LAND OF PLENTY IN THE MEDITERRANEAN SEA

TEXT: KLAUS WILHELM

At the Max Planck Institute for Marine Microbiology in Bremen, Marcel Kuypers' and Nicole Dubilier's departments are unraveling the mysteries of seagrass meadows. Their discoveries have been astonishing: microorganisms play a crucial role in the fitness, productivity, and element fluxes of plants, not just on land, but also in the sea.

If you enjoy swimming in the sea or walking along the shore, you might be familiar with seagrass as unpleasant debris: the long, gray-green leaves often wash ashore en masse in the fall and after storms. At the coast of the Baltic Sea, for example, a thick carpet of seagrass is part of the beach scene – just like the dredgers that remove the plant debris. On the other hand, researchers, are just beginning to understand the real value of these underwater meadows.

Seagrass meadows are common in the shallow, coastal regions of the temperate and tropical seas worldwide. They cover about 600,000 square kilometers, an area larger than France. They are the foundation for an ecosystem that is home to many animals including endangered species like sea turtles, seahorses, and manatees, and provide a safe nursery for several species of fish.

Seagrasses are flowering plants and, unlike algae, form true roots. These roots keep them firmly anchored in the seabed protecting the coasts from erosion. Seagrass meadows produce vast amounts of oxygen and, every year, take up millions of tons of carbon dioxide, which they store as 'blue carbon.'

These underwater meadows are thus not only of immense ecological importance, but they also play a key role in the global climate. Until recently, relatively little was known about the microorganisms associated with seagrass. In their joint work, scientists at the Max Planck Institute for Marine Microbiology in Bremen have now shed light on the matter. Their expeditions to the Mediterranean Sea provide astonishing new insights. In particular, the question of how carbon storage in the ocean actually occurs has not been unveiled so far. The "Symbiosis" department of the Max Planck Institute in Bremen, headed by Director Nicole Dubilier, has now revealed a mechanism. Several years ago, research group leader Manuel Liebeke analyzed the metabolome of gutless worms native to a seagrass meadow off Elba in the Mediterranean Sea. The metabolome includes all of an organism's or ecosystem's molecules that are somehow involved in metabolism – amino acids, sugars, fats, and many more. To everyone's amazement, the researcher detected enormous amounts of plant sugars in the worms. Where could they have come from?

"We suspected the sugars came from the seagrass," Liebeke says. To investigate the source, the researchers sampled water from the root environment of seagrasses at several locations – off the coast of Elba, but also in the Caribbean. "To sample interstitial water from different regions next to and below the seagrass, we penetrate the sediment with a thin metal lance and use a large syringe-type collector," the scientist described the procedure. The samples are then shipped frozen to the Bremen laboratory as quickly as possible for analysis. The crux: the salt in the seawater would interfere with the measurements of the metabolites in the sample.

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PHOTO: HYDRA MARINE SCIENCES GMBH

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Playground for marine researchers: scientists from Bremen's Max Planck Institute for Marine Microbiology study seagrass beds of the species *Posidonia oceanica* off the coast of Elba.

Intimate partnership: microscopic images show a cross-section of a seagrass root (top) and a section of the root interior (bottom) showing the symbionts (in pink).

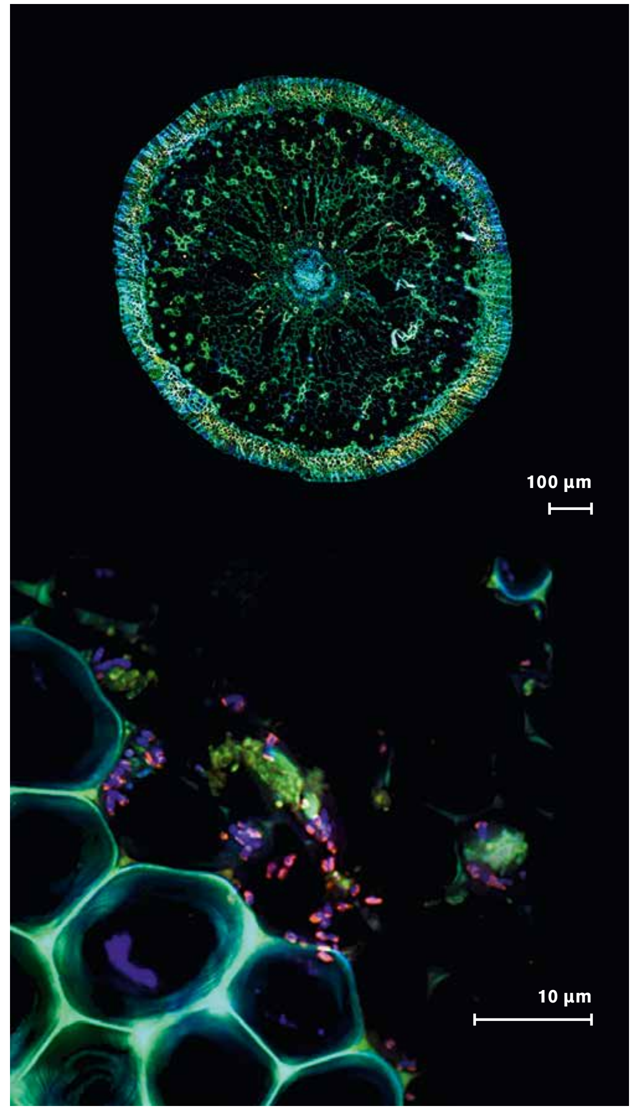


IMAGE: MPI FOR MARINE MICROBIOLOGY/DANIELA TIENKEN/SOEREN AHMERKAMP

Dubilier's colleague at the time, Maggie Sogin, took the lead in developing a method to initially remove the troublesome salt. The sample is then transferred to a gas chromatograph coupled to a mass spectrometer that records the chemical compounds in the pore water sample. This snapshot reveals which molecules are present and in what quantities. "In our pore water samples, there was always a gigantic peak that came from the sugar sucrose," says Sogin, who now works at the University of California, Merced. Never before had such masses of sugar been detected in the sea: "It is eighty times more than what is usually found in seawater," explains Dubilier. To put it into perspective: the researchers estimate that globally there are one million tons of sucrose in the sediment beneath all the seagrass beds in the world's oceans – a massive amount of sugar.

An overflow valve for energy

Physiologically, the release of sugars by the seagrass is most likely a mechanism to manage excess energy – similar to an overflow valve. But how can so much sucrose accumulate in an ecosystem? In the largely oxygen-rich environments on land, legions of microorganisms immediately pounce on the sugar and break it down. Most of the sediment in the root zone of seagrass meadows – called the rhizosphere – is, however, free of oxygen. But there are enough specialists among the microbes that can also cope with this. It took a while for the scientists to come up with the solution to the riddle. An insight from their worm research proved helpful: one of their colleagues had discovered that microbial symbionts living in gutless worms can break down phenols – chemical substances found in red wine, dark chocolate, tea, and berries, and are known for their antibiotic properties. This put the Bremen team on the right track: could the seagrass be producing phenols that prevent bacteria from devouring the sugars? The researchers launched a series of experiments that clearly proved that the seagrass actually produces phenols and releases them into the sediment, along with the sugar! These substances prevent many species of bacteria from utilizing sucrose. However, some microorganisms have adapted to the low-oxygen but phenol-rich situation and can still use the sugar. Thanks to this ability, they live unrivaled in a land of plenty.

The team of the "Biogeochemistry" department, led by director Marcel Kuypers, has been researching seagrass beds for about ten years. Kuypers' coworker Wiebke Mohr and her colleagues recently found out why seagrass can thrive in the Mediterranean despite its low nutrient content: in its roots, it maintains a symbiosis with a bacterium that provides the plant with the nitrogen it needs. The habitat of many seagrasses is low in nutrients for much of the year. In its molecular form, nitrogen is abundant in the ocean, but seagrasses are unable to utilize it in this form. The reason the plants grow luscious is due to their microscopic helpers that fix the dissolved nitrogen gas within the roots and make it available to the plants in a usable form. Together with colleagues from Hydra Marine Sciences in Bühl and the Swiss Aquatic Research Institute Eawag, the Bremen team investigated the organization of this intimate relationship between the seagrass and the bacterium. Until that point, the assumption was that the fixed nitrogen came from bacteria living in the sediment of the rhizosphere. “We’ve demonstrated that this relationship is much closer,” Mohr says. “The bacteria live right there in the roots of the seagrass.”

Nitrogen-fixing bacteria

This is the first time such a close symbiosis with nitrogen-fixing bacteria has been shown in seagrasses. Until now, it was only known from land plants: in particular, agriculturally important species such as legumes, wheat, and sugarcane receive nitrogen with the help of bacteria and in return provide them with carbohydrates and other nutrients. A very similar exchange of metabolites also takes place between the seagrass and its symbionts.

The bacteria that live in the plant roots are a new discovery. Mohr and her team named them *Celerinatantimonas neptuna*, after their host, the Neptune grass *(Posidonia oceanica)*. Relatives of *C. neptuna* have also been found in marine algae, such as kelp. “About a hundred million years ago, seagrasses colonized the sea from land. Back then, they probably adopted the bacteria from the large algae,” suspects Mohr. “They copied the system that was highly successful on land. To survive in low-nutrient seawater, they acquired marine symbionts.” The studies by the Bremen team bridge the entire ecosystem from seagrass productivity to the symbionts responsible for it in the root system. Research methods such as on-site oxygen measurements are revealing the productivity of the seagrass meadow. Special microscopy techniques such as fluorescence in situ hybridization (FISH) make it possible to color-code individual bacterial species and locate symbionts among the root cells of seagrasses. The bacteria’s activity can be detected in the NanoSIMS, a state-of-the-art mass spectrometer. Genome and transcriptome analyses reveal the mechanisms likely to be important for host-symbiont interactions.

SUMMARY

Seagrasses produce vast amounts of sucrose, which they store in the sediment. This sugar forms a large carbon reservoir for otherwise climate-damaging carbon dioxide.

Seagrasses’ roots house symbiotic bacteria that supply the plants with vital nitrogen.

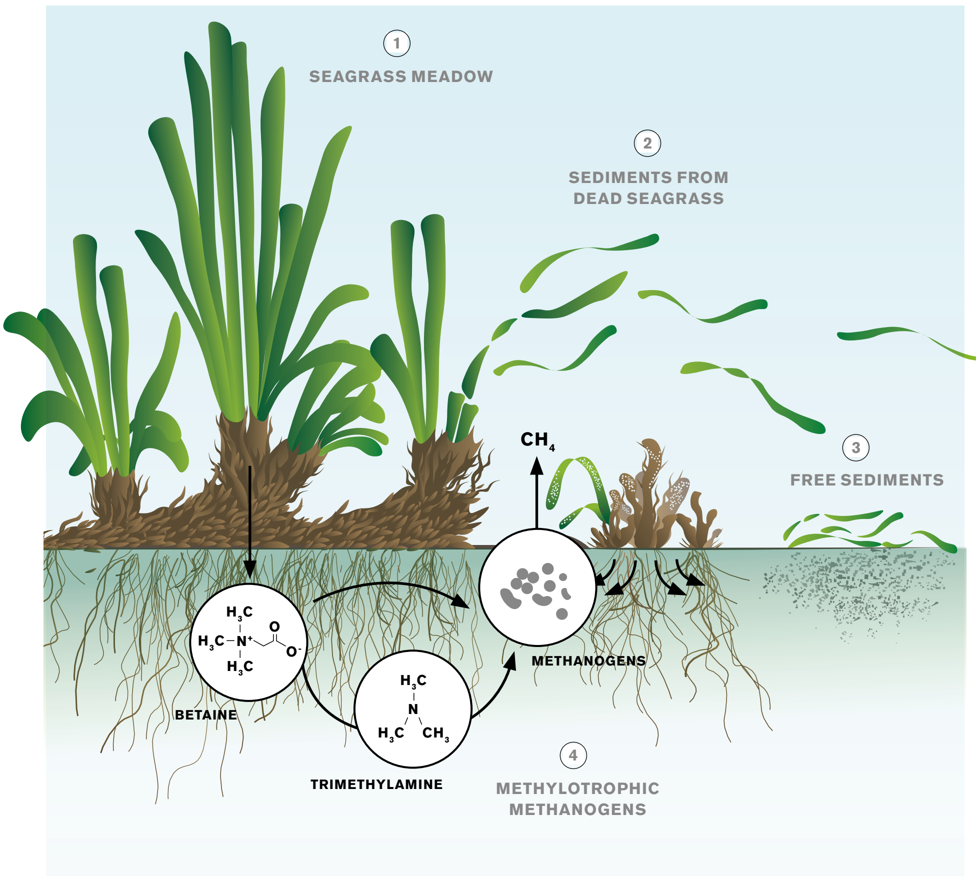
Other microbes contribute to the production of methane – a greenhouse gas – in seagrass sediments. This methane is released even when the plants have already died

The study also provided evidence that the seagrass community maintains a seasonal rhythm – depending on the supply of nutrients in the coastal waters. In winter and spring, the nutrients available in the water and sediment are enough for the seagrasses. “The symbionts are then present sporadically in the roots of the plants but are probably not very active,” Mohr says. In summer, when the sunlight increases, more and more algae grow and consume the nutrients. Then nitrogen also becomes scarce. At this stage, the symbionts take over and provide the seagrass with the nitrogen they need. This is how seagrasses can reach their largest growth in summer, when nutrients are most scarce in the environment.

Seagrass beds play an important role in the carbon cycle and, therefore, in the climate for two reasons: they are highly productive ecosystems and at the same time, a natural source of methane. As a PhD student in the “Greenhouse Gases” research group led by Jana Milucka, Sina Schorn is investigating the activity of methane-producing microorganisms including those in the seagrass beds of the Mediterranean Sea. Just like their land-based relatives, seagrasses form large peat deposits beneath the sediment surface during their growth. On land, peat releases large amounts of methane during the microbial decomposition of the organic material. The same happens underwater: methane, the simplest hydrocarbon, is also a greenhouse gas – and a far more potent one than carbon dioxide. In addition to protecting the climate, seagrass beds hence also have a climate-damaging impact that partially offsets the blue carbon effect. It was unclear for a long time, how the methane is formed. Schorn and her colleagues initially suspected that in seagrass beds, the gas is produced in the same way it is in terrestrial ecosystems. This made it all the more a surprise when the researchers took a closer look at the mechanisms of methane production: “In the sediments of seagrass beds, methane is produced only from a specific group of organic compounds,” the microbiologist explains. These so-called methylated compounds are produced by the seagrass plant itself. Specialized microorganisms – the methanogenic archaea – then take care of the conversion to methane.

Methane from dead plant matter

The methylated compounds include betaine, for example – a molecule that helps seagrasses cope with the fluctuating salinity of seawater, and its breakdown products, such as methylamines. Because methanogenic microorganisms can use methylamines



1) Neptune grass *(Posidonia oceanica)* produces a variety of methylated compounds that get broken down by microorganisms. Methane (CH_4) is produced in the process.

2) Plant parts in the sediments of dead seagrass beds release methylated compounds over long periods of time.

3) Detached seagrass leaves deposited on free sediments are also a source of methylated molecules.

4) A diverse community of microbes – called methylotrophic methanogens – lives in the sediment releasing methane directly from methylated compounds (such as betaine) or from their breakdown products (such as trimethylamine).

GRAPHIC: GCO, SCHORN, S., AHMERKAMP S. ET AL. DIVERSE METHYLOTROPHIC METHANOGENIC ARCHAEA CAUSE HIGH METHANE EMISSIONS FROM SEAGRASS MEADOWS. PNAS, VOL. 119 | NO. 9, MARCH 1, 2022

directly, methane production in seagrass beds is highly efficient and methane is released very quickly. As if through a straw, the gas passes from the seabed, through the plant tissue, and into the water. Because seagrasses only grow in shallow marine areas, microorganisms have little opportunity to break down the methane before it escapes into the atmosphere. “Moreover, the methane literally gets washed out of the sediment by the force of the waves,” says Milucka.

In the course of their study, the Bremen researchers sampled productive seagrass beds but also one that had already died. The latter revealed another surprise: “The rates of methane production there were similar to those in an intact seagrass meadow,” Milucka explains. “The reason for this sustained methane release is probably that the methylated compounds persist in plant matter for a very long time.” They were even still found in plant tissue that had died more than two decades ago.

Taken together, the Bremen scientists’ findings show that seagrass beds play an important yet widely underestimated role in climate change. At the same time, the underwater meadows are under severe threat: as nearshore habitats, they are particularly affected by human-induced changes. Above all, they are affected by overfertilization of the oceans from agriculture and aquaculture. Large amounts of phosphorus and nitrogen lead to excessive algae growth: the seagrasses can no longer obtain sufficient light and die off. In addition, more and more recreational boats anchor on seagrass beds and holes torn into the vegetation often do not close again. At a growth rate of only a few centimeters per year, underwater meadows regenerate very slowly.

The greatest threat, however, is global warming: Neptune grass, for example, is very sensitive to heat. Just a few more degrees will induce physiological stress in the plants and increase their die-off rate. “We’re currently experiencing a die-off of seagrass beds worldwide with devastating effects on coastlines,” says Milucka. This is fatal for the climate for two reasons: “Our findings show that after the plants die, carbon dioxide is no longer sequestered from the atmosphere and stored in the sediment as blue carbon. On top of that, methane continues to be released.”

Coastal regions as carbon reservoirs

The new findings make it clear that seagrass beds deserve far greater attention than they have received so far.

The Bremen-based scientists continue to work closely across multiple groups to improve our understanding of this fascinating habitat. Within the German Marine Research Alliance (DAM), the Max Planck Institute for Marine Microbiology is involved in a variety of projects focusing on coastal regions – by investigating, for example, how much carbon can be stored in seagrass meadows on German coasts. For this purpose, several institutions have joined forces in the sea4soCiety project.

Max Planck researchers Manuel Liebeke and Jana Geuer are involved in analyzing which carbonaceous substances the plants produce and release into the water: "We've already measured hundreds of different compounds," Liebeke says. Next, they aim to find out how stable these molecules are under stress, such as under strong UV light or at elevated temperatures. This is crucial for the fate of carbon: "The idea is to target coastal ecosystems that produce as many stable carbon compounds as possible over the long term," Liebeke explains. Reforestation of destroyed areas could also be conceivable if the coastal conditions allow it. With long-term planning, the Bremen researchers are also ensuring the future of their research on seagrass beds. To this end, a new research collaboration has just been launched in the western Mediterranean on the island of Mallorca. With half a million Euros funding from the Max Planck Society, the Portocolom lighthouse will serve as an outpost for Bremen's seagrass research in the future. Together with the Mediterranean Institute for Advanced Studies IMEDEA in Esporles, the German and Mallorquin scientists are planning long-term observations and detailed studies of the seagrasses and the habitat's many inhabitants. As the Max Planck Institute in Bremen has shown impressively, it's not just forests on land that count for climate protection – but also marine ecosystems such as underwater seagrass meadows.

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GLOSSARY

ARCHAEA
Unicellular microorganisms without a nucleus. Along with bacteria and eukaryotes, they form a third domain of life within biology.

BLUE CARBON
The carbon stored in marine/coastal ecosystems.

RHIZOSPHERE
Region of the soil that is directly surrounding the plant roots.

SUCROSE
Disaccharide consisting of one molecule each of glucose and fructose.

PHOTO: ACHIM MULTHAUPT FOR MPG

Out of the sea and into the laboratory: Sina Schorn examines samples of Neptune grass *(Posidonia oceanica)* collected off the coast of Elba. Her doctoral thesis is researching methane-producing microorganisms in seagrass beds.