# Methanogenic Archaea dominate mature heartwood habitats of Eastern Cottonwood (*Populus deltoides*)

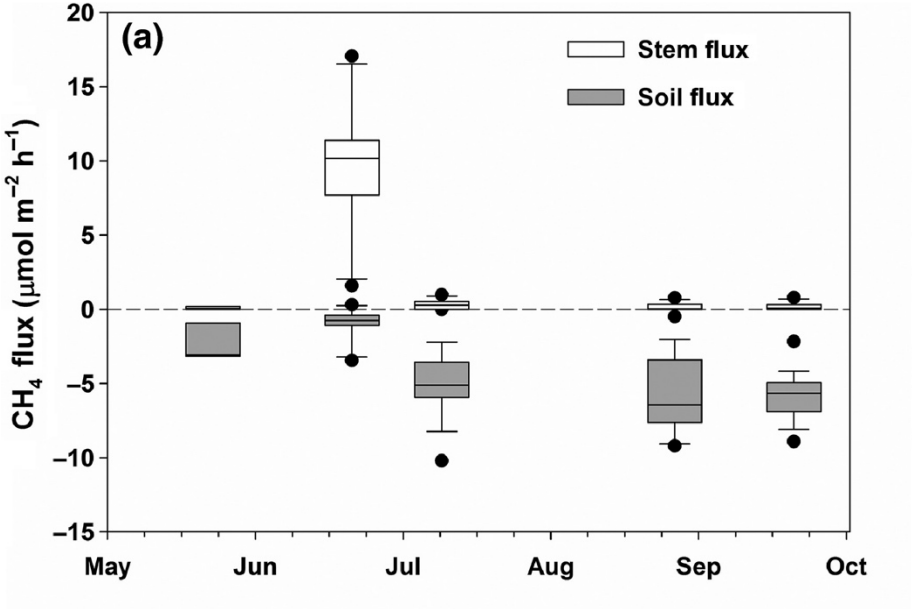
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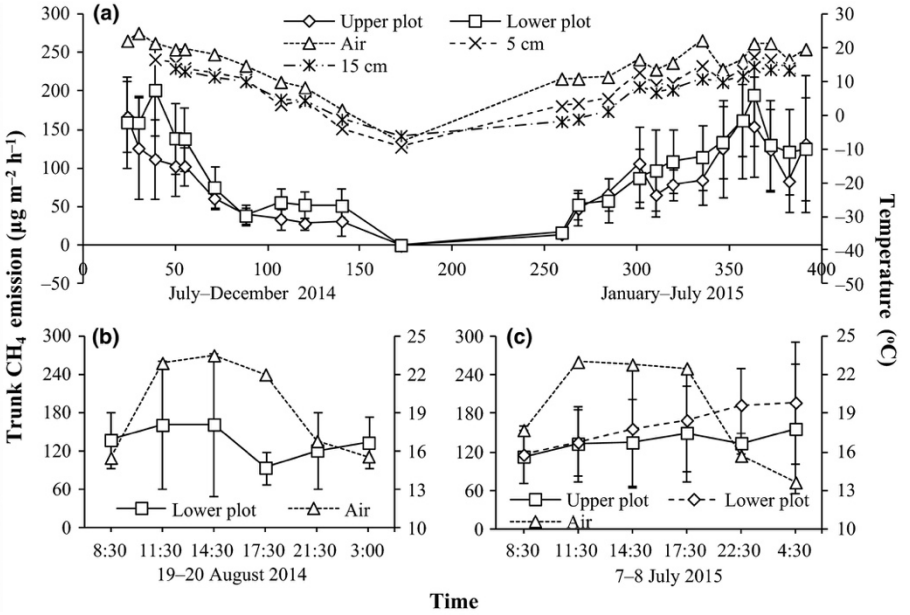
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# A “new” source for missing methane?

- Current global modeling approaches cant account for up to 20% of methane
- A flurry of recent studies are showing are showing that appreciable methane can be emitted from the trunks of living trees that is unaccounted by current models




Pitz & Megonigal, 2017, New Phytologist
(7 upland tree species in varying seasonal and diurnal patterns)




Wang et al., 2016, New Phytologist
(High flux rates in *Populus davidiana* during growing season)

# Historically microbiologists had also examined wood communities

- In 1970s and 80s there was also a flurry of studies from anaerobic microbiologists studying organisms from wood

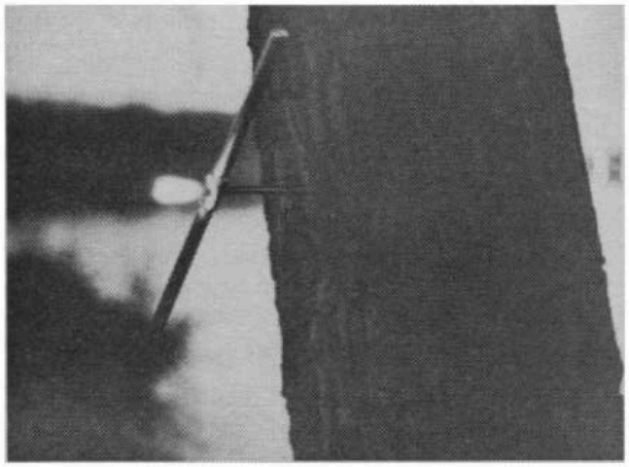
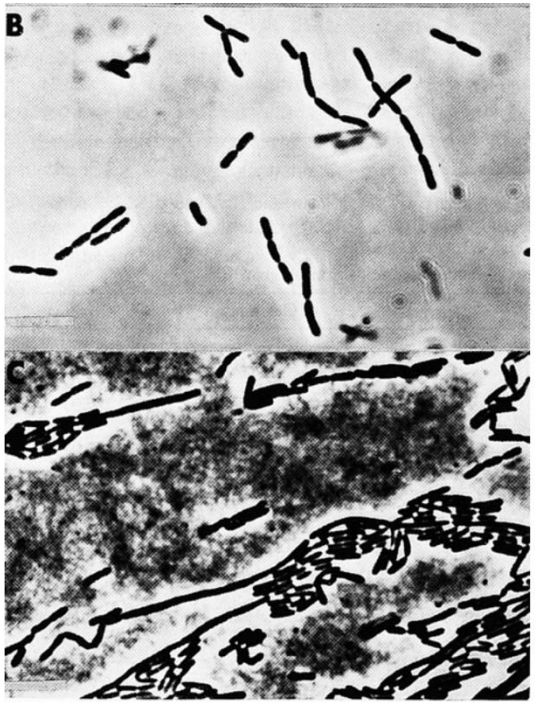


Fig. 1. Ignited methane gas emanating from a hollow increment borer bit drilled 20 cm into a cottonwood tree located on the shore of Lake Wingra, Wisconsin. Photograph is from a time exposure taken at night.

Zeikus & Ward, 1974, Science
(Methane producted from *Populus deltoides* in situ and in wood incubations)




Zeikus & Henning, 1975, Antonie van Leeuwenhoek
(isolated *Methanobrevibacter arbophilicus* sp. nov. from *Populus deltoides*)

TABLE 1. *Comparison of pectinolytic and heterotrophic microbial populations in representative wetwoods*

| Tree | Population | Total no.[a] Pectinolytic | Total no.[a] Heterotrophic |
|---|---|---|---|
| *P. deltoides* 19 | Anaerobes | $3.0 \times 10^6$ | $3 \times 10^6$ |
| *P. deltoides* 2 | Aerobes | $3 \times 10^4$ | $1.5 \times 10^6$ |
| | Anaerobes | $6 \times 10^4$ | $2 \times 10^6$ |
| *P. deltoides* 4 | Aerobes | $5.6 \times 10^5$ | $5.6 \times 10^6$ |
| | Anaerobes | $2 \times 10^5$ | $2 \times 10^6$ |
| *U. americana* 79 | Aerobes | $1.1 \times 10^5$ | $7 \times 10^5$ |
| | Anaerobes | $2.3 \times 10^5$ | $8 \times 10^7$ |

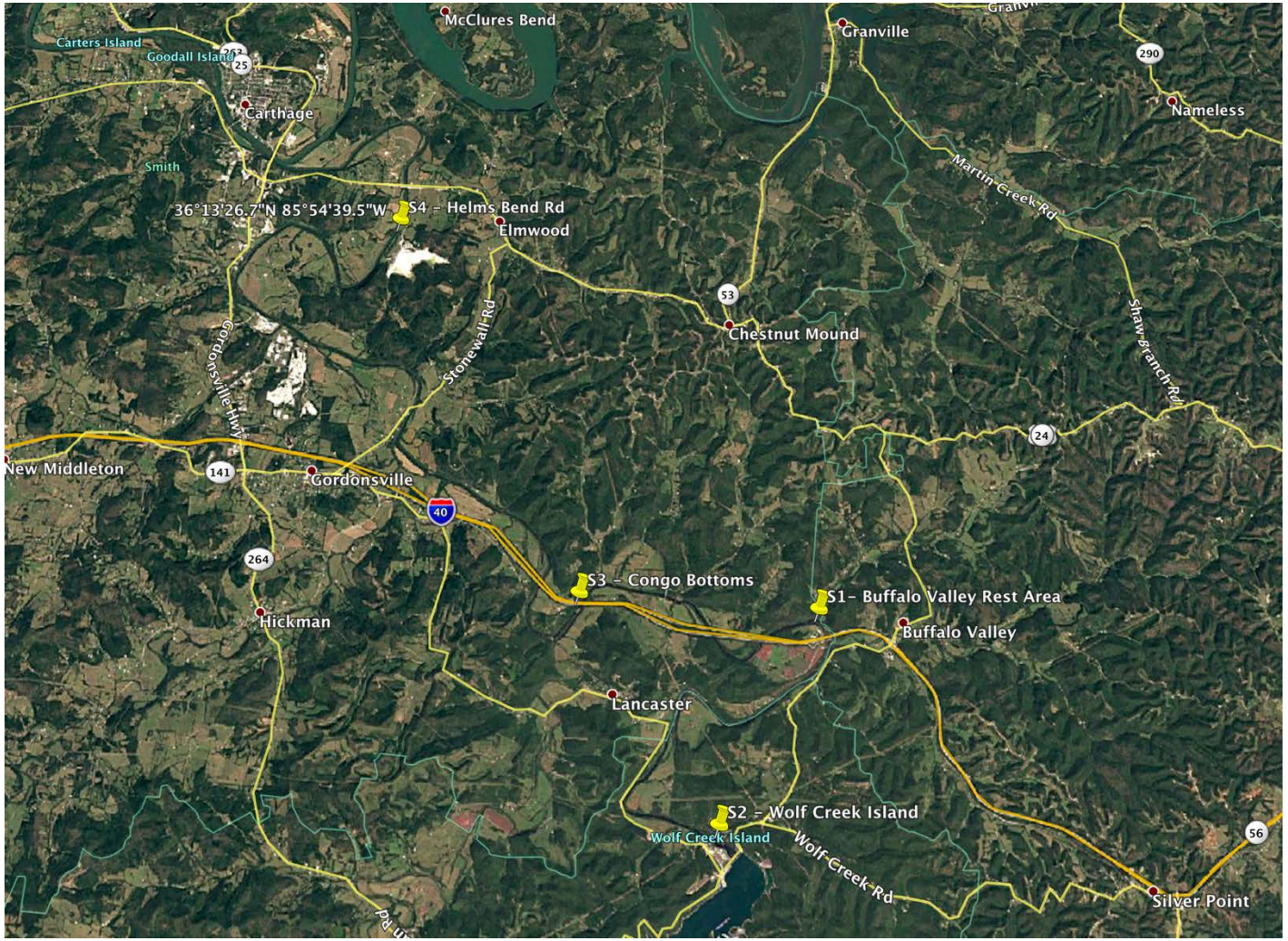
Schink et al., 1981, AEM
(Showed pectin degraded by wood *Clostridium* isolates and produced methane from in mixed cultures)

# What can rRNA gene-based molecular ecology tell us about these communities?

- Dan Yip (former Post-Masters Assoc.) and I decided to examine this in *Populus deltoides*
- Sampled wood cores from trees on Caney Fork River locations my lab had previously studied
- Hypothesized that wood microbiome would vary with:
  - Tissue type: Heartwood vs. sapwood
  - Across locations: Multiple trees from four separate locations
  - Tree characteristics: Tree diameter, wood pH, wood water content
- With a long term goal to better understand the fundamental mechanisms, as well as provide indicators and data that may be useful to improved model predictions

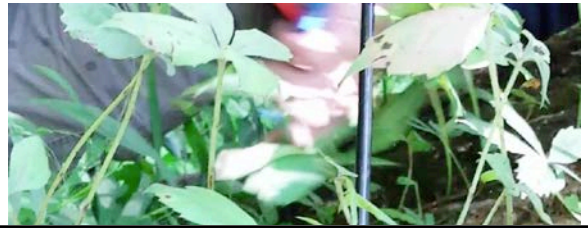
# Sampling Locations

- Four sites spanning ~22 river miles along Caney Fork River, TN
- Sites varied in tree size (DBH), canopy dominance & soil types



# Some samples of sampling observations

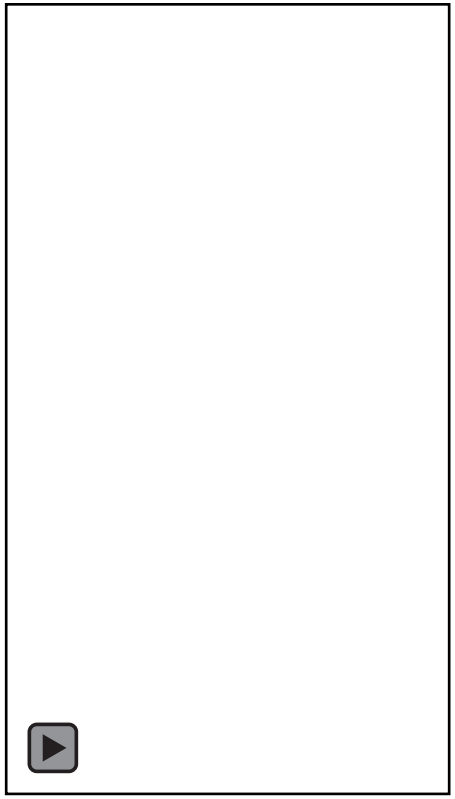
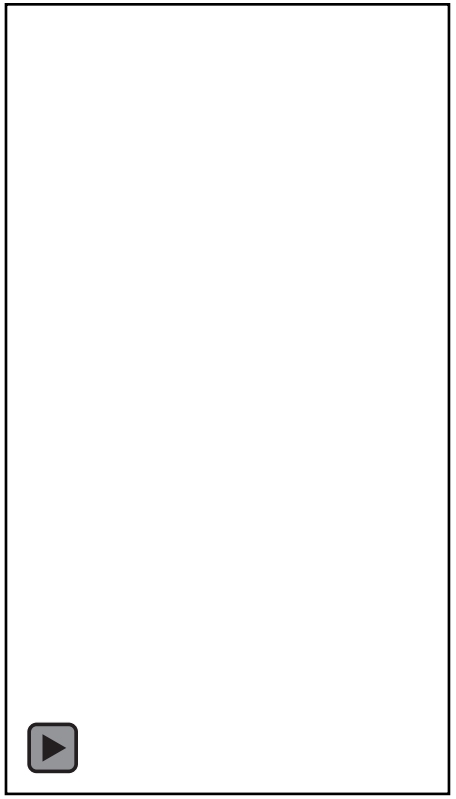
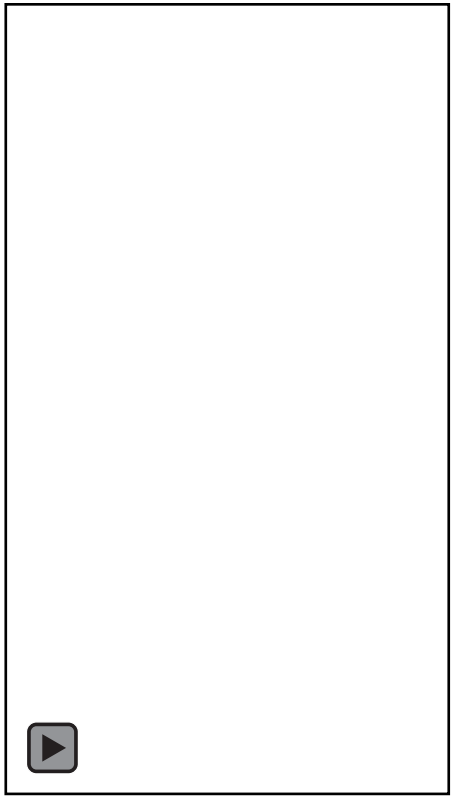
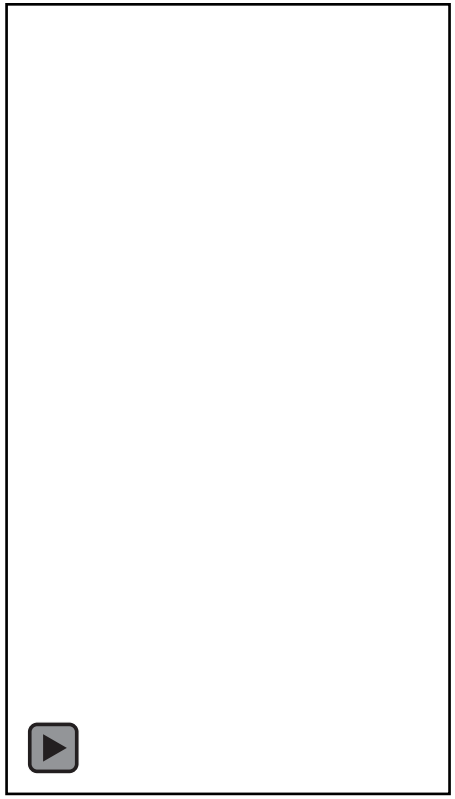
- Trees varied in response to coring: wet, bubbling, gassy, and…





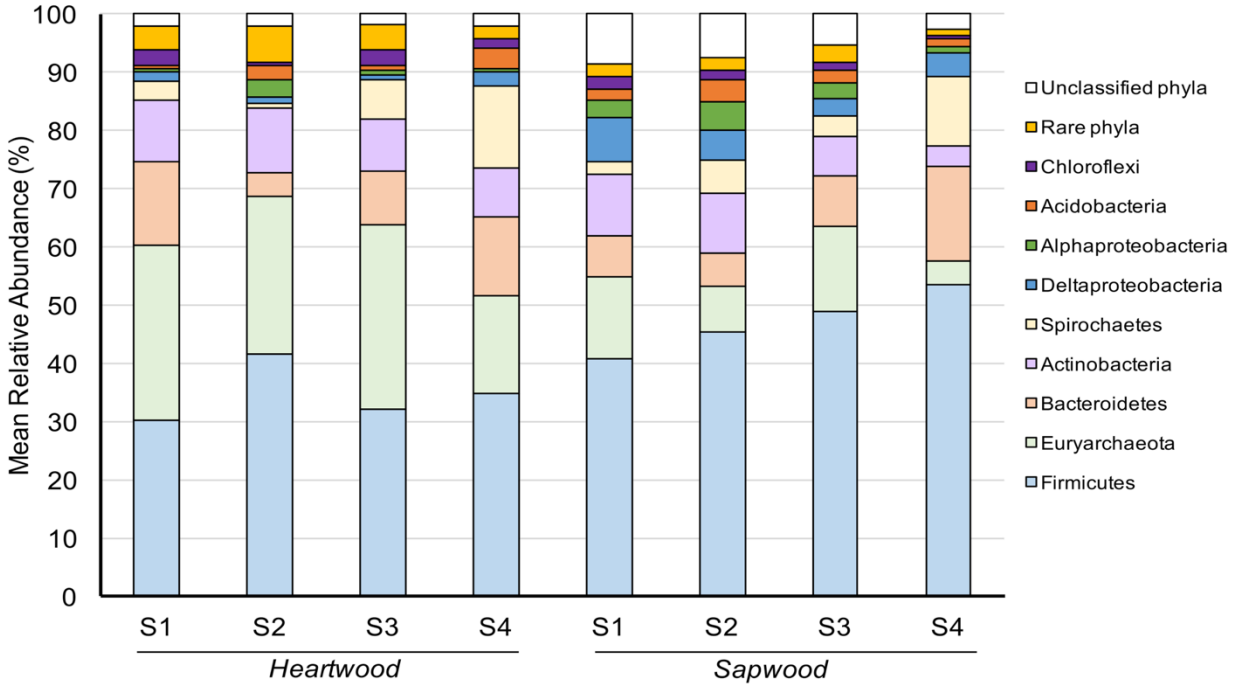
# Sampling observations (cont.)

- … FIRE!

# Methanogens dominate heartwood habitats

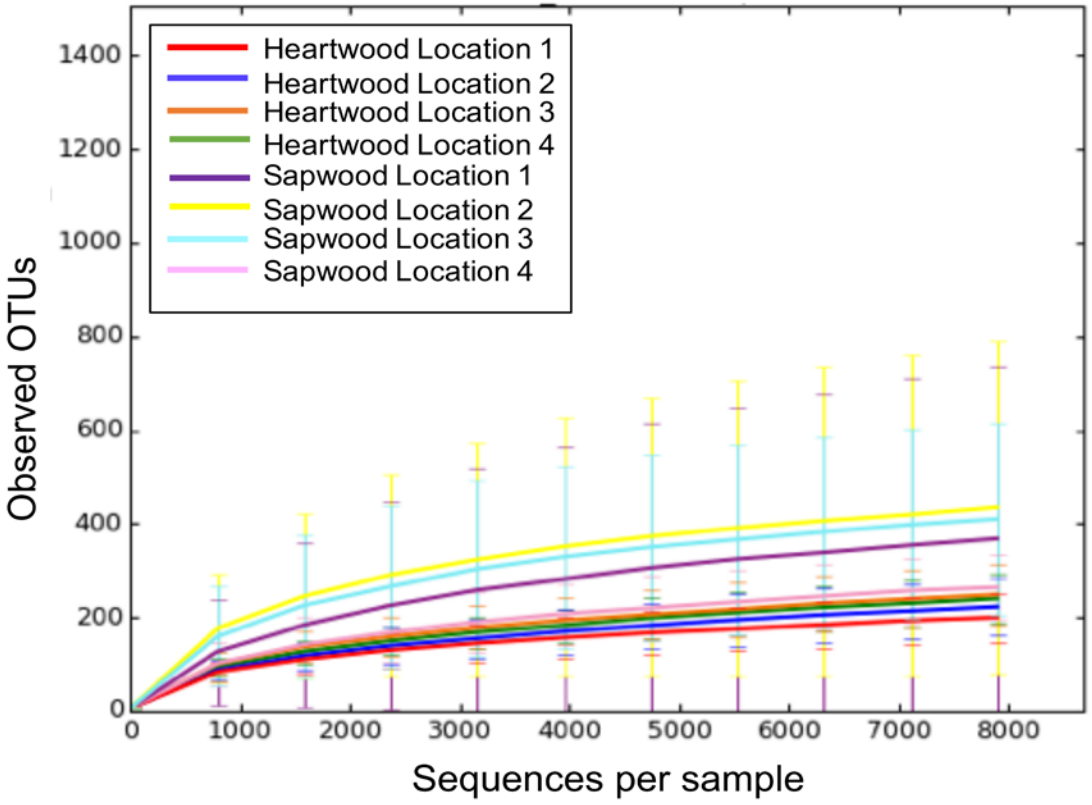
- Methanogens present in all samples, som > 50% rel. abundance
- Heartwood habitats contain more methanogens than sapwood
- Other prominent groups include Firmicutes, Acidobacteria Spirochaetes, Alpha-proteobacteria, Delta-proteobacteria, etc

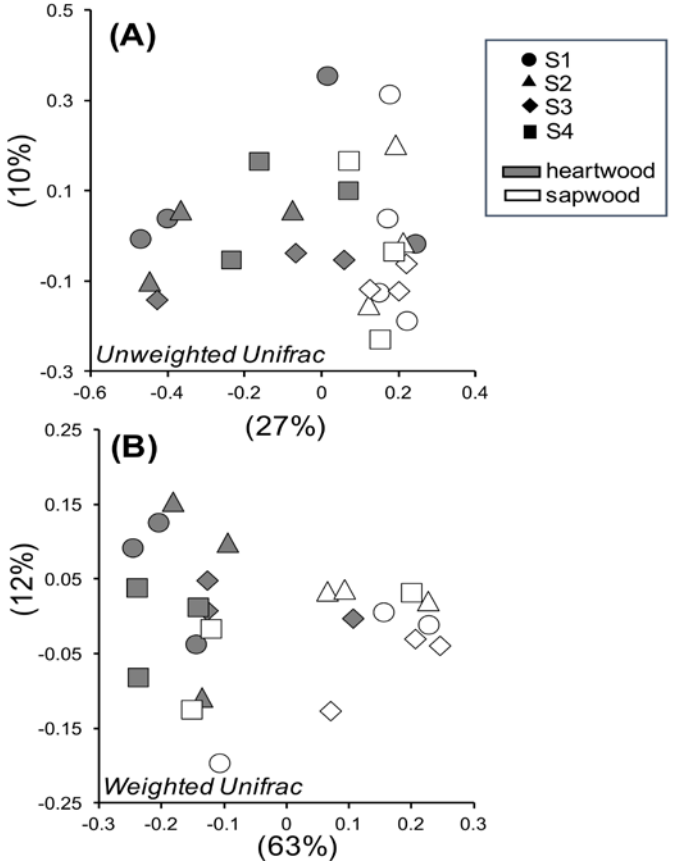



Average composition by site and tissue type

# Overall communities structured by habitat

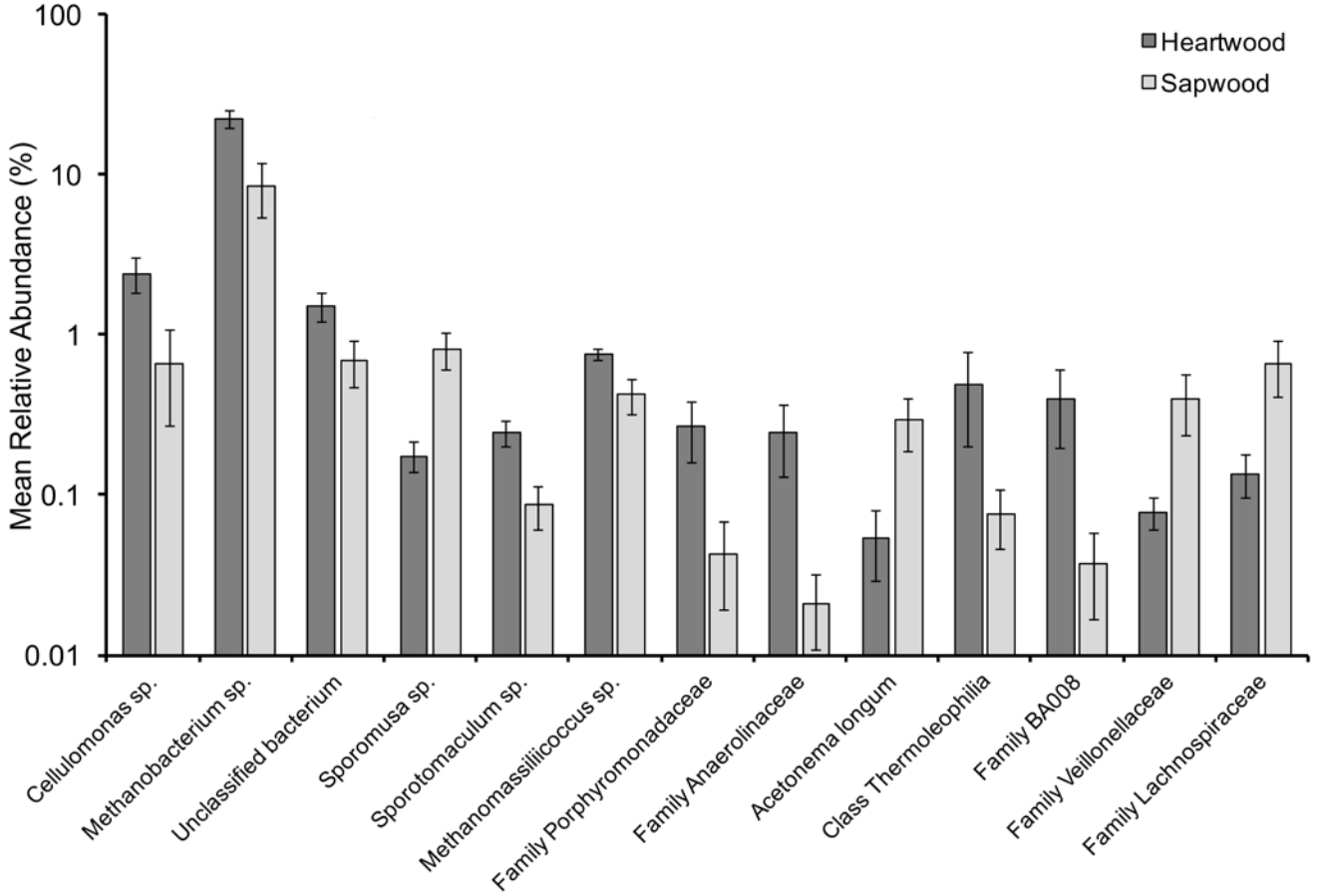
- Heartwood vs. sapwood habitat effects are much greater than those of sampling site origin, for both alpha-diversity (richness) and beta-diversity (composition) patterns



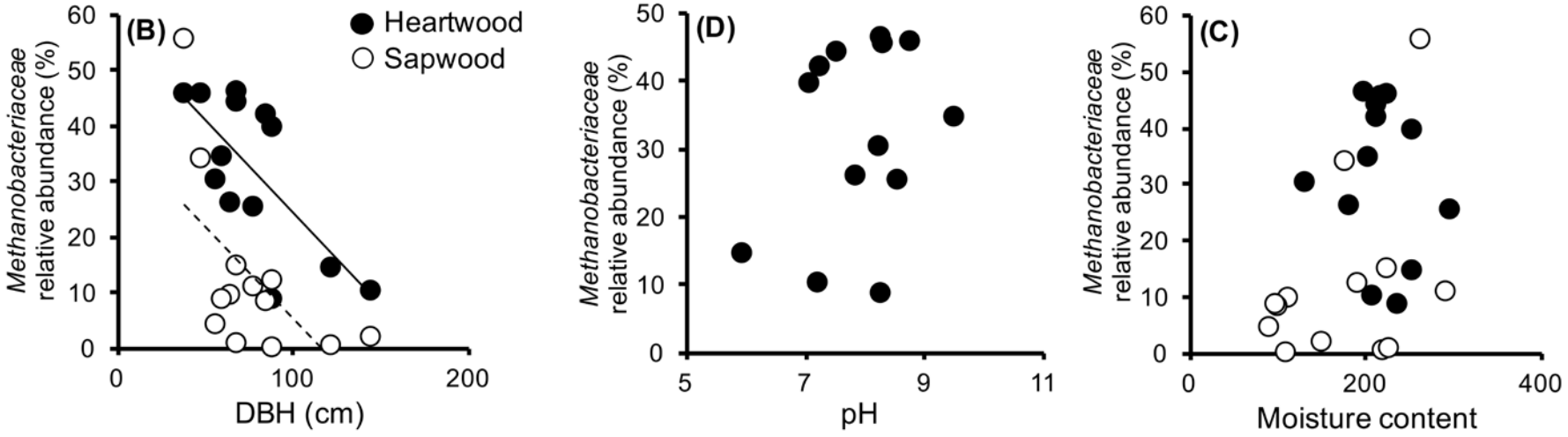
# Typically anaerobic OTUs are prominent and differ by habitat

- SIMPER analyses showing heartwood vs sapwood habitats differ in abundance of numerous prominent OTUs.
- Classification of some unresolved, but many of the groups are typified by anaerobic and fermentative metabolisms



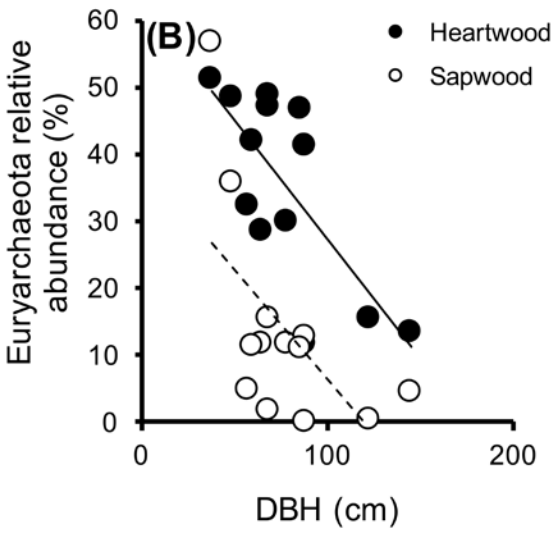
# Tree characteristics correlate with abundance of different groups

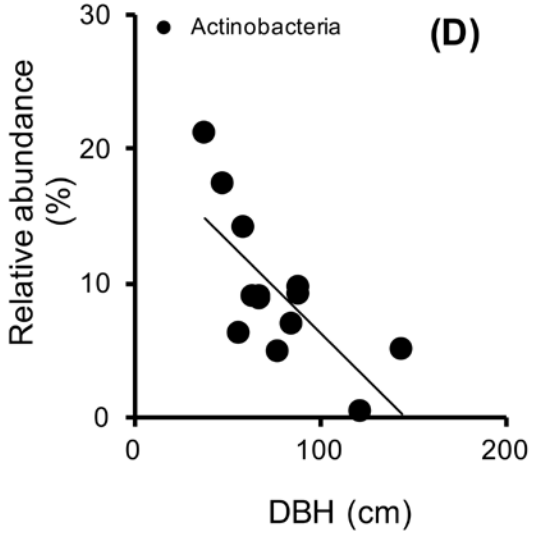
- Relative abundance of dominant methanogens (most *Methanobacterium* sp.) is negatively related to tree diameter (DBH), and not significantly related to pH and moisture content

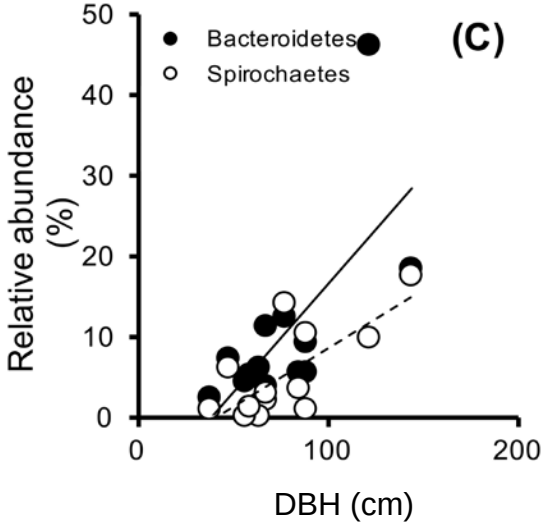


# Tree characteristics correlate with abundance of different groups

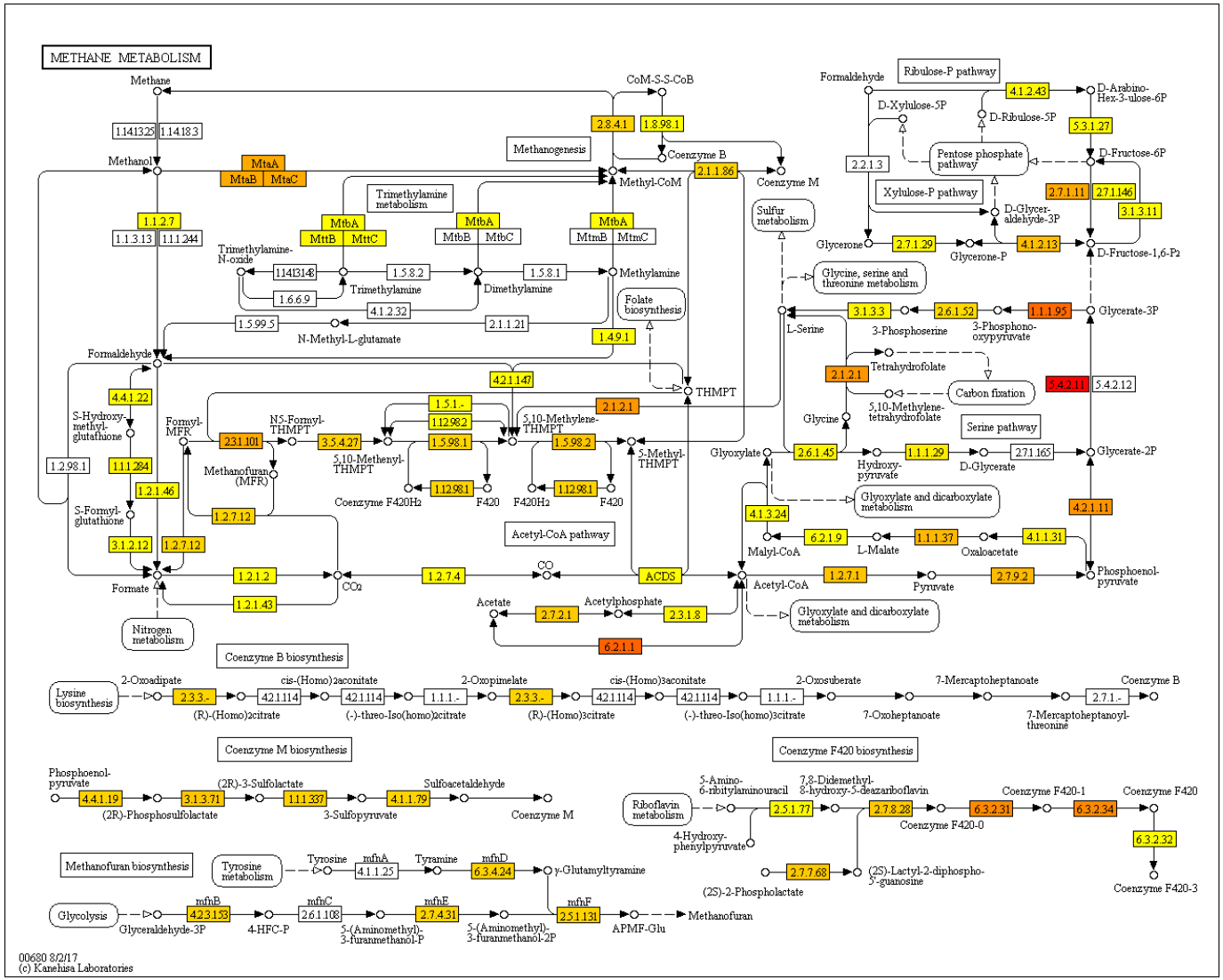
- DBH correlates with other prominent groups as well including: negatively with overall Euryacheayota & Actinobacteria; and positively with abundance of Bacteriodetes and Spirochaetes



# *Predicted* metagenomes (PICRUSt ) show evidence for acetoclastic and hydrogenotrophic pathways



# Conclusions: What can rRNA-based molecular ecology tell us about these communities?

- Heartwood habitats are dominated by methanogens; most prominently a *Methanobacterium* spp. (not *Methanobrevibacter* like Ziekus and Henning, 1975)
- The wood community differs by tissue type from heartwood to sapwood
- Several groups of organisms and dominant OTUs are correlated with tree diameter (DBH)
  - Tree diameter is also correlated with wood pH. DBH may be a proxy for age, carbon quality or other unknown factors that are driving these relationships

# Future Directions?

- Need studies to examine wider range of potential tree characteristics as predictors
- Need studies to examine direct links between community composition, their activity and methane flux rates (e.g. RNA based, diurnal to seasonal studies)
- Methods development needed to incorporate metagenomics approaches with the high host DNA background (we have some working for roots, but not wood)
- There is a potential for N fixation in these systems that is even less well studied!



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