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PATHWAYS OF NITROGEN AND CARBON TRANSFORMATION IN SOILS AND WASTEWATER AS REVEALED BY MICROBIAL GENOMES: IMPLICATIONS FOR GREENHOUSE GAS EMISSIONS

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Abstract: This poster shows the combined use of omics technologies and computational analysis to decipher mechanisms of production of greenhouse gases in agricultural soils and wastewater systems. An analysis of microbial genomes suggests that over 25 enzymatic reactions can influence N2O formation in these systems. Computational modelling allows us to explore how carbon and nitrogen inputs in farm soils and wastewater influence enzymatic reactions that affect greenhouse gas formation by microbes, in contrast to the "black box" approach traditionally adopted for such estimates. The analysis reveals that the biodegradability of soluble carbon sources as well as the profile of nitrifying and denitrifying microbial species significantly influence the rate of reactions producing N_2O. Also the analysis identifies carbon and nitrogen input strategies as well as target chemical reactions to reduce N_2O formation.

Keywords: Biological nitrogen removal, nitrous oxide, metabolic modelling, metabolomics

Introduction

Agricultural soils and wastewater treatment facilities emit gases such as nitrous oxide (N_2O).[1] These emissions cause nitrogen and carbon losses as well as negative atmospheric greenhouse and ozone depleting effects.[2,3] Figure 1 presents a percentage break down of New Zealand‟s greenhouse gas (GHG) emissions. Sixty teragrams (Tg) of CO_2 equivalents are emitted in New Zealand per year. From this amount, carbon dioxide (CO_2) is the most emitted greenhouse gas (68 %), although N_2O is the most concerning emissions due its 300 times stronger than CO_2 greenhouse and ozone depleting effects.[2,3]

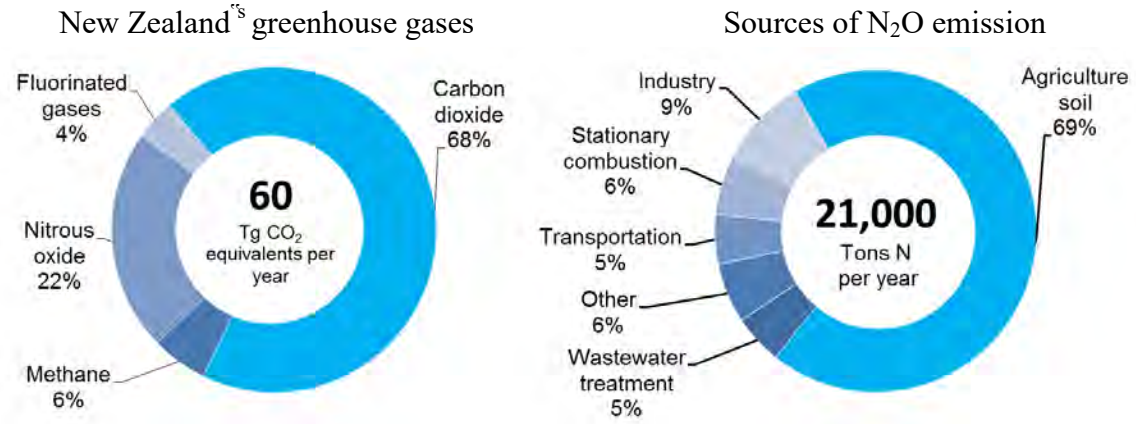

Figure 1. New Zealand‟s greenhouse gas emissions as in 2013 according to The Ministry of Environment.[1]

Activity of nitrifying and denitrifying microbes is the source of these emissions.[4] The most important environmental factors influencing N_2O emissions in nitrification and denitrification processes are dissolved oxygen, nitrite, ammonium and organic carbon concentrations.[5] However its not know how soil and wastewater conditions influence the biochemical pathways of N_2O formation. Moreover it is unknown if it is possible to control such emissions. Quantification of N_2O producing pathway activity in regard to specific environmental conditions has been done only in a few studies.[6-8] It follows therefore that quantitative relationships between N_2O emission, production pathway rates and soil and water bodies conditions are necessary to be able to define guidelines for emission prevention Consequently, the aim of this research is to establish the relationships between the activity of N_2O production pathways and environmental conditions (e.g. electron donor and acceptor availability) in BNR bioreactors by using stoichiometric metabolic network (SMN) modelling and metabolite profiling of nitrifying and denitrifying microbes.

Material and Methods

A combination of innovative computational (SMN modelling) and experimental microbial culturing and biochemical analyses (metabolomics) was conducted (Figure 2).

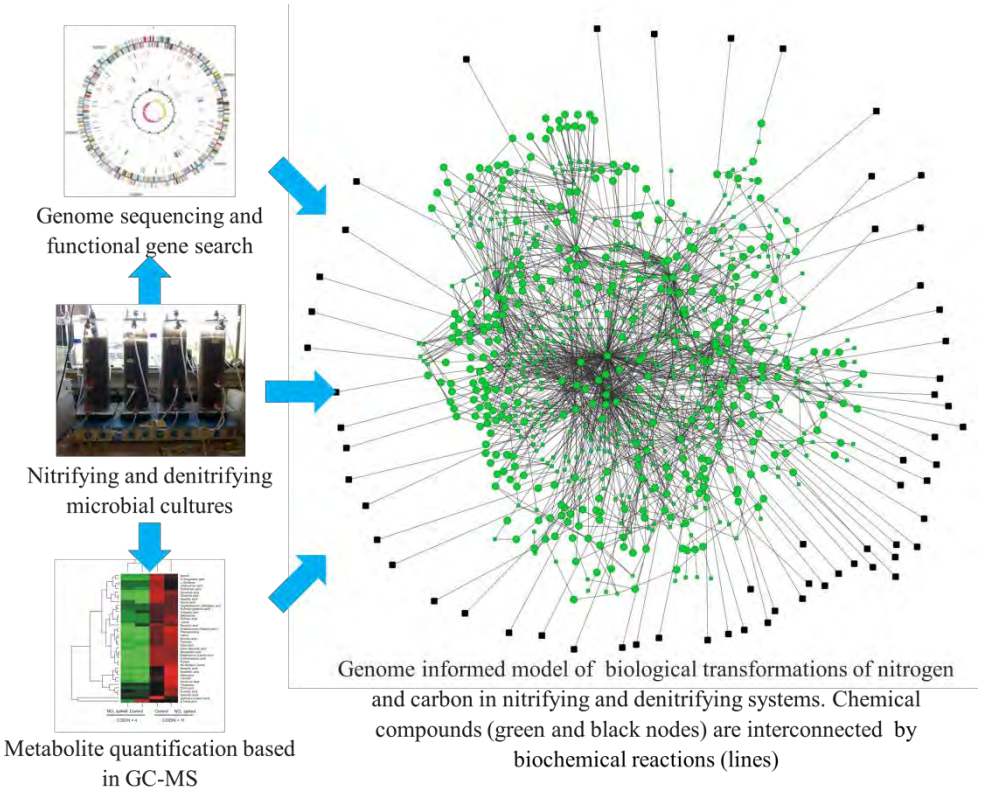

Figure 2: Implemented research pipeline

Three SMN models for biochemical reactions and metabolites formed during energy production metabolism of 1) *Nitrosomonas europaea*, 2) nitrifying microbial community, and 3) denitrifying community, were constructed by following the procedure described by Thiele and Palsson (2010),[9] using organism-specific genomic and biochemical information from metabolic pathway databases KEGG and MetaCyc (respectively accessible at http://www.genome.jp/kegg/ and http://metacyc.org/).

Analysis of mixed microbial cultures from farm soil and wastewater were conducted in laboratory. Rates of consumption and production of nutrients were measured in laboratory scale bioreactors. Metabolomics analysis was done only for denitrifying cultures. Relative abundance of the intracellular metabolites in these cultures was measured following the protocol described in Smart et al (2010)[10] using GC-MS analysis of methyl chloroformate (MCF) derivatives from biomass samples.

The metabolic models were used to estimate the rates of nitrogen and carbon transformations using experimental measurements described above as input data as follows. Experimental data observed in laboratory scale cultures of *N. europaea*, nitrifying mixed communities and denitrifying mixed communities was analysed with its corresponding SMN model. Flux balance analysis was applied to estimate the unknown rates of metabolic network reactions by using values of consumption rates of substrates (i.e. oxygen, ammonium, nitrate) as model input.

Results and Conclusions

A total of 25 enzymatic reactions were identified to influence the rate of N_2O production by nitrifying and denitrifying microbes. The simulations of pure cultures (*N. europaea*) show that N_2O production results from electron flow imbalances in nitrifying cells, and that electron carriers (cytochromes and ubiquinone) play a key role in transferring electron equivalents to N_2O and NO formation reactions. The simulations with mixed nitrifying cultures reveal two key aspects of N_2O formation in nitrifying microbial communities (Figure 3): (i) microbes can lower N_2O emissions by dissipating NO (a N_2O precursor molecule) through reactions catalysed by cytochromes and flavohemoglobins in both ammonia and nitrite oxidizing bacteria (Figure 2); and (ii) the structure (i.e. the richness and abundance of species) of the microbial community influences the amount of N2O produced and emitted, i.e. nitrifying microbial communities dominated by *Nitrosomonas eutropha* produce more N_2O than the ones dominated by *Nitrosomonas europaea*. GC-MS metabolite profiling of denitrifying mixed cultures showed that hard to degrade organics and endogenous respiration stimulate higher production of N_2O, due the establishment of a chronic lack of electron equivalents to completely reduce NO_3^- to N_2.

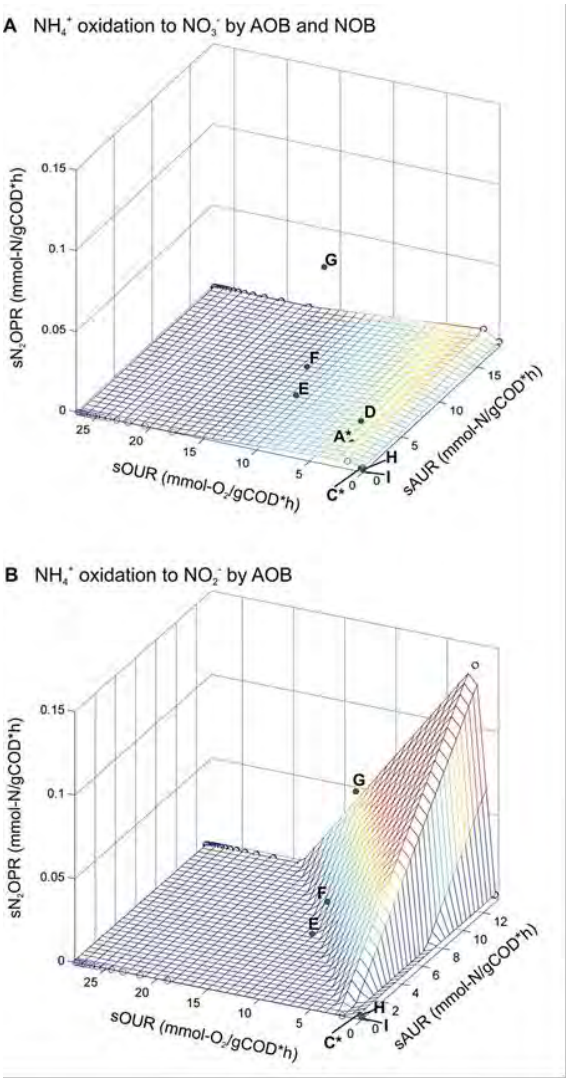

Figure 3. Fitting nitrifying mixed cultures data using the nitrifying community Experimental oxygen and ammonium uptake rates (respectively sOUR and sAUR) were used as model input data to estimate specific N_2O production rates. Experimental data is represented with nodes and letters while the meshes represent data estimated by the model.

This study concludes that operational conditions that promote imbalances between the cell‟s electron donors and electron acceptors cause N_2O formation (Figure 4). Specifically, in nitrification processes, a build-up of electron equivalents leads to N_2O formation, while in denitrification processes the deficiency of electron equivalents promotes N_2O accumulation.

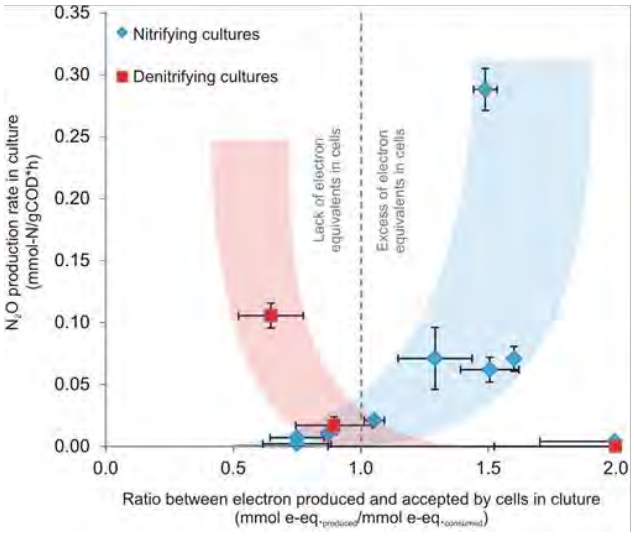

Figure 4. N_2O production rate related to the ratio between electrons produced and accepted by the cultured cells.

Research contribution

- For the first time, the complex biochemical pathway of nitrogen and carbon transformation occurring in nitrifying and denitrifying systems is starting to be revealed.
- The developed approach is currently being used to design nutrient application strategies to mitigate N_2O emissions and NO_3^- leaching agriculture soils, aquifers and wastewater treatment plants.

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