The microbiomes of human excrement composting: toward safe human waste cycling for closed-system horticulture

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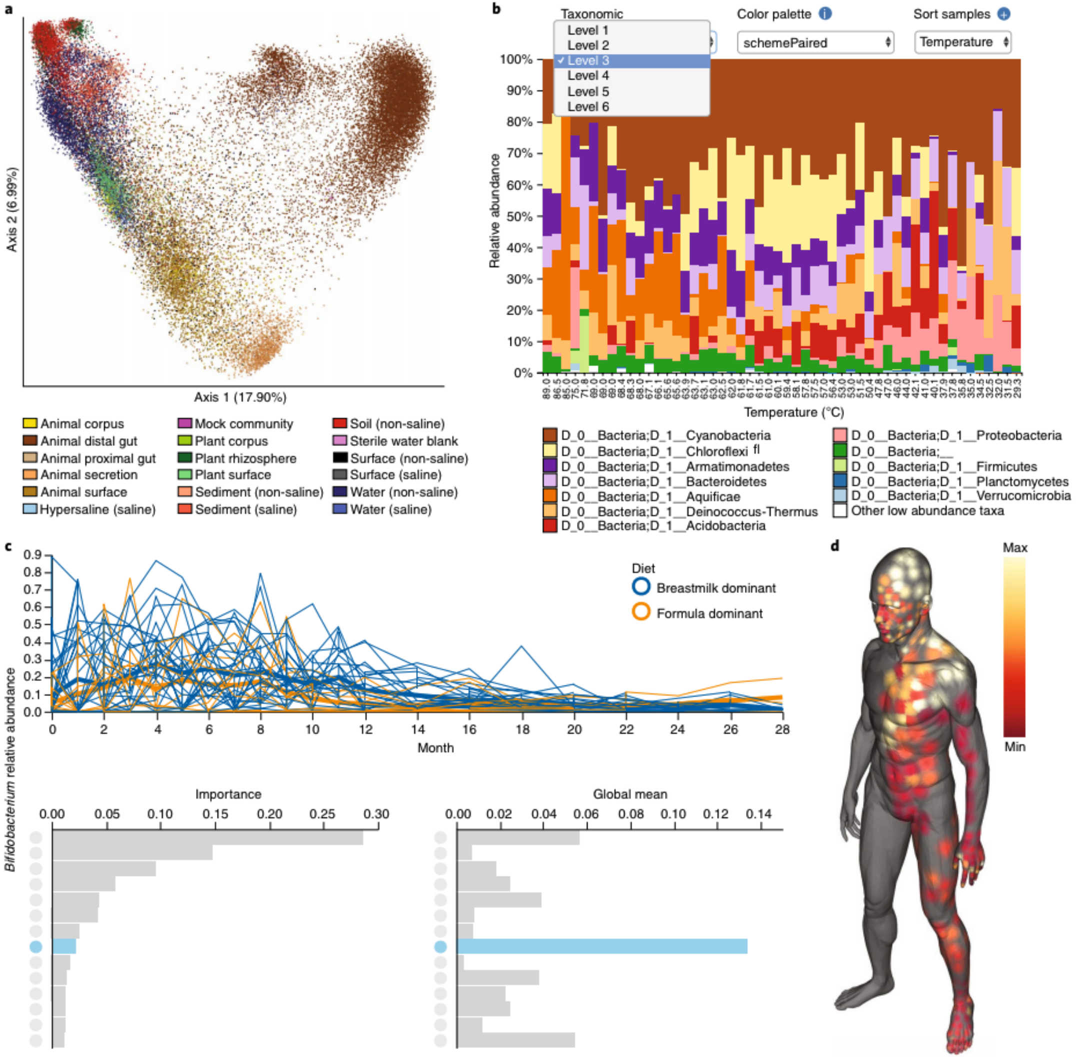
These slides: http://bit.ly/4nyGgC5

nature
biotechnology

Correspondence | Published: 24 July 2019

Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2

Evan Bolyen, Jai Ram Rideout, Matthew R. Dillon, Nicholas A. Bokulich, Christian C. Abnet, Gabriel A. Al-Ghalith, Harriet Alexander, Eric J. Alm, Manimozhiyan Arumugam, Francesco Asnicar, Yang Bai, Jordan E. Bisanz, Kyle Bittinger, Asker Brejnrod, Colin J. Brislawn, C. Titus Brown, Benjamin J. Callahan, Andrés Mauricio Caraballo-Rodríguez, John Chase, Emily K. Cope, Ricardo Da Silva, Christian Diener, Pieter C. Dorrestein, Gavin M. Douglas, Daniel M. Durall, Claire Duvallet, Christian F. Edwardson, Madeleine Ernst, Mehrbod Estaki, Jennifer Fouquier, Julia M. Gauglitz, Sean M. Gibbons, Deanna L. Gibson, Antonio Gonzalez, Kestrel Gorlick, Jiarong Guo, Benjamin Hillmann, Susan Holmes, Hannes Holste, Curtis Huttenhower, Gavin A. Huttley, Stefan Janssen, Alan K. Jarmusch, Lingjing Jiang, Benjamin D. Kaehler, Kyo Bin Kang, Christopher R. Keefe, Paul Keim, Scott T. Kelley, Dan Knights, Irina Koester, Tomasz Kosciolek, Jorden Kreps, Morgan G. I. Langille, Joslynn Lee, Ruth Ley, Yong-Xin Liu, Erikka Loftfield, Catherine Lozupone, Massoud Maher, Clarisse Marotz, Bryan D. Martin, Daniel McDonald, Lauren J. McIver, Alexey V. Melnik, Jessica L. Metcalf, Sydney C. Morgan, Jamie T. Morton, Ahmad Turan Naimey, Jose A. Navas-Molina, Louis Felix Nothias, Stephanie B. Orchanian, Talima Pearson, Samuel L. Peoples, Daniel Petras, Mary Lai Preuss, Elmar Pruesse, Lasse Buur Rasmussen, Adam Rivers, Michael S. Robeson II, Patrick Rosenthal, Nicola Segata, Michael Shaffer, Arron Shiffer, Rashmi Sinha, Se Jin Song, John R. Spear, Austin D. Swafford, Luke R. Thompson, Pedro J. Torres, Pauline Trinh, Anupriya Tripathi, Peter J. Turnbaugh, Sabah Ul-Hasan, Justin J. J. van der Hooft, Fernando Vargas, Yoshiki Vázquez-Baeza, Emily Vogtmann, Max von Hippel, William Walters, Yunhu Wan, Mingxun Wang, Jonathan Warren, Kyle C. Weber, Charles H. D. Williamson, Amy D. Willis, Zhenjiang Zech Xu, Jesse R. Zaneveld, Yilong Zhang, Qiyun Zhu, Rob Knight & J. Gregory Caporaso



Editorial | Open access | Published: 16 September 2024

Microbiome multi-omics can accelerate human excrement composting research

Jeff Meilander, Mary Jewell & J. Gregory Caporaso

Microbiome **12**, Article number: 174 (2024) | Cite this article

3013 Accesses | **1** Citations | **3** Altmetric | Metrics

JOURNAL ARTICLE

Microbiome science of human excrement composting

Jeff Meilander, J Gregory Caporaso

The ISME Journal, Volume 18, Issue 1, January 2024, wrae228, https://doi.org/10.1093/ismejo/wrae228

Published: 09 November 2024 **Article history** ▾

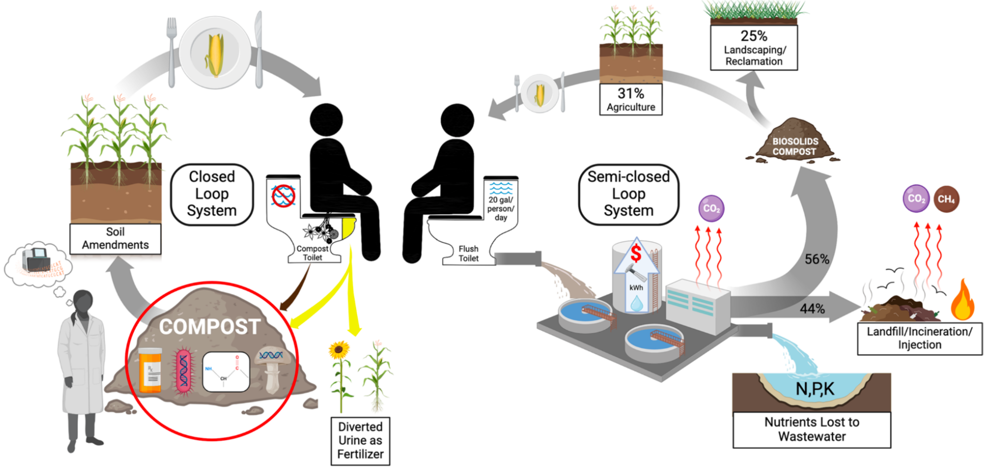
JOURNAL ARTICLE

Upcycling human excrement: the gut microbiome to soil microbiome axis

Jeff Meilander, Chloe Herman, Andrew Manley, Georgia Augustine, Dawn Birdsell, Evan Bolyen, Kimberly R Celona, Hayden Coffey, Jill Cocking, Teddy Donoghue ... Show more

ISME Communications, Volume 5, Issue 1, January 2025, ycaf089, https://doi.org/10.1093/ismeco/ycaf089

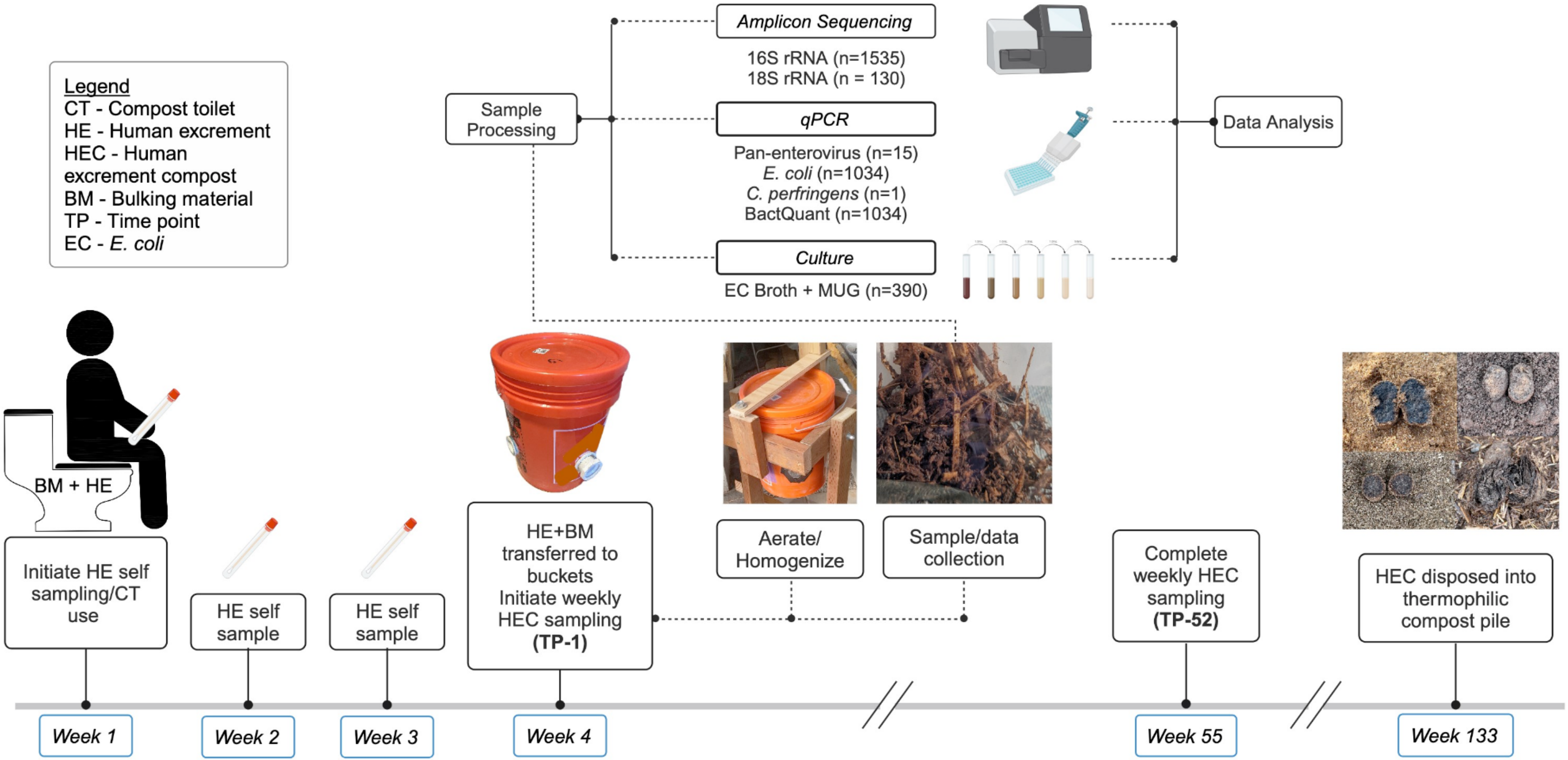
Published: 29 May 2025 **Article history** ▾



Jeff Meilander, PhD

Microbiomes of human excrement composting: initial experiment

Legend
CT - Compost toilet
HE - Human excrement
HEC - Human excrement compost
BM - Bulking material
TP - Time point
EC - *E. coli*



Sample Processing

Amplicon Sequencing
16S rRNA (n=1535)
18S rRNA (n = 130)

qPCR
Pan-enterovirus (n=15)
E. coli (n=1034)
C. perfringens (n=1)
BactQuant (n=1034)

Culture
EC Broth + MUG (n=390)

Data Analysis

BM + HE

Initiate HE self sampling/CT use

HE self sample

HE self sample

HE+BM transferred to buckets
Initiate weekly HEC sampling
(TP-1)

Aerate/ Homogenize

Sample/data collection

Complete weekly HEC sampling
(TP-52)

HEC disposed into thermophilic compost pile

Week 1

Week 2

Week 3

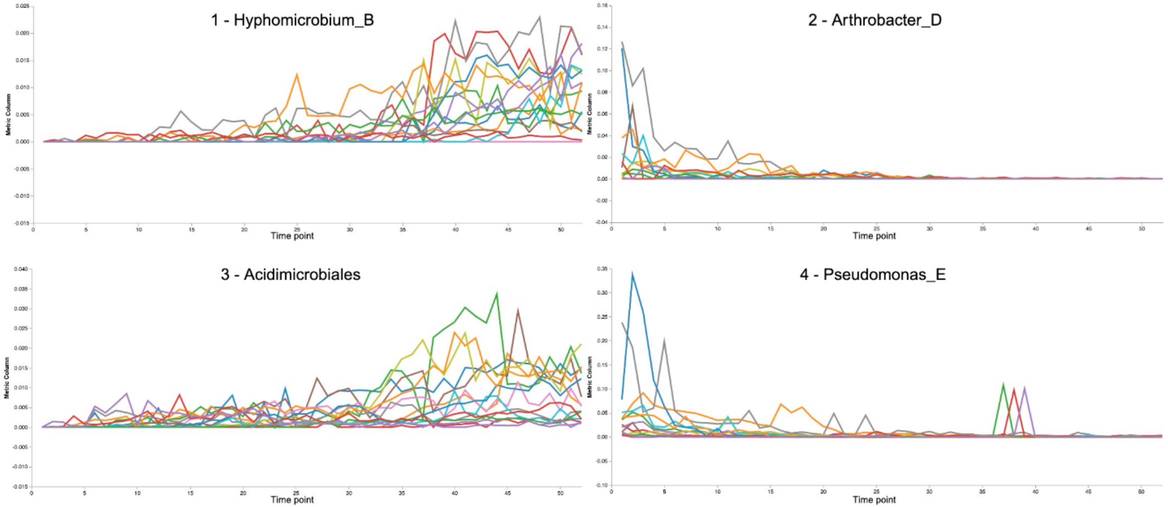
Week 4

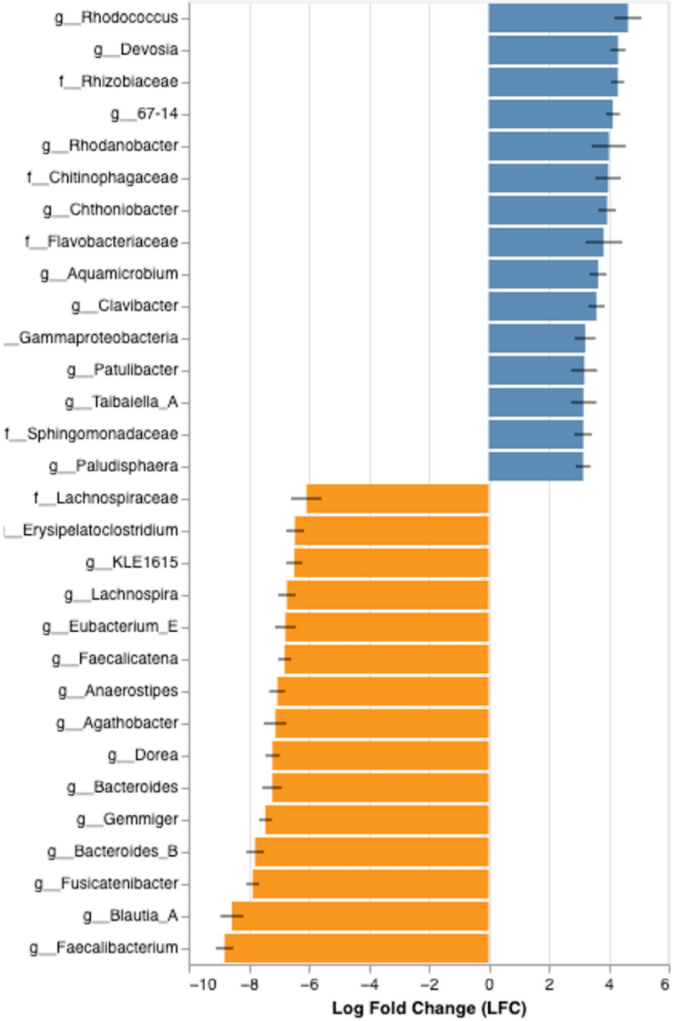
Week 55

Week 133

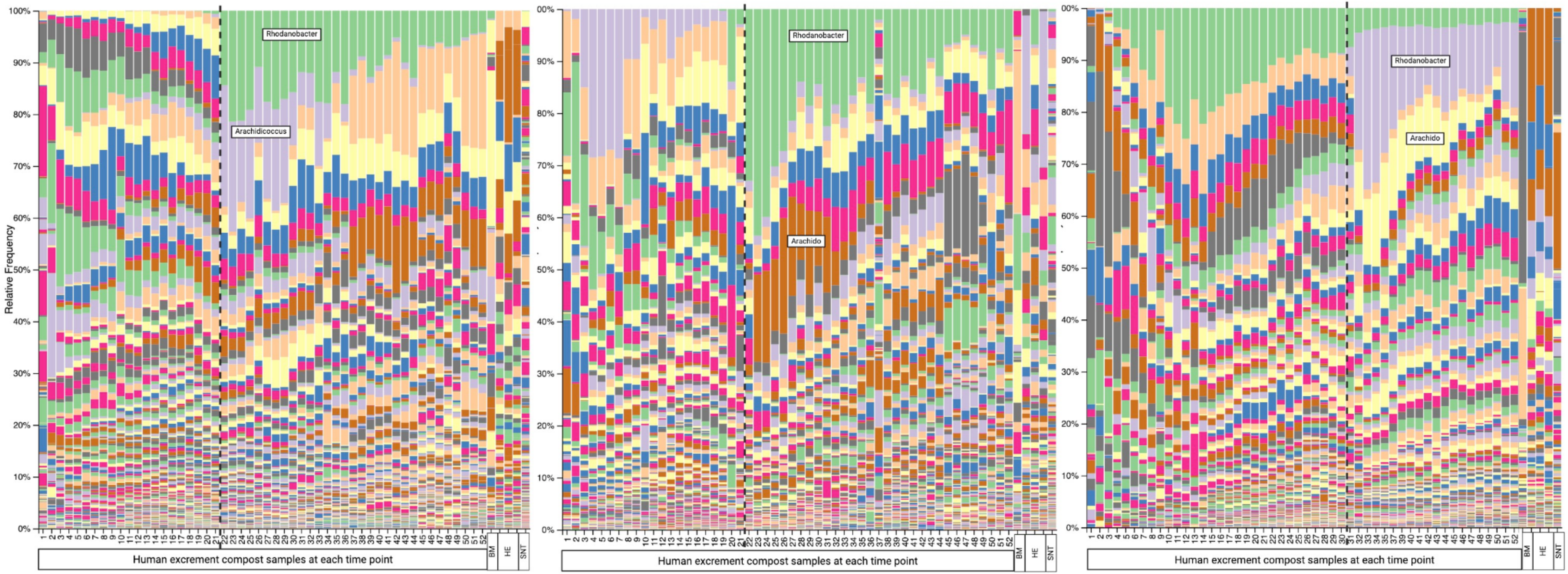
We observe significant:

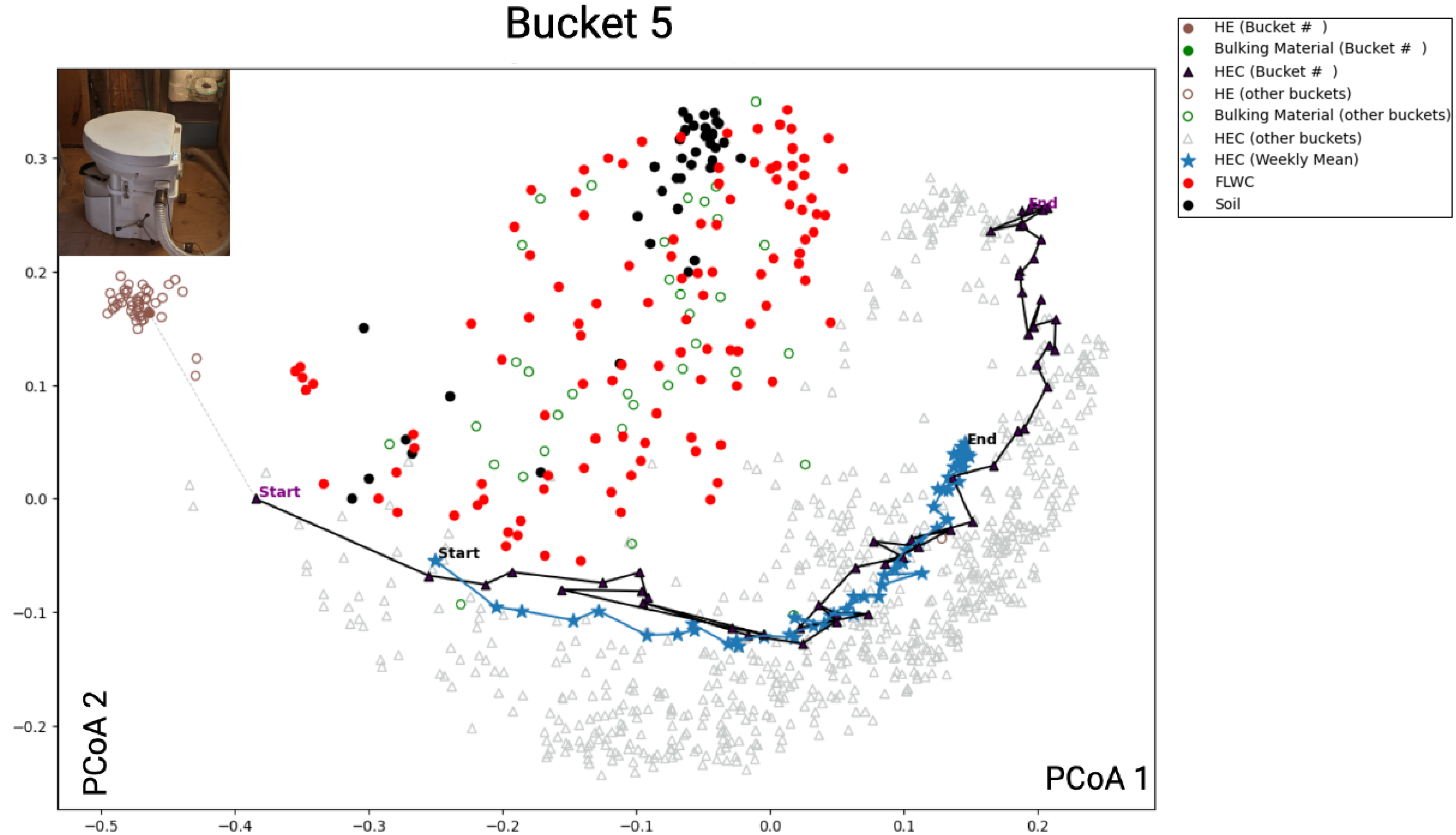
- increases in abundances of soil-associated microorganisms, and
- decreases in abundance of fecal-associated microorganisms.



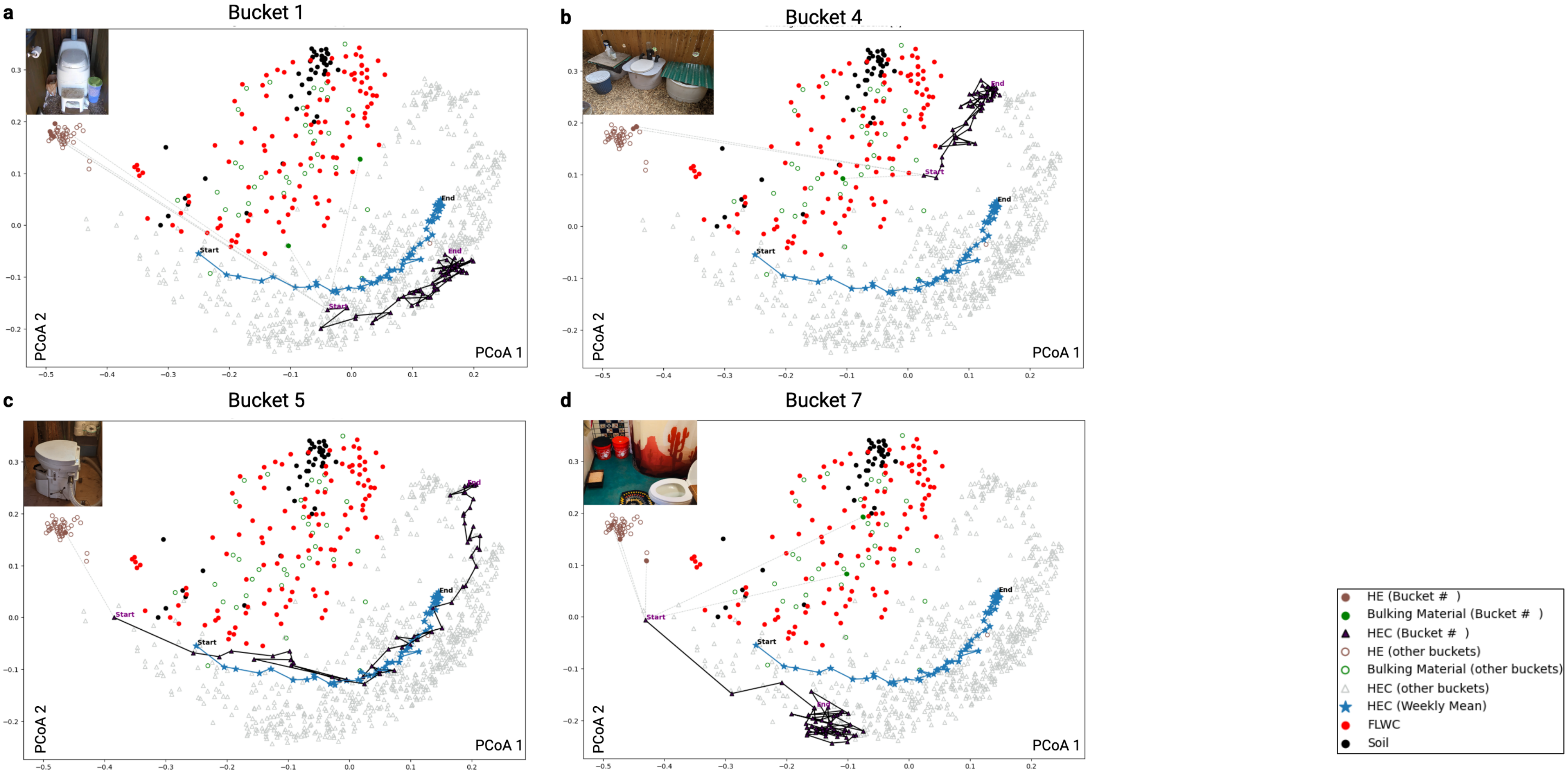


The “gut-to-soil microbiome axis” in three buckets.

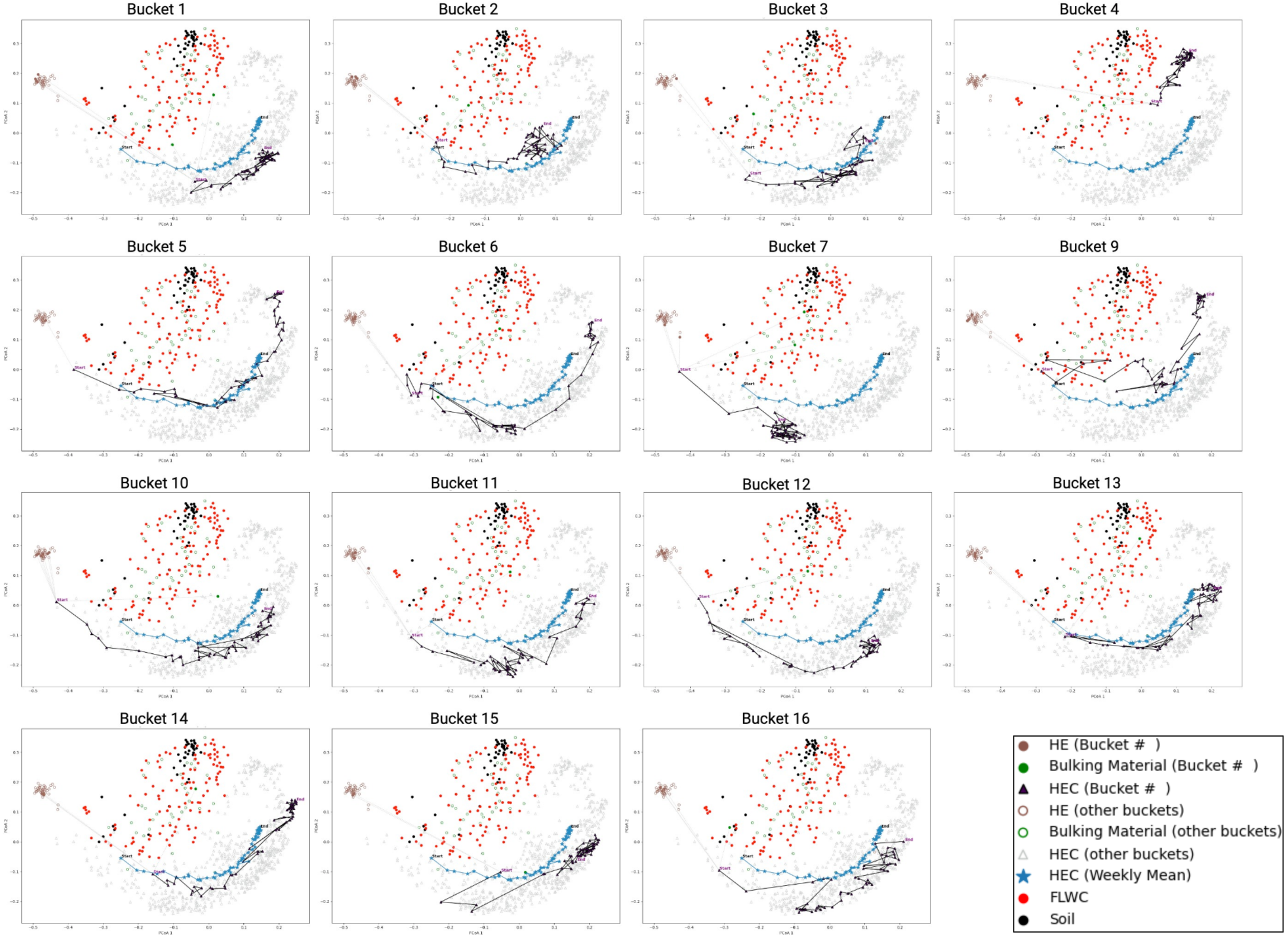



Bucket 5
HE (Bucket #)
Bulking Material (Bucket #)
HEC (Bucket #)
HE (other buckets)
Bulking Material (other buckets)
HEC (other buckets)
HEC (Weekly Mean)
FLWC
Soil
Start
End
Start
End
PCoA 2
PCoA 1
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.5
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2

All buckets progress along the gut-to-soil trajectory, though at different rates and to different extents.

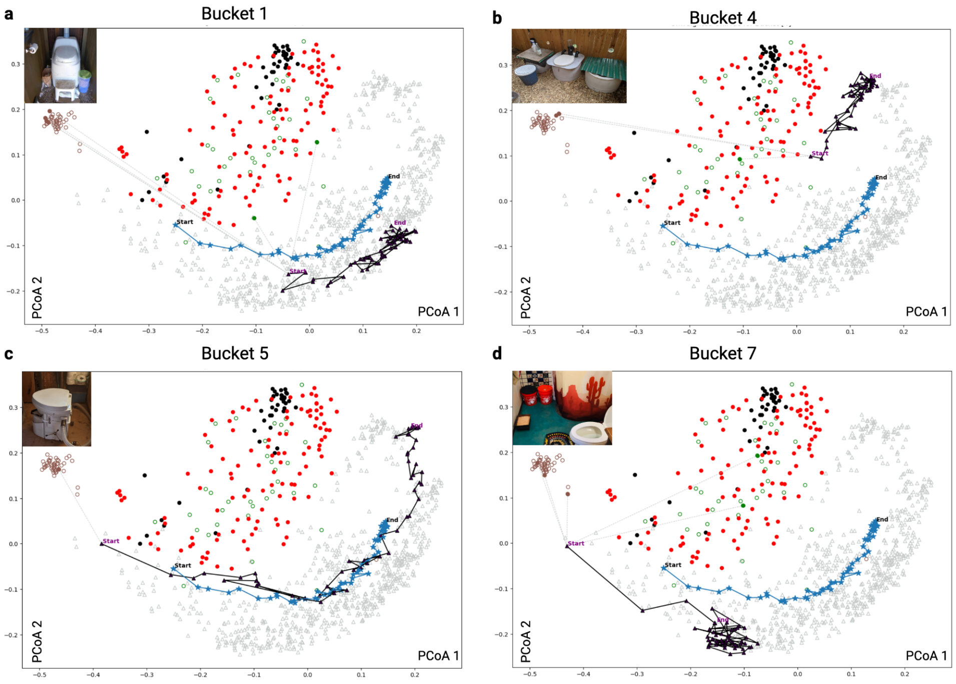


All buckets progress along the gut-to-soil trajectory, though at different rates and to different extents.

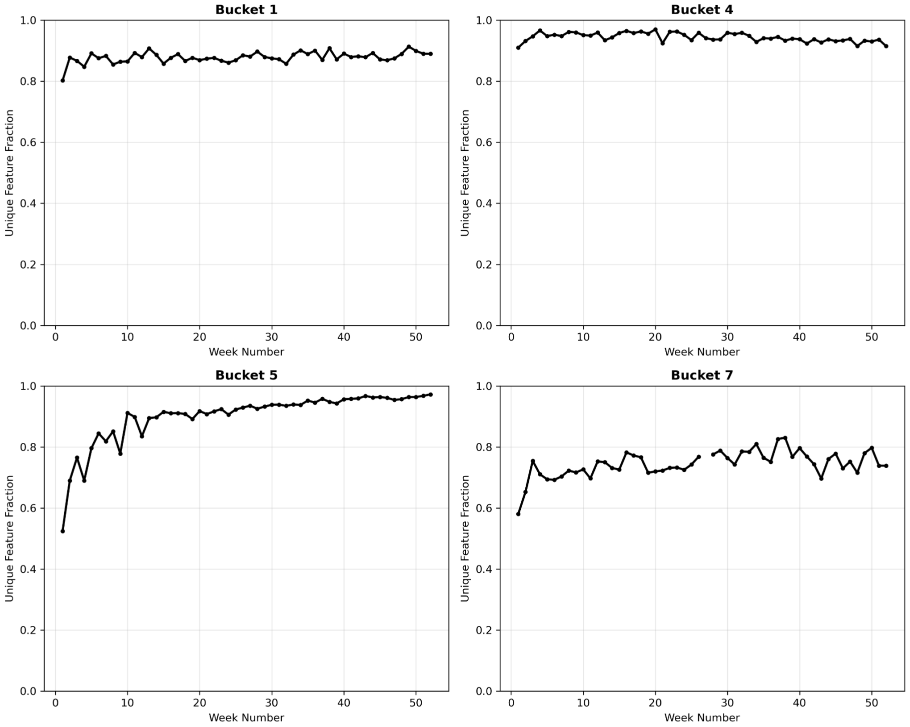


Most of the microbes involved in composting do not appear to be present in the source samples (fecal and bulking materials).

Where do they come from?
Would the reaction proceed without them?

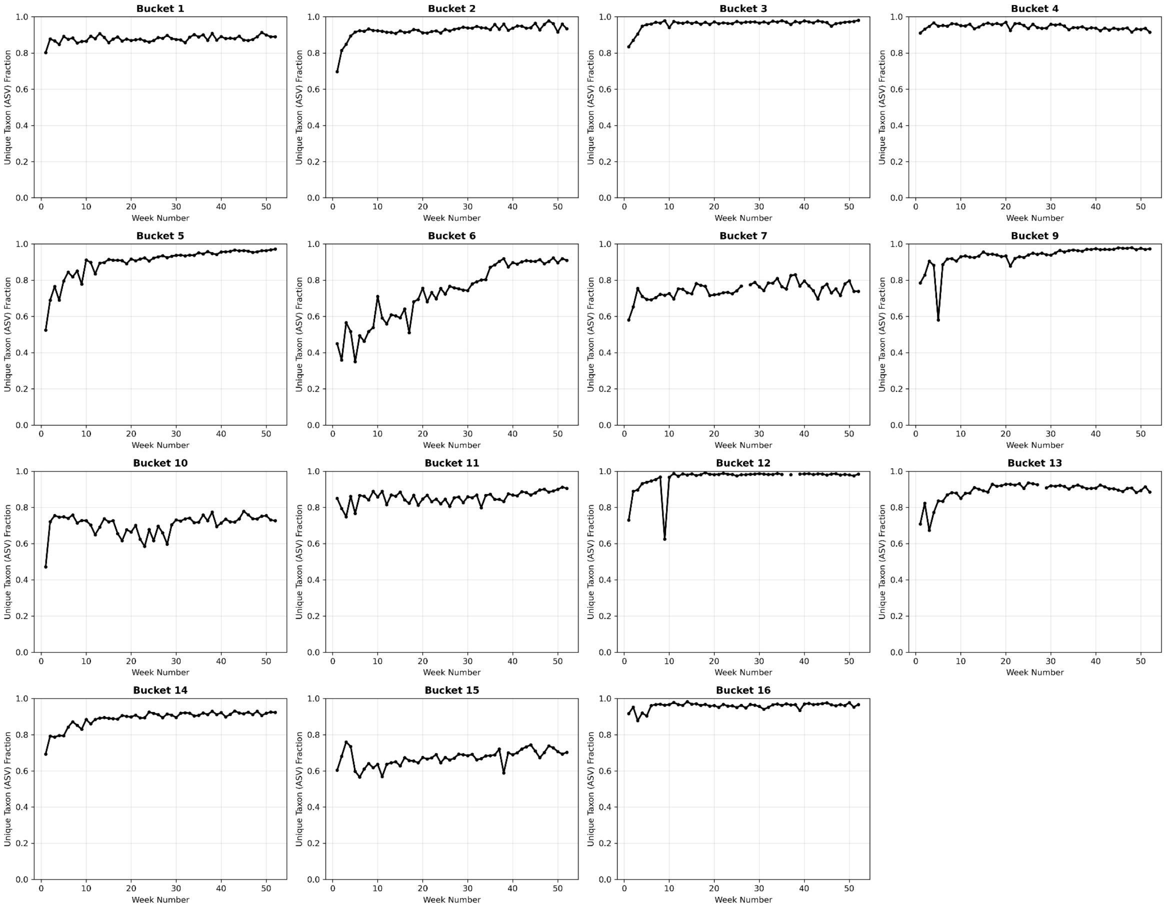


Fraction of microbes observed by week that are **not observed in the source materials**.



Bass Becking hypothesis of microbial biogeography (1934): “everything is everywhere: but the environment selects”.

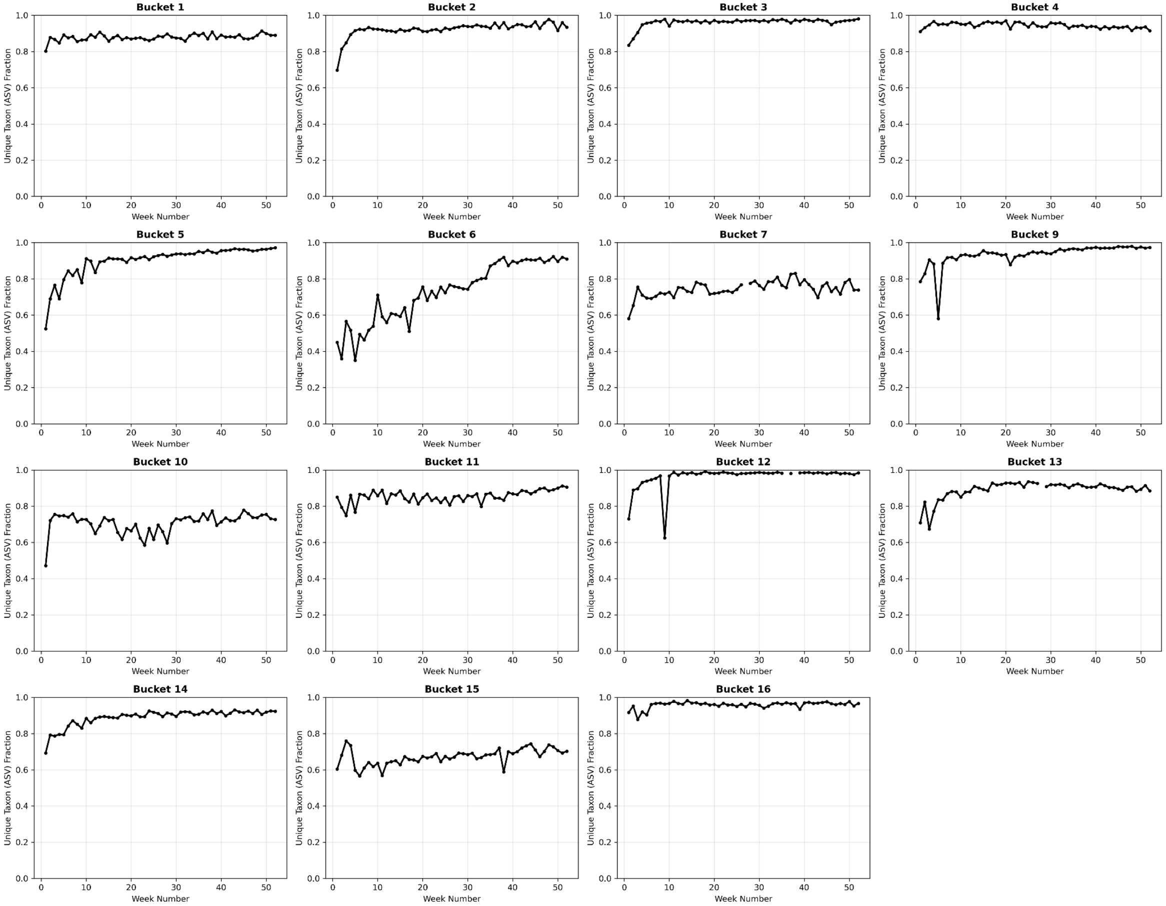
Fraction of microbes observed by week that are **not observed in the source materials.**



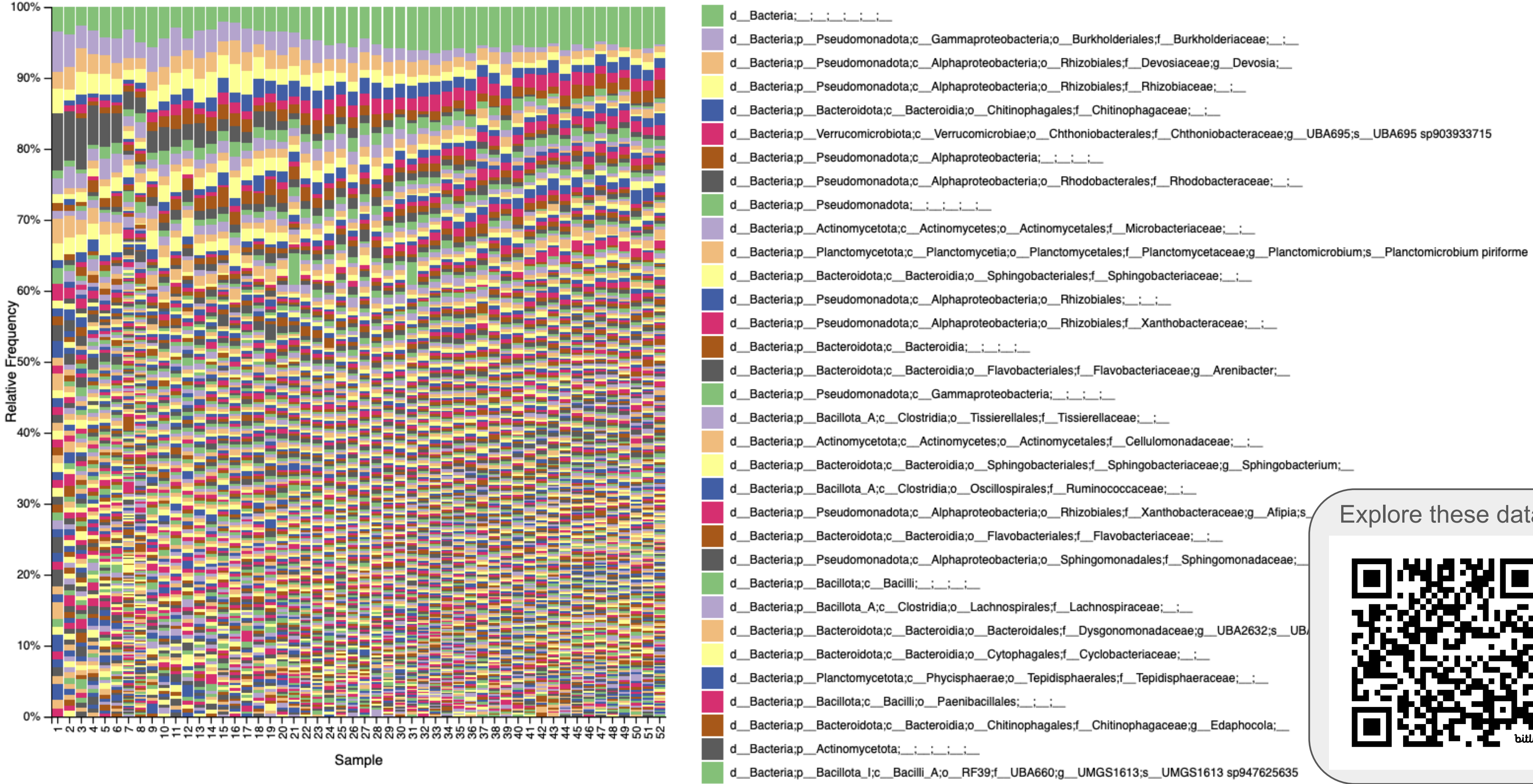
Fraction of microbes observed by week that are **not observed in the source materials.**

Bass Becking hypothesis of microbial biogeography (1934): “everything is everywhere: but the environment selects”.

This is useful for conceptualizing microbial dispersion on Earth. But if we want to compost off-Earth, we should bring the microbes who drive the reaction.



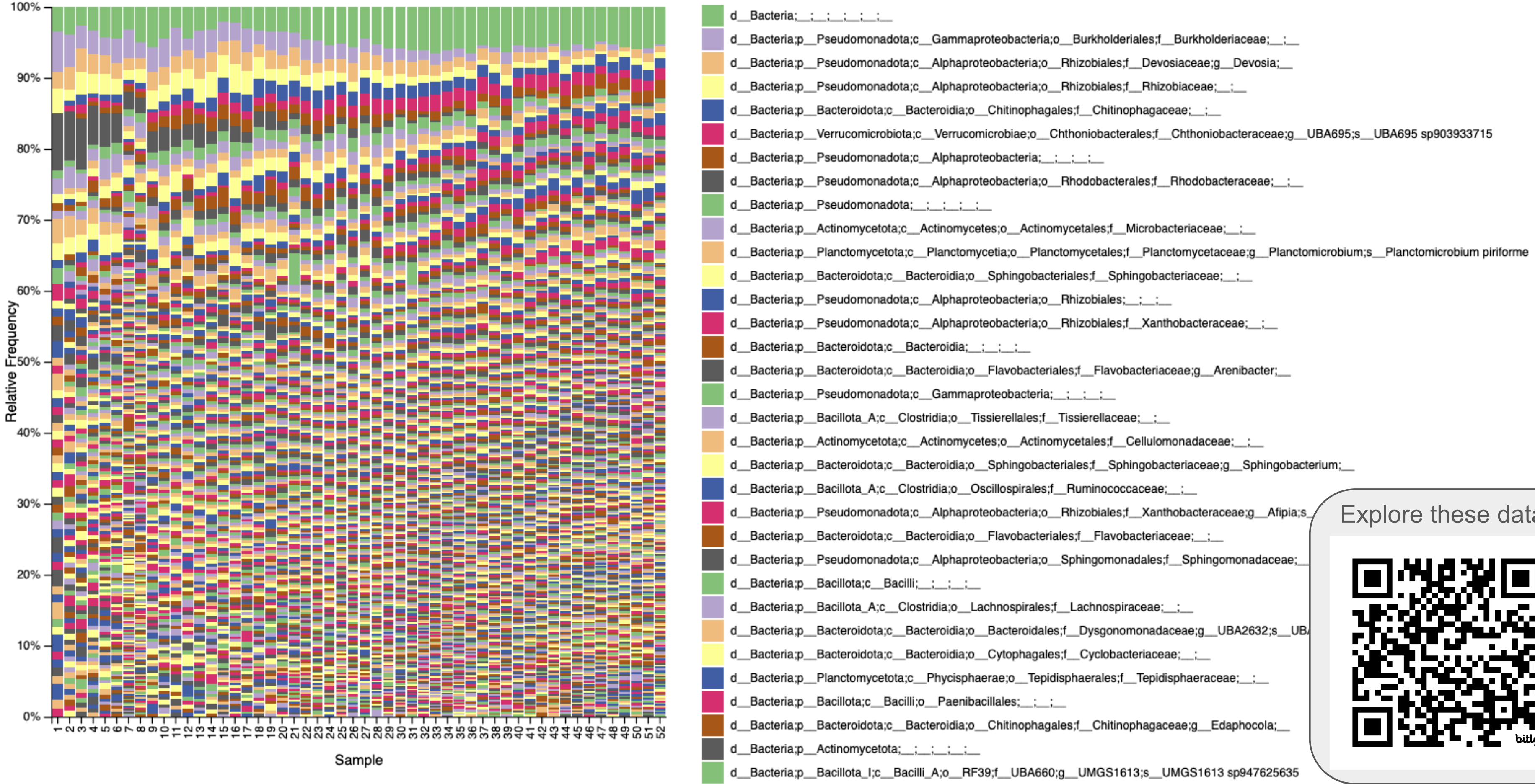
Bucket 5: Unique species relative to source samples by week of composting (or, a space composting packing list)



Explore these data:

bitly

Many of these appear to be uncharacterized organisms, or “microbial dark matter”



Explore these data:

bitly

On-going experiments

- Thermophilic composting of eight biological replicates with microbial activity tracking (microbial metatranscriptomics).

Photo credit: Greg Caporaso

On-going experiments

- Thermophilic composting of eight biological replicates with microbial activity tracking (microbial metatranscriptomics).
- Vermicomposting (i.e., worm-based composting), including sequencing of worm gut microorganisms.

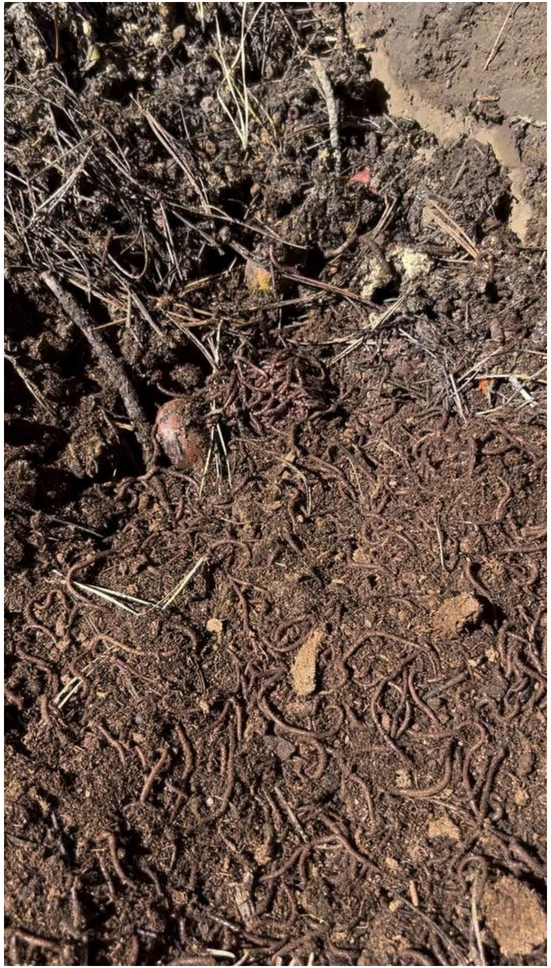


Photo credit: Jeff Meilander

On-going experiments

- Thermophilic composting of eight biological replicates with microbial activity tracking (microbial metatranscriptomics).
- Vermicomposting (i.e., worm-based composting), including sequencing of worm gut microorganisms.
- Plant growth experiments in simulated lunar and martian regolith.

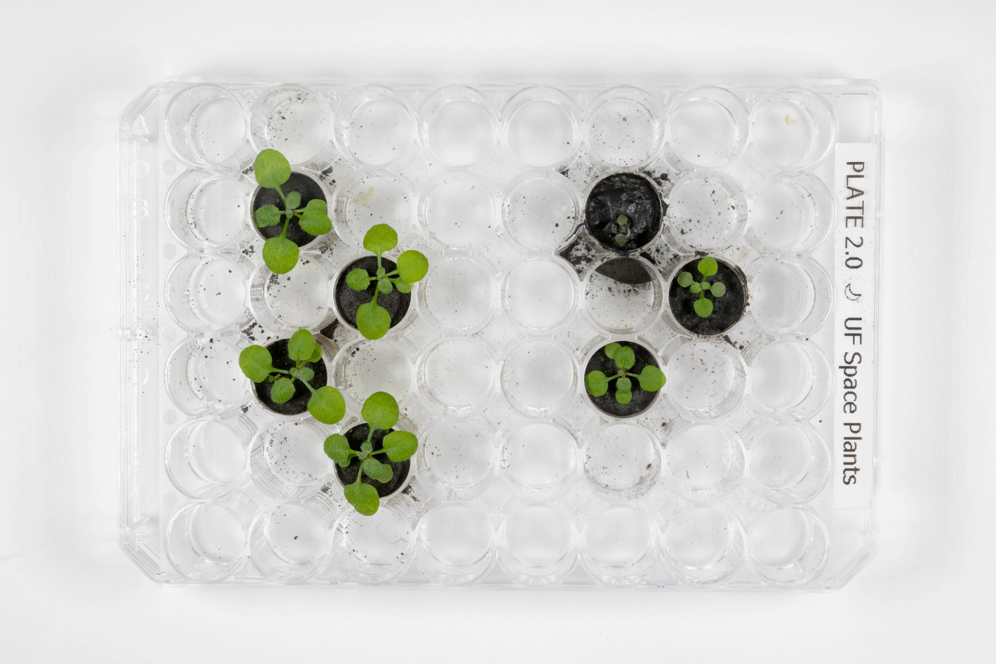


Image source: https://www.nasa.gov/humans-in-space/scientists-grow-plants-in-lunar-soil/
UF/IFAS photo by Tyler Jones

On-going experiments

- Thermophilic composting of eight biological replicates with microbial activity tracking (microbial metatranscriptomics).
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- Plant growth experiments in simulated lunar and martian regolith.
- Sequencing with Oxford Nanopore sequencing technology, as deployed on ISS.

Image source:
https://nanoporetech.com/products/sequence/minion

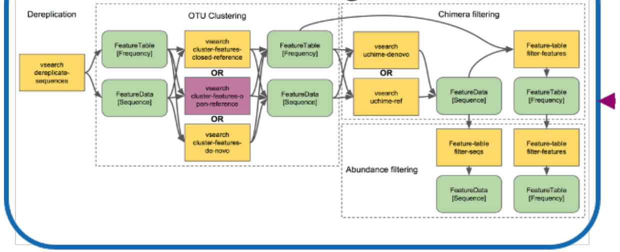
On-going experiments

- Thermophilic composting of eight biological replicates with microbial activity tracking (microbial metatranscriptomics).
- Vermicomposting (i.e., worm-based composting), including sequencing of worm gut microorganisms.
- Plant growth experiments in simulated lunar and martian regolith.
- Sequencing with Oxford Nanopore sequencing technology, as deployed on ISS.
- What else? We are seeking collaboration!

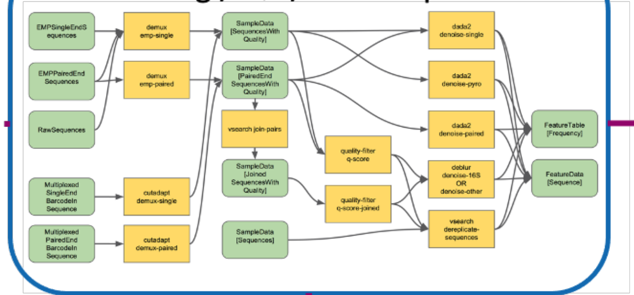
NAU **C**ompost **R**esearch **A**pplications **P**rogram

Extensive options for **amplicon sequencing analysis**.
Learn more at: https://amplicon-docs.qiime2.org

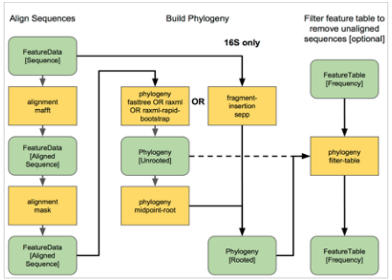
Clustering


Dereplication
vsearch dereplicate-sequences
OTU Clustering
FeatureTable [Frequency]
FeatureData [Sequence]
vsearch cluster-features-closed-reference
OR
vsearch cluster-features-open-reference
OR
vsearch cluster-features-de-novo
FeatureTable [Frequency]
FeatureData [Sequence]
Chimera filtering
vsearch uchime-denovo
OR
vsearch uchime-ref
FeatureData [Sequence]
Feature-table filter-features
FeatureTable [Frequency]
Abundance filtering
Feature-table filter-seqs
Feature-table filter-features
FeatureData [Sequence]
FeatureTable [Frequency]

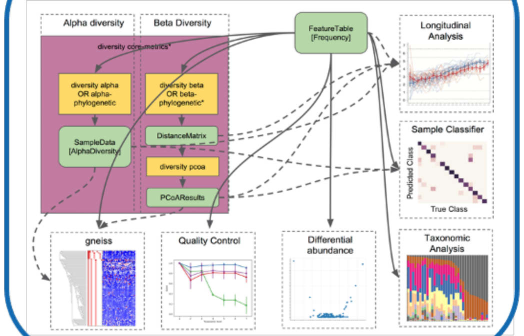
Denoising / QC / Demultiplex


EMPSingleEndSequences
EMPPairedEnd Sequences
RawSequences
Multiplexed SingleEnd BarcodeIn Sequence
Multiplexed PairedEnd BarcodeIn Sequence
demux emp-single
demux emp-paired
cutadapt demux-single
cutadapt demux-paired
SampleData [SequencesWithQuality]
SampleData [PairedEndSequencesWithQuality]
vsearch join-pairs
SampleData [JoinedSequencesWithQuality]
SampleData [Sequences]
quality-filter q-score
quality-filter q-score-joined
dada2 denoise-single
dada2 denoise-pyro
dada2 denoise-paired
deblur denoise-16S OR denoise-other
vsearch dereplicate-sequences
FeatureTable [Frequency]
FeatureData [Sequence]

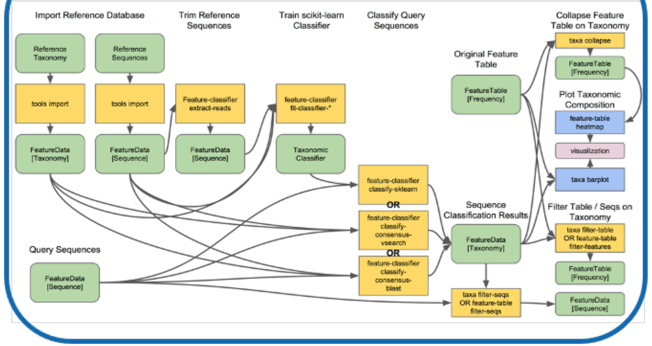
Alignment / Phylogenetics


Align Sequences
Build Phylogeny
Filter feature table to remove unaligned sequences [optional]
16S only
FeatureData [Sequence]
alignment mafft
FeatureData [Aligned Sequence]
alignment mask
FeatureData [Aligned Sequence]
phylogeny fasttree OR raxml OR raxml-rapid-bootstrap
OR
fragment-insertion sepp
Phylogeny [Unrooted]
phylogeny midpoint-root
Phylogeny [Rooted]
FeatureTable [Frequency]
phylogeny filter-table
FeatureTable [Frequency]

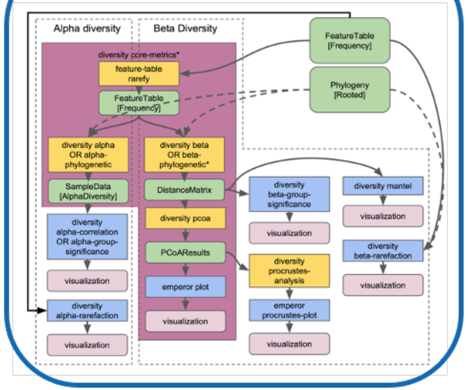
Stats / ML / Visualization


Alpha diversity
Beta Diversity
diversity core-metrics*
diversity alpha OR alpha-phylogenetic
SampleData [AlphaDiversity]
diversity beta OR beta-phylogenetic*
DistanceMatrix
diversity pcoa
PCoAResults
FeatureTable [Frequency]
Longitudinal Analysis
Sample Classifier
Predicted Class
True Class
gneiss
Quality Control
Differential abundance
Taxonomic Analysis

Taxonomy Classification

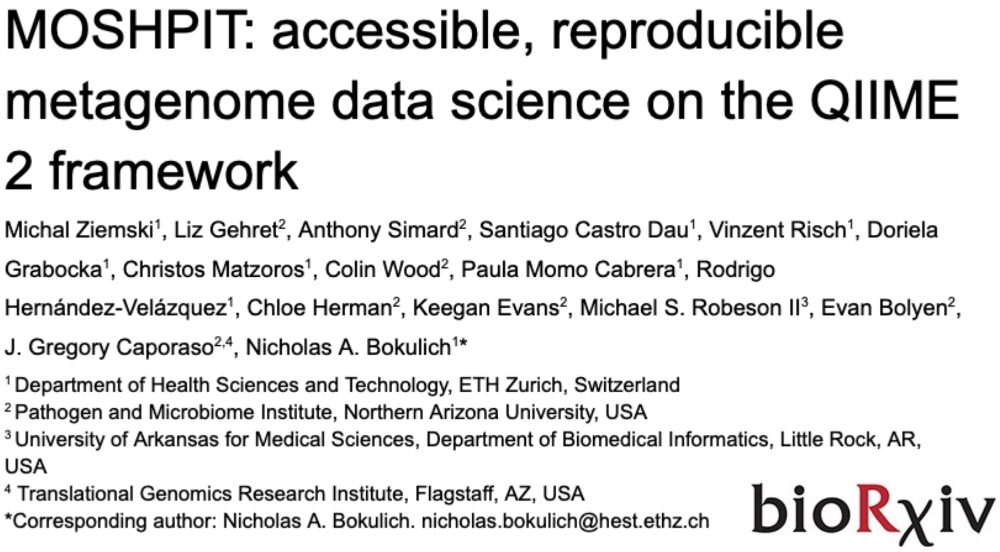

Import Reference Database
Trim Reference Sequences
Train scikit-learn Classifier
Classify Query Sequences
Collapse Feature Table on Taxonomy
Reference Taxonomy
Reference Sequences
tools import
tools import
FeatureData [Taxonomy]
FeatureData [Sequence]
Feature-classifier extract-reads
FeatureData [Sequence]
feature-classifier fit-classifier-*
Taxonomic Classifier
Query Sequences
FeatureData [Sequence]
feature-classifier classify-sklearn
OR
feature-classifier classify-consensus-vsearch
OR
feature-classifier classify-consensus-blast
Original Feature Table
FeatureTable [Frequency]
Sequence Classification Results
FeatureData [Taxonomy]
taxa filter-seqs OR feature-table filter-seqs
taxa collapse
FeatureTable [Frequency]
Plot Taxonomic Composition
feature-table heatmap
visualization
taxa barplot
Filter Table / Seqs on Taxonomy
taxa filter-table OR feature-table filter-features
FeatureTable [Frequency]
FeatureData [Sequence]

Diversity Analysis


Alpha diversity
Beta Diversity
FeatureTable [Frequency]
Phylogeny [Rooted]
diversity core-metrics*
feature-table rarefy
FeatureTable [Frequency]
diversity alpha OR alpha-phylogenetic
SampleData [AlphaDiversity]
diversity alpha-correlation OR alpha-group-significance
visualization
diversity alpha-rarefaction
visualization
diversity beta OR beta-phylogenetic*
DistanceMatrix
diversity pcoa
PCoAResults
emperor plot
visualization
diversity beta-group-significance
visualization
diversity procrustes-analysis
emperor procrustes-plot
visualization
diversity mantel
visualization
diversity beta-rarefaction
visualization

End-to-end **metagenome data analysis**, including options for both read-based and assembly-based analysis.

Learn more at: https://moshpit.qiime2.org

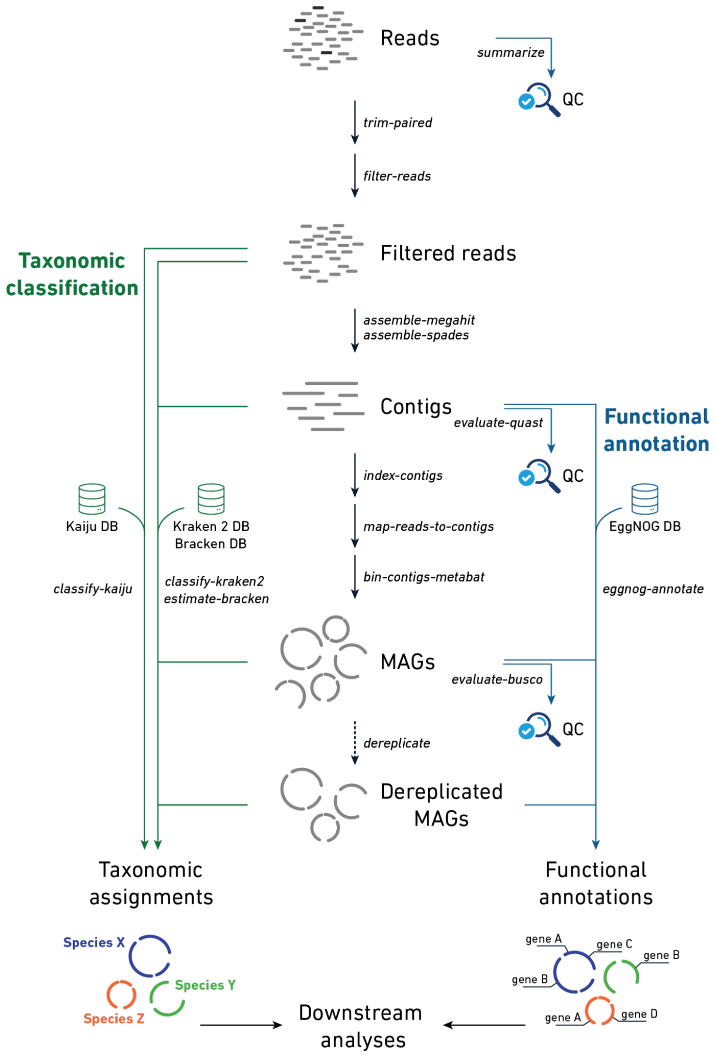


MOSHPIT: accessible, reproducible metagenome data science on the QIIME 2 framework

Michal Ziemski[1], Liz Gehret[2], Anthony Simard[2], Santiago Castro Dau[1], Vinzent Risch[1], Doriela Grabocka[1], Christos Matzoros[1], Colin Wood[2], Paula Momo Cabrera[1], Rodrigo Hernández-Velázquez[1], Chloe Herman[2], Keegan Evans[2], Michael S. Robeson II[3], Evan Bolyen[2], J. Gregory Caporaso[2,4], Nicholas A. Bokulich[1*]

[1] Department of Health Sciences and Technology, ETH Zurich, Switzerland
[2] Pathogen and Microbiome Institute, Northern Arizona University, USA
[3] University of Arkansas for Medical Sciences, Department of Biomedical Informatics, Little Rock, AR, USA
[4] Translational Genomics Research Institute, Flagstaff, AZ, USA
*Corresponding author: Nicholas A. Bokulich. nicholas.bokulich@hest.ethz.ch

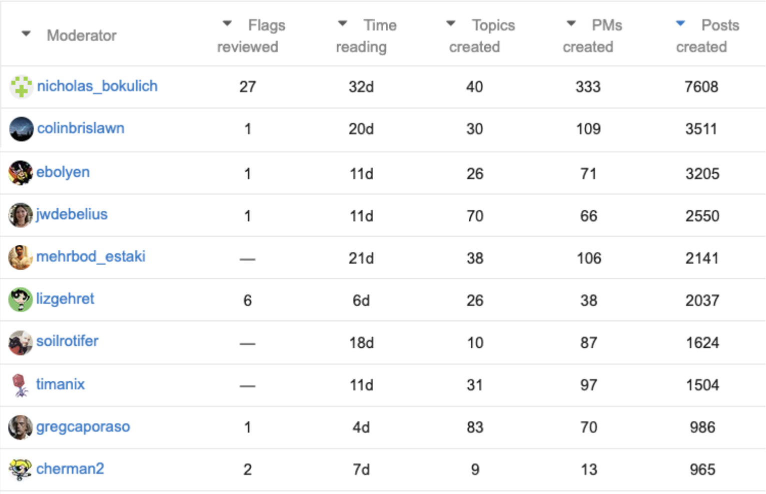
bioRχiv



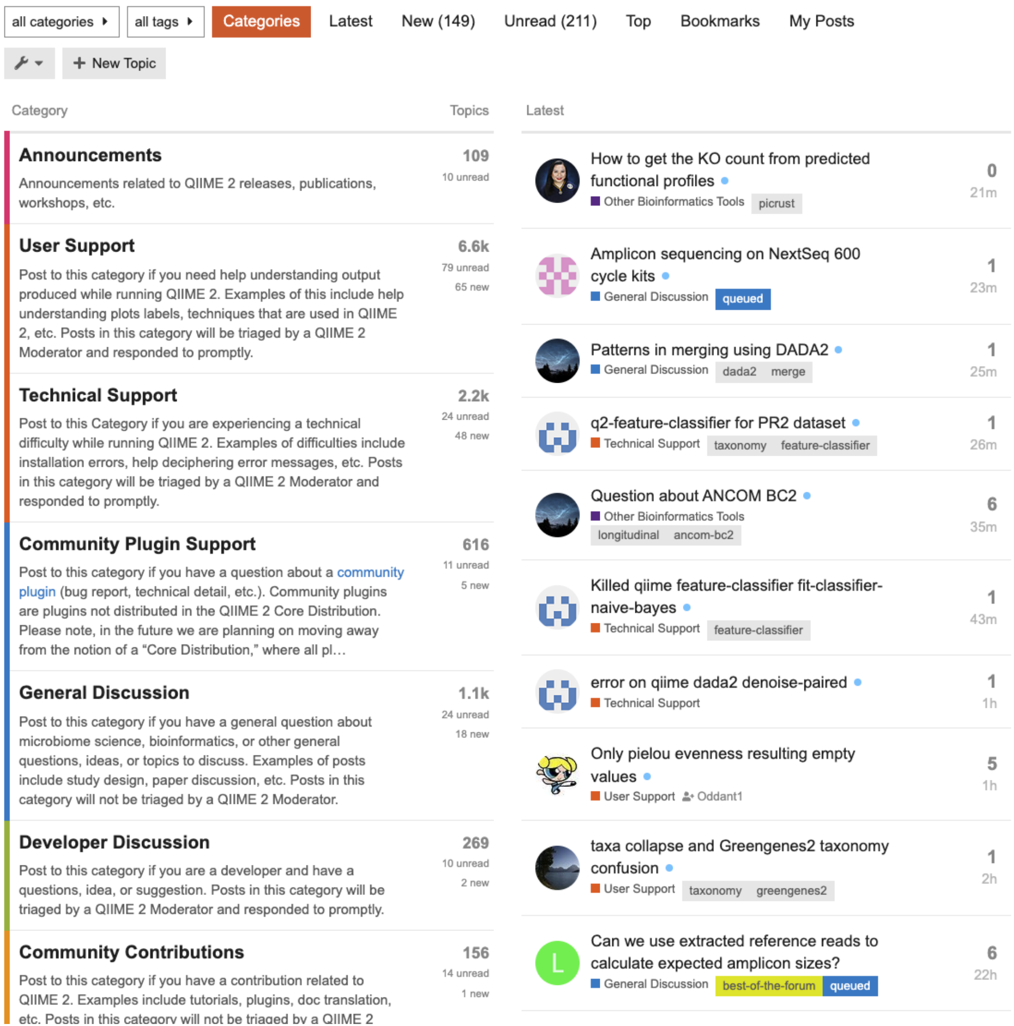
Nearing a decade of free, high-quality support on the qiime2forum

https://forum.qiime2.org
[Community Code of Conduct]()

Top ten contributing moderators.



Moderator	Flags reviewed	Time reading	Topics created	PMs created	Posts created
nicholas_bokulich	27	32d	40	333	7608
colinbrislawn	1	20d	30	109	3511
ebolyen	1	11d	26	71	3205
jwdebelius	1	11d	70	66	2550
mehrbod_estaki	—	21d	38	106	2141
lizgehret	6	6d	26	38	2037
soilrotifer	—	18d	10	87	1624
timanix	—	11d	31	97	1504
gregcaporaso	1	4d	83	70	986
cherman2	2	7d	9	13	965



Category	Topics
Announcements Announcements related to QIIME 2 releases, publications, workshops, etc.	109 10 unread
User Support Post to this category if you need help understanding output produced while running QIIME 2. Examples of this include help understanding plots labels, techniques that are used in QIIME 2, etc. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.	6.6k 79 unread 65 new
Technical Support Post to this Category if you are experiencing a technical difficulty while running QIIME 2. Examples of difficulties include installation errors, help deciphering error messages, etc. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.	2.2k 24 unread 48 new
Community Plugin Support Post to this category if you have a question about a community plugin (bug report, technical detail, etc.). Community plugins are plugins not distributed in the QIIME 2 Core Distribution. Please note, in the future we are planning on moving away from the notion of a "Core Distribution," where all pl…	616 11 unread 5 new
General Discussion Post to this category if you have a general question about microbiome science, bioinformatics, or other general questions, ideas, or topics to discuss. Examples of posts include study design, paper discussion, etc. Posts in this category will not be triaged by a QIIME 2 Moderator.	1.1k 24 unread 18 new
Developer Discussion Post to this category if you are a developer and have a questions, idea, or suggestion. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.	269 10 unread 2 new
Community Contributions Post to this category if you have a contribution related to QIIME 2. Examples include tutorials, plugins, doc translation, etc. Posts in this category will not be triaged by a QIIME 2	156 14 unread 1 new

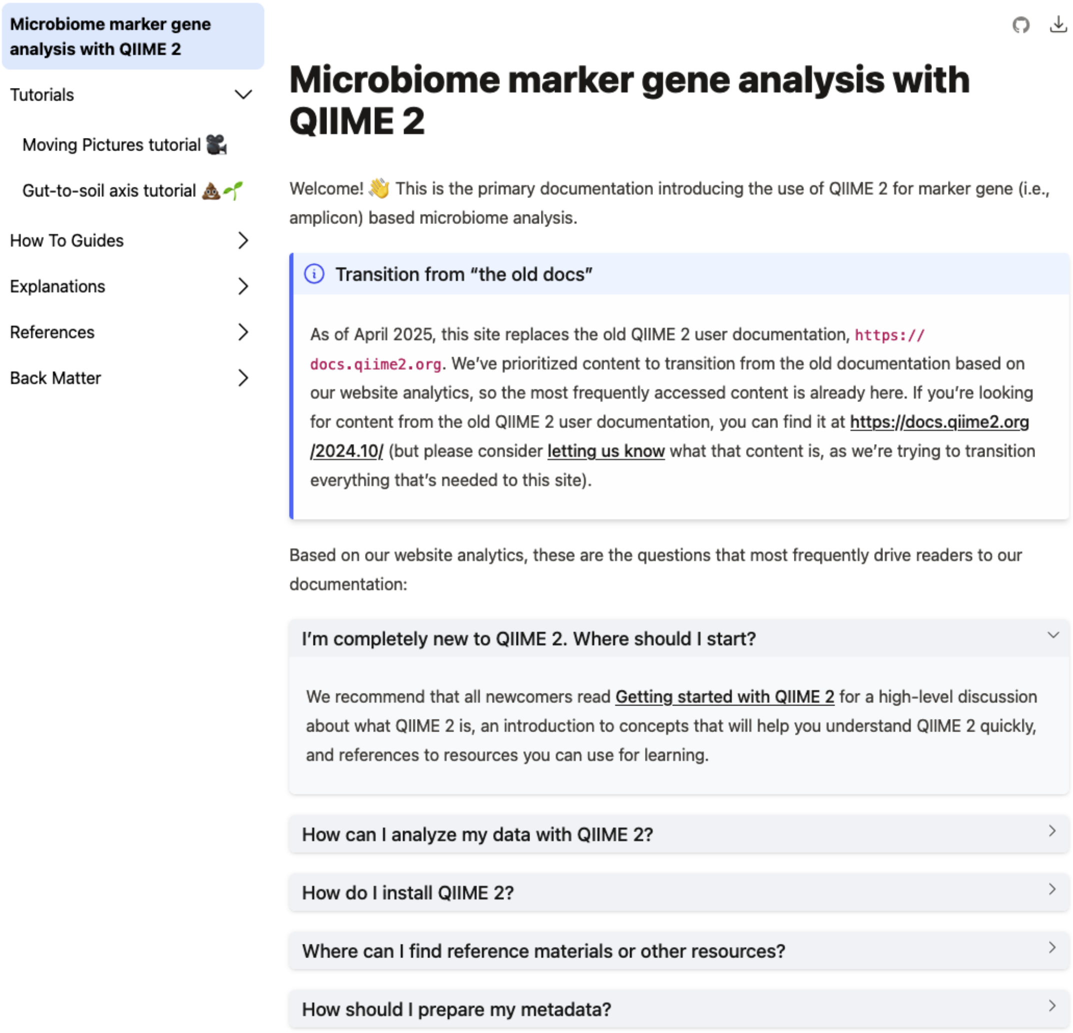
Latest		
How to get the KO count from predicted functional profiles Other Bioinformatics Tools · picrust	0	21m
Amplicon sequencing on NextSeq 600 cycle kits General Discussion · queued	1	23m
Patterns in merging using DADA2 General Discussion · dada2 merge	1	25m
q2-feature-classifier for PR2 dataset Technical Support · taxonomy feature-classifier	1	26m
Question about ANCOM BC2 Other Bioinformatics Tools · longitudinal ancom-bc2	6	35m
Killed qiime feature-classifier fit-classifier-naive-bayes Technical Support · feature-classifier	1	43m
error on qiime dada2 denoise-paired Technical Support	1	1h
Only pielou evenness resulting empty values User Support · Oddant1	5	1h
taxa collapse and Greengenes2 taxonomy confusion User Support · taxonomy greengenes2	1	2h
Can we use extracted reference reads to calculate expected amplicon sizes? General Discussion · best-of-the-forum queued	6	22h

Lean microbiome data science with the **“gut-to-soil microbiome axis”** tutorial.

Teaches microbiome concepts and data science skills in the context of **human waste composting microbiome data**.

Ideal for self-study, classrooms learning, workshops, and study groups.

https://amplicon-docs.qiime2.org



Microbiome marker gene analysis with QIIME 2

Welcome! 👋 This is the primary documentation introducing the use of QIIME 2 for marker gene (i.e., amplicon) based microbiome analysis.

Transition from “the old docs”

As of April 2025, this site replaces the old QIIME 2 user documentation, `https://docs.qiime2.org`. We’ve prioritized content to transition from the old documentation based on our website analytics, so the most frequently accessed content is already here. If you’re looking for content from the old QIIME 2 user documentation, you can find it at https://docs.qiime2.org/2024.10/ (but please consider letting us know what that content is, as we’re trying to transition everything that’s needed to this site).

Based on our website analytics, these are the questions that most frequently drive readers to our documentation:

I’m completely new to QIIME 2. Where should I start?

We recommend that all newcomers read Getting started with QIIME 2 for a high-level discussion about what QIIME 2 is, an introduction to concepts that will help you understand QIIME 2 quickly, and references to resources you can use for learning.

How can I analyze my data with QIIME 2?

How do I install QIIME 2?

Where can I find reference materials or other resources?

How should I prepare my metadata?

Acknowledgements

Caporaso Lab

Jeff Meilander
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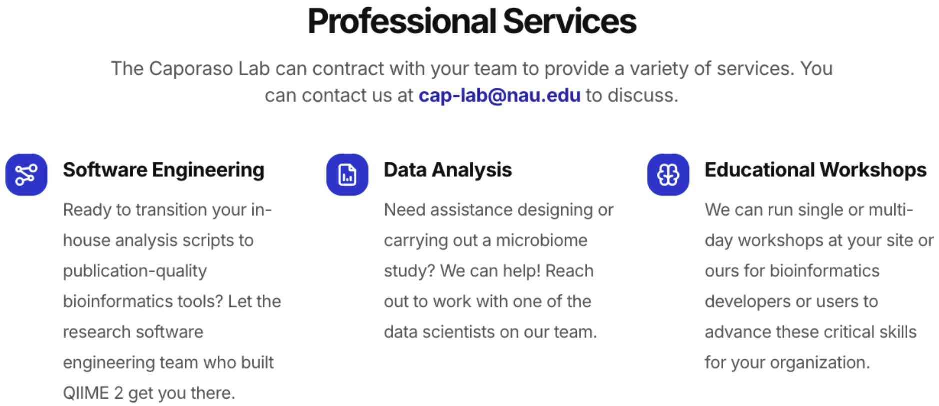


Professional Services

The Caporaso Lab can contract with your team to provide a variety of services. You can contact us at **cap-lab@nau.edu** to discuss.

Software Engineering

Ready to transition your in-house analysis scripts to publication-quality bioinformatics tools? Let the research software engineering team who built QIIME 2 get you there.

Data Analysis

Need assistance designing or carrying out a microbiome study? We can help! Reach out to work with one of the data scientists on our team.

Educational Workshops

We can run single or multi-day workshops at your site or ours for bioinformatics developers or users to advance these critical skills for your organization.

These slides:

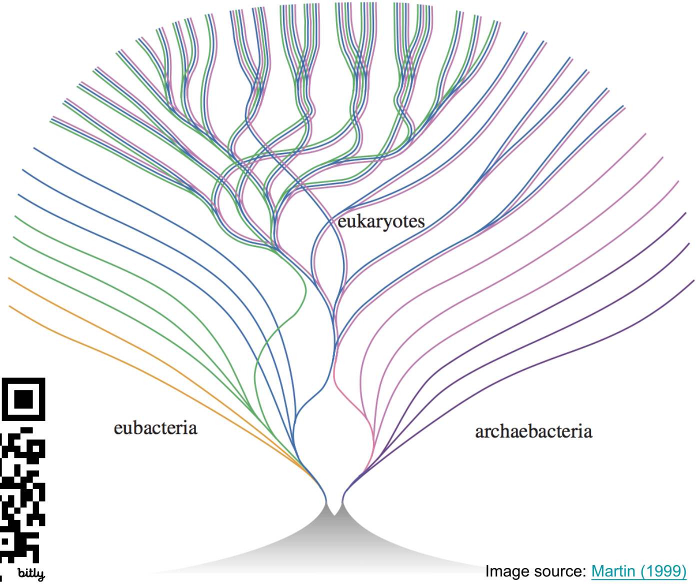
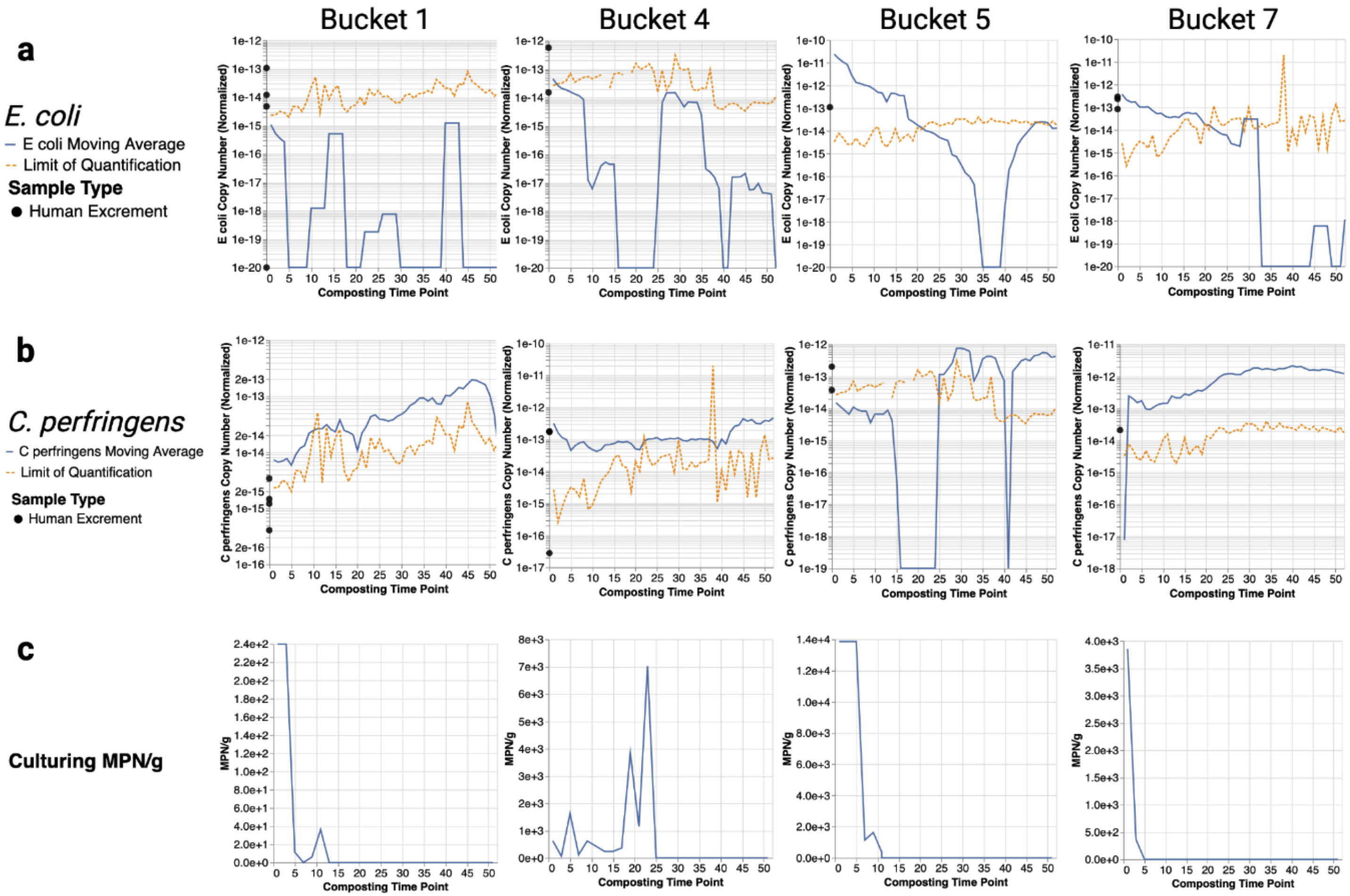
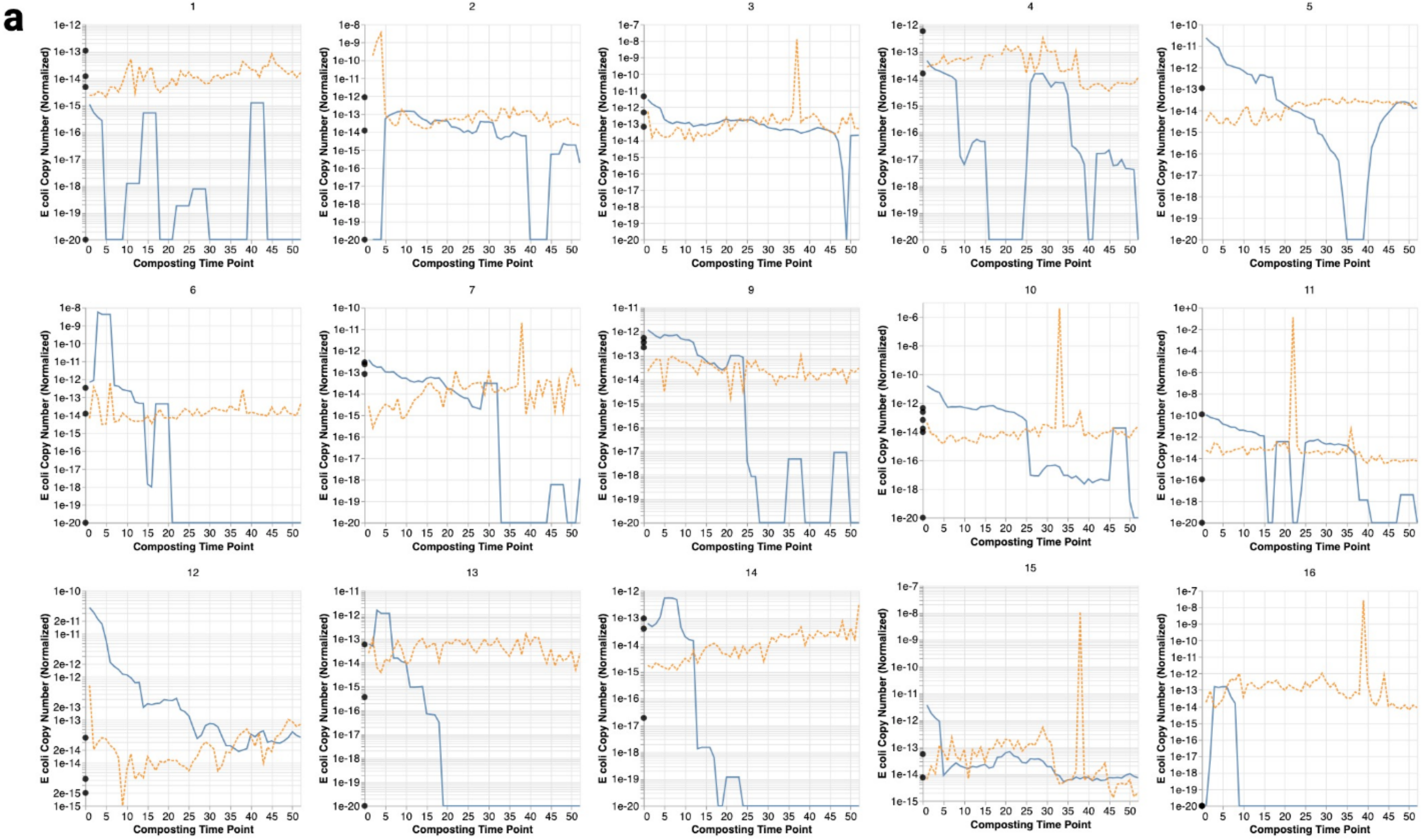


Image source: Martin (1999)

qPCR and culturing track known pathogens



qPCR assays show *E. coli* below our limit of detection in all buckets by end of experiment.



qPCR assays show spore former <u>*C. perfringens* remains detectable</u> at end of experiment.