

BACKGROUND

Following application of nitrogen fertilizers, organic and/or inorganic nitrogen compounds enter the biogeochemical nitrogen cycle and may be converted to one of nine possible oxidation states.

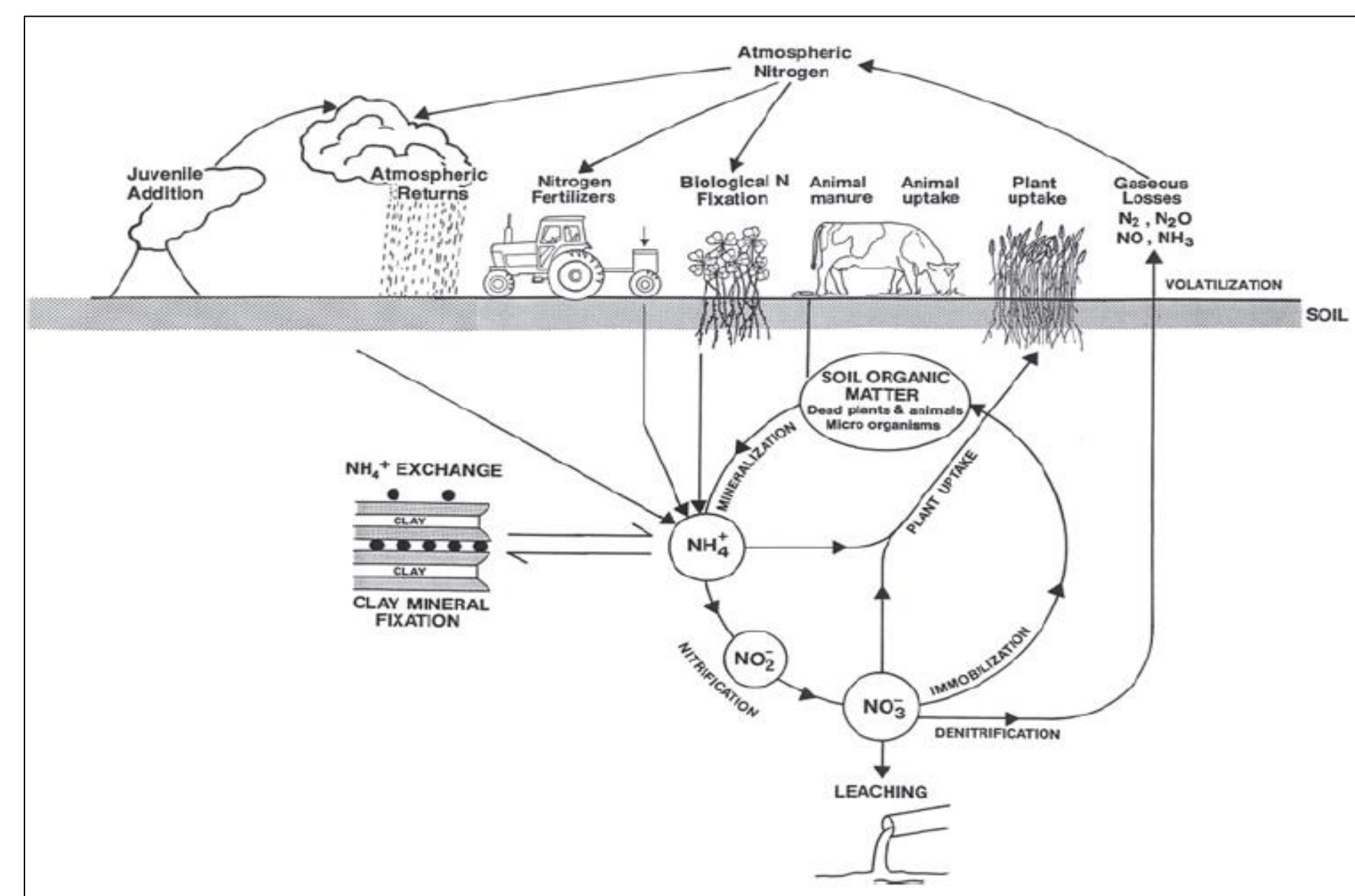


Figure 1. The Nitrogen Cycle, from Cameron, 2013.

Two forms, nitrate (NO_3^-) and nitrous oxide (N_2O), are of particular concern. Nitrate in groundwater has been linked to a number of human health hazards as well as degradation and eutrophication of aquatic ecosystems (Carpenter, 1998; Galloway, 2003; Lunau, 2012; Ward, 2005). Nitrous oxide, N_2O , is a biogeochemical byproduct of nitrate reduction. Modern emissions of this powerful greenhouse gas (Wrage, 2001) are largely correlated to agricultural fertilizer use. N_2O is also the leading modern contributor to stratospheric ozone depletion (Ravishankara, 2009). Of the many factors controlling biogeochemical denitrification rates and nitrous oxide production, the impact of trace antibiotics that can be introduced via manure fertilizers (Figure 2) remains poorly understood, particularly at sub-detection limit concentrations.

OBJECTIVES

The primary objective of this research is to assess whether sub-detection limit exposure to antibiotics ($\text{ng}\cdot\text{kg}^{-1}$ or $\text{ng}\cdot\text{L}^{-1}$) has the potential to significantly impact denitrification in anaerobic soil environments.

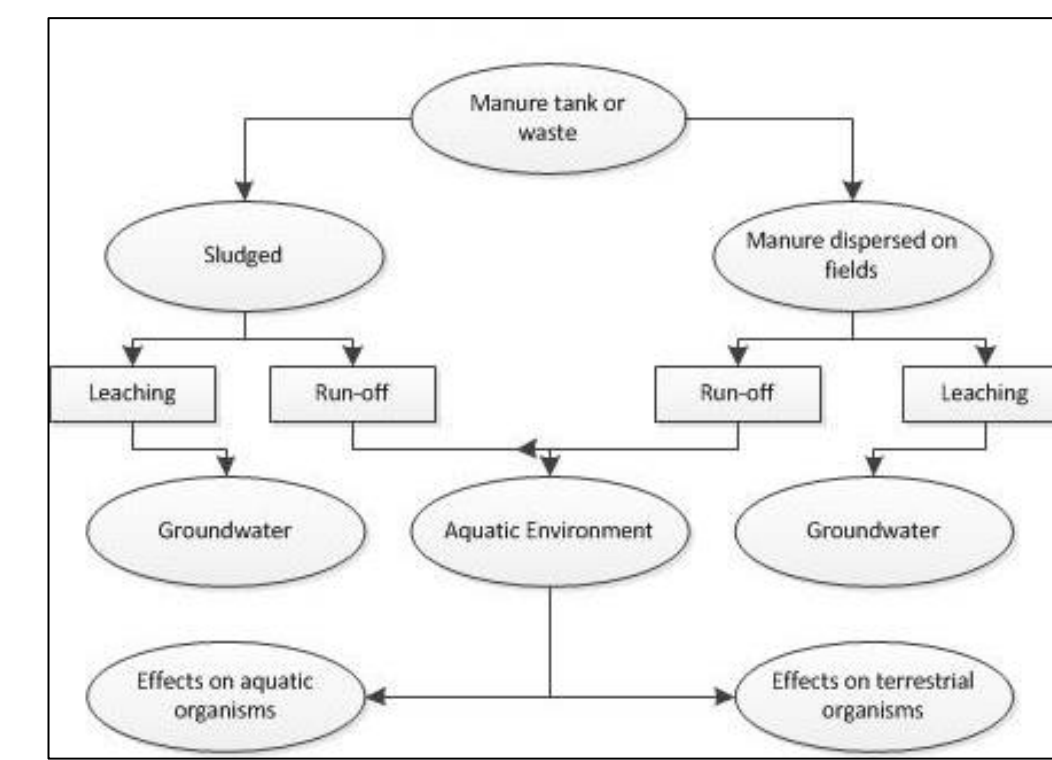


Figure 2. Potential pathways for veterinary antibiotics to enter terrestrial and aquatic environments. Adapted from Sarmah, 2006.

SOIL AND SEDIMENT SAMPLING

Soil samples for the first experiment were collected at an agricultural site along the Upper Indian River Bay (Figure 3) near Milford, Delaware.

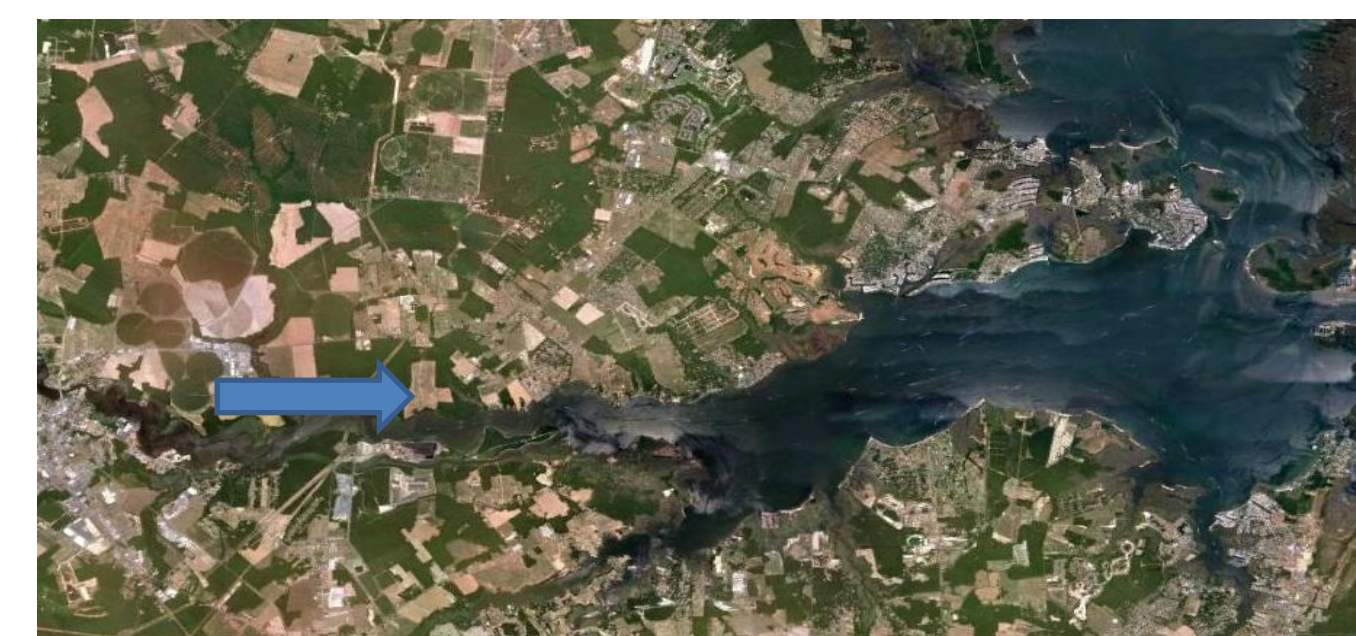


Figure 3. Soil and sandy aquifer material were collected at Bull's Eye Farm, Milford, Delaware.

METHODS

Incubation experiments were performed to investigate the impact of four veterinary antibiotics a soil. After a 10-day pre-incubation to establish anaerobic conditions, 10 gram soil samples were spiked with 0 (control) or 0.001-1.0 ng/g (experimental) narasin, gentamicin, sulfamethoxazole, or sulfadiazine. Three samples from each group were extracted over a period of five days and the extractable nitrate concentration determined using a Seal AQ2 Discrete Nutrient Analyzer.

A saturated column experiment was executed to evaluate whether continuous exposure to 1 $\text{ng}\cdot\text{L}^{-1}$ sulfamethoxazole significantly modifies the removal of nitrate under saturated flow conditions. The columns were packed with sandy aquifer material collected from the saturated zone. For two weeks, influent to the columns contained glucose

with 0.1 mM nitrate and the effluent concentration reached a steady state value of 40% (C/C_0) within 3 days. After two weeks, influent to three columns was spiked with 1 $\text{ng}\cdot\text{L}^{-1}$ sulfamethoxazole. Effluent samples were collected every six hours 1 day prior to spiking the experimental columns and continued for an additional 3.5 days. Nitrate was quantified by ion chromatography (Dionex, AS14A-5 μm column).

RESULTS

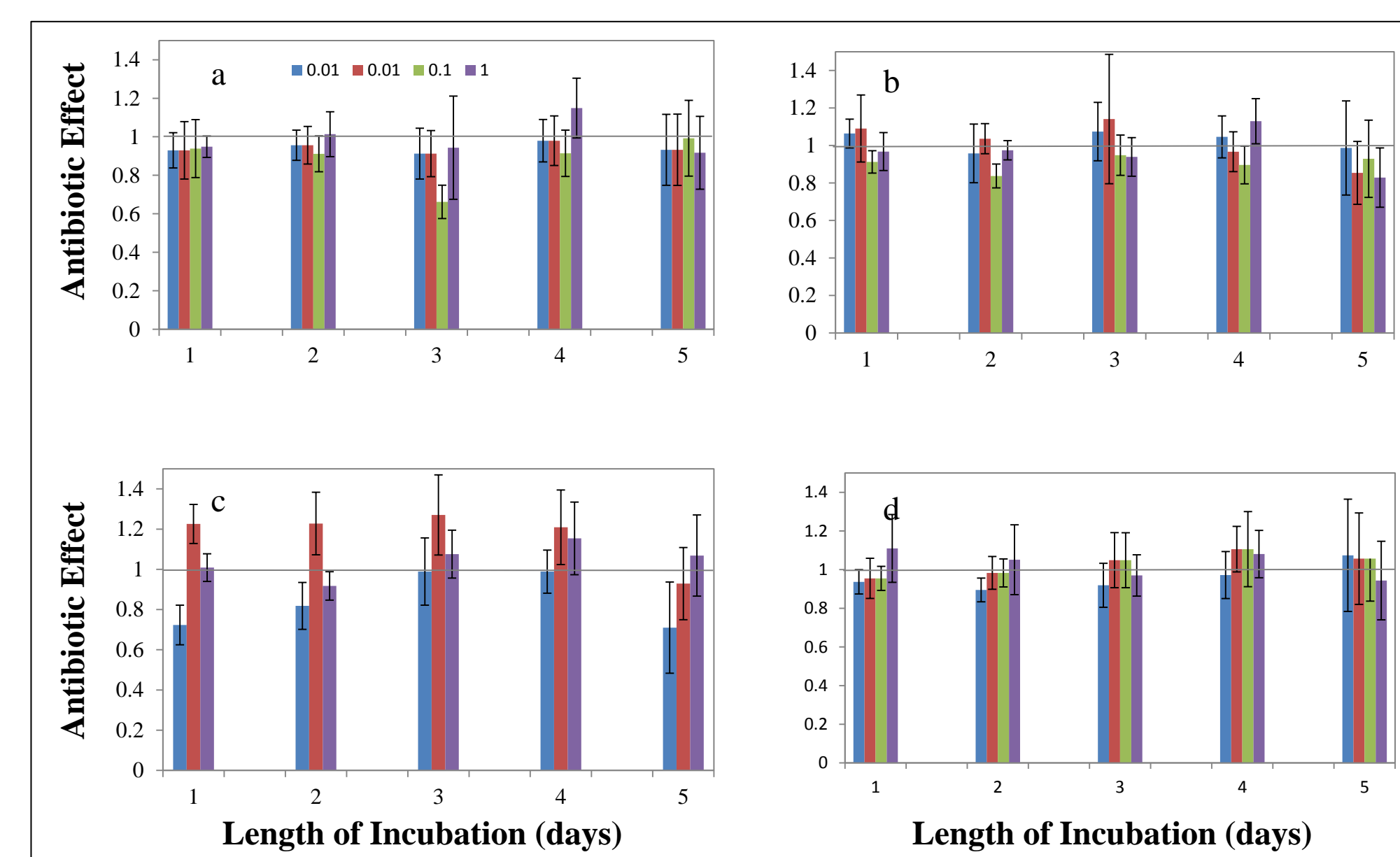


Figure 4. The antibiotic effect in soils exposed to 0.001-1 $\text{ng}\cdot\text{g}^{-1}$ narasin (a), gentamicin (b), sulfamethoxazole (c), or sulfadiazine (d). Values above gray line indicate that nitrate reduction was inhibited and values below the line are evidence of stimulated nitrate removal, relative to the control.

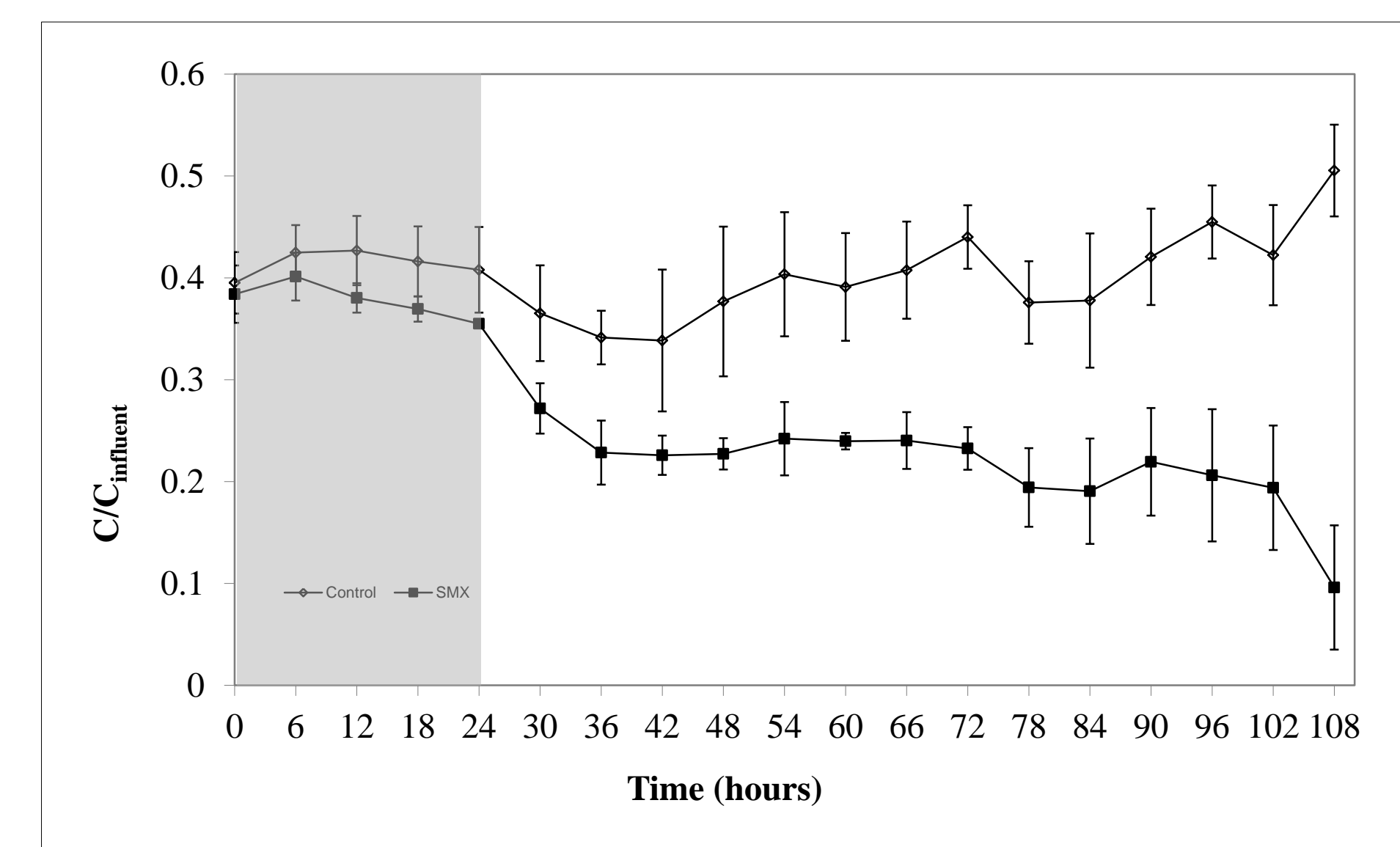


Figure 5. Results of column experiment in which control columns (open diamond) received 1 mM nitrate and 0.4 mM glucose at a constant rate of 1 m/day . Experimental columns received an identical through shaded portion of the chart, after which 1 ng/L sulfamethoxazole was added.

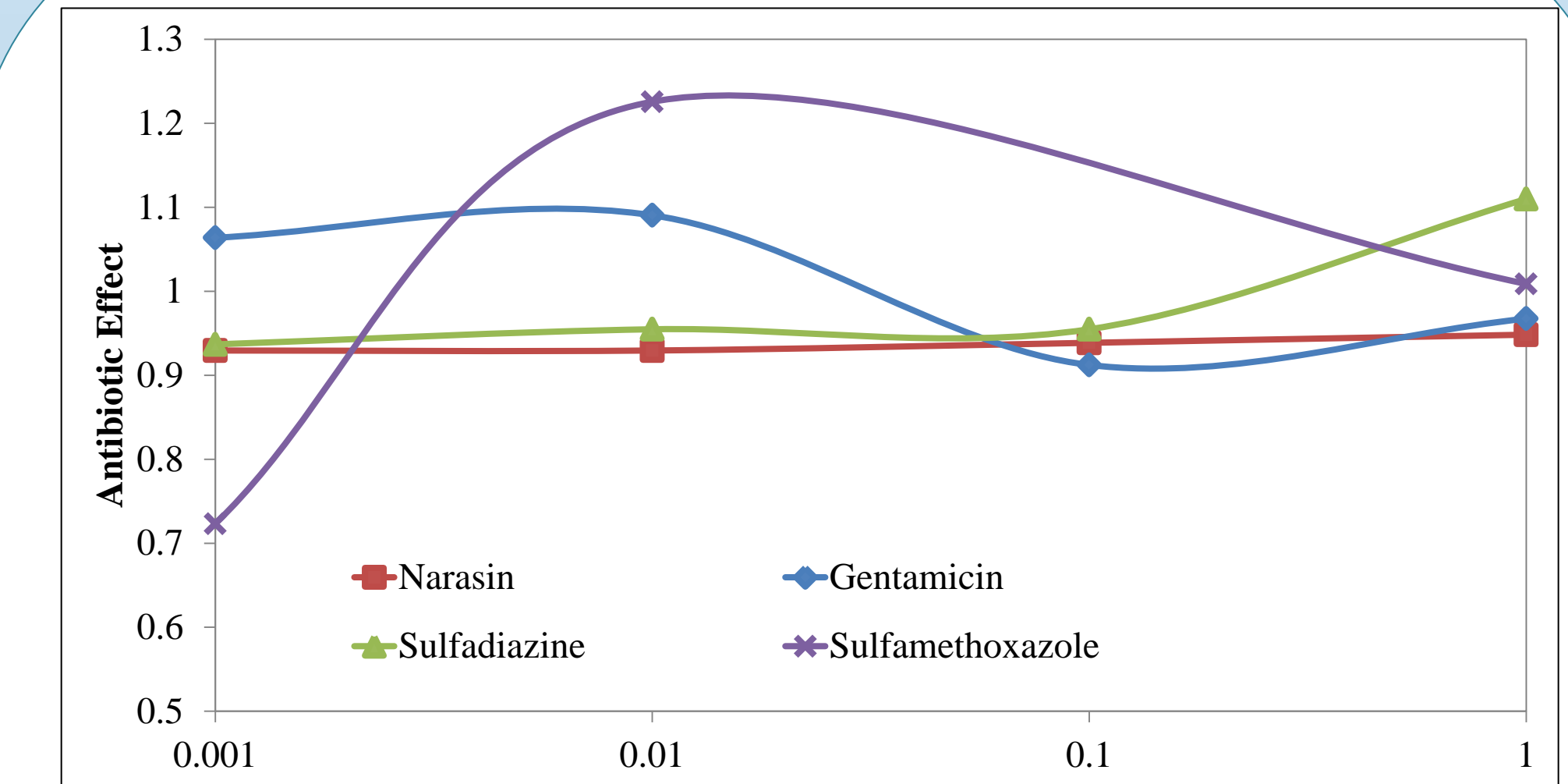


Figure 6. Dose response curve for extractable nitrate concentrations in soils 1 day after antibiotics were added.

CONCLUSIONS

Nitrate reduction in anaerobic soils exposed to veterinary antibiotics at or below detection limits was strongly affected by sulfamethoxazole but less so by the other antibiotics tested. Interestingly, the 1 $\text{ng}\cdot\text{L}^{-1}$ column test points to stimulated nitrate reduction, an effect also observed in soils incubated with sulfamethoxazole at the lowest tested dose (1 $\text{ng}\cdot\text{kg}^{-1}$). The dose response curve constructed for sulfamethoxazole (Figure 6) is highly suggestive of hormesis or J-curve response, a model recently championed by Calabrese (2005). Since N_2O is a product of denitrification, additional study is warranted to determine whether N_2O flux is similarly affected.

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