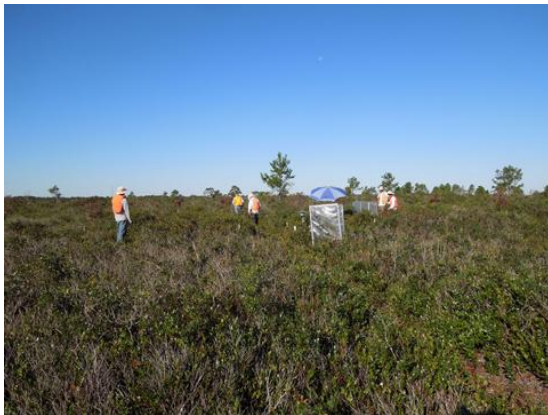
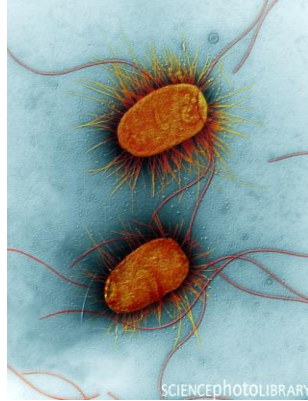


# Microbial community responses to nutrient enrichment in wetland soils



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# INTRODUCTION

## Do microbes respond to nutrients in wetland soils ?

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### **Nutrient cycling critical to key WETLAND FUNCTIONS:**

- Primary productivity, water quality and carbon cycling

### **Microbes control nutrient cycling in wetlands**

- BUT responses to altered nutrients are poorly understood

### **Microbial communities respond to nutrients in UPLAND soils**

- to + N across habitats at continental scale (*Ramirez et al. 2012 GCB*)

### **BUT response to nutrients in WETLANDS is less clear...**

- No relationships observed in freshwater peatlands and salt marshes  
(*Hartman et al. 2008, Bowen et al. 2011 ISMEJ*)

#### **RESEARCH OBJECTIVE:**

Assess response of soil microbes (bacteria and fungi) to nutrient additions in replicated field experiments across contrasting wetland types

# STUDY DESIGN

## Nutrient amendments in contrasting wetland types

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### Used sites and plots from Differential Nutrient Limitation study:

- NSF #0816593: Richardson, Neubauer, Sundareshwar



#### **Tidal Freshwater Marsh (GA):**

Altamaha River marsh (GA coastal LTER)

Soil N:P = 32:1

N – Limited plants



#### **Tidal Salt Marsh (SC):**

North Inlet estuary

Soil N:P = 15:1

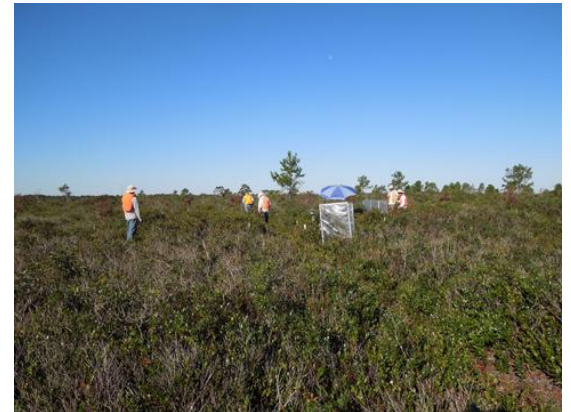
N – Limited plants

#### **Pocosin Bog (NC):**

Croatan N.F.

Soil N:P = 66:1

P – Limited plants





# STUDY DESIGN

## Nutrient amendments in contrasting wetland types

---

### Fertilization Treatments:

- Control
- + N
- + P
- + N, P

### **3 replicate Plots / Treatment**

(12 plots / Site) x 3 sites

### **Fertilized in months:**

April, July, Oct

### **Fertilizer applied in years:**

2009, 2010, 2011



**Fertilizer levels vary by Site to account for differences in ambient levels**

# METHODS

## Determination of microbial community responses

---

### Soil sampling: (July 2011)

- 2 cores / Plot pooled  
(10 cm depth x 7 cm dia.)
- Roots wet sieved, picked
- Soil Homogenized
- Hierarchically sub-sampled  
into 0.5 g composite



### Soil DNA extraction:

- MO BIO Ultra Clean DNA extraction kit



### DNA Sequencing:

- **16S (Bacteria)** and **28S (Fungi)** rDNA
- amplicon **454 GLS FX Titanium** (pyrosequencing)



### Informatics:

- *QIIME, RDP,*
- *also MG-RAST, Mothur, R...*



# METHODS

## Overview of Analysis Approach

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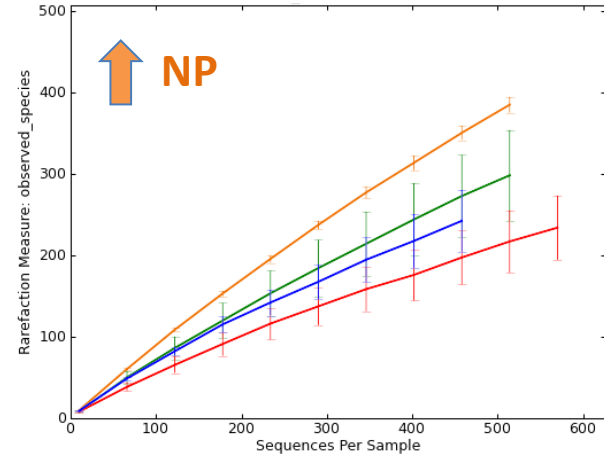
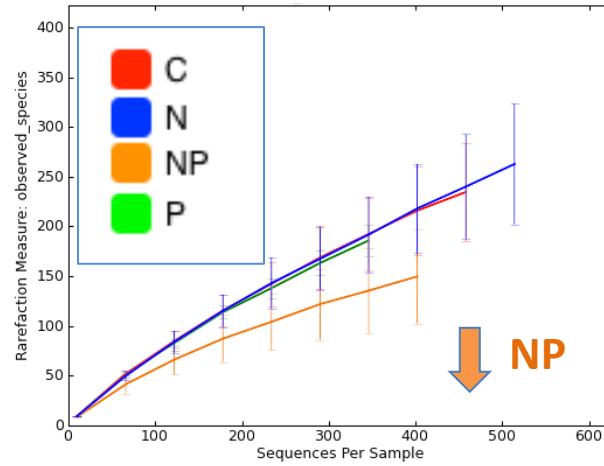
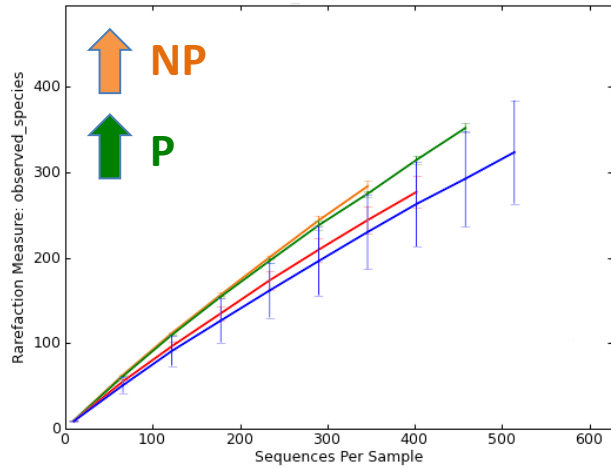
### ANALYSES PRESENTED:

- 1) Diversity of microbial communities**
  - Bacteria and Fungi
  
- 2) Community composition and Treatment responses**
  - a) Bacteria
  - b) Fungi
  
- 3) Relations with environment and C cycling**

# RESULTS

## 1) Diversity of Microbial Communities

### BACTERIA:

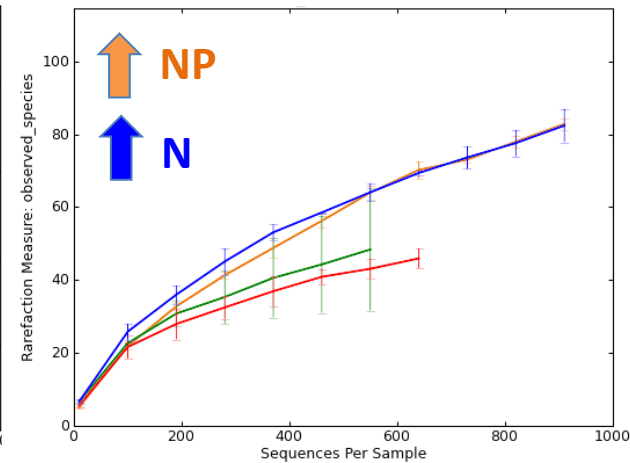
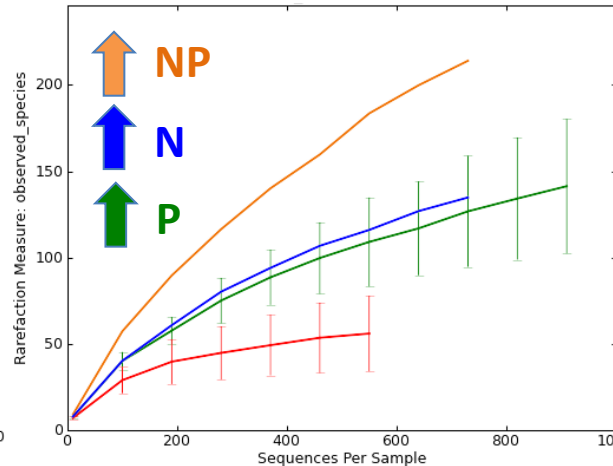
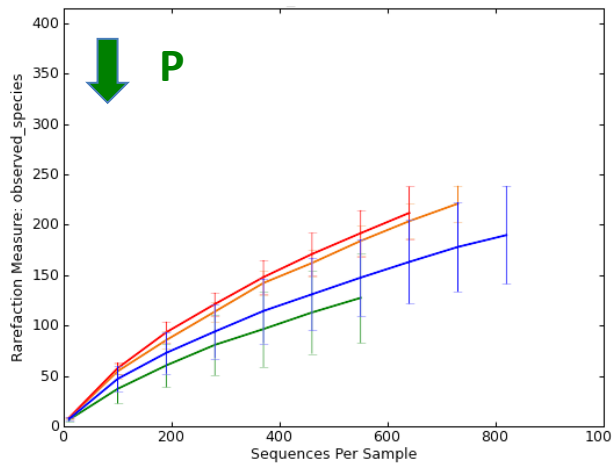


GA

NC

SC

### FUNGI:

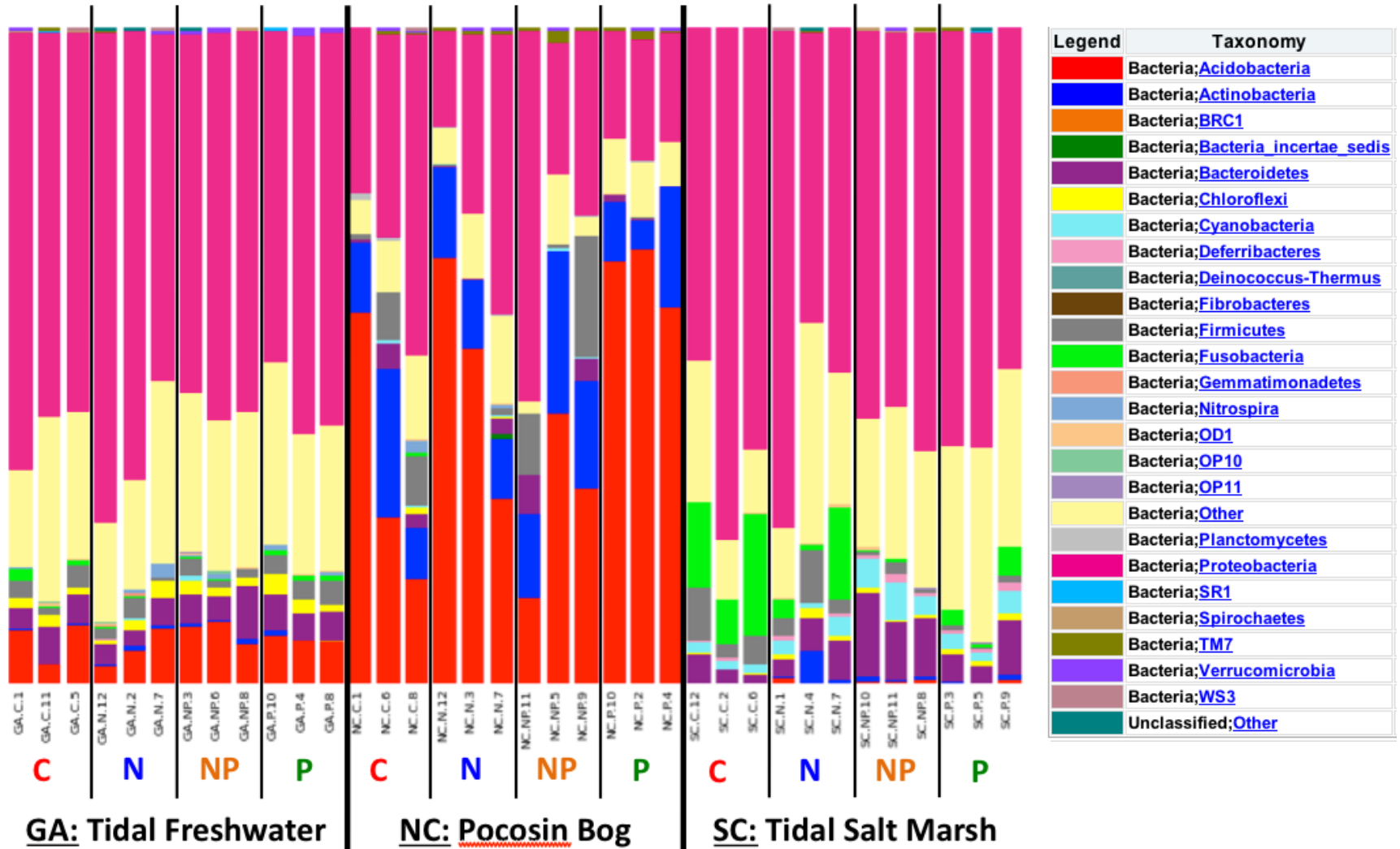


# RESULTS

## 2. a) Bacterial Community Composition

**PHYLA:** 26 taxonomic groups

~ 600 seqs. per plot



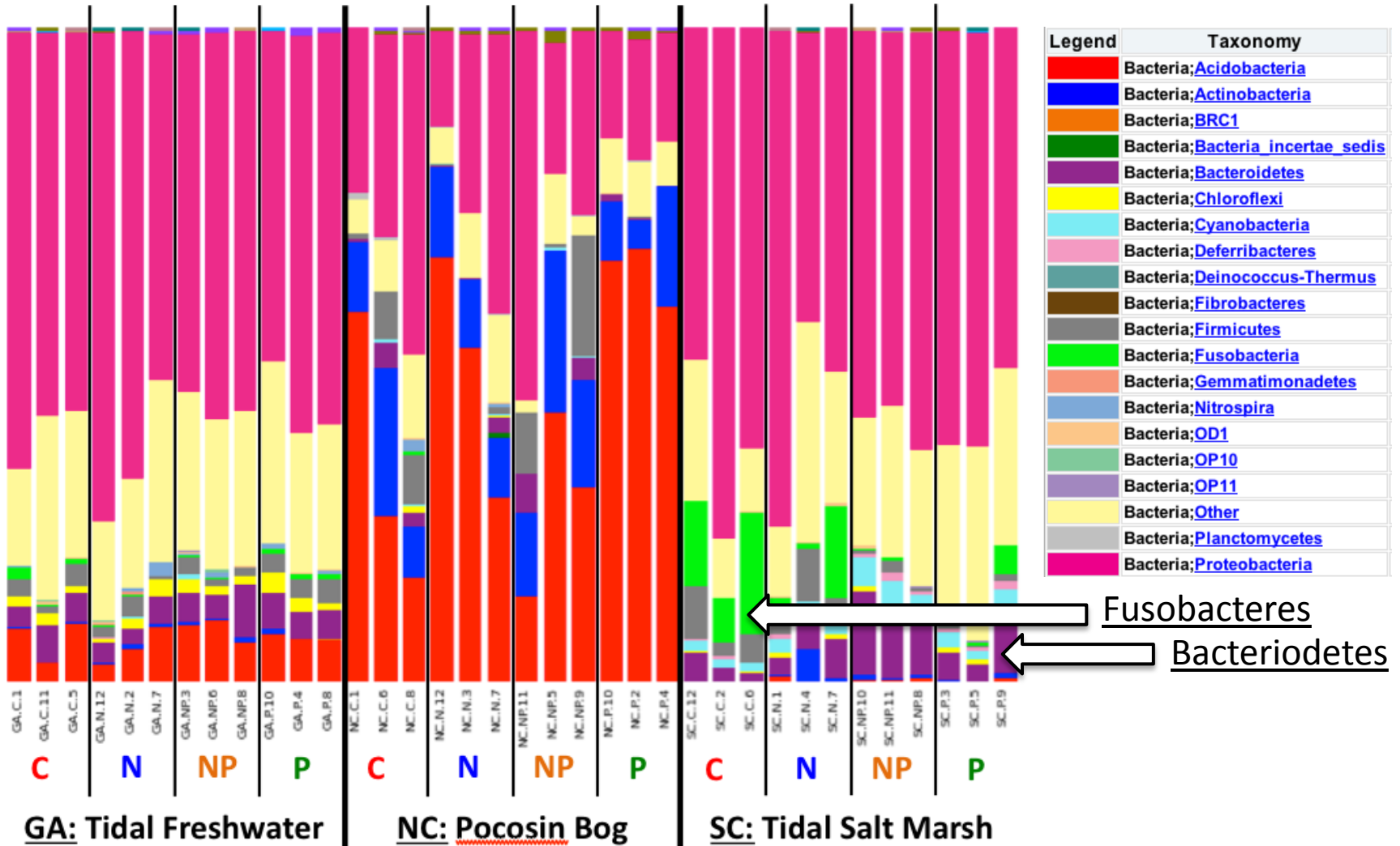


# RESULTS

## 2. a) Bacterial Community Composition

**PHYLA:** 26 taxonomic groups

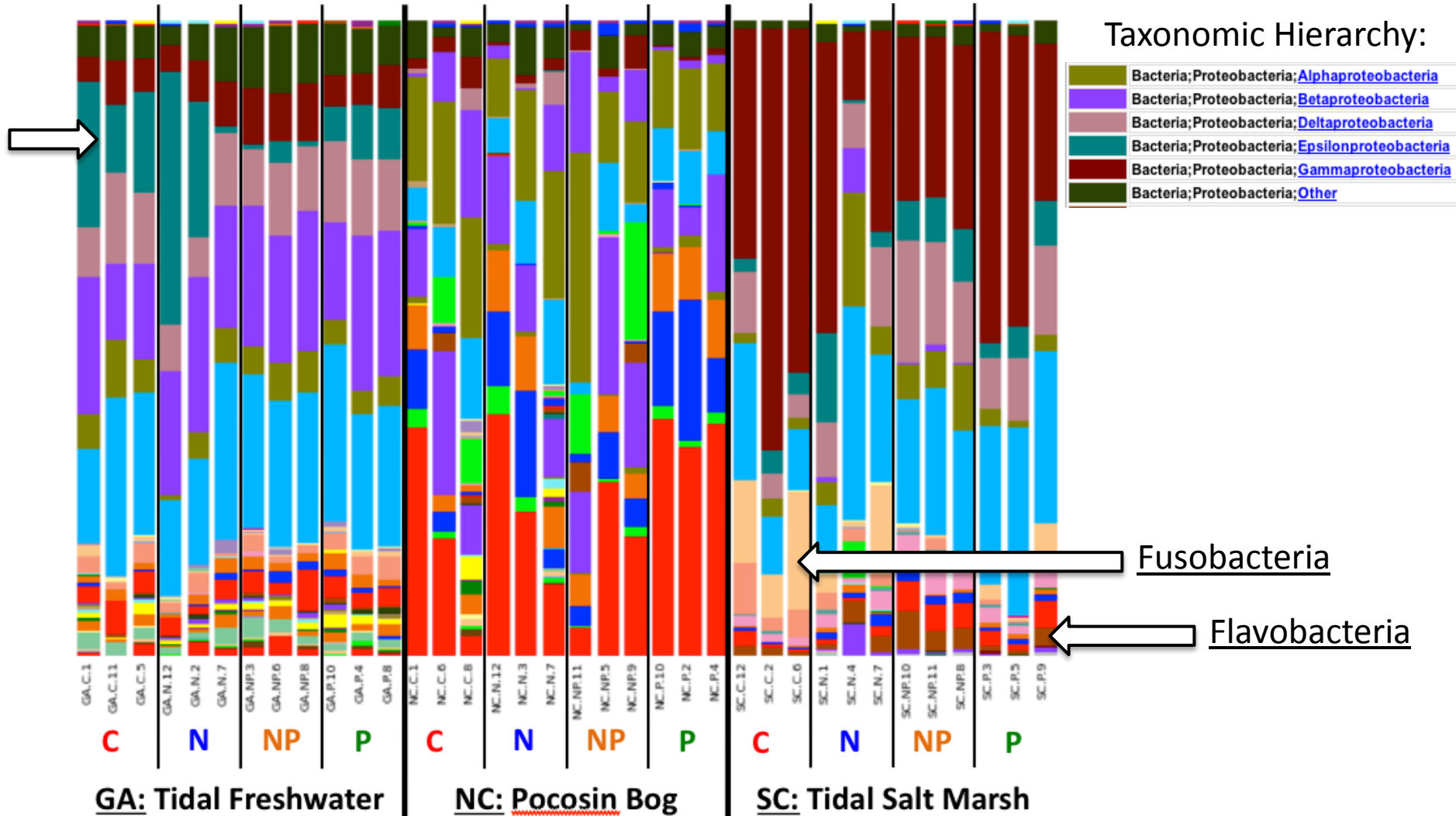
~ 600 seqs. per plot



# RESULTS

## 2. a) Bacterial Community Composition

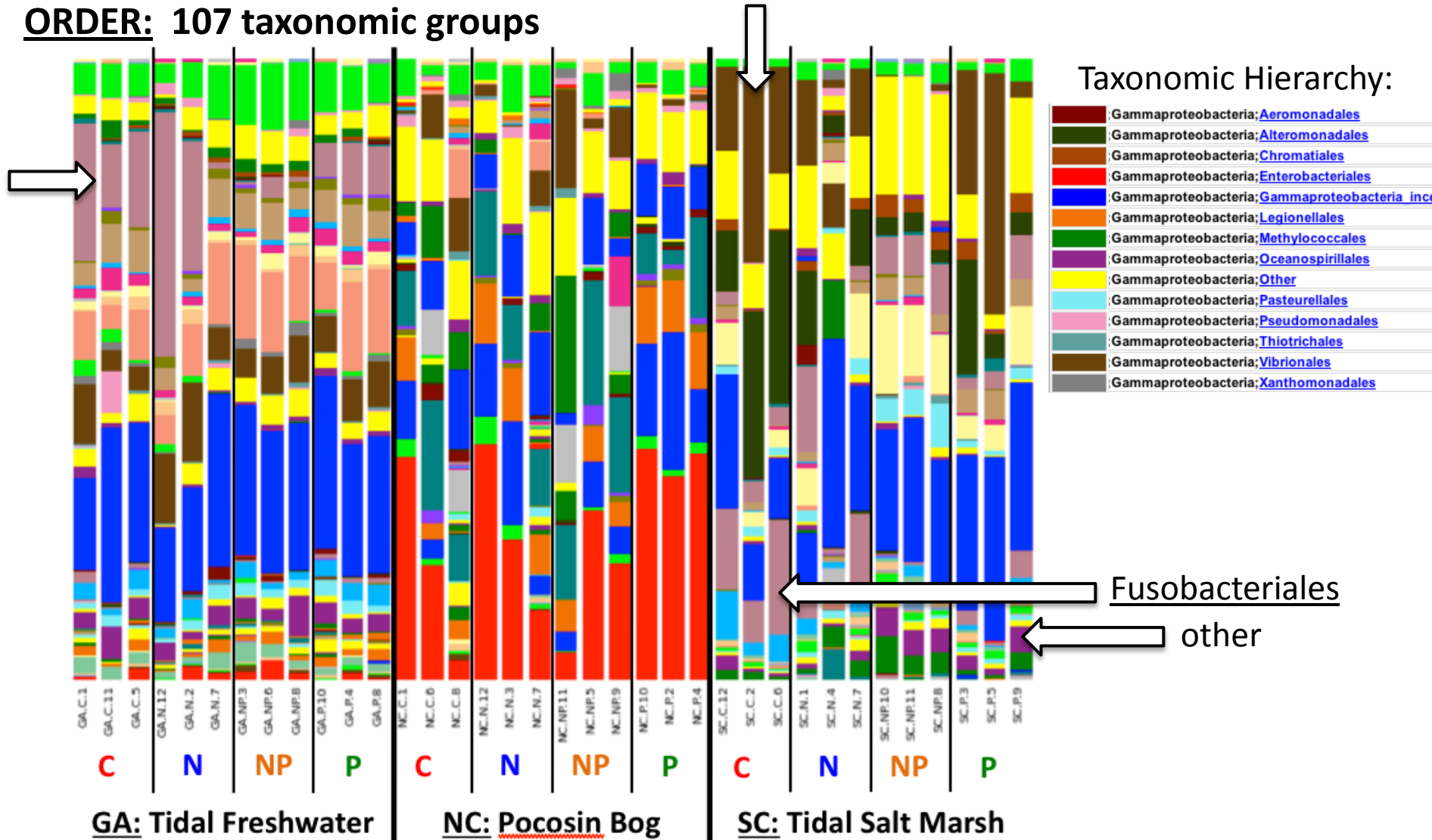
**CLASS:** 65 taxonomic groups



# RESULTS

## 2. a) Bacterial Community Composition

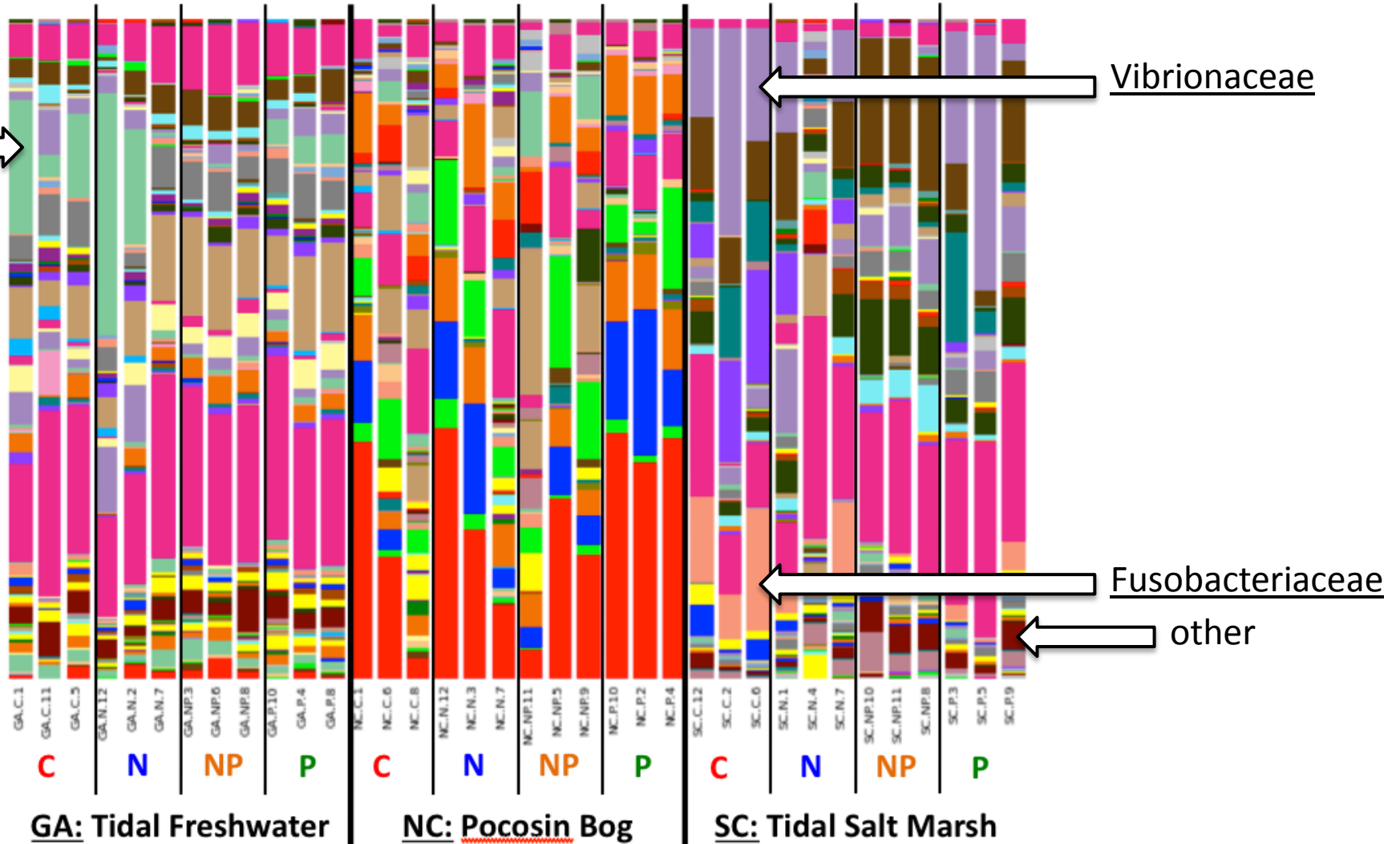
**ORDER:** 107 taxonomic groups



# RESULTS

## 2. a) Bacterial Community Composition

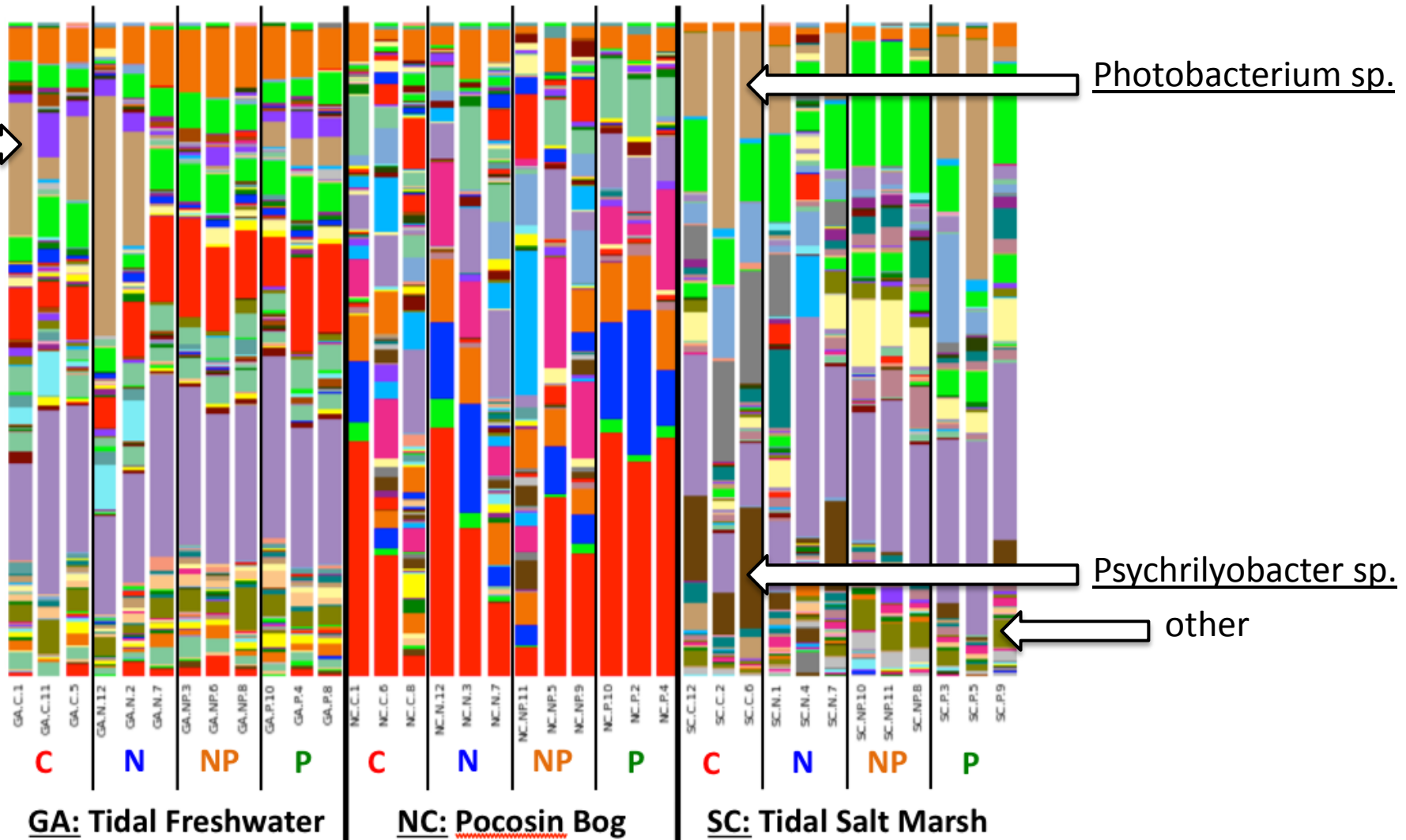
**FAMILY:** 204 taxonomic groups



# RESULTS

## 2. a) Bacterial Community Composition

**GENUS: 391 taxonomic groups**



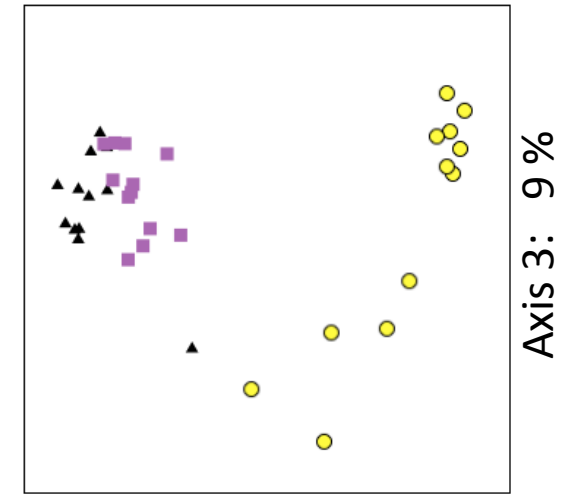
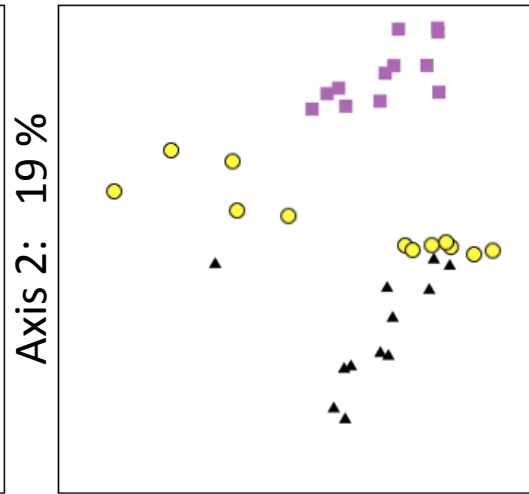
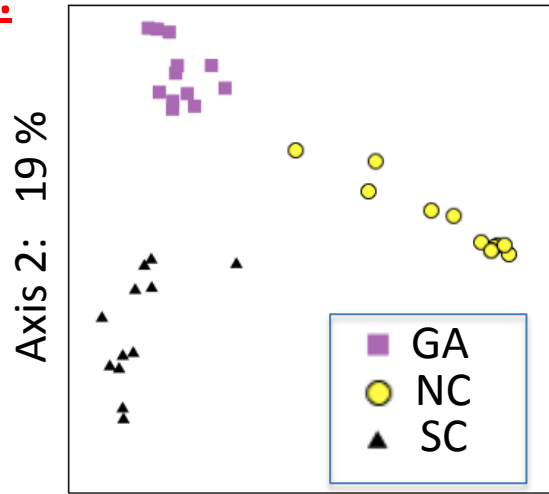


# RESULTS

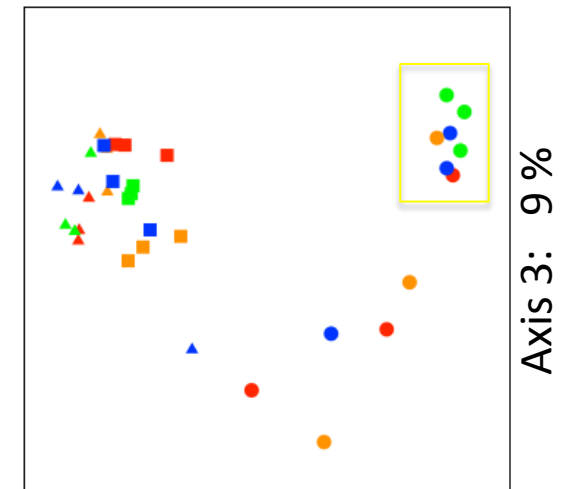
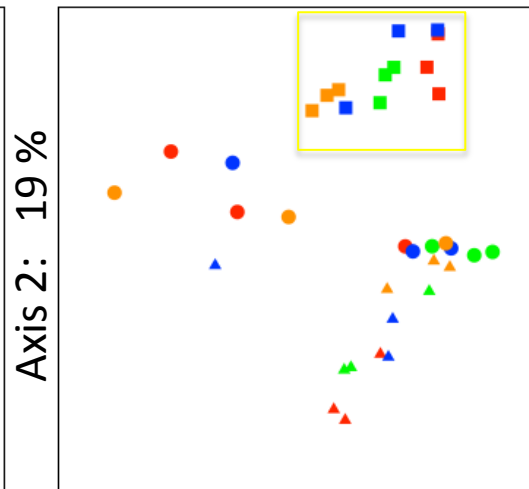
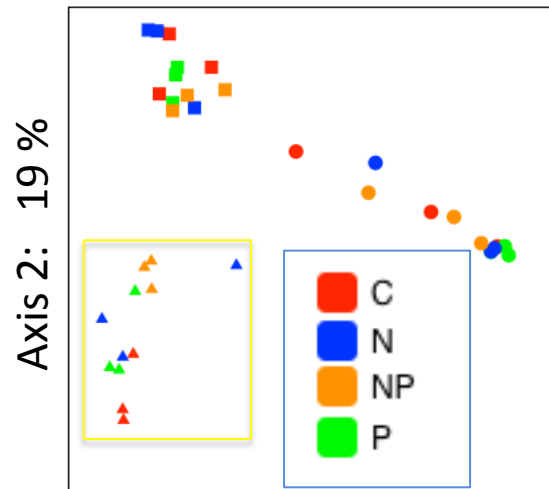
## 2. a) Bacterial Community Composition

### Ordination of communities by UNIFRAC (sequence distance)

by SITE:



by TREAT:



# RESULTS

## 2. a) Bacterial Community Composition

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Statistical differences in BACTERIAL communities by **ANOSIM:**  
(Non-parametric bootstrapped distance-based ANOVA)

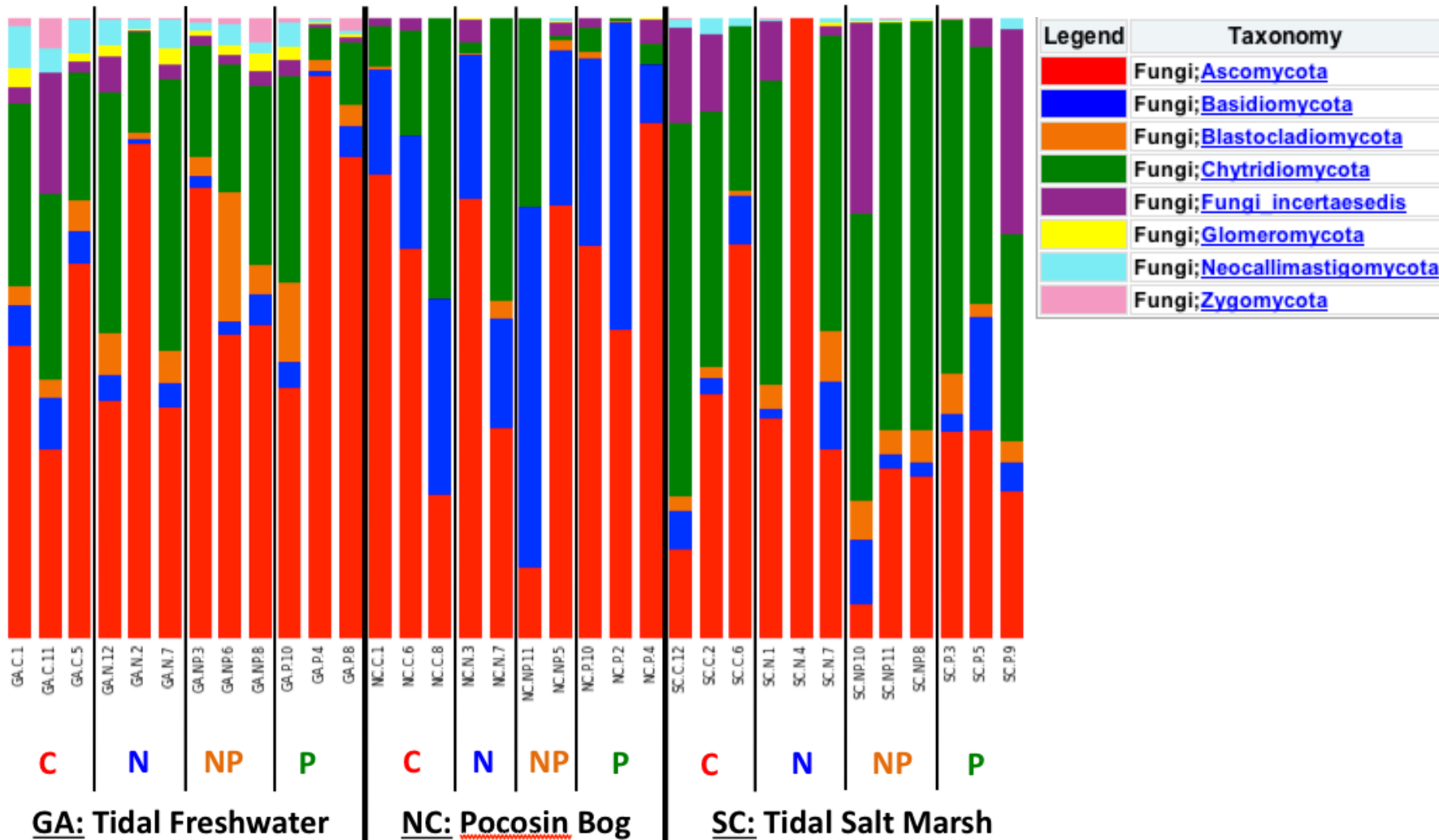
<b>analysis</b>	<b>site</b>	<b>R</b>	<b>p</b>
Site	<b>all</b>	<b>0.911</b>	<b>0.001</b>
Treat   site	<b>GA</b>	<b>0.352</b>	<b>0.037</b>
	<i>NC</i>	<i>0.090</i>	<i>0.246</i>
	<b>SC</b>	<b>0.494</b>	<b>0.005</b>

# RESULTS

## 2. b) Fungal Community Composition

**PHYLA:** 8 taxonomic groups

~ 900 seqs. per plot

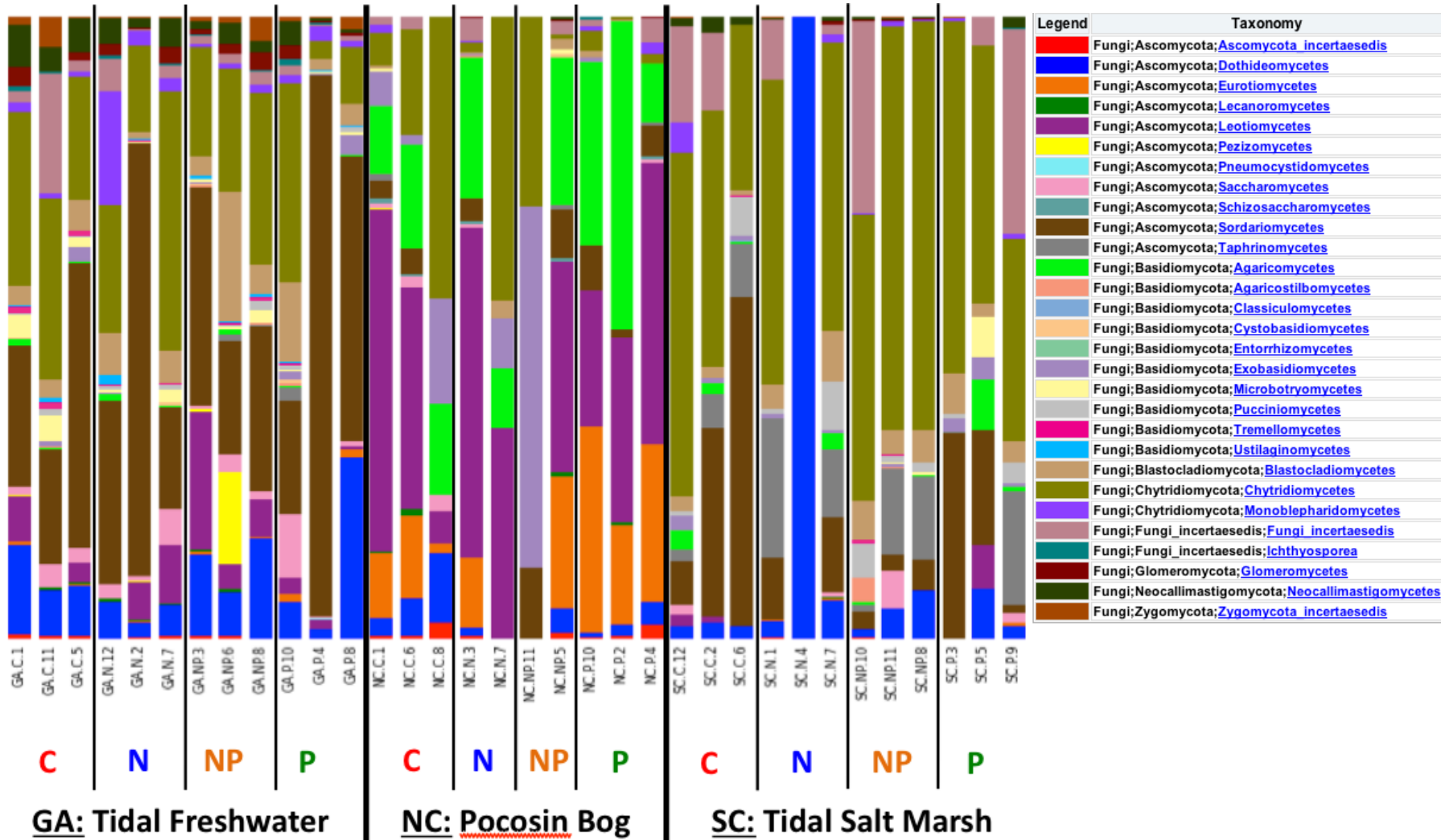


# RESULTS

## 2. b) Fungal Community Composition

**Class:** 29 taxonomic groups

...417 genera



# RESULTS

## 2. b) Fungal Community Composition

---

Statistical differences in FUNGAL communities by ANOSIM:  
(Non-parametric bootstrapped distance-based ANOVA)

<b>analysis</b>	<b>site</b>	<b>R</b>	<b>p</b>
Site	<b>all</b>	<b>0.699</b>	<b>0.001</b>
Treat   site	<i>GA</i>	<i>-0.068</i>	<i>0.693</i>
	<i>NC</i>	<i>0.000</i>	<i>0.426</i>
	<i>SC</i>	<i>0.095</i>	<i>0.284</i>



# RESULTS

## 3. a) Relationships between communities and environment

Correlation between **BACTERIAL communities** and **environment** by **MANTEL's tests**: (Non-parametric bootstrapped distance-based regression)

Data shown are Mantel's correlation ( $r$ ), only significant results shown:

Soil Var.	<u>ALL SITES</u>		<u>Partial by site</u>		
	Partial	Pure Partial	GA	NC	SC
C	0.60	0.49			
N	0.43	0.46			
P	0.27	0.22		0.32	0.44
Ext. NH <sub>4</sub>	0.14				
Ext. NO <sub>3</sub>	0.16				
Ext. P			0.39		
pH	0.64	0.13			
% Moisture	0.34	0.15			
C:N	0.58	0.20			
C:P	0.62	0.33		0.32	
N:P	0.50	0.27		0.54	
Ext N:P					

Bacterial communities within sites linked primarily with soil **PHOSPHORUS**

# RESULTS

## 3. a) Relationships between communities and environment

---

Correlation between **FUNGAL communities** and **environment** by **MANTEL's tests**: (Non-parametric bootstrapped distance-based regression)

Data shown are Mantel's correlation ( $r$ ), only significant results shown:

Soil Var.	ALL SITES		Partial by site		
	Partial	Pure Partial	GA	NC	SC
C	0.55	0.54			
N	0.55	0.54			
P					
Ext. NH <sub>4</sub>					
Ext. NO <sub>3</sub>					
Ext. P					
pH	0.26				0.40
% Moisture	0.22	0.10			
C:N	0.17				
C:P	0.25	0.21		0.39	
N:P	0.34	0.31		0.67	
Ext N:P					

# RESULTS

## 3. b) Relationships between communities and C cycling

### Correlation between microbial communities and C cycling by MANTEL's tests:

Data shown are Mantel's correlation ( $r$ ), only significant results shown:

#### BACTERIA:

<u>ALL SITES</u>		<u>Partial by site</u>		
<u>C cycling</u>	<u>Partial</u>	<u>GA</u>	<u>NC</u>	<u>SC</u>
CO <sub>2</sub> (ring)	0.17			
NEE				
GEP	0.24			
P_synth				
CO <sub>2</sub> (Dark)	0.24			
CH <sub>4</sub>	0.16		NA	NA
MBC	0.23			

#### FUNGI:

<u>ALL SITES</u>		<u>Partial by site</u>		
<u>C cycling</u>	<u>Partial</u>	<u>GA</u>	<u>NC</u>	<u>SC</u>
CO <sub>2</sub> (ring)	0.28			
NEE				
GEP				
P_synth				
CO <sub>2</sub> (Dark)				
CH <sub>4</sub>			NA	NA
MBC	0.53			

BUT, CO<sub>2</sub> (ring), NEE, GEP, CO<sub>2</sub> (Dark) did not respond to Treatment within Sites

- Only responses within site were P\_synth (GA), MBC (SC)

# CONCLUSIONS

## Summary, implications and future work

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### Microbial diversity

- Response to nutrients varies by site
- potential BACTERIAL:FUNGAL interactions

### FUNGAL communities did not clearly respond to nutrients

### Wetland BACTERIAL communities do respond to nutrients

- Especially in marshes (GA and SC)
- Response appears most strongly linked to PHOSPHORUS
- Opposite of marsh plant limitation by **NITROGEN**

May indicate Differential Nutrient Limitation of plants and microbes

### Carbon cycling weakly linked with microbes

- **But C cycling did not respond to nutrient addition**
- Could test against nutrient responsive microbial taxa separately

(Using Indicator Species Analysis – *Dufrene and Legendre 1997 Ecol. Mongr.*)

# ACKNOWLEDGMENTS

Many thanks to...

---

## PIs / Co-authors

- Curt Richardson, Scott Neubauer, P. V. Sundareshwar

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- Johnathan Bills (Soil sampling, chemical analysis)
- Mengchi Ho (Licor CO<sub>2</sub>, Site Photos)
- Wes Willis (Chemical analysis)

## Neubauer Lab

- NEE, GEP, P\_Synth, CO<sub>2</sub> (Dark), CH<sub>4</sub> data

## Duke Mycology Lab

- Rytas Vilgalys and Greg Bonito  
(DNA Lab space and advising)

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**QUESTIONS ?**