# The Effects of Ultra Low Narasin Exposure on Soil Nitrogen Processing

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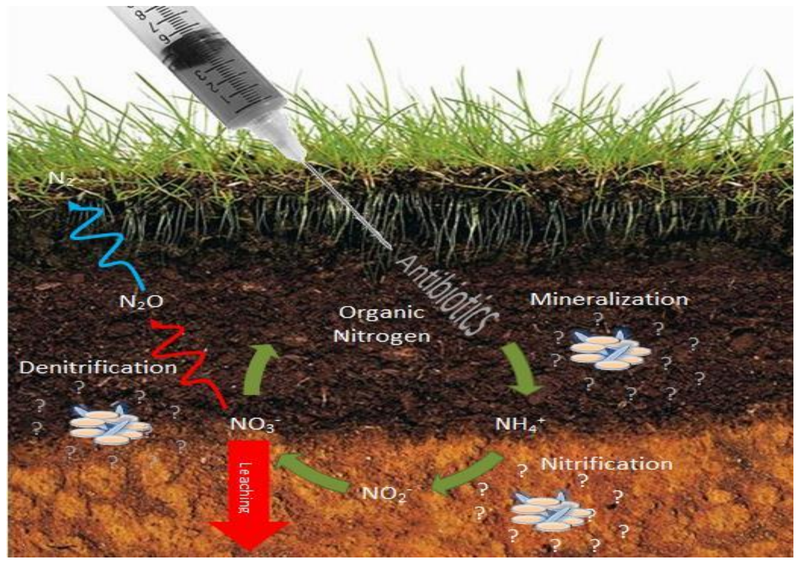


Figure 1. Processes potentially affected by antibiotics in soil.

## INTRODUCTION

Animal excrement and wastewater often contain trace concentrations of un-metabolized veterinary and human antibiotic compounds. When these are used in agriculture for fertilizer and/or irrigation, these compounds have the potential to affect microbial activity in soils. The objective of this research is to evaluate the impact of trace Narasin exposure on nitrification, denitrification, and $N_2O$ flux rates.

Figure 2. Soil incubation chambers equipped with headspace sampling ports.

## EXPERIMENTAL METHODS

In replicates of six, 75 g air-dried soil (sandy loam) was treated with 0-1000 ng/kg Narasin and a 50:50 mixture of $KNO_3$:$(NH_4)_2SO_4$ in amounts equivalent to 100 kg N hectare$_{-1}$. At each dose, 3 samples were enriched with $^{15}N$-$NO_3^-$ and 3 with $^{15}N$-$NH_4^+$ (10% atom excess). Total volumetric water content was brought to 40% Water-Filled Pore Space (Experiment 1) and 60% Water Filled Pore Space (Experiment 2) and the soils were placed in gas-tight jars (Figure 2) and allowed to incubate for 3 days at room temperature. Soil extracts (2M KCl) and headspace samples were collected daily and used to quantify NO3, $NH4^+$, and $N_2O$ flux.

## EFFECTS ON MINERAL NITROGEN

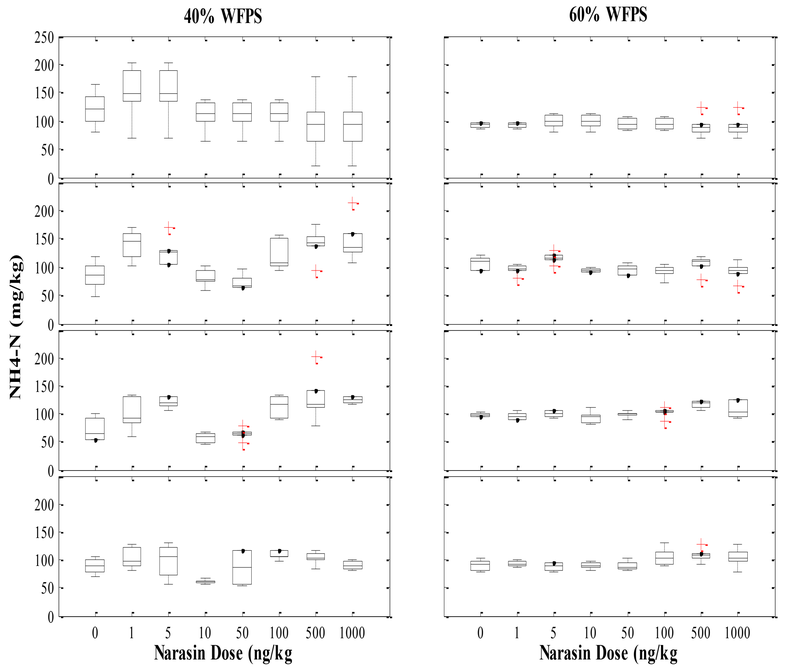


Figure 3. Box-whisker plots illustrating extractable $NH_4^+$ over the course of soil incubations. At 40% WFPS, a statistically significant dose response was observed on Day 1 (p = 2.58E-06), Day 2 (p = 1.3E-07), and Day 3 (p = 1.66E-03) where the concentration of $NH_4^+$ in treated soils exceeded that of untreated soils at all but the 10 and 50 ng kg$_{-1}$ Narasin doses. At 60% WFPS, a statistically significant dose response on Day 1 (p = 0.00), Day 2 (p = 00005), and Day 3 (p = 0.004). Here, $NH_4^+$ was depleted relative to the control at all doses on Day 1 but equal to or in excess of the control on Days 2 and 3.

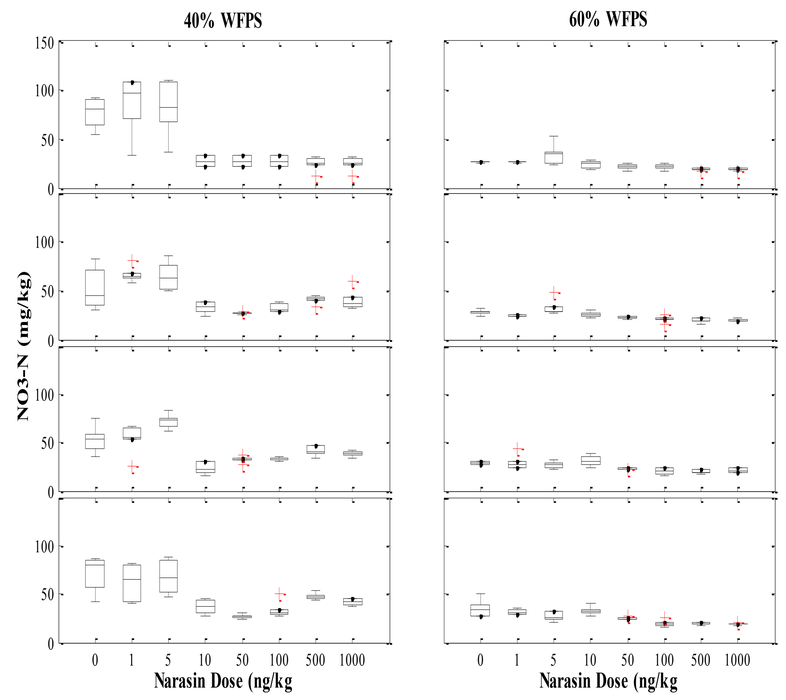


Figure 4. Box-whisker plots illustrating extractable NH4+ over the course of soil incubations. At 40% WFPS, a statistically significant dose response was observed on Day 1 (p = 4.83E-08), Day 2 (p = 1.2E-11) ,and Day 3 (p = 2.34E-08) where the concentration of $NO_3^-$ in treated soils was depleted relative to the control at Narasin doses greater than 10 ng kg$^{-1}$. A more distinctly linear pattern in which increasing dose correlates to decreased $NO_3^-$ was observed at 60% WFPS, with statistically significant dose-responses observed on Day 1 (p = 6.62E-07), Day 2 (p = 2.34E-06), and Day 3 (p = 3.62E-09).

## EFFECTS ON $N_2O$ FLUX

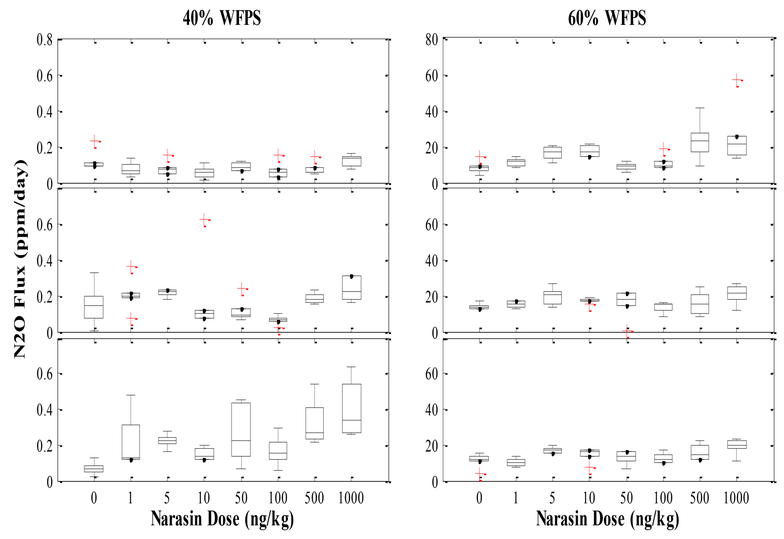


Figure 5. Box-whisker plots illustrating $N_2O$ flux (ppm/day). At both 40% and 60% WFPS, increasing antibiotic doses correlate to increasing N2O flux rates. At the lower water content, the significance of this effect is delayed until Day 3 (p = 0.006) whereas the pattern is immediately observed in soils raised to 60% WFPS (p = 0.0006), muted on Day 2 (p = 0.115), and observed again on Day 3 (p = 0.0007).

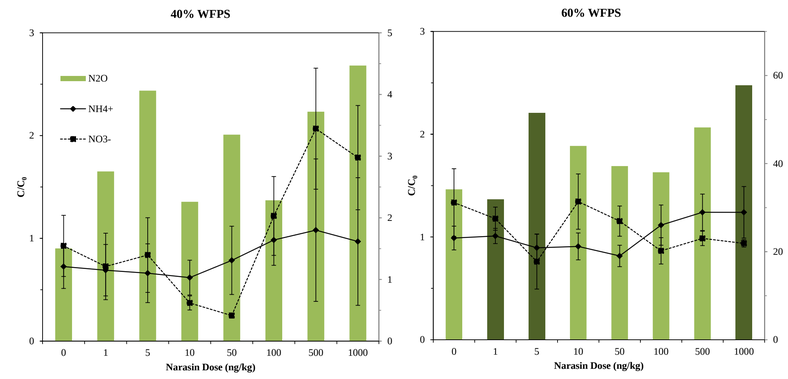


Figure 6. A comparison of mineral nitrogen on Day 3 ($C/C_0$) to total N2O flux shows little appreciable correlation between mineral N species and $N_2O$ flux rate in drier soils, but NO3- and N2O show similar trends at 60% WFPS for all but the 500 and 1000 ng kg$^{-1}$ doses.

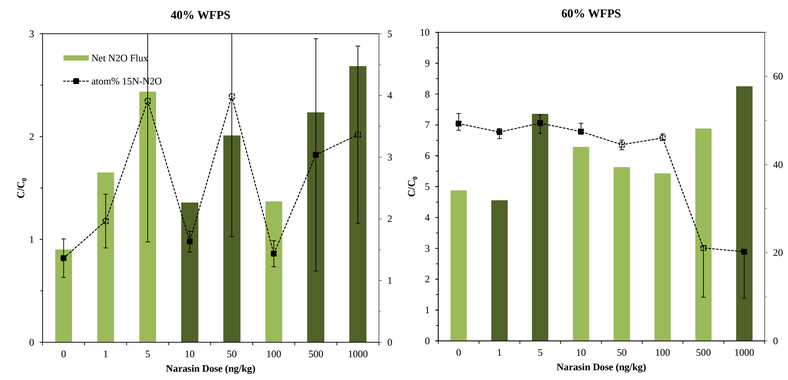


Figure 7. The $^{15}N$ isotopic enrichment of $N_2O$ from soils that were treated with $^{15}N$-$KNO_3$ strongly correlate to total $N_2O$ accumulation on Day 3, suggesting that denitrification is the primary $N_2O$ source. The distinct disagreement between $^{15}N$-$N_2O$ and total $N_2O$ flux at 60% WFPS, 500 and 1000 ng kg$^{-1}$ doses implies significant dilution of the $NO_3^-$ pool by mineralization and nitrification, coupled with denitrification.