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The utility of environmental DNA from sediment and water samples for recovery of observed plant and animal species from four Mojave Desert springs

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Abstract

Mojave Desert springs are fragile ecosystems, hosting endemic plants and animals, which are threatened by the increasing human demand for water and climate change. To develop management practices that will protect the groundwater-dependent ecosystems at Mojave Desert springs, real-time, low-cost biodiversity monitoring, and assessments are required. Environmental DNA (eDNA) metabarcoding uses DNA shed from organisms (e.g., skin cells, feces, and pollen) that are present in water, air, soil, or sediment samples to assess community composition. This approach can increase the detection sensitivity for rare and elusive species, compared with expensive and time-consuming conventional methods, which also require taxonomic expertise. This study tests the effectiveness of eDNA techniques in capturing the observed Mojave Desert spring biodiversity in the winter and spring of 2019 at four distinct, naturally occurring springs. We also test the utility of sample types (water vs. sediment) for capturing biodiversity. We found that each of the four Mojave Desert springs supports a unique biological community. Sediment samples contained the greatest biodiversity, but all sample types captured species observed in the field by humans or camera traps. We also found no statistical difference in species richness captured in winter and spring except for the Cytochrome Oxidase I marker, for which winter had greater biodiversity. This study supports the use of eDNA metabarcoding as an effective tool to mirror observation by human observers of ecological communities in desert springs. The study demonstrates the importance of appropriately timing eDNA field sampling, primer selection, and using field-based surveys of wildlife and plants in addition to eDNA detection. This study also identified gaps in reference sequence databases for Mojave biodiversity and encourages collaboration of eDNA researchers with managers for effective conservation management plans.

KEYWORDS
biological monitoring, conservation of water resources, ecological communities, endangered species, environmental DNA, invasive species, metabarcoding, natural springs

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1 | INTRODUCTION

Desert springs are recognized as globally important biodiversity hotspots because of their productivity and diversity (Bogan et al., 2014) and because they serve as evolutionary and ecological refugia (Davis et al., 2013). Desert springs also support endemic, rare, or relictual species of plants, fish, and invertebrates (Davis et al., 2017). Despite the recognized environmental importance of spring ecosystems, their ecosystem function is threatened by water loss caused by groundwater extraction for human consumption, agriculture, and a variety of industrial and recreational uses (Davis et al., 2017). Species found at springs can be extirpated by livestock use (Davis et al., 2017; Unmack & Minckley, 2008) and the introduction of invasive non-native species and their parasites (Unmack & Minckley, 2008). These problems, in combination with climate change, are degrading the ecological function and biodiversity of springs (Vörösmarty et al., 2010; Woodward et al., 2010).

Mojave Desert springs are ecologically important and under threat. They support a unique biological community composed of endemic plants and animals, including crenophilic organisms (Pavlik, 2008; Ricketts & Imhoff, 2003; Sada & Lutz, 2016). A high proportion of biodiversity in the Mojave Desert is dependent and concentrated around desert springs due to the scarcity of surface water across the landscape (Fraga, 2017; Randall et al., 2010). Major threats to Mojave Desert springs include negative impacts to spring habitats by changes in land use, such as the development of residential, commercial, agricultural, or industrial facilities; changes to ecosystem function by introduced non-native species such as burros and fish (e.g., mosquito fish, goldfish); and the extraction of groundwater from aquifers that support spring flow. Mojave Desert springs are included in the 2016 Land Use Plan Amendment (LUPA) of the Desert Renewable Energy Conservation Plan (DRECP). As of October 2020, the LUPA stood as the most current, legally binding document intended to govern the management of public lands by the Bureau of Land Management (BLM) in the California deserts (CEC, 2016). Mojave Desert groundwater extraction as a water resource for human use in a drought-stricken California and the competing interests of preserving ecological communities is an ongoing issue (Love & Zdon, 2018). In a clear and recent example, the Cadiz project has proposed to extract groundwater from the desert for human use in coastal Orange County and the project brings into question how groundwater extraction would impact biodiversity at springs located closest to the point of extraction (Sizek, 2018).

The management of biodiversity has traditionally relied on field-based visual surveys by taxonomic experts. These efforts are time-consuming, expensive, and require numerous individuals with specific taxonomic expertise for species identification and count estimates that can be difficult to obtain (Bogich et al., 2008). Field surveys conducted at a single point in time are unlikely to provide a complete understanding of the use of springs by plants and animals (imperfect detection, MacKenzie, 2005), as many species that are dependent on these water resources visit springs infrequently (Rosenstock et al., 2004). For example, migratory birds use them as a stopover during a period of long-distance migration (Airola et al., 2019; Baldassarre et al., 2019; Lavee & Safriel, 1989) and annual or ephemeral plants are only present seasonally when there is soil moisture (Bilbrough & Caldwell, 1997). There are also gaps in basic spring knowledge such as spring classification (e.g., identifying spring type, rheocrene, and limnocrenic, Springer et al., 2008; Stevens et al., 2020) and effective low-cost monitoring methods that encompass all taxonomic groups of biodiversity. For Mojave Desert springs, general information and data are sparse and the hydrology and biology of many springs are understudied and poorly understood (Love & Zdon, 2018). Therefore, introducing advancements in molecular techniques to monitor desert springs could benefit the development of conservation plans.

Environmental DNA (eDNA) metabarcoding utilizes trace extracellular DNA from saliva, feces, skin cells, pollen, gametes, or living organisms present in the environment to assess biodiversity (Taberlet et al., 2012). The method of eDNA metabarcoding allows for sequencing and assignment of a diverse community of species at any given location based on an environmental sample (Deiner et al., 2017; Ruppert et al., 2019). The application of eDNA has proved to be rapid and efficient for surveying biodiversity in ecosystems (Thomsen & Willerslev, 2015). Although studies have focused on using eDNA in springs (Amin et al., 2018; D'Auria et al., 2018; Vörös et al., 2017), including desert springs (Paulson & Martin, 2019), few freshwater studies have focused on the detection of organisms across diverse taxonomic groups (Belle et al., 2019). Similarly, eDNA sediment and water samples have been compared in only a few studies but have consistently found sedimentary eDNA to have a lower decay rate and higher concentration of eDNA (Buxton et al., 2018; Turner et al., 2015). The increased degradation rate of eDNA in water samples, as compared to sediment, may further limit its ability to detect species that occur infrequently (Buxton et al., 2018; Ostberg et al., 2018). The use of both sample types provides robust information of species distribution (Sakata et al., 2020), potentially detecting different species due to differences in degradation.

Environmental DNA metabarcoding holds promise to address information gaps about community composition and can be useful in the biomonitoring of inaccessible habitats. Mojave Desert Springs are difficult to access and survey due to their remote locations, difficult terrain and extreme environmental conditions (Sada & Lutz, 2016). Therefore, we initiated a small-scale study to explore the use of environmental DNA metabarcoding as a low-cost, non-invasive monitoring tool that may provide similar information about community composition relative to more expensive and time intensive observational studies. We sampled four different types of Mojave Desert springs in winter and spring 2019 to capture a snapshot of biodiversity and compare diversity patterns recovered by sediment and water samples. Finally, we compare our eDNA findings with direct observations (botanical and camera trap surveys) and make recommendations on the use of eDNA for monitoring and habitat management.

2 | MATERIALS & METHODS

2.1 | Study sites

We sampled four spring sites located within the Mojave Desert in California (Figure 1). Ahn spring (Figure 1a) is small (33 sq. cm at its largest) and is located in the Portal Ridge Preserve, 18 miles west of Lancaster (Hanford, 2015). Big Morongo Canyon springs (Figure 1b; https://www.bigmorongo.org/) is a 31,000-acre desert oasis composed of streams and wetlands that is half a mile southeast of the town of Morongo Valley, in Big Morongo Canyon, and is located in San Bernardino and Riverside counties (Richert, 2002). Bonanza spring (Figure 1c) is the largest spring in the Clipper Mountains within the newly established Mojave Trails National Monument of San Bernardino County and supports a substantial riparian area (Zdon et al., 2018). Hummingbird spring (Figure 1d) is a local, perennial perched spring in the Marble Mountains of San Bernardino County (Zdon et al., 2018) and has an aluminum wildlife guzzler that contains the spring's outflow.
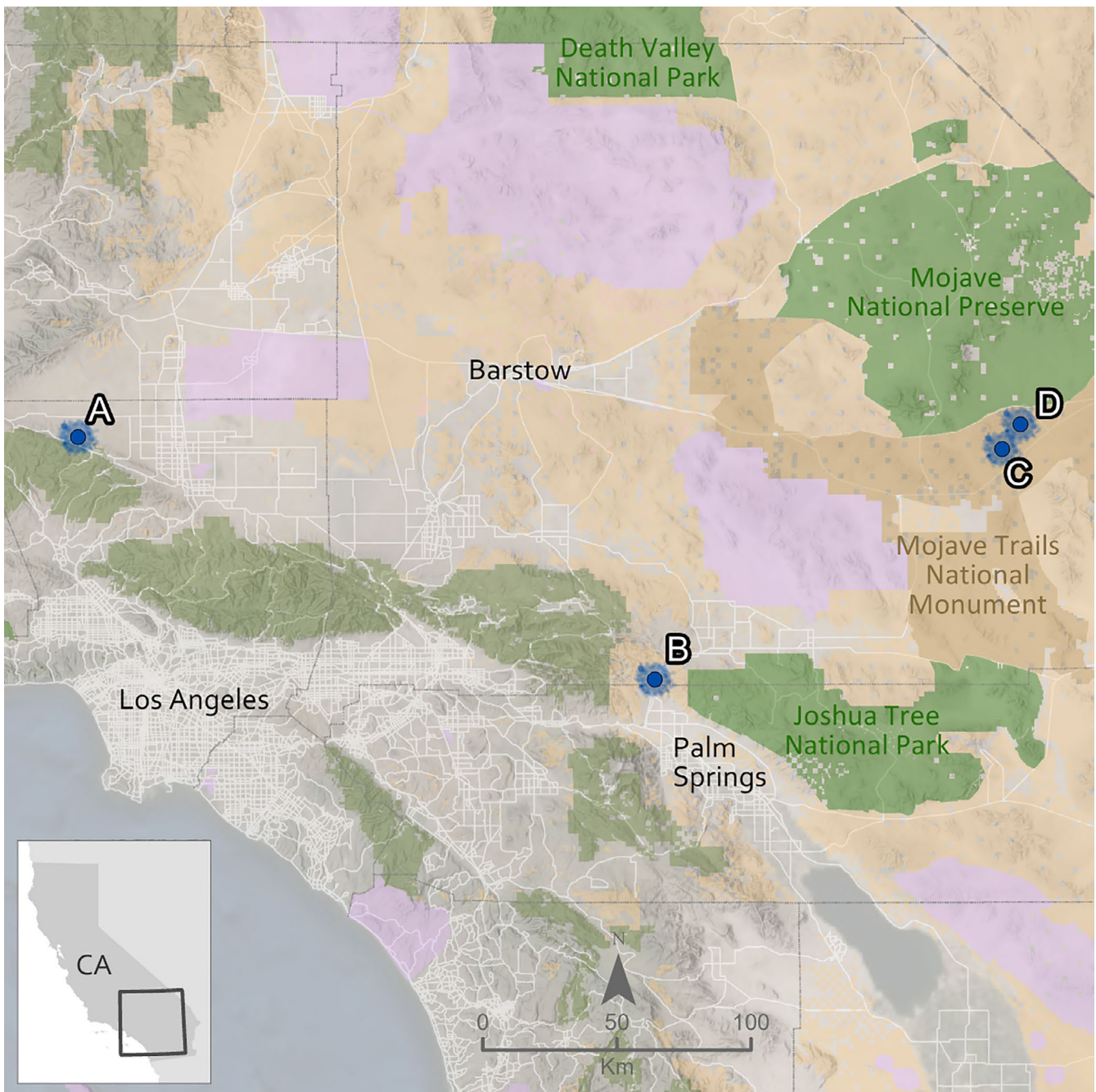

FIGURE 1 Mojave Desert springs sampling locations. (a) Ahn Spring, (b) Big Morongo Canyon Springs, (c) Bonanza Spring, and (d) Hummingbird Spring. Federal land management is shown in green—US National Park Service, light green—US Forest Service, brown—US National Monument, beige—US Bureau of Land Management, and pink—US Department of Defense. County boundaries are shown in gray, and roads are shown in white

2.2 | Collection of field survey data for plants and animals

Botanical surveys were conducted at each of the four springs either in October 2018 or October 2019. We also included historical herbarium specimen records to provide a more complete list of plants at each site. Commercial camera traps were deployed from 2017–2019 at Ahn spring, by the reserve manager Vern Biehl, and collected images were processed to generate a list of observed animal species. The system was usually "windowed" (switched off) between the hours of 9 a.m. to 4 p.m. to reduce saturating the memory cards and exhausting batteries. Photographs that were out of focus, poorly exposed, or contained images too small to identify were deleted. We also obtained photos from a camera trap set for 24 hr between January and February 2020 to capture organisms that were visiting the spring during the day and not captured with the other camera trap dataset. Pictures were taken 15 s after the camera initially detected movement. Big Morongo Canyon Preserve has an inventory of birds, plants, and animals (https://www.bigmorongo.org/ecosystem/), and we also consulted with the reserve manager (Meg Foley) for confirmation of species detected with eDNA. For Bonanza and Hummingbird springs, we provided a list of species detected using eDNA for confirmation of presence by the Bureau of Land Management biologist and rangeland specialist. We also utilized iNaturalist observations to confirm the presence of species within the Mojave Desert between January and February 2020.

2.3 | Sampling and DNA extraction

Sampling for water and sediment was conducted between January and March 2019 for winter and in May 2019 for spring. The number of samples chosen per spring varied based on spring size and was intended to capture additional taxa from microhabitat variation such as a dry creek bed, a pond, and flowing creeks. We sampled three sites at Ahn spring, nine sites at Big Morongo Canyon springs, nine sites at Bonanza spring, and three sites at Hummingbird spring (Table S1).

The water samples collected in the winter and spring differed in the number of biological replicates collected per site, with one sample replicate taken per site in winter and three sample replicates taken per site in spring. Proper measures were implemented in the field and in the laboratory due to the sensitivity of eDNA to cross-contamination and contamination by exogenous DNA (e.g., materials used one time and disposed, gloves changed between sample collection, Deiner et al., 2015; Goldberg et al., 2016). Water samples consisted of one liter of water using 40 ml Falcon Tubes for transfer into Enteral Feeding bags (Covidien) and filtered within six hours through 0.22 μm Sterivex millipore filters (Millipore) using a Masterflex® L/S portable Peristaltic pump (Cole-Parmer). A 3 ml syringe was used to remove any remaining water in the sample, and samples were transported on ice to UCLA and placed in a −20°C freezer. DNA was extracted from each sample within 1 week of field sampling using a DNeasy Tissue and Blood Qiagen Kit (Qiagen) with a modified protocol (Spens et al., 2017). Proteinase K and ATL buffer were directly added to the filter and incubated overnight. To remove inhibitors, samples were processed with the Zymo One Step Inhibitor Removal Kit (Zymo Research). For samples with replicates, DNA was pooled after extraction and processed alongside samples with single replicates. We included four extraction blanks in this study.

One paired sediment sample was collected for most sites with three biological replicates in 2.0 ml cryotubes (Table S1). In locations without water, soil samples were taken. Samples were kept on ice, transported to UCLA and placed in a −80°C freezer. Samples were then thawed on ice and the biological replicates pooled. DNA was extracted using the Qiagen DNeasy PowerSoil Kit (Qiagen) following the manufacturer's protocol, which included in four extraction blanks. Sample volumes extracted are consistent with those used by the Earth Microbiome Project and previously published protocols (Marotz et al., 2018; Turner et al., 2015).

Water and sediment samples for each site were treated separately for the library preparation and although pooling replicates for each sample type reduces the detection of rare taxa, it does not substantially affect comparisons of communities (Sato et al., 2017; Zhang et al., 2020). Critically, pooled replicate samples can provide information about the presence of species that were observed by traditional methods, a major goal of our study. In addition, we reduced the effect of rare taxa in our diversity analysis by considering only Family Level classification and including only Families present in 30% of our samples (see below for details).

2.4 | Illumina library prep and sequencing

Two rounds of PCR were utilized to amplify targeted metabarcodes from eDNA extracts, and 2 libraries were prepared for Illumina sequencing (Olds et al., 2016). Each DNA extract was amplified with three previously published primer sets (CO1, Geller et al., 2013, Leray et al., 2013; PITS, Gu et al., 2013; Ve16S, Evans et al., 2016; Table S2). Primers for each of the three assays were ordered from Integrated DNA Technologies (Iowa) as they were originally published, with the exception of the Ve16S-F primer, which was synthesized without the last base (A) from its original published sequence to reduce previously identified mismatches based on alignments to fish species (unpublished). Additionally, Illumina Nextera Transposase Adapters were included on the 5′ end of the synthesized primer pairs: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG (forward) or GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (reverse). The 20 μl PCR mixes were as follows: 4 μl of 5X GoTaq Flexi Buffer (Promega), 0.4 μl of 10 mM dNTPs, 1.6 μl of 25 mM MgCl$_2$, 1 μl of 10 μM forward primer, 1 μl of 10 μM reverse primer, 8 μg of Bovine Serum Albumin (BSA, 20 mg/ml, VWR, Pennsylvania), 0.15 μl of GoTaq G2 Flexi DNA Polymerase (Promega), 4 μl of DNA extract, and 5.85 μl of sterile water. Cycling protocols for each assay began with an initial 3-min denaturing step at 95°C and ended with a single extension step at 72°C for 10 min. The three-step cycling involved

denaturation at 95°C for 30 s, annealing for 45 s (temperatures and cycle numbers given in Table S2), and elongation at 72°C for 60 s. PCR products of Plant_ITS2 (PITS), mlCOIintF/jgHCO2198 (CO1), and Ve16S were pooled in one mix at equal amounts and cleaned with Mag-Bind® TotalPure NGS (Omega Bio-Tek Inc) magnetic beads at a ratio of 1(beads):1(DNA) and following the manufacturer's recommendations. The DNA concentration of a subset of pooled amplicons was quantified with the Qubit dsDNA HS Assay (Life Technologies).

The second round of PCR added sample-specific dual-indexes and used the PCR products from the first round of PCR as the template. Each pooled PCR amplicon mix was used as a template for a different sample; consequently, one library was generated for each eDNA sample. The 30 μl second round PCR mix was as follows: 6 μl of 5X GoTaq Flexi Buffer (Promega), 0.6 μl of 10 mM dNTPs, 2.4 μl of 25 mM MgCl$_2$, 1.5 μl of 10 μM forward primer, 1.5 μl of 10 μM reverse primer, 0.15 μl of GoTaq G2 Flexi DNA Polymerase (Promega), 3 μl of the pooled PCR amplicon mix, and 14.85 μl of sterile water. The forward and reverse primers included the remaining Illumina adaptor sequence, AATGATACGGCGACCACCGAGATCT ACAC[i5]TCGTCGGCAGCGTC (forward) and CAAGCAGAAGACG GCATACGAGAT[i7]GTCTCGTGGGCTCGG (reverse), and Nextera DNA indexes (i5 and i7) for dual-indexing (Illumina, 2020). PCR products were cleaned with Mag-Bind® TotalPure NGS (Omega Bio-Tek Inc) magnetic beads at a ratio of 0.8(beads):1(DNA) and following the manufacturer's recommendations to remove fragments below 300 base pairs (bp). The DNA concentration of each library was quantified with the Qubit dsDNA HS Assay (Life Technologies) so the library could be pooled to equal molar concentrations for Illumina MiSeq sequencing used a MiSeq Reagent Kit v3 (600-cycle, 2X300 bp paired end).

2.5 | Positive and negative controls

For positive controls we sampled clownfish species (*Amphiprion*) that do not occur in the Mojave Desert. A 500 ml water sample was collected from aquaria housing only two species of clownfish, the tomato clownfish (*Amphiprion frentatus*), and the ocellaris clownfish (*Amphiprion ocellaris*), in the finfish department at the Oceanic Institute of Hawai'i Pacific University. The mock community water sample was vacuum filtered through a single 47 mm GN-6 Metricel 0.45 μm MCE membrane disc filter (Pall Corporation); filters were submerged in 700 μl of Longmire's buffer (Longmire et al., 1997) in a 2 ml microcentrifuge tube and stored at room temperature. DNA extraction for the mock community sample followed the protocol outlined in Renshaw et al. (2015) starting with Phenol-Chloroform-Isoamyl alcohol (25:24:1). Illumina library prep for the mock community sample was performed alongside the eDNA samples from the Mojave Desert. In addition to the extraction blank negative controls, a single PCR negative control was included. For metabarcoding PCRs, sterile water was used in place of template DNA. We used the positive and negative controls to monitor for contamination that could have occurred during laboratory handling steps.

2.6 | Data analysis

We used the Anacapa QC and DADA2 module (with default parameters) to run quality control, metabarcode reads sorting, and ASV determination for raw sequences (Curd 2019). The Anacapa classifier (with default parameters) was used to taxonomically assign ASVs. Briefly, ASVs were determined using the default settings in DADA2 v1.6 (Callahan et al., 2016) as implemented by Anacapa. Taxonomic calls for ASV were determined using a modified Bayesian Least Common Ancestor (Gao et al., 2017) algorithm implemented in Anacapa using default settings and a Bootstrap Confidence Cutoff of 60. For each metabarcode and sample type combination (e.g., CO1 water samples), we removed contamination from the sample ASVs using R package decontam (version 3.4.2; Davis et al., 2018) at alpha 0.1. Datasets were processed by metabarcode marker and sequencing run. The ASVs found to be contaminants were removed from the dataset (see Table S3 for details). As there are typically numerous ASVs per taxonomic call, we did not remove non-contaminate ASVs given the same taxonomic call as a contaminated ASV. The remaining ASVs were then collapsed by a unique taxonomic path (Domain, Phylum, Class, Order, Family, Genus, Species), and samples not assigned a taxonomic path were removed from the analysis. Because the soil samples (4) were collected in a dry stream bed, we treated them as sediment samples and the final sediment and water datasets were merged for further analysis (r library Phyloseq version 1.32.0; McMurdie & Holmes, 2013). The datasets were merged to provide a fuller representation of all of the species present in the Mojave Desert springs, as the different biological substrates (e.g., water and sediment) directly influence the biotic composition (Hermans et al., 2018; Koziol et al., 2019), which improves the comparison to observational data. Analyses include linear models and Permutational Multivariate Analysis of Variances (PERMANOVA), which are described below. Based on plots of family recovery by sequencing effort, the combined datasets were rarefied 10 times to an even depth and rarefactions were averaged using R library ranacapa (Kandlikar et al., 2018; Figure S1; CO1 and PITS: 5,000 reads). Rarefied datasets were used for alpha diversity and beta diversity analyses.

We explored patterns of alpha and beta diversity across samples binning taxonomy entries to Family levels for CO1 and PITS. We did not run alpha or beta diversity analysis on the Ve16S dataset due to low numbers of taxa and low overlap in taxa between samples. Family level analyses were chosen because the classifier is more accurate at higher levels of taxonomic resolution (Curd 2019; Edgar, 2018), and the large number of Unicellular organisms included in these datasets makes manual curation of reads to species unfeasible. For CO1, PITS, and Ve16S sequences, we explored species-level data for phylum Chordata (CO1 and Ve16S) and Streptophyta (PITS). We also explored the relationship between our sequencing results and CRUX database coverage for Ve16S and PITS metabarcodes, by aligning the ASVs generated for this study with CRUX reference sequences of taxa associated with Mojave springs using the Geneious Alignment (Geneious 9.1.5; http://www.geneious.com, Kearse et al., 2012).

2.7 | Alpha diversity

Alpha diversity was measured as the number of families (CO1, PITS) found in each sample. We did not use an abundance-based measure because relative abundance is problematic using eDNA (Yates et al., 2019). We assessed differences in the family counts using linear models where we initially included the categorical variables spring location, sample type, and season as independent variables and used an AIC stepwise selection model to determine the best final model (r packages Mass version 7.3–53, Ripley et al., 2013; and Fox et al., 2012). We tested the final model for Homogeneity of Variance using Levene's test with multiple independent variables as implemented by R library car (version 3.0-0; Fox & Weisberg, 2018).

2.8 | Beta diversity

The Beta diversity analysis for CO1 excluded taxa not present in at least 30% of the samples and with fewer than four reads per sample (McMurdie & Holmes, 2013; Phyloseq) to minimize the effects of taxa with small means and large CVs on ordinations. For PITS, we excluded taxa not present in 10% of the samples and with fewer than three reads per sample, because there were relatively few common families across samples. The resulting datasets were converted to Jaccard distance matrices (chosen because eDNA data provide poor abundance information) and used for subsequent analyses. We visualized beta diversity using Non-metric multidimensional scaling (NMDS, Phyloseq) and explored the differences in these eDNA communities using PERMANOVA (ADONIS, r library Vegan version 2.5-6) as implemented in Phyloseq (Dixon, 2003). Specifically, we determined the effects of two categorical variables: (a) spring location; and (b) sampling season. Prior to running PERMANOVA analyses, we determined that the Homogeneity of multivariate dispersions for spring and season did not differ ($p > .05$) using BetaDisper as implemented in Vegan.

3 | RESULTS

3.1 | Database sequence coverage

ASV sequence coverage and CRUX database coverage varied for Ve16S and PITS metabarcodes. We found differences in length between the forward and reverse reads generated for each metabarcode locus. The Ve16S forward reads were on average 11 bases longer than Ve16S reverse reads, and the PITS forward reads were on average 59 bases longer than reverse reads (Table S4). For each marker (excluding CO1, where we obtained species identification for a total of 13 bird species), we aligned all ASV sequences with the CRUX reference database sequence of species relevant to the Mojave. For Ve16S, most of the reference sequence spanned the length of the expected metabarcode amplicon (Supplemental File S1). Therefore, we maintained equivalent reference database coverage for the Ve16S forward and reverse ASV sequences. However, many PITS reference sequences did not cover the 3′ end of the expected amplicon. Consequently, we had good coverage of forward ASV sequences but incomplete coverage for PITS ASV reverse sequences (Supplemental File S2). Therefore, we relied on forward sequences for assignment to species for PITS and sequences were verified using BLAST (Altschul et al., 1990; see Tables S5–S7) on the GenBank nucleotide database (Benson et al., 1993) for all markers. Sequencing statistics are available in Table S8.

3.2 | Databases and species detection

We compared Mojave Desert springs biota sequence data to current reference sequence databases and found noted gaps in barcode databases, which sometimes led to poor or unknown taxonomic assignment (Tables S5–S7). Based on expected species list (Table S9), the barcode Ve16S had 42% (134/317) of expected animal species present in the sequence database, CO1 had 86% (273/317) of animal species present, and PITS had 43% (220/512) of plant species present. This could be related to poor species-level taxonomic resolution (e.g., CO1 poorly resolves rodents in the Cricetidae and PITS for plant families Rosaceae, Fabaceae, Asteraceae, etc.; Tables S5 and S6) and unknown or unidentified taxa. We treated boar and cow observations as contaminants as they are commonly found in laboaotory reagents (Leonard et al., 2007). Human detections, mock marine community organisms, and taxa not known to occur at site (e.g., organisms not found in the continent) were also treated as contamination.

The resolution of taxonomic assignments and composition detected varied considerably for each of the three metabarcode primers used in this study. The Vertebrate 16S primer appeared ideal in capturing vertebrate diversity to the species level and detected 25 different species (8% of the total animals; 25/317) including amphibians (3), birds (4), carnivores (5), ungulates (2), reptiles (2), and lagomorphs (2) with varying resolution for species of rodents (7 species and 8 genera; Figure 2, Table S5). The

FIGURE 2 Vertebrate species of conservation interest detected with Vertebrate 16S marker at four Mojave Desert springs. All of the species at Ahn Spring were confirmed by managers as present by human observation or camera trap data (underlined species) with the exception of American bullfrog, Northern Pacific Tree Frog, and Bighorn sheep. Shared species between sites are bolded. All species at Big Morongo Canyon Springs were confirmed by managers as present by human observation with the exception of Big eared woodrat, which have never been observed and American bullfrogs not observed since 2009. Species with asterisks represent organisms that were incorrectly assigned to species or could not be assigned to species level but managers were able to provide a species identification. All species at Bonanza and Hummingbird springs were confirmed to be present with the exception of northern pacific tree frog and dogs (species designated with hashtags). The images of organisms are from iNaturalist observations

Ahn Spring (17)

Reptilia
Western fence lizard

Aves
California scrub jay
Dark-eyed junco

Amphibia
Red spotted toad
Northern Pacific treefrog#
American bullfrog#

Mammalia
Dog
Coyote
Bighorn sheep#
Mule deer
Black tailed jackrabbit
Deer mouse
Desert woodrat
Vole sp.
Big-Eared woodrat
Kangaroo rat
Squirrel sp.

Squirrel sp.

California Scrub Jays

Mule deer

Big-Eared Woodrat

Big Morongo Canyon Springs (20)

Amphibia
Red spotted toad
California tree frog*
American bullfrog#

Aves
California scrub jay

Mammalia
Racoon
Dog
Coyote
Bighorn sheep
Mule deer
Kit fox
Black tailed jackrabbit
Cottontail rabbit
Deer mouse
Desert kangaroo rat*
Merriam's kangaroo rat
Desert woodrat
California vole*
Big eared woodrat#
Desert pocket mouse*
California ground squirrel*

Merriam Kangaroo rat

American Bullfrog

Black-tailed Jackrabbit

Red-spotted toad

Bonanza Spring (14)

Amphibia
Red spotted toad
Northern Pacific treefrog#
American Bullfrog

Mammalia
Coyote
Bighorn sheep
Mule deer
Kit Fox
Bobcat
Black tailed jackrabbit
Cottontail rabbit
Long-tailed pocket mouse
Desert woodrat
Big eared woodrat
Squirrel sp.

Bobcat

Bighorn sheep

Long-tailed Pocket mouse

Desert Woodrat

Hummingbird Spring (17)

Reptilia
Common side-blotched lizard

Amphibia
Red spotted toad
Northern Pacific treefrog#
American bullfrog

Aves
Mourning dove
Crow

Mammalia
Dog#
Coyote
Kit Fox
Bobcat
Black tailed jackrabbit
Cottontail rabbit
Long-tailed pocket mouse
Desert woodrat
Vole sp.
Big eared woodrat
Squirrel sp.

Common side-blotched lizard

Squirrel sp.

Vole sp.

Kit fox

CO1 marker is designed to capture metazoan taxa (vertebrates and invertebrates), with a 5% (15/317) detection rate for vertebrates. Detection of vertebrates using CO1 was particularly sensitive for birds (13) and amphibians (2) (Table S6; Figure 3) but did not capture the species diversity of mammals or reptiles (Table S6).
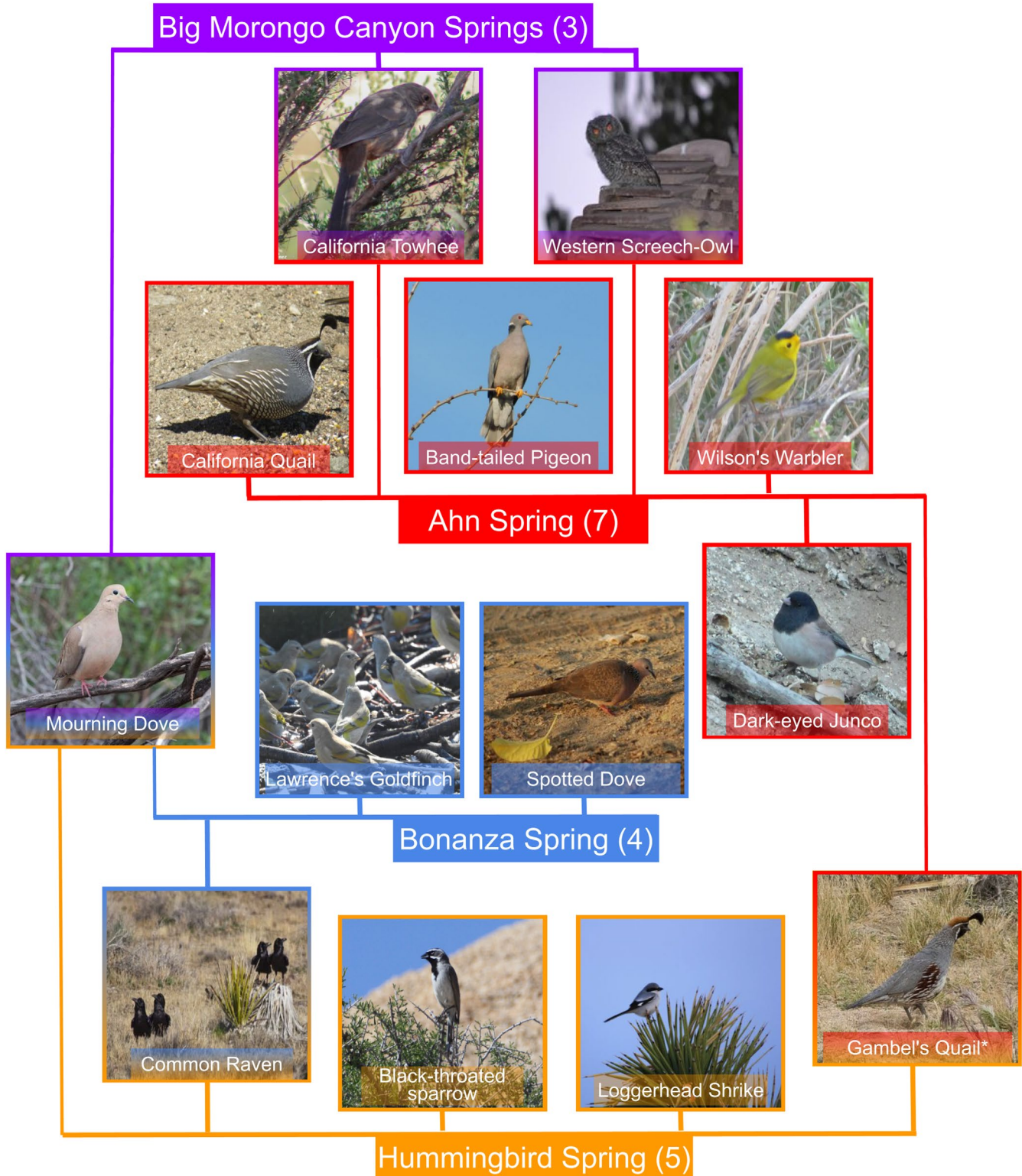
FIGURE 3 Bird species detected with environmental DNA with CO1 marker at four Mojave Desert springs. Each color represents a spring location, maroon for Big Morongo Canyon Spring, red for Ahn Spring, blue for Bonanza Spring, and orange for Hummingbird Spring. All bird species detected at Ahn Spring were confirmed as present with Camera trap data except for Gambel's quail (represented with asterisk, instead observed Mountain quail). The bird species at the remaining location were all confirmed to be present by reserve managers or biologists. The images of organisms are from iNaturalist observations

The comprehensive list of plant species at Big Morongo Canyon springs allowed us to more accurately compare eDNA identification with human-observed species. We resolved taxonomic assignment

of plants to species level for 75% (59/79) of eDNA detections and 18% (14/79) to genus level (Table S7). There were 33 plant species detected with eDNA which had never been observed (including

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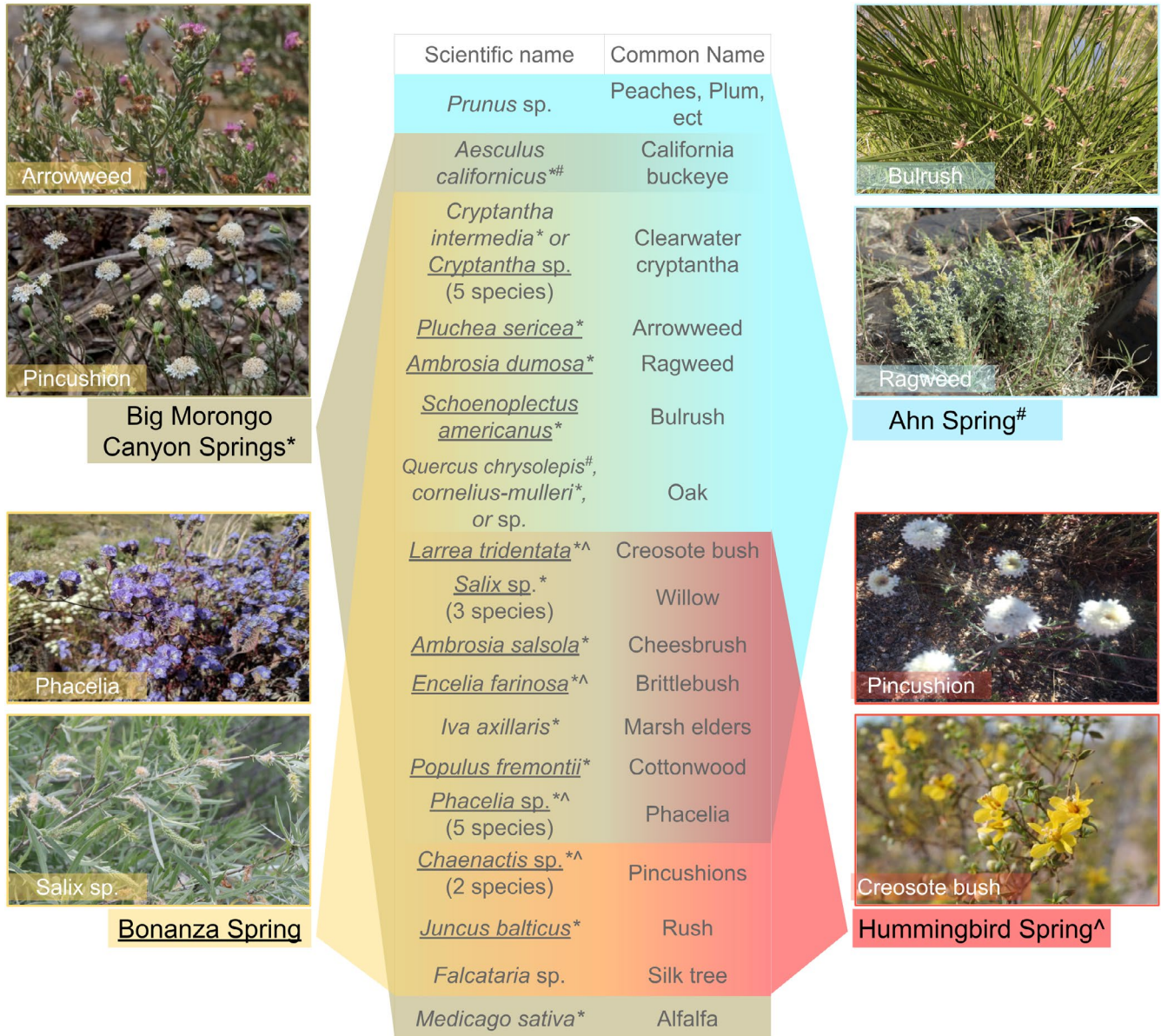
FIGURE 4 Plant species detected with environmental DNA and confirmed to be present at four Mojave Desert springs. Each color represents species present at each spring location, with light yellow for Bonanza spring, grey for Big Morongo Canyon spring, aqua for Ahn spring, and red for Hummingbird spring. Plant species scientific name validated by field surveys are marked by special symbols; with Bonanza Spring as underlined, Big Morongo Canyon Spring with an asterisk*, Ahn spring with a hashtag #, and Hummingbird Spring with a carrot ^

sesame, common peas, and eucalyptus) and 6 uncallable sequences (not shown; Table S7). The plant species and field observations also provided information to successfully match identification to the species level, for example Muller's oak (*Quercus cornelius-mulleri),* California Ash (*Fraxinus dipetala*), and perennial ryegrass alkali goldenbush (*Isocoma acradenia*) (Figure 4; Table S7). For Bonanza spring, species assignment rate was 71% (54/76) and 29% (22/76) to genus, with 32% (24/76) observed and six uncallable sequences (Table S7). For Hummingbird spring, species assignment rate was 65% (39/60) and 35% (21/60) to genus, with 46% (28/60) observed and five uncallable sequences (Table S7). Ahn spring, species assignment rate was 62% (36/58) and 31% to genus (18/58), with 3% observed (2/58) and three unassigned sequences (Table S7).

3.3 | Agreement between observations and eDNA detection of spring species

Based on communication with land managers, we were able to confirm the species list generated by eDNA for vertebrates and plants at all four springs (Figures 2–4 and Tables S5–S7) and resolve classifications to species-level identification for five sequences (represented by asterisks, Figure 2). We also confirmed vertebrate species through camera trap data at Ahn spring (Table S10) and plant species at the remaining springs through botanical surveys (Table S11). In general, each spring has a unique community composition (Ahn spring 24 animals, 14 plants; Big Morongo Canyon springs 23 animals, 16 plants; Bonanza spring 18 animals, 15 plants, and Hummingbird spring 22

animals, 10 plants; Figures 2 and 3) but shared four vertebrate species (eDNA data; Red spotted toad, Coyote, Black tailed jackrabbit, and Desert woodrat; Figure 2). The most similar vertebrate communities were shared between Bonanza and Hummingbird springs (12 species; Figures 2 and 3), and vegetation communities were most similar between Bonanza and Big Morongo Canyon springs (12 species; Figure 4).

3.4 | Agreement between observations and eDNA detection of Vertebrates

At Ahn spring, the agreement between eDNA and the camera trap data for vertebrates was low at 23% (11/48 species, Figures 2 and 3; Table S10), with eDNA contributing an additional nine observed species and three other species that have not been previously observed but are known from the area (e.g., American bullfrog, North Pacific tree frog, and bighorn sheep; Figure 2). Big Morongo Canyon springs had a high level of agreement at 92% (22/24) with previous observations and communication with the reserve manager contributed to reassigning the species identification of the Northern Pacific Tree frog to the California tree frog. The two exceptions included the invasive American bullfrog and native big eared woodrats. The American bullfrog was only detected in sediment samples (Table S5) and was observed 10 years ago but thought to be now locally extirpated. Big eared woodrat eDNA was detected in multiple water and sediment samples, despite never having been observed (Figure 2; Table S5). By comparing the lists generated by eDNA detections for Bonanza and Hummingbird springs to validations by the resident biologist, we found a 94% (17/18 species) and 86% (16/22 species) match for animals at the two springs, respectively. The red spotted toad was not initially verified at Bonanza springs; however, a recent survey by the Bureau of Land Management biologist, confirmed this species was present through observations and mating calls. The North Pacific tree frog has never been observed but has a strong eDNA signal in water at Bonanza and in sediment at Hummingbird springs (Table S5). There was also uncertainty surrounding squirrel identifications at Hummingbird spring (Table S5).

3.5 | Agreement between observations and eDNA detection of Plants

For the analysis of plant taxa present in 10% of the samples, we recovered 18 common species and found seven species were shared across the four springs and eight species were shared between Big Morongo Canyon springs and Bonanza spring (Figure 4). We found low concordance between eDNA and botanical surveys. At Ahn spring, botanical surveys and eDNA had a match rate of 14% (2/14; botanical survey observations/eDNA detected species), with more species detected with eDNA (14 species vs. 2; Figure 4). For Big Morongo Canyon springs, we compared eDNA detections to botanical surveys and plant species list and there was a 94% (16/17 species)

agreement. In Bonanza spring, there was an 80% (12/15) concordance between botanical surveys and eDNA detected species, with one species not expected based on its range distribution (*Quercus* sp.; Figure 4). The vegetation at Hummingbird spring had 40% congruence (4/10) between eDNA detected species and plant surveys and one species (*Quercus* sp.) was out of range (Figure 4).

3.6 | Alpha diversity

The alpha diversity pattern observed for the CO1 marker was highest at Ahn spring followed by Big Morongo Canyon springs, Bonanza spring, and Hummingbird spring (Figure S2). There was higher diversity in sediment samples (Figure S3) and slightly higher diversity in the winter (Figure S5). We identified the variables associated with differences in alpha diversity using a linear model that included spring location, sample type, season, and the interactions between all pairs of variables and between all three variables. We found differences in alpha diversity by sample type ($p = .0112$), the interaction between sample type and spring location ($p = .0186$) and the interaction between sample type, spring location, and season ($p = .0021$) were all significant. This suggests that sample type, spring location, and season are drivers of differences in the number of families found within samples.

The alpha diversity patterns observed for the PITS marker differed from CO1. The PITS marker showed the lowest diversity at Ahn spring and Big Morongo Canyon springs had the highest, followed by Bonanza and Hummingbird springs (Figure S2). PITS showed higher diversity in water than sediment (Figure S3). PITS also had a higher diversity in the spring season than winter (Figure S5). We identified the variables associated with differences in alpha diversity using a linear model that included spring location, sample type, season, and the interactions between spring location and season. We found significant differences in alpha diversity by sample type ($p = 3.709e-08$), and the interaction between season and spring ($p = .00012$). This suggests that sample type, spring location, and season are drivers of differences in the number of families found within samples.

3.7 | Beta diversity

We used NMDS ordination to assess beta diversity patterns among the four Mojave Desert springs (Figure 5). PERMANOVA shows a unique community composition by spring for both markers (CO1, $p < 9.999e-05$, perm = 10,000; PITS, $p < 9.999e-05$, *perm = 10,000*; Figure 5) when controlling for season or sample type as random effects on community composition. When controlling for the effect of spring location, we detected community differences based on season for CO1 ($p = .0196$, perm = 10,000) but not PITS ($p = .2807$, perm = 10,000). However, the effects of season were marginal when controlling for the random effects of sampling type for CO1 ($p = .0507$, perm = 10,000) but did not differ for PITS ($p = .3773$, perm = 10,000). Consequently, the results suggest that spring

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location and possibly season or sampling time influence biodiversity patterns, with spring location showing the greatest effect.

4 | DISCUSSION

This pilot study explores the use of eDNA metabarcoding for detecting biodiversity at four different Mojave Desert springs. The Mojave Desert is divided into six distinct subregions (Webb et al., 2009), and these springs are a small representation of Mojave Desert springs types. They vary in seasonal flow, depth, geographic location, and structural modification by humans. We find eDNA metabarcoding to be a promising supplemental method in combination with traditional methods to assess biodiversity of Mojave Desert Springs. For each spring, the levels of agreement between eDNA and observational data varied (23%–94% for animals and 14%–94% for common plants at the species level). We found high concordance at springs that have existing species-level inventories (Big Morongo Canyon, Bonanza and Hummingbird springs, in order of completeness). We compared eDNA detections to camera trap results and found camera traps were more effective at capturing biodiversity (48 species captured using camera traps vs. 20 species using eDNA, and an overlap of 11 species) and showed a peak of diversity in the summer, a season during which we have no eDNA data. This finding suggests the need for more frequent eDNA sampling covering all seasons. We find each Mojave Desert spring supports a unique ecological community (PERMANOVA, $p < 9.999e-05$ for CO1 and PITS markers) with low overlap in animal species and more overlap in vegetation in large and unmodified springs (Big Morongo Canyon and Bonanza springs).

The use of three genetic markers captured biodiversity across Domains, including species of conservation interest (e.g., Bighorn sheep, *Ovis canadensis*) and invasive species (e.g., American bullfrog,

Lithobates catesbeianus). However, our results suggest the need for building more complete reference barcode databases on Mojave Desert biota and additional taxonomic ground surveys for comparisons to eDNA metabarcoding. The results show the importance of using multiple genetic markers, which target biodiversity across distinct groups (e.g., Ve16S, CO1, and PITS) and within them (e.g., Ve16S for vertebrates and CO1 for birds) to reduce primer bias and increase the likelihood of capturing diversity. We also recognize the need to develop study-specific primers and tools to explore primer selection a priori, for example incomplete coverage for the PITS reverse primer for plant species of the Mojave Desert.

We demonstrate that spring location, sample type, and season significantly influence biodiversity patterns of each spring. The results comparing sample types (water vs. sediment), revealed a general trend of higher levels of biodiversity in sediment samples, with partial overlap in community composition with water samples. We did not detect seasonal turnover in the ecological community between winter and spring with eDNA, which may be due to the limited sampling size or single sampling event per season. This study highlights the importance of collaboration between scientists using eDNA metabarcoding to monitor and survey ecological communities and managers or taxonomic experts to validate results with traditional methods.

4.1 | Agreement between eDNA detected spring biodiversity and observational data

The collaborative efforts of eDNA scientists, managers, taxonomic experts, and resident biologists were critical for the interpretation and validation of the eDNA results. The availability of existing species lists allowed a sensitive assessment of the agreement between eDNA
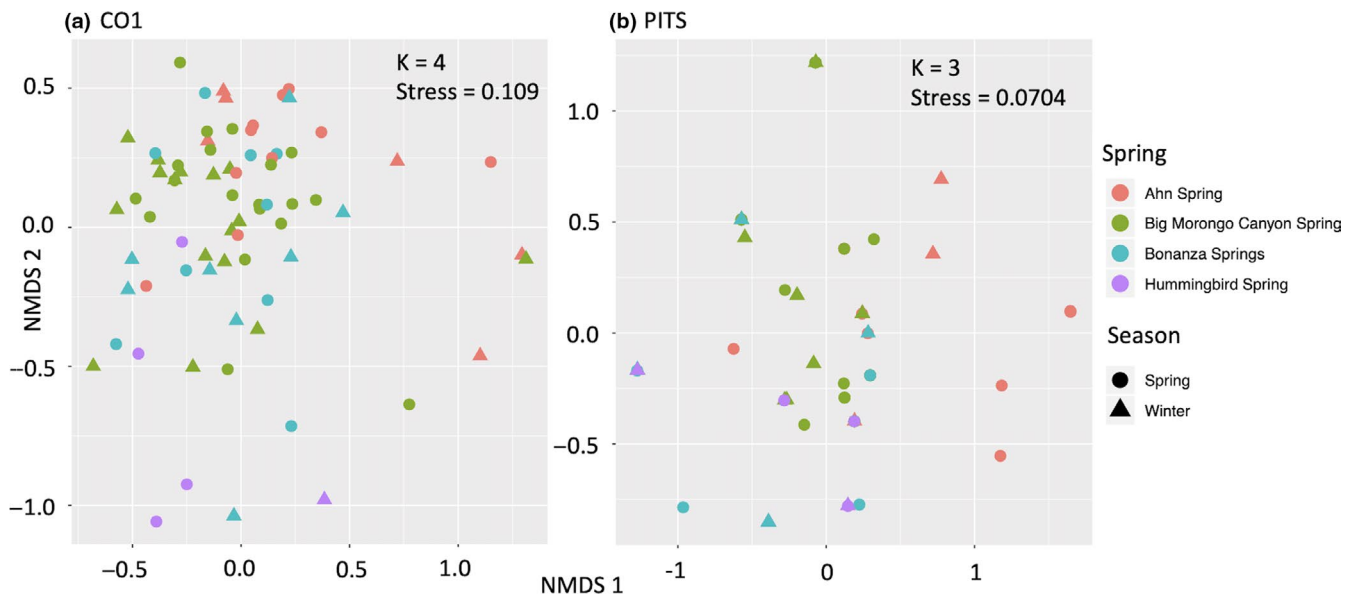

FIGURE 5 Beta diversity plot of the first two axes from nonmetric multidimensional scaling of Jaccard distance matrices at the Family level for COI and PITS for combined water and sediment samples. Each dataset includes only taxa with more than three reads and present in at least 30% of the reads. (a) CO1 includes 20 families. (b) PITS includes six families

detections and observations. For example, long-term biodiversity monitoring is being conducted at the Big Morongo Canyon springs by the community science group Friends of Big Morongo Canyon Preserve (volunteers composed of naturalists and outdoors enthusiasts), and this effort has generated extensive species lists for animals, birds, and plants. As a result, we were able to better assess the validity of eDNA results and found high agreement for both animals (92%) and plants (75% all eDNA detections or 94% for most common plants) at this site. Big Morongo Canyon springs have the most diverse ecological community, a reflection of being located within a transition zone between the low elevation, high temperature Colorado Desert, and the high elevation, low temperature Mojave Desert (Richert, 2002). In contrast, Bonanza and Hummingbird spring are found 9.6 km apart on the slopes of the Clipper Mountains in the south-eastern Mojave subregion and are managed by the Needles Bureau of Land Management office. The agency has recently initiated field surveys for animals and plants with the goal of building inventory species lists for springs under their jurisdiction. These recent efforts facilitated our assessments between eDNA detections and field observations of animals (86%–94%) and plants (40%–80%). Lastly, despite the small size, arid environment, isolation, and high elevation (1,153 m) of Ahn spring, the spring sustains a wide variety of wildlife. The spring serves as a year-round source of water with the main source of precipitation (1,000 mm/year) in the form of snow during the winter months (Ball & Izbicki, 2004). The camera trap data of Ahn spring over a seven-year period suggest a summer peak in the diversity of vertebrate taxa visiting springs (Daniel Potter, personal communication), mirroring findings from previous seasonal taxon-targeted eDNA studies (Buxton et al., 2018; Stewart et al., 2017) and aligning with the known breeding season (Stewart, 2019) of Mojave species. Our single eDNA sampling effort in other seasons led to poor agreement of vertebrates between the two methods (23%) and suggests increased sampling intensity, including summer, may allow us to better assess seasonal differences in wildlife communities present at springs. The same is true for plants, which will require botanical surveys during the winter and spring wet seasons, as the shallow nature and low flow of Ahn spring creates unsuitable conditions for spring dependent plant communities (Davis et al., 2017). Our results show that eDNA metabarcoding provides similar taxon lists at Mojave Desert springs, especially for animals, as other methods. However, eDNA metabarcoding requires some ground-truthing with traditional observational methods to assess the best sampling schemes and to determine taxa not detected by a low level eDNA survey.

4.2 | Databases and markers

The biggest impediment for this study was the lack of sequence information of Mojave Desert spring organisms in public databases (>45% for plants and animals for informative markers), a common occurrence in understudied (Sinniger et al., 2016; Wangensteen et al., 2018) and aquatic ecosystems (in Europe, Weigand et al., 2019), which led to unresolved taxonomic assignments at the species level. This study identifies existing gaps in barcode libraries for Mojave Desert biota and taxonomist

ground surveys (e.g., plants and rodents), limiting the conclusions drawn from eDNA metabarcoding biodiversity assessments for environmental management of Mojave Desert spring ecosystems. Future efforts and resources will need to focus on creating reference databases for commonly used barcodes by prioritizing sequencing efforts based on species lists generated by ground surveys (e.g., camera trap images and botanical surveys) from voucher specimens (e.g., deWaard et al., 2019) and include advanced technology (e.g., artificial intelligence of camera trap data, Norouzzadeh et al., 2018; Tabak et al., 2019).

In general, each of the three genetic markers applied in this study was successful at capturing biodiversity present at each of the four Mojave Desert springs. However, each marker had limitations. The Vertebrate 16S marker was ideal for detecting vertebrate species, including mammals, fish, amphibians, and reptiles, but rarely detected birds. This limitation may represent primer bias to certain DNA sequences (Deiner et al., 2017), primer mismatch despite wobble, or polymerase bias (Nichols et al., 2018). Interestingly, COI was a catch-all marker, sensitive to groups that may have gone undetected by other markers (e.g., Vertebrate 16S and birds), but poor with other groups (rodents in the Cricetidae). These findings reinforce the importance of using multiple primers that target the same taxonomic group to minimize primer bias and increase the likelihood of capturing the true diversity in a sample (Alberdi et al., 2017). Specifically, the use of a single marker would have reduced the detection of bird diversity at the springs. This taxonomic bias is particularly relevant for conservation management of the Mojave Desert as new solar facilities are proposed, which are known to be detrimental to birds (Kagan et al., 2014). For example, solar facilities sited in close proximity to open water are known to have caused 70 mortalities of 26 species of birds due to collisions with structures or burning over 10 months (McCrary et al., 1986). The PITS marker was useful in detecting common plant species, which is surprising given the complexities in identifying plant markers useful in distinguishing between plant species (Fazekas et al., 2009). We find that eDNA metabarcoding detected wetland-associated plants (e.g., *Iva axillaris*, *Populus fremontii*, *Juncus* sp., and *Pluchea sericea*), as well as annuals and wind pollinated plants associated with neighboring arid ecosystems (e.g., *Quercus* sp., *Cryptantha* sp., *Phacelia* sp). In desert ecosystems, certain plants will only establish and germinate during periods of high soil moisture (Morton et al., 2011) and therefore sampling and surveying during or after the rainy season will better reflect true levels of plant biodiversity. Future studies should focus on developing, testing, comparing, and improving molecular markers for plants. Specifically, to select for markers that detect spring dependent plant communities (Fazekas et al., 2009) as well as animals (e.g., 16S, Smith et al., 2020; 12S, Furlan et al., 2020).

4.3 | Taxonomic diversity captured by sample type collected

We compared the levels of organismal diversity captured by sample type namely, water or sediment. The varied taxonomic composition based on the substrate sampled has previously been attributed to taxonomic differences in suitability and occupation

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bias, and consequently, any single substrate underestimates total diversity (Koziol et al., 2019). We find that biodiversity tends to be higher in sediment than in water samples which may be due to eDNA being bound to sediments and persisting longer due to a slower degradation rate (Barnes et al., 2014). In water, eDNA degrades more quickly and is detectable for days to weeks (Dejean et al., 2011; Goldberg et al., 2013) depending on biotic and abiotic factors (Barnes et al., 2014). A previous study comparing water and sediment detection found that the probability of detecting eDNA from water exceeded that from sediment, but this study only focused on one species (great crested newt; Buxton et al., 2018). Our finding of higher diversity in sediment can also be explained by the presence of more living organisms in the form of microbes and meiofauna (minute invertebrates) in sediment samples (~98%, not shown; Traunspurger & Majdi, 2017) and markers that can capture this ecological community (e.g. CO1). Previous studies have suggested that eDNA in water samples captures more recent site occupancy (Turner et al., 2015) but this can be influenced by temperature and concomitant microbial activity, which increases DNA release from plant matter within aquatic sediments (Poté et al., 2009). This difference may explain higher alpha diversity in water than sediment for PITS. We recommend the sample type selected should be determined by the specific objectives of future studies.

4.4 | Seasonal fluxes in biodiversity detection

We tested differences in eDNA detection in spring versus winter samples and found no statistically significant ($p > .05$) difference in detection of species with the exception of PITS. The results showed winter had an increase in biodiversity observed for COI, a pattern that could have been caused by deposition of allochthonous eDNA from outside the study area (Jerde et al., 2016) or potentially by rainstorms prior to sampling (Bista et al., 2017). It is possible that the environmental conditions experienced between winter and spring in 2019 were insufficient to cause seasonal turnover of eDNA community diversity at Mojave Desert springs. Persistent and undisturbed springs are stable ecosystems because they are not exposed to variability in temperature, discharge, and water chemistry (McCabe, 1998), potentially sustaining a similar community structure between the two seasons.

4.5 | Limitations of the study

This small study provides support for eDNA metabarcoding as an effective method for capturing the community composition of Mojave Desert springs; however, future studies will need to address the limitations of this study. For example, a single sampling event in each season represents a snapshot of diversity, whereas longitudinal eDNA studies often sample multiple times in a season (Berry et al., 2019; Milhau et al., 2019; Zhang et al., 2019), improving the detections

of seasonal variation in species composition and biodiversity. Additionally, the pooling of biological replicates reduces the signal and detection of rare species (Sato et al., 2017; Zhang et al., 2020), which are often of conservation concern (Franklin et al., 2019) and a single PCR replicate captures less taxonomic diversity than duplicates or triplicates (Beentjes et al., 2019). The small sample size of springs ($n = 4$) and representation of spring types (large, naturally flowing, and unmodified springs vs. small or modified springs) directly affects vegetation comparisons, as water availability may be limiting the support of a riparian plant community (Sada et al., 2005) and indirectly animal communities. Future studies will benefit from including multiple samples per season, treating each biological replicates as individual samples, duplicate or triplicate PCR replications, and comparisons across similar spring types for a more robust study.

4.6 | Conservation implications

Current plans for the development of renewable energy facilities, water extraction projects, and other industrial or commercial uses of Mojave Desert resources require that the biological communities of Mojave Desert springs be well-documented and monitored to ensure their long-term conservation management as required by law. Springs are known to provide water and support important habitat for rare and endangered species of birds such as the Least Bell's Vireo (*Vireo bellii pusillus*), rare plants such as Tecopa's bird's beak (*Chloropyron tecopense*) and alkali ivesia (*Ivesia kingii var. kingii*; Fraga, 2019), and wide-ranging mammals of conservation concern such as bighorn sheep (*Ovis canadensis nelsoni*). However, the presence of and use by specific plants and animals varies by spring and monitoring these resources over a vast landscape is time and cost prohibitive. The use of eDNA holds promise for conservation practitioners and land managers to more efficiently monitor Mojave Desert springs. Our study demonstrates the importance of appropriately timing eDNA field sampling, carefully selecting eDNA primers, and including field-based survey information of wildlife and plants in conjunction with eDNA detection to maximize detection of rare or special status species, and better document biodiversity at desert springs more broadly.

AUTHOR CONTRIBUTION

MP, EC, KE, MAR, RD, DP, NF, JM, JS, RKW, and SSP have made major contributions to at least one of the following: (a) the conception or

design of the study, (b) the acquisition, analysis, or interpretation of the data, and (c) writing of the manuscript.

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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