Agricultural Wastes are affecting the abundance of denitrifying community and the short term N_2O emissions

M. Omirou[1,] I. Anastopoulos[1,2], C. Stephanou[1,3] I.M. Ioannides[1]

[1]Department of Agrobiotechnology, Agricultural Research Institute, Nicosia, Cyprus
[2] Department of Chemistry, University of Cyprus, Nicosia, Cyprus
[3]Molecular Genetics Thalassaemia Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
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Presenting author e-mail: michalis.omirou@ari.gov.cy

Introduction

There is an urgent need to develop strategies that can reduce the negative effect of agriculture on climate. In particular, reducing the use of N-chemical fertilizers and sustainable use of agricultural wastes, as organic amendments could be a valuable approach for the reduction of GHG and to sustain systems productivity (Aguilera et al., 2013). Indeed, there is a large body of literature showing that the application of organic wastes increases soil fertility and subsequently crop productivity through the improvement of soil porosity, aggregate stability, cations exchange capacity and nutrient levels (Gómez-Muñoz et al., 2017; Kaur et al., 2008; Meyer et al., 2014). However, organic amendments contribute directly to N_2O emissions through N-compounds present in the organic matter and indirectly through their effects on soil properties. Moreover, several laboratory and field studies reported contradictory results regarding N_2O emissions from soils received organic amendments, and this was depended on the type of applied amendment. For example, animal manures, pig slurries and green manure caused higher N_2O emissions than composts and bio-char (Li et al., 2016). Postharvest industry produces high quantities of wastes and there is an urgent need for their management to reduce the associated environmental risks. Citrus industry is one of the most important agricultural industries in Mediterranean region. Particularly for Cyprus, 120000 metric tn of citrus are produced and more than 90% are processed. From those, 25000 tn are used for the production of juices, thus creating a substantial amount of waste that has to be managed. A possible management strategy is the incorporation of these wastes into soils in order to increase organic carbon and to support plant nutrition in annual crops by providing nutrients, such as nitrogen. In this study we address the effect of agricultural wastes application on the diversity of soil bacterial community, the abundance of denitrifiers as well as soil direct GHG emissions. We quantified short term N_2O emissions as well as the relative bacterial abundances and diversity over a 34 days period in a microcosm study using ammonium nitrate, agricultural wastes and non-treated soils. We anticipated that the use of post-harvest industry wastes increases soil bacterial community diversity, the abundance of microbial guilds that are associated with N2O production and reduction, thereby affecting N_2O gas emissions. Finally, we hypothesized that the application of ammonium nitrate causes higher N_2O emissions than soils treated with agricultural wastes.

Materials and Methods

The experimental set-up of the microcosm study included 5 treatments: soil + banana peels (BP), soil + orange peels (OP), soil + mandarin peels (MP) and soil + ammonium nitrate fertilizer (F) and untreated soil (C) with 3 replicates each. For this purpose, 240 g of air-dried soil was placed in 1500 mL glass bottles and water was added to achieve 70% water holding capacity. N_2O fluxes were measured in sealed jars by using linear regression of the N_2O concentration change over time (dC/dt). Briefly, headspace gas samples were collected at 15-minute intervals over a 60-minute closure time using a gas-tight syringe and analysed in a GC-MS (Agilent 5975, MSD). Soil NO_3^- was analyzed colorimetrically on a UV-Visible Spectrophotometer (ThermoScientific). The NO_3^- contents were determined from 10 g of soil extracted with 2 M KCl. Genetic indicators of soil bacteria (16S rRNA gene), and different populations of denitrifiers (nirK, nirS, nosZ clade I and II genes) were detected by quantitative PCR. Microbial community composition was determined by Next-Generation 16S rRNA Amplicon Sequencing.

Results and Discussion

Type of amendment, time and their interaction had a significant effect on cumulative N_2O gas emissions and varied widely among treatments ranging from 5 to 159 ng/g soil. Cumulative N_2O emission in fertilized soil was 22.7, 5.3, 9.9 and 10.2 times higher than that measured in control, BP, MP and OP soils, respectively. Interestingly, N_2O emission from soils treated with organic amendment was depended on the type of the added waste as well as time. Soil cumulative CO_2 emission was gradually increased in all treatments during the course of the experiment. CO_2 emission in control and ammonium nitrate treated soil was similar and was substantially lower compared to that measured in soils received organic amendments. The cumulative CO_2 emission is clearly depended on the amendment type, and emissions derived from soil treated with MP was higher than those treated with BP and OP. At the end of the experiment, the higher cumulative CO_2 emission within soils that received organic amendments was noticed in MP soil followed by BP and OP soil, respectively. Both type of amendment and time had strong and interactive effects on the bacterial Shannon index in the soil. The lowest diversity was observed five days after the initiation of the experiment in soils that received organic amendments; while the highest was noticed in non-treated soils (C) and NH_4NO_3 treated soils (F). The lowest diversity was measured in OP treated soils followed by BP and MP treated soils. Non-metric Dimensional Scaling (NMDS) based on Bray-Curtis distance revealed that the bacterial diversity varied with time and type of amendment addition (Figure 3). These effects were also supported by PERMANOVA analysis, which showed that time, treatment as well as their interaction had a significant effect on the soil bacterial community ($p<0.001$). In detail, this study revealed that the abundance of α-, β-, γ-proteobacteria and Bacilli was substantially higher in soils received organic amendments compared to control and fertilized soils. These findings indicate that the incorporation of agricultural wastes increases soil C, thereby stimulating the proliferation of copiotrophic species. The lower N_2O emissions found in soils incubated with agricultural wastes were associated to the substantial increase of the abundance of the complete N_2O reducers. Interestingly, agricultural wastes caused a substantial reduction of the relative abundance of bacterial taxa associated with N_2O emissions in soil.

Conclusion

This study proves evidence that agricultural wastes could be integrated in a general nutrient management strategy in agricultural ecosystems with reduced N_2O emissions through shifts of the diversity of microbial community as well as the abundance of N_2O reducers.

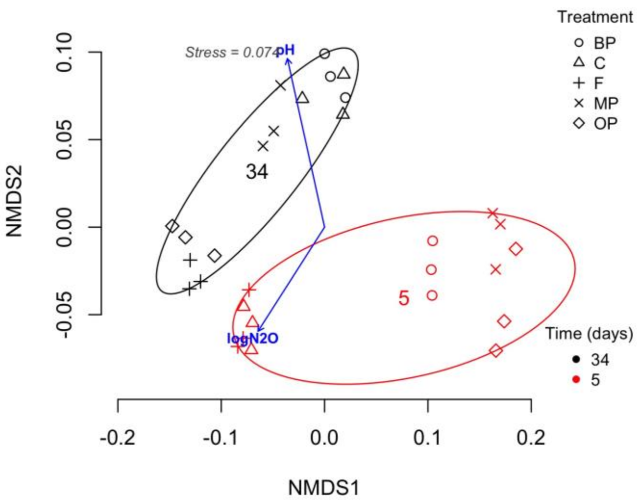

Figure 1. Non-metric Multidimensional Scaling (NMDS) ordination of the bacterial community in soils 5 and 34 days after the application of the treatments.

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