

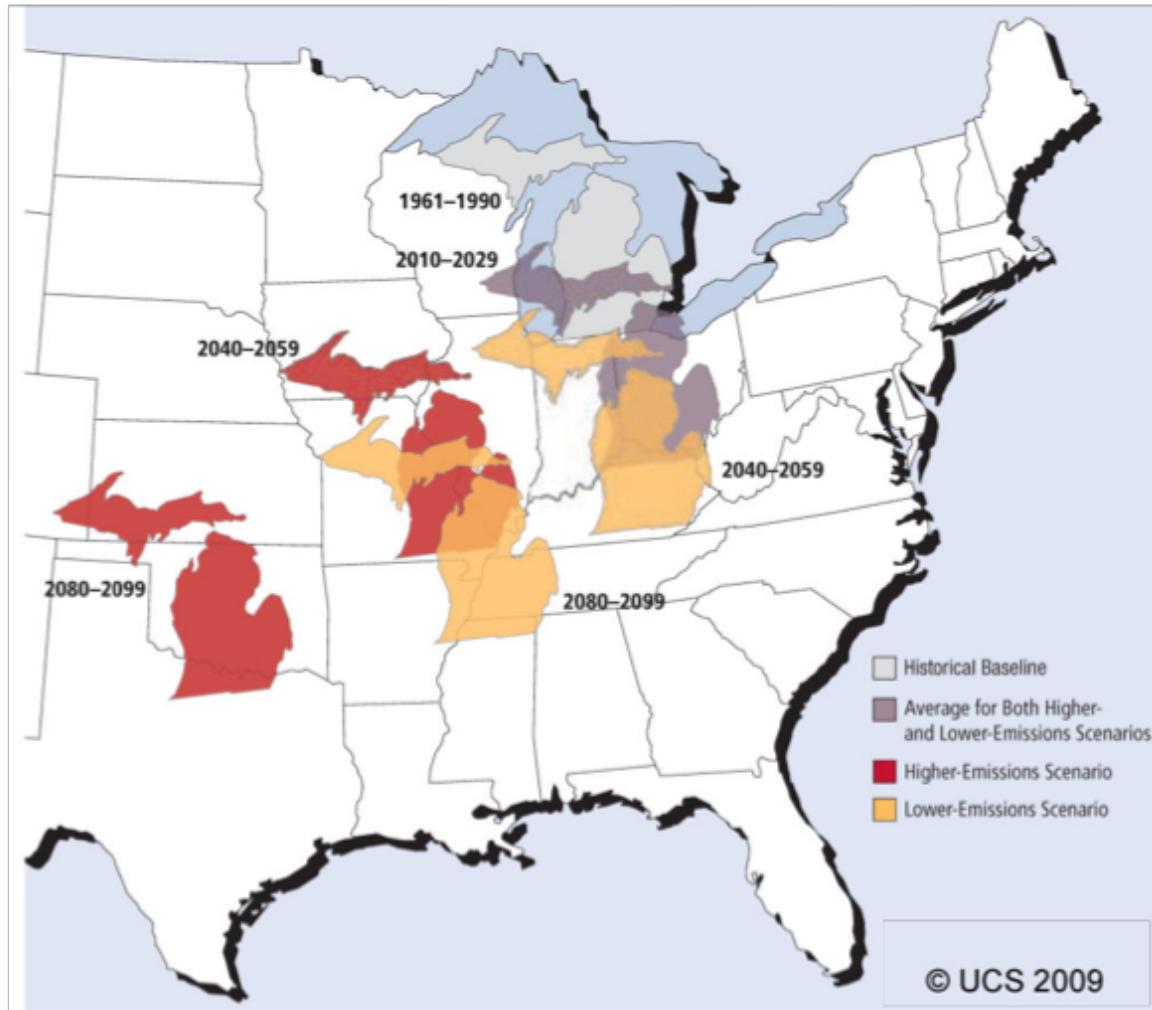
Effects of agricultural management on microbial communities and associated greenhouse gas fluxes: A study with metagenomics

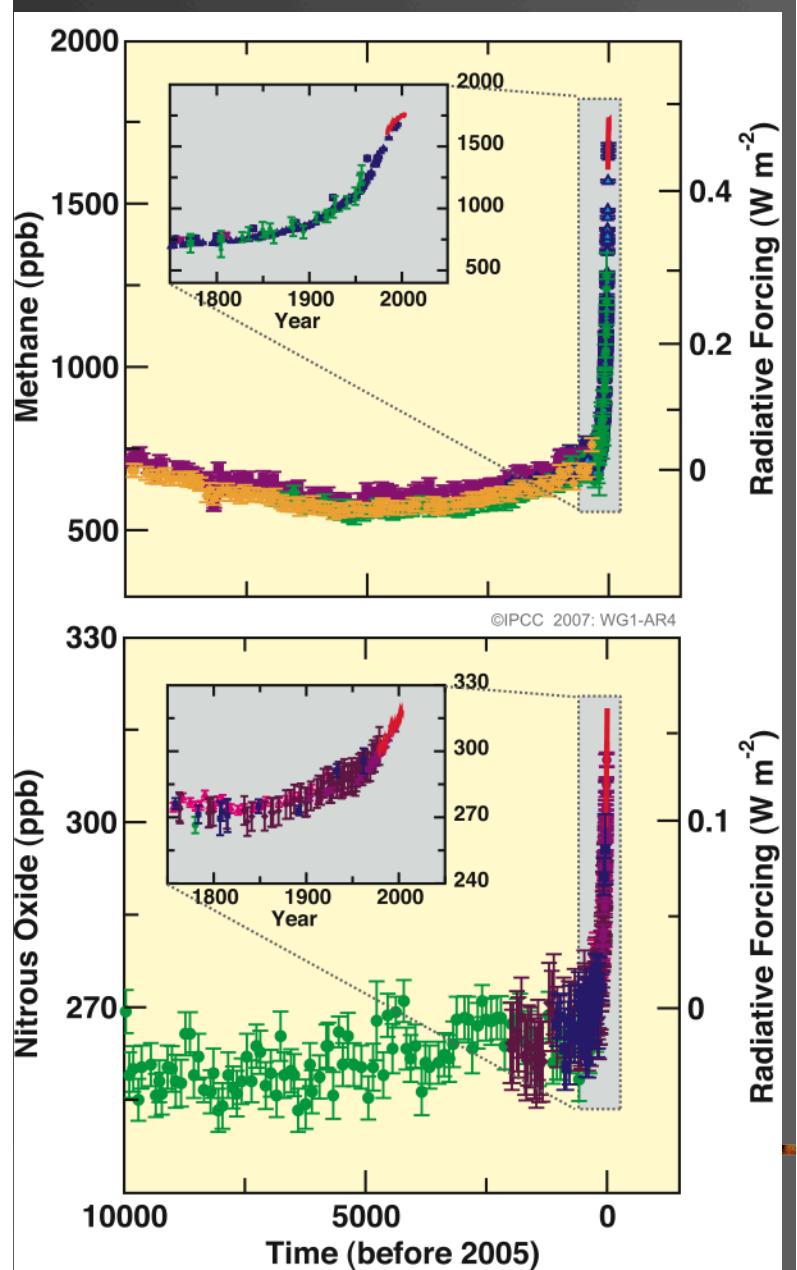
Tracy K. Teal

@tracykteal

Data Carpentry, Michigan State University, UC Davis

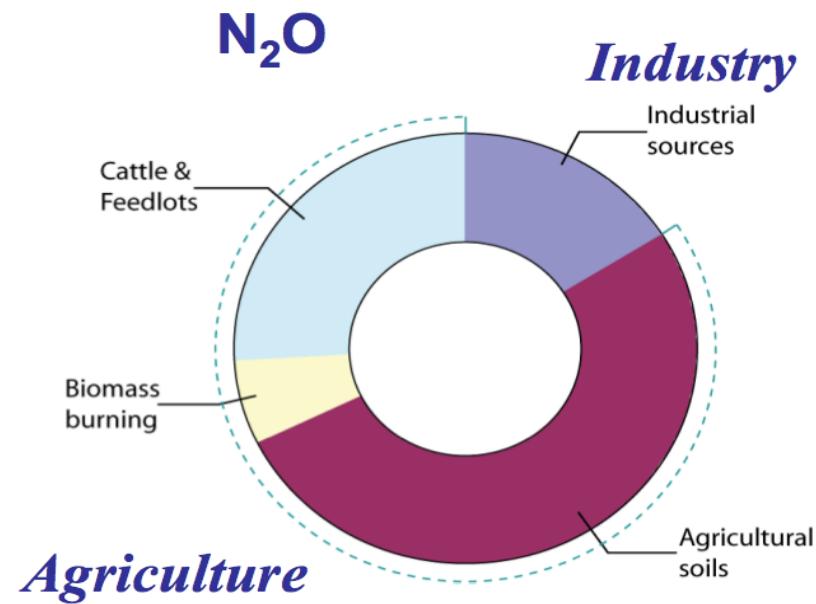
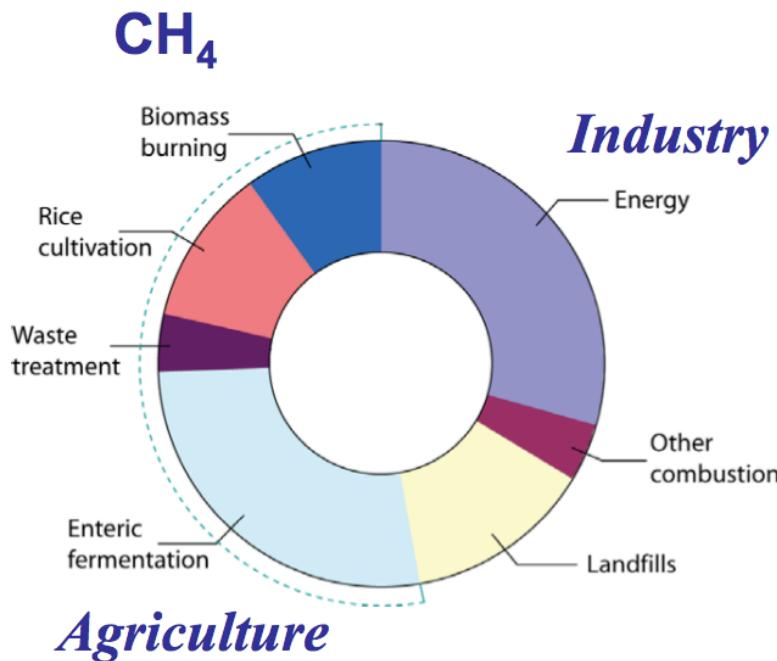
The climate is changing



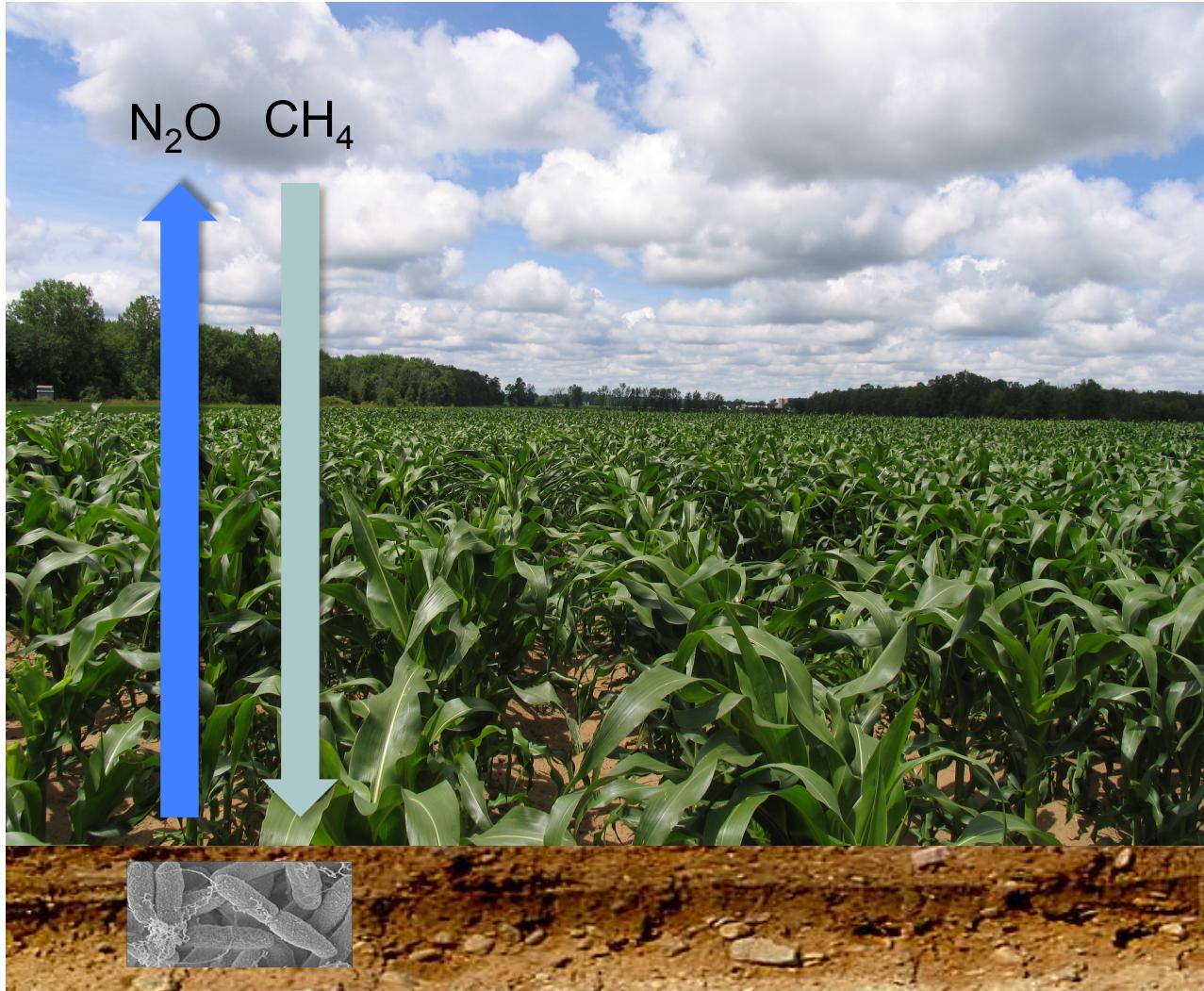


- Dramatic increases in CH_4 and N_2O in the last 200 years
- High Global Warming Potential (GWP) due to time in the atmosphere and infrared absorption

Land use change and intensive agriculture increase greenhouse gas fluxes



Microbes are the primary mediators of methane and nitrous oxide



Land management, sustainability and microbial communities

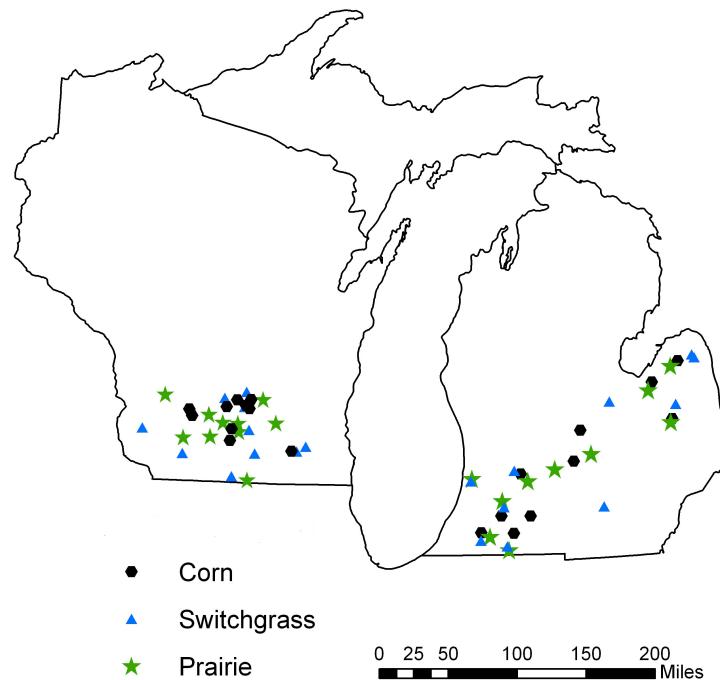
- How do microbial communities change with land management?
- What is the relationship between denitrifying bacteria and N₂O in agricultural soils?
- How long does it take for a community to recover from agriculture? Do all groups recover in the same way?

Experiment sites

Kellogg Biological Station LTER



GLBRC Extensive Sites



How do microbial communities change with land management?

Kellogg Biological Station LTER

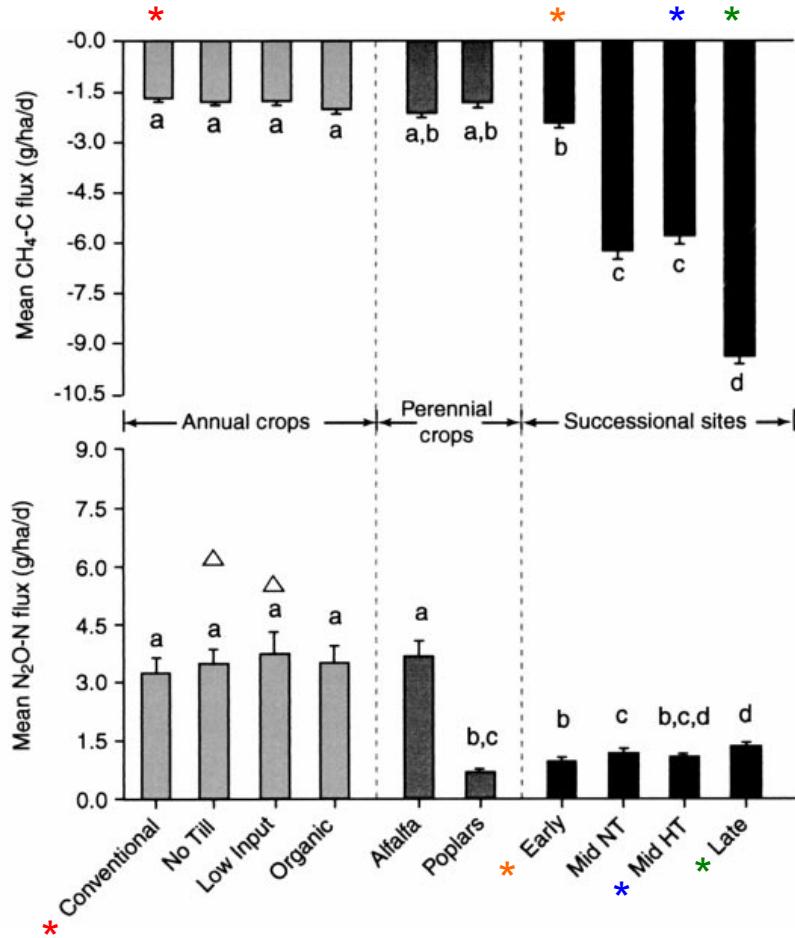
- ✖ Vicente Gomez-Alvarez
- ✖ Tom Schmidt



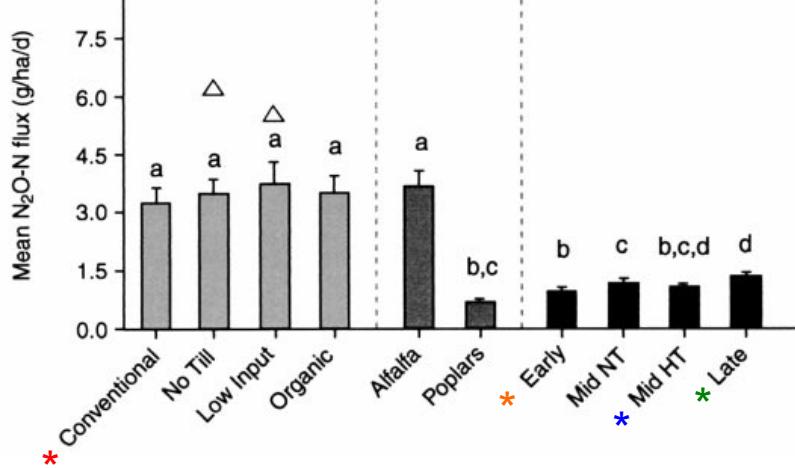
How do microbial communities change with land management?

Kellogg Biological Station LTER

Methane



Nitrous oxide



Robertson et al, 2000



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

Sampling design

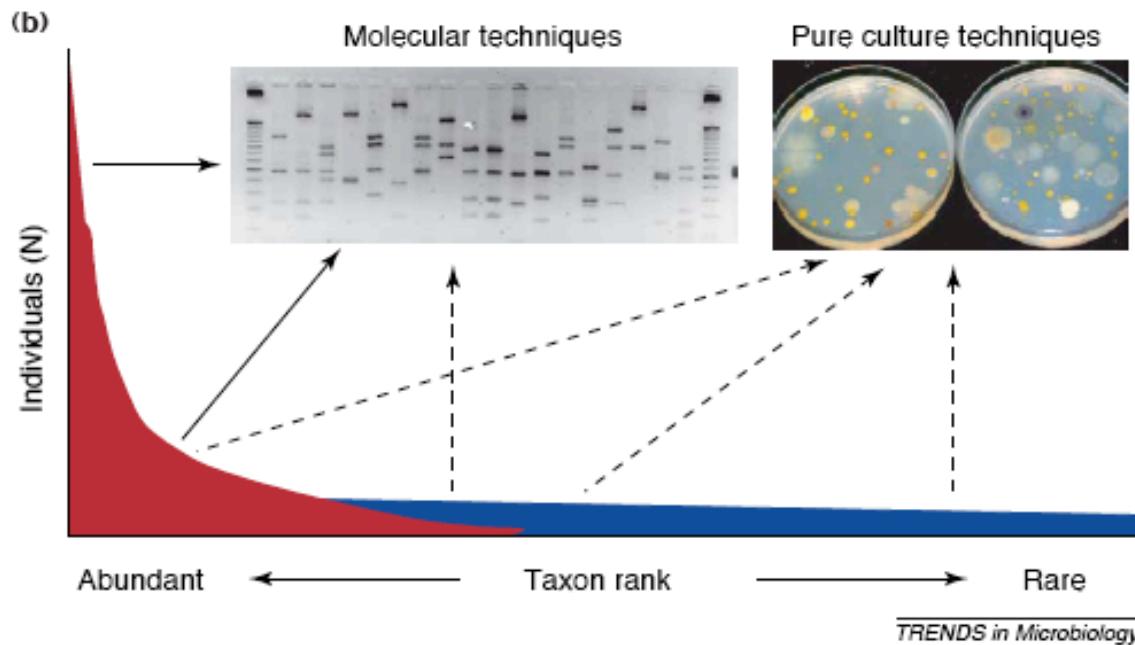
Sampled two plots of 4 treatments
In two years – 2008 and 2009

- 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

Microbial surveys



Why metagenomics?

[Bacteria are the] dark matter of the biological world.

- E.O. Wilson

Information about the taxonomic and functional structure of the microbial community – a way of ‘seeing’ the microbial community in a depth we have never been able to before

Targeted metagenomics and Shotgun metagenomics

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



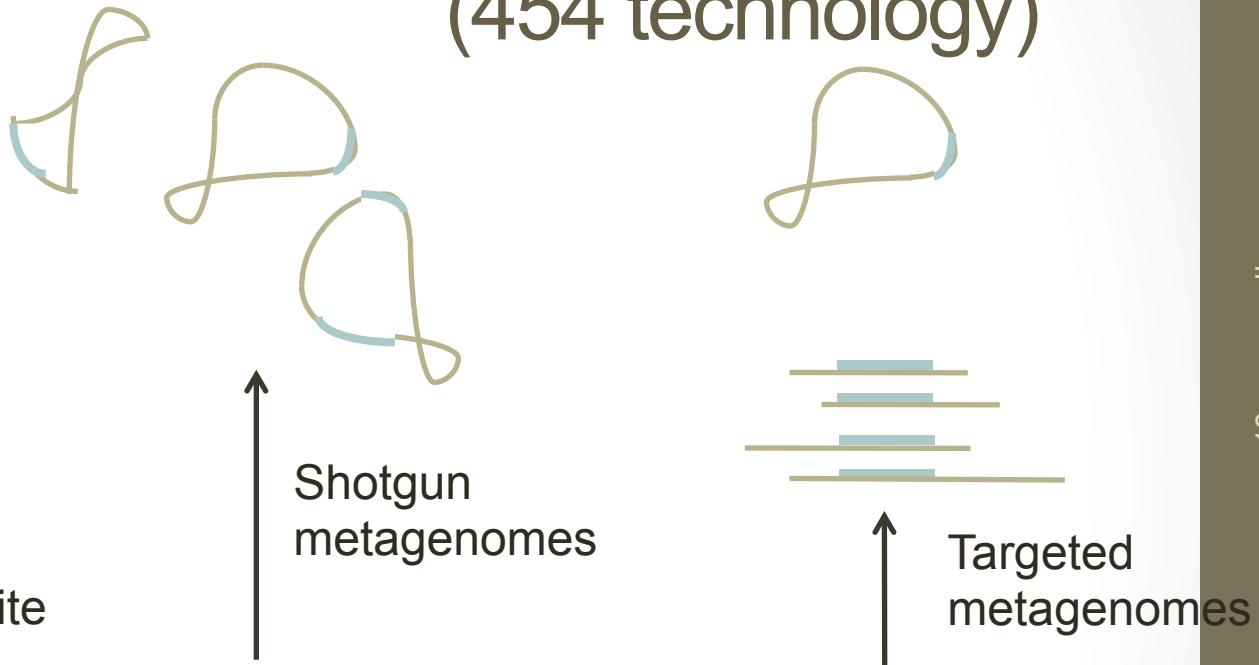
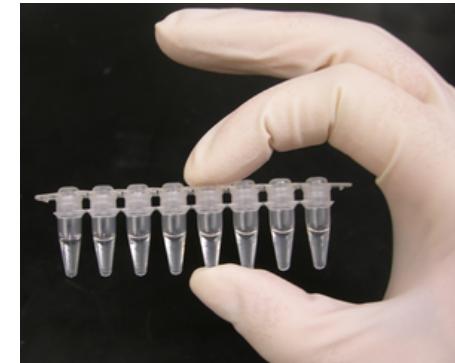
↓ Collect, composite
and sieve

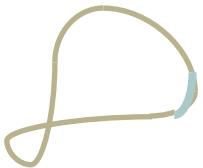


Extract
DNA
→

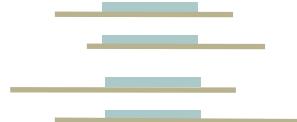


PCR
→

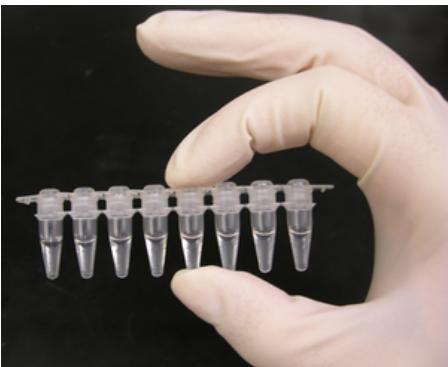




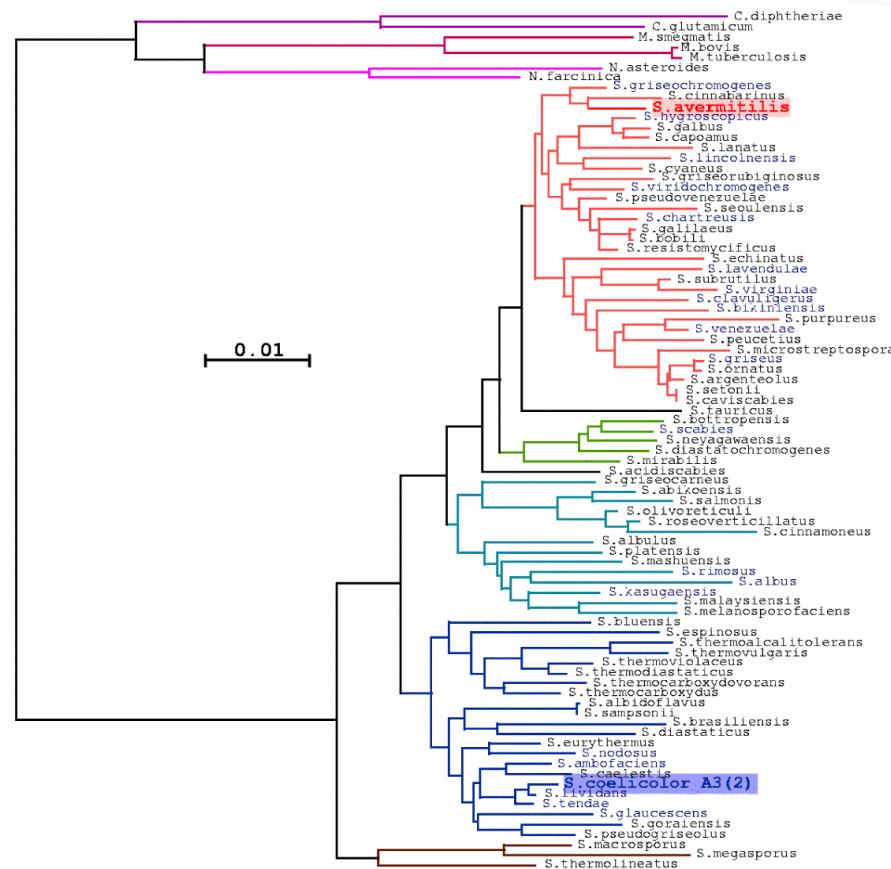
16S → Taxonomic composition



↑ Targeted
metagenomes



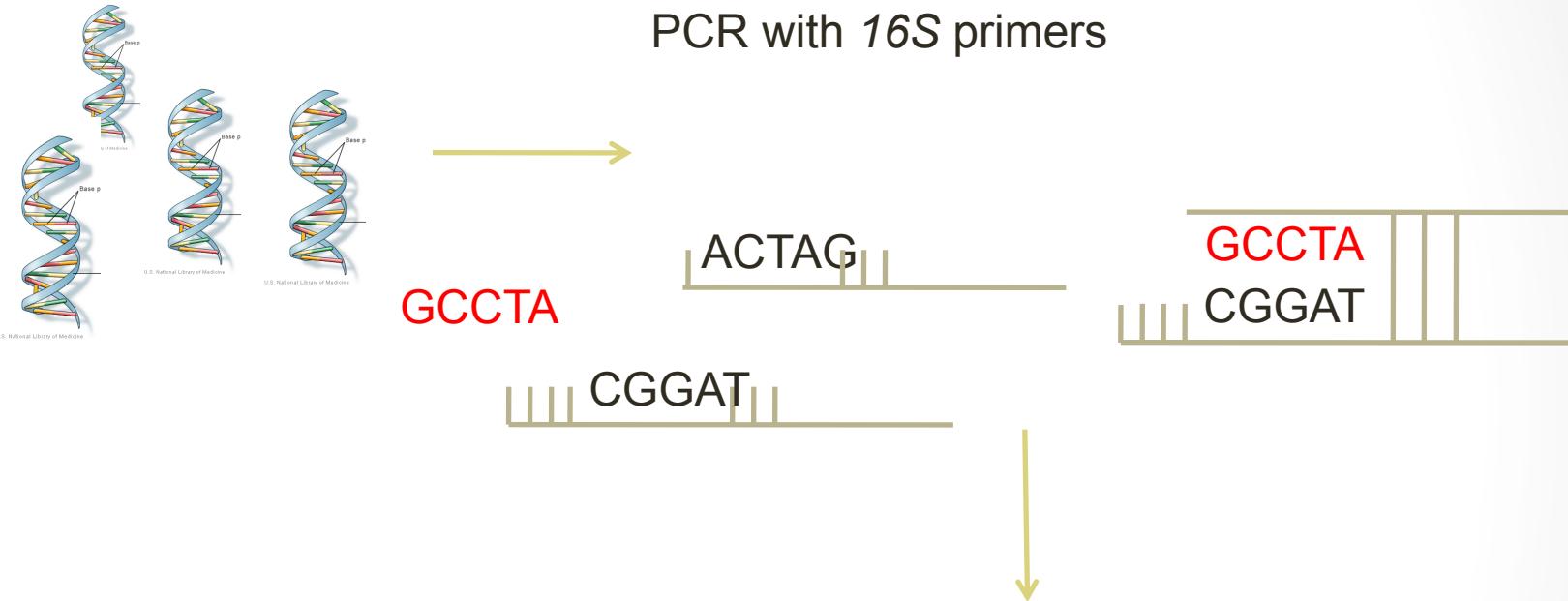
PCR



Who's there and are there differences between treatments?

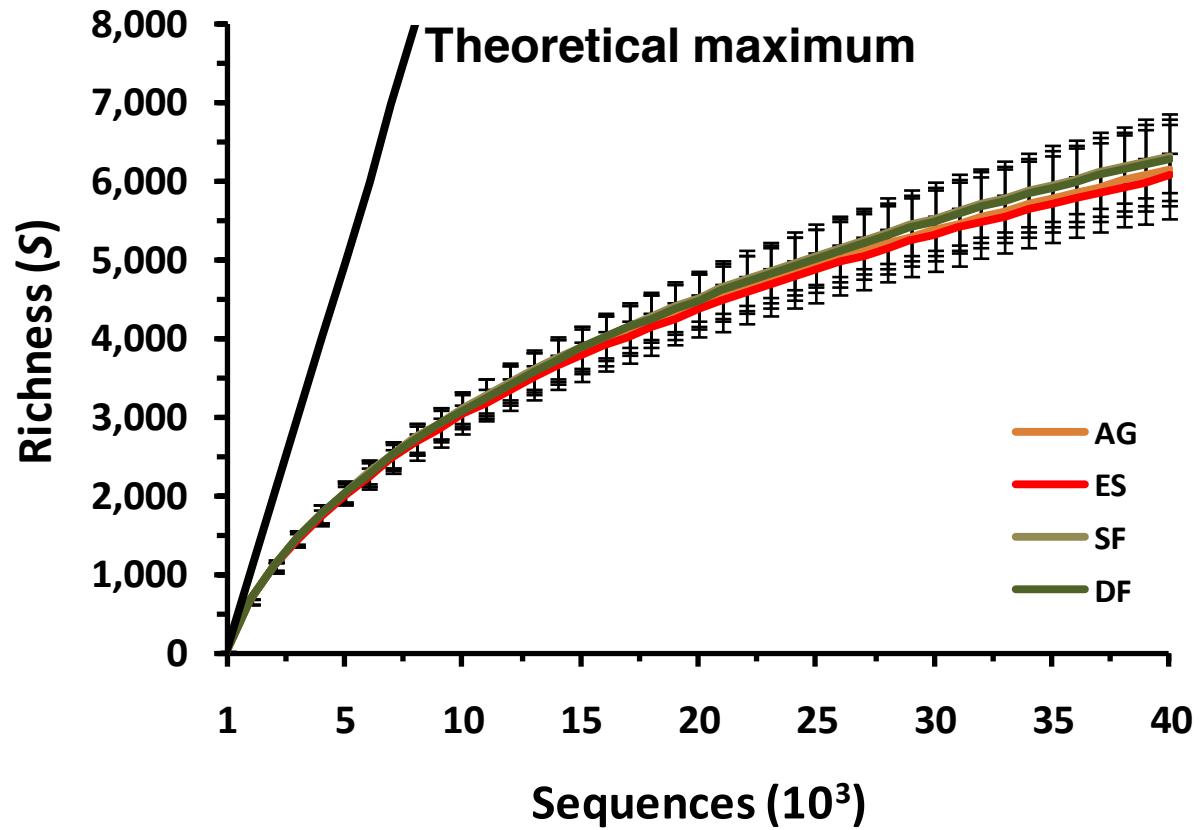
Null hypothesis – no difference in community composition between treatments

16S Amplicon sequencing

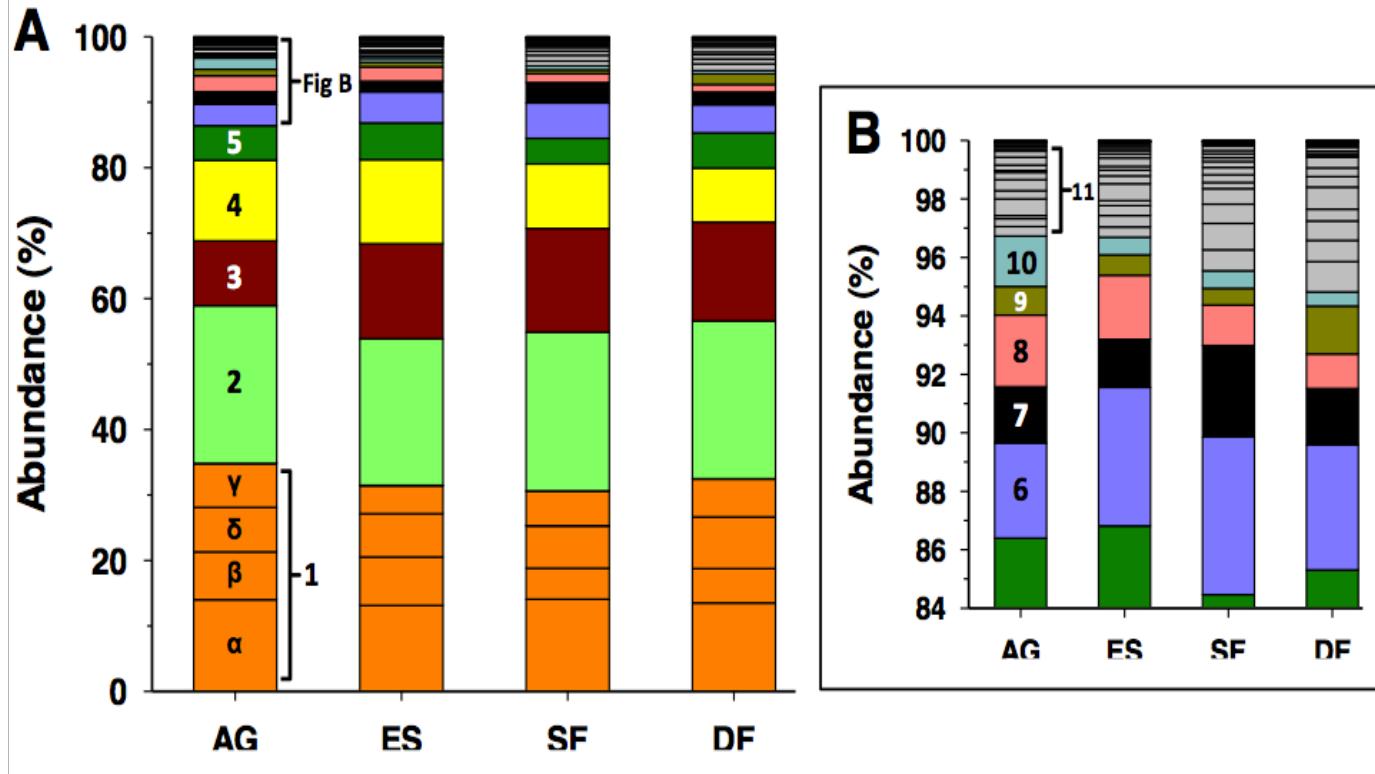


- Sequence 1 CGGATGCAGGTTAACGAATTGCCCTAACGCGG...
- Sequence 2 CGGATGCAGGTTAACGAATTGCCCTTGAACGCAG...
- Sequence 3 CGGATGCAGGTTAACGAATTGCCCTTGAACGCAG...

Soil microbial communities are extremely diverse



Who's there and differentiating communities



- (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.

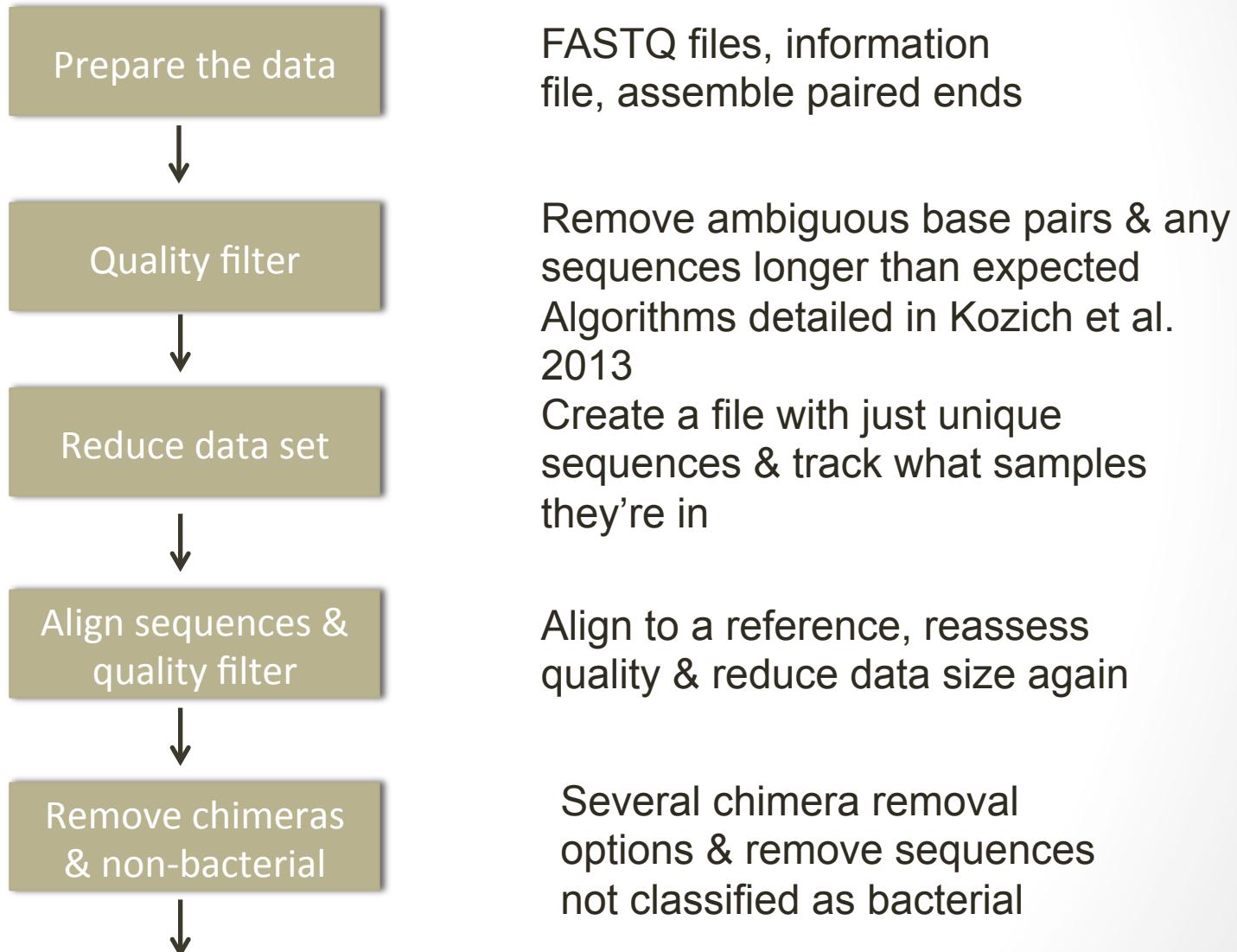
OTU based analysis

mothur and QIIME

- Both aim to enable advances in microbial ecology and are actively maintained and developed
- Both are open source and on github
- Both require alignment
- QIIME is ... mothur is not an acronym
- While QIIME connects multiple tools, mothur re-implements algorithms, so that it is all one program
- mothur in C++, QIIME connections in Python
- QIIME has mothur and mothur has Unifrac, but the default behavior for mothur is to do clustering based on sequence distance
- mothur's clustering can be very memory intensive, Uparse as used in QIIME requires less memory

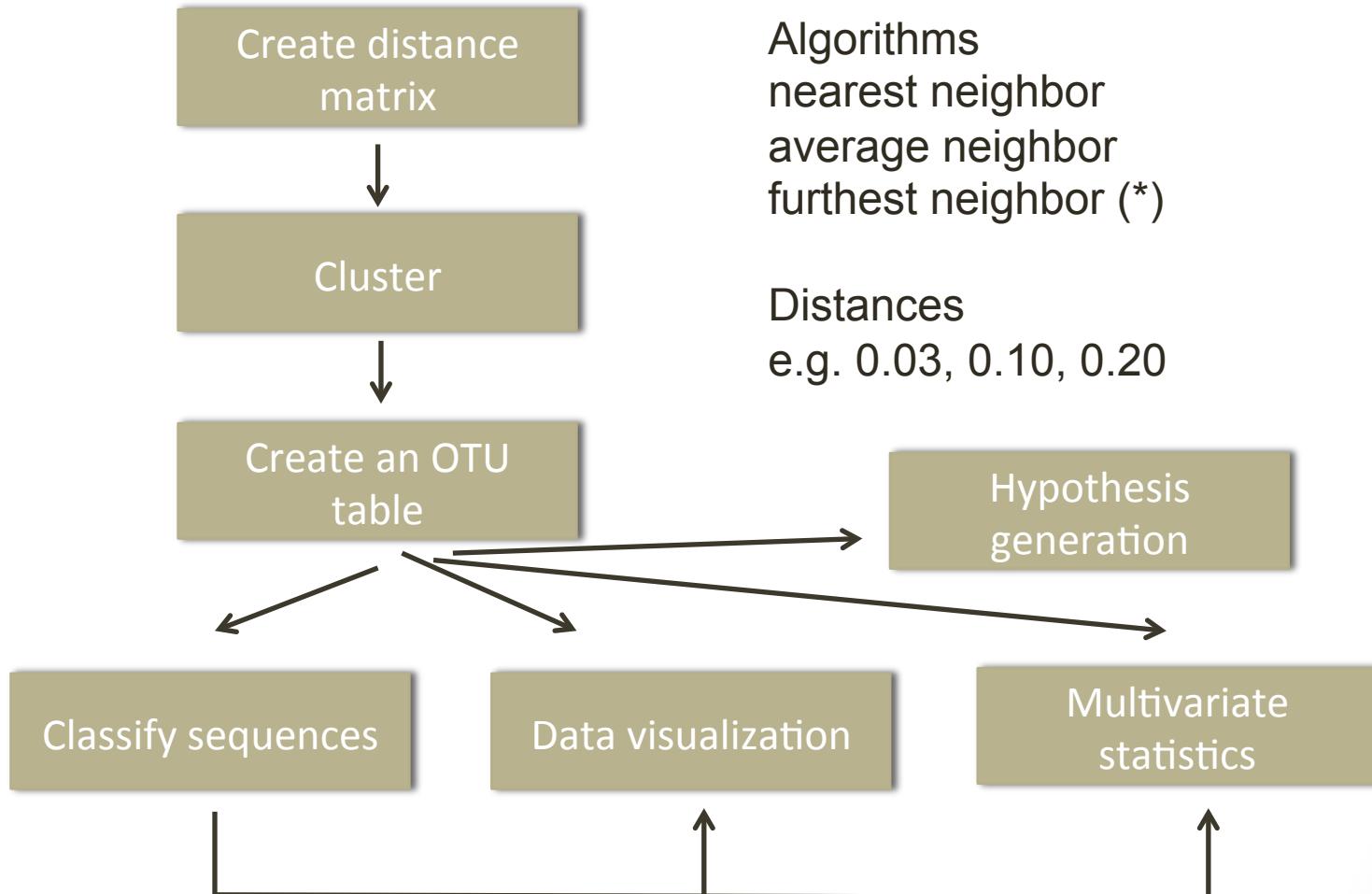
mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)

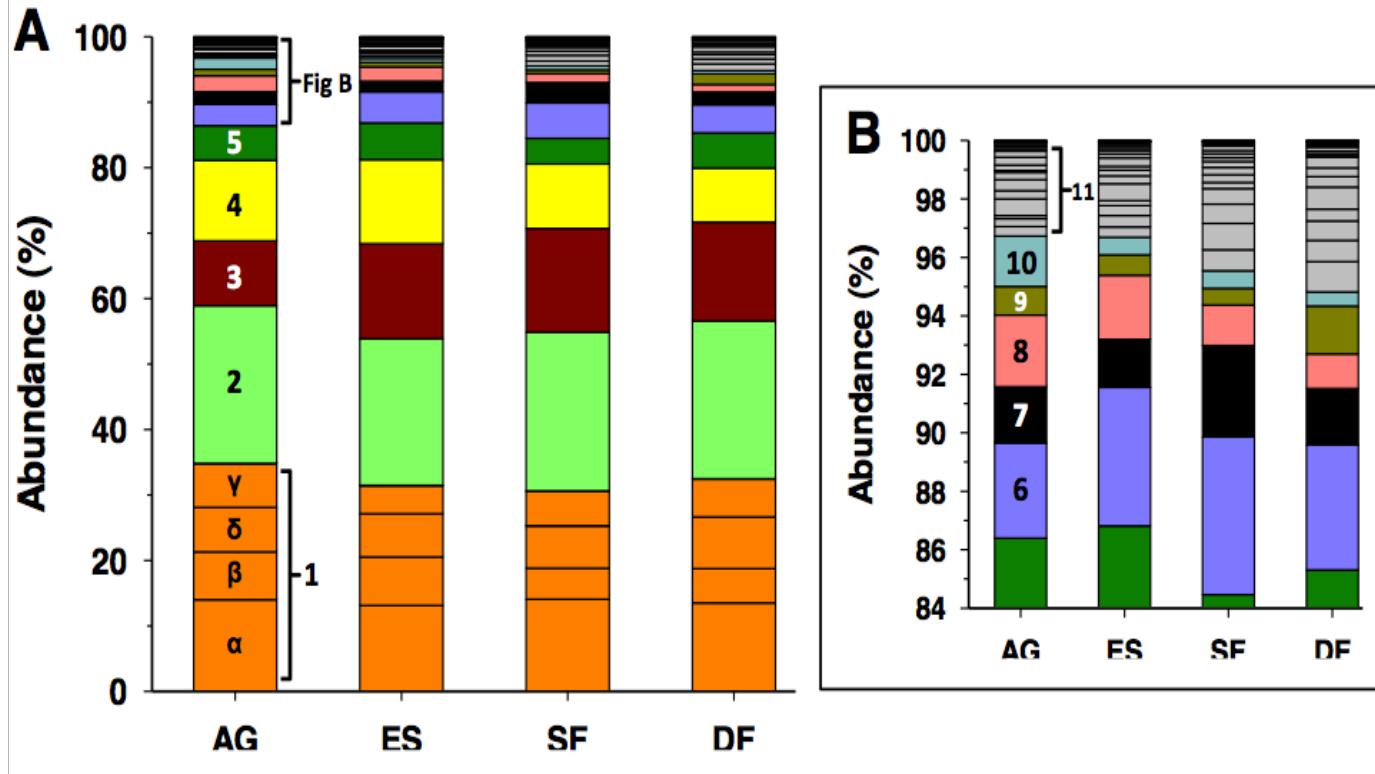


mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)



Who's there and differentiating communities

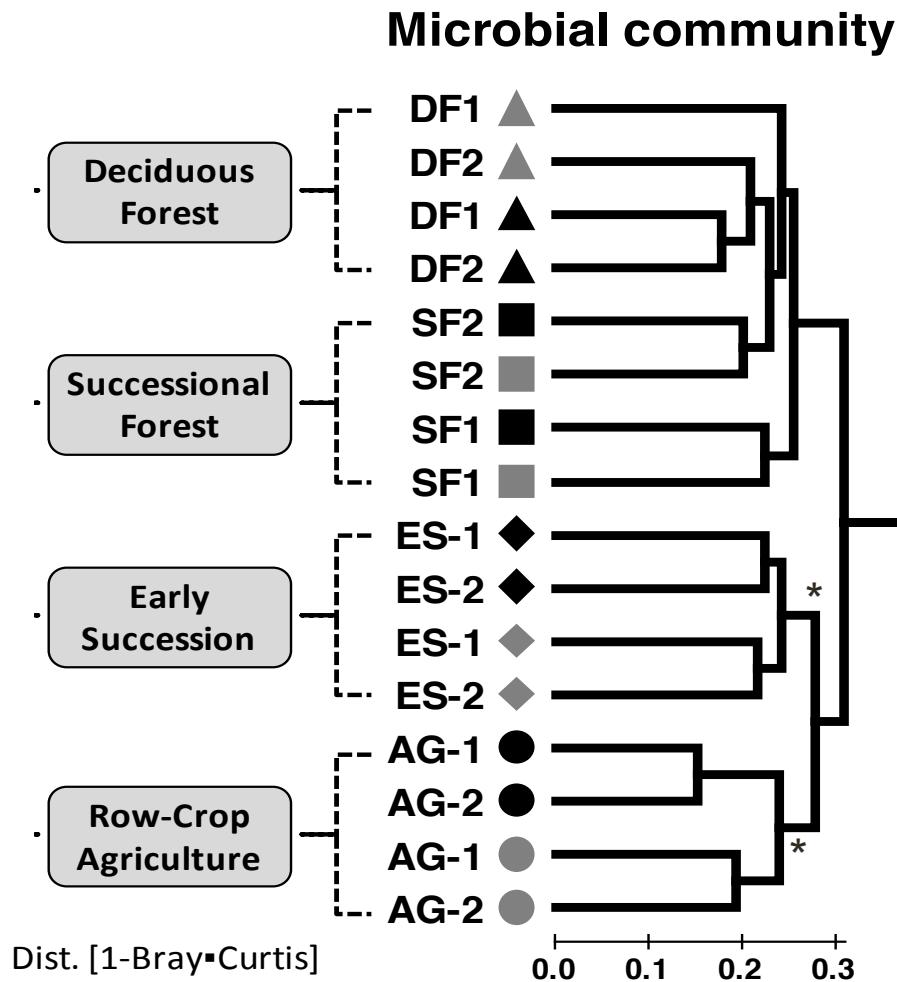


- (1) Proteobacteria [class added], (2) Acidobacteria, (3) Verrucomicrobia, (4) Actinobacteria, (5) Bacteroidetes, (6) Planctomycetes, (7) Chloroflexi, (8) Gemmatimonadetes, (9) Nitrospirae, (10) Firmicutes, and (11) 30 additional phylum corresponding to less than 4% of the total distribution.

OTU Table

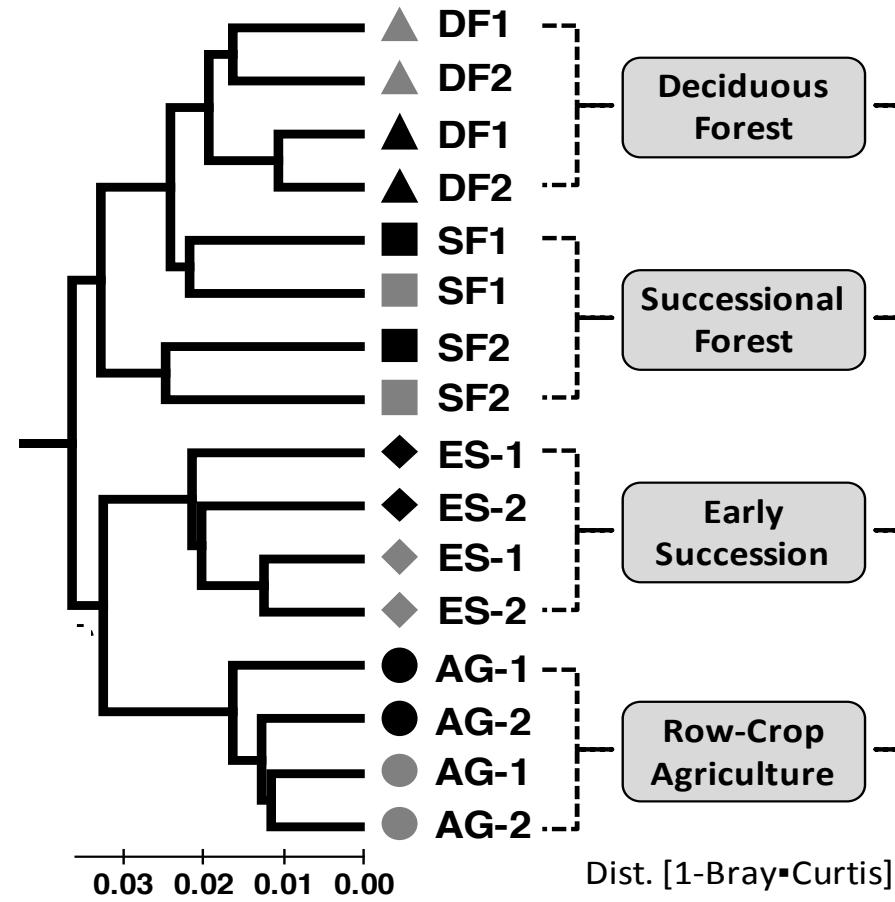
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	T1-1_2008	T1-1_2009	T1-2_2008	T1-2_2009	T7-1_2008	T7-1_2009	T7-2_2008	T7-2_2009	SF1_2008	SF1_2009	SF2_2008	SF2_2009	DF1_2008	DF1_2009	DF2_2008	DF2_2009
OTU00003	1	2	1	8	10	7	2	1	280	244	321	344	63	86	59	44
OTU00005	33	19	24	24	8	15	23	24	7	9	8	14	7	6	3	8
OTU00006	86	149	27	24	32	41	69	109	95	118	112	123	137	157	112	136
OTU00007	55	117	65	92	92	94	100	137	40	59	34	37	11	19	58	50
OTU00008	61	71	4	1	21	15	3	1	56	60	96	111	44	46	108	123
OTU00009	28	57	24	23	6	12	16	32	13	21	55	51	69	70	28	31
OTU00010	169	201	71	78	48	43	106	130	10	13	17	18	118	140	44	55
OTU00011	4	18	13	10	8	8	20	23	8	7	19	23	39	45	7	11
OTU00012	57	76	65	78	31	29	72	107	8	4	28	33	32	57	15	21
OTU00013	8	15	0	0	6	5	0	1	121	165	185	198	20	15	66	76
OTU00014	118	150	57	79	85	80	69	93	49	49	89	89	98	105	63	77
OTU00015	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
OTU00016	71	105	26	36	59	76	42	61	26	44	37	46	111	134	54	41
OTU00017	79	94	37	30	113	160	51	73	68	64	105	123	22	20	95	100
OTU00018	48	58	75	131	21	26	177	250	2	0	17	9	14	10	3	8
OTU00019	28	54	62	71	12	12	93	89	1	2	5	4	0	0	3	4

Taxonomic composition of bacterial communities changes with treatment

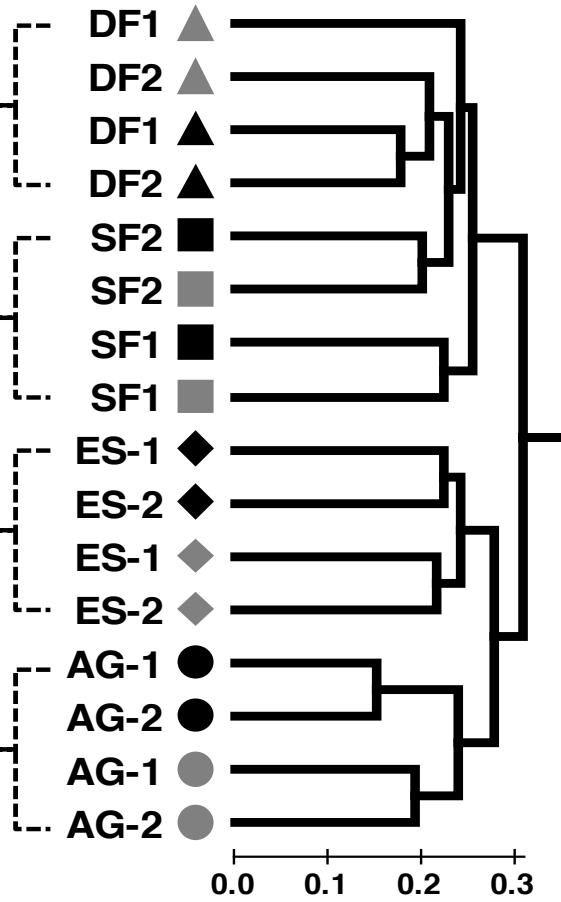


Biogeochemistry and bacterial community change concomitantly

Soil properties



Microbial community

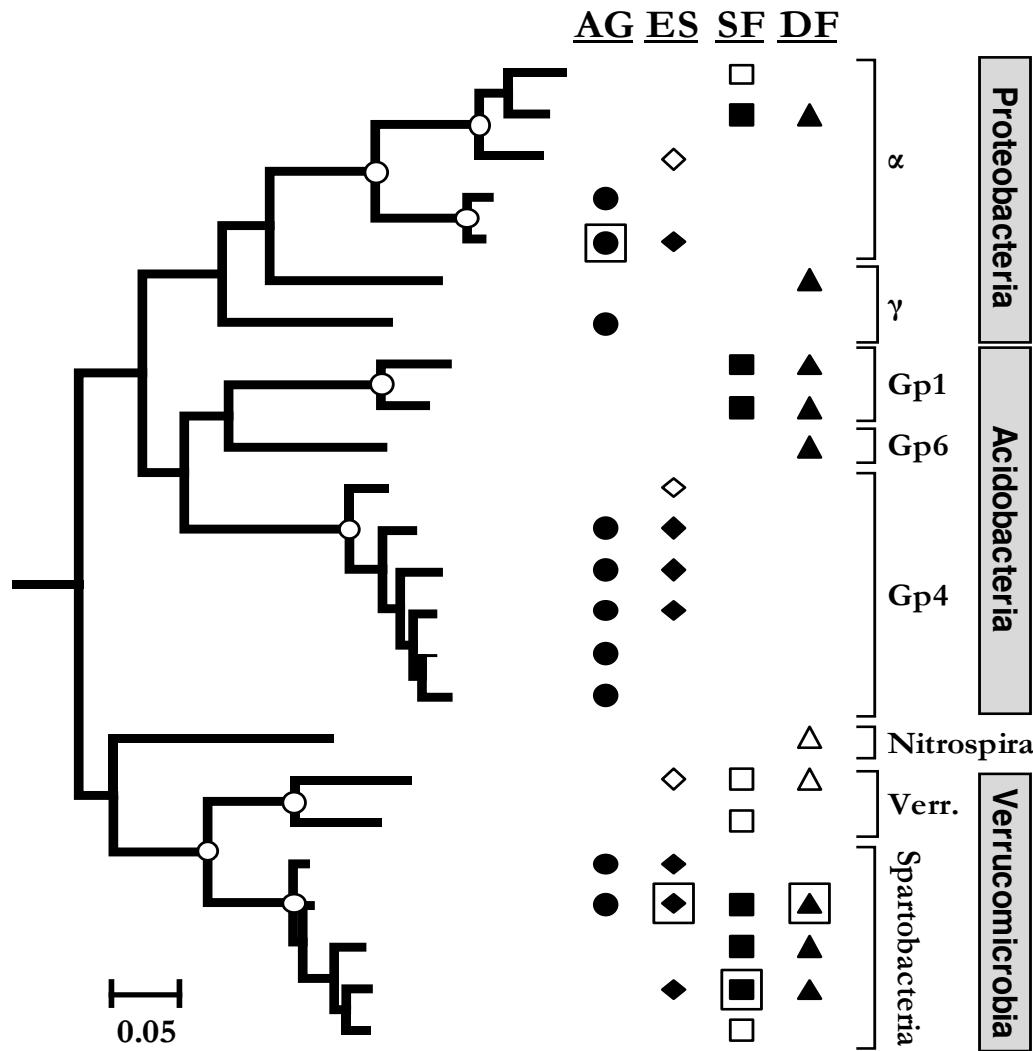


OTU Table

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	T1-1_2008	T1-1_2009	T1-2_2008	T1-2_2009	T7-1_2008	T7-1_2009	T7-2_2008	T7-2_2009	SF1_2008	SF1_2009	SF2_2008	SF2_2009	DF1_2008	DF1_2009	DF2_2008	DF2_2009
OTU00003	1	2	1	8	10	7	2	1	280	244	321	344	63	86	59	44
OTU00005	33	19	24	24	8	15	23	24	7	9	8	14	7	6	3	8
OTU00006	86	149	27	24	32	41	69	109	95	118	112	123	137	157	112	136
OTU00007	55	117	65	92	92	94	100	137	40	59	34	37	11	19	58	50
OTU00008	61	71	4	1	21	15	3	1	56	60	96	111	44	46	108	123
OTU00009	28	57	24	23	6	12	16	32	13	21	55	51	69	70	28	31
OTU00010	169	201	71	78	48	43	106	130	10	13	17	18	118	140	44	55
OTU00011	4	18	13	10	8	8	20	23	8	7	19	23	39	45	7	11
OTU00012	57	76	65	78	31	29	72	107	8	4	28	33	32	57	15	21
OTU00013	8	15	0	0	6	5	0	1	121	165	185	198	20	15	66	76
OTU00014	118	150	57	79	85	80	69	93	49	49	89	89	98	105	63	77
OTU00015	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
OTU00016	71	105	26	36	59	76	42	61	26	44	37	46	111	134	54	41
OTU00017	79	94	37	30	113	160	51	73	68	64	105	123	22	20	95	100
OTU00018	48	58	75	131	21	26	177	250	2	0	17	9	14	10	3	8
OTU00019	28	54	62	71	12	12	93	89	1	2	5	4	0	0	3	4

Select the top 20 most abundant OTUs in each treatment

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

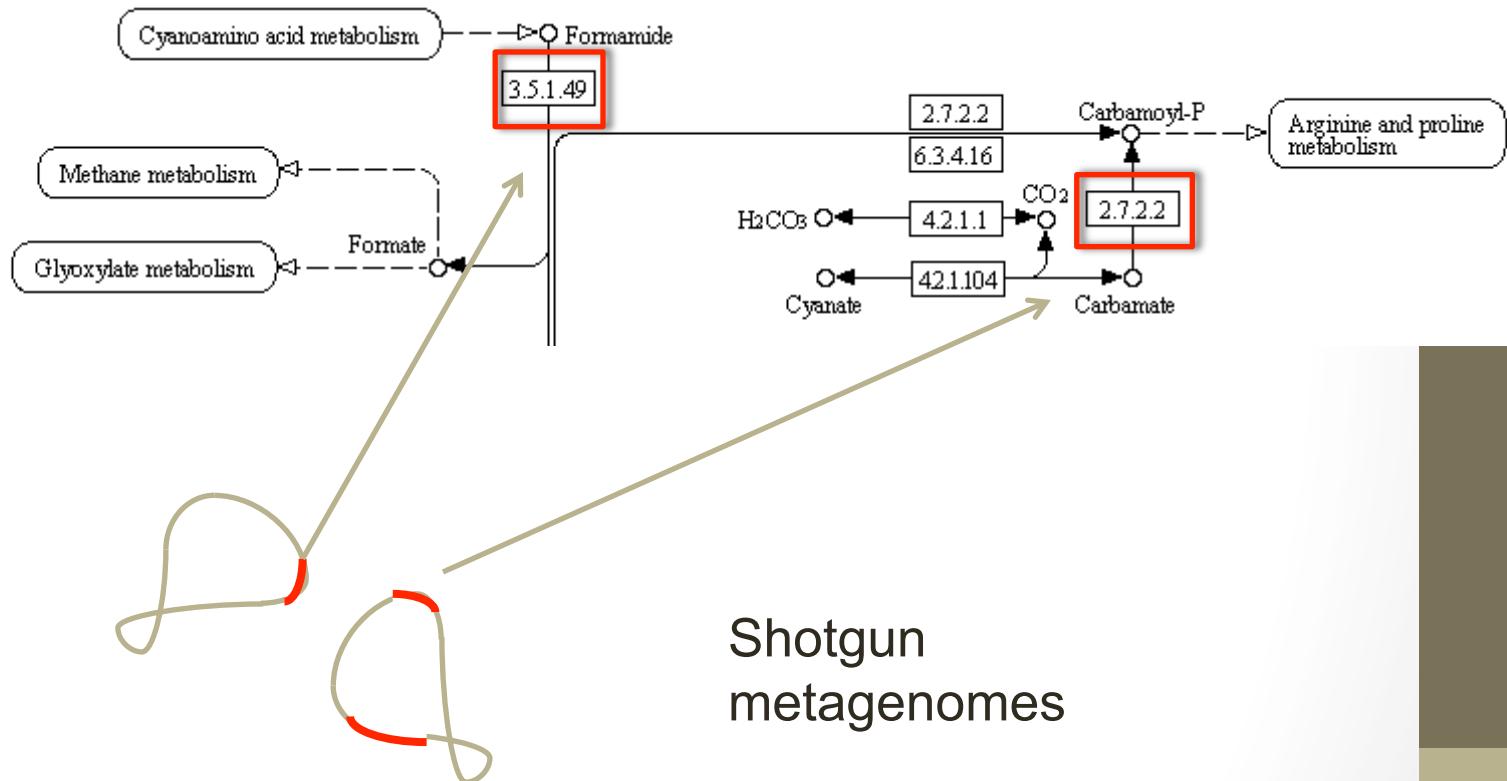
The important thing to understand is not just who's there, but what are they doing. What is their role in ecosystem functioning, particularly in greenhouse gas production or consumption?

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

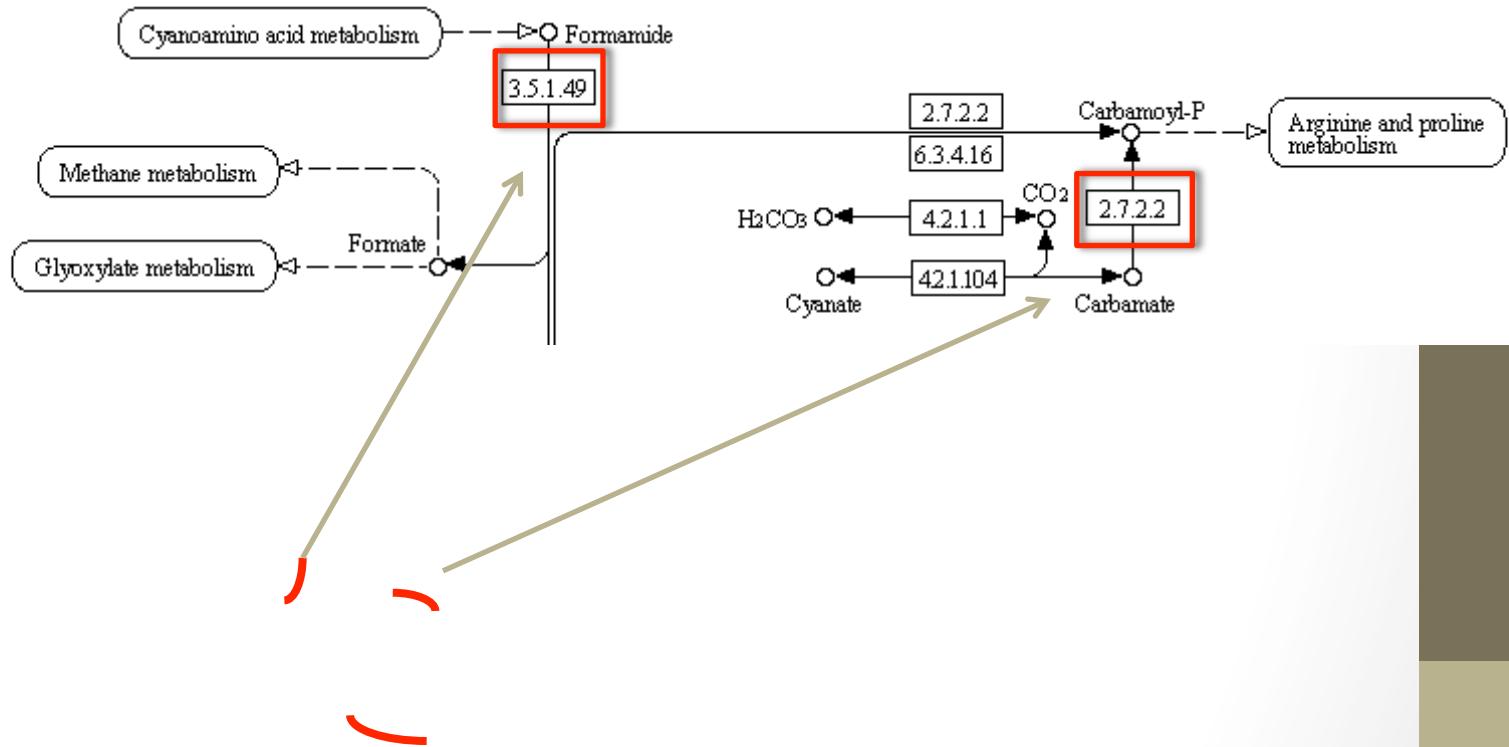
Caveats with metagenomic sequencing

- 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

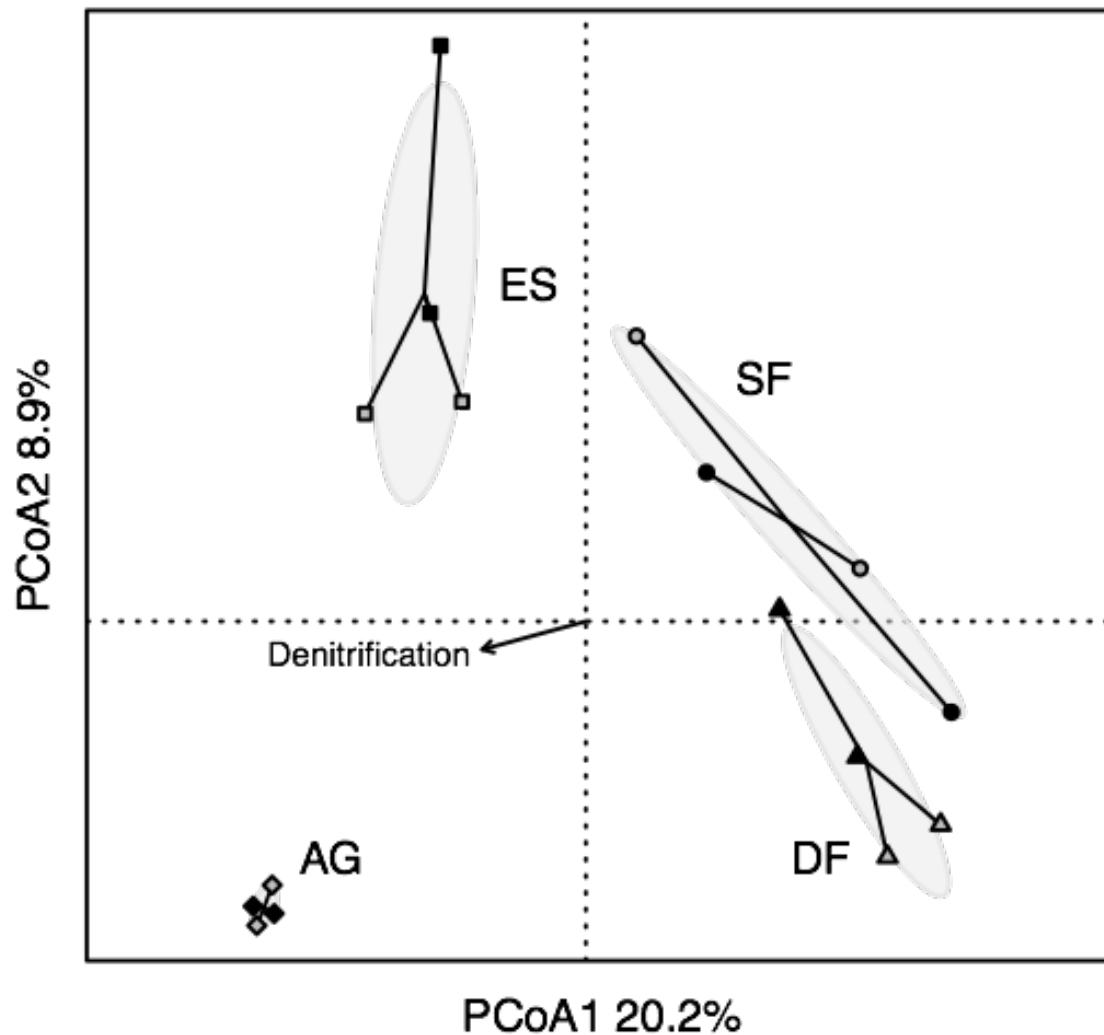


Annotation via MG-RAST or other Blast-based approaches

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



(36)

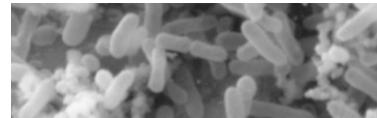
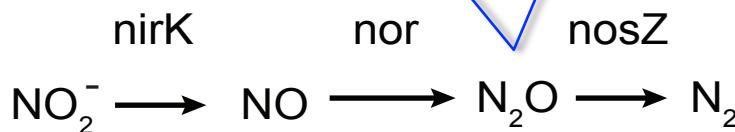
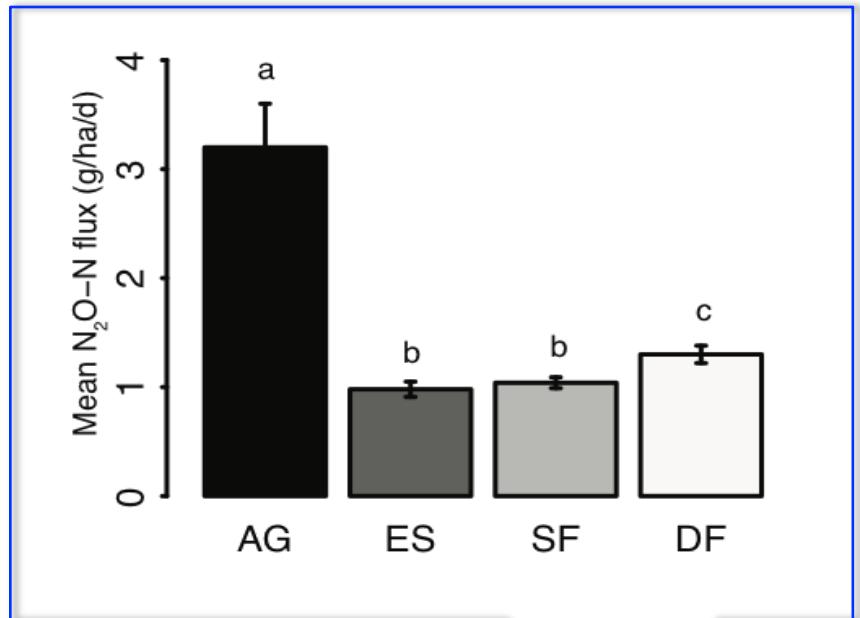
Analysis of 7058 genes annotated by MG-RAST against the SEED database

Functional potential of communities differs with treatment

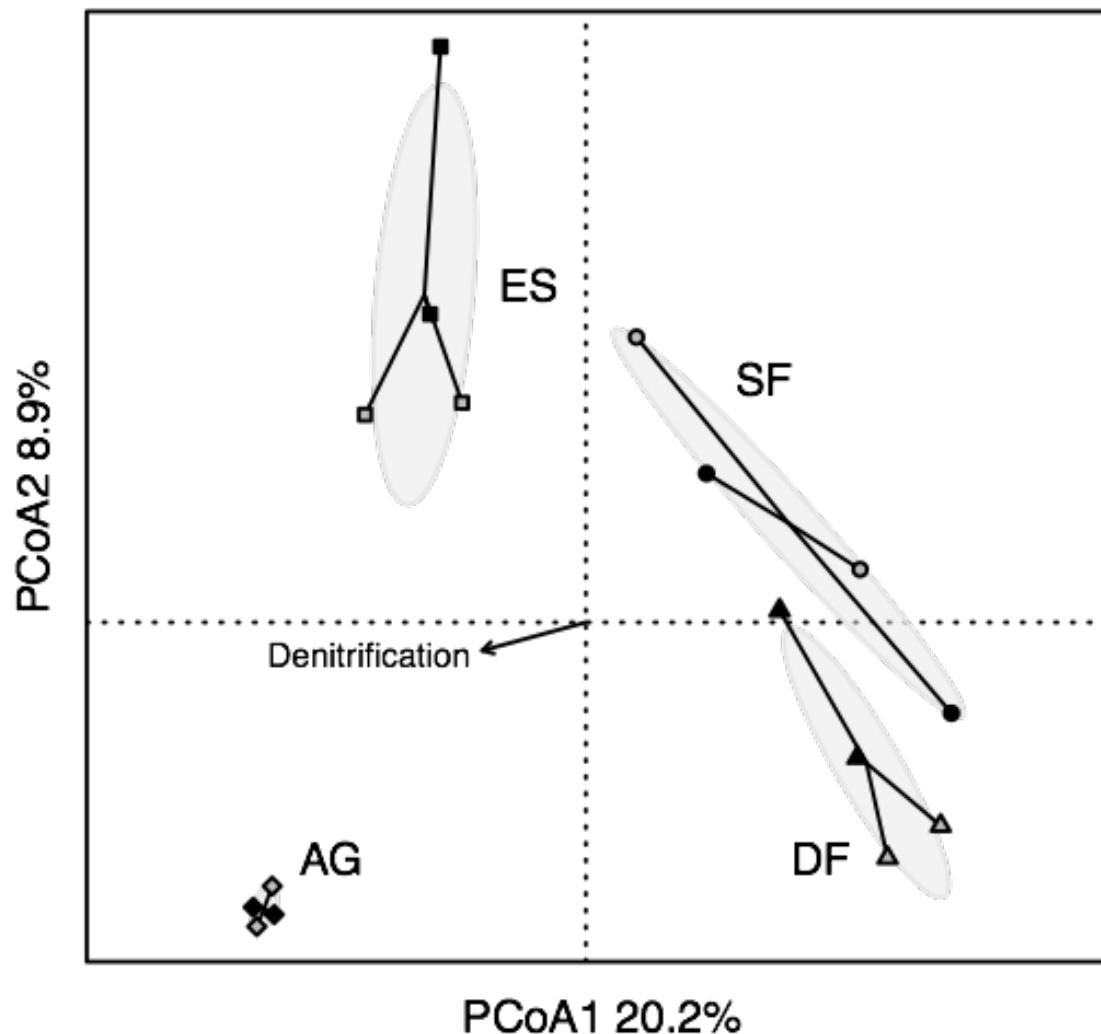
- Shotgun metagenomics can be used to look at the functional potential of a sample. It reflects only what's present in the soil, not what metabolism is occurring.
- There are differences in the functional potential of the communities that parallel that of taxonomic composition and soil biogeochemistry

What is the relationship between denitrifying bacteria and N₂O in agricultural soils?

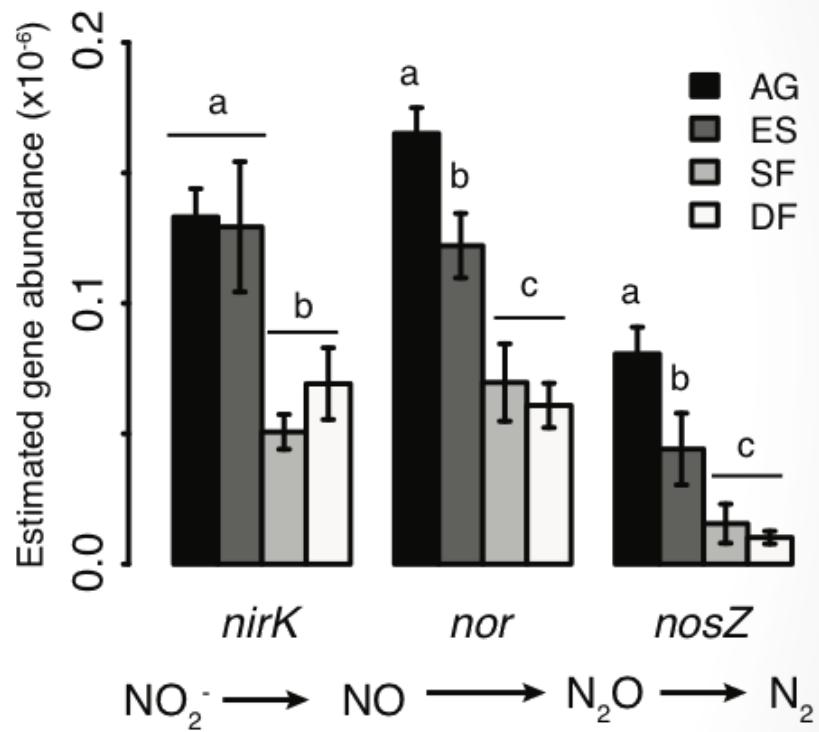
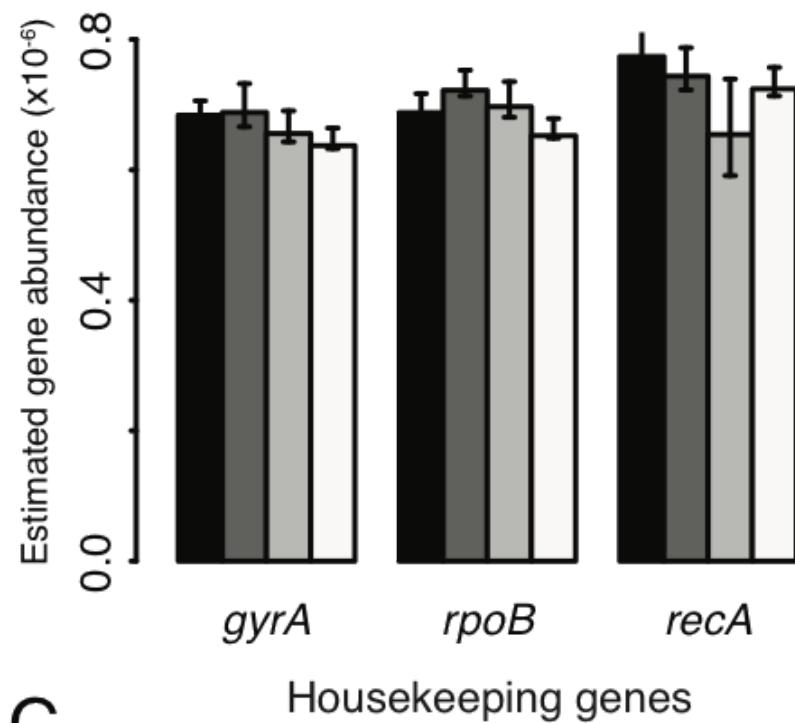
Denitrifying microbes



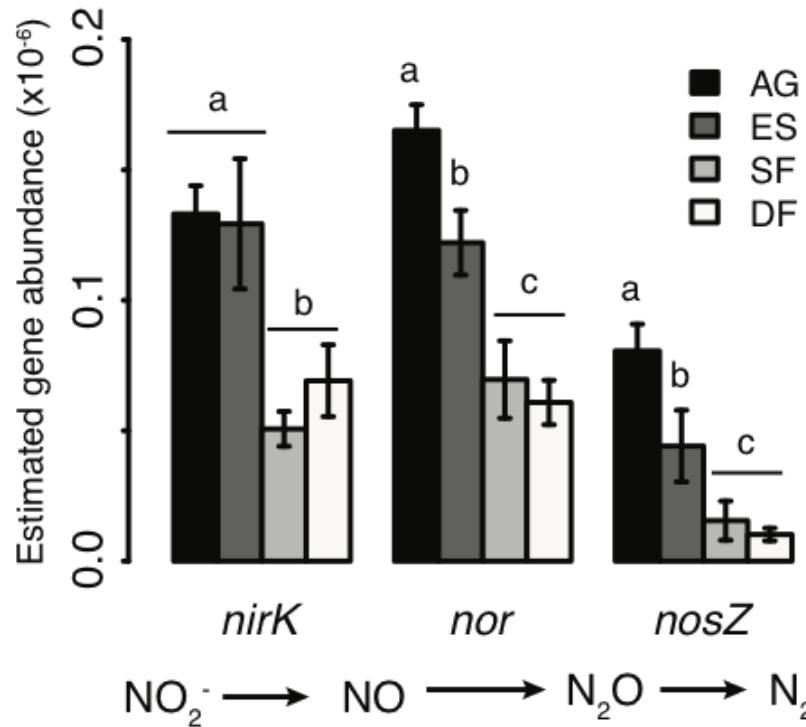
Denitrification contributes to the differentiation of communities



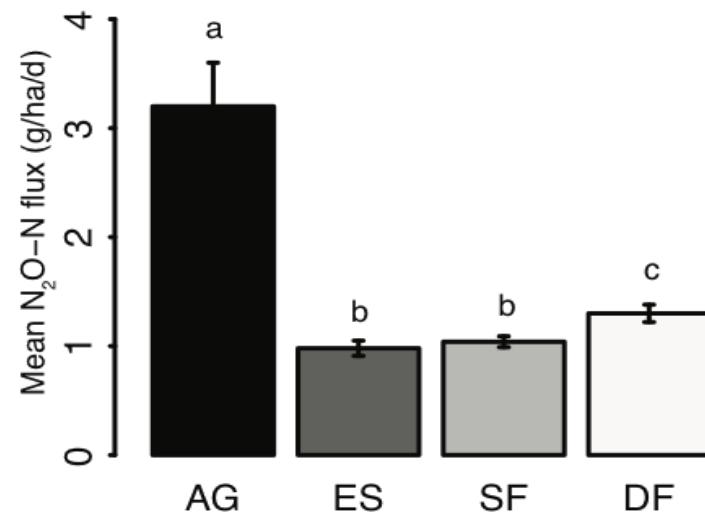
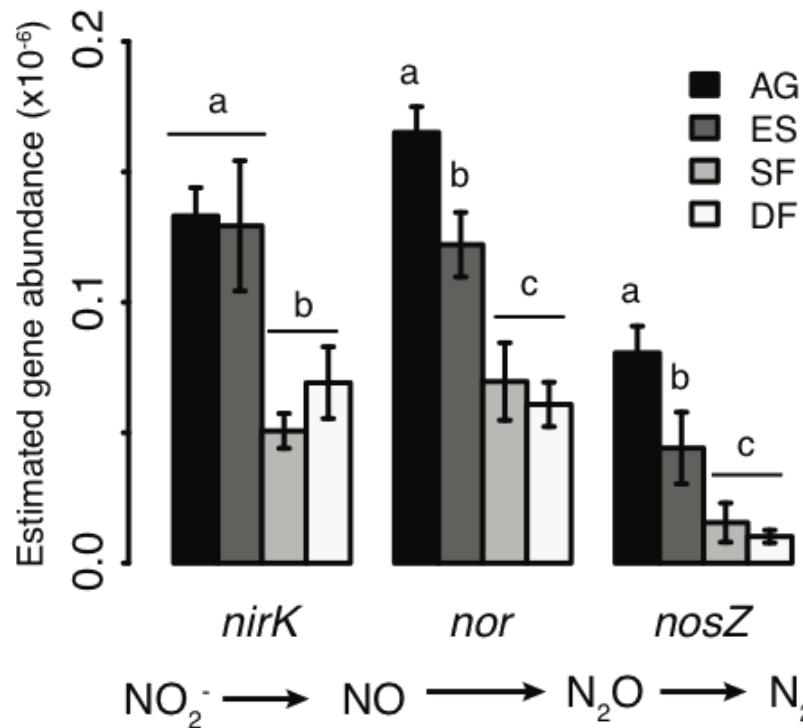
More denitrification potential in Ag soils



Not only abundance contributes to N₂O flux



Not only abundance contributes to N₂O flux

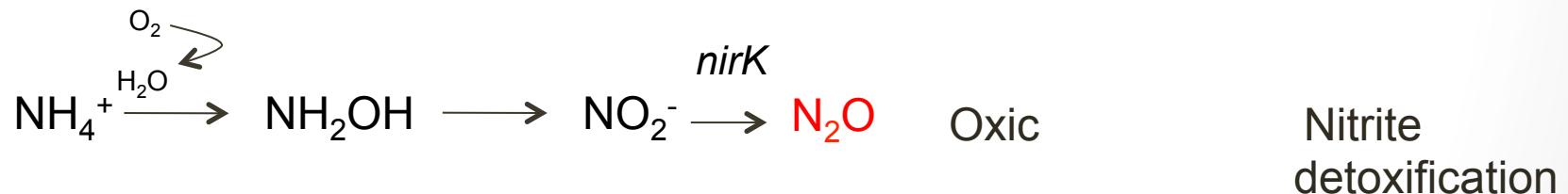


Types of denitrification

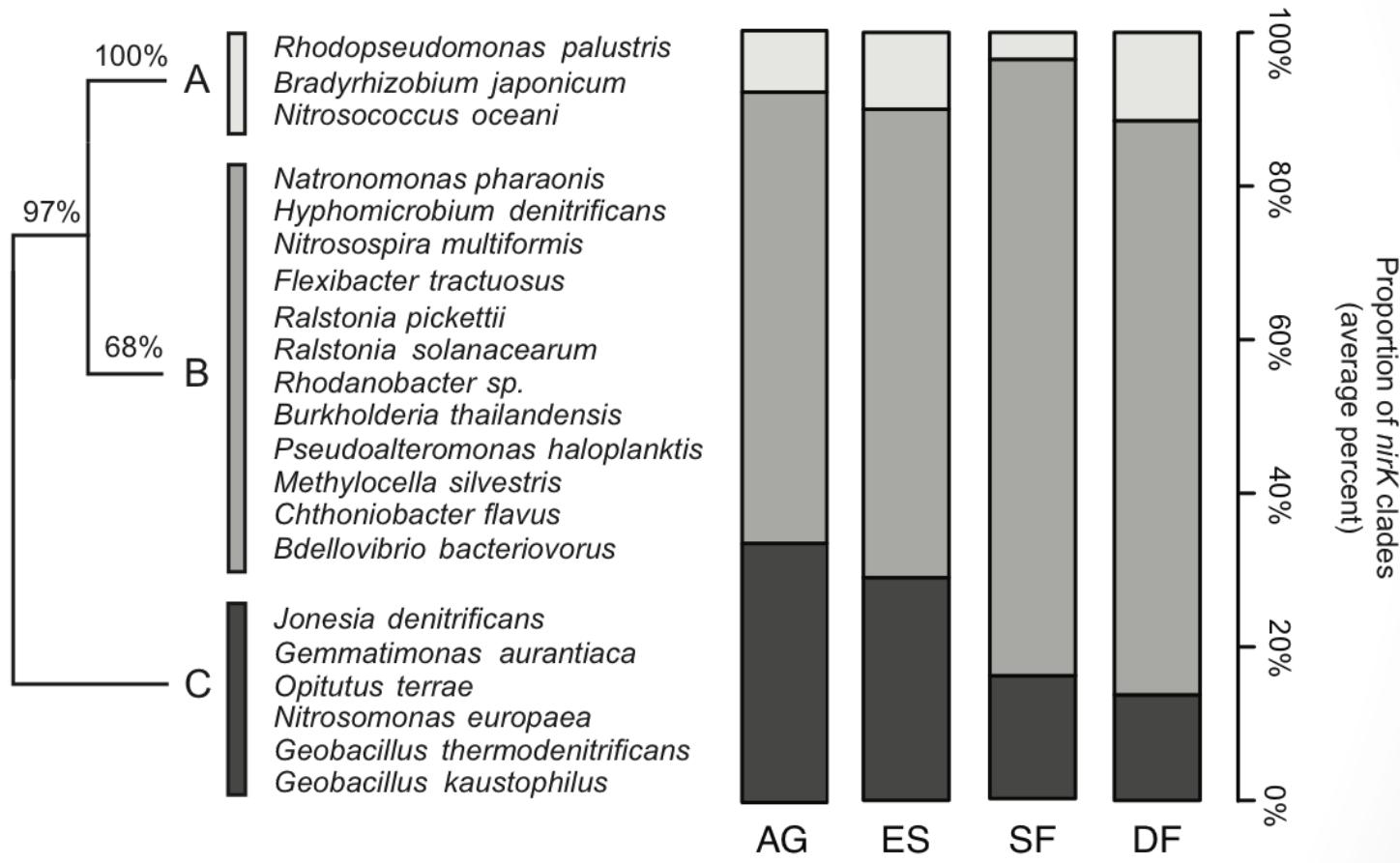
Heterotrophic denitrification



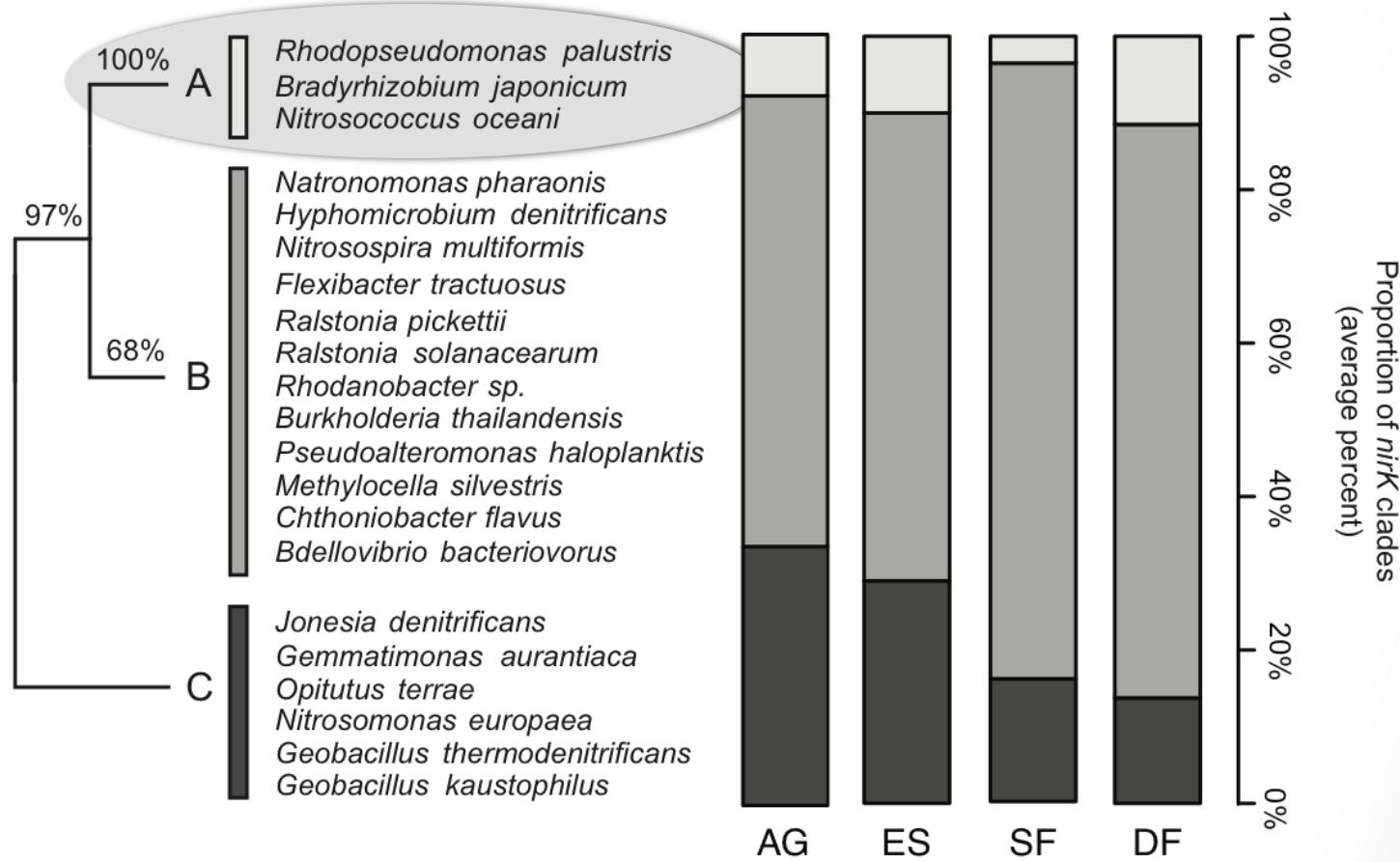
Autotrophic denitrification (AOBs)



Variability in denitrifier composition



High denitrifier diversity

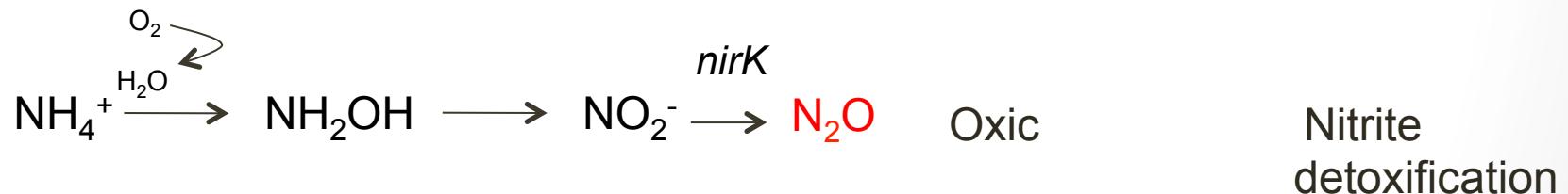


Types of denitrification

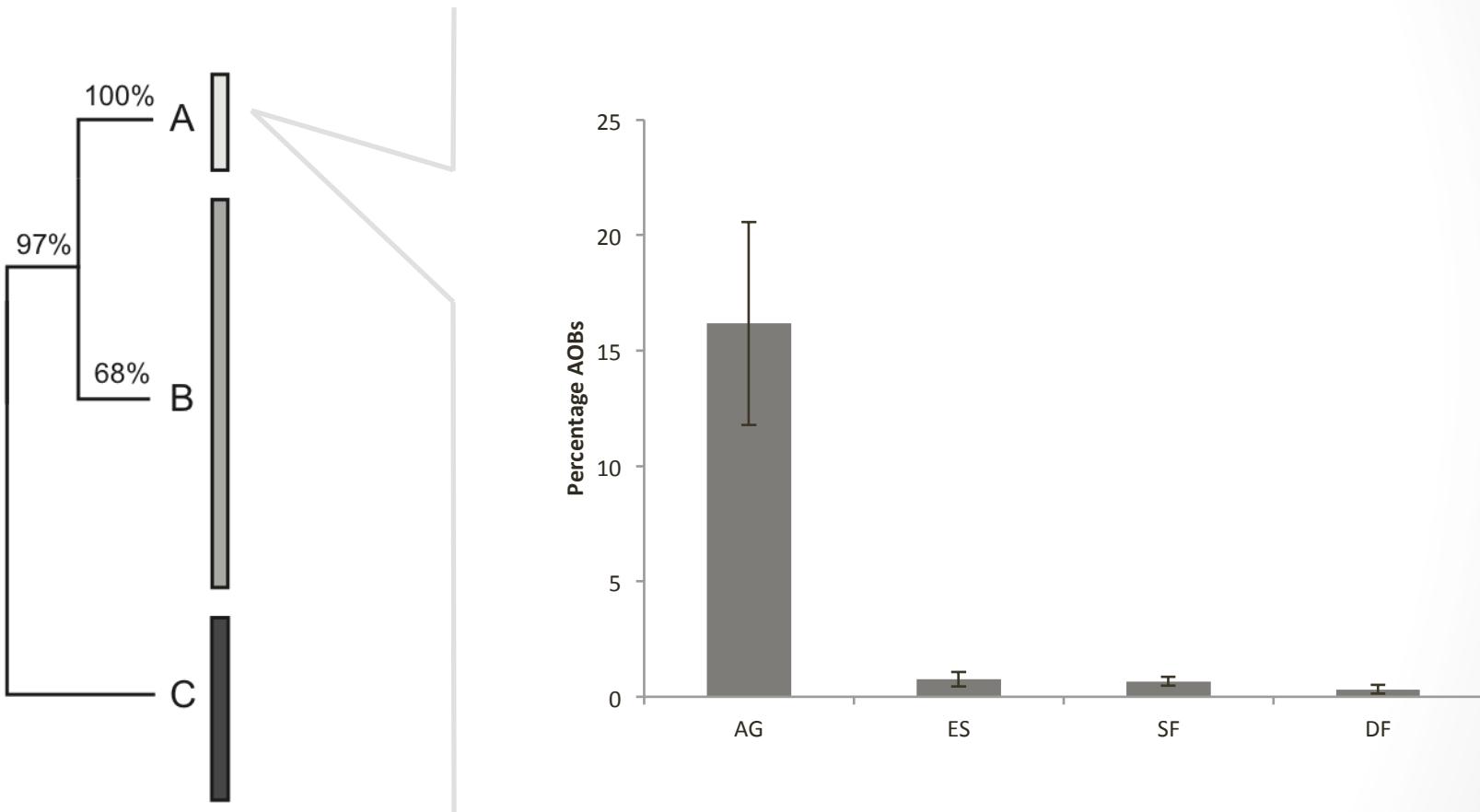
Heterotrophic denitrification



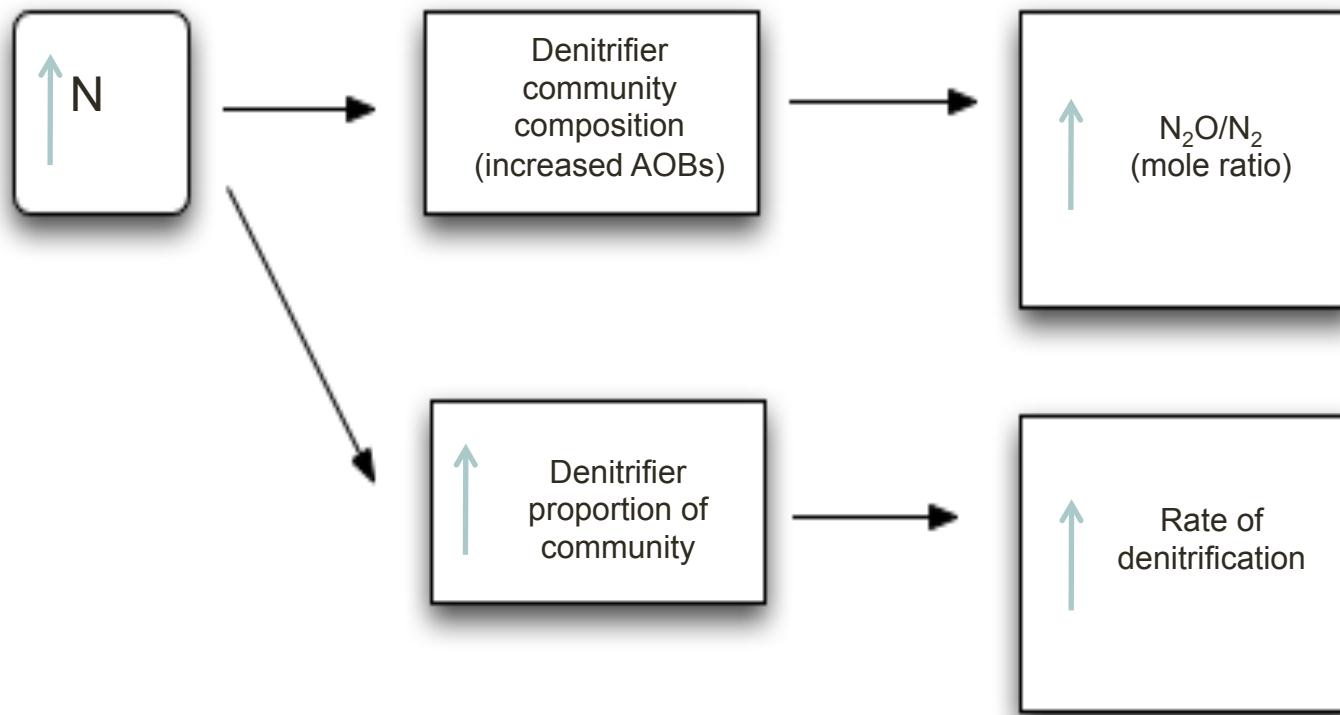
Autotrophic denitrification (AOBs)



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



Microbial communities in Ag sites with higher nitrogen availability are also poised for N_2O production



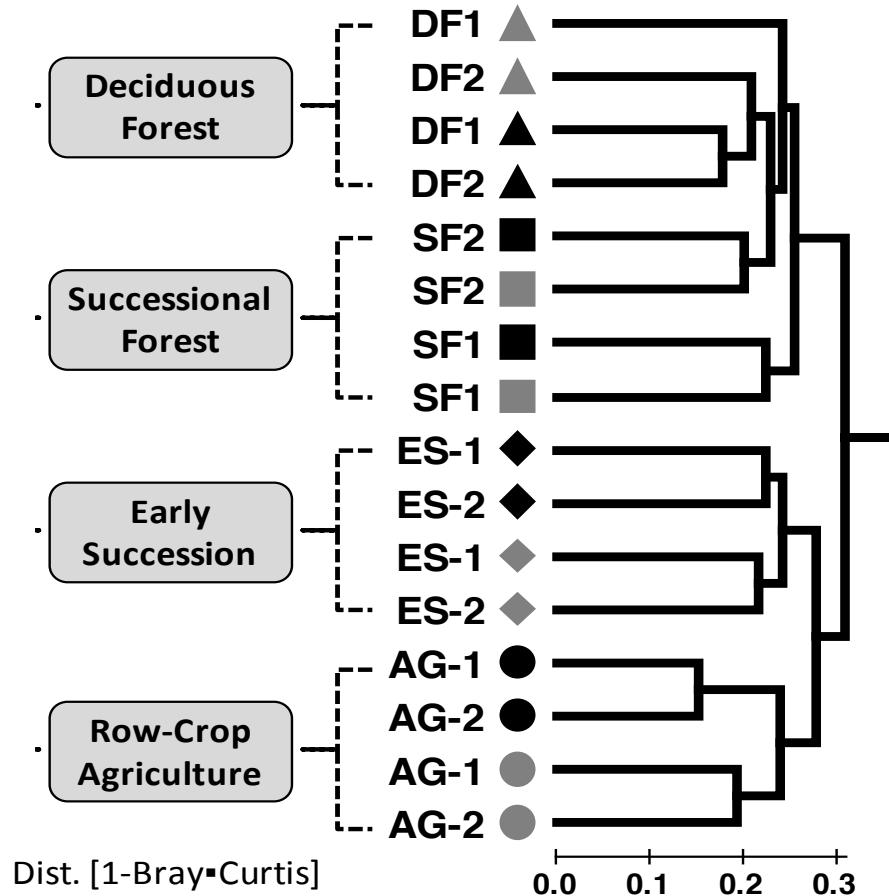
What is the relationship between denitrifying bacteria and N₂O in agricultural soils ?

- 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?

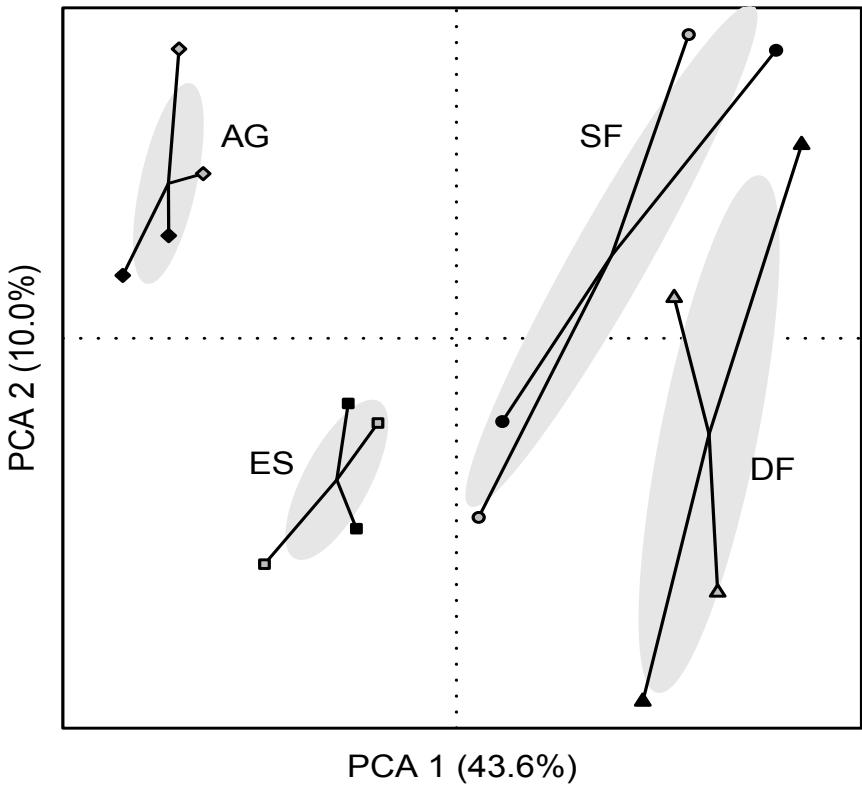
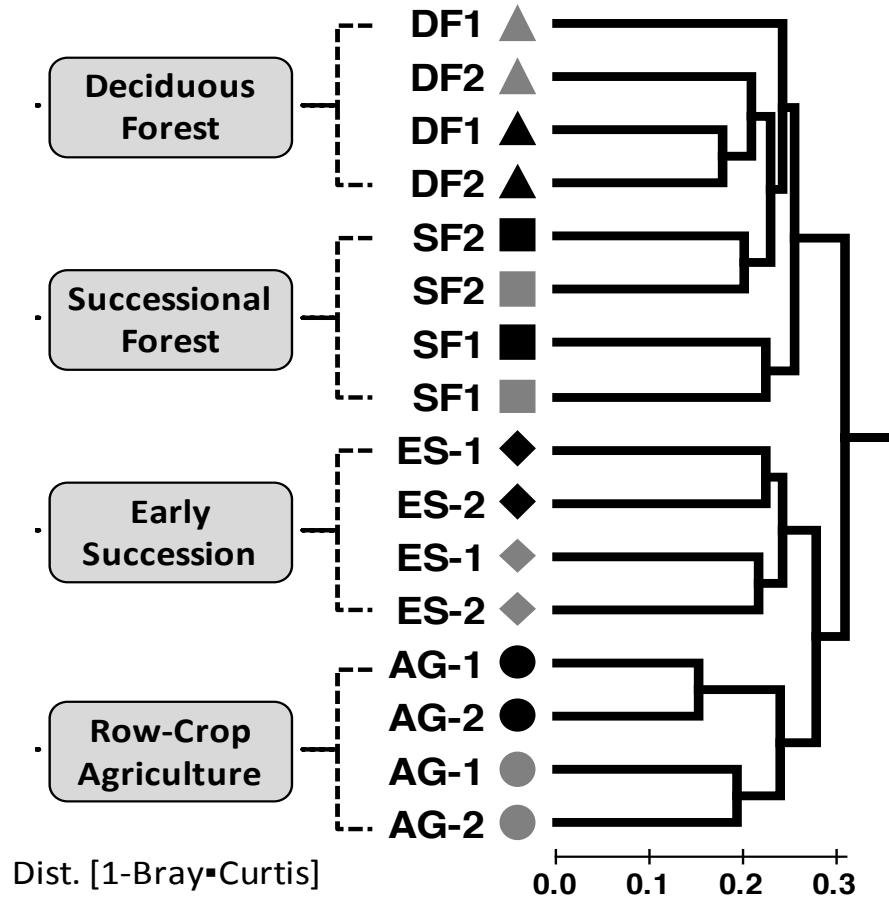
More than 20 year recovery for bacterial community

Microbial community

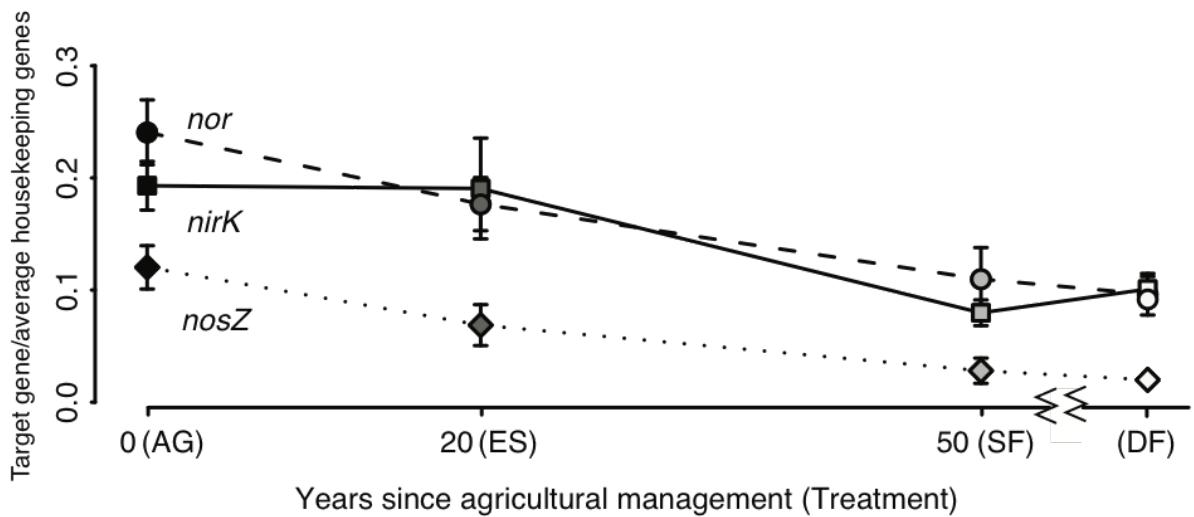


More than 20 year recovery for bacterial community

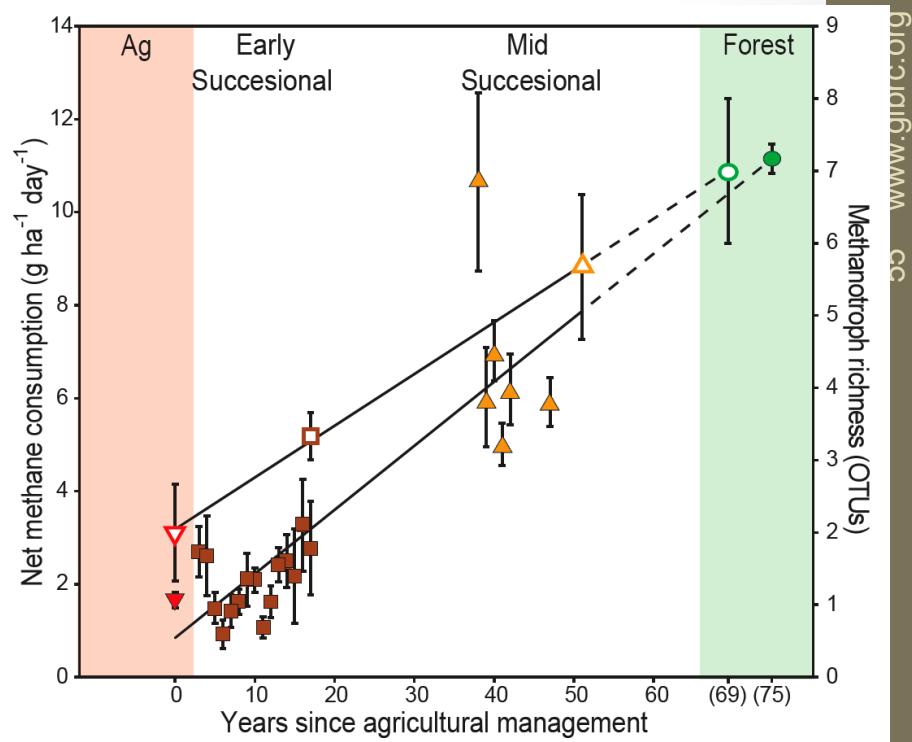
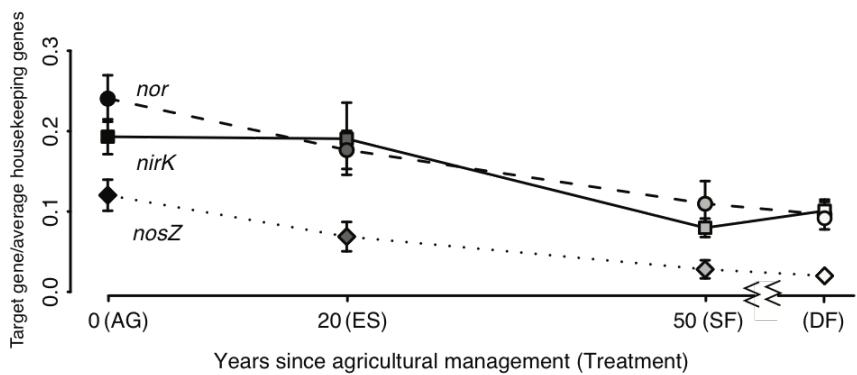
Microbial community



More than 20 year recovery for denitrifiers



Methanotrophs take more than 40 years to recover



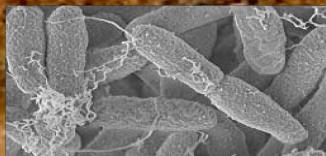


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Ben Roller
Tom Schmidt

✖ Funding
✖ DOE GLBRC
✖ NSF
✖ NSF Postdoctoral Fellowship

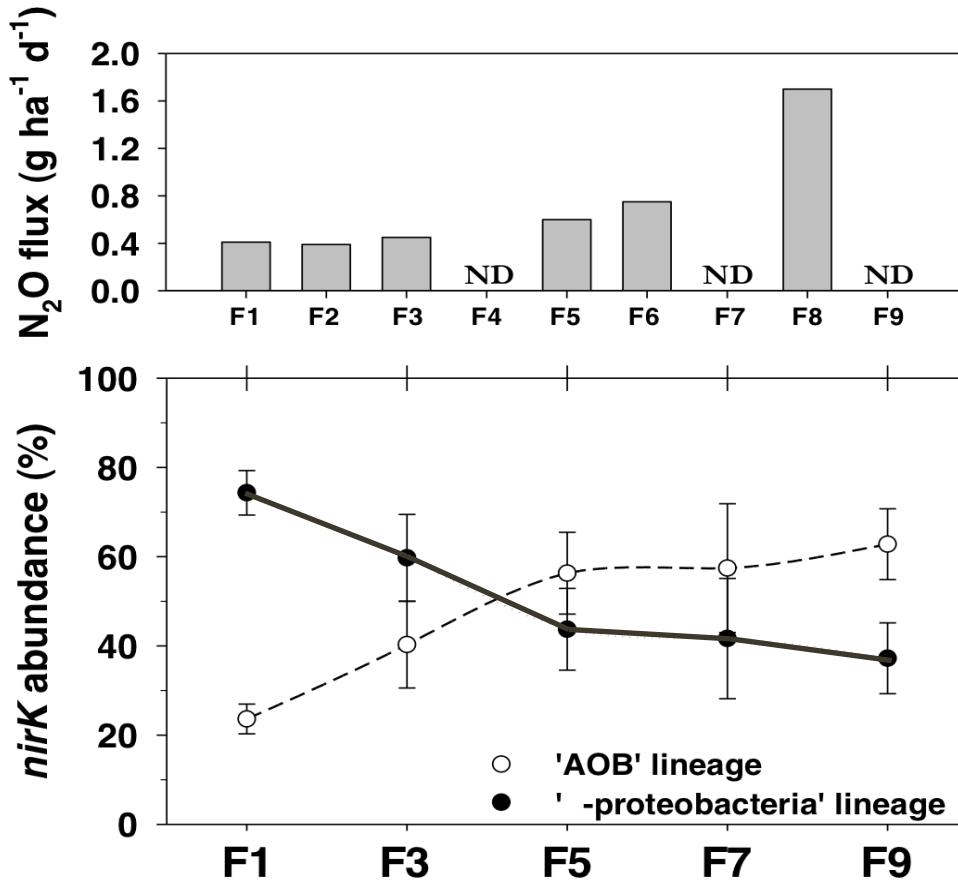


N_2O CH_4

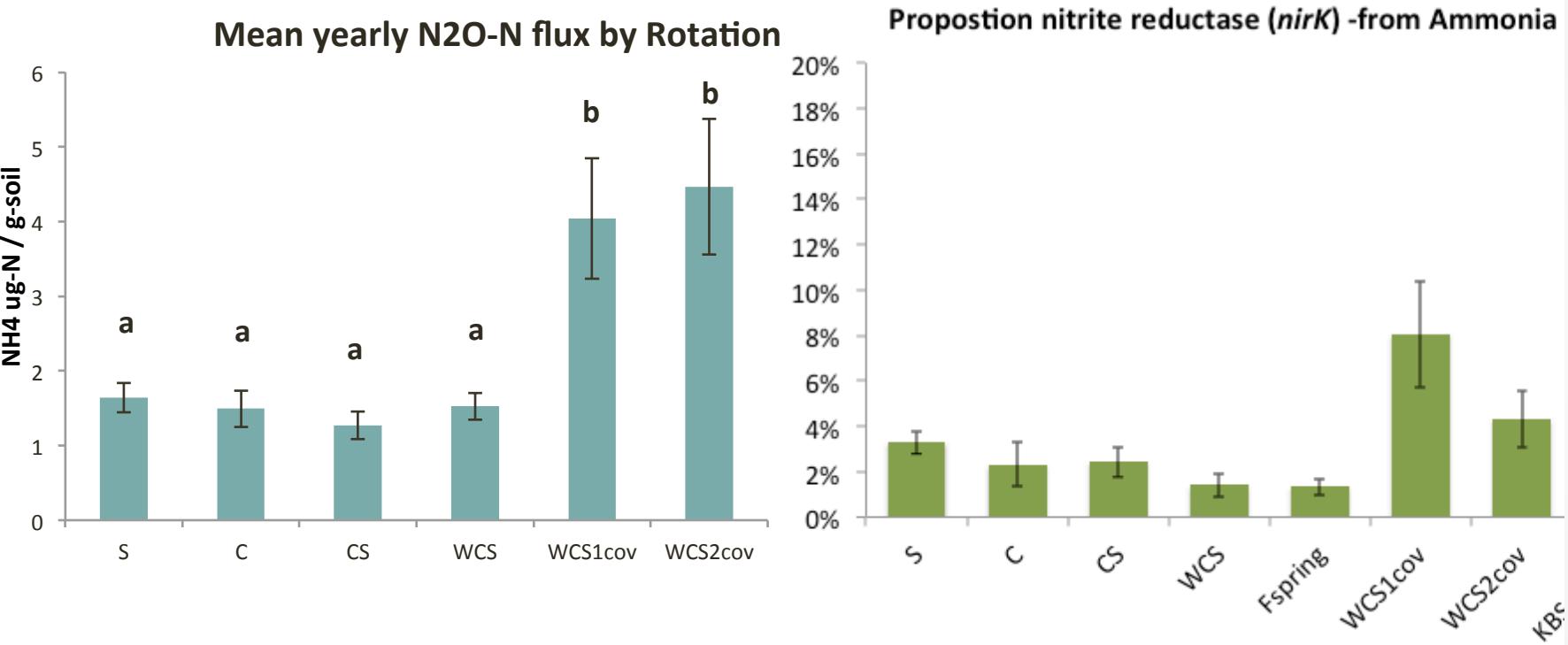


More on denitrifiers

AOB proportion correlates with fertilization level and N₂O production

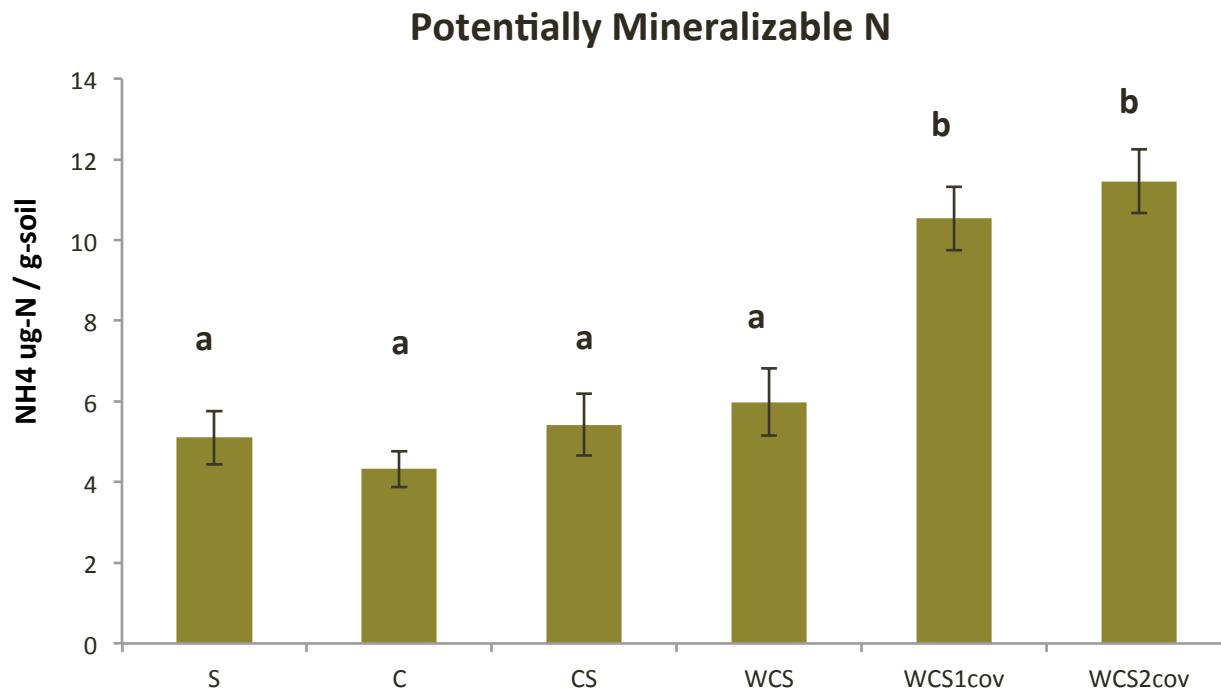


AOB proportion correlates with cover crops and N₂O production

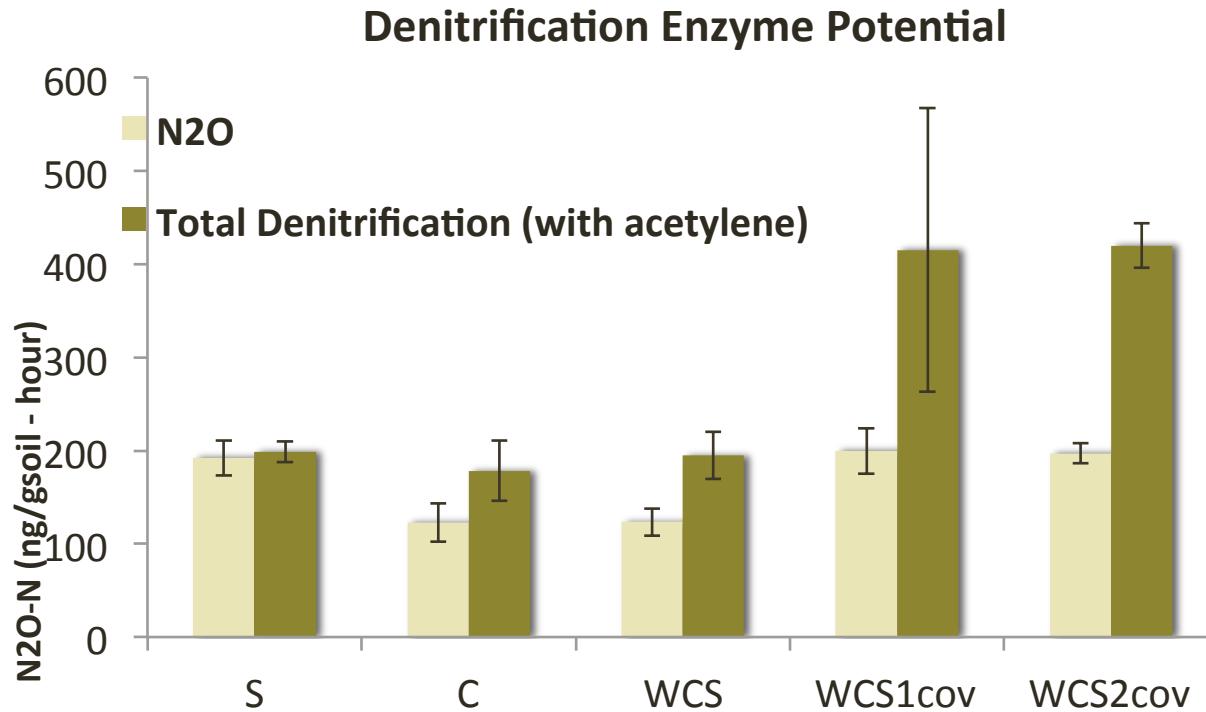


(60)

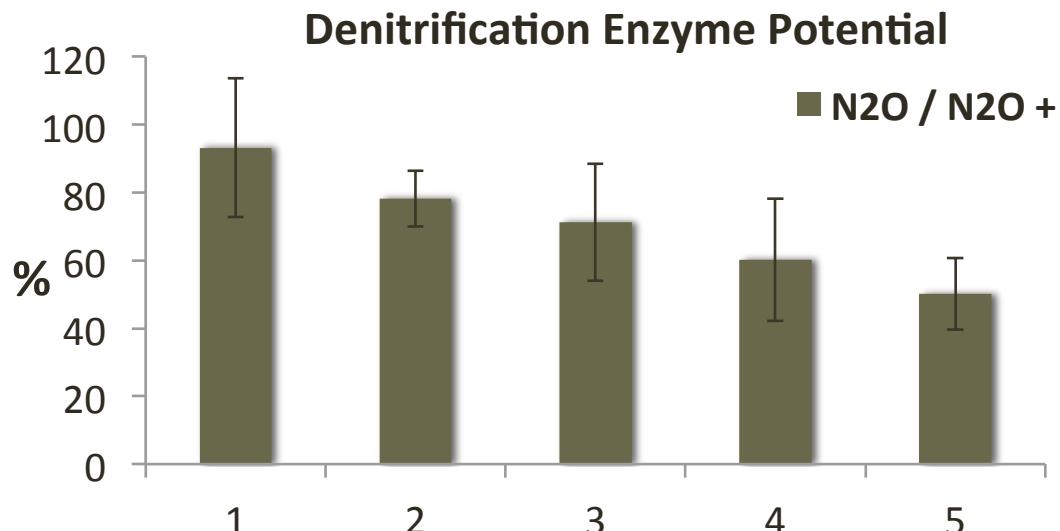
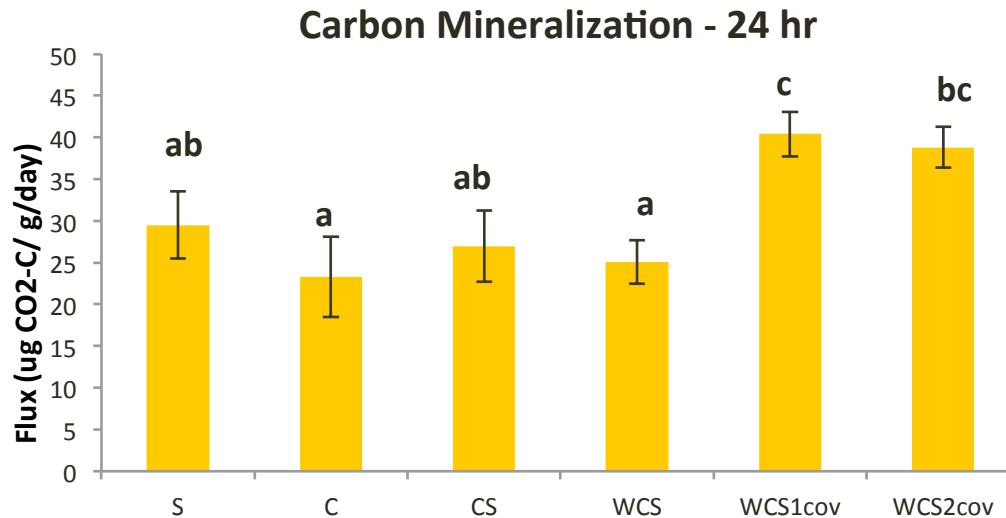
Suggests source of nitrogen is not important for AOBs or N_2O production



Heterotrophic denitrification (no AOBs)

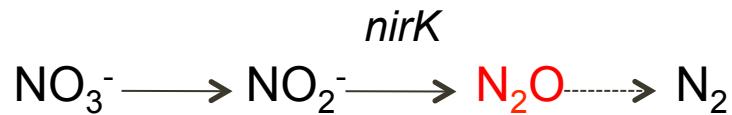


Carbon availability also important



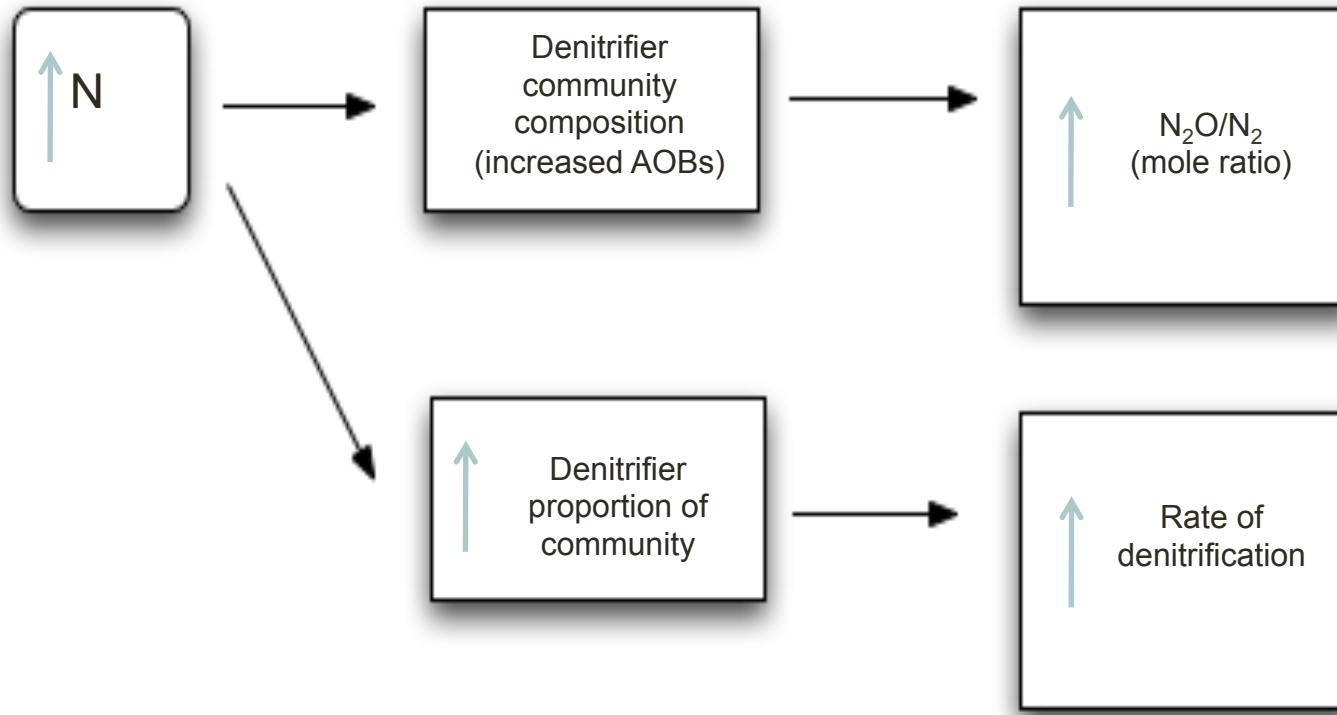
Types of denitrification

Heterotrophic denitrification



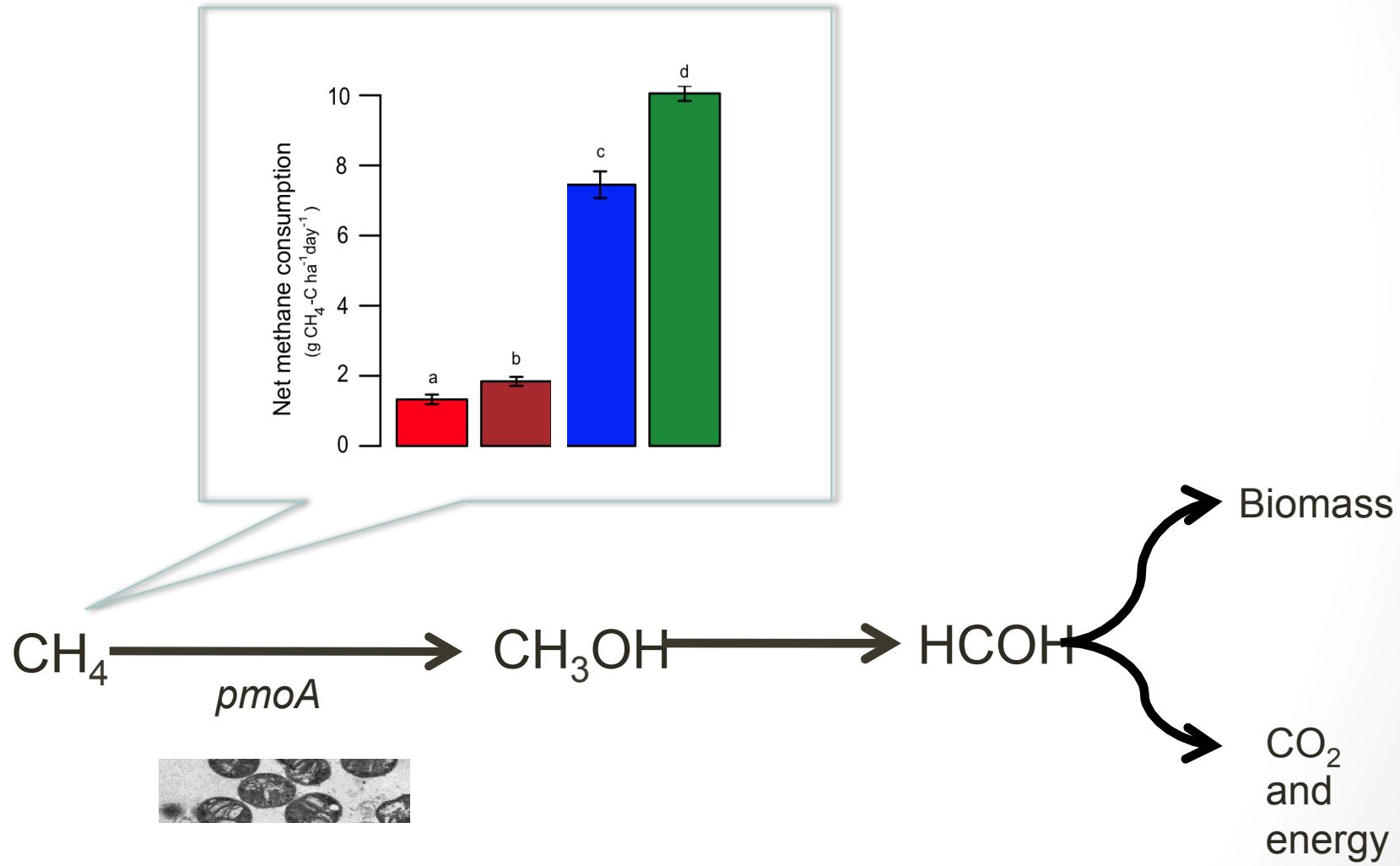
Conditions	Function
Anoxic	Energetics

More denitrifiers and more with the potential just produce N₂O but ratios and mechanisms still not understood

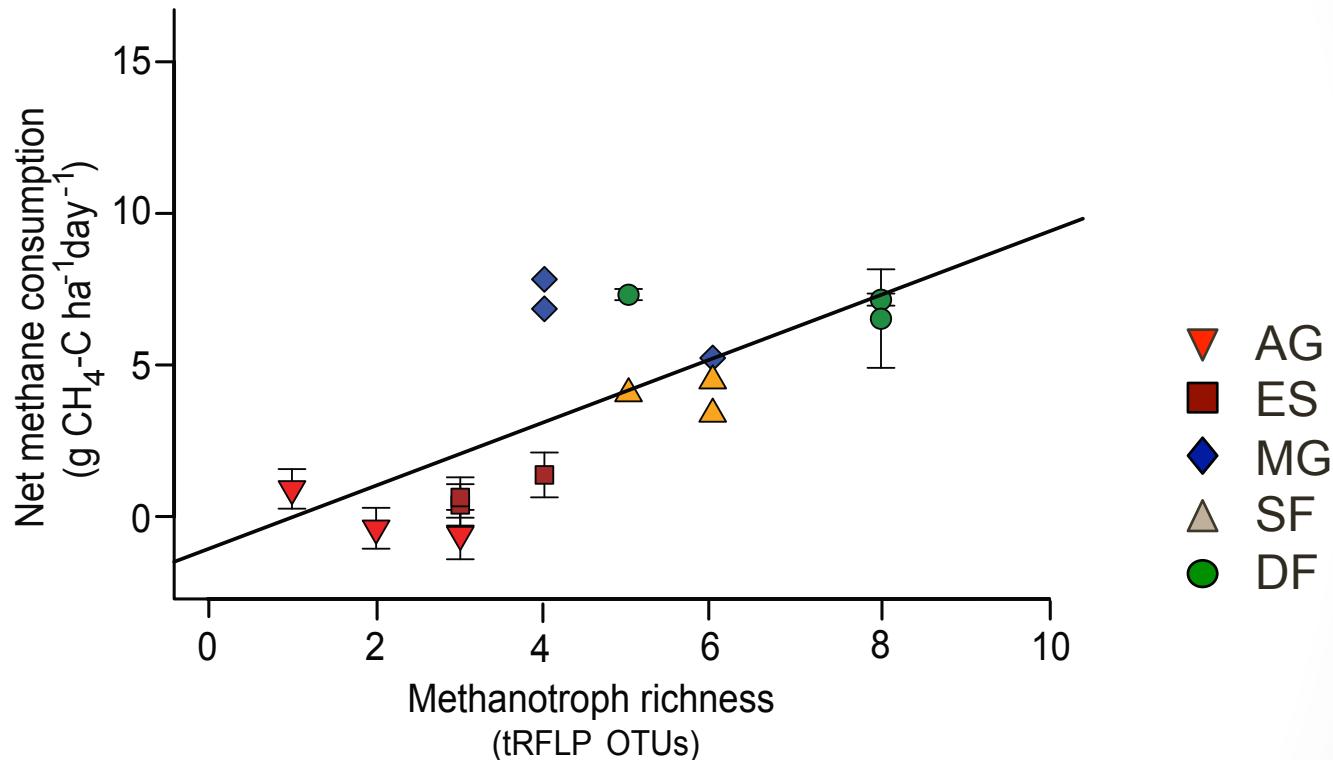


Methanotrophs

Methanotrophs use methane as a sole source of carbon and energy



Methanotroph richness correlates with methane consumption

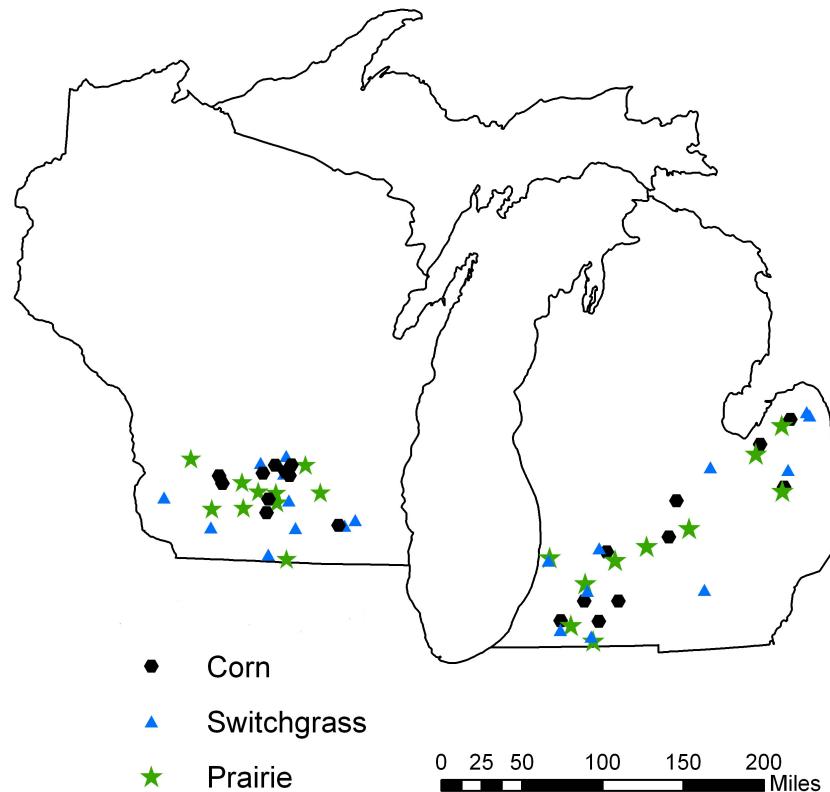


Levine, Teal, Robertson & Schmidt 2010

- Uri Levine
- Bernard Schroeter

Methane in biofuel crops

GLBRC Extensive Sites



Clone libraries

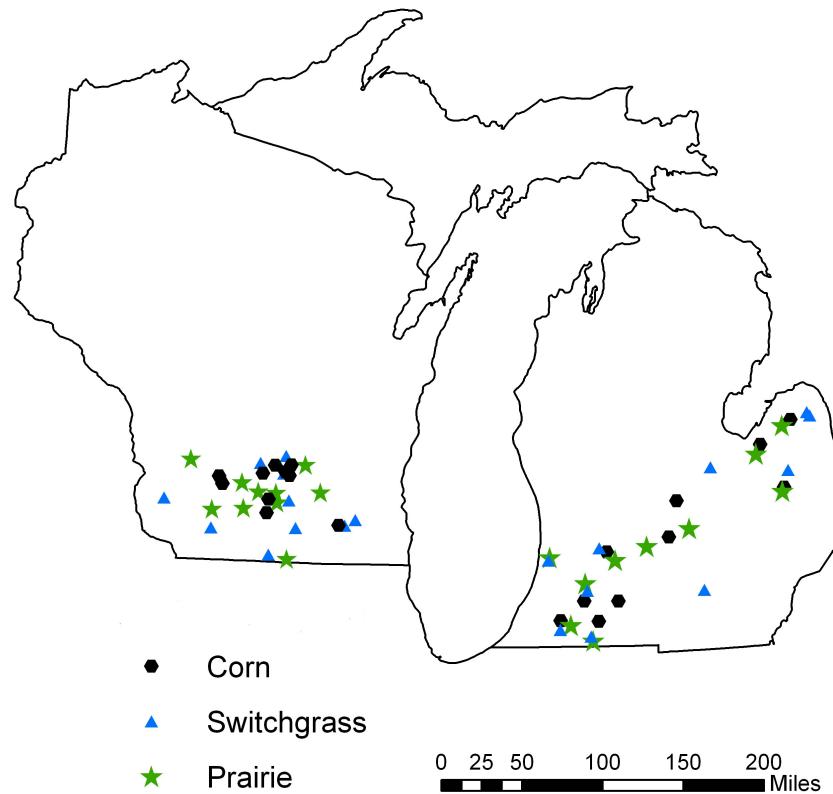
Classification of *pmoA* / *amoA*

GFClassify –
Ribosomal Database Project
Jordan Fish

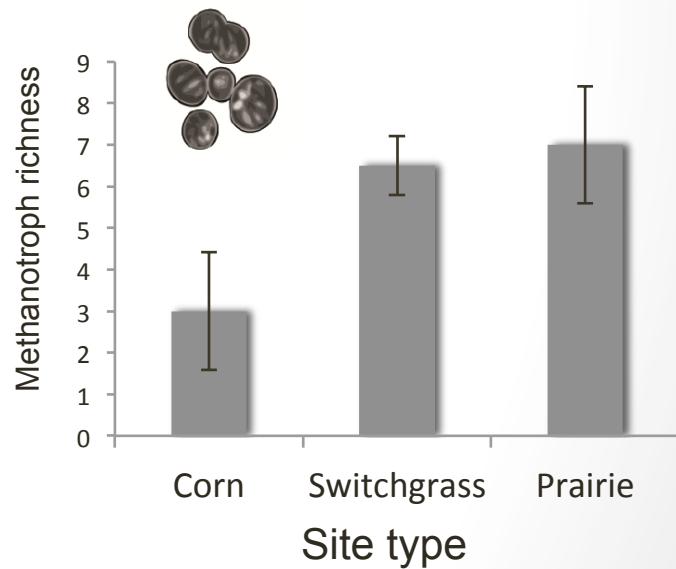
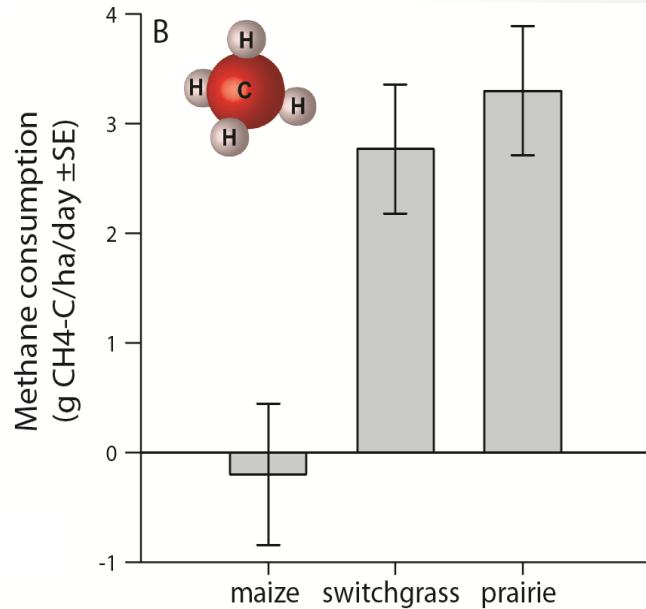
Leilei Ruan and G. Philip Robertson

Methane in biofuel crops

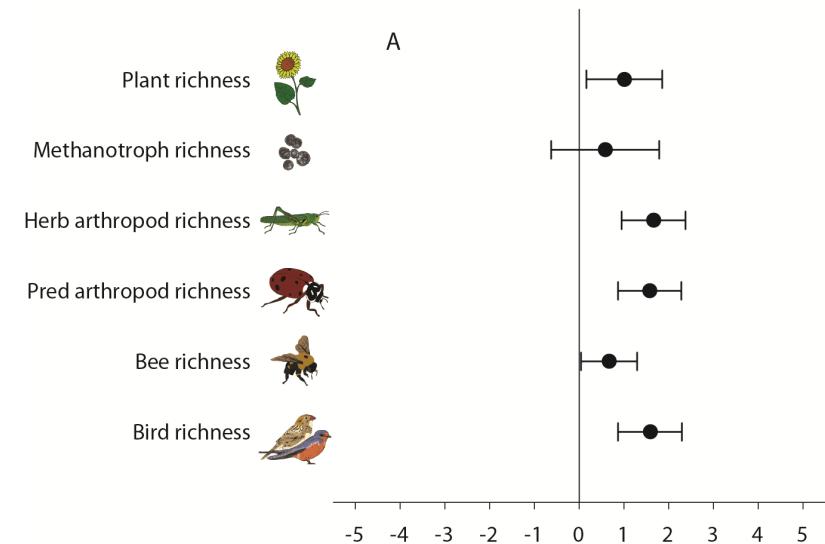
GLBRC Extensive Sites



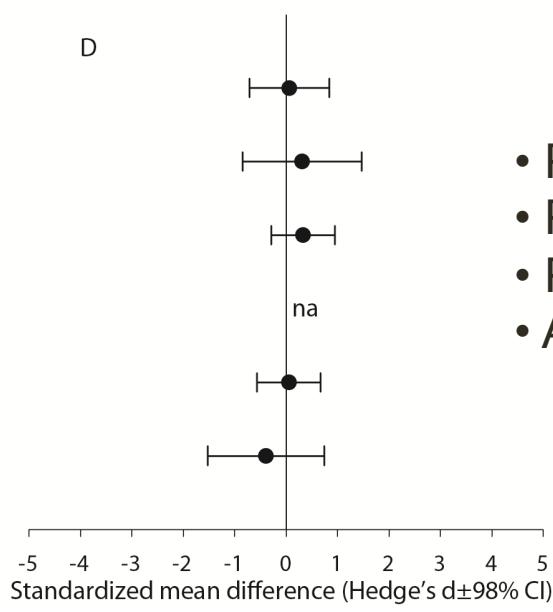
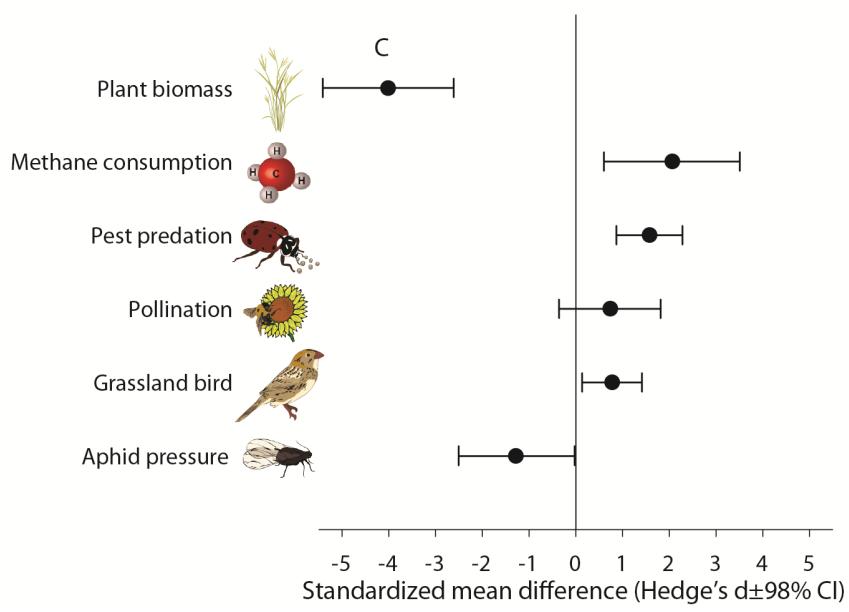
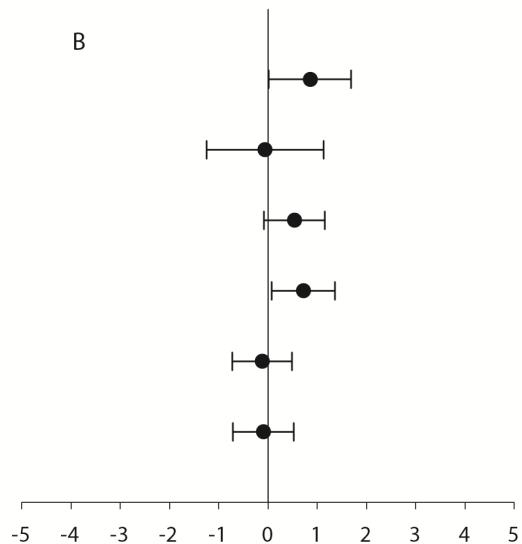
✗ Leilei Ruan and G. Philip Robertson



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

