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

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How to cite this manuscript

If you make reference to this version of the manuscript, use the following information:

Kirk, M. F., Martini, A. M., Breecker, D. O., Colman, D. R., Takacs-Vesback, C., & Petsch, S. T. (2012). Impact of commercial natural gas production on geochemistry and microbiology in a shale-gas reservoir. Retrieved from <http://krex.ksu.edu>

Published Version Information

Citation: Kirk, M. F., Martini, A. M., Breecker, D. O., Colman, D. R., Takacs-Vesback, C., & Petsch, S. T. (2012). Impact of commercial natural gas production on geochemistry and microbiology in a shale-gas reservoir. *Chemical Geology*, 332-333, 15-25.

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Digital Object Identifier (DOI): doi:10.1016/j.chemgeo.2012.08.032

Publisher's Link: <http://www.sciencedirect.com/science/article/pii/S0009254112003919>

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Abstract [310 words]

19
20 We consider the effect that commercial gas production has had on microbiology and
21 water and gas geochemistry in the northern producing trend of the Antrim Shale, an
22 unconventional gas reservoir in the Michigan Basin, USA. We analyzed gas, water, and
23 microbial biomass samples collected from seven wells in 2009 and compared our findings to the
24 result of analyses performed as early as 1991 on samples collected from the same wells. We also
25 examined production records associated with six wells. Water production has decreased sharply
26 over time and is currently at 0.2 to 14.6% of peak levels. While this has happened, the chemical
27 and isotopic composition of gas and water produced from the wells has shifted. The proportion
28 of CO₂ has increased by as much as 15 mole% while CH₄ content has correspondingly
29 decreased. Isotopically, the $\delta^{13}\text{C}$ and δD values of CH₄ decreased for most wells by averages of
30 1.3‰ and 9‰, respectively, while $\delta^{13}\text{C}$ values of CO₂ increased for most wells by an average of
31 1.7‰. Alkalinity in the water from each well decreased by 10 mM on average and SO₄²⁻ content
32 increased from below 50 μM to over 200 μM on average in water from each well with initial
33 values. Microorganisms most closely related to CO₂-reducing methanogens were the most
34 abundant group in archaeal clone libraries and SO₄²⁻ reducers were the most abundant group in
35 bacterial libraries. In contrast, no SO₄²⁻ reducers were identified in a nucleic acid-based analysis
36 of a sample collected in 2002 from one of the wells we sampled. Our results show that
37 commercial gas production has not only caused chemical and isotopic changes in water and gas
38 in the Antrim Shale but also an increase in the abundance of SO₄²⁻-reducing microorganisms, a
39 change that can ultimately have a negative impact on biogenic CH₄ formation. Processes that can
40 explain these changes include ongoing biogeochemical reactions, groundwater flow, gas
41 desorption, and open-system degassing.

42

43 **Keywords:** sulfate reduction, methanogenesis, Antrim Formation, Michigan Basin,

44 unconventional natural gas reservoir, black shale

45

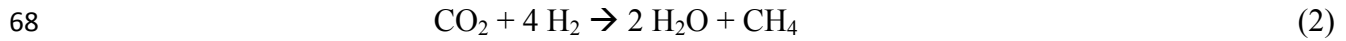
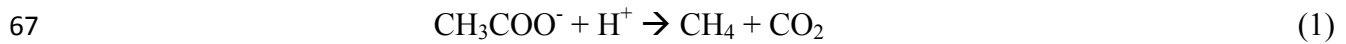
46 **1. Introduction**

47 Unconventional natural gas reservoirs such as fractured organic-rich shale are becoming
48 increasingly important energy resources. Natural gas provides a major source of energy for the
49 U.S., accounting for more than 20% of the energy supply (NETL, 2009). The rate of gas
50 consumption, however, increasingly exceeds the rate of domestic production. Greater production
51 from fractured organic-rich shale can help make up some of this imbalance (NETL, 2009).

52 Moreover, although carbon dioxide (CO₂) is emitted during gas combustion, natural gas is the
53 cleanest fossil fuel. Compared to coal, for example, natural gas combustion emits about half as
54 much CO₂ per joule of energy produced, as well as far lower NO_x, SO_x, heavy metals and
55 particulate matter. Producing a greater percentage of our energy from natural gas at the expense
56 of oil and coal, therefore, would be environmentally advantageous (White et al., 2003).

57 In many unconventional natural gas reservoirs, a significant portion of the gas formed
58 biologically as microbial communities degraded sedimentary organic matter (e.g., Bates et al.,
59 2011; Flores et al., 2008; Formolo et al., 2008; Martini et al., 1996; McIntosh et al., 2008;
60 McIntosh et al., 2002; Schlegel et al., 2011; Scott et al., 1994; Strapoć et al., 2008; Su et al.,
61 2005; Waldron et al., 2007; Warwick et al., 2008). Biological formation of methane (CH₄), the
62 primary component of natural gas, requires a consortium of microorganisms. Fermentative and
63 syntrophic *Bacteria* degrade complex organic matter and ultimately produce substrates that can
64 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:



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 $\geq 97\%$ 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 archaeal 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

180 to Vienna Standard Mean Ocean Water (VSMOW). The precision of CH₄ and water isotope
181 values reported by Martini et al. (1996; 1998), are identical to the errors in our analysis.

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

186

187 *2.5. Field station records*

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

192 Water and gas produced from multiple wells are delivered to each field station. The field stations
193 in our dataset were fed by 22 wells on average. Withdrawals from the individual wells sampled
194 for this study were estimated by dividing the total monthly gas and water production by the
195 number of online wells in each field. It should be noted, however, that production levels can vary
196 significantly among the wells in an individual field and our data do not constrain this variation.

197 We could not evaluate, therefore, the extent to which the values we calculated accurately depict
198 production levels for the wells we sampled. Nonetheless, the values we calculated still provide a
199 useful measure of the average trends in water and gas production over time for the wells
200 associated with each field station.

201 **3. Results**

202 *3.1. Microbial community composition*

203 Chao1 values based on OTUs defined at $\geq 97\%$ sequence identity were greater for
204 *Bacteria* than *Archaea* in all samples, indicating greater richness for *Bacteria* than *Archaea* at
205 that similarity level (Fig. 1). Richness was greatest for *Archaea* in the 2009 sample with
206 intermediate salinity and greatest for *Bacteria* in the 2009 sample with the lowest salinity.
207 Strongly asymptotic rarefaction curves for each *Archaea* clone library (Supplementary Content
208 Fig. 1) indicate that the archaeal community was adequately sampled. Similarly, rarefaction
209 curves for bacterial clone libraries from well 22 and the 2002 sample from well 150 were also
210 asymptotic. Curvilinear rarefaction curves for bacterial clone libraries from well 147 and the
211 2009 sample from well 150, however, indicate that additional sequencing would be needed to
212 fully characterize the bacterial community in the water produced from those wells.

213 Taxonomic classification places all *Archaea* clones in the *Euryarchaeota* (Fig. 1A),
214 which contains the methanogens and extreme thermophiles and halophiles (Takacs-Vesbach et
215 al., 2001). Within this phylum, the majority of the archaeal clones obtained from 2009 samples
216 grouped within two orders of methanogenic microorganisms: *Methanomicrobiales* (59%) and
217 *Methanobacteriales* (33%). *Methanobacteriales* clones were most abundant in the lowest salinity
218 sample and *Methanomicrobiales* clones were most abundant in the highest salinity sample (Fig.
219 1A). Cultured members of these orders reduce CO₂ typically with H₂ as their electron donor,
220 although some can use formate or secondary alcohols (Bonin and Boone, 2006; Garcia et al.,
221 2006). *Methanosarcinales*, the only order of methanogens that contains species capable of using
222 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.

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

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

266

267 *3.3. Chemical and isotopic composition of gas*

268 Similar to the observed changes in water production and composition, the amount of gas
269 being produced and its chemical and isotopic composition has shifted since the wells were
270 developed. Field station records show that gas production has decreased and that the proportion
271 of CO₂ in produced gas has increased by an average of 13 mol% while overall natural gas
272 production has steadily declined (Fig. 2).

273 Analysis of gas samples collected for this study show a similar result to the field station
274 records. Compared to samples collected initially, the CH₄ content of the gas samples we
275 collected decreased by 11 mol% on average in five wells while CO₂ content increased by an
276 equivalent amount (Supplemental Content Table SC3). Gas wetness [$C_1/(C_2+C_3)$] values in the
277 samples were generally lower than initial values. Wetness averaged 1001 compared to 1432
278 initially. Although the composition of gas shifted as gas production declined, CH₄ is still by far
279 the largest component. The mean CH₄ content of the samples we collected was 82 mol%
280 compared to 86 mol% initially.

281 Shifts in the δD value of CH₄ correspond to those observed in water. As the δD value of
282 water decreased, the δD value of CH₄ also largely decreased (Fig. 5A). With the exception of one
283 sample with values that were not considerably different from those measured initially (>4‰), the
284 δD values of CH₄ were lower in all of the samples by 9‰ on average. The average difference
285 between the δD of CH₄ and water in the samples we collected was 172‰, which is not
286 significantly different from the value measured initially, 171‰, based on a Student's T test (P
287 0.735).

288 Similarly, the $\delta^{13}C$ value of CH₄ also decreased for most wells (Fig. 5B). One sample had
289 CH₄ $\delta^{13}C$ values that did not differ from initial values by more than combined analytical error
290 (>0.2‰). The remaining six samples had $\delta^{13}C$ values that were 1.3‰ lower on average. The $\delta^{13}C$

291 values of CO₂ measured in gas samples increased for most wells (Fig. 5B). Four samples had a
292 CO₂ δ¹³C value 1.7‰ higher on average. Of the remaining three samples, one did not differ from
293 initial values by more than combined analytical error (>0.2‰) and two decreased by 0.3 and
294 2.5‰. The fractionation factor (α_c) between δ¹³C values of CO₂ and CH₄ calculated for each
295 sample we collected was 1.076 on average, where α_c is expressed as:

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

296
297 (3)

298 This value is very similar to that observed in the samples collected from the wells initially,
299 1.074. Nonetheless, these averages are statistically different based on a Student's T test (P =
300 0.045).

301

302 **4. Discussion**

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

309

310 *4.1. Pathway of CH₄ formation*

311 Using isotopic evidence, Martini and others (1996; 1998) interpreted that CH₄ over much
312 of the northern producing trend in the Antrim Shale was generated by CO₂-reducing

313 methanogens. The results of our isotopic analyses are consistent with those findings. Where CH₄
314 is produced by CO₂ reduction, the δD value of CH₄ 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 CH₄ and water are
317 approximately twice as large where CH₄ is produced by acetate fermentation (Schoell, 1980;
318 Whiticar et al., 1986; Woltemate et al., 1984). Similarly, CH₄ produced by CO₂ 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 CH₄ by
324 CO₂ reduction in the archaeal clone libraries we generated suggests that CO₂-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 H₂ (Madigan et al., 2003), implying that acetate is being
342 generated within the shale. During organic matter degradation, production of acetate relative to
343 H₂ increases as a result of the activity of acetogenic microorganisms, *Bacteria* that consume H₂
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 CH₄ is present (Martini et al., 2003) and H₂ oxidation appears to have largely fueled
351 formation of CH₄.

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 SO₄²⁻ reducers and syntrophic acetate oxidizers. The limited availability of SO₄²⁻ until recently
355 has likely restricted the activity of SO₄²⁻ 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 CH₄ 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., Strapoc 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 CH₄ 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 (Magonigal 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 ($\text{Cl}^- < 1 \text{ M}$) and low- δD , low- $\delta^{18}\text{O}$ 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 methanogenesis 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- $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ values.
465 This change, however, is largely inconsistent with our results. Instead, $\delta^{18}\text{O}$ values were slightly
466 heavier in most cases, consistent with inflow that has a greater component of basin brine ($\delta^{18}\text{O}$ -
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

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

483

484 *4.5. Shift in gas geochemistry*

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

493 As the proportion of CO_2 has increased, our results show that the isotopic composition of
494 CO_2 and CH_4 has shifted. Similar to the observed shifts in water geochemistry and microbiology,
495 these shifts may have occurred because gas is being drawn into each well from a different
496 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 CO₂ is simply being drawn into those wells from a zone with CO₂ that has a lower δ¹³C
521 than the CO₂ that was initially present, additional CO₂ must have been generated that has a δ¹³C
522 more consistent with organic matter (i.e., lower). Parallel shifts in the δ¹³C of CO₂ and CH₄ and
523 the δD of water and CH₄ are consistent with continued CH₄ 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 CH₄ there would be consistent with changes in the
526 isotopic composition of both CO₂ and water.

527 Unlike the possibilities outline above, CH₄ oxidation does not appear to be a primary
528 control on the isotopic composition of either CH₄ or CO₂. During CH₄ oxidation, isotopically
529 depleted CH₄ is preferentially oxidized (Barker and Fritz, 1981; Holler et al., 2009). This effect
530 would increase the δ¹³C value of residual CH₄ and decrease the δ¹³C value of CO₂, 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

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

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

551

552

553 **5. Conclusions**

554 Our results show that (1) gas being commercially produced in the field area today was
555 still primarily produced by CO₂ reduction, (2) SO₄²⁻ concentration and the abundance of SO₄²⁻-
556 reducing microorganisms have increased, changes that may ultimately allow SO₄²⁻ reducers to
557 displace methanogens, and (3) in addition to SO₄²⁻, other changes in the chemical and isotopic
558 composition of water and gas in the shale have also occurred. These changes in microbiology
559 and geochemistry can be explained by ongoing biogeochemical reactions and processes driven
560 by commercial gas production, including groundwater flow, gas desorption, and open-system
561 degassing.

562 These findings highlight the complex array of processes that can influence geochemistry
563 and microbiology during commercial gas production and multiple areas where additional
564 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

572 **Acknowledgements**

573 We thank members of the Gas Technology Institute New Albany Shale consortium for
574 helpful comments and reviews. We are grateful to two anonymous reviewers for helpful
575 comments that improved this manuscript. We also thank Thomas Naughton and the personnel of
576 energy companies that assisted us with field work, Maarten de Moor and Zach Sharp for helpful
577 discussions, and Tim Maness for field station data. This work was supported by both RPSEA
578 funding through the Ultra-Deepwater and Unconventional Natural Gas and Other Petroleum
579 Resources program and the American Chemical Society-Petroleum Research Fund grant to
580 Martini.

581

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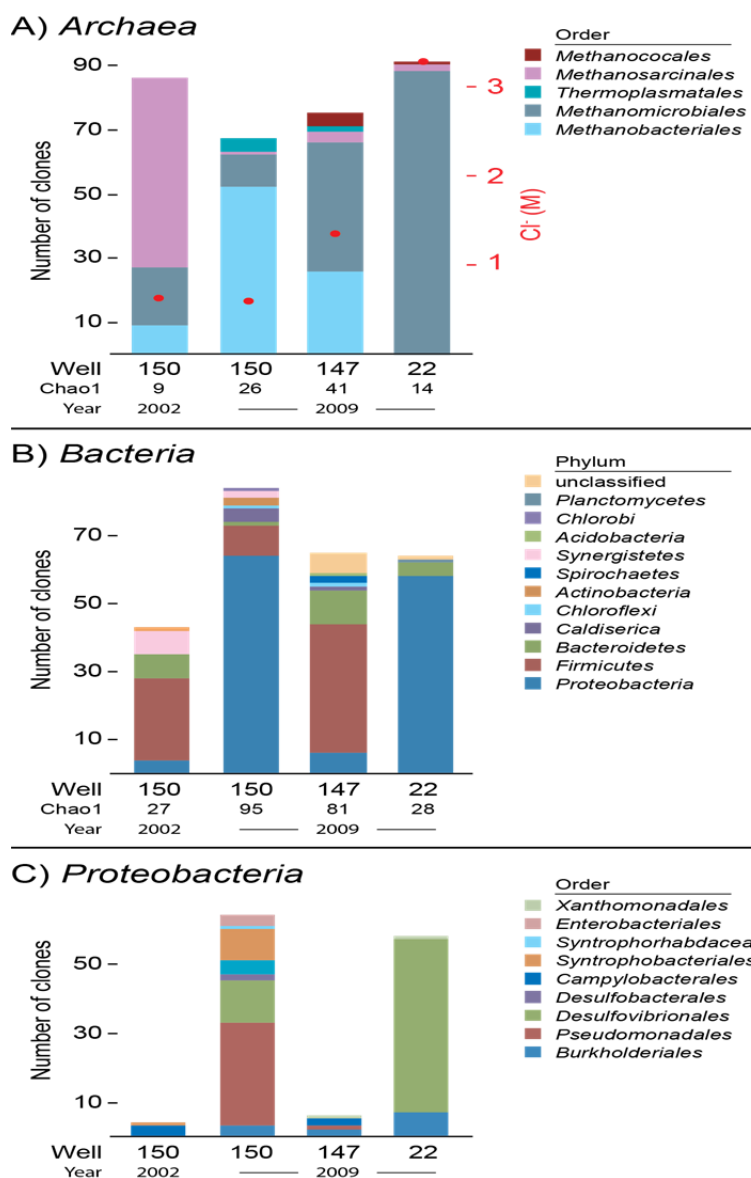
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754 **Figures**

755 Figure 1



756

757 Figure 1. Taxonomic distribution of clones detected in samples from well 150, 147, and 22.

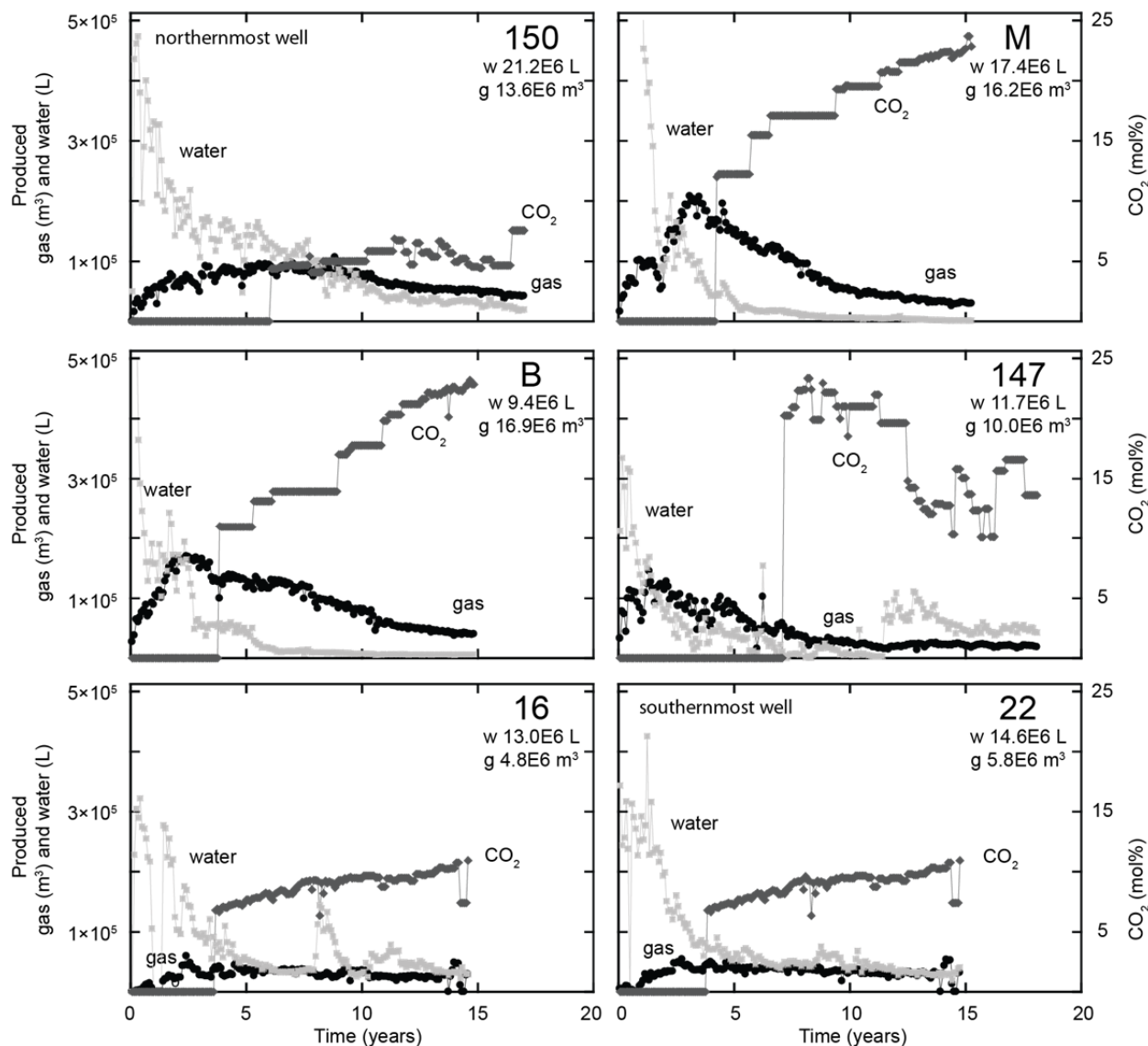
758 Chart (A) shows the distribution of archaeal clones at the order level, (B) shows bacterial clones

759 at the phylum level, and (C) shows proteobacterial clones at the order level. Chao1 richness

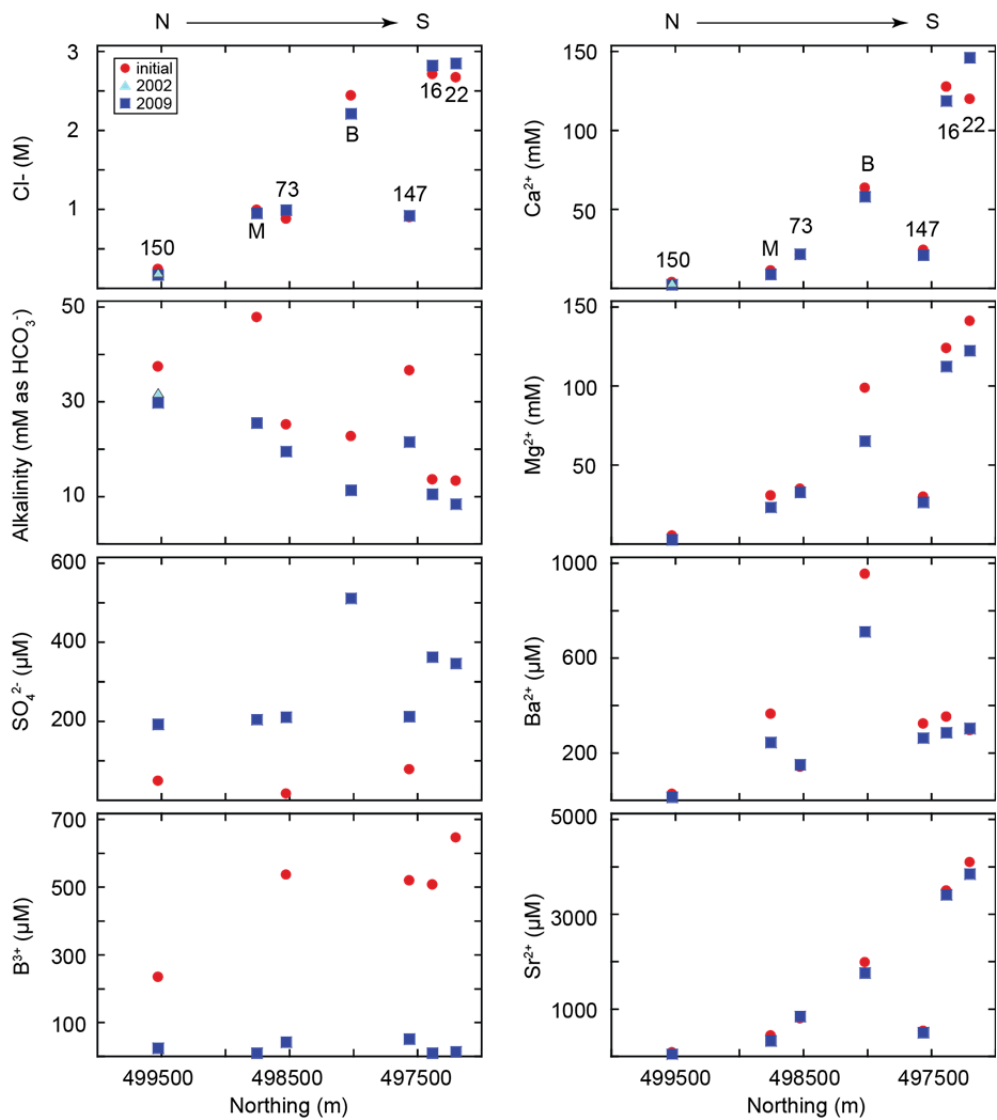
760 estimates based on OTUs defined at $\geq 97\%$ sequence identity are listed for each library under the

761 charts for *Archaea* and *Bacteria*. Cl^- concentration is plotted on the chart showing *Archaea*.

762 Figure 2



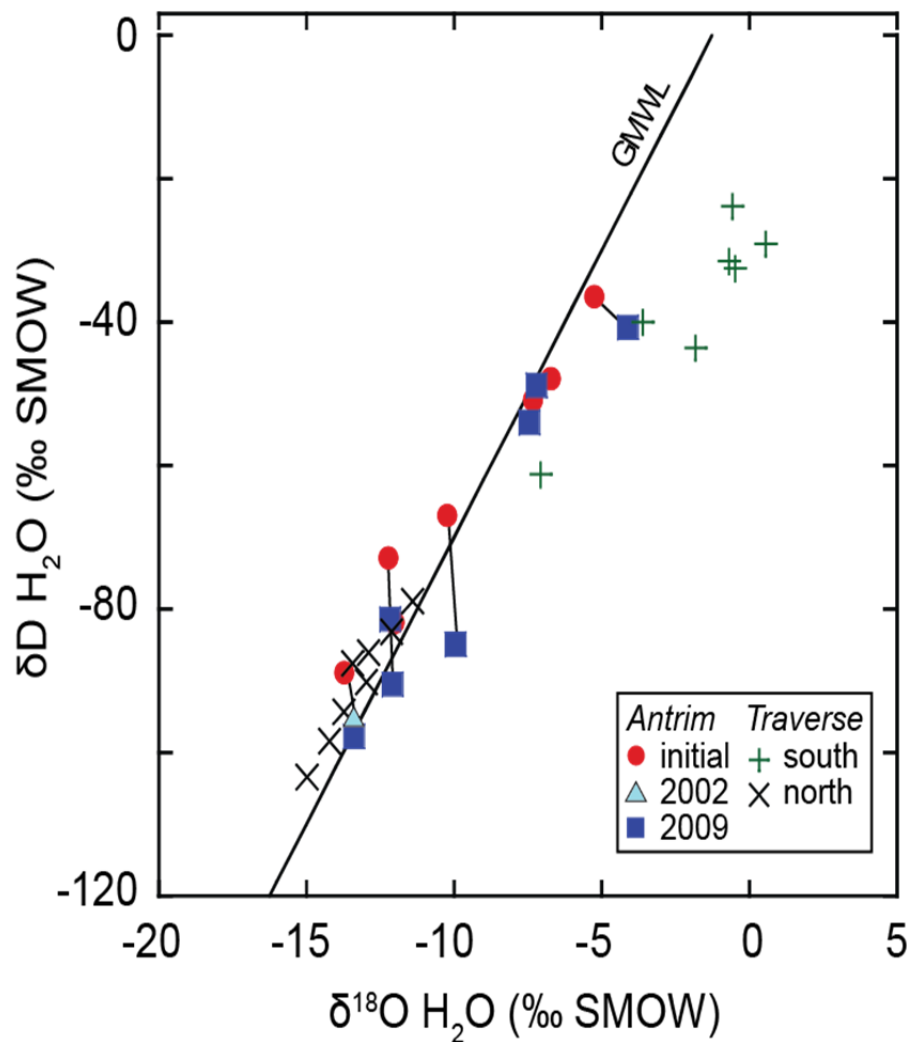
763
 764 Figure 2. Variation in water and gas production and gas CO₂ content over time at field stations
 765 supplied by wells 150, M, B, 147, 16, and 22. Data are plotted relative to the date each field
 766 station came online (t = 0) and normalized to the number of wells in the field. The graphs 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.



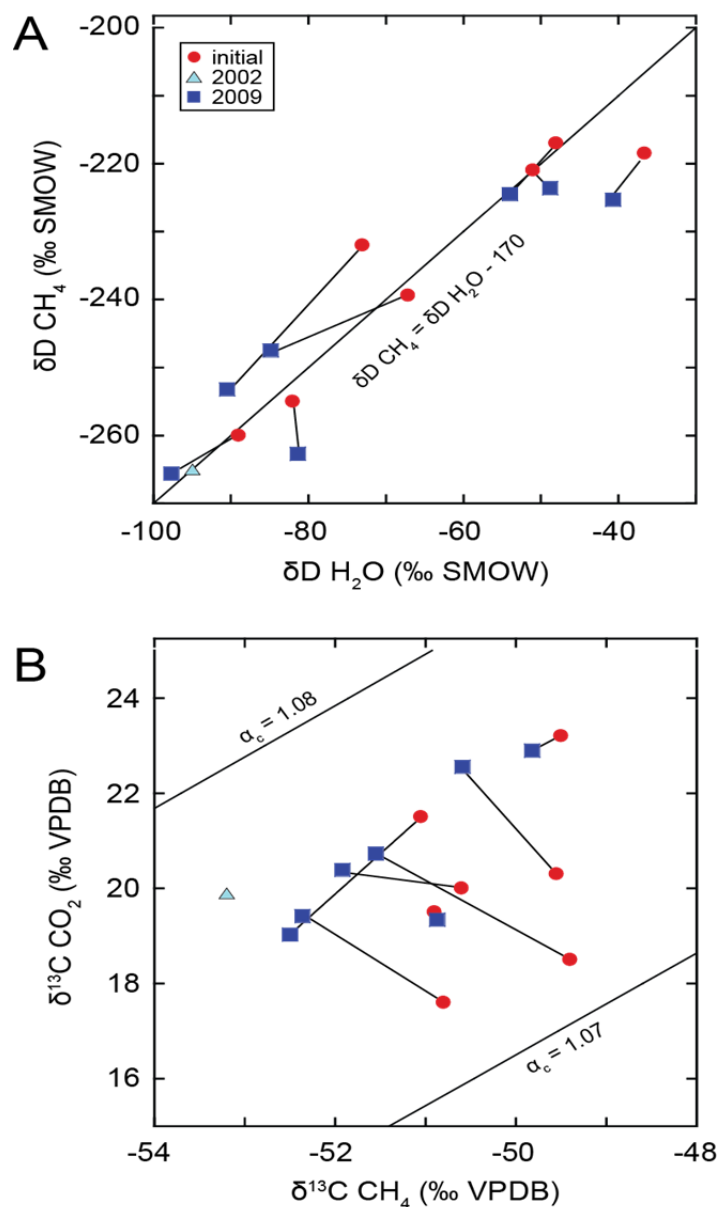
771

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

773



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



782

783 Figure 5. Variation in (A) the hydrogen isotope composition of CH₄ relative to co-produced

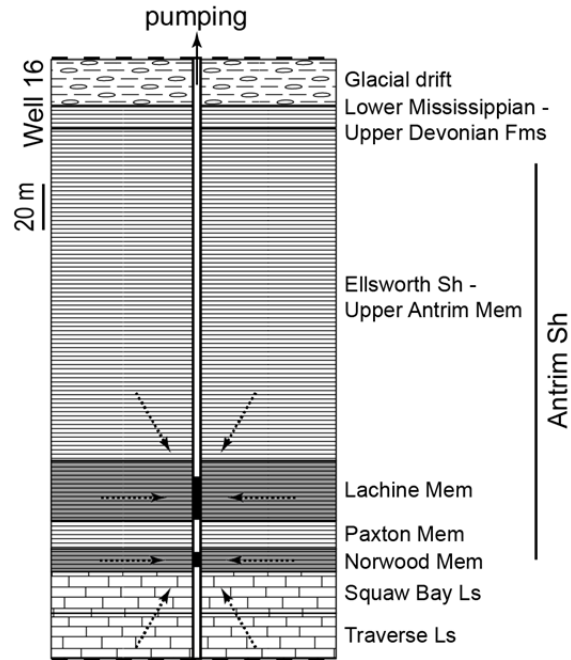
784 water and (B) the carbon isotope composition of CH₄ relative to CO₂. The δD value of CH₄ was

785 lower than the δD value of water by 172‰ (samples collected in 2009) and 171‰ (samples collected

786 initially), on average. The average fractionation factor (α_c) between CO₂ and CH₄ carbon

787 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

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

¹Analysis 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.

²Archaeal DNA amplified using 25F (5'-CYG GTT GAT CCT GCC RG-3') AND 958R (5'-YCC GGC GTT GAM TCC AAT T-3')

³Archaeal 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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