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Effects of tetracycline on water quality, soil and gases in aerated and unaerated Leachfield mesocosms

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10 We examined the effects of tetracycline (TET) addition on the function of mesocosms representing aerated and unaerated septic system
leachfields. Replicate mesocosms ($n = 3$) were filled with soil and either vented to a leachfield (LEACH) or aerated intermittently to
maintain an O_2 level of $\sim 0.21 \text{ mol mol}^{-1}$ (AIR). All mesocosms were dosed every 6 h for 10 d with 3 cm of domestic wastewater
amended with 5 mg TET L^{-1} . Water quality parameters, headspace gas composition, and soil properties were measured prior to and
15 during the dosing period, and for 42 days after the last antibiotic dose. No significant effect of TET was observed on the pH, level
of dissolved O_2 or dissolved organic carbon (DOC) in drainage water from either treatment. In contrast, levels of Fe^{2+} and SO_4 in
drainage water from LEACH mesocosms decreased in response to TET dosing, with lower levels persisting until Day 52. Persistent
increases were observed in the level of NO_3 in drainage water from AIR lysimeters and in NH_4 in LEACH mesocosms in response
to TET additions. Removal of total P and DOC removal were unaffected by TET dosing in either treatment. Nitrogen removal in
20 AIR mesocosms decreased during the TET dosing period, returning to pre-dosing values by Day 52. In contrast, TN removal in
LEACH mesocosms increased during TET dosing, returning to pre-dosing values by Day 52. The composition of headspace gases
in AIR mesocosms was not affected by tetracycline dosing. TET dosing resulted in significant increases in soil NH_4 concentration in
LEACH mesocosms, whereas significant decreases were apparent in AIR mesocosms. Elevated levels of H_2S and CH_4 in the headspace
of LEACH mesocosms coincided with TET dosing and returned to pre-dosing levels when antibiotic dosing ceased. The effects of
25 tetracycline leachfield mesocosms differed as a function of aeration. Although most effects were transient, with values returning to
pre-dosing levels after a 6-week recovery period in both treatments, persistent effects were observed in LEACH mesocosms.

Keywords: Domestic wastewater, water quality, intermittent aeration, on-site wastewater treatment systems, antibiotics.

Introduction

Antibiotics are widely used therapeutically in human and veterinary medicine, as well as non-therapeutically in animal production. Many antibiotics are excreted unchanged^[1] and are frequently found in wastewater^[2–4] and surface waters.^[1,5] This is of concern because of the potential effects of antibiotics on human and animal reproduction, development of antibiotic resistance in pathogenic bacteria^[6], and disruption of ecosystem functions.^[7,8]

35 Tetracycline has been detected in wastewater^[9] and there is evidence that tetracycline antibiotics may impact nitrogen removal from wastewater in treatment plants. Halling-Sorensen^[10] conducted a study examining the effects of tetracycline, chlortetracycline, and oxytetracycline on nitrifying bacteria in activated sludge. Although tests with tetra-

cycline were not performed, results indicated that chlortetracycline and oxytetracycline inhibited nitrification.^[10] Like centralized municipal wastewater treatment plants, soil-based wastewater treatment systems rely on the activities of a complex community of organisms to improve water quality. With nearly 25% of households in the United States using on-site wastewater treatment systems (OWTS) for treatment of domestic wastewater^[11] there is a clear need to understand the extent to which antibiotics can affect the water quality functions performed by these systems.

45 Despite their potential for adverse effects on soil-based treatment systems, there appear to be no studies of the effects of antibiotics in these soils. We present the results of a mesocosm-scale study using soil-filled lysimeters with headspace that was either vented to a conventional leachfield trench (LEACH) or intermittently aerated (AIR). The AIR treatment represented an alternative technology for soil-based treatment systems that improves hydraulic function.^[12] In a previous pilot-scale study^[13] intermittent aeration significantly improved water quality relative to

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unaerated soil, with higher removal rates for BOD₅, fecal coliforms, and total nitrogen.

65 Septic tank effluent amended with tetracycline was used to dose the mesocosms four times a day for a period of 10 days. Tetracycline was chosen as the test antibiotic because (i) it is excreted 80–90% unchanged^[14] and therefore has a high probability of reaching leachfield soil as well as
 70 groundwater via wastewater, (ii) it persists in soils,^[15,16] (iii) it can continue to exhibit antibacterial activity while bound to the soil,^[17] and (iv) there is evidence that it may affect microbial processes that are important for improvement of water quality, such as nitrification.^[10]

75 In a companion study, we examined the effects of tetracycline on antibiotic resistance and removal of fecal indicator bacteria in aerated and unaerated leachfield mesocosms.^[18] The objectives of the present study were to examine the effects of tetracycline on the composition of headspace gases,
 80 water quality parameters, and removal rates of nitrogen, phosphorus, and dissolved organic carbon from domestic wastewater in unaerated and intermittently aerated leachfield soil mesocosms.

Materials and methods

85 Research facility

The study was conducted in a pilot-scale research facility built adjacent to a two-story, two-family home in southeastern Connecticut, (Fig. 1). The home was fitted with a conventional septic system (4733 L [1250 gallon] max. capacity) which was not pumped during the course of this study. A detailed description of the facility can be found in Potts et al.^[13] Septic tank effluent (STE) was diverted to an HDPE storage tank (1,325 L; residence time = 2 d) located in a climate controlled (17–19°C) room above the
 90 lab. Every 6 h, effluent from the storage tank was pumped to dosing tanks in the lab. The temperature of STE ranged from 20.4°C to 24.8°C.

The wastewater (12 cm d⁻¹) flowed by gravity from the dosing tanks into a series of stainless steel lysimeters containing 30 cm of a mixture of B and C horizon soil (sandy-skeletal, mixed, mesic Typic Udorthent; 92% sand, 8% silt) and a headspace of approximately 20 cm. The soil used was typical of that used for construction of leachfields in Rhode Island, and was obtained from the Peckham Farm Research Area in Kingston, RI.
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Lysimeters began receiving wastewater on 13 August 2003 at a rate of 4 cm d⁻¹. On 22 June 2004, the dosing rate was increased to 12 cm d⁻¹, and remained constant for the duration of the experiment.

110 Treatments

The headspace of LEACH mesocosms was vented to a conventional septic system leachfield, while the headspace of

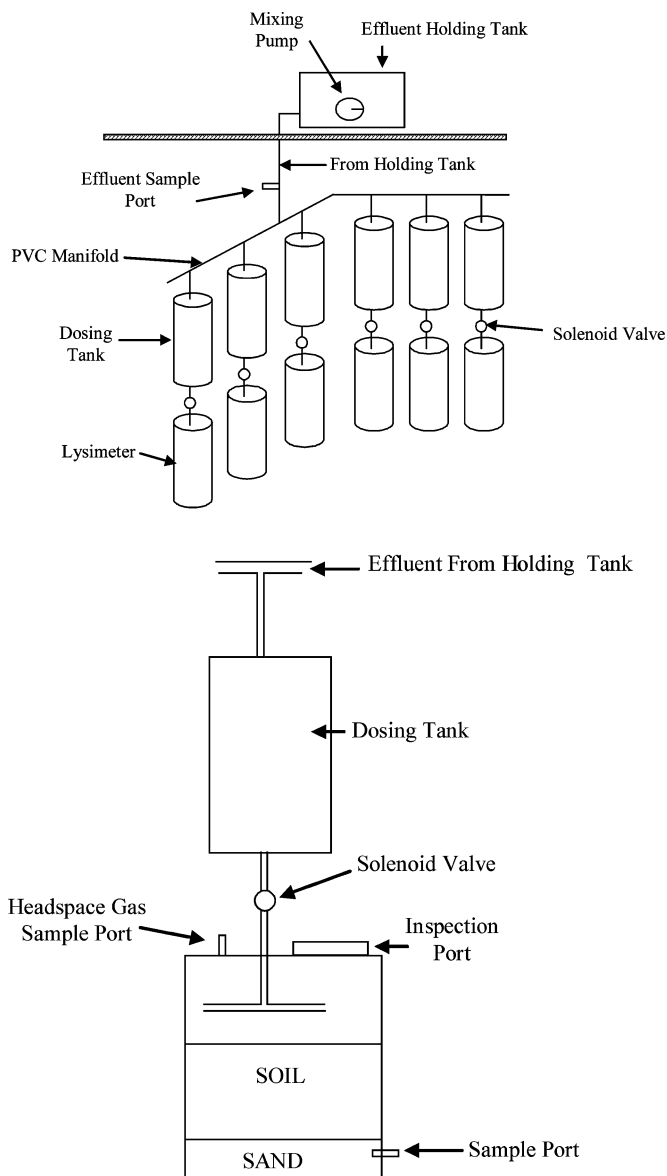


Fig. 1. Schematic diagram of laboratory facility (top) and leachfield mesocosms (bottom) employed in this study. Drawings are not to scale.

AIR lysimeters was periodically supplied with air to maintain an O₂ level of 0.21 mol mol⁻¹. The headspace of AIR mesocosms had a positive pressure (2.5–6.7 kPa) as a result of aeration. Treatments were replicated three times.
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Tetracycline dosing

Mesocosms were dosed with STE containing 5 mg tetracycline L⁻¹ for a period of 10 days beginning on 13 July 2005. The concentration of tetracycline was determined based on two people taking 1,000 mg of tetracycline day⁻¹, assuming a household of 2.57 people^[11] producing 192 L of wastewater per person per day.^[19] Every 6 h, a peristaltic pump (Thomas Scientific, Swedesboro, NJ) delivered ~30 mL of
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125 a 500 mg tetracycline L⁻¹ solution to a horizontal distri-
 bution pipe at the surface of the lysimeters, simultaneously
 with the STE dose (~2.8 L). The tetracycline solution was
 stored in a sterile polypropylene bottle, and kept at ~8°C us-
 130 ing a thermoelectric water chiller (Coolworks[®], San Rafael,
 CA).

Soil sampling

Soil samples were collected on Days 0, 10, and 52 approxi-
 mately 2 h after the 9 a.m. dosing event. Soil was sampled
 to a depth of 4 cm using surface-sterilized, cut-off, 60-mL
 135 plastic syringes (2.75-cm i.d.). Samples were stored in ster-
 ile Whirl-Pak bags, transported on ice to the laboratory in
 Kingston, RI and stored at 4°C.

Water sampling

140 Drainage water from the lysimeters was collected in auto-
 claved, polypropylene screw-cap bottles that were purged
 with N₂ gas to prevent reactions involving O₂. The tem-
 perature of drainage water from the mesocosms ranged
 from 18.4°C to 18.9°C. To ensure that water samples were
 145 exposed to the same atmosphere as that found in the
 headspace of lysimeters, two lengths of Tygon tubing were
 inserted into the cap, one connected to the lysimeter for
 collection of sample and the other vented to the headspace
 of the lysimeter. STE samples were collected from a valve
 in the input stream (Fig. 1). Samples were transported on
 150 ice to the laboratory in Kingston, RI and stored at 4°C.

Soil analyses

Soil moisture was determined gravimetrically by drying 5
 g (fresh weight) soil at 105°C for 24 h. Soil pH was de-
 155 termined using a 1:10 (w/v) soil/water ratio^[20] and mea-
 sured with a combination pH electrode and a UB-10 pH
 meter (Denver Instruments, Denver, CO). Extractable NO₃
 and NH₄^[21] and bicarbonate extractable PO₄^[22] were ana-
 lyzed colorimetrically using an automated nutrient ana-
 lyzer (Flow Solution IV, Alpkem, College Station, TX).
 160 Sulfate was extracted^[23] and analyzed using the barium
 chloride turbidimetric method^[24] using a model UV160U
 spectrophotometer (Shimadzu, Columbia, MD). Particle
 size distribution of soil was determined using the hydrom-
 eter method.^[25]

165 Water analyses

Analyses were performed according to the methods de-
 scribed by the American Public Health Association.^[24] Im-
 mediately following collection, the Fe²⁺ concentration of
 water samples was determined using EM Quant iron test
 170 strips (EM Industries, Gibbstown, NJ). Dissolved oxygen
 (DO) was measured using the azide modification of the
 Winkler titration using a LaMotte test kit (LaMotte Co.,

Chestertown, MD). The remaining water was kept on ice
 during transport to the laboratory in Kingston, RI. The pH
 of water samples was determined immediately upon arrival
 175 to the lab using a combination pH electrode and a UB-10
 pH meter (Denver Instruments, Denver, CO). A portion
 of unfiltered sample was reserved and frozen for analysis of
 total N (TN) and total P (TP) using the persulfate digestion
 method.^[24] The remaining sample was passed through a nylon
 180 filter (0.45-μm pore size, 47-mm dia., Osmonics, Inc.,
 Minnetonka, MN) and the filtrate stored at 4°C. Sulfate
 concentrations were measured using the barium chloride
 turbidimetric method. Concentrations of NO₃, NH₄, and
 185 PO₄ were determined colorimetrically using an automated
 nutrient analyzer (Flow Solution IV).

The dissolved organic carbon (DOC) concentration
 was measured using a TOC-5000A (Shimadzu). DOC
 was determined instead of BOD₅ due to the possible in-
 190 hibitory effects of tetracycline on microbial activity, which
 is necessary to determine BOD₅ using the respirometric
 method.^[24] DOC analysis enabled us to determine changes
 in dissolved organic C regardless of the effects of tetracy-
 cline on the microbial community.

Sampling and analysis of headspace gases

195 The concentration of CO₂, CH₄, O₂, and H₂S in the
 headspace of AIR and LEACH lysimeters was measured
 using a portable soil gas monitor using infrared (CO₂),
 catalytic bead (CH₄), galvanic (O₂), and electrochemical
 (H₂S) sensors (SoilAir Technology, East Longmeadow,
 200 MA). Gases were drawn at a rate of 0.057 m³ h⁻¹ (2.0 std
 ft³ h⁻¹) for approximately 60 s, with measurements made
 ~ 2 h after a dosing event.

Statistical Analyses

A one-way repeated measures ANOVA was used to evaluate
 205 significant changes in water quality parameters in drainage
 water and soil cores from LEACH and AIR lysimeters
 (SigmaStat for Windows, V. 2.03, SPSS, Chicago, IL). The
P-value for all analyses was <0.05.

Results

Headspace gases

Tetracycline dosing had no apparent effect on the composi-
 tion of headspace gases in the AIR treatment. Levels of O₂
 were at or near 0.209 mol mol⁻¹ on all sampling dates, with
 215 low (0.0008 to 0.0044 mol mol⁻¹) levels of CO₂ observed
 during the course of the study (Fig. 2). The concentration
 of CH₄ in the headspace of AIR mesocosms did not ex-
 ceed 1.62 × 10⁻⁴ mol mol⁻¹ and H₂S was absent from the
 headspace on all sampling dates (Fig. 2).

In LEACH mesocosms the level of O₂ in the headspace
 220 generally increased over the course of the experiment, from

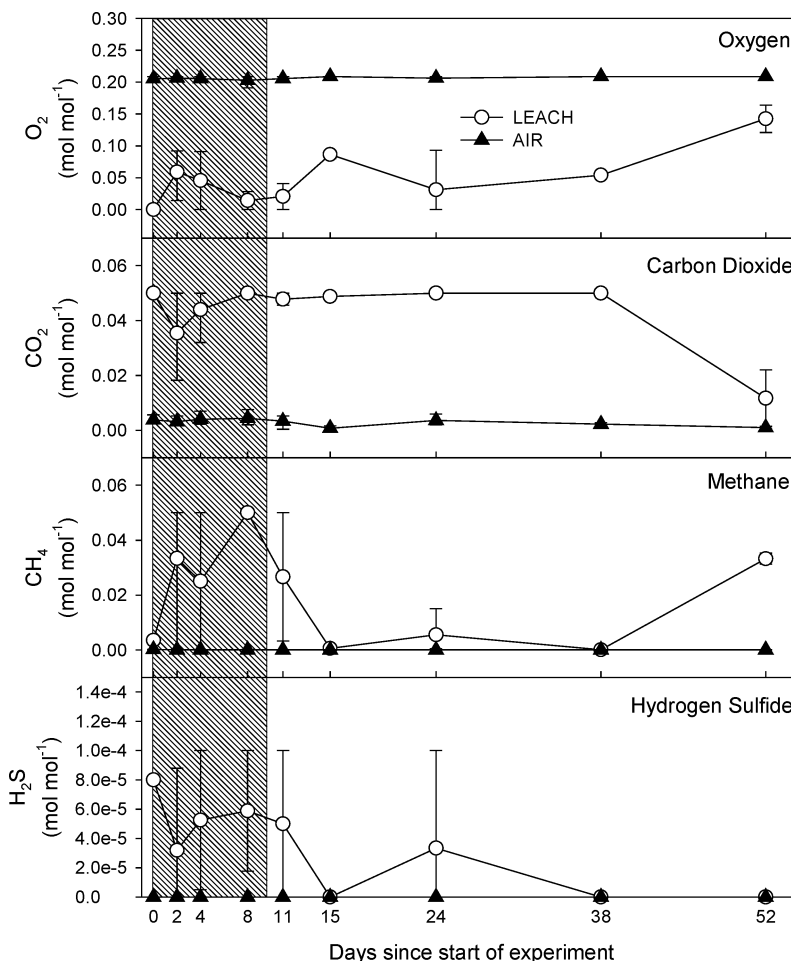


Fig. 2. Mean ($n \leq 3$) concentration of O₂, CO₂, CH₄, and H₂S in the headspace of intermittently aerated (AIR) and unaerated (LEACH) mesocosms. Shaded area indicates tetracycline dosing period. Bars indicate range of values.

near 0 mol mol⁻¹ on Day 0 to 0.13 mol mol⁻¹ on Day 52 (Fig. 2). Levels of CO₂ in LEACH mesocosm were lowest on Day 2, returning to pre-dosing levels by Day 8. The concentration of CH₄ in the headspace of LEACH mesocosms increased during the tetracycline dosing period, reaching a maximum of 0.05 mol mol⁻¹ (upper detection limit) on Day 8, decreasing to pre-dosing levels (8.0×10^{-5} mol mol⁻¹) by Day 15. Levels of H₂S in the LEACH treatment were highest on Day 0 (1.0×10^{-4} mol mol⁻¹; upper detection limit) and declined during the tetracycline dosing period, remaining below those on Day 0 for the duration of the experiment.

Statistical analyses could not be performed on headspace gas data from the LEACH treatment because on most sampling days one or more lysimeters had no headspace due to ponding of STE.

Drainage water constituents

The pH of drainage water from LEACH mesocosms ranged from 6.9 to 7.1 and did not change significantly during the study (Fig. 3; Table 1). The pH of drainage water from

AIR mesocosms declined during the TET dosing period, remaining significantly lower than pre-dosing values until the end of the experiment. Antibiotic dosing had no significant effect on levels of DO in drainage water in either treatment (Fig. 3; Table 1).

Levels of SO₄ in drainage water from LEACH mesocosms decreased during the antibiotic dosing period, remaining significantly lower than initial values until the end of the experiment (Fig. 3; Table 1). Sulfate levels in drainage water from AIR mesocosms increased significantly after dosing for 2 days and between Day 24 and 38, returning to pre-dosing levels by Day 52 (Fig. 3; Table 1).

The level of Fe²⁺ in drainage water from LEACH mesocosms decreased during the tetracycline dosing period from an initial value of 42 mg L⁻¹ to 20 mg L⁻¹ remaining below pre-dosing levels for the remainder of the experiment (Fig. 3; Table 1). Reduced iron was absent from drainage water from AIR mesocosms on all sampling dates.

The concentration of NH₄ in drainage water from LEACH mesocosms increased during and after the antibiotic dosing period, with the highest levels observed on Day 24, and values remained higher than those on Day 0 for

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Table 1. Significant differences in pH, dissolved O₂, SO₄, Fe²⁺, NH₄, and NO₃ in drainage water from unaerated (LEACH) and intermittently aerated (AIR) leachfield mesocosms before (Day 0), during (Day 2–8) and after (Day 11–52) dosing with tetracycline

Parameter	Treatment	Days since start of experiment								
		0	2	4	8	11	15	24	38	52
pH	LEACH	a ¹	a	a	a	a	a	a	a	a
	AIR	a	abcdef	abcdef	abcdef	bcdef	bcdef	bcdef	bcdef	bcdef
DO	LEACH	a	a	a	a	a	a	a	a	a
	AIR	a	a	a	a	a	a	a	a	a
SO ₄	LEACH	abcd	abcd	abcd	acd	abcd	abcd	cd	b	cd
	AIR	edef	bf	acdef	acdef	acdef	acdef	acde	cdef	acde
Fe ²⁺	LEACH	a	a	a	a	a	a	a	a	a
	AIR	a	a	a	a	a	a	a	a	a
NO ₃	LEACH	a	a	a	a	a	a	a	a	a
	AIR	a	acde	bcde	bcde	bcde	abcde	bcde	abcde	abcde
NH ₄	LEACH	ad	a	ad	abcd	abcd	abcd	bcd	abcd	bcd
	AIR	a	a	a	a	a	a	a	a	a

¹Values within a parameter and treatment with the same letter are not significantly different.

265 the duration of the experiment (Fig. 4; Table 1). Dosing with TET had no significant effect on NH₄ concentration in drainage water from AIR lysimeters (Fig. 4; Table 1). In contrast, NO₃ levels increased steadily during the tetra-

cycline dosing period, with values on Days 4 through 24 that were significantly higher than on Day 0. No effects of TET were observed in drainage water from LEACH 270 mesocosms.

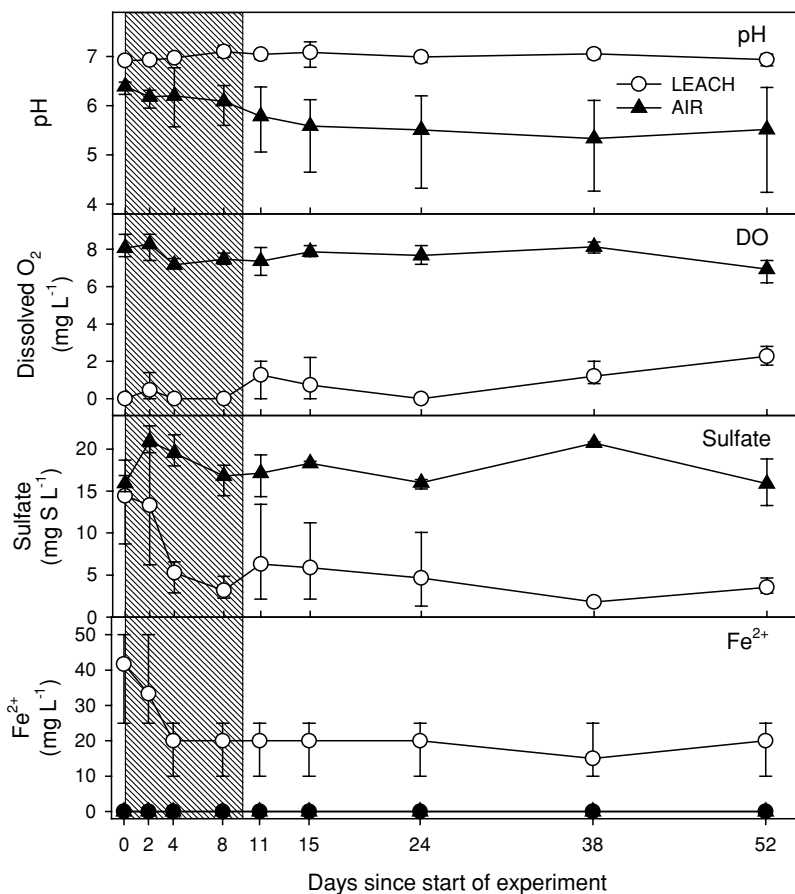


Fig. 3. Mean (n = 3) values of pH, and concentration of dissolved O₂, SO₄, and Fe²⁺ in drainage water from intermittently aerated (AIR) and unaerated (LEACH) mesocosms. Shaded area indicates tetracycline dosing period. Bars indicate range of values.

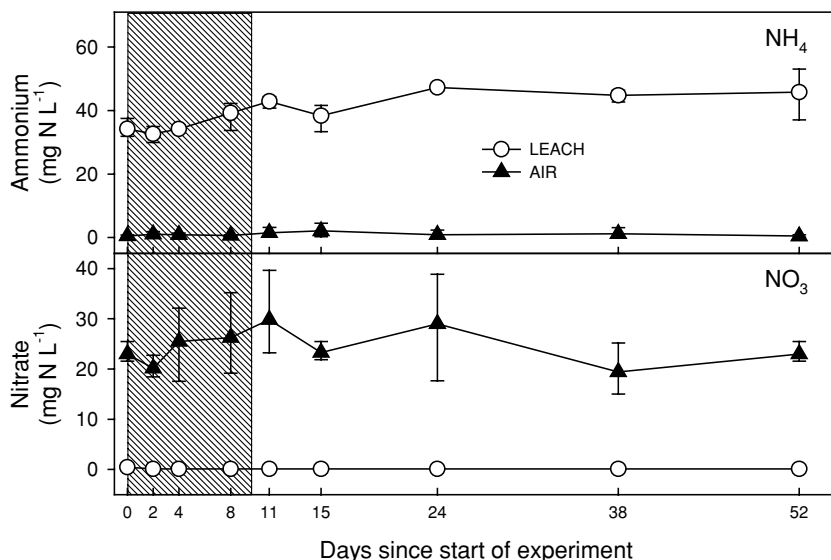


Fig. 4. Mean ($n = 3$) concentration of NH_4 and NO_3 in drainage water from intermittently aerated (AIR) and unaerated (LEACH) mesocosms. Shaded area indicates tetracycline dosing period. Bars indicate range of values.

275 Tetracycline dosing did not have a significant effect on the PO_4 concentration in drainage water from either LEACH or AIR treatments (data not shown). Similarly, no effect of TET addition was observed on levels of DOC in drainage water from either treatment over the course of the experiment (data not shown).

Constituent removal rates

Removal rates for DOC in the AIR treatment ranged from 72.9 to 94.0%, and from -42.2% (net gain) to 28% (net loss) in the LEACH treatment (Fig. 5; Table 2). Significantly

280 lower DOC removal rates were observed on Day 4 and 15 in the LEACH treatment, with values returning to pre-dosing levels by Day 52. Tetracycline had no significant effect on DOC removal in the AIR treatment.

285 Total N removal in LEACH mesocosms ranged from -0.2% (net gain) to 52% (net loss) (Fig. 5; Table 2). The removal rate increased to 25% four days after antibiotic dosing commenced, declining to initial values by the end of the dosing period. A second removal maximum of 52% was observed on Day 24, with TN removal subsequently returning to pre-dosing levels by Day 52.

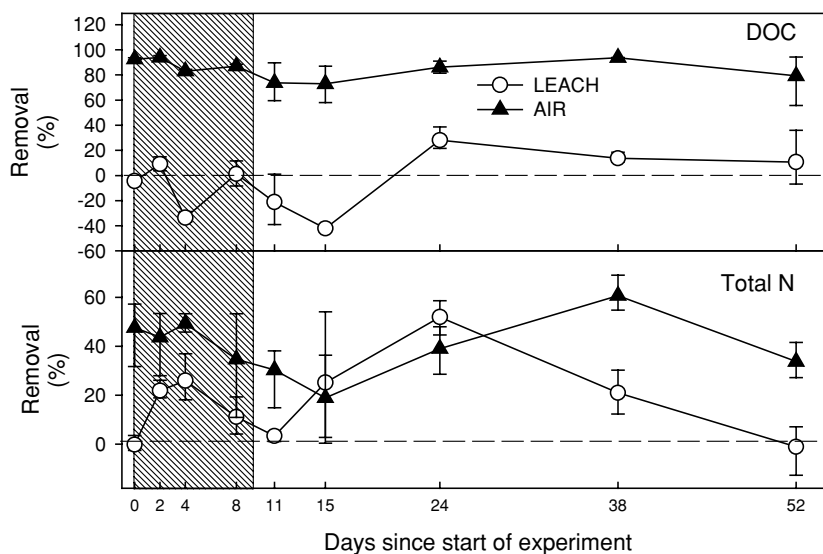


Fig. 5. Mean ($n = 3$) removal rates for dissolved organic C (DOC) and total N (TN) in intermittently aerated (AIR) and unaerated (LEACH) mesocosms. Shaded area indicates tetracycline dosing period. Bars indicate range of values.

Table 2. Significant differences in total N (TN), total P (TP), and dissolved organic C (DOC) removal rates in unaerated (LEACH) and intermittently aerated (AIR) leachfield mesocosms before (Day 0), during (Day 2–8) and after (Day 11–52) dosing with tetracycline.

Parameter	Treatment	Days since start of experiment								
		0	2	4	8	11	15	24	38	52
Total N	LEACH	ac ¹	abc	abc	ac	ac	abc	b	abc	ac
	AIR	a	a	a	a	a	b	ab	b	ab
Total P	LEACH	a	a	a	a	a	a	b	a	a
	AIR	acd	acd	acd	acd	ad	abcd	bc	ad	c
DOC	LEACH	abcdefg	abcdefg	adefg	abcdefg	abcef	abcd	abcdefg	abcdefg	abcdefg
	AIR	a	a	a	a	a	a	a	a	a

¹Values within a parameter and treatment with the same letter are not significantly different.

290 Removal rates for total N ranged from 19.0% to 60.8%
 in the AIR treatment, with maxima observed on Days 4
 (49.3%) and 38 (60.8%), and minima on Day 15 (19.0%)
 (Fig. 5; Table 2). There appeared to be no significant effect
 on TN removal during TET dosing in AIR mesocosms, but
 295 significantly lower removal rates were observed after dosing
 ceased (Fig 5; Table 2).

Total P removal rates in LEACH mesocosms ranged
 from 40.4% to 75.4%, whereas removal rates in the AIR
 mesocosms ranged from 49.2% to 73.2% (data not shown).
 300 Removal of TP in LEACH and AIR treatments was
 not significantly affected during the tetracycline dosing
 period.

Soil properties

305 Tetracycline dosing did not have a significant effect on soil
 moisture content, soil pH, or extractable NO₃, PO₄ or SO₄
 levels in the AIR or LEACH treatments (Table 3). In con-
 trast, levels of extractable NH₄ in soil from the LEACH
 treatment were significantly higher on Days 11 and 52 than
 on Day 0, whereas in the AIR treatment, soil NH₄ levels
 310 were significantly lower than pre-dosing values on both of
 these sampling dates (Table 3).

Discussion

The absence of an effect of TET on DOC removal in ei-
 ther treatment was surprising given that TET is a broad-
 spectrum antibiotic, inhibiting protein synthesis in wide a
 range of G⁺ and G⁻ bacteria. [26] The lack of effect may
 be due to the relatively low concentration of antibiotic
 (5 mg L⁻¹) used in this study. In a companion experiment
 Atoyan et al. [18] determined that the concentration of TET
 necessary to inhibit the growth of *E. coli* in soil was 400
 320 mg L⁻¹ for both AIR and LEACH treatments. Further-
 more, periodic input of STE to the mesocosms introduces
 a new population of microorganisms capable of organic C
 mineralization, which can negate the inhibitory effects of
 325 tetracycline on those members of the microbial community
 that are sensitive to low levels of the antibiotic.

Transient increases in the level of CH₄ in the headspace of
 LEACH mesocosms may have resulted from the enhanced
 activity of methanogenesis and/or inhibition of methane
 consumption caused by TET addition.
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In a study conducted by Schnell and King [27] methane
 oxidation in a forest soil was rapidly inhibited by the ad-
 dition of chlortetracycline. Similarly, tetracycline inhibited
 consumption of methyl bromide—a structural analog of
 methane—by 45% in a forest soil at a concentration of

Table 3. Mean (n = 3) values of moisture, pH, and concentration of extractable SO₄, NH₄, NO₃, and PO₄ in soil from LEACH and AIR mesocosms

Treatment	Day	Moisture (g g ⁻¹)	pH	SO ₄ (μg S g ⁻¹)	NH ₄ (μg N g ⁻¹)	NO ₃ (μg N g ⁻¹)	PO ₄ (μg P g ⁻¹)
LEACH	0	0.28 (0.04)	7.26 (0.17)	9.8 ± 0.6	61.4 (1.5)	0.2 (0.1)	69.5 (11.1)
	11	0.25 (0.06)	7.27 (0.04)	13.2 ± 6.7	77.3* (4.8)	0.7 (0.4)	64.2 (13.2)
	52	0.24 (0.01)	7.40 (0.07)	8.7 ± 3.5	75.8* (4.5)	0.4 (0.1)	36.1 (35.9)
AIR	0	0.24 (0.03)	5.85 (0.08)	25.4 ± 20.7	13.9 (4.3)	16.9 (5.0)	107.3 (14.0)
	11	0.25 (0.02)	5.86 (0.61)	10.7 ± 3.0	2.4* (2.9)	30.7 (24.8)	111.5 (21.8)
	52	0.30 (0.05)	5.99 (0.56)	10.7 ± 2.4	1.4* (1.1)	28.6 (10.4)	101.6 (44.9)

Samples were taken prior to the first dose of tetracycline (Day 0), immediately after the last dose of tetracycline (Day 11), and after a period of system recovery (Day 52). Values in parentheses represent one standard deviation.

*Indicates significant difference from Day 0.

335 25 mg TET L⁻¹.^[28] Elevated methane levels may also be
 the result of enhanced methanogenesis due to reduced com-
 petition for C substrates. For example, Lovely and Phillips
 [29] observed that methanogens and sulfate-reducing bacte-
 340 ria compete with iron-reducing bacteria for electron donors
 such as acetate. We observed a decrease in Fe²⁺ concentra-
 tions during the tetracycline dosing period (Fig. 3), as well
 as increase levels of CH₄ and H₂S over the same period
 (Fig. 2), suggesting that methanogens and sulfate-reducing
 345 bacteria may have been able to out-compete iron-reducing
 bacteria due to inhibition by tetracycline.

Thiele-Bruhn^[30] found that TET at concentrations of
 3 to 231 mg kg⁻¹ soil inhibited Fe reduction in different
 soils. Atoyán et al.^[18] estimated the concentration of TET
 adsorbed in the soil of mesocosms to be about 38 mg kg⁻¹,
 350 within the range of inhibitory values reported by Thiele-
 Bruhn.^[30] The effects of TET on sulfur transformations are
 less clear. Levels of SO₄ decreased significantly during TET
 dosing and remained low until the end of the experiment,
 suggesting that sulfate reduction may have been enhanced
 355 (Fig. 3). However, levels of H₂S during TET dosing did not
 rise above initial values, and declined to below detection
 limits by Day 15 (Fig. 2), suggesting that enhanced sulfate
 reduction may not be involved.

The effects of tetracycline on methane levels in LEACH
 360 mesocosms appear to require regular inputs of the antibi-
 otic, since these declined to pre-dosing levels after dosing
 stopped. In contrast, the inhibitory effects of TET on SO₄
 and Fe²⁺ levels were apparent weeks after dosing ceased.
 Differences in duration of effects suggest that if suppres-
 365 sion of competing populations was responsible for elevated
 CH₄ levels, it was not the only mechanism in play. Inhibi-
 tion of methane oxidizers by TET may be more important
 to methane dynamics than suppression of competition by
 sulfate and iron-reducing bacteria.

370 Net nitrogen removal in AIR mesocosms most likely
 takes place via denitrification.^[13,31] During aeration, NH₄
 in the soil is oxidized to NO₃ and denitrified to N₂O and
 N₂^[31] when oxygen-depleted and organic C-rich STE in-
 puts come in contact with the soil. Nitrogen removal rates
 375 in AIR mesocosms decreased significantly between Day 0
 and 15, coincident with tetracycline dosing. Nitrate levels
 in drainage water also increased during this period, and
 soil NO₃ levels were significantly higher on Days 11 and
 52 than on Day 0, suggesting that inhibitory effects on
 380 denitrification – rather than nitrification – were likely re-
 sponsible for lower rates of N removal during TET dos-
 ing. Halling-Sorensen^[10] found that the effective concen-
 tration for inhibition of 50% the nitrification activity of a
 pure culture of *Nitrosomonas europaea* was 4 mg L⁻¹, close
 385 to the concentration employed in our experiment. The ab-
 sence of an effect of TET on nitrate levels in our study may
 be ascribed to nitrification by antibiotic-resistant nitrifying
 species and/or adsorption of the antibiotic to soil partic-
 390 les, which could reduce its effectiveness. Inhibitory effects
 of TET on denitrification have been reported previously.

For example, Borriello et al.^[32] found that the denitrifier
Pseudomonas aeruginosa was more susceptible to six antibi-
 otics, including TET, under aerobic than anaerobic condi-
 tions. This differential effect of the antibiotic is likely due to
 the fact that the antibacterial effects of TET are through in-
 395 hibition of protein synthesis, which prevents growth.^[26] In-
 termittent aeration is thought to promote bacterial growth
 in the mesocosms,^[33] which may make some processes more
 susceptible to the effects of TET. Increases in TN removal
 400 rate between Day 15 and 38 to pre-dosing levels suggests
 recovery of denitrification, likely due to the periodic input
 of denitrifiers in STE.

In contrast to the inhibitory effects of TET on N re-
 moval observed in AIR mesocosms, N removal rates in
 the LEACH treatment increased from nearly 0% on Day 0
 405 to a maximum of 26.0% on Day 4. This effect of TET
 was unexpected because TN removal is minimal under
 the anaerobic conditions characteristic of conventional
 leachfields^[19] and was very low in LEACH mesocosms in
 previous experiments.^[13] Nitrate levels in drainage water
 410 from LEACH mesocosms remained below detection limit
 for the duration of the experiment, suggesting that denitri-
 fication was not involved. Enhanced anaerobic NH₄ oxida-
 tion, referred to as anammox, may explain increases in N
 removal in LEACH mesocosms. This process, carried out
 415 by bacteria belonging to the group planctomycetes, results
 in the conversion of NH₄ and nitrite to dinitrogen gas.^[34]
 The anammox process has been shown to be involved in
 N removal in wastewater treatment.^[35] Alternatively, pro-
 420 duction of N₂O by ammonia-oxidizing bacteria under low
 O₂ tensions^[36] may account for N removal in LEACH meso-
 cosms. Preliminary analysis of the soil bacterial community
 of LEACH mesocosms based on cloning and sequencing of
 16S rRNA gene fragments indicates the presence of vari-
 425 ous ammonia-oxidizing bacteria (data not shown). How-
 ever, our data do not allow us to explain how TET could
 enhance N removal via either anammox or ammonia oxida-
 tion.

The lack of response of TP removal to tetracycline addi-
 tions in AIR and LEACH treatments was expected. Phos-
 430 phorus removal from STE is largely an abiotic process that
 involves the reaction of phosphate with aluminum and iron
 oxides under acidic conditions^[19,37] or calcium under al-
 kaline conditions,^[19] forming insoluble complexes in soil.

435 Conclusions

The effects of TET in leachfield mesocosms appear to be
 a function of aeration and of the particular processes in-
 volved. For example, removal of DOC and total P were un-
 affected by antibiotic dosing in both aerated and unaerated
 440 treatments, suggesting that there is little interaction among
 the antibiotic, the processes responsible for removal, and
 level of aeration. Thus, these water quality functions ap-
 pear unlikely to be affected by inputs of TET in soil-based
 treatment systems.

445 In contrast, levels of methane increased temporarily in
 response to TET under unaerated conditions, whereas Fe²⁺
 and sulfate levels were reduced well beyond the antibiotic
 addition period. No such effects were observed in aerated
 mesocosms. In addition, TET had contrasting effects on
 450 nitrogen removal, with transient enhancement observed in
 unaerated mesocosms and transient decreases in aerated
 mesocosms. These results indicate that the effects of TET
 on the microorganisms that control the concentration of
 certain gases and solutes depend on the availability of O₂.
 455 Our results suggest that if TET is introduced for a period
 of 10 days at a dosage rate equivalent to two adults tak-
 ing 1,000 mg d⁻¹, its positive and negative effects on water
 quality will be limited mainly to the dosing period. Fur-
 thermore, despite the negative effects of TET on N removal
 460 under aerated conditions, the resulting drainage water has
 a lower N concentration than observed in a conventional
 leachfield.^[19] Effects beyond the antibiotic dosing period—
 observed for Fe²⁺ and SO₄—may dissipate with contin-
 ued additional inputs of wastewater, which introduce a new
 465 population of microorganisms capable of anaerobic and/or
 aerobic metabolism with every dose. Long-term effects may
 also be dictated by the fate of TET in leachfield soil. Tetracy-
 cline is subject to abiotic degradation, and some of the prod-
 ucts have bactericidal properties.^[38,39] Tetracycline also ad-
 470 sorbs to soil^[40] and the sorbed antibiotic may retain some
 of its antibacterial activity.^[17] Thus, TET and/or its degra-
 dation products may have the potential to affect susceptible
 microorganisms long after inputs have ceased.

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