# The effects of tetracycline on bacterial communities responsible for denitrification and nitrous oxide production in agricultural soils

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## Introduction

The extensive use of antibiotics in veterinary medicine for livestock production and subsequent application of animal manure to agricultural land as part of sustainable farm waste management, contribute to the progressive accumulation of antibiotics to the surrounding terrestrial ecosystem. A substantial proportion of administered antibiotics is excreted as active metabolites in soils where they often persist and remain bioavailable (Sarmah *et al*., 2006). Soil microbes fulfil critical roles in biogeochemical cycling and organic matter degradation, and they have been shown to respond to traces of antibiotics in soils through changes in their community structure, composition and function (Grenni *et al*., 2017).

Microbial denitrification drives the removal of eco-toxic nitrogen (N) compounds in soil as inert dinitrogen ($N_2$) gas in the atmosphere through a series of soluble [nitrite ($NO_2^-$) and nitrate ($NO_3^-$)] and gaseous intermediates [nitric oxide (NO) and nitrous oxide ($N_2O$)]. The process of denitrification is carried out by a diverse group of microbes, and it is of particular ecological interest since $N_2O$ is a major greenhouse gas and currently the leading contributor to ozone depletion (Richardson *et al*., 2009). Studies demonstrated the effect of various antibiotics on denitrification and $N_2O$ production (Devries *et al*., 2015). However, their impact on the functional microbial groups, which directly produce/consume $N_2O$ from soils has scarcely been addressed (Semedo *et al*., 2018).

The tetracycline class of antibiotics is heavily used in animal health management in Cyprus. It comprises one of the most frequently detected antibiotics in soils with reported concentrations often within the ng.kg-1 to mg.kg-1 range (DeVries and Zhang, 2016). The present study evaluates the effect of tetracycline on N losses and $N_2O$ flux over time in soil collected from a local agroecosystem, and investigates whether exposure to tetracycline influences bacterial abundance and structure with emphasis on denitrifying communities.

## Materials and Methods

The experiment was carried out in pots containing 1.35 kg soil. Following cultivation with oregano (*Oregano officinalis*), soil samples were treated with $NH_4NO_3$ fertilizer (100 mg.kg-1) and tetracycline (0.1 mg.kg-1) (FT sample), and incubated over a period of 30 days. The water content was re-adjusted daily to 60%. The study included two experimental controls; soil samples left untreated (UC) and treated with fertilizer (FC). Each soil management practice was replicated thrice. $N_2O$ 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 10-minute intervals over a 60-minute closure time on an $N_2O$ Gas Analyzer (ThermoScientific). Soil N contents ($NH_4^+$, $NO_2^-$ and $NO_3^-$) were analyzed colorimetrically on a UV-Visible Spectrophotometer (ThermoScientific). The $NO_3^-$ and $NH_4^+$ contents were determined from 10 g of soil extracted with 2 M KCl. The $NO_2^-$ content was determined from 25 g of soil extracted with 0.015 M chlorate using the shaken-slurry method. The rate of $NH_4^+$ oxidation was calculated as the linear increase in $NO_2^-$ content from 0 to 6 h. Genetic indicators of soil bacteria (16S rRNA gene), and different populations of denitrifiers (nirK, nirS, nosZ clade I and II genes) and nitrifiers (AOA and AOB genes) were detected by quantitative PCR. Microbial community structures were determined by Next-Generation 16S rRNA Amplicon Sequencing.

## Results and Discussion

Fluxes of $N_2O$ were higher in the first 4 days after fertilizer treatment and decreased steadily in the following days to reach levels similar to the UC for the remaining incubation period (~0.04 μg N $m^{-2}$ $h^{-1}$). Tetracycline treatment had the highest flux peak on day 4, which was at a significantly lower level than in FC (0.78±0.13 vs. 1.01±0.13 μg N $m^{-2}$ $h^{-1}$), Figure 1. Corresponding to the decline in $N_2O$ production rate, $NO_3^-$ concentration was higher in FT than in FC at every sampling point during the 30 days of incubation. The presence of tetracycline did not affect the $NH_4^+$ content, whereas a significant increase by 1.2-fold in $NO_2^-$ was observed on day 30. Furthermore, tetracycline was shown to affect the soil bacterial abundances with a direct effect in nitrifying functional guilds. The abundance of amoA genes from ammonia-oxidizing bacteria (AOB) increased markedly in fertilized soil samples, suggesting that AOB dominated over ammonia-oxidizing archae (AOA) in high N conditions. The AOB-amoA gene abundances in FC and FT samples increased dramatically between days 0 and 9, followed by a significant decrease on day 30. The relative abundances of the denitrification genes provided

evidence that the soil samples were dominated by nirK gene-containing denitrifying bacteria. Although, both nirK and nirS genes catalyze nitrite reduction, they are suggested to function under different environmental conditions. Fertilizer application affected the abundance of nirK-type denitrifiers during incubation time with a substantial decrease on day 9, while the presence of tetracycline resulted in a lower abundance on day 30, which was in line with an observed decrease in $N_2O$ flux. The introduction of fertilizer or tetracycline to soil did not yield significant changes in the abundances of nosZ gene-carrying bacteria. However, the abundance of the nosZ II-type denitrifiers decreased during time in both FC and FT samples.

Conclusion

This is the first study to evaluate the effect of tetracycline on agricultural soil $N_2O$ production and denitrifying genes abundance in the presence of oregano, an aromatic plant. These findings demonstrated that the common practice of administering antibiotics to livestock can have important, unintended impacts on soil bacteria and the N transformation processes they mediate in agroecosystems.

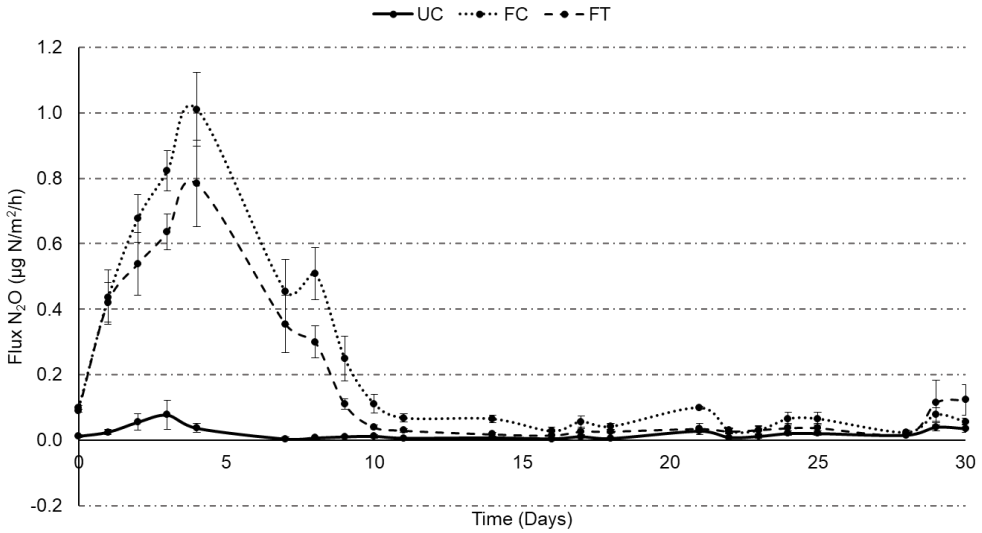


**Figure 1.** $N_2O$ flux measurements from untreated soil (UC), fertilizer-treated soil (FC) and fertilizer-treated soil polluted with tetracycline (FT) over an incubation period of 30 days. Each treatment was performed in triplicate. Data represents mean ± standard error.

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