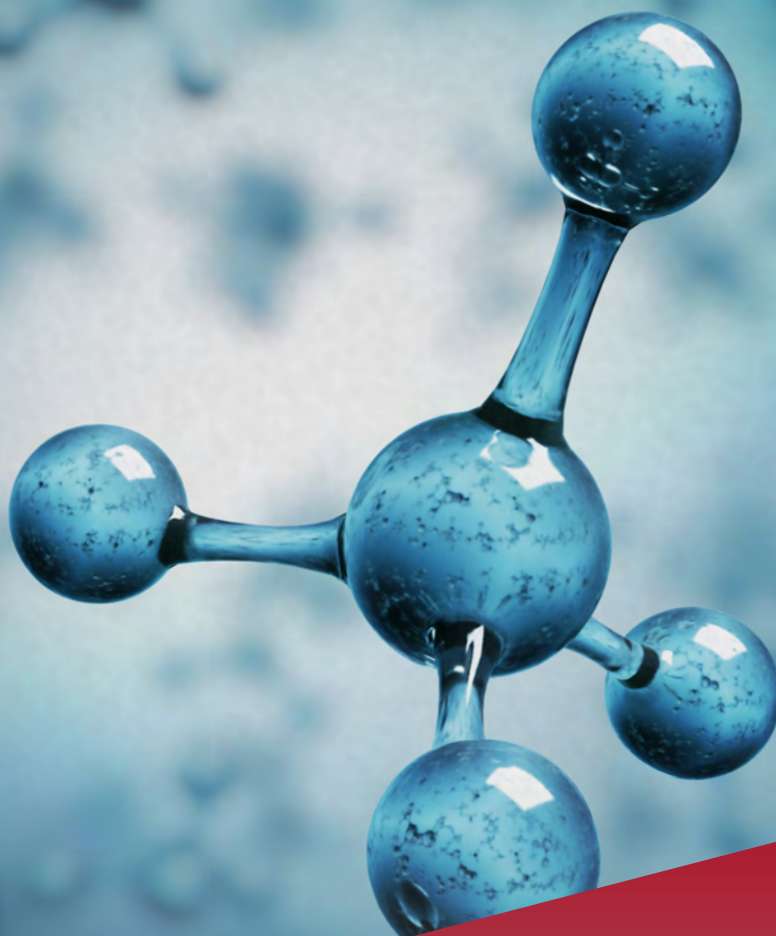


# COLLOQUIUM REPORT



## The Role of Microbes in Mediating Methane Emissions



AMERICAN  
SOCIETY FOR  
MICROBIOLOGY



# The Role of Microbes in Mediating Methane Emissions

Report on an American Academy of Microbiology (Academy) and The American Geophysical Union (AGU),  
Colloquium held on May 31 & June 1, 2023.

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# Executive Summary

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Increased greenhouse gases leading to climate change are recognized as the main driver of record-breaking global heatwaves, which threaten human health and well-being. Microorganisms are important producers and consumers of major greenhouse gases, including methane ( $\text{CH}_4$ ).  $\text{CH}_4$  is ~80 times as potent as  $\text{CO}_2$  on a mass basis at trapping heat in the atmosphere over a 20-year period, significantly contributing to a warming planet. As the most abundant organisms on Earth, microbes can make enormous contributions to the planet's climate by mitigating  $\text{CH}_4$  emissions.

This report is based on the deliberations of experts who participated in a colloquium on 31 May and 1 June 2023, organized by the American Academy of Microbiology, the honorific leadership group and think tank within the American Society for Microbiology (ASM), and the American Geophysical Union (AGU). These experts came from diverse disciplines and sectors to articulate opportunities to use microbes to mitigate  $\text{CH}_4$  emissions from four main sources: enteric fermentation in ruminants, animal wastes, rice paddies, and landfills. The participants highlighted knowledge gaps and potential strategies that harness microbial processes to mediate global warming and address climate change. The report states the recommendations of the colloquium participants for the scientific community as the next step to further our understanding of these topics.



# Introduction

**Climate change** is altering the planet and threatens humanity. Burning of fossil fuels, deforestation, and rapid population growth have contributed to climate change by increasing the concentrations of **greenhouse gases** in the atmosphere (USGRCP 2017). Heat retained by greenhouse gases has caused global temperatures to rise and alter climate in every region of the world, which can negatively affect human health, food production, and global economies (IPCC 2022). While human activities are largely responsible for the rise in greenhouse gas (GHG) emissions, microorganisms are major drivers of natural biogeochemical cycles that produce and consume these gases. While small, microorganisms are the most abundant organisms on Earth, and their contributions to the planet's climate are enormous because of their sheer numbers.

Microbes include **bacteria, archaea, viruses, fungi, algae,** and **protists** found in diverse ecosystems across the planet. Some microbes produce and consume the three greenhouse gases that have contributed most to global warming: **carbon dioxide** (CO<sub>2</sub>),

**methane** (CH<sub>4</sub>), and **nitrous oxide** (N<sub>2</sub>O) (Table 1). Microbes can thus help accelerate or slow the rise in global temperatures. Better understanding of microbes and microbial processes that add or remove greenhouse gases from the atmosphere can help us mitigate climate change's negative effects.

Earth's primary greenhouse gas most affected by human activity is CO<sub>2</sub>, which is responsible for 50% of the anthropogenic **greenhouse effect**, while CH<sub>4</sub> accounts for 30% (International Energy Agency 2022). Since pre-industrial times, CH<sub>4</sub> concentrations in the atmosphere have increased by 260%, compared to a 50% rise in CO<sub>2</sub> concentrations during that same time (International Energy Agency 2022). The average lifetime of CH<sub>4</sub> in the atmosphere is about 12 years, compared to hundreds of years for CO<sub>2</sub>. Within a 100-year time frame, each ton of CH<sub>4</sub> absorbs about 30 times more heat than per ton of CO<sub>2</sub> and in a 20-year time frame that increases to about 80 times more heat absorbed by CH<sub>4</sub> (Jackson et al. 2020). As a result, reducing CH<sub>4</sub> emissions is an effective way to slow greenhouse

**Table 1.** Lifetimes and global warming potentials (GWPs) of greenhouse gases produced and consumed by microorganisms (adapted from Table 7.1.5, Climate change 2021: the physical science basis, contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change); global warming potential is the factor that quantifies the heat trapping potential of each GHG relative to that of carbon dioxide (CO<sub>2</sub>) per unit mass.

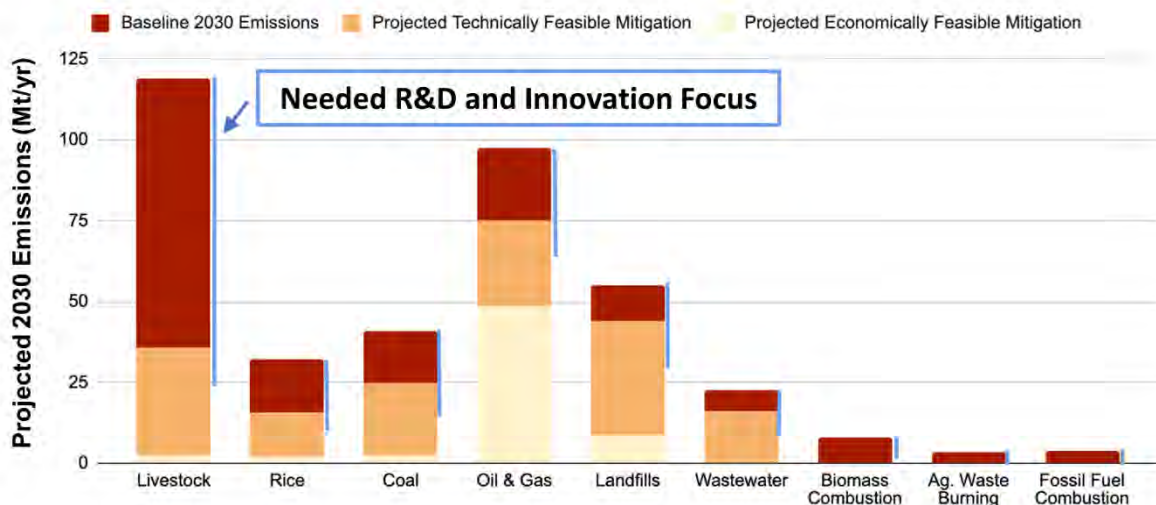
Greenhouse gas	Lifetime (Years)	Global Warming Potential (over 20 years)	Global Warming Potential (over 100 years)
CO <sub>2</sub>	Hundreds	1	1
CH <sub>4</sub>	12	80-83	27-30
N <sub>2</sub> O	109	273	273



warming in the near term, alongside CO<sub>2</sub> reduction efforts that will take much longer periods of time. A focus on CH<sub>4</sub> is not meant to defer or replace efforts to reduce CO<sub>2</sub> and N<sub>2</sub>O emissions, as mitigation of all greenhouse gases is urgently needed. Rather, the emphasis here on CH<sub>4</sub> is to ensure that this highly effective tool to slow the rate of warming is not overlooked and is rigorously pursued.

To aid in this endeavor and help achieve the Paris Agreement temperature goals, the global community launched the Global Methane Pledge in 2021 at the 26th United Nations Climate Change Conference of the Parties (COP26) in Glasgow. This initiative aims to reduce global CH<sub>4</sub> emissions by at least 30% from 2020 levels by 2030. To date, more than 100 countries have signed the pledge and more than 50 have developed national CH<sub>4</sub> action plans (U.S. Department of State). Curbing CH<sub>4</sub> emissions is considered the most effective strategy to limit global warming and its negative effects on society in the short term,

but reaching this goal will take novel approaches and innovative solutions. Most of the efforts so far on the Global Methane Pledge have focused on the fossil fuel industry, where significant abatement strategies are already relatively low cost. However, to meet the ambitious pledge target, emission abatement will also be needed in agricultural and waste management sectors, where the CH<sub>4</sub> sources are predominantly microbial. Fortunately, the diverse metabolic capabilities of microorganisms can be harnessed to reduce global CH<sub>4</sub> emissions, but most potential abatement options in these sectors are not yet economically or technologically viable (Fig. 1). Investment in research and development is urgently needed in agricultural and waste management sectors to develop microbial solutions that can help reduce CH<sub>4</sub> emissions from these sectors. Abating CH<sub>4</sub> emissions now and in the next two decades will also reduce future positive feedbacks to climate change, including warming-induced emissions of CH<sub>4</sub> and other greenhouse gases from natural ecosystems.

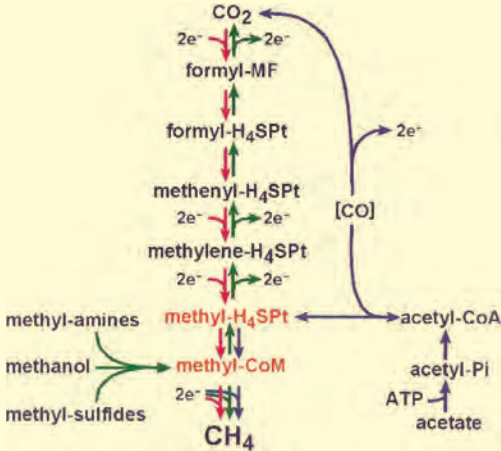
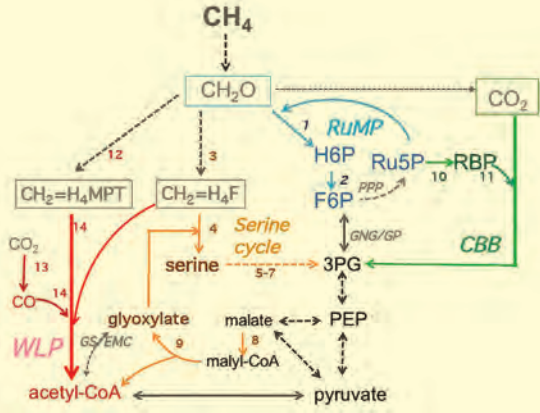


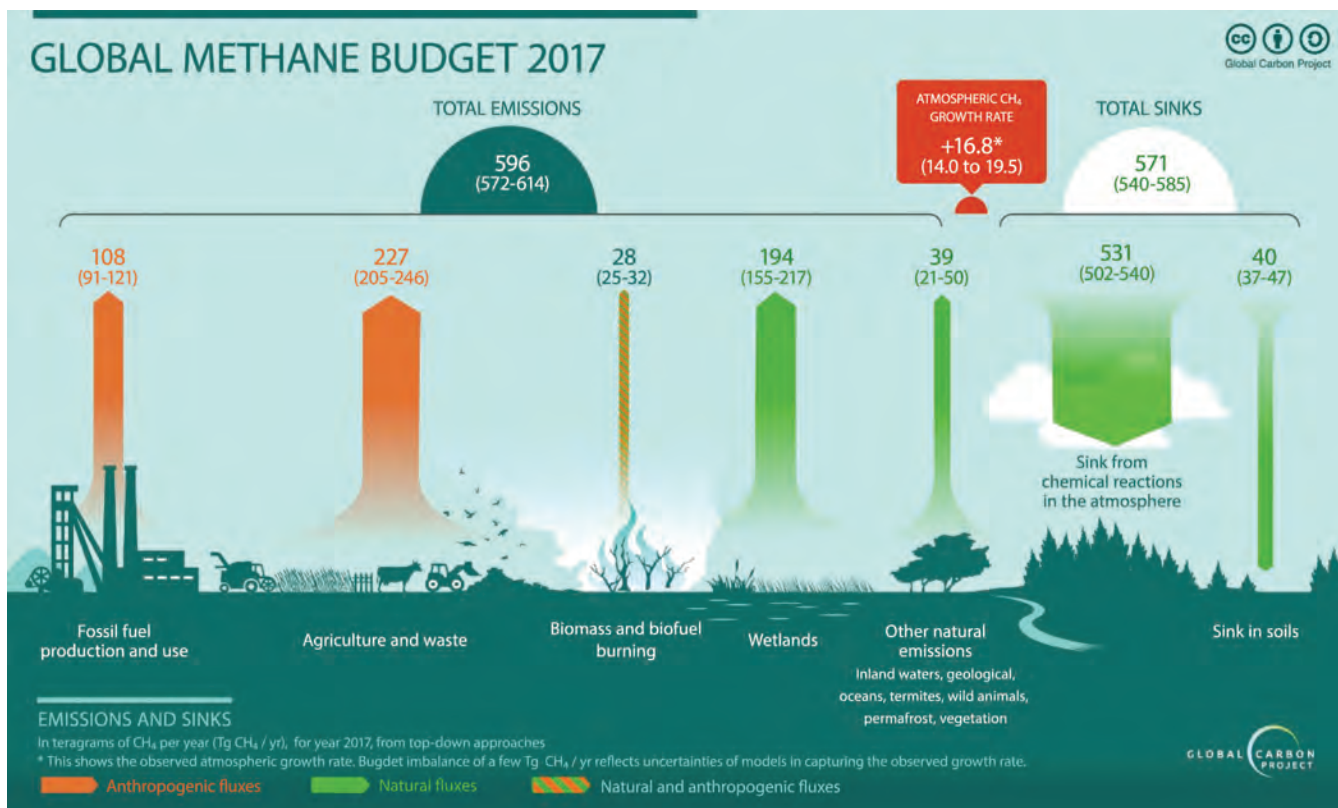
**Figure 1.** Projected 2030 global anthropogenic CH<sub>4</sub> emissions by sector, stratified by the portions that are economically feasible at no net cost (light beige), technically but not yet economically feasible (orange), and for which mitigation is not expected to be either technically or economically feasible (red). The blue lines highlight the emissions where research and development and innovation are urgently needed to develop mitigation options, most of which would require improved management of methanogenesis or methanotrophy in agricultural and waste management sectors. Drawn based on data in Ocko et al. 2021.

# Microbes as Methane Sources and Sinks

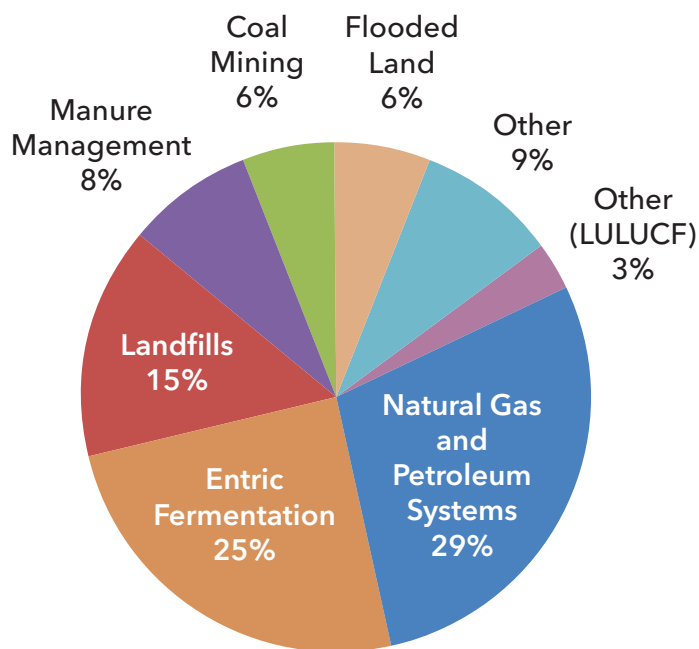
Methanogens and methanotrophs are microbes that produce and consume CH<sub>4</sub>, respectively. These groups have different evolutionary histories and grow in contrasting environments. Methanogens are archaea that grow in the absence of oxygen (anoxic environments), while methanotrophs are bacteria, many of which respire oxygen (oxic environments). Many terrestrial environments such as wetlands and rice paddies contain oxic and anoxic

microsites that either are spatially close to each other or change over time. The coexistence of methanogens and methanotrophs in a single ecosystem can be explained by spatial and temporal variations in oxygen availability. Reduced CH<sub>4</sub> emissions can be achieved either by preventing or suppressing CH<sub>4</sub> production (**methanogenesis**) or by promoting CH<sub>4</sub> uptake and conversion to CO<sub>2</sub> (**methanotrophy**) in sinks. Basic microbiological knowledge of these processes, as well as their application, can be developed and applied for CH<sub>4</sub> reduction efforts.

Methanogenesis	Methanotrophy
<p>Methanogenesis is the production of CH<sub>4</sub> by members of the domain archaea using fermentation products generated by other microbes during degradation of plant or animal matter (Fig. 2). Methanogenesis requires anoxic conditions and a low redox potential, such as in animal digestive tracts (particularly the rumen), saturated soils, wetlands, deep sediments, animal waste lagoons, and compost piles. Methanogenesis occurs by three general pathways (Berghuis et al. 2019). Hydrogenotrophic methanogens obtain energy by using H<sub>2</sub> to reduce CO<sub>2</sub> and produce CH<sub>4</sub>. Aceticlastic methanogens split acetate, oxidizing the carbonyl portion of the molecule to CO<sub>2</sub> and reducing the methyl portion to CH<sub>4</sub>. Methylotrophic pathways involve CH<sub>4</sub> production from the methyl portions of compounds such as methanol, methylamines, and dimethylsulfide. All three pathways have the energy conserving terminal step catalyzed by the methyl reductase enzyme shown in red.</p>  <p><b>Figure 2.</b> Methanogenesis biochemical pathways. Figure from Galagan et al. 2002.</p>	<p>Methanotrophy is the oxidation of CH<sub>4</sub> to CO<sub>2</sub> of ten under oxic conditions by taxonomically diverse bacteria possessing CH<sub>4</sub> monooxygenase (MMO) enzymes (Fig. 3). Two forms of MMO have been found: a copper-containing membrane-bound form (pMMO) and a cytoplasmic iron-containing form (sMMO). Methanotrophs have wide physiological diversity, with varying O<sub>2</sub> requirements, preferred CH<sub>4</sub> concentrations, temperature preferences, the ability to express either form of MMO, and the ability to fix N<sub>2</sub>. In anoxic environments containing nitrate, iron, manganese, or sulfate, CH<sub>4</sub> may also be oxidized to CO<sub>2</sub> by anaerobes capable of reducing these compounds to oxidize CH<sub>4</sub>, i.e., essentially methanogenesis in reverse (Caldwell et al. 2008).</p>  <p><b>Figure 3.</b> Main pathways for carbon assimilation by methanotrophs. RuMP, assimilatory ribulose mono-phosphate pathway (blue); serine cycle (orange); CBB, Calvin cycle (green); WLP, the reductive acetyl-CoA (Wood-Ljungdahl) pathway (red). Figure from Kalyuzhnaya et al. 2019.</p>



**Figure 4.** The global CH<sub>4</sub> budget for year 2017 based on natural sources and sinks (green), anthropogenic sources (orange), and mixed natural and anthropogenic sources (hatched orange-green). Image from Jackson et al. 2020.



**Figure 5.** Source of U.S. CH<sub>4</sub> Emissions. LULUCF stands for Land Use, Land-Use Change, and Forestry. Image from U.S. Environmental Protection Agency.

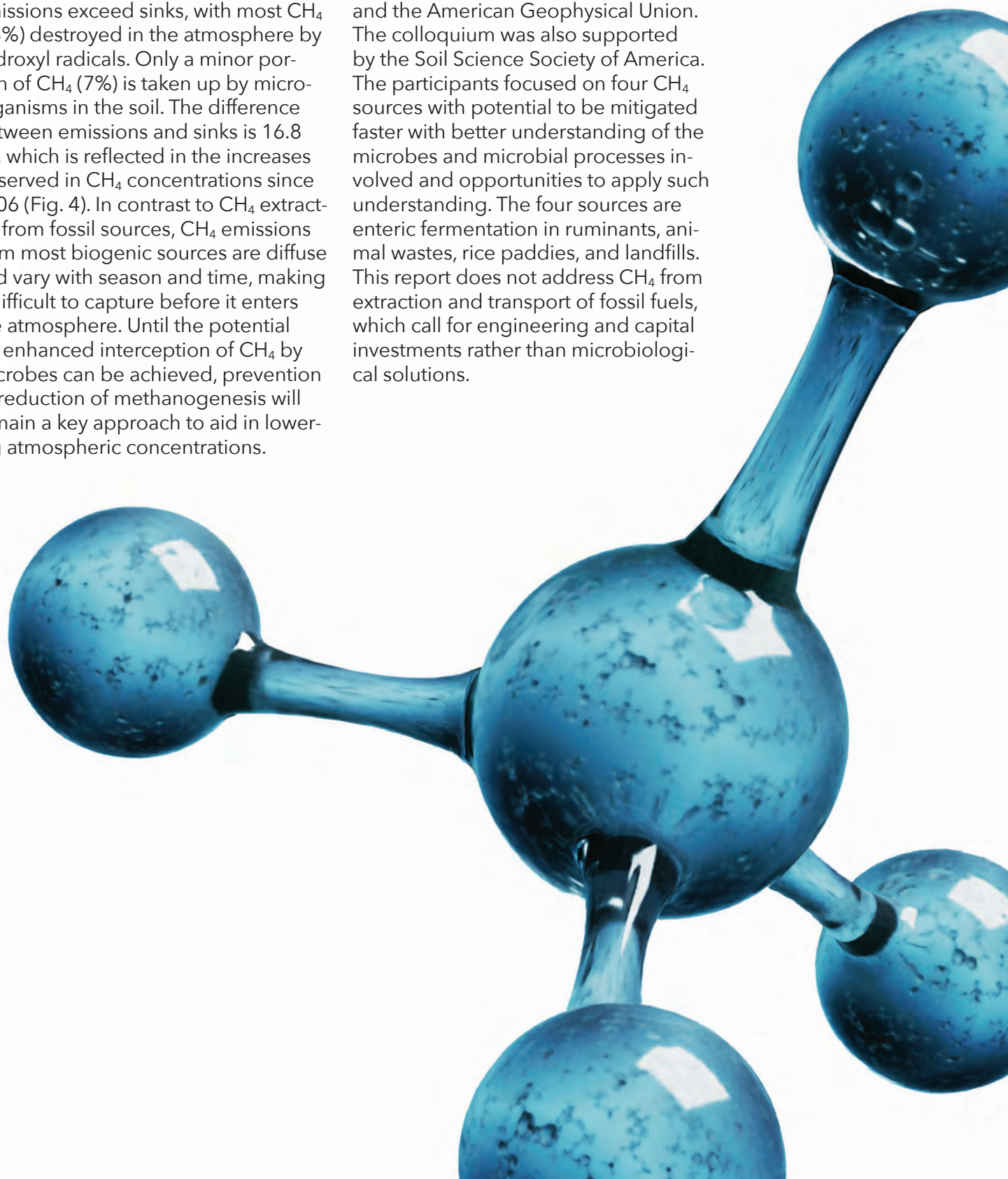
All CH<sub>4</sub> is produced through anaerobic microbial decomposition of organic matter but differs according to the time during which decomposition and CH<sub>4</sub> formation occurs. Fossil CH<sub>4</sub> is a non-renewable energy resource because it was formed from organic matter decomposition that occurred millions of years ago after being concentrated and buried by geological processes. Biogenic CH<sub>4</sub> is formed by contemporary microbial processes and is considered a renewable energy resource if it can be collected and captured. Since 2006, CH<sub>4</sub> concentrations in the atmosphere have risen rapidly, with annual emissions from biogenic sources now estimated to be three times greater than CH<sub>4</sub> emissions from fossil fuels (Schaefer et al. 2016; Lan et al. 2021).

The total flux of CH<sub>4</sub> entering the atmosphere every year is estimated at 600 Tg (1015 g) (Fig. 4). Anthropogen-



ic sources are estimated to account for 60% of  $\text{CH}_4$  emissions, although there are large uncertainties in global  $\text{CH}_4$  budgets. As observed in the U.S., about two-thirds of the anthropogenic  $\text{CH}_4$  emissions come from agricultural and waste sources, while one-third comes from fossil fuels (Fig. 5). Annual emissions exceed sinks, with most  $\text{CH}_4$  (33%) destroyed in the atmosphere by hydroxyl radicals. Only a minor portion of  $\text{CH}_4$  (7%) is taken up by microorganisms in the soil. The difference between emissions and sinks is 16.8 Tg, which is reflected in the increases observed in  $\text{CH}_4$  concentrations since 2006 (Fig. 4). In contrast to  $\text{CH}_4$  extracted from fossil sources,  $\text{CH}_4$  emissions from most biogenic sources are diffuse and vary with season and time, making it difficult to capture before it enters the atmosphere. Until the potential for enhanced interception of  $\text{CH}_4$  by microbes can be achieved, prevention or reduction of methanogenesis will remain a key approach to aid in lowering atmospheric concentrations.

This report is based on the deliberations of experts in microbial sciences who participated in a colloquium on 31 May and 1 June 2023, organized by the American Academy of Microbiology, which is the honorific leadership group and think tank within the American Society for Microbiology, and the American Geophysical Union. The colloquium was also supported by the Soil Science Society of America. The participants focused on four  $\text{CH}_4$  sources with potential to be mitigated faster with better understanding of the microbes and microbial processes involved and opportunities to apply such understanding. The four sources are enteric fermentation in ruminants, animal wastes, rice paddies, and landfills. This report does not address  $\text{CH}_4$  from extraction and transport of fossil fuels, which call for engineering and capital investments rather than microbiological solutions.



# Enteric Fermentation in Ruminants

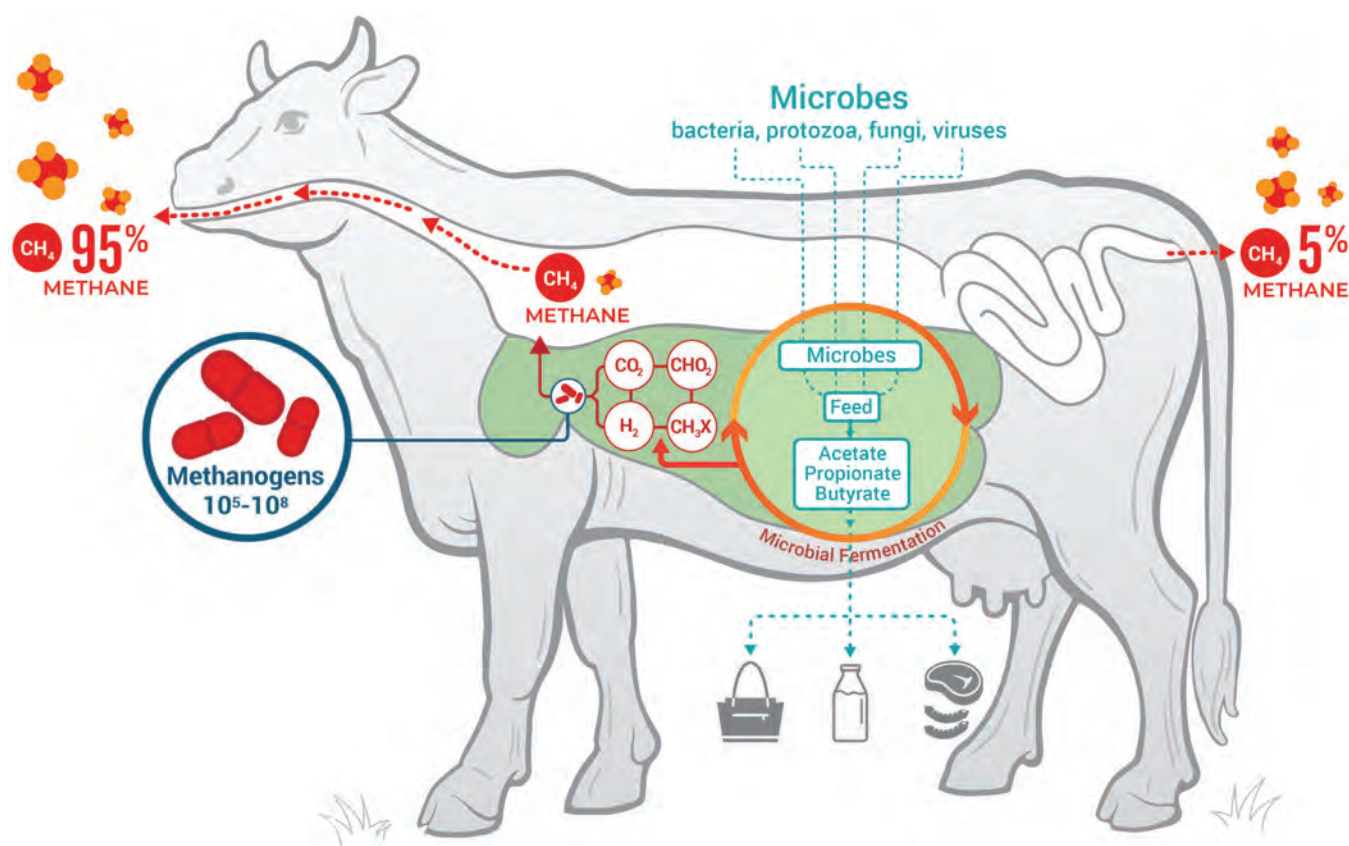
## Overview of CH<sub>4</sub> Emissions from Enteric Fermentation in Ruminants

The **ruminant** is a specialized and evolutionarily advanced animal that uses fermentation in a foregut to break down plant materials that other animals cannot digest (Fig. 6). Ruminants are characterized by their large pre-gastric foregut, known as the rumen, that maintains a diverse but concentrated community of anaerobic bacteria, methanogenic archaea, ciliate protozoa, and anaerobic fungi responsible for a variety of degradative and fermentative reactions. The **rumen microbiome** is characterized by its high population density, wide diversity, and

complexity of microbial interactions. The rumen contains representatives of all three domains of life (Bacteria, Archaea, and Eukarya) as well as an abundant **phage** population.

Bacteria and methanogenic archaea have the greatest numbers in the rumen, but a variety of ciliate protozoa and anaerobic fungi are also abundant. Ciliate protozoa can contribute as much as half of the microbial biomass in the rumen. Bio-degradable organic matter, mainly plant cell wall polymers and other

**Figure 6.** Microbial digestion via fermentation occurs in the rumen and is where the majority of CH<sub>4</sub> is produced. Figure from Glasson et al. 2022.



dietary polysaccharides, are converted in the rumen into microbial biomass and short-chain fatty acids, primarily acetic, propionic and butyric acids, and occasionally non-volatile lactic acid, as well as CO<sub>2</sub>, CH<sub>4</sub>, and ammonia. The mutualistic microbial fermentation in the rumen is based on digestion of the plant cell wall by cellulase and hemicellulase enzymes and synthesis of microbial proteins often from poor-quality dietary (forage) protein and non-protein nitrogen mainly via ammonia as precursor. Rumen microbes also synthesize vitamins B and K and break down toxic compounds like phytotoxins and mycotoxins. In turn, the host animal provides a mechanism for the selection and harvesting of feed, maintaining a high level of nutrient supply, temperature regulation, and buffers pH through addition of saliva and absorption and removal of acidic end products of fermentation. The rumen also provides an osmotically controlled environment for microbes and removes soluble inhibitory end products of digestion as well as undi-

gested particulate matter (residence time 1–2 days).

Rumen fermentation produces large volumes of gas that are eliminated mostly via eructation (belching). CH<sub>4</sub> emissions from enteric fermentation in livestock, mainly ruminants, account for the single largest agricultural source of anthropogenic greenhouse gas emissions. These considerations have led to an increase in efforts to identify technologies to mitigate ruminant CH<sub>4</sub> emissions by redirecting electron flow (energy) using a number of strategies. Cattle can produce between 250 and 500 L of CH<sub>4</sub> per day (equivalent to 175–350 g of CH<sub>4</sub> per day). Gas composition in the rumen is approximately 65% CO<sub>2</sub>, 27% CH<sub>4</sub>, and 7% N<sub>2</sub> with traces of oxygen and hydrogen. The traces of H<sub>2</sub> are evident at the early stages of the feeding cycle when fermentation rates are highest with fresh intake of carbohydrates. CH<sub>4</sub> eliminated by eructation represents a loss of between 3% and 12.5% of the digestible energy available to the host animal.

**Table 2.** Global numbers of domestic ruminants in 2021.

<b>Continent and country</b>	<b>Cattle</b>	<b>Buffalo</b>	<b>Goats</b>	<b>Sheep</b>	<b>Camelids*</b>	<b>Equids#</b>
<b>Africa</b>	373,000	1,263	481,000	416,965	34,270	42,160
<b>Asia</b>	467,505	200,183	571,891	571,213	8,631	28,278
<b>Oceania</b>	35,010	0.2	4,374	93,822	-----	351
<b>Australia</b>	24,431	-----	3,934	68,047	-----	219
<b>Europe</b>	114,709	482	15,157	121,652	7	500
<b>North America</b>	104,847	-----	2,616	5,961	-----	11,123
<b>South America</b>	372,855	2,045	23,679	63,827	8,631	17,515
<b>World total</b>	1,530,000	203,939	1,111,284	1,284,851	47,927	111,036

Large animal inventory (in thousands of head) for Ruminant and Non-ruminant animals in 2021. Data obtained from FAOSTAT (<https://www.fao.org/faostat/en/#home>)

\* Camelids = Camels + Other camelid species

# Equids = Asses+ Horses + Mules/Hinnies



Rumen methanogens have been recognized as belonging to the phylum Euryarchaeota, but new lineages of methanogens have been discovered (Berghuis et al. 2019). Methanogens are unique in their metabolic ability to convert a range of fermentation products to  $\text{CH}_4$ . They possess the enzymes and co-factors that transfer electrons from molecular hydrogen ( $\text{H}_2$ ) and formate ( $\text{HCOO}^-$ ) ultimately to  $\text{CH}_4$ , which is the primary electron sink disposed of through eructation. If methanogenesis is inhibited,  $\text{H}_2$  and/or formate accumulate in the rumen, so the ability of primary fermenters to regenerate electron carriers may become blocked, thereby disrupting microbial metabolism and growth. Consequently, methanogens help maintain the metabolic efficiency of primary fermenters, but the  $\text{CH}_4$  they produce represents a loss of energy to the animal.

In the rumen, hydrogenotrophic methanogens use  $\text{H}_2$  to reduce  $\text{CO}_2$ , and this pathway is thought to account for about two-thirds of terminal electron flow in the rumen. Some electron flow is facilitated by the process of interspecies hydrogen trans-

fer, which is the mutually beneficial unidirectional transfer of  $\text{H}_2$  gas from  $\text{H}_2$ -producing to  $\text{H}_2$ -utilizing bacteria. This coupled reaction maintains low partial pressures and makes  $\text{H}_2$  transfer thermodynamically feasible. Production of the reduced fermentation acids, propionate and butyrate, are also significant electron sinks in the rumen ecosystem and account for about one-third of the electrons generated during rumen microbial metabolism (19% and 14%, respectively). This electron disposal route is highly beneficial to the host animal, because virtually all of the propionate and butyrate is absorbed from the intestinal tract to support energy metabolism in the host animal.

Worldwide, cattle are the most numerous large ruminants (Table 2) and their numbers have increased due to rising demand for meat as countries undergo development. Together, small ruminants (goats and sheep) exceed the numbers of cattle and are particularly numerous in Africa and Asia. Wild ruminants are estimated to number 75,000 and thus form only a small portion of all ruminant animals (Hackman and Spain 2010).





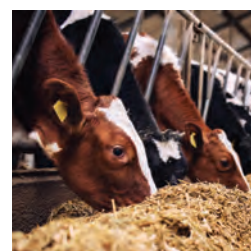
## Knowledge Gaps

A major knowledge gap is not understanding which methanogens are the key players in enteric CH<sub>4</sub> formation. Methanogens belonging to the order *Methanobacteriales*, such as *Methanobrevibacter* species (which utilize H<sub>2</sub> + CO<sub>2</sub> and/or formate), are thought to predominate, followed by a small percentage of *Methanosphaera* species (which reduce methanol with H<sub>2</sub>) and methylamine-utilizing *Methanomassiliicoccaceae* (Danielsson et al. 2017). The latter two groups constitute methylotrophic methanogens and, albeit at lower abundance, are thought to be responsible for a greater share of ruminal methanogenesis than previously thought (Poulsen et al. 2013; Sollinger et al. 2018). Notably, RNA-based approaches have revealed that methylotrophic methanogens are metabolically more active than hydrogenotrophic *Methanobrevibacter* species (Pitta et al. 2021; Pitta et al. 2022). Studies have shown that populations of the genus *Methanobrevibacter* are inversely correlated to *Methanosphaera* in the rumen and that methylotrophic methanogens may outcompete hydrogenotrophic *Methanobrevibacter* owing to their lower requirements and thresholds for H<sub>2</sub> in the rumen (Thauer et al. 2008; Feldewert et al. 2020). In contrast, the abundance of methylotrophic methanogens is limited by the availability of methyl-donating substrates in the rumen (Pitta et al. 2022b).

While the amount of CH<sub>4</sub> formed is dependent on the distribution of CO<sub>2</sub>-utilizing and methylotrophic methanogens, the diversity of methanogens is dependent on several factors such as genetics, dietary composition, the amount of feed consumed and the rate at which the feed is digested, feeding behavior, and other dietary factors; most interactions are poorly understood and require further investigation. For example, ruminant diets rich in

choline, pectin, and glycine betaine are rapidly fermented by microbes to generate several methyl compounds, supporting a greater abundance of methylotrophic methanogens during the first few hours post-feeding but only on a restricted range of fresh natural pasture grazing in temperate environments (Sollinger et al. 2018; Pitta et al. 2021). The amount of CH<sub>4</sub> belched out from cattle is much higher during the first six hours after feeding, implying that methylotrophic methanogens may be contributing significantly to total CH<sub>4</sub> formation (Hristov and Melgar 2020). Interestingly, ruminants with a low-CH<sub>4</sub>-yield phenotype are enriched in *Methanosphaera* whereas the higher-CH<sub>4</sub> emitters have an abundance of *Methanobrevibacter* populations (Shi et al. 2014; Stepanchenko et al. 2023). How diet and animal genetics interact to shape methanogen communities also merits further research.

A second major knowledge gap is how the entire rumen microbiome responds to feed additives that inhibit methanogenesis. Molecular hydrogen released by H<sub>2</sub>-producing bacteria is a key intermediate that allows re-oxidation of already reduced co-factors to continue fermentation in the rumen (Ungerfeld et al. 2015). The high affinity of methanogens for H<sub>2</sub> gives them the competitive advantage to capture most of this H<sub>2</sub>, preventing other bacteria from using it. Inhibiting methanogens will cause increases in dissolved H<sub>2</sub> concentrations, which in turn alters upstream fermentation pathways. Some of these effects are transient while others persist longer term and ultimately affect the animals' health and productivity. Examples of shifts include a reduction in acetate, an increase in succinate and ethanol, and changes in H<sub>2</sub>-utilizing bacteria (Greening et al. 2019). Reductive acetogenesis is one of the most beneficial reductive processes not only because acetate



**The amount of CH<sub>4</sub> belched out from cattle is much higher during the first six hours after feeding.**

is the major fatty acid used by the animal for energy metabolism, but because it also mitigates CH<sub>4</sub> formation and sequesters CO<sub>2</sub> into acetate. Strategies that promote the flow of reducing substrates produced by fermenters away from methanogenesis and into alternative electron sinks have been a key target for rumen microbiologists. Even if effective feed additives can be developed, however, their deployment would only be applicable to large commercial operations but not grazing systems.

Interactions between ruminant diets and rumen microbiomes are of increased interest, particularly the response of H<sub>2</sub>-producing bacteria when methanogens are inhibited and how these changes interact with alternate H<sub>2</sub> sinks and other microbes. Previous attempts to obtain a full accounting of alternative reduced products have been unsuccessful (Ungerfeld et al. 2015). Whole-microbiome shifts coupled with metabolomics must be investigated to understand the short-term and longer-term impacts of methanogen inhibition on rumen function. Knowledge of factors influencing microbial dynamics in the rumen will aid the development of CH<sub>4</sub> mitigation strategies. One factor is the rate at which CH<sub>4</sub> is formed. For example, CH<sub>4</sub> formation via methylotrophic pathways occurs much faster than the CO<sub>2</sub> pathway, as the former involves extraction of methyl groups and then direct transfer to coenzyme M (Neil et al. 1978). In the CO<sub>2</sub> pathway, electrons are carried through several one-carbon compounds before methyl coenzyme M is formed. This may also explain why methylotrophic methanogens proliferate soon after feeding whereas CO<sub>2</sub> utilizing methanogens become active after several hours post-feeding since this time frame coincides with maximum volatile fatty acids and H<sub>2</sub> production (Pitta et al.

2021). CH<sub>4</sub> mitigation strategies can affect these pathways in different ways over different time frames.

A second factor is the amount and fate of unconsumed H<sub>2</sub> when methanogens are inhibited. Generally, four moles of H<sub>2</sub> are required to reduce one mole of CO<sub>2</sub> and form one mole of CH<sub>4</sub>, which is the case in the CO<sub>2</sub>-reducing pathway. However, the requirement of only one mole of H<sub>2</sub> to form one mole of CH<sub>4</sub> by methylotrophic methanogens has been ignored because the latter methanogens have been thought to play a minimal role in rumens (Kelly et al. 2019). The amount and fate of H<sub>2</sub> spared will depend on methanogen diversity dynamics, different H<sub>2</sub> flux mechanisms, and the relative availability of acceptor species (CO<sub>2</sub> versus methyl groups).

The third factor is the balance between specific methanogens and the prevalence of their syntrophic partners. It has been hypothesized that specific H<sub>2</sub>-producing bacteria may form cohorts with certain methanogens based on interspecies H<sub>2</sub> transfer mechanisms leading to the presence of distinct cohorts influencing host phenotype for CH<sub>4</sub> emissions (Kaplan-Shabtai et al. 2020). Besides syntrophic partners, eukaryotes can impact overall amounts of CH<sub>4</sub> produced. However, there are few studies of the role of eukaryotes such as fungi or ciliate protozoa in the rumen (Huws et al. 2018; Hagen et al. 2020). Overall, knowledge of methanogen diversity as well as the rumen microbiome as a whole is critical not only for CH<sub>4</sub> mitigation but for rumen function and performance of the animal host.

## Research Prospects and Challenges

When addressing the key microbial players of enteric CH<sub>4</sub> formation, a

major barrier is that a fraction of the diversity of bacteria and archaea found in the rumen microbiome have representative isolates (Seshadri et al. 2018). The majority of rumen microbiota are yet to be isolated for two key reasons. First, many microorganisms are obligately dependent on others that provide one or more essential nutrients for growth. These dependencies can often be eliminated by supplementing the growth medium with rumen fluid, which has been shown to increase cultivation efficiency substantially (Zehavi et al. 2018). Second, plating efficiency, fast growth, and high yields enforce a strong bottleneck for the cultivation of environmental microorganisms under laboratory conditions. The development of new microfluidic devices and in situ physiological approaches have eliminated many of these hurdles and promise to facilitate the cultivation of a larger fraction of the rumen microbiome (Hatzenpichler et al. 2020). The availability of uncultivated metagenome assembled genomes recovered from environmental sequences offer unprecedented insights into the uncultured majority that could help inform future cultivation strategies; however, there remains a paucity of environmental samples from diverse hosts, diets, and geographical areas, obscuring a larger hidden diversity (Stewart et al. 2019).

Anaerobic fermentation of complex polysaccharides in the rumen proceeds in a stepwise fashion that often leads to the production of hydrogen as a metabolic intermediate. The steady-state level of hydrogen acts as a thermodynamic lever and controls the flow of metabolites in the rumen. If the hydrogen partial pressure is high, the fermentation of sugars, alcohols, and acids becomes thermodynamically unfavorable. In contrast, as the hydrogen partial pressure drops, methanogenesis becomes thermody-

namically less favorable. Under these circumstances, alternative metabolites like lactate might replace hydrogen to sustain continued fermentation of feeds. Detailed studies of known and novel metabolites that can serve as redox-active intermediates would be informative in devising strategies to inhibit methanogenesis.

CH<sub>4</sub> produced in the ruminant digestive tract accounts for a substantial flux of both carbon and hydrogen and in theory could serve as a carbon and energy source for methanotrophic microbes. However, essentially nothing is known regarding the presence and activity of methanotrophs in this habitat. Culture-independent and culture-dependent methods can potentially identify aerobic methanotrophs (e.g., within the ruminant's oral cavity or on the ruminal wall, where O<sub>2</sub> may be available) or anaerobic methanotrophs (e.g., in the liquid or solid contents of the rumen or hindgut, where redox potentials are low). Establishment or enhancement of methanotrophs within the rumen may represent a target for enhanced activity to decrease net CH<sub>4</sub> emissions.

There are still unknowns about the distribution of methanogen diversity in the rumen and its contribution to total methanogenesis. Several studies report the effects of different inhibitors on enteric CH<sub>4</sub> emissions, but less so on individual methanogenic species. Most studies are limited to 16S rRNA genes or methyl coenzyme M reductase gene *mcrA* copy numbers of methanogens, or quantitative changes in a few individual methanogens using real-time reverse transcription polymerase chain reaction (rtPCR) (Pitta et al. 2021). It is generally accepted that methanogens may require advanced omics approaches to identify differences in individual methanogens in the rumen (Shi et al. 2014; Wallace et al. 2015; Pitta et al.



2022). It has been reported that the potent inhibitor 3-nitrooxypropanol (3-NOP) was more effective in inhibiting hydrogenotrophic *Methanobrevibacter*, even at smaller doses, while large doses were needed to inhibit methylotrophic methanogens (Duin et al. 2016; Pitta et al. 2022). Some studies have reported that while halogenated compounds such as bromoform and chloroform are effective in inhibiting CH<sub>4</sub> formation, prolonged exposure to halogenated compounds may induce the emergence of resistance mechanisms such as reductive dehalogenation (Glasson et al. 2022). For example, halogenated compounds inhibit the transfer of methyl compounds by interfering with methyltransferase enzymes. Higher doses or prolonged exposure could enable methyltransferases to perform cobalamin-mediated dehalogenation of active compounds to bypass the inhibitory effects. How these halogenated compounds interfere with individual methanogens remains to be elucidated. Therefore, the dose and effectiveness of inhibitors to mitigate CH<sub>4</sub> will depend on the methanogens present, and it becomes important to under-

stand how different methanogens interact with inhibitors to understand the short-term and longer-term effects on the rumen microbiota. Additionally, studies are needed on the use of combinations of inhibitors, particularly those having different modes of action that may act synergistically.

Strategies to inhibit CH<sub>4</sub> production should ideally be coupled to production of metabolites that can be used as energy sources (such as propionate) or anabolic precursors (such as cellular lipids or other components of biomass) by the host animal. Studies aimed at quantifying CH<sub>4</sub> emissions should include parallel measurements of animal performance to identify feeding and management strategies that maximize economically important production traits (particularly milk, meat, or wool production and feed efficiency).

## Potential Strategies

In the next decade, dietary feed additives or drugs to reduce CH<sub>4</sub> production (grams per day) or yield (grams per kilogram of dry matter





intake) are technologies that will likely be used to lower CH<sub>4</sub> emissions from ruminant systems. Two classes of compounds are being considered: rumen modifiers and direct inhibitors. Rumen modifiers are feed additives (i.e., ingredients) that modify rumen microbial metabolism and communities. Examples of rumen modifiers with the potential to reduce methanogenesis include unsaturated and medium-chain fatty acids, ionophores, redox mediators, secondary plant metabolites and extracts (e.g., essential oils, tannins, and saponins), lactate-producing probiotics, and biochar. Although rumen modifiers are often described solely within the context of carbohydrate fermentation and volatile fatty acid production (Firkins and Mitchell 2023), these compounds may influence biohydrogenation, redox balance, microbial protein synthesis and nitrogen use, pH homeostasis, hydrogen transfer, and methanogenesis. The modes of action for rumen modifiers must be defined, and possible modes include lowering ruminal methanogen or protozoal populations and enhanced propionate production.

In contrast, direct inhibitors of ruminal methanogenesis target specific enzymes. Two examples include 3-NOP and halogenated CH<sub>4</sub> analogues including bromoform. 3-NOP inhibits methyl-coenzyme M reductase, which controls the terminal catalytic reaction of CH<sub>4</sub> production. Bromoform, which can be fed to cattle as a component of *Asparagopsis taxiformis* seaweed or in pure form, interacts with reduced vitamin B12 to lower the activity of the cobamide-dependent methyl-transferase reaction of methanogenesis. Dietary 3-NOP supplementation reduces methanogenesis by approximately 32% in dairy cattle (Kebreab et al. 2023), although the efficacy of reduction is impacted by neutral detergent fiber and fat contents of the diet. Feeding *A. taxiformis* has been shown in some cases to reduce enteric CH<sub>4</sub> emissions by more than 80% (Wasson et al. 2022), but such results are inconsistent.

Widespread adoption of rumen modifiers or direct inhibitors may be limited by difficulties in scalability and the need for daily administration or alternative modes of delivery. Inhibito-



ry compounds risk the potential accumulation of hazardous residues that may compromise human food safety, rumen microbial adaptation may occur with long-term feeding, and there may be unknown influences of such technologies on animal health. The scientific community lacks a clear understanding of how rumen modifiers or direct inhibitors influence CH<sub>4</sub> emissions when fed in parallel or alternating sequence. Other poorly understood factors are the efficacy and safety of these compounds as impacted by other dietary ingredients, nutrition levels, microbial dysbiosis caused by extreme heat, and bacterial and host genetics. The use of multi-omics approaches is likely to reveal how the rumen environment changes when inhibitors are administered over both short and long terms. Research must also focus on how these compounds influence fecal nutrient and microbial composition as well as CH<sub>4</sub> and N<sub>2</sub>O emissions from manures.

**Enhanced efficiency of meat and milk production in developed countries has been shown to reduce CH<sub>4</sub> intensity.**

Enhanced efficiency of meat and milk production in developed countries has been shown to reduce CH<sub>4</sub> intensity (i.e., grams of CH<sub>4</sub> per kilogram of meat or milk). In India, Pakistan, and sub-Saharan Africa, recent projections suggest an increase in ruminant inventories to meet a growing demand for dairy-sourced foods

(OECD/FAO 2021). In these regions of the world, CH<sub>4</sub> emissions per unit of fat- and protein-corrected milk produced are greater than European and North American nations (Mazzetto et al. 2022). Improvements in animal genetics (e.g., crossbreeding), ration balancing programs (e.g., better feed chemistry and established nutrient requirements for indigenous breeds), management (e.g., heat abatement and synchronized breeding), and veterinary care are needed to enhance the efficiency of nutrient use for ruminant systems in these localities. Such efforts are of paramount importance, considering the high density of ruminants and humans in the developing world, with high incidence of malnutrition and low household incomes that are unlikely to support the use of CH<sub>4</sub> mitigation technologies without gains in animal performance.

Alternative strategies to reduce enteric CH<sub>4</sub> emissions from live-stock systems include vaccines and breeding programs for low-emitting animals. Such practices best suit the needs of pastoral and rangeland systems by reducing the need for daily human intervention. A limited number of studies have investigated the use of anti-methanogen vaccines to lower CH<sub>4</sub> emissions from ruminants (Baca-González et al. 2020). *In vivo* and *in vitro* studies have demonstrated results varying between a 20% increase to a 69% decrease in CH<sub>4</sub> emissions (Baca-González et al. 2020). Several challenges exist such as ensuring immunoglobulin binding to methanogen surface epitopes and achieving a high secretion of anti-methanogenic *Archaea* IgG and IgA antibodies from blood to saliva. Genetic selection for low-CH<sub>4</sub>-emitting cattle may be a global solution to reduce CH<sub>4</sub> emissions from the livestock sector. Recent studies have revealed moderate heritability of low-CH<sub>4</sub> phenotypes in both



dairy cattle (Manzanilla-Pech et al. 2022) and sheep (Pinares-Patiño et al. 2013). However, reductions in neutral detergent fiber digestibility and modified rumen fermentation are potential consequences which have not been fully investigated (Løvendahl et al. 2018; Stepanchenko et al. 2023). Further research to identify specific host markers that correlate with CH<sub>4</sub> emission phenotypes is necessary to allow robust screening of host populations for further breeding experiments. Additional research is also needed to determine the underlying mechanisms by which these markers facilitate decreased CH<sub>4</sub> emissions by the host's ruminal microbial community.

## Mitigation Opportunities

The vast numbers of ruminants worldwide make reducing CH<sub>4</sub> emissions enormously challenging but also potentially enormously impactful. Prospects for mitigating emissions from animals in confinement clearly have greater potential than from grazing animals. Colloquium participants identified opportunities for CH<sub>4</sub> mitigation through the acquisition and application of microbiological knowledge to act upon in short, mid-range, and long terms.

### Now

- Support academic training in anaerobic microbiology and physiology to expand the number of scientists skilled in isolating and characterizing the many uncultured rumen microorganisms that could be made available in international culture collections.
- Prioritize research on multi-omics studies to direct the flow of reducing substrates produced by fermenters away from methanogenesis and into alternative energetically favorable electron sinks.

- Perform expanded rumen microbiome studies to elucidate the relationship of microbial species, especially on the role of ciliate protozoa and anaerobic fungi in the rumen. This can include comparing transcriptomes and metabolites in high- and low-CH<sub>4</sub>-emitting animals to gain insight into which microbes may contribute to lower CH<sub>4</sub> emissions.
- Collaborate with industry to develop new methanogen inhibitors supported by investigating their interactions with diet and animal genetics.

### Next 5–10 years

- Expand physiological research and understanding about the rumen microbiome. This includes understanding how inhibitors impact the rumen microbial community and ways to decrease CH<sub>4</sub> emissions that do not negatively affect the host animal's health or production.
- Optimize livestock feed to reduce overall CH<sub>4</sub> emissions.
- Genetic selection for low-CH<sub>4</sub>-emitting ruminants and mobilization of breeding programs to increase availability of low-emitting breeds.
- Develop and evaluate vaccines against methanogens and rumen protists.
- Examine the capacity of aerobic and anaerobic methanotrophs to re-oxidize a portion of the CH<sub>4</sub> generated in the digestive tract.



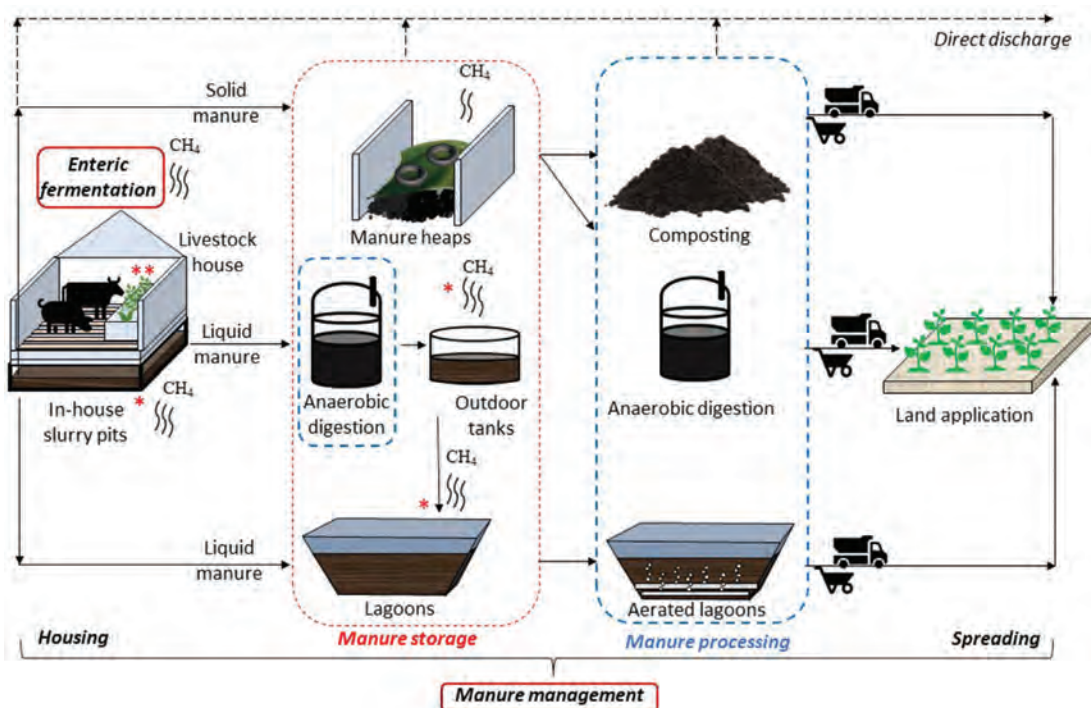
# Animal Wastes

## Overview of CH<sub>4</sub> Emissions from Animal Wastes

Manure is defined collectively to include both dung and urine produced by livestock (IPCC 2006). Manure management refers to all activities, decisions, and components used to handle, store, and dispose of feces and urine from livestock with the goal of preserving and recycling the nutrients in the livestock production system (IPCC 2006) (Fig. 7). This includes manure handling and collection in buildings, storage, processing, treatment, and application to land as well as deposition in pastures and on rangelands (Varma et al. 2021). Globally, manure management contributes about 10% of agricultural CH<sub>4</sub>, but in

confined livestock production systems (e.g., dairies and piggeries) with liquid manure management, this proportion can exceed 50% of agricultural CH<sub>4</sub> depending on climate (Owen and Silver 2015). Global estimates of emissions from all livestock manure management are about 10 Tg of CH<sub>4</sub>-C (Wolf et al. 2017).

CH<sub>4</sub> may be produced via microbial degradation of soluble lipids, carbohydrates, organic acids, and proteins present in manures. The decomposition of manure under anaerobic conditions during storage and treatment produces CH<sub>4</sub>.



**Figure 7.** Manure management in livestock production and potential mitigation targets for CH<sub>4</sub> emission. An asterisk indicates targets for additives to mitigate CH<sub>4</sub> emission from liquid manure storage in manure management. A double asterisk indicates feed additives as targets to mitigate CH<sub>4</sub> emission from enteric fermentation. Slurry can also be stored directly in an outside storage without prior anaerobic digestion. Figure from Ambrose et al. 2023.



- *Solid.* When manure is handled as a solid (e.g., in stacks, pits or piles) or when it is deposited on pastures and rangelands, it tends to decompose under mostly aerobic conditions, resulting in less CH<sub>4</sub> production. However, compaction in solid manure heaps may create anaerobic conditions that increase the potential for CH<sub>4</sub> emissions.
- *Liquid.* Livestock production systems generate liquid slurry, a mixture of feces and urine from housed livestock, mixed with bedding material and cleaning water. Storage of slurry is required to enable the spreading in the field at appropriate times to supply nutrients to crops. Manure management in cold climates requires storage capacity for at least 6 months in the United States (U.S. EPA). In Ontario, Canada, at least a 240-day capacity is required (OMAFRA). Thus, a major part of the slurry is transferred from housings to outdoor stores such as storage ponds and anaerobic earthen lagoons (Kupper et al. 2020). Slurries from lagoons generally have a lower dry matter and greater surface area compared to tanks, leading to higher emission potential. Up to 30% of the manure solids entering these systems are non-biodegradable and accumulate as sludge at the bottom of the storage system.
- *Biogas or anaerobic digestion.* Anaerobic degradation (AD) of organic compounds to simple substances by complex microbial consortia can be separated into four steps as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Hassa et al. 2018). The final biogas composition consists mainly of CH<sub>4</sub> (50%–70%) and CO<sub>2</sub> (30%–50%) and is determined based on feedstock composition

and operational parameters (e.g., temperature and pH) (Khoshnevisan et al. 2021). While AD is highly promoted due to the simultaneous stabilization of livestock manure and bioenergy production, the investment cost for such technology is a major barrier for its wide application.

CH<sub>4</sub> emission from manure management is largely dependent on ambient temperature and the composition and management practices of manure, including treatment, storage, and application methods (Petersen et al. 2013). According to the U.S. EPA greenhouse gas inventory, beef and swine contribute 55.9% and 32.4% of the total CH<sub>4</sub> from manure management (2.5 million metric tons CO<sub>2</sub> equivalents), respectively (Liu et al. 2021). However, estimates of CH<sub>4</sub> emissions from manure management may be low.

Methanogenesis is temperature dependent. In cold climates, where slurry freezes, higher emissions have been observed during spring thaw due to trapped CH<sub>4</sub> and de novo CH<sub>4</sub> production as temperatures increase above 0°C (Leytem et al. 2017). A study in Manitoba, Canada, found that CH<sub>4</sub> emissions from stored swine manure were lower than IPCC 2019 guidance due to manure freezing in winter and the short warm season, effectively limiting the rate of methanogenesis (VanderZaag et al. 2022). CH<sub>4</sub> emissions from soils are also temperature dependent and positively correlated with mean annual temperatures (Owen and Silver 2015).

Incomplete removal of slurry in farm-scale storages and lagoons can enhance CH<sub>4</sub> emissions due to the aged slurry acting as an inoculum (Wood et al. 2014). Mixing of solid manure piles and addition of fresh manure increased CH<sub>4</sub> emissions (Leytem et al. 2017).

## Why N<sub>2</sub>O is Important to Consider with CH<sub>4</sub> Mitigation

Both CH<sub>4</sub> and N<sub>2</sub>O are potent greenhouse gases with global warming potentials of approximately 80 and 273 times that of CO<sub>2</sub>, respectively, on a 20-year timescale (Table 1). In the atmosphere, CH<sub>4</sub> molecules are destroyed by hydroxyl ions and persist for 12 years on average, while N<sub>2</sub>O molecules persist for an average of 109 years. As a result, CH<sub>4</sub> mitigation efforts that result in higher N<sub>2</sub>O production will cancel out some or all of the climate benefits of reducing CH<sub>4</sub>.

Care must be taken when developing biological CH<sub>4</sub> removal strategies to avoid conditions that encourage microbial N<sub>2</sub>O production. The efficiency of biological CH<sub>4</sub> consumption technologies in natural or engineered systems relies on appropriate nutrient availability, including nitrate (NO<sub>3</sub><sup>-</sup>) or ammonium (NH<sub>4</sub><sup>+</sup>) as a nitrogen source. However, when NO<sub>3</sub><sup>-</sup> concentrations are high and when O<sub>2</sub> becomes limiting, such as in landfills and flooded soils, microbial activities will convert NO<sub>3</sub><sup>-</sup> into N<sub>2</sub>O, exchanging one greenhouse gas for a more potent one.

### Conditions Leading to Increased Risk of Microbial N<sub>2</sub>O Emission

Increased emission of N<sub>2</sub>O from manure storage, flooded soils, and landfills is connected mainly to nitrogen availability (high), copper availability (low), C:N ratio (low), and redox potential (moderate to low) (Lee et al. 2023; Chang et al. 2021). Nitrification and denitrification are the dominant microbial pathways for N<sub>2</sub>O production along with abiotic reactions of N oxides (Stein 2020). Interestingly, certain types of methanotrophs can also use NO<sub>3</sub><sup>-</sup> as a terminal electron acceptor under hypoxic conditions to release N<sub>2</sub>O while consuming CH<sub>4</sub> (Kits et al. 2015). High NO<sub>3</sub><sup>-</sup> levels in landfill and manure-amended soils can also slow methanogenesis, leading to lower CH<sub>4</sub> emissions under conditions that also promote N<sub>2</sub>O production (Lee et al. 2023). Thus, the main strategies to promote CH<sub>4</sub> removal while preventing N<sub>2</sub>O emission from landfill and agricultural soils are to simultaneously avoid fertilizers and amendments that promote high NO<sub>3</sub><sup>-</sup>

concentrations, maintain a high C:N ratio, ensure adequate copper availability, and limit anoxia.

### *Rice Paddies and Manure-Amended Fields: Nitrogen, Carbon, and Redox*

Rice paddies are especially strong emitters of both CH<sub>4</sub> and N<sub>2</sub>O due to the combined factors of high nitrogen availability, low C:N ratio, and periodic anaerobicity (Kritee et al. 2018). In efforts to curb CH<sub>4</sub> emissions from rice cultivation, farmers began to practice intermittent flooding of paddies, which resulted in up to 30–45 times more N<sub>2</sub>O emitted than that measured from continuously flooded fields, due to the promotion of nitrification and denitrification activities because high levels of nitrogen were applied (Kritee et al. 2018). The logical conclusion was that careful co-management of nitrogen, carbon, and water is required to keep both CH<sub>4</sub> and N<sub>2</sub>O emissions under control as high nitrogen availability, low C:N ratio, low copper availability, and low O<sub>2</sub> availability are the culprits that support microbial GHG emissions. Moderation in applying animal wastes to upland fields as well helps avoid spikes in N<sub>2</sub>O emissions following precipitation or irrigation events.

### *Landfills: Avoiding N-Spikes*

Landfills follow the same theme where CH<sub>4</sub> and N<sub>2</sub>O emissions increase when conditions favor high rates of methanogenesis, nitrification, and/or denitrification. To create an effective CH<sub>4</sub> biofilter, landfill soils and cover vegetation require a low-nitrogen management regime, proper aeration, and gas flow, among other engineering considerations (Gebert et al. 2022). However, even with careful engineering, spikes of N<sub>2</sub>O emissions can occur, particularly when aerobic processes like nitrification and abiotic N oxides reactions are stimulated from an overload of available nitrogen. Thus, to maintain control over both CH<sub>4</sub> and N<sub>2</sub>O emissions, constant surveillance and adjustment of total N, C:N, copper, and redox potential are essential practices.

Total CH<sub>4</sub> emissions per livestock unit from anaerobic digestors have been suggested to be about 10% of the emissions from anaerobic lagoons (Owen and Silver 2015). It has been suggested that CH<sub>4</sub> emissions from digestate storage are substantially lower compared to untreated manure, because part of the degradable matter (volatile solids) is consumed inside the biodigester, followed by a further decrease after solid separation (Maldaner et al. 2018). However, predicting CH<sub>4</sub> emissions from digestates is complicated by the fact that manure is often co-digested with other wastes, and how this affects emissions during storage depends on the substrate composition, as well as process parameters and storage conditions. Furthermore, fugitive CH<sub>4</sub> losses from biogas facilities including digestate storage and leakage during abnormal operations have been shown to have a significant impact on the efficiency of these operations (Balde et al. 2022).

In biogas production, the slurry (i.e., the digestate) needs a storage period of at least 3–6 months before field application. When stored, significant amounts of CH<sub>4</sub> can also be released. Baral et al. concluded that greenhouse gas emissions from manure management chains must focus on mitigating CH<sub>4</sub> emissions during manure storage, even in systems that incorporate AD (Baral et al. 2018).

## Knowledge Gaps

There are several pertinent knowledge gaps needed for reducing emissions from animal wastes. First, the influence of rumen fermentation management on subsequent manure land applications is not well defined. How microbial communities within manure transform as a result of changes in rumen fermentation processes is unknown, but they can have

serious consequences. Variabilities in feedstock quality can perturb microbial activities, sometimes jeopardizing the entire anaerobic digestion process. As for anaerobic digestors, the ideal microbial compositions and their ratios tailored for different feedstocks remain elusive. This gap in knowledge includes potentially unidentified microbial strains that could revolutionize digestion efficiency.

Second, the current understanding of emissions from manure is derived mainly from laboratory studies, with scant field-based data, which often report higher emissions. More field-based studies are needed from more diverse locations. Most research predominantly targets developed countries, which leaves gaps in understanding for developing regions and how cultural values and local customs impact waste management. Understanding the viability of sustainable practices in low-income areas will help inform global **climate-smart** practices.

Third, while research underscores composting as an efficient solid waste treatment method for carbon and nitrogen conservation and limiting greenhouse gas emissions, there remains a fundamental dearth in systemwide knowledge, especially concerning its scalability and applicability across diverse climatic and socioeconomic contexts. Comprehensive large-scale studies, supplemented by life cycle assessment and economic analysis, are essential to bridge this knowledge gap.

Fourth, the application of compost products for soil health and carbon sequestration is well documented but there remain some unresolved questions (Kutos et al. 2023). The longevity of inherent carbon and nitrogen in these products and their subsequent impact on a soil's



**Understanding the viability of sustainable practices in low-income areas will help inform global climate-smart practices.**

capacity to retain carbon and nitrogen, especially in relation to  $\text{N}_2\text{O}$  emissions and soil carbon sequestration by soil microbes, remains uncertain. To enhance composting management practices, the role of microbiology, particularly the deliberate introduction or enrichment of methanotrophs, stands out. Despite increasing research and development emphasis on managing methanotrophs in compost piles, there is a notable knowledge gap in this foundational area. This highlights the pressing need for detailed experimental research. Moreover, a holistic life cycle perspective addressing  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions from solid waste composting and its ensuing soil application is notably lacking (although see DeLonge et al. 2013).

The link between awareness campaigns, education, and sustainable practice adoption requires more study. Effective strategies for raising awareness in areas with greater adoption potential are under-documented. How stakeholders, from the public to policymakers, view and tackle waste management is vital due to its significant effect on mitigation outcomes.

## Research Prospects and Challenges

$\text{CH}_4$  emissions from manure management are largely dependent on regionality, as farm wastes, climate, management practices, and regulation differ globally (Wolf et al. 2017; Petersen et al. 2013). Additionally, varied feedstocks lead to heterogeneous manure characteristics and microbial populations with unknown impacts on overall  $\text{CH}_4$  emissions. This poses a challenge when designing strategies as each local environment and its inputs must be taken into account. This is especially important in the context of  $\text{N}_2\text{O}$

emissions, since  $\text{CH}_4$  mitigation actions may lead to increased  $\text{N}_2\text{O}$  (Box 1). For example, acidification can decrease  $\text{CH}_4$  emissions from slurry ponds, although slurry acidification may increase emissions of  $\text{N}_2\text{O}$  (Fangueiro et al. 2015). Thus, research is needed to understand the entire life cycle of animal wastes, starting with the animals' feedstock and digestive microbiome to manure storage and application, all the while tracking the relation between  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions throughout.

Anaerobic digestion and conversion of  $\text{CH}_4$  into alternative products are additional possible research prospects. Biogas from anaerobic digestion is mainly used for energy production, but gas fermentation offers an alternative means to utilize  $\text{CH}_4$  and  $\text{CO}_2$  for production of liquid fuels (e.g., sustainable aviation fuels, chemicals, materials, or proteins).  $\text{CH}_4$  and  $\text{CO}_2$  can also be reformed to syngas, which can be utilized by acetogens that also use  $\text{CO}_2$  with  $\text{H}_2$  (Köpke and Simpson 2020). Acetogenic gas fermentation is already being carried out at commercial scale and methanotrophic gas fermentation has been shown at pilot and demonstration scales (Köpke and Simpson 2020). Nevertheless, there is a lack of pilot infrastructure in the U.S. Another challenge to overcome is the common (mis)perception that anaerobic systems are technically difficult. Although some expertise is required, it can be provided through various outreach programs.

## Potential Strategies

While enteric fermentation is generally assumed to account for the majority of  $\text{CH}_4$  emissions, manure management is a significant contributor and, in some regions, accounts for over 50% of cattle emissions. Physical and chemical strategies can be used



to limit these emissions using better manure management can have an immediate impact on atmospheric CH<sub>4</sub> concentrations. Because the majority of these emissions come from liquid storage where a strongly anaerobic environment usually develops, physical strategies such as use of impermeable covers, such as lids, plastic film, and tiles, and vegetable oil, formation of natural crusts, aeration, and pasteurization can lower CH<sub>4</sub> emissions (Kupper et al. 2020; Ambrose et al. 2023). Chemical strategies such as acidification, addition of oxidizing agents, antimicrobial agents, and flocculating agents have also been identified as valuable tools for reducing emission rates from storage systems (Ambrose et al. 2023). Cost effectiveness, ease of implementation, and downstream consequences for land application of slurries and biogas production are all factors that need to be considered (Ambrose et al. 2023).

Diverting livestock manure to composting can lower emissions from anaerobic lagoons. Emissions were reduced by approximately 66% in one California study (Vergara and Silver 2019). Anaerobic digestion for energy production can also reduce emissions from manure management. Estimates from life cycle assessments vary widely ranging from 5% to 92% reduction in global warming potential (Adghim et al. 2020). However, there is still considerable uncertainty regarding fugitive emissions from anaerobic digestors. Given the high CH<sub>4</sub> production rates of anaerobic digestors, fugitive emissions can still provide an important emissions source. One meta-analysis estimated that fugitive emissions from biodigestors increase dairy CH<sub>4</sub> releases by 1.4% relative to storage in slurry ponds (Miranda et al. 2015).

Most manure is currently wasted, contributing to climate change and

exacerbating anthropogenic footprints on the environment. Compared to liquid manure management, anaerobic digestion is highly preferred due to the simultaneous stabilization of livestock manure and biogas production but requires some investment cost. Recent years have seen a strong development of anaerobic digestion units worldwide (Akhiar et al. 2020). There are around 20,000 biogas plants in the European Union (EU) (European Biogas Association, IFEU 2022), though a recent study suggests this is less than 3% of what is possible with most untapped potential in livestock farming (Bumharther et al. 2023). Specific drivers in the EU include requirements to increase renewable energy sources and high state incentives for renewable energies that led to a rapid increase of anaerobic digestion of 69% from 2010 (Akhiar et al. 2020). In many developing nations, anaerobic digestion is still at an early stage. More public awareness and stronger commitment from stakeholders like the government and investors are required (Roopnarai et al. 2017). While anaerobic digestion is a mature technology that originated in the 1970s, opportunities exist for further optimization of efficiency, more economic small-scale biogas plants, and integration with gas fermentation (Bumharther et al. 2023).

## Mitigation Opportunities

Reducing CH<sub>4</sub> emissions from animal wastes calls for increased on-farm awareness of stored slurries as CH<sub>4</sub> sources and more direct utilization of manures as feedstocks for composting or processing as stabilized fertilizers. Colloquium participants recognized efforts that affect upstream processes, like dietary and rumen microbiome modification, may also affect waste characteristics related to greenhouse gas emissions.



**Diverting livestock manure to composting can lower emissions.**

## Now

- Increase research on chemical additives, such as acidification, physical barriers, and the combination of mitigation strategies.
- Promote grazing management over confined feeding operations to encourage waste dispersal as much as possible in cattle production.
- Build infrastructure to divert livestock manure from anaerobic lagoons to composting or anaerobic digestion.
- Standardize protocols for production and application of composts to foster sustainability and minimize CH<sub>4</sub> emissions.
- Raise awareness of the need to prevent gas leakage from existing storage systems and digestors.
- Avoid adding fresh manures to stored manures, which act as methanogenic inoculum.

- Conduct models to look at management practices and outcomes to optimize the approach for each area and under a variety of climatic conditions.

## Next 5-10 years

- Reinforce incorporation of manure management as an integral component of climate-smart agriculture.
- Develop a holistic life cycle perspective addressing CH<sub>4</sub> and N<sub>2</sub>O emissions from solid waste composting and its ensuing soil application.
- Research the effect of inoculating manure storage with methanotrophs, and/or prebiotic additions to enrich for desirable methanotrophs, on reducing overall CH<sub>4</sub> emissions.
- Develop urban-rural linkages to expand use of animal manures in municipal composting programs.



# Rice Production

## Overview of CH<sub>4</sub> Emissions from Rice Production

Rice is a staple food and critical source of calories and nutrients for more than half the world's population, especially in many vulnerable nations of Asia and Africa (Zeigler and Barclay 2008; Fukagawa and Ziska 2019). Globally, average annual rice production in 2018–2020 was 509 million metric tons (Mt), exceeded only by wheat (753 Mt) and maize (1151 Mt) (Fig. 8). By 2030, global rice production is predicted to increase to 567 Mt (OECD/FAO 2021). Rice is also the third largest crop by harvested area (greater than 160 million hectares) after wheat and maize (Grogan et al.

2022). Increased rice production is a result of both increases in cultivated area (Davidson et al. 2018) and average yields that rose from 1.8 to 4.6 t/ha between 1961 and 2019 (Bin Rahman and Zhang 2023). These increased yields have been driven by improved rice varieties combined with more intensive farming practices (Zeigler and Barclay 2008).

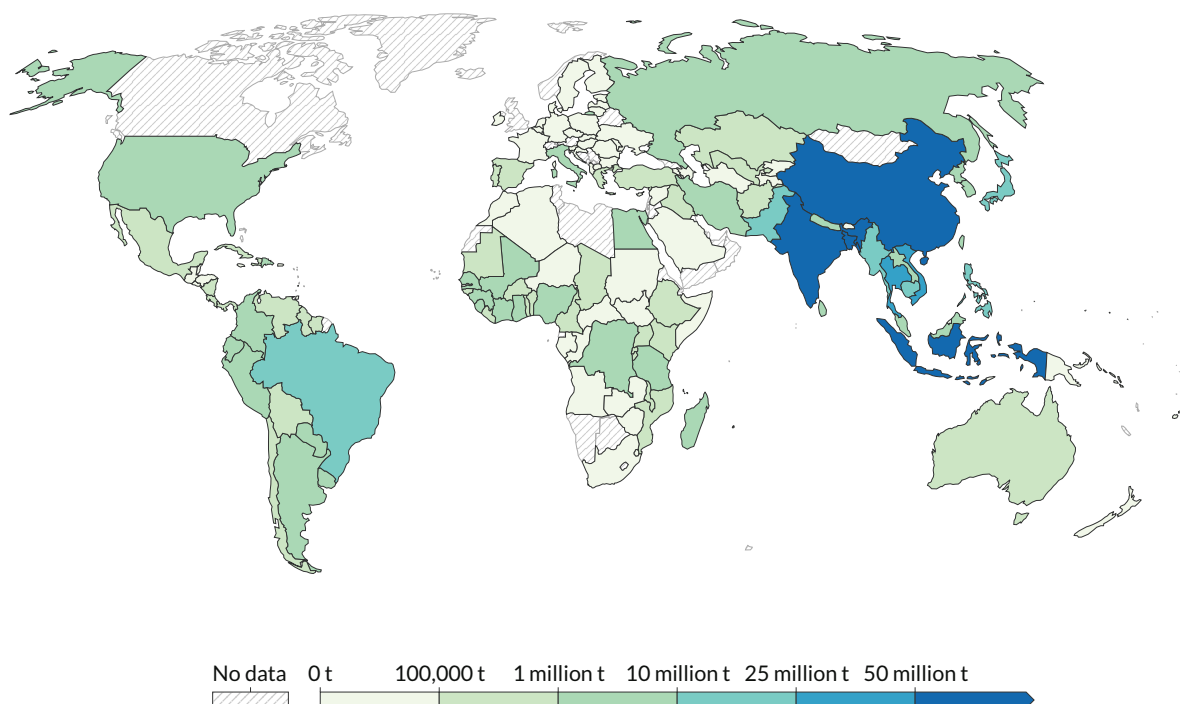
Rice is a semi-aquatic plant conventionally cultivated in low-lying or terraced wetlands (i.e., flooded paddy fields) that can be either rain fed or irrigated. The practice of flooding serves

**Figure 8.** Rice is produced throughout the globe. Source: <https://ourworldindata.org/grapher/rice-production>.

### RICE PRODUCTION, 2021

Rice production is measured in tonnes.

Our World  
in Data



Data source: Food and Agriculture Organization of the United Nations

OurWorldInData.org/agricultural-production | CC BY

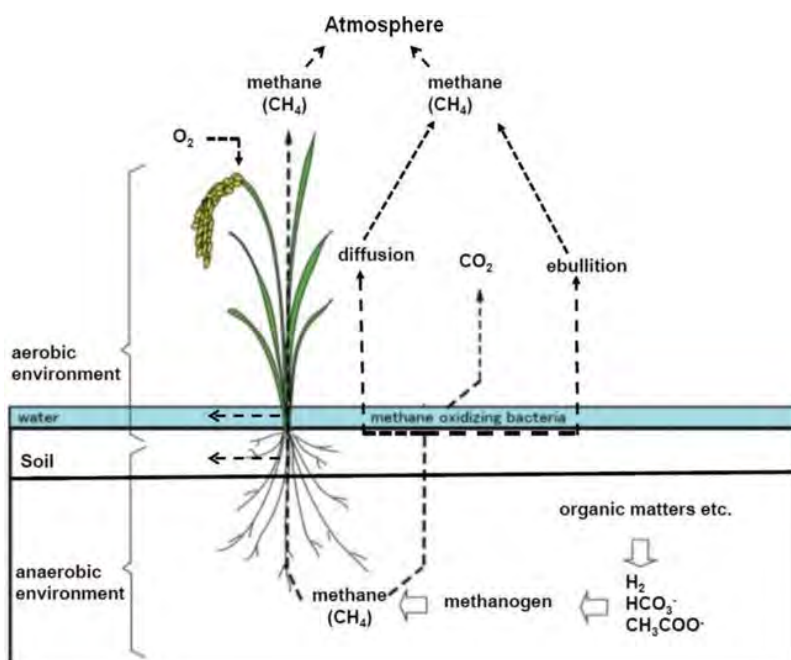


as a cornerstone of optimal rice production as it prevents drought stress, provides optimum supply of fertilizer, controls weeds/pests, and buffers against temperature extremes. On the downside, rice cultivation in flooded paddies is water intensive and leads to high  $\text{CH}_4$  release to the atmosphere (Bo et al. 2022) (Fig. 9).  $\text{CH}_4$  from paddies and other wetlands is the product of anaerobic decomposition of organic matter culminating in  $\text{CH}_4$  production by methanogenic archaea (Conrad 2007). The water regime, which under inundated conditions results in anoxia and low redox potential, is the most important controller of  $\text{CH}_4$  production in paddy soils, but other factors including soil texture and temperature, organic amendments, planting method, and plant variety also play a role (Zhang et al. 2016; Nikolaisen et al. 2023; Yan et al. 2009).

A variety of water-saving farming approaches for rice cultivation have been developed that are suitable for unflooded soils (Tuong and Bouman 2003). As a result, rice is grown in diverse agroecosystems ranging from permanently flooded to aerobic soils, commonly referred to as upland

or dryland cultivation. These tend to have much lower yields (approximately 1 t/ha) compared with flooded rice production (approximately 6–8 t/ha), but new aerobic high-yielding rice cultivars are being developed (Bernier et al. 2008; Saito et al. 2018). **Alternate wetting and drying (AWD)** of paddy fields is another approach, which increases the redox potential of the soil and leads to reduced methanogenesis (Asch et al. 2023; Bo et al. 2022; Nikolaisen et al. 2023; Weller et al. 2016; Zhang et al. 2016). Maintaining soil redox values between  $-150$  and  $+150$  mV is optimal because this range minimizes microbial activities leading to emissions of both  $\text{CH}_4$  and  $\text{N}_2\text{O}$  (Yu and Patrick 2004). Similarly, ridge and furrow irrigation decrease overall flooding and lead to reduced  $\text{CH}_4$  emissions (Hao et al. 2016). The trade-off of aerobic rice cultivation is increased  $\text{N}_2\text{O}$  production when nitrogen fertilizer is applied above optimal rates at inopportune times during the flooding cycle, which can offset the climate benefits of reduced  $\text{CH}_4$  emissions, particularly in the longer term (Kritee et al. 2018; LaHue et al. 2016). It is known that proper fertilizer timing is critical to reducing emissions.

**Figure 9.**  $\text{CH}_4$  production in rice. Image from Mahabubur Rahman & Yamamoto 2021.



Zhang et al. estimated that  $\text{CH}_4$  emissions from rice increased 85% from 1901 to 2010, resulting primarily from expansion of the rice cultivation area, but also from elevated atmospheric  $\text{CO}_2$  and nitrogen fertilizer use (Zhang et al. 2016). Global  $\text{CH}_4$  emissions during the 2008–2019 decade were 30 [25–38] Tg  $\text{CH}_4$  per year (Saunois et al. 2020), which was a decrease from about 45 Tg per year in the 1980s (IPCC 2021). The recent decrease has been attributed to plateauing of rice cultivation in Asia, but also to a northward trend in rice cultivation in China where  $\text{CH}_4$  production is lower (Bin Rahman and Zhang 2023; Saunois et al. 2020; Chen et al. 2013). Whether these decreases have resulted in part

from an increase in aerobic rice cultivation is not clear because the extent to which upland or AWD has replaced traditional methods in Asia is not well documented.

## Knowledge Gaps

Due to the global significance of rice as a food crop, we have a fundamental understanding of the flow of carbon in rice paddy systems. Flooding rice paddies creates anoxic conditions that result in anaerobic degradation of organic matter, terminating in the production of  $\text{CH}_4$  (Conrad 2007). Approximately two-thirds of methanogenesis in rice paddies is derived from acetate and one-third from  $\text{CO}_2$  reduction with molecular hydrogen (Conrad 1999). Methanogens come by these substrates as the products of either primary or secondary fermentation. In primary fermentation, organic matter inputs are broken down into monosaccharides and eventually fatty acids, alcohols, acetate,  $\text{CO}_2$ , and  $\text{H}_2$ . In secondary fermentation, fatty acids and alcohols from primary fermentation are converted into acetate and  $\text{H}_2$  by so-called syntrophs, which rely on methanogens consuming  $\text{H}_2$  to create a low enough partial pressure that the reactions remain favorable. These organisms are especially difficult to culture due to their obligate symbiotic metabolisms (McInerney et al. 2009).

There are also limited oxic habitats in rice paddies in which aerobic metabolism can occur. Aerobic methanotrophs, which normally couple aerobic respiration to  $\text{CH}_4$  oxidation, play a crucial role in regulating the amount of  $\text{CH}_4$  that reaches the atmosphere. It is estimated that  $\text{CH}_4$  emissions from rice paddies would be 10%–60% higher without aerobic methanotrophs in these zones acting as a biofilter and oxidizing  $\text{CH}_4$  to  $\text{CO}_2$  (Conrad 2009). They inhabit a shallow oxic layer on the surface

of the soil as well as a thin film surrounding rice roots, which is oxygenated via gas exchange through the rice plants' aerenchyma (open air channels in the plant) (Conrad 2007; Kludze et al. 1993; Jackson and Armstrong 1999).

Under low- $\text{O}_2$  conditions, certain methanotrophs are capable of engaging in fermentation-based methanotrophy, where  $\text{CH}_4$  assimilation is coupled with a highly efficient pyrophosphate-mediated glycolytic pathway (Kalyuzhnaya et al. 2013). More recently, nitrate-dependent anaerobic methanotrophs have been cultured from rice paddy soils (Vaksmas et al. 2017). The anaerobic methanotrophic archaeum *Candidatus Methanoperedens nitroreducens* couples the oxidation of  $\text{CH}_4$  to the reduction of nitrate to nitrite, while the bacterium *Candidatus Methylomirabilis oxyfera* couples the oxidation of  $\text{CH}_4$  to the further reduction of nitrite to  $\text{N}_2$  gas (Haroon et al. 2013). Although their contributions to  $\text{CH}_4$  oxidation are much smaller when compared to their aerobic counterparts, they have been estimated to oxidize around 0.001  $\mu\text{g}$  of carbon per gram of dry soil per hour, which, integrated over the large volume of anoxic soil, is likely significant (Fan et al. 2019).

Finally, some microbes lower  $\text{CH}_4$  emissions by competing with methanogens for resources. Nitrate reducers draw from monosaccharide pools when nitrate is briefly available early in the growing season. Iron and sulfur reducers can also act as a sink of acetate and  $\text{H}_2$ , reducing the magnitude of these substrates available for methanogenesis (Chidthaisong and Conrad 2000).

Despite this general understanding of the fundamental processes underpinning  $\text{CH}_4$  cycling in rice paddies, there are still several key knowledge

gaps preventing us from implementing CH<sub>4</sub> mitigation strategies. Much of what we know about the microbiology of CH<sub>4</sub> cycling in rice paddies is built on studies of cultured isolates and amplicon-based field surveys (Ferry 1992; Joulain et al. 1998; Lieberman and Rosenzweig 2004; Vaksmaa et al. 2017; Breidenbach and Conrad 2014; Lee et al. 2015; Reim et al. 2012). Amplicon surveys using the 16S rRNA marker rely on databases to infer the functional potential of microbial species, which are limited by the availability of isolate genomes. Many other studies use the methyl coenzyme M reductase *mcrA* or CH<sub>4</sub> monooxygenase *pmoA* genes to describe methanogen and methanotroph diversity but fail to elucidate the full causal web of carbon cycling (Ma et al. 2012; McDonald and Murrell 1997). Understudied taxa of interest include syntrophic fermenters and their methanogenic partners, iron and sulfur reducers that compete with methanogens for acetate and H<sub>2</sub>, and microbes involved in anaerobic CH<sub>4</sub> oxidization (McInerney et al. 2009; Hori et al. 2009; Wind et al. 1999).

Genome-resolved metagenomic and metatranscriptomic studies are emerging, but many more are necessary to elucidate the identity and physiology of key carbon cycling community members in diverse rice paddy environments (Zhang et al.

2023; Masuda et al. 2018). Due to the wide range of climates and soil types in which rice is cultivated, rice paddy soil communities exhibit considerable variation (Conrad 2007; Zhang et al. 2017; Edwards et al. 2015). It remains uncertain whether a solitary engineered strain would be viable across diverse paddy systems. Therefore, an in-depth comprehension of the eco-physiology of rice paddy microbes becomes imperative for the purpose of devising microbial interventions. Metatranscriptomic studies in particular will be essential for understanding the immediate response of the soil community to interventions (e.g., alternative wetting and drying) and for devising an accurate estimate of the DNA community representing living cells (Carini et al. 2016). Incorporating spike-in control sequences as an internal standard further enables the estimation of gene copies expressed per gram of dry soil, facilitating a more meaningful correlation with environmental metadata (e.g., CH<sub>4</sub> fluxes) than what can be achieved through relative abundance analysis alone.

Comprehensive investigations encompassing entire ecosystems and involving many types of micro- and macro-organisms are essential for gaining a deeper understanding of carbon cycling within rice paddies. This entails examining the intricate interactions between rice plants, fungi (both straw





degraders and arbuscular mycorrhizal fungi, which may prevent plant desiccation and increase nutrient uptake), prokaryotes, and various predators (e.g., nematodes, protozoa, and viruses) (Panneerselvam et al. 2018; Liu et al. 2008; Murase et al. 2008; Williamson et al. 2017). When considering the introduction of microbial interventions (e.g., application of engineered high-efficiency methanotrophs), their successful establishment and survival within the ecosystem while evading predation become crucial factors (Pandey et al. 2013). Additionally, continuous monitoring of intervention effectiveness is imperative, necessitating the development of user-friendly and scalable  $\text{CH}_4$  flux measurement equipment to facilitate this process. Finally, gaining deeper insights into rice plants and their capacity to recruit specific microbial communities offers significant potential for the development of low- $\text{CH}_4$  cultivars, fostering a more sustainable and climate-smart approach to rice cultivation (Su et al. 2015; Jiang et al. 2017).

## Research Prospects and Challenges

Flooded rice fields are a significant source of  $\text{CH}_4$  in agriculture and the main target of mitigating efforts to address climate change (Adviento-Borbe 2020). Floodwater management can effectively influence net

$\text{CH}_4$  emissions. Two common flood management methods are cascade or conventional flow through systems and multiple inlet rice irrigation. Furrow irrigation and AWD are increasingly adopted water management practices (Lampayan et al. 2015).

Alternate wetting and drying methods include draining rice paddy fields one or more times through management practices, such as midseason drainages, intermittent flooding, and percolation control (Sanchis et al. 2012; Xu et al. 2015). In furrow irrigation, plastic pipes are laid out at the top of the graded field and holes opened so that the water flows in the furrows between existing beds from a previous soybean or maize crop. Alternate wetting and drying practices implement wet and dry cycles one or more times during the growing season in zero- to precision-graded fields. Considerable field studies have reported that AWD or furrow irrigation reduced seasonal  $\text{CH}_4$  emissions by 48% to 84% (Runkle et al. 2019; Karki et al. 2021). However, temporarily drying the rice field may result in increased  $\text{N}_2\text{O}$  emissions, creating a trade-off between  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions. Several field studies reported cumulative  $\text{N}_2\text{O}$  emissions increased by 3–37 times in intermittent flooding relative to  $\text{N}_2\text{O}$  emissions in continuous flooding, suggesting no net decrease in total global warming potentials of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions (Liao et al. 2020;







Rice plants influence  $\text{CH}_4$  emissions by supplying plant-derived carbon substrates to support microbial processes

Lagomarsino et al. 2016). It has been demonstrated in commercial rice farms that  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions were effectively reduced without yield penalty when water and fertilizer nitrogen management were applied efficiently (Karki et al. 2023). Thus, multiple benefits of non-continuous flooding practice in rice cultivation can be achieved by careful timing of aerobic cycles, during either the vegetative or reproductive stage, and optimal nitrogen fertilization (LaHue et al. 2016). The effect of higher  $\text{CO}_2$  in the atmosphere may also have unpredictable effects. In a greenhouse experiment, for example, rice plants grown under doubled  $\text{CO}_2$  had more root biomass and lower  $\text{CH}_4$  emissions relative to controls (Schrope et al. 1999). Increased temperatures, soil heterogeneity, and varied management are all factors contributing to variable  $\text{CH}_4$  flux.

Rice plants influence  $\text{CH}_4$  emissions by supplying plant-derived carbon substrates to support microbial processes (Wassmann and Aulakh 2000; Conrad 2007). Many studies have examined the large diverse microbial community involved in  $\text{CH}_4$  emissions, and considerable knowledge and experimental data are available to further understand and model the rice root-microbiome- $\text{CH}_4$  emission interactions in paddy rice fields (Krüger et al. 2001; Reichardt et al. 2001; Edwards et al. 2015; Fernández-Baca et al. 2021). Recently, several studies that focus on complex interactions between rice plants and microbes during drained conditions and high-yielding hybrid cultivars have been gaining more attention because of their feedback on  $\text{CH}_4$  emissions and rice productivity. For instance, Santos-Medellin et al. reported a compositional shift characterized by an increase of Actinobacteria (e.g., *Streptomyces*) in the endosphere communities of rice

root microbiota that impacted root microbial recovery during a prolonged dry cycle (Santos-Medellin et al. 2021). Unlike in low-CH<sub>4</sub>-emitting hybrid rice cultivar (CLXL745), the root microbiome of the high-CH<sub>4</sub>-emitting inbred rice cultivar (Sabine) was enriched both in methanogens and in taxa associated with fermentation and acetogenesis, processes that support methanogenesis (Liechty et al. 2020).

While hybrid rice cultivars can produce more CH<sub>4</sub> compared to inbred cultivars through more biomass and stronger rooting systems, several field studies reported that hybrid rice cultivars decreased CH<sub>4</sub> emission rate due to enhanced CH<sub>4</sub> oxidation and/or reduced CH<sub>4</sub> transport capacity (Lu et al. 2000; Ma et al. 2010; Kim et al. 2018). More specific studies on microbes with methanogenesis- and methanotrophy-related functions across potentially high-yielding and widely used cultivars can help formulate mechanistic explanations for the differences in CH<sub>4</sub> emissions and yield responses under a wider range and more desirable crop growth conditions. Additionally, such potential effects of rice cultivars on methanogens and methanotrophs may be used to aid the development of breeding programs that mitigate CH<sub>4</sub> emissions from paddy rice cultivation.

In-depth research on microbial genetic and metabolic networks controlling major stress (salinity, heat, and water) in rice plants is needed to better understand the crop response under stress and recovery conditions. Attempts should be conducted under experimental environments that represent the complex nature of a rice production farm. The discovery of a new microbiome that is directly linked to the reduction of CH<sub>4</sub> emissions and grain development that has superior field performance could

potentially act as a sink for CH<sub>4</sub> in rice and sustain yield at the same time (Davamani et al. 2020).

Rice farmers have greater opportunities to reduce CH<sub>4</sub> emissions by adopting practices such as AWD and the use of low-emitting cultivars without major changes in their farming systems and additional farm cost. However, farmers' adoption remains low despite effective outcomes and possible savings from the implementation of these mitigating strategies because of the risks of yield loss, grain quality, nutrient recovery, weeds and disease susceptibility, and difficulties to manage practices in a commercial-sized rice field. Farmer reluctance to change practices in the face of uncertain consumer acceptance are additional socioeconomic obstacles. Finally, national carbon market-based trading schemes that allow farmers to obtain and trade credits by implementing mitigation practices are lacking. Such programs include AWD credits obtained from CH<sub>4</sub> reduction that can be certified by an authorized third party and sold using offset brokers and traders (Smith et al. 2007).

## Potential Strategies

Potential strategies to mitigate CH<sub>4</sub> emissions from rice paddies fall into several categories, including water management, growth of low-CH<sub>4</sub>-emitting and drought-tolerant rice cultivars, application of alternative substrates to outcompete methanogenesis (e.g., sulfate and ferric iron), microbial inoculants (e.g., methanotrophs), and co-culturing of rice and aquatic macrofauna (Aulakh et al. 2001; Wassman et al. 1993). These strategies mitigate CH<sub>4</sub> emissions by either (1) impeding the conditions that promote methanogenesis, namely, low-oxygen content/high water content (due to slower diffusion of oxygen into water than through air),

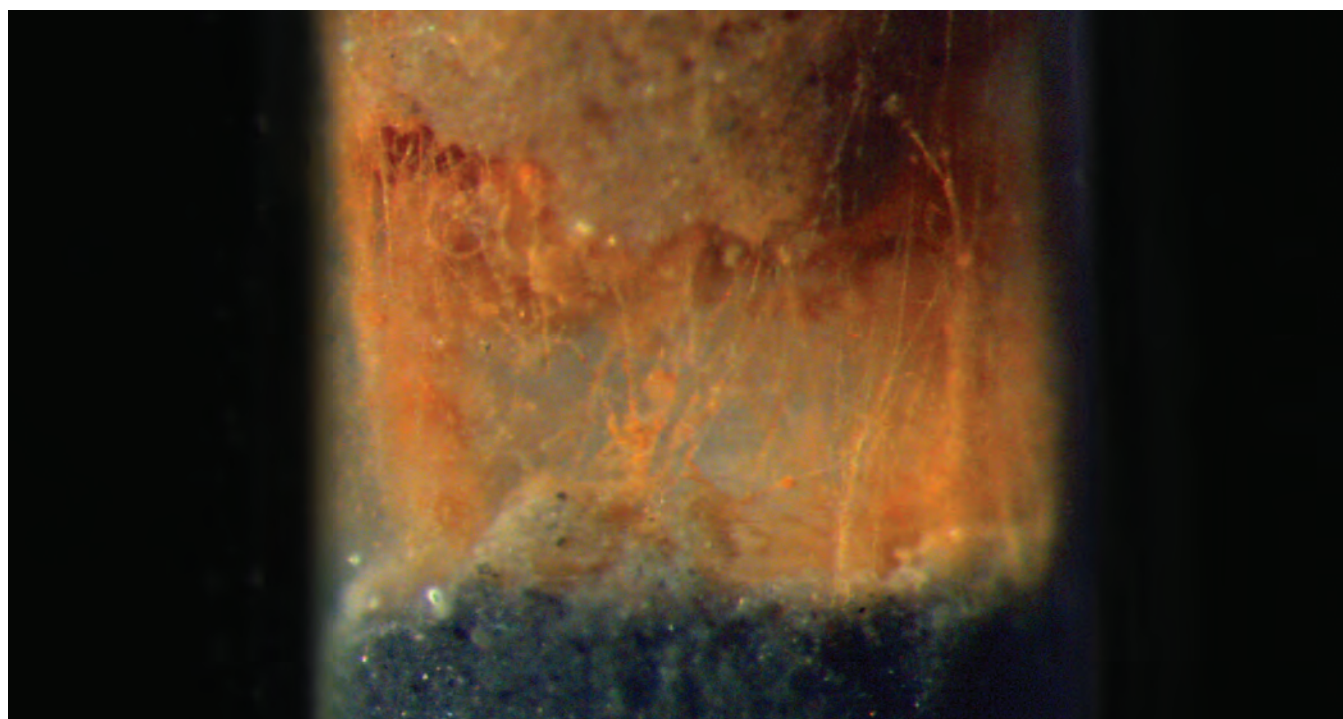


**Cable bacteria have recently been shown to decrease CH<sub>4</sub> emissions by 93% when introduced into rice paddy soils**

high organic matter, and absence of alternative electron acceptors and/or (2) enhancing conditions for methanotrophy, such as high oxygen content, in order to oxidize the CH<sub>4</sub> before it can be released to the atmosphere. As noted, water/irrigation management, such as alternate wetting and drying, reduces methanogenesis because methanogenic archaea are obligate anaerobes. These irrigation strategies are highly effective for reducing CH<sub>4</sub> emissions, with up to 80% lower emissions compared to continuous flooding when carefully timed (Souza et al. 2021; Sanchis et al. 2012; Yagi et al. 2019; Zhang et al. 2016 and references therein). Another method to reduce water demand and associated CH<sub>4</sub> emissions is to grow water-saving and drought-resistant rice cultivars, which are already commercially available (Luo 2010). Higher-yielding rice cultivars can also reduce CH<sub>4</sub> emissions in high organic carbon soils by increasing root porosity, allowing increased oxygen infiltration, and thereby stimulating CH<sub>4</sub> oxidation (Jiang et al. 2017). Rice cultivars can also have varying

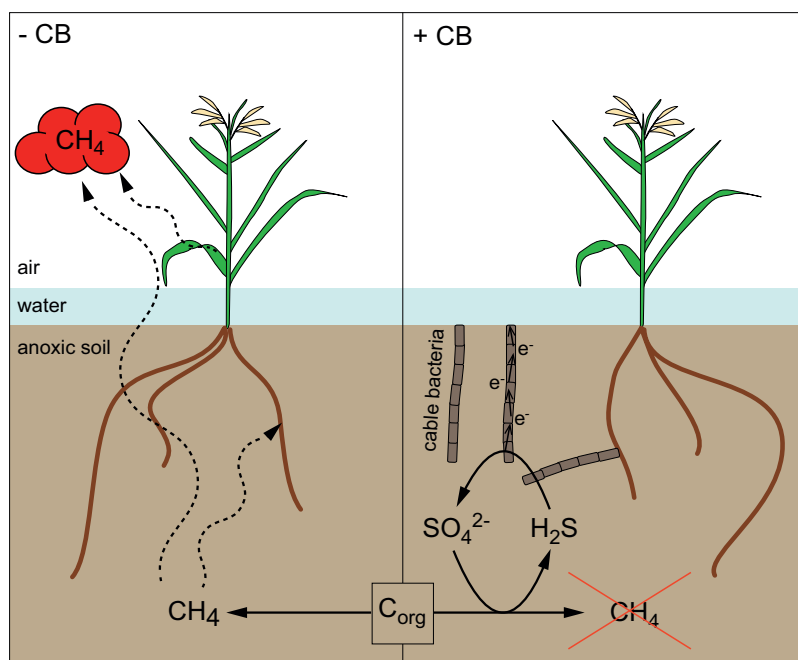
gas transport characteristics and can be selected for morphologies that limit radial oxygen loss and CH<sub>4</sub> loss (Zheng et al. 2018; Butterbach-Bahl et al. 1997).

The second set of mitigation practices is targeted to reduce the supply of exogenous organic matter inputs to the rice soil. It has long been known that application of rice straw as an organic fertilizer can stimulate CH<sub>4</sub> emissions due to high organic loading (Sass et al. 1991) and that therefore alternative rice straw removal methods, or application times (e.g., during the non-rice growing season), are recommended to lower CH<sub>4</sub> emissions (Sanchis et al. 2012; Wang et al. 2023; Yagi et al. 2019). The burning of rice straw and application of biochar to rice fields is also associated with higher CH<sub>4</sub> and nitric oxide emissions, even though other greenhouse gases such as N<sub>2</sub>O may decline (Zhang et al. 2010), although subsequent studies have reported variable results (Dong et al. 2021; Wang et al. 2019). Rice cultivars such as *SUSIBA2* can alter the distribution of photosynthetic products,



thereby allocating less organic carbon to the roots where methanogenesis is most prevalent (Du et al. 2021; Su et al. 2015).

A third strategy is application of alternative substrates (sulfate or ferric iron) to rice paddies to stimulate activity of other anaerobic microorganisms that will outcompete methanogens for substrates (e.g., acetate and hydrogen). Sulfate additions to rice paddies has long been known to be effective in mitigating  $\text{CH}_4$  emissions by promoting growth of sulfate reducing bacteria that outcompete methanogens for acetate or hydrogen (Denier van der Gon et al. 2001). The recent discovery of filamentous cable bacteria that can transport electrons over multi-centimeter distances and span from anoxic to oxic soil to couple sulfide oxidation by molecular oxygen suggests a potential promising strategy for recycling of sulfide back to oxidized forms of sulfur in rice paddies (Scholz et al. 2020). In this way, cable bacteria are able to continuously oxidize sulfide. Sulfate-reducing bacteria then utilize the continuously replenished sulfate pool to draw down acetate and hydrogen, depleting the pool of necessary substrates for methanogenesis. Cable bacteria have recently been shown to decrease  $\text{CH}_4$  emissions by 93% when introduced into rice paddy soils in a greenhouse experiment, but further field testing is needed (Scholz et al. 2020) (Fig. 10). Cable bacteria are commonly associated with marine sediments and therefore inoculation of sulfur-amended rice paddies with cable bacteria or sediments might be required to bring cell numbers to a threshold with detectable activity. Addition of ferric iron to promote iron-reducing bacteria that outcompete methanogens has also been demonstrated to reduce  $\text{CH}_4$  emissions (Jackel and Schnell 2000). However, alternative substrates at



**Figure 10.** Cable bacteria reduce  $\text{CH}_4$  emissions from rice-vegetated soils. Source: Scholz et al. 2020.

high levels can have adverse effects on rice plants and should be implemented with caution and further testing.

Another approach to remove  $\text{CH}_4$  from the soil would be to introduce the protein complexes necessary for  $\text{CH}_4$  monooxygenase (MMO) enzyme activity into plant cells. Based on mathematical modeling and the use of modern biotechnologies, Strand et al. proposed expressing MMO enzymes into eukaryotic mitochondria to support the reconstruction of such enzymatic machinery in plants (Strand et al. 2022). This strategy is technically challenging to pursue; however, some success in expression of MMO enzymes in plants has been recently documented (Arah and Kirk 2000; Lieberman and Rosenzweig 2004; Spatola Rossi et al. 2023). Employing gene-edited rice as one step to achieve net zero  $\text{CH}_4$  emissions will have risks and uncertainties and therefore must be evaluated and regulated by government agencies. In the U.S., genome-edited (GE) crops and products are assessed and regulated under health, safety, and environmental laws (Wolt and Wolf 2018).



Possible regulatory restrictions may include toxicities, pest occurrence, reduced productivity, and public resistance to GE crops, which likely slow down the development of novel microbial approach to mitigate CH<sub>4</sub> emissions in rice fields.

An emerging strategy in recent years is the concept of reducing CH<sub>4</sub> emissions via co-culturing of rice and aquatic fauna farmed for human consumption, such as duck, fish, crayfish, crabs, frogs, and prawns. Many of the rice-animal co-cultures were shown to emit lower CH<sub>4</sub> emissions than rice paddies without the animals, with the exception of the rice-fish combination, which was associated with higher CH<sub>4</sub> emissions (Bashir et al. 2020; Cui et al. 2023).

## Mitigation Opportunities

CH<sub>4</sub> emissions from continuously flooded rice production systems can be lowered with adoption of irrigation practices that use AWD periods. The geographic diversity of soils, rice production, and fertilization management make it challenging to specify which changes in farming practices will minimize net greenhouse gas production for any given region. Colloquium participants recognized opportunities for mitigation with different time frames for implementation.

### Now

- Optimize water management practices. Implementing AWD practices requires a cohesive plan that integrates irrigation and drainage system refinement alongside water balance modeling and nitrogen management to ensure optimal efficacy.

- Discourage practice of leaving straw in rice fields.
- Use water-saving and drought-resistant rice cultivars and expand research on rice genetics.
- Expand studies on the interactions of AWD, organic carbon inputs, and fertilizer inputs on both CH<sub>4</sub> and N<sub>2</sub>O emissions.

### Next 5-10 years

- Expand research on and investment in design of alternative substrates (such as iron) to promote the growth of anaerobic microorganisms that outcome compete methanogens.
- Continue to breed and select for rice cultivars that are low-CH<sub>4</sub> emitters and genetically engineer rice cultivars to be low-CH<sub>4</sub> emitters.
- Collaborate with industry and farmers to develop sustainable and climate-smart products (such as inoculum of cable bacteria and/or methanotrophs) to apply to rice paddies.
- Assess the impacts of long-term AWD irrigation on soil organic carbon stocks as seasonal CO<sub>2</sub> emissions may increase during the dry cycle.

# Landfills

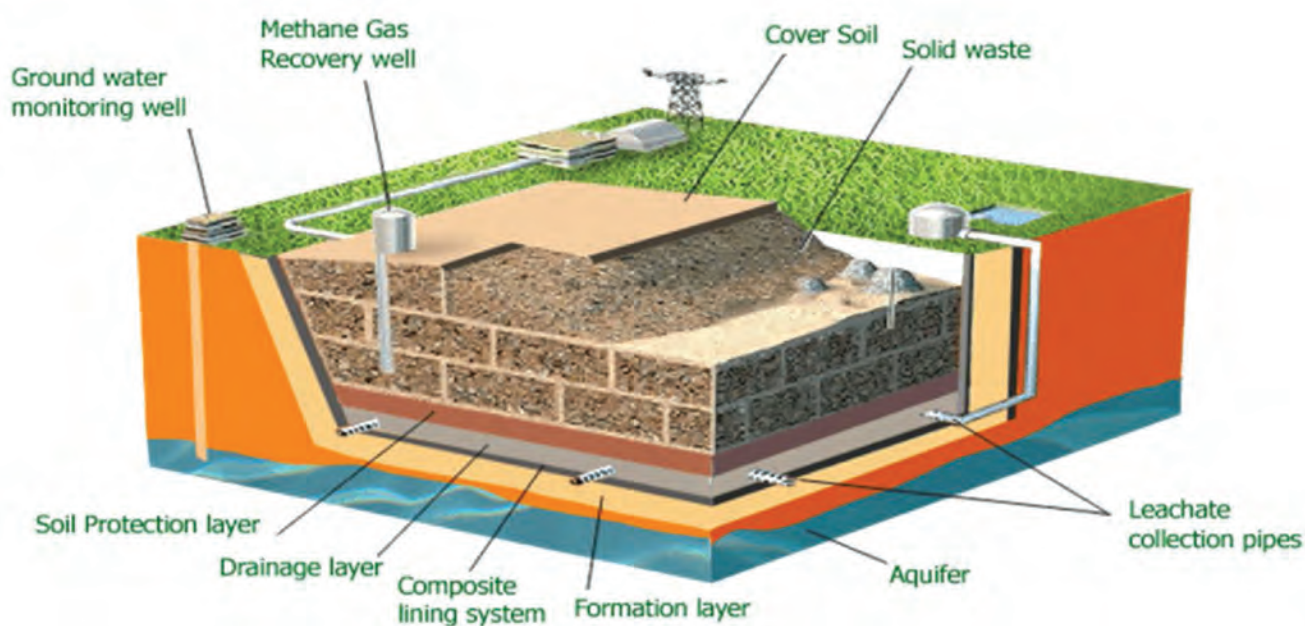
## Overview of CH<sub>4</sub> Emissions from Landfills

Modern landfills are highly engineered sites to reduce environmental harm, particularly groundwater contamination, resulting from the burial of waste. They are typically constructed as “cells” (approximately 5–10 ha each) with impermeable basal and side liners that collect landfill liquids (leachate) above the bottom liner for subsequent removal/treatment. Landfills can have multiple cells resulting from both horizontal and vertical expansions. They incorporate engineered biogas collection networks installed incrementally during the operational life of the site using vertical wells or horizontal collector pipes. Landfills also incorporate systems for monitoring/control of biogas (CH<sub>4</sub> + CO<sub>2</sub>) generated from the anaerobic microbial decomposition of

biodegradable waste fractions such as paper, food waste, and garden waste (Fig. 11). Collected gas is either flared (burned off) or used as a source of renewable energy.

Landfills are the third-largest source of human-related CH<sub>4</sub> emissions in the U.S., accounting for approximately 14% of the total greenhouse gas emissions in 2021 (U.S. EPA 2023). The exact number of landfills in the U.S. is not well defined. The EPA reported a total of over 2,637 U.S. landfills as of March 2023 (<https://www.epa.gov/lmop/landfill-gas-energy-project-data>). When CH<sub>4</sub> concentrations are high enough, landfill gas (LFG) can be recovered by collecting it for energy generation (biogas). At lower concentrations, gas is burned

**Figure 11.** Cross-section of a modern landfill. Image from Khoo et al. 2019.



Detailed analyses of methanotrophic, methanogenic, and fermentative populations are also essential.

off (flared) because it is not economically feasible to capture and utilize. The properties of surface covers and landfill age can all affect the amounts of “fugitive”  $\text{CH}_4$  that escapes from a landfill. Landfill  $\text{CH}_4$  emissions at a particular site depend on the thickness and properties of the various cover materials, site operational activities including the presence of an engineered gas recovery system, and the site latitude/longitude location (which determines daily climate- and soil microclimate-related  $\text{CH}_4$  oxidation rates in cover materials). A better understanding of soil-microclimate controls on methanotrophy for modeling landfill emission estimates is needed for a more accurate estimate of  $\text{CH}_4$  emissions from landfills in the U.S.

## Knowledge Gaps

Landfills function as pulse-fed bioreactors that vary spatially at multiple scales within systems and across systems geographically (Sauk and Hug 2022). The microbial activities they support also vary temporally in response to numerous factors (Liu et al. 2019). Spatial and temporal patterns of methanogenesis,  $\text{CH}_4$  oxidation, and  $\text{CH}_4$  emission are understood in a general sense, but few studies have established linkages among the mosaic of habitats within landfills and  $\text{CH}_4$  dynamics at system levels. This limits efforts to model the behavior of individual systems and to deploy targeted approaches for management. Greater use of envi-

ronmental geological methods, e.g., ground-penetrating radar (Dawrea et al. 2021), coupled with in situ and ex situ biogeochemical and molecular ecological approaches could provide a wealth of new insights that would facilitate goals of reducing  $\text{CH}_4$  effluxes and leachate pollution.

More detailed analyses of methanotrophic, methanogenic, and fermentative populations are also essential for understanding how best to manage  $\text{CH}_4$  dynamics. While numerous studies have used targeted gene analyses to establish community compositions with different degrees of temporal and spatial resolution, linking populations, functions, and measured activities has been a greater challenge (Liu et al. 2019; Yang et al. 2021; Sauk and Hug 2022). Functions for some well-characterized genera can be readily assigned, but in many instances the functions of identified taxa remain unknown, as well as for the large fraction of as yet uncultivated taxa. Increased application of deeply sequenced metagenomes and metagenomic genome assembly is essential for resolving both functions and interactions among populations, with the latter especially important due to the complex, poorly understood interdependencies that exist within communities (Meyer-Dombard et al. 2020; Dang et al. 2023).

In spite of considerable research, the use of cover soils to completely elim-





inate CH<sub>4</sub> efflux remains an elusive goal (Chanton et al. 2011). In part, this reflects distinct differences between methanotrophs that consume CH<sub>4</sub> at high concentrations in the lower levels of cover soils and those that are active at lower concentrations near the soil surface. The latter remain largely uncultured and poorly known, but it is evident that methanotrophs near the surface respond differently to disturbances and other stresses than those at depth. Differential microbial dynamics have not been adequately considered in cover soil design and management. Similarly, soil CH<sub>4</sub> flow paths in landfills can vary substantially over space and time, resulting in varied microbial processes and oxidation “hot spots.” Too little is known about CH<sub>4</sub> transport in complex matrices to predict flow behavior in landfills and how this modifies cover soil properties as microbial habitat in support of CH<sub>4</sub> uptake capacity (Gebart and Perner 2015).

Major gaps exist in our understanding of methanotrophic dynamics in complex communities in situ. Vascular plants have been promoted for use in phytocapping, which stabilizes soil structure and improves water management (Lamb et al. 2014), but the mechanisms by which plants enhance CH<sub>4</sub> oxidation are uncertain. Plants can affect microbial processes directly and indirectly, both within the rhizosphere and in the bulk soil (Shangjie et al. 2023). Plant-methanotroph interactions also involve poorly

understood relationships with other microbes, including those that oxidize ammonium and potentially toxic volatile organic compounds. Methylobionts’ interactions with plants can also contribute to CH<sub>4</sub> oxidation, by producing essential co-factors (Shangjie et al. 2023). *Ex situ*, culture-based and molecular ecological approaches will be needed to identify and understand interactions that can accelerate and sustain CH<sub>4</sub> oxidation and distinguish those from interactions that can reduce activity.

Mitigation of landfill CH<sub>4</sub> emissions largely occurs through a well-documented aerobic process. However, anaerobic oxidation of CH<sub>4</sub> (AOM) has long been suspected to occur in at least some landfills coupled to sulfate, nitrate/nitrite, Fe<sup>3+</sup>, or Mn<sup>4+</sup> (Parsaeifard et al. 2020). Recently, both sulfate and nitrate/nitrite-coupled AOM has been observed using <sup>13</sup>C-CH<sub>4</sub> to trace activity and molecular ecological methods to determine which populations were involved (Chi et al. 2022; Xu and Zhang 2022). However, the extent to which this process occurs in other cover soils and might be exploited to reduce CH<sub>4</sub> fluxes to oxic soils is largely unknown, and controls of AOM and its relevant populations remain unexplored. A focused effort is needed to understand how AOM can be promoted sustainably, which electron acceptors are most effective, and what adverse outcomes might arise (e.g., sulfide or N<sub>2</sub>O production).





## Research Prospects and Challenges

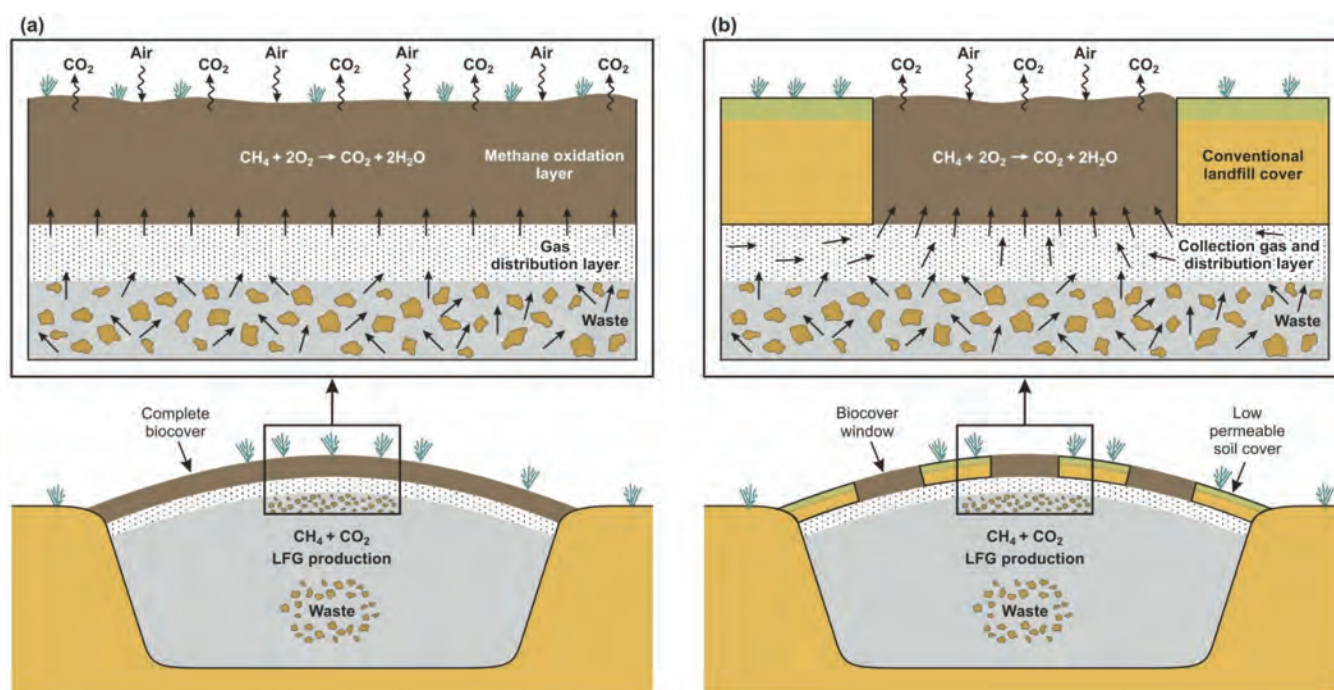
The ability to determine the efficacy of landfill design or modification in reducing CH<sub>4</sub> emissions requires accurate measurements at the whole-landfill scale, which is technically challenging. Small-scale measurements of CH<sub>4</sub> flux using small chambers across the surface of a landfill can vary by as much as six orders of magnitude (Bogner et al. 1995; Chanton et al. 2011a) so that scaling up from a low number of chamber-based measurements may underestimate whole-landfill emissions (Monster et al. 2019). Cover defects or erosion can create hot spots responsible for most emissions (Gebert et al. 2016; Bogner et al. 1997). In one study, 5% of the landfill surface was responsible for 50% of the emissions (Gonzalez-Vallencia et al. 2016). Gas connections or leachate wells can also be hot spots, and if they are not measured, they can contribute to underestimates (Scheutz et al. 2011; Fredenslund et al. 2010). In some cases, portions of the landfill surface can even serve as a sink for atmospheric CH<sub>4</sub> (Bogner et al. 1995; Stern et al. 2007; Abichou et al. 2006). Different approaches for measuring whole landfill emissions are described in several reports (Monster et al. 2019; Goldsmith et al. 2012; Lando et al. 2017; Innocenti et al. 2017; Bel Hadj Ali et al. 2020).

Variability in landfill CH<sub>4</sub> emissions is caused by the thickness and properties of soil covers, the presence and condition of a gas recovery system, and the efficiency of microbial CH<sub>4</sub> oxidation within the soil cover (Bogner et al. 2011). Whalen et al. reported that soil methanotrophic communities collected from landfills had the ability to rapidly oxidize CH<sub>4</sub> at concentrations as low as 1 ppmv (atmospheric CH<sub>4</sub> is nearly 2 ppmv) (Whalen et al. 1990). The extent to which

CH<sub>4</sub> within the cover is oxidized is a function of climatic conditions, cover type, and CH<sub>4</sub> loading from the decomposing wastes below (Chanton et al. 2011a). Reductions in CH<sub>4</sub> entering the bottom of the cover soil increase the overall percentage of CH<sub>4</sub> oxidized in the cover, although the absolute CH<sub>4</sub> consumption rate may decrease. Thus, an effective gas collection system may be the most effective mechanism to increase the percentage of CH<sub>4</sub> oxidized. The temperature optimum for most known methanotrophs is 30°C and as such, CH<sub>4</sub> oxidation is lower during colder winter conditions and higher in summers (Whalen et al. 1990; Czepiel et al. 1996; Klussman and Dick 2000; Chanton and Liptay 2000).

Other factors affecting CH<sub>4</sub> oxidation in landfill covers include moisture content (optimum of 10%–15% [wt/wt] water) (Whalen et al. 1990; Czepiel et al. 1996). Some moisture is required to support biological activity of the CH<sub>4</sub> oxidizing bacteria, but too much moisture will limit gaseous diffusion of oxygen into the cover and reduce bacterial metabolic rates. Another factor is atmospheric pressure and how it changes. Decreasing atmospheric pressure resulted in large emissions, while increasing pressure yielded less emissions, with up to a 35-fold variation in daily emissions due to changes in atmospheric pressure (Gebert and Groengroeft 2006; Rachor et al. 2013; Xu et al. 2014). It is possible that in landfills with modern effective gas collection systems this pressure effect may be reduced, although there are no recent studies to confirm this.

Groundwater protection from landfills calls for minimizing leachate volume, and landfill covers are often constructed of clay to shed rain water. However, water-shedding covers reduce oxygen entry and limit activity of methanotrophs. Covers that allow

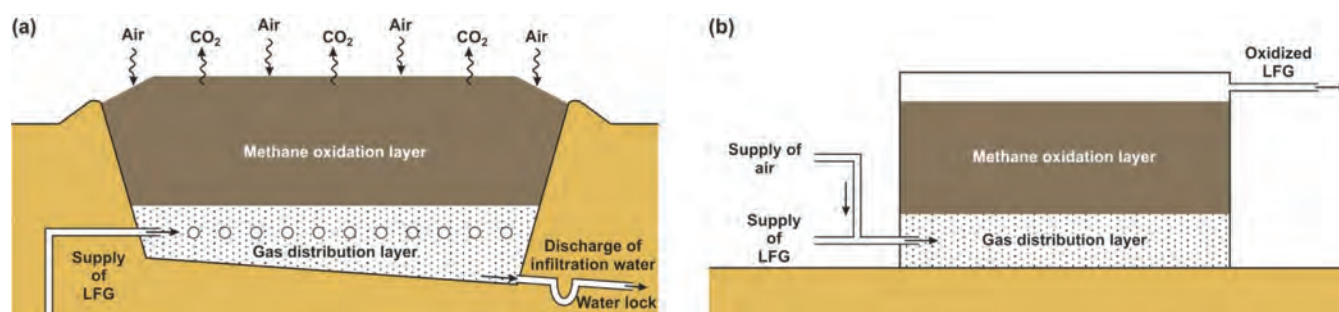


oxygen diffusion, water retention, and methanotrophic activity are classified as **biocovers**, **biowindows**, or **biofilters** (Fig. 12 and 13). Although similar in purpose, the designs of these systems have significant differences.

First, biocovers rely on passive transport of  $\text{CH}_4$  and oxygen and generally consist of a (10–30)-cm-thick gas distribution layer of gravel and a  $\text{CH}_4$  oxidation layer which is 50–100 cm (Kjeldsen and Scheutz 2019).  $\text{CH}_4$ -oxidation layers may consist of composted yard and kitchen waste mixed with sand or some other mineral soil. In addition, biocovers can be constructed solely of either compost or a mineral soil alone. Of these options, organic

material provides water-holding capacity and gas permeability, but mineral soils may be more resilient over time because they do not decompose and do not demand as much oxygen through aerobic decomposition of the compost (Gebert and Gröngroft 2006; Gebert et al. 2022). Finally, the use of biochar as a biocover material has been receiving increased attention as this has high porosity and high water holding potential and is nitrogen rich, thus facilitating greater and more uniform methanotrophic growth that allows for more effective gas distribution and reduced clogging (Sadavisam and Reddy 2014; Sadavisam and Reddy 2015; Reddy et al. 2014). Although biocovers are useful, they

**Figure 12.** An example of (A) a full surface biocover and (B) a biowindow. Image from Kjeldsen and Scheutz 2019.



**Figure 13.** Biofilters in (A) an open bed and (B) a closed bed with pump mode. Image from Kjeldsen and Scheutz 2019.

require prior design and planning and are an integral part of the landfill cover system. For landfills with pre-existing cover systems, passive biowindows can be retroactively constructed. Biowindows are higher permeability areas dug into previously installed impermeable covers to create surface zones with greater  $\text{CH}_4$  oxidation potential. For example, Scheutz et al. showed that by installing compost biowindows, whole landfill  $\text{CH}_4$  emissions were reduced by 30% (Scheutz et al. 2011). Although controlling the flow of  $\text{CH}_4$  to biowindows and achieving a more even distribution of gas remains challenging, these systems can achieve high (~75%)  $\text{CH}_4$  oxidation efficiency (Pecorini and Iannelli 2020).

Finally, biofilters can be installed either above ground or below grade where  $\text{CH}_4$  and air are piped from below (Duan et al. 2022). In these systems,  $\text{CH}_4$  and air can be actively provided, allowing for better control of  $\text{CH}_4$  to oxygen ratios to ensure higher and more extensive  $\text{CH}_4$  removal. (Thomassen et al. 2019; Farrokhzadeh et al. 2017).

Whereas biocovers consist of soils, compost, or mixtures that support methanotrophic communities, phytocovers consist of soil-based biocovers planted with vegetation that can also assist in  $\text{CH}_4$  reduction. Evapotranspiration by plants in phytocovers helps reduce rainfall infiltration and leachate production through the processes of vegetative interception and transpiration (Lamb et al. 2014). The proliferation of roots in a healthy rhizosphere zone results in more aggregated and porous topsoil, which promotes oxygen diffusion and creates more belowground biomass to enhance biological  $\text{CH}_4$  oxidation (Abichou et al. 2015). If transpiration is sufficiently high, soil remains unsaturated, and oxygen diffuses more easily into the

soil through open pores, supporting bacterial methanotrophy. However, methane dissolved in water can also be transported to leaves and ultimately to the atmosphere via the transpiration stream. In addition, if soil is waterlogged, open aerenchyma tissue can develop in plants and serve as a conduit for diffusive or pressurized flow of methane to the atmosphere (Vroom et al. 2022). Endophytic methanotrophs have been detected within tissues of diverse plants and may help reduce such methane emissions to the atmosphere (Stepniewska et al. 2018). In drier environments, a healthy root zone can aid with water storage, enhancing the soil environment for methanotrophs, supporting soil stability, reducing dust, and potentially resulting in a harvestable product (Lamb et al. 2014; Abichou et al. 2015; Shah 2021). Abicou et al. modeled phytocover-based  $\text{CH}_4$  oxidation in Nebraska, U.S., and determined that a phytocover could oxidize 100% of  $\text{CH}_4$  loadings up to roughly  $5 \text{ g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ , although oxidation efficiency fell off with higher loading rates (Abicou et al. 2015).

## Potential Strategies

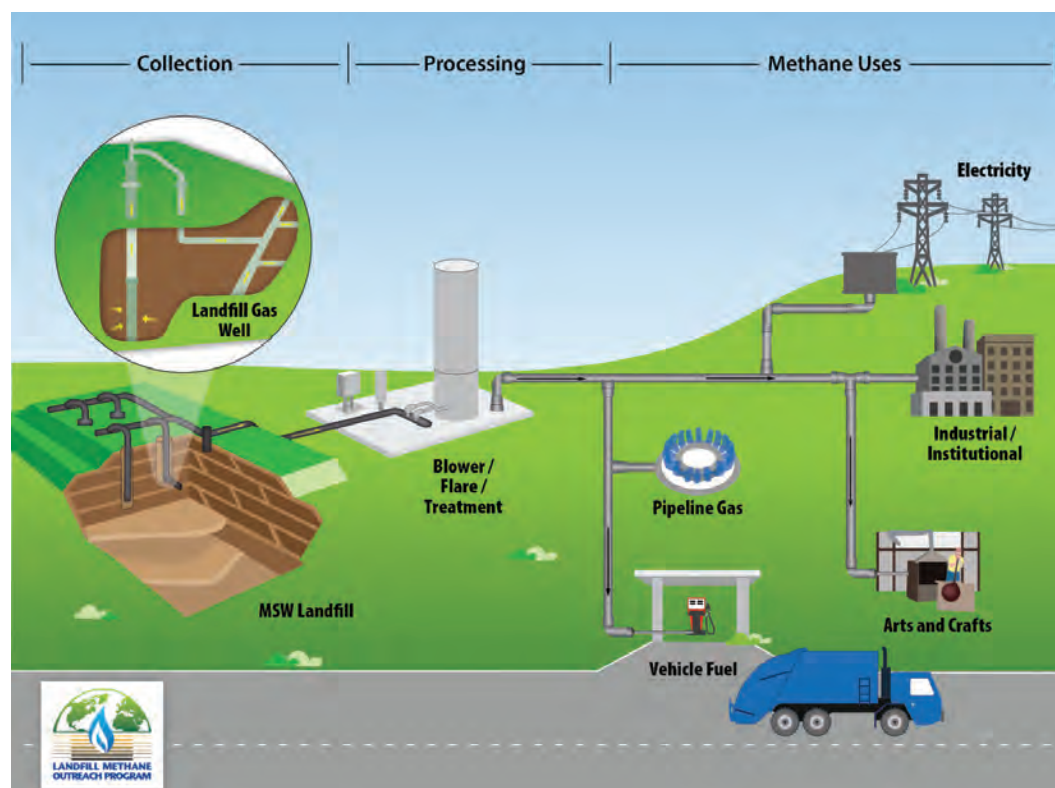
Current efforts are underway to take steps to reduce  $\text{CH}_4$  emissions from landfills and create value-added products at the same time. The U.S. Methane Emissions Reduction Action Plan is launching initiatives across the major  $\text{CH}_4$ -producing sectors including municipal solid waste (MSW) landfills (White House Office of Domestic Climate Policy 2021). One national-scale goal is capturing 70% of  $\text{CH}_4$  emissions from U.S. landfills and a second goal is to reduce food loss and waste by 50% by 2030, thereby keeping that organic material (which constitutes ~24% of total material) from fueling landfill  $\text{CH}_4$  production and emission. The EPA has also developed the voluntary Landfill Methane Outreach Program to con-



nect industry stakeholders and waste officials with resources supporting implementation and funding of cost-effective projects developing landfill gas (LFG) as an alternative to fossil fuel.

CH<sub>4</sub> captured via gas collection systems installed in landfills has multiple uses, such as heat and electricity generation, industrial product feedstock, and injection of cleaned and purified renewable natural gas into natural gas pipelines (Fig. 14). These end uses differ in their requirements for removal of contaminants such as water, CO<sub>2</sub>, siloxane/hydrogen sulfide, and oxygen from LFG (U.S. EPA 2021). Although more complex than direct use of LFG for heat or electricity, processes harnessing microbial capabilities for cleaning LFG or for generation of bioproducts from LFG are promising. For example, Chidambarampadmavathy et al. argue that production of biodegradable bioplastic polyhydroxybutyrate (PHB) by methanotrophs using waste CH<sub>4</sub> from landfills can be cost effective in Australia, providing a

“cradle-to-cradle” approach to plastic production (Chidambarampadmavathy et al. 2017). Further, it has been shown that methanotrophy can be used to create lipids, a precursor for biodiesel production, so as to generate protein that can be used as an animal feed supplement (Semrau et al. 2010; Pieja et al. 2017; Lee et al. 2016). Some have provided a proof-of-concept pilot-scale test of the capacity of a chemolithotrophic sulfur-oxidizing consortium, immobilized on a biotrickling filter, to remove the corrosive contaminant H<sub>2</sub>S from LFG while maintaining the energy quality of the purified biogas (Ibrahim et al. 2022). In addition, taking a purely chemical approach, Merkouri et al. have suggested that chemical synthesis of dimethyl ether and methanol from landfill gas, via syngas, can be profitable (Merkouri et al. 2022). The site-specific challenges for establishing and scaling such approaches remain daunting particularly in developing countries, where incentives for collection and utilization of LFG do not yet exist. However, in the



**Figure 14.** Landfill CH<sub>4</sub> can be processed for multiple end uses, including electricity, industrial uses, arts and crafts, pipeline gas, and vehicle fuel. Image from <https://www.epa.gov/lmop/basic-information-about-landfill-gas>.



U.S., EPA data indicate more than 500 projects utilizing LFG are operating, with the vast majority targeting electricity generation (U.S. EPA 2023).

The *United Nations Global Methane Assessment* notes that for the waste sector, up to 60% of measures that could be implemented to reduce CH<sub>4</sub> emissions are either negative cost or low cost, including net economic benefit from LFG capture and use for heat and electricity (Singh et al. 2022). However, in many low- and middle-income countries, municipal waste is disposed of in large garbage dumps that are not properly engineered landfills. Little is known about the CH<sub>4</sub> emissions from these dumps, but because they likely include organic wastes and no engineered mechanisms to collect CH<sub>4</sub> or to oxidize it in properly designed landfill covers, it is likely that these dumps are significant sources of CH<sub>4</sub> globally. Closing these dumps and converting them to proper landfills or building new landfills would be an effective strategy to reducing uncontrolled emissions from waste dumps.

## Mitigation Opportunities

Reducing CH<sub>4</sub> emissions from landfills is a shared responsibility among waste-generating community members, local and regional governments, and private contractors. Socioeconomic networks can utilize knowledge about optimal conditions for microbial processes to achieve substantial CH<sub>4</sub> reductions.

### Now

- Increase installation of gas collection infrastructure in all new landfills to improve efficiency of methanotrophs.
- Expand research and deployment of biocovers, biowindows, and biofilters in landfills.
- Increase research into microbial

community composition/activity both within landfills and in engineered systems for CH<sub>4</sub> removal to better understand and thus control CH<sub>4</sub> production and consumption. Such studies should explicitly consider microbe-microbe and plant-microbe interactions that either inhibit methanogenesis and/or enhance methanotrophy.

- Facilitate more formalized collaborations among scientists, landfill managers and operations organizations, industrial leaders, and venture capital groups to accelerate adoption of novel strategies to reduce overall CH<sub>4</sub> emissions from landfills.
- Conduct coordinated public education campaigns to reduce the amount of organic wastes entering landfills, which can include encouraging backyard and larger-scale composting efforts.

### Next 5-10 years

- Develop novel microbial strategies to utilize captured landfill gas to enhance CH<sub>4</sub> valorization, i.e., conversion of CH<sub>4</sub> to higher-value products such as bioplastics, biofuels, and animal feed supplements.
- Enhanced measurements and models of CH<sub>4</sub> emissions at the whole-landfill scale that explicitly consider microbial, geographical, infrastructural, and seasonal differences between sites.
- Review waste management approaches in low- and middle-income countries to identify opportunities and prioritize appropriate technologies for converting waste dumps into engineered landfills and to incorporate biogas utilization where feasible.

# Conclusions

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The record-breaking heatwaves of summer 2023 highlighted the urgent threat that extended and extreme heat events pose to human health, food security, and well-being. Slowing the increased rise of CH<sub>4</sub> concentration in the atmosphere today will have a strong near-term effect on global temperature, making fulfilling the Global Methane Pledge vital for addressing the negative impacts of climate change. Fortunately, microbes can help to address this crisis by both consuming CH<sub>4</sub> and releasing less CH<sub>4</sub> into the atmosphere.

The colloquium participants discussed four main sectors where microbial actions can be harnessed to mitigate CH<sub>4</sub> emissions and identified knowledge gaps and opportunities for microbial solutions. The recommendations below are mitigation strategies considered by participants to yield the most impactful outcomes.

- **Enteric Fermentation in Ruminants.** Enteric fermentation accounts for a quarter of anthropogenic CH<sub>4</sub> emissions. Greater knowledge of the relationships among microbial species in the rumen microbiome will be necessary to advancing methanogen inhibitors, adjusting ruminant feedstocks, and developing vaccines against rumen methanogens.
- **Animal Wastes.** Over 10% of agricultural CH<sub>4</sub> emissions comes from animal wastes and manure management. A better understanding of the manure and soil microbiome from field experiments and modeling studies will be impera-

tive for optimizing microbial communities to reduce CH<sub>4</sub> emissions.

- **Rice Production.** As one of the top global food staples, holistic research on the soil microbiome in relationship with the rice plant will be key to implementing the application of microbial inoculants (such as cable bacteria), managing alternate wetting and drying strategies, and co-culturing of rice and aquatic animals and alternative substrates to outcompete methanogenesis in rice paddies.
- **Landfills.** Landfills are the third-largest source of human-related CH<sub>4</sub> emissions in the U.S. Expanded characterization of landfill microbiome community structure and function, especially with regard to plants in phytocovers, will be important when optimizing landfill covers that effectively lower CH<sub>4</sub> emissions. Additionally, microbial conversion of landfill gas into valuable products will create financial incentives needed to increase research and development on microbial solutions to address CH<sub>4</sub>.

Microbes are central to each of the four main sectors discussed. The participants agreed a stronger foundational knowledge of the microorganisms involved in each is important for harnessing microbial solutions. However, the inability to culture or have pure cultures for many of the microbes found in the rumen, manure, rice paddies, or landfills presents a major barrier for increased knowledge of the physiology of each microbe. Metagenomics and meta-

**A better understanding of how microbiome communities interact with agricultural crops can enable the next generation of microbe-based mitigation opportunities.**

transcriptomics provide routes for elucidating microbiome members and their activities, respectively, and more of these studies are needed. Additionally, harmonized data standards should be developed to promote data sharing about different microbiomes. More research is also needed to develop genetic tools for more model organisms that are representative of the microbes found in rumen, manure, rice paddies, and landfills. Establishing repositories for microbiomes found in these environments can also improve the basic understanding of which microbes are the major players in each environment and how each member contributes to the overall CH<sub>4</sub> production. Understanding how microbiomes adapt to changes in their environment such as pH, increased precipitation, and warming will be especially important as climate change leads to global environmental changes. This in turn can help to inform CH<sub>4</sub> mitigation strategies, such as developing methanogen inhibitors for ruminants or methanotroph inoculants in rice production.

Expanded foundational microbial knowledge can spur innovation and support for more climate-smart agricultural practices. A better understanding of how microbiome communities interact with agricultural crops can enable the next generation of microbe-based mitigation opportunities. For example, new knowledge and/or discovery of microbiomes that are directly linked to crop production and CH<sub>4</sub> emissions are extremely important in the development of effective means to mitigate CH<sub>4</sub> emissions in rice agroecosystems, such as cable bacteria inocula. Additionally, rumen enteric fermentation plays a crucial role in influencing agricultural livestock waste microbiomes. Microbiology research focused on modifying the rumen microbiome to reduce meth-

anogenesis has potential benefits to improve feed efficiency and induce downstream effects on manure quality and microbial composition that subsequently impact soil health and CH<sub>4</sub> emissions. Therefore, microbiologists need to work with agricultural scientists, ecologists, and farmers to learn how best to utilize and implement microbial solutions to improve current agricultural practices. These microbe-focused, climate-smart approaches will make the food system more resilient and sustainable to environmental change.

Microbiologists' deep knowledge of microbial processes, metabolism, and physiology will be vital to inform microbe-based solutions for reducing CH<sub>4</sub> emissions across various sectors. However, addressing climate change will require diverse expertise from multiple sectors and disciplines. Transdisciplinary research groups that share data and address research issues holistically, understanding that microbes both affect and are affected by the whole ecosystem, will be critical. Besides academic research groups, microbiologists need to build stronger relationships with stakeholders and the private sector. Better communication with both can hasten innovation that is financially and economically viable and will be actually adopted by end users. This includes collaborating with community stakeholders, understanding their values and priorities, and then co-designing research aims and solutions with them. Colloquium participants also invited the scientific community to consider inequitable impacts of climate change on vulnerable populations and co-design their research based on the needs and priorities of the communities most affected by climate change.

The climate crisis is certain and already has begun to negatively affect human



well-being. Increasing temperature harms human health and food production. Thus, it is imperative for humans to harness microbes' unique abilities to reduce CH<sub>4</sub> emissions to slow global

warming and environmental changes. The opportunities outlined in this report provide a foundation for building a more resilient and sustainable future to combat climate change.

# Major Recommendations

## Recommendations to Expand Foundational Knowledge of Microbes for Mitigating Methane

- Expand academic training in anaerobic microbiology and physiology to increase the number of scientists skilled in isolating and characterizing methanogens.
- Prioritize work on determining rumen microbiomes supporting hydrogen budgets for lower methane emissions from ruminants.
- Establish microbiome collection repositories for the community and continue research for a deeper understanding of the relationship among microbes in the microbiomes of the rumen, animal wastes, agricultural crops, and landfills.
- Increase investment in genetic tools and non-model organism development.

## Recommendations to Incorporate Microbial Understanding into Methane-reducing, Climate-smart Agriculture Practices

- Expand research on plant-microbe and animal-microbe interactions to inform future agricultural practices.
- Document performance of extant microbial inocula and identify management practices that enhance survival and persistence of inocula in agricultural soils.

- Investigate use of redox-active compounds to add to manure to reduce overall CH<sub>4</sub> emissions.
- Recognize current efforts and work with agricultural producers to identify climate-smart practices.

## Recommendations to Increase Transdisciplinary Collaboration Across Sectors

- Demonstrate the value and support further investments of transdisciplinary research consortia that harmonize data collection to allow for greater data sharing and take a holistic approach to addressing climate change.
- Strengthen partnerships with industry and the private sector to advance research and innovation, especially on microbe-based products.
- Foster science communication efforts to encourage microbiologists to highlight the importance of microbes in climate change and demonstrate the need for microbial solutions to reduce CH<sub>4</sub> emissions with diverse stakeholders, including other scientists, media, public officials, etc.
- Collaborate with community stakeholders to understand cultural values and local customs to co-develop research priorities and solutions.

# Glossary

**Algae:** photosynthetic, eukaryotic organisms mainly found in aquatic environments.

**Alternate wetting and drying (AWD):** water management practice for rice production to reduce overall water usage.

**Archaea:** single-cell organisms that lack a nucleus (known as a prokaryote) that are similar but evolutionarily distinct from bacteria; many have been found in extreme environments.

**Bacteria:** microscopic organisms that have only one cell and no nucleus.

**Biocover:** porous material layer laid directly on top of a landfill, which is then covered by an oxidizing layer of mature compost.

**Biofilters:** porous material layer as well as an organic material (often compost) layer to oxidize CH<sub>4</sub>.

**Carbon dioxide (CO<sub>2</sub>):** greenhouse gas associated with global warming.

**Ciliate protozoa:** large single-celled eukaryotic microorganisms that carry out anaerobic fermentation.

**Climate change:** a long-term change in Earth's, or a specific region's, climate.

**Climate-smart agriculture:** practices for managing livestock and crops to be more resilient to the effects of climate change and more sustainable by reducing greenhouse gas emissions from agriculture.

**Fungi:** eukaryotic organisms that belong to the kingdom fungi; they can be unicellular or multicellular.

**Global warming:** the increase in Earth's average surface temperature due to rising levels of greenhouse gases.

**Greenhouse effect:** the trapping of the sun's warmth by greenhouse gases in a planet's atmosphere, resulting in increased surface temperature.

**Greenhouse gas:** gases in the atmosphere that absorb radiation and trap heat.

**Methane (CH<sub>4</sub>):** a greenhouse gas 80 times more potent at absorbing heat in the atmosphere than CO<sub>2</sub> on a 20-year timescale.

**Methanogenesis:** formation of CH<sub>4</sub> by microbes.

**Methanotrophy:** consumption of CH<sub>4</sub> by microbes as a sole carbon and energy source.

**Microbial metabolism:** all the biochemical reactions that provide microorganisms the energy and nutrients needed to grow and reproduce.

**Nitrous oxide (N<sub>2</sub>O):** greenhouse gas about 300 times more potent at absorbing heat in the atmosphere than CO<sub>2</sub> on a 20-year timescale.

**Phage:** virus that infects a bacterial cell, also known as bacteriophage.

**Phytocover:** soil-based biocovers planted with vegetation.

**Radiative Forcing:** change in energy flux in the atmosphere. Positive values indicate that Earth is receiving more incoming energy from sunlight than it radiates to space, which causes warming.

**Ruminant:** grazing mammals that acquire nutrients from plants by microbes fermenting it in a specialized stomach prior to digestion.

**Rumen microbiome:** collection of microbes that live in the rumen stomach that ferment plant products for energy.

**Virus:** microscopic infectious agent that replicates only inside the living cells of an organism.

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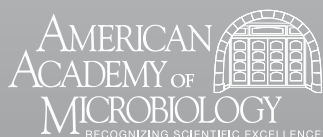
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