.008%	0.010%	0.019%	0.717%	0.012%	0.002%	0.001%	0.002%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae	0.615%	0.031%	0.036%	0.014%	0.110%	0.033%	0.085%	2.710%	3.176%	0.448%	9.814%	0.044%	5.555%	0.033%	0.373%	0.165%	0.507%	0.114%	0.086%	1.597%	0.546%	1.214%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.004%	0.006%	0.004%	0.005%	0.003%	0.004%	0.004%	0.296%	0.744%	0.233%	0.260%	0.005%	0.002%	0.001%	0.003%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfohalobiaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.038%	0.121%	0.000%	0.001%	0.000%	0.001%	0.002%	0.001%	0.001%	0.000%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfomicrobiaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	4.300%	0.325%	0.538%	0.101%	0.544%	0.109%	0.644%	6.233%	7.069%	3.496%	0.563%	1.593%	13.890%	11.930%	0.365%	0.082%	0.528%	0.261%	0.073%	0.151%	1.314%	0.183%

k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.148%	0.000%	0.001%	0.000%	0.000%	0.015%	0.001%	0.000%	0.001%	0.001%	0.000%	0.000%	0.001%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__	0.018%	0.007%	0.001%	0.000%	0.003%	0.000%	0.799%	0.001%	1.177%	0.016%	0.001%	0.004%	0.004%	0.005%	0.009%	0.005%	0.014%	0.003%	0.002%	0.004%	0.007%	0.005%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae	0.141%	0.243%	1.132%	0.115%	0.662%	0.166%	1.231%	0.062%	0.096%	0.046%	0.067%	0.064%	0.040%	0.083%	1.858%	23.376%	2.125%	1.553%	0.105%	0.063%	0.068%	0.081%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae	0.014%	0.044%	0.018%	0.019%	0.008%	0.008%	0.000%	0.000%	0.002%	0.013%	0.004%	0.001%	0.002%	0.000%	0.007%	0.017%	0.008%	0.003%	0.001%	0.000%	0.004%	0.001%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae	7.373%	71.726%	30.679%	17.320%	5.554%	37.325%	0.761%	2.527%	10.219%	0.587%	13.155%	7.378%	1.124%	21.558%	12.489%	7.563%	17.101%	1.617%	5.373%	0.623%	6.811%	21.823%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;Other	0.064%	0.027%	0.011%	0.033%	0.010%	0.020%	0.001%	0.006%	0.003%	0.000%	0.002%	0.000%	0.002%	0.003%	0.005%	0.028%	0.031%	0.009%	0.001%	0.000%	0.002%	0.002%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__GW-28;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.015%	0.041%	0.001%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0.004%	0.000%	0.003%	0.000%	0.000%	0.002%	0.000%	0.000%	0.005%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NKB15;f__	0.131%	0.002%	0.004%	0.002%	0.003%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Sva0853;f__JTB36	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.036%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae	1.124%	0.046%	0.027%	0.034%	0.015%	0.031%	0.005%	0.007%	0.008%	0.004%	0.003%	0.008%	0.005%	0.003%	0.041%	1.068%	0.465%	1.276%	0.003%	0.007%	0.006%	0.004%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.001%	0.003%	0.002%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae	3.158%	4.744%	59.071%	78.311%	37.542%	59.760%	33.529%	56.490%	67.423%	51.203%	70.336%	75.644%	48.328%	20.630%	50.127%	4.248%	14.399%	29.662%	50.281%	93.536%	88.484%	71.498%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.002%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__;f__	0.004%	0.002%	0.000%	0.002%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.008%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae	0.081%	0.003%	0.003%	0.002%	0.003%	0.005%	0.001%	0.000%	0.002%	0.001%	0.001%	0.001%	0.001%	0.002%	0.000%	0.001%	0.310%	0.000%	0.033%	0.001%	0.001%	0.001%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae	0.011%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	0.884%	0.027%	0.042%	0.019%	0.054%	0.022%	0.003%	0.001%	0.023%	0.029%	0.006%	0.022%	0.007%	2.427%	0.006%	0.002%	0.000%	0.001%	0.009%	0.006%	0.001%	0.005%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae	0.004%	0.002%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae	0.007%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoracaceae	0.011%	0.010%	0.007%	0.003%	0.003%	0.002%	0.013%	0.004%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Endozoicimonaceae	0.028%	0.002%	0.015%	0.002%	0.005%	0.000%	0.555%	0.001%	0.003%	0.002%	0.001%	0.001%	0.001%	0.002%	0.003%	0.005%	0.001%	0.001%	0.000%	0.003%	0.002%	0.002%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0.067%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae	0.039%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae	0.032%	0.003%	0.000%	0.000%	0.003%	0.000%	0.010%	0.001%	0.000%	0.000%	0.010%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae	0.714%	0.050%	0.099%	0.045%	0.169%	0.030%	0.131%	0.060%	0.145%	0.138%	0.057%	0.029%	0.004%	0.517%	0.008%	0.004%	0.003%	0.017%	0.230%	0.048%	0.009%	0.039%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	3.197%	0.087%	0.076%	0.081%	0.153%	0.070%	0.015%	0.009%	0.121%	0.181%	0.065%	0.024%	0.006%	1.408%	0.257%	0.006%	0.021%	0.005%	0.180%	0.036%	0.017%	0.055%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae	0.050%	0.002%	0.000%	0.002%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	0.159%	0.007%	0.004%	0.003%	0.005%	0.003%	0.027%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.000%	0.001%	0.000%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	6.506%	0.121%	0.110%	0.085%	0.199%	0.093%	0.006%	0.003%	0.021%	0.051%	0.010%	0.026%	0.003%	1.349%	0.003%	0.002%	0.001%	0.001%	0.059%	0.004%	0.001%	0.002%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Proteobacteria;Other;Other;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.002%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%
k__Bacteria;p__SAR406;c__AB16;o__;f__	0.071%	0.000%	0.003%	0.000%	0.003%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__SAR406;c__AB16;o__noFP_H7;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.006%	0.007%	0.008%	0.004%	0.017%	0.005%	0.006%	0.004%	0.050%	2.564%	0.191%	2.049%	0.008%	0.017%	0.007%	0.019%
k__Bacteria;p__Spirochaetes;c__[Leptospirae];o__[Leptospirales];f__Sediment-4	0.347%	0.009%	0.007%	0.002%	0.003%	0.007%	0.001%	0.000%	0.000%	0.000%	0.002%	0.001%	0.001%	0.001%	0.011%	0.219%	0.059%	0.092%	0.001%	0.001%	0.000%	0.001%
k__Bacteria;p__Spirochaetes;c__MVP-15;o__PL-11B10;f__	0.060%	0.000%	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.001%	0.000%	0.002%	0.001%	0.000%	0.228%	0.005%	0.030%	0.139%	0.000%	0.000%	0.002%	0.001%
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Sphaerochaetales;f__Sphaerochaetaceae	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.008%	0.001%	0.001%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	0.011%	0.000%	0.000%	0.000%	0.000%	0.000%	0.038%	0.020%	0.034%	0.016%	0.025%	0.034%	0.033%	0.032%	3.009%	3.307%	20.249%	5.241%	0.048%	0.061%	0.026%	0.037%
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Aminiphilaceae	2.468%	0.091%	0.133%	0.166%	0.092%	0.065%	0.038%	1.426%	0.045%	0.026%	0.207%	0.016%	1.529%	0.005%	0.013%	0.129%	0.141%	0.107%	0.028%	0.012%	0.152%	0.119%
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Dethiosulfovibrionaceae	1.011%	0.150%	0.239%	0.224%	0.268%	0.146%	10.727%	11.483%	0.572%	0.825%	0.770%	0.869%	6.356%	23.121%	0.047%	0.151%	0.076%	0.382%	0.355%	0.421%	0.202%	0.318%
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Thermovirgaceae	2.118%	0.101%	0.445%	0.291%	0.475%	0.098%	0.302%	2.675%	0.021%	0.037%	0.304%	0.041%	1.318%	0.018%	0.024%	0.039%	0.009%	0.071%	0.300%	0.020%	0.292%	0.754%
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;Other	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.004%	0.004%	0.007%	0.003%	0.008%	0.009%	0.003%	0.473%	0.008%	1.823%	1.644%	0.065%	0.009%	0.005%	0.006%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.013%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Thermotogae;c__Thermotogae;o__Thermotogales;f__Thermotogaceae	0.099%	0.000%	0.000%	0.000%	0.003%	0.003%	0.002%	0.008%	0.002%	0.004%	0.006%	0.006%	0.004%	0.007%	0.017%	2.297%	0.084%	1.508%	0.061%	0.013%	0.010%	0.007%
k__Bacteria;p__TM7;c__TM7-1;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae]	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Puniceicoccales;f__Puniceicoccaceae	0.011%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__Verrucomicrobia;c__Verruco-5;o__MSBL3;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.005%	0.017%	0.002%	0.001%	0.001%	0.002%	0.010%	0.002%	0.000%	0.001%	0.000%	0.000%	0.005%	0.003%	0.000%	0.004%
k__Bacteria;p__WPS-2;c__;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__WS1;c__;o__;f__	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.008%	0.000%	0.000%	0.000%	0.000%

k__Bacteria;p__WS3;c__PRR-12;o__GN03;f__	0.021%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__WS3;c__PRR-12;o__PBS-III-9;f__	0.032%	0.003%	0.000%	0.000%	0.000%	0.002%	0.000%	0.000%	0.001%	0.000%	0.001%	0.000%	0.000%	0.001%	0.001%	0.002%	0.037%	0.161%	0.000%	0.000%	0.000%	0.000%
k__Bacteria;p__WS5;c__;o__;f__	0.000%	0.003%	0.000%	0.000%	0.000%	0.000%	0.001%	0.003%	0.000%	0.056%	0.000%	0.002%	0.000%	0.000%	0.000%	0.005%	0.000%	0.000%	0.000%	0.008%	0.002%	0.001%
k__Bacteria;p__WWE1;c__[Cloacamonae];o__[Cloacamonales];f__MSBL8	0.467%	0.005%	0.004%	0.003%	0.005%	0.003%	0.015%	0.022%	0.018%	0.015%	0.016%	0.010%	0.016%	0.015%	0.043%	1.280%	0.150%	18.661%	0.283%	0.061%	0.021%	0.061%
Unassigned;Other;Other;Other;Other	1.124%	0.214%	0.492%	0.151%	0.181%	0.146%	0.276%	0.565%	0.312%	0.491%	0.268%	0.182%	0.516%	1.641%	0.438%	0.528%	1.760%	1.016%	0.382%	0.175%	0.092%	0.149%

Appendix 5 – Cherokee basin well data

	Spud Date	Completion Date	GL Elev. (ft)	GL Elev. m	Total Depth (ft)	depth m	bottom elevation m msl	County	Section	Township	Range	Latitude	Longitude	TDS
DATA FROM KIRK. ET AL. 2015														
Ben Hinkle 30-1	5/17/2004	6/1/2004	840	256.0	808	246.3	9.8	Labette	30	32S	19E	37.2363692	-95.3589479	57815.9
Theodore Housel 28-1	6/15/2004	6/28/2004	946	288.3	1137	346.6	-58.2	Montgomery	28	31S	17E	37.3230323	-95.5442702	83276.4
Baugher Trust 1-11	6/6/2004	6/29/2004	914	278.6	836	254.8	23.8	Labette	11	31S	19E	37.3600367	-95.2965286	48878.2
Middleton 9-1	1/22/2004	1/29/2004	1036	315.8	1162	354.2	-38.4	Neosho	9	30S	18E	37.4551268	-95.4356287	58715
Nunnenkamp 5-3	12/13/2006	12/18/2006	821	250.2	1193	363.6	-113.4	Wilson	5	29S	16E	37.5553595	-95.6824155	98227.1
Triplett 25-2	8/19/2008	8/26/2008	982	299.3	1105	336.8	-37.5	Neosho	25	29S	18E	37.4917915	-95.3891812	51571.7
RWJ Farms 29-2	4/18/2008	4/23/2008	932	284.1	1296	395.0	-110.9	Wilson	29	28S	16E	37.5788816	-95.6766792	68341.7
Lester Arthur 26-2	4/11/2007	4/19/2007	1051	320.3	1273	388.0	-67.7	Neosho	26	28S	17E	37.5822550	-95.5230407	41447.4
Jerry Brant 3-4	8/20/2008	8/27/2008	960	292.6	1115	339.9	-47.2	Neosho	3	29S	18E	37.5508170	-95.4229279	70431.3
William Stich 1-1	1/19/2005	2/10/2005	958	292.0	1014	309.1	-17.1	Neosho	1	29S	18E	37.5492966	-95.3813417	n/a
Joseph Stich 8-1	11/22/2004	12/3/2004	966	294.4	979	298.4	-4.0	Neosho	8	29S	19E	37.5346976	-95.3549924	35600.6
MIH Alexander 18-3	11/27/2007	12/4/2007	966	294.4	997	303.9	-9.4	Neosho	18	27S	20E	37.6906333	-95.2541504	42884.2
King Farms 5-1	1/4/2008	1/10/2008	904	275.5	994	303.0	-27.4	Neosho	5	28S	19E	37.6360933	-95.3495515	39936.6
Teleconnect Inc. 23-1	9/10/2007	9/27/2007	900	274.3	913	278.3	-4.0	Neosho	23	28S	19E	37.5950501	-95.2947392	n/a
Kepley RA 23-1	1/26/2006	2/9/2006	985	300.2	1212	369.4	-69.2	Neosho	23	28S	17E	37.5966104	-95.5093611	41681.6
Cheyney Land 24-4	8/26/2008	9/2/2008	945	288.0	1090	332.2	-44.2	Neosho	24	28S	18E	37.5886442	-95.3961138	58393.2
NEW DATA														
Marion Bailey 2-2	2/21/2002	6/1/2002	924	281.6	1065	324.6	-43.0	Neosho	2	29S	18E	37.5551577	-95.4119878	56250.2
William Stich 1-2	2/15/2008	2/26/2008	951	289.9	1043	317.9	-28.0	Neosho	1	29S	18E	37.5463718	-95.3855583	72458.1

Appendix 6 – Cherokee basin water chemistry

	Sample Date	Gas (psi)	pH	Temp °C	Temp K	Conductivity (mS)	Alk (CaCO3) mg/L	HCO3 mg/L	Cl mg/L	Br mg/L	SO4 mg/L	Mg mg/L	Ca mg/L	K mg/L	Na mg/L	Fe mg/L
DATA FROM KIRK. ET AL. 2015																
Ben Hinkle 30-1	11/13/13	15	7.15	17.7	290.85	49.9	284.30	346.50	35710.00	172.00	4.44	1013.00	1200.00	68.00	17650.00	83.90
Theodore Housel 28-1	11/13/13	4	6.75	17.5	290.65	66.2	361.30	440.50	48790.00	292.00	10.72	1912.00	1336.00	118.00	23620.00	30.70
Baugher Trust 1-11	11/13/13	31	6.98	17.5	290.65	56.5	192.70	234.90	28840.00	125.00	5.16	785.00	966.00	73.00	14920.00	12.20
Middleton 9-1	11/13/13	12	6.73	19.3	292.45	57.9	266.90	325.20	35930.00	116.00	4.98	795.00	1835.00	152.00	17830.00	21.40
Nunnenkamp 5-3	11/12/13	20	6.55	17.2	290.35	66.6	176.70	215.40	58540.00	268.00	0.67	1830.00	2345.00	123.00	28050.00	16.50
Triplett 25-2	11/12/13	10	6.81	20.7	293.85	59.9	260.20	317.30	29900.00	103.00	4.03	649.00	1492.00	116.00	15560.00	6.60
RWJ Farms 29-2	11/12/13	12	7.01	25.3	298.45	75.7	197.20	240.40	39890.00	132.00	3.87	675.00	1591.00	184.00	20790.00	3.10
Lester Arthur 26-2	11/12/13	6					249.70	304.40	24310.00	120.00	3.16	501.00	468.00	65.00	13200.00	8.40
Jerry Brant 3-4	11/12/13	7	6.78	15.2	288.35	79.6	312.80	381.30	44080.00	174.00	5.34	1240.00	1724.00	95.00	20860.00	36.50
William Stich 1-1	11/12/13															
Joseph Stich 8-1	11/11/13	6	6.76	28.2	301.35	46.4	425.20	512.50	21300.00	72.00	5.14	346.00	707.00	93.00	11900.00	0.40
MIH Alexander 18-3	11/11/13	< 1	7.54	17	290.15	55.8	237.20	289.20	26220.00	113.00	6.86	695.00	866.00	80.00	13670.00	1.70
King Farms 5-1	11/11/13	< 1	7.55	17.7	290.85	51.8	176.20	214.80	23440.00	95.00	4.97	486.00	837.00	79.00	12670.00	3.30
Teleconnect Inc. 23-1	11/11/13															
Kepley RA 23-1	11/11/13	5	7.39	17.6	290.75	54.3	215.50	263.00	24240.00	102.00	3.00	523.00	844.00	79.00	13110.00	24.40
Cheyney Land 24-4	11/11/13	8	7.1	16.2	289.35	70.8	166.70	203.20	35680.00	151.00	4.66	859.00	1466.00	99.00	18260.00	62.60
NEW DATA																
Marion Bailey 2-2	2/12/2015	n/a	6.67	15.5	288.65	51.2	133.20	162.40	33058.01	108.16	1.50	1283.00	1415.90	190.00	19898.00	n/a
William Stich 1-2	2/5/2015	n/a	7.45	16.9	290.05	59.4	248.10	302.40	43785.00	129.41	1.20	850.00	1008.00	297.00	25837.00	n/a

Appendix 7 – DNA concentrations (NanoDrop)

REAL ID	Exp. 1 Description	Conc. (ng/µl)	260/280	260/230
1-OA1	Stich (post-reactor): FW + oil	0.83	1.33	0.14
Stich	Stich well site (pre-reactor): FW	0.44	0.31	0.51
1-OB1	Stich (post-reactor): FW	0.65	0.61	0.24
1-OH1	Stich (post-reactor): FW + waste	2.93	1.61	0.28
1-OA4	Bailey (post-reactor): FW + oil	-0.09	0.49	-0.04
Bailey	Bailey well-site (pre-reactor): FW	0.44	2.00	0.10
1-OB4	Bailey (post-reactor): FW	2.66	1.08	0.67
1-OH4	Bailey (post-reactor): FW + waste	2.77	1.24	0.31

REAL ID	Exp. 2 Description	Conc. (ng/µl)	260/280	260/230
2-CA1	Stich II (post-reactor): FW + coal	6.42	1.31	0.60
2-CB1	Stich II (post-reactor): FW	4.75	1.65	0.29
2-CH1	Stich II (post-reactor): FW + waste	5.43	1.20	0.57
2-CA4	Bailey (post-reactor): FW + coal	7.62	1.52	0.38
2-CB4	Bailey (post-reactor): FW	5.41	1.50	0.37
2-CH4	Bailey (post-reactor): FW + waste	3.89	1.55	0.41

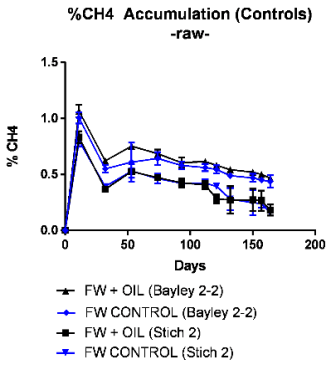
REAL ID	Exp. 3 Description	Conc. (ng/µl)	260/280	260/230
3-SB1	Middleton 9-1 (Control)	6.42	1.15	0.56
3-SB2	Middleton 9-1 (Acetate)	4.86	1.33	0.46
3-SB3	Middleton 9-1 (Methanol)	6.33	1.65	0.57
3-SB4	Middleton 9-1 (H2)	5.96	1.49	0.48
3-SN1	Stich (Control)	4.72	2.09	0.31
3-SN2	Stich (Acetate)	3.97	0.75	0.47
3-SN3	Stich (Methanol)	7.12	1.36	0.61
3-SN4	Stich (H2)	3.93	1.79	0.41

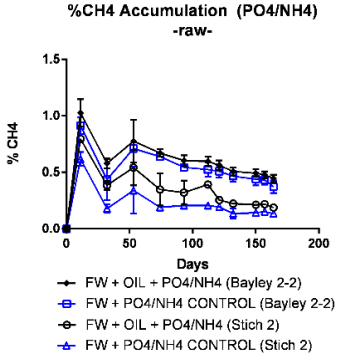
Appendix 8 – Additional resources

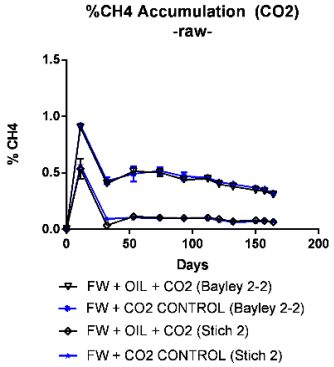
Raw Temporal variation of headspace CH_4 (exp. 1&2)

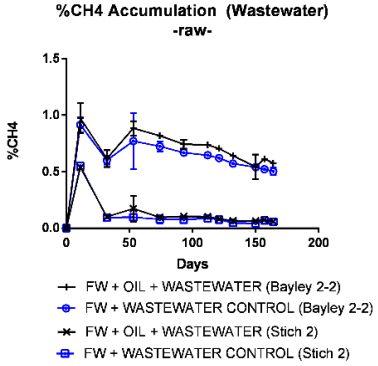
Temporal variation of methane in the headspace of cultures at the end of the incubation for Experiment 1 (left) and Experiment 2 (right). For both charts, the blue line indicates the relative treatment or FW control. Standard deviation from mean is plotted. These data points were un adjusted.

Oil-based









Coal-based