Science of the Total Environment 842 (2022) 156768

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Subsurface microbial communities as a tool for characterizing regional-scale groundwater flow

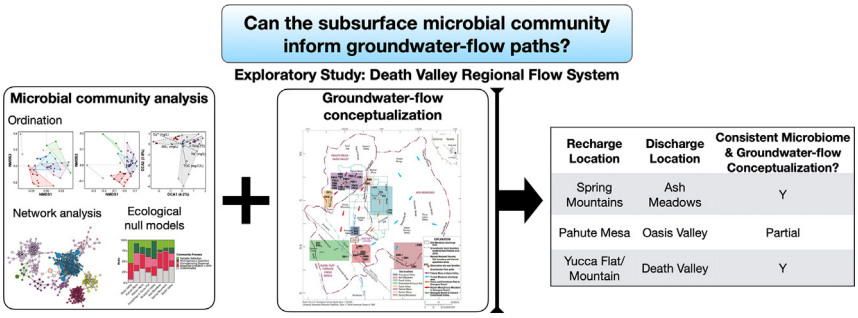
Nancy Merino [a,*], Tracie R. Jackson [b], James H. Campbell [c], Annie B. Kersting [a], Joshua Sackett [d,e,f], Jenny C. Fisher [d,g], James C. Bruckner [d], Mavrik Zavarin [a], Scott D. Hamilton-Brehm [d,h], Duane P. Moser [d,e,**]

[a] Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, United States
[b] Nevada Water Science Center, U.S. Geological Survey, Boulder City, NV 89005, United States
[c] Department of Natural Sciences, Northwest Missouri State University, Maryville, MO 64468, United States
[d] Division of Earth and Ecosystems Sciences, Desert Research Institute, Las Vegas, NV 89119, United States
[e] Division of Hydrologic Sciences, Desert Research Institute, Las Vegas, NV 89119, United States
[f] School of Life Sciences, University of Nevada, Las Vegas, NV 89154, United States
[g] Biology Department, Indiana University Northwest, Gary, IN 46408, United States
[h] Department of Microbiology, Southern Illinois University Carbondale, Carbondale, IL 62901, United States

HIGHLIGHTS

- The subsurface microbial community is consistent with known hydraulic connections.
- Location plays a major role in microbial community variation.
- Network analysis of common microbes corroborated recharge and discharge areas.
- Ecological null models provided insight into community assembly patterns.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jurgen Mahlknecht

Keywords:
Fractured-rock aquifer
Subsurface microbial ecology
Microbial community assembly
Regional-scale aquifer microbial community
Deep biosphere

ABSTRACT

Subsurface microbial community distribution patterns are influenced by biogeochemical and groundwater fluxes and may inform hydraulic connections along groundwater-flow paths. This study examined the regional-scale microbial community of the Death Valley Regional Flow System and evaluated whether subsurface communities can be used to identify groundwater-flow paths between recharge and discharge areas. Samples were collected from 36 sites in three groundwater basins: Pahute Mesa–Oasis Valley (PMOV), Ash Meadows (AM), and Alkali Flat–Furnace Creek Ranch (AFFCR). Microbial diversity within and between communities varied by location, and communities were separated into two overall groups that affiliated with the AM and PMOV/AFFCR basins. Network analysis revealed patterns between clusters of common microbes that represented groundwaters with similar geochemical conditions and largely corroborated hydraulic connections between recharge and discharge areas. Null model analyses identified deterministic and stochastic ecological processes contributing to microbial community assemblages. Most communities were more different than expected and governed by dispersal limitation, geochemical differences, or undominating processes. However, certain communities from sites located within or near the Nevada National Security Site were more similar than expected and dominated by homogeneous dispersal or selection. Overall, the (dis)

Abbreviations: AFFCR, Alkali Flat Furnace Creek Ranch; AM, Ash Meadows; DVRFS, Death Valley Regional Flow System; NNSS, Nevada National Security Site; PMOV, Pahute Mesa–Oasis Valley; UGTA, Underground Test Area.

* Correspondence to: N. Merino, Lawrence Livermore National Lab, 7000 East Ave, Livermore, CA 94550, United States.
** Correspondence to: D.P. Moser, Division of Hydrologic Sciences, Desert Research Institute, Las Vegas, NV 89119, United States.
E-mail addresses: merino4@llnl.gov (N. Merino), Duane.Moser@dri.edu (D.P. Moser).

http://dx.doi.org/10.1016/j.scitotenv.2022.156768
Received 31 January 2022; Received in revised form 26 May 2022; Accepted 14 June 2022
Available online 21 June 2022

similarities between the microbial communities of DVRFS recharge and discharge areas supported previously documented hydraulic connections between: (1) Spring Mountains and Ash Meadows; (2) Frenchman and Yucca Flat and Amargosa Desert; and (3) Amargosa Desert and Death Valley. However, only a portion of the flow path between Pahute Mesa and Oasis Valley could be supported by microbial community analyses, likely due to well-associated artifacts in samples from the two Oasis Valley sites. This study demonstrates the utility of combining microbial data with hydrologic, geologic, and water-chemistry information to comprehensively characterize groundwater systems, highlighting both strengths and limitations of this approach.

1. Introduction

Groundwater plays an essential role in providing water for societal needs and maintaining ecosystem health. As the global hydrologic cycle shifts with climate change, groundwater quality and quantity are increasingly affected, necessitating sustainable groundwater-management practices. These practices require a regional-scale understanding of the groundwater system. Regional-scale groundwater flow is typically characterized using geologic, water-level, water-chemistry, and aquifer-testing data (Neuman, 2005; Ye et al., 2016). However, groundwater systems also are impacted by and serve as distinct habitats for microbial communities (Chapelle, 2000; Flynn et al., 2013; Griebler et al., 2014). Thus far, groundwater microbial communities remain an underutilized data source to understand hydraulic connections between recharge and discharge areas.

Groundwater flow is governed largely by recharge and discharge rates and the hydraulic properties of the saturated rocks. Groundwater-flow paths are derived from hydraulic gradients between areas of recharge (e.g., precipitation in high topographic regions) and discharge (e.g., springs in low topographic regions) (Tóth, 1963; Tóth, 1999; Freeze and Witherspoon, 1967). Geologic units also exert control on groundwater-flow pathways. For example, the juxtaposition of permeable geologic units against impermeable units downgradient results in a hydraulic barrier to groundwater flow. The sources of groundwater and travel times along groundwater-flow paths can be informed by water-chemistry data (Woessner, 2022; Cook, 2022; Devlin, 2022).

The groundwater microbial community also can be used to characterize groundwater systems and validate groundwater-flow paths. This concept dates back to the 1890s when studies began using microbes as tracers to understand subsurface pathogen transport and to delineate groundwater-flow paths through injection and recovery experiments (Harvey, 1997). With the advent of the genomics era and the reducing costs of high-throughput sequencing, it may now be possible to use the subsurface microbial community to aid in groundwater characterization. Unattached (planktonic) microbes may be transported through aquifers along groundwater-flow paths through pore spaces and fractures (Walvoord et al., 1999; Amalfitano et al., 2014; Zhang et al., 2020). Several abiotic and biotic factors impact subsurface microbial movement (Ginn et al., 2002; Gerba et al., 2015), including groundwater-flow rates, hydraulic barriers, pore size, microbial adhesion processes, and the physiological state of microbial cells.

Subsurface microbial transport may impact microbial community assemblages through space and time and could result in more similar or dissimilar communities. Microbial community assembly can be evaluated with ecological null models (Stegen et al., 2012, 2013, 2015) (see Table S1 for terms and definitions used for ecological null models) that may inform groundwater characterization. For example, high microbial-dispersal rates (i.e., 'homogenizing dispersal') may provide a signature for tracking hydraulic connections through the co-occurrence of microorganisms in recharge and discharge areas. In contrast, hydraulic barriers may prevent or impede microbial transport and mixing ('dispersal limitation'), such as in the hard-rock aquifers of Brittany (France) (Maamar et al., 2015), causing microbial communities to diverge over time or along flow paths through stochastic ecological processes (Zhou and Ning, 2017). Biogeochemical and redox conditions also drive community assembly, selecting for adaptable microorganisms (Maamar et al., 2015; Hug et al., 2015; Fillinger et al., 2019), and are known as deterministic processes that occur when conditions are consistent ('homogeneous selection') or fluctuating ('variable selection'). Consistent environmental conditions lead to less divergence in the community, whereas fluctuating conditions can perturb microbial abundances and diversity. Ecological null models can identify the dominating ecological processes that impact microbial community assembly between two communities. Along with other analytical approaches to analyze microbial community data, the groundwater microbial community could be an advantageous tool to supplement other geologic and hydrologic datasets to provide a comprehensive understanding of regional groundwater flow.

This study assesses the potential of using subsurface microbial community patterns as a supplemental dataset to characterize regional-scale groundwater flow. The Death Valley Regional Flow System (DVRFS) microbial community was used as a test case because, over the past few decades, hundreds of boreholes and wells have been drilled in the DVRFS to monitor water levels and water quality. Thus, the DVRFS provides a unique opportunity to identify novel microbial community patterns on a regional-scale and to determine whether microbial community patterns are consistent with regional-scale groundwater flow-paths (Halford and Jackson, 2020). The aims of this study were to (1) evaluate the groundwater bacterial and archaeal community distribution patterns with alpha- and beta-diversity analyses and network analysis, (2) identify the assembly processes dominating the community patterns using ecological null models, and (3) use the microbial community patterns and assembly processes to assess any (dis)similarities along groundwater-flow paths. To achieve these aims, we collected samples from recharge and discharge areas and hypothesized that microbial (dis)similarities, co-occurrences, and assembly patterns may infer and support the most recent groundwater conceptualization (Halford and Jackson, 2020).

2. Materials and methods

2.1. Site description

The DVRFS is a 100,000 km^2 region that is part of the Great Basin physiographic province in southern Nevada and California (Fig. 1). The study area includes three groundwater basins in the DVRFS: Pahute Mesa–Oasis Valley (PMOV), Ash Meadows (AM), and Alkali Flat–Furnace Creek Ranch (AFFCR) (Fig. 1) (Halford and Jackson, 2020). The Nevada National Security Site (NNSS, formerly Nevada Test Site) intersects these three groundwater basins. Land-surface elevations range from −86 to 3600 m relative to sea level. Principal geologic units are grouped into four categories: Paleozoic carbonate rocks, Tertiary volcanic rocks, Cenozoic basin fill, and undifferentiated, low-permeability siliciclastic and granitic rocks (Halford and Jackson, 2020).

Long-term water-level and water-quality monitoring have occurred in the study area. A total of 828 underground nuclear tests were detonated beneath the NNSS from 1951 to 1992 (U.S. DOE, 2015). Underground nuclear testing prompted a long-term U.S. Department of Energy environmental management program, known as the Underground Test Area (UGTA) Activity, to assess and monitor potential groundwater transport of radionuclides. Extensive hydrogeologic investigations (Halford and Jackson, 2020; Moreo et al., 2003; Moreo and Justet, 2008) also were conducted by the U.S. Geological Survey to evaluate water levels and groundwater-withdrawal rates. Several groundwater studies (Halford and Jackson, 2020; Winograd and Pearson, 1976; Winograd et al., 1998; Thomas et al., 2020, 2013;

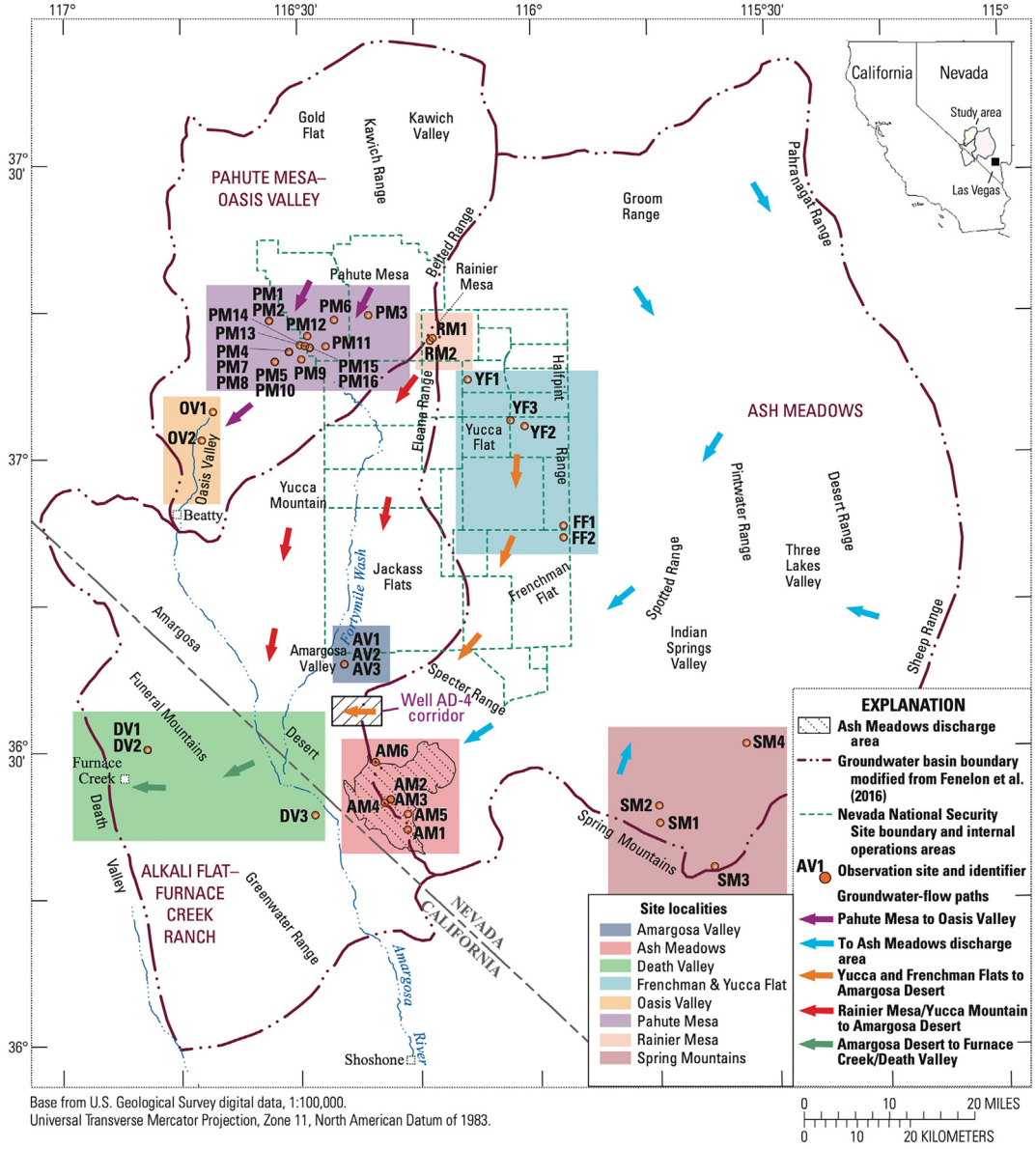


Fig. 1. Map of groundwater basins, locations, sampling sites, and groundwater-flow paths within the study area.

Anderson et al., 2006; Bushman, 2008; Belcher et al., 2009, 2019; Bushman et al., 2010; Fenelon et al., 2020; Fenelon et al., 2016; Nelson and Mayo, 2014; Warix et al., 2020) have evaluated flow paths in the DVRFS.

This study evaluates groundwater-flow paths using the most recent groundwater conceptualization of the DVRFS (Halford and Jackson, 2020; Jackson et al., 2021) (Fig. 1). The five regional groundwater-flow paths evaluated in this study are: (1) Pahute Mesa to Oasis Valley; (2) Spring Mountains to Ash Meadows discharge area; (3) Frenchman and Yucca Flat to Amargosa Desert; (4) Rainier Mesa/Yucca Mountain to Amargosa Desert; and (5) Amargosa Desert to Death Valley (Halford and Jackson, 2020) (Fig. 1). In the PMOV basin, Pahute Mesa is the primary recharge area contributing to discharge from Oasis Valley (Jackson et al., 2021). In the AM basin, most recharge is derived from the Spring Mountains and Sheep Range, with lesser recharge amounts from the Groom, Pahranagat, Desert, Pintwater, and Spotted Ranges. Groundwater from the Spring Mountains and other ranges in the AM basin moves southward and westward to high-volume springs of the Ash Meadows discharge area, the largest oasis in the Mojave Desert (Laczniak et al., 2001). Groundwater from Yucca and Frenchman Flats moves into the Amargosa Desert through the well AD-4 corridor north of Ash Meadows discharge area (Fig. 1). The well AD-4 corridor hydraulically connects transmissive carbonate rocks in the AM basin with transmissive basin fill in the Amargosa Desert (see Section 4.3 for more details). Groundwater from Rainier Mesa and the Yucca Mountain area moves southward into the Amargosa Desert. Most groundwater in the Amargosa Desert moves westward and discharges at Furnace Creek in Death Valley.

2.2. Sample collection and geochemical analysis

Between 2008 and 2014, a total of 42 samples were collected from wells, mine vent holes, or springs. These samples were collected from 36 sites across the DVRFS (Fig. 1, Table S2). The number of sites per location varied (Table S2) based on accessibility [Amargosa Valley (sites [n] = 1, samples [s] = 3), Ash Meadows discharge area (n = 7, s = 7), Death Valley (n = 3, s = 3), Frenchman Flat (n = 2, s = 2), Oasis Valley (n = 2, s = 2), Pahute Mesa (n = 12, s = 16), Rainier Mesa (n = 2, s = 2), Spring Mountains (n = 4, s = 4), and Yucca Flat (n = 3, s = 3)]. The location Amargosa Valley is within the Amargosa Desert. For well samples, groundwater generally was pumped at high rates (hundreds of L/min) for hours-to-days prior to collection of geochemical and microbial samples to minimize

wellbore artifacts. In a few cases, well water samples were obtained by lower rate "jack pumps" (in-line submerged pump) or from static water columns with discrete sampler deployments (i.e., "bailers"). The mine water samples (U12n-10 [RM1] and U12n-vent2 [RM2]) were collected from flooded workings by bailer from hundreds of meters overhead via vertical ventilation holes. Spring samples from Ash Meadows and Spring Mountains were collected by pumping with a peristaltic pump and sterile platinum-cured silicone tubing (Masterflex LS-15) inserted as deeply into the spring orifice as possible via a 24′ (7.3 m) telescoping probe to minimize surface associated influences. For the site in the Amargosa Valley (4PD), water was pumped continuously over a few weeks, with samples collected on days 1, 9, and 23. At four sites on Pahute Mesa (ER-20-8 [PM15, PM16], ER-EC-13 [PM10, PM5], ER-EC-15 [PM4, PM7, PM8], and PM-3 [PM1, PM2]), samples were collected via pumping from discrete piezometers screened at multiple different depths, as listed in Table S2, because there may be microbial community differences with depth (Moser et al., 2014).

Cells for DNA extraction were collected on 0.22 μm Sterivex™ filters (EMD Millipore, U.S.A.) with the filtrate retained for geochemical analysis and the filters transported on dry ice and stored at −80 °C. The DNA extraction and sequencing methods are described in Supplementary methods Section 1.1. The method and volume of filtration for cells varied depending on the circumstance: (1) ranging from tens to hundreds of liters collected via a pressurized sampling manifold (~0.5 bar) at the wellhead for most pumped samples; or (2) two to four liters filtered offsite for bailed samples or samples containing radioactive elements. Nevares Deep Well 2 (DV1) is artesian and was sampled at ambient pressure (0.4 bar) via a dedicated sampling port at the wellhead. Most geochemical analyses reported here were processed as part of the UGTA groundwater sampling and analysis program (Farnham, 2020). Otherwise, the geochemical analyses were performed at the DRI Water Analysis Laboratory (Reno, NV) or at Princeton University T.C.O. (Princeton, NJ) (Moser et al., 2014), as described in Supplementary methods Section 1.2.

2.3. Statistical analyses of the geochemical data

The geochemical dataset (Table S2, color scheme in Table S3) used in this paper contains information on location, depth, and geochemical measurements. Several geochemical parameters contained missing and left-censored (i.e., below detection limit) values. To reduce the number of missing values, some missing values were substituted with geochemical data collected from the same site on a different sampling date. Substituted data were obtained from the UGTA Activity Environmental Management Project database (Farnham, 2020). Although many of these values were measured between the 1970s and 1990s, the geochemical parameters have remained generally stable over the past few decades (Farnham, 2020). A piper diagram was created using the Geochemist's Workbench Release 11.0.8 (Aqueous Solutions LLC, USA) with a subset of the geochemical data (Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}, HCO_3^{-}). Principal component analysis (PCA) also was used to visualize the sample variation through dimensionality reduction, as described in Supplementary methods Section 1.3.

2.4. Quality control of the 16S rRNA amplicon sequences

Raw sequences were processed through QIIME 1 and DADA2 v1.12.1 (Callahan et al., 2016), as described in Supplementary methods Section 1.4 and using similar methods as Fischer et al. (2022). Because there were two different sequencing batches, samples were separated by batch and processed through DADA2 separately to account for potential error rate model differences. This resulted in amplicon sequence variants (ASVs) unique to each batch, which may have been caused by technical or biological differences (e.g., sample preparation, human processing, PCR amplification, sequencing errors, and multiple 16S rRNAs per cell). However, many ASVs from both batches were closely-related, as determined by inspection of a phylogenetic tree and sequence alignment, and were subsequently clustered into OTUs in QIIME 2 (Bolyen et al., 2019) at the 97 % identity level with the function vsearch cluster-features-denovo. OTU clustering at the 97 % identity level can provide comparable overall results as ASVs on a broad-scale (Louca et al., 2019; Glassman and Martiny, 2018; Joos et al., 2020; Moossavi et al., 2020; Nearing et al., 2018; Prodan et al., 2020). Taxonomy was assigned to the clustered OTUs using the QIIME 2 function feature-classifier classify-sklearn with a pretrained-classifier SILVA v138 database for OTUs from 515F/806R region of 16S rRNA sequences (Quast et al., 2013; Yilmaz et al., 2014; Bokulich et al., 2018, 2020). The OTU and taxonomy table were then imported to phyloseq v1.16.2 (McMurdie and Holmes, 2013), and contaminant OTUs removed, as described in Supplementary methods Section 1.4. Based on rarefaction curves generated using the R package iNEXT (Hsieh et al., 2016), sufficient sequencing depth was achieved for each sample (Fig. S1).

2.5. Microbial community analyses

Alpha diversity (diversity within samples) indices were computed with phyloseq (function estimate_richness) and picante v1.8.2 (Kembel et al., 2010) using a phylogenetic tree generated as described in Supplementary methods Section 1.5. The means between each categorical variable were evaluated using the non-parametric Mann-Whitney test with false discovery rate *p*-value adjustment. Phylogenetic relatedness also was determined by calculating the standardized effect size (SES) of Faith's PD (phylogenetic diversity), MPD (mean pairwise distance), and MNTD (mean nearest neighbor phylogenetic distance) (see Table S1 for definitions), as described in Supplementary Methods.

Beta diversity (diversity between samples) was evaluated using five approaches for comparison: (1) non-metric multidimensional scaling (NMDS) ordination on unweighted and weighted UniFrac distances, (2) DEICODE robust Aitchison principal-component analysis (RPCA) (Martino et al., 2019) in QIIME 2 with auto-RPCA, (3) centered-log ratio (CLR) PCA (Gloor et al., 2017), (4) phylogenetic isometric-log ratio (PhILR) PCA (Silverman et al., 2017), and (5) canonical correspondence analysis (CCA). Permutational analysis of variance (PERMANOVA) marginal tests with 999 permutations were conducted with R function adonis2 on the NMDS ordinations and homogeneity was checked with R function betadisper.

Deterministic and stochastic ecological processes were evaluated using the β-nearest taxon index (βNTI) and Raup-Crick (Bray-Curtis) (RCBC) metrics, following null model analyses (Stegen et al., 2012, 2013, 2015; Zhou and Ning, 2017; Dini-Andreote et al., 2015; Danczak et al., 2020) (see Table S1 for definitions). Only for these metrics, communities were rarefied to the lowest sequencing depth. βNTI is used to identify deterministic processes by quantifying phylogenetic turnover. First, species were randomly shuffled across the tips of the phylogeny, also known as the between-community mean-nearest-taxon-distance (βMNTD) metric, to obtain a null distribution after repeating the shuffling 999 times. Subsequently, the significance is evaluated by calculating the difference between the observed βMNTD and mean null distribution, with $\beta NTI < -2$ (less phylogenetic turnover; homogeneous selection) and $\beta NTI > 2$ (more phylogenetic turnover; variable selection) as significant (Stegen et al., 2012). Insignificant βNTI suggests that the observed compositional differences are due to stochastic ecological processes (Hardy, 2008), further determined by RCBC. RCBC is based off of community composition and OTU abundances, and follows Raup–Crick (Raup and Crick, 1979) to probabilistically assemble local communities, followed by quantification with Bray–Curtis (Bray and Curtis, 1957) to obtain a null distribution after repeating 9999 times (Stegen et al., 2013; Danczak et al., 2020; Chase et al., 2011). The RCBC null distribution is then standardized between −1 and +1 and significant values determined as $RCBC < -0.95$ (homogeneous dispersal) and $RCBC > 0.95$ (dispersal limitation). Ecological null models also assumes that phylogenetic relatedness is associated with ecological niche differences (Stegen et al., 2012; Fillinger et al., 2019; Dini-Andreote et al., 2015), which was confirmed for the DVRFS microbial community using Mantel correlograms, following Dini-Andreote et al. (2015). A significant ($P < 0.05$) positive

correlation was observed across relatively short phylogenetic distances (Fig. S2).

Network analysis was performed using SPIEC-EASI (SParse InversE Covariance Estimation for Ecological Association Inference; Kurtz et al., 2015) on commonly observed OTUs among all samples, excluding Oasis Valley samples, to ensure robust results. 'Common OTUs' were defined as those with low coefficient of variation across samples (≤ 3; community standard deviation divided by community mean) (McMurdie and Holmes, 2013) and observed at least two times in more than two samples (466 total OTUs). The glasso (sparse inverse covariance selection) and Meinshausen–Buhlmann (MB; neighborhood selection) method were evaluated with the Stability Approach to Regularization Selection (StARS) and bounded-StARS (bstars) selection criteria. Similar results were observed for both selection criteria. Since a wider degree distribution range was observed for glasso (Fig. S3), the glasso bstars approach was chosen for subsequent analysis in igraph (Csardi and Nepusz, 2006). OTUs with weak connection (edge weight < 0.02) were removed. Various clustering algorithms were performed to define clusters within the network, and the unsupervised Louvain clustering algorithm (Clauset et al., 2004; Blondel et al., 2008) was chosen based on the highest modularity score of 0.694 among all the algorithms. The 16 clusters were then manually inspected; clusters with only two connections were removed and nearby connected clusters with the same overall location associated with OTUs were combined. Cytoscape (https://cytoscape.org/) was used for visualization and network analysis.

2.6. Data availability

The 16S rRNA gene amplicon sequences were deposited at GenBank under the accession KEWZ00000000. The version described in this paper is the first version, KEWZ01000000. Scripts used to analyze and create figures of the geochemistry and microbial community are available at https://github.com/LLNL/2022_DVRFS_microbiome.

3. Results

3.1. Sample description, study limitations, and biases

A total of 42 samples were collected from 36 sites (Fig. 1, Tables S2–S3) that encompass the AM, AFFCR, and PMOV groundwater basins. Samples were collected opportunistically based on site availability, and most samples were collected from recharge and discharge areas within their respective groundwater basins. Due to our sampling limitations, the influence of seasonality, changes in recharge events, or hydraulic dynamics could not be determined on the temporal dynamics of subsurface microbial communities. This has been documented in previous studies of the Hainich Critical Zone Exploratory fractured aquifer system (Yan et al., 2021), a landfill-leachate contaminated aquifer (Abiriga et al., 2021), and a tar-oil contaminated aquifer (Pilloni et al., 2019). However, the very long residence times and deep flow paths of water in this system would argue against short-term effects other than from pumping (Halford and Jackson, 2020). There also are sample biases in this study, including the number of samples per location, sample collection method, and two sequencing batches. The specifics of these biases are described in Section 2.2 and were considered when analyzing the microbial community data.

3.2. Geochemistry

The major-ion chemistry of study sites were grouped into three broad categories: Ca-Mg-HCO_3, Na-HCO_3, and NaCl dominated waters (Fig. 2A), which were determined from a piper diagram (Fig. S4) and concur with previous reports (Thomas et al., 2013; Belcher et al., 2009; Belcher et al., 2019; Warix et al., 2020; Harrill and Bedinger, 2020). Principal component analysis (PCA) indicates that Ca-Mg-HCO_3-type waters cluster together, whereas the Na-HCO_3-type and NaCl-type waters group together. Notably, all 42 samples distinctly clustered into carbonate and volcanic rock types (Fig. 2A). Rock types were assigned based on the predominant rock type found at the depth sampled. Most samples also clustered by location, with the largest variation observed for samples collected from Yucca Flat, Rainier Mesa, and Oasis Valley (Fig. 2B). The water chemistry of Yucca Flat samples (YF1–YF3) is highly variable because of different hydrogeologic settings. YF1 (UE-2ce-WW-Nash), YF2 (U-3cn5-Bilby), and YF3 (UE-3E-4-Aleman) were sampled from wells completed in carbonate rock, carbonate and volcanic rocks, and volcanic rocks, respectively. Rainer Mesa samples (RM1 [U12n-10], RM2 [U12n-vent2]) likely do not cluster together because of the water geochemistry. RM1 has higher SO_4^{2-}, Na^+, and SiO_2^- concentrations (influenced by a local granite intrusion and pyrite oxidation leading to high sulfate) compared to RM2 (Fig. 2C, Table S2). Oasis Valley samples (OV1, OV2) likely do not cluster together because of higher SO_4^{2-} and total organic carbon (TOC) concentrations observed in OV1.

3.3. Description of groundwater bacterial and archaeal groundwater microbial community

The groundwater microbial community consisted of 5124 unique operational taxonomic units (OTUs) that were identified from 1,267,990 reads (Tables S4–S5). The final number of reads for OTUs per sample ranged from 2790 to 123,420 (average = 30,190) (Table S4). Because a higher number of reads was observed for samples sequenced in batch 2 (average = 90,352; batch size = 7) than batch 1 (average = 18,158; batch size = 35), subsequent analyses considered sequence batch effects. Among the OTUs identified, only 43 OTUs were present in more than ten sites. The most commonly observed taxa include those from genera *Pseudomonas*, *Curvibacter*, *Phenylobacterium*, *Thiobacillus*, and *Hydrogenophaga*. Although community structure varied from site to site, the overall community was composed mainly of Bacteria (49–100 %), and most sites were dominated by the phyla Proteobacteria (average = 42 %), Bacteroidota (15 %), Firmicutes (13 %), and Desulfobacterota (11 %) (Fig. S5). Within Proteobacteria, only Alphaproteobacteria and Gammaproteobacteria classes were observed. The archaeal relative abundances averaged 7 % for all sites, and the highest abundances (51 %) were observed in sample DV3 (Inyo-BLM 1).

Diversity within communities (alpha diversity) largely varied based on location and location type (Figs. 3 and S6). Although there were significant differences ($P < 0.05$) between the means of some categories (e.g., springs and wells) (Fig. S6), the standardized effect size (SES) of all alpha diversity metrics suggests that the differences could be affected by species richness (number of OTUs in each sample) (Fig. 3) (Pavoine et al., 2013; Swenson, 2014; Sandel, 2018). SES also can describe the phylogenetic relatedness within communities, and for most communities sampled, the SES-PD and SES-MNTD were negative and significantly different than the null model ($P < 0.05$), indicating that OTUs within each community were more closely related than expected by chance.

3.4. Microbial community variability by location

Differences in community composition between samples (beta diversity) were mostly explained by location. PERMANOVA by location- and geochemical-type variables revealed that location significantly (PERMANOVA $P = 0.003$; ANOSIM $P = 0.001$) explained the most variance of any factor (~27 %) for the NMDS weighted UniFrac (Table 1). NMDS ordination of unweighted (Fig. 4A) and weighted UniFrac (Fig. 4B) distances both identified that Oasis Valley microbial communities are distinct from other study-area microbial communities. The following clusters appear to group together for both ordinations, even when removing potentially biased samples (e.g., sequence batch): (1) Ash Meadows and Spring Mountains, and (2) Pahute Mesa and Frenchman and Yucca Flat. Rainier Mesa and Death Valley microbial communities differ between unweighted and weighted UniFrac NMDS ordinations, suggesting that both OTU relative abundances and phylogeny are important factors for the sites sampled. In addition to location, sampling depth relative to the water table ($P = 0.001$) significantly contributed to the variation but explained only ~6 %.

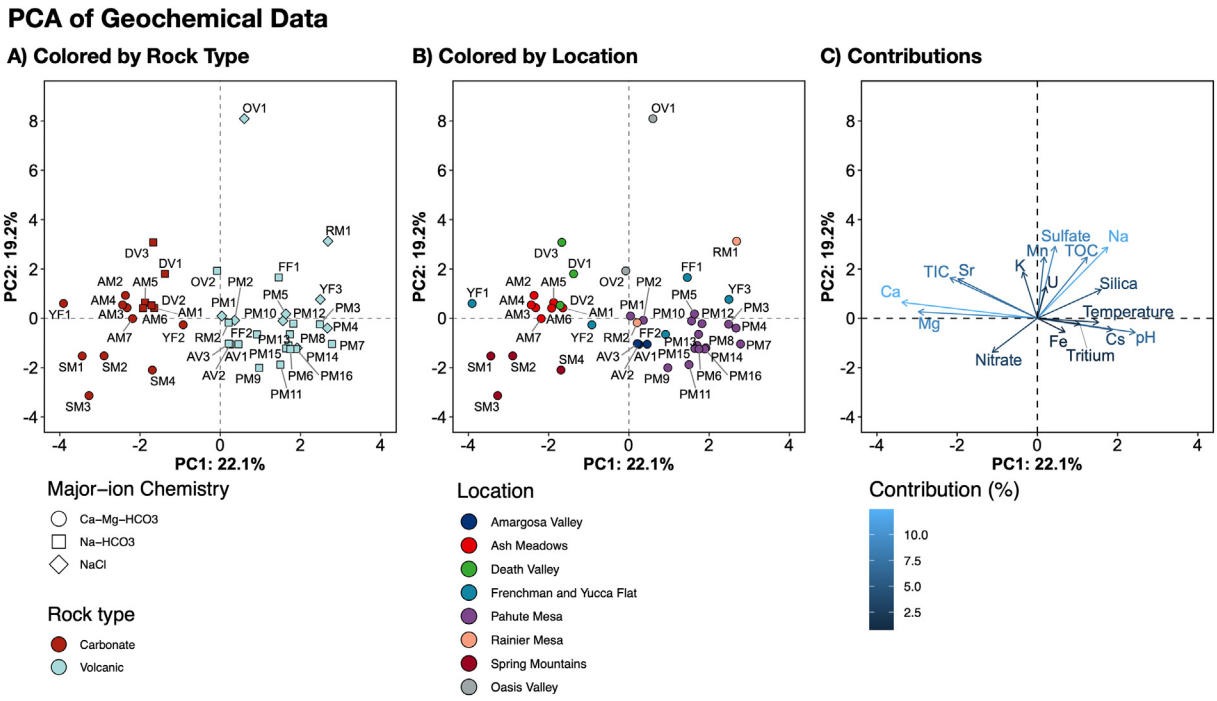


Fig. 2. Principal components analysis (PCA) of geochemical data. (A) Variation of samples by major-ion chemistry and rock type. (B) Variation of samples by location. (C) Contribution of each geochemical variable (n = 17) used in the PCA.

Geochemical-type variables minimally explained community variation (Table 1, Fig. 4C). For NMDC ordinations, rock type is the most significant contributor (~9 %; P = 0.002), followed by TOC (~6 %, P = 0.026) and temperature (~6 %, P = 0.002). Unlike location-type variables, the PERMANOVA of geochemical-type variables is significantly influenced by sequence batch runs (P = 0.039) and sampling-method approaches (P = 0.020). However, constrained ordination using CCA (Fig. 4C) confirmed that rock type, TOC, and temperature are significant ($P < 0.05$) factors determining the microbial community variation. Oasis Valley samples were removed before performing CCA because of their distinct geochemical and microbial compositions. Although CCA only explained ~8 % of the total community variation, the microbial communities could be clustered by rock type and location. Notably, similar to the geochemical ordination (Fig. 2), the two rock type clusters (carbonate and volcanic) followed along the vectors for calcium, nitrate, and sodium. Within location, CCA also revealed that the community variation may be explained by certain geochemical-type variables (e.g., Fig. 4C; Pahute Mesa communities appear to vary along a temperature gradient). Thus, the combined results of CCA and NMDS ordinations indicate that, on a regional-scale, although microbial

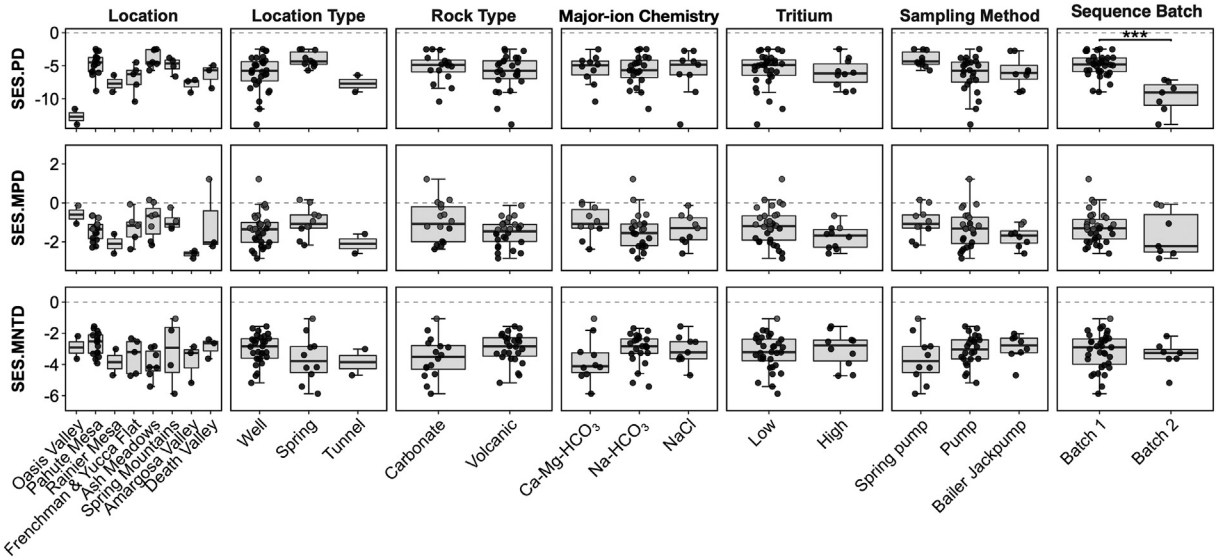


Fig. 3. Standardized effect size (SES) of Faith's PD (SES-PD), mean pairwise distance (SES-MPD), and mean nearest neighbor phylogenetic distance (SES-MNTD). Asterisks indicate p-value significance: *P < 0.05, **P < 0.01, ***P < 0.001. Black circles indicate samples with communities that are significantly (P < 0.05) different from the null distribution, whereas red circles are not significantly different from the null distribution.

Table 1
PERMANOVA of the weighted UniFrac beta diversity ordination using location and geochemical parameters.

Interest	[X][a]	Sum sqrs	R^2	Pseudo *F* statistic	P-value	Betadisper[b] *P*-value	ANOSIM[b] R-value	ANOSIM P-value
Location	**Location**	**0.488**	**0.266**	**2.104**	**0.003**	**0.168**	**0.418**	**0.001**
	Depth (m)[c]	**0.105**	**0.058**	**3.180**	**0.001**	**0.672**		
	Sampling method[d]	0.063	0.035	0.957	0.416	0.464	0.080	0.169
	Sequence batch[e]	0.039	0.021	1.177	0.244	0.662	0.190	0.091
	Residual	0.994	0.543					
	Total	1.831	1					
Geochemistry	**Rock type**[f]	**0.158**	**0.086**	**4.661**	**0.002**	**0.056**	**0.296**	**0.001**
	Temperature (°C)	**0.101**	**0.055**	**2.990**	**0.002**	**<0.001**		
	TOC (mg-C/L)	**0.108**	**0.059**	**3.190**	**0.026**	**0.290**		
	pH	0.046	0.025	1.363	0.148	0.477		
	Tritium[g]	0.040	0.022	1.178	0.244	0.115	−0.117	0.887
	Sampling method[d]	**0.122**	**0.067**	**1.806**	**0.020**	**0.464**	**0.080**	**0.169**
	Sequence batch[e]	**0.063**	**0.034**	**1.847**	**0.039**	**0.662**	**0.190**	**0.091**
	Residual	1.119	0.611					
	Total	1.831	1					

[a] PERMANOVA was conducted on the weighted UniFrac beta diversity distribution (Fig. 4B). [X] refers to the formula: adonis2([data] ~ [X], perm = 999, by = "margin"). [X] considers all listed variables for each 'Interest'. [data] is the distance matrix derived from the weighted UniFrac. For example, adonis2([data] ~ Location + Depth + Sampling Method + Sequence Batch, permutations = 999, by = "margin"). Bolded variables are significant ($P < 0.05$ for PERMANOVA and/or ANOSIM).
[b] Betadisper (homogeneity condition) and ANOSIM (analysis of similarity for categorical variables) R functions were used on individual variables.
[c] Sample collection depth from the water table.
[d] Samples were collected by pump, jack pumps (in-line submerged pump), or bailer. For location variables, 'sampling method' was used as a proxy for 'location type' (well, spring, or tunnel) due to overlaps in metadata for spring and groundwater sampling methods.
[e] The majority of samples were sequenced in Batch 1 and seven samples sequenced in Batch 2 (Table S2).
[f] Samples were categorized by rock type based on host rock (carbonate or volcanic) at the depth sampled.
[g] Tritium concentrations were split into 'High' (≥100 Bq/L) and 'Low' (<100 Bq/L) categories.

community variation can be explained more by location, geochemical conditions play an important role.

Other ordinations, including DEICODE RPCA (Martino et al., 2019), CLR PCA, and PhILR PCA (Silverman et al., 2017) (Fig. S7), that take into account the sparse, compositional nature of microbial community datasets (Gloor et al., 2017) were evaluated to compare against the NMDS and CCA ordinations. Consistent with the NMDS ordinations, Oasis Valley communities were identified as a separate group for all three ordinations (Fig. S7A, D, G). However, this result may be attributed to a noticeable separation of samples by sequence batch for CLR (Fig. S7E) and PhILR PCA (Fig. S7H). Sequence batch did not seem to impact DEICODE RPCA (Fig. S7B), likely because the DEICODE algorithm utilizes the geometric mean of log-transformed nonzero data and conducts matrix completion (Martino et al., 2019). After removing potentially biased samples (i.e., Batch 2 and tunnel-collected samples), both CLR and PhILR PCA ordinations also identified the two groups observed for NMDS ordinations: (1) Ash Meadows and Spring Mountains, and (2) Pahute Mesa and Frenchman and Yucca Flat (Fig. S7F and I). In contrast, these two groups were not observed for DEICODE RPCA (Fig. S7C) and may be the result of the likely highly-ranked (Martino et al., 2019) nature of the microbial community dataset, in which samples contain few similar microbes depending on location or other unknown factors not included in the metadata. Highly-ranked datasets also might be caused by unforeseen local-scale geochemical gradients that impact the microbial community and influence the resultant RPCA ordination (Martino et al., 2019). In contrast, the unweighted and weighted UniFrac NMDS ordination may accurately represent the microbial community and has been demonstrated to achieve high clustering accuracy (Martino et al., 2019; Weiss et al., 2017; Wright et al., 2021).

Network analysis of the 'common OTUs' identified nine clusters and revealed that co-occurring taxa generally subsisted within the same location (Fig. 5, Fig. S8 depicts the ratio of reads per location for each OTU). For network analysis, OV samples were removed because beta-diversity and geochemical analyses indicate that OV1 and OV2 were microbially and geochemically distinct from all other locations. Five clusters (NNSS 1–5) represent OTUs that co-occurred within or near the NNSS sites (Pahute Mesa, Rainier Mesa, and Frenchman and Yucca Flat). Each NNSS 1–5 cluster contains different 'common OTUs' (Table S7). Cluster AV (Amargosa Valley) was categorized separately from the NNSS clusters since the majority of OTUs co-occurred only in Amargosa Valley. In addition, there is a high degree (average = 22.8) of connectivity within cluster AV, which

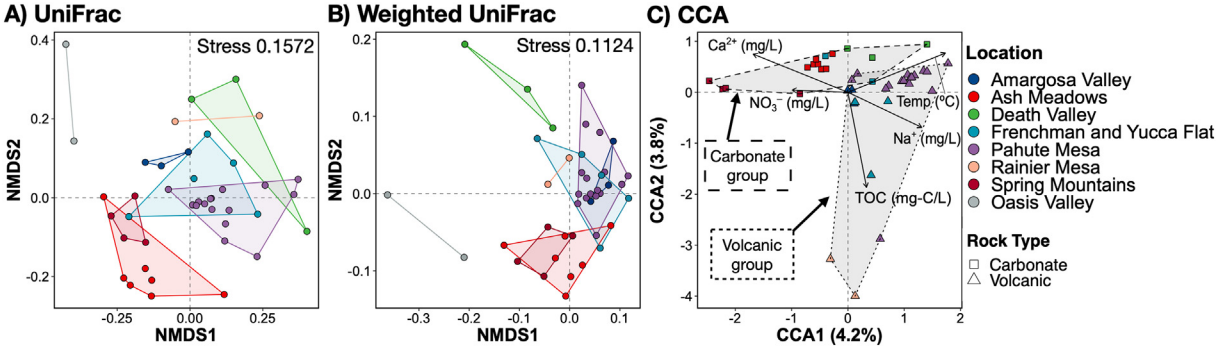


Fig. 4. NMDS (Non-metric multidimensional scaling) ordinations of the planktonic microbiome. Beta diversity was evaluated using NMDS ordination [(A) unweighted UniFrac and (B) Weighted UniFrac] and (C) CCA ordination [with OV samples removed; vectors show the significant ($P < 0.05$) geochemical variables contributing to the variance]. Samples are colored by location. Rock type also is specified for CCA ordination.

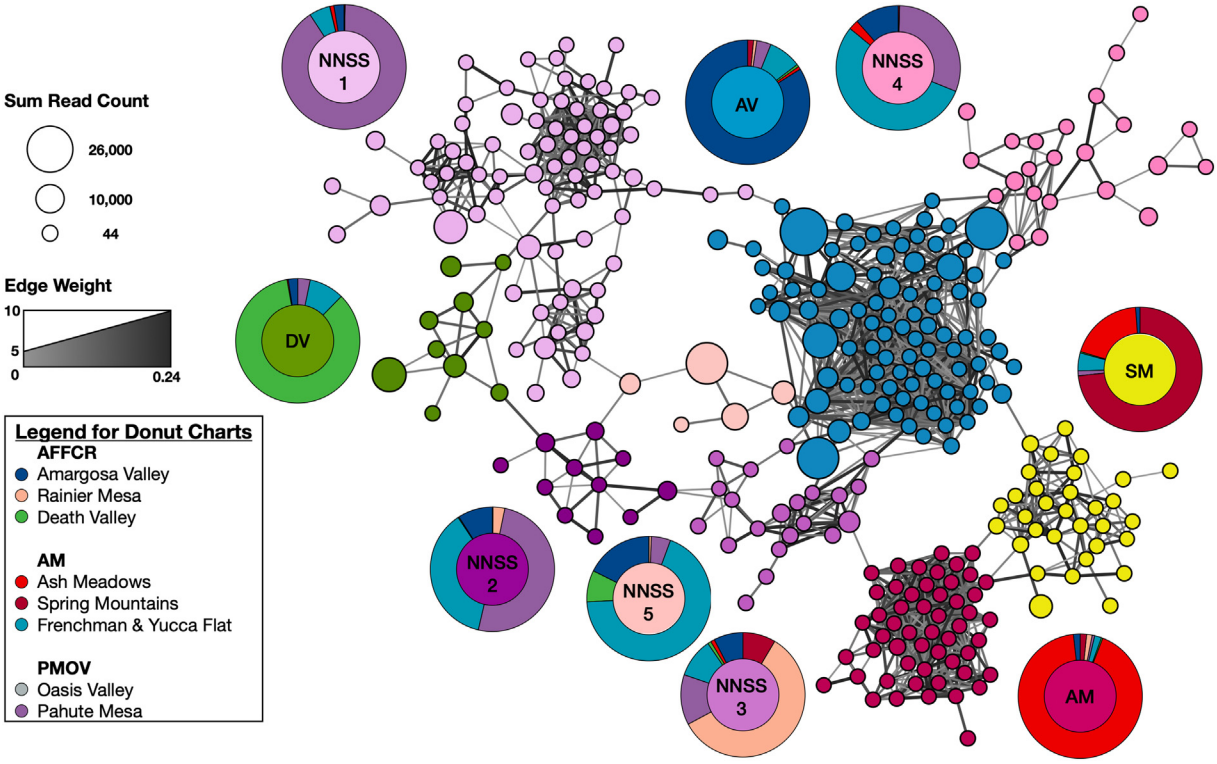


Fig. 5. Co-occurring 'common OTUs' cluster by location. Nodes are colored by cluster and represent the overall location associated with a set of OTUs. Nine clusters were identified: NNSS 1 = Nevada National Security Site 1 (light purple; representative of Pahute Mesa communities), NNSS 2 (dark purple; Frenchman & Yucca Flat and Pahute Mesa), NNSS 3 (purple; Rainier Mesa), NNSS 4 (pink; Frenchman & Yucca Flat and Pahute Mesa), AV = Amargosa Valley (blue), AM = Ash Meadows (red), SM & OV = Spring Mountains and Oasis Valley (yellow), DV = Death Valley (green), and OV = Oasis Valley (grey). The ratio of reads per location for each cluster is represented by a donut chart, and Fig. S8 depicts the ratio of reads per location for each OTU (node). The size of the node is based on the total number of reads for that OTU. Edge weights are shown as thickness and darkness of the connection between two nodes (e.g., thicker and darker edges indicate a stronger connection).

may be attributed to the collection of three samples pumped at the same site (4PD [AV1–3]) over time from 1 to 23 days. This suggests there is some degree of temporal consistency within this site, and potentially in other sites. In comparison, the degree of connectivity for the five NNSS clusters average between 3.2 and 7.7 (Table S6). Cluster AM also has a high average degree (19.3) of connectivity and three nodes connect cluster AM to the rest of the network via cluster SM.

3.5. Ecological processes that influence microbial community variation

Null models (see Table S1 for definitions) were used to identify the ecological processes that drove microbial community assembly and could influence community variation. This approach has been applied to communities in surface water (Wang et al., 2013), soil (Dini-Andreote et al., 2015; Wang et al., 2013; Liu et al., 2017), and the subsurface (Stegen et al., 2012, 2013, 2015; Fillinger et al., 2019; Danczak et al., 2020; Wang et al., 2013; Beaton et al., 2016; Danczak et al., 2018). There are two null modeling steps to identify deterministic and stochastic ecological processes: βNTI and RCBC, as described in Section 2.5. Overall, stochastic and undominated processes governed the microbial communities most (Fig. 6 inset), with deterministic processes only contributing ~25 % (except for Oasis Valley at ~50 %). Although certain locations had limited samples in our study, the assembly processes, taken as a whole, support the clusters identified by ordination analyses. The microbial communities of Spring Mountains, Ash Meadows, and Oasis Valley were governed largely by variable selection (βNTI > 2), dispersal limitation (|βNTI| < 2 and RCBC > 0.95), and undominated processes (|βNTI| < 2 and |RCBC| < 0.95) when compared against other communities (Fig. 6). For microbial communities within and near the NNSS, homogeneous dispersal (|βNTI| < 2 and RCBC < −0.95) and homogeneous selection (βNTI < −2) were more prominent. For example, Pahute Mesa communities were more similar (homogeneous selection) and had less turnover (homogenizing dispersal) than expected by chance. This signifies high dispersal rates and comparable geochemical conditions across Pahute Mesa that forced local communities to be more similar.

4. Discussion

In this study, we hypothesize that groundwater flows through hydraulically connected fractures and enables the dispersal of planktonic microorganisms, sometimes over considerable distances. Along a given flow path, geochemical conditions evolve with rock-water interactions or mixing of disparate fluids within interconnected pore spaces. These conditions can impart selective pressures on transported microorganisms and influence the local community assembly patterns. Fluid conduits also can become clogged over time and inhibit microbial dispersal, such as from tectonic influences (Uprety et al., 2017; Kim et al., 2020; Morimura et al., 2020), secondary mineral infilling, or the overgrowth of microbial biofilms at biogeochemical hotspots (Jackson et al., 2021; Lumban Gaol et al., 2021; Baveye et al., 1998). Separated communities may change with time into disparate compositions because of stochastic processes (e.g., genetic or ecological drift) (Zhou and Ning, 2017). Ecological processes can be quantified (Stegen et al., 2012, 2013, 2015), and in combination with microbial diversity and co-occurrence patterns, we assessed whether regional microbial community patterns are consistent with regional groundwater-flow paths (Fig. 1). Microbial community patterns are compared to the following regional groundwater-flow paths (Halford and Jackson, 2020): (1) Spring Mountains to Ash Meadows discharge area; (2) Frenchman & Yucca Flat to Amargosa Desert; (3) Rainier Mesa to Amargosa Desert; (4) Pahute Mesa to Oasis Valley; and (5) Amargosa Desert to Death Valley (Fig. 1).

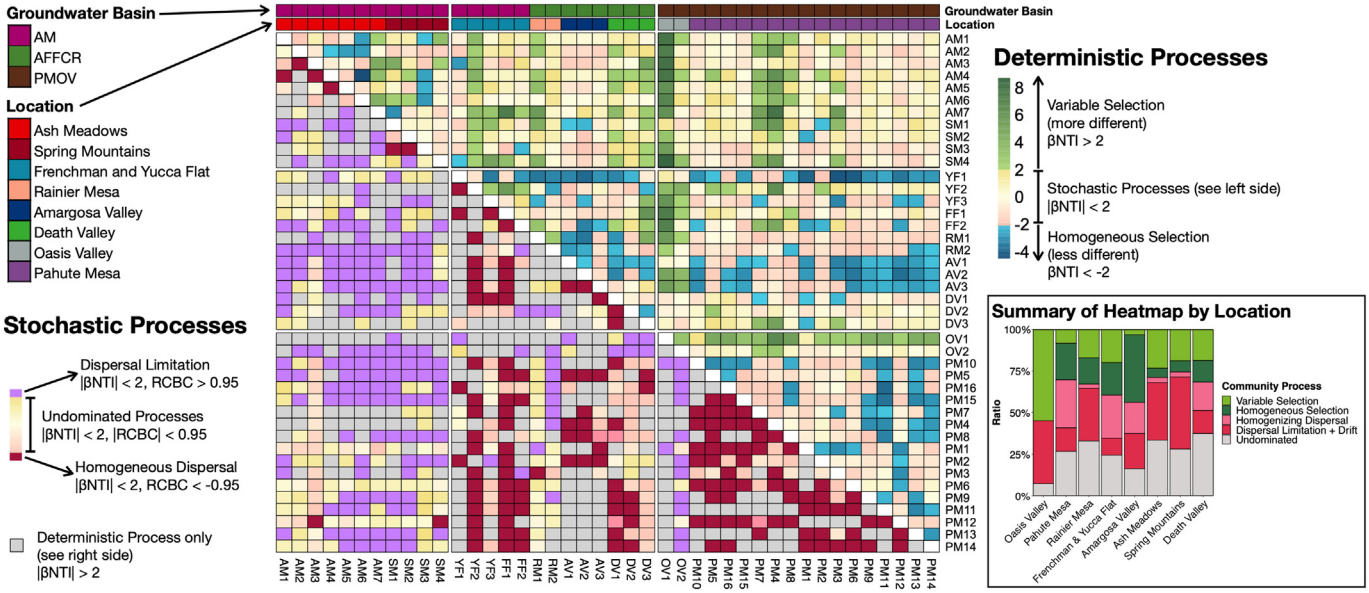


Fig. 6. Heatmap of ecological processes dominating microbial community composition between locations. Deterministic and stochastic community assembly processes were quantified using null models (Moreo and Justet, 2008; Winograd and Pearson, 1976; Winograd et al., 1998). Deterministic processes (right side of heatmap) include variable selection (green; βNTI > 2) and homogeneous selection (blue; βNTI < −2). When |βNTI| < 2, the phylogenetic relatedness between two communities did not differ significantly than expected by chance, and stochastic processes dominate (see left side of heatmap). Stochastic processes include homogenizing dispersal (red; |βNTI| < 2 and RCBC < −0.95), dispersal limitation and drift (purple; |βNTI| < 2 and RCBC > 0.95), and undominated (|RCBC| < 0.95) processes (Table S8; Table S1 for definitions). Ratios of the ecological processes were obtained for each overall location (inset).

4.1. Microbial community dispersal and evolution from Spring Mountains to Ash Meadows

The Spring Mountains (recharge area) to Ash Meadows (discharge area) groundwater-flow path (Fig. 1) likely transports planktonic microorganisms. In the AM Basin, the microbial community is similar between the recharge area (Spring Mountains) and Ash Meadows discharge area (Fig. 4). This result was corroborated with network analysis (Fig. 5), which identified 'common OTUs' co-occurring in the Spring Mountains and Ash Meadows discharge area (e.g., 'common OTUs' in cluster SM). The separation of 'common OTUs' into two clusters (AM and SM; clusters previously described in Section 3.4) suggests that other factors impacted the communities observed in each location. Indeed, null model analyses identified three major ecological forces that can explain differences between the Ash Meadows and Spring Mountains communities: selection processes, dispersal limitation, and undominating processes (Fig. 6). This is not surprising given the differences in elevation and geochemistry (Fig. 2) that arise from the evolution of groundwater along the flow path. Notably, these two communities are separated in the CCA ordination (Fig. 4C), likely due to differences in calcium and nitrate. In addition, the most-recent groundwater characterization study of the DVRFS (Halford and Jackson, 2020) identified other sources that contribute to Ash Meadows discharge, including the Sheep, Desert, Pintwater, and Spotted Ranges (Fig. 1).

4.2. Microbial community similarities within the NNSS and at Amargosa Valley

Microbial communities at sites within and near the NNSS were relatively similar (Amargosa Valley, Frenchman and Yucca Flat, Pahute Mesa, Rainier Mesa; Fig. 4), despite locations occurring within different groundwater basins (Fig. 1). Network analysis (Fig. 5) also identified co-occurring 'common OTUs' within these locations, and five disparate NNSS clusters of 'common OTUs' were identified (Cluster NNSS 1–5). The five NNSS clusters connected to the network via cluster AV (Amargosa Valley; within Amargosa Desert), cluster DV (Death Valley), or cluster AM (Ash Meadows).

Similarities between the microbial communities of NNSS sites with Amargosa Valley suggest the communities have adapted to similar environmental conditions or have dispersed via a groundwater flow connection towards Amargosa Valley. Many communities between these locations were dominated by homogenizing dispersal or homogeneous selection (Fig. 6), demonstrating the communities are more similar than expected by chance. Network analysis also grouped 'common OTUs' with putatively diverse metabolisms within cluster AV. The four most abundant OTUs in cluster AV were putative sulfur-oxidizing (e.g., *Thioalkalispiraceae*, *Hydrogenophilaceae*), methanotroph (e.g., *Methylomonas*), and iron-oxidizing (e.g., *Gallionellaceae*) microbes. Some OTUs are likely metabolically flexible, capable of mixotrophic or facultative anaerobic growth, such as those within family *Hydrogenophilaceae*. The diversity of potential metabolisms present in cluster AV is consistent with the geochemical mixing of multiple groundwater sources in the Amargosa Desert. Groundwater in the Amargosa Desert is derived largely from the AFFCR and AM groundwater basins (Halford and Jackson, 2020), in which three recharge areas converge in the Amargosa Desert: (1) Yucca Mountain and nearby upland areas in the AFFCR groundwater basin; (2) Yucca Flat in the AM groundwater basin; and (3) infiltration from the Amargosa River and Fortymile Wash in the AFFCR basin (Fig. 1). This mixed groundwater then flows towards the Furnace Creek discharge area in Death Valley.

4.3. The Death Valley microbial community likely supports the basin-fill flow conceptualization

There are two conceptualizations for groundwater flow towards Death Valley: (1) the deep-carbonate flow conceptualization; and (2) the basin-fill flow conceptualization (Fig. 7). Previous groundwater studies suggested that Death Valley discharge was sourced from a deep-carbonate flow path that passed beneath the Ash Meadows discharge area and Amargosa Desert (Belcher et al., 2019; Faunt et al., 2010; Bredehoeft and King, 2010) (Fig. 7). In contrast, the most recent groundwater study by Halford and Jackson (2020) determined that the basin-fill flow conceptualization best explains water levels, water chemistry, and aquifer-testing data. The basin-fill flow conceptualization interprets groundwater flow as moving

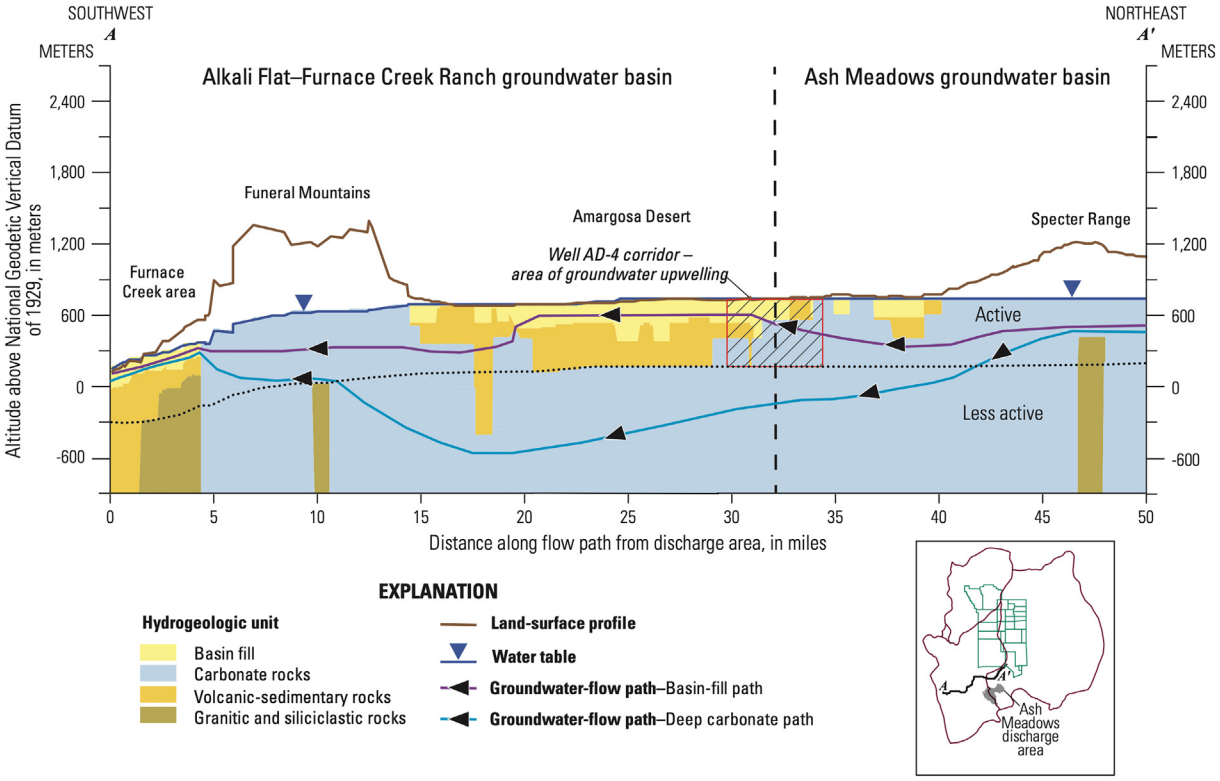


Fig. 7. Conceptualized groundwater-flow paths towards Death Valley (Furnace Creek area). Figure modified from Halford and Jackson (2020).

through shallow basin-fill, volcanic, and carbonate rocks towards Furnace Creek–the principal discharge area in Death Valley (Fig. 7).

The Death Valley microbial community better supports hydraulic connections proposed in the basin-fill conceptualization, rather than the deep-carbonate flow conceptualization. The Death Valley microbial community consists of samples collected from three carbonate wells: DV1 (Nevares Deep Well 2) and DV2 (Nevares) in the Furnace Creek discharge area; and DV3 (Inyo-BLM 1) in the central Amargosa Desert (Fig. 1). For the Death Valley microbial community to support the deep-carbonate flow conceptualization, microbial communities are expected to be related between Death Valley, Ash Meadows, and the Spring Mountains. Instead, NMDS ordination (Fig. 4A and B), network analysis (Fig. 5), and ecological null models (Fig. 6) suggest that Death Valley microbial communities are more related to Amargosa Valley and NNSS locations, rather than to Ash Meadows and Spring Mountains. Although there may be some similarity between Death Valley communities and Ash Meadows in the CCA ordination (Fig. 4C), this analysis only accounts for ~8 % of the total community variation. Moreover, homogeneous dispersal or selection contributed to community turnover between Death Valley, NNSS locations, and Amargosa Valley (Fig. 6; Table S8). This suggests that these communities were more similar than expected by random chance. In contrast, dispersal limitation and variable selection played a role in community turnover between Death Valley and Ash Meadows/Spring Mountains, which suggests a limited hydraulic connection between these areas. Network analysis indicates that cluster DV is closely associated with the NNSS clusters, particularly NNSS1 and NNSS2, and 'common OTUs' observed in Death Valley are also found in NNSS clusters and cluster AV (Fig. 5).

4.4. The Death Valley microbial community may support groundwater compartmentalization into shallow and deep zones

Halford and Jackson (2020) posit that the DVRFS is compartmentalized into two parts: (1) a shallow, high-transmissivity part within 500 m of the water table where nearly all flow occurs; and (2) a deep, less active, low-transmissivity part that has limited interaction with the shallow part (Halford and Jackson, 2020). The Death Valley microbial community appears to support a compartmentalized groundwater system.

Nevares wells (DV1/DV2) have ~100 m sampling depths and are conceptualized as occurring within the active, high-transmissivity part of the groundwater system. In contrast, Inyo-BLM 1 (DV3) was sampled at ~600 m depth, and is in low-transmissivity rock (~9–84 m^2 per day) (Halford and Jackson, 2020; Cutillo and Bredehoeft, 2011); thus, Inyo-BLM 1 (DV3) is conceptualized as occurring within the deep, less active, low-transmissivity part of the groundwater system. Inyo-BLM 1 (DV3) and Nevares Deep Well 2 (DV1) have similar geochemical conditions (Fig. 2, Table S2), including temperature and sulfate concentrations. Despite similar aqueous chemistries, Thomas et al. (2013) suggested hydraulic isolation between the microbial communities of Inyo-BLM 1 (DV3) and Nevares Well and Spring.

In this study, NMDS and CCA ordinations (Fig. 4) appears to concur with Thomas et al. (2013), with more similarity between Nevares wells (DV1/DV2) compared to Inyo-BLM1 (DV3). There also are large phylogenetic and compositional differences. Only eight OTUs were present in both Nevares Deep Well 2 (DV1) and Inyo-BLM 1 (DV3); and only two OTUs were present in both Nevares (DV2) and Inyo-BLM 1 (DV3). In contrast, 256 OTUs were present in both Nevares communities (DV1/DV2). The Inyo-BLM 1 (DV3) community consisted of a large population of putative methanogens (~50 %), followed by sulfate reducers (~31 %). In contrast, probable sulfate reducers composed most of the population at Nevares (DV1/DV2). Community differences may be impacted by unmeasured geochemical factors, such as higher pressures at depth in Inyo-BLM 1 (DV3) compared to the shallow Nevares wells (DV1/DV2). These deterministic differences were not apparent using ecological null models (Fig. 6), and more samples are required to confirm the null models between Inyo-BLM 1 (DV3) and Nevares wells (DV1/DV2).

4.5. Lack of similarity between Pahute Mesa and Oasis Valley

The microbial community of Oasis Valley is compositionally and phylogenetically distinct from all other study-area locations (Fig. 4). Oasis Valley

OTUs represented ~39 % of the total OTUs sampled in this study, of which ~85 % were observed only in Oasis Valley. The lack of microbial similarity between Pahute Mesa and Oasis Valley does not reflect regional groundwater-flow paths in the PMOV basin (Fig. 1; Bushman et al., 2010; Fenelon et al., 2020). However, the two Oasis Valley samples may not be representative of regional groundwater in this location as supported by the presence of many soil-associated and aerobic microbes. Moreover, the geochemistry of OV1 is distinct from other locations (Fig. 2), with relatively high TOC concentrations (37 mg-C/L). The combination of microbial (Fig. 4; taxonomic composition) and geochemical (Fig. 2) data suggest that the two Oasis Valley samples are likely more impacted by land-surface-associated factors, owing to their relatively shallow depths and siting within the alluvium of the Amargosa River floodplain. Hence, these sites are probably disconnected hydrologically from the regional aquifer and conceptually can be regarded as hyporheic. This observation demonstrates the utility in supplementing geologic and hydrologic datasets with microbial community data for a comprehensive evaluation of groundwater samples.

The Pahute Mesa microbial community consists of wells that were sampled within the Pahute Mesa recharge area (PM3; PM6; PM11; PM12) and wells immediately downgradient of the recharge area (PM1; PM2; PM4; PM5; PM7–PM10; PM13–PM16) (Fig. 1). Pahute Mesa communities are similar by NMDS and CCA ordinations (Fig. 4), and many 'common OTUs' co-occur within Pahute Mesa (cluster NNSS 1) (Fig. 5). Moreover, the two ecological processes dominating between Pahute Mesa communities include homogeneous dispersal and homogeneous selection (Fig. 6). Microbial dispersal may be influenced by the flow system at Pahute Mesa, which is dominated by high groundwater-flow velocities (Fenelon et al., 2016; Jackson et al., 2021). Groundwater flow in this area occurs through highly transmissive volcanic rocks, and tritium plumes from Pahute Mesa have only traveled <4 km beyond the NNSS borders since 2021, with an advective transport velocity of ~84 m/yr (Halford and Jackson, 2020). Taken together, these observations suggest that microbial dispersal in the PMOV basin is possible at least within the Pahute Mesa recharge location.

5. Future outlook

This study demonstrates that the regional-scale groundwater microbial community is a relevant data source; however, there were many limitations, as noted in Section 3.1, and future studies are needed. For example, the findings in this study suggest there could be a detection limit to identifying similar microbial communities between recharge and discharge areas, which may be correlated with groundwater-flow rates, transmissivity, or time. Areas with relatively fast-flowing groundwater and high transmissivity are more likely to have similar microbial communities (e.g., Ash Meadows and Spring Mountains). In contrast, areas with low transmissivity (e.g., deep-carbonate flow towards Death Valley) may impede large microbial migration from recharge to discharge areas, such that deterministic factors and genetic evolution outcompete dispersal. Future studies that quantify and correlate microbial community patterns with hydrogeologic factors and perturbations (e.g., groundwater-flow rate, transmissivity, time-scale) are needed. Temporal replicates will also help establish the microbial community variation within each site and may reveal potential seasonal-, pumping-, or recharge-associated perturbations. Moreover, groundwater microbial community samples collected along a groundwater-flow path and at various depths can identify potential candidates for use as a microbial 'tracer' by providing insight into the persistence of specific microbial species. This spatial data also will help to confirm that 'common OTUs' in recharge and discharge zones can be used for helping characterize groundwater flow. While our study approach was cost-effective (amplicon sequencing), it was limited to microbial abundance information and the bacterial/archaeal community. Future studies can combine other approaches, such as obtaining microbial functions/activity and metabolites, to provide additional insight into hydrobiogeochemical dynamics and fluxes.

6. Conclusion

The DVRFS microbial community patterns were mostly consistent with the regional-scale groundwater-flow conceptualization. Overall, microbial communities within recharge and discharge areas connected by a flow path were similar (e.g., Spring Mountains and Ash Meadows), and location was the most significant variable differentiating between the communities. Notably, communities within and near the NNSS were similar, although groundwater flow from Pahute Mesa towards other NNSS sites is limited. Network analysis also demonstrated that 'common OTUs' clustered together by location, and in particular, clusters of 'common OTUs' found within and near the NNSS were connected via a cluster composed of Amargosa Valley OTUs. These OTUs represented a range of putative metabolisms, indicative of the mixing of various groundwater sources at Amargosa Valley, which is consistent with the most recent groundwater conceptualization of the DVRFS. Ecological null model analyses also identified locations in which communities were relatively similar due to deterministic and stochastic processes, and largely corroborated the other microbial community analyses conducted in this study. However, the microbial community patterns contradicted the hydraulic connection between Pahute Mesa (recharge) and Oasis Valley (discharge) in the PMOV groundwater basin, probably reflecting a lack of direct hydrologic connectivity between the upgradient regional flow system and discharge zone wells that were available for sampling. Overall, this exploratory study demonstrates that regional-scale groundwater microbial community patterns can be used to supplement geologic and hydrologic data in characterizing groundwater flow.

Credit authorship contribution statement

NM analyzed the 16S rRNA gene amplicon sequences and compiled the geochemical data. DPM served as principal investigator for the projects under which the samples were collected. SHB, JCB, JS, and JCF contributed to the collection, processing, and extraction of the samples. All authors wrote the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Navarro-Interra for coordination with NNSS field sampling and on-site measurements. We thank Rachel Lindvall and Sarah Roberts (LLNL); Ron Hershey and John Healey (Desert Research Institute); Jeff Sanchez and Robert Zella (U.S. Geological Survey); and Robert Goodwin, Russ Shelton, Jeff Wurtz, and others from the UGTA Program for onsite logistical support at NNSS. Special thanks to Bill Wilborn, former UGTA subproject director, for his interest and support of our work. Thanks to Terry Fisk, Richard Friese, Josh Hoines, Genne Nelson, and Kevin Wilson (U.S. National Park Service); Alisa Lembke and the Inyo County Planning Commission; John Bredehoeft and Michael King (The Hydronamics Group, LLC); Darrell Lacy, Jamie Walker, John Klenke, Levi Kryder and others from the Nye County Nuclear Waste Repository Program Office; John Healey, Brad Lyles, Patty Montgomery, Molly Devlin, and Brittany Kruger (DRI); and the Nature Conservancy for site access and logistical assistance with the Death Valley, Amargosa Valley and Oasis Valley sites. Thanks to the DRI Water Lab, T.C. Onstott (Princeton University) and Barbara Sherwood Lollar (University of Toronto) for supplemental chemistry analysis. We also thank Yongqin Jiao (LLNL) for invaluable discussions; and Jeff Kimbrel and Juliet Johnston (LLNL), the Bioinformatics Virtual Coordination Network (BVCN), especially Haley Sapers and Liz Suter, and the LLNL Data Science Institute Consulting Service (DSICS), especially Jason Bernstein and Aram Avila-Herrera, for their advice in analyzing the microbial communities.

Funding sources

This research was partially funded by the Office of Biological and Environmental Research of the U.S. Department of Energy (DOE) as part of the Subsurface Biogeochemical Research Program under Work Proposal Number SCW1053, *Biogeochemistry of Actinides* and under DOE Contract Number SC0005306, *Radiochemically-Supported Microbial Communities*. Partial funding was also provided by the US DOE Environmental Remediation Sciences Program under Grant Number FG02-07ER64406, *Characterizations of Microbial Communities in Subsurface Nuclear Blast Cavities of the Nevada Test Site*; NSF GoLife DEB 1441646, *Collaborative research: Untangling the Deep Genealogy of Microbial Dark Matter*, and NSF EPSCOR OIA 1826734, *RII Track-2 FEC: Single Cell Genome-to-Phenome: Integrating Genome and Phenome Analyses of Individual Microbial Cells in Complex Microbiomes*; NASA Astrobiology Institute's *Life Underground* project (NNA13AA92A); NWRPO (10-023), *Characterization of Indigenous Microorganisms from Nye County Wells and Assessment of their Utility as Hydrologic Tracers*; The Hydrodynamic Group, *Characterization of Indigenous Microorganisms from Deep Wells and Assessment of their Utility as Hydrologic Tracers*. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 (IM#: LLNL-JRNL-823248). Thanks also to the Alfred P. Sloan Foundation's Deep Carbon Observatory (DCO) for sequencing and postdoc support (APS 2013-10-03) through their Census of Deep Life (CoDL) and Deep Life Community (DLC) programs. JS was partially supported by a Nevada NASA Space Grant Consortium Graduate Fellowship (NNX15AI02H).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156768.

References

Abiriga, D., Jenkins, A., Alfsnes, K., Vestgarden, L.S., Klempe, H., 2021. Spatiotemporal and seasonal dynamics in the microbial communities of a landfill-leachate contaminated aquifer. FEMS Microbiol. Ecol. 97, fiab086.

Amalfitano, S., et al., 2014. Groundwater geochemistry and microbial community structure in the aquifer transition from volcanic to alluvial areas. Water Res. 65, 384–394.

Anderson, K., Nelson, S., Mayo, A., Tingey, D., 2006. Interbasin flow revisited: the contribution of local recharge to high-discharge springs, Death ValleyCA. J. Hydrol. 323, 276–302.

Baveye, P., Vandevivere, P., Hoyle, B.L., DeLeo, P.C., de Lozada, D.S., 1998. Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials. Crit. Rev. Environ. Sci. Technol. 28, 123–191.

Beaton, E.D., et al., 2016. Local and regional diversity reveals dispersal limitation and drift as drivers for groundwater bacterial communities from a fractured granite formation. Front. Microbiol. 7.

Belcher, W.R., Bedinger, M.S., Back, J.T., Sweetkind, D.S., 2009. Interbasin flow in the Great Basin with special reference to the southern Funeral Mountains and the source of Furnace Creek springs, Death Valley, California, U.S. J. Hydrol. 369 (14).

Belcher, W.R., Sweetkind, D.S., Faunt, C.C., Pavelko, M.T., Hill, M.C., October 13, 2019. An update of the Death Valley regional groundwater flow system transient model, Nevada and California. U.S. Geological Survey, 2017.

Blondel, V.D., Guillaume, J.-L., Lambiotte, R., Lefebvre, E., 2008. Fast unfolding of communities in large networks. J. Stat. Mech. Theory Exp. 2008, P10008.

Bokulich, N.A., et al., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90.

Bokulich, N., Robeson, M., Kaehler, B., Dillon, M., 2020. bokulich-lab/RESCRIPt: 2020.6.1. July 31, 2020. https://doi.org/10.5281/zenodo.3945228.

Bolyen, E., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland Forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349.

Bredehoeft, J., King, M., 2010. Potential contaminant transport in the regional carbonate aquifer beneath Yucca Mountain, NevadaUSA. Hydrogeol. J. 18, 775–789.

Bushman, M., 2008. Contribution of Recharge Along Regional Flow Paths to Discharge at Ash Meadows, Nevada. Brigham Young University.

Bushman, M., Nelson, S.T., Tingey, D., Eggett, D., 2010. Regional groundwater flow in structurally-complex extended terranes: an evaluation of the sources of discharge at ash meadowsNevada. J. Hydrol. 386, 118–129.

Callahan, B.J., et al., 2016. DADA2: high-resolution sample inference from illumina amplicon data. Nat. Methods 13, 581–583.

Chapelle, F.H., 2000. The significance of microbial processes in hydrogeology and geochemistry. Hydrogeol. J. 8, 41–46.

Chase, J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., Inouye, B.D., 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2, art24.

Clauset, A., Newman, M.E.J., Moore, C., 2004. Finding community structure in very large networks. Phys. Rev. E 70, 066111.

Cook, P., 2022. Introduction to Isotopes and Environmental Tracers as Indicators of Groundwater Flow (GW Project, 2020) April 4.

Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. Int. J. Complex Syst. 10.

Cutillo, P.A., Bredehoeft, J.D., 2011. Estimating aquifer properties from the water level response to earth tides. Groundwater 49, 600–610.

Danczak, R.E., Johnston, M.D., Kenah, C., Slattery, M., Wilkins, M.J., 2018. Microbial community cohesion mediates community turnover in unperturbed aquifers. mSystems 3.

Danczak, R.E., et al., 2020. Ecological assembly processes are coordinated between bacterial and viral communities in fractured shale ecosystems. mSystems 5.

Devlin, J.F., 2022. Groundwater Velocity (Groundwater Project, 2020).

Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. 112, E1326–E1332.

Farnham, I.M., 2020. The Nature and State of Groundwater Contamination at the Nevada National Security Site: What Have We Learned from Decades of Groundwater Analysis? in WM2020.

Faunt, C.C., Blainey, J.B., Hill, M.C., D'Agnese, F.A., O'Brien, G.M., 2010. Death Valley Regional Groundwater Flow System, Nevada and California-Hydrogeologic Framework and Transient Groundwater Flow Model. https://doi.org/10.3133/pp1711.

Fenelon, J.M., Halford, K.J., Moreo, M.T., 2016. Delineation of the Pahute Mesa–Oasis Valley groundwater basin, Nevada. U.S. Geological Survey.

Fenelon, J.M., Sweetkind, D.S., Laczniak, R.J., February 13, 2020. Groundwater Flow Systems at the Nevada Test Site, Nevada: A Synthesis of Potentiometric Contours, Hydrostratigraphy, and Geologic Structures. 2010. U.S. Geological Survey.

Fillinger, L., Hug, K., Griebler, C., 2019. Selection imposed by local environmental conditions drives differences in microbial community composition across geographically distinct groundwater aquifers. FEMS Microbiol. Ecol. 95.

Fischer, Fabian, Merino, Nancy, Sugnaux, Marc, Huguenin, Gérald, Nealson, Kenneth H., 2022. Microbial community diversity changes during voltage reversal repair in a 12-unit microbial fuel cell. Chem. Eng. J. Volume 446 (4), 137334.

Flynn, T.M., et al., 2013. Functional microbial diversity explains groundwater chemistry in a pristine aquifer. BMC Microbiol. 13, 146.

Freeze, R.A., Witherspoon, P.A., 1967. Theoretical analysis of regional groundwater flow: 2. Effect of water-table configuration and subsurface permeability variation. Water Resour. Res. 3, 623–634.

Gerba, C.P., Pepper, I.L., Newby, D.T., 2015. Microbial transport in the subsurface. Environmental Microbiology, Third Elsevier, pp. 319–337.

Ginn, T.R., et al., 2002. Processes in microbial transport in the natural subsurface. Adv. Water Resour. 26.

Glassman, S.I., Martiny, J.B.H., 2018. Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. mSphere 3, e00148-18.

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome datasets are compositional: and this is not optional. Front. Microbiol. 8.

Griebler, C., Malard, F., Lefébure, T., 2014. Current developments in groundwater ecology—from biodiversity to ecosystem function and services. Curr. Opin. Biotechnol. 27, 159–167.

Halford, K.J., Jackson, T.R., 2020. Groundwater Characterization and Effects of Pumping in the Death Valley Regional Groundwater Flow System, Nevada and California, with Special Reference to Devils Hole U.S. Geological Survey.

Hardy, O.J., 2008. Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. J. Ecol. 96, 914–926.

Harrill, J.R., Bedinger, M.S., March 11, 2020. Death Valley Regional Groundwater Flow System, Nevada and California—Hydrogeologic Framework and Transient Groundwater Flow Model. 2010. U.S. Geological Survey.

Harvey, R.W., 1997. Microorganisms as tracers in groundwater injection and recovery experiments: a review. FEMS Microbiol. Rev. 20, 461–472.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). Methods Ecol. Evol. 7, 1451–1456.

Hug, L.A., et al., 2015. Aquifer environment selects for microbial species cohorts in sediment and groundwater. ISME J. 9, 1846–1856.

Jackson, T.R., Fenelon, J.M., Paylor, R.L., 2021. Groundwater Flow Conceptualization of the Pahute Mesa–Oasis Valley Groundwater Basin, Nevada—A Synthesis of Geologic, Hydrologic, Hydraulic-property, and Tritium Data. U.S. Geological Survey.

Joos, L., et al., 2020. Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. BMC Genomics 21, 733.

Kembel, S.W., et al., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464.

Kim, H., et al., 2020. Impact of earthquake on the communities of bacteria and archaea in groundwater ecosystems. J. Hydrol. 583, 124563.

Kurtz, Z.D., et al., 2015. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput. Biol. 11, e1004226.

Laczniak, R.J., et al., 2001. Ground-water Discharge Determined From Estimates of Evapotranspiration, Death Valley Regional Flow System, Nevada and California. https://doi.org/10.3133/wri014195.

Liu, C., et al., 2017. Long-term nitrogen addition affects the phylogenetic turnover of soil microbial community responding to moisture pulse. Sci. Rep. 7.

Louca, S., Mazel, F., Doebeli, M., Parfrey, L.W., 2019. A census-based estimate of Earth's bacterial and archaeal diversity. PLoS Biol. 17, e3000106.

Lumban Gaol, C., Ganzer, L., Mukherjee, S., Alkan, H., 2021. Investigation of clogging in porous media induced by microorganisms using a microfluidic application. Environ. Sci. Water Res. Technol. 7, 441–454.
Maamar, S.B., et al., 2015. Groundwater isolation governs chemistry and microbial community structure along hydrologic flowpaths. Front. Microbiol. 6.
Martino, C., et al., 2019. A novel sparse compositional technique reveals microbial perturbations. mSystems 4.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8, e61217.
Moossavi, S., Atakora, F., Fehr, K., Khafipour, E., 2020. Biological observations in microbiota analysis are robust to the choice of 16S rRNA gene sequencing processing algorithm: case study on human milk microbiota. BMC Microbiol. 20, 290.
Moreo, M.T., Justet, L., 2008. Update to the Ground-Water Withdrawals Database for the Death Valley Regional Ground-water Flow System, Nevada and California, 1913-2003. Geological Survey, U.S.
Moreo, M.T., Halford, K.J., Camera, R.J.La, Laczniak, R.J., 2003. Estimated Ground-water Withdrawals From the Death Valley Regional Flow System, Nevada and California, 1913-98. Geological Survey, U.S.
Morimura, S., Zeng, X., Noboru, N., Hosono, T., 2020. Changes to the microbial communities within groundwater in response to a large crustal earthquake in Kumamoto, southern Japan. J. Hydrol. 581, 124341.
Moser, D.P., et al., 2014. Radiochemically-supported Microbial Communities. A Potential Mechanism for Biocolloid Production of Importance to Actinide Transport. May 25, 2021. Desert Research Institute, Las Vegas, NV (United States). https://doi.org/10.2172/1213048.
Nearing, J.T., Douglas, G.M., Comeau, A.M., Langille, M.G.I., 2018. Denoising the denoisers: an independent evaluation of microbiome sequence error-correction approaches. PeerJ 6, e5364.
Nelson, S.T., Mayo, A.L., 2014. The role of interbasin groundwater transfers in geologically complex terranes, demonstrated by the Great Basin in the western United States. Hydrogeol. J. 22, 807–828.
Neuman, S.P., 2005. Trends, prospects and challenges in quantifying flow and transport through fractured rocks. Hydrogeol. J. 13, 124–147.
Pavoine, S., Gasc, A., Bonsall, M.B., Mason, N.W.H., 2013. Correlations between phylogenetic and functional diversity: mathematical artefacts or true ecological and evolutionary processes? J. Veg. Sci. 24, 781–793.
Pilloni, G., et al., 2019. Dynamics of hydrology and anaerobic hydrocarbon degrader communities in a tar-oil contaminated aquifer. Microorganisms 7, 46.
Prodan, A., et al., 2020. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. PLOS ONE 15, e0227434.
Quast, C., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.
Raup, D.M., Crick, R.E., 1979. Measurement of faunal similarity in paleontology. J. Paleontol. 53, 1213–1227.
Sandel, B., 2018. Richness-dependence of phylogenetic diversity indices. Ecography 41, 837–844.
Silverman, J.D., Washburne, A.D., Mukherjee, S., David, L.A., 2017. A phylogenetic transform enhances analysis of compositional microbiota data. eLife 6, e21887.
Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. 6, 1653–1664.
Stegen, J.C., et al., 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. 7, 2069–2079.
Stegen, J.C., Lin, X., Fredrickson, J.K., Konopka, A.E., 2015. Estimating and mapping ecological processes influencing microbial community assembly. Front. Microbiol. 6.
Swenson, N.G., 2014. Functional and Phylogenetic Ecology in R. 1st ed. Springer.
Thomas, J.M., et al., 2013. Using water chemistry, isotopes and microbiology to evaluate groundwater sources, flow paths and geochemical reactions in the Death Valley flow system, USA. Procedia Earth Planet. Sci. 7, 842–845.
Thomas, J.M., et al., 2020. Geochemical and Isotopic Interpretations of Groundwater Flow in the Oasis Valley Flow System, Southern Nevada. 2003. U.S. Department of Energy. https://doi.org/10.2172/806667 March 26.
Tóth, J., 1963. A theoretical analysis of groundwater flow in small drainage basins. J. Geophys. Res. 1896–1977 (68), 4795–4812.
Tóth, J., 1999. Groundwater as a geologic agent: an overview of the causes, processes, and manifestations. Hydrogeol. J. 7, 1–14.
U.S. DOE, 2015. United States Nuclear Tests: July 1945 through September 1992. U.S. Department of Energy.
Uprety, S., et al., 2017. The effect of the 2015 earthquake on the bacterial community compositions in water in Nepal. Front. Microbiol. 8.
Walvoord, M.A., et al., 1999. Groundwater flow and geochemistry in the southeastern San Juan Basin: implications for microbial transport and activity. Water Resour. Res. 35, 1409–1424.
Wang, J., et al., 2013. Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. ISME J. 7, 1310–1321.
Warix, S.R., Rademacher, L.K., Meyers, Z.P., Frisbee, M.D., 2020. Groundwater geochemistry and flow in the Spring Mountains, NV: implications for the Death Valley regional flow system. J. Hydrol. 580, 124313.
Weiss, S., et al., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5.
Winograd, I.J., Pearson, F.J., 1976. Major carbon 14 anomaly in a regional carbonate aquifer: possible evidence for megascale channeling, south central Great Basin. Water Resour. Res. 12, 1125–1143.
Winograd, I.J., Riggs, A.C., Coplen, T.B., 1998. The relative contributions of summer and cool-season precipitation to groundwater recharge, Spring Mountains, NevadaUSA. Hydrogeol. J. 6, 77–93.
Woessner, W.W., 2022. Groundwater-surface Water Exchange (GW Project, 2020) April 4.
Wright, R.J., Langille, M.G.I., Walker, T.R., 2021. Food or just a free ride? A meta-analysis reveals the global diversity of the plastisphere. ISME J. 15, 789–806.
Yan, L., et al., 2021. Groundwater bacterial communities evolve over time in response to recharge. Water Res. 201, 117290.
Ye, M., Wang, L., Pohlmann, K.F., Chapman, J.B., 2016. Evaluating groundwater interbasin flow using multiple models and multiple types of data. Groundwater 54, 805–817.
Yilmaz, P., et al., 2014. The SILVA and "All-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648.
Zhang, Y., et al., 2020. Microbial community composition in deep-subsurface reservoir fluids reveals natural interwell connectivity. Water Resour. Res. 56, e2019WR025916.
Zhou, J., Ning, D., 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81.