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MOVING DENITRIFYING BIOREACTORS BEYOND PROOF OF CONCEPT

Fecal Bacteria, Bacteriophage, and Nutrient Reductions in a Full-Scale Denitrifying Woodchip Bioreactor

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Abstract

Denitrifying bioreactors using woodchips or other slow-release carbon sources can be an effective method for removing nitrate (NO_3^-) from wastewater and tile drainage. However, the ability of these systems to remove fecal microbes from wastewater has been largely uninvestigated. In this study, reductions in fecal indicator bacteria (*Escherichia coli*) and viruses (F-specific RNA bacteriophage [FRNA phage]) were analyzed by monthly sampling along a longitudinal transect within a full-scale denitrifying woodchip bioreactor receiving secondary-treated septic tank effluent. Nitrogen, phosphorus, 5-d carbonaceous biochemical oxygen demand ($CBOD_5$), and total suspended solids (TSS) reduction were also assessed. The bioreactor demonstrated consistent and substantial reduction of *E. coli* (2.9 $\log_{10}$ reduction) and FRNA phage (3.9 $\log_{10}$ reduction) despite receiving highly fluctuating inflow concentrations [up to 3.5×10^5 MPN (100 mL)$^{-1}$ and 1.1×10^5 plaque-forming units (100 mL)$^{-1}$, respectively]. Most of the removal of fecal microbial contaminants occurred within the first meter of the system (1.4 $\log_{10}$ reduction for *E. coli*; 1.8 $\log_{10}$ reduction for FRNA phage). The system was also efficient at removing NO_3^- (>99.9% reduction) and TSS (89% reduction). There was no evidence of consistent removal of ammonium, organic nitrogen, or phosphorus. Leaching of $CBOD_5$ occurred during initial operation but decreased and stabilized at lower values (14 g O_2 m^{-3}) after 9 mo. We present strong evidence for reliable microbial contaminant removal in denitrifying bioreactors, demonstrating their broader versatility for wastewater treatment. Research on the removal mechanisms of microbial contaminants in these systems, together with the assessment of longevity of removal, is warranted.

Core Ideas

- Denitrifying bioreactors are a technology for nitrate removal from wastewater.
- We show a full-scale bioreactor can also remove fecal bacteria and viruses.
- Fecal bacteria and viruses were reduced by >2.9 $\log_{10}$.
- Median effluent concentration of *E. coli* was 20 MPN (100 mL)$^{-1}$.
- Median effluent concentration of F-specific RNA phage was 3 PFU (100 mL)$^{-1}$.

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Extensive research has shown that denitrifying bioreactors can be an effective, low-cost, and simple technology for reducing nitrogen (N) from septic tank effluent and drainage water (Robertson et al., 2005; Robertson et al., 2008; Schipper et al., 2010a; Christianson et al., 2012). They generally comprise beds, walls, or layers (Schipper et al., 2010b) of porous, carbon-rich media (commonly woodchips) through which nitrified effluent or agricultural drainage water is passed. During passage through the carbon-rich media, nitrate (NO_3^-) is converted into nitrogen gas (N_2) by microbial denitrification (Robertson, 2000; Greenan et al., 2006; Gibert et al., 2008; Schipper et al., 2010b). In a comparative study, Oakley et al. (2010) concluded that denitrifying bioreactors, preceded by a sand filter, performed better than any other onsite wastewater treatment technology in reducing N loads. To date these systems have been designed to target a single contaminant—NO_3^-—but their efficacy in removing other wastewater contaminants such as fecal microbes has been largely uninvestigated.

Removal of microbial contaminants from septic tank effluent and tile drainage is important from a health perspective because the disposal of poorly treated septic tank effluent or tile drainage can result in the potential transmission of infectious disease via waterborne pathogenic microorganisms (Graun, 1985; Gerba and Smith, 2005; Asano et al., 2007). Elevated concentrations of fecal bacteria and viruses have been detected in surface and groundwater located downstream of septic tanks, animal feeding operations, and land receiving animal waste application (Viraraghavan, 1978; Charles et al., 2003; Soupir et al., 2006; Sapkota et al., 2007). Because drinking and irrigation water is frequently sourced from waterbodies that receive upstream inputs of human or animal waste, these elevated concentrations present a serious public health concern. Therefore, there is a widespread need for appropriate on-site technologies that can reduce the risk of fecal pathogen contamination.

The ability of bioreactors to reduce microbial contaminants has been briefly assessed by Robertson et al. (2005) and Tanner et al. (2012), who reported 0.2 to 1.9 $\log_{10}$ reductions in *E. coli* with passage through a denitrifying bioreactor. This indicated

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Abbreviations: $CBOD_5$, 5-d carbonaceous biochemical oxygen demand; FRNA phage, F-specific RNA bacteriophage; HSSF, horizontal subsurface flow; MPN, most probable number; PFU, plaque-forming units; TSS, total suspended solids.

that these systems can reduce microbiological contaminant loads. However, the datasets reported were limited, with only 10 data points scattered over a period of 3 yr (Robertson et al., 2005) or only annual median reduction of *E. coli* reported (Tanner et al., 2012). Additionally, systems were solely analyzed in terms of their inlet and outlet concentrations. Consequently, there was little information about the distance over which *E. coli* was removed, which is critical if bioreactors are to be designed to remove microbial contaminants. Furthermore, both studies solely measured changes in indicator bacteria and did not consider viruses. Viruses, however, pose an important health risk because they are present in large numbers in wastewater (Yates, 1985; Simmons and Xagoraraki, 2011), have the ability to migrate over long distances through the subsurface (Keswick and Gerba, 1980), and have high potential to initiate waterborne infections (Graun, 1985; Leclerc et al., 2002). Consequently, enteric viruses have been recognized as a significant cause of waterborne disease outbreaks, with Norwalk-like viruses as one of the major causes of waterborne illnesses worldwide (Leclerc et al., 2002; Hrudey and Hrudey, 2007). Therefore, determining the ability of denitrifying bioreactors to remove viruses is important for assessing their capacity to reduce waterborne disease risks.

Due to differences in size, shape, survival characteristics, and susceptibility to disinfection, *E. coli* is unlikely to be a good model for the removal of viruses (Leclerc et al., 2000). Bacteriophage (viruses that infect bacteria) are commonly used to assess human enteric virus removal because direct detection and enumeration of pathogenic viruses is costly and time consuming. A specific group of bacteriophages that have particularly attractive features as models of human enteric viruses are F-specific RNA bacteriophages (FRNA phages). F-specific RNA bacteriophages are commonly excreted in human feces, and their physical structure, composition, and morphology closely resemble those of many human enteric viruses (Leclerc et al., 2000; Grabow, 2001). They have therefore been widely used in studies on wastewater virus transport and removal (Sinton et al., 2002; Hijnen et al., 2005; Zhang and Farahbakhsh, 2007; Aronino et al., 2009; Marti et al., 2011; De Luca et al., 2013) and are widely accepted as a model organism for viruses.

To address the paucity of information in relation to fecal microbial removal within bioreactors, we studied an operational full-scale denitrifying bioreactor receiving secondary-treated septic tank effluent initially established in 2013 for NO_3^- removal. We extended the performance evaluation to include an investigation into the removal of bacterial and viral fecal microbial contaminants, *E. coli*, and FRNA phage. Information about the distance over which *E. coli* and FRNA phage were removed was acquired by sampling along a longitudinal transect within the bioreactor. Additionally, reduction in the major constituents of typical domestic wastewater, such as nutrients (nitrogen, phosphorus) and organic load (total suspended solids [TSS] and 5-d carbonaceous biochemical oxygen demand [$CBOD_5$]), were quantified. This study allows us to assess the potential complementary use of denitrifying bioreactors for microbial contaminant and nutrient removal as well as organic load reduction in onsite wastewater treatment systems.

Materials and Methods

Study Site

In this study, we made use of a full-scale denitrifying bioreactor constructed in May 2013 at the Livestock Improvement Corporation, Newstead, New Zealand. The bioreactor consisted of a trapezoidal bed (20 m top length, 7 m top width, side slope of ~1:1 [width/height], 1.0 m depth, and zero bottom slope) lined with polyethylene and filled with woodchips (*Pinus radiata* D. Don, 10–30 mm in size) (Fig. 1). A 150-mm-deep layer of planting media consisting of sand and coconut peat was placed over the top of a geotextile mesh overlaying the woodchip and was planted with *Carex virgata Sol*. Ex. Boott and *Cyperus ustulatus* A. Rich. The roots of the plants did not penetrate the geotextile mesh and therefore remained restricted to the surficial layer of growth media.

The bed received effluent from a research station consisting of wastewater from laboratories and ablution blocks serving approximately 500 people during the majority of the year. The system was designed and sized based on required nitrate removal taking into account an anticipated increase in flow rate into the system as a result of an expected increase in occupancy. Before discharge into the bed, the effluent was pretreated by passage through a septic tank and a recirculating textile filter (AdvanTex AX100, Orenco Systems Inc.). Effluent entered the denitrification bed through a slotted plastic arch vault with inspection

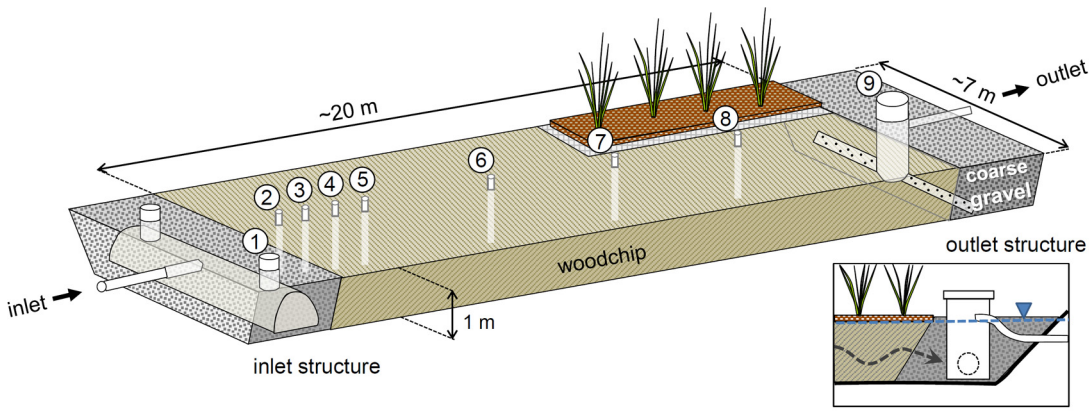

Fig. 1. Schematic of the denitrifying bioreactor, indicating Sampling Points 1 through 9, the inlet structure (a slotted plastic arch vault with inspection risers set in coarse gravel), and the outlet structure (a slotted collection pipe set in coarse gravel connected to a sump with a standpipe). The overlaying planted coconut peat and sand layer is only partially shown.

risers at each end and exited through a slotted collection pipe connected to an outlet sump at the other end of the bed. The water level in the bed was controlled by a standpipe in the outlet sump keeping the water level in the system at 1 m above the bottom of the trench, near the surface of the woodchip media. After construction of the trench, four PVC sampling wells (50 mm diameter, 900 mm length) were installed at even intervals (of ~4 m) along the length of the bed pipe to allow for sampling along the longitudinal transect. In February of 2015, three additional PVC sampling wells (30 mm diameter, 900 mm length) were installed at even intervals (of ~1 m) between the inlet and first sampling well.

Sampling and Analysis

From August 2013 to June 2015, bimonthly sample collections were made, each consisting of two grab samples from an inspection risers at the inlet (Sampling Point 1 in Fig. 1) and the outlet sump (Sampling Point 9). Samples were immediately placed on ice for transport for subsequent analysis. All samples were analyzed for *E. coli* (most probable number [MPN] count in EC MUG Broth), total suspended solids (TSS) (filtration, gravimetric), $CBOD_5$ (incubation for 5 d at 20°C, dissolved oxygen meter), total Kjeldahl nitrogen (phenyl/hypochlorite colorimetry discrete analyzer), ammoniacal nitrogen (NH_4–N; phenyl/hypochlorite colorimetry by flow injection analyzer), total phosphorus (TP) (ascorbic acid colorimetry), and total oxidized nitrogen (NO_x–N; automated cadmium reduction by flow injection analyzer) using standard methods (APHA, 2012). The inlet and outlet analysis was extended in June 2014 to February 2015 to include sulfate (SO_4^{2-}) (filtered sample, ion chromatography).

Additional monthly grab samples were taken at Sampling Wells 4 through 8, located along the longitudinal transect of the denitrifying bioreactor, and analyzed for NO_x–N. Because nitrite (NO_2^-) levels are often much lower than nitrate (NO_3^-), NO_3^-, and NO_x–N (the sum of NO_3^- and NO_2^-) were considered to be approximately equivalent for the purposes of this assessment.

From February to June 2015, sampling was extended to include analysis of *E. coli* and FRNA phage along the longitudinal transect of the denitrifying bioreactor. On a monthly basis, grab samples were collected from the inlet riser (Sampling Point 1), intermediate sampling wells (Sampling Points 2–8), and outlet sump (Sampling Point 9). Samples for *E. coli* and FRNA phage were collected on separate days. Samples were analyzed for *E. coli* (MMO–MUG test using Colilert; IDEXX Laboratories), FRNA phage (double-layer agar technique), and NO_x–N (automated cadmium reduction by flow injection analyzer) using APHA (2012) methods. The FRNA methods were adapted to improve the level of detection in low concentration samples [<100 plaque-forming units (PFU) (100 mL)$^{-1}$] by increasing the sample volume to 50 mL and adding this to 50 mL of top agar, which was then distributed over six plates lowering the detection limit to 2 PFU (100 mL)$^{-1}$.

Flow Rate, Theoretical Hydraulic Residence Time, and Temperature

Total daily flow rate was measured before the inlet of the system using an electromagnetic flow meter (MagMaster, ABB Limited). Nominal (or theoretical) hydraulic retention time (nHRT) in the bed was calculated as nHRT = $(V_s\, n)/Q$, where V_s is the saturated volume of the bed, Q is the flow rate, and n is the primary porosity of the woodchip media. The primary porosity of the woodchip media was assumed to be 0.7 (Schipper et al., 2010b).

Spot measurements of temperature within the bioreactor were measured on a monthly basis using a calibrated meter (model WP81, TPS Pty.). As a result of a change in sampling protocol, no temperature measurements were conducted from January 2014 to August 2014.

Statistical Analysis

As a result of the sampling frequency used (i.e., periodic sampling of all sampling wells occurred on the same day), outlet concentrations did not necessarily correspond to the inlet concentrations sampled on the same day. It was, therefore, not possible to precisely calculate contaminant reduction for each month. Reduction was consequently calculated as the difference between the average inlet and outlet concentration throughout the complete period of monitoring. When the data were nonnormal, reduction was calculated on a median basis. Microbial removal was calculated as median $\log_{10}$ reduction. The values for the detection limits were used for censored data when concentrations were below detection limit. The Shapiro–Wilk's W test of normality was conducted to test if a distribution could be considered to be normal (Statistica version 12, StatSoft Inc.). Subsequently, differences between the concentrations of the microbiological and physiochemical parameters at the inlet and those at the outlet were tested for significance by ANOVA (for normal distributions) or Mann–Whitney test (for nonparametric distributions) (Statistica version 12, StatSoft Inc.). *P* values of <0.05 were considered significant.

Results

Flow Rate and Temperature

Flow rate through the denitrifying bioreactor varied with weekly and seasonal work patterns and subsequent laboratory and ablution block usage. Daily inflows varied between 0 and 29.9 m^3 from August 2013 to June 2015, with an average influent flow rate of 10.0 $m^3\ d^{-1}$ (SD, 6.8 $m^3\ d^{-1}$), which approximately equals the use of 10 households (five persons, 1000 L d^{-1}). The average hydraulic residence time (HRT) in the denitrifying bioreactor was calculated to be ~8 d.

The average water temperature within the bioreactor ranged between 13 and 23°C, with the highest temperatures recorded in summer (February and March) and the lowest temperatures in winter (July and August).

Microbial Contaminant Reduction

The denitrifying bioreactor achieved a significant reduction in *E. coli* between the inlet and outlet, resulting in a median reduction of 2.9 $\log_{10}$ ($P < 0.01$) (Table 1). Although inlet concentrations of *E. coli* varied greatly through time [from ~500 to 3.5×10^5 MPN (100 mL)$^{-1}$] (Fig. 2), reduction of *E. coli* was consistent over the 2-yr period with 90% of all *E. coli* concentrations in the outlet being <350 MPN (100 mL)$^{-1}$ and a median outflow concentration of 20 MPN (100 mL)$^{-1}$ (Table

Table 1. Summary of contaminant concentrations for the inlet and outlet of the denitrifying bioreactor from August 2013 to June 2015.

Contaminant†	Inlet			Outlet			Reduction	*P* value‡
	n	Mean or median	SD or 90th percentile	n	Mean or median	SD or 90th percentile		
Escherichia coli, MPN (100 mL)$^{-1}$	42	**1.6×10^4**§	**9.2×10^4**	41	**20**	**350**	2.9 $\log_{10}$¶	**<0.01**
F-RNA phage, MPN (100 mL)$^{-1}$	8	**2.2×10^4**	**3.4×10^4**	8	**3**	**23**	3.9 $\log_{10}$¶	**<0.01**
TN, g m^{-3}	35	95.2	27.2	40	59.7	21.7	37.3%	<0.01
NO_x–N, g m^{-3}	35	31.2	24.5	40	0.0	0.0	99.9%	<0.01
NH_4–N, g m^{-3}	35	57.1	18.2	38	50.3	16.8	11.8%	0.11
Organic N, g m^{-3}	33	8.5	15.2	36	5.0	13.4	41.0%	0.34
TP, g m^{-3}	23	16.8	2.8	23	15.7	2.4	6.8%	0.06
SO_4^{2-}, g m^{-3}	10	15.6	3.1	10	15.0	2.4	4.2%	<0.01
TSS, g m^{-3}	40	51.7	8.4	42	6.7	6.3	87.0%	<0.01
$CBOD_5$, g m^{-3}	42	**54.8**	**8.0**	40	**8.0**	**8.9**	85.3%	**0.81**

† $CBOD_5$, 5-d carbonaceous biochemical oxygen demand; FRNA phage, F-specific RNA bacteriophage; MPN, most probable number; TN, total nitrogen; TP, total phosphorus; TSS, total suspended solids.

‡ Obtained with ANOVA or Mann–Whitney test as appropriate.

§ Bold values indicate situations where median, 90th percentile, and *P* values obtained with a Mann–Whitney test are given.

¶ Reduction efficiencies for *E. coli* and F-RNA phage are expressed as $\log_{10}$ removals.

1). However, on two occasions concentrations above 500 MPN (100 mL)$^{-1}$ were recorded (Fig. 2). The longitudinal survey of *E. coli* revealed that most of the removal occurred within 1 m from the inlet (Sampling Point 1) (Fig. 3), with a median reduction of 1.4 $\log_{10}$ reduction. The average hydraulic retention time of the wastewater at this distance was approximately 6 h (SD, 12 h). Annual median *E. coli* reduction was 2.7 $\log_{10}$ ($P < 0.01$) in the first year of operation (August 2013 to July 2014) and 3.1 $\log_{10}$ ($P < 0.01$) in the second year of operation (August 2014 to June 2015).

F-specific RNA bacteriophage inlet concentrations fluctuated from approximately 2.7×10^3 to 1.1×10^5 PFU (100 mL)$^{-1}$ (Fig. 2). The median inlet concentration was 2.2×10^3 MPN (100 mL)$^{-1}$ (Table 1). Overall, the denitrifying bioreactor achieved a 3.9 median $\log_{10}$ reduction in FRNA phage. Median outlet concentrations were very low at 3 PFU (100 mL^{-1}). Figure

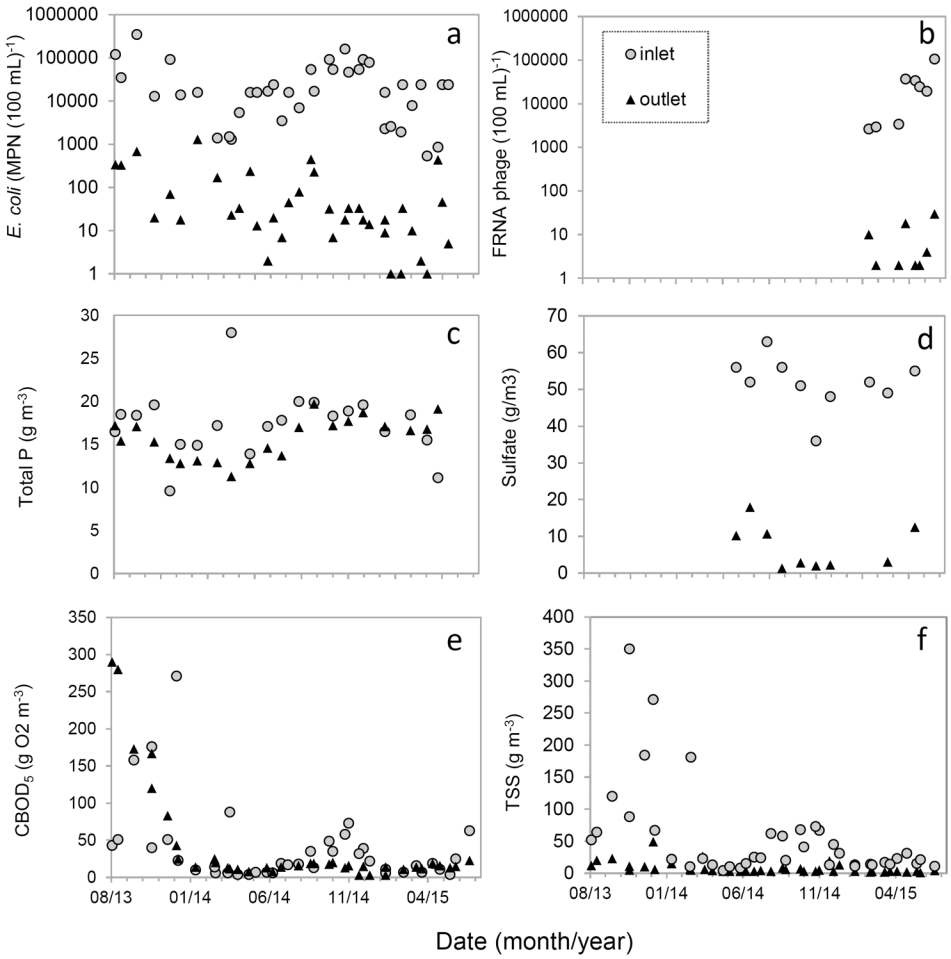

Fig. 2. Inlet and outlet concentrations for (a) *Escherichia coli*, (b) F-specific RNA bacteriophage (FRNA phage), (c) total phosphorus, (d) sulfate, (e) 5-d carbonaceous biochemical oxygen demand ($CBOD_5$), and (f) total suspended solids (TSS) between August 2013 and June 2015. For *E. coli* and FRNA phage, the *y* axis is a $\log_{10}$ scale. MPN, most probable number.

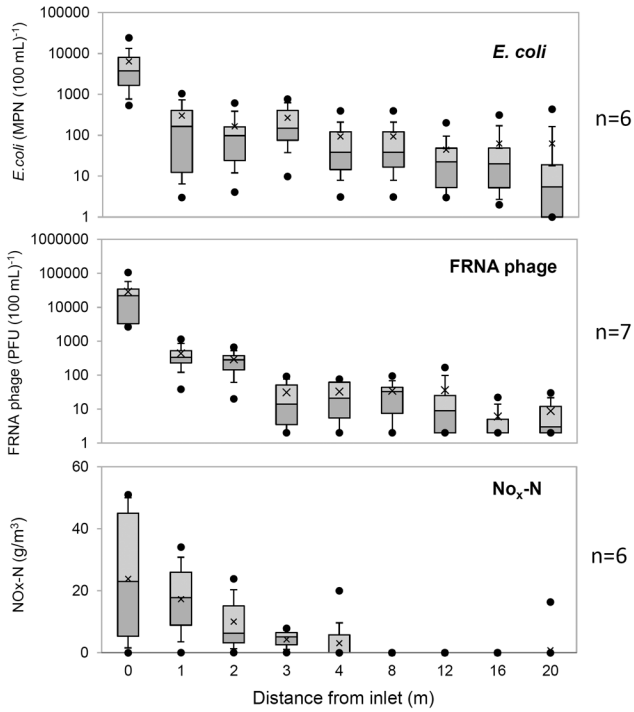

Fig. 3. Box and whisker plot of *Escherichia coli*, F-specific RNA bacteriophage (FRNA phage), and total oxidized nitrogen (NO_x–N) concentrations along the longitudinal transect of the denitrifying bioreactor measured between January 2015 and June 2015. Lines within the boxes are median values, the bottom and top of the boxes are the 25th and 75th percentiles, and error bars are the 10th and 90th percentiles. Dots represent the minimum and maximum values of the data, crosses represent the mean concentrations, and n refers to the sample size for each sampling well. For *E. coli* and FRNA phage, the *y* axis is a $\log_{10}$ scale.

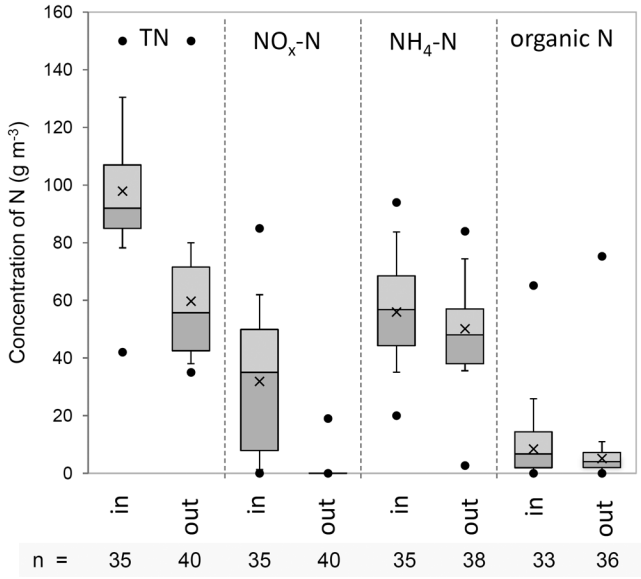

Fig. 4. Box and whisker plot of inlet and outlet concentrations for different N species. Lines within the boxes are median values, the bottom and top of the boxes are the 25th and 75th percentiles, and error bars are the 10th and 90th percentiles. The dots represent the minimum and maximum values of the data, the crosses represent the mean concentrations, and n refers to the sample size. TN, total nitrogen; NO_x-N, total oxidized nitrogen; NH_4-N, ammoniacal nitrogen.

3 shows that, similar to *E. coli*, most of the removal (1.8 median $\log_{10}$ reduction) FRNA phage occurred by the first sampling well (~1 m from the inlet). Near complete removal (3.2 median $\log_{10}$ reduction) was achieved before well 4 (at ~3 m from the inlet), which represents an average hydraulic retention time of about 1 d (SD, 2 d).

Nutrients and Organic Load Reduction

Concentrations of N species in the inlet and outlet of the denitrifying bioreactors are given in Fig. 4. Total N loads entering the bioreactor varied with time, ranging from 42 to 134 g N m^{-3}. Additionally, composition of inlet N loads varied greatly with time, with NO_x–N inlet concentrations varying from 0.002 to 74 g NO_x–N m^{-3} (Fig. 4). Nitrate was the major form of N removed from the effluents passing through the bed (Fig. 2 and 4), with outlet concentrations generally below 0.02 g m^{-3} (with the exception of two outliers) and a median reduction of over 99.9% (Table 1). Average nitrate mass removal rate, calculated from the difference between the mass of NO_x–N at the inlet and Sampling Well 4 (at ~4 m from the inlet) divided by the volume of bioreactor up to this sampling well, was ~14 g N m^{-3} d^{-1}. The system received substantial NH_4^+ and organic N at the inlet. For these N species, the mean reduction was calculated to be 12 and 39%, respectively, but this was not a statistically significant reduction (Table 1). During the first 17 mo of measurements, a significant reduction in phosphorus concentration (~14%) was observed as effluent passed through the denitrifying bioreactor (Fig. 2). After this period, phosphorus outlet concentrations increased, resulting in an overall mean reduction of 7% for the entire monitoring period (P = 0.06). A substantial decrease in SO_4^{2-} concentration (94% reduction) was obtained between inlet and outlet wells (Fig. 2).

The denitrifying bioreactor effluent had high $CBOD_5$ (>100 g O_2 m^{-3}) during the first 9 mo after start-up (Fig. 2). Subsequently, $CBOD_5$ decreased and stabilized at much lower values (mean outlet concentration, 14 g O_2 m^{-3}). After stabilization, the system achieved a significant reduction in $CBOD_5$ load of 40% (P = 0.04). The system was able to substantially reduce TSS (87%), with a 90th percentile value of 18 g m^{-3} at the outlet (P < 0.01) (Table 1; Fig. 2).

Discussion

Microbial Contaminant Reduction

This study demonstrated that a significant reduction of *E. coli* of around three orders of magnitude can be achieved by passing secondary-treated, nitrified effluent through a denitrifying bioreactor. These findings are supported by studies by Robertson et al. (2005) and Tanner et al. (2012), who also reported substantial reductions in *E. coli* within denitrifying bioreactors. However, quantitative comparison of *E. coli* reduction between these studies is challenging due to differences in experimental conditions (e.g., system size, nominal hydraulic retention time, and inlet concentration). Nevertheless, Tanner et al. (2012) reported slightly higher median outlet concentrations [70–1250 CFU (100 mL) $^{-1}$] and lower median $\log_{10}$ reductions (1.2–1.9 $\log_{10}$) for smaller bioreactor systems (1.8 m^3) with nominal retention times of 7 and 10 d, respectively. In the work by Robertson et al. (2005), the majority (79%) of all denitrifying bioreactor outlet samples had no detectable *E. coli* [<10 CFU (100 mL) $^{-1}$]. These systems, however, received relatively low *E. coli* loads

[up to 2000 CFU (100 mL^{-1})]. In our full-scale system, most of the reduction in *E. coli* in the denitrifying bioreactor occurred within the first meter from the inlet (at Sampling Well 2). It is therefore likely that this bioreactor has the capacity to manage substantially higher loads (i.e., higher concentrations or shorter hydraulic retention times).

The removal performance of the bioreactor compared favorably with other passive technologies for wastewater treatment that are suggested as appropriate solutions for reducing pathogen loads from wastewater, such as treatment wetlands. Subsurface flow wetlands have been found to reduce microbial populations with varying but significant degrees of effectiveness. In general, reduction of *E. coli* achieved by this full-scale denitrifying bioreactor was at the upper end of the range (1.3–3.1 log_{10} reduction) reported in literature for horizontal subsurface flow (HSSF) wetlands (Green et al., 1997; Ottová et al., 1997; Decamp and Warren, 2000; Mantovi et al., 2003; Molleda et al., 2008). It is likely that there is a greater capacity for removal in the bioreactor system under investigation as outlet concentrations generally remained low and steady despite fluctuating inflow concentrations, with median and 90th percentile concentrations for *E. coli* of 20 and 350 MPN (100 mL) $^{-1}$, respectively, in the final effluent. This demonstrates the resilience of these systems for microbial contaminant removal.

In contrast to findings by Robertson et al. (2005), the denitrifying bioreactor in this study was not able to consistently reduce *E. coli* concentrations to near zero (i.e., below detection limit). The observed background concentration could be the result of the production of fecal indicator bacteria by animals that frequent the treatment system (Kadlec and Wallace, 2009) or the result of regrowth of *E. coli*, which has been observed in aquatic environments (Gerba, 2000; Ishii et al., 2006). Effluent concentrations from the bioreactor would generally be suitable for subsurface irrigation (WHO, 2006). To achieve concentrations for safe reuse within gardens or homes, where there is potential for human contact, effluent would require a greater degree of disinfection.

Due to fluctuations in inflow concentration, no pronounced seasonality effects for *E. coli* removal could be detected. Some HSSF wetlands display seasonal effects for fecal coliform removal, with lower efficiencies at lower water temperatures (Rivera et al., 1995). The effect of seasonality on microbial reduction efficiency in denitrifying bioreactors should be investigated further under more controlled conditions.

Although some evidence for *E. coli* removal has previously been documented, there are no data available on the removal of viruses within denitrifying bioreactors. This study demonstrated that denitrifying bioreactors can also achieve significant and consistent reduction in FRNA phage. Because enteric viruses can behave similarly to FRNA phages in wastewater treatment processes (Grabow, 2001), the results of this study indicate that denitrifying bioreactors could also remove enteric viruses from wastewater.

Compared with *E. coli*, there is very limited information on the removal of FRNA phage in onsite treatment systems such as HSSF wetlands. In the literature, generally poorer removal rates for FRNA phage are reported for HSSF wetlands, with the degree of effectiveness between systems varying widely from −0.1 to 3.5 log_{10} reduction (Gersberg et al., 1987; Barret et al., 2001). Therefore, reductions in FRNA phage achieved by the full-scale denitrifying bioreactor in the present study exceeded the upper limit found in literature for HSSF wetlands. Similar to *E. coli*, outlet concentrations of FRNA did not appear to be dependent on inflow concentration. Log_{10} reduction in FRNA phage was therefore affected by inflow concentration. Because near complete reduction (3.2 median log_{10} reduction) in FRNA phage occurred by the fourth sampling well (at ~3 m from the inlet), the system is expected to be able to cope with higher loads.

Nitrate removal in denitrifying bioreactors has been shown to decline with time (Robertson et al., 2008; Moorman et al., 2010). Extended studies are required to determine if microbial contaminant removal decreases as the bioreactor matures. In the current study there was no obvious decline in removal rate of *E. coli* or FRNA phage with time. In contrast, Tanner et al. (2012) observed an apparent decrease in *E. coli* removal performance with maturation of denitrifying bioreactors over 1 yr. The long-term ability of denitrifying bioreactors to remove microbial contaminants from wastewater will depend on the main removal mechanisms. An understanding of these processes is needed to improve prediction of microbial contaminant removal in denitrifying bioreactors and to define standards for effective design of denitrifying bioreactors for microbial contaminant removal. For nitrate removal, a supply of carbon to denitrifying bacteria from woodchip is essential (Schipper et al., 2010b). Microbial contaminant removal mechanisms could include a variety physical, chemical, and biological processes, such as predation, adsorption, filtration, and die-off (Schijven and Hassanizadeh, 2000; Stevik et al., 2004). Further research on removal mechanisms of bacteria and viruses, how long these will remain active, and how they are affected by factors such as seasonality, loading rate, and inflow concentration is warranted.

Nutrient and Organic Load Reduction

As expected, the denitrifying bioreactor was effective in removing NO_3^- from wastewater. The mass removal rate of 14 g N m^{-3} d^{-1} is at the high end of removal rates recorded for denitrifying bioreactors (Schipper et al., 2010b). The removal rate is expected to decrease as carbon depletes with maturation of the system (Schipper et al., 2005; Robertson et al., 2008; Moorman et al., 2010). However, throughout the period of data collection, denitrification in the bioreactor was likely nitrate limited rather than C limited. The observed removal of SO_4^{2-} was in keeping with complete NO_3^- removal, which allowed SO_4^{2-} reduction (Schipper et al., 2010b). Robertson et al. (2005), Schipper et al. (2010a), and Tanner et al. (2012) also reported no significant removal of NH_4^+ or organic N with passage through the denitrifying bioreactor. In contrast to findings by Schipper et al. (2010a), a small but significant reduction (~14%) in phosphorus concentration was observed as effluent passed through the denitrifying bioreactor. This reduction, however, only occurred in the first 17 mo and could be a result of initial phosphorus immobilization in microbial biomass and adsorption to the woodchip media with subsequent saturation or of phosphorus release from the woodchip or microbial biomass after 17 mo. To improve phosphorus removal, the incorporation of phosphorus-adsorbing compounds in denitrifying bioreactors should be assessed.

The high outlet $CBOD_5$ during the first 9 mo of operation of the denitrifying bioreactor was likely the result of leaching of soluble organic constituents from the woodchips, which may result in undesirable oxygen consumption in receiving waters (Robertson et al., 2005; Schipper et al., 2010a). The gradual decrease and stabilization of $CBOD_5$ in the outlet over time indicate that $CBOD_5$ loss is likely to be a temporary concern. The reduction and subsequent low TSS and $CBOD_5$ concentrations at the outlet make the effluent readily amenable to disinfection via chlorination or ultraviolet lamps (Leverenz et al., 2006).

Conclusions

This study demonstrated that, in addition to significant reduction in NO_3^- loads, denitrifying bioreactors are effective at reducing bacterial and viral concentrations of secondary-treated, nitrified septic tank effluent. Substantial reductions in TSS were also achieved. Leaching of $CBOD_5$ out of denitrifying bioreactors should be expected during the first months of operation; however, this is a short-term concern. Although the hydraulic loads entering the bioreactor varied substantially and influent bacterial and viral concentrations were often quite high and variable over time, the outlet concentrations generally remained low and stable but would require further disinfection for safe reuse of wastewater where there is potential for human contact. The low TSS and $CBOD_5$ outlet concentrations make the effluent readily amenable to further disinfection via chlorination or ultraviolet lamps. Despite high levels of NO_3^- removal, there was no evidence of removal of NH_4^+ or organic N during passage through the bioreactor. Although removal of phosphorus was observed, the overall reduction was relatively small and decreased with time. Overall, we present strong evidence for microbial contaminant removal in denitrifying bioreactors. To improve prediction of microbial contaminant removal in denitrifying bioreactors and to support the development of effective design criteria of denitrifying bioreactors for microbial contaminant removal, longer-term studies under well-controlled conditions are needed to identify the dominant microbial removal mechanisms, the longevity of removal and the influence of seasonality, loading rate, and inflow concentration on removal.

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