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MICROBIAL PLANET

EST. 2019

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Inside the Paper: What About the Microbes?

Or, how a seminar I barely understood turned into the first new research project from the Chase Lab at SMU.

By Alexander B. Chase, Ph.D.[1]
[1]Department of Earth Sciences, Southern Methodist University, Dallas, TX

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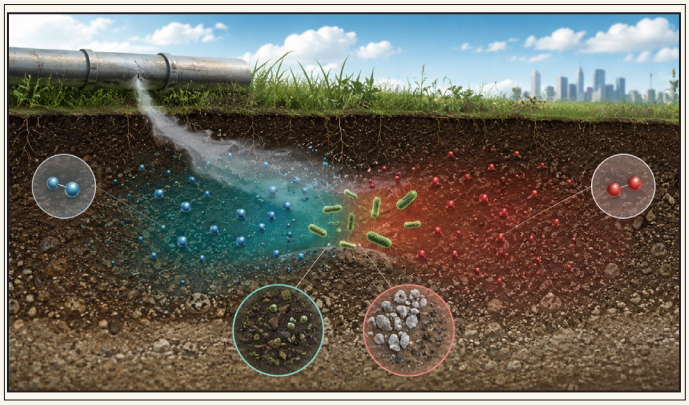

Methane diffusion creates a CH_4–O_2 boundary coupling microbial oxidation with soil carbon transformation.

This paper[1] started, appropriately enough, with me knowing almost nothing about the thing we were studying.

Not long after I arrived at SMU, Kathleen "Kate" Smits (another relatively new faculty member at the university and now the Solomon Professor for Global Development in Civil and Environmental Engineering) gave a seminar in our Earth Sciences department about subsurface natural gas leaks. Kate studies how gas moves through soils after leaking from underground infrastructure: where it goes, how quickly it moves, and why some leaks are surprisingly difficult to detect at the surface.

This was a world I had never really thought about.

I knew methane as a greenhouse gas. I knew methane could also be a microbial substrate. But I knew of methane and its relationship to microbes from very specific places, like wetlands, marine sediments, landfills, and other places where microbial ecologists tend to encounter **lots** of methane.

What I had not thought much about was that methane is a resource we use every day, from natural gas extracted from subsurface fossil-fuel reservoirs. To transport these gases, we have built extensive networks of pipelines beneath our feet. But these pipes aren't perfect and are prone to leaks, and the question I never really thought much about was: what happens when relatively small amounts of natural gas quietly escapes from them into otherwise ordinary, oxygenated soils.

So, while I sat in the audience listening to Kate explain this climate hazard, and how she and her team investigate the gas transport, soil diffusivity, leak detection, and subsurface methane plumes, admittedly things way beyond my understanding! So many equations, there was a reason I became a biologist, not a physicist!

But then she said something I found very interesting. That some of the leaking methane is accounted for in their models as being consumed by the soil microbes. At first, I wasn't shocked by this, microbes have been on the planet for billions of years, and in that time, have evolved ways to innovate metabolic pathways and consume/transform nearly every chemical on Earth. But the more I thought about it, the more interesting and confused I became. These leaky soils aren't these weird high-methane places where we know of methanotrophs, they are regular soils, soils that became contaminated with this invasive hydrocarbon. After her seminar, I approached Kate and asked one question:

Tell me more about these microbes.

> **"Research Area 1: Methane Sinks and Sources: Research on atmospheric and ecosystem methane sinks is needed to better understand the potential to enhance natural sinks for atmospheric methane removal; determine removal scales required for climate impacts; and enable monitoring, reporting, and verification of atmospheric methane removal technologies."**
>
> *— from the National Academy of Sciences report on "A Research Agenda Toward Atmospheric Methane Removal"*

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The following summer, we went to a controlled field testbed in Fort Collins, CO where Kate and her team could do something that is nearly impossible with an accidental leak: control the gas release, measure how methane moved through the soil, and densely sample the physical structure of the resulting subsurface plume. Instead of discovering a leak ***after*** the fact and trying to reconstruct what happened, we could watch one develop.

And because I had successfully inserted myself into the project, we of course collected soil samples.

Then came the fun part (at least for me).

From my limited knowledge of methanotrophs at the time, I knew many can be incredibly difficult to culture and grow. Papers suggested they took months (!!!) to replicate, needed highly specialized media, and complex lab apparatus for gas chambers to supply with methane gas. My lab was still being built at SMU and we did not have the infrastructure in place to try really any of this. Although, I did have an army of absolutely amazing and eager undergraduates try to isolate them using the first product of methane oxidation, methanol. We hypothesized that if they could grow on methanol, they might also be able to convert methane to this cellular intermediate. But we knew this was a long shot.

Instead, we generated metagenomes, a culture-independent approach where we do not need to actually grow the microbes in the lab. We just extract all the environmental DNA from the soil samples and look at the immense genetic diversity of the microbiome. Obviously, I started searching for methane monooxygenases (MMOs), the enzymes that initiate aerobic methane oxidation by converting methane to methanol. My expectation was fairly straightforward: find the methane-oxidizing bacteria, see whether they were enriched near the leak, and determine whether microbial methane consumption could explain some of the attenuation we were seeing.

Except the sequences I found were not really what I expected…

We found monooxygenase genes. Plenty of interesting ones, actually. But many were associated with organisms that did not resemble the methanotrophs I knew. They kept mapping toward Actinomycetota and other lineages that, at the time, made me suspicious that I was simply looking at enzymes with other hydrocarbon substrates. Basically, a related enzyme that is very similar to MMOs but targeted other related hydrocarbons, like propane or ethane.

My initial interpretation was simple: Well, maybe the microbes aren't doing anything ***that*** interesting. So I put the project aside.

This is one of those parts of science that rarely makes it into the final paper. Sometimes the data immediately produce a beautiful story: your hypotheses align with the experimental setup, the results support those hypotheses, and you write the paper. But most of the time, I find that you stare at a folder on your computer for several months and hope that either you or biology eventually becomes smarter.

In this case, biology won (it usually does).

Over the next couple of years, papers began appearing that expanded the known diversity of aerobic methane-oxidizing bacteria. In 2022, researchers demonstrated aerobic methanotrophy in a cave-associated *Mycobacterium*, extending known aerobic methanotrophy beyond the bacterial groups in which it had traditionally been studied[2].

Suddenly those strange genes in our metagenomes looked a lot less strange.

So we went back to our data.

And that is where this project really started moving.

Our metagenomes contained Actinomycetota-affiliated monooxygenase systems, including in *Rhodococcus*-like genomes, recovered from samples positioned precisely where methane and oxygen overlapped in the soil. That part is important! In order to do aerobic methane oxidation, the microbes require both "ingredients": methane as the substrate and oxygen for methane activation and respiration. The physical structure of the leak therefore created a relatively narrow ecological opportunity—a CH_4–O_2 boundary where methane oxidation was possible.

That became the central idea of the pa-

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per: methane attenuation was not occurring uniformly across the site. It was organized by the physical transport of gases through soil, which created a spatially restricted zone where microbial metabolism could operate. Our public data and analyses capture that coupling between the methane plume, oxygen availability, soil conditions, microbial genes, and geochemical responses.

But there was still a problem.

Metagenomes tell us what organisms could do. They do not prove that they actually did it.

This is where being a microbial ecologist who had recently moved into an Earth Sciences department became *very* useful.

Methane is not just microbial food. It is also carbon.

And the methane in conventional natural gas carries information about where that carbon came from. Much conventional natural gas ultimately derives from buried organic material transformed over geological timescales through thermal maturation. That carbon can have a stable-isotope composition distinct from the carbon circulating through contemporary plants and soils (Figure).

Which meant we had a tracer!

If microbes really were oxidizing leaked methane, some of that methane-derived carbon should move into other soil carbon pools. Rather than relying only on genes saying, trust us, methane oxidation could happen here, we could ask whether the carbon itself had left a fingerprint.

There was only one slight complication:

I am not a stable-isotope geochemist.

Fortunately, I work down the hall from one.

I started talking with Neil Tabor, an Earth Sciences professor at SMU who works on soils, paleoclimate, and stable-isotope geochemistry. We sent samples through SMU's Stable Isotope Laboratory and measured soil carbon and nitrogen concentrations and isotope compositions. The lab has been doing light stable-isotope measurements at SMU since 1981, so this was a particularly convenient moment to discover that my microbiology problem was also a geochemistry problem.

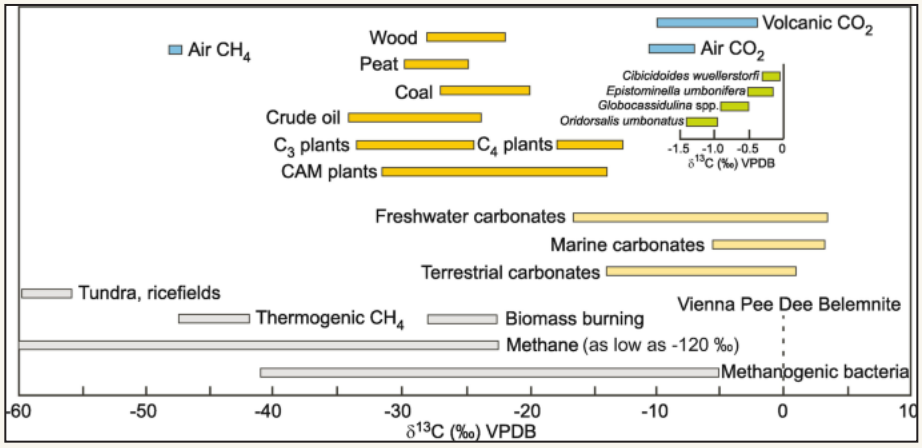
Then Neil asked another question: What about the carbonates?

This was a second moment in the project when someone from another discipline completely changed how I was thinking about the system. Methane does not simply disappear when a microbe oxidizes it. Its carbon is transformed, ultimately producing CO_2 and dissolved inorganic carbon that can interact with the mineral and carbonate pools in soil.

So Neil measured those too.

And there it was.

The carbonate pool changed systematically across the methane plume. Together with the organic-carbon isotope patterns, the metagenomes, and the physical gas measurements, those data connected processes that we had initially been treating separately. The public dataset shows striking spatial variation in carbonate abundance and carbon-iso-



Different pools of carbon and different forms of carbon have varying $\delta^{13}C$ isotope values From Encyclopedia of Earth Sciences Series (EESS)

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tope composition across the experiment.

That is the part of this paper I like most.

A diffusion-driven CH_4–O_2 boundary structures methane oxidation and carbon transformation in upland soils is technically a paper about a controlled natural gas leak. But to me, it became a paper about why environmental systems are difficult to understand if we insist on dividing them into disciplines.

Methane leaking from a pipe does not know whether it belongs to civil engineering, geology, geochemistry, atmospheric science, or microbiology.

It diffuses through pore spaces.

It encounters oxygen.

It changes soil chemistry.

Microbes oxidize it.

Its carbon moves into other pools.

And all of those processes happen simultaneously.

Our paper argues that methane attenuation in these aerated upland soils emerges from that coupling. The physical environment determines where methane and oxygen meet; that boundary structures where methane oxidation can occur; and microbial metabolism then contributes to measurable changes in soil carbon chemistry.

For me, it is also the first paper from a research project that began after I started the Chase Lab at SMU. I did not arrive intending to work on underground natural gas leaks. The project happened because someone studying a completely different part of the Earth system gave a seminar, I asked a very microbial-ecologist question, and neither of us immediately knew the answer.

That seems like a pretty good model for science.

And now, naturally, I have many more questions.

The candidate methane oxidizers we found are particularly interesting because these are not microbes living in a deep-sea methane seep or another environment where methane provides a relatively persistent resource. They occur in ordinary upland soils where methane availability may be intermittent; appearing during a leak and disappearing again when that leak stops.

So how do they survive between methane pulses? Are their metabolic strategies adaptations to fluctuating hydrocarbon availability? Do we see the same organisms at chronic low-level leak sites elsewhere? How widespread are these Actinomycetota-associated methane-oxidation systems? And could organisms adapted to consuming methane at relatively low concentrations eventually be useful for methane mitigation or biotechnological capture?

There is already substantial interest in finding methanotrophs capable of oxidizing methane efficiently at concentrations far below those typical of classic methane-rich environments, in part because those organisms could be useful for mitigating diffuse emissions. Our results suggest that intermittently exposed soils may be an interesting place to look.

I do not know the answers yet.

Which, thankfully, is how this project started in the first place.

This project also had two other amazing co-authors, Navodi Jayarathne, a former graduate student turned postdoc in Kate's lab who led the fieldwork and gas transport data collection, and Motahare Haghighatjoo, my first graduate student in my lab.

CITATIONS

1. AB Chase, JRRN Jayarathne, M Haghighatjoo, NJ Tabor, KM Smits. 2026. A Diffusion-Driven CH4–O2 Boundary Structures Methane Oxidation and Carbon Transformation in Upland Soils. *Environmental Science & Technolnogy*.
2. RJ Van Spanning, Q Guan, C Melkonian, J Gallant, L Polerecky, JF Flot, BW Brandt, M Braster, PI Espinoza, JW Aerts, et al. 2022. Methanotrophy by a *Mycobacterium* Species That Dominates a Cave Microbial Ecosystem. *Nature Microbiology*.