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# Succession in native groundwater microbial communities in response to effluent wastewater

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by

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## DEDICATION

This thesis is dedicated to the members of Team Deco- Dr. David Jones, Ph.D., Sam Auyeung, and Richard Dinon. Dave introduced me to Dr. Garey and encouraged me to pursue a graduate education in something I'm passionate about, subterranean ecosystems. Without you, this journey never would have happened. Sam is the warm hug and glue that holds Team Deco together. The many laughs she encouraged have helped maintain my sanity during this process. Finally, my incredible husband, Richard Dinon. Without your support, encouragement, and all the home-cooked dinners you've prepared, there's no doubt in my mind this endeavor would have been far more arduous.

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## ABSTRACT

Aquifer storage and recovery techniques are used globally to mitigate increasing demands for groundwater. Microcosms were used to evaluate the effect of wastewater effluent that may be used for aquifer storage and recovery injection on native aquifer microbial communities. Sulfur Springs water was used as the source of aquifer water. The microcosms were 100% spring water, 100% unchlorinated filtered wastewater effluent, or one of three mixtures of unchlorinated filtered wastewater effluent and spring water. The mixture microcosms were 50%, 30%, and 10% unchlorinated filtered wastewater effluent with the balance as spring water. The effluent water was UV treated prior to assembling the microcosms. Sampling was carried out at 0, 2, 4, and 6 days and 5 replicates were run for each mixture. Water chemistry and 16S microbiome analyses were carried out. In terms of water chemistry, spring water and 10% effluent were nearly indistinguishable, 30% and 50% effluent appeared midway between pure spring water and pure effluent. The microbial communities present in the effluent are distinct from the spring water and it appears a third type of community develops over time when effluent and spring water are mixed together. In mixtures of spring and effluent water, there appeared to be a succession of dominant taxa within the microbial communities. At high effluent concentrations (30% and 50% effluent), the succession was more rapid than at low effluent concentrations (10% effluent) but after six days, all the communities in the mixed microcosms had the same single most dominant taxon,

*Rheinheimera.* There were three other highly abundant taxa found in all three mixtures of effluent and spring water at the end of the mixing experiment. The UV treatment did not completely or permanently inactivate the microbial communities from the effluent, so it is difficult from this study to conclude the relative impact of mixing two different microbial communities from the relative impact of mixing water with two very different nutrient profiles.

# CHAPTER ONE: INTRODUCTION

Artificial recharge, also known as managed aquifer recharge, is the engineered introduction of water into aquifers. This water management tool has been used internationally for decades to mitigate the increasing demand for groundwater usage particularly in regions with seasonal fluctuations of precipitation and groundwater demand, such as Florida (Singer et al. 1993). Water intended for use in these programs can be sourced from rainfall, surface water from rivers or lakes, anthropogenic sources such as irrigation systems, and wastewater (Jódar-Abellán et al. 2017). Pathogenic microbiota can be found in these water sources. One of the commonly used techniques in managed aquifer recharge is aquifer storage and recovery (ASR). In this method, water ranging in quality from potable to raw wastewater is injected into the aquifer and is intended to be extracted for use at a later time, typically when water demands increase. Operations that utilize ASR often expect water quality improvements upon extraction, so it is often investigated as an additional method of water treatment (Page et al. 2015). Geochemical processes combined with groundwater flow rates, temperature, pH, microorganism size, density of microorganisms, native groundwater microbial community composition, and available nutrient concentrations and fluctuations can all influence changes in both nutrient concentrations and pathogen abundance within the injected water during ASR (Bradford et al. 2014).

Subsurface microbial populations rely on the presence of ions, such as sulfate, to serve as electron acceptors for anaerobic cellular respiration. As a result, these communities have a major role in the geochemistry of groundwater because long water residence times in aquifers can render groundwater anoxic. Contaminants can be biodegraded by native microbial communities if the metabolic functions present utilize the elements or a portion of the compound and if the concentration introduced to the community is not toxic or inhibitory to microbial functions (Caracciolo et al. 2012; Flynn et al. 2013). Some compounds are more easily degraded by microbes than others, probably due to steric hindrance, or the inability of microbial enzymes to bind to elements needed for nutrient cycling (Racz and Goel 2009). Generally, organisms will use the energy source most easily attainable. If another energy source exists in the environment, more complex compounds are less likely to be biodegraded.

Ecological patterns observed between plants and animals can also be applied to microorganisms, but on a shorter timescale. Many microbial communities are resilient in the long-term regardless of short-term variability in that when exposed to a perturbation event, such as the introduction of contaminants, they can rebound to the original state, close to the original state, or recover to another stable variation of the original state (Fuhrman et al. 2015). Some communities may not exhibit resilience and never recover in terms of community structure or function after a disturbance (Shade et al. 2012), while others may be resistant to a disturbance and remain unchanged (Allison and Martiny 2008). The presence of a native diverse and abundant community is key in

the ability of a community to respond to contaminants introduced into an ecosystem (Allison and Martiny 2008; Caracciolo et al. 2012).

As ASR use increases, many studies focus on biodegradation potential of microbial communities typically with specific compounds (Caracciolo et al. 2012; Deng et al. 2016), use of ASR as an additional water treatment option for pathogen and nutrient removal (Page et al. 2015; Zhang et al. 2016), and the potential public health risks associated with injecting non-potable wastewater into regional aquifers (Page et al. 2015). The potential response of microbial communities in short-residence-time aquifers after exposure to wastewater effluent is currently unknown. Subsurface groundwater has been estimated to have $5x10^{27}$ planktonic bacterial cells in existence on Earth (McMahon and Parnell 2013), but these microbial communities are poorly characterized (Korbel et al. 2017). Approximately $3.0x10^{29-30}$ cells exist in formed biofilms attached in the subsurface substrates on Earth (Wagner et al. 2007). As a result, subsurface microbial communities are key drivers in global biogeochemical reactions (Flemming and Wuertz 2019; Battin et al. 2016), and altering these communities could impact ecosystems beyond the subsurface.

Advances in DNA sequencing technology have provided valuable new tools that are useful in the characterization of groundwater microbial communities. Studies that investigate aquifer storage and recovery have not described in detail how the native microbial communities in the aquifer respond to injected wastewater (Fackrell et al. 2016, Jodar-Abellan et al. 2017). The way in which these communities can respond to disturbances likely depend on the structure and metabolic functional capabilities of the

native microbial communities. It is also suggested that the mechanisms in which the different genera interact within established networks can impact the recovery time of both the chemistry of the water and the microbial community structure (Feng et al. 2016). The mechanisms of competition and succession in environmental microbial communities have been reviewed by Hibbing et al. (2010), Sub et al. (2016) and others.

Simpson-diversity is used to describe taxonomic diversity, but it includes relative abundance of the taxa. With Simpson-diversity, a higher value means lower diversity. The Shannon-diversity index is used to describe taxonomic diversity within a community. It accounts for both abundance and evenness of the community, and can correlate with the ability of a community to be resistant or resilient after disturbance (Feng et al. 2016). However, loss of microbial community diversity ensures a reduction of community functionality (Delgade-Baquerizo et al. 2015), which could lead to a decline or inability of the community to respond to disturbances.

The purpose of this study was to address the response of native aquifer planktonic microbial communities after exposure to filtered effluent wastewater. It was hypothesized that the planktonic microbial community will not be resistant to unchlorinated filtered wastewater effluent and changes in community structure will be observed in communities exposed to the effluent. Microbes have been known to respond to environmental contaminants (Fang and Barcelona 1998), and microbial communities can temporarily alter structure to promote the metabolic functions needed in the current environment (Fuhrman et al. 2015, Flemming and Wuertz 2019).

In this 6-day study, microcosms were used to introduce wastewater effluent to oligotrophic aquifer recharged groundwater that contained native unattached microbial communities. Planktonic microbes were used in this study because water column samples are more readily available compared to attached biofilms or sedimentary communities, although non-planktonic communities could contribute to the overall response within the aquifer. Conducting this study on a microcosm level allowed many environmental factors that could have influenced the native microbial communities, such as groundwater movement, to be eliminated. The native microbial community structure was expected to shift in response to effluent water and then return to its original state.

## CHAPTER TWO: MATERIALS AND METHODS

### *2.1 Sample Collection*

Herein "unchlorinated filtered wastewater effluent" will be referred to as "effluent." Effluent was collected from Howard F. Curren Advanced Wastewater Treatment Plant (Tampa, FL, USA). Effluent samples were collected immediately after the denitrification phase in the wastewater treatment facility. Prior to sample collection, three 20-L polypropylene carboys were washed and autoclaved. Each carboy was rinsed with the effluent water 3 times prior to being filled. Carboys were completely filled and sealed to prevent air exposure, and transported to the lab. Samples were stored out of direct light until UV-irradiation treatment. Based on visual inspection, the effluent was more turbid considering it was collected after the denitrification filtration phase.

Aquifer water issuing from Sulphur Springs in Tampa, Florida was collected using sterile 1-L polypropylene bottles. Samples were collected underwater by scientific scuba divers in accordance with the American Academy of Underwater Sciences (AAUS) auspices directly from the spring vent. Each bottle was flushed with inert helium gas three times prior to the final sample collection. Samples were placed on ice immediately after collection and were transported to the lab.

### *2.2 Experimental Design*

Prior to mixing the effluent with spring water, a Viqua Pro30 UV system was used for microbial inactivation of the effluent water alone. The effluent was treated at a

flow rate of approximately 5 liters per minute at a 253.7 nm UV dosage. All microcosm samples were in 1-L polypropylene bottles with no headspace upon closing the bottle. Five different water ratios were used: 100% effluent (1000 mL), 100% spring water (1000 mL), 10% effluent (100 mL) mixed with 90% spring water (900 mL), 30% effluent (300 mL) mixed with 70% spring water (700 mL), and 50% effluent (500 mL) mixed with 50% (500 mL) spring water. The microcosm samples were processed on days 0, 2, 4, and 6. On each of those days, 200 mLs were used for chemical analysis, and the remaining 800 mLs were filtered for DNA. Five replicates per water ratio per sampling day were used for a total of one hundred microcosms (Table 1). Each microcosm was wrapped in aluminum foil to avoid exposure to light and stored in darkness at room temperature (~23 $^{o}$C) until harvested for water chemistry analysis and filtration for DNA sequencing. Immediately prior to harvest, samples were placed on ice.

**Table 1**: Microcosm experimental design by water type and sampling day

| Microcosm Experiment Design | | | | | |
|---|---|---|---|---|---|
| | 100% Effluent Water | 100% Spring Water | 10% Effluent, 90% Spring Water | 30% Effluent, 70% Spring Water | 50% Effluent, 50% Spring Water |
| Day 0 | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates |
| Day 2 | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates |
| Day 4 | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates |
| Day 6 | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates | 5 Replicates |

### *2.3 Nutrient Chemistry*

Three of the five replicates were used for chemical analysis. Upon harvest, 50 ml aliquots of sample from each of three replicates were stored in sterile 50 ml conical vials for total organic carbon analysis (TOC). Aliquots of 150 ml from three out of five

total replicates were transferred to acid-washed autoclaved glass jars for water chemistry analysis. A HACH DR3900 spectrophotometer (Loveland, CO, USA) was used to measure concentrations of ammonium (HACH; Method 10205), alkalinity (HACH; Method10239), nitrate (HACH; Method 8171), nitrite (HACH; Method 10207), phosphorus (HACH; Method 8190), sulfide (HACH; Method 8131), and sulfate (HACH; Method 10248). TOC was measured on a Shimadzu TOC-V autosampler using manufacture's standard protocol (Columbia, Maryland).

*2.4 Microbial Community Analysis*

All five replicates from each treatment were filtered separately onto sterile 0.2 μm filters for microbial community DNA sequencing. Once sample filtration was completed, the 0.2 μm filters were aseptically transferred to sterile 15 ml conical vials and stored at -20 °C until DNA extraction. Qiagen PowerSoil kits (Germantown, MD) were used following the manufacturer's protocol to extract DNA. Thermo Scientific Fisher Nanodrop Lite spectrophotometer (Waltham, Ma) was used to quantify/confirm successful extraction. Samples were prepared and shipped to Applied and Biological Materials, Inc. (Richmond, BC, Canada) for sequencing using an Illumina MiSeq platform. Earth Microbiome 515F and 806R primers were used to target the V4-V5 region (Shunsuke et al. 2014, Chappidi et al. 2019). More than 3.6 million sequences representing 100 samples were generated and analyzed for this study and resulted in the identification of 108,977 distinct operational taxonomic units (OTUs).

*2.5 Bioinformatics*

A known-DNA mock community was assembled using *Methanococcus maripaludis* (ATCC 43000D-5), *Thermococcus gorgonarius* (ATCC 700654D-5), and a six-strain mixture (ATCC MSA 3000). The mock community was sequenced and processed via Mothur software version 1.40 (Schloss et al. 2009) to determine the error rate for our samples. The error rate was determined to be 0.025%.

Mothur software version 1.40 (Schloss et al. 2009) was used via the USF research cluster computer to remove sequences that were longer than 310 base pairs, as well as non-prokaryotic and ambiguous sequences for all samples and replicates. The USF research computing infrastructure could not analyze all of sequence data at once. Therefore, a feature in Mothur was used to randomly subsample sequences to 20% of total. The VSEARCH algorithm in Mothur was used to remove chimeric sequences- where two different fragments of DNA have ligated together. Bacterial sequences were compared to the Silva v132 database for identification, and clustered into OTUs with 97% similarity. OTUs with fewer than 20 sequences were omitted from the analysis to avoid possible artifacts from potential sequencing errors (Brown et al. 2015). Outlier OTUs with significantly low sequence reads were removed. All samples were rarefied to the lowest number of sequences per sample at 10065 sequences as the presence of rare species were not a concern in this study (McMurdie and Holmes 2014). OTUs were classified using the Bayesian classifier with a bootstrap cutoff equal to 80 (Werner et al. 2012). GenBank query was used to provisionally identify OTUs that could not be identified with the Silva Database within the top 7300 OTUs. GenBank queries with less

than 95% match were classified as unknown. R version 1.1.383 was used to randomly subsample replicates to ensure replicate numbers were identical across all samples.

Potential metabolic function for each provisionally-identified genus was assigned by performing a literature review. All provisionally identified genera were categorized as aerobic, anaerobic, or facultative bacteria. All facultative aerobic and anaerobic bacteria were classified together, and strict aerobes and anaerobes were classified independently. The nutrient cycle that each provisionally-identified genus was likely to utilize was also identified from the literature. Nutrient reducers and oxidizers were classified separately based on the nutrient; for example, nitrogen reducers were independently classified from nitrogen oxidizers. Genera with established pathogenic species were labeled as potentially pathogenic for the purposes of this study, and included both plant and animal potential pathogens. Shannon and Simpson indices were calculated according to genus in using the Vegan package (V.2.5-4) to determine the taxonomic diversity of each sample. Sample richness was determined by the number of unique OTUs in each sample.

### *2.6 Statistical Analysis*

Sequence abundances of all OTUs for all water types and replicates were analyzed using Primer-e version 7 software (Primer-e Ltd., Devon, United Kingdom). Data were square root transformed and clustered using Bray-Curtis dissimilarity prior to being graphed as a principal coordinate analysis (PCoA). In order to compare changes in the microbial community metabolic functions in each water type over the duration of

the incubation period, relative abundances for each unique provisionally-identified genus were averaged prior to principal coordinate analysis. These data were square root transformed, normalized, and clustered using Euclidean distance and visualized with principal coordinate analyses (PCoA).

Aqueous geochemistry was analyzed using R software (R Core Team 2017). Three replicates were used to perform two-tailed t-tests to determine significant differences between sampling days for each water ratio. Averaged replicate values were uploaded to Primer-e (Primer-e Ltd., Devon, United Kingdom). All values were square-root transformed, normalized, and clustered using Euclidean distance prior to being graphed as a PCoA. Bio-Env (BEST) analysis was used to quantify significant relationships between biological and nutrient chemistry data.

# CHAPTER THREE: RESULTS

## *3.1 Geochemistry*

Aqueous geochemistry of effluent was distinct when compared to the other water types (Table 2). The BEST routine is a component of the Primer-e software package that determines correlations between chemical and community analyses. BEST correlation analysis suggests ammonium as the most important variable with the strongest correlation between community and chemical data (Table 3).

Spring water was measured as anoxic at 0 mg/L of dissolved oxygen at the collection depth, but has been previously found to be either anoxic or microoxic (Scharping et al. 2018). Alkalinity decreased from day 0 to day 6 in microcosms with spring water, 10% effluent, and 30% effluent, and increased from day 0 to day 4 in microcosms with 50% effluent. Alkalinity was not statistically different between 100% effluent and 100% spring water microcosms on day 0 ($p>0.05$), but was statistically different by sampling day 6 ($p<0.0001$). Alkalinity was only statistically different between 100% spring water and 10% effluent on sampling day 6 ($p=0.036$).

Ammonium concentrations increased from day 0 to day 6 in microcosms with all water ratios ($p<0.05$) (Table 2). Ammonium concentrations were statistically different when 100% effluent was compared to all other water types by sampling day. Microcosms with 100% spring water and 10% effluent were not distinct in terms of ammonium concentrations on sampling days 0 and 2, but were statistically different on

days 4 and 6 ($p=0.00081$ and $p=0.00107$, respectively). Both 30% and 50% effluent microcosms had distinct ammonium concentrations compared to 100% spring water on all sampling days ($p<0.001$).

Sulfate concentrations decreased from day 0 to day 6 in all water mixture microcosms except those with 50% effluent (Table 2). Spring water and effluent microcosms were distinct on sampling day 0 ($p=0.003$), but were not statistically different on sampling day 2, 4, or 6 ($p>0.005$). Effluent microcosms were distinct by sampling day 6 when compared to microcosms with 50% effluent ($p=0.01$) and 10% effluent microcosms ($p=0.01$).

Nitrate concentrations decreased in effluent microcosms from sampling day 0 to day 6 ($p=0.004$). Microcosms with 30% effluent water had an increase in nitrate concentrations from sampling day 0 to day 4 ($p=0.04$). Effluent and spring water microcosms did not have distinct nitrate concentrations on sampling days 0 or 2, but were found statistically different on days 4 and 6 ($p<0.005$). Spring water microcosms had statistically distinct nitrate concentrations when compared to 30% and 50% effluent microcosms on sampling days 4 and 6 ($p<0.005$). Microcosms with 10% effluent did not have distinct nitrate concentrations when compared to spring water microcosms (Table 2).

Nitrite concentrations decreased in microcosms with 100% effluent water from sampling day 0 to 4 ($p=0.02$), spring water from sampling day 0 to 6 ($p=0.04$), and 50% effluent water from sampling day 0 to 6 ($p=0.0002$). Nitrite concentrations decreased from day 0 to day 2 in microcosms with 10% effluent water ($p=0.03$), and

remained constant afterwards. Microcosms with effluent water had distinct nitrite concentrations from all other water types on sampling days 0, 2, and 4 ($p<0.0001$). On sampling day 6, effluent microcosms were distinct from spring water microcosms ($p<0.04$), but not distinct from 10%, 30%, or 50% water mixture microcosms ($p>0.05$).

Phosphorus concentrations increased from sampling day 0 to day 6 in microcosms with 50% effluent water ($p=0.026$). Microcosms with effluent water were distinct from all other water mixtures on all sampling days ($p<0.01$), and microcosms with spring water were distinct from all other water mixtures on all sampling days ($p<0.003$) (Table 2).

Total Organic Carbon (TOC) concentrations decreased in microcosms with spring water from sampling day 0 to day 6 ($p=0.03$), and increased in microcosms with 50% effluent from sampling day 0 to day 6 ($p=0.02$). All microcosms with effluent water had distinct TOC concentrations from all other water microcosms on all sampling days ($p<0.0001$). All spring water microcosms had distinct TOC concentrations from the other water mixtures ($p<0.001$), except microcosms with 10% effluent water on sampling day 0.

### *3.2 Microbial community*

The amount of DNA recovered from each sample is shown in Table 4. The V4-V5 region of the 16S rRNA gene was amplified and sequenced in order to determine the

biodiversity of each water sample. Following rarefaction, 15,871 unique OTUs and 885,720 total sequences were reported by Mothur.

Shannon and Simpson indexes were calculated according to genus in RStudio (V.1.1.383) using the Vegan package (V.2.5-4) to determine the Shannon diversity of each sample (Table 5). Sample richness was determined by the number of unique OTUs in each sample (Table 5c).

A Principal coordinate analysis plot of all 108,977 unique OTUs produced by Mothur software reveals a distinct microbial community in unchlorinated filtered effluent water that doesn't change much during the incubation period (Figure 1). The spring water microbial community doesn't change as much during incubation as the communities of the mixed water microcosms. The top 30 provisionally-identified genera with relative abundances and replicates for each water type and sampling day can be found in the Supplementary Tables 1-20.

PCoA analysis of the estimated function of the top 30 provisionally-identified genera is provided in Figure 2. The percentage of potentially pathogenic bacteria of the top 30 provisionally-identified genera in unchlorinated filtered effluent water, spring water, 10% unchlorinated filtered effluent water, 30% unchlorinated filtered effluent water, and 50% unchlorinated filtered effluent was as high as 25%, 24.5%, 29%, 26%, and 27% respectively (Table 6).

**Table 2:** Aqueous geochemistry measurements with each water type by sampling day.

| | Alkalinity (mg/L) | Ammonia (mg/L) | Phosphorus (mg/L) | Nitrate (mg/L) | Nitrite (mg/L) | Sulfide (ug/L) | Sulfate (mg/L) | TOC (mg/L) |
|---|---|---|---|---|---|---|---|---|
| **100% Effluent** | | | | | | | | |
| Day 0 | 206 ± 15 | 0.227 ± 0.019 | 8.31 ± 0.07 | 4.18 ± 0.13 | 0.520 ± 0.009 | 0 ± 0 | 311 ± 3 | 10.31 ± 0.21 |
| Day 2 | 183 ± 1 | 0.299 ± 0.001 | 8.67 ± 1.08 | 2.51 ± 0.37 | 0.533 ± 0.010 | 0 ± 0 | 279 ± 7 | 10.26 ± 0.09 |
| Day 4 | 192 ± 1 | 0.667 ± 0.100 | 8.59 ± 0.20 | 3.65 ± 0.09 | 0.549 ± 0.005 | 8 ± 2 | 239 ± 12 | 10.59 ± 0.07 |
| Day 6 | 187 ± 2 | 1.200 ± 0.235 | 9.05 ± 0.49 | 2.85 ± 0.28 | 0.414 ± 0.124 | 18 ± 7 | 249 ± 5 | 10.78 ± 0.21 |
| **100% Spring Water** | | | | | | | | |
| Day 0 | 166 ± 9 | 0.001 ± 0.001 | 0.54 ± 0.03 | 2.66 ± 1.01 | 0.013 ± 0.002 | 1 ± 1 | 354 ± 6 | 3.26 ± 0.13 |
| Day 2 | 146 ± 2 | 0.005 ± 0.001 | 0.55 ± 0.02 | 2.66 ± 1.63 | 0.008 ± 0.003 | 0 ± 0 | 284 ± 9 | 2.83 ± 0.12 |
| Day 4 | 165 ± 5 | 0.008 ± 0.003 | 0.58 ± 0.02 | 1.33 ± 0.10 | 0.013 ± 0.001 | 3 ± 2 | 260 ± 8 | 2.98 ± 0.04 |
| Day 6 | 144 ± 3 | 0.010 ± 0.001 | 0.50 ± 0.02 | 1.34 ± 0.11 | 0.008 ± 0.002 | 1 ± 1 | 237 ± 3 | 2.84 ± 0.08 |
| **10% Effluent** | | | | | | | | |
| Day 0 | 170 ± 14 | 0.009 ± 0.007 | 1.18 ± 0.02 | 1.44 ± 0.08 | 0.071 ± 0.001 | 0 ± 0 | 320 ± 8 | 3.50 ± 0.09 |
| Day 2 | 153 ± 6 | 0.018 ± 0.004 | 1.19 ± 0.03 | 1.50 ± 0.31 | 0.062 ± 0.003 | 0 ± 0 | 313 ± 12 | 3.46 ± 0.06 |
| Day 4 | 166 ± 1 | 0.042 ± 0.002 | 1.24 ± 0.00 | 1.32 ± 0.06 | 0.072 ± 0.002 | 0 ± 0 | 284 ± 29 | 3.55 ± 0.04 |
| Day 6 | 158 ± 5 | 0.037 ± 0.003 | 1.16 ± 0.02 | 1.42 ± 0.12 | 0.071 ± 0.000 | 0 ± 0 | 268 ± 5 | 3.55 ± 0.10 |
| **30% Effluent** | | | | | | | | |
| Day 0 | 170 ± 2 | 0.075 ± 0.002 | 3.00 ± 0.16 | 1.89 ± 0.04 | 0.216 ± 0.002 | 1 ± 1 | 316 ± 8 | 5.34 ± 0.14 |
| Day 2 | 175 ± 8 | 0.089 ± 0.002 | 3.02 ± 0.14 | 3.04 ± 0.96 | 0.216 ± 0.001 | 0 ± 0 | 319 ± 10 | 5.40 ± 0.03 |
| Day 4 | 168 ± 7 | 0.121 ± 0.014 | 3.06 ± 0.17 | 2.18 ± 0.12 | 0.218 ± 0.001 | 0 ± 0 | 261 ± 2 | 5.35 ± 0.04 |
| Day 6 | 155 ± 4 | 0.135 ± 0.000 | 3.10 ± 0.18 | 1.94 ± 0.05 | 0.217 ± 0.000 | 0 ± 0 | 259 ± 3 | 5.21 ± 0.02 |
| **50% Effluent** | | | | | | | | |
| Day 0 | 164 ± 5 | 0.101 ± 0.000 | 3.69 ± 0.25 | 2.54 ± 0.19 | 0.283 ± 0.001 | 0 ± 0 | 289 ± 13 | 6.28 ± 0.08 |
| Day 2 | 164 ± 3 | 0.170 ± 0.002 | 3.83 ± 0.20 | 2.17 ± 0.29 | 0.278 ± 0.002 | 4 ± 4 | 282 ± 13 | 6.81 ± 0.13 |
| Day 4 | 174 ± 1 | 0.217 ± 0.010 | 4.42 ± 0.01 | 2.51 ± 0.10 | 0.316 ± 0.004 | 0 ± 0 | 267 ± 31 | 6.73 ± 0.05 |
| Day 6 | 164 ± 8 | 0.292 ± 0.006 | 4.31 ± 0.07 | 2.63 ± 0.07 | 0.258 ± 0.002 | 0 ± 0 | 297 ± 13 | 6.85 ± 0.17 |

**Table 3:** BEST Correlation Analysis of Biota and Geochemistry

| **BEST Correlation Analysis: Biota and Geochemistry** | |
|---|---|
| **Correlation** | **Chemistry Variable** |
| 0.691 | Ammonium |
| 0.74 | Ammonium, TOC |
| 0.732 | Ammonium, Phosphorus |
| 0.727 | Alkalinity, Ammonium, Phosphorus, TOC |
| 0.72 | Alkalinity, Ammonium, Phosphorus, Sulfate, TOC |

**Table 4:** Quantification of extracted DNA

| Sampling Day | Sample | Result (mcg/L) |
|---|---|---|
| 0 | 100% Effluent | 11.8 ± 1.6 |
| 2 | 100% Effluent | 24.8 ± 15.6 |
| 4 | 100% Effluent | 14.7 ± 5.2 |
| 6 | 100% Effluent | 5.2 ± 3.1 |
| 0 | 100% Spring | 34.3 ± 3.9 |
| 2 | 100% Spring | 51.3 ± 17.2 |
| 4 | 100% Spring | 50.7 ± 8.6 |
| 6 | 100% Spring | 29.3 ± 3.9 |
| 0 | 10% Effluent | 32.7 ± 10.5 |
| 2 | 10% Effluent | 47.2 ± 5.8 |
| 4 | 10% Effluent | 29.3 ± 14.0 |
| 6 | 10% Effluent | 34.6 ± 4.3 |
| 0 | 30% Effluent | 29.5 ± 1.8 |
| 2 | 30% Effluent | 42.3 ± 19.5 |
| 4 | 30% Effluent | 31.4 ± 11.8 |
| 6 | 30% Effluent | 34.2 ± 9.3 |
| 0 | 50% Effluent | 13.5 ± 0.2 |
| 2 | 50% Effluent | 42.8 ± 21.5 |
| 4 | 50% Effluent | 18.0 ± 2.3 |
| 6 | 50% Effluent | 13.9 ± 2.6 |

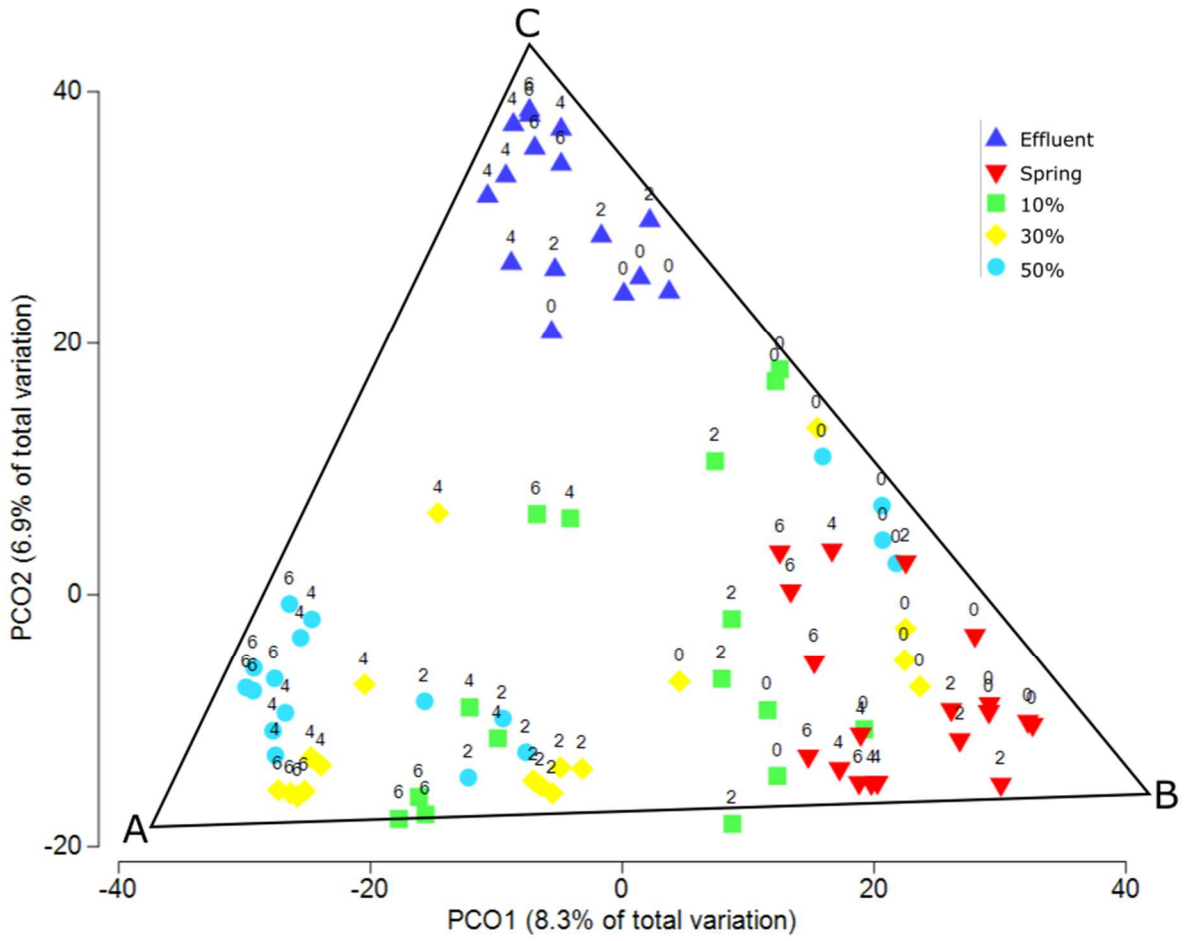


**Figure 1:** Principal coordinate analysis of all OTUs by water type and sampling day. Each point on the plot represents one replicate. The number by the symbol represents the day the sample was processed (day 0, 2, 4, or 6). The blue triangles near label "C" represent the microbial communities in the effluent water, and the red triangles near label "B" represent the communities in spring water. Note that the mixtures of effluent and spring water (light blue, yellow, and green) from early in the study mostly appear along the CB axis and likely represent simple mixing while those from later in the study fall near the label "A" on the triangle and likely represent new communities that grew as a result of the mixing.

**Table 5:** (a) Shannon Diversity Index for all water types by sampling day, (b) Simpson Diversity Index for all water types by sampling day, (c) Genera richness for all water types by sampling day

(a)

| Shannon Diversity Index | | | | | |
|---|---|---|---|---|---|
| | Effluent | Spring | TEN | THIRTY | FIFTY |
| Day 0 | 6.272 ±0.365 | 5.153 ± 0.089 | 6.093 ± 0.266 | 5.894 ± 0.207 | 5.980 ± 0.126 |
| Day 2 | 4.485 ± 1.147 | 5.467 ± 0.178 | 5.957 ±0.189 | 5.607 ± 0.162 | 5.523 ± 0.216 |
| Day 4 | 4.861 ± 0.433 | 5.858 ± 0.261 | 6.144 ± 0.299 | 5.816 ± 0.194 | 5.619 ± 0.077 |
| Day 6 | 4.621 ± 0.186 | 6.191 ± 0.137 | 6.194 ± 0.243 | 5.740 ± 0.158 | 5.896 ± 0.094 |

(b)

| Simpson Diversity Index | | | | | |
|---|---|---|---|---|---|
| | Effluent | Spring | TEN | THIRTY | FIFTY |
| Day 0 | 0.991 ± 0.008 | 0.983 ± 0.003 | 0.993 ± 0.007 | 0.990 ± 0.008 | 0.989 ± 0.003 |
| Day 2 | 0.902 ± 0.137 | 0.986 ± 0.005 | 0.991 ± 0.009 | 0.9887 ± 0.005 | 0.986 ± 0.008 |
| Day 4 | 0.971 ± 0.011 | 0.984 ± 0.007 | 0.992 ± 0.005 | 0.988 ± 0.007 | 0.986 ± 0.004 |
| Day 6 | 0.965 ± 0.009 | 0.992 ± 0.005 | 0.993 ± 0.004 | 0.988 ± 0.009 | 0.989 ± 0.006 |

(c)

| Richness | | | | | |
|---|---|---|---|---|---|
| | Effluent | Spring | TEN | THIRTY | FIFTY |
| Day 0 | 398 ± 189 | 477 ± 68 | 482 ± 112 | 576 ± 133 | 541 ± 91 |
| Day 2 | 394 ± 182 | 488 ± 70 | 479 ± 99 | 517 ± 55 | 534 ± 49 |
| Day 4 | 352 ± 169 | 629 ± 111 | 550 ± 112 | 482 ± 122 | 506 ± 25 |
| Day 6 | 244 ± 46 | 564 ± 84 | 580 ± 86 | 536 ± 27 | 570 ± 51 |

**Table 6**: Percentage of estimated metabolic functions of microbial communities in each water type by sampling day

| | % Aerobic | % Anaerobic | % Facultative | % Potential Pathogen | % Found in fecal samples | % WWTP | % Sulfur Oxidizer | % Sulfur Reducer | % Nitrogen Oxidizer | % Nitrogen Reducer | % Denitrifier | % Nitrogen Fixation | % Nitrate Assimilation | % Photosynthetic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **100% Effluent** | | | | | | | | | | | | | | |
| **Day 0** | 54.2 | 4.0 | 37.6 | 25.0 | 4.7 | 1.9 | 30.3 | 1.2 | 1.1 | 72.5 | 48.9 | 3.1 | 10.4 | 1.0 |
| **Day 2** | 31.7 | 0.3 | 67.0 | 16.7 | 1.3 | 0.6 | 63.2 | 0.0 | 0.7 | 81.8 | 71.0 | 3.3 | 0.4 | 0.7 |
| **Day 4** | 41.1 | 1.9 | 31.0 | 20.7 | 0.6 | 1.3 | 24.0 | 0.0 | 0.0 | 61.1 | 31.4 | 0.9 | 0.5 | 0.8 |
| **Day 6** | 49.1 | 1.9 | 31.0 | 20.7 | 0.6 | 1.3 | 24.0 | 0.0 | 0.5 | 59.4 | 37.2 | 2.6 | 2.1 | 3.2 |
| **100% Spring** | | | | | | | | | | | | | | |
| **Day 0** | 45.9 | 3.4 | 46.6 | 17.4 | 2.6 | 3.7 | 34.2 | 0.0 | 0.0 | 67.4 | 47.8 | 6.3 | 1.1 | 0.6 |
| **Day 2** | 42.5 | 3.2 | 48.8 | 22.8 | 2.2 | 2.7 | 39.6 | 0.0 | 0.0 | 71.7 | 58.4 | 7.2 | 2.3 | 0.0 |
| **Day 4** | 50.5 | 1.6 | 44.9 | 19.5 | 1.5 | 2.1 | 30.8 | 0.0 | 0.0 | 63.3 | 43.2 | 7.2 | 2.5 | 0.6 |
| **Day 6** | 46.3 | 1.6 | 49.8 | 24.5 | 1.2 | 1.3 | 37.5 | 0.6 | 0.7 | 72.6 | 50.7 | 7.5 | 2.9 | 0.0 |

**Table 6 (Continued)**: Percentage of estimated metabolic functions of microbial communities in each water type by sampling day

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10% Effluent** | | | | | | | | | | | | | | |
| **Day 0** | 43.3 | 3.2 | 49.9 | 21.2 | 2.8 | 3.2 | 38.8 | 0.0 | 0.0 | 76.5 | 52.8 | 6.9 | 2.0 | 0.0 |
| **Day 2** | 50.1 | 3.8 | 49.1 | 25.0 | 4.0 | 1.5 | 32.8 | 0.0 | 0.0 | 7.1 | 48.9 | 11.0 | 2.5 | 1.1 |
| **Day 4** | 51.6 | 1.6 | 44.6 | 29.1 | 0.6 | 0.5 | 33.7 | 0.0 | 0.7 | 71.6 | 53.2 | 12.8 | 1.1 | 0.0 |
| **Day 6** | 51.6 | 0.8 | 43.8 | 26.3 | 1.0 | 0.6 | 32.0 | 0.0 | 0.7 | 65.8 | 49.6 | 12.6 | 1.2 | 1.5 |
| **30% Effluent** | | | | | | | | | | | | | | |
| **Day 0** | 46.0 | 4.8 | 45.2 | 17.5 | 4.1 | 3.5 | 35.6 | 0.0 | 0.0 | 68.8 | 45.0 | 8.8 | 1.6 | 1.0 |
| **Day 2** | 54.2 | 7.2 | 42.3 | 26.1 | 8.8 | 1.2 | 33.9 | 0.0 | 0.6 | 65.9 | 42.9 | 14.3 | 0.0 | 0.9 |
| **Day 4** | 54.1 | 1.1 | 40.1 | 15.3 | 1.3 | 1.1 | 30.8 | 0.4 | 1.2 | 56.5 | 41.1 | 20.1 | 1.7 | 0.0 |
| **Day 6** | 51.7 | 0.0 | 46.5 | 21.6 | 0.9 | 1.4 | 35.2 | 0.0 | 0.9 | 69.1 | 49.0 | 14.8 | 1.0 | 0.0 |
| **50% Effluent** | | | | | | | | | | | | | | |
| **Day 0** | 55.4 | 2.5 | 39.8 | 22.9 | 2.6 | 3.7 | 30.0 | 0.0 | 0.0 | 64.7 | 44.6 | 6.4 | 2.5 | 1.4 |
| **Day 2** | 56.0 | 2.8 | 38.5 | 27.4 | 3.1 | 0.0 | 33.5 | 0.0 | 0.0 | 69.2 | 48.3 | 13.9 | 1.6 | 1.3 |
| **Day 4** | 70.6 | 0.0 | 30.0 | 21.5 | 1.0 | 3.4 | 22.2 | 1.5 | 1.8 | 60.9 | 38.5 | 19.8 | 2.6 | 0.6 |
| **Day 6** | 59.5 | 1.1 | 36.3 | 22.6 | 1.5 | 1.3 | 27.3 | 0.0 | 0.0 | 57.2 | 42.7 | 23.1 | 1.6 | 0.0 |

## CHAPTER FOUR: DISCUSSION

The effluent was treated with a UV system that was designed to inactivate microbes at a flow rate of 120 L/min. This is a small-scale version of the devices used in the waste treatment industry (Friedler et al. 2011; Perez et al. 2017; Salgot and Folch 2018). Ultraviolet (UV) irradiation causes DNA damage that can render microbes inactive (Lindenauer and Darby 1993). DNA mutations caused by UV-C are cis-syn cyclobutane-pyrimidine dimers (Thoma 1999). Inactivation, or the inability to replicate, occurs almost immediately in most microbes, but reactivation can occur in many bacterial strains in as little as 2 hours (Zimmer-Thomas et al. 2007).

The effluent samples for this study were treated at the much lower flow rate of 5 L/min. There was little evidence that the microbial communities were completely inactivated. For example, the water chemistry associated with microbial communities in the 100% effluent microcosms should not have changed much if the microbial communities were inactive but the large increases in ammonia (0.227 to 1.2 mg/L; $p=0.0043$) and sulfide (0 to 18 μg/L; $p=0.023$) observed over the course of the study suggests they remained active or became reactivated. Increases in ammonium could be due to decomposition or nitrification within the microcosm (Plunkett et al. 2020). The amount of DNA extracted from the 100% effluent appears to be lower than the other microcosms in the study ($P= 0.002$). It has been shown that UVC can crosslink DNA to

protein within bacterial cells which reduces the amount of pure DNA that can extracted (Smith et al. 1962).

In this microcosm study, the Shannon-diversity within the 100% effluent water microcosms dropped immediately upon incubation (Table 5a; $p=0.0389$). Although oxygen concentrations were not measured, effluent water was exposed to oxygen during the sample collection. 100% effluent was collected from a spigot into a large air-filled carboy. Oxygen concentrations during incubation likely decreased as a result of the activity of the microbial community present, thus reducing the richness and Shannon diversity as obligate-aerobic organisms could not thrive in a low-oxygen environment. Increased ammonium concentrations ($p=0.0043$) and decreased nitrate concentrations ($p=0.004$) from day 0 to day 6 (Table 2) in 100% effluent samples further suggests low concentrations of oxygen within the microcosms. In contrast, the Shannon diversity ($p<0.001$) and richness ($p=0.015$) of 100% spring water increased during incubation. The oxygen concentration of the 100% spring water was near zero as reported previously (Sharping et al. 2018), so unlike the 100% effluent, the microorganisms present in the 100% spring water microcosms did not have to adjust to changes in oxygen concentrations during incubation. The increase in Shannon diversity could be explained by rarer genera becoming more common within the microcosm because of decreased competition compared to their natural environment or changes in water chemistry promoting their growth. Some of the nutrients in the 100% spring water changed from day 0 to day 6 (Table 2) with P-values ranging from 0.0001 to 0.043.

The Shannon diversity of the microbial communities from the 10% and 30% mixture microcosms did not change significantly during the course of incubation, but the diversity index for communities in the 50% microcosms declined ($p<0.001$). Richness only changed significantly in the 50% microcosms from day 4 to day 6 ($p=0.018$). Richness of the microcosms with 10% effluent fluctuated slightly but insignificantly and were comparable on sampling days 0 and 6. The lack of change over time of many of the measured nutrients (e.g. nitrate, nitrite, sulfate, phosphorus, and TOC; see Table 3 and Fig. 3) in the mixed samples suggests that the microbial communities were not changing rapidly enough in the time frame of the experiment to alter those nutrient concentrations. The Simpson Index values in all cases were very close to the value of one. This suggests (Nagendra 2002) that the communities in this study are each dominated by only a few taxa as summarized in Table 7 and described more completely in Supplementary Tables 1-20.

An analysis of estimated functions of the top 30 unique provisionally-identified genera (Figure 2) indicates that 100% effluent community function was quite different from that of 100% spring water and that of all the mixtures of spring and effluent water throughout the course of the experiment. This can be seen in Figure 2 where a diagonal line clearly separates the function of 100% effluent communities from the function of the communities in all the other samples. This is corroborated by the analysis of the water chemistry discussed above. The function of mixtures of spring and effluent water more closely resemble those of 100% spring water with the possible exception of the day four and day six 50% mixtures, which are nearer to the function of

communities from effluent water on days two, four and six. The function of the community from the 100% effluent sample on day 0 does not cluster with the community function of 100% effluent on days 2, 4 and 6 (see Figure 2). This suggests that the function of the communities in 100% effluent changed throughout the course of the experiment which was not reflected in Figure 2, but is consistent with the decrease in Shannon Diversity ($p=0.0004$ comparing day 0 to day 6) observed in the effluent samples for days 2, 4 and 6 compared with day 0 (Table 5).

Figure 1 provides an overview of all communities for all samples and replicates. The ABC triangle was overlaid on the PCoA plot for discussion purposes. Points nearest triangle point C are microbial communities from 100% effluent (dark blue triangles) from all sampling days. Points nearest triangle point B are mostly microbial communities from 100% spring water (red inverted triangles) from all days. The position of the communities from 100% effluent or 100% spring water from all days suggests that these communities by themselves are relatively stable over the course of the experiment. Microbial communities from microcosms of mixtures of effluent and spring water found along the BC axis most likely represent simple mixing of the different communities from the 100% spring water and 100% effluent water and represent microcosms sampled on day 0 and day 2. Points representing communities from microcosms of more concentrated effluent mixtures (30% and 50%) appear to move toward point A even at day 2 of the incubation. The less concentrated effluent mixture (10%) appears to change more slowly, not moving toward point A until days 4 and 6. This suggests that it generally takes several days for the mixed samples to form the

new communities nearer to point A on the plot. The observed changes in mixed water communities most likely represents a succession in the dominant taxa within the communities. Although the communities near point A could be stable, because of the short duration of the experiment, it is possible that the succession would have continued if the experiment had been longer.

A summary of the 10 most abundant genera from communities of each sample observed during the course of the study are shown in Figure 4. The designation "Unknown" refers to 16S sequences that could not be identified by comparing to known bacterial sequences. Each instance of unknown could contain multiple taxa and thus are not discussed further. More than 5 of the 10 most abundant identified genera for all water types on sampling day 0 were different by day 4 or 6, with the exception of those found in the 10% effluent microcosms and the 100% spring water microcosms. Microcosms with 10% effluent maintained 6 of 10 most abundant genera on all sampling days, as expected with the lower dose of effluent in the mixture. Microcosms with 50% effluent experienced the greatest changes in the 10 most abundant genera as none of the most abundant taxa present on sampling day 0 were present on day 6, with the exception of those genera classified as "unknown." This is likely an indicator that the 50% effluent microcosm communities were disturbed more than the communities with less effluent and thus were changing more rapidly.

In the 100% effluent microcosms, *Salinibacter* was the most abundant identifiable genus present on sampling day 0. By sampling days 4 and 6, *Salinibacter* declined in abundance likely because it is obligately aerobic and any available oxygen in

the microcosms was likely metabolized during incubation. *Chryseobacterium*, *Massilia*, and *Pedobacter* were the most abundant genera on sampling day 4 and 6. *Chryseobacterium* is commonly found in soil or water sources and may have anti-fungal properties (Abarca et al. 2018). The genus *Massilia* have members that are strictly aerobic, and can found in drinking waters. *Pedobacter* can metabolize carbon or nitrogen for energy. From sampling day 4 to sampling day 6, *Pedobacter* increased while *Massilia* decreased in abundance likely because of their metabolic needs. *Pedobacter* could thrive in microcosms with higher carbon or nitrogen available in the effluent water (Table 2).

*Thiobacillus* and *Sulfurimonas* genera were most abundant in microcosms with 100% spring water on all sampling days. *Thiobacillus* and *Sulfurimonas* genera are facultative sulfur oxidizers and nitrogen reducers. These samples originated from micro- or anoxic spring water and were never exposed to oxygen during the course of the experiment, so it is not unexpected that they changed the least.

In microcosms with 10% effluent, many of the most abundant taxa remained as such. Comparable to the 100% spring water concentrations, *Thiobacillus* and *Sulfurimonas* were of the most abundant taxa on sampling days 0 and 2, but unlike 100% spring water these genera decreased in relative abundance by sampling day 6 (Figure 4). This trend can also be observed in Figure 1 in that points from 10% effluent water (green squares) were farther from point A on the triangle than the other mixture microcosms (30% and 50% effluent microcosms).

Microcosms with 30% effluent experienced more change than those with 10% effluent. *Thiobacillus*, *Sulfurimonas,* and *Salinobacter* were the most common on day 0, but by day 6 they were largely replaced by *Rhenherieinema* but *Comamonas* was also prevalent. *Comamonas* is commonly found in soil, water and sewage sludge and is a known androgen degrader (Chen et al. 2016). Some isolates have been implicated in benzene degradation (Jiang et al. 2015). *Comamonas* is highly motile (Farooq et al. 2017) and thus could be implicated in microbial community succession (Hibbing et al. 2010).

Microcosms with 50% effluent experienced the greatest changes in the most abundant taxa. *Thiobacillus* was the most abundant genus on sampling day 0, but was quickly replaced with *Rheinheimera* on sampling days 2, 4, and 6. Few taxa present on the earlier sampling days were still present on the later sampling days (Figure 4).

In all cases of mixing spring water with effluent, the new community that developed was dominated by a bacterium identified as belonging to the genus *Rheinheimera*. This genus has members known to inhibit other bacteria and ciliates in microbial communities and has been found in soil, freshwater and marine ecosystems (Chiellini et al. 2019). Inhibition of other microbes by *Rheinheimera* appears to be caused in a variety of ways including the generation of l-lysine oxidase, but the most important mechanism appears to be the production of diketopiperazine factors and other compounds (Sun et al. 2016) that have anti-quorum sensing activity in microbial communities. The disruption of quorum sensing pathways can prevent competing microbes from producing their own anti-microbials (Hibbing et al. 2010). It has been

shown that these kinds of anti-microbial compounds are related to succession in ecological niches. *Rheinheimera* was found at low abundance in both 100% spring water (ranked 15$^{th}$ on day 2, see Supplementary Table 6) and 100% effluent (ranked 20$^{th}$ on day 0, see Supplementary Table 1) so the origin of the *Rheinheimera* found in the communities from the mixed water microcosms is not known. On sampling day 6, *Novosphingobium* and *Sediminibacterium* were present among the most abundant taxa in all three mixed water microcosms.

Species belonging to the genus *Novosphingobium* have been found in groundwater samples (Lee et al. 2014) as well as wastewater treatment facilities. Some species of *Novosphingobium* have been shown to degrade certain aromatic compounds, estrogen compounds, and can be nitrogen fixers (Liu et al. 2018, Addison et al. 2007). *Sediminibacterium* species have been isolated from freshwater environments, including aquifers (Jiang et al. 2019), and sewage sediment (Song et al. 2017, Ayarza et al. 2014). Members of this genus can be aerobic and motile. Both *Novosphingobium* and *Sediminibacterium* have been isolated from groundwater environments with high concentrations of arsenic and could be involved in arsenic mobilization (Jiang et al. 2019).

The 100% spring water communities changed very little over the 6-day experiment. For example, *Thiobacillus* and *Sulfurimonas* were ranked 1$^{st}$ and 2$^{nd}$ in the community throughout the 6 days. In contrast, there appeared to be a major change in the community from the 100% effluent water over time in that *Chryseobacterium* and *Pedobacter* became the most highly abundant taxa by day six.

At the end of the six-day incubation period of mixed water samples, the most abundant taxa present were generally far less abundant in the original effluent or spring water samples (Figure 4). For example, while *Rheinheimera* ranked 20th in 100% effluent and 54th in 100% spring water on day 0, it ranked 1st on day 6 of all three mixing experiments. Members of the Rheinheimera genus have mechanisms that can inhibit surrounding mircoorganisms, such as anti-quorum sensing as noted in X species/strain (reference).

Similar results were found for *Chryseobacterium*, *Massilia*, *Comamonas*, *Novosphingobium* and *Sediminibacterium* (Figure 4), ranging in rank from 12th – 45th in communities of either 100% spring water or 100% effluent on day one but rising to the 10 most abundant taxa in the water mixtures by day 6. One exception was *Thiobacillus*, which was either 1st or 2nd in abundance in 100% spring water and ranked 10th in the 10% effluent mixture on day 6. Most commonly, taxa that were dominant at the end of the mixing experiments were far less abundant than at the beginning indicating that community succession occurred in the microcosms of the mixed spring and effluent water communities.

**Table 7:** Sampling day 0 abundance of the most abundant Genera present on sampling day 6 for all water types

| **Highly Abundant End-point Taxon** | **Effluent Day 0 Rank** | **Spring Day 0 Rank** |
|---|---|---|
| *Rheinheimera* | 20 | 54 |
| *Chryseobacterium* | 13 | 29 |
| *Massilia* | 12 | 18 |
| Comamonas | 18 | 16 |
| *Novosphingobium* | 30 | 15 |
| *Sediminibacterium* | 45 | 21 |

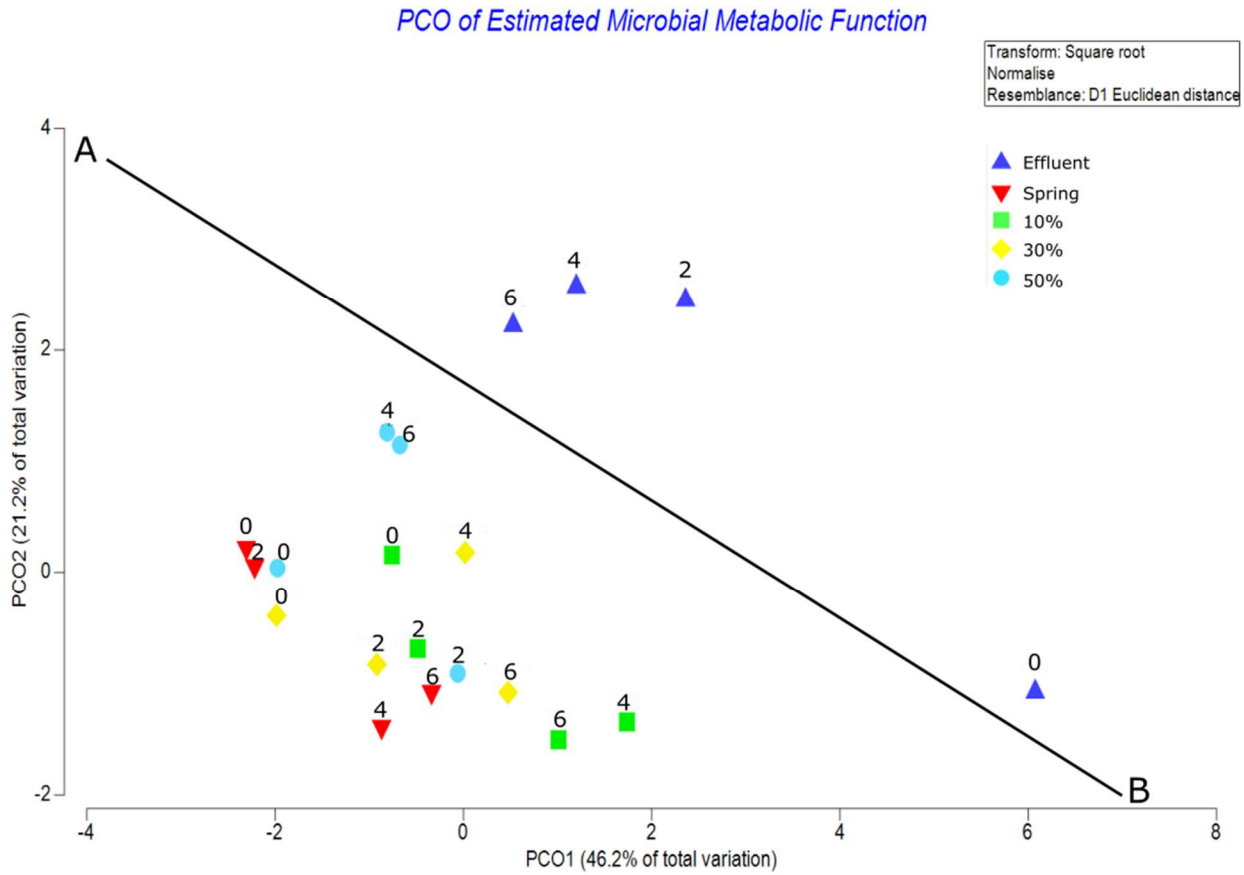


**Figure 2:** Principal coordinate analysis of estimated microbial metabolic function by water type and sampling day. Note that the effluent samples (blue triangles) are the most distinct from the spring (red triangles), while the mixtures of spring water and effluent (light blue, yellow, green) fall nearer to spring water than to effluent, with the possible exception of the 50% mixture (light blue circles) on days 4 and 6 of the experiment.

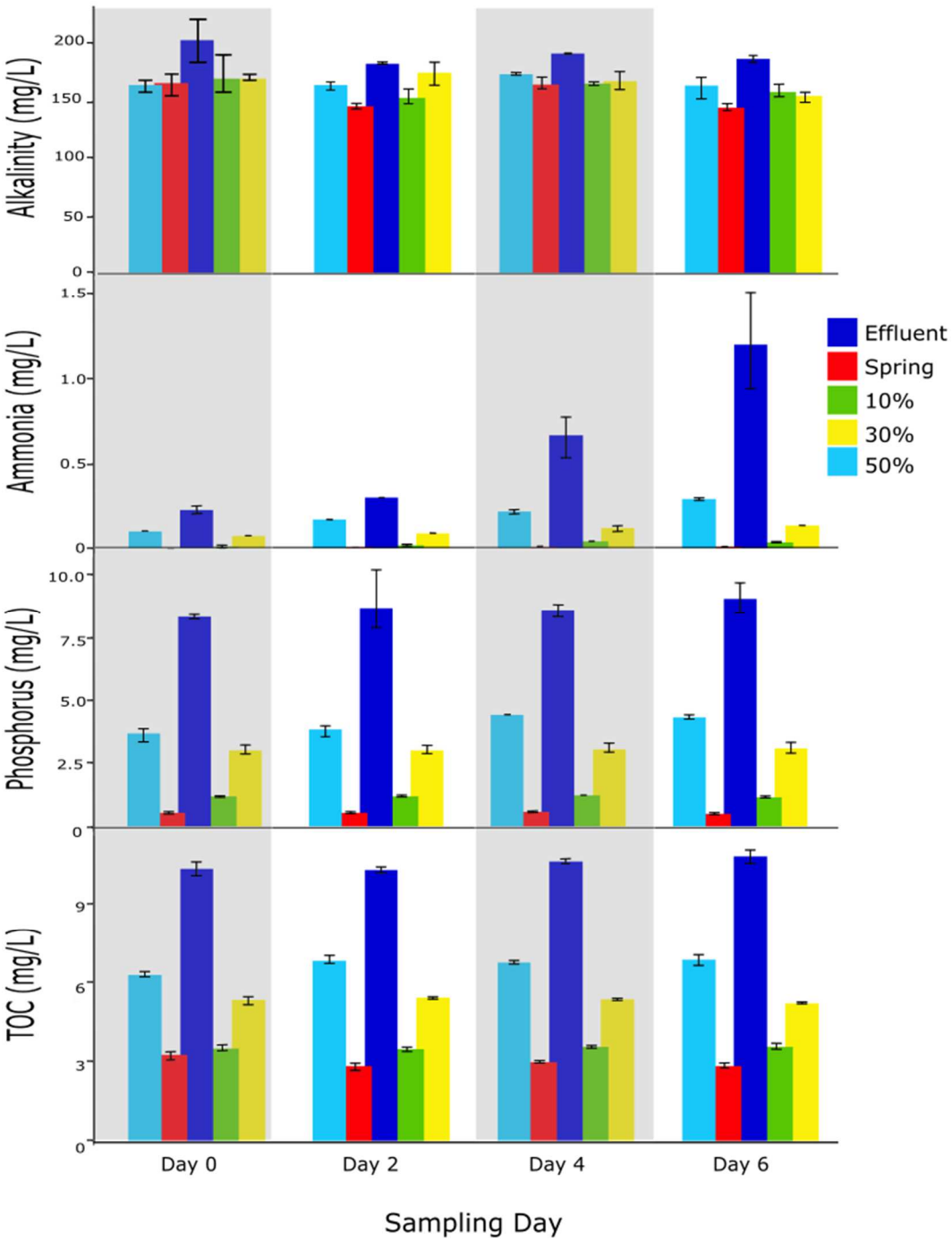


**Figure 3a:** Alkalinity, Ammonia, Phosphorus, and Total Organic Carbon results for all water types by sampling day.

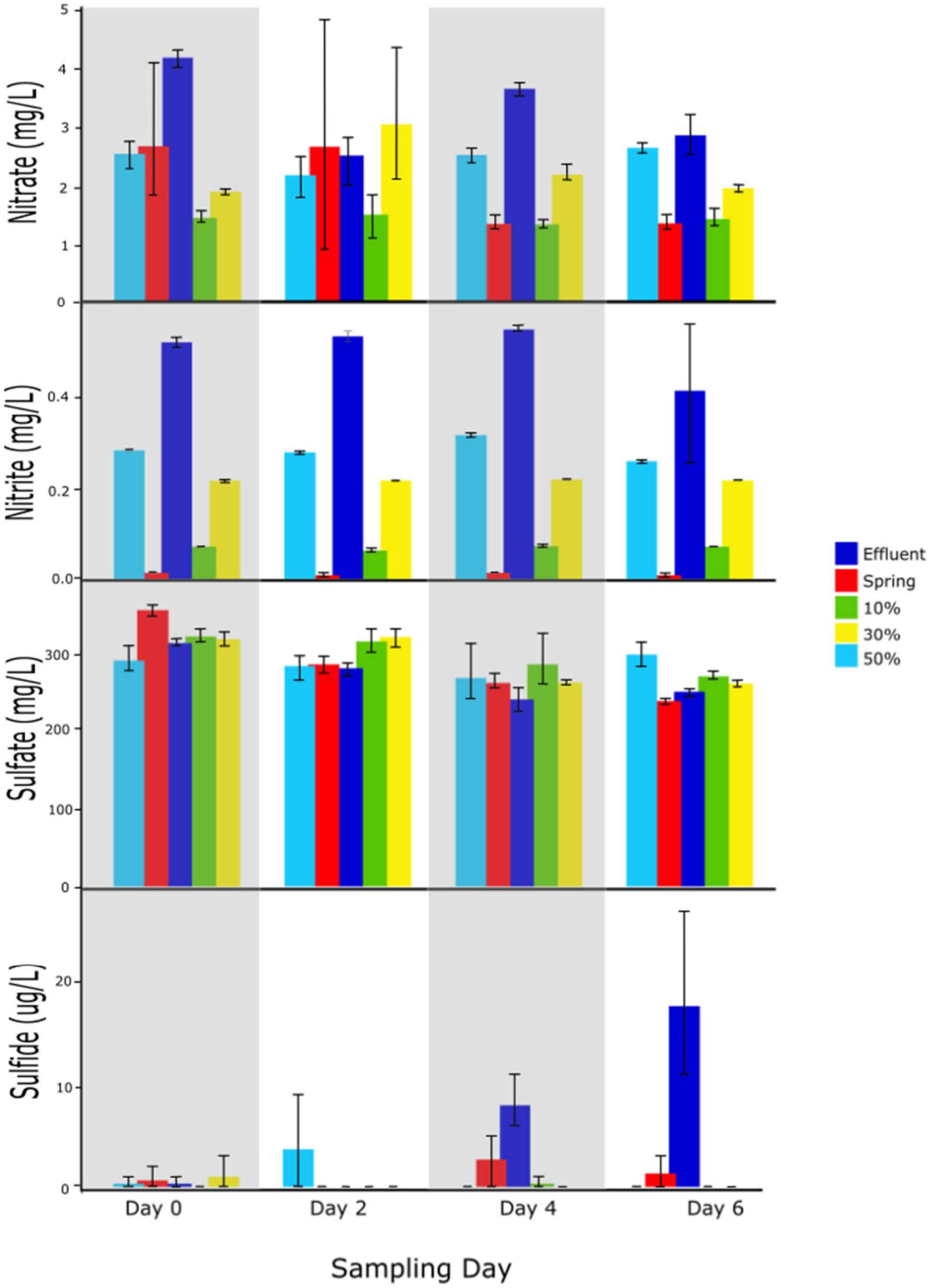


**Figure 3b:** Nitrate, Nitrite, Sulfate, and Sulfide results for all water types by sampling day

| Effluent Day 0 | Effluent Day 2 | Effluent Day 4 | Effluent Day 6 |
|---|---|---|---|
| **Unknown 2168** | Massilia 4466 | Chryseobacterium 3417 | Chryseobacterium 2979 |
| **Salinibacter 1976** | **Unknown 1124** | Massilia 1739 | Pedobacter 1536 |
| Pseudomonas 895 | **Salinibacter 915** | Pedobacter 995 | Massilia 1249 |
| Derxia 338 | Pseudomonas 841 | **Unknown 924** | **Unknown 838** |
| Burkholderia 228 | Haliangium 180 | Hydrogenophaga 740 | Bacillus 800 |
| Salinivenus 212 | Marivirga 152 | Haliangium 461 | Hydrogenophaga 571 |
| Haliangium 158 | Comamonas 103 | Leeia 284 | Dechloromonas 271 |
| Sulfurimonas 133 | Sulfurimonas 102 | Sphingobium 115 | **Salinibacter 182** |
| Prevotella 129 | Salinivenus 84 | **Salinibacter 89** | Klebsiella 139 |
| Marivirga 108 | Lactobacillus 71 | Sulfurimonas 50 | Azospira 102 |
| **Spring Water Day 0** | **Spring Water Day 2** | **Spring Water Day 4** | **Spring Water Day 6** |
| **Sulfurimonas 3836** | **Thiobacillus 3635** | **Thiobacillus 2863** | **Thiobacillus 2408** |
| **Thiobacillus 3665** | **Sulfurimonas 2833** | **Unknown 1679** | **Unknown 1882** |
| **Unknown 927** | **Unknown 1029** | **Sulfurimonas 869** | **Sulfurimonas 633** |
| Aquaspirillum 237 | Aquaspirillum 274 | Sulfuricurvum 535 | Curvibacter 549 |
| Alviniconcha 103 | **Salinibacter 226** | **Salinibacter 367** | **Salinibacter 519** |
| **Salinibacter 90** | Curvibacter 182 | Curvibacter 363 | Candidatus Planktophila 235 |
| Lutibacter 64 | Alviniconcha 164 | Thiothrix 335 | Pseudomonas 229 |
| Thiothrix 41 | Comamonas 90 | Tahibacter 164 | Comamonas 209 |
| Pseudomonas 39 | Sulfuricurvum 68 | Comamonas 147 | Caulobacter 178 |
| Lutiella 38 | Pseudomonas 60 | Novosphingobium 146 | Candidatus Kentron 129 |
| **10% Effluent Water Day 0** | **10% Effluent Water Day 2** | **10% Effluent Water Day 4** | **10% Effluent Water Day 6** |
| **Thiobacillus 1826** | **Thiobacillus 1647** | **Unknown 1811** | **Unknown 1711** |
| **Unknown 1537** | **Unknown 1118** | **Thiobacillus 1026** | **Rheinheimera 675** |
| Sulfurimonas 1404 | **Salinibacter 1026** | **Rheinheimera 984** | **Salinibacter 507** |
| **Salinibacter 1135** | Sulfurimonas 909 | **Salinibacter 793** | **Comamonas 434** |
| Pseudomonas 409 | **Curvibacter 581** | **Curvibacter 616** | **Curvibacter 432** |
| **Comamonas 272** | **Rheinheimera 486** | Sulfurimonas 435 | Novosphingobium 358 |
| **Curvibacter 249** | Pseudomonas 390 | **Comamonas 308** | Sediminibacterium 285 |
| **Rheinheimera 192** | **Comamonas 174** | Novosphingobium 239 | Herbaspirillum 190 |
| Cloacibacterium 112 | Chryseobacterium 170 | Candidatus Planktophila 146 | Candidatus Planktophila 187 |
| Salinivenus 109 | Cloacibacterium 136 | Pseudomonas 113 | **Thiobacillus 156** |
| **30% Effluent Water Day 0** | **30% Effluent Water Day 2** | **30% Effluent Water Day 4** | **30% Effluent Water Day 6** |
| Thiobacillus 2271 | Curvibacter 1411 | Rheinheimera 1612 | Rheinheimera 1387 |
| Sulfurimonas 2199 | Rheinheimera 978 | **Unknown 1415** | **Unknown 1365** |
| **Unknown 1196** | Cloacibacterium 768 | **Comamonas 630** | **Comamonas 520** |
| Salinibacter 541 | Thiobacillus 764 | Salinibacter 524 | Acidovorax 350 |
| Curvibacter 389 | **Unknown 632** | Herbaspirillum 181 | Mesorhizobium 346 |
| Pseudomonas 283 | Sulfurimonas 517 | Pseudomonas 180 | Sediminibacterium 272 |
| Aeromonas 199 | **Comamonas 318** | Novosphingobium 133 | Candidatus Planktophila 163 |
| Cloacibacterium 138 | Chryseobacterium 220 | Sulfurimonas 127 | Novosphingobium 149 |
| Alviniconcha 108 | Tabrizicola 84 | Candidatus Planktophila 104 | Polynucleobacter 112 |
| **Comamonas 102** | Pseudorhodobacter 63 | Sediminibacterium 91 | Herbaspirillum 109 |
| **50% Effluent Water Day 0** | **50% Effluent Water Day 2** | **50% Effluent Water Day 4** | **50% Effluent Water Day 6** |
| Thiobacillus 2847 | Rheinheimera 1189 | Rheinheimera 2688 | Rheinheimera 1890 |
| Sulfurimonas 2198 | Curvibacter 841 | **Unknown 1087** | **Unknown 1724** |
| **Unknown 1349** | Comamonas 832 | Comamonas 604 | Massilia 502 |
| Salinibacter 703 | **Unknown 671** | Salinibacter 239 | Sediminibacterium 364 |
| Pseudomonas 208 | Arcobacter 610 | Acidovorax 141 | Comamonas 292 |
| Derxia 207 | Thiobacillus 535 | Pararheinheimera 104 | Mesorhizobium 255 |
| Aquaspirillum 139 | Chryseobacterium 510 | Polynucleobacter 96 | Fluviicola 168 |
| Burkholderia 115 | Cloacibacterium 380 | Fluviicola 84 | Chitinophaga pinensis 128 |
| Alviniconcha 95 | Massilia 338 | Herbaspirillum 84 | Novosphingobium 124 |
| Salinivenus 92 | Sulfurimonas 304 | Sediminibacterium 75 | Acidovorax 107 |

**Figure 4:** Most abundant genera for each water type on all sampling days. Genera highlighted changed in abundance during incubation. Genera that are both highlighted and bolded experienced changes in abundance on all four sampling days.

# CHAPTER FIVE: CONCLUSIONS

The goal of this study was to evaluate the response of a native aquifer microbial community after exposure to effluent that could be used for deep well injection during aquifer storage and recovery processes. Microbial community changes within the effluent microcosms suggests the UV treatments were less effective than expected. In mixtures of spring and effluent water, there appeared to be a succession of dominant taxa within the microbial communities. At high effluent concentrations (30% and 50% effluent), the succession was more rapid than at low effluent concentrations (10% effluent) but after six days, all the communities from the microcosms made from mixing effluent and spring water had the same single most dominant taxon, *Rheinheimera.* There were three other highly abundant taxa found in all three mixtures of effluent and spring water at the end of the mixing experiment. Because the UV treatment did not completely or permanently inactivate the microbial communities from the effluent water, it is difficult from this study to conclude the relative impact of mixing two different microbial communities from the relative impact of mixing water with two very different water quality profiles. A control experiment with sterilized effluent water should be incorporated in any further studies.

A new microbial community appears to form when effluent is mixed with spring water, but longer and more complex microcosm studies would be beneficial to determine the resilience of the native aquifer microbial communities.

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