

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<sub>2</sub>O 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<sub>3</sub>:(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in amounts equivalent to 100 kg N hectare<sup>-1</sup>. At each dose, 3 samples were enriched with <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> and 3 with <sup>15</sup>N-NH<sub>4</sub><sup>+</sup> (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 NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup>, and N<sub>2</sub>O flux.

## EFFECTS ON MINERAL NITROGEN

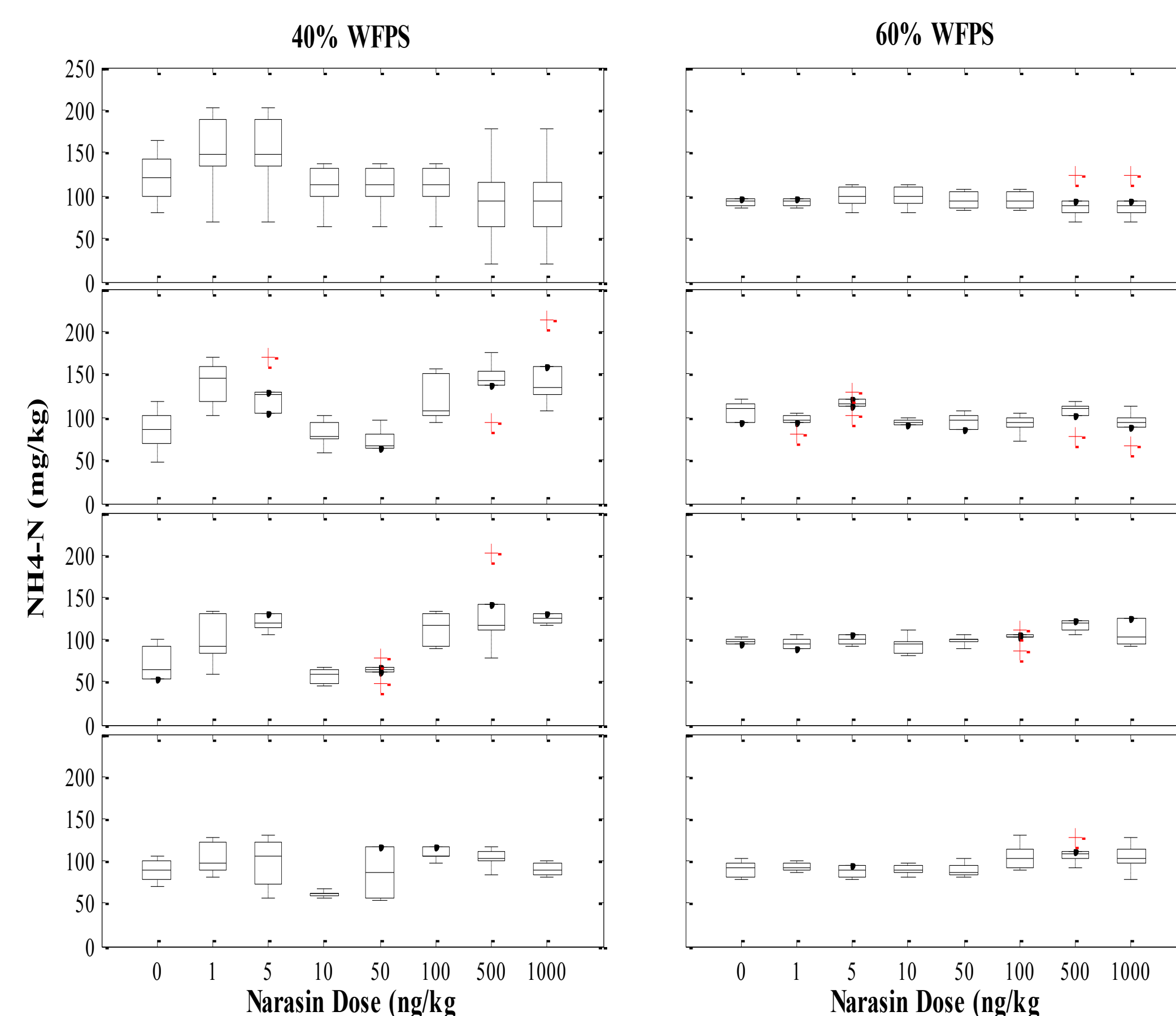


Figure 3. Box-whisker plots illustrating extractable NH<sub>4</sub><sup>+</sup> over the course of soil incubations. At 40% WFPS, a statistically significant dose response was observed on Day 1 ( $p = 2.58E-06$ ), Day 2 ( $p = 1.3E-07$ ), and Day 3 ( $p = 1.66E-03$ ) where the concentration of NH<sub>4</sub><sup>+</sup> in treated soils exceeded that of untreated soils at all but the 10 and 50 ng kg<sup>-1</sup> Narasin doses. At 60% WFPS, a statistically significant dose response on Day 1 ( $p = 0.00$ ), Day 2 ( $p = 0.0005$ ), and Day 3 ( $p = 0.004$ ). Here, NH<sub>4</sub><sup>+</sup> 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.

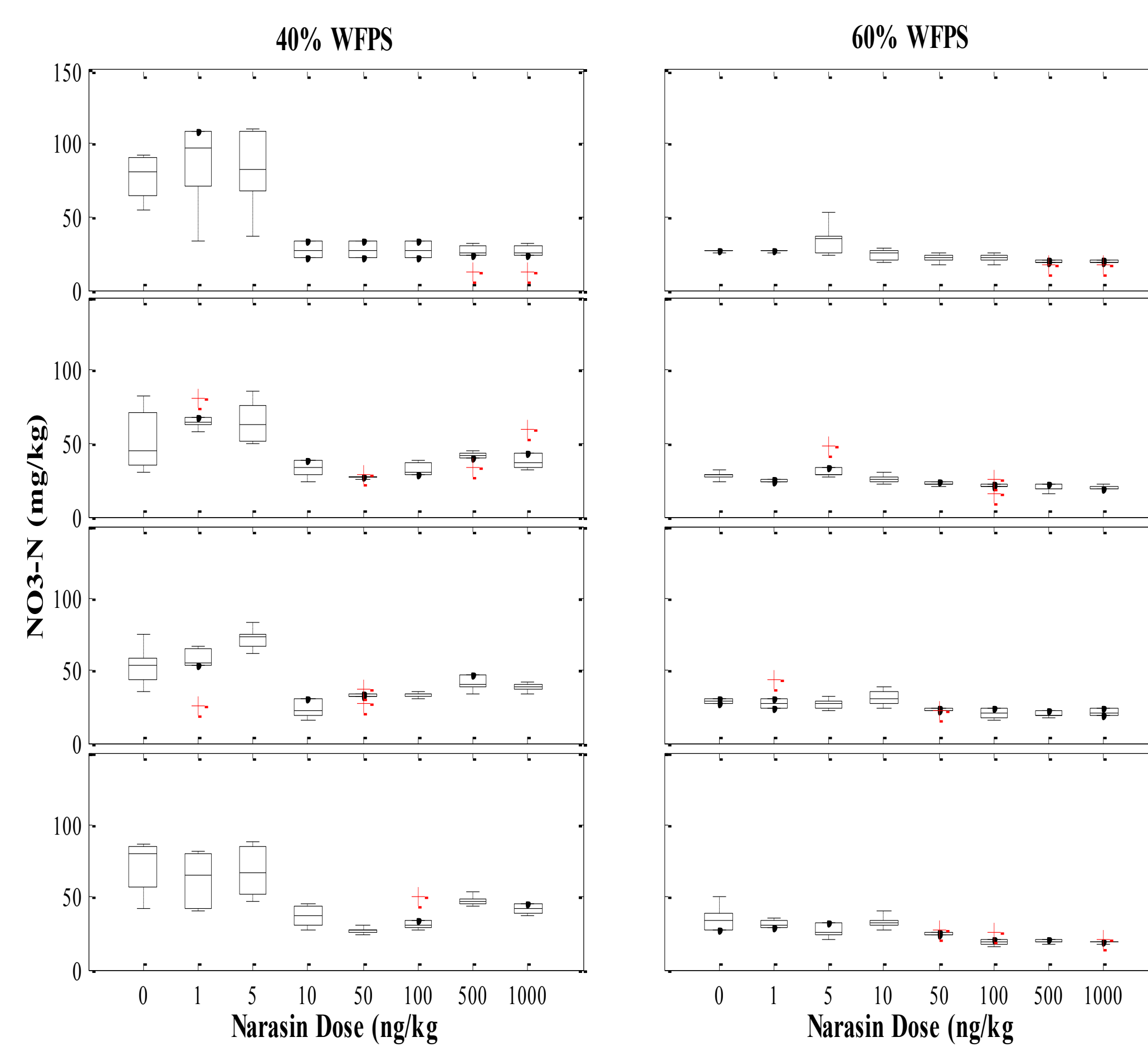


Figure 4. Box-whisker plots illustrating extractable NH<sub>4</sub><sup>+</sup> over the course of soil incubations. At 40% WFPS, a statistically significant dose response was observed on Day 1 ( $p = 4.83E-08$ ), Day 2 ( $p = 1.2E-11$ ), and Day 3 ( $p = 2.34E-08$ ) where the concentration of NO<sub>3</sub><sup>-</sup> in treated soils was depleted relative to the control at Narasin doses greater than 10 ng kg<sup>-1</sup>. A more distinctly linear pattern in which increasing dose correlates to decreased NO<sub>3</sub><sup>-</sup> 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<sub>2</sub>O FLUX

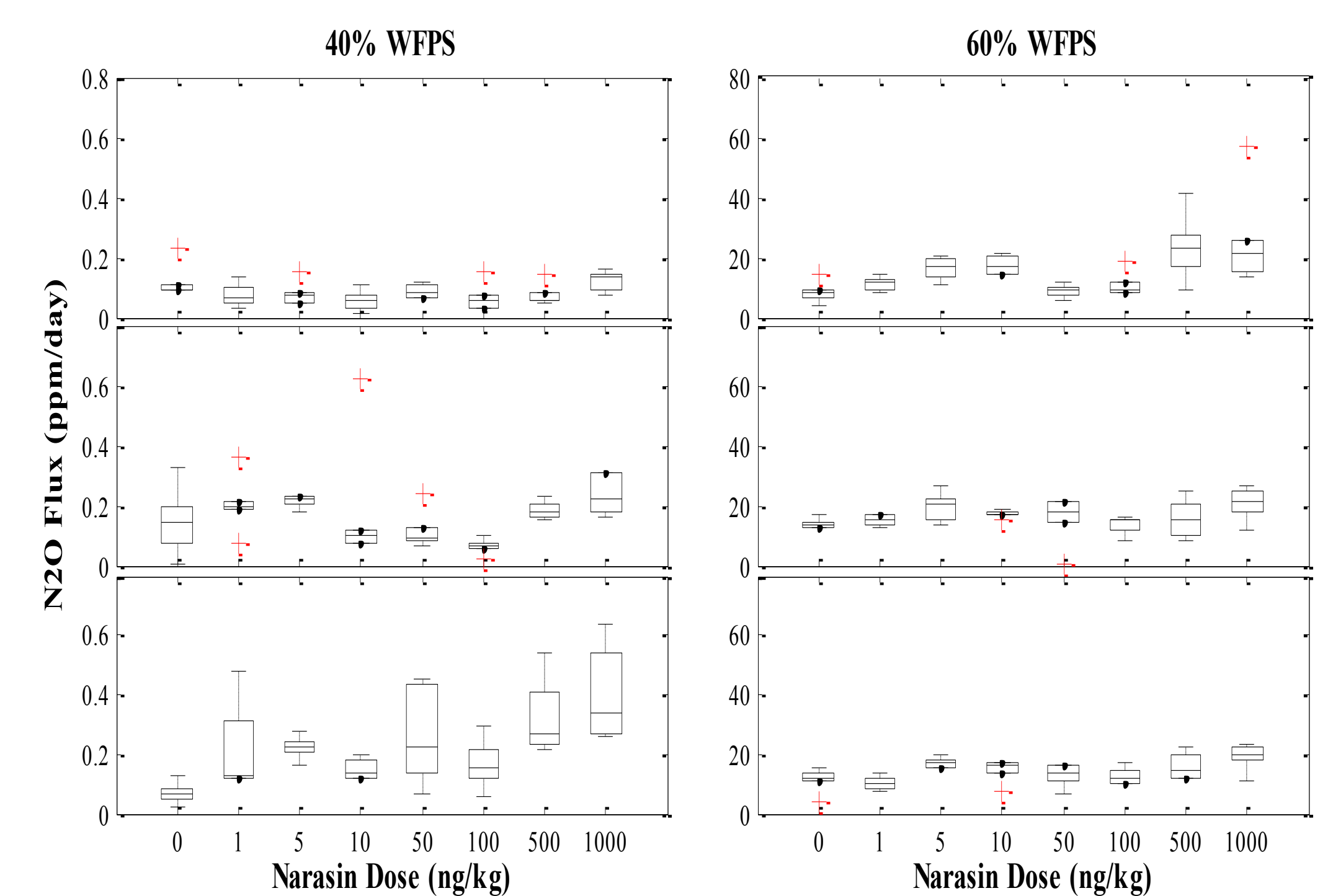


Figure 5. Box-whisker plots illustrating N<sub>2</sub>O flux (ppm/day). At both 40% and 60% WFPS, increasing antibiotic doses correlate to increasing N<sub>2</sub>O flux rates. At the lower water content, the significance of this effect is delayed until Day 3 ( $p = 0.006$ ) whereas the pattern is immediately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

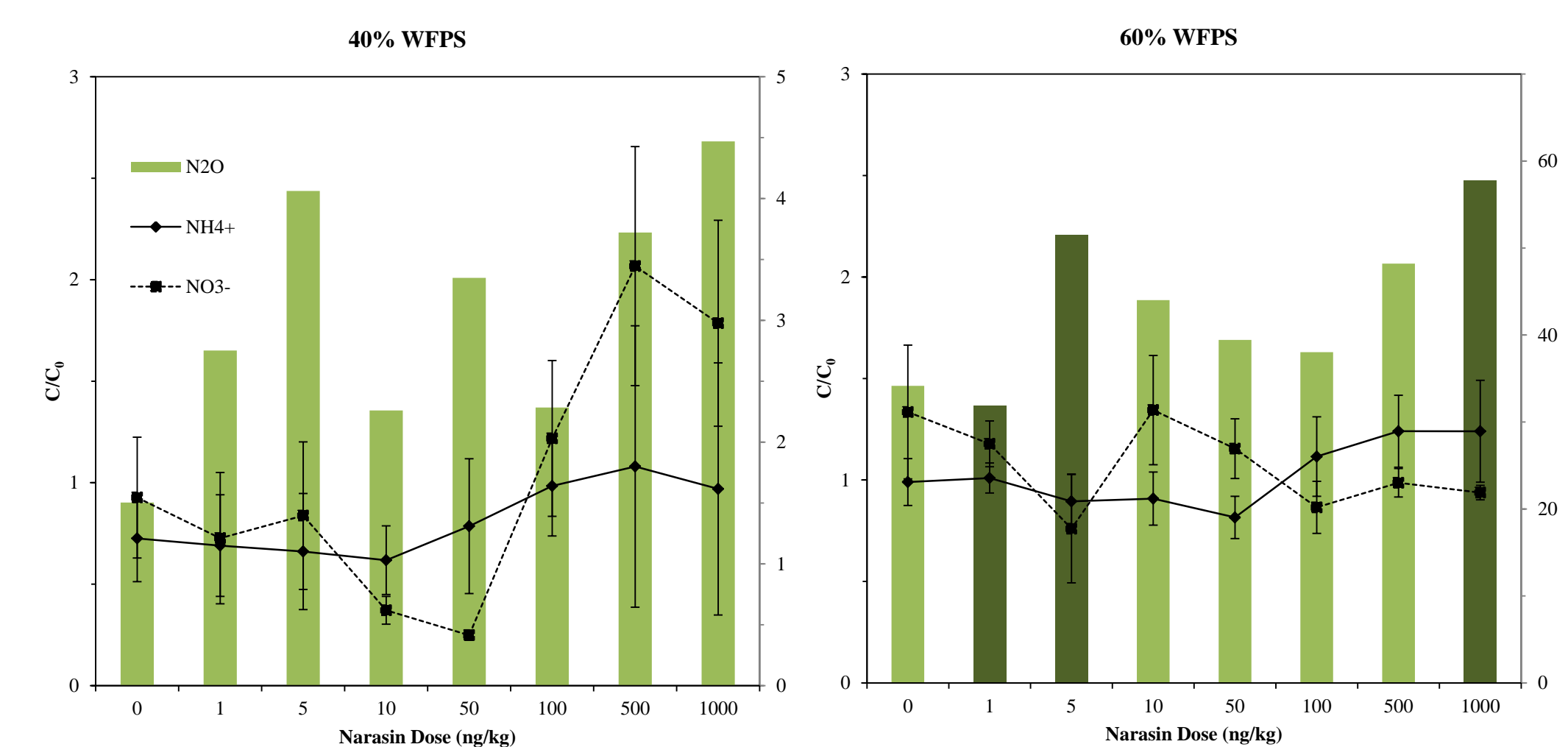


Figure 6. A comparison of mineral nitrogen on Day 3 (C/C<sub>0</sub>) to total N<sub>2</sub>O flux shows little appreciable correlation between mineral N species and N<sub>2</sub>O flux rate in drier soils, but NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O show similar trends at 60% WFPS for all but the 500 and 1000 ng kg<sup>-1</sup> doses.

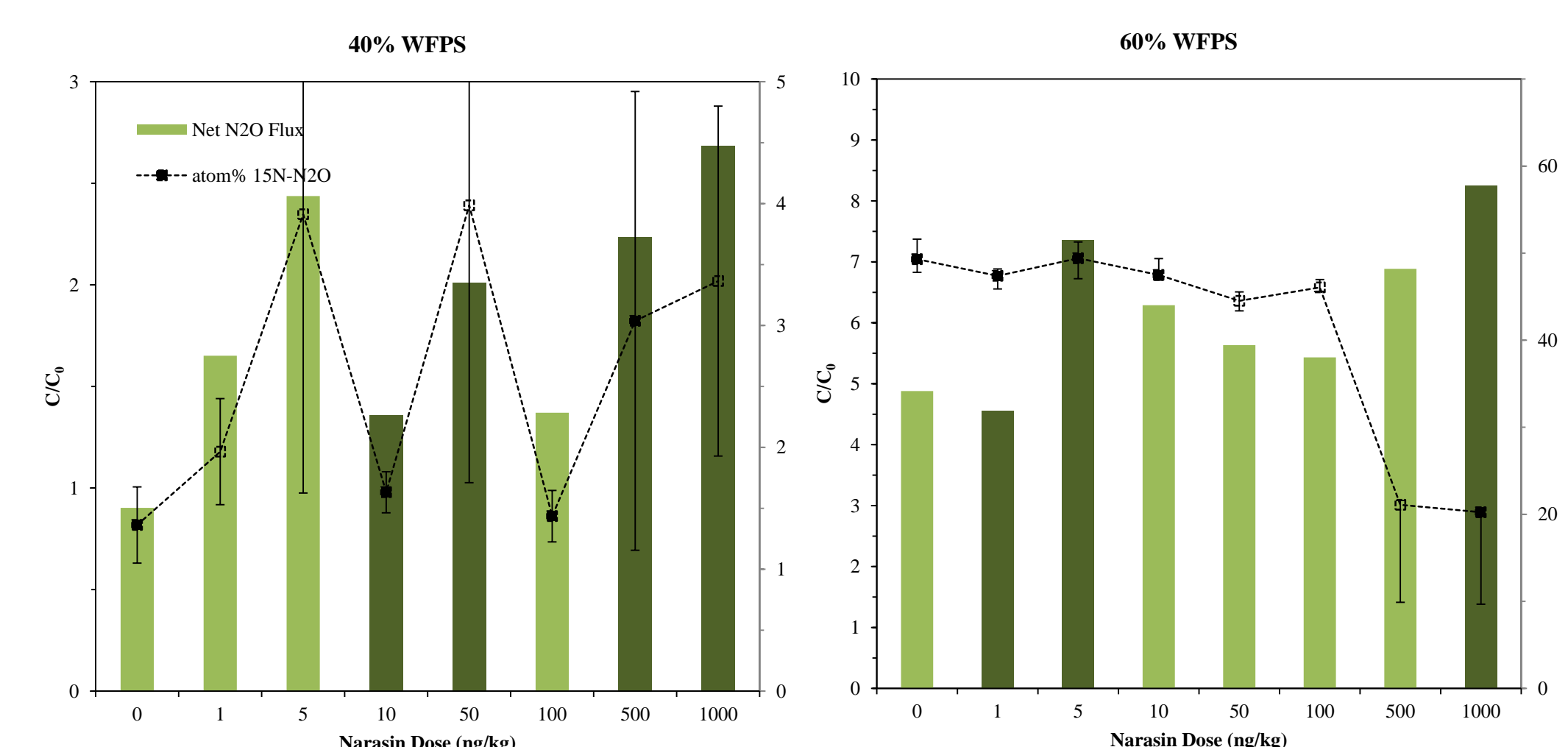


Figure 7. The <sup>15</sup>N isotopic enrichment of N<sub>2</sub>O from soils that were treated with <sup>15</sup>N-KNO<sub>3</sub> strongly correlate to total N<sub>2</sub>O accumulation on Day 3, suggesting that denitrification is the primary N<sub>2</sub>O source. The distinct disagreement between <sup>15</sup>N-N<sub>2</sub>O and total N<sub>2</sub>O flux at 60% WFPS, 500 and 1000 ng kg<sup>-1</sup> doses implies significant dilution of the NO<sub>3</sub><sup>-</sup> pool by mineralization and nitrification, coupled with denitrification.