RESEARCH ARTICLE

Microbial community composition of a hydrocarbon reservoir 40 years after a CO₂ enhanced oil recovery flood

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One sentence summary: Microbial community composition was compared between samples affected by a CO₂-EOR flood and those from areas that were outside or stratigraphically above the flood region to determine if CO₂-EOR flooding impacted the microbial community, or if the reservoir was able to “reset” back to pre-flood conditions.

Editor: Tillmann Lueders
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ABSTRACT

Injecting CO₂ into depleted oil reservoirs to extract additional crude oil is a common enhanced oil recovery (CO₂-EOR) technique. However, little is known about how in situ microbial communities may be impacted by CO₂ flooding, or if any permanent microbiological changes occur after flooding has ceased. Formation water was collected from an oil field that was flooded for CO₂-EOR in the 1980s, including samples from areas affected by or outside of the flood region, to determine the impacts of CO₂-EOR on reservoir microbial communities. Archaea, specifically methanogens, were more abundant than bacteria in all samples, while identified bacteria exhibited much greater diversity than the archaea. Microbial communities in CO₂-impacted and non-impacted samples did not significantly differ (ANOSIM: Statistic R = -0.2597, significance = 0.769). However, several low abundance bacteria were found to be significantly associated with the CO₂-affected group; very few of these species are known to metabolize CO₂ or are associated with CO₂-rich habitats. Although this study had limitations, on a broad scale, either the CO₂ flood did not impact the microbial community composition of the target formation, or...
INTRODUCTION

As atmospheric CO₂ concentrations increase worldwide, strategies to reduce this greenhouse gas are becoming necessary to curb global climate change. One popular method for carbon utilization and/or storage is CO₂-enhanced oil recovery (CO₂-EOR). CO₂-EOR involves injecting CO₂ into depleted crude oil reservoirs in order to extract residual oil from a formation. This process typically leaves around 30% of the injected CO₂ in the target formation, thus presenting the potential to curb CO₂ emissions, while the remaining 70% is recycled back to the surface (Melzer 2012).

Increasing energy demand, depletion of oil reservoirs and rising CO₂ levels in the atmosphere are driving the initiation of more CO₂ injection projects. Changes in subsurface microbiology due to CO₂ injection can impact the long-term fate and transport of the injected CO₂, as well as impact CO₂ injectivity and possibly alter target formation and cap rock lithology (e.g. Giese et al. 2009). This injected CO₂ also may also be converted by in situ methanogens into additional natural gas (e.g. Sugai et al. 2012), making the injection more financially appealing and potentially converting a greenhouse gas into a usable fuel source. Therefore, it is increasingly important to understand the long-term impacts of CO₂ injection on reservoir microbial communities. Recent work has demonstrated that the innate microbial community composition may change after a CO₂ injection, albeit varying environments (Mu et al. 2014; Wilkins et al. 2014; Peet et al. 2015; Kirk et al. 2016), and cells may die as a result of CO₂ dissolving into cell membranes (e.g. White, Burns and Christensen 2006). Other research has determined that microbial communities can live and thrive in high CO₂ conditions like those experienced during CO₂-EOR (Freedman, Tan and Thompson 2017; Probst et al. 2017). Furthermore, microbial populations have been documented to change after CO₂ injection compared to water-flooded portions of the same reservoir (Liu et al. 2015), but no study has analyzed microbial community composition of a reservoir decades after CO₂ injection has stopped. Even though changes in microbial communities can impact a CO₂ injection project, very few large-scale projects have monitored microbiological changes in situ (e.g. Michael et al. 2010). Furthermore, studies with a microbiological component mostly involve geochemical modeling and/or simply examine isolates from high-CO₂ natural analogue environments, such as hot springs (e.g. West et al. 2011; Kirk et al. 2016); few studies target the actual microbial communities living in geologic CO₂ sequestration or CO₂-EOR reservoirs.

The Olla Oil Field in the LaSalle Parish, Louisiana, USA has been previously studied due to its high microbial methanogenesis activity compared to surrounding oil fields. This was initially hypothesized to be due to a CO₂-EOR flood in the 1980s (McIntoosh et al. 2010; Shelton et al. 2014, 2016a, 2016b). Shelton et al. (2014) determined that the CO₂ flood was not the cause of the increased methanogenesis observed in the Olla Field; therefore, the crude oil composition and the microbiology of the Olla Field and surrounding oil fields were also examined in an attempt to determine the drivers of increased methanogenic activity (Shelton et al. 2016a, 2016b). This present study analyzes how the CO₂-EOR flood may have changed microbial community structures in the Olla Oil Field. The microbial composition of the samples collected from CO₂-affected production wells (n = 2) are compared to samples from unaffected portions of the same target sand (n = 5) and unaffected younger strata in the same oil field (n = 2). This study is unique in that it allows for an assessment of in situ microbiology almost 40 years after a CO₂ flood, providing an opportunity to determine how long-term CO₂ injection and cessation may impact the innate microbial communities. This study is also the first of its kind in analyzing microbial communities in situ post-CO₂ injection and cessation.

METHODS

Formation water from 9 different wells that produce from the Olla Oil Field in LaSalle Parish, Louisiana, USA was collected in August of 2014 (Fig. 1). Water samples from continuously pumping wells were collected in sterile 1 L glass bottles and filtered through sterile 0.22 µm Sterivex GP filter units (Millipore®, Billerica, MA USA) using a GeoPump (Geotech Environmental Equipment, Inc. Denver, CO, USA) and sterile plastic Nalgene tubing until the filters clogged. Filters were immediately frozen on dry ice and kept frozen until analysis at the University of Colorado Next Generation Sequencing Facility. DNA was extracted at the University of Colorado at Boulder using a MO BIO Powersoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). Slices of the Sterivex filters were added directly to the bead tubes of the MO BIO kit in order to extract DNA from the filter units. The 515-F (5′-GTGCACCMGGCGGTAA-3′) and 806-R (5′-GGACTACHVGGGTWTCTAAAT-3′) 16S rRNA gene primer pair (Fierer et al. 2012) was used during amplification; these primers included Illumina adapters and error-correcting 12-bp barcodes. A GoTaq® Hot Start PCR Master Mix (Promega, Madison, WI, USA) was used for PCR. Thermal cycling (in a 25 µL reaction) consisted of initial denaturation at 94°C, annealing at 50°C for 30 s, extension at 70°C for 30 s and a final extension at 72°C for 10 min. Gel electrophoresis was used to confirm amplification, and all PCR products were quantified using the PicoGreen dsDNA assay. Samples were pooled together in equimolar concentrations, and the amplified DNA was sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA), running 2 × 250 base pair (bp) chemistry.

Sequenced DNA was processed downstream using a joint QIIME (Caporaso et al. 2010) and UPARSE pipeline (Edgar 2013) as discussed in Shelton et al. (2016a). Demultiplexing was performed in QIIME, while the remainder of the downstream processing was performed using UPARSE. Quality filtering was performed with a maxee value of 0.5, sequences were dereplicated and singletons were removed from the dataset prior to determining and assigning phylotypes (all in UPARSE). Taxonomic units were mapped to operational taxonomic units (OTUs) at a minimum of 97% similarity (typically greater) using the GreenGenes 13,8 (http://greengenes.secondgenome.com) database.

All subsequent steps were performed in R (R Core Team 2015), including contaminant removal, where any sequences matching mitochondria and/or chloroplast phylotypes were eliminated from the dataset, as well as any phylotypes identified at greater than 5% in the blank (i.e. control) samples (8 contaminant OTUs...
Figure 1. Map of sample locations, Louisiana, USA. The small green square on the map of Louisiana annotates the location of the inset map.

were identified and removed from the dataset). This resulted in 180,773 (n = 9) total sequences, with the minimum number of sequences per sample being 5829 (sample O4). Therefore, the entire sample set was then randomly subsampled to 5000 sequences per sample. All subsequent statistical analyses were performed in R using the vegan (Oksanen et al. 2014), bioDist (Ding, Gentleman and Carey 2017), RAM (Chen, Simpson and Levesque 2016) and indicspecies (De Caceres and Jansen 2016) packages. All methods requiring a distance matrix were performed using a Bray–Curtis dissimilarity matrix. Sequences reads for each sample were deposited into the National Center for Biotechnology Information Short Read Archive (SRA) under BioProject PRJNA310850 and BioSample accession numbers SAMN04457241 and SAMN04457231 - SAMN04457237.

The Olla Field was injected with CO2 from 1983 until 1986 via eight injection wells (Shelton et al. 2014). Due to heterogeneous CO2 flooding of the 2800’ sand—the target formation—of the Olla Oil Field, only parts of the 2800’ sand were impacted by the injected CO2. Two samples, O3 and O4, were collected from wells that produced injected CO2 during the CO2-EOR flood (deemed the CO2-affected wells; Shelton et al. 2014). Seven additional wells, O5, O6, O7, O8, O18, O26 and O27, were sampled that were not affected by the CO2-EOR flood as they never produced injected CO2 during the EOR project (Shelton et al. 2014).

Two of the CO2-unaffected wells, O26 and O27, produce from strata stratigraphically above the 2800’ sand, which was not impacted by the CO2 flood. Samples from strata younger than the 2800’ sand were added to the study to increase the robustness of the sample set, even though the hydrochemistry and lithology of this younger strata are slightly different than the 2800’ sand. Although these two samples were located in different strata than the other CO2-unaffected samples, they were statistically similar in regard to hydrogeochemical parameters (Shelton et al. 2016a; see Tables SI–2 and SI–3 (Supporting Information) for hydrologic and gas geochemical data). Therefore, this was used as justification to group the samples together. The remaining 5 CO2-unaffected wells, O5, O6, O7, O8 and O18, produce from portions of the 2800’ sand that were not impacted by injected CO2. Detailed information about the study site, background and sampling methods can be
Deferribacter bacteria have been identified in crude oil reservoirs, greater than 6.2% abundance in any of the nine samples. The dance bacterial OTUs; no single bacterial OTU was identified at movingaceae diversethanarchaea: 847 different bacterial OTUs were identified sp. and the order NRA6 of the class Methanocalculus sp. (Greene, Patel and Sheehy 1997). However, only one has been associated with high CO2 environments, Deferribacter sp., which is typically identified in hydrothermal vents growing autotrophically off of CO2 (Slobodkina et al. 2009; Takai et al. 2003). Alicyclobacillus sp. is usually associated with fruit juices (e.g. Chang and Kang 2004), BA021 has been identified in anaerobic digestors (e.g. Wang, Hou and Su 2017), while A. lwofii is typically associated with animal environments (e.g. Debarry et al. 2007). In some cases, when considered together, these low abundance bacterial OTUs dominate the community compositions of some of the samples collected (Fig. 2).

There are no obvious differences between the CO2-affected (O3 and O4) and CO2-unaffected (n = 7) samples when looking at the alpha diversity metrics and the general microbial community composition. Any major observed variation exists mostly in the low abundance bacterial communities. Unfortunately, concentrations of DNA were not measured and therefore, differences in total biomass could not be compared between the CO2-affected and CO2-unaffected samples.

A previous study by Gulliver, Gregory and Lowry (2016) compared the microbial communities of different samples that they exposed to different partial pressures of CO2, emulating CO2 sequestration conditions in a saline aquifer and a depleted crude oil formation. The study found no relationship between microbial community diversity and the partial pressure of CO2 (pCO2) in formation water associated with an oil field (similar to that of the Olla Field), while in the saline aquifer, microbial diversity decreased with increasing pCO2 (Gulliver, Gregory and Lowry 2016). An additional study by Gulliver, Lowry and Gregory (2014) also found that increasing pCO2 over a specific threshold initiated decreases in microbial community diversity in the target formation. The CO2-affected samples in this study generally had higher Shannon Diversity values than the unaffected samples, opposite to what was observed in the Gulliver, Gregory and Lowry (2016) study for a saline aquifer and the oil field formation water. Conversely, the Gulliver, Gregory and Lowry (2016) study found that changes in the microbial community were site-specific (e.g. aquifer versus oil field formation water) and highly dependent on pH. The microbial community in the oil field formation water shifted from Pseudomonas in no- to low-pCO2 samples to Escherichia in the high pCO2 samples (Gulliver, Gregory and Lowry 2016). This study did not identify any dominating Escherichia OTUs in the CO2-affected samples; however, Pseudomonas was present in higher abundances in the CO2-affected versus the CO2 unaffacted samples (Fig. 2), albeit not at a statistically significant difference.

Beta diversity

In order to determine if the microbial composition of the CO2-affected samples were distinct compared to the CO2-unaffected samples, the data were analyzed using a variety of statistical methods. As shown in Fig. 2, no obvious grouping of the CO2-affected samples can be observed in a dendrogram based on hierarchical clustering of Bray–Curtis distances between the sites (Fig. 2). The O3 and O4 samples were separated into two different, distinct clusters, both being more similar to CO2-affected samples than to each other.

The same result occurred when performing a principal coordinates analysis (PCoA) on the dataset. The dataset was evaluated 4 different ways in order to determine if a relationship exists between the microbial communities of the CO2-affected samples versus the CO2-unaffected samples. Four different PCoA plots were constructed: one at the OTU level that included both the identified archaea and
Figure 2. Coupled dendrogram (based on hierarchical clustering of Bray–Curtis distances between the sample locations) and a species abundance-based heat map. Dark green indicates a greater % abundance while white indicates a smaller % abundance. The Shannon Diversity Index, sample richness and Pielou Evenness after rarefaction (at the species rank) are also listed. ‘All Other’ indicates all of the remaining identified OTUs that were present at less than 3% abundance in at least one sample.

Table: Rarefied Species Rank Dataset

| OTU    | O7 | O6 | O8 | O18 | O3 | O4 | O26 | O27 |
|--------|----|----|----|-----|----|----|-----|-----|
| Methanothermococcus | 42.7 | 2.2 | 37.8 | 51.7 | 14.2 | 37.8 | 61.1 | 46.1 | 74.1 |
| Methanohalophilus     | 0.0 | 69.0 | 0.4 | 0.8 | 0.5 | 5.8 | 0.1 | 0.1 | 0.0 |
| Methanolobus          | 0.0 | 0.2 | 0.0 | 0.8 | 13.2 | 0.4 | 0.2 | 1.5 | 0.5 |
| Chitinophagaceae      | 6.6 | 1.0 | 1.0 | 0.2 | 1.4 | 0.1 | 0.0 | 0.4 | 0.1 |
| Alocyclobacillus      | 1.2 | 0.4 | 4.8 | 3.8 | 5.0 | 2.8 | 0.7 | 1.2 | 0.5 |
| Unclassified BA021    | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Kozitobacter          | 3.0 | 0.6 | 1.5 | 0.4 | 2.0 | 1.3 | 0.0 | 0.0 | 0.0 |
| Unclassified Thermovirgaceae | 0.0 | 5.1 | 0.1 | 0.0 | 1.0 | 3.5 | 5.0 | 9.9 | 7.6 |
| Acinetobacter         | 5.2 | 1.4 | 1.2 | 1.4 | 4.0 | 0.2 | 0.3 | 0.2 | 0.2 |
| Pseudomonas           | 2.2 | 0.9 | 3.5 | 2.8 | 5.9 | 3.8 | 0.5 | 2.1 | 0.4 |
| All Other             | 39.1 | 19.2 | 49.7 | 38.1 | 52.8 | 44.3 | 32.1 | 37.7 | 11.1 |

To confirm the lack of significant differences between the CO2-affected and CO2-unaffected samples, an ANOSIM test was also performed on the four different scenarios, testing the CO2-affected (n = 2) samples against the CO2-unaffected (n = 7) samples (Fig. 3). None of the ANOSIM results indicated a significant difference between the microbial community composition of the CO2-affected versus the CO2-unaffected samples for the data. Scenarios considered were all taxa at the OTU level (Statistic R = -0.2597, Significance = 0.769), just the bacteria at the OTU level (Statistic R = -0.2208, Significance = 0.785), just the archaea at the OTU level (Statistic R = 0.1494, 0.269), and all taxa at the Order level (Statistic R = -0.2338, Significance = 0.779).

These three pieces of evidence—the dendrogram of Bray–Curtis distances, PCoA and ANOSIM tests—indicate that there is no statistical difference between the total microbial community compositions of the CO2-affected samples compared to the CO2-unaffected samples. It is important to note that these data cannot confirm whether or not the microbial communities in this reservoir were ever impacted by the injected CO2, meaning that the microbial communities of the impacted areas of the reservoir may not have changed during the CO2 flood to begin with. However, these wells did produce injected CO2 during the EOR project (Shelton et al. 2014), confirming that the well areas of the CO2-affected wells were impacted by injected CO2. If the microbes were indeed modified during the CO2 flood, these results suggest that reservoirs impacted by a CO2 flood (or perhaps a CO2 leak) may have the ability to rebound back to their pre-flood microbial composition. Furthermore, geochemical data found in Table SI–2 (Supporting Information) also provide evidence for the rebounding of these CO2-affected wells back to their pre-injection condition. Many hydrologic parameters of formation water change during a CO2 injection. The pH decreases, the alkalinity increases, dissolved iron content increases and the δ18O-H2O decreases (Kharaka et al. 2006; Zheng et al. 2012). These geochemical and isotopic changes are not observed in our sample set (Table SI–2, Supporting Information): the pH values for O3 and O4 are near neutral and close to those of the CO2-unaffected samples, the alkalinity values of O3 and O4 are high, but not significantly higher than the CO2-unaffected wells, iron was below the detection limit for most samples, and the δ18O-H2O values of the CO2-affected wells are similar to those of the CO2-unaffected wells. This evidence supports the conclusion that the CO2-affected portions of the reservoir may have reverted back to pre-flood conditions, given the CO2-unaffected wells are currently representative of pre-flood conditions.

the identified bacteria (Fig. 3A), one at the order level that included the identified archaea and bacteria (Fig. 3B), one at the OTU level that only considered the identified bacteria (Fig. 3C) and one at the OTU level that only considered the identified archaea. The datasets were evaluated this way due to the alpha diversity statistics; as there was much more bacterial diversity than archaeal diversity, the archaea could have been masking any bacterial differences between the CO2-affected and CO2-unaffected samples. However, the CO2-affected samples (O3 and O4) neither cluster together nor do they cluster distinctly from the CO2-unaffected samples in any of the four scenarios (Fig. 3).
Another issue to consider is that the small sample size may have also skewed the results, as this study only sampled two CO2-affected wells. Therefore, it is difficult to statistically determine if the two wells were initially impacted in the same way by the CO2 flood, or if their impact was site-specific as was observed by Gulliver, Gregory and Lowry (2016). Further, it is difficult to determine with our experiment design if the microbial communities in O3 and O4 shifted as a result of the CO2 flood in the same manner or in a different way, meaning that the microbiology of O3 and O4 may have been modified in different ways by the CO2 flood.

**Indicator species for CO2-affected wells**

Although the composition of the total microbial community suggested that the overall community composition of the CO2-affected versus the CO2-unaffected wells was not statistically different, additional tests were performed in order to see if any OTUs could serve as indicator species in the CO2-affected samples. An indicator species (R package indicspecies::multipatt) test was performed on the group of the two CO2 affected samples (O3 and O4) against the CO2 unaffected samples (O5, O6, O7, O8, O18, O26 and O27). The test resulted in 14 statistically significant indicator species associated with the CO2 affected samples, but no OTUs associated with the CO2 unaffected samples (Table 1). These 14 organisms are Desulfitobacter sp., Sporotomaculum sp., Corynebacterium sp., Syntrophomonas sp., Flavobacterium frigidarium, an organism from the order Ellin6067 and the order Burkholderiales and organisms from the families Pasteurellaceae, Sporichthycaceae, Ellin6513, Methylophilaceae, Pseudomonadaceae, Oralobacteraceae and Enterobacteriaceae (Tables 1 and 2).

These indicator species were present in statistically higher abundance in the CO2-affected samples versus the CO2-unaffected samples (if at all), and could be indicative of the species that thrived in CO2-flooded conditions. The increased abundance of these indicator species is likely not due to differences in geology or hydrochemistry, as all of these wells...
have very similar lithology and water chemistry parameters (Shelton et al. 2014, 2016b). Of these 14 indicator species, to our knowledge, only four have been previously observed in or associated with CO2-rich environments: Desulfotobacter sp., Pseudomonadaceae, Burkholderiales and Enterobacteriaceae (Morozova et al. 2011; Frerichs et al. 2014; Mu et al. 2014; Octavia and Lan 2014; Gulliver, Gregory and Lowry 2016; Ham et al. 2017). The other 10 organisms, Sporotomaculum sp., Corynebacterium sp., Syntrophomonas sp., F. frigidarium, Ellin6067, Pasteurellaceae, Sporichthyaceae, Ellin6513, Methylophilaceae and Oxalobacteraceae, have been identified in a variety of environments, including methanogenic sludge; sewage; in animals, human materials and plants; waterlogged soils and soils in general; marine sediment; surface waters; and aquifers (Tamura, Hayakawa and Hatano 1999; Humphry et al. 2001; Qiu et al. 2003; Baldani et al. 2014; Doronina, Kaparullina and Trotsenko 2014; Kim et al. 2014; Schink and Muñoz 2014; Stackebrandt 2014; Tauch and Sandbott 2014).

A previous study by Gulliver, Gregory and Lowry (2016) examined how various pCO2 concentrations impacted the microbial communities of formation waters from a saline aquifer and an oil field. That study found that Pseudomonas dominated their low-pCO2 samples, while Escherichia dominated the high-pCO2 sample. As Escherichia was not present in their 0 MPa pCO2 samples, this suggests that Escherichia thrives in CO2-rich environments. If the Enterobacteriaceae identified as an indicator species in this study are of the Escherichia genus, then this could imply that, when the CO2 flood occurred, Escherichia may have dominated, or at least became more prevalent, in the CO2 affected areas of the aquifer. Given that current pCO2 and CO2 concentrations for the Olla Field were, at the time of sampling, much lower than typical injection conditions (Shelton et al. 2014), it appears that the presence of Pseudomonadaceae as an indicator species for the CO2-affected wells is in agreement with the study by Gulliver, Gregory and Lowry (2016); however, neither Pseudomonadaceae nor Enterobacteriaceae dominate (i.e. are >10% abundance) the samples. This may be because sufficient time has elapsed since the CO2 flood, allowing these CO2-affected areas of the reservoir to revert back to close to ‘pre-injection’ conditions, or to microbial compositions similar to the unaffected portions of the reservoir.

Morozova et al. (2011) found that total bacterial cell counts in a CO2-flooded saline aquifer initially decreased by 50% during the CO2 flood, but rebounded up to 75% of the original pre-flood cell counts after a period of 5 months of CO2 flooding. Sulfate-reducing bacteria (SRB), specifically Desulfohalobium utahense, increased in concentration 5 months after the CO2 flood (Morozova et al. 2011). The SRB identified as an indicator species for CO2-affected wells in this study were Desulfotobacter sp., which are within the same phylum as D. utahense but not more closely related. Furthermore, cell counts of archaea initially increased after the CO2 flood, but, after 5 months of CO2 flooding, no archaea were identified in the CO2-affected formation waters. In contrast to the findings presented by Morozova et al. (2011), the CO2-affected wells in the present study are both dominated by archaea. However, the CO2 flood in the Olla Field ceased ca. 1 year after it was initiated, and high CO2 concentrations were not maintained in the Olla Field over the past 40 years (Shelton et al. 2014).

A study by Mu et al. (2014) monitored microbiological changes before and after a 4 day CO2 injection into a saline aquifer. They observed a dramatic increase in the relative abundances of Comamonas and Sphingobium ca. 30 days after the end of the 4 day CO2 injection. Burkholderiales, which contains the genus Comamonas, is significantly associated with the CO2-affected samples in this study. If the Burkholderiales OTU identified is of the genus Comamonas, this would be in agreement with the study by Mu et al. (2014) and this significant association may indeed be due to the CO2 flood. The Mu et al. (2014) post-CO2 injection samples also clustered together on a PCoA plot, unlike the samples for this study. This may be due to drastic changes to pH caused by active CO2 flooding, which others have speculated may be the main cause for microbiological changes to the target formation during a CO2 flood (Xu et al. 2010). The pH for the water samples collected for this study were close to 7 (Shelton et al. 2014), and had obviously rebounded back to near-neutral

### Table 1. Results of the indicator species analysis (R package indicspecies::multipatt). All identified operational taxonomic units (OTUs) are associated with the CO2-affected samples. The analysis was performed at the OTU level on the identified bacteria, the identified archaea and the whole rarefied dataset. No archaea were identified as indicator species.

| Species | Stat | P value | Stat | P value |
|---------|------|---------|------|---------|
| Only bacteria (OTU rank) | | | OTU rank | |
| Desulfotobacter sp. (OTU 778) | 0.999 | 0.031 | 0.999 | 0.024 |
| Sporotomaculum sp. (OTU 3911) | 0.998 | 0.031 | 0.998 | 0.024 |
| Ellin6067 (OTU 142) | 0.997 | 0.031 | 0.997 | 0.024 |
| Pasteurellaceae (OTU 4562) | 0.997 | 0.031 | 0.997 | 0.024 |
| Sporichthyaceae (OTU 1093) | 0.993 | 0.031 | 0.993 | 0.024 |
| Ellin6513 (OTU 141) | 0.988 | 0.031 | 0.988 | 0.024 |
| Syntrophomonas sp. (OTU 1683) | 0.985 | 0.031 | 0.985 | 0.024 |
| Methylophilaceae (OTU 4351) | 0.978 | 0.031 | 0.978 | 0.024 |
| Pseudomonadaceae (OTU 3861) | 0.975 | 0.031 | 0.975 | 0.024 |
| Pseudomonadaceae (OTU 18 730) | 0.965 | 0.031 | 0.965 | 0.024 |
| Oxalobacteraceae (OTU 2315) | – | – | 0.954 | 0.046 |
| Flavobacterium frigidarium (OTU 15 553) | 0.938 | 0.031 | 0.938 | 0.024 |
| Enterobacteriaceae (OTU 8894) | – | – | 0.936 | 0.046 |
| Burkholderiales (OTU 3883) | 0.922 | 0.031 | 0.922 | 0.024 |
| Corynebacterium sp. (OTU 860) | – | – | 0.916 | 0.05 |
Table 2. Indicator species correlated to the CO₂-affected samples, including information about the physiology, habitat and any evidence for living in a CO₂-rich environment. The references for the data provided in the columns for each row are also provided.

| Indicator species          | Habitat                           | Known CO₂ relationship | Optimal salinity | Metabolism                                      | Oxygen requirements | References                                           |
|----------------------------|-----------------------------------|------------------------|------------------|-------------------------------------------------|--------------------|------------------------------------------------------|
| Desulfitobacter sp.        | Heating system pipes              | Only with general      | D. alkalitolerans: 0–5% (w/v) NaCl [0–0.5%] | Fermentative; sulphite-reducing               | Anaerobic           | (Nielsen, Kjeldsen and Ingvorsen 2006; Morozova et al. 2011) |
| Sporotomaculum sp.         | Anoxic environments               | No                     | S. hydroxybenzoicum: 0–0.2% (w/v) NaCl Not given | Fermentative; possibly benzate-degrading     | Anaerobic           | (Brauman et al. 1998)                                |
| Ellin6067                  | Soils                             | No                     | Presumably salty (e.g. body fluid) Not given for S. brevicatena | Heterotrophic; facultatively ammonia-oxidizing | Aerobic             | (Ye et al. 2016)                                     |
| Pasteurellaceae            | Generally animal-borne Soils      | No                     | Presumably salty (e.g. body fluid) Not given for S. brevicatena | Heterotrophic; facultatively ammonia-oxidizing | Aerobic             | (Naushad et al. 2015)                                |
| Ellin6513                  | Soils                             | No                     | Not given        | Heterotrophic; acidophilic                      | Aerobic             | (Beulig et al. 2014, Wegner and Liesack 2017)         |
| Syntrophomonas sp.         | Anoxic habitats                   | No                     | Not given        | Syntrophic; fatty-acid oxidizing                | Anaerobic           | (McInerney et al. 2015; Frerichs et al. 2014)        |
| Methylphilaceae            | Surface waters, mud, activated    | No                     | Methylotenera mobilis: No growth above 0.1% (w/v) NaCl | Obligate or restricted facultative methylotrophs; methylamine or methanol utilizing | Aerobic             | (Kalyuzhnaya et al. 2006; Doronina, Kaparullina and Trotsenko 2014) |
| Pseudomonadaceae           | Widespread                        | Yes if 'Pseudomonas'   | Varied           | Heterotrophic; varies by species                | Aerobic or facultatively anaerobic | (Freirehs et al. 2014; Gulliver, Gregory and Lowry 2016) |
| Oxalobacteraceae           | Plants, soils and waters          | No                     | Herbaspirillum psychrotolerans: 0–0.5% (w/v) NaCl | Heterotrophic; can be pathogenic               | Mostly aerobic or facultative aerobic; Oxalobacter is strictly anaerobic | (Bajerski et al. 2013; Baldwin et al. 2014) |
| Flavobacterium frigidarium | Antarctic marine sediments        | No                     | Growth in up to 10% (w/v) NaCl | Psychrophilic, xylanolytic and laminarinolytic | Aerobic             | (Humphry et al. 2001)                                |
| Enterobacteraceae          | Widespread, typically in guts of animals | Yes if 'Escherichia' | Varies based on genus | Nitrate-reducing, glucose fermentation; may be pathogenic | Facultatively anaerobic | (Octavia and Lan 2014; Gulliver, Gregory and Lowry 2016) |
| Burkholderiales            | Widespread                        | Yes if 'Comamonas'     | Varied            | Heterotrophic; possibly aromatics-degrading     | Varies by genus     | (Offre et al. 2008; Mu et al. 2014; Tong et al. 2015; Probst et al. 2017) |
| Corynebacterium sp.        | Widespread                        | No                     | Varies based on genus | Chemoorganotrophic, fermentative and oxidative, may be pathogenic | Aerobic or facultatively anaerobic | (Tauch and Sandbote 2014)                             |

Since the active CO₂ flood in the 1980s, which would have lowered the pH in the formation.

Ham et al. (2017) compared two naturally CO₂-rich sites and one low-pCO₂ control site as an analog for long-term microbiological changes to reservoirs impacted by CO₂ flooding. They found sequences close to Comamonadaceae in one CO₂-rich site and taxa related to Anaerolineaceae, Nitrospirae and methanogens in the other CO₂-rich site. This study identified Burkholderiales as significantly associated with the CO₂-rich sites, similar to the Ham et al. (2017) finding that Comamonadaceae was associated with one group of CO₂-rich sites. However, these sites were a mix of surficial springs and shallow groundwater wells, which provide geochemical conditions that are not comparable to the sites in this study.

Taken together, our study provides some evidence that the CO₂ flood in the Olla Field impacted the microbial community...
structure in CO2-affected regions of the formation. Although the majority of the microbial populations identified in both the CO2-affected and CO2-unaffected samples were similar (e.g., most samples were dominated by Methanothermococcus spp.), several low abundance taxa were significantly more abundant in the CO2-affected samples compared to the CO2-unaffected samples. Some of these OTUs have been linked to CO2-rich environments; however, their low overall % abundance in the CO2-affected wells may suggest that any long-term microbial changes to a formation due to a CO2 flood or leak would be minor or even insignificant. It is important to note that only groundwater was sampled in this study and our findings cannot account for the response of attached microbial populations associated with biofilms or the rock matrix to the CO2 flood. Under ideal conditions, sampling techniques that can evaluate biofilms or rock-attached microbial populations such as cores or diffusive samplers (e.g., Barnhart et al. 2013) would be utilized. Unfortunately, due to core material being unavailable, these methods could not be applied.

CONCLUSIONS

In summary, the present study provides a field-scale representation of how the microbial community structure of a formation may recover from a CO2 flood or a CO2 leak and how native microbial communities may change years after the cessation of a CO2 flood. To the authors’ knowledge, this is the only study performed at this time scale (ca. 40 years after flood). No large-scale variation was present when comparing the major microbial communities identified in the CO2-affected and CO2-unaffected samples. However, certain lower abundance OTUs were identified in the CO2-affected samples that were significantly less abundant or absent in the CO2-unaffected samples. These OTUs were found to be similar to taxa that have been shown to thrive in CO2-rich environments. Due to their low % abundance here, this may provide evidence for the microbiology of a formation to return to pre-injection conditions after a CO2 flood ceases or if a CO2 leak would be stopped. At a minimum, the results of this study show that the microbial community of the CO2-affected wells is not significantly different than the community identified in the CO2-affected samples after 40 years post-CO2 injection. This has wide implications for both CO2-EOR operations and CO2 leaks into shallow aquifers due to CO2 sequestration. The results of this study suggest the potential for any possible microbial effects from or responses to changing the concentration of CO2 in an aquifer or hydrocarbon-bearing formation to rebound back to pre-injection conditions if the CO2 concentration was returned to baseline conditions.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

ACKNOWLEDGMENTS

This work was supported by the U.S. Geological Survey’s Energy Resources Program (Walter Guidroz, Program Coordinator). We thank XTO Energy for access to wells, and Elliott Barnhart, Carla Brezinski and Gary Mahon for thorough reviews of this manuscript, which greatly increased the quality of this work. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The Licensed Material constitutes ‘a work of the United States government’ as defined by the U.S. Copyright Act) 17 U.S.C. sec. 101; 17 U.S.C. sec. 105) and is not subject to copyright protection in the United States. However, copyright in a foreign country may apply.

Conflicts of interest. None declared.

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