

Greenhouse Gas from the Garden

It is commonly thought that methane forms either chemically, at high pressure or temperature, or as a product of microbial activity. But there are also other ways. Junior scientists working with **Frank Keppler** from the **Max Planck Institute for Chemistry** in Mainz discovered unexpected sources of methane: plants, fungi, soil – and even meteorites.



Illuminating stress: Plants release higher levels of methane under UV light. The precursors are contained in the substance pectin, found in the cell wall.

TEXT **MAREN EMMERICH**

The man doesn't exactly seem like a revolutionary. Nevertheless, he is the one who turned conventional thinking around on the chemistry of methane in 2006: not only do microorganisms in bogs, moors and the stomachs of ruminants produce methane, but plants, too, release that most common of all carbon compounds. Moreover, they do this under aerobic conditions, in the presence of oxygen – thus contradicting a second and long-standing paradigm about the formation of the molecule known primarily as swamp gas. Now Frank Keppler and his fellow scientists have fanned the fire: fungi are also sources of methane, according to the new insights gleaned in the laboratory at the Max Planck Institute for Chemistry in Mainz. But first things first.

MICROBES AS SOURCES OF GREENHOUSE GAS

Ten years ago, the chemistry of methane was well understood. Composed of carbon and four hydrogen atoms and having a greenhouse gas effect some 25 times as high as that of carbon dioxide, scientists then knew of only two ways for this substance to form. A single group of microorganisms is responsible for about two-thirds of the roughly 600 million metric tons that reach the atmosphere globally each year. These methanogenic single-celled organisms, known as Archaea, colonize systems that lack elemental oxygen, such as rice fields, moors and the digestive tracts of animals. They survive in these environments on the energy released when hydrogen and carbon dioxide react to form methane and water. The remain-

below | Lavender (*Lavandula angustifolia*) produces more methane when the researchers gently compress the leaves or cut them up (left). If they nourish the plants with methionine tagged with an isotope, it can be detected in the methane. The scientists compare the methane emissions of various plants in the lab, including rosemary (*Rosmarinus officinalis*) (right).

right | On the trail of greenhouse gas: Katharina Lenhart and Frank Keppler discuss a new experimental setup with which they want to measure methane emissions of plants.



ing third of gases results from chemical reactions that take place under very high pressure or at high temperatures, for instance in the Earth's crust or during forest fires or wildfires.

No one questioned that this methane cycle was closed and complete when Keppler returned to Heidelberg from a research residency at Queen's University in Belfast, where he had done his Ph.D. a few years previously. Up to that time, the young geochemist had investigated primarily monohalo-methanes. These compounds play an important role in the natural destruction of ozone in the stratosphere. They consist of three hydrogen atoms and a chlorine or bromine atom bound to a central carbon atom. Keppler and his colleagues had determined that plants release bromomethane and chloromethane, especially as they mature.

"The transition from bromomethane or chloromethane to methane isn't a big step chemically," explains Keppler. "You simply exchange chlorine or

bromine for hydrogen. This suggests that methane can theoretically form from these compounds. Initially, however, we lacked the means to investigate this analytically." That changed with Keppler's move to Thomas Röckmann's group at the Max Planck Institute for Nuclear Physics in Heidelberg. "There, I simply took the plant material that was also known to release bromomethane and chloromethane," he reports. "And lo and behold, we did indeed detect methane."

SUNLIGHT STIMULATES AND FUELS THE FORMATION OF METHANE

Even at just 30 degrees Celsius, both freshly cut leaves and dried ones produce measurable amounts of methane, according to the results of the first lab experiments. When the scientists repeated the experiment at various temperatures, they made an interesting observation: each increase of 10 degrees Celsius doubled the speed at which

methane was released – right up to a temperature of 70 degrees Celsius.

From this, it became clear that the process couldn't involve enzymes. Enzymes are proteins that accelerate biochemical reactions in all living organisms. However, the enzymes required for methane production lose their structure, and thus their effect, above 42 degrees Celsius. It made hardly any difference whether the scientists used fresh leaf matter or previously sterilized samples. However, the rate of emission was many times higher if the plants were exposed to sunlight beforehand.

The article about the findings that the team working with Frank Keppler and Thomas Röckmann published in the journal *NATURE* in 2006 broke with the general view that there were only two mechanisms of methane formation. "This work triggered a real storm that went on for half a year. We received countless requests for interviews and e-mails of every sort. Some of these were euphoric about the discovery, but



there was also angry hostility expressed," Keppler recalls.

A number of critics suspected that the methane was formed in the soil by microorganisms, and the plants served as chimneys through which the gas escaped. Keppler has no doubts about this, either. "But there is an additional process that takes place in the plant itself," says the scientist. He and his colleagues have since gathered proof of this. This proof stems from studies in which the researchers tagged molecules with stable isotopes. As a result, individual atoms within these marker molecules have a different mass than their common counterparts in the environment. Heavy hydrogen, also known as deuterium, is one well-known example of a stable isotope.

To prove that plants actually release methane themselves, the scientists in Keppler's research group manufactured pectin molecules – plant sugars that contain the precursors of methane. The scientists tagged these compounds with

in the pectin molecules with deuterium. When they exposed the pectin molecules tagged with deuterium to UV light, methane formed containing these heavy hydrogen atoms. This proved unequivocally that the plant pectin released methane in a purely chemical process stimulated by UV radiation.

Other research groups came to similar conclusions in their investigations of the extent to which stress responses in general cause plants to give off methane. Regardless of whether the scientists triggered the stress by UV light, injured the plants by cutting them, or cut off their oxygen supply – the response was a consistent increase in methane formation.

To date, scientists can only postulate about the mechanism through which the stress-induced formation of methane arises. They assume that the cells unload excess electrons that have accumulated, for instance due to oxygen deprivation, on methyl groups – thus reducing them to methane. Ac-

cordingly, the temperature dependency the researchers observed in their experiments arises from the fact that chemical reactions proceed faster at higher temperatures than at lower ones.

THE ORIGIN: A VITAL AMINO ACID

However, even if the hypothetical mechanism itself were confirmed, this would not explain everything. "There is still an additional methane pathway that involves not just plants, but fungi as well," explains Frank Keppler. Methionine appears to play a key role in this process. This amino acid is an important component in the metabolism of all organisms.

Keppler's team member Katharina Lenhart, among others, reverted to isotope-tagged methionine when she investigated the formation of methane by fungi. Like pectin, methionine contains a methyl group that can easily react to form methane under certain circumstances. "We tagged this methyl group



in the methionine with a heavy carbon atom,” explains Katharina Lenhart. “Then we fed the tagged methionine to the fungi under sterile conditions. We subsequently took gas samples from the closed vessel and investigated the concentration of the methane as well as its isotope signature.”

A DISCOVERY WITH BROAD IMPLICATIONS

The scientists could clearly demonstrate through these experiments that the addition of tagged methionine leads to an enrichment of tagged methane in the gaseous phase. “There is only one explanation for this. The methyl group of the methionine has been split off and transformed into methane,” says the plant ecologist.

In contrast to plants, which also form methane through the purely chemical, stress-induced process, fungi form the gas mainly from methionine, according to current scientific knowledge.

This mechanism proceeds only as long as the fungi and plants are alive. “After we killed the fungi by boiling them, we could no longer measure any methane emission,” reports Katharina Lenhart. It therefore apparently involves a biochemical process rather than a purely chemical one. Otherwise, the process would continue in the dead plant matter. The scientists were able to exclude the possibility that methanogenic microorganisms play any role, since they made sure in the initial stages that neither bacteria nor Archaea were present.

The discovery could have broad implications, as the amino acid methionine occurs in all living systems. “Now of course we are trying to establish through experiments whether methionine can function as a fundamental methyl group donor for methane in plants, fungi, algae and mammals,” explains Frank Keppler, alluding to the future plans for his research group.

The amount of the gas released by these sources is secondary for him.

What interests Keppler is filling the gaps in the course books. It’s clear from his findings thus far that there are additional methane pathways, ones that depart from established models, ones that run neither via microbes nor via high pressures or extreme temperatures.

“The paradox is that these processes take place under aerobic conditions,” says the chemist. Methane, the most strongly reduced organic compound of all, should be formed under conditions of oxygen starvation. “But now we’ve found processes that take place in oxygenated environments and represent a third pathway. Now we need to investigate this system step by step.”

These additional methane pathways aren’t restricted to the Earth. After all, methane isn’t released only from organic substances in the Earth’s soil by photochemical processes. The scientists have also observed formation of the gas in meteorites that contain several percent carbon. “Methane could be formed

left | The scientists in Mainz measure the isotope composition of methane with the aid of a mass spectrometer. Using the isotope's signature, they can determine which process formed the gas.

below | From forest to lab: White-rot fungi (*Phanerochaete chrysosporium*) flourishing on spruce (left). The researchers cultivate it in glass vessels to measure the methane emissions. In contrast, determining how much methane plants and fungi produce in the wild, such as glowing yellow stagshorn (*Calocera viscosa*, right), is difficult.



this way on Mars, for example, without life forms playing a role," Keppler believes. Contrary to the Earth, Mars doesn't possess a protective ozone layer that shields it from the majority of incident UV radiation. For that reason, this process is probably even more relevant there than it is here.

OTHER POSSIBILITIES DISREGARDED

As to the question of why many additional sources of methane could have remained undiscovered for so long, Keppler identifies two reasons. "For one, there is, of course, the common school of thought that Archaea produce methane – which they do, indeed, do on a large scale. That prevented many people from even beginning to consider other possibilities at all." He sees the second reason as having to do with analysis techniques. Compared to the methane concentrations in the atmosphere, often it is only trace amounts

that are released by plants, fungi and soil. "You have to exclude all the other sources very carefully to say with certainty that these sources actually do form methane."

Despite this, the scientist was never in doubt about his findings. Keppler undertook all aspects of the plant studies himself – from taking the samples right through to carrying out the measurements. He is so experienced with analysis that no one does a better job. However, he is also willing to admit mistakes. "What I'm not happy about, in retrospect, is the extrapolation we calculated in 2006," concedes the scientist.

At that time, he and his co-authors assumed that all plants form methane at the same rate they measured in their lab experiments. They projected that a total of 60 to 240 million metric tons of methane per year could originate from plants. The upper figure would correspond to 40 percent of the total global budget – it was clear to Keppler

and his fellow scientists that the rate at which plants form methane couldn't lie in this order of magnitude.

THE RELATIONSHIPS ARE MORE COMPLEX THAN ASSUMED

To their chagrin, many scientists still cite this upper value today. "What they fail to take into account, unfortunately, is that this extrapolation was modified over the course of time, just like any other result," Keppler says with regret. "We have since come a good deal further along and are examining the underlying mechanism." And it's becoming increasingly clear that the relationships in nature are significantly more complex than originally assumed.

"Plants and fungi don't occur in nature as isolated monocultures," says Katharina Lenhart. "A great many bacteria live right on the plant's surface. Some of these are, no doubt, types that oxidize methane, and thus destroy the



Methane producers from space: Meteorites also release greenhouse gas when the researchers irradiate them with UV light. Markus Greule takes a sample to determine the concentration.

molecule.” Methane is also broken down in plants. These degradation processes haven’t been included in any of the budgetary calculations up to now. So it’s entirely possible that current calculations are underestimating the ultimate order of magnitude for all methane sources and sinks.

Frank Keppler assumes that the disregarded methane degradation processes could make a 5 to 10 percent difference in the total budget. “If this is the case, then we need additional sources to balance the budget, of course,” says the researcher. “That has no impact on the total methane content in the atmosphere, but it would certainly affect the magnitude of individual sources and sinks.” With stress-induced formation of methane and the biochemical pathway via methionine, it still isn’t clear whether Frank Keppler’s group has identified all the sources that compensate for the degradation processes that were previously neglected.

Due to the large number and complexity of the processes that need to be taken into account for methane formation and degradation, Keppler and his team don’t want to risk making any fur-

ther predictions at the moment about how their discoveries might affect the overall atmospheric methane budget.

In any case, Frank Keppler and Katharina Lenhart don’t think that restricting formation of methane in plants and fungi as a way to constrain greenhouse gases is a good idea. These processes have always been going on; they don’t originate from human activities. “If you wanted to stop man-made climate change, you would have to intervene elsewhere,” Katharina Lenhart believes. “It wouldn’t make sense to employ any sort of means to prevent plants or fungi from giving off methane.” “If we wanted to reduce methane emissions, eating less meat would be considerably easier,” adds Frank Keppler. “That would have a much more noticeable effect.”

DESCRIBING THINGS THAT WERE PREVIOUSLY INCONCEIVABLE

The scientists see it as their mission to first understand how these processes actually proceed in nature. “It helps that we have natural scientists of all sorts in our group, from geologists and

plant ecologists to food chemists and physicists,” says Frank Keppler. “Otherwise, we wouldn’t be able to shed light on all the facets of such a broad topic.”

What fascinates him about methane is that it is the most commonly occurring organic compound anywhere – whether in the atmosphere or in the Earth’s crust. Scientists have also repeatedly established a relationship between this simple molecule and the origin of life. Stanley Miller employed it, for instance, in his famous experiment in 1953, when he replicated the early conditions on Earth in his lab. “Describing things that were previously inconceivable holds a certain fascination,” suggests Keppler. “Just like the headwind you encounter when you go up against the prevailing school of thought,” adds Katharina Lenhart. She experienced this for the first time with her study of fungi. Frank Keppler has since become accustomed to it. Nevertheless, he still remembers well how stressful he found the resistance he initially encountered to his work on the formation of methane by plants.

“For example, the Florists Association called us up and claimed that we

were disgracing their industry and said we should retract the publication. And then there were the people who had planned vacations in the rainforest and asked if they needed to be afraid of being poisoned." He laughs about these experiences today. "But faced with the situation, you do feel some responsibility. It was quite stressful to deal with the first wave of reactions. However, I also learned a lot from it all. And it brought me into contact with people who think differently than what the traditional textbooks teach."

Katharina Lenhart thinks it's great that her boss is unimpressed by conventional opinions and, to the contrary, that he's prepared to ask what underlies them. Keppler himself describes the approach that he also follows in his other scientific projects thus: "I take a look at the processes in the world

around me, and I regard patterns that catch my eye as intriguing and interesting. Then I check whether these processes have already been described in studies. And if they haven't, I plan a new research project."

This process inevitably results in him describing things that don't please everyone. Keppler doesn't see himself as a revolutionary. "Though I do sometimes get the impression that I am," he adds with a grin. ◀

TO THE POINT

- Methane originates via a chemical pathway, at high pressure or temperature, or as a product of microbial activity. Plants, fungi and even meteorites also emit this greenhouse gas.
- Plants produce methane when stressed, for instance under UV irradiation. Part of the gas originates from pectin, a component of the cell wall. Unlike microorganisms, plants produce methane in the presence of oxygen.
- Plants and fungi also release methane from methionine, an amino acid that is part of the metabolism of all organisms.
- Carbonaceous meteorites give off methane under UV irradiation, too. That could play an important role on Mars. The existence of methane thus doesn't constitute proof of life on the red planet.



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