Who Needs Oxygen?

Denitrification is an important process that occurs in just about every ecosystem on Earth, from land to sea, and from warm climates to cold climates. Denitrification is part of our planet's nitrogen cycle. Bacteria turn nitrates—an important fertilizer—into nitrogen gas. This process helps remove extra nitrates from wastewater, which helps prevent harmful algae blooms. The denitrification process involves several steps, each of which produce intermediate compounds. Intermediate compounds are produced in one step and consumed in a later one. One of these intermediates is a strong greenhouse gas—nitrous oxide (N_2O)—which can be detrimental when it escapes into the atmosphere. Due to the importance of denitrification in our natural world, denitrification has been an area of interest for scientists for a long time.

In previous studies, scientists noticed that many bacteria involved in denitrification have the enzyme needed for the last step in denitrification (Step 4 in **Figure 1** below) but do not have the enzyme needed for Step 2. There is only a tiny amount of nitrous oxide in the air, compared with quite a bit of nitrate in the soil. So although you might think that a cell relying on denitrification for energy might die without this enzyme, many cells can get just enough energy to survive, grow, and divide only by performing the Step 4 reaction. Scientists wondered under what conditions do bacteria survive while missing the Step 2 enzyme? Could these bacteria consume N_2O from the environment, acting like greenhouse gas vacuums?

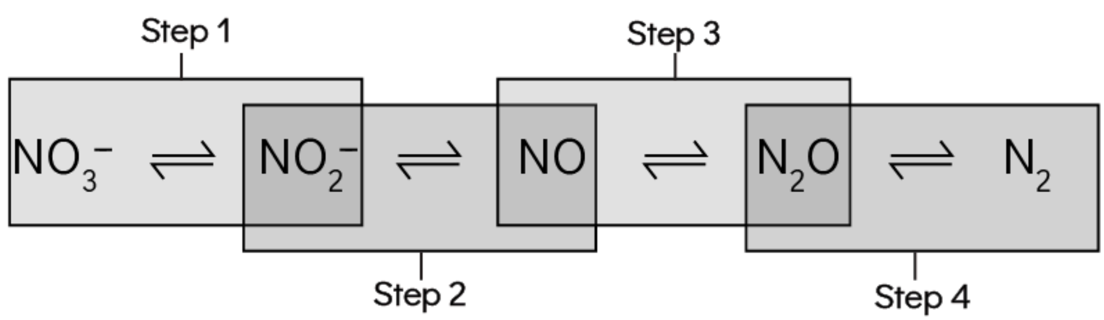

Figure 1. Denitrification. The denitrification process converts nitrate (NO_3^-) to nitrogen gas (N_2) in four stages. Each step in the process is catalyzed by reaction-specific enzymes. **Important!** The reactions shown are **not** balanced.

To examine this, researchers from Norway and the United Kingdom conducted a few different experiments looking at how the concentrations of NO (the reactant typically used to form N_2O in denitrification) and N_2 (the product formed from N_2O) varied over time and at different temperatures. The soil bacteria they studied were *Paracoccus denitrificans*.

In all of the experiments, the scientists cut off the O_2 supply to the bacteria cells, which would force the bacteria to use denitrification reactions instead of oxygen-dependent processes to produce energy. They also labeled the cells in two ways. First, they genetically modified the cells so that they would glow red if they had the Step 2 enzyme. They also gave the bacteria glowing green pigment molecules. If a cell was growing and dividing, these molecules would be spread out among its offspring, and so the green glow would fade over time. Cells that were not dividing would keep their bright green glow. A summary of the indicators are shown in the table below:

If the cells…	then they...	
	are dividing	have the Step 2 enzyme
Glow bright green	✖	✖
Lose their bright green glow	✔	✖
Glow red	✔	✔

Experiment A: How Does Denitrification Work at High Temperature? First, the scientists kept bacteria cells at 30°C and studied how fast denitrification was occurring by giving the cells a known amount of NO_2^-, the reactant in Step 2, and measuring the amounts of NO and N_2 nitrogen compounds produced. The results of these measurements are shown in **Figure 2.**

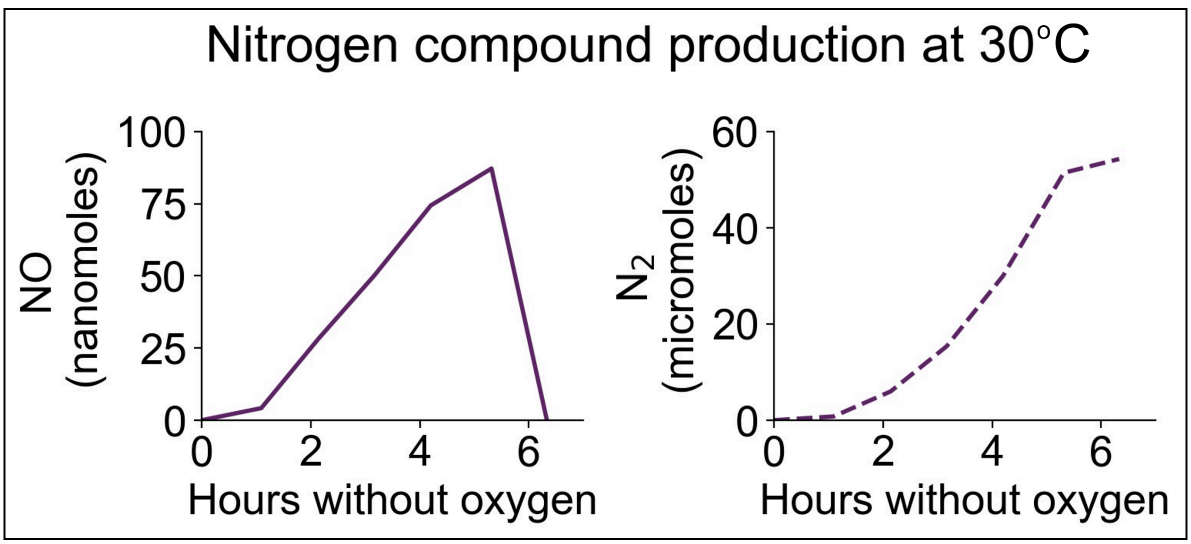

Figure 2. Amount of Nitrogen Compounds Produced by *P. denitrificans* at 30°C. During denitrification, NO is produced as an intermediate (the product of Step 2). Later, NO is used up in Steps 3 and 4 where N_2 is produced as a final product. Notice that the units of NO in the left panel and N_2 in the right panel are different. This is because NO is being made in Step 2 and consumed in Step 3 while N_2, as the final product, builds up over time. *Source:* Figure modified from Lycus et al. 2018.

All of the cells in this experiment glowed red, so they all had the Step 2 enzyme and were performing all steps in denitrification. Since all cells were doing denitrification, they all had energy to grow and divide, so the scientists were not surprised to see the green light fade over time. Denitrification had turned out to be pretty straightforward at high temperatures. But, how would this change under other environmental conditions?

Experiment B: How Does Denitrification Work at Low Temperatures? The scientists repeated their earlier experiment, but this time, they turned the temperature down to a cooler 17°C. The production of NO and N_2 in this experiment is shown in **Figure 3**.

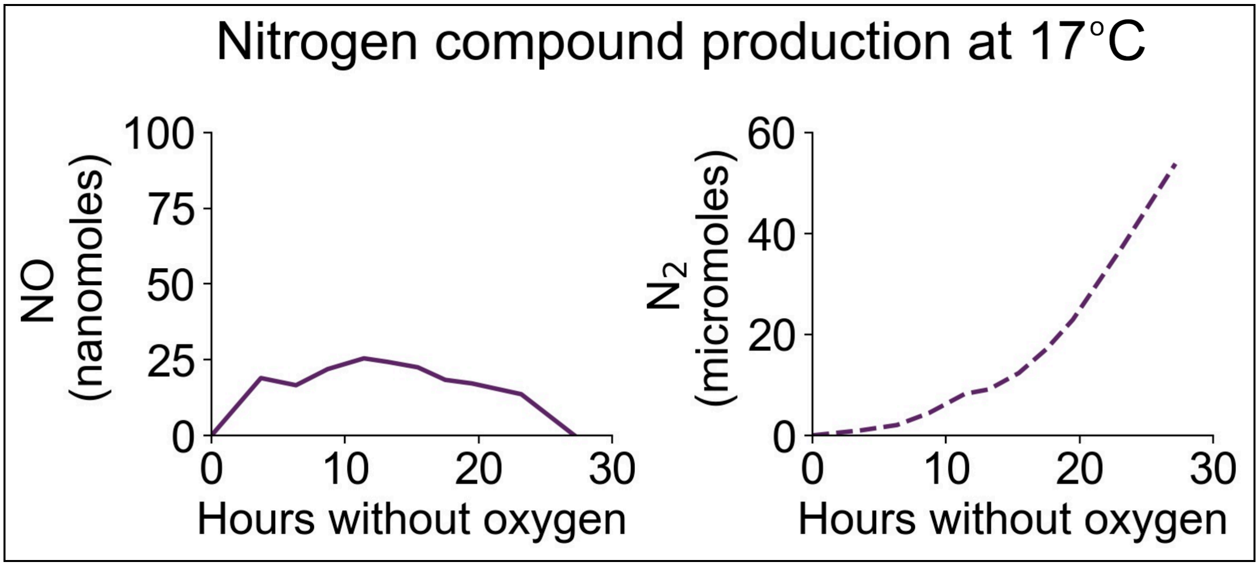

Figure 3. Amount of nitrogen compounds produced by *P. denitrificans* at 17°C. At the lower temperature the scientists studied (17°C), the soil bacteria still converted NO to N_2, but it took much longer. Like in **Figure 2**, the units of NO and N_2 are different. *Source:* Figure modified from Lycus et al. 2018.

At the cooler temperature, the bacteria produced NO and N_2 much more slowly. But this time, even though the green glow faded from all cells, only *some* cells glowed red! These results mean that all the cells were growing and dividing, regardless of whether or not they were performing the whole denitrification process!

Bacteria That Can Help the Environment

So, how did the cells without the Step 2 enzyme manage to grow and divide? Well, in yet another experiment, the scientists showed that bacteria could take N_2O from the environment and use it to make N_2. Most likely, the cells without the Step 2 enzyme were catching N_2O gas as it escaped from the cells with the Step 2 enzyme.

Together, these experiments let the scientists conclude that, given the right environment, *P. denitrificans* could use up extra N_2O made by other sources. In the future, the scientists hope to find ways to help clean up human-made N_2O pollution using bacteria like *P. denitrificans* that occur naturally in the soil.

Reference

Lycus, Pawel, Manuel Jesús Soriano-Laguna, Morten Kjos, David John Richardson, Andrew James Gates, Daniel Aleksanteri Milligan, Åsa Frostegård, Linda Bergaust, and Lars Reier Bakken. 2018. "A bet-hedging strategy for denitrifying bacteria curtails their release of N_2O." *PNAS* 115, no. 46 (November): 11820-11825. https://doi.org/10.1073/pnas.1805000115.

BiteScientist Profiles

Kelsey Lucas is a marine scientist fascinated by how the amazing behaviors animals do arise from the inner workings of their bodies and the physical laws that govern them. By understanding animal behavior holistically, she strives to better understand how nature works around us and how we can better care for, connect to, and learn from it. In her free time, she likes to volunteer for science education causes like BiteScis or at museums, and to be outdoors running, hiking, and sketching.

Kristen L. Cacciatore is a physics and chemistry teacher in Boston. She loves helping students apply what they learn in science classes to their everyday lives, like using physics to understand how to hit a baseball further or applying chemistry to make better tasting cookies. When she's not teaching, Kristen enjoys traveling far and wide, being outdoors, and reading books about everything.