

Article

Mesocosm- and Field-Scale Evaluation of Lignocellulose-Amended Soil Treatment Areas for Removal of Nitrogen from Wastewater

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Abstract: Non-proprietary N-removal onsite wastewater treatment systems are less costly than proprietary systems, increasing the likelihood of adoption to lower N inputs to receiving waters. We assessed the capacity of non-proprietary lignocellulose-amended soil treatment areas (LCSTAs)—a 45-cm-deep layer of sand above a 45-cm-deep layer of sand and sawdust—to lower the concentration of total N (TN) in septic tank effluent (STE) at mesocosm and field scales. The mesocosm received wastewater for two years and had a median effluent TN concentration of 3.1 mg/L and TN removal of 60–100%, meeting regulatory standards of 19 mg/L or 50% removal. Removal varied inversely with temperature, and was lower below 10 °C. Removal was higher in the mesocosm than in five field sites monitored for 12–42 months. Median effluent TN concentration and removal met the standard in three continuously-occupied homes but not for two seasonally-occupied homes. Sites differed in temporal pattern of TN removal, and in four of five sites TN removal was greater—and effluent TN concentration lower—in the LCSTA than in a control STA containing only sand. The performance of non-proprietary LCSTAs was comparable to that for proprietary systems, suggesting that these may be a viable, more affordable alternative for lowering N inputs to receiving waters.

Keywords: onsite waste water treatment systems; nitrogen removal; lignocellulose-amendment; temperature effects



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1. Introduction

Coastal communities often rely on onsite wastewater treatment systems (OWTS; also known as septic systems) to treat residential wastewater. Conventional OWTS—consisting of a septic tank with gravity flow of effluent to a pipe-on-stone soil treatment area (STA), also called a drainfield—are not designed to remove nitrogen (N) from wastewater, leading to excess N inputs to ground and coastal waters. High levels of nitrate (NO₃[−]) in groundwater present human health risks in areas where this is the main source of drinking water [1]. Nitrogen pollution in coastal waters can lead to eutrophication, hypoxia, the death of fish and benthic communities [2], and harmful algal blooms [3]. These effects, in turn, have a negative impact on coastal ecosystem services, resulting in limits to public use of estuaries (e.g., recreation, fishing and shellfishing, and aquaculture) and restricting habitat for marine organisms.

Proprietary, commercially-available advanced OWTS are designed for improved removal of target contaminants associated with conventional OWTS. They are used in areas sensitive to contamination of surface and ground water with pathogens, excess organic carbon (C), and/or nitrogen. The latter, referred to as N-removal OWTS, rely on sequential

processes similar to those of a wastewater treatment plant with biological N removal (BNR). These involve the introduction of air to promote microbial oxidation of ammonia to nitrate, followed by an anoxic step to promote microbial denitrification, which results in N loss as N_2 and N_2O . These systems are capable of removing a minimum of 50% of the N in septic tank effluent (STE), which is the regulatory standard in many localities in the U.S., although they can lower TN concentration even further [4–7]. The systems cost between \$15,000 and \$33,000 to engineer and install [8] for a typical four bedroom home, with yearly operation and maintenance costs of \$250 to \$350 [9] and electrical costs from \$75 to \$250 each year. This is a non-trivial financial burden that is a barrier to their adoption by many homeowners.

Lignocellulose-amended soil treatment areas (LCSTAs, also referred to as N bioreactors) are a non-proprietary alternative to N-removal OWTS to lower the concentration of N in wastewater before it is discharged to the underlying soil and groundwater [10–15]. Septic tank effluent is dispersed to a layer of sand where ammonia is nitrified. The nitrified effluent percolates on to an underlying layer of sand amended with lignocellulosic material (sawdust or wood chips), where the high water retention capacity of the LC materials promotes hypoxic/anoxic conditions, and organic C from the LC serves as a C and energy source for microbial denitrification. Robertson et al. [14] have shown that these systems are effective at removing N after continuous use for 15 years, and that the rate of C loss from the lignocellulose layer is <1% per year. These STAs can vary considerably in terms of materials (e.g., sand type, LC source) and depth and extent of confinement of the nitrification and denitrification layers. Versions of these STAs have been used successfully to lower N concentration under field conditions [10,11]. They are currently approved in parts of the USA, including for general use in the state of Florida [16], and are being considered for approval as alternative technologies for N reduction in Barnstable County, Massachusetts [17]; Suffolk County, New York [18]; and in the state of Rhode Island [19]. Recently, Gobler et al. [10] reported that LCSTAs with a bottomless configuration treating STE in private homes in Suffolk County, NY were capable of producing a median effluent TN concentration of 9.1 mg/L and 88% TN removal, suggesting a promising future for this non-proprietary technology.

Here, we examine the capacity of a mesocosm-scale LCSTA system to remove N from STE and compare it to the N removal capacity of five LCSTA systems serving households under field conditions in Barnstable County, MA. The LCSTA consists of a 45-cm layer of sand over a 45-cm layer of sand mixed with sawdust (1:1 by volume). Because sand beds can remove N from STE [20–22], the presence of a control STA in all the field systems, consisting of a 90-cm-deep layer of sand adjacent to the LCSTA and receiving wastewater from the same septic tank, allowed us to assess the extent to which LC affects N removal relative to the STA configuration currently approved for use in Massachusetts [23]. The location of the systems tested in our study is approximately 240 km NE of Suffolk County, allowing for the evaluation of system effectiveness in a cooler region. This is particularly important in light of the temperature dependence of the performance of these systems reported by others [13,14].

2. Materials and Methods

2.1. Mesocosm

A mesocosm representing an LCSTA was built in October 2014 at the Massachusetts Alternative Septic System Testing Center (MASSTC) in the town of Sandwich, MA, which receives an average annual rainfall of about 114 cm. Influent wastewater for the mesocosm consisted of untreated sewage from a nearby wastewater treatment plant. The mesocosm (Figure 1) had a cross-sectional area of 31.22 m² (3.66 m × 8.53 m) and was dosed with influent at a rate of 833 L d⁻¹ (26.7 L m⁻² d⁻¹) from November 2014 to October 2016. The mesocosm had a 15-cm layer of top soil above a Geomat™ system (Geomatrix Systems, LLC, Old Saybrook, CT, USA), a subsurface, low-profile dispersal system that delivers wastewater through orifices in pressurized PVC lateral pipes surrounded by plastic fila-

ments and a geotextile membrane, which increases the contact area of wastewater with the underlying sand media. Wastewater was dispersed to a 45-cm-deep layer of silica sand mined from local glacial outwash deposits (0.30 mm effective particle size; D10) underlain by a 45-cm-deep layer of sand mixed with hardwood sawdust (mostly *Quercus* sp.; 1:1 by volume). A pan lysimeter placed below the sand/sawdust layer was used to collect effluent samples for analysis. The lysimeter was designed so that the percolate flows through the sampling container continuously. As such, the container is filled with an amount of percolate that is at least three times its volume over the course of a day. This eliminates the need for purging prior to sampling since new percolate mixes with that already in the sampling container. Samples were collected every two weeks over the course of two years.

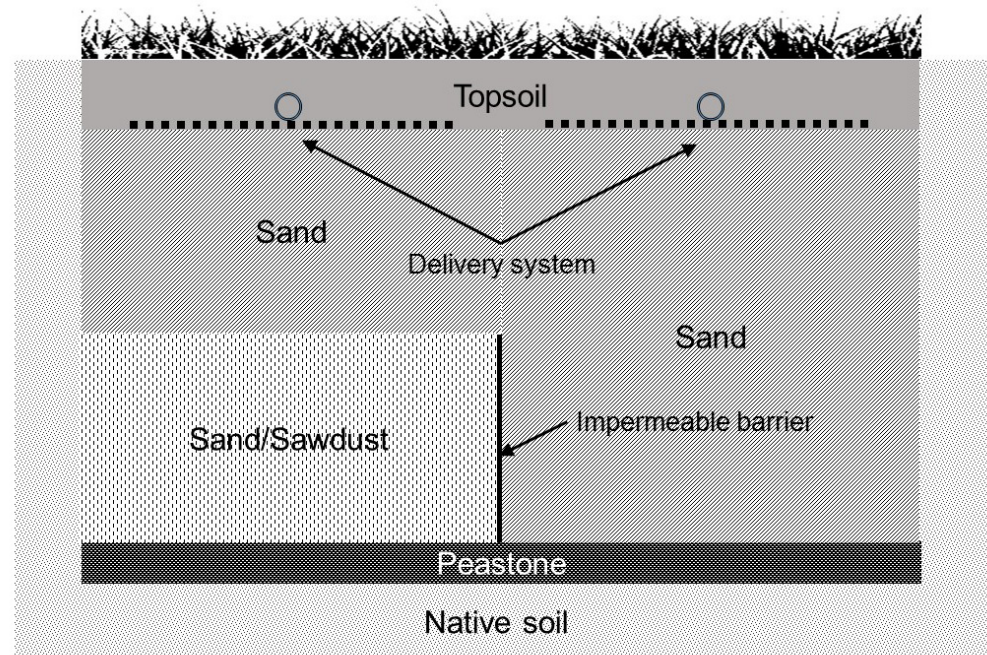


Figure 1. Schematic diagram of a field soil treatment area showing the experimental side with a sand layer over a sand/sawdust layer, and the control side consisting of a single sand layer. The two sides are separated at the bottom by an impermeable barrier. The mesocosm had the same configuration as the experimental side. Drawing is not to scale.

2.2. Field Systems

Field systems were installed between 2017 and 2019 in five private homes in southeastern Massachusetts, in the towns of Acushnet (Acushnet), North Dartmouth (Gaffney), Westport (Main), and Falmouth (Chappaquoy and Sippewissett). The area receives between 110 and 120 cm of rainfall annually. The systems were designed by private engineering firms and installed by independent contractors, with both design and installation overseen by MASSTC personnel. The homes were occupied by one to three people, with three of the homes continuously occupied, and the other two occupied seasonally (Table 1). All five systems had the same main components (Figure 1): (1) a conventional, two-compartment, 5678 L concrete septic tank for primary treatment, with a detention time of 6.2 to 8.4 days; (2) a 3785 L concrete pump tank (for surge flow storage and equalization) with a mechanical pump used to deliver a consistent time-dosed volume of STE to (3) a GeoMat™ dispersal system [24] that was placed 25 to 90 cm below the ground surface. A 15 cm layer of top soil (silt loam or sandy loam, depending on the site) was placed above the dispersal system. A Trumeter model p2-4906 event timer (Trumeter Co., Inc., Coconut Creek, FL, USA) was mounted next to or installed along with a programmable logic controller to count the number of doses to the STA surface, which allowed us to calculate wastewater flow and detention time based on the pump delivery rate.

Table 1. System and site characteristics for systems installed in five private homes in southeastern Massachusetts (USA) in the towns of Acushnet (Acushnet), North Dartmouth (Gaffney), Westport (Main), and Falmouth (Chappaquoit and Sippewissett).

Characteristic	System				
	Acushnet	Chappaquoit	Sippewissett	Gaffney	Main
Occupancy Pattern	Year-round	Seasonal (May–September)	Seasonal (June–September)	Year-round	Year-round
Installation Date	April 2018	April 2017	November 2017	May 2019	September 2019
Number of Occupants	2 adults, 1 infant	2 adults	1 adult	2 adults	2 adults
Average Daily Flow, Occupied (L/d)	825	303	423	416	306

Each STA was split into a control (CTL) and an experimental (EXP) area. The CTL STA was designed in accordance with the Massachusetts State Environmental Code [23] and consisted of a 90 cm-deep layer of silica sand mined from local glacial outwash deposits (0.30 mm effective particle size; D10). The EXP STA consisted of a 45 cm-deep layer of the same medium sand above a 45 cm-deep layer of medium sand amended with hardwood sawdust (mainly *Quercus* spp.; 1:1 by volume) (Figure 1). The bottom of the STA was underlain by a layer of washed, locally sourced pea stone gravel (~9.5 mm dia.) that was in contact with native soil. A 45 cm-high plastic sheet extending from the bottom of the STA to the top of the sand/sawdust layer separated the EXP and CTL areas. A pan lysimeter like that used for the mesocosm was placed at the base of each STA to collect effluent samples for analysis. Samples were collected every 2 to 6 weeks when the homes were occupied. There was a longer hiatus between sampling dates during the first half of 2020 due to government-mandated travel restrictions associated with the COVID-19 pandemic. The systems were sampled for 1 to 3.5 years, depending on the system.

2.3. Sampling and Analysis

Samples of influent for the mesocosm were collected from a cement trough that fed a series of mesocosms at the MASSTC facility, whereas influent for the field systems was collected from the system pump tank. Effluent from both the mesocosm and field systems was collected from the pan lysimeters underlying the STA. A small, 12 V pump (16 L min⁻¹; Whale Marine, Bangor, Northern Ireland) with 9.5 mm-dia. plastic tubing was used to sample influent and effluent, with a small amount of liquid discharged to waste prior to sample collection.

Measurements of dissolved oxygen (DO), pH, and temperature were made by dipping a probe from a YSI 556 Multi-Probe System (YSI, Inc., Yellow Springs, OH, USA) directly into the pump tank. The concentration of total nitrogen (TN) in influent and effluent was calculated from the sum of total Kjeldahl nitrogen (TKN) and NO₃⁻ and NO₂⁻. Measurement of TKN was made using US EPA Method 351.2. Ion chromatography was used to determine the concentration of NO₃⁻ and NO₂⁻ (US EPA Method 300.0) [25,26], and the concentration of NH₄⁺ was measured colorimetrically following US EPA Method 350.1 [27]. Measurements of alkalinity, BOD₅, and total suspended solids (TSS) were made using US EPA Method 405.1, US EPA Method 310.1, and US EPA Method 160.2, respectively [28–30].

2.4. Statistical Analyses

Regression analyses and *t*-tests were carried out using SigmaPlot V.12.5 (Systat Software, Inc., San Jose, CA, USA).

3. Results and Discussion

3.1. Mesocosm

The regulatory performance standards for advanced N removal systems in Massachusetts require an effluent TN concentration of 19 mg/L or lower, which is meant to approximate a 50% N reduction [23,31]. The median ($n = 95$) value for TN removal in the experimental mesocosm was 93.4% during the first two years of operation, with a median influent TN concentration of 42 mg/L ($n = 95$) and a median final effluent TN concentration of 3.1 mg/L ($n = 97$). Removal of TN fluctuated over the course of the experiment (Figure 2), with lower removal and higher effluent TN concentration during winter and early spring, and higher removal and lower effluent TN concentration during summer and early fall, generally following variation in effluent temperature. Nitrogen removal was always greater than 50%, and the TN concentration in effluent was below the 19 mg/L standard on 96 out of 97 sampling events (Figure 2). Increases in effluent TN concentration generally co-occurred with increases in effluent NO_3^- and NH_4^+ levels. These results confirm the effectiveness of this LCSTA design at removing TN from wastewater at the mesocosm scale.

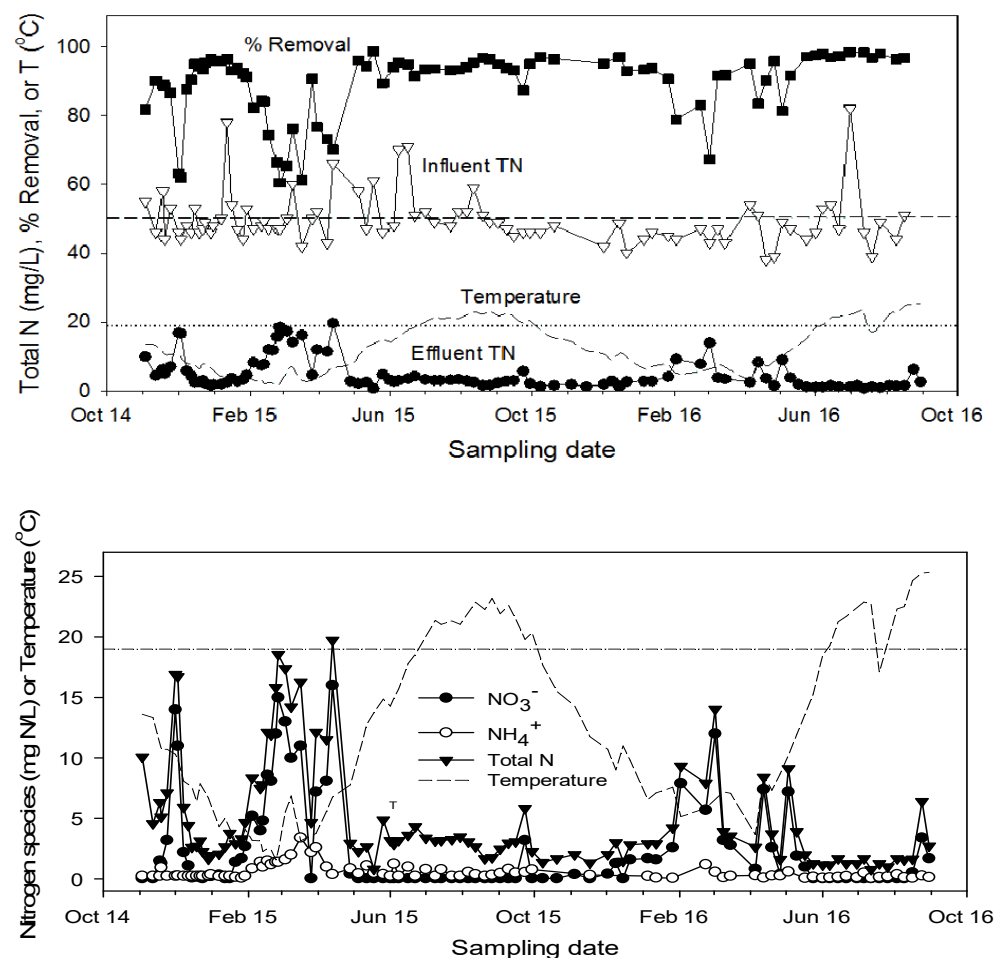


Figure 2. **Top:** Changes in total N concentration in influent wastewater and effluent water, % total N removal, and temperature in a lignocellulose STA mesocosm as a function of time. Dashed line indicates 50% removal; dotted line indicates 19 mg TN/L standard. **Bottom:** Changes in concentration of ammonium, nitrate and total N in effluent, and in effluent temperature in a lignocellulose STA mesocosm as a function of time. Dashed line indicates 19 mg TN/L standard.

Analysis of the relationship between temperature and N species showed that the concentration of NO_3^- , NH_4^+ , and TN in effluent was inversely proportional to temperature,

whereas % TN removal decreased inversely with decreasing temperature (Figure 3). The inflection point for the relationship was between 5 and 10 °C for all four parameters and was most clearly defined for NO_3^- . There was no apparent relationship between any of the other effluent properties measured and either the concentration of N species or TN removal.

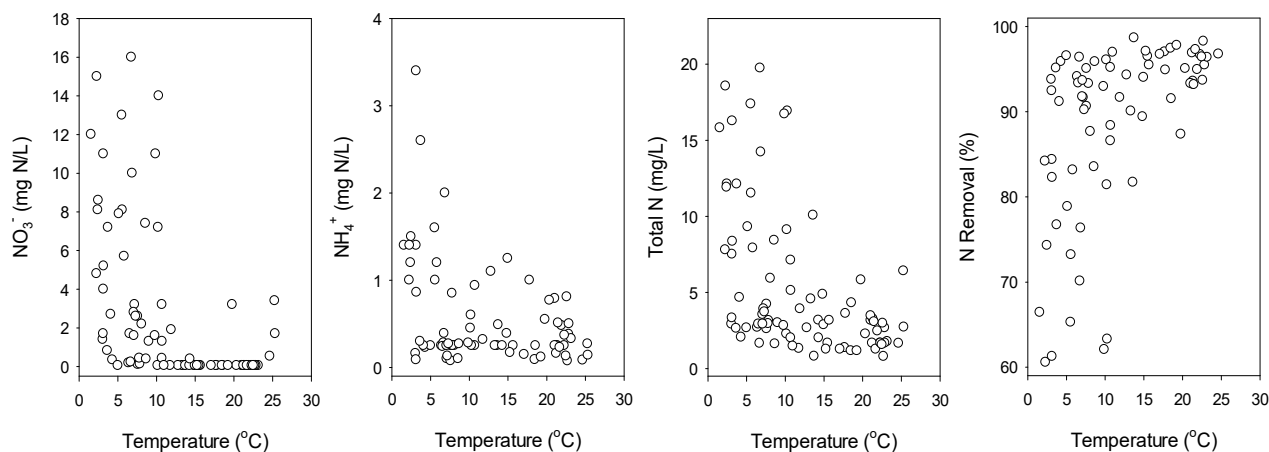


Figure 3. Relationship between % N removal, nitrate, ammonium, and total N concentration in effluent and temperature in the mesocosm.

Our results suggest that both nitrification and denitrification are affected by temperature in the mesocosm. If only nitrification was impacted negatively by low temperature, we would expect the concentration of NH_4^+ (but not NO_3^-) in the effluent to increase once the temperature is sufficiently low. In contrast, if only denitrification was affected by low temperature, we would expect the concentration of NO_3^- (but not NH_4^+) to increase as the temperature decreases. Increases in the concentration of both N species between February and May 2015 and February and April of 2016, when the temperature was lowest, suggests that both processes were affected. Some instances of high NO_3^- levels without increased NH_4^+ levels when the temperature was still in the lower range (e.g., April–June 2016) may indicate greater sensitivity of denitrification to lower temperature.

The temperature dependence of N removal in LCSTAs has been reported previously [13,14]. More recently, Gobler et al. [10] reported that there was significantly lower TN removal in mesocosms of the same design during the winter season (January–April) tested at the same facility as the one in our study. They suggested that denitrification was negatively affected by low temperatures, whereas nitrification in the sand layer was unaffected. They propose that increased solubility of oxygen in water with lower temperatures increases the concentration of dissolved oxygen (DO) in the denitrification layer, resulting in preferential use of O_2 over NO_3^- as a terminal electron acceptor by denitrifiers [10]. As stated earlier, our data suggest that this was this case in some instances, but both processes generally appeared to be similarly affected by low temperatures. The discrepancy may be due to differences in the temporal scales used for data analyses, involving evaluation of temperature dependence of N removal at the week scale in our case versus analysis of differences in N removal at the seasonal scale by Gobler et al. [10]. However, the absence of time-resolved performance data in Gobler et al. [10] prevents us from reaching a conclusion regarding the reasons for differences in performance.

3.2. Field Systems

The median value for TN removal for the experimental (EXP) STA in field systems was above 50% for all five systems, with continuously-occupied sites having a higher removal rate than those that were seasonally-occupied (Figure 4). Median TN removal values were higher for the EXP than the control (CTL) STAs for all sites except for Main, where the two values were similar. When the N removal capacity of field EXP STAs was compared to

that for the mesocosm, the median TN concentration in effluent from the mesocosm was considerably lower, and %N removal higher, than for the field systems.

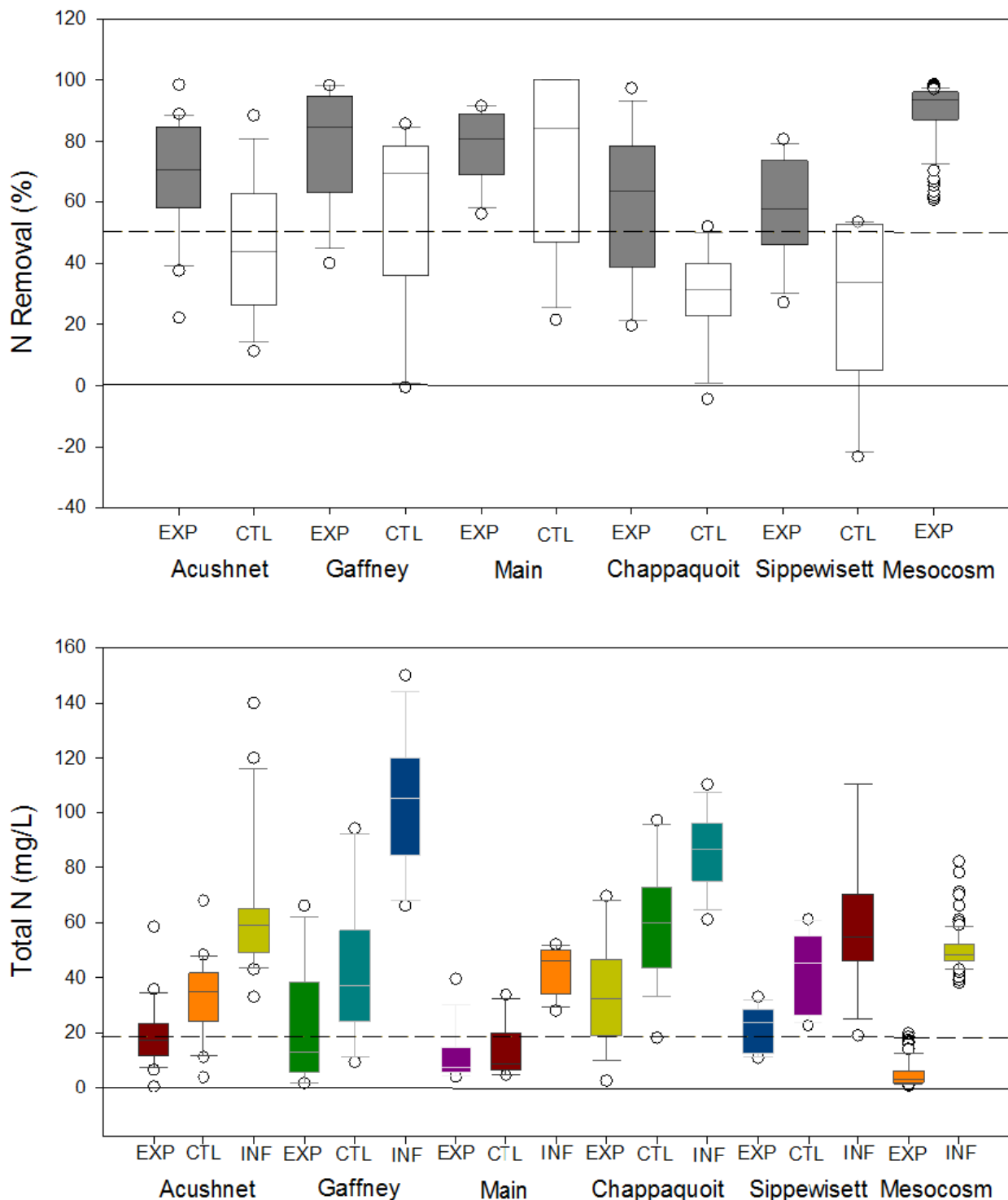


Figure 4. **Top:** Median and interquartile range of % total N removal values ($n = 13$ to 25) in the sawdust-amended (EXP) and unamended (CTL) soil treatment areas (STA) for field systems. Values ($n = 97$) for a mesocosm representing an experimental STA are included for comparison. Dashed line indicates 50% TN removal standard. **Bottom:** Median and interquartile range of total N concentration values ($n = 13$ to 25) for in influent (INF; septic tank effluent) and effluent from EXP and CTL STAs for field systems. Values ($n = 96$) for a mesocosm representing an experimental STA are included for comparison. Dashed line indicates 19 mg TN/L standard.

The median concentration of TN in effluent was below 19 mg/L for all of the EXP STA in systems that were in continuous use, whereas it was above the regulatory level for both seasonally-used systems (Figure 4). This is in contrast to proprietary advanced N-removal OWTS, for which Ross et al. [7] found no difference in performance between those serving homes occupied continuously and those occupied seasonally. Furthermore, the communities of nitrifying and denitrifying bacteria in proprietary advanced N-removal OWTS that served seasonally-occupied homes were similar to those of continuously-occupied homes [32]. Wigginton et al. [33] found that the communities of nitrifying and denitrifying bacteria at the Acushnet, Sippewissett, and Gaffney sites were more similar in the summer, when all three systems were receiving wastewater inputs, than in the spring, when only the Acushnet site was receiving wastewater and the other two systems were dormant. The differences in performance between LCSTA systems in seasonally-occupied homes and those under continuous occupancy may be related to differences in the timing of wastewater inputs, which may in turn affect the structure and composition of nitrifying and denitrifying bacterial communities, as suggested by Wigginton et al. [33].

The median TN concentration in effluent from the CTL STA was higher than that from the EXP STA for all five systems, and was higher than the regulatory standard for all sites except for Main, where it was below the standard. The median concentration of TN in influent varied considerably among field sites (45 to 105 mg/L) and was generally higher than for the mesocosm (42 mg/L). None of the influent properties measured, including TN level (Table 2), appeared to influence effluent N levels consistently across sites.

Table 2. Mean and standard deviation (sd) values of total suspended solids (TSS), alkalinity, 5-day biochemical oxygen demand (BOD₅), dissolved oxygen (DO), pH, temperature, and total N for field systems. Units are mg/L except for temperature (°C) and pH. ND = not determined.

Property	System									
	Sippewissett		Chappaquoit		Main		Gaffney		Acushnet	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
TSS	39	23	34	11	128	105	50	20	80	83
Alkalinity	288	74	388	81	ND	ND	520	60	232	42
BOD ₅	80	61	253	162	103	74	82	74	192	79
DO	3.3	3.9	1.4	1.6	0.3	0.3	0.3	0.8	1.1	1.5
pH	6.8	0.5	7.0	0.3	6.8	0.3	7.8	0.4	7.5	0.7
Temp.	14.4	6.9	17.7	3.1	15.2	5.4	13.0	5.0	17.2	6.2
Total N	60.7	26.5	87.3	15.9	43.1	8.1	103.8	24.4	63.3	25.8

The concentration of TN in effluent and %TN removal for all field systems fluctuated with time (Figure 5). Furthermore, despite being installed within a 20-km radius, and thus subject to similar precipitation and temperature patterns, the systems had unique temporal patterns of effluent TN concentration. Analysis of % TN removal in CTL vs. EXP STAs within a system using a two-tailed paired *t*-test showed that removal was significantly higher in the EXP STA in four of the systems, with no statistically significant differences between STAs in the fifth site (Main).

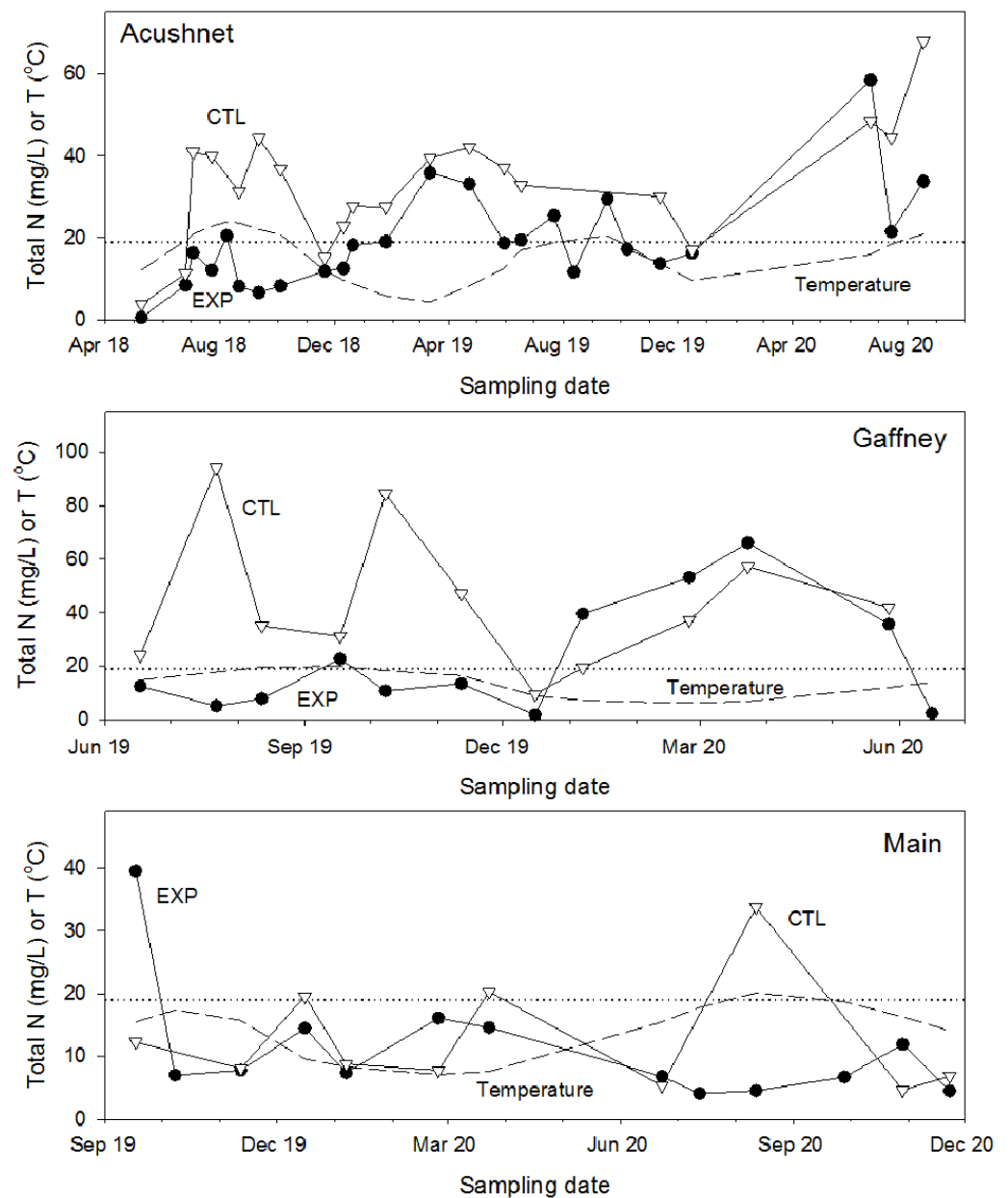


Figure 5. Total nitrogen concentration and temperature of effluent from the sawdust-amended (EXP) and unamended (CTL) sides of the soil treatment area for continuously-occupied systems. Dotted line indicates 19 mg TN/L standard.

Within the continuously-used systems, effluent TN levels in EXP and CTL in Acushnet followed similar patterns over time, with values in the CTL STA consistently higher than in the EXP STA for 30 months (Figure 5). In contrast, effluent TN levels at Gaffney were different between EXP and CTL for the first six months after installation, with much lower values for EXP, whereas EXP and CTL had the same temporal pattern for the subsequent six months of operation. At Main, a third temporal pattern was apparent, with a similar TN concentration in effluent from EXP and CTL on most sampling dates, and TN levels generally below the 19 mg/L threshold in both STAs.

Within the seasonally-used systems, the effluent TN concentration from EXP and CTL at Chappaquoit generally followed the same temporal pattern during the initial two periods of occupation in mid-summer, with EXP values much lower than for the CTL (Figure 6). By the third occupation period, CTL and EXP TN concentration was similar, and

much higher than in previous seasons. In contrast, temporal variation of effluent TN at Sippewissett was similar for EXP and CTL, but the concentration of TN was much higher for the latter.

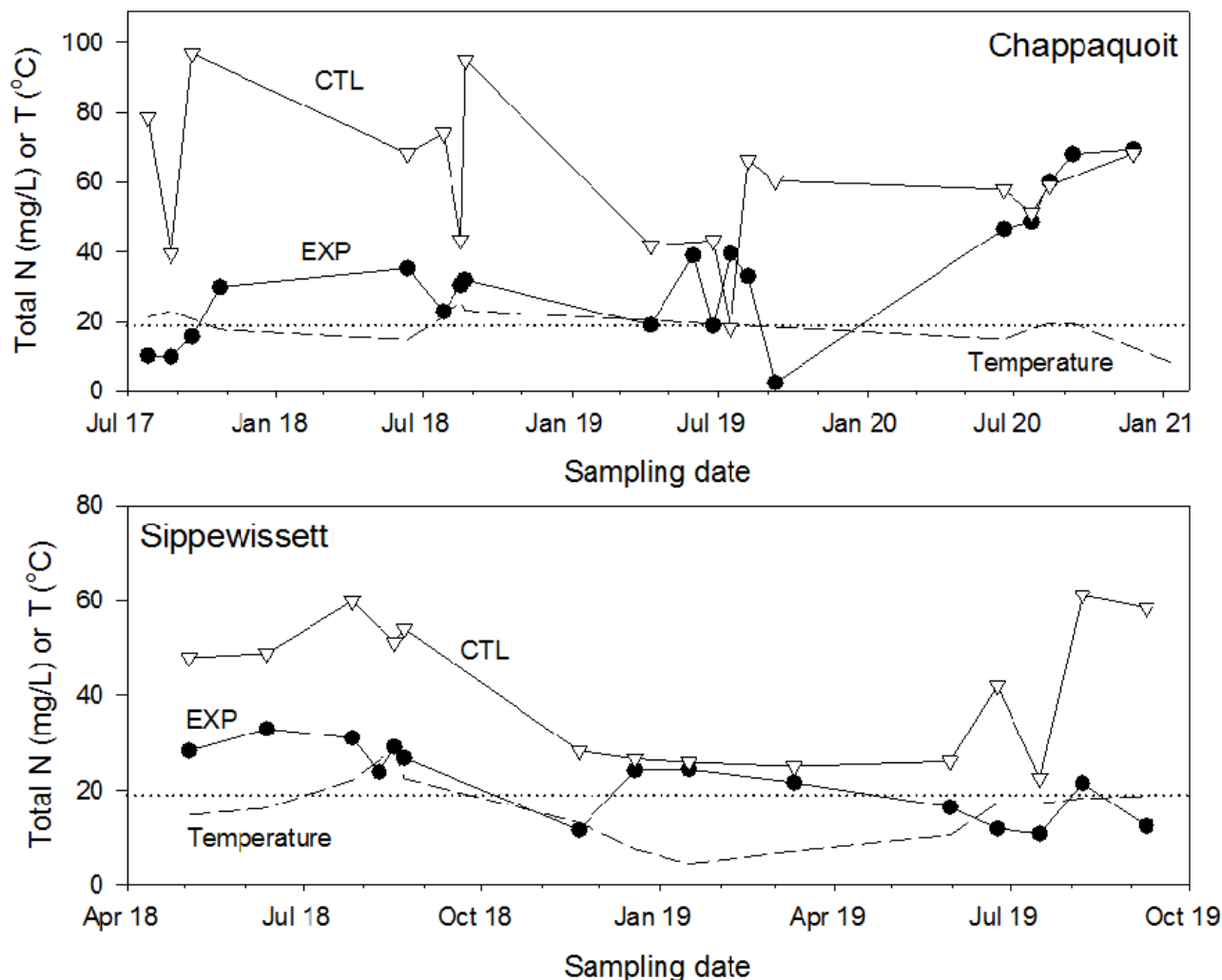


Figure 6. Total nitrogen concentration and temperature of effluent from the sawdust-amended (EXP) and unamended (CTL) sides of the soil treatment area for seasonally-occupied systems. Dotted line indicates 19 mg TN/L standard.

Our results show that N removal from influent wastewater takes place in both CTL and EXP conditions, although the extent of removal was greater in EXP in most systems. Nitrogen removal has been shown to take place in sand beds and sandy soil in the absence of lignocellulose amendments [20–22]. For example, using undisturbed soil mesocosms representing different types of STA in sandy skeletal soil, Cooper et al. [20] reported N removal of 12.0% for pipe and stone STAs receiving septic tank effluent, and between 4.8% and 5.4% in STAs receiving effluent from a sand filter. The absence of time-resolved N-removal data, and of a CTL STA, for the field systems in Gobler et al. [10] prevents comparison with our results.

The assumption behind the design of the EXP STA is that the sand layer provides conditions conducive to aerobic, autotrophic nitrification, and the lignocellulose mixed with the sand provides organic C and hypoxic/anoxic conditions for heterotrophic anaerobic denitrification, leading to greater N removal. However, these are unlikely to be the only processes at work under these conditions [33]. The processes that contribute to N removal may be similar in CTL and EXP conditions, with the differences in removal resulting from differences in the relative contribution of different processes. In addition, because the CTL

STA receives influent with a high concentration of organic C, there could be removal of N through coupled nitrification/denitrification in microsites with aerobic and anaerobic regions. A similar mechanism was proposed by Gobler et al. [10] to explain N removal in the nitrification layer of LCSTAs.

As is the case here, greater TN removal in mesocosms than in field LCSTAs was reported by Gobler et al. [10]. Although the mesocosm and the field systems in our study were all in southeastern Massachusetts, and thus exposed to similar environmental conditions (e.g., temporal variation in temperature and precipitation), a number of other factors likely contributed to differences in performance between field and mesocosm systems, as well as among field systems. There were, for example, considerable differences in the TN concentration in influent water among the field sites, and between the field sites and the mesocosm (Figure 4). In addition, landscaping practices, such as fertilizer amendments, the presence of grass and mowing practices, can result in extraneous inputs of N to the STA that are not accounted for in our measurements. Differences in depth of soil above the infiltrative surface can affect inputs of water as well as dynamics of O₂ in gaseous and dissolved forms. Home occupancy patterns affect the composition of wastewater as well as daily flow and dosing frequency, which impact water-filled porosity, the spatial distribution of oxygen and redox conditions, and the availability of organic C sources. The presence of medications, such as antibiotics, in septic tank effluent can affect the structure and function of the microbial community in the STA [34,35]. All of these factors can affect microbial N removal processes and likely account for some of the difference among field systems, and between field and mesocosm systems.

The effects of temperature, which were a significant factor in controlling temporal differences in N removal in the mesocosm (Figure 3), were much less apparent in field systems. Although temporal variation did appear to follow seasonal changes in temperature for both EXP and CTL STAs, analysis of the data showed there was no clear relationship between temperature and total N concentration for any of the five field systems when data were analyzed by individual system. In contrast, when data for all five systems were analyzed in the aggregate, there was a significant linear relationship between %TN removal and temperature ($r^2 = 0.150$; $p < 0.05$; $n = 52$), with removal increasing with increasing temperature.

The median and range of effluent TN concentrations for the field systems (Figure 4) are within the values reported for proprietary advanced N-removal systems in other areas of southern New England [5,7] and in Suffolk County, NY [9,18]. These proprietary systems employ mechanical pumps and aeration devices to promote sequential nitrification and denitrification of septic tank effluent, with N removal taking place primarily as N₂ gas from denitrification prior to effluent dispersal to an STA. For example, median effluent TN values for 42 individual proprietary systems (four different technologies) serving private homes within the Greater Narragansett Bay watershed (sampled monthly for 18 months) ranged from 5 to 42 mg TN/L [5]. More recently, Ross et al. [7] reported median effluent TN values for 47 proprietary systems serving private homes in a single community in southern Rhode Island (four different technologies sampled quarterly over 32 months) that ranged from 7 to 35 mg N/L.

Median effluent TN values from the three systems serving continuously-occupied homes in our study (Acushnet, Gaffney and Main), monitored for 12 to 28 months, were between 7 to 19 mg/L (mean = 17.8; median = 13.5 for the three systems). These values are higher than reported for three LC STAs with the same design serving continuously-occupied homes in Suffolk County, NY [10], which were monitored for 9 to 16 months and had a median effluent TN concentration of 9.1 mg/L. In addition, the median value of TN removal for the three continuously-occupied systems in our study was 76% (Figure 4), whereas those in Suffolk County, NY had a median TN removal of 88%. The reasons for the disparity in performance between the systems in our study and those in Suffolk County are unclear. Differences in the concentration of TN in influent wastewater were small, and the values were slightly higher for Suffolk County—73 mg/L vs. 69 mg/L in our

study (Table 2)—and thus unlikely to explain the differences in performance. It may be that these are driven partly by differences in the magnitude and temporal distribution of precipitation, which could result in differences in dilution and establishment of anoxic conditions.

Because temperature affects TN removal in LCSTAs [13–15] (Figure 3), we examined climatic differences between Barnstable and Suffolk County as an explanation for difference in performance. Suffolk County is approximately 250 km SW of Barnstable County and experiences warmer temperatures in winter and summer. For example, the average winter minimum and maximum high temperatures in Suffolk County are -3.9 and 3.3 °C, whereas in Barnstable County they are -5.0 and 2.8 °C. Average minimum and maximum high temperatures in summer in Suffolk County are 20.0 and 27.8 °C, whereas in Barnstable County they are 13.3 to 25.6 °C. To further examine temporal differences in soil heating between the two regions, we compared growing degree days (GDD)—a measure of heat accumulation above a base temperature over time—using an online calculator [36]. We used a base temperature for microbial N removal of either 5 or 10 °C, based on the observed temperature dependence of N removal in the mesocosm (Figure 3), with GDD estimates based on temperature averages for each area for the past 15 years, using the period from 1 January to 15 November 2021 for comparison. At a base temperature of 5 °C, the number of GDD for Barnstable County is estimated at 2569, and 2725 for Suffolk County, a 6.1% difference; at a base temperature of 10 °C, the number of GDD is estimated at 1500 for Barnstable and 1637 for Suffolk County, a 9.0% difference. Because microbial activity tends to vary exponentially with temperature, these differences in GDD can translate to large differences in N removal by LCSTAs between the two regions, helping to explain performance differences between systems.

4. Conclusions

Data from mesocosm-scale testing suggests that the STA design we evaluated is highly effective for N removal. Although N removal was temperature-dependent, the systems consistently met regulatory standards for effluent TN concentration and % TN removal over the course of two years. In four of the five field systems, tested over 1 to 3.5 years, the median effluent TN concentration and %TN removal in the EXP treatment was significantly different from the CTL and the systems were capable of meeting regulatory standards. However, their performance was less consistent and effective than that of the mesocosm. There was considerable temporal variation in performance within and among field systems, likely due to factors such as number and demographic characteristics of occupants, occupancy patterns, and medication use, as well as differences in the concentration of influent TN. Performance was lower than for field systems with the same design in Suffolk County, NY, possibly due to differences in the magnitude and temporal variations in temperature between the two regions. Robertson et al. [14,15] found that these types of systems remove N effectively and function hydraulically after 15 years of continuous use, with a C loss rate of $<1\%$ per year, suggesting that concerns about their long-term performance may not be warranted. Our results suggest that LCSTA systems are capable of delivering performance comparable to proprietary systems, and point to the need for testing of their performance in situ to ascertain their long-term capacity to reduce N inputs to receiving waters under prevailing climatic conditions.

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References

1. Ward, M.H.; Jones, R.R.; Brender, J.D.; De Kok, T.M.; Weyer, P.J.; Nolan, B.T.; Villanueva, C.M.; Van Breda, S.G. Drinking water nitrate and human health: An updated review. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1557. [CrossRef] [PubMed]
2. Bergondo, D.L.; Kester, D.R.; Stoffel, H.E.; Woods, W.L. Time-series observations during the low sub-surface oxygen events in Narragansett Bay during summer 2001. *Mar. Chem.* **2005**, *97*, 90–103. [CrossRef]
3. Anderson, D.M.; Gilbert, P.M.; Burkholder, J.M. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* **2002**, *25*, 704–726. [CrossRef]
4. Amador, J.A.; Görres, J.H.; Loomis, G.W.; Lancellotti, B.V. Nitrogen loading from onsite wastewater treatment systems in the Greater Narragansett Bay (Rhode Island, USA) watershed: Magnitude and reduction strategies. *Water Air Soil Pollut.* **2018**, *229*, 65. [CrossRef]
5. Lancellotti, B.V.; Loomis, G.W.; Hoyt, K.P.; Avizinis, E.; Amador, J.A. Evaluation of nitrogen concentration in final effluent of advanced nitrogen-removal onsite wastewater treatment systems (OWTS). *Water Air Soil Pollut.* **2017**, *228*, 383. [CrossRef]
6. Oakley, S.M.; Gold, A.J.; Oczkowski, A.J. Nitrogen control through decentralized wastewater treatment: Process performance and alternative management strategies. *Ecol. Eng.* **2010**, *36*, 1520–1531. [CrossRef]
7. Ross, B.N.; Hoyt, K.P.; Loomis, G.W.; Amador, J.A. Effectiveness of advanced nitrogen-removal onsite wastewater treatment systems in a New England coastal community. *Water Air Soil Pollut.* **2020**, *231*, 1–10. [CrossRef]
8. Suffolk County. Septic Improvement Program, 4 Bedroom Quote List I/A OWTS Vendor List Contact Information. 2018. Available online: <https://www.ohamptonny.gov/DocumentCenter/View/2426/Vendor-Ranking-Document-PDF> (accessed on 3 August 2021).
9. Sohngen, J.; Jobin, J.; Priolo, J. 2018 Report on the Performance of Innovative and Alternative Onsite Wastewater Treatment Systems. 2019. Available online: https://www.suffolkcountyny.gov/Portals/0/FormsDocs/health/EnvironmentalQuality/2018_Performance_Evaluation_of_IAOWTS_Appendices_11-18-2019.pdf (accessed on 3 August 2021).
10. Gobler, C.J.; Waugh, S.; Asato, C.; Clyde, P.M.; Nyera, S.C.; Graffam, M.; Brownawell, B.; Venkatesan, A.K.; Goleski, J.A.; Price, R.E.; et al. Removing 80–90% of nitrogen and organic contaminants with three distinct passive, lignocellulose-based on-site septic systems receiving municipal and residential wastewater. *Ecol. Eng.* **2021**, *161*, 106157. [CrossRef]
11. Hazen and Sawyer Environmental Engineers & Scientists. Florida Onsite Sewage Nitrogen Reduction Strategies Study. Evaluation of Full Scale Prototype Passive Nitrogen Reduction Systems (PNRS) and Recommendations for Future Implementation—Volume I of II. Prepared for: Florida Department of Health, Division of Disease Control and Health Protection, Bureau of Environmental Health, Onsite Sewage Programs. 2015. Available online: http://www.floridahealth.gov/environmental-health/onsite-sewage/research/_documents/rrac/hazensawyervolireportrmall.pdf (accessed on 3 August 2021).
12. Robertson, W.; Cherry, J. In situ denitrification of septic-system nitrate using reactive porous media barriers: Field trials. *Ground Water* **1995**, *33*, 99–111. [CrossRef]
13. Robertson, W.; Blowes, D.; Ptacek, C.; Cherry, J. Long-term performance of in situ reactive barriers for nitrate remediation. *Ground Water* **2000**, *38*, 689–695. [CrossRef]
14. Robertson, W.; Vogán, J.; Lombardo, P. Nitrate removal rates in a 15 year old permeable reactive barrier treating septic system nitrate. *Groundwater Monit. Remed.* **2008**, *28*, 65–72. [CrossRef]
15. Robertson, W. Nitrate removal rates in woodchip media of varying age. *Ecol. Eng.* **2010**, *36*, 1581–1587. [CrossRef]
16. State of Florida Department of Health. Chapter 64E-6, Florida Administrative Code Standards for Onsite Sewage Treatment and Disposal Systems; Florida Administrative Code & Florida Administrative Register: Tallahassee, FL USA, 2018. Available online: <https://www.flrules.org/gateway/ChapterHome.asp?Chapter=64e-6> (accessed on 30 July 2021).

17. Barnstable County Department of Health and Environment. *The Performance Monitoring of Non-Proprietary Layered Systems Presently under Site-Specific Pilot Approvals*; Annual Report; Massachusetts Department of Environmental Protection: Sandwich, MA, USA, 2018. Available online: https://www.eastham-ma.gov/sites/g/files/vyhlf4371/f/uploads/annualreport_2018_layeredsystems.pdf (accessed on 30 July 2021).
18. Jobin, J.; Priolo, J. *Draft 2018 Report on the Performance of Innovative and Alternative Onsite Wastewater Treatment Systems*; Suffolk County Department of Health Services: Great River, NY, USA, 2020.
19. Rhode Island Department of Environmental Management. *Alternative & Experimental Technologies*; Rhode Island Department of Environmental Management (RIDEM): Providence, RI, USA, 2021.
20. Cooper, J.A.; Morales, I.; Amador, J.A. Nitrogen transformations in different types of soil treatment areas receiving domestic wastewater. *Ecol. Eng.* **2016**, *94*, 22–29. [[CrossRef](#)]
21. Gill, L.W.; O’Súilleabháin, C.; Misstear, B.D.; Johnston, P.M. Comparison of stratified sand filters and percolation trenches for on-site wastewater treatment. *J. Environ. Eng.* **2009**, *135*, 8–16. [[CrossRef](#)]
22. Holden, S.A.; Stolt, M.H.; Loomis, G.W.; Gold, A.J. Seasonal variation in nitrogen leaching from shallow–narrow drainfields. In *Proceedings of the 10th Symposium on Individual and Small Community Sewage Systems*, Sacramento, CA, USA, 21–24 March 2004.
23. Massachusetts Department of Environmental Protection. *The State Environmental Code, Title 5: Standard Requirements for the Siting, Construction, Inspection, Upgrade and Expansion of on-Site Sewage Treatment and Disposal Systems and for the Transport and Disposal of Septage*. Available online: <https://www.mass.gov/regulations/310-CMR-15-state-environmental-code-title-5-standard-requirements-for-the-siting> (accessed on 30 July 2021).
24. Wigginton, S.K.; Loomis, G.W.; Amador, J.A. Greenhouse gas emissions from lignocellulose–amended soil treatment areas for removal of nitrogen from wastewater. *Sci. Total Environ.* **2020**, *744*, 140936. [[CrossRef](#)] [[PubMed](#)]
25. O’Dell, J.W. *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, USA, 1993.
26. Pfaff, J.D. *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, USA, 1993.
27. O’Dell, J.W. *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, USA, 1993.
28. USEPA. *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, USA, 1983.
29. USEPA. *Method 350.1 Alkalinity by Titration*; US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory: Cincinnati, OH, USA, 1983.
30. USEPA. *Method 405.1 Biochemical Oxygen Demand*; US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory: Cincinnati, OH, USA, 1983.
31. Heufelder, G.R.; Rask, S.; Burt, C. Performance of Innovative Alternative Onsite Septic Systems for the Removal of Nitrogen in Barnstable County, Massachusetts 1999–2007. Available online: <https://buzzardsbay.org/etistuff/bched--alternative--septic-sytems--2007.pdf> (accessed on 30 July 2021).
32. Ross, B.N.; Wigginton, S.K.; Cox, A.H.; Loomis, G.W.; Amador, J.A. Influence of season, occupancy pattern, and technology on structure and composition of nitrifying and denitrifying bacterial communities in advanced nitrogen–removal onsite wastewater treatment systems. *Water* **2020**, *12*, 2413. [[CrossRef](#)]
33. Wigginton, S.K.; Amador, J.A. Structure and composition of nitrifying and denitrifying bacterial communities of lignocellulose–amended soil treatment areas receiving wastewater. *Appl. Environ. Microbiol.* **2021**, in press.
34. Atoyan, J.A.; Staroscik, A.M.; Nelson, D.R.; Patenaude, E.L.; Potts, D.A.; Amador, J.A. Microbial community structure of a leachfield soil: Response to intermittent aeration and tetracycline additions. *Water* **2013**, *5*, 505–524. [[CrossRef](#)]
35. Patenaude, E.L.; Atoyan, J.A.; Potts, D.A.; Amador, J.A. Effects tetracycline on water quality, soil and gases in aerated and unaerated leachfield mesocosms. *J. Environ. Sci. Health, Part A* **2008**, *43*, 1054–1063. [[CrossRef](#)]
36. Climate Smart Farming. CSF Growing Degree Day Calculator. Available online: <http://climatesmartfarming.org/tools/csf-growing-degree-day-calculator/> (accessed on 30 July 2021).