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Impact of commercial natural gas production on geochemistry and microbiology in a shale-gas reservoir

Matthew F. Kirk[1,5,*], Anna M. Martini[1], Daniel O. Breecker[2], Daniel R. Colman[3], Cristina Takacs-Vesbach[3], Steven T. Petsch[4]

[1]Department of Geology, Amherst College, Amherst, MA 01002, USA

[2]Department of Geological Sciences, University of Texas, Austin, TX 78712, USA

[3]Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

[4]Department of Geosciences, University of Massachusetts Amherst, Amherst, MA 01003, USA

[5]Current address: Department of Geology, Kansas State University, Manhattan, KS 66506-3201

[*]Corresponding author: matthew.f.kirk@gmail.com, office +1 785-532-6724, fax +1 785-532-5159

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Abstract [310 words]

We consider the effect that commercial gas production has had on microbiology and water and gas geochemistry in the northern producing trend of the Antrim Shale, an unconventional gas reservoir in the Michigan Basin, USA. We analyzed gas, water, and microbial biomass samples collected from seven wells in 2009 and compared our findings to the result of analyses performed as early as 1991 on samples collected from the same wells. We also examined production records associated with six wells. Water production has decreased sharply over time and is currently at 0.2 to 14.6% of peak levels. While this has happened, the chemical and isotopic composition of gas and water produced from the wells has shifted. The proportion of CO_2 has increased by as much as 15 mole% while CH_4 content has correspondingly decreased. Isotopically, the $\delta^{13}C$ and δD values of CH_4 decreased for most wells by averages of 1.3‰ and 9‰, respectively, while $\delta^{13}C$ values of CO_2 increased for most wells by an average of 1.7‰. Alkalinity in the water from each well decreased by 10 mM on average and SO_4^{2-} content increased from below 50 μM to over 200 μM on average in water from each well with initial values. Microorganisms most closely related to CO_2-reducing methanogens were the most abundant group in archaeal clone libraries and SO_4^{2-} reducers were the most abundant group in bacterial libraries. In contrast, no SO_4^{2-} reducers were identified in a nucleic acid-based analysis of a sample collected in 2002 from one of the wells we sampled. Our results show that commercial gas production has not only caused chemical and isotopic changes in water and gas in the Antrim Shale but also an increase in the abundance of SO_4^{2-}-reducing microorganisms, a change that can ultimately have a negative impact on biogenic CH_4 formation. Processes that can explain these changes include ongoing biogeochemical reactions, groundwater flow, gas desorption, and open-system degassing.

Keywords: sulfate reduction, methanogenesis, Antrim Formation, Michigan Basin, unconventional natural gas reservoir, black shale

1. Introduction

Unconventional natural gas reservoirs such as fractured organic-rich shale are becoming increasingly important energy resources. Natural gas provides a major source of energy for the U.S., accounting for more than 20% of the energy supply (NETL, 2009). The rate of gas consumption, however, increasingly exceeds the rate of domestic production. Greater production from fractured organic-rich shale can help make up some of this imbalance (NETL, 2009). Moreover, although carbon dioxide (CO_2) is emitted during gas combustion, natural gas is the cleanest fossil fuel. Compared to coal, for example, natural gas combustion emits about half as much CO_2 per joule of energy produced, as well as far lower NO_x, SO_x, heavy metals and particulate matter. Producing a greater percentage of our energy from natural gas at the expense of oil and coal, therefore, would be environmentally advantageous (White et al., 2003).

In many unconventional natural gas reservoirs, a significant portion of the gas formed biologically as microbial communities degraded sedimentary organic matter (e.g., Bates et al., 2011; Flores et al., 2008; Formolo et al., 2008; Martini et al., 1996; McIntosh et al., 2008; McIntosh et al., 2002; Schlegel et al., 2011; Scott et al., 1994; Strąpoć et al., 2008; Su et al., 2005; Waldron et al., 2007; Warwick et al., 2008). Biological formation of methane (CH_4), the primary component of natural gas, requires a consortium of microorganisms. Fermentative and syntrophic *Bacteria* degrade complex organic matter and ultimately produce substrates that can be used by methanogenic *Archaea* as energy sources (Conrad, 1999). Methanogens primarily use

65 acetate (CH_3COO^-) or dihydrogen (H_2) as their substrate (Conrad, 1999), producing CH_4 by
66 acetate fermentation or CO_2 reduction, respectively:

67 $$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad (1)$$

68 $$CO_2 + 4\ H_2 \rightarrow 2\ H_2O + CH_4 \quad (2)$$

69 How these microbial processes are affected by commercial production of natural gas is
70 unclear. Gas is stored in shale reservoirs primarily by adsorption to the solid matrix (Scott et al.,
71 1994). To harvest the gas, water is pumped out of the formation, which lowers pressure adjacent
72 to the borehole and allows the gas to desorb (Martini et al., 2003). We hypothesize this process
73 could impact microbial activity by drawing water into the shale that has a different composition
74 than the water present before development. Such changes may affect subsurface microbes
75 because, while microbes affect the composition of their environment by driving reactions
76 forward, the environment also influences microbial activity by providing electron donors and
77 acceptors and other nutrients (Jin and Bethke, 2007). Potential shifts in water composition driven
78 by pumping, therefore, may impact microbial activity and ultimately CH_4 formation.

79 This study examines how commercial production of natural gas has affected
80 geochemistry and microbiology in the Devonian Antrim Shale along the northern margin of the
81 Michigan Basin. Waldron et al. (2007) found evidence that commercial gas production there is
82 causing SO_4^{2-} concentration to increase, a change that could negatively impact methanogenesis.
83 This finding warrants further study to fully evaluate how geochemistry has changed and identify
84 consequences for microbial activity, information that has implications for the sustainability of
85 gas production in unconventional gas reservoirs. The Antrim Shale provides an ideal field site to
86 examine this question; the formation was one of the earliest shale-gas reservoirs to be developed
87 (Curtis, 2002) and conditions soon after widespread development are well documented (see data

88 available in Martini et al., 1998). Furthermore, most of the gas produced commercially along the
89 northern edge of the basin (i.e., the northern producing trend) is biogenic (Martini et al., 1996;
90 Martini et al., 1998). Our analysis is constrained by data gathered soon after widespread
91 development of the northern producing trend in the early 1990s, data collected from one well in
92 2002, and data we collected in 2009.
93
94 **2. Materials and methods**
95 *2.1. Commercial gas wells*
96 We selected seven wells along the northern producing trend that had originally been
97 sampled in the early 1990s and re-sampled them during January, 2009. One well, ID# 150, was
98 also sampled again in 2002. Data collected from the initial set of samples were published in
99 Martini et al. (1996; 1998) and Walter et al. (1996). Data from 2002 samples were published in
100 Martini et al. (2005) and Formolo et al. (2008). Site numbers used in this study are consistent
101 with those used in Martini et al. (1998), with the exception of two wells, B and M, which were
102 not included in that publication.
103 Information about each well is summarized in the Supplemental Content (Table SC1). An
104 annotated map showing the location of each well accompanies the online version of this article.
105 Additional maps showing regional variation in pore water composition are available in Martini et
106 al. (1998) and Waldron et al. (2007).
107
108 *2.2. Sample collection*
109 Temperature and pH measurements were made in the field for a subset of wells. Gas
110 samples were collected for compositional and isotopic analyses in Isotubes® (Isotech

111 Laboratories, Inc.). Water samples were collected for chemical and isotopic analyses and
112 microbial analyses in acid-washed and sterile bottles, respectively. Chemical and isotopic
113 samples were filtered using 0.22 μm nylon syringe filters. Cation samples were preserved at pH
114 < 2 with trace-metal grade HNO_3. Microbial biomass samples were collected by filtering water
115 through sterile 25 mm 0.22 μm mixed cellulose-ester filter membranes. The samples were stored
116 in sterile 2 mL microcentrifuge tubes and preserved with 0.2 mL of sucrose lysis buffer
117 (Giovannoni et al., 1990). All sample filtration and preservation was performed within 12 hours
118 of sample collection rather than immediately in the field due to adverse weather conditions.
119 Samples were stored on ice in the field. In the lab, water samples were stored at 4°C and
120 microbial samples at –20°C.
121
122 *2.3. Microbial analysis*
123 Microbial biomass samples collected from wells 22, 147, and 150 were selected for
124 nucleic acid-based analysis. These wells were selected because they produce water with high,
125 intermediate, and low salinity. Previous research has shown that salinity is an important
126 constraint on microbial community composition in the northern producing trend (Waldron et al.,
127 2007). These wells, therefore, allow us to examine microbial communities across the range of
128 geochemical conditions present. Microbial biomass was also previously sampled from well 150
129 in 2002 and analyzed using methods similar to those we employed, which are described in
130 Formolo et al. (2008).
131 DNA was extracted from the filters using a MoBio ultra-clean soil DNA kit. The
132 alternative protocol described by the manufacturer was used to limit DNA shearing during the
133 extraction. 16S rRNA genes were amplified from the environmental DNA using universal

134 primers 8F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT
135 ACG ACT T-3') and archaeal primers 109F (5'-ACK GCT CAG TAA CAC GT-3') and 915R
136 (5'-GTG CTC CCC CGC CAA TTC CT-3') (Grosskopf et al., 1998). PCR products were
137 purified using a Wizard DNA gel purification kit and ligated into a TOPO TA vector. Cloning
138 and sequencing was performed at the Washington University Genome Center. For each
139 sample/primer pair, partial sequences were collected from 96 clones. Low quality sequence reads
140 were excluded from subsequent analyses, leaving 213 bacterial sequences and 239 archaeal
141 sequences, which all exceeded 300 bp in length.
142 Sequences were aligned using the Greengenes NAST aligner (DeSantis et al., 2006a) and
143 checked for chimeras using Bellerophon (DeSantis et al., 2006b). Operational taxonomic units
144 (OTUs) were defined at ≥97% sequence identity using mothur (Schloss et al., 2009). Mothur was
145 also used to identify representative sequences for each OTU and calculate rarefaction curves and
146 Chao1 values for each clone library, which provide a measure of richness defined at the OTU
147 level (Hughes et al., 2001). To evaluate which bacterial and achaeal groups were present in the
148 samples, the taxonomy of representative sequences for each OTU was assessed using a naïve
149 Bayesian rRNA classifier and an 80% confidence threshold (Wang et al., 2007). We also
150 employed this same procedure to classify sequences obtained from well 150 in 2002.
151 Sequences obtained from well 150 samples collected in 2002 were deposited in the
152 GenBank database under accession numbers EF117331-EF117417 and EF117512-EF117553.
153 Sequences obtained from the samples collected in 2009 were deposited under accession numbers
154 JX472462-JX472913.
155
156 *2.4. Chemical and isotopic analysis*

157 Alkalinity was determined using Gran alkalinity titrations. Cl^- and SO_4^{2-} concentrations
158 were measured at a precision of 2% using a Dionex AS50 ion chromatograph equipped with a
159 CD20 conductivity detector, an ASRS 300 suppressor, and an IonPac AS14 column and AG14
160 guard column. Cl^- was measured directly from diluted samples and SO_4^{2-} was measured in
161 samples that were treated with Dionex OnGuard II Ag cartridges to remove Cl^-. Na^+, Ca^{2+}, Mg^{2+},
162 and Sr^{2+} were measured at 3% precision and K^+ at 5% precision using a Leeman Labs ICP-AES.
163 A suite of trace elements in each sample was measured using an Agilent 7500ce ICPMS. The
164 instrument operated in reaction gas mode for select elements to eliminate mass interference.
165 Samples and standards were acidified with Optima high-purity nitric acid to 3% by volume prior
166 to analysis. Results were adjusted based upon recovery of a multi-element internal standard
167 (SPEX CertiPrep).
168 Gas compositional and isotopic analyses and water isotopic analyses were performed at
169 Isotech Laboratories, Inc. Gas composition was measured using gas chromatography. Hydrogen
170 isotopic compositions of CH_4 and water were measured using dual-inlet isotope ratio mass
171 spectrometry (DI-IRMS) at 2‰ precision. Oxygen isotopic compositions of water and carbon
172 isotopic compositions of CH_4, CO_2, dissolved inorganic carbon (DIC), and ethane were analyzed
173 with DI-IRMS at 0.1‰ precision, with the exception of ethane sampled from wells 147, 150, and
174 M. In those samples, ethane carbon isotope compositions were measured using gas
175 chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) at a precision of
176 0.3‰. Water samples were prepared for isotopic analysis using the Indiana zinc method for
177 hydrogen, CO_2 equilibration for oxygen, and acid digestion for DIC. All isotopic compositions
178 are reported in standard δ notation. Carbon isotopic compositions are reported relative to Vienna
179 Pee Dee Belemnite (VPDB) and hydrogen and oxygen isotope compositions are reported relative

to Vienna Standard Mean Ocean Water (VSMOW). The precision of CH_4 and water isotope values reported by Martini et al. (1996; 1998), are identical to the errors in our analysis.

For this study, we did not consider differences between values we measured and the initial values to be important unless they differ by more than the potential analytical error of the initial and recent value combined. The limited amount of data available precluded a rigorous statistical analysis of each parameter.

2.5. Field station records

To evaluate gas and water production over time at the field site, we obtained field station records from the Michigan Public Services commission for six of the wells we sampled. The records start when the field stations first came online and extend through 2007. A complete record was not available for the well field containing well 73.

Water and gas produced from multiple wells are delivered to each field station. The field stations in our dataset were fed by 22 wells on average. Withdrawals from the individual wells sampled for this study were estimated by dividing the total monthly gas and water production by the number of online wells in each field. It should be noted, however, that production levels can vary significantly among the wells in an individual field and our data do not constrain this variation. We could not evaluate, therefore, the extent to which the values we calculated accurately depict production levels for the wells we sampled. Nonetheless, the values we calculated still provide a useful measure of the average trends in water and gas production over time for the wells associated with each field station.

3. Results

3.1. Microbial community composition

Chao1 values based on OTUs defined at ≥97% sequence identity were greater for *Bacteria* than *Archaea* in all samples, indicating greater richness for *Bacteria* than *Archaea* at that similarity level (Fig. 1). Richness was greatest for *Archaea* in the 2009 sample with intermediate salinity and greatest for *Bacteria* in the 2009 sample with the lowest salinity. Strongly asymptotic rarefaction curves for each *Archaea* clone library (Supplementary Content Fig. 1) indicate that the archaeal community was adequately sampled. Similarly, rarefaction curves for bacterial clone libraries from well 22 and the 2002 sample from well 150 were also asymptotic. Curvilinear rarefaction curves for bacterial clone libraries from well 147 and the 2009 sample from well 150, however, indicate that additional sequencing would be needed to fully characterize the bacterial community in the water produced from those wells.

Taxonomic classification places all *Archaea* clones in the *Euryarchaeota* (Fig. 1A), which contains the methanogens and extreme thermophiles and halophiles (Takacs-Vesbach et al., 2001). Within this phylum, the majority of the archaeal clones obtained from 2009 samples grouped within two orders of methanogenic microorganisms: *Methanomicrobiales* (59%) and *Methanobacteriales* (33%). *Methanobacteriales* clones were most abundant in the lowest salinity sample and *Methanomicrobiales* clones were most abundant in the highest salinity sample (Fig. 1A). Cultured members of these orders reduce CO_2 typically with H_2 as their electron donor, although some can use formate or secondary alcohols (Bonin and Boone, 2006; Garcia et al., 2006). *Methanosarcinales*, the only order of methanogens that contains species capable of using acetate, contributed little to the total *Archaea* clone library (3%) obtained from 2009 samples.

223 This result differed considerably from the results obtained from the 2002 sample from well 150,
224 in which most clones (69%) grouped within *Methanosarcinales* (Fig. 1A).
225 Taxonomic classification of *Bacteria* shows that most of the clones obtained from 2009
226 samples are contributed from the phyla *Proteobacteria* (60%), *Firmicutes* (22%), and
227 *Bacteroidetes* (7%), but that numerous other groups are also represented (Fig. 1B). Within the
228 *Proteobacteria*, most of the clones grouped within the orders *Desulfovibrionales* (48%) and
229 *Pseudomonadales* (24%) (Fig. 1C). Members of the *Desulfovibrionales* are primarily SO_4^{2-}
230 reducers (Garrity et al., 2005). Clones grouping within *Desulfovibrionales* were particularly
231 abundant in the sample collected from well 22, in which they accounted for 78% of the total
232 bacterial clones. *Pseudomonadales* includes the genus *Pseudomonas*, which comprises a group
233 of species that are ubiquitous in soil and water ecosystems and capable of using a wide variety of
234 organic and inorganic compounds (Moore et al., 2006). Results obtained from the sample
235 collected in 2002 from well 150 contain similar groups of *Bacteria* as observed in 2009 samples.
236 Unlike the 2009 sample from well 150, however, no sequences grouping with *Desulfovibrionales*
237 were present in the 2002 clone library.
238
239 *3.2. Chemical and isotopic composition of water*
240 Field station records demonstrate that water production has decreased sharply over time
241 since the wells were developed. Water production peaked within the first five years of
242 production for all of the wells and both peak and cumulative levels were highest in the wells
243 furthest north (Fig. 2). Current levels of water production range from 0.2 to 14.6% of peak
244 levels.

Although water production has declined, pH, salinity, and bulk chemical composition has changed relatively little (Fig. 3; Supplemental Content Table SC2). As with the original samples, the samples we analyzed were Na-Cl type water with near-neutral to mildly acidic pH and salinity generally increasing southward (i.e., basinward). Some aspects of the groundwater composition were different, however. Alkalinity decreased in all of the wells by an amount ranging from 3.1 to 22.3 mM. Ca^{2+} concentration decreased in five of seven wells by 1.5 to 8.9 mM. Mg^{2+} content decreased in all of the wells by 2.1 to 33.7 mM. SO_4^{2-} concentrations were higher, averaging 290 μM compared to 48 μM in the three samples that had reported SO_4^{2-} concentration initially. The groundwater we sampled also generally had a higher concentration of K^+ and dissolved Mn and Fe and a lower concentration of Sr^{2+}, B^{3+}, and Ba^{2+}.

Concurrent with these compositional changes and the decline of water production, the isotopic composition of the water and DIC also changed. Compared to initial samples, water δD values we measured differed by more than combined analytical error (>4‰) in samples from five of the seven wells. In those samples, δD values were 11‰ lower on average than the values measured initially (Fig. 4). In contrast to δD, $\delta^{18}O$ values were higher than initial values in nearly half of the samples. Three samples had $\delta^{18}O$ values that were not different from the initial values by more than the combined analytical error (>0.2‰), one sample had a $\delta^{18}O$ that was 0.8‰ lower, and three samples had $\delta^{18}O$ values that were 0.6‰ higher on average (Fig. 4). DIC $\delta^{13}C$ values differed by more than combined analytical error (>0.2‰) in all four samples that had initial values. On average, the $\delta^{13}C$ value of DIC decreased 2.7‰ in two wells and increased 1‰ in the other two (Supplemental Content Table SC2).

3.3. Chemical and isotopic composition of gas

Similar to the observed changes in water production and composition, the amount of gas being produced and its chemical and isotopic composition has shifted since the wells were developed. Field station records show that gas production has decreased and that the proportion of CO_2 in produced gas has increased by an average of 13 mol% while overall natural gas production has steadily declined (Fig. 2).

Analysis of gas samples collected for this study show a similar result to the field station records. Compared to samples collected initially, the CH_4 content of the gas samples we collected decreased by 11 mol% on average in five wells while CO_2 content increased by an equivalent amount (Supplemental Content Table SC3). Gas wetness [$C_1/(C_2+C_3)$] values in the samples were generally lower than initial values. Wetness averaged 1001 compared to 1432 initially. Although the composition of gas shifted as gas production declined, CH_4 is still by far the largest component. The mean CH_4 content of the samples we collected was 82 mol% compared to 86 mol% initially.

Shifts in the δD value of CH_4 correspond to those observed in water. As the δD value of water decreased, the δD value of CH_4 also largely decreased (Fig. 5A). With the exception of one sample with values that were not considerably different from those measured initially (>4‰), the δD vales of CH_4 were lower in all of the samples by 9‰ on average. The average difference between the δD of CH_4 and water in the samples we collected was 172‰, which is not significantly different from the value measured initially, 171‰, based on a Student's T test (P 0.735).

Similarly, the $\delta^{13}C$ value of CH_4 also decreased for most wells (Fig. 5B). One sample had CH_4 $\delta^{13}C$ values that did not differ from initial values by more than combined analytical error (>0.2‰). The remaining six samples had $\delta^{13}C$ values that were 1.3‰ lower on average. The $\delta^{13}C$

values of CO_2 measured in gas samples increased for most wells (Fig. 5B). Four samples had a CO_2 $\delta^{13}C$ value 1.7‰ higher on average. Of the remaining three samples, one did not differ from initial values by more than combined analytical error (>0.2‰) and two decreased by 0.3 and 2.5‰. The fractionation factor (α_c) between $\delta^{13}C$ values of CO_2 and CH_4 calculated for each sample we collected was 1.076 on average, where α_c is expressed as:

$$\alpha_c = \frac{(\delta^{13}C_{CO_2} + 1000)}{(\delta^{13}C_{CH_4} + 1000)} \quad (3)$$

This value is very similar to that observed in the samples collected from the wells initially, 1.074. Nonetheless, these averages are statistically different based on a Student's T test (P = 0.045).

4. Discussion

Our results demonstrate that considerable changes in the geochemistry and microbiology of co-produced water and gas have occurred since widespread development of the Antrim northern producing trend. In the sections that follow, we discuss how ongoing biogeochemical reactions within the shale coupled with processes driven by commercial gas production could have contributed to these changes. These findings have implications for the sustainability of commercial gas production in unconventional gas reservoirs.

4.1. Pathway of CH_4 formation

Using isotopic evidence, Martini and others (1996; 1998) interpreted that CH_4 over much of the northern producing trend in the Antrim Shale was generated by CO_2-reducing

313 methanogens. The results of our isotopic analyses are consistent with those findings. Where CH_4
314 is produced by CO_2 reduction, the δD value of CH_4 is typically about 160‰ +/-10% lower than
315 the surrounding water (Nakai et al., 1974; Schoell, 1980), which is comparable to the value we
316 observed (Fig. 5A). In comparison, differences between the δD values of CH_4 and water are
317 approximately twice as large where CH_4 is produced by acetate fermentation (Schoell, 1980;
318 Whiticar et al., 1986; Woltemate et al., 1984). Similarly, CH_4 produced by CO_2 reduction is
319 generally associated with relatively high fractionation factors ($\alpha_c > 1.06$), comparable to those
320 we observed (Fig. 5B), whereas lower values ($\alpha_c < 1.06$) are typical of acetate fermentation
321 (Whiticar et al., 1986).

322 The results of our nucleic acid-based analysis are consistent with our interpretation based
323 on isotopic results. The dominance of phylotypes with cultured relatives that produce CH_4 by
324 CO_2 reduction in the archaeal clone libraries we generated suggests that CO_2-reducing
325 methanogens are the most abundant *Archaea* in the shale. This result also compares favorably
326 with the results of previous studies that analyzed *Archaea* in the Antrim Shale using molecular
327 techniques. Although the clone libraries published in Formolo et al. (2008), Waldron et al.
328 (2007), and Martini et al. (2005) contained a higher percentage of clones grouping with
329 *Methanosarcinales* than our libraries, clones grouping in *Methanomicrobiales* and
330 *Methanobacteriales* were found to be more abundant overall than those grouping in
331 *Methanosarcinales* in those studies.

332 The relative abundance of sequences in a clone library does not necessary accurately
333 represent the abundance of the species corresponding to those sequences in the environment due
334 to both PCR (Suzuki and Giovannoni, 1996) and sampling bias (Flynn et al., 2008). Similarly,
335 interpreting pathways of microbial methanogenesis based on isotopic analysis may be less

336 definitive than originally thought (e.g., Bates et al., 2011; deGraaf et al., 1996; Waldron et al.,
337 1998). Nonetheless, both of these lines of independent evidence are in agreement, providing
338 compelling support of our interpretation.

339 These findings highlight a gap in our understanding of electron flow in the Antrim Shale;
340 the fate of acetate remains unresolved. The ultimate products of organic matter degradation
341 generally include both acetate and H_2 (Madigan et al., 2003), implying that acetate is being
342 generated within the shale. During organic matter degradation, production of acetate relative to
343 H_2 increases as a result of the activity of acetogenic microorganisms, *Bacteria* that consume H_2
344 and produce acetate. Most of the clones that grouped within the phylum *Firmicutes* (31 of 42;
345 Fig. 1B) also grouped within the genus *Acetobacterium* based on our taxonomic analysis and
346 indeed, a more rigorous analysis than we performed concluded that acetogens were in fact
347 present in the northern producing trend (Formolo et al., 2008). Not only is acetate likely being
348 generated in the shale, therefore, but its relative importance as a substrate for microbial activity
349 may be even greater as a result of acetogenesis. Despite this, acetate has not accumulated where
350 microbial CH_4 is present (Martini et al., 2003) and H_2 oxidation appears to have largely fueled
351 formation of CH_4.

352 This apparent lack of acetate consumption by methanogens can be explained if some
353 group of microorganisms other than methanogens is consuming acetate. Possibilities include
354 SO_4^{2-} reducers and syntrophic acetate oxidizers. The limited availability of SO_4^{2-} until recently
355 has likely restricted the activity of SO_4^{2-} reducers (see Section 4.3). Syntrophic acetate oxidizers,
356 however, could be active within the shale where the reaction is energetically favorable.
357 Consistent with this possibility, clones in the library from well 150 that grouped within the Order
358 *Syntrophobacterales* (Fig. 1C) also grouped within the genus *Smithella* based on our taxonomic

359 analysis. Gray et al. (2011) found evidence that *Smithella* species were responsible for
360 syntrophic acetate oxidation in methanogenic oil-degrading microcosms.
361 In addition to these possibilities, the apparent lack of acetate consumption by
362 methanogens could also be explained if our isotopic and nucleic acid-based analyses
363 underestimate CH_4 contributions from acetate-fermenting methanogens or if some unknown sink
364 for acetate exists within the shale. Uncertainty regarding the fate of acetate has also been
365 observed in many other anoxic environments (Conrad, 1999), including unconventional gas
366 reservoirs (e.g., Strąpoć et al., 2008). Additional research is needed to fully elucidate the
367 pathways of electron flow through these systems.
368
369 *4.2. Shifts in archaeal community composition*
370 Differences in the composition of the archaeal clone libraries collected from well 150 in
371 2002 and 2009 suggest that the abundance of *Methanosarcinales* species adjacent to that well has
372 decreased over time while the abundance of *Methanobacteriales* species has increased. This shift
373 may have occurred because methanogens adjacent to the well continue to generate CH_4 and
374 changes in the environment as a result of commercial gas production favor *Methanobacteriales*
375 species over *Methanosarcinales* species. It is also possible, however, that cells are simply being
376 transported to the well by groundwater movement from a different zone within the subsurface
377 than they were in 2002 (Fig. 6). In other words, a different population of planktonic cells was
378 sampled in 2009 than 2002 because the source(s) of groundwater flowing to the well as a result
379 of gas production has changed over time.
380 In addition to both of these possibilities, differences in the molecular techniques used to
381 analyze *Archaea* could have also contributed to the differences in community composition.

382 Archaeal 16S rRNA genes in the 2002 sample were amplified using a different primer set than
383 the primer set that we used, potentially leading to differences in amplification efficiency between
384 studies that may have favored *Methanosarcinales* species in the 2002 sample. Moreover, unlike
385 our own PCR reactions, they used nested reactions to amplify archaeal DNA, which can
386 introduce bias if too many cycles are used in the first round of amplification (Park and Crowley,
387 2010).

388 We examined the potential impact of differences in primer choice using the Ribosomal
389 Database Project Probe Match tool (Cole et al., 2009). The probes were tested in pairs, as they
390 were used, and the database search was restricted to sequences with data that span the
391 *Escherichia coli* region targeted by both sets of primers (8 to 1000). Compared to the primers we
392 used, the primers used to amplify archaeal DNA from the 2002 sample matched a much smaller
393 portion of the *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* sequences
394 tested (Table 1). Of the three groups, furthermore, the primer set used for the 2002 sample
395 matched considerably more *Methanosarcinales* sequences than *Methanobacteriales* and
396 *Methanomicrobiales* sequences. These findings strongly suggest that differences in primer
397 efficiency contributed to the differences in archaeal community composition observed between
398 the 2002 and 2009 samples.

399

400 *4.3. Shifts in bacterial community composition*

401 Our molecular results indicate that SO_4^{2-}-reducing species are increasing in abundance in
402 the northern producing trend. SO_4^{2-}-reducing species were undetected in the analysis of the
403 sample collected from well 150 in 2002 by Martini et al. (2005) but accounted for a considerable
404 portion of our clone library for that well (Fig. 1C). Amplification conditions used in that study

405 for *Bacteria* were nearly identical to those we used, implying that differences in the methods are
406 less likely to contribute to the differences observed in the bacterial clone libraries than the
407 archaeal libraries. In addition to well 150, furthermore, analysis of samples from other wells in
408 the northern producing trend also did not detect SO_4^{2-} reducers using molecular techniques
409 (Formolo et al., 2008).

410 This shift in the composition of the bacterial community is consistent with the increase in
411 SO_4^{2-} levels we observed. Where SO_4^{2-} concentration exceeds as little as 30 μM in freshwater
412 sediments, SO_4^{2-} reducers can hold acetate and H_2 concentrations below levels necessary for
413 methanogen populations to grow (Lovley and Klug, 1986; Ward and Winfrey, 1985). In saline
414 environments, this threshold may be as high as 2 mM (Megonigal et al., 2005). Threshold
415 concentrations ranging between both extremes are likely important in the northern producing
416 trend, where the gradient in groundwater salinity is very steep (Martini et al., 1998; McIntosh et
417 al., 2002). SO_4^{2-} reducers may be increasing in abundance in the shale, therefore, because SO_4^{2-}
418 reducers have begun to actively grow and compete with methanogens for substrates as SO_4^{2-}
419 concentration has increased. Similar to *Archaea* now present, however, groundwater movement
420 may also be transporting these cells into the shale along with SO_4^{2-} from zones within the
421 subsurface that differ from those supplying water when the wells were previously sampled. Both
422 of these possibilities may contribute to the observed changes in bacterial community
423 composition.

424

425 *4.4. Shifts in groundwater geochemistry*

426 Our results demonstrate that the chemical and isotopic composition of water in the shale
427 has shifted considerably in most of the wells since they were initially sampled. Relatively dilute

428 ($Cl^- < 1$ M) and low-δD, low-$\delta^{18}O$ water recharged the Antrim Shale during melting of
429 Pleistocene glaciers (McIntosh et al., 2002). Modern groundwater flow in the Great Lakes
430 region, however, is largely restricted to shallow glacial drift aquifers near the surface (McIntosh
431 et al., 2011; McIntosh and Walter, 2006). These changes within the past two decades, therefore,
432 were likely caused by groundwater inflow in response to pumping to extract natural gas rather
433 than the natural movement of groundwater in the basin.

434 Groundwater seeping into the Antrim likely originates from multiple sources. We
435 hypothesize that most of this inflow, however, originates from the underlying Traverse
436 Formation (Fig. 6). The distribution of aquifers and aquitards is a major control on fluid
437 migration along the Michigan Basin margin (McIntosh et al., 2002). The Antrim Shale is capped
438 by brown Mississippian shales and the Ellsworth shale, which has a much lower intrinsic
439 permeability than the Antrim (Ryder, 1996), The Antrim is underlain by Devonian carbonate
440 aquifer systems. Silurian-Devonian aquifers such as the Traverse Formation were the primary
441 path of freshwater recharge into the overlying Antrim Shale during melting of Pleistocene
442 glaciers (Eberts and George, 2000; McIntosh et al., 2002). This relatively high permeability
443 formation may also serve as the primary route of groundwater flow into the Antrim as a result of
444 commercial gas production.

445 Shifts in SO_4^{2-} and alkalinity levels we observed support this hypothesis. The increase in
446 SO_4^{2-} concentration we observed may reflect the presence of anhydrite in the Traverse Formation
447 immediately beneath the Antrim Shale. Wilson and Long (1993) measured groundwater SO_4^{2-}
448 levels ranging as high as 6.3 mM with an average at 1.2 mM in the Traverse Formation. The
449 decrease in alkalinity levels we observed is consistent with the low alkalinity content of the
450 Traverse Formation. The highest alkalinity reported by Wilson and Long (1993) was 2.6 mM as

451 HCO_3^-. Alkalinity levels from zones of microbial methanogensis in the Antrim Formation along
452 the northern margin of the Michigan Basin generally exceed 10 mM (McIntosh et al., 2004).
453 The extent to which changes in the isotopic composition of formation water support this
454 hypothesis is less clear. The isotopic composition of water in the Michigan Basin varies widely
455 (Martini et al., 1998; McIntosh et al., 2002). This variation reflects mixing between a ^{18}O-
456 enriched basin brine end-member and recharge from low-δD, low-$\delta^{18}O$ Pleistocene glacial
457 meltwater and modern precipitation. The decrease in δD values we observed, therefore, is
458 consistent with inflow of water that has a greater proportion of meltwater and/or modern
459 precipitation than the water present when the initial samples were collected. Because the
460 Traverse Formation was a source of low δD recharge to the Antrim Shale during the Pleistocene,
461 further inflow from the Traverse would likely continue to lower δD values. Indeed isotopic
462 values reported by McIntosh et al. (2006) for the Traverse Formation along the northern edge of
463 the Michigan basin range to lower values than those we observed in the Antrim Shale (Fig. 4).
464 Such a shift in δD values would likely also be accompanied by a decrease in $\delta^{18}O$ values.
465 This change, however, is largely inconsistent with our results. Instead, $\delta^{18}O$ values were slightly
466 heavier in most cases, consistent with inflow that has a greater component of basin brine ($\delta^{18}O$-
467 enriched), such as that sampled by Wilson and Long (1993) from the Traverse Formation further
468 south within the basin (Fig. 4). These inconsistencies imply that groundwater mixing as a result
469 of pumping is not the only control on the isotopic composition of water in the shale.
470 Coupled with changes caused by groundwater inflow, open-system groundwater
471 degassing may have also contributed to the changes we observed. Zhou et al. (2005) showed that
472 open-system groundwater degassing as a result of commercial gas production is fractionating
473 noble gases in coal in the San Juan Basin, USA. We hypothesize that this process could also

affect the isotopic composition of groundwater by extracting water vapor through unsaturated pore space adjacent to the wells. Similar to evaporation, this process would enrich the isotopic composition of the residual water and may have a greater impact on $\delta^{18}O$ than δD. Similarly, open-system degassing of CO_2 could also affect the composition of water by causing the pH of aqueous solutions to increase and thereby driving precipitation of carbonate minerals and a decrease in alkalinity (Dreybrodt et al., 1992). This impact would be consistent with the observed decreases in alkalinity, Ca^{2+}, and Mg^{2+} levels. More research is needed to fully evaluate the impact that pumping has on the chemical and isotopic composition of groundwater in unconventional reservoirs.

4.5. Shift in gas geochemistry

Both field station records and compositional analysis of the samples we collected demonstrate that CO_2 has increased relative to CH_4 in the gas produced in the field area. This finding is consistent with those of Martini et al. (2003), who concluded that CO_2 increases over time due to differences in the ability of each gas to adsorb. CH_4 and CO_2 compete for the same adsorption sites, with CO_2 being more strongly adsorbed than CH_4 (Arri et al., 1992; Weniger et al., 2010). As a result, the proportion of adsorption sites filled with CO_2 increases as formation pressure decreases during commercial gas production, ultimately causing CO_2 to account for an increasing proportion of the produced gas.

As the proportion of CO_2 has increased, our results show that the isotopic composition of CO_2 and CH_4 has shifted. Similar to the observed shifts in water geochemistry and microbiology, these shifts may have occurred because gas is being drawn into each well from a different location than it was when the initial samples were collected. Like water, the isotopic composition

497 of gas varies sharply in the Antrim Shale along the northern edge of the basin (McIntosh et al.,
498 2004). Drawing gas from different zones over time, therefore, would cause the isotopic
499 composition of produced gas to shift. Parallel shifts in the δD values of water and CH_4 that we
500 observed are consistent with this interpretation. The fractionation factor between water and CH_4
501 remained constant as the δD of water changed, providing evidence that the co-produced water
502 was present when the CH_4 formed. The water and gas, therefore, may have been drawn toward
503 the well simultaneously from the same location.

504 In addition to changes in gas source, many other processes may have also contributed to
505 changes in the isotopic composition of CO_2 and CH_4 including fractionation associated with
506 desorption and continued microbial activity. Light isotopologues generally desorb more easily
507 and have higher diffusion coefficients than heavy isotopologues (Xia and Tang, 2012; Zhang and
508 Krooss, 2001). These processes would cause the gas to get heavier over time during commercial
509 production as light isotopologues would be withdrawn preferentially following initial
510 development of a reservoir. This process may indeed explain the observed shift in the $\delta^{13}C$ of
511 CO_2 but not CH_4, possibly reflecting differences in the extent to which those gases adsorb to
512 organic matter. A recent study concluded that CH_4 fractionation in response to adsorption and
513 diffusion is limited under geological conditions (Xia and Tang, 2012). If this is true for CH_4 but
514 not CO_2, then it could at least partially explain the changes in α between the recent and initial
515 samples.

516 Continued microbial activity could have contributed to changes in the isotopic
517 composition of CO_2 and CH_4 by generating both CO_2 and CH_4 under conditions that are more
518 consistent with an open system than they were before development. The decrease in the $\delta^{13}C$ of
519 CO_2 produced from wells 73 and B is consistent with CO_2 generation within the last 20 years.

520 Unless CO_2 is simply being drawn into those wells from a zone with CO_2 that has a lower $\delta^{13}C$
521 than the CO_2 that was initially present, additional CO_2 must have been generated that has a $\delta^{13}C$
522 more consistent with organic matter (i.e., lower). Parallel shifts in the $\delta^{13}C$ of CO_2 and CH_4 and
523 the δD of water and CH_4 are consistent with continued CH_4 formation in wells 147 and B. If
524 methanogenesis continues to occur at a significant rate in the volume sampled by those wells,
525 changes in the isotopic composition of CH_4 there would be consistent with changes in the
526 isotopic composition of both CO_2 and water.

527 Unlike the possibilities outline above, CH_4 oxidation does not appear to be a primary
528 control on the isotopic composition of either CH_4 or CO_2. During CH_4 oxidation, isotopically
529 depleted CH_4 is preferentially oxidized (Barker and Fritz, 1981; Holler et al., 2009). This effect
530 would increase the $\delta^{13}C$ value of residual CH_4 and decrease the $\delta^{13}C$ value of CO_2, the opposite
531 of what we observed in most wells.

532

533 *4.6. Potential impact of hydraulic fracturing*

534 Hydraulic fracturing within the wells we sampled does not appear to have caused the
535 changes in geochemistry and microbiology that we observed. Each of the wells included in this
536 study were stimulated soon after the wells were drilled (Supplemental Content Table SC1).
537 Stimulation was accomplished using nitrogen foam, acid solutions, and sand; an approach used
538 in many other wells in the northern producing trend of the Antrim Shale (Milici, 1993). All of
539 the samples collected initially from the wells included in this study were collected at least 3
540 months after stimulation. Moreover, there is no record of well re-working for any of the wells
541 between the initial sampling dates and the final sampling dates based on personal communication

with well operators and well records obtained from the Michigan Department of Environmental Quality.

If wells were completed near those we sampled during the period of time between collection of our initial and final samples, however, it is possible that hydraulic fracturing could have caused some of the changes we observed. The water, chemicals, and dissolved gases injected into the shale for hydraulic fracturing could have ultimately mixed with pore water flowing to the wells we sampled via natural and induced fractures. Considering the potential that this process has to impact biological processes within shale-gas reservoirs, future research is warranted to examine the biological implications of hydraulic fracturing in more detail.

5. Conclusions

Our results show that (1) gas being commercially produced in the field area today was still primarily produced by CO_2 reduction, (2) SO_4^{2-} concentration and the abundance of SO_4^{2-}-reducing microorganisms have increased, changes that may ultimately allow SO_4^{2-} reducers to displace methanogens, and (3) in addition to SO_4^{2-}, other changes in the chemical and isotopic composition of water and gas in the shale have also occurred. These changes in microbiology and geochemistry can be explained by ongoing biogeochemical reactions and processes driven by commercial gas production, including groundwater flow, gas desorption, and open-system degassing.

These findings highlight the complex array of processes that can influence geochemistry and microbiology during commercial gas production and multiple areas where additional research is needed. These findings also have important implications for commercial gas

565 production. They imply that the practices used currently for commercial gas production from
566 fractured shale can ultimately shorten the lifespan of an unconventional natural gas play by
567 creating conditions that favor growth of microorganisms that can compete with methanogens for
568 substrates. Future development in unconventional gas reservoirs should consider the chemical
569 composition of water in adjacent formations and the potential of those formations to serve as a
570 source of water inflow in response to pumping.
571

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Figures

Figure 1

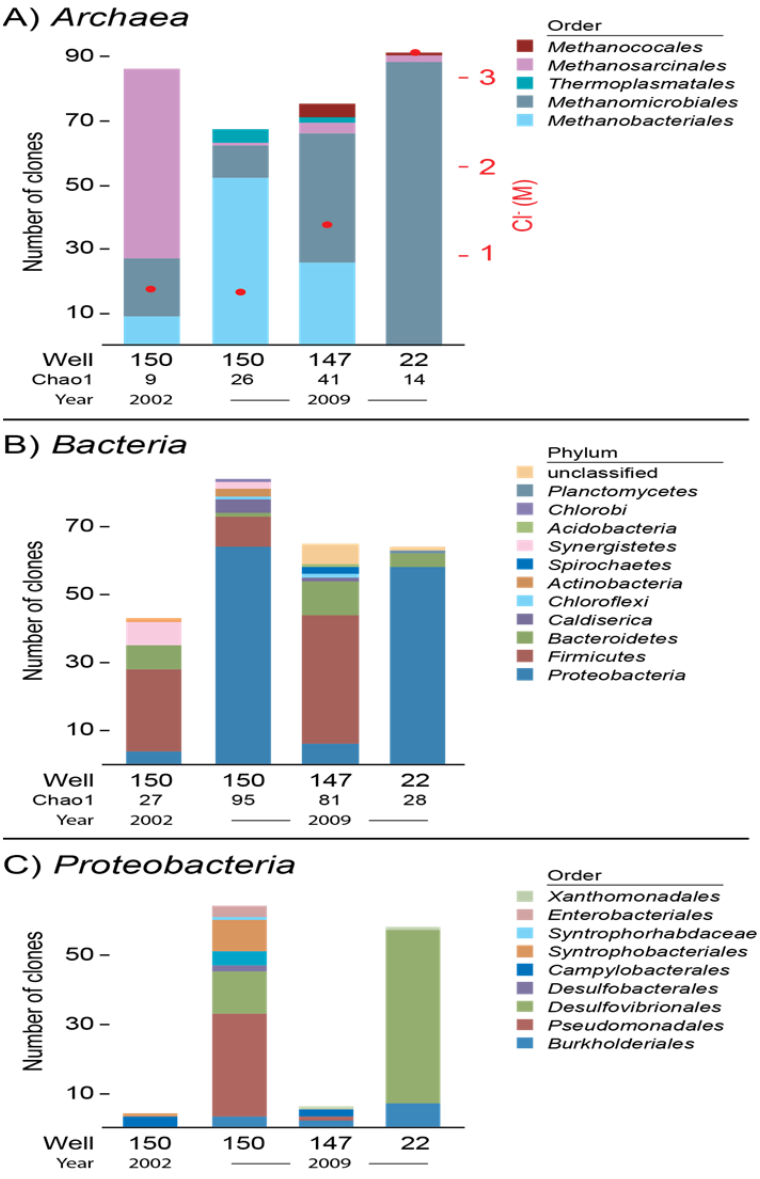
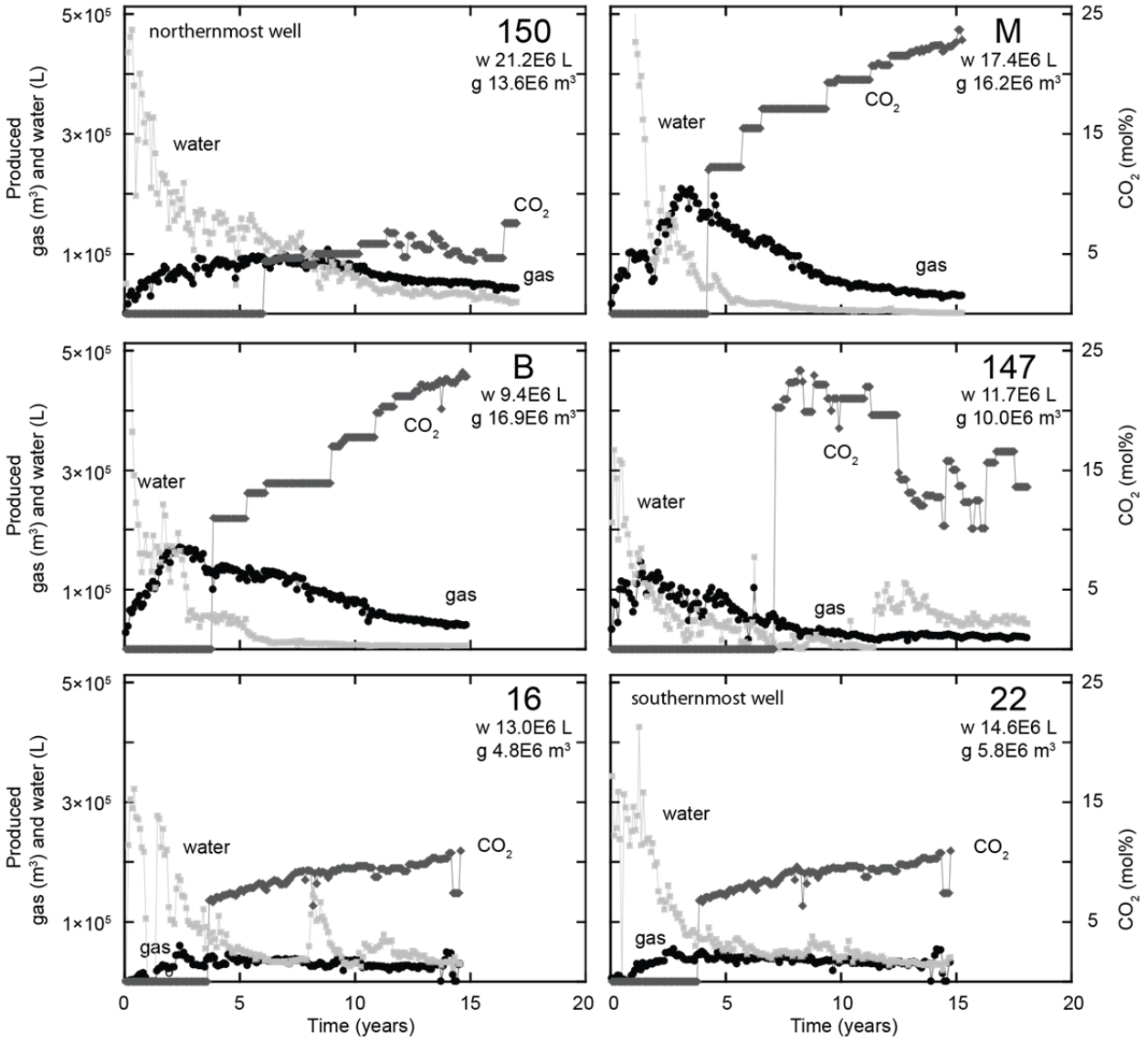


Figure 1. Taxonomic distribution of clones detected in samples from well 150, 147, and 22. Chart (A) shows the distribution of archaeal clones at the order level, (B) shows bacterial clones at the phylum level, and (C) shows proteobacterial clones at the order level. Chao1 richness estimates based on OTUs defined at ≥97% sequence identity are listed for each library under the charts for *Archaea* and *Bacteria*. Cl^- concentration is plotted on the chart showing *Archaea*.

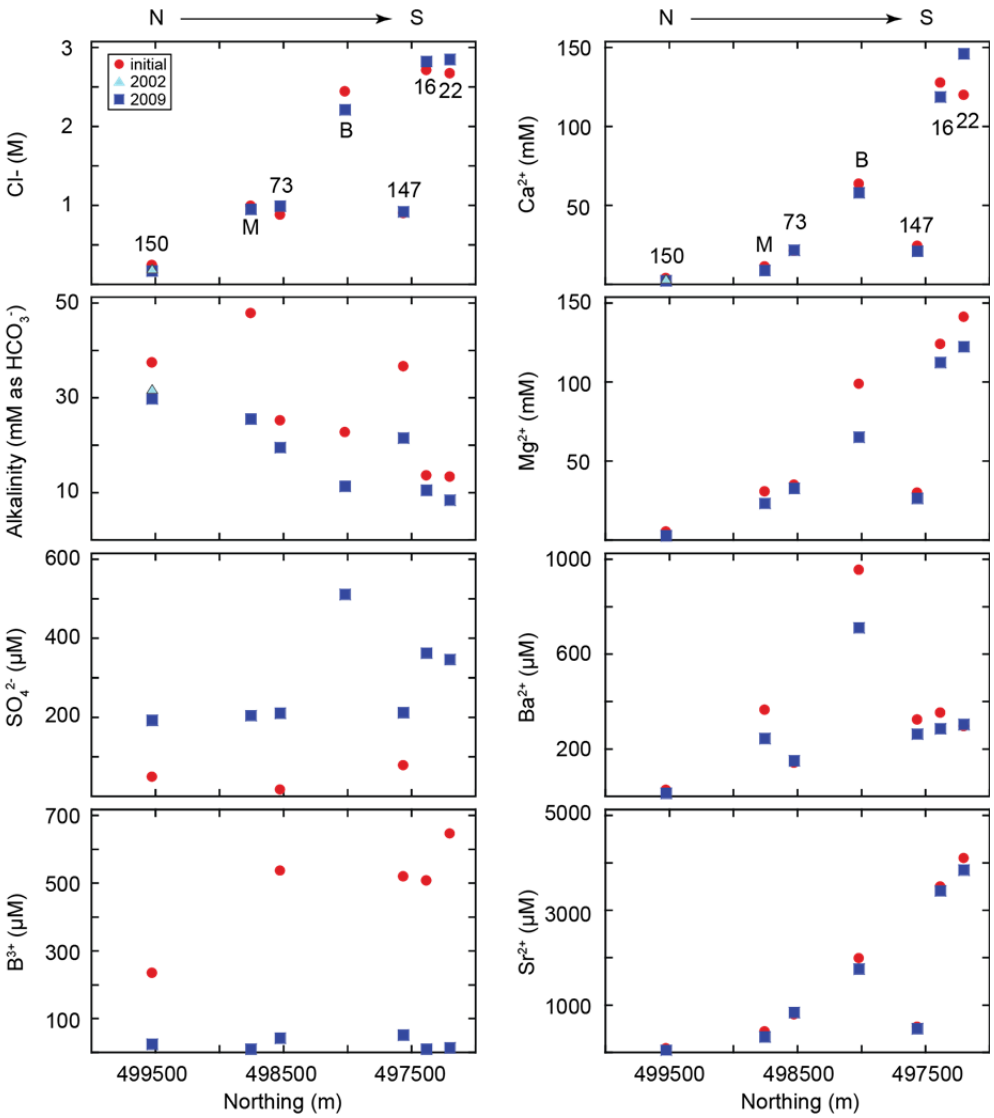
762 Figure 2



763

764 Figure 2. Variation in water and gas production and gas CO_2 content over time at field stations
765 supplied by wells 150, M, B, 147, 16, and 22. Data are plotted relative to the date each field
766 station came online (t = 0) and normalized to the number of wells in the field. The graphs are
767 ordered from north to south as indicated on the figure. Cumulative water (w) and gas (g)
768 volumes produced at each field station are provided in the upper right corner of each figure.
769 These values are also normalized to the number of wells in the field.

770 Figure 3

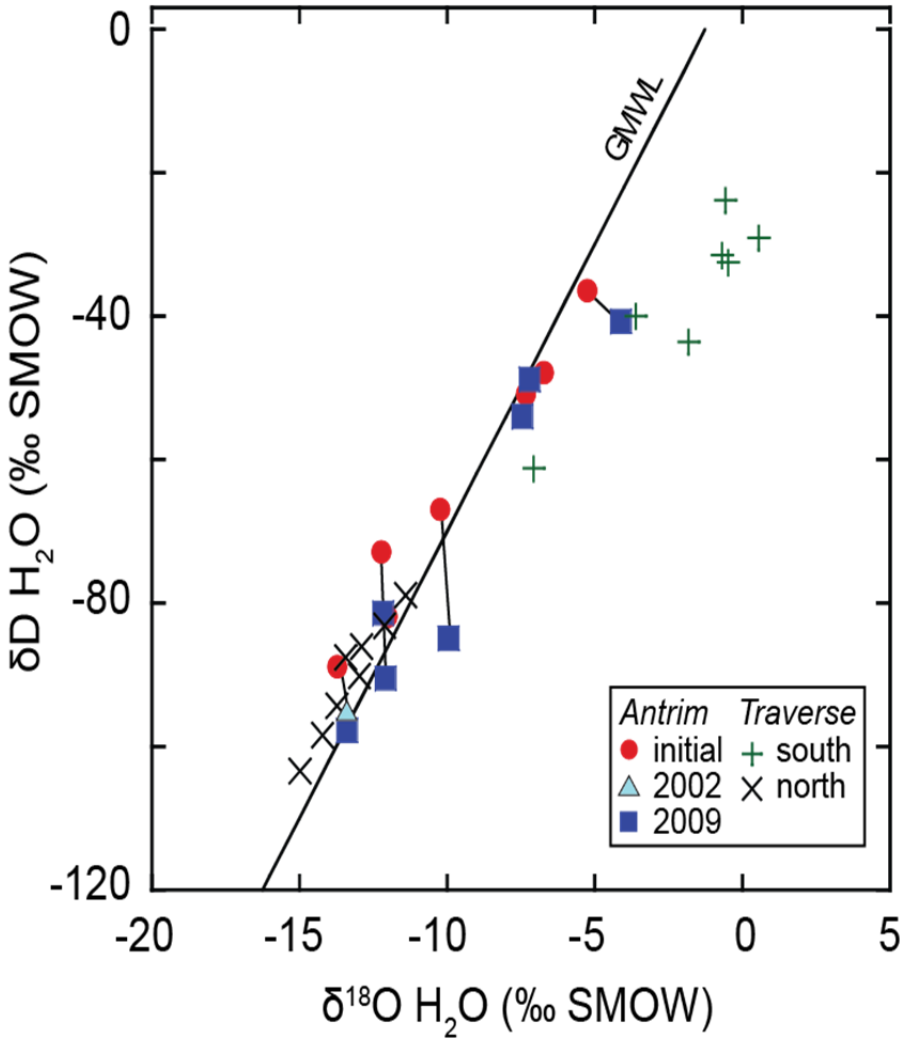


771

772 Figure 3. Variation in aqueous chemistry with distance north to south.

773

774 Figure 4



775

776 Figure 4. Variation in the isotopic composition of water relative to the global meteoric water line
777 (GMWL; Craig, 1961). Also plotted are data collected from the Traverse Formation along the
778 northern margin of the basin by McIntosh and Walter (2006) and further south by Wilson and
779 Long (1993).

780

Figure 5

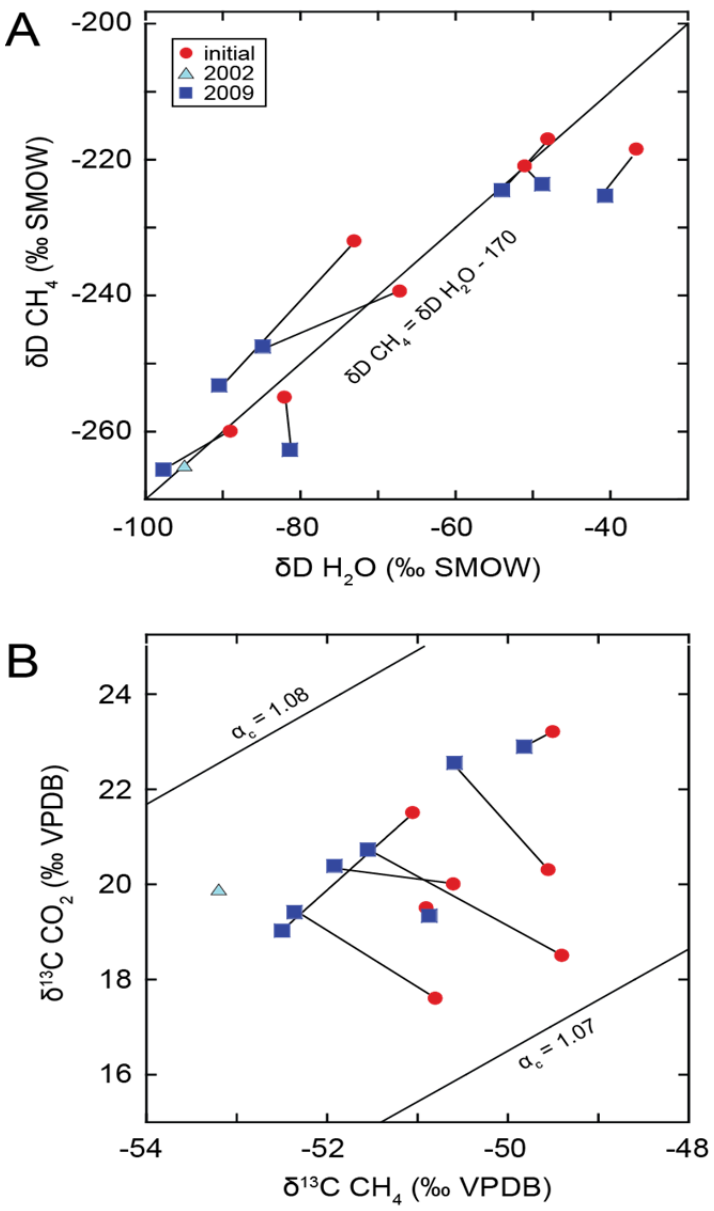
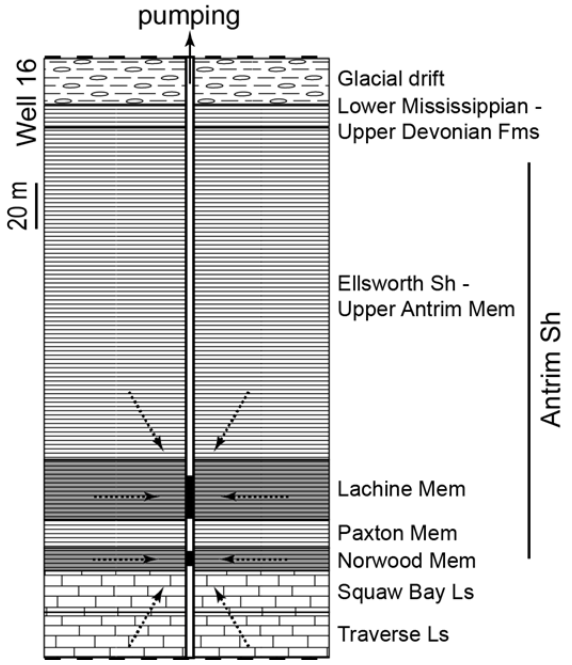


Figure 5. Variation in (A) the hydrogen isotope composition of CH_4 relative to co-produced water and (B) the carbon isotope composition of CH_4 relative to CO_2. The δD value of CH_4 was lower than the δD value of water by 172‰ (samples collected in 2009) and 171‰ (samples collected initially), on average. The average fractionation factor (α_c) between CO_2 and CH_4 carbon isotopes was 1.076 in 2009 samples and 1.074 in the samples collected initially.

788 Figure 6



789

790 Figure 6. Schematic showing possible sources of groundwater inflow into the Antrim Shale as a
791 result of pumping. The stratigraphy shown was interpreted from electric well logs for well 16
792 (Walter et al., 1996) and is similar to the stratigraphy observed in all of the wells we sampled.
793 The Lachine and Norwood Members of the Antrim Shale have the highest organic matter content
794 (0.5-24 wt.% TOC) and are the main targets for commercial gas production (Martini et al.,
795 1998). Well perforations coinciding with the depth of these members are shown in black in the
796 well bore. The upper Devonian and lower Mississippian formations above the Antrim include the
797 Coldwater, Red Rock, Sunbury, Berea, and Bedford. The glacial drift is 202 m thick at the well
798 site and the Traverse Limestone exceeds 66 m in thickness. The Ellsworth Shale has a much
799 larger fracture spacing than the Antrim, Squaw Bay, and Traverse formations (Ryder, 1996). As
800 a result, the Ellsworth has a lower intrinsic permeability, which likely limits groundwater flow
801 from that formation as a result of pumping.

Table 1. Results of probe match analysis

	Total[1]	2002 sample[2]		2009 sample[3]	
Order	sequences	matches	%	matches	%
Methanobacteriales	189	29	15%	168	89%
Methanomicrobiales	563	34	6%	520	92%
Methanosarcinales	999	322	32%	813	81%

[1]Analysis performed using the Ribosomal Database Project Probe Match tool (Cole et al., 2009) with the database restricted to sequences containing data in the *E. coli* region from 8 to 1000.
[2]Archaeal DNA amplified using 25F (5'-CYG GTT GAT CCT GCC RG-3') AND 958R (5'-YCC GGC GTT GAM TCC AAT T-3')
[3]Archaeal DNA amplified using 109F (5'-ACK GCT CAG TAA CAC GT-3') and 915R (5'-GTG CTC CCC CGC CAA TTC CT-3')

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