Linking soil microbial community dynamics to N2O emission after bioenergy residue amendments

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Summary

Recycling crop and industrial residues is a sustainable agricultural management practice, which also helps to improve soil structure and to increase the stock of nutrients. However, the addition of residues to agricultural fields causes disturbances to the soil ecosystem and to the soil microbial community in general. Until now, information about the magnitude and duration of these disturbances is scarce. Vinasse is a major by-product generated by the sugarcane biofuel industry. It is a source of microbes, nutrients and organic matter and often it is recycled as fertilizer. There is evidence that the application of vinasse together with mineral nitrogen (N) fertilizers in sugarcane fields affect the composition, functions and dynamics of the soil microbiome, thereby enhancing the emission of nitrous oxide (N_2O). However, it is still poorly understood how vinasse (and straw) affect the dynamics of the soil microbiome and the mechanisms that control the high N_2O emissions.

The research described in this thesis firstly addressed how organic residues - vinasse and sugarcane straw - added together with N fertilizer affect the soil microbial community structure and function and N_2O emission in a short-term experiment (Chapter 2). Vinasse and straw, both induced changes in the soil microbial community composition and potential functions, but straw additions triggered the stronger changes in particular related to functions involved in decomposition of different C-compounds. Functions related to spore-producing microorganisms were overrepresented in the vinasse treatment, which could be related with the presence of vinasse-inhabiting microorganisms and their survival in the soil. All additional residues increased the abundance of microorganisms related to nitrogen metabolism and N_2O emissions. However, treatments with vinasse plus straw applications showed highest N_2O emissions.

To investigate the capacity of the soil microbiome to recover from the impact of vinasse, or the potential invasion of the vinasse-inhabiting microorganisms, total microbial community analyses were performed during an entire sugarcane season (389 days) (Chapter 3). Vinasse, N fertilizer or a combination of both were applied in sugarcane plantations in which the soil was covered with straw, a common practice. Vinasse caused significant changes in the resident soil microbial community. However, these changes were restricted to a short period as the resident microbial community was able to recover. The invasive bacteria present in the vinasse were unable to survive in the soil and disappeared after one month, except of members of the Lactobacillaceae family that persisted in the soil even after one year. This study is the first to show the persistence of vinasse-inhabiting bacteria in soil, and further studies elucidating the ecological functions of these invaders in soil are urgently needed. Despite this, the resident soil microbial community was highly resilient to vinasse and N fertilizer application.

The higher abundance of bacteria of the order of Nitrosomodales in the treatments with organic residues was evidence that nitrification was one of the main pathways responsible for the N_2O production (Chapter 2). Therefore, I investigated the role of nitrification and denitrification in the N_2O production in straw-covered soils amended with concentrated and non-concentrated vinasses before or at the same time as N fertilizer at different time points of the sugarcane cycle in two seasons (Chapter 4). Independent of the (rainy or dry) season, the microbial processes involved in N_2O production were nitrification by ammonia-oxidizing bacteria (AOB) and archaea and denitrification by bacteria and fungi. The contribution of each process differed and depended on soil moisture, soil pH, and N sources. However, *amo*A-AOB and fungal *nir*K were the most important genes

related to N_2O emissions overall, which indicates that nitrification by AOB and denitrification by fungi are likely to be the main microbial-driven processes linked to N_2O production in tropical soil receiving straw and vinasse applications. Despite the increment in the AOB abundance in the soils receiving vinasses and N fertilizer, the diversity and the community structure of AOB did not change and was dominated by *Nitrosospira sp.* (Chapter 5). In addition, the application of vinasse 30 days prior to N fertilizer reduced N_2O emissions by 37-65%.

In conclusion, the research presented in this thesis showed for the first time the successional changes in the soil microbial community composition and functions after vinasse, straw and N fertilizer applications as well as the links of the dynamics of the soil microbiome with N_2O emissions. Also, it was the first time that the invasion potential of vinasse-inhabiting microbes was determined. A practical result of this research is that vinasse application 30 days before N fertilizer applications reduced the N_2O emissions. These results highlight the importance and limitations of recycling crop residues and fertilizer management and can be used as a reference and a practical tool to develop good management practices during organic fertilization as part of sustainable sugarcane production systems.