Linking Ecosystem Function to Methanogen Community Structure in Peatland Soils

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Microbial ecology: a core question
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• Does microbial community structure explain ecosystem function beyond the effect of environmental factors?
Microbial ecology: a core question

• Does microbial community structure explain ecosystem function beyond the effect of environmental factors?

• Does spatial and temporal variation in microbial diversity and activity contribute to the control of key biogeochemical processes?
Methanogens: a small community, an essential function
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• A microbial group with a distinct, unique function and (relatively) low diversity
Methanogens: a small community, an essential function

- A microbial group with a distinct, unique function and (relatively) low diversity

- Two distinct biochemical pathways that are phylogenetically distributed within the methanogens, and can be quantified independently via radioisotope labeling
Polymers e.g., polysaccharides, proteins

Monomers e.g., monosaccharides, amino acids

Organic Acids and Alcohols

Acetate

Inorganic Alternative Electron Acceptors (Anaerobic Respiration)

Fermenters

Hydrogenotrophic Methanogens

Acetoclastic Methanogens

CO₂ + H₂

CH₄

Aerobic Heterotrophs

CO₂

Methanotrophs

CH₄

Aerobic

Anaerobic

Figure courtesy of Dr. Jason Keller, Chapman University
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Methanogenesis pathways are taxonomically distinct.
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\[
\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\]

\[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2
\]
Methanogenesis pathways are taxonomically distinct

$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$

$^{14}\text{CO}_2 + 4 \text{H}_2 \rightarrow ^{14}\text{CH}_4 + 2\text{H}_2\text{O}$

Addition of radioactive substrate allows direct quantification of each pathway!
Climate change context: northern peatlands
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- 33% of terrestrial soil carbon
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- Substantial methane flux
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• 33% of terrestrial soil carbon

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• A distinct hydrogeomorphological gradient that harbors a diversity of methanogen communities
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• 33% of terrestrial soil carbon

• Substantial methane flux

• A distinct hydrogeomorphic gradient that harbors a diversity of methanogen communities

• Vulnerable to large increases in mean annual temperature in the coming decades
Northern peatlands: a variety of communities and functional characteristics

pH

hydrology

methane pathway

ombrotrophic

minerotrophic
Northern peatlands: a variety of communities and functional characteristics

- low pH
- neutral pH
- ombrotrophi
- minerotrophi
- hydrology
- methane pathway
Northern peatlands: a variety of communities and functional characteristics

- low precipitation
- pH
- neutral groundwater
- hydrology
- methane pathway

ombrotrophic

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Northern peatlands: a variety of communities and functional characteristics

- Ombrotrophic
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- Low precipitation
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- Hydrology
- Methane pathway

- Hydrogenotrophic
- Acetoclastic
Study Sites
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University of Notre Dame Environmental Research Center (UNDERC)

Crystal Falls, MI (most ombrotrophic site)
Study Sites

ombrotrophic

• Bog
• Bog
• Poor Fen
• Intermediate Fen
• Cedar Swamp
• Rich Fen

minerotrophic
Study Sites

Sampling:

• 5 events (two in 2009, three in 2010)

• 5 replicate samples from each site

• Samples incubated at average in situ temperature with $^{14}$C-labeled bicarbonate tracer
Hydrogenotrophic Methanogenesis by Sampling Event

Methane Production (umol/d/g dry soil)

Acetoclastic Methanogenesis by Sampling Event

Methane Production (umol/d/g dry soil)
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• the relative proportions of the two pathways within sites did not change significantly over time, however.
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• the relative proportions of the two pathways within sites did not change significantly over time, however

• as expected, there was a significant shift in dominant pathway across the gradient, with acetoclastic methanogenesis becoming increasingly dominant in more minerotrophic sites
• In 2010, the same spatial and temporal pattern in total methanogenesis was observed.
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However, acetoclastic methanogenesis did not significantly vary with season in any site!
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the increase in total methane was instead driven by an explosive increase in hydrogenotrophic methanogenesis, which increased significantly in all sites in the summer and fall!
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• however, acetoclastic methanogenesis did not significantly vary with season in any site!

• the increase in total methane was instead driven by an explosive increase in hydrogenotrophic methanogenesis, which increased significantly in all sites in the summer and fall!

• what could account for this inter-annual variability in hydrogenotrophic methanogenesis?
Acetoclastic Methane Production vs Water Table Depth

Hydrogenotrophic Methane Production vs Water Table Depth
Water table depth appears to **strongly** effect hydrogenotrophic methanogenesis.
Acetoclastic methanogenesis varied primarily with gradient position, and did not vary significantly between years or (generally) throughout the growing season.
Pathways Summary

• Acetoclastic methanogenesis varied primarily with gradient position, and did not vary significantly between years or (generally) throughout the growing season

• Hydrogenotrophic methanogenesis, on the other hand, varied tremendously between 2009 and 2010, becoming dominant in nearly every site in the latter year, which was unusually wet
Pathways Summary

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• Hydrogenotrophic methanogenesis, on the other hand, varied tremendously between 2009 and 2010, becoming dominant in nearly every site in the latter year, which was unusually wet.

• Could the substantially different spatial and temporal dynamics of the two pathways be explained by the phylogenetically distinct methanogens carrying them out?
Community Structure Analysis: \textit{mcrA}, functional gene marker about town

- \textit{mcrA} is a gene that codes the alpha-subunit of methyl coenzyme-M reductase (MCR), which catalyzes the terminal step of methanogenesis in \textit{all methanogens}
- Only one copy per genome, simplifying quantification

\textit{Ermler et al. 1997}
Preliminary Community Data

• DNA was extracted from one core from each site taken in May 2010

• mcrA was amplified from each core using PCR, and the PCR amplicons were cloned and sequenced via Sanger sequencing

• the resulting sequence libraries were trimmed, aligned, and binned into operational taxonomic units (putative genera) using the MOTHUR microbial genomics software package
Collector's Curves, Genera

Number of OTUs

Number of sequences

B1
B2
PF
IF
CS
RF
Collector's Curves, Genera

Number of OTUs vs. Number of sequences

Bray-Curtis Cluster Analysis
Community Conclusions

- The structure of the methanogen community in each of the six study sites is significantly different.
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• This difference appears to be driven by the ombrotrophic-minerotrophic gradient
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• All communities dominated by putative hydrogenotrophs
Structure and Function

- The interannual variability in methanogenesis within the peatland sites appears to be driven by changes in hydrogenotrophic methanogenesis rates
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• Putative hydrogenotrophs are dominant in all May 2010 community samples
Structure and Function

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• Putative hydrogenotrophs are dominant in all May 2010 community samples

• Acetoclastic methanogenesis correlate very strongly to gradient position, while hydrogenotrophic methanogenesis does not
Hypotheses
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• H1: Acetoclastic methanogenesis in northern peatlands is primarily driven by hydrogeomorphic conditions, resulting in a strong spatial pattern of different rates, performed by a relatively small but robust community acetoclasts
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• H2: Hydrogenotrophic methanogenesis in northern peatlands is primarily driven by dynamic or opportunistic changes in hydrogenotroph community activity by season, resulting in a strong temporal pattern of rate variation
Future Directions
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• Thoroughly sample the *mcrA* DNA of each site during all seasons, to determine if the total communities remain stable throughout the growing season (H1) using high-throughput sequencing (454 pyrosequencing)
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• Use SEM and NMS to compare effects of community and hydrogeomorphic context on the two pathways (H1)
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• Thoroughly sample the *mcrA* DNA of each site during all seasons, to determine if the total communities remain stable throughout the growing season (H1) using high-throughput sequencing (454 pyrosequencing)

• Use SEM and NMS to compare effects of community and hydrogeomorphic context on the two pathways (H1)

• Thoroughly sample *mcrA* mRNA from each site and sampling event, to determine if the transcriptional activity of functional methanogen groups fluctuates in sync with the rates of their associated pathways (H2)
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