



Microbiology

October 30,2020

A newly discovered (microscopic) global source of methane

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This Break was edited by Max Caine, Editor-in-chief - TheScienceBreaker

Methane is a potent greenhouse gas. Recent studies challenge the dogma that biogenic methane is produced solely in the absence of oxygen. Blue-green algae (Cyanobacteria) are photosynthetic microbes that are found in all illuminated environments on Earth and produce half the oxygen we breathe. We show that Cyanobacteria produce methane with potential significance to global climate.



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The average temperature on Earth rose dramatically during the last century. This is due to human activity, which led to the increased atmospheric concentration of certain gases, typically called greenhouse gases. These gases increase the solar heat trapped by our planet. The greenhouse gas methane is of great interest, as its atmospheric concentration has more than doubled within the last century. Nowadays, methane is the second most important anthropogenic greenhouse gas after carbon dioxide.

Most methane in the atmosphere originates from biological processes in sediments, soils, and digestive systems of ruminants. In these environments, in the absolute absence of oxygen, methane is produced by a unique group of microorganisms, methane-producing Archaea For decades, the biological source of methane was exclusively attributed to these microorganisms. Recently, however, this concept has been challenged by multiple studies showing that other organisms also produce methane, and surprisingly in the presence of high oxygen concentrations. First reported in 2011, and repeatedly ever since, researchers found abnormally high levels of methane in lakes and oceans coinciding with high concentrations of Cyanobacteria. Cyanobacteria, also known as blue-green algae, use light to fix carbon-dioxide into biomass via photosynthesis. Such a microorganism thrives in every illuminated environment on Earth. They produce over 50 % of the oxygen we breathe but are also notorious for releasing toxins into our waters. It has been





recently reported that Cyanobacteria can break down certain phosphorus-containing molecules, releasing methane in the process. However, we used the following three approaches, to investigate if freshwater, marine, and terrestrial Cyanobacteria can release methane independently of such precursors.

First, we asked whether Cyanobacteria convert carbon-dioxide taken up during photosynthesis into methane. For this purpose, we used carbon-dioxide enriched with a heavy form of carbon (a stable isotope). Such "heavy" carbon-dioxide was added to the cyanobacterial cultures. Thus, if Cyanobacteria indeed produce methane from carbon-dioxide, the methane molecules should also be heavier due to the labeling.

Second, we wanted to see when during the day do Cyanobacteria produce methane and at which rates. Therefore, we connected the cyanobacterial cultures to a device that can continuously analyze the concentration of gases dissolved in water, following the changes in methane, oxygen, and carbon-dioxide concentration in each culture every 10 seconds for several days.

Third, to check if our cyanobacterial cultures contain any intruding Archaea that may produce methane via the "classical" pathways, we used different molecular markers to search for such organisms.

The results supported our hypothesis that Cyanobacteria emit methane independent of any precursor other than carbon-dioxide. First, heavy methane was detected in all cases where Cyanobacteria were fed with heavy carbon-dioxide. This showed a direct link between methane formation and the photosynthesis process in which light is used to assimilate carbon-dioxide. Second, following the methane generation for several days, we observed that as soon as we turned the light on, methane was emitted parallel to the expected release of oxygen. This strengthens the previous result as it shows a tight connection between light and methane emission.

Third, our tests showed that in most cultures there were no other organisms present than Cyanobacteria. Only in a few cultures, we found a very low abundance of potentially methaneproducing Archaea. However, the production of methane simultaneously with the presence and production of oxygen suggests these are not responsible for the measured methane as oxygen strongly inhibits methane production by Archaea.

So far, Cyanobacteria were an unaccounted source of methane on a global scale. Though Cyanobacteria occur everywhere on Earth, it is difficult to upscale the methane production rates obtained from our laboratory experiments to a global scale. There are several gaps in the global methane budget for which Cyanobacteria could account for. Our study shows once more that we don't know as much as we would like to about where methane comes from – and our Earth is about to fall over the edge of a climate "cliff".

During the last two decades, we have witnessed an increase in the number of occurrences, duration, and cell density of cyanobacterial "blooms" in waters. This is driven, among others, by an increase in average water temperature due to global warming. The methane released by these Cyanobacteria during photosynthesis or by other documented pathways directly contributes to further warming, thus possibly forming a spiral feedback loop. We now aim to measure cyanobacterial methane emission in nature and understand what environmental factors drive the different rates observed in the laboratory.