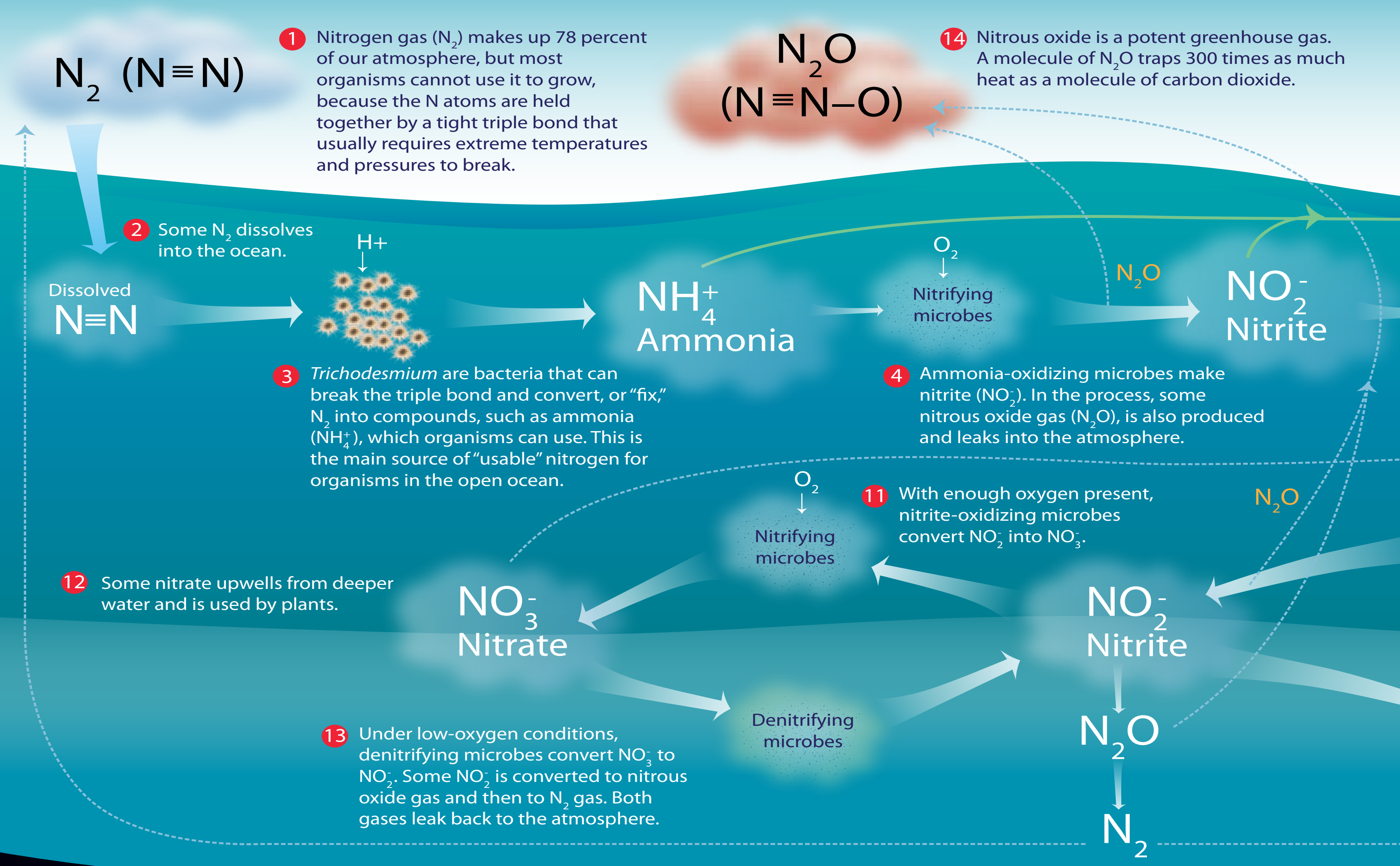


① Nitrogen gas (N_2) makes up 78 percent of our atmosphere, but most organisms cannot use it to grow, because the N atoms are held together by a tight triple bond that usually requires extreme temperatures and pressures to break.

② Some N_2 dissolves into the ocean.

③ *Trichodesmium* are bacteria that can break the triple bond and convert, or "fix," N_2 into compounds, such as ammonia (NH_4^+), which organisms can use. This is the main source of "usable" nitrogen for organisms in the open ocean.

④ Ammonia-oxidizing microbes make nitrite (NO_2^-). In the process, some nitrous oxide gas (N_2O), is also produced and leaks into the atmosphere.

⑪ With enough oxygen present, nitrite-oxidizing microbes convert NO_2^- into NO_3^-.

⑫ Some nitrate upwells from deeper water and is used by plants.

⑬ Under low-oxygen conditions, denitrifying microbes convert NO_3^- to NO_2^-. Some NO_2^- is converted to nitrous oxide gas and then to N_2 gas. Both gases leak back to the atmosphere.

⑭ Nitrous oxide is a potent greenhouse gas. A molecule of N_2O traps 300 times as much heat as a molecule of carbon dioxide.

Tracking nitrogen's elusive and essential

Chemical detective work reveals where nitrogen goes

By Carly Buchwald

Humans often seem to be unable to fix a problem without creating a new one. We invented DDT to kill mosquitoes and stop the spread of malaria, but almost caused the extinction of bald eagles and other birds. For industry and transportation, we developed fossil fuels, whose greenhouse gas emissions are now causing global climate change. And we invented a way to extract nitrogen from the air to make fertilizers that bolstered agriculture and helped feed hungry populations. But ultimately that has upset the balance of nitrogen, especially in the oceans, causing a rash of new dilemmas that we now confront.

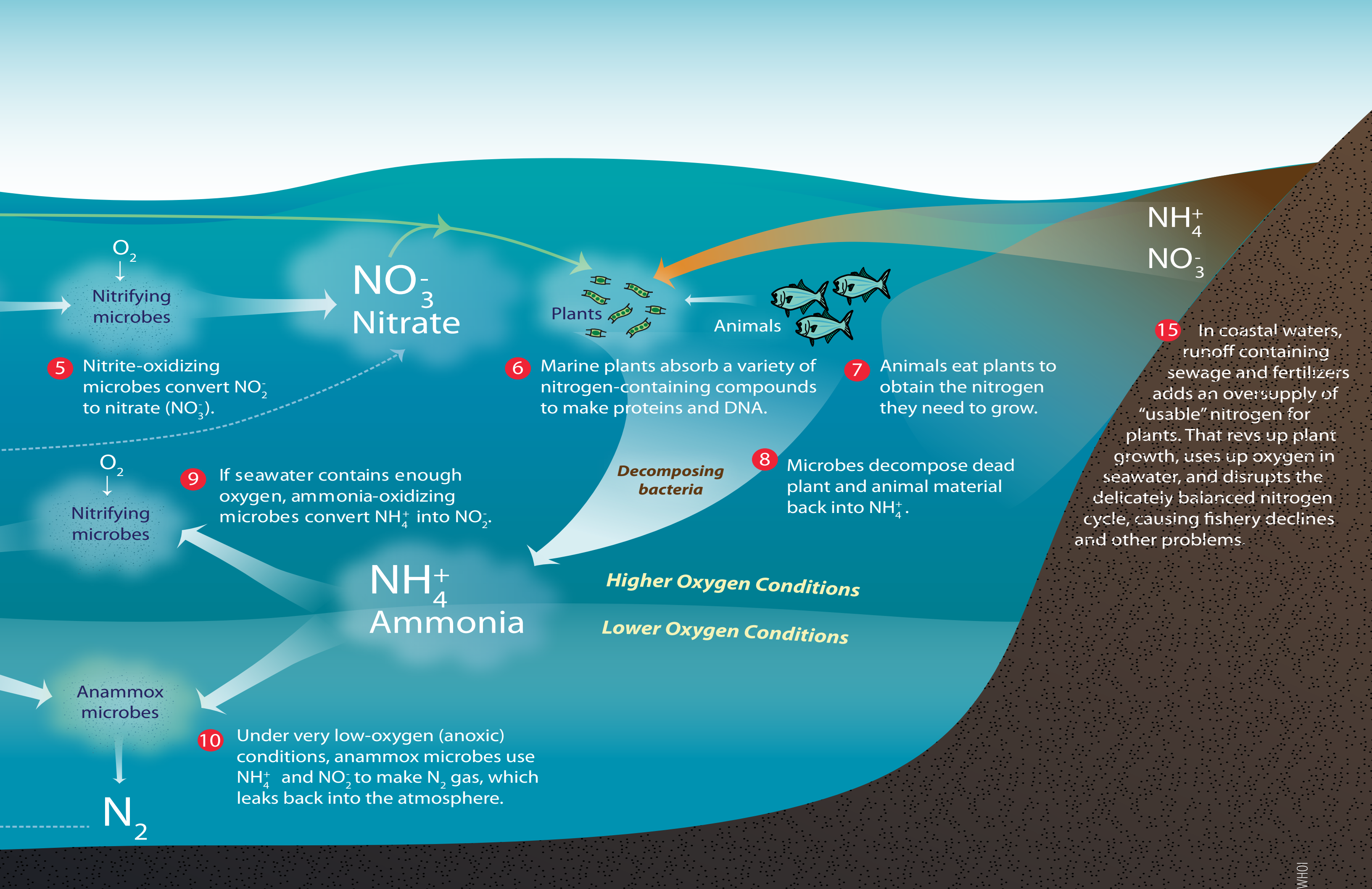
Fertilizers have leaked into the oceans through wastewater and runoff from farms and lawns, loading coastal waters with excess nitrogen that fertilizes the rampant growth of marine plants. The decomposition of these multitudes of phytoplankton removes oxygen from seawater, creating oxygen-poor "dead zones" where fish cannot live. The situation also may be leading to the increased production of nitrous oxide, a gas that traps heat 300 times more efficiently than carbon dioxide and also destroys ozone.

Any attempt to restore the nitrogen balance on our planet requires far more understanding than we now have of how nitrogen moves through the environment. To find solutions to these problems, we first need to determine how much nitrogen is entering the ocean, what happens to it once it gets there, and how much eventually gets removed or recycled back to the atmosphere. But tracking nitrogen—as it gets incorporated into various chemical compounds in the air, in the ocean, and in organisms—has been a daunting challenge.

Nitrogen's journey through the oceans is largely mediated by microbes. To live and grow, a variety of microbes use nitrogen in different ways, metabolically transforming it into diverse chemical compounds. They cycle it quickly and often concurrently in the same places in the vast ocean, so that we humans can't easily measure the chemical processes that are taking place.

But we're trying. As a graduate student in the MIT/WHOI Joint Program, I am working on a new method using a natural tag to follow nitrogen's trail. The tag that I measure can record nitrogen's chemical life history: where it came from, what chemical reactions it has undergone, and how and where it ends up.

(Continued on page 8)



5 Nitrite-oxidizing microbes convert NO_2^- to nitrate (NO_3^-).

6 Marine plants absorb a variety of nitrogen-containing compounds to make proteins and DNA.

7 Animals eat plants to obtain the nitrogen they need to grow.

8 Microbes decompose dead plant and animal material back into NH_4^+.

9 If seawater contains enough oxygen, ammonia-oxidizing microbes convert NH_4^+ into NO_2^-.

10 Under very low-oxygen (anoxic) conditions, anammox microbes use NH_4^+ and NO_2^- to make N_2 gas, which leaks back into the atmosphere.

15 In coastal waters, runoff containing sewage and fertilizers adds an oversupply of "usable" nitrogen for plants. That revs up plant growth, uses up oxygen in seawater, and disrupts the delicately balanced nitrogen cycle, causing fishery declines and other problems.

trail through the world's ocean

Katherine Joyce, WHOI

Nitrous oxide: a greenhouse gas that bears watching

By Caitlin Frame

There's a greenhouse gas whose concentration is on the rise because of human activities. But it's not the one you'd expect. It's nitrous oxide (N_2O), also known as laughing gas. It's been accumulating in the atmosphere since the 1700, and it's powerful and persistent. One molecule of N_2O has the same greenhouse warming power of 300 molecules of carbon dioxide. Once that N_2O molecule gets into the upper atmosphere, it can stay there for more than 100 years before it's destroyed naturally.

Fortunately, air has about 1,000 times less N_2O than carbon dioxide. But the rise in N_2O has accelerated over the past two decades. And while we know where the excess carbon dioxide is coming from, we don't know precisely how N_2O is produced. That's information we'll need to know in order to curb future N_2O production.

"As the U.S. gets serious about controlling greenhouse gas emissions, we need figure out how and where N_2O is made," said Kevin Kroeger, a scientist at the U.S. Geological Survey (USGS) in Woods Hole.

Every year an estimated 17 million tons of nitrogen are released to the atmosphere as N_2O, according to the International Panel on Climate Change. The source of more than half of this is probably soil, where scientists have tended to focus their studies. The rest comes from the ocean—where measurements have been harder to make. But as we take a closer look, we are realizing that N_2O production in the ocean is more important than we used to think.

"People are realizing that soil isn't the end of the N_2O story," said USGS scientist John Crusius. Kroeger, Crusius, and colleagues John Bratton and Eric Sundquist are leading efforts to measure coastal N_2O emissions. The USGS team seeks to answer questions such as: Where is the coastal N_2O coming from and how much is there?

At the Woods Hole Oceanographic Institution (WHOI), we're investigating other pieces of the puzzle: Which organisms are making N_2O in the ocean? How are they doing it? Are ocean conditions changing to produce more N_2O?

In the oceans and in soil, the primary players are microbes, which as they go about their lives, transform certain nitrogen compounds into N_2O. Humans have dumped huge amounts of these compounds into the soil and coastal waters, sending the microbes into N_2O overdrive.

(Continued on page 9)

Chemical detective work reveals where nitrogen goes

(Continued from page 6)

Nitrogen gas (N_2) makes up 78 percent of our atmosphere, but most organisms cannot utilize this source of nitrogen to grow, because the nitrogen atoms are held together by a tight triple bond so strong that it requires extreme temperatures and pressures to break.

In the early 20th century, German scientist Fritz Haber won the Nobel Prize for inventing a method to rip this bond apart and create other nitrogen-containing compounds that are more available for industrial use. He needed to use temperatures of 932° to 1,832°F (500° to 1,000°C) and pressures 100 to 250 times atmospheric pressure to break the triple bond.

The Haber process starts with so-called "non-fixed" forms of nitrogen, which aren't easily usable—nitrogen gas (N_2), for example, or other nitrogen-containing gases such as nitrous oxide (N_2O) and nitric oxide (NO). The process converts them into easily usable "fixed" forms of nitrogen, such as ammonia (NH_3), nitrate (NO_3^-), or nitrite (NO_2^-), which are essential nutrients for organisms to grow.

The Haber process provided a new supply of "fixed" nitrogen to make fertilizers that are now responsible for sustaining one-third of the world's population. In the future, however, with rising population and demands for food and energy, human-made sources of nitrogen in the environment are projected to double the natural input to the environment, reported two of the world's leading nitrogen cycle experts, Nicolas Gruber of the Swiss Federal Institute of Technology and James N. Galloway of the University of Virginia, in the journal *Nature* in 2008.

Which brings us to our current predicament. Agricultural runoff can overload coastal waters with nitrogen. Every year, for example, a mother lode of nitrogen drains into the Mississippi River, mostly from agricultural lands, and pours into the Gulf of Mexico. It stimulates a phytoplankton bloom and then an oxygen-depleted dead zone covering an area almost the size of New Jersey.

Nutrient-rich, oxygen-poor waters like these support a frenzy of nitrogen cycling, in which certain bacteria use nitrates as an energy source. As they eat the nitrates, they form nitrites as an intermediate chemical byproduct. What happens to the nitrites is key. They can either be converted back to nitrates and reused as food by other microbes. Or they can be converted to nitrogen gas and released back to the atmosphere. How can we follow what happens to the nitrites?

Isotopes to the rescue

With the help of my Ph.D. advisor, Karen Casciotti, I'm developing a way to use isotope ratios to track different chemical transformation processes involving nitrogen. Isotopes are atoms of the same element that have different weights. In my case, I study ^{14}N and the heavier ^{15}N, as well as two isotopes of oxygen: ^{16}O and ^{18}O. The key is that in chemical reactions, atoms with different weights behave a little differently.

Imagine your mom asks you to move 20 bricks from a pile of 100 bricks in the driveway to the basement. Some weigh 14 pounds, let's say, and the others weigh 15 pounds. Which bricks will you choose to move? The light ones, of course! After you are done moving your 20 bricks, the pile in the basement will have a larger ratio of light to heavy bricks than the pile in the driveway.

Similarly, microbes moving nitrogen around with their biochemical reactions usually pick lighter or heavier isotopes of nitrogen. So different microbes using different chemical reactions will produce end products with different ratios of heavy and light isotopes. We call this phenomenon an "isotope effect," and if we can measure these isotope effects, we can distinguish the reactions that produce them.

Sorting out the nitrogen cycle

In the Watson Laboratory at Woods Hole Oceanographic Institution, I'm conducting experiments with marine nitrite-oxidizing microbes first discovered in the 1970s by Stanley Watson (after whom the building is named) and John Waterbury, a scientist emeritus on the floor below me. Waterbury became an expert at culturing marine microbes like these and maintains a "library" of microbes in the Watson Lab that other scientists can use in their experiments. Nitrite-oxidizing microbes convert nitrite back into nitrate, and they are the only natural source of nitrate to the oceans.

In my experiments, I measured the isotope ratios of nitrogen and oxygen atoms in nitrate produced by these microbes from nitrite. We've discovered something unique about these mirobes—they actually prefer to move the heavy isotopes, ^{15}N. That's unexpected and interesting, but more important, it reveals something new about the oceanic nitrogen cycle. This information helps us track chemical pathways and determine how much nitrite is recycled naturally back into nitrate, which is to reused by other microbes in the ocean, and how much is converted into nitrogen or nitrous oxide gas, which is released back into the atmosphere.

Our experiments offer the promise that we can perform similar isotopic analyses of other chemical reactions that are going on in the ocean and begin to reveal exactly what is happening to the extra nutrients we are adding to our oceans. Can we continue to "fix" nitrogen into fertilizers without disrupting the natural environment? We won't know until we better understand exactly how human activities are affecting the nitrogen cycle. And that's where I can help.

Carly Buchwald was supported by the J. Seward Johnson Fund.

Tom Kleindinst, WHOI

Carly Buchwald was born and raised in Massachusetts. She attended MIT as an undergraduate, and by the time she finishes her Ph.D., she'll have spent a decade at the institute. She was an avid cheerleader (and football watcher) from middle school through college. She also likes scuba diving, and her love of the ocean began at a young age on family diving vacations around the world. She first visited WHOI to do a high school science project on hydrothermal vents and met geochemist Meg Tivey, a leader in the field. She knew she'd be back—and sure enough in 2006, she became an undergraduate summer student fellow in geochemist Karen Casciotti's laboratory studying microorganisms involved in nitrogen cycling. Her summer project piqued her interest in nitrogen isotopes, so she returned as a graduate student.

Nitrous oxide: a greenhouse gas that bears watching

(Continued from page 7)

When people add nitrogen-rich fertilizers to farmland and lawns, the nitrogen dissolves in groundwater and runoff that eventually flows into the ocean. In heavily settled coastal areas, nitrogen also seeps out of septic tanks (human waste, like other kinds of manure, is rich in nitrogen) or vaporizes over land and rains out or blows out to sea.

Coastal areas represent a small part of the total ocean, but the human impact on them is disproportionately high. Heavily populated coastlines such as Cape Cod get hit with a particularly heavy nitrogen load.

So far, the USGS group has focused on the intertidal zone, the region between low and high tide. A lot of N_2O could be coming out of this area, but it has been overlooked because most N_2O measurements are either made in soil or in seawater.

"Coastal (N_2O) fluxes in general are not that well understood and may be underappreciated," Crusius said. The USGS team's preliminary measurements in West Falmouth Harbor on Cape Cod show that in some areas, N_2O is released from sediments at a rate that's 60 times faster than previous reports on other coastal areas.

Compared to the open ocean, the conditions on the coast are less uniform and change quickly. The trick for these scientists will be to make enough measurements across a large enough area and over a long enough time span to be able to say how much N_2O is leaking out of the zone annually.

To catch N_2O as it's produced, the USGS team puts airtight chambers over sand exposed at low tide and then measures how quickly the gas builds up.

Unusual suspects

At WHOI, I am taking a closer look at the microbial "personalities" involved in making N_2O. It turns out that there are two major groups of microbes that can make N_2O. These two groups carry out very different chemical processes, but they depend on each other because they are part of a larger nitrogen cycle that needs them both to continue spinning (see page 32).

The two groups of N_2O-making microbes are called "nitrifiers" and "denitrifiers." The nitrifiers convert ammonia (NH_3) to nitrite (NO_2^-), a process that gives off a small amount of N_2O and gives the microbes the energy to convert carbon dioxide into their cell components (a lot like plants do during photosynthesis).

Nitrifiers can only do this conversion when oxygen is around. The relationship between oxygen and nitrifier-produced N_2O isn't straightforward though. For reasons we don't fully understand yet, when oxygen levels in their vicinity drop, the nitrifiers actually increase their N_2O production.

The consequences could be significant. The excess nitrogen that people are adding to coastal waters can stimulate blooms of tiny marine plants (phytoplankton), whose decomposition ultimately depletes the oxygen in the water. The result is a high-nitrogen, low-oxygen environment that encourages nitrifiers to make more N_2O.

Unlike the nitrifiers, the other N_2O producers, the denitrifiers, have trouble doing their thing when there's oxygen around. To get energy, they change nitrate (NO_3^-) to nitrogen gas (N_2). N_2O is an intermediate produced during that chemical reaction and some of it can leak out of the denitrifiers' cells and into the environment.

Which microbes made what?

So what is the "sweet spot" combination of conditions that make the nitrifiers and denitrifiers produce the most N_2O? To figure this out, we need to know where these two groups of microbes tend to thrive and how each contributes to overall N_2O production.

This isn't easy, because in many areas where N_2O is produced, it's possible for nitrifiers and denitrifiers to coexist. Furthermore, most N_2O detection methods can't distinguish the N_2O molecules made by nitrifiers from N_2O molecules made by denitrifiers.

I am working with Karen Casciotti, an associate scientist at WHOI, to figure out ways to distinguish which N_2O molecules come from which bacteria. To do this, we're looking at the isotopes of the nitrogen and oxygen atoms in the N_2O molecules. Isotopes are varieties of the same element that have slightly different masses. For N_2O, we're interested in two different isotopic flavors of nitrogen (^{14}N and ^{15}N).

We use a powerful mass spectrometer to separate N_2O that contains the heavier nitrogen isotope (^{15}N) from N_2O made from the lighter isotope (^{14}N). It turns out that in the N_2O molecule (with a chemical structure of $N\equiv N{-}O$), nitrifiers tend to put relatively more ^{15}N into the central nitrogen ($N\equiv{}^{15}N{-}O$) than denitrifiers do. We can measure that slight preference for ^{15}N, giving us a way to tell nitrifier N_2O apart from denitrifier N_2O.

We started making these measurements on N_2O produced by bacteria grown in our lab. Now we're applying the same technique to seawater samples that I collected on a recent cruise across the Atlantic, from Brazil to Namibia, on the research ship *Knorr*.

The cruise took us through marine environments with low and high levels of nutrients, and the cruise data will help us understand how these different conditions influence the rate and mechanisms of N_2O production. Casciotti also has plans to study the controls on coastal N_2O production at the Martha's Vineyard Coastal Observatory.

Although studying N_2O is tricky, new methods are making it possible for us to track the elusive gas. That's good news if we want don't want this greenhouse gas to have the last laugh.

Caitlin Frame has been supported by a National Science Foundation Graduate Research Fellowship and a National Defense Science and Engineering Grant.

Caitlin Frame grew up in Brooklyn, N.Y., where one of her first connections to the ocean was the Gowanus Canal. It was once so polluted, it actually stank, but intense community efforts have helped clean it up —you can actually see (live) fish in it now. Frame received a B.A. in biology from Harvard University and is working on a doctorate in marine chemistry in the WHOI/MIT Joint Program. She was drawn to microbiology "to observe things that are hidden in plain sight," she said. Along the way, she said she's gotten encouragement from amazing science teachers and hopes to pay the favor forward. Beyond her research, "I'm a winter sports fan," she said. "Skiing, hockey—if it's outside and it's cold, I like it."