Variation in groundwater geochemistry and microbial communities in the High Plains aquifer system, south-central Kansas

by

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Abstract

Groundwater from the High Plains aquifer is vital for food production and a growing human population in the Great Plains region of the United States. Understanding how groundwater quality is changing in response to anthropogenic and natural processes is critical to effectively managing this resource. Our study considers variation in groundwater geochemistry in the Great Bend Prairie aquifer, a portion of the High Plains aquifer in southcentral Kansas. We collected samples during summer 2016 from 24 monitoring wells and compared our results to data collected previously from the same wells from 1979 to 1987. We sampled 13 wells screened in the upper portion of the aquifer (avg. depth 72 ft), 10 wells screened near the aquifer base (avg. depth 141 ft), and one well screened in underlying bedrock. Compared to initial samples, samples we collected tended to have higher total dissolved solids (TDS) and nitrate content, particularly those we collected from the upper aquifer. Compared to initial samples, TDS was 78 mg/L higher in samples we collected from the upper aquifer and 373 mg/L lower in samples we collected from the aquifer base on average. Nitrate exceeded the U.S. standard for public supplies of drinking water (10 mg/L as N) in seven of the samples we collected, compared to only two samples collected previously. Compared to previous samples, nitrate concentrations were 9.5 and 3.9 mg/L as N higher on average in samples collected from the upper aquifer and aquifer base, respectively. Based on a mixing analysis, variation in the salinity of our samples primarily reflects the dilution of natural Permian brines by freshwater recharge throughout the area. However, salinity decreases observed in four samples reflects flushing of initial oil brine contamination over time, salinity increases in two samples may be due to evapotranspiration, and salinity increases in two samples may reflect migration of oil-brine contamination towards the site. Stable nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios in our samples primarily fall

within the range typical of nitrification of ammonium-based fertilizers with potential contributions from manure or sewage. In our analysis of the microbial community, we observed groups capable of denitrification, including genera within *Nitrospirae, Firmicutes,* and *Proteobacteria*. Despite their presence, our results demonstrate that water quality in the aquifer has degraded over the past 30 to 40 years due to nitrate accumulation.

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Chapter 1 - Introduction

Groundwater from the High Plains aquifer is vital for food production and a growing human population in the Great Plains region of the United States. Groundwater from the High Plains aquifer is used to grow over a quarter of the Nation's agricultural products (Gurdak et al., 2009). In addition, the High Plains aquifer provides drinking water to over 2.3 million people, a majority of whom live in rural areas (Gurdak et al., 2009). In 2000, approximately 97% of total withdrawals from the High Plains aquifer were used for irrigation and 2% were used for public water supply (Smidt et al., 2016; Maupin and Barber, 2005). A small percentage of withdrawals for drinking water accounts for about 82% of the population living within the boundaries of the High Plains aquifer (Gurdak et al., 2009).

In this agriculturally dominated region, agricultural activities have the potential to alter surface and groundwater quality. Common agrarian practices like the application of animal wastes or pesticides and fertilizers can change concentrations of dissolved constituents including chloride, nitrate, and hydrogen ions (pH) (Bohlke, 2002). Run-off of excess nutrients from fertilizers or animal waste can also cause eutrophication of surface waters, causing algal blooms and hypoxia. Some agricultural pesticides and fertilizers can affect trace element mobility. For example, previous researchers have found evidence that agricultural nitrate contamination can mobilize uranium and selenium (Nolan and Weber, 2015; Gates et al., 2009).

Hydrocarbon production also covers an extensive portion of the Great Plains region (Blondes et al., 2017). Oil and gas extraction produces large quantities of formation waters that commonly contain high concentrations of dissolved salts, trace metals or radionuclides (USGS, 2017). Currently, the typical disposal technique is subsurface injection to maintain the pressure of producing reservoirs and enhance recovery (USGS, 2017). In the past, this produced water

was intentionally or accidentally discharged on the surface, or left in unlined pits to evaporate. These practices caused salt scarring and surface and groundwater contamination in many locations (USGS, 2017).

The ultimate impact on groundwater quality from agricultural activities and hydrocarbon production depends in part on the biogeochemical reactions of microorganisms in response to perturbed conditions in the aquifer. Microorganisms have some of the most diverse biochemical metabolisms on Earth and are capable of aerobic and anaerobic respiration of oxygen, sulfur, nitrogen, carbon dioxide, and metals (e.g. iron and magnesium). Microbial communities can increase or decrease the amount of nitrate through nitrification or denitrification reactions. Microbes can also increase the mobility of hazardous trace elements through oxidative dissolution of trace element bearing minerals (Nolan, 2015). Understanding how microorganisms respond to environmental factors is vital for developing useful bioremediation strategies and sustainable farming practices that can be applied to similar areas.

In this study, we examined variation in groundwater geochemistry within the Big Bend Groundwater Management District No. 5 (GMD 5) in a portion of the Central High Plains aquifer in south-central Kansas. Our goals were to identify how concentrations of dissolved constituents have changed over time and how they may affect microbial communities. We collected and compared our geochemical data with data from Whittemore (1993) to determine any changes in groundwater parameters. We analyzed the composition of microbial communities in our study area and compared microbial community composition to groundwater geochemistry. When groundwater movement is relatively slow and natural attenuation rates are limited, groundwater quality could be negatively impacted for decades or millennia (Gurdak et al., 2009). Understanding how groundwater quality in the High Plains aquifer changes in response to

natural and anthropogenic activities is important for societal health, the sustainability of the agricultural industry, and conservation of this limited resource.

Chapter 2 - Geologic Setting

The High Plains aquifer is approximately 450,000 km^2 and underlies eight states from South Dakota to Texas. The High Plains aquifer can be divided into the Northern High Plains, the Central High Plains and the Southern High Plains aquifers (KGS, 2012). Many refer to the High Plains aquifer as the Ogallala aquifer, mainly because the Ogallala Formation is the largest geologic unit underlying the High Plains region. However, other hydraulically connected units in the High Plains aquifer include the Brule Formation, the Arikaree Group, and overlying Quaternary sediments (Fig. 2.1).

The Central High Plains aquifer is approximately 79,000 km^2 and covers most of the western half of Kansas. This portion is further divided into sub-regional aquifer systems that include the Ogallala aquifer, and the Great Bend Prairie aquifer and Equus Beds in south-central Kansas. GMD 5 covers most of the Great Bend Prairie aquifer (Fig. 2.2).

System	Series	Geologic Unit	Thickness (ft.)
QUATERNARY	Pleistocene and Holocene	Valley-fill deposits	0 to 60
		Dune sand	0 to 300
		Loess	0 to 250
	Pleistocene	Unconsolidated alluvial deposits	0 to 550
TERTIARY	Miocene	Ogallala Formation	0 to 700
		Arikaree Group	0 to 1,000
	Oligocene	White River Group: Brule Formation	0 to 700
		White River Group: Chadron Formation	
CRETACEOUS	Upper	Undifferentiated rocks	0 to 8,000
	Lower	Undifferentiated rocks	0 to 700
JURASSIC	Middle and Upper	Undifferentiated rocks	0 to 600
TRIASSIC	Upper	Dockup Group	0 to 2,000
PERMIAN	Lower and Upper	Undifferentiated rocks	300 to 3,000

Figure 2.1 | Stratigraphic column of hydraulically connected units in light blue and bedrock units in white (modified from USGS, 2012; modified from Gutentag and others, 1984).

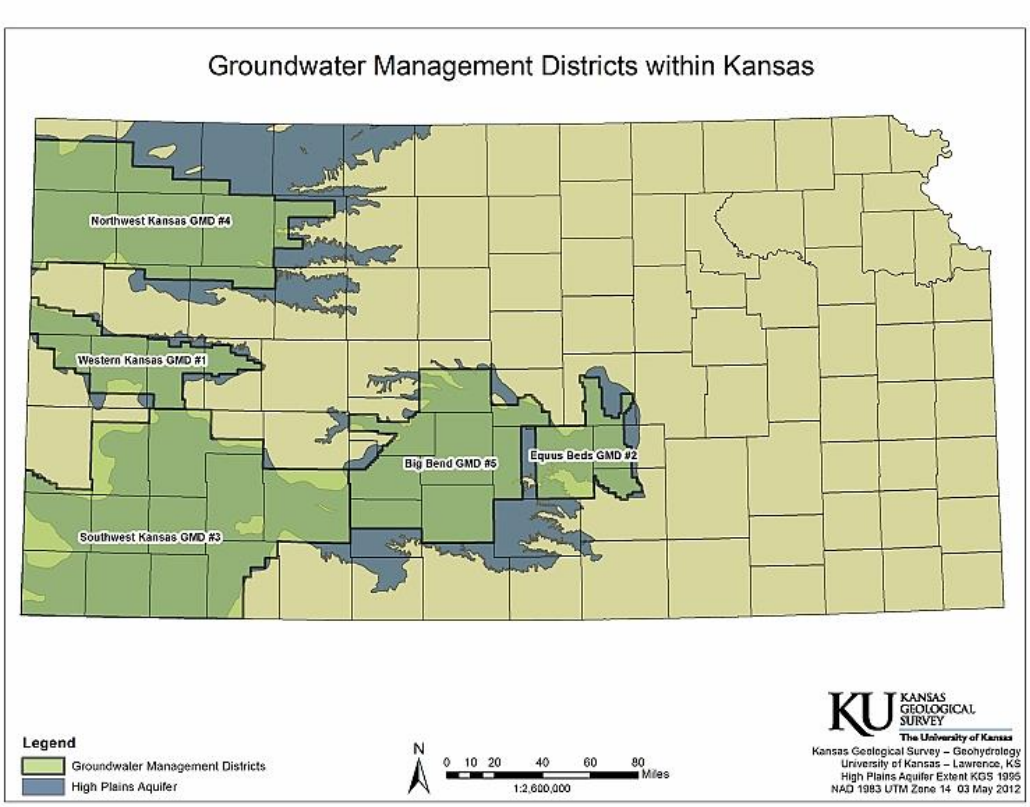

Figure 2.2 | There are five GMDs throughout Kansas for the conservation and management of water resources; GMD 5 is in south-central Kansas and covers the Great Bend Prairie aquifer (KGS, 2012).

An observation well network was constructed in GMD 5 from 1979 to 1987 by the Kansas Geological Survey (KGS) and GMD 5 at 52 sites in Stafford, Pratt, Reno, and Rice Counties (Fig. 2.3) (Rosner, 1988; Whittemore, 1993). Observation wells are located roughly on township corners (approximately every 10 km) (Sophocleous and Ma, 1998). Most of these locations included three wells: one screened within the bedrock, one screened in the lower portion of the aquifer near the base, and one screened in the shallow, upper portion of the aquifer (Fig. 2.4). A few locations included a fourth well, referred to as the intermediate level well, screened near the interface between fresh and saline water (Whittemore, 1993).

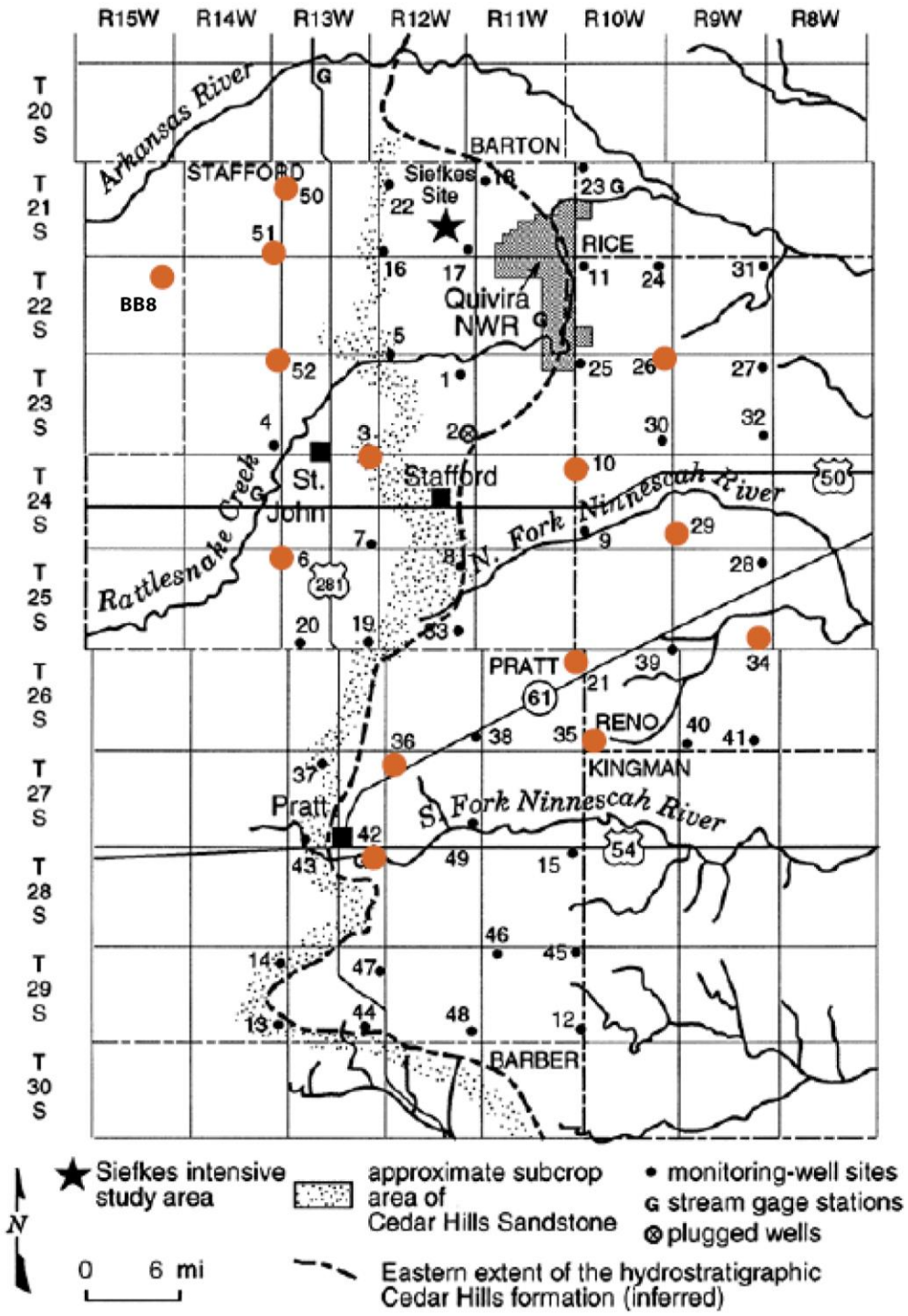

Figure 2.3 | GMD 5 and KGS observation well network map. Orange dots depict the wells we sampled in summer 2016. Map modified from Buddemeier (1994).

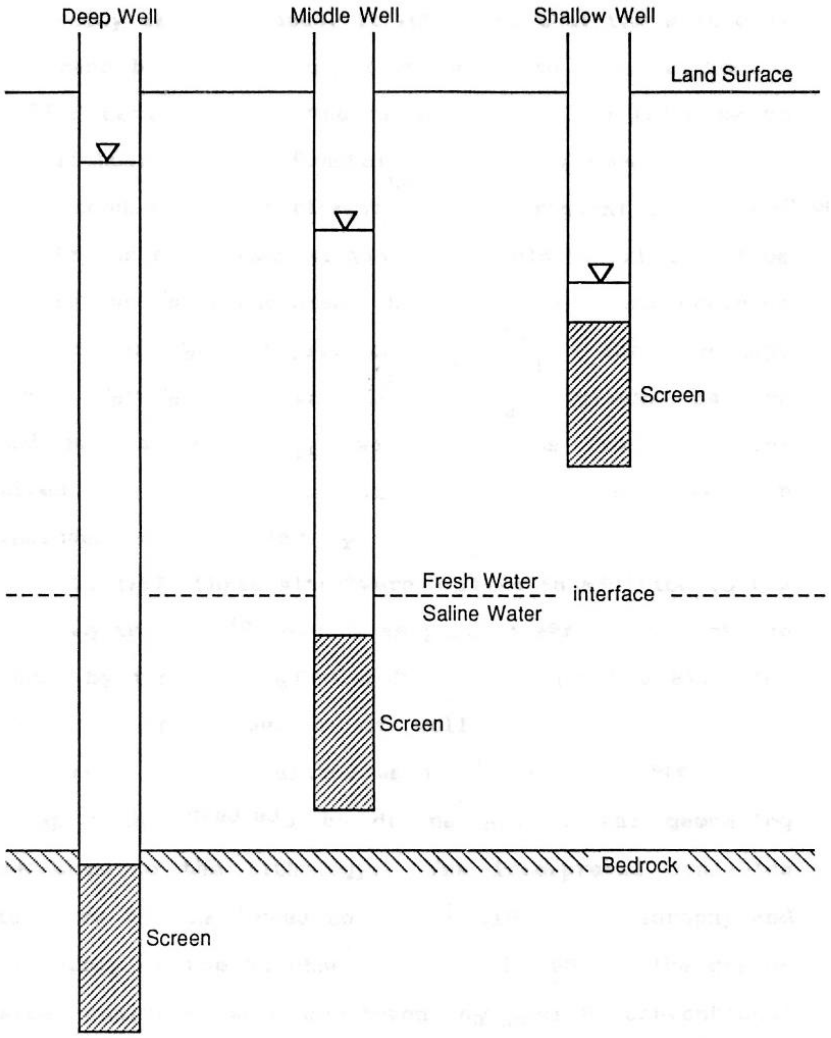

Figure 2.4 | Illustration of the typical layout of wells at each well site within the observation well network; figure not to scale. Reproduced with permission. (Rosner, 1988).

Quaternary and Tertiary units make up the principle water-bearing units in south-central Kansas (Fig. 2.4). The stratigraphy in this area consists of dune sands, loess, and gravel with lenses of clay, silt, and caliche that unconformably overly Permian and Cretaceous bedrock (Rosner, 1988). Much of the Quaternary alluvium was deposited by the Arkansas River and other streams, carried down from the Rocky Mountains (Rosner, 1988; Fader and Stulken, 1978). Underlying the Quaternary alluvium are Tertiary deposits, consisting of unconsolidated silt and fine sand, interbedded with gravel and caliche (Fader and Stulken, 1978). Groundwater within

the aquifer is generally of good quality with total dissolved solids (TDS) content < 500 mg/L. Groundwater can be of poorer quality in northeastern parts and deeper portions of the aquifer from the dissolution of evaporites within Permian or Cretaceous bedrock. (Fader and Stulken, 1978). Natural saline water within deeper portions of the aquifer can intrude into fresh groundwater in shallower portions of the aquifer; the observation well network in GMD 5 was originally installed to monitor natural saltwater intrusion (Rosner, 1988). In addition to natural salinity within portions of the aquifer, this area is dominated by agricultural land use and past

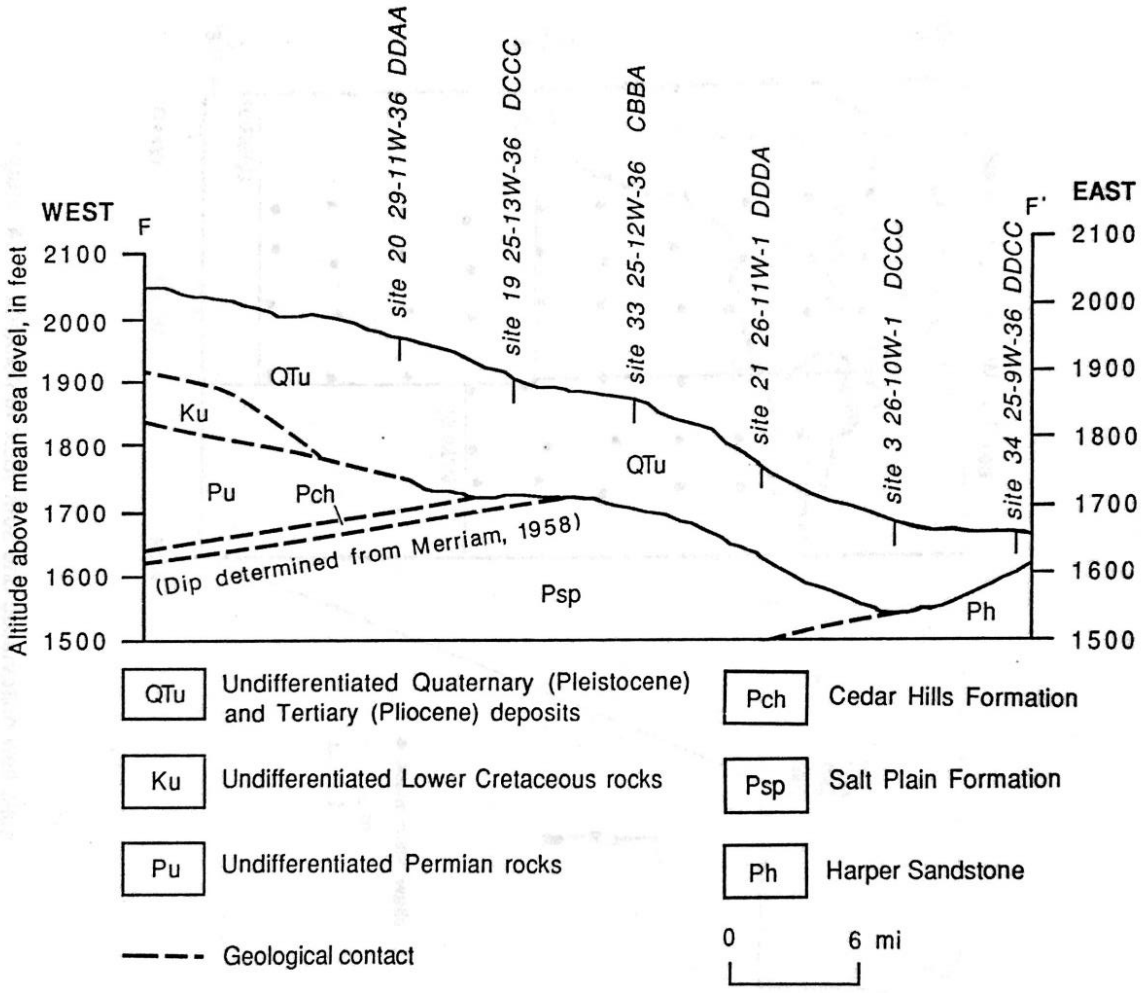

Figure 2.5 | General geologic cross-section of the Great Bend Prairie area. Quaternary and Tertiary units make up the aquifer in this area. Natural saline water from the dissolution of Permian evaporates can intrude into freshwater in the shallower portions of the aquifer, reproduced with permission (Rosner, 1988).

and present producing oil and gas wells. Every township within our field site has one or at least part of an oil or gas field (Fig. 2.5) (Whittemore, 1993).

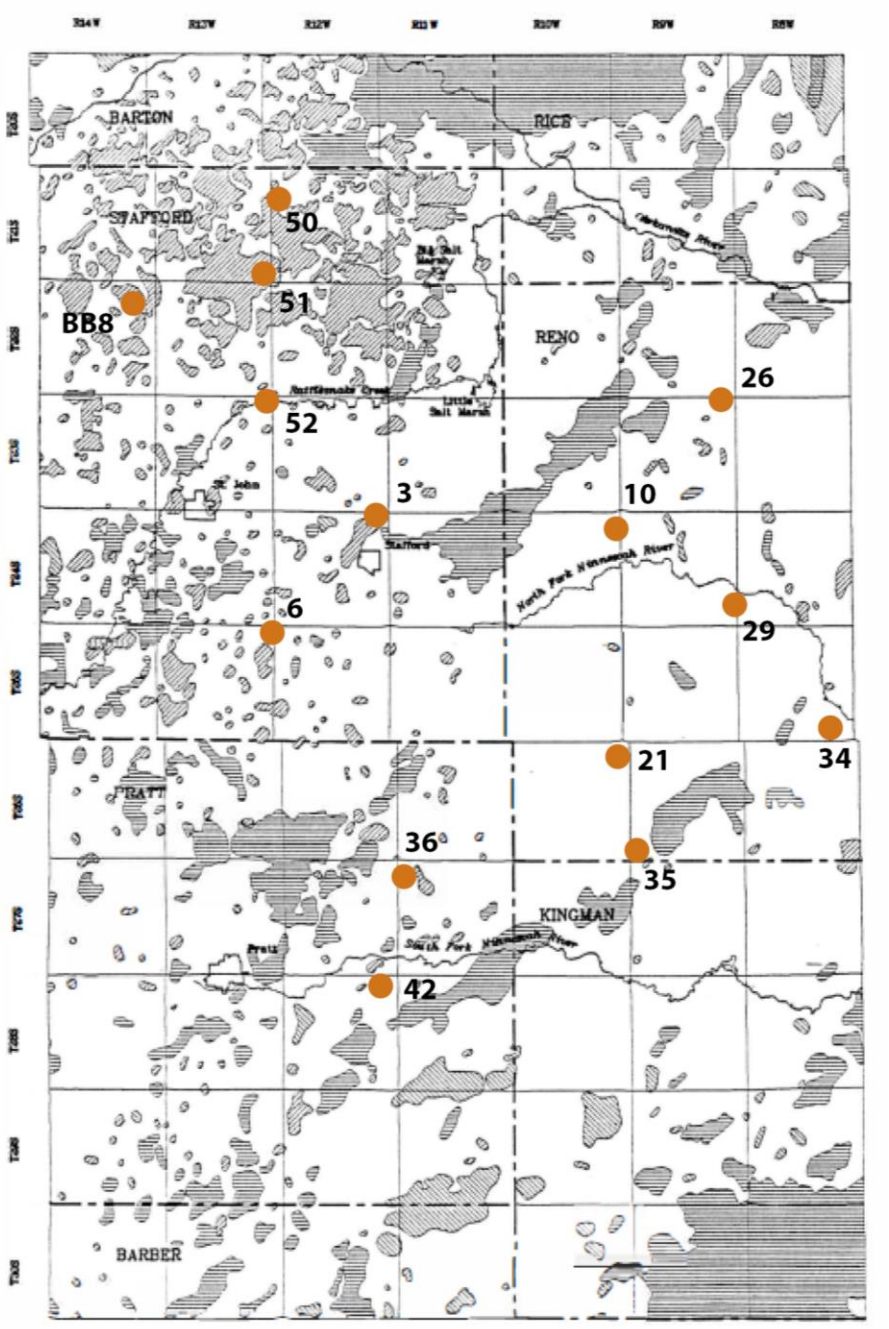

Figure 2.6 | Oil and gas fields underlying the field area; orange dots depict the location of the wells we sampled in summer 2016 and their proximity to oil and gas fields. Map modified from Whittemore (1993).

The Great Bend Prairie aquifer is mostly unconsolidated and unconfined. Like the change in surface elevation, the regional flow of groundwater is generally from west to east (Whittemore, 1993). There are four major streams in the area to which the aquifer discharges: the Arkansas River, Rattlesnake Creek, and the North and South Fork of the Ninnescah River (Fig. 2.2) (Whittemore, 1993; Rosner 1988). Precipitation is the main form of recharge to the aquifer, with an average rate of approximately 25-30 in/year (Goodin et al., 2004). The depth of the water table ranges from less than 25 ft to 50 ft in most of the area and 50–100 ft in southwest portions of the aquifer (Fig. 2.6) (KGS, 2012). Saturated thickness ranges from 100–150 ft in most of the area but can be as much as 150–200 ft in southwestern and northern portions. Around the outer edge of the Great Bend Prairie aquifer, saturated thickness is less than 50 ft (Fig. 2.7) (KGS, 2012). Average hydraulic conductivity in the Great Bend Prairie area generally ranges from 50–250 ft/day (KGS, 2012).

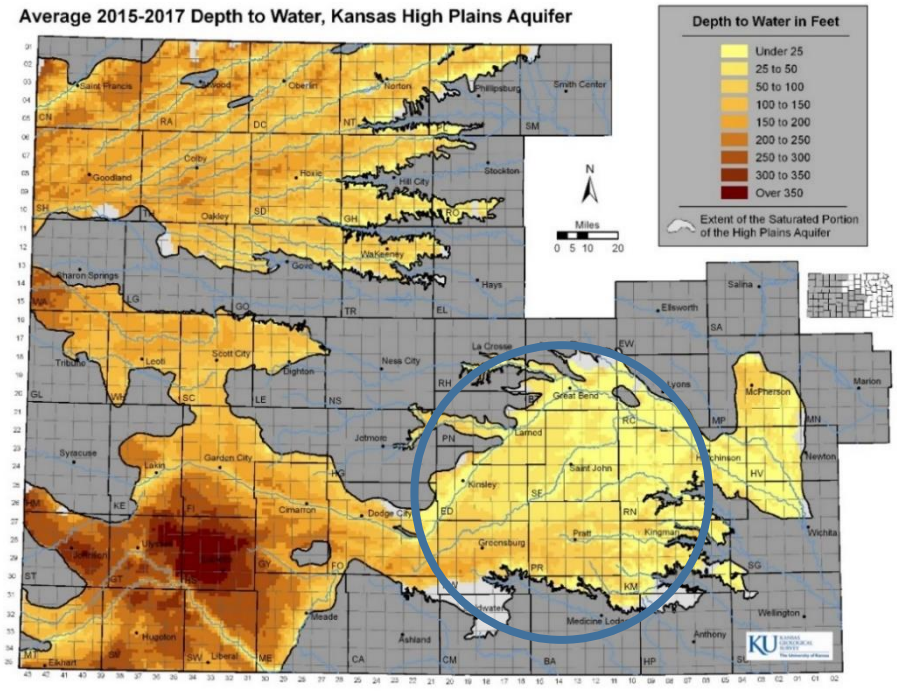

Figure 2.7 | Average saturated thickness (2015-2017) for areas of the High Plains aquifer and Great Bend Prairie aquifer in Kansas (KGS, 2012). The circled area represents the general location of the GMD 5 observation well network.

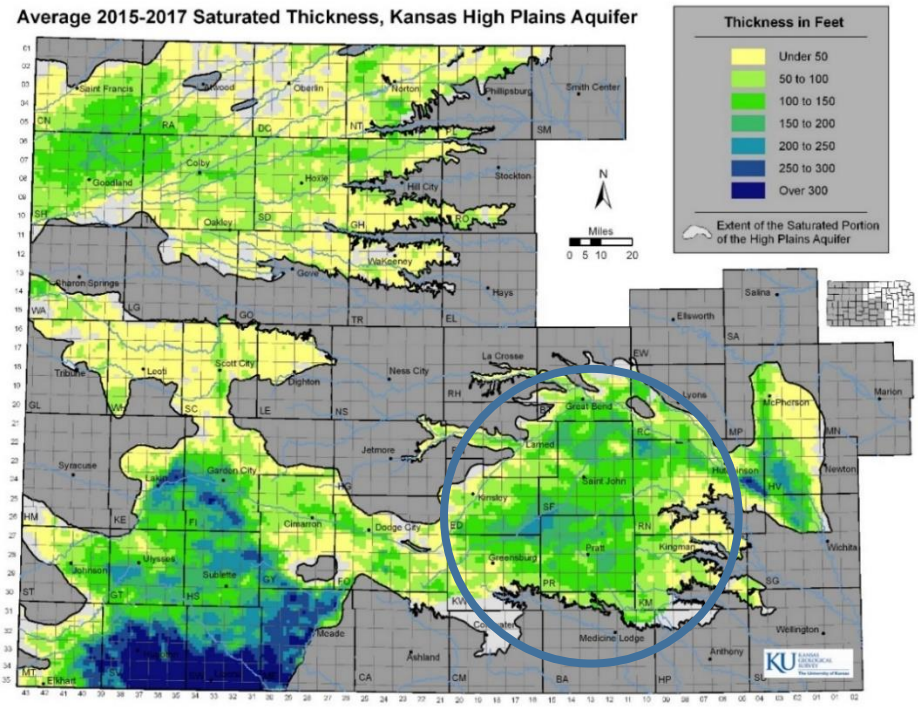

Figure 2.8 | Average depth to water (2015-2017) for areas of the High Plains aquifer and Great Bend Prairie aquifer in Kansas (KGS, 2012). The circled area represents the general location of the GMD 5 observation well network.

Chapter 3 - Methods

Field Methods

We collected groundwater samples during summer 2016 from 24 monitoring wells in GMD 5. The 24 well-sites were chosen to serve as an accurate reflection of the overall groundwater quality within GMD 5. Each well-site was numbered according to the installation date; we chose wells at sites: 3, 6, 10, 21, 29, 34, 35, 36, 42, 50, 51, 52, and BB8 (Fig. 2.3). Wells were chosen based on either the date of installation, or the proximity to surface water, towns, or oil and gas fields. Of the wells we sampled, 13 were screened in the upper portion of the aquifer (average depth 72.2 ft), 10 were screened in the aquifer base (average depth 141.1 ft), and one was screened in the underlying bedrock (depth 54.1 ft). We purged the wells at low flow rates (approximately 1 to 1.5 gal/min) prior to sampling. Groundwater temperature, pH and electrical conductivity was measured using an Oakton PC-300 meter. Samples were collected after temperature, pH, and electrical conductivity had stabilized over three consecutive readings five minutes apart.

Groundwater samples for cation, anion, and trace element concentrations were filtered (0.45 μm) in the field and stored in Nalgene™ bottles. Sample bottles were filled completely and with the least amount of head space possible. Cation samples were preserved with concentrated trace metal grade nitric acid to a pH < 2. Dissolved organic carbon (DOC) samples were filtered in the field using pre-washed glass-fiber membrane filters (0.7 μm). DOC samples were collected in amber glass bottles, and preserved with concentrated hydrochloric acid to a pH < 2. Dissolved oxygen (DO) levels were measured using a LaMotte Dissolved Oxygen Test Kit. Filters and plugs used for microorganism samples were sterilized prior to sampling using an autoclave

(120°C for 30 min). Microorganisms were collected by taking up water with a sterile 60 mL syringe and pushing it through a mixed cellulose ester (MCE) filter membrane (0.22 μm) until the filter membranes became clogged (Kirk et al., 2015). Microorganism samples were preserved using 0.2 mL of sucrose lysis buffer and plugged with a luer lok plug before being placed in individual Whirl-Paks (Giovannoni et al., 1990; Kirk et al., 2015). All samples were stored on ice in the field and moved to the laboratory refrigerator upon return.

Geochemical Analysis

Most geochemical analyses was conducted in the Department of Geology as Kansas State University. Total alkalinity was measured using burette titration with 10 mL of sample, a 0.02 N sulfuric acid titrant, and the USGS Gran alkalinity titration calculator (USGS, 2013; Kirk et al., 2015). We used an ICS-1100 Ion Chromatograph (IC) (Thermo Fisher Scientific™) to measure concentrations of cations and anions. TDS was calculated as the sum of inorganic constituent concentrations; we estimated a value for dissolved silica content by using values from initial samples collected by Whittemore (1993). The charge balance errors for all samples are < 4% except for the sample from well 10D for which the error is 5.8%.

Trace element samples were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) by the Redox Biology Center in the Department of Biochemistry at the University of Nebraska-Lincoln. Groundwater samples were analyzed for stable nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios of nitrate on a Trace Gas-GVI IsoPrime-Isoptope Ratio Mass Spectrometer (TG-IRMS) by the Environmental Isotope Laboratory in the Department of Earth and Environmental Sciences at the University of Waterloo. Results are expressed in delta notation relative to atmospheric air for nitrogen ($\delta^{15}N$ AIR) and Vienna

Standard Mean Ocean Water for oxygen ($\delta^{18}O$ VSMOW) with precisions of +/-0.3‰ and +/-0.8‰, respectively.

Microbial Community Analysis

Total microbial community DNA was extracted from 17 filtered microorganism samples using a Power Soil ® DNA Isolation Kit (MO BIO). The summarized and modified 20-step procedure was completed for each sample (Appendix C) (Kirk et al., 2015). DNA concentration and purity was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific™) from the Integrated Genomics Facility at Kansas State University. The spectrophotometer equipment and corresponding software was initialized for nucleic acid analysis and 1 µL of DNA extraction was placed on the pressure reservoir. Absorbance data was collected and hard copies were printed at the facility. This procedure was done a number of times for multiple samples to assess whether DNA extraction was successful enough to send to MR DNA® Laboratory.

MR DNA® Laboratory was contracted to amplify and sequence 16S rRNA genes in the samples. The 16S rRNA gene was amplified using universal prokaryotic primers 519F (CAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) (Wuchter et al., 2013). DNA was sequenced for 17 samples; eight samples were from wells screened within the deep portion of the aquifer, eight samples were from wells screened within the shallow portion of the aquifer and one sample was from a well screened within the bedrock. Raw sequencing data was processed using the Python 2 software QIIME (Quantitative Insights into Microbial Ecology) v. 1.8.0 (Caporaso et al. 2010; Kirk et al., 2015). Several steps for the installation of the

QIIME Virtual Box, QIIME, and subsequent script necessary for processing of sequencing data are listed in Appendix C.

Statistical Analysis

Geochemical data were analyzed using basic functions and graphing capabilities of Excel 2016 and GraphPad Prism 6. Trace elements, microbial community and geochemistry correlations were analyzed for statistical significance using the Spearman's Rho rank order correlation and Man-Whitney test software package available through GraphPad Prism 6. Spearman's Rho rank order correlation coefficient (ρ) is a non-parametric value for two variables that can assess linear or non-linear relationships and ranges from -1 to +1 (GraphPad 2015). A P-value less than 0.05 is considered statistically significant. Microbial communities were also analyzed using a nonparametric Mann-Whitney test to determine any significant differences between two unpaired groups. If the separate mean values for both groups are significantly different, a P-value will be less than 0.05 (GraphPad 2015).

Chapter 4 - Results

Geochemistry

Samples from the shallow portion of the aquifer tended to have lower temperature, pH and TDS content than samples from the deeper portion of the aquifer (Table 4.1; Appendix B). Temperature and pH values averaged 16.4°C and 7.30, respectively, for upper aquifer samples and 17.1°C and 7.42 for samples collected from the aquifer base. Average TDS content, calculated as the sum of major ion concentrations, was 509 ± 299 mg/L in the shallow portion of the aquifer and 4,155 ± 7,138 mg/L in the deep portion of the aquifer. The sample we collected from the bedrock portion of the aquifer had a temperature of 22.4°C, a pH of 7.58, and 222 mg/L TDS content, although much higher salinities were observed in groundwater from other bedrock portions of the aquifer.

	BEDROCK n=1	**AVG. DEEP** n=10	**AVG. SHALLOW** n=13
T (°C)	22.4	17.06	16.38
pH	7.58	7.42	7.30
C (µs/cm)	339	6,974	929.23
Alk | $CaCO_3$	134.1	169.82	187.78
F^-	0.40	0.48	0.35
Cl^-	6.39	2,194	125.4
Br^-	0.04	0.24	0.14
SO_4^{2-}	13.35	282.89	25.85
Na^+	23.63	1,331	73.29
K^+	1.37	24.83	3.83
Mg^{2+}	9.87	25.12	7.12
Ca^{2+}	26.86	140.33	79.30
Sr^{2+}	1.62	11.52	3.73
HCO_3^-	163.51	206.95	228.88
NO_3-N	7.30	4.44	12.56
PO_4-P	b.d.l.	b.d.l.	b.d.l.
NH_4-N	b.d.l.	b.d.l.	b.d.l.
NO_2-N	b.d.l.	b.d.l.	b.d.l.
TDS	222.2	4,155	509.23

Table 4.1 | Average groundwater parameters for samples we collected during summer 2016. Units are in mg/L unless stated.

Concentrations of dissolved constituents including chloride, bromide, sulfate, sodium, potassium, magnesium and calcium were highest in wells screened within the deeper portion of the aquifer on average (Table 4.1; Fig. 4.1). Differences in TDS between shallow and deep portions of the aquifer primarily are due to variation in chloride and sodium concentration. Chloride concentration ranged from 5.1 to 427 mg/L in

the shallow portion of the aquifer and 35.5 to 12,958 mg/L in the deep portion of the aquifer. Similarly, sodium concentration ranged from 11.7 to 410.7 mg/L in the shallow aquifer and from 40 to 7,816 mg/L in the deep portion.

Nitrate concentrations were highest in wells screened within the shallow portion of the aquifer. Values ranged from 0.5 to 52.3 mg/L as N in the shallow portion of the aquifer and 1.6 to 10.8 mg/L as N in the deep portion of the aquifer. Nitrate concentration exceeded the U.S. standard for public supplies of drinking water (10 mg/L as N) in seven of the samples we collected (6C, 21C, 34B, 42C, 50B, 50C and 51B) (Appendix A). Six out of these seven samples were collected from the shallow portion of the aquifer; 50B was the only well screened within the deep portion of the aquifer with a nitrate concentration above the standard.

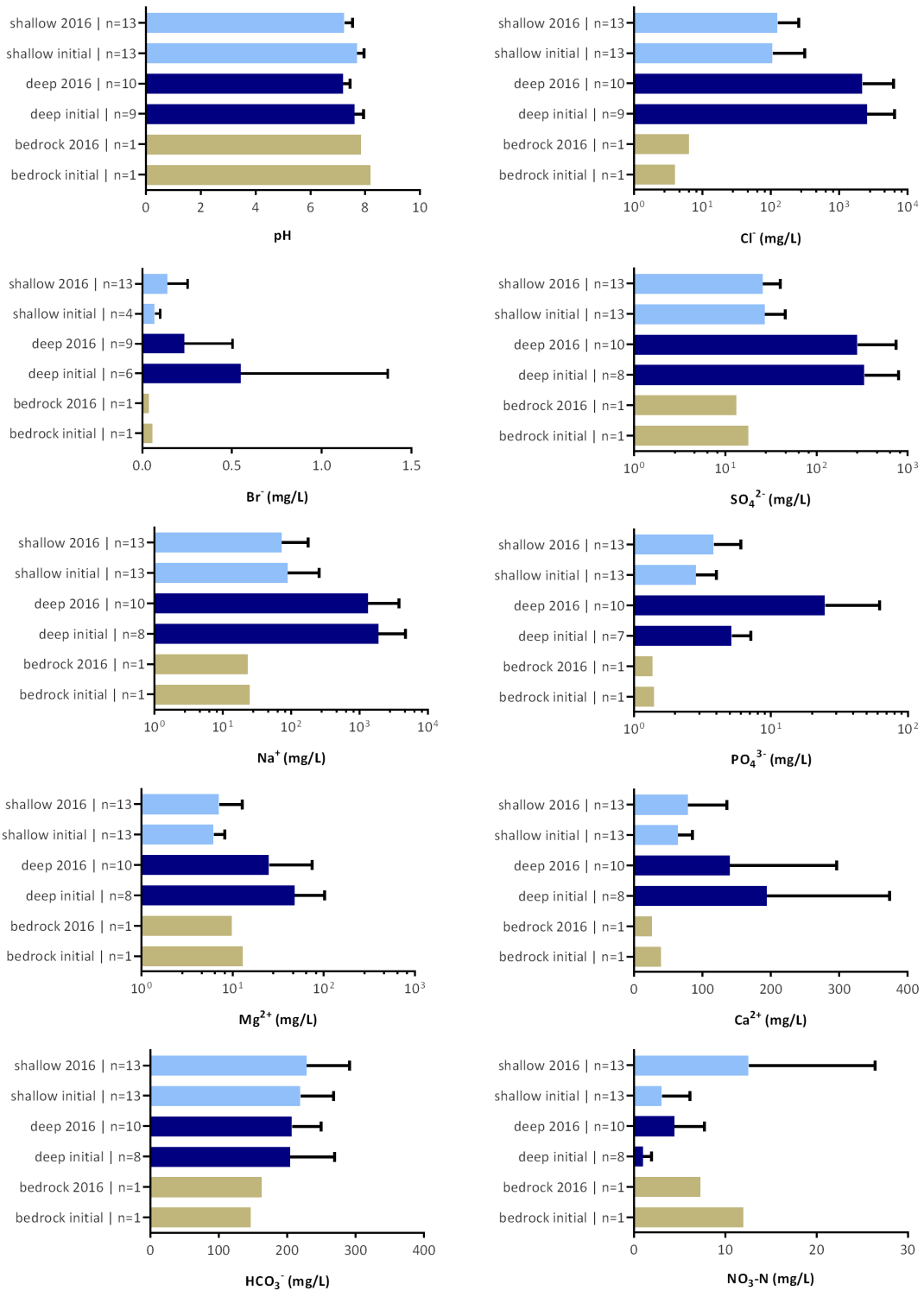

Figure 4.1 | Mean and standard deviation of groundwater parameters for initial samples collected by Whittemore (1993) and samples we collected during summer 2016. Light blue bars represent shallow 2016 and initial samples, dark blue bars represent deep 2016 and initial samples and tan bars represent bedrock 2016 and initial samples.

Trace element concentrations fall below the U.S. EPA drinking water standards for most of the samples we collected (Fig. 4.2; Appendix A). The sample we collected from 10D had a uranium concentration of 61.16 µg/L, exceeding the U.S. EPA Maximum Contaminate Level (MCL) of 30 µg/L. The sample from 21C had a barium concentration of 2.46 mg/L, exceeding the MCL of 2 mg/L. Lead concentrations exceeded the U.S. EPA Action Level of 15 µg/L in 21 out of 24 samples. Samples we collected from 10C, 26B, 42B, 50B, and BB5HA had manganese

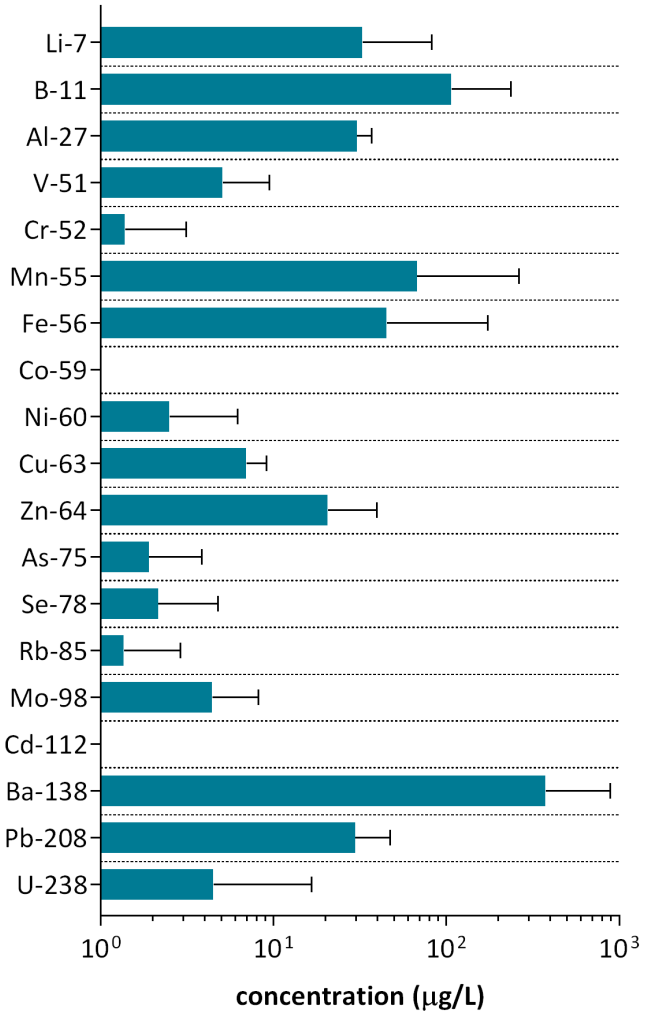

Figure 4.2 | Mean and standard deviation of trace element concentrations for groundwater samples collected during summer 2016.

concentrations above the U.S. EPA Secondary MCL (SMCL) of 0.05 mg/L, and samples from 42B had an iron concentration above the SMCL of 0.3 mg/L.

Stable nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios of nitrate can be used to identify nitrate sources in groundwater (Fig. 4.3; Appendix B) (Xue et al., 2009). The isotopic composition of nitrogen varies among different nitrate sources including atmospheric nitrogen, soil, chemical fertilizers and manure. We found that most of our samples fall within the $\delta^{15}N$ range of 0 to +10‰ and the $\delta^{18}O$ range of -10 to +20‰, ranges typical of nitrification of ammonia-based fertilizers, with varying contributions from manure.

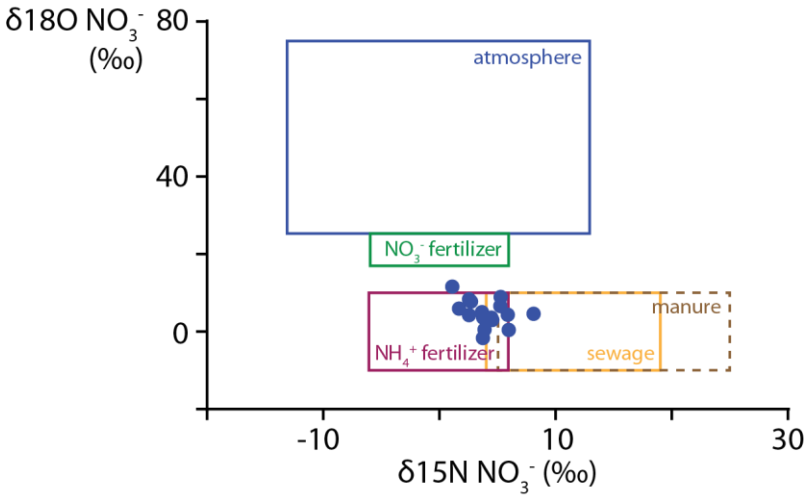

Figure 4.3 | Samples we collected are identified by blue dots. Stable nitrogen and oxygen isotope ratios of nitrate fall within the range typical of nitrification of ammonium-based fertilizers with potential contributions from manure and/or sewage. Isotopic composition ranges plotted according to Xue et al. (2009).

Microbial Community Composition

On average, bacteria from the phylum *Protobacteria* had the highest relative abundance for all wells screened within the bedrock, deep and shallow portions of the aquifer (Table 4.2; Fig. 4.4). *Proteobacteria* are the largest and most physiologically diverse of all bacteria phyla and include six major classes. Genera from the classes *Gammaproteobacteria* (12.6%), *Deltaproteobacteria* (9.89%), *Alphaproteobacteria* (9.42%), and *Betaproteobacteria* (7.01%) had the highest average relative abundance within the *Proteobacteria* phylum. Several genera of nitrifying and denitrifying, iron oxidizing, sulfur reducing and anoxygenic phototrophic groups are within the *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria* classes (Madigan et al., 2012).

Within the *Gammaproteobacteria* class and *Moraxellaceae* family, relative abundance was highest on average for the genus *Acinetobacter* (6.15%). Sequences grouping in *Acinetobacter* were particularly abundant in wells 50C (49.7%) and 50B (26.1%). Species of *Acinetobacter* are relatively common in soil, water and on human skin. *Acinetobacter* species are strictly aerobic, non-fermentative and are involved in the degradation of organic matter (Madigan et al, 2012; AWWA 2006). Other notable genera within the *Gammaproteobacteria* class include *Pseudomonas* (avg 1.1%). *Pseudomonas* is another common, strictly aerobic bacterium found throughout the environment.

Within the *Alphaproteobacteria* class, relative abundance was highest on average for genera from the *Rhodospirillaceae* family (4.2%) and was highest for deep wells 21B (9.8%) and 10C (9.5%). Genera from the *Rhodospirillaceae* family are considered purple non-sulfur bacteria capable of fermentative or anaerobic respiration (Madigan et al., 2012). The highest relative abundance within the *Rhodospirillaceae* family was an unclassified genus; other genera we see in our samples include *Phaeospirillum* and *Inquilinus.*

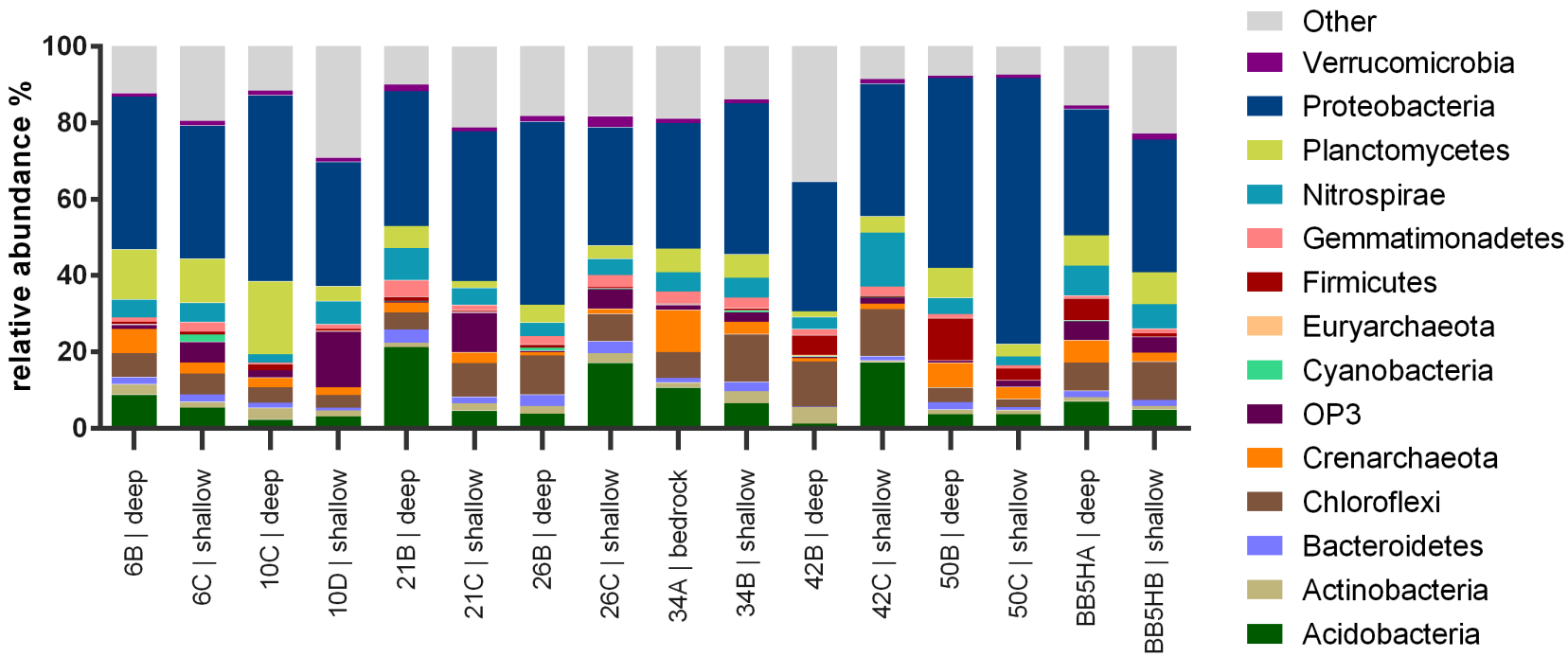

Figure 4.4 | Relative abundance in microorganisms by phylum for wells screened within the bedrock, deep, and shallow portions of the aquifer. Samples were collected during summer 2016.

PHYLUM	AVG. RELATIVE ABUNDANCE (%) n=17	AVG. SHALLOW RELATIVE ABUNDANCE (%) n=8	AVG. DEEP RELATIVE ABUNDANCE (%) n=8	BEDROCK RELATIVE ABUNDANCE (%) n=1
Proteobacteria	39.69	39.53	40.70	32.82
Chloroflexi	7.45	7.72	7.26	6.77
Acidobacteria	7.23	7.79	6.25	10.62
Planctomycetes	6.98	5.34	8.72	6.22
Nitrospirae	5.67	5.79	5.44	5.07
Crenarchaeota	3.39	2.42	3.40	11.08
OP3	3.37	5.66	1.34	1.27
Firmicutes	2.02	0.83	3.43	0.33
Gemmatimonadetes	1.84	1.96	1.56	3.06
Actinobacteria	1.82	1.66	2.05	1.29
Bacteroidetes	1.79	1.67	1.98	1.21
Verrucomicrobia	1.18	1.36	1.00	1.21
Cyanobacteria	0.28	0.39	0.19	0.08
Euryarchaeota	0.06	0.05	0.07	0.04

Table 4.2 | Average relative abundance of microorganisms by phylum for wells screened within the bedrock, deep and shallow portions of the aquifer. Samples were collected during summer 2016.

Within the *Deltaproteobacteria* class, genera from the *Syntrophobacteraceae* family (3.0%) had the third highest relative abundance within the *Proteobacteria* phylum on average. Relative abundance of *Syntrophobacteraceae* was highest for shallow wells 34B (6.1%) and 26C (5.8%). *Syntrophobacteraceae* genera are strictly anaerobic and are generally considered sulfate reducers (Madigan et al., 2012). The highest relative abundance within the *Syntrophobacteraceae* family was an unclassified genus; other genera we see in our samples include *Syntrophobacter*.

Within the *Betaproteobacteria* class, relative abundance was highest on average for genera from the *Neisseriales* (2.3%) and *Burkholderiales* (1.7%) orders. Species within the -*Neisseriales* order are obligate aerobes and relative abundance was highest in well 42C (10.3%) (Madigan et al., 2012). Relative abundance in genera within the *Burkholderiales* order was highest in shallow well-site 21C (9.1%). Species within the *Burkholderiales* order include groups capable of nitrogen fixation, nitrification, and iron or sulfur oxidation (Madigan et al., 2012). The highest relative abundance within the *Burkholderiales* order include genera *Cupriavidus*, *Delftia* and *Hydrogenophaga*.

Genera within the *Chloroflexi* (7.45%) and *Acidobacteria* (7.23%) phyla had a higher average relative abundance than the *Betaproteobacteria* and *Zetaproteobacteria* classes. Relative abundance in *Chloroflexi* was highest in shallow wells 34B (12.5%) and 42C (12.3%). Some species of *Chloroflexi* are capable of iron and nitrate reduction under anaerobic conditions (Kawaichi et al., 2013). The highest relative abundance within *Chloroflexi* were unclassified genera and other classified groups include genera within the *Dehalococcoidaceae* family. Only a few known species of *Acidobacteria* are known and ones that are characterized include aerobic and anaerobic chemoorganotrophs (Madigan et al., 2012). Relative abundance in *Acidobacteria* was highest in wells 21B (21.2%) and 26C (17.2%). The highest relative abundance within *Acidobacteria* were unclassified genera within the *Solibacteres* class in addition to a few uncharacterized classes.

Genera within the *Planctomycetes* phylum had an average relative abundance of 7.0% for all wells and was highest in deep wells 10C (19.1%) and 6B (13.1%). Genera within the *Planctomycetes* phylum are capable of anaerobic ammonium oxidation and relative abundance

was highest for unclassified genera and unclassified species within the *Phycisphaerae* class and *Pirellulaceae* family (Falkiewicz-Dulik et al., 2015).

Genera within the *Nitrospirae* (5.7%) phylum had the fourth highest relative abundance for all wells on average. Relative abundance in *Nitrospirae* was highest for wells 42C (14.1%) and BB8 (9.6%). *Nitrospirae* genera are the most abundant nitrifying bacteria in nature and some genera are also capable of denitrification through iron oxidation (Hedrich et al., 2011; Madigan et al., 2012). Relative abundance was highest for unclassified genera within *Nitrospirales* order; other genera include *Nitrospira* and other unclassified genera within the *Nitrospiraceae* family.

Genera within the *Crenarchaeota* phylum had an average relative abundance of 3.4% for all wells and was highest for the bedrock well 34A (11.1%). The highest relative abundance within the *Crenarchaeota* phylum was for species within the *Nitrosopumilus* genera followed by many unclassified genera. Species within the *Nitrosopumilus* genus are autotrophic ammonia oxidizing chemolithotrophs (Madigan et al., 2012).

Genera within the OP3 phylum had an average relative abundance of 3.4%. The OP3 phylum is still considered a candidate phylum and genera have yet to be cultured. This phylum was first observed through gene sequencing from samples collected from the Obsidian pool hot spring, rich in iron, sulfide, carbon dioxide, and hydrogen in Yellowstone National Park (Kumar and Saravanan, 2011). Compared to deep wells, relative abundance in genera from the OP3 phylum was significantly higher for shallow wells, but relative abundance was highest for highest for shallow well 10D (14.6%).

Genera within the *Firmicutes* phylum had an average relative abundance of 2% for all wells and was highest for deep well 50B (11.13%). Relative abundance was highest for genera within the *Bacillales* and *Clostridiales* orders. Genera within the *Bacillales* order are aerobic or

facultatively aerobic; genera present in our samples include mostly unclassified species and species within the Exiguobacterium genus (Madigan et al., 2012) Some genera within the Bacillales order can oxidize manganese or reduce uranium (VI) (Sathiyanarayanan et al., 2016). Genera from the *Clostridiales* order are fermentative; genera we observed include *Clostridium* and *Desulfosporosinus*. Species of *Bacillales* and *Clostridiales* are endospore-forming which usually occurs when there is a lack of nutrients or other environmental factors are unfavorable for growth, e.g. high metal concentrations (Madigan et al., 2012; Sathiyanarayanan et al., 2016). Compared to shallow wells, genera within the *Firmicutes* phylum were significantly higher for deep wells.

Chapter 5 - Discussion

Changes in Groundwater Geochemistry

By comparing the results of our groundwater analysis to groundwater parameters collected by Whittemore (1993), we can evaluate how groundwater geochemistry has changed since initial samples were collected between 1979 and 1987. On average, concentrations of dissolved constituents increased slightly in the shallow portion of the aquifer and decreased in the aquifer base (Figure 4.1; Appendix B). Average TDS content was 78 mg/L higher in samples we collected from the shallow portion of the aquifer and 373 mg/L lower in samples we collected from the aquifer base, compared to initial samples. However, there were no significant differences in TDS content for samples we collected from the shallow and deep portions of the aquifer, compared to initial TDS values from shallow and deep portions of the aquifer.

Most importantly, our results show that nitrate concentrations increased by an average of 9.5 and 3.9 mg/L as N in the shallow and deep portion of the aquifer, respectively. As stated in the results, seven of our samples had nitrate concentrations above the U.S. standard for public supplies of drinking water (10 mg/L as N). In contrast, only two samples collected by Whittemore (1993) had nitrate concentrations above the standard. Consequently, nitrate and salinity increases, especially in the shallow aquifer, represent significant degradation of groundwater quality in the aquifer over the past 30 to 40 years.

To better understand the changes in salinity, we applied mixing relationships established by Whittemore (1993, 1995), which are based on chloride concentrations and bromide/chloride ratios (Appendix A) (Fig. 5.1-5.2; Table 5.1). Fresh surface water and groundwater will typically have a chloride concentration between 0.1 to 100 mg/L and a bromide/chloride ratio between 0.0003 to 0.1 (3 to 1000 when ratios are multiplied by 10,000) (Whittemore, 1995). Natural

brines from the dissolution of evaporite minerals will typically have chloride concentrations ranging from 10,000 to 250,000 mg/L and a bromide/chloride ratio ranging from 0.00006 to 0.0005 (0.6 to 5 when ratios are multiplied by 10,000) (Whittemore, 1995). Oil and gas brines will typically have a chloride concentration ranging from less than 10,000 mg/L to 270,000 mg/L and a bromide/chloride ratio ranging from 0.0005 to 0.04 (5 to 400 when ratios are multiplied by 10,000) (Whittemore, 1995). Using this approach, we can differentiate changes in the chloride concentration of groundwater in response to the natural dissolution of brines from Permian bedrock, contamination from oil field brines, and evapotranspiration of soil moisture followed by infiltration to the groundwater.

In like manner to samples collected by Whittemore (1993), most of the samples we collected fall within the zone of mixing between freshwater and natural Permian brines for the Great Bend Prairie area (16 out of 22) (Fig. 5.1). Of the samples that fell within the zone of mixing, seven (3A, 3B, 21C, 34A, 34B, 35C and 42C) had increasing chloride concentrations and decreasing bromide/chloride ratios, and one (52B) had both an increasing chloride concentration and bromide/chloride ratio, compared to initial samples. An increasing chloride concentration in these eight samples suggests an increase in salinity with less freshwater mixing near the well (Fig. 5.1). Out of the 16 samples that plot within the zone of mixing, three (6B, 29C and 51B) had decreasing chloride concentrations and increasing bromide/chloride ratios and three (21B, 29B and 35B) had both decreasing chloride concentrations and bromide/chloride ratios, compared to initial samples (Fig. 5.1). A decreasing chloride concentration in these six samples suggest chloride dilution by the mixing of freshwater. Two samples (42B and 50B) plot nearly on the upper limit for the mixing zone and may suggest something other than simple mixing between freshwater and Permian brines (Fig. 5.1). Overall, a significant portion of

samples we collected show that changes in chloride concentration can mainly be attributed to the natural mixing of freshwater and Permian brines.

The approximate upper limit for natural dissolved nitrate concentrations throughout the study area is approximately 4 mg/L as N (Whittemore, 1993). For samples that fell within the zone of natural mixing, nitrate concentrations exceeded 4 mg/L as N in 10 out of 16 samples and exceeded the U.S. standard for public supplies of drinking water (10 mg/L as N) in five out of 16 samples. All 16 samples that plot within the zone of natural mixing were taken from sites located on the edge of an irrigated field, downgradient of one or multiple irrigation systems, or within proximity to row crops. Nitrate concentrations increased substantially for shallow wells 21C (increased from 2.92 mg/L as N to 52.3 mg/L as N), 34B (increased from 0.5 mg/L as N to 11.6 mg/L as N), 42C (increased from 3.81 mg/L as N to 11.2 mg/L as N), and 51B (increased from 2.75 mg/L as N to 12.6 mg/L as N) (Table 5.1). Nitrate concentrations above the natural background concentration suggests agricultural inputs of nitrate.

Although most samples fell within the natural zone of mixing, samples that plot nearly on the upper limit for the mixing zone (42B and 50B) may suggest something other than simple mixing between freshwater and natural Permian brines. The sample from 42B showed very minimal change in chloride concentration and a significant increase in bromide/chloride ratio, which may suggest the migration of oil-brine contaminated groundwater to the site. The sample from 42B was collected from a deep well within proximity of an irrigation system, however, nitrate concentrations were below 4 mg/L as N. Site 42B is to the south of an oil well and two salt water disposal wells, which may be the source of oil-brine contaminated groundwater. The sample from 50B showed very minimal change in bromide/chloride ratio and an increase in chloride concentration. The well location is surrounded by four irrigation circles (Appendix A)

and had a nitrate concentration greater than the U.S. standard for public supplies of drinking water. The location of 50B suggests that return flow of irrigation water affected by evapotranspiration could have caused the chloride increase. Although both sites are still within the mixing zone between freshwater and natural Permian brines, their location and changes may indicate factors additional to simple mixing are affecting chloride concentrations and bromide/chloride ratios.

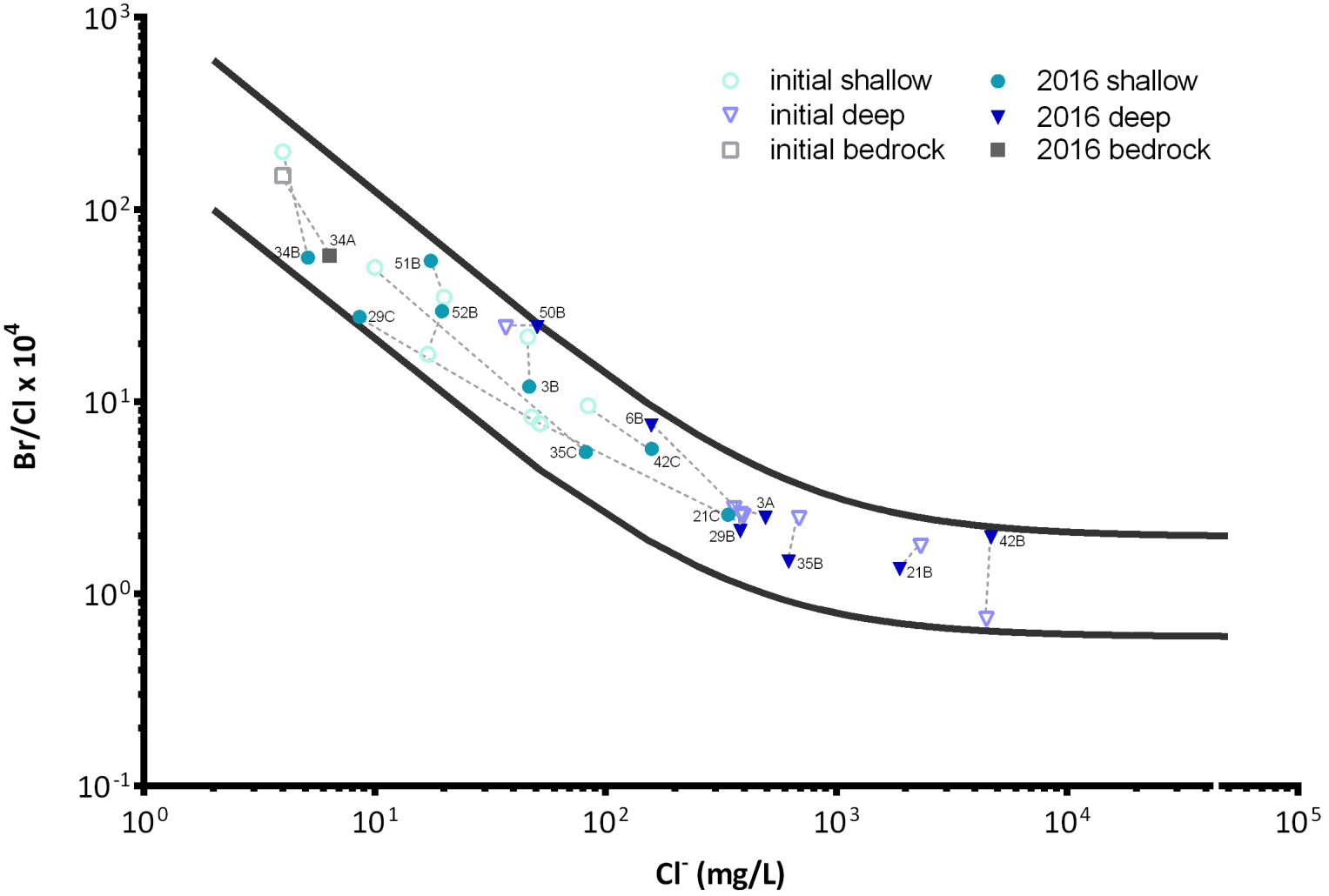

Figure 5.1 | 16 out of 22 samples fall within the natural mixing zone of freshwater and Permian saltwater for the Great Bend Prairie area. Samples were collected during summer 2016 and mixing curves were creating using the geochemical methods of Whittemore (1993) (Appendix A).

Samples that fell outside of the natural zone of mixing suggest additional factors affecting groundwater chemistry, in addition to the natural mixing between freshwater and natural Permian brines (6 out of 22 samples) (Fig. 5.2). Our results show that six wells plot outside of the natural zone of mixing, compared to four wells sampled previously by Whittemore (1993). Three out of six samples we collected (6C, 10D and 26C) had chloride concentrations that decreased slightly; the bromide/chloride ratios decreased in sample 6C, increased a little in 26C, and stayed about the same in 10D, compared to initial samples (Table 5.1). Whittemore (1993) identified an oil-brine contribution to the total chloride concentration in samples from all three of these wells. Changes in these three samples may indicate flushing of the initial oil-brine contamination over time. Water we sampled from well 10C showed relatively small increase in chloride concentration but a decrease in the bromide/chloride ratio compared to the initial sample from this well. Whittemore (1993) also discerned an oil-brine contamination contribution to chloride in this well water. The sample we collected may represent flushing of initial oil-brine contamination by slightly more saline water with a natural source of chloride.

Three out of the four samples (6C, 10C, and 26C) with initial oil-brine contributions identified by Whittemore (1993) had nitrate concentrations less than 4 mg/L as N and now have nitrate concentrations greater than 4 mg/L as N (Table 5.1; Appendix A). Nitrate concentrations for 6C were slightly above the standard for public supplies of drinking water (nitrate increased from 4.3 mg/L as N to 10.3 mg/L as N) (Table 5.1). Site 6 is located downgradient of irrigated fields. Site 10 has irrigation systems to the north and south of the well, but the site is not directly downgradient of irrigation systems and site 26 is not directly downgradient of irrigation systems but is located on the edge of row crops. Nitrate concentrations above the natural background suggests agricultural inputs of nitrate.

Two samples initially fell inside the natural zone of mixing and from our results, now plot outside the natural zone of mixing (36D and 50C). Samples from 50C show an increasing chloride concentration and a minor decrease in bromide/chloride ratio. The nitrate concentration from 50C was above the standard for public supplies of drinking water (increased from 10.6 mg/L as N to 28.8 mg/L as N) (Table 5.1). Well 50C is surrounded by four center pivot irrigation systems which may indicate agricultural inputs such as the infiltration of irrigation return flow from fertilized fields that had been affected by evapotranspiration (Appendix A). In addition, the sample collected from the deep well within the same well-nest (50B) fell nearly on the upper limit for the mixing zone and may reflect similar agricultural inputs contributing to the chloride concentration.

Samples from 36D show an increasing chloride concentration and a decreasing bromide/chloride ratio. Nitrate concentration for 36D was above 4 mg/L as N and was greater than in the initial sample from this well (an increase from 0.89 mg/L as N to 5.5 mg/L as N) (Table 5.1). Site 36 is not near irrigated cropland but is in an oil field area with oil wells and injection wells for enhanced oil recovery to the south and north of the site. This location suggests that there is a possibility that brine spills and brine line leaks might have contributed to the chloride increase. The nitrate concentration was much lower in the sample from well 36D than in 50C; the nitrate increase might represent the impact of the west to east migration of fertilizer impacted groundwater from non-irrigated cropland within a half-mile to the west. Substantial areas of irrigated fields lie a few miles to the west; there is also the possibility that regional migration of groundwater impacted by irrigation return flow from this area could contribute to the nitrate and chloride increase at well 36D.

Overall, our results indicate that initial oil-brine contamination identified by Whittemore (1993) in four sites (6C, 10C, 10D and 26C) has been flushing since these wells were sampled previously by Whittemore (1993). Two sites (42B and 36D) that were not initially affected by oil-brine contamination show changes that may reflect the migration of groundwater contaminated by oil-brine leaks or brine spills towards the site. In addition, these two sites are within proximity to oil and gas fields, oil wells, or injection wells. We see agricultural inputs of nitrate; nitrate concentrations were above 4 mg/L in five out of six sites that fell outside of the zone of natural mixing. Agricultural inputs such as infiltration of irrigation return flow from fertilized fields affected by evapotranspiration has affected the chloride concentration in at least two sites (50B and 50C). These two shallow and deep wells are nearly in the middle of four center pivot irrigation systems and had nitrate concentrations above the standard for public supplies of drinking water (Appendix A) (Table 5.1). Even though we see that initial oil-brine contamination has been flushing over time, there may be oil-brine contamination impacting additional sites that was not seen initially. In addition to the number of sites impacted by agricultural inputs of nitrate, fertilized fields affected by evapotranspiration may also be affecting chloride concentration in at least two sites.

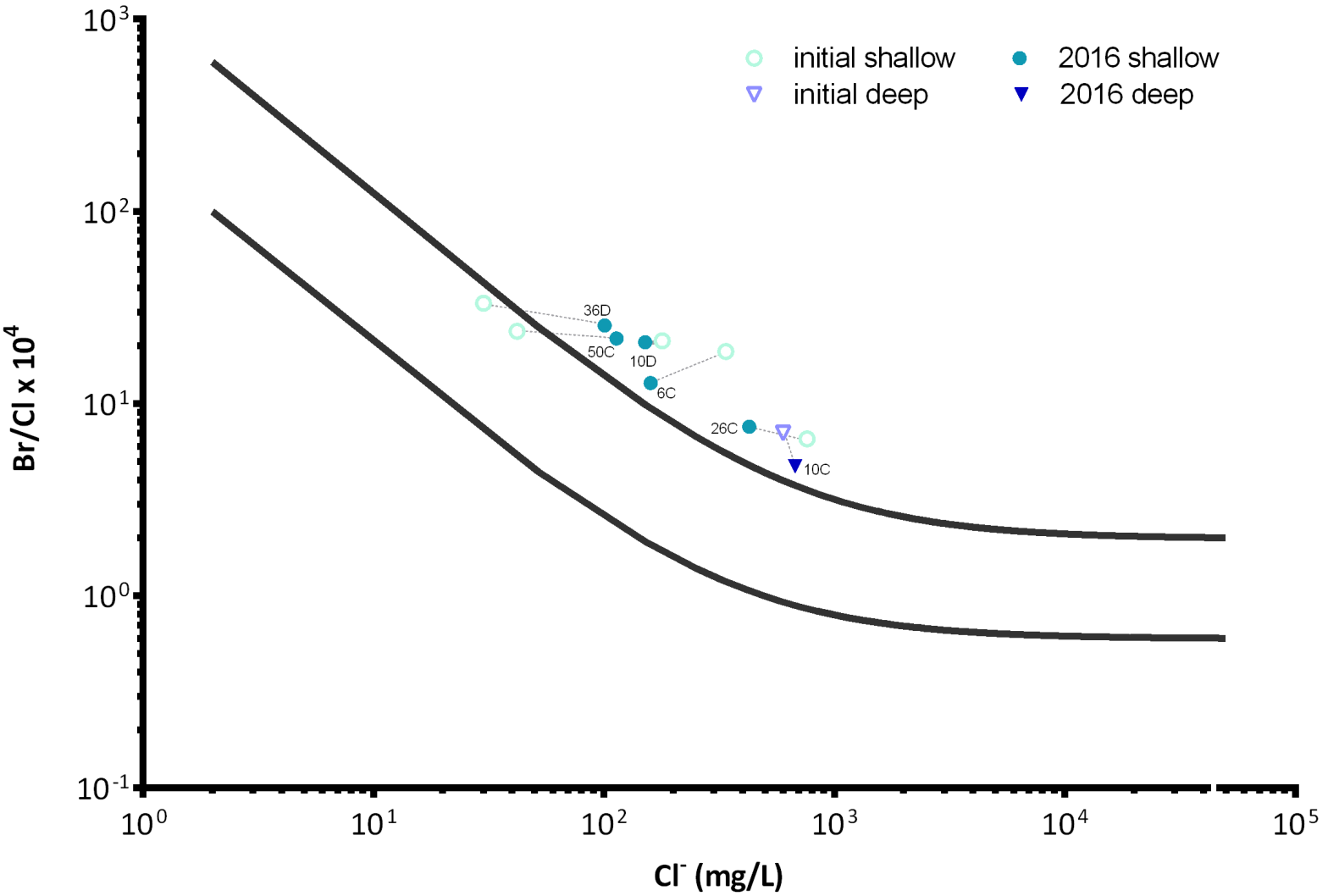

Figure 5.2 | 6 out of 22 samples that we collected during summer 2016 fall outside of the natural mixing zone of freshwater and Permian saltwater for the Great Bend Prairie area. These points represent a factor additional to simple mixing that affects the chloride concentration or bromide/chloride ratio. Mixing curves were creating using the geochemical methods of Whittemore (1993) (Appendix A).

Well-site	Type	Δ Years	NO_3-N	Δ NO_3-N	Cl^-	Δ Cl	Br^-	Δ Br^-	Br/Cl x 10^4	Δ Br/Cl x 10^4
3A	deep	**33**	**6.5**	**+6.43**	492.8	**+131**	0.12	**+0.02**	2.5	**-0.284**
3B	shallow	**32**	1.3	**+1.30**	46.7	**+0.69**	0.06	**-0.04**	11.97	**-9.77**
6B	deep	**33**	1.6	**-0.05**	158.2	**-237**	0.12	**+0.02**	7.5	**+4.97**
6C	shallow	**32**	**10.3**	**+6.03**	159.4	**-178.6**	0.20	**-0.43**	12.80	**-5.84**
10C	deep	**33**	**4.2**	**+4.04**	676.9	**+78.9**	0.32	**-0.1**	4.7	**-2.31**
10D	shallow	**32**	0.5	**+0.15**	151.1	**-27.85**	0.32	**-0.07**	20.8	**-0.39**
21B	deep	**33**	1.9	**+0.53**	1,885	**-434**	0.25	**-0.16**	1.3	**-0.42**
21C	shallow	**32**	**52.3**	**+49.38**	340.1	**+292**	0.09	**+0.05**	2.6	**-5.75**
26B	deep	**33**	**9.0**	**+8.30**	12,958	**+598**	b.d.l.	**-2.2**	-	-
26C	shallow	**32**	**7.1**	**+6.47**	427.4	**-334**	0.32	**-0.18**	7.6	**+1.01**
29B	deep	**33**	3.1	**+0.80**	384.3	**-1.73**	0.08	**-0.02**	2.1	**-1.48**
29C	shallow	**32**	**8.0**	**+1.92**	8.6	**-43.42**	0.02	**-0.02**	27.6	**+19.93**
34A	bedrock	**33**	**7.3**	**-4.7**	6.4	**+2.39**	0.04	**-0.02**	57.5	**-92.46**
34B	shallow	**31**	**11.6**	**+11.55**	5.1	**+1.14**	0.03	**-0.05**	56.2	**-143.79**
35B	deep	**33**	2.5	**+0.67**	621.9	**-67.1**	0.09	**-0.08**	1.5	**-1.00**
35C	shallow	**32**	**9.4**	**+3.80**	82	**+72**	0.04	**-0.005**	5.5	**-44.54**
36D	shallow	**32**	**5.5**	**+4.64**	100.7	**+70.69**	0.26	**+0.16**	25.5	**-7.88**
42B	deep	**33**	3.3	**+3.29**	4,682	**+212**	0.92	**+0.59**	2.0	**+1.22**
42C	shallow	**32**	**11.2**	**+7.39**	158.4	**+74.43**	0.09	**+0.01**	5.7	**-3.84**
50B	deep	**29**	**10.8**	-	50.6	**+13.62**	0.12	**+0.03**	24.4	**+0.04**
50C	shallow	**29**	**28.8**	**+18.24**	113.5	**+71.53**	0.25	**+0.15**	21.85	**-1.96**
51B	shallow	**29**	**12.6**	**+9.85**	17.5	**-2.52**	0.09	**+0.02**	54.18	**+19.18**
52B	shallow	**29**	**4.7**	**+2.94**	19.6	**+2.59**	0.06	**+0.03**	29.61	**+11.96**
BB8*	deep	**32**	1.6	-	35.5	-	0.10	-	29.38	-

Table 5.1 | Nitrate, chloride, and bromide concentrations, and bromide/chloride ratio for samples we collected during summer 2016. The change in nitrate, chloride, and bromide concentrations, and bromide/chloride ratios compared to data collected by Whittemore (1993). The number of years since the initial samples were taken are also listed for each site. *no initial

Controls on Trace Element Mobility

The changes in groundwater we observed have the potential to impact the mobility of hazardous trace elements. For example, previous studies have found evidence that agricultural nitrate addition has mobilized uranium and selenium in portions of the High Plains aquifer (Gates et al., 2009; Nolan and Weber, 2015). To evaluate whether similar effects are occurring in GMD5, we examined the relationship between trace element concentration and levels of nitrate and chloride using Spearman's rho rank order correlation tests (Graph Pad, 2015) (Table 5.2).

	vs. NO_3-N		vs. Cl^-	
	rho	P	rho	P
Li-7	-0.3353	0.1092	**0.8974**	**<0.0001**
B-11	-0.1348	0.5300	**0.6235**	**0.0011**
Al-27	-0.3153	0.1334	0.18	0.4000
V-51	0.142	0.5079	-0.1501	0.4840
Cr-52	-0.2134	0.3167	0.3462	0.0975
Mn-55	-0.2527	0.2336	0.3913	0.0586
Fe-56	**-0.541**	**0.0063**	**0.4652**	**0.0220**
Co-59	-0.2857	0.1759	**0.4678**	**0.0211**
Ni-60	-0.09132	0.6713	0.3904	0.0593
Cu-63	-0.2009	0.3465	**0.4261**	**0.0379**
Zn-64	0.03262	0.8797	**-0.4443**	**0.0296**
As-75	-0.0848	0.6936	0.05913	0.7837
Se-78	-0.2405	0.2577	-0.1026	0.6333
Rb-85	**-0.441**	**0.0310**	**0.5821**	**0.0028**
Mo-98	**-0.4223**	**0.0398**	**0.4139**	**0.0444**
Cd-112	0.0187	0.9309	0.1826	0.3931
Ba-138	0.384	0.0640	**-0.6504**	**0.0006**
Pb-208	-0.05175	0.8102	-0.1991	0.3509
U-238	-0.157	0.4637	0.2796	0.1857

Table 5.2 | Significant correlations between trace element concentrations and nitrate and chloride concentrations for 2016 samples. Spearman's Rank-Order correlation (rho) and orange-bolded P-values are < 0.05 and considered statistically significant.

We found a few significant relationships between nitrate and trace element concentrations but numerous significant relationships between chloride and trace element concentrations (Table 5.2). Orange highlighted P-values and corresponding Spearman's Rho rank order correlation coefficients are considered statistically significant (P-value < 0.05). Nitrate appears to share a significant negative correlation with iron, rubidium, and molybdenum. Chloride shares a significant positive correlation with lithium, boron, iron, cobalt, copper, rubidium, and molybdenum and a significant negative correlation with zinc and barium.

Whether the relationships of nitrate with rubidium and molybdenum are meaningful is unclear. The relationships between iron and nitrate, however, likely reflects aquifer microbial interactions. Nitrate respiration can typically provide microorganisms with more energy than iron reduction (Bethke et al., 2011). Thus, where nitrate is available, microbial iron reduction is typically repressed, which can limit the accumulation of ferrous iron in groundwater.

A significant negative correlation between iron and nitrate may reflect the ability of nitrate reducers to outcompete iron reducers for energy sources where nitrate is available (Bethke et al., 2011). Alternatively, it may also reflect iron oxidation as a pathway to denitrification. In anoxic subsurface environments, certain chemolithotrophs can use iron as an electron donor (iron oxidation) and nitrate as an electron acceptor (nitrate reduction). Indeed, some groups that we see in our microbial community analysis contain genera capable of iron oxidation including *Nitrospirae* (avg 5.7%), *Firmicutes* (avg 2.0%), and *Burkholderiales* (avg 1.7%). Relative abundance of genera within these groups was much higher than the average in a few wells (Hedrich et al., 2011).

A significant positive correlation between chloride and many trace elements suggests that Permian brine within deeper portions of the aquifer simply has a greater concentration of those elements. Indeed, elements such as lithium and boron are commonly associated with sedimentary brines (Ayotte et al., 2011). The significant relationship between chloride and iron suggests that saline groundwater in the field area is generally more reduced, compared to fresh or dilute groundwater, and better able to support denitrification. Over the pH range of our samples, ferric iron is largely insoluble, therefore, dissolved iron in our samples is likely ferrous iron. The higher ionic strength of saline waters compared to freshwaters, could increase the solubility of

minerals, and could also be a partial explanation for higher concentrations of selected trace elements.

Results of our geochemistry and microbial community analysis are consistent with the conclusions that deeper, more saline groundwater is generally more reduced and has a greater capacity to support denitrification. Samples we collected from deep wells had an average chloride concentration of 2,434 mg/L and only one sample from deep wells had a nitrate concentration greater than the U.S. standard for drinking water. Moreover, relative abundance of *Firmicutes*, a phylum of bacteria capable of denitrification through iron oxidation was significantly higher in wells screened within the deeper portion of the aquifer (Fig. 4.4). These observations provide further evidence that deeper portions of the aquifer are better able to support denitrification.

Previous research by Gates et al. (2009) found that agricultural nitrate addition to groundwater in the intensely irrigated alluvial valley of the Arkansas River in Colorado can mobilize selenium. Parts of the Arkansas River valley in Colorado are underlain by Cretaceous bedrock containing substantial selenium concentrations. Some microorganisms can respire selenium minerals by reducing dissolved oxygen, nitrate, or selenite, thereby increasing the concentrations of dissolved selenium in groundwater. Once selenium is mobilized in groundwater, it can potentially reach surface water and bioaccumulate through the food chain (Gates et al, 2009). A lack of significant correlation between nitrate and selenium suggests that nitrate addition to the aquifer is not triggering selenium mobilization, a relationship observed in the heavily irrigated alluvial valley of the Arkansas River in Colorado (Table 5.2) (Gates et al, 2009). Parts of the Arkansas River valley in Colorado are underlain by Cretaceous bedrock contain substantial selenium concentrations. The bedrock units underlying the Great Bend Prairie

and the sediments within the High Plains aquifer of the Great Bend Prairie may not have high enough selenium concentrations for a similar process to occur as in Colorado.

Previous research completed by Nolan and Weber (2015) in the High Plains aquifer found that agricultural nitrate addition can lead to secondary uranium contamination through of number of abiotic and microbially mediated processes including denitrification. A lack of significant correlation between nitrate and uranium suggests that nitrate addition to the aquifer is not contributing to uranium mobilization, even though we see this relationship established elsewhere in the High Plains Aquifer (Nolan et al. 2015). We did find that one well (10C) had a uranium concentration above the MCL. However, we found no significant correlations between uranium concentration and relative abundance of microorganisms capable of uranium oxidation.

Controls on Microbial Community Composition

In addition to trace elements, the observed temporal and spatial differences in groundwater geochemistry also have the potential to impact the composition of aquifer microbial communities. Microorganisms impact their environment by catalyzing oxidation-reduction reactions. However, environmental factors can influence microbial communities by providing nutrients, energy sources, and stresses (Bethke et al., 2011). Thus, the perturbed chemistry of the aquifer also has the potential to alter the microbial community. To evaluate this possibility, we examined relationships between microbial community composition and concentrations of nitrate and chloride. Our geochemical analysis indicates these parameters vary primarily in response to agricultural activities and natural mixing relationships, respectively. We also consider the relationship between community composition and well depth, assuming that agricultural impacts would generally be greatest in shallow portions of the aquifer.

There were no clear relationships between the relative abundance of microbial communities and the location of wells for most groups of microorganisms we identified. A Mann-Whitney test indicated that relative abundance of sequences classified in the phyla of *Proteobacteria, Chloroflexi*, *Acidobacteria, Planctomycetes, Nitrospirae* and *Crenarchaeote* was not significantly different for shallow and deep wells (Graph Pad, 2015; Table 4.2). However, there were significant differences in relative abundance in genera from the *Firmicutes* phylum and unclassified OP3 phylum for shallow and deep wells. A Mann-Whitney test indicated that relative abundance in genera from the *Firmicutes* phylum was significantly greater (P-value = 0.0104) for deep wells (median = 1.51) than for shallow wells (median = 0.5), and relative abundance in genera from the OP3 phylum was significantly greater (P-value = 0.0047) for shallow wells (median = 4.6) than for deep wells (median = 0.76) (Graph Pad, 2015). The variation in the relative abundance of certain phyla with depth in the aquifer suggests separate oxic and anoxic microbial zones.

Our analysis of nitrate isotopes indicates that nitrification of ammonium based fertilizer is the primary source of nitrate to the groundwater we sampled. As such, we might expect to find a positive correlation between nitrate concentration and the relative abundance of groups containing nitrifiers. However, we do not. We did notice that relative abundance of *Proteobacteria* and nitrate concentration tended to increase together ($r = 0.83$, P-value = 0.0154). Conversely, there were no significant positive correlations between nitrate concentration in shallow wells and classes within the *Proteobacteria* phylum or any other phylum capable of nitrification. Thus, nitrification may largely be occurring in the overlying soils. Consistent with this, we see that ammonium and nitrite concentrations were below our detection limits. Other

factors that could contribute to the lack of a correlation include variation in nitrate sources, denitrification, and the complexity of the nitrifying community.

Our microbial community analysis indicates that groups capable of denitrification are present in the aquifer, especially within deep portions where denitrification is more likely to occur. We see groups within the *Nitrospirae* (5.7%) and *Firmicutes* (2.0%) phyla, and *Burkholderiales* (1.7%) order capable of denitrification. It's possible that nitrate addition to the aquifer over the past 30+ years has stimulated growth for these types of microorganisms due to the increase in available substrate. However, we found no significant correlations between relative abundance of denitrifying groups and nitrate or iron concentrations. Most of the wells we sampled contained a significant amount of dissolved oxygen and thus many wells were dominated by strictly aerobic bacteria. Nitrate addition is more likely to stimulate growth of denitrifiers where dissolved oxygen is limited.

Formation water salinity and specifically chloride concentration has been observed as a major environmental control on microbial community composition in other systems (Kirk et al., 2015). However, in samples we collected, there were no obvious significant relationships between salinity and relative abundance in microorganisms. It would most likely be necessary to perform a more comprehensive beta diversity analysis and collect more samples to determine whether there are any significant relationships between salinity and microbial community composition. Our current dataset lacks statistical power for such analyses and more sampling would be necessary.

Sources of Uncertainty

We estimated the precision of our ion chromatography data as twice the standard deviation of values obtained from replicate analysis of quality control samples (Gill, 1997). We evaluated detection limits for each ion measured by ion chromatography as described in the U.S. EPA method 300.1.

Wells within the observation well network and GMD were installed from 1979 to 1987 and we do not know the installation details behind every site. The cement in the annular seals of some observation wells may have deteriorated over time, thereby allowing recharge or irrigation water to reach shallow groundwater much faster than it typically would. In addition, the typical irrigation wells in the area have gravel pack in the annular space up to within 10 to 20 ft of the ground surface, which can allow near surface water to drain to the aquifer. Many past oil and gas field wells that were abandoned may not have been properly sealed or documented.

We sampled wells during a dry, hot, summer, typically when irrigation occurs most frequently. Groundwater parameters fluctuate throughout different times of the year, therefore, groundwater parameters that we collected may only be an accurate reflection of the aquifer in this area over a typical summer. In some sampling sites groundwater temperature would falsely increase as the pump hose sat in the sun. In later samples, we used a heat blanket to cover the pump hose but consequently, a few of our samples may not have accurate groundwater temperatures.

The number of samples collected and analyzed was limited by time and funding. Increasing the number of samples collected would be a better reflection of overall microbial communities and changes in geochemistry throughout this portion of the aquifer. Some statistical analyses couldn’t be done due to a lack of microorganism samples with nitrate concentrations

above the standard (10 mg/L). There may be a small amount of contamination due to sample handling. Staphylococcus is a normal inhabitant of human skin and generally does not occur in groundwater systems, however, relative abundance of the genus Staphylococcus ranged from 0 to 0.19% for all samples. Some of our samples for stable isotope ratios of nitrate arrived at the facility broken and were unable to be analyzed.

Conclusions

Within the Great Bend Prairie aquifer and GMD 5, groundwater quality in the shallow portion of the aquifer has degraded over the past 30 to 40 years due to increases in salinity and nitrate. Nitrate concentrations were greater than the U.S. standard for public supplies of drinking water in seven of the samples we collected, compared to only two samples collected previously by Whittemore (1993). Stable nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios indicate that nitrate addition to the aquifer is primarily due to nitrification of ammonia-based fertilizers. Average TDS levels for wells screened within the shallow portion of the aquifer were higher on average in samples we collected, compared to previously collected samples. Mixing analysis suggests that salinity mostly varies in response to natural mixing between dilute recharge water and brine from the dissolution of evaporite minerals within the Permian bedrock. We did observe a decrease in chloride concentration for four sites that suggests flushing of initial oil brine contamination over time, and an increase in chloride concentration for two sites that may suggest that oil-brine spills or brine line leaks might have contributed to the chloride increase. We also observed a chloride increase in two sites that may be attributed to the irrigation return flow of fertilized fields affected by evapotranspiration.

Unlike other portions of the High Plains aquifer, nitrate accumulation does not appear to be increasing the mobility of uranium or selenium or any other trace elements we examined. A significant negative correlation between iron and nitrate, and a significant positive correlation between iron and chloride generally suggests that groundwater within the deeper portion is more reduced and has the potential to better support microbially mediated denitrification, compared to the shallow portion of the aquifer.

Microbial community analysis shows a diverse community of microorganisms are present. We see numerous phyla capable of aerobic respiration and other metabolic processes including denitrification, nitrification, iron oxidation, sulfate reduction, and even groups capable of methanogenesis and fermentation. Whether microbial communities have changed in response to surface inputs and changing groundwater geochemistry is unclear from the present analysis. More samples would be needed in order to accurately reflect microbial communities throughout the aquifer and to provide more statistical power for analysis.

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Appendix A - Supporting Information

Oxidation-Reduction Reactions

Microbial metabolism involves obtaining chemical energy from oxidation-reduction reactions which involve the transfer of electrons from one reactant to another. The energy obtained from these reactions can be stored within the cell, used for the construction of enzymes or nucleic acids, or for other basic life functions (Chappelle, 1993). In aquifers, microorganisms are generally distributed in zones based on the amount of energy obtained from redox reactions, also known as the thermodynamic ladder (Bethke et al., 2011). Traditional models for redox zonation generally assumes that species like oxygen, nitrate, iron (III), sulfate, and carbon dioxide are depleted sequentially (Bethke et al., 2011). Sometimes this traditional model is an oversimplification; it has been observed that iron reduction and methanogenesis can occur simultaneously as well as iron reduction and sulfate reduction (Bethke et al., 2011).

Bromide/Chloride Mass Ratios

Bromide/chloride mass ratios can be used to determine sources of groundwater salinity. Bromide and chloride are both halide ions with similar chemical properties in aqueous solutions. Both ions are conservative in groundwater, meaning they do not undergo decay or sorption processes, nor do they partake in oxidation-reduction reactions. Sorption processes in soil can change chloride and bromide concentrations, but in such low amounts that the effects of these processes are ignored when using these techniques to identify sources of chloride in groundwater (Whittemore, 1995).

Methods for identifying salinity sources are based on calculating mixing curves for bromide/chloride versus chloride plots while still considering additional geochemical data for

each individual well site like nitrate concentration (Whittemore, 1995). Mixing curves assume conservative mixing of varying concentrations of chloride and bromide for high and low chloride end points using the following equation (Whittemore, 1995):

$$C_{mix} = C_1 V + C_2(1 - V)$$

Where C_{mix}, C_1, and C_2 are concentrations of bromide or chloride in the two end points and in the mixture and V is the volume fraction (Whittemore, 1995). Figure A.1 shows the zone of natural mixing between freshwater and Permian saltwater from the dissolution of halite for groundwater within the Great Bend Prairie area (Whittemore, 1993).

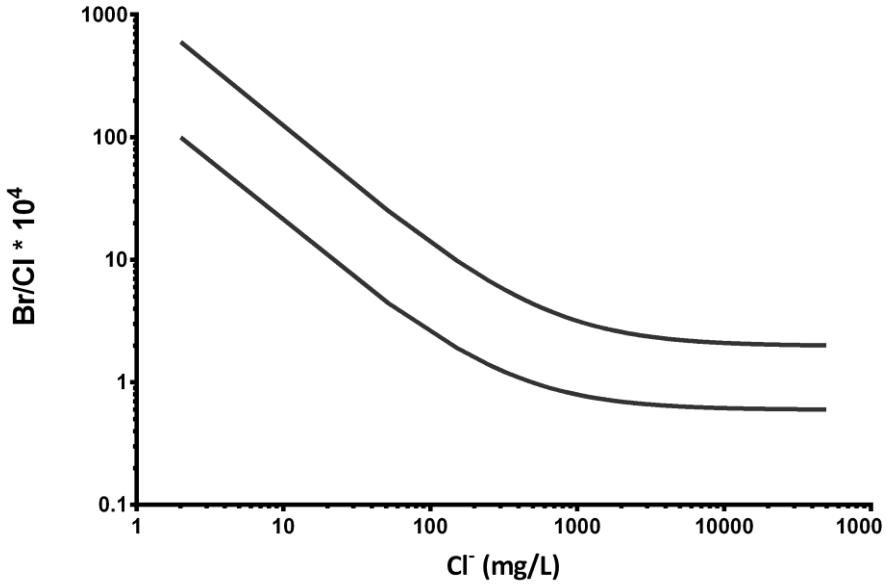


Figure A.3 | Two conservative mixing curves represent the zone of natural mixing between freshwater and Permian saltwater from the dissolution of halite within the Great Bend Prairie portion of the High Plains aquifer (Whittemore, 1993; Whittemore, 1995).

In our field area, natural saltwater from the dissolution of Permian evaporites (e.g. halite) will have low bromide/chloride mass ratios and high chloride concentrations due to the chemical composition of the salt. Oil brines in our study area generally have higher bromide/chloride ratios due to mixing with other evaporite affected water or bromide additions from the decomposition of organic matter during the formation of oil and gas (Fig. A.2) (Whittemore 1993). Samples collected by Whittemore (1993) fall primarily within a narrow band representing the natural mixing zone between freshwater and Permian saltwater from the dissolution of halite for the Great Bend Prairie area. Samples that plot outside of the narrow band represent additional contributions of chloride outside of the natural mixing expected for the area (e.g. oil brines or increases in salinity from evapotranspiration).

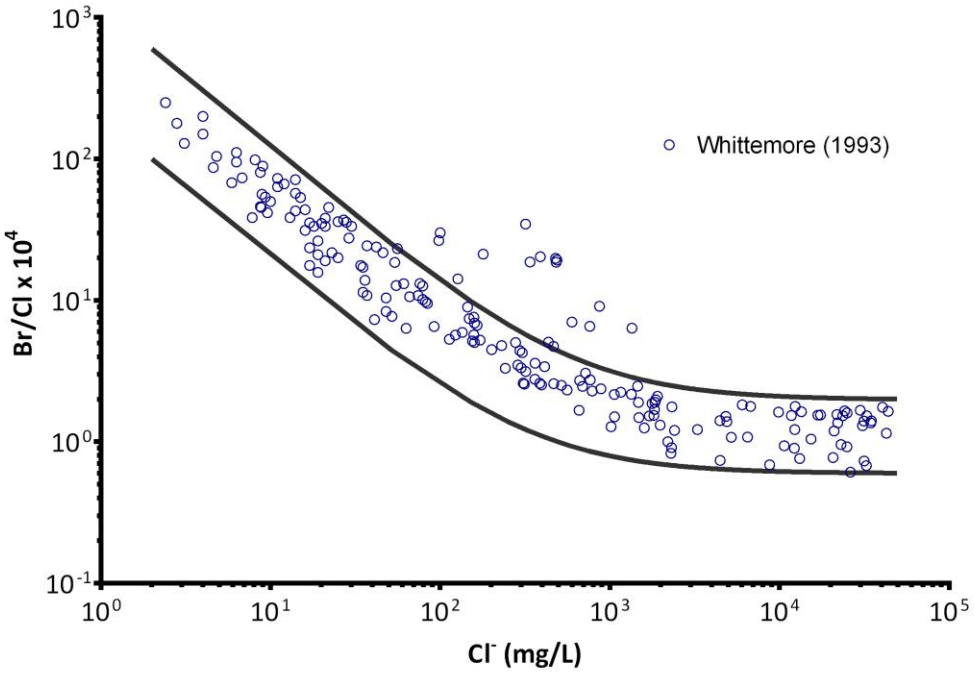
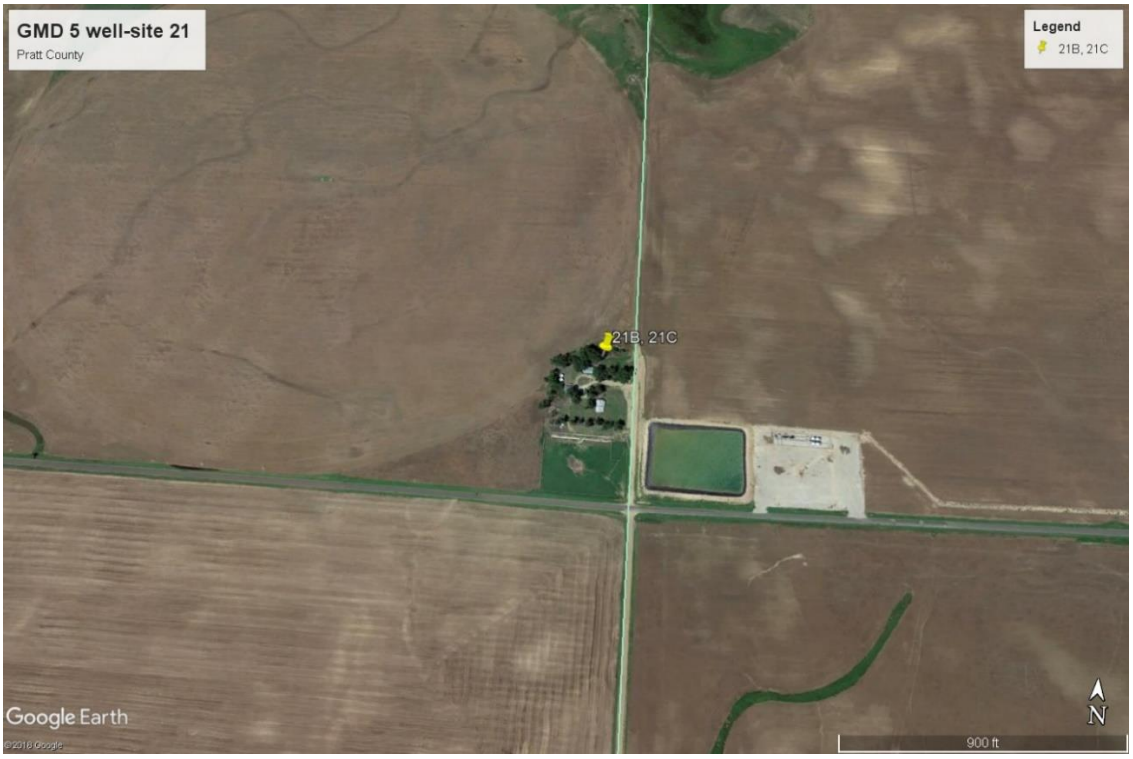


Figure A.4 | Groundwater samples collected by Whittemore (1993) generally fall within a narrow band representing the natural mixing between fresh groundwater and Permian saltwater from the dissolution of evaporite minerals. Modified from Whittemore (1993).

Appendix A – 2017 | Google Earth image well-site locations

Appendix A – 2017 | Google Earth image well-site locations



Appendix A – 2017 | Google Earth image well-site locations

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Appendix A – 2017 | Google Earth image well-site locations

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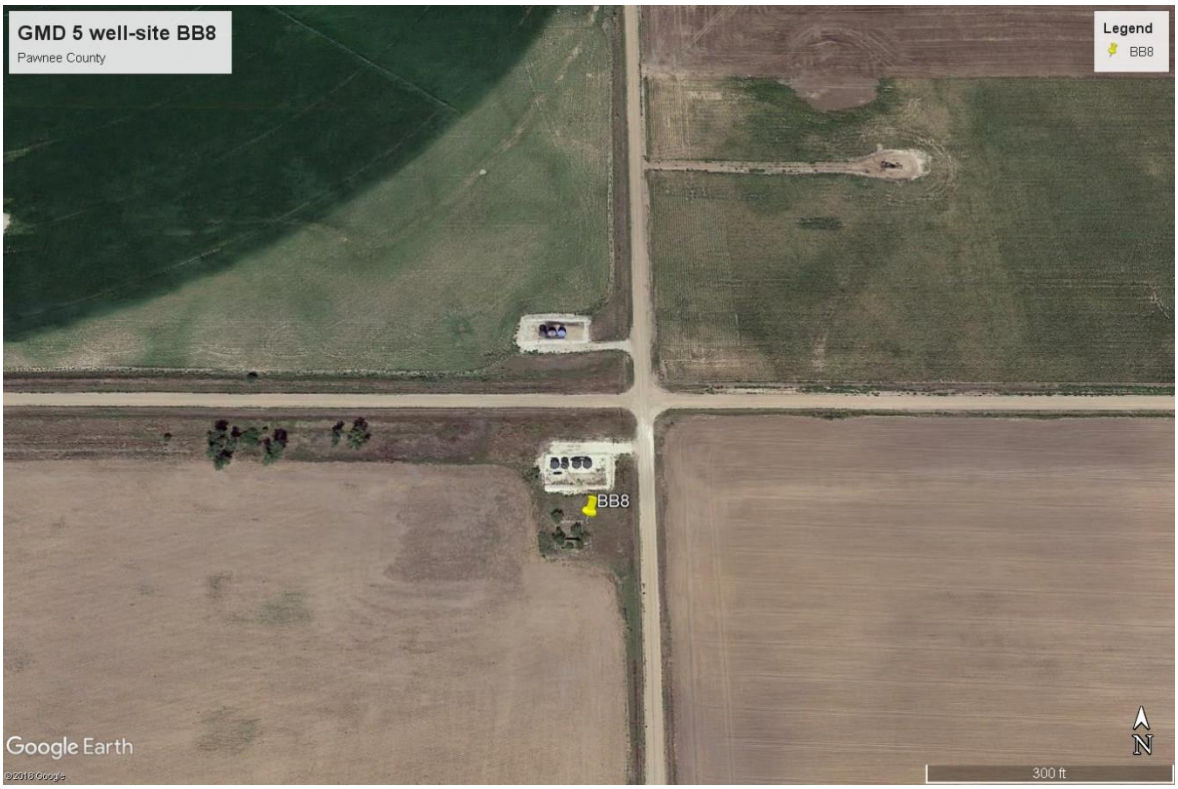
Appendix A – 2017 | Google Earth image well-site locations



Appendix B - Geochemistry

Sample	Date	Location	County	Depth to Water (ft)	Total Depth (ft)	Group	T (°C)	C (µS/cm)	pH (field)	pH (lab)
3A*	6/23/16	SEC36 T23S R13W	Stafford	32.83	155	deep	17.7	2170	7.5	7.08
3B*	6/23/16	SEC36 T23S R13W	Stafford	28.94	73	shallow	16.5	575	7.51	7.57
6B	6/3/16	SEC6 T25S R13W	Stafford	16.32	147	deep	16.9	873	7.43	7.38
6C	6/3/16	SEC6 T25S R13W	Stafford	20.93	70.5	shallow	16.3	1032	7.45	7.41
10C	6/22/16	SEC6 T24S R10W	Reno	24.68	110	deep	17.7	2780	7.33	7.12
10D	6/22/16	SEC6 T24S R10W	Reno	22.2	82	shallow	17.5	886	7.43	7.34
21B	5/31/16	SEC1 T26S R11W	Pratt	31.59	125.6	deep	16.3	6540	7.24	7.32
21C	5/31/16	SEC1 T26S R11W	Pratt	31.18	43.2	shallow	15.2	2170	6.81	6.78
26B	6/2/16	SEC1 T23S R10W	Reno	15.81	126.8	deep	16.1	36900	7.2	7.13
26C	6/1/16	SEC1 T23S R10W	Reno	12.33	65.1	shallow	15.8	2180	7.57	7.65
29B	6/2/16	SEC36 T24S R10W	Reno	43.2	128	deep	17.4	1650	7.76	7.65
29C	6/2/16	SEC36 T24S R10W	Reno	44.27	66.8	shallow	17.1	331	7.02	7.07
34A	6/1/16	SEC36 T25S R9W	Reno	11.27	54.15	bedrock	22.4	339	7.58	7.86
34B	6/1/16	SEC36 T25S R9W	Reno	10.25	35.65	shallow	16.6	373	7.58	7.71
35B	6/1/16	SEC31 T26S R10W	Reno	32.44	165	deep	17.4	2600	7.51	7.48
35C	5/31/16	SEC31 T26S R10W	Reno	29.04	73.8	shallow	16.1	759	7.16	7.09
36D	6/1/16	SEC6 T27S R12W	Pratt	34.21	97.2	shallow	16.1	811	7.33	7.2
42B	6/23/16	SEC1 T28S R13W	Pratt	20.6	167.5	deep	17.1	15040	7.31	6.9
42C	6/23/16	SEC1 T28S R13W	Pratt	15.79	106	shallow	16.6	901	7.43	6.99
50B	6/24/16	SEC6 T21S R13W	Stafford	28.59	136	deep	18.2	639	7.34	6.9
50C	6/24/16	SEC6 T21S R13W	Stafford	28.34	52.2	shallow	15.9	1095	7.02	7.14
51B	6/22/16	SEC36 T21S R14W	Stafford	21.35	106	shallow	15.9	484	7.29	6.86
52B	6/22/16	SEC6 T23S R13W	Stafford	34.33	109.3	shallow	17.4	483	7.34	7.37
BB8	6/23/16	SEC3 T22S R15W	Pawnee	53.18	150	deep	15.8	557	7.54	7.08

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Water Type	Alk (meq/L)	Alk as $CaCO_3$ (mg/L)	DO	DOC	Calcite	Silica**	TDS
3A*	Na-Cl	3.32	166.4	0.40	<0.4	1.478	20	1102.1
3B*	Ca-HCO_3	3.87	193.5	0.60	<0.4	1.468	16	316.0
6B	Ca-Cl	2.9	145.3	2.82	<0.4	1.414	23	446.9
6C	Ca-Cl	3.44	172	3.42	<0.4	1.624	20	541.5
10C	Na-Cl	4.13	206.5	0.40	0.80	1.284	56	1512.7
10D	Ca-Cl	3.83	191.5	0.70	0.65	1.646	16	488.4
21B	Na-Cl	3.65	182.5	3.00	<0.4	0.572	28	3497.8
21C	Ca-Cl	4.95	248	5.60	1.18	1.172	28	1087.1
26B	Na-Cl	3.99	199.9	0.37	<0.4	1.11	22	23002.3
26C	Na-Cl	5.69	284.6	3.10	n/a	0.4743	24	1139.5
29B	Na-Cl	2.36	118.2	4.30	n/a	1.212	25	848.0
29C	Ca-HCO_3	2.07	103.6	7.40	<0.4	0.1792	26	204.5
34A	Ca-HCO_3	2.68	134.1	8.30	0.41	0.7954	26	222.2
34B	Ca-HCO_3	2.4	120.3	9.20	<0.4	0.9443	25	231.5
35B	Na-Cl	4.17	208.6	6.10	<0.4	1.003	26	1315.3
35C	Ca-HCO_3	3.91	195.9	7.05	0.77	1.042	23	402.7
36D	Ca-HCO_3	4.27	213.8	7.00	0.57	1.615	24.8	439.3
42B	Na-Cl	2.2	110.1	0.10	<0.4	0.8619	18	9136.3
42C	Na-Cl	2.88	144.2	4.20	<0.4	0.9811	28	520.5
50B	Ca-HCO_3	3.52	176.3	0.60	0.86	1.139	25	402.0
50C	Ca-HCO_3	4.77	238.9	4.00	1.09	1.15	26	668.0
51B	Ca-HCO_3	3.04	152.2	2.65	<0.4	0.714	20	303.0
52B	Ca-HCO_3	3.65	182.7	3.40	0.41	1.133	21	277.9
BB8	Ca-HCO_3	3.68	184.4	0.55	<0.4	1.442	n/a	290.0

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.
**Dissolved silica values were estimated by using values obtained by Whittemore (1993).

Sample	F^-	Cl^-	Br^-	HCO_3^-	CO_3^{2-}	NO_2^-	NO_2^--N	NO_3^-	NO_3^--N	PO_4^{3-}	PO_4^{3-}-P	SO_4^{2-}
3A*	0.4	492.8	0.12	202.6	99.6	b.d.l.	b.d.l.	28.6	6.5	b.d.l.	b.d.l.	51.7
3B*	0.4	46.7	0.06	236.1	116.1	b.d.l.	b.d.l.	6.0	1.3	b.d.l.	b.d.l.	17.9
6B	0.3	158.2	0.12	176.9	87.0	b.d.l.	b.d.l.	7.3	1.6	b.d.l.	b.d.l.	19.6
6C	0.3	159.4	0.20	209.9	103.2	b.d.l.	b.d.l.	45.8	10.3	b.d.l.	b.d.l.	29.3
10C	0.5	676.9	0.32	252.0	123.9	b.d.l.	b.d.l.	18.5	4.2	b.d.l.	b.d.l.	88.9
10D	0.4	151.1	0.32	233.7	114.9	b.d.l.	b.d.l.	2.1	0.5	b.d.l.	b.d.l.	34.4
21B	0.5	1885.5	0.25	222.7	109.5	b.d.l.	b.d.l.	8.2	1.9	b.d.l.	b.d.l.	202.6
21C	0.2	340.1	0.09	302.0	148.5	b.d.l.	b.d.l.	231.5	52.3	b.d.l.	b.d.l.	22.3
26B	b.d.l.	12958.4	b.d.l.	243.4	119.7	b.d.l.	b.d.l.	39.8	9.0	b.d.l.	b.d.l.	1274.9
26C	0.6	427.4	0.32	347.1	170.7	b.d.l.	b.d.l.	31.3	7.1	b.d.l.	b.d.l.	52.9
29B	0.4	384.3	0.08	144.0	70.8	b.d.l.	b.d.l.	13.5	3.1	b.d.l.	b.d.l.	47.1
29C	0.3	8.6	0.02	126.3	62.1	b.d.l.	b.d.l.	35.5	8.0	b.d.l.	b.d.l.	14.1
34A	0.4	6.4	0.04	163.5	80.4	b.d.l.	b.d.l.	32.3	7.3	b.d.l.	b.d.l.	13.3
34B	0.4	5.1	0.03	146.4	72.0	b.d.l.	b.d.l.	51.3	11.6	b.d.l.	b.d.l.	15.2
35B	0.3	621.9	0.09	254.4	125.1	b.d.l.	b.d.l.	11.0	2.5	b.d.l.	b.d.l.	50.5
35C	0.2	82.0	0.04	238.5	117.3	b.d.l.	b.d.l.	41.6	9.4	b.d.l.	b.d.l.	13.6
36D	0.3	100.7	0.26	260.5	128.1	b.d.l.	b.d.l.	24.5	5.5	b.d.l.	b.d.l.	16.0
42B	0.9	4682.44	0.92	134.2	66.0	b.d.l.	b.d.l.	14.7	3.3	b.d.l.	b.d.l.	1028.2
42C	0.3	158.4	0.09	175.7	86.4	b.d.l.	b.d.l.	49.6	11.2	b.d.l.	b.d.l.	30.7
50B	0.5	50.6	0.12	214.8	105.6	b.d.l.	b.d.l.	48.0	10.8	b.d.l.	b.d.l.	36.4
50C	0.3	113.5	0.25	291.0	143.1	b.d.l.	b.d.l.	127.7	28.8	b.d.l.	b.d.l.	55.9
51B	0.5	17.5	0.09	185.5	91.2	b.d.l.	b.d.l.	55.6	12.6	b.d.l.	b.d.l.	19.8
52B	0.4	19.6	0.06	222.7	109.5	b.d.l.	b.d.l.	20.8	4.7	b.d.l.	b.d.l.	13.7
BB8	0.5	35.5	0.10	224.5	110.4	b.d.l.	b.d.l.	6.9	1.6	b.d.l.	b.d.l.	28.9

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Na^+	NH_4^+	NH_4^+-N	K^+	Ca^{2+}	Mg^{2+}	Sr^{2+}
3A*	302.2	b.d.l.	b.d.l.	14.9	82.5	4.2	4.9947
3B*	49.0	b.d.l.	b.d.l.	4.8	53.5	3.0	2.6311
6B	45.2	b.d.l.	b.d.l.	3.4	88.3	11.5	3.1385
6C	78.2	b.d.l.	b.d.l.	3.5	86.9	11.4	3.3422
10C	419.8	b.d.l.	b.d.l.	21.3	95.0	5.5	6.088
10D	78.3	b.d.l.	b.d.l.	8.3	78.0	4.4	b.d.l.
21B	1135.7	b.d.l.	b.d.l.	7.3	87.2	27.8	5.151
21C	43.6	b.d.l.	b.d.l.	1.8	239.2	21.2	10.412
26B	7816.9	b.d.l.	b.d.l.	51.4	525.2	163.8	30.31
26C	410.7	b.d.l.	b.d.l.	2.6	14.1	3.8	0.9573
29B	244.9	b.d.l.	b.d.l.	4.5	48.7	6.3	2.4105
29C	18.4	b.d.l.	b.d.l.	1.9	32.8	3.4	1.4442
34A	23.6	b.d.l.	b.d.l.	1.37	26.9	9.87425	1.6242
34B	11.7	b.d.l.	b.d.l.	1.0	43.3	4.7	1.8863
35B	417.6	b.d.l.	b.d.l.	3.9	46.4	12.4	b.d.l.
35C	23.2	b.d.l.	b.d.l.	1.7	89.5	6.8	3.7305
36D	40.8	b.d.l.	b.d.l.	3.9	88.1	8.0	3.9728
42B	2840.1	b.d.l.	b.d.l.	123.8	310.6	13.4	37.164
42C	90.8	b.d.l.	b.d.l.	7.6	62.3	3.2	3.2103
50B	57.0	b.d.l.	b.d.l.	9.0	66.7	3.1	b.d.l.
50C	48.5	b.d.l.	b.d.l.	3.9	127.4	15.8	5.7108
51B	36.0	b.d.l.	b.d.l.	4.3	54.6	3.3	b.d.l.
52B	23.7	b.d.l.	b.d.l.	4.5	61.3	3.5	b.d.l.
BB8	40.0	b.d.l.	b.d.l.	8.8	52.7	3.3	2.9227

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Li-7	B-11	Al-27	V-51	Cr-52	Mn-55	Fe-56	Co-59	Ni-60	Cu-63	Zn-64	As-75	Se-78	Rb-85	Mo-98	Cd-112	Ba-138	Pb-208	U-238
3A	29.19	77.74	31.07	1.03	1.50	947.07	55.63	0.30	5.71	7.17	59.23	0.91	-1.00	1.30	16.37	0.32	92.67	48.30	1.67
3B	13.71	48.22	27.87	2.83	0.80	31.99	23.70	-0.09	0.78	7.40	31.83	2.06	0.52	0.40	4.23	0.26	227.70	60.77	1.03
6B	20.22	61.12	31.73	3.20	1.03	1.74	2.83	0.24	1.94	4.97	31.20	0.72	3.32	1.47	4.47	0.25	247.40	9.67	2.83
6C	18.05	63.00	26.37	3.20	0.93	1.45	1.10	-0.13	0.68	4.43	12.37	0.54	1.44	0.97	3.11	0.18	269.97	18.77	3.30
10C	31.33	102.51	33.77	1.60	0.23	158.20	8.63	0.01	1.70	12.20	34.63	3.35	-0.49	5.37	9.38	0.28	134.20	70.87	1.43
10D	20.20	54.94	29.10	0.87	0.17	13.59	147.40	0.01	0.82	7.60	6.70	0.32	0.79	1.10	4.18	0.21	319.37	55.10	61.17
21B	50.18	195.60	31.03	5.83	3.90	3.15	92.33	0.43	18.79	10.10	4.87	1.86	4.51	1.47	8.21	0.20	54.97	16.63	1.60
21C	20.97	55.48	32.63	7.07	0.40	1.51	9.37	0.08	4.07	11.27	13.60	1.26	0.05	0.73	0.45	0.18	2457.33	25.37	2.70
26B	239.17	652.20	26.57	1.43	0.70	131.77	29.73	0.10	1.40	6.63	-16.93	0.90	2.71	6.60	3.16	0.44	39.33	17.93	4.20
26C	20.57	106.60	25.93	16.40	1.43	1.26	8.13	-0.11	1.73	6.30	-0.10	2.23	0.47	0.40	6.04	0.18	62.77	15.33	1.53
29B	22.09	59.88	57.07	10.70	8.10	1.00	2.63	0.13	0.61	6.93	4.80	1.48	2.49	2.17	2.82	0.15	118.60	14.90	1.70
29C	8.23	39.53	24.93	6.20	0.67	1.09	6.23	-0.08	1.20	4.50	22.43	1.75	0.47	0.27	0.73	0.20	306.93	20.53	0.20
34A	8.02	67.43	35.30	15.00	1.23	0.99	1.07	-0.11	0.72	4.83	31.23	4.48	2.84	0.77	0.74	0.18	487.40	18.13	1.10
34B	6.84	57.42	29.50	8.63	0.70	1.69	4.43	-0.13	0.76	4.47	52.10	1.93	2.19	0.50	0.44	0.30	518.53	20.23	0.87
35B	29.95	120.70	31.77	8.07	1.47	1.30	15.10	-0.10	1.57	5.60	34.10	1.79	3.13	0.87	2.36	0.22	105.97	12.83	1.77
35C	9.82	39.31	27.90	6.63	0.37	0.75	2.40	-0.08	1.09	4.90	16.93	1.26	0.12	0.60	0.48	0.14	1247.00	16.20	1.40
36D	23.32	67.82	30.03	8.63	1.10	1.21	-0.17	0.17	1.38	4.80	45.33	2.29	3.85	1.00	2.25	0.29	594.00	21.83	2.97
42B	123.17	298.07	29.93	0.83	1.30	169.43	622.87	0.64	2.23	8.20	-9.97	3.15	0.63	1.93	9.32	0.18	50.20	30.53	1.93
42C	12.57	66.31	29.13	4.70	0.93	3.88	0.47	-0.14	0.83	7.83	5.17	1.40	1.66	0.63	2.07	0.18	228.60	28.57	1.03
50B	11.51	69.54	28.63	0.30	0.07	132.63	1.03	-0.04	2.12	8.60	17.23	9.62	-1.39	0.93	8.33	0.14	102.67	49.03	0.70
50C	17.63	64.44	20.63	3.43	3.97	2.16	4.73	1.32	4.12	7.53	25.63	0.48	1.09	0.23	3.78	0.40	544.50	16.00	4.53
51B	17.13	66.89	29.60	1.33	0.23	1.15	-2.00	-0.11	0.59	5.33	25.83	0.27	10.56	0.63	3.67	0.23	279.40	49.87	1.90
52B	15.55	59.73	29.00	2.37	1.23	3.26	22.20	-0.06	3.16	6.73	40.37	0.91	2.51	0.47	6.26	0.14	368.13	47.13	3.67
BB8	16.70	71.20	31.47	1.63	0.70	11.04	27.70	-0.08	2.19	8.97	5.97	0.75	7.30	1.97	3.20	0.14	139.93	28.80	2.77

Units are in µg/L.
*Well 3 is labeled BB5H in the field.

	$\delta^{15}N \mid NO_3$ (AIR ± 0.3‰)		$\delta^{18}O \mid NO_3$ (VSMOW ± 0.8‰)	
Sample	Result	Repeat	Result	Repeat
3A*	-	-	-	-
3B*	3.63	3.94	3.64	3.53
6B	3.88	3.67	-1.63	-1.59
6C	2.74	-	7.82	-
10C	-	-	-	-
10D	0.71202123	1.54099339	11.30629363	11.8646494
21B	-	-	-	-
21C	5.28	-	8.96	-
26B	-	-	-	-
26C	8.09	8.14	4.49	4.76
29B	5.91	6.04	0.54	0.40
29C	4.38	4.60	3.53	3.65
34A	4.35	4.81	2.99	3.13
34B	3.73	3.63	4.80	5.26
35B	3.60	4.20	0.23	0.84
35C	3.89	3.83	3.80	4.04
36D	5.91	-	4.41	-
42B	2.50	2.63	7.84	8.92
42C	-	-	-	-
50B	-	-	-	-
50C	5.35	5.15	6.56	6.62
51B	2.62	2.51	4.83	3.77
52B	1.36	2.04	5.93	6.00
BB8	-	-	-	-

*Well 3 is labeled BB5H in the field.

Sample	Date	Group	pH (lab)	C (µS/cm)	Alk as $CaCO_3$	TDS
3A*	10/23/78	deep	7	1660	239	910
3B*	10/23/78	shallow	7.1	521	172	299
6B	10/24/78	deep	7.4	5010	507	3082
6C	10/24/78	shallow	7.8	695	129	404
10C	11/1/79	deep	7.8	2700	381	1457
10D	11/1/79	shallow	7.5	1020	297	n/a
21B	6/15/83	deep	7.9	8020	511	4513
21C	1/10/82	shallow	7.7	590	270	344
26B	11/21/83	deep	7.3	34500	1922	22850
26C	2/17/83	shallow	7.9	3170	97	1721
29B	12/8/83	deep	7.6	1500	100	818
29C	10/1/81	shallow	7.5	484	133	307
34A	5/24/83	bedrock	8.2	379	153	253
34B	1/4/83	shallow	8	388	161	224
35B	3/8/83	deep	7.6	2670	249	1476
35C	3/7/83	shallow	7.6	440	202	276
36D	12/23/82	shallow	7.6	475	155	296
42B	4/26/83	deep	7.9	14200	1546	8745
42C	4/26/83	shallow	8	650	191	387
50B	8/20/87	deep	8	600	n/a	n/a
50C	8/19/87	shallow	7.8	600	244	360
51B	8/6/87	shallow	7.8	500	193	303
52B	8/6/87	shallow	7.9	440	183	264

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Date	F^-	Cl^-	Br^-	HCO_3^-	NO_2^-	NO_2^--N	NO_3^-	NO_3^--N	PO_4^{3-}	PO_4^{3-}-P	SO_4^{2-}
3A*	10/23/78	0.5	382	b.d.l.	244	b.d.l.	b.d.l.	0.09	0.02	0.15	0.05	44
3B*	10/23/78	0.4	36	b.d.l.	245	b.d.l.	b.d.l.	0.22	0.05	0.52	0.17	18
6B	10/24/78	0.4	1660	b.d.l.	197	b.d.l.	b.d.l.	7.48	1.69	0.21	0.07	165
6C	10/24/78	0.6	99	b.d.l.	195	b.d.l.	b.d.l.	19.04	4.3	0.12	0.04	23
10C	11/1/79	0.6	677	b.d.l.	277	b.d.l.	b.d.l.	0.62	0.14	0.34	0.11	87
10D	11/1/79	0.4	192	b.d.l.	231	b.d.l.	b.d.l.	1.42	0.32	0.15	0.05	20
21B	6/15/83	0.3	2320	0.41	222	b.d.l.	b.d.l.	5.89	1.33	0.18	0.06	249
21C	1/10/82	0.3	29	b.d.l.	265	b.d.l.	b.d.l.	12.84	2.9	0.18	0.06	23
26B	11/21/83	0.3	12360	2.2	225	b.d.l.	b.d.l.	3.06	0.69	2.24	0.73	1320
26C	2/17/83	0.6	780	b.d.l.	324	0.049	0.015	2.70	0.61	1.01	0.33	73
29B	12/8/83	0.4	386	0.1	74	b.d.l.	b.d.l.	10.01	2.26	0.09	0.03	40
29C	10/1/81	0.4	45	b.d.l.	164	b.d.l.	b.d.l.	27.02	6.1	n/a	n/a	23
34A	5/24/83	0.4	4	0.06	147	b.d.l.	b.d.l.	53.15	12	0.18	0.06	18
34B	1/4/83	0.3	2.5	b.d.l.	124	b.d.l.	b.d.l.	0.09	0.02	0.21	0.07	59
35B	3/8/83	0.4	689	0.17	248	b.d.l.	b.d.l.	8.02	1.81	0.12	0.04	59
35C	3/7/83	0.3	11	b.d.l.	219	b.d.l.	b.d.l.	24.80	5.6	0.31	0.1	16
36D	12/23/82	0.3	29	0.08	223	b.d.l.	b.d.l.	3.94	0.89	0.31	0.1	22
42B	4/26/83	0.4	4470	0.33	151	b.d.l.	b.d.l.	0.09	0.02	0.49	0.16	730
42C	4/26/83	0.3	77	b.d.l.	188	b.d.l.	b.d.l.	16.83	3.8	0.18	0.06	29
50B	8/20/87	b.d.l.	37	0.09	n/a	b.d.l.	b.d.l.	n/a	n/a	n/a	n/a	b.d.l.
50C	8/19/87	0.3	42	0.1	213	b.d.l.	b.d.l.	46.95	10.6	0.46	0.15	17
51B	8/6/87	0.4	20	0.07	242	b.d.l.	b.d.l.	11.96	2.7	0.08	0.026	20
52B	8/6/87	0.4	17	0.03	223	b.d.l.	b.d.l.	7.79	1.76	0.44	0.144	11

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Date	Na^+	NH_4^+	NH_4^+-N	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}
3A*	10/23/78	252	n/a	n/a	2.9	12	76	0.6
3B*	10/23/78	40	n/a	n/a	2.8	5.4	60	0.3
6B	10/24/78	940	n/a	n/a	5.1	31	152	1.4
6C	10/24/78	96	n/a	n/a	2.7	5.2	43	0.03
10C	11/1/79	412	n/a	n/a	3.9	21	118	1
10D	11/1/79	87	n/a	n/a	3.7	11	101	0.6
21B	6/15/83	1610	0.13	0.1	5.6	32	152	1.1
21C	1/10/82	15	0.13	0.1	1.3	6.7	97	0.3
26B	11/21/83	8350	0.13	0.1	n/a	148	526	7.2
26C	2/17/83	643	0.13	0.1	4.2	6.6	28	0.2
29B	12/8/83	272	0.26	0.2	8.7	3.2	35	1.4
29C	10/1/81	53	n/a	n/a	2.2	4.4	46	0.2
34A	5/24/83	25	b.d.l.	b.d.l.	1.4	13	40	0.2
34B	1/4/83	14	0.13	0.1	1	5.2	56	0.2
35B	3/8/83	478	b.d.l.	b.d.l.	3.6	15	75	0.5
35C	3/7/83	15	0.13	0.1	1.5	5.3	72	0.2
36D	12/23/82	43.6	0.10	0.08	3.2	4.4	54.9	0.21
42B	4/26/83	2900	0.13	0.1	6.3	121	420	5.5
42C	4/26/83	69	0.13	0.1	2	6.3	66	0.3
50B	8/20/87	n/a	n/a	n/a	n/a	n/a	n/a	n/a
50C	8/19/87	27	0.13	0.1	4.1	9.6	82	0.3
51B	8/6/87	34	0.13	0.1	4.1	5.7	68	0.3
52B	8/6/87	23	0.13	0.1	4.1	5.1	65	0.3

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Group	Year	pH (lab)	C (µS/cm)	Alkalinity as $CaCO_3$	TDS
Δ 3A*	deep	38	0.08	510	-72.6	192.1
Δ 3B*	shallow	38	0.47	54	21.5	17.0
Δ 6B	deep	38	-0.02	-4137	-361.7	-2635.1
Δ 6C	shallow	38	-0.39	337	43	137.5
Δ 10C	deep	37	-0.68	80	-174.5	55.7
Δ 10D	shallow	37	-0.16	-134	-105.5	n/a
Δ 21B	deep	33	-0.58	-1480	-328.5	-1015.2
Δ 21C	shallow	34	-0.92	1580	-22	743.1
Δ 26B	deep	33	-0.17	2400	-1722.1	152.3
Δ 26C	shallow	33	-0.25	-990	187.6	-581.5
Δ 29B	deep	33	0.05	150	18.2	30.0
Δ 29C	shallow	35	-0.43	-153	-29.4	-102.5
Δ 34A	bedrock	33	-0.34	-40	-18.9	-30.8
Δ 34B	shallow	33	-0.29	-15	-40.7	7.5
Δ 35B	deep	33	-0.12	-70	-40.4	-160.7
Δ 35C	shallow	33	-0.51	319	-6.1	126.7
Δ 36D	shallow	34	-0.4	336	58.8	143.3
Δ 42B	deep	33	-1	840	-1435.9	391.3
Δ 42C	shallow	33	-1.01	251	-46.8	133.5
Δ 50B	deep	29	-1.1	39	n/a	n/a
Δ 50C	shallow	29	-0.66	495	-5.1	308.0
Δ 51B	shallow	29	-0.94	-16	-40.8	0
Δ 52B	shallow	29	-0.53	43	-0.3	13.9

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	F^-	Cl^-	Br^-	HCO_3	NO_2^-	NO2-N	NO_3^-	NO3-N	PO_4^{3-}	PO4-P	SO_4^{2-}
3A*	-0.06	110.78	n/a	-41.45	b.d.l.	b.d.l.	n/a	6.43	b.d.l.	b.d.l.	7.74
3B*	0.01	10.69	n/a	-8.89	b.d.l.	b.d.l.	n/a	1.30	b.d.l.	b.d.l.	-0.12
Δ 6B	-0.08	-1501.85	n/a	-20.07	b.d.l.	b.d.l.	n/a	-0.05	b.d.l.	b.d.l.	-145.42
Δ 6C	-0.28	60.39	n/a	14.87	b.d.l.	b.d.l.	n/a	6.04	b.d.l.	b.d.l.	6.35
Δ 10C	-0.09	-0.07	n/a	-25.03	b.d.l.	b.d.l.	n/a	4.04	b.d.l.	b.d.l.	1.95
Δ 10D	0.03	-40.85	n/a	2.67	b.d.l.	b.d.l.	n/a	0.15	b.d.l.	b.d.l.	14.43
Δ 21B	0.21	-434.51	-0.16	0.69	b.d.l.	b.d.l.	n/a	0.53	b.d.l.	b.d.l.	-46.37
Δ 21C	-0.06	311.10	n/a	37.00	b.d.l.	b.d.l.	n/a	49.38	b.d.l.	b.d.l.	-0.66
Δ 26B	n/a	598.35	n/a	18.43	b.d.l.	b.d.l.	n/a	8.30	b.d.l.	b.d.l.	-45.10
Δ 26C	0.04	-352.57	n/a	23.15	b.d.l.	b.d.l.	n/a	6.47	b.d.l.	b.d.l.	-20.05
Δ 29B	-0.04	-1.73	-0.02	69.98	b.d.l.	b.d.l.	n/a	0.80	b.d.l.	b.d.l.	7.10
Δ 29C	-0.14	-36.42	n/a	-37.71	b.d.l.	b.d.l.	n/a	1.92	b.d.l.	b.d.l.	-8.85
Δ 34A	0.00	2.39	-0.02	16.51	b.d.l.	b.d.l.	n/a	-4.70	b.d.l.	b.d.l.	-4.65
Δ 34B	0.06	2.64	n/a	22.42	b.d.l.	b.d.l.	n/a	11.55	b.d.l.	b.d.l.	-43.80
Δ 35B	-0.06	-67.07	-0.08	6.41	b.d.l.	b.d.l.	n/a	0.67	b.d.l.	b.d.l.	-8.51
Δ 35C	-0.06	71.00	n/a	19.55	b.d.l.	b.d.l.	n/a	3.80	b.d.l.	b.d.l.	-2.37
Δ 36D	-0.03	71.69	0.18	37.51	b.d.l.	b.d.l.	n/a	4.64	b.d.l.	b.d.l.	-5.98
Δ 42B	0.54	212.44	0.59	-16.78	b.d.l.	b.d.l.	n/a	3.29	b.d.l.	b.d.l.	298.23
Δ 42C	-0.03	81.43	n/a	-12.29	b.d.l.	b.d.l.	n/a	7.39	b.d.l.	b.d.l.	1.70
Δ 50B	n/a	13.62	0.03	n/a	b.d.l.	b.d.l.	n/a	n/a	b.d.l.	b.d.l.	n/a
Δ 50C	-0.04	71.53	0.15	78.02	b.d.l.	b.d.l.	n/a	18.24	b.d.l.	b.d.l.	38.90
Δ 51B	0.10	-2.52	0.02	-56.53	b.d.l.	b.d.l.	n/a	9.85	b.d.l.	b.d.l.	-0.18
Δ 52B	0.00	2.59	0.03	-0.31	b.d.l.	b.d.l.	n/a	2.94	b.d.l.	b.d.l.	2.66

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

Sample	Na^+	NH_4^+	NH4-N	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}
3A*	50.23	n/a	n/a	12.00	-7.81	6.52	4.39
3B*	9.03	n/a	n/a	1.98	-2.38	-6.53	2.33
Δ 6B	-894.83	n/a	n/a	-1.71	-19.54	-63.73	1.74
Δ 6C	-17.84	n/a	n/a	0.84	6.20	43.85	3.31
Δ 10C	7.77	n/a	n/a	17.36	-15.53	-23.00	5.09
Δ 10D	-8.67	n/a	n/a	4.64	-6.60	-22.96	
Δ 21B	-474.34	n/a	n/a	1.71	-4.17	-64.79	4.05
Δ 21C	28.64	n/a	n/a	0.54	14.54	142.18	10.11
Δ 26B	-533.08	n/a	n/a	n/a	15.80	-0.83	23.11
Δ 26C	-232.34	n/a	n/a	-1.62	-2.76	-13.93	0.76
Δ 29B	-27.07	n/a	n/a	-4.17	3.09	13.70	1.01
Δ 29C	-34.64	n/a	n/a	-0.35	-0.96	-13.18	1.24
Δ 34A	-1.37	n/a	n/a	-0.03	-3.13	-13.14	1.42
Δ 34B	-2.34	n/a	n/a	-0.02	-0.49	-12.74	1.69
Δ 35B	-60.40	n/a	n/a	0.33	-2.56	-28.56	
Δ 35C	8.18	n/a	n/a	0.19	1.46	17.52	3.53
Δ 36D	-2.81	n/a	n/a	0.70	3.57	33.15	3.76
Δ 42B	-59.89	n/a	n/a	117.48	-107.60	-109.39	31.66
Δ 42C	21.82	n/a	n/a	5.57	-3.14	-3.69	2.91
Δ 50B	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Δ 50C	21.47	n/a	n/a	-0.24	6.23	45.42	5.41
Δ 51B	2.04	n/a	n/a	0.23	-2.37	-13.37	n/a
Δ 52B	0.67	n/a	n/a	0.35	-1.63	-3.72	n/a

Units are in mg/L unless noted.
*Well 3 is labeled BB5H in the field.

ICS-1100 Ion Chromatograph (IC) Detection Limits

	F^-	Cl^-	Br^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}
MDL	0.06	1.54	0.08	0.13	0.016	0.48	0.71	0.22	0.13	0.16	0.19	0.50	0.17

Units are in mg/L unless noted.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) Detection Limits

Sample	Li-7	B-11	Al-27	V-51	Cr-52	Mn-55	Fe-56	Co-59	Ni-60	Cu-63	Zn-64	As-75	Se-78	Rb-85	Mo-98	Cd-112	Ba-138	Pb-208	U-238
DL	0.0918	0	0.48	0.0053	0.023	0.019	0.188	0.0104	0.0936	0.013	4.16	0.0114	1.074	0.002	0.0032	0.0026	0.006	0.285	0.0006

Units are in µg/L.

Appendix C - Microorganism Analysis

DNA Extraction

Total microbial community DNA was extracted from 17 filtered microorganism samples using a Power Soil ® DNA Isolation Kit (MO BIO). DNA extraction included a summarized and modified 20-step procedure using solutions (C1-C6) included in the Power Soil ® DNA Isolation Kit; the 20-step process was completed for each sample. The detailed extraction procedure was modified for this individual procedure by Kirk et al., 2015 and is as follows (MO BIO Laboratories, Inc., 2016.):

1. The MCE filter membranes were shredded on a sterile petri dish using a sterilized scalpel. The shredded filter material was then placed inside a PowerBead tube provided in the kit. The PowerBead tube contained a buffer that began the dissolution of humic acids and protected nucleic acids from degradation.
2. The PowerBead tube was then gently vortexed.
3. Making sure that there were no preexisting precipitates in Solution C1, 60 µL of the solution was added to the PowerBead tube. Solution C1 contained SDS (sodium dodecyl sulfate) and other detergents required for cell lysis that break down fatty acids and lipids associated with the cell membrane of many organisms.
4. The modified Althernative Lysis Methods section of the manual is as follows: The Power Bead tube was then vortexed for 2 minutes and heated to 70°C for 10 minutes. Then vortexed for 2 minutes and heated to 70°C for 10 minutes and then vortexed another 2 minutes.
5. After vortexing, the PowerBead tubes were centrifuged at 10,000 x g for 30 seconds at room temperature.
6. PowerBead tubes were removed from the centrifuge and supernatant was transferred to a sterile 2 mL collection tube.
7. 250 µL of solution C2 was added to the sterile 2 mL collection tube. Solution C2 included a patented Inhibitor Removal Technology ® that includes a reagent to precipitate non-DNA organic/inorganic material, e.g. humic substances, cell

debris, and proteins. The 2 mL tubes were then vortexed for 5 seconds and incubated at 4°C for 5 minutes.

8. The tubes were then centrifuged at 10,000 x g for 1 minute at room temperature. This process left a small pellet at the bottom of the 2 mL tube that was white and gel-like in appearance which contained non-DNA organic/inorganic materials.
9. Avoiding the pellet, up to but no more than 600 µL of supernatant was then moved to a clean 2 mL collected tube.
10. 200 µL of solution C3 was added to the 2 mL collection tube. This solution was similar to solution C2 and is the second process to remove any additional non-DNA organic and inorganic material. The tube was then vortexed briefly and incubated at 4°C for 5 minutes.
11. The tubes were then centrifuged at 10,000 x g for 1 minute at room temperature and up to but no more than 750 µL of supernatant was transferred to a clean 2 mL collection tube, avoiding the pellet.
12. Solution C4 was gently shook before use and 1,200 µL of the solution was added to the supernatant and vortexed for 5 seconds. Solution C4 is a highly concentrated salt solution which allows the binding of DNA, but not the binding of non-DNA organic/inorganic materials which may still be present.
13. Approximately 670 µL was then loaded onto a Spin Filter (included in the kit) and centrifuged at 10,000 x g for 1 minute at room temperature. The Spin Filter, which can be removed from the tube, was carefully removed using sterile tweezers and the flow through was discarded. The Spin Filter was then replaced in the tube and this process was repeated a total of three times. In this process the DNA is selectively bound to the silica membrane in the Spin Filter from the highly concentrated salt solution. DNA is bound to the filter membrane and non-DNA organic/inorganic materials can pass through.
14. 500 µL of Solution C5 was added to the Spin Filter and the tube was centrifuged at 10,000 x g for 30 seconds at room temperature. Solution C5 is an ethanol based wash solution used to clean the DNA bound to the silica filter membrane in the Spin Filter. This washed any remaining salt, humic acid, and other

contaminates, and allowed the DNA to stay bound to the silica membrane. After samples were centrifuged, the flow through was discarded.

15. The samples were then centrifuged again at 10,000 x g for 1 minute at room temperature to remove any residual Solution C5.
16. The Spin Filter was then removed and placed in a sterile 2 mL collection tube, while careful not to splash remaining C5 solution on the spin filter.
17. 75 µL of Solution C6 was added to the center of the filter membrane, making sure that the entire filter membrane was saturated. This solution released the DNA from the Spin Filter. The DNA that was bound in the highly concentrated salt solution is released by Solution C6 which is salt lacking.
18. The collection tube was then centrifuged at 10,000 x g for 30 seconds at room temperature.
19. The Spin Filter was discarded and the collection tube contained the DNA ready for any downstream use.
20. The DNA extractions were stored frozen (-20° to -80°C).

DNA Sequencing

Raw sequencing data was processed using the Python 2 software QIIME (Quantitative Insights into Microbial Ecology) v. 1.8.0 (Caporaso et al. 2010; Kirk et al., 2015). Several steps for the installation of QIIME Virtual Box, QIIME, and subsequent script necessary for the processing of sequencing data are as follows:

1. The QIIME Virtual Box was downloaded and installed using the QIIME Virtual Box installation instructions (QIIME, 2015). The QIIME Virtual Box provides a functioning QIIME full install inside a Ubuntu Linux virtual machine. QIIME was initialized through the QIIME Virtual Box and the command window was opened.
2. Demultiplexing – *split_libraries.py* – This command used the raw data provided by MR DNA® Laboratory to identify low-quality sequences and extract out only the samples found in the mapping file provided by MR DNA® Laboratory.
3. Picking out the Operational Taxonomic Unit (OTU) – *pick_dsumme_novo_otus.py* – This command aligns the sequences, bins them into OTUs, creates a phylogenetic tree using

FastTree, and assigns a consensus taxonomy to each OTU. The output file for this command in a BIOM-formatted OTU table that is used in many later analyses.

4. Filtering OTUs – *filter_otus_from_otu_table.py* – This command removes OTUs with only one representative sequence and leaves OTUs with at least two sequences.
5. Summarize the taxonomy of sequences in each sample – summarize_taxa_through_plots.py – This command outputs HTML-formatted charts that display the taxonomy of each sample (kingdom, phylum, class, order, family, genus). The raw data is output in tab-delimited text files. By summarizing the taxonomy of sequences in each sample we can look at the relative abundance, or the percentage of a particular microorganism relative to the total number of microorganisms within the aquifer, for each well-site.
6. Evaluating alpha diversity – *alpha_rarefaction.py* – This command generated rarefied OTU tables, computed measures of alpha diversity (the diversity within a sample) for each rarefied OTU table, collated alpha diversity results and generated alpha rarefaction plots.

Appendix D - Relative Abundance Data

Raw sequencing data will be uploaded into the MG RAST database and the information needed to access the data will be available if requested.