

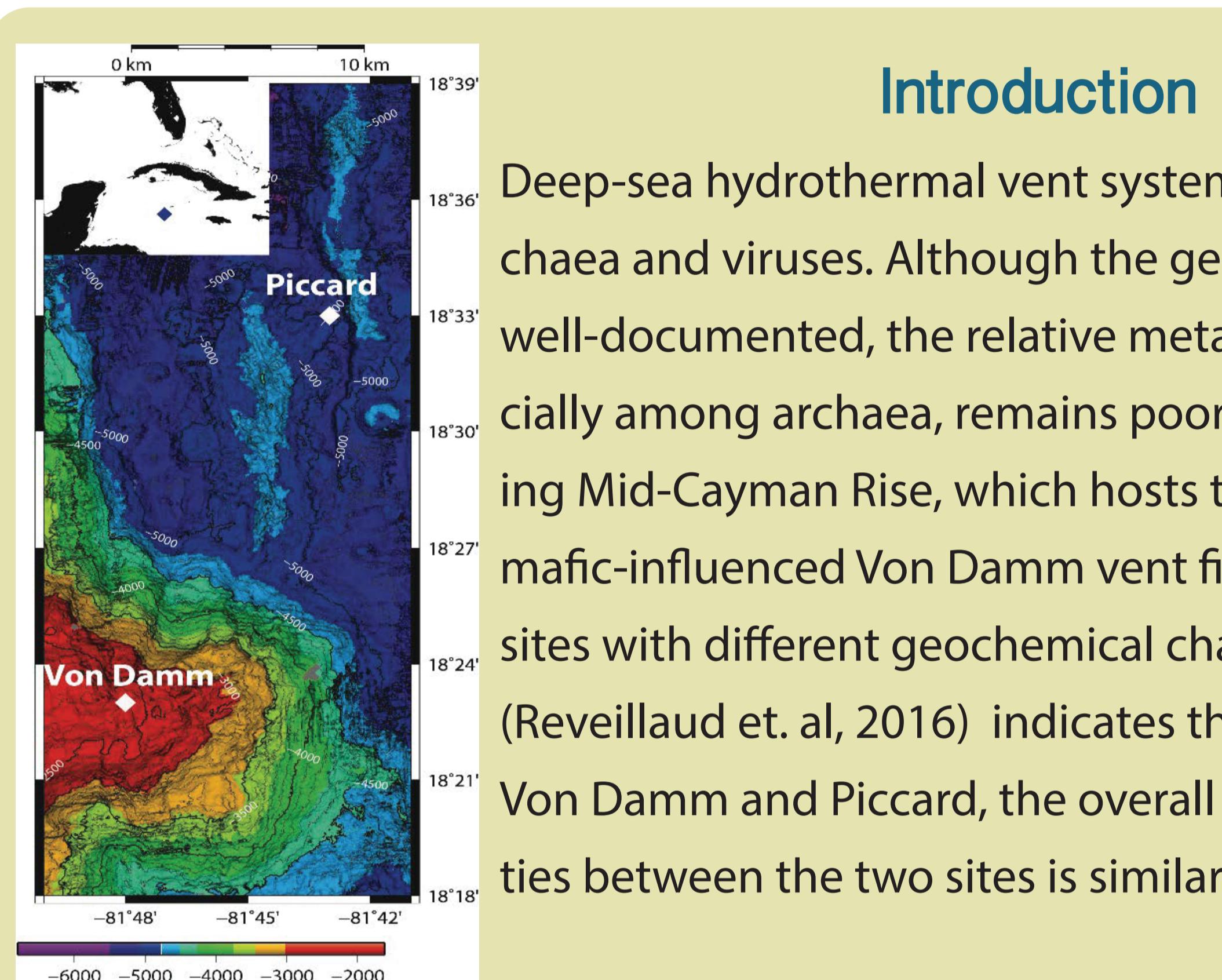
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# Motivating Questions

1. Which metabolic genes are most highly expressed at the community level?
  2. Which taxonomic groups (esp. archaea) have the highest rates of overall gene expression?
  3. What is the metabolic potential (esp.methanogenesis) of each taxonomic group?
  4. What nutrient cycling relationships define community-level methane metabolism?



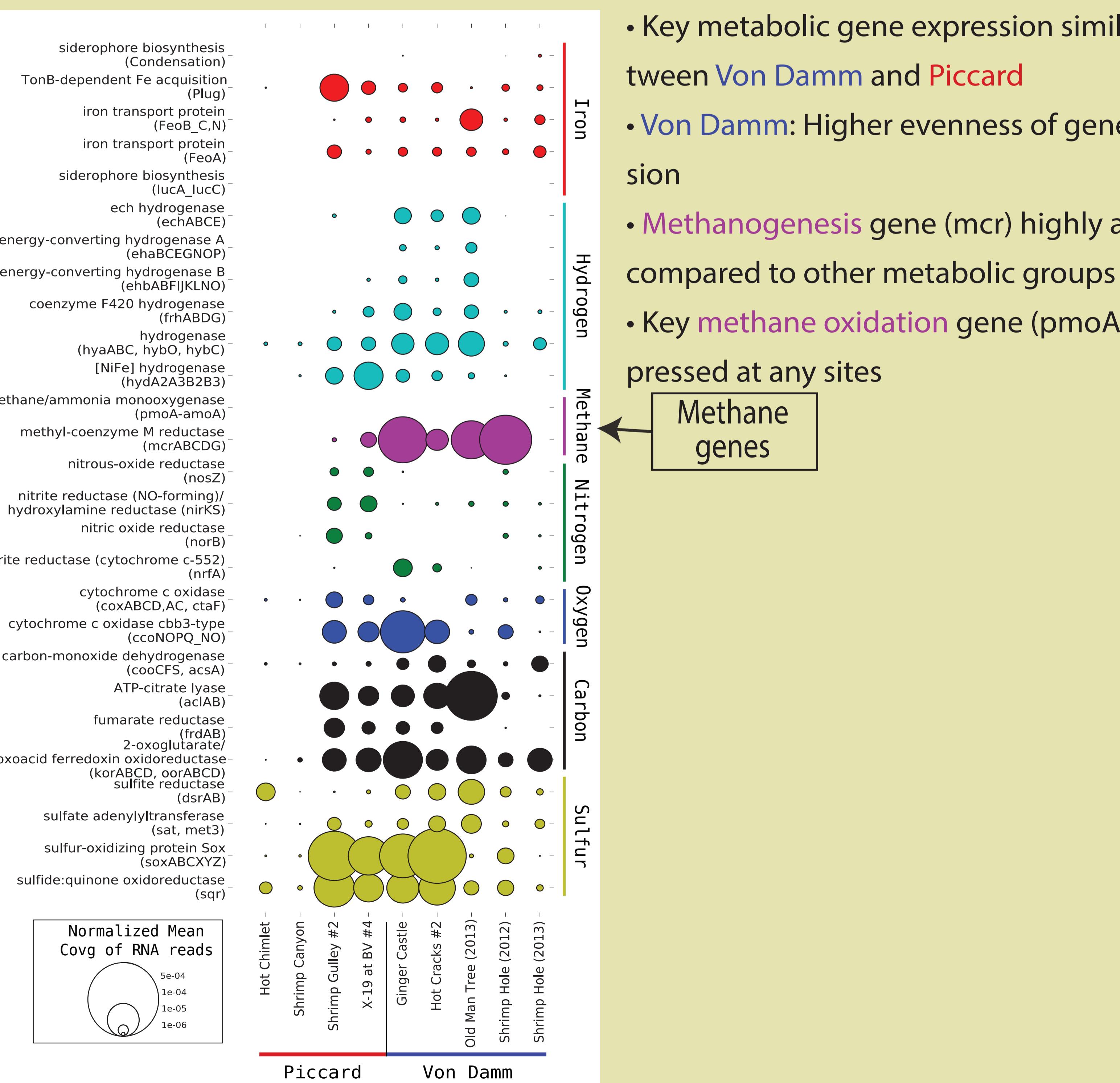
# Introduction

Deep-sea hydrothermal vent systems host a wide diversity of bacteria, archaea and viruses. Although the geochemical conditions at these vents are well-documented, the relative metabolic activity of microbial lineages, especially among archaea, remains poorly characterized. The deep, slow-spreading Mid-Cayman Rise, which hosts the mafic-influenced Piccard and ultra-mafic-influenced Von Damm vent fields, allows for the comparison of vent sites with different geochemical characteristics. Previous metagenomic work (Reveillaud et. al, 2016) indicates that despite the distinct geochemistry at Von Damm and Piccard, the overall functional profile of microbial communities between the two sites is similar.

## Methods

Sequencing of metagenomes and metatranscriptomes was conducted on an Illumina Hi Seq 1000. Reads were assembled with IDBA (Peng et. al, 2010). Mapping was done with Bowtie2 (Langmead and Salzberg, 2012). Genes were called using Prodigal (Hyatt et. al, 2010) and annotated using the IMG-JGI pipeline and the KO (Kanehisa and Goto, 2000) and Pfam (Finn et. al, 2016) databases . We used anvi'o v2.3.2 (Eren et. al, 2015) for supervised binning of the metagenome-assembled genomes (MAGs) based on coverage and tetranucleotide frequency. We also used anvi'o v2.3.2 for the pangenomic analysis. Figures were generated using matplotlib (Hunter, 2007) and seaborn.

# Community-Level Metabolism



# Community-Level Metabolism

- Key metabolic gene expression similar between Von Damm and Piccard
  - Von Damm: Higher evenness of gene expression
  - Methanogenesis gene (mcr) highly active compared to other metabolic groups
  - Key methane oxidation gene (pmoA) not expressed at any sites

# Pangenomics and Metabolic Potential

**Group I: Core methanogenesis. MAGs contain majority of genes for 1+ methanogenesis pathways**

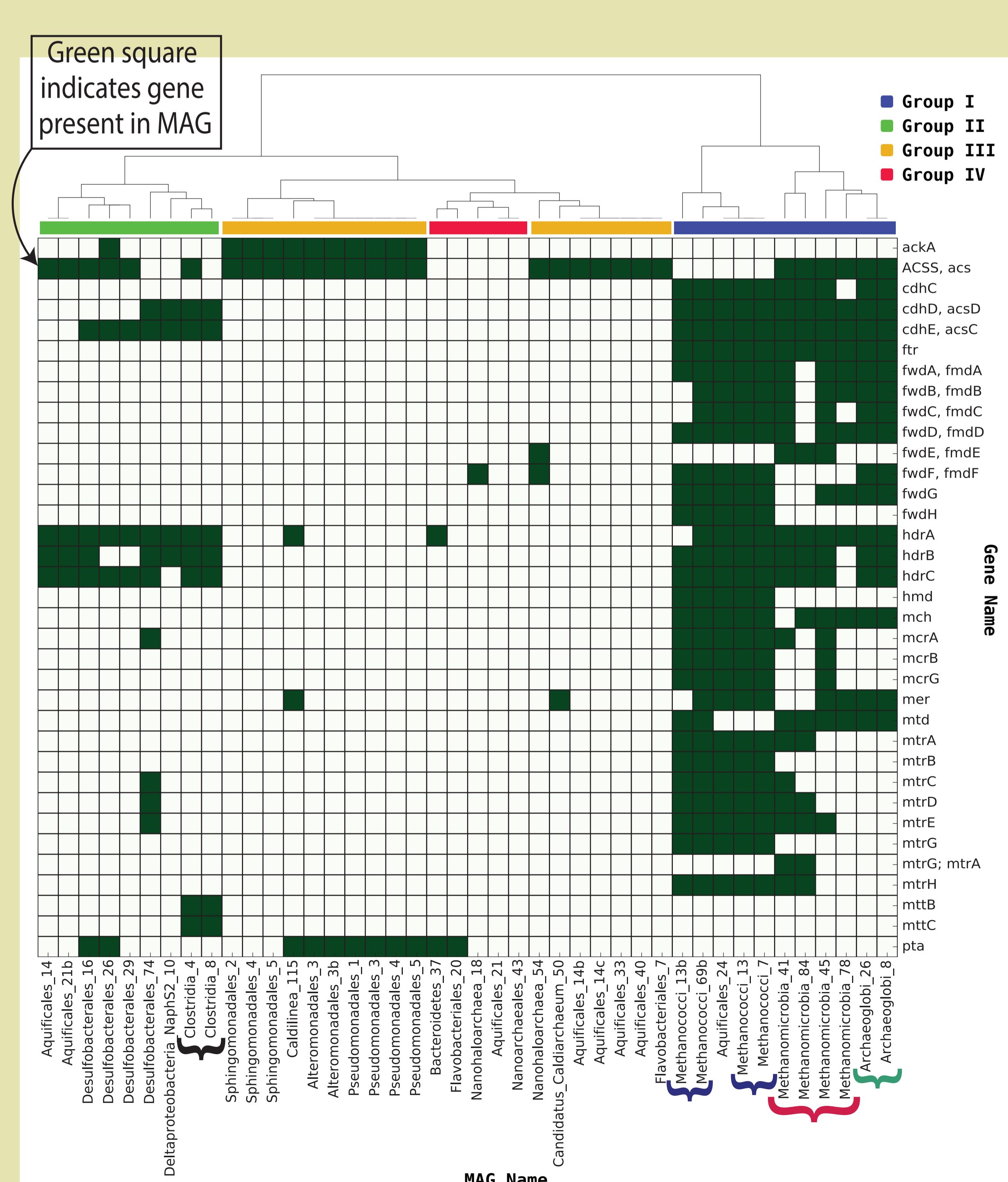
- Methanomicrobia: FULL acetate => methane , partial CO<sub>2</sub> => methane
  - Methanococci: partial CO<sub>2</sub> => methane , partial acetate=>methane
  - Archaeoglobi: partial CO<sub>2</sub> => methane , partial acetate=>methane

**Group II: Accessory methanogenesis. MAGs contain 1-3 genes for 1+ pathways**

- Clostridia (Bacteria): partial trimethylamine => methane (only MAC contributing to this pathway)

**Group III: MAGs contain genes for first steps of ace-**

- Group IV: MAGs do not contain genes sufficient for a step in any methanogenesis pathway**



# Conclusions

- Supervised binning yielded 73 total MAGs with completeness >70% and redundancy <10%
  - **56 MAGs (a):**
    - **Piccard** and **Von Damm** vent fields show clear differences in MAG expression
    - MAGs do not cluster based on taxonomy  - **Archaeal MAGs (b):**
    - Archaeoglobi 26 most highly and commonly expressed
    - Methanomicrobia: All MAGs only active at Shrimp Hole site
    - Methanococci: MAGs variously active at Ginger Castle, Shrimp Canyon, Hot Chimlet
    - No obvious correlations between vent geochemistry/vent field and community-level expression of metabolic genes in the Mid-Cayman Rise
    - At the community level, genes for methane oxidation and sulfur redox most highly expressed
    - Nautiliales and Sulfurovum are most active taxa overall
    - Archaeoglobi are most active archaea
    - Despite having similar methane metabolism profiles, Methanococci & Methanomicrobia show different expression levels across sample sites
    - Previously identified methanogens Methanococci and Methanomicrobia (Evans et. al, 2015) control last step of methanogenesis (mcr gene)
    - In terms of metabolic potential for methanogenesis, Archaeoglobi, Methanomicrobia and Methanococci are generalists rather than specialists
    - Output of Archaeoglobi methane metabolism likely feeds into CO<sub>2</sub> => methane pathway of Methanococci and Methanomicrobia
    - Methanococci and Methanomicrobia likely require Clos-tridia for trimethylamine=>methane metabolism
    - Bacteria of metabolic Groups II and III (see Fig. 3) may contribute to acetate=>methane metabolism in Archaeoglobi, Methanococci, and Methanomicrobia

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## Acknowledgements

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