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WASTE MANAGEMENT

Mechanisms of Ammonium Transformation and Loss in Intermittently Aerated Leachfield Soil

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Abstract

Optimization of N removal in soil-based wastewater treatment systems requires an understanding of the microbial processes involved in N transformations. We examined the fate of $^{15}NH_4^+$ in intermittently aerated leachfield mesocosms over a 24-h period. Septic tank effluent (STE) was amended with $^{15}NH_4Cl$ to help determine N speciation and distribution in drainage water, soil, and headspace gases. Our results show that 5.7% of the ^{15}N was found in soil, 10.0% in drainage water, and 84.3% in the gas pool. Ammonium accounted for 41.7% of the soil ^{15}N pool, followed by NO_3^- (29.2%), organic N (21.7%), and microbial biomass N (7.5%). In drainage water, NO_3^- constituted ~80% of the ^{15}N pool, whereas NH_4^+ was absent from this pool. Nitrous oxide was the dominant form of ^{15}N in the gas phase 6 h after addition of $^{15}NH_4^+$–amended STE to the mesocosms, after which its mass declined exponentially; by contrast, the mass of $^{15}N_2$ was initially low but increased linearly with time to become the dominant form of ^{15}N after 24 h. Analysis based on the isotopic enrichment of $^{15}N_2O$ and $^{15}N_2$ indicates that nitrification contributed 98.8 and 23.1% of the $^{15}N_2O$ flux after 6 and 24 h, respectively. Our results show that gaseous losses are the main mechanism for NH_4^+ removal from wastewater in intermittently aerated soil. In addition, nitrification, which is generally not considered a significant pathway for N loss in soil-based wastewater treatment, is an important source process for N_2O.

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Conventional onsite wastewater treatment systems (OWTS), particularly those that are failing hydraulically or otherwise functioning improperly, have the potential to degrade ground, surface, and coastal water quality. Excess nitrogen inputs from OWTS to groundwater can be detrimental to human health and can cause eutrophication and hypoxia in freshwater and coastal marine ecosystems (Oakley et al., 2010). These systems are designed with the dual goals of pathogen removal and water dispersal below ground in a soil absorption trench (also known as a drainfield or leachfield). Nitrogen removal from wastewater is not the primary function of conventional OWTS, and removal rates in the soil vary from 0 to 30% (USEPA, 2002). Onsite wastewater treatment systems are ubiquitous in unsewered rural and suburban areas of the United States and are found in watersheds sensitive to N pollution. Thus, identifying the mechanism(s) of N removal is an important aspect of soil-based wastewater treatment to improve this function.

The biogeochemical processes by which N is removed in conventional OWTS and the microorganisms involved have not been identified. In the absence of direct mechanistic information, N removal in conventional OWTS is generally attributed to denitrification in the soil below the infiltrative surface (Crites and Tchobanoglous, 1998), which produces gaseous forms of N that are readily lost to the atmosphere. The process involves the sequential reduction of NO_3^- to N_2O and N_2 by heterotrophic microorganisms under anoxic conditions:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO\,(g) \rightarrow N_2O\,(g) \rightarrow N_2\,(g)$$

Denitrification requires NO_3^-, which is not present in septic tank effluent (STE). Production of NO_3^- involves sequential oxidation of NH_4^+, the main form of N present in STE, to NO_3^-, which can be performed by a group of autotrophic bacteria and archaea (Taylor et al., 2012) under oxic conditions (Fig. 1).

Soil in the leachfield undergoes periods of anoxia (resulting from inputs of anoxic STE) followed by periods of limited O_2 availability after infiltration and percolation of STE, conditions that may support NH_4^+ oxidation to NO_3^-, providing the requisite electron acceptor for denitrifiers once anoxic conditions are

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Abbreviations: BOD_5, 5-day biochemical oxygen demand; OWTS, onsite wastewater treatment system; STE, septic tank effluent; TN, total nitrogen.

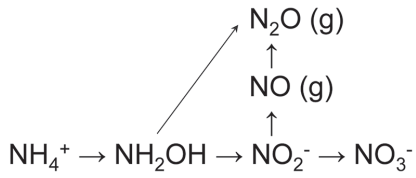

Fig. 1. Pathways for production of N_2O via nitrification.

re-established. Nitrification can also produce increasing amounts of N_2O as the availability of O_2 decreases (Fig. 1) (Bollmann and Conrad, 1998). As such, N_2O production by nitrification may be an important process for net removal of N in soil absorption systems. Nevertheless, studies that measure N_2O production in the leachfield of conventional systems attribute it exclusively to denitrification (e.g., Degen et al., 1991; Hagedorn and Reneau, 1994). In a conventional leachfield, the frequency and duration of oxic and anoxic periods can vary widely, depending on daily, weekly, and seasonal patterns of water use. Temporal differences in water use patterns and wastewater properties may translate into variable N removal rates in leachfield soil, affecting the ability of OWTS to protect groundwater.

The sequence of events leading to N loss in the leachfield of a conventional OWTS has been inferred from the constraints imposed by the microbial processes likely to be involved and by measured changes in the chemical composition of STE as it moves through the soil (e.g., Wilhelm et al., 1994; McCray et al., 2005). Assuming this sequence is correct, a better understanding of the specific mechanisms involved may help to optimize system conditions to improve N removal.

In previous studies we have shown that intermittent aeration of leachfield mesocosms receiving domestic STE have N removal rates ranging from 25 to 75%, depending on the soil type and dosing rates (Potts et al., 2004; Amador et al., 2007). More recently we have shown that N removal rates similar to those in the laboratory are observed in conventional OWTS under field conditions when intermittent aeration is used (Amador et al., 2010). We have hypothesized that intermittent aeration enhances N removal by promoting nitrification (during the aeration phase), followed by denitrification (as STE infiltrates and percolates through the soil), with net N losses attributed to N_2 and N_2O produced by denitrification (Potts et al., 2004). In the present study, we used $^{15}NH_4^+$–amended STE to test this hypothesis by tracking the transformations and fate of ^{15}N within intermittently aerated leachfield mesocosms.

Materials and Methods

Experimental Facility

The study was conducted at a wastewater research facility in Westbrook, Connecticut consisting of a two-family home that housed three to six people and a pilot-scale laboratory adjacent to the house. The house had a conventional septic system built in 1996, with a septic tank capacity of 4733 L. Septic tank effluent was pumped to a climate-controlled room (17–19°C) above the laboratory and stored in a 1325-L high-density polyethylene tank with a residence time of approximately 2 d. Septic tank effluent in the storage tank was continuously mixed and evenly distributed to a series of high-density polyethylene dosing tanks (30.5-cm i.d.; 45.7 cm high) in the laboratory (Fig. 2). Approximately 2.8 L of STE was retained within the dosing tanks and gravity-fed to a series of sealed stainless steel mesocosms (36.0-cm i.d.; 61.0 cm high) filled with soil and sand to represent a leachfield. The mesocosms were dosed with STE every 6 h at a rate of 12 cm d^{-1}. The frequency and volume of dosing was computer-controlled, as was the timing and frequency of aeration. Intermittent aeration of the soil was representative of SoilAir technology (Potts, 2000). Air was pumped for 30 min into the headspace of the mesocosms using a blower to maintain ambient O_2 levels (0.20–0.21 mol mol^{-1}), followed by a 60-min period with the blower off. Levels of O_2 in the headspace of the mesocosms were checked using a portable soil gas monitor (SoilAir Technology).

Mesocosms were filled with 7.5 cm of no. 4 silica sand (diam. 1.40 mm; uniformity coefficient <1.8) (U.S. Silica Co.) overlain by a mixture of soil (30-cm deep) from the B and C horizons of a moderately well drained Hinckley sandy loam (Typic Udorthent) from Kingston, Rhode Island. The soil is typical of that used for leachfield construction in Rhode Island and had a pH of 5.6, an organic matter content of 0.25 g kg^{-1}, and a particle size distribution of 92% sand and 8% silt. Three replicate mesocosms were used in our experiment.

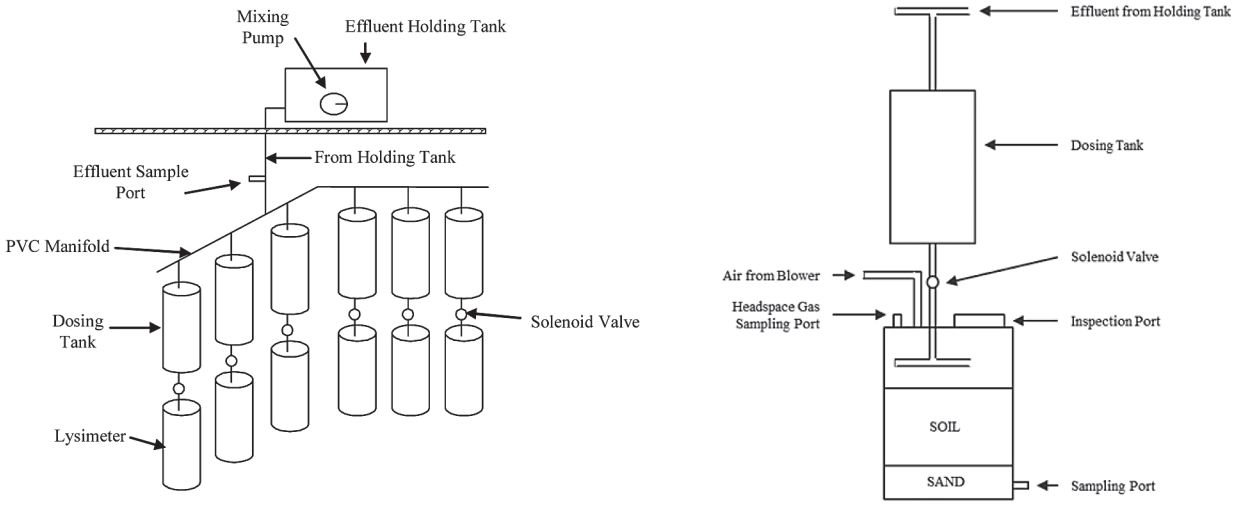

Fig. 2. Schematic diagram of laboratory facility (left) and mesocosms (right) used in this study. Drawings are not to scale (after Patenaude et al., 2008).

Operational History

The ^{15}N tracer addition experiment was conducted in the context of a long-term experiment on the effects of aeration on leachfield mesocosms. Over the course of this experiment the mesocosms had an average N removal rate of >50% (Amador et al., 2007). The STE dosing rate was 4 cm d^{-1} (1 gal. ft^{-2} d^{-1}) from 13 Aug. 2003 until 22 June 2004, after which it was increased to 12 cm d^{-1} (3 gal. ft^{-2} d^{-1}) to optimize conditions for N removal (Amador et al., 2007) and remained at this rate during the ^{15}N tracer experiment, which was conducted on 13 and 14 Oct. 2004.

^{15}N Tracer Addition

^{15}N-ammonium (as $^{15}NH_4Cl$, ~98+ At.%) (Isotec Chemical Co.) was mixed in a plastic carboy with 22.9 L of STE to a final concentration of 1 mg ^{15}N L^{-1} (~3.8 mg $^{15}NH_4Cl$ L^{-1}) and added to the mesocosms at 9:00 AM on 13 Oct. 2004. Automated dosing was suspended, and mesocosms were dosed manually once with 2.8 L of ^{15}N-amended effluent. The automatic dosing schedule resumed after the manual dosing event, with the next dose of STE applied to the mesocosms 6 h after the manual dose.

Sampling

Drainage water from the mesocosms was collected in covered plastic containers kept on ice during collection. After collection for 24 h, the containers holding the water samples were weighed to determine the recovered volume, and subsamples were frozen and stored for subsequent analysis. A portion of the drainage water was filtered through a glass fiber filter (GF/F, Millipore Corp.) and the filtrate was frozen for subsequent analysis.

A gas-tight, 50-mL syringe was connected to the headspace sampling port fitted with a rubber septum (Fig. 2) and was used to mix headspace gases by repeated pumping for 1 min. After mixing, a 12-mL sample of headspace gases was removed every 6 h just before a dosing event and stored in pre-evacuated vials (Labco Ltd.). The temperature in the headspace was measured before sampling using an electronic thermometer.

Soil samples were taken at the end of the 24-h water collection period. A slide-hammer fitted with a surface-sterilized (70% methanol) plastic sleeve (2.54-cm diam., 35-cm long) was used to obtain two 30-cm-deep cores from each mesocosm. Soil from both cores was mixed to make one composite sample per mesocosm. Composited soil samples were placed in sterile Whirl-Pak bags and stored at 4°C until analyzed.

Analyses

Soil and water samples were processed within 24 h of collection. The concentration of NH_4^+ and NO_3^- in STE, mesocosm drainage water, soil extracts, and extracts for soil microbial biomass N determination was measured colorimetrically by the Cd reduction method using an automated nutrient analyzer (Flow Solution IV, Alpkem Corp.). The total N (TN) concentration in STE and drainage water was determined using unfiltered samples that were oxidized using the persulfate digestion method (APHA, 1998). The pH of water samples was determined using a combination pH probe and a UB-10 pH meter (Denver Instruments).

Gas samples were analyzed for N_2O and N_2 using a purge-and-trap gas sampling system with a cryogenic column immersed in liquid nitrogen connected to a Hewlett-Packard Model 5890A gas chromatograph equipped with a 2-m Porapak Q column and electron capture detector at 350°C (for N_2O analysis) and a 2-m calcium chabazite column with a thermal conductivity detector at 20°C (for N_2 analysis) (An and Joye, 1997).

The method of Keeney and Nelson (1982) was used to extract NH_4^+ and NO_3^- from soil, and the extracts analyzed as described above. Soil microbial biomass N was determined using the fumigation-extraction method (Voroney and Paul, 1984), the extracts were filtered and oxidized by alkaline persulfate oxidation (Cabrera and Beare, 1993), and the NO_3^- concentration in the filtrate was determined as described above.

Soil samples to be analyzed for total N and ^{15}N enrichment were dried overnight at 105°C, ground with a mortar and pestle, weighed, and compacted into tins (5 mm diam. × 9 mm). Prepared samples were sent to the Stable Isotope Facility, University of California-Davis for ^{15}N analysis.

Diffusion of ^{15}N in Water and Soil Extracts

Dissolved ions ($^{15}NH_4^+$ and $^{15}NO_3^-$) in water samples and soil extracts were diffused using the acidified disk diffusion method (Khan et al., 1998). Samples for $^{15}NH_4^+$ analysis were amended with MgO to release the $^{15}NH_4^+$ as $^{15}NH_3$. Samples for $^{15}NH_4^+$ and $^{15}NO_3^-$ analysis were amended with Devarda's alloy to reduce the $^{15}NO_3^-$ to $^{15}NH_4^+$ and then with MgO to release the $^{15}NH_4^+$ as $^{15}NH_3$. The solution in the diffusion units was heated to 50°C on a griddle placed at a 1° angle (Khan et al., 1998). To maximize recovery, the solution heating time was adjusted based on the sample volume and mass of N in the sample. The resulting $^{15}NH_3$ was collected on an acidified (0.2 mol L^{-1} sulfamic acid solution) filter paper suspended within the diffusion unit. The diffusates were dried in a vacuum desiccator, packaged into tins (5-mm-diameter × 9-mm), and sent to the Stable Isotope Facility, University of California-Davis for ^{15}N analysis. For analysis of total ^{15}N in water samples, samples were digested using the persulfate method (APHA, 1998), which oxidizes organic N to NO_3^-, and diffused as described above.

^{15}N Analysis of Diffusates, Gas, and Soil Samples

All analyses of ^{15}N enrichment in water and soil extract diffusates, headspace gas samples, and soil samples were performed at the Stable Isotope Facility, University of California-Davis. Total N content of soil and ^{15}N enrichment of water and soil diffusates and soil samples was determined using continuous flow isotope ratio mass spectrometry (PDZEuropa) after sample combustion and conversion to N_2 at 1000°C using an on-line elemental analyzer (PDZEuropa ANCA-GSL) as described by Mulvaney (1993).

Concentrations of N_2O and N_2, as well as isotopic enrichment of $^{15}N_2$ and $^{15}N_2O$ gas samples, were determined on a dual inlet isotope ratio mass spectrometer (PDZ Europa 20–20 IRMS) after gas chromatography (PDZEuropa TGII trace gas analyzer) to separate N_2 and N_2O as described by Mosier and Schimel (1993).

^{15}N Calculations

The measured mass of ^{15}N added to each mesocosm was 2700 μg ^{15}N, and the measured natural abundance ^{15}N enrichment

was subtracted from all values. The isotope effect (fractionation) has been reported to be between 0.0068 and 0.0071 At.% for microbially mediated nitrification and denitrification (Delwiche and Steyn, 1970). Choi and Ro (2003) reported that the upper limit for fractionation of ^{15}N was 0.006 At.% in unsaturated soils and 0.002 At.% in saturated soils. Because the ^{15}N enrichment values in our experiment were considerably higher than reported values for fractionation, it was not considered significant in our experiment.

The recovered ^{15}N in the gas pool was calculated as follows:

$$\text{Mass}^{15}\text{N} = \frac{\text{At.\%}}{100} C_{\text{gas}} V_{\text{gas}} \quad [1]$$

where C_{gas} is the concentration of N (mg L^{-1}) in headspace, and V_{gas} is the headspace volume (L).

The headspace volume in the mesocosms was assumed to remain constant while the blower was off due to dewatering as a result of air flow and pressure during aeration.

The recovered ^{15}N in the water pool was calculated as follows:

$$\text{Mass}^{15}\text{N} = \frac{\text{At.\%}}{100} C_{\text{water}} V_{\text{water}} \quad [2]$$

where C_{water} is the concentration of N (mg L^{-1}) in drainage water, and V_{water} is the volume of drainage water (L) recovered from mesocosm over 24 h.

The recovered ^{15}N in the soil pool was calculated as follows:

$$\text{Mass}^{15}\text{N} = \frac{\text{At.\%}}{100} C_{\text{soil}} M_{\text{soil}} \quad [3]$$

where C_{soil} is the concentration of N (mg N g^{-1}) found in mesocosm soil, and M_{soil} is the mass of soil in mesocosms (g). A measured bulk density of 1.30 g cm^{-3} was used to calculate the mass of soil in the mesocosms.

Statistical Analyses

A one-way repeated measures ANOVA was used to evaluate significant differences among sampling times. Means separation was accomplished using Tukey's test (SPSS, 1995). All statistical tests were evaluated at the $P < 0.05$ confidence level.

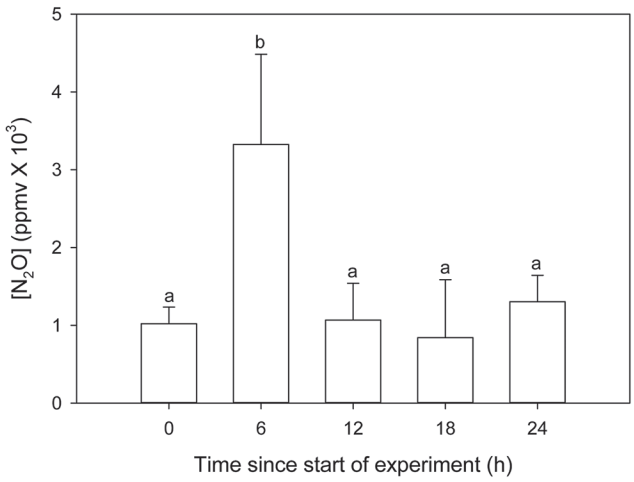

Fig. 3. Mean (n = 3) concentration of N_2O in the headspace of intermittently aerated leachfield mesocosms as a function of time. Bars represent 1 SD. Values with the same letter were not significantly different ($P > 0.05$).

Results

Gas Phase

At the start of the experiment, the concentration of N_2O in the headspace of the mesocosms ranged from 800 to 1300 ppmv (Fig. 3). Six hours after the start of the experiment, there was a statistically significant increase in the concentration of N_2O, which ranged between 2500 and 4800 ppmv, with N_2O levels in the headspace returning to initial values after 12 h. The enrichment of N_2O with ^{15}N was highest (0.90454 excess At.%) after 6 h, decreasing exponentially thereafter (Fig. 4). There were no significant differences in enrichment between 6 and 12 h, whereas values at 18 and 24 h were significantly different

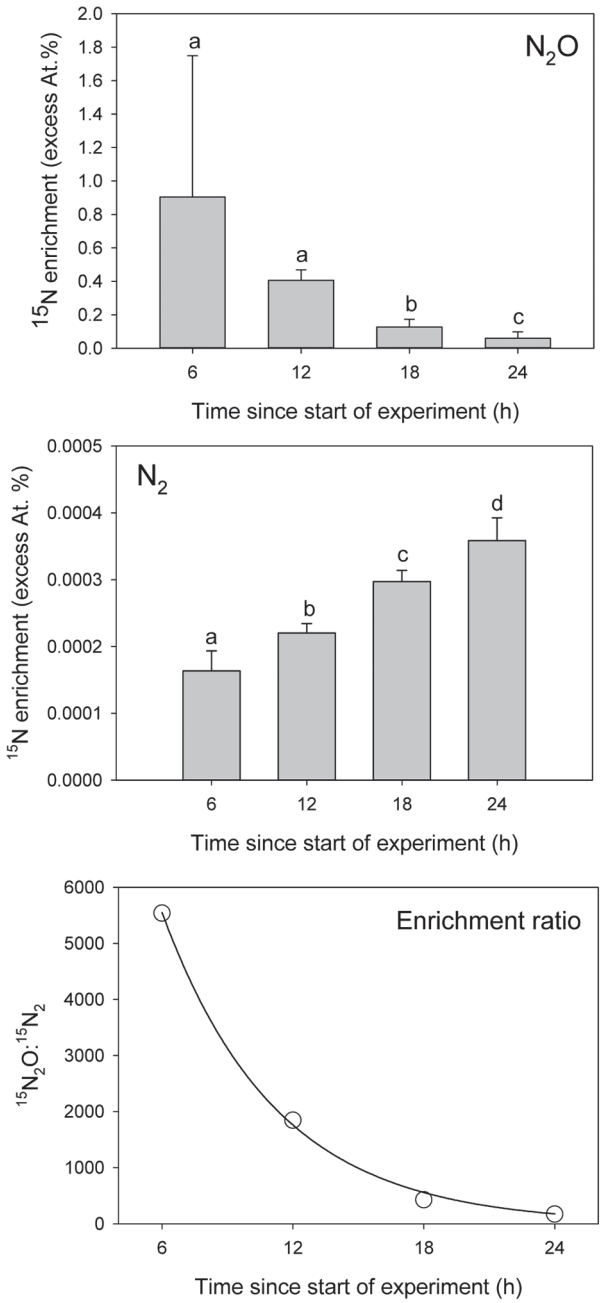

Fig. 4. Mean (n = 3) enrichment of $^{15}N_2O$ and $^{15}N_2$ in the headspace of intermittently aerated leachfield mesocosms and $^{15}N_2O/^{15}N_2$ enrichment ratio as a function of time. Bars represent 1 SD. Values with the same letter were not significantly different ($P > 0.05$).

from those at 6 h. Nitrogen-15 enrichment of the N_2 pool was considerably lower than for N_2O and increased linearly throughout the experiment from 0.000163 excess At.% at 6 h to 0.000359 excess At.% at 24 h (Fig. 4). Significant differences were observed among all sampling times. The ratio of N_2O and N_2 enrichment with ^{15}N decreased exponentially as a function of time (Fig. 4).

The mass of $^{15}N_2O$–N in the headspace was highest at 6 h (~400 μg $^{15}N_2O$–N mesocosm^{-1}), declining exponentially to ~15 μg $^{15}N_2O$–N mesocosm^{-1} after 24 h (Fig. 5). By contrast, the mass of $^{15}N_2$–N in the headspace increased linearly with time from 40 μg $^{15}N_2$–N mesocosm^{-1} after 6 h to a maximum of 80 μg $^{15}N_2$–N mesocosm^{-1} after 24 h. The fraction of ^{15}N gases comprised by N_2O declined linearly with time from a maximum of 92% at 6 h to a low of 15% at 24 h (Fig. 5).

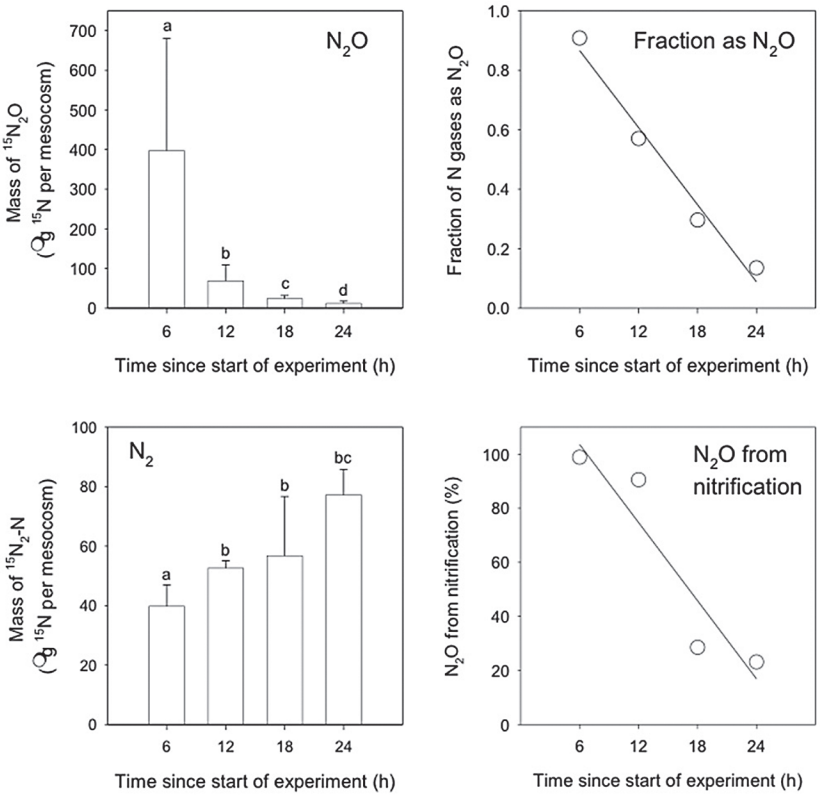

Fig. 5. Mean (n = 3) mass of ^{15}N as N_2O and N_2 in the headspace of intermittently aerated leachfield mesocosms, fraction of ^{15}N gases as N_2O, and mass ratio of $^{15}N_2O$ and $^{15}N_2$ as a function of time. Bars represent 1 SD. Values with the same letter were not significantly different (P > 0.05).

Soil

The soil had a moisture content (mean ± SD) of 20.0 ± 3.0%, a total C concentration of 4.6 ± 0.6 g C kg^{-1}, a TN concentration of 0.3 ± 0.1 g N kg^{-1} (Table 1), and a pH of 5.9 ± 0.8. At the end of the 24-h experimental period, the mass of ^{15}N associated with TN and inorganic N ($^{15}NH_4^+ + {}^{15}NO_3^-$) in soil was 120 and 85 μg ^{15}N mesocosm^{-1}, respectively (Fig. 6). The mass of ^{15}N associated with extractable NH_4^+ and NO_3^- was 50 and 35 μg ^{15}N mesocosm^{-1}, respectively. The amount of ^{15}N in soil microbial biomass was 9 μg ^{15}N mesocosm^{-1}.

Water

Septic tank effluent had a BOD_5 of 200 mg L^{-1}, a pH of 6.85, and a temperature of 17.7°C. The concentration of TN in STE was 24.4 mg L^{-1}, with an NH_4^+ concentration of 19.0 mg L^{-1}, which accounted for 78% of the TN pool (Table 1). Nitrate was not detected in STE. Drainage water from the mesocosms, collected over the 24-h experimental period, had a TN concentration of 20.4 mg L^{-1}, an NH_4^+ concentration of 0.1 mg L^{-1} (<1% of TN), and a nitrate level of 16.5 mg L^{-1} (81% of TN). The organic N pool (3.8 mg L^{-1}) accounted for ~19% of TN. The mass of ^{15}N associated with TN in drainage water was 314 μg ^{15}N per mesocosm, and the inorganic N pool had a ^{15}N mass of 233 μg mesocosm^{-1}, with nearly all of it as NO_3^- (Fig. 6).

Discussion

Of the ~2,700 μg ^{15}N added to each mesocosm, we were able to account for ~1,200 μg (44.4%) (Fig. 7). Of the ^{15}N we could account for, the largest fraction was found in the gas pool (70%), followed by the water (22%) and soil (8%) pools. Because the pH of the drainage water was acidic, gas-phase losses as $^{15}NH_3$ are unlikely. The unaccounted ^{15}N is most likely associated with $^{15}N_2$ and $^{15}N_2O$. For example, drainage water likely contained dissolved $^{15}N_2$ and $^{15}N_2O$, which we did not measure. Additional unmeasured gaseous losses of $^{15}N_2O$ and $^{15}N_2$ likely took place from the headspace of the mesocosms as a result of intermittent aeration events, which removed gases from the headspace and soil. Because we sampled headspace gases every 6 h (just before a dosing event), our results represent only those gases produced over the 60 min between the last aeration event and the next STE dosing event. As such, a portion of the ^{15}N-labeled gases in the headspace was not accounted for in our measurements.

Assuming that all of the unaccounted ^{15}N (~1500 μg per mesocosm) was associated with the gas phase, which is a reasonable assumption given the potential loss pathways, and that our analysis of soil and water captured all of the nongaseous forms of ^{15}N present in these two compartments, we estimate that, 24 h after addition of $^{15}NH_4^+$ to the mesocosms, 5.7% of the added ^{15}N was found in the soil, 10.0% in the drainage water, and 84.3% in the gas phase. The loss of ^{15}N in the gas phase was higher

Table 1. Mean (n = 3) concentration of nitrogen in different pools in septic tank effluent and drainage water and in soil from intermittently aerated leachfield mesocosms.

N pool	Concentration in		
	Septic tank effluent	Drainage water	Soil
	mg N L^{-1}		mg N kg^{-1}
Total N	24.4 (0.7)†	20.4 (2.5)	300 (100)
NH_4^+	19.0 (0.5)	0.1 (0.1)	0.9 (1.0)
NO_3^-	0.0 (0.0)	16.5 (2.4)	4.9 (9.6)
Microbial biomass N	ND‡	ND	23.4 (11)

† Values in parentheses are 1 SD.

‡ Not determined.

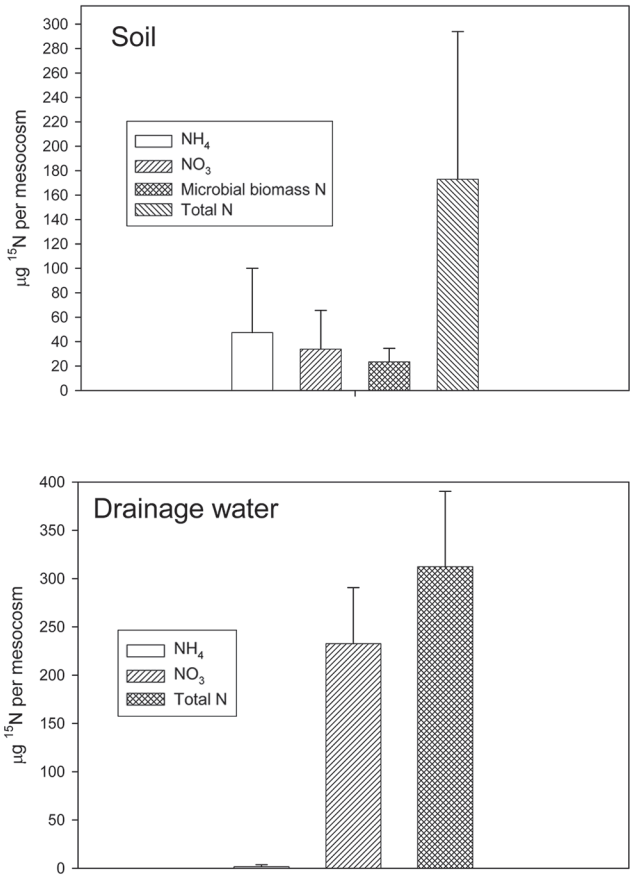

Fig. 6. Mean (n = 3) mass of ^{15}N in soil (as total N, inorganic N, NH_4^+, NO_3^-, and microbial biomass) and drainage water (total N, NH_4^+, and NO_3^-) from intermittently aerated leachfield mesocosms 24 h after dosing with $^{15}NH_4^+$–amended septic tank effluent. Bars represent 1 SD.

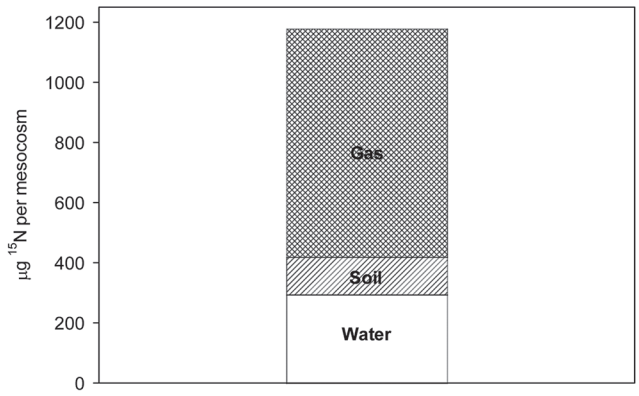

Fig. 7. Distribution of ^{15}N recovered in the water, soil, and gas pools of intermittently aerated leachfield mesocosms 24 h after dosing with $^{15}NH_4^+$–amended septic tank effluent. Values are means (n = 3).

than that calculated from the difference between TN in STE and in drainage water (Table 1) in part because our experiment examined the fate of $^{15}NH_4^+$, whereas STE and drainage water contain both inorganic and organic N forms.

Our results show that microbial uptake is a minor fate for $^{15}NH_4^+$ in intermittently aerated mesocosms, accounting for 0.3% of added ^{15}N and 7.5% of the ^{15}N pool in soil. These results indicate that the main role of the leachfield soil microbial community with respect to N is transformation rather than storage.

The difference between total ^{15}N and the sum of $^{15}NH_4^+$ and $^{15}NO_3^-$ in drainage water represents ^{15}N-labled organic N. This pool of ^{15}N, which accounts for ~20% of the ^{15}N in drainage water, may include nitrogenous organic metabolites excreted by microorganisms and ^{15}N-labeled live and dead microorganisms.

The presence of $^{15}NO_3^-$ in soil and drainage water indicates that nitrification (Fig. 6) is an important pathway for transformation of NH_4^+ in intermittently aerated mesocosms, as demonstrated in previous studies (Potts et al., 2004; Amador et al., 2007; Amador et al., 2008). The total soil inorganic N pool had a NO_3^-/NH_4^+ ratio of 5.4, whereas this ratio was 0.8 for the ^{15}N-labeled pool, suggesting that the extent of the oxidation of $^{15}NH_4^+$ to $^{15}NO_3^-$ was less than for the unlabeled pool within the 24-h duration of our experiment. By contrast, the NO_3^-/NH_4^+ ratio was much closer in drainage water (165 and 199 for the total and ^{15}N-labeled pools, respectively), indicating that the extent of $^{15}NH_4^+$ oxidation to $^{15}NO_3^-$ in water was similar for the labeled and unlabeled pools. Slower rates of NH_4^+ oxidation in soil may account for these differences.

Production of N_2O in soil is associated mainly with nitrification and denitrification, whereas N_2 is produced exclusively via denitrification. The alternating oxic (due to aeration) and anoxic (as a result of STE dosing) conditions in the mesocosms could result in production of N_2O from nitrification (oxic conditions) followed by N_2 and N_2O production by denitrification (anoxic conditions), with the two processes separated in time. Nitrification and denitrification may also have co-occurred in soil microsites (Sexstone et al., 1985). Our data allow us to test hypotheses on the sources of N_2O in our mesocosms. If production of $^{15}N_2$ and $^{15}N_2O$ occurs strictly via sequential nitrification–denitrification, and if $^{15}N_2O$ is produced only by denitrification, we would expect (i) the concentrations of $^{15}N_2O$ and $^{15}N_2$ to increase with time as the concentration of $^{15}NO_3^-$ from oxidation of $^{15}NH_4^+$ increases and (ii) the ratio of ^{15}N enrichment of N_2O and N_2 to remain constant throughout the experiment because both gases would be formed from the same pool of $^{15}NO_3^-$. Our results do not support the hypothesis that denitrification is solely responsible for production of nitrogenous gases. Although the mass and enrichment of $^{15}N_2$ increased linearly with time, the mass of $^{15}N_2O$ and its enrichment declined exponentially with time (Fig. 3). Consequently, rather than remaining constant, the mass and enrichment ratio of $^{15}N_2O$:$^{15}N_2$ declined over time (Fig. 4). Although sequential nitrification and denitrification are clearly taking place, as evidenced by production of $^{15}N_2$ from added $^{15}NH_4^+$, our results suggest that $^{15}N_2O$ production results both from denitrification and nitrification.

We estimated the relative contribution of nitrification and denitrification to N_2O production using the approach described by Khalil et al. (2004). This approach assumes that N_2 emitted by the soil has the same isotopic composition as the N_2O and that the ratio of N_2O to N_2 produced by denitrification is constant under the same conditions. We used an N_2O:N_2 ratio of 0.12 for our calculations based on the results of a separate experiment in which we measured $^{15}N_2$ and $^{15}N_2O$ production from anaerobic mesocosms receiving STE amended with $^{15}NH_4^+$ (data not shown). Based on this analysis, the contribution of nitrification to the N_2O pool was highest at 6 h (98.8%), declining to 23.1%

at 24 h (Fig. 5). Our results are in agreement with studies that show that the contribution of nitrification to the flux of N_2O depends on O_2 availability (Bollmann and Conrad, 1998). The alternating oxic and anoxic conditions in our mesocosms support nitrification and denitrification, both of which contribute to N_2O production, and to gaseous losses of N from these systems.

The relative importance of nitrification and denitrification to N_2O production, and thus net removal of N from wastewater, has implications for the design and management of soil-based wastewater treatment systems. Production of N_2O by nitrification requires NH_4^+ (the dominant species of N in STE) as an electron donor, does not require organic C, and proceeds under limited O_2 availability. By contrast, production of N_2O via denitrification requires NO_3^- as an electron acceptor, organic C as an electron donor, and anoxic conditions. Thus, soil-based wastewater treatment systems that provide oxic and anoxic conditions sequentially in the same soil volume may be more effectiveat removing N in the gas form than systems that provide only oxic or anoxic conditions. Optimizing wastewater and drainfield conditions for maximum gaseous N losses via nitrification and denitrification for soils with differing texture and structure by regulating the amount and timing of O_2 availability and the timing of anoxic conditions and organic carbon sources should help to ameliorate N pollution of ground, surface and coastal waters by OWTS.

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