Development and Analysis of a Stable, Reduced Complexity Model Soil Microbiome

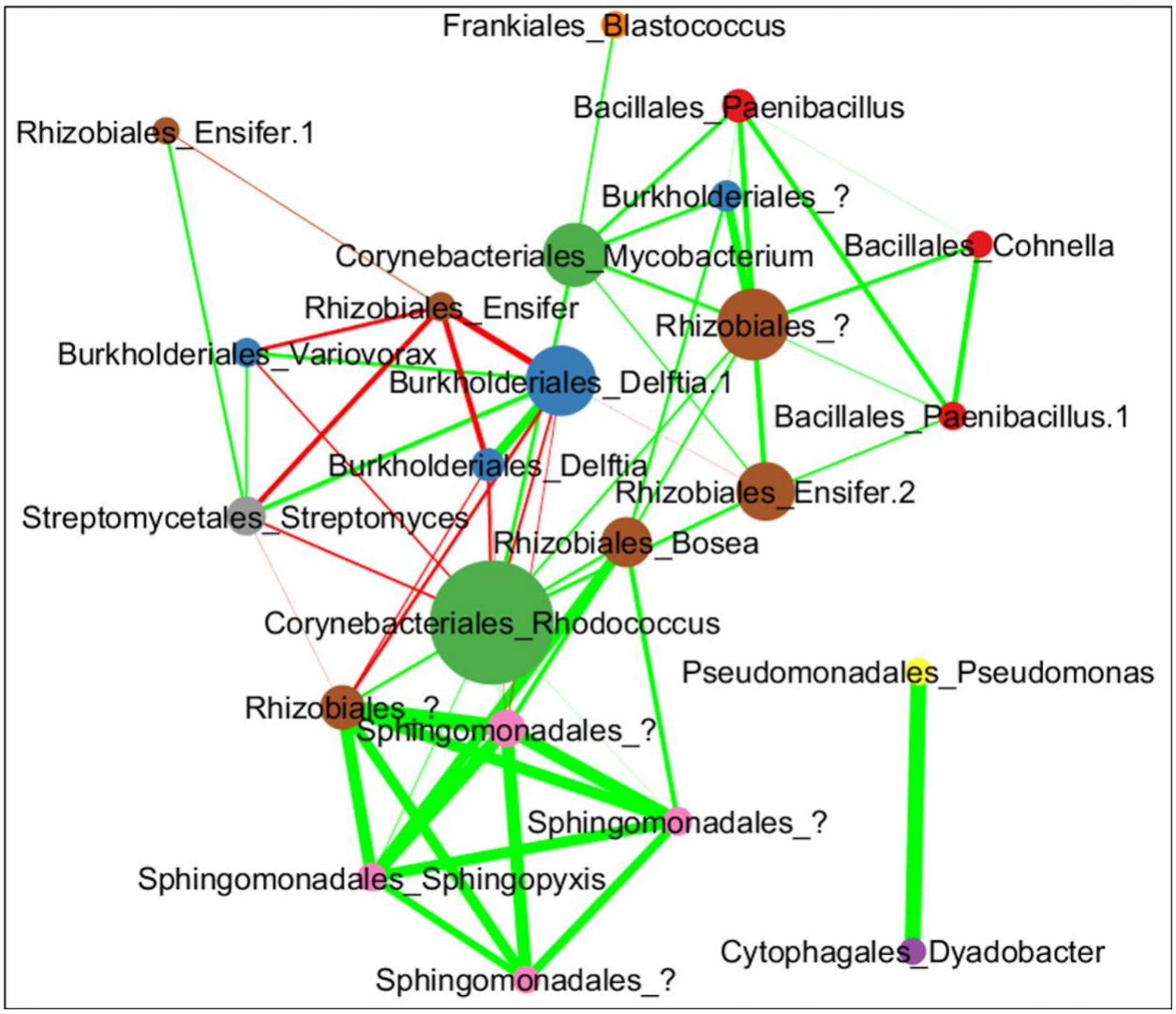
Ryan McClure,[a#] Dan Naylor,[a] Yuliya Farris,[a] Michelle Davison,[a] Sarah Fansler,[a] Kirsten S. Hofmockel,[a,b] Janet K. Jansson.

[a]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA 99352, United States
[b]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50010, United States

ABSTRACT

The soil microbiome is central to the cycling of carbon and other nutrients and to the promotion of plant growth. Despite its importance, analysis of the soil microbiome is difficult due to its sheer complexity, with thousands of interacting species. Here, we reduced this complexity by developing model soil microbial consortia that are simpler and more amenable to experimental analysis but still represent important microbial functions of the native soil ecosystem. Samples were collected from an arid grassland soil and microbial communities were enriched on agar plates containing chitin as the main carbon source. Chitin was chosen because it is an abundant carbon and nitrogen polymer in soil that often requires the coordinated action of several microorganisms for complete metabolic degradation. Several soil consortia were derived that had tractable richness (30-50 OTUs) with diverse phyla representative of the native soil, including Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia. The resulting consortia could be stored as glycerol or lyophilized stocks at -80° C and revived while retaining community composition, greatly increasing their use as tools for the research community at large. One of the consortia that was particularly stable was chosen as a model soil consortium (MSC-1) for further analysis. MSC-1 species interactions were studied using both pairwise co-cultivation in liquid media and during growth in soil under several perturbations. Co-abundance analyses highlighted interspecies interactions and helped to define keystone species, including Rhodococcus and Rhizobiales taxa. These experiments demonstrate the success of an approach based on naturally enriching a community of interacting species that can be stored, revived, and shared. The knowledge gained from querying these communities and their interactions will enable better understanding of the soil microbiome and the roles these interactions play in this environment.

https://doi.org/10.25584/WAIsoCMSC1/1635272



FIELD SITE

Washington State University Irrigated Agriculture Research and Extension Center (IAREC)
Prosser, WA, USA

PARENT METAGENOME

WA-TmG.1.0

https://doi.org/10.25584/WAIsoCMSC1/1635272

BIOSAMPLE RECORD

Identifiers: BioSample Name: WA-IsoC_MSC1.1.0

Organism: soil metagenome (unclassified sequences; metagenomes; ecological metagenomes)

Package: MIMARKS: specimen, soil; version 5.0

Attributes:

collection date	2018-05-02
depth	0-20 cm
elevation	300 m
broad-scale environmental context	ENVO:01000177 (grassland biome)
local-scale environmental context	ENVO:00005750 (grassland soil)
environmental medium	ENVO:00001998 (soil)
geographic location	GAZ:00003937: GAZ:22223879
latitude and longitude	46.25 N 119.73 W

Description: Chitin enriched soil community collections isolated from the IAREC field site in Prosser, WA.

BioProject: PNNL Soil Microbiome SFA 'Phenotypic Response of the Soil Microbiome to Environmental Perturbations'

AMPLICON STRATEGY

Sampling Method: 16S rRNA Amplicon (Sequencing)

Isolation Method: DNeasy PowerSoil Kit

Data Source: Illumina MiSeq System

DATASET TAGS

soil microbiome, consortia, model microbiome, chitin degradation, Amplicon, 16S, soil microbiology, WA, IAREC, earth systems science, biology, chitin enrichment

https://doi.org/10.25584/WAIsoCMSC1/1635272

DATA PACKAGE CONTENTS

MIMARKS Environmental/Soil Standard Information (metadata)

- WA-IsoC_MSC1.1.0_MIMARKS.survey.soil.version.5.0 (.xlsx)

  Minimum information about a marker gene sequence (MIMARKS) use is for any type of marker gene sequences, eg, 16S, 18S, 23S, 28S rRNA or COI obtained from cultured or voucher-identifiable specimens. Organism cannot contain the term 'metagenome'. This package includes attributes defined by the Genome Standards Consortium (GSC) to formally describe and standardize sample metadata.

Amplicon 16S Data

- WA-IsoC MSC1.1.0 Amplicon 16S Dataset Key (.xlxs)

  TAB 1 - 16S_Initial Consortia

  TAB 2 - 16S_Reconstitution Consortia

  TAB 3 - 16S_Isolates of Consortia

  TAB 4 - 16S_MSC1 in Soil

- WA-IsoC MSC1.1.0 Initial Consortia [11.36 GB; 768 items]

  Full & Sectioned Communities A-H; Weeks 1-8; Dilutions 10^{-2}, 10^{-3}, 10^{-4} (TAB 1)

  Soil samples were collected in 100 g portions (3 replicates), sieved (4 mm), added to 250 ml Mason Jars containing powdered chitin. Sterile deionized water was added to establish field capacity (24% soil moisture), weighing jars weekly (adding water as needed) to ensure that the soil moisture remained constant. Samples were incubated for 7 months (20 °C) in the dark, with respiration used to monitor bioactivity once a week. 1 g soil liquid cultures were serial diluted and 10^{-2} ,10^{-3} and 10^{-4} dilutions were spread onto agar plates containing soil extract and 100 ppm shrimp shell chitin (chitin/soil extract agar). Resulting soil suspensions were centrifuged 30 min at 5000 g and the supernatants were filtered using a 0.22 micron filter to produce the soil extract. After one week of incubation at 20 °C the collective growth on each plate was re-plated onto a new plate. These represent "Full" communities A-H, where the entire community was re-plated. To enrich "Sectioned" communities, a 100 µL aliquot of each dilution was spread onto a full chitin/soil extract agar plate. After one week of growth at 20 °C this plate was divided into 8 sections and each section was re-plated onto a new plate (Part communities A-H). Both Full and Sectioned plating approaches were employed to explore possible heterogeneity in the content and structure of developing communities across the agar surface. The communities were re-plated weekly for 22 weeks, with samples collected for amplicon analysis every week for the first 8 weeks.

- WA-IsoC MSC1.1.0_Reconstitution Consortia [2.05 GB; 146 items]

  Generation 2 Communities; Glycerol Replating; Before/After Lyophilization; Full & Part ¼ Sections (TAB 2)

  Stored microbial biomass was collected from plates and resuspended in soil extract liquid, adjusting the O.D.600 to 0.35. Cell suspensions were diluted 1:10 in soil extract and 25 µL aliquots added to

cryotubes followed by addition of 25 µL of 50% glycerol. Lyophilized stocks of 50 µL aliquots 1:10 dilutions were dried for 24 h in a Benchtop Freeze Dryer Lyophilizer (2.5 L). Both glycerol and lyophilized stocks were stored at -80 °C. Consortia regrowth from stocks, glycerol or lyophilized stocks were spread with an inoculum loop onto a 1/4th section of a chitin/soil extract agar plate and incubated at 20 °C for one week.

- WA-IsoC MSC1.1.0 Isolates of Consortia [1.7 GB; 118 items]

  Soil Community isolates; Isolation plating on +/- chintin, R2A agar; Isolates 1-30; (TAB 3)

  Soil community was plated onto chitin/soil extract agar and incubated at 20 °C. Colonies were re-plated onto chitin/soil extract agar plates and incubated at 20 °C until sufficient biomass was available for amplicon analysis. The resultant colonies were isolated, DNA was collected and 16S amplicon analysis was used to putatively identify isolates. Glycerol stocks were made of all isolates by resuspending isolated colonies in sterile soil extract liquid and adding glycerol to a final concentration of 25% before placing in a -80 °C freezer for storage. The MSC-1 consortium was also grown on other media to collect additional axenic constituent strains. The additional media included: 1) R2A agar, 2) agar with 100 ppm chitin and 10% soil extract (diluted in water), 3) agar made with 100 ppm chitin and no soil extract (replacing with water), and 4) agar made with soil extract but no chitin.

- WA-IsoC MSC1.1.0 MSC1 in Soil [5.24 GB; 560 items]

  Model Soil Consortium-1 (MSC-1); 4 Growth Treatments; Weeks 1-5 (TAB 4)

  MSC-1 was grown on chitin/soil extract agar for one week at 20 °C. Resulting MSC-1 growth was collected and resuspended in soil extract to an O.D. of 0.35. This resuspension was then diluted 1:10 using soil extract and 800 µL of this dilution was added to 8 g of sterile field soil (sterilized by autoclaving and pre-wet with 1.2 mL soil extract 48 h before the start of the experiments). The following treatments were applied to the sterile soil incubations: 1) Standard: sterile soil wet to 25% (v/v) with soil extract, incubated at 20 °C with 100 ppm chitin; 2) high temperature (HighTemp): same as Standard but incubated at 37 °C; 3) Low Temperature (LowTemp): same as Standard but incubated at 10 °C; 4) No chitin (NoChitin): same as Standard but lacking chitin; 5) Salt stress (SaltStress): same as standard but supplemented with 100 mM NaCl; 6) incubation with 2,4-Dichlorophenoxyacetic acid (Herbicide): same as Standard but supplemented with 15 ppm of 2,4-Dichlorophenoxyacetic acid (2,4 D) per gram of soil. Each sterile soil incubation condition was performed with 5 replicates. Sub-samples (0.25 g) were collected once a week for amplicon analysis

Model Microbial Community R Markdown

- c2manuscript_Rscripts.Rmd (.rmd; 1 item)

A series of model microbial consortia were generated through plating dilutions of native soil on a media of soil extract with chitin and analyzed using the following code in the R markdown file for modeling "Reproducible Storage, Reconstitution and Analysis of a Model Microbial Community Representing a Marginal Soil"

https://doi.org/10.25584/WAIsoCMSC1/1635272

Supplemental Material

Supplemental Figures (8 figures, 1 table)

- **SI Figure 1. Respiration of native soil with chitin.** Respiration levels of native soil grown in dark at 20 °C for several months is indicted. The orange line represents respiration levels with no chitin added. The grey line represents respiration with chitin added to 10 ppm. The yellow line represents respiration with chitin added to 50 ppm. The blue line represents respiration with chitin added to 100 ppm. Error bars indicate standard deviation.

- **SI Figure 2. Reconstitution of consortia.** Phylum level representation of each consortium is shown for the original parent consortia after 22 weeks on plates (Original), and in each of 4-5 glycerol or lyophilized stocks.

- **SI Figure 3. Reconstitution of consortia.** Class level representation of each consortium is shown for the original parent consortia after 22 weeks on plates (Original), and in each of 4-5 glycerol or lyophilized stocks.

- **SI Figure 4. Reconstitution of consortia.** Order level representation of each consortium is shown for the original parent consortia after 22 weeks on plates (Original), and in each of 4-5 glycerol or lyophilized stocks.

- **SI Figure 5. Bray-Curtis distances of reconstitution of consortia.** Each parent consortium is shown as a colored square, colors for each consortium are shown on the right. Each glycerol stock is shown as a colored circle with colors that match its parent consortium. Each lyophilization stock is shown as a colored triangle with colors that match its parent consortium.

- **SI Figure 6. Parent/stock comparisons of consortia for glycerol storage.** Bray-Curtis distance is on the y-axis for the reconstituted stock, the parent community and the distance from the other six parent communities. A star indicates NOT significantly different (p-value > 0.05).

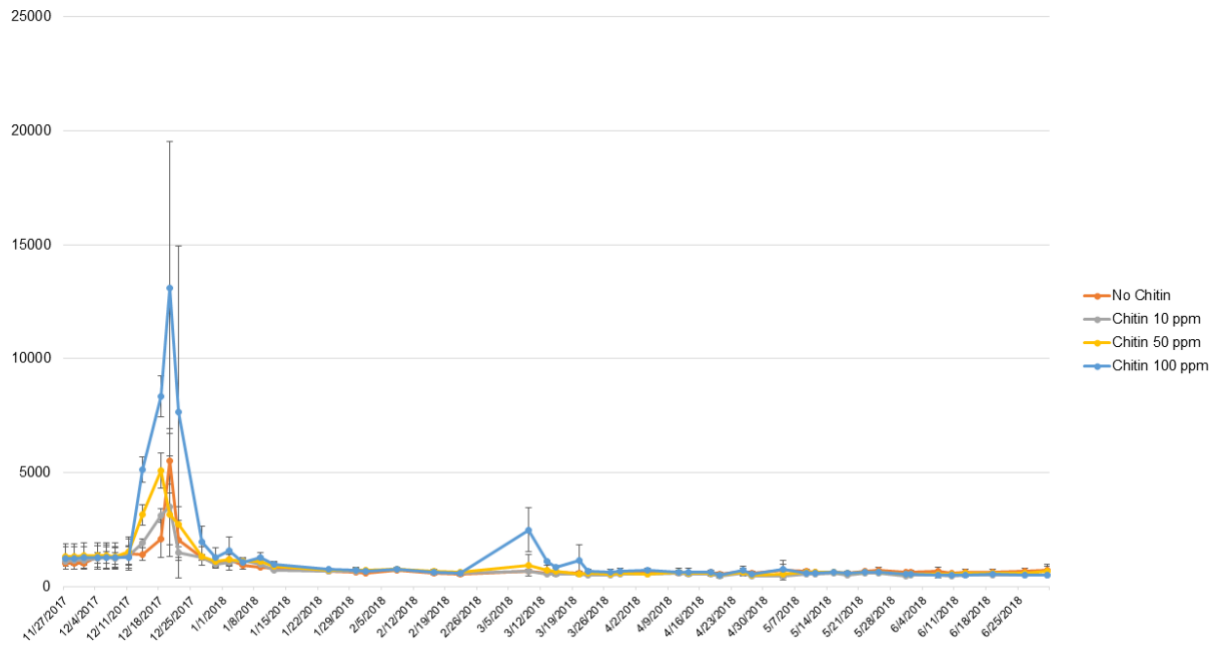
- **SI Figure 7. Parent/stock comparisons of consortia for lyophilization storage.** Bray-Curtis distance is on the y-axis for the reconstituted stock, the parent community and the distance from the other six parent communities. A * indicates NOT significantly different (p-value > 0.05).

- **SI Figure 8. Reconstitution of Generation 2 of consortia.** Consortia are shown on the x-axis with type shown along the top. Bray-Curtis distances are shown on the y-axis.

https://doi.org/10.25584/WAIsoCMSC1/1635272

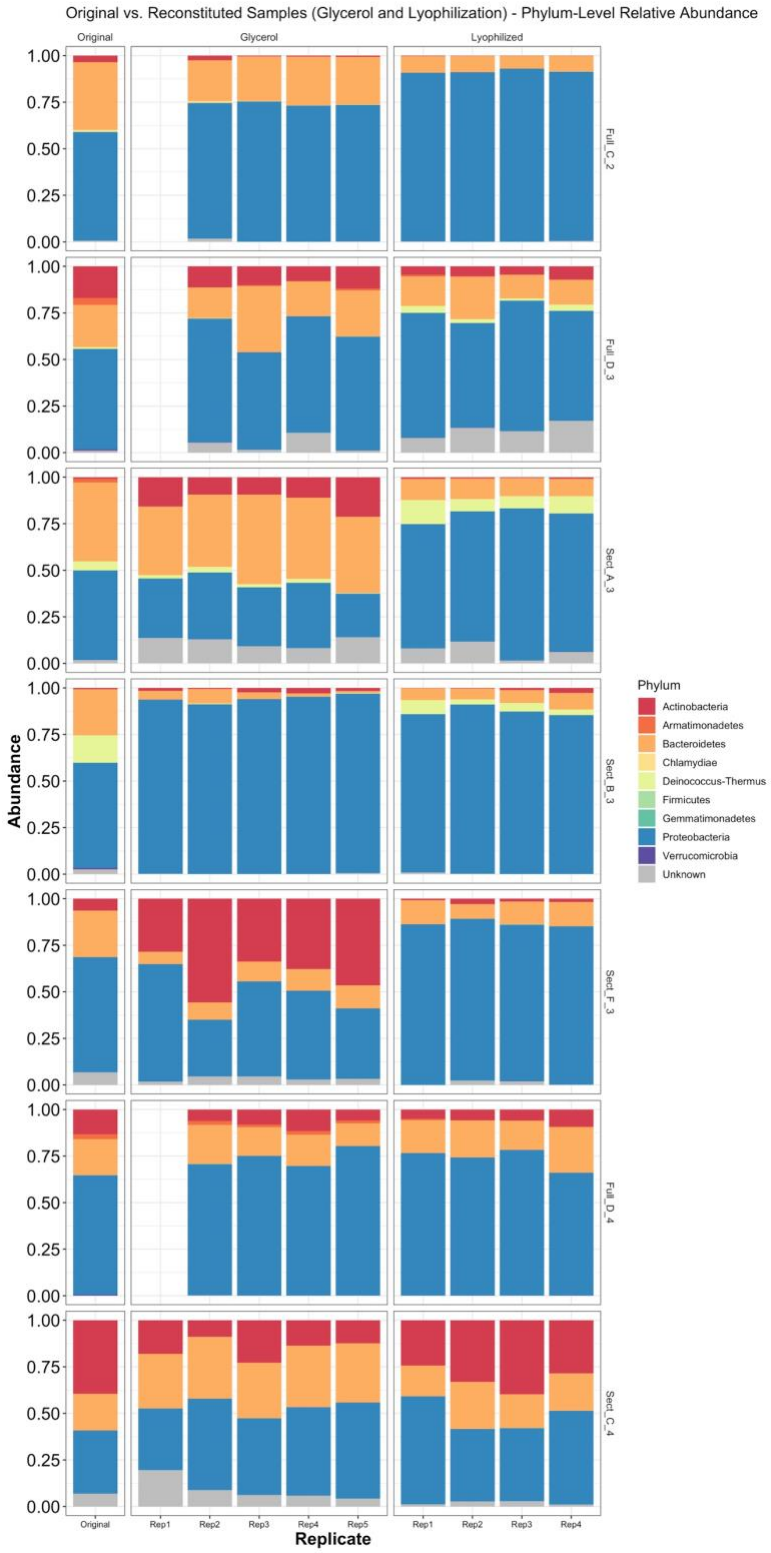
- **SI Table 1. Taxonomic makeup of MSC-1.** *Based on V4 amplicon data, it could not be distinguished which of these Ensifer species matches to the Ensifer isolate (see attached .xlsx file on DataHub data download page).

SI.1



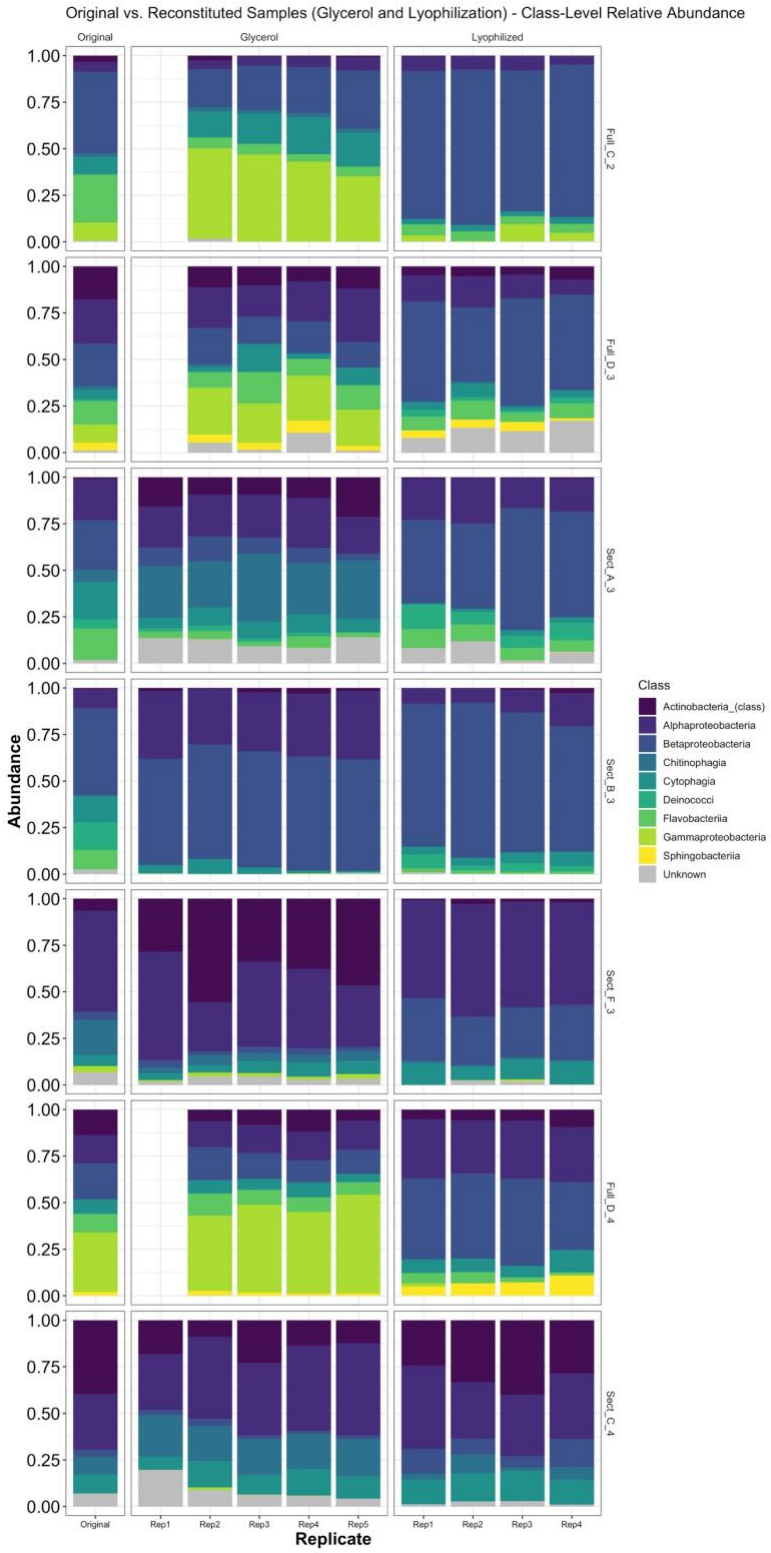
https://doi.org/10.25584/WAIsoCMSC1/1635272

SI.2



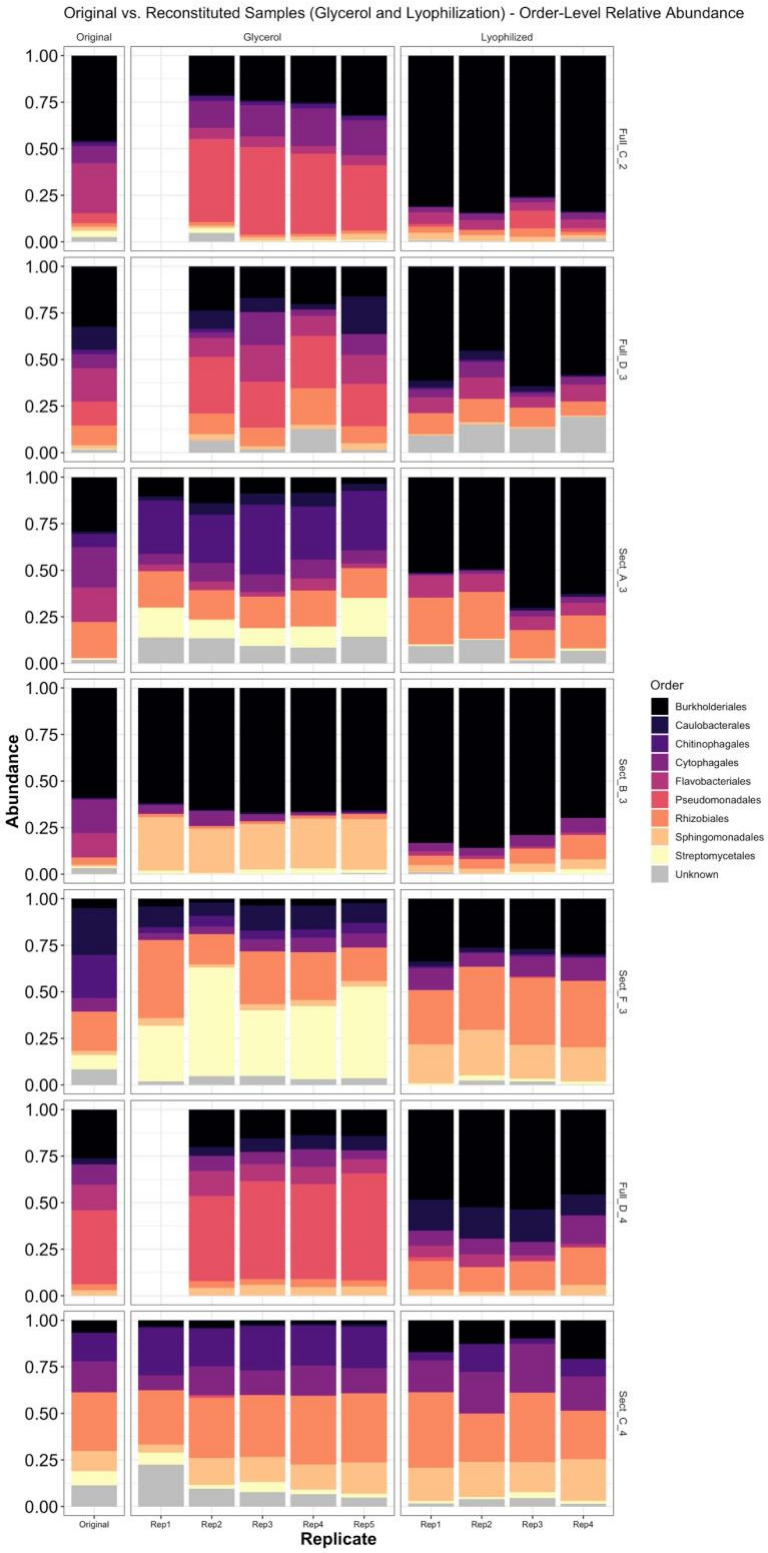
https://doi.org/10.25584/WAIsoCMSC1/1635272

SI.3



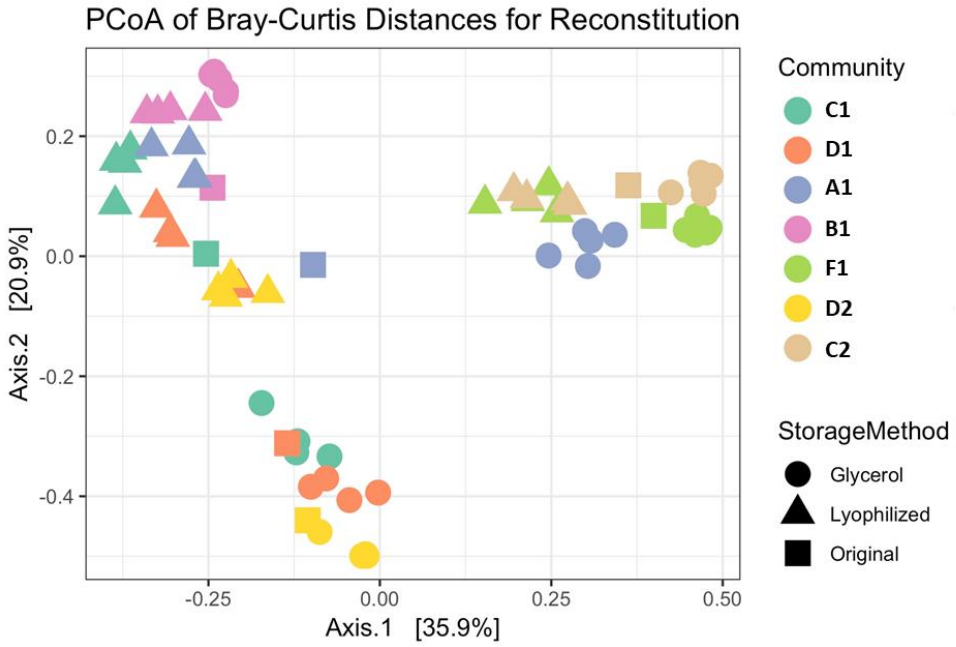
https://doi.org/10.25584/WAIsoCMSC1/1635272

SI.4

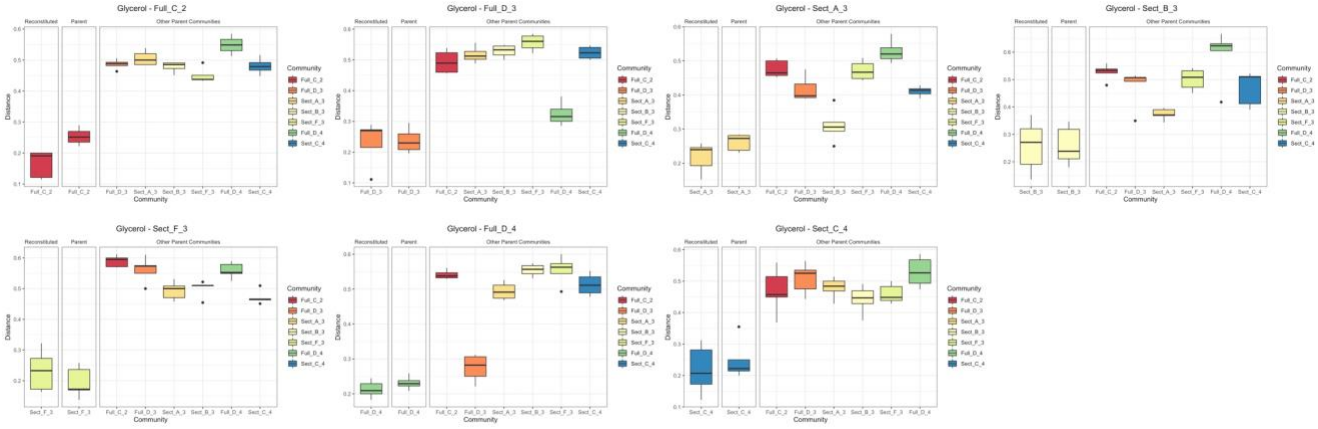


https://doi.org/10.25584/WAIsoCMSC1/1635272

SI.5

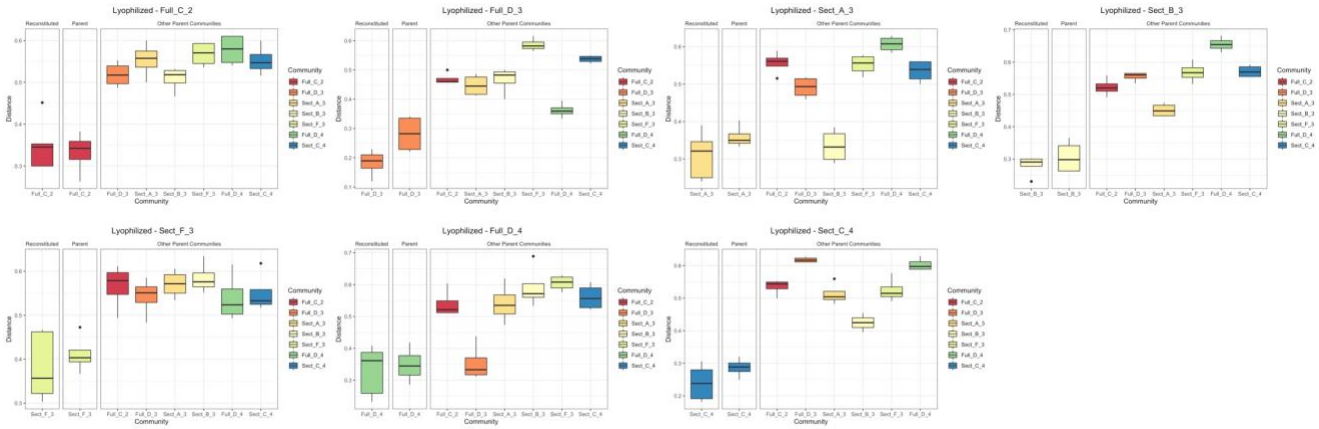


SI.6

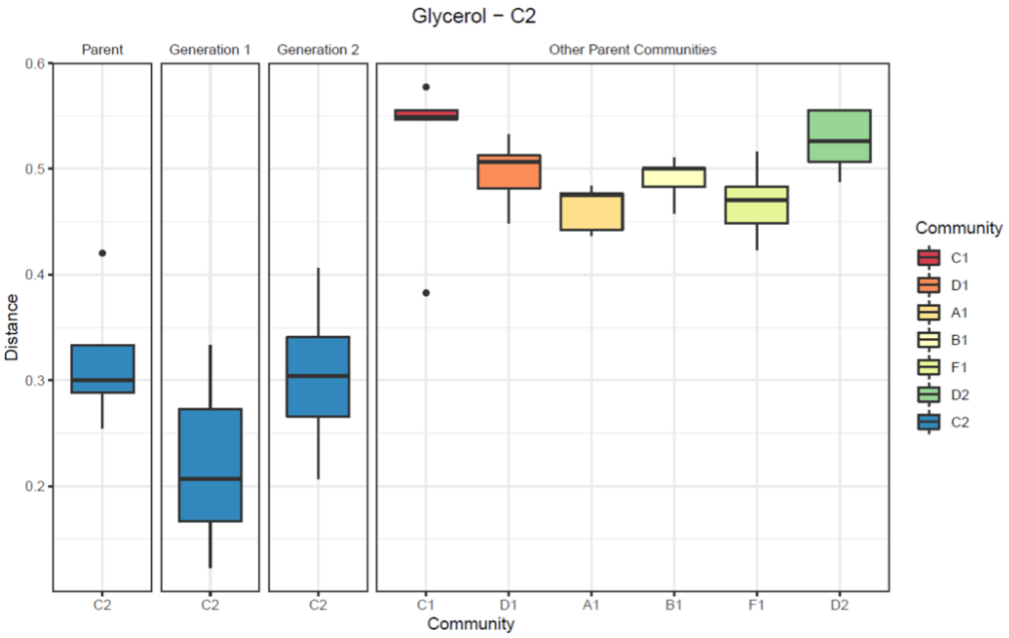


https://doi.org/10.25584/WAIsoCMSC1/1635272

SI.7



SI.8



https://doi.org/10.25584/WAIsoCMSC1/1635272

Acknowledgements

This research was supported by the Department of Energy Office of Biological and Environmental Research (BER) and is a contribution of the Scientific Focus Area “Phenotypic response of the soil microbiome to environmental perturbations.” This research was also supported by Environmental Molecular Sciences Laboratory (EMSL) open call proposal number 48784, Dissecting and Deciphering of the Soil Microbiome. EMSL is a U.S. Department of Energy (DOE) Office of Science user facility sponsored by the DOE’s Office of Biological and Environmental Research and located at PNNL. A portion of the research was conducted using PNNL Institutional Computing (PIC) resources and partially supported by the Microbiomes in Transition Initiative under the Laboratory Directed Research and Development Program at PNNL. PNNL is a multiprogram national laboratory operated by Battelle for the U.S. DOE under contract DE-AC05-76RL01830.

https://doi.org/10.25584/WAIsoCMSC1/1635272