Proceedings of the workshop on "Climate change, reactive nitrogen, food security and sustainable agriculture"
15-16 April, 2019 Garmisch-Partenkirchen, Germany

How can microbiologists help with N_2O mitigation?

Lars R Bakken

Faculty of chemistry biotechnology and food science, Norwegian university of Life Science, Norway

Email lars.bakken@nmbu.no

Keywords: bacteria, denitrification, microbiology, physiology, respiration.

Extended Abstract

In the biosphere, microbes are the primary producers of N_2O, but also the primary consumers of this climate gas, reducing it to harmless N_2. Thus, the emissions of N_2O is controlled by microbial physiology as constrained by a plethora of physical and chemical constraints of the natural environment. These constraints are normally absent in laboratory cultures of microbes, which has been the primary tool for unravelling their physiology, pathways of N-transformations, and regulatory biology. One may question, whether the knowledge gained through such culturing is relevant for understanding the physiology of organisms in their natural environment, as eloquently phrased by Winogradsky already in 1923: "*..one cannot challenge the notion that a microbe cultivated sheltered from any living competitors and luxuriously fed becomes a hot-house culture, and is induced to become in a short period of time a new race that could not be identified with its prototype without special study*" [1]. Although Winogradsky's suspicion has been justified by a wealth of experimental evidence, my claim is that pure culture experiments still are invaluable source of new information that helps to understand, predict and mitigate N_2O emission. I will demonstrate this by four examples: clarification of nitrification driven N_2O emission, modulating denitrification by liming, promising mitigation by rhizobia and by enriching biodigestates with N_2O reducing bacteria. Some of this has been reviewed more thoroughly by Bakken and Frostegård [2], hence I limit the number of references to a minimum in the present text.

Nitrification

Nitrification results in N_2O emission, primarily as a "side product" of ammonia oxidation, both by ammonia oxidizing bacteria (AOB) and –archaeae (AOA) [3]. Both AOA and AOB have been studied in pure cultures, which have demonstrated that the N_2O yield (Y_{N2O} = N_2O-N as % of oxidized N) is 0.1-1% for AOB and 0.01-0.1 for AOA, and this contrast has also been confirmed by experiments with soils dominated with AOB and AOA [4]. Soils with high pH and at high ammonia concentrations favor AOB over AOA, thus Y_{N2O} is enhanced by high fertilizer doses, and this effect is further aggravated by the fact that Y_{N2O} by AOB themselves increase with ammonium concentration [5]. The implications would be that slow release ammonium fertilizers would result in less N_2O emission from nitrification.

Nitrifier denitrification

In theory, AOB can also produce N_2O by denitrification, since they are equipped with genes for both nitrite reductase (NIR) and nitric oxide reductase (NOR). This has spurred an interest

by biogeochemists for nitrifier denitrification as an N_2O source. There is little doubt that they can produce N_2O via NIR and NOR, but the production is miniscule even under hypoxic conditions, and comes to a complete halt under anoxic conditions. Therefore, the term nitrifier denitrification gives wrong connotations, and the experimental evidence suggests that it is rather a pathway for redox balancing at high ammonium concentrations [5,6]. A valid method to determine the rate of nitrifier denitrification in soil has not yet been invented (see [2] and references therein).

Denitrification
N_2O is a free intermediate in denitrification, i.e. the stepwise reduction $NO_3^-\rightarrow NO_2^-\rightarrow NO\rightarrow N_2O\rightarrow N_2$, catalyzed by the four enzymes NAR/NAP, NIR, NOR and NOS, respectively. The pathway is used by many heterotrophic microorganisms to sustain their respiratory metabolism in the absence of O_2. The N_2O/N_2 product stoichiometry of the process depends strongly on the activity of NOS (relative to that of the other enzymes). Many denitrifying organisms have a truncated denitrification pathway; lacking the gene for 1-3 of the four enzymes, and this has been taken to suggest that the propensity of soils to emit N_2O can be predicted by gene abundance [7]; more specifically the *nir*/*nosZ* abundance ratio. No consistent relationship between N_2O emission and denitrification gene abundance has been found, however, despite numerous attempts. A plausible explanation is that a majority of active denitrifiers in soils have both *nir* and *nosZ*, hence the N_2O/N_2 product ratio is controlled by their regulatory phenotypes as affected by environmental factors. Among the environmental factors controlling the N_2O/N_2 product ratio of denitrification, the soil pH plays a prominent role: this was discovered already in 1954 [8], but not understood, hence forgotten and rediscovered several times. Recent investigations of the phenomenon, both in the model strain *Paracoccus denitrificans* in soils have clarified that low pH cause high N_2O/N_2 products during anoxic spells is by impeding the synthesis of functional NOS [8]. Thus, the synthesis of functional NOS takes longer time in acid soils than in soils with neutral pH. But once synthesized successfully, NOS functions well at low pH! This explains why the N_2O reduction in drained soils is effectively impeded by low pH, but it also explains why peats and permanently waterlogged soils may effectively reduce N_2O even at low pH.

This opens for mitigating N_2O emissions from farmed soils by increasing the soil pH beyond the minimum for crop growth, either by liming or by biochar. There is ample evidence that this effectively reduces N_2O emission, and implementation of this mitigation option is strongly recommended. The most attractive approach is to lime soils beyond the minimum pH for crop growth, simply because liming is already an established agronomic practice. An objection could be that liming causes emissions of CO_2, at least if adopting IPCC's assumption that all the carbonate-C is emitted as CO_2. This is wrong, however: when liming a moderately acidic soil, less than 50% of the carbonate-C is emitted as CO_2, and the net effect of liming a soil with pH $\geq$6 is CO_2 sequestration rather than emission [10]. This means that the recommended additional lime to increase pH above the minimum for adequate crop growth causes CO_2 sequestration, rather than CO_2 emission! A revision of IPCC's recommendations for national GHG budgets is clearly needed to pave the way for this mitigation option.
Another objection to liming has been that it accelerates the mineralization of soil organic

carbon, but here is no evidence for this [11].

Engineering the soil denitrification
Is it possible to manipulate the denitrifying communities of soils to enhance N_2O reduction, thus lower their N_2O emission? Recent research has opened two feasible options:

One is to inoculate legumes with rhizobia with strong expression of NOS, as demonstrated by Itakura *et al.* [12]. Recent investigations of rhizobia in our lab (Frostegård *et al.*, unpublished) unraveled that naturally occurring strains are highly variable: some lack *nosZ*, hence those are net producers of N_2O, while others are full-fledged denitrifiers with a strong NOS activity. This underscores that the choice of inoculant when introducing "new" legumes in areas that lack indigenous symbionts is important: if choosing an inoculant without nosZ, the N_2O emission will be enhanced, and this symbiont will establish itself in the soil (effectively competing with a second inoculant!).

A second option is to grow N_2O reducing bacteria in digestates from biogas production systems, which will reduce N_2O emission from soils fertilized with the digestates. We have provided the first proof of concept in our lab (Jonassen *et al.*, unpublished), demonstrating fast growth of N_2O reducers in digestates, reaching cell densities of $2*10^9$ g^{-1} dryweight. Fertilization with this digestate (~10 mg dryweight g^{-1} soil) resulted in marginal N_2O emission from denitrification compared to the control (digestate without N_2O reducing bacteria).

Acknowledgements: I thank all my colleagues in NMBU Nitrogen group for their direct and indirect contribution (webpage https://www.nmbu.no/en/research/groups/nitrogen), the research council for providing funding, and the fertilizer Company Kingenta for generous sponsoring of our research group.

Accreditation: This paper was given at the workshop on Climate Change, Reactive Nitrogen, Food Security and Sustainable Agriculture which took place in Garmisch-Partenkirchen, Germany, on 15-16 April 2019, and which was sponsored by the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems whose financial support made it possible for the author to participate in the workshop.

OECD disclaimer
The opinions expressed and arguments employed in this paper are the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its Member countries.

References

[1] Dworkin M: **Sergei Winogradsky: a founder of modern microbiology and the first microbial ecologist.** *Fems Microbiol Rev 2012*, **36**: 364-379.

[2] Bakken L, Frostegård Å: **Sources and sinks for N_2O, can microbiologist help to mitigate N_2O emissions?** *Env Micr* 2017**, 19**:4801-4805

[3] Butterbach-Bahl K, Baggs E, Dannemann M, Kiese R, Zechmeister-Boltenstern S: **Nitrous oxide emissions from soils: how well do we understand the processes and their controls?** *Phil Trans R Soc B* 2013**, 368: 1-13.** http://dx.doi.org/10.1098/rstb.2013.0122

[4] Hink L, Nicol GW, Prosser JI: **Archaea produce lower yields of N_2O than bacteria during aerobic ammonia oxidation in soil.** *Env Microbiol* 2016, **19**:4829-4937**.**

[5] Hink L, Lycus P, Gubry-Rangin C, Frostegård Å, Nicol G, Prosser J, Bakken LR: **Kinetics of NH3-oxidation, NO-turnover, N2Oproduction and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers.** *Env Micr* 2017, **19**: 4882-4896.

[6] Stein L: **Insights onto the physiology of ammonia-oxidizing microorganisms**. *Current Opinions in Chemical Biology*. 2019, online https://doi.org/10.1016/j.cbpa.2018.09.003

[7] Graf DRH, Jones C, Hallin S: **Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N_2O Emissions**. *PLoS ONE* 2014, **9**(12): e114118. doi:10.1371/journal.pone.0114118

[8] Wijler J, Delwiche CC: **Investigations on the denitrifying process in soil**. *Plant Soil* 1954, **5**:155–169. http://dx.doi.org/10.1007/BF01343848.

[9] Liu B, Frostegård Å, Bakken LR: **Impaired Reduction of N2O to N2 in acid soils is due to a posttranscriptional interference with the expression of nosZ**. *mBio* 2014, **5**(3) doi:10.1128/mBio.01383-14.

[10] Hamilton SK, Kurzman AL, Arango C, Jin L, Robertson GP: **Evidence for carbon sequestration by agricultural liming**. *Global Biogeochemical Cycles, 2007,* **21**:1-12. http://refhub.elsevier.com/S0065-2113(16)30059-1/sbref0615

[11] Kunhikrishnan A, Thangarajan R, Bolan NS, Xu Y, Mandal S, Gleeson DB, SeshadriB, Zaman M, Barton L, Tang C, Luo J, DalalR, Ding W, KirkhamMB, Naidu R: **Functional Relationships of soil acidification, liming, and greenhouse gas flux**. *Adv. Agron* 2016, **139**:3-54. http://dx.doi.org/10.1016/bs.agron.2016.05.001

[12] Itakura M,Yoshitaka, Uchida Y, Akiyama H, Hoshino YT, Shimomura Y, Morimoto S, Tago K, Wang Y ,Hayakawa C, Uetake Y, Sánchez C, Eda S, Hayatsu M, Minamisawa K: **Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation**. *Nature Climate Change* 2013, **3**:208-212.