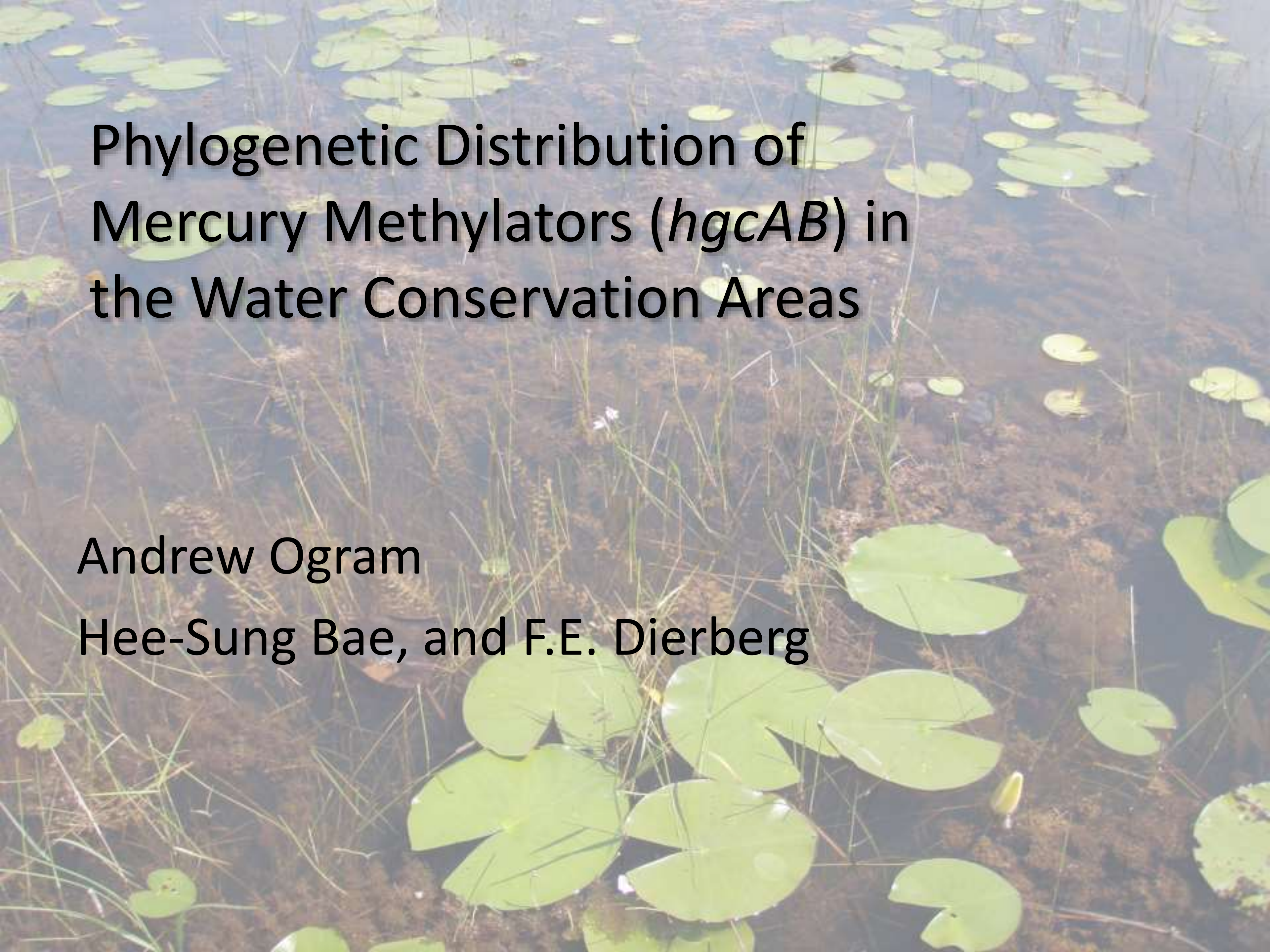


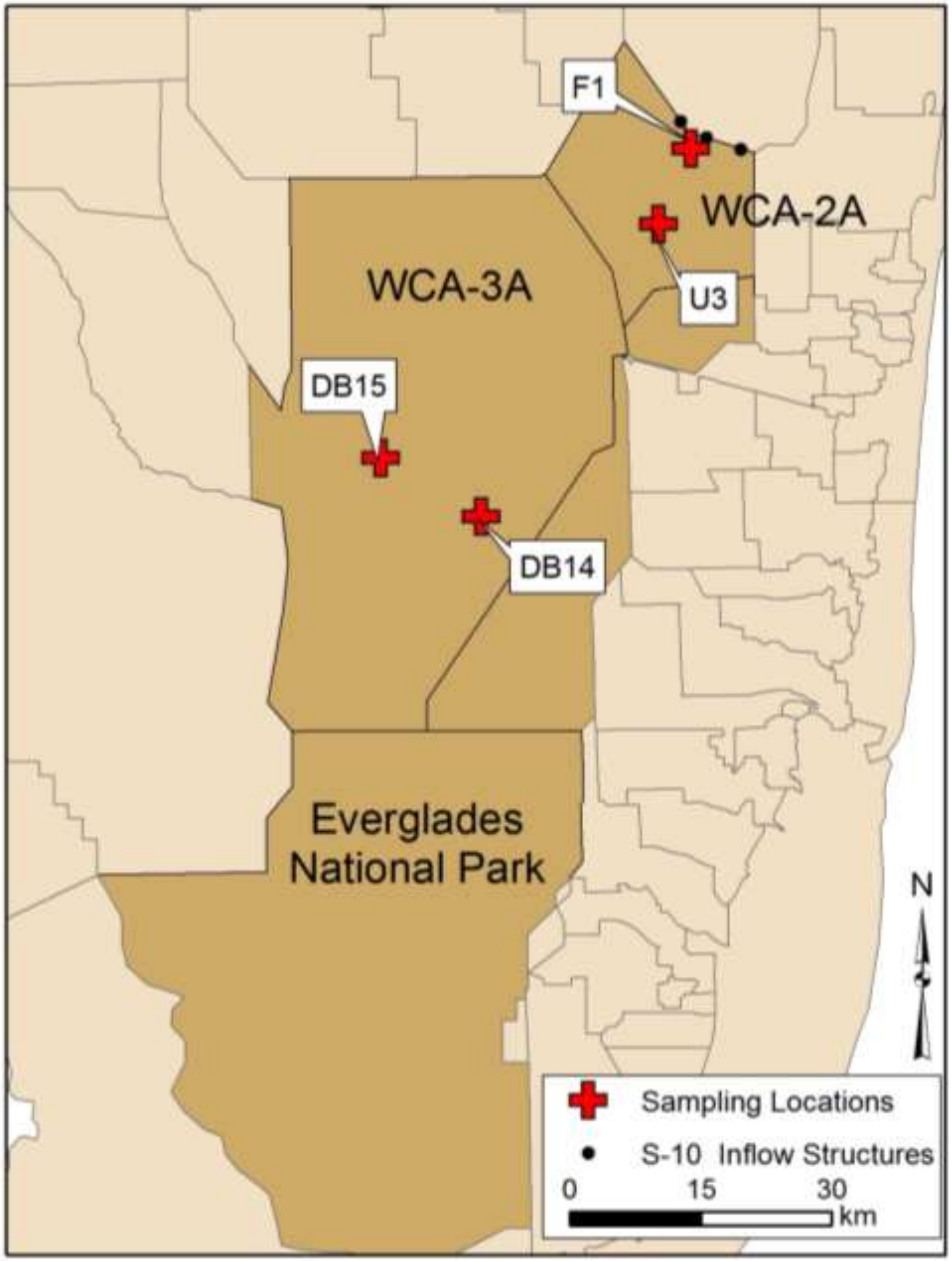
Phylogenetic Distribution of Mercury Methylators (*hgcAB*) in the Water Conservation Areas

Andrew Ogram

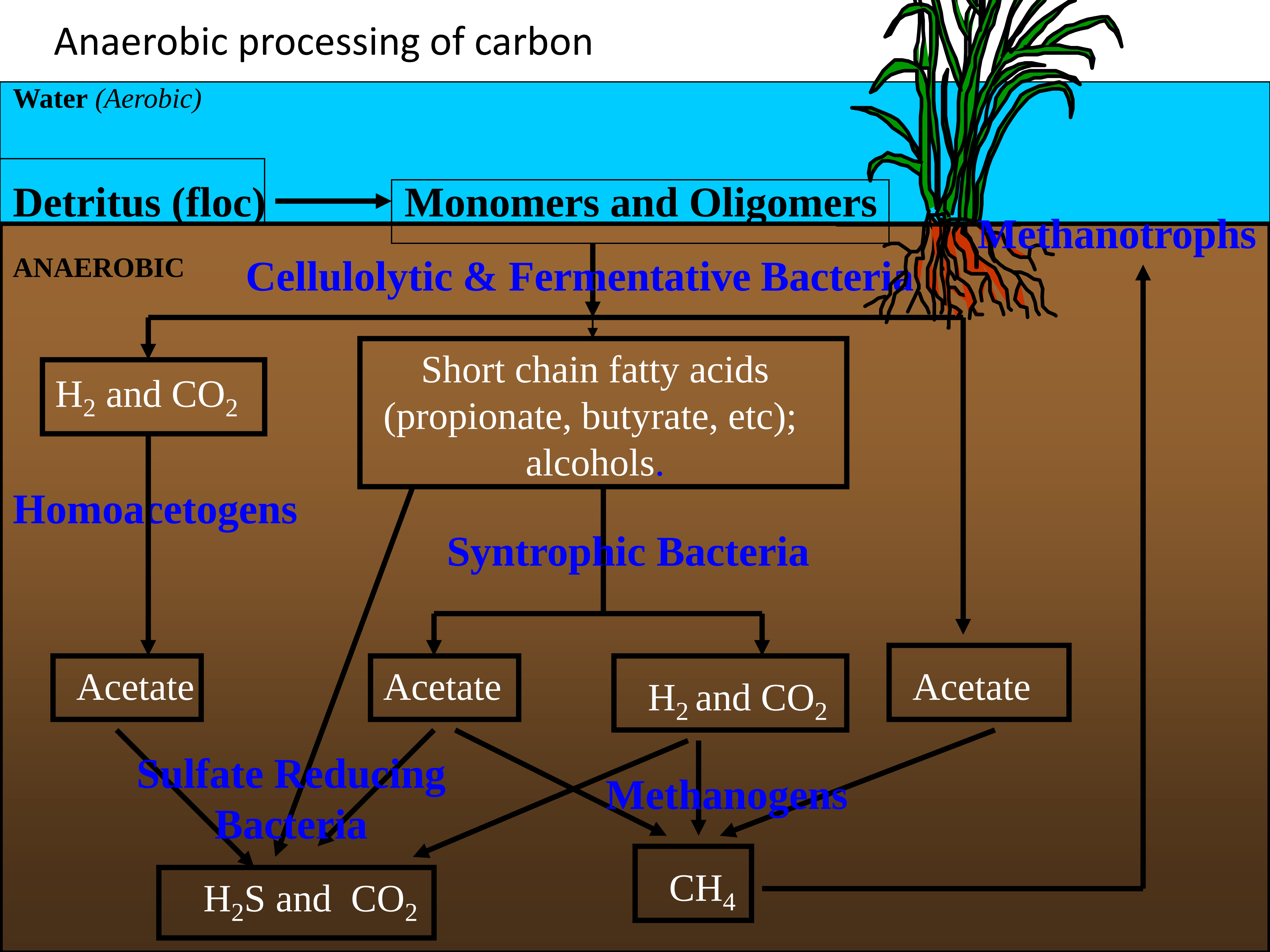
Hee-Sung Bae, and F.E. Dierberg

Outline

- The anaerobic carbon cycle in the Everglades
- Peaceful coexistence: sulfate reduction vs methanogenesis
- Distribution of *hgcAB* in different compartments down the gradient.
- *Disclaimer: genes do not indicate activities!*


F1
WCA-2A
U3
WCA-3A
DB15
DB14
Everglades National Park
N
Sampling Locations
S-10 Inflow Structures
0
15
30
km

Anaerobic processing of carbon



Water *(Aerobic)*

Detritus (floc)

Monomers and Oligomers

Methanotrophs

ANAEROBIC

Cellulolytic & Fermentative Bacteria

H_2 and CO_2

Short chain fatty acids (propionate, butyrate, etc); alcohols.

Homoacetogens

Syntrophic Bacteria

Acetate

Acetate

H_2 and CO_2

Acetate

Sulfate Reducing Bacteria

Methanogens

H_2S and CO_2

CH_4

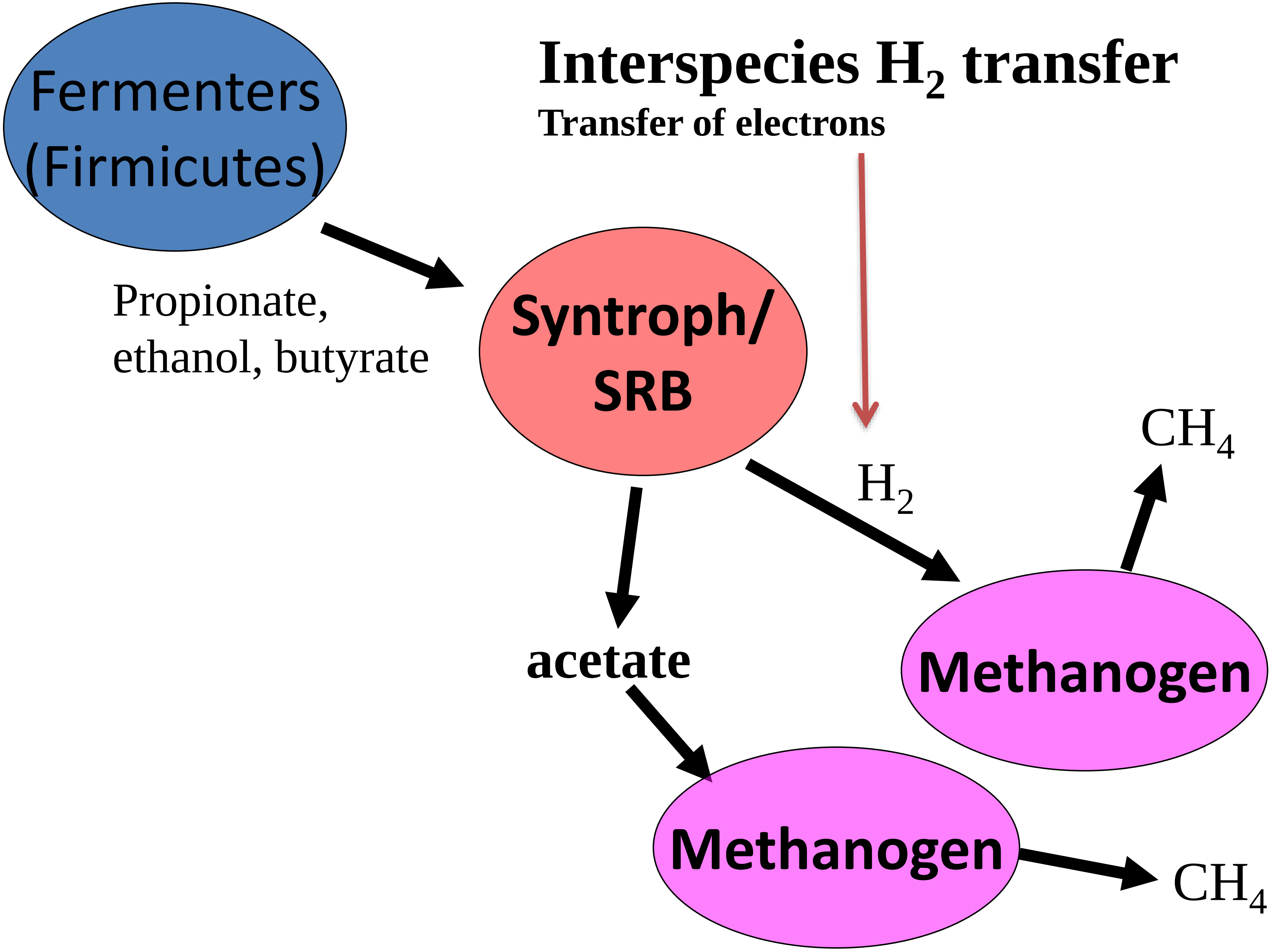
More carbon is mineralized via methane production than via sulfate reduction in soil lab incubations

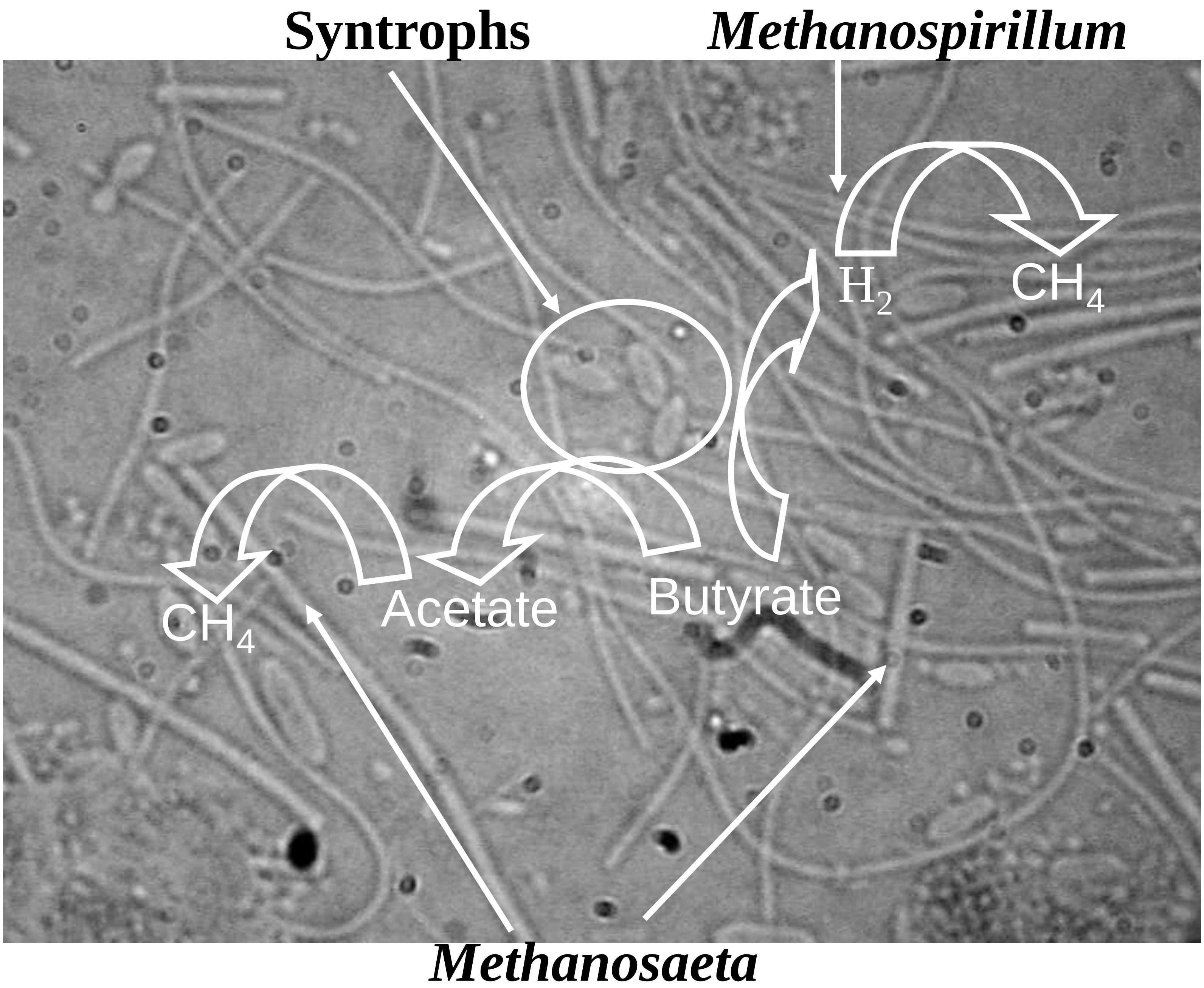
Characteristics	F1	F4	U3
		CH_4 production rate $(\mu\text{mol}\times\text{g soil}^{-1}\times\text{d}^{-1})$	
Intact soils	3.1±3.2	0.9±0.9	<0.1
Acetate	5.3±1.4	2.7±0.5	0.4±1.2
H_2/CO_2	11.6±3.5	5.6±2.9	2.1±1.0
		SO_4^{2-} reduction rate $(\mu\text{mol}\times\text{g soil}^{-1}\times\text{d}^{-1})$	
Intact soils	0.5±0.4	<0.01	0.03±0.04
Acetate	1.4±0.3	0.1±0.1	0.07±0.07
H_2/CO_2	1.2±1.0	<0.01	0.14±0.17

Bae et al., 2015

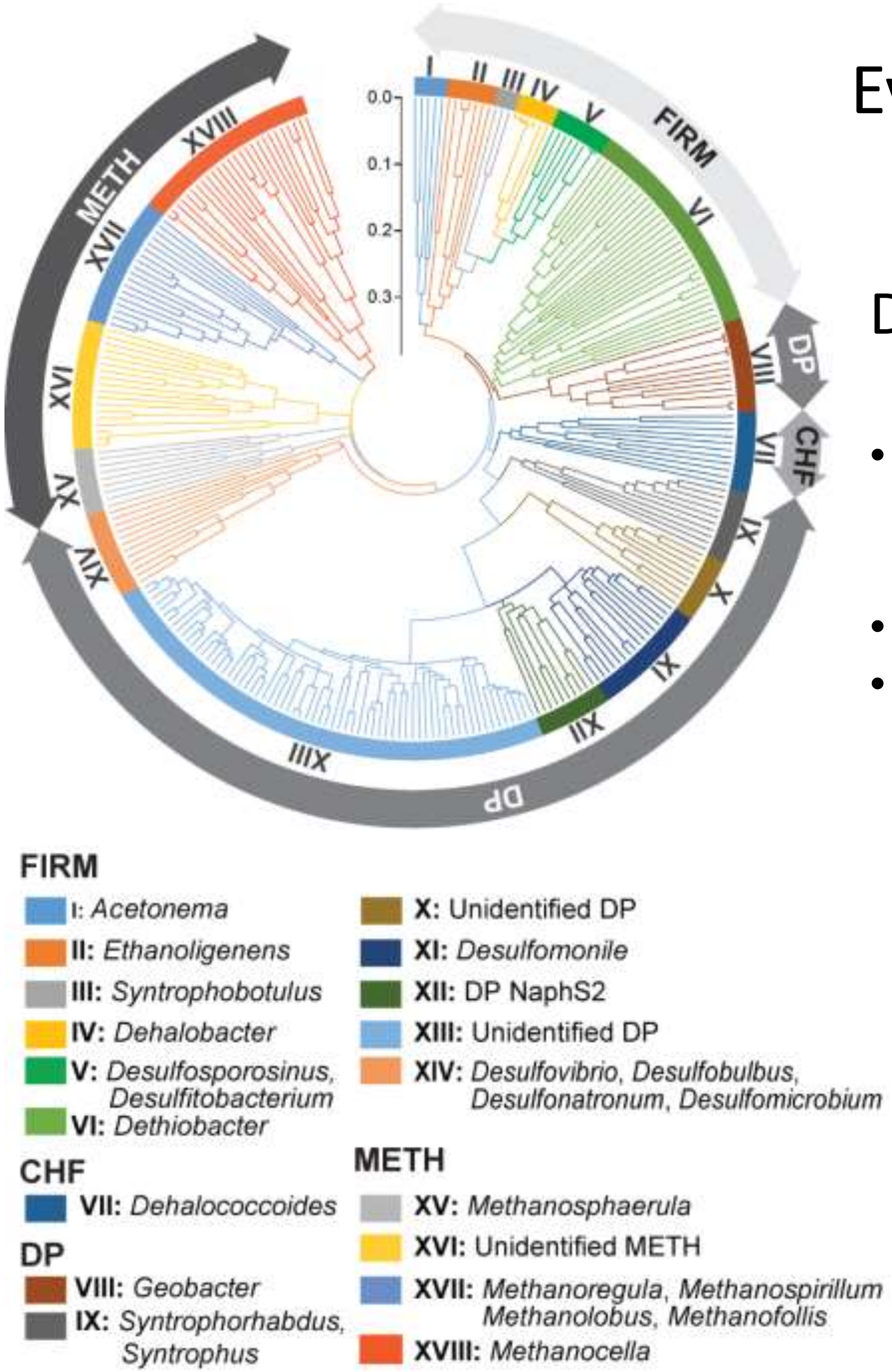
Interspecies H_2 transfer

Transfer of electrons




Syntrophs
Methanospirillum
H_2
CH_4
Acetate
Butyrate
CH_4
Methanosaeta

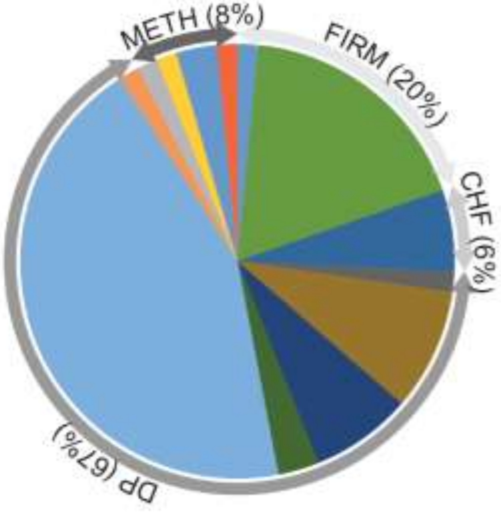
Everglades *hgcAB* phylogeny



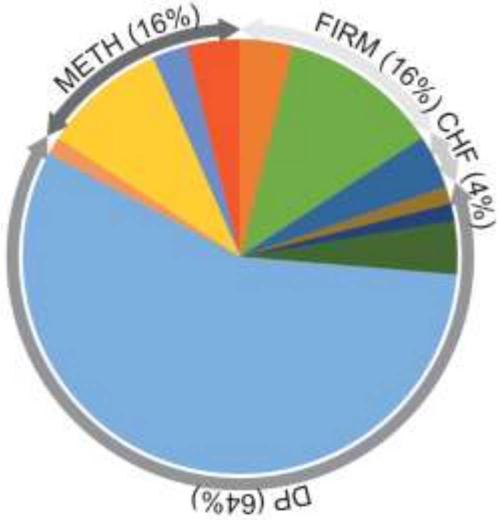
Dominant groups include:

- Deltaproteobacteria (SRB, FeRB)
  - Most affiliated with syntrophs.
  - Can also reduce sulfate
- Methanogens
- Firmicutes (Primary fermenters and *Dethiobacter*)

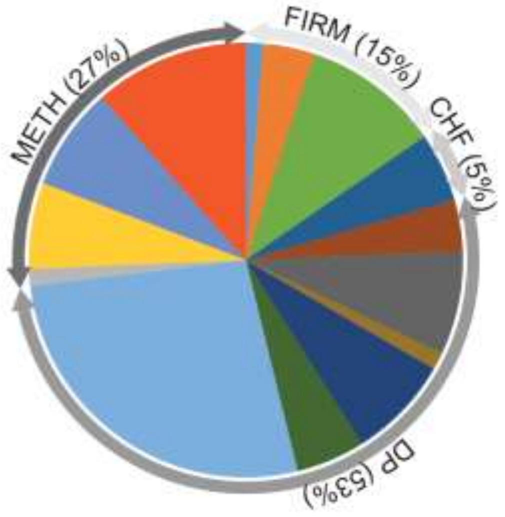
hgcAB distribution in soil down the gradient



F1



U3



DB15

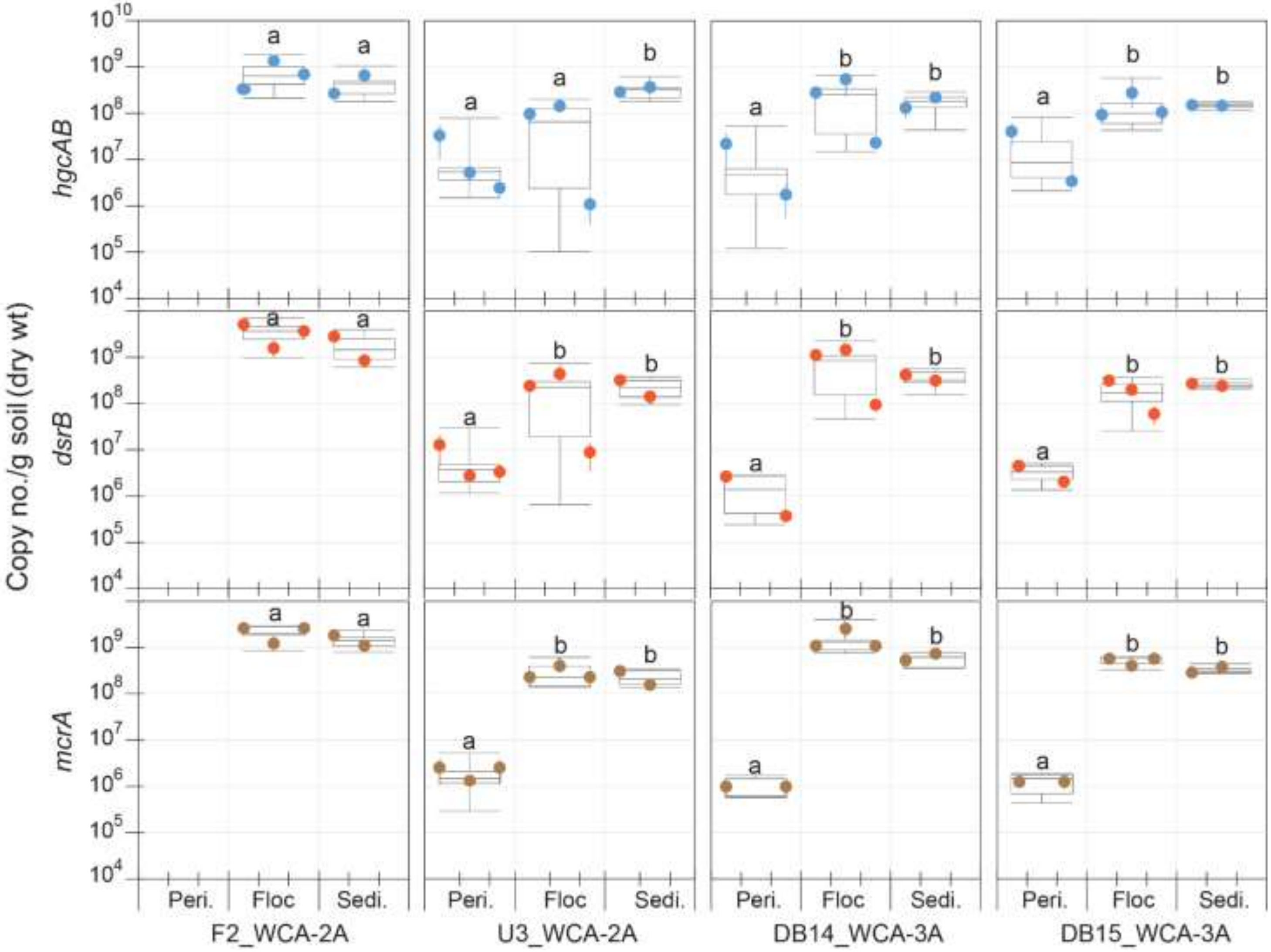
DOC: SO_4^{2-} *16.8 (F2)* *9.1* *691*

Methanogen component increases

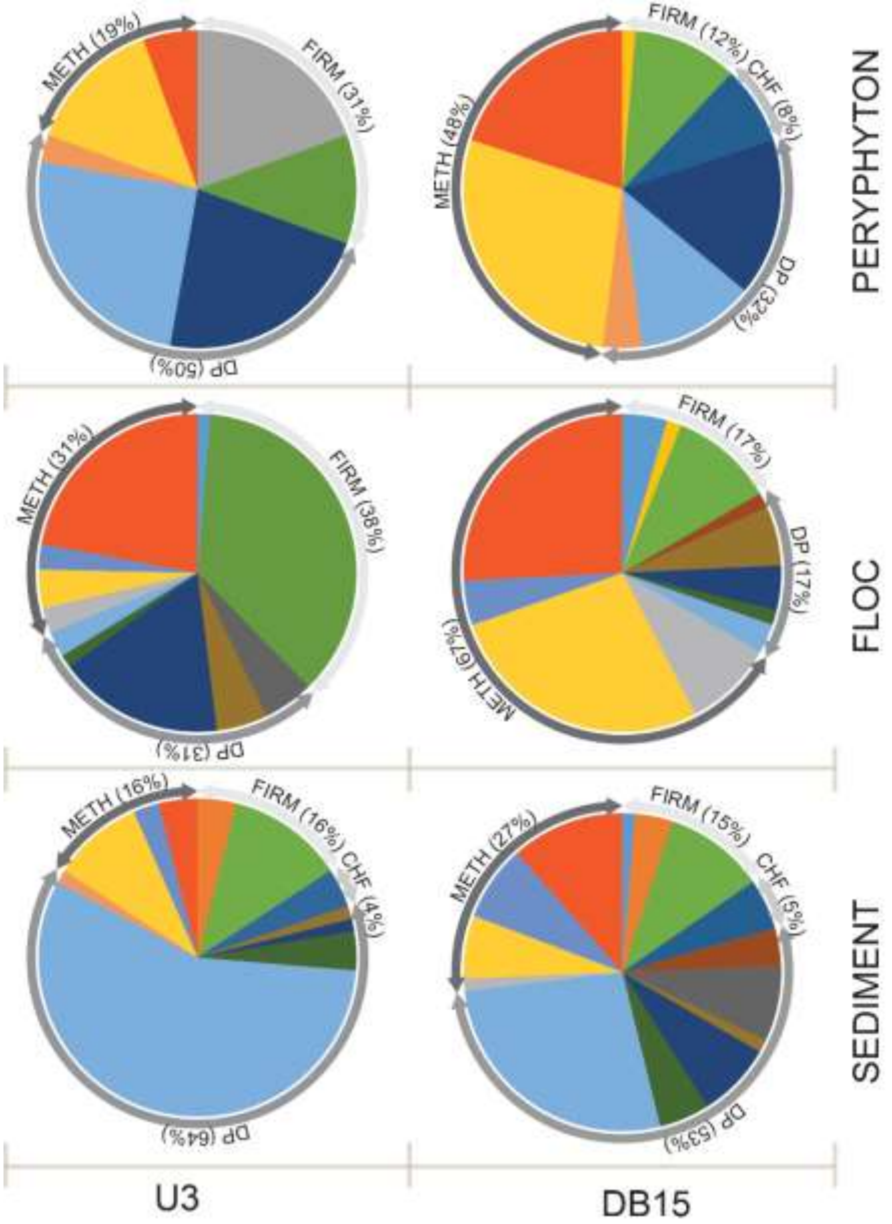
Firimicutes dominated by *Dethiobacter* (NOT SRB)

Complex ecosystem including soil, floc, and floating periphyton

Numbers of mercury methylators (*hgcAB*), SRB (including syntrophs) (*dsrB*) and methanogens (*mcrA*). Generally higher in floc and soil than periphyton.



Methanogens and deltaproteobacteria dominate



Methanogens increase in importance at DB15

Methanogens and Firmicutes increase in importance. Firmicutes dominated by *Dethiobacter*.

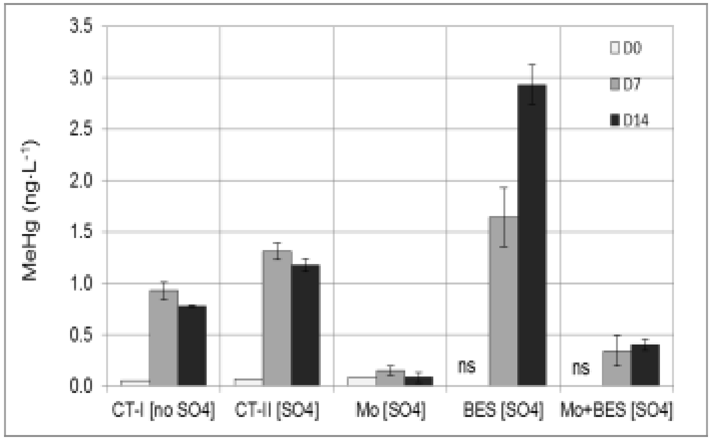
DP (SRB/Syntrophs) dominate at both sites, with increasing methanogen component at DB15

Summary

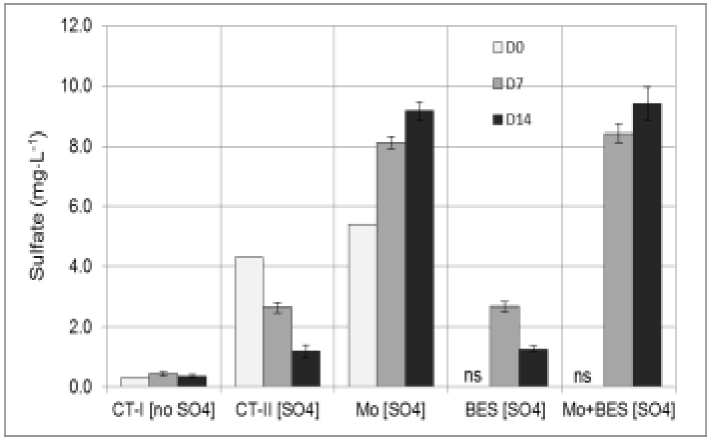
- Understanding ecological context yields insight into potential dominant processes that drive methylation.
- Mercury methylators can be viewed in the context of carbon cycle.
- Significant differences in distribution of dominant methylators in different areas and different compartments.
- Deltaproteobacteria (SRB, inc. syntrophs) dominate in WCA-2A
- Methanogens and firmicutes more important in floc, periphyton than soil. More important in DB15 (WCA-3A) than WCA-2A.
- *Presence of genes does not indicate activities.*

Slurry incubations with WCA-3A soil and selective inhibitors

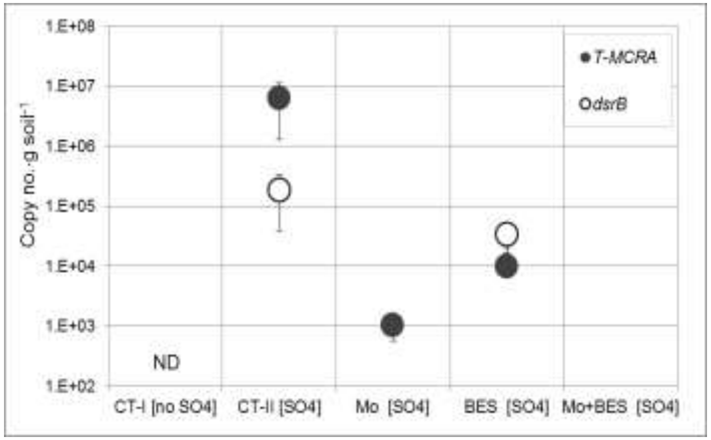
MoO_4^{2-} *inhibits SR*
BES inhibits methanogens



Mo ↓ MM
BES ↑ MM
BES + Mo ↓ MM



Mo ↓ SR
BES no effect SR
BES +Mo ↓ SR

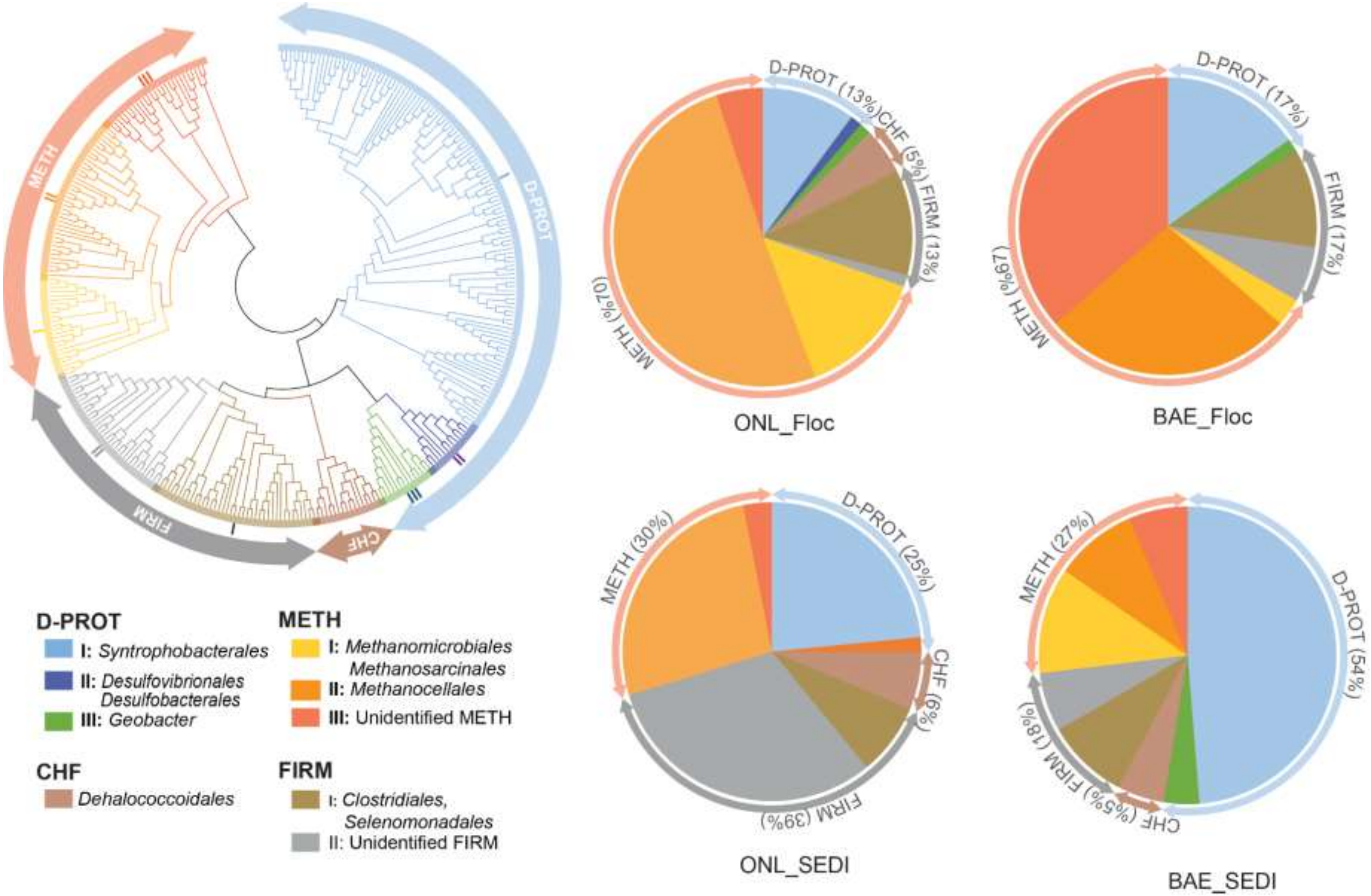


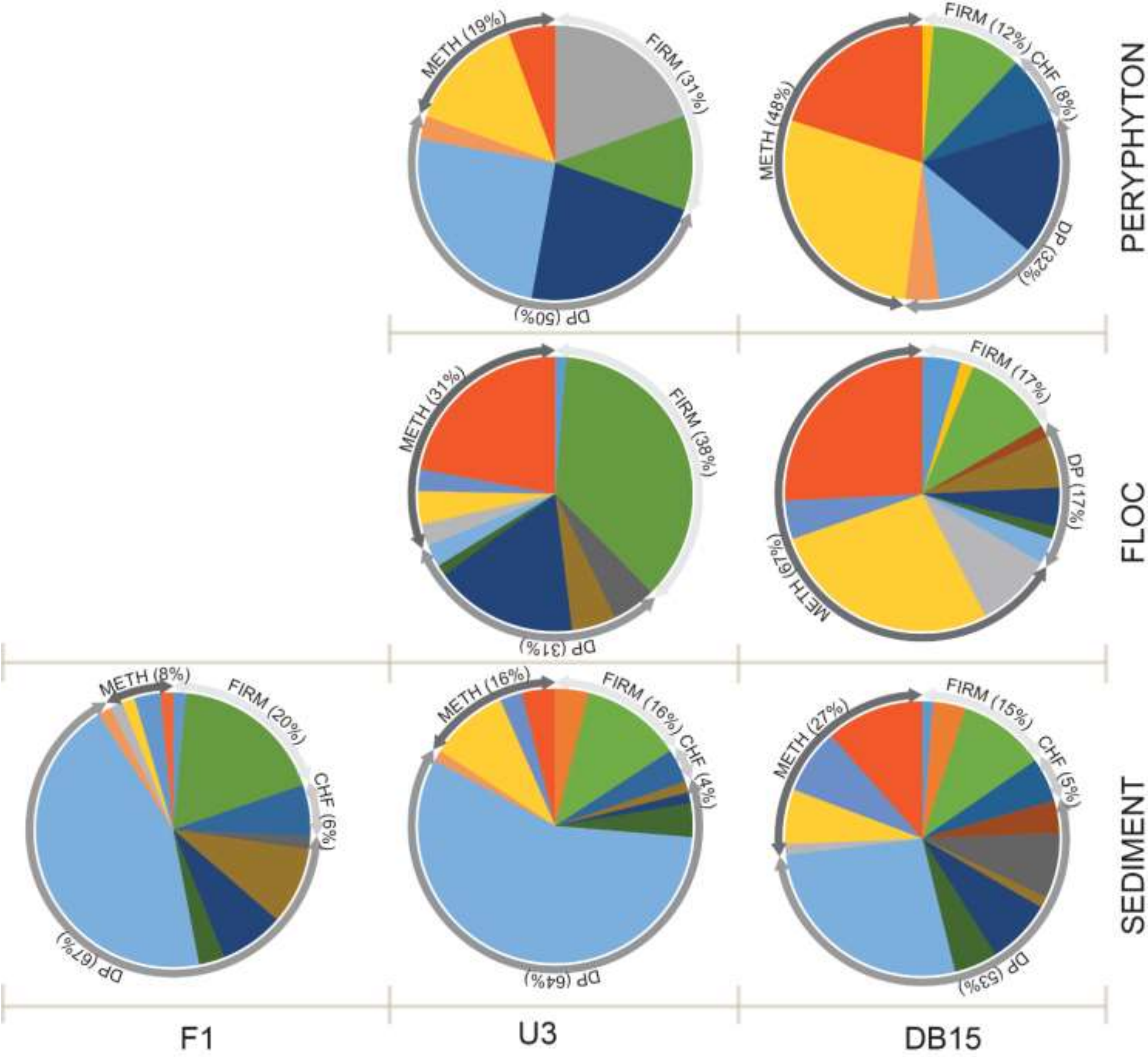
SO_4^{2-} ↑ *dsrB*
MoO_4^{2-} ↓ *mcrA +dsrB*
BES ↓ *mcrA*
BES +Mo ↓ *mcrA +dsrB*

Characteristics	F1	F4	U3	W3
Coordinates	26°21′39″N, 80°22′10″W	26°18′59″N, 80°23′01″W	26°17′15″N, 80°24′41″W	26°02′35″N, 80°49′39″W
Soil chemistry[a]				
TP (g/kg)	1.28±0.24[b]	0.60±0.26	0.25±0.04	0.38±0.02
TN (g/kg)	31±3	34±3	27±5	36±1
TC (g/kg)	442±31	424±29	385±69	424±4
Porewater chemistry[c]				
Acetate (µM)	19.5±9.9	25.9±33.8	17.1±16.4	ND
H_2 (µM)	0.33±0.10	0.28±0.20	0.28±0.14	ND
SO_4^{2-} (µM)[d]	56±86	74±110	39±35	≤4
CH_4 production rate (µmol·g soil^{-1}·d^{-1})[e]				
Intact soils	3.1±3.2	0.9±0.9	<0.1	ND
Acetate	5.3±1.4	2.7±0.5	0.4±1.2	ND
H_2/CO_2	11.6±3.5	5.6±2.9	2.1±1.0	ND
SO_4^{2-} reduction rate (µmol·g soil^{-1}·d^{-1})[e]				
Intact soils	0.5±0.4	<0.01	0.03±0.04	ND
Acetate	1.4±0.3	0.1±0.1	0.07±0.07	ND
H_2/CO_2	1.2±1.0	<0.01	0.14±0.17	ND

Bae et al., 2015

Bae *hgcAB* primers compared with ORNL primer set (DB 15)




METH (19%)
FIRM (31%)
DP (50%)
FIRM (12%)
CHF (8%)
METH (48%)
DP (32%)
PERYPHYTON
METH (31%)
FIRM (38%)
DP (31%)
FIRM (17%)
DP (17%)
METH (67%)
FLOC
METH (8%)
FIRM (20%)
CHF (6%)
DP (67%)
METH (16%)
FIRM (16%)
CHF (4%)
DP (64%)
METH (27%)
FIRM (15%)
CHF (5%)
DP (53%)
SEDIMENT
F1
U3
DB15