Designing intact soil core incubation experiments for evaluating the correlation between anoxic microsite abundance and methane emissions

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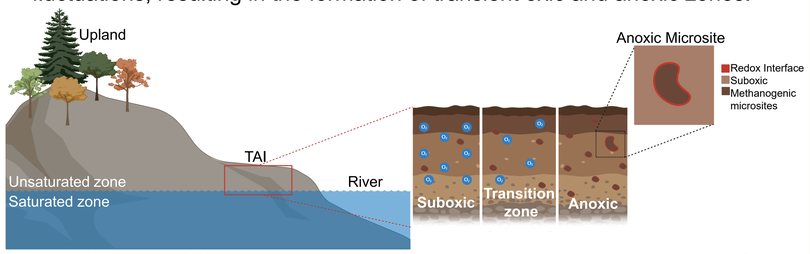
Introduction

- Terrestrial-aquatic interfaces (TAIs), such as riverine floodplains, are dynamic transitions between land and water that experience frequent redox fluctuations, resulting in the formation of transient oxic and anoxic zones.



Conceptual diagram of TAI showing the transition from unsaturated zone to saturated zone. Oxygen availability changes with depth, moisture levels and soil properties, limited by slow diffusion under saturated conditions. Locally, methanogens, a single-celled microorganism belonging to the domain Archaea that is active in low-oxygen environments, may produce methane.

- Anoxic microsites are localized oxygen-depleted (i.e., anoxic) inclusions within an otherwise bulk oxic soil environment, formed by rapid microbial respiration and limited oxygen (O_2) diffusion.
- Sequential chemical reactions culminate in methanogenesis, contributing to methane (CH_4) emissions in riparian floodplain environment.
- Methane generation is dependent on bulk soil properties such as moisture, redox, texture, soil organic matter, and the presence of terminal electron acceptors, such as iron and sulfate.

While field measurements indicate moisture as a driving factor for methane generation, confounding factors like variability in moisture levels, soil carbon content, and the presence of methane-consuming microbial communities prohibit the quantification of methane fluxes from anoxic microsites.

Research Objectives and Hypotheses

Specific Aims

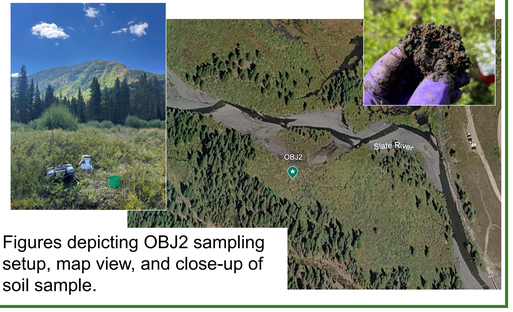
- **Measure CH_4 flux** from triplicate intact soil cores under controlled anoxic incubation conditions, with constant soil moisture and the same bulk soil properties. Controlled anoxic conditions limit the activity of methanotrophs, who are predominantly aerobic.
- **Compare CH_4 flux** from same location, under high moisture conditions.
- **Correlate the relationship between CH_4 flux and anoxic microsite abundance**.
- **Evaluate** the impact of sub-dm soil property variations on microsite formation and on CH_4 flux.

Hypothesis

- The abundance and characteristics of anoxic microsites affect CH_4 emissions, even under consistently anoxic conditions and same moisture.

Research Site: Slate River Floodplain, CO

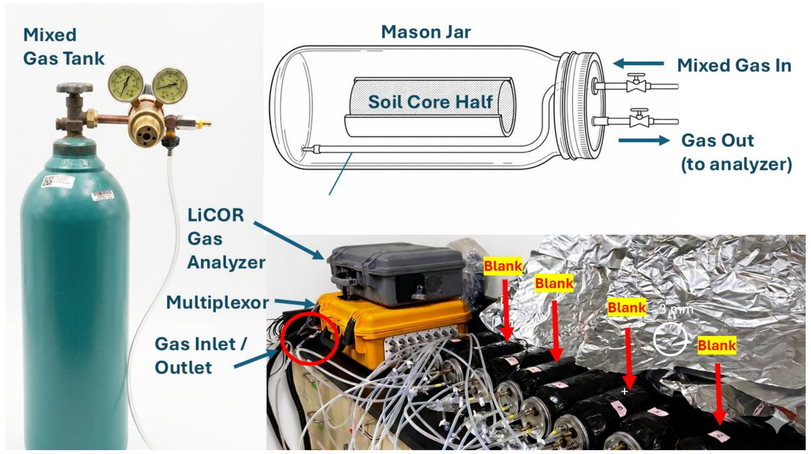
Our study site is located within the **Slate River floodplain** in the Upper Colorado River Basin. **OBJ2**, the focus of this study, is a sampling location in the transition zone adjacent to the river bank, experiencing dynamic seasonal changes.
June: **waterlogged soils**
September: **dry soils**



Figures depicting OBJ2 sampling setup, map view, and close-up of soil sample.

Experimental Setup & Incubation Design

- The LI-COR gas analyzer combined with the LI-8250 Multiplexer was used to measure CH_4 fluxes from soil cores incubated in sealed Mason Jars.
- The LI-COR setup was modified to enable continuous flow: compressed N_2 replaces the standard purge pump, flowing at ~0.5 L/min through each jar.
- Continuous N_2 flow flushes produced gases; CH_4 does not accumulate and measurements are representative of fluxes from the soil.
- Sealed-jar incubations underestimate flux by about 33% (Pihlatie et al. 2013).



A total of 4 intact cores collected from the same site at Slate River OBJ2 were incubated.

Within site variability

- Triplicate cores (Core 1-3) collected from the site at the same time
- All three cores had statistically similar bulk gravimetric moisture (~30%).

Between season variability

- One core from the same site, but collected in September
- Core 4 had almost double bulk moisture (56%) than Cores 1-3.

Paired analysis of microsite abundance v. methane flux

- Cores were split in two-halves; one for incubation, and one for imaging.
- Imaging with multiple energy u-X-Ray fluorescence mapping allows quantification of microscale redox heterogeneities like anoxic microsites.
- Bulk soil data before and after incubation were collected.

Main findings & Conclusions

Sample	Avg CH_4 flux (nmol m^{-2} s^{-1}) Field Data	Avg CO_2 flux (µmol m^{-2} s^{-1}) Field Data	CH_4 flux (nmol kg^{-1} s^{-1}) Incubation	CO_2 Flux (µmol kg^{-1} s^{-1}) Incubation
Core 1	-0.14027	0.91415	0.0288	0.1056
Core 2	-0.35611	1.29953	0.0474	0.2237
Core 3	-0.37144	1.37217	0.0360	0.2161
Core 4	0.03755	0.656	0.0474	0.2310

Field to incubation comparison

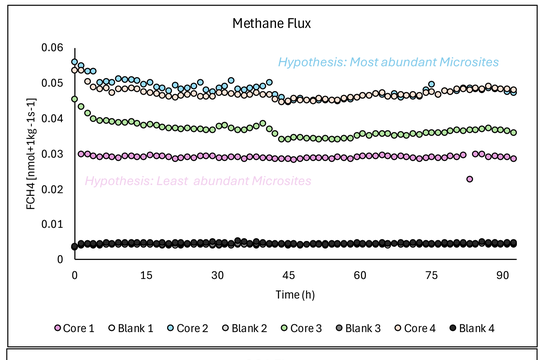
- Methane flux was positive in all core incubations, even if the same site was a methane sink (CH_4 consumed > CH_4 produced) in-situ.
- Controlled anoxic conditions increased net methane fluxes.

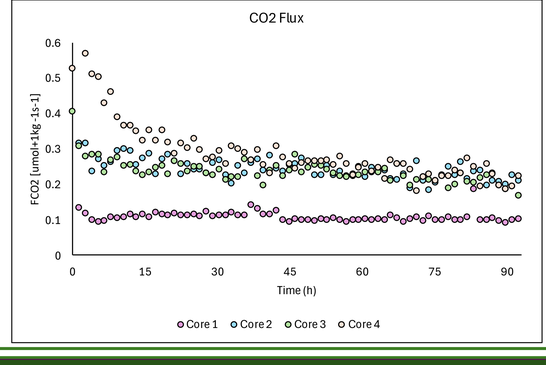
Within site variability

- The triplicate cores (Core 1-3) showed variable levels of methane generation when incubated, despite having the same moisture before and during the experiment.

Between season variability

- Core 4 showed a less pronounced increase in methane flux between the in-situ and the incubation measurement.
- Because of the increased moisture of OBJ2 in June, the soil was already oxygen-limited in the field, promoting methanogenesis and limiting methanotrophy, and leading to a positive CH_4 flux.
- The complete oxygen limitation during incubation, increased CH_4 flux by 20%, indicating redox sensitivity even under saturated conditions.





Conclusions:

- We confirmed that bulk anoxia creates conditions favorable to methanogenesis.
- To explain the variation in methane production potential observed in the field, a mechanistic understanding of carbon dynamics and redox heterogeneity is necessary.

Next steps:

- Upcoming imaging and bulk soil analysis will be used to test the hypothesis that differences between the three cores are due to the abundance and characteristics of anoxic microsites
- Measurements to be collected:
  - Imaging of reduced iron (FeS) of paired half cores
  - Bulk iron concentrations, soil texture, soil organic carbon

Acknowledgments

Slate River sampling team for collecting cores used in the study - Vincent Noël, Elizabeth Paulus, Kenneth Celis, Kathryn Farber, Amrita Bhattacharyya.
SSRL/SLAC LOAMS program and beamline staff. Special thanks to Vincent Noël and Caileb Travier for help handling the cores and using the lab space.
LI-COR Environmental. (2025). Using the LI-8250 multiplexer (Publication No. 19441). LI-COR, Inc.
Funding: DOE

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U.S. DEPARTMENT OF ENERGY | Office of Science