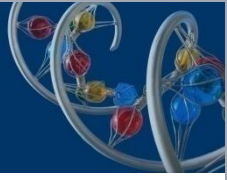


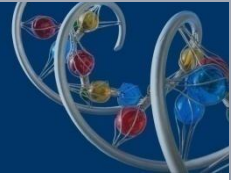
Microbial communities in restored freshwater wetlands

Susannah Green Tringe
DOE Joint Genome Institute
INTECOL, June 5, 2012





- **Project motivation and background**
 - **Metagenomics and carbon cycling**
 - **Study site, sampling plan, biogeochemistry**
 - **Methane production**
- **Sequence-based analyses**
 - **16S rRNA pyrotag profiling**
 - **Shotgun metagenomics**

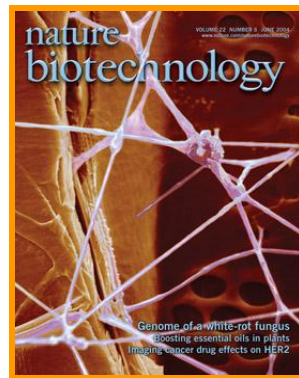


 **DOE Bioenergy
Research Centers**

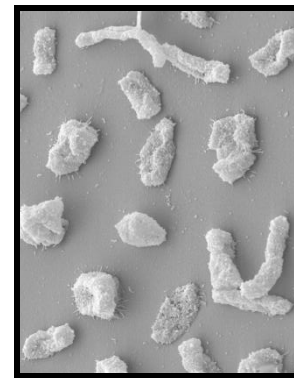
**Community
Sequencing
PROGRAM**



Plants



Fungi

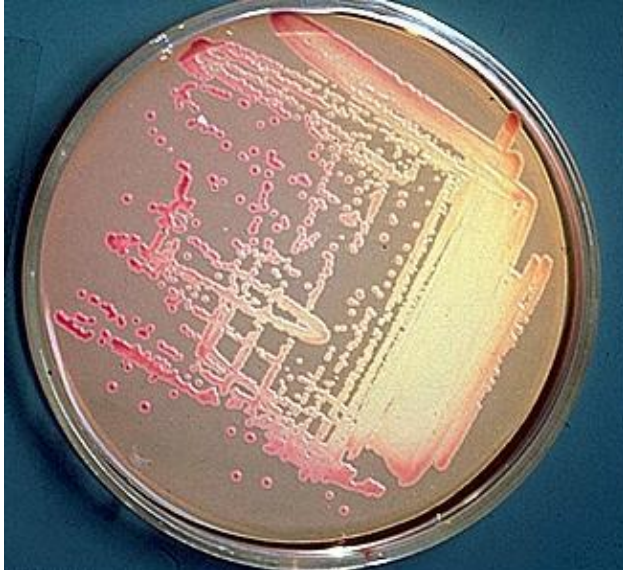
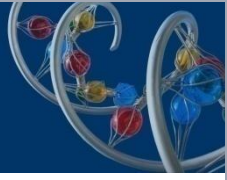


Microbes

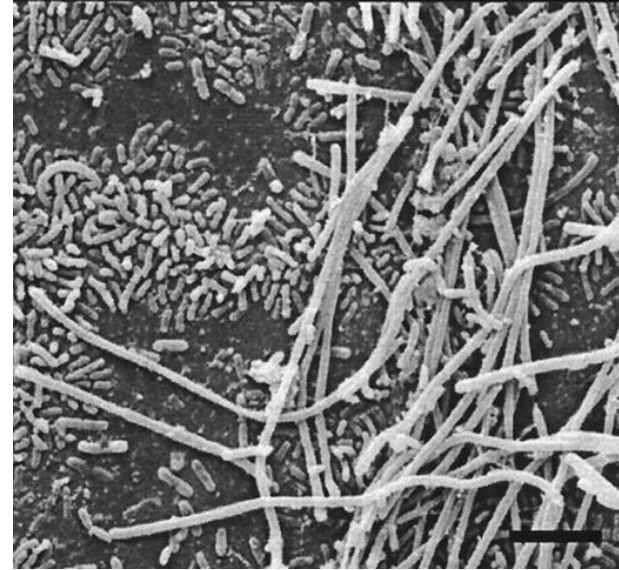


Metagenomes

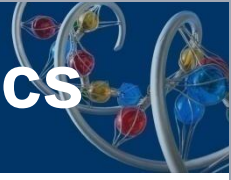
What is metagenomics?



Genomics



Metagenomics



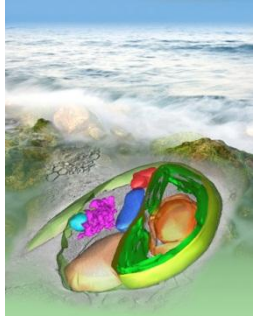
Permafrost metagenome



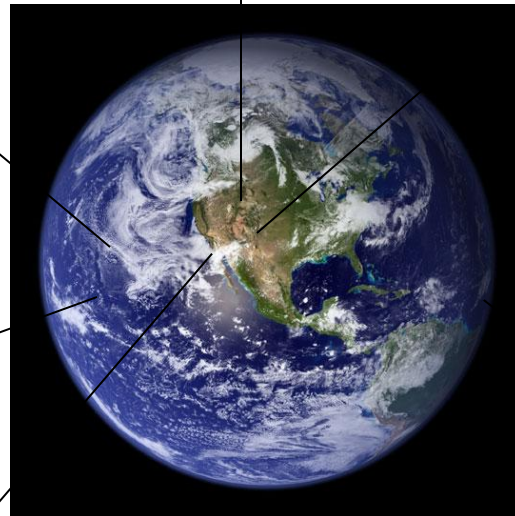
Prairie soil metagenome



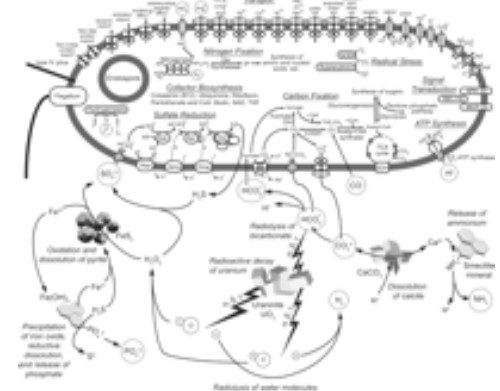
Micromonas sp.



(Science)



Deep subsurface ecosystem



(Science)

Phaeodactylum (diatom)



(Nature)

Lake Washington Methylophils



(Nat Biotech)

Why study wetlands?

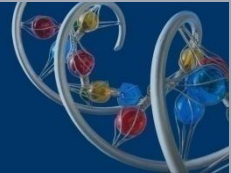
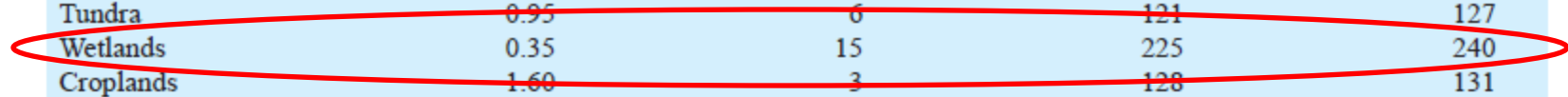


Table 1: Global carbon stocks in vegetation and soil carbon pools down to a depth of 1 m.

Biome	Area (10 ⁹ ha)	Global Carbon Stocks (Gt C)		
		Vegetation	Soil	Total
Tropical forests	1.76	212	216	428
Temperat				159
Boreal fo				559
Tropical				330
Temperate grasslands	1.25	9	295	304
Deserts and semideserts	4.55	8	191	199
Tundra	0.95	6	121	127
Wetlands	0.35	15	225	240
Croplands	1.60	3	128	131
Total	15.12	466	2 011	2 477

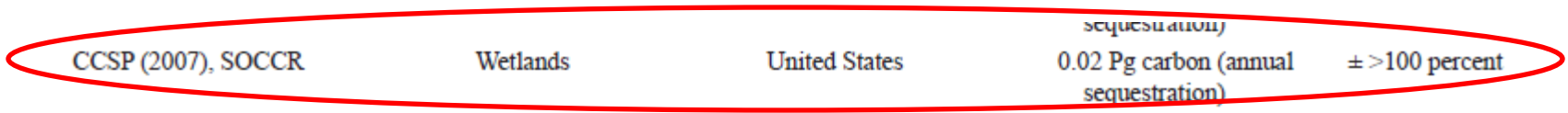
Note: There is considerable uncertainty in the numbers given, because of ambiguity of definitions of biomes, but the table still provides an overview of the

Wetlands store a lot of carbon (IPCC, 2000)

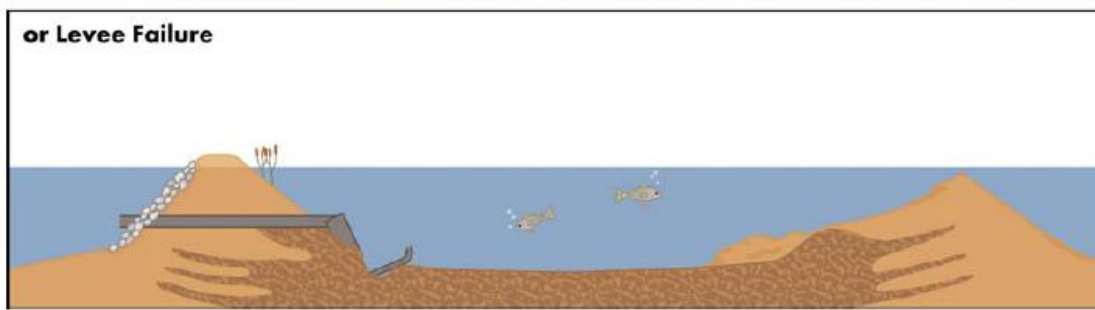
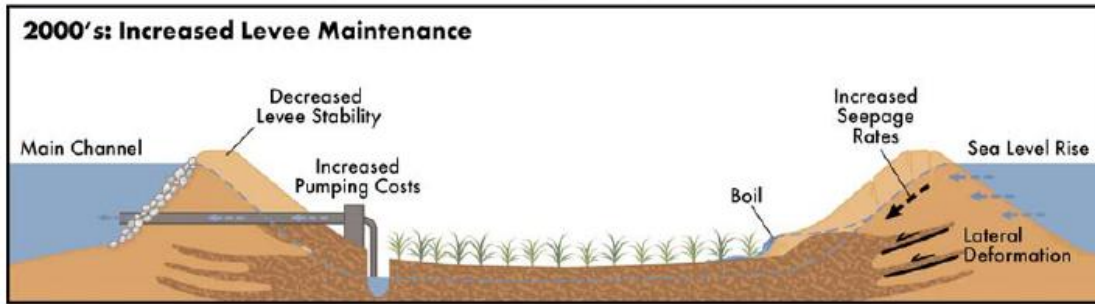
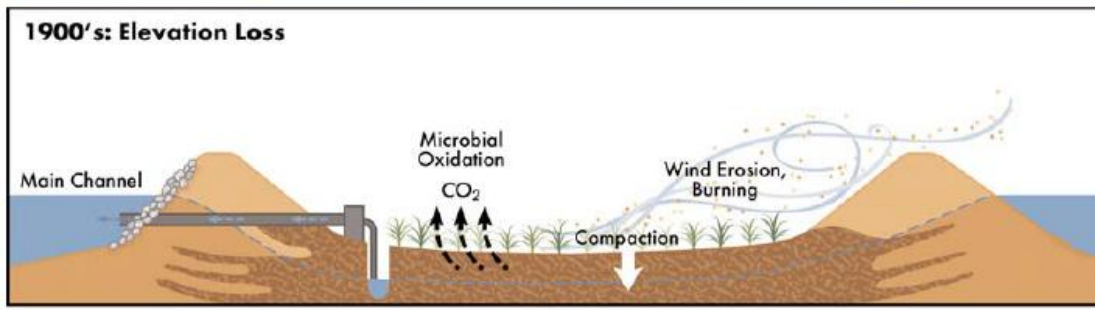
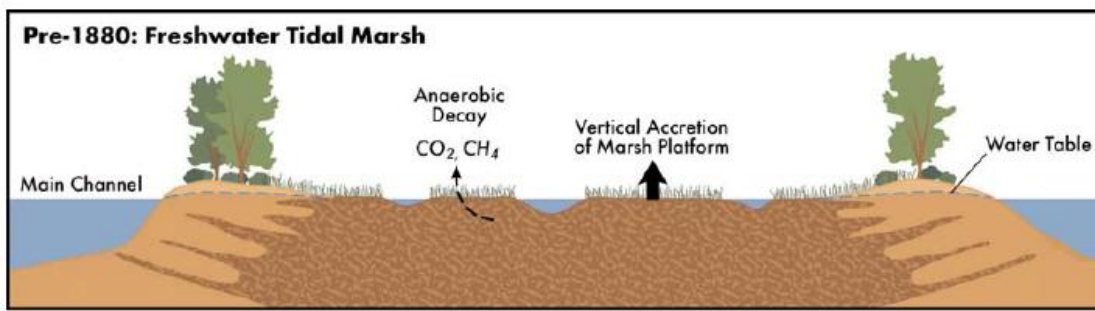
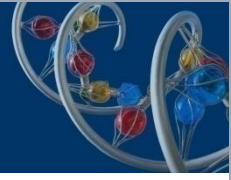


Assessment and source	Assessment components	Geographic scope	Quantity (stock or flux)	Uncertainty (95 percent confidence level)
CCSP (2007), SOCCR	Wetlands	United States	0.02 Pg carbon (annual sequestration)	± >100 percent

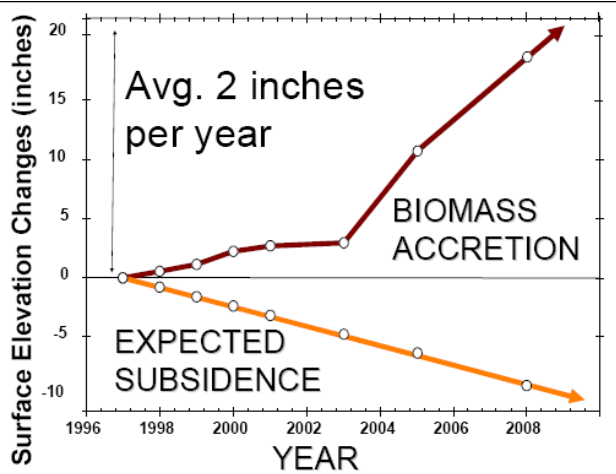
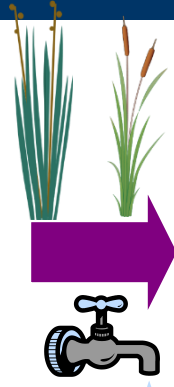
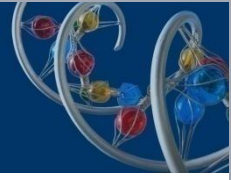
...but their sequestration potential is uncertain (USGS, 2010)



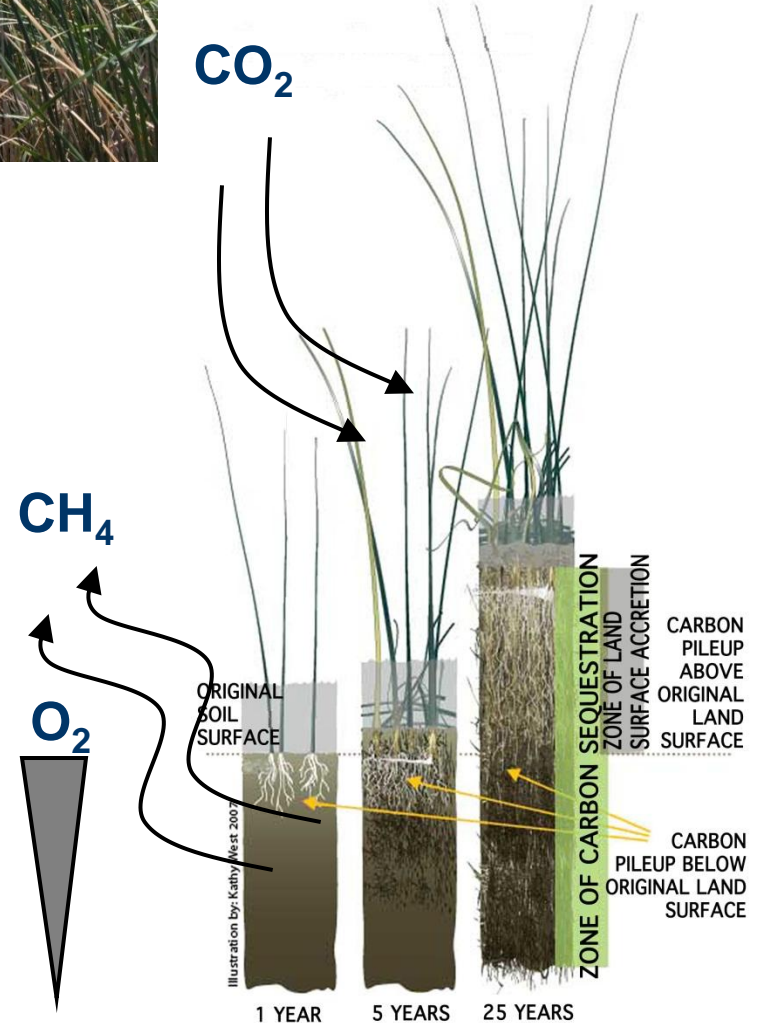
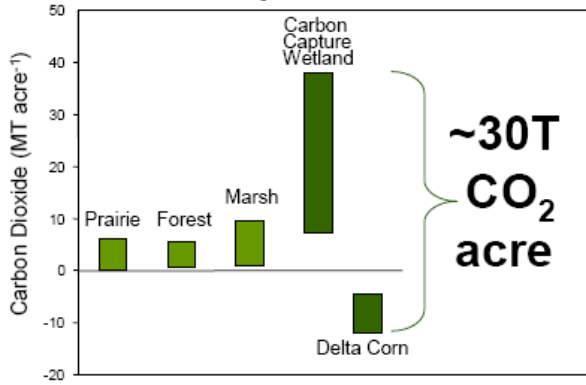
Peat island subsidence



Wetland "carbon farming"

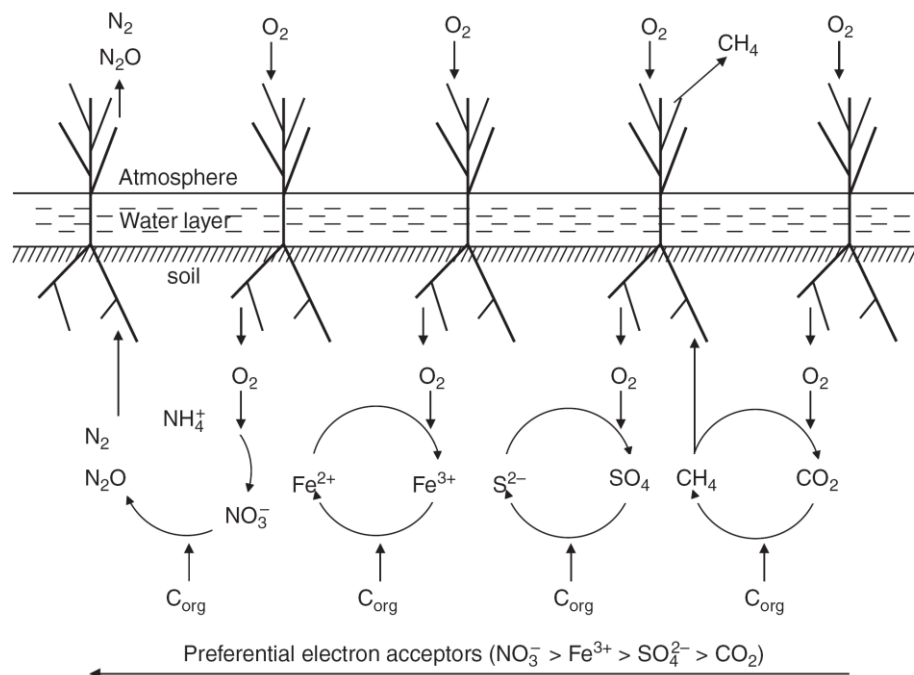


Lisamarie Windham-Myers
(talk Tues pm)

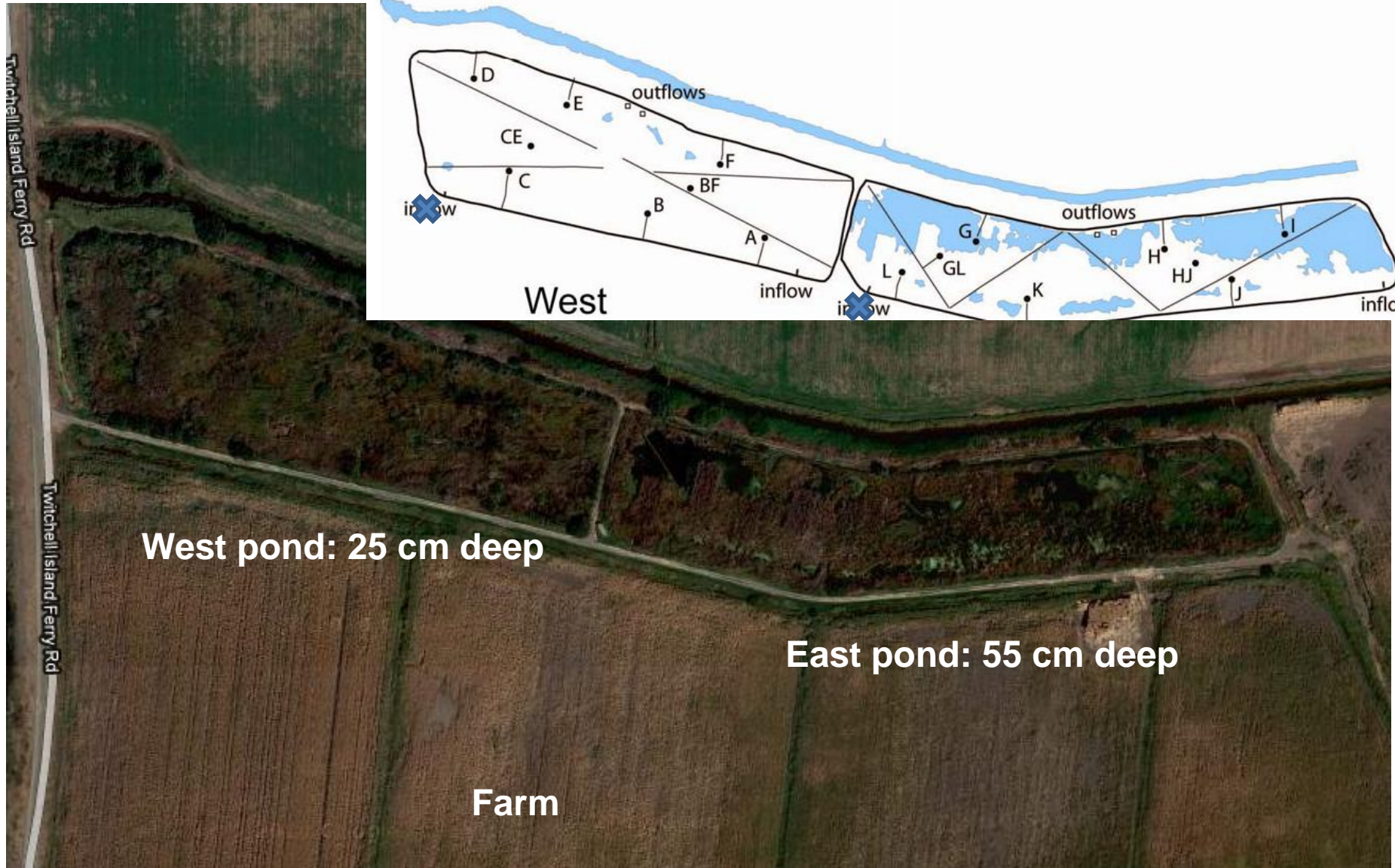
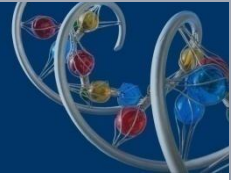




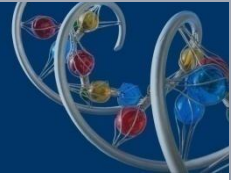
- Plant biomass decomposition
- Denitrification
- Mn(IV) reduction
- Fe(III) reduction
- Sulfate reduction
- Methanogenesis
- Methane oxidation (aerobic or anaerobic)



How do these processes impact “carbon farming”?



Sampling site gradients



Site

A

B

C/L

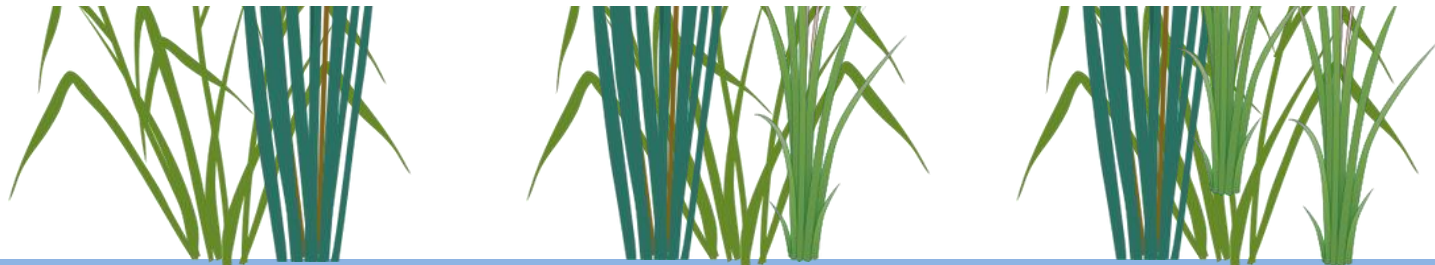
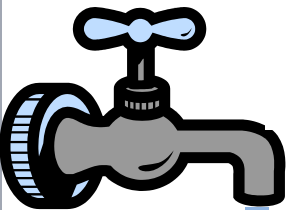
Methane
flux



Water
inlet



Does microbial community composition change with nutrient gradients, primary production and methane release?



Peat
accretion

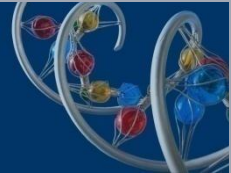


Oxygen,
Nitrate,
Sulfate



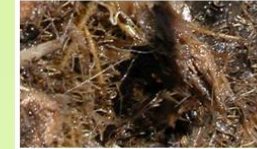
Water
outflow





Sample Collection

Three Sample Types



Bulk decomposed



Cattail rhizome



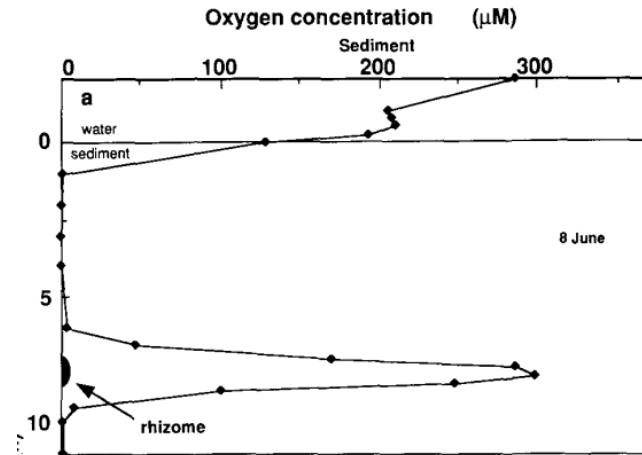
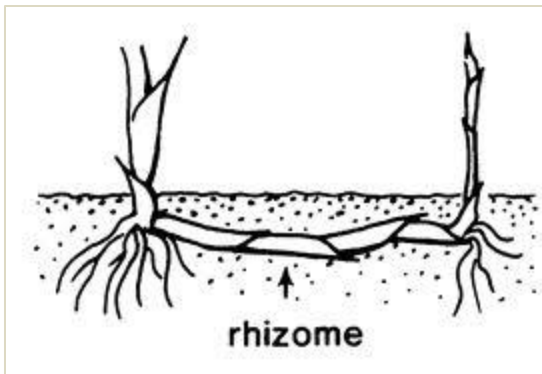
Tule rhizome



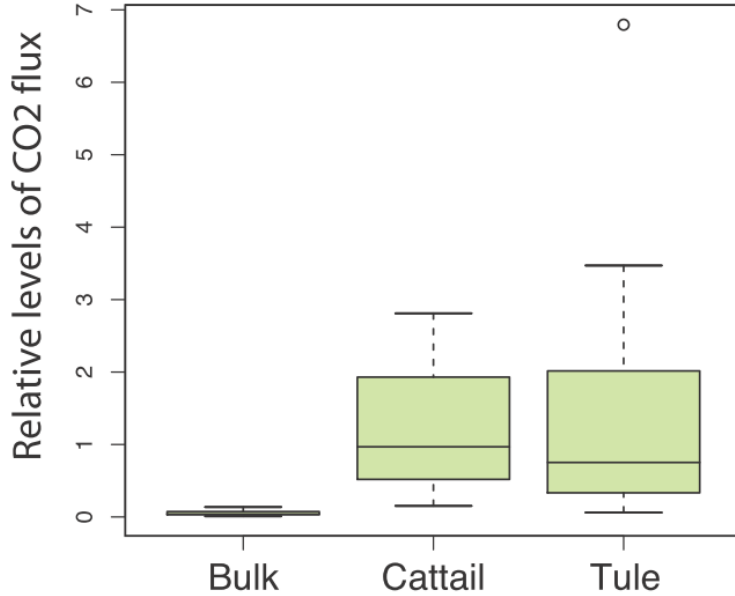
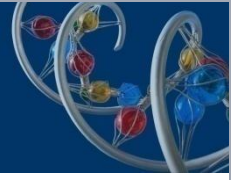
Sampling



Sediment Core

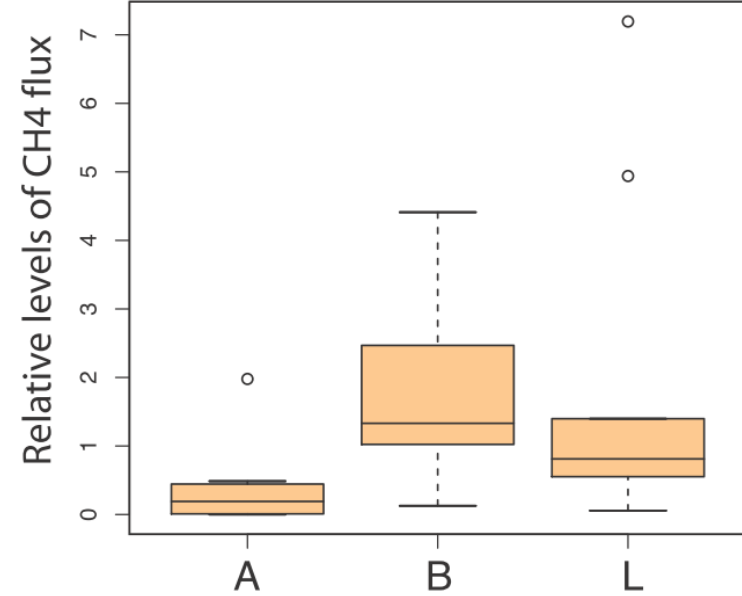


Caffrey & Kemp 1991



CO₂ Flux

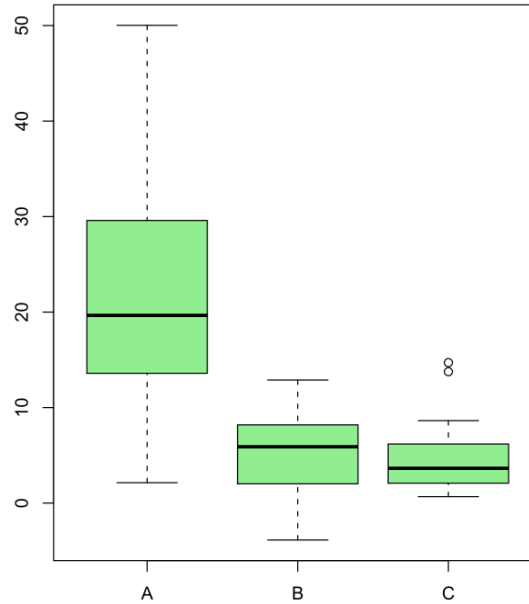
Bulk < Cattail ≈ Tule



CH₄ Flux

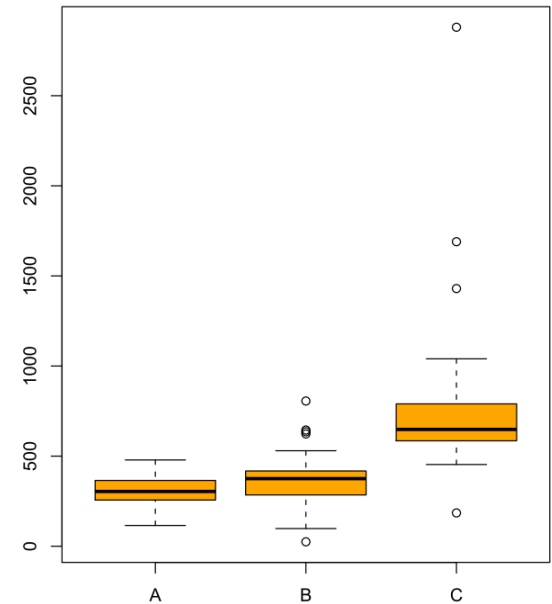
Site A < Site B ≈ Site L

- Data from Mark Waldrop, USGS



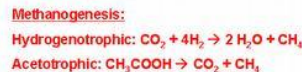
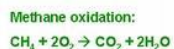
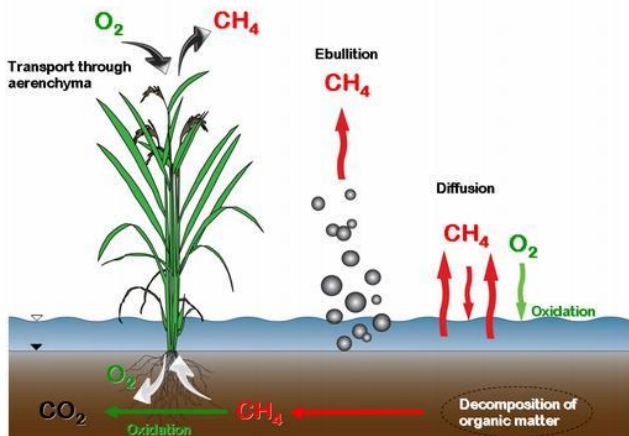
Net CO2 Uptake

Site A > Site B ≈ Site C

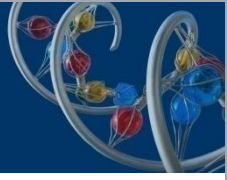


Net CH4 Emission

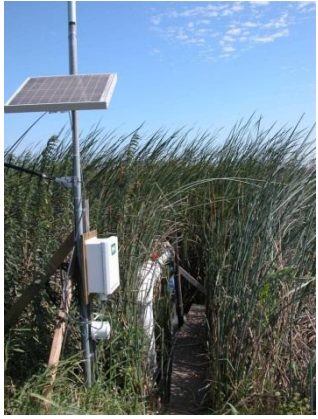
Site A ≈ Site B < Site C



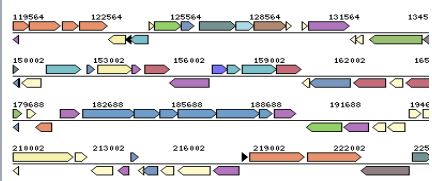
- Data from the USGS team



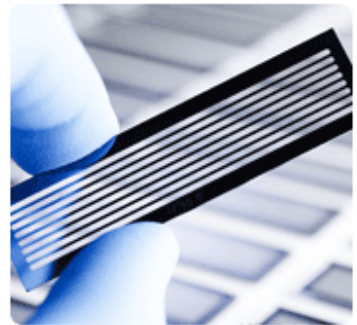
- Project motivation and background
 - Metagenomics and carbon cycling
 - Study site, sampling plan, biogeochemistry
 - Methane production
- **Sequence-based analyses**
 - **16S rRNA pyrotag profiling**
 - **Shotgun metagenomics**



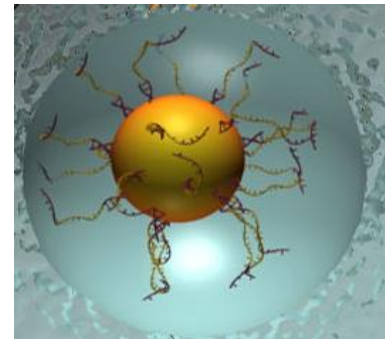
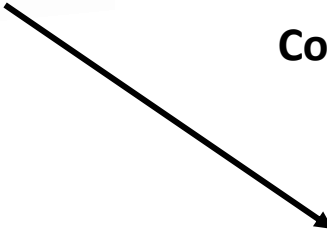
Shotgun Metagenome



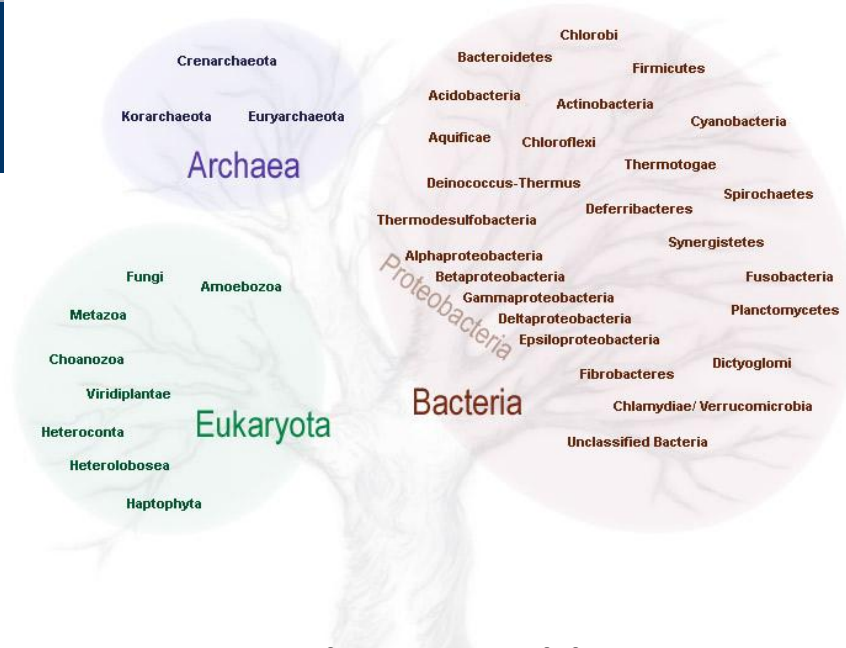
Functional analysis

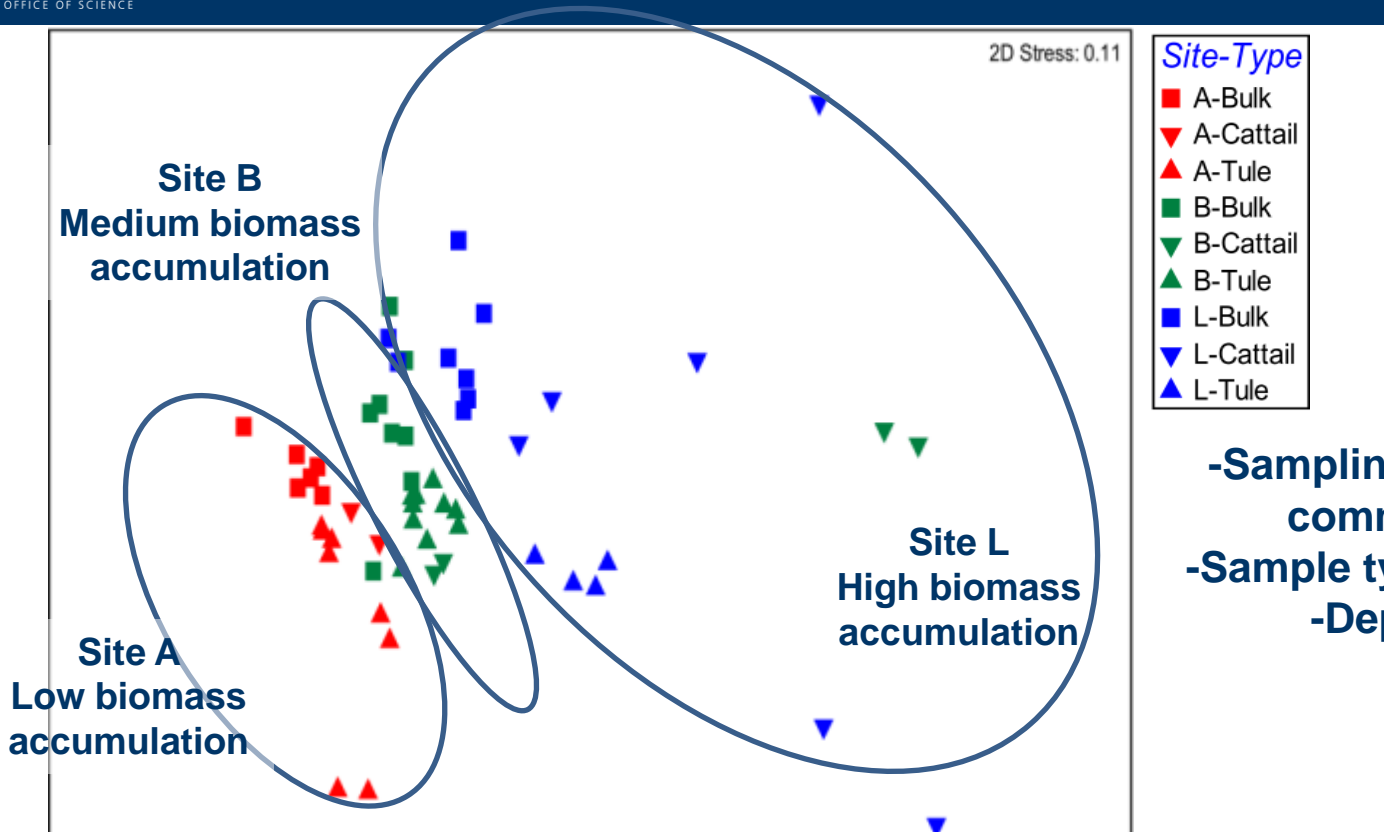


Illumina shotgun sequencing



454 Titanium Pyrotag sequencing





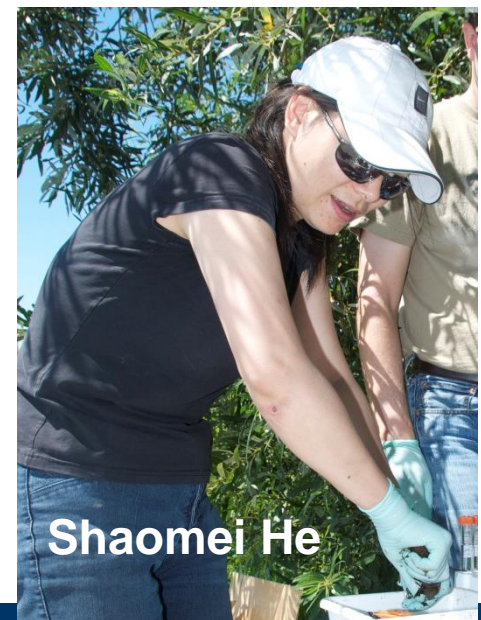
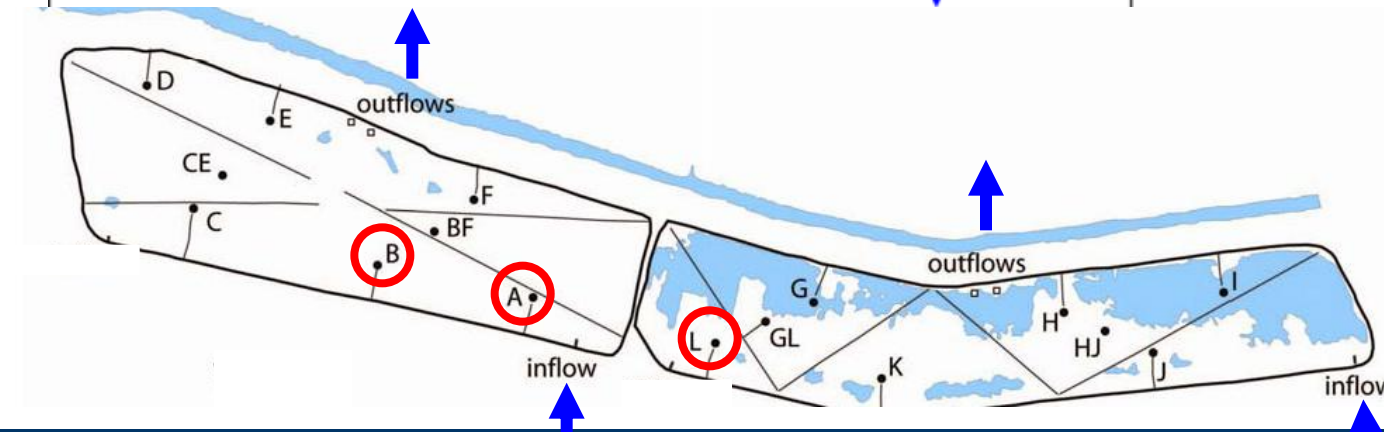
2D Stress: 0.11

- Site-Type*
- A-Bulk
 - ▼ A-Cattail
 - ▲ A-Tule
 - B-Bulk
 - ▼ B-Cattail
 - ▲ B-Tule
 - L-Bulk
 - ▼ L-Cattail
 - ▲ L-Tule

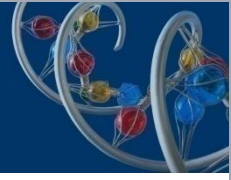
February 2011

-Similar results in August

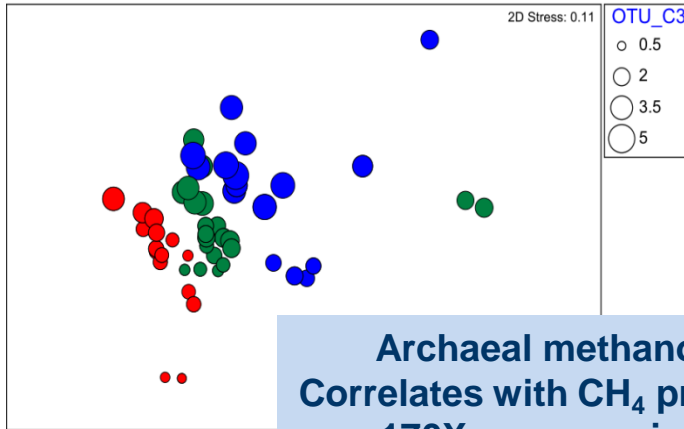
- Sampling site is major driver of community composition
- Sample type is next largest factor
- Depth effect is subtle



Shaomei He

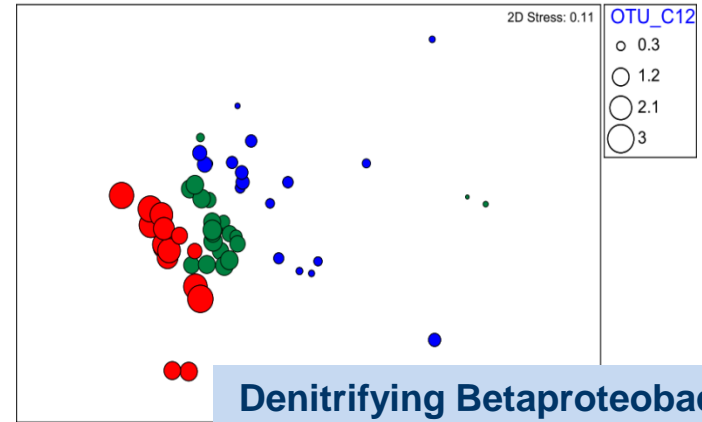


Methanoregula OTU



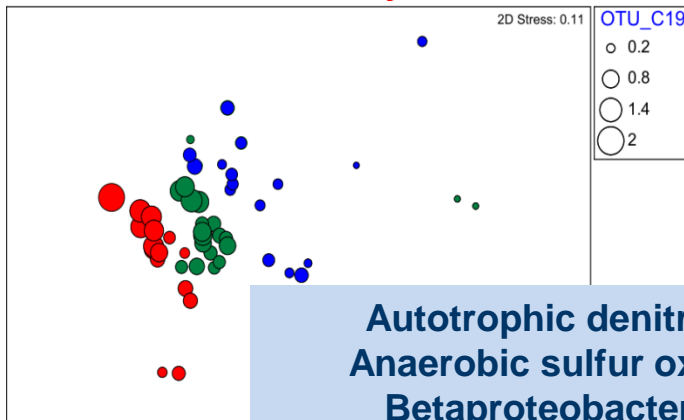
Archaeal methanogen
Correlates with CH₄ production
170X coverage in one
metagenome dataset

Dechloromonas OTU



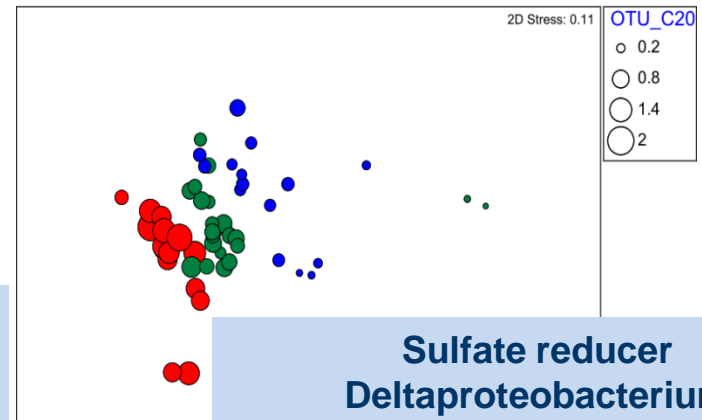
Denitrifying Betaproteobacterium
Correlates with nitrate abundance

A Thiobacillus denitrificans OTU

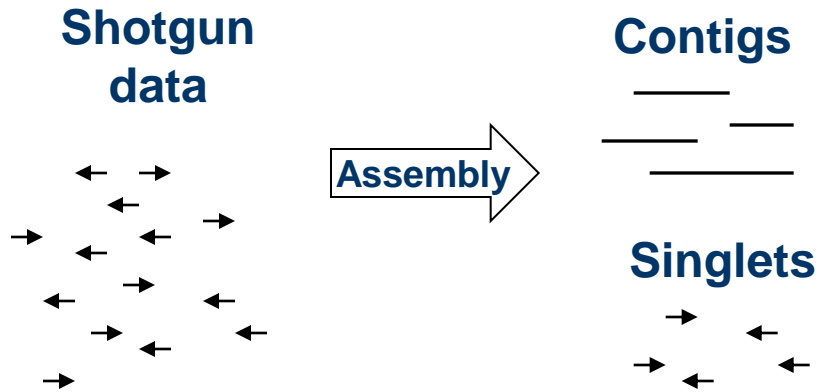
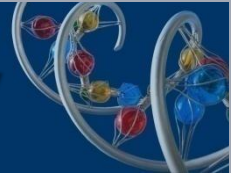


Autotrophic denitrifier /
Anaerobic sulfur oxidizer
Betaproteobacterium
Correlates with nitrate abundance

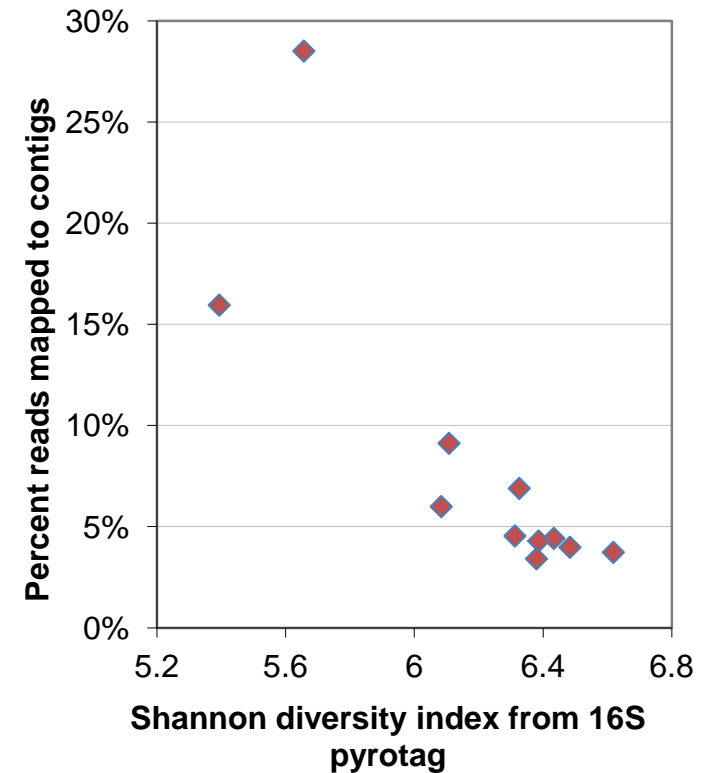
A Desulfobacteraceae OTU



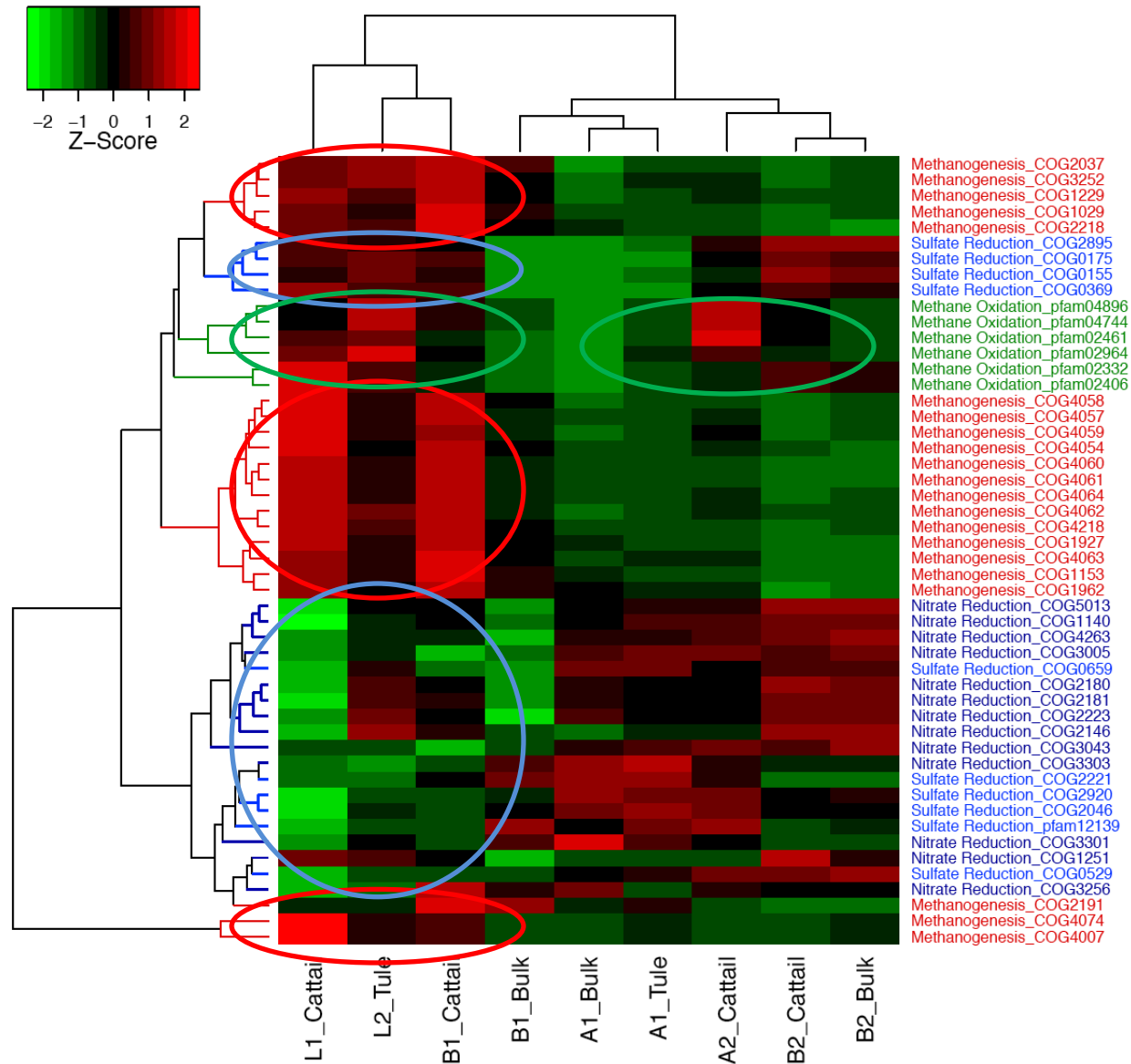
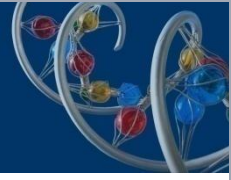
Sulfate reducer
Deltaproteobacterium
Correlates with sulfate abundance



Sample	Total bases generated (Gbp)	Contig N50 (bp)	Contig max length (bp)	Total base in contig (Mbp)	Percent reads mapped to contig (%)
A1_Bulk	59.9	456	55,504	208	9.12
A2_Cattail	58.8	389	85,839	84	3.73
A1_Tule	57.0	432	33,581	129	5.99
B1_Bulk	53.7	437	128,213	153	6.89
B2_Bulk	54.6	375	12,499	61	3.41
B1_Cattail	60.2	541	144,255	291	15.95
B2_Cattail	61.5	364	42,624	91	3.98
B2_Tule	53.9	373	37,498	106	4.42
L1_Bulk	60.3	439	89,191	70	4.54
L1_Cattail	59.5	779	246,650	297	28.51
L2_Tule	52.2	389	221,244	112	4.29



➤ **More complex community, less assembly**



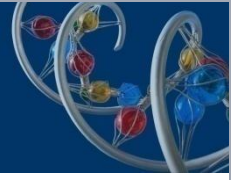
- Methanogenesis_COG2037
- Methanogenesis_COG3252
- Methanogenesis_COG1229
- Methanogenesis_COG1029
- Methanogenesis_COG2218
- Sulfate Reduction_COG2895
- Sulfate Reduction_COG0175
- Sulfate Reduction_COG0155
- Sulfate Reduction_COG0369
- Methane Oxidation_pfam04896
- Methane Oxidation_pfam04744
- Methane Oxidation_pfam02461
- Methane Oxidation_pfam02964
- Methane Oxidation_pfam02332
- Methane Oxidation_pfam02406
- Methanogenesis_COG4058
- Methanogenesis_COG4057
- Methanogenesis_COG4059
- Methanogenesis_COG4054
- Methanogenesis_COG4060
- Methanogenesis_COG4061
- Methanogenesis_COG4064
- Methanogenesis_COG4062
- Methanogenesis_COG4218
- Methanogenesis_COG1927
- Methanogenesis_COG4063
- Methanogenesis_COG1153
- Methanogenesis_COG1962
- Nitrate Reduction_COG5013
- Nitrate Reduction_COG1140
- Nitrate Reduction_COG4263
- Nitrate Reduction_COG3005
- Sulfate Reduction_COG0659
- Nitrate Reduction_COG2180
- Nitrate Reduction_COG2181
- Nitrate Reduction_COG2223
- Nitrate Reduction_COG2146
- Nitrate Reduction_COG3043
- Nitrate Reduction_COG3303
- Sulfate Reduction_COG2221
- Sulfate Reduction_COG2920
- Sulfate Reduction_COG2046
- Sulfate Reduction_pfam12139
- Nitrate Reduction_COG3301
- Nitrate Reduction_COG1251
- Sulfate Reduction_COG0529
- Nitrate Reduction_COG3256
- Methanogenesis_COG2191
- Methanogenesis_COG4074
- Methanogenesis_COG4007

Samples with more **methanogenesis** genes have less dissimilatory sulfate/nitrate reduction genes

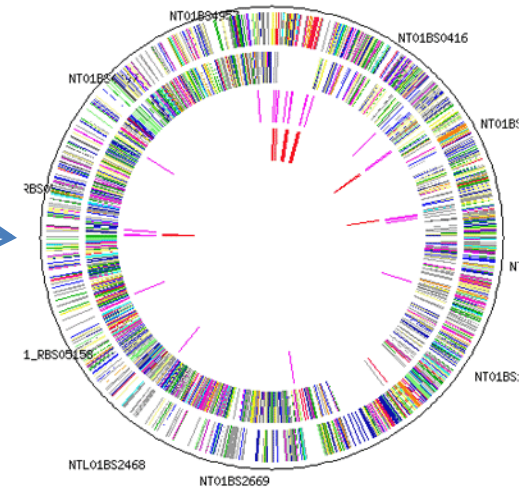
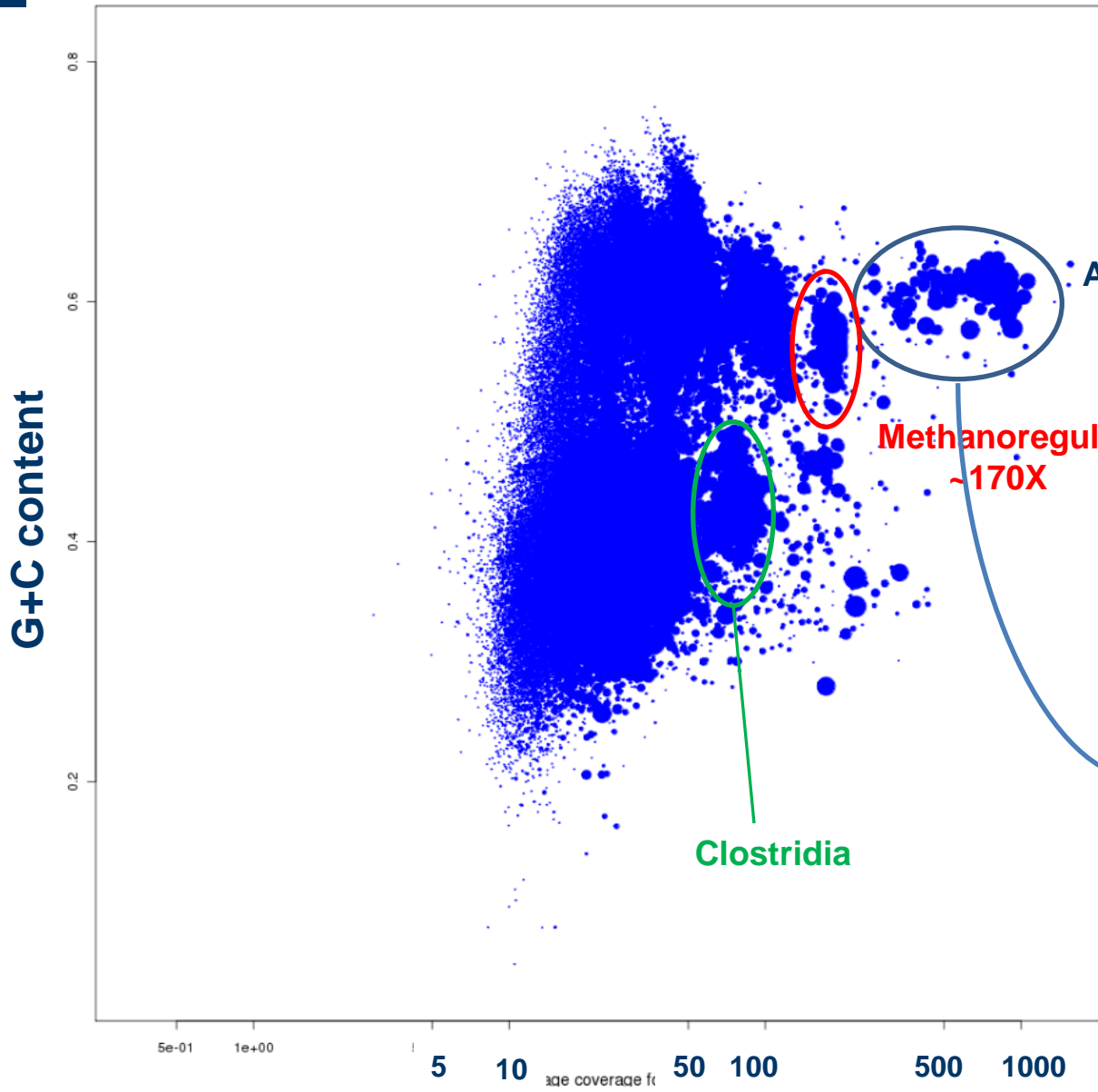
Methane oxidation genes were more abundant in rhizomes

Difference between replicate cores can be large

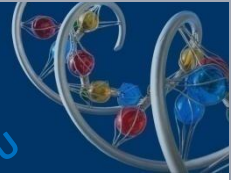
Metagenome assembly



Contigs average fold coverage vs. GC



Draft methanogen genome

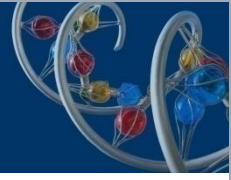


M. boonei
Wetland OTU

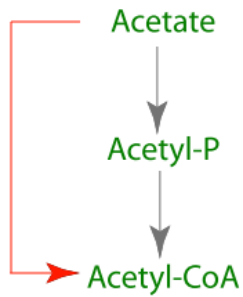
COG ID	Function Name	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met	Met
COG0016	Phenylalanyl-tRNA synthetase alpha subunit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0048	Ribosomal protein S12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0049	Ribosomal protein S7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0051	Ribosomal protein S10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0052	Ribosomal protein S2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0072	Phenylalanyl-tRNA synthetase beta subunit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0080	Ribosomal protein L11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0081	Ribosomal protein L1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0087	Ribosomal protein L3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0088	Ribosomal protein L4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0090	Ribosomal protein L2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0091	Ribosomal protein L22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0092	Ribosomal protein S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0093	Ribosomal protein L14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0094	Ribosomal protein L5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0096	Ribosomal protein S8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0097	Ribosomal protein L6P/L9E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0098	Ribosomal protein S5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0099	Ribosomal protein S13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0100	Ribosomal protein S11	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
COG0103	Ribosomal protein S9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0127	Xanthosine triphosphate pyrophosphatase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0149	Triosephosphate isomerase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0164	Ribonuclease HII	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0184	Ribosomal protein S15P/S13E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0185	Ribosomal protein S19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0186	Ribosomal protein S17	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
COG0197	Ribosomal protein L16/L10E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0200	Ribosomal protein L15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0244	Ribosomal protein L10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0256	Ribosomal protein L18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0343	Queuine/archaeosine tRNA-ribosyltransferase	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2
COG0504	CTP synthase (UTP-ammonia lyase)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0532	Translation initiation factor 2 (IF-2; GTPase)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0533	Metal-dependent proteases w/ chaperone activity	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
COG0541	Signal recognition particle GTPase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

High coverage and low redundancy of the draft genome

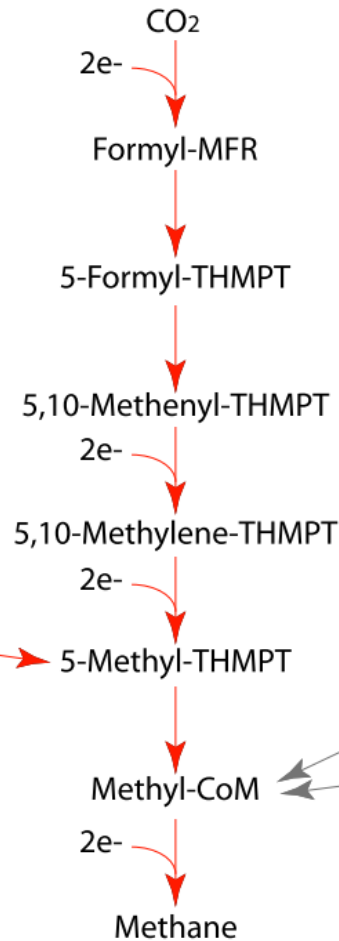
Methanogenesis Pathways



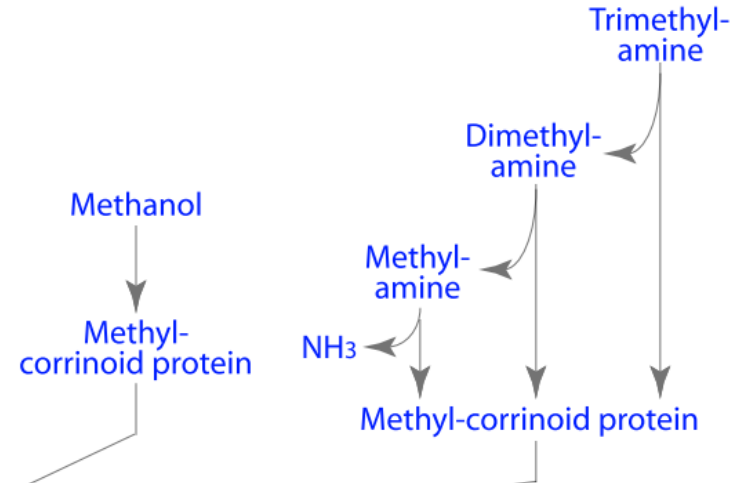
Acetoclastic



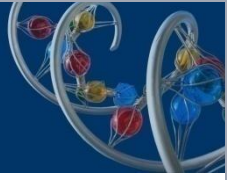
Hydrogenotrophic



Methylotrophic



Red: present in wetland *Methanoregula*
Grey: absent in wetland *Methanoregula*



- **Conclusions**
 - **Microbial community composition varies with location within the site, sample type, and depth**
 - **Higher methane production is reflected in higher abundance of archaeal methanogens and methanogenesis genes; similar patterns for other functional guilds**
- **Future directions**
 - **Analyzing metagenome data to reconstruct metabolic pathways on the organism and community level**
 - **Metatranscriptome sequencing to identify active pathways**
 - **Characterizing community succession in newly restored wetlands**



Early Career Research Award Program



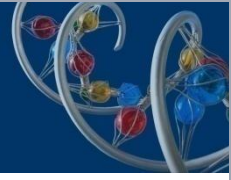
JGI Community Sequencing Program



US Geological Survey



Also: Mark Waldrop, Stephanie Malfatti, Tijana Glavina del Rio and many others at JGI



The focus of this Community Sequence Program (CSP) includes:

- **Plant and Plant-Microbe interactions**
- **Microbial emission and capture of greenhouse gases**

And exploit JGI's capabilities in:

- **Single-Cell Genomics**
- **DNA Synthesis**

CSP Microbial quarterly call:

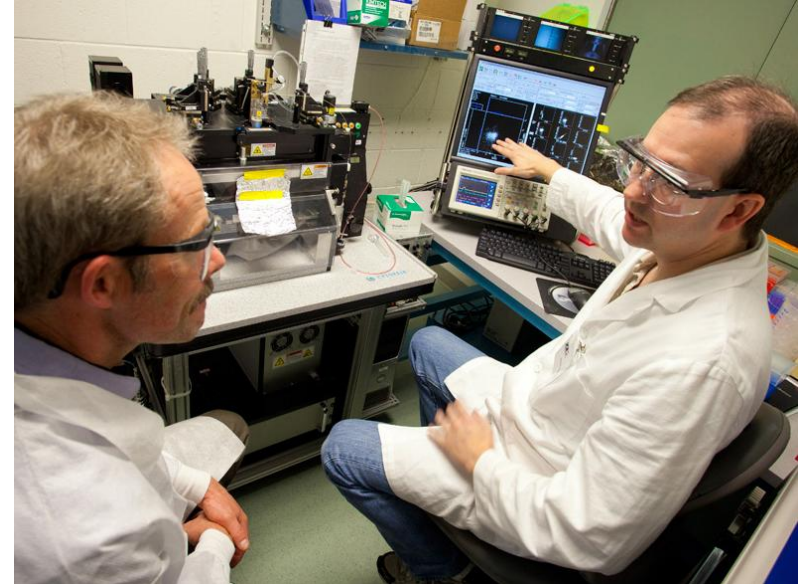
- **Bacterial and Archaeal isolates and single cells**
- **Bacterial and Archaeal resequencing**
- **Bacterial and Archaeal RNA sequencing**

Upcoming deadlines:

- **June 12, 2012**
- **September 11, 2012**



- Spend your sabbatical at JGI
- Gain direct access to:
 - *DNA datasets*
 - *Analysis pipelines*
 - *New technologies*
 - *JGI personnel*
- Flexible length of stay
- Can also accommodate students &/or post-docs



jgi.doe.gov/whoweare/visiting-scientist-program.html

Distinguished Postdoctoral Fellow in Genomics

The ideal applicant would directly leverage JGI experimental and/or computational resources for massive-scale genomic research and sequence-based science of relevance to the DOE. The fellowship allows recent graduates with a Ph.D. (or equivalent) to acquire further scientific training at one of the leading facilities for genomic research and to develop professional maturity for independent research.