Study reveals dynamics of microbes and nitrate

August 7 2014, by James Hathaway

Human tampering with global carbon balances has received massive public attention because of its effects on global warming, but we pay less attention to another set of chemical processes we are similarly disrupting: human input to the nitrogen cycle. Unfortunately, the story of nitrogen transformations in the biosphere is also less understood.

In modern times, humans developed the technology to turn nitrogen gas in the atmosphere into a biologically available form to be used as fertilizer. Before this, bio-available or "fixed" nitrogen was only created sparingly by natural forces such as lightning and nitrogen-fixing bacteria, while some fixed nitrogen was always being eliminated by denitrifying bacteria, which returned nitrogen to the atmosphere. This change to the planet's chemical balance has been critical because now the majority of life-essential nitrogen comes from human, not environmental, sources. Run-off from this systematic massive input of fixed nitrogen in managed natural and engineered environments (through agriculture and aquaculture), has lead to immediate problems like eutrophication in lakes and reservoirs and the oceans (with resulting dead zones), but also has long-term effects on the atmosphere.

Like carbon, nitrogen is naturally cycled and mediated in local ecosystems and integrated environmental processes by global cycling – facilitated by microbial communities. It has been well established that microbes take biologically usable nitrate ($NO3$ -) and process it in one of two critically important pathways – denitrification, which returns it to biologically inert nitrogen gas ($N2$,), or ammonification, which turns nitrate into ammonium (NH_4^+) and keeps the nitrogen biologically useable.

Because microbial ecosystems are vastly complex and difficult to monitor in nature, the environmental factors that determine whether microbial communities facilitate either the denitrification or the ammonification processes have been poorly understood. A variety of conditions—including temperature, pH, the carbon to nitrogen ratio, and sulfide concentration—have been suggested as potential determining elements but measurements taken directly from the environment have yielded conflicting or incomplete evidence.

Now, by performing 15 long-term experiments with a microbial community sampled from nitrate-filtering sediments found in a sandy tidal flat, researchers have been able to conclusively test the effect of controlled conditions and specific nutrient supplies on the fate of nitrate in nitrogen-cycling processes. The dynamics of structure and activity of the community were carefully monitored under controlled experimental conditions, using a suite of sophisticated bioinformatics techniques including metagenomics, transcriptomics and proteomics.

"I think our study differs in that we started with a really biodiverse sample that can do denitrification or ammonification and really let natural selection do its job," said Marc Strous, professor of geoscience at the University of Calgary and leader of the study. "We really get the best competitors in both pathways. This kind of study was not possible before the era of metagenomics because the whole system was simply too complex to study."

"The technology also allowed us to do continuous cultivation, where we are really able to mimic the conditions that occur in nature, but with the variables controlled," Strous noted.

The study will appear in the August 8 issue of *Science*. Strous' co-authors are Beate Kraft, Halinina E. Tegetmeyer, Timothy G. Ferdelman and Jeanine S. Geelhoed from the Max Planck Institute for Marine Microbiology, Ritin Sharma and Robert L. Hettich from the University of Tennessee-Oak Ridge National Lab Graduate School of Genome Science and Technology, and Martin G. Klotz, Department of Biological Sciences at the University of North Carolina at Charlotte.

The researchers found that three specific initial factors were conclusively responsible for determining the denitrification or ammonification of the nitrate supply, regardless of other conditions: the nitrite to nitrate ratio, the carbon to nitrogen ratio, and – hitherto unknown - the time it takes the microbes to duplicate themselves as a community (their "generation time"). Further, the team found that different specific cohorts of bacteria achieved system dominance to perform the processes, depending on the set conditions.

"The natural sample we used was from an area of sandy tidal flat, where nitrate flushes from fertilizer runoff pass through. The tides cause water movement through the sediments, so there is movement and disturbance and change," noted UNC Charlotte's Martin Klotz, an authority on microbial enzymes that process nitrogen. "Everything that survives there as a community has to be prepared for huge fluctuations. There are lots of little specialists that work together."

"There are very few micro-organisms that have in one cell all the tools to do either complete direction of these reactions," Klotz said. "It is usually different populations that translate to these bins that do one or the other and they are favored by environmental signals. Either there is energy and reductant available for them to make a living or they respond to different cues, such as pH, temperature, and so on. "

Strous notes that it really comes down to the basic chemical pathways and their energetics, which, depending on conditions, allow different assemblages of bacteria to work together and do their part in the process. The three factors identified by Strous' team's experiments are the critical switches that determine whether it is the denitrification or the ammonification process that dominates the microbial ecosystem, in that the factors select specific bacteria that succeed in getting major parts in the process.

"The factors we found that drive the processes are very generic," Strous said. "The findings imply that these can be applied to any ecosystem. It's really a generalizable result because the factors seem to act directly on the basic pathways."

Among the three factors the experiment identified as being critical in determining the process, the ratio of the two oxidized forms of fixed nitrogen, nitrate (NO3-) or nitrite (NO2-), is the most basic because when nitrite is more abundant (nitrate can change to nitrite and vice-versa), the process that dominates is inevitably denitrification.

"Nitrite accepts three electrons in denitrification and six in ammonification," Strous pointed out. "In both cases, the supply is there, but the difference between the way the denitrifiers respire them is that out of each electron accepted they are able to conserve more of the energy – less energy gets lost as heat, per electron."

When nitrite is more abundant, the denitrifying bacteria dominate because they are more efficient and faster. The fundamental advantage of the speed and efficiency of the denitrification process also contributes in part to the second critical factor the study found – microbial generation time. Regardless of the ratio of the fixed nitrogen supply, when conditions encouraged rapid regeneration (generation time of less than 1.7 days), then the denitrifying bacterial groups came to dominate (either rapidly or eventually), but slow growth conditions favored ammonifying bacteria.

The same kind of fundamental chemical energy reasoning seems to help explain the third critical factor the team identified – the carbon to nitrogen ratio.

"As has already been proposed in the literature, the carbon to nitrogen ratio is an important factor because, based on the bioenergetics, carbon limitation will favor denitrification – denitrifying organisms will have higher productivity under those conditions. Whereas the ammonifying microorganisms will have higher productivity when the nitrogen is limited. That follows from the way these organisms respire these compounds," Strous said.

"Carbon limited means that these organisms have less to eat. You need carbon for making biomass," Klotz added. "A good portion of these guys are fermenters and they produce products that serve others as reductant and energy sources – hydrogen, formate, acetate. If that is not happening because the carbon is low, then only the fast and cheap will prevail, which perform predominantly denitrification."

Though the findings are fundamental, Strous and Klotz both stress that gaining a more conclusive understanding of dynamics of the microbial systems in the environment that process the new, human-caused abundance of fixed nitrogen has important implications.

"When you look at the coastal systems we studied," Strous said, "those are actually fairly important in terms of getting rid of the nutrients in Europe.

"We understand this better now and this understanding is not trivial because the outcome of this competition is really important in determining primary productivity—because ammonium from ammonification is directly re-usable by the primary producers and with denitrification the nitrogen is mainly lost. So this hard-to-study bacterial competition is really important in determining that primary productivity. In any modeling you do on how human impact causes global change, this is a very important piece of the puzzle."

Klotz stresses how the study shows the importance of having new "omics" tools – metagenomics, transcriptomics, proteomics, etc. – to help study in real time the metabolic activity and changes going on in complex bacterial communities.

"We looked for what genes were being expressed, what proteins were there and we could correlate and assign inventory to lifestyles of organisms. We had taxonomic markers because we could assign N-cycle relevant genes to known genomes, so we knew who we could attribute the processes to," he noted.

"What was most interesting for me is I could localize in different taxonomic branches the different enzymes that participate at the different conditions," Klotz said. "I could distinguish between enzymes that are known to be involved in these processes. We could watch how a microbial ecosystem's biochemical modules compete under real conditions."

More information: "The environmental controls that govern the end product of bacterial nitrate respiration" *Science*, www.sciencemag.org/lookup/doi/ … 1126/science.1254070

Provided by University of North Carolina at Charlotte