

A photograph of a forest with a stream in the foreground, overlaid with a semi-transparent dark box containing text. The forest consists of tall, thin trees, some with green foliage and some with yellowing leaves, suggesting an autumn setting. The stream is small and flows through the center of the frame, surrounded by fallen leaves and green plants. The text is centered within the dark box.

# Identifying the microbial guilds responsible for methylmercury cycling in wetlands

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Ida Tjerngren  
Erik Björn





Wetlands are often “hot spots” for Hg methylation and methylmercury accumulation

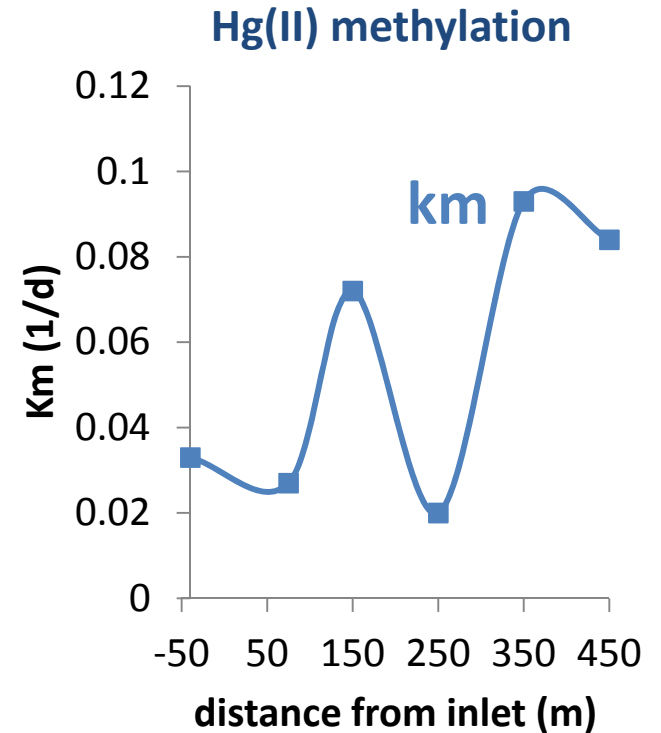
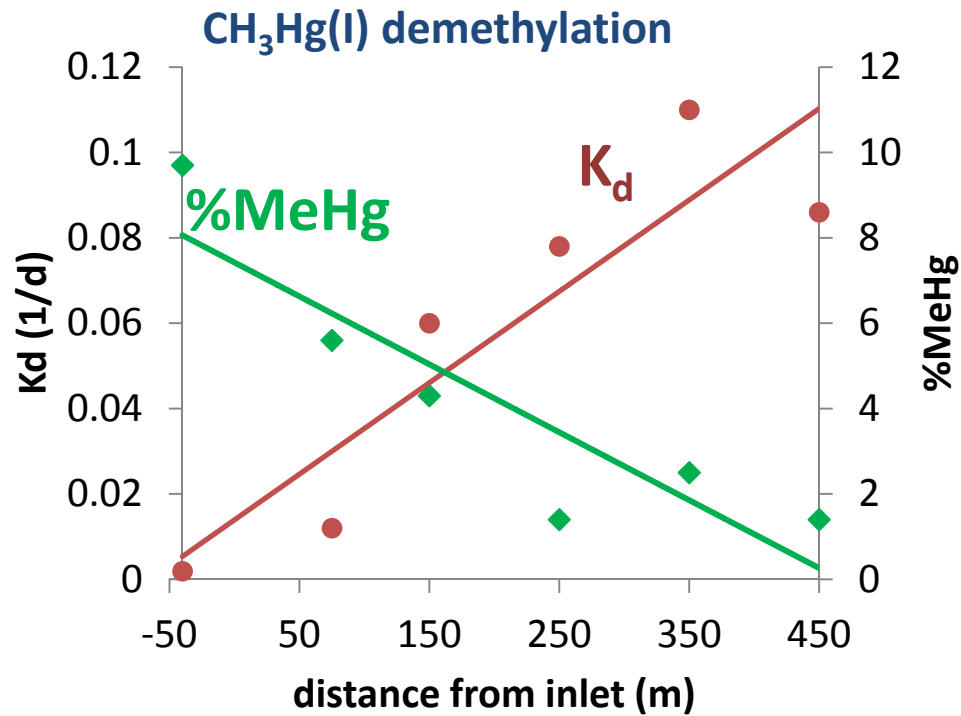


Alder swamps appear to be a net sink for MeHg

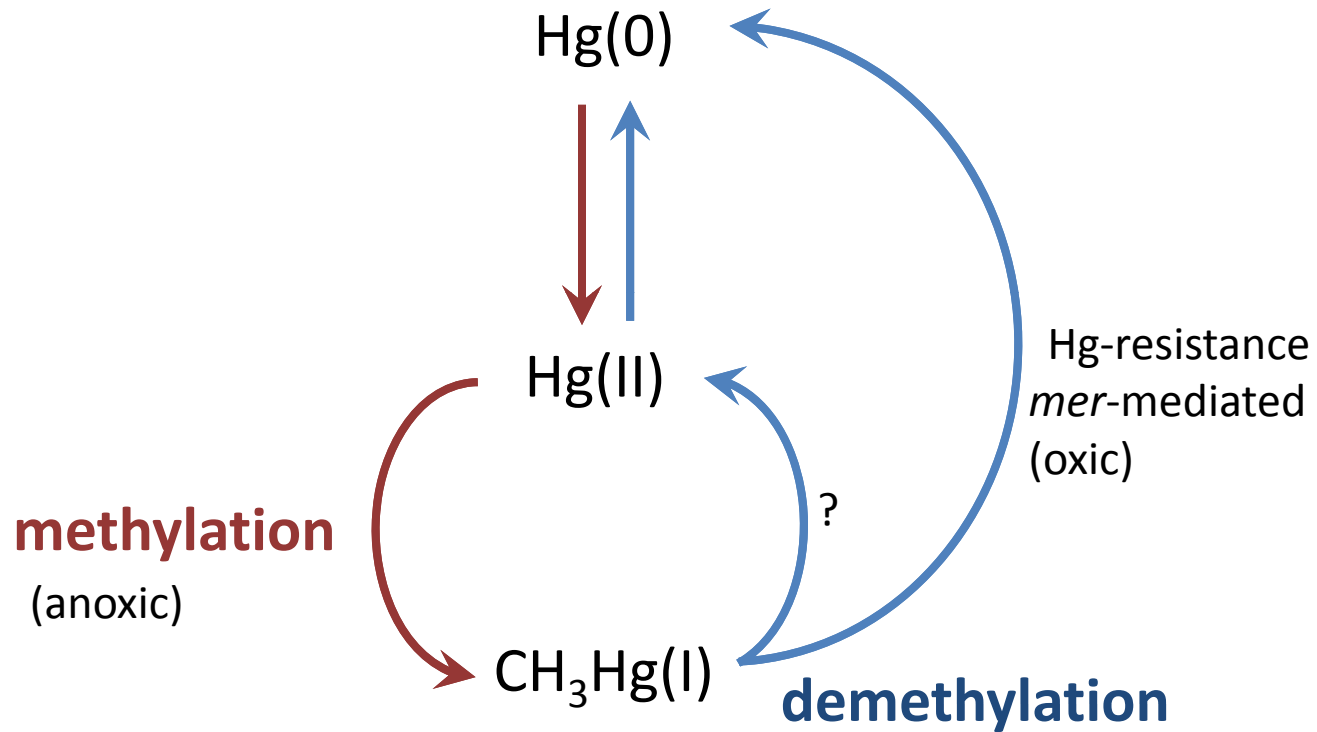


# Demethylation limits MeHg accumulation

2008

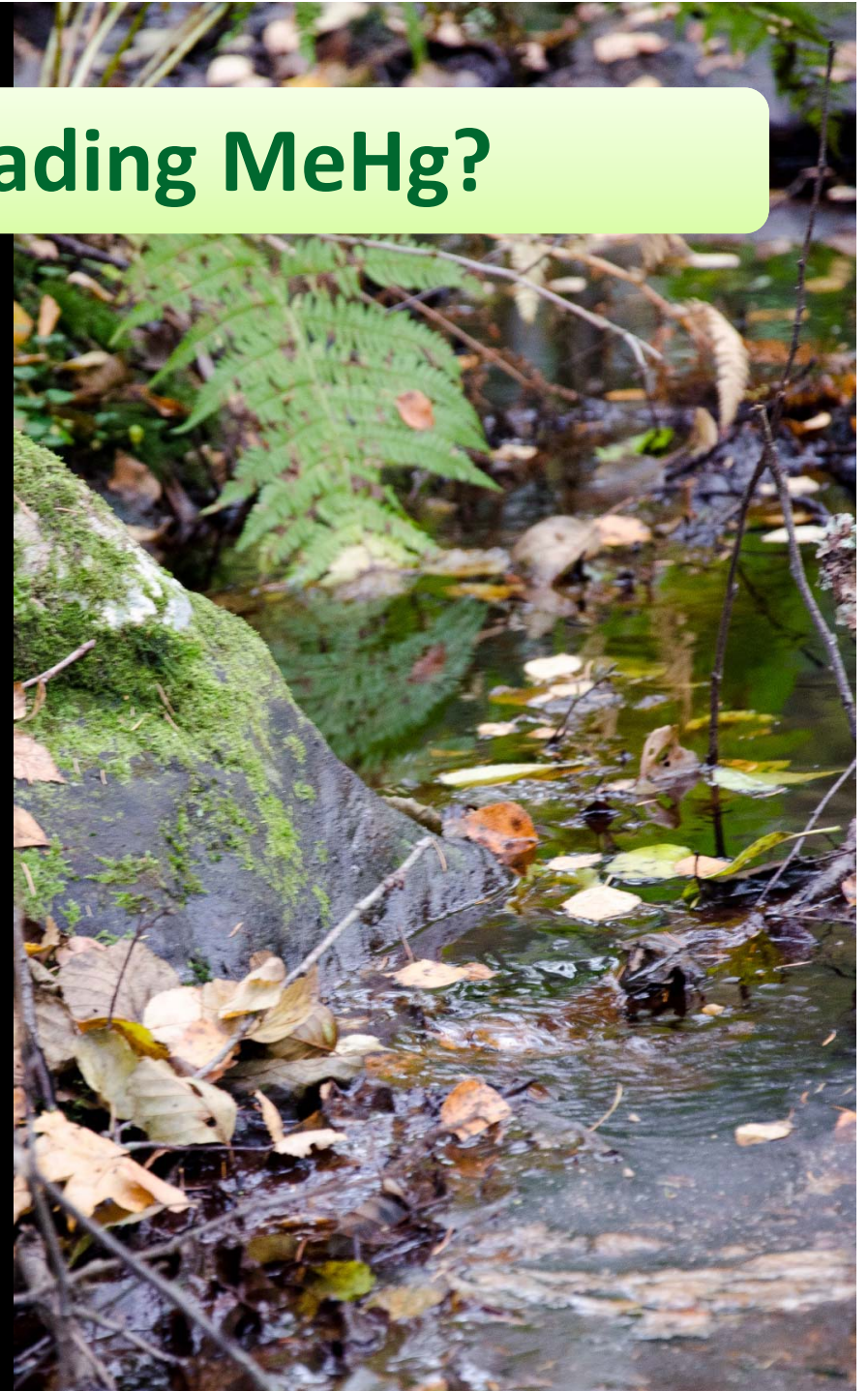


# Bacterial Hg cycle

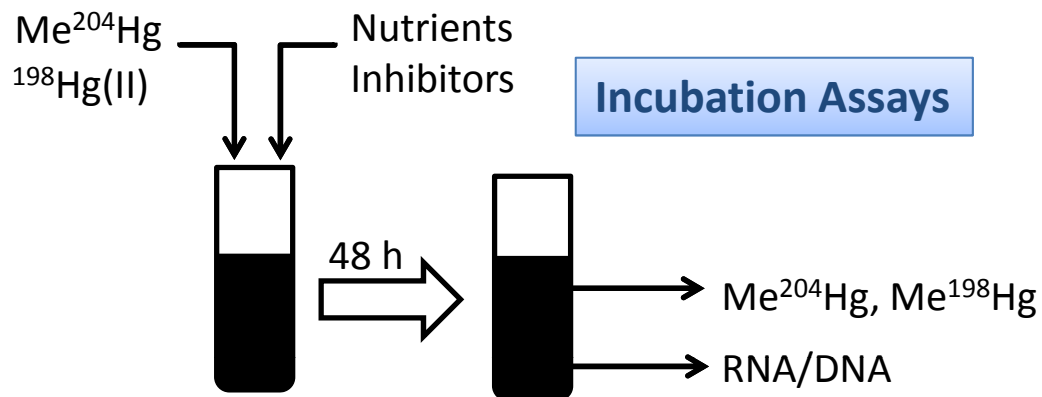


# Who is producing/degrading MeHg?

- Activity measurements
  - Nutrient additions
  - Specific inhibitors
- Molecular signals (RNA/DNA)
  - Gene expression
  - Diversity
  - Abundance
- Culturing techniques



# Which microbial group(s) influence MeHg accumulation?



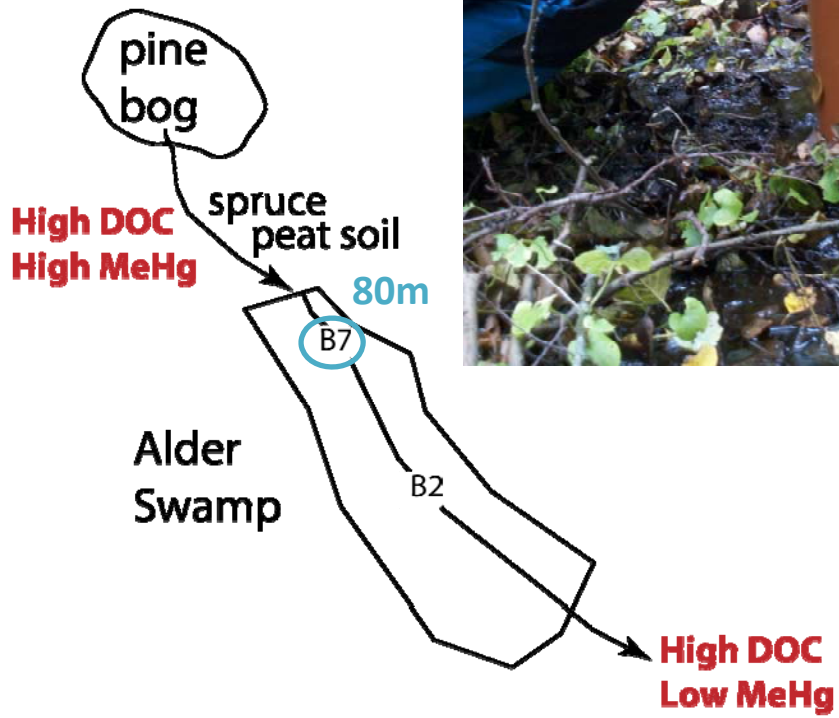
Microbial Guild	Activity measurements		Molecular approach	
	Inhibitor	Nutrient additions	Guild Specific gene/transcript	Hg methylation gene/transcripts
FeRB	-	Fe(III), Mn(IV)	16S rRNA	<i>hgcA</i>
SRB	MoO <sub>4</sub>	Sulfate	<i>dsrB</i>	<i>hgcA</i>
Methanogens	BES	-	<i>mcrA</i>	<i>hgcA</i>



# EdB7

High methylation;  
Low demethylation

pH 5.2

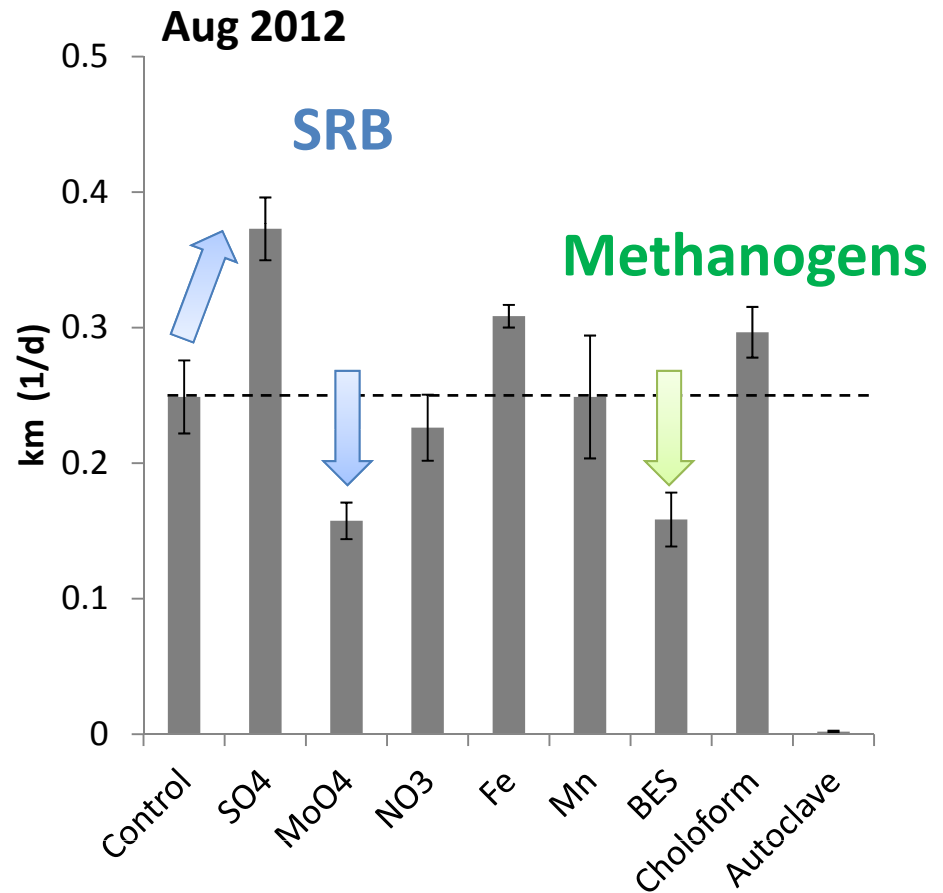
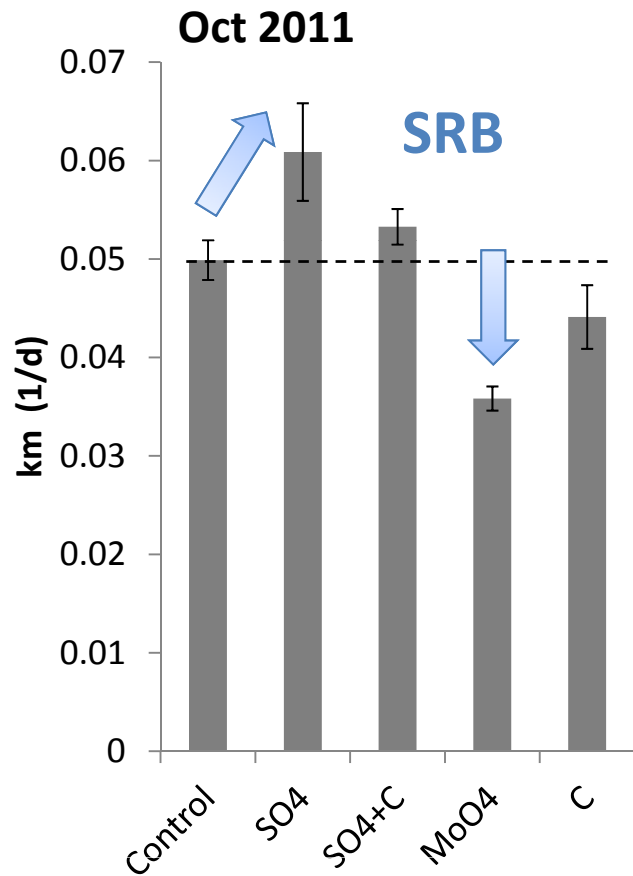




# Potential methylation rate constants

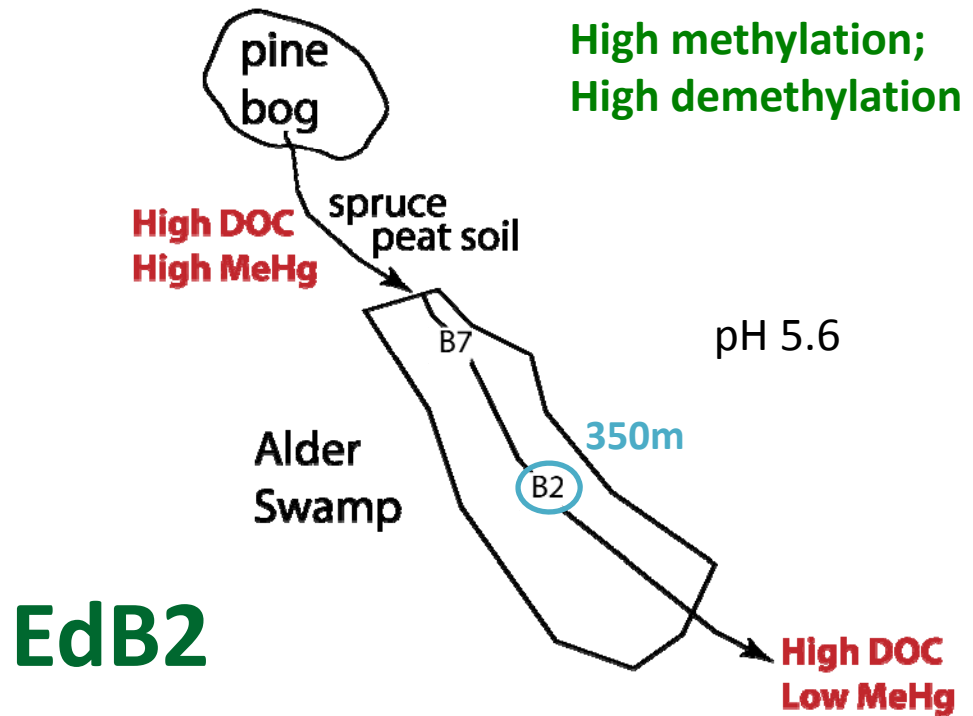
B7, 80 m from inlet

~10% ambient as  $^{198}\text{Hg}(\text{II})$   
48 h incubations (anaerobic)



**FeRB *Geobacter* species?**

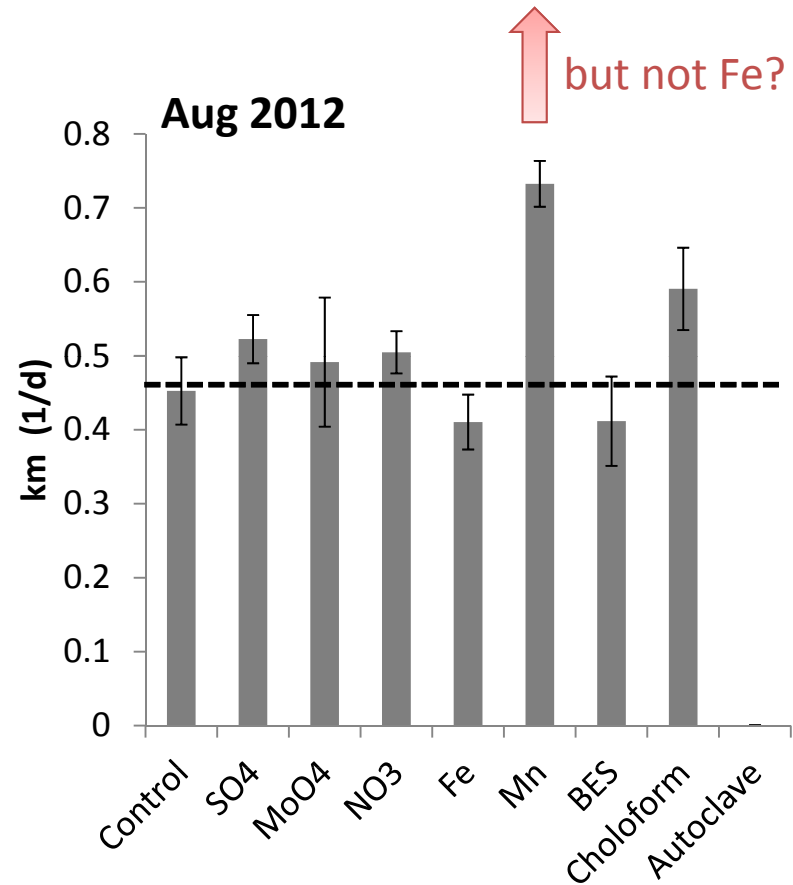
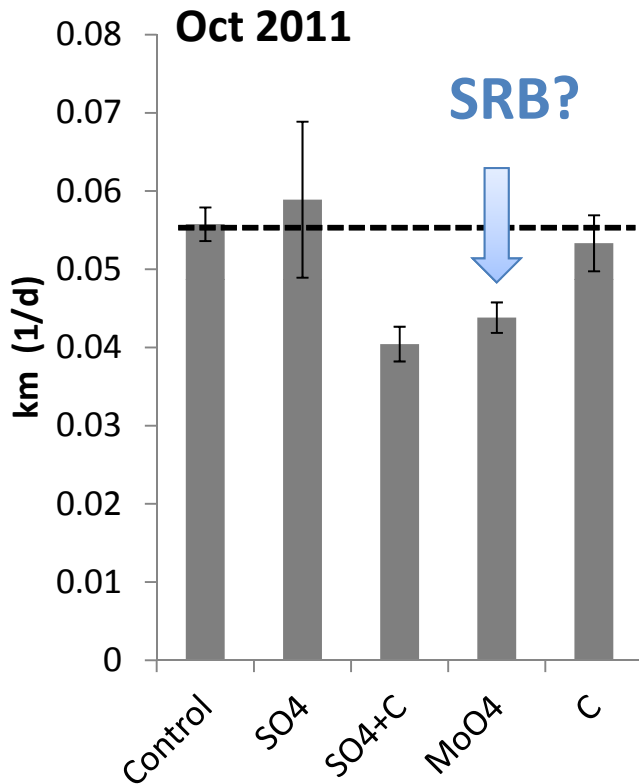






# Potential methylation rate constants

B2, 350 m from inlet

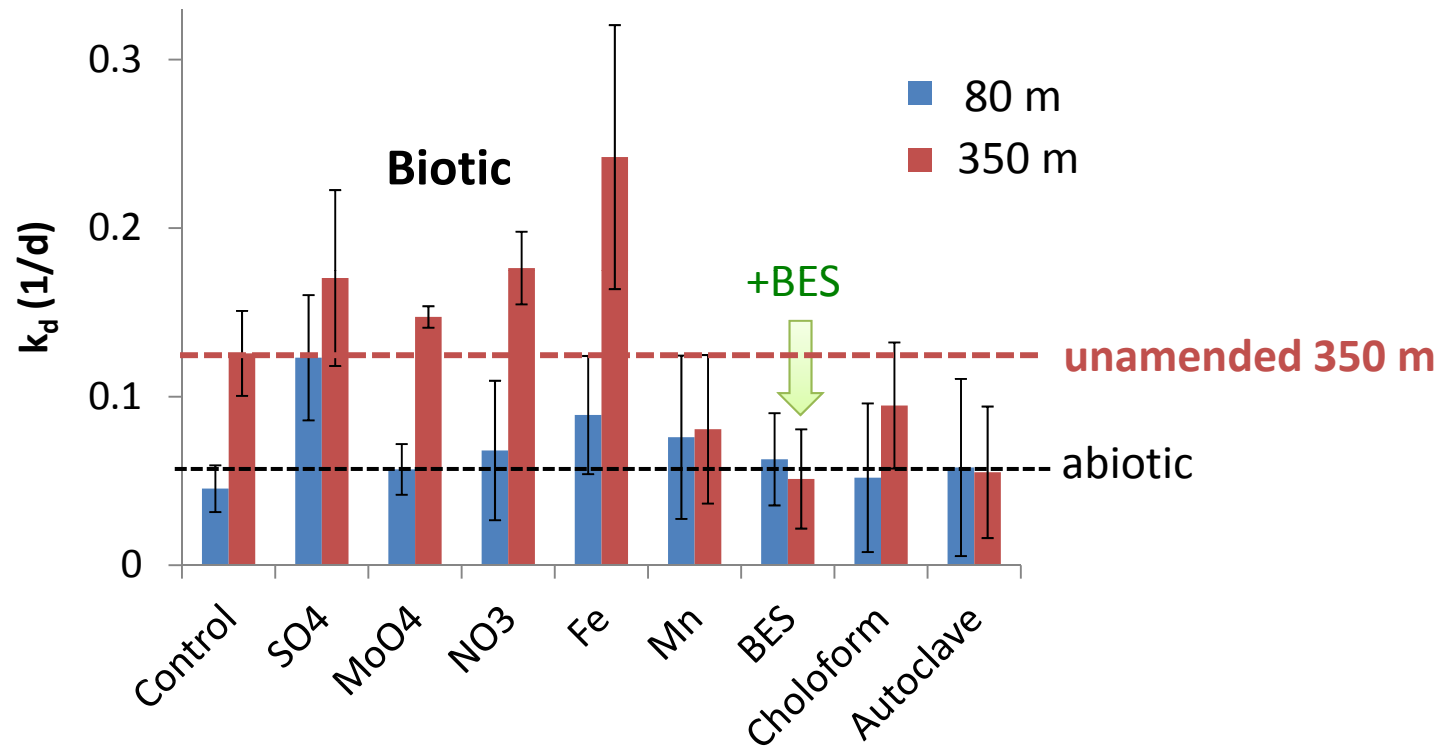


**FeRB *Geobacter* species?**



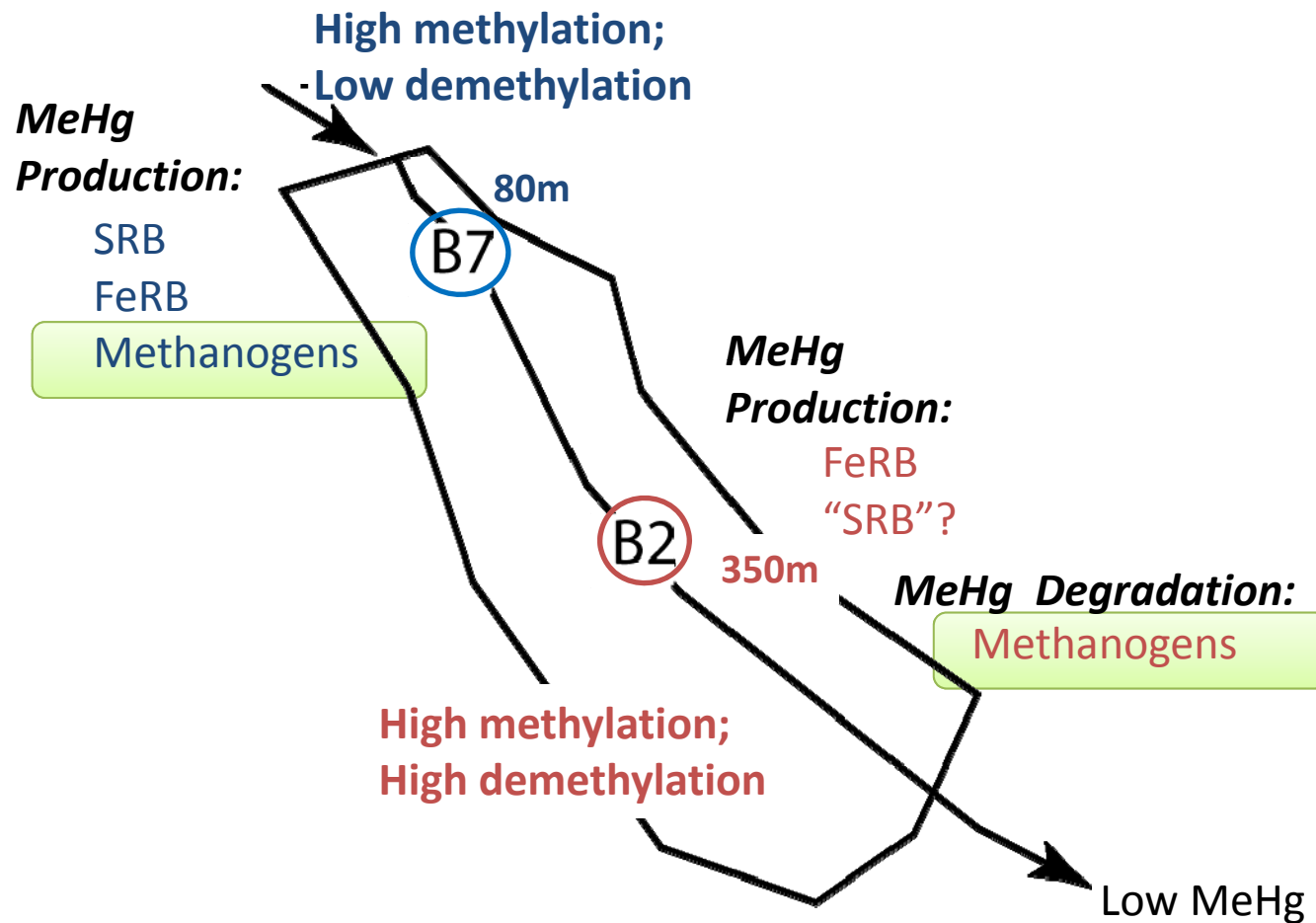
# Demethylation

~15% ambient as  $\text{Me}^{204}\text{Hg}(\text{II})$   
48 h incubations (anaerobic)



Methanogens implicated in the demethylation of MeHg

# Microbial guilds implicated in MeHg cycling



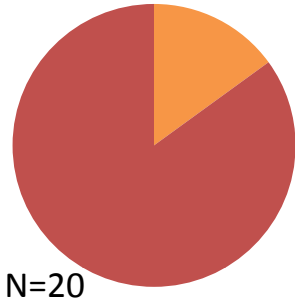


# Methanogen diversity in incubations

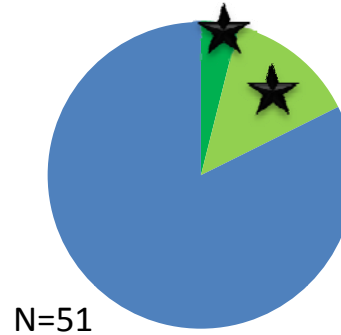
**McrA**

*mcrA* transcripts

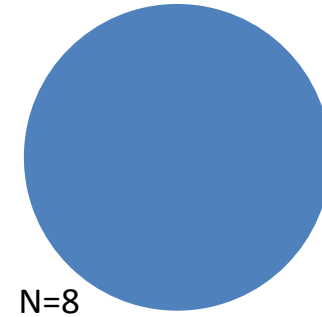
**80 m, t0 genes**



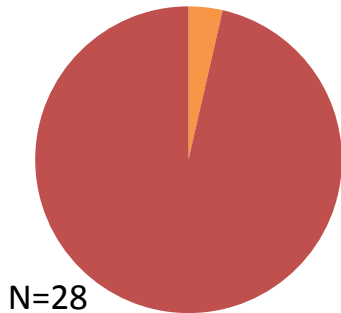
**B7 t48 C**



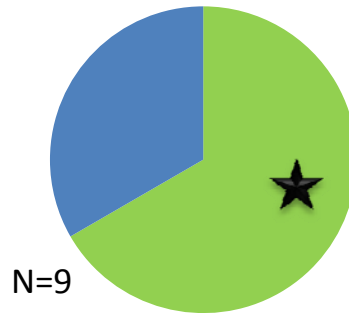
**B7 t48 SO4**



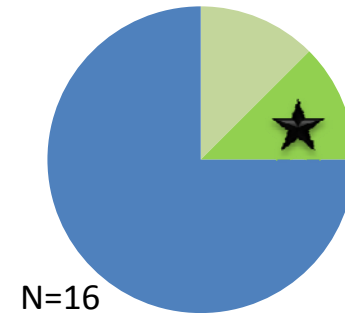
**350 m, t0 genes**



**350 m, t0**



**350 m, t48 +SO4**



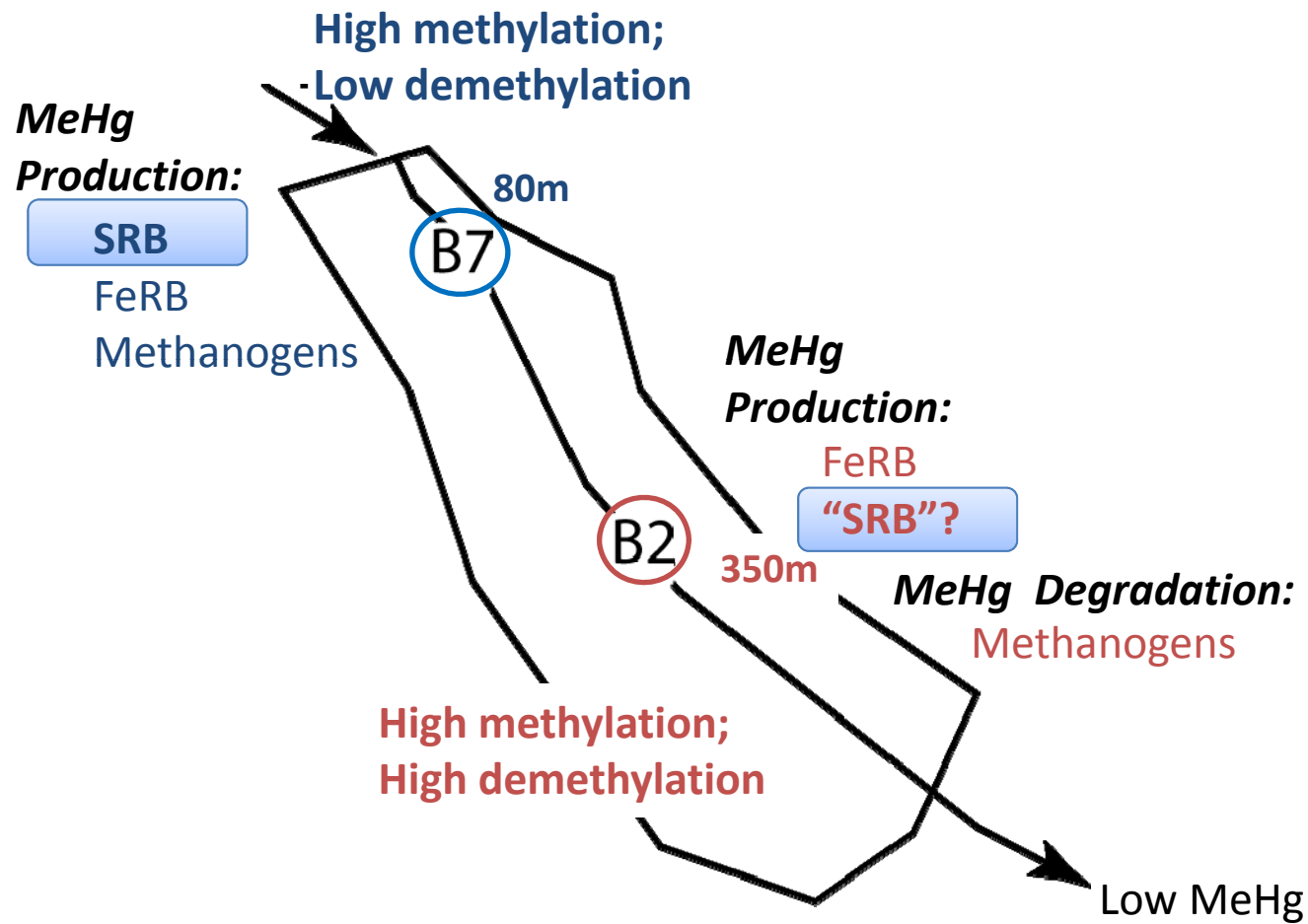
**Methanobacteria**

- Methanococci
- Methanobacteria

**Methanomicrobia**

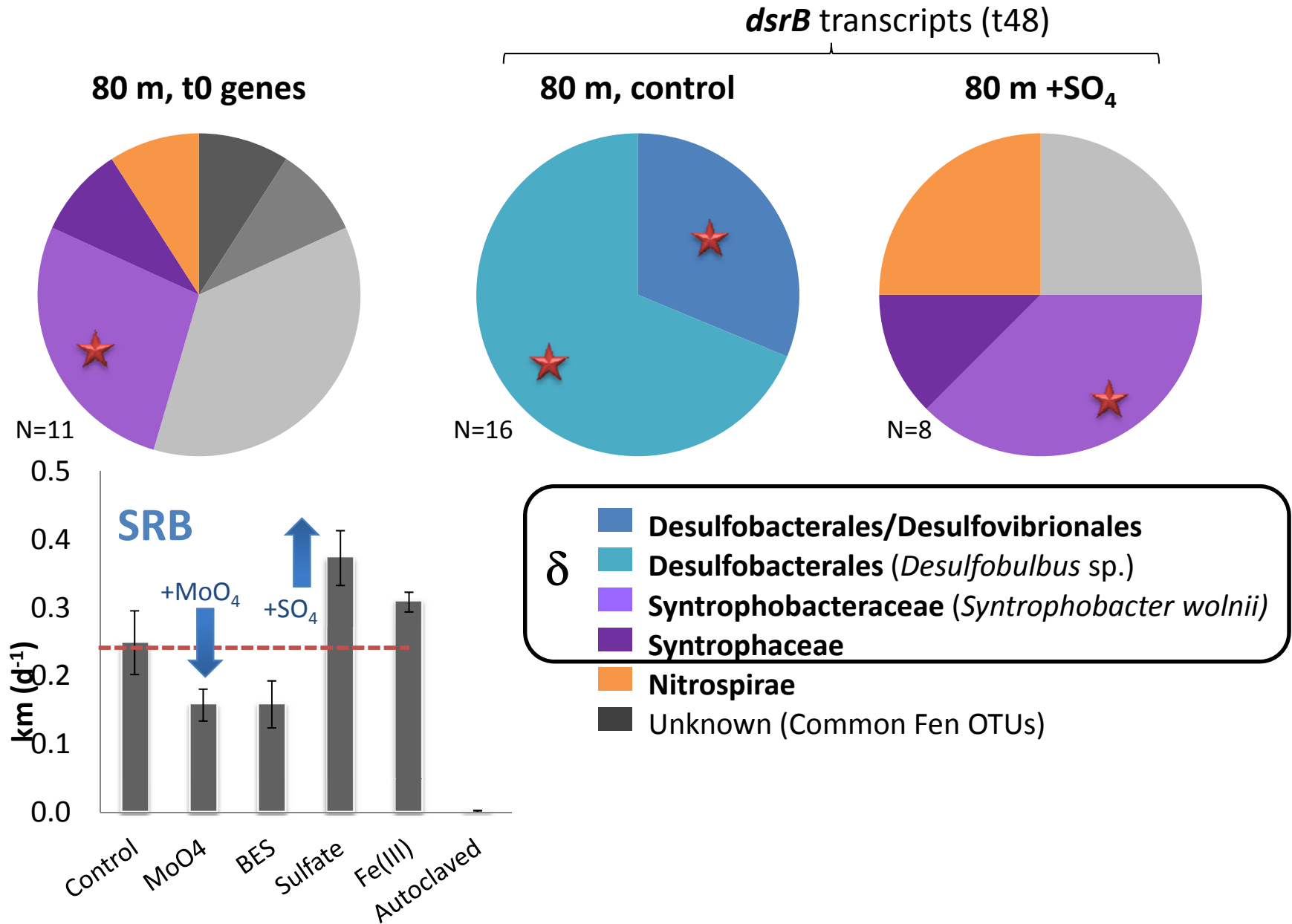
- Methanocellaceae
- Methanoregulaceae
- Methanosarcinaceae
- Methanosarcinaceae

# Microbial guilds implicated in MeHg cycling



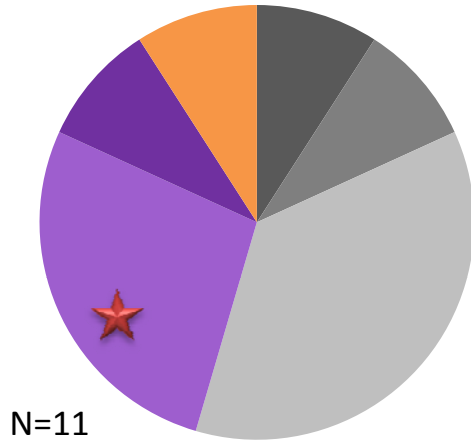


# SRB diversity in incubations (*dsrB*)

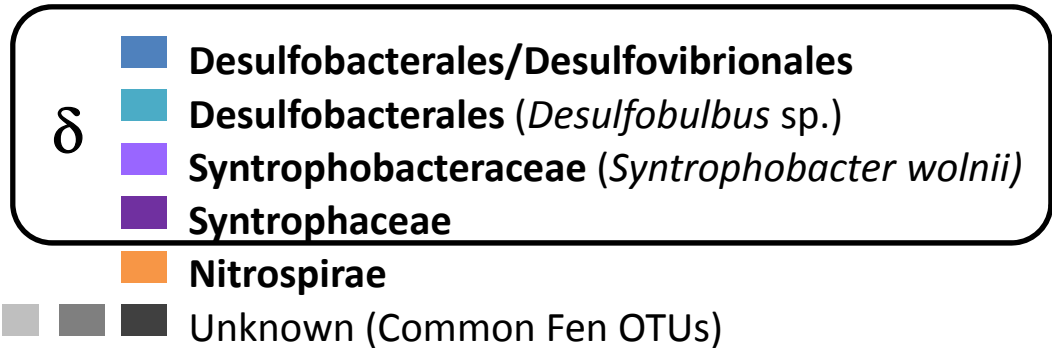
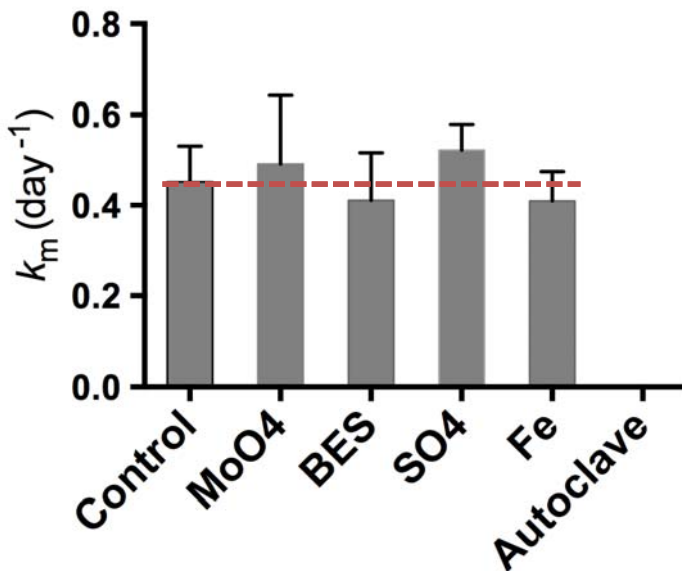
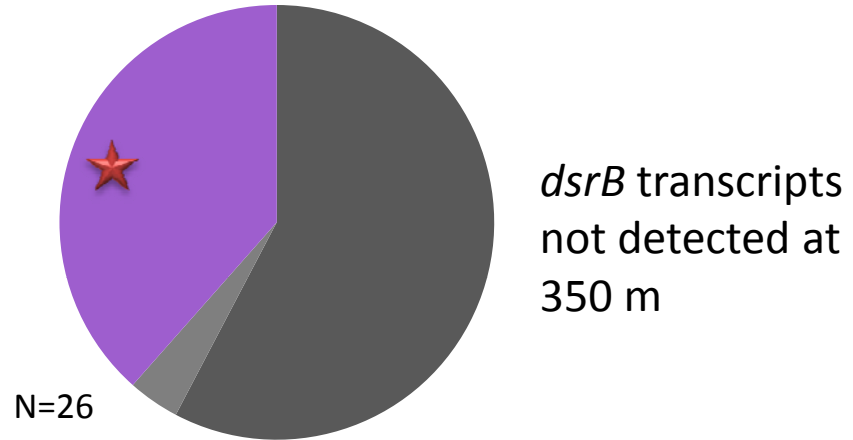


# SRB diversity in incubations (*dsrB*)

80 m, t0 genes



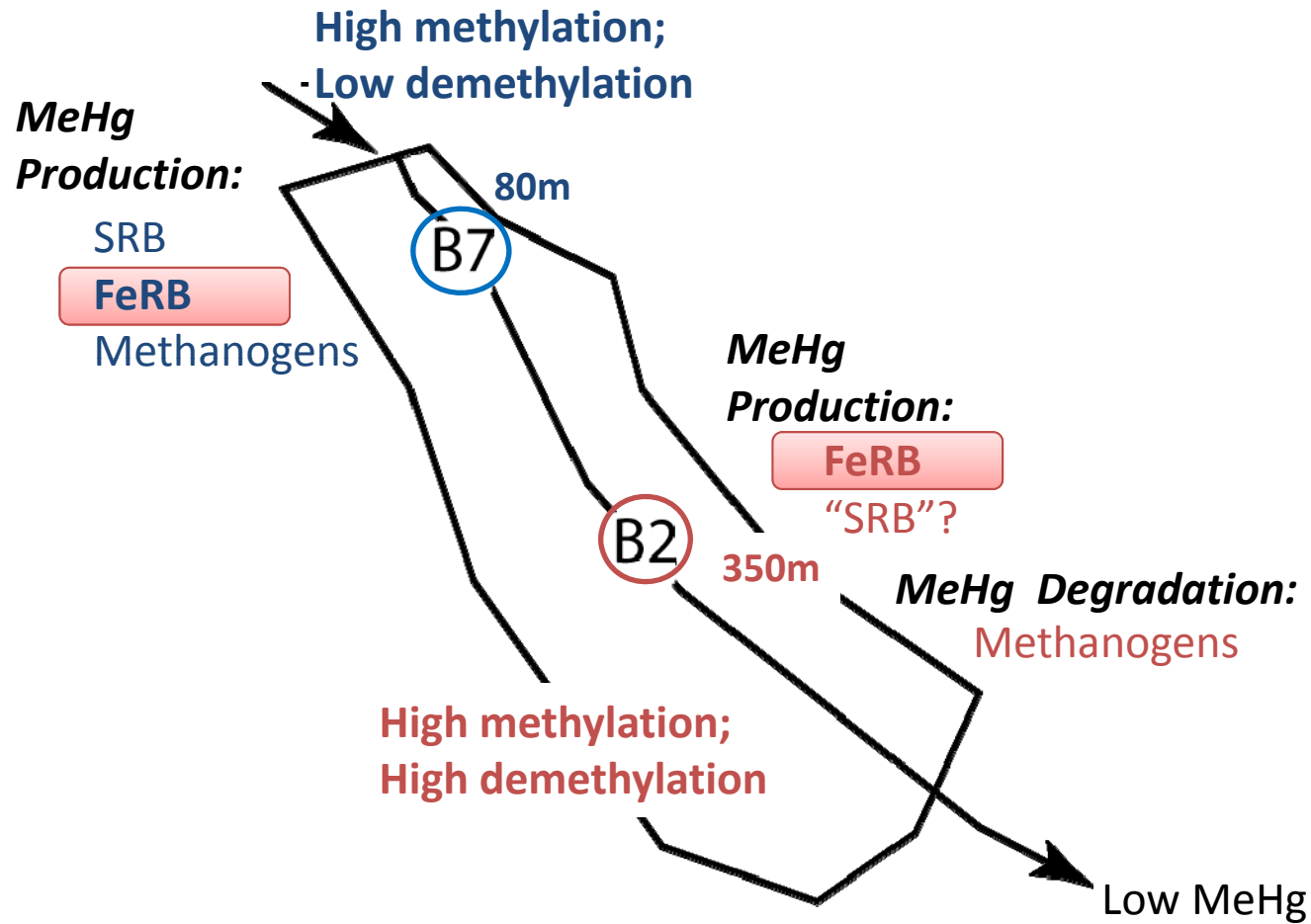
350 m, t0 genes



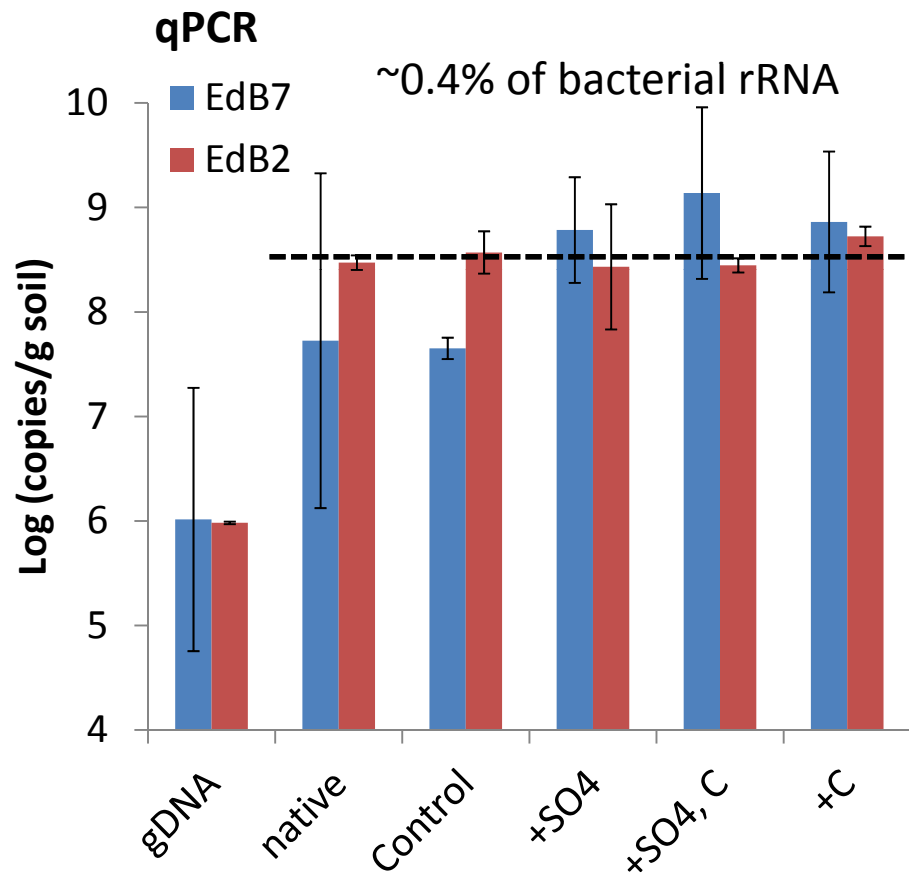
MPNs: 2 – 3 x 10<sup>4</sup> cells/g soil



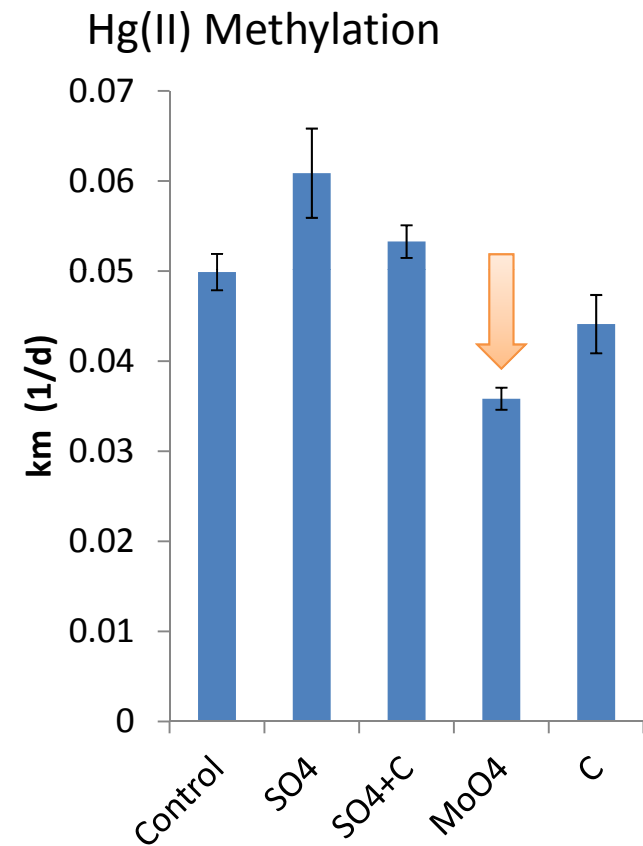
# Microbial guilds implicated in MeHg cycling



# *Geobacter* (FeRB) are abundant and active



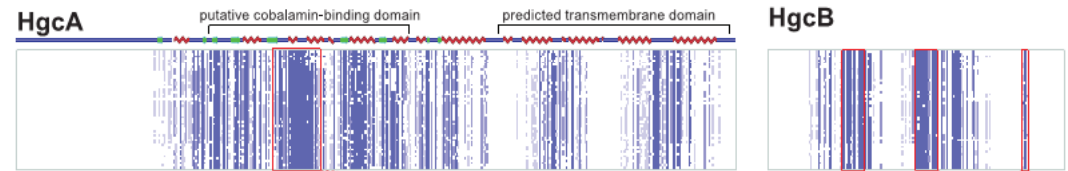
~10<sup>11</sup> copies eubacterial rRNA





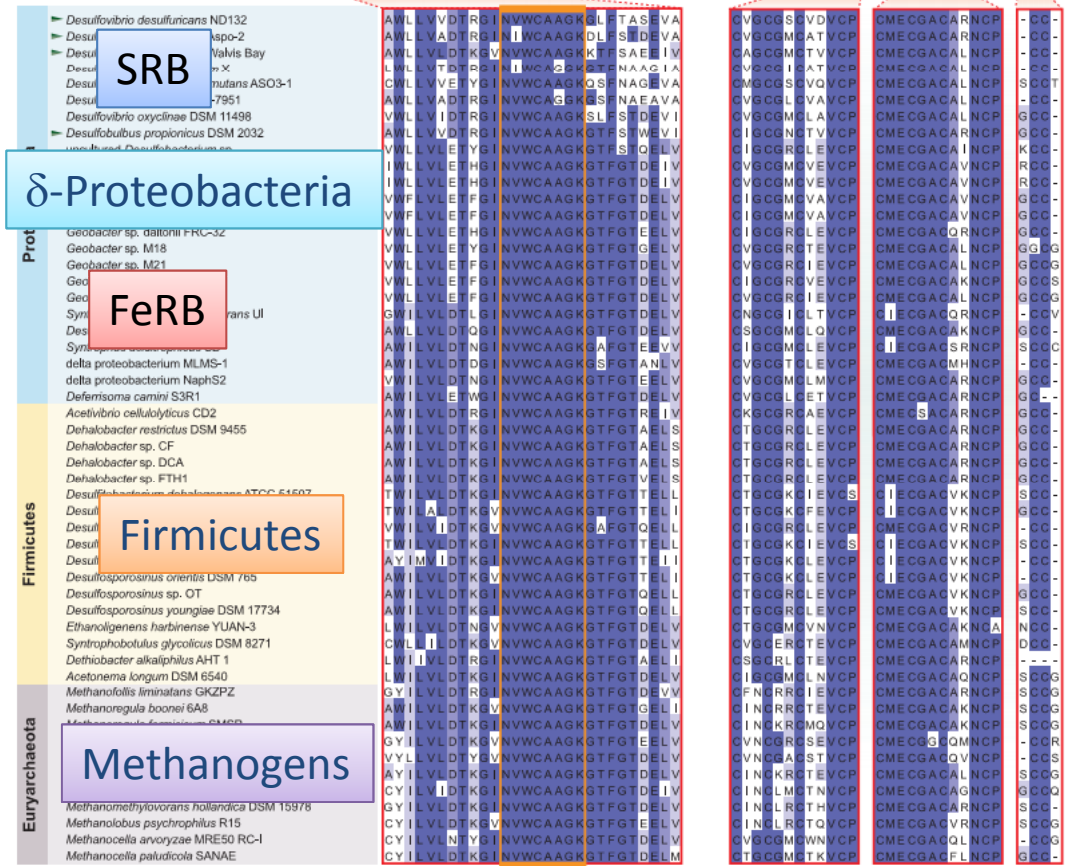
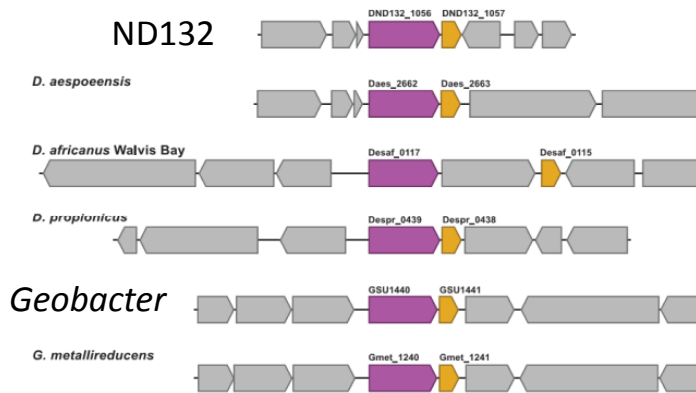
# The Genetic Basis for Bacterial Mercury Methylation

Jerry M. Parks,<sup>1\*</sup> Alexander Johs,<sup>2\*</sup> Mircea Podar,<sup>1,3\*</sup> Romain Bridou,<sup>4</sup> Richard A. Hurt Jr.,<sup>1</sup> Steven D. Smith,<sup>4</sup> Stephen J. Tomanicek,<sup>2</sup> Yun Qian,<sup>2</sup> Steven D. Brown,<sup>1,5</sup> Craig C. Brandt,<sup>1</sup> Anthony V. Palumbo,<sup>1</sup> Jeremy C. Smith,<sup>1,5</sup> Judy D. Wall,<sup>4</sup> Dwayne A. Elias,<sup>1,5†</sup> Liyuan Liang<sup>2†</sup>



HgcA = corrinoid protein

HgcB = ferredoxin



$\delta$ -Proteobacteria

Firmicutes

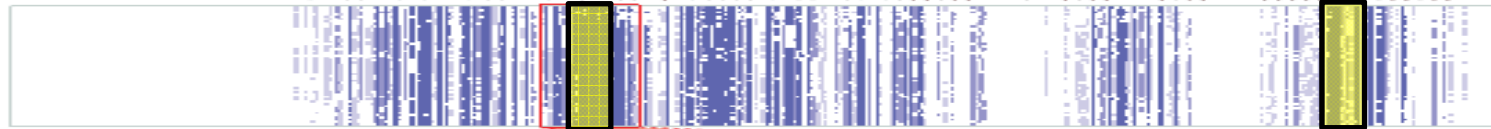
Methanogens

predicted cap helix [4Fe-4S] [4Fe-4S]

# HgcA

## Cobalamin-binding domain

## transmembrane domain



GSU1440\_1/1-325  
Gmet\_1240\_1/1-323  
Gura\_0480\_1/1-318  
Gbem\_1183\_1/1-340  
Geob\_2483\_1/1-324  
Desde\_2772\_1/1-334  
Desdi\_0780\_1/1-334  
Desaci\_1621\_1/1-334  
Desor\_2652\_1/1-334  
DCF50\_p1170\_1/1-385  
Sgly\_2352\_1/1-352  
Ethha\_0975\_1/1-369  
Dnd132\_1056\_1/1-338  
Daes\_2662\_1/1-334  
Despr\_0439\_1/1-339  
Dbac\_0376\_1/1-346  
Desaf\_0117\_1/1-331  
Desti\_1022\_1/1-339  
SYN\_00351\_1/1-396  
Mhun\_0876\_1/1-342  
Mpsy\_0587\_1/1-356  
MCP\_0718\_1/1-331  
Mboo\_0422\_1/1-345  
Mpal\_1034\_1/1-320

```
GPGT P V V V T A N Y K M S Y D S V R W E L A G R D I W L L V L E T G I N V W C A A G K G T F G T D E I V
GPED P V V V T A N Y K M S Y D L V R R E L S G R N V W F L V L E T G I N V W C A A G K G T F G T D E L V
TRAD P V V V T A N Y K M S Y D L V R R S L A G R N V W L L V L E T G I N V W C A A G K G T F G T D E L V
DAEA P V V V T A N Y K M S F D L V R K A L A G R N V W L L V L E T G I N V W C A A G K G T F G T D E L V
TGS D Q V V V T A N Y K M S F D I V R N E L A G R N V W L L V L E T G I N V W C A A G K G T F G T E E L V
NEN S P V L V T A N Y K M S F D S L R K E L S G L D T W I L V L D T G I N V W C A A G K G T F G T T E L I
DGS S P V L V T A N Y K L T F D G L R K K L T G L D T W I L A L D T G V N V W C A A G K G T F G T T E L
DKS S P V F V S A N Y K M S F D S L R K E L S G F D A Y I M V I D T G I N V W C A A G K G T F G T T E I
DPNA P V L V T A N Y K M T F D A L R K E L T D L D A W I L V L D T G V N V W C A A G K G T F G T T E L
DEHS E V L V T A N Y R M T F D A L R K E L A G L Q A W I L V L D T G I N V W C A A G K G T F G T A E L
DRT S P V L V S A N Y K L T F D S L R K E L S A L N C W L L I L D T G V N V W C A A G K G T F G T D E L V
AEN S P V L V T A N Y K L T F D S L R K E L A G L S L W I L V L D T G V N V W C A A G K G T F G T D E L V
DRT S P V L V T A N Y K L T F D T L R E R L T S I D A W L L V V D T G I N V W C A A G K G L F T A S E V A
TSD S P V I V T G N Y K L T F D A V R E L S D L D A W L L V A D T G I N I W C A A G K D L F S T D E V A
GNQ S A V L V S A N Y K L S F D A L R H Q L G G L D A W L L V V D T G I N V W C A A G K G T F S T W E V
GPEA P V I V T A N Y K L T F D T V R F T L P G R D L W L L V T D T G I N I W C A A G K G T F N A A G I A
DAQ S P V I V T A N Y K L T F D A L R K E L S G L N A W L L V L D T G V N V W C A A G K K T F S A E E I V
DAAS P V L V T A N Y K M S F D S L R S A L P G S S A W L L V L D T G I N V W C A A G K G T F G T D E L V
DESAP V L V S A N Y K L S F D C L R E A L P G R T A W I L V L D T G I N V W C A A G K G A F G T E E V I
DADS P V F A S A N Y T I S F D T L R L N L K G F D A Y I L V L D T G I N V W C A A G K G T F G T D E L V
NADS P V L V S A N Y T L S F D A V R S A L A G T D C Y I L V L D T G V N V W C A A G K G T F G T E E L V
TAES P V L V T A N Y T L S F D A L R S A L K D V D C Y I L V L D T G I N V W C A A G K G T F G T D E L V
TBES P V I V T A N Y T L S F D A V R S A L G R T D A W I L V L D T G V N V W C A A G K G T F G T G E L
DRTA Q V I V T A N Y T L T F D A V R S A L D G I D V V L L V L D T G V N V W C A A G K G T F G T D E L V
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Geobacter sp.

Fermicutes

SRB  
Syntrophs

Methanogens

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330 340 350 360 370 380 390 400
GSU1440_1/1-325 --G-MVSLHEVAALFFITPAVCSFLALTF TGSTPFTSRSGVKKEMRVALPVMIAALVAGLTSWLFGSFLLS...
Gmet_1240_1/1-323 --G-ATGWLDAMALFLTVPVAFFLTUNFTGSTPFTSRSGVKKEMRLGLPMMAVSLLAGILSWIAARFF...
Gura_0480_1/1-318 --GGSWSVPVAIAAFLTLPVSAFYTLNFTGCTTFTSRSGVKKEMRLALPVMGALAVSALLVAGLFL...
Gbem_1183_1/1-340 --GEGWSLPVTLAAFLAFPAVTSFYTLNFTGCTTFTSKSGVKKEMRLGLPVLGGAIALAALLLVTVGRMLS...
Geob_2483_1/1-324 --GATWGLPATIGAFALPAVSAFYALNFTGCSYTSRTGVKREMRRALPLMGGAVALGVLLFVAGKICVIC...
Desde_2772_1/1-334 --YDPQYSMLQALGYVLVFPVSAYLAMNFTGSSYTSFSGVLKEMRIAIPAIIVSIFIGCVLILVNSFI...
Desdi_0780_1/1-334 --YEPYRSLQLQALGYILIFPAVSGYLANNFTGSSYTSFSGVLKEMRIAIPITIIISIVGCVLLLAGSFI...
Desaci_1621_1/1-334 --YAPQYGLLKALGYLLILPSSAYLAMNFTGSSYTSFSGVLKEMKTAIPAIISVVLGVILILVNSFI...
Desor_2652_1/1-334 --AANCYGLLRALGYMLLIPVSAYLAMNFTGSSYTSFSGVLKEMKTAIPAIISVVLGVILILVNSFI...
DCF50_p1170_1/1-385 --GELSYSWFRALGYLLVLPSSAYLANNFTGSSYTSFSGVMKEMKTAVPIAVTICIGCILILLSSFLGL...
Sgly_2352_1/1-352 --VS--DNLLLGIGYLLVLPSSAFLAMDFGSSYTSFSGVIKEMKLSLPIFILASVIGIVLVLIKSFMA...
Ethha_0975_1/1-369 --GLPQLGIRITLSFILLPAVSAYTAMNFTGSSYTSFSGVVKEMRVAIPAMLVSSILGAILLVSLVSSGLVETIL...
Dnd132_1056_1/1-338 --YLRVHWADPLALTLWATAVSAWQAMNFTGSTPYTSPSGVKEMRRGMLQALAAALAAAGLWLAGPFLG...
Daes_2662_1/1-334 --FAQTLGLLESAAALLWTTAASYLAMNFTGSTPYTSPSGVLEMRRAIPQAGAALCALILWIAAPLI...
Despr_0439_1/1-339 --LAARLAAVEQVALISWVLSLSYLANNFTGSTPFTSPSGVEYEMRRGLPQLAATCLALVWLWASPFLH...
Dbac_0376_1/1-346 --FPQLSAAAVTGAAGLWMSLASWLANNFTGSTPYTSPSGVEKEMRKAIPVLAGGTLAAILFVITGNFL...
Desaf_0117_1/1-331 --FAGGLGWLDELAMILAATVVSWYGLNYTGSSYTSFSGVVKELRRYMPAQAVLTLAALVAWLVSFFAA...
Desti_1022_1/1-339 --TGEWPNTELEAGAWILLITVVVSYLGMNFTGCSYTSLSGSKKEMKWAVPFIAGIVAGIALWVSSGFLA...
SYN_00351_1/1-396 --PLSLPRVLESFVSLFLVPAASYWAMKFTGSTPITSLSGVRKEMRRALPQIAGALIGLCWAAAKLLRVL...
Mhun_0876_1/1-342 --DLPEWALAAGAIVPLLIIPAVVAYLGLNFTGSTPYTSTRTGVKKEIFRYVPIMAVMTGIGSVIGIVLSIFRLAGVI...
Mpsy_0587_1/1-356 --IMPLWTDLLKSLVPLLIMPALTAYLALNFTGCTTFTSTRTGVKKEIYRYVPMVLMAGSGILILVLLGAIWLMVEI...
MCP_0718_1/1-331 --GSATLNAVAAAGYLLAMPPTVAYLALNFTGCTTFTSTRTGVRREIFTYIMPMAVMFGLGALIFIGLNVARFMGVA...
Mboo_0422_1/1-345 --PATDGLPVAIGLIRLFLFSAIVGYLALNFTGCTTFTSTRTGVKKEIFRYVPMALLAGLGIACWAVAGLLLYLGAGHA...
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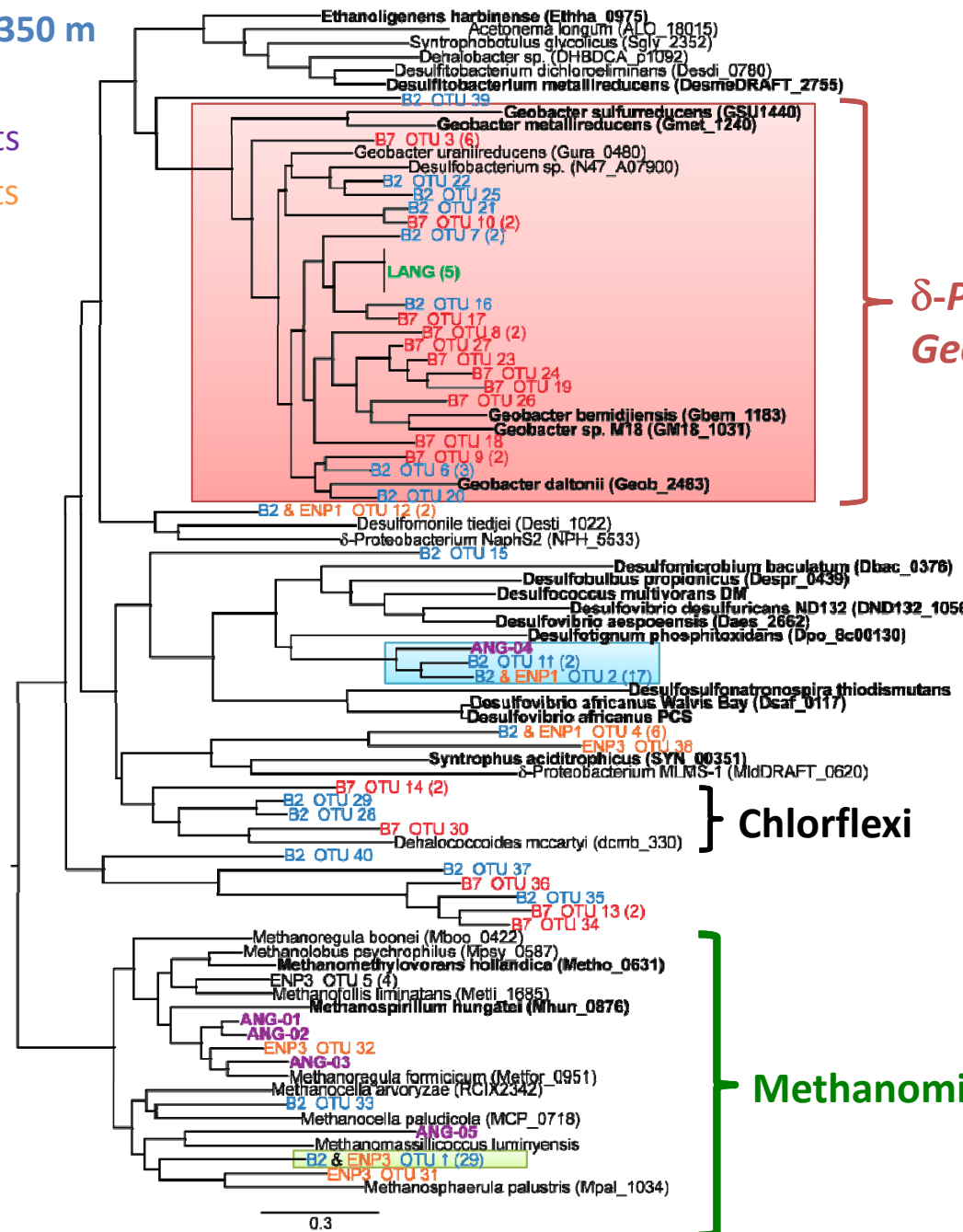
Alder swamp: 80 m, 350 m

Ombrotrophic bog

Humic lake sediments

Everglades sediments

Hgca



*δ-Proteobacteria*  
*Geobacter (FeRB)*

*δ-Proteobacteria*  
SRB

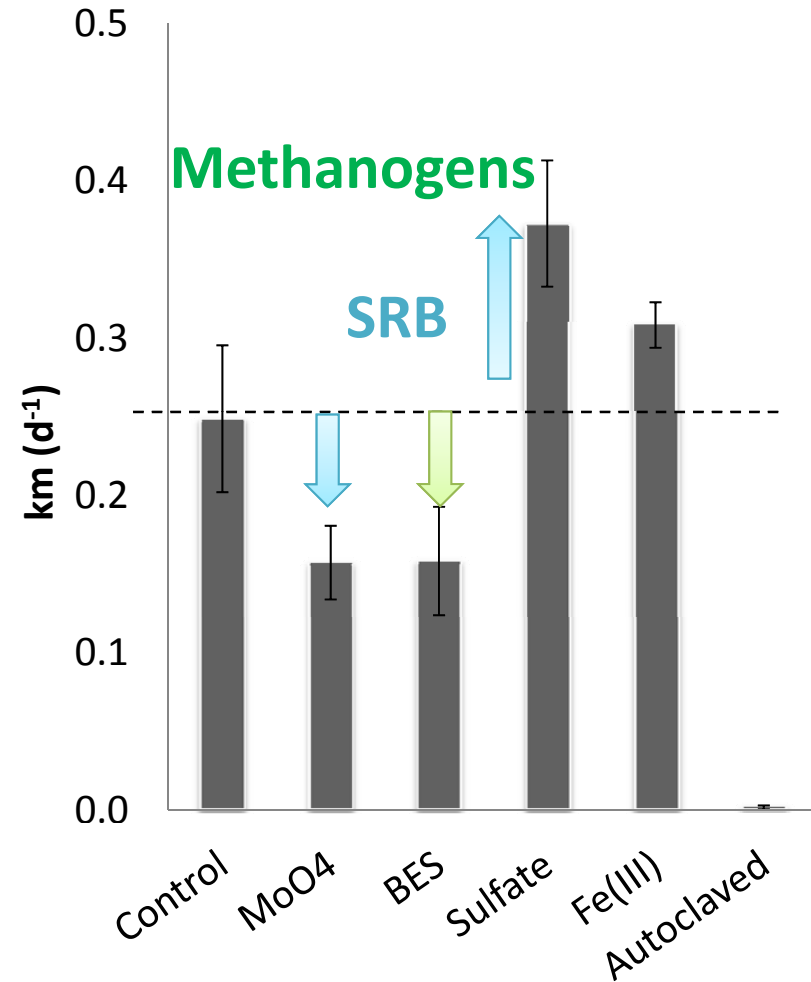
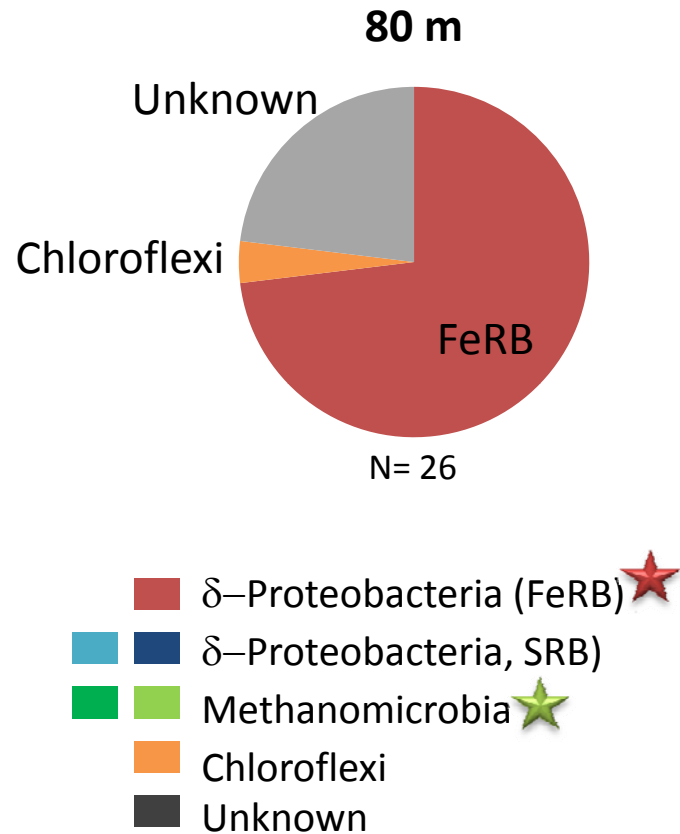
*δ-Proteobacteria*  
Syntrophs

Chlorflexi

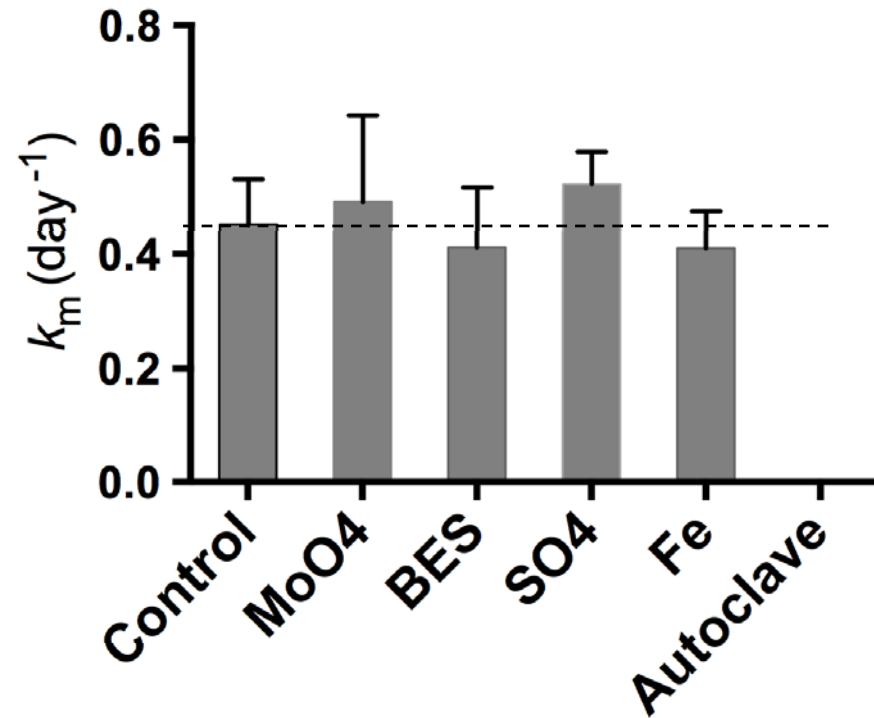
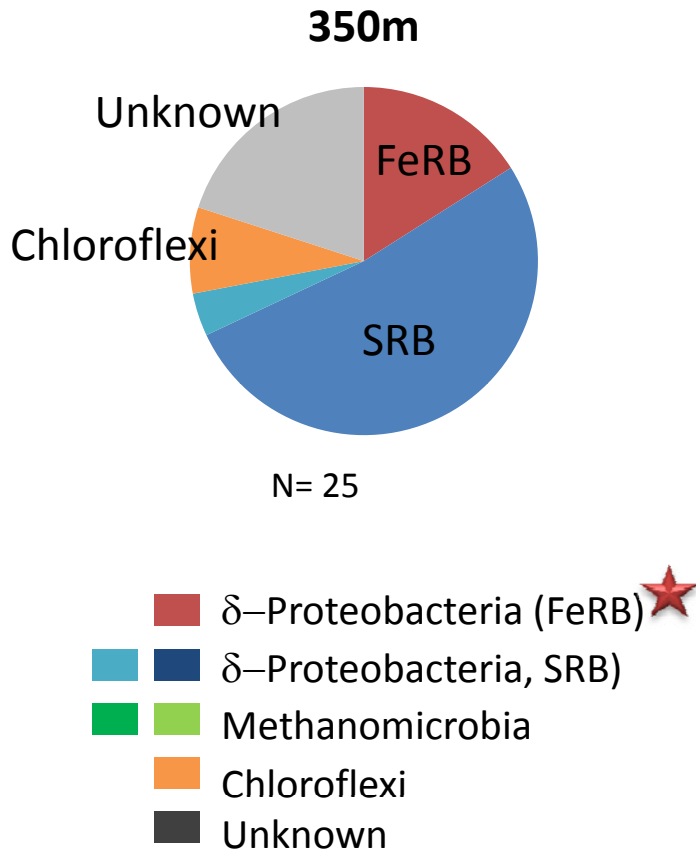
Methanomicrobia

0.3

# HgcA diversity in Edshult soils

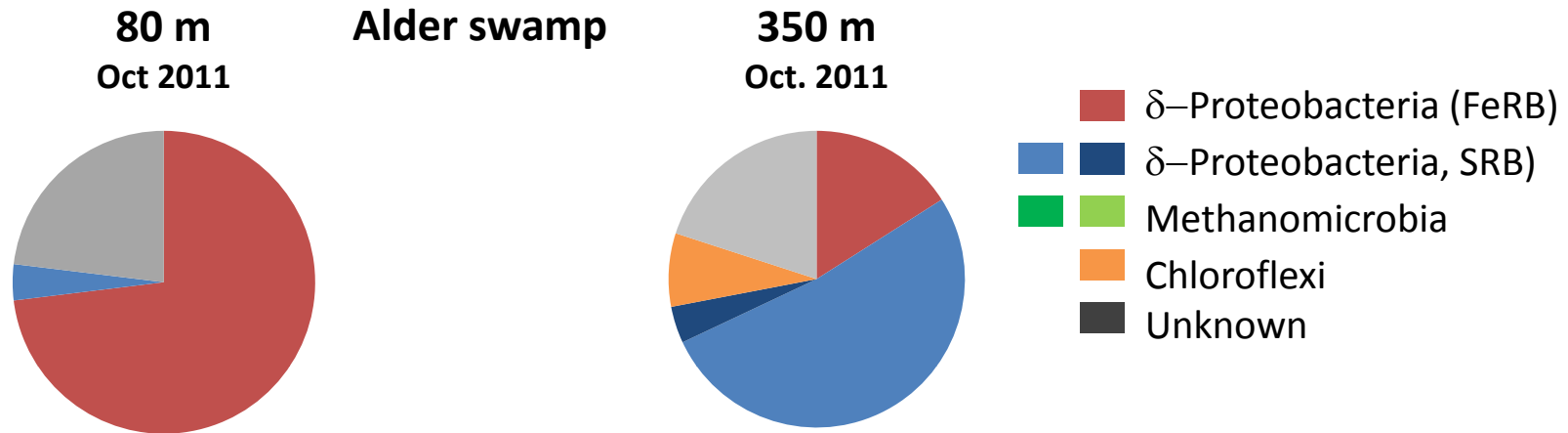


# HgcA diversity in Edshult soils

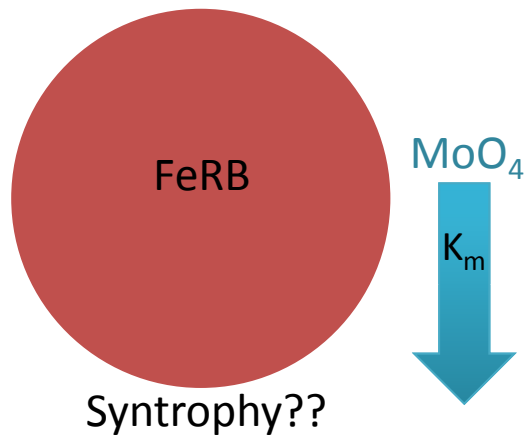




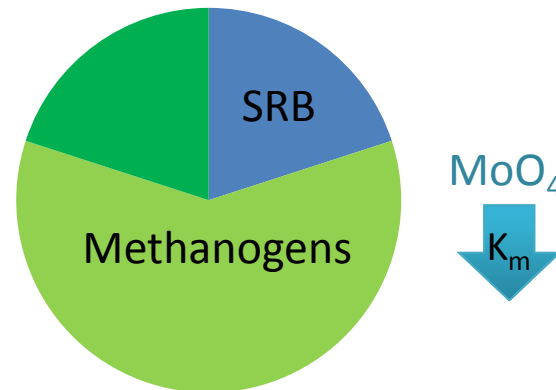
# HgcA diversity in various environments



**Ombrotrophic Bog**  
May 2009



**Humic Lake sediments**  
April 2009

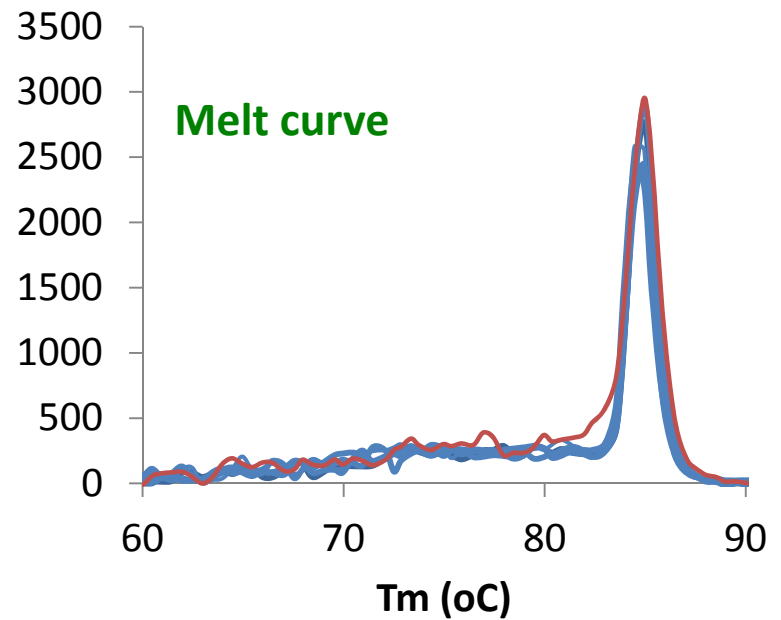
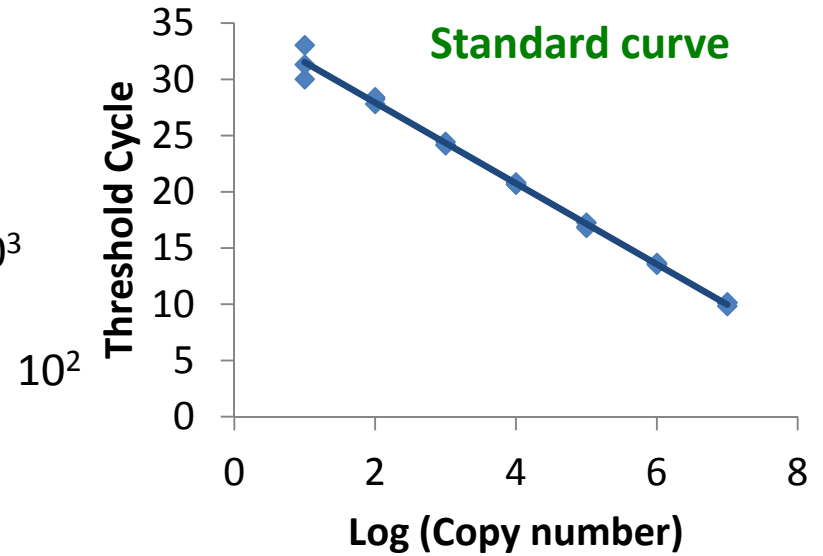
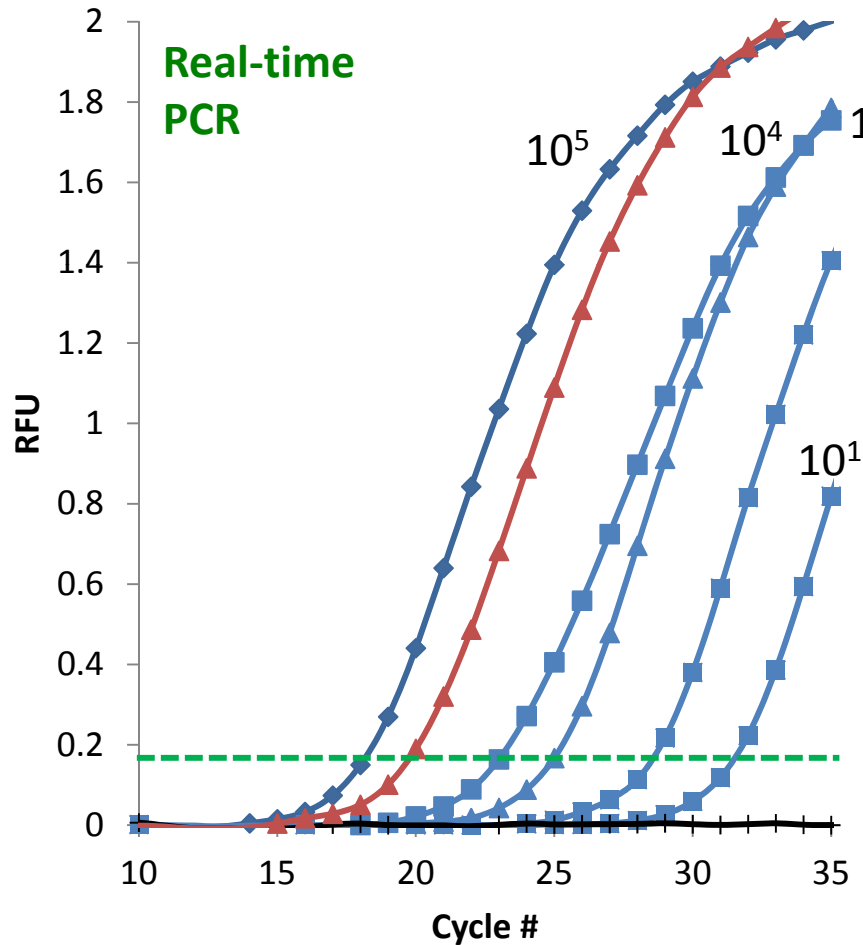


**What about abundance?**

# Quantitation of genes/transcripts

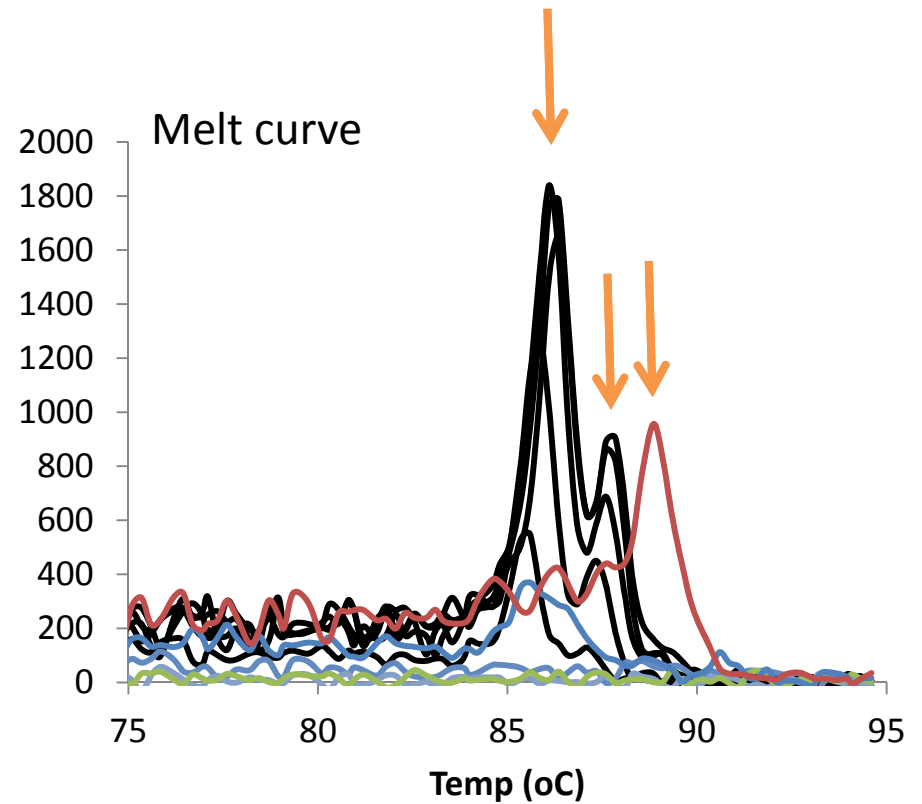
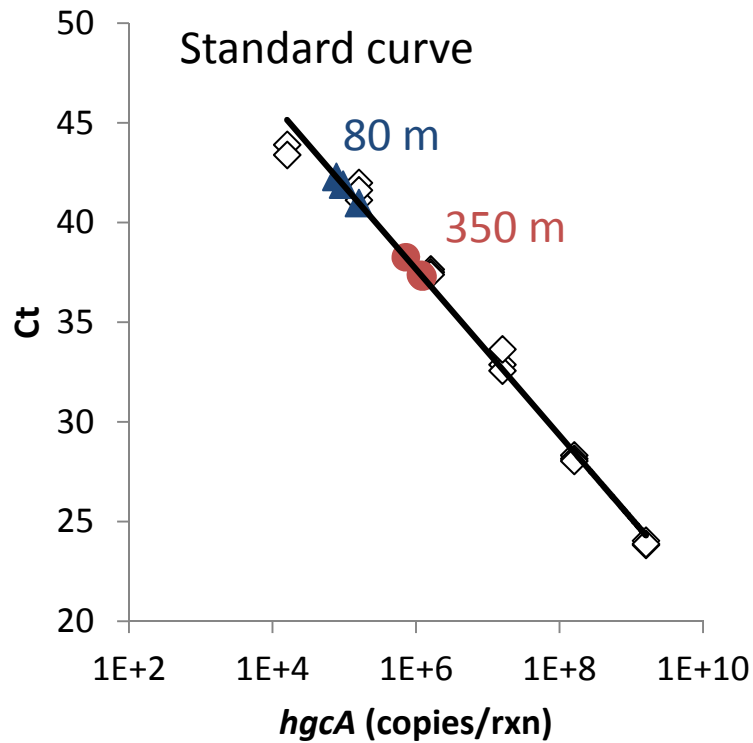
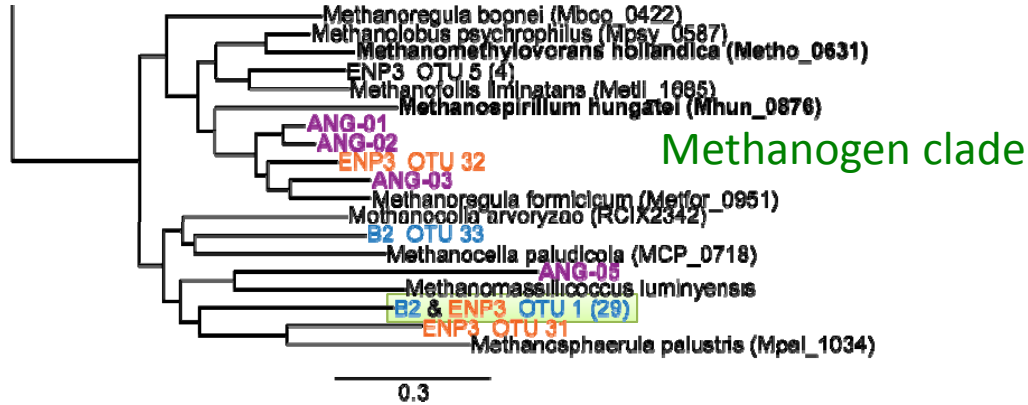
qPCR approach  
SYBR Green

**16S**





# hgcA gene abundance



# Summary

## 80 m Edshult High methylation, Low abiotic demethylation

Microbial Guild	Phyla	Km Activity	Guild specific transcripts	<i>hgcA</i> phylotypes
FeRB	<i>Geobacteraceae</i>	Methylation in +MoO <sub>4</sub> or BES	16S rRNA increased	Transcripts detected
SRB	δ-Proteos	~30% inhib MoO <sub>4</sub>	<i>dsrB</i>	
Syntrophs	δ-Proteos	Similar effects of MoO <sub>4</sub> & BES	<i>dsrB</i>	
Methanogens	<i>Methanomicrobia</i>	~30% inhib BES	<i>mcrA</i>	Transcripts detected

## 350 m Edshult High methylation, High biotic demethylation

Microbial Guild	Phyla	Km / Kd Activity	Guild specific transcripts	<i>hgcA</i> phylotypes
FeRB	<i>Geobacteraceae</i>	Methylation in +MoO <sub>4</sub> or BES	nd	Transcripts detected
Methanogens	<i>Methanomicrobia</i>	Demethylate (inhib. by BES)	<i>mcrA</i>	Genes detected (not transcripts)

# Various guilds contribute to MeHg production

