Ecological Engineering 161 (2021) 106157

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Removing 80%–90% of nitrogen and organic contaminants with three distinct passive, lignocellulose-based on-site septic systems receiving municipal and residential wastewater

Christopher J. Gobler[a,b,*], Stuart Waugh[a], Caitlin Asato[a], Patricia M. Clyde[a,b], Samantha C. Nyer[a,b], Molly Graffam[a,b], Bruce Brownawell[b], Arjun K. Venkatesan[a,c], Jennifer A. Goleski[b], Roy E. Price[a,b], Xinwei Mao[a,c], Frank M. Russo[a,c], George Heufelder[d], Harold W. Walker[a,1]

[a] New York State Center for Clean Water Technology, Stony Brook University, Stony Brook, NY 11794, USA
[b] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA
[c] Department of Civil and Environmental Engineering, Stony Brook University, Stony Brook, NY 11794, USA
[d] Massachusetts Alternative Septic System Test Center (MASSTC), Barnstable County Department of Health and Environment, Barnstable, MA 02630, USA

A R T I C L E I N F O

Keywords:
Passive onsite wastewater treatment
Lignocellulose biofilters
Organic contaminant removal
Nitrogen removal
Coupled nitrification denitrification

A B S T R A C T

Three distinct septic systems designed for onsite removal of nitrogen (N) from residential wastewater were installed at the Massachusetts Alternative Septic System Test Center (MASSTC) and at homes across Suffolk County (SC), New York. All configurations featured nitrifying sand beds coupled with denitrifying biofilters composed of 1) a lined, saturated sand and woodchip layer, 2) a saturated box filled with woodchips, or 3) an unlined, unsaturated sand and woodchip layer. Total N (TN) in final effluent discharge from the three systems at MASSTC over more than two years were 7.1 ± 7.8, 4.3 ± 4.2, and 6.9 ± 8.4 mg N L^{-1}, respectively representing TN reductions of 83%, 87%, and 84% from influent TN. Systems at MASSTC also removed on average 90.0–99.9% of 10 of 11 organic contaminants in pharmaceutical and personal care products, microbes indicative of pathogens, and biochemical oxygen demand. Over periods up to 16 months from start-up, effluent from three lined, one woodchip box, and three unlined systems in SC averaged 8.3 ± 9.2, 5.3 ± 3.7, and 8.7 ± 4.9 mg-TN L^{-1} representing removal rates of 90%, 94%, and 88%, respectively. For all systems, wastewater N was effectively nitrified year-round; N removal varied seasonally as denitrification attenuated in winter. Substantial quantities of TN were removed in the sand beds, likely due to denitrification in anoxic micro-zones. While elevated levels of carbon leached from the wood-based biofilters installed at MASSTC during the first 60 days of operation, no substantial decline in dissolved organic carbon or N removal was observed between the first 15 months of operation and the following 12 months. Collectively, the performance of these non-proprietary, passive systems suggest they may be a useful alternative septic system for protection of groundwater from elevated levels of N, organic contaminants, and pathogens.

1. Introduction

Nitrogen (N) pollution can initiate a cascade of negative ecosystem impacts in marine and fresh waters including harmful algal blooms, hypoxia, and acidification, as well as a deterioration in water clarity leading to loss of both seagrass beds and benthic biodiversity (Valiela et al., 1992; Howarth, 2008; Hattenrath et al., 2010; Gobler and Sunda, 2012; Gobler et al., 2012; Wallace et al., 2014). N pollution of drinking water has been associated with a variety of human health risks including methemoglobinemia in infants (Wolfe and Patz, 2002; Greer and Shannon, 2005) and epidemiological studies have suggested connections with non-Hodgkin's lymphoma (Ward et al., 1996) as well as multiple cancers (Weyer et al., 2001; Espejo-Herrera et al., 2016; Jones et al., 2016; Schullehner et al., 2018). Along with atmospheric

* Corresponding author at: New York State Center for Clean Water Technology, Stony Brook University, Stony Brook, NY 11794, USA.
E-mail address: Christopher.Gobler@stonybrook.edu (C.J. Gobler).
[1] Present address: Department of Civil and Environmental Engineering, Worcester Polytechnic Institute, Worcester, MA 01609, USA.

https://doi.org/10.1016/j.ecoleng.2021.106157
Received 9 April 2020; Received in revised form 21 December 2020; Accepted 11 January 2021
Available online 30 January 2021

deposition and fertilizer runoff, N loadings from residential wastewater delivered through cesspools and conventional septic systems to groundwater can be a primary source of N pollution to aquifers used for drinking water and to coastal oceans (e.g., Valiela et al., 1992; Latimer and Charpentier, 2010; Kinney and Valiela, 2011). Residential wastewater is also a source of organic contaminants from pharmaceuticals and personal care products to coastal ecosystems (Dougherty et al., 2010; Singh et al., 2010; Gaw et al., 2014), groundwater (Phillips et al., 2015; Hinkle et al., 2005) and drinking water (Schaider et al., 2014; Schaider et al., 2016).

In the last several decades, a number of proprietary and non-proprietary on-site residential technologies utilizing the biological process of nitrification coupled to denitrification have been installed to remove N from wastewater near its source (e.g., Robertson and Cherry, 1995; Oakley et al., 2010; Addy et al., 2016). Various configurations have been developed to sequence the oxidation and reduction steps involved in transforming reduced wastewater N, primarily in the form of the organic N and ammonium (NH_4^+), to inert dinitrogen gas (N_2) (Oakley et al., 2010; Addy et al., 2016). Many of these systems incorporate lignocellulose-based biofilters similar to those used in remediating nitrate (NO_3^-) in agricultural (e.g., Schipper et al., 2010) and stormwater (e.g., Lopez-Ponnada et al., 2017) applications. According to a comparative survey of 20 separate wastewater treatment systems, a simple nitrifying sand bed coupled to a lignocellulose-based denitrifying biofilter achieved the most reliable and lowest N concentration in final effluent (Oakley et al., 2010). The basic principle comprises nitrification of wastewater N as it percolates through a sand layer and subsequent denitrification in a biofilter as naturally occurring microbes utilize NO_3^- in the absence of oxygen to oxidize carbon in wastewater and from lignocellulose thereby converting NO_3^- to inert N_2 gas.

Despite previous reports of high rates of N removal by sand bed and lignocellulose biofilters (Oakley et al., 2010; Hirst and Anderson, 2015; Addy et al., 2016), important questions regarding such systems remain unanswered. The performance of differing designs receiving a common source of wastewater has not been explored. The dynamics of N speciation and associated parameters (e.g. dissolved oxygen (DO), alkalinity, and pH) at different stages of treatment as wastewater percolates through soil-based systems in residential settings has rarely been reported (Addy et al., 2016). Only several field studies have tested drainfields or alternative residential wastewater treatment systems for the removal of organic contaminants in pharmaceuticals and personal care products (Schaider et al., 2017).

This study addresses these knowledge gaps by describing three different designs of full-scale nitrogen removing biofilters (NRBs) which were installed at the Massachusetts Alternate Septic System Test Center in Barnstable County, MA (MASSTC) and at seven residential properties in Suffolk County, NY (SC). The objective was to provide a comparative analysis of performance of each different design in removing N species, 5 day biochemical oxygen demand (BOD_5), fecal coliform, and organic contaminants from residential wastewater and to investigate the determinants of differences in system performance. N removal performance was assessed cumulatively, seasonally and, because other researchers (e.g. Robertson, 2010) observed substantial decreases in TN removal efficiency of woodchip based biofilters from the first year of operation to subsequent years before stabilization, between the initial 15 months of operation and the following 12 months for each design. While two of these designs are similar to prior installations (Robertson and Cherry, 1995; Oakley et al., 2010; Hirst and Anderson, 2015; Addy et al., 2016), a third design consisting of a sand bed coupled to an accessible 'woodchip box' has not been reported on in the peer reviewed literature.

2. Methods

2.1. Description of NRB designs

For all systems used in this study, wastewater flows by gravity from a traditional septic tank to a pump chamber from where it is delivered to a sub-surface distribution system when a threshold water volume is reached (typically 2–8 times per day) by a low pressure pump (Fig. 1). Beneath the distribution system, septic tank effluent (STE) percolates through an unsaturated sand bed where NH_4^+ is converted to NO_3^- before reaching a biofilter in which NO_3^- is converted to N_2 under low oxygen conditions created as woodchips are remineralized (Fig. 1) (Robertson and Cherry, 1995; Oakley et al., 2010). The sand used was commercial concrete grade sand (classified as C-33 sand according to standards of ASTM International) and woodchips were generally 1–7 cm in length primarily from oak and pine trees.

The three NRB designs installed at MASSTC consist of a sand bed coupled to (1) a lined, saturated denitrification biofilter ("lined NRB") (Fig. 2a); (2) a denitrifying woodchip box ("woodchip box system"; Figs. 2b and 3) an unlined, unsaturated denitrification biofilter ("unlined NRB"; Fig. 2c). SC installations followed similar design plans. Design specifications for these systems are reported in Tables 1 and 2. The lined systems consist of ~15 cm of top-soil which covers a lateral Geomat™ or Infiltrator Quick4® distribution system (Fig. 2a). This layer is underlain by a 46 cm nitrifying C-33 sand layer at the base of which, depending on the installation, at least one pan lysimeter was placed. A 46 cm denitrifying layer of sand and woodchips blended as an equal mix by volume prior to installation lies beneath the sand beds (Fig. 2a). A 0.05 cm-thick polyethylene lining encases all sides and the bottom of the denitrification layer and channels wastewater from the liner bottom to a perforated PVC effluent pipe which extends to the height of the interface between the nitrification and denitrification layers. This structure creates a hydraulic pressure head which maintains the sand and woodchip layer in an anoxic, saturated condition (Fig. 2a). Maintaining the sand and woodchip layer in a saturated condition minimizes oxidation and therefore degradation of the woodchips over time (Wilson et al., 1993; Moorman et al., 2010). Treated wastewater is then channeled to a final dispersal system.

A second design facilitates replacement of woodchips by housing them in a box (specifications in Tables 1 and 2) accessible by a chimney with a removable cover buried adjacent to rather than underneath the sand bed (Fig. 2b). This system consists of sand bed 46 cm in height (same as described above) overlaying a gravel bed from which nitrified wastewater is collected in a polyethylene liner connected by PVC pipe to the bottom of a plastic tank filled with woodchips where wastewater flows upward through the tank to ensure constant saturation of woodchips before exiting through a pipe near the top of the box (Fig. 2b). As with the lined NRB, wastewater is then channeled to a final dispersal system.

A third, unlined NRB design is nearly identical to the lined system described except there is no plastic liner around the denitrification layer (Fig. 2c) and it is not necessarily continuously saturated. Unlike the lined and woodchip box NRBs, effluent from this system percolates directly to groundwater. Samples for wastewater analysis are collected by pan lysimeters placed at the bottom of each layer (Fig. 2c).

The NRBs installed at the MASSTC were operated by the Barnstable County Department of Health and Environment, Barnstable County, MA. Untreated wastewater was diverted from a sewage treatment plant to a shallow, common cement trough (influent source) from which it was pumped to septic tanks dedicated to individual systems. Sand bed surface areas for each NRB at MASSTC were 3.66 m wide by 8.53 m long and received 833 L of sewage per day delivered at a loading rate of 26.7 L m^{-2} d^{-1} from fall 2016 through August 2018 when the flow rates were increased to 1250 L d^{-1} yielding a loading rate of 40 L m^{-2} d^{-1} (Table 1). At these loading rates, the calculated hydraulic retention times (HRT) for the denitrification layer of the lined system were 5.8 and

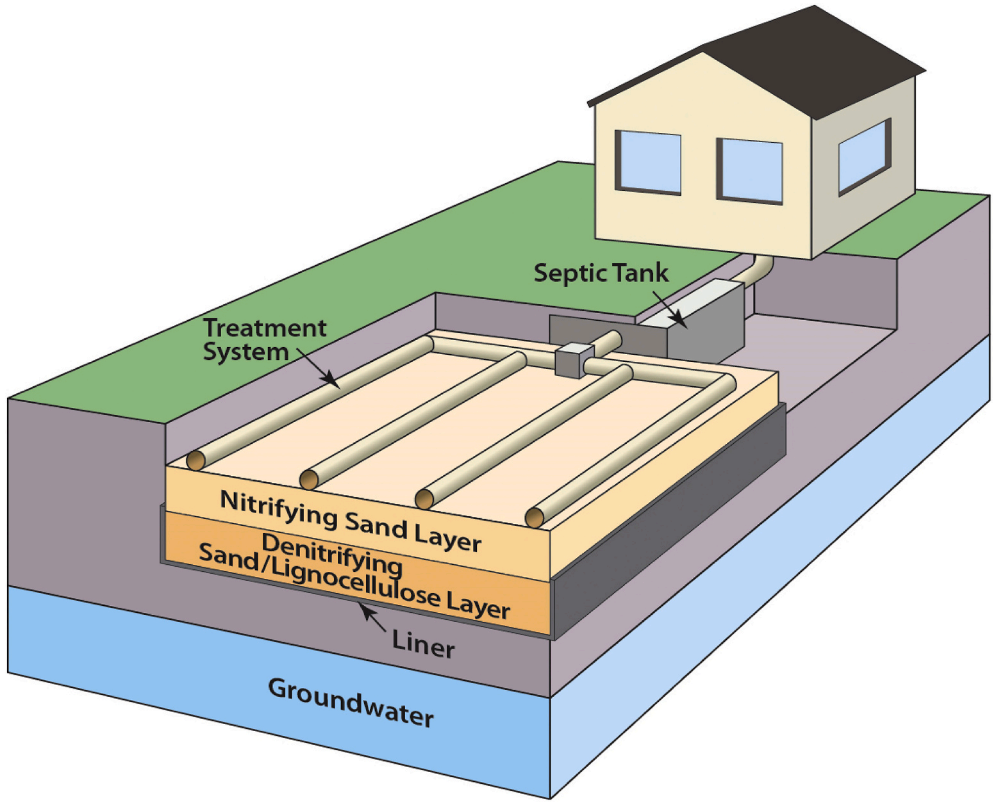

Fig. 1. Schematic of generalized nitrogen removing biofilter showing denitrifying woodchip-based biofilter underlying nitrifying sand bed. Mechanical and electrical components are limited to one pump which moves wastewater from septic tank to soil distribution system.

3.9 days and for the woodchip box were 2.0 and 1.4 days, respectively. The HRT of unsaturated layers was not calculated from system geometry because flow depends on many factors including hydraulic conductivity, pore sizes, and sand texture (Amador and Loomis, 2019).

Between April 2018 and September 2019, three lined NRBs, one woodchip box system, and three unlined NRBs were installed at SC residences. Design flows were scaled to the sizes of the residences (SC design flow per bedroom ~420 L per day) based on surface loading rates of 31 L m^{-2} d^{-1}, with the exception of one unlined system which was made shallower and dosed at 20 L m^{-2} d^{-1} to accommodate a higher groundwater level. At this design flow, HRT in the saturated denitrification layers of the lined systems were 6.8–7.9 days while the HRT in the woodchip box was 1.2 days.

2.2. Sampling and analyses

The MASSTC systems were sampled biweekly by MASSTC staff for ~2.25 years using submersible pumps. Influent was sampled from a common source (cement trough upstream of individual septic tanks). Final effluent was sampled from (1) a sump into which percolate from the liner drains for the lined system; (2) a pipe which drains treated effluent from the woodchip box and (3) pan lysimeters underlying the sand and woodchip layer of the unlined system. Temperature, DO, and pH were analyzed immediately on site (YSI 556 Multi-Probe System). Total nitrogen (TN) was calculated as the sum of total Kjeldahl nitrogen (TKN) measured via US EPA Method 351.2 and NO_3^- and NO_2^- measured by ion chromatograph according to US EPA Method 300.0 (O'Dell, 1993b; Pfaff, 1993). NH_4^+ was measured colorimetrically following US EPA Method 350.1 (O'Dell, 1993a). Alkalinity, BOD_5, and total suspended solids (TSS) were determined according to US EPA Method 405.1, US EPA Method 310.1, and US EPA Method 160.2, respectively (USEPA, 1983a, 1983b, 1983c). Fecal coliform bacteria were quantified using membrane filtration method SM9222D (Eaton et al., 2005). Dissolved organic carbon (DOC) was measured monthly between October 2017 and September 2018 on a Shimadzu TOC-L analyzer (USEPA, 1974). Denitrification is approximated as the difference between NO_3^- measured downstream of the sand beds (in pan lysimeters positioned at the bottom of sand beds in the layer NRBs, and in a pipe receiving nitrified percolate upstream of the woodchip box) less NO_3^- measured in final effluent; denitrification efficiency is calculated as denitrification divided by NO_3^- measured downstream o the sand beds. The significance ($p < 0.05$) of seasonal differences was assessed by comparing TN means of each NRB design in winter (January–April) against those from non-winter (May–December) periods by *t-test*; the significance ($p < 0.05$) of attenuation in TN removal performance over multi-year periods was assessed by comparing means from all systems in the first 15 months against those over the following 12 months of operation by *t-test*. Because NRB designs were not replicated at MASSTC, the significance of differences in TN in final effluent among each of the three NRB designs was not assessed.

Influent and effluent samples for SC systems were sampled monthly and lysimeters underneath/downstream of the sand beds were sampled quarterly from inception (1–16 month periods depending on site; Table 2). Samples were collected by peristaltic pumps. Influent samples from all systems were collected from pump chambers immediately downstream of each septic tank. For the lined NRBs, effluent was collected from a sump connecting the under-drain to the final dispersal system. Effluent from the woodchip box NRB was collected from a pump chamber dosing the final dispersal system. In the unlined NRBs, effluent samples were collected in pan lysimeters installed at the bottom of the denitrification layer. Upon collection, DO, temperature, and pH were analyzed (YSI ProODO for DO and temperature and Hach MM150 for pH); TKN, NO_3^- and NO_2^-, and NH_4^+ were measured via US EPA Method 351.2, US EPA Method 353.2, and US EPA Method 350.1 (O'Dell, 1993a, 1993b, 1993c), respectively. Alkalinity was measured titrimetrically following USGS I-2030-85 (Rounds, 2012). Fecal coliform bacteria were

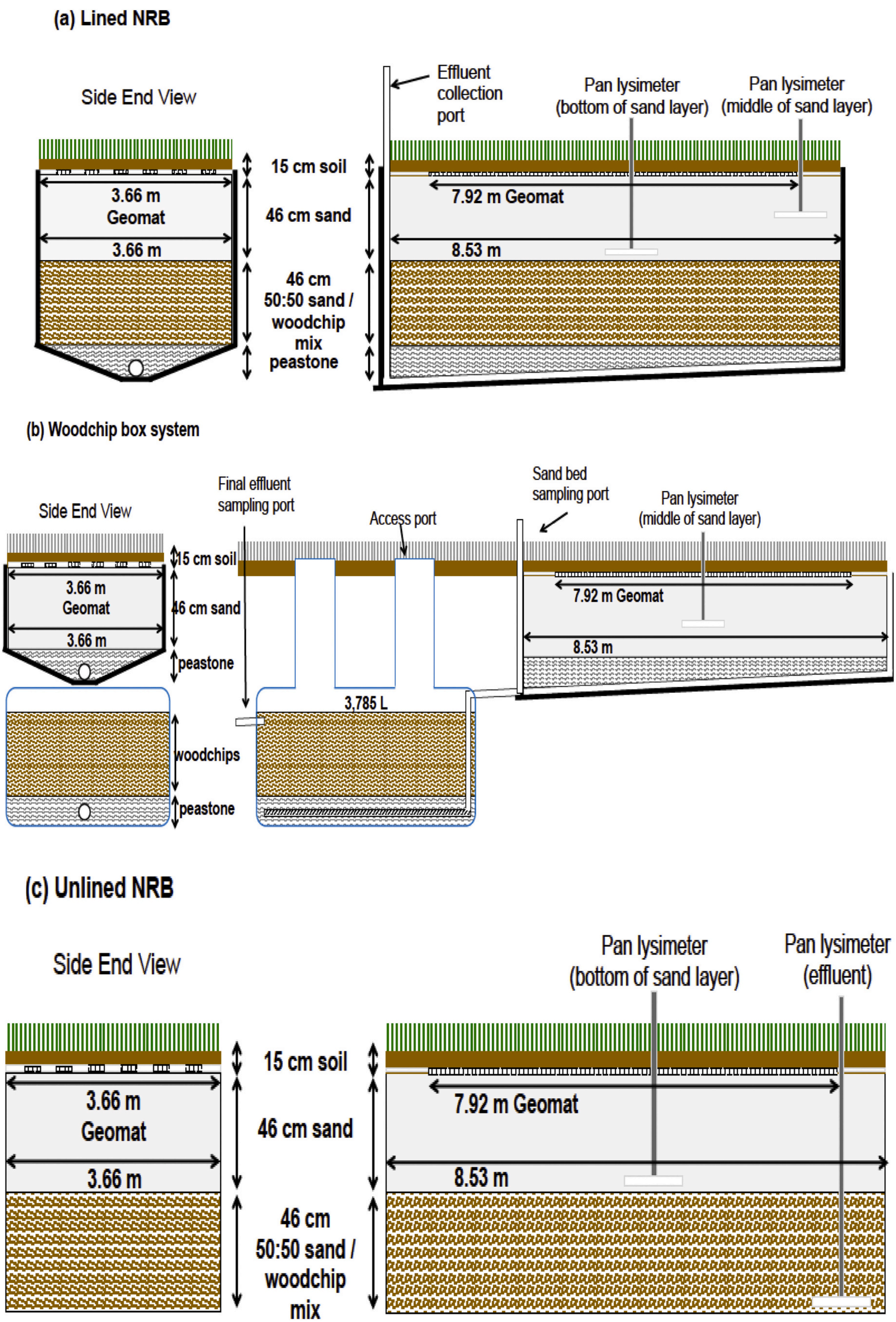

Fig. 2. Illustrations of three designs of nitrogen removing biofilters (NRB) installed at the Massachusetts Alternative Septic System Test Center: (a) lined NRB; (b) woodchip box system and (c) unlined NRB.

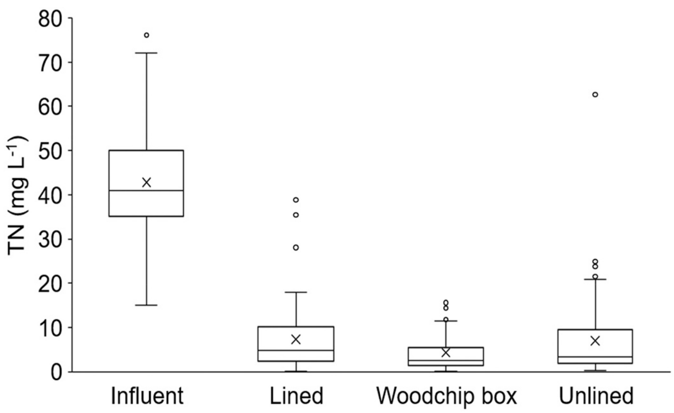

Fig. 3. Total nitrogen (TN) in wastewater influent and final effluent from three nitrogen reducing biofilter (NRB) designs at Massachusetts Alternative Septic System Test Center, Barnstable County, MA. Medians, indicated by mid-line in box, are 4.7, 2.6 and 3.4 mg-N L^{-1} for lined, woodchip box, and unlined systems; 75th percentiles, indicated by the top lines of the boxes, are 10.1, 5.4, and 9.2 mg-N L^{-1}. Bottom lines represent 25th percentiles, × symbols represent means, ○ symbols represent outlier points, and whiskers extend to the range of non-outlier data points (those falling within 1.5 times the interquartile range).

Table 1
Design parameters of nitrogen removing biofilters installed at Massachusetts Alternative Septic System Test Center.

Parameter		Lined	Woodchip box	Unlined
Design length (m)		8.53	8.53	8.53
Design width (m)		3.66	3.66	3.66
Nitrification filter depth (cm)		46	46	46
Denitrification layer depth (cm)		46	N/A	46
Denitrification layer volume (L)[a]		14,361	N/A	14,361
Denitrification tank volume (L)		N/A	3785	N/A
Design flow (L d^{-1})	Sep 2016–Aug 2018	830	830	830
	Aug–Dec 2018	1250	1250	1250
Normalized loading rate (L d^{-1} m^{-2})	Sep 2016–Aug 2018	27	27	27
	Aug–Dec 2018	41	41	41
HRT[b] (d^{-1}) of denitrifying phase	Sep 2016–Aug 2018	5.8	2	N/A
	Aug–Dec 2018	3.9	1.4	N/A

[a] Bulk volumes of denitrification phases of layered (lined and unlined) calculated geometrically. The woodchip box system has a tank rather than an underlying layer for denitrification.
[b] Hydraulic retention time (HRT) was calculated by assuming a porosity value of 0.45 for the lined denitrification layer and 0.5 for the woodchip box. The woodchip box was estimated to be filled to 9/10th of its height with woodchips. HRT was not calculated for the denitrification layer of unlined systems.

quantified using membrane filtration according to SM 9222D and BOD_5 was determined according to SM5210B (Eaton et al., 2005). N species, alkalinity, fecal coliform bacteria and BOD_5 measurements were performed by Stony Brook University laboratories certified under New York State Department of Health's Wadsworth Center's Environmental Laboratory Approval Program (ELAP).

2.3. Quantification of organic contaminants

Organic contaminants were quantified in the systems installed at MASSTC. Samples were taken from the lined and the woodchip box systems in September 2017 and in July 2018, and from the unlined system in July 2018. All samples were collected in cleaned amber glass bottles with Teflon liners, and transported on ice to the Trace Organic Chemical Mass Spectrometry lab at the School of Marine and Atmospheric Sciences at State University of New York, Stony Brook. Sample volumes were 500 mL for effluent samples and 100 mL for influent samples, as a balance of detectable concentrations for compounds of interest, and matrix level of the sample. Immediately upon arrival to the lab, each sample was filtered using a 0.45 μm glass fiber filter (Whatman, 47 mm).

Within 12 h of filtering, each sample was concentrated by solid-phase extraction (SPE) using 500 mg Oasis HLB (Waters) cartridges conditioned with two 4 mL washes of methanol followed by four 2 mL washes of Milli-Q water. The samples were then loaded on the cartridges under vacuum at a rate of 10 mL min^{-1}. After sample loading, each cartridge was rinsed with two 1 mL washes of Milli-Q water and then dried under vacuum. Dried cartridges were stored at −20 °C. Immediately prior to analysis, samples were extracted using three 2 mL aliquots of methanol followed by three 2 mL aliquots of methanol with 0.1% trifluoroacetic acid. The extracted samples were blown down to 100 μL under N_2 gas, at which point 100 μL methanol was added, and the samples were vortexed. Then 800 μL Milli-Q water was added, the samples were vortexed again, and then centrifuged at 3000 rpm for 5 min to remove any precipitates. Finally, the samples were transferred via Pasteur pipette to autosampler vials for analysis.

Organic contaminant samples were analyzed on an Agilent high performance liquid chromatograph coupled with an Agilent time-of-flight mass spectrometer (HPLC-TOF-MS) in the Chemistry Department of State University of New York, Stony Brook. All data was quantified using the Agilent MassHunter Qualitative and Quantitative Analysis programs. Methodological detection limits (MDLs) were calculated using the smallest detectable sample area for each compound and scaling it down to a signal to noise ratio of 3-to-1. The samples were analyzed in a single batch, in November 2018 using adjusted methods from Benotti and Brownawell (2007) optimized to the analysis instrument.

In brief: a Kinetex XB-C18 column (2.1 × 100 mm, 1.7 μm particle size) was used with a sample injection volume of 5 μL for all samples. HPLC solvent A was 75% methanol, 25% acetonitrile with 0.3% acetic acid, and solvent B was 100% water with 0.3% acetic acid. The samples

Table 2
Design parameters of nitrogen removing biofilters installed in Suffolk County.

Parameter	Lined #1	Lined #2	Lined #3	Woodchip box	Unlined #1	Unlined #2	Unlined #3
Design length (m)	12.07	9.30	6.40	12.07	14.33	15.85	15.85
Design width (m)	6.55	7.32	4.27	6.55	6.55	5.28	5.28
Nitrification filter depth (cm)	46	46	46	46	46	46	46
Denitrification layer depth (cm)	46	46	46	N/A	30	46	46
Denitrification layer (tank*) volume (L)	36,367	31,315	12,571	5678*	28,158	38,496	38,496
Design flow (L d^{-1})	2080	2080	830	2080	1666	1666	1666
Normalized loading rate (L d^{-1} m^{-2})	31	31	31	31	20	31	31
HRT[a] (d^{-1}) of denitrifying phase	7.9	6.8	6.8	1.2	N/A	N/A	N/A
Months in operation	16	5	1	7	16	9	9

[a] Hydraulic retention time (HRT) was calculated by assuming a porosity value of 0.45 for the lined denitrification layer and 0.5 for the woodchip box. The woodchip box was estimated to be filled to 9/10th of its height with woodchips. HRT was not calculated for the denitrification layers of unlined systems.

were eluted at 450 μL/min with a 40-min gradient beginning with 95% solvent A, 5% solvent B. The initial ratio was maintained for 2 min and then ramped linearly to 5% solvent A, 95% solvent B at 32 min, and ramped linearly again to 1% solvent A, 99% solvent B at 34 min. That ratio was maintained for 3 min before returning to the initial ratio by 40 min. Sample concentrations and removal efficiencies are reported here for 11 organic contaminants including antibiotics (trimethoprim and sulfamethoxazole), beta-blockers (metoprolol and atenolol), stimulants (caffeine and nicotine) and their primary human metabolites (paraxanthine and cotinine, respectively), an NSAID (acetaminophen), a mosquito repellant (DEET), and an antihistamine (diphenhydramine).

3. Results

3.1. Massachusetts alternate septic system test center installations

Between system installations during the fall of 2016 and December 2018, mean (± standard deviation) TN concentrations in final effluent for the lined, woodchip box, and unlined NRBs were 7.1 ± 7.8, 4.3 ± 4.2, and 6.9 ± 8.4 mg-N L^{-1}, respectively, representing TN reductions of 83%, 87%, and 84% from influent TN which averaged ~43 ± 10.5 mg-N L^{-1} (Fig. 3; Tables 3-5). Three quarters of effluent TN observations over the period fell below 10.1, 5.4, and 9.2 mg- N L^{-1} for the lined, woodchip box, and unlined systems, respectively.

As anoxic STE percolated through each sand bed, wastewater TKN was efficiently transformed to NO_3^- as only 3%–7% of TN remained as TKN in samples downstream of the sand beds (Tables 3-5) over the entire sampling period. Within sand bed effluent, TKN varied between 1.4 and 3.6 mg-N L^{-1} and comprised primarily organic N while NH_4^+ was consistently below 1 mg-N L^{-1} (Tables 3-5). A substantial portion of influent TKN (17%–37%) was not recovered as any form of dissolved N in samples downstream of the sand beds and was apparently removed to the gas phase (Tables 3-5).

Contrasting the first 15 months of operation with the subsequent 12 months, TN in final effluent of the unlined system decreased from 7.6 to 6.2 mg-N L^{-1} (Table 3); TN in final effluent of the woodchip box system and the unlined NRB increased from 2.9 to 6.5 mg-N L^{-1} (Table 4) and from 4.7 to 9.3 mg-N L^{-1} (Table 5). Collectively, the NRBs did not show a significant change between the two periods ($p > 0.05$).

Seasonal effects were observed for all three systems. The TN concentrations in final effluent during the January–April period ("winter") were 13.0, 8.2, and 14.6 mg-N L^{-1} for the lined, sand bed and woodchip

Table 3
Nitrogen transformations in lined nitrogen removing biofilter at Massachusetts Alternative Septic System Test Center. Units are mg-N L^{-1} except percentages. Month ranges are for entire sampling period.

	9/19/16–12/31/18	9/19/16–12/31/17	12/31/17–12/31/18	Jan–Apr	May–Dec
Influent					
TKN	42.8	37.2	48.2	38.5	44.5
Samples downstream of sand bed					
TKN	2.7	2.4	3.2	2.1	2.9
NO_3^-/NO_2^-	32.9	31.6	36.6	30.6	34.0
TN	35.6	34.0	39.8	32.7	36.9
Final effluent samples					
TKN	2.92	3.53	1.91	2.8	3.0
NO_3^-/NO_2^-	4.16	4.08	4.29	10.2	2.4
TN	7.08	7.61	6.20	13.0	5.4
Analysis					
TN removed in sand bed	7.14	3.17	8.4	5.7	7.5
Denitrification	28.8	27.6	32.3	20.4	31.6
Denitrification efficiency	87%	87%	88%	67%	93%
Fate of N as % influent TKN					
% N removed in sand bed	17%	9%	17%	15%	17%
% N denitrified	67%	74%	67%	53%	71%
% N final effluent	17%	22%	14%	34%	12%
Mass balance closure	101%	105%	99%	102%	100%

Table 4
Nitrogen transformations in woodchip box nitrogen removing biofilter at Massachusetts Alternative Septic System Test Center. Units are mg- N L^{-1} except percentages.

	9/19/16–12/31/18	9/19/16–12/31/17	12/31/17–12/31/18	Jan–Apr	May–Dec
Influent					
TKN	42.8	37.2	48.2	38.5	44.5
Samples downstream of sand bed					
TKN	1.5	1.5	1.5	1.5	1.5
NO_3^-/NO_2^-	25.4	23.6	28.6	23.1	26.5
TN	27.0	25.1	30.1	24.6	28.0
Final effluent samples					
TKN	1.67	1.64	1.73	1.54	1.72
NO_3^-/NO_2^-	2.59	1.27	4.77	6.68	2.03
TN	4.26	2.91	6.50	8.21	3.75
Analysis					
TN removed in sand bed	15.8	12	18.2	13.8	16.4
Denitrification	22.8	22.3	23.8	16.5	24.5
Denitrification efficiency	90%	95%	83%	71%	92%
Fate of N as % influent TKN					
% N removed in sand bed	37%	32%	38%	36%	37%
% N denitrified	53%	60%	49%	43%	55%
% N final effluent	10%	8%	13%	21%	8%
Mass balance closure	100%	100%	100%	100%	100%

Table 5
Nitrogen transformations in unlined nitrogen removing biofilter at Massachusetts Alternative Septic System Test Center. Units are mg- N L^{-1} except percentages.

	9/19/16–12/31/18	9/19/16–12/31/17	12/31/17–12/31/18	Jan–Apr	May–Dec
Influent					
TKN	42.8	37.2	48.2	38.5	44.5
Samples downstream of sand bed					
TKN	1.4	1.8	1.4	2.6	1.1
NO_3^-/NO_2^-	28.2	25.5	30.1	24.4	29.3
TN	29.7	27.3	31.5	27.0	30.4
Final effluent samples					
TKN	2.19	1.70	2.23	2.05	2.22
NO_3^-/NO_2^-	4.69	3.03	7.06	12.6	1.89
TN	6.88	4.73	9.28	14.65	4.11
Analysis					
TN removed in sand bed	13.1	9.9	16.7	11.4	14.1
Denitrification	23.6	22.5	23.1	11.8	27.5
Denitrification efficiency	83%	88%	77%	48%	94%
Fate of N as % influent TKN					
% N removed in sand bed	31%	27%	35%	30%	32%
% N denitrified	55%	61%	48%	31%	62%
% N final effluent	16%	13%	19%	38%	9%
Mass balance closure	102%	100%	102%	99%	103%

box, and unlined NRBs – significantly higher ($p < 0.05$) than the corresponding means for other periods of the year which were 5.4, 3.8, and 4.1 mg-N L^{-1} (Tables 3-5). The higher effluent TN occurred even though TKN in influent was lower in winter (38.5 mg-N L^{-1}) than in non-winter (44.5 mg-N L^{-1}). Consequently, percentage of N removal dropped from 88% to 66% of influent TKN for the lined NRB, from 92% to 79% from the woodchip box system and from 91% to 62% from the unlined NRB. Seasonally high TN concentrations comprised primarily NO_3^-. There were no large differences in TN removal in the nitrifying sand beds between winter and the remainder of the year. However, denitrification of NO_3^- was lower in winter for all systems (mean = 20.4, 16.5 and 11.8 mg-N L^{-1} for lined, woodchip box, and unlined systems) compared with corresponding averages for the rest of the year (mean = 31.6, 24.5 and 27.5 mg-N L^{-1}) over the ~2.25 years the NRBs were monitored (Table 3-5). Accordingly, denitrification efficiency (1-(average NO_3^- in final effluent)/(average NO_3^- in nitrified percolate)) decreased from 93, 94, and 92% for lined, woodchip box, and unlined systems, respectively, during May – December to 67, 71, and 48%, respectively, in winter (Tables 3-5).

After treatment operations commenced during the fall of 2016, BOD_5 in final effluent samples was ~90 mg-L^{-1} from the lined NRB and ~600 mg-L^{-1} from the woodchip box system (Fig. 5). The BOD_5 concentrations fell sharply to <30 mg L^{-1} after ~ one month for the lined NRB and after ~ two months for the woodchip box system; measurements of BOD_5 from the unlined NRB did not commence until after 12 months when concentrations had fallen to low levels. Over the entire monitoring period, BOD_5 averaged 12, 64, and 2.3 mg L^{-1} in the lined, woodchip box, and unlined systems compared with a level of 215 mg L^{-1} in wastewater influent. Excluding the first two months of operation, BOD_5 in final effluent for the woodchip box system averaged 16 mg L^{-1}.

The NRBs at MASSTC also efficiently removed TSS and indicator bacteria and altered alkalinity and pH levels (Table 6). Fecal coliform bacteria were reduced by more than five-orders of magnitude to a mean of 13, 18, and 21 colony forming units per 100 mL in effluent in the lined, woodchip box, and unlined NRBs, respectively, while TSS were lowered from >180 mg L^{-1} in influent to <35 mg L^{-1} in all systems (Table 6). Alkalinity decreased from 181 mg-$CaCO_3^-$ L^{-1} in influent to 47 (unlined), 49 (woodchip box), and 23 (unlined) mg-$CaCO_3^-$ L^{-1} downstream of the sand bed and lowered pH from 6.88 to 5.86, 6.26, and 5.58, respectively (Table 6).

Table 6
Mean (±S.D.) analyte concentrations in lined, woodchip box and unlined nitrogen removing biofilters (NRB) installed in Massachusetts Alternative Septic System Test Center over the period from inception fall 2016 to December 2018.

Location	Alkalinity	pH	TSS	BOD_5	Fecal coliform
Influent	182 ± 49	6.9	181 ± 61	215 ± 68	7.76 E06 ± 7.08 E06
Lined NRB					
Bottom, sand layer	56 ± 54	5.9			181 ± 107
Effluent	182 ± 50	6.3	12 ± 12	12 ± 21	31 ± 79
Woodchip Box NRB					
Bottom, sand layer	49 ± 17	6.3	10 ± 10	1 ± 1	250 ± 796
Effluent	117 ± 27	6.6	5 ± 3	64 ± 145	18 ± 31
Unlined NRB					
Bottom, sand layer	30 ± 24	5.6	63 ± 101		682 ± 2053
Effluent	168 ± 60	6.0	35 ± 38	2 ± 2	21 ± 56

Note: Alkalinity in mg L^{-1} as $CaCO_3$, pH, total suspended solids (TSS) in mg L^{-1}, five-day biochemical oxygen demand (BOD_5 in mg L^{-1}) and fecal coliform bacteria in colony forming units per 100 mL. BOD_5 in final effluent of woodchip box includes measurements from initial start-up; measured from two months after start-up, BOD_5 is 16 mg-L^{-1}.

The NRBs at MASSTC were also highly efficient at removing all organic contaminants measured including the antibiotics, trimethoprim and sulfamethoxazole, the beta-blocker, atenolol, the NSAID, acetaminophen, the mosquito repellant, DEET, the antihistamine, diphenhydramine, and the stimulants, caffeine and nicotine, together with their primary human metabolites, paraxanthine and cotinine. Removal of these compounds was 90% or greater (Fig. 6). The only compound not removed by >90% was the beta-blocker, metoprolol, which averaged 76 ± 12% removal across all systems sampled (Fig. 6). There were no substantial differences in removal efficiencies among the three different NRBs.

3.2. Suffolk County experimental installations

In SC, seven NRB prototypes (three lined, one woodchip box, and three unlined) were installed at individual residences between spring of 2018 and summer of 2019 and sampled monthly starting after roughly one month of operation. TN in STE averaged 81.2 ± 32.2, 93.5 ± 7.75, and 73.2 ± 27.4 mg-N L^{-1} in lined, woodchip box, and unlined systems, respectively, while TN in final effluent averaged 8.35 ± 9.20, 5.33 ± 3.65, and 8.74 ± 4.90 mg-N L^{-1} representing reductions of 90, 94, and 88% (Fig. 4). For three-quarters of sampling dates, the lined, woodchip box, and unlined NRBs produced effluent with less than 10.0, 8.15, and 11.6 mg-N L^{-1}, respectively. On average, the lined NRBs, woodchip box NRB, and unlined NRBs achieved fecal coliform reductions of four, three, and one order(s) of magnitude and BOD_5 reductions of 76%, 90%, and 44%, respectively (Table 7). Alkalinity also decreased in the sand beds compared to influent by 32%, 75%, and 30% for lined, woodchip box, and unlined systems (Table 7).

4. Discussion

The simple, mostly gravity-driven sand and woodchip biofilters described here remove 80%–90% of N, 90%–99% of organic contaminants and almost all BOD_5, TSS, and fecal coliform from STE. In addition, they remove >90% of wastewater phosphorous (Wehrmann et al., 2020). The suitability of individual designs to specific site conditions as well as relative performance and controls on N and organic contaminant removal are discussed below.

4.1. Design applications

Each of the three NRB designs presented has distinguishing features more or less suitable for different site characteristics. The effluent from

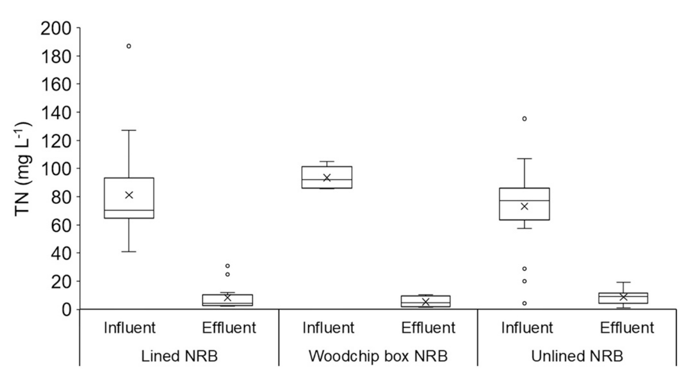

Fig. 4. Total nitrogen (TN) concentrations in final effluent and influent of nitrogen removing biofilters (NRBs) installed in Suffolk County. Medians, indicated by mid-line in box, are 4.2, 4.7, and 9.1 mg-N L^{-1} for the lined, woodchip box, and unlined systems, respectively; 75th percentiles, indicated by the top lines of the boxes, are 10.0, 8.2, and 11.6 mg-N L^{-1}. Bottom lines represent 25th percentiles, × symbols represent means, and whiskers span to the range of non-outlier data points (those falling within 1.5 times the interquartile range).

Table 7
Mean (±S.D.) analyte concentrations in lined, woodchip box and unlined nitrogen removing biofilters (NRB) installed in Suffolk County during 2018 and 2019.

Location	Alkalinity	pH	BOD_5	Fecal coliform
Lined NRBs				
Influent	264 ± 138	6.39	396 ± 157	121,000 ± 71,786
Bottom, sand layer	179 ± 108	6.38	30 ± 44	
Effluent	332 ± 133	6.33	96 ± 158	47 ± 66
Woodchip Box NRB				
Influent	417 ± 55	7.33	51 ± 27	31,000 ± 24,042
Bottom, sand layer	103	6.18	3 ± 0.4	
Effluent	260 ± 96	6.45	5 ± 2	86 ± 37
Unlined NRBs				
Influent	318 ± 93	6.94	201 ± 62	90,000 ± 88,198
Bottom, sand layer	224 ± 154	5.43	4 ± 2	
Effluent	380 ± 304	6.15	111 ± 159	2043 ± 5947

Note: Alkalinity in mg L^{-1} as $CaCO_3$, five-day biochemical oxygen demand (BOD_5 in mg L^{-1}), and fecal coliform bacteria in colony forming units per 100 mL.

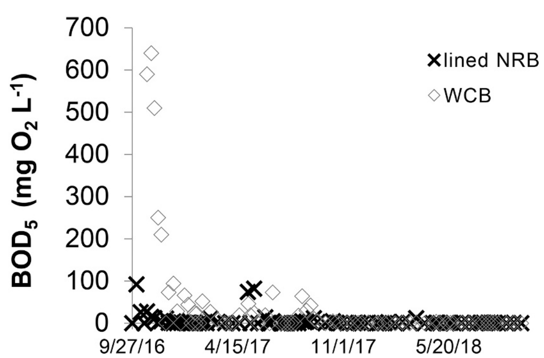

Fig. 5. Biological oxygen demand (BOD_5) in final effluent samples from start-up in fall 2016 through December 2018 for lined nitrogen removing biofilter (NRB) and woodchip box system at Massachusetts Alternative Septic System Test Center. Unlined NRB not shown as initial BOD_5 measurements were not recorded until after start-up.

the unlined NRB flows directly to groundwater whereas the lined and woodchip box NRBs require final disposal either to leaching rings, galleys, or a separate drainfield and, consequently, require more depth above groundwater than the unlined system. Since unlined NRBs do not require a liner, installation is simpler and less expensive compared with lined and woodchip box designs. While dosing multiple times a day will maintain water capacity in the sand and woodchip mix (Graffam, 2020), the unlined biofilter will likely be subject to continually changing degrees of oxygen saturation whereas the woodchips in the lined and woodchip boxes will remain continually anoxic. Consequently, a higher proportion of bioavailable carbon inventories may be consumed by aerobic remineralization than in denitrification over the life of the unlined system (Moorman et al., 2010). The difference in denitrification efficiency between lined and woodchip box NRBs compared to the unlined NRB (87% and 90% versus 83% from inception to December 2018) may reflect greater aerobic remineralization in the unlined system.

Multiple field and lab studies have reported initially high rates of N removal in wood-based biofilters declined during the first year of operation and then stabilized at lower levels in subsequent years (Robertson et al., 2008; Cameron and Schipper, 2010; Robertson, 2010; Addy et al., 2016). This attenuation has been attributed to lower inventories of labile carbon available after an initial period of rapid leaching of DOC in the early stage of biofilter operation (Robertson, 2010; Addy et al., 2016). Average N removal efficiencies in the lined system at MASSTC did not decline during the first 15 months of operation compared to the next 12 months (Table 3). For the woodchip box system and the unlined system, the percentage of NO_3^- removed declined slightly over this period (Tables 4-5) but not to the extent (>50%) reported by other researchers (Robertson, 2010; Addy et al., 2016). Uncertainty over carbon longevity (Robertson, 2010) is overcome in the woodchip box system which is designed so woodchips can be replaced as needed and therefore may extend the effective life of these systems far beyond estimates for buried woodchip biofilters (Robertson, 2010).

As near passive systems made primarily of natural materials (sand, wood), the parts and supplies for NRBs are inexpensive. The major expense of NRBs are labor costs associated with excavating sites and installing system components. Design advances that reduce costs will require smaller foot-prints and/or reduced installation times and may include recirculating flow and/or reuse of existing onsite infrastructure as permittable. The lignocellulose biofilter portion of NRBs could also be reduced in size and adapted as polishing units, perhaps as lignocellulose-amended drainfields or as upflow saturated woodchip boxes, to remove residual NO_3^- in effluent of some commercial innovative/alternative onsite wastewater treatment systems (I/A OWTS).

4.2. N removal performance comparisons

Differences in influent composition (Friedler and Butler, 1996; Whelan and Titamnis, 1982), loading rates (often unknown in residential settings) and environmental conditions make comparisons between onsite residential wastewater systems difficult. Similar N removal rates were obtained over six years for sand and woodchip biofilters installed below sand receiving septic drainage (74% N removal) and nitrified percolate (80% N removal) (Robertson and Cherry, 1995; Robertson et al., 2000); however, residence times for these systems were much longer (15–40 days) and influent N concentrations were more variable than for systems described here (Robertson et al., 2000). Additionally, these systems were seasonal summer residences whereas the systems described here operated continuously year-round in a temperate climate. Performance for all three designs described here also achieved broadly similar N removal to those reported by Hirst and Anderson (2015) for a NRB configuration with an unsaturated sand bed and saturated denitrifying biofilters prior to sulfur reduction in a subtropical environment (FL, USA). Combined, these reports offer substantial evidence that simple sand beds coupled to wood-based biofilters offer substantial benefits in TN removal from residential wastewater compared with presently available proprietary onsite wastewater systems (Oakley et al., 2010).

Influent TN concentrations in the NRB systems installed in Suffolk County (SC) were more than double the level at MASSTC. Actual flow rates were available for one NRB each for lined, woodchip box and unlined designs in SC (390, 214 and 644 L d^{-1}) and, at these loading rates, N was removed from STE at mean rates of 29, 19 and 45 g d^{-1} respectively. Corresponding rates at MASSTC were 32, 34 and 32 g d^{-1} for lined, woodchip box and unlined NRBs so the three designs' overall averages were similar, i.e., 31 g d^{-1} for SC NRBs and 33 g d^{-1} for MASSTC NRBs. Normalized to drainfield area, however, MASSTC N removal rates were higher (1.0, 1.1 and 1.0 g m^{-2} d^{-1}) than corresponding rates in SC (0.37, 0.20 and 0.48 g m^{-2} d^{-1}) because of the larger footprint of NRBs installed in SC.

4.3. Factors regulating N removal performance

The use of a common source of wastewater for the three NRBs at MASSTC afforded direct performance comparisons between the different NRB designs. Relative N removal performance among all systems was influenced by a number of factors including (1) quantity of TN removed in sand beds; (2) extent and duration of attenuation of denitrification in winter (i.e., temperature) and (3) in most, but not all cases, wastewater residence time in the biofilters. The mechanisms and environmental controls regulating N and organic contaminant removal

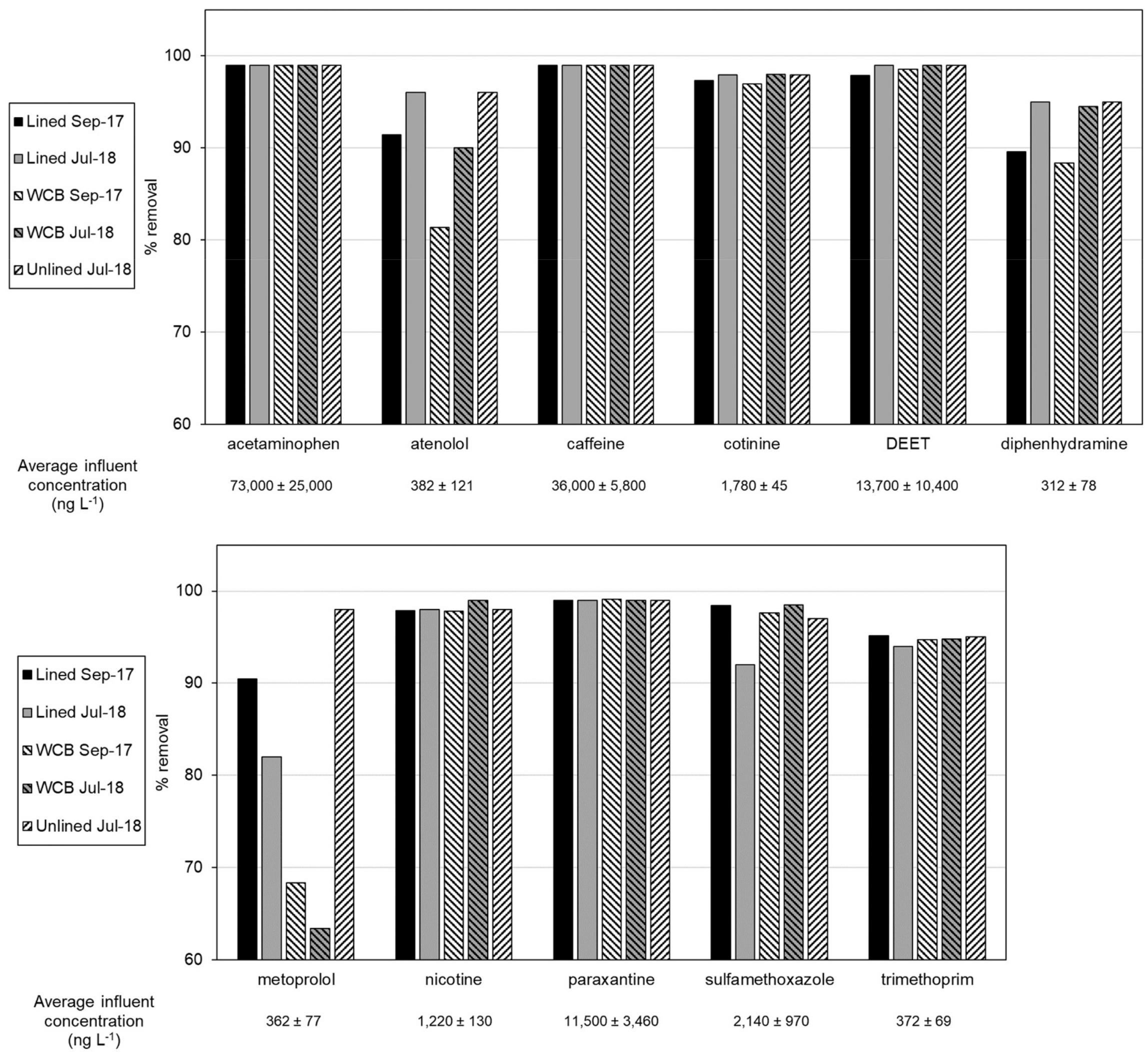

Fig. 6. Organic contaminant removal from nitrogen removing biofilters with three different designs (lined, woodchip box system and unlined) at the Massachusetts Alternate Septic System Test Center sampled in September 2017 and July 2018.

among installed NRBs are discussed below.

4.3.1. Biogeochemistry of sand beds

The simple process of percolation of wastewater through sand beds yielded highly efficient conversion of TKN to NO_3^- in all systems installed at MASSTC (mean > 95%) (Table 3-5). Alkalinity was not limiting as almost all NH_4^+ was removed by each system. Wastewater contains alkalinity (Lowe et al., 2009), and, based on a theoretical stoichiometric consumption rate of 7.1 g-$CaCO_3$/g-N as NH_4^+ oxidized to NO_3^- (Prosser, 1990; Li and Irvin, 2007), available alkalinity in wastewater should have only been sufficient to transform ~25 mg-N L^{-1} as NH_4^+ to NO_3^-. However, TKN decreased to <3 mg-N L^{-1} from >42.8 mg-N L^{-1} in influent in all cases, so either (1) the stoichiometric assumption does not apply to nitrification within sand beds, (2) incremental alkalinity was supplied by the sands themselves and/or (3) it was produced by denitrification in anoxic micro-zones within the sand beds (Gerardi, 2006). Across all systems in SC, mean influent alkalinity was ample and higher (264, 417, and 313 mg-$CaCO_3$ L^{-1} for lined, woodchip box, and unlined systems) than at MASSTC (182 mg-$CaCO_3$ L^{-1}) (Tables 6 & 7).

While the sand beds of all three NRB designs displayed substantial TN removal, there was more occurring within the sand bed of the woodchip box system at MASSTC (37%) than the other two NRB designs (17% lined and 31% unlined, respectively) (Tables 3-5). Removal of TN was also observed in the SC NRBs where loss in the sand layers averaged 58% in the lined systems, 62% in the woodchip box system, and 71% in the unlined system. Given the high levels of BOD_5 within these layers, there clearly exists an adequate carbon supply necessary for NO_3^- to be denitrified under anoxic conditions. Facultative microbes capable of denitrification in low oxygen environments have been found in effluent of sand beds (Langlois et al., 2020). Thus, TN removal in sand beds may have occurred by denitrification in anoxic micro-zones in sand beds using wastewater carbon as an electron donor (e.g. Waugh et al., 2020; Oh and Silverstein, 1999). It is not clear, however, why this process should have occurred more extensively or at a greater rate in the sand bed of the woodchip box system at MASSTC compared with those in the layered systems as all three sand beds have the same dimensions. The primary difference between the sand bed of the woodchip box system and those of the layered systems is that it was positioned above gravel drainage from which nitrified percolate was channeled to the woodchip box whereas the sand beds in the layered systems lie directly above the denitrifying sand and woodchip layers. Water infiltration is slowed when percolate moves from finer-textured to coarser-textured matrices due to differences in water potential (Amador and Loomis, 2019); whether this transition could increase the HRT of percolate in the sand bed enough to create an anoxic zone and thereby account for the difference in N removal compared with the layered systems is unknown. A final point regarding the sand beds is that, while Rambags et al. (2016) demonstrated biofilters comprised of sand and woodchips effectively remove BOD_5, TSS, and fecal coliform from wastewater, this study, consistent with Hirst and Anderson (2015), shows these pollutants can be largely removed in the sand beds alone (Tables 6 & 7).

4.3.2. Seasonality of NO_3^- removal in woodchip biofilters

Significant ($p < 0.05$) seasonal variation in N removal by NRBs was driven by lower denitrification rates during winter periods (January–April). Sand beds of the MASSTC NRBs nitrified nearly all of wastewater N through the entire year with no substantial temperature effects, while NO_3^- levels in effluent varied substantially and effluent TKN levels were little changed (~2 mg-N L^{-1}) between winter and non-winter periods (Tables 3-5). Temperature dependence of NO_3^- removal has been reported in field and pilot studies of wood-based biofilters (Robertson et al., 2000; van Driel et al., 2006; Cameron and Schipper, 2010; Addy et al., 2016). The seasonal attenuation of denitrification in winter may be attributed to lower demand for NO_3^- as an electron acceptor in heterotrophic activity because of the higher availability of more thermodynamically favorable dissolved oxygen in nitrified percolate which will be at higher concentrations due to increased solubility at colder temperatures. Higher ambient dissolved oxygen levels will require greater rates of bacterial respiration to create anoxic conditions needed for denitrification. In addition, lower temperatures may slow the metabolism of denitrifying bacteria and/or cellulolytic bacteria that provide dissolved carbon required for heterotrophic denitrification.

The difference in seasonal N removal in the sand beds compared with the woodchip biofilters (Table 3–5) may shed light on the mechanism controlling the seasonality in N removal. If TN was removed by denitrification in spatially or temporally anoxic microzones in the sand beds, then the required carbon could only have been delivered in the wastewater itself. Unlike carbon from woodchips, this carbon would have been available to denitrifying bacteria without the need for initial breakdown to DOC by cellulolytic bacteria. Directly bioavailable substances in wastewater, such as acetic acid, simple carbohydrates, alcohols, and amino acids could support heterotrophic denitrification (Henze et al., 1994). Given that TN removal did not slow in the sand beds in winter but did in each woodchip biofilter, it seems likely cold temperatures inhibited activity by cellulolytic bacteria rather than by denitrifying bacteria which seemed to perform at similar rates within sand beds year-round. The importance of cellulolytic bacteria in rendering C available for denitrifying bacteria is also suggested by the timescale of seasonal N removal (Table 3–5). Attenuation of denitrification did not show a direct monthly correlation with temperature but lagged by > one month (monthly data not shown) so N removal continued at a high rate through December, attenuated in January and did not increase again until May. This lagged relationship between N removal and temperature is consistent with the hypothesis denitrification varies directly with the bioavailability of C inventories which take time to accumulate or decrease based on production of temperature sensitive cellulolytic bacteria and consumption by denitrifying bacteria.

4.3.3. Residence time in woodchip biofilters

The woodchip box received less NO_3^- in nitrified percolate than the sand and woodchip biofilters of the layered systems at MASSTC but removed slightly more of it than the lined and unlined systems (90% against 87 and 83%, respectively) (Table 3–5). This result was achieved even though nitrified percolate had a substantially lower residence time in the woodchip box (1.4 d at a loading rate of 1250 L d^{-1}) than in the lined NRB (3.9 d at same loading rate; residence time in the biofilter of the unlined system was not calculated). Longer residence times generally have a direct relationship with NO_3^- removal (e.g., Addy et al., 2016; Martin et al., 2019). Consistent with these findings, the N removal from the lined SC system (residence time 7.8 d) operating for more than six months was higher than either the lined or woodchip box systems at MASSTC. The estimated volume of woodchips in the lined NRB (2.75 m^3 assuming porosity of sand and woodchips = 0.45 and sand: woodchip volume ratio of 1:1) is also greater than the volume of woodchips in the box (~1.70 m^3 assuming ratio of woodchips: total volume = 0.5) so the greater denitrification efficiency of the box is not caused by the volume of woodchips. The woodchips used in all NRBs were oak and pine of varying particle sizes; however, these variations likely do not account for performance differences as lab and field tests have not found a strong correlation between woodchip particle size and NO_3^- removal (van Driel et al., 2006; Cameron and Schipper, 2010; Schmidt and Clark, 2013). We hypothesize that the full strength of woodchips in the boxes offer a high concentration of organic carbon to support more rapid denitrification than the 50:50 mix of sand and woodchips in the other NRB designs.

4.4. Removal of organic contaminants in pharmaceuticals and personal care products

All three NRB designs removed >90% of 10 of 11 different pharmaceuticals, antibiotics, and other organic contaminants measured in wastewater influent at MASSTC (metoprotol removals were 82%, 63% and 98% for lined NRB, woodchip box system, and unlined NRBs, respectively). Some of the compounds measured here such as metoprolol and diphenhydramine have been found to have removals of <30% in conventional wastewater treatment plants (WWTPs; Jelic et al., 2010; Ryu et al., 2014). Other compounds including atenolol, DEET, paraxanthine, trimethoprim, and sulfamethoxazole which are often removed ~50%–80% in conventional WWTPs (Benotti and Brownawell, 2007; Jelic et al., 2010; Ryu et al., 2014; Schaider et al., 2017), were removed at 90%–>99% in all three NRBs at MASSTC. Compounds with reported removals near 90% in WWTPs, such as cotinine and nicotine (Benotti and Brownawell, 2007), were removed at 96%–>99%. The consistently highest removals in the NRBs were observed for acetaminophen and caffeine, removed at >99% in every system tested. These compounds often also have removals >99% in conventional WWTPs (Benotti and Brownawell, 2007; Ryu et al., 2014).

Relative to removal in septic drainfields and other onsite alternative residential wastewater treatment systems reported in Schaider et al. (2017) the three NRB installations at MASSTC achieved similar removal of acetaminophen, caffeine and paraxanthine (>99%) but far higher removal for the antibiotic sulfamethoxazole (>90% versus 40% and 28% for drainfields and alternative systems, respectively) and for DEET (>99% versus 88% for drainfields). For the antibiotic trimethoprim, removal was greater than in drainfields (>90% versus 60%) but slightly less than that in other alternate systems (>90% versus >99%). The antihistamine diphenhydramine showed similar removal in the three NRB installations (95% versus >99% for both drainfields and alternative systems) (Schaider et al., 2017).

Removal of organic contaminants in the NRB systems is hypothesized to be due to microbial activity within the nitrification zones as many nitrifying bacteria have been shown to efficiently degrade organic compounds (Eichhorn et al., 2005; Matamoros and Bayona, 2006; Ooi et al., 2018; Reis et al., 2020). The high levels of labile organic carbon in wastewater streams entering oxic, nitrifying sand layers may offer advantages in microbial degradation of organic contaminants over systems where BOD is removed prior to nitrification. Other compounds such as sulfamethoxazole have been found to have high removal by anaerobic digestion (Carballa et al., 2007). The design of the NRBs creates within one system, zones for targeting aerobic and anaerobic treatment. This setup, along with likely naturally occurring microenvironments throughout the NRB layers and adsorption sites on the sand and woodchips, clearly removes high levels of a variety of organic contaminants. Finally, the longer HRT in NRBs (days) compared to WWTPs (hours) may allow bacteria more time to degrade organic compounds.

5. Conclusions

This study supports the conclusions of Oakley et al. (2012) that a sand bed coupled to woodchip-based biofilter may offer compelling alternatives to proprietary, mechanized and energy-intensive innovative/

alternative onsite wastewater treatment systems (I/A OWTS). The designs described here have consistently reduced TN by 80%–90% and organic contaminants by 90%–99%. Additionally, they remove almost all BOD_5, TSS, fecal coliform and >90% of phosphorus (Wehrmann et al., 2020) from septic tank effluent. Commercially available I/A OWTS are typically more energy- and equipment-intensive, relying on pumps and aerators to create sequential oxic and anoxic conditions required to facilitate nitrification and denitrification. For I/A OWTS that generate residual NO_3^- in effluent, sand and woodchip or strictly woodchip biofilters may provide a useful 'polishing step' to reduce TN to <10 mg-N L^{-1}.

Credit author statement

Christopher J. Gobler: Conceptualization, Writing - Original Draft, Investigation, Formal analysis, Supervision, Project administration, Funding acquisition.

Stuart Waugh: Methodology, Writing - Original Draft, Investigation, Formal analysis, Data curation, Visualization.

Caitlin Asato: Methodology, Writing - Original Draft, Investigation, Formal analysis, Data curation, Visualization.

Patricia M. Clyde: Investigation, Writing - Original Draft, Visualization.

Samantha C. Nyer: Investigation, Writing - Original Draft.

Molly Graffam: Investigation, Writing - Review & Editing.

Bruce Brownawell: Formal analysis.

Arjun K. Venkatesan: Formal analysis.

Jennifer A. Goleski: Investigation.

Roy E. Price: Supervision, Formal analysis.

Xinwei Mao: Formal analysis.

Frank M. Russo: Supervision, Formal analysis.

George Heufelder: Methodology, Investigation, Resources.

Harold W. Walker: Funding, data analysis.

Declaration of Competing Interest

None.

Acknowledgements

This study was, and the NYS CCWT is, funded by the New York State Department of Environmental Conservation [DEC01-C00366GG-3350000]. The authors are grateful for the assistance of Damann Anderson, Josefin Hirst, and Steve Cluff from Hazen and Sawyer for their assistance in drafting the designs for these NRBs and David Potts from Geomatrix and Jose Amador from the University of Rhode Island for useful discussions on the design and functionality of NRBs. The authors also thank the Chemistry Department of Stony Brook University for use of the HPLC-TOF-MS instruments, as well as Dr. Béla Ruzsicska for his help with the instrumental analysis. In addition, the authors acknowledge Stony Brook University graduate students Zahra Maleki Shahraki and Sarah Lotfikatouli for their assistance in sampling and data analysis.

References

Addy, K., Gold, A.J., Christianson, L.E., David, M.B., Schipper, L.A., Ratigan, N.A., 2016. Denitrifying bioreactors for nitrate removal: a meta-analysis. J. Environ. Qual. 45 (3), 873–881. https://doi.org/10.2134/jeq2015.07.0399.

Amador, J.A., Loomis, G.W., 2019. Water in the soil treatment area. In: Soil-Based Wastewater Treatment. American Society of Agronomy, Incorporated, Soil Science Society of America, Incorporated, Crop Science Society of America, Incorporated, Madison, WI, pp. 133–158. https://doi.org/10.2134/sbwtreatment.

Benotti, M.J., Brownawell, B.J., 2007. Distributions of pharmaceuticals in an urban estuary during both dry-and wet-weather conditions. Environ. Sci. Technol. 41 (16), 5795–5802. https://doi.org/10.1021/es0629965.

Cameron, S.C., Schipper, L.A., 2010. Nitrate removal and hydraulic performance of carbon substrates for potential use in denitrification beds. Ecol. Eng. 36, 1588–1595. https://doi.org/10.1016/j.ecoleng.2010.03.010.

Carballa, M., Omil, F., Ternes, T., Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Res. 41 (10), 2139–2150. https://doi.org/10.1016/j.watres.2007.02.012.

Dougherty, J.A., Swarzenski, P.W., Dinicola, R.S., Reinhard, M., 2010. Occurrence of herbicides and pharmaceutical and personal care products in surface water and groundwater around Liberty Bay, Puget Sound, Washington. J. Environ. Qual. 39 (4), 1173–1180. https://doi.org/10.2134/jeq2009.0189.

van Driel, P.W., Robertson, W.D., Merkley, L.C., 2006. Denitrification of agricultural drainage using wood based reactors. Trans. ASAE 48, 121–128. https://doi.org/10.13031/2013.20391.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., Franson, M.A.H., 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.

Eichhorn, P., Ferguson, P.L., Pérez, S., Aga, D.S., 2005. Application of ion trap-MS with H/D exchange and QqTOF-MS in the identification of microbial degradates of trimethoprim in nitrifying activated sludge. Anal. Chem. 77 (13), 4176–4184. https://doi.org/10.1021/ac050141p.

Espejo-Herrera, N., Gràcia-Lavedan, E., Boldo, E., Aragonés, N., Pérez-Gómez, B., Pollán, M., Bosetti, C., 2016. Colorectal cancer risk and nitrate exposure through drinking water and diet. Int. J. Cancer 139 (2), 334–346. https://doi.org/10.1002/ijc.30083.

Friedler, E., Butler, D., 1996. Quantifying the inherent uncertainty in the quantity and quality of domestic wastewater. Water Sci. Technol. 33 (2), 65. https://doi.org/10.1016/0273-1223(96)00190-4.

Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc. B 369 (1656), 20130572. https://doi.org/10.1098/rstb.2013.0572.

Gerardi, M.H., 2006. Nitrifying bacteria. In: Wastewater Bacteria. Wiley, Hoboken, NJ, pp. 77–90. https://doi.org/10.1002/0471979910.

Gobler, C.J., Sunda, W.G., 2012. Ecosystem disruptive algal blooms of the brown tide species, *Aureococcus anophagefferens* and *Aureoumbra lagunensis*. Harmful Algae 14, 36–45. https://doi.org/10.1016/j.hal.2011.10.013.

Gobler, C.J., Burson, A., Koch, F., Tang, Y.Z., Mulholland, M.R., 2012. The role of nitrogenous nutrients in the occurrence of harmful algal blooms caused by *Cochlodinium polykrikoides* in New York estuaries (USA). Harmful Algae 17, 64–74. https://doi.org/10.1016/j.hal.2012.03.001.

Graffam, M., 2020. Hydro-Biogeochemical Dynamics in Woodchip-Based Subsurface Barriers: Implications for Nitrogen Removal and Pollution Swapping. Doctoral Dissertation. Stony Brook University (200pp).

Greer, F.R., Shannon, M., 2005. Infant methemoglobinemia: the role of dietary nitrate in food and water. Pediatrics 116 (3), 784–786. https://doi.org/10.1542/peds.2005-1497.

Hattenrath, T.K., Anderson, D.M., Gobler, C.J., 2010. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) Estuary. Harmful Algae 9 (4), 402–412. https://doi.org/10.1016/j.hal.2010.02.003.

Henze, M., Kristensen, G.H., Straube, R., 1994. Rate- capacity characterization of wastewater for nutrient removal processes. Water Sci. Technol. 27 (7), 101–107. https://doi.org/10.2166/wst.1994.0318.

Hinkle, S.R., Weick, R.J., Johnson, J.M., Cahill, J.D., Smith, S.G., Rich, B.J., 2005. Organic Wastewater Compounds, Pharmaceuticals, and Coliphage in Ground Water Receiving Discharge from Onsite Wastewater Treatment Systems near La Pine, Oregon: Occurrence and Implications for Transport; Scientific Investigation Report 2005–5055. U.S. Geological Survey, Reston, VA.

Hirst, J., Anderson, D., 2015. Backyard BNR: A passive nitrogen reduction system shows promising results for onsite wastewater treatment. Water Environ. Technol 27 (3), 40–43.

Howarth, R.W., 2008. Coastal nitrogen pollution: a review of sources and trends globally and regionally. Harmful Algae 8 (1), 14–20. https://doi.org/10.1016/j.hal.2008.08.015.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2010. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45 (3), 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010.

Jones, R.R., Weyer, P.J., DellaValle, C.T., Inoue-Choi, M., Anderson, K.E., Cantor, K.P., Krasner, S., Robien, K., Freeman, L.E.B., Silverman, D.T., Ward, M.H., 2016. Nitrate from drinking water and diet and bladder cancer among postmenopausal women in Iowa. Environ. Health Perspect. 124 (11), 1751–1758. https://doi.org/10.1289/EHP191.

Kinney, E.L., Valiela, I., 2011. Nitrogen loading to Great South Bay: land use, sources, retention, and transport from land to bay. J. Coast. Res. 27 (4), 672–686. https://doi.org/10.2112/JCOASTRES-D-09-00098.1.

Langlois, K., Gobler, C.J., Walker, H.W., Collier, J.L., 2020. Microbial communities in partially and fully treated effluent of three nitrogen-removing biofilters. J. Sustain. Water Built Environ 6 (2), 04020010. https://doi.org/10.1061/JSWBAY.0000912.

Latimer, J.S., Charpentier, M.A., 2010. Nitrogen inputs to seventy-four southern New England estuaries: application of a watershed nitrogen loading model. Estuar. Coast. Shelf Sci. 89 (2), 125–136. https://doi.org/10.1016/j.ecss.2010.06.006.

Li, B., Irvin, S., 2007. The comparison of alkalinity and ORP as indicators for nitrification and denitrification in a sequencing batch reactor (SBR). Biochem. Eng. J. 34 (3), 248–255. https://doi.org/10.1016/j.bej.2006.12.020.

Lopez-Ponnada, E.V., Lynn, T.J., Peterson, M., Ergas, S.J., Mihelcic, J.R., 2017. Application of denitrifying wood chip bioreactors for management of residential non-point sources of nitrogen. J. Biol. Eng. 11 (1), 16. https://doi.org/10.1186/s13036-017-0057-4.

Lowe, K.S., Tuchloke, M.B., Tomaras, J.M.B., Conn, K., Hoppe, C., Drewes, J.E., McCray, J.E., Munakata- Marr, J., 2009. Influent Constituent Characteristics of the Modern Waste Stream from Single Sources. Water Environment Research Foundation and IWA Publishing, London, UK.

Martin, E.A., Davis, M.P., Moorman, T.B., Isenhart, T.M., Soupir, M.L., 2019. Impact of residence time on nitrate removal in pilot-scale woodchip bioreactors. J. Environ. Manag. 237, 424–432. https://doi.org/10.1016/j.jenvman.2019.01.025.

Matamoros, V., Bayona, J.M., 2006. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ. Sci. Technol. 40, 5811–5816. https://doi.org/10.1021/es0607741.

Moorman, T.B., Parkin, T.B., Kaspar, T.C., Jaynes, D.B., 2010. Denitrification activity, wood loss and N_2O emissions over 9 years from a wood chip bioreactor. Ecol. Eng. 36, 1567–1574. https://doi.org/10.1016/j.ecoleng.2010.03.012.

Oakley, S.M., Gold, A.J., Oczkowski, A.J., 2010. Nitrogen control through decentralized wastewater treatment: process performance and alternative management strategies. Ecol. Eng. 36 (11), 1520–1531. https://doi.org/10.1016/j.ecoleng.2010.04.030.

O'Dell, J.W. (Ed.), 1993a. Method 350.1 Determination of Ammonia Nitrogen by Semi-Automated Colorimetry. US Environmental Protection Agency, Office of Research and Development, Environmental monitoring Systems Laboratory, Cincinnati, OH.

O'Dell, J.W. (Ed.), 1993b. Method 351.2 Determination of total Kjeldahl nitrogen by semi-automated colorimetry, Rev. 2.0. US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

O'Dell, J.W. (Ed.), 1993c. Method 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Oh, J., Silverstein, J., 1999. Oxygen inhibition of activated sludge denitrification. Water Res. 33 (8), 1925–1937. https://doi.org/10.1016/S0043-1354(98)00365-0.

Ooi, G.T., Tang, K., Chhetri, R.K., Kaarsholm, K.M., Sundmark, K., Kragelund, C., Christensson, M., Bester, K., Andersen, H.R., 2018. Biological removal of pharmaceuticals from hospital wastewater in a pilot-scale staged moving bed biofilm reactor (MBBR) utilising nitrifying and denitrifying processes. Bioresour. Technol. 267, 677–687. https://doi.org/10.1016/j.biortech.2018.07.077.

Pfaff, J.D., 1993. Method 300.0, Rev 2.1. Determination of inorganic anions by ion chromatography, Rev 2.1, US Environmental Protection Agency, Office of Research and Development. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Phillips, P.J., Schubert, C., Argue, D., Fisher, I., Furlong, E.T., Foreman, W., Gray, J., Chalmers, A., 2015. Concentrations of hormones, pharmaceuticals and other micropollutants in groundwater affected by septic systems in New England and New York. Sci. Total Environ. 2015 (512), 43–54. https://doi.org/10.1016/j.scitotenv.2014.12.067.

Prosser, J.I., 1990. Autotrophic nitrification in bacteria. In: Rose, A.H., Tempest, D.W. (Eds.), Advances in Microbial Physiology, vol. 30. Academic Press, Cambridge, MA, pp. 125–181. https://doi.org/10.1016/S0065-2911(08)60112-5.

Rambags, F., Tanner, C.C., Stott, R., Schipper, L.A., 2016. Fecal bacteria, bacteriophage, and nutrient reductions in a full-scale denitrifying woodchip bioreactor. J. Environ. Qual. 45, 847–854. https://doi.org/10.2134/jeq2015.06.0326.

Reis, A.C., Kolvenbach, B.A., Nunes, O.C., Corvini, P.F., 2020. Biodegradation of antibiotics: the new resistance determinants–part I. New Biotechnol. 54, 34–51. https://doi.org/10.1016/j.nbt.2019.08.002.

Robertson, W.D., 2010. Nitrate removal rates in woodchip media of varying age. Ecol. Eng. 36 (11), 1581–1587. https://doi.org/10.1016/j.ecoleng.2010.01.008.

Robertson, W.D., Cherry, J.A., 1995. In situ denitrification of septic-system nitrate using reactive porous media barriers: field trials. Ground Water 33 (1), 99–111. https://doi.org/10.1111/j.1745-6584.1995.tb00266.x.

Robertson, W.D., Blowes, D.W., Ptacek, C.J., Cherry, J.A., 2000. Long-term performance of in-situ reactive barriers for nitrate removal. Ground Water 38 (5), 689–695. https://doi.org/10.1111/j.1745-6584.2000.tb02704.x.

Robertson, W.D., Vogan, J.L., Lombardo, P.S., 2008. Nitrate removal rates in a 15-year-old permeable reactive barrier treating septic system nitrate. Ground Water Monit. Remediat. 28 (3), 65–72. https://doi.org/10.1111/j.1745-6592.2008.00205.x.

Rounds, S.A., 2012. Alkalinity and acid neutralizing capacity (ver. 4). In: U.S. Geological Survey Techniques of Water-Resources Investigations book 9, chap. A6., sec. 6.6, September 2012. (accessed 24 January 2019, from). http://pubs.water.usgs.gov/twri9A6/.

Ryu, J., Oh, J., Snyder, S.A., Yoon, Y., 2014. Determination of micropollutants in combined sewer overflows and their removal in a wastewater treatment plant (Seoul, South Korea). Environ. Monit. Assess. 186 (5), 3239–3251. https://doi.org/10.1007/s10661-013-3613-5.

Schaider, L.A., Rudel, R.A., Ackerman, J.M., Dunagan, S.C., Brody, J.G., 2014. Pharmaceuticals, perfluorosurfactants, and other organic wastewater compounds in public drinking water wells in a shallow sand and gravel aquifer. Sci. Total Environ. 468, 384–393. https://doi.org/10.1016/j.scitotenv.2013.08.067.

Schaider, L.A., Ackerman, J.M., Rudel, R.A., 2016. Septic systems as sources of organic wastewater compounds in domestic drinking water wells in a shallow sand and gravel aquifer. Sci. Total Environ. 547, 470–481. https://doi.org/10.1016/j.scitotenv.2015.12.081.

Schaider, L.A., Rodgers, K.M., Rudel, R.A., 2017. Review of organic wastewater compound concentrations and removal in onsite wastewater treatment systems. Environ. Sci. Technol. 51 (13), 7304–7317. https://doi.org/10.1021/acs.est.6b04778.

Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C., 2010. Denitrifying bioreactors - an approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36, 1533–1543. https://doi.org/10.1016/j.ecoleng.2010.04.008.

Schmidt, C.A., Clark, M.W., 2013. Deciphering and modeling the physicochemical drivers of denitrification rates in bioreactors. Ecol. Eng. 60, 276–288. https://doi.org/10.1016/j.ecoleng.2013.07.041.

Schullehner, J., Hansen, B., Thygesen, M., Pedersen, C.B., Sigsgaard, T., 2018. Nitrate in drinking water and colorectal cancer risk: a nationwide population-based cohort study. Int. J. Cancer 143 (1), 73–79. https://doi.org/10.1002/ijc.31306.

Singh, S.P., Azua, A., Chaudhary, A., Khan, S., Willett, K.L., Gardinali, P.R., 2010. Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicology 19 (2), 338–350. https://doi.org/10.1007/s10646-009-0416-0.

USEPA, 1974. Method 415.1 Organic Carbon, Total (Combustion Or Oxidation). US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

USEPA, 1983a. Method 160.2 Residue, Non-Filterable (Gravimetric, Dried at 103-105°C). US Environmental Protection Agency, Office of Research and Development, Environmental monitoring Systems Laboratory, Cincinnati, OH.

USEPA, 1983b. Method 350.1 Alkalinity by Titration. US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

USEPA, 1983c. Method 405.1 Biochemical Oxygen Demand. US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., Brawley, J., 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries 15 (4), 443–457. https://doi.org/10.2307/1352389.

Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal ocean acidification: the other eutrophication problem. Estuar. Coast. Shelf Sci. 148, 1–13. https://doi.org/10.1016/j.ecss.2014.05.027.

Ward, M.H., Mark, S.D., Cantor, K.P., Weissenburger, D.D., Correa-Villasenor, A., Zahn, S.H., 1996. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology 7 (5), 465–471. https://www.jstor.org/stable/3702144.

Waugh, S., Mao, X., Heufelder, G., Walker, H., Gobler, C.J., 2020. Nitrogen transformations and microbial characterization of soils from passive nitrogen removing biofilters. J. Sustain. Water Built Environ. 6 (2), 04020009 https://doi.org/10.1061/JSWBAY.0000907.

Wehrmann, L.M., Lee, J.A., Price, R.E., Heufelder, G., Walker, H.W., Gobler, C.J., 2020. Biogeochemical sequestration of phosphorus in a two-layer lignocellulose-based soil treatment system. J. Sustain. Water Built Environ. 6 (2), 04020002 https://doi.org/10.1061/JSWBAY.0000906.

Weyer, P.J., Cerhan, J.R., Kross, B.C., Hallberg, G.R., Kantamneni, J., Breuer, G., Jones, M.P., Zheng, W., Lynch, C.F., 2001. Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study. Epidemiology 11, 327–338. https://www.jstor.org/stable/3703710.

Whelan, B.R., Titamnis, Z.V., 1982. Daily chemical variability of domestic septic tank effluent. Water Air Soil Pollut. 17 (2), 131–139. https://doi.org/10.1007/BF00283296.

Wilson, M.A., Godfrey, I.M., Hanna, J.V., Quezada, R.A., Finnie, K.S., 1993. The degradation of wood in old Indian shipwrecks. Org. Geochem. 20 (5), 599–610. https://doi.org/10.1016/0146-6380(93)90026-8.

Wolfe, A.H., Patz, J.A., 2002. Reactive nitrogen and human health: acute and longterm implications. AMBIO 31 (2), 120–125. https://doi.org/10.1579/0044-7447-31.2.120.