

## From genomes to methane emission: targeting critical knowledge gaps in wetland soils

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**Project Goals:** The overarching objective of this early career research project is to identify the biogeochemical and genomic determinants of methane production, as well as the scale and physical distribution over which they operate, along freshwater wetland gradients. Here we use field and laboratory data to provide highly resolved information on small scale spatial heterogeneity in forcing and identify the soil conditions impacting methane emission. Results from this project will be integrated into biogeochemical models to resolve methane flux predictions at the terrestrial-aquatic interface.

### Abstract

Despite their relatively small land coverage, wetlands represent the largest natural source of atmospheric methane. Wetland emission budgets for this potent greenhouse gas are highly variable, with over 25% uncertainty. Accurately predicting net methane fluxes from wetlands depends on multiple interrelated geochemical, ecological, and metabolic constraints that are poorly understood, oversimplified, or missing in global biogeochemical models. This project studies the distribution, diversity, and gene expression of methane cycling microbial communities along wetland spatiotemporal gradients. The current paradigm is that oxygen inhibits methanogenesis and that most methane is produced from acetate or hydrogen; assumptions incorporated into global biogeochemical models today. In contrast, our wetland observations challenge these widely held views on microbial methane metabolism in soils.

Over two seasons, we have shown clear geochemical and biological evidence for 10 times greater methane production in well-oxygenated bulk soils of a freshwater wetland (1). Recovery of the first near-complete genomes for a novel methanogen species, *Candidatus Methanotherix paradoxum*, using metagenomic and metatranscriptomic sequencing showed that acetoclastic methane production is dominant in oxygenated soils. Moreover, this microorganism was a dominant member of the soil community across this wetland over a three-year period, but was also active in other methane emitting ecosystems, suggesting a global significance. Importantly, in this wetland, we estimated that up to 80% of methane fluxes could be attributed to methanogenesis in oxygenated soils. Additionally, we showed that this surface soil methanogen activity is relatively stable by season, but methanotroph activity decreases in summer, thereby a reduction in consumption, rather than increased production, may be an important contributor to increased methane fluxes observed in summer (2).

In addition to the dominant acetoclastic methanogen from surface oxygenated soils, our previous descriptions of archaeal diversity (3) revealed methylotrophic *Methanomassiliicoccales* were prevalent across the wetland over multiple years, especially in deeper anoxic soils. Given that little is known about the contribution from methylotrophic methanogenesis in soils, the microbial taxa, substrates, and possible contributions to methane production are here characterized. In contrast to other reports from wetland soils, we demonstrated that trimethylamine amended soils produce 60-fold greater methane than soils with endogenous methanogen substrates alone. NMR of porewater fluids indicated methanogen substrate availability varied by depth, with acetate and formate in higher concentration in surface soils, and methylotrophic substrates like TMA and methanol enriched in deeper soils. Consistent with the substrate profile, metatranscripts from *Methanomassiliicoccus* are three times higher in the deep soils. Metatranscript data also demonstrated that methanol, rather than methylamine, is the preferred substrate for these methanogens. Together our field and laboratory findings highlight that methylotrophic methanogenesis may be an underrepresented contributor to overall methane emissions in this system.

As a part of this DOE career award, we have developed an extensive database of over 1,900 genomes. We use this database, along with highly resolved spatial (cm scale) and temporal (monthly) sampling, to map the distribution of chemical and biological determinants of methane across wetland gradients. Toward this goal, in 2018 we completed an extensive field campaign, collecting over 700 soil samples from 9 sites every month for 6 months. Soil samples were paired to *in situ* methane measurements collected at the same cm-scale resolution, with data linked to overall methane fluxes. 16S rRNA gene analyses, along with untargeted soil metabolomics, will track methane cycling taxa distribution and substrate profiles across these samples. A JGI community sequencing project is currently generating 80 metagenomes and over 230 metatranscriptomes. Collectively, these data provide an unprecedented, high resolution view of methane production and consumption activities, resolved spatially and temporally across a wetland site. This research can facilitate the transition of climate models from treating methane flux in wetlands as a “black box”, primarily relying on overall flux rates, to more process or spatially oriented models.

## References

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