

## Methane consumption by bacteria living inside insect burrows

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### Background

Methane is a potent greenhouse gas and can be naturally produced by microbes. These microbes are known as methanogenic archaea, and they are found in many anoxic environments including wetlands (Liu & Whitman, 2008). Wetlands contribute approximately one third of all methane emissions to the atmosphere (Zhang et al., 2017). However, wetlands are incredibly diverse and the biological factors controlling methane emissions are not well understood. Beaver ponds are such a type of wetland ecosystem, and their number is rising in recent decades (Whitfield et al. 2015). The ponds are formed when beavers build dams along streams causing flooding that rapidly alters the landscape and ecology of the affected area (Naiman, 1988). A low water flow and deposition of sediment and organic matter make these wetlands an ideal habitat for methanogenic archaea.

A considerable amount of the methane that is produced in ponds and lakes is in turn consumed by another group of microbes that are called aerobic methanotrophs (Reis et al., 2022). Aerobic methanotrophs reduce methane emissions by using methane as their sole carbon and energy source (Hanson & Hanson, 1996). These methanotrophs are bacteria that oxidize methane using oxygen and thus often inhabit oxic-anoxic interfaces such as the surface of sediments. The surface area of lake sediments may be increased through the activity of bioturbating animals, including the larvae of Chironomid flies (Grey, 2016). Chironomids are found worldwide in freshwater habitats (Halpern & Senderovich, 2015). The larvae introduce oxygen through burrows in otherwise anoxic sediments. The walls of the burrows may represent an oxic-anoxic interface that provides methanotrophs with simultaneous access to both methane and oxygen.

A preliminary study conducted in northeastern Massachusetts found a beaver pond to exhibit uncharacteristically low levels of dissolved methane year-round compared to inflow streams (Figure 1). In addition, <sup>13</sup>C-carbon stable isotope data strongly suggests that the chironomid larvae dwelling in the pond sediment have a diet that is almost exclusively based on methanotrophic microorganisms (Figure 2). Biogenic methane is notably depleted in the <sup>13</sup>C carbon isotope, and thus stable isotope analyses can be used to trace methane across trophic levels. Taken these findings together, we hypothesize that the bioirrigation by Chironomid larvae introduces oxygen into the pond sediment and substantially increases microbial methane oxidation. The

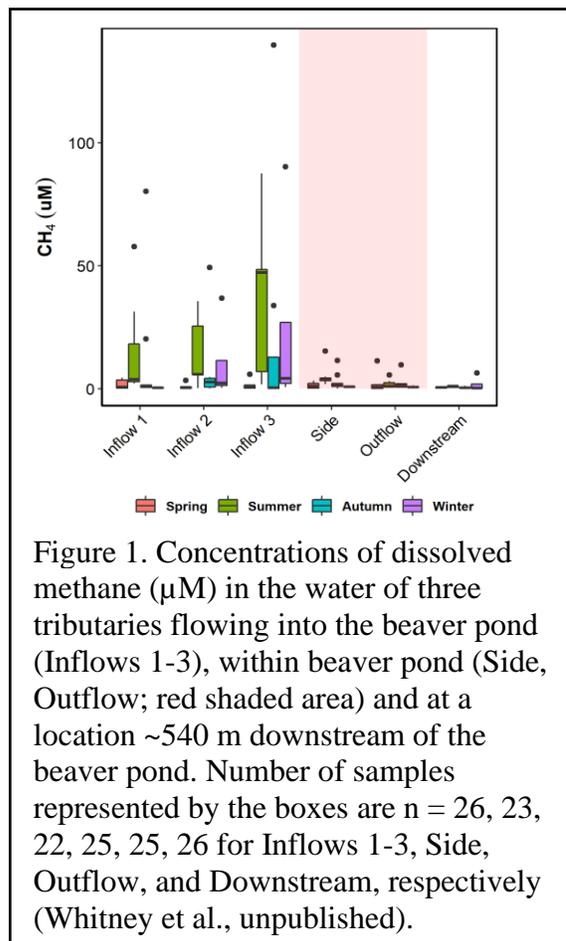
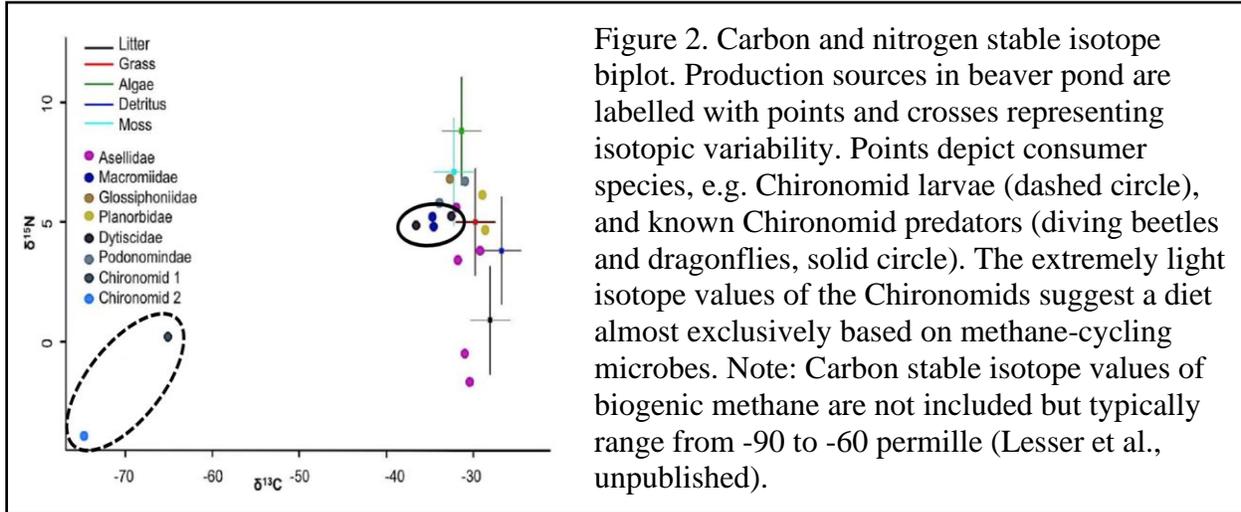


Figure 1. Concentrations of dissolved methane ( $\mu\text{M}$ ) in the water of three tributaries flowing into the beaver pond (Inflows 1-3), within beaver pond (Side, Outflow; red shaded area) and at a location ~540 m downstream of the beaver pond. Number of samples represented by the boxes are  $n = 26, 23, 22, 25, 25, 26$  for Inflows 1-3, Side, Outflow, and Downstream, respectively (Whitney et al., unpublished).

microbes that can live inside the burrows and burrow walls are “harvested” by the larvae and used as a food source. This removes methane from the system and suggests that methanogenesis-derived carbon may be an important contributor to the entire food web of the pond. To test this hypothesis PI Ruff received funding by the FICUS program partnering with the US Department of Energy to carry out laboratory experiments and analysis using  $^{13}\text{C}$ -labelled methane. The planned experiments are described below and will help us to better understand the role of biogenic methane as an energy source for insects and lake ecosystems.



### Statement of work

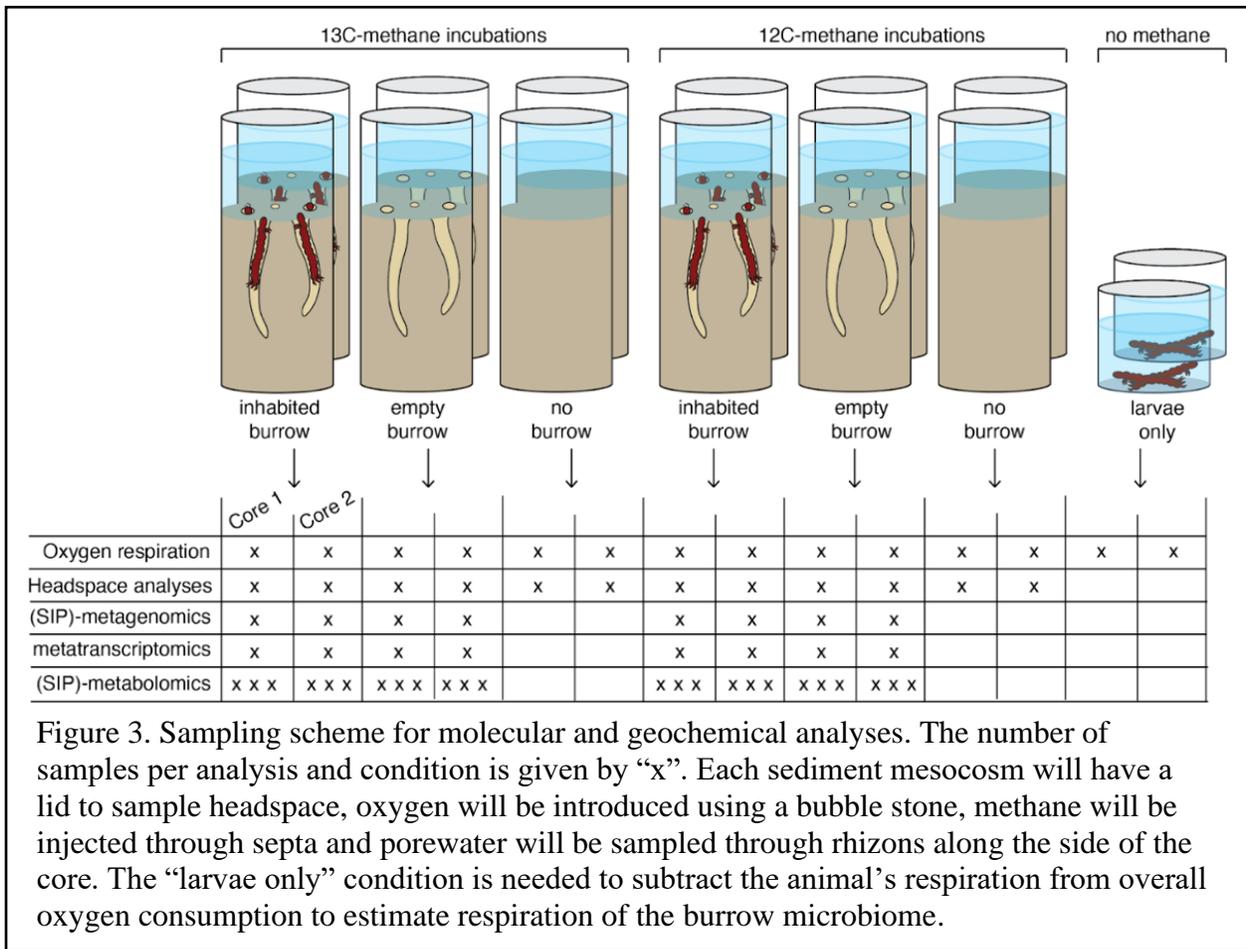
We hypothesize that the *burrowing and irrigation by Chironomid larvae increases methane oxidation in the sediment*. We will study the redox conditions and the spatial arrangement of microbes in the burrow and seek to determine whether bioirrigation increases methane oxidation in the burrow. Because it is difficult to study larvae in the environment under controlled conditions, we decided to use model ecosystems that can be monitored and fine-tuned. For this we will carefully retrieve sediment from the pond and set up laboratory mesocosms representing selected bioirrigation scenarios (Figure 3). The lab of PI Ruff has extensive experience with mesocosms, batch and continuous culture bioreactors and the methods and analyses that are needed to characterize the microbial communities (Bhatnagar et al., 2020; Kuloyo et al., 2020; Pérez Castro et al., 2021; Saad et al., 2017).

We will measure bulk oxygen and methane consumption in mesocosms i) with burrows and larvae, ii) with burrows without larvae, and iii) without burrows and without larvae, as well as iv) in controls, i.e., chambers that just contain live larvae and no burrows (Figure 3). Studying and comparing these four conditions will enable us to determine the effect of bioirrigation on methane removal and sediment respiration. To understand the methane oxidation, and elucidate potentially different oxidation pathways, in the full oxic condition (burrow + larva) versus the oxygen limited condition (burrow – larva) we plan to amend selected sediment cores with  $^{13}\text{C}$ -labelled  $\text{CH}_4$  (Figure 2).

We will detect  $^{13}\text{C}$ -enriched metabolites via Isotope-Ratio Mass Spectrometry and stable isotope probing (SIP) metabolomics (funded by FICUS grant to PI Ruff). We will identify the  $^{13}\text{C}$ -enriched microbial populations via SIP metagenomics and determine their gene expression via metatranscriptomics (FICUS funded). In addition, we will try to visualize the 3D-structure and organic matter content of the burrows using high-resolution micro-X-ray computed

tomography (FICUS funded). To unambiguously test whether active  $^{13}\text{C}$ -metabolizing methanotrophic microbes are grazed and consumed by the chironomid larvae we will visualize and quantify the  $^{13}\text{C}$ -enriched methanotrophs in samples of the burrow and the larva gut using a nanoscale Secondary Ion Mass Spectrometer (nanoSIMS, FICUS funded). PI James Nelson is an expert in stable isotope ecology and will lead this analysis (Nelson et al., 2019; James et al., 2019).

Together, these experiments will reveal how much oxygen and methane is consumed by the aerobic methanotrophs with and without bioirrigating activity, and how the methanotrophic activity and metabolic pathways differ under different oxygen conditions, as well as corroborate whether the methanotrophs are consumed by the larvae. All planned analyses are enabled by the FICUS grant awarded to PI Ruff, which is specifically designed to pay for analyses and instrument time, yet does not cover salaries, housing, and other research expenses. With this proposal I am asking for funds to cover the costs for my housing expenses, allowing me to conduct the described mesocosm experiments and sample collection in summer 2022. The sample analyses will be performed by collaborators at the Joint Genome Institute (JGI) and Environmental Molecular Science Laboratory (EMSL), also funded by the FICUS grant to PI Ruff. Subsequent data analyses will be performed by me and a team of collaborators. The described mesocosm experiments are one component of a larger effort to understand key hydro-biogeochemical processes that influence the cycling of carbon in beaver pond wetlands.



## **Completed Work**

Instrumental analysis for this project is funded through the 2022 Facilities Integrating Collaborations for User Science (FICUS) Grant sponsored by the U.S. Department of Energy and DOE Office of Science user facilities including the Joint Genome Institute (JGI) and Environmental Molecular Science Laboratory (EMSL). The samples I will be using will be collected from a coastal watershed in Northern Massachusetts which has been monitored since 2001 by the NSF Plum Island Estuary Long-Term Ecological Research Site (PIE-LTER). Some examples of work with great significance in this project include the analysis methane flux and stream characteristics at the headwaters of the beaver-engineered pond (Robison et al., 2021). Dissolved methane concentrations as well as preliminary stable isotope analysis from the pond are provided here (Figure 1, Figure 2). Additionally, multiple ponds have been assessed for benthic invertebrate diversity and biomass by Julia McMahon. Research conducted by students Anna Warsaw and Leah Pendl-Robinson in the Ruff lab in fall 2021 have helped to design the mesocosms and provided insights into methane-cycling microbes in the pond sediment.

## **How the Study Benefits Coastal Wetlands**

Beaver ponds have increased throughout North America in recent decades following re-introduction programs (Whitfield et al., 2015). My research will improve the interpretation of future field studies that estimate methane emissions from beaver ponds and provide new knowledge of how insect bioirrigation impact methane oxidation and emission. The sediment bioirrigation and consumption of methanotrophs by Chironomid larvae may represent an overlooked part of the methane cycle, and a methane sink of global relevance. The expected results could greatly enhance our understanding of methane emissions to the atmosphere and allow for more informed wetland management and conservation practices.

## **Budget**

The sample analyses for the project are fully funded through PI Ruff. Additionally, funds for travel have been allocated by PI Nelson. My only remaining expenses are for housing and meal costs. My goal is to live near the Marine Biological Laboratory for 90 days during the summer of 2022 to work in the lab of PI Ruff. Housing is quite expensive in this area during the summer with dorms being the most affordable option. I will use this grant to offset the costs of using my personal funds for a shared dorm room (\$4,500) and meals (\$1,350).

## **Plans for Sharing Research Results with a Larger Audience**

The results and findings of this research will be shared with the scientific community via peer-reviewed publications as well as during national and international conferences such as the Annual Meeting of the Ecological Society of America and the International Symposium on Microbial Ecology for which PI Ruff is a conference chair 2022 and Early Career Committee member since 2021. The findings will also be shared with the public during outreach events including the Woods Hole Science Stroll, webinars, and social media. PIs Nelson and Ruff have an excellent track record both for publishing in top-tier journals in their fields as well as for disseminating their research to a wider public.

## **References**

Bhatnagar, S., Cowley, E.S., Kopf, S.H., Pérez Castro, S., Kearney, S., Dawson, S.C., Hanselmann, K., & Ruff, E.S. (2020). Microbial community dynamics and coexistence in a sulfide-driven phototrophic bloom. *Environmental Microbiome*, 15, Article 3.  
<https://doi.org/10.1186/s40793-019-0348-0>

- Grey, J. (2016). The Incredible Lightness of Being Methane-Fuelled: Stable Isotopes Reveal Alternative Energy Pathways in Aquatic Ecosystems and Beyond. *Frontiers in Ecology and Evolution*, 4, Article 8. <https://doi.org/10.3389/fevo.2016.00008>
- Halpern, M., & Senderovich, Y. (2014). Chironomid Microbiome. *Microbial Ecology*, 70, 1-8. <https://doi.org/10.1007/s00248-014-0536-9>
- Hanson, R.S., Hanson T.E. (1996). Methanotrophic Bacteria. *American Society for Microbiology*. 60(2) 439-471. <https://doi.org/10.1128/mr.60.2.439-471.1996>
- Kuloyo, O., Ruff, S.E., Cahill, A., Connors, L., Zorz, J.K., Hrabe de Angelis, I., Nightingale, M., Mayer, B., & Strous, M. (2020). Methane oxidation and methylotroph population dynamics in groundwater mesocosms. *Environ Microbiology*, 22(4), 1222-1237. <https://doi.org/10.1111/1462-2920.14929>
- James, W.R., Lesser, J.S., Litvin, S.Y., & Nelson, J.A. (2019). Assessment of foodweb recovery following restoration using resource niche metrics. *Science of The Total Environment*, 771, Article 134801. <https://doi.org/10.1016/j.scitotenv.2019.134801>
- Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, 1125, 171-189. <https://doi.org/10.1196/annals.1419.019>
- Naiman, R. J., Johnston C.A., & Kelley J.C. (1988). Alteration of North American Streams by Beaver: The structure and dynamics of streams are changing as beaver recolonize their historic habitat. *BioScience*, 38(11), 753-762. <https://doi.org/10.2307/1310784>
- Nelson, J.A., Johnson, D.S., Deegan, L.A., Spivak, A.C., & Sommer, N.R. (2019). Feedbacks between nutrient enrichment and geomorphology alter bottom-up control on food webs. *Ecosystems*, 22(2), 229-242. <https://doi.org/10.1007/s10021-018-0265-x>
- Pérez Castro, S., Borton, M.A., Regan, K., Hrabe de Angelis, I., Wrighton, K.C., Teske, A.P., Strous, M., & Ruff, E.S. (2021) Degradation of biological macromolecules supports uncultured microbial populations in Huaymas Basin hydrothermal sediments. *The ISME Journal*, 15, 3480-3497. <https://doi.org/10.1038/s41396-021-01026-5>
- Reis, P.C., Thottathill, S.D., & Prairie, Y.T. (2022). The role of methanotrophy in the microbial carbon metabolism of temperate lakes. *Nature Communications*, 13, Article 43. <https://doi.org/10.1038/s41467-021-27718-2>
- Robison, A. L., Wollheim, W. M., Turek, B., Bova, C., Snay, C., & Varner, R.K. (2021). Spatial and temporal heterogeneity of methane ebullition in lowland headwater streams and the impact on sampling design. *Limnology and Oceanography*, 66(12), 4063-4076. <https://doi.org/10.1002/lno.11943>
- Saad, S., Bhatnagar, S., Tegetmeyer, H.E., Geelhoed, J.S., Strous, M., & Ruff, S.E. (2017). Transient exposure to oxygen or nitrate reveals ecophysiology of fermentative and sulfate-reducing benthic microbial populations. *Environmental Microbiology*, 19(12), 4866-4881. <https://doi.org/10.1111/1462-2920.13895>
- Whitfield, C.J., Baulch, H.M., Chun, K.P., & Westbrook, C.J. (2015). Beaver-mediated methane emission: The effects of population growth in Eurasia and the Americas. *AMBIO*, 44, 7-15. <https://doi.org/10.1007/s13280-014-0575-y>
- Zhang, Z., Zimmermann, N. E., Stenke, A., Li, X., Hodson, E. L., Zhu, G., Huang, C., & Poulter, B. (2017). Emerging role of wetland methane emissions in driving 21st century climate change. *Proceedings of the National Academy of Sciences*, 114(36), 9647-9652. <https://doi.org/10.1073/pnas.1618765114>