Microbial communities as resilient systems: from one to one billion

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MBL Microbial Diversity

Resilience

1. Interacting parts make up a system.

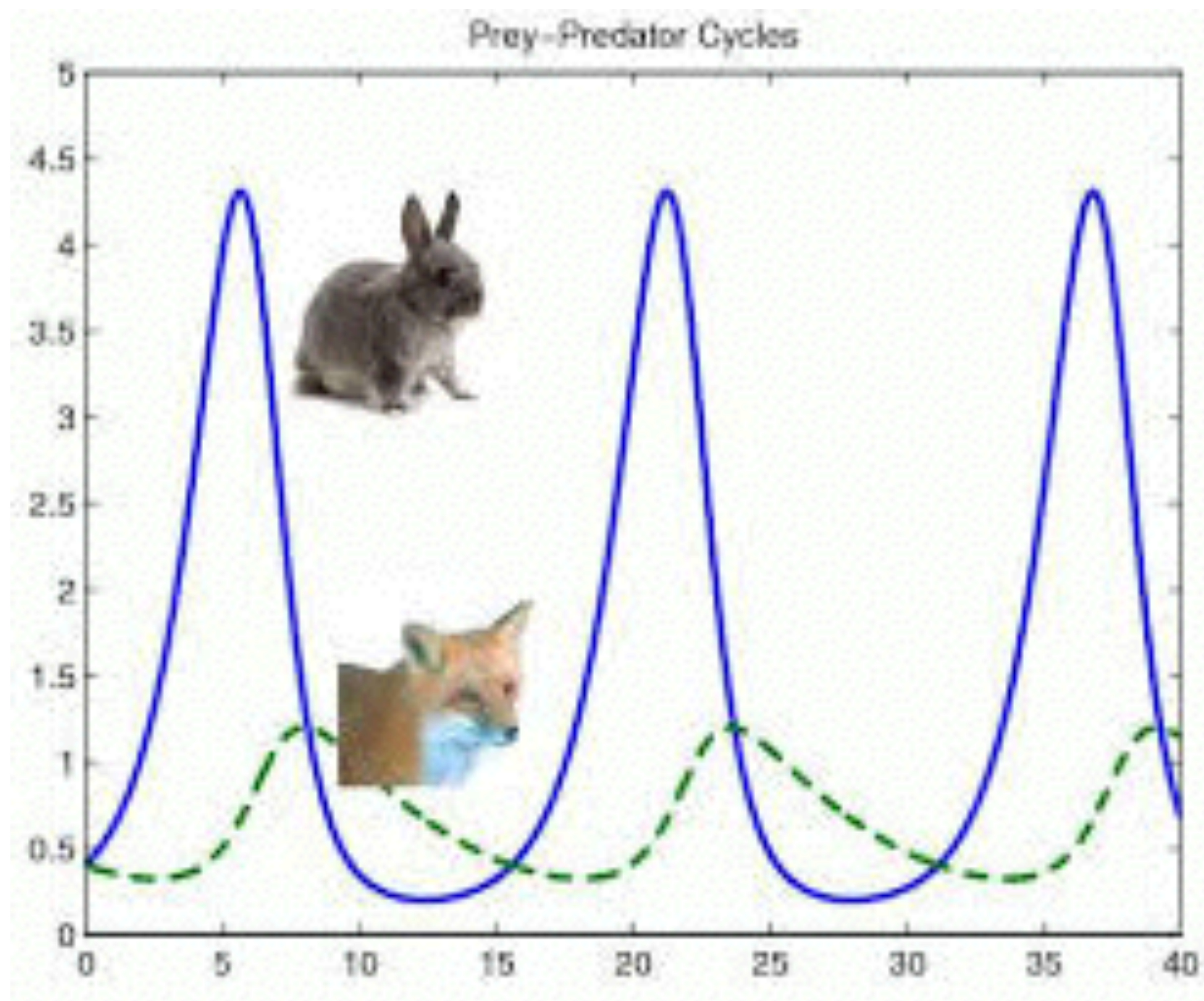
2. The system has some adaptive capacity.

The system has the ability to withstand, recover, or reorganize in response to environmental perturbations.

Resilient communities

Community is maintained but the system structure may not be

Lotka-Volterra interspecific competition (1932)



Communities of microbes & their interactions

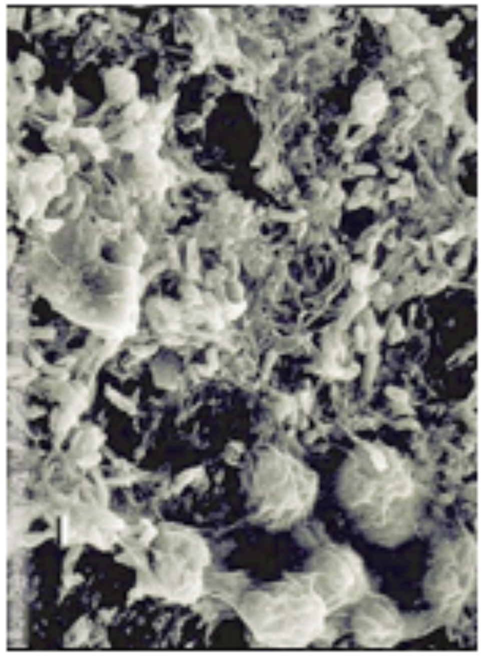
Putting the informatics back into bio

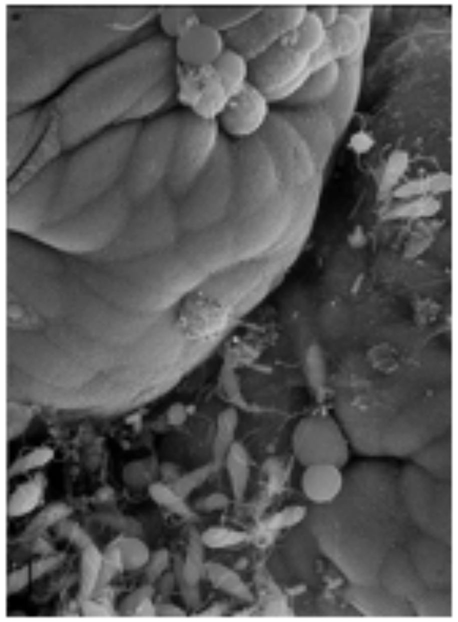
Single-species *Shewanella oneidensis* biofilm

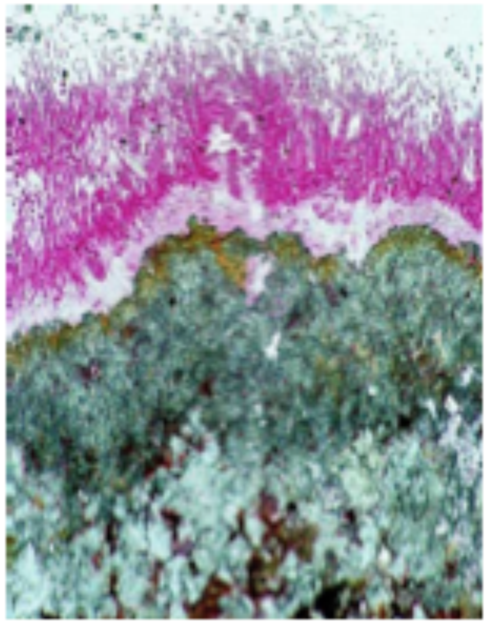
The role of SoxR in response to phenazines

Microbial communities and greenhouse gas fluxes in response to agricultural land management

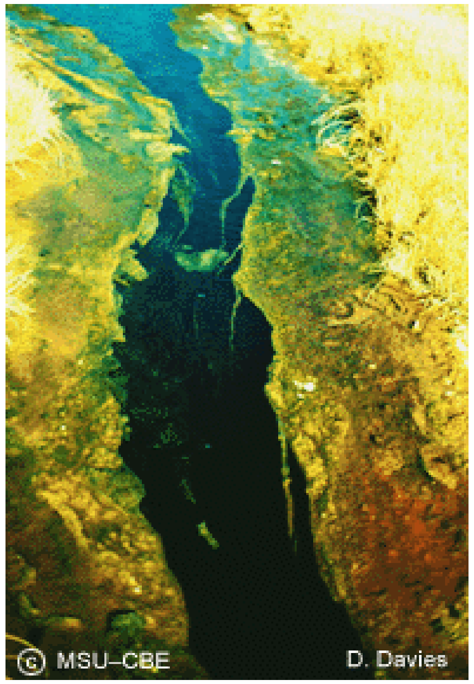
Bacteria live in communities called biofilms

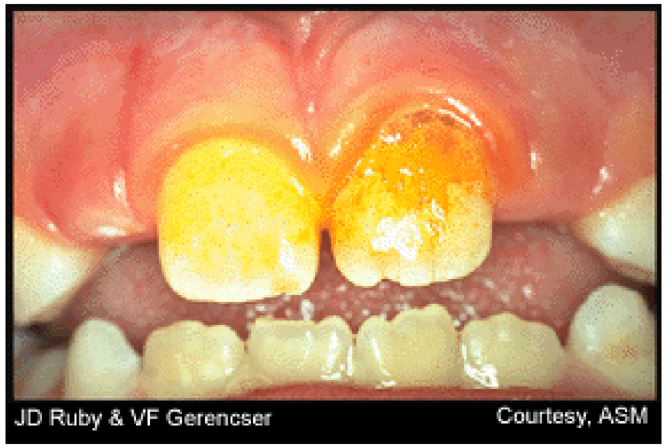


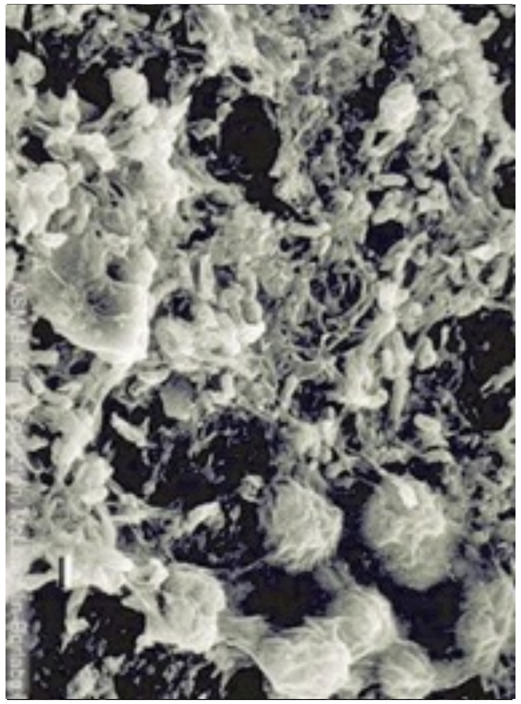


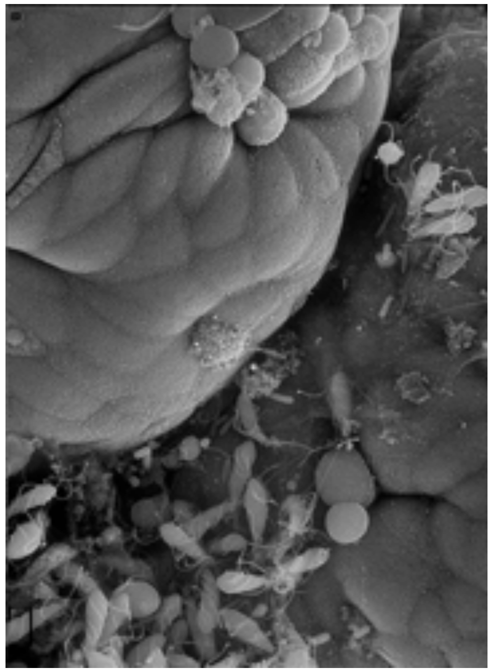


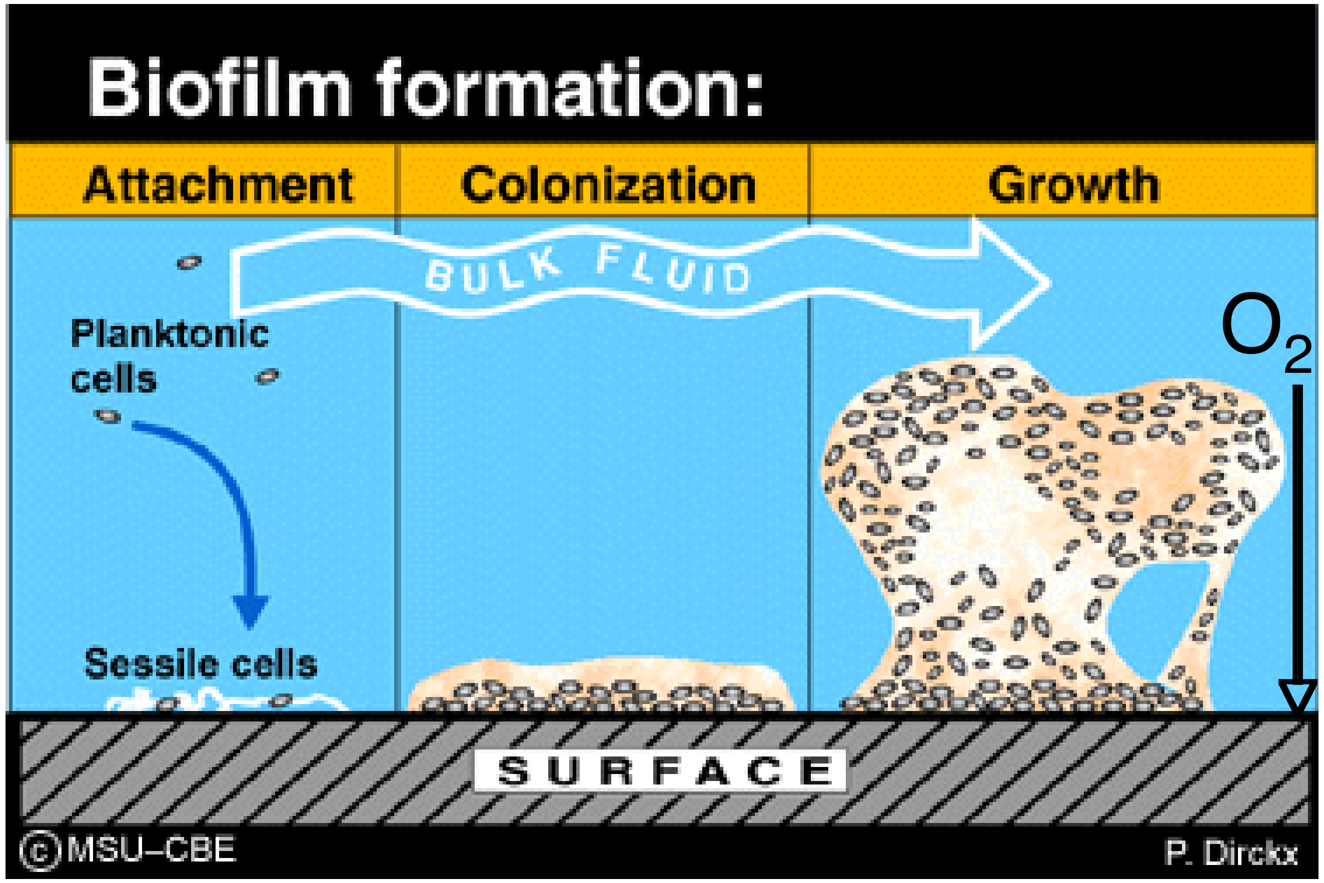
Biofilms
Spatially stratified microbial communities

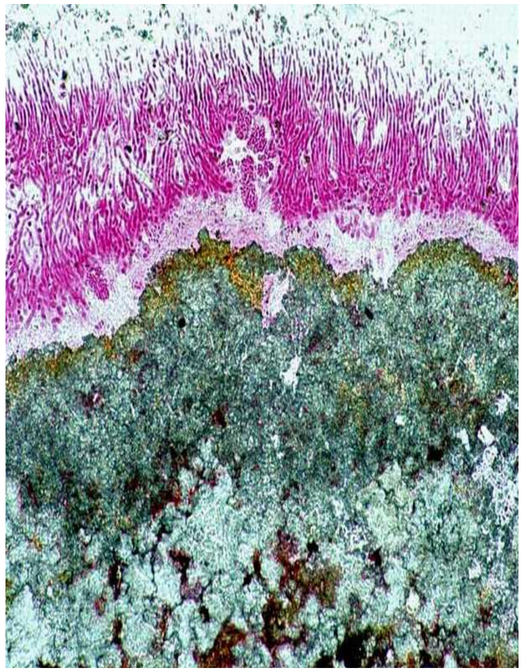




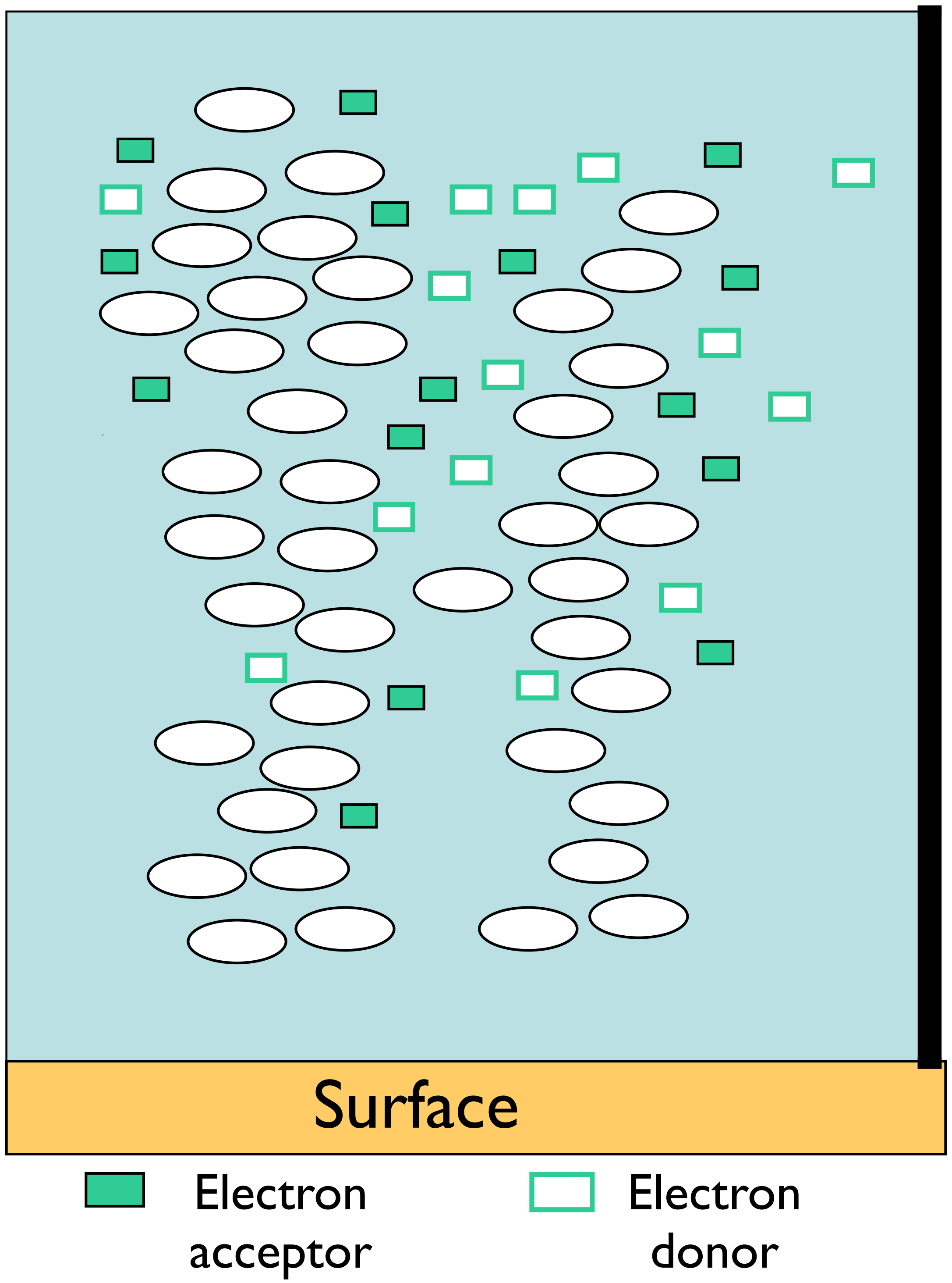


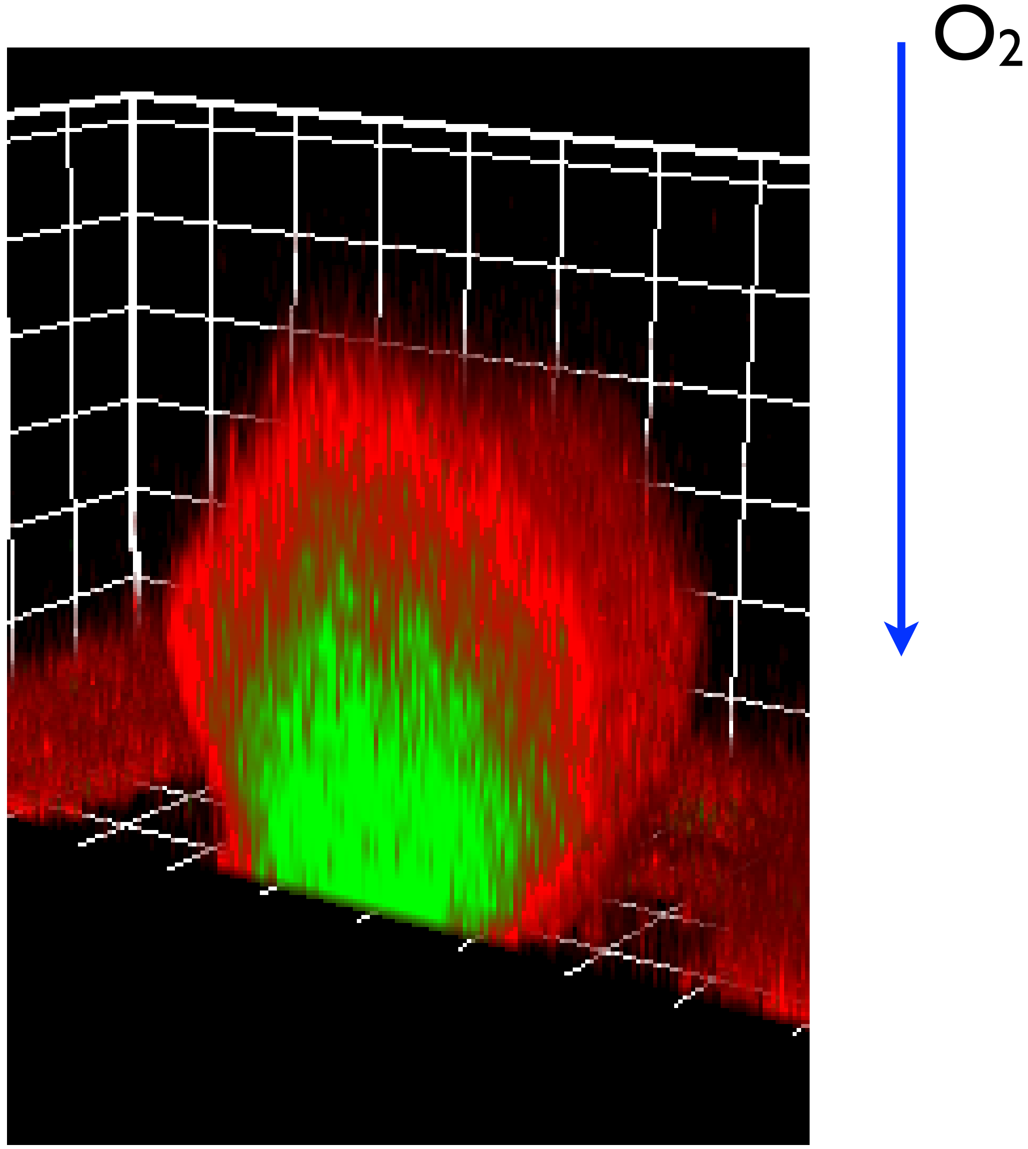






Heterogeneity within biofilms





Spatial organization in biofilms

- What is the spatial and temporal stratification of growth and metabolic states within a biofilm?

- Does availability of nutrients affect growth and metabolic stratification and can this stratification respond and change dynamically?

Shewanella oneidensis MR-1

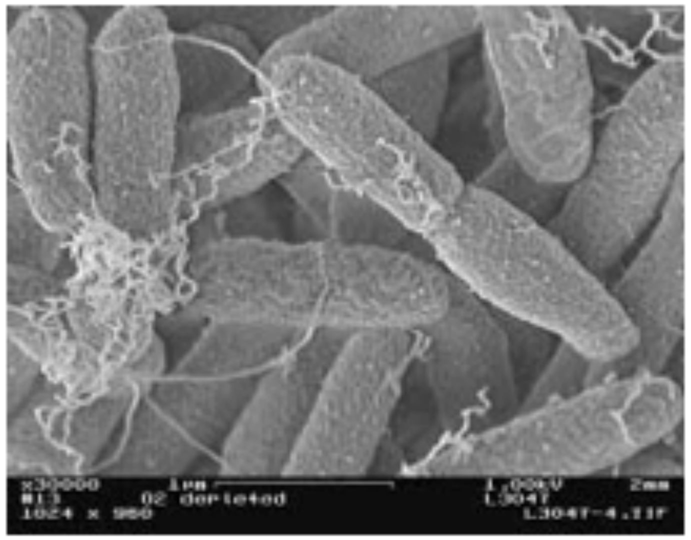
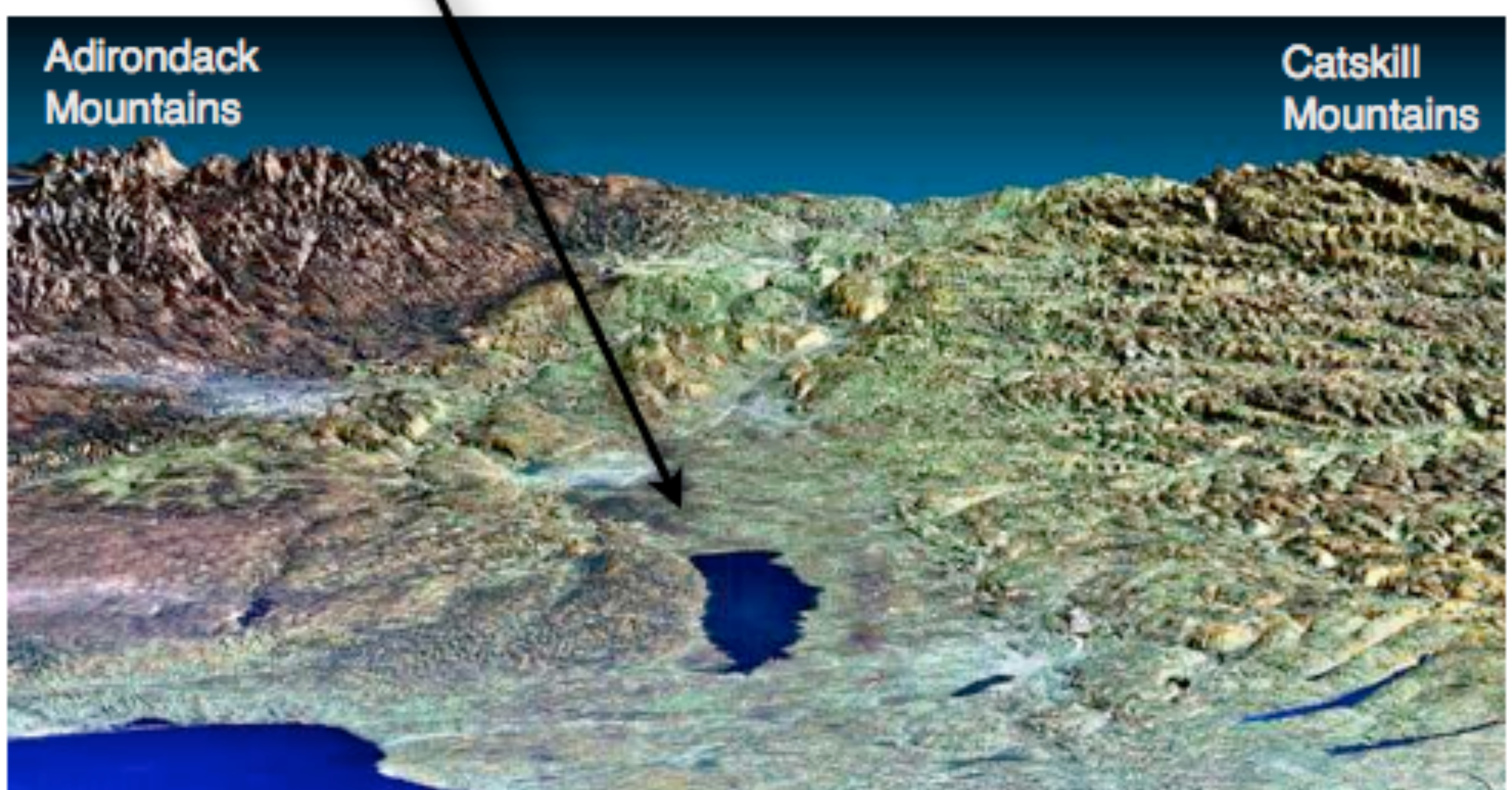


Image: PNNL

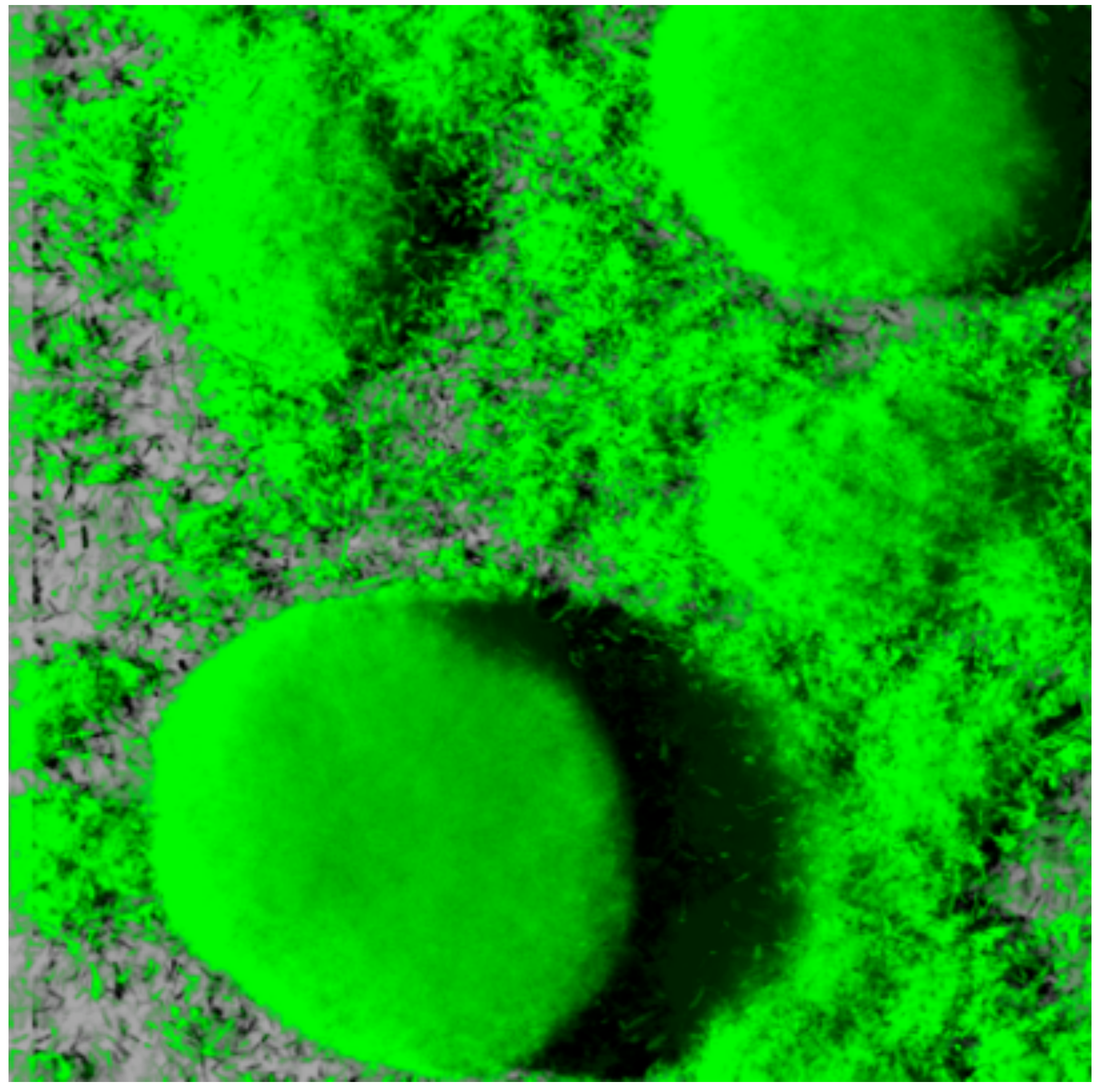
- Facultative anaerobe
- Versatile respiration
- Forms biofilms
- Metabolism well-characterized
- Genome sequenced
- Manipulated genetically

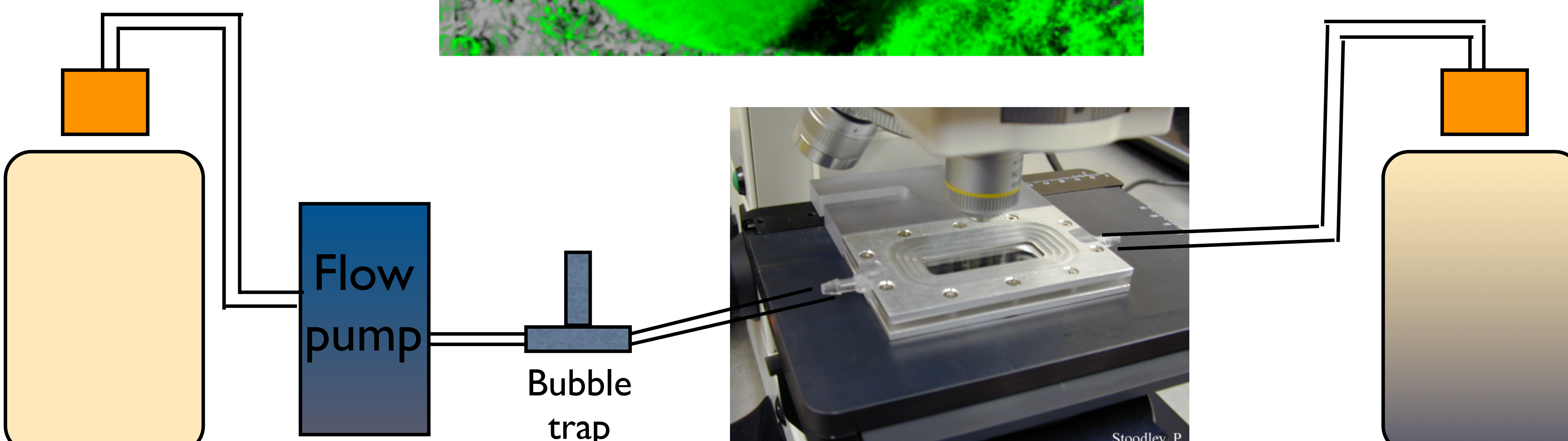


Lake Ontario

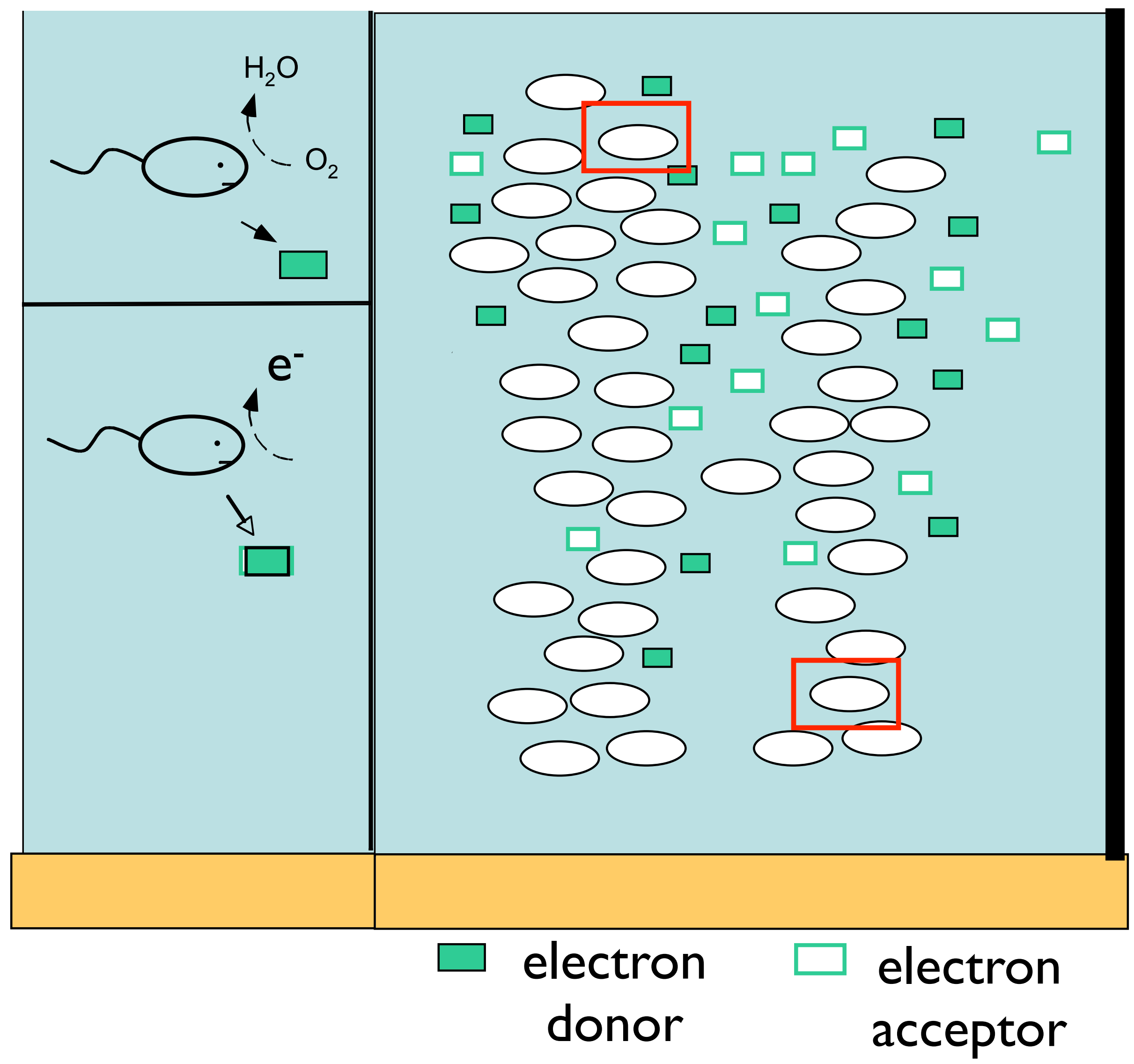
Photo: NASA

Experimental setup

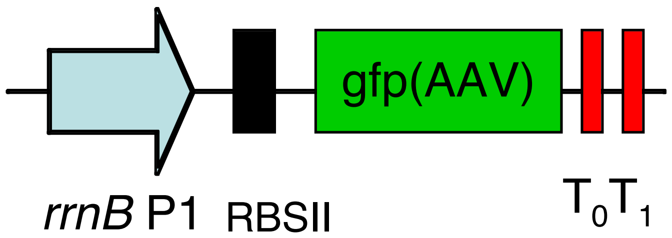




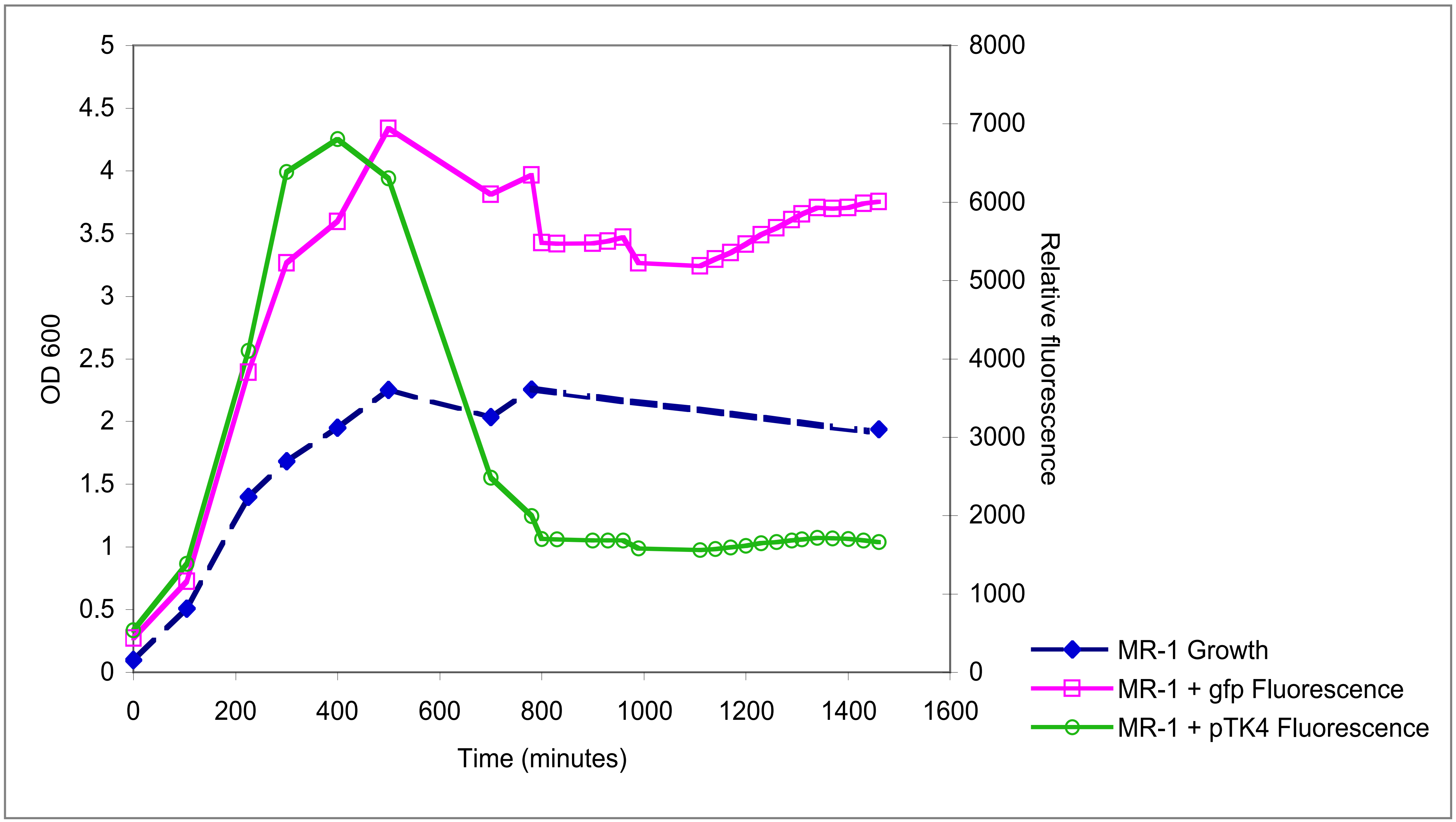
What is the growth state of cells in a biofilm?



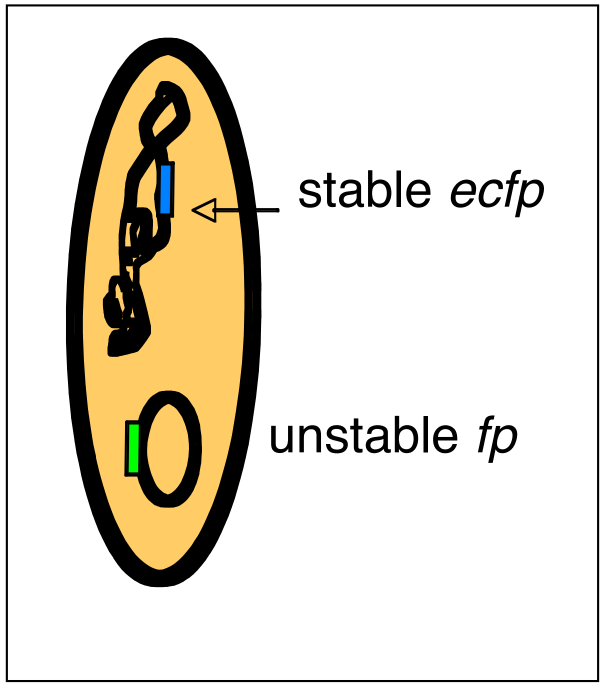
In vivo reporter of growth activity

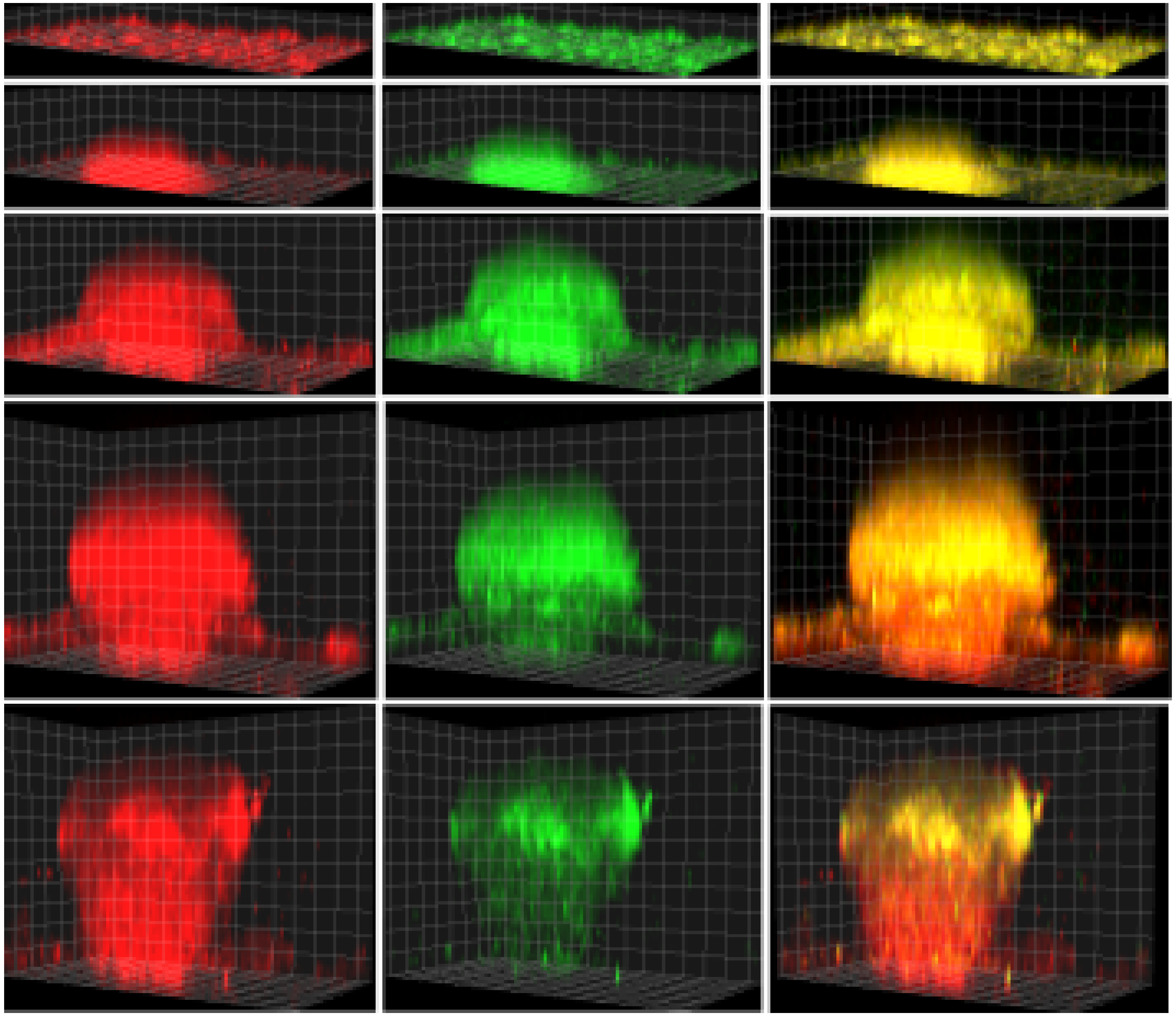


A reporter system for ribosomal RNA expression is correlated with growth activity



Cells have variable growth state at different regions of the biofilm



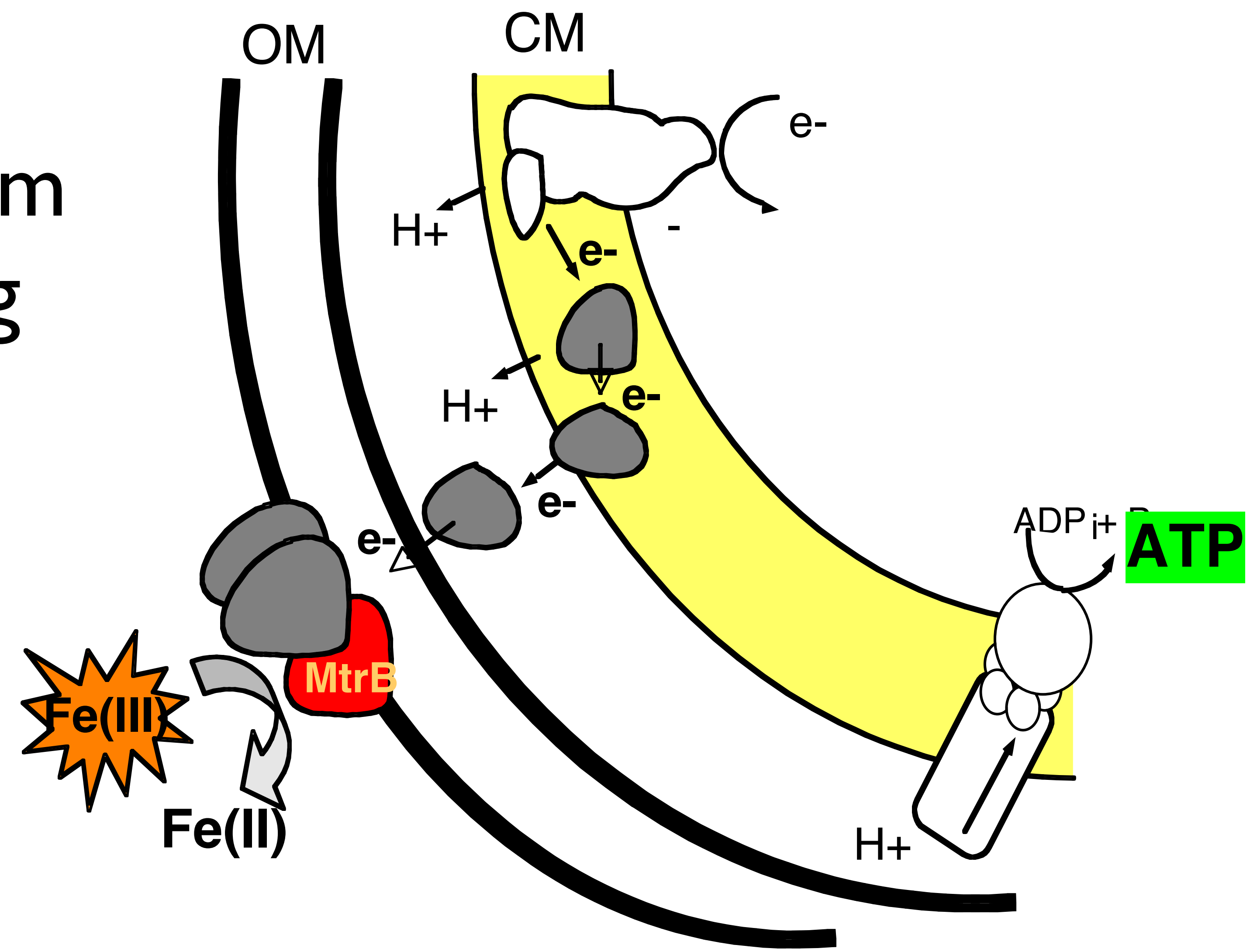


All cells Growth Overlay

Are cells responding to local microenvironments?

A reporter for metabolism linked to oxygen sensing

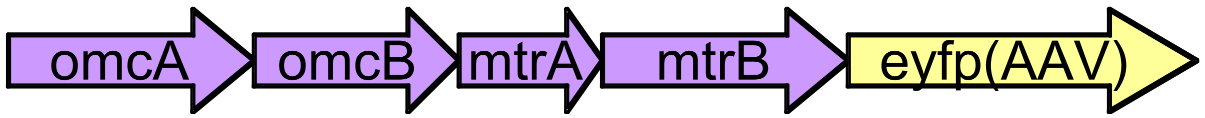
mtrB

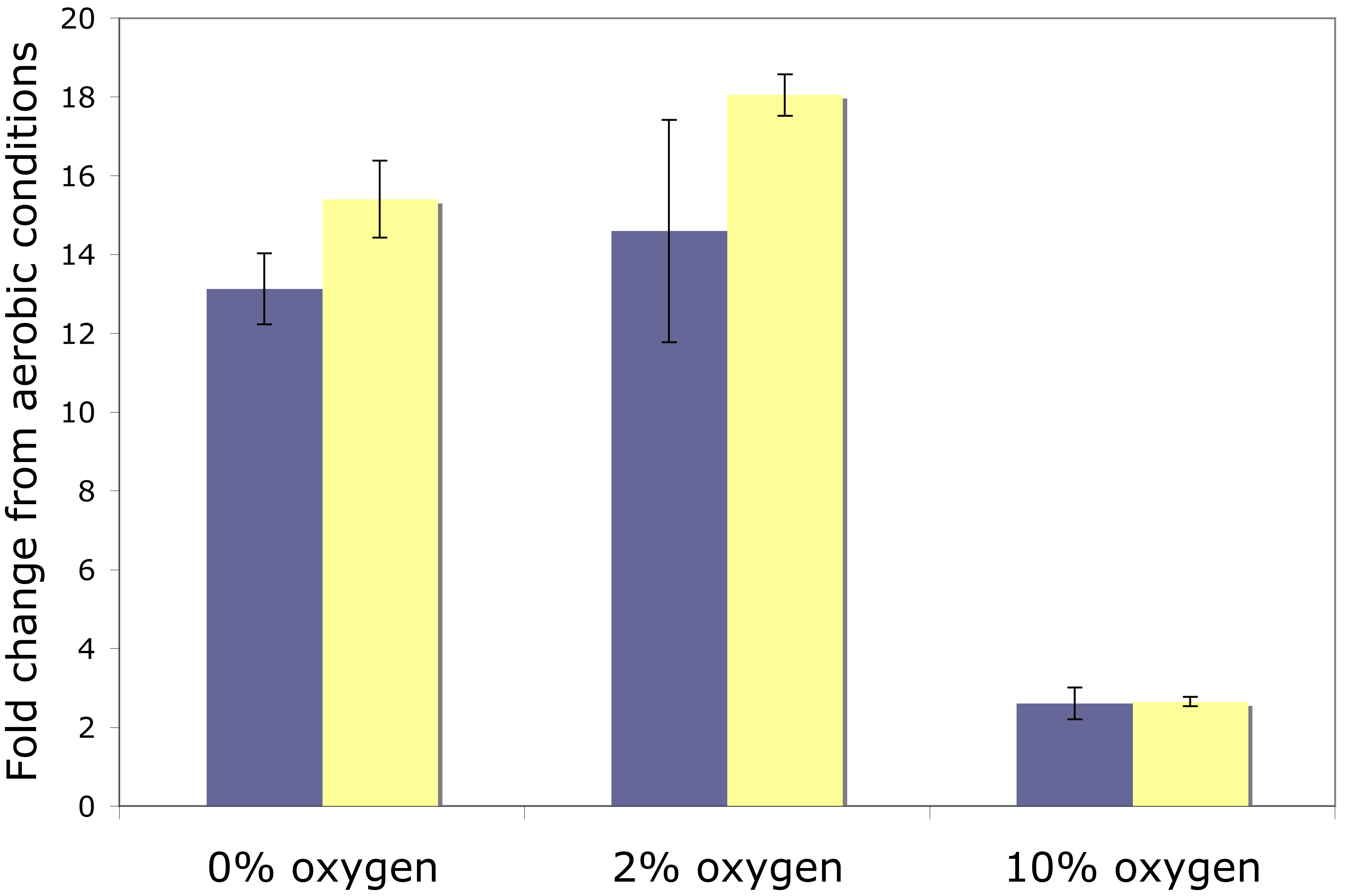


mtrB expression implies 2 things:

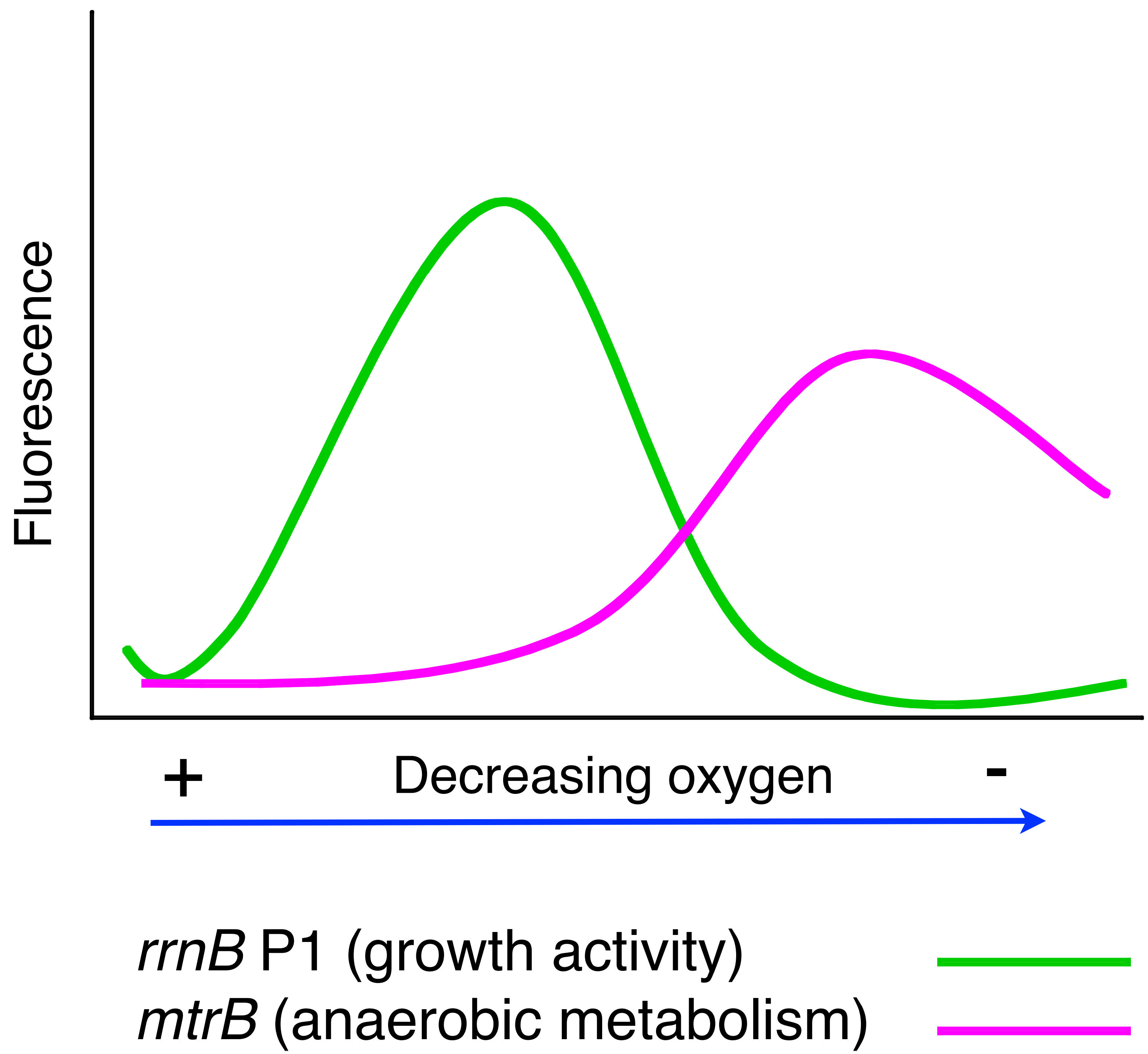
1. O_2 status within a biofilm
2. Active metabolism under low O_2 conditions

YFP is expressed from *mtrB* under anaerobic conditions

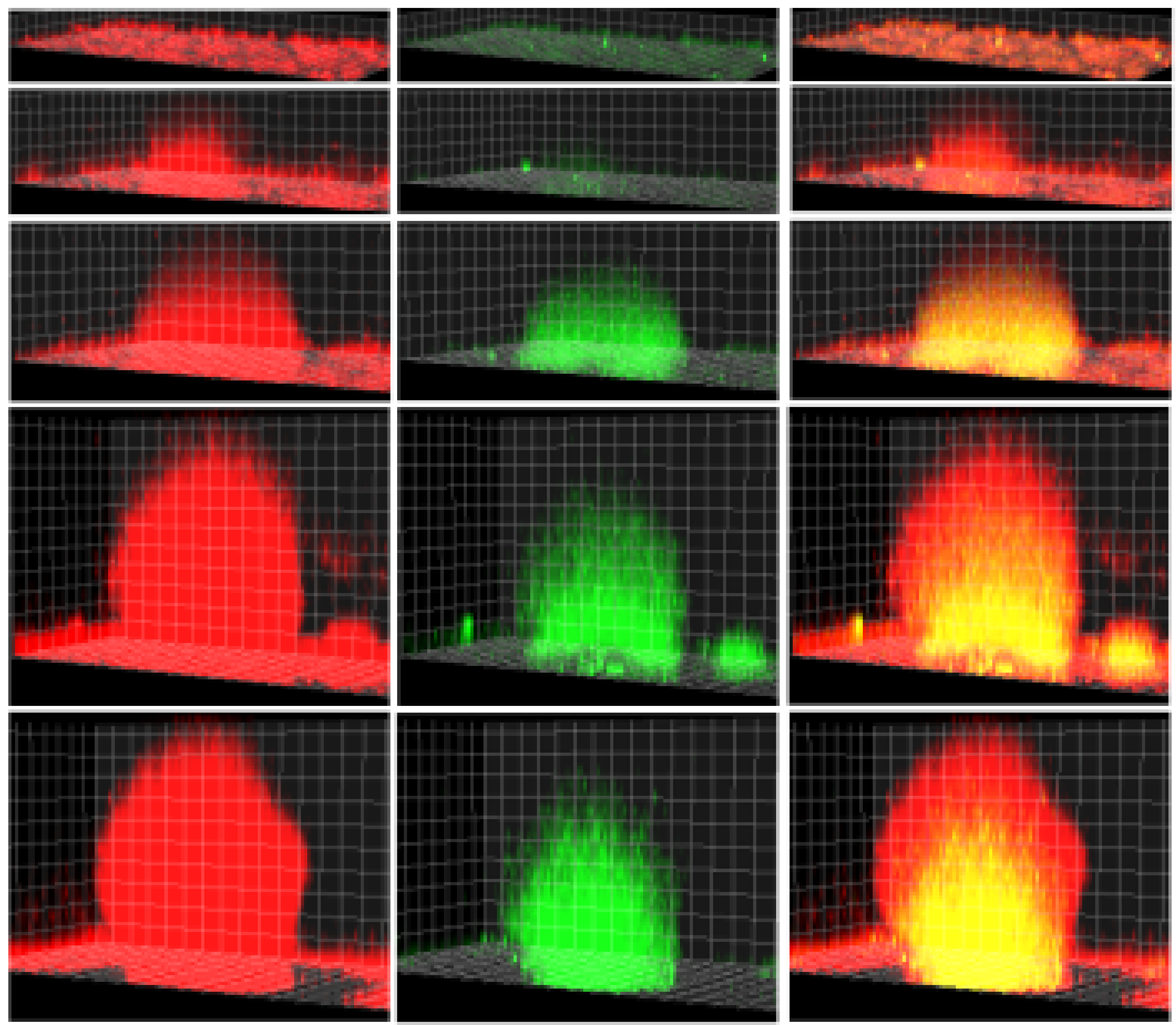




Expectations for expression under different oxygen conditions

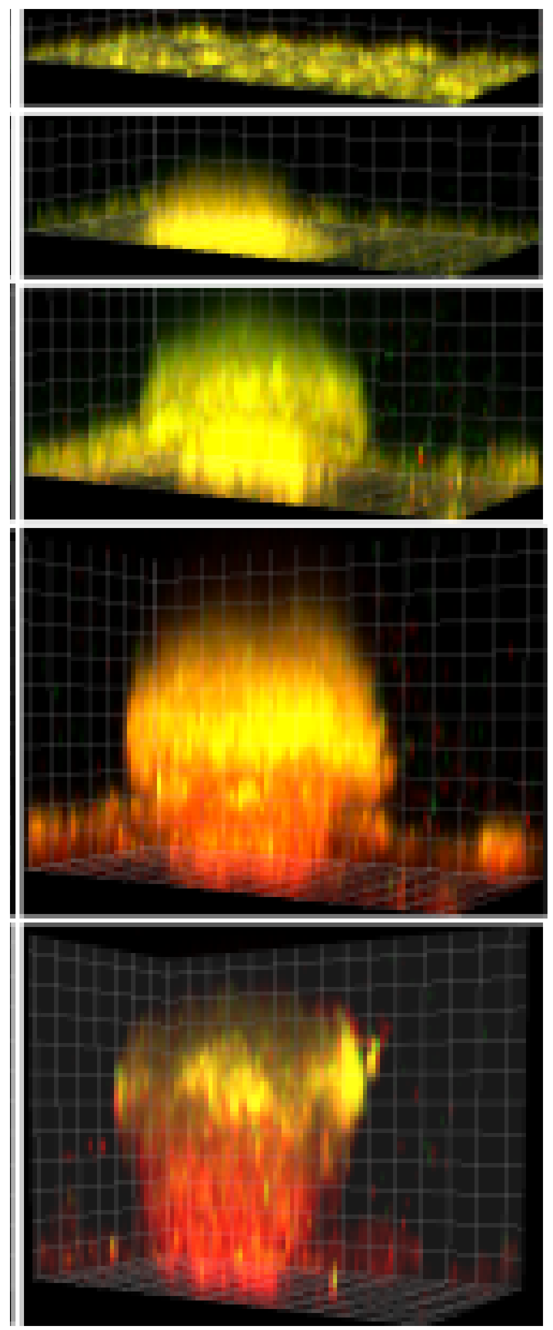


Distribution of *mtrB* expression indicating response that changes with oxygen conditions

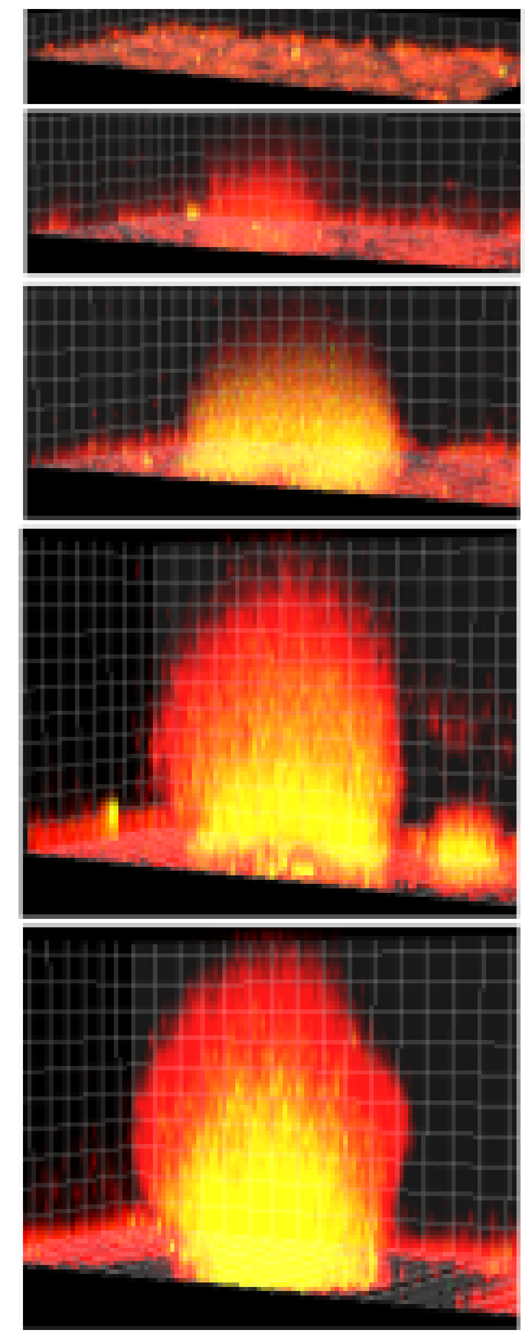


All cells *mtrB* Overlay

Cells have variable growth state and metabolism at different regions of the biofilm



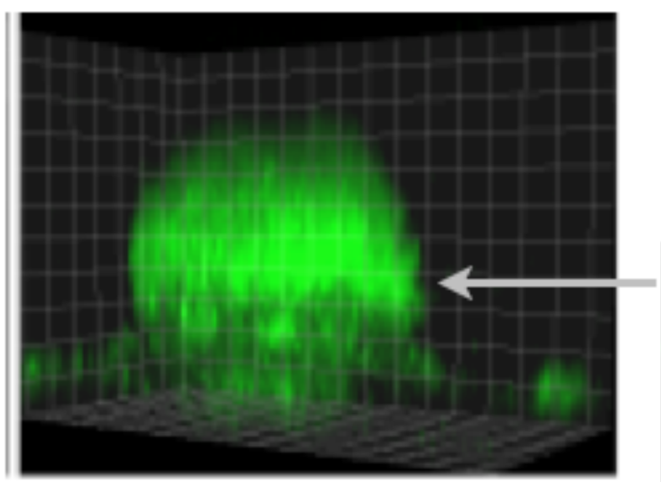
Growth activity

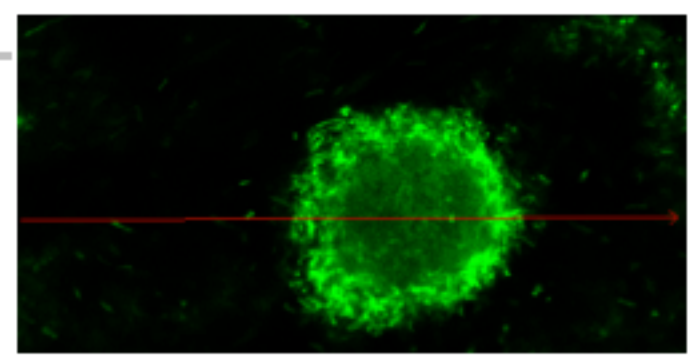


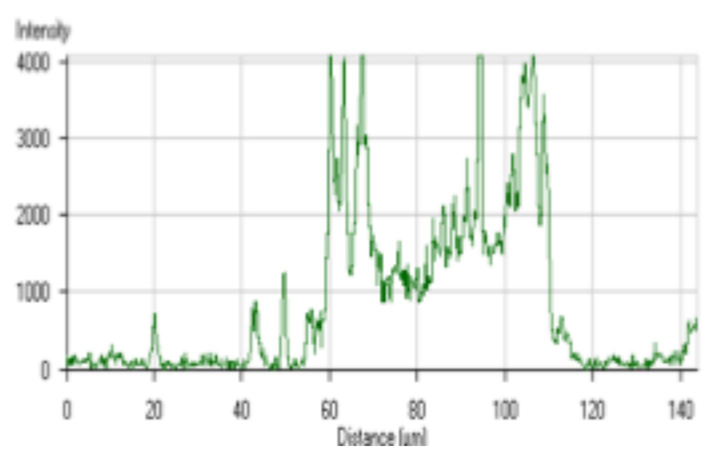
Oxygen metabolism

Quantitative analysis of fluorescence distribution and intensity

"When you employ the microscope, shake off all prejudice, nor harbor any favorite opinions; for, if you do, 'tis not unlikely fancy will betray you into error, and make you see what you wish to see." - The Microscope Made Easy, Henry Baker, "Cautions in Viewing Objects", 1742

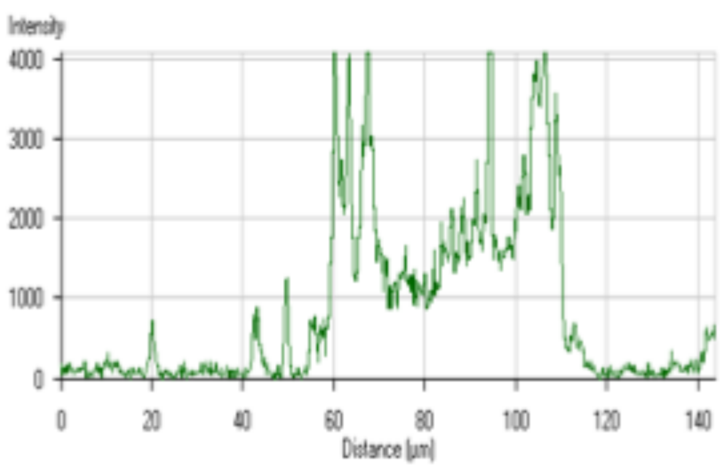






- extract fluorescent intensity profile
- average 9-pixel bins
- plot fluorescence intensity versus distance from center
- bin structures into representative sizes and average

Quantitative analysis of fluorescence distribution and intensity

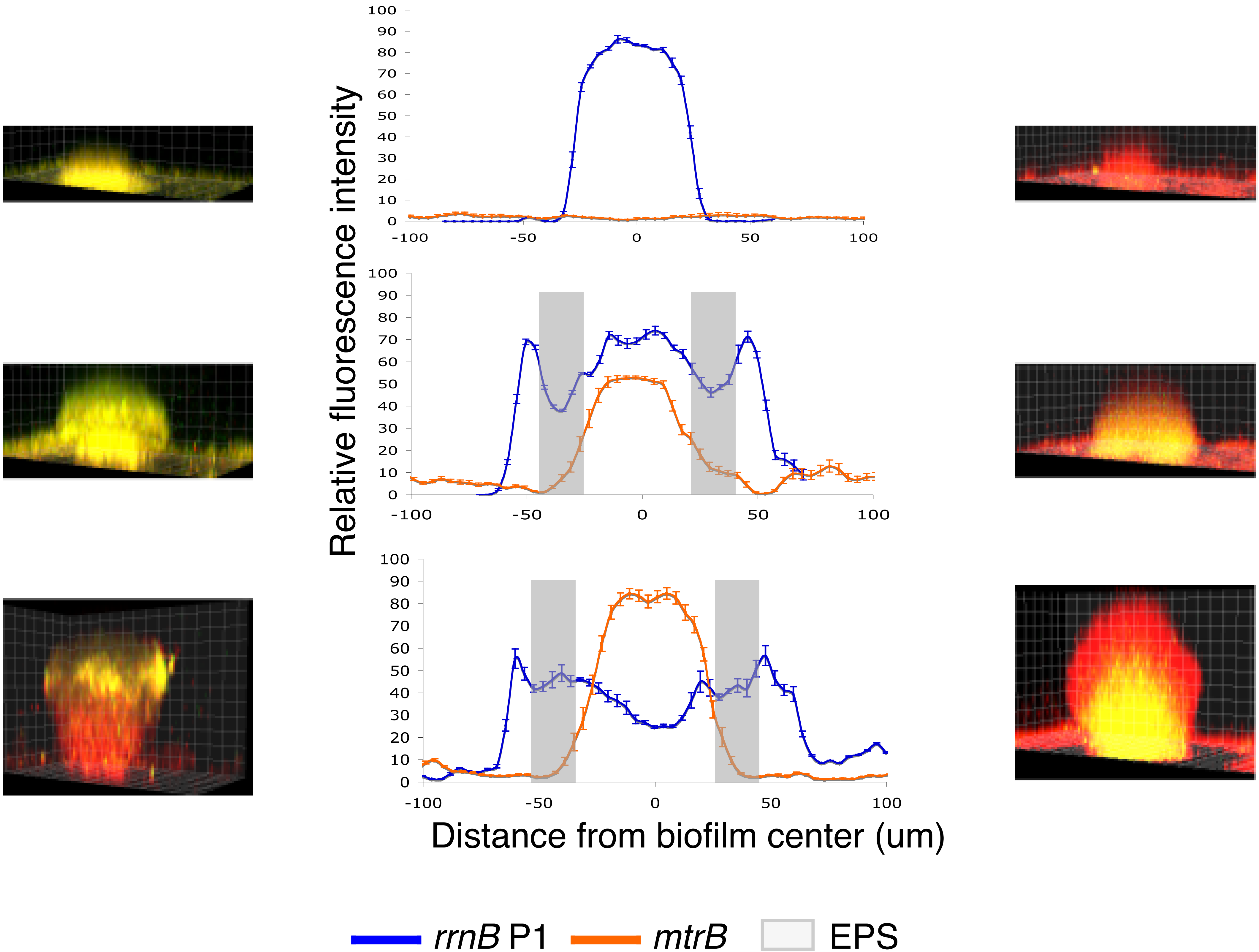


Intensity plot binned

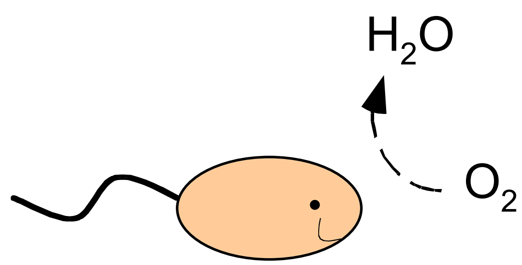
Intensity plot distance from center

Intensity plot distance from center averaged

Patterns of growth and activity correlate with colony size



Does oxygen availability affect biofilm stratification?



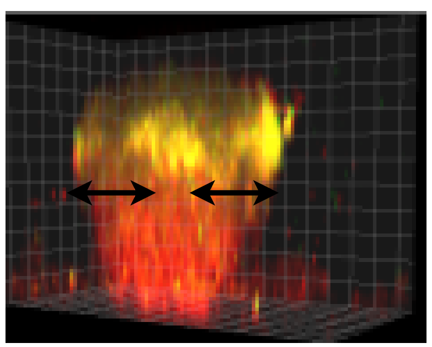
Rate of O_2 consumption by *Shewanella* under stationary phase conditions = 5.53mg O_2/L*s

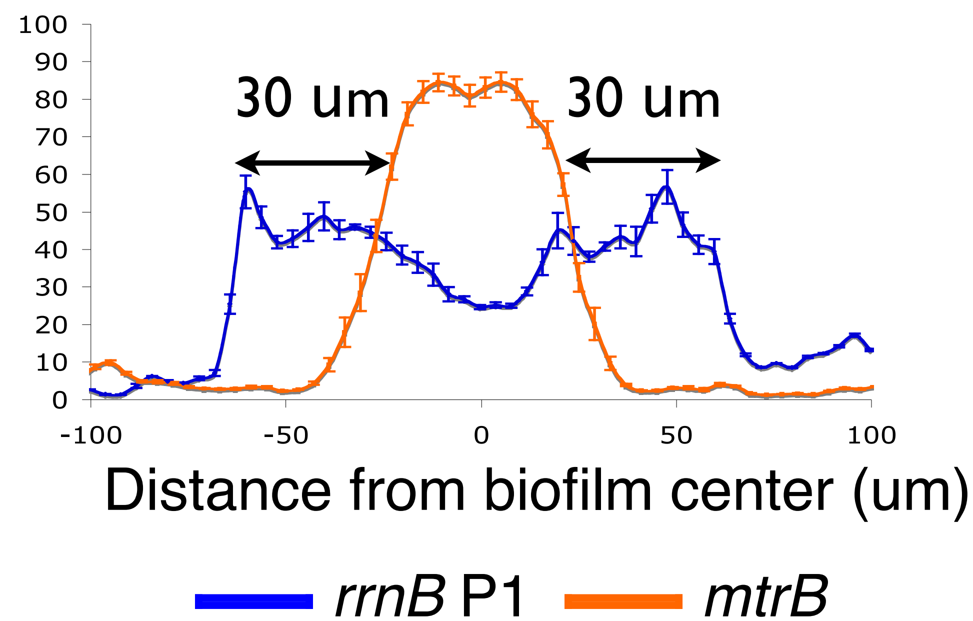
Use a reaction/diffusion equation $R_{min} = (6*D_e*S_0/K_0)^{1/2}$

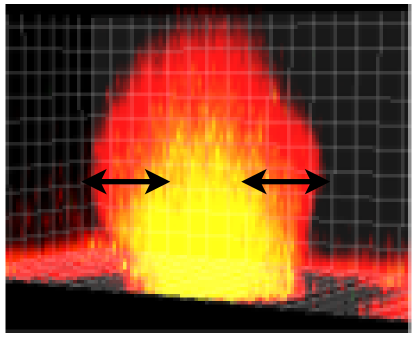
Predict that oxygen will diffuse ~30um into the biofilm

Oxygen diffusion correlates with growth activity

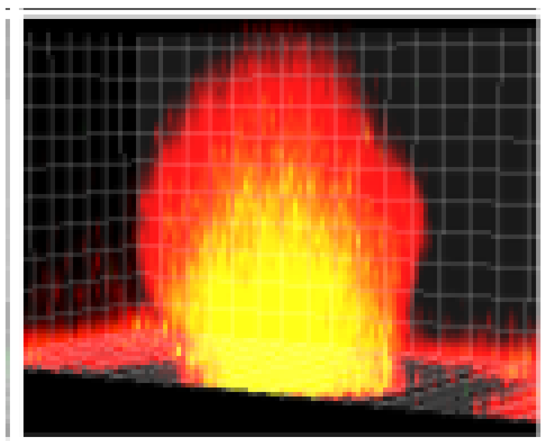
Predict that oxygen will diffuse ~30um into the biofilm



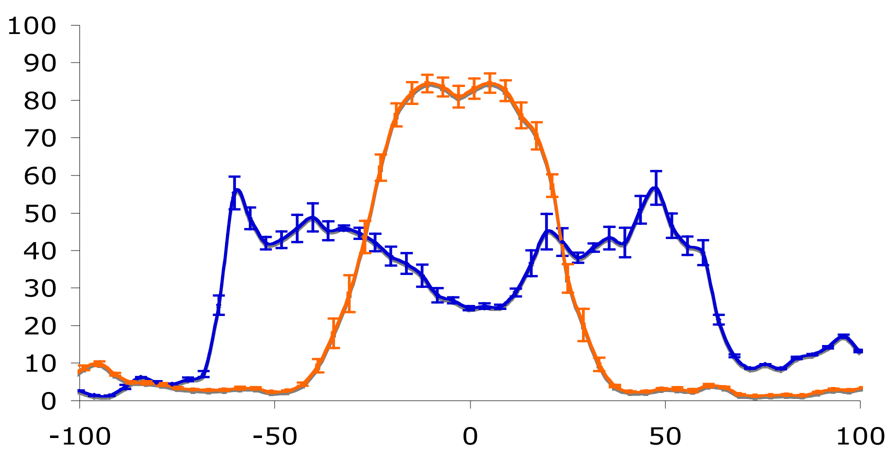




Summary



Cell growth and metabolism is heterogeneous within the biofilm. Growth can be decoupled from metabolism.



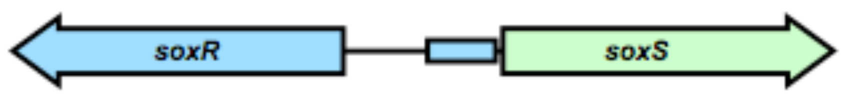
Quantitative profiles show that patterns of growth and metabolism are consistent and predictable based on structure size and oxygen concentrations

Cells are dynamically regulating their response to different environmental conditions

SoxR

SoxR responses differ between bacteria

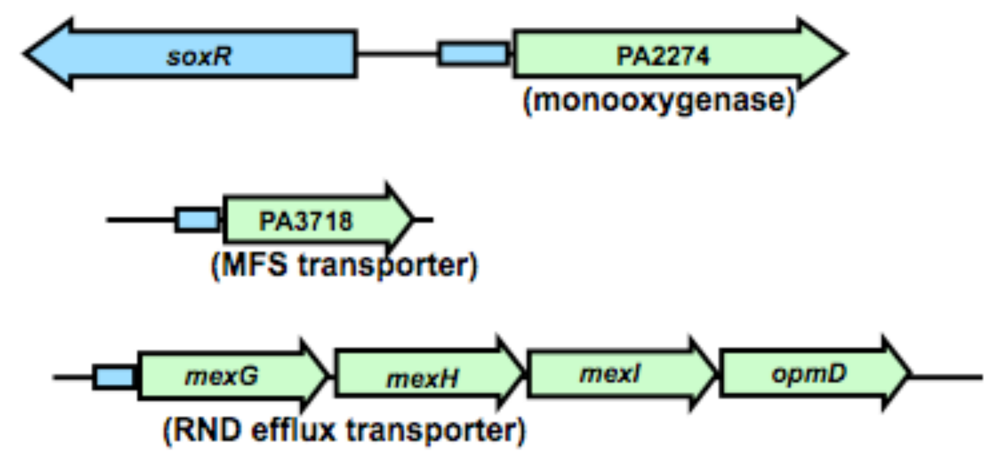
E. coli



Δ*soxR*: sensitive to paraquat

Superoxide response

P. aeruginosa



Δ*soxR*: insensitive to paraquat

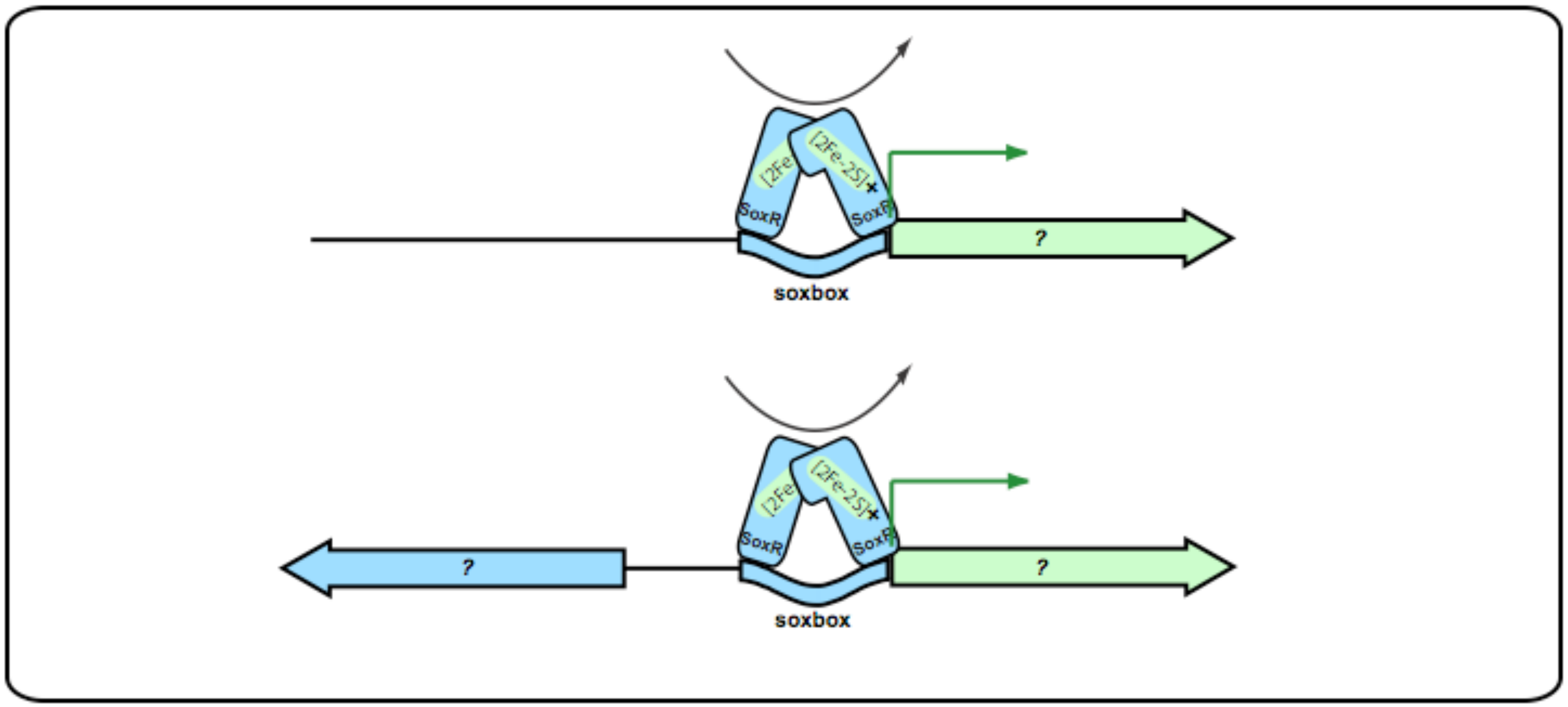
Phenazine response

Kobayashi *et al.*, J. Biochem., 2004
Palma *et al.*, Infection and Immunity, 2005

Lars Dietrich

How ubiquitous is SoxR and what types of genes does it regulate?

Pseudomonads



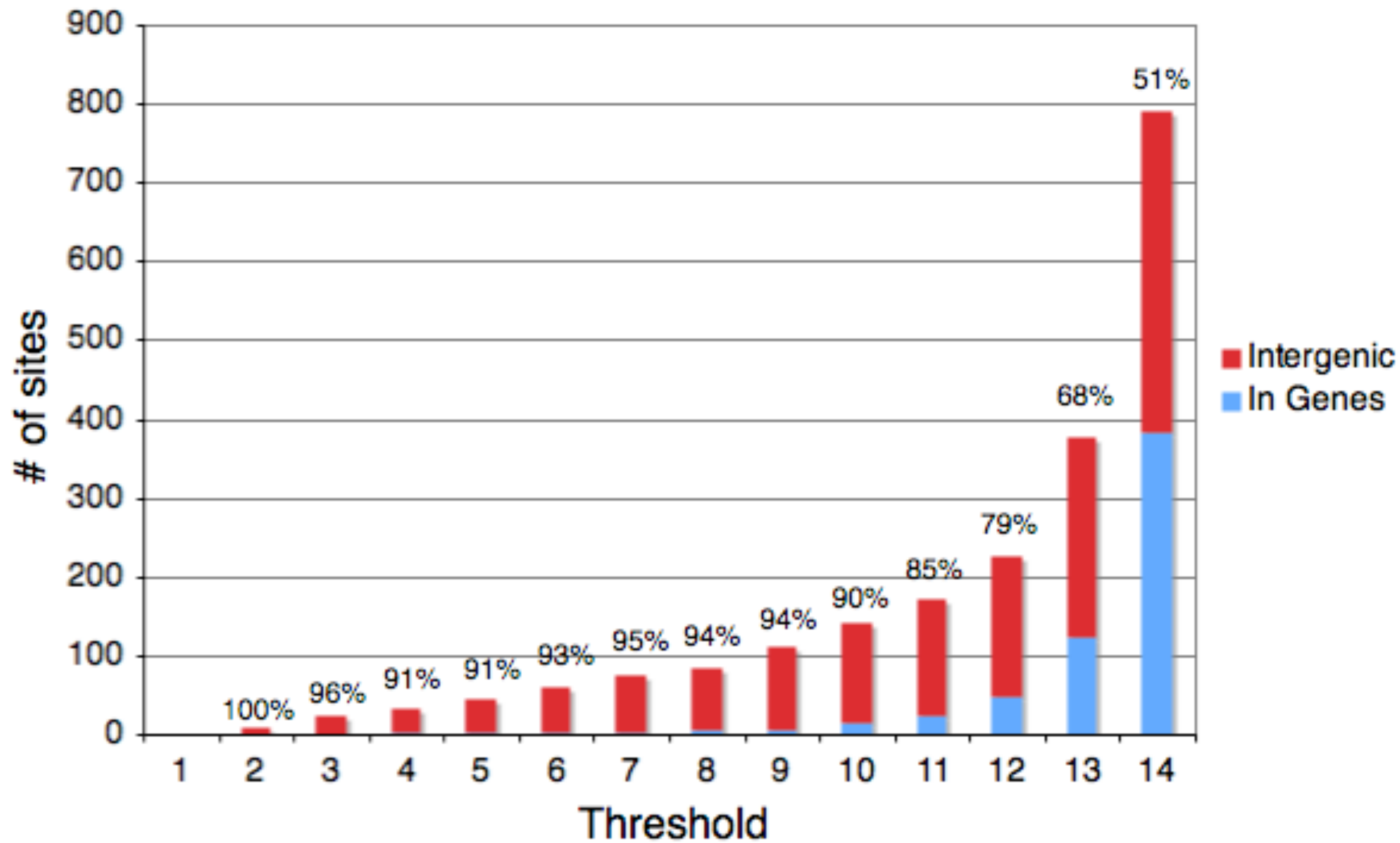
"We cannot exclude the possibility that a completely new oxidative defense system may have evolved in Pseudomonas species, thus rendering SoxR-dependent system obsolete or taking over the control of genes that were formerly SoxR regulon." Park et al 2006

Bioinformatics approach to finding SoxR regulated genes

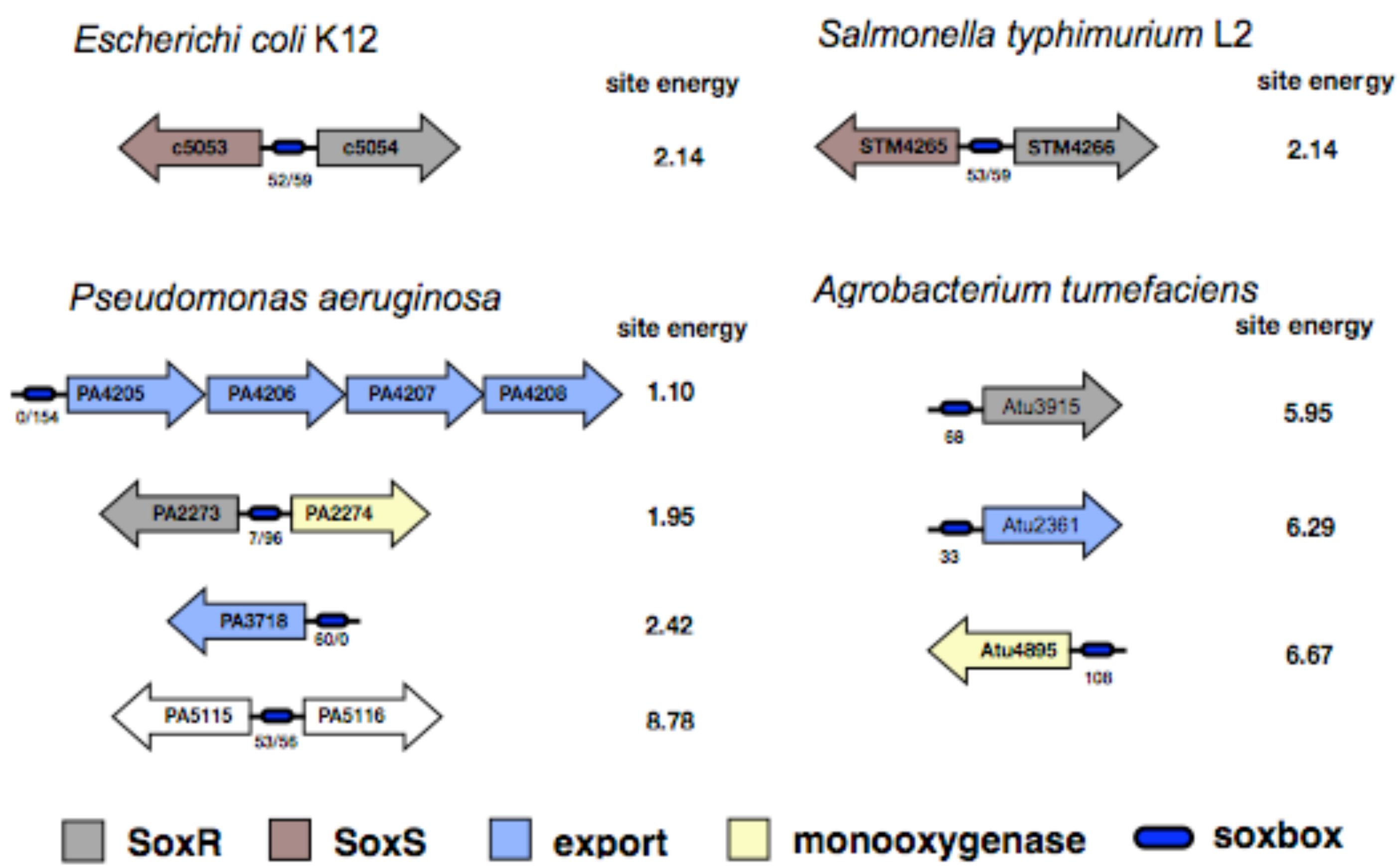
Pseduomonas aeriginosa PAO1 PA2274 (from Pseudomonas.com)	TTGACCTCAAGTTTGCTTGAGGTTTT
Pseduomonas aeriginosa PAO1 PA4205 (from Pseudomonas.com)	TTGACCTCAACTTAACTTGAGGTTTT
Pseduomonas aeriginosa PAO1 PA3718 (from Pseudomonas.com)	TTTACCTCAAGTTAACTTGAGCTATC
Escherichi coli from Kobayashi_Tagawa2004)	TTTACCTCAAGTTAACTTGAGGAATT
Xanthomonas axonopodis from Kobayashi_Tagawa2004	TTGACCTCAACTTAGGTTGAGGCAGG
Chromobacterium axonopodis from Kobayashi_Tagawa2004	TTGACTTCAAGTTAACTTGAACTTTG

Reliability of the PWM

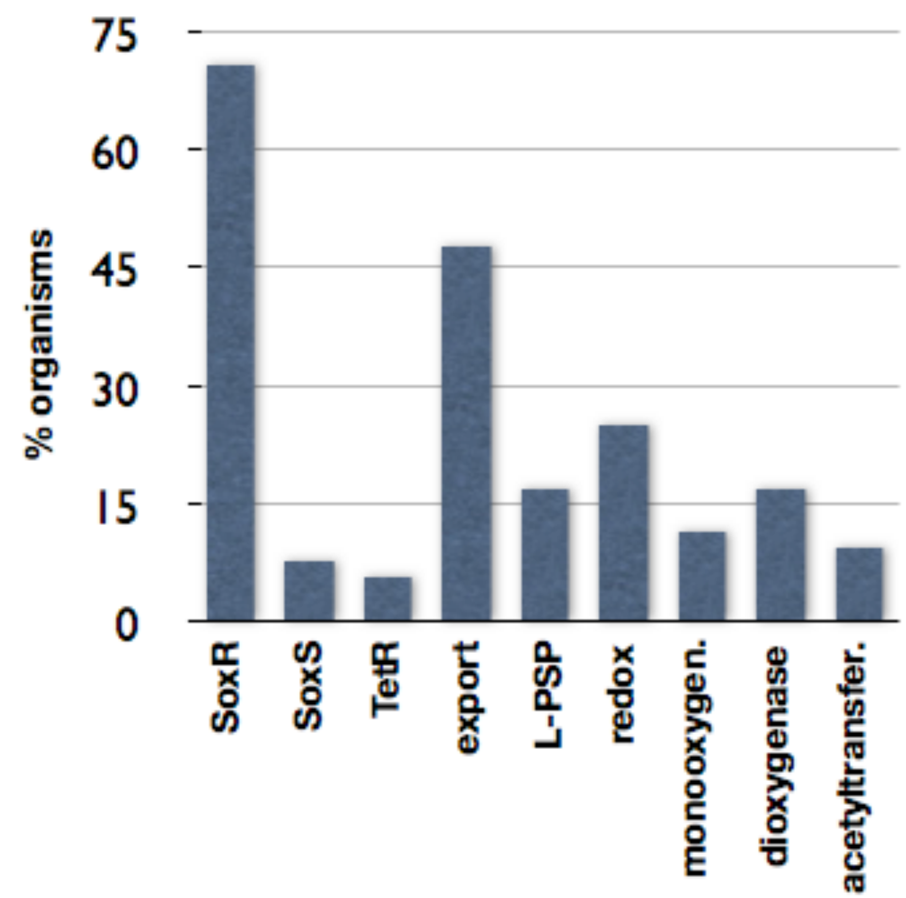
Intergenic versus in genes



Predictions of soxboxes match data from the literature

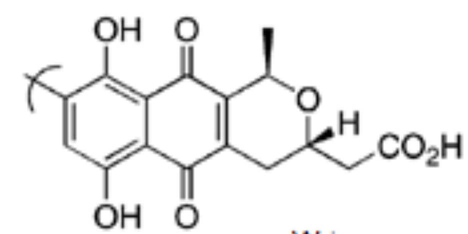


Genes not associated with superoxide stress are more highly represented



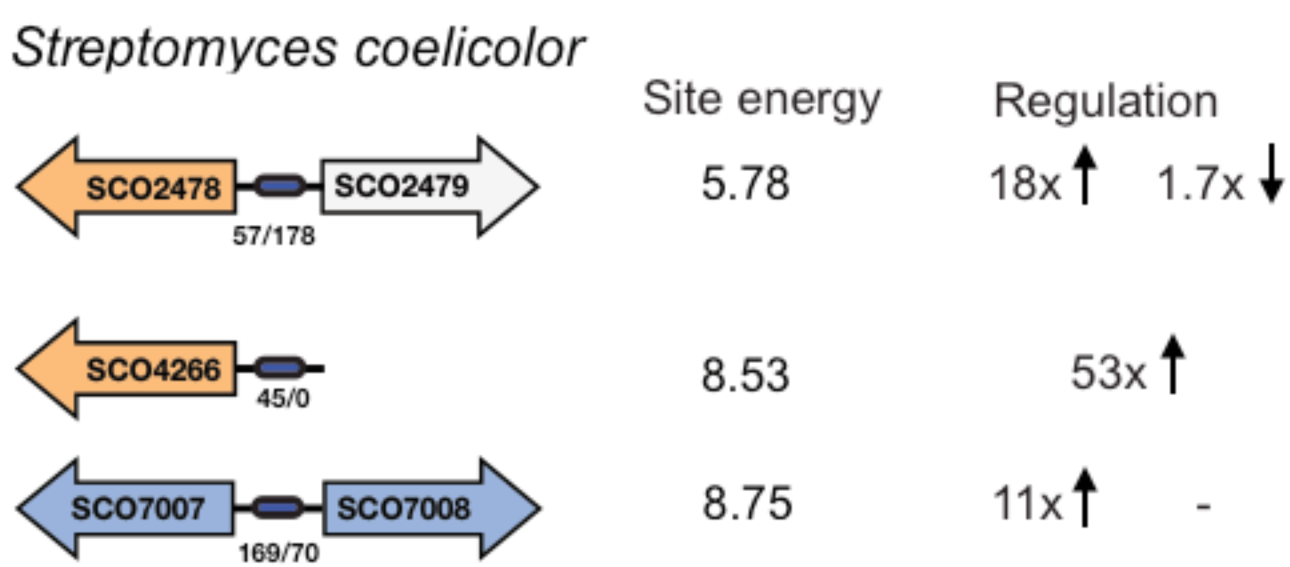
Testing soxbox predictions in *Streptomyces coelicolor*

Actinorhodin

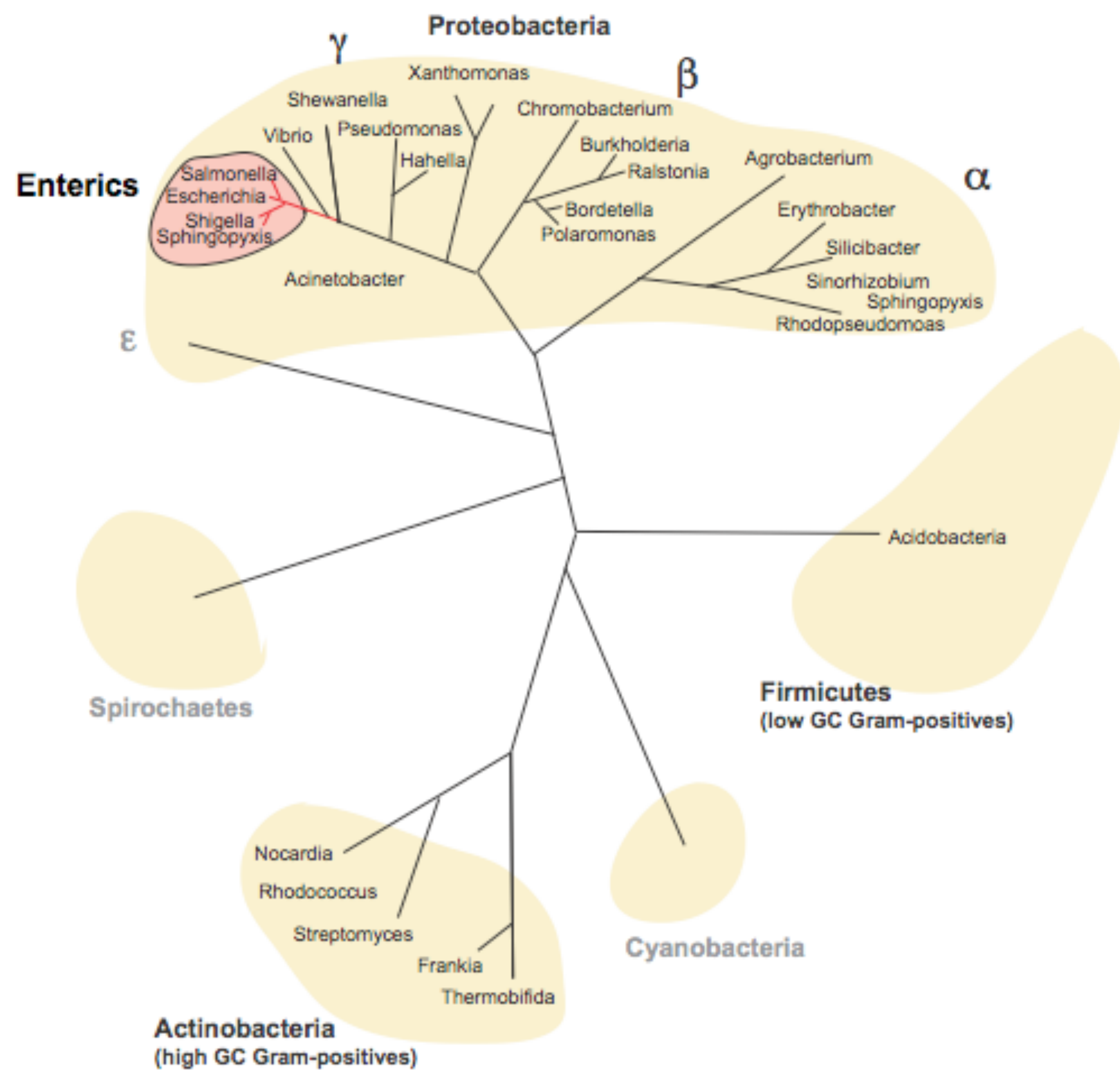


Weissman

In the presence of actinorhodin: ***wild type*** versus **Δ*act***

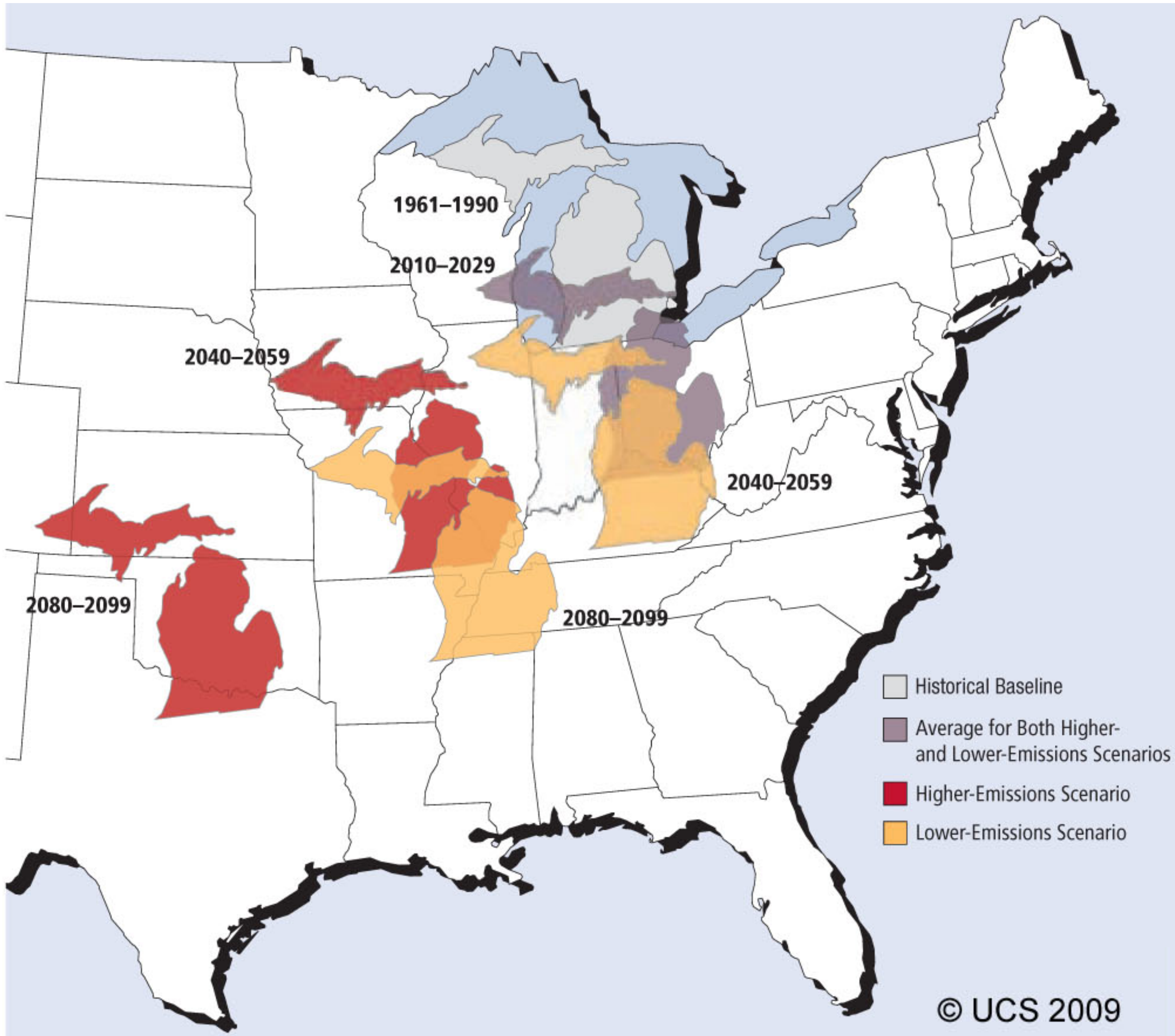


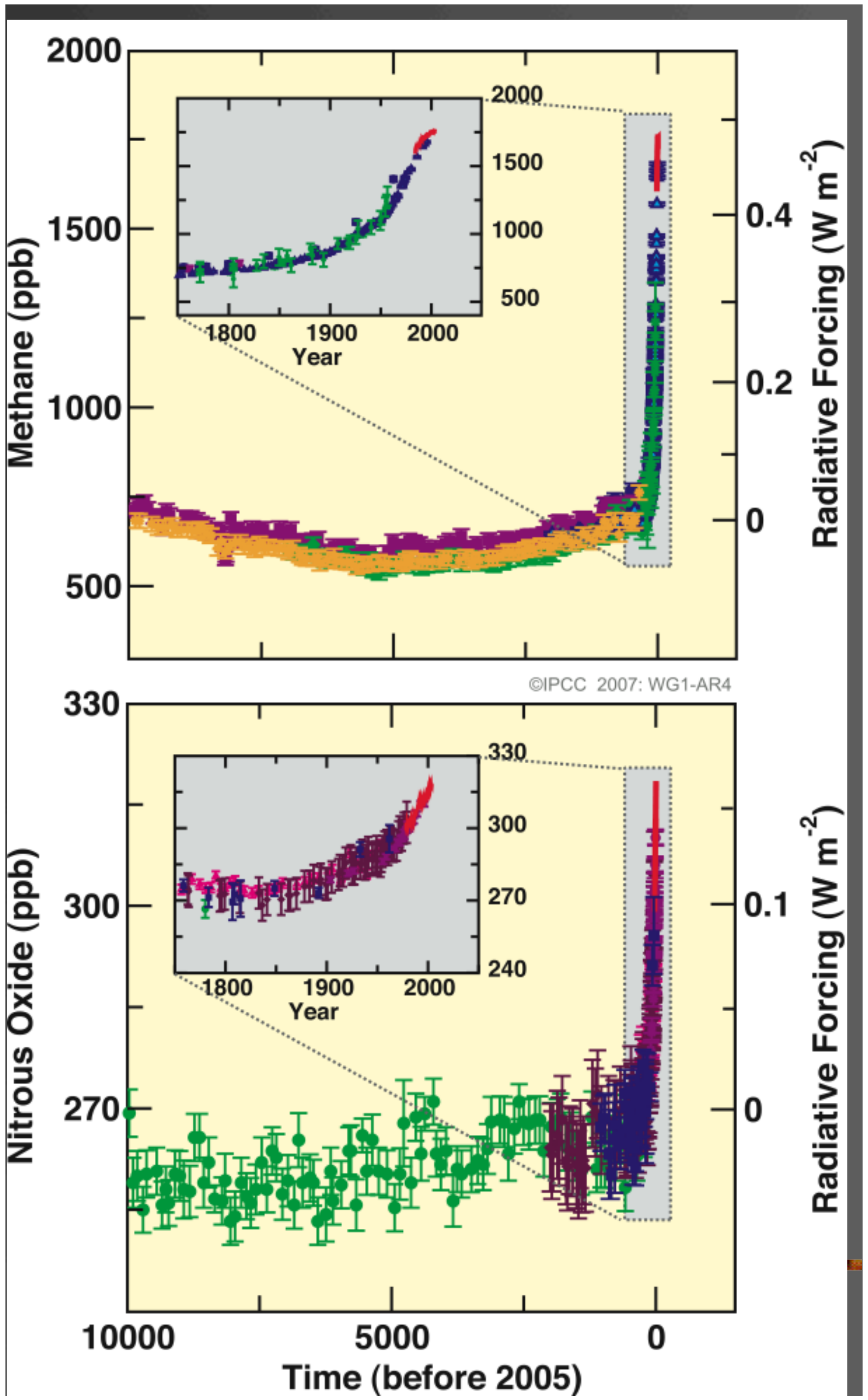
SoxRS paradigm seems limited to enterics



The effects of land use change on microbial communities and greenhouse gas fluxes

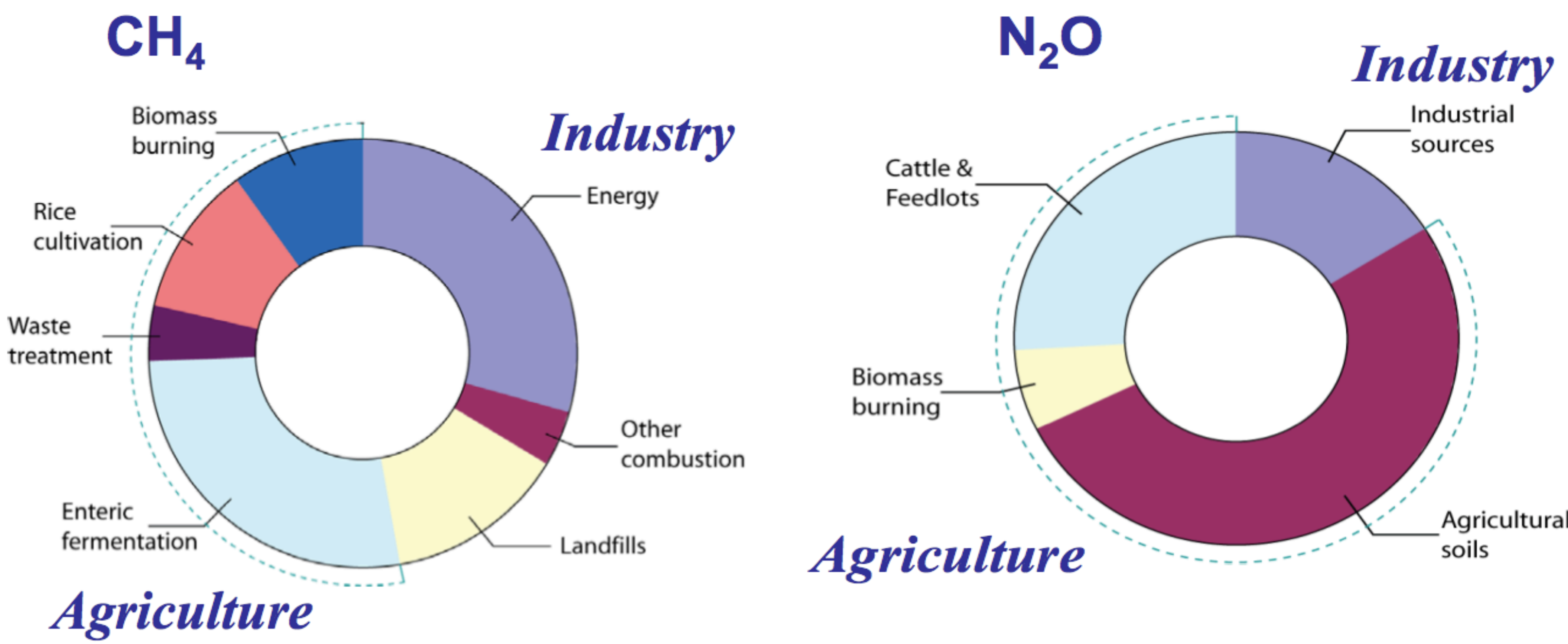
Climate change is happening



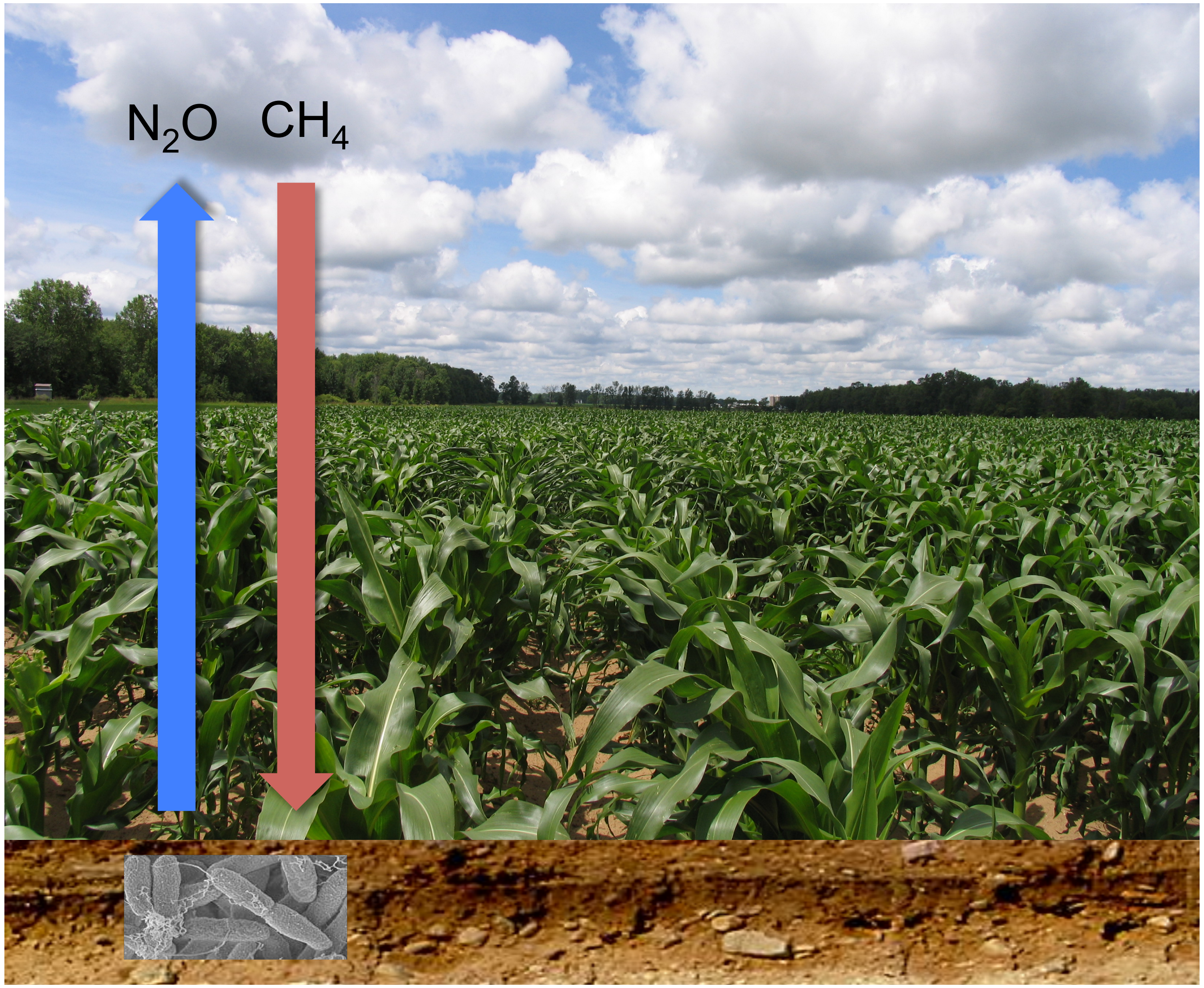


- Dramatic increases in CH_4 and N_2O in the last 200 years
- High Global Warming Potential (GWP) due to time in the atmosphere and infrared absorption

Much of this shift is due to land use change and intensive agriculture



Microbes are the primary mediators of methane and nitrous oxide



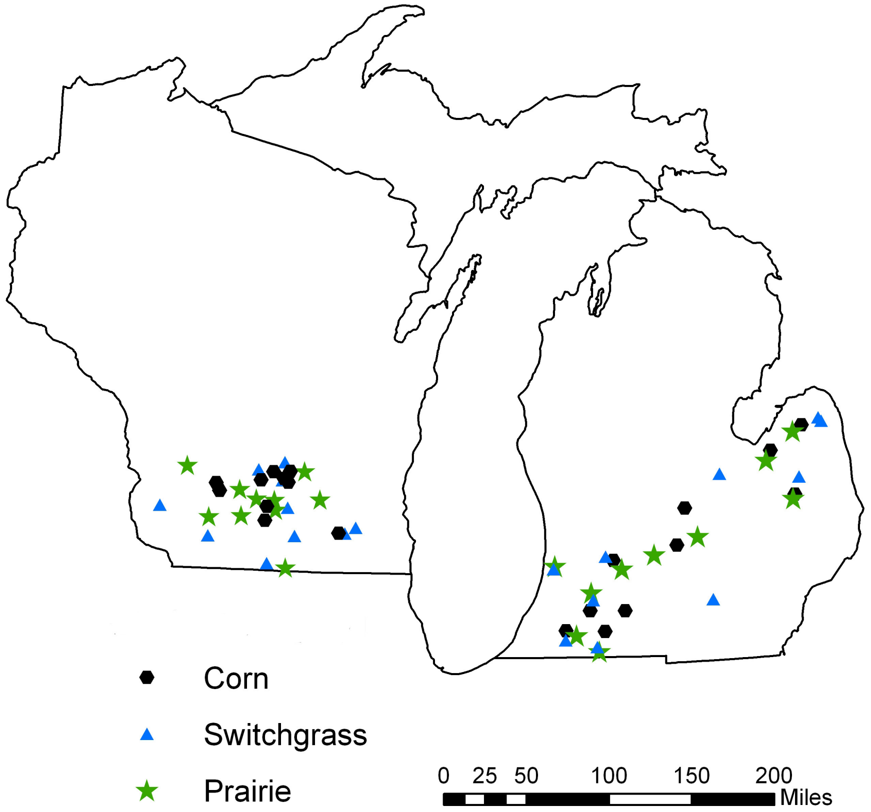
How do microbial communities change with land management?

What is the relationship between denitrifying bacteria and N_2O in agricultural soils?

Experiment sites

Kellogg Biological Station LTER

GLBRC Extensive Sites



How do microbial communities change with land management?

Kellogg Biological Station LTER

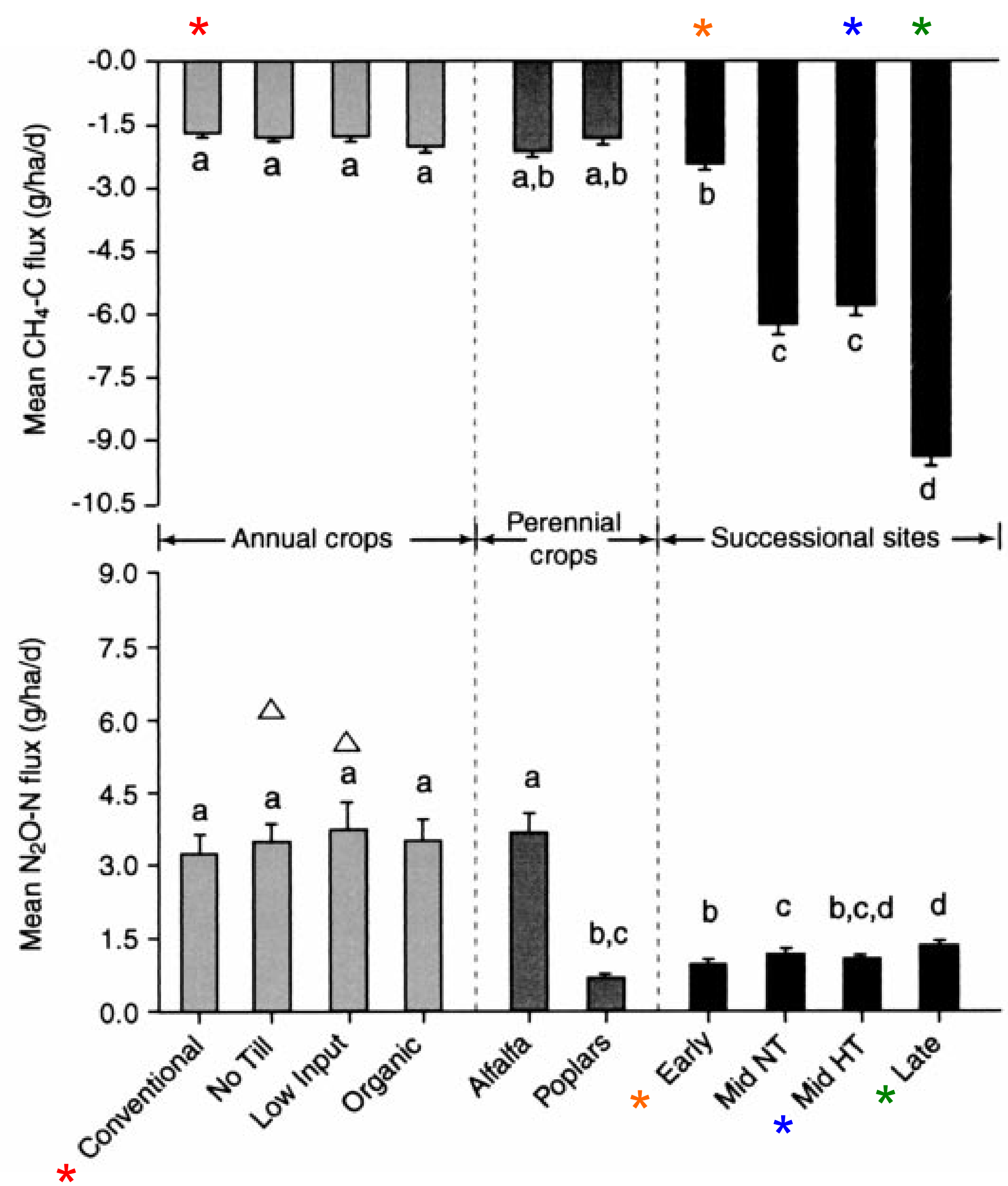
- Vicente Gomez-Alvarez
- Tom Schmidt

How do microbial communities change with land management?

Kellogg Biological Station LTER

Methane

Nitrous oxide



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

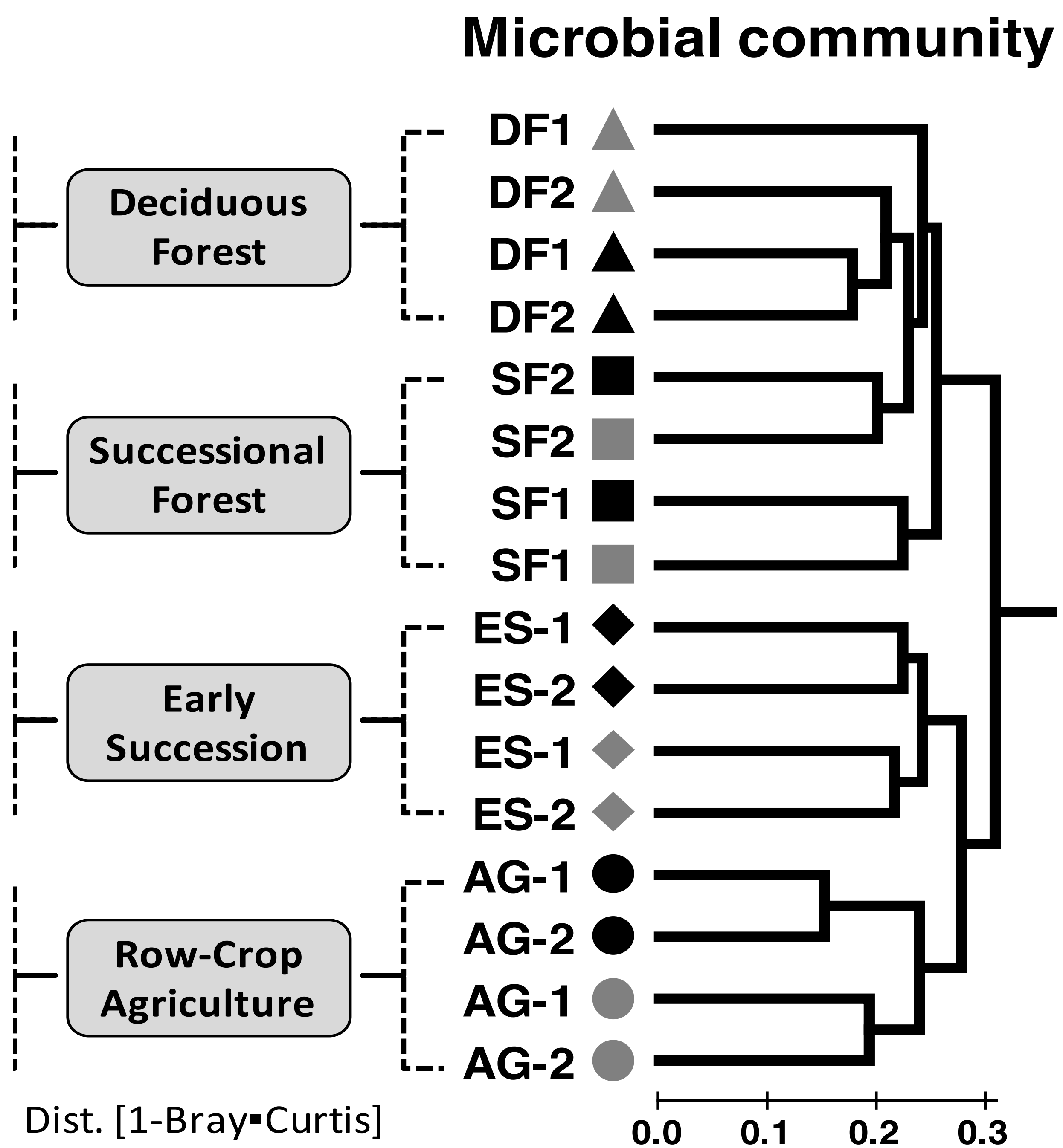
Sampling design

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

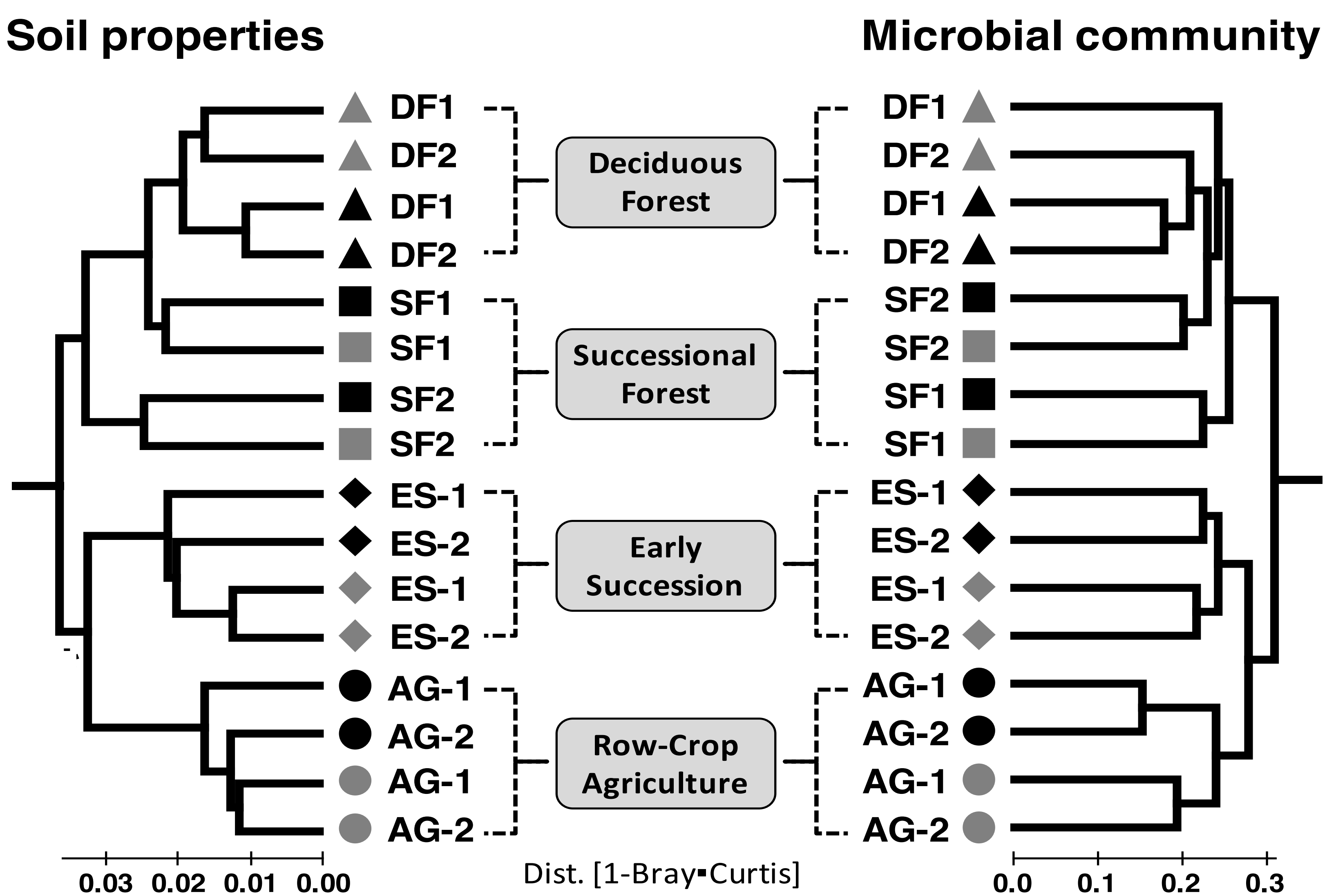
- 10 cm cores
- Soil from 5 flags pooled and sieved
- Soil frozen after being collected
- Microbial community analysis
- Soil analysis

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

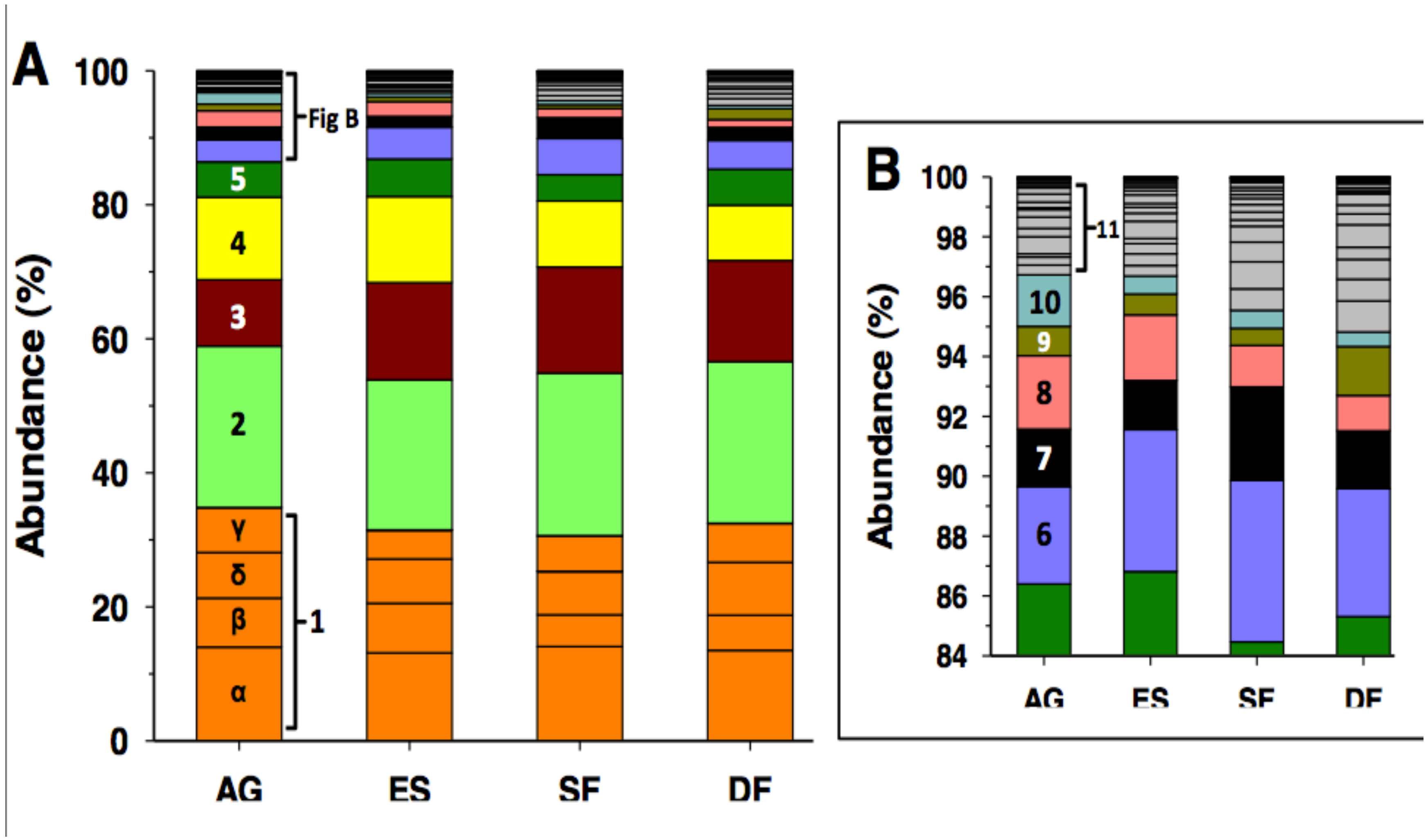
Taxonomic composition of bacterial communities changes with treatment



Biogeochemistry and bacterial community change concomitantly

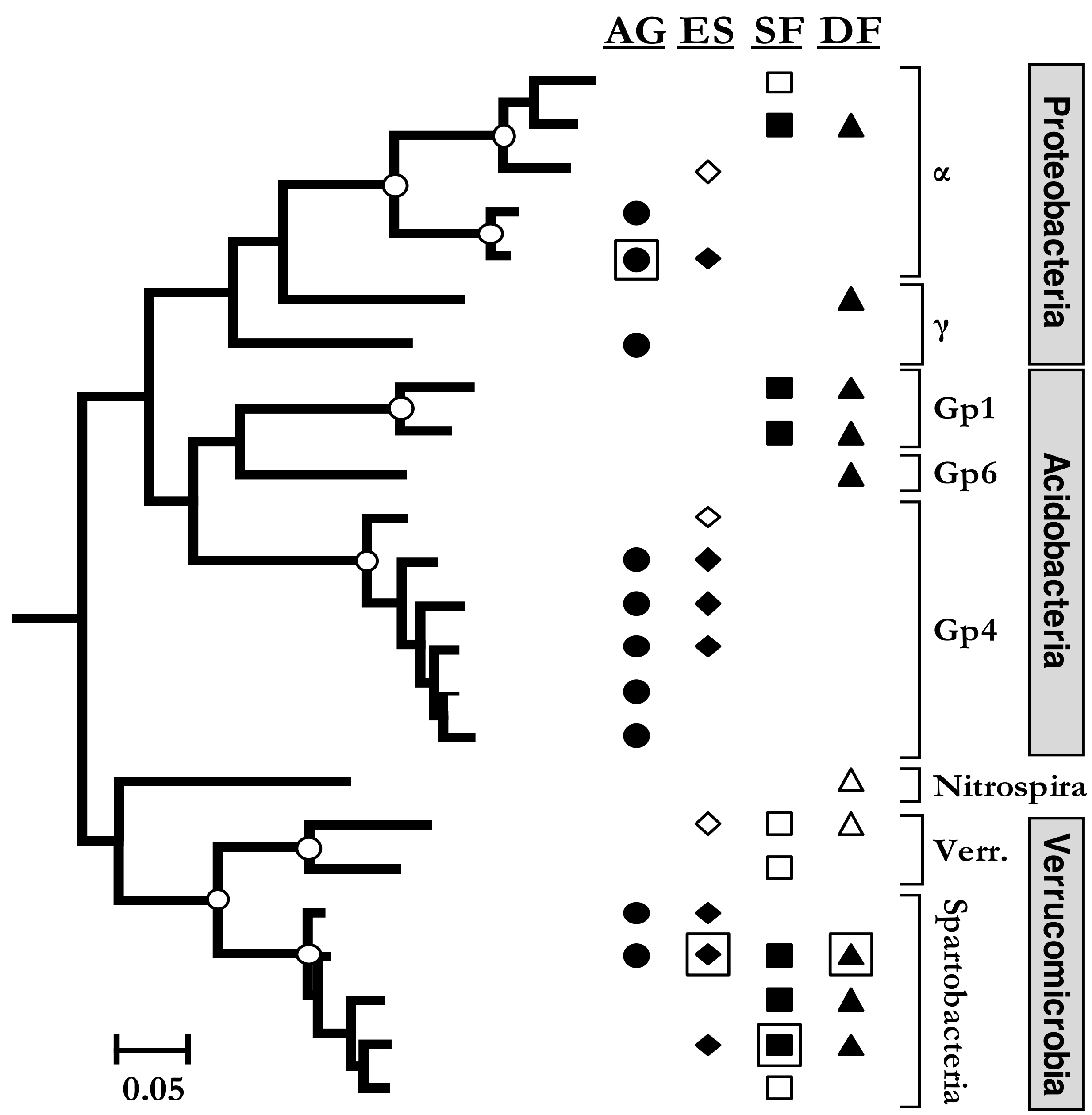


Who’s there and differentiating communities



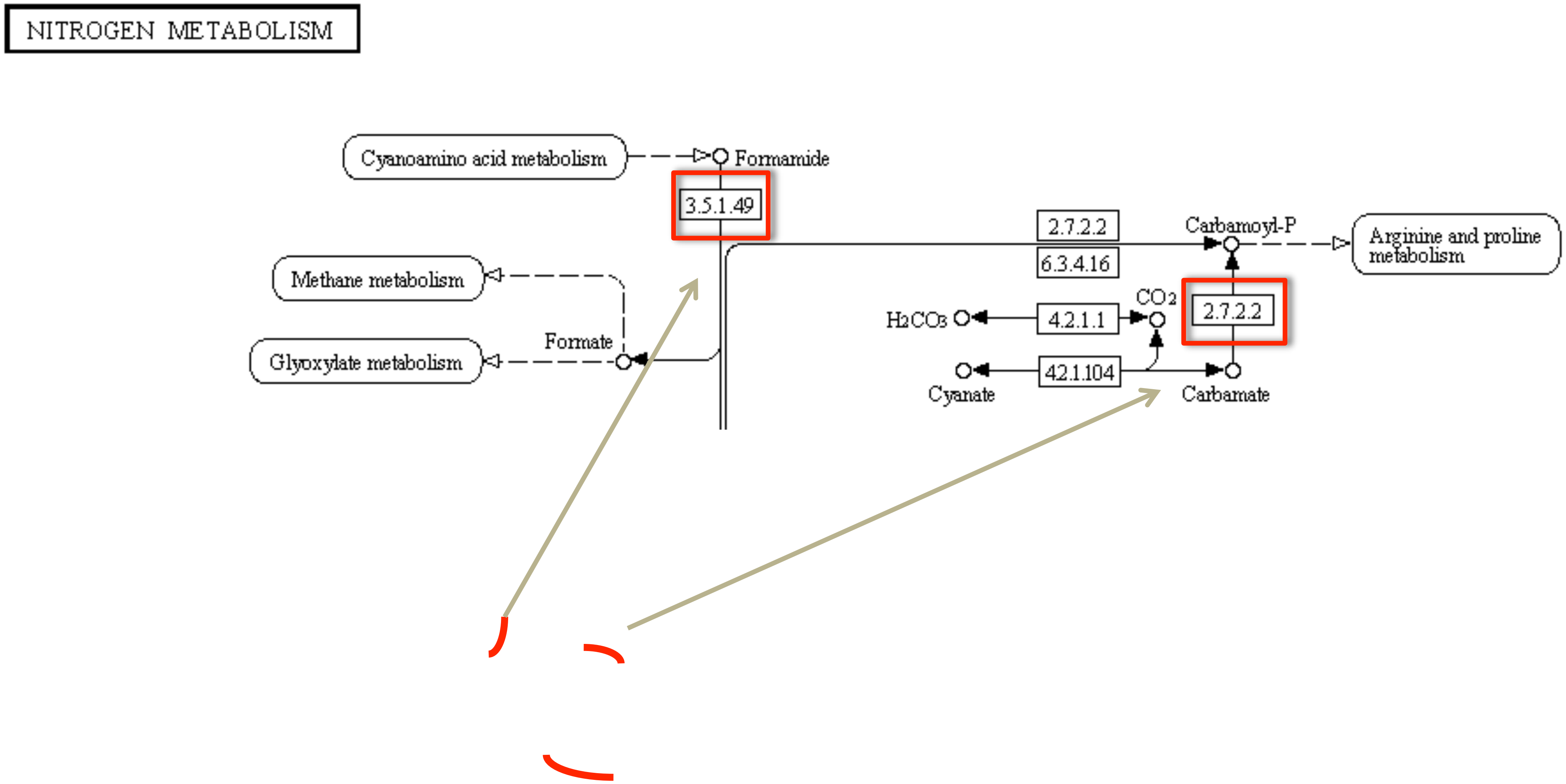
(1) Proteobacteria [class added], (2) Acidobacteria, (3) Verrucomicrobia, (4) Actinobacteria, (5) Bacteroidetes, (6) Planctomycetes, (7) Chloroflexi, (8) Gemmatimonadetes, (9) Nitrospirae, (10) Firmicutes, and (11) 30 additional phylum corresponding to less than 4% of the total distribution.

Shifts within phyla differentiate communities



Metagenomics is terrible, but why we use it anyway

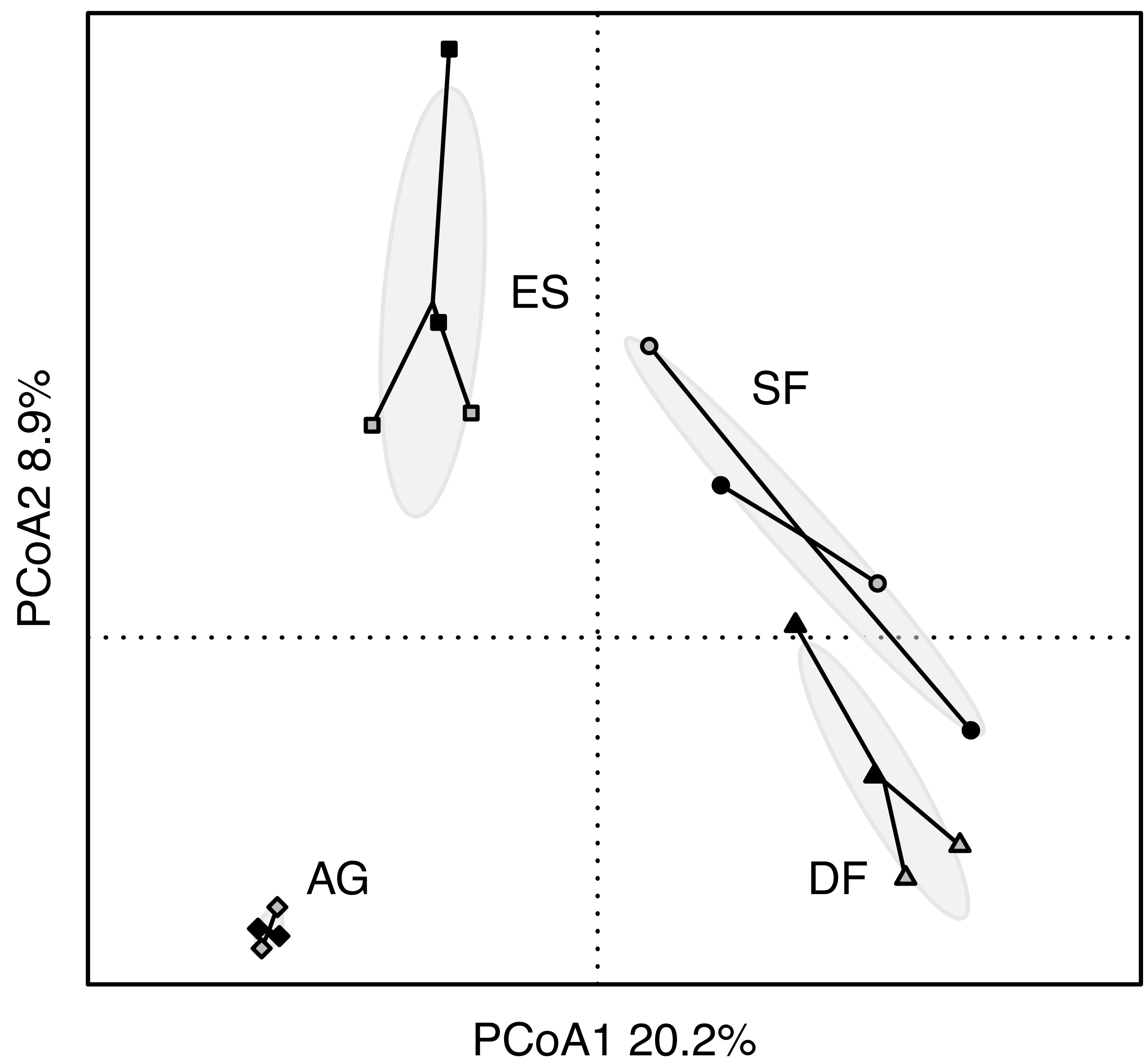
Annotate shotgun reads



Matrix of normalized gene abundance by treatment

Function	Subsystem	T1R1_2008	T1R2_2008	T1R1_2009	T1
(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	

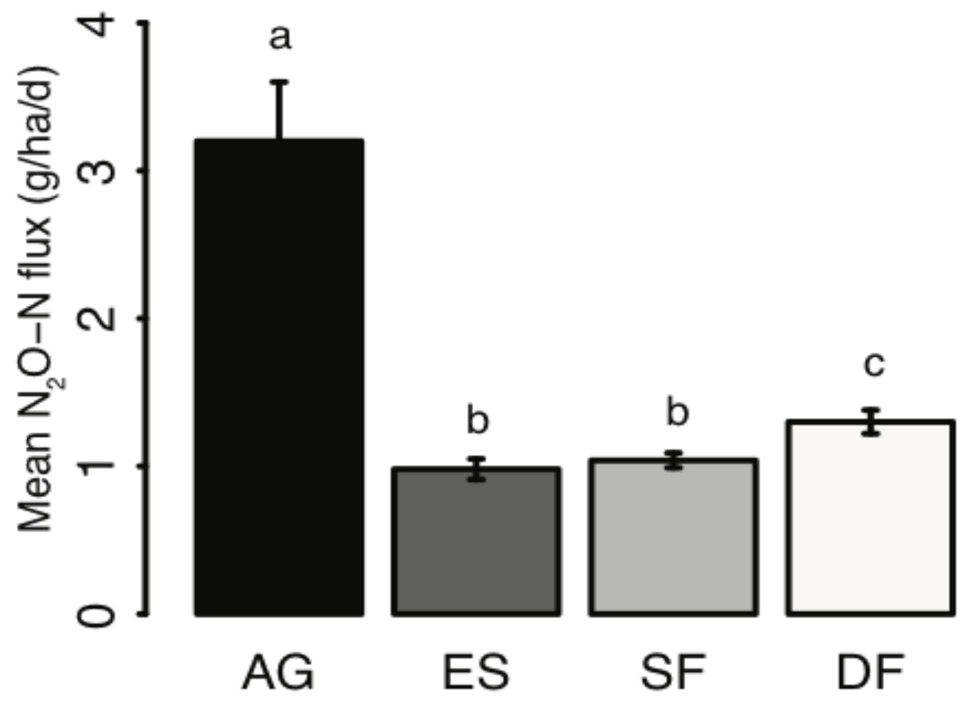
Functional potential changes with land management



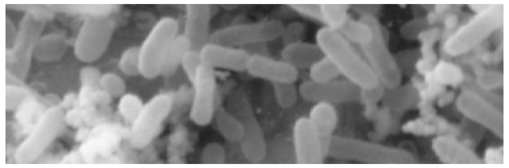
How do microbial communities change with land management?

- Bacterial community taxonomic composition and functional potential changes with land use
- Communities change concomitantly with soil biogeochemistry
- Community composition differs in agriculture from forested sites

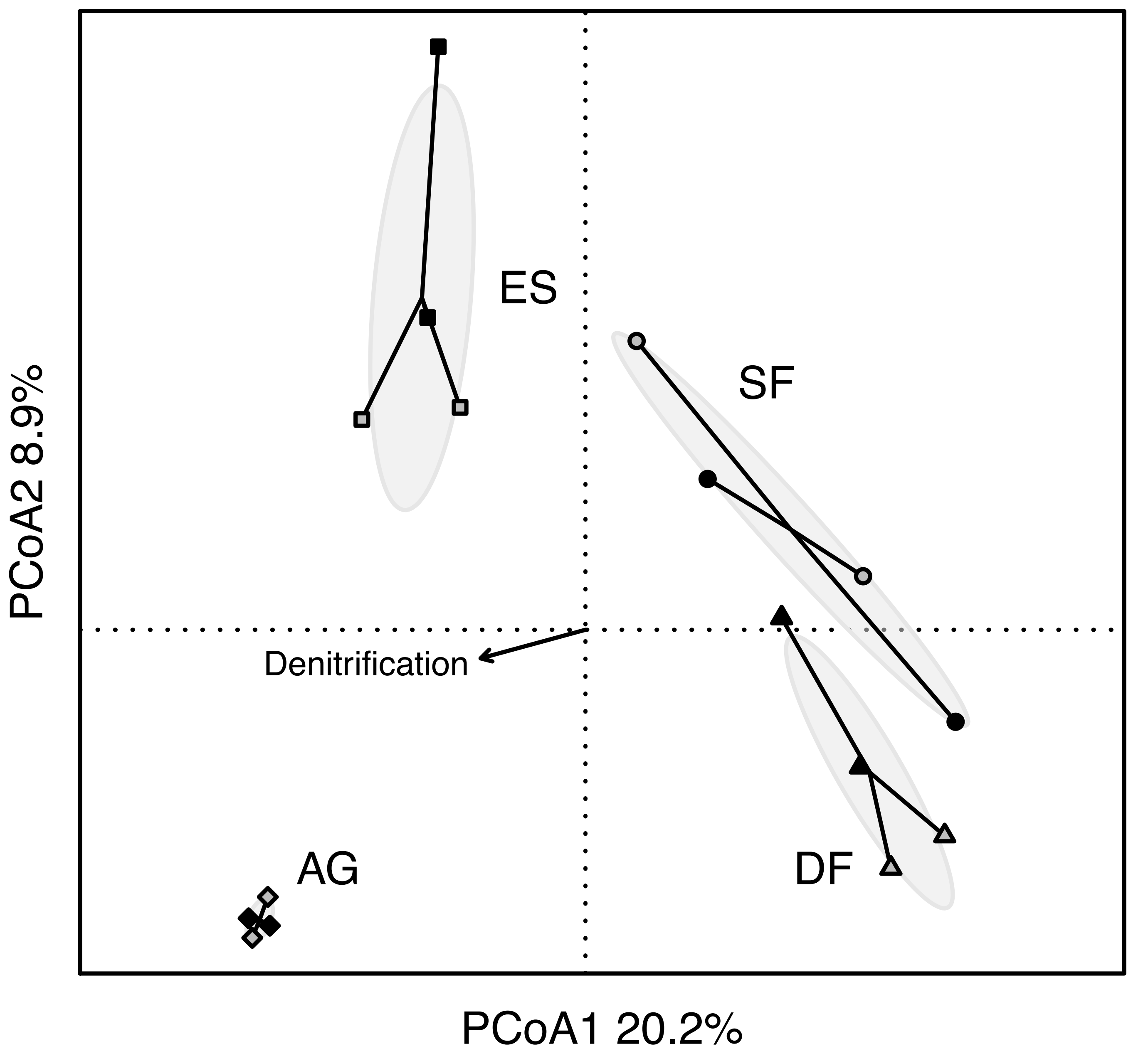
Denitrifying microbes



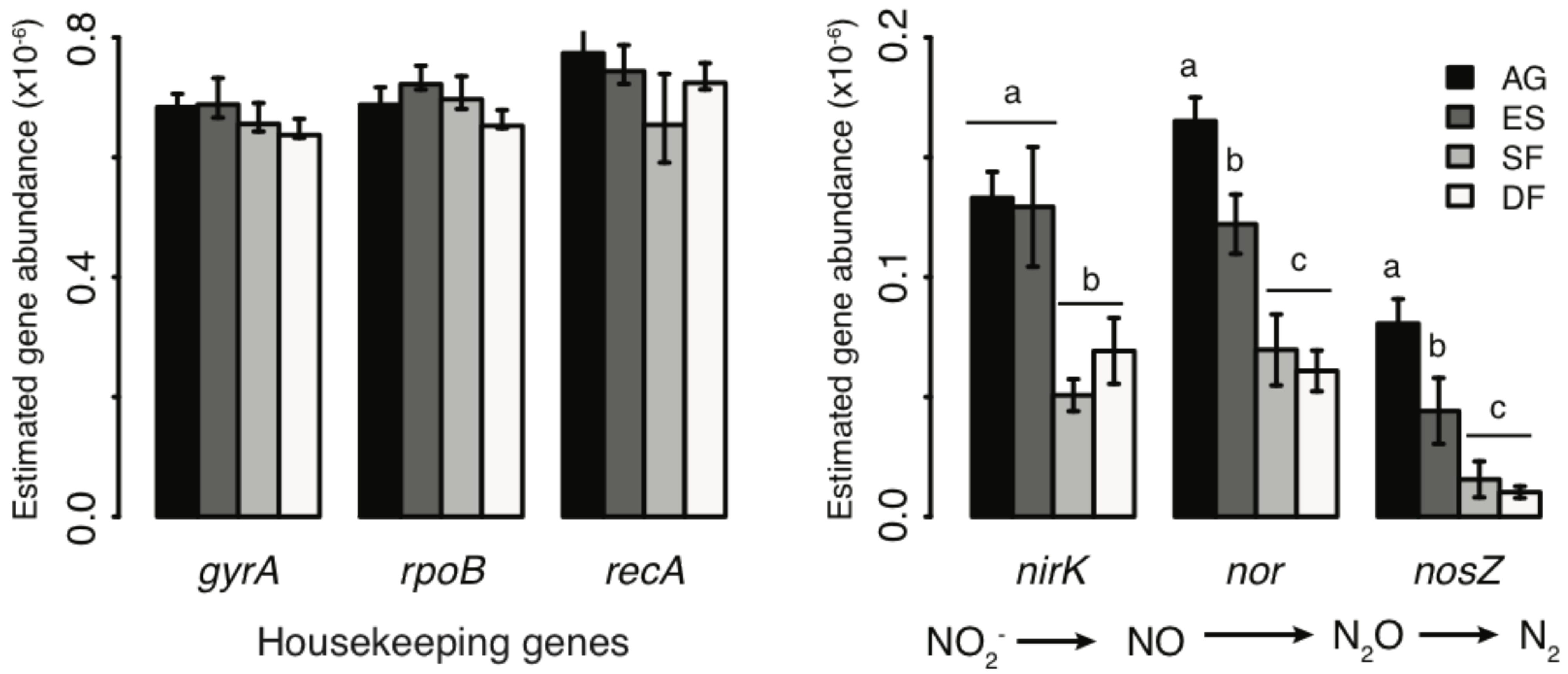
$$NO_2^- \xrightarrow{\text{nirK}} NO \xrightarrow{\text{nor}} N_2O \xrightarrow{\text{nosZ}} N_2$$



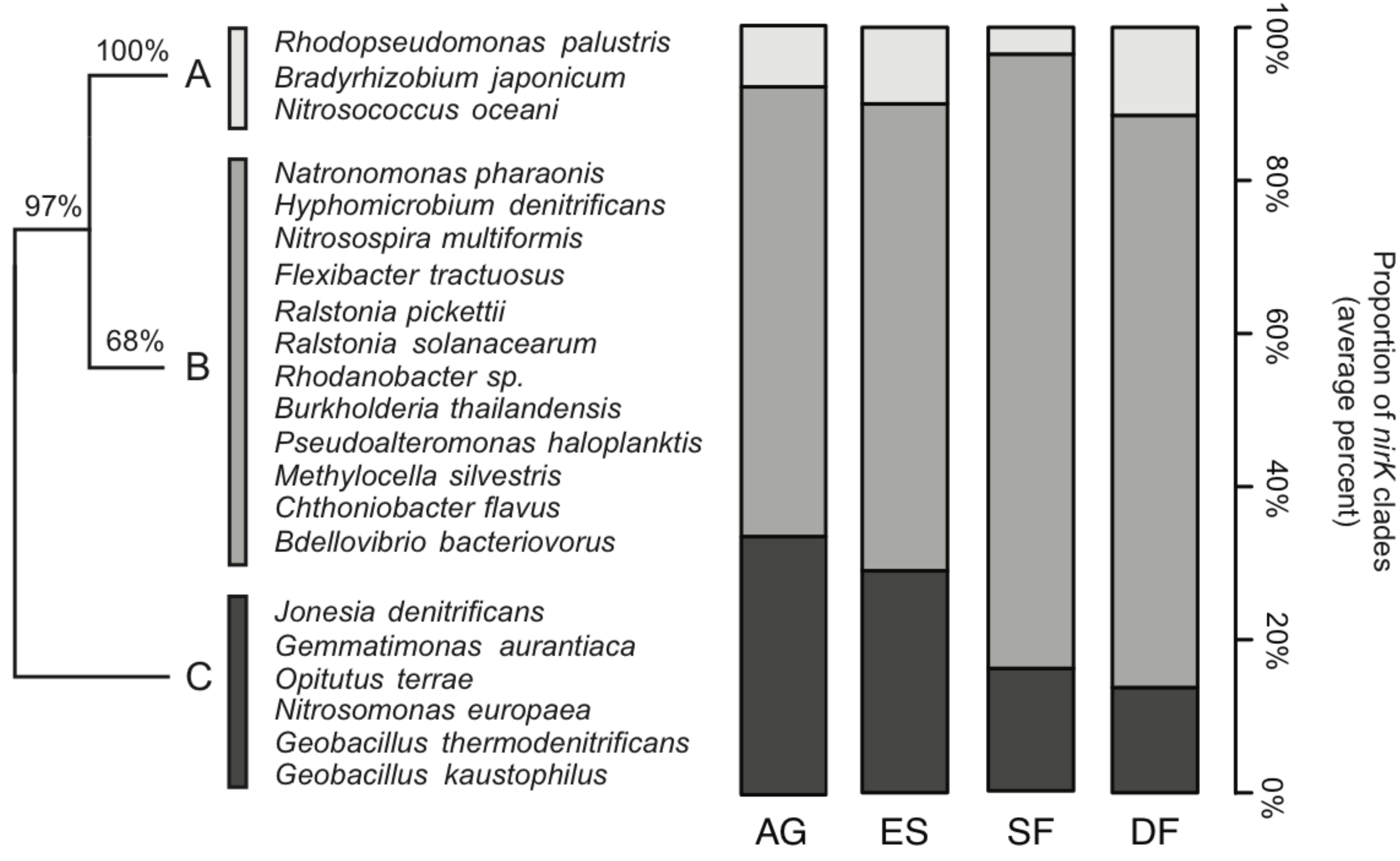
Nitrogen metabolism contributes to the differentiation of communities



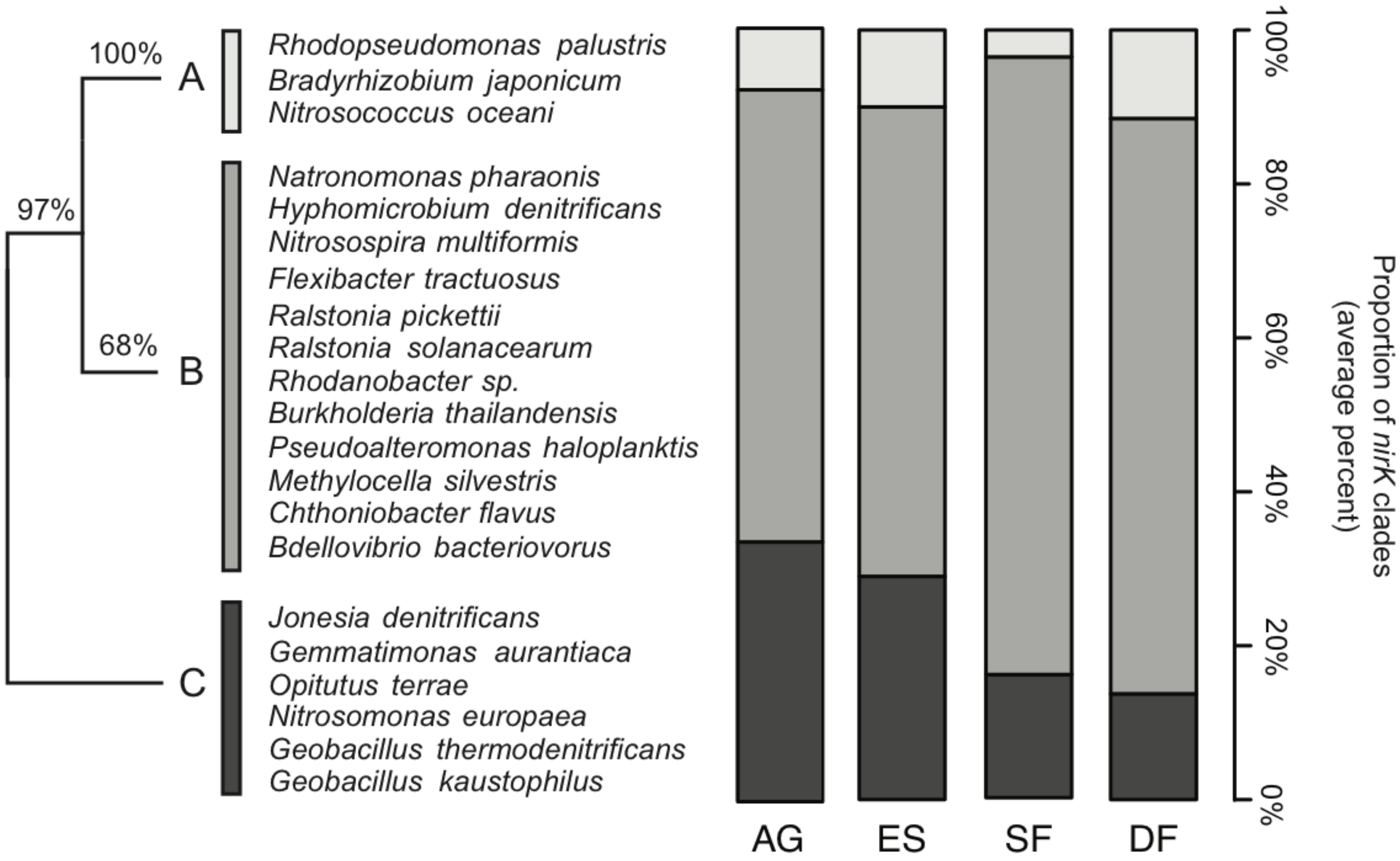
More denitrification potential in Ag soils



Denitrifier composition also changes



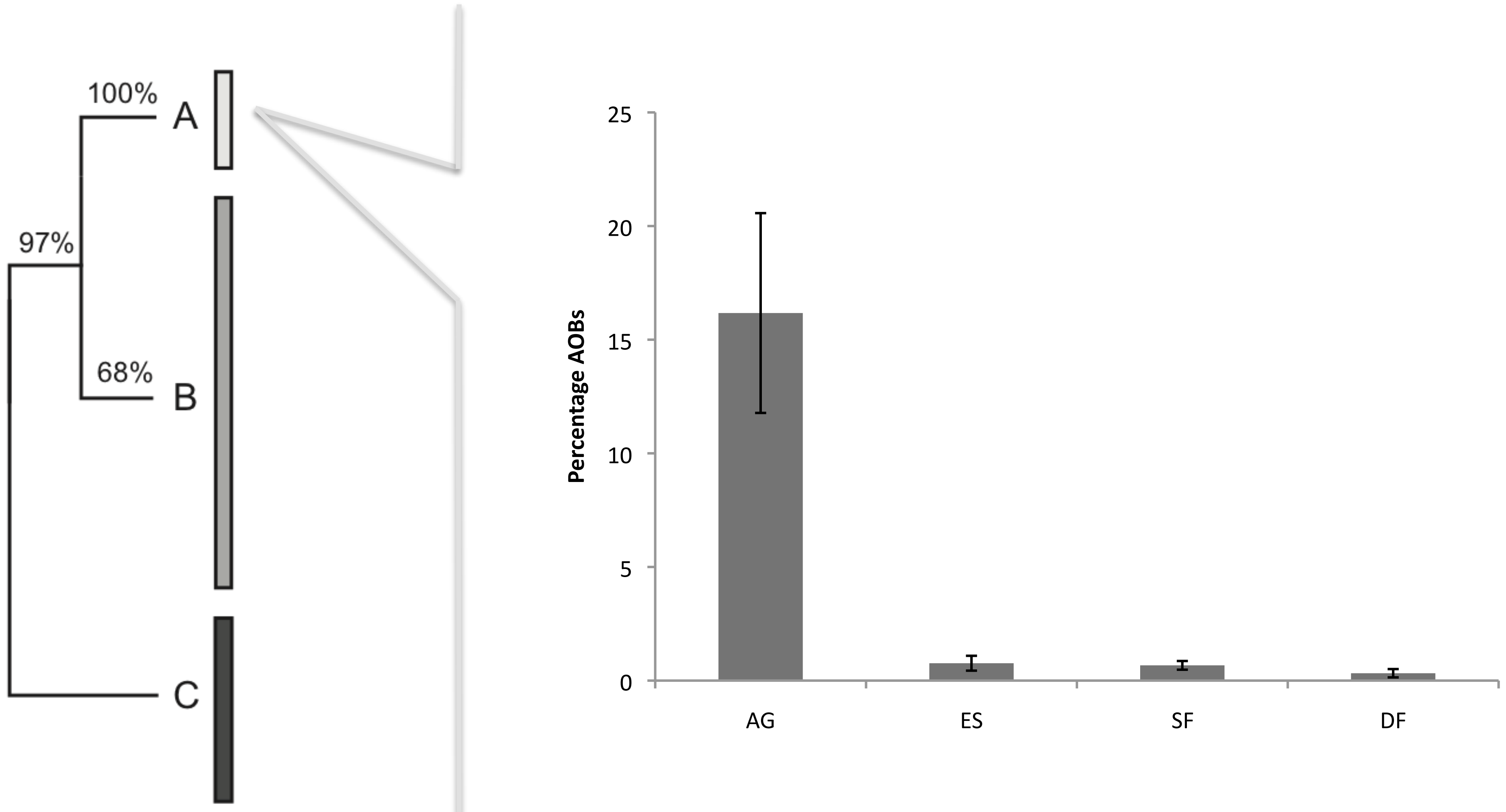
High denitrifer diversity



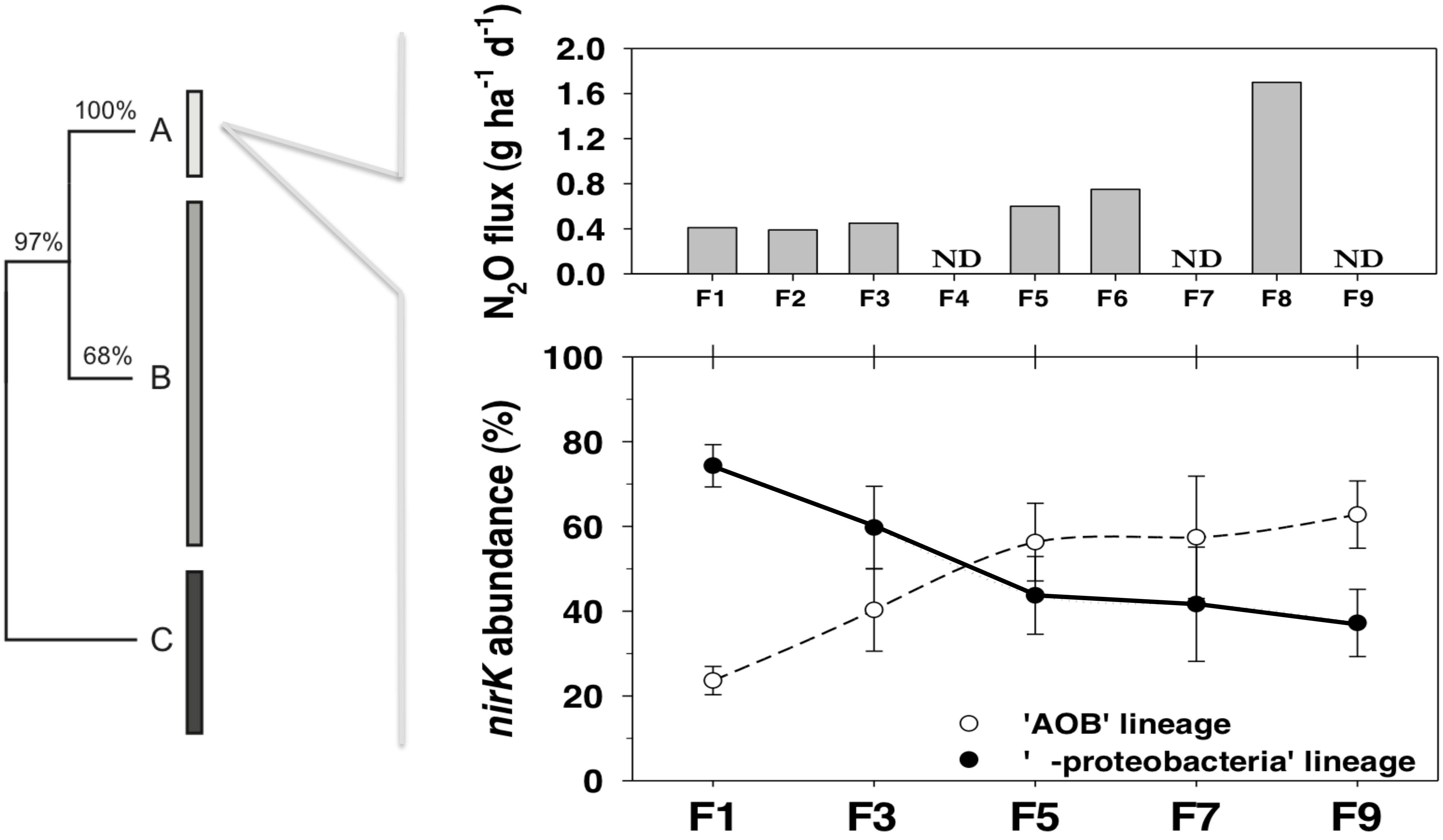
Types of denitrification

	Conditions	Function
<u>Heterotrophic denitrification</u> $NO_3^- \longrightarrow NO_2^- \xrightarrow{\textit{nirK}} N_2O \dashrightarrow N_2$	Anoxic	Energetics
<u>Autotrophic denitrification (AOBs)</u> $NH_4^+ \xrightarrow{O_2\ \ H_2O} NH_2OH \longrightarrow NO_2^- \xrightarrow{\textit{nirK}} N_2O$	Oxic	Nitrite detoxification

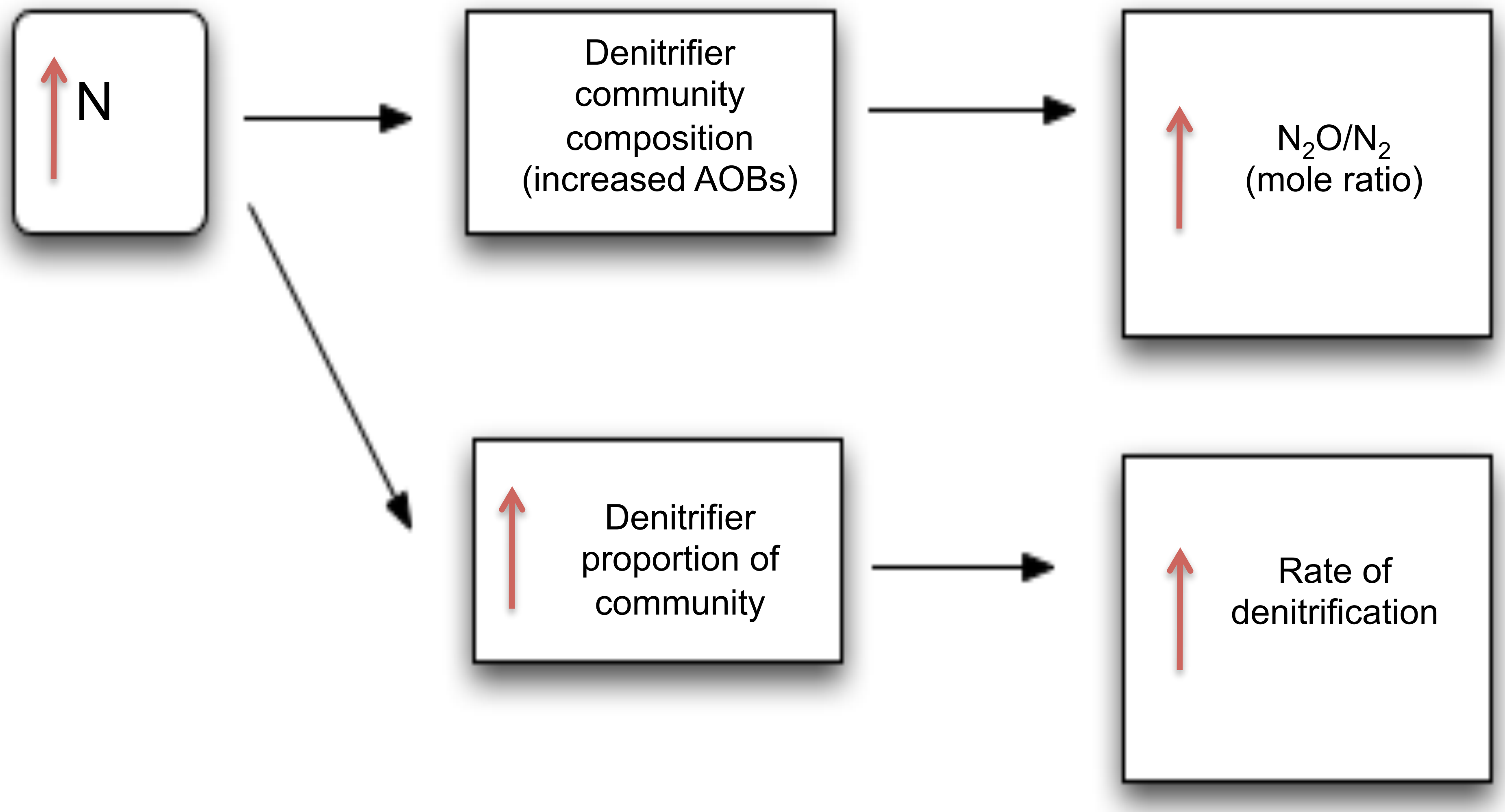
AOB proportion increased significantly in AG



AOB percentage correlates with fertilization

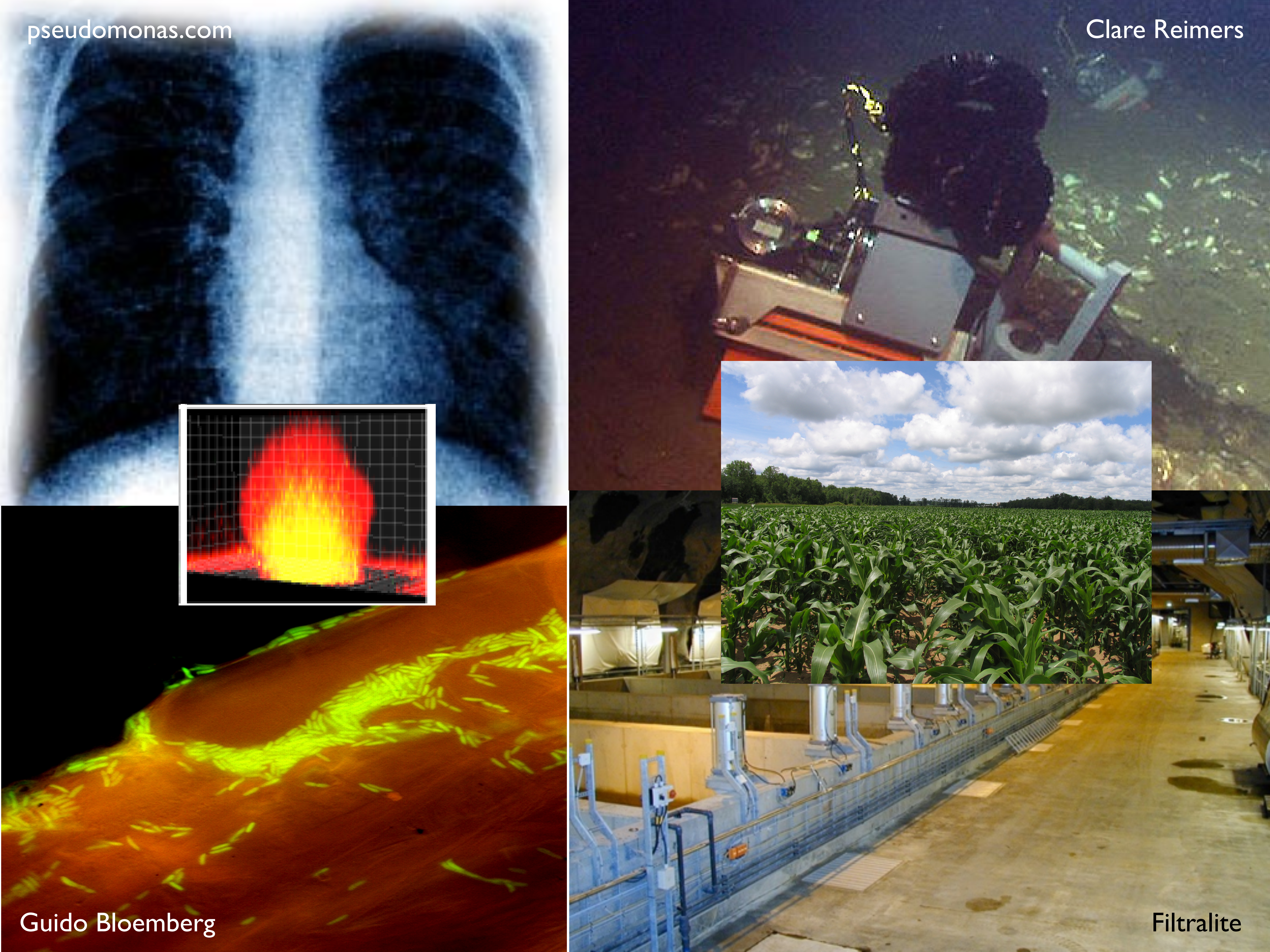


Microbial communities in agriculture are poised for denitrification



What is the relationship between denitrifying bacteria and N_2O 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


pseudomonas.com
Clare Reimers
Guido Bloemberg
Filtralite

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Lars Dietrich

Dr. Tom Schmidt

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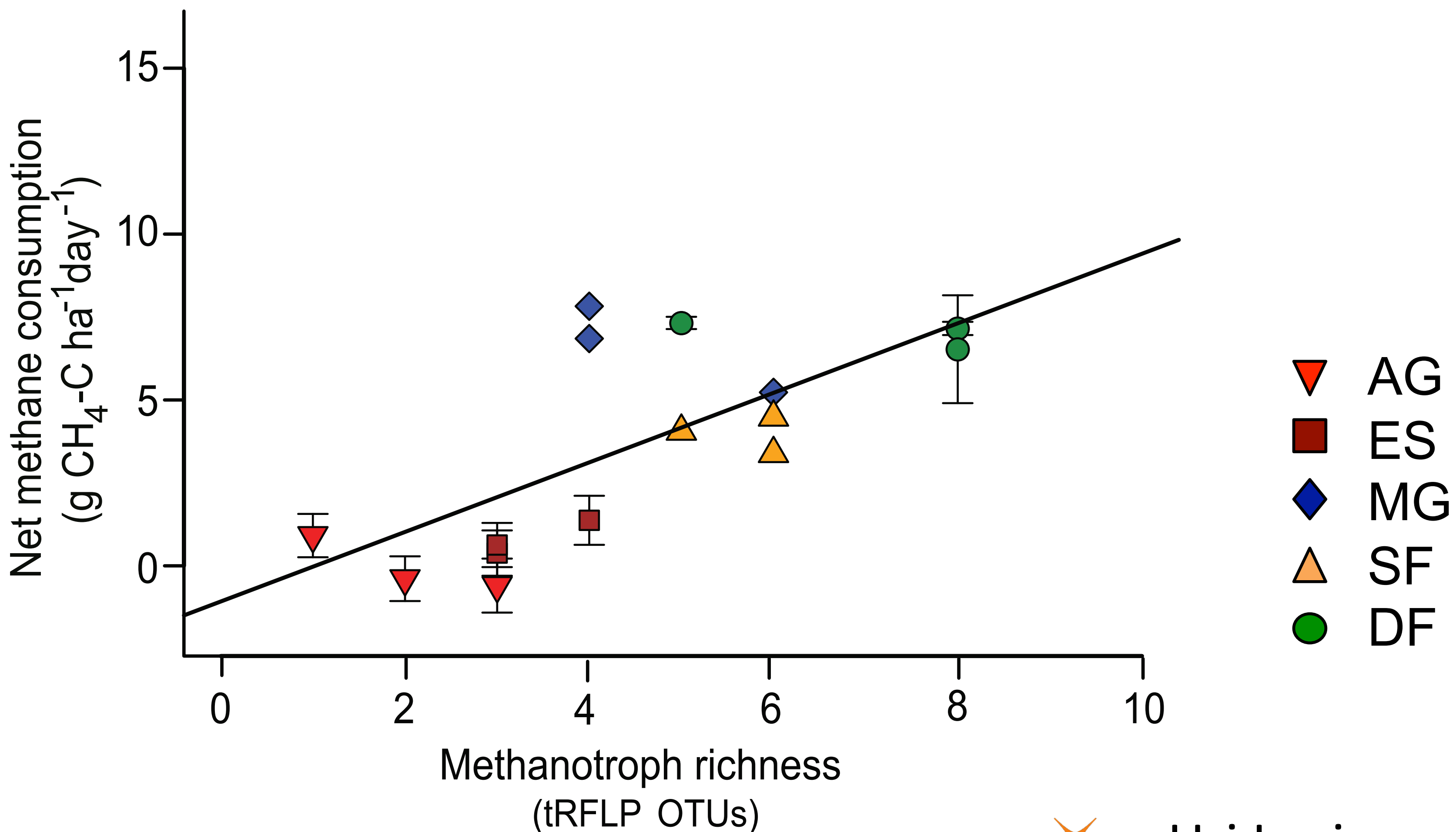
Bernard Schroeter
John Dover
Zarraz Lee
Brendan O'Neill
Ben Roller
Tom Schmidt

- Funding
  - DOE GLBRC
  - NSF
  - NSF Postdoctoral Fellowship

Methanotroph richness correlates with methane consumption

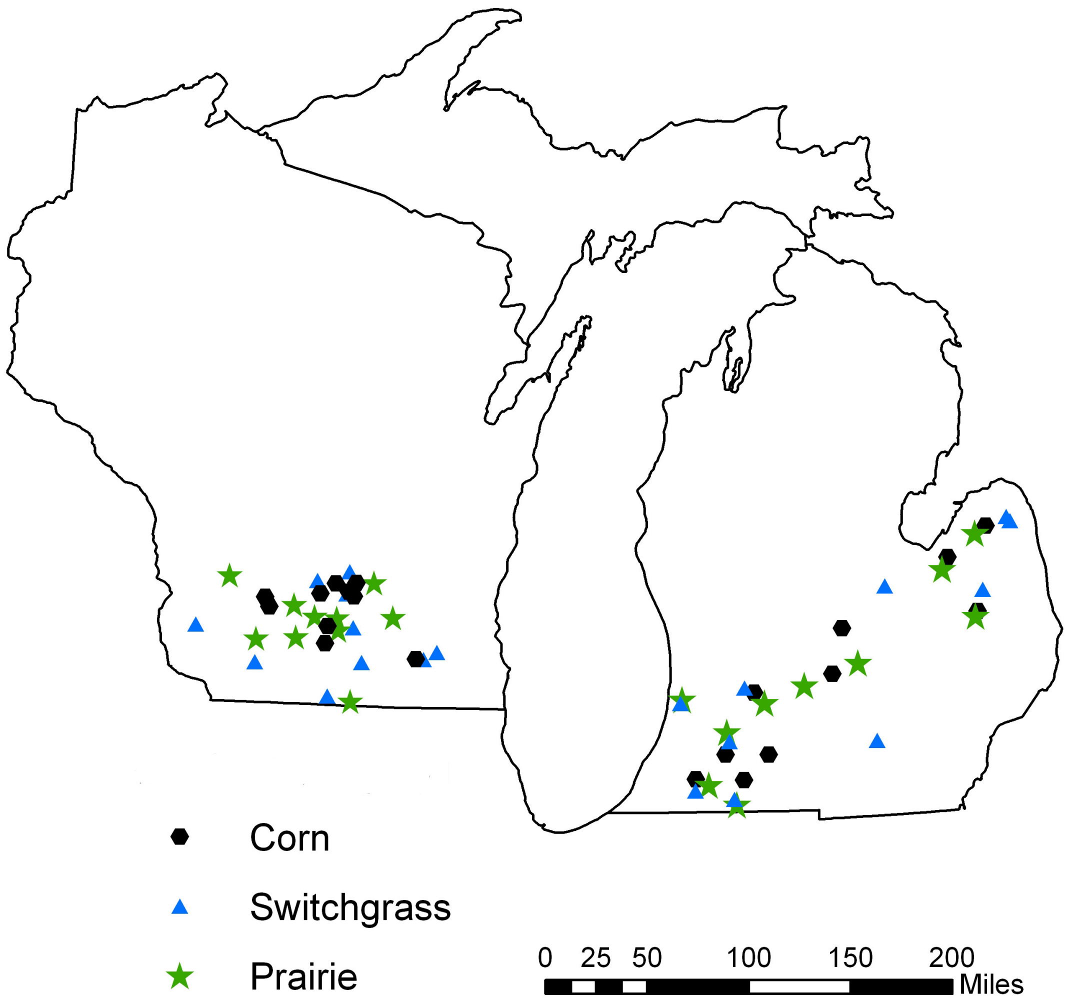


- Uri Levine
- Bernard Schroeter

- Levine, Teal, Schmidt 2010

Methane in biofuel crops

GLBRC Extensive Sites



- Leilei Ruan and G. Philip Robertson

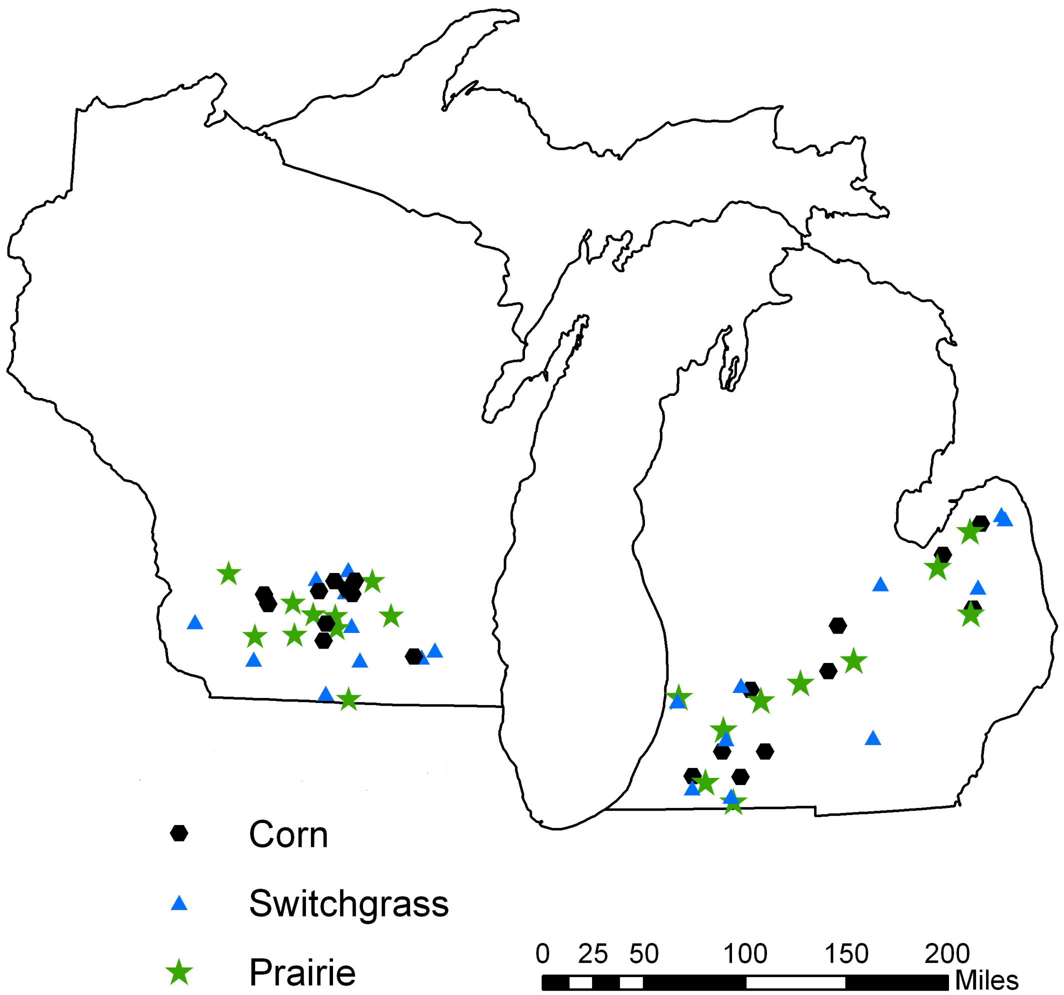
- Clone libraries
  - Classification of *pmoA* / *amoA*

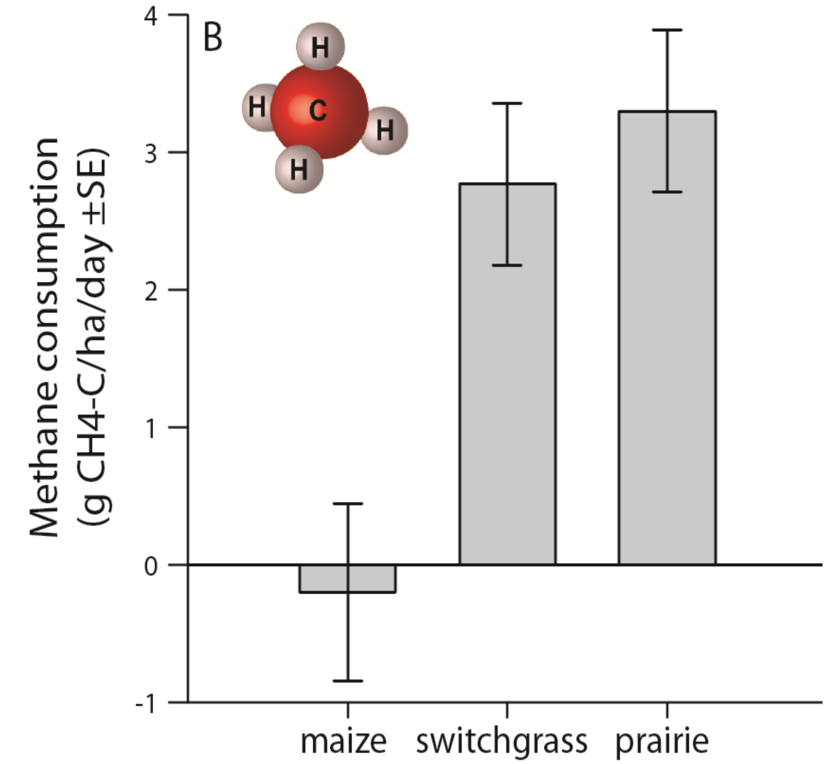
GFClassify –

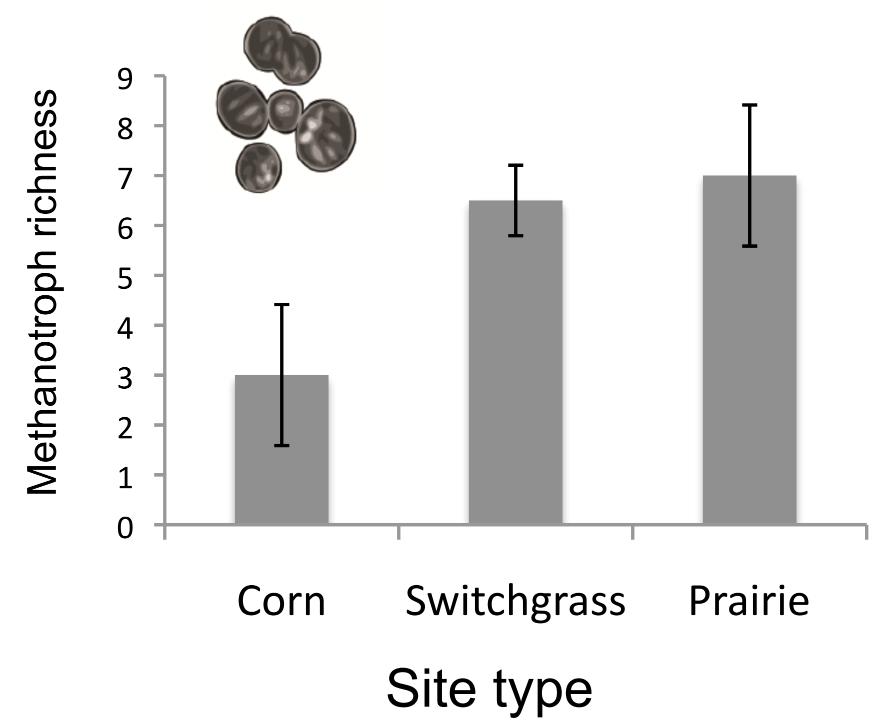
Ribosomal Database Project
Jordan Fish

Methane in biofuel crops

GLBRC Extensive Sites

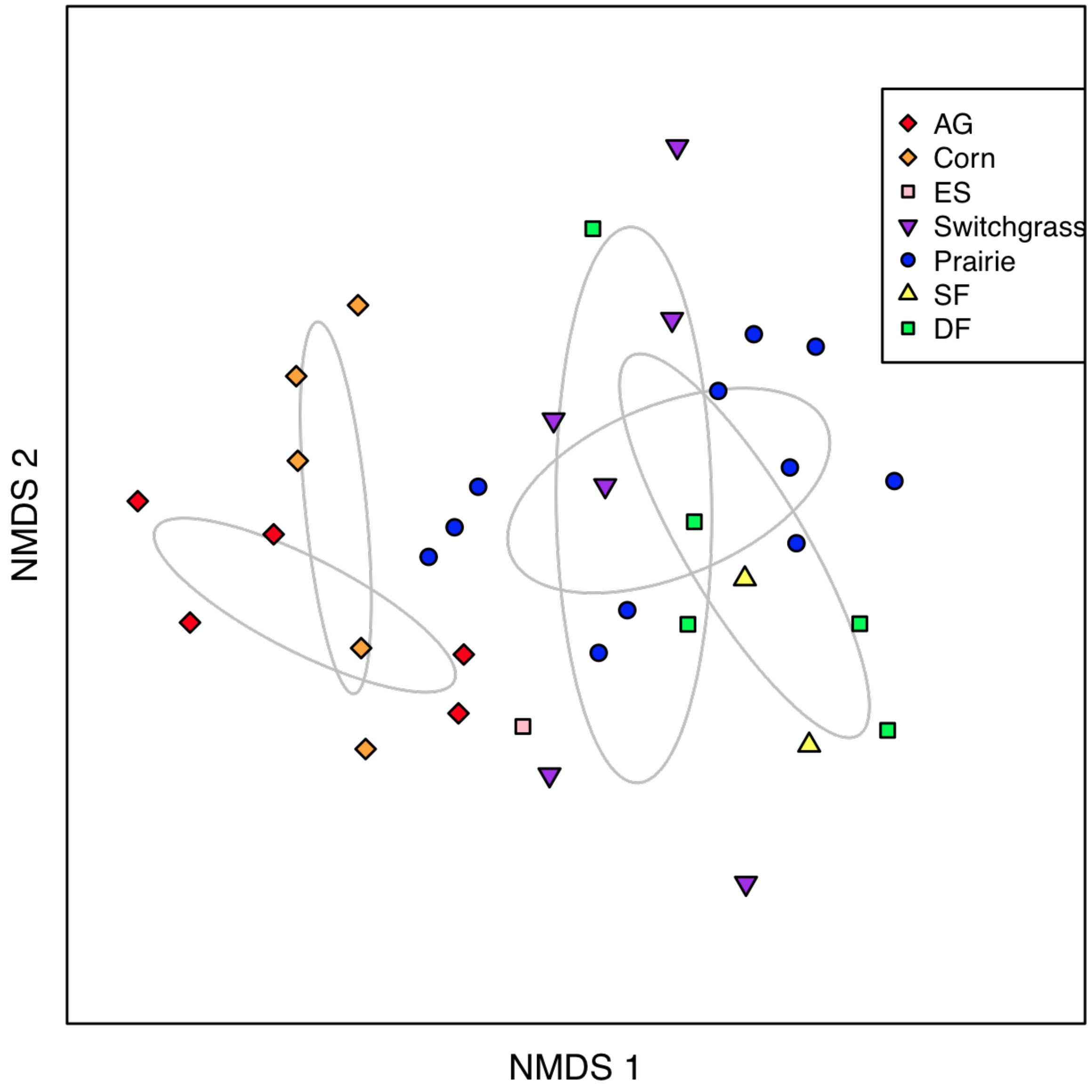


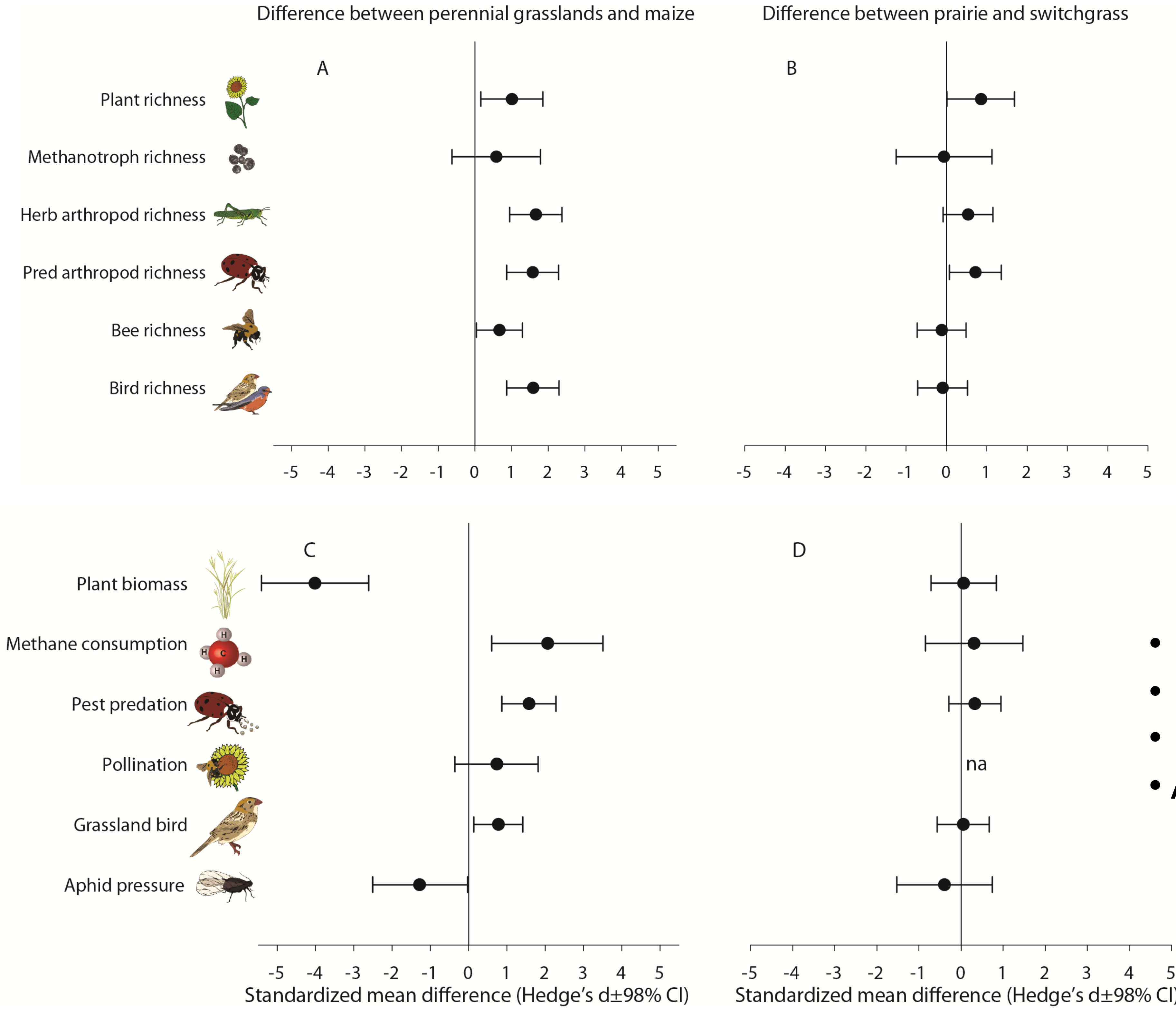




- Leilei Ruan and G. Philip Robertson

Methanotroph composition changes with agricultural management





- Productive
- Perennial
- Polycultures
- Appropriate Placement

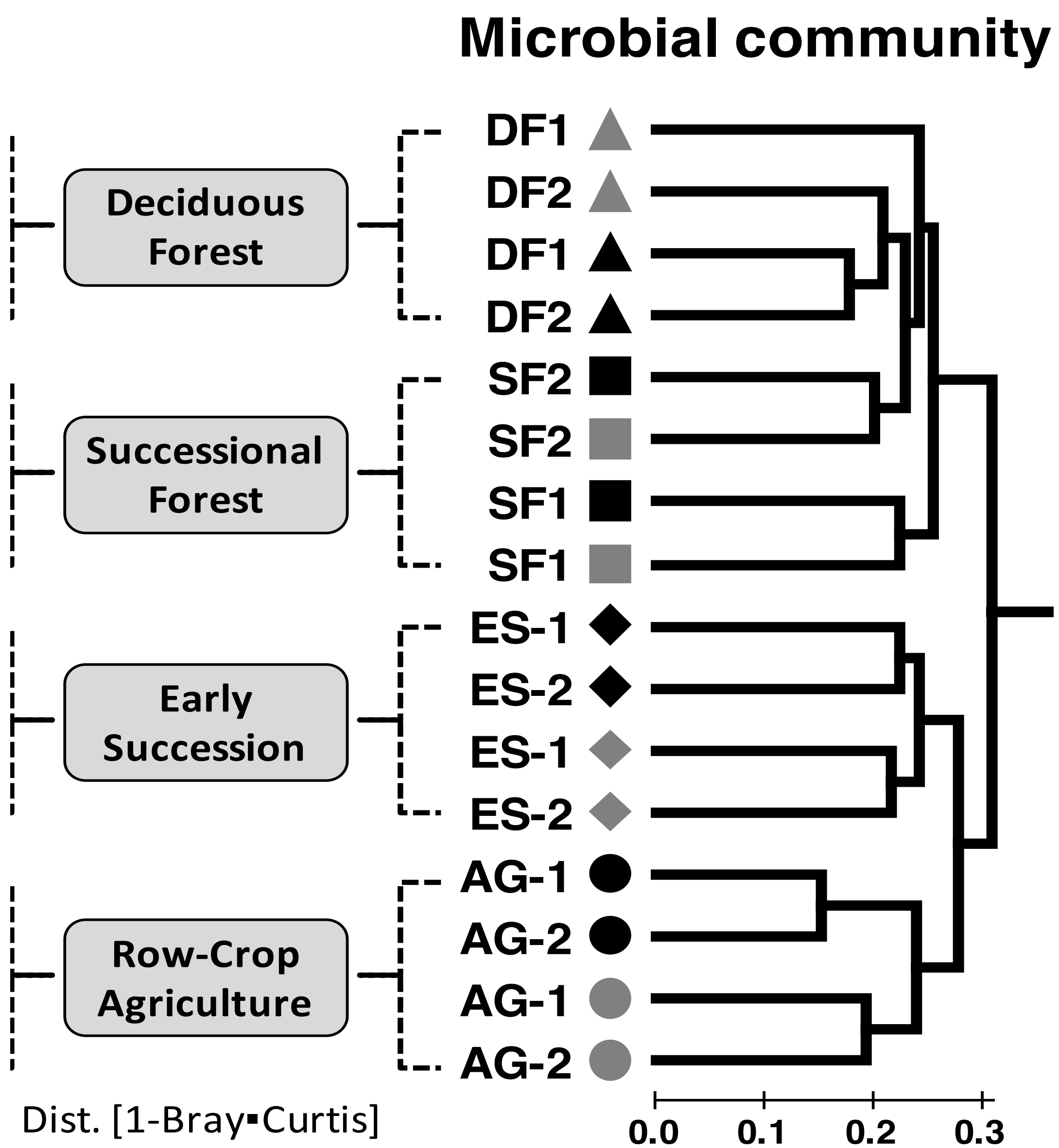
Werling et al, submitted

What is the relationship between denitrifying bacteria and N_2O, and methane oxidizing bacteria and CH_4 in upper Midwest soils?

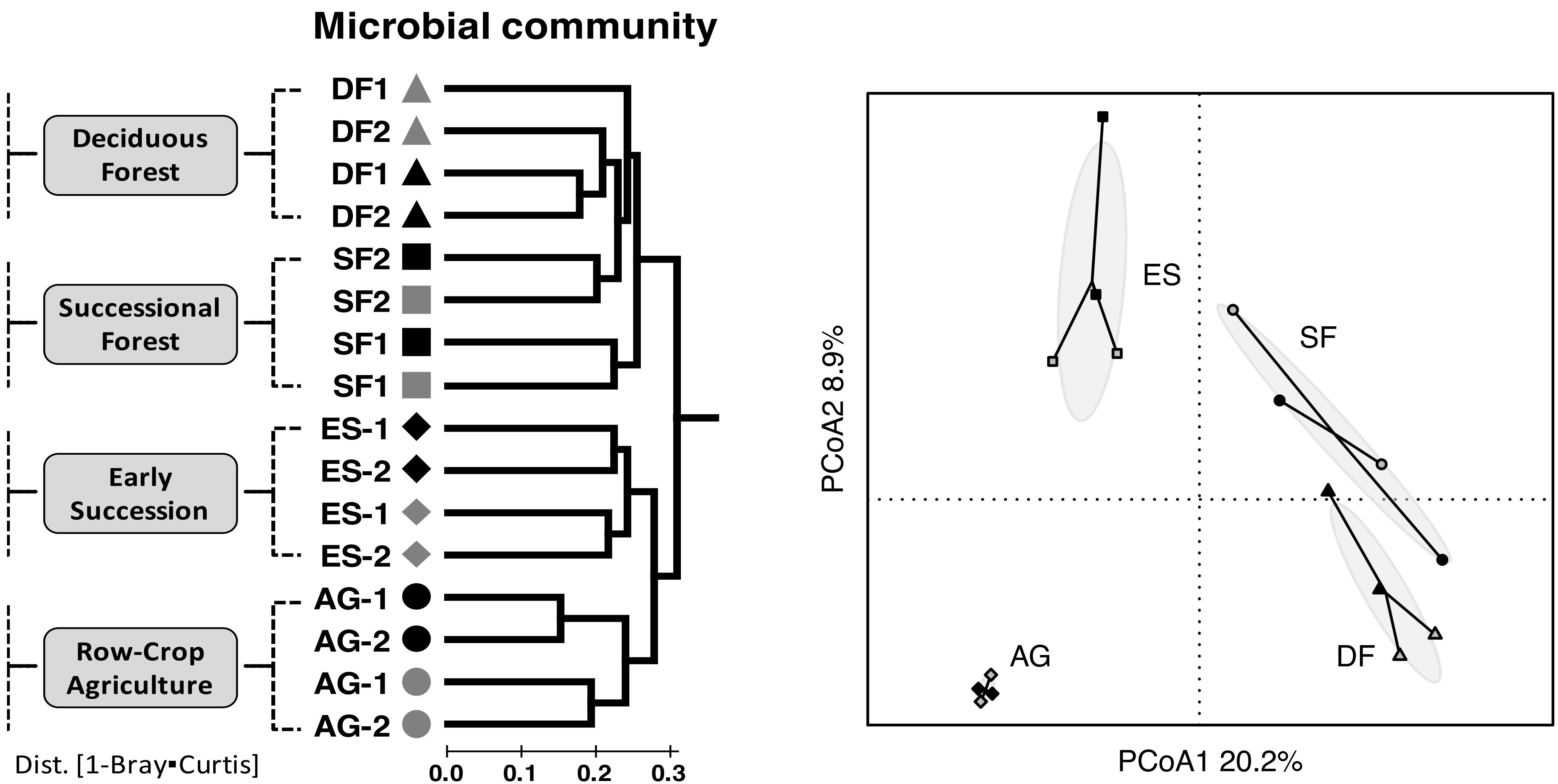
- Denitrifier abundance and composition changes with agricultural management. Communities more diverse than previously thought and poised for denitrification.
- Methanotroph richness and composition also changes with agricultural management and correlates with methane consumption
- May be potential for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

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

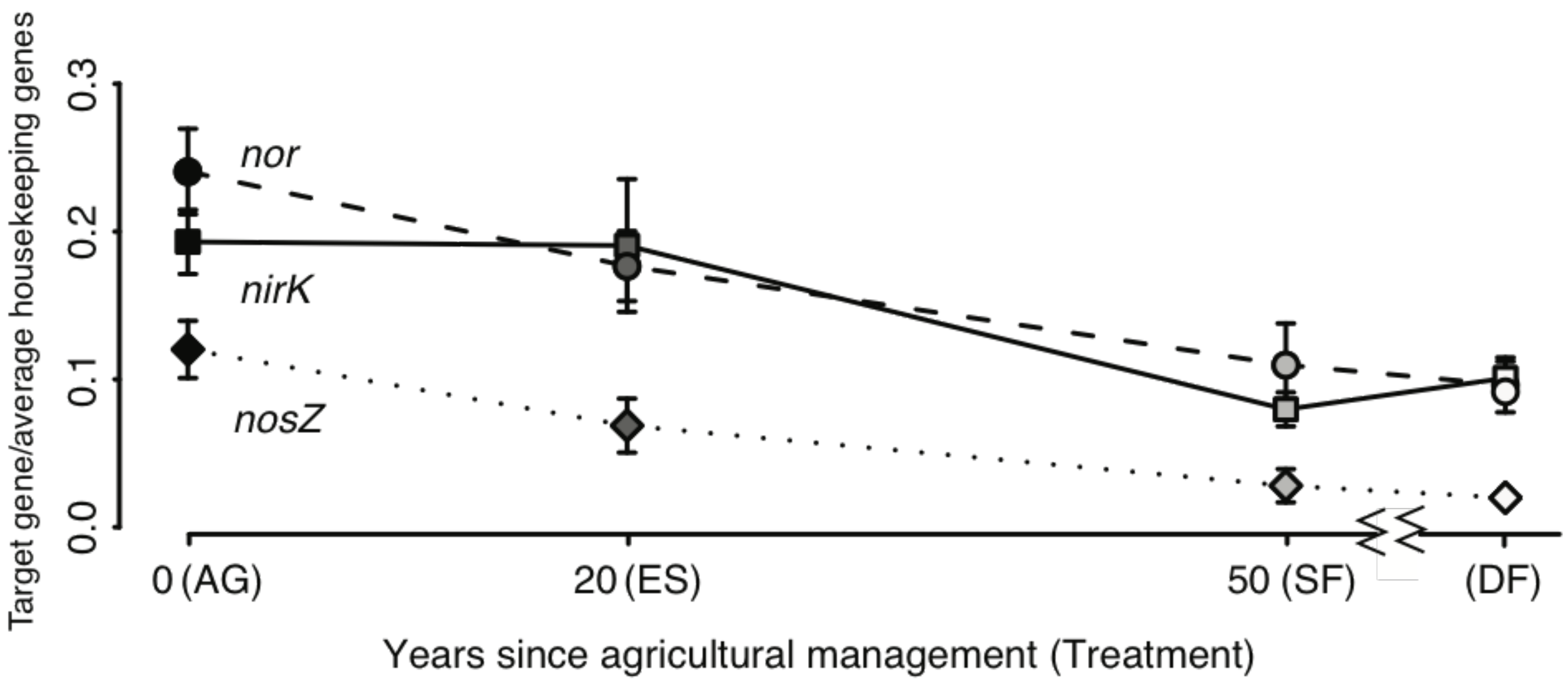
More than 20 year recovery for bacterial community



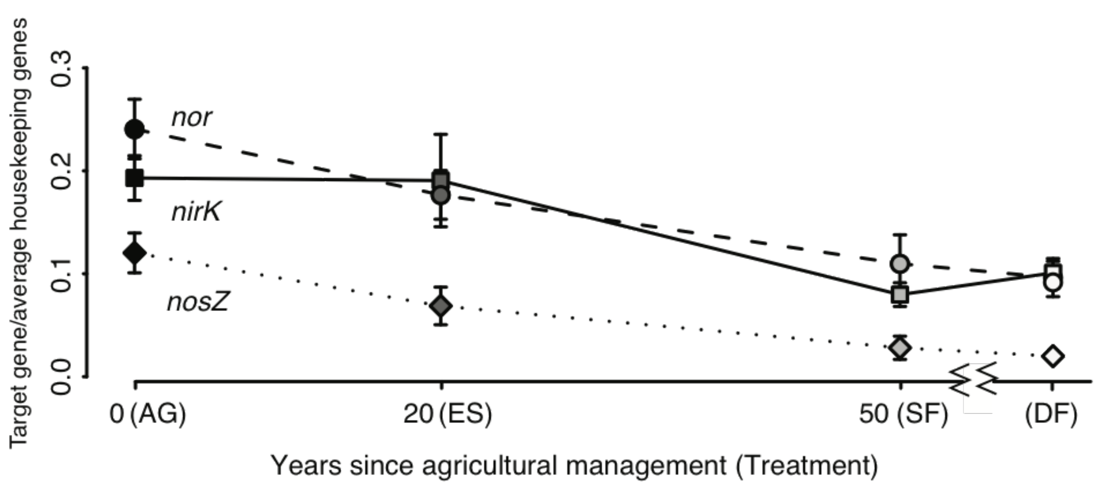
More than 20 year recovery for bacterial community

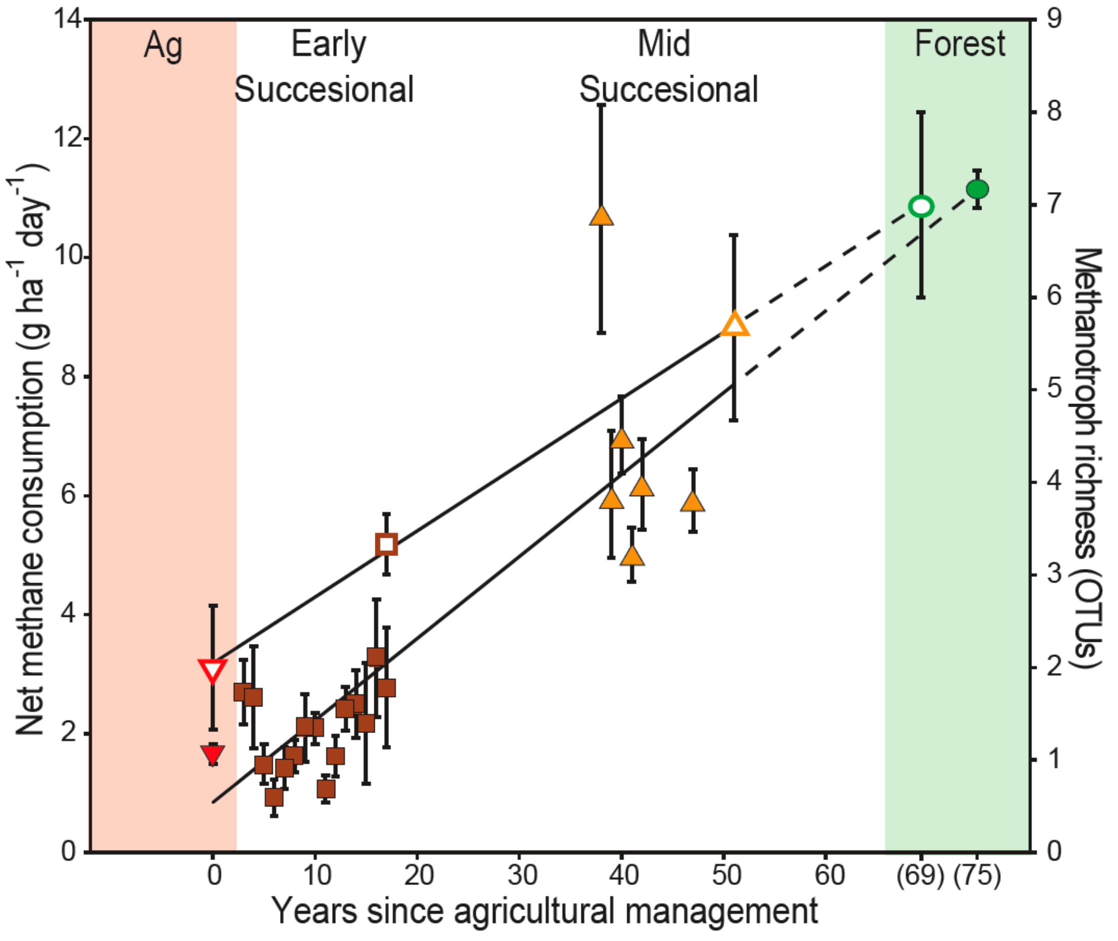


More than 20 year recovery for denitrifiers



Methanotrophs take more than 40 years to recover





Properties contributing to resilience

1. Responsive, regulatory feedbacks

Single-species *Shewanella oneidensis* biofilm

2. Diversity and redundancy

Soil microbial communities and greenhouse gas fluxes

3. Modular networks

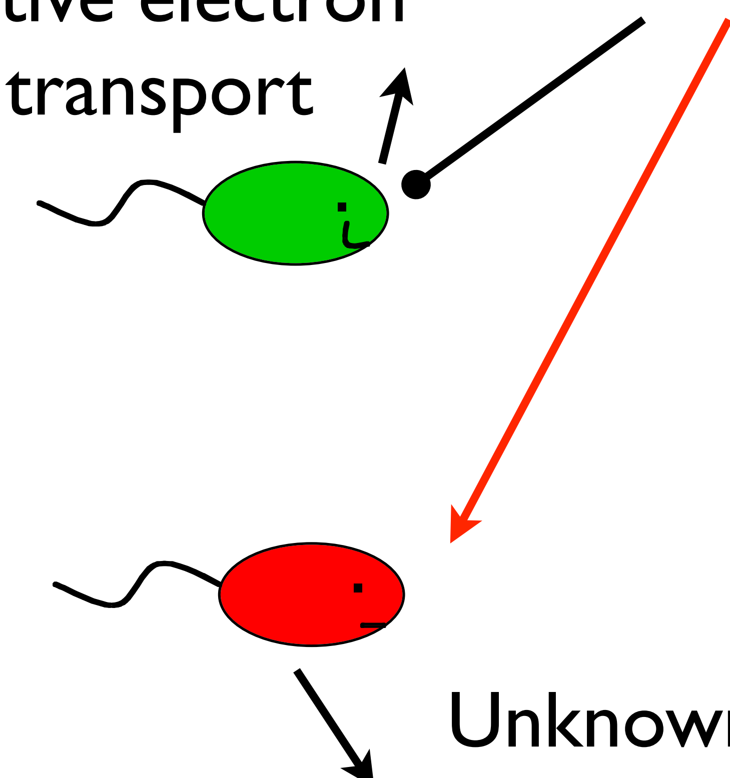
Future directions

Live/Dead stain may not be accurate

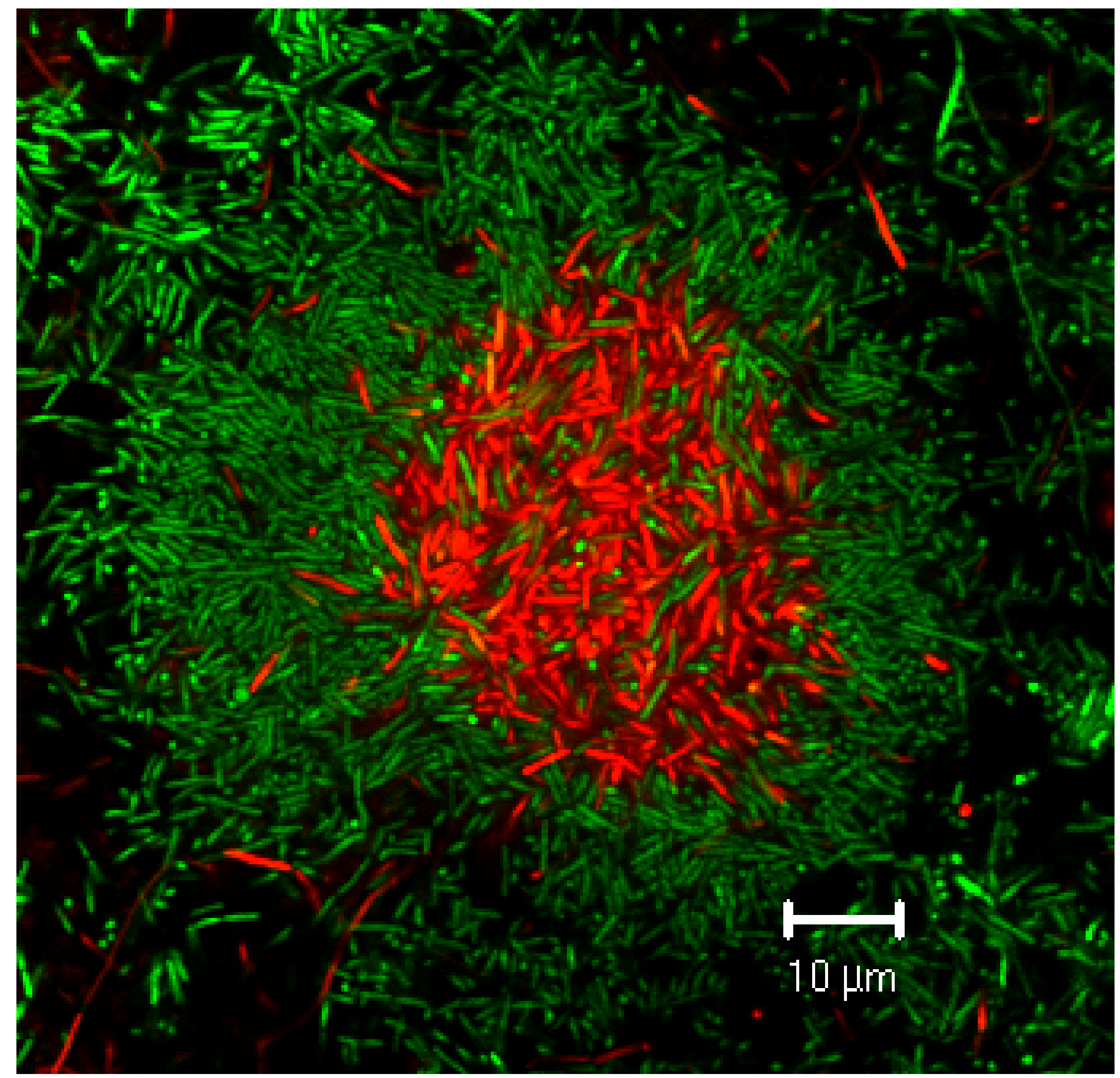
Dead stain

Propidium iodide

Active electron transport

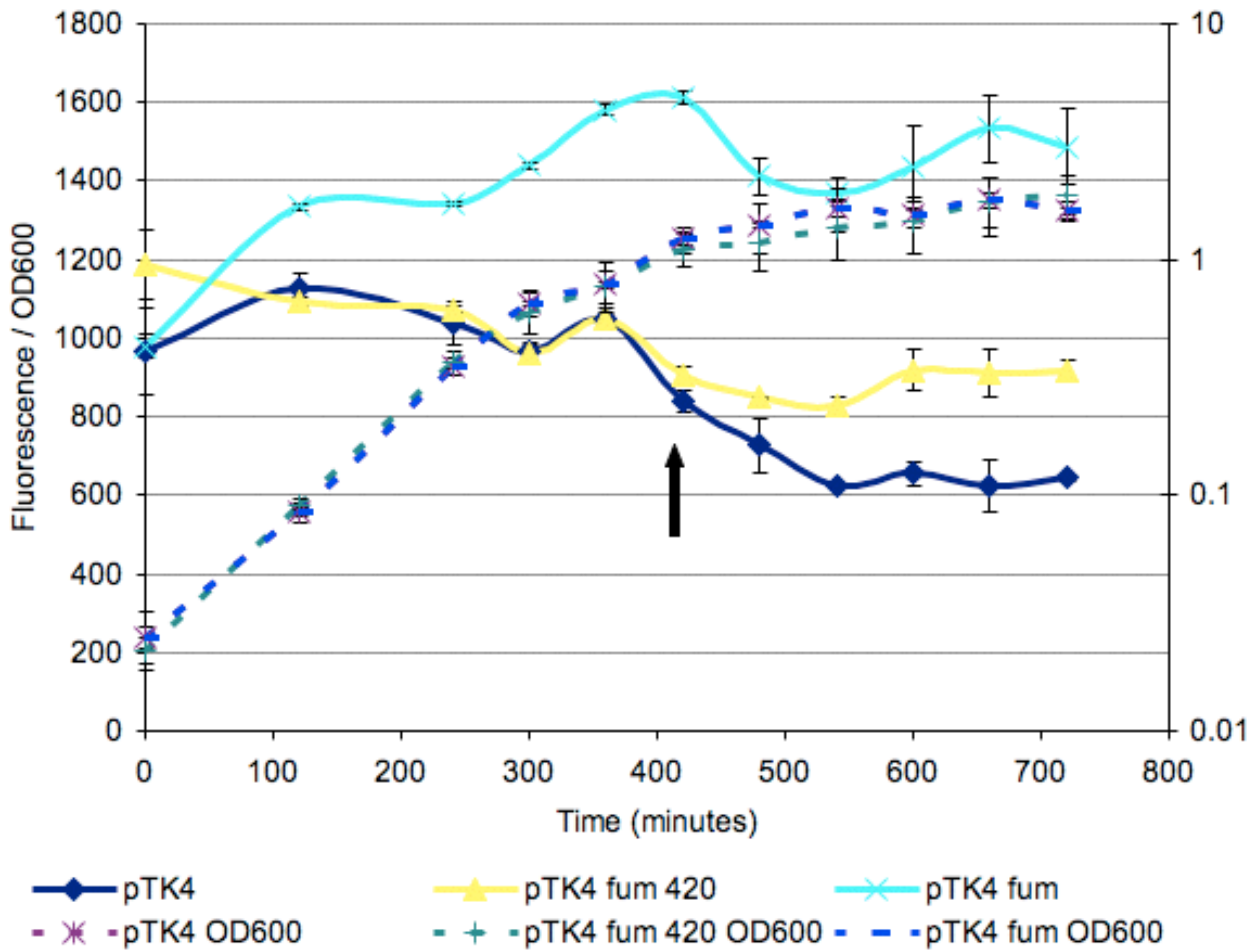


Unknown levels of electron transport

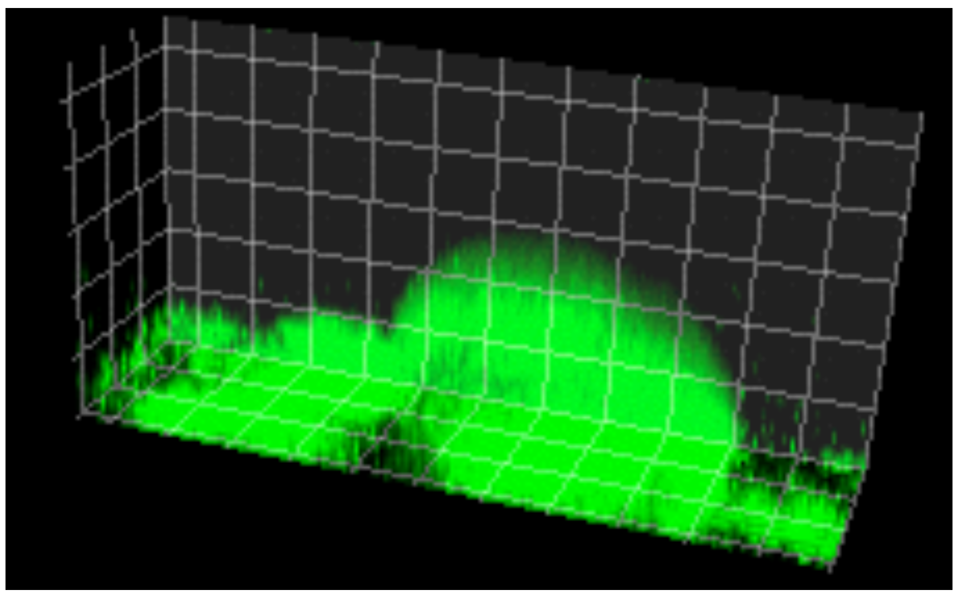


Teal et al, 2006

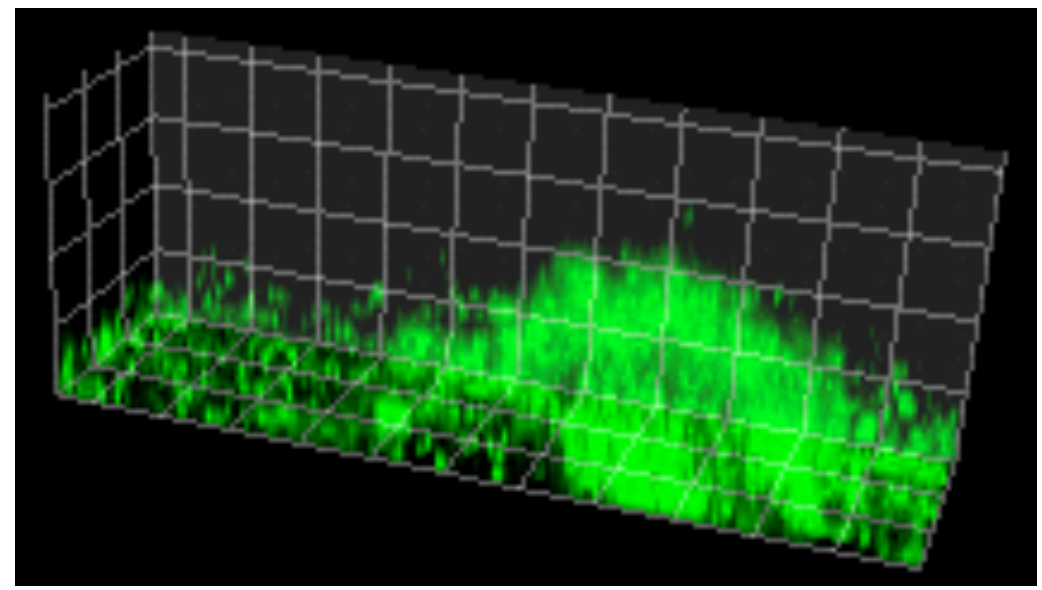
Addition of an alternative electron acceptor affects cell growth state



Addition of an electron donor does not have the same effect



Before



20mM lactate

Colony size not significantly different, but intensity is lower due to decreased number of cells