UNRAVELING THE SOURCES AND SINKS OF NITROUS OXIDE (N_2O) IN SUSTAINABLE BIO-BASED AGRICULTURE

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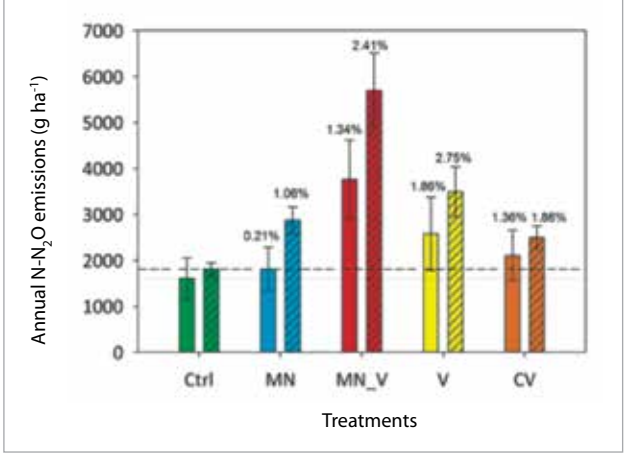

Figure 1. Annual nitrous oxide emissions from soils with sugarcane and respective emission factors (%). Bars are the standard errors. Reference lines are the calculated emissions from the control (Ctrl) treatments without straw (dotted line) and with straw (dashed line). Ctrl: no nitrogen addition; MN: mineral nitrogen fertilization; MN_V: mineral nitrogen plus vinasse fertilization; V: vinasse; CV: concentrated vinasse. Transversal lines: treatments with straw left on soil

One of the main concerns of biomass production for bio-energy is the potential positive feedbacks of crop production to global change, particularly in the form of greenhouse gas emissions (CH_4, CO_2, N_2O – GHGs). Nitrous oxide (N_2O) is a significant by-product of agricultural intensification, primarily due to the application and transformation of inorganic Nitrogen (N) fertilizers. In our previous and ongoing studies with sugarcane crop we have observed that not only nitrogen fertilizer in soil increases N_2O emissions but also the combination of N and vinasse (by-product of ethanol) (Carmo et al. 2013; Pitombo et al, 2015). Soil microorganisms are central to these transformations and thus regulate the loss or retention of inorganic N, including N_2O. Biological emissions of N_2O are mainly controlled by two microbial processes: nitrification and denitrification. However, we have limited understanding of how these processes are regulated in complex systems such as soils under crops for bio-energy. Prior work has often focused on individual microbial species that contribute to each process and on ecosystem scale parameters such as organic matter content, soil texture, pH, soil N status and precipitation. What is lacking is an approach that combines fine scale mechanistic details on the physiology of key functional groups of the N cycling microbial community and their interaction with their environment and each other. Our goal is to combine advanced omic technologies (meta-genomics, -transcriptomics and -proteomics) to determine the key players in the biogeochemical cycling of N, with a specific focus on organisms involved in denitrification in a model of sustainable sugarcane biomass production system.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A series of field experiments measuring GHGs to provide data for life cycle analyses are in the scope of other FAPESP projects coordinated by the members of the same research group. The use of molecular techniques in these field experiments has stated that the microorganisms used as models for nitrification and denitrification are not representing the ones present in the field (Pitombo et al. 2015; *Fig. 1*). Specifically in sugarcane fields it has being suggested that the microorganisms present in the vinasse may increase the release of N_2O from soil (*Figure 2*). On the other hand, potential N_2O reducers were highlighted by linking N_2O release with microbial abundance in soil. Contemporary GHG gas models presume that N_2O to N_2 reduction (i.e., the final step of the denitrification pathway) is the major attenuation process controlling N_2O flux to the atmosphere. Then, it is in what the main aim of this research is focused on.

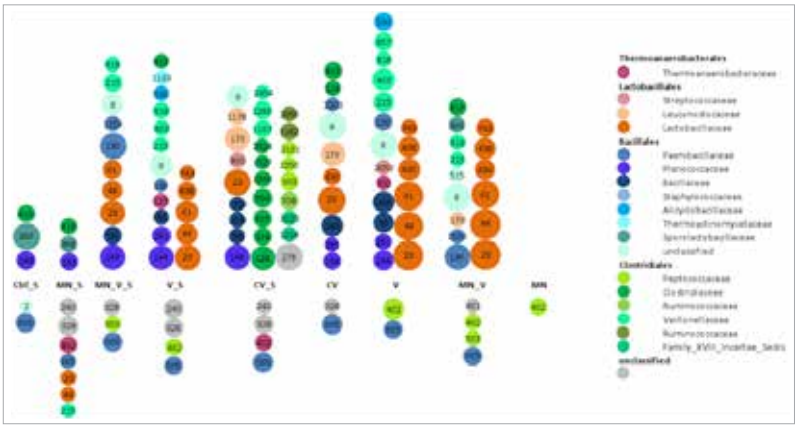

Figure 2. Effect of different treatments on OTUs belonging to Firmicutes other phylum. Numbers represent the OTU identification. Color groups at Family. Circle size indicates the fold-change of the respective OTU when compared to the control treatment (Ctrl). Overrepresented OTUs are in the upper part of the plot and underrepresented OTU are in the below part of the plot. Ctrl_S: no N with straw; MN_S: mineral nitrogen with straw; MN_V_S: mineral nitrogen plus vinasse with straw; V_S: vinasse with straw; CV_S: concentrated vinasse with straw; CV: concentrated vinasse; V: vinasse; MN_V: mineral nitrogen plus vinasse; MN: mineral nitrogen.

For a more detailed understanding of the processes related with N_2O release, we brought to the lab the microorganisms to date used as models and the ones that are being pointed out as important drivers of nitrogen redox processes (e.g. *Pseudomonas sp.*, *Lactobacillus sp.*, *Nitrosospira sp.*, *Nitrososphaera sp.*, *Anaeromyxobacter sp.*). Most of the strains have the genome already sequenced and they will be used as template to verify which ones are active and which steps are they developing in the soil to contribute to lower or higher N_2O emissions. A microorganism which requires high ammonium concentrations and uses oxalic acid as carbon source was present in all models explaining N_2O fluxes from the soil under different management practices in the work of Pitombo et al. (2015) and now it was included in the mock community under study.

The mock community has being introduced in microcosms and submitted to variation of conditions simulating the environmental and management factors which most likely control the N_2O releases. Among these factors are soil moisture; nitrogen availability and speciation; straw; vinasse; and copper availability in soil. To date, no study considering soil copper content and N_2O reduction has being published despite it has being pointed out as determining factor for the syntheses of the most studied nitrous oxide reductase.

The deep understanding of the interactions between the microorganisms and their roles on nitrogen cycle at genomic and postgenomics level will provide knowledge to promote a more efficient fertilizer use with lower N_2O emissions. Recycling vinasse in the fields as fertilizer is essential to keep the ethanol lifecycle as close as possible. As vinasse holds a huge biotechnological potential, the perspectives are that it might be used as media to develop probiotic products addressed to lower N_2O emissions and N fixation in the fields.

MAIN PUBLICATIONS

Pitombo LM, Carmo JB do, Cantarella H, Rossetto R, Hollander M, Lopez MV, Kuramae EE. 2015. Exploring soil microbial 16S rRNA sequence data to increase carbon yield and nitrogen efficiency of a bioenergy crop. *Global Change Biology. Bioenergy*, DOI: 10.1111/gcbb.12284.

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