Microbial Diversity of the Hell Creek Watershed at the Tri-Faith Community in Nebraska, Based on 16S rRNA Gene Amplicon Sequencing

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ABSTRACT  Hell Creek’s watershed is a historically important native land area located in Omaha, Nebraska, that includes Hell Creek and an adjacent flood plain. This initial microbial analysis showed that even though samples were isolated from the same watershed area, there were significant differences between the creek itself and the nearby pond.

The Hell Creek watershed area was once an established prairie, savanna, and forest, before development of homes and business roads caught up with the watershed in the past two decades. This significantly reduced the ability to absorb rainwater, making Hell Creek more flood-prone and a possible source of harmful coliform bacteria (1, 2). The Hell Creek area is also the location of the globally unique Tri-Faith Commons (https://www.tenxtenstudio.com/trifaith; 3), and with this new establishment, a restoration of the native watershed area has been started. This includes selected plantings that promote local wildlife and rain gardens that reduce runoff. Hell Creek runs north to south diagonally through the area, which also includes a nearby stand-alone reservoir pond (Fig. 1).

We isolated samples from both the creek and the nearby pond in April 2020. Two pond samples were taken from the center of the pond, ~6 m apart (HC1 and HC2), while a third pond sample was taken near the edge of the pond (HC3). A fourth sample (HC4) was taken from Hell Creek itself, near the pond (41°14′37.15″N, 96°7′2.42″W) (Fig. 1). Samples were collected in sterile collection tubes and immediately transferred to the lab, where they were stored at 4°C. The next day, 10 ml of each sample was centrifuged for 15 min at 16,000 × g to form a biomass pellet. Total DNA was extracted using the PureLink microbiome DNA purification kit (Invitrogen). Utilizing Qubit and NanoDrop technologies, we determined the quality and quantity of DNA, showing A260/A280 ratios of 1.76 (HC3) to 1.94 (HC4). A 16S rRNA amplicon sequencing library was prepared for each sample, following the 16S Metagenomic Sequencing Library Preparation protocol (Illumina; https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Amplicon primers targeting the V3 and V4 region (4) including the Illumina adapter overhang sequences are described in the Illumina library prep protocol and were synthesized by Sigma. The samples were sequenced using a 1.8-pM library with an Illumina Miniseq instrument. Paired-end (2 × 150 bp) sequencing generated 1,714,184 (HC1), 1,744,434 (HC2), 1,649,932 (HC3), and 1,539,232 (HC4) reads. The primer sequences were removed, and reads with a low quality score (average score, <20) were filtered out using the FASTQ toolkit within BaseSpace version 2.2.0 (Illumina). The 16S Metagenomics application (version 1.0.1) within BaseSpace was used to perform a taxonomic classification, which uses an Illumina-curated version of the GreenGenes taxonomic database and the RDP naive Bayes taxonomic classification algorithm with an accuracy of >98.2% at the species level.

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Paired reads were used for a separate 16S metagenomic analysis for each individual sample. An aggregate comparison and tierarchal clustering of the taxonomic classification results of all 4 samples were also performed within the 16S metagenomic analysis (Fig. 1, insert). Default parameters were used for all software unless otherwise noted.

All samples had more than 88% of the reads identified at the genus level. All three of the pond samples contained abundant representation of the *Verrucomicrobiaceae* (~15%) and *Rhodobacteraceae* (>10%) families, which were nearly absent from the HC4 creek sample (Fig. 1, insert). In contrast, HC4 contained substantial amounts (~10%) of *Intrasporangiaceae* (of the *Actinobacteria* phylum [6]), which were found to be less than 1.5% of organisms in the pond samples. All of the samples contained substantial amounts of chloroplast (algal) (~30%) and *Comamonadaceae* (~10%), but few to no typical coliform bacteria were identified.

According to a Shannon species diversity analysis (7, 8) run within the 16S Metagenomics application in BaseSpace, each of the samples contained over 2,000 potential species, with substantial differences between the creek and pond water isolates. This establishes a good baseline for further studies of microbiological diversity and ecological impacts of the restoration of this locally important watershed.

**Data availability.** The 16S rRNA gene amplicon data sets have been deposited at DDBJ/ENA/GenBank under BioProject number PRJNA627532 and can be accessed with the BioSample numbers SRS6521435 (HC1), SRS6522831 (HC2), SRS6523000 (HC3), and SRS6523533 (HC4).

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