Impact of nitrogen fertilization on the soil microbiome and nitrous oxide emissions

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The use of N fertilizers has increased worldwide in the past century. While this increased input of N has increased food productivity, it has also contributed to decreases in biodiversity, soil quality and environmental health, including increases in greenhouse gas emissions. These emissions in agricultural soils are largely carried out by the soil microbiome, or the microorganisms living in the soil and transforming N fertilizers to different forms. Here, the overall research aim was to gain detailed insight into the effects of nitrogen fertilizer schemes, including long term fertilization, on soil microbial communities. To do this, I applied next-generation sequencing technology and associated bioinformatics analyses to field experiments in the Netherlands and in Brazil.

In Chapter 2 I found that long-term fertilization with lime, ammonium nitrate (N), super phosphate (P) and NPK resulted in "habitats" with different soil conditions in Dutch hay meadows. In these different fertilizer habitats, the taxonomic makeup of the soil bacterial community, or the community composition and diversity differed from the control plots by the varying abundance of the different taxa; however, potential functions did not differ between treatments. This suggests that each treatment affected the soil bacterial community as a whole by being associated with altered abundances of taxa, but not at a broad functional level, with the gene potential remaining the same across treatments. Nitrogen fertilization alone resulted in the most significant changes in the soil bacterial community, in which the abundances of the Actinobacteria phyla were higher compared to the other treatments. This is in line with the results from other studies, which identifies the Actinobacteria as a copiotrophic phyla, or a taxa well able to succeed under high-nutrient conditions. This suggests differences in decomposition rates in each habitat, with decomposition and associations by the bacterial community affected especially under N fertilization.

In Chapter 3, I extended the investigation from Chapter 2 to include the plant and soil fungal communities in the long-term fertilized fields. I found that the plant and soil fungal but not the plant and soil bacterial and soil fungal and bacterial communities varied similarly across the plots. The plant community was less diverse in the NPK compared to the other treatments, which was thought to be due to the success of high-nutrient-adapted grasses in the plant communities in this habitat. In addition, the liming treatment was associated with higher diversity in all three communities, which was likely due to the increased availability of nutrients from the higher pH. Regarding the plant and fungal community compositions, these were different in the NPK plots compared to the control plots, and our co-variation analysis supported their interdependence, with different potential co-

varying taxonomic groups identified. This suggested that the plant and soil fungal communities are closely interconnected in these fields, suggesting more ecological connections between these communities compared to either with the soil bacterial community. Further, the bacterial community appeared not to co-vary with either the plant nor the fungal communities, even though the macroorganisms are known to have specific associated bacterial communities. These results point to interesting questions in the differences in the ecological communities in each habitat due to interconnected effects of the fertilizers and nutrient availability on the plant and soil microbial communities.

In Chapter 4 and 5 I investigated the short-term effects of nitrogen fertilizers on the soil microbial community and N_2O emissions. Urea and urea with nitrification inhibitor treatments were evaluated over 256 days for the effect on the bacterial communities based on *16S rDNA* sequencing (Chapter 4), and on the ammonia-oxidizing subset of the bacterial community based on *amoA* sequencing (Chapter 5). Overall bacterial community compositions were unaffected by treatment, at least at phylum-level taxonomic resolution based on DNA. Furthermore, the bacterial community diversity was not affected by treatment. Moreover, we concluded that the nitrification inhibitors successfully inhibited the N_2O emissions. When looking specifically at the *amoA*-containing bacterial (AOB) OTUs, however, the abundances of different ammonia-oxidizing bacterial species indeed differed between treatments. Further, we identified a Nitrosospira-like AOB as the likely N_2O-emitter in the plots under urea fertilization, and also showed that the abundances of this bacteria decreased in the treatments with the nitrification inhibitors DMPP and DCD. These chapters indicated that short-term nitrogen fertilization with urea did not affect the soil bacterial community composition at a high taxonomic resolution, but did lead to differences at the OTU-level. Further, the ammonia-oxidizing bacterial community had low diversity in these soils, which might be due to the low levels of ammonia that are normally present. Most interestingly, we identified cohorts of ammonia- and nitrite-oxidizing OTUs that were associated with different soil factors, suggesting a picture of the nitrifying bacterial community in these soils.

In Chapter 6 I detailed for the first time the bacterial assemblage of sugarcane vinasse, which is widely used as a potassium fertilizer in conjunction with nitrogen fertilizers during sugarcane management. Targeting concerns over greenhouse gas emissions during the fertirrigation practice, I evaluated the potential presence of genes from the main N_2O-producing microbial pathways from 21 metagenome-assembled vinasse bacterial genomes (MAGs). The main genera I uncovered from the vinasse MAGs were Lactobacillus, Megasphaera and Mit-

suokella, and these had mainly denitrification gene potential. Interestingly, these had varying presence of denitrification genes, suggesting that the different vinasses used as fertilizer can be a source of bacteria with different N_2O-producing gene potential, potentially influencing the actual emissions of N_2O. Further, the potential presence of antibiotic-resistance genes was found across almost all the MAGs; this reinforced the idea that the bacterial component of vinasse is mainly the contaminants of the bioethanol production cycle, which often includes an antibacterial sterilizing step. Further, this result raises the visibility of the potential for horizontal gene transfer potential in sugarcane soil and subsequent risks for public health and crop productivity. This work also highlighted the necessity to include measures of the varying biotic component of vinasse in research of greenhouse gases from this system.

In conclusion, metagenomic and bioinformatic analyses were applied to data from field experiments investigating the effects of nitrogen deposition on the soil microbiota and related soil physicochemical factors. This thesis sheds light on the complex and interconnected changes within the soil microbial community upon nitrogen fertilization. Two separate studies in the Netherlands and Brazil demonstrated the effect of long-term nitrogen deposition on the soil bacterial community at coarse taxonomic levels, as well as the effect of short-term fertilization on soil microbes at the OTU level. This research underscores the idea that nitrogen fertilization in fields affects not only crop production, but the soil microbial community composition and the ecology of the communities in these fields, which can have long-term effects on crop productivity. Last, genome binning from bacterial DNA sequences extracted from sugarcane vinasse revealed 21 potential bacterial contaminants of the bioethanol production process. Since vinasse is widely used as a fertilizer, especially in conjunction with nitrogen fertilizers, for sugarcane in Brazil, this research paved the way for future studies linking the genetic potential of vinasse bacteria, vinasse and nitrogen fertilization, and field emissions of N_2O.