Methanogenesis in Oxygenated Soils is a Substantial Fraction of Wetland Methane Emissions

Jordan Angle[1], Timothy Morin[2,3], Lindsey Solden[1], Adrienne Narrowe[4], Garrett Smith[1], Mikayla Borton[1,3], Camilo Rey-Sanchez[2,3], Rebecca Daly[1], David Hoyt[5], William Riley[6], Christopher Miller[4], Gil Bohrer[2,3], **Kelly Wrighton**[1,3*] (kwrighton@gmail.com)

[1] Department of Microbiology, The Ohio State University, Columbus, OH 43210 USA; [2] Department of Civil, Environmental and Geodetic Engineering, The Ohio State University, Columbus, OH 43210 USA; [3] Environmental Science Graduate Program, The Ohio State University, Columbus, OH 43210 USA; [4] Department of Integrative Biology, University of Colorado Denver, Denver, CO 80217; [5] Pacific Northwest National Laboratory, Richland, WA 99352 US; [6] Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Project Goals:

Despite their relatively small land coverage, wetlands represent the largest source of atmospheric CH_4 (20-40%). However, variations in these wetland emission budgets are high, with over 25% uncertainty. Accurately predicting net CH_4 fluxes from these systems depends on multiple interacting geochemical, ecological, and metabolic constraints that are poorly understood, oversimplified, or missing in global biogeochemical models[12-14]. ***The overarching objective of my early career proposal is to identify the biogeochemical and genomic determinants impacting methane production, and the scale and physical distribution over which they operate, along freshwater wetland gradients***. Using field investigations, methanogen types, activity, and responses to geochemical conditions will be determined along seasonal and spatial gradients (**Objective 1**). Using laboratory microcosms, the formation of anoxic microsites and their capacity to facilitate methanogenic activity in wetland soils will be simulated (**Objective 2**). Lastly, these field and laboratory data will be used for multi-scale, process-level evaluation of an ecosystem biogeochemical model (the upcoming CLM-coupled version of *ecosys*) that accommodates these newly identified processes and parameterizes representation of these processes along relevant environmental gradients (**Objective 3**).

Abstract text:

The current paradigm, widely incorporated in soil biogeochemical models, is that microbial methanogenesis can only occur in anoxic habitats. Recent reports present an alternative view that in some ecosystems methanogenesis also occurs in oxic soils, lakes, and marine systems, a concept known as the methane paradox. Here we use porewater and greenhouse-gas flux measurements to provide clear evidence for methane production in oxygenated soils from a freshwater wetland. A comparison of oxic to anoxic soils revealed up to ten times greater methane production and nine times more methanogenesis activity in oxygenated soils. Metagenomic and metatranscriptomic sequencing recovered the first near complete genomes for a novel methanogen species, and showed acetoclastic production from this organism was the dominant methanogenesis pathway in oxygenated soils. This organism, *Candidatus* Methanothrix paradoxon, is prevalent across methane emitting ecosystems, suggesting a global significance. Moreover, in this wetland, we estimated that up to 80% of methane fluxes could be attributed to methanogenesis in oxygenated soils. Together our findings challenge a widely-held

assumption about methanogenesis, with significant ramifications for global methane estimates and Earth system modeling.

Funding statement:

This material is based upon work supported by Early Career Award from the U.S. Department of Energy to K.C.W., Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018022. W.J.R. was supported by the Director, Office of Science, Office of Biological and Environmental Research of the US Department of Energy under Contract No. DE-AC02-05CH11231 as part of their Regional and Global Climate Modeling program. Graduate student support was provided by the National Sciences foundation via Graduate Research Fellowship (to G.J.S.) and a Doctoral Dissertation Improvement Grant (to T.H.M.), and by the Department of Energy Office of Science Graduate Research Program, Solicitation 2 (to T.H.M.). Porewater metabolites (NMR) were performed using EMSL, a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research supported by DOE contract No. DE-AC05-76RL01830. DNA and RNA sequencing was conducted by the US Department of Energy Joint Genome Institute, a DOE Office of Science User Facility that is supported by the Office of Science of the US Department of Energy under Contract No. DE-AC02-05CH11231. Flux observations were conducted with support from the US Department of Energy's Office of Science, Ameriflux Management project.