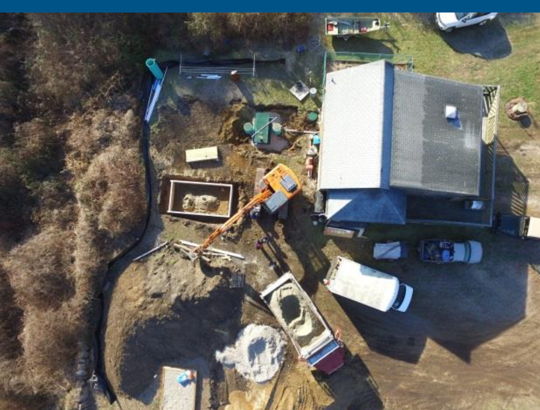
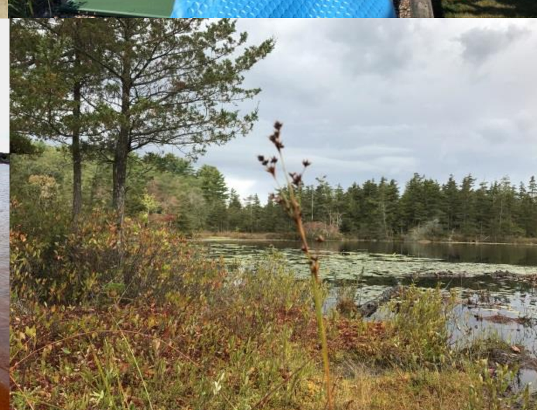
RIDEM APPLICATION FOR ALTERNATIVE OWTS TECHNOLOGY: NITROGEN REDUCING LAYERED SOIL TREATMENT AREA

Town of Charlestown, R.I.
On-Site Wastewater Management Program

IN PARTNERSHIP WITH
URI LABORATORY OF SOIL ECOLOGY AND MICROBIOLOGY AND
THE NEW ENGLAND ON-SITE WASTEWATER TRAINING PROGRAM

March 2, 2021




LSEM
Laboratory of Soil Ecology & Microbiology
University of Rhode Island

CHARLESTOWN
TOWN OF CHARLESTOWN

NEOWTP

UNIVERSITY OF
Rhode Island

<u>Cover Figures from top left</u>

1.) Drone photo of a nitrogen reducing OWTS installed to replace a failing metal tank system located less than 100-feet from a coastal wetland under the EPA SNEP Grant, summer 2019

2.) Nitrogen reduction efficiency sampling of a N-reducing OWTS in Charlestown, Summer 2019

3.) Presentation of N-reducing technology efficiency monitoring to the Charlestown Town Council, March 2019

4.) Ninigret Pond, back barrier flat

5.) Freshwater wetlands in Salt Ponds Critical Resource Area protected by the Charlestown EPA SNEP Grant

6.) Installation of a conventional OWTS to replace a failing system, spring 2019

Town of Charlestown, R.I.
On-Site Wastewater Management Program

A DEPARTMENT OF CHARLESTOWN PUBLIC WORKS

RIDEM Application for Alternative OWTS Technology (Experimental)

PREPARED BY: MATT DOWLING, WASTEWATER PROGRAM MANAGER

VISION STATEMENT

Protecting the quality of Charlestown's drinking water, groundwater and surface water resources for public health and environmental management by using septic systems as a cost effective alternative to a municipal sewer system.

MISSION STATEMENT

The Charlestown Onsite Wastewater Management Program is committed to serving the needs of Charlestown residents, businesses, and visitors by protecting our groundwater quality, the only source of drinking water in Charlestown, and surface water quality through the management of on-site wastewater treatment systems (OWTS) while providing funding, educational outreach, and technical assistance to property owners; and facilitating future economic growth balanced with resource protection.

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Via email
March 2, 2021

TOWN OF CHARLESTOWN
Via Electronic Mail & USPS
CN-21-005

TOWN OF CHARLESTOWN

4540 SO. COUNTY TRAIL
CHARLESTOWN,
RHODE ISLAND

Tel (401) 364-5030
Fax (401) 364-1238

Rhode Island Department of Environmental Management
Office of Water Resources
OWTS Program, 235 Promenade Street
Providence, RI 02908-5767

RE: Application for Alternative OWTS Technology – Experimental, REV-3
Nitrogen Reducing Layered Soil Treatment Area

Program Director and OWTS Staff:

The Town of Charlestown and our partners the University of Rhode Island (URI) Laboratory of Soil Ecology and Microbiology (LSEM) and the New England On-Site Wastewater Training Program (NEOWTP) have received the second round of comment from RIDEM regarding the January 22, 2021 *Application for Alternative OWTS Technology – Experimental, Nitrogen Reducing Layered Soil Treatment Area*. Our working group has prepared the attached application as revised to the RIDEM.

The application seeks RIDEM approval for pilot assessment of experimental N-reducing OWTS technology utilizing Layered Soil Treatment Area (LSTA). LSTA is a non-proprietary, passive, lower cost on-site wastewater N-reduction method that treats septic tank effluent by creating sequential aerobic and anaerobic zones within the soil treatment area. Extensive piloting assessment of this technology has been conducted by the Barnstable County Department of Health and the Environment (BCDHE) at the Massachusetts Alternative Septic System Test Center (MASSTC) and Suffolk County, NY. Further, the wastewater treatment method is approved by the State of Connecticut as "Passive N Reduction" OWTS.

Existing N-reducing OWTS technologies are an effective method to mitigate and reduce nutrient loading from on-site wastewater. However, these systems are often costly and complex. The development of non-proprietary, lower cost, effective N-reducing technologies such as LSTA will provide an alternative option for property owners and will foster additional upgrades from older conventional and substandard OWTS within these watersheds.

Charlestown and our partners propose to replace between three and ten substandard / failing OWTS in the coastal zone of Charlestown with LSTA technology and monitor effluent for at least two years to assess its efficacy for N-reduction. Project findings will be compiled with results from other regional collaborators and on-going piloting projects to ultimately seek approval for LSTA as an approved N-Reducing Technology in RI. These tasks are being funded entirely with public investment through the Town of Charlestown specifically for the greater public good. We are collaborating with the nationally recognized OWTS research experts and soil scientists at URI, BCDHE, MASSTC and Suffolk County, NY to ensure the most recent and peer reviewed scientific data and methods are employed.

Given the implications of RIDEM OWTS Rule §§ 6.41(F)(c) and the requirement for escrow funds to manage potential experimental system replacement, our initial project has been reduced to half scope. The OWTS Rules also explicitly refer to <u>any</u> developer of <u>any</u> I&A technology as a "vendor" and require manufacturers of a proprietary technology to apply through a complex approval process. To foster innovation, the Town urges RIDEM to modify the OWTS Rules to streamline N-reducing OWTS technology approvals and to consider a separate approvals process for non-proprietary or "field built"

OWTS technology assessments. These are often conducted by research institutions, nonprofits, or others with limited funds from public sources with the intended goal of not for profit, but for expanding protection of environmental resources and public health. We would advocate for such a process in the next OWTS Rule modification to foster innovation of N-reducing OWTS technology in RI. Revisiting the 2016 EPA funded Data Share Agreement would allow for strongly vetted technologies to receive regional approval for N reduction, this would open the marketplace and lower costs.

We consider RIDEM a partner in this process, not only the regulatory agency charged with siting and reviewing technological OWTS data for approval, but as the governmental body responsible for protection for the state's groundwater and surface water resources, the end goal of this project. We believe that with the assessment of this OWTS design method and subsequent approval, a lower cost N-reducing option would be available in certain circumstances based on lot size and groundwater table elevation. A lower cost, effective N-reducing technology will facilitate a higher upgrade rate of older substandard and conventional OWTS and reduce N loading in the critically impacted watersheds of all of Rhode Island.

We look forward to working with RIDEM as a partner in this process. Please contact me with any questions or comments regarding this application.

Sincerely,
Town of Charlestown

Matthew J. Dowling
Onsite Wastewater Manager, Environmental Scientist

CC: The Honorable Charlestown Town Council
Charlestown Wastewater Management Commission
Janet Coit, RIDEM Director
Mark Stankiewicz, Charlestown Town Administrator
Wyatt Brochu, Esq., Charlestown Town Solicitor
Jane Weidman, Charlestown Town Planner
George Loomis, NEOWTP
Alissa Cox, PhD, NEOWTP
Jose Amador, PhD, URI LSEM
Justin Jobin, Suffolk County, NY Department of Health
George Heufelder, BCDHE MASSTC
Brian Baumgaertel BA, REHS/RS, Senior Environmental Specialist MASSTC

<u>Required Revisions for REV-3 Experimental Technology Application and LSTA Draft Guidance Document dated January 22, 2021</u>

1. Revise application proposal and guidance document to reflect a statewide required separation to the SHWT of 2' and 4' to Ledge. Separation to the SHWT and Ledge shall be measured from the receiving soil surface or bottom of peastone layer.

Revised Application Section IV A as such:

> "The infiltrative surface for the LSTA shall be the bottom of the peastone below the 18-inch ASTM C33 sand/sawdust denitrifying layer. LSTA vertical separation distance to seasonal high water table (SHWT) shall be measured from the base of the infiltrative surface and shall be a minimum of two (2) feet statewide, as approved for previously installed LSTA in Massachusetts by BCDHE. If bedrock is encountered, the infiltrative surface shall be at least four (4) feet from the restrictive layer. SHWT and soil characteristics shall be determined by an approved RIDEM Soil Evaluation."

Revised Application Section IV D as such

> "The LSTA design criteria provides more enhanced separation distance to SHWT than existing RIDEM approved PSND or GeoMat design criteria which allows for a two (2) foot separation distance to SHWT in native soils. In the case with LSTAs there is three feet of effluent treatment provided by media within the LSTA."

Revised Guidance Document Page 9 as such:

> "The infiltrative surface for the LSTA shall be the bottom of the peastone below the 18-inch ASTM C33 sand/sawdust denitrifying layer. LSTA vertical separation distance to seasonal high water table (SHWT) shall be measured from the base of the infiltrative surface and shall be a minimum of two (2) feet statewide, as approved for previously installed LSTA in Massachusetts by BCDHE. If bedrock is encountered, the infiltrative surface shall be at least four (4) feet from the restrictive layer. SHWT and soil characteristics shall be determined by an approved RIDEM Soil Evaluation."

2. Revise O&M section of proposed guidance document to include a requirement to obtain 4 quarterly samples every 5 years to demonstrate continued compliance with performance standards. Indicate that media replacement or system replacement will be required if performance standards cannot be maintained.

Revised Guidance document Page 12 to include the new statement:

> "Subsequent to the final monitoring report, a series of four quarterly samples shall be collected and analyzed according to the referenced protocol for each LSTA starting the fifth operating year of each LSTA installed under the experimental

approval. Sampling results shall be submitted to RIDEM for evaluation of performance standards."

3. Revise guidance document and application proposal to include a specific performance standard for each key parameter (i.e. Total Nitrogen, BOD5, TSS, and Oil and Grease). Highlight the fact that performance data must achieve the required standards on a yearly average basis for each parameter. The standards that must be met to move forward with a Class Two or Class One approval are as follows: Total Nitrogen 19 mg/l, BOD 30 mg/l, TSS 30 mg/l, and Oil and Grease 5 mg/l.

<u>Revised Application Section V1.3 to include the following:</u>

"Sample results for key regulated parameters shall be compared to the following performance standards:

Analyte	Performance Standard (mg/L)
Total Nitrogen	19
BOD5	30
TSS	30
FOG	5

Any fats, oil or grease (FOG) present in untreated OWTS effluent that is not sequestered in the septic tank will be absorbed and entrapped within the initial one to two inches of aerobic sand media, similar to single pass sand filter. Further, LSTA shall be utilized only for residential applications where FOG is typically not a factor affecting effluent treatment. Requirement of FOG analysis in this application is an unproductive utilization of fiscal resources. However, one FOG sample per LSTA will be collected during the second monitoring event six months into each system operation. If FOG is detected above the performance standard of 5 mg/L, subsequent confirmatory sampling will be implemented."

and,

"Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored."

<u>Revised Application Section VIII, Section 1.1 of the Guidance Document as such:</u>

"During experimental trials, forward flow shall be determined at each site visit using elapsed pump cycle meter. STE and final effluent shall be sampled for DO, temperature, BOD5, TSS, pH, TN, Nitrate, Nitrite, Ammonium, Alkalinity, and FOG and TKN (reported by equivalent analysis provided by subtraction). STE shall be collected from septic tank pump basin and final effluent shall be collected from the pan lysimeter installed within the LSTA. Samples shall be collected using standard procedures by Town of Charlestown staff and partners and will be transported on ice under chain of custody protocol to LSEM for laboratory analysis. Sample results for key regulated parameters shall be compared to the following performance standards:

Performance Standards

Analyte	Performance Standard (mg/L)
Total Nitrogen	19
BOD5	30
TSS	30
FOG	5

Analysis of fats, oil and grease (FOG) is not warranted. Any fats, oil or grease (FOG) present in untreated OWTS effluent that is not sequestered in the septic tank will be absorbed and entrapped within the initial one to two inches of aerobic sand media, similar to single pass sand filter. Further, LSTA shall be utilized only for residential applications where FOG is typically not a factor affecting effluent treatment. Requirement of FOG analysis in this application is an unproductive utilization of fiscal resources. However, one FOG sample per LSTA will be collected during the second monitoring event six months into each system operation. If FOG is detected above the performance standard of 5 mg/L, subsequent confirmatory sampling will be implemented.

pH data from existing installations in Barnstable County summarized as part of this application indicate that of the seven monitored LSTA OWTS, a total of 184 observations of final LSTA effluent pH were collected. Average pH measurement was 6.5 with a mean of 6.4 and a standard deviation of 0.74. The maximum recorded pH measurement was 9.6 and the minimum was 4.4. The pH of final effluent is considered to be equivalent of that with any other approved OWTS technology and pH warrants no additional protocols or mitigation measures as part of this experimental assessment.

Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored. Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Two years following the final system sampling protocol, a final monitoring report shall be submitted to RIDEM from the Town and LSEM to discuss results, conclusions and next steps including compliance with RIDEM OWTS Rule 6.41.F.2.c & d.

Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Two years following the final system sampling protocol, a final monitoring report shall be submitted to RIDEM from the Town and

LSEM to discuss results, conclusions and next steps including compliance with RIDEM OWTS Rule 6.41.F.2.c & d.

Subsequent to the final monitoring report, a series of four quarterly samples shall be collected and analyzed according to the referenced protocol for each LSTA starting the fifth operating year each LSTA installed under the experimental approval. Sampling results shall be submitted to RIDEM for evaluation of performance standards."

4. The last paragraph in page 8 of the proposed guidance document indicates that a flat loading rate of 0.7GPD/sq.ft. is proposed and alternative loading rates may be assessed. To account for tight soils that may be encountered, and to prevent potential hydraulic failure of the LSTA leaching systems, revise the guidance document to require compliance with OWTS Rule 6.33. Please also indicate that the maximum allowable loading rate is 0.7 GPD/sq.ft.

Based on subsequent discussions with RIDEM the following appears appropriate in Section VI.2 of the Guidance Document:

'LSTA effluent analysis from existing installations indicates that water quality is commensurate to that from a RIDEM approved advanced wastewater treatment unit, including BOD and TSS. Therefore, our standard loading rate of 0.70 gal/ft2/day is considered a conservative rate."

5. It is noted that the guidance document was revised to clearly show that systems may be designed using 9 inches or 18 inches of the ASTM-C-33 sand only layer. Although the study period will be used to evaluate the effectiveness/viability of both depths to guide future design modifications, the DEM would like you to reconsider this approach before you respond. Although the Experimental Technology requirements do not specifically prohibit this approach, introducing too many variables may limit the ability for this technology to be granted approval for unlimited statewide use in the future. Consider modifying the proposal to utilize a streamlined design that is consistent for all installations. This approach will allow for potential future approval of the proposed technology under the Class Two or Class One A/E Technology criteria. To meet these standards, if that is the long-term goal, a specific the number of systems, performance data, and utilization of a consistent design approach is necessary to satisfy OWTS Rule 6.41.D. If ramp up time is required for performance results to be achieved, edits to the proposed monitoring period of 2 years may also be required to set the stage for a future Class Two or Class One approval.

Deleted reference to modifying aerobic zone thickness as part of this assessment in Section VII of the Guidance Document and in Section IV of the Application.

6. Establish deadline/timeframe of six months for completion of final report for each system following completion of the 2-year monitoring period. The final report must discuss

results, conclusions, and next steps including compliance with OWTS Rule 6.41.F.2.c&d. Revise application and guidance document accordingly.

Revised Section VI.C.3 of the application as such:

"Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored. Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Within six months of the completion of the two year LSTA sampling protocol for each LSTA installed under this approval, efficacy data and operational summaries shall be submitted to RIDEM in report format to detail results, conclusions and next steps including compliance with OWTS Rule 6.41.F.2.c&d."

Revised Guidance Document VIII.1.1 to include:

"Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored. Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Within six months of the completion of the two year LSTA sampling protocol for each LSTA installed under this approval, efficacy data and operational summaries shall be submitted to RIDEM in report format to detail results, conclusions and next steps including compliance with OWTS Rule 6.41.F.2.c&d."

7. Remove reference to new construction or alteration permit types on Page 9, Section 3 of the proposed guidance document.

Removed

8. Provide final copy of revised guidance document as a stand-alone pdf file. Also submit a revised application in hard copy format (1 copy) by mail. Submit electronic copies also via SharePoint site established by DEM OWTS. To share documents electronically please visit this SharePoint link where you can paste documents for review by DEM OWTS Program staff: https://rigov.sharepoint.com/:f:/r/sites/dem-ep-owr/Shared%20Documents/OWTS%20-%20AE%20Technology%20Program/Nitrogen%20Reducing%20LSTA?csf=1&web=1&e=CFlil2

Submitted as Required.

NOTE: Modified "EXAMPLE LSTA SEPTIC SYSTEM DETAIL" and EXAMPLE LSTA SEPTIC SYSTEM DETAILS" to reflect updated SHWT setback

TOWN OF CHARLESTOWN

Comments received by the Town of Charlestown from RIDEM on December 8, 2020 and specific responses as referenced in the January 22, 2021 Revised RIDEM Nitrogen Reducing LSTA Experimental OWTS Application and Guidance Document.

1. The application must be revised to address OWTS Rule 6.41.F.2.c&d; revise application materials and guidance document accordingly.

   Discussion included as new subsections into the <u>Application (Section IV.B)</u> and <u>Guidance Document (Section VI.3.1)</u> detailing program for compliance with specified rules.

2. Clarify specific separation required separation distances to WT and Ledge proposed from bottom of pea stone layer for all areas of the state, including Critical Resource Areas as established in OWTS Rule 6.42.

   Details and justification included in <u>Application under Section IV.A</u> and in the <u>Guidance Document under Section VI.2</u> explaining infiltrative surface design point and seasonal high water table separation.

3. Discuss as part of the revised proposal how the longevity of the carbon source will be evaluated beyond the initial 2-year study period. Performance monitoring and criteria need to be proposed and triggers for media replacement established beyond this 2-year period.

Carbon media longevity is discussed in a new section in the <u>Application under</u> <u>Section IV.F</u>

4. Indicate exactly how design loading rates will be determined, establishing a cap of 0.7 GPD/sq.ft. Create clear guidance on this as part of the application and guidance document revisions. See OWTS Rule 6.33.B as a starting point and refine from there.

   Loading rates discussions have been modified and improved to clearly explain and justify loading rate for LSTA. See <u>Application Sections IV.A and IV.D</u> and <u>Guidance Document Sections VI and VI.2.</u>

5. Revise guidance document to clearly show that systems may be designed using 9 inches or 18 inches of the ASTM-C-33 sand only layer. Study period should evaluate effectiveness/viability of both depths to guide future modifications to experimental approval.

   Revised to specify modifications to uppermost ASTM 33 sand only aerobic zone thickness in a subset of experimental sites in the <u>Application under Section IV.A</u> and in the <u>Guidance Document under Section VII.</u>

6. Specify minimum fill perimeter of 10 feet.

   Included language in the <u>Application in Section IV.A</u> and in the <u>Guidance Document in Section VII</u>.

7. Establish deadline/timeframe for completion of final report for each system based on 2-year monitoring period. Final report must discuss results, conclusions, and next steps including compliance with OWTS Rule 6.41.F.2.c&d.

   Added discussion in <u>Section VI.C.3 in the Application</u> and <u>Section VII in the Guidance Document</u>

8. The proposed guidance document must be revised to include all design information and monitoring requirements outlined in the application.

   Updated both documents to include necessary design and monitoring information as required.

9. TKN and O&G should be sampled and reported for each system monthly for first two years, this should not be optional for consistency and for simplicity reasons.

   Updated <u>Application</u> for clarification in <u>Section VI.C.3</u> and <u>Guidance Document Section VIII.1.1</u>

10. The example site plan does not match the proposal in terms of media and layer depths for the system configuration. Revise accordingly.

    Revised both site plan and specification sheet.

11. Discuss as part of application revisions and guidance document how a low pH discharge to subsurface will be evaluated. Modify study to evaluate any potential adverse impacts of this to the environment and human health.

    Updated <u>Application</u> for clarification in <u>Section VI.C.3</u>, no modifications amended to Guidance Document.

12. Earlier studies indicate that the sand/sawdust layer has the potential to settle over time, establish a method to evaluate this as part of the study of the experimental systems to feed forward any future O&M recommendations.

    Included language in Section IV.F of the Application and VIII.1 of the Guidance Document

13. Revise guidance document to clearly indicate that this technology is appropriate for residential use only and may be utilized for design flows equal to or less than 345GPD (Maximum 3 Bedroom Use).

    Application and Guidance Document revised throughout to indicate LSTA shall be implemented for OWT Repair Application for total daily flows not exceeding 460-gallons per day, or 4-bedroomom design.

14. The proposal includes incorporation of Geomat technology for wastewater distribution - clarify **in guidance document** how system design /sizing will be completed vs how system sizing is typically done using the Geomat technology. It is imperative to eliminate any ambiguity on this point.

    Sizing is clarified in Section IV.A of the Application and VI and VI.2 of the Guidance Document

15. In order to maintain the non-proprietary nature of the proposal the application and subsequent study of the LSTA systems would be strengthened by incorporating several distribution technology types. Revise application and guidance document to allow the use of several types of wastewater distribution technologies including existing non-proprietary technologies such as a PSND as outlined in OWTS Rule 6.37.D. Be clear about which existing and approved technologies are compatible and address any specific design guidance that may apply to each configuration proposed.

    PSND is now specifically mentioned as the other option for effluent dispersal for LSTA throughout Application and Guidance Document.

16. Provide final copy of revised guidance document and revised application in hard copy format (3 copies) by mail. Submit electronic copies also via SharePoint site established by DEM OWTS. To share documents electronically please visit this SharePoint link where you can paste documents for review by DEM OWTS Program staff: https://rigov.sharepoint.com/:f:/r/sites/dem-ep-owr/Shared%20Documents/OWTS%20-%20AE%20Technology%20Program/Nitrogen%20Reducing%20LSTA?csf=1&web=1&e=CFlil2

    OK

17. Please also copy and paste the original application materials dated September 1, 2020 in this same SharePoint site folder. This is the safest means of transferring files to the DEM per our IT department.

    OK

APPLICATION FORM FOR ALTERNATIVE/EXPERIMENTAL TECHNOLOGY

Rhode Island Department of Environmental Management
Onsite Wastewater Treatment Systems Program
Office of Water Resources
235 Promenade Street, Providence, RI 02908-5767
Tel. (401) 222-3961; Email: DEM.OWTS@dem.ri.gov
www.dem.ri.gov/septic

INNOVATIVE OR ALTERNATIVE TECHNOLOGY:

☐ Alternative System or Technology ☐ System Component ☒ Experimental System

*Fee schedule on next page

INNOVATIVE OR ALTERNATIVE TECHNOLOGY or COMPONENT – CLASS

☐ Class One ☐ Class Two

COMPANY NAME:
Town of Charlestown, RI

MAILING ADDRESS (STREET) (CITY/TOWN, STATE)
4540 South County Trail, Charlestown, RI 02813

TECHNOLOGY NAME:
Nitrogen Reducing Layered Soil Treatment Area OWTS

TELEPHONE NUMBER:
(401) 364-5030

CONTACT PERSON:
Matthew Dowling

EMAIL:
mdowling@charlestownri.org

If applicant is not the manufacturer, indicate authority to distribute technology:

☐ The applicant has sole authority to distribute or authorize distribution of the subject technology in Rhode Island.

☐ The applicant does not have sole authority to distribute or authorize distribution of the subject technology in Rhode Island and has enclosed herewith a letter from the manufacturer authorizing the applicant to seek AE technology approval.

I CERTIFY THAT THE INFORMATION ABOVE AND ATTACHED HERETO WAS PREPARED IN ACCORDANCE WITH THE PROCEDURES PRESCRIBED IN RIDEM "250-RICR-150-10-6 RULES ESTABLISHING MINIMUM STANDARDS RELATING TO LOCATION, DESIGN, CONSTRUCTION AND MAINTENANCE OF ONSITE WASTEWATER TREATMENT SYSTEMS", AND THAT THE INFORMATION IS TRUE, ACCURATE AND COMPLETE.

SIGNATURE OF APPLICANT Matthew J. Dowling DATE March 2, 2021

JOB TITLE Onsite Wastewater Program Manager / Environmental Scientist

**PLEASE MAKE CHECK PAYABLE TO THE RHODE ISLAND GENERAL TREASURER. MAIL CHECK, APPLICATION FORM AND SUBMITTALS TO: THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT, OFFICE OF WATER RESOURCES, 235 PROMENADE STREET, PROVIDENCE, RI 02908

***THIS APPLICATION IS TO BE USED TO APPLY FOR APPROVAL OF A TECHNOLOGY OR COMPONENT ONLY. IT IS NOT TO BE USED TO APPLY FOR A PERMIT TO INSTALL THE TECHNOLOGY OR COMPONENT AT A SITE.

APPLICATION CHECKLIST

THE FOLLOWING ITEMS MUST BE INCLUDED WITH YOUR SUBMISSION:

- [ ] A complete application form and all attachments (1 digital copy and 3 hardcopies);
- [ ] Proper fee in accordance with OWTS Rule 6.54.B, see table below. Make checks payable to the "Rhode Island General Treasurer";

Alternative OWTS or Technology:	**Fee:**
Class One	$1,000.00
Upgrade from Class Two to Class One	$500.00
Class Two	$1,000.00
Alternative OWTS Component:	
Class One	$200.00
Class Two	$300.00
Experimental OWTS or Technology	$2,000.00
Approval Modification	$200.00

Note: Carefully review submittal requirements.

INCOMPLETE APPLICATIONS: APPLICANTS WILL BE INFORMED OF DEFICIENCY AND THE APPLICATION WILL NOT BE REVIEWED UNTIL ALL REQUIRED SUPPLEMENTAL MATERIAL HAS BEEN SUBMITTED.

Complete all data and forward the complete package to:

RHODE ISLAND DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
OFFICE OF WATER RESOURCES
OWTS PROGRAM
235 PROMENADE STREET
PROVIDENCE, RI 02908-5767

And
DEM.OWTS@dem.ri.gov

Questions pertaining to the application process should be directed to the OWTS program at (401) 222-3961 or DEM.OWTS@dem.ri.gov.

SUBMITTAL REQUIREMENTS FOR ALTERNATIVE OR EXPERIMENTAL TECHNOLOGY OR COMPONENT APPLICATION

Answer all the following questions. Your answers must be in the sequence presented below and in outline format. Use separate sheets as necessary. If a question does not require a response, please write in "NOT APPLICABLE" and a justification as to why no response is required. Please attach additional sheets if more space is required.

I. Technology Information

A. Technology trade name and/or model number(s).

Nitrogen Reducing Layered Soil Treatment Area - (LSTA)

B. Description of the theory behind proposed technology.

Proprietary nitrogen (N) reducing onsite wastewater treatment systems (OWTS) can be an effective means of lowering N loading to critically sensitive watersheds. However, cost of these technologies is often cited as a major barrier to more widespread implementation as a regional watershed scale N-loading reduction strategy. Nitrogen-reducing layered soil treatment area (LSTA) OWTS technology is a non-proprietary method of facilitating sequential nitrification and denitrification of residential strength septic tank effluent (STE) utilizing only the drainfield and no other secondary treatment components. The treatment train consists of a two-compartment septic tank with a hanging pump vault in the second compartment, and the layered soil treatment area (STA). The treatment process is passive, using only one pump to time-dose STE to the LSTA surface. Because the LSTA is a single pass media filter (similar to a single pass sand filter (SPSF) and bottomless sand filter (BSF)), it does not require wastewater to be recirculated between multiple compartments or actively aerated. The LSTA configuration relies only on stratification of aerobic and anaerobic carbon-amended zones within the STA, leveraging the microbial communities to sequentially nitrify and denitrify the incoming N in the septic tank effluent within the two layers of the LSTA.

An LSTA, also referred to elsewhere as a “layer cake” STA, promotes sequential nitrification and denitrification as wastewater passes vertically through the LSTA profile. This is accomplished by constructing the LSTA in two layers: a top 18-inch thick layer of ASTM C-33 sand (nitrification layer; where aerobic conditions promote autotrophic nitrification) above an 18-inch thick layer of ATCM C-33 sand mixed with lignocellulosic material (sawdust/wood chips) denitrification layer, where anaerobic conditions exist and provides a carbon source as an electron donor supporting populations of heterotrophic microbes and facilitating denitrification.

A layer of peastone at the interface with native soil helps retain moisture in the denitrification layer, further promoting anaerobic conditions. In this non-proprietary design, STE is time-dosed to the top of the sand layer (in a manner similar to a single-pass sand filter/bottomless sand filter), where passive aerobic conditions allow ammonium (NH_4^+) to be oxidized to nitrate (NO_3^-). The nitrified effluent subsequently infiltrates into the underlying denitrification layer, where the water content is higher, as the lignocellulos materials used to amend the sand have a higher water-holding capacity and slows down the diffusion of oxygen (O_2). This denitrifying layer also has a higher concentration of dissolved organic carbon (C) from the lignocellulose wood products, which serves as a C source for heterotrophic denitrification, and helps keep O_2 levels low as a result of microbial oxidation of organic C (Amador and Loomis, 2018).

Many of the concepts and components associated with the LSTA already exist under the current RIDEM Rules Establishing Minimum Standards Relating to Location, Design, Construction and Maintenance of Onsite Wastewater Treatment Systems (OWTS Rules) and are very familiar to the OWTS design and installation community, including; programmable timer, timed-dosing, pressure dosing, two-compartment tank with hanging pump vault, surge storage capacity in the tank, sand filtration, bottomless filters and ASTM C-33 sand media. **The only new concept is the mixing of sand and lignocellulos and its placement beneath the upper sand layer in the LSTA.** Wastewater professionals familiar with SPSF, pressurized shallow narrow drainfield (PSND) and BSF will recognize commonly used materials and components in the LSTA design.

This method of onsite wastewater treatment has proven highly effective at N-reduction in field trials conducted in the Northeast United States over the last several years.

The results of these field trials warrant additional field studies in Rhode Island (RI) to further assess the efficacy of LSTA as a standalone N-reducing OWTS technology in RI. Further, assessments of the technology with reductions in vertical and horizontal footprints, adapting LSTA for more effective use in coastal estuarine watersheds and as a potential alternative to BSF in some applications should be assessed. Therefore, we have submitted the attached RIDEM Application for Experimental Technology approval to conduct experimental installations of the LSTA in RI, with the goal of providing homeowners and the onsite wastewater community with a cost-effective alternative to currently approved proprietary N-reducing systems.

C. Statement of Claim (if applicable) – state the effluent concentration and/or percent pollutant reductions you claim this technology can consistently achieve.

Nitrogen Reduction: 50% reduction of TN from residential strength wastewater and a preponderance of final effluent N concentrations of 19 mg/L or less

D. What classification are you applying for and give justification as to why the technology belongs in the requested class.

This application is for "*Experimental OWTS Classification*" in accordance with Section 250 of the Rhode Island Code of Regulations (RICR) 250-150-10-6.41 of the OWTS Rules. We proposed to install between 4 and 10 experimental OWTS utilizing LSTA to evaluate final effluent for total N (TN) percent reduction and to confirm that a preponderance of final effluent TN concentrations are nineteen (19) milligrams per liter (mg/L) or less. Sampling of STE and the final effluent (below the LSTA and before final dispersal to native soil) will be conducted for each experimental LSTA installation for a period of no less than two years.

Based upon positive treatment performance results from systems installed in the greater Southern New England region, we feel the LSTA technology is capable of meeting the criteria set forth in 250-RICR-150-10-6.41 (Heufelder, 2017, Sohngen et al., 2019, Heufelder, 2020; Cox et al., 2020; Wigginton et al., 2020; Langlois et al., 2020).

II. Approval/Denial History

A. Approvals from: (Include copies of all approved letters, conditions, restrictions, and contact person).

1. Rhode Island:

None Approved

2. Other state approvals:

The technology is currently approved for general use by the Connecticut Department of Health Connecticut as Passive Nitrogen Reduction (PNR) in accordance with Section VI(C) of the *Connecticut On-site Sewage Disposal Regulations and Technical Standards for Subsurface Sewage Disposal Systems* dated January 1, 2018. The configuration of PNR can be installed in Connecticut as described in this application.

LSTA is currently also approved by Massachusetts Department of Environmental Protection (MASSDEP) for Site Specific Piloting Assessment.

3. Other jurisdictions:

<u>Experimental/Piloting LSTA Installations in Barnstable County, MA</u>, currently under assessment by MASSTC, (Heufelder, 2017; Waugh et al., 2020; Wigginton et al., 2020; Cox et al., 2020; and Langlois et al., 2020).

<u>Modified Experimental Installations in Suffolk County, New York</u>, termed Nitrogen Reducing Biofilters, Currently under assessment by Suffolk County Dept. of Health Services and Stony Brook University, (NY State Center for Clean Water Technology, 2016, Sohngen et al., 2019, Pigott et al., 2020).

<u>Modified Experimental Installations in Florida</u>, assessed and published by Chang et al., 2010 and Anderson and Hirst, 2015.

<u>Modified Experimental Installations in Central Ontario, Canada</u>, Assessed and published findings, (Robertson and Cherry, 1995; and Robertson et al., 2000; Robertson et al., 2008; Suhogusoff et al., 2019).

<u>Modified Experimental Installations in Sweden</u>, (Dalahmeh et al., 2019)

<u>Modified Experimental Installations in Brazil</u> (Suhogusoff et al., 2019)

B. Denials from: (Include copies of all denial letters, reasons for denial and contact person).

1. Rhode Island:

None

2. Other states:

None

3. Other jurisdictions:

None

III. Performance Data

A. What are you trying to achieve by use of the innovative or alternative technology?

Conduct treatment performance assessment in RI to confirm findings from jurisdictions within the Northeast US region of a passive OWTS soil treatment area design that facilitates reduction of TN from residential strength wastewater by at least 50% and that a preponderance of treated effluent TN concentrations are nineteen (19) mg/L or less. Develop a non-proprietary cost-effective alternative N reduction technology that encourages more widespread adoption of use to help protect groundwater and N-sensitive water resources of the State.

B. How does the technology or component compare in performance to applicable conventional technologies contained in RIDEM's OWTS RULES?

LSTA is a novel, passive and lower cost onsite wastewater treatment method that consists of layering a STA to specifically facilitate N reduction. The design time-doses STE over a buried stratified soil treatment area as previously described. The treatment train design is simple, has several commonly used components already familiar to wastewater professionals, and differs from SPSF and BSF technology only in the stratified modification of the filter.

Like the SPSF, the LSTA is buried and receives STE. Comparable the BSF, the LSTA is also bottomless. The LSTA differs from both these other media filters, in that it is specifically designed to remove N from wastewater. It also differs from any conventional STA in that same regard. The design loading rate to the LSTA (0.7 g/sf/d) is less than the low rate SPSF (1.25 g/sf/d) as well as the high rate SPSF (2.0 g/sf/d), so hydraulic failure at the top of the sand media is highly unlikely with a managed and maintained system as required. Refer to Section IV(C) of this application for detailed loading rate specifications.

Data from Barnstable County, MA indicate that LSTA, when implemented as proposed here, is a highly effective means of TN reduction from residential strength STE by at least 50%. The Massachusetts Alternative Septic System Test Center (MASSTC) and Barnstable County Department Health and Environment (BCDHE) data indicate an average of 70% TN reduction from seven pilot sites in Barnstable over an operating period of two years.

Further, recent installations of seven piloted LSTA's locally termed Nitrogen Reducing Biofilters (NRB) in Suffolk County, New York have demonstrated the capability of reducing residential strength wastewater TN concentrations to below 6 mg/L (Sohngen et al., 2019 and Pigott et al., 2020).

C. Data to support applicant's claims:

There have been numerous OWTS configurations utilizing lignocellulose media as a carbon source for enhancing a saturated, anaerobic denitrification zone as part of final effluent management. These include installations and assessments in Florida, New York, Massachusetts, Ontario Canada, Brazil and Sweden.

However, the designs from these various locations have differed somewhat. The specific design methods proposed here have been installed and assessed by BCDHE, MASSTC and the University of Rhode Island (URI) Laboratory of Soil Ecology and Microbiology (LSEM).

Specifically, LSTA installations assessed by Fine, 2017; Cox et al., 2020; Heufelder, 2020, Wigginton et al., 2020; and Langlois et al., 2020 (**Attachment 1**) and research currently in progress from George Heufelder at MASSTC and Jose Amador, PhD, at LSEM form the basis for this experimental OWTS configuration and our application to RIDEM.

1. Number of installations tested.

Background

The earliest published use of layering aerobic and anaerobic treatment using lignocellulose media for the treatment of N from OWTS was conducted by Robertson and Cherry (1995). These researchers constructed OWTS using similar methods proposed here and found that after one year of operation, between 60% and 100% TN was removed from wastewater with influent TN concentrations up to 125 mg/L. The researchers also found that in the aerobic zone near complete nitrification of NH_4^+ to NO_3^- was observed and the anaerobic carbon zones were highly effective at denitrification, reducing influent nitrate concentrations from 125 mg/L NO_3^- to between 1.2 mg/L and <0.005 mg/L. Subsequent analyses of their OWTS design indicated that after 15 years of operation, the system provided nearly complete removal of influent NO_3^-N. However, the results also indicated that the functionality of the denitrification zone is temperature dependent (Robertson et al., 2008). The longevity of the carbon source was also examined and determined to be consumed at a rate of <1% annually (Robertson, 2000).

Based on the findings of Robertson and Cherry (1995), Robertson (2000), Robertson et al. (2008) and subsequent successes of experimental designs in Florida assessed as part of the Florida Department of Health's Passive Nitrogen Removal Study, BCDHE commenced LSTA mesocosm assessments at the MASSTC in Massachusetts in 2014.

Initial mesocosm design and testing results indicated that when loaded with residential strength wastewater at 0.7 gallons per square foot per day, near complete nitrification of wastewater is feasible in the upper 18" aerobic portion of an LSTA in three different soil types assessed for the nitrification zone - sand (100% MA Title V sand; consisting of ASTM C-33 sand), loamy sand (70% sand 30% silt), and sandy loam (60% sand 40% silt). Beneath the 18" aerobic zone, an 18" layer of 50/50% mixture of sand and lignocellulose material (typically sawdust) mixed by volume was placed. This layer remained saturated as nitrified percolate from the aerobic zone was applied via drip distribution. Further, alkalinity and pH were determined to remain at levels within the mesocosm conducive to fostering complete nitrification. Results indicated final effluent concentrations of TN of <5 mg/L. Results of these first MASSTC assessments are attached as **Attachment 2** –- *BCDHE MASSTC Progress Report on the investigation of non-proprietary means of removing nitrogen in onsite septic systems, July – August 2014 and October –December 2014* Cape Cod Commission (2015).

Based on the positive outcomes of N removal from the mesocosm analysis, BCDHE installed a small scale, 25 square foot test LSTA at the MASSTC facility as the first field trial mark up in October 2014. The field trial LSTA was first dosed with septic tank effluent and sampled in November 2014. Results indicated some initial N concentration fluctuations but were compelling to warrant further study. Subsequently, a full scale 15ft X 30ft LSTA was installed at the MASSTC in November 2014 and became operational in December 2014. This LSTA received STE and was routinely sampled through November 2016.

Both the small scale and full-scale installations utilized loamy sand as the 18-inch upper nitrification (aeration) zone. Following the first full scale test design, four more test designs were installed at MASSTC between 2016 and 2017. Modifications made included using Massachusetts Title V (ASTM C-33) sand as the media base for both aerobic zone and the sand base of the anaerobic zone.

Other various design modification and alterations were implemented including lining portions of the LSTA with impermeable liners, various extents of saturation and various sand/silt and cellulose ratio mixes. The results indicated that the design entitled "*Design #5*" which used 18-inches of standard MA Title V ASTM C-33 sand above an 18-inch thick layer of 1:1 ratio by volume of sand and sawdust, with a layer of peastone below the saturated layer and an impervious liner on the sidewalls of the soil treatment area proved to be the most cost effective and allowed for >75% reduction in TN with a final effluent average concentration of 9.9 mg/L. Further, 5-day biological oxygen demand (BOD_5) averaged <15 mg/L.

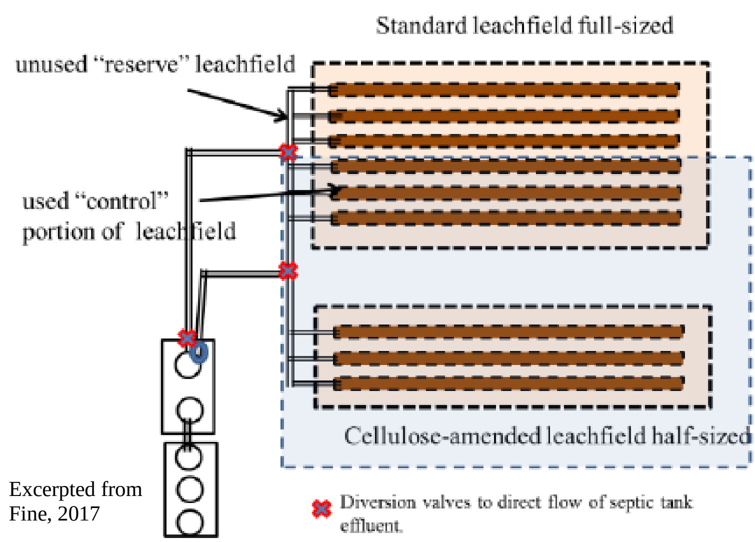
Results are summarized in Fine, 2017 in **Attachment 1** – *Continued Operation of the Massachusetts Alternative Septic System Test Center and the Investigation of Passive Nitrogen Removal Strategies for Onsite Septic Systems – Project 15-07 319* (Fine, 2017).

The LSTA concept was further assessed on a larger scale as part of a US Environmental Protection Agency (EPA) Southeast New England Program (SNEP) grant partnership with URI LSEM. The grant program proposed the installation of up to 12 LSTA demonstration piloting sites in Barnstable County for residential field trial assessments in 2016.

Under the SNEP grant, there were ultimately ten (10) residential installations of LSTA

OWTS configurations in Barnstable County between 2017 and 2019. Three of the most recent installations have yet to be sampled based on inadequate occupation by residents and COVID-19 pandemic issues. All LSTA systems were installed using low pressure timed-dosing of STE. Each treatment train consists of a 1,500-gallon two compartment septic tank, a 1,000-gallon pump tank that allows for timed-dosing STE to the LSTA utilizing GeoMatrix Systems, LLC GeoMat shallow drainfield material. Note that, in Massachusetts a separate pump tank is required for pressure dosing systems, but this would not be necessary for systems in Rhode Island. The researchers note that although the proprietary dispersal system GeoMat was utilized to achieve as low a profile installation as possible, a non-proprietary pressurized dosing technique to disperse STE to the LSTA could be used. For comparative analytical purposes, BCDHE installed a full-scale control (36" Title V sand) STA at all sites but shut off half the laterals to half of the control drainfield, in addition to a half-sized LSTA. Daily flow from the dwelling was evenly divided between the experimental LSTA and the control STA, to allow for a direct comparison of treatment efficacy among the control and experimental LSTA technology.

The typical LSTA pilot design layout as detailed:



The LSTA in cross section is installed as below:

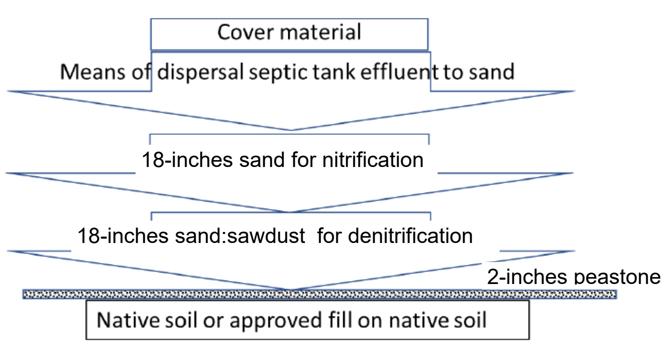


Figure 1 Conceptual representation of layered system for denitrifying percolate beneath onsite septic system soil absorption systems.

Excerpted from Heufelder 2020

BCDHE Field Pilot Installation Design and Testing

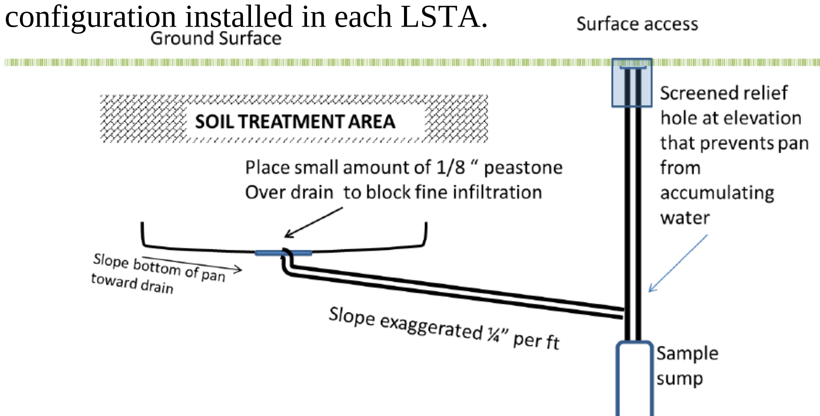
Seven field installations have been monitored for a period of two years. The sites are identified and data is summarized in **Section C4**. Full site information and data analysis are provided in detail in Heufelder, 2020 in **Attachment 1** *–2019 Annual Report to The Massachusetts Department of Environmental Protection-The performance monitoring the non-proprietary layered systems presently under Site-Specific Pilot Approvals* (Heufelder, 2020).

2. Duration of tests.

Testing of the LSTA at these seven residential sites was conducted by BCDHE from 2017 through 2020 and is ongoing; data are summarized in **Attachment 1,** Heufelder, 2020**.**

3. Condition of tests.

Each LSTA installed as part of BCDHE's assessment was designed with pan lysimeters installed directly below the base of the LSTA. Emphasis from sampling assays was obviously placed on N as this was the basis of the study. Samples were collected and analyzed monthly by BCDHE staff and submitted to Commonwealth Certified laboratories including the Barnstable County Department of Health and Environment Laboratory. Below is a schematic from BDCHE detailing the pan lysimeter configuration installed in each LSTA.



Excerpted from Heufelder, 2020

4. Analytical Results.

Results from the seven installed LSTAs in Barnstable County Massachusetts indicate substantial N reduction in final effluent. N reduction results varied between sites, typically based on beginning STE strength and system use. Data are summarized in the **Table 1** below. Average STE TN concentration was 67.0 mg/L and ranged from 96.6 mg/L (Gaffney site) to 47.3 mg/L (Cummings site). Average final effluent TN concentrations collected from LSTA at the base of the sand/sawdust layer were 19.1 mg/L and ranged from 31.9 mg/L (Falmouth seasonal site) to 4.3 mg/L (Little Harbor seasonal site). Percent TN removal averaged 70% and ranged from 91% (Little Harbor seasonal site) to 60% (Westport Main Road site).

	Total Nitrogen Levels (mg/L) as determined by calculation TKN + nitrate + nitrite											
	STE	STE upper 95%CI	STE lower 95% CI	Control	Control upper 95% CI	Control lowere 95% CI	Sawdust	Sawdust upper 95% CI	Sawdust lower 95% CI	Days monitored	Data points	% removal based on concentration
Acushnet (year round (2-3 pers.)	55.4	63.6	47.7	31	37	25.4	17.3	20.6	13.9	586	22	69%
Falmouth (Sippiwissett)(sporadic 1-2 pers.)	64.3	77.3	51.2	39.3	46.7	34.8	21.2	25	17.5	494	16	67%
Westport (Main Rd) (year round 2 pers.)	38.7	46.1	31.1	12.2	17.4	8.2	15.3	23.7	6.9	188	7	60%
S. Dartmouth (Gaffney) (year round 2 pers.	96.6	108.3	84.9	43.9	60.9	26.8	23.2	36.9	9.5	280	10	76%
West Falmouth (Little Harbor - Seasonal)	79.4	92.4	66.4				7.3	10.3	4.3	410	7	91%
Chappaquoit Falmouth (Seasonal)	87.6	98.8	76.5	60.6	74.1	47	31.9	41.3	22.5	775	7	64%
Westport (Cummings Lane) (year round 2 pers.)	47.3	59.2	35.4	36.9	46.2	27.6	17.5	26.6	8.4	600	13	63%
Averages	67.0			37.3			19.1			476	12	70%

Detailed data analysis indicates that performance of LSTA is temperature dependent with reduction in N-removal efficiencies identified at temperatures below 10°C. The primary process limitation at these temperatures appears to be the denitrification portion of the process as opposed to the nitrification biology which appears to be less impacted. Concurrent research at the MASSTC suggests that nitrification generally is minimally impacted even at temperatures as low as 5°C (Heufelder, 2020).

By comparison, in a recent study conducted by Ross (2020), 50 residential sites in Charlestown, RI utilizing four RIDEM approved N-reducing OWTS technologies were monitored for N-removal efficiency over a two-year period. Results indicate that on average, TN effluent concentrations ranged from 13.2 mg/L to 33.8 mg/L, depending on technology. TN effluent concentrations from FAST systems ranged from 2.6 to 62.3mg/L, AdvanTex AX-20 from 0.6 to 87.4 mg/L, AdvanTex RX-30 from 3.0 to 60.7 mg/L and Norweco various models from 5.5 to 60.6 mg/L.

Suffolk County, NY Department of Health Services

The Center for Clean Water Technology (CCWT) at Stony Brook University is researching and testing LSTA defined by Suffolk County Department of Health Services (SCDHS) Division of Environmental Quality as Nitrogen Reducing Biofilters (NRB) at MASSTC as well as the new CCWT test center that opened in 2019. CCWT has installed seven full-scale NRBs in Suffolk County under Experimental Use approval as of December 31, 2019. Three pilot NRB's were installed at the MASSTC in 2016 and seven full scale systems were installed at private residences in Suffolk County from 2017-2019 with additional installations planned for 2020. The NRBs installed in Suffolk County, NY were installed in three configurations, unlined, lined and box) and were monitored once the system reached steady state. The unlined configuration is consistent with the design proposed under this Experimental Technology Application.

In Suffolk County, field installed pilot NRB systems have been capable of reducing nitrogen to below 6 mg/L. Pilot testing will be expanded in both scope and scale needed on year-round residences in Suffolk County. Further refinement of NRB's is required in order to bring the installation costs to affordable levels.

Monthly laboratory analytical data collected from residential NRB installations from 2018 through 2019 are summarized below.

Table 5: SCDHS 2019 NRB Sample Results

NRB Technology	# of Systems as of 12/31/2019	# of Samples as of 12/31/2019	AVG TN mg/L
Unlined NRB (grab samples)	3 (1 has no occupants)	27	10.7 mg/L
Lined NRB	3	19	11.4 mg/L
Box NRB	1 (2 pending)	5	4.0 mg/L

Table excerpted from Pigott et al., 2020

The initial proposal for the Town of Charlestown's LSTA program was to install eight LSTA. However, the RIDEM OWTS Rules classify the Town as a "Technology Vendor" which establishes a requirement for funds held in reserve for LSTA replacement if deemed necessary. This requirement ultimately reduces the project scope by half. As such, and in an effort to expand the LSTA installations for assessment, the Town of Charlestown has partnered with Suffolk County, NY, URI LSEM and Stonybrook University and has successfully been awarded funding under a Restore America's Estuaries National Estuary Program Coastal Watershed Grant Program in 2021 to install four (4) LSTA OWTS as designed in this application in Suffolk County, NY to expand efficacy testing in our region. These installations will commence in 2021.

1. **Persons, research entities, private companies, or governmental agencies that collected, analyzed or evaluated samples or results. (Third party testing is highly desirable).**

LSTA OWTS sampling protocols and analytical results described above were performed by BCDHE, URI LSEM and SCDHS personnel. All samples were submitted for laboratory analysis at state certified laboratories including the lab located at BCDHE and CCWT at Stonybrook University. Data were analyzed by MASSTC, URI LSEM and SCDHS personnel.

Note: If there is more than one model of this technology, please label the performance data to indicate which model was used for each test.

IV. Design Criteria

A. Design and materials of the proposed technology.

The Town of Charlestown is proposing to implement LSTA as designed by the BCDHE and installed at their Barnstable County pilot sites detailed above and in **Attachment 1** Heufelder, 2020, with the following modifications:

1) The experimental LSTA systems will not include valved control (sand-only) STAs. We believe through the work conducted by BCDHE, enough data exists to indicate that LSTA is a viable means to reduce STE N concentrations and a

control STA installation is not necessary.

2) The LSTA design will replace the 1,000-gallon pump tank in the BCDHE designs with a hanging screened pump vault located in the second compartment of a 1,500 gallon, 2-compartment septic tank, which will reduce both the system's cost and footprint. The minimum septic tank size is 1,500 gallons and the maximum design daily flow shall be 460 gallons.

Systems will be installed at dwelling units with full time occupancy to ensure analysis of N-reduction throughout all seasons. Since data indicate that LSTA efficiency is maximized in warmer summer months, future approval of this technology will benefit Charlestown's seasonally occupied dwellings in the coastal zone which are typically only occupied during warmer periods of the year. Nearly 2/3 of the dwelling units in the RIDEM delineated Critical Resource Area (CRA) within Charlestown's jurisdictional boundary are occupied seasonally. Similar occupancy regimes are common throughout the statewide RIDEM CRA where N-reducing technology OWTS are required for any new OWTS installation.

Residential dwelling units utilized for experimental technology installation will be limited to four-bedroom occupancy, 460-gallons daily total flow or less as designed. Site soil conditions and seasonal high groundwater table shall be determined by a RIDEM Class IV Licensed Soil Evaluator for any LSTA installation.

The LSTA systems are timed-dosed systems and will be designed using a 1,500-gallon, two-compartment septic tank equipped with a hanging screened pump vault. A surge storage volume of at least 75-gallons per bedroom will be factored into the design to provide surge flow protection to the LSTA. STE will be pressure dosed to the LSTA using a GeoMat 1200 dispersal system or a pressurized shallow narrow drainfield (PSND) as a distribution mechanism at a loading rate of 0.70 gallons per square foot per day. Dosing to the LSTA will be controlled by a programmable timer with an elapsed time meter and event counter capable of logging normal operational and alarm events. Effluent dosing orifice sizing shall be 3/16-inch and shall be spaced according to the RIDEM pressurized drainfield design guidance in 250-RICR-150-10-6.36 through 6.38 of the RIDEM OWTS Rules.

LSTA sizing for each site will be designed on total daily design flow based upon the number of bedrooms and the design loading rate to the LSTA of 0.70 $gal/ft^2/day$. Sizing is based on conventional STA design parameters by applying 0.70 gallons per square foot per day ($gal/ft^2/day$) loading rate to the dwelling total daily flow using GeoMat 1200 and PSND for effluent dispersal. Note that the single loading rate specified here differs from loading rates detailed in the 2016 Geomatrix Systems, LLC Rhode Island GeoMat Design Manual and 250-RICR-150-10-6.37.D.

The infiltrative surface for the LSTA shall be the bottom of the peastone below the 18-inch ASTM C33 sand/sawdust denitrifying layer. LSTA vertical separation distance to seasonal high water table (SHWT) shall be measured from the base of the infiltrative surface and shall be a minimum of two (2) feet statewide, as approved for previously installed LSTA in Massachusetts by BCDHE. If bedrock is encountered, the infiltrative surface shall be at least four (4) feet from the restrictive layer. SHWT and soil

characteristics shall be determined by an approved RIDEM Soil Evaluation.
STE will be dispersed in a minimum of 12 doses and a maximum of 24 doses per day. Controls shall be installed as signal rated floats and include a high-water alarm and peak enable control. All design specifications shall comply with the RIDEM Guidelines *Pressurized Drainfields* according to *250-RICR-150-10-6.3 of the RIDEM OWTS Rules*, except where otherwise specified herein. Orifice spacing shall not exceed 24 inches, and dosing volume will be no more than 0.25g/orifice/dose.

LSTA installation will incorporate practices similar to the site preparation for BSF or single pass sand filter. LSTA will be designed using 18-inches of ASTM C-33 sand over 18-inches of a 1:1 ratio by volume ASTM C-33 sand and untreated sawdust sourced from local sawmills and lumber yards. All sand used in the LSTA shall be in a damp state when added to the LSTA. Sand / sawdust mixture shall be mixed at a location with a clean hard surface (concrete or asphalt) to eliminate contamination with soil/dirt/debris at the construction site. The mixture will then be transported to the site for installation. The sand/sawdust mixture shall be placed in nine-inch lifts and compacted with a standard duty forward plate compactor in a single pass. The sand/sawdust layer will overlie a two-inch layer of double washed peastone as a textural break to help maintain saturated and anoxic conditions in the denitrifying sand/sawdust layer. The sidewalls of the LSTA will be lined with impervious 30-mil polyethylene / PVC liner to prevent effluent outflow in loose or friable soil conditions and enhance saturated conditions in the denitrification layer. Pan lysimeters shall be installed in each LSTA according to the attached guidance document.

A layer of geotextile landscape fabric will be placed immediately above the pressurized dispersal system to preclude the migration of fine material into the LSTA. Cover material consisting of six to eight inches of loam, loamy sand or sandy loam shall then be placed above the effluent dispersal lines.

A minimum fill perimeter of 10 feet shall be maintained from the invert elevation of the pressure drainfield laterals.

Design specifications and site preparation methods are also described including LSTA installation photographs in **Section IX** – Draft Guidance Document.

B Compliance with Section 250-RICR-150-10-6.41F.2.c-d of OWTS Rules

Upon approval, the Town of Charlestown will manage and oversee the installation of LSTA OWTS within the Town's jurisdictional boundary. All installations conducted under this approval shall be implemented solely to replace failing or substandard septic systems under RIDEM "OWTS Applications for Repair" and no increases in flow or new construction activities shall be part of the approval.

For each installation, the Town of Charlestown will comply with the Section 250-RICR-150-10-6.41.F.2.c-d, the RIDEM OWTS Rules. To ensure compliance, the Town of Charlestown will establish funds held in reserve equivalent to installing one Orenco Systems, Inc., AdvanTex AX-20 Nitrogen Reducing OWTS Pod as part of the LSTA treatment train. Costs to design, install and procure the AX-20 pod and necessary equipment are estimated not to exceed $20,000 for each experimental system installed.

These funds will be utilized by the Town to repair replace or take any other action required if the Department determines that the LSTA fails to meet the performance claims after two years or is found to be a failed OWTS.

With each RIDEM OWTS Application for Repair submitted under this approval, a signed statement detailing fiscal responsibility to repair, replace or modify the experimental technology if it fails to perform as designed shall be included. The signed statement will clearly state who is responsible for the cost of repairing, replacing or modifying the OWTS and the specific funds held in reserve shall be identified as part of each signed statement.

C Information on structural, electrical and mechanical components.

LSTA is passive system and consists of commonly used and readily procured components found locally in Rhode Island. The electrical controls, timer and panel box are those commonly used for a single pass sand filter (SPSF). The 1,500-gallon septic tank and hanging pump vault are the same used for a SPSF and other commercial technologies. The distribution manifold and laterals are very similar to those used for SPSF, PSND and BSF. ASTM C33 sand is used for other approved technologies and is readily available. Sawdust is available at lumber yards and sawmills. A control panel will control the pump to time-dose effluent to the pressurized distribution network in the LSTA. The drainfield distribution network will consist of 12-inch GeoMat 1200 laterals, and 1-inch dimeter PVC pipe with 3/16-inch orifices or PSND using 3/16-inch orifices.

D Leachfield sizing and justification.

Installations of LSTA in Barnstable County have determined 0.70 gal/ft2/day to be the ideal loading to generate conditions necessary for peak LSTA N reduction efficacy. The lowest loading rate allowed by RIDEM for advanced pressurized drainfield are for Category 9 soils, extremely firm lodgement till. These rates are 1.5 gal/ft^2/day for Category 1-time dosed systems and 1.0 gal/ft^2/day for Category 2 systems. These loading rates are designed for wastewater that has been treated through an advanced wastewater treatment unit. LSTA effluent analysis from existing installations indicates that water quality is commensurate to that from a RIDEM approved advanced Category 2 wastewater treatment unit, including BOD and TSS. Therefore, our standard loading rate of 0.70 gal/ft^2/day is considered a conservative rate.

The LSTA design criteria provides more enhanced separation distance to SHWT than existing RIDEM approved PSND or GeoMat design criteria which allows for a two (2) foot separation distance to SHWT in native soils. In the case with LSTAs there is three feet of effluent treatment provided by media within the LSTA.

E Design restrictions or limitations.

Sizing will be similar to a conventional OWTS drainfield, pipe on stone (trenches) or flow diffusers. No reduction in STA sizing would be allowed since STE is untreated prior to the LSTA portion of the OWTS. Small lots where new construction or alteration permits are requested may not meet required setbacks for LSTA installation. Further LSTA use may be limited at marginal sites where vertical separation to water table or restrictive layers does not allow for necessary setback.

F Longevity of Carbon Source

The longevity of the carbon source in LSTA was conclusively determined to be consumed at a rate of <1% annually (Robertson, 2000). Further evaluation of the longevity of the carbon source is not warranted as findings have been peer reviewed. Settling of media has not been observed in any of the existing installations. Given the temporal scale of carbon degradation, LSTA settling should not occur as a result of carbon removal. Incorrect installation could result in settling over time and installers and designers should be trained and approved to design and install LSTA. Infield observations as part of routine O&M will record physical observations of LSTA conditions.

G Typical layout, plans and cross sections.

See **Attachment 3**

H Precautions needed for noise or odor control during initial start-up or long-term operation.

System is a single pass timed-dosed media filter design and no blowers or fans are used. Noise or odor control is not an issue.

I Other.

V. Installation Criteria

A. Construction restrictions or limitations.

Installations of LSTA shall follow safe practices as outlined by OSHA and as determined by the designated Competent Person on the construction site.

B. **Aesthetics or appearance considerations**.

The LSTA system is buried and components are all below grade and resemble a single pass sand filter from the surface. Therefore, there are minimal aesthetic or visual appearance concerns, aside from usual lateral cleanout access ports and septic tank riser lids. Final grading requirements would be very similar to SPSF and conventional STA and include homeowner education on appropriate landscaping choices within 10’ of the drainfield (e.g. no water-loving shrubs or trees, or plants with extensive root systems).

VI. Operation and maintenance/cost/monitoring requirements.

A. Operation and maintenance

1. Details of the required operation and maintenance/inspection procedures.

a. Applicant's recommended frequency for maintenance.

Operation of the LSTA is simple since the technology is single pass and requires only one pump. Under normal operation, annual O&M visits are required. As part of this experimental approval, systems will be inspected and sampled at least monthly.

b. Extent of required maintenance.

Annual maintenance involves pump and float inspection, tank inspection, obtaining elapsed time meter and pump cycle count data from panel, and inspection of LSTA area. Annually, laterals will also be flushed, snaked and flushed again, in accordance with pressurized drainfield maintenance requirements. Hanging pump vault, pump, and float cleaning will be required, and the O&M service provider shall conduct this cleaning on an as-needed basis as would be done with other technologies.

c. Technical qualifications for required operation and maintenance personnel.

Completion of INSP-100 at the New England Onsite Wastewater Training Program (NEOWTP) is required to provide O&M personnel with a background in basic OWTS function, and inspection and maintenance competency. Additionally, completion of LSTA operation and maintenance training class conducted through the NEOWTP will be required. These programs will be offered to practitioners by utilizing what we learn from the experimental installation sites

2. Availability of parts/system components in the case of failure or routine maintenance.

Operation of the LSTA is rather simple since the technology requires only one pump. All components and parts are readily available and often used as replacement parts for other already approved technologies.

3. Long term reliability and life expectancy of individual components and the entire system.

Long term viability of LSTA is no different than any other approved RIDEM OWTS. Preliminary research regarding the longevity of the LSTA carbon source indicates at least a multiple decadal temporal scale. As with all technologies utilizing pumps and float switches, these would need to be replaced as needed on the LSTA system.

4. Describe any warranties or guarantees.

LSTA is non-proprietary therefore no warranties or guarantees are applicable. Warranties from the manufacturers of pumps, floats, and panel boxes components may apply as with other technologies.

5. **Precautions needed for noise or odor control.**

System is single-pass technology with one timed-dosed effluent pump. Noise or odor control is not an issue.

B. Cost Analysis (Complete breakdown)

1. **Design cost estimates.**

< $2,000 including soil evaluation and all permitting costs

2. **Typical construction/installation cost estimates.**

< $20,000 - including 1,500-gallon tank, pump vault, pump, programmable timer, wiring, piping, sand, sawdust, GeoMat or PSND for effluent dispersal, excavator and site restoration.

3. **Operation and maintenance cost estimates, including chemicals, labor, routine maintenance.**

< $200/year

4. **Energy cost estimates. (i.e. annual electric bill, etc.)**

< $90/year (similar to a SPSF)

C. Monitoring

1. **If monitoring is required, what is your recommended monitoring schedule?**

Annual O&M. As previously stated, as part of this Experimental Approval all sites will be visited at least monthly and samples will be collected. Laterals will be flushed, snaked, and flushed annually.

2. **What are the recommended sampling ports/locations?**

STE should be sampled from the pump vault and final treated effluent is sampled through pan lysimeters installed at the base of the LSTA.

3. **What do you recommend for analysis? (e.g. nitrogen, phosphorus, pathogens, etc.)**

During experimental trials, forward flow shall be determined at each site visit. STE and final effluent shall be sampled for DO, temperature, BOD_5, TSS, pH, TN, Nitrate, Nitrite, Ammonium, Alkalinity and TKN (reported by equivalent analysis provided by subtraction). STE shall be collected from septic tank pump basin and final effluent shall be collected from the pan lysimeter installed within the LSTA. Samples shall be collected using standard procedures by Town of Charlestown staff and partners and will be transported on ice under chain of custody protocol to LSEM for laboratory analysis. Sample results for key regulated parameters shall be compared to the performance standards in the following table.

Performance Standards

Analyte	**Performance Standard (mg/L)**
Total Nitrogen	19
BOD5	30
TSS	30
FOG	5

Any fats, oil or grease (FOG) present in untreated OWTS effluent that is not sequestered in the septic tank will be absorbed and entrapped within the initial one to two inches of aerobic sand media, similar to single pass sand filter. Further, LSTA shall be utilized only for residential applications where FOG is typically not a factor affecting effluent treatment. Requirement of FOG analysis in this application is an unproductive utilization of fiscal resources. However, one FOG sample per LSTA will be collected during the second monitoring event six months into each system operation. If FOG is detected above the performance standard of 5 mg/L, subsequent confirmatory sampling will be implemented.

pH data from existing installations in Barnstable County summarized as part of this application indicate that of the seven monitored LSTA OWTS, a total of 184 observations of final LSTA effluent pH were collected. Average pH measurement was 6.5 with a mean of 6.4 and a standard deviation of 0.74. The maximum recorded pH measurement was 9.6 and the minimum was 4.4. The pH of final effluent is considered to be equivalent of that with any other approved OWTS technology and pH warrants no additional protocols or mitigation measures as part of this experimental assessment.

Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored. Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Within six months of the completion of the two year LSTA sampling protocol for each LSTA installed under this approval, efficacy data and operational summaries shall be submitted to RIDEM in report format to detail results, conclusions and next steps including compliance with OWTS Rule 6.41.F.2.c&d.

VII. Failure History

A. Report any failures to date.

None

B. Cause of failure and how determined.

NA. Hydraulic failure is no more likely than for any other RIDEM approved STA.

C. Correction/System modifications undertaken to remedy failures.

NA

VII Draft Guidance Document

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ATTACHMENT 1

RELEVANT LSTA PUBLICATIONS, RESEARCH AND ANALYTICAL DATA

(In order of relevance and number of citations)

Heufelder, 2020
Fine, 2017
Langlois et al., 2020
Wigginton et al., 2020
Cox et al., 2020

2019 Annual Report to

The Massachusetts Department of Environmental Protection

The performance monitoring of the non-proprietary layered systems presently under Site-Specific Pilot Approvals

REPORT BY

Barnstable County Department of Health and Environment

Massachusetts Alternative Septic System Test Center

Submitted

June 2020

Contact person:

George Heufelder, M.S.,R.S – Cell 508-737-8848

WWTPO Registration Number 7591 4-M Full

Executive Summary

Seven soil treatment systems were installed as part of a demonstration project to determine the efficacy of enhancing the area beneath wastewater dispersal with a sustainable carbon source made up of sawdust for the purpose of facilitating denitrification during percolate passage toward the groundwater. In all but one of the cases, a control portion of the soil treatment area that met full compliance with 310 CMR 15.00 (Title 5) was installed and monitored for comparison. Each of the control and the treated portions of the system received half of the facility's daily flow. On average and based on concentration differences between septic tank effluent and percolate beneath the amended soil treatment areas, this modification removed ~70% of the total nitrogen compared with ~44% removed in the control portions of the treatment areas. A summary table of the amended portion of the systems is presented below.

	Total Nitrogen Levels (mg/L) as determined by calculation TKN + nitrate + nitrite											
	STE	STE upper 95%CI	STE lower 95% CI	Control	Control upper 95% CI	Control lowere 95% CI	Sawdust	Sawdust upper 95% CI	Sawdust lower 95% CI	Days monitored	Data points	% removal based on concentration
Acushnet (year round (2-3 pers.)	55.4	63.6	47.7	31	37	25.4	17.3	20.6	13.9	586	22	69%
Falmouth (Sippiwissett)(sporadic 1-2 pers.)	64.3	77.3	51.2	39.3	46.7	34.8	21.2	25	17.5	494	16	67%
Westport (Main Rd) (year round 2 pers.)	38.7	46.1	31.1	12.2	17.4	8.2	15.3	23.7	6.9	188	7	60%
S. Dartmouth (Gaffney) (year round 2 pers.	96.6	108.3	84.9	43.9	60.9	26.8	23.2	36.9	9.5	280	10	76%
West Falmouth (Little Harbor - Seasonal)	79.4	92.4	66.4				7.3	10.3	4.3	410	7	91%
Chappaquoit Falmouth (Seasonal)	87.6	98.8	76.5	60.6	74.1	47	31.9	41.3	22.5	775	7	64%
Westport (Cummings Lane) (year round 2 pers.)	47.3	59.2	35.4	36.9	46.2	27.6	17.5	26.6	8.4	600	13	63%
Averages	67.0			37.3			19.1			476	12	70%

Detailed analyses of the data indicate that performance of the system is temperature dependent with reduction in nitrogen-removal efficiencies indicated at temperatures below 10^{o}C. The primary process limitation at these temperatures appears to be the denitrification portion of the process as opposed to the nitrification biology which appears to be less impacted. Concurrent research at the Massachusetts Alternative Septic System Test Center suggest that nitrification generally is minimally impacted even at temperatures as low as 5^{o}C.

Still remaining are questions relating to the longevity of the carbon source in this particular layering approach. Study is ongoing.

Of particular note is the fact that standard soil treatment systems in our area exhibit percolate pH levels below 6 pH units and suggest that this parameter is not a valid comparator of performance for this technology. Low pH remaining in percolate is likely the effect of generally low alkalinity levels and higher (> 60 mg/L) total nitrogen levels in the wastewater. The nitrification process, converting the ammonia to nitrate, subsequently uses the alkalinity (the chemolithotroph bacteria perfroming this conversion use the carbonate as a carbon source) and reduces the pH due to the resulting production of acidic conditions that cannot be buffered by the reduced alkalinity.

Introduction

The following serves as an annual report on a number of systems allowed under the site-specific Pilot Approval allowance. The systems involve a modification of a standard soil absorption system by placing a layer of organic matrix within the profile of fill installed in accordance with 310 CMR 15.255: Construction in Fill. Specifically, a layer of sand mixed with sawdust at an approximate ratio of 1:1 by volume is placed beneath an 18-inch layer of sand fill which fully meets the above-referenced requirement specification. In each case, the bottom of "the system" for purpose of determining compliance with 310 CMR 15.212: Depth to Groundwater is the bottom-most extent of the sand/sawdust layer or an underlying layer of peastone beneath that layer.

The technology reported on herein has been variously named "layered system", "layer cake", "sawdust system" and others. It is a non-proprietary strategy for attenuating nitrogen by facilitating the denitrification of nitrified percolate beneath a soil adsorption system. The soil(fill) profile sequence is illustrated below.

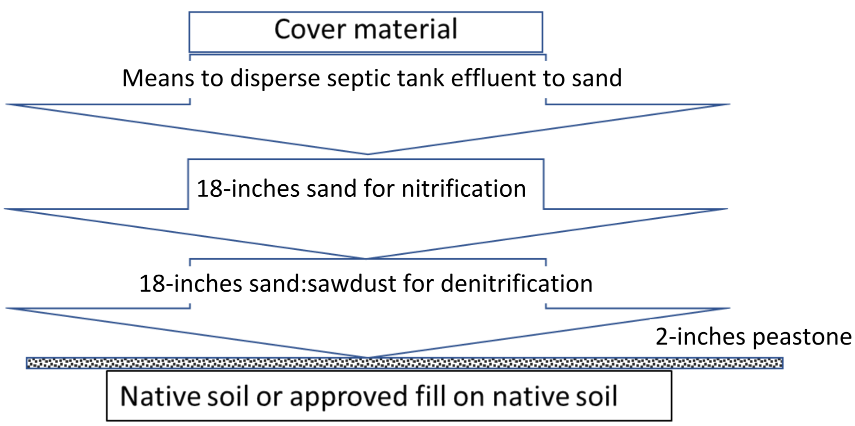


Figure 1 Conceptual representation of layered system for denitrifying percolate beneath onsite septic system soil absorption systems.

Simply described, the layered system is a soil absorption system installed in compliance with 310 CMR 15.000 with the exception that a portion of the fill material used contains organic material which is not allowed by 310 CMR 15.255 (3) which states "The fill shall be comprised of clean granular sand, be free from organic matter…". The sawdust by nature is classed as organic matter.

Use of this layered strategy derives from work by Robertson (2010; 2005; 2000) and others. Systems reported here were piloted under various demonstration grants and were supported partially by federal or state funds for the purpose of researching this strategy as a simple, sustainable means of attenuating nitrogen from onsite septic systems. The goal is to attenuate the impacts of wastewater-derived nitrogen on sensitive marine embayments in the Commonwealth and elsewhere. Although some commercially-available products are used in the systems reported on herein, the use of any particular products (notably the means of effluent dispersal to the sand layers) is not exclusive and substitutions will likely achieve similar results if properly adapted for pressure distribution.

Sampling

Sampling at all systems was from pan lysimeters placed below the soil absorption system. These sampling ports, by their nature, may not always yield sufficient volume for all assays. *The emphasis and priority for assays was placed on the nitrogen species since in each case of the installations, no relief from SAS sizing requirements was requested*. In all but one of the systems reported a portion of the soil absorption system (SAS) was constructed that complied with 310 CMR 15.00 and acted as a controlled comparison; that is, a portion of the soil absorption system did not contain any sawdust in the lower portion (18 inches) of the ~38-inch profile of required fill. Two pan lysimeters were placed in both the treated and control portion of the system and the collection from each was composited to use as a representation of system or control performance. Pan lysimeters are described and illustrated in Appendix A.

All samples were taken by staff of Barnstable County Department of Health and Environment and all analyses were performed at Commonwealth Certified laboratories including the Barnstable County Department of Health and Environment Laboratory. All data collected are reported in Appendices B-E. The following summaries focus on the nitrogen component of the wastewater since this was the primary reason for their installation. The discussion below begins with those systems where the residence is most consistently occupied and for which we have collected the most comprehensive data.

System Installations and Results

System #1 South Main St., Acushnet

This year-round residence had two occupants until July 2018 when an additional one person (infant) came to reside. The average daily flow approximated 222 gallons (840 liters). Samples have been taken monthly until December 2019, when the house became unoccupied. The average Total Nitrogen (TN) beneath the sawdust amended system was 17.3 mg/L (13.9 – 20.6 mg/L, p=.05) representing a 69% reduction in TN (figure 2). The average TN beneath the control portion of the system was 31.0 mg/L (25.4 – 36.7 mg/L, p=.05) representing a 44% reduction in TN. The average influent TN concentration as determined at the septic tank effluent was 55.4 mg/L (47.7 – 63.6 mg/L, p=.05). All data collected are presented in Appendix B.

Periods of less TN reduction in the sawdust amended portion of the system appear related to temperature with significant inefficiencies in denitrification noted at temperatures below 10°C. As in most of the soils-based systems, nitrification (the necessary precursor for denitrification) appears less impacted by the lower temperatures. Studies at the Massachusetts Alternative Septic System Test Center indicate that nitrification reduction occurs at temperatures below 5°C which are rarely encountered in soils-based household wastewater treatment.

Summary of performance – Overall 69% reduction in TN compared with 44% reduction in control.

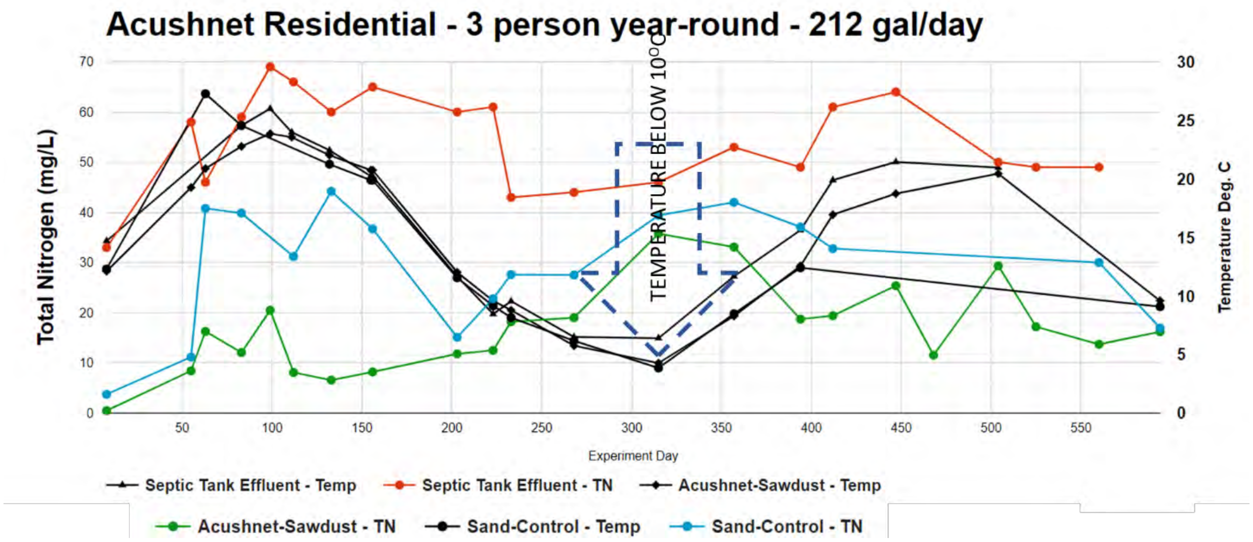


Figure 2 Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at South Main Street, Acushnet during 2018 - 2019.

System #2 Gaffney Rd, North Dartmouth

This system, installed in May 2019, has been monitored monthly through June 2020. The two occupants are year-round residents. Of particular note at this property is the high influent wastewater strength in relation to TN (average 96.6 mg/L, 84.9 – 108.3 mg/L, p= .05). The overall average TN concentration beneath the sawdust amended portion of the system was 23.2 mg/L (9.5 – 36.9 mg/L) indicating a 76% removal (figure 3). As with the previously discussed system, temperatures below 10°C significantly impact performance. At temperatures above 10°C (n=6), the average TN in lysimeters beneath the sawdust amended portion of the system was 8.4 mg/L (3.5 – 13.3 mg/L, p= .05) representing a 91% removal rate. The overall average TN in the control portion of the system was 43.9 mg/L (26.8 – 60.9 mg/L, p=.05) representing a 55% removal.

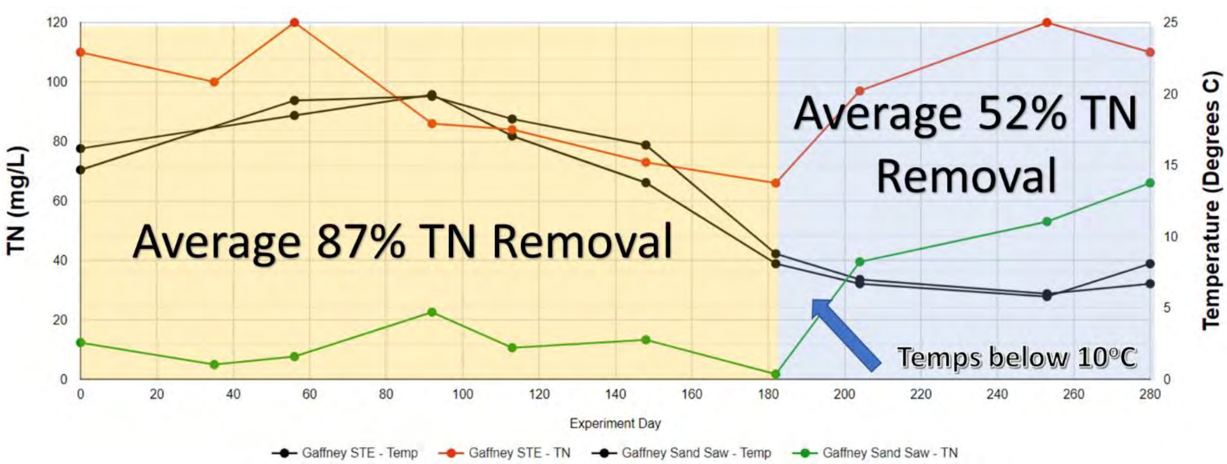


Figure 3 Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Gaffney Rd, North Dartmouth May 2019 – March 2020.

Summary of performance – Overall 76% reduction in TN compared with 55% reduction in control.

System #3 Sippewissett Rd. Falmouth

This system was installed in May 2018 and has been sporadically occupied year-round. Fifteen samples have been taken to date (figure 4).

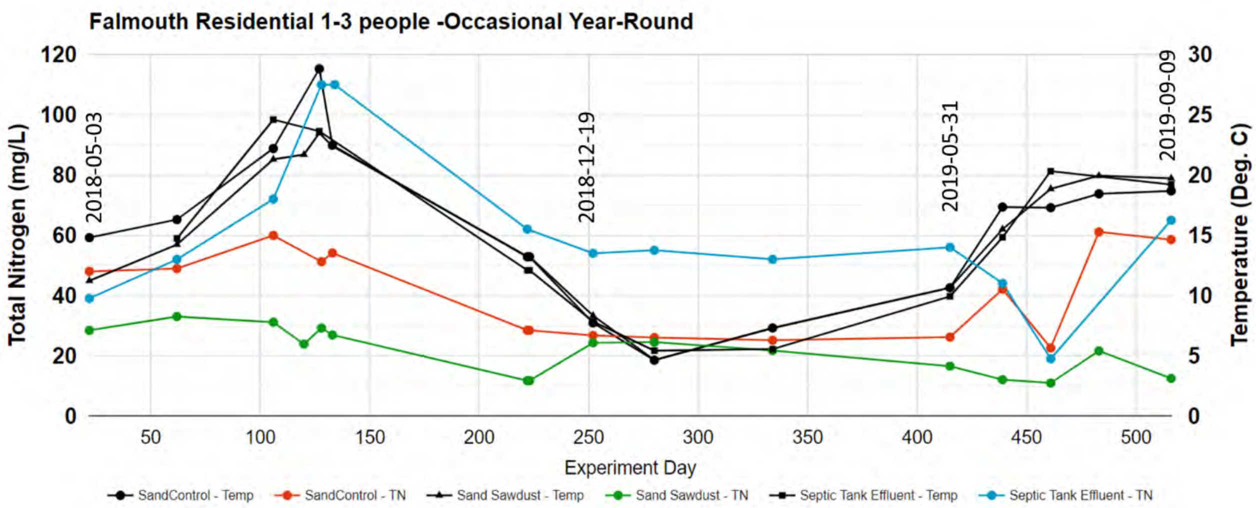


Figure 4 Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Sippewissett Rd, Falmouth May 2018 – September 2019.

Septic tank effluent averaged 64mg/L TN (51 – 77 mg/L, p=.05), and the control portion of the system show a percolate concentration of 39.3 mg/L (34.8 – 46.7 mg/L, p=.05) indicating a 39% attenuation. The amended portion of the soil treatment area released an average of 21.2 mg/L TN (17.5 – 25.0 mg/L, p=.05). This represents a 67% TN removal. Again, the least TN removal occurs when influent or septic tank effluent temperatures are less than 10°C, when the performance of the amended soil area is only slightly better than the control portion of the system.

Summary of performance – Overall 67% reduction in TN compared with 39% reduction in control.

System #4 Main Rd., Westport

Monitoring of this system was initiated in September and only three samples were taken prior to the natural reduction in temperature to below 10°C that occurred in December – March. Although samples were taken in December, 2019, the flow into the system as indicated by the numerical count of doses between November 12 and December 16 was very limited and suggested that the occupants were not present for a significant time during that period (less than one dose per day to the soil treatment area). It is therefore interesting that relatively high total nitrogen levels were noted during this sampling period (figure 5).

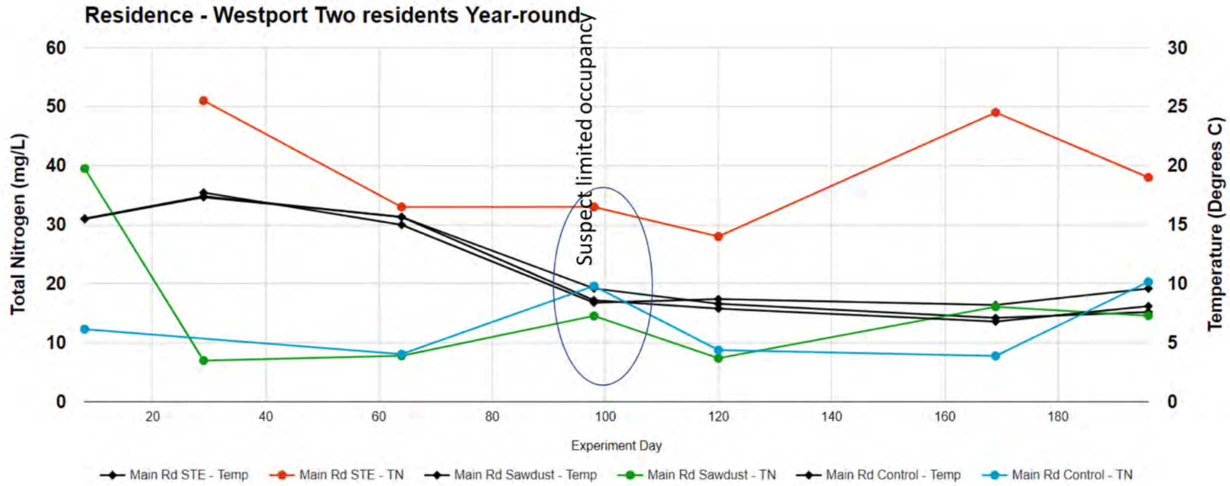


Figure 5. Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Main Road in Westport September 2019 - March 2020.

System #5 Chappaquoit Rd, Falmouth

This system, installed in 2017, is occupied seasonally by at least two individuals and is connected to a cottage occupied occasionally during the summer. It has been sampled at least four times during each occupied season. The system was also the subject of a study from the University of Rhode Island on greenhouse gas emissions.

Figure 6 . Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Chappaquoit Road in Falmouth July 2017 - September 2019

During the first two seasons monitored, this system showed a 75% reduction in nitrogen in the amended portion of the soil treatment area compared to a 21 % reduction in the unamended portion, despite a high (average 90 mg/L TN) influent nitrogen. However, during the third season, the performance of the amended portion of the system was erratic (figure 6) and in July and September 2019 percolate from the amended portion of the system actually had a higher TN than the unamended portion. Overall, with and average TN influent of 87.6 mg/L TN (76.5 – 98.8 mg/L, p=.05), the amended portion of the system averaged 31.9 mg/L TN (22.5 – 41.3 mg/L TN, p=.05) and the unamended portion averaged 60.6 mg/L TN (47.0 – 74.1 mg/L TN, p=.05) reflecting a 64% and 31% reduction of influent nitrogen respectively.

Summary of performance – Overall 64% reduction in TN compared with 31% reduction in control.

System #6 Cummings Lane, Westport

This system serves a two-unit residential property where the main house (which has an adjacent seasonally-occupied cottage) is occupied year round. There are at least two occupants. We discovered some initial problems with the installation in that the pump chamber was receiving (and to some extent still receives groundwater input under heavy precipitation) was receiving groundwater. This was subsequently pumped to the soil treatment area. There is some indication also that the there is some seasonal water table issues in the soil treatment area. Despite the aforementioned issues, the sawdust-amended portion of the system on average leached less than half the total nitrogen as the control portion of the system. The septic tank effluent, as measured at the pump chamber showed a TN of 47.3 mg/L (35.4 – 59.2 mg/L, p=.05). The control portion of the system released 36.9 mg/L TN (27.6 – 46.2 mg/L, p=.05) compared with the release of 17.5 mg/L TN (8.4 – 26.6 mg/L, p=.05) from the sawdust-amended portion of the system. Overall the removal in the amended portion of the system, using the above averages, was 63% TN removal (figure 7). With a singular exception, temperatures below 10^{o}C predict lower nitrogen removal rates.

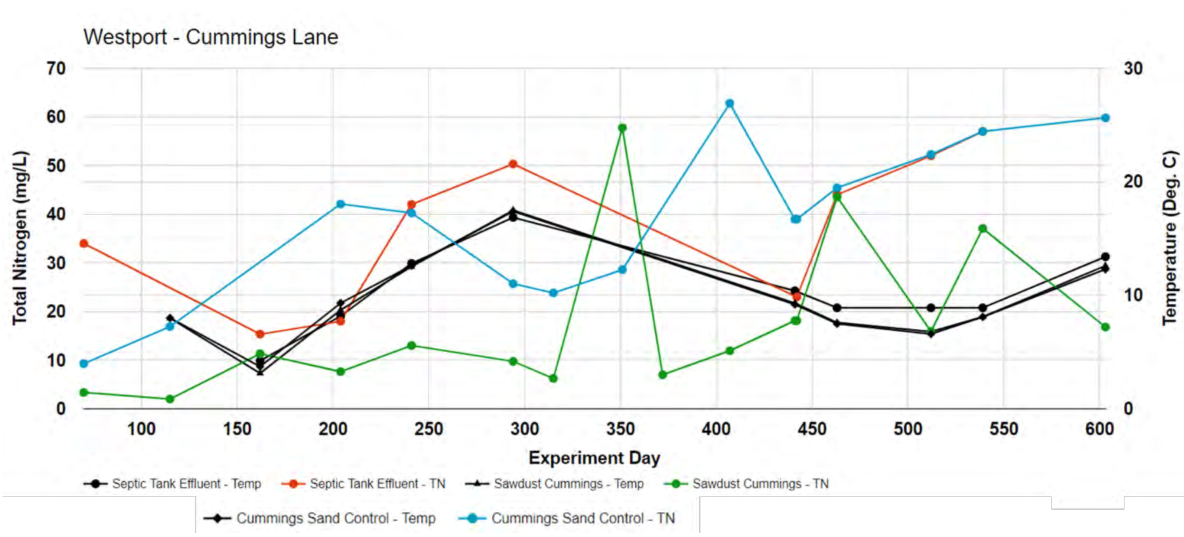


Figure 7 Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Cummings Lane in Westport December 2018 – May 2020.

Summary of performance – Overall 67% reduction in TN compared with 22% reduction in control.

System #7 Little Island Road, West Falmouth

This system was installed in 2017, but due to unfortunate circumstances having to do with the owners' health and intermittent occupation of the property only seven samples have been taken (Appendix H).

For all seven samples taken in 2018- 2019, the average TN of the influent was 79 mg/L TN (51 – 108 mg/L, p=.05 – figure 8). Since there is no control trench installed at this location treatment of the sand/sawdust can only be compared with the septic tank effluent. The percolate collected beneath the system showed an average TN of 7.3 mg/L (4.3 – 10.3 mg/L, p=.05). This represented 91% removal of TN. Collectively these data suggest that seasonal and sporadic use does not appreciably impact system performance during at least three years of operation in this mode.

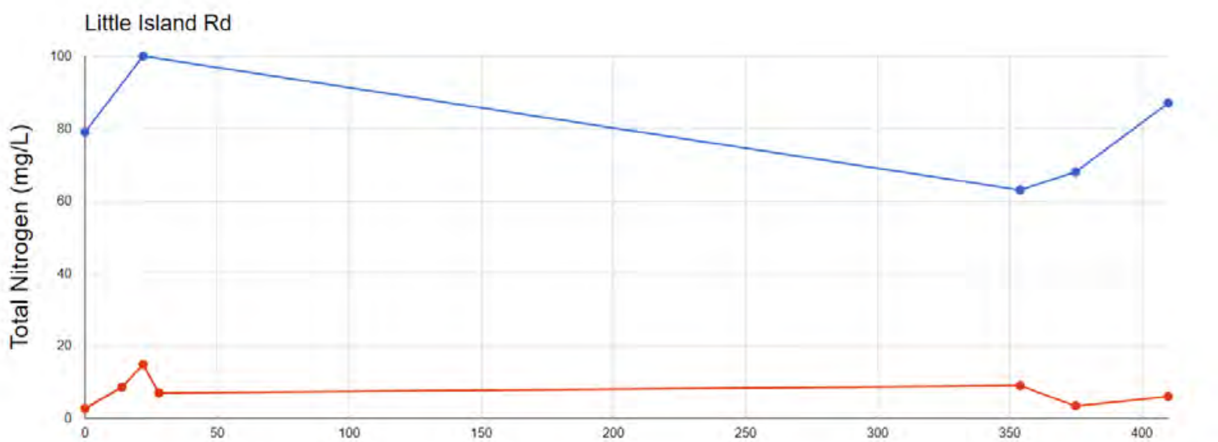


Figure 8. Total nitrogen in percolate beneath a sawdust amended layered and unlayered portion of the soil absorption system at Little Harbor Road in Falmouth July 2018 - September 2019

Special Note

It should be noted the percolate beneath both the standard SAS and the SAS supplemented with sand/sawdust matrix occasionally exhibit pH values less than 6.0. This is likely due to the low alkalinity of our wastewater and the geologic setting. The authors question the use of this parameter as a regulatory benchmark when being applied to soils-based systems.

Systems installed but not yet monitored

A number of layered systems have been installed but have not been monitored due to occupancy of the residents or inadequate flow to have gone through the system and presented a sample. In addition, the present pandemic has prevented the visits to some locations. The locations are as follows with the associated reasons for sample absence.

Juniper Point Falmouth – inadequate time before pandemic to sample.
53 Long Beach Road, Wareham – No consistent occupancy
Drift Rd., Westport – Low occupancy and inadequate time before pandemic to sample.

Operation and Maintenance

All systems installed under the Site-Specific Pilot Approval have low pressure distribution components as part of their treatment. Each time in the case of Systems # 1-6, the electrical panel was opened, checked for problems such as leaks and electrical problems, and the meter reading (dose counts) was recorded for the purpose of calculating the nitrogen loads. In addition, the pressure-dosed field was walked and inspected. During each sampling event, pump chambers were inspected on all systems. No problems were observed.

The Little Island Road system has an electrical panel using programmable logic controllers which were not inspected (located just inside a barn and visible from the pump chamber), however the system field was walked, inspected and all functions appear to be normal. Field operation at the Test Center suggests that the pressure filters installed after the discharge pump in Systems# 1 – 3 should be removed and cleaned every two years. The low BOD and TSS values in the pump chambers suggest that this guideline remains appropriate for residential operation. During the next year, filters will be removed and cleaned.

Conclusion:

These results appear promising that the simple layering of organic matter that serves as a source of carbon, can reduce nitrogen in percolate from soil absorption systems. However, there still remain questions regarding the longevity of the media and degree to which the sawdust will settle over time.

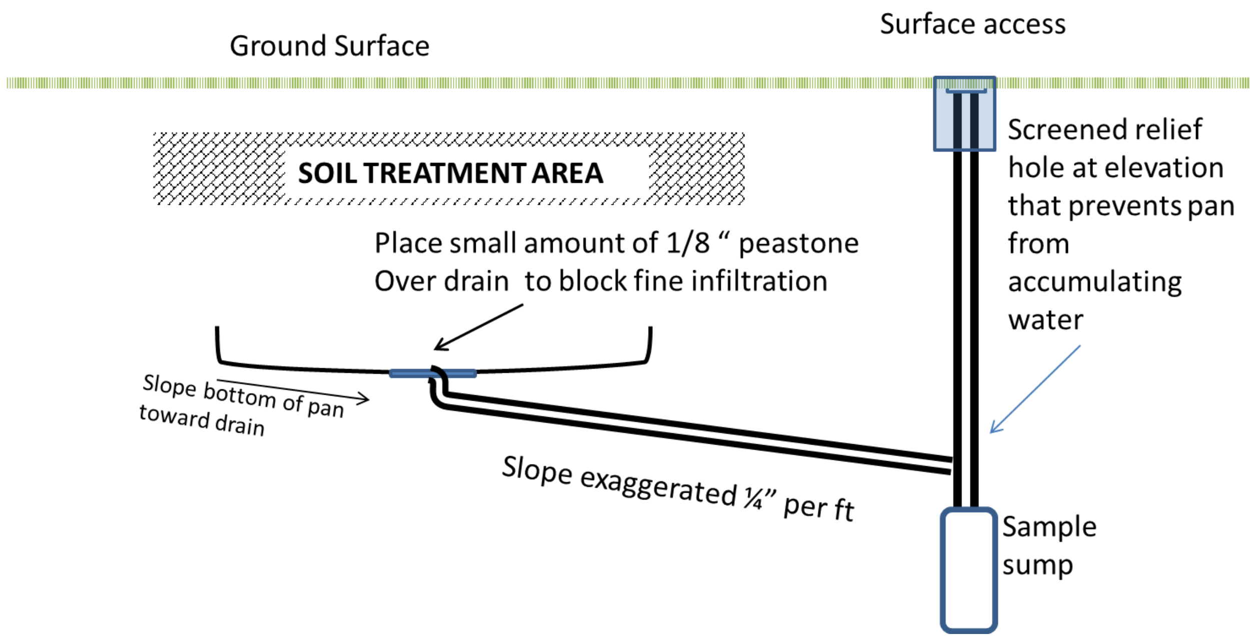
Literature Cited

Robertson, W. et al., 2000. Long-term performance of in situ reactive barriers for nitrate remediation. *Ground Water*, 38(5), pp.689–695.

Robertson, W., 2010. Nitrate removal rates in woodchip media of varying age. *Ecological Engineering*, 36(11), pp.1581–1587.

Robertson, W. & Cherry, J., 2005. In situ denitrification of septic-system nitrate using reactive porous media barriers: field trials. *Ground Water*, 33(1), pp.99–111.

APPENDIX A – ILLUSTRATION OF PAN LYSIMETER SAMPLING PORT



Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2018-05-09	Acushnet-Sawdust	26	0.125			4.0	0.05	0.025	5.3	12.1	0.51	0.5	
2018-06-25	Acushnet-Sawdust	47	0.51	500		1.0	0	0.24	5.0	19.3	8.2	8.4	83
2018-07-03	Acushnet-Sawdust	48	0.3			2.1	0.29	0.025	4.4	20.9	16	16.3	
2018-07-23	Acushnet-Sawdust	80	2.8			2.2	0.05	0.025	5.6	22.8	12	12.1	
2018-08-08	Acushnet-Sawdust	67	6.4			2.1	5.5	0.025	6.0	23.9	15	20.5	
2018-08-20	Acushnet-Sawdust					2.6			6.8	23.6			
2018-08-21	Acushnet-Sawdust	90	1.5				0.61	0.3			7.2	8.1	
2018-09-10	Acushnet-Sawdust					1.2			5.9	22.0			
2018-09-11	Acushnet-Sawdust	110	1.3	20			0.76	0.24			5.6	6.6	
2018-10-03	Acushnet-Sawdust					1.2			5.8	20.8			
2018-10-04	Acushnet-Sawdust	110	0.5			1.2	5.3	0.025	5.8	20.8	2.9	8.2	
2018-11-20	Acushnet-Sawdust	81	0.125			3.1	9.6	0.025	5.6	12.0	2.2	11.8	
2018-12-10	Acushnet-Sawdust	83	0.28			2.2	11	0.025	5.9	9.6	1.5	12.5	
2018-12-20	Acushnet-Sawdust	47	1.3		1	5.2	16	0.025	5.8	8.8	2.2	18.2	23
2019-01-24	Acushnet-Sawdust	30	2.6				15	0.025	5.9	5.8	4	19.0	
2019-03-12	Acushnet-Sawdust	29	9			4.4	27	0.18	6.0	4.3	8.6	35.8	
2019-04-23	Acushnet-Sawdust	27	1.9		1	0.6	31	0.097	5.6	8.3	2	33.1	5
2019-05-30	Acushnet-Sawdust	44	3		1	1.9	14	0.14	5.3	12.6	4.6	18.7	16
2019-06-17	Acushnet-Sawdust	46	0.062			3.0	18	0.025	6.0	17.0	1.4	19.4	
2019-07-22	Acushnet-Sawdust	54	1.9			0.6	22	0.4	5.7	18.8	3	25.4	
2019-08-12	Acushnet-Sawdust	170	2				6.6	0.17			4.8	11.6	
2019-09-17	Acushnet-Sawdust	67				3.7	27	0.37	6.5	20.5	2	29.4	
2019-10-08	Acushnet-Sawdust	93					15	0.22			2.2	17.2	
2019-11-12	Acushnet-Sawdust	62					12	0.13			1.6	13.7	
2019-12-16	Acushnet-Sawdust	37				1.1	15	0.025	5.6	9.6	1.2	16.2	
2018-05-09	Sand-Control	18	0.125			6.7	3.4	0.025	5.4	12.3	0.36	3.8	
2018-06-25	Sand-Control	64	2.9				6	0.003			5.2	11.2	
2018-07-03	Sand-Control	15	0.125				38	0.025	6.1	27.3	2.8	40.8	
2018-07-23	Sand-Control	10	0.57				37	0.36	6.1	24.6	2.5	39.9	
2018-08-21	Sand-Control	5	0.125				29	0.025			2.2	31.2	
2018-09-10	Sand-Control					5.3			6.2	21.3			
2018-09-11	Sand-Control	8.6	0.125				42	0.003			2.2	44.2	
2018-10-03	Sand-Control					4.7			5.1	19.9			
2018-10-04	Sand-Control	11	0.125			4.7	35	0.025	5.1	19.9	1.7	36.7	
2018-11-20	Sand-Control	33	0.125			8.3	14	0.025	6.3	11.6	1.1	15.1	
2018-12-10	Sand-Control	9.8	0.38			7.7	20	0.025	5.7	9.2	2.8	22.8	
2018-12-20	Sand-Control	8.9	1.1		1	9.1	26	0.025	5.4	8.2	1.6	27.6	19
2019-01-24	Sand-Control	4.4	0.73				26	0.025	5.5	6.2	1.5	27.5	

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2019-03-12	Sand-Control	1	4.7			7.3	35	0.025	5.2	3.9	4.4	39.4	
2019-04-23	Sand-Control	1	1.7		1	1.9	40	0.025	6.1	8.5	2	42.0	5
2019-05-30	Sand-Control	15	6		1	2.9	30	0.025	5.4	12.4	7.1	37.1	61
2019-06-17	Sand-Control	1	0.04				31	0.025	5.4		1.8	32.8	
2019-11-12	Sand-Control	10					28	0.025			2	30.0	
2019-12-16	Sand-Control	15				9.3	16	0.025	5.7	9.1	0.96	17.0	
2018-05-09	Septic Tank Effluent	170		78		2.1			9.6	14.7	33	33.0	120
2018-06-25	Septic Tank Effluent	220		260		0.5			8.0		58	58.0	22
2018-07-03	Septic Tank Effluent	210	42			1.6			8.5		46	46.0	40
2018-07-23	Septic Tank Effluent	220	44			1.7			8.1	24.6	59	59.0	
2018-08-08	Septic Tank Effluent	290	52			0.1			7.3	26.0	69	69.0	
2018-08-20	Septic Tank Effluent					0.4			7.7	24.0			
2018-08-21	Septic Tank Effluent	300	51								66	66.0	150
2018-09-10	Septic Tank Effluent					0.6			6.9	22.5			
2018-09-11	Septic Tank Effluent	250	52								60	60.0	68
2018-10-03	Septic Tank Effluent					0.1			7.9	20.2			
2018-10-04	Septic Tank Effluent	260	49		54	0.1			7.9	20.2	65	65.0	56
2018-11-20	Septic Tank Effluent	240	43		220	0.6			6.9	11.7	60	60.0	280
2018-12-10	Septic Tank Effluent	230	44			1.7			6.6	8.5	61	61.0	
2018-12-20	Septic Tank Effluent	190		230		3.3			7.8	9.6	43	43.0	9
2019-01-24	Septic Tank Effluent	170							7.8	6.5	44	44.0	
2019-03-12	Septic Tank Effluent	180				6.4			7.3	6.4	46	46.0	
2019-04-23	Septic Tank Effluent	200		140		0.4			7.2	11.7	53	53.0	19
2019-05-30	Septic Tank Effluent	220		250		1.0			7.2	15.6	49	49.0	40
2019-06-17	Septic Tank Effluent	260				0.8			7.0	19.9	61	61.0	
2019-07-22	Septic Tank Effluent	280				0.2			6.9	21.5	64	64.0	
2019-08-12	Septic Tank Effluent	290									56		
2019-09-17	Septic Tank Effluent					2.2			7.0	21.0	50	50.0	
2019-10-08	Septic Tank Effluent										49	49.0	
2019-11-12	Septic Tank Effluent										49	49.0	

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2019-06-17	Gaffney Sand Control	91	1.2		68		4	0.025	6.5	14.6	20	24.0	58
2019-07-22	Gaffney Sand Control	220	0.125		1		84	0.025			10	94.0	
2019-08-12	Gaffney Sand Control	250	0.64		8	2.7	32	0.16	6.4	19.2	2.8	35.0	230
2019-09-17	Gaffney Sand Control	190					27	0.35			3.7	31.1	
2019-10-08	Gaffney Sand Control	160					81	0.29			3.1	84.4	
2019-11-12	Gaffney Sand Control	210				4.8	45	0.16	6.1	16.0	1.8	47.0	
2019-12-16	Gaffney Sand Control	34				-0.1	6.6	0.025	7.9	8.1	2.8	9.4	
2020-01-07	Gaffney Sand Control	200				8.7	18	0.025	6.6	7.0	1.3	19.3	
2020-02-25	Gaffney Sand Control	200					33	0.025			4.2	37.2	
2020-03-23	Gaffney Sand Control	45					53	0.19			4	57.2	
2019-06-17	Gaffney Sand Saw	160	2.3		230	1.7	0.23	0.12	5.8	14.7	12	12.4	25
2019-07-22	Gaffney Sand Saw	320	0.125		210		0.11	0.17			4.7	5.0	2.4
2019-08-12	Gaffney Sand Saw		0.125			1.5	1.6	0.95	6.0	19.5	5.1	7.7	42
2019-09-17	Gaffney Sand Saw	350			84	2.6	9.3	1.3	6.2	19.8	12	22.6	200
2019-10-08	Gaffney Sand Saw	340			42	1.9	4.2	0.13	6.9	18.2	6.3	10.6	83
2019-11-12	Gaffney Sand Saw	300			13	1.5	3.1	0.21	7.3	16.4	10	13.3	54
2019-12-16	Gaffney Sand Saw	10			1	2.6	0.12	0.025	6.3	8.8	1.6	1.7	54
2020-01-07	Gaffney Sand Saw	210			2.3	4.8	32	0.62	6.3	7.0	6.9	39.5	430
2020-02-25	Gaffney Sand Saw	170			1	5.7	50	0.36	6.4	6.0	2.7	53.1	160
2020-03-23	Gaffney Sand Saw	33			1.5	7.9	63	0.15	6.2	6.7	2.9	66.1	26
2019-06-17	Gaffney STE	520				0.7			7.7	16.2	110	110.0	
2019-07-22	Gaffney STE	460		120							100	100.0	6.2
2019-08-12	Gaffney STE	580		43		0.2			7.4	18.5	120	120.0	62
2019-09-17	Gaffney STE			41		2.6			7.5	20.0	87	86.0	38
2019-10-08	Gaffney STE			3.7		0.1			8.1	17.1	84	84.0	48
2019-11-12	Gaffney STE			110		0.1			7.0	13.8	73	73.0	55
2019-12-16	Gaffney STE			20		-0.1			7.9	8.1	66	66.0	76
2020-01-07	Gaffney STE			46		0.4			8.0	6.7	97	97.0	49
2020-02-25	Gaffney STE			270		-0.3			8.1	5.8	120	120.0	62
2020-03-23	Gaffney STE			82		0.6			8.1	8.1	110	110.0	55

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2018-05-03	Sand Sawdust	130	11	150		4.8	14	0.41	6.2	11.2	14.0	28.4	21
2018-06-12	Sand Sawdust	150	8.6	36		0.4	20	0.99	6.4	14.2	12.0	33.0	22
2018-07-26	Sand Sawdust	140	3.2			2.2	24	0.92	6.3	21.3	6.2	31.1	
2018-08-09	Sand Sawdust	120	0.37			2.7	20	0.76	6.1	21.7	3.1	23.9	
2018-08-16	Sand Sawdust					2.4			6.4	23.5			
2018-08-17	Sand Sawdust	110	0.125				26	1.1			2.1	29.2	
2018-08-22	Sand Sawdust	120	0.125			1.4	24	0.98	6.4	22.4	1.9	26.9	
2018-11-19	Sand Sawdust	150	0.125			5.0	9.6	0.025	6.5	13.2	2.1	11.7	
2018-11-20	Sand Sawdust	150	0.125			5.0	9.6	0.025	6.5	13.2	2.1	11.7	
2018-12-19	Sand Sawdust	72	0.125		1	10.2	24	0.025	6.6	8.3	0.3	24.3	
2019-01-16	Sand Sawdust	88	0.125				23.6	0.025	6.8	4.6	0.9	24.5	
2019-03-11	Sand Sawdust	70	0.125			10.8	20	0.025	5.9	7.3	1.7	21.7	
2019-05-31	Sand Sawdust	100	0.25			5.5	15	0.025	6.2	10.6	1.5	16.5	
2019-06-24	Sand Sawdust	99	0.02			6.2	11	0.025	6.0	15.5	1.0	12.0	
2019-07-16	Sand Sawdust	110	3.1			1.2	8.2	0.025	6.4	18.9	2.7	10.9	
2019-08-07	Sand Sawdust	94	0.26			1.2	18	0.21	5.9	19.9	3.4	21.6	
2019-09-09	Sand Sawdust	150	0.77			3.3	6.9	0.065	5.6	19.7	5.5	12.5	
2018-05-03	SandControl	78	2.2	72		7.3	42	1.8	5.9	14.8	4.2	48.0	
2018-06-12	SandControl	63	0.26	6.8			46	0.32	6.6	16.3	2.6	48.9	
2018-07-26	SandControl	34	0.125			5.9	58	0.23	6.1	22.2	1.7	59.9	
2018-08-16	SandControl					6.4			5.9	28.8			
2018-08-17	SandControl	46	0.29				50	0.025			1.2	51.2	
2018-08-22	SandControl	41	0.125			2.5	52	0.025	6.1	22.5	2.1	54.1	
2018-11-19	SandControl	92	0.125			8.6	27	0.025	6.7	13.2	1.4	28.4	
2018-11-20	SandControl	92	0.125			8.6	27	0.025	6.7	13.2	1.4	28.4	
2018-12-19	SandControl	100	0.125		1	11.7	25	0.025	6.5	7.7	1.7	26.7	
2019-01-16	SandControl	54	0.125				23.6	0.025	6.7	4.6	2.4	26.0	
2019-03-11	SandControl	67	0.125			13.0	24	0.025	6.1	7.3	1.1	25.1	
2019-05-31	SandControl	49	0.25				24	0.15	6.9	10.6	2.0	26.2	
2019-06-24	SandControl	0.11	1.4			9.0	41	0.11	6.4	17.3	1.0	42.1	
2019-07-16	SandControl	81	6.5			2.2	17	0.19	6.1	17.3	5.4	22.6	
2019-08-07	SandControl	1	3.3			4.2	56	1.3	5.9	18.4	3.8	61.1	
2019-09-09	SandControl	15	0.125			8.1	57	0.13	4.4	18.7	1.4	58.5	

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2018-05-03	Septic Tank Effluent	240		150							39.0	39.0	20
2018-06-12	Septic Tank Effluent	290		41		2.0			6.7	14.7	52.0	52.0	33
2018-07-26	Septic Tank Effluent	270				2.9			7.2	24.6	72.0	72.0	
2018-08-16	Septic Tank Effluent					1.7			6.5	23.6			
2018-08-17	Septic Tank Effluent	400									110.0	110.0	
2018-08-23	Septic Tank Effluent										110.0	110.0	
2018-11-19	Septic Tank Effluent	330				3.0			6.7	12.1	62.0	62.0	
2018-11-20	Septic Tank Effluent	330				3.0			6.7	12.1	62.0		
2018-12-19	Septic Tank Effluent	270		48		4.0			7.3	7.8	54.0	54.0	65
2019-01-16	Septic Tank Effluent	280							7.5	5.4	55.0	55.0	
2019-03-11	Septic Tank Effluent	340				13.6			7.1	5.5	52.0	52.0	
2019-05-31	Septic Tank Effluent	400				0.5			7.0	9.9	56.0	56.0	
2019-06-24	Septic Tank Effluent	240				2.7			6.6	14.8	44.0	44.0	
2019-07-16	Septic Tank Effluent	130				0.3			6.4	20.3	19.0	19.0	
2019-09-09	Septic Tank Effluent	260				2.4			5.7	19.2	65.0	65.0	

Sample Date	Sample Location	Alkalinity	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2019-09-17	Main Rd Control	180			1.5	4.0	0.3	5.82	15.5	8	12.3	
2019-10-08	Main Rd Control	200			2.3	1.7	0.33	6.01	17.3			
2019-11-12	Main Rd Control	22			0.3	0.7	0.025	5.87	15.7	7.4	8.1	
2019-12-16	Main Rd Control	150			3.2	18.0	0.31	6.19	9.4	1.3	19.6	
2019-12-16	Main Rd Control				12.4			6.66	8.5			
2020-01-07	Main Rd Control	170			2.3	6.8	0.28	6.26	7.9	1.7	8.8	
2020-02-25	Main Rd Control	64			5.4	6.6	0.18	6.03	6.8	1	7.8	
2020-03-23	Main Rd Control	130			1.4	16.0	0.12	6.12	8.1	4.2	20.3	
2019-09-17	Main Rd Sawdust				3.2	35.0	1.6	5.97	15.5	2.9	39.5	14
2019-10-08	Main Rd Sawdust	220		610	0.5	1.0	1.8	6.09	17.4	4.2	7.0	46
2019-11-12	Main Rd Sawdust	260		320	0.3	2.3	2.7	6.23	15.7	2.8	7.8	28
2019-12-16	Main Rd Sawdust	160		22	0.4	12.0	0.54	6.2	9.6	2	14.5	160
2020-01-07	Main Rd Sawdust	96		8.2	0.3	5.6	0.39	6.25	8.3	1.4	7.4	97
2020-02-25	Main Rd Sawdust	200		3.6	0.1	14.0	0.31	6.3	7.1	1.8	16.1	35
2020-03-23	Main Rd Sawdust	46		1.5	2.5	12.0	0.096	6.02	7.6	2.5	14.6	180
2019-10-08	Main Rd STE		51		0.5			7.19	17.7	27	51.0	250
2019-11-12	Main Rd STE		65		0.4			6.06	15.0	33	33.0	170
2019-12-16	Main Rd STE		27		0.8			7.06	8.4	33	33.0	40
2020-01-07	Main Rd STE		46		0.6			7.05	8.7	28	28.0	44
2020-02-25	Main Rd STE		92		0.3			7.22	8.2	49	49.0	24
2020-03-23	Main Rd STE		53		0.2			6.97	9.6	38	38.0	240

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2017-07-26	Chap Sawdust	480	0.51		810	1.38	0.5	1.9	6.5	21.4	8	10.4	
2017-08-24	Chap Sawdust	470	7.4		200	1.46	0.1	0.001	6.8	23.2	10	10.1	
2017-09-19	Chap Sawdust	370	7.5				2.6	2.2			11	15.8	
2017-10-23	Chap Sawdust	300	0.62		2.8	2.24	29.0	0.01	6.5	17.8	1	30.0	63
2018-06-12	Chap Sawdust	210	0.78	2.7		1.41	32.0	0.85	6.5	14.9	2.6	35.4	25
2018-07-26	Chap Sawdust	220	0.125			5.99	20.0	0.025	6.2	21.5	3	23.0	
2018-08-16	Chap Sawdust								6.3	26.2			
2018-08-17	Chap Sawdust	240	0.125				26.0	0.025			4.5	30.5	
2018-08-22	Chap Sawdust	250	0.125				28.0	0.38			3.7	32.1	
2018-08-22	Chap Sawdust					2.25			6.4	23.3			
2019-04-08	Chap Sawdust	250	0.125				16.0	0.025			3.1	19.1	
2019-05-31	Chap Sawdust	230	0.25				36.0	0.025			3.1	39.1	
2019-06-24	Chap Sawdust	270	0.53				16.0	0.025			2.9	18.9	
2019-07-16	Chap Sawdust	180	0.38				39.0	0.025			0.55	39.6	
2019-08-07	Chap Sawdust	200	4				26.0	0.16			6.9	33.1	
2019-08-07	Chap Sawdust	270	0.125				61.0	0.21			3.1	64.3	
2019-09-09	Chap Sawdust	160	0.4				74.0	0.29			2.6	76.9	
2017-07-26	Chap Control	300	1.1				0.1	76			2.6	78.7	
2017-08-24	Chap Control	200	2.2		21	5.54	33.0	0.35	6.9	22.1	6	39.4	
2017-09-19	Chap Control	260	2.7				92.0	0.001			5	97.0	70
2018-06-12	Chap Control	180	0.32	1		5.49	66.0	0.025	6.2	16.0	2.3	68.3	
2018-07-26	Chap Control	160	0.125			6.7	72.0	0.025	6.7	22.2	2.2	74.2	
2018-08-17	Chap Control	100	0.125				42.0	0.025			1.6	43.6	
2018-08-22	Chap Control					2.99			6.4	22.5			
2018-08-23	Chap Control	140	0.125				94.0	0.025			1.1	95.1	
2019-04-08	Chap Control	89	0.125				41.0	0.025			0.89	41.9	
2019-06-24	Chap Control	18	0.21				42.0	0.025			1.3	43.3	
2019-07-16	Chap Control	200	0.125				18.0	0.025			0.13	18.2	
2019-08-07	Chap Control	11	1.3				64.0	0.025			2.4	66.4	
2019-09-09	Chap Control	1	0.71				59.0	0.14			1.4	60.5	
2017-09-19	Chap STE	470	92	440							93	93.0	37
2017-10-23	Chap STE	540	92	160		2.36			7.1	16.6	110	110.0	21
2018-06-12	Chap STE	340		160		2.25			6.7	13.9	77	77.0	43

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2018-07-26	Chap STE	350									97	97.0	
2018-08-16	Chap STE					1.37			6.7	23.5			
2018-08-17	Chap STE	290									73	73.0	
2019-04-08	Chap STE		60								61	61.0	
2019-05-31	Chap STE	360	71								78		
2019-06-24	Chap STE	350	83			4.46			7.5	18.1	90	90.0	
2019-07-16	Chap STE	400									120		
2019-09-09	Chap STE										100	100.0	

Sample Date	Sample Location	Alkalinity	NH4	BOD5	CBOD5	DO	NO3	NO2	pH	Temp	TKN	TN	TSS
2018-12-10	Cummings Sand Control	300	0.1				6.8	1.30			1.2	9.3	
2019-01-24	Cummings Sand Control	250	17.0				0.9	0.03	6.9	8.0	16	16.9	
2019-03-12	Cummings Sand Control	280	11.0			3.4			6.6	3.7	9.6		
2019-04-23	Cummings Sand Control	210	9.8		1	4.7	34.0	0.09	7.0	9.3	8	42.1	2
2019-05-30	Cummings Sand Control	240	3.3		3.5	2.2	36.0	0.05	6.8	12.6	4.2	40.3	11
2019-07-22	Cummings Sand Control	320	2.1		1	0.3	22.0	0.03	6.1	17.4	3.7	25.7	4
2019-08-12	Cummings Sand Control	470	18.0		8.2		1.7	0.14			22	23.8	180
2019-09-17	Cummings Sand Control	330					2.4	0.21			26	28.6	
2019-11-12	Cummings Sand Control	480					0.7	0.11			62	62.8	
2019-12-16	Cummings Sand Control	310				0.0	2.2	0.76	6.4	9.2	36	39.0	
2020-01-07	Cummings Sand Control	340				1.7	2.3	0.12	6.1	7.5	43	45.4	
2020-02-25	Cummings Sand Control	190				0.0	38.0	0.28	6.3	6.6	14	52.3	
2020-03-23	Cummings Sand Control	59				0.3	22.0	2.00	6.0	8.1	33	57.0	
2020-05-26	Cummings Sand Control					-0.2			6.2	12.3			
2018-12-10	Sawdust Cummings	260	0.1				0.1	0.03			3.3	3.4	
2019-01-24	Sawdust Cummings	270	0.4				0.1	0.03	5.7	8.0	1.9	2.0	
2019-03-12	Sawdust Cummings	250	8.3			3.1	2.7	0.64	5.7	3.1	8	11.3	
2019-04-23	Sawdust Cummings	280	5.5		120	0.5	0.9	0.03	6.6	8.7	6.7	7.7	13
2019-05-30	Sawdust Cummings	330	9.1		80	0.8	2.0	0.03	6.7	12.5	11	13.0	40
2019-07-22	Sawdust Cummings	400	8.2		300	0.6	4.6	0.16	5.8	17.5	5	9.8	5.4
2019-08-12	Sawdust Cummings	360	1.5		190		0.7	0.05			5.5	6.3	68
2019-09-17	Sawdust Cummings	390			61		3.2	0.56			54	57.8	160
2019-10-08	Sawdust Cummings	380			73		2.1	0.03			4.9	7.0	140
2019-11-12	Sawdust Cummings	470			57		2.5	0.05			9.4	12.0	73
2019-12-16	Sawdust Cummings	370			19	0.0	3.0	0.13	6.5	9.3	15	18.1	130
2020-01-07	Sawdust Cummings	300			43	0.2	1.4	0.14	6.4	7.6	42	43.5	71
2020-02-25	Sawdust Cummings	430			1	-0.1	2.9	0.03	6.8	6.8	13	15.9	230
2020-03-23	Sawdust Cummings	67			16	0.2	0.1	0.03	6.0	8.1	37	37.1	120
2020-05-26	Sawdust Cummings					-0.2			6.4	12.6			
2018-12-10	Septic Tank Effluent		30.0								34	34.0	
2019-03-12	Septic Tank Effluent		13.0			4.3	0.1	0.31	7.0	4.2	15	15.4	
2019-04-23	Septic Tank Effluent	200		92		0.6			7.2	8.2	18	18.0	59
2019-05-30	Septic Tank Effluent	220		220		0.2			7.0	12.8	42	42.0	36
2019-07-22	Septic Tank Effluent	270	38.0	140		0.2	2.3	0.03	6.6	16.9	48	50.3	3.3
2019-08-12	Septic Tank Effluent	340		370							69	69	54
2019-09-17	Septic Tank Effluent			56							62	62	25
2019-10-08	Septic Tank Effluent			60							94	94	37
2019-11-12	Septic Tank Effluent			190							54	54	23
2019-12-17	Septic Tank Effluent			30		-0.2			6.8	10.4	23	23.0	130
2020-01-07	Septic Tank Effluent			73		0.2			7.2	8.9	44	44.0	150

2020-02-25	Septic Tank Effluent			120		-0.4			7.1	8.9	52	52.0	57
2020-03-23	Septic Tank Effluent			54		0.1			7.2	8.9	57	57.0	110
2020-05-26	Septic Tank Effluent					-0.4			7.3	13.4			

Sample Date	Sample Location	Alkalinity	NH4	DO	NO3	NO2	pH	TKN	TN
2019-09-09	Sand-Sawdust	47			4.1	0.34		1.5	5.94
2019-08-05	Sand-Sawdust	51			1.9	0.025		1.4	3.325
2019-07-15	Sand-Sawdust	73			7.4	0.025		1.5	8.925
2018-08-23	Sand-Sawdust	67	0.125		5.5	0.025		1.4	6.925
2018-08-17	Sand-Sawdust	82	0.125		13	0.025		1.7	14.725
2018-08-16	Sand-Sawdust			5.01			6.68		
2018-08-09	Sand-Sawdust	54	0.125		5.7	0.025		2.8	8.525
2018-07-26	Sand-Sawdust	68	0.78		0.05	0.025		2.5	2.575
2019-09-09	Septic Tank Effluent							87	87
2019-08-05	Septic Tank Effluent	78			1.2	0.025		68	68
2019-07-15	Septic Tank Effluent							63	63
2018-08-17	Septic Tank Effluent	370						100	100
2018-08-16	Septic Tank Effluent			1.5			6.89		
2018-07-26	Septic Tank Effluent	280	69					79	79

Continued Operation of the Massachusetts Alternative Septic System Test Center and the Investigation of Passive Nitrogen Removal Strategies for Onsite Septic Systems

(and Continued Operation of the Test Center to Investigate Proprietary Technologies)

Project 15-07 319

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This project has been financed with Federal Funds from the Environmental Protection Agency (EPA) to the Massachusetts Department of Environmental Protection (the Department) under an s. 319 competitive grant. The contents do not necessarily reflect the views and policies of EPA or of the Department, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

Executive Summary

This project follows upon Project 14-03/319 and demonstrates the efficacy of non-proprietary strategies for the reduction of nitrogen from discharges of onsite septic systems with primary focus on soils-based treatment. Five full-scale systems were installed and tested at the Massachusetts Alternative Septic System Test Center and designed to receive 220 gallons per day, the equivalent of the full design capacity of a two-bedroom house in the Commonwealth of Massachusetts. The designs closely followed work done under the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) Project[1] and work performed by others[2–4], with some modifications that anticipated regional and climate differences. All designs incorporated the use of lignocellulose or wood products (sawdust, mulch or woodchips) into the treatment process in a passive manner. Passive is defined by the fact that only one liquid pump is used. In all designs tested, this sole pump is used to distribute septic tank effluent to a low-pressure, time dosed Soil Treatment Area (STA aka. leachfield) comprised of 18 inches of sandy media to facilitate nitrification. Three designs position a layer of sandy media mixed in a ratio of 1:1 by volume with sawdust or wood mulch beneath the above-referenced nitrifying layer and maintain a saturated condition using an impervious liner (DESIGN 1,2 &3). One system conveys the nitrified percolate from the 18-inch depth of sand media to a box of woodchips (DESIGN 4). The final design underlays the nitrification layer with a 1:1 mixture (by volume) of sand and wood mulch in a free draining condition unrestricted by an impervious liner (DESIGN 5).

Generally, all designs achieve at least a 50% removal of nitrogen throughout the year, even in colder months. DESIGN 1,2,3 &5 exhibit clear seasonal trends and achieve levels Total Nitrogen (TN) levels less than 10 mg/L when temperature of the influent is above 10^{o}C. When temperatures are greater than 15^{o}C, percolate TN levels are often less than 5 mg/L TN which corresponds to greater than 85% removal. DESIGN 4, which diverts nitrified effluent to a container of woodchips exhibited an overall average TN of 3.6 mg/L (2.3 – 4.8 mg/L, *p= .05*) which reflects a 90% removal rate. This system was less affected by temperature. A major question relating to the longevity of carbon source that supports denitrification could not be anwered here, however literature reveiwed herein suggests that the longevity of saturated designs (1,2,3,4) would be expressed in decades. The unstaurated DESIGN 5, although appealing due to its simplicity and the fact that no final disposal area is required, requires further research to determine its longevity. Three of the systems (3,4 and 5) will continue to be the object of study from Stony Brook University, whose research efforts in the coming year will hopefully clarify some of these questions.

This project and Project 14-03/319 has encouraged significant interest by industry and non-proprietary researchers in using the principles demonstrated here and was the basis for securing a large demonstration grant from the Southeast New England Coastal Watershed Restoration Program of USEPA which is installing selected designs in summer 2017.

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Introduction

This report is a companion document with "Investigation of Passive Nitrogen Removal Strategies for Onsite Septic Systems at the Massachusetts Alternative Septic System Test Center (and Continued Operation of the Test Center to Investigate Proprietary Technologies) Project 14-01 319. The informational setting and definition of need is explained in that document. In summary, these two projects proceed from investigations of low-impact, sustainable and economical ways to treat wastewater for nitrogen in an onsite setting using non-proprietary means. In the Project 14-01/319, we endeavored to investigate the simplest means of interrupting the downward movement of percolate beneath a soil absorption system following the oxidation of ammonia (nitrification) with a source of carbon to promote denitrification. In that configuration, there is simply a layer of carbon (lignocellulose) mixed with sand or silty-sand positioned beneath the nitrifying strata of the system (figure 1). In the present study, we report on the use of a saturated layer of carbon:media mix located beneath the nitrification strata (figure 2), one additional unsaturated design, and an additional design diverting nitrified effluent to a box reactor of woodchips.

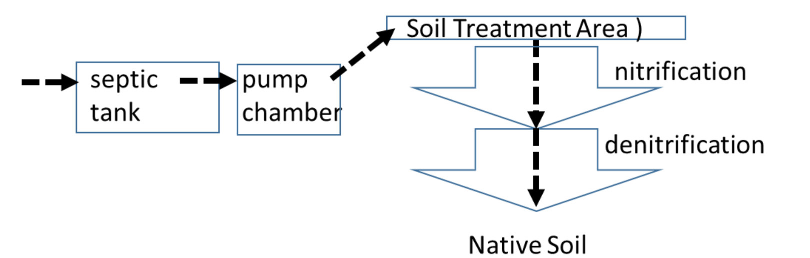


Figure 1. Schemata of "unsaturated" configuration that was the subject of PROJECT 14-01/319. Further experimentation with this method is also presented herein.

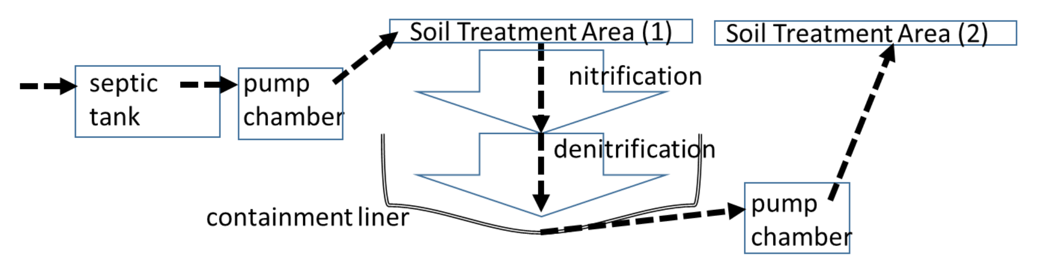


Figure 2. Diagrammatical representation of initial designs used under Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) studies.

The saturated designs copy closely those used in the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) Project[1] with one exception. In the FOSNRS designs, a pump chamber is then required to deliver the final effluent to a second Soil Treatment Area (STA). We have omitted this portion of the design to focus on the denitrification treatment and to determine what further treatment (if any) would be required following exit from the lined portion of the system and prior to final disposal.

Project Description

This project reports on five full scale (220 gallon/day) systems using four concepts:

- DESIGN 1 - A saturated system started in December 2014 and operated until November 2016 (loamy sand as a nitrifying layer) (figure 3);
- DESIGN 2 - Operation of the above following replacement of the loamy sand with ASTM C33 Sand (sand that meets the requirements for standard fill under 310 CMR 15:255 – "Title 5" fill) (figure 4);
- DESIGN 3 - A saturated system as directly above installed with support from Stony Brook University and substituting "Long Island Sand" for the sand in both layers and "Long Island mulch" as a substitute for sawdust (figure 4 modified as described);
- DESIGN 4 -A nitrification layer underdrained and diverted to a box of woodchips (figure 5), and;
- DESIGN 5 - An unsaturated system similar in dimensions to the silty-sand – sawdust system reported in Project 14-01 319 but substituting sand and sawdust from Long Island, New York sources (figure 6).

Results

DESIGN 1- A saturated system started in December 2014 and operated until November 2016

This design follows closely designs used in the Florida Department of Health Onsite Nitrogen Reduction Strategies Study (FOSNRS) Stage 1 portion of a design. In that study, a drip dispersal system was used in conjunction with an 18-inch layer of sand for the nitrifying portion of the system and a nine-inch layer of a sawdust-sand mix was used for the underlying saturated denitrifying portion of the system. In the FOSNRS, a further polishing upflow reactor using elemental sulfur and oyster shell mixture was used to facilitate autotrophic denitrification prior to discharge to a disposal area. In the present study only the first reactor area was tested and contained an 18-inch layer of sawdust-sand mixture underlying an 18-inch layer of loamy sand. Dispersal to the top of this reactor bed was accomplished by a low-pressure distribution system using GeoMat™ (figure 3). A final disposal area was not included in the present study, however data from the discharge will be used to determine the requirements for final disposal. The decision to use loamy sand (a commercial blend of sand and soil used in golf courses) was based on previous work at MASSTC that had shown that loamy sand preserved the alkalinity of the percolating wastewater (necessary for ammonia oxidation or nitrification) and buffered changes in pH. This was thought necessary for facilitating complete nitrification (a necessary precursor for denitrification) and maintaining a near-neutral pH (also thought necessary for complete nitrification). The assumptions that adequate alkalinity and pH neutrality were required conditions is based on generally-accepted stoichiometry. These assumptions are challenged by work presented below, but are presented here to explain the initial attempts to meet them.

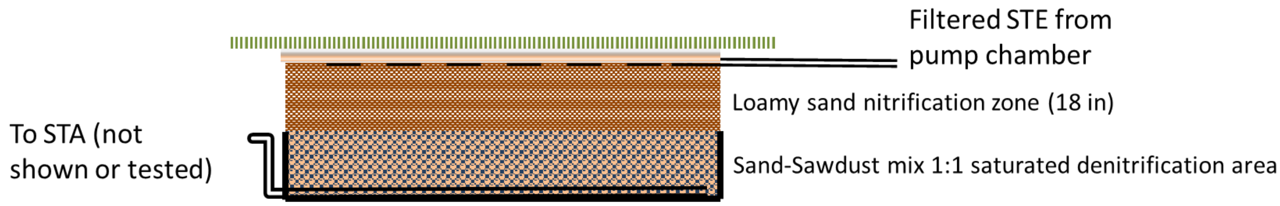


Figure 3. Saturated denitrification system design using a containment liner. Note the nitrifying layer is a loamy sand (60-440 Sand/soil, New England Specialty Soils, 435R Lancaster St Leominster, MA 01453). STA = Soil Treatment Area or leaching facility, STE = Septic tank effluent. DESIGN 1

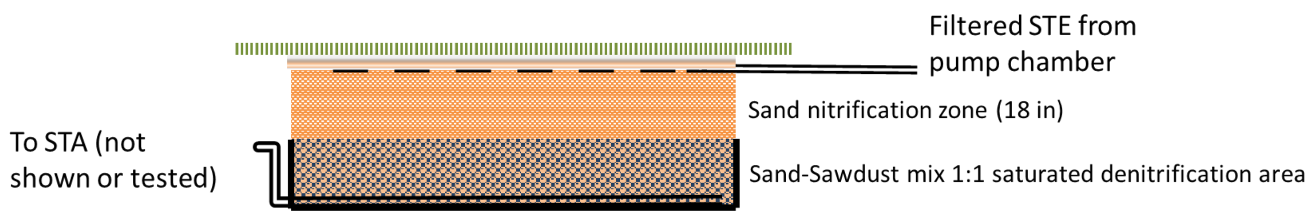


Figure 4. Saturated denitrification system design using containment liner. Note that nitrifying layer uses ASTM C-33 Sand (design 2) or sand provided by Stony Brook University and originating from Long Island, New York. STA = Soil Treatment Area or leaching facility, STE = Septic tank effluent. DESIGN 2 and DESIGN 3

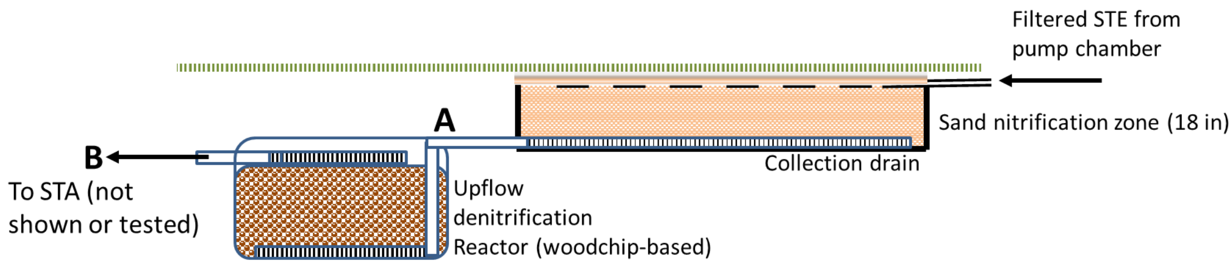


Figure 5. Denitrifying configuration with nitrifying STA percolate diverted through a container of lignocellulose. STA = Soil Treatment Area or leaching facility, STE = Septic tank effluent. A and B denote sampling locations. DESIGN 4

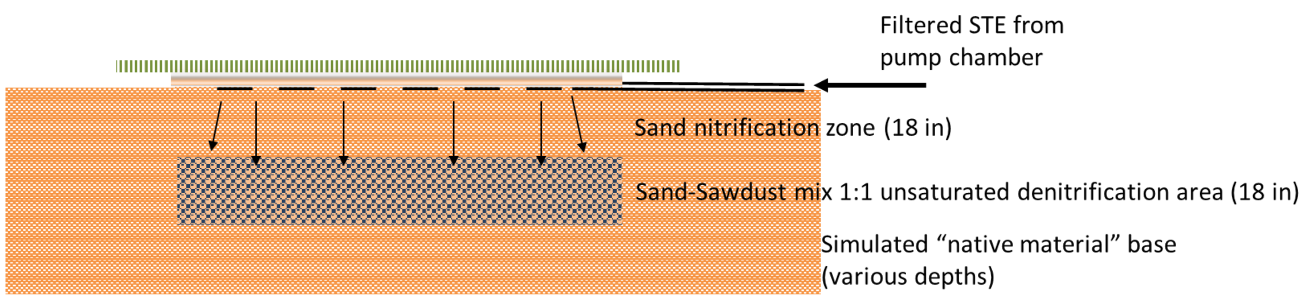


Figure 6. Unsaturated system design constructed in sand provided by Stony Brook University and originating from Long Island, New York. STE = Septic tank effluent. DESIGN 5

For over nine months following startup of the system, the Total Nitrogen (TN) levels in the final effluent of this system generally remained below 5 mg/L. Following this period, the nitrate in the percolate from the nitrifying layer of loamy sand began to decrease concurrent with an increase in ammonia (figure 7). Since the major processes in denitrification prerequire the oxidation of ammonia to nitrate, the major increases in TN were due to the ammonia passing through the denitrification layer unchanged (figure 8).

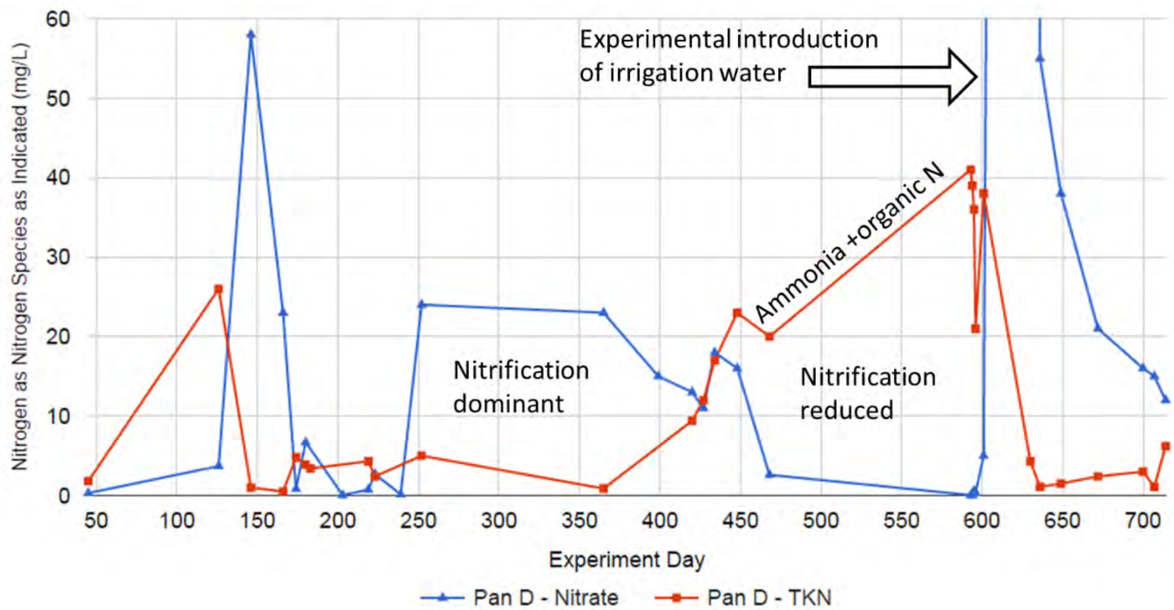


Figure 7. Nitrate and Total Kjeldahl Nitrogen (TKN) collected at the bottom of the nitrifying layer in the saturated denitrification system design using a containment liner (see figure 3). Note extremely high (>300 mg/L) nitrate following a rapid introduction of irrigation water.

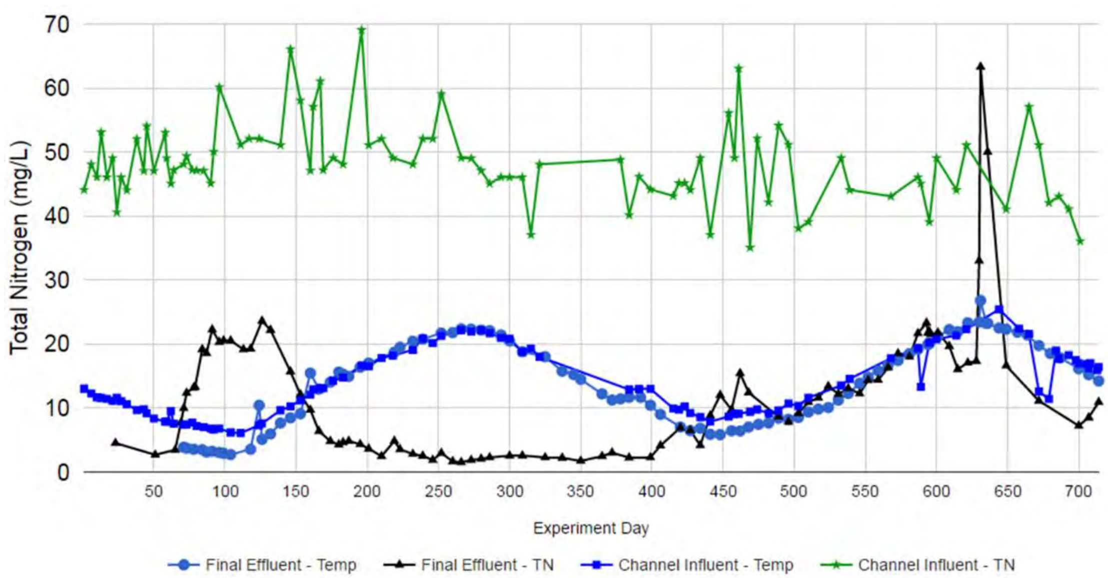


Figure 8. Total nitrogen concentrations with temperature in the saturated denitrification system design using a containment liner (see figure 3).

Because all other functioning systems exhibited decreasing nitrogen with increasing air and influent temperature, it was decided to interrupt the flow to the system and excavate selected areas for inspection. In addition, it appeared that some of the areas were not freely draining and some areas of wastewater surfacing were observed. On July 27, 2016, approximately 600 days following startup, inspection revealed that the soil column under the distribution system was not freely draining and the soil remained wet between dosing cycles. It was thought that this likely was the cause for decreased nitrification. On August 3, 2016 following a two-week period of no influent supply we conducted an experiment which involved the watering of the top of the system with approximately 300 gallons (nominally one gallon per square foot of area) of tap water within 30 minutes. The results were sudden and striking with nitrate levels elevating to 300 mg/L in the pan lysimeter located at the interface of the nitrification and denitrification interface (figure 9). The data suggest a rapid response by nitrifying bacteria to mobilized ammonia released from the wastewater soil interface as a result of the irrigation flooding with tap water.

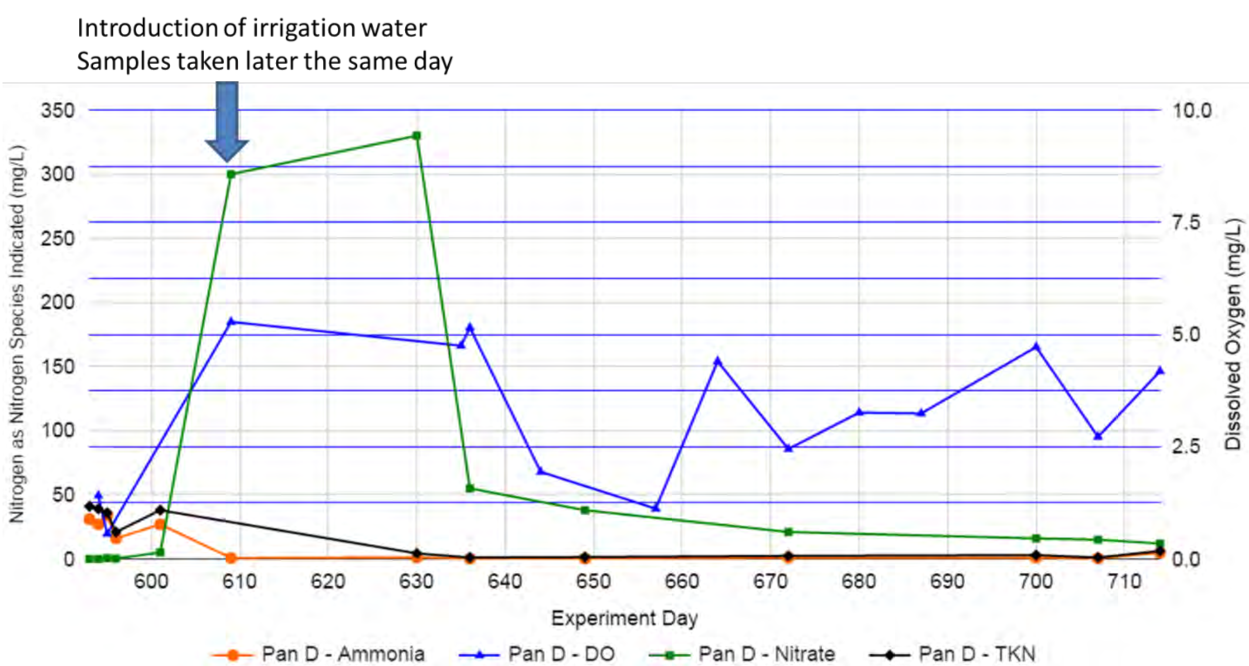


Figure 9. The response of the unsaturated layer of the saturated system to an intense irrigation event on August 3, 2016.

The response to the pulse of nitrate passing through the system was observed at the final discharge point approximately three weeks following the irrigation event (figure 10). Even challenged with > 300 mg/L nitrate-nitrogen, the denitrification layer reduced the nitrate by at least 80%.

The system was subsequently run under normal influent flow until November 15, 2016 when flow was stopped due to an anticipated forensic excavation with Stony Brook University. It was decided that since the loamy sand showed signs of hydraulic stress in spring 2016, the nitrification layer would be replaced with ASTM C33 sand to closely approximate an installation of a saturated system for Stony Brook University which would use sand from Long Island that met the ASTM C33 specifications.

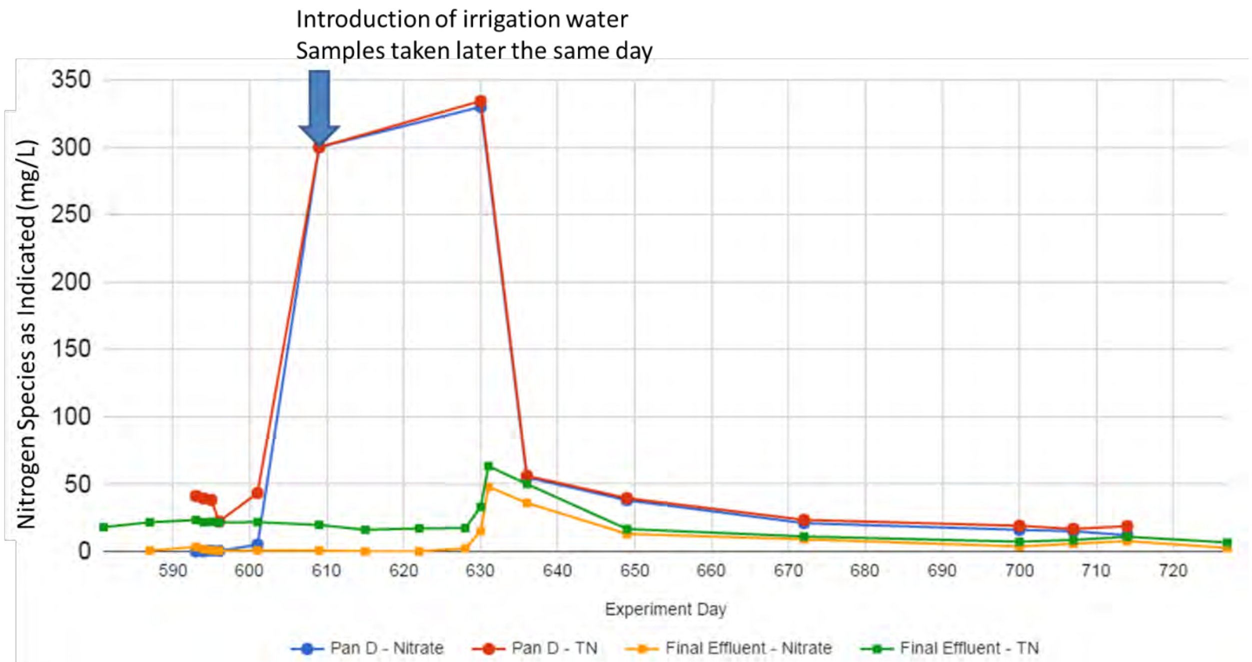


Figure 10. Comparison of nitrogen species between the nitrification layer (as represented by the pan lysimeter (Pan D) at the nitrification-layer denitrification layer boundary) and the final discharge from the denitrification layer during a selected period of operation.

CONCLUSION – DESIGN 1

The cause of the hydraulic stress and diminished nitrification during the spring of 2016 is undetermined. The hydraulic loading rate of 0.5 gal./sq. ft./day was initially within the loading rates specified in the soil type by the Massachusetts Code (Title 5 – 310 CMR 15.242). During the excavation, split-ring permeameter test run at the wastewater/soil interface indicated an acceptance rate of 10 – 15 min/inch. This rate compared favorably with the assumed rate of 10 min/inch at installation, again supporting the appropriateness of the loading rate by the prior-cited requirement. There were minor construction faults observed (a few low areas where wastewater collection was observed), however these areas could not account completely for the observations of reduced nitrification. At the time of excavation, researchers from Stony Brook University took many core and bacteria samples which will be examined in the coming months.

The decision of Stony Brook to install a system with similar design but using materials from Long Island, and to use sand in the nitrification layer (as opposed to the loamy sand used above), compelled this project to modify the design in November 2016. Leaving the denitrification layer in place, we removed the nitrification layer and replaced the loamy sand with locally-sourced "Title 5 sand" that met the specifications of ASTM C33. The results of this modification are presented below.

DESIGN 2 - Operation of the above following replacement of the loamy sand with ASTM C33 Sand.

In November-December 2016, the cover, distribution system and nitrification layer of the above system (DESIGN 1) was removed and numerous soil core samples were taken and are being analyzed for bacteria species by Stony Brook University. The Loamy sand was replaced with standard "Title 5" sand fill meeting the same specifications as ASTM C-33. The hydraulic loading was resumed at approximately 0.5 gal/sq. ft./day based on the areal coverage of the bed (areal area ≈450 sq. ft., daily load ≈220 gallons). The reduction in total nitrogen of this rebuilt system followed a similar pattern as the original installation (figure 11).

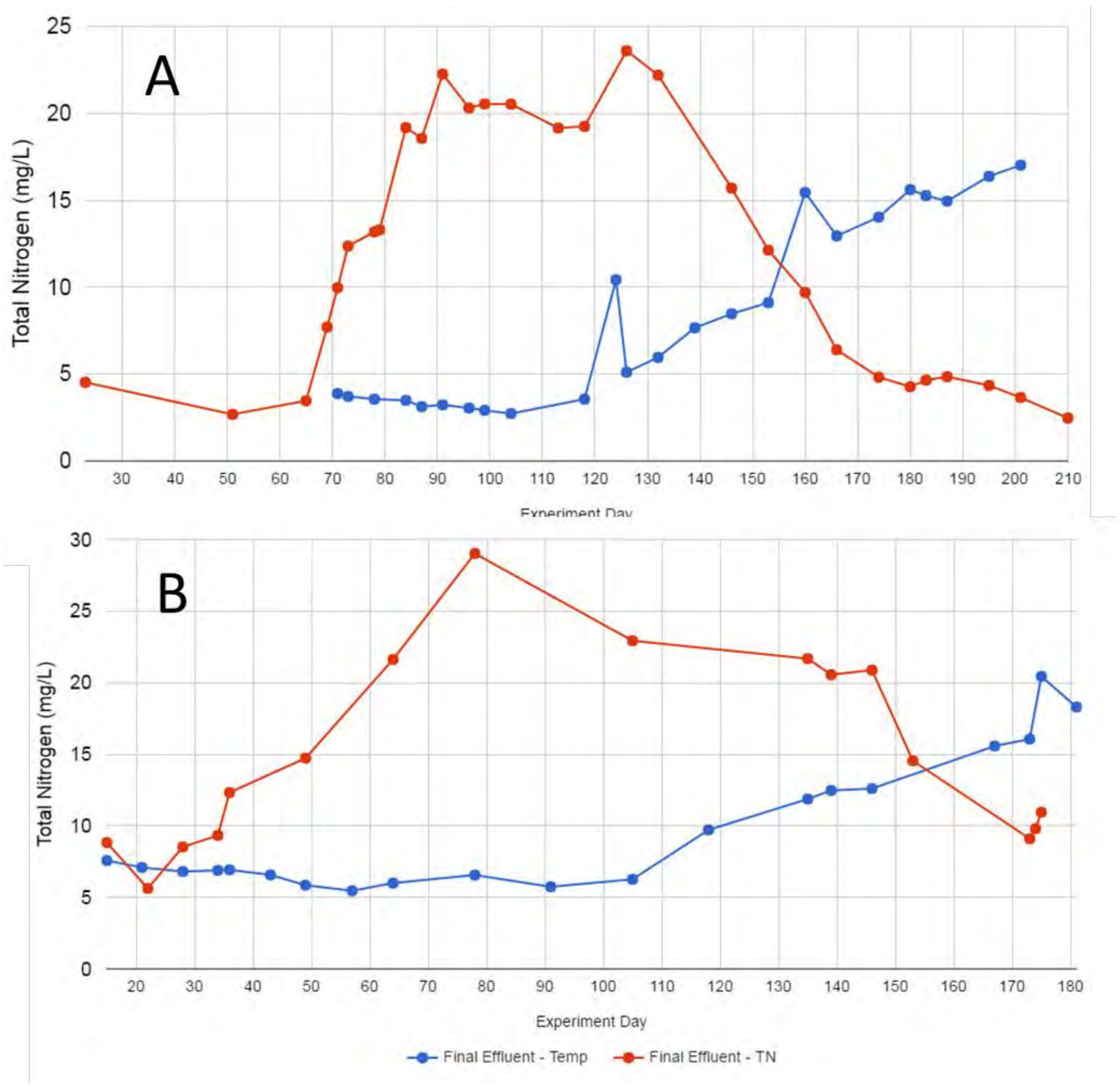


Figure 11. Comparison of the "start-up" nitrogen removal between DESIGN 1 (A – Operated December 2014 – November 2016 containing loamy sand as nitrifying layer) and DESIGN 2 (B – Operated since December 2016 containing ASTM C-33 sand as the nitrifying layer). Note similar time periods for removal of TN to levels below 10 mg/L.

Since both systems were started at the same time of year (going into winter), their start up performance can be compared. The data suggest that the loamy sand and the sand perform similarly, showing a level of 10 mg/L TN is achieved within 160-170 days of startup.

A closer inspection of the data shows that the first two months of operation, there is an approximate 50% reduction in TN which is likely due to the uptake of bacteria during the growth phase and its retention in the organisms themselves.

This system will remain in operation and will be monitored for at least the next two years.

CONCLUSION - DESIGN 2

As previously discussed, the reasons for the hydraulic stress in DESIGN 1 were not determined, but we posit that the blended soil (loamy sand) changed drainage characteristics over time due to unknown factors involving both the physical (migration of fine materials and subsequent reduction in hydraulic capacity) or the biology (growth of clogging organisms at a rate exceeding their senescence and decline). DESIGN 2 was an attempt to standardize the nitrification layer with a known and accepted media that is required in Massachusetts Regulations (310 CMR 15.000 – Title 5). In addition, this modification will allow a direct comparison with DESIGN 3 which is the same design using materials sourced from Long Island.

DESIGNS 3 – 5 Origins

This and previous Project 14-01 319 generated significant interest by Stony Brook University (SBU), Center for Clean Water Technology who was tasked with investigating non-proprietary means for nitrogen removal in Suffolk County (Long Island), New York. Following a design charrette with consultants Hazen-Sawyer (who was instrumental in completing the FOSNRS), University of Rhode Island personnel (George Loomis - University of Rhode Island, Research and Extension Soil Scientist and the Director of the New England Onsite Wastewater Training Center and José A. Amador – Professor Laboratory of Soil Ecology and Microbiology University of Rhode Island), researchers from Stony Brook University, regulators from Suffolk County and others, it was decided that this Design 2 should be duplicated using Long Island-sourced material. With support from SBU, the following three systems were installed at MASSTC in late-July and August 2016. In addition, the unsaturated design like those reported on previously (Project 14-01/319) was also duplicated using Long Island sourced materials. Finally, a design in which a shallow drainfield percolate is diverted in an upflow fashion through a container of woodchips was also installed and tested. Most of the monitoring for these systems was supported under this project and we advance this as a key achievement in leveraging this project funding with the construction and limited sampling support from SBU.

DESIGN 3 - A saturated system similar to DESIGN 2

This design substitutes “Long Island Sand” for the sand in both layers and “Long Island mulch” in place of the sawdust. It was installed with support from Stony Brook University. This system was installed in July 2016 and began operation in August.

Similar to DESIGN 1 and DESIGN 2, we observed a negative correlation between temperature and overall nitrogen removal in this saturated system (figure 12).

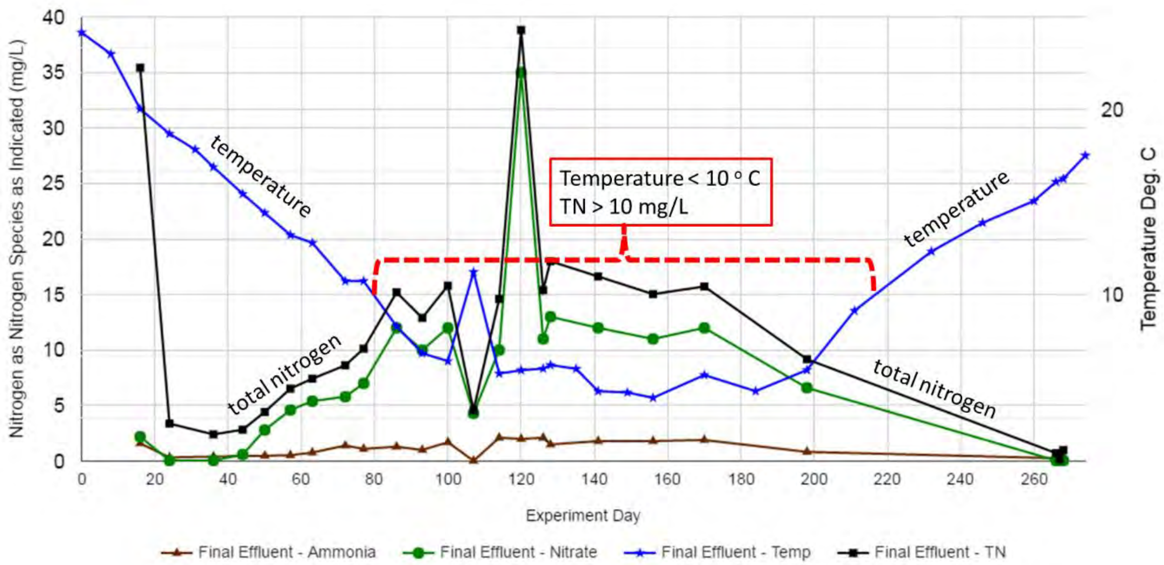
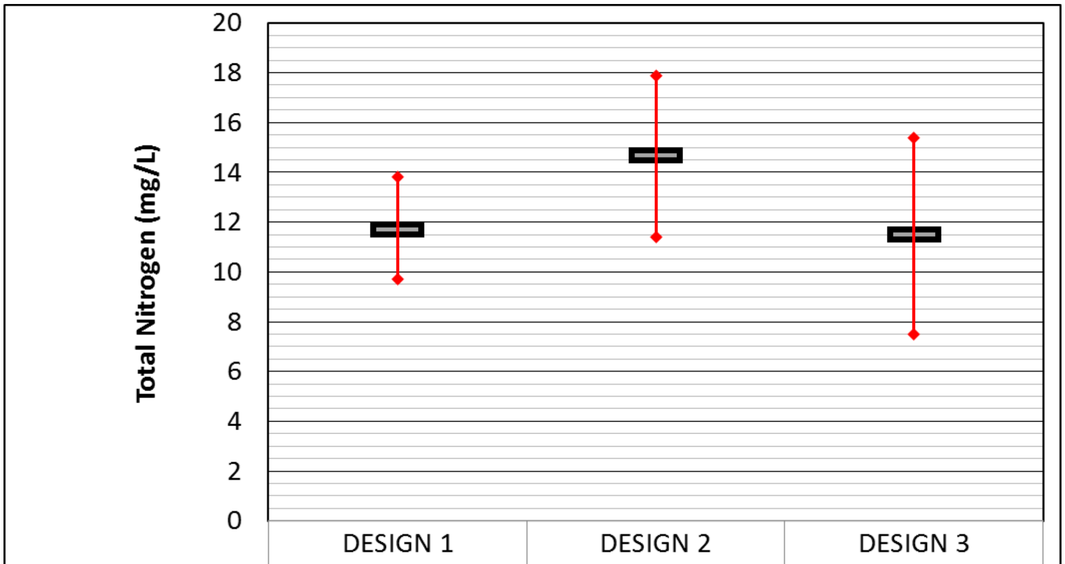


Figure 12. Concentration of selected nitrogen species with temperature at the discharge of the saturated system installed with support from Stony Brook University at MASSTC in July 2016. Shaded area denotes TN < 10 mg/L.

The data suggest that discharge TN concentrations below 10 mg/L occurs when the temperature of the discharge exceeds 10ºC. This relationship between temperature and TN concentration was like that observed in DESIGN 1 (figure 11 a). The data also indicate that the nitrification is not limiting during the colder months as over 75% (67 – 94%) of the TN in the discharge is made up of nitrate and only 10% (5 – 14%) was made up of ammonia. Thus, it appears that the condition limiting the denitrification in the colder months is the reduction of nitrate to nitrogen gas and not the prerequisite oxidation of the ammonia to nitrate. An examination of the dissolved oxygen levels in the discharge reveal that, excluding a single aberrant value, the average oxygen concentration in the effluent was 0.27 mg/L (0.18 – 0.36 mg/L, *p=.05*) which would appear to support the reduced conditions necessary to reduce the nitrate. We conclude that the denitrification step is more sensitive to temperature than the nitrification step in this design type.

CONCLUSION - DESIGN 3

This design, like DESIGN 1 and DESIGN 2, relies on a saturated zone of sand and sawdust for denitrification. All these designs exhibit a seasonal reduction in performance for nitrogen removal with DESIGN 2 (the most recently installed and started up during late autumn) having the most profound loss of performance in the first winter (figure 13). In saturated designs such as these, there is less question as to the longevity of the sawdust used, with most estimates exceeding 50 years.



	DESIGN 1	DESIGN 2	DESIGN 3
Upper CI (p=.05)	13.8	17.9	15.4
Lower CI (p =.05)	9.7	11.4	7.5
Mean	11.7	14.7	11.5

Figure 13. Comparison of Total Nitrogen at discharge points for DESIGNS 1, 2 and 3 (mean TN and 95% Confidence Limits). CI = Confidence Interval.

Although the saturated design system is minimally complex to construct and has a more-proven denitrification media longevity, the system as described in the three designs above requires an area for final effluent/percolate disposal. Potential designs for this final disposal area vary and may be an area that surrounds or is adjacent to the containment area and variously sized. In the Florida locations, a complete second STA was constructed. Since the wastewater exiting the containment area after denitrification following a start-up period is generally < 20 mg/L $BOD_{5\text{-day}}$, the final disposal soil-contact area can be greatly reduced (hydraulic loading rate can be increased). The exception to this was DESIGN 1 in which there was a four-month period where the average $BOD_{5\text{-day}}$ was 144 mg/L (< 10 mg/l at all other times). In Long Island, New York, officials are considering the use of existing cesspools as a final disposition for the discharge of this design, thus maximizing the use of existing infrastructure and minimizing the cost to the homeowner.

DESIGN 4 - A nitrification layer underdrained and diverted to a box of woodchips.

This design offers the opportunity to closely inspect each of the nitrification and denitrification processes, since each process occurs in a separate container in the system and there is a discrete sampling point between the two processes. The nitrification area is basically a full-sized underdrained soil absorption system that can be considered a slow-rate sand filter. That bed contains 18 inches of sand sourced from Long Island and that meets the specifications of ASTM C-33 and is hydraulically loaded at 1.2 gal/sq.ft./day[a]. A bottom drain in this area diverts the percolate to the bottom of a 1500-gallon tank filled with oak woodchips. Samples were taken following the nitrification bed prior to the

[a] This loading rate is calculated on the actual contact area of the wastewater dispersal device (GeoMat™) and the soil and not the areal area of the system.

diversion to the wood chip container (figure 5 sampling location “A”). Samples taken at this location indicate that despite low temperatures during late autumn and winter, nearly complete nitrification continued with most of the TN observed as nitrate and minimal ammonia present in percolate (figure 14).

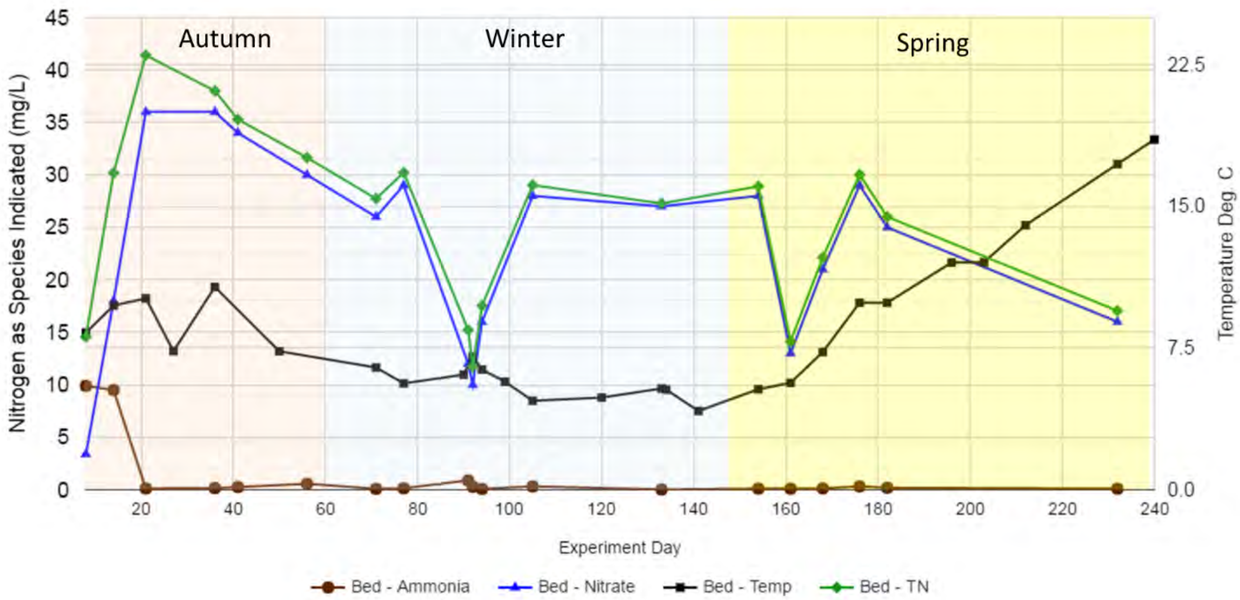


Figure 14. Nitrogen species in percolate beneath nitrification bed in DESIGN 4. Samples taken prior to discharge to the denitrification container (figure 5 location “A”).

The two periods of depressed TN occurred shortly after rainfall events of at least two inches. These data suggest that in soils-based denitrification systems, nitrification is not the limiting condition even during colder months of the year. Nitrogen concentrations in the discharge of the *denitrification portion* (location B – figure 5) of the system averaged 3.6 mg/L (2.3 – 4.8 mg/L, *p= .05*). The remaining TN in the discharge was primarily comprized of Total Kjeldahl Nitrogen (TKN), about 5 – 20% of which is ammonia. There are three notable exception to this trend (figure 15). In each of these instances, nitrate is the main constituent of the TN. These events were related to precipitation events prior to or during the sampling. We posit that the precipitation results in increased flow rates through the denitrification chamber and hence decreased residence time during which denitrification could occur. Despite this apparent vulnerability of the technology to upset, >90% of the observed values were < 10 mg/L TN and >70% of the TN values were < 5 mg/L TN.

CONCLUSION DESIGN 4

This design exhibited the most stable denitrification among the designs tested. Despite influent temperatures (percolate from the nitrification bed) below 5°C, denitrification continued. The decreases in performance related to precipitation events apppeared shortlived. As with the other designs, this design would require a structure for final disposition of effluent,

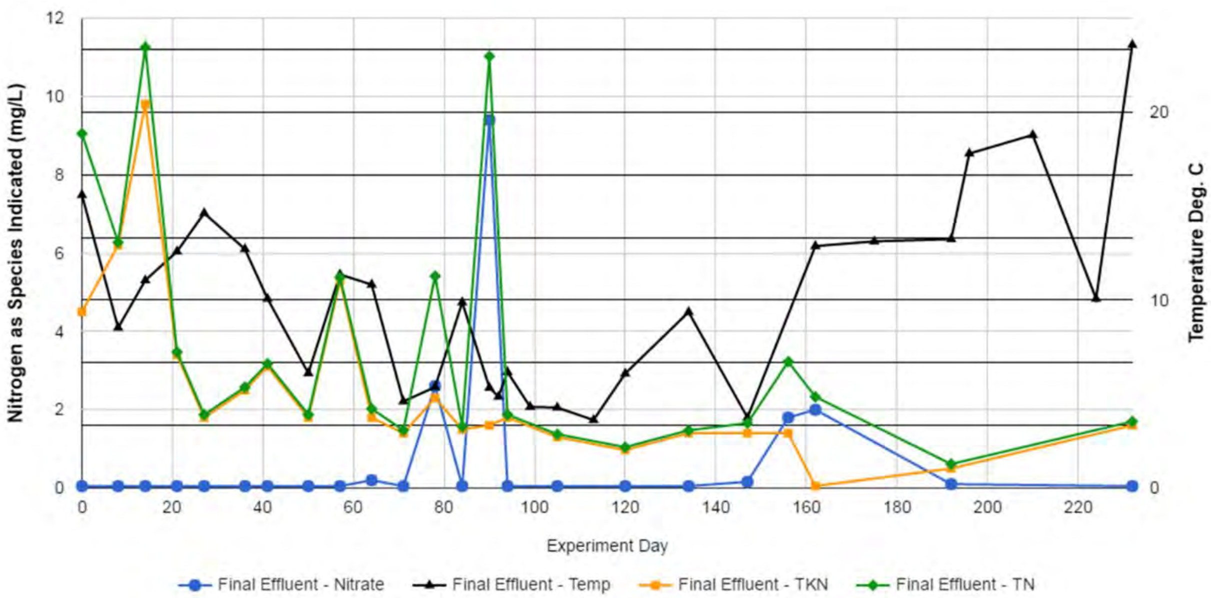


Figure 15. Nitrogen levels in discharge from denitrification area of DESIGN 5.

A major advantage to this design is the accessibility of the denitrification media for inspection and replacement. A disadvantage of the system as tested is the need for a final disposal area. In Long Island, the sponsors of the installation at Stony Brook University are considering the possibility of using existing cesspools on a property as a final disposition for the system discharge. To examine this potential, we examined the Biochemical Oxygen Demand ($BOD_{5\text{-day}}$) of the effluent since we posit that the wood-based carbon source may add significantly to the BOD of the final effluent. During the first four months of operation, the average of five BOD measurements was 440 mg/L. Thirteen subsequent measurements taken from November 2016 to June 2017 show and average of 36 mg/L. We conclude that more research should be performed regarding the sustainability of smaller final soil absorption systems for this technology, however data would suggest at least a 50% reduction in final STA could be sustained with this quality of effluent.

DESIGN 5 - Unsaturated system similar in dimensions to the silty-sand-sawdust system reported in Project 14-01 319 but substituting sand and sawdust from Long Island, New York sources.

The final design examined under this project was installed with support from Stony Brook University and closely followed the silty-sand-sawdust system reported previously under Project 14-01 319. The major difference between the two designs is the substitution of standard sand fill for the silty sand and the percentage of sand:sawdust (figure 16). DESIGN 5 contained a 1:1 mix sand:sawdust in the denitrification layer, while the system reported on in Project 14-01 319 used a 1:5 sawdust:sand-silt media mix. Design 5, along with sampling locations is illustrated in figures 16 and 17. Paired lysimeters were installed on each side of the system to enable some controlled experiments involving precipitation to be conducted in the summer of 2017. This design is the simplest design to install in the field since it does not involve the use of containment liners and is simply a modification of the fill material used beneath a low pressure dosed dispersal system.

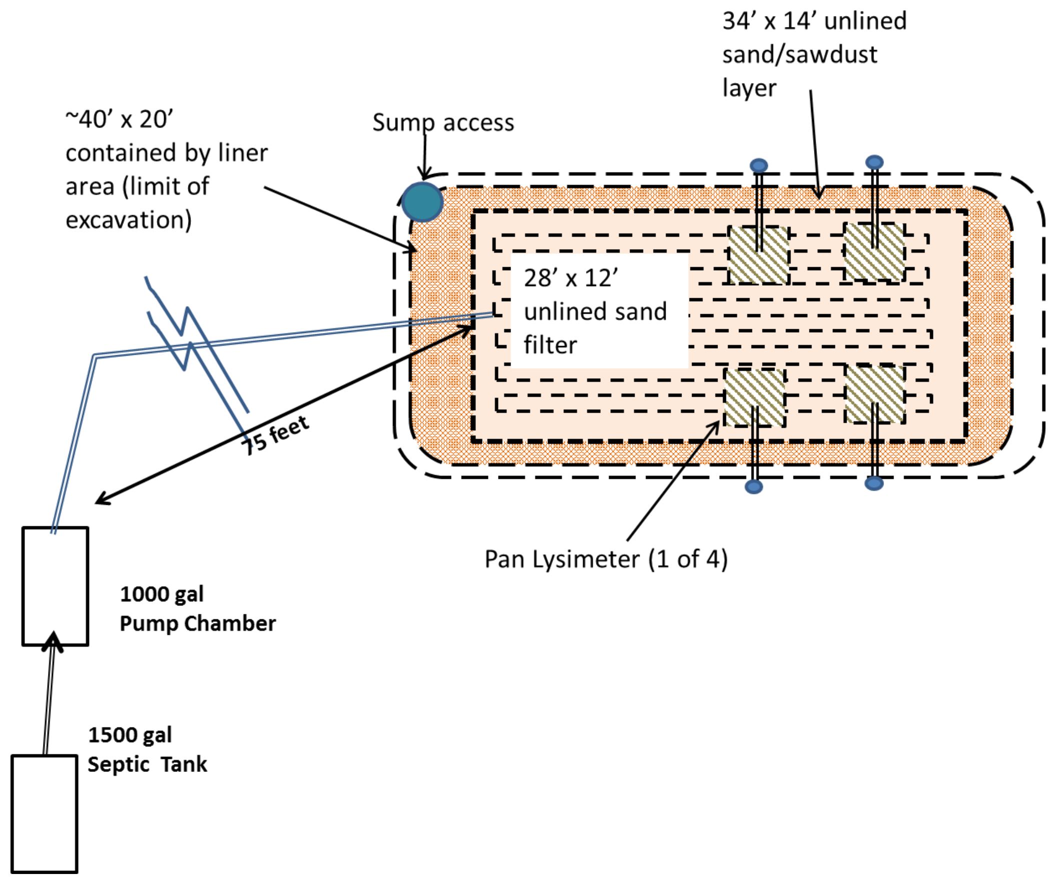


Figure 16 Illustration DESIGN 5 showing component and sampling locations.

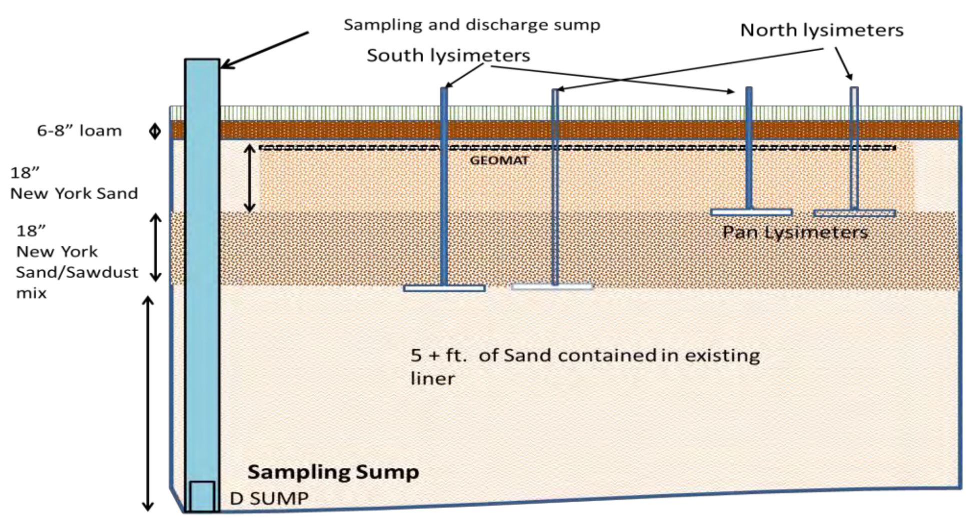


Figure 17 Profile illustration of DESIGN 5 - Unsaturated flow system.

Total nitrogen (TN) concentration for the first 250 days from samples collected in the sump under the entire system averaged 9.9 mg/L (8.5 – 11.3 mg/L, *p=.05*, n= 26)[b]. Although some seasonality is suggested (figure 18), this will be confirmed only after another year of operation. TN samples collected directly beneath the system in a pan lysimeter averaged 6.4 mg/L (4.7 – 8.2 mg/L, *p=.05*, n=21) are significantly different than the sump (*p=0.003*), indicating that the sump had higher concentrations of nitrogen than the lysimeter. While we would expect that there might be some differences due to the collection area of the sump (20' x 40') compared with the pan lysimeter (2' x 2') we have no explanation for why the sump would have higher TN levels unless some short-circuiting of percolate flow occurred around two 4-inch pipes that were left in the test cell from previous testing.

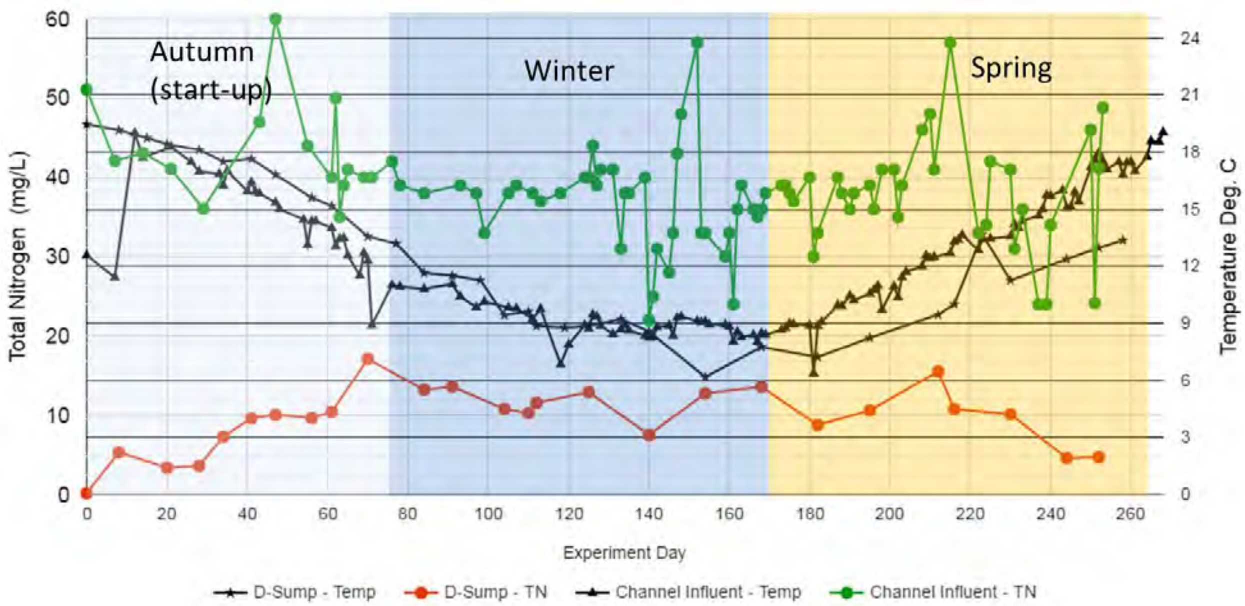


Figure 18. Total Nitrogen (TN) concentration in percolate beneath DESIGN 5, an unsaturated flow system installed at MASSTC. "D-Sump" = percolate beneath the system, "Channel Influent" == the raw wastewater supplied to the septic tank.

As with the other designs, it appears that nitrification is not limiting, even during the colder months. Sump samples beneath the system show that the TN present is predominantly in the form of nitrate with ammonia comprising <3% of the nitrogen on average (figure 19). The remaining organically bound nitrogen constitutes an average of 6% of the TN in the percolate.

CONCLUSION – DESIGN 5

In comparison with DESIGNS 1 – 4, DESIGN 5 is the simplest to construct in the field since no liners are required and basically an 18-inch layer of standard fill material is substituted with a mixture of fill material and sawdust. In addition, and in contrast to the other designs, this design does not require a separate facility for the final disposition of the treated effluent. Since this system has only been in operation since August 2016, we cannot yet determine the long-term performance. As opposed to the

[b] First value of 0.2 mg/L was not used in this calculation as it was considered luxury uptake by organisms.

saturated designs (DESIGN 1 – 4), the sawdust/cellulose in this design would appear to be more vulnerable to aerobic decomposition.

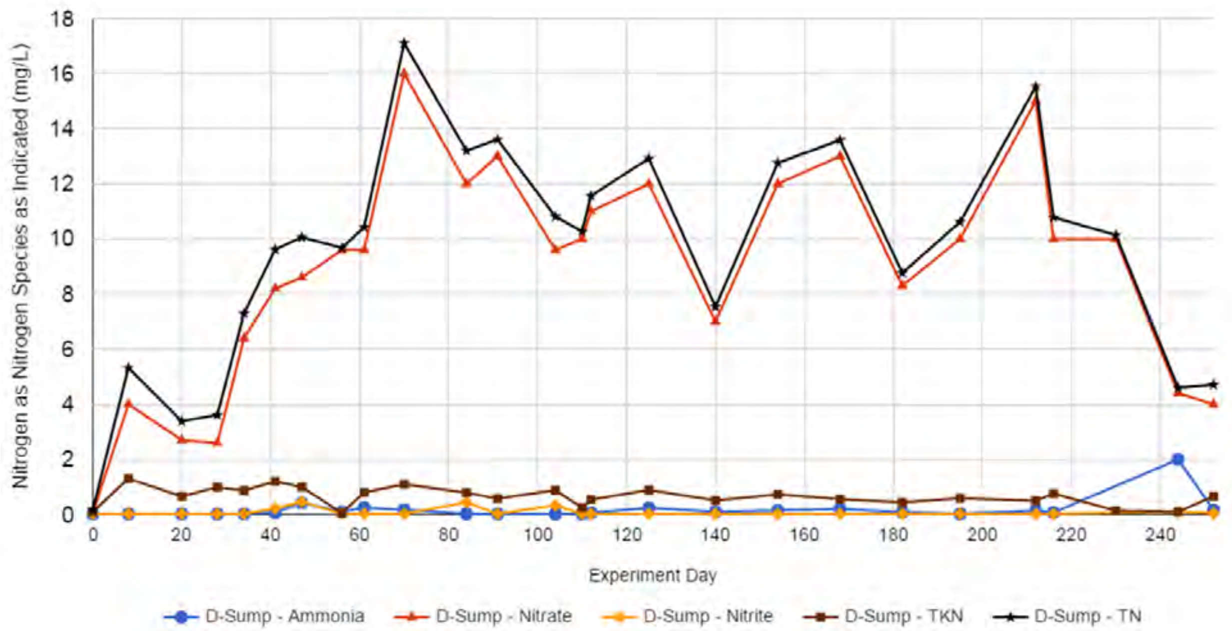


Figure 19. Nitrogen concentrations (mg/L) by species in effluent (percolate) of DESIGN 5.

The release of carbon dioxide in the aerobic process could reduce the long-term ability of the sawdust to provide carbon for the organisms responsible for denitrification. To address this possible shortcoming, collaborators on this project convened a design charrette on October 25, 2016 at the University of Rhode Island. Participants used a newly developed data display tool to review all data collected to that point by this project and Project 14-01 319. The group decided that the following design modifications would be introduced to field installations to enhance anoxia in the denitrification layer of the system.

- A layer of peastone gravel will be placed immediately below the denitrification layer. Since the sand :sawdust layer is finer textured than the gravel beneath it, there will be a restriction in the water flow and an area of saturation above the gravel layer[5]. This saturation will occlude oxygen and oxygen transfer and hopefully enhance the anoxia.
- An impervious vertical liner will be placed around the denitrification layer. This liner will further restrict the exchange of oxygen from the adjacent soil areas.

These modifications are not expected to introduce significant complexity to the design since the practice of layering peastone and installing impervious barriers are common practice in septic system installation. These changes were summarized in an informational flyer for designers and is included in Appendix 1.

STUDY CONCLUSIONS AND FURTHER STEPS

We conclude from the present project and Project 14-01/319 that there is potential for significant nitrogen removal from onsite septic systems by making simple modifications to the Soil Treatment Area -STA (aka drainfields) using various configurations of lignocellulose and sand media. Following a review of the extensive work done by the Florida Department of Health[1] and discussions with those researchers and others, this project endeavored to install and test the simplest, most cost-effective and sustainable means of achieving nitrogen removal from onsite septic systems. The understanding gained from the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) Project and work by others[2,3,6], informed our decision to test the five systems reported on herein at the Massachusetts Alternative Septic System Test Center. These efforts are also in collaboration with Center for Clean Water Technology - Stony Brook University and the Suffolk County New York Health Department and offer an unprecedented opportunity to involve academic researchers and regulators in our efforts.

Each design was installed in full-scale, which we considered to be a minimum of 220 gallons per day. Beyond this flow, we consider the required sizing of the system to progress in linear fashion to all single-family home applications and possibly beyond. For example, a system design required for 440 gallons per day, which is equivalent to a four-bedroom house requirement under Massachusetts regulations, would be twice the areal area as the systems that we tested, which were sized equivalent to requirements for a two-bedroom system under Massachusetts regulations.

Among the most significant findings of this work is the documentation of the differences in results compared with the Florida studies, which are likely due climate differences. Since all the wastewater processes involving nitrogen transformations are controlled by temperature, we expected that the final extent of denitrification might be variable throughout the year at our latitude and show a diminishment of performance during the colder months. This was verified in each system to some extent with DESIGN 4 (nitrifying bed diverted to a container of saturated woodchips) exhibiting the least reduction in performance in the cold weather. Another significant finding was that in the colder weather the limiting step in the denitrification process was the reduction of nitrate to nitrogen gas. This is contrary to the common belief that nitrification or the oxidation of ammonium to nitrate is the limiting step in denitrification in cold climates. This common belief, likely deduced from larger wastewater treatment technologies, is apparently not true for soils-based denitrification processes. This knowledge will be used to consider design changes that might optimize the overall denitrification.

Four of the designs tested under this project require final disposition of effluent. Our studies indicate that DESIGN 2 and DESIGN 3 (both saturated systems with sand as the primary media), could be served by reduced-size final disposal areas since their final effluent $BOD_{5\text{-day}}$ (BOD) averaged < 15 mg/L, which would meet the criteria for many states' reduced sizing requirements. DESIGN 1 (a saturated system similar to DESIGN 2 and 3, but which used a loamy sand in the nitrification layer, exhibited a five-month period high BOD (40 – 240 mg/L) which will require consideration of a larger final disposal area, as did DESIGN 4. Since these designs require a final area for disposal, future efforts will involve a determination of the configuration for these final disposal areas, focusing on optimizing total system size and reducing costs.

DESIGN 5 was the simplest design and holds the promise of being the most economical system investigated. Although early in its testing (9 months), this design indicates that the TN can be reduced by ≈75%. Longer-term testing will be needed to evaluate project this system's performance. Data suggest however that this system will perform comparable to the silt-sand-sawdust system reported on in Project 14-01 319. These findings are the basis of a grant request under the EPA SNEP Coastal Watershed Restoration which will be installing 12 systems in the next two years for the evaluation of this technology. The questions regarding the longevity of the media remain to be addressed, however some researchers indicate that carbon depletion will occur over decades[6].

Continued Operation of the Massachusetts Alternative Septic System Test Center (MASSTC)

During the two projects referenced, MASSTC continued to sponsor both standardized testing for the onsite wastewater industry and research and development efforts sponsored by private parties. New technologies are now in development that have been encouraged by our efforts to develop non-proprietary ones supported under Projects 14-03/319 and 15-07/319 . Some of these technologies are using cellulose-based denitrification in part in response to efforts supported under these grants. MASSTC is presently also involved in two "spinoff" grants from the Massachusetts Clean Energy Center (CEC) that encourages businesses involved in wastewater products that relieve large infrastructure of some of the wastewater loads. These are grants given to the companies involved and MASSTC participation involves providing consultation and test-bed facilities. CEC is also engaged in determining the viability of MASSTC and other potential test-bed sites for their ability to encourage innovation and economic development. It appears from the investment from the private industry and research being conducted, such as was supported by the two grants, that MASSTC is a beneficial public-private partnership which allows the Commonwealth the advantage of having a facility to answer some research questions regarding their regulations pertaining to onsite wastewater and watershed management for contaminants, while concurrently serving as a facility at which private industry can research, develop and test products to address those same needs.

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Appendix 1

Construction Summary for Layered Soil Treatment Area (LSTA) to be installed under the Demonstration Project

A primer for board of health members, septic system designers and installers

Massachusetts Alternative Septic System Test Center

Construction Summary for Layered Soil Treatment Area (LSTA) to be installed under the Demonstration Project

A primer for board of health members, septic system designers and installers

Note: *The following describes a demonstration project by which Barnstable County Department of Health and Environment (BCDHE) in collaboration with others, intends to install modified Soil Treatment Areas (STA) alternately known as leachfields at various residential pilot locations to test their effectiveness. The following describes various aspects of the project and is meant for health agents, system designers and system installers.*

What is a Layered Soil Treatment Area (LSTA)?

A LSTA is basically a leachfield that is placed in layers, using materials that allow for the successive nitrification and denitrification of septic tank effluent as it percolates through the layers.

The Barnstable County Department of Health and Environment in collaboration with others and with information gleaned from many sources, has been experimenting with various configurations of LSTA at the Massachusetts Alternative Septic System Test Center over the past few years. We have received funding from various sources to place Pilot Systems at residences. To minimize the risk of failure at the pilot locations, certain design features have been incorporated in these pilot project sites and are described below.

Ideal sites for consideration of the layered system.

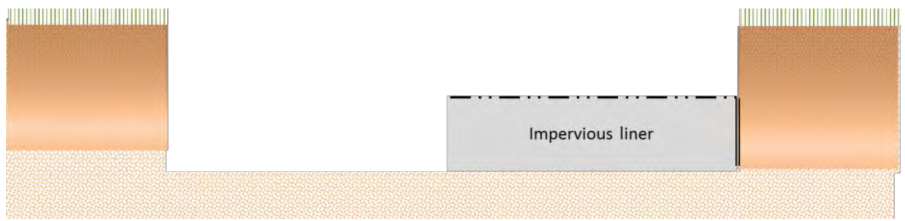
The ideal site for a pilot LSTA installation is one that enables a strip-out to an elevation of four feet below existing grade. In this excavation 18 inches of a sand-sawdust mix is first placed over a two-inch layer of washed pea stone followed by 18 inches of “Title 5” sand. Atop the sand layer, the distribution system will be placed (shallow pressurized drainfield product, drip dispersal). The placement of a liner/barrier around the lower sand/sawdust layer is also required. The sequence would be as follows:

STEP 1

Excavate areal area required to a depth of at least four feet.

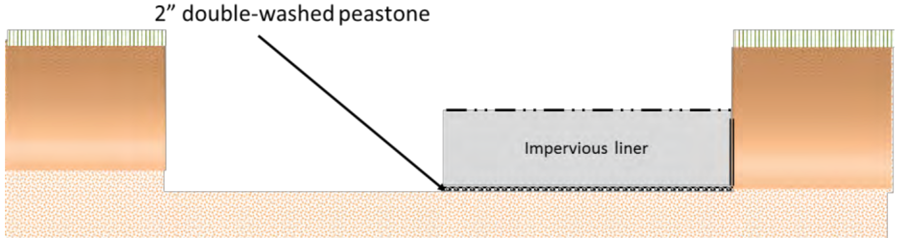
STEP 2

Place 20 mil impervious liner around perimeter of excavation ONLY ON THAT PORTION OF THE SYSTEM DESIGNATED TO RECEIVE THE PEA STONE AND THE SAND/SAWDUST MIXTURE. Hold in place with geotextile staples or other suitable method.



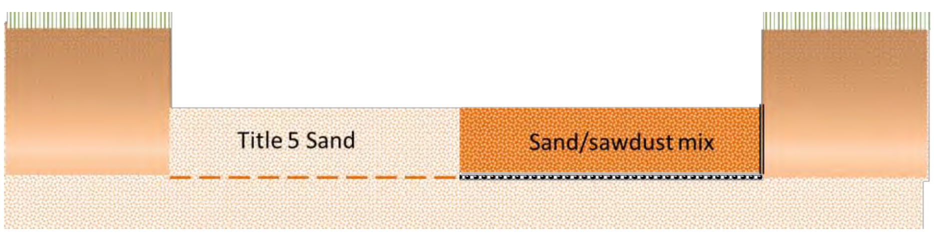
STEP 3

Place 2 inches of double-washed pea stone under portion of system that will receive the sand/sawdust.



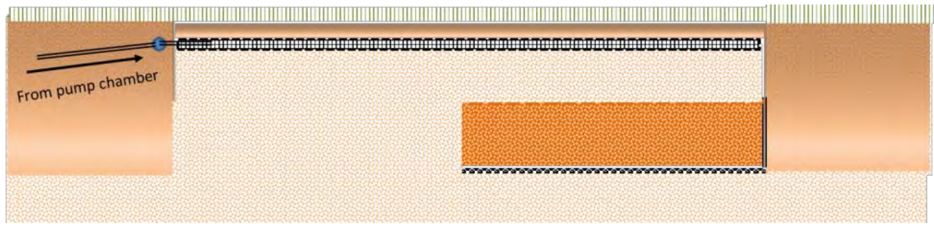
STEP 4

Place 18 inches of sand/sawdust mix in excavation (use light plate compactor after 12 inches and again at final grade to obtain 18 inches ONLY IN AREA DESIGNATED FOR TREATMENT. Fill the adjacent area with Title 5 sand.



Step 5

Place Title 5 sand to an elevation appropriate to the distribution method (drip, shallow pressurized drainfield, GeoMat™) 18 inches in depth above the sand/sawdust layer and install distribution system and cover.



Why is the sawdust in only half of the STA?

You will read above that certain measures are being taken to minimize the risk of placing this pilot system at their residence. The design team decided that splitting the system into halves has two advantages. Foremost, in the unlikely event that the sawdust mixture causes a hydraulic failure, the homeowner will still have the remaining Title 5 system to disperse wastewater. Secondly, the halving of the system will allow a comparison between the amended STA with a standard Title 5 system.

Is there another way to minimize the risk to the homeowner?

Yes. There are two configurations possible in the pilot. The ideal situation is where an installation of a complete Title 5 system and an additional half sized system with an amended STA. The two possibilities are sketched below.

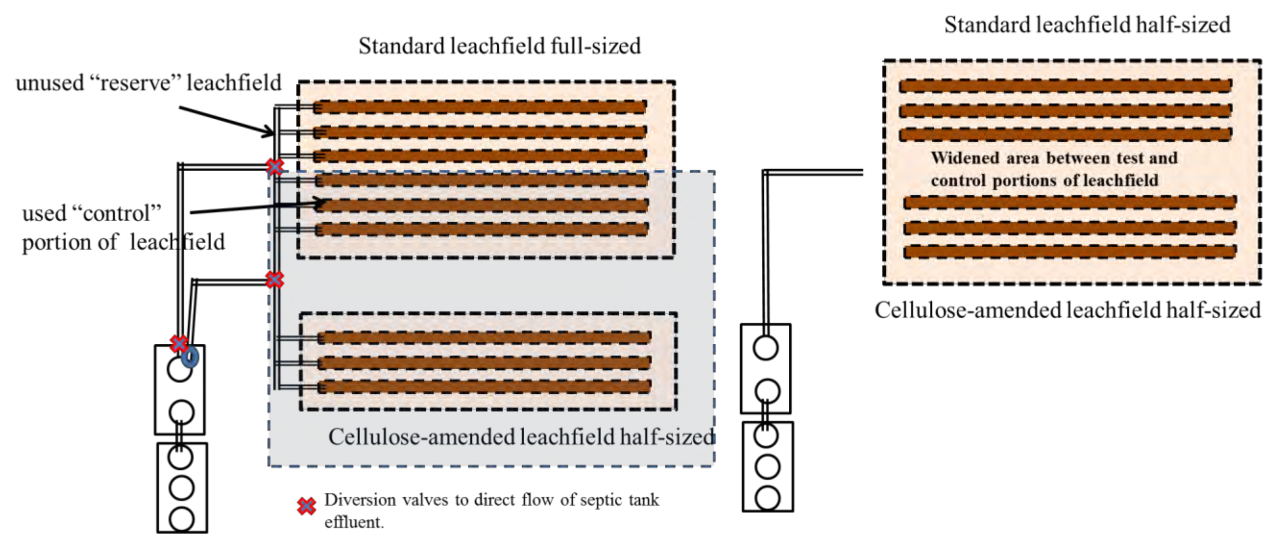


Figure A **Figure B**

Figure A above shows the situation where one-half of the Title 5 system is used in conjunction with a layered STA (LSTA). In the event of a failure of the LSTA portion, a few "diversion" valves as shown above are turned and the homeowner is left with a full-sized Title 5 system. In the event of a failure in the situation shown in Figure B above, the homeowner would have a half-capacity system.

Homeowners that choose to have a configuration like Figure B above will be asked to sign a waiver that releases the County, designer and the contractor from all liability in the event of a failure in the amended section of the STA. This is because if the amended portion of the STA fails hydraulically, the responsibility to replace the section of the STA would be the homeowner's. This legal paperwork is presently being drafted.

What about sampling of the system?

Under the grant, samples will be taken monthly for two years. Samples will be taken from the pump chamber as well as from a series of pan lysimeters under the system. In addition, water use and pump-run counters will be checked during each sampling event. Following the period of the grant, the homeowner will be responsible for causing an annual inspection of the system and any monitoring required by the Commonwealth's DEP. We anticipate that annual monitoring will be required and annual inspection and adjustment of the low-pressure distribution system will be needed. A checklist for this requirement is being prepared.

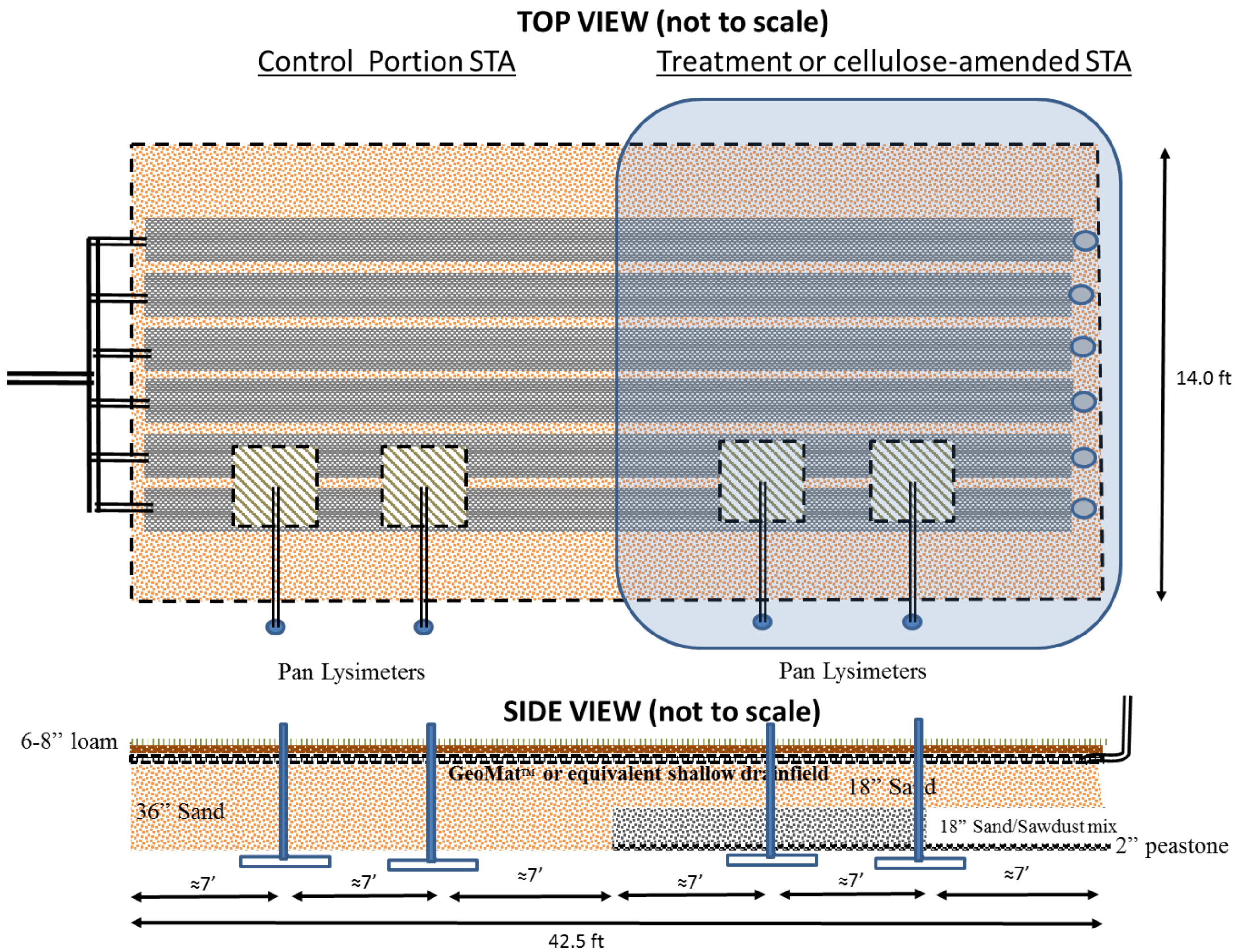
During each installation, pan lysimeters will be placed at four places (two under each of the STA and LSTA). Pan lysimeters are essentially "pans" that collect percolating water and convey it to a collection point. The placement of pan lysimeters is illustrated in a typical system below.

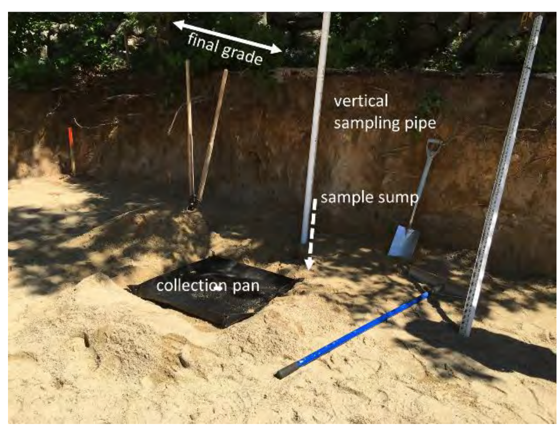
Will there be some assistance with permitting?

As part of the SNEP Grant, our partners at the Buzzards Bay Coalition have been helping with permitting. Korrin Petersen will attend the meeting of the board of health to answer questions, as may George Heufelder with Barnstable County Department of Health and Environment. We will also be providing assistance for system designers. In some cases, we will meet with the homeowners to make sure they understand the experimental nature of the project and their responsibilities for long-term operation and maintenance.

Long Term Maintenance?

As mentioned above and in accordance with Title 5, all pressure dosed systems must be maintained annually. The homeowners in this program will be informed and must agree to this and any other monitoring requirements. The systems will be registered with the Barnstable County Tracking Program and there will be online access to the information for your board of health.





Pan lysimeters will be fabricated and installed by staff of Barnstable County Department of Health and Environment. The vertical sampling port will be protected by a standard curb box. Other sampling ports may be required.

Other inspection ports and sampling devices may be installed. These installations may suspend fill operations for short periods of time and will be installed by personnel of the Barnstable County Department of Health and Environment.

Please remember

As we have said all along, the systems installed under this program are experimental. While we have taken design steps to minimize the risk of harm to the public health and environment, there is still some risk that the system may not perform to the expected standard. Some homeowners who allow a system sized at 1.5 x the design flow as described above will bear little risk of having to replace their system if there is some hydraulic failure (since we can merely turn a few valves and have a fully-complying leachfield. Others who install the system as in Figure B above will be signing a waiver noting that they will be responsible for any repairs necessary to the non-complying portion of the system should it fail by Title 5 criteria.

For designers, we will be available to consult on your design plans. In addition, any pressure dosed system designs that incorporate a Perc-Rite® Drip Dispersal System or a low pressure-dosed system using GeoMat® will have assistance from Oakson, Inc. or GeoMatrix LLC respectively. Other low-pressure dosed dispersal means over the nitrifying layer will be considered.

Barnstable County Department of Health and Environment will be holding some introductory sessions on the technology and the results in your area. If you are interested in attending one of these sessions, please send an email to George Heufelder at the email address below.

Project Partners

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Appendix 2 – 6
Raw Data

Key

DO = dissolved oxygen in mg/L

TKN = Total Kjeldahl Nitrogen in mg/L

Temp = Temperature in degrees Celsius

CBOD = 5-day Carbonaceous Biochemical Oxygen Demand

BOD = 5-day Biochemical Oxygen Demand

TSS = Total Suspended Solids (mg/L)

Confidence Interval = 95%

Upper CI = Mean or Average + Confidence Interval

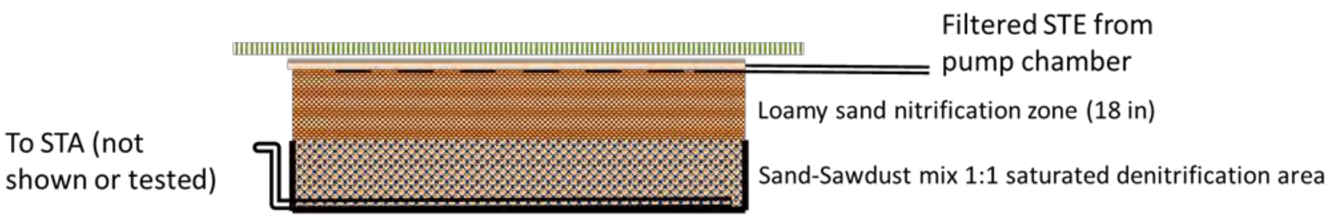
Lower CI = Mean or Average - Confidence Interval

Count = Number of Observations

Ph – report in Ph Units

*** all nitrogen parameters reported in milligrams per liter (mg/L) - nitrogen

Appendix 2
Raw Data
DESIGN 1



DESIGN 1 - Raw Data

Saturated System Using Loamy Sand Nitrification Layer

Sample Date	Alkalinity	Ammonia	BOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2014-12-02									4.3	
2014-12-12									2.9	
2014-12-25					2.30	0.025			2.2	4.5
2015-01-22					0.16	0.025			2.5	2.7
2015-02-05		2.30			0.06	0.005			3.4	3.5
2015-02-09	400	5.90			0.01	0.003			7.7	7.7
2015-02-11	410	8.30		0.11	0.03	0.240	6.40	3.9	9.7	10.0
2015-02-13	410	9.20		0.18	0.36	0.003	6.57	3.7	12.0	12.4
2015-02-18	430	12.00		0.21	0.16	0.022	6.39	3.6	13.0	13.2
2015-02-19	420	11.00			0.05	0.250			13.0	13.3
2015-02-24	440	17.00		0.12	0.05	0.130	6.32	3.5	19.0	19.2
2015-02-27	450	16.00		2.07	0.40	0.160	6.30	3.1	18.0	18.6
2015-03-03	440	19.00		1.44	0.05	0.210	6.38	3.2	22.0	22.3
2015-03-09		18.00		0.57	0.05	0.260	6.37	3.1	20.0	20.3
2015-03-12		17.00		0.31	0.34	1.200	6.16	2.9	19.0	20.5
2015-03-17		17.00		0.36	0.05	0.480	6.31	2.7	20.0	20.5
2015-03-26	320	15.00			0.05	0.110			19.0	19.2
2015-03-31	400	16.00		0.25	0.05	1.200	6.65	3.6	18.0	19.3
2015-04-06				4.23			6.60	10.4		
2015-04-08	430	15.00		0.22	1.90	2.700	6.50	5.1	19.0	23.6
2015-04-14	450	13.00	31	0.19	2.80	4.400	6.38	6.0	15.0	22.2
2015-04-21				0.25			6.47	7.7		
2015-04-28	750	10.00	63	0.09	1.20	0.500	6.57	8.5	14.0	15.7
2015-05-05	480	7.20	110	0.10	0.22	0.910	6.50	9.1	11.0	12.1
2015-05-12	390	6.10	170	0.67	0.05	0.750	6.82	15.5	8.9	9.7
2015-05-18	430	4.00	240	0.06	0.10	0.300	6.32	13.0	6.0	6.4
2015-05-26		2.10	220	0.12	0.05	0.580	6.41	14.0	4.2	4.8
2015-06-01	320		150	0.18	0.05	0.025	6.32	15.6	4.2	4.3
2015-06-04	370	1.30	190	0.08	0.05	1.200	6.35	15.3	3.4	4.7
2015-06-08	390	1.20	210	0.07	0.05	1.400	6.27	15.0	3.4	4.9
2015-06-16	450	1.40	260	0.24	0.05	1.500	6.36	16.4	2.8	4.4
2015-06-22	450	1.40	210	0.10	0.05	1.100	6.32	17.0	2.5	3.7
2015-07-01	420	1.20	180		0.05	0.025			2.4	2.5
2015-07-10	380	0.70	190	0.13	0.05	1.100	6.30	18.7	3.7	4.9
2015-07-14	400	0.87	240	0.04	0.05	1.100	6.31	19.5	2.4	3.6
2015-07-23	440	0.25	160	0.25	0.05	0.770	6.43	20.4	2.0	2.8
2015-07-30	460	0.25	140	0.04	0.05	0.600	6.36	20.8	1.9	2.5
2015-08-06	460	0.54	110		0.05	0.025			1.8	1.9
2015-08-12	470	0.46	120	0.04	0.81	0.260	6.37	21.7	1.9	3.0
2015-08-20	470	0.25	81	0.10	0.05	0.025	6.43	21.8	1.6	1.7
2015-08-26	450	0.66	74	0.19	0.05	0.076	6.24	22.4	1.4	1.5
2015-09-02	450	0.62	62	0.10	0.05	0.025	6.48	22.3	1.8	1.9
2015-09-09	460	0.74	60	0.19	0.14	0.025	6.33	22.2	1.9	2.1
2015-09-15	140	0.84	46	0.20	0.05	0.025	6.38	22.1	2.2	2.3
2015-09-23				0.12			6.40	21.4		
2015-09-29	450	1.20	27	0.05	0.22	0.025	6.42	20.5	2.3	2.5
2015-10-08	390		11	0.07	0.05	0.025	6.46	18.8	2.5	2.6
2015-10-24				0.06	0.05	0.025	6.53	18.0	2.2	2.3
2015-11-04			14	0.07	0.49	0.025	6.50	15.8	1.7	2.2
2015-11-12				0.11			6.38	15.2		
2015-11-17	360	0.77	14	0.08	0.05	0.083	6.50	14.5	1.6	1.7

DESIGN 1 - Raw Data

Saturated System Using Loamy Sand Nitrification Layer

Sample Date	Alkalinity	Ammonia	BOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2015-12-02				0.15	0.78	0.091	6.52	12.2	1.6	2.5
2015-12-09	360			0.20	0.61	0.110	6.04	11.3	2.3	3.0
2015-12-15				0.13			5.99	11.4		
2015-12-21				0.12	0.43	0.025	6.27	11.7	1.8	2.3
2015-12-29				0.17			6.41	11.7		
2016-01-05	260		7	0.17	0.45	0.025	6.64	10.4	1.8	2.3
2016-01-12	330	0.49		0.24	1.90	0.025	6.28	9.0	2.2	4.1
2016-01-26	320	1.30		0.21	4.50	0.025	6.27	7.0	2.4	6.9
2016-02-02	320		5.2	0.27	3.10	0.025	6.54	6.5	3.4	6.5
2016-02-09	300		3.1	0.15	0.05	0.025	6.87	6.8	4.1	4.2
2016-02-16	290		4.8	0.16	3.80	0.026	6.36	5.9	5.0	8.8
2016-02-23	280	3.20	7	0.14	8.20	0.025	6.38	5.9	3.8	12.0
2016-03-02	290	3.20	5.1	0.11	5.40	0.025	7.03	6.4	4.3	9.7
2016-03-08		5.20		0.15	7.50	0.025	6.23	6.4	7.9	15.4
2016-03-15	310	3.70	6.9	0.86	8.10	0.025	5.93	7.0	4.3	12.4
2016-03-22				0.14			6.06	7.5		
2016-03-29				0.13			6.05	7.7		
2016-04-05	370	5.10	8.3	0.13	2.20	0.025	6.04	8.4	6.4	8.6
2016-04-12	370	4.90	5	0.19	1.80	0.025	5.96	8.2	6.0	7.8
2016-04-19	370	7.40	10	0.26	0.05	0.025	5.97	8.5	9.1	9.2
2016-04-26	420	7.50	10	0.18	1.30	0.025	5.96	9.4	9.6	10.9
2016-05-03	460	9.20		0.13	0.58	0.025	6.31	9.8	11.0	11.6
2016-05-10	450			0.14	0.38	0.025	6.20	10.1	13.0	13.4
2016-05-17		9.50	12	0.28	0.18	0.025	6.01	11.2	12.0	12.2
2016-05-24		9.80		0.24	0.05	0.025	6.49	12.3	13.0	13.1
2016-06-01		9.00	22	0.14	0.24	0.025	5.87	13.9	12.0	12.3
2016-06-07		9.20	20	0.12	0.32	0.025	5.57	14.8	14.0	14.3
2016-06-14		11.00	22	0.21	0.35	0.025	5.83	15.8	14.0	14.4
2016-06-21		9.40	13	0.27	1.30	0.025	5.94	16.7	15.0	16.3
2016-06-28		12.00		0.11	1.50	0.025	6.14	17.4	17.0	18.5
2016-07-06		13.00	10	0.14		0.025	5.88	18.5	17.0	18.0
2016-07-12		16.00	27	0.10	0.64	0.025	6.09	19.2	21.0	21.7
2016-07-18		13.00	5.3		3.30	0.025			20.0	23.3
2016-07-19		13.00	12	0.13	1.70	0.025	5.76	19.9	20.0	21.7
2016-07-20		16.00		0.05	0.94	0.025	5.84	20.1	21.0	22.0
2016-07-21		17.00			0.48	0.025			21.0	21.5
2016-07-26		14.00		0.25	0.76	0.025	5.98	21.2	21.0	21.8
2016-08-03		15.00	16	0.22	0.64	0.025	6.11	22.3	19.0	19.7
2016-08-09		13.00	9.3	0.13	0.05	0.025	5.91	21.9	16.0	16.1
2016-08-16		13.00	7.3	0.09	0.05	0.025	6.43	23.3	17.0	17.1
2016-08-22		7.20			2.30	0.025			15.0	17.3
2016-08-24		10.00	14	0.40	15.00	0.025	6.56	23.4	18.0	33.0
2016-08-25		7.10		1.48	48.00	0.310	6.73	26.8	15.0	63.3
2016-08-29				0.34			6.62	23.2		
2016-08-30		7.60	5.1	0.15	36.00	0.025	6.78	23.2	14.0	50.0
2016-09-07				0.20			6.52	22.5		
2016-09-12		2.20		0.05	13.00	0.025	6.77	22.3	3.6	16.6
2016-09-20				0.10			6.46	21.9		
2016-09-27				0.05			6.60	21.4		
2016-10-05		3.10	1	0.16	9.00	0.025	6.38	19.8	2.1	11.1
2016-10-13				0.22			6.37	18.5		

DESIGN 1 - Raw Data

Saturated System Using Loamy Sand Nitrification Layer

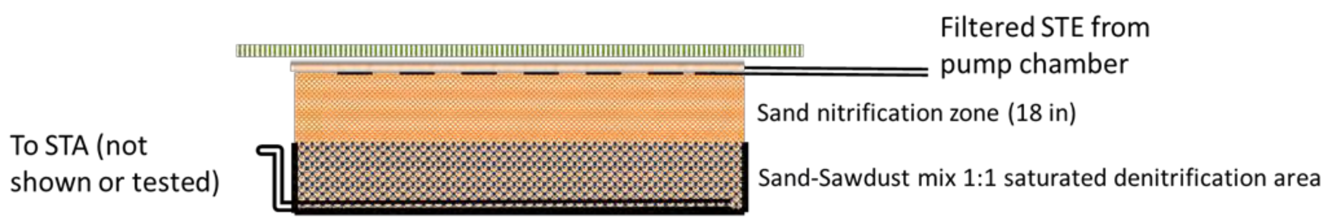
Sample Date	Alkalinity	Ammonia	BOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-10-20				0.11			6.20	17.9		
2016-11-02		2.10	1	0.10	3.70	0.025	6.83	16.1	3.5	7.2
2016-11-08		2.80	1	0.18	5.70	0.025	6.19	15.2	2.8	8.5
2016-11-15		2.20	1	0.18	7.70	0.025	6.13	14.2	3.2	10.9
2016-11-28	250	2.70	1		2.60	0.025			4.0	6.6

Count	53	77	57	93	90	91	93	93	93	91
Average	396	7.36	64	0.27	2.44	0.280	6.32	13.9	8.9	11.7
Median	410	7.20	16	0.15	0.35	0.025	6.36	14.8	6.0	10.0
Std Dev	86	5.82	80	0.51	6.68	0.627	0.27	6.6	6.9	10.0
Confidence Interval	23	1.30	21	0.10	1.38	0.129	0.05	1.3	1.4	2.1
Upper CI	419	8.66	85	0.37	3.82	0.409	6.38	15.2	10.3	13.8
Lower CI	373	6.06	43	0.16	1.06	0.151	6.27	12.5	7.5	9.7

DESIGN 1 Lysimeter at interface of nitrification and denitrification layer - "PAN D"
RAW DATA

Sample Date	Alkalinit	Ammonia	BOD5	DO	Nitrat	Nitrite	pH	Temp	TKN	TN
2016-11-15		4.70		4.18	12.00	0.520	5.88	11.4	6.2	18.7
2016-11-08		0.50		2.72	15.00	0.650		13.0	1.1	16.8
2016-11-02		0.93		4.72	16.00	0.025		13.5	3.0	19.0
2016-10-20				3.24				17.7		
2016-10-13				3.26				16.9		
2016-10-05		0.92		2.45	21.00	0.025		18.6	2.4	23.4
2016-09-27				4.40			6.50	20.2		
2016-09-20				1.12			5.86	22.3		
2016-09-12		0.28			38.00	0.025			1.5	39.5
2016-09-07				1.94			6.17	22.8		
2016-08-30		0.19		5.15	55.00	0.025	6.96	24.8	1.1	56.1
2016-08-29				4.75			6.97	24.7		
2016-08-24		1.20			330.00	0.025			4.3	334.3
2016-08-03		0.72		5.28	300.00	0.025	6.22	23.1		300.0
2016-07-26		27.00			5.00	0.310			38.0	43.3
2016-07-21		16.00			0.32	0.880			21.0	22.2
2016-07-20		34.00		0.56	0.59	1.600	6.59	25.6	36.0	38.2
2016-07-19		27.00		1.41	0.05	0.270	6.59	29.1	39.0	39.3
2016-07-18		31.00			0.05	0.058			41.0	41.1
2016-03-29				3.22			6.24	8.2		
2016-03-15	260			5.58	2.60	0.025	6.14	7.1	20.0	22.6
2016-02-23	250			2.92	16.00	0.320	6.13	4.7	23.0	39.3
2016-02-09	250			3.14	18.00	0.200	6.65	3.9	17.0	35.2
2016-02-02	250			5.12	11.00	0.025	6.12	4.4	12.0	23.0
2016-01-26	270				13.00	0.460			9.4	22.9
2016-01-05	190	0.03			15.00	0.220				15.2
2015-12-02					23.00	0.110			0.9	24.0
2015-08-26				0.25			6.56	24.3		
2015-08-12		1.30		0.04	24.00	0.110	6.51	22.8	5.0	
2015-07-30				0.55	0.14	0.025	6.81	22.8		
2015-07-14		0.34		0.27	2.70	0.025	6.62	22.3	2.4	5.1
2015-07-10		0.70		0.12	0.75	0.110	6.66	21.2	4.3	5.2
2015-06-24					0.05	0.300				
2015-06-22				7.39			6.69	19.1		
2015-06-04		0.25		1.22			6.76	15.7	3.4	
2015-06-01		0.25		0.24	6.70	0.025	6.67	18.1	3.9	10.6
2015-05-26		0.39		0.07	0.83	0.060	6.80	16.1	4.8	5.7
2015-05-18	330	0.21		1.85	23.00	0.025	6.64	16.3	0.5	23.5
2015-04-28		0.71		0.70	58.00	0.930	6.67	9.1	1.0	59.9
2015-04-21				1.38			6.67	9.1		
2015-04-08	390	23.00			3.70	0.440			26.0	30.1
2015-01-16	350	0.40			0.30	0.025			1.8	2.1
Count	9	24		31	31	31	26	31	28	28
Average	282	7.2		2.56	32.64	0.254	6.50	17.1	11.8	47.0
Median	260	0.7		2.45	12.00	0.110	6.61	18.1	4.6	23.5
Std Dev	62	11.7		2.03	76.94	0.356	0.31	7.0	13.3	77.8
Confidence Interval	41	4.7		0.72	27.09	0.125	0.12	2.5	4.9	28.8
Upper CI	323	11.9		3.27	59.72	0.379	6.62	19.5	16.7	75.9
Lower CI	242	2.5		1.84	5.55	0.129	6.38	14.6	6.8	18.2

Appendix 3
Raw Data
DESIGN 2



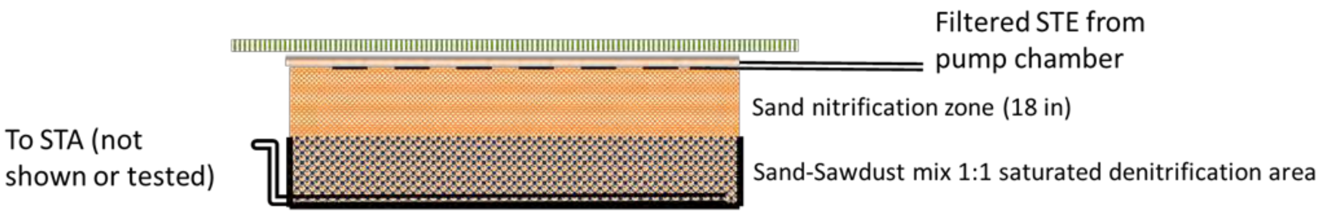
DESIGN 2 Saturated system with sand as nitrification layer

Sample Date	Alkalinity	Ammonia	BOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN	TSS
2017-01-04	160	0.53	1	0.52	7.6	0.025	6.05	7.57	1.2	8.8	23
2017-01-10				0.33			5.8	7.09			
2017-01-11	180	1.6	1		2.7	0.025			2.9	5.6	
2017-01-17	200	5.3		0.97	1.6	0.025	6.05	6.8	6.9	8.5	
2017-01-23		5.4	1	0.15	0.2	0.025	6.66	6.89	9.1	9.3	
2017-01-25		9	0	1.23	1.3	0.025	6.4	6.92	11	12.3	
2017-02-01				0.34			5.79	6.57			
2017-02-07		12	1	0.06	1.7	0.025	6.57	5.85	13	14.7	
2017-02-15				0.05			6.93	5.46			
2017-02-22		15	1	0.2	4.6	0.025	6.28	6	17	21.6	
2017-03-08		12	1	0.21	15	0.025	6.11	6.56	14	29.0	
2017-03-22				0.24			5.95	5.74			
2017-04-05		4.6		0.33	16	0.33	5.69	6.26	6.6	22.9	
2017-04-18				0.21			5.64	9.71			
2017-05-05		1.3	1	0.36	21	0.18	5.45	11.87	0.5	21.7	
2017-05-09		0.28		0.35	19	0.36	5.61	12.47	1.2	20.6	
2017-05-16		0.42		0.3	20	0.31	5.62	12.6	0.57	20.9	
2017-05-23					13	0.14			1.4	14.5	
2017-06-06				0.42			5.97	15.57			
2017-06-12		0.25		0.51	8.8	0.05	5.37	16.06	0.05	8.9	
2017-06-12		0.25			8.8					9.1	
2017-06-13		0.2			9.7	0.05			0.05	9.8	
2017-06-14				0.51			6.02	16.44			
2017-06-14	150	0.22		3.02	9.6	0.05	6.17	20.44	1.3	11.0	
2017-06-20				0.06			6.59	18.3			
Count	4	16	8	21	17	16	21	21	16	17	
Average	173	4.3	1	0.5	9.4	0.1	6.0	10.1	5.4	14.7	
Median	170	1.5	1	0.3	8.8	0.0	6.0	7.1	2.2	12.3	
Std Dev	22	5.1	0	0.6	6.9	0.1	0.4	4.8	5.7	6.8	
Confidence Interval	22	2.5	0	0.3	3.3	0.1	0.2	2.0	2.8	3.2	
Upper CI	194	6.8	1	0.8	12.7	0.2	6.2	12.1	8.2	17.9	
Lower CI	151	1.8	1	0.2	6.2	0.0	5.9	8.0	2.6	11.4	

Pan D - Lysimeter at interface of the nitrification and denitrification layer

Sample Date	Alkalinity	Ammonia	BOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN	TSS
2017-01-10							5.36	5.77			
2017-01-11					0.05	0.025			0.75	0.83	
2017-01-23		0.34		8.35	0.05	0.025	6.14	9.31	0.98	1.06	
2017-02-01				9.55			6.31	5.44			
2017-02-07				9.55	1.1	0.025	6.31	5.44	29	30.1	
2017-05-05				5.96	40	0.93	5.79	13.63	1	41.9	

Appendix 4
Raw Data
DESIGN 3



DESIGN 3 Saturated System Using Long Island - Sourced Materials

Sample Date	Alkalinity	Ammonia	BOD5	CBOD5	DO	Fecal coli	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-09-19					0.09				6.58	24.2		
2016-09-27					0.05				6.68	23.0		
2016-10-05		1.60	92		0.11		2.20	0.025	6.43	20.0	33.2	35.4
2016-10-13		0.33	26		0.08		0.05	0.025	6.65	18.7	3.3	3.4
2016-10-20					0.08				6.57	17.8		
2016-10-25		0.41	28		0.13		0.05	0.025	6.51	16.9	2.3	2.4
2016-11-02		0.44	15		0.12		0.60	0.430	6.97	15.4	1.8	2.8
2016-11-08		0.48	12		0.19		2.80	0.025	6.29	14.4	1.6	4.4
2016-11-15		0.53	1		0.09		4.60	0.025	6.43	13.2	1.9	6.5
2016-11-21		0.77	5		0.08		5.40	0.025	6.35	12.8	2.0	7.4
2016-11-30		1.40	11		0.14		5.80	0.025	6.44	10.7	2.8	8.6
2016-12-05		1.10	1		0.12		7.00	0.025	6.51	10.7	3.1	10.1
2016-12-14					0.19				6.40	8.3		
2016-12-14		1.30	4		0.19		12.00	0.025	6.40	8.3	3.2	15.2
2016-12-21					0.30				6.11	6.8		
2016-12-21		1.00	4		0.30		10.00	0.400	6.11	6.8	2.5	12.9
2016-12-28		1.70	2		0.29		12.00	0.460	6.42	6.4	3.3	15.8
2017-01-04		0.03	1		7.70		4.30	0.025	5.59	11.2	0.3	4.6
2017-01-11		2.10	1		0.30		10.00	0.800	5.91	5.7	3.8	14.6
2017-01-17	140	2.00			0.92		35.00	0.025	6.10	5.9	3.8	38.8
2017-01-23		2.10	1		0.26		11.00	1.000	5.99	6.0	3.4	15.4
2017-01-25		1.50			0.28		13.00	1.000	6.29	6.2	4.0	18.0
2017-02-01					0.27				6.11	6.0		
2017-02-07		1.80	1		0.15		12.00	0.330	6.70	4.8	4.3	16.6
2017-02-15					0.02				6.86	4.7		
2017-02-22		1.80	11		0.23	9	11.00	0.240	6.05	4.4	3.8	15.0
2017-03-08		1.90	3		0.24	4	12.00	0.025	5.87	5.7	3.7	15.7
2017-03-22					0.25				6.00	4.8		
2017-04-05		0.83			0.23		6.60	0.380	6.01	5.9	2.2	9.2
2017-04-18					0.16				6.15	9.1		
2017-05-04		1.40		74	0.20		0.10	0.420	5.86	11.9	6.6	7.1
2017-05-09		0.43			0.28		0.05	0.025	6.07	12.3	28.0	28.1
2017-05-16		0.33		82	0.24		0.05	0.025	6.23	12.3	29.0	29.1
2017-05-23		0.05			0.22		0.20	0.025	6.36	13.9	2.7	2.9
2017-06-06		0.36	14	14	1.04		0.10	0.050	6.33	15.1	0.8	1.0
2017-06-12		0.26			1.12		0.05	0.050	5.87	16.1	0.6	0.7
2017-06-13		0.22					0.05	0.050			0.1	0.2
2017-06-14	220	0.40			0.40		0.05	0.050	6.50	16.3	0.9	1.0
2017-06-20	220	0.31		4.7	0.02		0.05	0.025	6.56	17.5	1.3	1.4
Count		30	19	4	38		30	30	38	38	30	30
Average		0.96	12	43.7	0.45		5.94	0.202	6.30	11.3	5.3	11.5
Median		0.80	4	44.0	0.21		4.45	0.025	6.34	11.0	3.0	8.9
Std Dev.		0.69	21	40.0	1.23		7.34	0.292	0.30	5.5	8.5	10.3
Conficence Interval		0.25	9	39.2	0.39		2.63	0.105	0.10	1.7	3.1	3.7
Upper CI		1.21	22	82.8	0.84		8.56	0.307	6.39	13.1	8.4	15.2
Lower CI		0.72	3	4.5	0.06		3.31	0.097	6.20	9.6	2.3	7.8

DESIGN 3

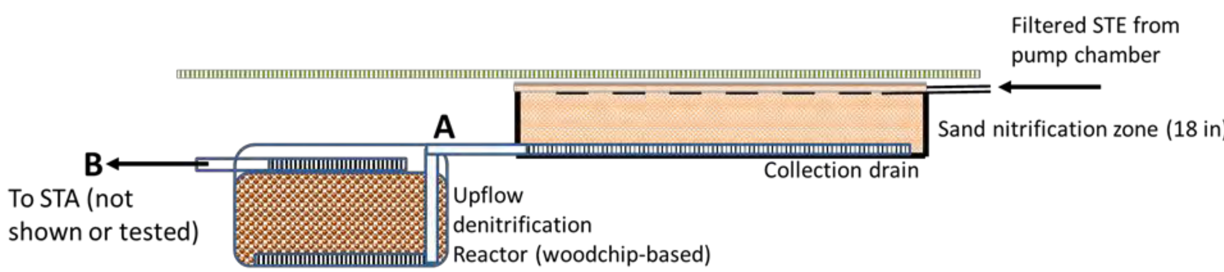
Lysimeter Sampling Ports

Port 1 18" deep
Port 2 6" deep

Sample Date	Sample Location	Ammonia	DO	Fecal coli	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-09-27	Port 1	0.7			18	5.6			3.6	27.2
2016-10-05	Port 1	0.52	0.96		0.05	0.5	6.24	20	2	2.55
2016-10-13	Port 1	1.5	0.24		18	9.9	6.12	17.8	4.8	32.7
2016-10-20	Port 1		0.22				6.01	17.47		
2016-10-25	Port 1	0.4	1.36		35	5.3	5.83	16.65	2.2	42.5
2016-11-02	Port 1	0.14	1.06		40	2	6.27	14.36	2	44
2016-11-08	Port 1	0.11	1.15		42	2.2	6.06	13.44	1.5	45.7
2016-11-15	Port 1	0.61	1.23		39	1.7	5.67	11.95	2.4	43.1
2016-11-21	Port 1	0.12	1.43		40	0.52	5.63	12.14	1.5	42.02
2016-11-30	Port 1	2.8	1.78		18	0.025	6.06	9.43	5.4	23.425
2017-01-11	Port 1	2.6	6.73		21	1.5	5.73	4.56	4.2	26.7
2017-01-23	Port 1	0.43	3.8		37	1	5.45	5.47	1.6	39.6
2017-01-25	Port 1	0.42	4.04		23	0.71	5.57	5.77	2.6	26.31
2017-02-01	Port 1		4.88				5.5	5.11		
2017-02-07	Port 1	0.32	5.48		38	0.025	5.96	4.17	2	40.025
2017-02-15	Port 1		5.41				6.49	3.91		
2017-02-22	Port 1	0.08	5.08		34	0.054	5.45	4.54	1.5	35.554
2017-03-08	Port 1	4.3	5.08		35	0.025	5.45	4.54	1.5	36.525
2017-05-04	Port 1	0.38	3.29		39	0.077	5.53	12.46	1	40.077
2017-05-23	Port 1		2.13				6.09	14.67		
Count		16	19		16	16	19	19	16	16
Average		0.96	2.91		29.8	1.95	5.8	10.4	2.49	34.2
Median		0.43	2.13		35.0	0.86	5.8	12.0	2	38.1
Std Dev		1.22	2.12		12.0	2.74	0.3	5.6	1.31	11.0
Confidence Interval		0.60	0.95		5.9	1.34	0.1	2.5	0.64	5.4
Upper CI		1.56	3.87		35.7	3.29	6.0	13.0	3.13	39.7
Lower CI		0.37	1.96		24.0	0.60	5.7	7.9	1.85	28.8

Sample Date	Sample Location	Ammonia	DO	Fecal coli	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-09-19	Port 2		3.85				6.92	24.17		
2016-09-27	Port 2	15			39	5.1			18	62.1
2016-10-05	Port 2	19	0.8		43	8.7	6.45	19.46	15	66.7
2016-10-13	Port 2	11	0.26		10	8.4	6.5	16.85	18	36.4
2016-10-20	Port 2		0.31				6.19	17.22		
2016-10-25	Port 2	5.5	1.14		9.1	24	5.94	16.27	8.5	41.6
2016-11-02	Port 2	8.7	1.81		8.3	18	6.56	13.42	11	37.3
2016-11-08	Port 2	6	1.21		9	20	5.96	12.42	6.6	35.6
2016-11-15	Port 2	4.5	1.9		11	21	5.78	10.86	7.5	39.5
2016-11-21	Port 2	4.2	0.8		16	18	6.45	11.31	5.8	39.8
2016-11-30	Port 2	13	4.15		8.3	4.9	6.21	8.45	15	28.2
2017-01-23	Port 2	12	2.77		16	2	5.89	5.69	15	33
2017-02-15	Port 2		4.15				6.68	3.45		
2017-02-22	Port 2	6.2	3.61	130	24	2.1	5.67	3.42	7.9	34
2017-03-08	Port 2	0.058	1.66	23	23	1.5	5.76	4.31	6.9	31.4
2017-05-04	Port 2	2.4	0.81		33	0.57	5.46	13.35	2	35.57
Count		13	15		13	13	15	15	13	13
Average		8.27	1.9		19.2	10.33	6.2	12.0	10.6	40.1
Median		6.20	1.7		16.0	8.40	6.2	12.4	8.5	36.4
Std Dev		5.43	1.4		12.3	8.59	0.4	6.2	5.1	11.4
Confidence Interval		2.95	0.7		6.7	4.67	0.2	3.1	2.8	6.2
Upper CI		11.23	2.7		25.9	15.00	6.4	15.2	13.3	46.3
Lower CI		5.32	1.2		12.5	5.66	5.9	8.9	7.8	33.9

Appendix 5
Raw Data
DESIGN 4



DESIGN 4 Nitrifying Bed Section

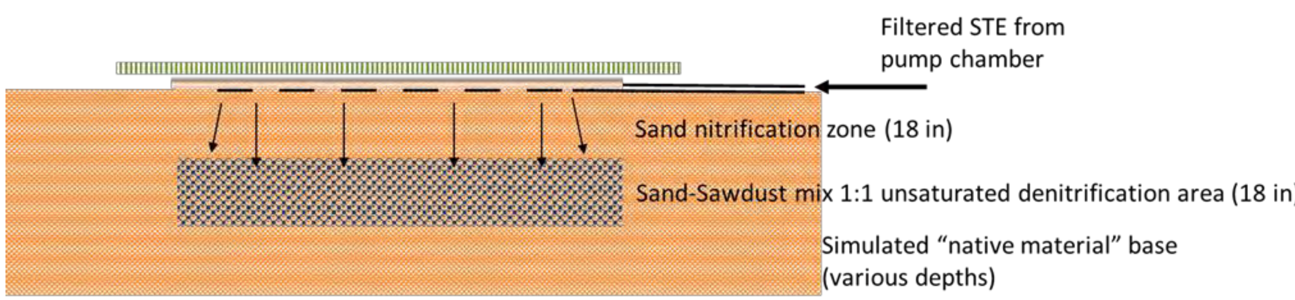
Sample Date	Alkalinity	Ammonia	CBOD	DO	Fecal coli	Nitrate	Nitrite	pH	Temp	TKN	TN	TP	TSS
2016-11-02		9.90	4	9.78	27	3.40	0.160	7.23	8.3	11.0	14.6		
2016-11-07		11.00				14.00	1.200			12.0	27.2		
2016-11-08	140	9.50	1	9.27	11	18.00	2.200	7.11	9.8	10.0	30.2		
2016-11-15	44	0.12	1	8.25	33	36.00	3.600	6.44	10.1	1.8	41.4		
2016-11-21		0.10	1	8.34	23	34.00	1.100	7.00	7.3	0.9	36.0		9
2016-11-30	42	0.16	1	9.16	33	36.00	0.600	6.42	10.7	1.4	38.0		
2016-12-05	53	0.25	1			34.00	0.480			0.8	35.3		
2016-12-14				11.98				6.37	7.3				
2016-12-20	41	0.57	1		33	30.00	0.340			1.3	31.6	2.9	24
2017-01-04	42	0.09	1	10.78	56	26.00	0.140	5.80	6.5	1.6	27.7	2.2	3
2017-01-10	40	0.14	1	10.78	7	29.00	0.025	6.31	5.6	1.2	30.2	2.4	4
2017-01-23		0.82	1	9.84		25.00	0.250	6.34	6.1	1.5	26.8	1.9	8
2017-01-24	48	0.89	5.5		5600	12.00	0.530			2.7	15.2	2.7	21
2017-01-25		0.28	2	0.61	1200	10.00	0.250	6.31	7.1	1.5	11.8	2.6	12
2017-01-27		0.08	1	8.66	20	16.00	0.025	6.22	6.4	1.5	17.5	2.2	9
2017-02-01		0.06	2.1	8.18	880	23.00	0.025	6.14	5.7	0.7	23.7	1.6	6
2017-02-07	40	0.32	1	10.03	11	28.00	0.025	6.62	4.7	1.0	29.0	1.8	5
2017-02-22		0.09	1	10.87	1	29.00	0.025	5.09	4.9	1.2	30.2	2.3	4
2017-03-07	30	0.03	1	10.88	5	27.00	0.025	5.70	5.4	0.3	27.3	2.3	1
2017-03-08				10.63	2000			5.77	5.3				
2017-03-15				12.54	3			5.93	4.2				
2017-03-21	16	0.67	1		15	29.00	0.025			1.8	30.8		4
2017-03-28	26	0.11	1	11.52	3	28.00	0.025	6.08	5.3	0.9	28.9	2.0	1
2017-04-04	40	0.10	1	10.61	140	13.00	0.290	5.88	5.7	0.8	14.1	2.2	12
2017-04-11	46	0.13	1	10.70	5	21.00	0.025	5.76	7.3	1.1	22.1	1.6	4
2017-04-19	40	0.32	1	11.20	1	29.00	0.025	5.93	9.9	1.0	30.0	2.1	4
2017-04-25	42	0.19	1	8.93	7	25.00	0.025	5.85	9.9	1.0	26.0	2.3	14
2017-05-09	67	0.15	1	8.66	20	15.00	0.025	5.95	12.0	1.9	16.9	1.9	9
2017-05-17	60	0.10	1	7.93	140	12.00	0.025	5.75	12.0	0.1	12.1	2.8	5
2017-05-25	53	0.05	1	9.74	12	20.00	0.025	6.32	14.0	1.7	21.7	2.5	2
2017-06-01	76	0.05	1		15	15.00	0.085			1.3	16.4	2.6	5
2017-06-07	76	0.10	1		360	17.00	0.025			0.9	17.9	2.8	18
2017-06-14	77	0.10	1	8.84	6	16.00	0.050	6.45	17.2	1.0	17.1	2.4	14
2017-06-21	64	0.05	1	7.11	27	5.00	0.140	6.64	18.5	0.6	5.7	2.6	19

Count	23	31	30	27	30	31	31	27	27	31	31	23	25
Average	52.3	1.178	1.32	9.475	28	21.79	0.38	6.2	8.4	2.14	24.3	2.3	9
Median	44	0.13	1	9.78		23	0.05	6.22	7.29	1.2	26.75	2.3	6
Std Dev	24.79	2.995	0.99	2.22		8.964	0.758	0.474	3.755	3.003	8.671	0.37	6.49
Confidence Interval	10.13	1.054	0.35	0.837		3.155	0.267	0.179	1.416	1.057	3.052	0.15	2.54
Upper CI	62.44	2.232	1.67	10.31		24.94	0.647	6.379	9.834	3.197	27.36	2.44	11.2
Lower CI	42.17	0.124	0.97	8.638		18.63	0.113	6.022	7.001	1.083	21.26	2.14	6.14

DESIGN 4 Final Discharge

Sample Date	Alkalinity	Ammonia	BOD	DO	Fecal coli	Nitrate	Nitrite	pH	Temp	TKN	TN	TSS
2016-10-25		0.77	590	2.23		0.05	4.500	5.52	15.59	4.5	9.05	
2016-11-02		2.10	640	7.24		0.05	0.025	5.36	8.53	6.2	6.275	
2016-11-08	100	5.40	510	6.04		0.05	1.400	5.39	11.05	9.8	11.25	
2016-11-15	110	0.20	250	5.74		0.05	0.025	6.08	12.58	3.4	3.475	
2016-11-21	120	0.11	210	3.76		0.05	0.025	6.14	14.62	1.8	1.875	
2016-11-30	120	0.30	1	4.81		0.05	0.025	6.48	12.71	2.5	2.575	
2016-12-05	140	0.25	73	5.68		0.05	0.025	6.57	10.07	3.1	3.175	
2016-12-14	130	0.09	94	8.75		0.05	0.025	6.54	6.11	1.8	1.875	
2016-12-21	130	0.25	28	6.50		0.05	0.025	7.08	11.36	5.3	5.375	
2016-12-28	140	0.11	1	6.65		0.20	0.025	6.77	10.82	1.8	2.025	
2017-01-04	130	0.07	66	5.35		0.05	0.025	6.25	4.61	1.4	1.475	
2017-01-11	120	0.18	44	6.49		2.60	0.510	6.74	5.37	2.3	5.41	
2017-01-17	110	0.15		7.27		0.05	0.025	6.75	9.88	1.5	1.575	
2017-01-23		0.07	25	7.28		9.40	0.025	6.94	5.34	1.6	11.025	
2017-01-25				6.56				6.64	4.86			
2017-01-27		0.17	23	7.52	110	0.05	0.025	6.72	6.17	1.8	1.875	
2017-02-01				7.50				6.40	4.32			
2017-02-07		0.18	52	8.02		0.05	0.025	7.06	4.28	1.3	1.375	
2017-02-15				8.46				7.09	3.62			
2017-02-22		0.10	27	8.00		0.05	0.025	6.68	6.09	1.0	1.045	
2017-03-08		0.12	8	7.10	1	0.05	0.025	6.60	9.36	1.4	1.475	
2017-03-21				7.81		0.16	0.097	6.18	3.75	1.4	1.657	
2017-03-30						1.80	0.025			1.4	3.225	
2017-04-05		0.18		7.84	66	2.00	0.280	6.58	12.87	0.1	2.33	
2017-04-18			19	5.65				6.78	13.12			10
2017-05-05		0.24	46	6.08		0.10	0.013	6.49	13.24	0.5	0.613	
2017-05-09		0.13	19	7.70		0.05	0.025	5.90	8.88	1.9	1.975	10
2017-05-16		0.20	24	0.55		0.05	0.025	6.93	17.79	1.9	1.975	
2017-05-23		0.05		4.06		0.05	0.025	6.95	18.78	1.0	1.025	
2017-06-06		0.16	19	6.16		0.10	0.170	5.29	10.07	0.1	0.37	
2017-06-14	140	0.05		4.06		0.05	0.050	7.32	23.57	1.6	1.7	
Count	12	25	22	30	3	27	27	30	30	27	27	2
Average	124.2	0.46	125.9	6.23	59.0	0.6	0.278	6.47	9.98	2.3	3.2	
Median	125.0	0.17	36.0	6.53	66.0	0.1	0.025	6.59	9.98	1.8	2.0	
Std Dev	13.114	1.11	196	1.858	54.836	1.875	0.888	0.54	4.96	2.1	3.0	
Confidence Interval	7.4	0.43	81.8	0.66	62.1	0.7	0.335	0.19	1.77	0.8	1.1	
Upper CI	132	0.90	208	6.89	121	1.3	0.613	6.67	11.75	3.1	4.3	
Lower CI	117	0.03	44	5.56	0	-0.1	-0.058	6.28	8.21	1.5	2.1	

Appendix 6
Raw Data
DESIGN 5



DESIGN 5 Pan Lysimeters beneath nitrifying area

Sample Date	Sample Locatio	Alkalinity	Ammonia	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-10-05	Port 1		0.28	0.13	13.00	0.025	6.37	21.37	1.0	14.0
2016-10-13	Port 1		0.63	0.33	0.05	0.440	6.28	19.40	6.0	6.5
2016-10-20	Port 1			0.72			6.26	18.82		
2016-10-25	Port 1		0.87	0.52	2.00	0.025	6.33	19.25	2.1	4.1
2016-11-02	Port 1		0.28	3.13	34.00	0.380	6.67	16.31	1.2	35.6
2016-11-08	Port 1		0.10	1.75	37.00	0.400	6.23	15.35	0.3	37.7
2016-11-15	Port 1		0.16	3.15	33.00	1.100	5.99	14.05	1.4	35.5
2016-11-21	Port 1		4.40	4.92	34.00	0.750	6.00	13.61	1.1	35.9
2016-11-30	Port 1		1.10	5.24	37.00	0.025	5.62	11.25	0.1	37.1
2017-01-11	Port 1		2.90	5.57	33.00	1.100	5.29	5.52	3.9	38.0
2017-01-17	Port 1		4.10	9.21	5.40	0.025	5.18	7.37	5.6	11.0
2017-01-23	Port 1		4.30	5.55	5.90	0.025	5.30	5.66	4.1	10.0
2017-02-15	Port 1			2.82			6.08	4.30		
2017-02-22	Port 1		4.30	3.17	27.00	0.025	4.92	4.20	5.7	32.7
2017-03-08	Port 1		4.20	4.42	29.00	0.025	5.35	5.69	7.7	36.7
2017-05-09	Port 1		0.07	2.70	30.00	0.025	5.29	13.21	1.4	31.4
2017-05-16	Port 1		0.07	2.11	13.00	0.025	5.75	13.58	0.6	13.6
2017-05-23	Port 1		0.05	0.80	12.00	0.160	6.22	15.36	1.0	13.2
2017-06-06	Port 1		0.49	1.17	13.00	0.050	5.89	16.34	1.0	14.0
2017-06-14	Port 1	29	0.33	2.50	16.00	0.050	5.49	17.70	1.1	17.2
2017-06-20	Port 1	32	0.25	0.76	11.00	0.025	5.61	19.38	1.2	12.2

Count			19	21	19	19	21	21	19	19
Average			1.52	2.89	20.28	0.246	5.82	13.22	2.4	23.0
Median			0.49	2.70	16.00	0.025	5.89	14.05	1.2	17.2
Std Dev			1.80	2.29	12.86	0.362	0.48	5.62	2.3	12.7
Confidence Interval			0.68	1.24	9.12	0.111	2.49	5.66	1.1	10.3
Upper CI			2.20	4.12	29.40	0.357	8.30	18.88	3.5	33.3
Lower CI			0.84	1.65	11.16	0.136	3.33	7.57	1.3	12.6
Sample Date	Sample Locatio	Alkalinity	Ammonia	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2017-05-04	Port 1a		0.69	4.10	28.00	0.092	5.19	14.32	1.0	29.1
2017-05-09	Port 1a		0.10	2.71	28.00	0.025	5.52	13.63	0.1	28.1
2017-05-16	Port 1a		0.09	0.61	18.00	0.080	6.07	13.67	1.8	19.9
2017-05-23	Port 1a		0.42	0.73	16.00	0.025	5.95	15.51	1.4	17.4
2017-06-06	Port 1a			0.44			6.22	16.08		
2017-06-14	Port 1a	60	1.40	0.56	12.00	0.050	5.77	17.78	1.9	14.0
2017-06-14	Port 1a	60	1.40	0.56	12.00	0.050	5.77	17.78	1.9	14.0
2017-06-20	Port 1a	58	1.10	0.81	12.00	0.025	5.83	19.48	1.8	13.8

Count			7	8	7	7	8	8	7	7
Average			0.74	1.32	18.00	0.050	5.79	16.03	1.4	19.5
Median			0.69	0.67	16.00	0.050	5.80	15.80	1.8	17.4
Std Dev			0.57	1.35	7.21	0.028	0.32	2.16	0.7	6.6
Confidence Interval			0.42	0.93	5.34	0.020	0.22	1.49	0.5	4.9
Upper CI			1.16	2.25	23.34	0.070	6.01	17.53	1.9	24.4
Lower CI			0.17	-0.03	10.79	0.022	5.47	13.87	0.7	12.8

DESIGN 5 Samples From Lysimeters 2 and 2A (beneath denitrification layer)

Sample Date	Sample Location	Alkalinity	Ammonia	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-10-05	Port 2		2.00	0.08	0.05	0.025	6.49	21.7	7.0	7.1
2016-10-13	Port 2		0.91	0.14	0.05	0.025	6.51	20.1	2.1	2.2
2016-10-20	Port 2			0.12			6.43	19.2		
2016-10-25	Port 2		1.20	0.01	0.05	0.025	6.49	19.1	7.3	7.4
2016-11-02	Port 2		0.66	0.27	0.05	0.760	6.69	17.1	3.3	4.1
2016-11-08	Port 2		0.99	0.21	0.05	0.025	6.41	16.1	2.1	2.2
2016-11-15	Port 2		0.47	0.28	0.05	0.025	6.31	14.8	2.8	2.9
2016-11-21	Port 2		0.47	0.26	1.90	0.025	6.25	13.9	1.7	3.6
2016-11-30	Port 2		0.78	0.2	0.05	0.025	6.24	12.1	3.1	3.2
2017-01-11	Port 2		0.50	5.04	3.70	0.240	5.99	5.9	2.6	6.5
2017-01-23	Port 2		1.20	5.12	4.90	0.025	6.15	6.1	2.9	7.8
2017-01-25	Port 2		1.10	4.99	3.80	0.025	6.17	6.1	3.1	6.9
2017-02-01	Port 2			4.31			6.03	6.2		
2017-02-07	Port 2		1.20	4.94	5.40	1.800	6.5	5.3	2.6	9.8
2017-02-15	Port 2			3.08			6.76	4.9		
2017-02-22	Port 2		0.96	3.32	9.20	0.025	5.65	4.5	2.1	11.3
2017-03-08	Port 2		1.20	4.72	4.30	0.025	5.82	6.2	3.2	7.5
2017-03-22	Port 2		0.88	0.75	8.20	0.025	5.73	6.2	2.3	10.5
2017-04-05	Port 2		0.79	0.75	13.00	0.350	5.73	6.2	2.5	15.9
2017-04-18	Port 2		1.40	0.51	9.30	0.610	5.83	9.0	2.8	12.7
2017-05-04	Port 2		0.57	0.76	0.56	0.011	5.76	12.2	2.7	3.3
2017-05-09	Port 2		0.26	0.44	5.00	0.640	5.75	12.9	2.5	8.1
2017-05-16	Port 2		0.27	0.35	0.32	0.025	6.03	13.2	2.3	2.6
2017-06-06	Port 2		0.56	0.22	0.10	0.083	6.03	16.1	0.2	0.3
2017-06-14	Port 2	200	0.69	0.28	0.05	0.050	6.34	16.9	1.6	1.7
2017-06-20	Port 2	190	0.58	0.09	0.05	0.025	6.31	18.5	1.3	1.4
Count			23	26	23	23	26	26	23	23
Average			0.85	1.59	3.05	0.213	6.17	11.9	2.8	6.0
Median			0.79	0.40	0.56	0.025	6.21	12.5	2.6	6.5
Std Dev			0.41	2.00	3.83	0.413	0.32	5.7	1.5	4.1
Confidence Interval			0.17	0.77	1.56	0.169	0.12	2.2	0.6	1.7
Upper CI			1.02	2.35	4.61	0.382	6.29	14.1	3.4	7.7
Lower CI			0.45	-0.41	-0.78	-0.200	5.85	6.3	1.2	2.0
Sample Date	Sample Location	Alkalinity	Ammonia	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2017-03-22	Port 2a		0.48	2.36	19.00	0.025	5.71	5.2	1.9	20.9
2017-04-05	Port 2a		0.25	1.33	23.00	0.420	5.62	6.2	1.2	24.6
2017-04-18	Port 2a		0.51	0.83	18.00	0.930	5.67	9.0	1.8	20.7
2017-05-04	Port 2a		0.03	0.46	8.60	0.180	5.59	12.1	1.0	9.8
2017-05-09	Port 2a		0.13	0.39	0.05	0.002	5.73	12.8	1.9	2.0
2017-05-16	Port 2a		0.44	0.36	0.05	0.025	6.08	13.2	1.6	1.7
2017-06-06	Port 2a		0.97	0.36	0.10	0.050	6.05	15.6	2.2	2.4
2017-06-14	Port 2a	220	1.00	0.25	0.05	0.050	6.30	16.5	1.5	1.6
2017-06-20	Port 2a	190	0.92	0.04	0.05	0.025	6.31	18.5	1.8	1.9
Count			9	9	9	9	9	9	9	9
Average			0.53	0.71	7.66	0.19	5.90	12.1	1.7	9.5
Median			0.48	0.39	0.10	0.05	5.73	12.8	1.8	2.4
Std Dev			0.37	0.72	9.75	0.31	0.29	4.6	0.4	9.8
Confidence Interval			0.24	0.47	6.37	0.20	0.19	3.0	0.2	6.4
Upper CI			0.76	1.18	14.03	0.39	6.09	15.1	1.9	15.9
Lower CI			0.16	-0.01	-2.09	-0.12	5.61	7.6	1.3	-0.3

DESIGN 5 Sump (Final Discharge)

Sample Date	Alkalinity	Ammonia	CBOD5	DO	Nitrate	Nitrite	pH	Temp	TKN	TN
2016-10-05		0.03	1	0.10	0.05	0.025	5.73	19.5	0.1	0.2
2016-10-13		0.03	1	0.38	4.00	0.025	5.46	19.2	1.3	5.3
2016-10-20				0.43			5.38	18.7		
2016-10-25		0.03	1	0.47	2.70	0.025	5.53	18.4	0.7	3.4
2016-11-02		0.03	1	0.67	2.60	0.025	6.69	18.1	1.0	3.6
2016-11-08		0.03	1	1.42	6.40	0.025	6.52	17.5	0.9	7.3
2016-11-15		0.07	1	2.23	8.20	0.220	5.99	17.6	1.2	9.6
2016-11-21		0.42	1	2.24	8.60	0.460	6.04	16.8	1.0	10.1
2016-11-30		0.10	1	2.48	9.60	0.025	6.16	15.6	0.1	9.7
2016-12-05		0.25	1	2.54	9.60	0.025	6.13	15.2	0.8	10.4
2016-12-14		0.17	1	3.52	16.00	0.025	6.14	13.6	1.1	17.1
2016-12-21				3.00			6.40	13.2		
2016-12-28		0.03	1	2.46	12.00	0.440	6.25	11.7	0.8	13.2
2017-01-04		0.03	1	4.07	13.00	0.025	6.08	11.5	0.6	13.6
2017-01-11				4.78			6.23	11.3		
2017-01-17	130	0.03		8.30	9.60	0.330	6.27	9.4	0.9	10.8
2017-01-23		0.03		2.86	10.00	0.025	6.25	9.6	0.3	10.3
2017-01-25		0.07		5.29	11.00	0.025	6.25	8.9	0.5	11.6
2017-02-01				4.16			6.08	8.8		
2017-02-07		0.24	1	2.88	12.00	0.025	6.42	8.9	0.9	12.9
2017-02-15				5.38			6.50	9.2		
2017-02-22		0.10	1	5.88	7.00	0.025	6.04	8.6	0.5	7.5
2017-03-08		0.15		4.72	12.00	0.025	5.91	6.2	0.7	12.8
2017-03-22		0.20		3.90	13.00	0.025	5.88	7.8	0.6	13.6
2017-04-05		0.08		5.60	8.30	0.025	5.59	7.2	0.4	8.8
2017-04-18		0.03		5.24	10.00	0.025	5.67	8.2	0.6	10.6
2017-05-04		0.14	1	4.43	15.00	0.026	5.21	9.5	0.5	15.5
2017-05-09		0.07		4.33	10.00	0.025	4.99	10.0	0.8	10.8
2017-05-16		0.07		0.61	9.40	0.063	5.99	13.7	0.1	9.5
2017-05-23		0.05		2.93	10.00	0.025	5.79	11.3	0.1	10.2
2017-06-06		2.00	2	1.62	4.40	0.100	5.86	12.4	0.1	4.6
2017-06-14	120	0.16		2.13	4.00	0.050	6.02	13.0	0.7	4.7
2017-06-20	120	0.05	1	0.88	3.70	0.025	6.09	13.4	0.3	4.0

Count		28	17	33	28	28	33	33	28	28
Average		0.17	1.06	3.09	8.65	0.08	5.99	12.53	0.62	9.34
Median		0.07	1.00	2.88	9.60	0.03	6.04	11.66	0.63	10.11
Std Dev		0.37	0.24	1.96	3.95	0.12	0.38	3.96	0.35	4.02
Confidence Interval		0.14	0.12	0.67	1.46	0.05	0.13	1.35	0.13	1.49
Upper CI		0.30	1.17	3.76	10.11	0.12	6.12	13.88	0.75	10.83
Lower CI		0.03	0.94	2.42	7.18	0.03	5.86	11.18	0.49	7.85

Microbial Communities in Partially and Fully Treated Effluent of Three Nitrogen-Removing Biofilters

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Abstract: In the United States, 24% of single-family homes have on-site wastewater treatment systems (OWTS). Not only is the proportion much higher in some areas, but also most of the OWTS provide no nitrogen removal. An innovative alternative to such OWTS are nitrogen-removing biofilters (NRBs), passive two-layer systems designed to select nitrifying (top layer) and denitrifying (bottom layer) microbial assemblages from incoming microorganisms to remove nitrogen from household wastewater by sequential nitrification-denitrification. Little is known about the microbial ecology of NRBs, or even about best practices for investigating NRB microbiology. This study characterized microbial communities of wastewater passing through three NRBs that differed in construction and nitrogen-removal efficiency by sampling nondestructively at four times over 1 year. Microbial assemblages collected from pan lysimeters buried within NRBs and from final effluent were distinct from the influent community, indicating environmental conditions in NRBs were selecting specific microbial communities. Principal coordinate analysis (weighted UniFrac) showed extensive overlap of microbial communities from different systems, layers, and times, as well as significant relationships between microbial community structure and NRB function (nitrogen transformation and removal). Genus-level analysis revealed differences between systems in dominant nitrifiers and that denitrification is likely driven by different bacteria than typically dominate in wastewater treatment plants. Replicated experiments and alternative sampling approaches will be necessary to elucidate whether differences in microbial communities between systems reflected environmental selection due to differences in NRB design, and how much stochastic processes affect NRB microbial community structure. **DOI: 10.1061/JSWBAY.0000912.** *This work is made available under the terms of the Creative Commons Attribution 4.0 International license, http://creativecommons.org/licenses/by/4.0/.*

Introduction

In the United States, household wastewater is typically treated in wastewater treatment plants (WWTPs) serving large sewersheds (Census Bureau 2011). In areas where large-scale WWTPs are not available, on-site wastewater treatment systems (OWTS) provide decentralized wastewater treatment. In the northeastern United States, nearly 40% of homes have OWTS, well above the national average of 24% (Census Bureau 2011). OWTS typically have two major components: septic tank and soil treatment unit (STU) (Lusk et al. 2017). An important role of the STU is harnessing soil microbial processes for the removal of potentially polluting nutrients like nitrogen (USEPA 2011; Lusk et al. 2017). In Suffolk County on Long Island, New York, approximately 70% of single-family households have OWTS consisting of a septic tank with no STU (Suffolk County 2015), and therefore, no nitrogen removal is carried out, endangering drinking water quality and contributing to ecosystem degradation by harmful algal blooms (Anderson et al. 2008; Bleifuss et al. 1998; Gobler et al. 2012; Kinney and Valiela 2011; LaRoche et al. 1997; Wakida and Lerner 2005).

One potential solution for areas with widespread OWTS and excess nitrogen entering the environment is the addition of nitrogen-removing biofilters (NRBs), partially engineered STUs designed to remove nitrogen from household septic tank effluent (STE) (Fig. 1). In a NRB, STE is evenly dispersed onto a sand layer, intended to provide oxic conditions promoting nitrification (conversion of ammonium to nitrate), and flows by gravity into a saturated or unsaturated sand-lignocellulose layer, intended to provide anoxic conditions and a long-lasting organic carbon source promoting denitrification (conversion of nitrate to nitrogen gas). Sequential nitrification-denitrification in engineered oxic-anoxic conditions is a crucial design aspect to many modern WWTPs (Henze 2000; Henze et al. 1987; Noredal Throbäck 2006).

The basic premise of the NRB design is that the sand layer will select for a nitrifying microbial community, and the sand-lignocellulose layer will select a microbial community that can denitrify using volatile fatty acids (VFAs) provided by decomposition of the lignocellulose (woodchips or sawdust) by cellulolytic bacteria (Leschine 1995). In addition to environmental selection, the process by which specific microbes or microbial functional groups are selected from a larger microbial community by environmental conditions (Baas-Becking 1934), random or stochastic processes may also structure wastewater treatment microbial communities (Curtis and Sloan 2006; Isazadeh et al. 2016; Langenheder and Székely 2011; Ofiţeru et al. 2010; Woodcock et al. 2007; Zhou et al. 2014, 2013). Determining the relative importance of these processes to NRB microbial community structure is important because the composition of these assemblages determines which

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Note. This manuscript was submitted on February 19, 2019; approved on October 9, 2019; published online on March 4, 2020. Discussion period open until August 4, 2020; separate discussions must be submitted for individual papers. This paper is part of the ***Journal of Sustainable Water in the Built Environment***, ©ASCE, ISSN 2379-6111.

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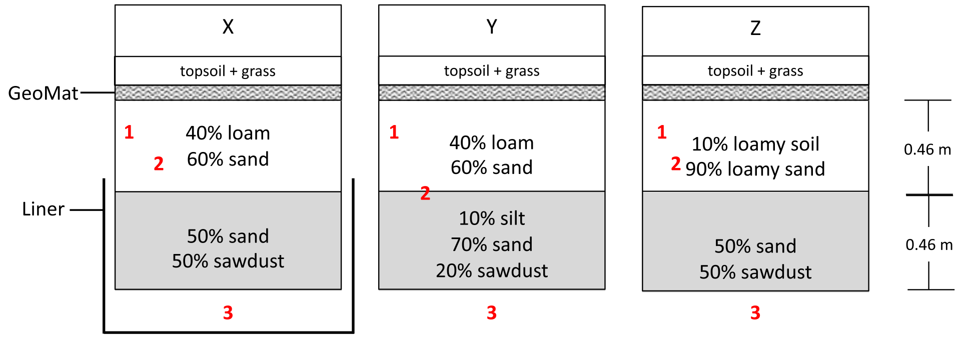

Fig. 1. Materials and configuration of each nitrogen removing biofilter at the Massachusetts Alternative Septic System Testing Center. Samples were collected in January, April, July, and November 2016.

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metabolic pathways may occur in the system and finally the quality of treated wastewater (Cydzik-Kwiatkowska and Zielińska 2016). Additionally, there are many possible variations in the details of design and construction of NRBs, and how such variations may alter microbial selection processes, how microbial community structure may vary stochastically across systems constructed in the same way, and how differences in microbial communities will relate to the desired system function of nitrogen removal are open questions that need to be answered before large-scale deployment of NRBs.

The microbial communities entering and leaving a NRB can be sampled nondestructively by collecting particulate matter from STE and NRB effluent, respectively, on filters. Sampling microbial communities at intermediate depths within NRBs to characterize the nitrifying and denitrifying layers separately is more challenging because removing the matrix material (perhaps by coring the sand and sand-lignocellulose mix) is likely to disrupt system function by exposure to the atmosphere or causing preferential flow through the cored region. To allow repeated intermediate sampling of NRBs without damage to the NRB itself, pan lysimeters were installed in several full-scale test systems both within the nitrifying layer and at the interface between the nitrifying and denitrifying layers. The pan lysimeters are plastic pans that collect liquid flowing through the system to a specific depth, and they can be sampled nondestructively by pumping the collected liquid up via a sampling port (Jemison and Fox 1992).

Using influent, effluent, and pan lysimeter samples, the system function in three NRBs of different design are herein described. It is then explored whether pan lysimeter samples reveal the presence of distinct microbial communities at different depths, how the sampled microbial communities differ among the three systems and vary over time, and whether the structure of microbial communities collected from each NRB is related to the nitrogen-removal function of that NRB. Based on these analyses, it is evaluated for the first time whether nondestructive pan lysimeter sampling offers an effective way to monitor the function of NRBs after widescale deployment.

Materials and Methods

NRB Specifications, Sample Collection, and DNA Extraction

Three NRBs (Fig. 1) were sampled for this study at the Massachusetts Alternative Septic System Testing Center (MASSTC), a unique facility to test wastewater treatment technologies in a setting geologically similar to Long Island without inconveniencing homeowners or endangering the local groundwater. As shown in Fig. 1, STE was evenly distributed to the sand layer via a GeoMat just below the soil surface. Each system was sampled from two pan lysimeters within the system, denoted in Fig. 1 by numbers 1 (first lysimeter, 15 cm below the top of the sand layer in each system) and 2 (intermediate lysimeter, 30 cm below the top of the sand layer in Systems X and Z and at the interface between the two layers in System Y). Final effluent samples were collected from a sump at the bottom of the system, denoted by number 3.

MASSTC also provided a single source of wastewater influent to multiple NRBs, allowing differences observed to be attributed to NRB design, rather than variability in the wastewater source. Systems X and Z were 4.6×7.6 m in area, and System Y was 4.6×9.8 m. Systems X and Y had a loamy sand (40/60 loam/sand) nitrification layer, and System Z had a 10/90 loamy soil/sand nitrification layer. Systems X and Z had a 50/50 sand/sawdust denitrification layer, and System Y had a 10/70/20 silt/sand/sawdust denitrification layer. All sand was ASTM C33 (ASTM 2018) sand with <2% fines, all mixtures were by volume, and each layer was 0.46 m in depth. The sand-lignocellulose layer of System X was enclosed in an impermeable liner to make it permanently saturated, whereas Systems Y and Z were unlined. Systems X and Y were installed December 2014, and System Z was installed December 2015. STE was delivered to each system through a GeoMat FLAT system (Old Saybrook, Connecticut), which ensured even distribution over the entire NRB top surface at a rate of 24.43 $1\ m^{-2}\ day^{-1}$. The GeoMat FLAT system (GeoMatrix LLC 2018) had a core of fused, entangled plastic filaments with a geotextile fabric bonded to one side. A pressure distribution line was installed on top of the core and covered with another layer of geotextile fabric, which was in turn covered with approximately 20 cm of topsoil. Raw influent was diverted from the nearby Joint Base Cape Cod Wastewater Treatment Plant, treating wastewater from the Otis Air National Guard Base on the Massachusetts Military Reservation, via an influent channel before solids settled in individual septic tanks providing STE to each system.

Two different sets of samples were collected. First, regular weekly to biweekly sampling of influent, NRB pan lysimeters, and effluent was performed to closely monitor nitrogen removal by each system. Second, NRB pan lysimeter and effluent samples for more detailed chemical analyses and microbial community characterization were collected by the New York State Center for Clean Water Technology (CCWT) in January, April, July, and November 2016 to capture seasonal variation, as weather permitted. Pan lysimeters were purged 24 h before collection, so all pan lysimeter samples represent 24-h composites.

When possible, influent wastewater samples were collected from the raw wastewater channel before it entered individual septic tanks, and STE was collected from the outflow of individual septic tanks to examine any effects of processes occurring in the septic tank (such as settling and oxygen and carbon consumption) on the incoming microbial communities. Liquid samples were transported on ice and filtered in lab within 12 h or immediately filtered in the field by passing 20–30 mL of each liquid sample through a 0.2-μm-pore 47-mm-diameter polycarbonate filter (Osmonics, Macungie, Pennsylvania). The filters were placed in 2 mL cryovials, then frozen and transported in liquid nitrogen if necessary and stored at −80°C until DNA was extracted using a MO BIO Powersoil kit (Qiagen, Hilden, Germany). Extracted DNA was quantified by PicoGreen (Invitrogen, Carlsbad, California) then stored at −80°C xuntil being sent out for sequencing.

Wastewater Chemistry

Total Kjeldahl nitrogen (TkN) and Nitrite-N plus Nitrate-N (NOx) in the raw influent channel, pan lysimeters, and NRB effluent samples were measured approximately weekly throughout 2016 by the Barnstable County Department of Health and Environment following EPA Methods 351.2 [(TkN) USEPA 1993a] and 300.0 [(NOx) USEPA 1993c]. Total nitrogen (TN) was calculated by adding TkN and NOx. In most effluent samples, ammonium [(EPA 350.1) USEPA 1993b] was also measured (details are given in the Supplemental Data). Influent and effluent samples were not always collected on the same days, so weekly and monthly TN removal were calculated using weekly averaged influent and effluent TN concentrations (starting on Sunday of each calendar week in 2016).

Several additional parameters were measured in each of the influent, STE, pan lysimeter, and effluent samples used for microbial community characterization. Total suspended solids (TSS) [(EPA 160.2) USEPA NERL/MCEARD 1971], alkalinity [(EPA 310.1) USEPA NERL/MCEARD 1978], biological oxygen demand (BOD) [(EPA 405.1) USEPA NERL/MCEARD 1974], and dissolved organic carbon (DOC) [following the manufacturer's instructions for the Shimadzu ASI-L Autosampler (Kyoto, Japan)] were measured by members of CCWT. Phosphate, sulfate, and iron were also measured [phosphate and sulfate analysis described by Lee (2017) and Wehrmann et al. "Biogeochemical sequestration of phosphorus in a two-layer lignocellulose-based soil treatment system," working paper, Stony Brook University, Stony Brook, New York; iron analysis described by Price et al. "Behavior of iron and other metals in a lignocellulose-based biofilter for onsite wastewater treatment," working paper, Stony Brook University, Stony Brook, New York; details are given in the Supplemental Data). Seasonal sampling time point was used as metadata: January (influent samples collected in February, no significant difference between sampling times, ANOVA $p = 0.11$) denoted by 1, April by 2, July by 3, and November by 4. Layer was a stand-in variable used as metadata for the path of wastewater through the system: untreated influent denoted by 1, STE by 2, first lysimeter by 3, second lysimeter by 4, and final effluent by 5.

Sequencing and Sequence Analysis Methods

Microbial community structure was determined by sequencing 16S small subunit ribosomal RNA (rRNA) amplicons, performed by MR DNA Lab (Shallowater, Texas) following their standard protocol. Primers A519F (3′-CAGCMGCCGCGGTAA-5′) and 802R (3′-TACNVGGGTATCTAATCC-5′) were used to amplify the 16S V4 variable region (Caporaso et al. 2011; Tremblay et al. 2015). Samples were amplified with a barcoded forward primer and HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. Polymerase chain reaction (PCR) products from multiple samples were pooled together in equal proportions and purified using calibrated Ampure XP beads (Beckman Coulter, Indianapolis). Then the pooled and purified PCR product was used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol and sequenced as a paired-end set of reads.

Sequence processing was carried out in QIIME2 (version 2017.12) (Caporaso et al. 2010) run through a 64-bit Virtual Box (VB) (version 5.2.4, Oracle, Redwood Shores, California). After the raw fastq files from MR DNA were demultiplexed in QIIME2, the R package dada2 (Callahan et al. 2016) dereplicated and merged all sequences into an abundance table of 99% identity amplicon sequence variants (ASV). Sequences per sample ranged from 23,843 to 81,759 (mean 45,674 and standard deviation 13,181). All samples were rarefied to 23,843 sequences per sample (discussed in the "Results" section).

The ASV abundance table and representative set of sequences were analyzed through various QIIME2 commands, automated with C + + in the text editor Atom version 1.27.0 (GitHub, San Francisco), using the compiler package atom-gpp. All automated commands can be found online (Langlois 2018). Briefly, the ASV table was rarefied, the representative set was aligned and used to produce a phylogenetic tree, and principal coordinate analysis (PCoA) was performed using the weighted UniFrac distance metric (Lozupone and Knight 2005). Rarefaction and PCoA plots were generated with the R package vegan. The vegan package function envfit was also used to fit environmental metadata to the PCoA ordination. A multiresponse permutation procedure (MRPP) was performed in PC-Ord version 5.10 (McCune and Mefford 2006) to determine nonparametric pairwise differences.

ASV were assigned taxonomic identifications from the Silva database (132 release). Taxonomic identifications were aggregated at the genus level using R in RStudio (version 1.0.143) to create genus abundance tables. To relate microbial community composition to potential microbial function, the genera were placed into potential functional groups based on physiological studies on the type species for each genus. At time of writing, the most recent curated database can be found online (Langlois 2019). Data visualizations were generated using the R package ggplot2 (Wickham 2016).

Results

NRB System Function

During 2016, regular weekly to biweekly monitoring of three full-scale test NRBs (Fig. 1) (details have been given in the "Materials and Methods" section) showed that TN in the influent ranged from 28.82 to 62.07 $\mathrm{mg\,N\,L^{-1}}$, averaging 45.79 $\mathrm{mg\,N\,L^{-1}}$ (standard deviation 6.61 $\mathrm{mg\,N\,L^{-1}}$). On average, System X effluent was 12.64 $\mathrm{mg\,N\,L^{-1}}$ (standard deviation 5.50 $\mathrm{mg\,N\,L^{-1}}$), consisting mostly of ammonium in spring and summer (Fig. 2). System Y effluent was 8.59 $\mathrm{mg\,N\,L^{-1}}$ (standard deviation 2.40 $\mathrm{mg\,N\,L^{-1}}$), consisting almost entirely of NOx for the entire year (Fig. 2). System Z effluent was 8.14 $\mathrm{mg\,N\,L^{-1}}$ (standard deviation 6.00 $\mathrm{mg\,N\,L^{-1}}$), switching from primarily NOx in the first half of the year to primarily ammonium in the second half of the year (Fig. 2).

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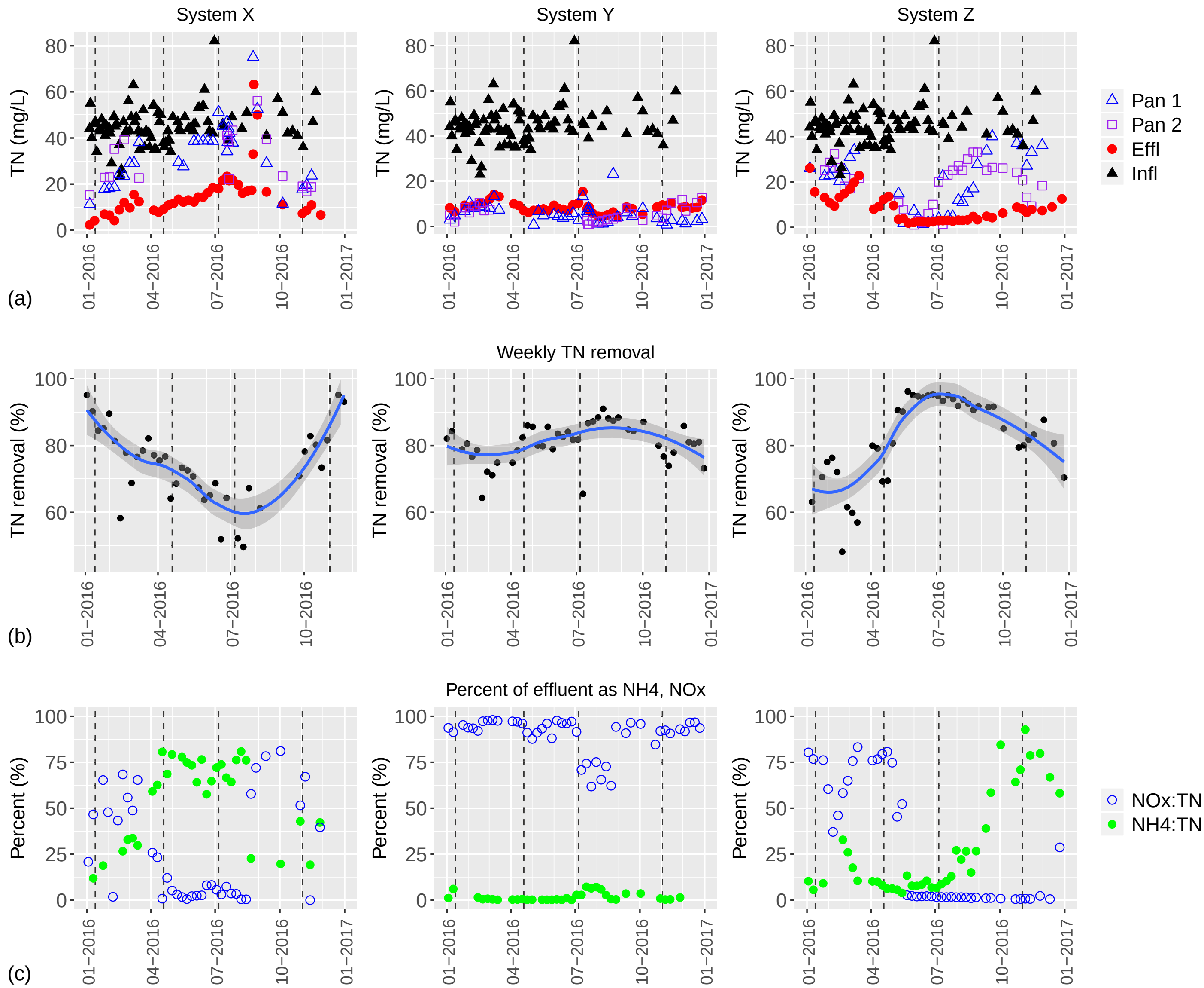

Fig. 2. (a) Total nitrogen (mg L^{-1}) in the raw wastewater influent (solid triangles), the first pan lysimeter (open triangles), second pan lysimeter (open squares), and final treated effluent of each system (solid circles); (b) weekly TN removal for each system and its associated Loess smoothing curve; and (c) effluent ammonium (solid circles) and nitrite+nitrate (NOx) (open circles) as proportion of effluent TN.

TN removal by Systems X and Z fluctuated seasonally, but in opposite patterns, from lows near 60% during summer for System X and winter for System Z to highs over 90% during winter for System X and summer for System Z (Fig. 2). During System X's lowest TN removal rates, the effluent was >50% ammonium, whereas during System Z's lowest TN removal rates, the effluent was primarily NOx (up to 80%) (Fig. 2). TN removal by System Y was more stable at approximately 80% year-round (Fig. 2). Nearly total TN removal was often observed in the first and intermediate lysimeters in System Y, then some TN was gained in the effluent for most of the year ($p < 0.001$ and $p = 0.001$ for effluent versus first and intermediate lysimeters respectively, Student's t-test). Shorter periods when most or all of the TN removal was observed in the lysimeter samples also occurred in Systems X and Z (Fig. 2).

Considering just the seasonal samples used for microbial community analysis, the stand-in variable for the flow of wastewater through the NRB, layer (described in the "Materials and Methods" section), was negatively correlated with BOD and concentrations of phosphate, ammonium, and TN, indicating the expected decline in these parameters as wastewater moved through the NRBs (Table 1). More DNA was extracted from influent than from lysimeter and effluent samples, reflected in strong positive correlations among DNA, BOD, phosphate, and ammonium. NOx was positively correlated with sulfate (Table 1) and negatively correlated with alkalinity, consistent with the usage of alkalinity by nitrification.

Microbial Community Structure

The number of 16S V4 amplicon sequence variants (ASV, 99% identity) detected in each sample initially ranged from 191 to 1,476, with both extremes found in shallow lysimeter samples (Fig. S1a). Although there was not a strong relationship between sequencing depth and ASV richness ($R^2 = 0.08$), each sample was rarefied to the minimum sequencing depth, 23,843 sequences per sample, which had very little effect on ASV richness (Fig. S1b). Although the range of ASV richness in each system was similar, System X had a greater proportion of high-richness samples than Systems Y and Z. Systems X and Z showed a seasonal progression

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Table 1. Environmental metadata correlation matrix

Metadata	System	Layer	Sampling event	DNA (μg/L)	Ammonium (mg/L)	Nitrite+nitrate (NOx) (mg/L)	Nitrite (mg/L)	Total nitrogen (TN) (mg/L)	Weekly TN removal	Monthly TN removal	Phosphate (mg/L)	Sulfate (mg/L)	Dissolved organic carbon (mg/L)	Alkalinity (mg/L)	BOD (mg/L)	TSS (mg/L)	Iron (mg/L)
System	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Layer	0.406	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sampling event	−0.007	−0.162	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DNA (μg/L)	−0.029	−0.507	0.184	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Ammonium (mg/L)	−0.09	−0.535	0.293	0.678	1	—	—	—	—	—	—	—	—	—	—	—	—
Nitrite+nitrate (NOx) (mg/L)	0.007	0.071	−0.099	−0.37	−0.37	1	—	—	—	—	—	—	—	—	—	—	—
Nitrite (mg/L)	0.076	−0.003	−0.15	−0.094	−0.112	0.228	1	—	—	—	—	—	—	—	—	—	—
Total nitrogen (TN) (mg/L)	−0.321	−0.596	0.101	0.271	0.588	0.342	0.074	1	—	—	—	—	—	—	—	—	—
Weekly TN removal	0.043	0.099	0.42	−0.22	−0.039	0.155	0.076	−0.035	1	—	—	—	—	—	—	—	—
Monthly TN removal	0.05	0.063	−0.45	0.056	−0.039	0.082	0.305	0.108	−0.595	1	—	—	—	—	—	—	—
Phosphate (mg/L)	−0.217	−0.652	0.239	0.701	0.786	−0.207	−0.046	0.558	−0.123	−0.064	1	—	—	—	—	—	—
Sulfate (mg/L)	−0.056	−0.075	−0.24	−0.117	−0.325	0.663	0.123	0.044	0.051	0.049	−0.03	1	—	—	—	—	—
Dissolved organic carbon (mg/L)	−0.12	0.127	−0.599	−0.038	−0.101	0.098	0.273	0.084	−0.218	0.437	−0.103	0.015	1	—	—	—	—
Alkalinity (mg/L)	−0.068	0.253	−0.057	0.009	0.178	−0.586	−0.117	−0.105	−0.078	0.071	−0.105	−0.664	0.119	1	—	—	—
BOD (mg/L)	−0.263	−0.617	0.271	0.702	0.739	−0.226	−0.071	0.478	−0.039	−0.121	0.824	−0.129	0.027	−0.047	1	—	—
TSS (mg/L)	0.023	−0.108	0.235	0.178	0.013	−0.393	−0.102	−0.074	0.241	−0.222	0.018	−0.472	−0.071	0.242	0.026	1	—
Iron (mg/L)	0.316	0.404	0.117	−0.101	−0.16	−0.214	−0.04	−0.295	0.064	−0.052	−0.232	−0.361	−0.095	0.25	−0.192	0.261	1

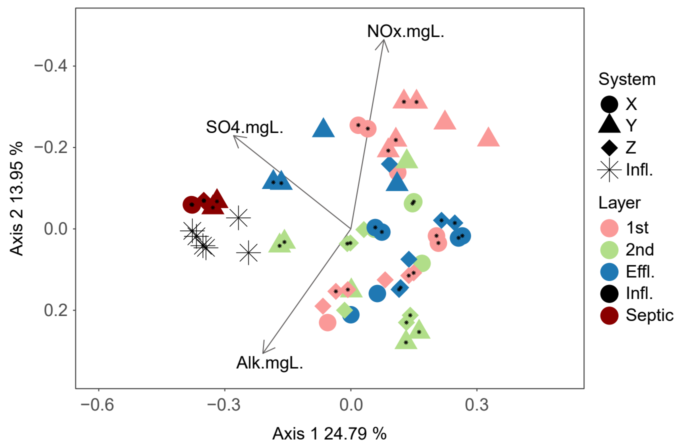

Fig. 3. Principal coordinate analysis ordination of all samples, including raw influent and septic tank effluent, using the weighted UniFrac distance metric. Vectors represent metadata correlated with Axis 1 and Axis 2 with a p-value <0.01.

from less richness in January and April to more richness in July and November; there was a clear break between the two seasons. System Y showed no seasonal progression in ASV richness. The STE samples had much lower ASV richness than many lysimeter samples, and in System Z, there was generally greater ASV richness in the deeper lysimeter and effluent than in the shallow lysimeter samples. All sequences can be accessed at NCBI Sequence Read Archive (SRA), Accession #PRJNA553796.

The weighted UniFrac PCoA explained 39% of the variance in microbial community structure on the first two axes, Axis 1 and Axis 2 (Fig. 3). Samples from July and November were sequenced in duplicate, and in general the replicate sequencing results plotted very close together and sometimes appear as one point (replicate samples indicated by small asterisks in the symbols in Fig. 3). The distance between points represents the dissimilarity of samples to one another; closer samples are less dissimilar to each other than samples far apart. Replicate samples that appear as one point suggests that the sequencing method contributed little to the variation observed. Raw influent and STE samples grouped to the left of samples collected within the NRB along Axis 1 (Fig. 3), and MRPP confirmed a significant difference between all influent (raw plus STE) and all other samples ($p < 0.001$). This ordination was significantly associated with system function parameters alkalinity, sulfate, and NOx (Table 1, Fig. 3, and Supplemental Data), although the NRB samples did not form clear groups by system, layer, or season.

To focus on differences among NRB samples, the PCoA was repeated excluding influent samples (Fig. 4). However, the first two axes accounted for only 34.5% of the variance, and despite significant correlations with alkalinity, NOx, and sulfate, the sample types were interspersed rather than clustered by layer or system (colored polygons) or sampling time (Fig. 4). Samples from the first lysimeter spread more along Axis 1, whereas samples from the effluent spread more along Axis 2 (left-hand side of Fig. 4). A few samples from System Y separated along Axis 1 and a few samples from Systems X and Z separated along Axis 2 (right-hand side of Fig. 4). The first six axes of the ordination accounted for 67% of the variance, but no pair of axes clearly separated samples by layer, system, or sampling time (Figs. S2 and S3). NOx and TSS were significantly associated with Axes 2 and 3 ($p < 0.05$), sulfate was significantly associated with Axis 3($p < 0.05$), and weekly TN removal of the system was significantly associated with both Axis 5 ($p < 0.05$) (Supplemental Data).

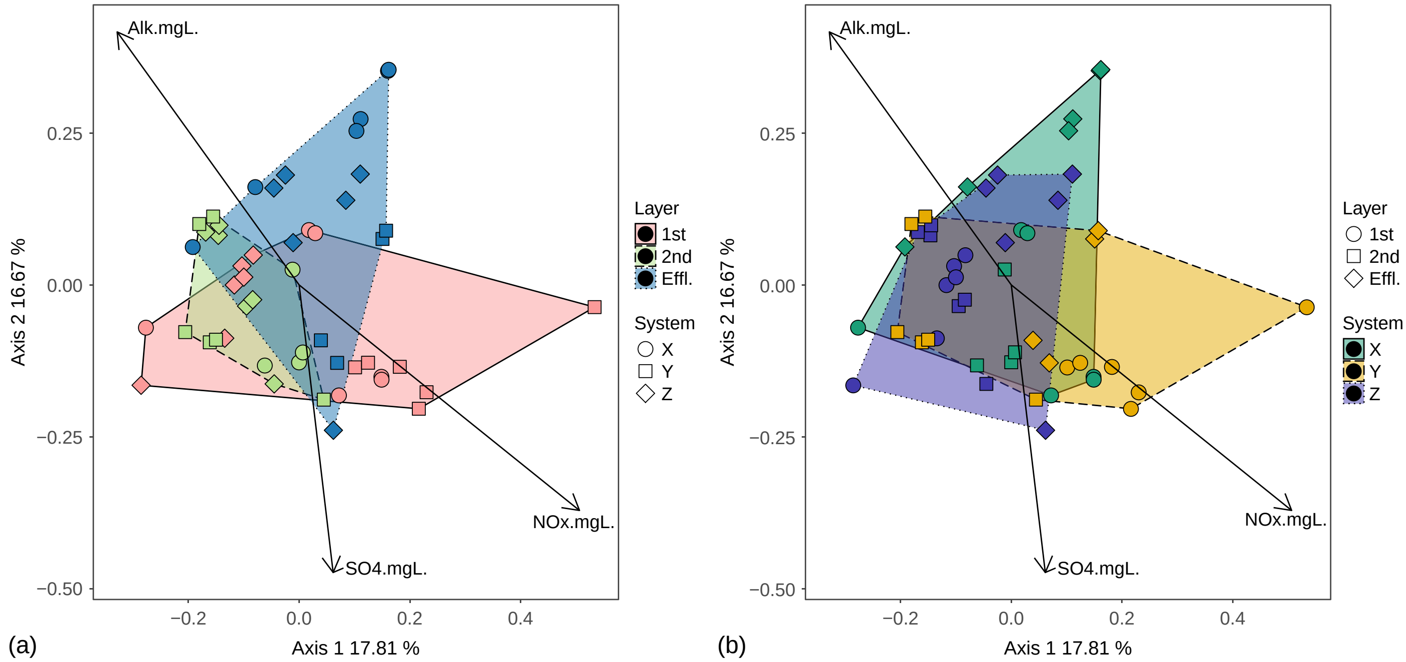

Fig. 4. Principal coordinate analysis ordination of all NRB lysimeter and effluent samples (Systems X, Y, and Z) using the weighted Unifrac distance metric: (a) polygons represent groups of the first lysimeter samples (solid line), the second lysimeter samples (dashed line), and the final effluent (dotted line); and (b) polygons represent groups of System X (solid line), System Y (dashed line), and System Z (dotted line). Metadata correlated to Axis 1 and Axis 2 with a *p*-value below 0.05 are displayed.

Functional Groups Involved in Nitrogen Removal: Taxonomy and Relative Abundance

The 16S rRNA ASV from potential ammonia-oxidizing microbes (AOM) and nitrite-oxidizing bacteria (NOB) contributed up to 5% of total sequences recovered from the lysimeters and effluent of all three NRBs, compared to less than 0.5% of total sequences recovered from influent and STE samples, and showed high temporal variation in all systems [Fig. 5(a)]. In Fig. 5, bubble size corresponds to the relative percent that each genus contributed to overall sequence abundance of the sample. Raw influent samples are shown in the far left column, followed by Systems X, Y, and Z in individual columns. Dashed lines separate the STE (far left), first lysimeter, second lysimeter, and final effluent (far right). Sample name is denoted by abbreviated sampling months, followed by the sampling port (Infl = raw influent, STE = septic tank effluent, pan1 = first lysimeter, pan2 = second lysimeter, and Effl = effluent).

Five genera of AOM were detected [Fig. 5(a) and Supplemental Data]. The richness and evenness of the AOM assemblage varied markedly among and within NRB systems, with no clear patterns except perhaps for NRBs to be dominated either by *Candidatus Nitrosotalea* and *Nitrosomonas* (System X and Z) or by *Candidatus Nitrosoarchaeum* and *Candidatus Nitrosotenuis* (System Y), with *Nitrosospira* dominating only in a few samples from Systems Y and Z [Fig. 5(a)]. The only AOM detected entering NRBs in the influent were *Candidatus Nitrosotalea* and *Candidatus Nitrosotenuis*, both at extremely low relative abundance (<0.1%, Supplemental Data). *Nitrospira* was the only NOB detected entering NRBs in the influent (Supplemental Data), and also was often the dominant NOB in the lysimeter samples, although its relative abundance varied greatly between and within systems [Fig. 5(a)]. The only other NOB identified, *Candidatus Nitrotoga*, was often the dominant or codominant NOB in NRB effluent samples [Fig. 5(a) and Supplemental Data].

Potential denitrifiers were found in all samples, including influent and STE, often as a large proportion of the total community (>20%) [Fig. 5(b)]. The potential denitrifier assemblage entering NRBs via STE was dominated by *Arcobacter* (up to nearly half of all 16S sequences recovered) [Figs. 5(b) and S4]. In general, the denitrifier assemblage in the NRB samples was more diverse (greater richness and evenness) than influent assemblages, with *Rhodoferax*, *Sulfuritalea*, and *Dechloromonas* the most consistently dominant potential denitrifiers [Fig. 5(b)]. Cellulolytic bacteria were detected as a small proportion of total sequences (2% or less, typically less than 0.5%) [Fig. 5(c) and Supplemental Data]. *Ruminiclostridium* and *Anaerolinea* were the two dominant genera. Microbial functional groups that could compete with heterotrophic denitrifiers for VFAs produced by cellulolytic microbes include sulfate-reducing bacteria (SRB), iron reducers, and methanogens. Potential iron-reducing bacteria were not detected in any sample. Two genera of SRB, *Sulfurospirillum* and *Desulfosporosinus*, were detected sporadically at low relative abundance (<2%) [Fig. 5(c) and Supplemental Data]. Four genera of potential methanogens were detected sporadically and contributed 0.5% or less to total sequence abundance when they were detected [Fig. 5(c) and Supplemental Data]. The potential for internal cycling of methane and sulfur in NRBs was indicated by the detection of seven genera of potential methanotrophs, with *Methylomonas* reaching up to 8% of total sequences [Fig. 5(c)], and the sulfur-oxidizing genus *Thiobacillus*.

Discussion

Each of the full-scale test NRBs examined here removed at least 50% of the influent TN throughout 2016, although each also exhibited a different amplitude and timing of seasonal variation in TN removal (Fig. 2). It is critical to understand the basis for such variation in performance in order to ensure the best use of this technology in OWTS. The pan lysimeters installed in these NRBs provided insight into this variation by enabling measurement of

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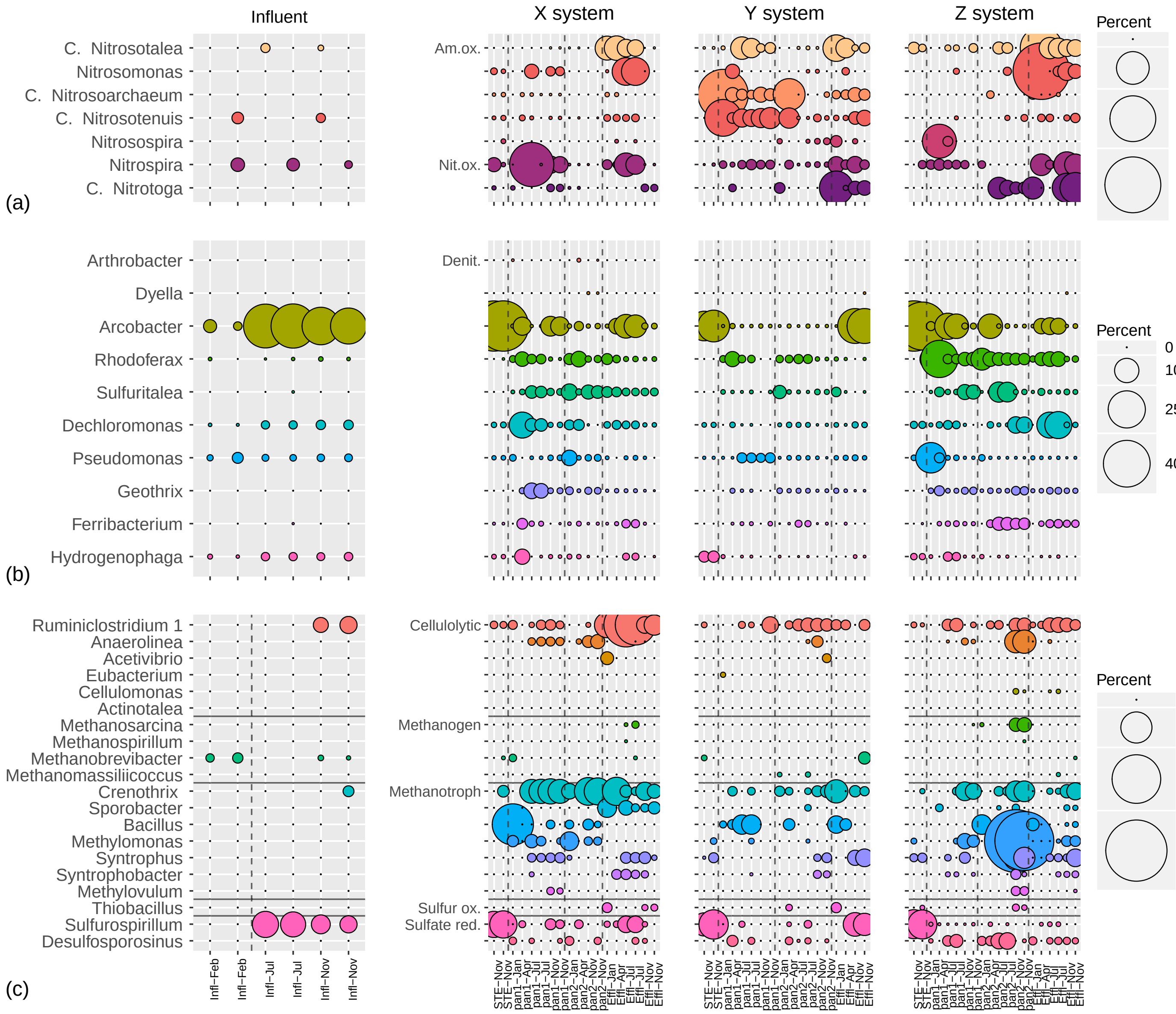

Fig. 5. Genera of each potential functional group displayed as a percent of total sequences in each sample: (a) genera of potential ammonia oxidizers and nitrite oxidizers; (b) a selection of potential denitrifiers; and (c) genera of potential cellulolytic, methanogenic, methanotrophic, sulfur-oxidizing, and sulfur-reducing microbes from bottom to top separated by solid lines.

target chemical species (i.e., nitrogen) at intermediate depths (Fig. 2) so that the function of the two layers could be evaluated independently. The apparent gain of TN between depths sometimes observed could reflect spatial heterogeneity within the NRB because the first and second lysimeters were horizontally offset from each other, and each sampled a small portion of the total NRB area.

Temporal variation of influent composition or input of freshwater to the NRB by precipitation, combined with the time required to move through the system, could also contribute to unexpected increases of TN with depth. TN removal was most consistent in System Y, which was limited by denitrification (effluent TN mainly in the form of NOx) year-round (Fig. 2). TN removal in Systems X and Z were also limited mainly by denitrification in winter, but then limited by nitrification (effluent TN mainly in the form of ammonium) in spring and summer (System X) or autumn (System Z) (Fig. 2). The negative association between alkalinity (Alk) and nitrite+nitrate (NOx) (Table 1), which is to be expected from the consumption of alkalinity during nitrification (Brewer and Goldman 1976), could point to alkalinity supply as an important design consideration.

TN removal in System Y always occurred mainly in the top sandy layer, and high TN removal in the top layer was also often observed in Systems X and Z during their periods of maximal TN removal (Fig. 2). The removal of TN within the top sandy layer is likely to reflect the presence of anoxic microzones that support denitrification hotspots (Groffman et al. 2009; McClain et al. 2003; Parkin 1987; Sexstone et al. 1985; Zausig et al. 1993) in which heterotrophic denitrification could be supported by wastewater organic carbon. Thus, although denitrification sometimes occurred mainly in the sand-lignocellulose layer (System X spring through autumn, System Z late summer through autumn), denitrification in the sand layer was likely often a major pathway of TN removal by these NRBs.

In general, differences between microbial communities could reflect either the deterministic selection of distinct communities by distinct environmental conditions, or random processes such as the founder effect and drift, or a mixture of both deterministic and random processes (Dini-Andreote et al. 2015; Zhou et al. 2013). All microbial assemblages collected from NRB lysimeters and effluent were distinct from raw wastewater influent and STE communities (Fig. 3), and there were clear differences between NRB and STE samples in the relative abundance of the dominant genera in many functional groups [Figs. 5(a–c)], indicating that environmental selection in NRBs produced an assemblage distinct from the immigrating wastewater community. To remove TN by sequential nitrification-denitrification as wastewater flows through the system, NRBs are expected to select microbial communities performing nitrification in the top layer and denitrification in the bottom layer.

Naively, a nitrifying community selected in the sand layer might be expected to contain a relatively greater proportion of nitrifying bacteria, and a denitrifying community selected in the sand-lignocellulose layer might be expected to contain a relatively greater abundance of denitrifiers and cellulolytic bacteria. Contrary to such simplistic expectations, there was not a consistently greater relative abundance of nitrifiers in the pan lysimeter samples or of denitrifiers in the final effluent samples [Figs. 5(a and b)]. Weighted UniFrac ordinations including all members of the microbial community detectable by the used methods revealed that microbial community structure was significantly correlated with chemical parameters associated with system function (NOx and alkalinity), again suggesting that environmental selection was important in structuring NRB microbial communities (Figs. 3, 4, S2, and S3).

However, these visualizations also did not reveal obvious grouping of samples by system, layer, or season. In the analysis of NRB samples alone, no single PCoA axis captured more than 18% of the total variance in microbial community structure, and four axes were required to explain just half of the variance (Fig. 4). The additional axes generally separated one or a few samples from the rest [e.g., Axis 3 separated the sample collected from the first Y lysimeter in April, and Axis 4 separated samples collected from the second Y lysimeter in July and the effluent of Y in November (Figs. S2 and S3)]. The more consistent function of System Y implies it maintained more constant internal conditions, but microbial communities from System Y appeared to vary just as much as those from Systems X and Z. The lack of a clear distinction between pan lysimeter and effluent microbial communities could reflect relatively weak effects of environmental selection on the microbes moving through the NRB layers and/or limitations of these sampling methods. Altogether, this may suggest that whole-community analyses include substantial noise from variation in members of the microbial community not directly related to the transformation of nitrogen, and that more sophisticated methods of investigating the most relevant portion of the community, such as functional gene analysis, will be required to gain deeper insight into the relationship between microbial community structure and the system function of nitrogen removal.

The most abundant ammonia-oxidizing microbes (AOM) within the X and Z NRBs [Fig. 5(a)] were *Candidatus Nitrosotalea*, an ammonia-oxidizing archaea commonly found in soils (Herbold et al. 2017; Lehtovirta-Morley et al. 2011), and *Nitrosomonas*, one of the most abundant AOM found in wastewater treatment systems (Dalahmeh et al. 2014; Ward 2012). In System Y, *Candidatus Nitrosoarchaeum* and *Candidatus Nitrosotenuis* dominated; whether this difference is related to the more consistent performance of System Y or to its particular design are points for future study. The dominant NOB was consistently *Nitrospira*, which is also one of the most common NOB in WWTPs (Atoyan et al. 2013; Dalahmeh et al. 2014; Guo et al. 2015; Ju and Zhang 2015; Tomaras et al. 2009; Ward 2012).

The most abundant denitrifying genus in influent and STE, *Arcobacter*, includes species that are aerotolerant (Vandamme et al. 1992), pathogenic to animals and humans (Collado and Figueras 2011), and contribute to the denitrification process (Heylen et al. 2006). The intention of NRB design is that cellulolytic bacteria will decompose lignocellulose in the sand-lignocellulose layer, producing VFAs to support heterotrophic denitrification. However, the potential denitrifiers that became most dominant in NRB samples have more flexible metabolism than the chemo-organoheterotrophic nitrate respiration targeted by NRB design. For example, *Rhodoferax* can use iron reduction as an alterxnative to nitrate reduction (Finneran et al. 2003), and *Sulfuritalea* couples nitrate reduction with thiosulfate oxidation (Kojima and Fukui 2011). The selection of these organisms in NRBs, as opposed to typical WWTP denitrifiers like *Zoogloea*, *Clostridium, Acidovorax*, and *Pseudomonas* (Guo et al. 2015; Wang and Chu 2016), suggests the biogeochemistry of NRBs is distinct. The authors speculate that the supply of VFAs may limit chemo-organoheterotrophic denitrification, selecting more metabolically diverse denitrifiers in NRBs than in WWTPs. Not much is known about either of the dominant cellulolytic genera detected, *Ruminiclostridium* and *Anaerolinea* [Fig. 5(c)] (Sheng et al. 2016; Yamada et al. 2006); therefore, no conclusions can be drawn about how they may influence NRB microbial community structure or function.

Although the functions of greatest interest in application of NRBs have to do with nitrogen transformation and removal, NRBs can be expected to support complex microbial communities performing a variety of other metabolic activities that may interact with desired nitrogen transformations. For example, methanogens may compete with denitrifiers for VFAs (Hendriksen and Ahring 1996; Percheron et al. 1999), but were detected only sporadically and at low abundance relative to denitrifiers [Figs. 5(b and c)]. Sulfate-reducing bacteria may also compete with denitrifiers and methanogens for VFAs (Westermann and Ahring 1987), and the positive correlation between nitrite+nitrate (NOx) and sulfate (Table 1) suggests both functional groups may have access to abundant electron acceptors at the same time, but again potential sulfate-reducing bacteria were detected only sporadically and at low abundance relative to potential denitrifiers [Figs. 5(b and c)].

In the larger context of wastewater treatment, NRBs also remove additional potential pollutants, including phosphorus, trace metals, and organic pollutants (Gobler et al. "Lignocellulose-based biofilters for onsite wastewater treatment that remove more than 90% of nitrogen and organic contaminants," working paper, Stony Brook University, Stony Brook, New York; Price et al. "Behavior of iron and other metals in a lignocellulose-based biofilter for onsite wastewater treatment," working paper, Stony Brook University, Stony Brook, New York; Wehrmann et al. "Biogeochemical sequestration of phosphorus in a two-layer lignocellulose-based soil treatment system," working paper, Stony Brook University, Stony Brook, New York). Polyphosphate-accumulating bacteria (PABs) are usually found in wastewater treatment systems involved in biological nutrient removal and thrive in conditions with frequently changing redox status (Streichan et al. 1990; Van Loosdrecht et al. 1997). A single potential PAB genus, *Candidatus Accumulibacter*, was detected in all systems at low relative abundance (1% or less), mostly in effluent samples (Supplemental Data). Whether samples with relatively high abundance of PABs are indicative of fluctuating redox conditions in NRBs is an important question for future study.

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Conclusion

Microbial communities collected from the flow-through of three NRBs constructed with different materials were distinct from those of incoming wastewater, supporting the hypothesis that environmental selection shapes NRB microbial communities. When comparing the entire microbial community, there was extensive overlap among and variance within systems; broadly, microbial communities from System Y showed consistently less overlap with Systems X and Z, and System Y exhibited a different pattern of nitrogen removal from Systems X and Z. When comparing subsets of the microbial community, such as the nitrifying and denitrifying assemblages, clear differences among systems emerged. System-specific differences could be due to different NRB materials or configurations selecting for specific microbial groups or due to stochastic processes like the founder effect. Predicted differences between communities collected within or just below the upper sand layer versus those collected below the lower sand-lignocellulose layer were not detected, potentially due to stochastic processes. This uncertainty will be the focus of future replicated experiments characterizing the NRB matrix microbial community, which is likely responsible for most of the system function.

Nevertheless, microbial communities collected from pan lysimeters without disrupting NRB function revealed differences between individual NRBs and suggested that a subset, rather than the whole community, exhibited a relationship to overall system function. Measuring the chemical composition of pan lysimeter samples offers an effective method for monitoring the performance of NRBs, and if future studies can identify the appropriate subset of the microbial community that best aligns with system function, nondestructive pan lysimeter sampling could also become an effective NRB microbial community monitoring tool.

Acknowledgments

Samples were collected by KL or members of CCWT. Postsequence processing and microbial analysis was by KL. Manuscript was written by KL and JC. Advisory and administrative support provided by CG and HW. Thanks to CCWT and to STRIDE for supporting KL. Thanks to the Seawulf high performance computer cluster for computational support. All MASSTC data provided by George Heufelder and MASSTC crew. Thanks to Drs. Laura Wehrmann and Roy Price for providing phosphate, sulfate, and iron data, respectively. Thanks to all CCWT for enthusiastic collaboration around collection of samples and measurement of parameters used here as metadata: Samantha Roberts, Molly Graffam, Zoe Smith, Patricia Clyde, Zahra Maleki Shahraki, and Sara Lofti. This study was, and the NYS CCWT is, funded by the New York State Department of Environmental Conservation.

Supplemental Data

Figs. S1–S4 and spreadsheets containing the potential functional groups of the microbial community, metadata used in analyses, nitrogen data used in analyses, PCoA associations derived from analyses (discussed in the "Materials and Methods" section), and abundance table of ASV in each sample before rarefaction are available online in the ASCE Library (www.ascelibrary.org).

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Science of the Total Environment 744 (2020) 140936

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Greenhouse gas emissions from lignocellulose-amended soil treatment areas for removal of nitrogen from wastewater

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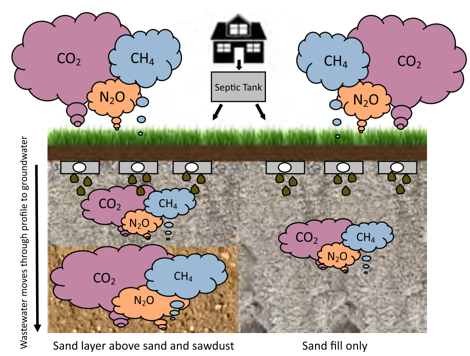
HIGHLIGHTS

- Experimental and Control STAs emitted similar amounts of GHGs to the atmosphere.
- Experimental and Control STAs emitted more GHG than an STA not receiving wastewater.
- Subsurface GHG concentrations were higher in Experimental than in Control STAs.
- The flux of all three gases were correlated with subsurface GHG levels and soil temperature and moisture.
- Emissions from STAs ranged from 30.4 to 1938.4 gCO2e capita^{-1} day^{-1}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 May 2020
Received in revised form 9 July 2020
Accepted 11 July 2020
Available online 15 July 2020

Editor: Huu Hao Ngo

Keywords:
Nitrous oxide
Methane
Carbon dioxide
Septic systems
Onsite wastewater treatment
Nitrogen removal

ABSTRACT

Lignocellulose-amended, layered soil treatment areas (STAs) remove nitrogen (N) passively from wastewater by sequential nitrification and denitrification. As wastewater percolates through the STA, the top sand layer promotes nitrification, and the lower, lignocellulos-amended sand layer promotes heterotrophic denitrification. Layered STAs can remove large amounts of N from wastewater, which may increase their emissions of CO_2, N_2O, and CH_4 to the atmosphere. We measured greenhouse gas (GHG) flux from sawdust-amended (Experimental) and sand-only (Control) STAs installed in three homes in southeastern Massachusetts, USA. The Experimental STAs did not emit significantly more GHGs to the atmosphere than Control STAs receiving the same wastewater inputs, and both Control and Experimental STAs emitted more CO_2 and N_2O – but not CH_4 – than soil not treating wastewater. Median (range) flux (μmol m^{-2} s^{-1}) for all homes for the Control STAs was 7.6 (0.8–23.0), 0.0001 (−0.0004–0.004), and 0.0008 (0–0.02) for CO_2, CH_4 and N_2O, respectively, whereas values for the Experimental STAs were 6.6 (0.3–24.3), 0 (−0.0005–0.005), and 0.0004 (0–0.02) for CO_2, CH_4 and N_2O, respectively. Despite the absence of differences in flux between Control and Experimental STAs, the Experimental STA had significantly higher subsurface GHG levels than the Control STA, suggesting microbial consumption of excess gas levels near the ground surface in the Experimental STA. The flux of GHGs from Experimental and Control STAs was controlled chiefly by temperature, soil moisture, and subsurface GHG concentrations. Total emissions (gCO_2e capita^{-1} day^{-1}) were higher than those reported by others for conventional STAs, with mean values ranging from 0 to 1835 for septic tanks, and from 30 to 1938 for STAs. Our results suggest that, despite a higher capacity to remove N from wastewater, layered STAs may have limited impact on air quality compared to conventional STAs.

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https://doi.org/10.1016/j.scitotenv.2020.140936
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1. Introduction

Coastal communities threatened by nutrient pollution from septic systems often require advanced onsite wastewater treatment systems (OWTS) that reduce C and N loads to sensitive ground and coastal waters to a greater extent than is possible with conventional OWTS (Bellone et al., 2017; FDOH, 2018; MassDEP, 2013; Murray et al., 2018; RIDEM, 2020). Like conventional systems, advanced OWTS rely on microbial processes that produce carbon dioxide (CO_2) and methane (CH_4) to remove organic C from wastewater as part of primary treatment. In addition, most advanced septic systems provide conditions that support enhanced microbial transformations of wastewater C and N to gaseous forms that are released to the atmosphere, including the greenhouse gases CO_2, CH_4, and nitrous oxide (N_2O).

Although septic systems treat wastewater for over 20% of US households (U.S. Cenus Bureau, 2006) and produce GHG through microbial pollutant removal, quantification of their greenhouse gas emissions has received little attention (Truhlar et al., 2016). A number of recent studies have begun to address this knowledge gap and indicate that septic systems can be important contributors of GHGs (Diaz-Valbuena et al., 2011; Dubber and Gill, 2014; Truhlar et al., 2016, 2019; Somlai-Haase et al., 2017; Somlai et al., 2019). As such, onsite wastewater treatment should be better integrated into global GHG emission estimates. For example, Truhlar et al. (2016) estimated the carbon footprint for a septic system (including emissions from the septic tank, roof venting system, and STA) to be 0.27 tCO_2e capita $^{-1}$ year^{-1}, equivalent to 1.5% of the U.S. total annual per capita carbon footprint (The United Nations Statistical Division, 2017; Truhlar et al., 2016).

A more complete understanding of the contribution of septic systems to global GHG emissions requires that we quantify emissions from all treatment train components. This includes the STA, where emissions to the atmosphere can result from physical processes, such as outgassing of gases dissolved in effluent, and from in situ microbial production and consumption of gases (Somlai et al., 2019). Values of GHG flux from soils treating wastewater vary widely. Diaz-Valbuena et al. (2011) reported consistently similar concentrations of N_2O, CO_2, and CH_4 between flux chambers above a conventional STAs and ambient atmospheric samples. In contrast, Truhlar et al. (2016) found STAs can be a substantial source of N_2O – but not CO_2 or CH_4 – relative to soils not receiving wastewater, with flux values ranging from −0.012 to 0.17 g N_2O capita^{-1} day^{-1}. Somlai et al. (2019) observed higher median CO_2 flux from STAs than from native soil, with STA values ranging from 0.66 to 22.72 μmol m^{-2} s^{-1}. Beyond developing a sense of the magnitude of GHG emissions from STAs, a better understanding of the factors governing the direction and magnitude of GHG fluxes in STAs would help to design systems that minimize emissions without compromising treatment.

The STA can be engineered to enhance N removal from septic tank effluent. One design, often referred to as a Layered STA, involves vertically stratifying treatment processes, with a top layer of sand to promote oxic conditions that support nitrification placed above a layer of sand mixed with lignocellulosic material (e.g., sawdust or mulch), which promotes anoxic conditions and provides a source of C and energy for heterotrophic denitrification.

The Layered design contrasts markedly with that for a conventional STA, which consists of a pipe-on-stone trench underlain with either native soil or sand that promotes oxic conditions and lacks a C source for denitrification, and is not designed to remove N. In contrast, Layered STAs can lower the total N concentration in septic tank effluent by as much as 85% (Heufelder, 2017). They are also non-proprietary, passive technologies, that provide a much less costly alternative to manufactured trade named advanced N-removal OWTS. As such, they represent a cost-effective solution to lowering N pollution in coastal areas. Assessing their GHG emissions will help evaluate their impact on the atmosphere relative to existing onsite N-removal technologies, which are net sources of GHG (Brannon et al., 2017; Ross et al., 2020).

We quantified emissions of CO_2, CH_4, and N_2O from Layered (also referred to as Experimental) and Control STAs, as well as the septic tank, at three single-family homes in Barnstable County, Massachusetts, USA. One home was occupied year-round, whereas the other two were occupied only in summer. We also measured subsurface concentrations of CO_2, CH_4, and N_2O to further characterize GHG dynamics patterns in the STAs. Finally, we examined the role of system and environmental variables in controlling both emissions and subsurface GHG dynamics. We expected that CO_2, CH_4, and N_2O emissions would be higher from the Layered STA than the Control STA, since the former is designed to increase heterotrophic denitrification, which should increase CO_2 and N_2O production. We also expected CH_4 would be produced at higher rates in the saturated lower layer of the STA, where anoxic conditions would promote methanogenesis. Finally, we expected that GHG flux from both the Control and Experimental STAs would be controlled by soil physical properties such as temperature, oxygen concentration, and belowground GHG concentration, since these factors modulate emissions in other soils (Davidson et al., 1998; Risk et al., 2002).

2. Methods

2.1. System design

We monitored three Layered STAs installed between April 2018 and July 2019 in the towns of Acushnet and Falmouth (referred to as Acushnet, Sippewissett, and Chappaquoit) in Massachusetts, USA. All three systems had the same main components (Fig. 1): (1) a conventional, two-chamber, 5678 L septic tank for primary treatment and (2) a 3785 L pump chamber (for surge flow storage) with a mechanical pump to deliver consistent doses of effluent to (3) a GeoMat™ leaching system. The latter is a subsurface, low-profile dispersal system that delivers wastewater through orifices in laterals (PVC pipes) surrounded by plastic filaments and geotextile fabric, which increases the contact area of wastewater with the underlying soil. Site and system characteristics are detailed in Table 1.

Each STA was split into a Layered – also referred to as Experimental – and a Control side. The Control STA was designed in accordance to Massachusetts State Environmental Code (MassDEP, 2013) and consisted of a 90 cm-deep layer of medium sand (0.30 mm effective particle size; D_{10}). The Experimental STA consisted of a 45 cm-deep layer of sand on top of a 45 cm-deep layer of sand amended with sawdust (primarily *Quecus spp.*; 1:1 by volume) (Fig. 1). The Acushnet site had an additional (Reserve) STA that was identical to the Control STA but did not receive wastewater: it served as a failsafe in the event of hydraulic failure of the Experimental STA (Fig. 1).

2.2. Gas flux measurements

We measured the flux of GHG at the soil surface and from septic tanks using a Picarro model G2508 cavity ring-down spectroscopy analyzer that simultaneously measures CO_2, CH_4, and N_2O flux in real time (Brannon et al., 2016). We measured GHG fluxes at the Acushnet site in July and December 2018 and April and July 2019, in May and July 2019 at the Sippewissett site, and in April and July 2019 at the Chappaquoit site. On every site visit we made at least seven gas flux measurements: three from the Experimental side, three from the Control side, and one from either the septic tank or pump chamber (Fig. 1). At Acushnet, we made three flux measurements from the Reserve STA in July 2019.

To measure gas flux at the soil surface, we used an opaque, closed PVC soil gas chamber (i.d. = 28 cm, height = 18 cm, fitted with a stainless steel pressure equilibration device) that was placed on a PVC collar (i.d. = 26.5 cm, height = 11.5 cm) and connected to the gas analyzer via nylon tubing. The chamber was fitted with a rubber silicon gasket to form an air-tight seal when it was placed on the collar. The collars were inserted ~10 cm into the soil on the day we measured fluxes. We

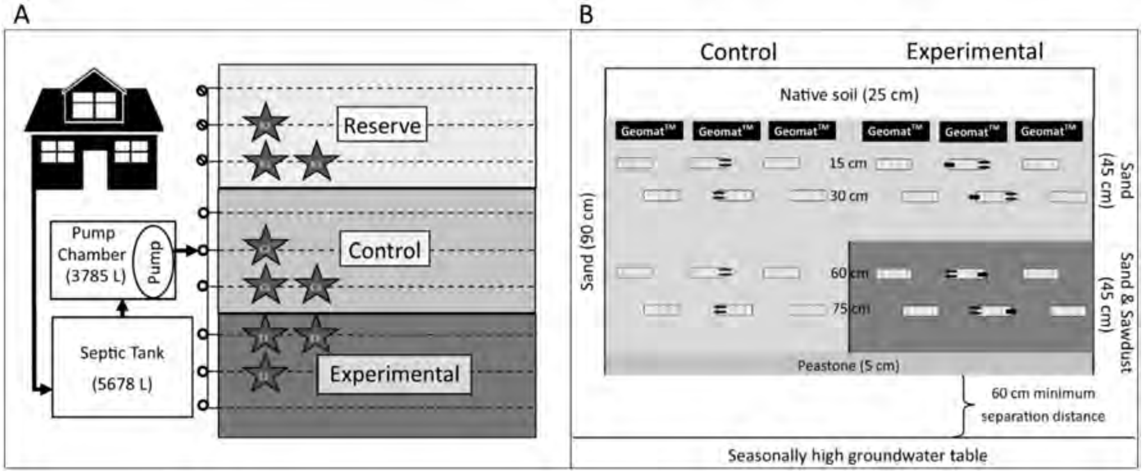

Fig. 1. (A) Schematic diagram of the soil treatment area (STA) at the Acushnet site. The STA was divided into thirds and gas flux measurements were made at three points in each STA: Experimental (E1-E3), Control (C1-C3), and Reserve (R1-R3). (B) Cross-section of the Experimental and Control STAs at the Acushnet site showing the placement of arrays of subsurface gas diffusion chambers, moisture probes, and temperature probes. Probes were installed during system construction at 15 cm, 30 cm, 60 cm, and 75 cm below the infiltrative surface. Geomat™ is the low-profile dispersal system used. Double dashed lines (=) indicate moisture probes, single dashed lines (−) indicate temperature probes. Figures not to scale.

measure GHG flux at the water surface of the septic tank or pump chamber as described in Brannon et al. (2017). A Hobo® data logger (Onset, Bourne, MA) was installed inside the flux chamber to measure the air temperature every 15 s.

The flow rate of gas from the chamber to the analyzer was ~223 standard cm^3 min^{-1} and the change in concentration over time was measured every second. Gas concentration data were collected for 5 to 15 min. The gas flux was calculated as outlined in Martin and Moseman-Valtierra (2015). We assigned a flux value of 0 to measurements with no statistically significant change in gas concentration over time. Calculations and assumptions for daily per capita conversions can be found in the Supplementary Materials.

2.3. Subsurface gas concentration, temperature, and moisture content

We measured the concentration of soil gases (CO_2, CH_4, N_2O, and O_2) as well as temperature and moisture content at the Acushnet site. Gas diffusion chambers were installed during STA construction at 15, 30, 60, and 75 cm below the infiltrative surface in six arrays: three in the Experimental STA and three in the Control STA (Fig. 1). The chambers were made of well screen PVC (i.d. = 2.5 cm; length = 17.1 cm; Atlantic-Screen Inc., Milton, DE), which allowed for the movement of soil gases while preventing water accumulation. The chambers were connected to the ground surface using nylon tubing, with a three-way stopcock valve placed on the end of the tubing that was closed to the atmosphere between sampling events.

We attached EC-5 soil moisture sensors (METER Group, Pullman, WA) to four of the gas diffusion chambers in one array each in the Experimental and Control STAs. We also attached Hobo® temperature probes (Onset, Bourne, MA) to the same four chambers as the moisture probes in the Experimental STA (Fig. 1). Moisture and temperature measurements were recorded every 30 min. The gas diffusion chambers, and associated moisture and temperature probes were installed during system construction using a wooden frame that stabilized the chambers and held them at the correct depth while media was added to build the STA.

Before sampling soil gases, we purged the system by removing 300 mL of gas using an air-tight, 60 mL syringe. The pore space gases

Table 1
System and site characteristics for Acushnet, Chappaquoit, and Sippewissett sites located in Barnstable County, MA, USA.

Characteristic	Site		
	Acushnet	Chappaquoit	Sippewissett
Occupancy	Year-round	Seasonal (May–September)	Seasonal (June–September)
Installation date	April 20, 2018	April 10, 2017	November 16, 2017
No. of occupants	2 adults, 1 infant	2 adults	1 adult
Average flow when occupied (L day^{-1})	872	375	450
Depth of soil above Geomat™ (cm)	25	25	90
Type and width (m) of Geomat	1200 (0.3)	3900 (1)	3900 (1)
Total no. of Geomats	9	4	4
Space between laterals (m)	0.3	0	0
Soil treatment area dimensions [1]			
Length (m)	7.62	13	10.7
Width (m)	6.64	4.3	3.9
Depth (m)	0.9	0.9	0.9
Area (m^2)	14	54.6	41.73
Vegetation cover and management	Lawn grasses (*Festuca sp., Lolium sp., Poa sp.*); mown to ~5 cm height	Lawn grasses (*Festuca sp., Lolium sp., Poa sp.);* mown to ~5 cm height	Very little vegetation; no management prior to summer, when vegetation was ~1 m high

were then mixed by drawing and expelling a 60 mL gas volume five times. After mixing we transferred a 20 mL sample of the gases into pre-evacuated glass vials fitted with rubber septa and an aluminum collar, which were stored immersed in water until analysis. An additional 60 mL gas sample was collected for analysis of O_2 concentration.

Soil greenhouse gas concentrations were measured on a Shimadzu Gas Chromatograph-2014 Greenhouse Gas Analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). A flame ionization detector (FID) was used to analyze CO_2 and CH_4 and an electron capture detector (ECD) was used to analyze samples for N_2O. The flow rate of the ultrapure N_2 used as carrier gas was 25 mL min^{-1}. Instrument temperatures were 250 °C for the FID, 325 °C for the ECD, 80 °C for the column, and 100 °C for the injection port.

To measure the concentration of oxygen, we injected a 60 mL of sample into a flow-through cell connected to an O_2 probe at a rate of ~1 mL s^{-1} to obtain percent O_2 (Cooper et al., 2015). Calculations to converted oxygen concentration in the gas phase to dissolved oxygen (DO) are found in Supplementary Materials.

2.4. Statistical analyses

We used RStudio (Version 1.2.1335; R Core Team, 2012) to perform all data analysis. We used a two-way ANOVA to determine differences in GHG flux among seasons and STA type at Acushnet and to examine the differences in GHG flux among the subsurface sampling depths and STA type. We used Student's *t*-tests to determine differences in flux between Layered and Control STAs at the seasonal sites and to assess differences in GHG flux between periods of use and nonuse at the seasonal sites. We examined the relationship between subsurface gas concentrations, soil temperature (average temperature during measurement), dissolved oxygen (DO) and GHG flux by constructing multiple linear models in RStudio. For all statistical analyses, we used a confidence interval of 95%.

3. Results & discussion

3.1. GHG emissions from the septic tank

We measured CO_2, CH_4, and N_2O from the primary treatment tank to compare emissions from different parts of the treatment system. Carbon dioxide flux from the septic tank at Acushnet, was consistently low with values ranging from 0 to 2.9 μmol m^{-2} s^{-1}. We did not detect a significant N_2O flux except during winter 2018, when the flux value was 0.0002 μmol m^{-2} s^{-1}. There was no significant CH_4 flux from the septic tank during summer 2018, three months post-installation. We subsequently observed a consistently high CH_4 flux from the tank, ranging from 0.24 to 0.82 μmol m^{-2} s^{-1} (Fig. 2). At the seasonally-occupied sites GHGs tank emissions were between one and three orders of magnitude higher than at Acushnet during both periods of use (summer) and non-use (spring) (Fig. 2).

Daily per capita emissions for all gases from the septic tank at Acushnet were lower than others have reported from conventional septic tanks, but those from the seasonal sites were much higher (Diaz-Valbuena et al., 2011; Somlai-Haase, 2019), and were more comparable to values for roof vents reported by Truhlar et al. (2016). Differences in CO_2 flux among sites and studies may be due to differences in the curing time of concrete septic tanks, since longer curing times result in less formation of calcium carbonate from the reaction of CO_2 with calcium hydroxide present in cement (Balayssac et al., 1995; Dias, 2000; Han et al., 2011). The tank at Acushnet had a shorter curing time and the wastewater had higher pH than at the other sites (data not shown), suggesting that increased calcium carbonate formation caused the low CO_2 flux. Differences in CH_4 flux are likely due to variations in development of a scum layer – a C-rich layer of oils and fats that accumulates on the wastewater surface between maintenance visits – and the stochastic nature of CH_4 off-gassing (Grinham et al., 2011; Somlai-Haase, 2019; Windsor et al., 1992).

3.2. GHG emissions from the soil treatment area

3.2.1. Carbon dioxide

Carbon dioxide flux from the Layered and Control STAs at the Acushnet site were not significantly different regardless of sampling period (Fig. 3). When flux values from the Control and Layered STA were included, the median CO_2 flux for the whole study period was 11 μmol m^{-2} s^{-1} and ranged from 1.56 to 24.3 μmol m^{-2} s^{-1}. The CO_2 flux in summer 2019 was nearly twice that in summer 2018, likely because the system was installed 75 days before summer 2018 sampling and the microbial community and soil hydraulic properties were not yet stable. In addition, CO_2 flux was higher during both summers than during winter or spring. We did not observe significantly different CO_2 emissions between the Control and Experimental STAs at either of the seasonally-occupied sites, and these were also higher in the summer than in the spring (Fig. S1). Truhlar et al. (2019) did not observe significant seasonal differences in CO_2 emissions from conventional STAs, but they did observe a general decrease in flux in late fall and early winter. Seasonal differences in our systems may be due to the use of a low-profile dispersal system, which places the infiltrative surface closer to the ground surface and thus less buffered from variations in temperature than a conventional STA with an infiltrative surface placed deeper in the soil profile.

The median CO_2 flux (11.24 μmol m^{-2} s^{-1}) in the Reserve STA at Acushnet – measured in summer 2019 – was significantly lower than

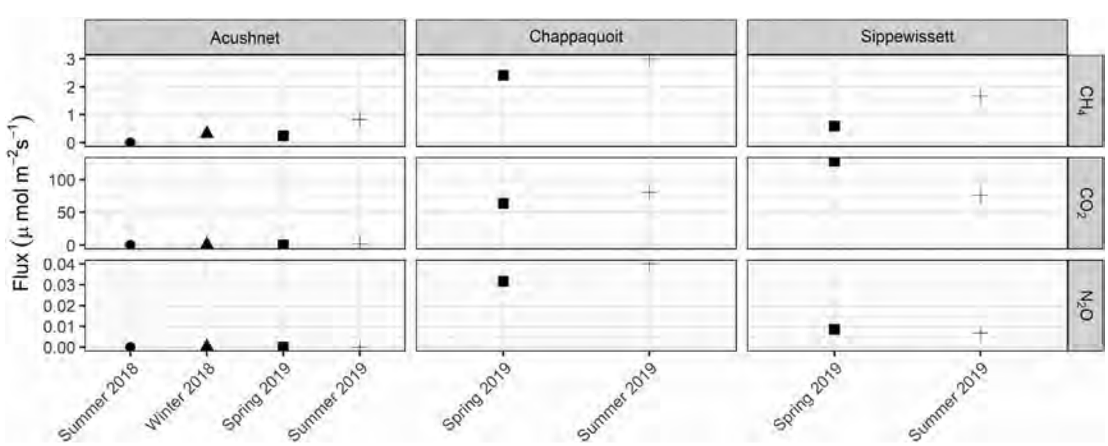

Fig. 2. CH_4, CO_2, and N_2O flux at the water surface of the septic tank at the Acushnet, Chappaquoit, and Sippewissett sites. Each symbol represents a single flux measurement.

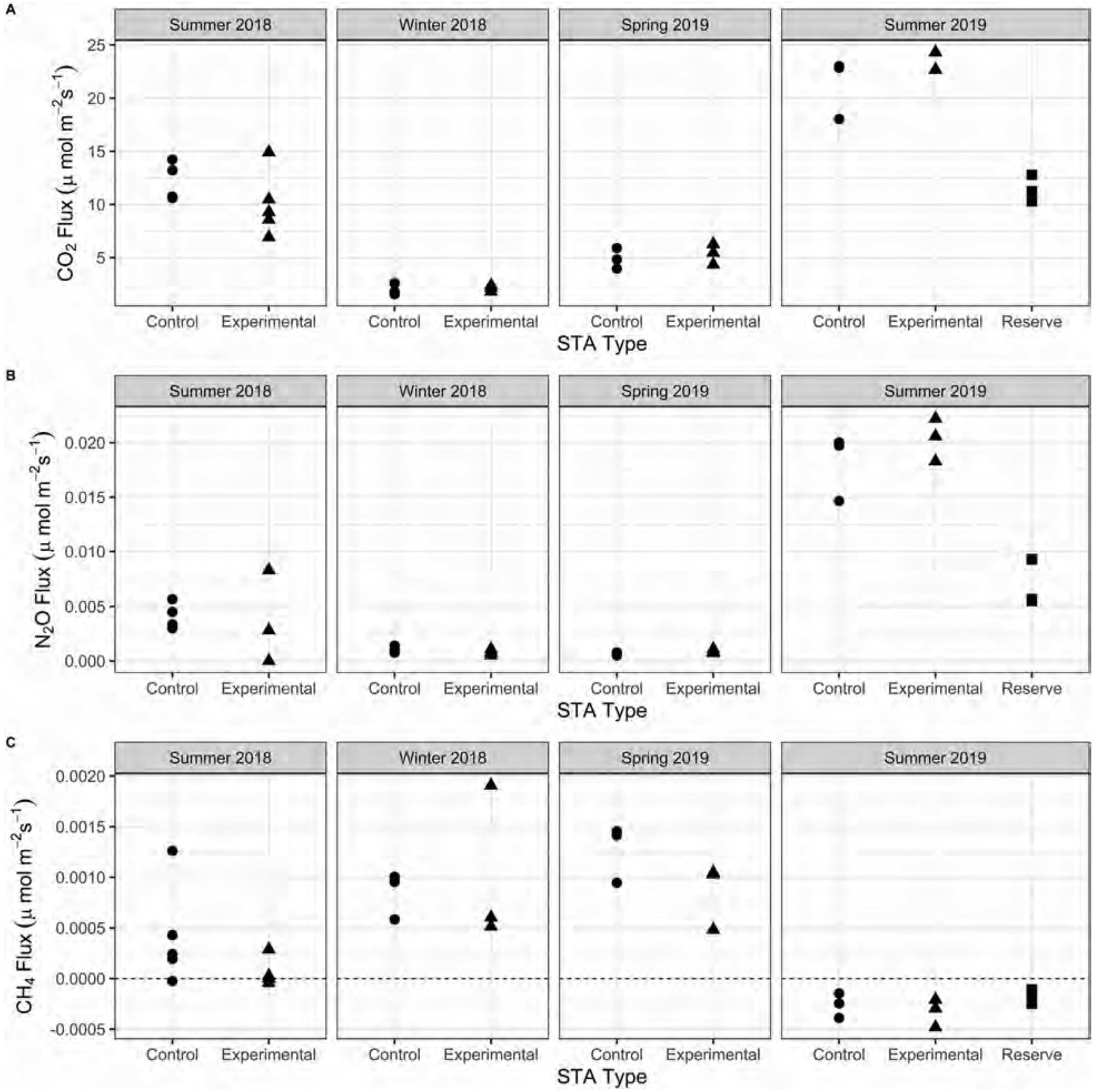

Fig. 3. Flux of (A) CO_2, (B) N_2O, and (C) CH_4 from Control and Experimental STAs measured between spring 2018 and summer 2019 at the Acushnet site. Additional flux measurements were made in the Reserve STA in summer 2019. Five measurements were made from each STA in summer 2018 and three measurements were made from each STA on other sampling dates.

for the Control (22.9 μmol m^{-2} s^{-1}) or the Layered (22.7 μmol m^{-2} s^{-1}) STAs (Fig. 3). The Reserve STA is filled with carbon-poor, biochemically-inactive sand and did not receive wastewater, resulting in lower microbial activity that produces less CO_2, compared to both native soil and STAs receiving wastewater. In contrast, Diaz-Valbuena et al. (2011) observed that CO_2 concentrations above a conventional STAs were the same as ambient atmospheric concentrations, and Truhlar et al. (2016, 2019) did not observe significantly different CO_2 emissions from STAs and native soils. Somlai-Haase et al. (2017) and Somlai et al. (2019) also observed similar CO_2 flux between native and STA soils, but they did observe higher CO_2 flux from STAs on some sampling events.

The daily per capita CO_2 emissions from the STA at the three homes ranged from 0.28 to 24.3 μmol m^{-2} s^{-1} and were slightly above the range reported by Somlai et al. (2019) and Somlai-Haase (2019) for conventional systems in Ireland.

3.2.2. Nitrous oxide

Nitrous oxide flux at Acushnet ranged from 0 to 0.018 μmol m^{-2} s^{-1}, with no significant differences observed between the Control and Layered STA for any of the sampling dates (Fig. 3). We also did not observe significant differences between the Control and Experimental STAs at the seasonally-occupied sites (Fig. S1). The N_2O flux was low in winter 2018 and spring 2019 for both the Control and Layered STA, with values ranging from 0.0008 to 0.001 μmol m^{-2} s^{-1}. Flux values were slightly higher in summer 2018 ranging from 0 to 0.008 μmol m^{-2} s^{-1} (Fig. 3) and they were an order of magnitude higher than previous dates in the summer of 2019, with values ranging from 0.01 to 0.02 μmol m^{-2} s^{-1}. The same temporal pattern was observed for CO_2 flux and is likely driven by higher temperatures once the microbial community becomes established in the STA.

The median flux of N_2O in the Reserve STA at Acushnet (0.006 μmol m^{-2} s^{-1}) – measured in summer 2019 – was significantly lower than in the Control and Experimental STAs, where median flux values were both 0.02 μmol m^{-2} s^{-1} (Fig. 3). In contrast, Diaz-Valbuena et al. (2011) did not observe N_2O concentrations differing between ambient atmospheric samples and from air above conventional STAs. Differences depth to the infiltrative surface between our systems and conventional STAs may be responsible for differences in N_2O flux. Truhlar et al. (2016) observed significantly higher N_2O emissions above conventional STAs than above control soils, as we did. Higher N_2O flux from the STAs observed by us and others suggests that soil-based wastewater treatment may be an important source of N_2O.

Across all sites, times, and STAs, N_2O flux ranged from 0 to 0.02 μmol m^{-2} s^{-1} (equivalent to 0 to 0.47 g N_2O $capita^{-1}$ day^{-1}). Although some our lowest values are comparable to those reported in previous studies (Diaz-Valbuena et al., 2011; Somlai-Haase, 2019; Truhlar et al., 2016), most are higher than previously reported, especially during summer 2019. The low-profile design of the dispersal systems may explain this. The proximity of the oxic layer, where N_2O can be produced by ammonia oxidation, and of the infiltrative surface to the soil surface, may result in higher N_2O flux than for systems where the infiltration surface is at a lower depth, with a higher volume of soil where N_2O may be consumed before it is emitted to the atmosphere. The location of the oxic layer relative to the ground surface may also explain the consistently low values of N_2O flux we observed at Sippewissett (Fig. S1), where fill material over the infiltrative surface is nearly four times deeper than at the other sites (Table 1). Cooper et al. (2016) also observed higher N_2O concentrations near the surface of mesocosms with low-profile dispersal systems compared to mesocosms with conventional STAs.

3.2.3. Methane

Methane flux values at Acushnet ranged from −0.0005 to 0.002 μmol m^{-2} s^{-1}, with no significant differences between the Layered and Control STAs on any sampling date (Fig. 3). Similarly, there were no significant differences in the CH_4 emissions between the Control and Experimental STAs in the seasonally-occupied sites (Fig. S1). Methane emissions had seasonal patterns that were opposite to those for CO_2 and N_2O, with higher values in the spring and winter (0.0005 to 0.002 μmol m^{-2} s^{-1}) than in the summer (−0.0005 to 0.001 μmol m^{-2} s^{-1}). The latter may be due to differences in the temperature sensitivity of microbial consumption and production of CH_4 in surface soil. Methane oxidation decreases significantly at temperatures under 10 °C (Le Mer and Roger, 2001), while methanogenesis can continue in the septic tank – increasing the level of dissolved CH_4 in effluent – where the water temperature was 12 °C in winter g, 6 °C higher than the soil (data not shown). As was the case for CO_2, Truhlar et al. (2019) did not observe seasonal differences in CH_4 flux, though they did observe that the STA acted as sink in the spring and fall, and as a source during the summer.

Unlike CO_2 and N_2O, the median CH_4 flux (μmol m^{-2} s^{-1}) for the Reserve STA (−0.00017) at Acushnet was not significantly different from the values for the Experimental (−0.00029) or Control STA (−0.00025) in summer 2019, when all three STAs acted as sinks (Fig. 3). Similarly, Truhlar et al. (2019) saw no differences in CH_4 flux between conventional STAs and native soil and that STAs frequently acted as CH_4 sinks.

Other studies have observed no net CH_4 flux from soils treating wastewater (Diaz-Valbuena et al., 2011), whereas we observed a wide range of flux values, depending on season and site. The range of CH_4 flux across all sites, dates, and STA types was −0.0005 to 0.005 μmol m^{-2} s^{-1} (−0.024 to 0.21 g CH_4 $capita^{-1}$ day^{-1}). Many of these values were within the range reported by others from conventional STAs (Somlai et al., 2019; Truhlar et al., 2016, 2019). Nevertheless, we observed some much higher flux values, especially during the winter and spring at Acushnet and in the summer at Chappaquoit. These high values could be explained by the low-profile dispersal systems in our STAs. It is likely that the soil above the low-profile dispersal system is not deep enough to allow for consumption of the CH_4 produced in the anoxic layer and that diffusing from the septic tank effluent dispersed to the STA. This is exacerbated in cold temperatures when CH_4 consumption decreases. The consistently low CH_4 flux at Sippewissett (Fig. S1) suggests that there is greater microbial consumption of the gas in the deeper soil above the dispersal system relative to the shallower soil in the other two systems (Table 1).

3.3. Subsurface concentrations of dissolved oxygen and GHG at Acushnet

At Acushnet, we measured subsurface concentrations of CO_2, N_2O, CH_4, and DO at four depths within the layered and control STAs to better describe patterns of gas production and consumption and to develop a better understanding of what controls GHG emissions of Layered systems.

3.3.1. Dissolved oxygen

In the Layered STA at Acushnet, median DO concentrations ranged from 3.7 mg L^{-1} at 75 cm in July 2019 to 11.9 mg L^{-1} in March 2019 at 30 cm. Median DO concentrations in the Control STA ranged from 6.9 mg L^{-1} at 30 cm in July 2019 to 13.6 mg L^{-1} in March 2019 at 15 cm (Fig. S2). Dissolved oxygen concentration decreased with increasing depth in the Layered STA, whereas DO levels in the Control STA varied little with depth. When compared across all dates, median DO values were significantly higher in the Control STA than in the Layered STA at the two lowest sampling points, where the Layered STA has the lignocellulose amendment, but no difference in DO was observed between STAs at the top two sampling depths, where both sides were filled with sand. These data suggest that the Layered STA acted as designed, lowering DO levels relative to the Control STA to promote denitrification.

Differences in DO concentration between the Layered and Control STAs were affected by season. There were no significant differences in DO levels between the control and layered STAs when the soil temperature fell below 7 °C, and we observed the lowest DO values during warm sampling dates, especially in the layered STA (Fig. S6). This is consistent with other seasonal DO observations in shallow groundwater and denitrifying reactive barriers (Datry et al., 2004; Warneke et al., 2011). Warm temperatures lower DO solubility, and DO levels decrease further due to increased microbial activity, which consumes DO and produces CO_2.

3.3.2. Carbon dioxide

Median subsurface levels of CO_2 in the control STA ranged from 2965 ppmv in March at 75 cm to 98,981 ppmv in June 2018 at 60 cm. In the Layered STA, CO_2 levels ranged from 420 ppmv in April at 75 cm to 171,168 ppmv at 75 cm in June 2018 (Fig. S3). When samples from all dates were considered, CO_2 levels were significantly higher in the Layered STA than in the Control STA at all depths except 15 cm. Carbon dioxide concentrations in the Control STA did not vary as a function of depth. In contrast, we found significantly lower CO_2 concentration at 15 cm than at 60 cm or 75 cm below the infiltrative surface – the denitrification zone – of the layered STA. High CO_2 concentrations in the denitrification zone and lower concentrations higher in the profile of the layered STA indicate CO_2 consumption in the nitrification zone. A small fraction of this could be attributed to chemoautotrophic organisms, such as nitrifiers, which use CO_2 as a carbon source (Robertson and Groffman, 2015). It is more likely that CO_2 is consumed as it reacts with silicate and carbonate compounds in the sand (Liu et al., 2011).

Carbon dioxide levels in the subsurface at Acushnet generally increased with increasing temperature, especially in the Layered STA (Fig. S6) and were notably high in June 2018, approximately six weeks after installation, when levels of the gas in the Layered STA were significantly higher than on any other date. While CO_2 concentrations in the Control STA were also highest in June 2018, they were significantly lower than in the Layered STA at 60 and 75 cm. This suggests that sometime during the first few months of installation a large amount of CO_2 was produced in the Layered STA, possibly by aerobic decomposition of the lignocellulose amendment before hypoxic/anoxic conditions were established.

3.3.3. Nitrous oxide

Subsurface N_2O levels were lowest in March 2019 at 15 cm, when median values were 11 and 19 ppmv in the Control and Layered STA, respectively. The highest N_2O levels were observed in July 2019, when the median N_2O value in the control STA was 160 ppmv at 15 cm and 197 ppmv at 75 cm in the Layered STA (Fig. S4). When all dates were considered, there were no significant differences in N_2O concentration with depth in either STA. We did, however, observe significantly higher concentrations of N_2O in the Layered STA compared to the Control STA, especially in the lower, carbon-amended layer (Fig. S4) where hypoxic/anoxic conditions support N_2O production by heterotrophic denitrification. Differences in N_2O levels between the Layered and Control STA are likely exacerbated in summer by warmer temperatures and low DO levels (Fig. S6), both contributing to higher denitrification rates (Korom, 1992; Sutton et al., 1975). During the coldest months, when temperatures at the bottom of the STA were consistently below 5 °C and DO levels were consistently above 11 ppmv, we did not observe significant differences in N_2O levels between the STAs.

3.3.4. Methane

Subsurface CH_4 levels were lowest in May 2019, when the median value in the Control STA was 3.8 ppmv at 75 cm and 4.4 ppmv at 60 cm in the Layered STA. The highest CH_4 concentrations were observed in November 2018 with median values 520 and 1832 ppmv at 75 cm in the Control and Layered STA, respectively (Fig. S5). Across all months and dates, subsurface CH_4 levels were significantly higher in the Layered than in the Control STA (Fig. S5) and differences in CH_4 concentrations between the two STAs were largest in August 2018, October 2018, and June 2019. There was no clear relationship between CH_4 concentration and depth in either STA. Subsurface CH_4 values were highest when temperatures were between 10 and 13 °C (Fig. S6). Methane concentration in both STAs was slightly elevated in the first two months after installation, otherwise it was very low except in November, when they were an order of magnitude higher than on all other sampling dates.

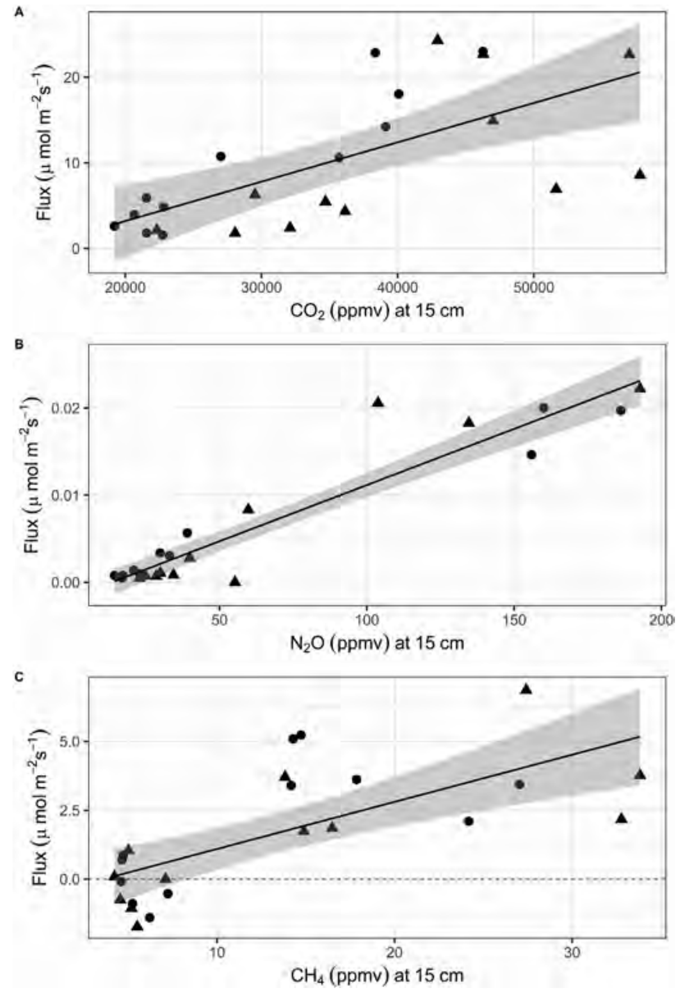

Fig. 4. Relationship between gas flux and gas concentration 15 cm below the infiltrative surface in Acushnet STA for (A) CO_2, (B) N_2O, and (C) CH_4. Circles represent measurements from the Control STA and triangles represent measurements from the Experimental STA. Grey shading represent 95% confidence interval.

3.4. Predictors of GHG flux at Acushnet

The flux of GHG from soil is partially controlled by soil physical properties (Ludwig et al., 2001; Oertel et al., 2016). Thus, we examined the relationship between surface GHG emissions and properties that may affect flux including soil temperature, soil DO, GHG levels just below the infiltrative surface, septic tank effluent water properties (total nitrogen, NH_4^+, NO_3^-, alkalinity, and pH) and nitrogen removal within the STAs. The models with the lowest AICs were those that included soil temperature, DO, and GHG concentrations at 15 cm below the infiltrative surface.

Using multiple linear regressions, we found that soil temperature, DO, and GHG gas concentrations at 15 cm below the infiltrative surface were the best predictors of emissions of all three GHGs, with R^2 values of 0.98 for CO_2 and N_2O flux, and 0.68 for CH_4 flux. Soil moisture and temperature are important drivers of GHG emissions from soils (Davidson et al., 1998; Oertel et al., 2016). Greenhouse gas concentrations at 15 cm below the infiltrative surface were always positively correlated with surface flux (Fig. 4). This, paired with our observation of significantly higher subsurface GHG concentrations in the Layered STA, makes the lack of difference in flux between the Control and Experimental STAs even more surprising.

Somlai et al. (2019) found that variation in CO_2 flux above STAs was mainly driven by daily fluctuations in soil temperature, in agreement with our results. Because CO_2 is a product of aerobic respiration, we expected to see a positive relationship between DO and CO_2; surprisingly, we observed high CO_2 flux associated with low DO (Fig. 5). The interaction of temperature and DO may explain these results: at higher temperatures, oxygen solubility decreases but microbial respiration increases, and thus CO_2 production increases (Oertel et al., 2016). The increased microbial activity further lowers DO concentration in soil pores (Oertel et al., 2016). At lower temperatures, DO is more available, but microbial activity is slower so we see a lower CO_2 at lower temperatures (Fig. 6).

The association of high CH_4 flux with low temperatures (Fig. 6) and high DO was also expected (Fig. 5) since these conditions are opposite of those that promote methanogenesis in soils (Le Mer and Roger, 2001). There are, however, reports of negative relationships between CH_4 flux and soil temperature in grasslands (Van Den Pol-van Dasselaar et al., 1998). The low CH_4 flux in the summer may be because of increased methane oxidation consumption in the surface soil when the temperature is warmer, while higher flux in cold months could be because CH_4 oxidizing bacteria slow their consumption, whereas methanogenesis is less affected by temperature changes (Le Mer and Roger, 2001).

High N_2O flux was associated with low DO levels (Fig. 5) and high temperatures (Fig. 6). Cold temperatures adversely affect denitrification and N_2O production in other STA types and in denitrification barriers designed to remove N based on the same mechanisms as Layered STAs (Robertson et al., 2008; Truhlar et al., 2019; Warneke et al.,

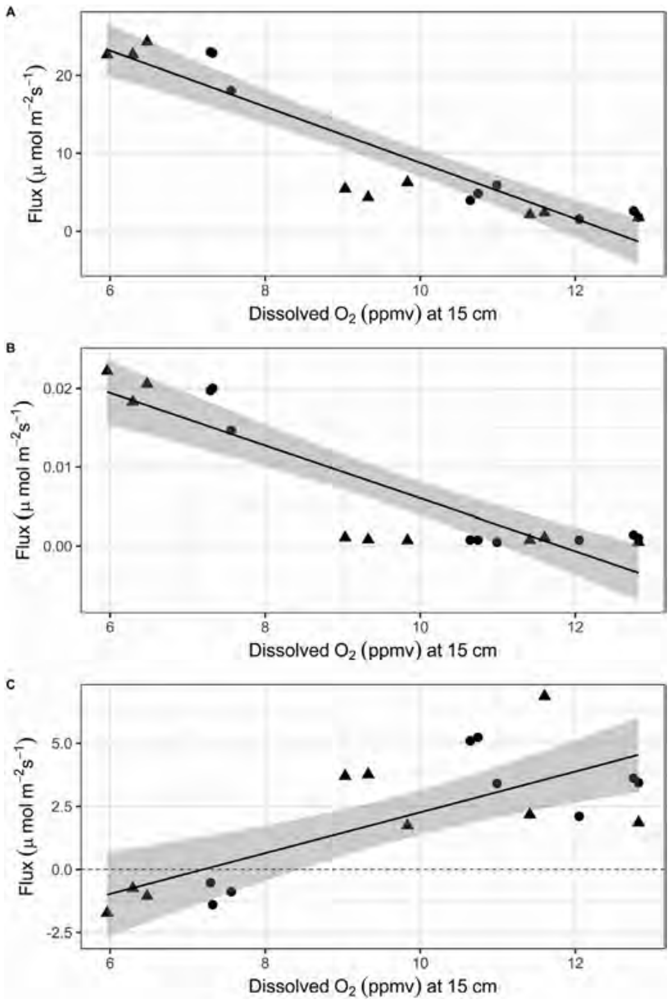

Fig. 5. Relationship between flux of (A) CO_2, (B) N_2O, and (C) CH_4 and dissolved oxygen concentrations 15 cm below the infiltrative surface in Acushnet STAs. Circles represent measurements from the Control STA and triangles represent measurements from the Experimental STA. Grey shading represent 95% confidence interval.

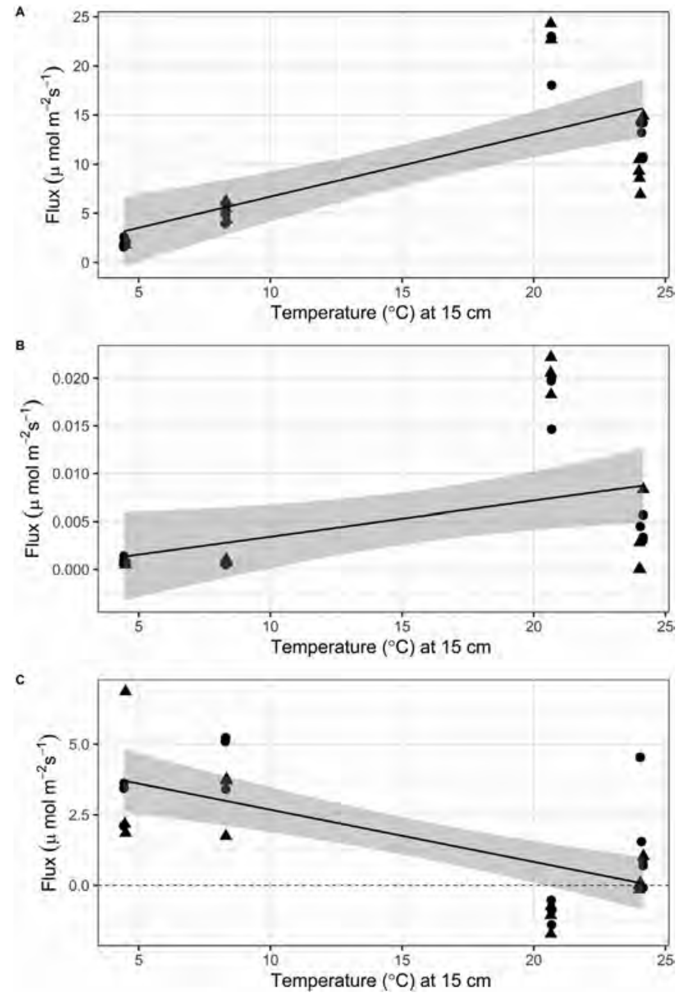

Fig. 6. Relationship between gas flux of (A) CO_2, (B) N_2O, and (C) CH_4 and soil temperature 15 cm below the infiltrative surface in Acushnet STA. Circles represent measurements from the Control STA and triangles represent measurements from the Experimental STA. Grey shading represent 95% confidence interval.

2011). Additionally, higher N_2O production is expected with low DO levels, since denitrification is limited by increasing oxygen levels (Korom, 1992).

3.5. Whole system emissions

Total GHG emissions from septic tanks at three homes ranged from 0 to 1835.4 gCO_2e capita^{-1} day^{-1}, and from 30.3 to 1938.4 gCO_2e capita^{-1} day^{-1} from the STAs. At Acushnet, emissions from the STA ranged from 48.2 ± 10 in winter to 579.9 ± 60 during the second summer of use (Table 2). While all the STAs were CH_4 sinks at some point, CH_4 consumption was never high enough to offset the GHG emissions produced by treatment. Many of the emission values we report here are much higher than those previously reported for conventional septic tanks or STAs (Table 2). This difference is likely because of our use of low-profile dispersal systems in the Control and Experimental STAs. These systems are designed to increase nutrient removal from wastewater but may result in high GHG emissions relative to conventional systems. The latter are installed deeper in the soil profile and have more soil separating the infiltrative surface and STA from the atmosphere. The reduced soil depth above the low-profle dispersal system used in,the Layered STA, lowers the opportunity for microbial consumption of GHGs relative to conventional STAs.

4. Conclusions

We did not observe a significant difference in GHG surface emissions between the Experimental Layered STAs and Control STAs at any of the sites during any sampling date. We did find evidence that onsite

Table 2
Total greenhouse gas emission in our study compared to published literature values for conventional systems. Values for this study are geometric mean and standard deviation per sampling date.

Study	Portion of treatment train	Total emissions g CO_2e cap^{-1}d^{-1}
This study (Acushnet site)[a]	Septic tank	0 to 74.4
	STA	48.2 ± 10 to 579.9 ± 60
Diaz-Valbuena et al. (2011)	Septic tank	310
	STA	Not different from ambient
Truhlar et al. (2016)	Roof vent	470
	STA	122
Somlai-Haase et al. (2017)	STA	78.2 (CO_2 only)
Somlai et al. (2019)	STA	83 (CO_2 and CH_4 only)
Somlai-Haase (2019)[b]	Septic tank	20
	STA	37

[a] Range of median values over four sampling dates.
[b] Average of two sites.

wastewater treatment does impact air quality, as both Control and Experimental STAs had significantly higher CO_2 and N_2O flux than a Reserve STA engineered identically, but not receiving wastewater inputs. We found that the Layered STA at Acushnet had higher GHG levels in the subsurface than the Control STA, but this did not translate to a difference in flux between the STAs, likely because of GHG consumption near the ground surface. These findings suggest that using lignocellulose-amended STAs to remove N from wastewater is unlikely to have adverse effects on air quality compared to other STAs with low-profile dispersal systems. Nevertheless, they may have higher GHG emissions than conventional STAs due to reduced soil depth above the infiltrative surface, which may lower the opportunity for microbial gas consumption. The GHG flux from the STA could be predicted based on subsurface temperature, DO level, and GHG concentration 15 cm below the infiltrative surface. This relationship may be used to estimate GHG emissions absent access to a continuous, real-time gas analyzer.

CRediT authorship contribution statement

Sara K. Wigginton: Conceptualization, Methodology, Formal analysis, Visualization, Investigation, Writing - original draft. **George W. Loomis:** Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition. **José A. Amador:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the homeowners for their willingness to participate. We also thank Bianca Ross, Alissa Cox, Fidda Janoudi, Connor Silverman, Owen Placido, Lincoln Dark, and Kelly Addy. This project was funded by the United States Environmental Protection Agency (USEPA) awarded to the Barnstable County Department of Public Health and the Environment by the USEPA Southeast New England Program for Coastal Watershed Restoration under Grant Agreement AWDO5317.

Although the information in this article has been funded wholly or in part by the United States Environmental Protection Agency, it has not undergone the Agency's publications review process and therefore may not necessarily reflect the views of the Agency; no official endorsement should be inferred. The viewpoints expressed here do not necessarily represent those of the United States Environmental Protection Agency, nor does mention of trade names, commercial products, or causes constitute endorsement or recommendation for use.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140936.

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STOTEN-140362; No of Pages 12

Science of the Total Environment 739 (2020) 140362

Contents lists available at ScienceDirect

Science of the Total Environment

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

Structure of greenhouse gas-consuming microbial communities in surface soils of a nitrogen-removing experimental drainfield

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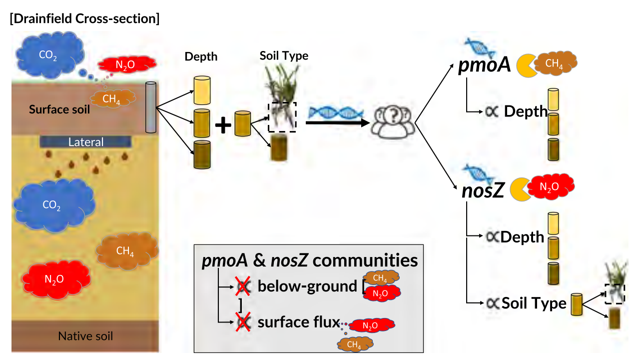
HIGHLIGHTS

- Greenhouse gas concentrations and flux not linked to CH_4 or N_2O consuming communities
- Methane oxidizing communities (*pmoA* gene) varied with depth in soil above drainfields.
- N2O reducing communities (nosZ gene) varied with depth and soil type above drainfield.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 January 2020
Received in revised form 13 May 2020
Accepted 17 June 2020
Available online xxxx

Editor: Ewa Korzeniewska

Keywords:
Wastewater
N-removing drainfield
Greenhouse gas
Microbial community
Nitrous oxide reductase
Particulate methane monooxygenase

ABSTRACT

Septic systems represent a source of greenhouse gases generated by microbial processes as wastewater constituents are degraded. Both aerobic and anerobic wastewater transformation processes can generate nitrous oxide and methane, both of which are potent greenhouse gases (GHGs). To understand how microbial communities in the surface soils above shallow drainfields contribute to methane and nitrous oxide consumption, we measured greenhouse gas surface flux and below-ground concentrations and compared them to the microbial communities present using functional genes *pmoA* and *nosZ*. These genes encode portions of particulate methane monooxygenase and nitrous oxide reductase, respectively, serving as a potential sink for the respective greenhouse gases. We assessed the surface soils above three drainfields served by a single household: an experimental layered passive N-reducing drainfield, a control conventional drainfield, and a reserve drainfield not in use but otherwise identical to the control. We found that neither GHG flux, below-ground concentration or soil properties varied among drainfield types, nor did methane oxidizing and nitrous oxide reducing communities vary by drainfield type. We found differences in *pmoA* and *nosZ* communities based on depth from the soil surface, and differences in *nosZ* communities based on whether the sample came from the rhizosphere or surrounding bulk soils. Type I methanotrophs (Gammaproteobacteria) were more abundant in the upper and middle portions of the soil above the drainfield. In general, we found no relationship in community composition for either gene based on GHG flux or below-ground concentration or soil properties (bulk density, organic matter, above-ground biomass). This is the first study to assess these communities in the surface soils above an experimental working drainfield, and more research is needed to understand the dynamics of greenhouse gas production and consumption in these systems.

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https://doi.org/10.1016/j.scitotenv.2020.140362
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1. Introduction

Onsite wastewater treatment systems (OWTS; also known as septic systems) are a common means of treating household wastewater in the United States and around the world. In the northeastern U.S. at least 25% of homes rely on OWTS (U.S. Environmental Protection Agency, 2002). Greenhouse gas (GHG) emissions are a byproduct of microbial transformations of wastewater constituents in wastewater (Brannon et al., 2017; Fernández-Baca et al., 2018, 2019; Truhlar et al., 2016). Production and consumption of GHG is driven by microbial processes that occur in septic tanks and drainfields (Amador and Loomis, 2018; Fernández-Baca et al., 2019; Somlai et al., 2019; Truhlar et al., 2016). In addition to carbon dioxide (CO_2), OWTS emit both methane (CH_4) and nitrous oxide (N_2O), potent GHGs with ~25 and ~300 times, respectively, the global warming potential of CO_2 (IPCC et al., 2014). Two particular processes are involved in the consumption - and thus possible mitigation - of GHGs:

1. Methane oxidation to CO_2 by methylotrophs and methanotrophs, which can be aerobic or anaerobic (Wang et al., 2017). Most aerobic methanotrophs rely on particulate methane monooxygenase (partially encoded by the gene *pmoA*) to perform the first step in methane oxidation, and are subdivided into type I and type II, which tend to have low and high affinities for methane, respectively (Chistoserdova et al., 2009; Knief, 2015).
2. Nitrous oxide reduction, which converts N_2O to harmless nitrogen (N) – carried out by the enzyme nitrous oxide reductase (partially encoded by *nosZ*).

Microbial consumption of GHGs is thought to happen in the soil above conventional drainfield infiltrative surfaces, and both *nosZ* and *pmoA* are ubiquitous in drainfield surface soils (Fernández-Baca et al., 2018). Previous studies have found that *pmoA* abundances are driven by CH_4 flux, and are greatest where aerobic drainfield surface soils intercept CH_4 produced deeper in the profile (Fernández-Baca et al., 2019). In contrast, *nosZ* abundances in drainfield surface soils were not found to be associated with N_2O flux (Fernández-Baca et al., 2018).

Grasses growing in lawns above shallow drainfields take up water and nutrients from wastewater dosed to the drainfield, resulting in lush green grass above laterals in stark contrast to grassy areas not located above a drainfield. The rhizosphere of these grasses has a different microbial community structure than the surrounding bulk soil, driven partly by the hypoxic regions surrounding roots, as the roots consume oxygen, and by C-rich root exudates (Philippot et al., 2013). Hypoxia, coupled with additional C sources, favors heterotrophic denitrification, so that the rhizosphere has a measurable impact on denitrifier community structure, increasing denitrifier abundance in the rhizosphere relative to surrounding bulk soil, and may affect the flux of N_2O (Henry et al., 2008).

In addition to GHGs, OWTS can contribute substantial quantities of N to the environment, posing threats to groundwater and surface water quality (Hoghooghi et al., 2016). Conventional OWTS are not designed to remove N from wastewater before it reaches groundwater (Cooper et al., 2015, 2016; Lancellotti et al., 2017), which results in high N loads delivered to coastal ecosystems (Amador et al., 2018). To address this problem, engineered advanced N-removal technologies are commonly required in coastal areas (BCDHE, 2014; Deschutes County, 2008; MDE, 2016; RIDEM, 2018). These proprietary technologies can cost homeowners more than twice as much as a conventional system, which presents a barrier to widespread adoption. A less costly, non-proprietary layered drainfield designed to remove N from septic tank effluent has been tested in Canada, Brazil and the US (NY State Center for Clean Water Technology, 2016; Robertson et al., 2000; Suhogusoff et al., 2019), and is currently undergoing field testing in Barnstable County, MA (NY State Center for Clean Water Technology, 2016). In contrast to conventional gravity-fed drainfields used in Massachusetts – which consist of sand media overlying native soil – the layered drainfield is pressure-dosed with septic tank effluent, and consists of layered media designed to facilitate passive N-removal. Nitrification takes place in an upper sand layer, and denitrification takes place in a lower layer consisting of a 1:1 mix (by volume) of sand and sawdust, providing a C source which is typically lacking in the deeper portions of conventional drainfields. This layered design, which borrows design principles from C-amended bioreactors commonly used in agriculture(van Driel et al., 2006) and groundwater remediation (Addy et al., 2016; Schipper et al., 2010), may change GHG flux patterns when compared to conventional systems, as the saturated C source could support methanogenesis, and could act as a source of N_2O from incomplete denitrification.

We examined the relationship among above- and below-ground GHG emissions and CH_4- and N_2O-consuming microbial communities as determined by *pmoA* and *nosZ*, respectively, in the surface soil above three types of drainfields serving the same household. One drainfield treats pressure-dosed septic tank effluent via a 90-cm sand layer (conventional system), and the other uses the layered experimental N-removing media described above. A third drainfield is identical to the conventional system, but is not in use. We hypothesized that surface soil community structure would vary in response to differences in below-ground CH_4 and N_2O concentration and differ with differences in surface GHG flux. Given that different types of aerobic methanotrophs favor different concentrations of methane and oxygen (Knief, 2015; Knief et al., 2003), we expected that methanotroph community structure would vary by drainfield type based on differences in methane concentrations. Additionally, we expected to find methanotroph communities to vary by depth in the surface soils of these drainfields, which likely experience a gradient of methane concentration, favoring high-affinity type I methanotrophs in the surface, and lower affinity type II methanotrophs deeper in the soil profile. Finally, we expected community structure to be different between rhizosphere and bulk soil in response to higher C availability and lower O_2 levels in the rhizosphere, likely providing conditions favorable for heterotrophic denitrifiers. To our knowledge, this is the first study to assess the methane- and nitrous oxide-consuming microbial community in the surface soil above an experimental N-removing drainfield.

2. Methods

2.1. System description

The OWTS in our study serves a single family (two adults, one young child) in Acushnet, MA, and was installed in April 2018. The system (Fig. 1) consists of a two-chamber, 5680-L septic tank, a 3785-L pump chamber with a mechanical pump that delivers a consistent dose of effluent (187.3 L per dose; ~870 L per day) to a GeoMat™ 1200 Leaching System (Geomatrix Systems, LLC, Old Saybrook, CT), a shallow pressurized network of PVC pipes above a 30-cm wide bed of plastic filaments, surrounded by geotextile fabric (Wigginton et al., 2020). The drainfield was divided into three sections (Fig. 1): (1) a reserve area, consisting of 3 GeoMat™ laterals above 90 cm of medium sand that is not dosed with wastewater; (2) a control area, consisting of 3 GeoMat™ laterals above 90 cm of sand dosed an average of 4.7 times a day; and (3) an experimental area, consisting of 3 GeoMat™ laterals on a 45-cm layer of sand underlain by a 45-cm layer of a 1:1 (by volume) mix of sand and sawdust (mainly *Quercus spp.*), dosed at the same time and with the same volume as the control area. An impermeable vertical plastic liner separates the lowest 45 cm of the control and experimental areas (Fig. 1). The entire drainfield is underlain by a 5-cm layer of peastone, designed to retain water above this interface and increase saturation and anoxic conditions of the lower layer of the drainfield.

Mean values for septic tank effluent constituents measured from system startup to the time of sampling fall within established values for residential wastewater (Amador and Loomis, 2018; Loomis and Kalen, 2014): 16.5 °C, 191.6 mg/L 5-day biochemical oxygen demand (BOD_5), 80.4 mg/L total suspended solids (TSS), 1.5 mg/L dissolved oxygen (DO), 55.1 PPM total nitrogen (TN), 228.8 mg alkalinity, and pH of 7.6.

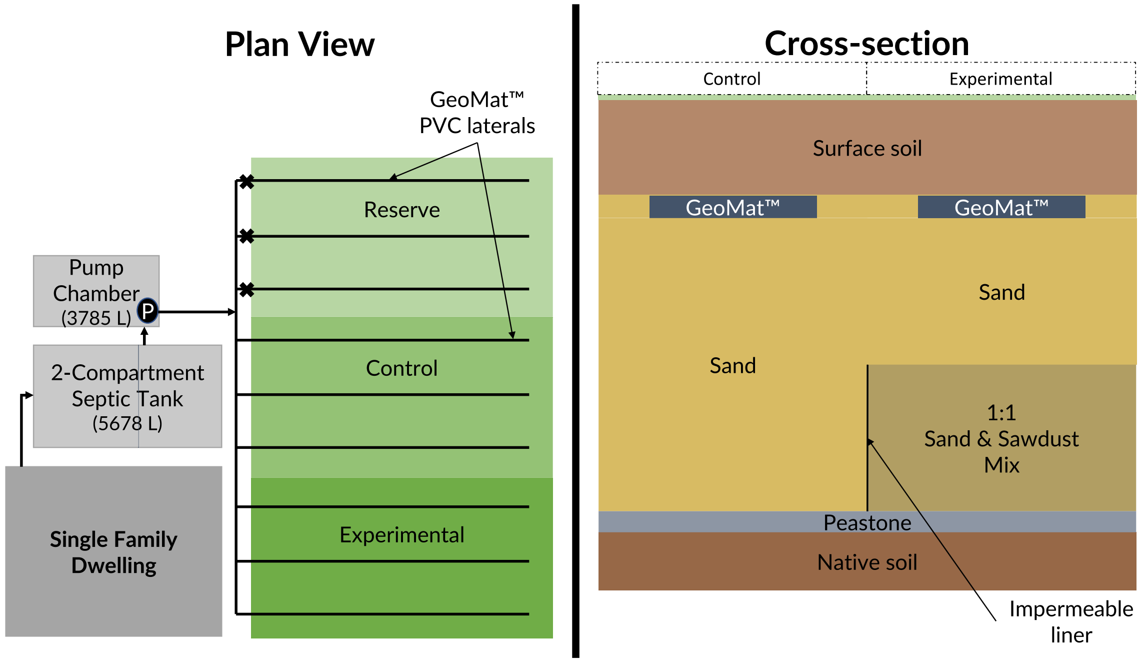

Fig. 1. Diagram of septic system layout for a single family dwelling (not to scale). Left: An aerial or plan view shows how wastewater moves from the dwelling to the septic tank, then to a pump chamber and is dosed to the drainfield. The drainfield is subdivided into three areas: Experimental (layered), Control (conventional) and Reserve (identical to conventional but not dosed with wastewater). Right: A cross section of a single lateral in the the control and experimental layered drainfields. Both drainfields are dosed with wastewater via the GeoMat™, which is located approximately 25 cm below the soil surface. The control drainfield media consists of a 90-cm layer of sand, while the experimental side consists of a 45-cm sand layer above a 45-cm layer containing a mix of sand and sawdust. Both sides are underlain by a 5-cm later of peastone, which sits above undisturbed native soil.

The system was sampled once in July 2019 to collect greenhouse gas and soil samples.

2.2. Below-ground GHG measurements

The control and experimental areas were instrumented with below-ground gas sampling chambers consisting of 17.1-cm-long and 2.5-cm-i.d. capped PVC well screen (Atlantic-Screen Inc., Milton, DE) that was connected to the surface via nylon tubing (Wigginton et al., 2020; Fig. 2). Chambers were installed approximately 15 cm below the GeoMat™ laterals, corresponding to a depth of ~38 cm from the ground surface. A three-way stopcock valve at the end of each tube, closed to the atmosphere when not being sampled, was used to collect gas samples (Wigginton et al., 2020).

Before we sampled below-ground gases, we purged the gas tubing and chambers. A 300-mL volume of gases was extracted with an air-tight 60-mL syringe and expelled to the atmosphere. Next we mixed pore space gases five times by drawing up a 60-mL gas sample and expelling the gases back into the gas chamber. A sample of the mixed gases was collected and stored in evacuated 20-mL glass vials with rubber septa that were submerged in water (Wigginton et al., 2020). Gas samples were analyzed on a Shimadzu Gas Chromatograph-2014 Greenhouse Gas Analyzer fitted with a methanizer (Shimadzu Scientific Instruments, Columbia MD), using either a flame ionization detector (250 °C, CO_2 and CH_4) or an electron capture detector (325 °C, N_2O). The column was kept at 80 °C and the injection port operated at 100 °C. The carrier gas was ultrapure N_2, flowing at a rate of 25 mL/min (Wigginton et al., 2020).

2.3. Surface GHG flux measurements

We measured greenhouse gas fluxes from the soil surface in three locations per drainfield type (Fig. 2) using a dark, closed PVC chamber (26.5 cm i.d., 13 cm height; Wigginton et al., 2020) connected to a model G2508 real-time cavity ring down spectroscopy analyzer (Picarro, Santa Clara, CA) via nylon tubing. The chamber, outfitted with a rubber gasket, was placed on a beveled ring made of 11.5-cm tall, 24.5-cm i.d. section of PVC pipe, which was pounded into the ground to a depth of ~10 cm. The chamber was left in place for 8 to 10 min at each sampling location (Brannon et al., 2016). Air temperature inside the chamber was measured continuously using a Hobo™ data logger (Onset, Bourne, MA).

Gas flux data were processed as described in Brannon et al. (2016, 2017) and Martin and Moseman-Valtierra (2015). Non-significant slopes ($p < 0.05$) were reported as no flux.

2.4. Surface soil properties

To characterize surface soil properties, we collected three soil cores in each drainfield type using aluminum cores (4.55-cm i.d., 15.1-cm tall). Cores were taken within 1 h after GHG flux measurements in the same area where these measurements were made (Fig. 2), transported in sealed bags on ice, and stored at 4 °C until analysis. For each core, we determined soil bulk density by dividing oven-dried (105 °C) core weight by the core volume (267.5 cm^3). We used the loss-on-ignition method (550 °C for 5 h) to determine organic matter content. To characterize the above-ground plant biomass, we cut vegetation at the top of each core at the soil surface and dried the vegetation at 60 °C for 2 weeks.

2.5. Soil samples for microbial analysis

Soil samples for microbial analyses were collected with a flame-sterilized coring device (2-cm i.d., 23-cm length) within 1.5 h of making greenhouse gas flux measurements in the same area where flux chambers were placed (Fig. 2). Cores were inserted into the ground vertically until resistance indicated we had reached the top of the geotextile fabric of the GeoMat™. Three cores were collected per treatment area (one core corresponding to each flux measurement location, see Fig. 2).

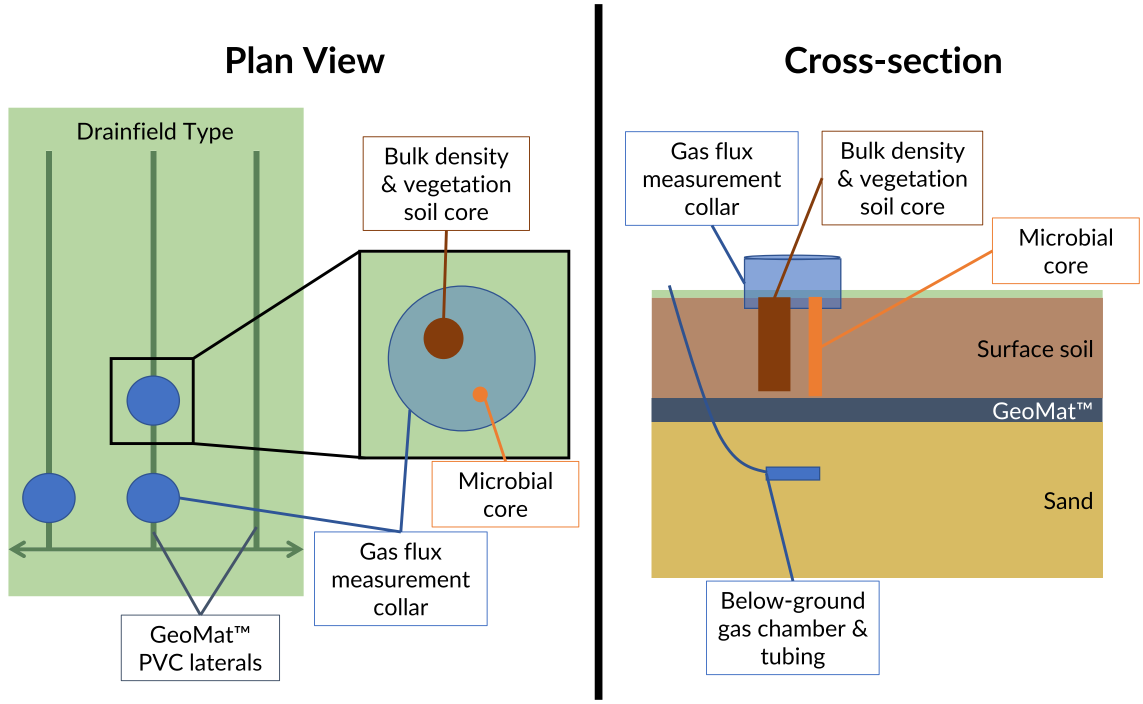

Fig. 2. Sampling scheme for greenhouse gas measurements, soil properties and microbial community analysis. Diagram is not to scale.

We used flame-sterilized tools to subdivide each core into upper (0–5 cm), mid (5–10 cm) and lower (10–15+ cm) sections. From each section we collected soil samples from the center of the core (to avoid cross-contamination) and placed them into sterile Whirlpack bags. We also separated visible roots and soil particles clinging to roots from "bulk" surrounding soils for each depth. Soil samples were transported on ice to the lab and stored at −80 °C until analysis.

2.6. Microbial community analysis

Community DNA was extracted from soil samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. 50 of the 54 possible microbial core subsamples collected in the field were included in our analysis, because four of the rhizosphere samples did not contain enough soil to extract at least 5 ng/μL DNA as determined using a NanoDrop™ spectrophotometer (Thermofisher Scientific, Waltham, MA). The DNA extracted from these 50 samples was used to amplify target gene sections.

We amplified *nosZ* and *pmoA* gene fragments in separate, single 50-μL reactions using primers *nosZ*1F and *nosZ*1662R (Throbäck et al., 2004; Wigginton et al., 2018), and *pmoA*A189cgF and *pmoA*mb661R (Costello and Lidstrom, 1999; Fernández-Baca et al., 2019), respectively. Primer pairs included a sequencing overhang (forward primer: TCGTCGGCA GCGTCAGATGTGTATAAGAGACAG, reverse primer: GTCTCGTGGGCTCG GAGATGTGTATAAGAGACAG) at the 5′ end. Supplemental material 1 contains primer sequences and PCR conditions for amplifying gene fragments. We examined PCR products on a 1% (w/v) ethidium bromide-stained agarose gel.

Amplicons were purified (Agencourt AMpure XP, Beckman Coulter, Indianapolis, IN), quantified (Invitrogen Qubit™ Flex Fluorometer, Thermo Fisher Scientific, Waltham, MA), indexed, and pooled equimolarly. Sequencing was performed on paired-end reads of 300 bp on an Illumina Miseq Next Generation Sequencer at the University of Rhode Island's Genetic Sequencing Center. Raw reads have been uploaded to the NCBI sequence read archive (SRA) database under accession number PRJNA604577.

2.7. Sequencing data analysis & statistics

We used QIIME 2 version 2019.10 (Bolyen et al., 2019) to process sequence reads and perform bioinformatic analyses. We demultiplexed and quality filtered raw reads with the q2-demux plugin, and then separated the pooled gene reads using the q2-cutadapt plugin. We used PCR primer sequences (Supplemental material 1) and their reverse complements for each gene to find and separate matching reads and trim off the primers. After this step, each gene amplicon was analyzed separately. Sequence read counts for each gene and each sample can be found in Supplemental material 1. For each gene amplicon, trimmed demultiplexed reads were joined, filtered and denoised using the q2-dada2 plugin (Callahan et al., 2016) and chimeras were removed, resulting in unique amplicon sequence variants (ASVs). We used the q2-alignment plugin to align ASVs with MAFFT (Katoh et al., 2002) to produce rooted and unrooted phylogenetic trees with FastTree 2 (Price et al., 2010).

To assign taxonomy with the q2-feature-classifier plugin (Bokulich et al., 2018), we created custom *nosZ* and *pmoA* databases. Briefly, we downloaded all FASTA sequences from the NCBI Nucleotide database matching the gene query in question ("*nosZ*"or "*pmoA*"), then converted the files to 'DNAFASTAFormat' to import into QIIME 2. We also downloaded a list of all accession numbers matching the same gene query from the NCBI Nucleotide database, and used Entrez Direct (*E-utilities on the UNIX Command Line*; Kans, 2013) to fetch taxonomy strings for each accession number. Taxonomy strings were formatted for import into QIIME 2. Using the imported sequences from NCBI for each gene, we extracted reference reads (via q2-feature-classifier) matching our PCR primer sequences (Supplemental material 1), and then trained the feature classifier with the appropriate taxonomy using a naïve Bayes approach. To assign taxonomy to our sequenced reads, we used the classify-sklearn command from the q2-feature-classifier plugin using our custom classifier for each gene.

We used the phyloseq package (v 1.31) in R (v 4.0.0; R Core Team, 2019) to explore alpha diversity and create taxonomic barplots and heatmaps in ggplot2 (v 3.3.0) from the QIIME2 outputs. Sequence reads were rarified with the vegan package (v 2.5-6) to a depth of 10 reads for *pmoA*, in order to preserve as many sequences as possible (five samples and 54 ASVs were dropped from the subsequent analysis as a result of rarefying). For *nosZ*, we rarified to a depth of 4510 reads after subsetting the data and retaining only taxa represented by at least 10 reads (two samples and 93 ASVs were removed in this step). We also used the vegan package to calculate Bray-Curtis dissimilarity matrices, and perform permutational multivariate analysis of variance (PERMANOVAS) using these distance matrices (with 999 permutations) to assess beta diversity via the Adonis function. We created dendrograms based on hierarchical clustering (hclust) with "average" distances, and visualized these with the ggdendro (v 0.1-20) package. The complete code for this analysis, including parameters used in each step in both QIIME2 and R, can be found at https://github.com/alissacox/GHG-cycling-genes.

We used R (v 4.4.0) to perform statistical analyses of soil properties, greenhouse gas fluxes and concentrations, and their relationships, which were plotted using the ggplot2 and ggpubr (v 0.2.4) packages. P values less than 0.05 were used as a cutoff to determine statistical significance.

3. Results & discussion

3.1. Surface soil properties

There were no significant differences among drainfield types for bulk density, organic matter content or above-ground biomass (Table 1), although visual inspection of each drainfield area showed much more lush grass above the control and experimental. Bulk density and above-ground biomass showed a statistically significant inverse relationship.

3.2. Greenhouse gases

All three drainfield types were net emitters of CO_2 and N_2O and net sinks for CH_4 (Fig. 3). Similar results have been reported for drainfields from conventional OWTS (Fernández-Baca et al., 2018, 2019; Somlai et al., 2019; Truhlar et al., 2016). The reserve area had the lowest CO_2 and N_2O emissions, and consumed methane at about the same rate as the control and experimental areas. Although the control and experimental drainfields had elevated N_2O and CH_4 concentrations relative to atmospheric levels 37 cm below the ground surface (Fig. 3), N_2O flux ranged from 0.01 to 0.02 μmol/m^2/h and CH_4 flux ranged from −1.73 to −0.53 μmol/m^2/h, suggesting consumption of these gases in surface soil. Previous studies of GHG fluxes from drainfield soils above conventional drainfields report ranges of −12 to 99 μmol/m^2/h for methane, and 0.14 to 14.11 μmol/m^2/h for nitrous oxide (Fernández-Baca et al., 2018, 2019).

Both N_2O and CO_2 fluxes were strongly positively correlated to above-ground biomass ($p = 0.006$ and $p = 0.003$, respectively). Additionally, multiple regression models indicate that flux values for each GHG were significantly correlated with bulk density and biomass, with positive relationships for CO_2 and N_2O, and an inverse relationship for CH_4 (p values of 0.02, 0.03 and 0.03, respectively).

Table 1
Mean and standard deviation (n = 3) of soil property values for the upper 15 cm of soil above the drainfield collected at each gas sampling point. There were no statistical differences among drainfield types based on Kruskal-Wallis testing.

Drainfield type	Bulk density (g/cm^3)		Organic matter (%)		Above-ground dry biomass (g/cm^2)	
	Mean	SD	Mean	SD	Mean	SD
Control	1.22	0.06	3.81	0.52	0.07	0.02
Experimental	1.27	0.05	4.15	1.41	0.08	0.01
Reserve	1.37	0.02	3.49	0.12	0.01	0.02

3.3. Microbial community richness, diversity and composition

3.3.1. pmoA

Our samples contained 161 unique *pmoA* sequences (or sequence variants). These 161 sequences were found a total of 3136 times across 36 samples (samples with 0 sequences were dropped from analysis; Supplemental material 1). Particulate methane monooxygenase amplicons in our dataset ranged from 265 to 440 basepairs, with a mean length of 317.5 bps and a median length of 297 bps. The mean frequency per sample was 64 (median = 25), with maximum frequency per sample of 550. Five of the 36 samples had fewer than 10 sequences, and were removed from the analysis after rarefaction. Rarefaction curves (Supplemental material 2) indicate that we had sufficient sampling depth, despite the low numbers of unique sequence variants. Fernández-Baca et al. (2018) reported similar numbers of *pmoA* sequences for communities in drainfield soils.

Analysis of alpha diversity shows that the number of observed sequence variants ranges from 1 to 27 across all samples (Supplemental material 3). The Shannon index, a measure of richness and diversity that accounts for both abundance and evenness of the taxa present (Shannon and Weaver, 1949), ranges from 0 to 2.8, and Simpson index values, which measure the sample richness by assessing the relative abundance of individual sequences (Simpson, 1949), range from 0 to 0.9. There were no significant differences in alpha diversity measures for drainfield type, depth, or soil type (bulk vs. rhizosphere), nor was alpha diversity significantly correlated with any soil properties or GHG flux or concentration values. In an analysis of upland methanotroph communities using the same primer pair in this study, only pH was found to have a significant influence on community distribution (Knief et al., 2003). Methane and oxygen concentrations, nutrient availability, temperature and soil moisture have also been described as drivers of methanotroph community structure (Knief, 2015). In contrast, Fernández-Baca et al. (2018) found no relationship between methanotroph communities and soil properties including volumetric water content, temperature or conductivity in a study of methane oxidizing communities in soil above conventional drainfields.

We also examined differences in the beta diversity of our samples using a Bray-Curtis distance matrix (Sørensen, 1948). Examination of distances using permutational multivariate of analysis of variances (Adonis) based on 999 permutations indicates that there were no significant differences in beta diversity among drainfield types. There were, however, significant differences by depth, exemplified by tendency of samples to cluster together based on depth in the dendrogram (Fig. 4 left), though visualization on a PCoA biplot based on the Bray-Curtis distance matrix does not yield insights on clustering patterns (Supplemental material 5). No other variable (soil properties, below-ground GHG concentration or above-ground GHG flux) showed a significant relationship with beta diversity patterns for *pmoA*.

The majority of the *pmoA* sequences (31.6%) are unidentified Bacteria, Methylococcaceae (27.4%), and unidentified Proteobacteria (17.7%; Fig. 5), in agreement with previously published *pmoA* sequence analyses (Sengupta and Dick, 2017). Unidentified Gammaproteobacteria (10.3%) and unidentified Bacteria from environmental samples (8.6%) are the next most abundant. Another 3.0% are unidentified members of the Methylocaccoales order, and 1.2% are in the Methylocystis family. *Methylobacter* sequences represent five of the unique *pmoA* sequence variants (0.2%) from our samples, and are only found in sample R3MB (Fig. 5). These findings are in line with those of (Fernández-Baca et al., 2018, 2019), who found similar taxonomic groups for methane oxidation in surface soils of conventional drainfields and drainfield mesocosms. These phylogenetic groups have also been reported for soils above capped landfills (Rai et al., 2019). Type I methanotrophs (Gammaproteobacteria; Hanson and Hanson, 1996) in this study appear to be more prevalent and abundant than type II methanotrophs, as described by Rai et al. (2019) and Sengupta and Dick (2017), including those in a conventional drainfield soil (Fernández-Baca et al., 2018).

Fig. 3. LEFT: Greenhouse gas flux measurements at the ground surface. The dashed line in the middle plot indicates 0 flux. RIGHT: Greenhouse gas concentrations in the control and experimental drainfields collected ~38cm below the ground surface. The reserve drainfield lacked gas sampling chambers.

Our results differ from those for a conventional drainfield soil, where the proportion of type II methanotrophs was greater than the proportion of type I methanotrophs (Fernández-Baca et al., 2018). However, our drainfield surface soils are quite shallow (<25 cm), and thus may have different gas diffusion dynamics than the soils above the deeper conventional systems (likely 90–120 cm) described by Fernández-Baca et al. (2018). This may explain why we had more type I methanotrophs (gammaproteobacterial). Type I methanotrophs are adapted to grow at low concentrations of methane, while type II (Alphaproteobacteria) are adapted to oxidize higher levels of methane and are more tolerant to low oxygen levels (Nazaries et al., 2013). *Methylocystis* spp., the only type II methanotroph present in our study, are oligotrophic (Knief and Dunfield, 2005), and were only present sporadically in our samples (Fig. 5).

While there were no differences in taxonomic composition among drainfield types, per sample taxonomic composition does appear to vary somewhat by depth: with the exception of sample R3LB, Gammaproteobacteria (Type I *pmoA*; Hanson and Hanson, 1996) appear to be restricted to middle and upper portions of the soil cores, mirroring patterns described in rice paddy soils (Lee et al., 2015) and in surface soils above drainfield mesocosms (Fernández-Baca et al., 2019). Fernández-Baca et al. (2019) hypothesized that decreasing oxygen levels below 8 cm depth may be responsible for the decline in aerobic methanotroph communities, which may explain what is occurring in this study. The *pmoA* primers used in this study do not amplify *pmoA* sequences from anaerobic methane oxidizers who couple this reaction to nitrite reduction (Luesken et al., 2011), and were not examined in this study. Anaerobic methane-oxidizing archaea (ANME-2), which are thought to use a form of reverse methanogenesis (Wang et al., 2017) - and don't have *pmoA* - were not assessed in this study either. The soil immediately above the GeoMat™ distribution system is likely to fluctuate between oxic and hypoxic/anoxic states during every dose of septic tank effluent, as the nutrients and carbon are degraded by aerobic organisms at the infiltrative surface, depleting oxygen temporarily. This cycling of oxygen concentration may make it difficult for type I methanotrophs to thrive in the deeper portions of the surface soil above the drainfield. This is in contrast to the oligotrophic *Methylocystis* spp. (Knief and Dunfield, 2005), whose distribution appears to be less constrained by depth, especially in the reserve portion of the drainfield (Fig. 5).

The composition of the methane oxidizing community does not vary among drainfield types; only sample depth is correlated with community composition of the methane oxidizers in drainfield surface soil. At installation, the surface soil microbial community across all three drainfield areas was similar, since the same soil was used to grade and landscape the surface. Although we measured elevated GHG concentrations – relative to background atmospheric levels – below the drainfield's PVC distribution network in the experimental and control drainfields, these differences do not appear to drive the surface methane oxidizer communities to diverge from the reserve portion of the drainfield. Although there was not much difference in methane flux in the three drainfield types (Fig. 3), it is unclear whether this is a function of the composition of the methane oxidation community or of other factors. The fact that the community is stratified by depth indicates that enough time has elapsed between system installation and our sampling event to establish stable, divergent communities. Monitoring of bacterial community dynamics in soil under controlled laboratory conditions indicates that soil microbial communities can become relatively stable in as few as 3–5 weeks, and maintain this stable composition for months thereafter (Zegeye et al., 2019). Therefore it is likely that the

Fig. 4. Beta diversity dendrograms based on "average" method of hierarchical clustering using Bray-Curtis distance matrices for pmoA (left) and nosZ (right). The length of the branches indicates distance between samples. pmoA samples are colored by depth and nosZ samples are colored by soil type. Sample codes indicate drainfield type (Control, Experimental or Reserve), replicate number, sampling depth (Upper, Middle or Lower) and soil type (Bulk or Rhizosphere).

communities we sampled in this study represent communities that have achieved relative stability in the ~14 months between installation and sampling.

There are several possible explanations for why the methane oxidizing community appears to vary with sampling depth, but not among drainfield types. The *pmoA* community stratified to reflect patterns typically found in drainfield soils, with more methanotrophs present at shallow depth and fewer occurring deeper in the profile (Fernández-Baca et al., 2018, 2019), but flux values may not vary sufficiently among drainfield types to result in differences in community structure among drainfields. It may be that the community we characterized via amplification of *pmoA* is not shaped or driven by methane levels in the sub-surface, a finding previously described by Fernández-Baca et al. (2018). Methane levels may not shape the communities in this study because the organisms containing *pmoA* are not relying on this gene for major metabolic functions. Our findings are in contrast to a study examining expression of *pmoA* in drainfield mesocosms, which did find communities to be shaped by methane levels (Fernández-Baca et al., 2019), though conditions in our study were likely more variable than in a mesocosm experiment using artificial wastewater. It is possible that our amplification and analysis methods (primer choice, analysis pipeline) inadvertently did not capture major players in this community. Our primers are specific to aerobic methanotrophs, and anaerobic methane oxidizers may be important members of methane oxidizing functional community in these drainfields. As mentioned earlier, *pmoA* in anaerobic methane oxidizers using DAMO process are not amplified with the primer set used in this study (Luesken et al., 2011), nor can our methods identify anaerobic methane-oxidizing archaea (ANME-2) relying on reverse methanogenesis. Finally, it is also possible that the ammonia volatilization following dosing of septic tank effluent may inhibit methane oxidizers directly above the infiltrative surface. This would only occur in the control and experimental drainfields that receive regular doses of wastewater, and may explain why more samples in the "lower" portion of our microbial cores in the control and experimental drainfields did not contain *pmoA* (Fig. 5).

3.3.2. nosZ

Our *nosZ* amplicons contained 5739 unique sequences, appearing a total of 673,960 times across all 50 samples. Sequence length ranged from 250 to 422 bps, with mean and median lengths of 408 and 414 bps, respectively. The per sample mean frequency was ~13,500, with a median of ~13,900 and a minimum and maximum frequency of about 3000 and ~31,000, respectively (Supplemental material 1). After

Fig. 5. Relative taxonomic abundance of methane oxidizing bacteria. Sample codes indicate drainfield type (Control, Experimental or Reserve), replicate number, sampling depth (Upper, Middle or Lower) and soil type (Bulk or Rhizosphere). One sequence variant in sample C1LB assigned to "Streptomyces" with 77% confidence was reclassified manually as "Bacteria".

rarefying, two samples were dropped from the analysis, along with 93 sequence variants.

Alpha diversity measures indicate a range of observed sequence variants from 67 to 380 per sample. Shannon's index ranges from 2.13 to 5.26, and Simpson's index ranges from 0.78 to 0.99 (Supplemental material 3), indicating greater diversity and richness of *nosZ* genes than for *pmoA*. This is to be expected, as *nosZ* is far more common, spanning a much wider number of phylogenetic groups than *pmoA*. There were no statistically significant differences in alpha diversity among drainfield type, or depth, nor was there a statistically significant relationship with GHG flux or below ground concentration of these gases. Though values for both alpha diversity indices are somewhat lower in the rhizosphere samples than in the bulk samples, these differences were not significant.

Based on PERMANOVA analysis with 999 permutations, there were no differences in beta diversity among drainfield types. There were, however, significant differences in beta diversity between bulk soil and rhizosphere *nosZ* communities, as well as among communities at different sampling depths. Previous research in conventional drainfield soils found greater *nosZ* abundance and variants in surface soils (Fernández-Baca et al., 2018). The dendrogram in Fig. 4 (right) shows clear clustering patterns based on soil type for *nosZ*, which can also be visualized in a PCoA biplot based on the Bray-Curtis distance matrix (Supplemental material 5). There were no other statistically significant relationships between *nosZ* beta diversity and any other variables.

In contrast to the *pmoA* taxonomic assignments, the vast majority of the sequence variants for our *nosZ* amplicons are classified as unknown Bacteria (46.9%) or unclassified Bacteria identified in environmental samples (40.6%; Fig. 6). The remaining sequences are all in the phylum Proteobacteria. The bulk of these sequences are in the alphaproteobacterial order, with many identified to genus: *Mesorhizobium* (5.3%), *Bradyrhizobium* (4.2%) and *Afipia* (1.9%). *Thauera* spp. (Zoogloeaceae) make up another 0.4% of the overall sequences, while *Azospirillium* spp. from environmental samples make up another 0.5%. Other taxa each account for less than 0.05% of the remaining sequences, and include the genera *Aeromonas* (the only Gammaproteobacterium in our samples), *Oligotropha*, *Zoogloea*, *Achromobacter*, *Pleomorphomonas*, *Hydrogenophaga*, *Sinorhizobium/Ensifer*, *Acidovorax*, *Agrobacterium*, and *Azospirillum*. Both *Thauera* and *Zoogloea* (genera in the Zoogloeaceae family) are common to wastewater treatment plants (Wang et al., 2012), and are able to utilize nitrate, nitrite and nitrous oxide as terminal electron acceptors, in addition to oxygen (Boden et al., 2017). The other Betaproteobacterial genera are also members of denitrifying families, and have been found in soil and/or onsite wastewater treatment systems (Ji et al., 2015; Orellana et al., 2014; Qin et al., 2020; Wigginton et al., 2018). In terms of Alphaproteobacteria, both the Bradyrhizobiales and Rhodospirillales orders contain genera occurring in soils and/or onsite wastewater treatment systems, and are known to complete at least some portion of the denitrification pathway (Bai et al., 2012; Orellana et al., 2014; Palmer and Horn, 2012; Wigginton et al., 2018).

Examining the taxonomic composition of the low abundance taxa shows that the reserve drainfield contains more samples with more low-abundance taxa than either of the other drainfield types (Fig. 6). Enrichment experiments in soil have shown that the original microbial community is reduced by growth on chitin-enriched soils (Zegeye et al., 2019), which may mirror the soils above the control and experimental drainfields which are dosed with N- and C-rich wastewater, and may have enriched for community members able to tolerate and thrive in these conditions in these areas. The lack of enrichment for particular

Fig. 6. Relative abundance of all nosZ sequences and their taxonomic assignments (A) and those representing <10% of the overall abundance by taxon (B). Sample codes indicate drainfield type (Control, Experimental or Reserve), replicate number, sampling depth (Upper, Middle or Lower) and soil type (Bulk or Rhizosphere).

members in the reserve portion may better reflect the original community composition of the topsoil used to for system installation. Interestingly, *Zoogloea* spp., common organisms in wastewater, are found in all drainfield types, even the reserve drainfield which was not dosed with wastewater.

nosZ is commonly found and expressed, even in organisms with incomplete or partial denitrification pathways (Albright et al., 2019; Lycus et al., 2018; Philippot et al., 2011). Thus many microbes are capable of performing the final step of denitrification, and potentially serve as a sink for N_2O (Conthe et al., 2019). The primers used in this study amplify a wider range of cultured and environmental *nosZ* organisms than other primers (Throbäck et al., 2004). The large number of unique sequence variants assigned to a variety of taxa supports this. Our results indicate that the differences in this study between below-ground N_2O concentration and surface flux (Fig. 3) are likely driven by active nitrous oxide reducers in surface soils, serving as an N_2O sink.

The ubiquity of *nosZ* among Bacteria is surprising, considering this conversion is not an energy-yielding process and there are many other electron-generating processes likely to be active in these soils. However, *nosZ* has been hypothesized to be able to effectively compete with other denitrifying reductases for electrons and thus be highly active and efficient, suggesting that organisms able to reduce N_2O may have a competitive advantage in environments with frequently fluctuating availability of electron donors and acceptors (Conthe et al., 2019). As described earlier, the soils directly above the infiltrative surface are likely to experience such cycling following every dosing event, as fresh anoxic wastewater makes contact with oxygen and the microbial communities in the immediate vicinity of the GeoMat™ distribution network. Given that *nosZ* may provide a competitive edge in this variable environment, the lack of relationship observed in this study between community composition and either N_2O flux or below-ground concentration is surprising, but has been reported in conventional drainfield soil communities (Fernández-Baca et al., 2018, 2019). It is possible, however, that the regular doses of wastewater create a gradient of available electron acceptors and donors for microbes in the soils above the drainfield, and may be what drives differences in beta diversity by depth. Long-term application of sewage sludge shapes *nosZ* communities to be different than soils amended with non-organic fertilizers, which is hypothesized to be a function of C contained in the sludge (Enwall et al., 2005). Furthermore, qualitative observations made during sampling indicate that our soil cores generally had fewer root hairs in the lower portions of the soil cores, possibly reducing some of the optimal habitat for these heterotrophic denitrifiers, and contributing to the observed differences by depth. However, the observed differences in *nosZ* communities based on soil type did support our hypothesis, indicating that the root exudates in the rhizosphere, a hypoxic area where oxygen is consumed rapidly by roots (Philippot et al., 2013), likely provide important carbon compounds to heterotrophic nitrous oxide reducers in the drainfield surface soils in this study. Similar findings have been described in agricultural systems (Hamonts et al., 2013; Henry et al., 2008).

Contrary to our expectations, we did not observe differences in methane oxidizing or nitrous oxide reducing communities among drainfield types, but found that beta diversity varied significantly with depth (*pmoA* and *nosZ*) and with soil type (and *nosZ* only). Given the complexity of microbial community dynamics across very fine scales in soils and soil aggregates (Wilpiszeski et al., 2019), it is possible that syntrophy among various community members plays an important role in these drainfield soils, and that GHG consumption is a function of more than just methane monooxygenase or nitrous oxide reductase. It is also possible is that the microbial community growing in the GeoMat™ and/or at the infiltrative surface also consumes GHGs generated in the drainfield below, affecting the concentration and flux of GHG in the soil above. Analyses of these communities and their functions could possibly help explain the observed difference between below-ground concentrations and net surface flux in this study.

4. Conclusions

We found the drainfield soils to be net emitters of CO_2 and N_2O, and net consumers of CH_4. Fluxes of each GHG were significantly correlated with soil bulk density and organic matter, but did not vary by drainfield type. When we examined methanotroph and nitrous oxide reducing communities in surface soils above drainfields, we found that methanotroph community composition varied with depth, with type I methanotrophs (Gammaproteobacteria) more abundant in the upper and middle portions of the soil samples. Neither drainfield type nor soil type (bulk vs. rhizosphere) were associated with significant differences in methanotroph communities, nor did GHG flux from the surface or GHG concentration below the infiltrative surface appear to have measurable effects on community composition. In contrast, nitrous oxide reducer community composition varied with soil type (bulk vs rhizosphere soil), but was not associated with drainfield type or any of the other soil properties or GHG surface flux or below-ground concentrations measured in this study.

To our knowledge, this is the first study to explore GHG consuming communities above an experimental passive N-removing drainfield. Onsite wastewater treatment in coastal regions is becoming more and more focused on N removal, and a better understanding of N-removing technologies' impacts on GHG emissions and the mechanisms involved is critical to understanding the true environmental impact and life cycle costs of these critical components of our wastewater infrastructure.

CRediT authorship contribution statement

Alissa H. Cox: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.**Sara K. Wigginton:**Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Validation, Writing - review & editing.**José A. Amador:**Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This project was funded by the US Environmental Protection Agency (USEPA) Southeast New England Program (under agreement 00A00249). Thanks to George Loomis, Owen Placido & Lincoln Dark for their assistance with collecting soil and greenhouse gas samples in the field. Special thanks to George Heufelder, Brian Baumgaertel and the crew at the Massachusetts Alternative Septic System Test Center for field assistance and wastewater sampling data. We are also extremely grateful to the homeowner for permission to access the property and collect samples. Although the information in this document has been funded wholly or in part by the USEPA, it has not undergone the Agency's publications review process and therefore, may not necessarily reflect the views of the Agency and no official endorsement should be inferred. The viewpoints expressed here do not necessarily represent those of the USEPA nor does mention of trade names, commercial products, or causes constitute endorsement or recommendation for use.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140362.

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ATTACHMENT 2

Cape Cod Commission, 2015

"BCDHE MASSTC Progress Report on the investigation of non-proprietary means of removing nitrogen in onsite septic systems, July – August 2014 and October –December 2014"

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GUIDANCE DOCUMENT FOR
NITROGEN REDUCING LAYERED SOIL TREATMENT AREA
ONSITE WASTEWATER TREATMENT SYSTEM

March 2, 2021

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I INTRODUCTION

Proprietary nitrogen (N) reducing onsite wastewater treatment systems (OWTS) can be an effective means of lowering N loading to critically sensitive watersheds. However, cost of these technologies is often cited as a major barrier to more widespread implementation as a regional watershed scale N-loading reduction strategy. Nitrogen (N) reducing layered soil treatment area (LSTA) OWTS technology is a non-proprietary method of facilitating sequential nitrification and denitrification of residential strength septic tank effluent (STE) utilizing only the drainfield and no other secondary treatment components. The treatment train consists of a two-compartment septic tank with a hanging pump vault in the second compartment and the layered soil treatment area (STA). The treatment process is passive, using only one pump to time-dose STE to the LSTA surface. Because the LSTA is a single pass media filter (similar to a single pass sand filter (SPSF) and bottomless sand filter (BSF)), it does not require wastewater to be recirculated between multiple compartments or actively aerated. The LSTA configuration relies only on stratification of aerobic and anaerobic carbon-amended zones within the STA, leveraging the microbial communities to sequentially nitrify and denitrify the incoming N in the septic tank effluent within the two layers of the LSTA.

II APPLICANT INFORMATION

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III TECHNOLOGY INFORMATION

An LSTA, also referred to elsewhere as a “layer cake” STA, promotes sequential nitrification and denitrification as wastewater passes vertically through the LSTA profile. This is accomplished by constructing the LSTA in two layers: a top 18-inch thick layer of ASTM C-33 sand (nitrification layer; where aerobic conditions promote autotrophic nitrification) above an 18-inch thick layer of ATCM C-33 sand mixed with lignocellulosic material (sawdust/wood chips) denitrification layer, where anaerobic conditions exist and provides a carbon source as an electron donor supporting populations of heterotrophic microbes and facilitating denitrification.

A layer of peastone at the interface with native soil helps retain moisture in the denitrification layer further promoting anaerobic conditions. In this non-proprietary design, STE is time-dosed to the top of the sand layer (in a manner similar to a single-pass sand filter/bottomless sand filter), where passive aerobic conditions allow ammonium (NH_4^+) to be oxidized to nitrate (NO_3^-). The nitrified effluent subsequently infiltrates into the underlying denitrification layer, where the water content is higher, as the lignocellulos materials used to amend the sand have a higher water-holding capacity, slowing down the diffusion of oxygen (O_2). This denitrifying layer also has a higher concentration of dissolved organic carbon (C) from the lignocellulose wood products, which serves as a C source for heterotrophic denitrification, and helps keep O_2 levels low as a result of microbial oxidation of organic C (Amador and Loomis, 2018).

Many of the concepts and components associated with the LSTA rely on exist under the current RIDEM Rules Establishing Minimum Standards Relating to Location, Design, Construction and Maintenance of Onsite Wastewater Treatment Systems (OWTS Rules) and are very familiar to the OWTS design and installation community, including; programmable timer, timed-dosing, pressure dosing, two-compartment tank with hanging pump vault, surge storage capacity in the tank, sand filtration, bottomless filters and ASTM C-33 sand media. **The only new concept is the mixing of sand and lignocellulos and its placement beneath the upper sand layer in the LSTA.** Wastewater professionals familiar with SPSF and BSF will recognize commonly used materials and components in the LSTA design.

This method of onsite wastewater treatment has proven highly effective at N-reduction in field trials conducted in the Northeast United States over the last several years.

The result of these field trials warrants additional field studies in Rhode Island (RI) to further assess the efficacy of LSTA as a standalone N-reducing OWTS technology in RI. Further, assessments of the technology with reductions in vertical and horizontal footprints, adapting LSTA for more effective use in coastal estuarine watersheds and as a potential alternative to BSF in some applications should be assessed. Therefore, we have submitted the attached RIDEM Application for Experimental Technology approval to conduct experimental installations of the LSTA in RI, with the goal of providing homeowners and the onsite wastewater community with a cost-effective alternative to currently approved proprietary N-reducing systems.

IV SUMMARY / DESCRIPTION

LSTA is a novel, passive and lower cost onsite wastewater treatment method that consists of layering a STA to specifically facilitate N reduction. The design time-doses STE over a buried stratified soil treatment area as previously described. The treatment train design is simple, has several commonly used components already familiar to wastewater professionals, and differs from SPSF and BSF technology only in the stratified modification of the filter.

Like the SPSF, the LSTA is buried and receives septic tank effluent. Comparable to the BSF the

LSTA is also bottomless. The LSTA differs from both these other media filters, in that it is specifically designed to remove N from wastewater. It also differs from any conventional STA in that same regard. The design loading rate to the LSTA, 0.70 gallons per square foot per day (gal/ft2/day) is less than the low rate SPSF (1.25 g/sf/d) as well as the high rate SPSF (2.0 g/sf/d), so hydraulic failure at the top of the sand media is highly unlikely with a managed and maintained system as required.

Data from Barnstable County, MA indicate that LSTA, when implemented as proposed here, is a highly effective means of TN reduction from residential strength STE by at least 50%. The Massachusetts Alternative Septic System Test Center (MASSTC) and Barnstable County Department Health and Environment (BCDHE) data indicate an average of 70% TN reduction from seven pilot sites in Barnstable over an operating period of two years.

V TERMS AND DEFINITIONS

- ASTM C-33 Sand - Sand that meets specific ASTM grading and quality requirements for concrete aggregates and/or the items that are added into the concrete in order to prepare it for use

- Barnstable County Department of Health and Environment (BCDHE) – Provides regulatory and programmatic management of OWTS in Barnstable County Massachusetts

- BOD5 - biochemical oxygen demand -five day. BOD5is determined by a five-day laboratory test which determines the amount of dissolved oxygen used by microorganisms in the biochemical oxidation (breakdown) of organic matter. BOD5concentrations are used as a measure of the strength of a wastewater

- Bottomless Sand Filter (BSF) - A timed-dosed sand filter used specifically as a dispersal / drainfield option for pretreated effluent which at least meets the BOD5 and TSS requirements of thirty (30) mg/L, and FOG of five (5) mg/l. The filter is intermittently pressure dosed with the effluent followed by periods of drying and oxygenation of the filter bed. Surge flow storage is achieved in the tank head space of the advanced treatment system or the bottomless sand filter dosing tank. Wastewater applied to the surface of a bottomless sand filter flows through that filter media once before infiltrating to the underlying native soils

- Charlestown On-Site Wastewater Management Program – A municipal OWTS management program established by Town Ordinance #210 under RI Gen L § 45-24.5-1 (2018) to protect the quality of Charlestown's drinking water, groundwater and surface water resources for public health and environmental management by using septic systems as a cost effective alternative to a municipal sewer system. The program is committed to serving the needs of Charlestown residents, businesses, and visitors by protecting our groundwater quality, the only source of drinking water in Charlestown, and surface water quality through the management of OWTS while providing funding, educational outreach, and technical assistance to property owners; and facilitating future economic growth balanced with resource protection

- Dosing - The pumped or regulated flow of wastewater

- Effluent - Liquid that is discharged from a septic tank, filter, or other onsite wastewater system component

- Experimental Technology - Any OWTS technology that does not meet the location, design or construction requirements as provided by the RIDEM OWTS Rules, but has been demonstrated in theory to meet the requirements of these rules and may not be in use in Rhode Island or elsewhere as an approved technology for wastewater treatment

- Filter fabric -Any man-made permeable textile material used with foundations, soil, rock, or earth

- Groundwater table - The upper surface of the zone of saturation in an unconfined aquifer; includes a perched groundwater table

- Laboratory of Soil Ecology and Microbiology (LSEM) – Laboratory located at the University of Rhode Island in Kingston, RI that conducts research focused on understanding the interplay among microorganisms, flora and fauna, and the physical environment, and how this affects the structure and composition of microbial communities and the biogeochemical processes they carry out. Learned knowledge is applied to address problems in the areas of wastewater treatment, soil quality, crop production, and ecosystem restoration, among others. The lab also focuses on science education, including novel pedagogical approaches to teaching soil science, and providing research opportunities

- Layered soil treatment area (LSTA) - A method of on-site wastewater treatment using a soil treatment area designed to facilitate sequential nitrification and denitrification of septic tank effluent

- Lignocellulose – Dry woody plant biomass consisting of cellulose, hemicellulose, and lignin.

- Maintenance - The regular cleaning of any concrete chamber, cesspool, septic tank, building sewer, distribution lines or any other component of an OWTS for the purpose of removing accumulated liquid, scum or sludge. The term, "maintenance," shall also be held to include regularly required servicing or replacement of any related mechanical, electrical, or other component equipment.

- Massachusetts Alternative Septic System Test Center (MASSTC) – Program conducted through BCDHE that conducts science-based performance assessment of various alternative and advanced OWTS technologies

- New England On-Site Wastewater Training Program (NEOWTP) - Located at the University of Rhode Island in Kingston, RI. NEOWTP offers classroom and field training experience for wastewater professionals, regulators, municipal and state officials, watershed groups, and homeowners. NEOWTP staff facilitated the design and installation of close to sixty advanced treatment demonstration and research systems installed in six

Rhode Island communities. These systems served as in-field training sites for practitioners both during (on the job site training) and after construction (tours of new technologies). Monitoring and evaluating the treatment performance of these systems has resulting in positive changes in onsite wastewater regulations and policy in the region. Working with the URI LSEM, NEOWTP researches optimizing treatment performance of advanced N removal technologies, soil-based wastewater treatment, the impact of climate variability and sea level rise on onsite wastewater treatment, and the use of vegetation to mitigate wastewater contaminants

- Nitrogen reducing technology - A wastewater treatment technology that is accepted by the RIDEM as capable of reducing the total nitrogen concentrations by at least fifty percent (50%) and meeting an effluent concentration of less than or equal to nineteen (19) mg/L

- O&M service provider - A professional who performs operation and maintenance on a wastewater treatment system

- Onsite wastewater treatment system (OWTS) - Any system of piping, tanks, dispersal areas, alternative toilets or other facilities designed to function as a unit to convey, store, treat or disperse wastewater by means other than discharge into a public wastewater system

- Pan Lysimeter - Also known as a zero tension lysimeter. A pan-like container filled with coarse material such as gravel installed beneath an OWTS soil treatment area. Percolate drains through the coarse material into the pan and is diverted into collection device allowing for access through a sampling port

- Pressurized shallow narrow drainfield (PSND) – An advanced pressure drainfield described in 250-RICR-150-10-6.37(D) of the RIDEM OWTS Rules

- RIDEM – The Rhode Island Department of Environmental Management

- RIDEM OWTS Rules - The rules and regulations Establishing Minimum Standards Relating to Location, Design, Construction and Maintenance of Onsite Wastewater Treatment System (the “OWTS Rules”)

- Septic tank - A watertight receptacle which receives the discharge of wastewater from a building sewer, and is designed and constructed to permit the deposition of settled solids, the digestion of the matter deposited, and the discharge of the liquid portion into the next treatment component or distribution box

- Septic Tank Effluent (STE) – Wastewater originating from the septic tank of an on-site wastewater treatment system

- Septic tank effluent pipe - The pipe that begins at the outlet of the septic tank or other treatment tank and extends to the next treatment component or distribution box

- Wastewater - Human or animal excremental liquid or substance, putrescible animal or vegetable matter or garbage and filth, including, but not limited to, water discharged from toilets, bathtubs, showers, laundry tubs, washing machines, sinks, and dishwashers. Both blackwater and graywater are considered wastewater under these rules

VI DESIGN CRITERIA

The Town of Charlestown is proposing to implement LSTA as designed by the BCDHE and installed at their Barnstable County pilot sites detailed above, with the following modifications:

(1) The experimental LSTA systems will not include valved control (sand-only) STAs. We believe through the work conducted by BCDHE, enough data exists to indicate that LSTA is a viable means to reduce STE N concentrations and control a STA installation is not necessary.

(2) The LSTA design will replace the 1,000-gallon pump tank in the BCDHE designs with a hanging screened pump vault located in the second compartment of a 1,500 gallon 2-compartment septic tank, which will reduce both the system's cost and footprint. The minimum septic tank size is 1,500-gallons and maximum design daily flow shall be 460 gallons per day, equivalent to a four-bedroom dwelling.

LSTA systems will be installed at dwelling units with full time occupancy to ensure analysis of N-reduction throughout all seasons. However, since data indicate that LSTA efficiency is maximized in warmer summer months, future approval of this technology will benefit Charlestown's seasonally occupied dwellings in the coastal zone which are typically only occupied during the warmer months of the year. Nearly 2/3 of the dwelling units in the RIDEM delineated Critical Resource Area (CRA) within Charlestown's jurisdictional boundary are occupied seasonally. Similar occupancy regimes are common in coastal zones throughout RI in the RIDEM Salt Ponds and Narrow River CRA's where N-reducing technology OWTS are required for any new OWTS installation.

Residential dwelling units utilized for this experimental technology installation will be limited to four-bedroom occupancy, 460-gallons designed daily total flow or less. Site soil conditions and seasonal high groundwater table shall be determined by a RIDEM Class IV Licensed Soil Evaluator for any LSTA installation.

LSTA OWTS are timed-dosed systems and will be designed using a 1,500-gallon, two-compartment septic tank equipped with a hanging screened pump vault. A surge storage volume of at least 75-gallons per bedroom will be factored into the design to provide surge flow protection to the LSTA. STE will be pressure dosed to the LSTA using GeoMat 1200 dispersal system or a pressurized shallow narrow drainfield (PSND) as a distribution mechanism at a loading rate of 0.70 gal/ft2/day. Dosing to the LSTA will be controlled by a programmable timer with an elapsed time meter and event counter capable of logging normal operational and alarm events. Effluent dosing orifice sizing shall be 3/16-inch and shall be spaced according to the RIDEM pressurized drainfield design guidance in 250-RICR-150-10-6.36 through 6.38 of the RIDEM OWTS Rules.

GeoMat 1200 or PSND is used as an effluent distribution system for LSTA. GeoMat sizing shall be 1-inch thick and 12-inches wide using 3/16-inch orifice sizing and designed in accordance with the *2016 Rhode Island Design Manual for GeoMat 1200 & 3900 Leaching Systems* and installed in accordance with the *2011 GeoMat Leaching System Installation Instructions* as applicable for LSTA described herein. PSND shall be designed using 3/16-inch orifice sizing and installed in accordance with the Advanced Pressure Drainfield guidance in 250-RICR-150-10-6.37.D as applicable to LSTA described herein.

Both GeoMat and PSND shall be installed using 3/16-inch orifice sizing and using a loading rate of 0.70 gal/ft2/day for all soil categories. These LSTA specific exceptions to standard GeoMat and PSND design must be clearly noted on all LSTA OWTS design plans.

LSTA sizing for each site will be designed on total daily design flow based upon the number of bedrooms and the loading rate of 0.70 gal/ft2/day. STE will be dispersed in a minimum of 12 doses and a maximum of 24 doses per day. Controls shall be installed as signal rated floats and include a high-water alarm and peak enable control. All design specifications shall comply with the RIDEM rules for Advanced Pressurized Drainfields according to 250-RICR-150-10-6.37of the RIDEM OWTS Rules. Orifice spacing shall not exceed 24 inches and dosing volume will be no more than 0.25 gal/orifice/dose. Pan lysimeters for final effluent collection purposes must be designed on all LSTA OWTS as detailed in **Section VII** of this Guidance Document.

1. Information on structural, electrical and mechanical components

LSTA is passive system and consists of commonly used and readily procured components found locally in Rhode Island. The electrical controls, timer and panel box are those commonly used for a single pass sand filter (SPSF). The 1,500-gallon septic tank and hanging pump vault are the same used for a SPSF and other commercial technologies. The distribution manifold and laterals are very similar to those used for SPSF, pressurized shallow narrow drainfields (PSND) and bottomless sand filters (BSF). ASTM C33 sand is used for other approved technologies and is readily available. Sawdust is available at lumber yards and sawmills. A control panel will control the pump to time-dose effluent to the pressurized distribution network in the LSTA. The drainfield distribution network will consist of 12-inch GeoMat laterals or PSND, and 1-inch dimeter PVC pipe with 3/16-inch orifices.

2. Leachfield sizing and justification.

Sizing is based on conventional STA design parameters by applying 0.70 gal/ft2/day loading rate to the dwelling total daily flow. GeoMat 1200 and PSND sizing shall be designed in accordance with applicable standards in the 2016 Geomatrix Systems, LLC Rhode Island GeoMat Design Manual and 250-RICR-150-10-6.37.D as amended and except where otherwise specified herein.

Installations of LSTA in Barnstable County have determined 0.70 gal/ft2/day to be the ideal loading to generate conditions necessary for peak LSTA N reduction efficacy. The lowest loading rate allowed by RIDEM for advanced pressurized drainfield are for Category 9 soils, extremely firm lodgement till. These rates are 1.5 gal/ft2/day for Category 1-time dosed systems and 1.0 gal/ft/day for Category 2 systems. These loading rates are designed for wastewater that has been treated through an advanced wastewater treatment unit. LSTA effluent analysis from existing installations indicates that water quality is commensurate to that from a RIDEM approved advanced wastewater treatment unit, including BOD and TSS. Therefore, our standard loading rate of 0.70 gal/ft2/day is considered a conservative rate. As part of this experimental assessment, we may assess alternative loading rates in these soils to determine the best conditions to facilitate maximum N reduction.

The infiltrative surface for the LSTA shall be the bottom of the peastone below the 18-inch ASTM C33 sand/sawdust denitrifying layer. LSTA vertical separation distance to seasonal high water table (SHWT) shall be measured from the base of the infiltrative surface and shall be a minimum of two (2) feet statewide, as approved for previously installed LSTA in Massachusetts by BCDHE. If bedrock is encountered, the infiltrative surface shall be at least four (4) feet from the restrictive layer. SHWT and soil characteristics shall be determined by an approved RIDEM Soil Evaluation.

The LSTA design criteria provides more enhanced separation distance to SHWT than existing RIDEM approved PSND or GeoMat design criteria which allows for a two (2) foot separation distance to SHWT in native soils without the addition of three feet of additional effluent treatment provided by LSTA.

3. Design restrictions or limitations.

All LSTA OWTS designs shall be limited to residential use only and not to exceed 460-gallons per day total flow or four-bedroom sizing to be commensurate in capacity with one Orenco, Inc., AdvanTex AX-20 Pod sized for a four-bedroom dwelling.

LSTA sizing will be similar to a conventional OWTS drainfield, pipe on stone (trenches) or flow diffusers. No reduction in STA sizing would be allowed since STE is untreated prior to the LSTA portion of the OWTS. LSTA use may be limited at marginal sites where vertical separation to water table does not allow for necessary vertical setback.

3.1 Compliance with Section 250-RICR-150-10-6.41F.2.c-d of OWTS Rules

Upon approval, the Town of Charlestown will manage and oversee the installation of LSTA OWTS within the Town's jurisdictional boundary. All installations conducted under this approval shall be implemented solely to replace failing or substandard septic systems under RIDEM "OWTS Applications for Repair" and no increases in flow or new construction activities shall be part of the approval.

For each installation, the Town of Charlestown will comply with the Section 250-RICR-150-10-6.41F.2.c-d of the RIDEM OWTS Rules. To ensure compliance, the Town of Charlestown will establish funds held in reserve equivalent to installing one Orenco Systems, Inc., AdvanTex AX-20 Nitrogen Reducing OWTS Pod as part of the LSTA treatment train. Costs to design, install and procure the AX-20 pod and necessary equipment are estimated not to exceed $20,000 for each experimental system installed. These funds will be utilized by the Town to repair replace or take any other action required if the department determines that the LSTA fails to meet the performance claims after two years of is found to be a failed OWTS.
With each RIDEM OWTS Application for Repair submitted under this approval, a signed statement detailing fiscal responsibility to repair, replace or modify the experimental technology if it fails to perform as designed shall be submitted. The signed statement shall clearly state who is responsible for the cost of repairing, replacing, or modifying the OWTS and the specific funds held in reserve shall be identified as part of each signed statement.

VII INSTALLATION CRITERIA

LSTA installation will incorporate practices similar to the site preparation for BSF or SPSF. LSTA will be designed using 18-inches of ASTM C-33 sand over 18-inches of a 1:1 ratio by volume ASTM C-33 sand and untreated sawdust sourced from local sawmills and lumber yards. All sand used in the LSTA shall be in a damp state when added to the LSTA. Sand / sawdust mixture shall be mixed at a location with a clean hard surface (concrete or asphalt) to eliminate contamination with soil/dirt/debris at the construction site. The mixture will then be transported to the site for installation. The sand/sawdust mixture shall be placed in nine-inch lifts and compacted with a standard duty forward plate compactor in a single pass.

The sand/sawdust layer will overlie a two-inch layer of double washed peastone as a textural break to help maintain saturated and anoxic conditions in the denitrifying sand/sawdust layer. The base of the LSTA excavation shall be scarified to provide an additional textural break. The sidewalls of the LSTA will be lined with impervious 30-mil polyethylene / PVC liner to prevent effluent outflow in loose or friable soil conditions and enhance saturated conditions in the denitrification layer. A layer of geotextile landscape fabric will be placed immediately above the dispersal system to preclude the migration of fine material into the LSTA. Cover material consisting of six to eight inches of loam, loamy sand or sandy loam shall then be placed above the effluent dispersal lines.

<u>Conceptual LSTA layout from BDCHE:</u>

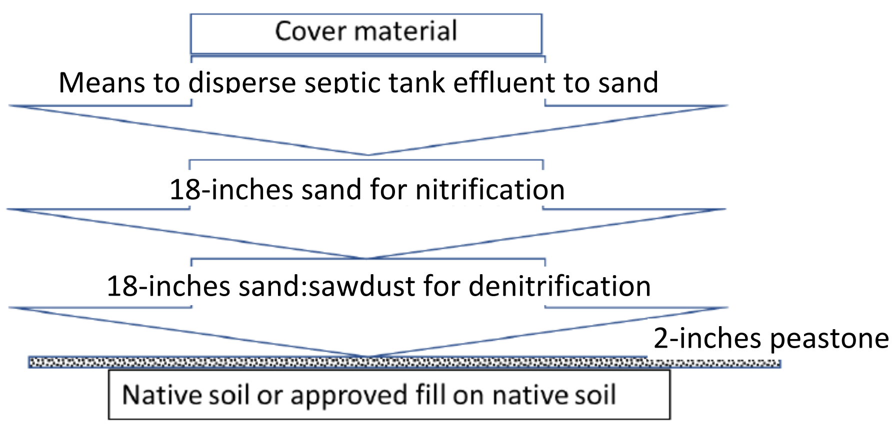
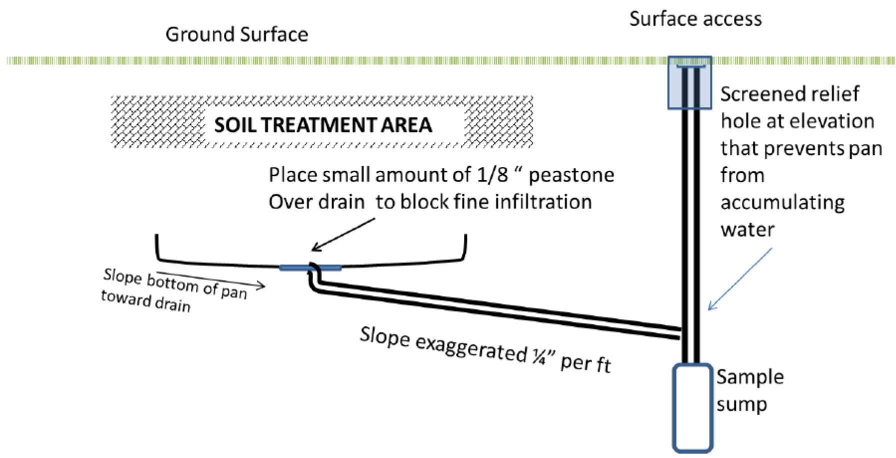


Figure 1 Conceptual representation of layered system for denitrifying percolate beneath onsite septic system soil absorption systems.

Excerpted from Heufelder 2020

A minimum fill perimeter of 10-feet shall be established to ensure the invert elevation of the infiltrative surface for the pressure drainfield option is extended for a minimum of 10-feet from the laterals.

A pan lysimeter with PVC sampling port shall be installed in each LSTA to provide sampling access to final effluent. Pan lysimeters shall be installed according to design criteria established by BCDHE detailed below:



VIII OPERATIONS AND MAINTENANCE/COST/MONITORING REQUIREMENTS

Operation of the LSTA is simple since the technology is single pass and requires only one pump. Under normal operation, annual O&M visits are required. As part of the Experimental approval, systems will be inspected and sampled at least monthly in accordance with the sampling protocol set for the in Section VI.C.3 of the LSTA RIDEM Application for Alternative / Experimental Technology and described below. Final reporting will be submitted to RIDEM based on data collected for two years from each LSTA OWTS as detailed of Section VI.C.3 of the referenced application and described here.

1 Extent of required maintenance

Annual maintenance involves pump and float inspection, tank inspection, obtaining elapsed time meter and pump cycle count data from panel, and inspection of LSTA area. Annually, laterals will also be flushed, snaked, and flushed again, in accordance with pressurized drainfield maintenance requirements. Hanging pump vault, pump, and float cleaning will be required, and the O&M service provider shall conduct this cleaning on an as-needed basis. Visual observations of site LSTA condition including yard maintenance activities, settling, surface water ponding or other issues with the LSTA shall be recorded.

1.1 Sampling Protocol

During experimental trials, forward flow shall be determined at each site visit using elapsed pump cycle meter. STE and final effluent shall be sampled for DO, temperature, BOD_5, TSS, pH, TN, Nitrate, Nitrite, Ammonium, Alkalinity, FOG and TKN (reported by equivalent analysis provided by subtraction). STE shall be collected from septic tank pump basin and final effluent shall be collected from the pan lysimeter installed within the LSTA. Samples shall be collected using standard procedures by Town of Charlestown staff and partners and will be transported on ice under chain of custody protocol to LSEM for laboratory analysis. Sample results for key regulated parameters shall be compared to the following performance standards:

Performance Standards:

Analyte	**Performance Standard (mg/L)**
Total Nitrogen	19
BOD5	30
TSS	30
FOG	5

Analysis of fats, oil and grease (FOG) is not warranted. Any fats, oil or grease (FOG) present in untreated OWTS effluent that is not sequestered in the septic tank will be absorbed and entrapped within the initial one to two inches of aerobic sand media, similar to single pass sand filter. Further, LSTA shall be utilized only for residential applications where FOG is typically not a factor affecting effluent treatment. Requirement of FOG analysis in this application is an unproductive utilization of fiscal resources. However, one FOG sample per LSTA shall be collected during the second monitoring event, six months into each system operation. If FOG is detected above the performance standard of 5 mg/L, subsequent confirmatory sampling will be implemented.

pH data from existing installations in Barnstable County summarized as part of this application indicate that of the seven monitored LSTA OWTS, a total of 184 observations of final LSTA effluent pH were collected. Average pH measurement was 6.5 with a mean of 6.4 and a standard deviation of 0.74. The maximum recorded pH measurement was 9.6 and the minimum was 4.4. The pH of final effluent is considered to be equivalent of that with any other approved OWTS technology and pH warrants no additional protocols or mitigation measures as part of this experimental assessment.

Regulatory applicability for meeting the performance standards shall be considered by achieving the required standards on a yearly average basis for each parameter at each system monitored. Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Within six months of the completion of the two year LSTA sampling protocol for each LSTA installed under this approval, efficacy data and operational summaries shall be submitted to RIDEM in report format to detail results, conclusions and next steps including compliance with OWTS Rule 6.41.F.2.c&d.

Monitoring shall be conducted for each installed LSTA for a period of no less than two years from system startup date. Two years following the final system sampling protocol, a final monitoring report shall be submitted to RIDEM from the Town and LSEM to discuss results, conclusions and next steps including compliance with RIDEM OWTS Rule 6.41.F.2.c & d.

Subsequent to the final monitoring report, a series of four quarterly samples shall be collected and analyzed according to the referenced protocol for each LSTA starting the fifth operating year of each LSTA installed under the experimental approval. Sampling results shall be submitted to RIDEM for evaluation of performance standards.

2. Technical qualifications for required operation and maintenance personnel

Completion of INSP-100 at the New England Onsite Wastewater Training Program (NEOWTP) is required to provide O&M personnel with a background in basic OWTS function, and inspection and maintenance competency. Additionally, completion of LSTA operation and maintenance training class conducted through the NEOWTP will be required. These programs will be offered to practitioners by utilizing the experimental installation sites.

3. Availability of parts/system components in the case of failure or routine maintenance

Operation of the LSTA is simple since the technology requires only one pump. All components and parts are readily available and often used as replacement parts for other already approved technologies.

4. Long term reliability and life expectancy of individual components and the entire system

Long term viability of LSTA is no different than any other approved RIDEM OWTS. Preliminary research regarding the longevity of the LSTA carbon source indicates at least a multiple decadal temporal scale (at least 30 to 40 years). As with all technologies utilizing pumps and float switches, these would need to be replaced as needed on the LSTA system.

5. Warranties or guarantees

LSTA is non-proprietary therefore no warranties or guarantees are applicable. Warranties from the manufacturers of pumps, floats, and panel boxes components may apply as with other technologies.

6. Precautions needed for noise or odor control

System is single pass technology with one timed-dosed effluent pump. Noise or odor control is not an issue.

IX TRAINING/QUALIFICATIONS

Designers – A RIDEM Soil Evaluation is required to be conducted by a RIDEM Licensed Class IV Soil Evaluator in accordance with 250-RICR-150-10-6.10 of the RIDEM OWTS Rules. OWTS design shall be completed by a RIDEM Licensed Class I or II Designer or a Professional Engineer who has completed an LSTA design training class training class conducted through the NEOWTP.

Installers – LSTA must be installed by a RIDEM Licensed OWTS Installer under direction of a Class I or II Designer or a Professional Engineer that meets the qualifications for LSTA design

O&M Service Providers - Completion of INSP-100 at the NEOWTP is required to provide O&M personnel with a background in basic OWTS function, and inspection and maintenance competency. Additionally, completion of LSTA operation and maintenance training class conducted through the NEOWTP will be required.

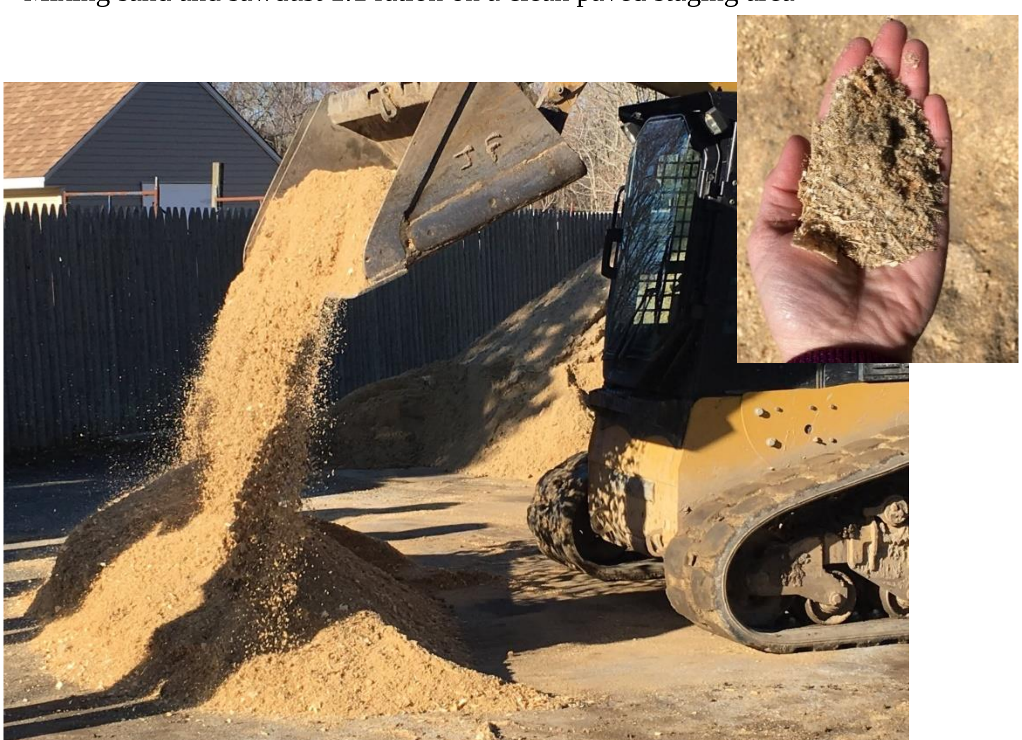
X DETAILS

See attached for scale example plan and detail spec sheet

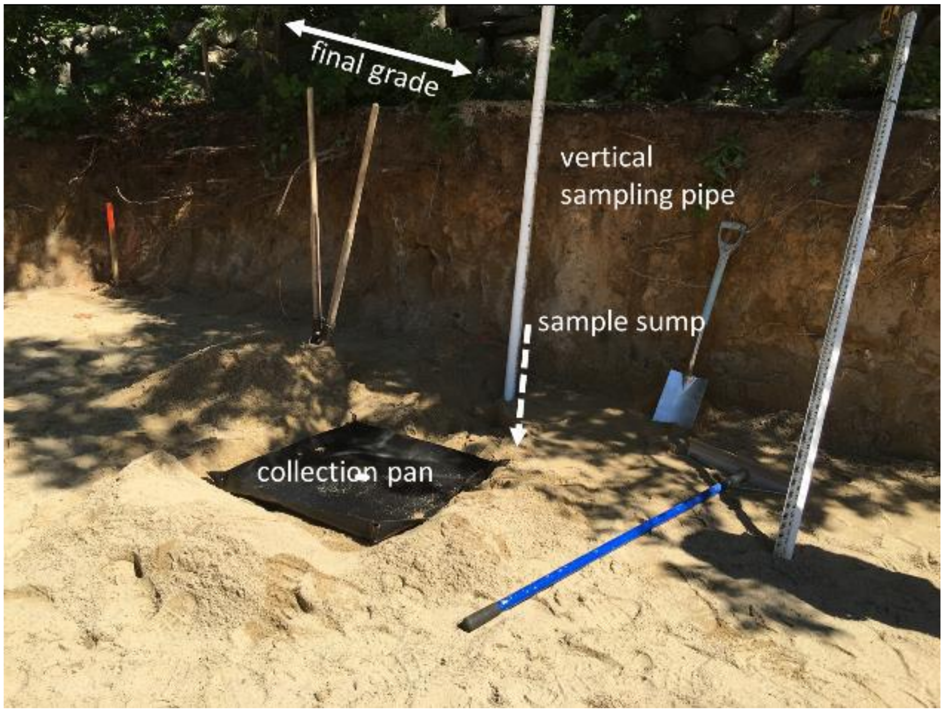
XI APPENDIX

Installation Photos – From a LSTA installed in Acushnet, Massachusetts 2018

Mixing sand and sawdust 1:1 ration on a clean paved staging area



Mixing sand and sawdust 1:1 ration on a clean paved staging area, a close-up of sawdust material used at this site



Pan lysimeter installation - final effluent sample collection equipment before installation of LSTA

Installing pan lysimeter final effluent sample collection equipment before installation of LSTA

Compacting in first layer of 1:1 mixture, see liner. Note: this demonstration project also used a control STA with sand only as shown on the left of the LSTA

"Walking in" lifts of aerobic zone sand, sampling equipment visible

"Walking in" lifts of aerobic zone sand, sampling equipment visible

“Walking in” lifts of aerobic zone sand, sampling equipment visible

Finishing the grade of lifts of aerobic zone sand, sampling equipment visible

Compacting and finishing the grade of lifts of aerobic zone sand, sampling equipment visible

Installing GeoMat above aerobic zone

Installing GeoMat above aerobic zone

Applying cover material above GeoMat

Cover material installed

Site restoration completed

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ATTACHMENT 3

EXAMPLE SITE PLAN
EXAMPLE EQUIPMENT DETAILS

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GENERAL & CONSTRUCTION NOTES - EXAMPLE

1. SYSTEM INSTALLER IS RESPONSIBLE FOR CONSTRUCTION IN ACCORDANCE WITH RIDEM OWTS RULES AND ALL OTHER APPLICBLE RULES, REGULATIONS AND STANDARDS
2. SYSTEM INSTALLER IS RESPONSIBLE TO CLEAR SITE WITH "DIG SAFE" PRIOR TO WORK AND SHALL BE RESPONSIBLE FOR IDENTIFYING AND LOCATING ALL UNDERGROUND UTILITIES
3. NO CHANGE TO THIS DESIGN IS ALLOWED WITHOUT THE PRIOR WRITTEN APPROVAL
4. THE SYSTEM AND ALL ASSOCIATED WORK MUST BE INSPECTED AND APPROVED BY RIDEM AND THE TOWN OF CHARLESTOWN
5. IF A "STRIP OUT" OF UNSUITABLE MATERIAL IS SPECIFIED THEN INSPECTIONS AS REFERENCED ABOVE WILL BE REQUIRED PRIOR TO THE PLACEMENT OF IMPORTED MATERIAL.
6. IF THE SYSTEM INSTALLER DISCOVERS VARIED SITE OR SOIL CONDITION, UNANTICIPATED GROUNDWATER ELEVATIONS, OR ANY CONDITION THAT IS INCONSISTANT WITH THE DESIGN THEY SHALL IMMEDIATELY NOTIFY THE TOWN OF CHARLESTOWN
7. FINISH GRADE SHALL SLOPE TO DRAIN (2% MIN.) OVER ALL SEPTIC SYSTEM COMPONENTS
8. THIS PLAN AND DESIGN ARE PREPARED AND INTENDED FOR THE SOLE PURPOSE OF THE SEPTIC UPGRADE. THE PLAN, DESIGN, AND INFORMATION CONTAINED HEREIN SHALL NOT BE USED FOR ANY OTHER PURPOSE.

DESIGN FLOW - EXAMPLE

RESIDENTIAL USE : 3 BEDROOMS AT 115 GPD/BEDROOM = 345 GPD REQUIRED

BOTTOM AREA AS DESIGNED = 493 S.F.

SIDEWALL AREA AS DESIGNED = 0 S.F.

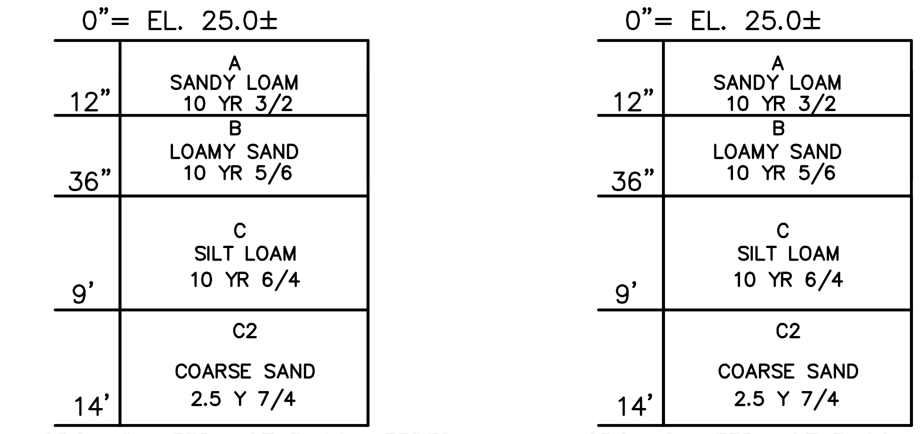
TOTAL LEACHING AREA PROVIDED = 15' X 33' LSTA = 495 S.F.

LOADING RATE = 0.7 GPD/S.F.

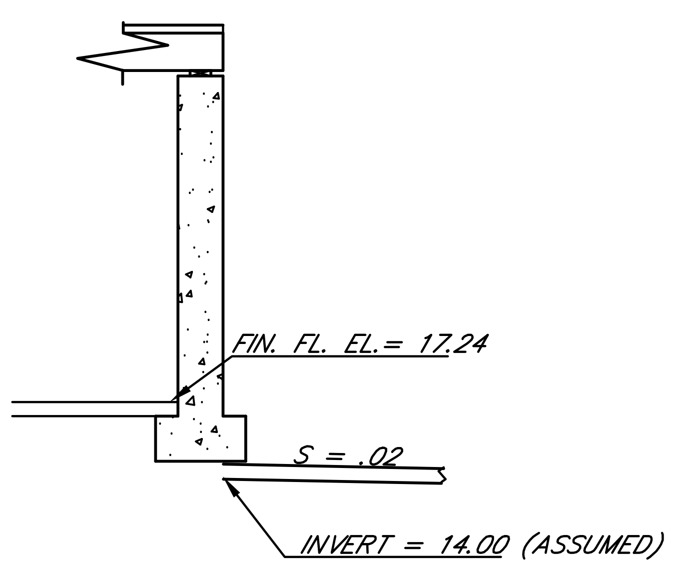
SYSTEM DESIGN CAPACITY = 345 GALLONS/DAY

NOTE: NO GARBAGE DISPOSAL ALLOWED WITH THIS DESIGN

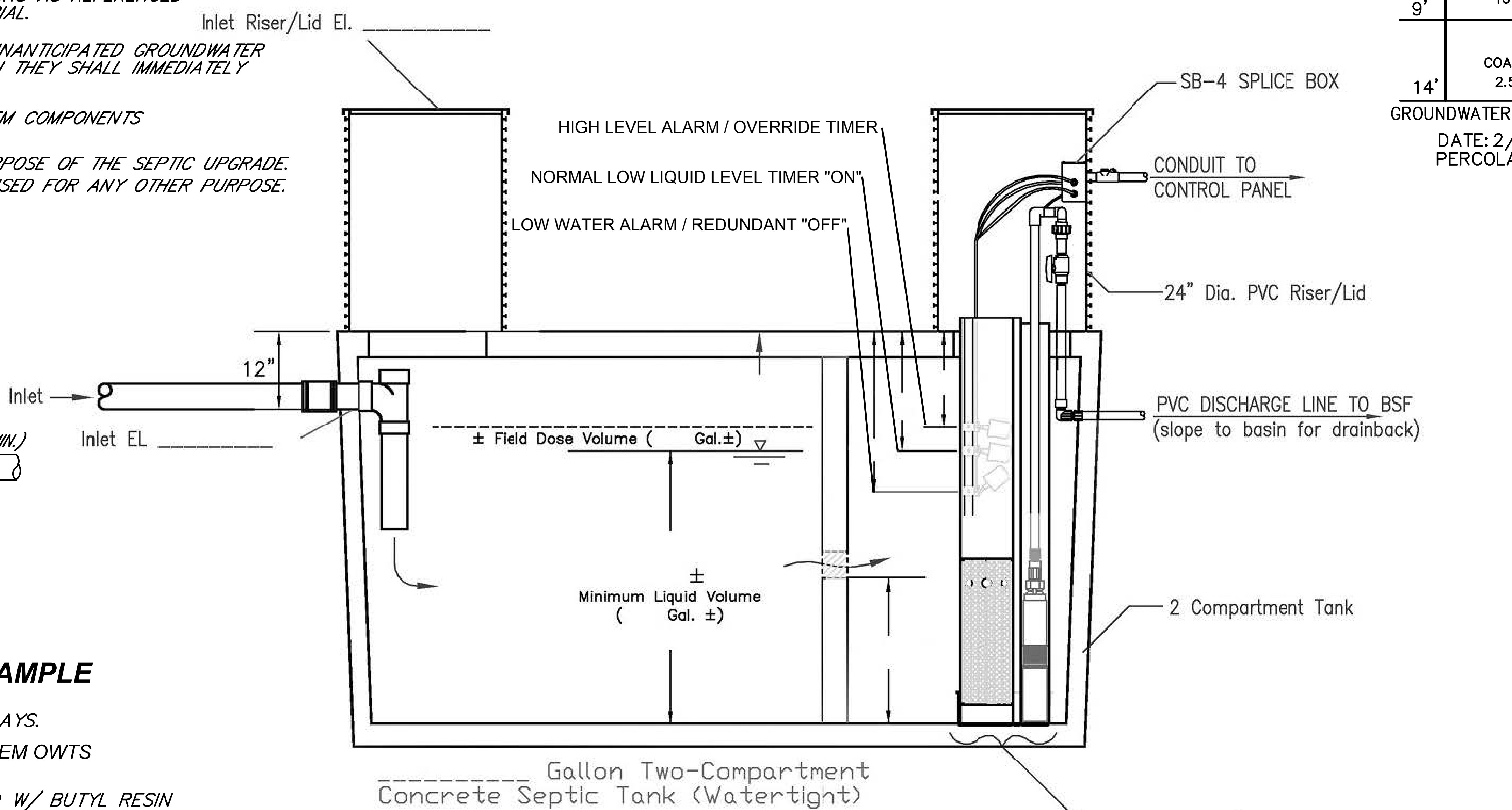
TEST PIT DATA - EXAMPLE



DATE: 2/25/16 SOIL EVALUATOR: DAVID H. MARTIN, P.E.
PERCOLATION RATE: 5 MIN./IN. WITNESSED BY: DAVID CARIGNAN FAL. B.O.H.



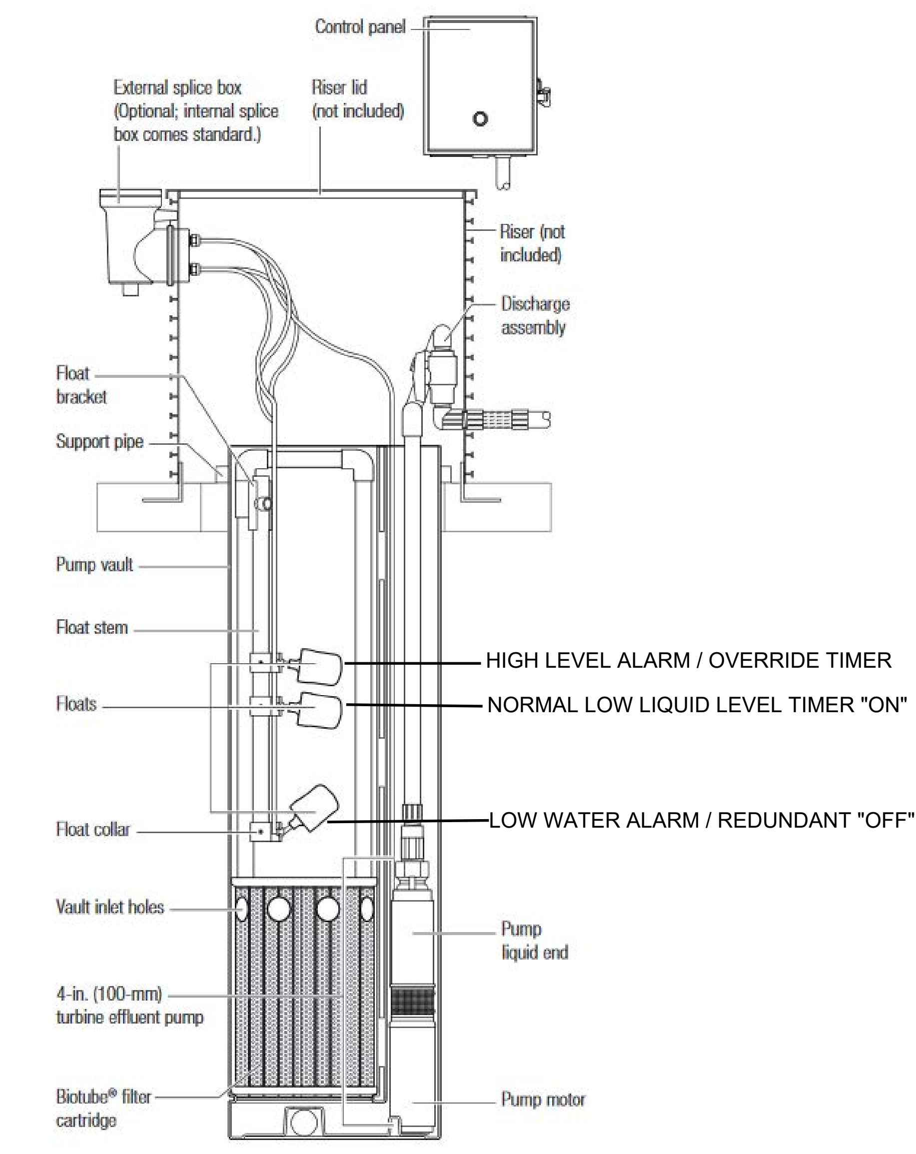
NOTE: FOR EXISTING DWELLINGS THE INVERT ELEVATION AT FOUNDATION SHALL BE FIELD VERIFIED BY INSTALLER PRIOR TO ANY CONSTRUCTION



SEPTIC TANK AND DISTRIBUTION BOX TO BE SET ON LEVEL STABLE COMPACTED BASE OF 6" LAYER OF CRUSHED STONE

1500 GALLON SEPTIC TANK (H-10)

NOT TO SCALE



Biotube® ProPak™ pump package components.

SPECIFICATIONS - EXAMPLE

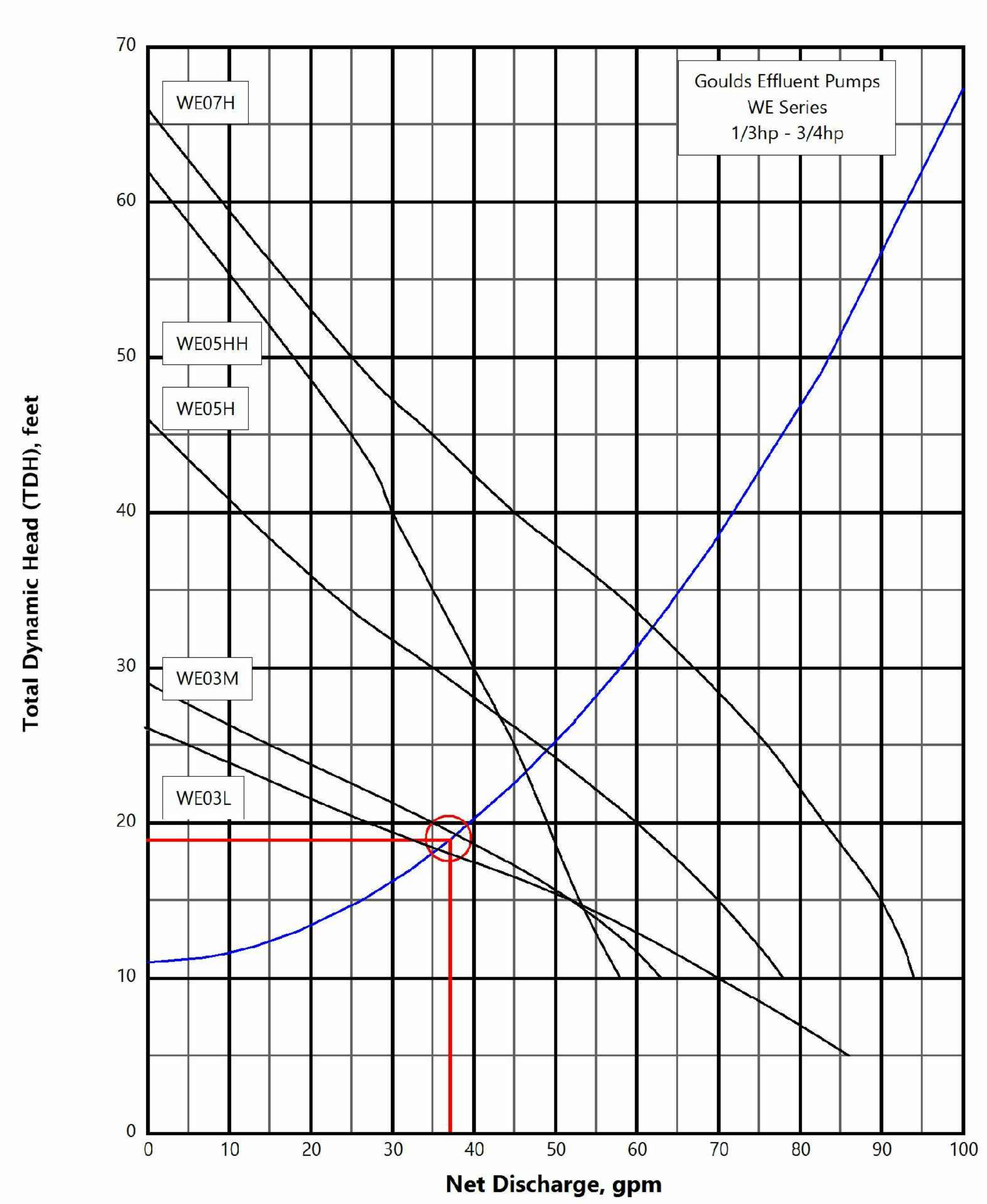
1.) CONCRETE 5,000 PSI AFTER 28 DAYS.
2.) CONSTRUCTION CONFORMS TO RIDEM OWTS RULES 250 RICR-15-10-6
3.) TONGUE & GROOVE JOINT SEALED W/ BUTYL RESIN
4.) REINFORCEMENT PER ASTM C1227-93.
5.) DESIGN LOADING AASHTO HS20
6.) WEIGHT 16,575 LBS

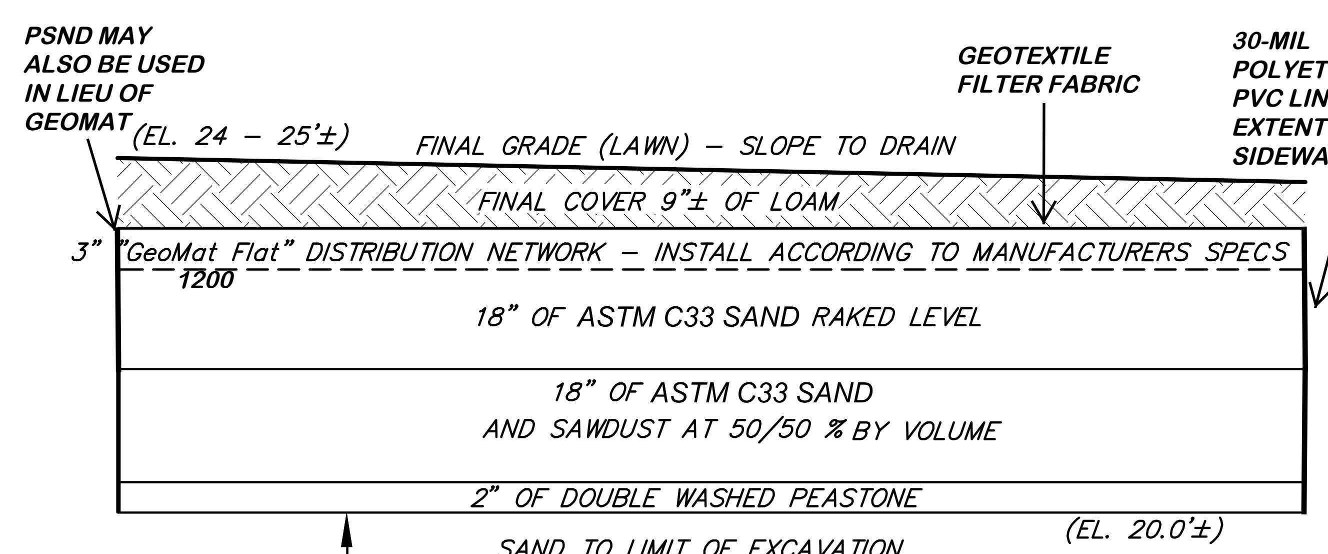
Calculations for a GeoMat System

Orifice Size	3/16	inches
Residual Head at Last Orifice	3.0	feet
Orifice Spacing	3.0	feet
Number of Laterals (all zones)	4	
Perforated Lateral Length	35.0	feet
Non-Perforated Lateral Length	0	feet
Lateral Line Size	1.00	inches
Lateral Pipe Class/Schedule	40	
Distributing Valve Model	None	
Manifold Length	10.0	feet
Manifold Line Size	2.00	inches
Manifold Pipe Class/Schedule	40	
Lift to Manifold	11.0	feet
Transport Length	60.0	feet
Transport Line Size	2.00	
Transport Pipe Class/Schedule	40	inches
Discharge Assembly Size	2.00	inches
Flow Meter	None	inches

Calculations

Minimum Flow Rate per Orifice	0.76	gpm
Number of Orifices per Zone	48	
Total Actual Flow Rate	37.1	gpm
Number of Lines per Zone	4	
% Flow Differential 1st and Last Orifice	8.4	%
Lift to Manifold	11.0	feet
Residual Head at Last Orifice	3.0	feet
Head Loss in Perforated Laterals	0.7	feet
Head Loss in Non-Perforated Laterals	0	feet
Head Loss Through Distributing Valve	0.0	feet
Head Loss in Manifold	0.1	feet
Head Loss in Transport Pipe	1.4	feet
Head Loss Through Discharge	2.8	feet
Head Loss Through Flow Meter	0.0	feet
Total Flow Rate	**37.1**	**gpm**
TDH	**18.9**	**feet**





INFILTRATIVE SURFACE 50% SEPARATION DISTANCE REDUCTION:
2.0 FEET TO ESHWT IN SALT PONDS OR NARROW RIVER CRA AND STATEWIDE
4.0 FEET TO BEDROCK

SECTION

(NOT TO SCALE)

PUMP AND PUMP CHAMBER NOTES

1. PUMP SHALL BE SINGLE SYSTEM INSTALLED ON A SLIDE RAIL SYSTEM
2. THE PUMP SYSTEM SHALL BE EQUIPPED WITH A CONTROL PANEL INSTALLED IN A READILY ACCESSABLE AREA.
3. THERE SHALL BE AN AUDIO/VISUAL ALARM MOUNTED IN AN AREA EASILY VISIBLE FROM THE OUTSIDE OF THE BUILDING.
4. ALL SLIDERAILS AND MOUNTING HARDWARE SHALL BE STAINLESS STEEL.
5. INSTALL PUMP SWITCHES AT THE ELEVATIONS INDICATED.
6. THE HATCH COVER SHALL BE WATERPROOF AND WATERTIGHT.
7. PUMP SHALL COMPLY WITH ATTACHED SPECIFICATIONS

1 - D.H Martin Engineering, Inc, Plan for Layer Cake Septic System for BCDHE at 239 Sippewisset Rd, Falmouth, MA, June 6, 2017 - DWG Name 16130DET3.DWG, 14 Quisset Ave, Box 741 Woods Hole, MA 02543, 774-836-0693, dhmartineng@gmail.com

EXAMPLE LSTA SEPTIC SYSTEM DETAILS

prepared for:

RIDEM OFFICE OF WATER RESOURCES
EXPERIMENTAL TECHNOLOGY
APPLICATION FOR LSTA

By Town of Charlestown RI
Modified from plan by D. H. MARTIN ENGINEERING, INC.[1]

SCALE: AS SHOWN	Town of Charlestown, RI Office of Wastewater Management 4540 South County Trail Charlestown, RI 02813	DATE: MARCH 2, 2021
DES./DRAWN.:		DWG. REV-003
APPROVED:		SHEET 2 OF 2

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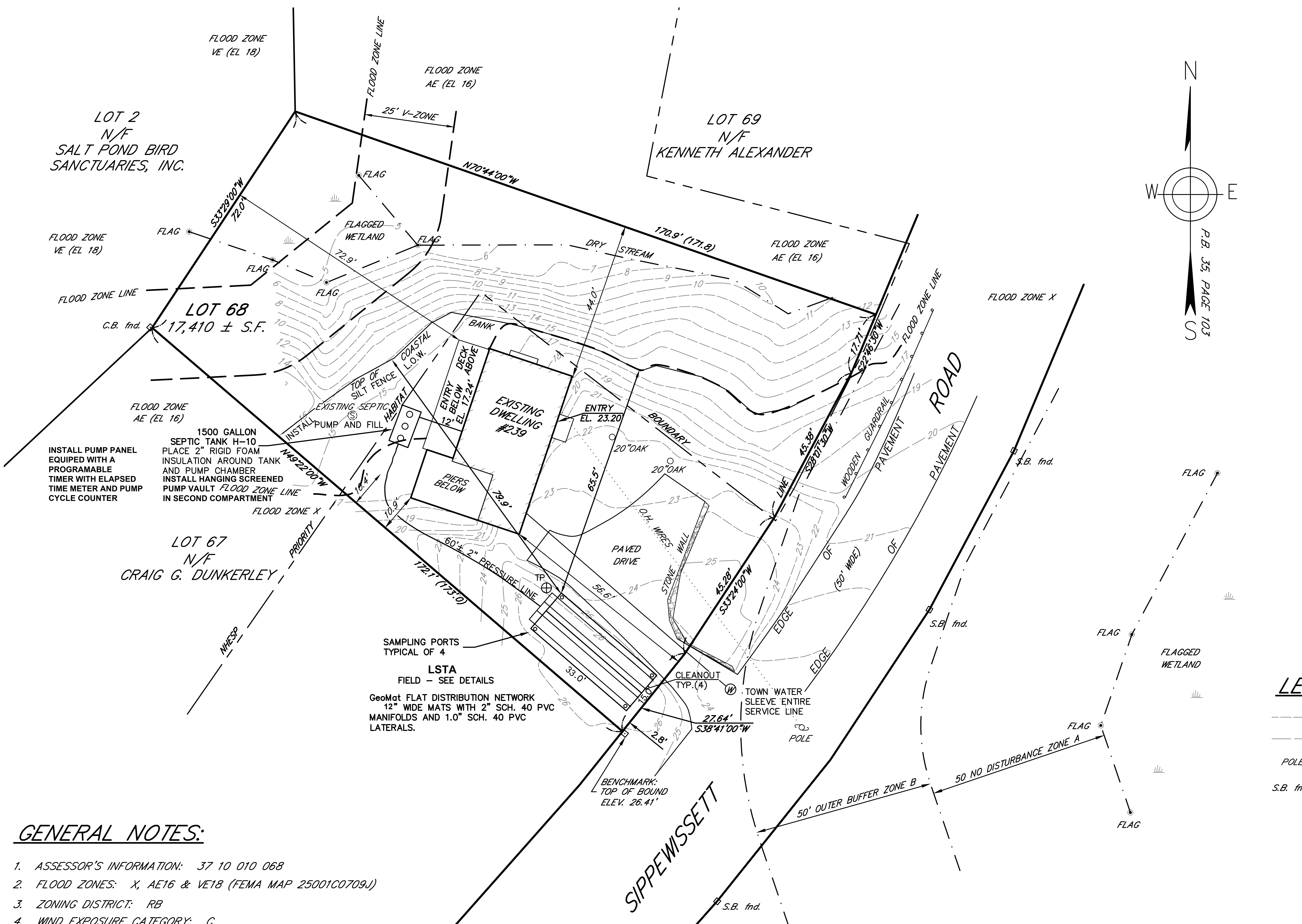

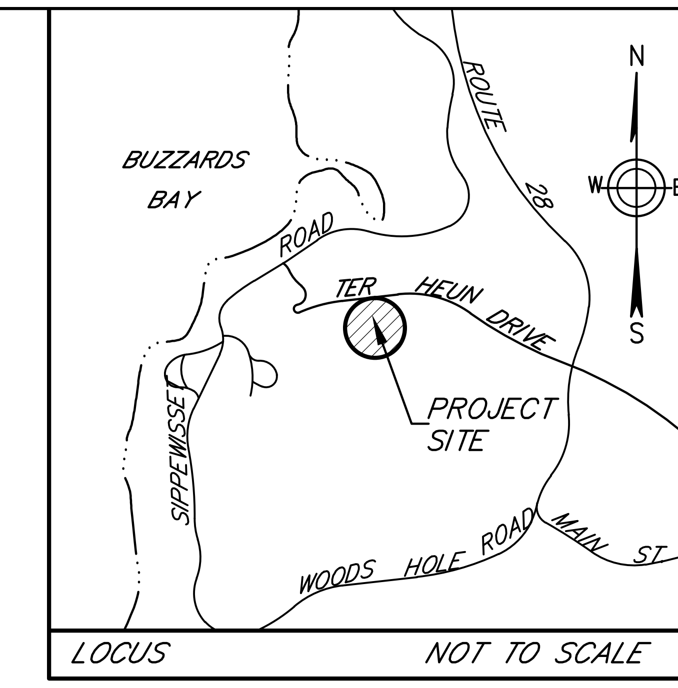

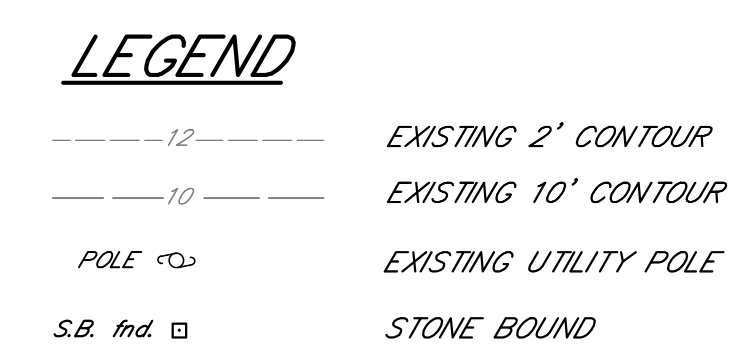

GENERAL NOTES:

1. ASSESSOR'S INFORMATION: 37 10 010 068
2. FLOOD ZONES: X, AE16 & VE18 (FEMA MAP 25001C0709J)
3. ZONING DISTRICT: RB
4. WIND EXPOSURE CATEGORY: C
5. OVERLAY DISTRICT: NHESP PRIORITY HABITAT
6. LOT COVERAGE BY:
   A. EXISTING STRUCTURES: 1,506 S.F./ 17,410 S.F. = 8.7 < 20%
   B. EXISTING STRUCTURES/PARKING/PAVING: 2,761 S.F./ 17,410 S.F. = 15.9%
7. WIND BORNE DEBRIS REGION: YES
8. STREET ADDRESS: 239 SIPPEWISSETT ROAD
9. ALL SETBACKS (NO ±): AS SHOWN
10. SEPTIC LOCATION: AS SHOWN
11. HOUSE NUMBER: 239
12. TOPOGRAPHIC INFORMATION COMPILED FROM AN ON THE GROUND SURVEY
13. ELEVATIONS SHOWN ARE BASED ON NORTH AMERICAN VERTICAL DATUM 1988.

VARIANCES REQUIRED:

1. LOCATE S.A.S. 2.8'± FROM FRONT LOT LINE (10' MIN. REQD.)
2. LOCATE S.A.S. 5' FROM SIDE LOT LINE (10' MIN. REQD.)

20 0 10 20 40

SCALE: 1 INCH = 20 FEET

1 - D.H Martin Engineering, Inc, Plan for Layer Cake Septic System for BCDHE at 239 Sippewisset Rd, Falmouth, MA, June 6, 2017 - DWG Name 16130SITE/DET3.DWG, 14 Quisset Ave, Box 741 Woods Hole, MA 02543, 774-836-0693, dhmartineng@gmail.com

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prepared for:
RIDEM OFFICE OF WATER RESOURCES
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APPLICATION FOR LSTA

By Town of Charlestown RI
Modified from plan by D. H. MARTIN ENGINEERING, INC.[1]

SCALE: 1"=20'	Town of Charlestown, RI Office of Wastewater Management 4540 South County Trail Charlestown, RI 02813	DATE: MARCH 2, 2021
DES./DRAWN.:		DWG. NAME: REV-003
APPROVED:		SHEET 1 OF 2