Tetracycline determines N2O fluxes through changes of microbial assemblages in a plant-soil system

M. Omirou[1], L. Philippot[2], I.M. Ioannides[1]
[1]Department of Agrobiotechnology, Agricultural Research Institute, Nicosia, 1516, Cyprus
[2]Université Bourgogne Franche-Comté, INRA, AgroSup Dijon, Agroécologie, 21000 Dijon, France
(E-mail: michalis.Omirou@ari.gov.cy)

Introduction

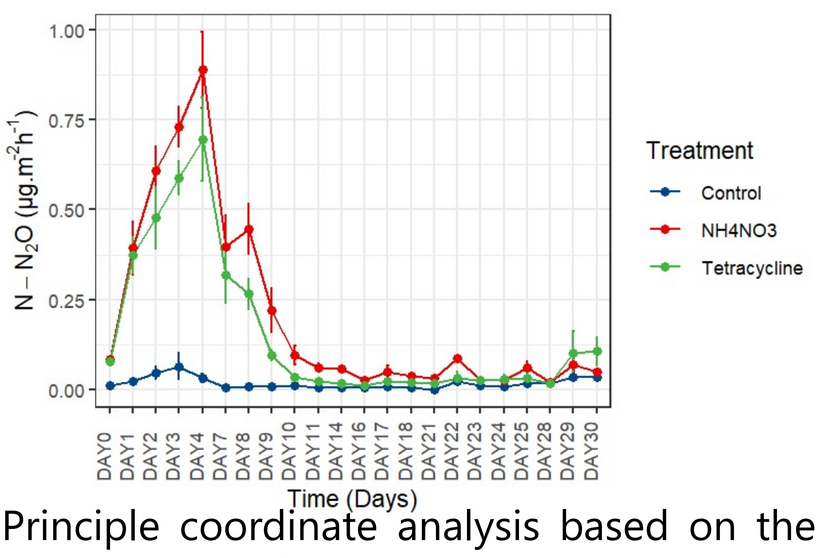
It is largely unknown how tetracycline results in changes to bacterial community composition and structure as well as in functional guilds involved in N-cycling when plants are present. In the current study, we examined the impact of tetracycline on N_2O emissions, the abundance of bacterial guilds involved in N cycling in bare and planted with *Origanum officinalis* soil microcosms, and the bacterial composition in both bulk and root samples.

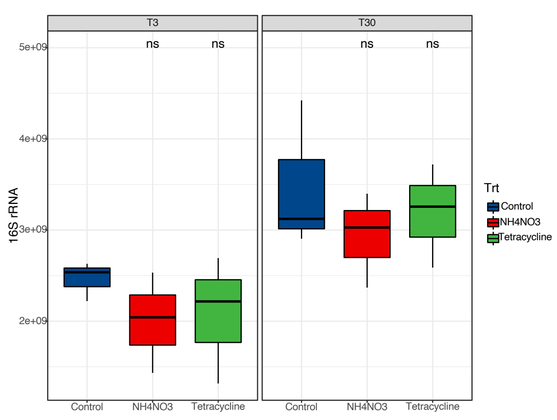
Material and Methods

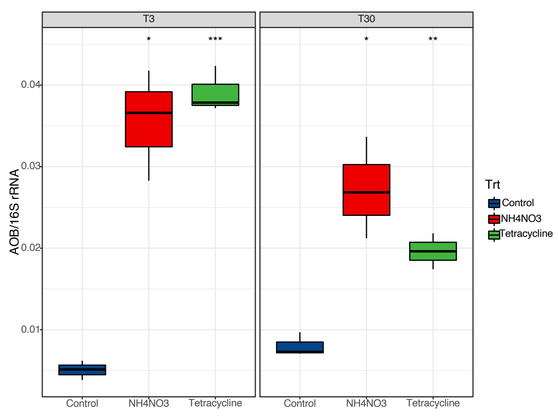
Totally, 1.4 kg of air-dried soil was weighted in glass incubation chambers and one plant of *Origanum vulgare* was transplanted. The soil received deionized water to achieve 60% of its water holding capacity. The chambers were pre-incubated for 7 days before the application of the treatments. In detail, antibiotic treated soils (TET) received tetracycline aquatic solution leading to a final concentration of the soil equal to 0.1 mg/kg and a nutrient solution of NH_4NO_3 to achieve final N addition of 100 mg/kg soil. Positive and negative control treatments were the same as described in the first experiment. In this experiment, N_2O fluxes were monitored for 30 days. The abundance of total bacteria and of bacteria involved in N-cycle was quantify by quantitative PCR (qPCR) with SYBR Green chemistry using the 16S as well as the AOA, AOB, nirK, nirS, nosZI and nosZII genes as molecular markers. The targeted metagenomic analysis was performed on a MiSeq platform.

Results and Discussion

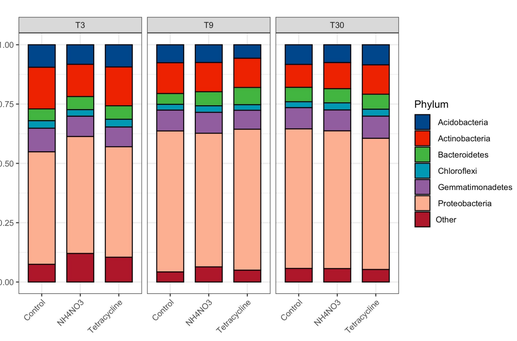
A peak of N-N_2O emissions was observed during the first 4 days after the initiation of the experiment in soils that received NH_4NO_3 irrespective of the presence of tetracycline. After the 4th day, a gradual reduction of daily emissions was noticed and 10 days after the initiation of the experiment the emissions were similar in all treatments. Overall, the different treatments and time had a significant effect on the microbial community abundance involved in the soil nitrification and denitrification. Bacterial community abundance increased during time but the treatments had no effect within each sampling point. The abundance of AOB increased tremendously after the fertilizer application but was unaffected to tetracycline presence.

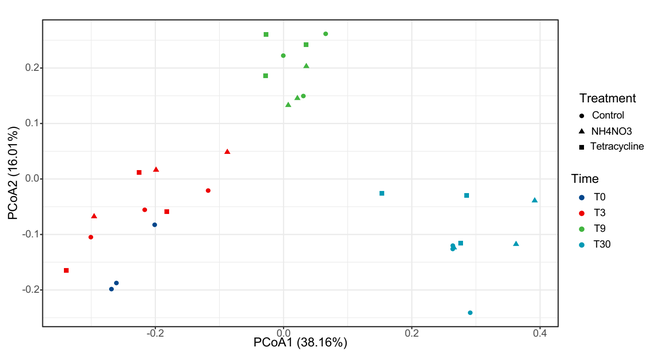


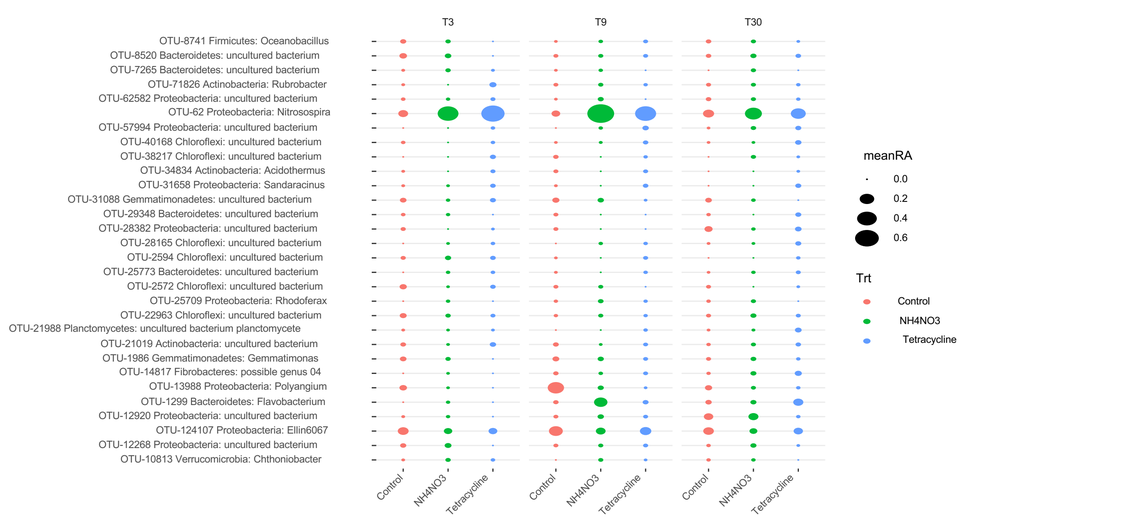




Principle coordinate analysis based on the OTUs with relative abundances higher than 2% revealed differences between bacterial communities during time both in bulk soil and plant root. These findings were further supported by non-parametric analysis of similarities (ANOSIM) and permutation multivariate analysis of variance (PERMANOVA) based on Brays-Curtis dissimilarity. The ANOSIM analysis showed that time significantly affected bulk soil bacterial composition (R=0.67, p<0.001) as well as root bacterial endophytes (R=0.68, p<0.001). The interaction effect of treatment and time was assessed by PERMANOVA analysis, but was not significant both in bulk soil and plant roots. The latter was verified by measuring the homogeneity of dispersion of the bacterial assemblages, which was not significant across the different treatments (F=0.312, p=0.72) as well as time (F=0.408, p=0.67). Random forest analysis revealed that 18 biomarkers belonging to Proteobacteria, Firmicutes, Bacteroidetes and Chloroflexi are significantly affected both from time and treatment. The abundance of these ASVs have been associated mainly by the presence of NH_4NO_3 but not tetracycline.







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