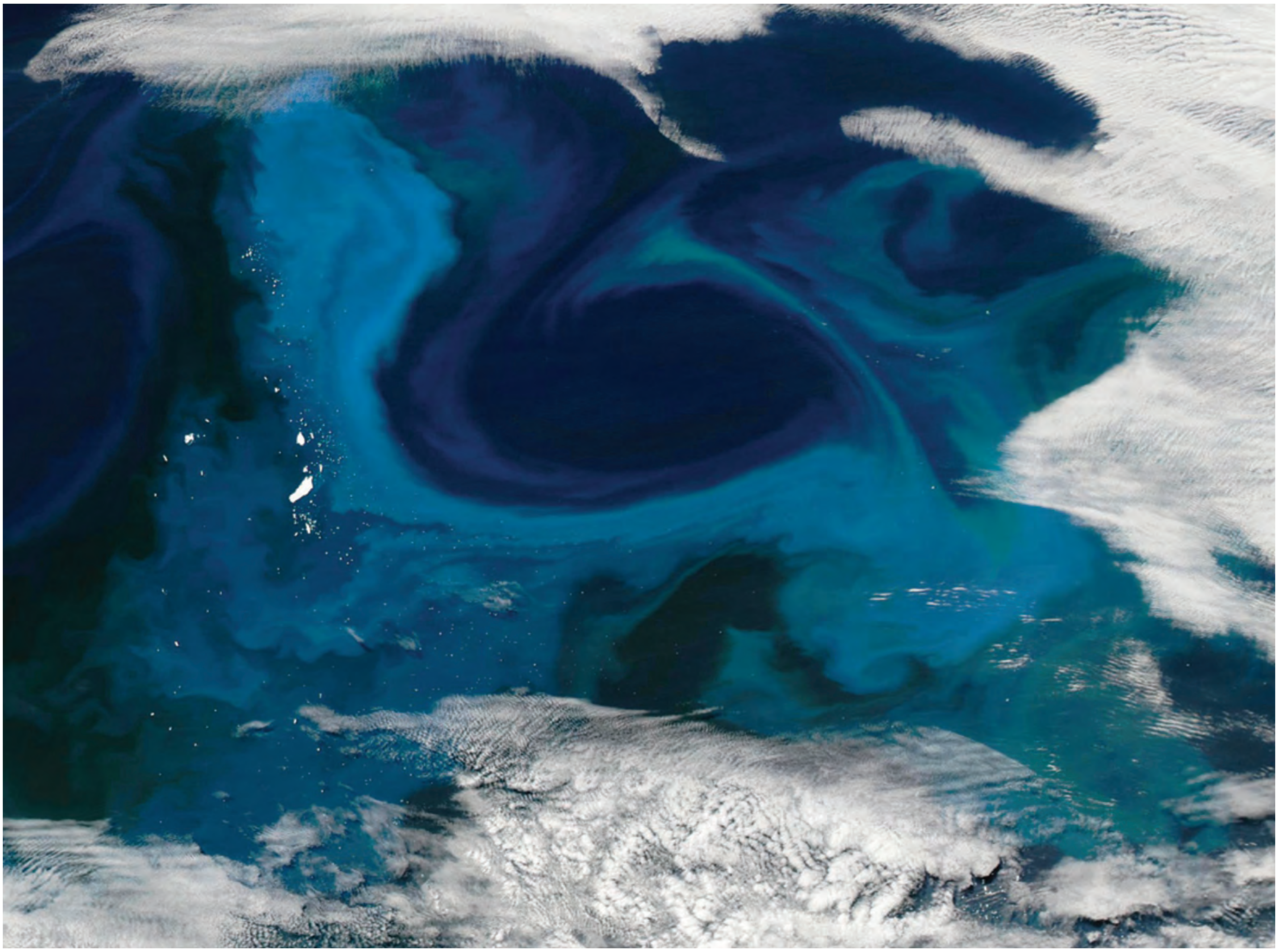

LAWRENCE BERKELEY NATIONAL LABORATORY/ SCIENCE PHOTO

Phytoplankton bloom (light blue) as seen in the Southern Ocean through a break in the clouds.

OCEAN SCIENCE

The power of plankton

Do tiny floating microorganisms in the ocean's surface waters play a massive role in controlling the global climate?

BY PAUL FALKOWSKI

The ocean is teeming with organisms so small you can't see them, populations of microorganisms called phytoplankton. Tiny they may be, but over recent decades these microscopic plant-like organisms have been shown to help drive the global carbon cycle. Further research by marine biologists is steadily revealing the important role of microorganisms and their genes, and raising new questions about how they evolved. Can we use this knowledge to help us restore balanced carbon cycling?

Colourful tropical fish flit among sea anemones in a coral reef. Anglers pose on deck with giant marlins. Porpoises play. The ocean's bounty of animal life has long provided people with food, adventure and a sense of awe and wonder. But none of it would be possible without the single-celled organisms called phytoplankton that float by the thousands in every drop of water in the top 100 metres of the sea.

Phytoplankton comprise two main groups: photosynthetic cyanobacteria and the single-celled algae that drift in the sunlit top layers of oceans. They provide food, directly or indirectly, for virtually every other marine creature. They emit much of the oxygen that permeates our atmosphere. Their fossilized remains, buried and compressed by geological forces, are transformed into oil, the dense liquid of carbon that we use to fuel our cars, trucks and buses. In addition, according to research that has only recently come into focus, they play a huge role in the cycling of carbon dioxide from the atmosphere to the biosphere and back, and this cycling helps to control Earth's climate.

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A CERTAIN RATIO

Early clues to the global importance of phytoplankton emerged in the 1930s. Over several research voyages, oceanographers had collected thousands of samples of sea water from the deep ocean (below a depth of 500 metres) around the

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world. They then measured the relative amounts of carbon, nitrogen and phosphorus — elements needed to construct essential cellular molecules — in both phytoplankton and the sea water. Alfred Redfield of Harvard University in Massachusetts realized that the proportions of these elements in the ocean were not haphazard. In every region of the ocean sampled, the ratio of nitrogen atoms to phosphorus atoms in the deep ocean was 16 to 1 — the same ratio as in the phytoplankton. Were the phytoplankton mirroring the ocean? Or were these tiny organisms determining the chemistry of the vast waters?

For more than 20 years, Redfield and others puzzled over why these ratios were identical. He eventually made a crucial conceptual leap, proposing in 1958 that phytoplankton not only reflected the chemical composition of the deep ocean, but created it[1]. He suggested that as phytoplankton and the animals that ate them died and sank to the bottom, along with those animals' faecal matter, microorganisms in the deep sea broke that material down into its chemical constituents, creating sea water with the same proportions of nitrogen and phosphorus.

"Phytoplankton not only reflected the chemical composition of the deep ocean, but created it."

The sea is not the only place where microorganisms shape the environment. Since Redfield's time, scientists have discovered that microorganisms also helped shape the chemical composition of our planet's air and land. Most dramatically, trillions of phytoplankton created the planet's breathable, oxygen-rich atmosphere.

By analysing a variety of minerals in rocks of known age, geologists discovered that for the first half of Earth's 4.6-billion-year history its atmosphere contained virtually no free oxygen — it only started accumulating 2.4 billion years ago. They found rocks containing fossilized cyanobacteria, or blue-green algae, whose present-day cousins perform a type of photosynthesis that uses the Sun's energy to split water into hydrogen and oxygen. There were no land plants to produce oxygen until almost 2 billion years after atmospheric oxygen levels first rose. It was the oxygen these photosynthetic microorganisms that created our oxygen-rich atmosphere.

Today, different groups of microorganisms, especially in the ocean, recycle waste produced by other microorganisms and use it to power global cycles of the elements most essential to life. Cyanobacteria and other groups also convert nitrogen gas (N_2) into ammonium (NH_4^+), fixing this essential nutrient in a form they can use to make the amino acids and proteins they need to build and maintain cells. Different microorganisms convert amino acids and other organic nitrogen compounds to nitrogen-containing gases, returning them to the atmosphere. And others help drive the recycling of different elements essential for life, including iron, sulphur and phosphorus.

Phytoplankton provide organic matter for the organisms that comprise the vast majority of marine life. They do this by consuming carbon dioxide that would otherwise dissolve in the sea water and make it more acidic. The organisms provide organic matter for the vast majority of the marine food chain. Removing carbon dioxide from water also allows more of it to diffuse in from the air, lowering atmospheric levels of the gas. In these ways, phytoplankton are crucial to the global carbon cycle, the circular path by which carbon atoms travel from the atmosphere to the biosphere, to the land and then back to the ocean.

CARBON CONFUSION

How do we know how individual elements such as carbon move through our vast oceans and the atmosphere? The first clues came in 1952, when a Danish ecologist named Einar Steeman-Nielsen introduced an important technique that would shed light on how carbon cycles in the ocean. It enabled scientists to measure an ocean ecosystem's primary productivity — the amount of organic matter that phytoplankton incorporate into their bodies through photosynthesis after meeting their own energy needs.

To make this measurement, Steeman-Nielsen added bicarbonate containing a radioactive isotope of carbon called carbon-14 to samples of sea water. When he exposed the samples to sunlight, the phytoplankton in the samples incorporated carbon-14 into their tissues. By isolating the phytoplankton and measuring the radioactive decay of carbon-14 in their cells, scientists could calculate the total amount of carbon dioxide fixed into organic matter.

Phytoplankton are the foundation of the ocean food web, providing organic matter for virtually all other marine creatures. Their primary productivity limits the growth of crustaceans, fish, sharks, porpoises and other marine creatures, just as the primary productivity of land plants limits the growth of elephants, giraffes and monkeys. By determining the productivity of phytoplankton, marine scientists can also determine how much carbon dioxide is being taken from the atmosphere.

For three decades, oceanographers used Steeman-Nielsen's carbon-14 technique to answer an important ecological question: how much organic matter do phytoplankton produce globally? The carbon-14 technique helped them measure how quickly phytoplankton were fixing carbon at thousands of sites across the globe, but the estimates of primary productivity they generated were far too low. They calculated that if the numbers were correct, the average phytoplankton in the ocean would take between 16 and 20 days to divide, but that didn't make sense to the biological oceanographers who were familiar with these organisms. The phytoplankton should have been growing much faster. Something was clearly wrong, but what?

Q&A Paul Falkowski

A slow-motion crisis

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THE VIEW FROM SPACE

In the late 1980s, chemist John Martin at the Moss Landing Marine Laboratory in California realized that the discrepancy occurred because of contamination. Most of the seawater samples taken over the previous three decades had been inadvertently contaminated by heavy metals from the black rubber O-rings used to seal the sampling devices. Rubber products are chemically treated during manufacture to give them the correct mechanical properties. This process, called vulcanization, involves treating them with sulphur containing some zinc and tiny amounts of lead. These metals leached from the O-rings and other components into the seawater samples, where they poisoned the phytoplankton. As a result, the measurements of primary production over three decades were compromised, causing scientists to seriously underestimate the importance of the world's oceans for the global carbon cycle.

Martin and others developed new sampling techniques that kept samples as free as possible of lead and other trace metals, allowing more accurate measurements of phytoplankton's primary productivity. But there was still a problem. Even with thousands of measurements of primary productivity in the world's oceans, most of the ocean was still not being observed in any given month or year. Mathematical methods could extrapolate from the primary productivity data to help fill in the gaps, but not well enough. No one really knew how much carbon the world's phytoplankton pulled from the water around them.

Obtaining reliable estimates of the ocean's primary productivity required a different approach. So scientists turned to data from the Coastal Zone Color Scanner (CZCS), launched into space on a NASA satellite, which was able to monitor the entire planet's phytoplankton populations each week.

The CZCS took advantage of the fact that oxygen-producing photosynthesis only occurs in organisms that have a pigment called chlorophyll a. This pigment enables the phytoplankton to absorb blue light, which would otherwise be scattered by the sea water. The more phytoplankton there are in an area of ocean, the more chlorophyll a there is and the darker the area appears from space. Oceanographers first calibrated the colour of the ocean in CZCS photographs with measures of primary productivity such as that developed by Steeman-Nielsen, and then used the colour measurements to obtain better mathematical estimates of phytoplankton productivity than were previously available. The results from several groups of scientists

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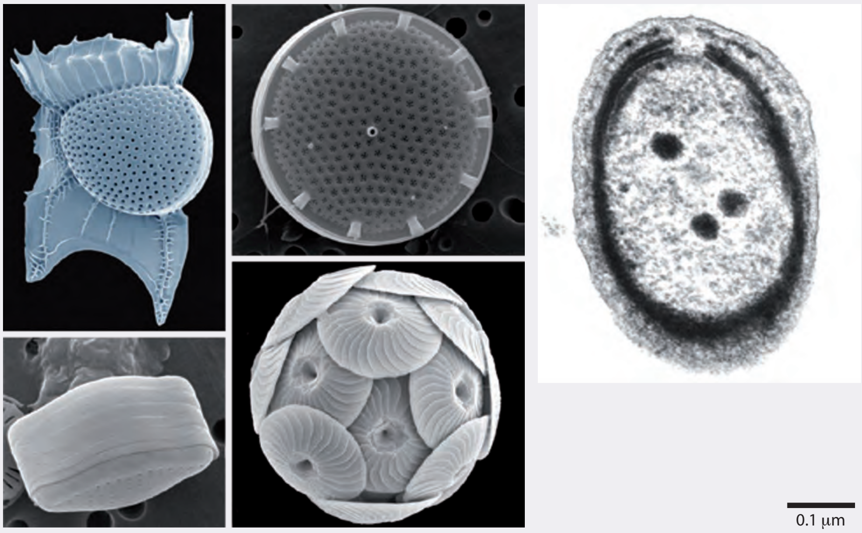
showed that the world's phytoplankton incorporated a stunning 45–50 billion tonnes of inorganic carbon into their cells, twice the highest previous estimate. The importance of phytoplankton in converting carbon dioxide into plant and animal tissue became clear.

How did phytoplankton's contribution compare with that of land plants? In 1998, a team from the Carnegie Institution of Science in Washington DC and my own lab at Rutgers University in New Jersey drew on data from the CZCS and other scientific satellites to find out.

We found that land plants incorporated 52 billion tonnes of inorganic carbon each year, just half as much as ecologists had previously estimated. Together, our results showed that we had vastly underestimated the global influence of the ocean's phytoplankton. Although they account for less than 1% of the photosynthetic biomass on Earth, phytoplankton contribute almost half of the world's total primary production, making them as important in modifying the planet's cycle of carbon and carbon dioxide as all the world's land plants combined[2]. This result surprised many ecologists, but the data were clear. The phytoplankton in our oceans are less visible than the trees and grasses we see in our daily lives, but their influence is profoundly underappreciated.

A BOUQUET OF PHYTOPLANKTON

Micrographs reveal phytoplankton's structural diversity and beauty.



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THE CARBON PUMP

Phytoplankton were so important to the planet's carbon cycle that we now needed to reconsider the fate of dead phytoplankton. Biologists set out to estimate the total biomass of phytoplankton and calculated that less than one billion tonnes of the single-celled microorganisms were alive in the ocean at any one time. There were 45 billion tonnes of new phytoplankton each year, 45 times more than their own mass at any given time. The phytoplankton would therefore have had to reproduce themselves entirely, on average, 45 times a year, or roughly once a week. In contrast, the world's land plants have a total biomass of 500 billion tonnes, much of it wood. The same calculations showed that the world's land plants reproduce themselves entirely once every ten years.

Phytoplankton have no roots, trunks or leaves. So what was happening to all the organic matter they were absorbing? Biologists considered two scenarios. In the first, all the phytoplankton in the sunlit top 100 metres of the ocean would be consumed in that layer by heterotrophs, animals and certain microorganisms that break down the phytoplankton's organic matter to obtain energy and nutrients to build their own tissues. This process would produce carbon dioxide. The carbon dioxide would be instantly available to be taken up by other phytoplankton, which would use it and the Sun's energy to grow. In this situation, carbon dioxide levels in the sunlit top layer of the ocean would be in a steady state, and none of the gas would be pumped to the deep ocean.

In a second scenario, the dead bodies of phytoplankton and some of the faecal material and bodies of the heterotrophs would sink slowly below the top 500 metres of the ocean. In the dark, cold waters below, scavengers and microorganisms would break down all this organic matter into its chemical constituents. Because those cold, deep waters rarely mix with the warm upper waters floating above, carbon dioxide and other simple nutrients would be stored in the deep ocean. A slow cycle of deep-ocean circulation would return this carbon dioxide-rich water to the surface centuries later, returning carbon dioxide to the atmosphere. In this scenario, the upper layer would act as a biological pump, sending carbon dioxide to the deep sea for hundreds of years.

In fact, both scenarios are occurring. In 2000, a team from my own lab and the University of Hawaii showed that phytoplankton and other organisms in the sunlit layer pump about 15% of the organic material produced each year to the deep sea[3]. Once there, about 0.1% of it gets buried in the seafloor, trapped in sediment. When conditions are right in Earth's crust, the fossil phytoplankton are turned into oil over a period of several million years.

> ***"There were more than 47,000 species in that one small area, and the biodiversity in the open ocean was immense"***

We have been using oil from fossil phytoplankton to fuel our cars and heat our homes for more than a century. Each year, we burn oil that took a million years to produce. This practice, along with our habit of burning fossil land plants in the form of coal, has pushed the atmospheric level of carbon dioxide to more than 390 parts per million (p.p.m.). This is 40% higher than before the industrial revolution and is driving global warming. The phytoplankton are still protecting us, however: if the phytoplankton in the upper ocean stopped pumping carbon down to the deep sea tomorrow, atmospheric levels of carbon dioxide would eventually rise by another 200 p.p.m. and global warming would accelerate further.

Ominously, global warming has begun to slow down this phytoplankton-driven pump. In a study I worked on that was led by Michael Behrenfeld, now at Oregon State University, researchers cross-checked satellite measurements of ocean chlorophyll with global climate measurements between 1997 and 2005. As the climate warmed between 1999 and 2005, we found that the upper layer of the ocean got warmer. Water becomes less dense as it warms and is more likely to float without mixing with the cold, nutrient-rich waters below. The warm top layer of these stratified waters therefore contained reduced nutrients, reduced phytoplankton growth, and diminished pumping to the deep sea. As our climate warms, we concluded, we can expect lower ocean carbon fixation in much of the world's oceans[4]. If that happens, it will alter ecosystems, diminish fisheries, and leave more carbon dioxide in the atmosphere. In a slightly more encouraging vein, carbon fixation could accelerate at high latitudes, such as the North Pacific, as its frigid waters warm.

GENOMICS AT SEA

Phytoplankton are clearly essential for the global cycling of carbon and other elements, but they are not the only microorganisms in the sea. How many other microorganisms are there in the oceans, and how are they making a living? For many years, no one knew how to address these questions. For scientists to study microorganisms in depth, they needed to grow them in laboratory cultures, but they could culture only a very small fraction of the microorganisms

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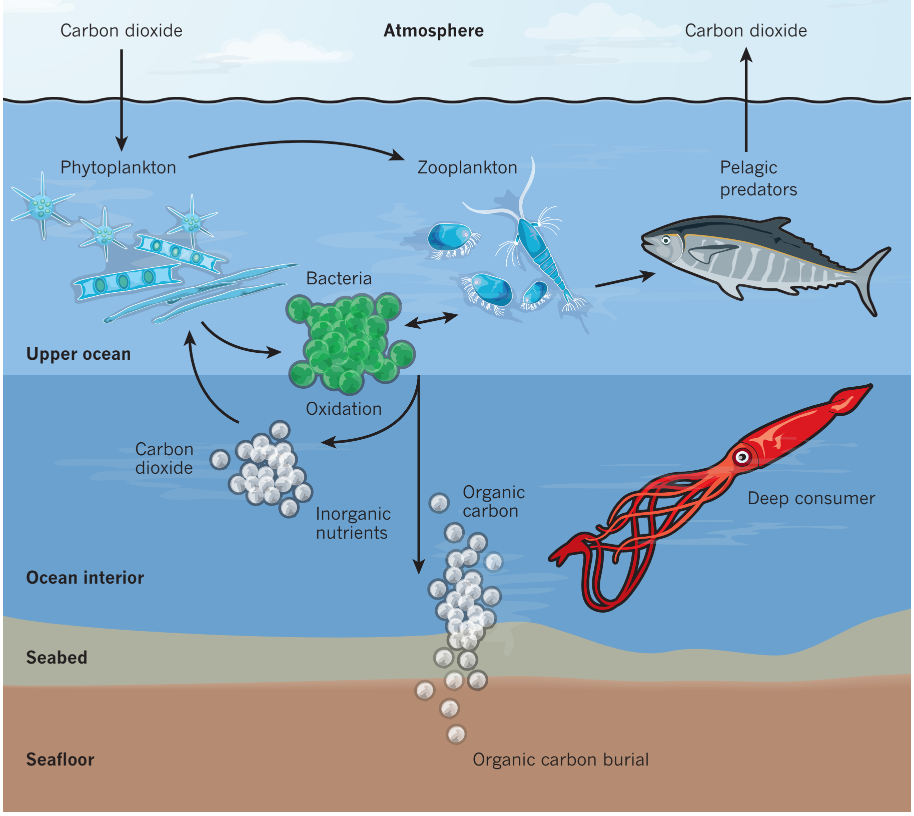
THE BIOLOGICAL PUMP

Phytoplankton drive a biological pump that uses the Sun's energy to move carbon from the atmosphere to the ocean interior, bringing down the atmospheric levels of carbon dioxide.



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they could see when they placed a drop of sea water under a microscope.

All that began to change in the 1990s, when marine microbiologists started using molecular biology techniques to survey the ocean's microbial biodiversity. They isolated bulk DNA from all the microbes in various samples of sea water. Then they used a technique called the polymerase chain reaction that allowed them to study all the samples of the gene that produced 16S ribosomal RNA, which every microorganism uses to manufacture proteins. Each variant 16S rRNA gene present indicated a different species of microorganism. These analyses typically revealed hundreds of microbial species in each sample of sea water.

In the early 2000s, biologists ramped up the biodiversity search using methods adapted from the human genome project. By then, molecular biologists had developed powerful techniques and computational methods that let them clone, sequence and analyse DNA thousands of times faster than before. Craig Venter, a molecular biologist and entrepreneur who had founded Celera Genomics, had helped developed one of those methods, called shotgun sequencing. In shotgun sequencing, an organism's DNA is broken randomly into many small segments and sequenced. Then a computer program finds regions of sequence overlap between the segments and uses them to stitch the segments together to reconstruct the original DNA sequence.

Not long after his team from Celera Genomics reported the first human genome sequence in 2000, Venter, an avid sailor, turned his attention to the sea. He sailed a research vessel to the Sargasso Sea, a well-studied area of the Atlantic Ocean off the coast of Bermuda, where his team collected hundreds of litres of sea water. They filtered the microbes out, isolated their DNA *en masse*, and began shotgun sequencing them on an almost industrial scale.

By determining the nucleotide sequence of more than 1.6 million cloned DNA fragments, they found evidence for 1,164 different microbial species in the seawater. They also estimated from statistical methods that even with their industrial-scale approach, they had failed to detect 98% of the species present. In other words, there were more than 47,000 species in just that one small area, and the microbial biodiversity in the open ocean was immense[5]. What's more, the Sargasso Sea is one of the ocean's least biologically active areas. Veter's study opened a door to large-scale genomics of the ocean itself, and by 2011 microbiologists had identified 20 million genes. This work has already found previously undiscovered forms of metabolism and new types of microorganisms.

Many of these genes are essential for the survival of the microorganisms, but about 1,500 genes are especially important. Some of these genes encode the proteins used in photosynthesis, which supplies the oxygen that keeps our atmosphere breathable and converts carbon dioxide to organic matter. Other genes encode enzymes that burn the organic matter with oxygen to create energy, returning the carbon dioxide and completing the cycle. Some encode enzymes that convert elemental nitrogen from the air to ammonia, which organisms can use to build tissues. Others encode enzymes that oxidize the nitrogen in the ammonia in several steps, regenerating the nitrogen. The enzymes encoded by these 1,500 genes do more than keep their organisms alive. Importantly, they oxidize and reduce the most abundant elements in organisms — hydrogen, nitrogen, sulphur, oxygen, carbon and phosphorus — allowing planetary-scale cycling that maintains an environment suitable to life as we know it.

MORE QUESTIONS

The more we learn, the more questions we have. Some of the questions are in the realm of basic biology. What evolutionary processes maintained such an extraordinary diversity of microbial species? Have microorganisms that play key biogeochemical roles gone undiscovered? How did these essential reactions evolve, and when did they become ubiquitous enough to influence the land, the oceans and the atmosphere worldwide?

Then there are the practical questions. As humanity pumps nitrogen into the oceans and carbon into the atmosphere, causing dead zones and disrupting the climate, how long can phytoplankton keep cleaning up our mess? Can we enlist phytoplankton genes to make hydrocarbons so we no longer have to drill for oil? Can we use other genes to help us harvest energy from the Sun? Can studying the diverse metabolic pathways of phytoplankton lead to ways to help them clean up oil spills or develop clean fuels that emit none of the carbon dioxide that drives climate change?

Ultimately, the microorganisms in the ocean will survive, as they have for billions of years, and they will help restore Earth to a biogeochemical steady state. If we can understand them better, perhaps we can help them help humanity survive as well. ■

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