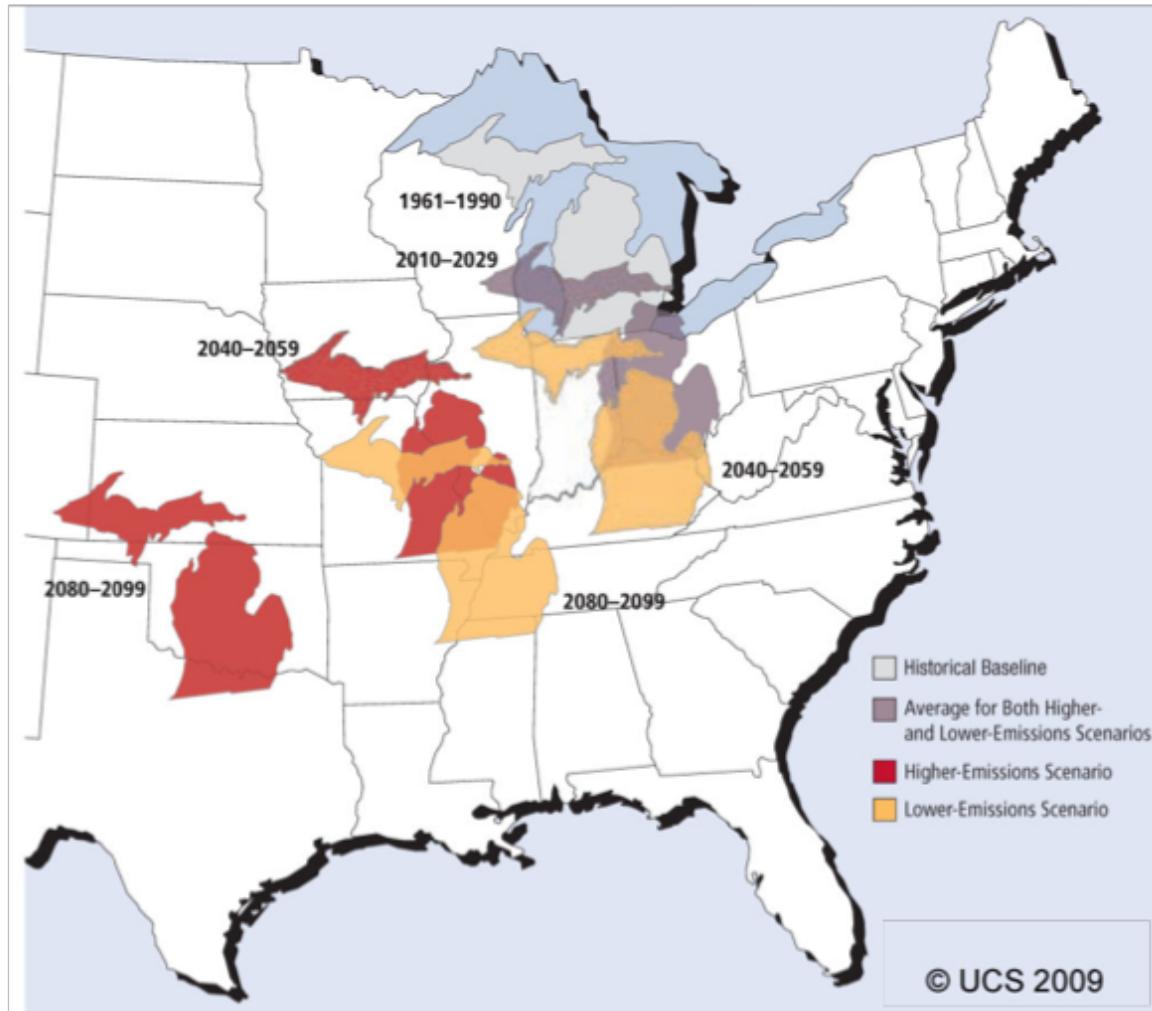
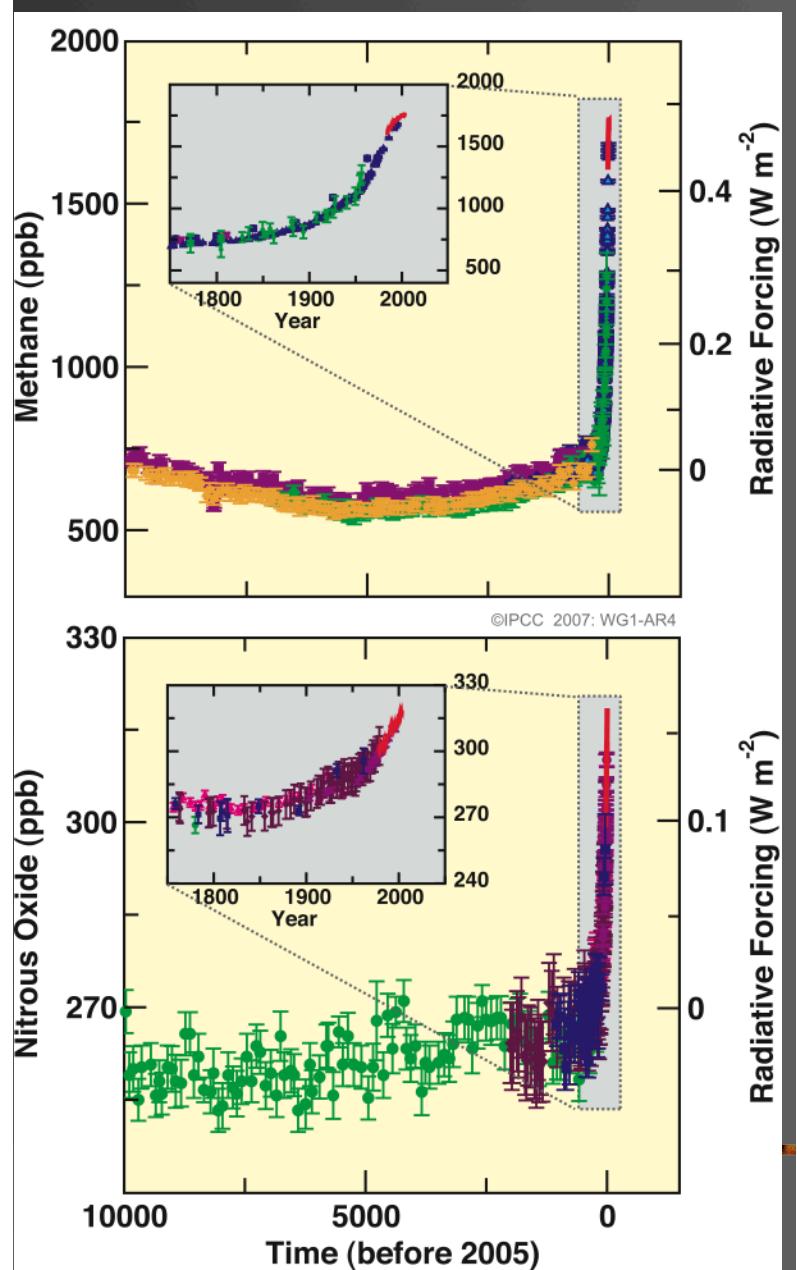


# Effects of agricultural management on microbial communities and associated greenhouse gas fluxes

Tracy K. Teal  
Data Carpentry  
Michigan State University  
UC Davis

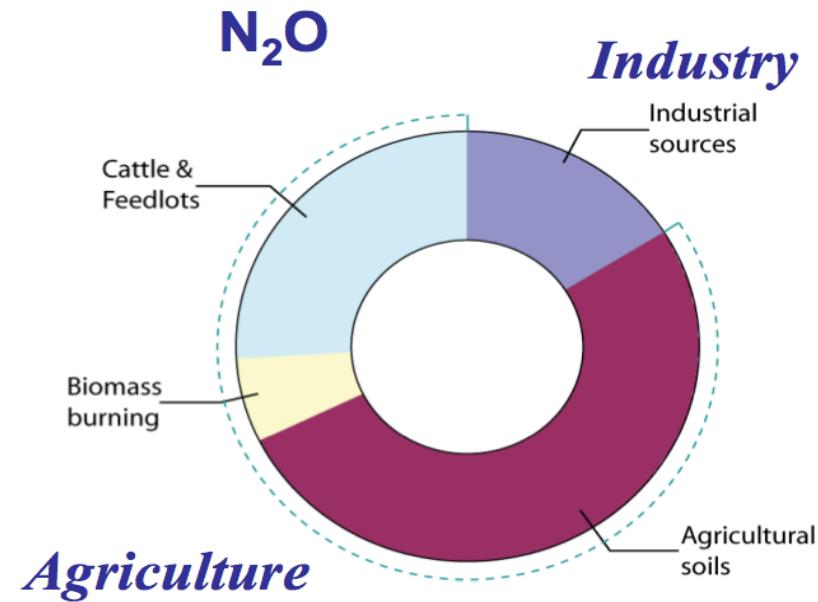
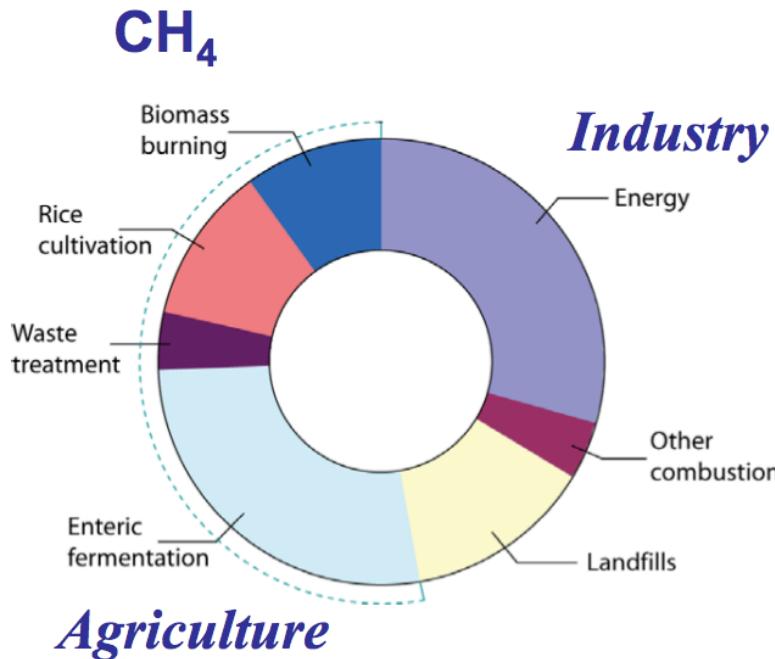
# The climate is changing





- Dramatic increases in  $\text{CH}_4$  and  $\text{N}_2\text{O}$  in the last 200 years
- High Global Warming Potential (GWP) due to time in the atmosphere and infrared absorption

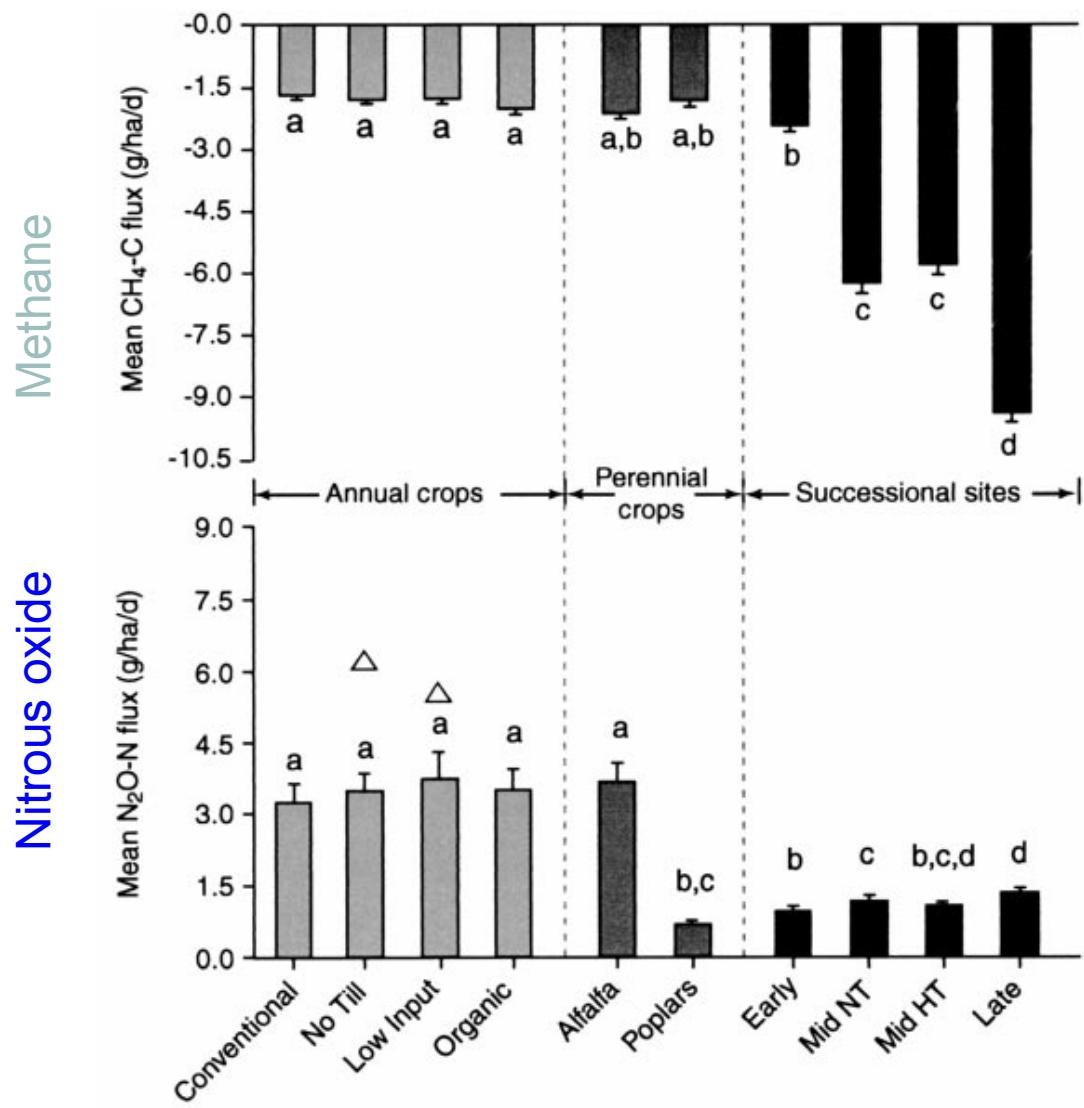
# Land use change and intensive agriculture increase greenhouse gas fluxes



# Experiment sites

Kellogg Biological Station LTER



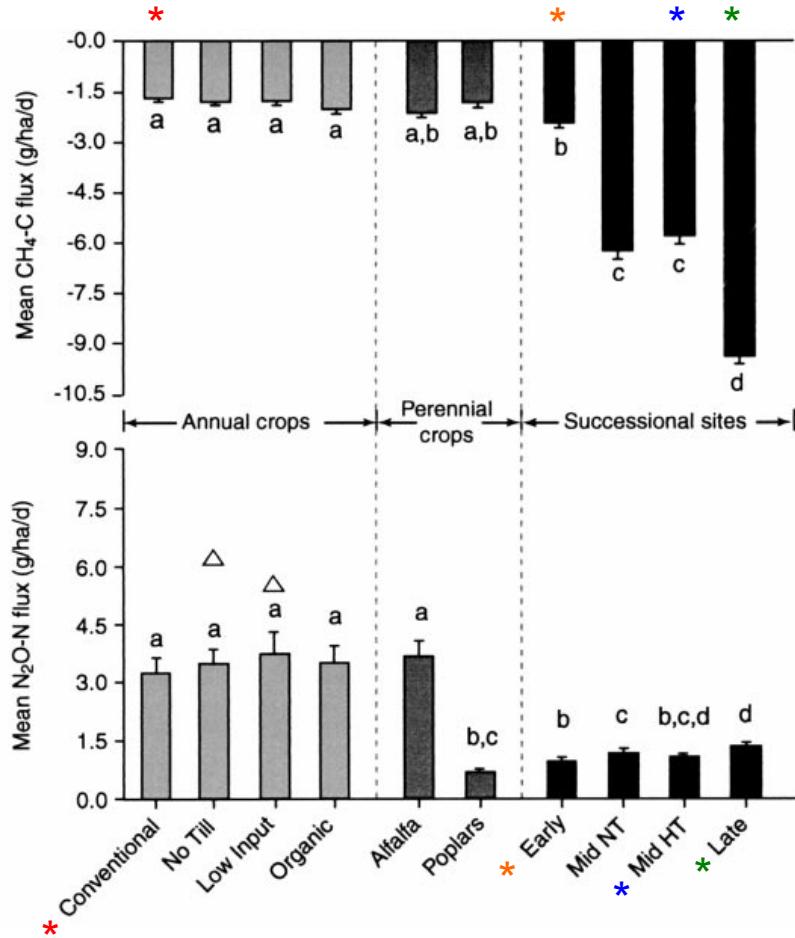


Robertson et al, 2000

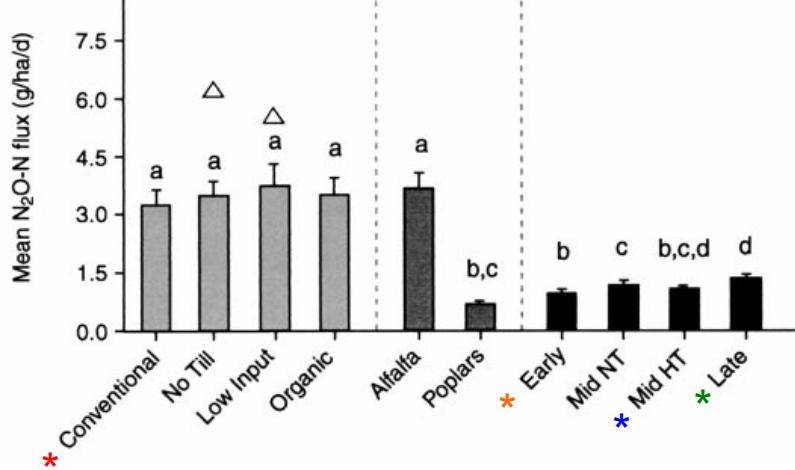
# How do microbial communities change with land management?

Kellogg Biological Station LTER

Methane



Nitrous oxide



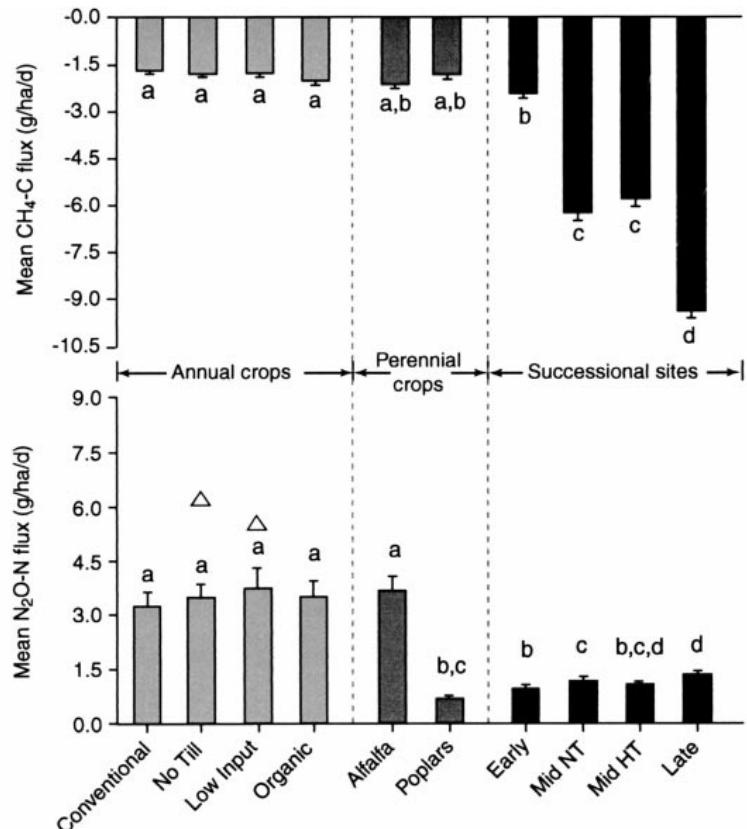
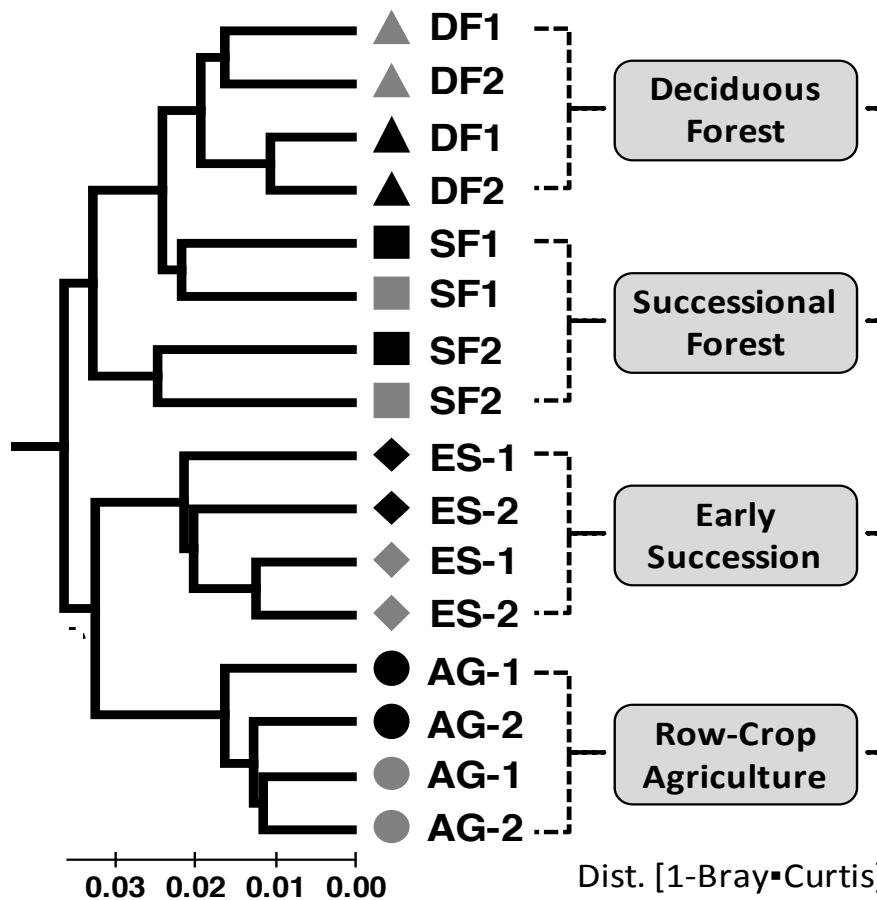
Robertson et al, 2000



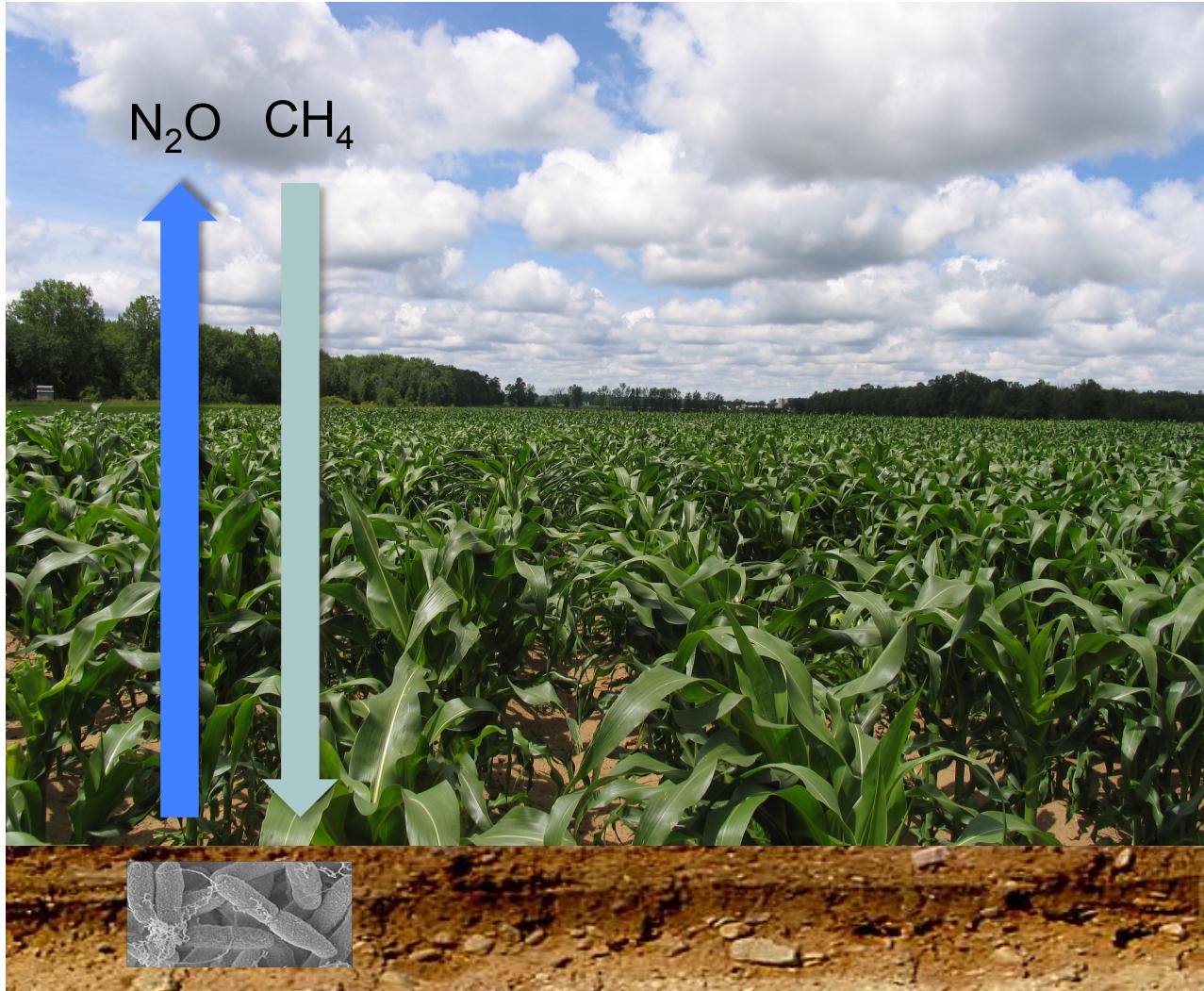
- AG      Conventional Agriculture
- ES      Early Successional
- SF      Successional Forest
- DF      Deciduous Forest

# Soil properties correlate with greenhouse gas fluxes

## Soil properties



# Microbes are the primary mediators of methane and nitrous oxide



# Land management, sustainability and microbial communities

- How do microbial communities change with land management?
- What is the relationship between denitrifying bacteria and N<sub>2</sub>O in agricultural soils?
- How long does it take for a community to recover from agriculture? Do all groups recover in the same way?

# How do microbial communities change with land management?

## Sampling design

Sampled two plots of 4 treatments  
In two years – 2008 and 2009

- 10 cm cores
- Soil from 5 flags pooled and sieved
- Soil frozen after being collected
- Microbial community analysis
- Soil analysis



- AG Conventional Agriculture
- ES Early Successional
- SF Successional Forest
- DF Deciduous Forest

# (Meta)genomics approach to survey the microbial community (454 technology)



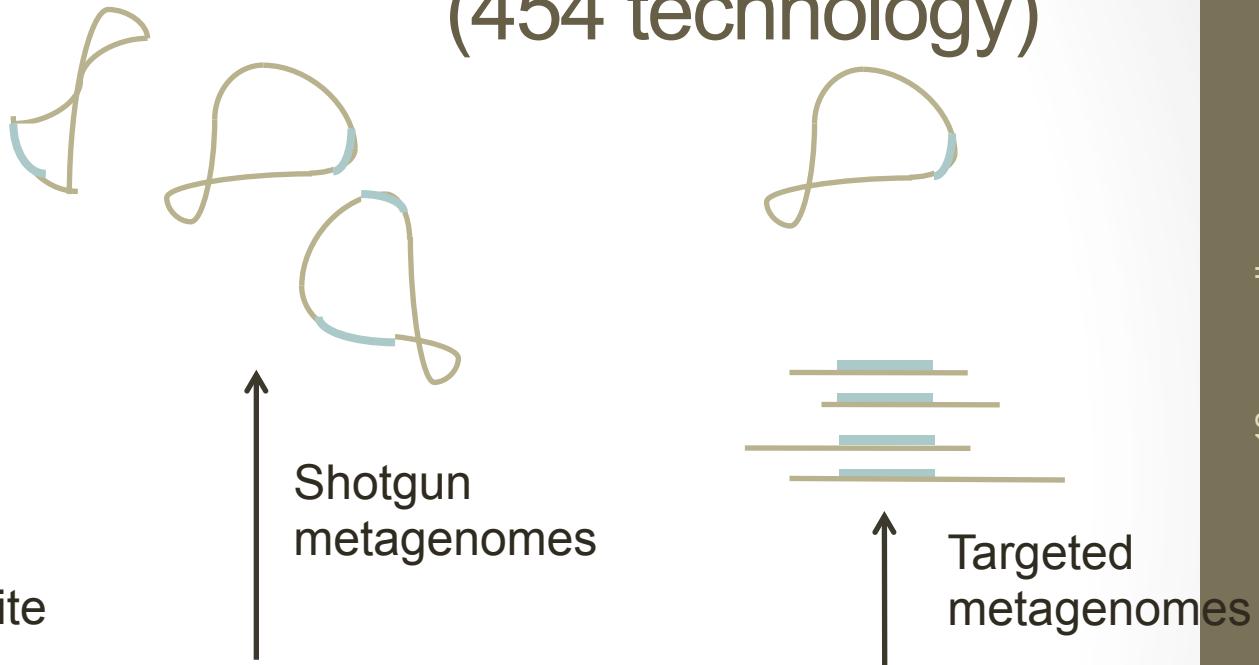
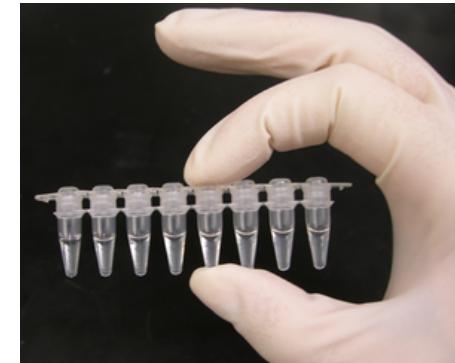
↓ Collect, composite  
and sieve

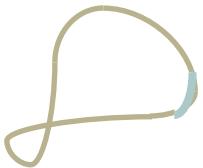


Extract  
DNA  
→

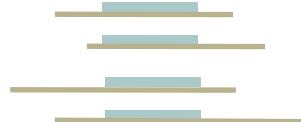


PCR  
→





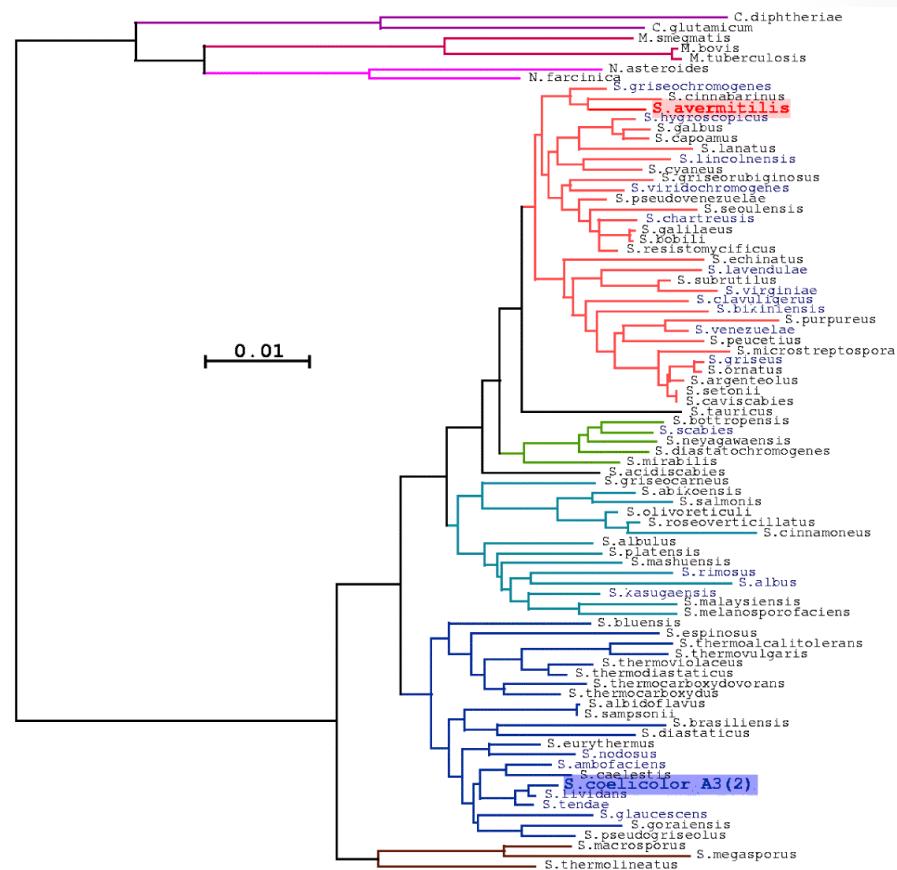
16S → Taxonomic composition



↑ Targeted  
metagenomes



PCR

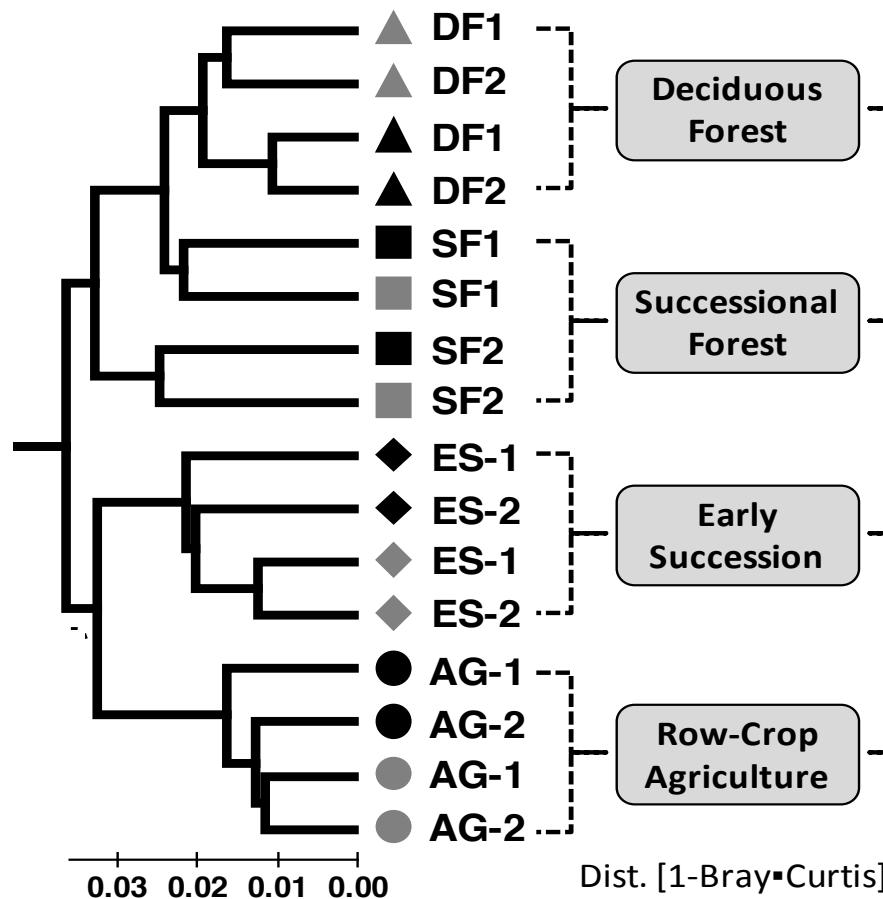


# Who's there and are there differences between treatments?

Null hypothesis – no difference in community composition between treatments

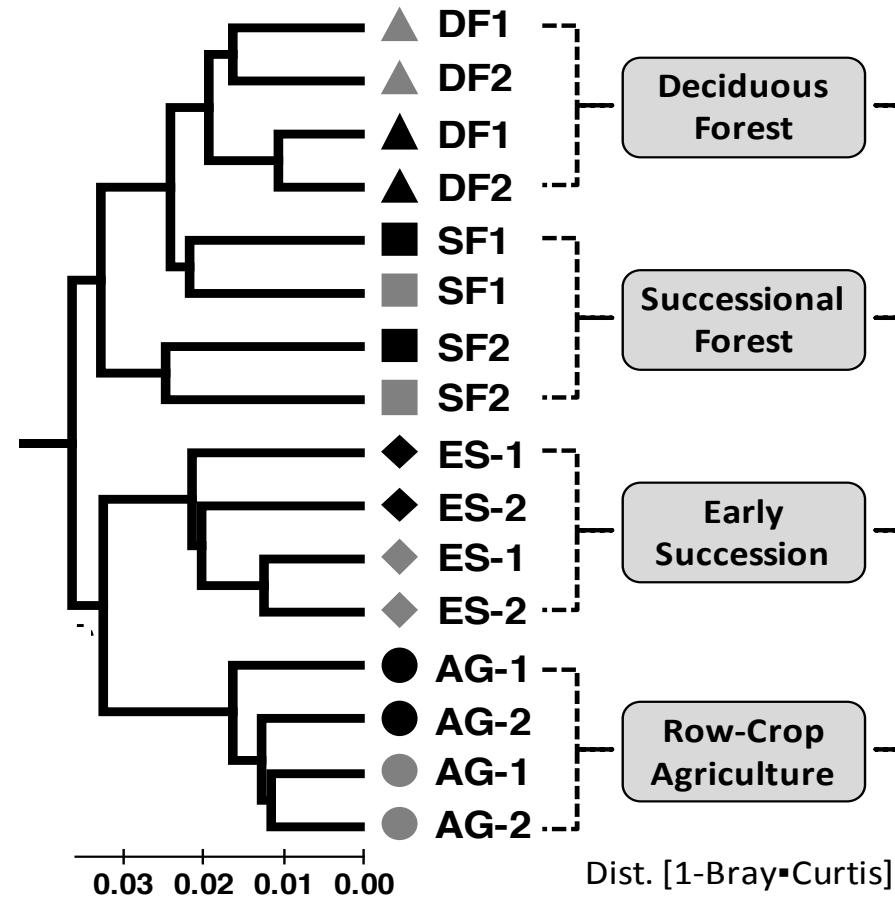
# Soil properties correlate with greenhouse gas fluxes

## Soil properties

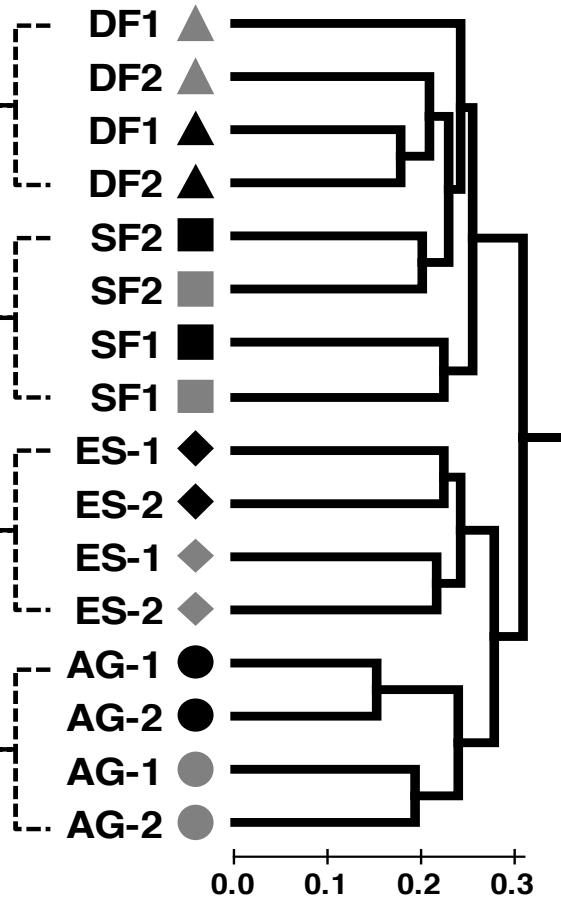


# Biogeochemistry and bacterial community change concomitantly

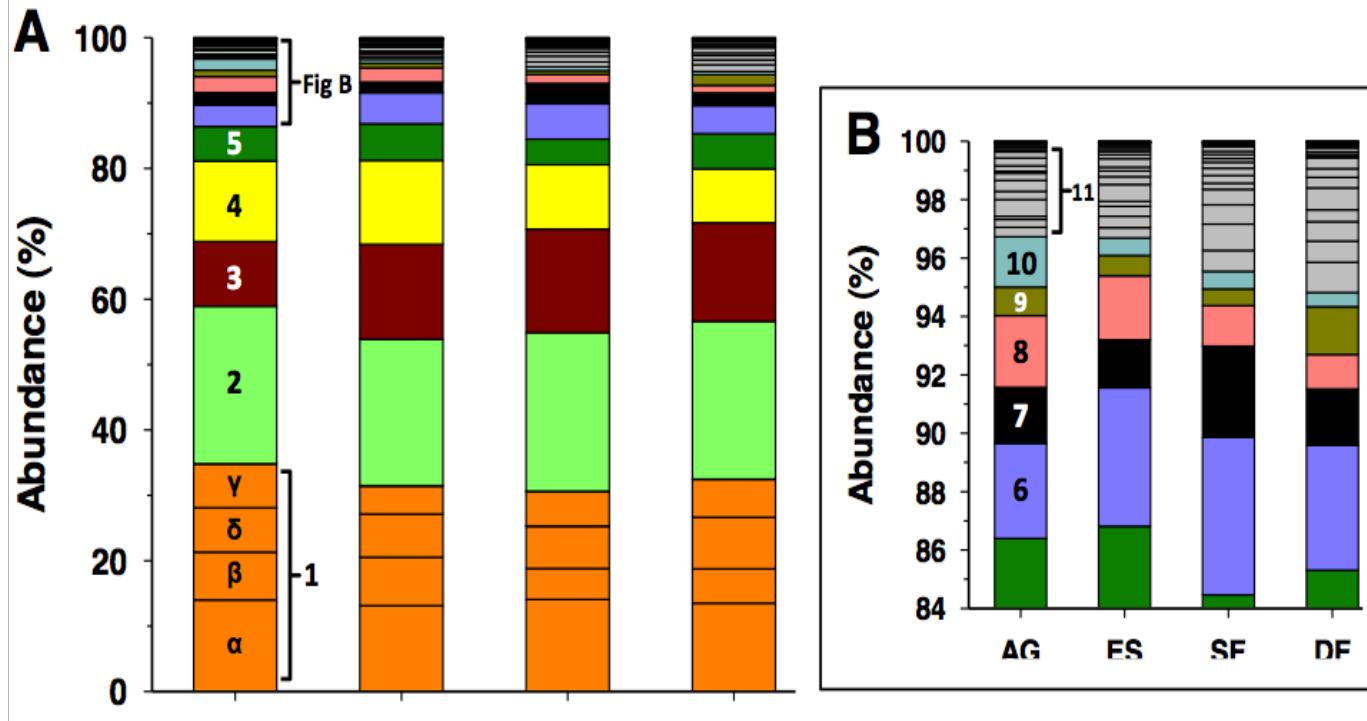
## Soil properties



## Microbial community

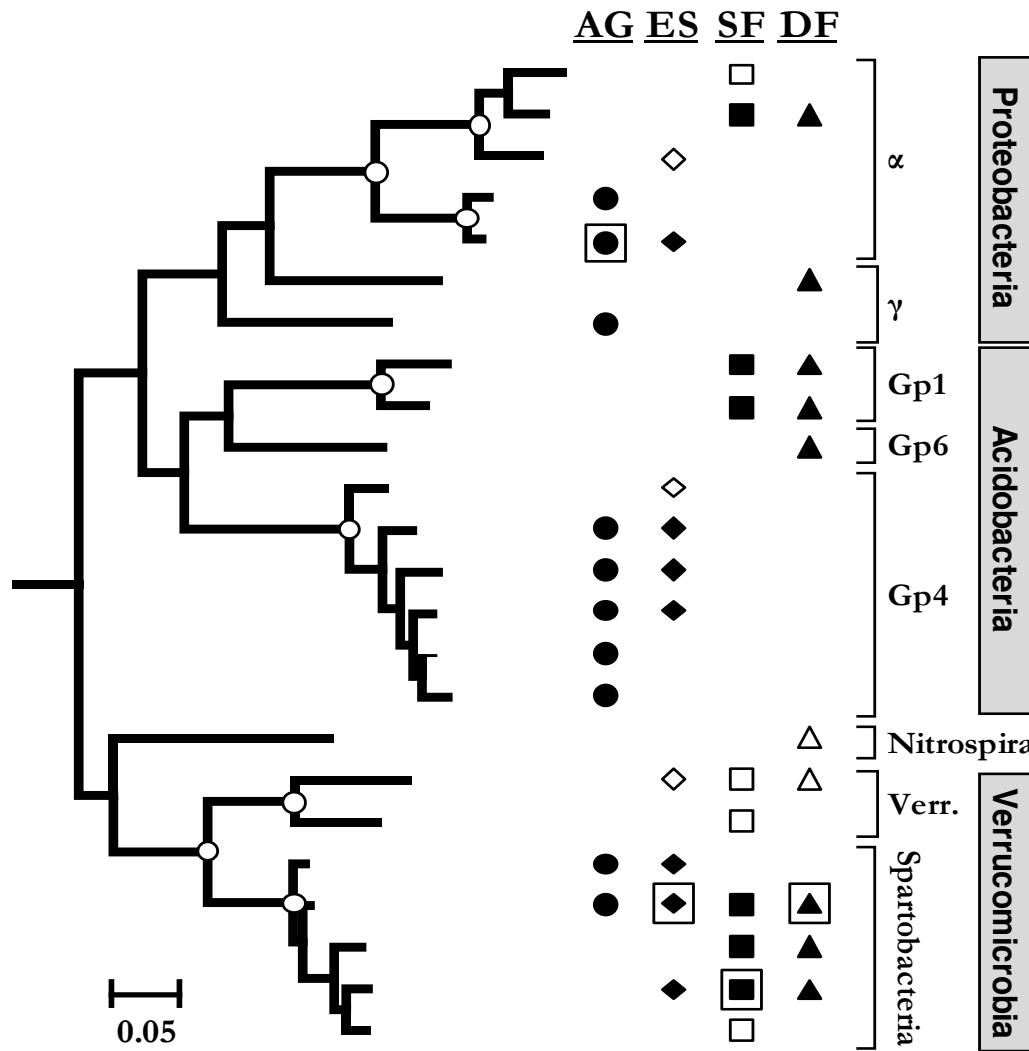


# What phyla are there?



- (1) Proteobacteria [class added], (2) Acidobacteria, (3) Verrucomicrobia,  
(4) Actinobacteria, (5) Bacteroidetes, (6) Planctomycetes, (7) Chloroflexi,  
(8) Gemmatimonadetes, (9) Nitrospirae, (10) Firmicutes, and  
(11) 30 additional phyla corresponding to less than 4% of the total distribution.

# Shifts within phyla differentiate communities



# Microbial community taxonomic composition varies between treatments

- Disproved null hypothesis – there is a difference in community composition between sites
- Communities change concomitantly with soil biogeochemistry
- Community composition differs in agriculture from forested sites

# Functional analysis of the community

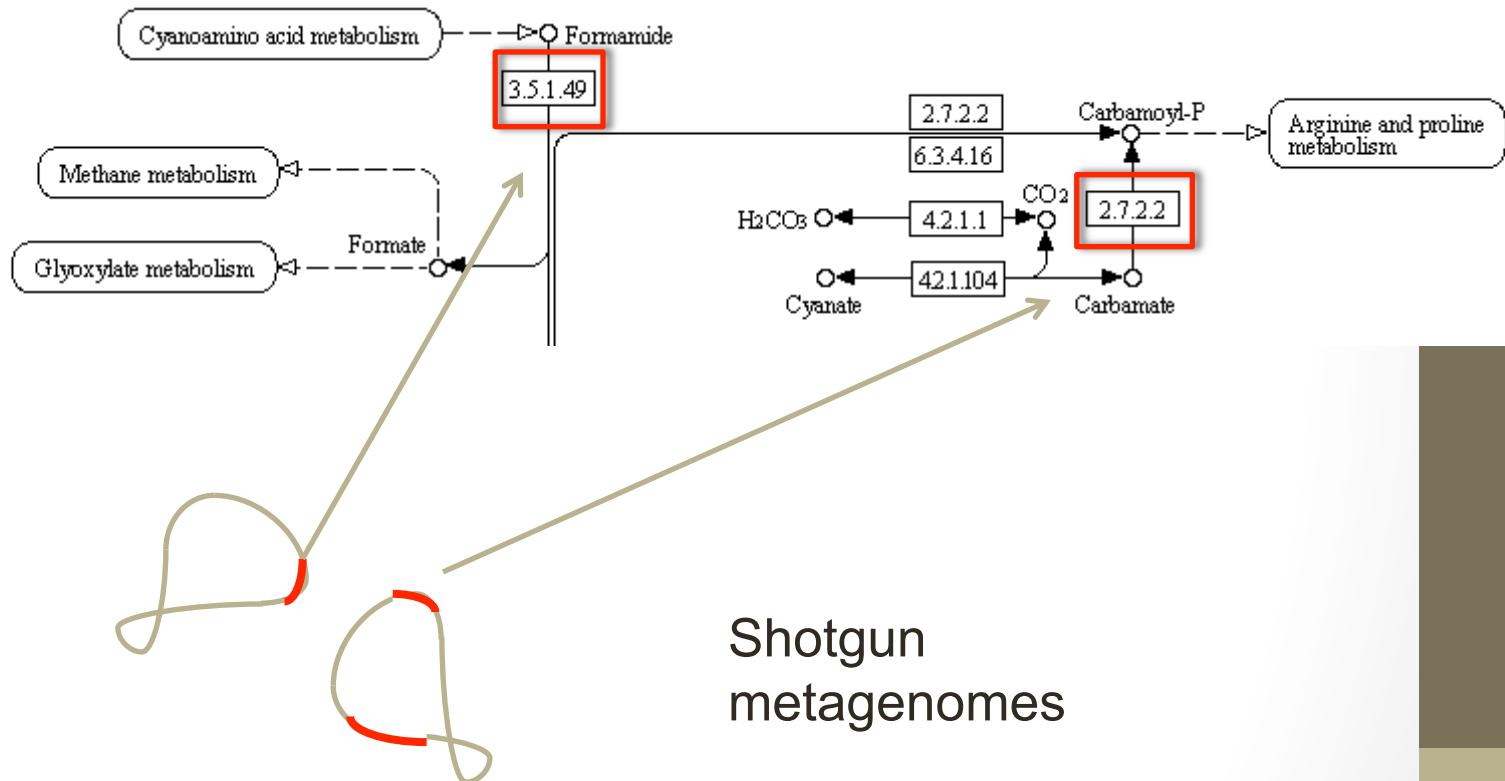
The important thing to understand is not just who's there, but what are they doing. What is their role in ecosystem functioning, particularly in greenhouse gas production or consumption?

## Challenges:

- Not many isolated or sequenced soil microbes
- Even closely related species can have different functional capacity
- Organisms not closely related can have similar functional capacity

# Functional potential of the microbial community

NITROGEN METABOLISM



Determining the composition and abundance of genes suggests the communities' functional potential

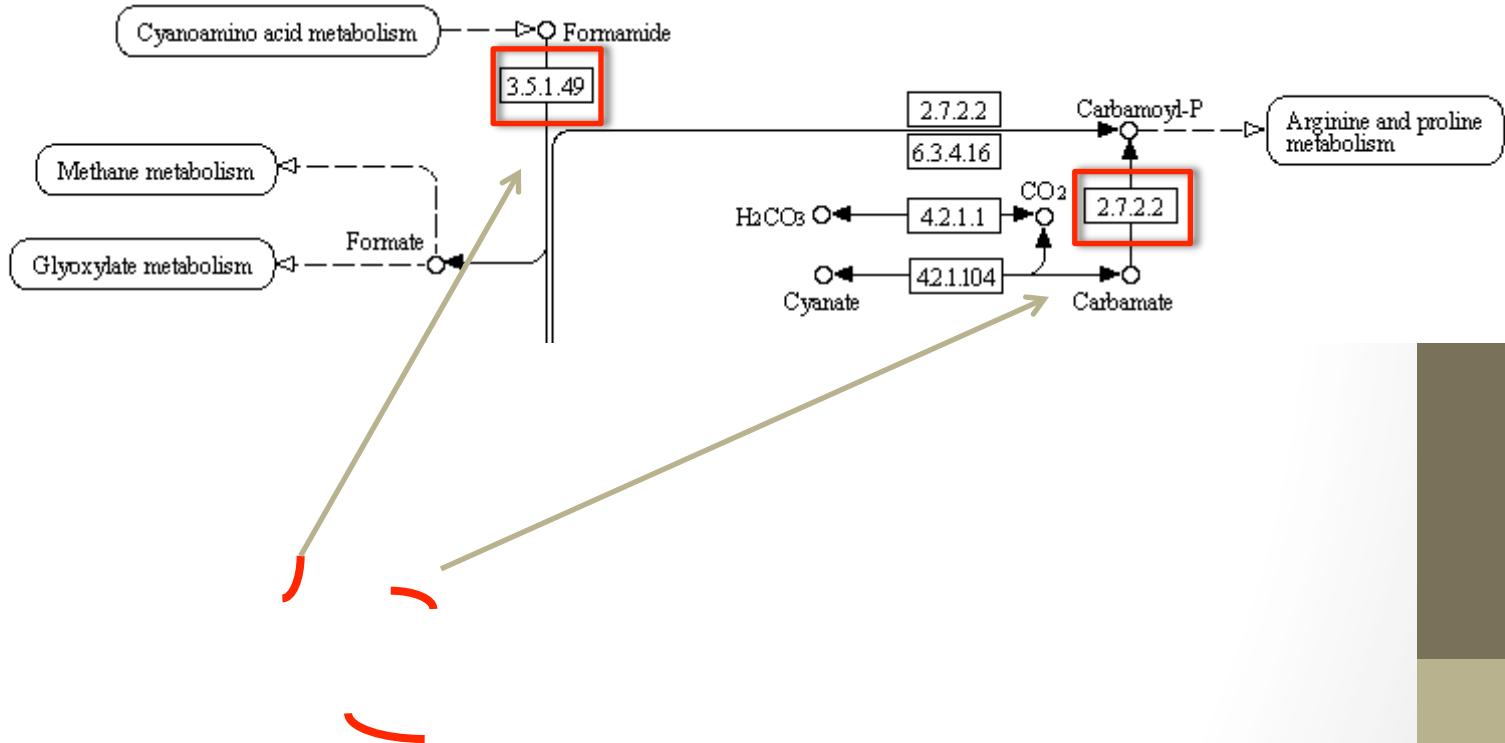
# Caveats with metagenomic sequencing

- Undersampling of communities
- Database limitations
- Sequencing active and dormant community
- Sequencing errors

# Why metagenomics is terrible and why we use it anyway

# Annotate shotgun reads

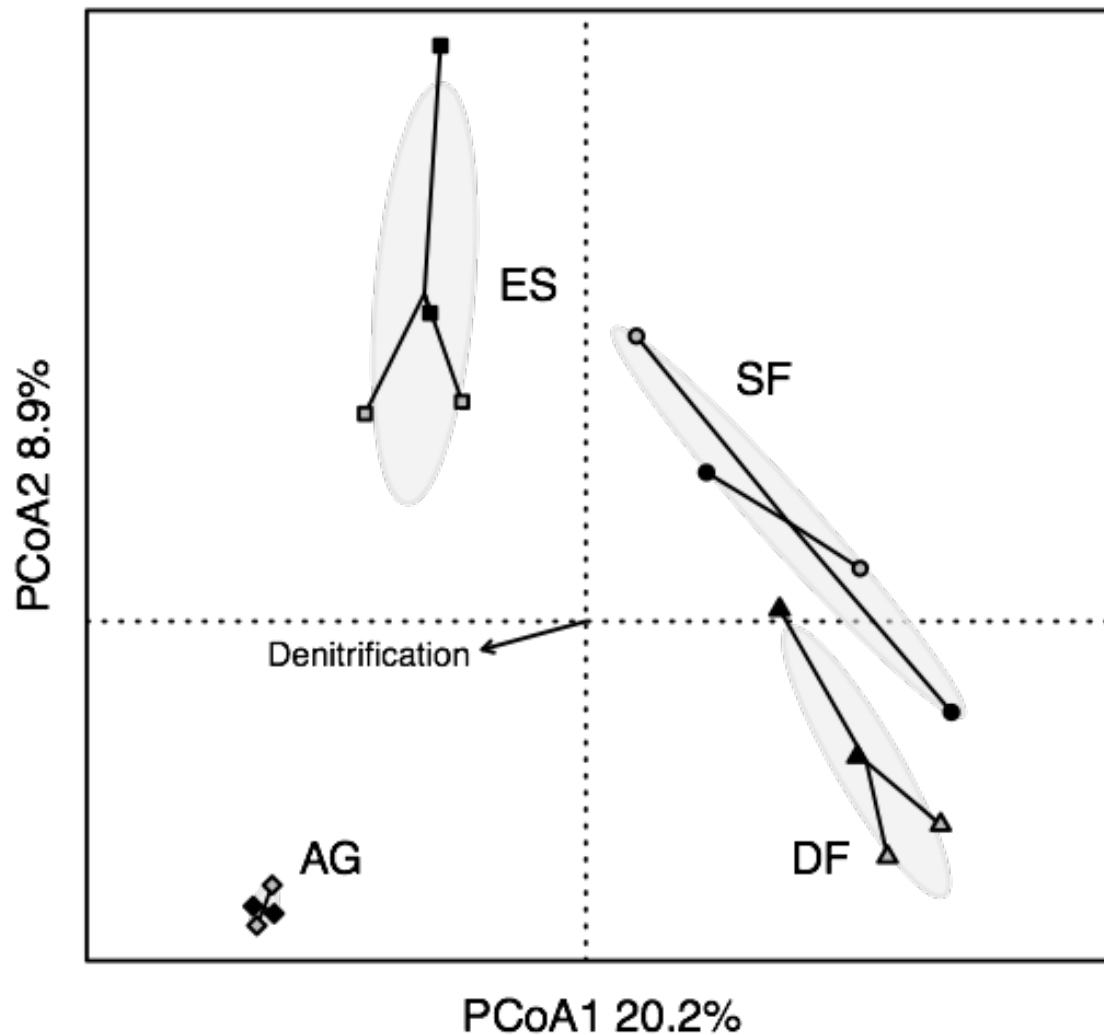
## NITROGEN METABOLISM



# Matrix of normalized gene abundance by treatment

| A   | B                           | C         | D         | E         | F         |
|---|-----------------------------|-----------|-----------|-----------|-----------|
| Function  | Subsystem                   | T1R1_2008 | T1R2_2008 | T1R1_2009 | T1R2_2009 |
| (R)-citramalate synthase (EC 2.3.1.182)   | Amino Acids and Derivatives | 84        | 103       | 53        |           |
| 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase (EC 1.13.11.54)              | Amino Acids and Derivatives | 9         | 4         | 8         |           |
| 1-pyrroline-4-hydroxy-2-carboxylate deaminase (EC 3.5.4.22)                       | Amino Acids and Derivatives | 21        | 17        | 17        |           |
| 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase (EC 2.3.1.89)    | Amino Acids and Derivatives | 28        | 23        | 21        |           |
| 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase (EC 2.3.1.117) | Amino Acids and Derivatives | 71        | 72        | 42        |           |
| 2,3-diketo-5-methylthiopentyl-1-phosphate enolase                                 | Amino Acids and Derivatives | 9         | 10        | 6         |           |
| 2,3-diketo-5-methylthiopentyl-1-phosphate enolase-phosphatase (EC 3.1.3.77)       | Amino Acids and Derivatives | 9         | 10        | 5         |           |
| 2,4-diaminopentanoate dehydrogenase (EC 1.4.1.12)                                 | Amino Acids and Derivatives | 1         | 0         | 0         |           |
| 2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase (EC 4.1.2.-)                      | Amino Acids and Derivatives | 62        | 42        | 38        |           |
| 2-Amino-2-deoxy-isochorismate synthase (EC 4.1.3.-)                               | Amino Acids and Derivatives | 2         | 8         | 0         |           |
| 2-Oxobutyrate dehydrogenase E1 (EC:1.2.4.1)                                       | Amino Acids and Derivatives | 0         | 1         | 2         |           |
| 2-Oxobutyrate oxidase, putative   | Amino Acids and Derivatives | 11        | 6         | 5         |           |
| 2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate synthase (EC 2.5.1.-)                | Amino Acids and Derivatives | 0         | 1         | 0         |           |
| 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase (EC 4.1.1.45)              | Amino Acids and Derivatives | 52        | 52        | 43        |           |
| 2-amino-3-ketobutyrate coenzyme A ligase (EC 2.3.1.29)                            | Amino Acids and Derivatives | 107       | 97        | 53        |           |
| 2-amino-4-ketopentanoate thiolase, beta subunit                                   | Amino Acids and Derivatives | 1         | 0         | 0         |           |
| 2-aminomuconate deaminase (EC 3.5.99.5)   | Amino Acids and Derivatives | 2         | 0         | 0         |           |
| 2-aminomuconate semialdehyde dehydrogenase (EC 1.2.1.32)                          | Amino Acids and Derivatives | 6         | 3         | 3         |           |
| 2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase                     | Amino Acids and Derivatives | 3         | 3         | 1         |           |
| 2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase related protein     | Amino Acids and Derivatives | 3         | 2         | 1         |           |

# Functional potential changes with land management



( 26 )

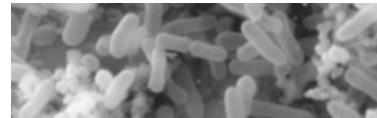
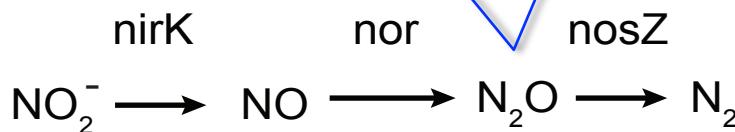
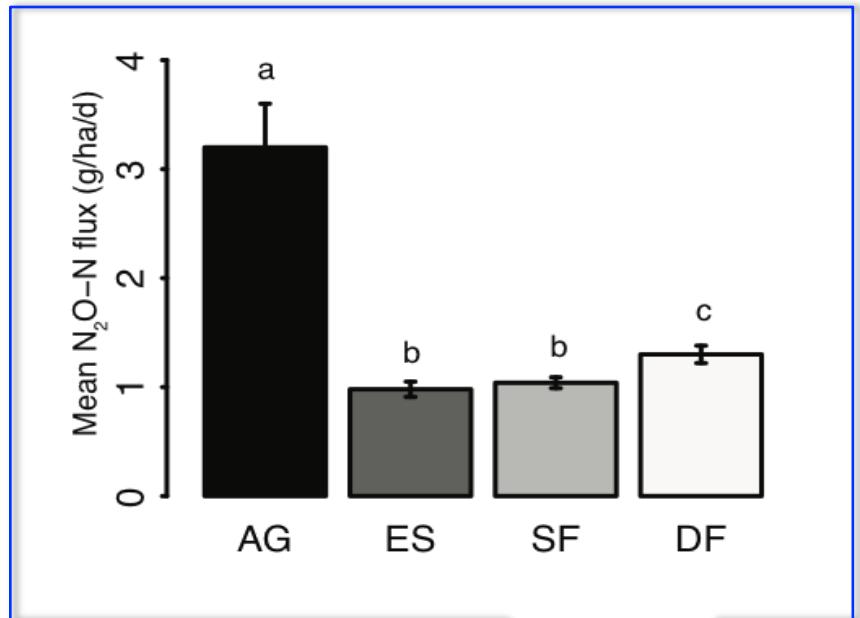
Analysis of 7058 genes annotated by MG-RAST against the SEED database

# Functional potential of communities differs with treatment

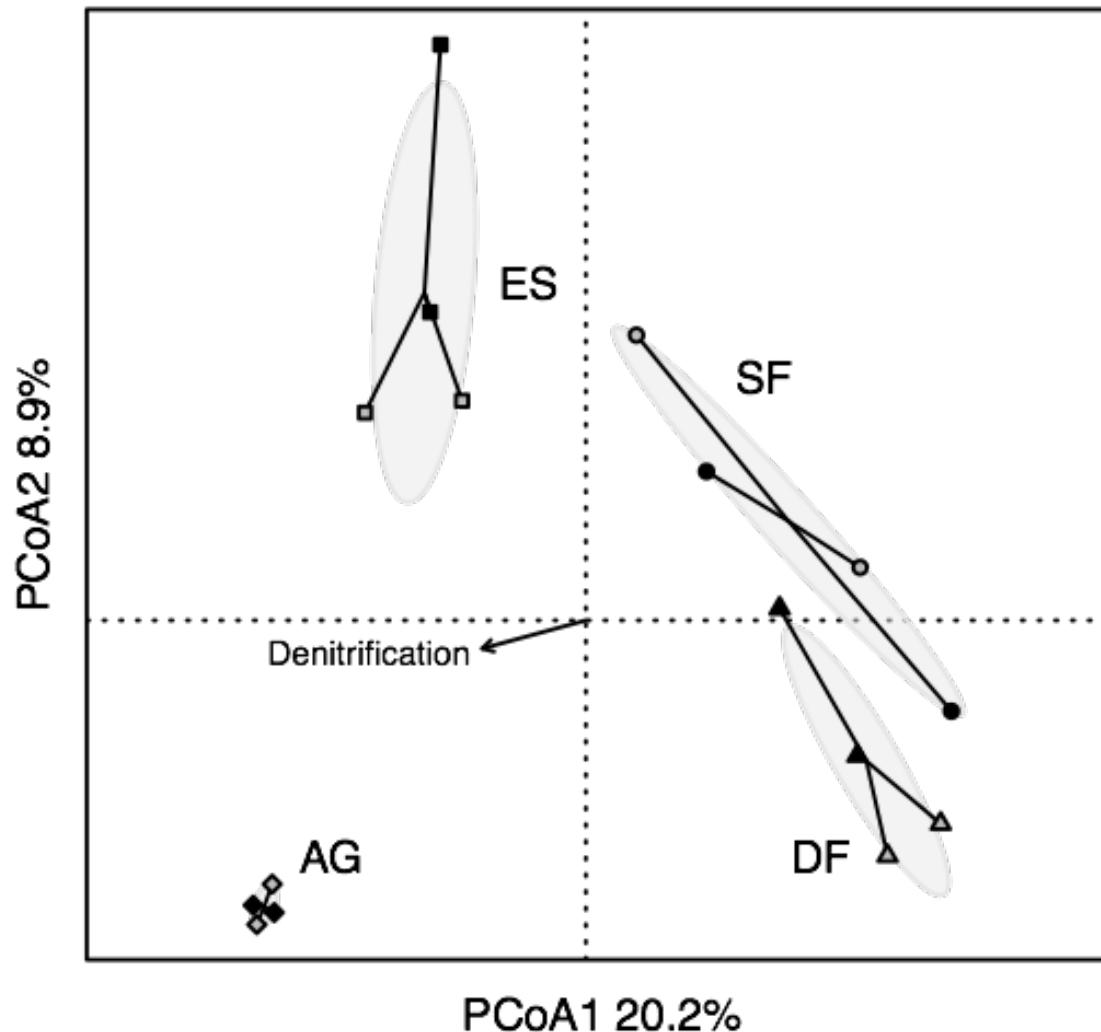
- Shotgun metagenomics can be used to look at the functional potential of a sample. It reflects only what's present in the soil, not what metabolism is occurring.
- There are differences in the functional potential of the communities that parallel that of taxonomic composition and soil biogeochemistry

What is the relationship between denitrifying bacteria and N<sub>2</sub>O in agricultural soils?

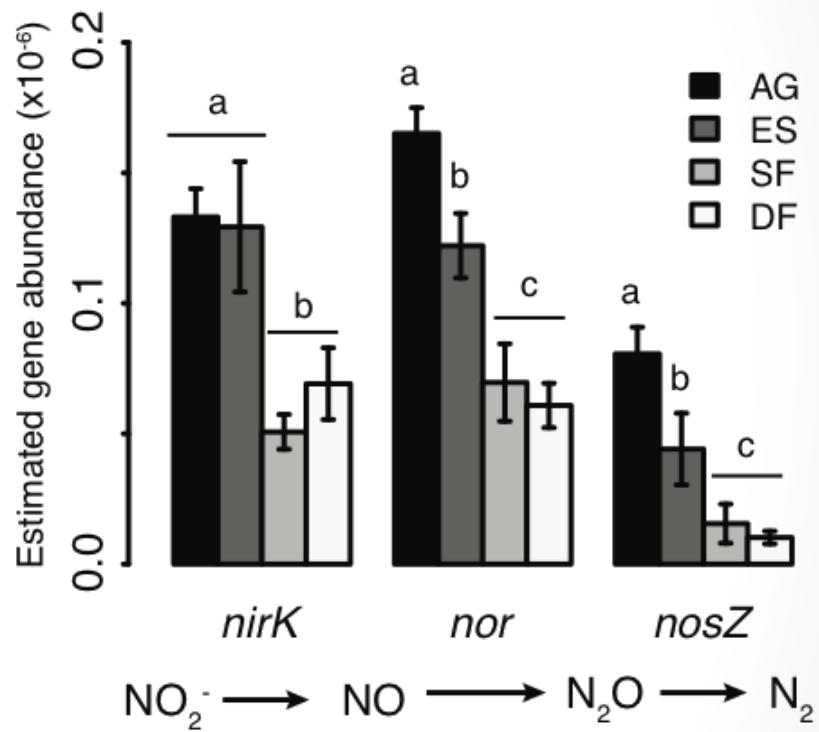
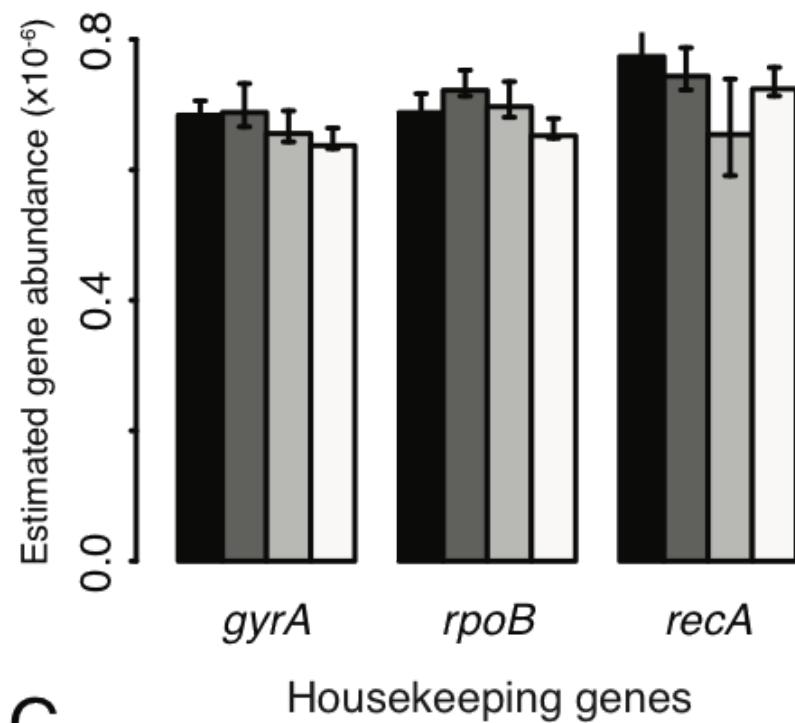
# Denitrifying microbes



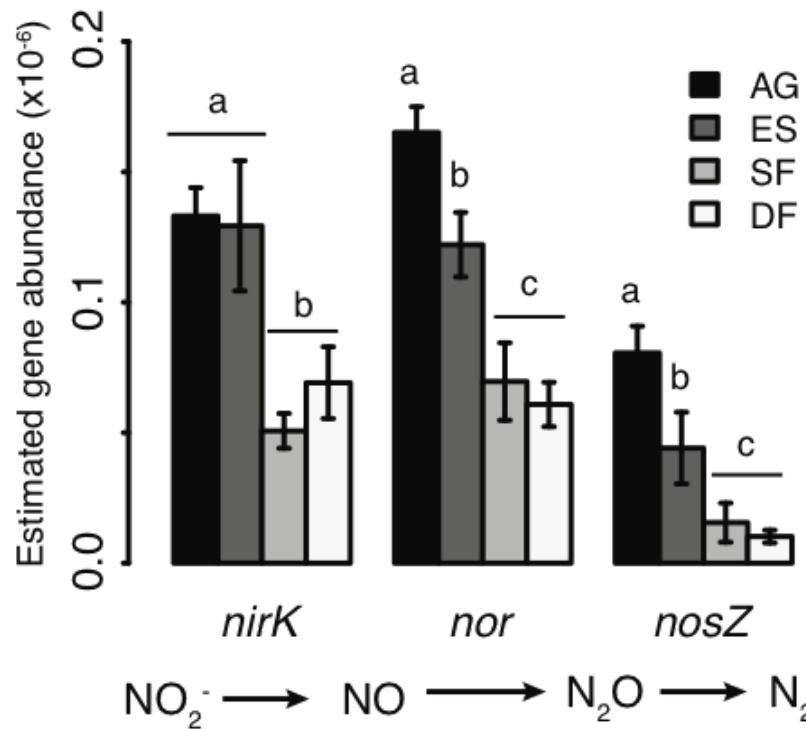
# Denitrification contributes to the differentiation of communities



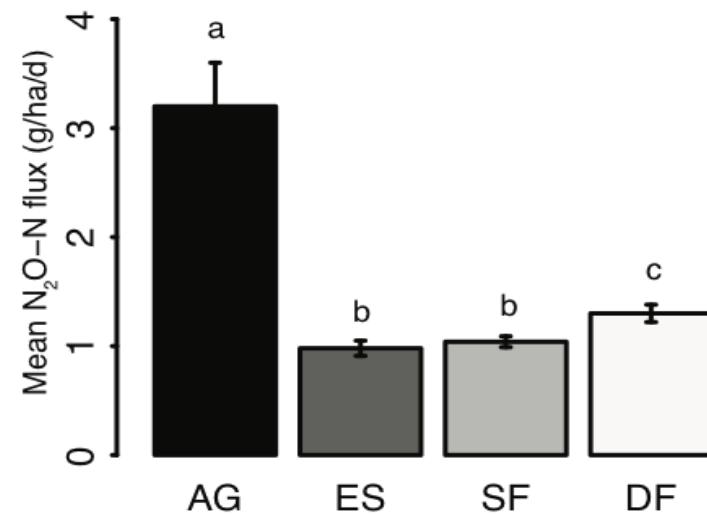
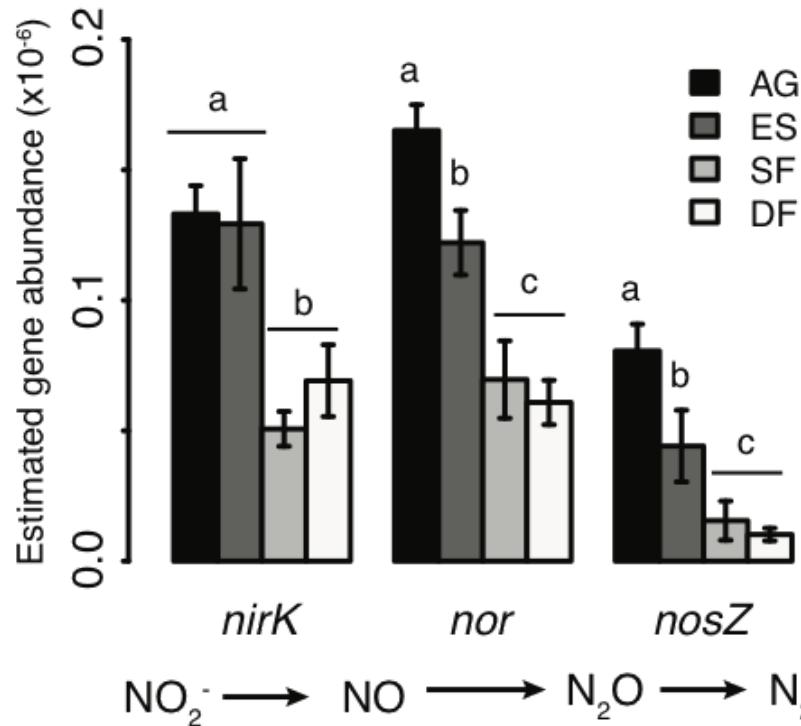
# More denitrification potential in Ag soils



# Not only abundance contributes to N<sub>2</sub>O flux



# Not only abundance contributes to N<sub>2</sub>O flux

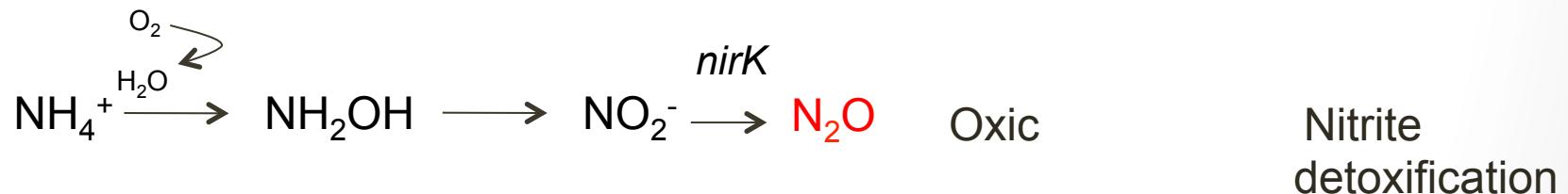


# Types of denitrification

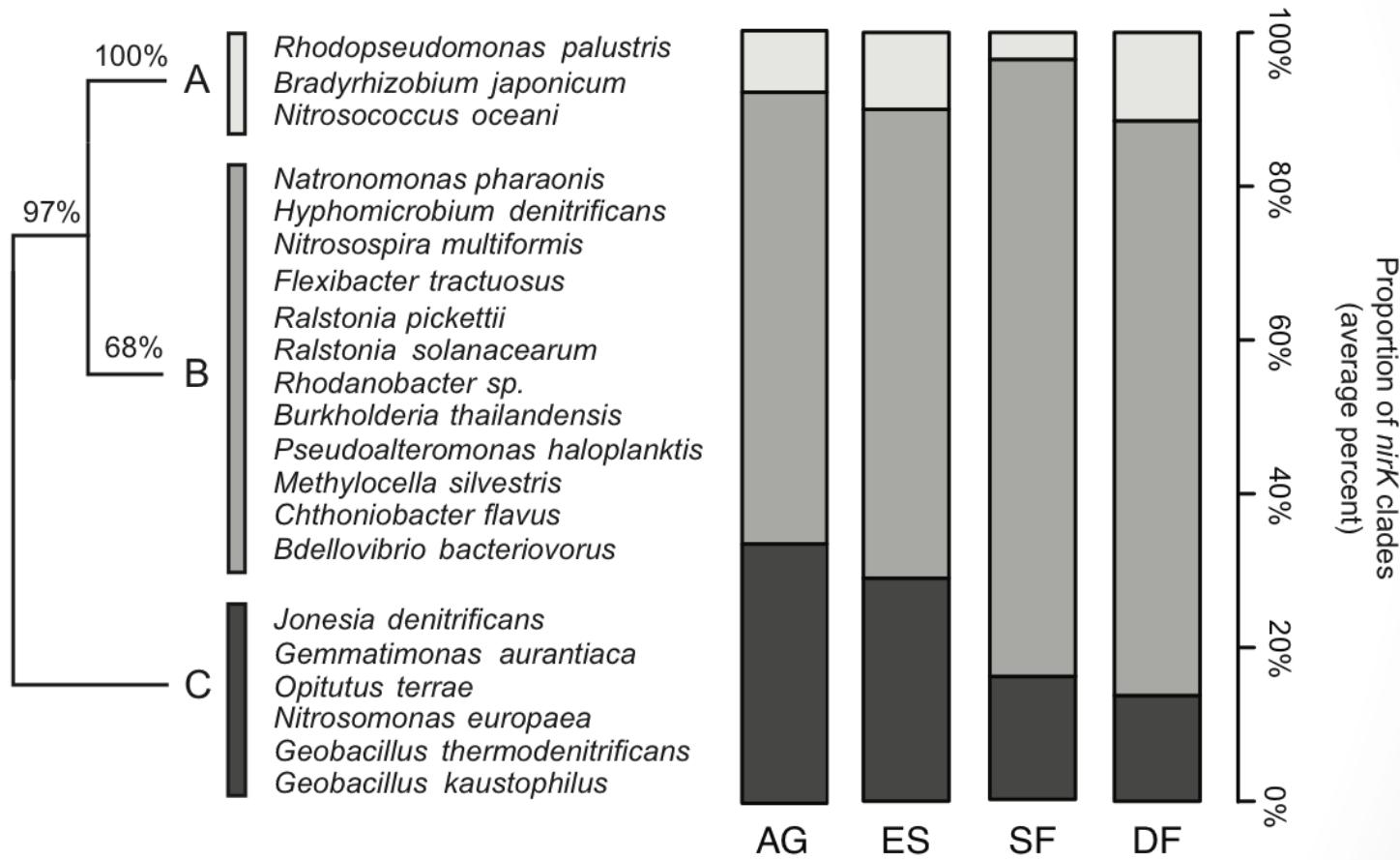
## Heterotrophic denitrification



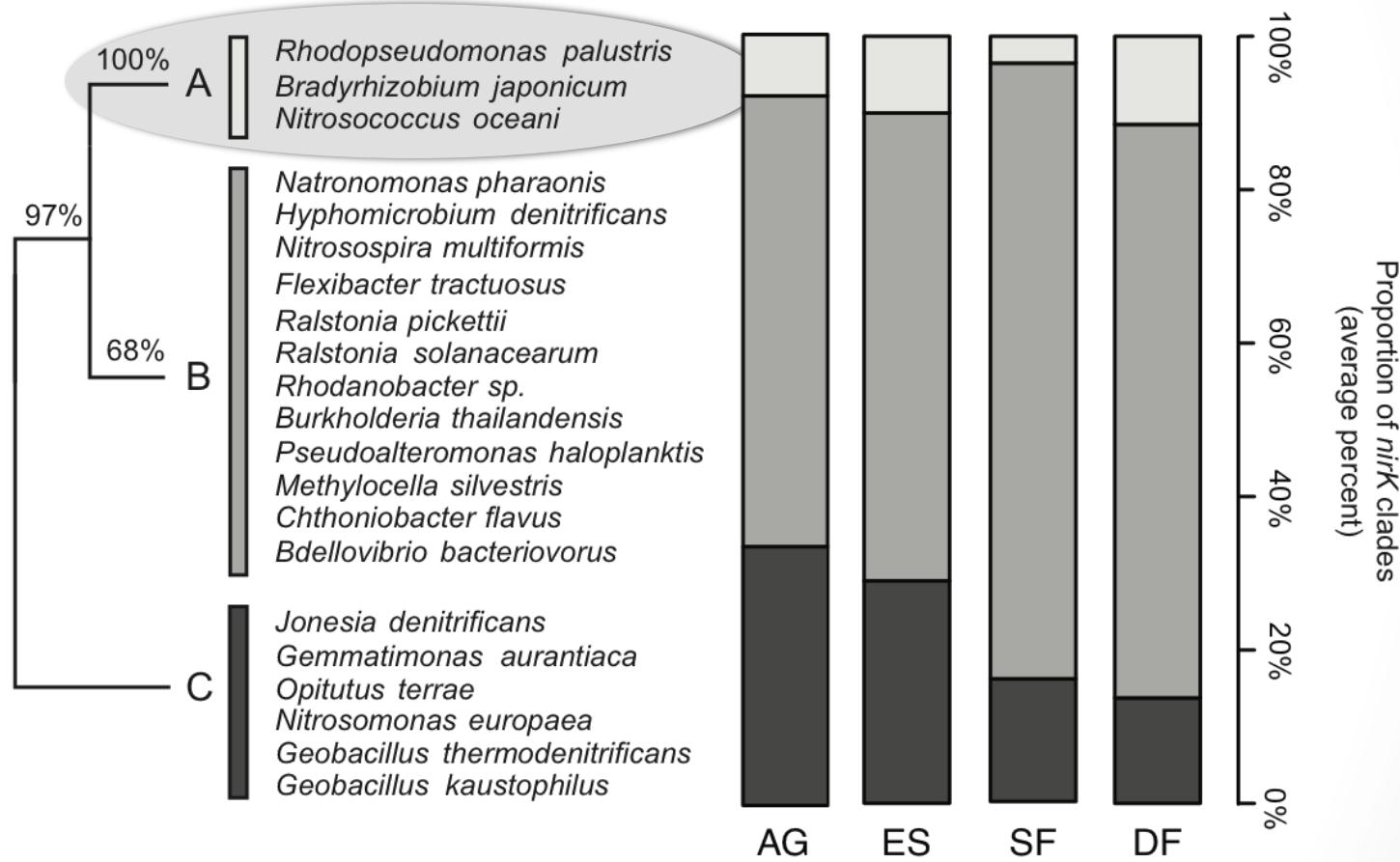
## Autotrophic denitrification (AOBs)



# Variability in denitrifier composition



# High denitrifier diversity

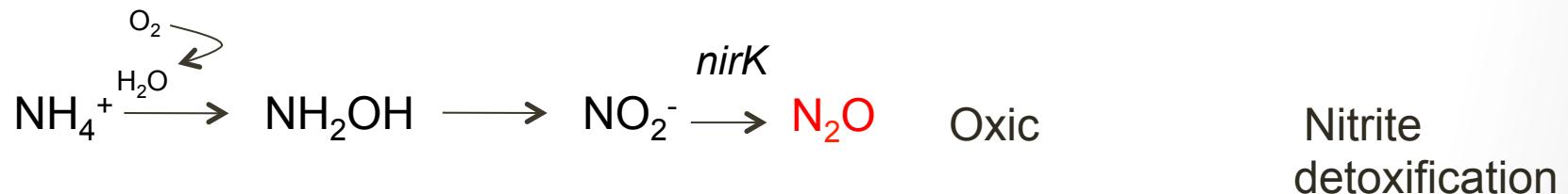


# Types of denitrification

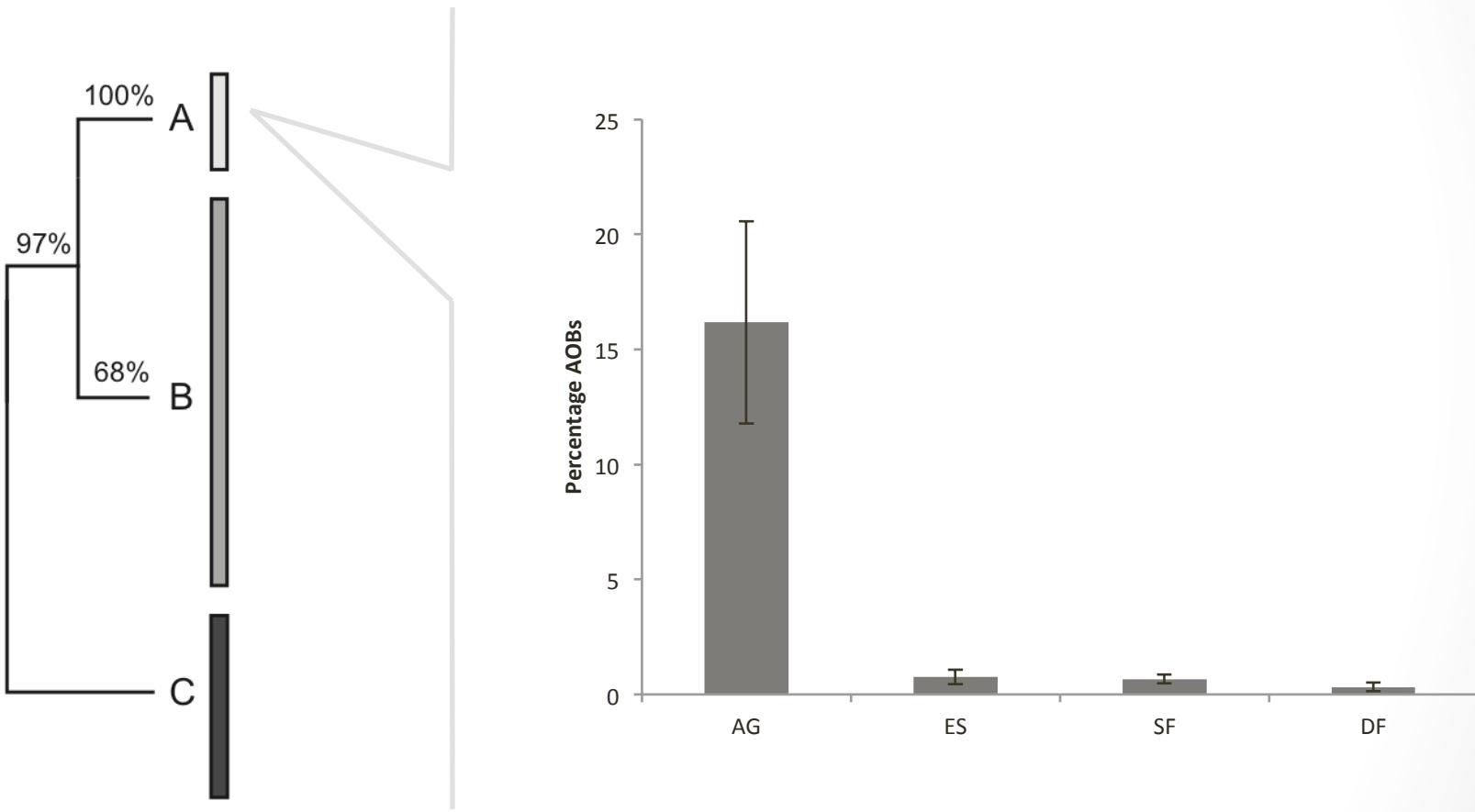
## Heterotrophic denitrification



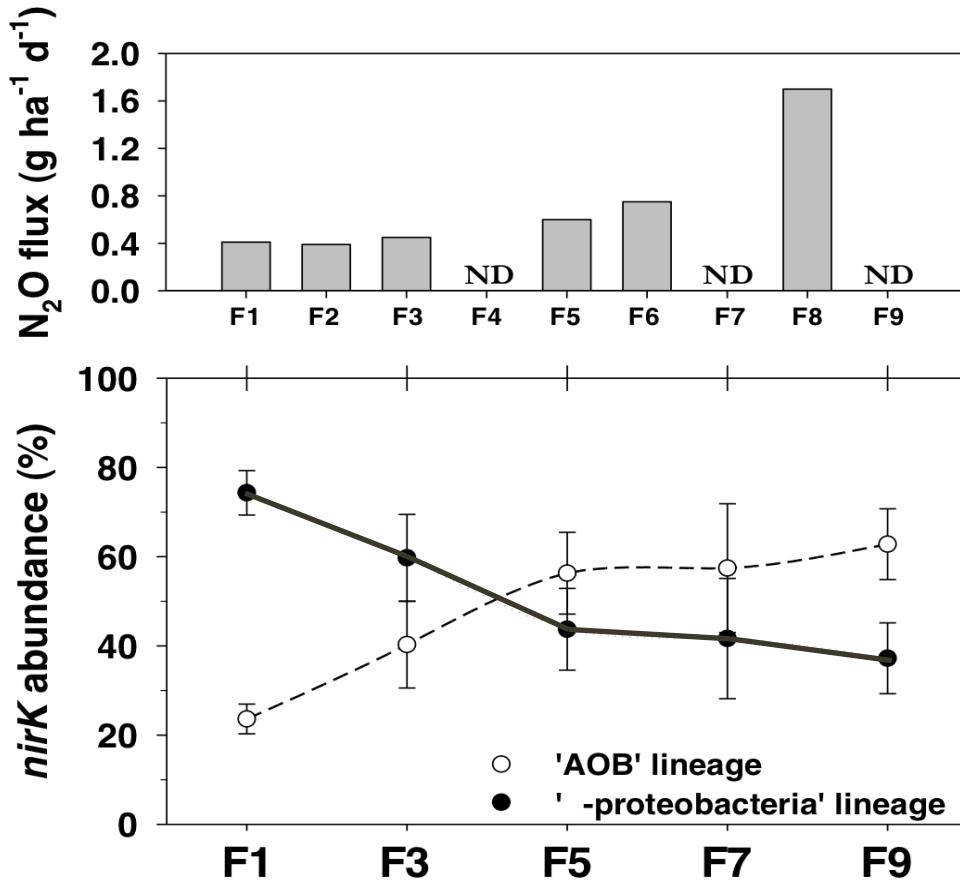
## Autotrophic denitrification (AOBs)



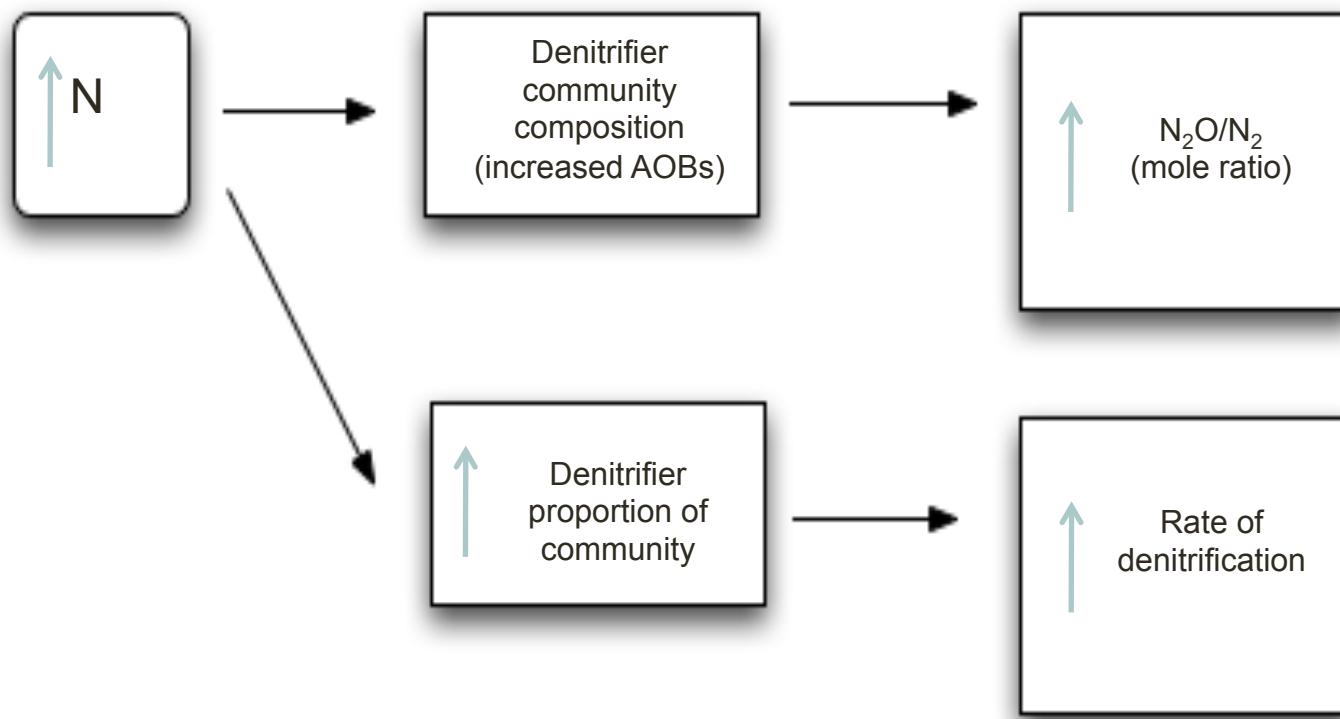
# Proportion of Ammonium Oxidizing Bacteria (AOB) increases significantly in AG



# AOB proportion correlates with fertilization level and N<sub>2</sub>O production



# Microbial communities in Ag sites with higher nitrogen availability are also poised for $\text{N}_2\text{O}$ production



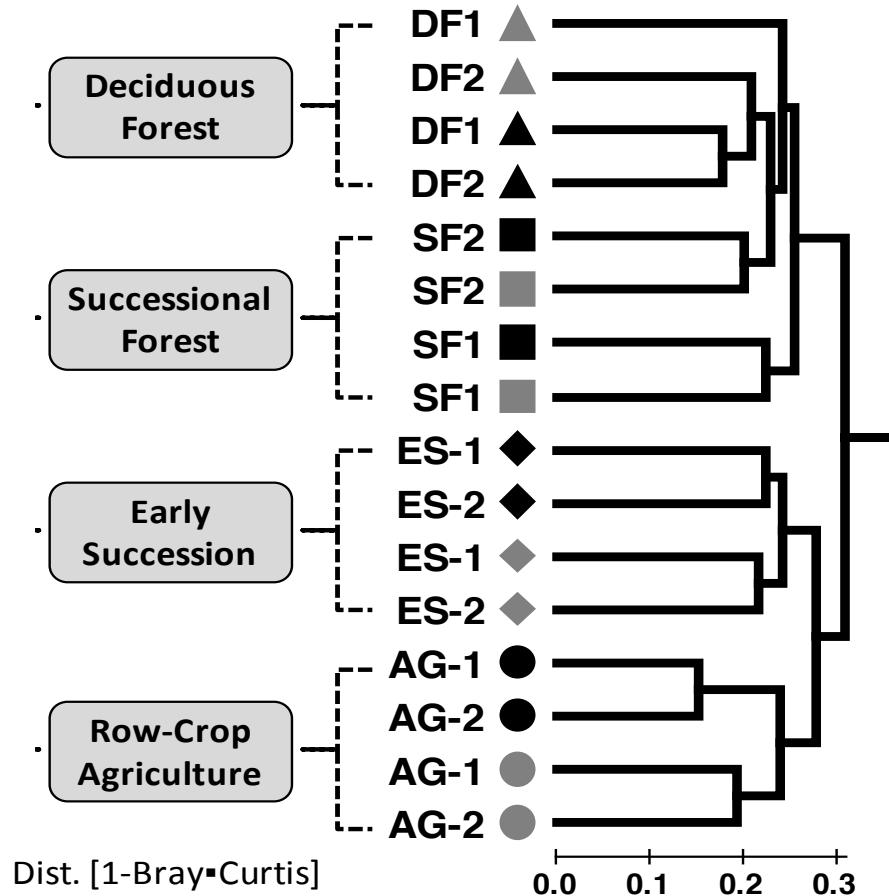
# What is the relationship between denitrifying bacteria and N<sub>2</sub>O in agricultural soils ?

- Denitrifier abundance and composition changes with agricultural management. Communities more diverse than previously thought and poised for denitrification.
- May be potential for microbial mediation

How long does it take for a community  
to recover from agriculture? Do all  
groups recover in the same way?

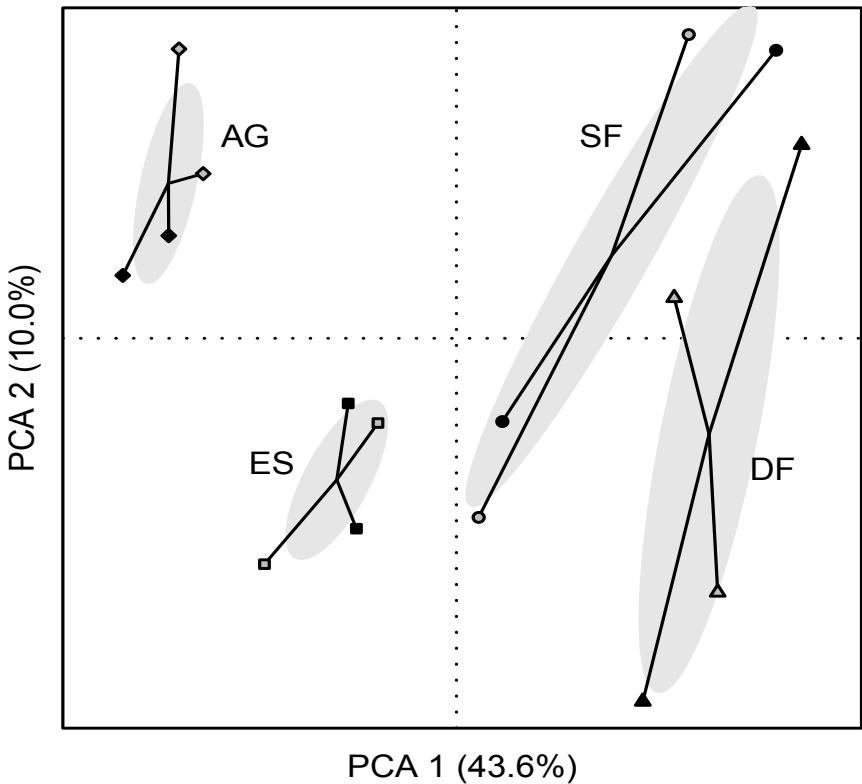
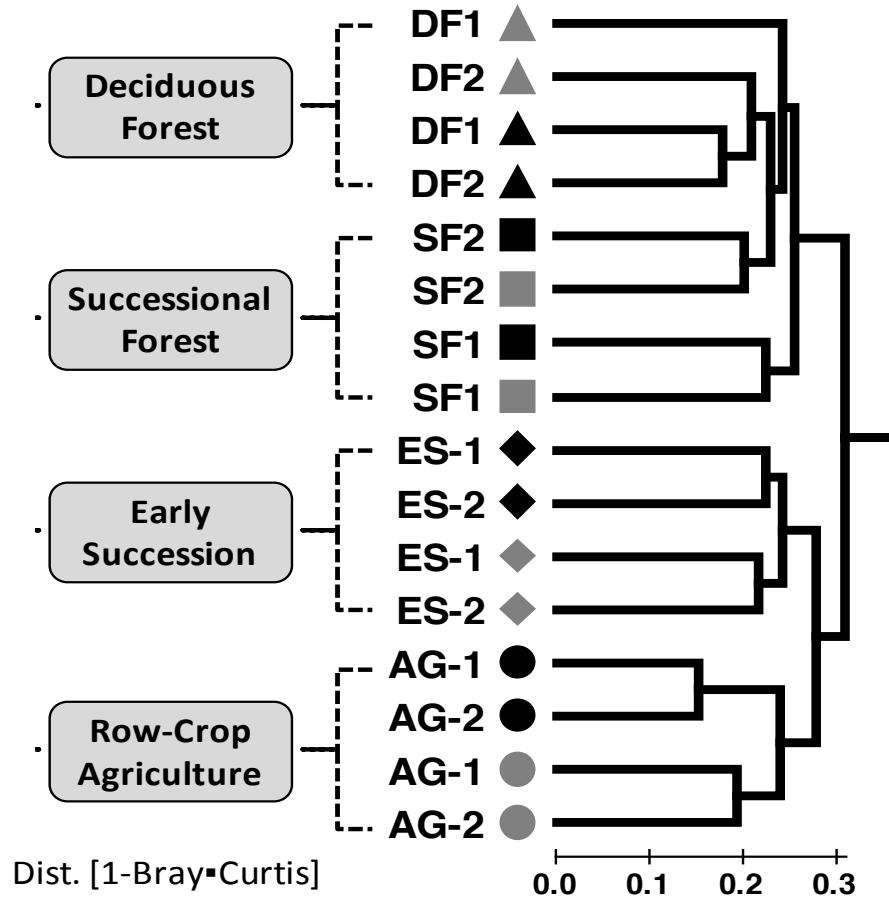
# More than 20 year recovery for bacterial community

**Microbial community**

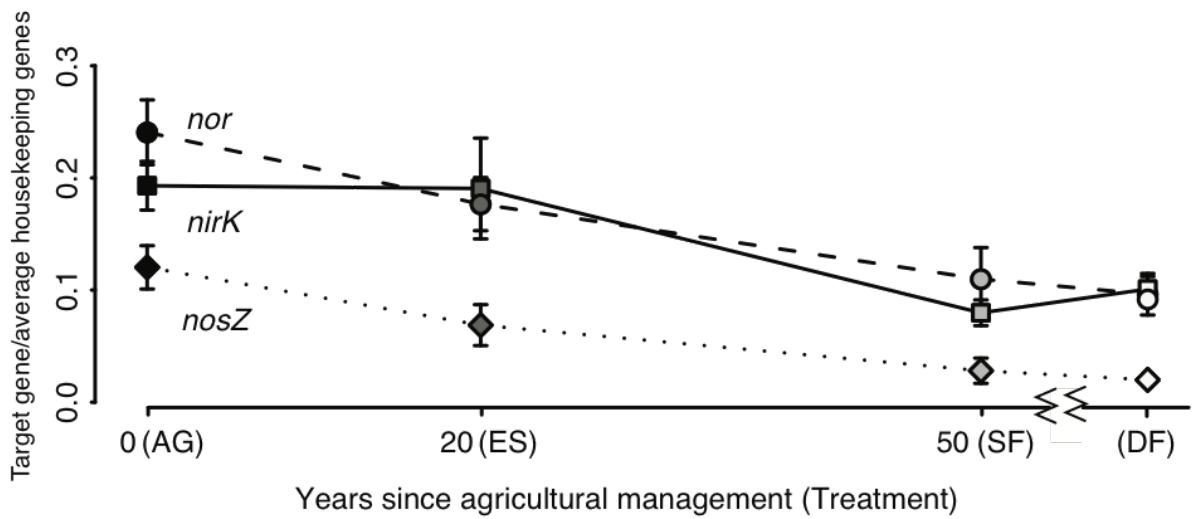


# More than 20 year recovery for bacterial community

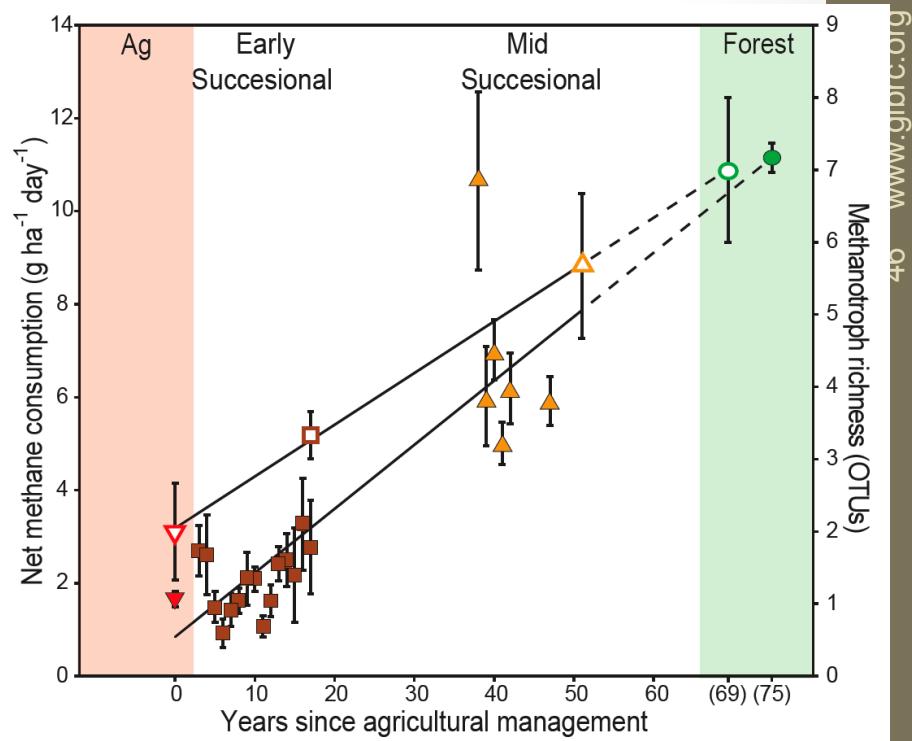
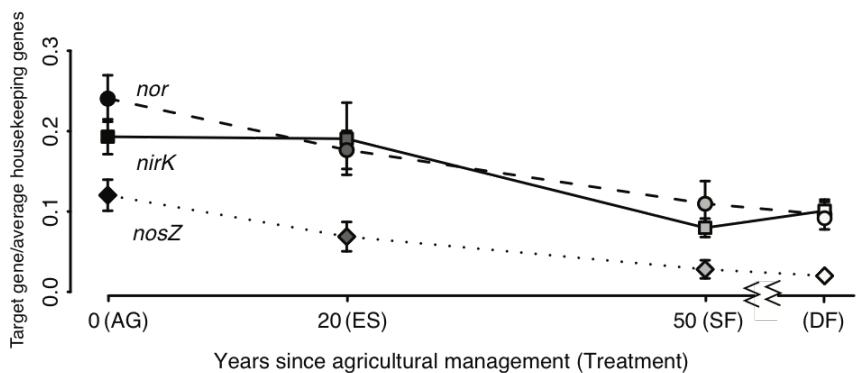
## Microbial community



# More than 20 year recovery for denitrifiers



# Methanotrophs take more than 40 years to recover



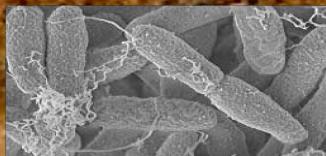


✖ Soil team  
Vicente Gomez-Alvarez  
Uri Levine  
Keara Towery  
Bernard Schroeter  
John Dover  
Zarraz Lee  
Brendan O'Neill  
Ben Roller  
Tom Schmidt

✖ Funding  
✖ DOE GLBRC  
✖ NSF  
✖ NSF Postdoctoral Fellowship

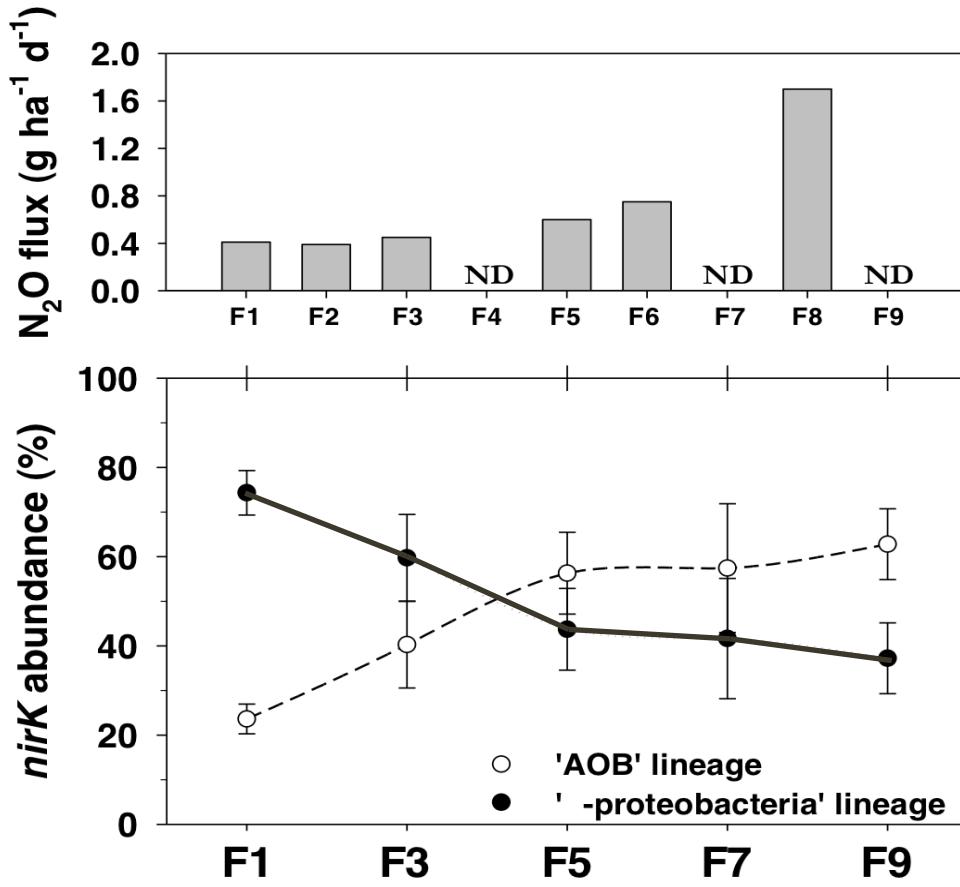


$\text{N}_2\text{O}$     $\text{CH}_4$

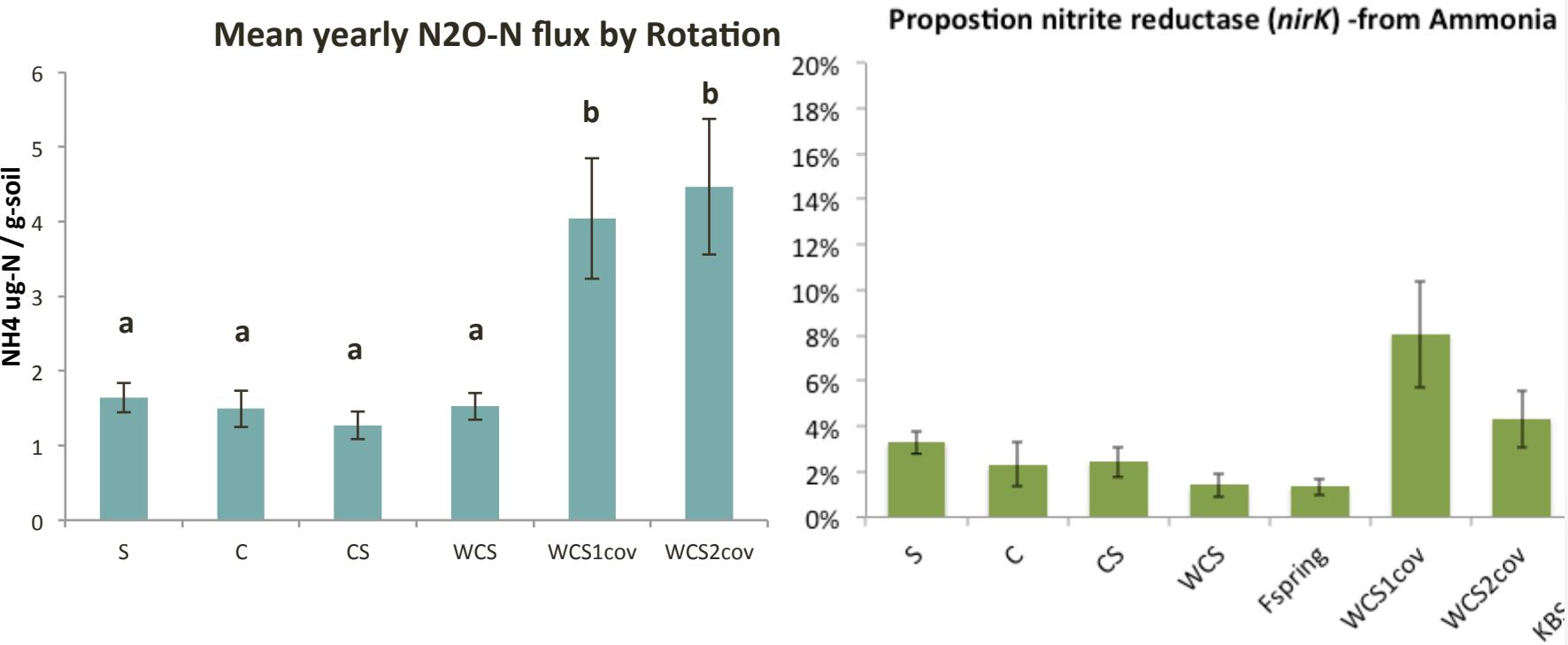


# More on denitrifiers

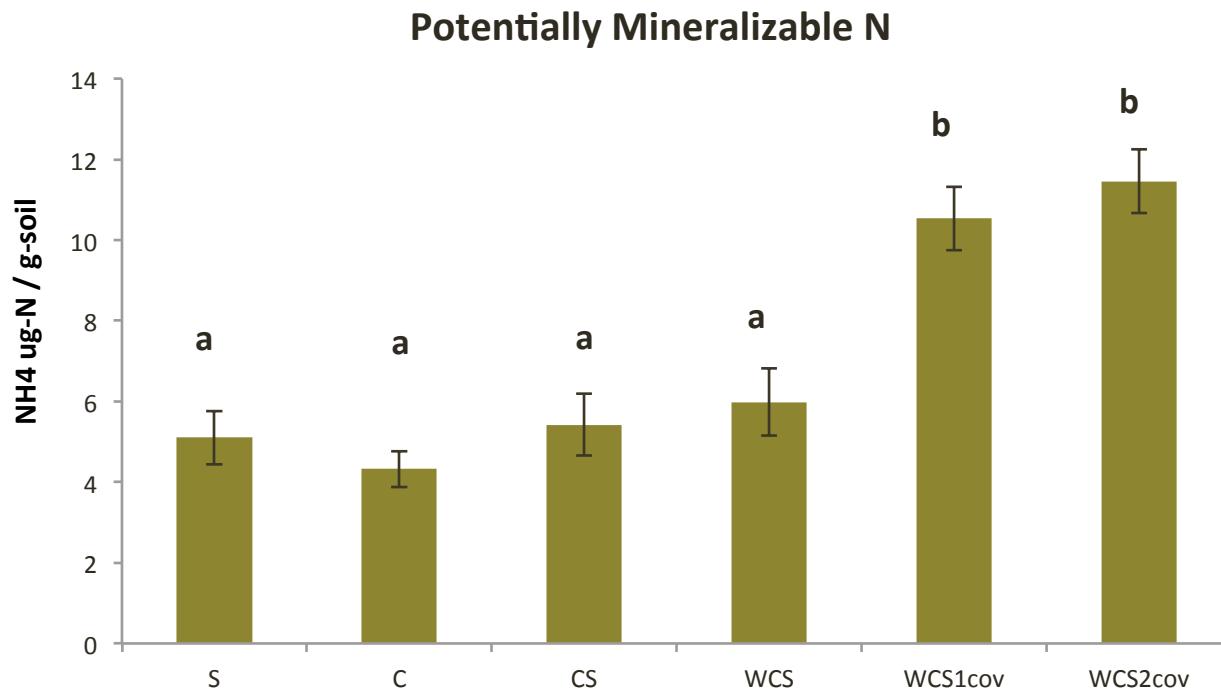
# AOB proportion correlates with fertilization level and N<sub>2</sub>O production



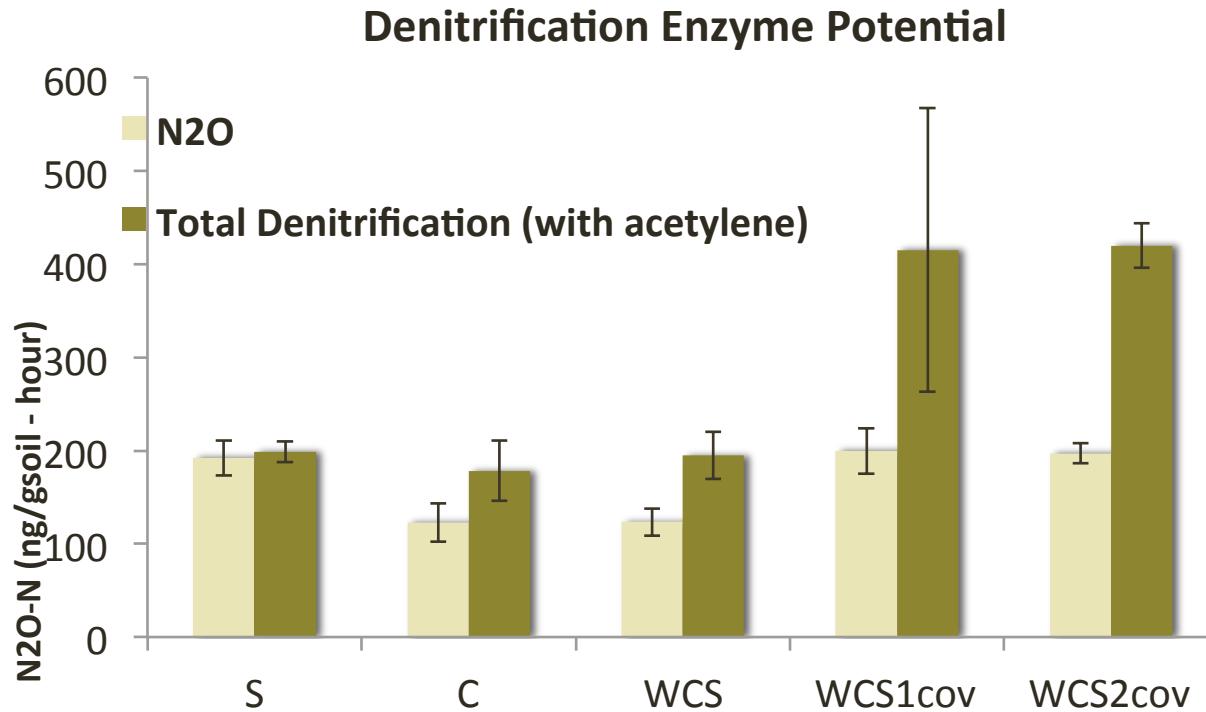
# AOB proportion correlates with cover crops and N<sub>2</sub>O production



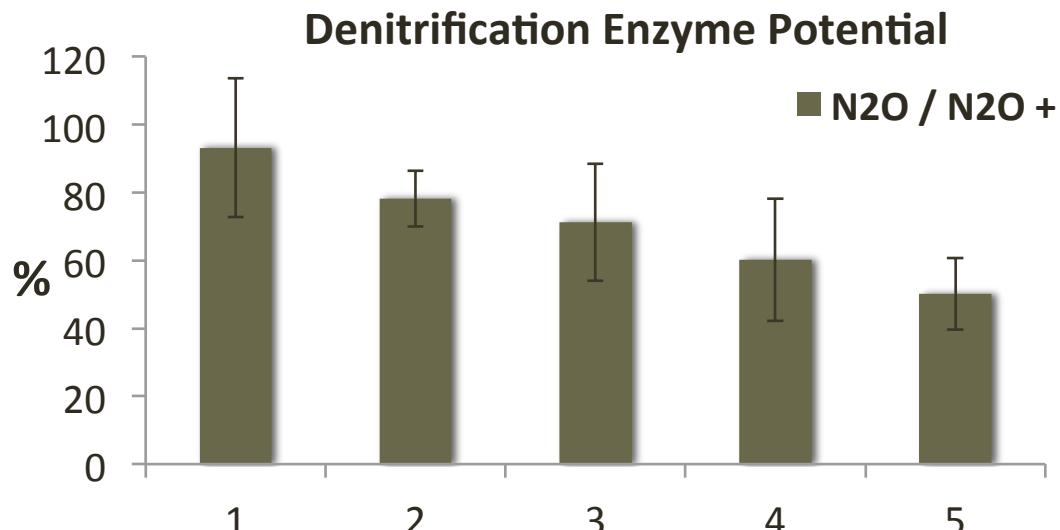
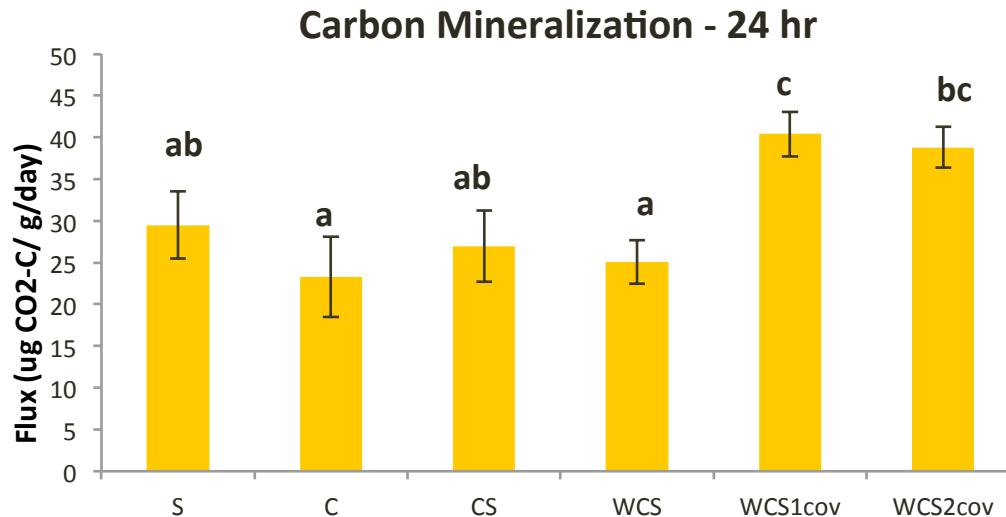
# Suggests source of nitrogen is not important for AOBs or $\text{N}_2\text{O}$ production



# Heterotrophic denitrification (no AOBs)

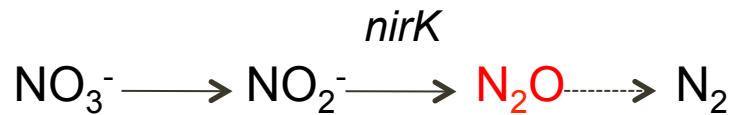


# Carbon availability also important



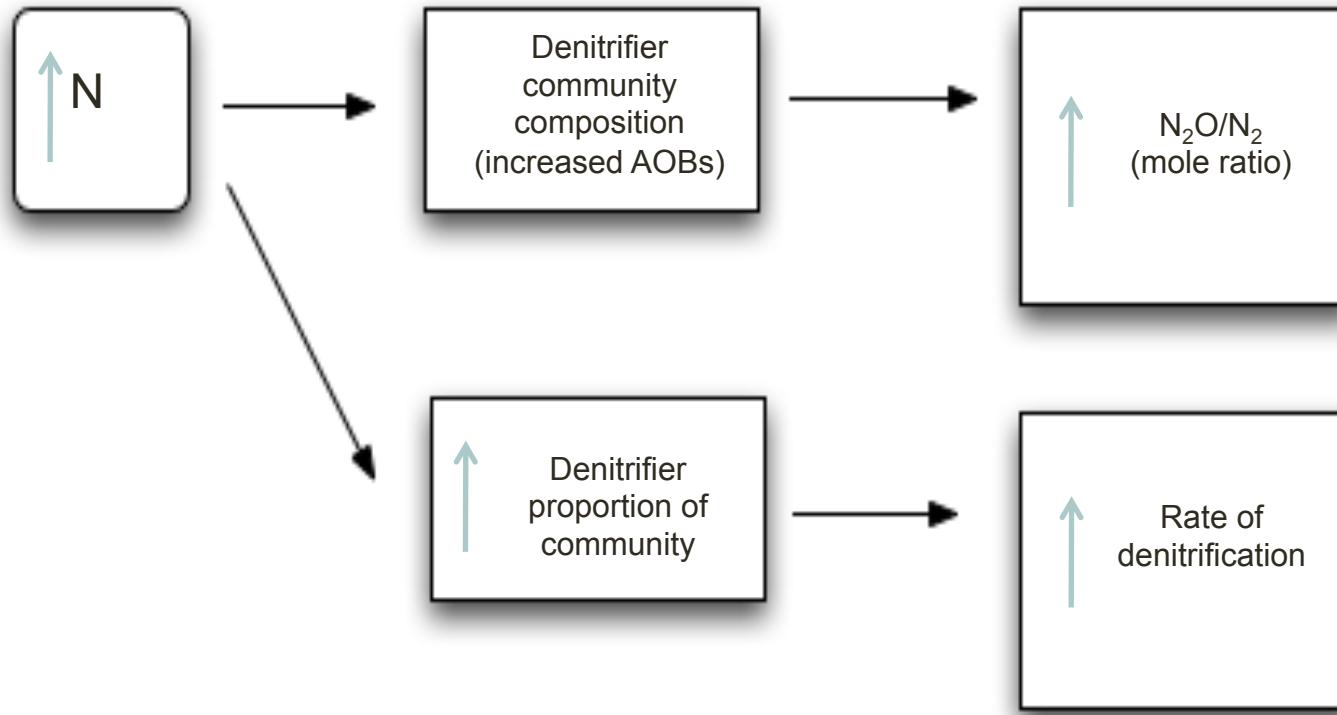
# Types of denitrification

## Heterotrophic denitrification



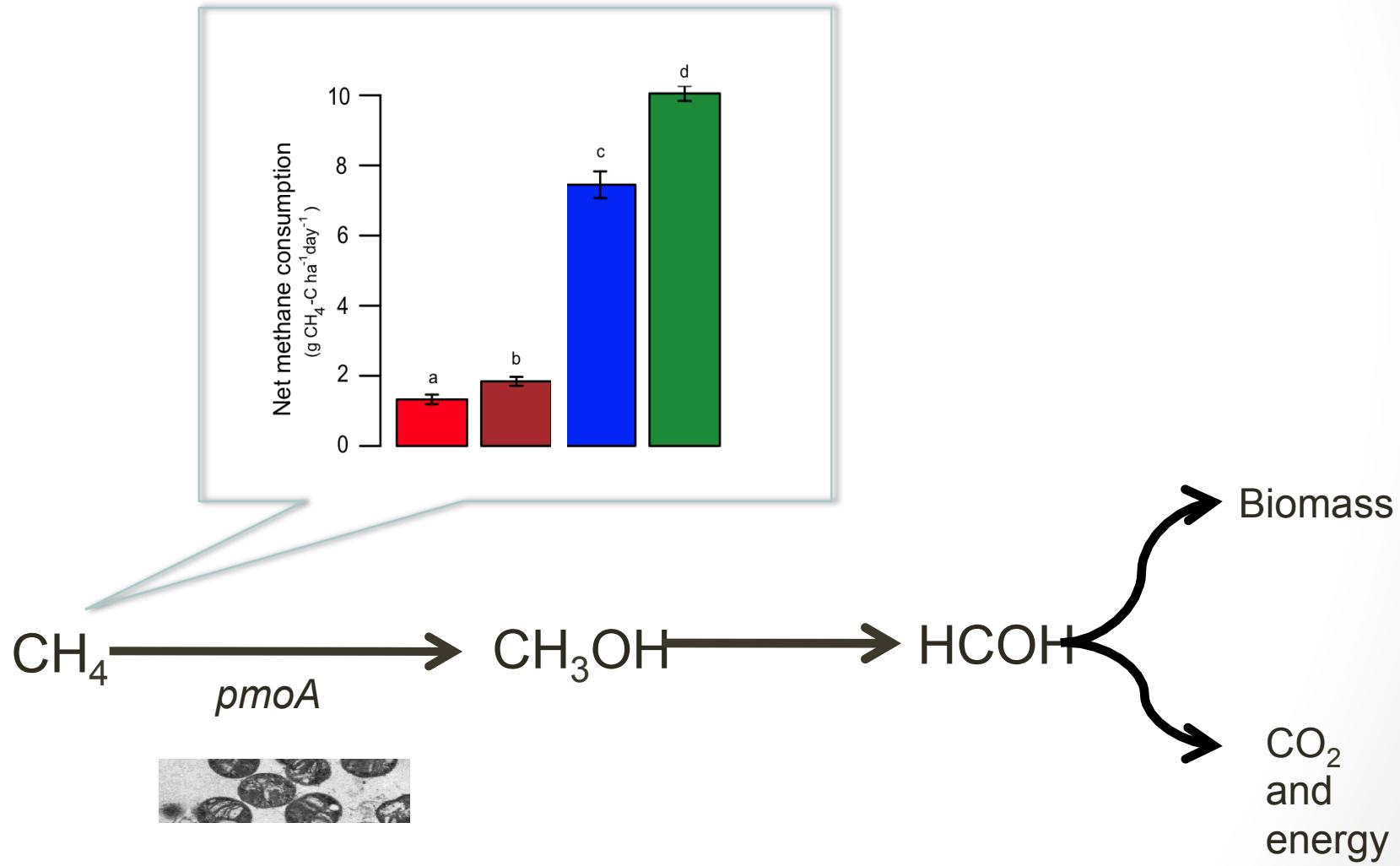
| Conditions | Function   |
|------------|------------|
| Anoxic     | Energetics |

# More denitrifiers and more with the potential just produce N<sub>2</sub>O but ratios and mechanisms still not understood

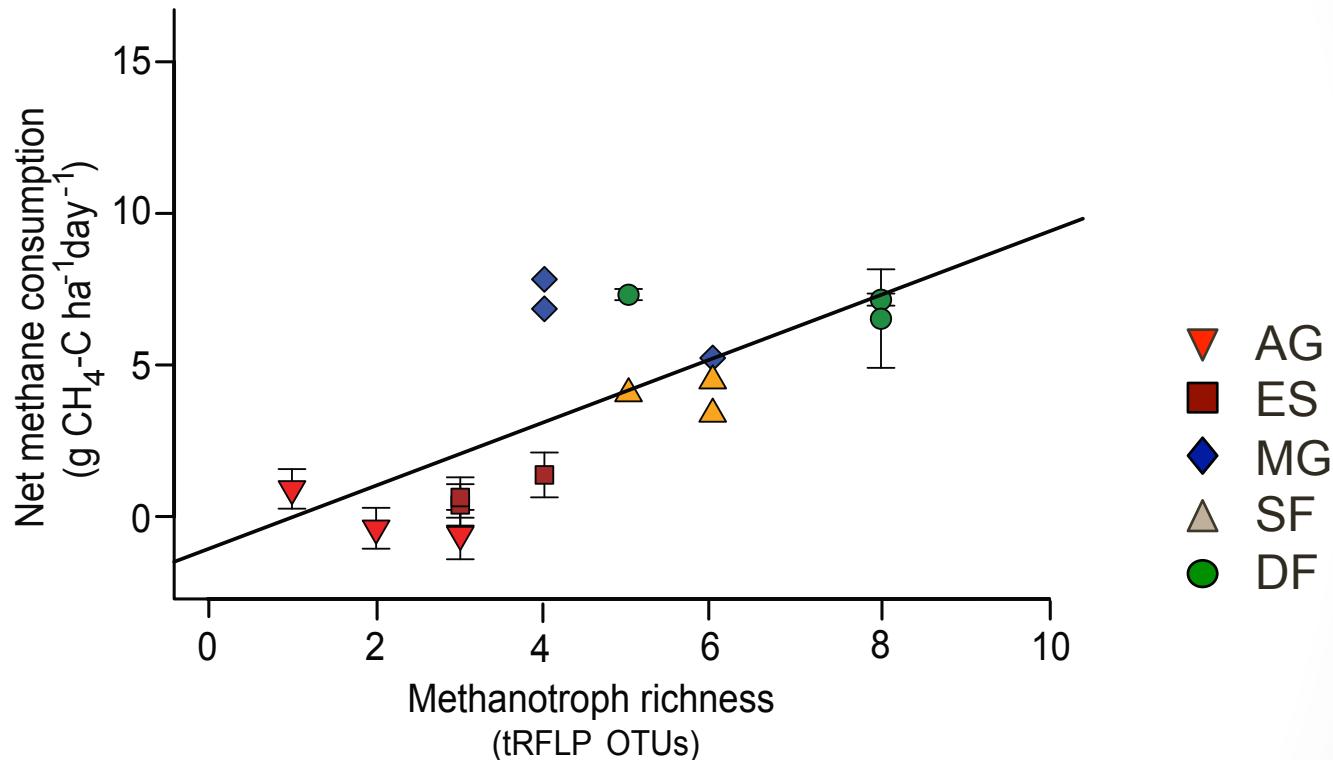


# Methanotrophs

# Methanotrophs use methane as a sole source of carbon and energy



# Methanotroph richness correlates with methane consumption

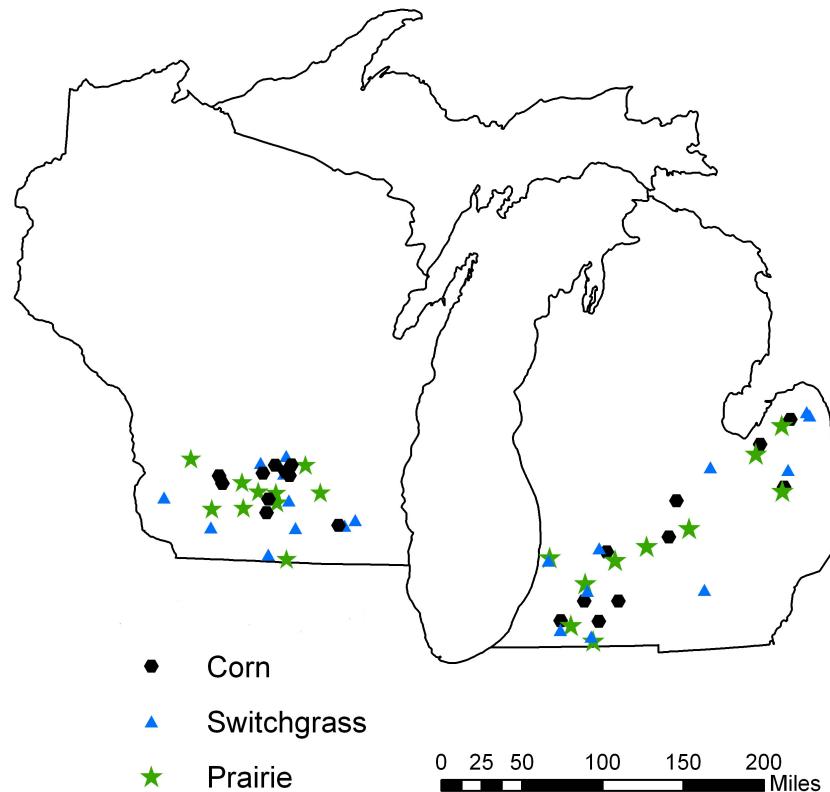


Levine, Teal, Robertson & Schmidt 2010

- Uri Levine
- Bernard Schroeter

# Methane in biofuel crops

GLBRC Extensive Sites



Clone libraries

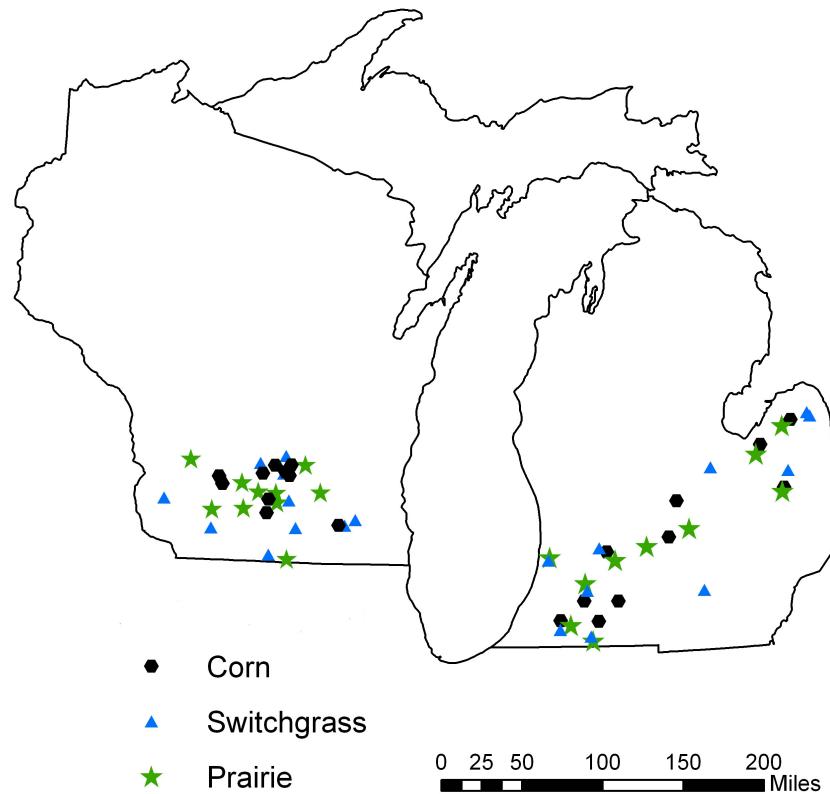
Classification of *pmoA* / *amoA*

GFClassify –  
Ribosomal Database Project  
Jordan Fish

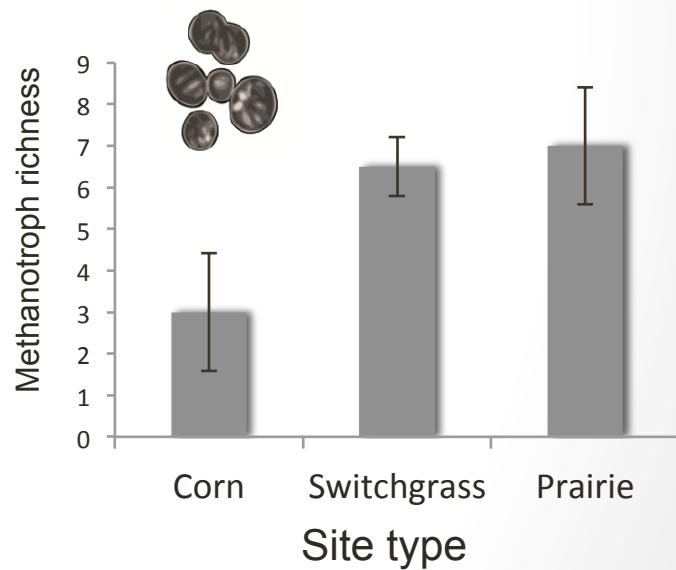
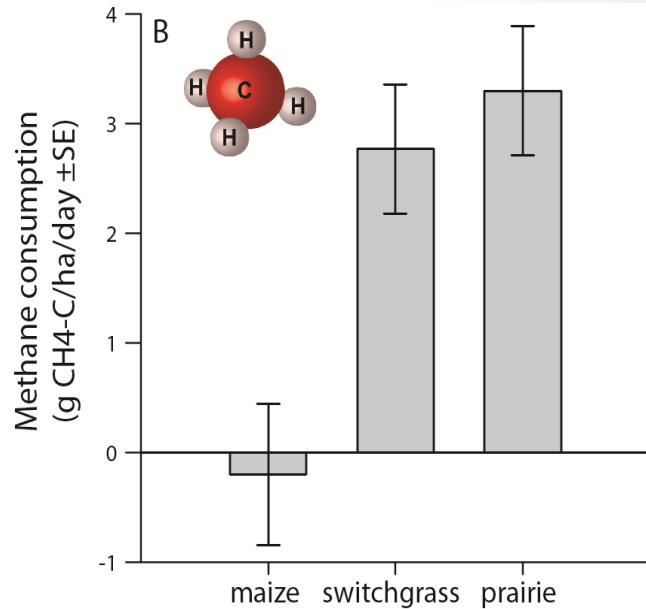
Leilei Ruan and G. Philip Robertson

# Methane in biofuel crops

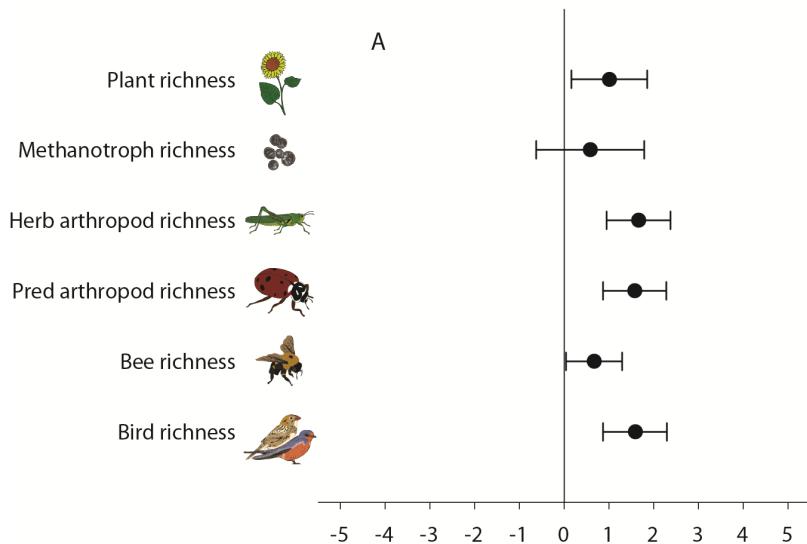
## GLBRC Extensive Sites



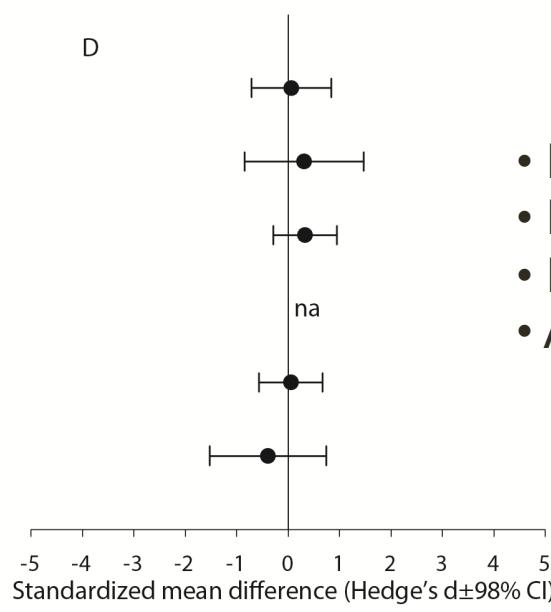
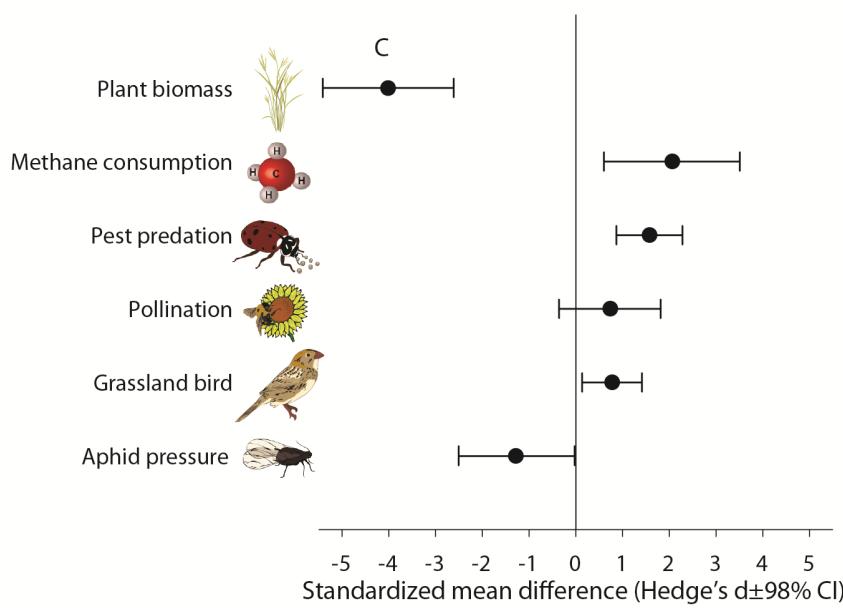
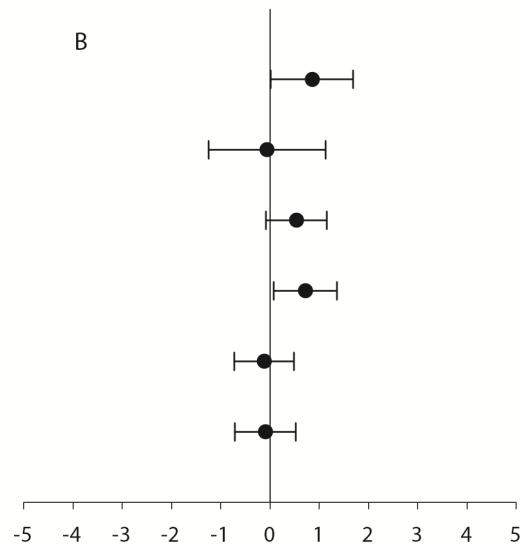
✗ Leilei Ruan and G. Philip Robertson



Difference between perennial grasslands and maize



Difference between prairie and switchgrass



- Productive
- Perennial
- Polycultures
- Appropriate Placement

# Amendments to recover methane oxidation

Enrichments consume methane at high concentrations

