Actionable problems to de-risk biological approaches to atmospheric methane removal

A synthesis of international expert voices from a November 2024 workshop

Homeworld Collective

Workshop supported by SPARK CLIMATE SOLUTIONS

Acknowledgments

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The Introduction and Executive Overview of Workshop Findings sections were drafted by Paul Reginato, with edits and feedback from Ariana Caiati, Sam Abernethy, and Paige Brocidiacono.

The problem statements were outlined during the workshop by workshop participants, then polished by Paul Reginato* and Rachel Strickman with feedback from workshop participants.

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Introduction

This report communicates a set of actionable problems limiting progress on biological methane removal (bioMR), as identified by 30 international experts at a workshop hosted by Homeworld Collective and Spark Climate Solutions in Fall 2024. It represents the voices of the workshop participants in a call to action to the broader research and funding communities, highlighting opportunities to de-risk technology pathways that could mitigate the impacts of methane emissions.

Motivation

Methane has an 80-fold stronger near-term warming effect compared to CO2, has caused ~30% of climate warming to date [1], and atmospheric levels continue to rise. If all known methane emissions mitigation strategies are implemented, a methane emissions gap of 25-120 MtCH4/yr is still expected in 2050 relative to a 2 ˚C warming scenario, resulting from amplified natural emissions caused by climate change [2].

Atmospheric methane removal (MR) technologies at the scale of 10 MtCH4/yr could substantially mitigate climate change, but are nascent and under-researched. MR is a potential way to close the methane emissions gap, and would have particularly strong impacts on near-term warming [2]. However, it is presently unclear whether any proposed MR approach can be scalable, climate beneficial after accounting for energy demands, or cost-effective [3]. Research is urgently needed to identify and derisk potential MR pathways by understanding their feasibility, scalability, trade-offs, and costs [3].

Several key MR pathways presently under consideration are biological [3]: engineered methane-removing plants [4], methane removal bioreactors [5], soil methanotrophy enhancement (Davidson, 2024;), and forest methanotrophy enhancement [6]. Methane oxidation by methanotrophic bacteria already accounts for atmospheric methane removal: 18–40 Tg CH_4 yr^{-1} via soil [7] and early estimates of 25-40 Tg CH_4 yr^{-1} via the woody surfaces of trees [8]. BioMR pathways aim to remove additional methane at 10 MtCH4/yr scale by enhancing methane uptake on managed lands or deploying mechanisms of methanotrophy in engineered systems.

The bioMR research community needs to grow, but few researchers and funders are aware of opportunities to contribute. Early leaders are conducting promising research, but the current pace of research is inadequate to derisk and develop bioMR technologies on the timescale necessary to feasibly mitigate severe harms resulting from methane-induced warming. While a 2024 report on methane removal by the US National Academies of Sciences, Engineering and Medicine (NASEM) [3] has done well to raise awareness about big-picture challenges, the specific open problems remain opaque to most researchers and funders.

Methodology

Homeworld Collective and Spark Climate Solutions convened international experts on bioMR and its underlying science to draft a set of problem statements describing actionable opportunities to move the field forward. Each problem statement conveys concrete goals which, if achieved, would constitute a solution to an important problem. After the workshop, Homeworld Collective polished the problem statements and circulated them for revision by workshop participants. The problem statements are published individually as distinct research opportunities in the Homeworld Problem Statement Repository, and are aggregated in this report.

Executive overview of workshop findings

Engineered methane-removing plants

The aim of engineering plants for MR is to express a methane-oxidizing enzyme in plant cells that removes atmospheric methane as air passes over the plants' leaves [4]. This approach is attractive due to the potential low cost of scaled implementation, since it is passive (no forced air) and would require little infrastructure and little to no operating costs with no energy input required. However, it faces technical hurdles: methane monooxygenase (MMO) enzymes – the only naturally-occurring methane-oxidizing enzymes – are notoriously difficult to engineer or express in non-native hosts, and methane-oxidizing activity was not detected in past efforts when an MMO was expressed in plants [9].

Workshop participants emphasized that the challenge of expressing MMO in plants stems from foundational challenges involved in working with MMO, especially the most active form of MMO, particulate MMO (pMMO). The problem statements on methane-removing plants therefore focused on improving our ability to study MMOs and engineered variants, and on engineering novel MMOs that are more straightforward to work with. Themes are:

- *MMO research tools:* Expressing MMO in a non-native context is likely to require optimization and modification to the native enzyme. However, MMO engineering is substantially hindered by the high cost and low throughput of current techniques for measuring the methane-oxidizing activity of MMO. Further, the function of particulate MMO (pMMO) – the most active type of MMO – seems to be very sensitive to subcellular physical conditions that current tools are unable to characterize. MMO engineering research could be majorly enabled by a novel low-cost, high-throughput technique for measuring MMO activity (PS1), and by tools for characterizing the native subcellular environment of pMMO (PS2).
- *Engineering novel MMOs:* A key hindrance to expressing active MMO in plants is the dependence of natural MMOs on native host biology. The ideal MMO for expression in diverse plant hosts would be agnostic to host biology, displaying robust methane oxidizing activity under a flexible range of hosts. The effort to express active MMO in plants could be streamlined by engineering host-independent MMO activity in a novel protein, whether it be a de novo protein or a variant of an existing one (PS3).

Methane removal bioreactors

The aim of MR bioreactors is to flow air through an engineered reactor system containing methanotrophs that remove trace methane from the air [5]. While methane bioreactors have been developed and commercialized for converting higher concentrations of methane into products, bioreactors that remove ultradilute methane (~2-1000 ppm) from air would require a different design and are at a very early stage of development (TRL 1-2) [5,10].

Workshop participants emphasized that it is presently unclear whether MR bioreactors could scale economically or result in a net climate benefit after accounting for energy usage, and exactly how methanotrophs could be deployed in reactors to reduce costs. The problem statements on MR bioreactors therefore focused on bioreactor design and enabling optimization of methanotrophs for use in an MR bioreactor, across the following themes:

- *MR bioreactor design, prototyping, and techno-economic analysis:* No well-defined MR

bioreactor design concept has yet been proposed, preventing assessment of feasibility and leaving engineering research without a big-picture target. MR bioreactor designs should be developed and evaluated via high-level techno-economic analysis (TEA) and life-cycle analysis (LCA) (PS4), to derisk and guide future research. To readily enable experimental exploration of novel designs in an intercomparable format, a standard testbed bioreactor should be developed and made available (PS5).

- *Enabling optimization of methanotrophs for an MR bioreactor:* Derisking or developing an MR bioreactor will require optimizing the methane uptake rate by methanotrophs within the reactor. However, optimization efforts are substantially hindered by a lack of methods for manipulating the relevant methanotroph taxa. Tools should be developed to study and engineer methanotrophs that oxidize trace methane (PS6) and the biofilms they form (PS7). A method to halt methanotroph growth while maintaining high methane oxidizing activity would also enable optimizations of bioreactors (PS8).

Forest methanotrophy enhancement

Recent research indicates that methanotrophs in and on the woody stems of upland trees may remove 10s of Mt of methane from the atmosphere annually [8], while showing that tree stems also emit methane depending on context [11]. As a proposed MR pathway, forest methanotrophy enhancement aims to increase methane uptake in managed forests [6]. Workshop participants emphasized that our present ability to identify levers for forest methanotrophy enhancement, and to estimate its scalability, is limited by a very incomplete state of understanding of the scale and mechanistic drivers of natural methane fluxes in trees. The problem statements therefore generally pointed toward solidifying the understanding of tree methane fluxes, across the following themes:

- *Data:* Tree methane fluxes have only been studied in a small subset of biomes, and where they have been studied, substantial within-biome variation in flux remains unexplained. The science of tree methane fluxes could be enormously empowered by a global measurement campaign to collect coarse-grained tree methane flux data with strong representation of biomes, environmental parameters, and key species (PS9).

- *Measurement:* Tree methane flux measurements are frequently difficult to intercompare, due to unquantified errors introduced by variation in measurement instrument design, environmental parameters, and the location of measurements along the soil-trunk-canopy system. It is crucial for the research community to establish standards for instrumentation, measurement, error-reporting, and data integration so that robust methane flux models can be developed through contributions from many studies across the globe (PS10).

- *Mechanisms:* The mechanistic factors governing methane uptake from trees are undercharacterized. Detailed field work to study the many parameters relevant to tree methane flux should be conducted at methane uptake hotspots that are identified by a first round of broad coarse-grained flux measurements (PS11). In parallel, the growth characteristics of atmospheric methane oxidizing bacteria should be studied (PS12), and methane uptake by those bacteria should be validated in the lab (PS13). The heritability of tree methane flux should also be studied, to understand whether breeding offers a potential lever for influencing the underlying mechanisms (PS14).

Soil methanotrophy enhancement

Soil methanotrophs are estimated to naturally remove 11-49 MtCH4 from the atmosphere annually [7]). As a proposed MR pathway, soil methanotrophy enhancement aims to increase atmospheric methane uptake by soil methanotrophs on managed land [12].

Workshop participants emphasized that our present ability to identify levers for soil methanotrophy enhancement, and to estimate its scalability, is limited by incomplete understanding of the mechanistic drivers of soil methane uptake. Problem statements therefore generally pointed toward solidifying the science of soil methane uptake across the following themes:

- *Data:* To effectively model soil methane uptake, more data is needed that links soil methane uptake rates to environmental conditions across key contexts, including under the effects of agricultural practices (PS15), in boreal snow (PS16), and during hot spots and hot moments of up to 10-fold increased uptake rates (PS17). Substantial progress could also be made by integrating existing published and unpublished soil methane uptake data (PS18).
- *Measurement:* The task of collecting and integrating the necessary soil methane uptake data is currently hindered substantially by the high cost and low intercomparability of measurements. Reducing the cost of sensors capable of distinguishing methane uptake from net methane flux (which also includes emissions) (PS19) and agreeing on standards for how methane uptake is measured (PS20) would enable rapid progress in understanding the soil methane sink and determining if it can be leveraged for MR.
- *Metabolisms and symbioses:* Indirect evidence suggests that trace methane metabolism may involve microbial consumption of additional substrates on top of methane or distribution of the metabolic steps across multiple organisms (PS 21). There is also indirect evidence that methanotrophs may associate with plant roots in upland environments (PS 22). Investigating these phenomena could uncover novel mechanistic dimensions to soil methane uptake that would inform models and could reveal MR levers.

Problem statements

The remainder of this white paper consists of the problem statements created through the Workshop on Biological Methane Removal hosted by Homeworld Collective and Spark Climate Solutions. The purpose of a problem statement is to convey a set of concrete goals which, if achieved, would constitute a solution to an important problem.

Each problem statement consists of three sections: 1) the Motivating Factor, which describes the big-picture reason why the problem statement is important; 2) the Specific Bottleneck, which describes a limitation that prevents progress on the Motivating Factor; and 3) the Actionable Goals, which describes concrete outcomes that are achievable by a research team and would solve an important problem by overcoming the Specific Bottleneck.

Each problem statement is intended to stand alone as a descriptor of a concrete research opportunity and its importance, and is published in the Homeworld Problem Statement Repository. Because the problem statements drive toward similar big-picture challenges, the Motivating Factor sections are similar across problem statements, particularly when they relate to the same bioMR pathway. A reader who is looking through numerous problem statements will naturally skim through the Motivating Factor sections, and focus on the Specific Bottlenecks and Actionable Goals.

Engineered methane-removing plants

1. Develop a sensitive and high-throughput assay for methane oxidation

Authors: Amy Rosenzweig, Eli Hornstein, Arjun Khakar, Verena Kriechbaumer, Jonas Wilhelm, Rachel Strickman, Paul Reginato

Motivating Factor

Novel technology for methane removal (MR) at 1-100 $MtCH_4$ scale is needed, particularly for oxidizing atmospheric concentrations (2 ppm) [2,3] and emissions at 2-1000 ppm that are too dilute to be scalably oxidized with existing technology [13].

Several MR strategies have been proposed that would leverage biological CH_4 oxidation in engineered contexts. One example is methane oxidation bioreactors [5]; another is engineered crops or managed trees expressing methane monooxygenase (MMO) in their leaves or roots, which could oxidize CH_4 in soil or ambient air [4,9].

Development of MMO-bearing plants or optimizing CH_4 oxidation in reactors will require engineering and/or heterologous expression of MMO. However, significant challenges limit such manipulation of MMO [14–16], preventing progress on such technologies.

Specific Bottleneck

A key challenge limiting progress on manipulating and characterizing MMO is a lack of a high-sensitivity and high throughput assay for facile measurement of methane oxidation to methanol at low methanol concentrations in cell culture or isolated MMO samples. Current protocols involve monitoring production of isotopically-labeled methanol by gas chromatography-mass spectrometry (GC-MS). This method, which requires serial delivery of the gaseous methane substrate to individual samples, is time-consuming, laborious, and relatively low-sensitivity (~10 μM lower limit of detection) [17]). The low throughput of this assay (100 samples per week, including controls and replicates) precludes efforts to engineer and screen new variants.

With these limitations on throughput and sensitivity, it is difficult to 1) distinguish MMO variants with different activities; 2) measure oxidation rates at very low methane concentrations (e.g. near atmospheric concentration); and 3) detect incremental progress towards methane oxidation in engineered or heterologous variants with very low activity. Our abilities to characterize MMO variants, build a sequence-function framework for MMO catalysis, develop heterologous MMO expression systems, and engineer de novo MMOs are thus curtailed significantly.

Actionable Goals

An assay should be developed to assay CH4 oxidation to methanol, with sensitivity to detect nM or sub-nM methanol and throughput to test at least 1000 samples per week (i.e., ~150 distinct test conditions, accounting for controls and replicates), or ideally 10000 samples per week. A-higher throughput assay enabling up to millions of measurements per week, perhaps using fluorescence-activated cell sorting (FACS) to read out methanol concentrations, is also desirable.

2. Optical biosensors and lipidomics for biophysical characterization of pMMO function

Authors: Amy Rosenzweig, Verena Kriechbaumer, Jonas Wilhelm, Arjun Khakar, Eli Hornstein, Rachel Strickman, Paul Reginato

Motivating Factor

Novel technology for methane removal (MR) at 1-100 $MtCH_4$ scale is needed, particularly for oxidizing atmospheric concentrations (2 ppm) [2,3] and emissions at 2-1000 ppm that are too dilute to be scalably oxidized with existing technology [13]. Several MR strategies have been proposed that would leverage biological CH_4 oxidation in engineered contexts. One example is methane oxidation bioreactors [5]; another is engineered crops or managed trees expressing methane monooxygenase (MMO) in their leaves or roots, which could oxidize CH_4 in soil or ambient air [4,9].

Development of MMO-bearing plants or optimizing CH_4 oxidation in reactors will require engineering, heterologous expression, and/or or context-specific characterization of MMO. However, significant challenges limit such manipulation of MMO, particularly the particulate MMO (pMMO), which is thought to have the highest affinity for CH_4 [15,18].

Specific Constraint

Native pMMO is embedded in intracytoplasmic membranes in ordered arrays of multi-subunit complexes [14]. While non-functional or low-activity protein complexes have been assembled outside the native host [9,19,20], pMMO with near-full function has not been expressed outside of its native host. Further, substantial open questions remain about how the native membrane environment provides the necessary biophysical conditions for pMMO to function, which hinder efforts to heterologously express and engineer high-activity pMMO [21]).

The membrane potential, ion concentrations, redox state, and pH in the native environment of pMMO are expected to substantially impact enzyme function, but are uncharacterized. Additionally, an elaborate lipid environment has been identified around pMMO in the intracytoplasmic membrane, in which at least 20% of the lipids have not been identified [14,19]. Unidentified lipids may have a role in pMMO function. Determining these unknown biophysical parameters in the native context of pMMO would likely aid engineering efforts involving pMMO.

Actionable Goals

To characterize biophysical factors in pMMO function, optical biosensors for voltage, pH, reactive oxygen species, and ions should be adapted for use in model methanotrophs with protocols for quantitative measurement through microscopy. These systems could include development of specific intracellular membrane localization tags for structures inside methanotrophs. Dye-based studies of the same factors, where applicable, should also be pursued.

To characterize the lipid environment in which pMMO is natively situated, lipidomics analysis (likely LC-MS based) of the pMMO array should be performed to comprehensively identify not only known lipid species, but also to identify lipids not present in libraries used for analysis.

The biophysical characterization studies described here would be further empowered if coupled with forward- and reverse-genetic studies of pMMO function. To enable such studies, tools must be developed for generating mutant libraries of pMMO in methanotrophs; that challenge is discussed in a related problem statement [15].

3. Design a de novo methane monooxygenase

Authors: Eli Hornstein, Arjun Khakar, Verena Kriechbaumer, Amy Rosenzweig, Jonas Wilhelm, Rachel Strickman, Paul Reginato

Motivating Factor

Novel technology for methane removal (MR) at 1-100 $MtCH_4$ scale is needed, particularly for oxidizing atmospheric concentrations (2 ppm) [2,3] and emissions at 2-1000 ppm that are too dilute to be scalably oxidized with existing technology [13].

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Development of MMO-bearing plants or optimizing CH_4 oxidation in reactors will require engineering, heterologous expression, and/or or context-specific characterization of MMO. However, significant challenges limit such manipulation of MMO [14,16,22], preventing progress on technologies leveraging MMO.

Specific Bottleneck

Expression of functional MMO in heterologous hosts is challenging for a variety of reasons. The particulate MMO (pMMO) is a membrane-bound multi-subunit complex. The biophysical conditions and electron delivery pathways needed for high MMO activity are undetermined, and are thought to be strongly coupled to host biology [14]. Heterologously-expressed pMMO has had low or no activity [9,20]. Further, the PmoC subunit of pMMO is toxic to cloning hosts [23], preventing generation of mutant libraries and substantially hindering efforts to engineer and study pMMO.

The soluble MMO (sMMO) is also a multi-subunit complex, for which functional heterologous expression is hindered by the need for chaperone proteins [24,25]. sMMO activity comparable to lower activities observed in its native context has been achieved in an optimized E. coli host [25], but those results are challenging to generalize due to host-specific biology.

An alternative pathway to MMO engineering and research would be to pursue de novo engineering of an MMO that is readily heterologously expressible with high activity.

Such an engineered MMO would be beneficial even if new research achieves a complete understanding of native MMO function, since it could help circumvent the challenges associated with heterologously expressing a functional multi-protein complex with dependencies on host biology.

Actionable Goals

De novo MMO proteins should be developed that achieve high methane oxidation rates (on par with native MMOs) and can function across diverse heterologous hosts. De novo MMOs would ideally be non-toxic single-domain monomeric proteins that rely minimally on host-specific biology for function, including folding and electron delivery.
High-throughput computation-intensive methods and well-known rational modifications might both be applied; for example, pMMO expression as a polycistron successfully avoided toxicity [9].

The mechanism by which the de novo MMO will obtain the reducing equivalents required for methane oxidation is critically important. The de novo MMO could be fused to a reductant-producing protein or localized to a cellular component with a supply of an appropriate reductant that is readily available across hosts. Characterization of alternative reductants from potential heterologous MMO hosts, in vitro or in natural methanotrophs, might be used to inform other aspects of protein design.

Consideration should also be given to maximizing access of de novo MMO to methane, which is non-polar and is more soluble in a lipid bilayer than the aqueous cytoplasm. Improved access could be achieved by engineering a de novo MMO as a membrane protein. Complementary host-engineering work to optimize methane accessibility could involve establishing lipid-rich environments at the localization region of the MMO.

Methane removal bioreactors

4. Develop and assess designs for methane removal bioreactors

Authors: Dominic Sauvageau, Will Sawyer, Allison Pieja, Rachel Strickman, Paul Reginato

Motivating Factor

If all known CH_4 emissions reduction approaches are implemented, a CH_4 emissions gap of ~25-120 $MtCH_4$/yr is still expected by 2050 compared to a scenario of <2 ˚C-consistent warming [2]. In particular, ~75% of CH_4 pollution is atmospheric (2 ppm) or area emissions below 1000 ppm, which are too dilute to be oxidized at scale using existing technologies [13]. Novel methane removal (MR) approaches for scalably oxidizing 2-1000 ppm CH_4 at 1-100 $MtCH_4$/yr are needed [2,3].

One MR approach under consideration involves flowing air through bioreactors containing methanotrophs, which are the only known passive catalyst of CH_4 oxidation under ambient conditions [5,18]. Any successful MR bioreactor technology will require a combination of process design optimization and deployment of methanotrophs in a format that accommodates the process design. While biofiltration is used to oxidize CH_4 in the concentration range of 10,000-50,000 ppm, it is presently unknown whether any MR bioreactor design can accomplish MR at 2-1000 ppm that is sustainable and economically feasible.

Specific Bottleneck

MR bioreactors have been proposed conceptually [5]. Adaptation of existing CH_4 reactor designs to handle 2-1000 ppm CH_4 is not expected to be economically feasible in absence of major breakthroughs [10], indicating that a scalable MR bioreactor would likely require a different system design oriented specifically to ultra-dilute concentrations. However, no well-defined MR bioreactor system design has been envisioned at scale or analyzed for sustainability and economic feasibility in the research literature.

Specifically, current discourse on MR bioreactors lacks new designs with an accompanying description of how air flow rates, air contactor format, biotechnology, and CH_4 mass transfer integrate into a system with defined energy usage, water usage, spatial footprint, and overall cost per unit of CH_4 oxidized. In absence of such designs and analyses, the technology community lacks clarity regarding the feasibility of MR bioreactors or a defined engineering goal to build toward.

Actionable Goals

Novel MR bioreactor designs should be developed, with accompanying life-cycle analysis (LCA) and techno-economic analysis (TEA) that enable estimation of feasibility at scale and identification of limiting factors on cost/sustainability. Such designs should incorporate existing knowledge of relevant industrial processes, biotechnology, fluid dynamics and mass transfer, as well as novel experiments. Experiments aimed at maintaining robust methanotroph communities or otherwise deploying methane oxidation catalysts (such as MMO), while maximizing CH_4 mass transfer, will be particularly helpful.

Designs should account for challenges associated with maximizing CH_4 mass transfer, minimizing energy usage, minimizing water usage, and maintaining a robust biocatalyst under process conditions, including delivering nutrients, preventing biofouling, and clearing accumulated biomass. TEA should account for costs associated with energy for forced air, nutrient delivery, facility construction

(capital cost), and facility downtime to deal with biofouling or biomass clearing. Biomass may be an economically useful output. LCA should account for energy-associated emissions, water usage, and facility footprint.

The ultimate goal of this work is to gain clarity on whether MR bioreactors are a scalable MR approach, and if so, to guide efforts to build them. An MR bioreactor design demonstrated at TRL 4 with encouraging TEA and LCA would be the strongest output.

5. A standardized testbed bioreactor for methane removal research

Authors: Dominic Sauvageau, Will Sawyer, Allison Pieja, Rachel Strickman, Paul Reginato

Motivating Factor

By 2050, if all known CH_4 emissions reduction approaches are implemented, a CH_4 emissions gap of ~25-120 $MtCH_4$/yr is expected compared to a scenario of <2 ˚C-consistent warming [2]. In particular, ~75% of CH_4 pollution is atmospheric (2 ppm) or area emissions below 1000 ppm, which are too dilute to be oxidized at scale using existing technologies [13]. Novel methane removal (MR) approaches for scalably oxidizing 2-1000 ppm CH_4 at 1-100 $MtCH_4$/yr are needed [2,3].

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Specific Bottleneck

There are numerous possibilities for utilizing methanotrophs in MR reactors. For example, the microbial strains or communities, nutrients provided, or microbial attachment surface may vary. Experimentation with these possibilities needs to be compared. However, the nascent MR bioreactor field has no standard for comparing different bioreactor configurations and process flows, hampering efforts to identify the optimal biology. Sensor design and measurement protocols also differ across experiments. Further, some groups with innovative biological ideas may not be readily able to develop testbeds (bench-scale prototype bioreactors). This lack of intercomparability and accessibility to prototyping apparatus limits the ability to find optimal solutions and identify progress via research across multiple labs.

Actionable Goals

A low-cost, readily-constructible standardized bioreactor testbed with explicit operating protocols should be designed. The design should integrate input from researchers representative of the MR reactor community, with a goal of accommodating flexible experimentation using consistent process design and implementation of sensors, actuators and control systems. A set of bioreactor testbeds should be fabricated and distributed to labs, with commitment from one or more project leads to fabricate and distribute additional testbeds if needed.

Standards should be agreed on for key performance metrics (e.g., methane consumption per unit microbial dry weight) and sets of standard experimental process parameters for benchmarking (e.g., temperature, pH, gas composition, gas flow rate). While various additional metrics and parameters will be relevant across experiments based on context, such standards would enable baseline comparability. A control experiment using a defined strain and experimental parameters should be established for use as initial validation of consistent use of the testbed across experimenters. As research results accumulate, an online repository of comparable results may be helpful.

6. Develop genetic tools for atmospheric methane oxidizing bacteria

Authors: Calvin A. Henard, Max Schubert, Mary Lidstrom, Henry Lee, Sukhwan Yoon, Rachel Strickman, Paul Reginato

Motivating Factor

By 2050, if all known CH_4 emissions reduction approaches are implemented, a CH_4 emissions gap of ~25-120 $MtCH_4$/yr is expected compared to a scenario of <2 ˚C-consistent warming [2]. In particular, ~75% of CH_4 pollution is atmospheric (2 ppm) or area emissions below 1000 ppm, which are too dilute to be oxidized at scale using existing technologies [13]. Novel methane removal (MR) approaches for scalably oxidizing 2-1000 ppm CH_4 at 1-100 $MtCH_4$/yr are needed [2,3].

One potential MR approach involves flowing air through bioreactors containing methanotrophs [5]. Other potential approaches include environmental methanotrophy enhancement in trees [6]or soils [12]. MR via reactors or environmental methanotrophy enhancement will require leveraging atmospheric methane oxidizing bacteria (atmMOB), i.e., methanotrophs able to grow on ~2 ppm methane [18,26–28]. However, we lack the biological understanding of atmMOB required to deploy them in a reactor, engineer their properties, or intervene on factors regulating their environmental activity.

Specific Bottleneck

atmMOB have distinct metabolisms and can grow with energy input below previous estimates of the lower bound for cell maintenance [26]. Many open questions remain about the mechanisms underlying their metabolism [26]) and the factors that govern their growth and activity [29]. It is also unclear how these organisms can be adapted to a reactor; whether their properties can be enhanced via manipulations of their genetics or growth environment; or whether their trace gas metabolism phenotypes can be reproduced in other organisms.

Progress is hindered by a lack of tools for genetically manipulating atmMOB, and by the long timescales required to isolate and cultivate them (e.g., 2 years of culture to obtain a pure strain [27]). Little research has explored whether genetic tools that work in other organisms could be adapted to atmMOB, or whether their growth could be accelerated. Developing such tools would be broadly enabling across research for mitigating the warming impacts of CH_4.

Actionable Goals

Work should be carried out to systematically test adaptation of existing genetic tools for use in atmMOB, in particular Methylocapsa and Methylocystis spp [26] or as-yet uncultured isolates. Example tools include methods for DNA delivery (e.g., electroporation, conjugation), DNA vectors (e.g. replicative plasmids, suicide plasmids), genome editing (e.g., CRISPR + homologous recombination), gene expression analysis (e.g., TnSeq), culture tools (e.g., antibiotic resistance genes), and manipulation of gene expression (e.g., CRISPRi).

The possibility of culturing trace CH_4-oxidizing methanotrophs at a faster growth rate or under more tractable media conditions by providing alternative energy and carbon sources (e.g., methanol, acetate, or other soluble substrates) should also be explored.

To ensure maximum utility from this tool-development work, tools should be developed in tandem with a scientific research project of importance for CH_4 mitigation applications.

7. Develop protocols for methanotrophic biofilm characterization

Authors: Jin Wang, Peter He, Richard Hamilton, Jessica Swanson, Daniel Goodwin, Mary Lidstrom, Calvin Henard, Max Schubert, Henry Lee, Sukhwan Yoon, Alex Tveit, Rachel Strickman, Paul Reginato

Motivating Factor

By 2050, if all known CH_4 emissions reduction approaches are implemented, a CH_4 emissions gap of ~25-120 $MtCH_4$/yr is expected compared to a scenario of <2 ˚C-consistent warming [2]. In particular, ~75% of CH_4 pollution is atmospheric (2 ppm) or area emissions below 1000 ppm, which are too dilute to be oxidized at scale using existing technologies [13]. Novel methane removal (MR) approaches for scalably oxidizing 2-1000 ppm CH_4 at 1-100 $MtCH_4$/yr are needed [2,3].

One MR approach under consideration involves flowing air through bioreactors bearing methanotrophs, which are the only known passive catalyst of CH_4 oxidation under ambient conditions [5].

Methanotrophic biofilms may have advantages or disadvantages for CH_4 oxidation efficiency at low concentrations (2 - 1000 ppm). Advantages may include highly concentrated cells, a protective environment, possible facilitation of CH_4 mass transfer, and efficient nutrient recycling. Disadvantages may include mass transfer inhibition and impediment of biomass removal from the reactor. However, we are unaware of research on the impacts of biofilms on CH_4 oxidation efficiency and ways to optimize them.

Specific Bottleneck

Control of methanotrophic biofilms may enable improved rates of CH_4 mass transfer and/or oxidation in bioreactors, for example by optimizing properties that increase CH_4 solubility, minimizing water layer thickness on the surface of the gas contactor, or maximizing cell density. Simultaneously, control over biofilms may enable prevention of thick biofilm accumulation that may inhibit mass transfer or gas flow through the reactor.

However, research on the potential role of biofilms in methanotrophic bioreactors is limited by a lack of foundational knowledge on the composition, properties and functions of methanotrophic biofilms and their impact on mass transfer. In turn, methanotrophic biofilm research is impeded by a lack of knowledge of the genes, regulatory mechanisms, and environmental factors underlying biofilm formation.

While some research has been done to develop methods to study and engineer biofilms, little has focused on methanotrophic biofilms, and standard methods for biofilm research are still evolving [30–32]. Application and development of such methods to characterize and control methanotrophic biofilms may unlock new approaches to efficient methane oxidation bioreactors across a range of applications, including oxidation of 2-1000 ppm CH_4.

Actionable Goals

Work should be undertaken to design and distribute protocols for studying methanotrophic biofilm establishment, properties, and function as they pertain to rates of CH_4 mass transfer and oxidation.

An initial goal is establishment of protocols for consistently producing methanotrophic biofilms as a research platform. Using such protocols, research should be conducted to characterize biofilm composition, structure, and heterogeneity; their impacts on CH_4 oxidation and mass transfer; and their

environmental and genetic controls. Key topics of study may include:

- biofilm composition (e.g., lipids, proteins, sugars), porosity, hydration, and their heterogeneity;
- physical processes governing transport of CH_4, nutrients, and cellular byproducts;
- properties governing the thickness of the water layer on top of the biofilm, or possibly allowing the biofilm to be in direct contact with gas;
- impacts of taxonomic composition, bioreactor surface chemistry, nutrients/ions, temperature, pH, methane concentration, and flow conditions on biofilm composition, structure and physical properties ([30–32]);
- impact of biofilm properties on methanotroph cell density, methane oxidation rate and growth rate
- genetics and regulatory mechanisms governing the above.

8. Halt methanotroph growth while maintaining high methane oxidation rate

Authors: Mary Lidstrom, Calvin A. Henard, Max Schubert, Henry Lee, Sukhwan Yoon, Rachel Strickman, Paul Reginato

Motivating Factor

By 2050, if all known CH_4 emissions reduction approaches are implemented, a CH_4 emissions gap of ~25-120 $MtCH_4$/yr is expected compared to a scenario of <2 ˚C-consistent warming [2]. In particular, ~75% of CH_4 pollution is atmospheric (2 ppm) or area emissions below 1000 ppm, which are too dilute to be oxidized at scale using existing technologies [13]. Novel methane removal (MR) approaches for scalably oxidizing 2-1000 ppm CH_4 at 1-100 $MtCH_4$/yr are needed [2,3].

One MR approach under consideration involves flowing air through bioreactors containing methanotrophs, which are the only known passive catalyst of CH_4 oxidation under ambient conditions [5,18]. Any successful MR bioreactor technology will require a combination of process design optimization and deployment of methanotrophs in a format that accommodates the process design. While reactor designs are still under active research, parallel work should investigate ways to adapt methanotroph biology to constraints likely to be imposed by any reactor design.

Specific Bottleneck

While methanotrophs are attractive methane oxidation catalysts, the low concentration in the atmosphere and resulting very low growth rate poses challenges to efficient MR reactor functioning. Crucially, at the very slow removal rates expected, biomass accumulation could eventually impede CH_4 mass transfer and increase the reactor pressure drop, which are key cost factors [33]. Biomass removal would also incur an operational cost and facility down time, and the low productivity per unit time likely precludes investing in harvesting technology for each MR unit. Further, disposal of waste biomass may cause life-cycle inefficiencies, particularly if it converts back into CH_4 in a landfill.

A possible solution would be to arrest methanotroph growth when cells have reached an optimal distribution, while maintaining high CH_4 oxidation activity. Methods for arresting cell growth while maintaining metabolic activity have been developed for other microbes and applications [34]. However, we currently lack the understanding of metabolism in non-growth-state methanotroph cultures/consortia that would be necessary to predict or control non-growth states and associated methane oxidation rates.

Actionable Goals

For methanotrophic cells/consortia consuming 2-1000 ppm CH_4, research should characterize environmental factors and physiologies that influence rates of growth and CH_4 oxidation, as well as mechanisms that support high CH_4 consumption with minimal growth. Non-growth mechanisms for dissipating excess energy resulting from CH_4 oxidation are of particular interest.

Relevant factors include nutrient availability (e.g., Cu, Fe, lanthanides, N) and reactor-relevant physical conditions (e.g, cell encapsulation or biofilms). Physiology varies with growth conditions, so experimental conditions should mimic a reactor and be consistent across complementary research efforts. Mass transfer limitations will strongly influence results, and must be clearly defined.

Ideally, cell subpopulations in growth, static, and death phases would be distinguished, but current methods for quantifying rates of growth and CH_4

oxidation rely on population-level measurements of CH_4/O_2 consumption and total biomass change [26,35].

Metabolic engineering approaches could also be explored for maintaining non-growth with high methane oxidation [36–40], for example by consuming the excess NADH expected to accumulate in absence of growth.

Forest methanotrophy enhancement

9. Survey tree methane flux across biomes globally

Authors: Jean-Francois Lamarque, Mari Pihlatie, Steven Strauss, Hinsby Cadillo-Quiroz, Luke Jeffrey, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. It is crucial to develop accurate CH_4 flux models for natural systems that enable prediction of future CH_4 dynamics. Further, on top of mitigating anthropogenic CH_4 emissions, we must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Trees are increasingly recognized as important players in the methane cycle [42]. Estimates indicate they mediate CH_4 emission [11,43,44] and atmospheric CH_4 uptake [8] at a scale of 10s of Mt/yr. As such, tree CH_4 fluxes should be incorporated into CH_4 cycle models and are a potential lever for sink maintenance, emissions mitigation, and MR [6]. However, we broadly lack an integrated understanding of how tree CH_4 flux varies across major world ecosystems, which prevents prediction of their future role in climate change and identification of which systems should be a focus for intervention.

Specific Bottleneck

Tree CH_4 fluxes have only been studied in a small subset of biomes. Strong flux variability between climatic regions has been observed, but drivers of variability are incompletely understood. Further, within-biome flux variability suggests that there may be significant cryptic or unknown sources and sinks.

Systematic collection of coarse-grained plant CH_4 flux data across biomes and key plant types would enable identification of flux hotspots and coldspots to inform models and enable site prioritization for subsequent mechanistic research toward emissions mitigation and MR.

Actionable Goals

A global survey of tree CH_4 fluxes across biomes should be performed to rapidly screen for hotspots and coldspots of CH_4 flux, covering the following axes:

- taxonomy: gymnosperms and angiosperms;
- vertical position along tree: lower tree stems, upper tree stems, branches, and leaves;
- latitude: tropical, temperate, and boreal regions;
- moisture: wetland and dryland forests;
- management: natural and managed forests, and major cultivated trees including pines, and eucalypts;
- seasonality: measurements should be distributed across the year;

In advance of the survey, existing tree CH_4 flux datasets should be identified, collated and synthesized in a literature review to inform survey design. Field CH_4 data collection standards should also be developed in advance, to ensure comparable measurements [45]. Survey design should also explore

the feasibility of remote sensing of positive and/or negative CH_4 fluxes.

As possible, the resulting survey data should be overlapped with existing large-scale datasets of important macro-scale drivers, such as land use, soil type, soil moisture, cumulative rainfall and temperature, history of fire/flood/disturbance, and other factors.

10. Standardize protocols and instrumentation for measuring methane flux in trees

Authors: Mari Pihlatie, Steve Strauss, Jean-Francois Lamarque, Hinsby Cadillo-Quiroz, Luke Jeffrey, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming[41]. It is crucial to develop accurate CH_4 flux models for natural systems that enable prediction of future CH_4 dynamics. Further, on top of mitigating anthropogenic CH_4 emissions, we must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13]..

Trees are increasingly recognized as important players in the methane cycle [42]. Estimates indicate they mediate CH_4 emission [11,43,44] and atmospheric CH_4 uptake [8] at a scale of 10s of Mt/yr. As such, tree CH_4 fluxes should be incorporated into CH_4 cycle models and are a potential lever for sink maintenance, emissions mitigation, and MR [6]. However, despite seminal work [8,11,42,46,47], we broadly lack the mechanistic models, empirical measurements, and integrative framework required to build predictive ecosystem-scale models.

Specific Bottleneck

Substantial coordinated effort is required to collect and integrate tree CH_4 flux data across global biomes to empower development of predictive models. However, there is no standard approach for measuring CH_4 flux or reporting possible sources of error, and methodologies vary across studies [48–53]. For example:

- measurements vary in their location across the soil-trunk-canopy system, frequency, distribution across seasons, and inclusion of other environmental parameters [54];
- designs vary for measurement chamber systems, resulting in various degrees of leakage and disturbance to plant processes; preventing accurate measurement of small fluxes [53];
- CH_4 buildup during incorrect measurement duration can cause errors [53];
- gas analyzers can be affected by environmental conditions (e.g. moisture, plant-emitted VOCs), leading to errors [55];

Further, there is no accepted approach for upscaling local measurements into ecosystem-scale models. Measurement and data integration standards should be developed and agreed on to enable coordinated research.

Actionable Goals

Standardized protocols should be developed for field measurement of CH_4 flux in trees. The work should:

- identify and define physical locations of key components in the soil-stem-canopy continuum to be compared across studies;
- list key environmental parameters to be measured alongside flux;
- inventory existing plant CH_4 flux measurement instruments and report on their characteristics, including configurations, accuracy, and sensitivity to humidity, temperature, and VOCs;
- identify instrument designs, materials, and protocols that enable reliable measurements on different parts of woody and herbaceous plants;

- provide guidance for reliable flux measurement, including instrument operation, measurement frequency, analyzer configurations, and instrument parameters such as diffusion capacity, reactivity, and internal gas mixing;
- standardize a data analysis workflow, from raw data to flux calculations and upscaling to ecosystems;
- provide guidance for navigating experimental tradeoffs (e.g., sensitivity, number of measurements, cost) under different scenarios (e.g. high vs. low flux, spatial extent).

This work should draw input from the tree CH_4 flux research community. Group working sessions or convenings may be helpful for achieving coordination and group buy-in.

11. Mechanistic study of tree methane flux hotspots globally

Authors: Mari Pihlatie, Steve Strauss, Jean-Francois Lamarque, Luke Jeffrey, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. It is crucial to develop accurate CH_4 flux models for natural systems that enable prediction of future CH_4 dynamics. Further, on top of mitigating anthropogenic CH_4 emissions, we must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Trees are increasingly recognized as important players in the methane cycle [42]. Estimates indicate they mediate CH_4 emission [11,43,44] and atmospheric CH_4 uptake [8] at a scale of 10s of Mt/yr. As such, tree CH_4 fluxes should be incorporated into CH_4 cycle models and are a potential lever for sink maintenance, emissions mitigation, and MR [6]. To develop interventions and accurate global models, we need mechanistic, process-based models accounting for key factors that influence CH_4 flux [56], particularly for flux hotspots. However, we broadly lack such process-based models at an actionable level of predictive power.

Specific Bottleneck

Despite seminal work [8,11,42,44,46,47,54], for most ecosystems, empirical measurements are lacking or incomplete for relating CH_4 flux to the many parameters relevant to CH_4 production, consumption, and movement in the soil-tree-air system [54]. Additionally, there are few controlled experiments to confirm observational measurements from scoping studies [57,58]. In-depth studies on plant CH_4 fluxes and drivers at flux hot spots would substantially advance our ability to construct actionable models of CH_4 dynamics in ecosystems and guide interventions.

Actionable Goals

Detailed studies of the many parameters relevant to tree CH_4 flux, with incorporation of controlled experimental confirmation of key findings, should be conducted at flux hotspots across natural and managed biomes globally. This work should begin with selecting representative sites based on identified hotspots in existing literature and coordination with any new research efforts launching to survey global biomes for flux hotspots [59]. Care should be taken to ensure measurements collected by collaborating teams are comparable, which would benefit from development of, and adherence to, standardized field sampling protocols [45]. The following themes should be explored:

- role of tree age, size, species, and position along stem or canopy;
- distinction of CH_4 transport vs. production in plant tissues;
- role of microbiome;
- partitioning of CH_4 emissions and consumption, and biotic and abiotic contributors;
- seasonal effects, and, where possible, effects of extreme events (fire, flood, etc.);
- wood density, physical soil properties, and hydrology;
- for managed ecosystems, role of agronomic activities, such as tillage, fertilization, weed control, and irrigation that affect plant growth and physiology.

12. Measure environmental growth rates of atmospheric methane oxidizing bacteria

Authors: Alexander Tøsdal Tveit, Pok Man (Bob) Leung, Marc Dumont, Hendrik Schaefer, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. We must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Soils and trees are estimated to uptake atmospheric CH_4 at 10s of $MtCH_4$/yr [7,8], and are a potential lever for sink maintenance, emissions mitigation, and MR [3,6,12]. Such uptake is presumed to be mediated by atmospheric methane-oxidizing bacteria (atmMOB), i.e., methanotrophs that can grow on ~2 ppm CH_4 [26,27]. Thus, interventions to atmospheric CH_4 uptake by soils and trees need to work by influencing atmMOB. atmMOB may also be a key component of bioreactors developed for MR [5]. It is therefore crucial to understand atmMOB growth dynamics in the environment and its limiting factors. However, atmMOB growth has not been studied outside of lab culture.

Specific Bottleneck

The slow growth rate of atmMOB (doubling time ~ weeks in culture [27]) is expected to be a key bottleneck for environmental CH_4 uptake and implementation of MR bioreactors. Growth rate will fundamentally limit how quickly environmental atmMOB can respond to interventions or recover from disturbance, and will limit the rate of microbial community establishment in reactors. While atmMOB have been cultured in the lab, growth rates in the environment may be substantially slower or faster, due to the presence of yet-unknown growth-inhibiting or growth-enhancing factors. The range of possible limiting factors is wide and includes physical disturbance, fertilizers, pesticides, and nutrient availability [60]. Research to quantify atmMOB growth rates and discover factors influencing growth using environmental samples in microcosm studies will provide foundational knowledge to inform method development for CH_4 emissions mitigation and MR.

Actionable Goals

The first goal is to establish a method for measuring atmMOB growth in microcosm studies, for example by using quantitative stable isotope probing (qSIP) with ^{18}O-H_2O in soil, bark or leaves. Method design will need to account for challenges associated with the slow growth rates, low abundance, and patchy distribution within samples of atmMOB.

In parallel, similar growth measurement experiments should be performed on pure cultures to calibrate the method. Such experiments could also reveal insights on the function of different cells during homeostasis vs early growth.

The second goal is to apply the growth-rate measurement method to examine the impact of environmental factors on atmMOB growth. A list of possible factors impacting atmMOB growth should be identified and prioritized, which may include: CH_4 concentration (0-2 ppm, or slightly above); history of physical disturbance; water content and tortuosity; presence of metals, nutrients, fertilizers, pesticides, and pollutants; presence of mixotrophic substrates (e.g., acetate, H_2, CO); and presence of other microbial species. Environmental sampling sites corresponding to these factors should be identified. Experimental designs should also be developed for performing controlled studies on the effects of these factors through lab manipulations in microcosms.

13. Lab validation and characterization of atmospheric methane uptake by microbes in the phyllosphere

Authors: Alexander Tøsdal Tveit, Pok Man (Bob) Leung, Hinsby Cadillo-Quiroz, Luke Jeffrey, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. It is crucial to develop accurate CH_4 flux models for natural systems that enable prediction of future CH_4 dynamics. Further, on top of mitigating anthropogenic CH_4 emissions, we must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Trees are increasingly recognized as important players in the methane cycle [42]. Estimates indicate they mediate CH_4 emission [11,43,44] and atmospheric CH_4 uptake [8] at a scale of 10s of Mt/yr. As such, tree CH_4 fluxes should be incorporated into CH_4 cycle models and are a potential lever for sink maintenance, emissions mitigation, and MR [6]. To develop interventions and accurate models, we need to understand the mechanism by which CH_4 is oxidized in trees. However, the mechanism behind atmospheric CH_4 uptake by trees is unknown.

Specific Bottleneck

Field measurements of atmospheric CH_4 uptake in upland tree stems [8] and leaves [61,62] suggest CH_4 oxidation by microbes in bark [44,46] or leaves. These microbes are presumed to be specialized atmospheric methane-oxidizing bacteria (atmMOB), which can grow on ambient (~2 ppm) CH_4 [26,27]. However, work to date has not confirmed phyllosphere (i.e., above-ground tree surface) uptake of atmospheric CH_4 in controlled lab experiments, demonstrated methanotrophy of atmospheric CH_4 in the caulosphere, or identified the responsible atmMOB. Further, putative atmMOB (Methylocystis spp.) have been found in mosses at a wide range of CH_4 concentrations, including non-detectable CH_4, though atmospheric CH_4 uptake was not investigated in that study [63].

The measurements required to confirm atmospheric CH_4 uptake and search for phyllospheric atmMOB are challenging [26,27]: the very low CH_4 uptake rate is difficult to distinguish from measurement noise; the very slow growth rate of atmMOB necessitates long experimental timelines; and the low abundance, patchy distribution, and lack of a characterized physical niche for putative phyllospheric atmMOB will make sampling difficult. Nonetheless, this fundamental work is required to enable further research into the limiting factors and requirements for atmospheric CH_4 uptake by trees, which may reveal CH_4 mitigation levers.

Actionable Goals

Work should begin with confirmation of phyllospheric CH_4 uptake in controlled lab experiments, which can achieve higher sensitivity than field measurements. Phyllosphere samples should be taken from upland tree stems and leaves across a series of heights, with inclusion of bark surfaces colonies by a range of macroepiphytes (lichen, moss). Instruments capable of ppb CH_4 sensitivity should be used to measure CH_4 dynamics of the samples in closed chambers, alongside negative controls.

Secondly, atmMOB should be confirmed as the origin of phyllospheric CH_4 uptake and specific species should be identified. Genomics technologies such as targeted amplicon (e.g. pmoA), deep metagenomic,

and single-cell genomic sequencing could be used to identify putative atmMOB, followed by DNA stable-isotope probing after long-term incubation with ^{13}C-CH_4 to confirm uptake from atmospheric CH_4 concentrations.

To accelerate detection or isolation of atmMOB in samples, methanotroph enrichment using above-atmospheric concentrations of CH_4 could be performed as an alternative to long-term incubation at atmospheric CH_4 levels, followed by culture-based confirmation of growth at atmospheric concentrations.

14. Measure the heritability of methane flux in plantation trees and crops

Authors: Steve Strauss, Jean-Francois Lamarque, Mari Pihlatie, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic CH_4 emissions, we must maintain natural CH_4 sinks, minimize natural emissions increases, and develop novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) and dilute (up to ~1000 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Plants are increasingly recognized as important players in the methane cycle [42]. Estimates indicate they mediate CH_4 emission [11,43,44] and atmospheric CH_4 uptake [8] at a scale of 10s of Mt/yr. Cultivated forests and crops therefore may constitute a lever for maintaining CH_4 sinks, mitigating CH_4 emissions, and MR, if their CH_4 fluxes can be systematically influenced [6]. However, no methods for influencing CH_4 flux in plants are currently known.

Specific Bottleneck

Natural variability in methane flux exists between [54] and within [64] plant species. For example, Gauci, 2024 notes that uptake by stems of individual trees was measured up to ~6-fold higher than the mean in their biomes. Understanding the cause of this variation and how to influence it may illuminate opportunities for novel CH_4 emissions mitigation or MR approaches. In particular, if the methane flux trait is heritable, then breeding or genetic modification for low- or negative-methane crop or tree lines [65,66] may be a tractable approach. While some of the variability could be related to genetically-controlled factors, such as wood density or secondary metabolites [54], the degree of genetic control over methane flux is entirely unknown. Clearer information on the heritability of the methane flux trait and its genetic correlations is needed to inform whether breeding or genetic methods toward CH_4 emissions mitigation and MR should be pursued.

Actionable Goals

The heritability of the CH_4 flux trait should be investigated in any major plantation species and annual crops that are established to have substantial CH_4 fluxes. For species where methane fluxes are low, and/or costly to measure, methods development for flux quantification might be a prerequisite to heritability studies (as precise, low-cost measurements are typically needed on numerous–usually hundreds–of individuals in each breeding experiment). Coordinated CH_4 flux measurements should be made across many genetic variants by piggybacking on established breeding trials across countries and programs, using consistent protocols to ensure intercomparability [45]. The resulting data should be used to estimate heritability, genetic associations, and correlation to other traits, which can be used to calculate whether methane fluxes can be sustainably changed via selective breeding or genetic modification without changing other important traits.

Soil methanotrophy enhancement

15. Map the effects of agricultural practices on soil methane uptake

Authors: Jeremy Semrau, Hinsby Cadillo-Quiroz, Katey Walter Anthony, Whendee Silver, Eric Davidson, Marc Dumont, Ruth Varner

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7]. Agricultural soils are under human management and often have suppressed CH_4 uptake relative to natural soils [6,7], suggesting methanotrophy enhancement in agricultural soils as a potential MR pathway. Even modest changes in areal CH_4 flux could have a substantial impact if achieved across a large fraction of the ~40% of Earth's land that is used for agriculture. However, while it is known that reversion of agricultural land to a natural ecosystem can recover some CH_4 uptake capacity [6,7], it is not clear what actions can be taken to increase CH_4 uptake in soils under sustained agricultural use [12].

Specific Bottleneck

Enhanced methanotrophy in agricultural soils might be achievable through land management practices that expand the soil methanotroph niche or improve access to CH_4 and/or O_2 [12]. Any monetary incentive for soil CH_4 uptake will be small compared to the costs and revenue in agriculture, even with a reasonable price on CH_4, so the most promising pathways for soil-based MR would likely consider enhanced methanotrophy as a co-benefit incorporated as a secondary incentive in agricultural management choices.

However, CH_4 uptake currently is difficult to account for in agricultural management, due to a lack of well-designed field experiments that study the effects of agricultural management practices on CH_4 uptake under conditions that represent major agricultural regions and cropping systems, apart from rice. More fundamentally, there is a lack of understanding of spatiotemporal patterns and biogeochemical/environmental drivers of methane uptake rates, because most studies only measure net CH_4 fluxes. Further, the utility of the limited data that has been collected is hampered by a lack of standards for measurement protocols and metadata sharing.

Actionable Goals

A systematic study should be performed to map the CH_4 uptake impacts of agricultural practices, using standardized measurement and data protocols. The necessary scale of such a study will require coordination of national and international research institutions, NGOs, and the agricultural private sector to develop a comprehensive research agenda. The research plan should include identification of a set of agricultural management practices, cropping systems, and geographies necessary to represent major agricultural activities and regions.

The plan should be accompanied by clear standards for measurement of CH_4 flux, soil properties, and microbial drivers, as well as standards for sharing and archiving data along with a defined set of metadata. This work could begin with a pilot study on a small

number (~3-5) of sites, which would serve as a model for a larger research framework, and a synthesis effort to collect and harmonize existing published and unpublished data that meets the necessary standards.

Results should be used to calculate estimated recovery times for atmMOB after disturbances and the rate of change of atmMOB populations in response to interventions.

16. Characterize the methane sink in boreal forest snow

Authors: Katey Walter Anthony, Hendrik Schaefer, Lisa Stein, Hinsby Cadillo-Quiroz, Mari Pihlatie, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Current large-scale climate predictions ignore potential changes in methane fluxes from natural sources [3], resulting in a potential for misrepresenting future climate scenarios. It is crucial to characterize natural CH_4 sources and sinks and their underlying drivers, in order to more accurately predict climate trajectories, anticipate the impacts of human activities on atmospheric GHG concentrations, and understand possibilities for enhancing CH_4 uptake as a form of MR.

Specific Bottleneck

The boreal forest dominates land surfaces north of 50° N. Boreal forest is considered a net CH_4 sink; however, this is based on observations made almost exclusively during the snow-free season [70,71]. CH_4 flux in boreal forest snow is uncharacterized in the scientific literature, but preliminary evidence collected by an author of this problem statement suggests that boreal forest snow is unique in supporting methanotrophy, while snow in other areas does not (Walter Anthony, unpublished), suggesting a potentially important unaccounted-for CH_4 sink.

Any CH_4 flux in boreal forest snow may be affected by changes in snow-cover patterns, treeline, temperature, snowfall, rainfall, and spring thaw timing resulting from climate change; forest fire recovery; deforestation; changes in deposition of methanotrophs into snow; and other limiting factors. In absence of knowledge of the overall sink, the microbes responsible for CH_4 uptake and their source, and limiting factors on CH_4 uptake, it is not possible to estimate the sink's overall importance, predict future changes in the sink, or reveal levers, if any, for enhancing CH_4 uptake.

Actionable Goals

The following outcomes are needed:

- Spatiotemporally-resolved measurements of methane fluxes in boreal snow should be performed across the snow-covered season over transects from boreal forests to tundra and across a range of anthropogenic influence.
- Microbial communities that may be responsible for CH_4 uptake in boreal forest snow should be identified. They should be compared to potential sources of inoculum, which might include plant debris [72], soil-derived aerosols, animal feces, or atmospheric deposition, informing strategies for inoculation or preservation of key sources during land management.
- Measurement of CH_4 flux and microbial communities should be compared to alpine and arctic tundra, which may not host methanotrophs.
- Limiting factors on CH_4 flux in boreal forest snow should be identified, which might include dispersal of inoculum, temperature or other physicochemical parameters that limit colonization and growth, inhibitory substances (e.g. pollutants), and availability of nutrients including trace elements needed for methanotrophy (Cu, Fe, lanthanides). This will require field and laboratory experiments. Results should be associated with anthropogenic and natural influences that may affect future changes to the sink or enable sink maintenance/enhancement (see second paragraph of Specific Constraint)

17. Characterize hot spots and hot moments of soil methane uptake

Authors: Ruth Varner, Whendee Silver, Eric Davidson, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7]. Further, agricultural soils, which are under human management and often have suppressed CH_4 uptake relative to natural soils [67], may be amenable to methanotrophy enhancement as potential MR pathway. A general model for soil CH_4 uptake is necessary in order to anticipate changes in the soil CH_4 sink resulting from climate change and human activities, and to explore the possibility of methanotrophy enhancement. However, the leading soil CH_4 uptake model remains underparameterized due to a lack of understanding of the controls on soil methanotrophy [68]. Models also lack sufficient validation across several major biomes [68], resulting in a lack of generality for predicting soil CH_4 uptake under diverse scenarios.

Specific Bottleneck

A particularly exciting line of inquiry for soil-based methanotrophy enhancement is characterization of hot spots and hot moments of CH4 uptake, where uptake rates are observed at 2- to 10-fold above background rates over a few days 73–76. Understanding the underlying drivers of hot spots and hot moments may illuminate intervention pathways for methanotrophy enhancement, but models of these phenomena and data for analyzing them is lacking.

The lack of data on hot spots and hot moments and their drivers is largely due to a lack of distributed, continuous CH4 flux measurements in the field accompanied by characterization of microbial, biogeochemical, and physicochemical factors. This situation is exacerbated by a lack of an integrated framework and community of practice for field-based researchers to explore existing data 77; most past and current soil CH4 flux research measures net CH4 flux (i.e., does not distinguish uptake from emission, which requires isotope tracers 78,79) and is biased toward analysis of net emissions. A coordinated research effort among soil CH4 researchers across many sites to characterize and model hot spots and hot moments of CH4 uptake, if successful, would substantially contribute to a generalized model of soil CH4 flux and may illuminate pathways for MR via soil-based methanotrophy enhancement.

Actionable Goals

Research should be undertaken to characterize hot spots and hot moments of CH4 uptake and their microbial, biogeochemical, and/or physicochemical drivers. This will involve distributed, continuous measurement of soil CH4 flux in a manner that distinguishes uptake from emission, alongside genomic and chemical characterization of relevant environmental factors, as well as analysis and interpretation of results toward a general model.

This research will require coordinated effort among research groups across geographies, as well as a standardized approach to measurement, data analysis, and interpretation of results. Given that few researchers currently perform this kind of work, it would be helpful to actively coordinate experts to roadmap a coordinated research agenda and establish standard protocols, which should include a list of priority field contexts and environmental parameters that the work should cover. An effective approach may include an initial pilot study to establish the research pipeline at a small number of high-priority sites.

18. Integrate soil methane flux data to inform methane uptake models

Authors: Ruth Varner, Hinsby Cadillo-Quiroz, Eric Davidson, Marc Dumont, Katey Walter Anthony, Jeremy D. Semrau, Lisa Stein, Whendee Silver, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7]. Further, agricultural soils, which are under human management and often have suppressed CH_4 uptake relative to natural soils [67], may be amenable to methanotrophy enhancement as potential MR pathway. A general model for soil CH_4 uptake is necessary in order to anticipate changes in the soil CH_4 sink resulting from climate change and human activities, and to explore the possibility of methanotrophy enhancement. However, the leading soil CH_4 uptake model remains underparameterized due to a lack of understanding of the controls on soil methanotrophy [68]. Models also lack sufficient validation across several major biomes [68], resulting in a lack of generality for predicting soil CH_4 uptake under diverse scenarios.

Specific Bottleneck

While we have a strong understanding of some environmental factors influencing soil CH_4 uptake (e.g., diffusion limitation, energy limitation, ammonium inhibition), much variation in uptake, including causes for hot and cold moments of uptake [73,75,76], remains unexplained. To construct a practically useful soil CH_4 uptake model, new large datasets are needed that describe CH_4 uptake and environmental factors under diverse conditions and interventions [68,77,80].

While new measurements are necessary, existing data can also be better integrated to inform model development. Published data exists in variable formats, preventing straightforward integration into a larger, richer, coherent database that may enable uncovering of relationships between CH_4 uptake and environmental factors that are not clear within the smaller original datasets produced by individual studies. Further, we strongly suspect that a substantial quantity of existing relevant data has not been published, owing to a historical research focus on soil CH_4 emissions, to the exclusion of uptake. Utilization of unpublished data is hindered by a lack of coordination amongst researchers and a major decline of public funding. We anticipate that these suboptimal data practices will persist in future CH_4 flux research in the absence of an intentional intervention.

Actionable Goals

Necessary outcomes include:

- Existing published and unpublished soil CH_4 flux data, emphatically including CH_4 uptake, should be systematically aggregated and integrated into a public database, along with data on relevant environmental factors. Expert input should

inform relevant environmental factors, which may include methanotrophic strains and microbial communities, temperature, moisture, pH, redox, micronutrients, contaminants, soil structure, soil type, geochemistry, degree of soil degradation, land use, and climate factors.

- The database should be analyzed to generate the highest-quality CH_4 uptake model to date.
- Based on the database contents and updated model, knowledge gaps should be identified to inform future research.
- The database should be maintained as a growing community resource.
- Standard methods for measurement and reporting of CH_4 flux and associated metadata (e.g., various environmental factors) should be established and communicated to the research community, to readily enable ongoing contributions.

19. Develop affordable sensors for atmospheric methane uptake

Authors: Ruth Varner, Whendee Silver, Eric Davidson, Hinsby Cadillo-Quiroz, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

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Specific Bottleneck

To understand soil CH_4 uptake mechanisms and construct practically useful CH_4 uptake models, new large datasets are needed, based on natural and controlled experiments that measure CH_4 uptake and associated drivers under diverse conditions and interventions [68]. Obtaining such datasets requires many measurements, using instrumentation that is sensitive below ambient CH_4 concentration (<2 ppm), distinguishes CH_4 uptake from net CH_4 flux, and operates continuously to capture hot and cold moments of flux. Existing instrumentation meeting these requirements leverages isotope tracers and pool dilution approaches [78,79].

While existing instrumentation is effective, it is prohibitively costly to deploy at the scale necessary to obtain the large datasets required to characterize CH_4 across the diverse conditions relevant to soil methanotrophy enhancement and develop a general CH_4 uptake model. Low-cost metal oxide sensors are available, but are unreliable below ~50 ppm; make measurements that are sensitive to moisture, temperature, and other gases; and are complicated to calibrate [81–83].

Actionable Goals

Low-cost (ideally <$10k) soil CH_4 sensors are needed that, a) distinguish CH_4 uptake from net emissions; b) are sensitive below 2 ppm CH_4; c) are robust to environmental conditions such as temperature, moisture, and other gases; and d) are not prohibitively burdensome to calibrate. Bringing sensor cost below $10k would have substantial impact, while cost below $1k would be transformational. Such sensors should be made maximally available to the research

community to quickly enable widespread deployment, and should be accompanied by standardized protocols for deployment and analysis that facilitate production of datasets that can be readily integrated [80].

The needs addressed by this problem statement may also be partially addressed by providing broader community access to existing instrumentation, to minimize instrument downtime. This might be accomplished through shared instrumentation centers, perhaps accompanied by a grant to purchase instrumentation that serves the community.

In addition, increased production of quality CH_4 uptake data may be enabled by encouraging researchers who do have access to isotope instrumentation to conduct measurements that distinguish CH_4 uptake from net flux, for instance through a commentary in AGU Advances or other widely distributed publication.

20. Standardize measurement of soil methane uptake kinetics

Authors: Eric Davidson, Ruth Varner, Whendee Silver, Hinsby Cadillo-Quiroz, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7]. Further, agricultural soils, which are under human management and often have suppressed CH_4 uptake relative to natural soils [67], may be amenable to methanotrophy enhancement as potential MR pathway. A general model for soil CH_4 uptake is necessary in order to anticipate changes in the soil CH_4 sink resulting from climate change and human activities, and to explore the possibility of methanotrophy enhancement. However, the leading soil CH_4 uptake model remains underparameterized and lacks sufficient validation across several major biomes [68], resulting in a lack of generality for predicting soil CH_4 uptake under diverse scenarios.

Specific Bottleneck

The kinetic parameters of CH_4 uptake are key inputs to CH_4 uptake models [68,69]. Soil methanotrophy kinetics are typically summarized as the K_M (half-saturation constant) and V_{max} (maximum rate) of methanotroph communities in soil samples. While analogous to the kinetic parameters of the same names used in enzyme kinetics, K_M and V_{max} measured in soil samples or field sites are properties of the methanotroph community in the sample or site, which can change when the community changes, and can change even in pure cultures due to cellular regulatory processes that are not fully understood, including in response to CH_4 concentration [68,69]. As a result, the kinetic parameters measured in soil incubations and field studies are expected to vary as a function of experimental procedures, including manipulations to the sample/site prior to measurement, introducing an incompletely-understood confounding factor into measured values.

Uncertainty in kinetic parameters of CH_4 consumption contributes significantly to uncertainty in models of CH_4 uptake: a recent study examining this uncertainty obtained a 37-fold variation in sink estimates based on models constructed using kinetic parameters aggregated from literature [68,69]. As data continues to be collected in service of generating better models, it is crucial that kinetic parameters be calculated based on protocols that are intercomparable and accurately reflect the true parameters of methanotroph communities in natural environments.

Actionable Goals

The fundamental requirements for obtaining useful kinetic parameters to inform models of CH_4 uptake are to clarify which measurement protocols reliably obtain parameters reflective of natural environments, and to consistently implement such protocols across research activities. Necessary outcomes to achieve these requirements include:

- A review of experimental and measurement protocols currently in use for estimating kinetic parameters of CH_4 consumption, accompanied by a description of the confounding factors the protocols introduce and their effectiveness in reflecting the parameters of natural environments. This will include literature review, but may also require additional experiments.

- Determination of standardized protocols for measurement and data interpretation that result in kinetic parameter estimates that are intercomparable and useful for developing general models of soil CH_4 uptake.
- Research community buy-in to implement standardized protocols in future research.

Such a standardization initiative may benefit from an initial convening of experts, expert-led community outreach for feedback, a high-visibility publication describing the standards and their value, and continued community outreach to encourage community buy-in on the value and adoption of the standards.

21. Characterize mixotrophy and syntrophy in atmospheric methane oxidation by methanotrophs

Authors: Jeremy D. Semrau, Marc Dumont, Lisa Stein, Katey Walter Anthony, and Hinsby Cadillo-Quiroz, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7], and recent work suggests that phyllospheric methanotrophy in upland trees may constitute a comparable sink [8]. Maintaining these sinks is crucial, and both are under investigation as possible MR pathways via methanotrophy enhancement [3,6,12]. Methanotroph communities in bioreactors for MR or oxidation of dilute methane streams are also under consideration [3,5]. A process-based framework for methanotrophy in natural and engineered contexts is required to design management and engineering strategies and estimate upper bounds of MR, but such a framework is lacking. A central open question is how microbial metabolism is able to utilize trace methane concentrations [26,27] that provide an energy source near or below the expected minimum requirements for cell maintenance [12].

Specific Bottleneck

Some evidence suggests that microbes utilize trace CH_4 via mechanisms outside of pure methanotrophy, such as mixotrophy or syntrophy, at least to some extent. For example, several methanotrophs have been found to subsist mixotrophically on atmospheric concentrations of H_2 and CO, alongside ~2ppm CH_4 [26,28]. Also, some CH_4-oxidizing microbes have been found to oxidize other alkanes and other 2- to 3-carbon substrates [88,89], monooxygenases specialized for other alkanes have been found to oxidize CH_4 [90], and alkane monooxygenase genes are known to be exchanged by horizontal gene transfer [91].

Thus, there is indirect evidence that CH_4 may be oxidized through mixotrophic or syntrophic microbial communities. For example, phyllosphere-associated, alkane-trophic organisms may fortuitously oxidize methane via alkane monooxygenases, which could enable those organisms to consume the resulting methanol as an additional substrate, or might form the basis of a syntrophic relationship with the surrounding microbial community. However, we lack the data required to estimate the extent and magnitude of these CH_4 oxidation mechanisms in natural systems, or to leverage them in the design of reactor systems to optimize economical CH_4 oxidation.

Actionable Goals

Experiments should be performed to determine the extent of mixotrophic and syntrophic consumption of low concentrations of methane (~2 ppm) by microbial communities, as well as the range of other substrates involved. These experiments may include microcosms, consortia constructed top-down by enrichment of natural communities, and, if possible, consortia constructed via bottom-up synthesis of defined members, ideally across many combinations of microbes via multiplex studies. Stable isotope probing (SIP) and omic technologies should be used to interrogate community structure, function, gene

expression, and signalling [92–94] underlying carbon flow (e.g., from ~2 ppm CH_4, small organic acids and alkanes, co-factors) and energy flow.

Experiments should also be performed to investigate co-oxidation of CH_4 by alkane monooxygenases present in soil or the phyllosphere, including confirmation of co-oxidation and measurement of rates and affinities. The fate of resulting methanol should also be investigated to determine if it is further oxidized, incorporated into biomass, and/or secreted into the environment.

22. Characterize methanotrophy in the upland rhizosphere

Authors: Katey Walter Anthony, Jeremy Semrau, Hinsby Cadillo-Quiroz, Lisa Stein, Ruth Varner, Hendrik Schaefer, Marc Dumont, Rachel Strickman, Paul Reginato

Motivating Factor

CH_4 emissions have contributed ~0.5 ˚C of global warming to date [1], and natural sources may increase via feedback to warming [41]. On top of mitigating anthropogenic emissions, we must maintain natural CH_4 sinks and explore the feasibility of novel methane removal (MR) approaches to oxidize atmospheric (2 ppm) CH_4 at 1-100 $MtCH_4$/yr scale [2,3,13].

Methanotrophy in upland soils is an important global CH_4 sink, with CH_4 uptake estimated at 11-49 $MtCH_4$ annually [7]. Further, agricultural soils, which are under human management and often have suppressed CH_4 uptake relative to natural soils [67], may be amenable to methanotrophy enhancement as potential MR pathway. A general model for soil CH_4 uptake is necessary in order to anticipate changes in the soil CH_4 sink resulting from climate change and human activities, and to explore the possibility of methanotrophy enhancement. However, the leading soil CH_4 uptake model remains underparameterized due to a lack of understanding of the controls on soil methanotrophy [68]. Models also lack sufficient validation across several major biomes [68], resulting in a lack of generality for predicting soil CH_4 uptake under diverse scenarios.

Specific Bottleneck

Rhizospheric methanotrophic communities are known to strongly influence CH_4 fluxes in high-CH_4 lowland soils [84–86]. It is reasonable to hypothesize that rhizospheric methanotrophs may also influence methane fluxes in upland soils, but this has not been investigated to our knowledge. Given that rhizospheric microbial communities play a substantial role in other biogeochemical cycles and are influenced by plant exudates and intra-plant microbial associations [87], modulation of rhizospheric methanotrophy by plant activity may underlie some variability in CH_4 uptake rates, may change as plant communities respond to climate change or human activity, and may constitute a lever for methanotrophy enhancement. Methanotrophy in the upland rhizosphere, and its relationship to CH_4 uptake, should be characterized.

Actionable Goals

An initial baseline assessment should be conducted to characterize the extent of methanotroph colonization and activity in the rhizosphere of upland plants, including their density and taxonomic distribution across the soil-plant gradient (rhizosphere, rhizoplane, and internal tissues) and changes over time, alongside atmospheric CH_4 flux. This will require a significant and coordinated sampling effort across biomes (e.g., crops, drylands, uplands, forest), should represent dominant plants and distinct plant metabolisms, and should capture gradients in relevant physical and biogeochemical parameters (e.g., soil humidity, pH, C:N, nutrient availability, soil CH_4 concentration, and trace metal speciation/distribution, amongst other factors).

In contexts where rhizospheric methanotrophy substantially influences CH_4 flux, a more complex assessment should be undertaken to uncover plant-mediated controls on microbiome structure, methanotroph density and activity, and CH_4 flux.

The required research described above will require work by multiple research groups and currently lacks a standardized approach. Therefore, it would be beneficial to coordinate a network of collaborators to design an approach to ensure comparability across results, as well as to conduct a literature review of existing relevant information and methods.

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