Safe Havens for Methane Eaters

Methane-oxidizing bacteria play an important role in our climate system. Research currently being carried out as part of the European METHECO project demonstrates how crucial the protection of the biotopes that provide a home for these microbes may be.

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Below him there is just bog. And the bridge “we built ourselves,” says Peter Frenzel. The professor from the Max Planck Institute for Terrestrial Microbiology in Marburg inches across the makeshift wooden platform into the expanses of the Estonian raised bog toward a seven-meter-long tree trunk, “that my colleague Edgar Karofeld and I sank last year” and that appears to be steady enough to be used as a mount for the micromanipulator. The device enables mechanical interventions in very small objects or organisms, such as bacteria. In this case, more specifically: methane-oxidizing bacteria. Based on a precisely defined chemical reaction, these bacteria use methane as a carbon source to build up cell mass and harness energy.

Such “methane oxidizers” are among the few organisms that thrive in nutrient-poor raised bogs, whose only source of nutrition is rainwater. An electrode clamped to the micromanipulator records the oxygen content of the biotope. “The oxygen content,” explains the energetic and agile professor, “reveals a lot about the processes that take place down there.” The curve of the oxygen profile enables the microbiologist from Marburg to identify the precise depth to which the oxygen penetrates through the layers of soil. The work of the methanotrophs starts at the boundary between these worlds with and without oxygen.

METHANOTROPHS CONTRIBUTE TO CLIMATE PROTECTION

And what important work it is. According to the leader of the biogeochemistry research group, to put it in a nutshell, methane oxidation by bacteria is “of global significance.” Methane that is not converted by the bacteria escapes from the soil into the atmosphere, where it takes effect as the second most important greenhouse gas. This, not least, is why Frenzel and nine other European research teams launched the METHECO project three years ago, the funding for which runs out in 2009. The objective is to describe methane oxidation in typical ecosystems in Europe as a model for biotic communities of bacteria, to record the dynamics of the associated processes, and to understand the microbial diversity of the methane oxidizers using the most advanced scientific methods available, including molecular biology approaches.

“We got off to a sluggish start due to bureaucratic obstacles,” says Frenzel, “but we have now made some progress in reaching an understanding of methane oxidation.” The Marburg-based researchers have succeeded in providing a better description than previously available of at least one crucial bolt of this process: the nutrient nitrogen. And, thanks to their meticulously designed experiments, they managed to track down a food chain that features the bacteria and so-called eukaryotic protozoa, such as amoebae, at its center. Finally, it emerges that the robustness of ecosystems with methane-oxidizing
bacteria varies according to their geographical latitude, methane concentrations, and various other factors. In response to these insights, Frenzel’s former colleague, Paul Bodelier, has set himself a new objective: to ensure that the biotopes that house the methanotrophs and thus play such an important role in our climate system are afforded the same protection as biotopes that contain other important flora and fauna.

First described scientifically in the 18th and 19th centuries by Allessandro Volta and John Dalton, methane is a gas from the group known as the hydrocarbons. However, methane was already known to the alchemists of the Middle Ages as “swamp gas,” the gas produced when stagnant water is combined with decaying organic matter. Methane was first produced in the laboratory by French scientist Marcellin Berthelot in 1856, and methane-producing bacteria were discovered by Dutch biologist Nicolaas Louis Söhngen in 1906. It is now clear that this gas is always produced when certain microorganisms decompose organic material. It is estimated that 500 million tons of methane are emitted worldwide every year.

RICE PADDIES HEAT UP THE GREENHOUSE

One source of methane generated by human activity is gaining in significance, namely cattle breeding. The gas is generated by bacteria in the stomachs and intestines of ruminants. However, the biggest sources of methane are wetlands, for example bogs and, in particular, man-made paddy fields, which are constantly on the increase. The methane-producing bacteria, or related protozoa, the archaea, find optimum conditions in these environments. This means that oxygen is a very scarce commodity here. These methane producers thus dominate the mud of flooded rice fields and emit large volumes of the gas into the atmosphere. Approximately 90 percent of the methane leaves the muddy soil of the rice field through the rice stalks, with the remaining 10 percent rising in the form of gas bubbles. Methane acts as a greenhouse gas in the atmosphere and has around 20 times more impact than its far more common counterpart carbon dioxide.

The crux of the matter, however, is that even more gas would escape from the rice fields and other methane-containing environments if the gas were not practically devoured by methane-oxidizing bacteria. “These microbes function like a biofilter that allows only a certain volume of methane to escape through it,” stresses Peter Frenzel. In some sediments, the microbes harness up to 95 percent of the methane formed, but “less in the paddy fields, around 20 to 30 percent” – maybe not as good, but something at least. Three of the key questions that arise in this context for Frenzel concern the identity of the factors that control the process, how they relate to the microbial biodiversity, and how stable these crucial methanotroph ecosystems are in terms of disturbances.
These topics were, and continue to be, among the focus issues of the METHECO project, which is aimed at biodiversity in general – a buzzword that is currently very much in vogue in science and the field of environmental policy. The loss of species and the often associated disruption of important biological processes are now seen as one of the most important issues of our time.

“Biodiversity is vaunted almost as a panacea for all of our environmental woes,” scoffs Frenzel, with the sober expression of a skeptic. Flora and fauna are subjected to minute scientific monitoring and are condensed into figures, equations, and indices – often uncritically and excessively, and accompanied by high political expectations.

Furthermore, such concepts of biodiversity, which originate from zoology and botany, are not likely to work in microbiology. Many microbial processes – carbon mineralization, for instance – are carried out by such a large number of bacterial groups that it would probably be impossible to extrapolate a relationship between a diversity of species and the process. International experts largely agreed on this point at a recent conference. Instead, for diversity projects, it makes far more sense to select a specific process, like methane oxidation, behind which lies a manageable concept of diversity. “Then we have a sure chance of learning something fundamental,” says Frenzel. “The identity of the species that play a crucial role in a process is more important for us than the overall number of species present.”

Speaking of species, in the case of microorganisms, the definition of a species poses another problem. There is still no agreement among experts in relation to the genetic and metabolic features of the bacteria that should be used to differentiate between species. At least some experts are currently focusing on the differences in the microbes’ so-called 16S-rRNA (ribosomal ribonucleic acid), which is one of the central components of the ribosome, the protein factory of life.

BACTERIA WITH A PREFERENCE FOR PARTICULAR BIOTOPES

In light of this information, it is not surprising that microorganisms have thus far been omitted from the biodiversity hype. Despite constituting a large part of the biomass and biodiversity on our planet and playing a crucial role in the biogeochemistry and thus functionality of ecosystems, they are largely disregarded in environmental research programs and in the debates surrounding global change. “It’s difficult to get a foot in the door there,” complains Frenzel. Moreover, because microbial communities have the reputation of being extinguishable, they appear to be omnipresent and extremely resistant to disturbances. In many cases, however, this reflects wishful thinking rather than reality. Spurred on by the new testing techniques available in the field of molecular biology, in the few cases that have been analyzed, microbiologists have found that microbial communities display a preference for certain biotopes and do not scatter themselves indiscriminately all over the planet.

Even the biotic communities of soil microbes that are actually highly flexible react sensitively to disturbances caused by agriculture, and this can lead to a kind of “genetic erosion” of the microorganisms. This, in turn, affects the stability of the entire system. Furthermore, certain groups of microorganisms with restricted habitats control many important environmental processes. “Under certain circumstances, if disturbed, the recovery of such microbial communities could take decades,” fears Frenzel. And even if an ecosystem eventually recovers, irreversible damage may have been inflicted. In view of all of this and the lack of knowledge in this area, a pioneering project like METHECO was long overdue.

Thus, since 2006, Frenzel and his colleagues from nine different countries have been undertaking regular expeditions and sampling soils from different ecosystems in a variety of regions. They
analyze forests and meadows whose methane-oxidizing bacteria absorb the gas from the air, and they examine soil from the banks of the Rhine, from natural wetlands such as raised bogs, and from paddy fields. The Marburg-based scientist has been to a variety of locations, including the Norwegian island of Spitsbergen in the Arctic, which sees a steady stream of international research teams – Europeans, Indians and Chinese.

MICROBIOLOGISTS NEED TO ARM THEMSELVES

The conditions are uncomfortable: icy temperatures and harsh winds, even in July. Frenzel and his team set out to do their daily work from the Koldewey Station, which is run by the Alfred Wegener Institute in Bremerhaven, but not without their weapons. “We set off with a rifle on our backs,” he reports, “the successor model of an old 98K with a hefty recoil.” First they do target practice to improve their accuracy, and then they turn their attention to science – this is everyday reality for the researchers up there, as polar bears can cross their paths at any time, “and you can’t dash back, either, as that would startle the geese.”

So Frenzel’s colleague Mette Svenning is seen with her weapon at her feet while the Max Planck researcher works with basic equipment on the “frozen-stiff” peat soil and goes stalking for methane-oxidizing bacteria. Frenzel is equipped with his micro-electrodes for measuring oxygen, a bag with syringes and needles, and his own gas chromatograph for the initial analyses. He collects soil cores that are frozen immediately in liquid nitrogen for molecular analyses of the bacterial genetic material. He quickly takes another few gas samples to see how the methane concentration in the soil changes.

Such data is important for the laboratory analysis that begins immediately after Frenzel’s return to the highlands of central Hesse. They provide a clear picture: two species that process methane thrive in the frost. After Frenzel’s analyses, it is also established that they oxidize methane and thus render it harmless to the Earth’s atmosphere. “These fellows are really active.” A comparison with the METHECO data now coming in from the other research groups reveals that the diversity of the methane-oxidizing bacteria increases the more southerly their location in Europe. Frenzel identifies a species-poor bacterial flora in the Arctic and extensive diversity in the Mediterranean with perhaps around 30 species. The rice paddies appear to be one of the habitats with the highest levels of diversity. This is one thing. The other is that the spectrum of species present in the communities differs based on the methane supply. The drier the habitat, the less methane is available, and the lower the level of species diversity.

However, the situation in the wetlands of Estonia is of particular interest to Frenzel. Despite the northerly location, high rates of methane-oxidizing bacteria can be observed, although it remains unclear as to how the biodiversity is organized there. All of the species there convert methane, the concentrations of which vary in the different habitats and are among the highest found in the wet habitats.

AMOEBAE ON THE HUNT FOR MICROBES

As part of the METHECO project, the scientists in Marburg also carried out a detailed study in recent years of the community of methane-oxidizing bacteria in an Italian rice cultivation area. They managed to track down a case of eating and being eaten – in other words, a food chain. The protagonists are eukaryotic protozoa (known as protists), such as amoebae, ciliates and flagellates, which literally graze on the methane-oxidizing bacteria. That, at least, is what Peter Frenzel and his colleague Jun Murase from the University of Nagoya in Japan assume. The two scientists have now definitive-
ly demonstrated this food chain with the help of an innovative laboratory experiment.

Murase isolated individual components of this ecosystem, consisting of the soil, the methanotrophs and numerous eukaryotic protozoa, from original soil samples taken from the paddy fields of Vercelli. From these “components,” he then created an artificial microcosm consisting of wafer-thin layers of sterile soil later colonized under controlled conditions with methanotrophs and protists. The biological processes of this ecosystem can be systematically measured using new techniques such as RNA stable isotope probing (SIP). The biologists use methane marked with heavy carbon that is processed by the bacteria. When the protists, in turn, have consumed the bacteria, they metabolize the carbon in the methane into their cellular substance. This entire process can now be reconstructed in the laboratory.

THE SEARCH FOR THE RELEVANT VARIABLES

With the help of further complex methodologies, Murase and Frenzel finally identified which bacteria represent a preferred source of food, and how the ecosystem changes as a result – in other words, whether the protists represent a key variable in methane-oxidizing systems. The results are clear: the protists graze on the methane-oxidizing bacteria and other microbes, and this alters the bacterial community. Certain amoebae, in particular, show a preference for certain species of methane oxidizers. “The pressure from the grazing protists regulates the composition of the ecosystem,” explains Frenzel. Ultimately, though, the rate of the methane oxidation does not change, even under increased pressure from the bacteria’s predators. “It would appear that other species jump in and take over the job,” concludes the microbiologist.
The question as to the factors that control the methane oxidation process thus remains unanswered. In any case, the protists appear to be out of the running as a variable here. Frenzel is now thinking in new directions. For example, the bacteria process the gas using an enzyme called methane monooxygenase, which occurs in two forms. One variant is called iron, the other copper. “There are even methanotrophic bacteria that build their own vehicle to make copper in the environment soluble and get it into their cells.” So could certain micronutrients such as copper help control and limit the process of methane oxidation?

NITROGEN FERTILIZERS SPELL RUIN FOR CERTAIN SPECIES

The nitrogen supply in the soil, on the other hand, has been deemed “a crucial variable for methane oxidation” for years now, at least under certain conditions. The methanotrophic species utilize nitrogen in a different form: some fix atmospheric nitrogen, and others also assimilate the nitrogen in ammonia, nitrate, amino acids or urea. The researchers are currently taking a closer look at the limiting factor for methane oxidizers from different biotopes, for example the rice paddies in Vercelli, in northern Italy, which the Marburg-based microbiologists now know inside out. Increasing volumes of rice are being consumed by ever-increasing numbers of people. Consequently, farmers are using more fertilizers than ever, including nitrogen-based fertilizers, to increase rice harvests. This could have repercussions for methane oxidation.

It was initially established that excess nitrogen hinders methane oxidation. This insight was taken as absolute dogma until Frenzel’s team discovered that it applies only under certain conditions. “If they have a lot of methane, they also tolerate an incredible amount of ammonium,” he says, “easily up to a hundred times as much.” However, with fertilizer use, the spectrum of the methane-oxidizing species is altered, causing changes in the ecosystem. Matthias Noll, Peter Frenzel, and Ralf Conrad, Director at the Max Planck Institute in Marburg, shipped rice soil from Vercelli to Hesse, applied fertilizer to some of it and none to the rest, and analyzed it using new molecular-genetic technology that can track down active methane oxidizers very accurately.

Two bacterial genera, namely *Methylocystis* and *Methylocaldum*, selected themselves out in the fertilized soil alone. The flood of nitrogen had absolutely floored all of the other species. Whether the species impoverishment causes the reduction in methane oxidation is not yet clear. In general,
however, the scientist concludes that the methane oxidation system is “extremely robust and largely resistant to stress” in most wet locations with high methane concentrations.

THE SENSITIVE SOULS AMONG THE METHANE EATERS

Nevertheless, as established by Werner Liesack’s Max Planck research team, methanotrophs from periodically flooded soils do appear to be more vulnerable. The microbiologists cultivated an organism that clearly possesses a completely new enzyme for methane oxidation – for use in the event that the methane concentration in the ecosystem fails. This variant of the bio-catalyst works far more effectively for this purpose than the more common enzyme. “This means that the relevant bacteria can survive at low methane concentrations.” The information available for methane oxidizers in predominantly dry habitats such as forest and meadow soils is scarce and provisional in nature. However, the initial METHECO analyses show that the organisms there are “really sensitive little souls” and “proper nitrogen fertilization annihilates them,” says Frenzel.

In the opinion of Paul Bodelier, it is these habitats that should be protected. Frenzel’s former colleague, who now works at the Netherlands Institute for Ecology in Niewersluis, sees “good reason to assume that such methane-oxidizing ecosystems could be damaged by environmental stressors in the future.” The biologist, who is also involved in the METHECO project, is thinking here of changes in the pH value of the soil, or mechanical faults. For example, when farmers plough the soil, the sensitive spatial arrangement of the microorganisms is disrupted in the long term. “Although I can’t prove it yet, I’m convinced that the methane oxidation suffers as a result.” The use of nitrogen fertilizers on these soils could have a similarly devastating effect on the ecosystem.

Not to mention climate change and its possible consequences, such as increasing drought or the thawing of the Arctic permafrost soil. No one can say at present how the microbial ecosystems in the permafrost would react to this change. After all, the Arctic permafrost contains around 14 percent of the global soil carbon, most of which could be released as methane under warmer climate conditions. In terms of climate modeling, Peter Frenzel now considers his work to be “basic research.” While methane oxidation plays a key role in the fight against the greenhouse effect, few climate models currently exist “that can depict these complex phenomena in the soil and the possible variables of methane oxidation.”

PROJECT
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