Biogeochemistry of the Antrim Shale Natural Gas Reservoir

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Abstract

The Antrim Shale, located in the Michigan basin, United States (U.S.), is a major U.S. shale play having produced over 2.5 Trillion Cubic Feet (Tcf) of unconventional shale natural gas as of 2010. The shallow nature of this formation sets it apart from other, more characterized unconventional shale gas plays. The depth of gas production of the Antrim ranges from approximately 150-600 meters and it is typically vertically drilled, contrary to deeper, horizontally drilled shales. A thorough understanding of the biogeochemistry and microbiology of this complex system will be advantageous for improving well performance, produced water management, and potential biocidal treatment as microbial community composition can vary substantially even amongst closely spaced wells. In this study, we analyzed produced water collected from nine different wells in the Antrim Shale by investigating the geochemical and microbial community composition of the produced water to gain greater insight into the overall biogeochemistry of this unique shale system. The majority of the wells from this study had high total dissolved solids (TDS) primarily composed of chloride and sodium, averaging 86,804 mg/L with a maximum 116,223 mg/L; however, three of the wells sampled along the northern margin of the basin exhibited significantly lower TDS ranging from 4,932-6,496 mg/L. Our microbial community analysis revealed relatively low abundance within our samples and high variability of the microbial community among the sampled wells. The majority of bacterial sequences were identified within *Proteobacteria*, *Firmicutes*, and *Actinobacteria* phyla and metagenomic sequencing revealed the low presence of *Methanobacteriaceae* within each sample. We also investigated potential microbial community drivers and found that TDS, sodium, chloride, iodide, bromide, ammonium, potassium, and strontium were significantly correlated with the observed microbial community. The varying geochemical conditions between wells demonstrates different subsurface environmental niches, potentially driving the heterogenous microbial communities we observed from well to well. This analysis suggests an important relationship between both well location and geochemistry and the observed microbial community that can persist in the

reservoir. Continued studies of the Antrim Shale will improve our understanding of the complex interdependencies of this ecosystem.

Keywords: Antrim Shale, unconventional natural gas reservoir, hydraulic fracturing, produced water microbiology, biogeochemistry

Introduction

Natural gas is the fastest growing fossil fuel worldwide with global consumption rising 2% from 2018 to 2019.[1] This fuel source is more environmentally sustainable and advantageous for mitigating climate change compared to other fossil fuels such as coal, as it produces significantly less toxic gases and yields lower CO_2 emissions for equivalent power generation.[2–4] Natural gas currently accounts for approximately 23% of global power generation with unconventional shale gas driving this growth and is subsequently projected to account for 30% of the world's natural gas production by 2040.[1,5] Much of these unconventional natural gas resources are harvested through hydraulic fracturing of organic-rich, low permeability shale formations.

During the hydraulic fracturing process, fluids, sand, and chemicals are injected at high pressure into bedrock formations to enhance formation permeability and increase natural gas production. These practices generate billions of gallons of 'produced water' across the entire shale play, composed of flowback water from the fracturing fluid as well as water that is released from the shale formation.[6] Produced water typically contains salts, metals, hydrocarbons, radionuclides, and various organic compounds, the composition of which varies both between and within shale plays.[6,7] Although microorganisms encounter extreme physicochemical conditions in shale reservoirs such as high salinity and pressure, variable temperatures, and the presence of chemical additives, certain extremophile microbes can also accumulate in well reservoirs and produced water.[8,9] Microorganisms present in produced water influence chemical processes that potentially compromise infrastructure and may result in water and waste management issues, increased production costs, and decreased well productivity.[7,10–12] Biocides are frequently used to minimize the growth of biofilms and other inhibitions brought on by microbial processes, however low diversity microbial populations persist.[6,13]

Several studies using 16S rRNA gene sequencing have characterized the microbiology of produced water from major shale plays in the United States. Studies investigating the Marcellus (Pennsylvania, United States), Barnett (Texas, United States) and Bakken (Nebraska, United States) Shales have identified sulfate reducing bacterial families including *Halanaerobiaceae* and *Desulfuromonadaceae,* and *Desulfovibrionaceae*.[14–17] These bacterial families are capable of biogenic sulfide production that can lead to microbially induced corrosion (MIC), which promotes deterioration and compromises the structural integrity of the well infrastructure.[14] Microbial ecology studies of the Marcellus and Barnett Shales produced and flowback water also identified *Pseudomonadaceae*, a bacterial family known for biofilm formation that can lead to clogging and decreased gas flow and extraction.[18,19] Optimal resource recovery from shale plays requires an understanding of the biogeochemical, microbial, and physical interdependencies of these complex systems to help mitigate deleterious microbial activity.

The Antrim Shale represents an important source of unconventional natural gas. This shale is located in the Michigan Basin (Midwest, United States) and extends approximately 39,000 square miles with the majority of the hydraulically fractured shale in Otsego and Montmorency counties located on Michigan's Lower Peninsula.[20] This organic-rich shale is approximately 380 million years old from the Devonian age and overlies the Traverse Formation.[21,22] The Antrim Shale has been one of the most actively drilled shales in the United States since the 1980s containing over 12,000 gas wells in the northern basin.[23] This shale play is also a source of CO_2 for Enhanced Oil Recovery (EOR) in the Northern Pinnacle Reef Trend.[24] Despite being an important source of unconventional natural gas deposits, the biogeochemistry of the Antrim Shale remains relatively uncharacterized. This Devonian aged shale is a stratigraphically shallower and less thick shale occurring at depths rarely exceeding 600 meters with wells that produce significant amounts of formation water that must be pumped out before gas production.[25] These shallow wells are predominantly vertically drilled in contrast

to deeper, horizontally drilled shales such as the Bakken or Marcellus Shales. Although parallels can be drawn between the Antrim Shale and other gas shale formations, the disparate characteristics of this shallow shale presents a unique environment with potential implications for natural gas extraction and biocidal treatments. Understanding the biogeochemistry and microbiology of Antrim Shale produced water can help optimize gas recovery strategies for this and similar hydrogeological systems.

A limited number of studies have investigated the geochemical and microbiology of the Antrim Shale, focusing on microbial gas production and methanogenic activity in the sedimentary basin.[4,26–30] Martini et al. 1996 and Martini et al. 2008 investigated geochemical and isotopic data collected from gas, water, and core materials of the northern margin of the Michigan basin that provided evidence that the majority of Antrim Shale gas is likely of biogenic origin.[26,31] This study proposed that there is substantial biological activity in the Antrim subsurface in which bacteria metabolize the organic compounds present to produce hydrogen and carbon dioxide that is then reduced by methanogens to produce methane.[26,27] A subsequent study performed by Waldron et al. 2007 collected pore water from wells in the methane producing zone of the Antrim Shale to investigate the relationship between archaeal diversity and salinity via enrichment cultures for which inoculum from each well was exposed to a salinity range of 0 to 4,000 mM Cl^-.[29] The results of this study suggests that the low diversity of methanogens that persist in the Antrim subsurface are constrained by the varying salinities of formation waters in the northern producing trend and that distinct methanogenic communities have evolved driven by the varying Cl- concentrations. Clone libraries constructed using 16S rRNA sequences isolated from two gas wells and archaeal phylotypes from Euryarchaeota were found to be most closely related to *Methanosarcinales* and *Methanomicrobiales* orders including previously identified halophilic methanogenic species.[29] Beyond sole examination of archaeal diversity within the Antrim Shale, very few studies have investigated the holistic prokaryotic communities of formation water of drilled Antrim wells.[4,30] In these previous studies, the

microbial community was examined using both cloning and sequencing analysis and the results demonstrated that the Antrim Shale formation water is dominated by *Proteobacteria*, *Fimicutes*, and *Bacteroidetes* with a high presence of anaerobic fermenting bacteria as well as sulfur-, sulfate-, and nitrate-reducing bacteria. The majority of archaeal diversity identified in these studies belonged to Euryarchaeota, and methanogenic microorganisms were detected in both studies. Well water chemistry was also analyzed in both studies; the pH of the Antrim wells assessed were near-neutral to mildly acidic and alkalinity ranged from 8.4 to 47.8 mM; TDS values for the sampled wells were not provided in these studies.[4,30] It is important to build upon these previous studies as microbial community composition and geochemistry can differ greatly due to geospatial variance, local differences in reservoir quality, hydraulic fracturing workflow, and well age. The limited studies of the Antrim Shale highlight the need for additional investigation to expand upon the geochemical profile of the shale coupled with a greater understanding of the microbiome of this region.[32,33]

The overall goal of this study was to shed light on the important microbial life and subsurface biogeochemistry of the shallow Antrim Shale by analyzing produced water samples from 9 wells in the northern region of the Michigan basin. We characterized the microbial community using qPCR, 16S rRNA sequencing, and shotgun metagenomic sequencing. We further characterized the geochemical parameters of the water including pH, alkalinity, TDS, and major ions to identify potential microbial community drivers of natural gas wells. This information will be used to better understand reservoir biogeochemistry and the potential implications of these processes for commercial infrastructure, gas production, and environmental impacts.

Materials and Methods

Sample Collection and Processing

Produced water was collected from 9 gas wells located in the hydraulically fractured Antrim Shale in Otsego and Montmorency County, MI in September 2019. Samples were frozen, transported to the lab, and stored at −20°C upon arrival until further analysis.[34] Produced water samples were then thawed and filtered through a 0.22 µm Millipore membrane (Millipore Inc.) for microbial community analysis and flow through was utilized for geochemical analysis.

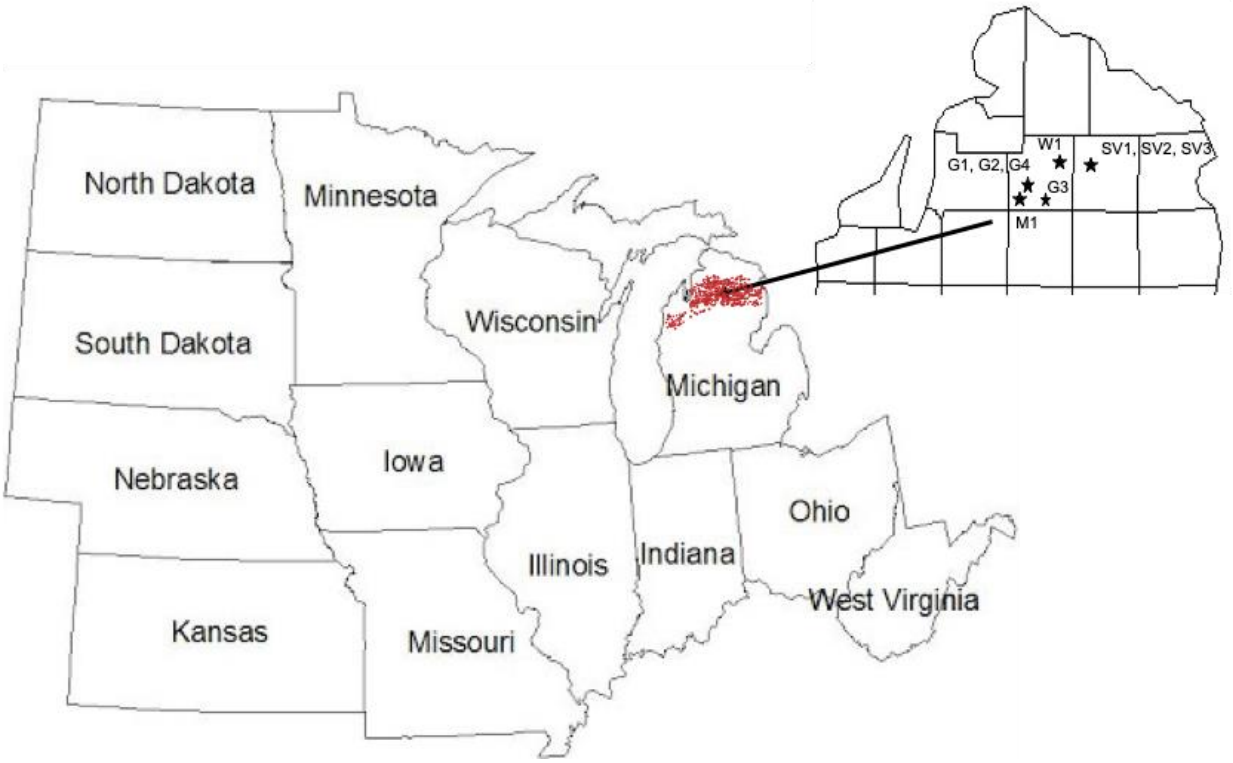

Figure 1. Map of the midwestern United States showing the general locations of the wells sampled for Antrim Shale produced water. Our sampled well locations in Northern Michigan are delineated with black stars and the red dots show the relative expansion of the Northern Michigan Antrim gas wells; location does not indicate the relative size or exact location of the reservoirs. Map is not drawn to scale.

Table 1. Well locations and descriptions.

Well ID	Location	Well Depth (m)	Production Type	Well Status	Well Age (yrs)
SV1	Montmorency	1636	Gas	Active	N/A
SV2	Montmorency	410	Gas	Active	28
SV3	Montmorency	421	Gas	Active	28
G1	Otsego	616	Gas	Active	30
G2	Otsego	614	Gas	Active	30
G3	Otsego	618	Gas	Active	30
G4	Otsego	631	Gas	Active	30
W1	Otsego	365-376	Gas	Shut-In	30
M1	Otsego	610	Gas	Active	26

Chemical Analyses

Produced water was measured for pH and titrated for alkalinity using a Hach digital titrator (Hach, Loveland, CO, United States). 15 mL volume of the filtered flow through was additionally syringe filtered using a 0.22 mm sterile polyethersulfone filter (Millipore, Inc.) and stored at 4 °C for ion chromatography (IC). Major cations were measured on a ThermoFisher (ThermoFisher, Waltham, MA) Dionex Integrion HPIC and anions were detected using a ThermoFisher (Thermofisher, Waltham, MA) ICS-5000+. An AS11-HC column was used to detect major anion, organic acids, and thiosulfate quantifications and a CS16 column for cation quantification. Calibration curves were created using serial dilutions from Dionex cation and anion IC standard stock solutions (Dionex, Thermofisher, Waltham, MA) freshly prepared within a week. Organic acids and thiosulfate calibration curves (Sigma Aldrich, St Louis, USA) were prepared and measured on IC the same day of sample measurement to prevent potential biodegradation or oxidation of standards. All samples were run in triplicate and the standard error of IC measurements was less than 3%. Additionally, every 10–20 samples, a cation or an anion and an organic acid control sample (Sigma Aldrich, St Louis, USA) with known certified concentrations was added during measurements and all control samples demonstrated an

accuracy within 95–105%. Total dissolved solid (TDS) concentrations were determined based on measured cation and anion concentrations (SI Table 1 and 2).

DNA Extraction and 16S rRNA Library Preparation and Sequencing

500 mL of produced water sample was thawed and filtered through a 0.22 μm Millipore membrane (Millipore) for initial processing. Collected biomass was digested with 10 mL of 20 mg/mL lysozyme for 1 hour at 37°C followed by DNA extraction using Qiagen DNeasy Powersoil kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A negative extraction control of 0.22 μm filtered Nanopure H_2O was also included. DNA was quantified using a Qubit dsDNA High Sensitivity Assay (Life Technologies, Carlsbad, CA). Samples from three wells were not sequenced at all as they failed DNA extraction or yielded low sequence counts due to low biomass. DNA was amplified using universal primers targeting the V4 region of the 16S rRNA gene as described by Caporaso et al. (2012) with the addition of Bovine Serum Albumin (BSA) (final concentration of 0.08 mg/mL) for enzyme stabilization and increased PCR yields. Negative controls were included for each PCR reaction. Following amplification, 16S rRNA gene PCR products were purified using AMPure beads clean up and quantified using the Qubit dsDNA High Sensitivity Assay Kit (Life Technologies, Carlsbad, CA) then visualized using an Agilent 2100 Bioanalyzer using the High Sensitivity DNA Kit (Agilent, Santa Clara, CA). All PCR negative controls and kit extraction controls were amplified and visualized. PCR negative controls had no amplification and the single extraction blank that contained amplicons was sequenced with our 16S rRNA library. Purified PCR products were pooled and diluted to a concentration of 2 nM. Diluted samples were then denatured using fresh 0.2 N sodium hydroxide for 5 min at room temperature and further diluted to 10 pMol library with hybridization buffer HT1 according to manufacturer's instructions (Illumina, San Diego, CA). The 10 pMol

library was spiked with 5% of 12.5 pMol PhiX control and sequenced using a 300 cycle V2 Nano kit on an Illumina MiSeq (Illumina, San Diego, CA).

Metagenome Library Preparation and Sequencing

Four Antrim samples yielded sufficient DNA for metagenomic library preparation using the Nextera XT library preparation kit (Illumina, San Diego, CA). Briefly, 1 ng of input sample DNA was tagmented with Illumina primers containing sequencing adapters and barcodes in a 12 cycle PCR step. PCR products were cleaned up using AMPure XP beads (Life Technologies, Carlsbad, CA). DNA libraries were normalized via pooling followed by quantification using Qubit (Life Technologies, Carlsbad, CA) and diluted to a concentration of 20-40 pM. The DNA library was then heated at 96^{o}C for 2 minutes to denature the DNA, cooled in an ice bath for 5 minutes, and sequenced using a 600 cycle V3 kit on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

Quantitative PCR

The microbial abundance for produced water collected from the Antrim Shale was determined using a Magnetic Induction Cycler (MIC) (Bio Molecular Systems, Upper Coomera, Australia) with 16S rRNA gene primers designed by Maeda et al. 2003.[35] Briefly, MIC qPCR reactions were run in triplicate with 1 μL of template DNA, 2X Bioline SensiFAST SYBR No-Rox master mix per sample (Boline, London, UK), 8.2 μL Molecular biology-grade H_2O per sample, and 0.5 μL reverse 16S rRNA gene primer and 0.5 μL forward 16S rRNA gene primer (400 nM concentration). Standard curves were generated using gBlocks Gene Fragments (Integrated DNA Technologies, Coralville, IA) and negative control samples were included in each amplification assay (SI Figure 1).

16S rRNA Data Analysis

16S rRNA gene single-end sequences were analyzed using Quantitative Insights into Microbial Ecology (QIIME) 2 pipeline version 2019.4.[36] Sequences were imported using EMPSingleEndSequences and demultiplexed using demux emp-single command. Sequences were then denoised and quality trimmed using the Deblur denoising software package wrapped in QIIME2. Reads were trimmed to 120 bps due to decreasing quality scores of bases at the end of the reads. A total of 45,274 16S rRNA gene sequences remained after quality control filtering. Amplicon sequence variants (ASVs) appearing in the extraction control were removed from all samples to minimize prevalence of contaminating amplicons. Q2-diversity plug-in was used to assess alpha diversity metrics including number of sequences, number of OTUs, Chao1, and Shannon indices (Table 3). Taxonomy was assigned using a pre-trained Naïve Bayes classifier and the q2-feature-classifier plugin that was trained on the Greengenes 13_8 99% OTUs.[25]

Quality controlled and normalized 16S rRNA was imported into R for further analysis.[37] The Vegan[38] package was used to both calculate Bray-Curtis dissimilarity distances for the Antrim Shale sequence library which was then utilized to perform a Mantel test to identify significant geochemical parameters. Vegan[38] was also used to generate a non-metric multidimensional scaling (NMDS) analysis.[38] Environmental parameters were fit onto the ordination plot using envfit in order to identify the strength of the correlation between geochemical parameters and the ordination. The statistical significance of the Mantel test and the environmental vectors was based on 999 permutations of the grouping, geochemical, and environmental data, respectively.

Metagenomic Data Analysis

Metagenomic reads were imported into kbase.us to access the quality of the raw sequence reads using FastQC.[39] Metagenomic reads were quality trimmed using the Trimmamatic application provided through kbase.us using the Total Sequence Quality provided by FastQC

report. Taxonomy of metagenomic reads was assessed using Kaiju and compared to the 16S rRNA taxonomy classifications.[40]

Results

Geochemistry of Produced Water from the Antrim Shale

Geochemical analysis of all Antrim Shale samples was performed to measure major ions in the Antrim Shale produced water, the range of pH and alkalinity, and identify potential geochemical microbial community drivers. Produced water pH was generally circumneutral, ranging from 6.4 to 7.9 (Table 2). The average values are within range of those reported for produced water analyzed from the Barnett, Bakken and Marcellus Shales.[9,16,17,41] Alkalinity had a wide range from 124 to 3120 mg/L with an average alkalinity of 974 mg/L and a median of 232 mg/L across all samples (Table 2).

The TDS for these samples ranged from 4,931 to 116,224 mg/L. It is noteworthy that the SV wells located in Montmorency county had TDS measurements significantly lower than the median of 86,018 mg/L. The majority of the TDS was represented by high concentrations of chloride and sodium (Table 2; SI Table 1 and 2), which is typical of produced water from hydraulic fracturing.[29] SV2 and SV3 wells had a lower TDS than is often reported for shale produced waters.[14,17,19,42]

Table 2. Geochemical measurements of Antrim Shale produced water samples and statistically significant ions associated with the observed microbial community.

Well ID	pH	Alkalinity (mg/L)	TDS (mg/L)	Cl^-	Br^-	I^-	Na	NH_4^+	K	Mg	Sr
SV1	7.5	2086	6,492	3591.4	5.88	0.1	2827.6	9.8	13.0	825	1.2
SV2	7.9	3120	5,638	2952.0	4.81	0.1	2602.9	7.1	11.8	28.11	1.3
SV3	7.7	2340	4,931	2622.1	4.45	0.1	2227.4	8.2	9.8	23.48	1.3
G1	6.8	213	22,265	13923.1	39.73	0.3	7671.4	8.7	39.1	402	25.2
G2	6.4	143	114,087	71312.2	258.47	1.2	35176	83.8	232.9	417	43.1
G3	7.0	124	94,755	59645.6	187.2	1.1	32277	33.9	196.7	953	38.7
G4	6.6	324	86,018	54385.0	158.48	0.9	29824	65.2	179.6	397	34.6
M1	6.7	179	116,224	73445.8	197.29	1.1	39432	31.0	220.7	376	46.7
W1	7.2	232	87,547	54859.6	133.12	1.0	31466	64.0	177.5	23.9	36.7

Microbial Abundance and Diversity of Produced Water

Microbial abundance of the Antrim region produced water samples was determined via qPCR. The microbial load for two of the six sequenced Antrim Shale samples were lower than the DNA extraction control and thus were not considered in our analyses. Remaining samples were at least 2.5 $\log_{10}$ 16S rRNA gene copies/mL above extraction control. Microbial abundance in the remaining four samples ranged from 10^7 to 10^{12} 16S rRNA gene copies/mL (Table 3; SI Figure 1). We assessed the microbial diversity of the collected water samples by calculating the number of sequences, richness, and diversity within each sample based on the ASV table obtained by 16S rRNA amplicon sequencing. The Chao1 index ranged between 14 and 81 and the Shannon diversity index ranged between 1.02 and 5.16 (Table 3). These alpha diversity values are similar to those observed in recent publications investigating produced water from shale-gas formations including the Bakken and Marcellus Shales.[10,16,17,43] SV1 and G2 wells showed the highest microbial diversity among all analyzed Antrim wells. SV2 had both the lowest measurements for diversity and number of ASVs (Table 3).

Table 3. Alpha Diversity results for Antrim Shale produced water determined by 16S rRNA sequencing.

Well ID	Number of sequences	Number of ASVs	Richness[R]	Diversity[D]	16S rRNA gene copies/mL
SV1	5648	32	81	4.51	12.25
SV2	1133	8	14	1.02	*
SV3	3389	12	22	2.02	11.16
G1	8873	24	51	3.87	7.33
G2	24456	44	80	5.16	*
W1	1775	9	14	2.55	10.88

* denotes samples that had concentrations lower than the DNA extraction control. [R]Chao 1 Index, [D]Shannon Diversity.

Microbial Community Composition of Produced Water

16S rRNA sequencing revealed a unique microbial community structure for each well with *Proteobacteria, Firmicutes*, *Actinobacteria,* and *Bacteroidetes* being the dominant phyla (Figure 2). The high variation observed among evaluated wells could be a potential result of distinctive drilling practices, fracturing histories, different fracking fluids, and unique reservoir biogeochemical properties. Our characterization of the Antrim Shale microbiome revealed a plethora of putative functions summarized in Table 4 such as fermentation, sulfate reduction, and methane production. ASVs within the strict anaerobic family *Syntrophaceae* were detected in all samples and overall the most prevalent ASV identified, accounting for almost 6% of all observed ASVs and 31.5%, 18.5%, and 12% of ASVs observed in W1, SV3, and G1 respectively. *Rhodocyclaceae* was the most prevalent ASV within a single sample, accounting for approximately 73.5% of the microbial community composition of the SV2 well. SV3 was strongly dominated by *Hydrogenphaga*, an aerobic hydrogen-oxidizer. The microaerophilic sulfur-degrader, *Sulfurospirillum,* was detected in several wells with the greatest abundance in W1. 16S rRNA sequencing identified methanogenic archaea in four of our wells, SV1, SV2, SV3, and G1 with ASVs most similar to the hydrogenotrophic methanogen, *Methanobacterium.*

G2 and SV1 displayed more diverse microbial community structures, with G2 having the greatest relative abundance of unique taxa amongst all sampled wells. The heterogeneity between wells suggests minimal well to well communication.

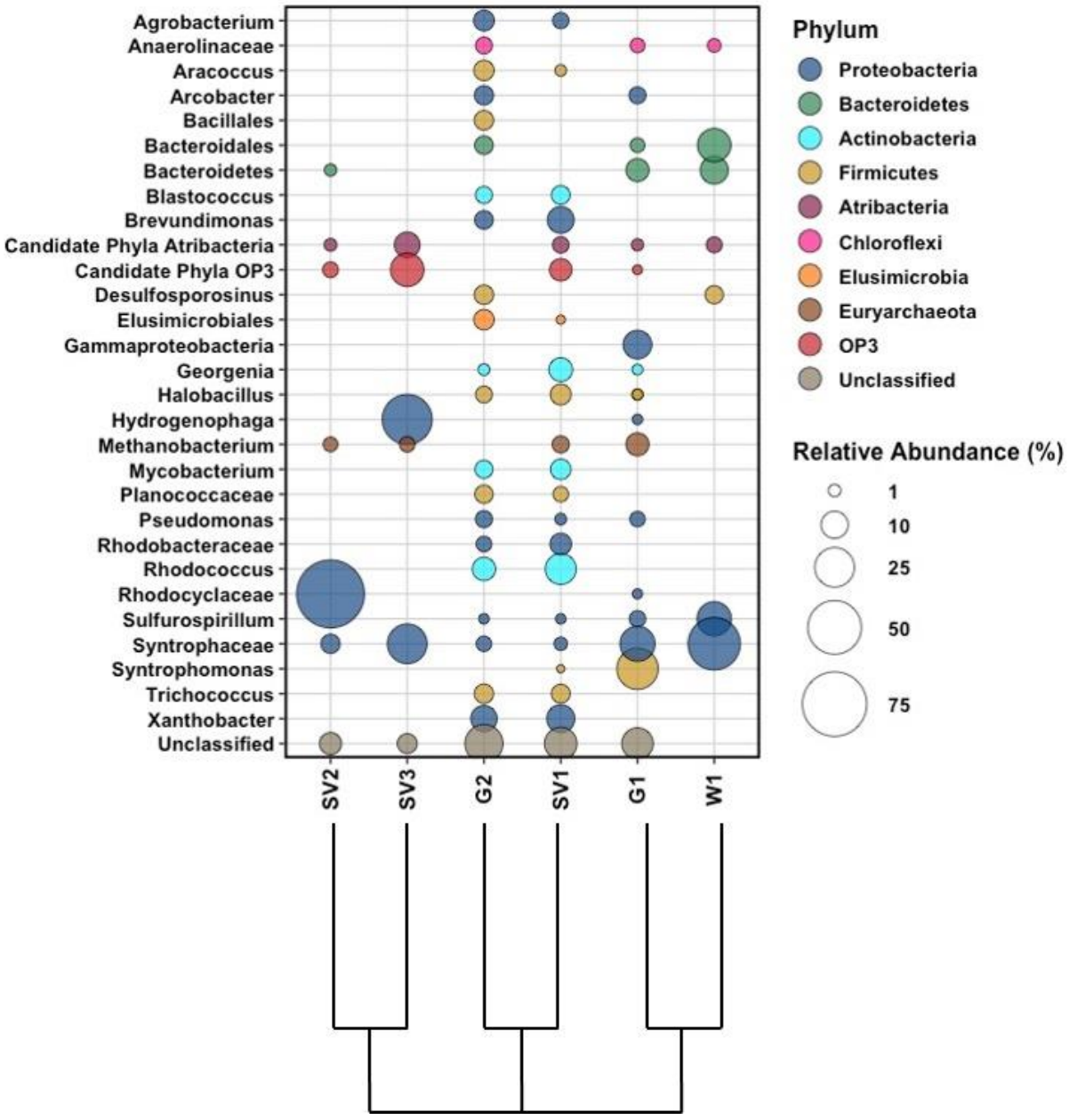

Figure 2. Bubble plot showing the relative abundance of the top 30 most abundant ASVs found across 6 produced water sampling sites with a beta-phylogenetic dendrogram displaying microbial community similarity. Bubble size correlates to relative abundance of the taxonomic family in the specific sample. Color shading of bubbles correlates to the phylum. Groups containing less than 100 ASVs were classified as “Other” and are not shown in the above plot.

Table 4. Putative functions of dominant microbial taxa in Antrim Shale wells.

Well	% of ASVs	Closest Identified Genus	Putative Function	Metabolism	Citations
W1	12.0%	*Sulfurospirillum*	Sulfur reducer	Microaerophilic	Goris and Diekert, 2016
G2	7.0%	*Xanthobacter*	Phenol Degrader	Aerobic	Wiegel, 2006
G1	17.3%	*Syntrophonmonas*	Fermenter	Anaerobic	Kuever, et al. 2014
G1	4.1%	*Methanobacterium*	Hydrogenotrophic methanogen	Anaerobic	Boone, 2015
SV3	31.2%	*Hydrogenophaga*	Hydrogen-oxidizing	Aerobic	Willems and Gillis, 2015
SV3	18.4%	*Syntrophaceae*	Sulfate-reducers, Fermenter	Anaerobic	Kuever, et al. 2014
SV2	73.5%	*Rhodocyclaceae*	(possible) nitrogen fixer	Aerobic	Oren, 2014
SV1	9.1%	*Rhodococcus*	Aromatics degrader	Aerobic	Alvarez, 2010

Our metagenomic analysis confirmed the taxonomic profile observed with 16S rRNA sequencing (Figure 3). The hydrogenotrophic methanogen, *Methanobacterium*, dominated the G1 well microbial community when assessed by 16S rRNA sequencing and was also identified in all four of the metagenomic samples. Hydrogenotrophic methanogens have been characterized in a wide array of subsurface environments including oil and gas fields.[44–46] The identification of methane-producing archaea in our samples further highlights the main role these archaea are playing in biogenic methane production of the Antrim Shale.

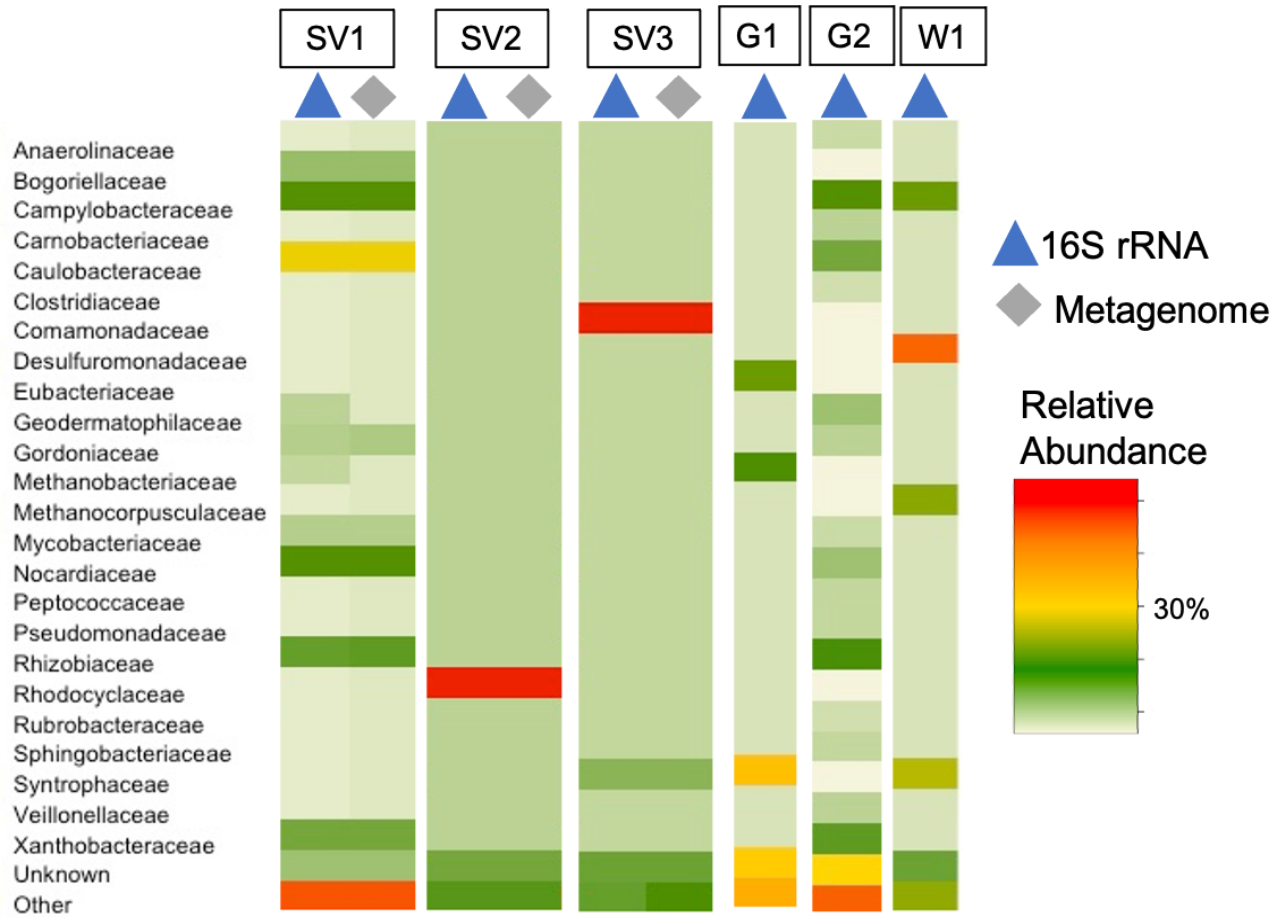

Figure 3. Heatmap comparing the relative abundances of the microbial orders in all experimental samples based on 16S rRNA amplicon sequencing and metagenomic sequence data.

To explore the relationship between the microbial community composition of each well and the observed geochemistry we utilized the vegan package in R to construct a non-metric multidimensional scaling (NMDS) plot of produced water samples with a stress value of 0.15.[38] This plot was constructed using Bray-Curtis distance calculated after rarifying sequence libraries. We observed a statistically significant relationship ($p \leq 0.05$) between the microbial community composition and the TDS and the following anions: chloride, bromide, and iodide and the following cations: sodium, ammonium, potassium, magnesium, and strontium (SI Figure

2; Table 2). There was no statistical significance observed for pH nor alkalinity. We also performed a Mantel Test using Bray-Curtis dissimilarity distances however, no statistically significant relationship was observed between the geochemistry and microbial ecology of individual wells. An analysis of similarities (ANOSIM) was performed to assess a potential correlation between well depth and well age and the observed microbial community; however, no significant correlation was found.

Discussion

Well Geochemistry is Influenced by Shallow Depths and Proximity to the Basin Center

Salinity and total dissolved solids, which can drive microbial community composition, were influenced by both the shallowness of the shale and location. The Antrim Shale is a shallow basin with the majority of our sampled wells being less than 650 meters deep (Table 1). The Antrim Shale spans approximately 400-600 km wide and dips 5-6 km deep towards the center of the Lower Peninsula of the state of Michigan.[47] Waldron et al. 2007 described the sharp salinity gradients of the Antrim Shale explaining that the gradients are more dilute along the margins of the basin and become more saline at the center.[29] We observed the phenomenon as wells G1, G2, G3, G4, and M1 are closer to the basin center and all have higher overall TDS; in addition, M1 had the highest overall TDS and was closest to the basin center (Figure 1; Table 2). The dominance of chloride and sodium detected in our samples are the key ions contributing to the higher overall TDS observed in the more centrally located wells. Prior studies investigating the geochemistry of this shale attributed the high salinity in part to the low fluid migration and diffusion within the Antrim Shale formation waters likely a result of low porosity within the sedimentary basin matrix.[29] SV1, SV2, and SV3 wells are located along the northern margin of the basin (Figure 1). Martini et al. 1996 previously used isotopic data of gas extracted from the Antrim Shale to show that methane production is greatest in the northern margin of the basin, supporting the theory that inorganic carbon derived from remineralization of organic

matter could be a reason for the elevated levels of alkalinity we observed in the SV1, SV2, and SV3 produced water samples.[27]

Produced Water Shows Unique Well-to-Well Microbial Composition with Important Implications for Shale Microbiology

Each of our produced water samples displayed a unique microbial community with different bacterial and archaeal families dominating and little evidence of well to well communication, contrary to what has been reported for other shale plays such as the Bakken.[17] The lack of interaction between wells could be due to the low porosity of the sedimentary basin and variation of reservoir quality with unique rock composition and fluid chemistry. Additionally, because these wells are shallow and vertically drilled, the wellbore only penetrates perpendicular to the surface compared to horizontally drilled wells that are developed off the vertical borehole with greater penetration into the geologic rock formation. The limited permeability of the shale coupled with reduced transfer from anthropogenically created fractures could have resulted in isolated evolution of microbial communities within the wells despite similar geospatial locations. Differing well history may also contribute to the inconsistent microbiology observed among these wells as variation in well injection fluids and production strategies can influence the microbial communities of each well.

Although much is still unknown about microbial communities in shale gas formations, several of the dominant microbial community members identified in our samples have been previously associated with environmental samples collected from natural gas reservoirs.[48,49] A variety of metabolisms ranging from aerobic, microaerophilic, and anaerobic were observed among the dominant families in these samples. Although the presence of aerobes may be surprising in a subsurface environment known to be mostly anoxic, several studies have identified aerobic metabolisms in natural gas geological environments. Species such as *Hydrogenophaga* have been found in relatively high abundance in typically anoxic subsurface

environments such as potential carbon storage sites.[50] This could suggest the presence of a low oxygen niche at an anoxic-oxic transition zone.[51,52]

Prior studies investigating the produced waters of the Antrim Shale reported samples dominated by *Proteobacteria, Firmicutes,* and *Bacteroidetes* with orders including *Pseudomonadales, Burkholderiales, Desulfovibrionales,* and *Campylobacterales* .[4,28,30] *Proteobacteria* were detected in all of our samples with 16S rRNA sequence data. However, *Bacteroidetes* and *Firmicutes* were only detected in G2, which had the greatest Shannon diversity and number of sequences among our samples (Table 3). The high species diversity found in G2 could potentially be explained by the high concentration of potential electron donors such as ammonium and bromide, and electron acceptors such as sulfate in this well. *Sulfurospirillum*, a microaerophilic sulfur reducer, was the dominant genus detected in the W1 well, and also detected in the G2 and SV1 wells.[53] Incubation experiments of Antrim Shale well waters with several fermentative and methanogenic substrates performed by Wuchter et al. 2013 observed 16S rRNA sequences similar to *Sulfurospirillum sp.*[30] These sulfur-cycling bacteria are responsible for sulfide production in other aqueous environments which can have important implication for metal cycling in the shale subsurface as well as potential contributors to well souring.[54]

Metagenomic sequencing revealed that two archaeal families, *Methanobacteriaceae* and *Methanocorpusculaceae,* classified as hydrogenotrophic methanogens, dominated G1 and W1 wells, respectively. Methanogenic archaea were detected at relatively low abundance (<1%) in all other metagenomic samples similar to what was observed in the 16S rRNA amplicon sequencing. This is consistent with other methanogenic communities in shales such as the Marcellus Shale in which methanogens often only account for <1-2% of the overall microbial community.[30,55] Early studies of the Antrim Shale by Martini et al. 1996 examined chemical and isotopic compositions of both gas and produced water and determined that microbial methanogenesis was responsible for the majority of methane in the shale.[27] Waldron et al. 2007

investigated the types of archaea that thrive in the saline Antrim subsurface and identified phylotypes similar to the hydrogenotrophic methanogen *Methanoplanus* in Antrim formation water and found that the largest number of methanogens in their enrichment cultures could utilize H_2 as an electron donor.[29] Similarly, Kirk et al. 2012 used archaeal-specific clone libraries to detect CO_2-reducing methanogens.[4] The high abundance of *Methanobacteriaceae* in G1 and low abundance detection in all other wells support the hypothesis by Waldron et al. 2007 that halotolerant hydrogenotrophic methanogens in the methane-producing zone are the main mode of methane production in this shale.

Study Implications and Limitations

The Antrim Shale is a top producing shale gas reservoir in the United States; however, the biogeochemistry of this system remains relatively uncharacterized. Understanding the produced water microbiology and geochemistry of this reservoir can aid in optimization and improvement of natural gas yields and overall understanding of oil and gas reservoir biogeochemistry. These findings highlight the unique microbial community and geochemistry of the Antrim Shale shallow basin. Our research contributes to the limited number of studies investigating the microbiology of the Antrim Shale and incorporates coupled geochemical and microbial datasets allowing for a comprehensive analysis of the subsurface biogeochemical cycling within the shale reservoir. In this work we characterized the microbial ecology and geochemistry from the largest number of Antrim Shale wells in a single study to date therefore elucidating important aspects of the microbial community structure and biogeochemical dynamics within this subsurface system. Microbial activity can have deleterious effects on shale gas recovery such as microbially induced corrosion (MIC), well souring, and biofilm formation leading to pore plugging and also prevent successful recycling of produced water.[56,57] Understanding the biological and geochemical characteristics of produced water will help predict and avert possible disruption to shale gas extraction by improving biocide treatment and future produced water management.

A primary objective of this study was to investigate the types of microbes present in the Antrim Shale reservoir and the diversity of the microbial communities within this system. Previous studies investigating produced waters from shale gas reservoirs have found that wells appear to be dominated by a single or relatively few taxonomic units. For example, studies using 16S rRNA sequencing found that samples from the Bakken Shale were heavily dominated by *Halanaerobiales, Bacillales,* and *Pseudomonadales* and studies investigating the Marcellus Shale and Permian Basin elucidated that a majority of the 16S rRNA gene sequences were classified within the order *Halanaerobiales*.[14,16,17,56] However, our taxonomic analysis of produced water revealed that there is substantial local variation among the microbial community across the nine samples with a variety of metabolic capabilities. This suggests that there is little to no inter-well communication. In addition, although there are geochemical similarities between wells, the microbiology remains disparate even among closely drilled wells especially on a more refined taxonomic level. This observation could be due to the shallow nature of the wells or varying hydraulic fracturing techniques; however, we did not recover enough DNA for 16S rRNA analysis from a subset of our samples. Further investigation of the physicochemical properties and the microbiology of wells across the Antrim Shale would be necessary to confirm potential drivers of the variability observed in our samples. These findings are a direct result of the analysis of produced water generated in the Antrim Shale, a unique unconventional natural gas reservoir with its own drilling operational platform, and therefore cannot be all used for direct interpretation of the biogeochemistry of other drilled oil and gas regions. However, these observations do demonstrate that a variety of microorganisms are able to survive in the harsh conditions of produced water (chemical treatments, presence of hydrocarbons, high TDS) beyond the well-studied anaerobic, halophilic genus *Halanaerobium* and contributes to a more comprehensive understanding of low abundance microbial communities in extreme hydrocarbon environments.

Beyond the study reported here, additional work, including additional sampling resolution, must be completed in order to better understand the complex biogeochemistry of hydraulically fractured wells. It is also important to continue the study of produced water in this region to further determine if factors such as seasonality, proximity to the basin center, or other unknown mechanistic and environmental parameters may influence the microbial community composition in the Michigan Basin.

Conclusions

This study investigated the biogeochemistry of produced water collected from nine wells in the Antrim Shale gas reservoir. The observed geochemistry and microbiology of this shale gas region possesses certain similarities to those reported for other unconventional natural gas plays such as high concentrations of chloride and sodium and TDS as a key driver of the microbial community; however, there appears to be little well to well communication amongst geospatially close wells.[17,43] This work contributes to the growing understanding of produced water microbiology in the engineered deep biosphere and reservoir biogeochemistry that is essential for understanding and improving natural gas recovery. Our comprehensive approach here provides insight into the microbial ecology of produced water and the associated geochemical parameters within the Antrim Shale well reservoirs.

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