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RESOURCES FOR LONG-TERM, CONTINENTAL-SCALE

MICROBIAL ECOLOGY

AND PRELIMINARY RESULTS FROM FOUR SITES

Dr. Kathryn Docherty, Assistant Professor, Western Michigan University

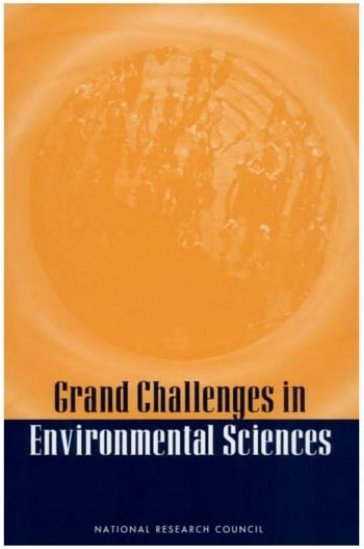
Grand Challenges in Environmental Sciences

Drivers of biological and ecological change

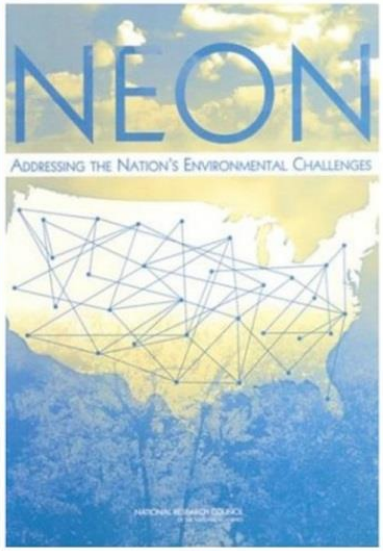
Drivers

- Climate change
- Land-use dynamics
- Invasive species

Interactions, Feedbacks



National Research Council
Press 2001 Washington DC



National Research Council
Press 2003 Washington DC

Effects on organisms, populations, and communities

Responses

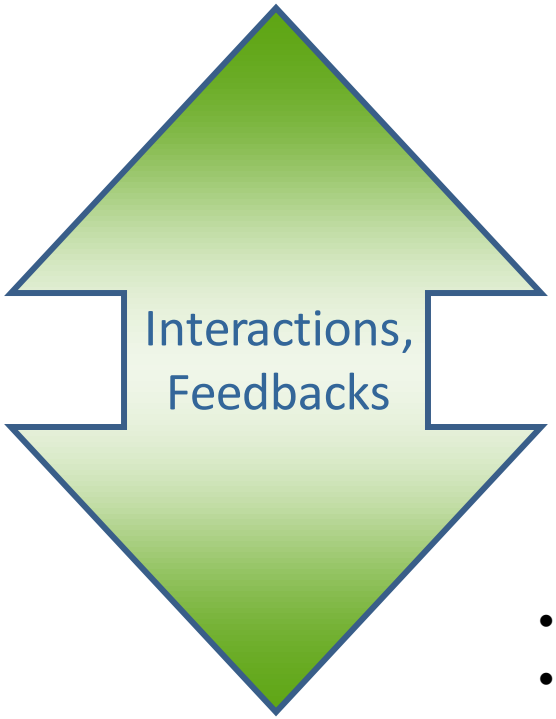
- Biogeochemical cycles
- Biodiversity and ecosystem function
- Hydrological forecasting
- Infectious diseases and environment

Grand Challenges in Environmental Sciences

Drivers of biological and ecological change

Drivers

- Climate change
- Land-use dynamics
- Invasive species



Information Infrastructure

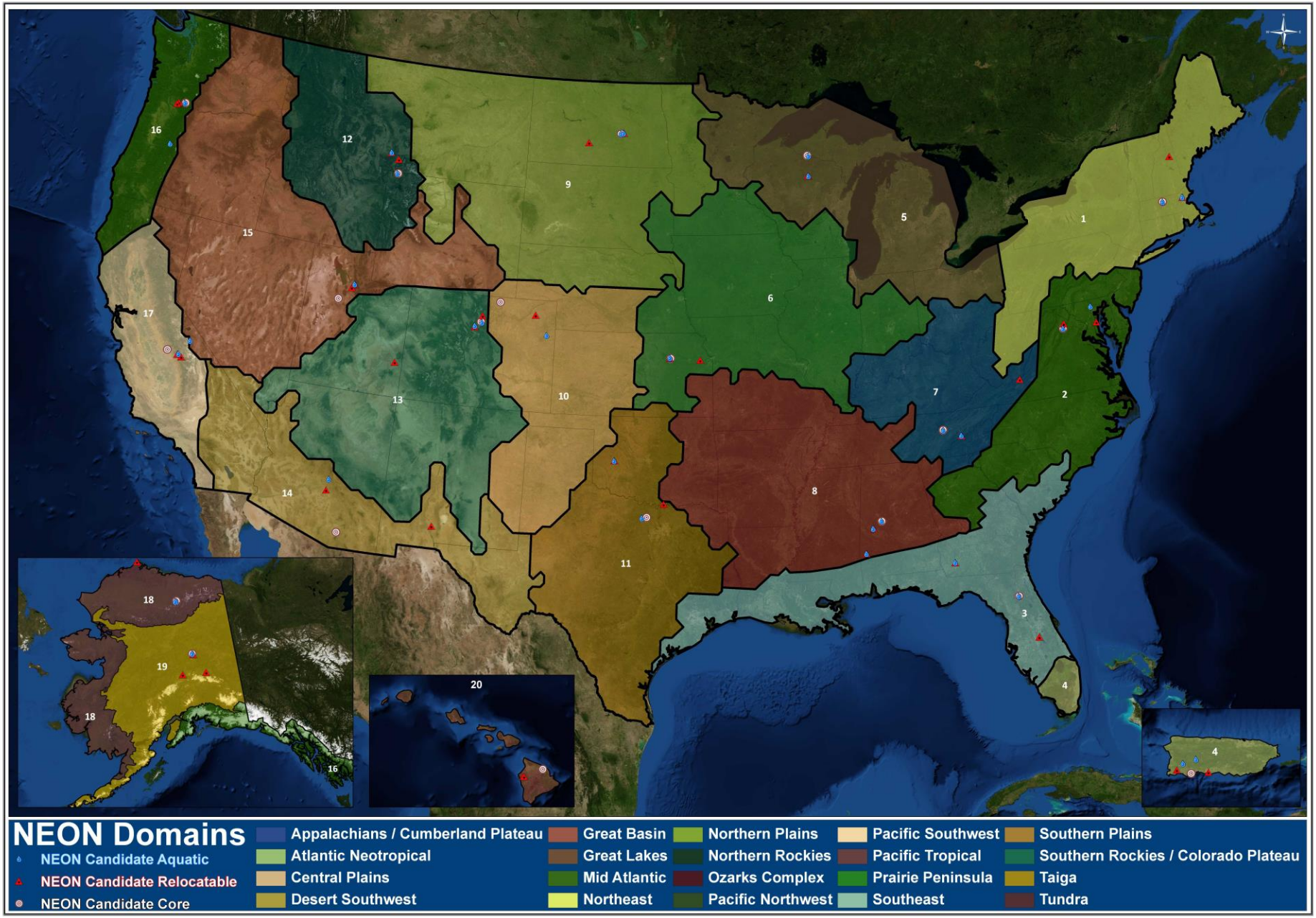
Physical Infrastructure

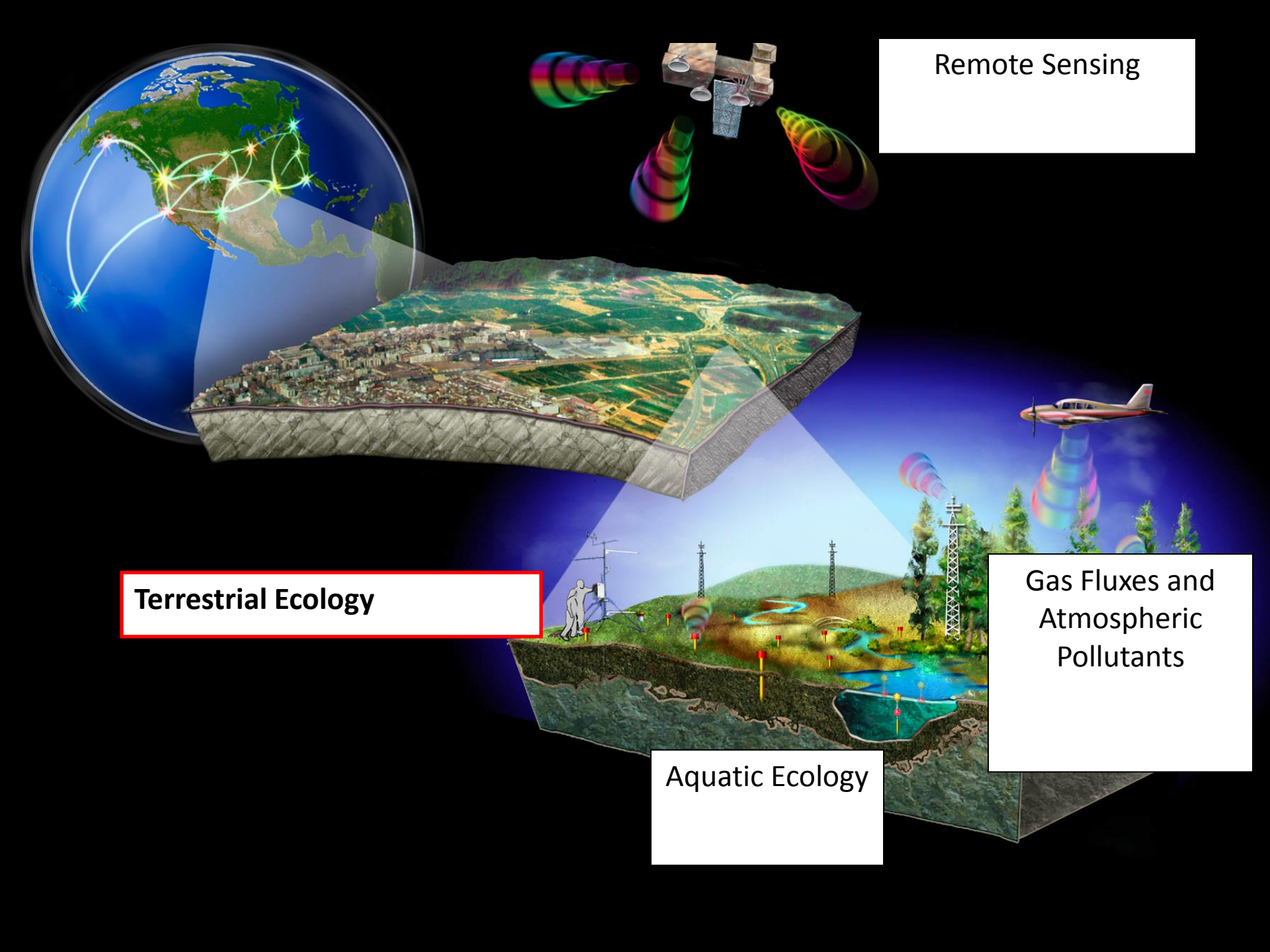
- Biogeochemical cycles
- Biodiversity and ecosystem function
- Hydrological forecasting
- Infectious diseases and environment

Effects on organisms, populations, and communities

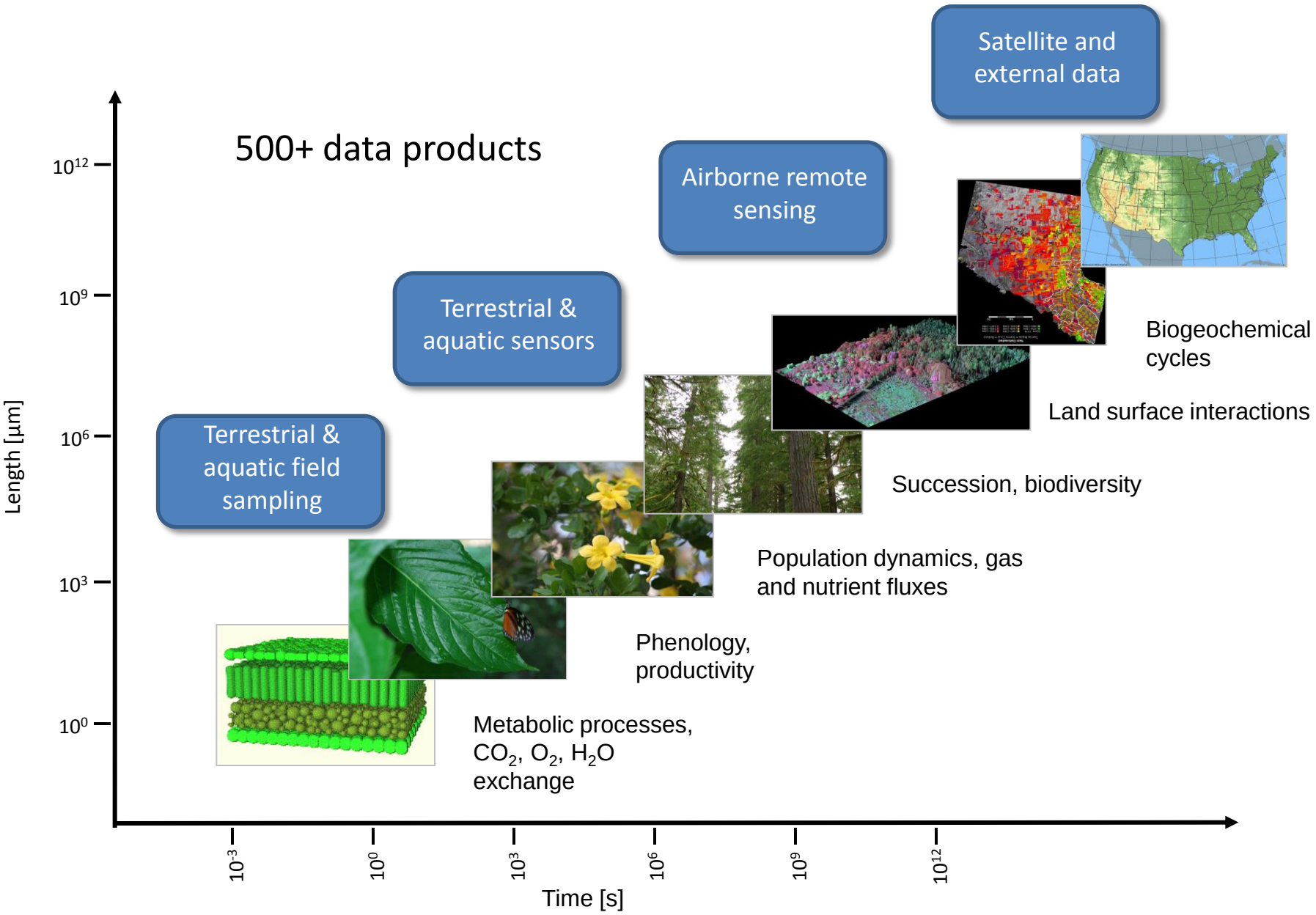
Responses

20 Eco-climatic domains, 3 sites/domain



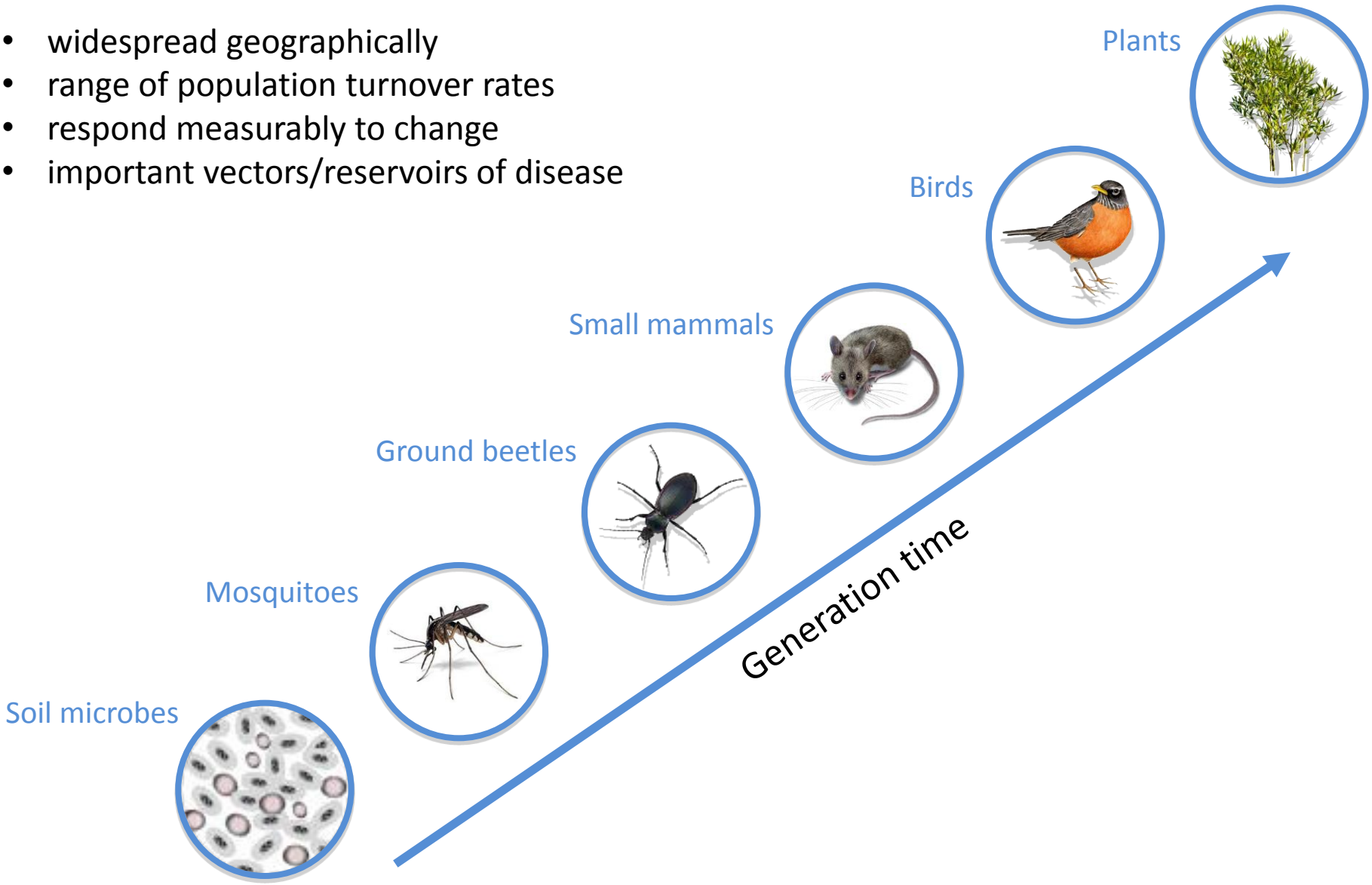

Remote Sensing
Terrestrial Ecology
Gas Fluxes and Atmospheric Pollutants
Aquatic Ecology

Scaling from Populations to Regions



Terrestrial “Sentinel Taxa”

- widespread geographically
- range of population turnover rates
- respond measurably to change
- important vectors/reservoirs of disease



NEON data are meant to…

FACILITATE SCIENTIFIC DISCOVERY

- Provide scientists with long-term background information at targeted sites
- Provide scientists with raw data to analyze/include in meta-analyses
- Provide scientists with stored samples to include in their own projects

ENABLE EDUCATION

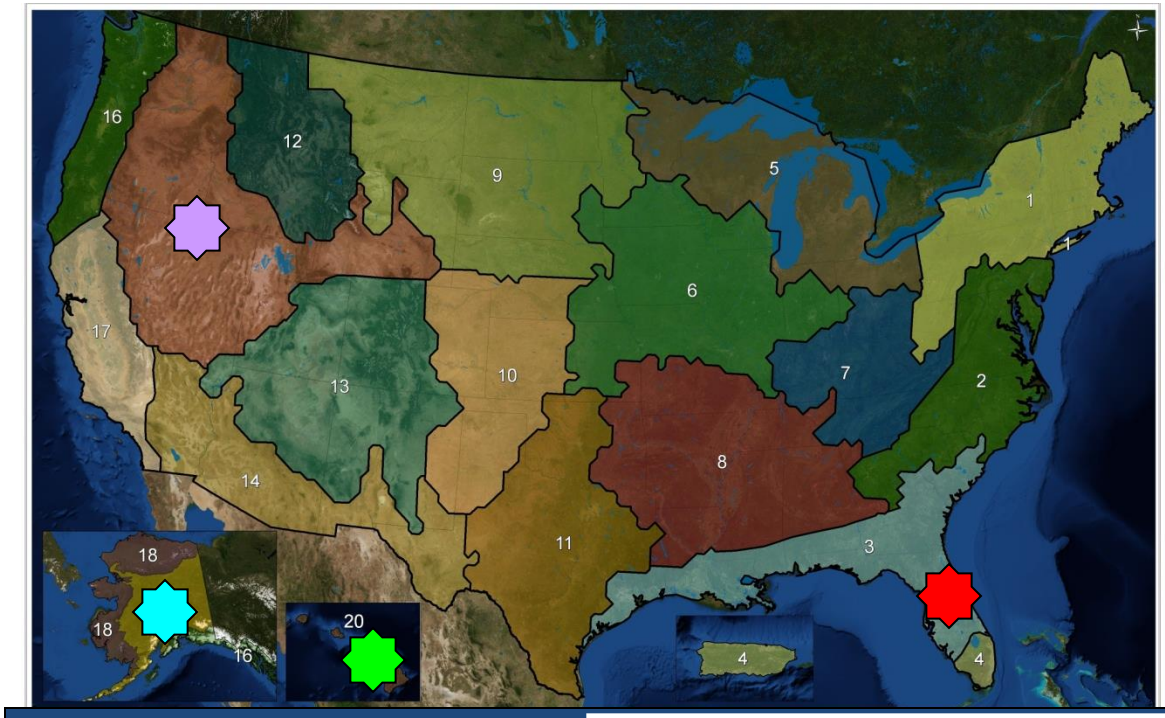
- Provide educators with raw and analyzed data for the classroom
- Provide citizen-science opportunities
- Provide policy-makers with interpreted data to help make informed political decisions

NEON's Soil Microbiology

How can soil microbes be incorporated into global change forecasting on a continental scale?

- Consistent seasonal and annual sampling
- Environmental metadata
- State-of-the-art sample analysis
- Quality control, data analysis
- Sample archive
- Integration with other NEON data
- 30 year + resource for microbial ecology

Soil Microbe Prototype



Soil Microbe Sampling

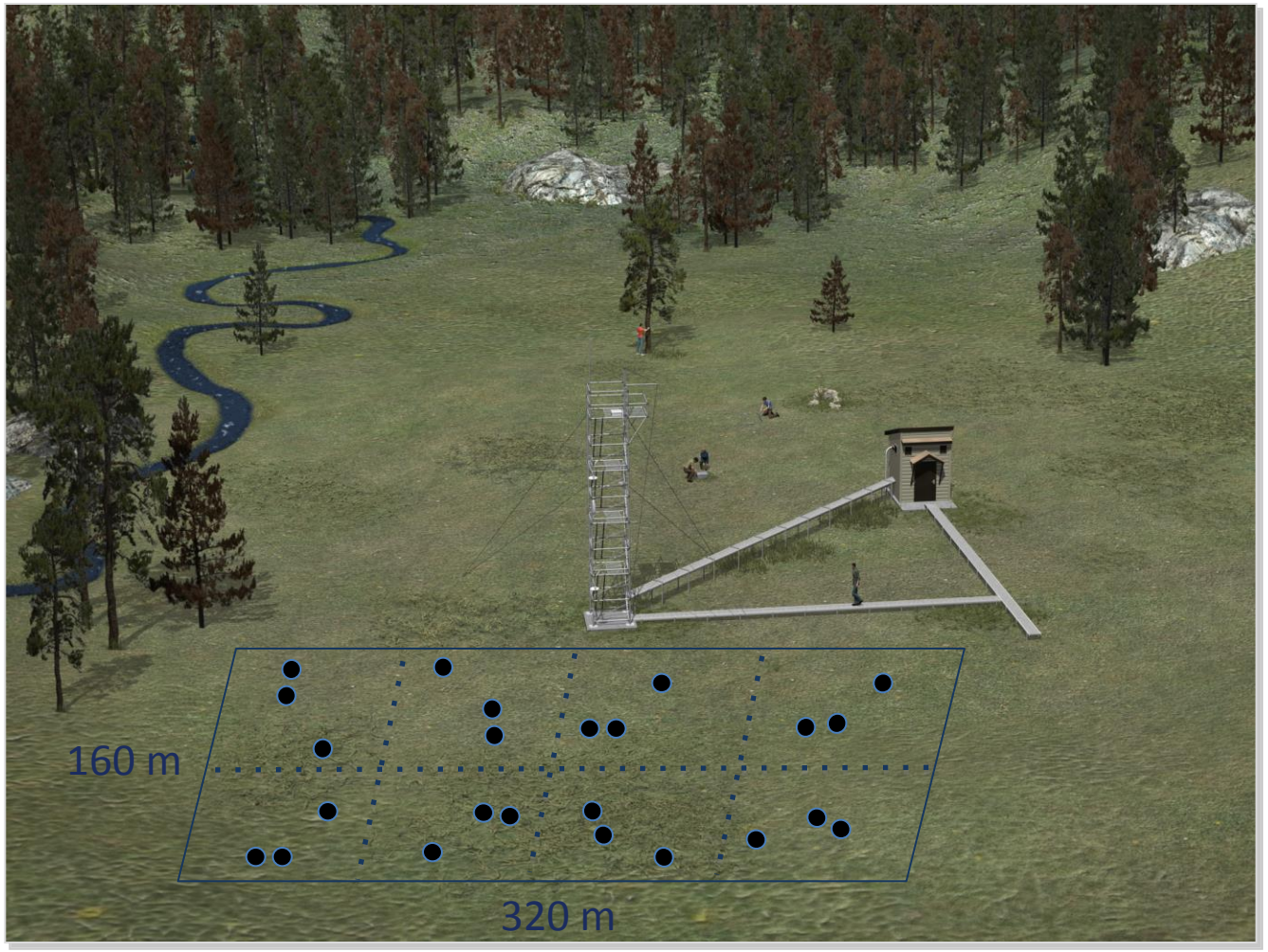
15 cm depth
(O & A horizon separate)

24 cores
(3/subplot)

1 composite core/subplot

4 times annually
(seasonal variation)

Frozen at -80°C and -20°C



Soil Measurements in 2009-2010

Soil Environmental Measurements

Soil Water Content (SWC)
Organic Matter Content (OM)
Percent Carbon (TC)
Percent Nitrogen (TN)
Nitrate (NO3-)
pH
Cation Exchange Capacity (CEC)
Potassium ions (K)
Calcium ions (Ca)
Magnesium (Mg)
Sodium (Na)
Chloride (Cl)
Sulfate (SO4)

Microbial Lipids (PLFA)

Total Microbial Biomass (TMB)
Fungal to Bacterial Ratio (FTB)
Gram Negative Lipids (GN)
Gram Positive Lipids (GP)
Actinobacterial Lipids (ACT)
Anaerobic Bacterial Lipids (ANA)
Arbuscurlar Mycorrhizal Fungal Lipids (AMF)
Saprophytic Fungal Lipids (SF)

Microbial Community Structure

Barcoded pyrosequencing with Roche Titanium Chemistry

- 16S rRNA (515f/816r)
- 18S rRNA (817f/1996r)

What kinds of questions can you address with these data?

University of Arizona
RNR696: Tools for Microbial Ecology
Instructor: Rachel Gallery

Western Michigan University
BIOS Independent Research
Instructor: Kathryn Docherty

What kinds of questions can you address with these data?

We know that pH is important for driving microbial community structure across distant sites, but what factors are important within a site?

Are there different microbial communities at different sites?

Do lipid-based measurements and 16S-/18S-based measurements yield similar results?

Do microbial communities change over time at one site vs. another?

Do important environmental factors change over time?

Does storage temperature matter to the microbial community?

Do microbial communities vary by soil core? Is community similarity a function of distance in some sites but not others?

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NEON metadata

What kinds of questions can you address with these data?

Here's where the pilot data became frustrating…

Why don't all the sites have cores that were stored at -80^{o}C and -20^{o}C?

For that matter, why do some sites have composite cores only and other sites have individual cores only and other sites have both composite and individual cores?

And why are there only 3 time points in Alaska instead of 4?

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Educational Advice Point #1

If you're going to use observatory data for a class or a meta-analysis, be flexible with your questions.

Coordinate with someone at NEON – they are there to help!

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What CAN we answer about storage temperature?

Does storage temperature influence 16S rRNA microbial communities from soils collected in Hawaii?

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Ecological Analysis Tools for Microbial Ecology (EATME)

http://mb3is.megx.net/eatme/

Pier Luigi Buttigieg
HGF-MPG Group for Deep Sea Ecology and Technology
Max Planck Institute for Marine Microbiology
pbuttigi@mpi-bremen.de

NMDS
ANOSIM
Permanova
CCA
RDA
PCoA
PCA
etc.

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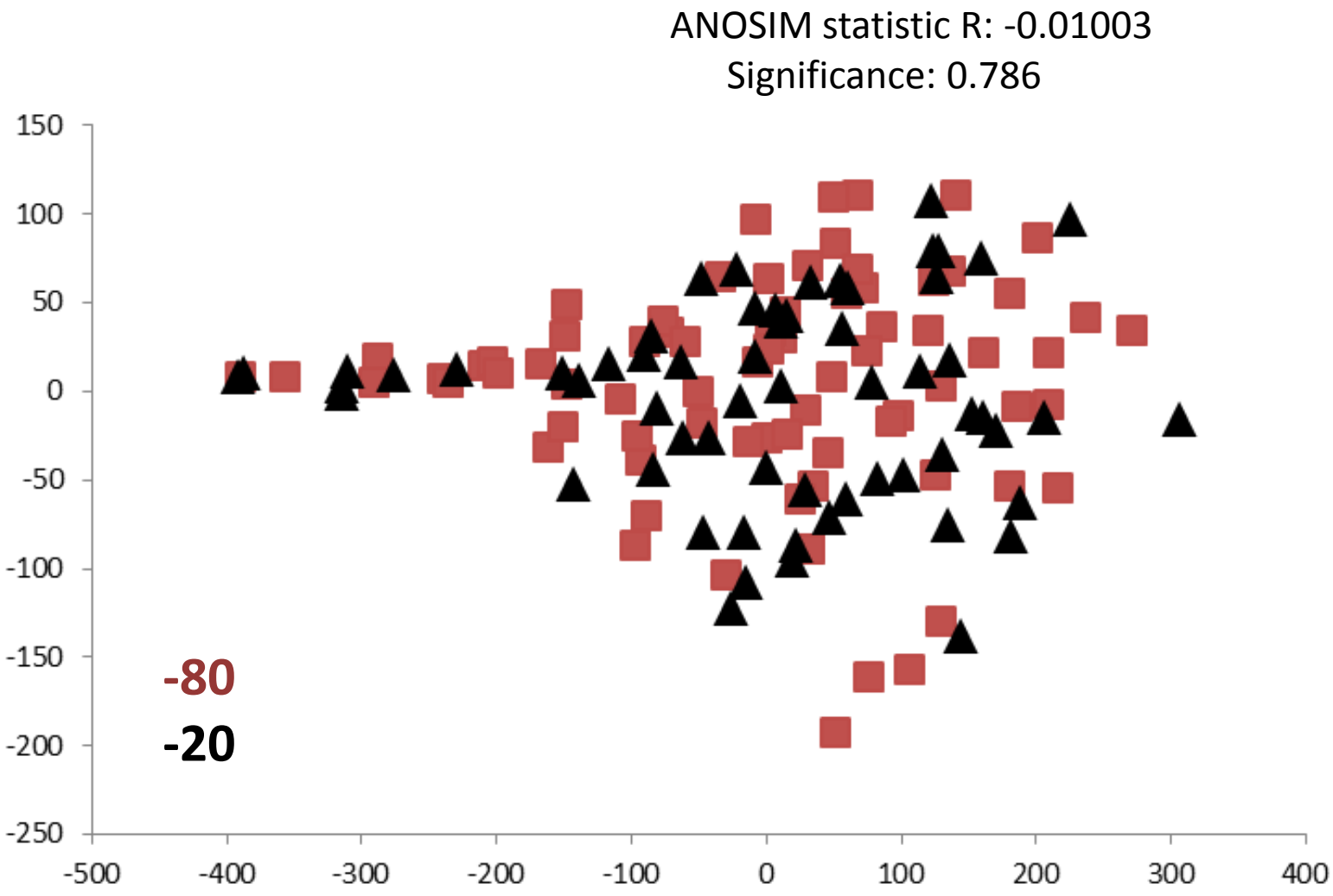
Ecological Analysis Tools for Microbial Ecology (EATME)

Example 1: Is there a difference in soil storage temperature for samples collected from Hawaii?

Open the file HI_Temperature 16S data.csv
Run NMDS

Open the file HI_Temperature Grouping Variables_ANOSIM.csv
Run ANOSIM

What CAN we answer about storage temperature?



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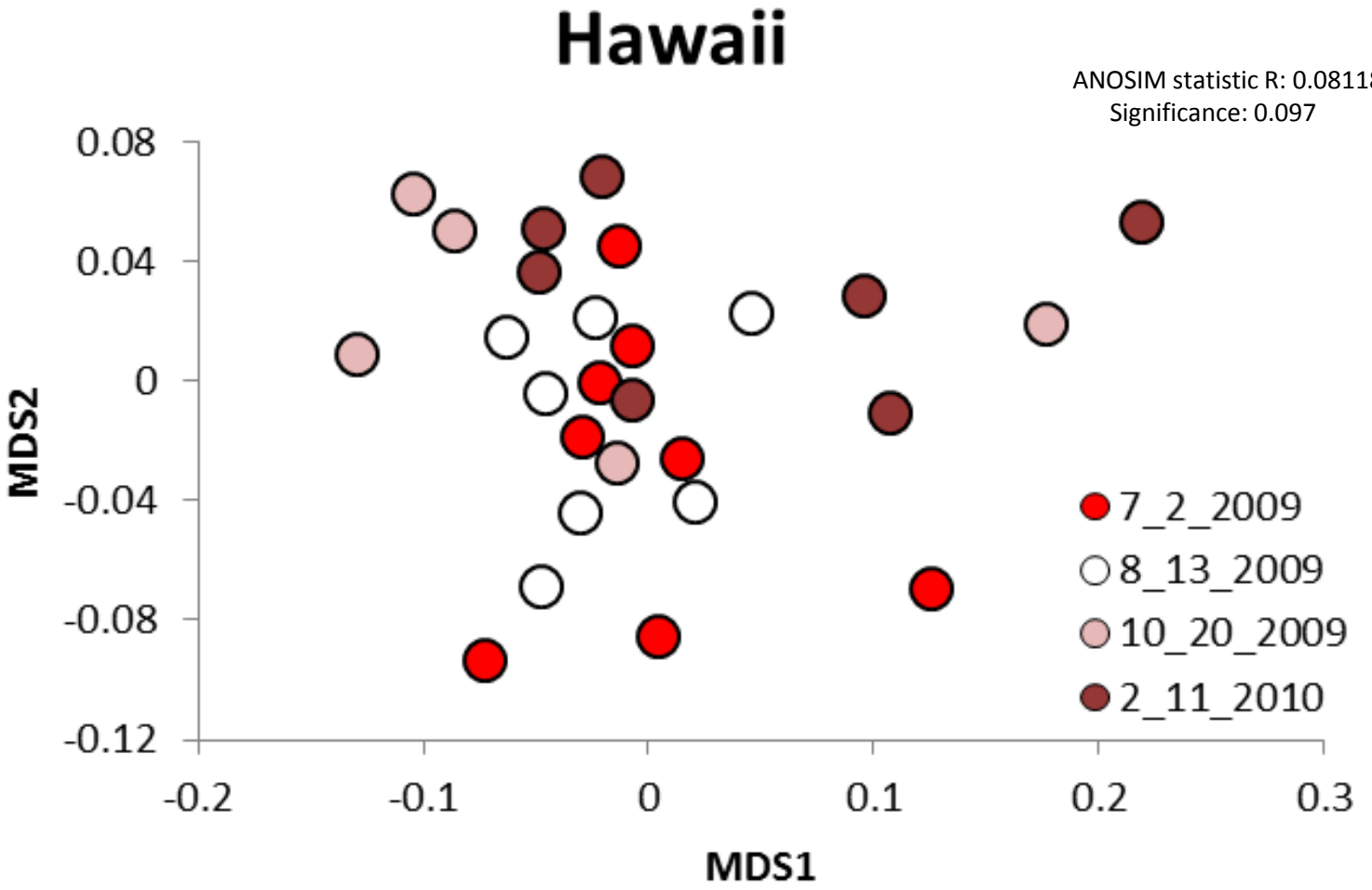
Exploring NEON Data

Example 2. Are there differences over time in microbial communities in Hawaii?

Things to consider:

1. Do you want to include unidentified taxa?
2. Should you calculate relative abundance or use raw OTU counts?
3. Do you want to use composite cores or individual cores or both?
4. How will you set up your grouping variables?

Are there differences over time in microbial communities in Hawaii?



Spring 2014 Class Questions

Using soil environmental data, microbial lipid data and 16S rRNA data:

1. Do sites differ at peak greenness?

2. Do individual sites differ over time?

3. Do Families within dominant soil Phyla differ over time?

Ok, now figure out how to do it.

Educational Advice Point #2

This type of class can be overwhelming for students, particularly early career grad students and undergraduates.

Everyone has a strength

TEAMWORK

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Everyone has a strength

TEAMWORK

Effective Team Leaders

Team Bioinformatics
Team Background Literature
Team Statistics

Facilitate Communication

Google Groups
iplant for data sharing
Dropbox
Clear weekly goals

Write Clear Protocols

Be able to explain what you did
Not having to re-invent the wheel
Video tutorials

Educational Advice Points #3

NEON data can be used to offer many types of classes

- Team-led investigative (graduate)
- In-depth community statistics
- Introductory statistics
- Intro to microbial ecology as part of undergraduate Microbiology

Educational Advice Points #4

Offer the grad-level version of a class to students in Biology and Computer Science and have them work together in teams

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Do sites differ at PEAK GREENNESS?

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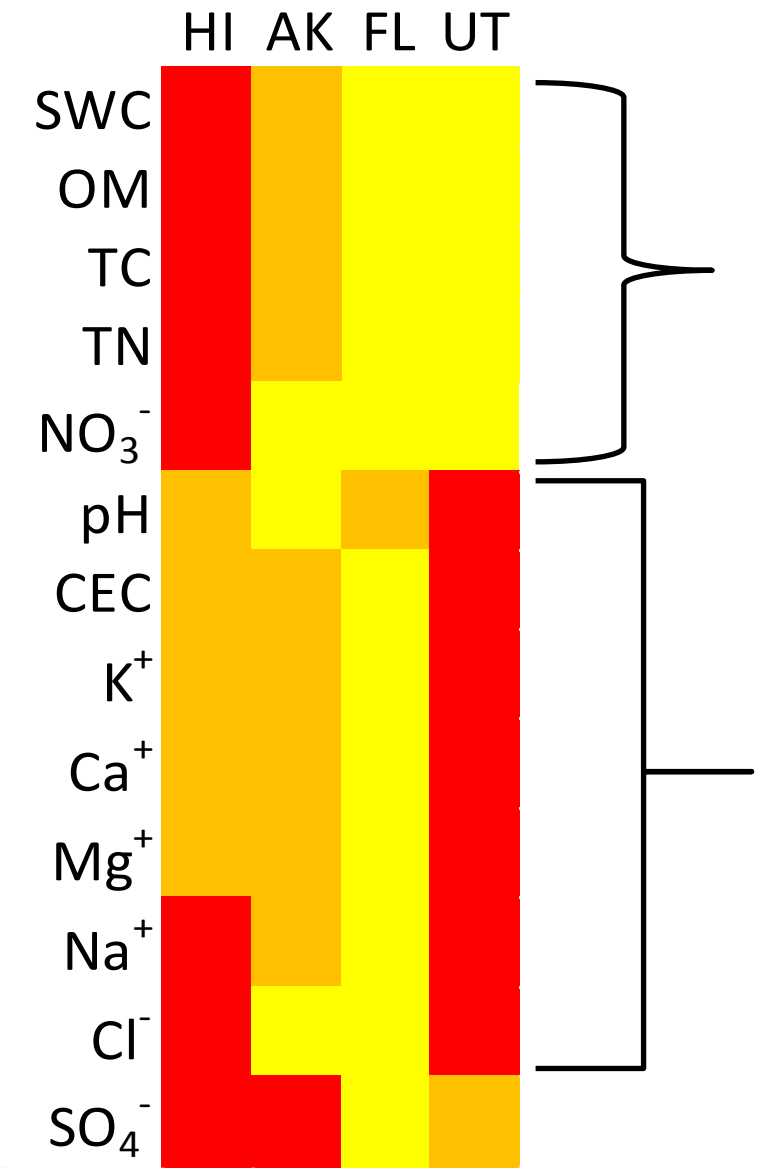
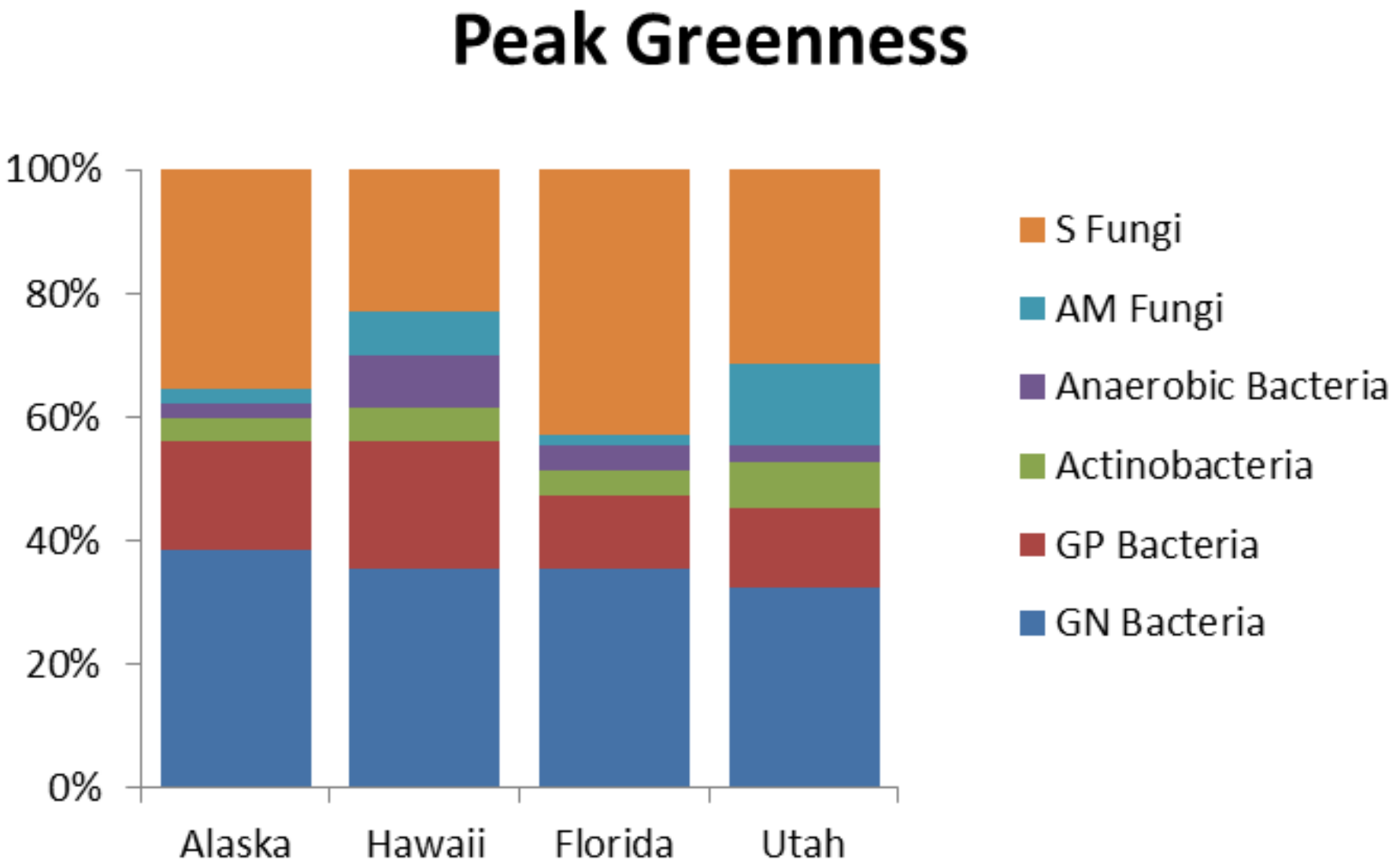
At Peak Greenness, there are environmental differences between the 4 sites



α=0.05

Docherty et al. in prep.

At Peak Greenness microbial lipid markers differ between the 4 sites



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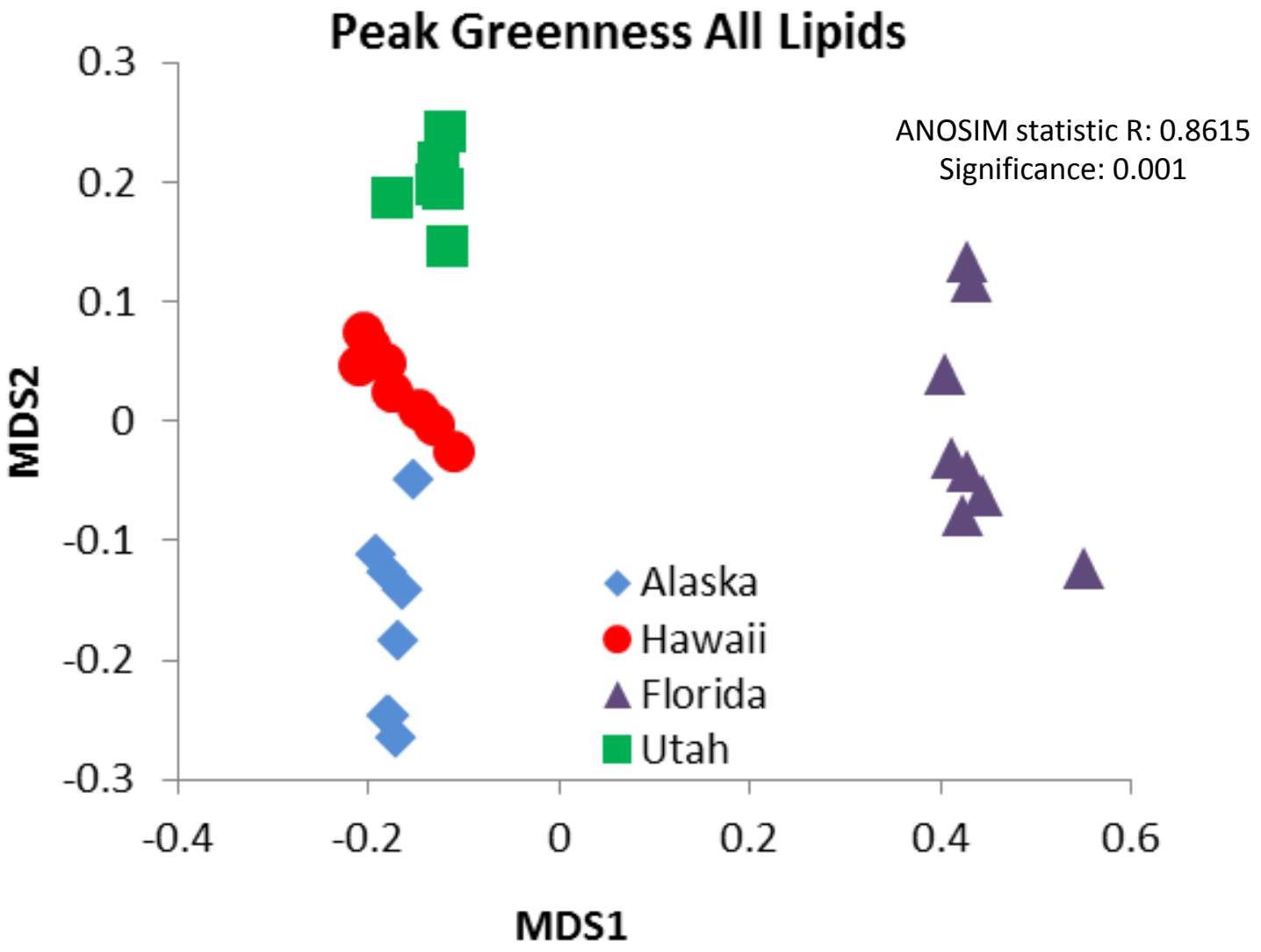

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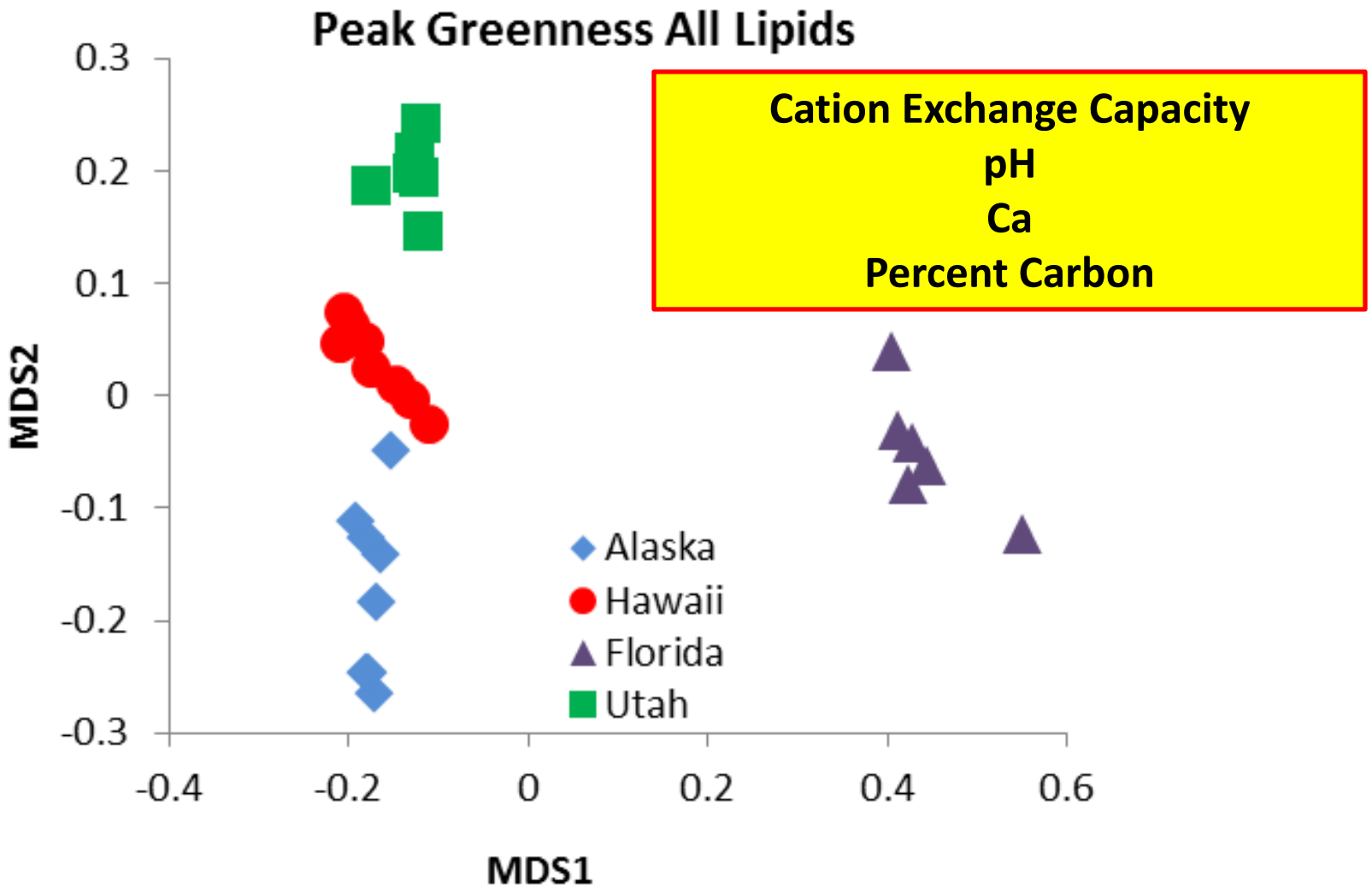

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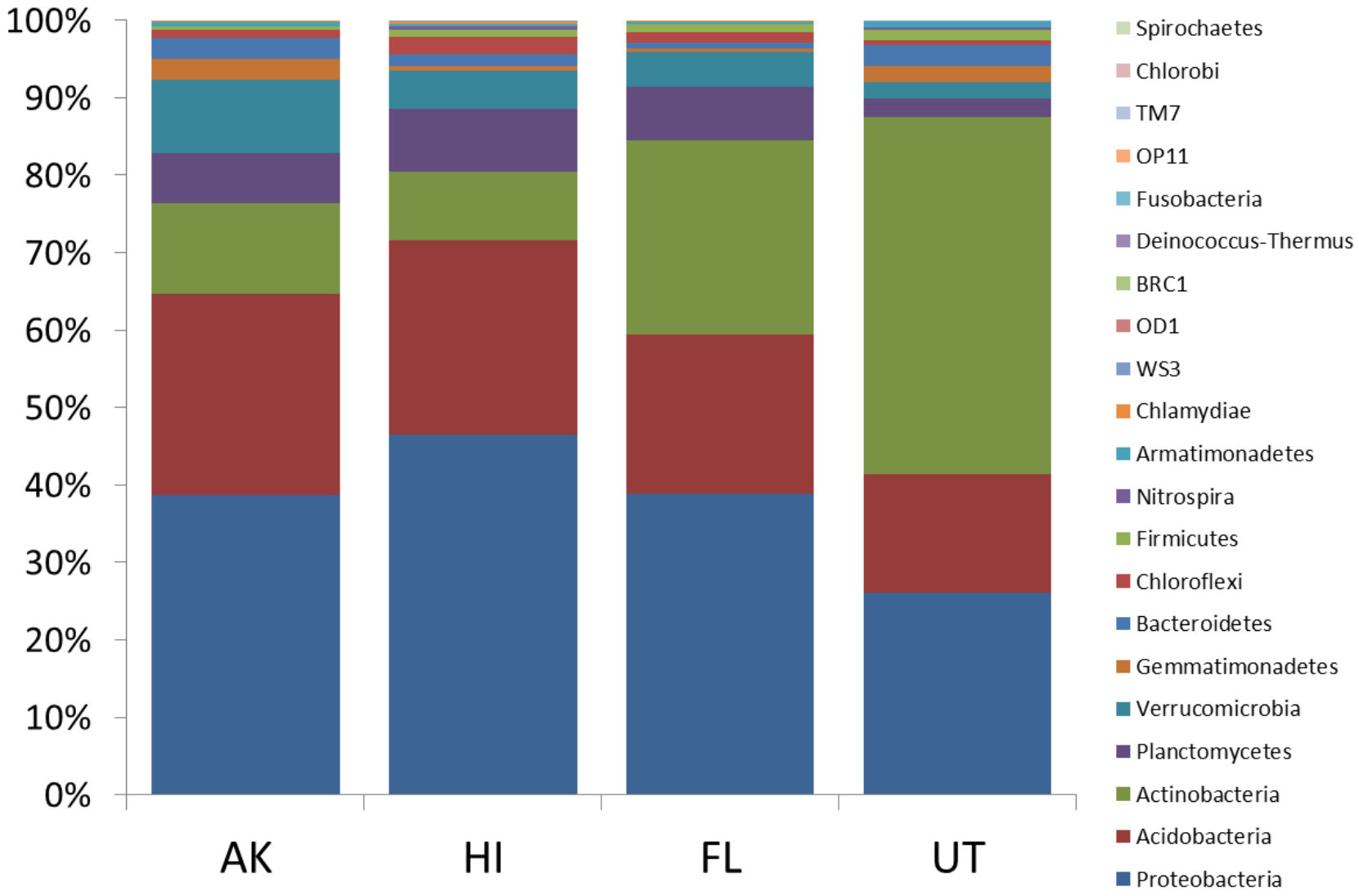
At Peak Greenness, 16S rRNA-based community structure differs between sites



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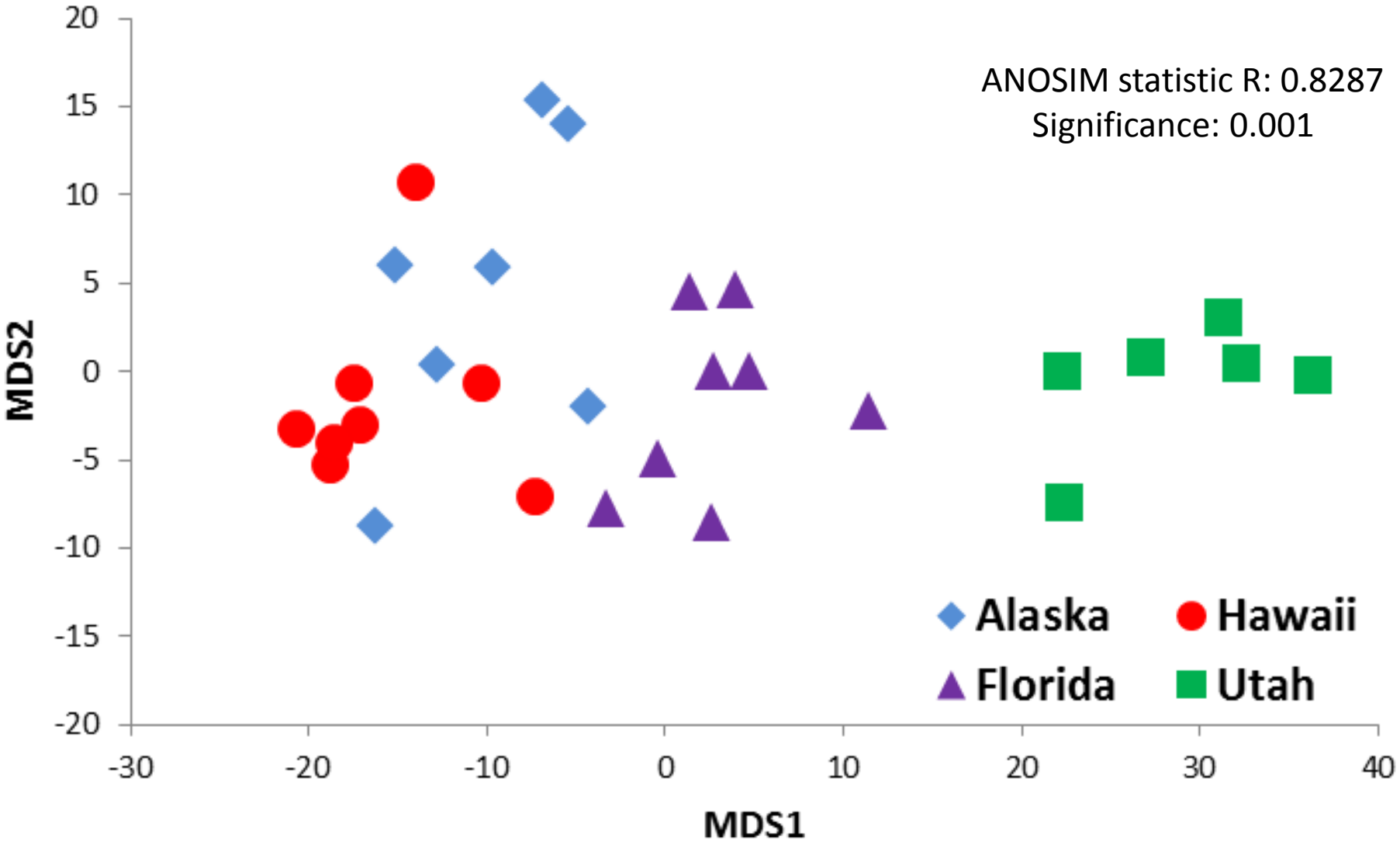

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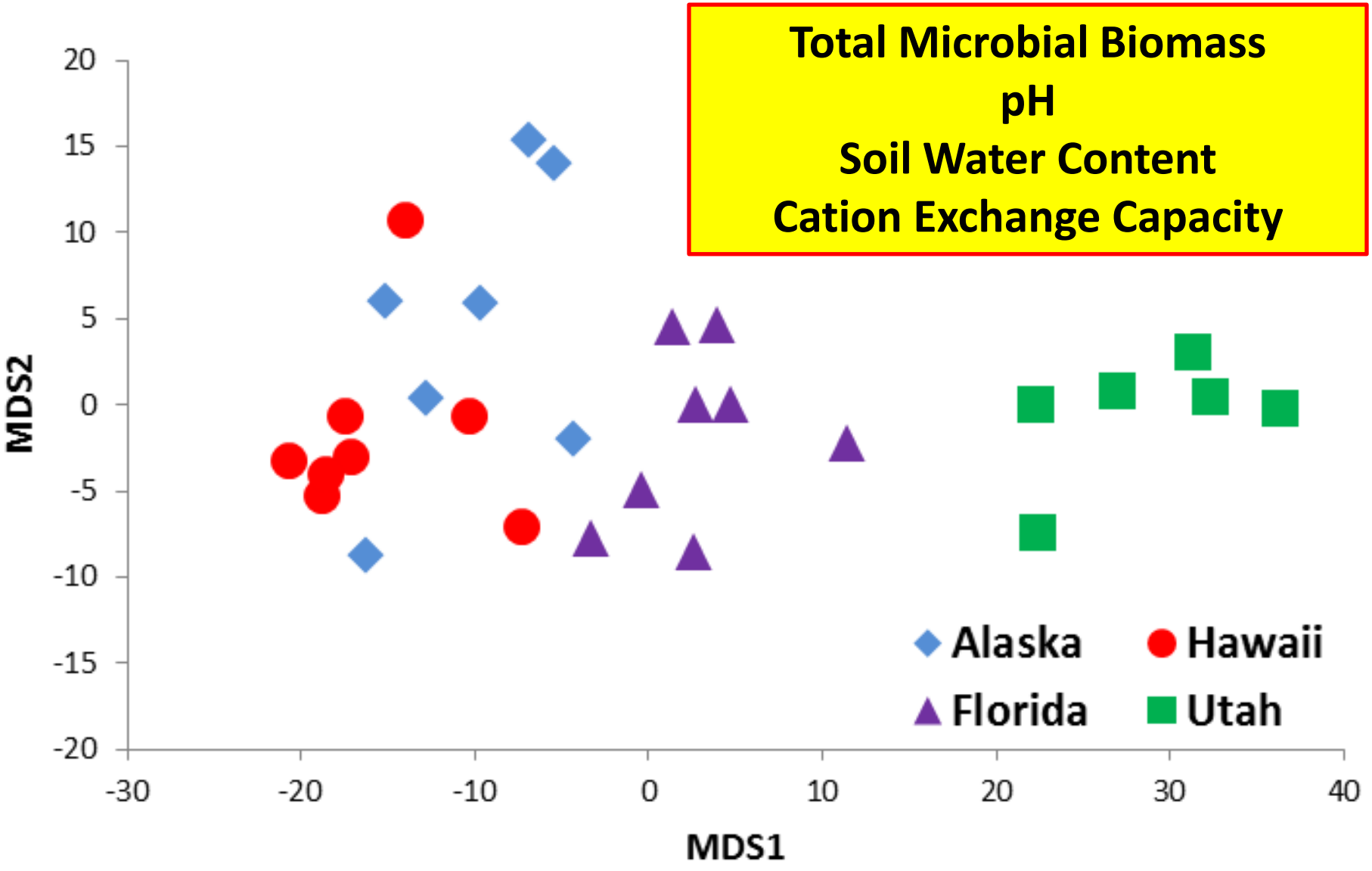

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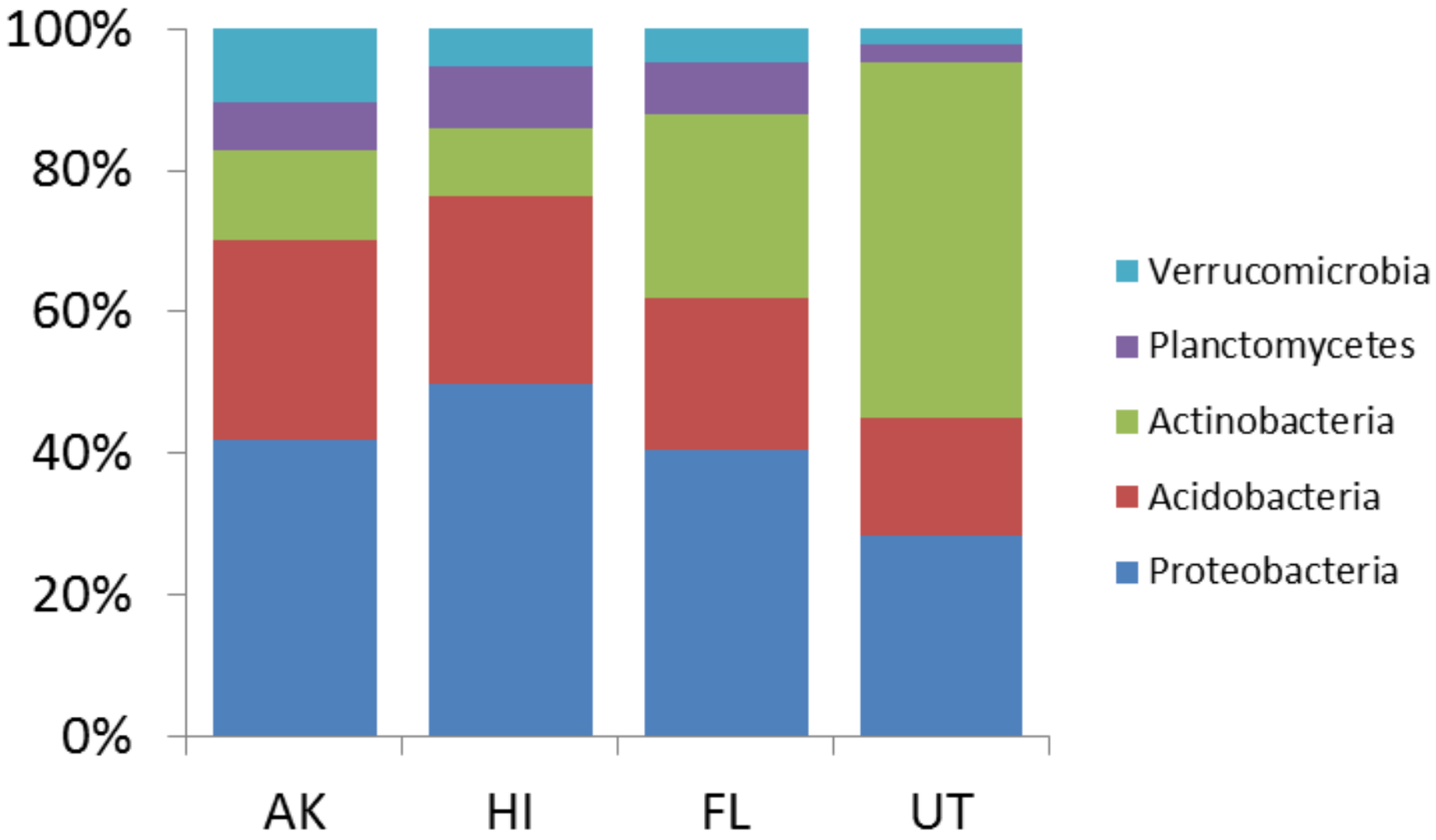
At Peak Greenness, 16S rRNA-based community differs by site (Top 5 Phyla)



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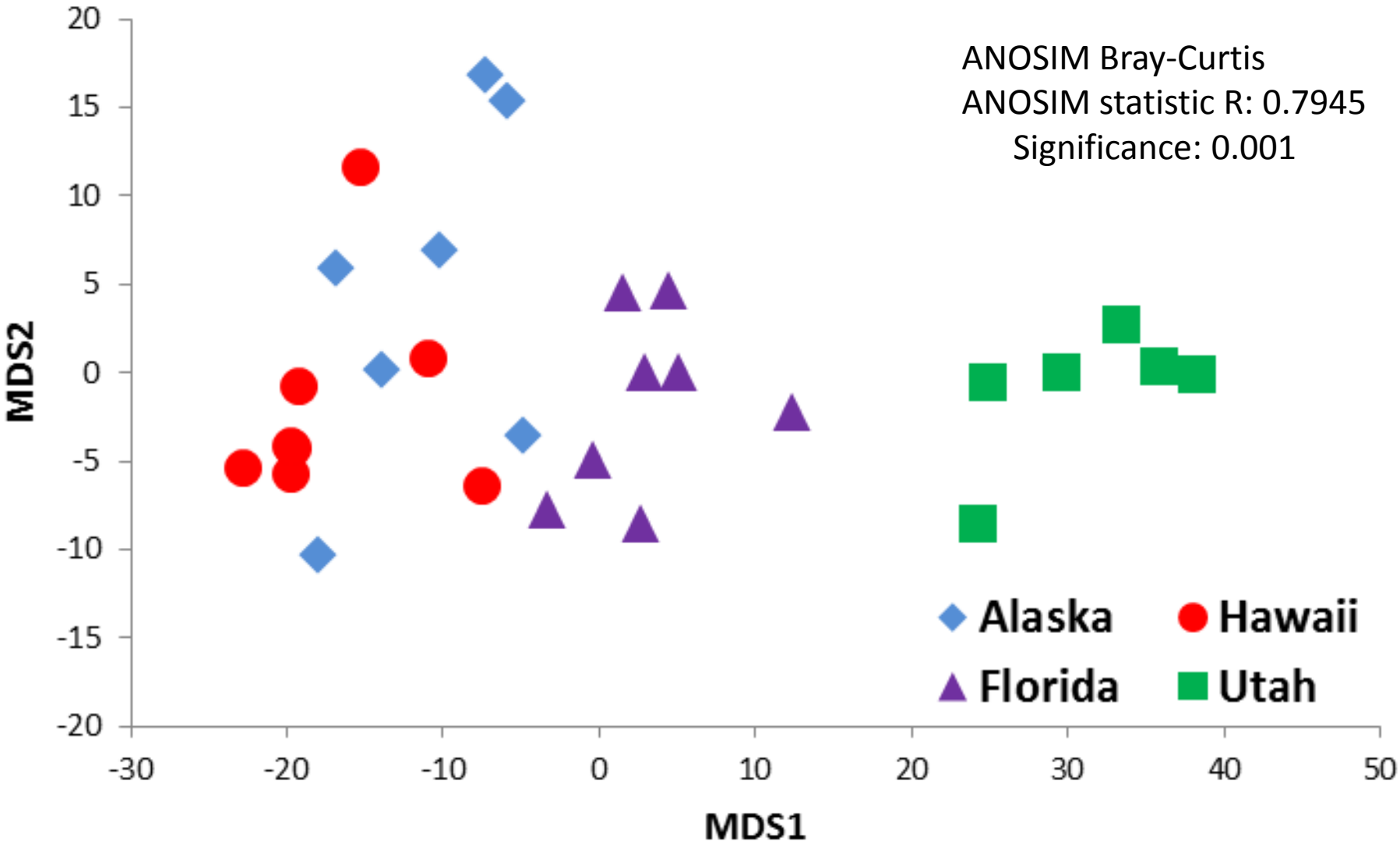

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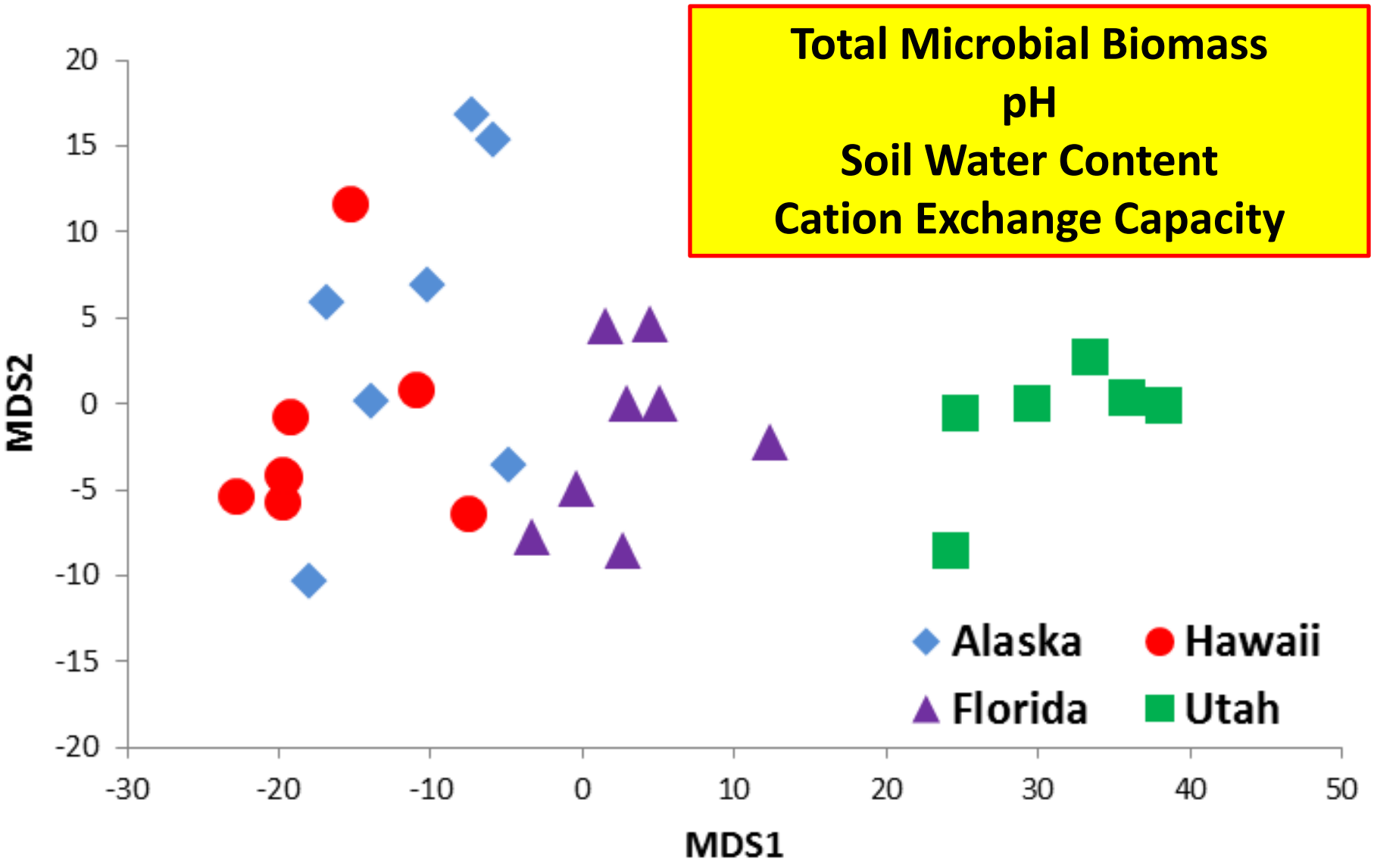

Docherty et al. in prep.

Do sites differ at PEAK GREENNESS?

Yes!

- Alaska and Hawaii are more similar, based on lipid and 16S data
- Florida and Utah differ
- According to lipid data, Utah is more similar to Alaska/Hawaii
- According to 16S data, Florida is more similar to Alaska/Hawaii

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Do sites differ OVER TIME?

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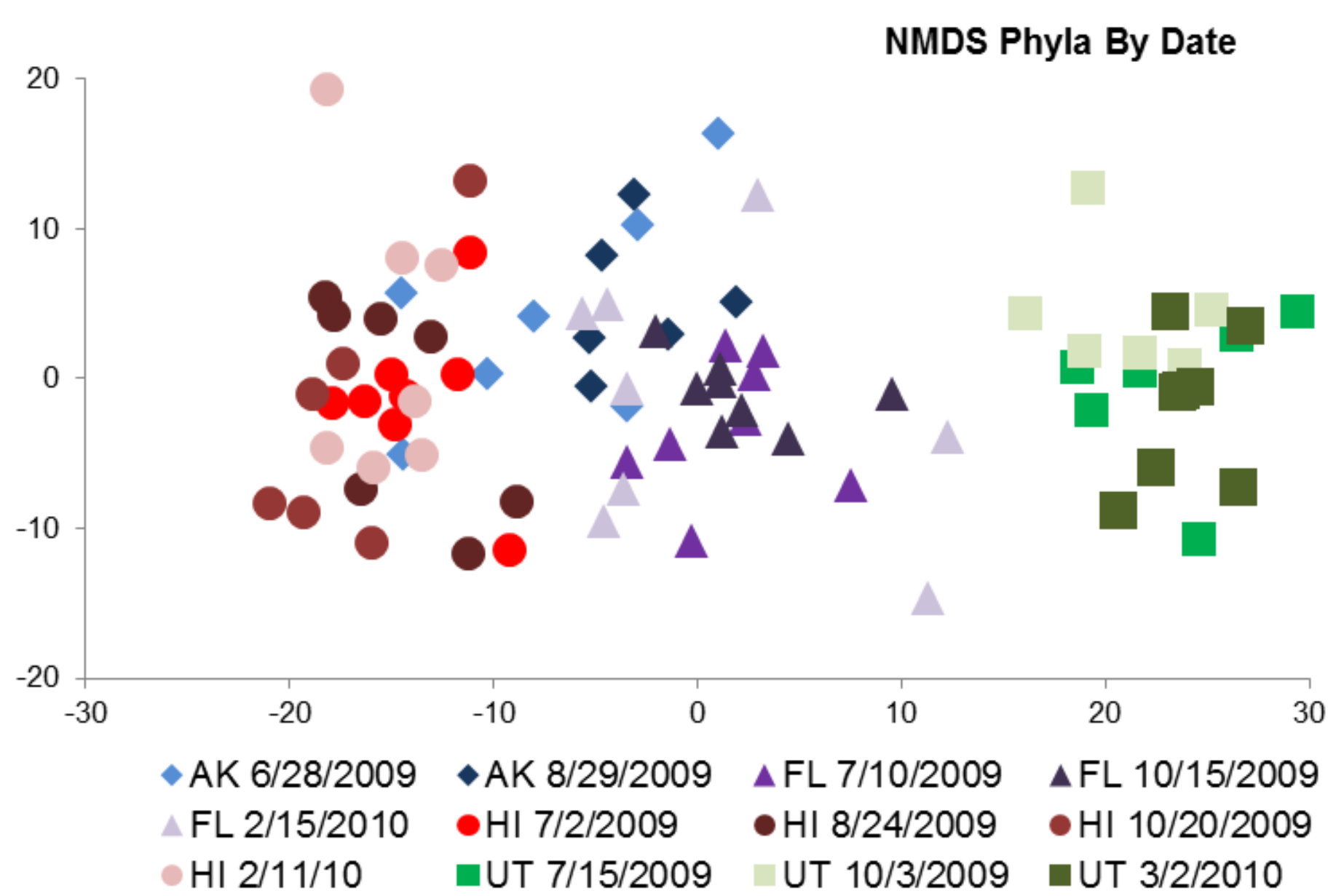
At all time points, 16S rRNA-based community differs by site



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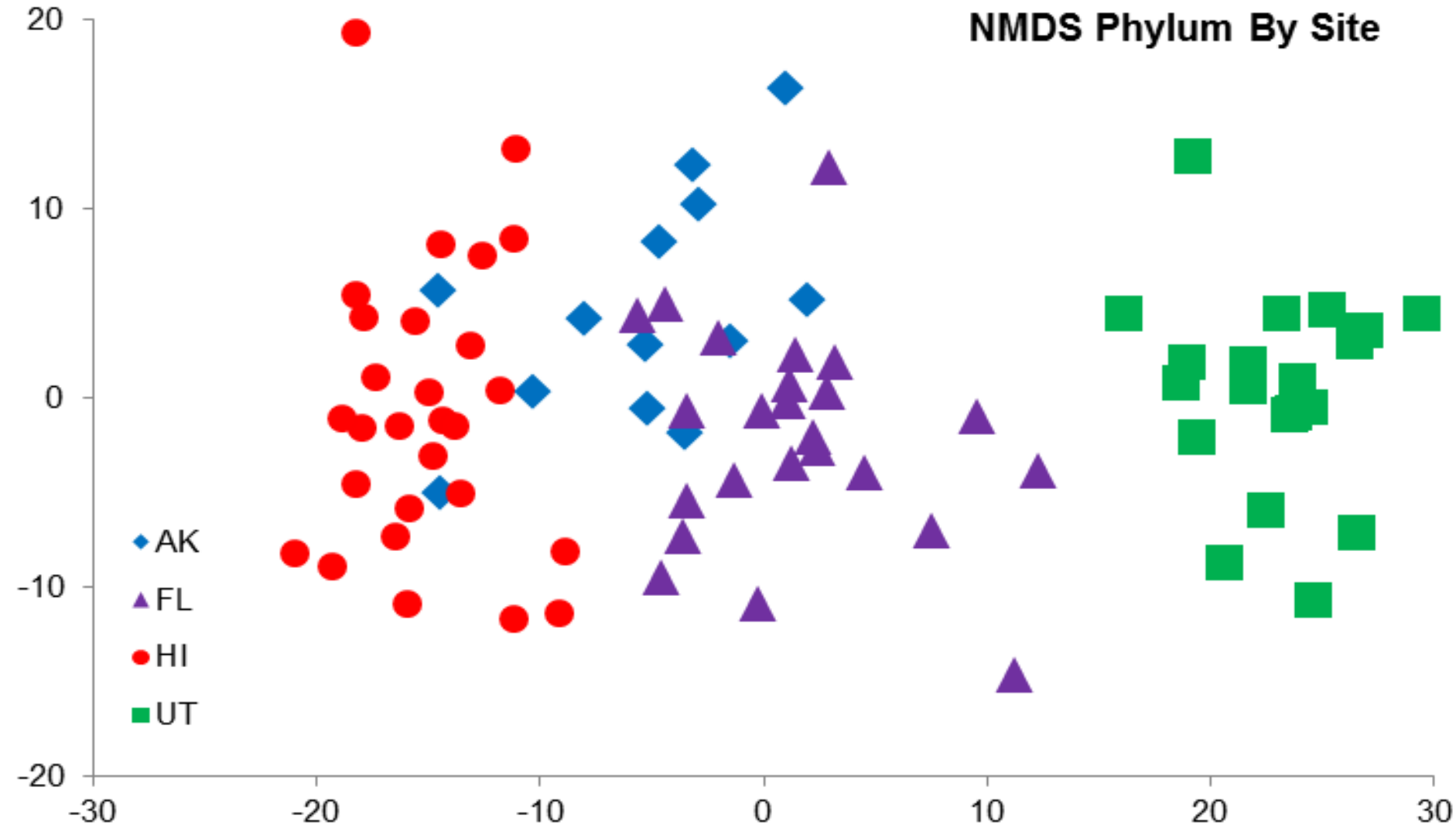
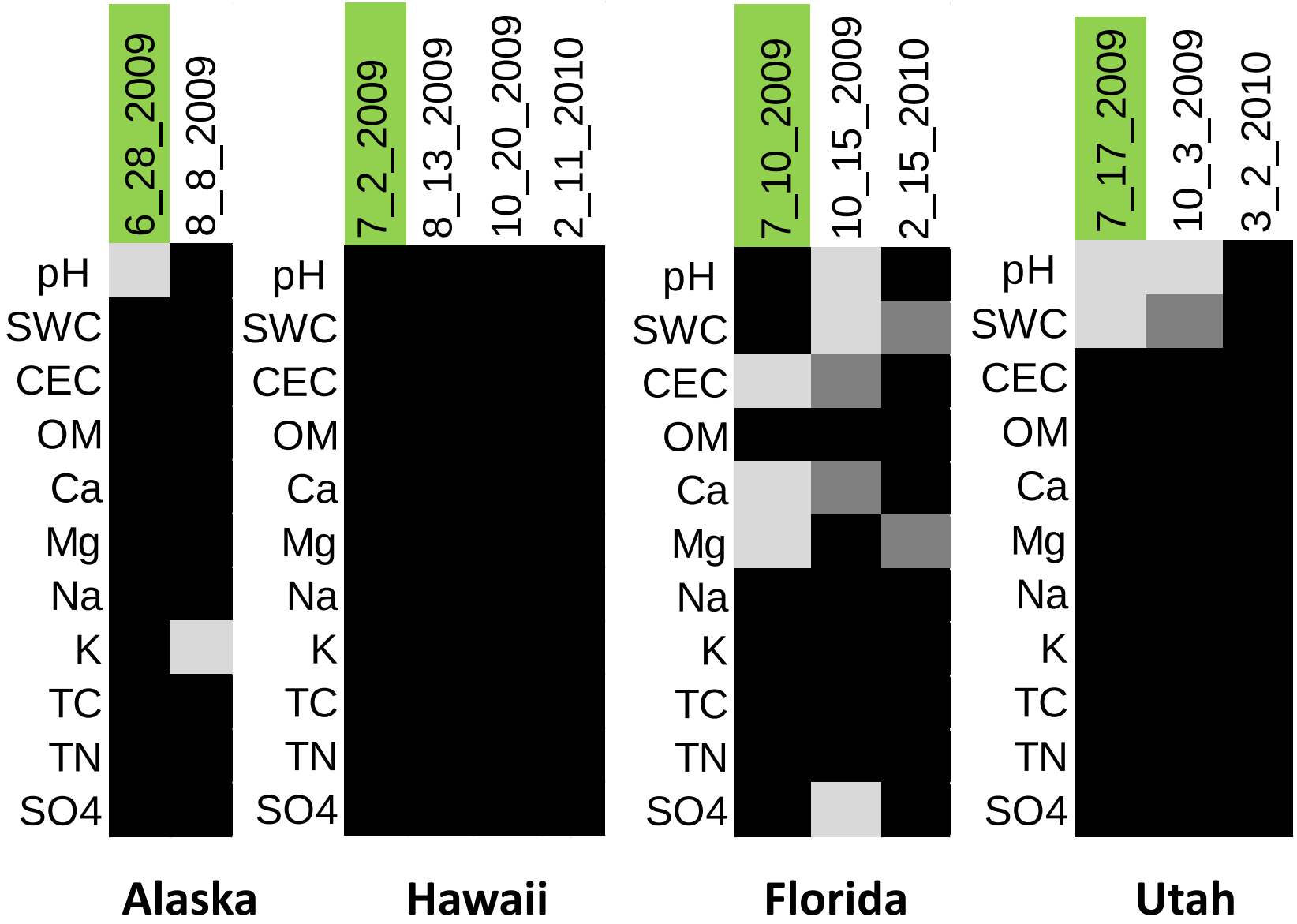
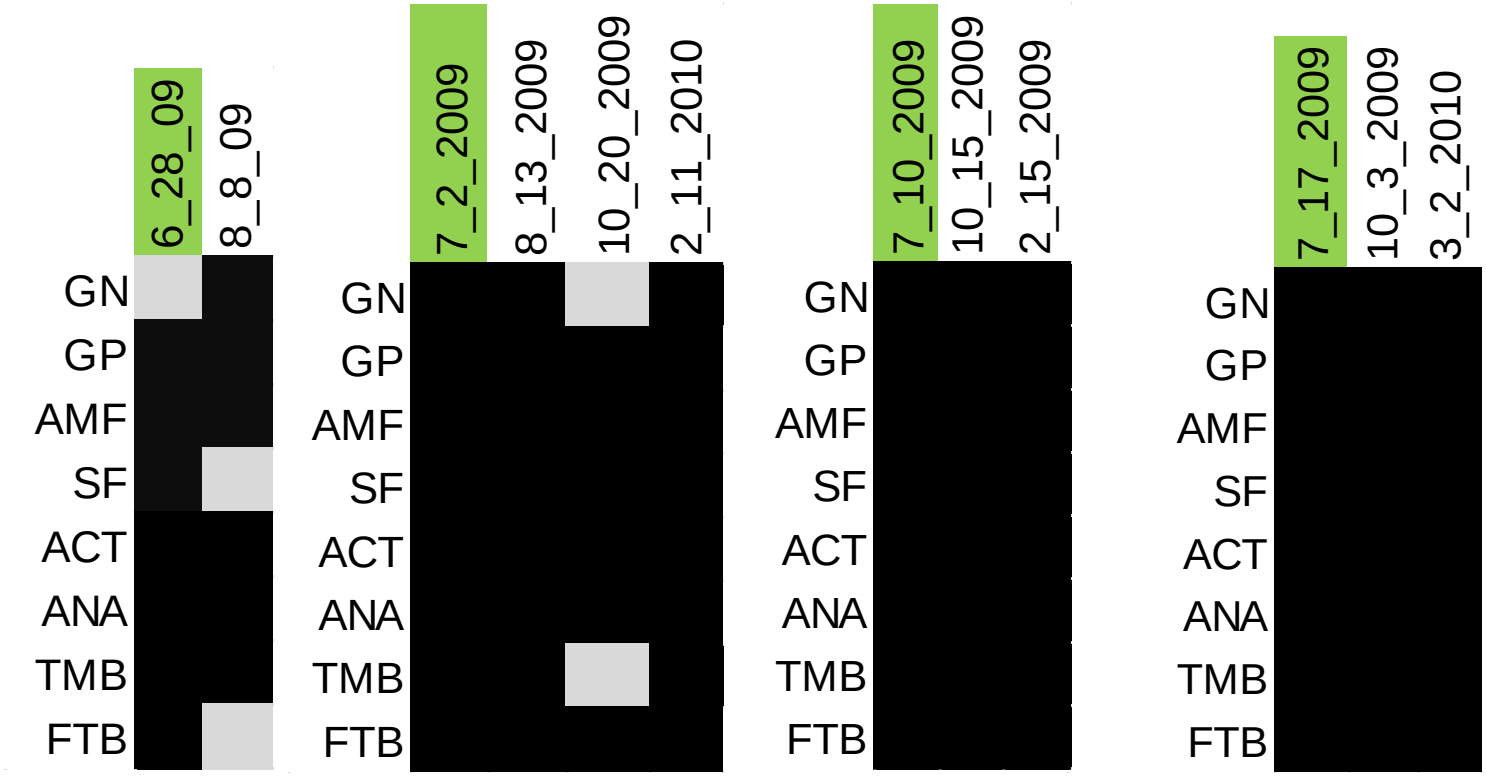


Variation in Environmental Variables Over Time

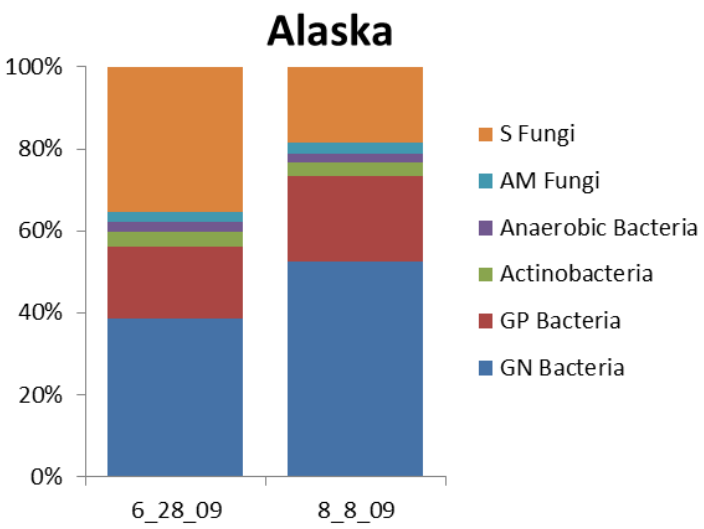


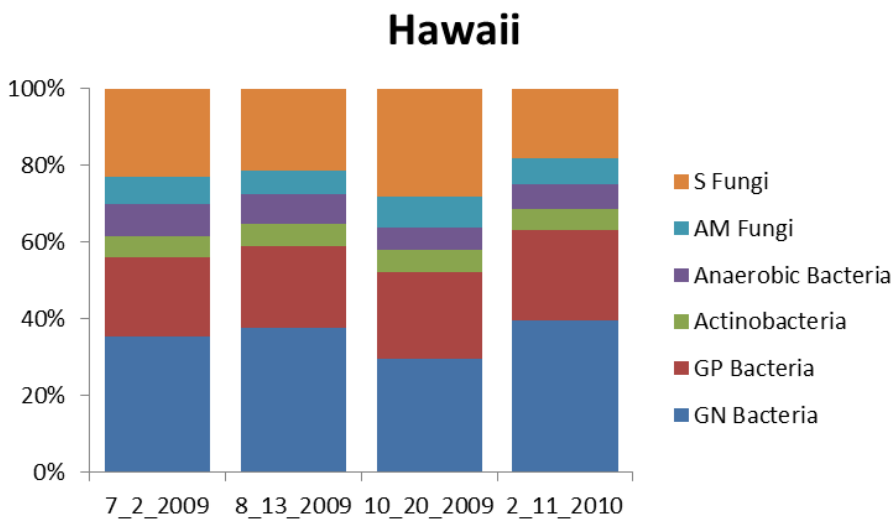
Variation in Microbial Lipids Over Time

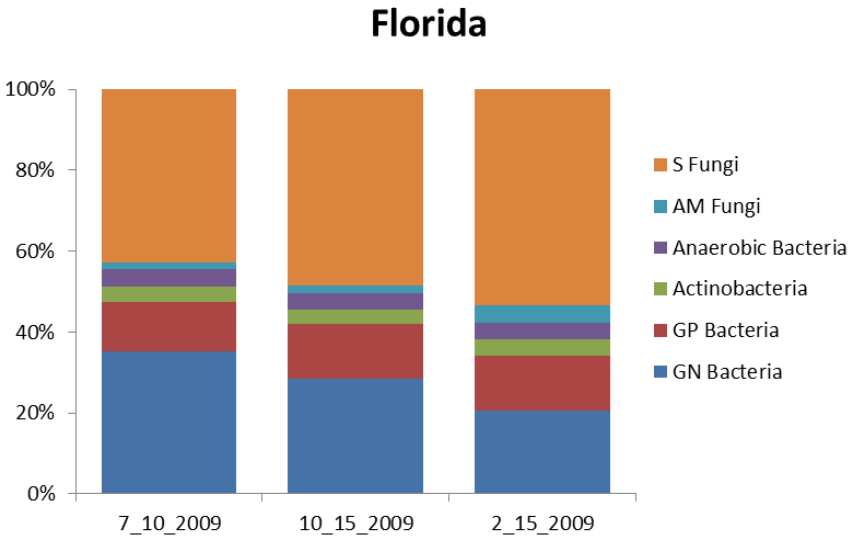


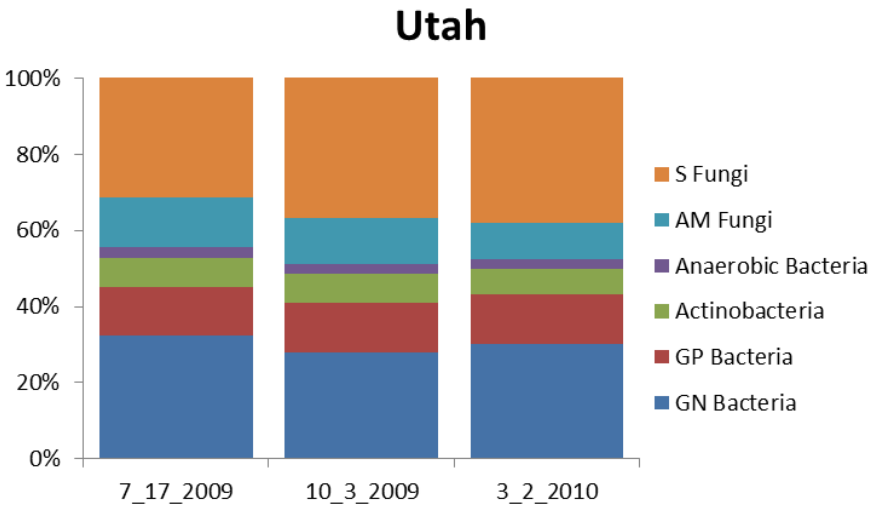
Docherty et al. in prep.

Defined Microbial Lipid Composition Over Time



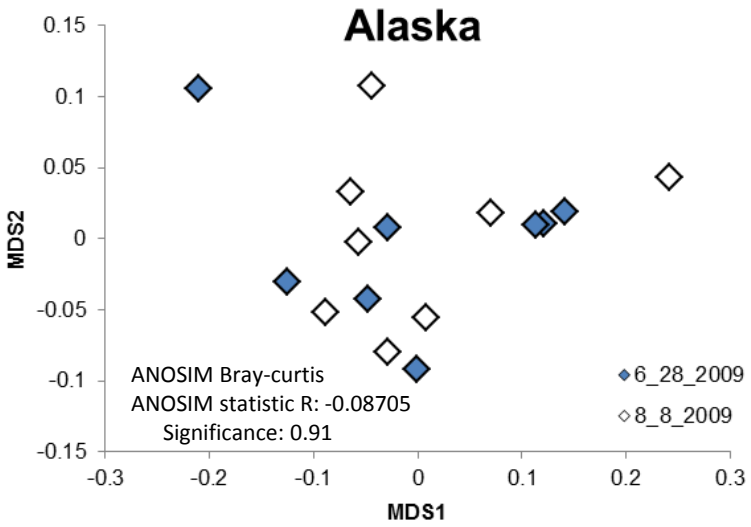






Docherty et al. in prep.

No Variation in Microbial Lipid Composition Over Time



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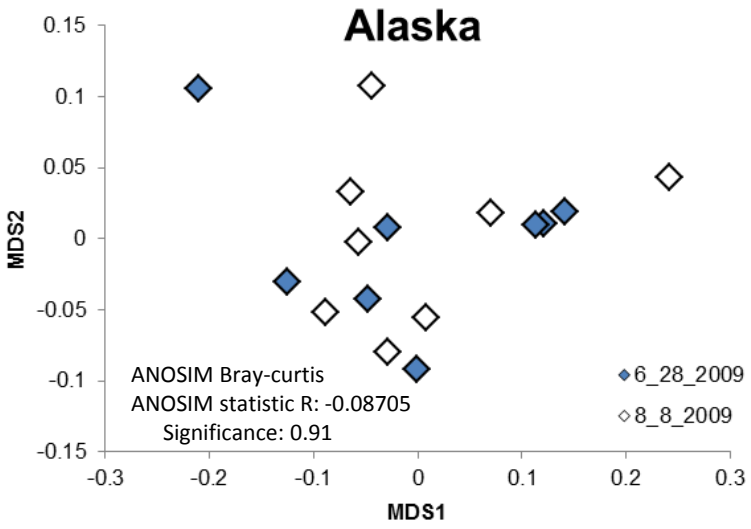

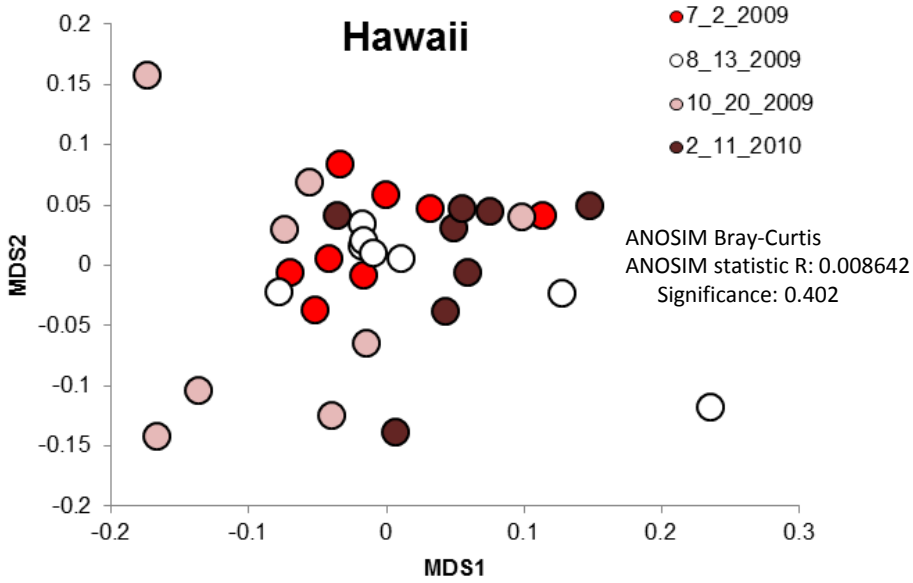

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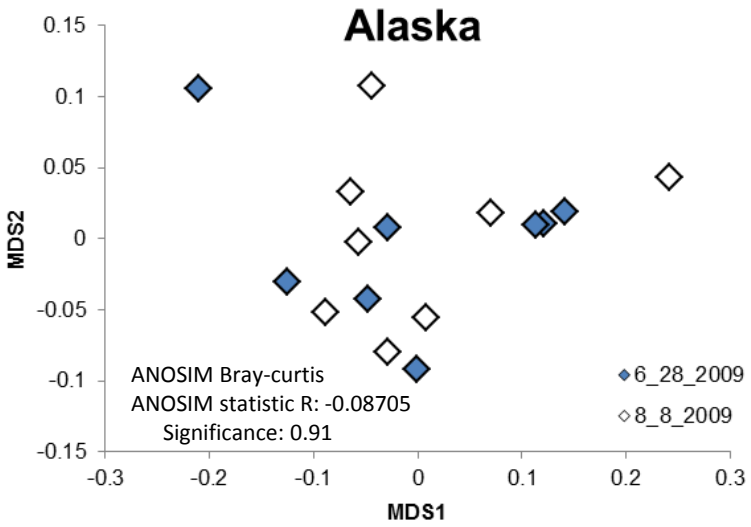

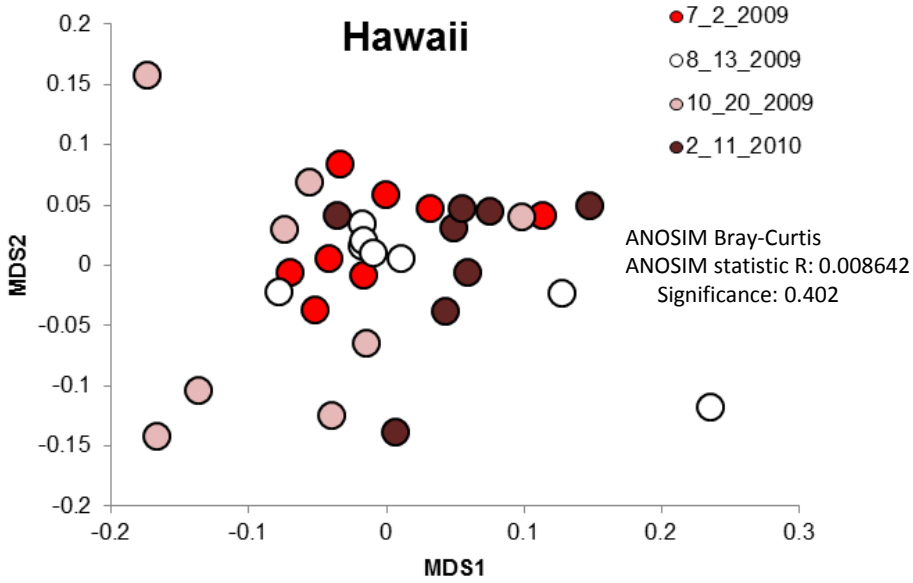

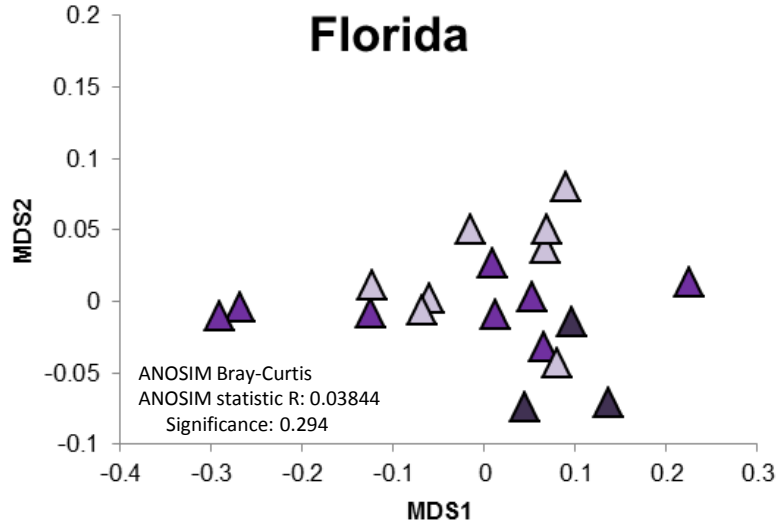

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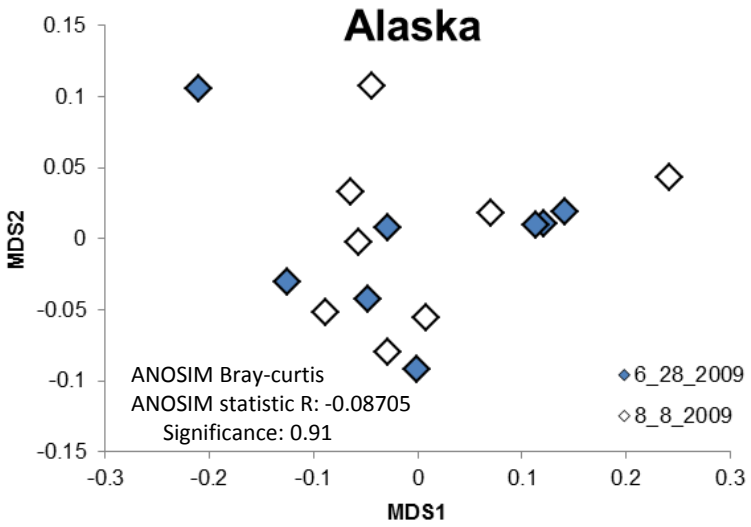

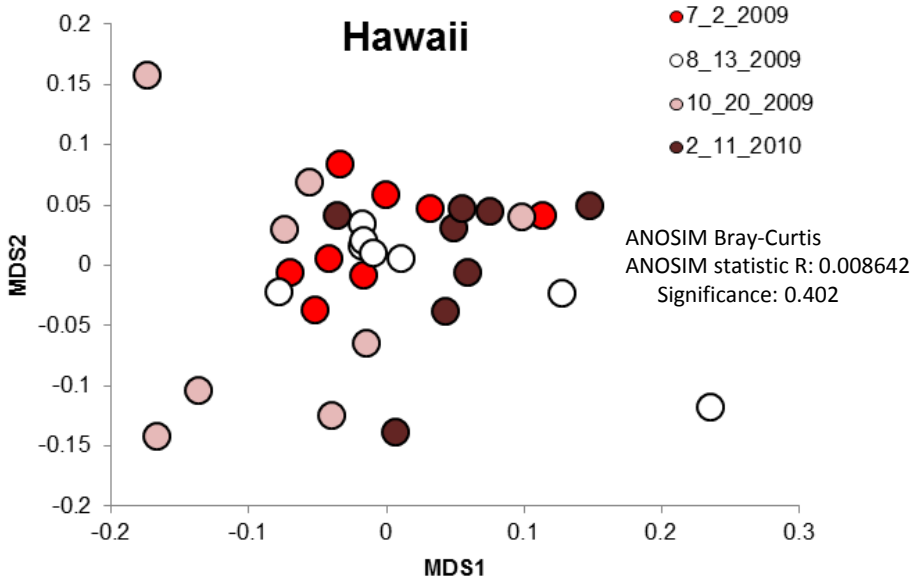

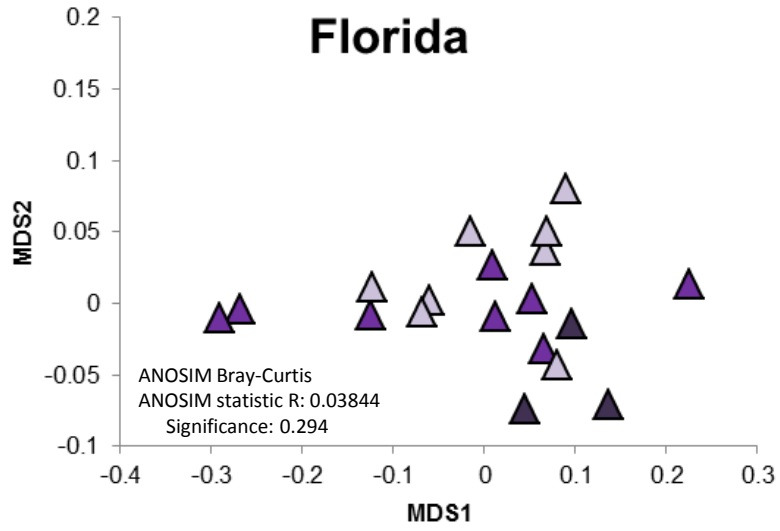

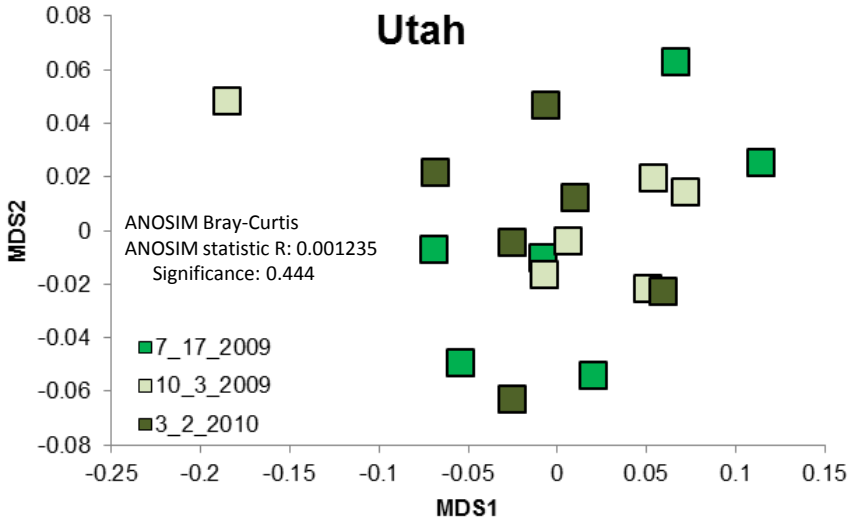

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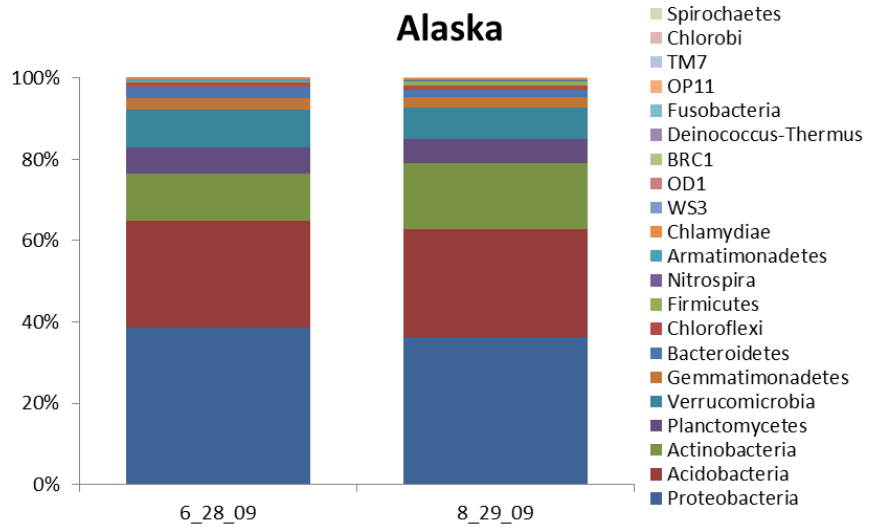
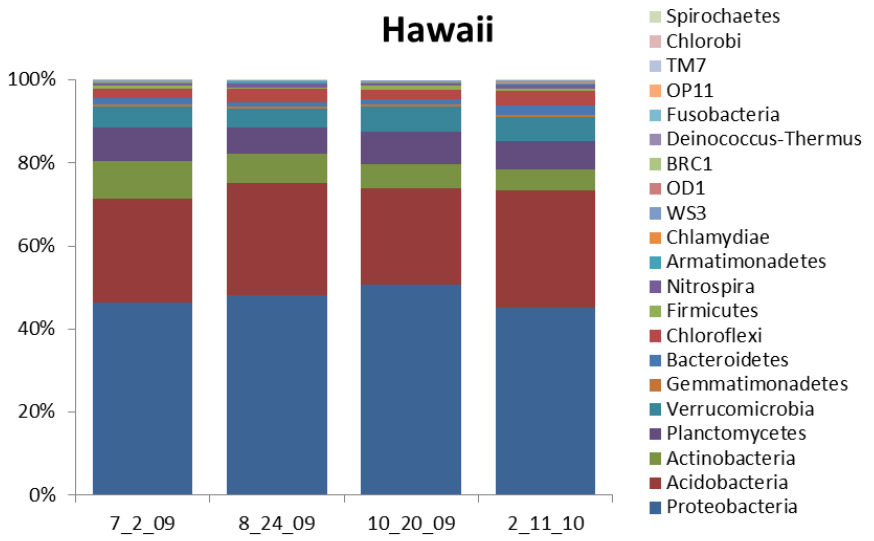
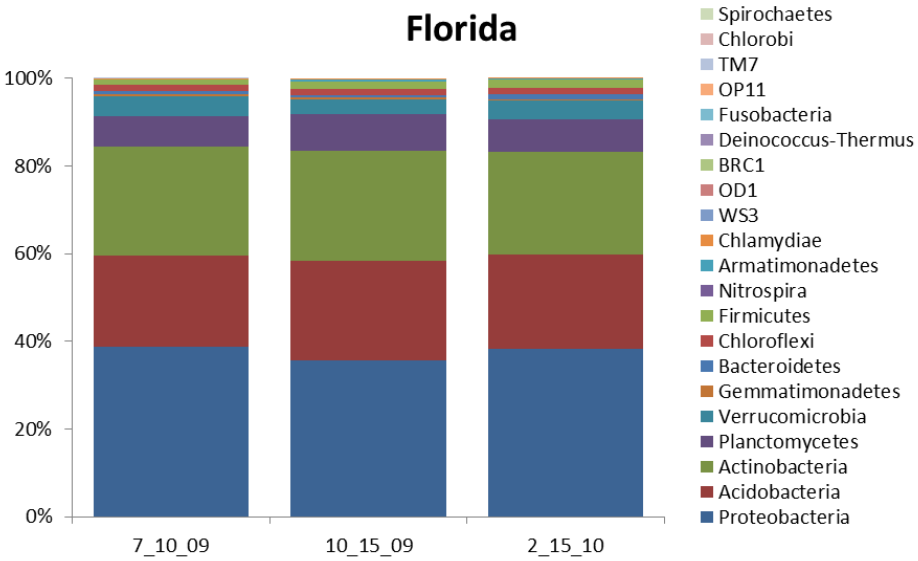
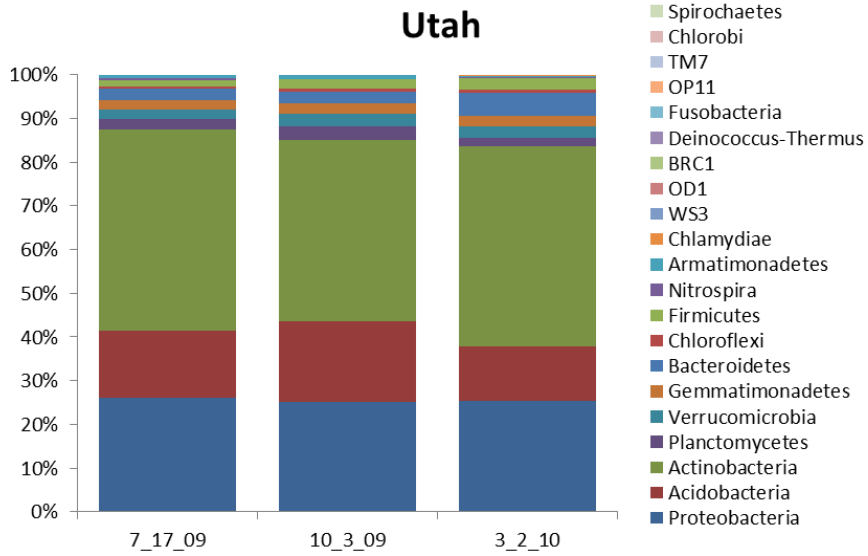
16S rRNA Microbial Community Composition Over Time



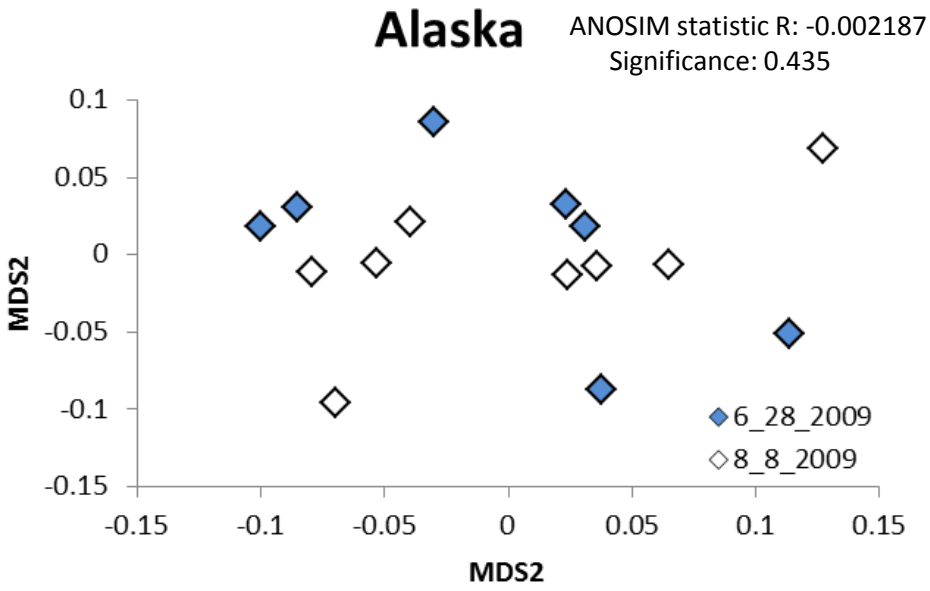




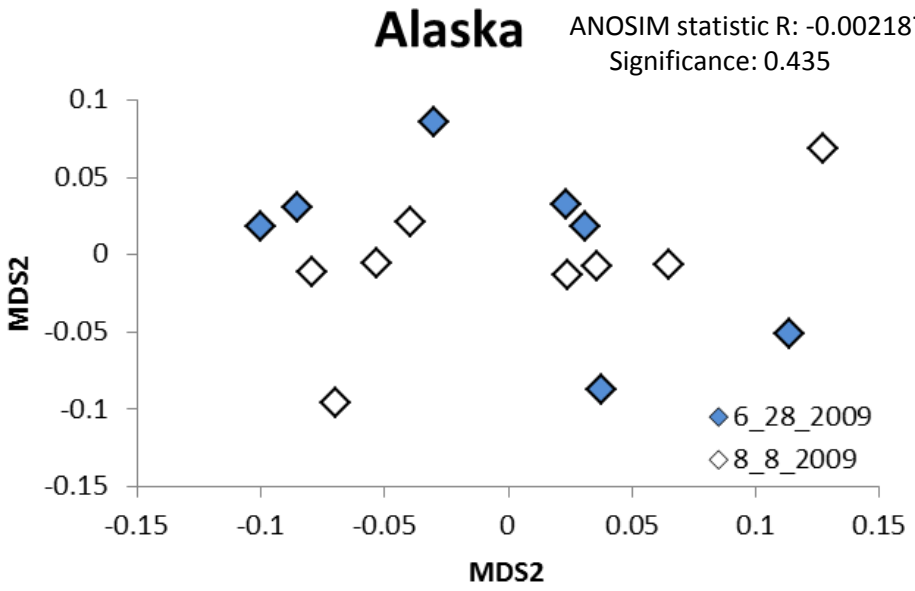


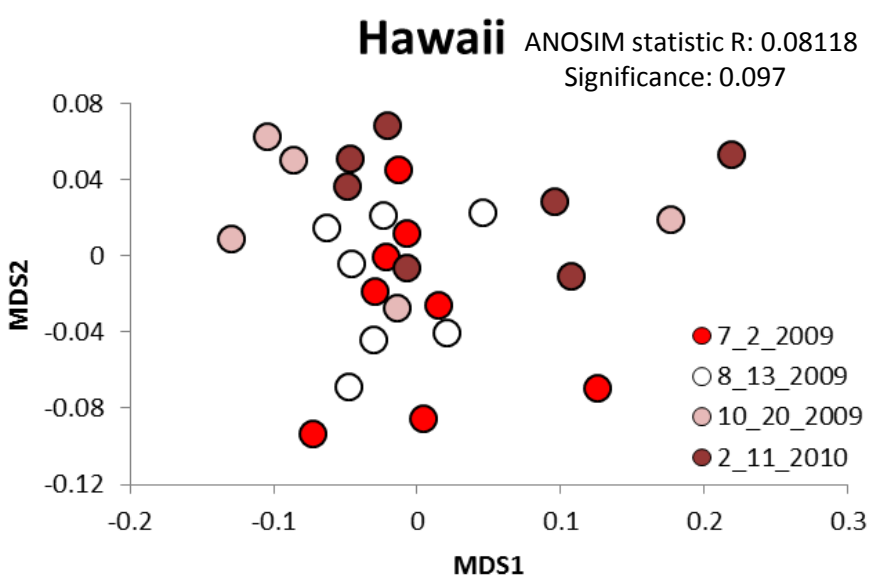
Docherty et al. in prep.

Variation in 16S rRNA Microbial Community Over Time in Florida Only



Variation in 16S rRNA Microbial Community Over Time in Florida Only

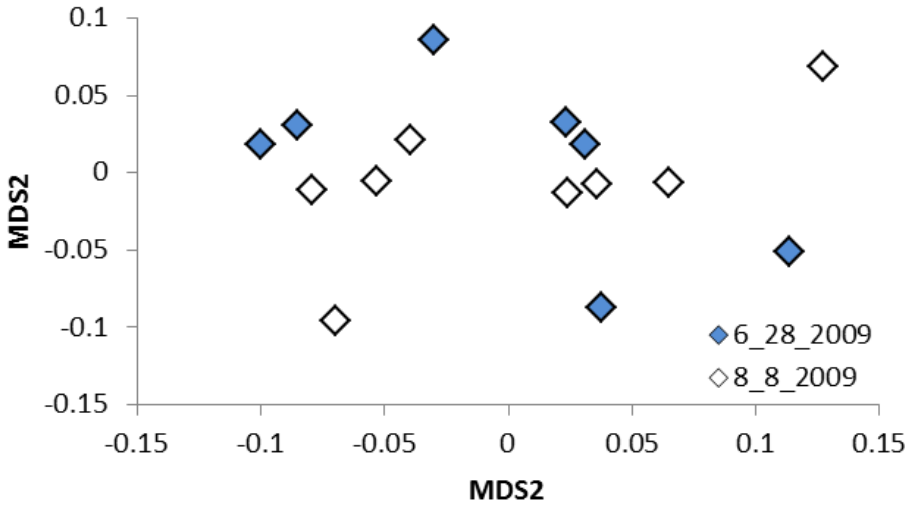




Variation in 16S rRNA Microbial Community Over Time in Florida Only

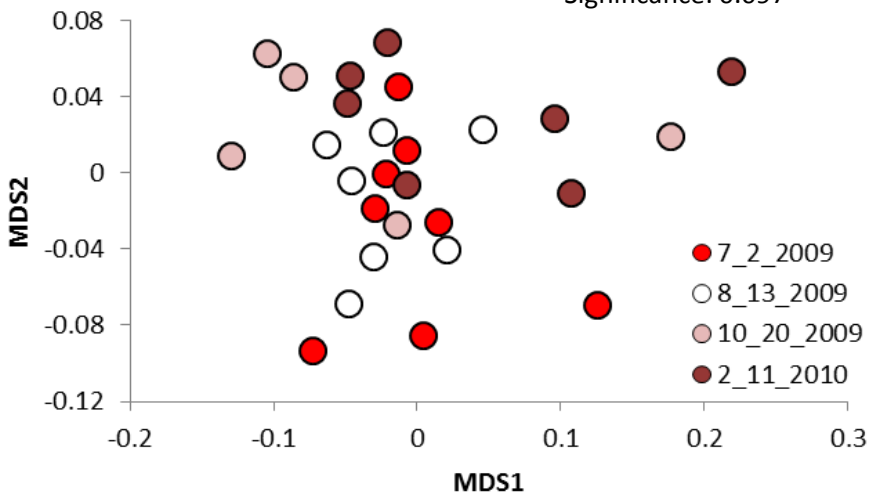
Alaska

ANOSIM statistic R: -0.002187
Significance: 0.435



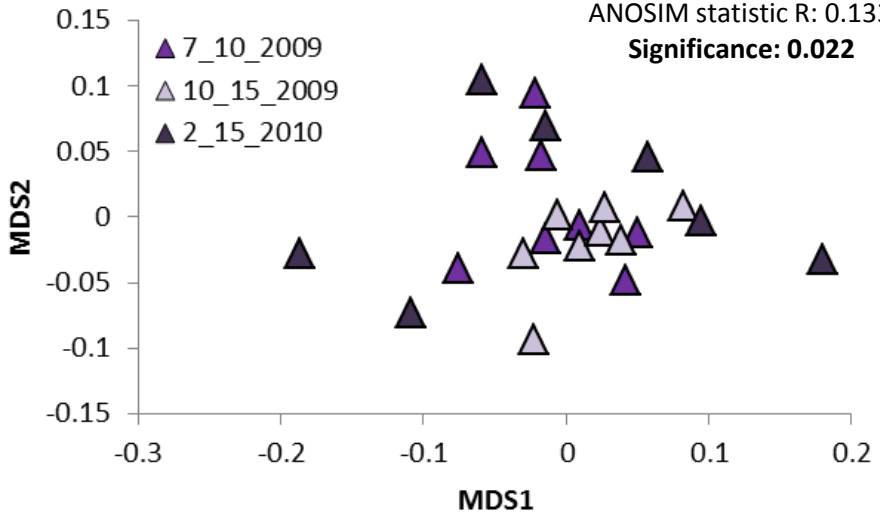
Hawaii

ANOSIM statistic R: 0.08118
Significance: 0.097



Florida

ANOSIM statistic R: 0.1336
Significance: 0.022



Variation in 16S rRNA Microbial Community Over Time in Florida Only

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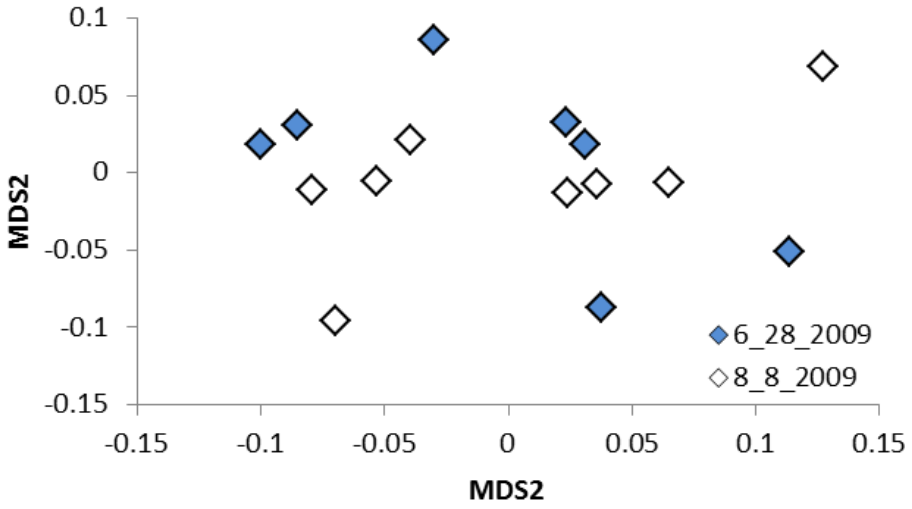

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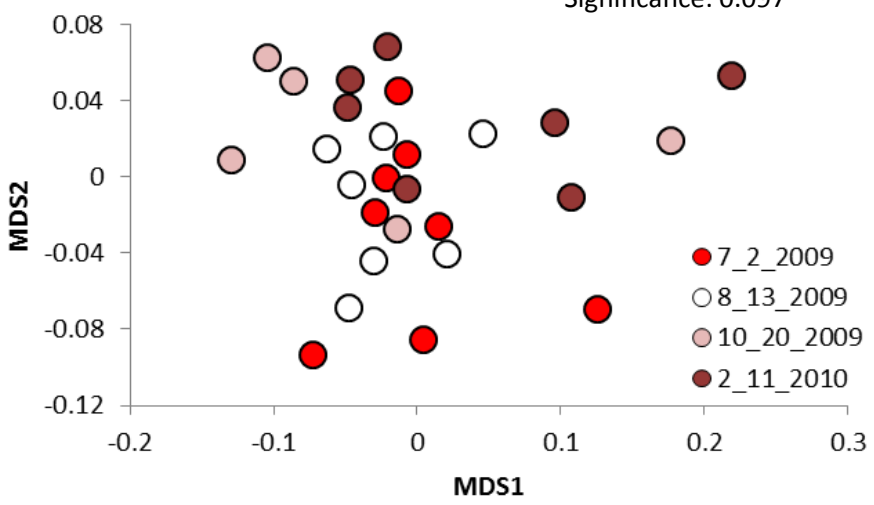

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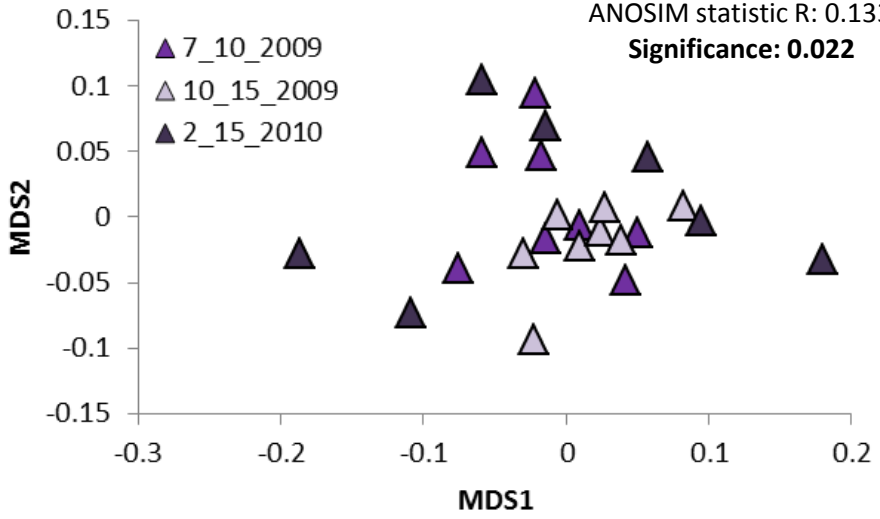
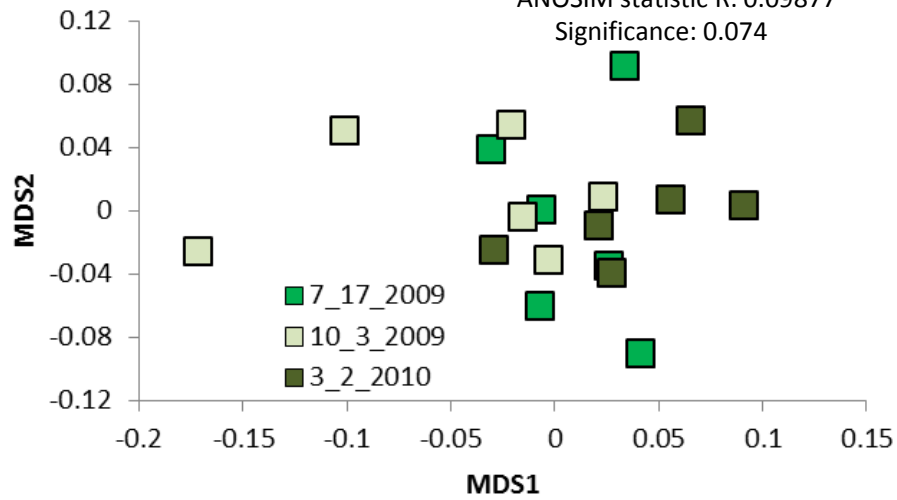


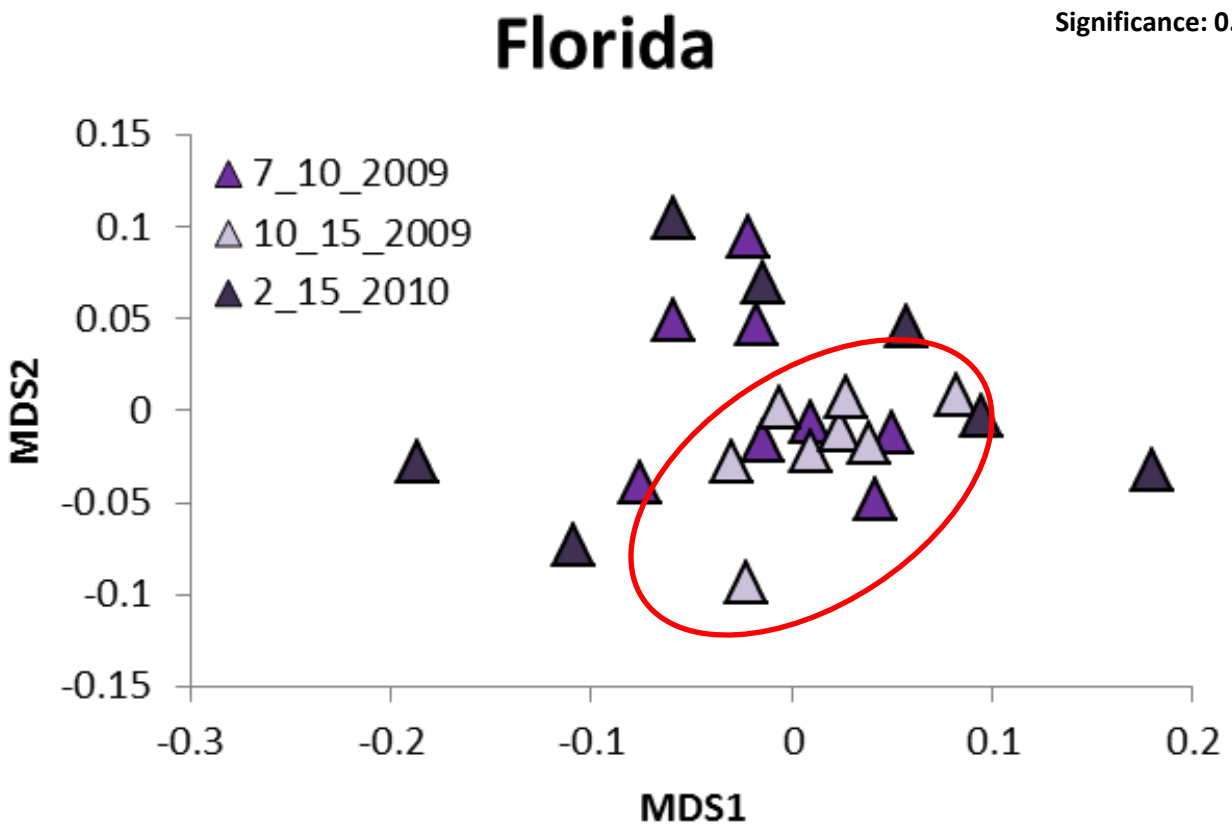
Utah

ANOSIM statistic R: 0.09877
Significance: 0.074

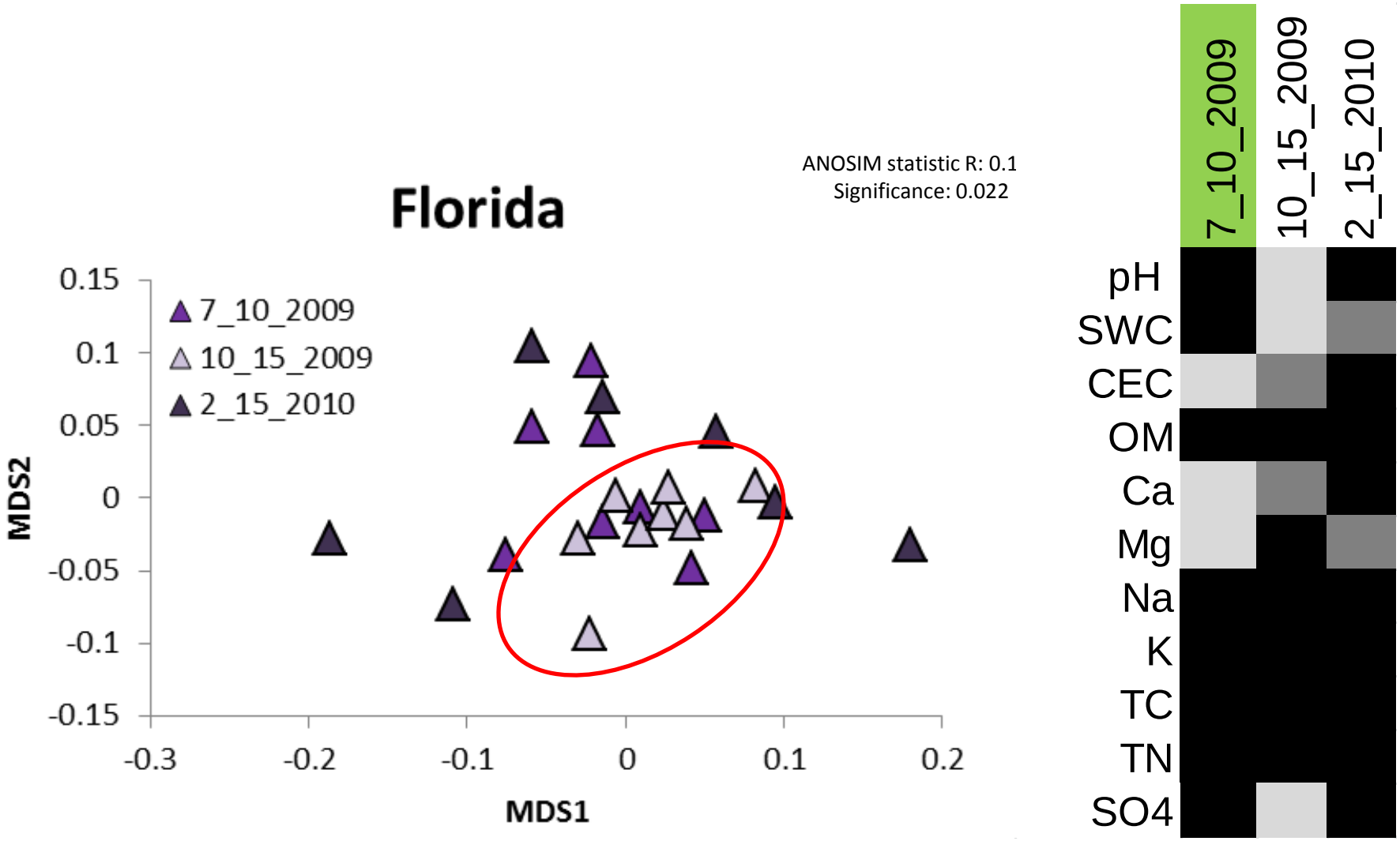


Tighter clustering in 16S rRNA Microbial Community in October

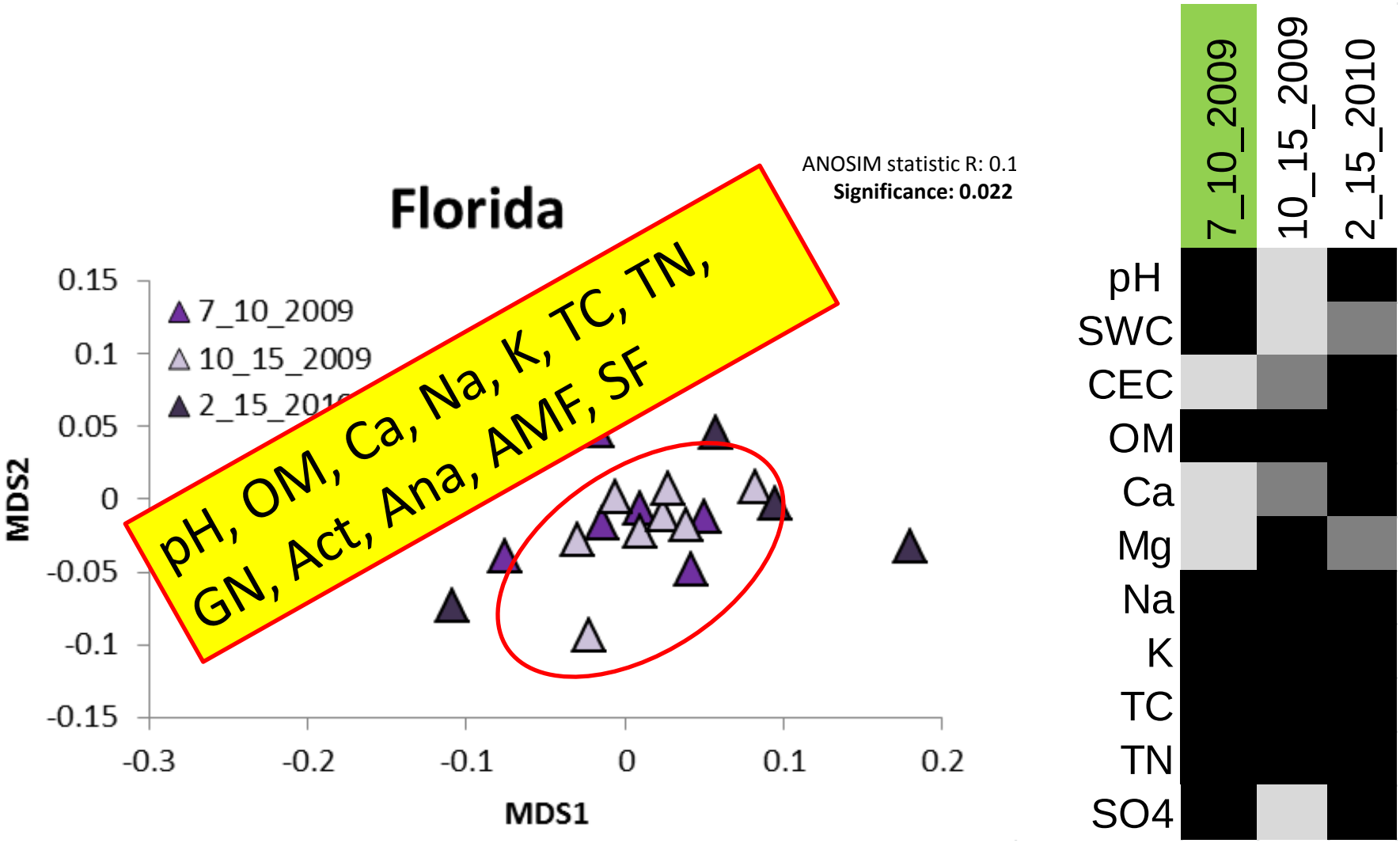
ANOSIM statistic R: 0.1336
Significance: 0.022



Tighter clustering in 16S rRNA Microbial Community in October



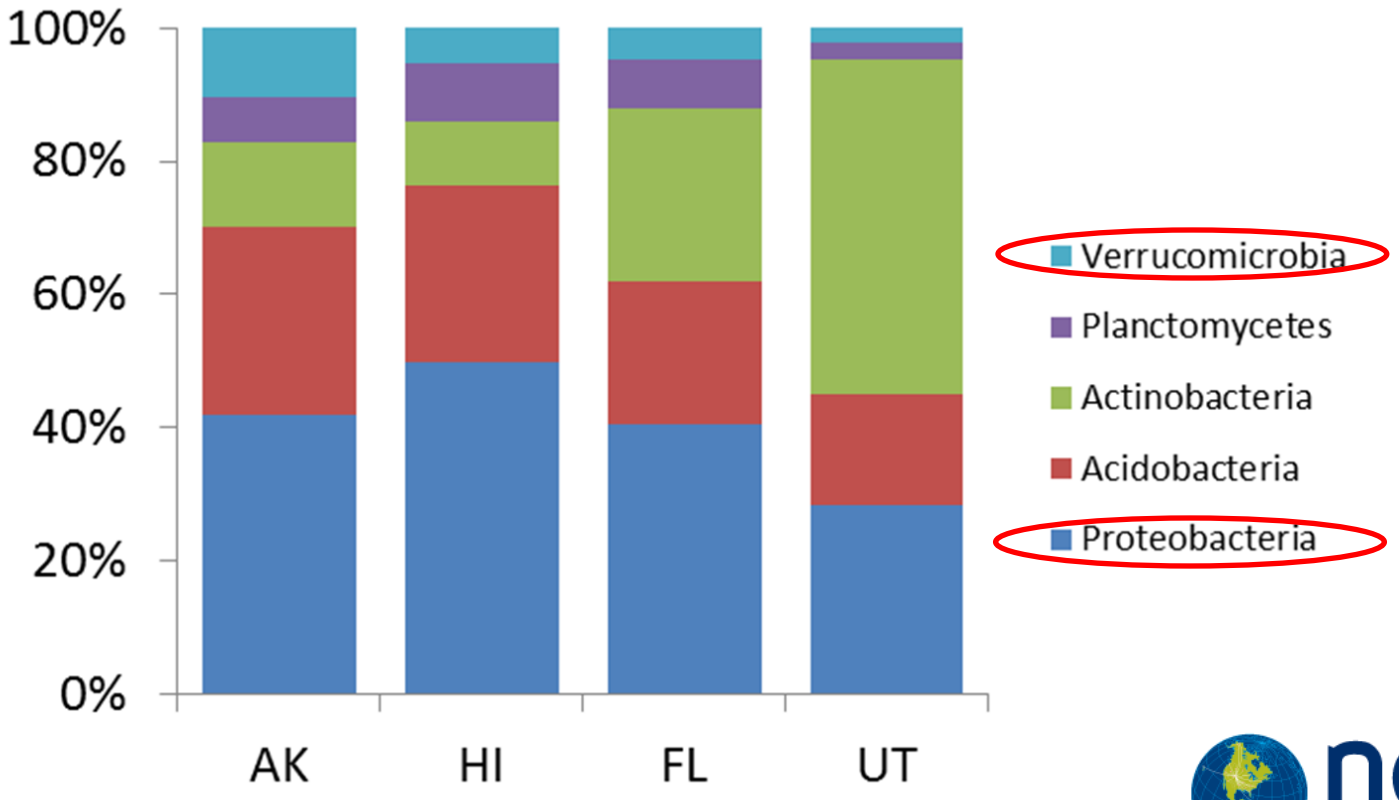
Tighter clustering in 16S rRNA Microbial Community in October



Do sites differ OVER TIME?

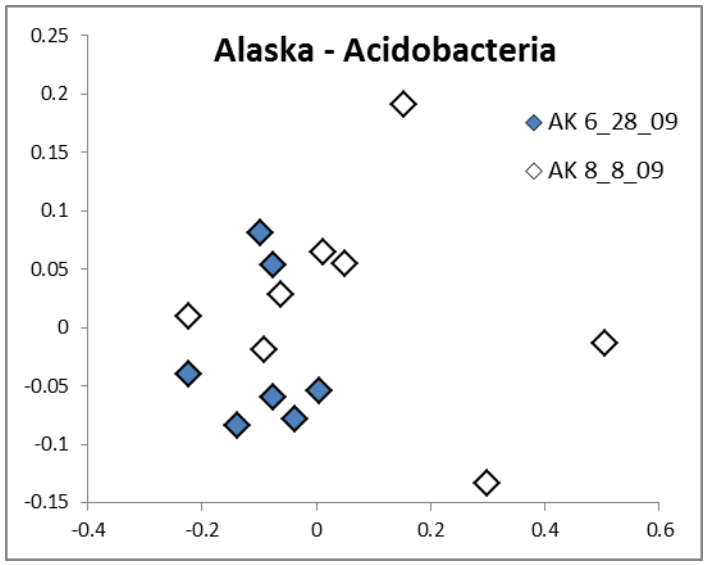
- Very little, and it depends on the site
- Florida is the only site that exhibited differences over time
- Differences were only seen with 16S data and not with lipid data

Does the composition of Families within Phyla differ by site/over time?

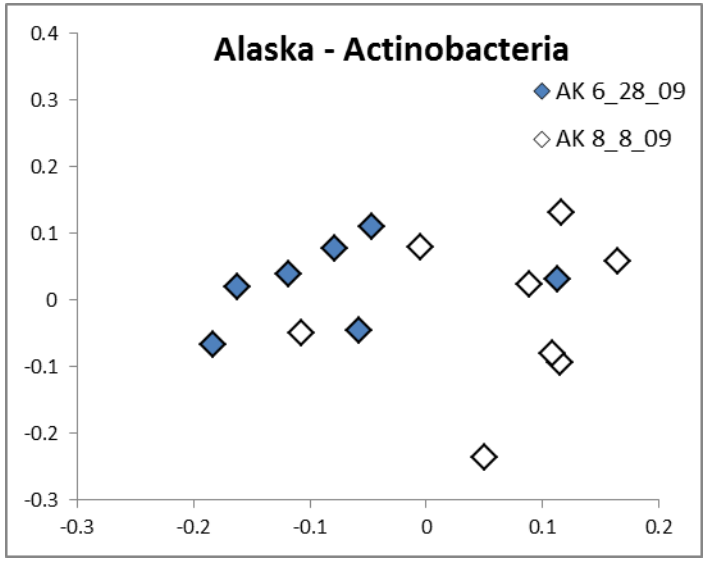


National Ecological Observatory Network

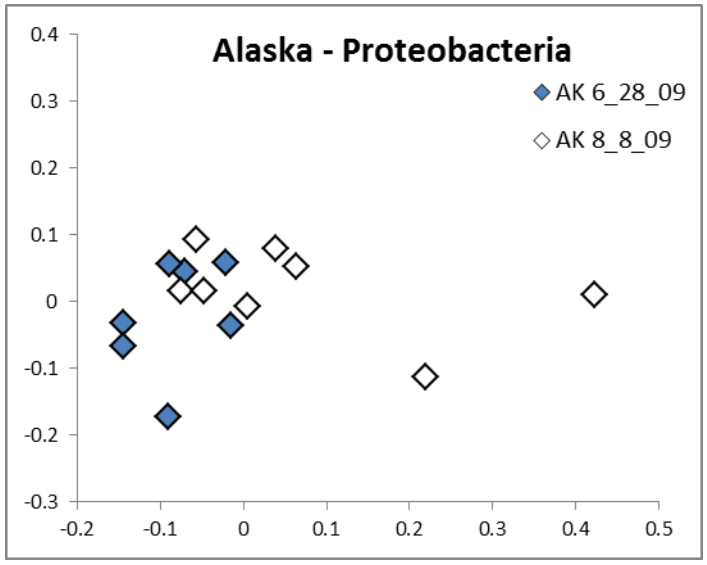
Alaska – Phyla Over Time



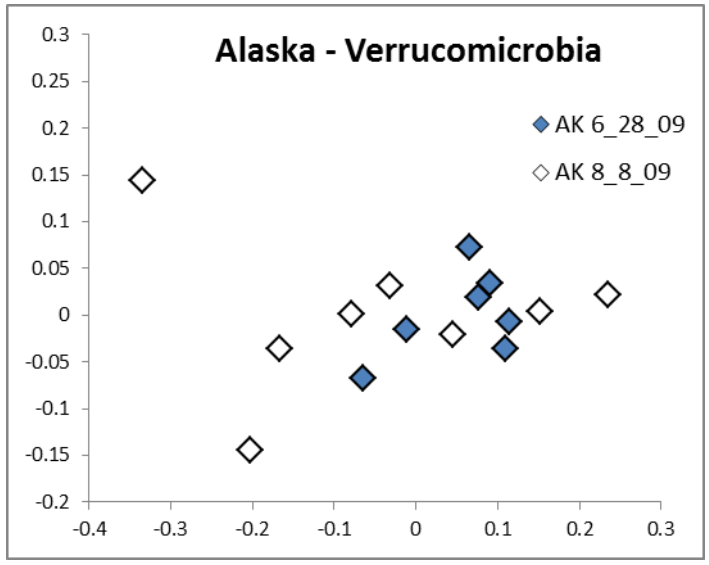
ANOSIM statistic R: 0.07216
Significance: 0.154



ANOSIM statistic R: 0.2682
Significance: 0.04

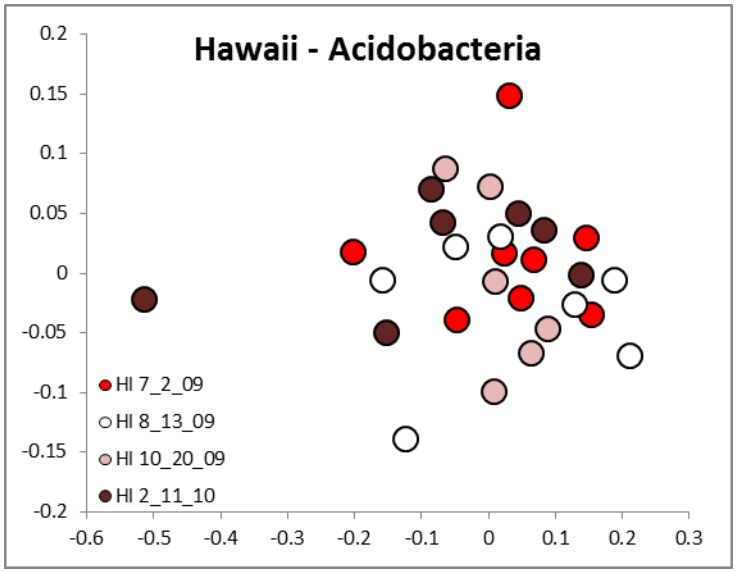


ANOSIM statistic R: 0.09402
Significance: 0.097

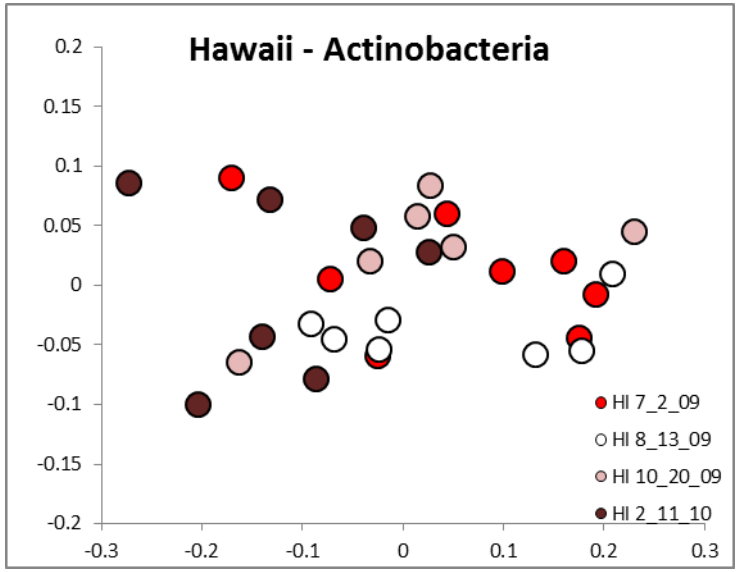


ANOSIM statistic R: 0.03863
Significance: 0.212

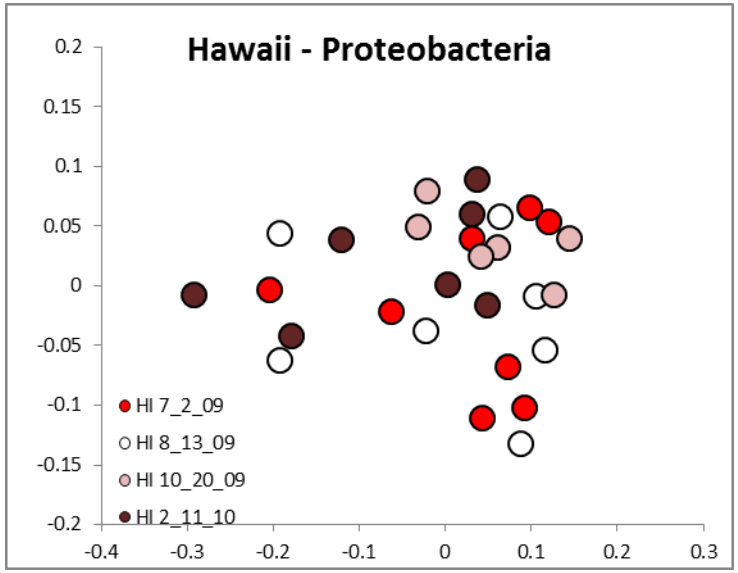
Hawaii – Phyla Over Time



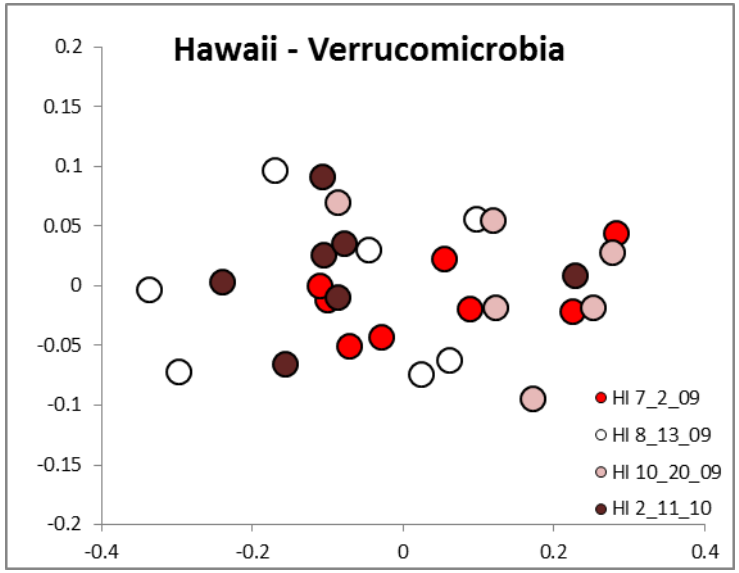
ANOSIM statistic R: -0.06228
Significance: 0.906



ANOSIM statistic R: 0.09231
Significance: 0.075

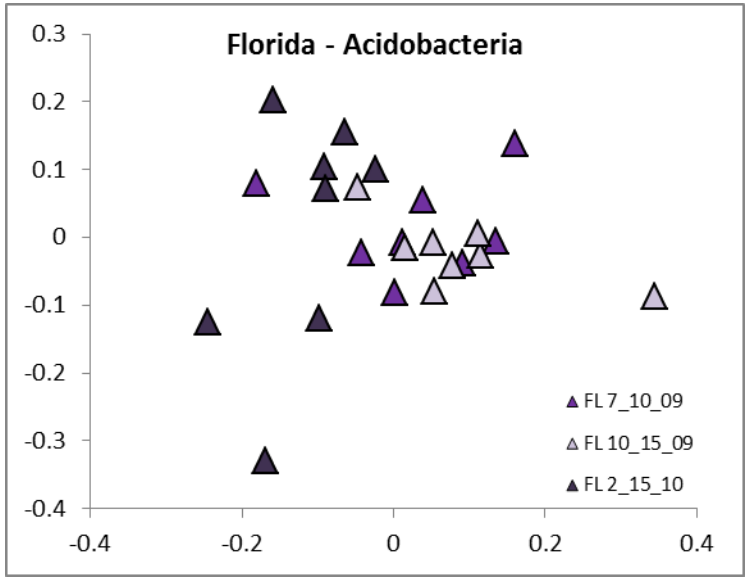


ANOSIM statistic R: -0.02718
Significance: 0.649

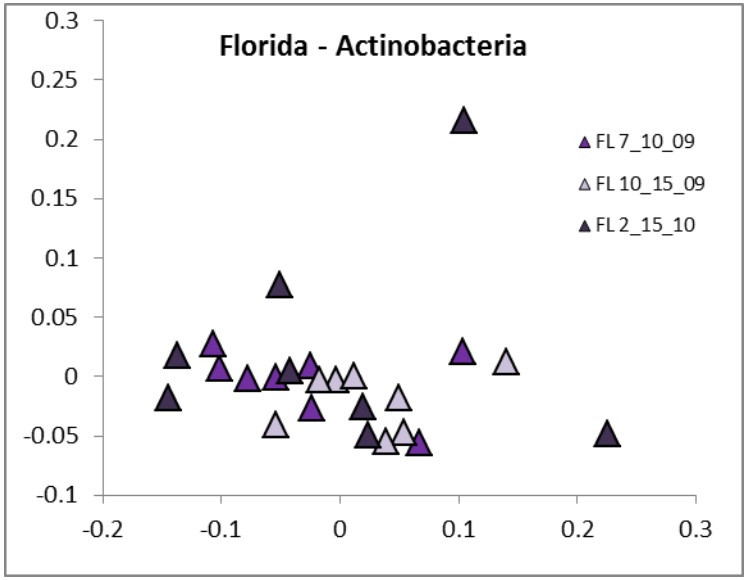


ANOSIM statistic R: 0.1217
Significance: 0.059

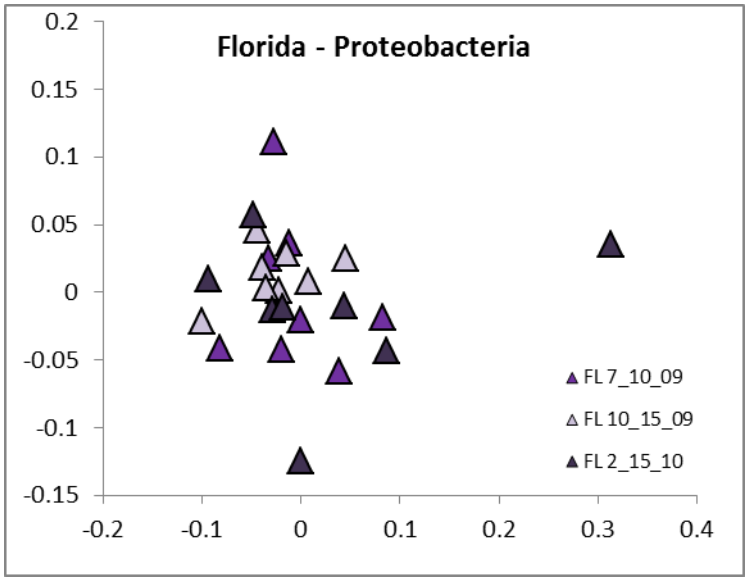
Florida – Phyla Over Time



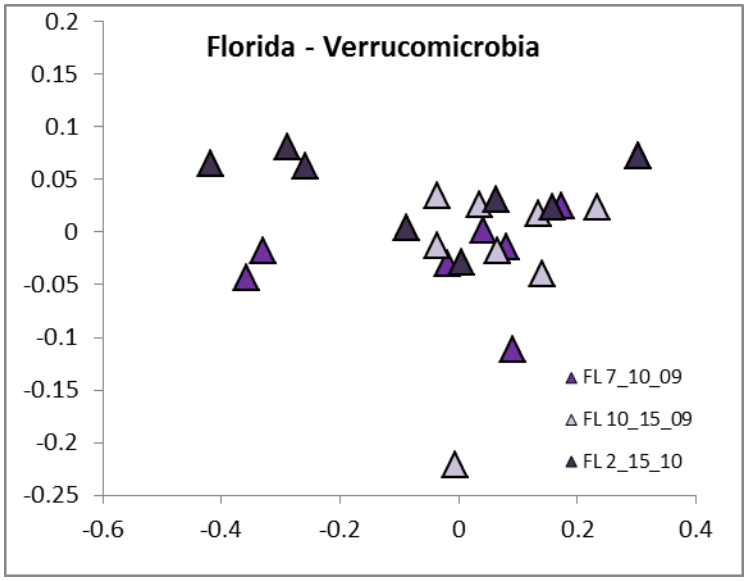
ANOSIM statistic R: 0.2057
Significance: 0.003



ANOSIM statistic R: 0.04861
Significance: 0.171

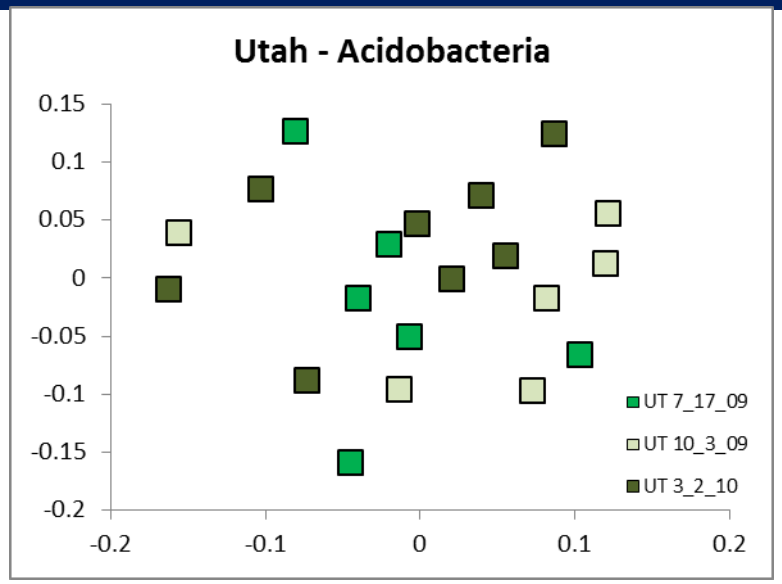


ANOSIM statistic R: 0.03968
Significance: 0.148

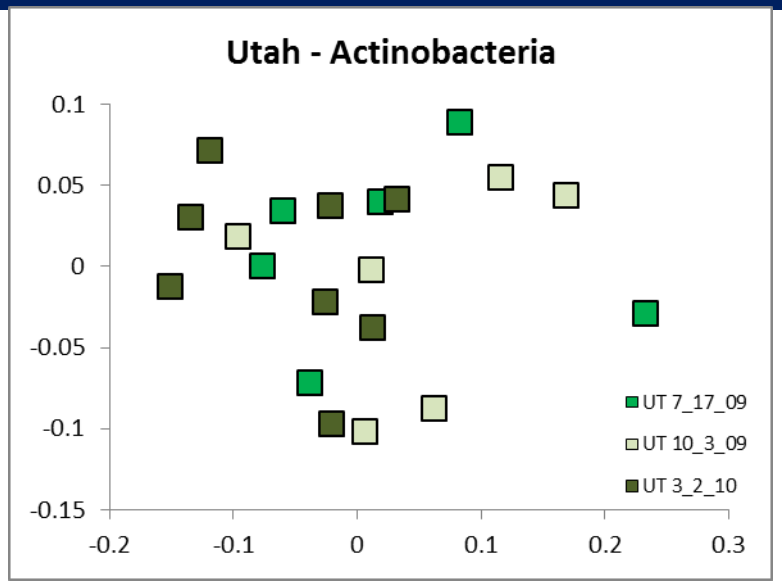


ANOSIM statistic R: -0.007502
Significance: 0.467

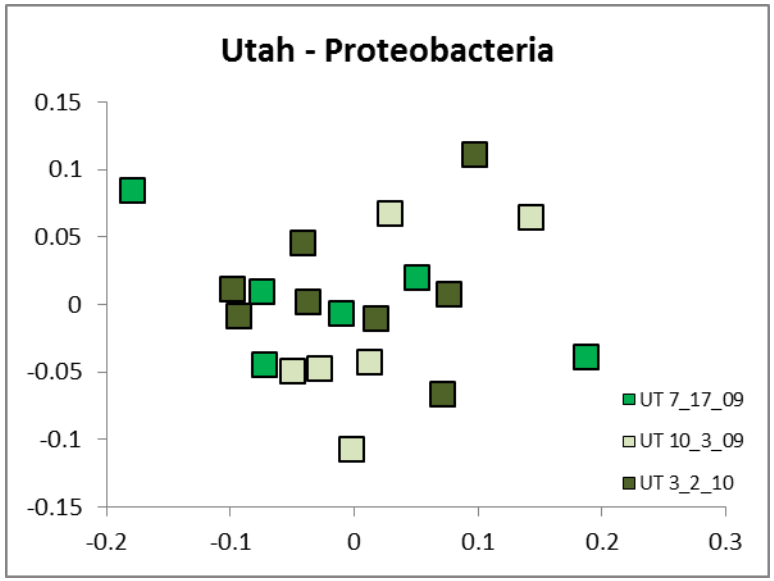
Utah – Phyla Over Time



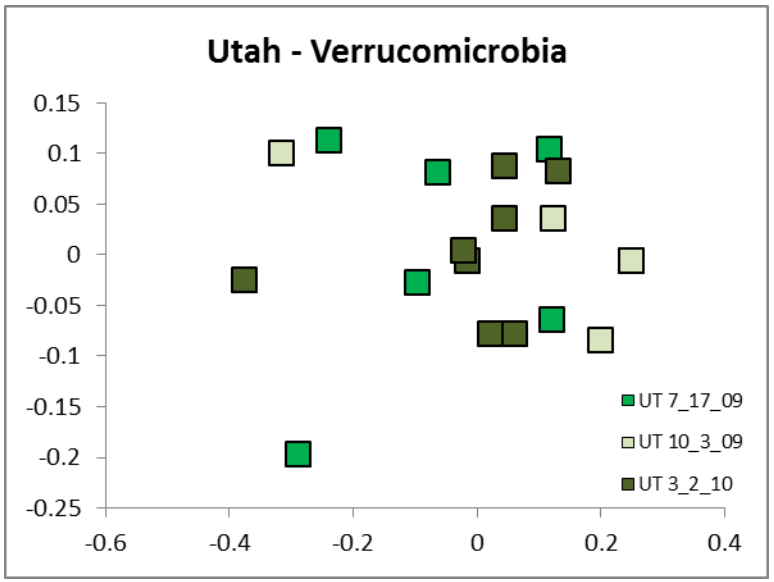
ANOSIM statistic R: -0.01385
Significance: 0.518



ANOSIM statistic R: 0.01489
Significance: 0.388



ANOSIM statistic R: 0.007576
Significance: 0.404



ANOSIM statistic R: 0.1057
Significance: 0.083

Does the composition of Families within Phyla differ by site/over time?

- There are very minor differences in some Phyla
  - Actinobacteria in Alaska
  - Verrucomicrobia in Hawaii
  - Acidobacteria in Florida
  - Nothing in Utah

Summary of Analyses

There are consistent differences and similarities between sites

16S and lipid data are fairly consistent, but exhibit some differences

There are few seasonal changes

Summary of Analyses

There are consistent differences and similarities between sites

There are few seasonal changes

NEON's main question: how does climate change impact soil microbial communities

Will we see these changes at peak greenness?
Will we only see changes at more extreme time points?

What do you think?

OPEN

The ISME Journal (2013) 7, 1493–1506

www.nature.com/ismej

ORIGINAL ARTICLE

A meta-analysis of changes in bacterial and archaeal communities with time

Ashley Shade[1], J Gregory Caporaso[2,3], Jo Handelsman[1], Rob Knight[4,5] and Noah Fierer[6,7]
[1]*Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA;* [2]*Department of Computer Science, Northern Arizona University, Flagstaff, AZ, USA;* [3]*Argonne National Laboratory, Argonne, IL, USA;* [4]*Department of Chemistry and Biochemistry and Biofrontiers Institute, University of Colorado, Boulder, CO, USA;* [5]*Howard Hughes Medical Institute, Boulder, CO, USA;* [6]*Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, USA and* [7]*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA*

Ecologists have long studied the temporal dynamics of plant and animal communities with much less attention paid to the temporal dynamics exhibited by microbial communities. As a result, we do not know if overarching temporal trends exist for microbial communities or if changes in microbial communities are generally predictable with time. Using microbial time series assessed via high-throughput sequencing, we conducted a meta-analysis of temporal dynamics in microbial communities, including 76 sites representing air, aquatic, soil, brewery wastewater treatment, human- and plant-associated microbial biomes. We found that temporal variability in both within- and between-community diversity was consistent among microbial communities from similar environments. Community structure changed systematically with time in less than half of the cases, and the highest rates of change were observed within ranges of 1 day to 1 month for all communities examined. Microbial communities exhibited species–time relationships (STRs), which describe the accumulation of new taxa to a community, similar to those observed previously for plant and animal communities, suggesting that STRs are remarkably consistent across a broad range of taxa. These results highlight that a continued integration of microbial ecology into the broader field of ecology will provide new insight into the temporal patterns of microbial and 'macro'-bial communities alike.
The ISME Journal (2013) **7,** 1493–1506; doi:10.1038/ismej.2013.54; published online 11 April 2013
Subject Category: Microbial population and community ecology
Keywords: similarity-decay; species–time relationship; beta diversity; 16S rRNA; turnover; high-throughput sequencing

Scale of Temporal Variation

"Several habitats had no or few communities that exhibited correlations between time and community structure.

For soil communities,
inter-annual changes would not be evident in this data set, as the study durations were > 6 months.

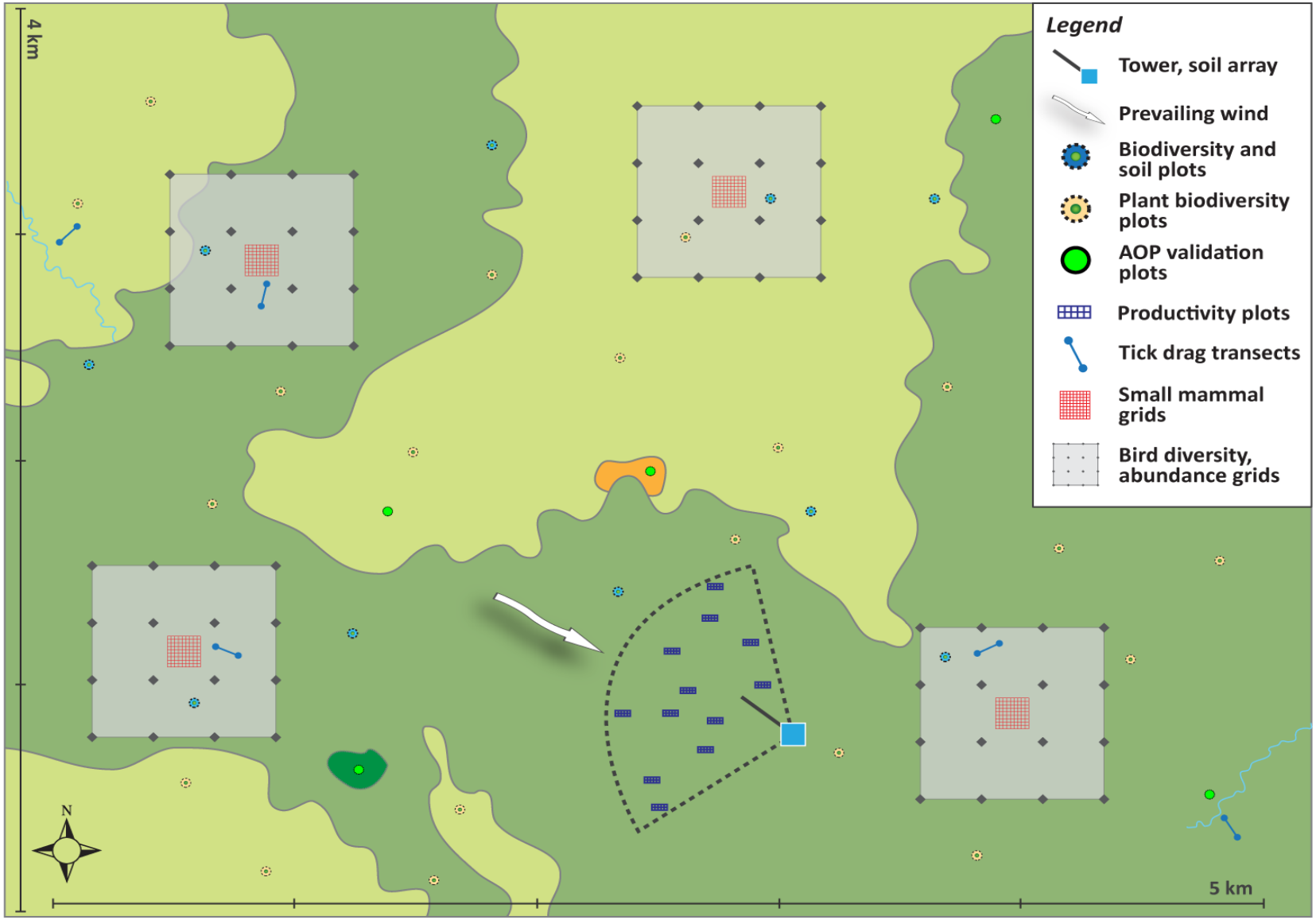
Thus, it may be that changes in soil communities are not correlated with time at the time scale included but that such relationships could become evident if we had time series extending across full years or multiple years."

Shade et al. 2013 ISME

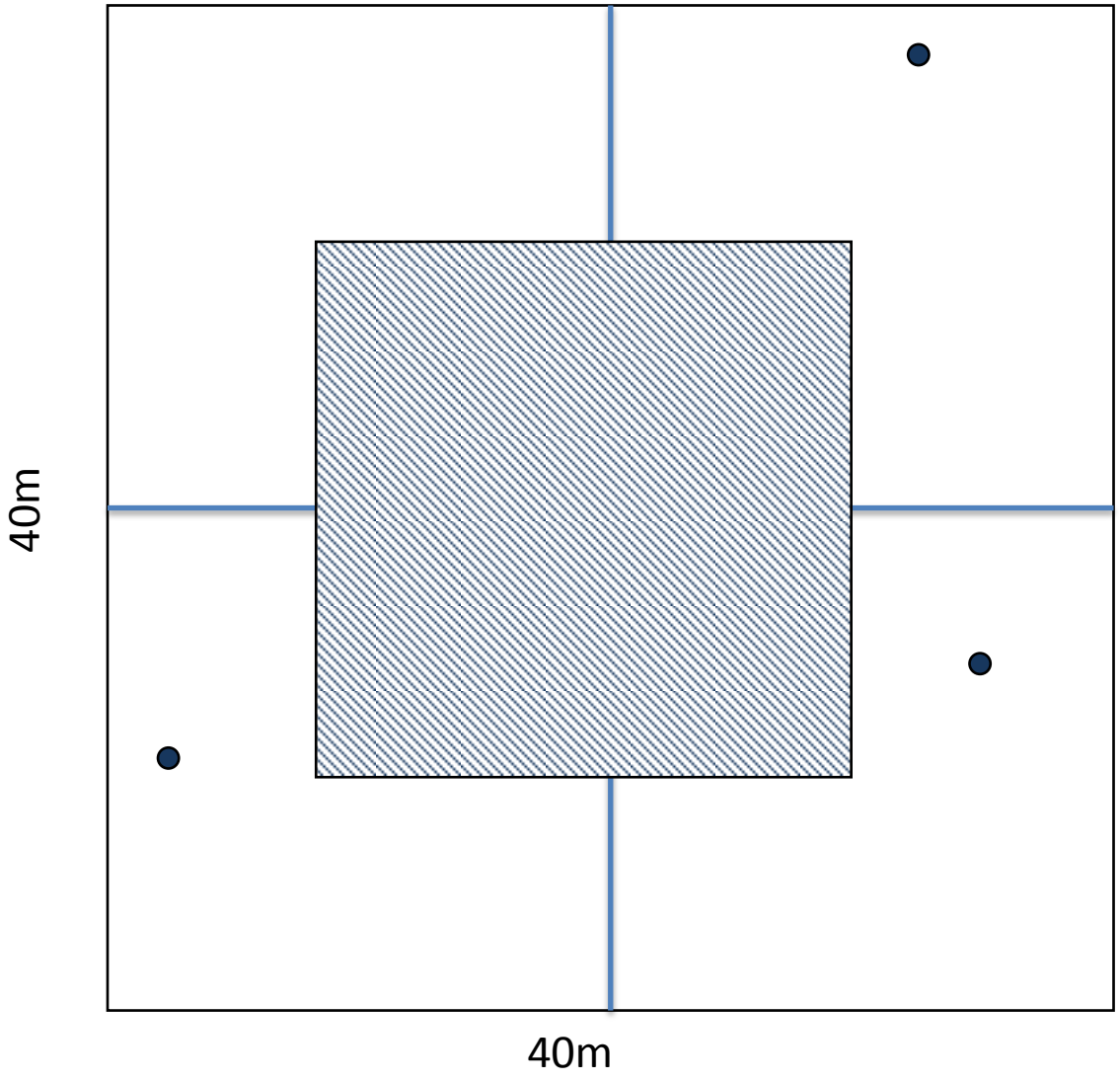
NEON Present and Future

Material	16S	ITS	Meta-transcriptome	Meta-genome	qPCR	Cell count
Soil	5400	5400	5400	1800	5400	0
Water	324	324	324	108	324	324
Sediment	324	324	324	108	324	324
Benthic	324	324	324	108	324	324
Total/yr	6372	6372	6372	2124	6372	972
Total	191160	191160	191160	63720	191160	29160

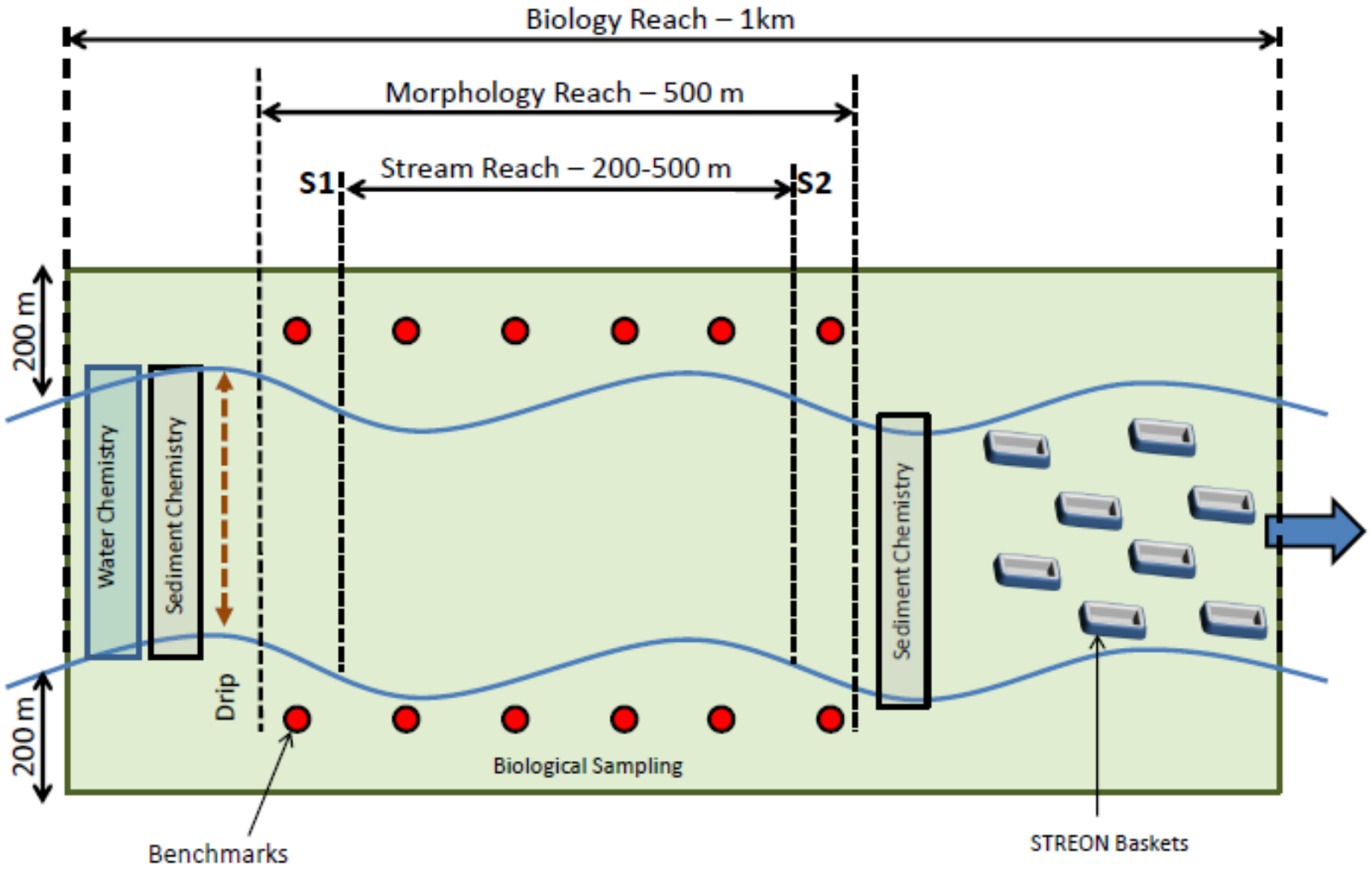
Co-location with Plant Biomass Sampling



New Sampling Design

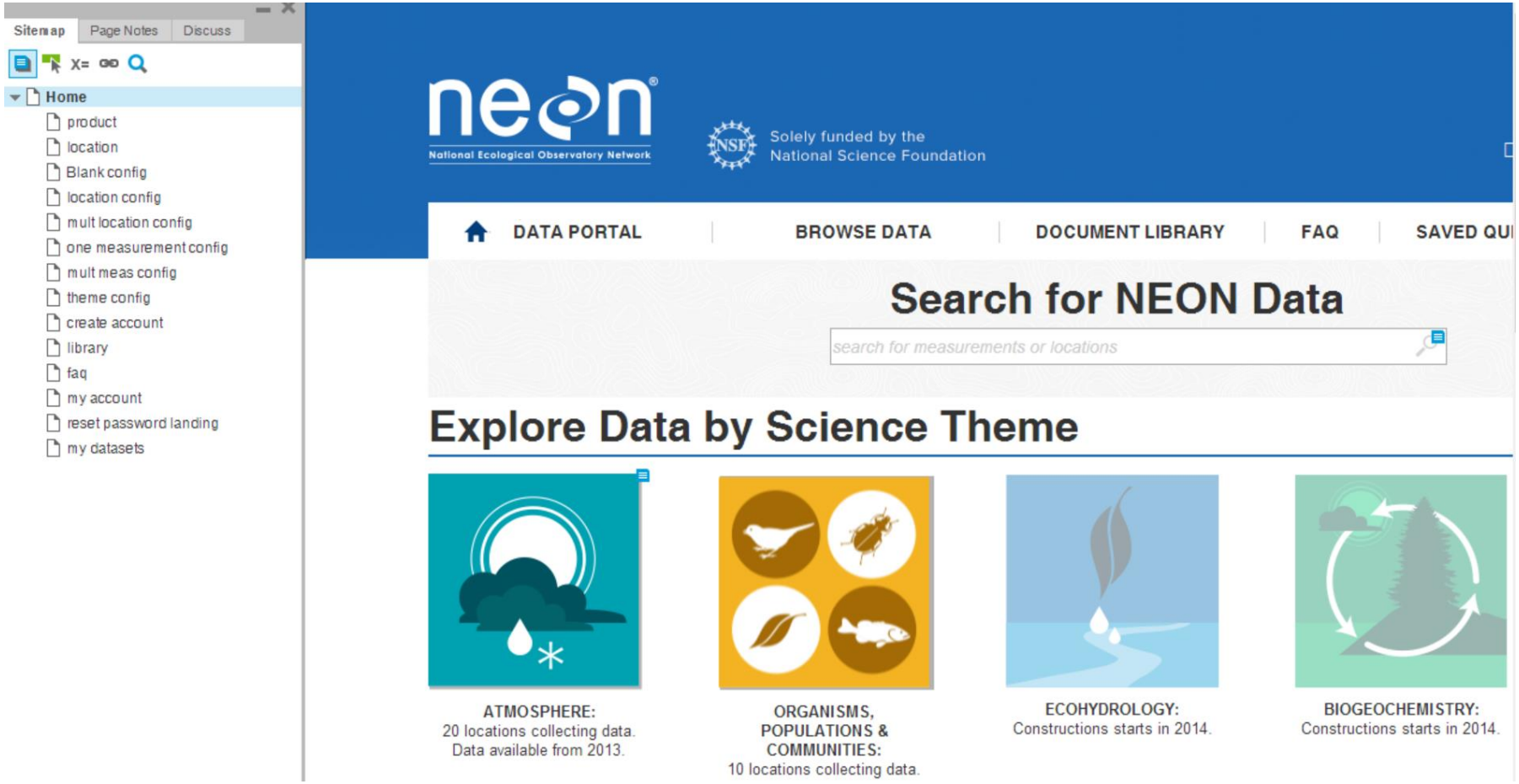


Aquatic Observation System (AOS) Field Design



NEON Data Portal

http://lz399t.axshare.com/#p=home



Who to Contact at NEON

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Dr. Stephanie Parker – Aquatic Microbial Sampling, sparker@neoninc.org

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Dr. Teri Balser

NEON

Dr. Jacob Parnell, NEON Microbe Working Group (G. King, L. Kinkel, L. Zeglin, C. Blackwood, N. Fierer, J. Gilbert, M. Allen, J. Tiedje, E. Triplett, D. Nemergut)

PERMANOVA results – Peak Greenness, all lipids

Some sort of RDA thing here

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
TMBT	1	3828.4	3828.4	24.4708	0.29676	0.001	***
phT	1	1715.7	1715.7	10.9664	0.13299	0.001	***
swcT	1	244.7	244.7	1.5644	0.01897	0.176	
cecT	1	3009.5	3009.5	19.2364	0.23328	0.001	***
omT	1	207.5	207.5	1.3261	0.01608	0.253	
caT	1	385.6	385.6	2.4647	0.02989	0.073	.
mgT	1	183.3	183.3	1.1716	0.01421	0.318	
naT	1	228.8	228.8	1.4628	0.01774	0.214	
kT	1	107.5	107.5	0.6872	0.00833	0.579	
tcT	1	390.5	390.5	2.4962	0.03027	0.061	.
tnT	1	114.1	114.1	0.7296	0.00885	0.554	
sulfT	1	138.3	138.3	0.8841	0.01072	0.469	

PERMANOVA – Florida 16S rRNA differences over time

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
pH	**1**	**42.37**	**42**	**0**	**0.03790**	**1**
SWC	1	113.83	114	0	0.10182	1
CECT	1	107.73	108	0	0.09637	1
OMT	**1**	**20.39**	**20**	**0**	**0.01824**	**1**
CaT	**1**	**52.35**	**52**	**0**	**0.04683**	**1**
Mg	1	152.51	153	0	0.13643	1
NaT	**1**	**40.61**	**41**	**0**	**0.03633**	**1**
K	**1**	**22.16**	**22**	**0**	**0.01982**	**1**
TCT	**1**	**19.63**	**20**	**0**	**0.01756**	**1**
TNT	**1**	**29.11**	**29**	**0**	**0.02604**	1
SO4	1	140.43	140	0	0.12562	1
GNT	**1**	**21.26**	**21**	**0**	**0.01901**	**1**
GP	1	78.20	78	0	0.06995	1
ACT	**1**	**41.88**	**42**	**0**	**0.03746**	**1**
ANA	**1**	**19.16**	**19**	**0**	**0.01714**	**1**
AMF1T	**1**	**44.73**	**45**	**0**	**0.04002**	**1**
SFT	**1**	**32.31**	**32**	**0**	**0.02890**	**1**
TMB	1	139.23	139	0	0.12455	1
Residuals	0	0.00	Inf		0.00000	
Total	18	1117.88			1.00000	

Harvard forest
All three Florida site
13 total sites

Andrea Thorpe – general questions
Stephanie Parker – aquatic microbial design
Eve Hinckley – terrestrial biogeochemistry

Co-locate sampling with other sampling (plant biomass, mammal traps, etc) which are distributed throughout the plot.

qPCR of 16S and ITS
Microscopic counts are for aquatic microbial sampling

Some of it metagenomics/metatranscroptomics might be available on MG-RAST
Some is on the QIIME database
Raw, unanalyzed data
NEON will make OTU tables available (processed data with spatio-temporal data associated)
Soil pH, soil moisture, soil temperature
Other variables, would have to be connected
Archiving soil samples, stored at -80
Not doing PLFA anymore