

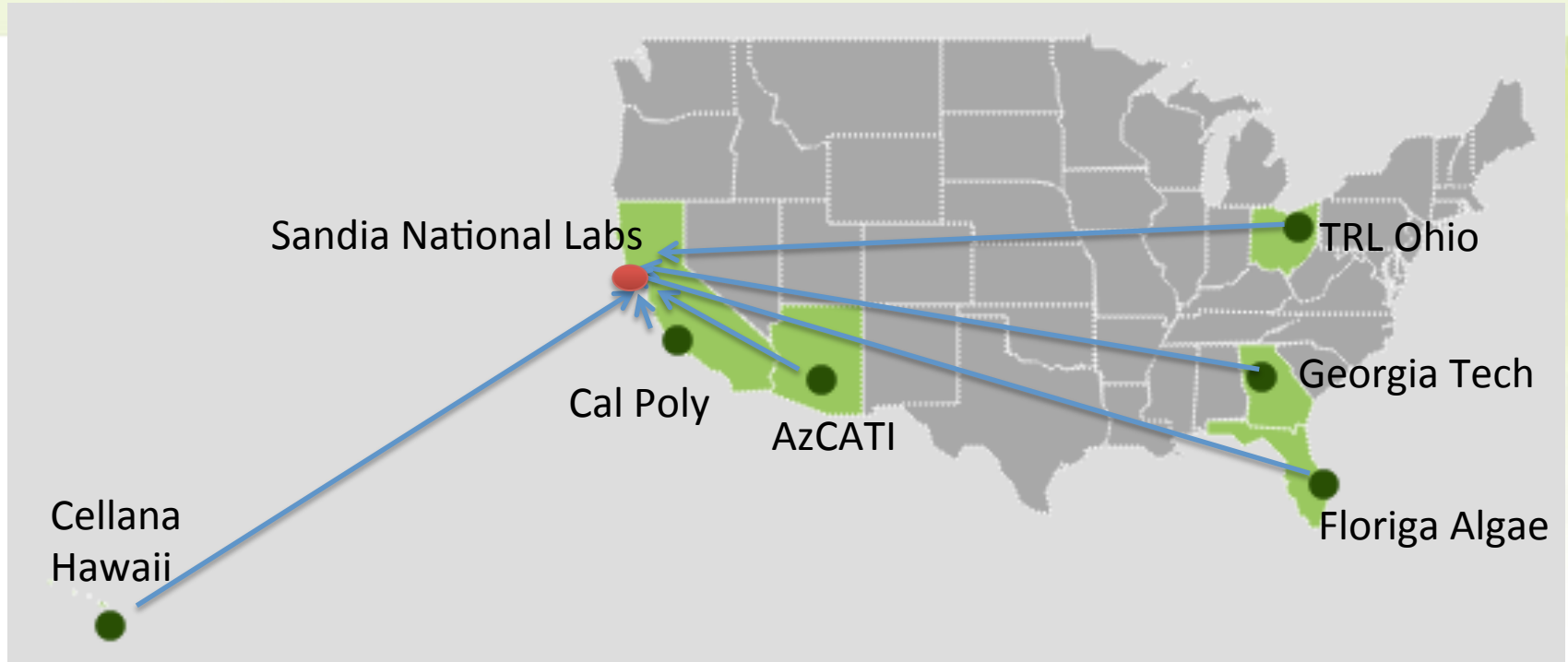


Title: Metagenomics of ATP³ Pond Samples

Kunal Poorey,
Todd W. Lane, Sandia National Labs

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ATP³ sites



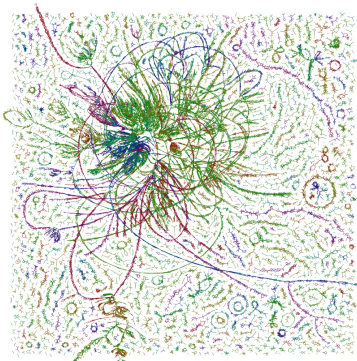
2



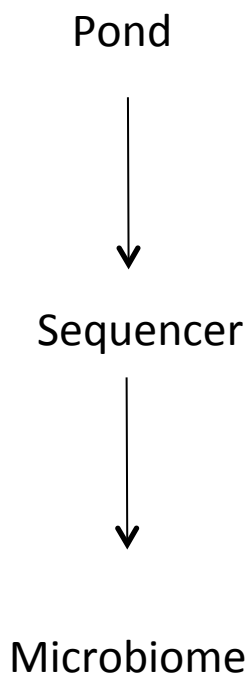
Sample acquisition

- Harvesting by centrifugation: 50 mL samples
- Resuspension in RNALater based on AFDW
- Storage at 4° overnight followed by -20°
- Shipment on wet ice.

Microbial community analysis will leverage work carried out in other BETO funded projects.



V Iverson/U of Washington



From “Pond Crash Forensics” and ATP³, we have developed methods for all the steps in the process

- Sample acquisition
 - Stabilization for shipment
- Nucleic acid extraction
 - Extraction bias
- Sequencing library prep
 - Plastid exclusion
 - Amplification bias
- Data analysis
- qPCR primer design

Microbial
community
sample

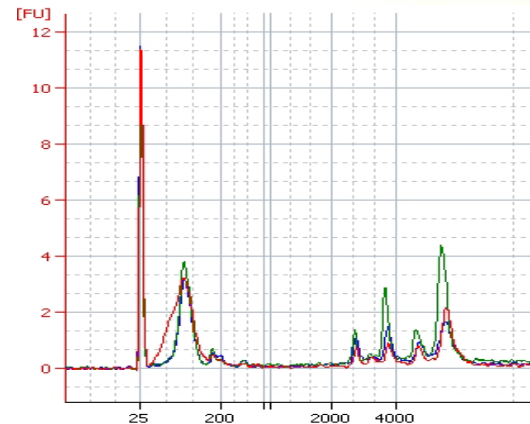
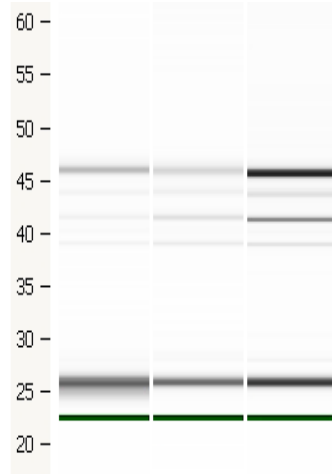
Extract
DNA

PCR amplification
indexing and
library preparation

16S based
sequencing

Analysis
pipeline

RNA from *N. salina* (Zymo Bact/Fungal Kit)

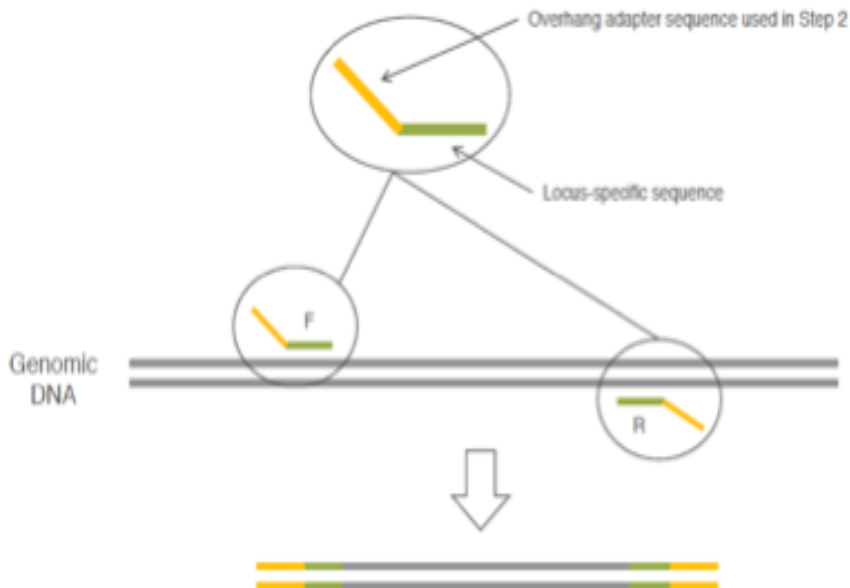


1. Cells in ESAW at 4C (3 weeks); **Red**
2. Cells in RNA Later, 4C (3 weeks); **Blue**
3. Cells in RNA Later, -20C (3 weeks); **Green**

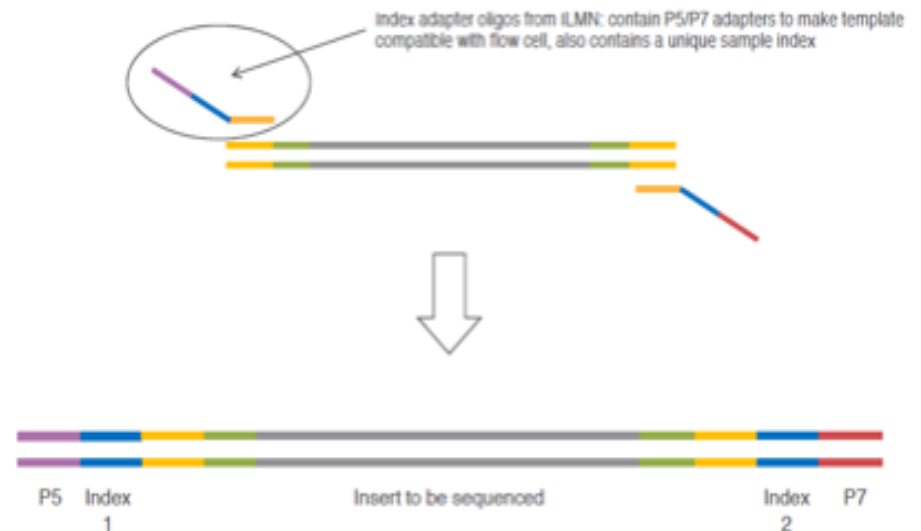
50 mL culture at AFDW (g/L) of	Milliliters of RNALater
0.13	0.625
0.25	1.25
0.38	1.88
0.5	2.5
0.75	3.75
1	5
1.25	6.25
1.5	7.5



Step 1: PCR to amplify regions of interest



Step 2: 2nd round of PCR to add Illumina indices and sequencing adapters



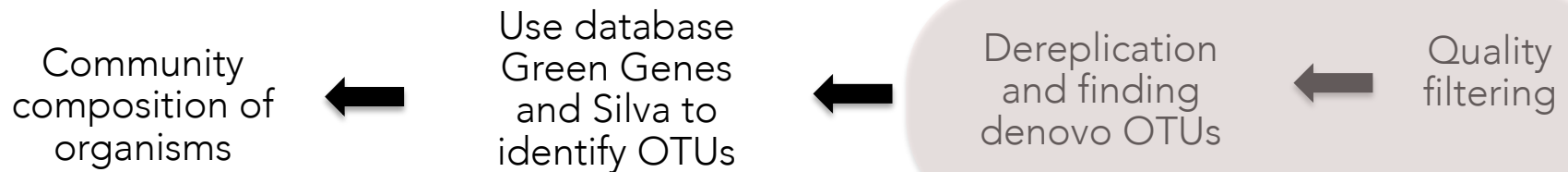
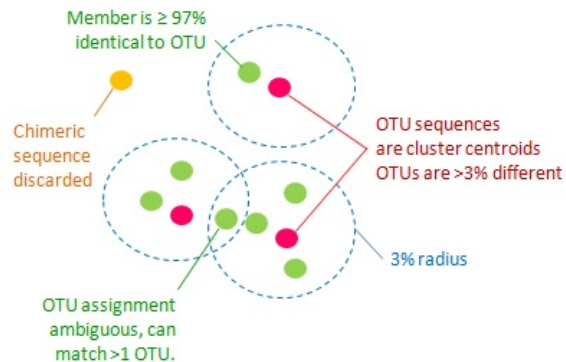


Quality filtering

Dereplication and finding denovo OTUs

Use database Green Genes and Silva to identify OTUs

Community composition of organisms





green
genes
16S rRNA gene database and
workbench compatible with ARB
greengenes.lbl.gov

& silva
high quality ribosomal RNA databases

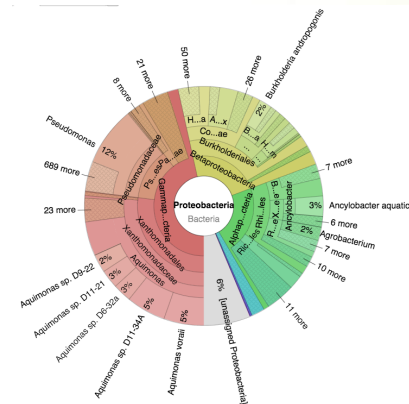
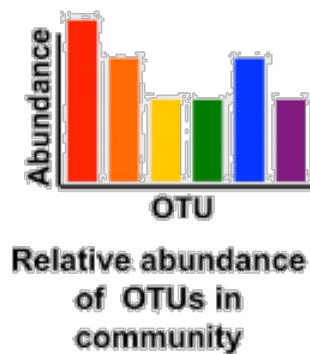
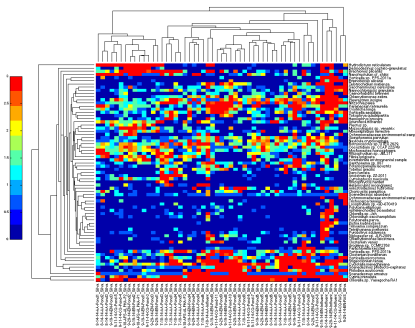
Community
composition of
organisms

Use database
Green Genes
and Silva to
identify OTUs

Dereplication
and finding
denovo OTUs

Quality
filtering





Sample Acquisition: leveraging the ATP³ protocol

Harvesting by centrifugation: 50 mL samples

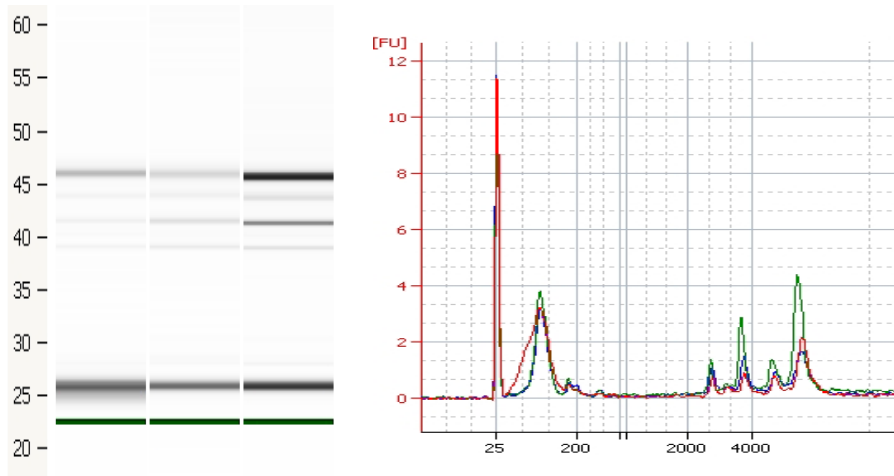
Resuspension in RNALater based on AFDW

Storage at 4° overnight followed by -20°

Shipment on wet ice.

50 mL culture at AFDW (g/L) of	Milliliters of RNALater
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RNA from *N. salina* (Zymo Bact/Fungal Kit)

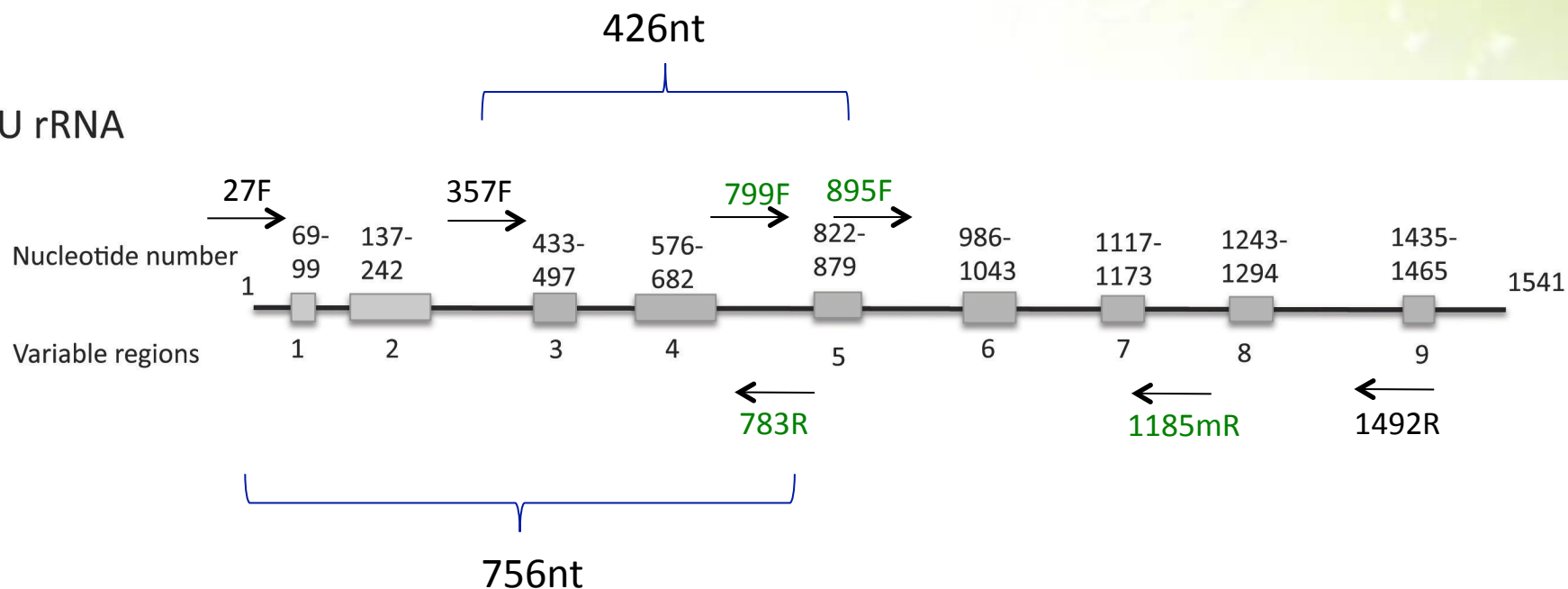


1. Cells in ESAW at 4C (3 weeks); **Red**
2. Cells in RNA Later, 4C (3 weeks); **Blue**
3. Cells in RNA Later, -20C (3 weeks); **Green**

SSU rRNA gene sequencing strategy (bacterial)

600nt kit (current)

SSU rRNA

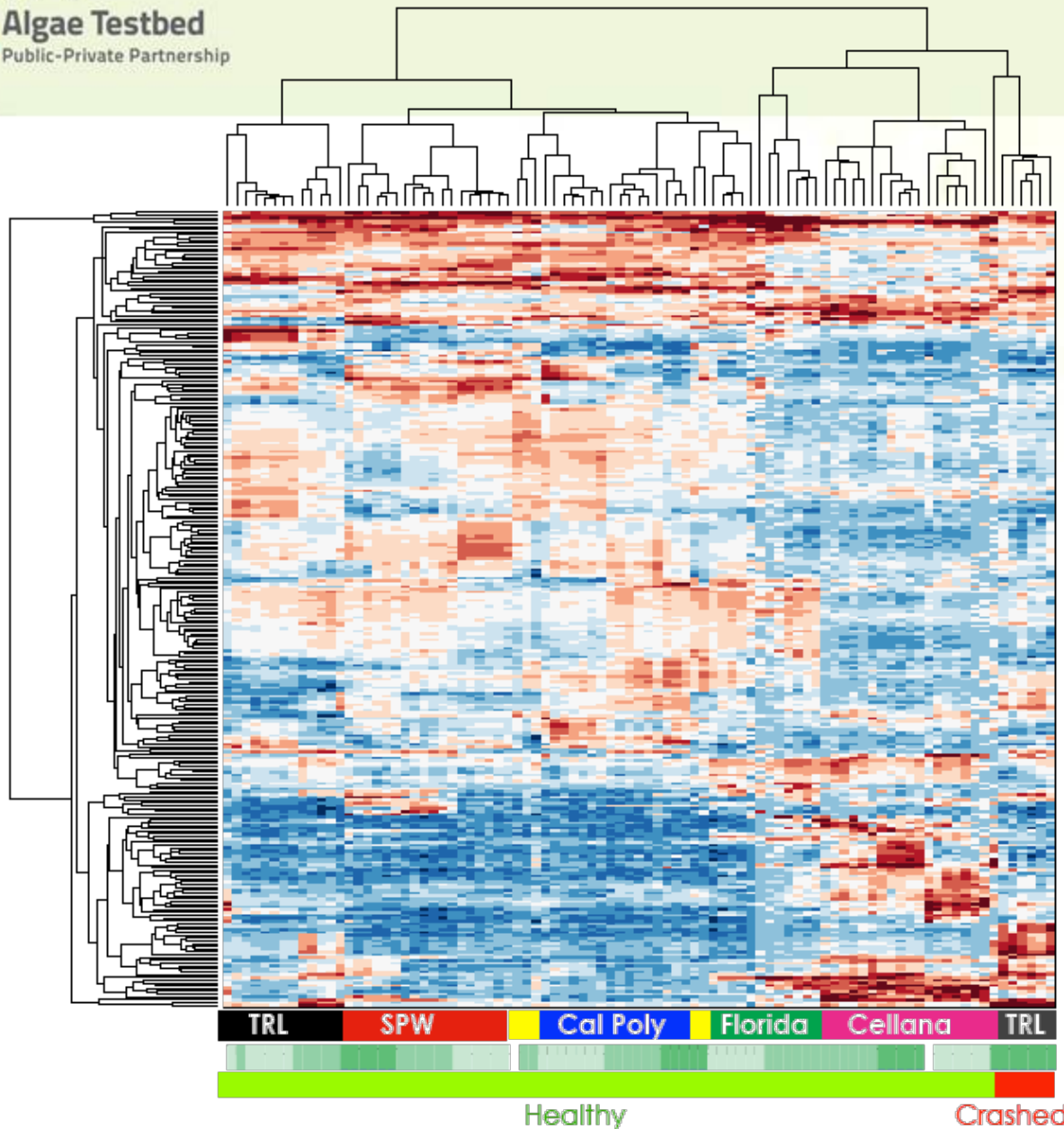


800nt kit (future)

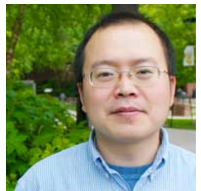
Data

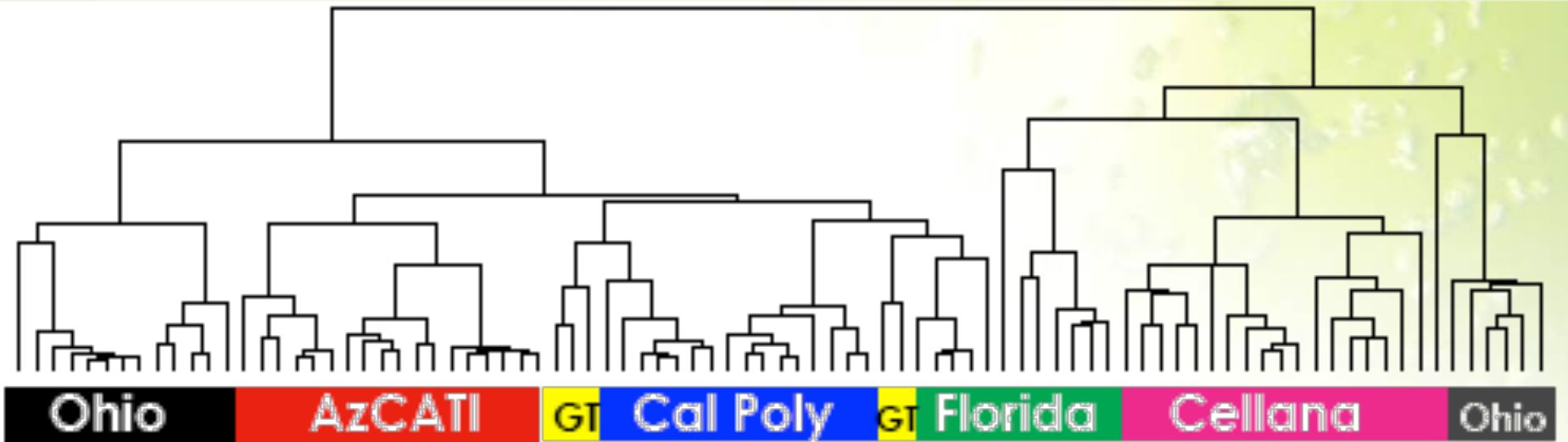
Data acquired and processed till now

		AzCATI	TRL	Cellana	CalPoly	GT	Florida
Spring	16S Library V3-V4	some	Processed	Processed	some	some	some
Spring	18S Library V4		Processed	Processed			
Summer	16S Library V3-V4			in queue	Processed		
Summer	18S Library V4			Processed	Processed		
Fall	16S Library V3-V4						
Fall	18S Library V4						

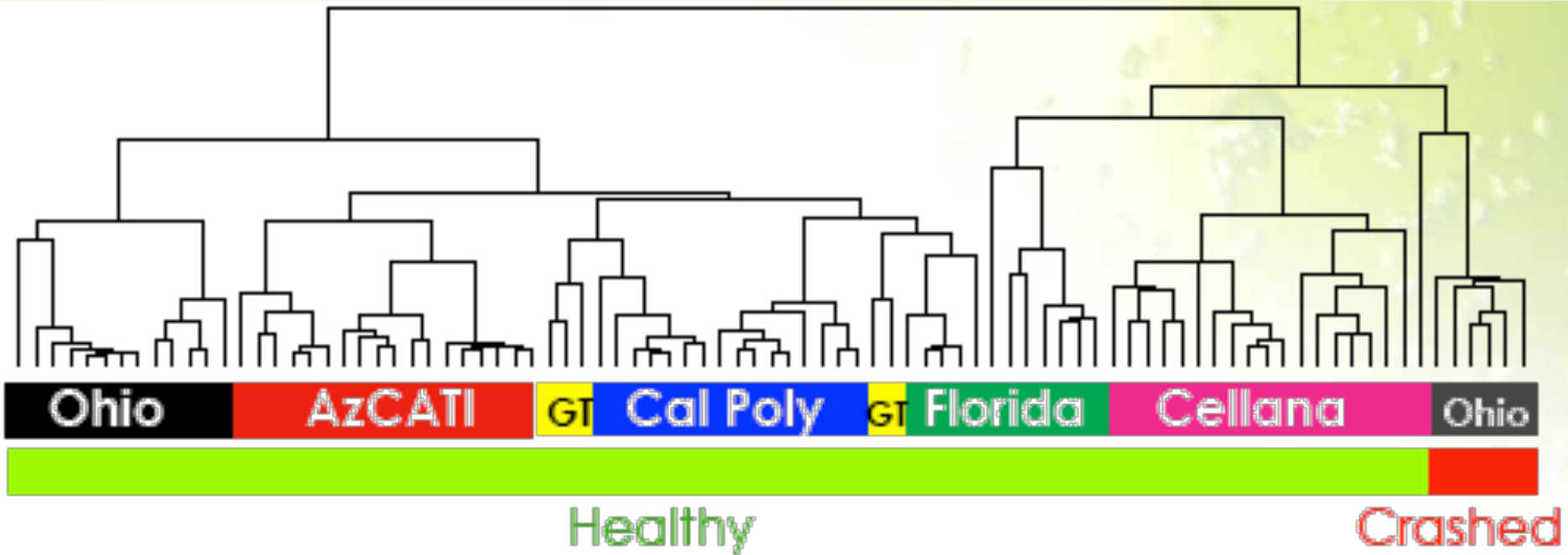


- Meta Summary for spring data from all sites
- Heatmap was made from clustering the classified OTU abundances for both species and samples.
- The Ponds from the same site cluster together

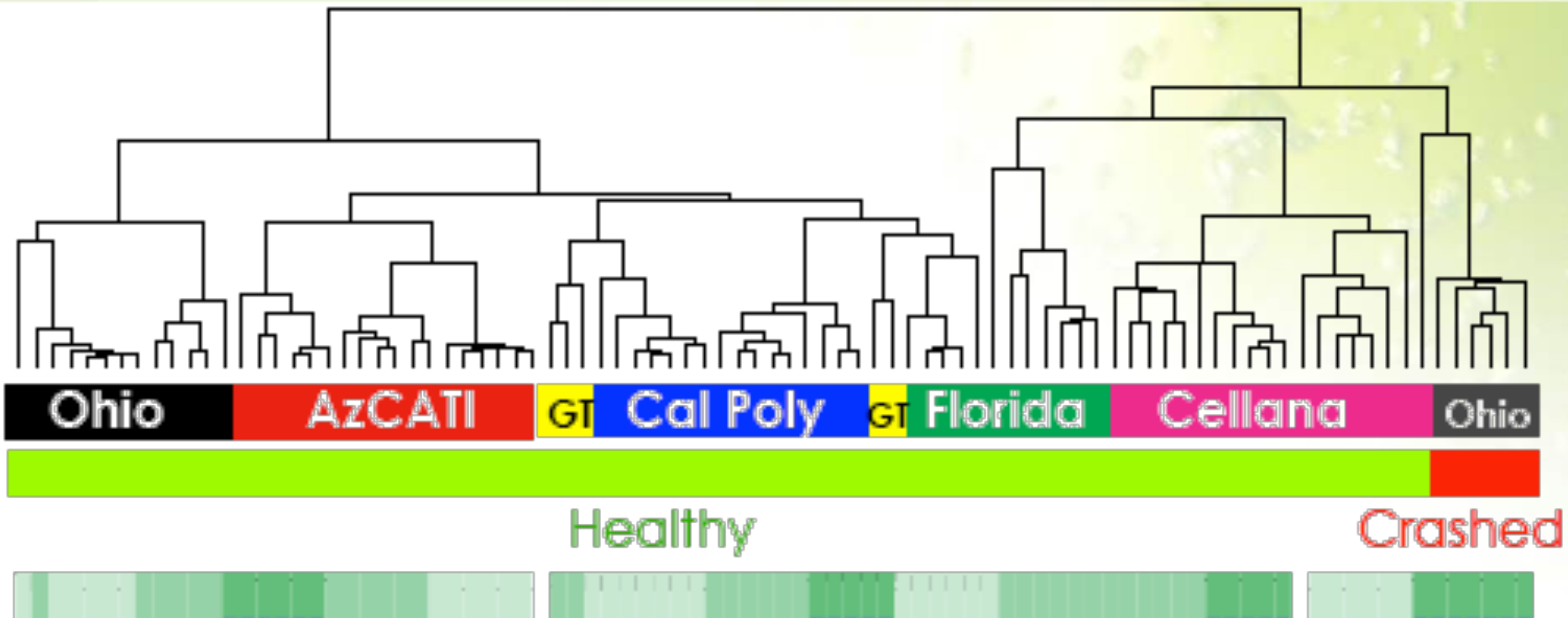




Bacterial communities can identify samples from the same site

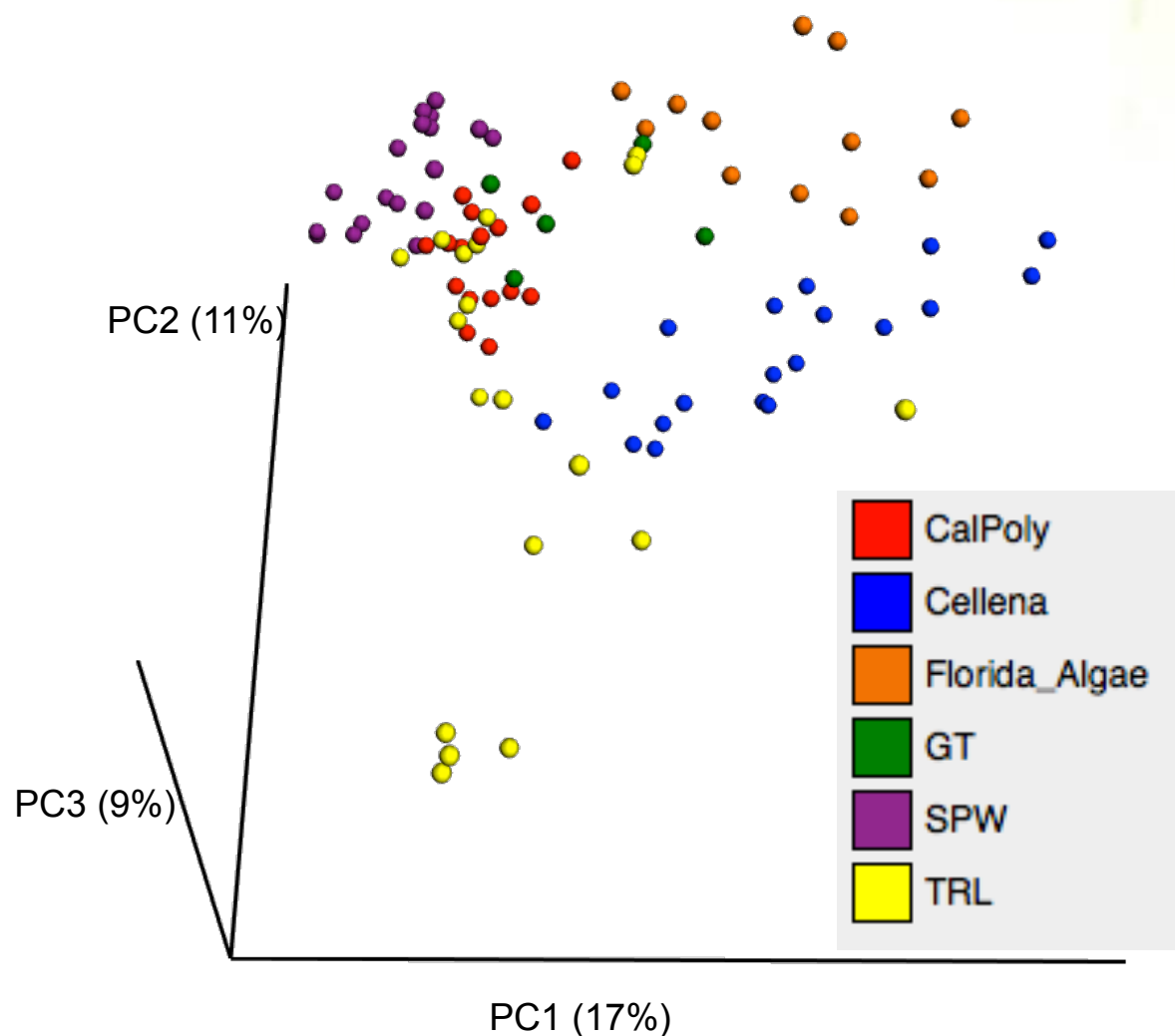


Crashed ponds had a different community structure than healthy ponds

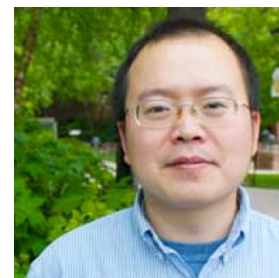


And within same sites the community structure gradually changed with time

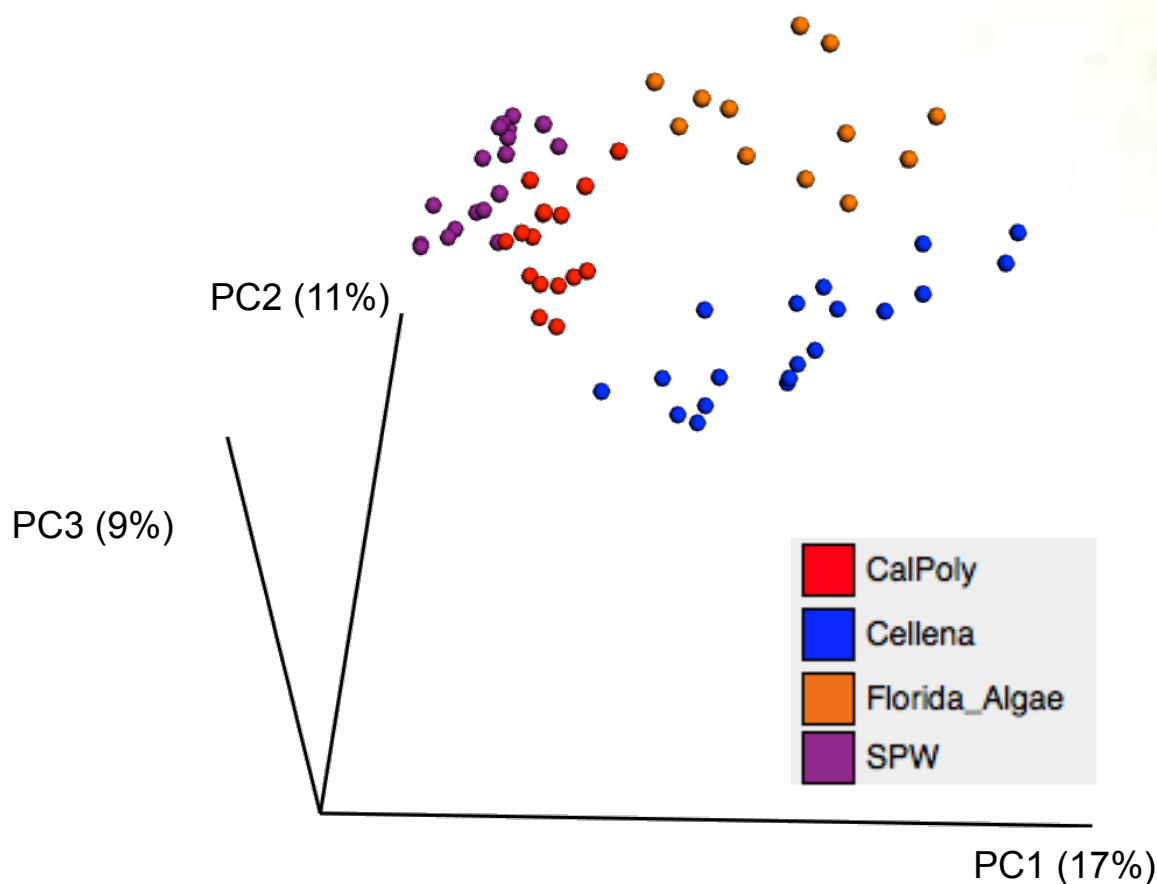
Principal Component Analysis (PCA)



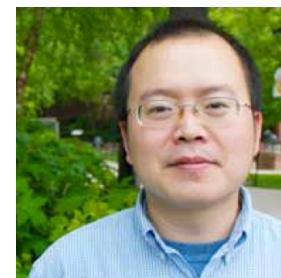
PCA analysis applied on the community reveals that each site has its own signature of communities



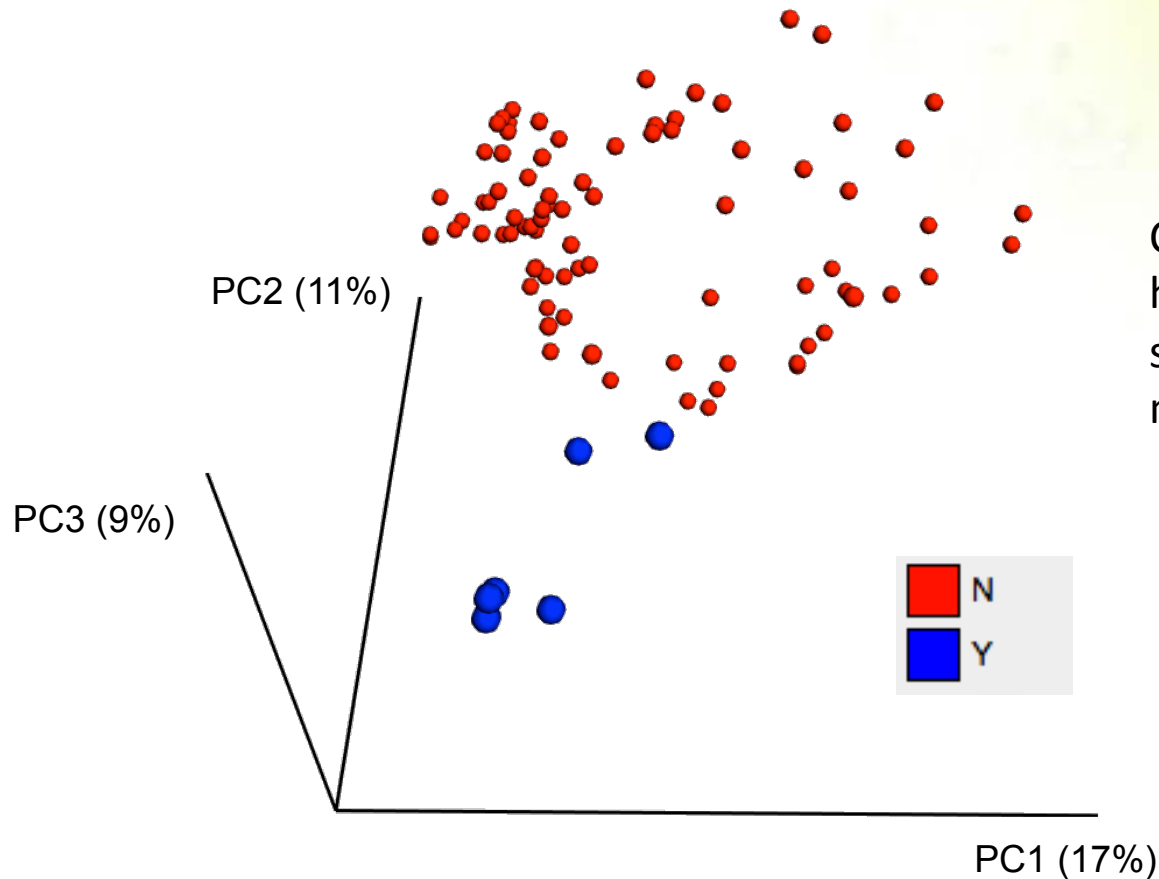
Principal Component Analysis (PCA)



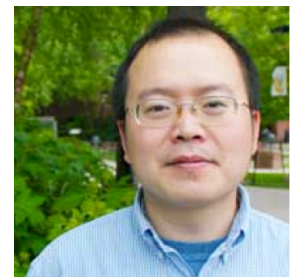
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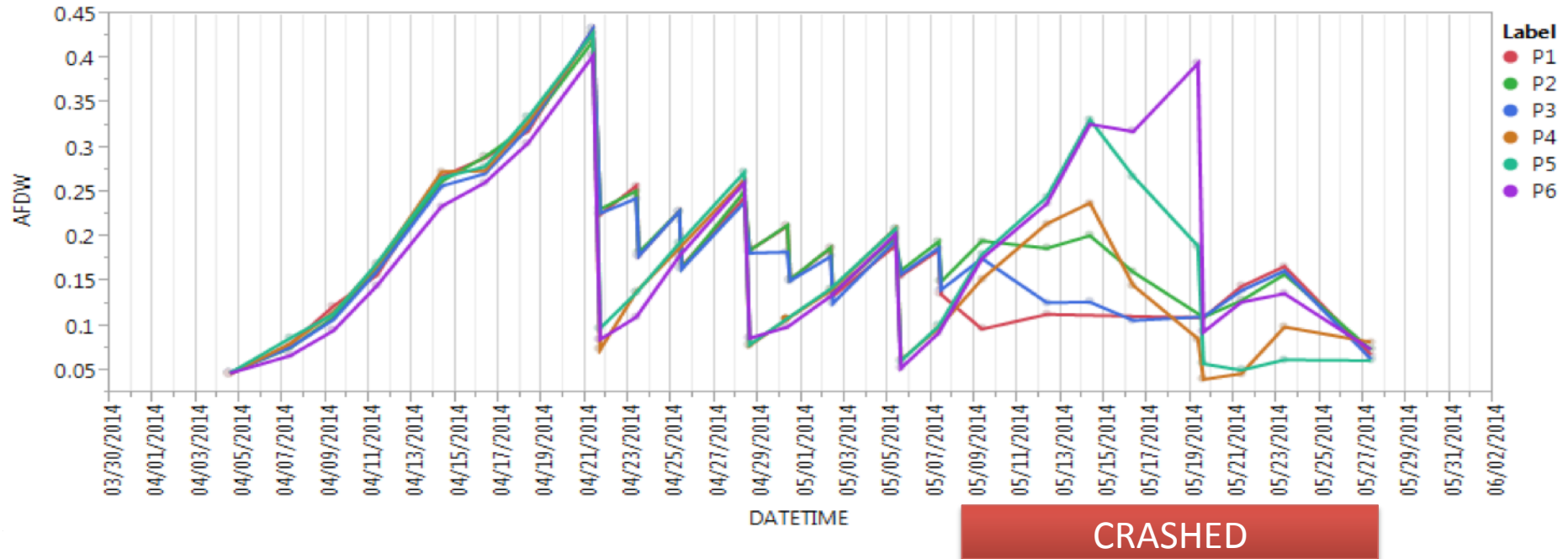
Principal Component Analysis (PCA)



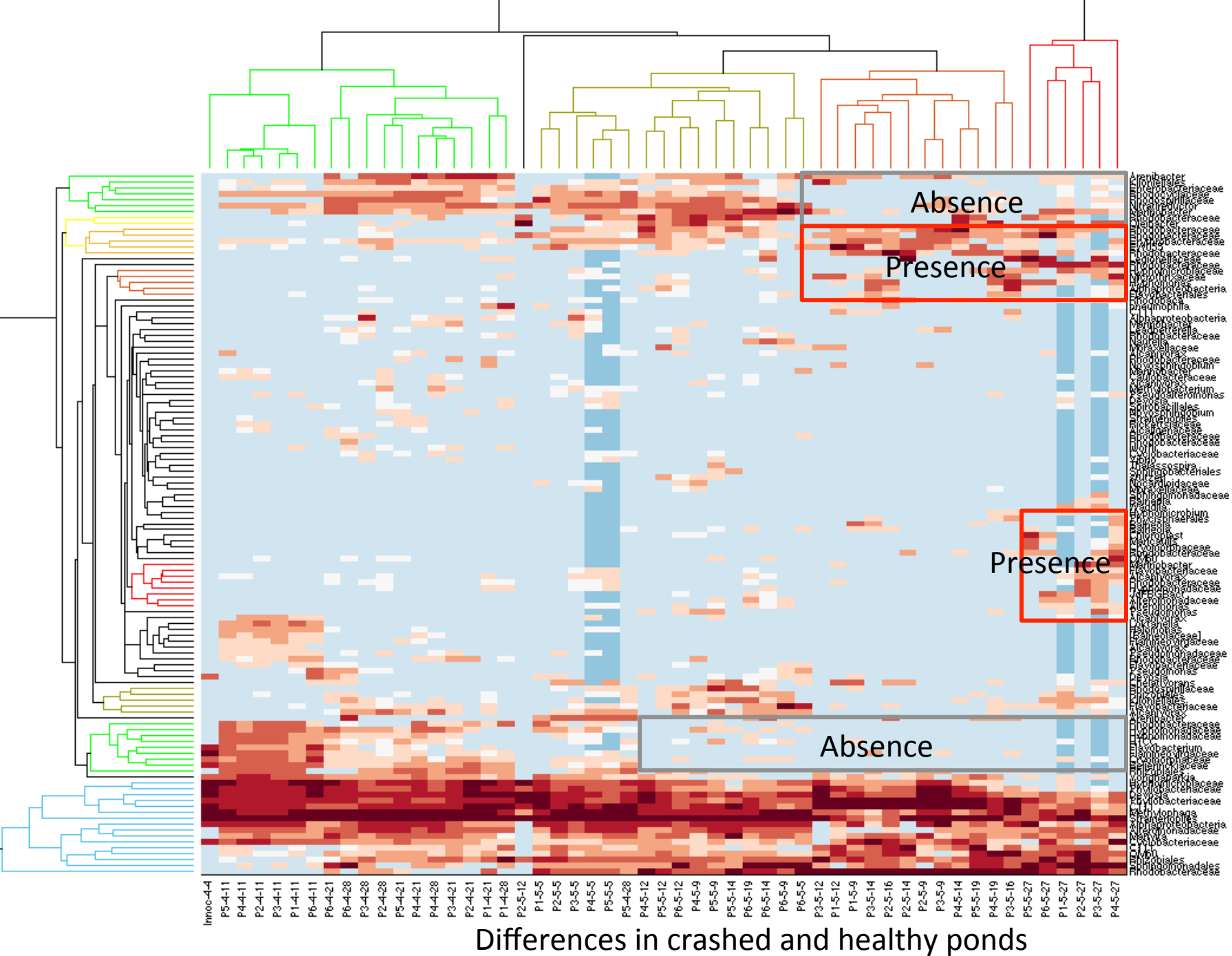
Crashed ponds
have its own
signature of
microbiome



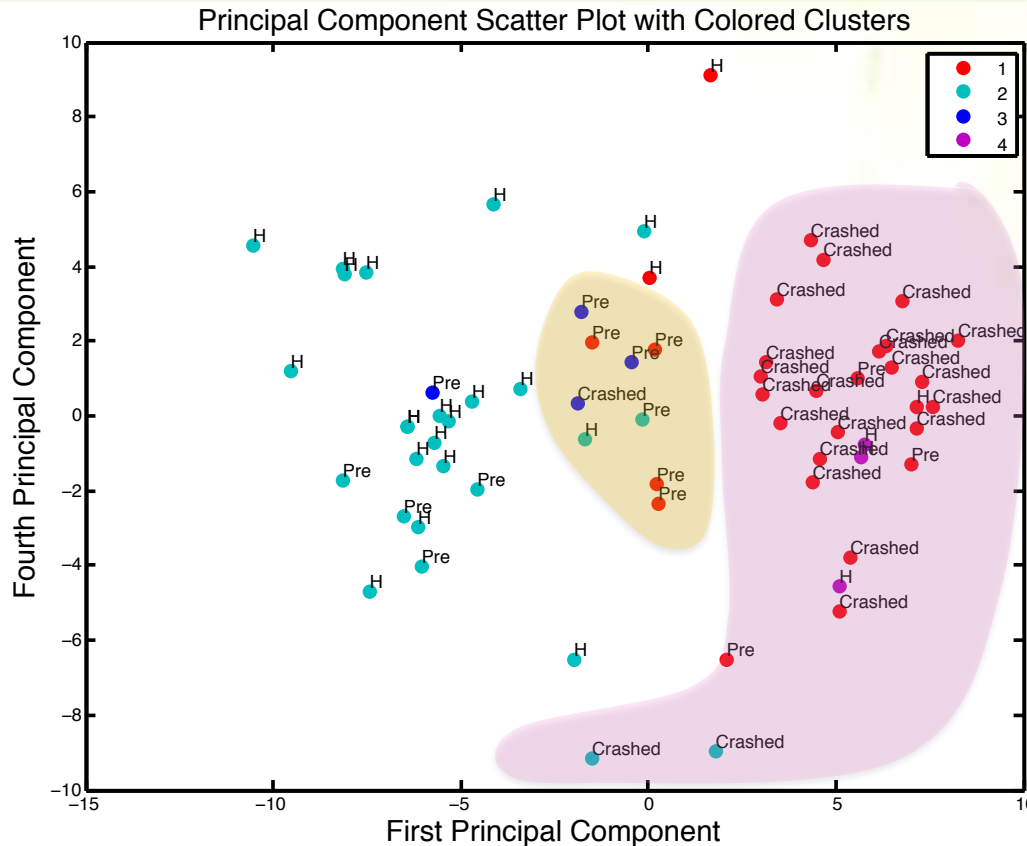
TRL Spring data



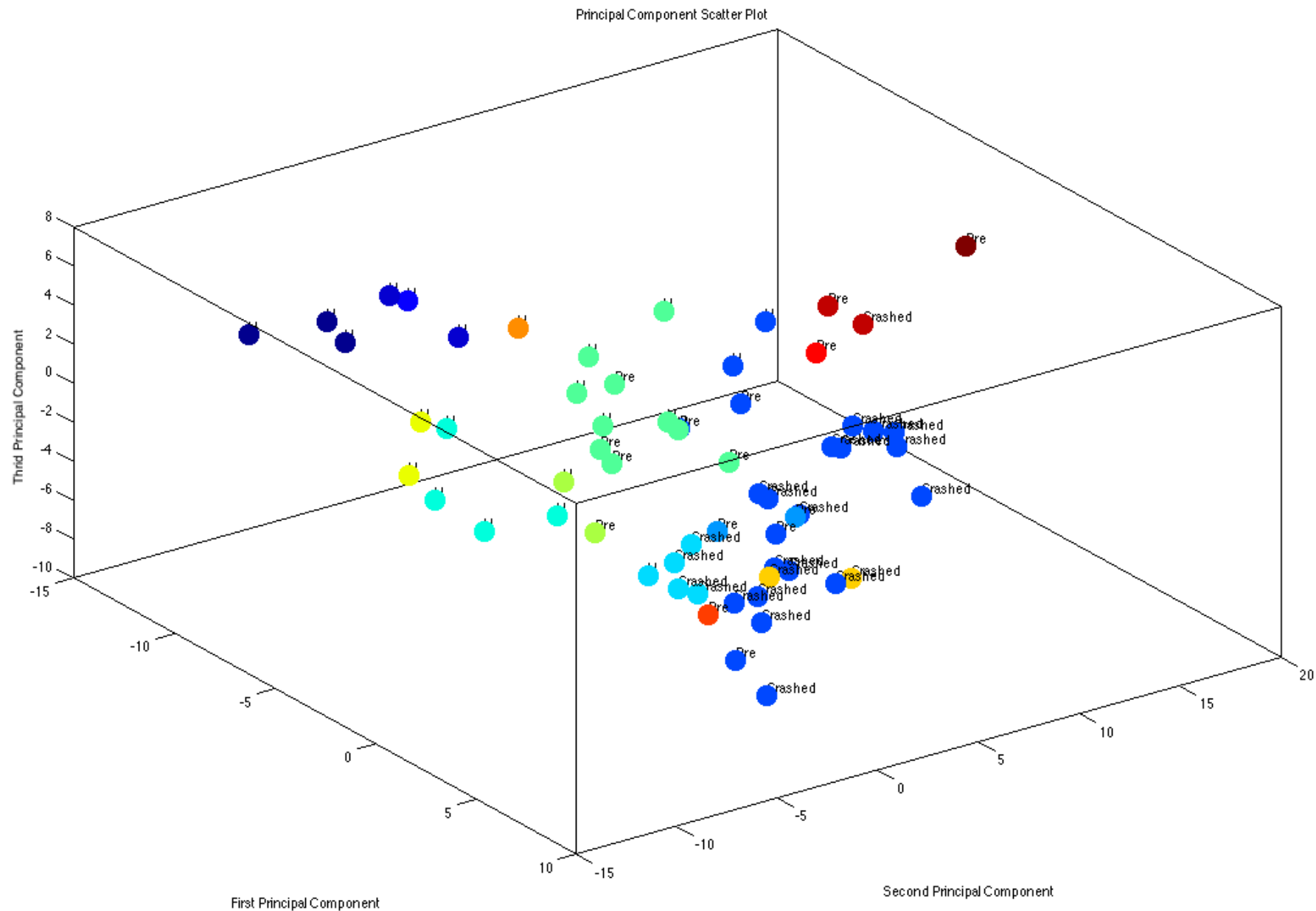
6 identical ponds from TRL Spring data



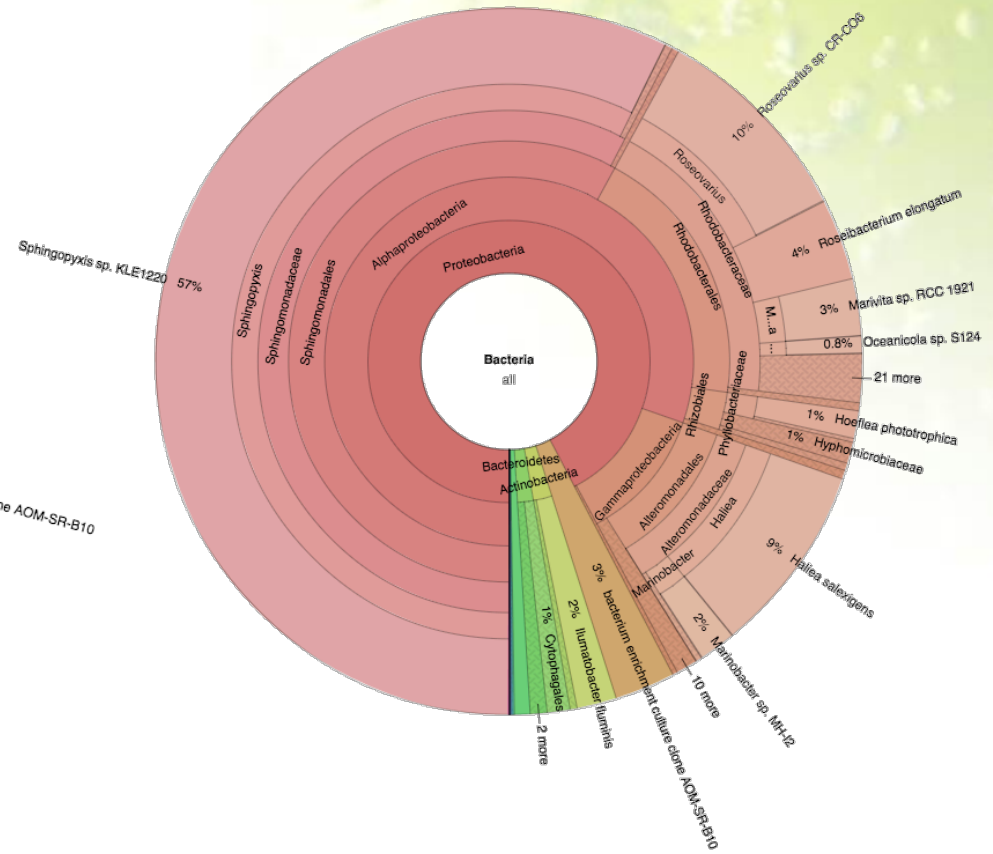
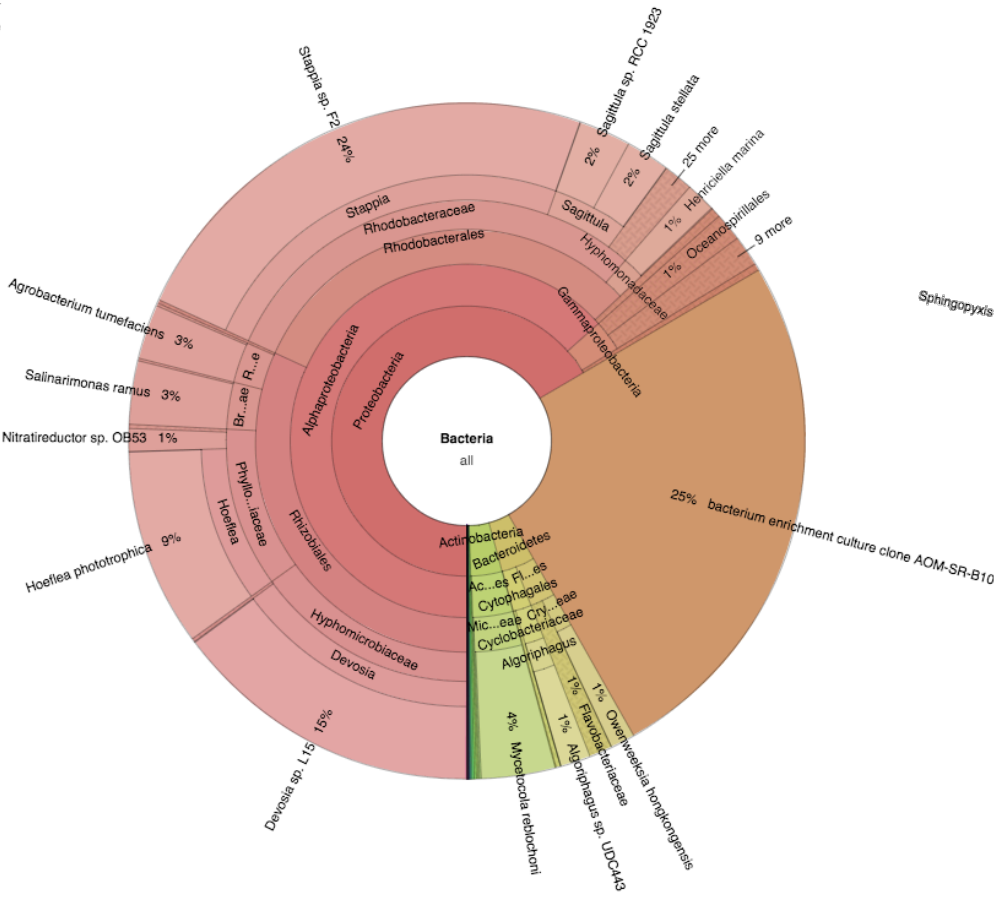
PCA Analysis



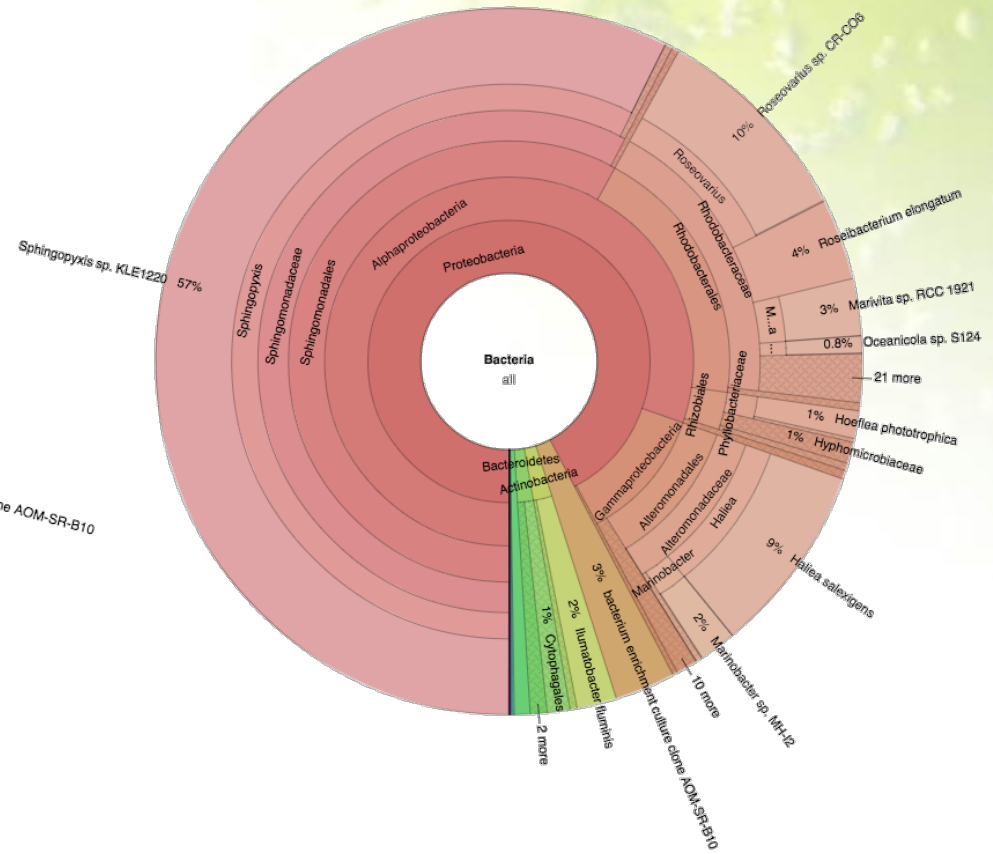
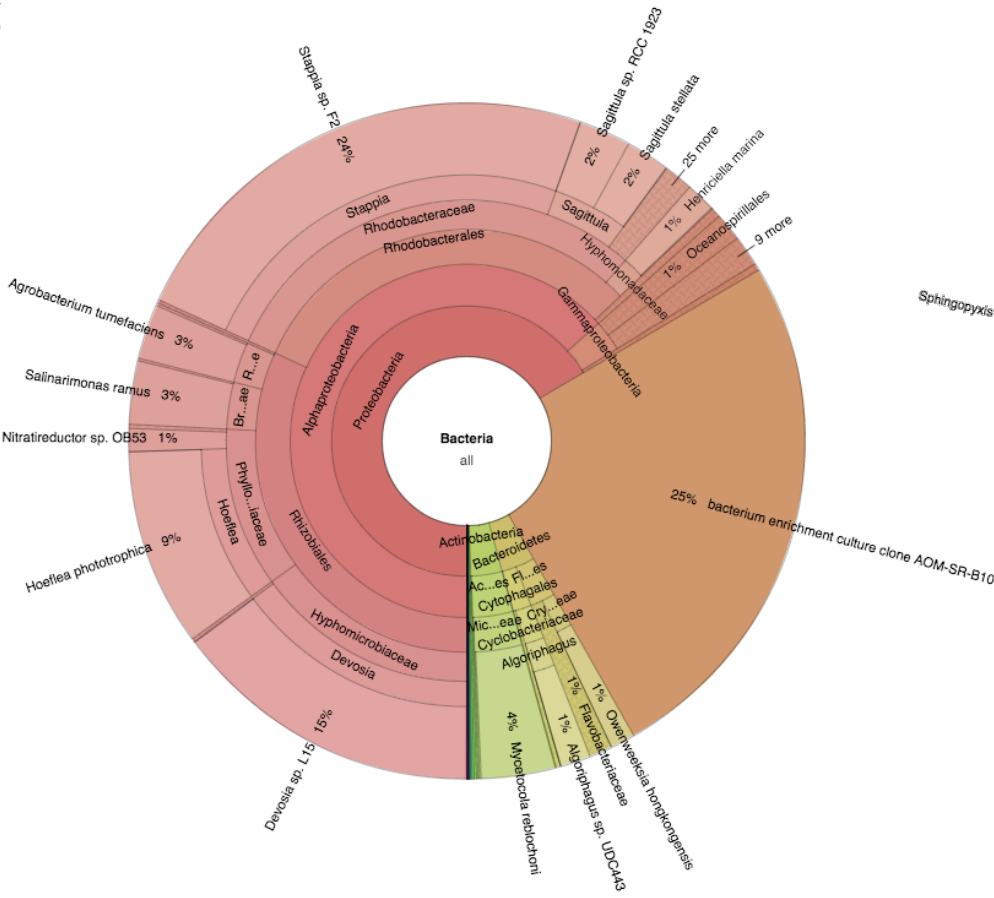
- Clustering and PCA show crashed ponds have similar community structure



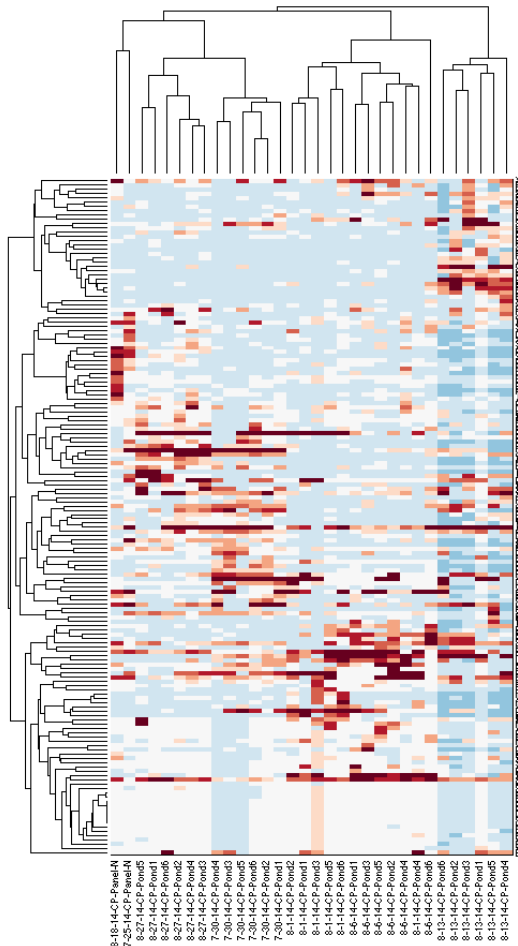
Krona Charts for OTU classification and xanomyxanomy



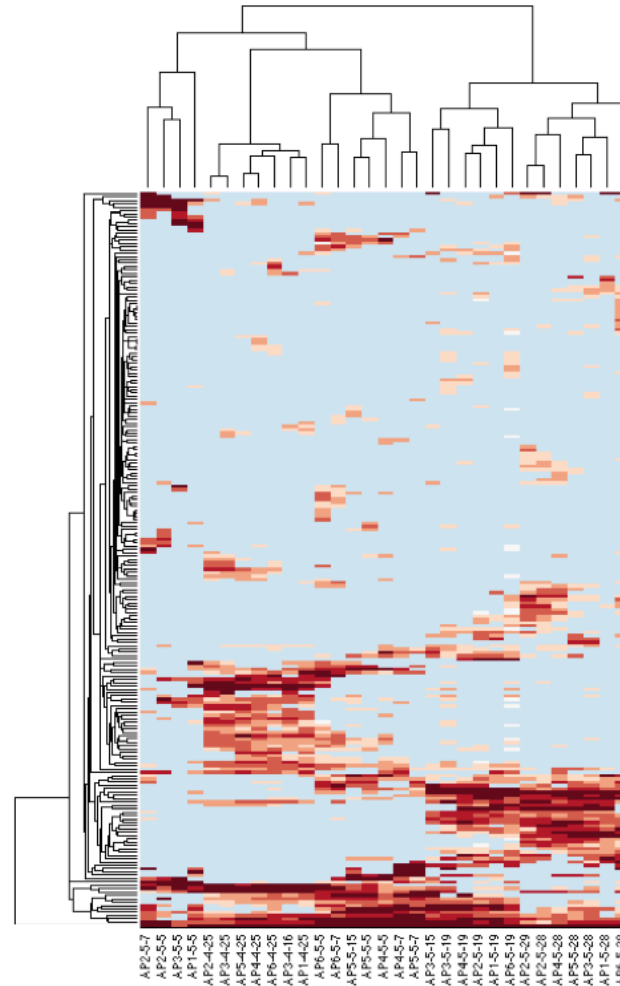
Krona Charts for OTU classification and xanomyxanomy



Cal Poly



Cellana



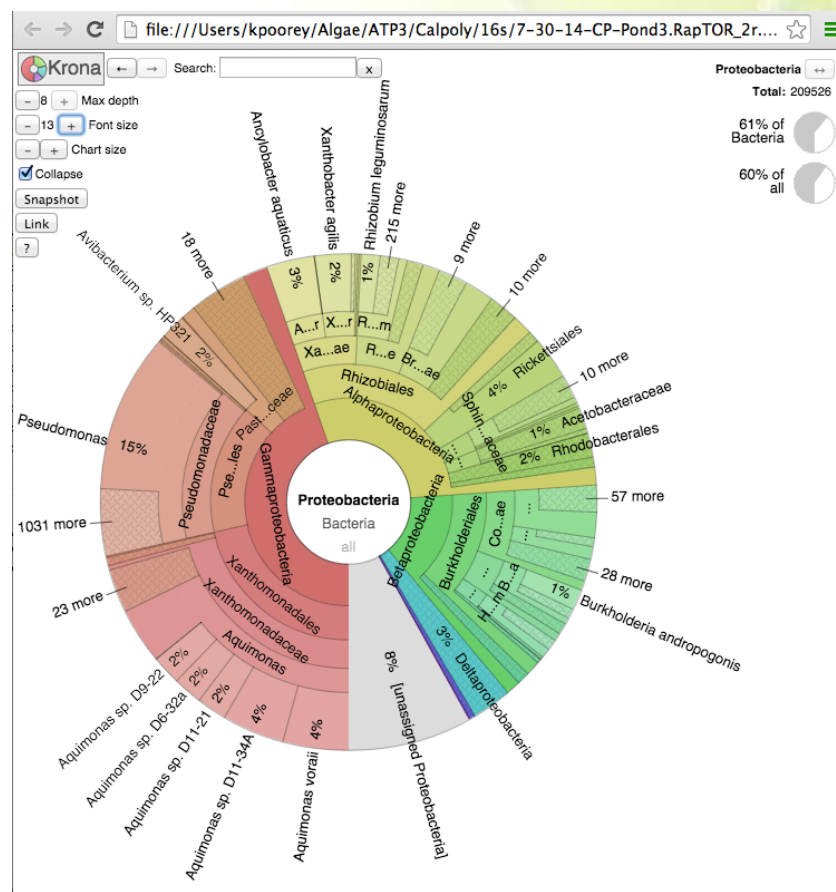
Similar clustering exercise was done for Cellana and CalPoly samples

- We found bacterial communities varying with time.

Data Deposition format

- Raw data
 - Raw reads uploaded to SRA
- Processed data
 - The taxonomy and abundances represented as Krona charts can be displayed at the website

html format



Data Deposition

Table of classified OTUs and their abundances can be displayed on websites as well

taxid in DB	Taxonomy	taxa	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
U41092.1.1792	Eukaryota;Eustigmatophyceae;Eustigmatales;Monodopsidacea	Nannochloropsis granulata;	13379	1	1	1	19644	21286
Y14950.1.1794	Eukaryota;Viridiplantae;Chlorophyta;Trebouxiophyceae;Chloro	Chlorella sp. Yanaqocha RA1;	3375		216	113	677	202
JF834543.1.1228	Eukaryota;Bacillariophyta;Bacillariophyceae;Thalassiosiphales;	Amphora sp. PP-2011;		1		1	1	1
DQ059583.1.1784	Eukaryota;Spirotrichea;Urostylida;Holostichidae;Holosticha;Ho	Holosticha diademata;	1					
EF165112.1.1687	Eukaryota;Chrysophyceae;Chromulinales;Chromulinaceae;Och	Ochromonas cf. gloeopara;	1		1		8	
FR865727.2.1770	Eukaryota;Viridiplantae;Chlorophyta;Chlorophyceae;Sphaerop	Scenedesmus armatus;	12		27	10	1	4
AY520448.1.1735	Eukaryota;Bicosoecida;Cafeteriaeae;Cafeteria;Cafeteria minin	Cafeteria minima;		6	13	3	1	17
AY622191.1.1753	Eukaryota;Metazoa;Arthropoda;Ostracoda;Podocopida;Cycloc	Cypria crenulata;			36	12	30	3
HM161745.1.1787	Eukaryota;Synurophyceae;Ochromonadales;Ochromonadacea	Poterioochromonas sp. Y4;	11		7			3
L27634.3.1794	Eukaryota;Labyrinthulomycetes;Thraustochytriaceae;Labyrinth	Labyrinthuloides minuta;						
EU106848.1.1693	Eukaryota;Bicosoecida;Caecitellus;Caecitellus sp. RCC1072;	Caecitellus sp. RCC1072;		2	14		21	63
M74497.1.1789	Eukaryota;Viridiplantae;Chlorophyta;Chlorophyceae;Sphaerop	Hydrodictyon reticulatum;	7		12	11	20	
JN120201.1.1541	Eukaryota;Oligohymenophorea;Opisthionectidae;Opisthionecta	Opisthionecta henneguyi;						
DQ514856.1.1722	Eukaryota;Bacillariophyta;Coscinodiscophyceae;Stephanodisca	Cyclotella meneghiniana;			5		1	
EU032356.1.1751	Eukaryota;Oligohymenophorea;Pleuronematida;Cyclidiidae;Cy	Cyclidium glaucoma;	1		9	1	8	1
JF489982.1.1752	Eukaryota;Eustigmatophyceae;Eustigmatales;Monodopsidacea	Nannochloropsis oceanica;						1
EU039885.1.1700	Eukaryota;Colpodea;Colpodida;Hausmanniellidae;Bresslauides	Bresslauides discoideus;						
U49911.1.1808	Eukaryota;Metazoa;Rotifera;Monogononta;Ploimida;Brachioni	Brachionus plicatilis;			11	2	9	
AF352222.1.2260	Eukaryota;Viridiplantae;Streptophyta;Zygnemophyceae;Desmi	Closterium moniliferum;						
HQ912576.1.1744	Eukaryota;Bacillariophyta;Coscinodiscophyceae;Stephanodisca	Cyclotella meneghiniana;						

Questions ?

- Sandia National Labs
 - **Todd Lane**
 - **Kunal Poorey**
 - **Deanna Curtis**
 - **Haifeng Gang**
 - **Pamela Lane**
 - **Kelly Williams**

