



## **Denitrification in *P. denitrificans* and wetland sediments; the effects of trace-metals on N<sub>2</sub>O emissions.**

Georgios Giannopoulos (1), Katherine Hartop (2), Heather Felgate (2), David Richardson (2), Bonnie Brown (3), and Rima Franklin (4)

(1) Environmental Technology Lab., Dept. Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece (george.z.giannopoulos@gmail.com), (2) Center for Molecular and Structural Biochemistry (CMSB), School of Biological Sciences, University of East Anglia, Norwich, UK (d.richardson@uea.ac.uk), (3) Ecological Genetics Lab., Dept. Biological Sciences, University of New Hampshire, Durham NH., USA (Bonnie.Brown@unh.edu), (4) Microbial Ecology Lab., Dept. Biology, Virginia Commonwealth University, Richmond VA., USA (rbfranklin@vcu.edu)

Certain microbes, when faced with a shortage of oxygen (O<sub>2</sub>) in their environment, are able to utilize nitrate (NO<sub>3</sub><sup>-</sup>) as an alternate electron acceptor for respiration. The most common and well-studied of the dissimilatory nitrate respiration pathways is denitrification, which converts nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite ions (NO<sub>2</sub><sup>-</sup>) to gaseous products including nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>). Each of these reductions is catalysed by a different enzyme, and not all microbes possess the full suite necessary to completely convert NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>. Denitrification enzymes require a metal co-factor; for example, the reduction of N<sub>2</sub>O to N<sub>2</sub> is catalysed by a Cu-containing nitrous oxide reductase (N2OR). Microbes with a complete denitrifying pathway not only have the additional pressure to reduce the accumulated denitrification intermediates (NO<sub>2</sub><sup>-</sup>, NO, and N<sub>2</sub>O) to inert N<sub>2</sub>, but also to effectively scavenge trace-metals from their environment. In a series of lab incubations, we examined the transcriptome of a model denitrifying organism (*Paracoccus denitrificans*) under aerobic and anaerobic conditions. Then we investigated the effects of metal depletion on *P. denitrificans* and metal additions on wetland sediments. We found that the transition from aerobic to anaerobic conditions is tightly regulated requiring only 200 genes. The majority of those genes are controlled by regulatory factors that respond to carbon, O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> environmental signals. Under low Cu availability N2OR remains non-functional and N<sub>2</sub>O accumulates, although N2OR is transcribed and translated. This disrupted denitrification pathway is comparable to organisms lacking N2OR. When considering the anaerobic environment of wetland sediments, we found that Mo, Fe, and Cu addition at μM levels notably enhanced denitrification and significantly reduced the accumulation of N<sub>2</sub>O. These biochemical process rate observations were concurrent with changes in the microbial group abundances for nitrite – and nitrous oxide – reducers, suggesting an ecological advantage for complete denitrifiers when electron acceptors are limited in the environment. Emerging from our studies, firstly, a denitrification regulatory model is proposed and secondly, trace-metal availability should be considered as an additional controlling factor when studying denitrification and N<sub>2</sub>O emissions.