PUMA Program for Unifying Microbiome Analysis

Christopher Dao

MIMG 109L

UCLA | Department of Microbiology, Immunology & Molecular Genetics

Topics

1. **UCLA MIMG 109L class**
    - **Research immersion in microbial ecology**

2. Overview of microbiome analysis
    - Data processing
    - Data analysis

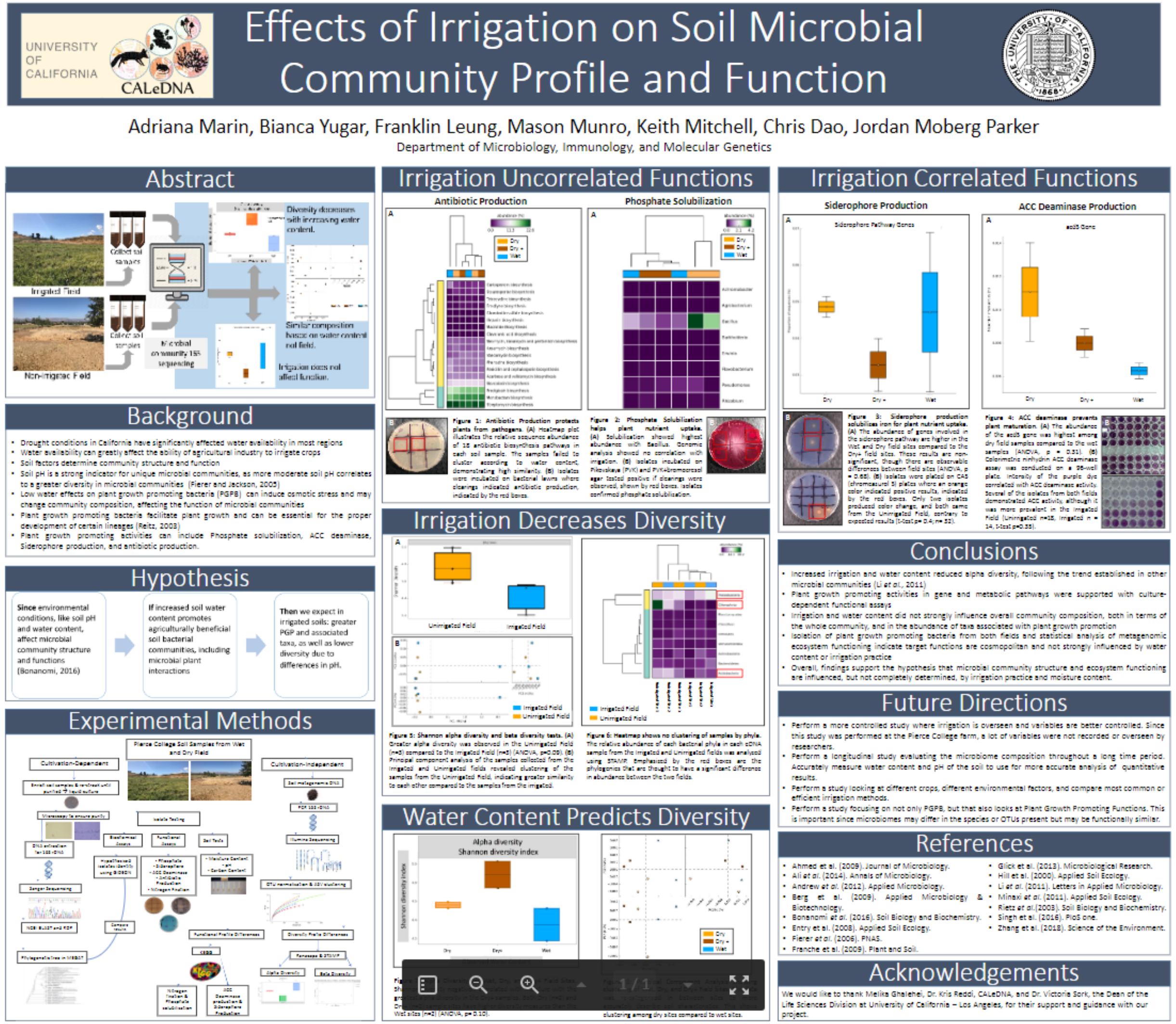
3. PUMA project

MIMG 109AL/BL – What’s it about?

Microbial ecology: how microorganisms interact with each other and their environment

- Biodiversity – isolate, identify, and quantify microorganisms in various habitats
  “WHO’S THERE?”
- Microbial activity – measure what microorganisms are doing in their habitats
  “WHAT ARE THEY DOING THERE?”

MIMG 109L Posters



Effects of Irrigation on Soil Microbial Community Profile and Function

Adriana Marin, Bianca Yugar, Franklin Leung, Mason Munro, Keith Mitchell, Chris Dao, Jordan Moberg Parker
Department of Microbiology, Immunology, and Molecular Genetics

UNIVERSITY OF CALIFORNIA CALeDNA

Abstract

Background

- Drought conditions in California have significantly affected water availability in most regions
- Water availability can greatly affect the ability of agricultural industry to irrigate crops
- Soil factors determine community structure and function
- Soil pH is a strong indicator for unique microbial communities, as more moderate soil pH correlates to a greater diversity in microbial communities (Fierer and Jackson, 2005)
- Low water effects on plant growth promoting bacteria (PGPB) can induce osmotic stress and may change community composition, affecting the function of microbial communities
- Plant growth promoting bacteria facilitate plant growth and can be essential for the proper development of certain lineages (Reitz, 2003)
- Plant growth promoting activities can include Phosphate solubilization, ACC deaminase, Siderophore production, and antibiotic production.

Hypothesis

Since environmental conditions, like soil pH and water content, affect microbial community structure and functions (Bonanomi, 2016)

If increased soil water content promotes agriculturally beneficial soil bacterial communities, including microbial plant interactions

Then we expect in irrigated soils: greater PGP and associated taxa, as well as lower diversity due to differences in pH.

Experimental Methods

Irrigation Uncorrelated Functions

Antibiotic Production

Figure 1: Antibiotic Production protects plants from pathogens. (A) Heatmap plot illustrates the relative sequence abundance of 18 antibiotic biosynthesis pathways in each soil sample. The samples failed to cluster according to water content, demonstrating high similarity. (B) Isolates were incubated on bacterial lawns where clearings indicated antibiotic production, indicated by the red boxes.

Phosphate Solubilization

Figure 2: Phosphate Solubilization helps plant nutrient uptake. (A) Solubilization showed highest abundance with Bacillus. Genomic analysis showed no correlation with irrigation. (B) Isolates incubated on Pikovskaya (PVK) and PVK+Bromocresol agar tested positive if clearings were observed, shown by red boxes. Isolates confirmed phosphate solubilization.

Irrigation Decreases Diversity

Figure 5: Shannon alpha diversity and beta diversity tests. (A) Greater alpha diversity was observed in the Unirrigated Field (n=3) compared to the Irrigated Field (n=3) (ANOVA, p=0.09). (B) Principal component analysis of the samples collected from the irrigated and Unirrigated fields revealed clustering of the samples from the Unirrigated Field, indicating greater similarity to each other compared to the samples from the irrigated.

Figure 6: Heatmap shows no clustering of samples by phyla. The relative abundance of each bacterial phyla in each cDNA sample from the Irrigated and Unirrigated fields was analysed using STAMP. Emphasized by the red boxes are the phylogenies that are thought to have a significant difference in abundance between the two fields.

Water Content Predicts Diversity

Figure [illegible] Diversity in Wet, Dry, and Dry+ Field Sites. Shannon [illegible] negatively correlated with [illegible] with the greatest alpha diversity in the Dry+ samples. Both Dry (n=2) and Dry+ (n=2) sample sites have higher diversity measures than the Wet sites (n=2) (ANOVA, p= 0.10).

Figure [illegible] Component Analysis [illegible] clustering of Wet, Dry, and Dry+ Field Sites. [illegible] was [illegible] in between sites to more accurately describe soil characteristics. This shows clustering among dry sites compared to wet sites.

Irrigation Correlated Functions

Siderophore Production

Figure 3: Siderophore production solubilizes iron for plant nutrient uptake. (A) The abundance of genes involved in the siderophore pathway are higher in the Wet and Dry field sites compared to the Dry+ field sites. These results are non-significant, though there are observable differences between field sites (ANOVA, p = 0.68). (B) Isolates were plated on CAS (chromeazurol S) plates where an orange color indicated positive results, indicated by the red boxes. Only two isolates produced color change, and both came from the Unirrigated Field, contrary to expected results (t-test p= 0.4; n= 32).

ACC Deaminase Production

Figure 4: ACC deaminase prevents plant maturation. (A) The abundance of the acdS gene was highest among dry field samples compared to the wet samples (ANOVA, p = 0.31). (B) Colorimetric ninhydrin ACC deaminase assay was conducted on a 96-well plate. Intensity of the purple dye correlated with ACC deaminase activity. Several of the isolates from both fields demonstrated ACC activity, although it was more prevalent in the Irrigated Field (Unirrigated n=18, Irrigated n = 14, t-test p=0.35).

Conclusions

- Increased irrigation and water content reduced alpha diversity, following the trend established in other microbial communities (Li *et al.*, 2011)
- Plant growth promoting activities in gene and metabolic pathways were supported with culture-dependent functional assays
- Irrigation and water content did not strongly influence overall community composition, both in terms of the whole community, and in the abundance of taxa associated with plant growth promotion
- Isolation of plant growth promoting bacteria from both fields and statistical analysis of metagenomic ecosystem functioning indicate target functions are cosmopolitan and not strongly influenced by water content or irrigation practice
- Overall, findings support the hypothesis that microbial community structure and ecosystem functioning are influenced, but not completely determined, by irrigation practice and moisture content.

Future Directions

- Perform a more controlled study where irrigation is overseen and variables are better controlled. Since this study was performed at the Pierce College farm, a lot of variables were not recorded or overseen by researchers.
- Perform a longitudinal study evaluating the microbiome composition throughout a long time period. Accurately measure water content and pH of the soil to use for more accurate analysis of quantitative results.
- Perform a study looking at different crops, different environmental factors, and compare most common or efficient irrigation methods.
- Perform a study focusing on not only PGPB, but that also looks at Plant Growth Promoting Functions. This is important since microbiomes may differ in the species or OTUs present but may be functionally similar.

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Acknowledgements

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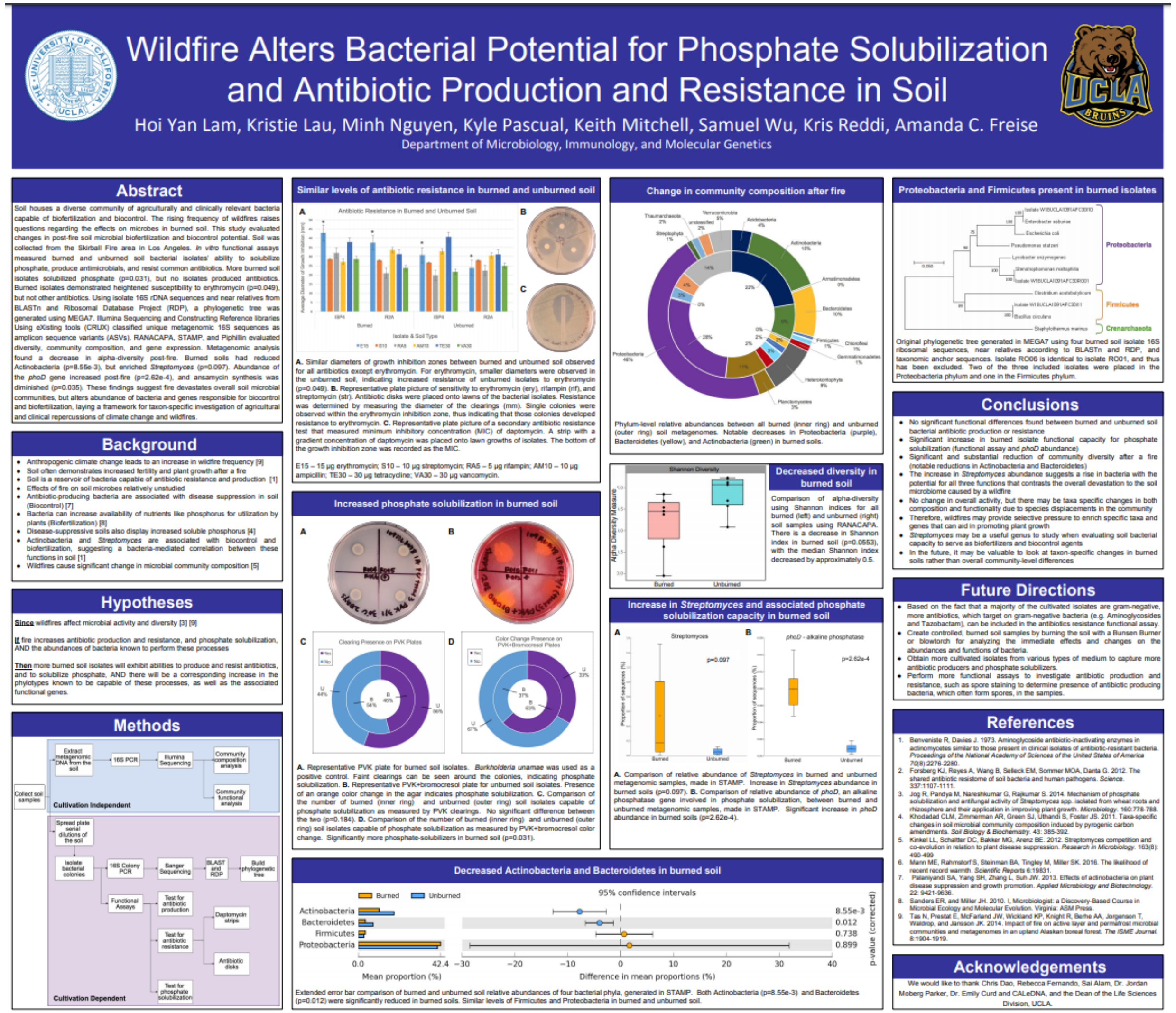

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UCLA BRUINS

Abstract

Soil houses a diverse community of agriculturally and clinically relevant bacteria capable of biofertilization and biocontrol. The rising frequency of wildfires raises questions regarding the effects on microbes in burned soil. This study evaluated changes in post-fire soil microbial biofertilization and biocontrol potential. Soil was collected from the Skirball Fire area in Los Angeles. *In vitro* functional assays measured burned and unburned soil bacterial isolates' ability to solubilize phosphate, produce antimicrobials, and resist common antibiotics. More burned soil isolates solubilized phosphate (p=0.031), but no isolates produced antibiotics. Burned isolates demonstrated heightened susceptibility to erythromycin (p=0.049), but not other antibiotics. Using isolate 16S rDNA sequences and near relatives from BLASTn and Ribosomal Database Project (RDP), a phylogenetic tree was generated using MEGA7. Illumina Sequencing and Constructing Reference libraries Using eXisting tools (CRUX) classified unique metagenomic 16S sequences as amplicon sequence variants (ASVs). RANACAPA, STAMP, and Piphillin evaluated diversity, community composition, and gene expression. Metagenomic analysis found a decrease in alpha-diversity post-fire. Burned soils had reduced Actinobacteria (p=8.55e-3), but enriched *Streptomyces* (p=0.097). Abundance of the *phoD* gene increased post-fire (p=2.62e-4), and ansamycin synthesis was diminished (p=0.035). These findings suggest fire devastates overall soil microbial communities, but alters abundance of bacteria and genes responsible for biocontrol and biofertilization, laying a framework for taxon-specific investigation of agricultural and clinical repercussions of climate change and wildfires.

Background

- Anthropogenic climate change leads to an increase in wildfire frequency [9]
- Soil often demonstrates increased fertility and plant growth after a fire
- Soil is a reservoir of bacteria capable of antibiotic resistance and production [1]
- Effects of fire on soil microbes relatively unstudied
- Antibiotic-producing bacteria are associated with disease suppression in soil (Biocontrol) [7]
- Bacteria can increase availability of nutrients like phosphorus for utilization by plants (Biofertilization) [8]
- Disease-suppressive soils also display increased soluble phosphorus [4]
- Actinobacteria and *Streptomyces* are associated with biocontrol and biofertilization, suggesting a bacteria-mediated correlation between these functions in soil [1]
- Wildfires cause significant change in microbial community composition [5]

Hypotheses

Since wildfires affect microbial activity and diversity [3] [9]

If fire increases antibiotic production and resistance, and phosphate solubilization, AND the abundances of bacteria known to perform these processes

Then more burned soil isolates will exhibit abilities to produce and resist antibiotics, and to solubilize phosphate, AND there will be a corresponding increase in the phylotypes known to be capable of these processes, as well as the associated functional genes.

Methods

Collect soil samples

Cultivation Independent

Extract metagenomic DNA from the soil → 16S PCR → Illumina Sequencing → Community composition analysis; Community functional analysis

Cultivation Dependent

Spread plate serial dilutions of the soil → Isolate bacterial colonies → 16S Colony PCR → Sanger Sequencing → BLAST and RDP → Build phylogenetic tree

Isolate bacterial colonies → Functional Assays → Test for antibiotic production; Test for antibiotic resistance (Daptomycin strips; Antibiotic disks); Test for phosphate solubilization

Similar levels of antibiotic resistance in burned and unburned soil

A. Similar diameters of growth inhibition zones between burned and unburned soil observed for all antibiotics except erythromycin. For erythromycin, smaller diameters were observed in the unburned soil, indicating increased resistance of unburned isolates to erythromycin (p=0.049). **B.** Representative plate picture of sensitivity to erythromycin (ery), rifampin (rif), and streptomycin (str). Antibiotic disks were placed onto lawns of the bacterial isolates. Resistance was determined by measuring the diameter of the clearings (mm). Single colonies were observed within the erythromycin inhibition zone, thus indicating that those colonies developed resistance to erythromycin. **C.** Representative plate picture of a secondary antibiotic resistance test that measured minimum inhibitory concentration (MIC) of daptomycin. A strip with a gradient concentration of daptomycin was placed onto lawn growths of isolates. The bottom of the growth inhibition zone was recorded as the MIC.

E15 – 15 µg erythromycin; S10 – 10 µg streptomycin; RA5 – 5 µg rifampin; AM10 – 10 µg ampicillin; TE30 – 30 µg tetracycline; VA30 – 30 µg vancomycin.

Increased phosphate solubilization in burned soil

A. Representative PVK plate for burned soil isolates. *Burkholderia unamae* was used as a positive control. Faint clearings can be seen around the colonies, indicating phosphate solubilization. **B.** Representative PVK+bromocresol plate for unburned soil isolates. Presence of an orange color change in the agar indicates phosphate solubilization. **C.** Comparison of the number of burned (inner ring) and unburned (outer ring) soil isolates capable of phosphate solubilization as measured by PVK clearings. No significant difference between the two (p=0.184). **D.** Comparison of the number of burned (inner ring) and unburned (outer ring) soil isolates capable of phosphate solubilization as measured by PVK+bromocresol color change. Significantly more phosphate-solubilizers in burned soil (p=0.031).

Change in community composition after fire

Phylum-level relative abundances between all burned (inner ring) and unburned (outer ring) soil metagenomes. Notable decreases in Proteobacteria (purple), Bacteroidetes (yellow), and Actinobacteria (green) in burned soils.

Decreased diversity in burned soil

Comparison of alpha-diversity using Shannon indices for all burned (left) and unburned (right) soil samples using RANACAPA. There is a decrease in Shannon index in burned soil (p=0.0553), with the median Shannon index decreased by approximately 0.5.

Increase in *Streptomyces* and associated phosphate solubilization capacity in burned soil

A. Comparison of relative abundance of *Streptomyces* in burned and unburned metagenomic samples, made in STAMP. Increase in *Streptomyces* abundance in burned soils (p=0.097). **B.** Comparison of relative abundance of *phoD*, an alkaline phosphatase gene involved in phosphate solubilization, between burned and unburned metagenomic samples, made in STAMP. Significant increase in *phoD* abundance in burned soils (p=2.62e-4).

Decreased Actinobacteria and Bacteroidetes in burned soil

Extended error bar comparison of burned and unburned soil relative abundances of four bacterial phyla, generated in STAMP. Both Actinobacteria (p=8.55e-3) and Bacteroidetes (p=0.012) were significantly reduced in burned soils. Similar levels of Firmicutes and Proteobacteria in burned and unburned soil.

Proteobacteria and Firmicutes present in burned isolates

Original phylogenetic tree generated in MEGA7 using four burned soil isolate 16S ribosomal sequences, near relatives according to BLASTn and RDP, and taxonomic anchor sequences. Isolate RO06 is identical to isolate RO01, and thus has been excluded. Two of the three included isolates were placed in the Proteobacteria phylum and one in the Firmicutes phylum.

Conclusions

- No significant functional differences found between burned and unburned soil bacterial antibiotic production or resistance
- Significant increase in burned isolate functional capacity for phosphate solubilization (functional assay and *phoD* abundance)
- Significant and substantial reduction of community diversity after a fire (notable reductions in Actinobacteria and Bacteroidetes)
- The increase in *Streptomyces* abundance suggests a rise in bacteria with the potential for all three functions that contrasts the overall devastation to the soil microbiome caused by a wildfire
- No change in overall activity, but there may be taxa specific changes in both composition and functionality due to species displacements in the community
- Therefore, wildfires may provide selective pressure to enrich specific taxa and genes that can aid in promoting plant growth
- *Streptomyces* may be a useful genus to study when evaluating soil bacterial capacity to serve as biofertilizers and biocontrol agents
- In the future, it may be valuable to look at taxon-specific changes in burned soils rather than overall community-level differences

Future Directions

- Based on the fact that a majority of the cultivated isolates are gram-negative, more antibiotics, which target on gram-negative bacteria (e.g. Aminoglycosides and Tazobactam), can be included in the antibiotics resistance functional assay.
- Create controlled, burned soil samples by burning the soil with a Bunsen Burner or blowtorch for analyzing the immediate effects and changes on the abundances and functions of bacteria.
- Obtain more cultivated isolates from various types of medium to capture more antibiotic producers and phosphate solubilizers.
- Perform more functional assays to investigate antibiotic production and resistance, such as spore staining to determine presence of antibiotic producing bacteria, which often form spores, in the samples.

References

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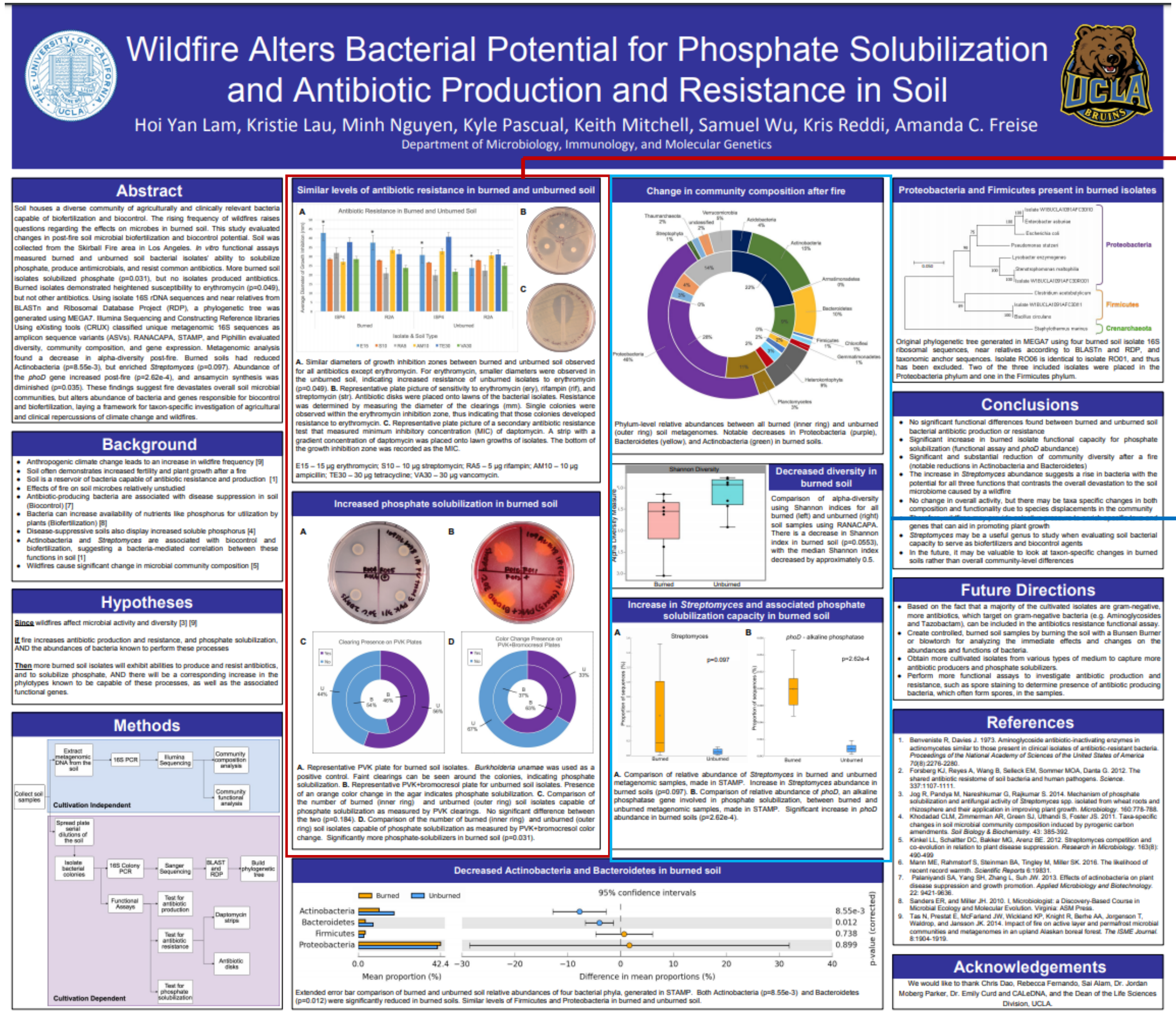

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Cultivation dependent analysis on cultured bacterial isolates

Cultivation independent analysis of microbial community profiles

Topics

1. UCLA MIMG 109L class
    - Research immersion in microbial ecology

2. **Overview of microbiome analysis**
    - **Data processing**
    - **Data analysis**

3. PUMA project

What is the microbiome?

- **Microbiome** – community of microorganisms (mainly bacteria) in any environment

- The microbiome has been studied in:
  - human samples
  - animal samples
  - soil samples
  - indoor surfaces etc., etc.

How can DNA sequencing techniques be used to study the microbiome?

Amplicon sequencing of the 16S gene

Use an molecular evolutionary clock!

16S rRNA gene

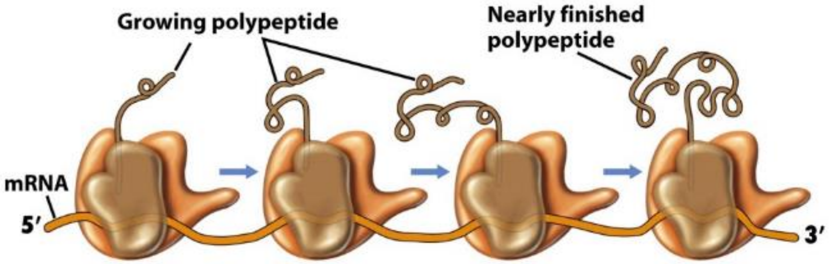

Figure 7-39 Brock Biology of Microorganisms 11/e
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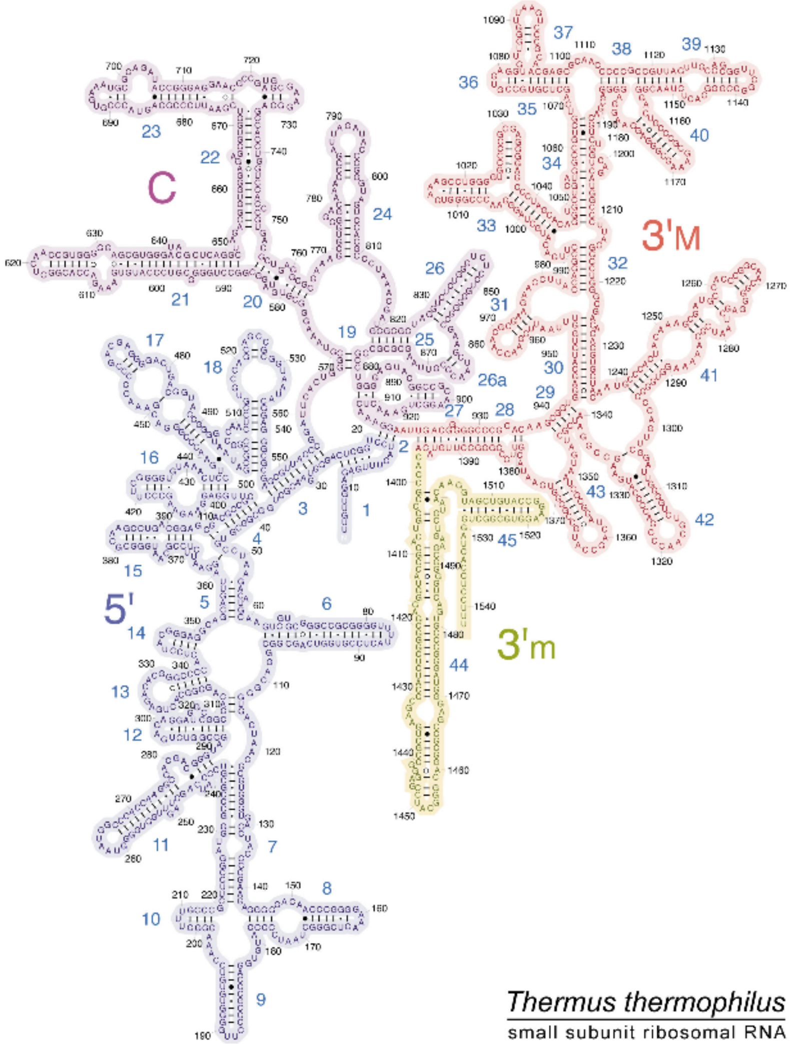
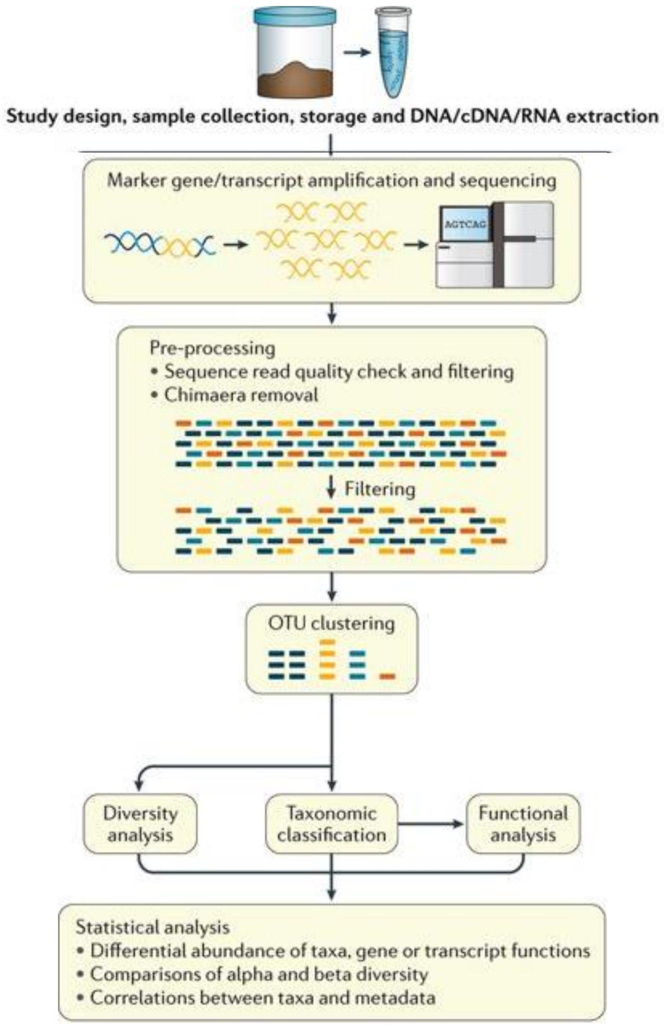


Thermus thermophilus
small subunit ribosomal RNA

Microbiome Analysis Flowchart



Study design, sample collection, storage and DNA/cDNA/RNA extraction

Marker gene/transcript amplification and sequencing

Pre-processing
- Sequence read quality check and filtering
- Chimaera removal

Filtering

OTU clustering

Diversity analysis

Taxonomic classification

Functional analysis

Statistical analysis
- Differential abundance of taxa, gene or transcript functions
- Comparisons of alpha and beta diversity
- Correlations between taxa and metadata

Claesson 2017

Topics

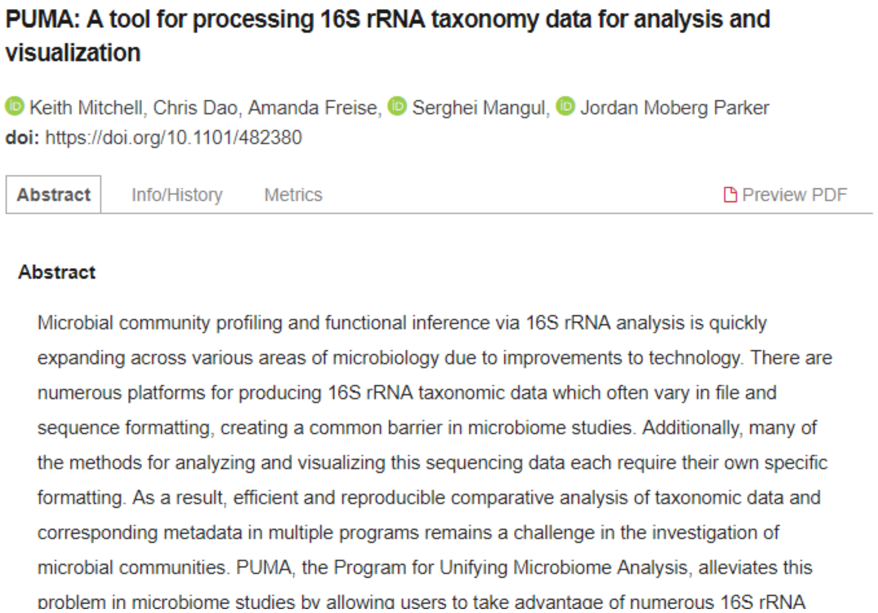
1. UCLA MIMG 109L class
   - Research immersion in microbial ecology

2. Overview of microbiome analysis
   - Data processing
   - Data analysis

3. **PUMA project**

PUMA: Program for Unifying Microbiome Analysis

UCLA
Department of
Microbiology, Immunology
& Molecular Genetics



PUMA: A tool for processing 16S rRNA taxonomy data for analysis and visualization

Keith Mitchell, Chris Dao, Amanda Freise, Serghei Mangul, Jordan Moberg Parker
doi: https://doi.org/10.1101/482380

Abstract

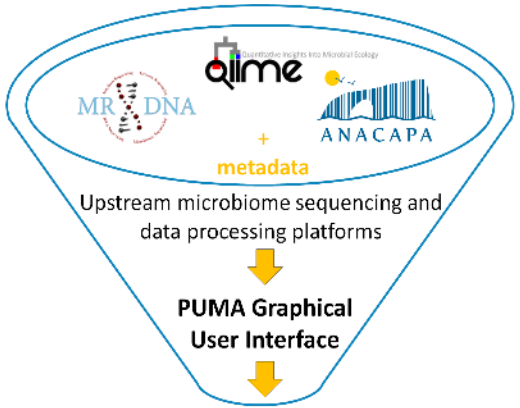
Microbial community profiling and functional inference via 16S rRNA analysis is quickly expanding across various areas of microbiology due to improvements to technology. There are numerous platforms for producing 16S rRNA taxonomic data which often vary in file and sequence formatting, creating a common barrier in microbiome studies. Additionally, many of the methods for analyzing and visualizing this sequencing data each require their own specific formatting. As a result, efficient and reproducible comparative analysis of taxonomic data and corresponding metadata in multiple programs remains a challenge in the investigation of microbial communities. PUMA, the Program for Unifying Microbiome Analysis, alleviates this problem in microbiome studies by allowing users to take advantage of numerous 16S rRNA

https://www.biorxiv.org/content/early/2018/11/29/482380

Mitchell 2018

PUMA: Program for Unifying Microbiome Analysis

Sequence processing/
taxonomic assignment platforms

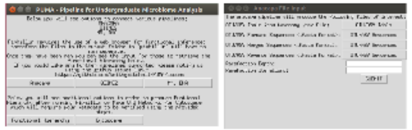


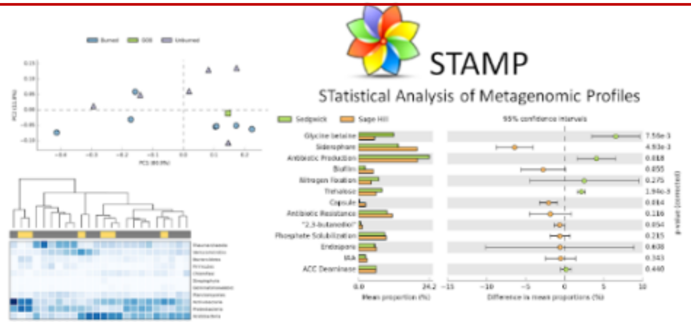
metadata

Upstream microbiome sequencing and data processing platforms

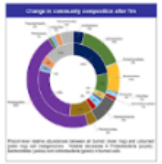
PUMA Graphical User Interface

PUMA



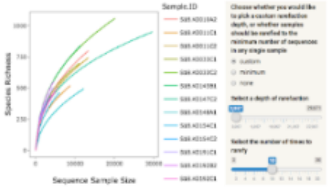


Differential abundance analysis of community and inferred functional profiles in STAMP

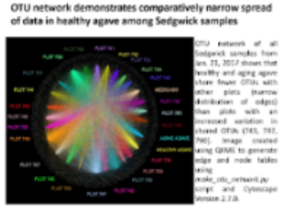


Community profiles with Excel Pivot tables

https://gauravsk.shinyapps.io/ranacapa/



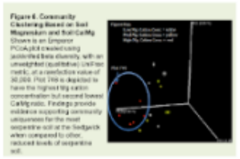
Data exploration in ranacapa (R based web app)



OTU network demonstrates comparatively narrow spread of data in healthy agave among Sedgwick samples

OTU network visualization in Cytoscape

Functional pathway visualization with KEGG and iPATH3



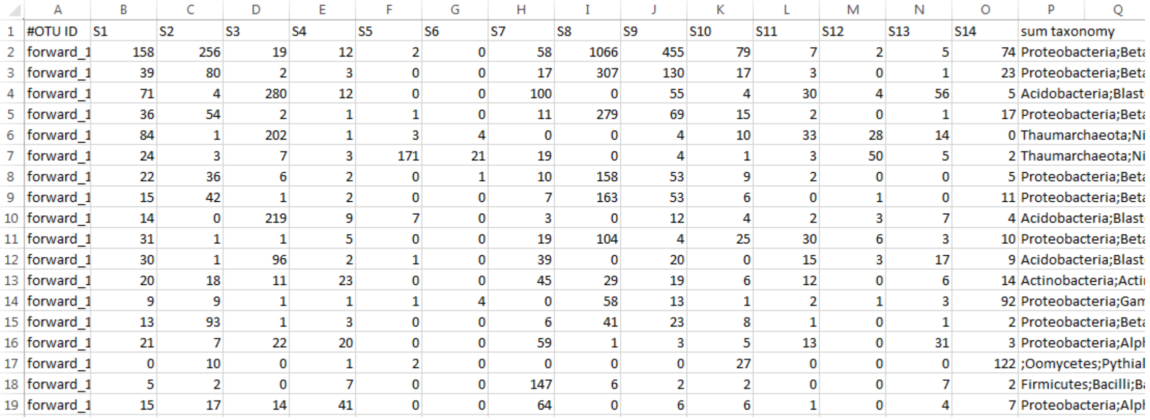
PCoA Phylogenetic Beta Diversity visualization in QIIME 2 (Emperor)

Statistical analysis and data visualization tools

PUMA: Program for Unifying Microbiome Analysis

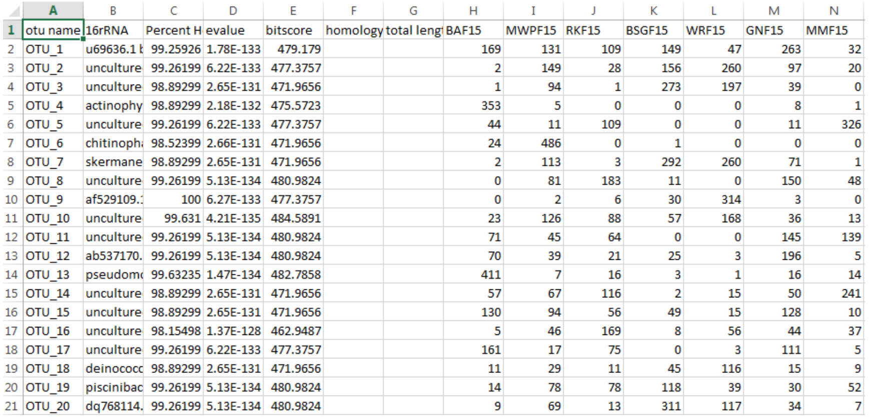
PUMA is a Python GUI tool that formats data from different sequence processing and taxonomic assignment platforms to use in statistical analysis and data visualization tools

Goal: allow researchers to conduct microbiome analyses without needing to use command-line interface or R/Python scripting



#OTU ID	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	sum taxonomy
forward_1	158	256	19	12	2	0	58	1066	455	79	7	2	5	74	Proteobacteria;Bet
forward_1	39	80	2	3	0	0	17	307	130	17	3	0	1	23	Proteobacteria;Bet
forward_1	71	4	280	12	0	0	100	0	55	4	30	4	56	5	Acidobacteria;Blast
forward_1	36	54	2	1	1	0	11	279	69	15	2	0	1	17	Proteobacteria;Bet
forward_1	84	1	202	1	3	4	0	0	4	10	33	28	14	0	Thaumarchaeota;Ni
forward_1	24	3	7	3	171	21	19	0	4	1	3	50	5	2	Thaumarchaeota;Ni
forward_1	22	36	6	2	0	1	10	158	53	9	2	0	0	5	Proteobacteria;Bet
forward_1	15	42	1	2	0	0	7	163	53	6	0	1	0	11	Proteobacteria;Bet
forward_1	14	0	219	9	7	0	3	0	12	4	2	3	7	4	Acidobacteria;Blast
forward_1	31	1	1	5	0	0	19	104	4	25	30	6	3	10	Proteobacteria;Bet
forward_1	30	1	96	2	1	0	39	0	20	0	15	3	17	9	Acidobacteria;Blast
forward_1	20	18	11	23	0	0	45	29	19	6	12	0	6	14	Actinobacteria;Acti
forward_1	9	9	1	1	1	4	0	58	13	1	2	1	3	92	Proteobacteria;Gam
forward_1	13	93	1	3	0	0	6	41	23	8	1	0	1	2	Proteobacteria;Bet
forward_1	21	7	22	20	0	0	59	1	3	5	13	0	31	3	Proteobacteria;Alp
forward_1	0	10	0	1	2	0	0	0	0	27	0	0	0	122	;Oomycetes;Pythial
forward_1	5	2	0	7	0	0	147	6	2	2	0	0	7	2	Firmicutes;Bacilli;B
forward_1	15	17	14	41	0	0	64	0	6	6	1	0	4	7	Proteobacteria;Alp

Anacapa data format



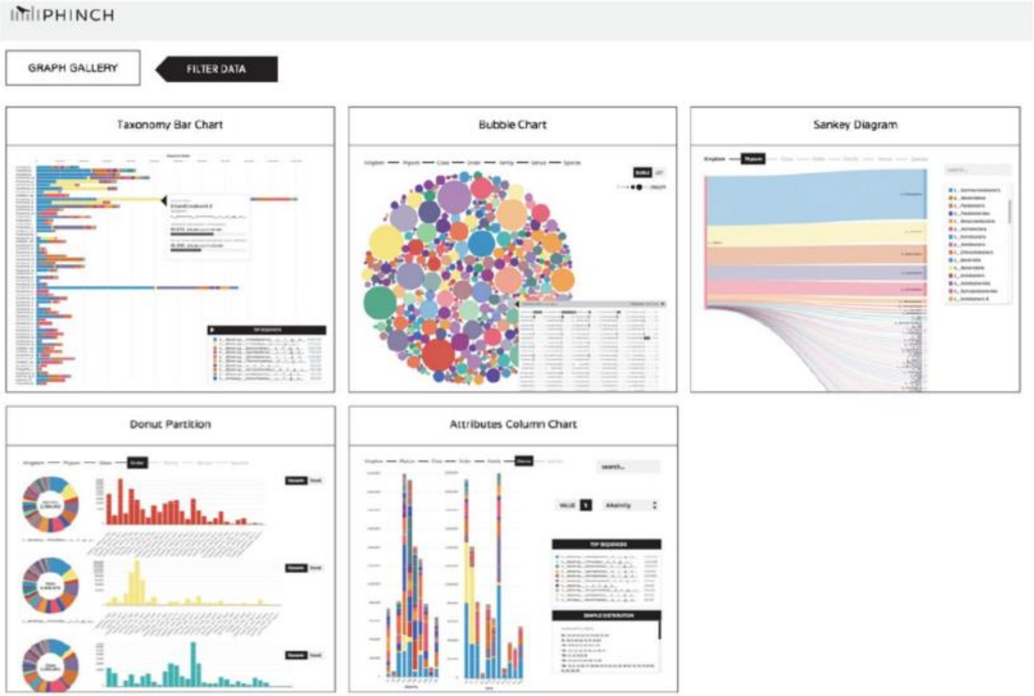
otu name	16rRNA	Percent H	evalue	bitscore	homology	total leng	BAF15	MWPF15	RKF15	BSGF15	WRF15	GNF15	MMF15
OTU_1	u69636.1	99.25926	1.78E-133	479.179			169	131	109	149	47	263	32
OTU_2	unculture	99.26199	6.22E-133	477.3757			2	149	28	156	260	97	20
OTU_3	unculture	98.89299	2.65E-131	471.9656			1	94	1	273	197	39	0
OTU_4	actinophy	98.89299	2.18E-132	475.5723			353	5	0	0	0	8	1
OTU_5	unculture	99.26199	6.22E-133	477.3757			44	11	109	0	0	11	326
OTU_6	chitinoph	98.52399	2.66E-131	471.9656			24	486	0	1	0	0	0
OTU_7	skermane	98.89299	2.65E-131	471.9656			2	113	3	292	260	71	1
OTU_8	unculture	99.26199	5.13E-134	480.9824			0	81	183	11	0	150	48
OTU_9	af529109.	100	6.27E-133	477.3757			0	2	6	30	314	3	0
OTU_10	unculture	99.631	4.21E-135	484.5891			23	126	88	57	168	36	13
OTU_11	unculture	99.26199	5.13E-134	480.9824			71	45	64	0	0	145	139
OTU_12	ab537170.	99.26199	5.13E-134	480.9824			70	39	21	25	3	196	5
OTU_13	pseudom	99.63235	1.47E-134	482.7858			411	7	16	3	1	16	14
OTU_14	unculture	98.89299	2.65E-131	471.9656			57	67	116	2	15	50	241
OTU_15	unculture	98.89299	2.65E-131	471.9656			130	94	56	49	15	128	10
OTU_16	unculture	98.15498	1.37E-128	462.9487			5	46	169	8	56	44	37
OTU_17	unculture	99.26199	6.22E-133	477.3757			161	17	75	0	3	111	5
OTU_18	deinococ	98.89299	2.65E-131	471.9656			11	29	11	45	116	15	9
OTU_19	piscinibac	99.26199	5.13E-134	480.9824			14	78	78	118	39	30	52
OTU_20	dq768114.	99.26199	5.13E-134	480.9824			9	69	13	311	117	34	7

MrDNA data format

Possible PUMA Projects

- Incorporate additional analysis tools as outputs of the pipeline:

Phinch



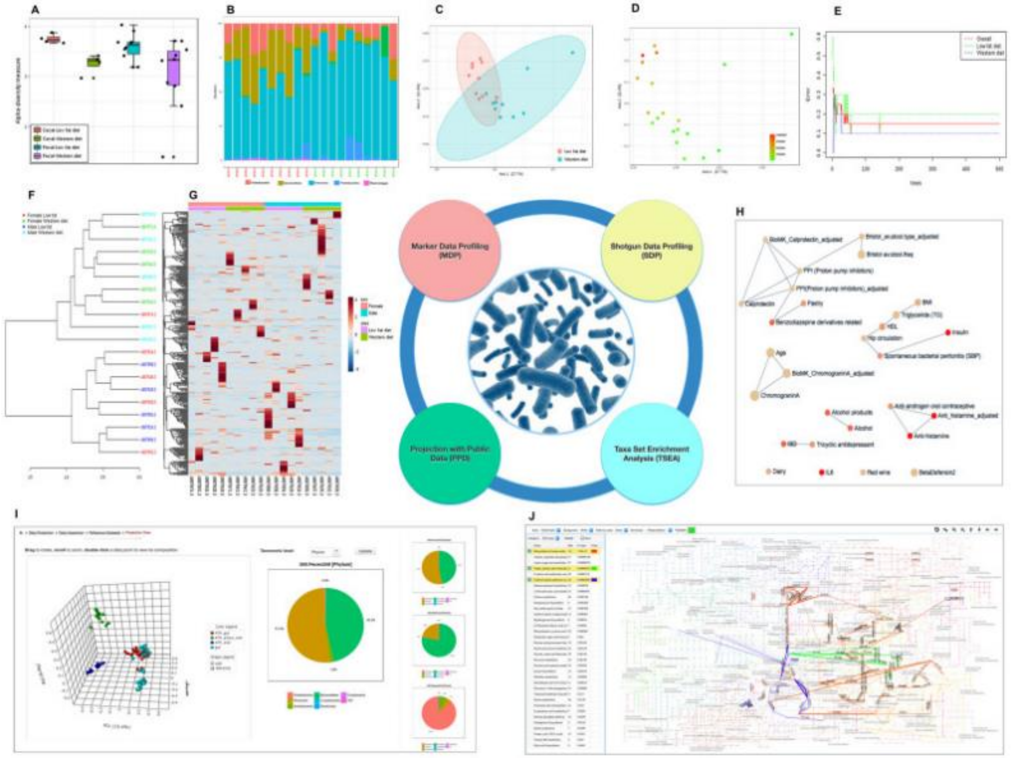
Exploratory data visualization

Bik 2014

Possible PUMA Projects

- Incorporate additional analysis tools as outputs of the pipeline:

MicrobiomeAnalyst



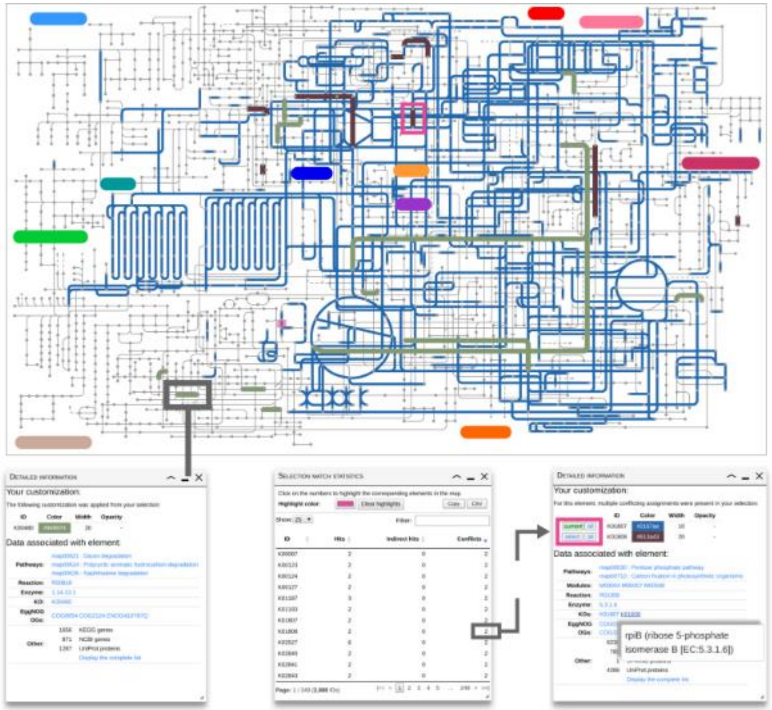
Diversity analysis
Differential abundance analysis

Dhariwal 2017

Possible PUMA Projects

- Incorporate additional analysis tools as outputs of the pipeline:

iPathway



Functional pathway visualization

Darzi 2018

Questions?

Links to tools cited

- https://pathways.embl.de/
- https://www.microbiomeanalyst.ca/
- http://phinch.org/