

# In Situ Enhancement and Isotopic Labeling of Biogenic Coalbed Methane

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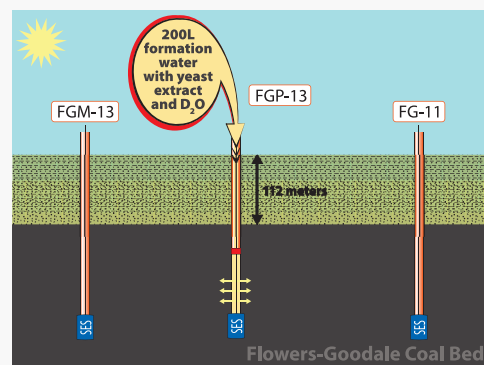


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**ABSTRACT:** Subsurface microbial (biogenic) methane production is an important part of the global carbon cycle that has resulted in natural gas accumulations in many coal beds worldwide. Laboratory studies suggest that complex carbon-containing nutrients (e.g., yeast or algae extract) can stimulate methane production, yet the effectiveness of these nutrients within coal beds is unknown. Here, we use downhole monitoring methods in combination with deuterated water ( $D_2O$ ) and a 200-liter injection of 0.1% yeast extract (YE) to stimulate and isotopically label newly generated methane. A total dissolved gas pressure sensor enabled real-time gas measurements (641 days preinjection and for 478 days postinjection). Downhole samples, collected with subsurface environmental samplers, indicate that methane increased 132% above preinjection levels based on isotopic labeling from  $D_2O$ , 108% based on pressure readings, and 183% based on methane measurements 266 days postinjection. Demonstrating that YE enhances biogenic coalbed methane production in situ using multiple novel measurement methods has immediate implications for other field-scale biogenic methane investigations, including in situ methods to detect and track microbial activities related to the methanogenic turnover of recalcitrant carbon in the subsurface.



**KEYWORDS:** biogenic methane, deuterium, subsurface environmental sampler, isotopic labeling, coalbed methane

## INTRODUCTION

Subsurface microorganisms have generated biogenic methane in many hydrocarbon environments including coal, organic-rich shale, and petroleum reservoirs at scales that impact global energy cycles.<sup>1–4</sup> Estimates suggest that biogenic gas accounts for one fifth of the world’s natural gas resources,<sup>5</sup> and over the past several decades, biogenic coalbed methane has been developed as an unconventional energy resource.<sup>1,3,6,7</sup> Laboratory investigations indicate that biogenic coalbed methane production can be enhanced with complex nutrients (e.g., yeast extract (YE) or algal cell components).<sup>1,3,8–11</sup> The use of nutrients from sources that remove carbon dioxide from the atmosphere (e.g., algae, plants) in coal beds and other hydrocarbon reservoirs could result in the enhanced generation of low-carbon renewable natural gas.<sup>9,11,12</sup> Laboratory results indicate that algae, cyanobacteria, yeast cells, and granulated YE increase coalbed methane production similarly<sup>11</sup> with 113% of the carbon added as amendment recovered as methane.<sup>13</sup> Companies (e.g., Luca Technologies, Next Fuel) have attempted field-scale injections with organic nutrients (including YE) in the Powder River Basin (PRB), USA, and other coal

beds worldwide, but have not published any results indicating that methane increased above preinjection levels.<sup>1</sup> To the authors’ knowledge, the only field-scale study to document an increase in methane due to nutrient injections occurred in a methane-free, sulfate-rich subbituminous coal bed so any methane measured could be considered “new”.<sup>14,15</sup>

Subsurface coal beds are complex systems where most of the methane is adsorbed in the coal matrix. The Langmuir isotherm is commonly used to determine the maximum adsorption of methane by the coal matrix of a specific coal sample.<sup>16–18</sup> Within the PRB and many other environments, carbon and hydrogen isotopic signatures (<sup>13</sup>C/<sup>12</sup>C and D/H) have been used to infer the origin of methane.<sup>19–22</sup> However, many factors contribute to the fractionation of these isotopes,

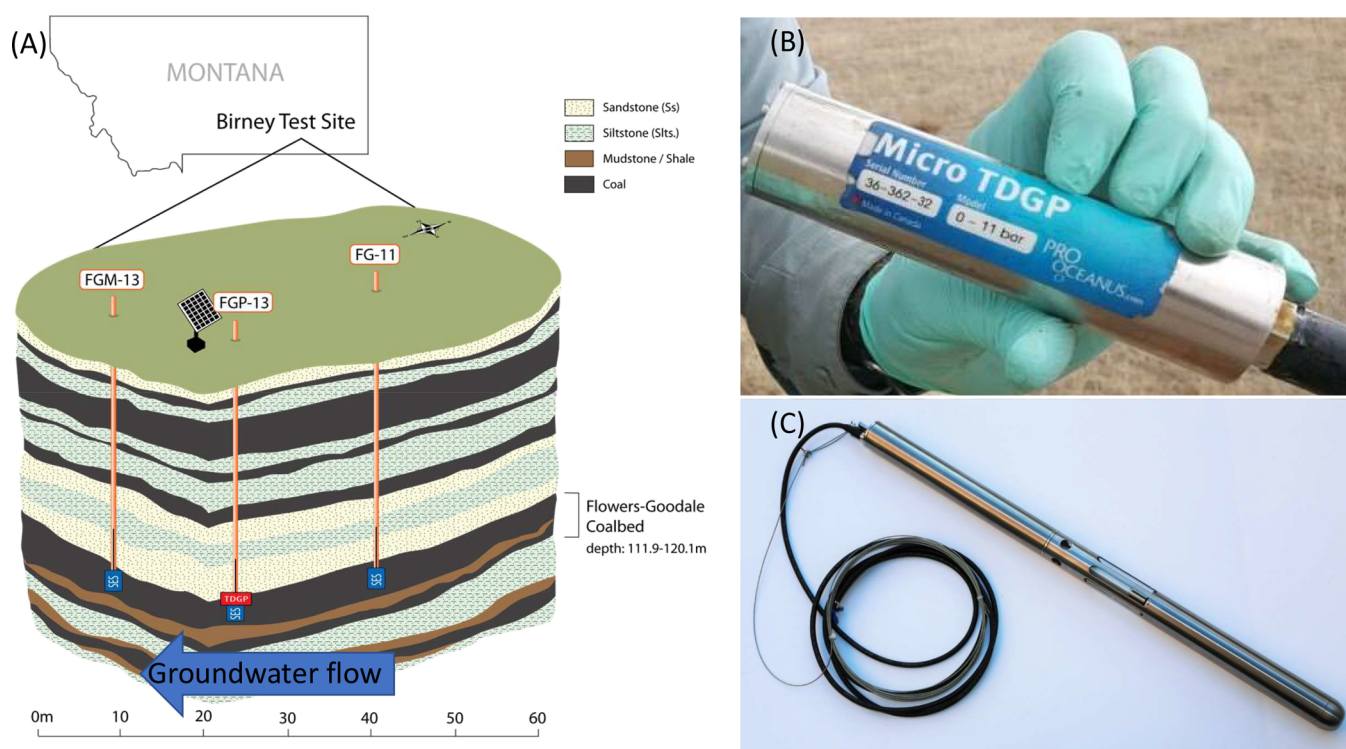
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**Figure 1.** (A) Schematic of the USGS Birney Test Site in the PRB, Rosebud County, Montana, showing location of wells and generalized stratigraphy. (B) Real-time total dissolved gas pressure sensor used to establish baseline equilibrium CBM concentrations in the Flowers-Goodale coal bed (photo credit, Elliott Barnhart, logo credit, Mark Barry). (C) SES used to obtain in situ gas samples and incubate coal down-well (photo credit: Randy Hiebert).

including the source of hydrogen and carbon, the age of the methane, oxidation (aerobic and anaerobic) reactions, and the mixed microbial community. This makes inferences about methane origin and generation time based solely on isotopic signatures problematic.<sup>23–25</sup> A nontoxic supplement to existing stable isotopic methods is the inclusion of deuterated water ( $D_2O$ ).<sup>26</sup> A recent proof of concept laboratory investigation using water, microorganisms, and coal from the Flowers-Goodale coal bed in the PRB indicated that the addition of deuterium can be used to quantitatively track methane increases because methanogenic pathways (i.e., acetoclastic, hydrogenotrophic, and methylotrophic) can incorporate deuterium from  $D_2O$  into methane based on the specific pathway and relative abundance of  $D_2O$  in the water.<sup>27</sup> Although this has been demonstrated in the laboratory and the idea has been patented,<sup>26</sup> to our knowledge, it has not yet been tested in a field-scale injection.

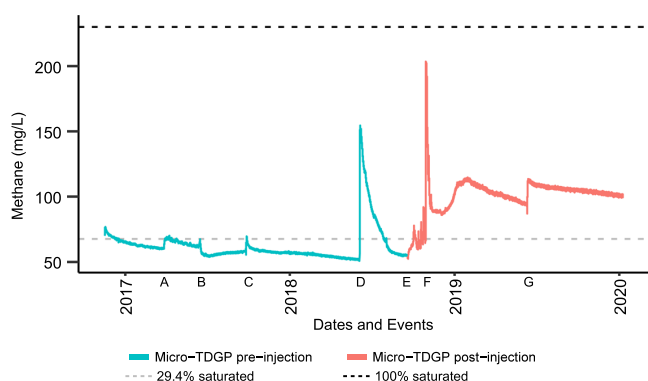
Investigating the isotopic changes in downhole fluids (e.g., water and methane gas) over time from deuterium addition requires advanced sampling procedures devoid of groundwater pumping, which would remove injected deuterium and impact subsequent samples. These new downhole (nonpumped) sampling technologies have provided critical insight into subsurface microbial communities generating methane and in situ geochemical changes.<sup>28–31</sup> However, what remains a key gap is the suitability and accuracy of these recent advances for measuring methane concentration in the subsurface.

Here, we utilize downhole sampling technology to measure methane enhancement following the addition of organic nutrients and deuterium in existing monitoring wells. This study took place in a hydrologically characterized U.S. Geological Survey field site (USGS; Birney Test Site) located

on Bureau of Land Management land with wells screened in the confined Paleocene Tongue River Member of the Fort Union Formation Flowers-Goodale coal (Figure 1A).<sup>32</sup> We combined downhole measurements obtained from a total dissolved gas probe (Pro-Oceanus Micro-TDGP) (Figure 1B) and a recently developed subsurface environmental sampler (SES) (Figure 1C)<sup>35</sup> with the standard gas assessment technology that includes core desorption and Langmuir isotherm saturation calculations to benchmark this new monitoring methodology.<sup>18</sup> From SES samples, conventional isotopic shifts ( $\delta^{13}C-CH_4$ ,  $\delta D-CH_4$ , and  $\delta^{13}C-CO_2$ )<sup>34–38</sup> were identified following the injection. Combined, these methods provide insight into in situ stimulation of methane production in coal beds following an injection of organic nutrients, the accuracy of downhole gas measurements, and the ability to use isotopic labeling as a tool to measure newly generated methane. The methods used and developed in this study have broad applications for measuring and tracking newly generated methane in many subsurface environments.

## METHODS

**Field Site Preparation for Injection.** The Flowers-Goodale coal bed at the Birney Test Site is a well described and characterized coal in the PRB.<sup>32</sup> A Micro-TDGP (Pro-Oceanus, Bridgewater, Canada, [www.pro-oceanus.com](http://www.pro-oceanus.com)) was installed in the Flowers-Goodale coal in the Flowers-Goodale Pump-13 (FGP-13) well within the well screen (116 m below land surface (bls)) and connected to a NexSens 3100-MAST data logger with an integrated cellular modem to transmit data in real-time every half hour (Figure 1A,B). The system began collecting data on December 16, 2016 and continued through January 9, 2020 (Figure 2).<sup>39</sup>



**Figure 2.** Micro-TDGP readings and pre (blue line) and postinjection (red line) from 2017 to 2020. The dashed lines represent when the water is 100% saturated with methane (black dashed line) and when the methane saturation of the water is equal to the preinjection methane saturation of the coal based on isotherm and core desorption analysis (gray dashed line). Events at the field site: (A) regional groundwater pumping; (B) injectivity test in FGP-13; (C) regional groundwater pumping; (D) groundwater pumping from FGP-13 and SES gas sample collected; (E) SES samples collected and YT/deuterium injection in FGP-13; (F,G) SES samples collected.

The dissolved gas pressure (DGP) range of the Micro-TDGP was 0 to 1099.7 kilopascals (kPa), and the output for the probe was an analog signal measured in milliamps (mA). The four-point factory calibration for the probe at 21 °C was set to 4 mA corresponding to 0 kPa and 20 mA equal to 1099.7 kPa of DGP.

Gas concentrations were calculated using Henry's Law with the following assumptions:

- $88 \pm 1.12\%$  of gas was methane based on previous dissolved gas analysis.<sup>32</sup>
- Atmospheric pressure at the Birney Test Site (~944 meters (m) elevation) was 90.2 kPa.
- Henry's Law constant for methane in water at 15.6 °C is 0.267 mg/L/kPa.<sup>40</sup>

**Langmuir Isotherm Analysis.** To compare concentrations of methane dissolved in formation water to methane sorbed in the coal bed, we analyzed core from the Flowers-Goodale coal bed at the Birney Test Site.<sup>39</sup> In 2010, a sample was chosen and desorbed (112.6–112.9 m bls:0.52 standard cubic centimeters of methane per gram of coal (Scm<sup>3</sup>/g) at 15.6 °C and 837 kPa).<sup>32</sup> A sample of this core was sent to RMB Earth Science Consultants, Ltd. (Delta, British Columbia, Canada) for low-pressure methane adsorption analysis.<sup>41</sup> There, the core was crushed to 60 mesh and placed in an equilibrium moisture bath for 21 days. Isotherm analyses

were conducted in low-pressure volumetric adsorption apparatus at a reservoir temperature of 15.6 °C. A known volume of gas was used to dose 100 g coal samples from the Flowers-Goodale coal bed, and the amount of gas adsorbed on equilibrated samples was determined using a series of pressure measurements.<sup>39</sup>

**SES Method.** The SES is a stainless steel sampling device that can open or close (thus collecting a gas or water sample in a sealed container) at a specific depth when deployed downhole (Figure 1C).<sup>33</sup> For this study, the SES was slowly lowered and opened ~5 m above the well screen and then lowered to the center of the well screen where it remained for several minutes before being closed and pulled up to the surface. Once retrieved, the SES chamber (now filled with 250 mL of formation water and dissolved gas) was used to collect baseline total dissolved gas pressure (TDGP) field readings 1 day prior to the injection (September 17, 2018) and twice postinjection (October 29, 2018 and June 11, 2019) from wells FGP-13, FGM-13, and FG-11 (Figure S1). The TDGP of the SES was measured using a Grainger (Lake Forest, Illinois) 0 to 1379 kPa ABS Case commercial pressure gage when the pressurized SES was returned to the surface.<sup>39</sup> The TDGP reading was then converted to total dissolved gas (TDG) based on Henry's Law using the assumptions discussed above. Following TDGP readings, an additional sample was collected, and the SES chamber was removed, placed on ice, and sent for bulk composition and stable isotope analysis of dissolved gases. Gas samples were collected for bulk composition and stable isotope analysis on June 5, 2018 (105 days preinjection) and September 17, 2018 (1 day preinjection) from well FGP-13 to establish baseline levels prior to the YE and D<sub>2</sub>O injection and collected twice postinjection (on October 29, 2018 (41 days postinjection) and June 11, 2019 (266 days postinjection)) from well FGP-13 ( $n = 4$  gas samples) (Table 1). Gas samples for bulk composition and stable isotope analysis were collected from all wells (FGP-13, FGM-13, and FG-11) on June 11, 2019 (266 days postinjection) to investigate the regional impact of the injection (Table 1).

A new laboratory methodology for sampling gas from the SES was developed. Briefly, an empty syringe was attached to one of the two ports in the bottom of the SES chamber, and a helium-filled syringe was attached to the other. There was no headspace gas in the SES chamber because they were under pressure and the concentration of methane in the aquifer was below saturation for the depth the samples were collected. With the SES chamber turned so that both ports pointed downward, an aliquot of helium was injected through one port while an equivalent amount of water was removed from the other. The water and headspace in the SES chamber were

**Table 1.** Methane in mg/L, Percent, and Isotopic Composition of Water from SES Samples from Wells FGP-13 (105 and 1 Days before the Nutrient Injection, and 41 Days and 266 Days after the Nutrient Injection) and FGM-13 and FG-11 (266 Days after the Nutrient Injection)

sample name	methane (mg/L)	GC-IRMS	GC-IRMS	GC-IRMS	GC-IRMS	GC-IRMS	TILDAS	TILDAS
		$\delta^{13}\text{C-CO}_2$	$\delta^{13}\text{C-CH}_4$	$\delta\text{D-CH}_4$	$\delta\text{D-H}_2\text{O}$	$\delta^{18}\text{O-H}_2\text{O}$	$\delta^{13}\text{C-CH}_4$	$\delta\text{D-CH}_4$
FGP-13 105 days preinjection	59							
FGP-13 1 day preinjection	92	-6.6	-67.38	-287.5	-136.4	-17.73	-66.51 ( $\pm 0.18$ )	-285.21 ( $\pm 0.08$ )
FGP-13 41 days postinjection	150	-5.2	-54.04	3361.5	8586.6	-17.28	-52.53 ( $\pm 0.22$ )	3761.01 ( $\pm 0.23$ )
FGP-13 266 days postinjection	170	-3.9	-63.77	1818.7	3957.5	-17.36		
FG-11 266 days postinjection	140	-6.0	-68.59	-277.7	-136.6	-17.85		
FGM-13 266 days postinjection	56	-8.9	-66.95	-283.8	-136.0	-17.69		

equilibrated for 2 h. After equilibration, the amount of headspace gas was determined by measuring the volume of excess headspace gas (above ambient atmospheric pressure) plus the volume of gas (helium) injected into the cylinder. After equilibration and sampling the headspace gas for gas chromatography (GC) and isotope analyses, the water in the cylinder was removed and weighed to determine the volume of water equilibrated with the headspace.

Stable isotopes were measured by gas combustion and isotope ratio mass spectrometry, and the bulk composition was measured using a headspace equilibration technique as previously described.<sup>42</sup> Methane isotopes ( $\delta^{13}\text{C}-\text{CH}_4$ ,  $\delta\text{D}-\text{CH}_4$ , and  $\delta^{13}\text{CH}_3\text{D}$ ) were analyzed using a tunable infrared laser direct absorption spectrometer (TILDAS) on FGP-13 samples collected 1 day preinjection and 41 days postinjection. Using a 413 m optical path length astigmatic Herriott cell and two quantum cascade lasers, the TILDAS scans the spectral regions of  $1090.46 \pm 0.1$  and  $1200.23 \pm 0.1$   $\text{cm}^{-1}$ , simultaneously measuring the five main isotopologues of methane.<sup>43</sup>

**Injection.** A 210 L tank and polypropylene tubing were purged with 95%  $\text{N}_2$ /5%  $\text{CO}_2$  gas, and one end of the tubing was lowered to 116.4 m in FGP-13 on September 18, 2018. On September 18, 2018, a 210 L tank was filled with approximately 196 L of formation water from nearby well Flowers-Goodale 2009 (FG-09),<sup>32</sup> 200 g of Bacto (Becton Dickinson, Franklin Lakes, NJ) YE dissolved in 3 L of deionized water, and 900 mL of 99.9%  $\text{D}_2\text{O}$  (Isowater, Ontario, Canada), bringing the total volume of injection fluid in the tank to  $\sim 200$  L. The SES and Micro-TDGP were removed from well FGP-13 prior to the injection. A pressure transducer (Level TROLL, in situ) was installed in FGP-13 to monitor water depth to keep the water level from raising above 9 m during the injection to avoid mixing in the borehole. The injection began on September 18, 2018 and was gravity fed taking 10 h to complete. After injection, approximately 9.5 L of fluid remained uninjected in the tubing which drained into well FGP-13 as the tubing was removed from the well. After injection ceased on September 19, 2018, the SES and Micro-TDGP were reinstalled in well FGP-13 and the transducer was removed. Overall,  $\sim 190.5$  L of fluid were injected at the well screen at an average injection rate of  $\sim 0.318$  L per minute.

## RESULTS AND DISCUSSION

### Establishing a Methane Baseline Prior to Injection.

Several methods were used to establish the in situ methane concentration prior to the injection. Standard gas assessment technology includes core desorption and Langmuir isotherm saturation calculations.<sup>18</sup> The Langmuir isotherm saturation from the Flowers-Goodale coal sample at the in situ temperature (15.6 °C) and pressure (at 17.6 kPa) was 1.77  $\text{Scm}^3/\text{g}$ . Dividing the in situ methane concentration measured from desorbing this core (0.52  $\text{Scm}^3/\text{g}$ ) by the maximum adsorbed concentration determined by the Langmuir isotherm (1.77  $\text{Scm}^3/\text{g}$ ) under reservoir conditions suggested that the coal bed was 29.4% saturated with methane gas when the core sample was obtained in 2010 prior to any stimulation.

Successive baseline measurements collected with the Micro-TDGP every half hour between November 16, 2017 and June 4, 2018 in well FGP-13 recorded an average methane concentration of  $59.4 \pm 5$  mg/L or 25.4  $\pm$  2.1% saturated (Figure 2).<sup>39</sup> Several minor methane fluctuations were recorded which coincided with four events including an

injectivity test and regional pumping (Figure 2). The average methane concentration ( $59.4 \pm 5.1$  mg/L) based on the Micro-TDGP was nearly equal to the SES direct methane measurement taken 105 days preinjection (59 mg/L or 25.2% saturated) (Table 1). This concentration was only slightly lower than the isotherm-calculated saturation of the coal bed 29.4% based on isotherm data (Figure 2). Overall, the Micro-TDGP and the SES gas composition measurement 105 days preinjection indicate that the baseline methane concentration within the water of the Flowers-Goodale coal bed at the Birney Test Site was approximately 60 mg/L or 26% saturated with methane preinjection. These measurements were very close to the methane saturation percentage calculated by the Langmuir Isotherm calculations (29.4% saturated) (Figure 2). More studies are needed relating isotherm results to downhole measurements to better understand site-specific conditions.

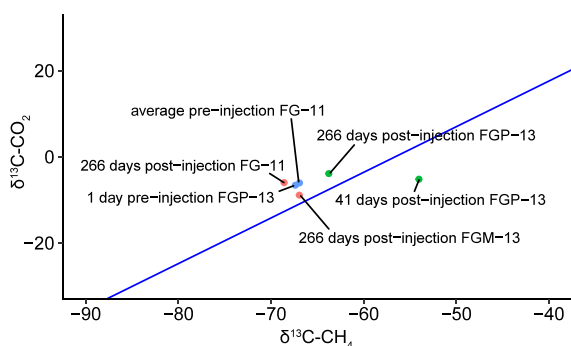
After pumping approximately three wellbore volumes of water (500 L) 105 days prior to the injection, methane increased from 51.8 to 153.7 mg/L (94.2 mg/L above the baseline, as measured by Micro-TDGP) and returned to the baseline approximately  $\sim 100$  days after pumping ceased (Figure 2). Drastic increases in dissolved gas due to gas migrating to the wellbore as a result of decreased pressure from pumping have been reported previously;<sup>30,44</sup> however, to the authors' knowledge, this is the first long-term report indicating the length of time needed to reach baseline prepumped levels. This long-term analysis indicates that following the standard three-wellbore-volume purging method prior to sampling dissolved gas for quantitative analysis could provide an overestimate of the in situ methane concentration for months following pumping.<sup>45,46</sup>

The average methane calculated from downhole SES pressure gage readings collected the day before the injection (September 17, 2018) was  $79.5 \pm 25.1$ ,  $68.7 \pm 15.2$ , and  $74.2 \pm 8.7$  mg/L for FGP-13, FGM-13, and FG-11, respectively (Figure S1). The SES methane concentration value from FGP-13 was 92 mg/L 1 day preinjection, which was within error of the SES reading in the field from the pressure gage ( $79.5 \pm 25.1$  mg/L) but significantly above the average measurements taken from the Micro-TDGP 1 day preinjection ( $55.4$  mg/L  $\pm$  0.13) (Table 1 and S2). Pumping that occurred nearby in well FG-09 to obtain Flowers-Goodale water for the injection could have temporarily lowered the water level and increased the gas pressure in well FGP-13 because of connectivity between the wells.<sup>32</sup>

Developing accurate downhole methane detection methodologies is increasingly important for energy development and greenhouse gas monitoring, and there are advantages and disadvantages to each of the different techniques used here to obtain gas measurements. For example, the Micro-TDGP can obtain many real-time TDGP measurements which can be used to indirectly calculate methane concentrations based on Henry's Law and the ideal gas law. The SES can be used to obtain TDGP readings in the field and directly measure methane and other gases using analytical techniques in the laboratory. A limitation of the SES is that measurements must be taken at the surface instead of downhole. Together, the Micro-TDGP and SES were complementary because the Micro-TDGP was monitored remotely and helped determine when detailed gas analysis would be advantageous with an SES measurement.

**Elevated Methane Levels Following Nutrient Injection.** Following the injection of YE and  $\text{D}_2\text{O}$  on September 18,

2018, the indirect measurements of methane from the Micro-TDGP and both direct and indirect measurements from the SES recorded significant methane concentration increases above any baseline values calculated from TDG and gas composition measurements (Figures 2, S1 and Table 1). The gas composition measurement from the FGP-13 SES indicated that the downhole methane concentration was 150 mg/L (90 mg/L above the 60 mg/L baseline) 41 days (October 29, 2018) after the injection while the average pressure reading taken in the field indicated that the methane concentration was 102 mg/L (42 mg/L above baseline) (Figure 2). Two days after the SES sample was collected (October 31, 2018), calculations from the Micro-TDGP measurements indicated a spike in methane to 200 mg/L (140 mg/L above baseline) (Figure 2). A few days later, the methane decreased to 90 mg/L (30 mg/L above baseline) and remained relatively constant for approximately 100 days and then increased rapidly, reaching 115 mg/L (55 mg/L above baseline) on January 30, 2019, and then slowly decreased to 94 mg/L (34 mg/L above the baseline) by June 9, 2019. The Micro-TDGP measured an increase to 113.2 mg/L (53 mg/L above baseline) on June 12, 2019 following SES retrieval from well FGP-13 (Figure 3). The average SES downhole TDG in well



**Figure 3.**  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  values collected from SESs in wells FGP-13, FG-11, and FGM-13. The blue points are values collected preinjection from well FGP-13 and FG-11. The green points are values collected postinjection from FGP-13, and the red points are from FG-11 and FGM-13 postinjection. The blue line is the 1.06 alpha fractionation line.

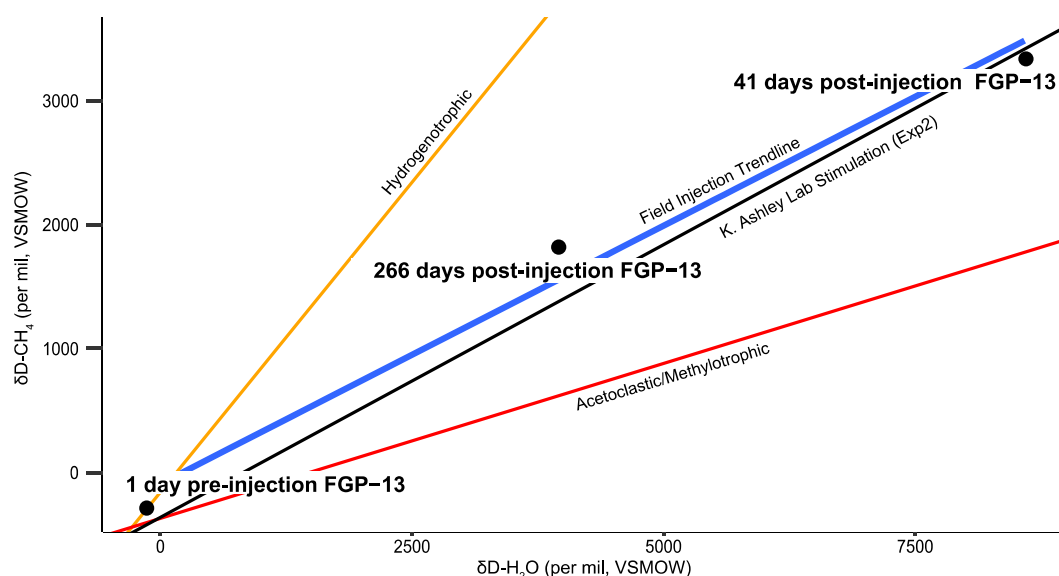
FGP-13 on June 10, 2019 (266 days postinjection) was 126  $\pm$  18 mg/L (Figure 2). The accompanying gas composition measurement from the SES indicated that the methane concentration was 170 mg/L (110 mg/L above the baseline) (Table 1). The methane concentration gradually decreased to 101 mg/L (41 mg/L above the baseline) on January 9, 2020.

These results indicate that the methane concentration downhole in FGP-13 remained elevated above the baseline for more than 16 months after the injection. The Micro-TDGP measured the largest concentration increase 134 days postinjection (114.5 mg/L methane or 91% above the baseline) and remained 68% elevated above the baseline 478 days postinjection when readings ceased. The SES gas pressure measurements and gas composition measurements indicated that the methane increased 108 and 183% respectively above the baseline 266 days postinjection (Figure S1 and Table 1). Although the methane measured from gas composition analysis was higher than the methane calculated from TDG measurements from the SES and Micro-TDGP, the trend was the same: dissolved gas concentration initially increased rapidly

after injection, and then more slowly, peaking at ca. day 267 postinjection. The SES was also used to collect samples from regional wells FG-11 (located  $\sim$ 14.5 m upgradient) and FGM-13 (located  $\sim$ 15 m downgradient) on the same days (September 17, 2018, October 29, 2018, and June 11, 2019) that samples were collected from FGP-13 to provide a regional perspective of the injection impact (Figures 2 and S1). The hydraulic conductivity in the Flowers-Goodale coal bed at the USGS Birney field site is very low (0.005 m/d) suggesting that the injectate only migrated a short distance from the injection point during this test.<sup>32</sup> On June 11, 2019 (226 days postinjection), the methane concentration was 140 mg/L and average methane concentration calculated from TDG from FG-11 was  $103.7 \pm 9$  mg/L, which was higher than the average preinjection measurement obtained from FG-11 of  $74.7 \pm 15$  mg/L (Table 1 and Figure S1). Previous gas measurements from FG-11 obtained on July 7, 2014 were 50 and 33 mg/L using the IsoFlask and inverted VOA method, respectively.<sup>32,42</sup> The increase in methane and dissolved gas outside of the injection well at well FG-11 was unexpected, especially because FG-11 is located 14.5 m upgradient from FGP-13.<sup>32,47</sup> These results suggest that newly generated gas migrated from FGP-13 upgradient to FG-11, although there is no evidence of newly generated gas in FG-11 based on isotopic results (Table 1, Figure 3). Previous laboratory and field research indicate that heavier isotopic volatile organic compounds diffuse slowly compared to isotopically light species.<sup>48</sup> Therefore, the isotopically lighter methane that was generated due to the YE injection could have migrated upgradient to well FG-11 while the isotopically heavier methane (i.e., higher  $\delta^{13}\text{C-CH}_4$  and  $\delta\text{D-CH}_4$  values) remained closer to the source at well FGP-13. The ability to inject  $\text{D}_2\text{O}$  and sample with the SES could provide the ability for more controlled future investigations on isotopic signatures and extent of methane migration. The methane concentration in well FGM-13 (downgradient from the injection well) was 56 mg/L 267 days postinjection, which was much lower than the concentration in FG-11 (140 mg/L) or FGP-13 (170 mg/L) on the same date, but similar to the initial baseline in FGP-13 (59 mg/L) (Table 1 and S1).

**SES Isotopic Analysis.** Previously published background  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  values produced from well FG-11 ( $-67.5 \pm 0.49\text{‰}$  and  $-6.07 \pm 0.12\text{‰}$ , respectively) were similar to the values collected for well FGP-13 preinjection and wells FGM-13 and FG-11 on day 266 postinjection (Table 1, Figure 3).<sup>32</sup> The  $\delta\text{D-H}_2\text{O}$ ,  $\delta^{18}\text{O-H}_2\text{O}$ , and  $\delta^{13}\text{C-CH}_4$  values from wells FGM-13 and FG-11 were all close to the FGM-13 preinjection measurements (Table 1). A slight variation observed in the  $\delta\text{D-CH}_4$  values, with sampled methane from FGM-13 and FG-11 measuring  $-283.8$  and  $-277.7\text{‰}$ , respectively, on day 266 postinjection compared to the preinjection value of  $-287.5\text{‰}$ . The variation observed in  $\delta\text{D-CH}_4$  values could simply be representative of natural variation, as all other isotopic entities measured were very close to preinjection conditions.

Isotopic shifts in both the  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  were observed on day 41 postinjection in well FGP-13: the  $\delta^{13}\text{C-CH}_4$  value increased by  $+13.4\text{‰}$  while the  $\delta^{13}\text{C-CO}_2$  value increased by  $+1.4\text{‰}$  (Figure 3). Measurements taken at 266 days postinjection in well FGP-13 indicate a mixing of two methane sources without much change in  $\delta^{13}\text{C-CO}_2$  values (Figure 3). Previous work indicates that the  $\delta^{13}\text{C}$  value of



**Figure 4.**  $\delta\text{D-H}_2\text{O}$  increased +8586.6‰ and  $\delta\text{D-CH}_4$  increased to +3361.5‰ 41 days postinjection and then decreased back toward preinjection levels 266 days postinjection. The fractionation of  $\delta\text{D-H}_2\text{O}$  and  $\delta\text{D-CH}_4$  was close to the predicted value based on the alpha fractionation line from the study by Ashley et al. (2021).

Flowers-Goodale coal from the Birney Test Site is  $-24.1\text{‰}$ , and YE used in that study had a  $\delta^{13}\text{C}$  value of  $-8.9\text{‰}$ .<sup>49</sup>

There are several potential causes for the isotopic shift in  $\delta^{13}\text{C-CH}_4$  (+13.4‰) that occurred sometime prior to the sample collection 41 days postinjection in FGP-13 (Table 1 and Figure 3). The first could be that during the early stages of stimulation, methanogens primarily derived carbon from the injected YE and then switched back to the other predominant C source, coal, later as the YE was depleted. However, this explanation seems unlikely based upon previous results (Davis et al., 2019) and low methane levels are detected with nutrient-only controls. It is more plausible that the nutrients stimulate primary and secondary coal-degraders that provide cometabolites to methanogens, and this process could have initiated before the day 41 sampling. Another possibility is that the stimulation fundamentally changed the energetics of the methanogenic metabolic pathway through large changes in substrate availability, and this alters the fractionation factor (i.e., less preference for lighter isotopes when the substrate is abundant).<sup>50</sup> Other laboratory experiments have shown similar trends with increasing  $\delta^{13}\text{C-CH}_4$ , but little change in  $\delta^{13}\text{C-CO}_2$  when a carbon source was added to stimulate methanogenesis.<sup>51</sup> The increase in  $\delta^{13}\text{C-CO}_2$  that we observed (+1.4‰) could be due to the high bicarbonate concentration and buffering capacity typical of PRB water,<sup>52</sup> or representative of natural variation.

The stable isotope values of formation water, or  $\delta\text{D-H}_2\text{O}$  and  $\delta^{18}\text{O-H}_2\text{O}$ , sampled from well FGP-13 1 day preinjection were  $-136.4$  and  $-17.7\text{‰}$ , respectively. The stable isotope values of free methane gas,  $\delta^{13}\text{C-CH}_4$  and  $\delta\text{D-CH}_4$ , sampled from well FGP-13 1 day preinjection were  $-67.4$  and  $-287.5\text{‰}$ , respectively. A previous  $\delta^{13}\text{C-CH}_4$  and  $\delta\text{D-CH}_4$  measurement taken from FG-11 on November 15, 2011, and duplicate measurements taken July 23, 2014, were similar to the FGP-13 preinjection values and averaged  $-66.9\text{‰} \pm 0.49$  and  $-289.6 \pm 2.26$ , respectively.<sup>32</sup>

Three of the four measured stable isotope tracers changed dramatically in well FGP-13 when sampled 41 days after the injection of YE and  $\text{D}_2\text{O}$ . In FGP-13,  $\delta\text{D-H}_2\text{O}$  increased to

+8586.6‰,  $\delta\text{D-CH}_4$  increased to +3361.5‰, and  $\delta^{13}\text{C-CH}_4$  increased to  $-54.0\text{‰}$  (Table 1, Figure 4). The postinjection  $\delta\text{D-H}_2\text{O}$  value of FGP-13 is approximately 1/3 of the value of deuterated water added (27000‰). The  $\delta\text{D-H}_2\text{O}$  and  $\delta^{13}\text{C-CH}_4$  values were above the quantification limit of the standards used for the instruments, and therefore, these results are estimates, though nearly the same values were detected by chromatographic separation followed by combustion and dual-inlet isotope ratio mass spectrometry and with a TILDAS<sup>53</sup> (Table 1). The observed isotopic changes in  $\delta\text{D-CH}_4$  could be achieved in one of two ways: direct deuterium exchange with water or uptake of deuterium during methane generation. Abiotic deuterium exchange with water at reservoir temperature of the Flowers-Goodale coal bed ( $15.6\text{ °C}$ ) would occur slowly (geologic timescales)<sup>54</sup> indicating the shifts that were observed were due to microbial activity. These are the first results that reveal the isotopic changes that occur from a  $\text{D}_2\text{O}$  injection into a subsurface system generating biogenic methane.

Recently, laboratory results indicated that it could be possible to quantify new methane generation by incorporating the isotopic changes that occur following  $\text{D}_2\text{O}$  injection (Figure S2).<sup>27</sup> The factor (0.44) below was experimentally derived from laboratory experiments using inoculum, coal, and formation water from the Flowers-Goodale coalbed at the Birney Test Site with  $R^2 = 0.997$ .<sup>27</sup>

$$\delta_{\text{new}} = 0.44 * \delta_{\text{mix}}(\text{H}_2\text{O}) - 361.9$$

Thus:

$$rV_2 = \frac{\delta_{\text{mix}} - \delta_{\text{old}}}{(0.44 * \delta_{\text{mix}}(\text{H}_2\text{O}) - 361.9) - \delta_{\text{mix}}}$$

Therefore, assuming  $\delta_{\text{mix}}(\text{H}_2\text{O})$  did not change significantly 41 days postinjection:

$$rV_2 = \frac{1818.7 - (-287.5)}{(0.44 * 8586.6 - 361.9) - 1818.7}$$

$$rV_2 = \frac{2106.2}{3416.2 - 1818.7} = 1.32$$

This indicates that at 266 days postinjection there was a 132% increase in new methane above the baseline methane levels. This calculation is less than the 183% increase at 266 days indicated by methane measured in the SES, but more than the increase calculated by any TDG calculations (Figure 1 and Table 1). Moving the SES up and down the well and sampling likely caused mixing and a release of methane from the surrounding coal bed because the SES has a diameter (~5 cm) only slightly smaller than the well (6.4 cm). This could have temporarily inflated the methane values from the SES when measured directly as was observed with the Micro-TDGP following SES sampling (Figure 2). The isotopic calculations required mixing to occur and the fractionation of  $\delta D-H_2O$  and  $\delta D-CH_4$  was close to the predicted value based on the alpha fractionation line from the laboratory deuterium investigation of Ashley et al. (2021) even though the laboratory experiment only reached 1500‰  $\delta D-H_2O$  (Figure 4).<sup>27</sup> The fractionation slope is surprisingly linear with the field results though at much higher  $\delta D-H_2O$  values (8586.6‰) 41 days postinjection; 266 days postinjection  $\delta D-H_2O$  and  $\delta D-CH_4$  values decrease back toward initial values (Figure 4). All isotopic values measured in FGP-13 were closer to preinjection conditions when sampled on day 266 than on day 41 for all tracers except  $\delta^{13}C-CO_2$  (Table 1 and Figure 4). The decrease in  $\delta D-CH_4$  was likely due to mixing (dispersive or advective) of the injection water and ambient formation water because most of the methane generation occurred within the first 4 months following the injection based on real-time data from the Micro-TDGP (Figure 2). The large shift in  $\delta D$  values observed indicates that much smaller quantities of deuterated water could be added in future studies/efforts with a similar quantitative result. However, the advantage of a larger amount of deuterated water addition is the ability for long-term (multiyear) monitoring. It is also possible to use lower purity  $D_2O$  and add more to the injection medium to reach the same concentration and save on costs as commercially available 70%  $D_2O$  is typically less than half the cost of 99.9%  $D_2O$ . This could provide an inexpensive alternative or supplement to traditional methane monitoring.

Quantitatively measuring biogenic methane enhancement in the subsurface is important for evaluating alternative natural gas resources and contributions to greenhouse gas emissions from subsurface environments. In the described study, we were able to investigate enhanced coalbed methane production from an injection of YE in the Flowers-Goodale coal bed in three new ways: (i) real-time analysis with a Micro-TDGP sensor that was compared with isotherm, core desorption, and SES samples, (ii) temporal pressurized downhole SES samples from multiple wells before and after the injection, and (iii) using  $D_2O$  to observe D-enriched methane due to microbial activity. Downhole samples collected with SESs indicate that methane increased 132% above preinjection levels based on isotopic labeling from  $D_2O$ , 108% based on pressure readings, and 183% based on methane measurements 266 days postinjection.

The techniques introduced and discussed herein can be used to monitor biogenic methane and the activity of subsurface microorganisms at in situ temperature and pressure from wells that are completed in any formation. The ability to inject  $D_2O$  and label newly generated methane to quantitatively calculate methane increases provides opportunities to quantify methane

production in other hard-to-reach environments such as deep shale reservoirs. The results could help constrain future groundwater models from coal beds and possibly other subsurface environments with biogenic methane generation. Overall, the methods tested and developed in this study provide new ways to investigate methane production and microbial activity in situ and suggest that microorganisms in coal beds are capable of actively generating significant quantities of methane with small injections of organic nutrients. This finding has immediate implications for understanding subsurface carbon cycling and the development of renewable low-carbon natural gas generation.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.1c05979>.

Additional information about the methane measured with different methodologies, SES measurements from wells, and an additional equation from Ashley et al., 2021 (PDF)

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## Notes

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