ABSTRACT

Title of Thesis: IN SITU AND LABORATORY STUDIES OF SOIL TREATMENT AREAS EXPERIENCING FLOODING

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Onsite wastewater treatment is used by over one in five American households to treat wastewater by soil biogeochemical transformations. In Maryland alone, 420,000 septic systems are in use primarily in rural and near coastal areas. Issues of sea level rise can threaten coastal infrastructure due to flooding damage that also can impact the ability of soil to efficiently treat nutrients found in wastewater. In this study, two onsite wastewater treatment systems with different soil types and treatment techniques were assessed in Anne Arundel County, Maryland. It was found that soil texture can impact the health of a soil in its function of treating wastewater, in addition to treatment techniques affecting inorganic nitrogen in the soil treatment area. To model the impacts of flooding damage to a soil treatment area, tidal flooding with fresh, brackish and saltwater was simulated in a laboratory-scale column study. The results from the month-long study showed decreases in the treatment efficiency for inorganic nitrogen and dissolved organic solids.

IN SITU AND LABORATORY STUDIES OF SOIL TREATMENT AREAS EXPERIENCING FLOODING

by

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Chapter 1: Introduction

Septic Systems usage in Maryland

Septic systems were first developed by John Mouras, a French engineer, in the 1860's, to deal with wastewater in decentralized systems [33]. He patented the technology around 1881, and a few years later around 1883, septic systems arrived in the United States of America. Septic systems started finding common usage after World War II, with an economic boom leading to more houses being built.

In the United States, there are currently more than 26.9 million septic systems that are used for wastewater treatment in primarily rural areas [34]. Septic systems are responsible for removing and reducing contaminants such as nitrogen, phosphorus, pathogens and suspended solids. In the state of Maryland, there are approximately 420,000 septic systems being used, and 52,000 of these systems are in the critical area, which is defined as being 1,000 ft within a tidal zone [28]. The Eastern Shore of Maryland consists of nine counties that discharge water into the Chesapeake Bay. Approximately 450,000 people live on the Eastern Shore of Maryland. In Maryland coastal communities that rely on septic systems, there is a need to ensure that their wastewater is being safely treated, and that the soil treatment areas of septic systems are consistently maintaining their function of treating wastewater.

Introduction to Septic Systems

A conventional septic system consists of a septic tank and a soil treatment area (drain field). In a septic tank, household wastewater undergoes primary treatment (Figure 3), where organic solids are anaerobically digested by heterotrophic microorganisms [27]. The drain field is considered secondary treatment, in which septic tank effluent undergoes nutrient transformations and pathogen removal, characterized by changes in biogeochemistry of the soil. Here, biofilms form on the soil particles approximately 30 to 60 cm below the infiltration point from the effluent pipe out of the septic tank (Figure 3).

Conceptual Model

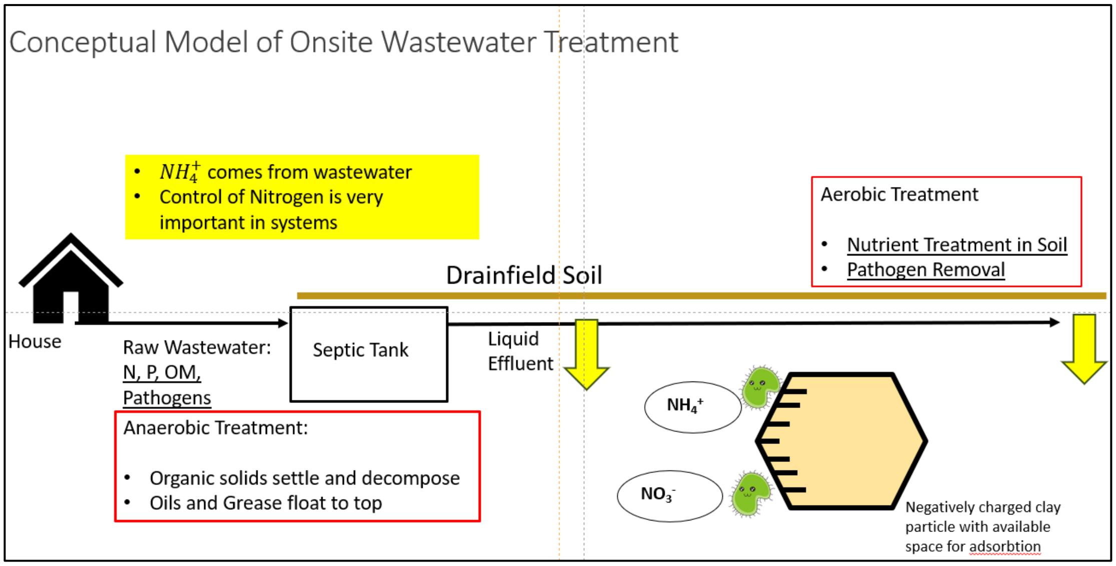


Figure 1: Septic System Conceptual Model

Nitrogen Loading into the Chesapeake Bay

The Eastern Shore of Maryland contributes about 6% by area of the drainage basins into the Chesapeake Bay, which translates to about 5 km^3/yr in terms of overall flow of water [1]. Nitrogen species in the waters of the Chesapeake Bay originates from a variety of sources such as agriculture, (de)-centralized wastewater treatment, forestry, and urban runoff, all contribute to loading into the Chesapeake Bay [2]. With the advent of commercial fertilizer use due to the industrialization of the Haber-Bosch process, nitrogen loading significantly increased [31], leading to an excess of nutrients in the Chesapeake Bay, causing issues today, such as harmful algal blooms and hypoxic waters [5]. The total nitrogen loading into the Chesapeake Bay comes from a variety of sources, with the majority from agricultural runoff (Figure 1) [2].

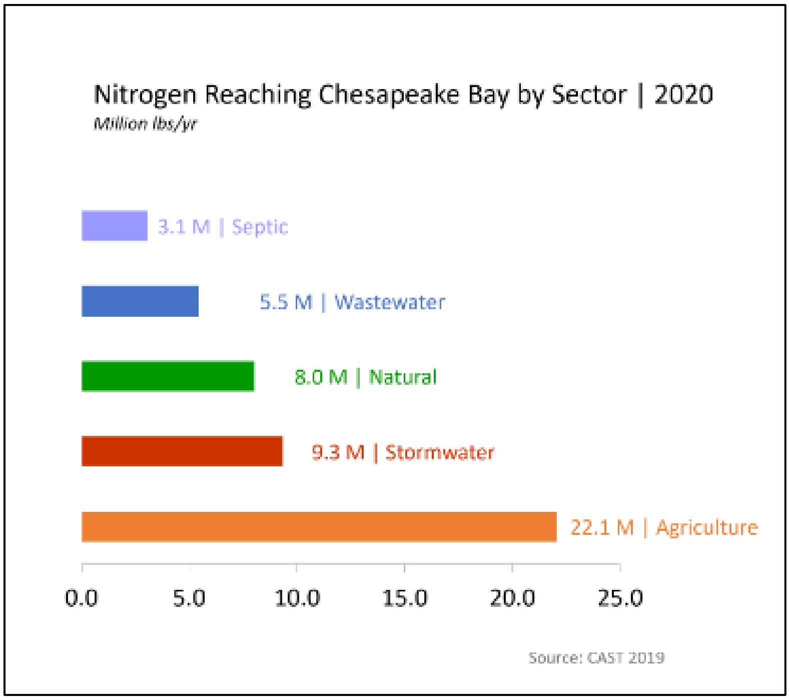


Figure 2: Total Nitrogen Reaching Chesapeake Bay by Sector for 2020 – Adapted from Maryland's 2020 Chesapeake Bay Annual Progress

Septic systems continue to be a major non-point source of concern, as the effluent from septic tanks after initial treatment contains NH_4^+, with the average amount being in the range 30-50 mg/L [27]. This NH_4^+ is converted into nitrate in the soil-treatment zone (i.e., drain field), by microbial processes. If the nitrate is not properly filtered and/or converted to nitrogen gas via denitrification, it can enter the groundwater, where it can have potential health impacts, with levels of >10 mg/L being harmful [3]. Potential harmful impacts include blue baby syndrome for infants (a condition caused by excessive nitrate ingestion, where the hemoglobin in blood is converted to methemoglobin, making it such that oxygen cannot be carried in the blood), as well as increased risk of cancer [3].

Using the Chesapeake Assessment Scenario Tool (CAST19) model, the total nitrogen load into the Chesapeake Bay originating from septic systems increased from 5.45 to 7.46 million pounds per year from 1985-2021 (Figure 2) [6]. With nitrogen loading from septic systems increasing over time, there can be an increased risk of nitrate toxicity in groundwater [3].

Modeled Nitrogen Loads to the Chesapeake Bay (1985-2021)

Loads simulated using CAST19 and jurisdiction-reported data on wastewater discharges. *The natural sector includes, in part, forests and wetlands which are preferable land use types with the lowest loading rates among sources.

VIEW CHART VIEW TABLE

Loads by Source Loads by Jurisdiction

Year	Agriculture	Developed	Wastewater	Septic	Natural	Atmospheric Deposition to Watershed	Atmospheric Deposition to Tidal Water
1985	156.28	27.36	94.54	5.45	48.51	15.90	21.52
2009	123.02	37.95	56.09	7.56	46.19	7.18	19.80
2021	117.05	40.27	29.97	7.85	45.31	0.88	16.19

Figure 3: Modeled Nitrogen Loads (10^6 lbs./yr) to the Chesapeake Bay (1985-2021)

Groundwater contributes about 54% of the total annual volume of water into the Chesapeake Bay, in addition to nitrate contributing about 50% of the total nitrogen [7]. As nitrate gets released into the groundwater, due to increased development and human activity, it can remain there for an average of 10 years [7]. The residence times of nitrate can vary due to the geology of the streams as well as land usage around the many streams that feed into the Chesapeake Bay [7]. With the increase of nitrogen loading into the Chesapeake Bay from septic systems over time (Figure 2), as well as high residence times of nitrate, it is important to treat nitrogen as much as possible in the soil treatment area of a septic system to reduce it from entering the waters of the Chesapeake Bay.

Leaching of nitrate occurs when the soil pores fill with water and the gravitational potential pulls the water downward. The percolating water carries soluble salts (including nitrate). It is less likely for nitrate to move through soils with a high clay content due to slower percolation and presence of more micropores, while sandy soils allow for an easier flow of water carrying soluble salts. In addition, the loading of salts can lead to swelling and shrinking in clay soils. This can create preferential flows paths leading to increased nitrate flushing [26].

Effects of Nitrogen Loading into water

Eutrophication mainly occurs due to excess nutrients entering waterways, allowing for phytoplankton and plants to grow, which subsequently become food/resources for fish and other invertebrates. This cycle continues until the biomass reaches a threshold, thus leading to the commonly seen algal blooms [8]. Once the algal blooms reach a threshold, they also start to die off leading to bacteria to decompose the algae, consuming oxygen and turning the waters hypoxic. This can render the services provided by this aquatic system negligible, leading to a loss of both plant and aquatic life, as well as loss of economic/cultural benefits provided by the water body. In a meta-analysis, it was found that the combined losses from eutrophication were around $2.2 billion annually in United States freshwaters [9]. Some of the chemical effects include increase in the pH of the water system, decrease in the dissolved oxygen level, and decreases in the aesthetic value of the water [11]. Since nitrate is often a limiting nutrient for water systems, the effort to reduce sources of nitrogen will have an important impact on the health of a water body [8]. Historically in the Chesapeake Bay, natural filters have existed that protect against nutrient loading, such as wetlands, riparian forests (forested areas near a body of water), and oysters (which naturally filter algae/nutrients from water) [10, 11].

Sea level rise predictions and effects on septic performance

In a 2008 report by the Climate Change Commission's Scientific and Technical Working Group *(STWG)* titled, *"Comprehensive Assessment of Climate Change Impacts in Maryland"*, it was projected that Maryland would experience a relative sea level rise (SLR) ranging from 2.7 ft (0.82 m) to a 3.4 ft (1.03 m) increase by 2100, dependent on greenhouse gas emission levels [13]. This SLR has specific consequences for Chesapeake Bay in terms of "tidal increases". With the rising sea levels, comes an increase in the volume of receiving water to the Chesapeake Bay, but more importantly, a change in the tidal range of the Chesapeake Bay. The report stated that a one-meter sea level rise would result in a 1.6 ft (0.487 m) increase in tidal range in much of the Maryland portion of the Chesapeake Bay [13]. With a larger tidal range, more water inundation in parcels along the Chesapeake Bay could occur, leading to issues with septic systems. In a study of SLR impacts on tidal range in the Chesapeake Bay region, researchers found that tidal range could decrease *if* low-lying land were allowed to flood [14]. The implications of this could mean that larger cities such as Washington, D.C. and Baltimore could have reduced the impacts of tidal flooding at the expense of low-lying areas.

In terms of septic systems, the higher water tables can affect the soil-based treatment area (drain field) by reducing the volume of unsaturated soil as well as oxygen available for nitrification. This in turn can result in an untreated effluent, containing a greater load of pathogens, and nutrients (nitrogen and phosphorus), to the receiving groundwater [15]. In the 2016 paper titled "*Hell and High Water: Diminished Septic System Performance in Coastal Regions Due to Climate Change"* researchers found that in batch experiments, total nitrogen removal was diminished due to climate change in shallow-narrow type drain fields, while total nitrogen removal marginally increased in conventional type drain fields [15]. In addition, for both types of drain fields, under climate change conditions, there was a reduction in the removal of pathogens.

Processes occurring in a Septic Tank

Septic tanks are underground chambers that receive the wastewater as it comes from the house, in which they are typically constructed of concrete and are made to be watertight. In conventional systems, septic tanks are anaerobic chambers, where the main function is to settle the organic solids that are initially present. A technology upgrade, such as aerobic pre-treatment, can pump air into the septic tank, allowing for the treatment of nitrogen in the septic tank, before it reaches the drain field, via nitrification (the microbial conversion of ammonium to nitrate). Pre-treated effluent can help reduce the burden placed on the soil in the drain field to treat the nitrogen, as certain soil types, such as sandy soils, cannot adequately treat or retain nitrogen [17].

Soil Properties of a Drain field

An important factor of soil in a drain field is the size and composition of the particles [17]. The pore size of a soil system, (which is a combination of the particle size and composition of a system) can influence the surface area available for biological transformations of organic matter. Due to the heterogeneity of soil matrices (Figure 4), ranging from sand to clay with varying content of organic matter, different pore sizes exist that can affect hydrologic movement of wastewater and influence the movement of gas flow through the pores of the soil [17].

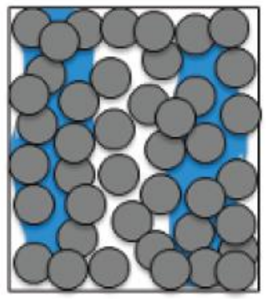
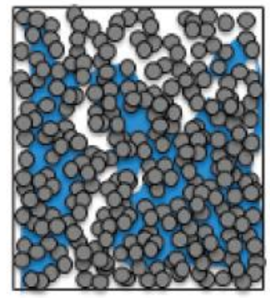
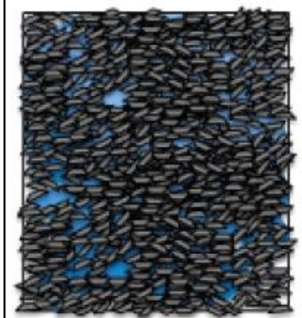
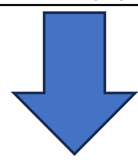
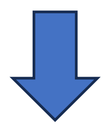
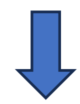
Dominant Particle	***Sand***	***Silt***	***Clay***
Size (mm):	0.05-2.00	0.002-0.050	<0.002
Water content at equal matric potential			
Macropores	+++	++	(+)
Mesopores	++	++	+
Micropores	(+)	++	+++
Percolation			

Figure 4: Hydrologic Properties of Various Soil Types and Pore distribution

Not all pores are the same size, contributing to a wide range of pore sizes. As illustrated in Figure 4, a sand aggregate, with the largest diameter, has the highest amount of macropores, which effectively transport water leading to a high percolation rate [17]. The slowest percolation rate is occurring in clay aggregates, since they have a larger amount of micropores that can retain more water. Another important concept in soil mechanics is pore connectivity, which describes how well soil pores are connected to each other. Diffusion of oxygen relies on the soil pore network to be connected, and nutrient transformations in soil need a water film network to also be connected.

<u>Effects of flooding and salinity on microbial processes in drain fields</u>

Effects of flooding on Soil Environment

Salinity ranges from 0 ppt to 50 ppt in areas around the Eastern Shore of Maryland [19]. The effect of flooding on the activity of microorganisms in soil can vary as soil can be heterogenous (Figure 5) [26]. A common effect from repeated flooding and inundation would be the conversion of a non-hydric soil to a hydric soil, creating an anoxic layer, meaning that oxygen flow through the soil pores would be greatly decreased [26]. A flooded soil as shown in Figure 5, would have the lowest amount of oxygen that could be able to move through the soil pore network. A decrease in the levels of oxygen in the soil system can reduce the ability of the soil nitrifying community to undergo nitrification, an aerobic reaction [15]. Another important impact of flooding is the ability for water to act as a solvent for ions and soluble compounds, increasing both the availability and free movement of metals, ions, and other nutrients [16]. This could mean that different salt ions can enter from flooding events and could infiltrate faster and farther down the soil profile. As the concentration of salt increases, it can be shown that the ability of a soil to aerate decreases, as the salt can accumulate in available pore space, decreasing the ability of air to move through the soil pores [23]. In addition, the physical structure of soil also becomes weaker due to increased stress from clay swelling from the loading of salts [23]. This in turn can affect the hydraulic conductivity of a soil, making it such that water flows through the saturated soil much more slowly, increasing the chances of the surfacing of wastewater effluent from the drainfield to the surface of the soil. The addition of salt in soil can also affect the solubility and ability of soil organic matter to be adsorbed by the pores of the soil by decreasing the number of available cation exchange sites that organic matter can bind to [23].

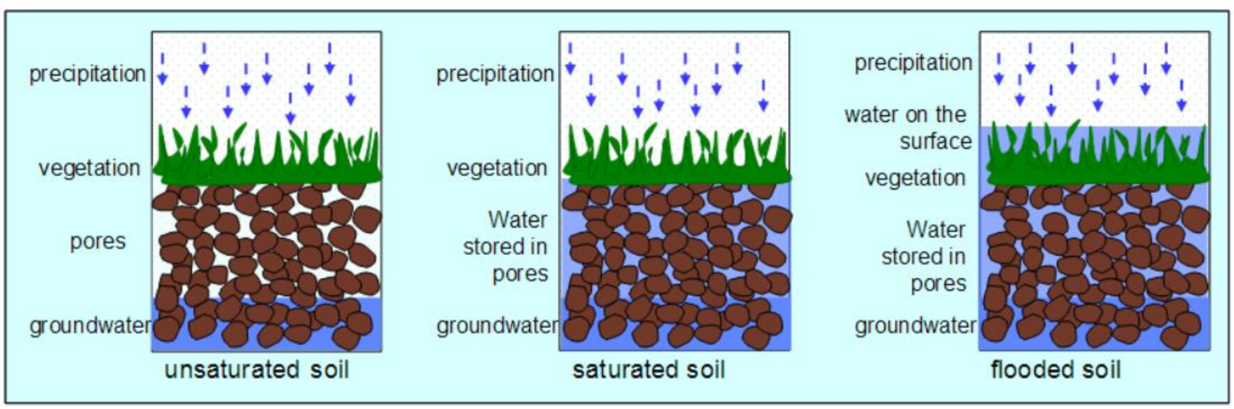


Figure 5: Conceptual Model of different saturation levels of soil. As the water in the soil increases, less oxygen becomes available.

Effects of flooding on microorganisms

Flooding can have a wide variety of effects on soil microorganisms, which can range from short to long term impacts.

A study in Louisiana was conducted by researchers to understand the effects of flooding; collected soil cores from different areas of an urban meadow in Baton Rouge along a four-meter elevation gradient. The cores were subjected to experimental flooding *ex-situ*, via addition of creek water poured over the surface of the cores [24]. The researchers found that differences in microbial communities were a result of the sampling locations and not a result from the impacts of flooding. The researchers used a Yue-Clayton theta similarity index, which considers similar and non-similar species when comparing samples [132]. However, even though the soil cores had different microbial communities, it was found that after flooding had occurred, both core groups emitted methane. This indicated that the soil redox environment had changed. If methane was produced it meant that the soil conditions changed from aerobic to anaerobic, meaning that oxygen was no longer the terminal electron acceptor. In short, what this means is that flooding can change redox conditions of the soil, which can affect the biochemical reactions that take place. In the context of nitrogen treatment in drainfields, nitrification is an aerobic reaction that requires oxygen to be a terminal electron acceptor. Since flooding can cause methane to become the terminal electron acceptor, this could reduce the treatment of NH_4 via the aerobic reaction of nitrification.

Another study focused on microbial resilience (defined as the ability of a microbial community to function after being impacted by a drying-rewetting cycle) and functional diversity in drying-rewetting cycles, in both nitrogen-treated soils and ambient forest soils [25]. It was found that in soils treated with nitrogen (via addition of granular NH_4NO_3), soil microbial populations were able to resist stress from drying-rewetting cycles, while ambient soils showed a decline in microbial biomass and resistance [25]. In addition, the effect of the drying and rewetting cycles suggested a notable decrease in the populations of nitrifying bacteria in soils subjected to frequent stress events, due to the NO_3^--N levels and nitrification rate decreasing. This could indicate that for septic system drain fields that rely on nitrifying bacteria to treat NH_4^+, there could be a reduction in nitrifying activity taking place when a flood event occurs. Even though soil microbial subjected to nitrogen could be able to resist stress during a flood event by potentially going dormant, a reduction in nitrifying activity could make for more untreated nitrogen moving through the soil profile.

Nuisance Flooding – frequency, impacts

The definition of nuisance flooding in accordance with §3-1001 of the Natural Resource Article of the Maryland Annotated Code is "*high tide* flooding that causes a public inconvenience." [22, 30].

According to the National Oceanic and Atmospheric Administration (NOAA), nuisance flooding is anticipated to continue with an increasing frequency over the next meteorological year with a national outlook of three to seven days per year, and by the year 2050, that number will be 45-70 days a year [22]. In Maryland, the number of days can range from 75-115 days in Annapolis on the Western Shore, to 60-100 days in Cambridge on the Eastern Shore [22]. Figure 6 illustrates how far inland nuisance flooding can occur.

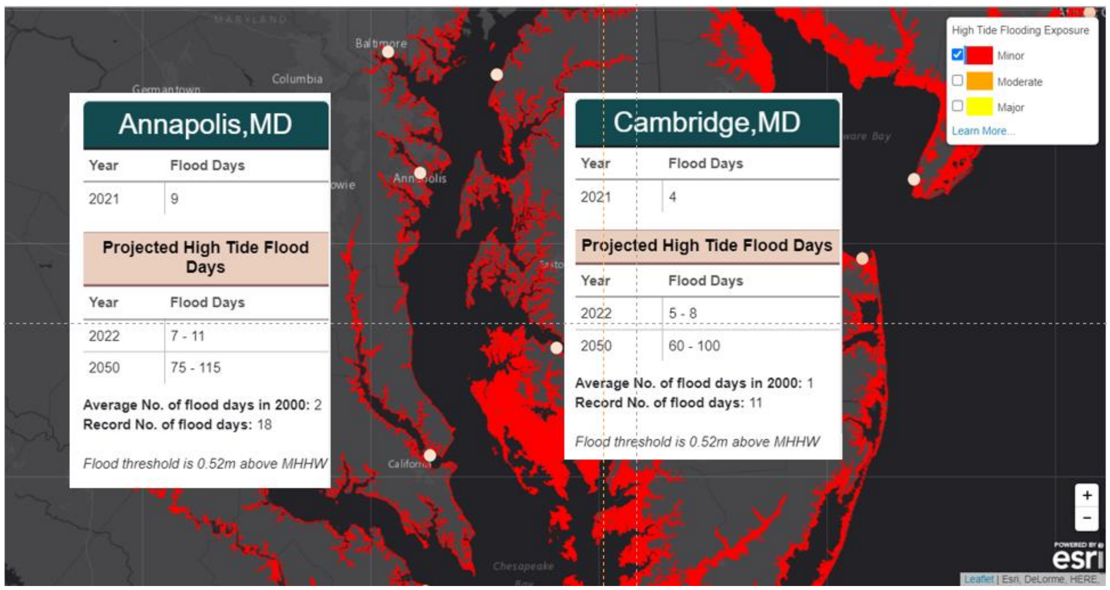


Figure 6: NOAA Map Displaying areas that can experience "Minor" Flooding Exposure on the Eastern Seaboard of the United States. The red layer displays "Minor" damage that can occur, which is defined by NOAA as "mostly disruptive, causing stormwater backups and road closures".

Objectives of the research project

The objectives of the research that was performed in this project were: 1. to understand the function of the soil treatment area of drain fields via studying inorganic nitrogen to assess nitrification. The treatment of nitrogen is an important function for wastewater soil treatment, and nitrification must be done by soil microorganisms to remove NH_4^+ in wastewater.

Nitrification in septic system drain fields was assessed in two separate studies:

- Field study - Two field sites assessed for transformation of inorganic nitrogen in the soil profile to understand the baseline of function.
- Column laboratory study - A soil column study was performed, where the soil was flooded intermittently with saline waters to evaluate the impacts on nitrification and the overall function of the system.

Lastly, the difficulties of septic system geospatial modeling, and the lack of consistent definitions of septic system failure between states. was discussed. The efforts of septic system geospatial modeling have not been attempted at a national scale, only city or county efforts have been undertaken. The studies reviewed all shared a common theme of a lack of centralized septic system parcel records, making it difficult to achieve an accurate picture of where septic systems are located. In addition, since septic systems are regulated at a local level, this can lead to difficulties in enacting policy and regulations at state or federal levels.

This is a part of an ongoing larger research project including researchers from the University of Maryland, George Mason University, nonprofit institutions such as Resources for the Future, as well as community partners from the MD-DC-VA area.

Chapter 2: Field Study

Introduction

Septic systems are used for domestic wastewater treatment by 20% of US households [76]. Each system can have its own setup and loading parameters, depending on the local conditions such as the soil matrix and proximity to water and depth to groundwater. They are commonly used in rural areas, where connection to municipal sewers cannot be made. This is due to municipal treatment plants being typically placed in urban or suburban areas, but they can also be found in urban areas to reduce infrastructure and energy costs [76].

Rising sea levels are threatening infrastructure along coastal areas in many areas including Maryland, where current projections are approximately one to two feet increase by 2050 and a potential to exceed more than four feet by 2100 [77]. This affects land usage and development. Since septic systems, and drain fields by extension, make use of the land via biochemical transformations in the soil, the need to mitigate flooding damage is becoming more pressing. Special attention is placed on the Chesapeake Bay, with 3.1 million pounds (1.4 million kg) of nitrogen annually being deposited to the Chesapeake Bay from septic systems alone (year 2020) [2].

One affected county, Anne Arundel, is located on the Western Shore of Maryland, and here the infrastructure is facing increasing impacts of sea level rise. Data from 2011 show that 7,238 septic systems of the 40,700 private systems in the county are at risk of inundation from zero to five (0 – 1.5 m) feet of sea level rise [78]. Regulation of nitrogen release for residential onsite wastewater treatment systems has not been put forward by Maryland Department of the Environment (MDE). However, for large treatment systems typical for commercial usage (> maximum flow of 5000 gpd), a nitrogen mass balance needs to be performed (meaning that total nitrogen must be analyzed near the groundwater), and for systems to not exceed 10 mg/L of nitrogen at the property line or at any point adjacent to the water. In Maryland, permits and oversight for septic systems and other onsite wastewater treatments are managed by individual county health departments, with counties following regulations set forth by the MDE, and can impose more stringent requirements.

According to the EPA, management programs for septic onsite wastewater treatment cannot be a “one-size fits all” approach, as different counties/regulated areas are subject to different soil types and matrices, and by extension can carry unique biogeochemical parameters [27]. These parameters can exist in a wide variety of permutations that can affect the efficiencies of the various treatment techniques employed in onsite treatment.

Similarly, transplanting programs from one locality to another based on the "success" of another management program, is also not sufficient grounds for implementation [27]. While the EPA does not issue enforceable guidelines, they suggest for management programs to limit nitrogen and other water quality parameters such as organic matter, total phosphorus, pathogens to be controlled and removed by the soil treatment system [27].

Septic systems are designed to reduce organic matter, nitrogen, phosphorus, and pathogens [27]. This can be achieved through filtration of pathogens in soil, and biochemical transformations of nitrogen, phosphorus, and organic matter. Soil texture differences can change key biogeochemical parameters, such as biological community composition or infiltration rate of wastewater [50, 62]. Studies have shown that clay rich soils are important for the treatment of nitrogen, while in another study fine sandy soils can be effective at treating total Kjeldahl nitrogen (TKN), but it can deliver NO_3^--N to the unsaturated zone [79, 35].

In Maryland, there are a total of 420,000 septic systems, and 52,000 of these systems are in the critical area within 1000 feet of tidal waters [28]. The state of Maryland requires onsite wastewater systems placed within these critical areas to be installed with a Best Available Technology (BAT) system, or to be upgraded to a BAT system, with the goal of decreasing nitrogen loading to the receiving groundwaters and subsequently to the Chesapeake Bay.

In this field study, the goal was to gain knowledge about field conditions for two septic systems and compare the two soil systems and the treatment of wastewater. The objectives were: 1) Characterize the design and operational parameters of the two systems including characterization of the soil and microbial communities and 2) describe the ongoing biological wastewater treatment processes and assess the health of the soil in the treatment area.

Two residential sites with unique BAT systems, a Class I Aerobic Pretreatment Unit in the Septic System followed by a shallow narrow Drainfield, and a Class IV soil distribution system categorized as a construction Sand Mound without aerobic pretreatment, were visited and samples were collected from each drain field for analysis of biogeochemical parameters.

In this chapter, the results from characterization of the two septic systems and drain fields will be described, an assessment of the ongoing biological treatment processes will be performed to assess if the drain fields are 'functioning'.

Methods

Description of Study Area

Soil samples were collected from two residential locations with different soil textures and treatment options on February 24th, 2023, with the help of Rich Piluk from Anne Arundel County Public Health Department. Figure 7 and Figure 11 display the location of two residences, while Table 1 and Table 2 share some soil parameters collected from the Web Soil Survey [58]. Figure 10 shows pictures from the day of sampling at Drainfield 1. Since both locations were residential onsite wastewater treatment systems, prior approval from homeowners was needed. Rich Piluk, who was assisting in the sampling efforts received prior approval from both homeowners. This fact also limited the ability to collect from different locations in the drainfield, as the process of removing soil cover and cutting through filter cloth and removing the gravel layer proved to be an intrusive process to both the soil systems and the homeowner's property.

Drain field 1 was characterized as a shallow narrow drain field with an area of 10 ft x 40 ft (3.05 m x 12.2 m) with an aerobic pretreatment unit to help reduce the nitrogen in the septic tank effluent before secondary treatment in the soil [55]. Drain field 2 was a sand mound system, with approximately two feet of sand underneath a one-foot-deep gravel bed and an additional one foot of cover, with the system having a 3:1 side slope [54].

In these two systems, the distribution of effluent was controlled by a pump linked to a float valve, i.e., when the volume in the septic tank exceeded a certain amount, the float would raise, and the effluent would be distributed to the soil. Starting at the distribution box, it would then proceed to flow both laterally via capillary action and downward via gravity.

Both systems had effluent ponded at the point of distribution resulting in an increased moisture content for the core samples than usual, and this could provide insight into the initial treatment of the effluent in the soil.

Drainfield 1

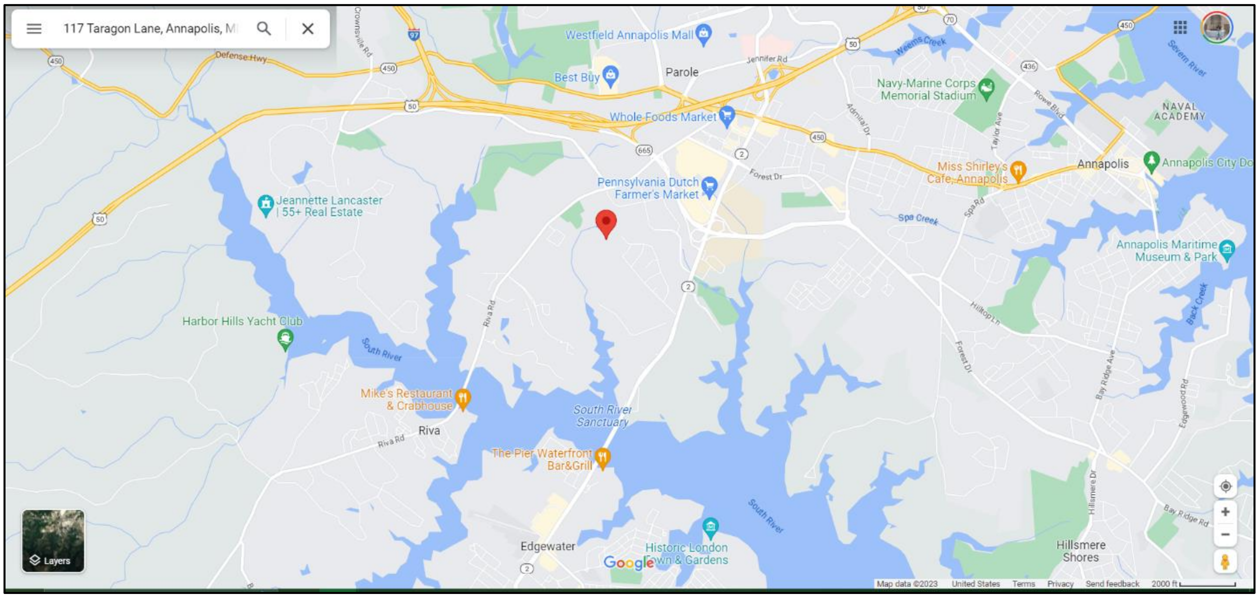


Figure 7: Location of Drainfield 1 - (Shallow-Narrow drainfield, has Aerobic Pretreatment Drainfield 1 Unit.

Table 1: Drainfield 1 Selected Parameters from Web Soil Survey

Parameter	Reported Value from 61 to 122 cm
Bed/Bath/Size	4 beds, 2.5 bath, Size: 2,048 sq.ft
Age	Started in 1997, so it is about 26 years old.
Effective Cation Exchange Capacity	7.8 meq / 100 g
pH 1:1	5.4
Bulk Density	1.63 g/cm^3
Clay	14.8 %
Sand	51.5 %
Silt	22.2 %
Organic Matter	0.4 %
Depth to Water Table	More than 80 in. (2.05 m)
Organic Matter Depletion	"Moderately High"

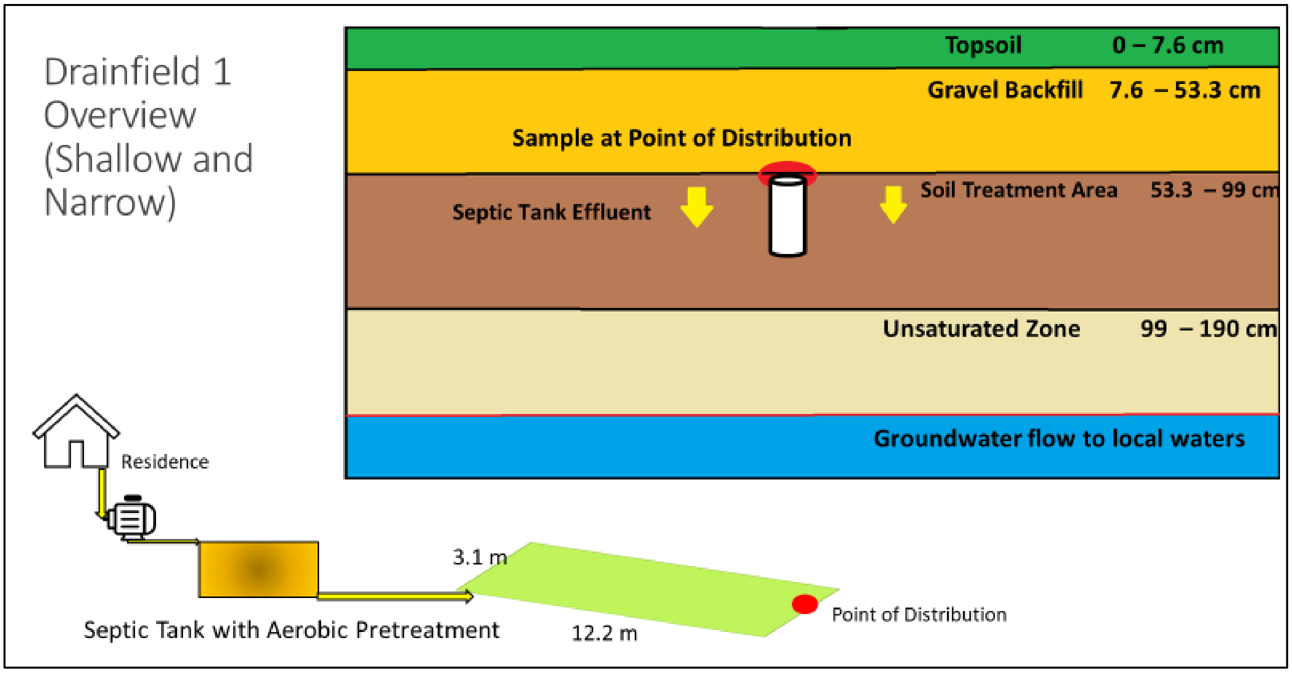


Figure 8: Conceptual Model of Drainfield 1

Figure 9: Conceptual Model of Soil Treatment Area of Drainfield 1

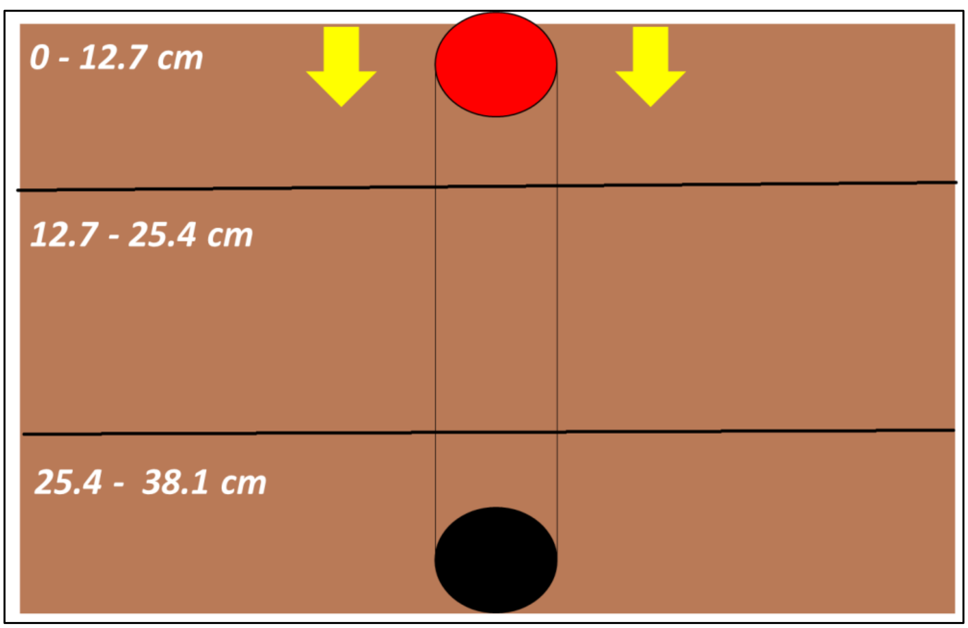


Figure 10: Drainfield 1 site visit Top Left: Digging through gravel. Top Right: Beginning of soil-water interface. Bottom Left: Ponded effluent at soil-water interface. Bottom Right: Collected cores in sample bags.

Drainfield 2

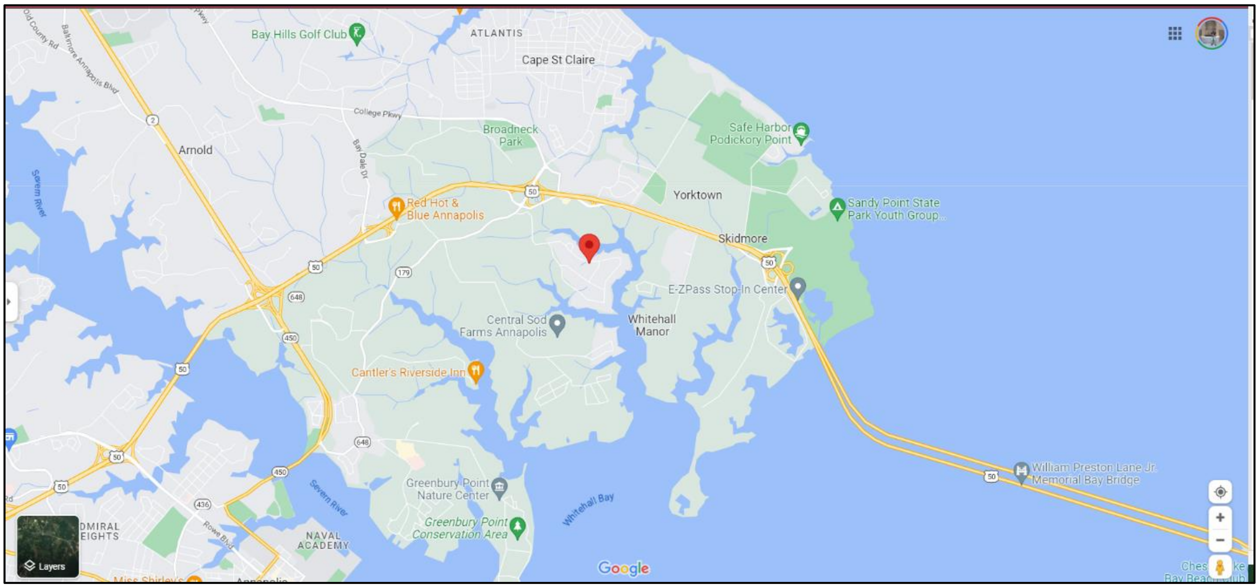


Figure 11: Location of Drainfield 2 - Constructed Sand Drainfield 2 system with no pretreatment.

Table 2: Drainfield 2 Selected Parameters from Web Soil Survey

Parameter	Reported Value from 61 to 122 cm
Bed/Bath/Size	4 beds, 3 bath, 2,592 sq.ft
Age	Started in 2000, so it is about 23 years old.
Effective Cation Exchange Capacity	1.8 meq / 100 g
pH 1:1	4.9
Bulk Density	1.55 g/cm^3
Clay	9 %
Sand	77.3 %
Silt	13.8 %
Organic Matter	0.69 %
Depth to Water Table	~ 20 – 40 in. (0.508 m – 1.02 m)
Organic Matter Depletion	"Moderately High"

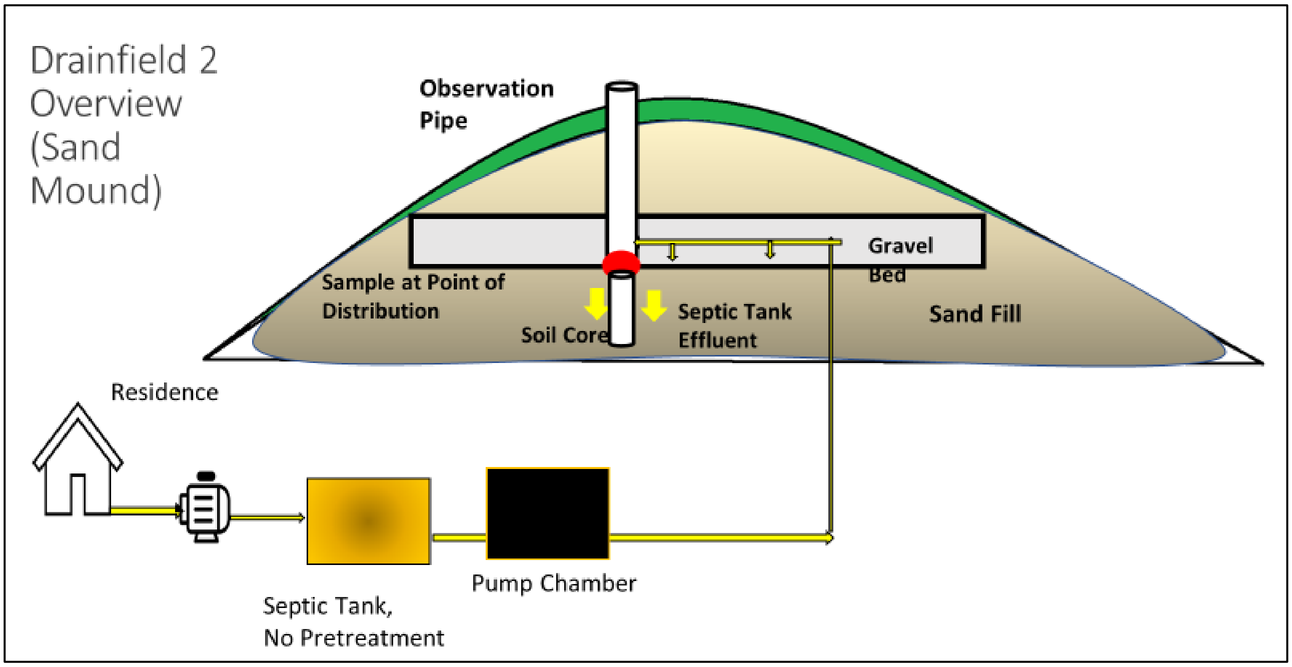


Figure 13: Conceptual Model of Drainfield 2

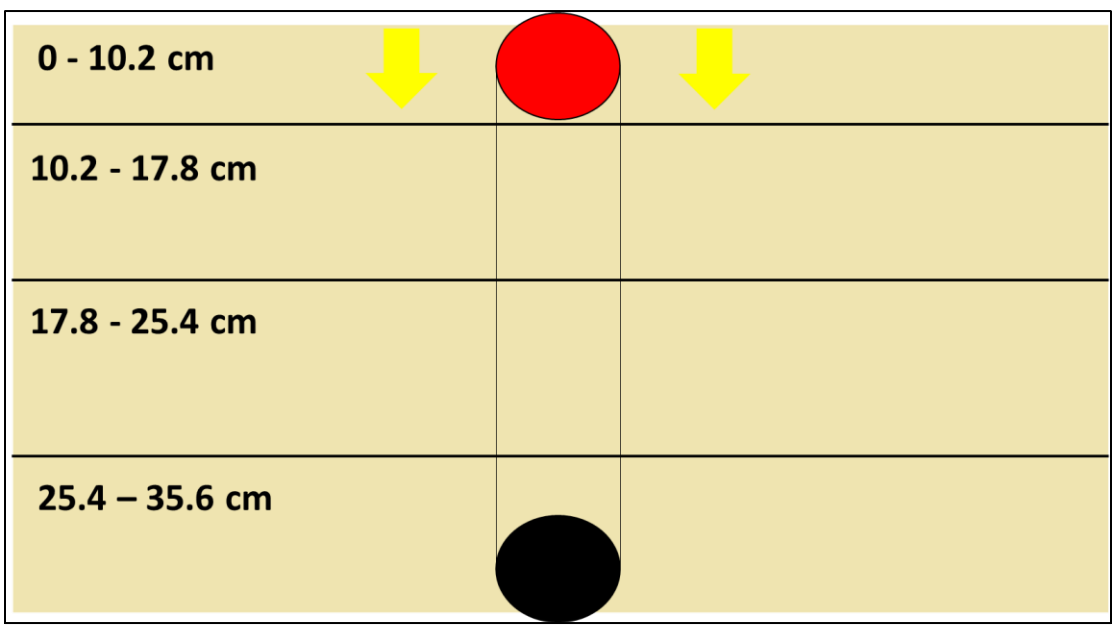


Figure 12: Conceptual Model of Soil Treatment Area of Drainfield 2

Soil sampling and laboratory analysis

Sampling instruments were kept as clean as possible before samples cores were collected at each location, with 70% ethanol-water mixture being sprayed on the soil core instrument and wiped down with clean paper towels. Samples were collected using a 3-1/4'' (8.25 cm) diameter soil auger (AMS, American Falls, United States) for Drainfield 1 and a 2-1/4'' (5.72 cm) diameter soil auger (AMS, American Falls, United States) for Drainfield 2 [81]. Samples were collected in fresh gallon-size plastic bags by being pushed into the plastic bag from the soil auger and placed on ice in a cooler to be transported back to the laboratory for analysis. Once the samples were returned to the laboratory, subsamples (each 6-10 g) were collected from each sample for analysis of moisture content, organic matter, number of nitrifying bacteria, inorganic nitrogen speciation, soil pH and soil electrical conductivity. The rest of the collected soil samples were stored at 4°C.

Abundance of nitrifying bacteria

The abundance of nitrifying bacteria in the soil samples from the two drain fields was determined via the Most Probable Number (MPN) analysis [44]. Briefly, a composite sample comprised of 3.3 g of soil from each sample site was added to 100 ml of a sterile 0.9% NaCl solution to desorb bacterial cells from the soil. After shaking at 100 rpm for 10 minutes, 1 mL of the supernatant was extracted with sterile pipette tips and distributed into 4 mL of sterilized bacterial growth media for nitrifying bacteria (ammonia and nitrite oxidizers). The bacterial growth media contained (in 1 L of DI water): 0.5 g $(NH_4)2SO_4$, 1.0 g K_2HPO_4, 0.03 g $FeSO_4 \cdot 7\ H_20$, 0.3 g NaCl, 0.3 g $MgSO_4 \cdot 7\ H_2O$, 1.0 g $CaCO_3$. The use of a pH indicator was used to qualitatively assess the media for nitrifying bacterial activity. The pH indicator was phenol red, which is red at 8.2 and becomes more yellow at reduced pH values. The media was adjusted to pH = 8.2 by addition of 0.1 M NaOH. The mL of media was then aliquoted into 15 ml tubes, which were sterilized in an autoclave and kept sealed and covered at 25 °C until use the same day. Sterile technique was best applied by spraying the workspace with 70% ethanol and 10% bleach. The soil supernatant of 1 mL, was serially diluted, starting from 10^{-1} mL and ending to 10^{-6} mL. Each dilution series had (n=3) replicates, and it was placed in a 30 °C incubator for 6 weeks. It's important to note that incubation at 30 °C is not representative of what is typically found in soil environments.

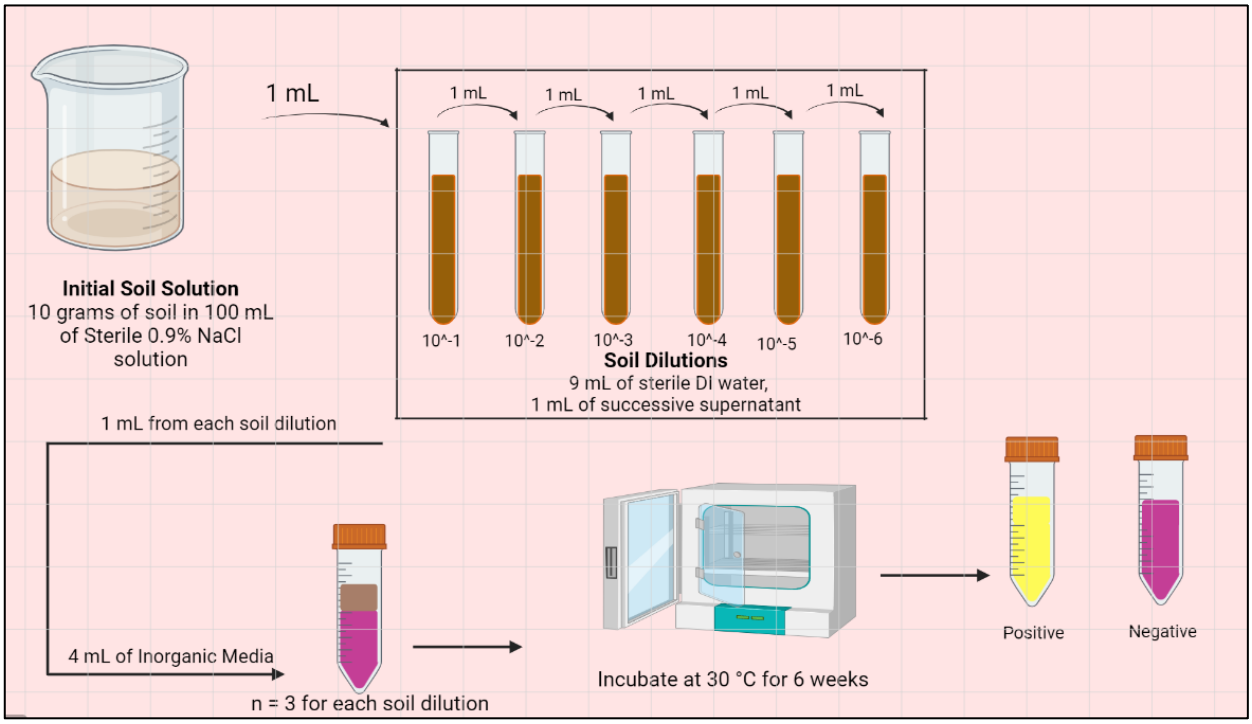


Figure 14: MPN via pH Indicator Conceptual Model

A positive control was used by sterilizing 100 g of soil from the 0 – 12.7 cm sample from Drainfield 1, by amending the soil with 100 mL of fresh wastewater influent, and 100 mL of sterile DI water [47]. The positive and negative controls were inoculated the same way as the soil samples, but only diluted to 10^{-1}, 10^{-3}, 10^{-6}, and not diluted to 10^{-2}, 10^{-4}, 10^{-5}.

The tubes were observed every two days and opened briefly to add oxygen into the system. The tubes were scored according to the FDA BAM table, and the sample calculation is provided in the field study appendix [72]. The high and low confidence intervals were taken from the same FDA BAM table and adjusted similarly for dry weight, with the FDA BAM table also in the field study appendix.

Moisture Content and Total Organic Matter

Moisture content was determined according to ASTM D2216-19 where 10 g of wet soil was weighed and dried at 105°C for a minimum of 24 h or until the weight did not change when measured [45]. The dried soil was subsequently used for determination of the Total Organic Matter content via the Loss on Ignition method, in accordance with ASTM D7348-21 [46]. This was done by placing the remaining soil samples (triplicate) in porcelain crucibles at 550°C for 2 h. The remaining mass was then determined.

Inorganic Nitrogen content in soil

The content of inorganic nitrogen in soil was determined according to UMD Agroecology's lab procedures [48]. Briefly, the soil was leached with a 2 M KCl solution. The KCl solution was prepared by taking 1 L of DI water and dissolving 150 g of KCl salt (Fisher Chemical, Fair Lawn, United States). Soil (6 g) was mixed with 30 mL of 2 M KCl, the slurry was shaking for 60 minutes in a 50 mL centrifuge tube after which the soil liquid was leached from the soil by filter paper by adding additional KCl solution. The leachate (30 ml) was frozen until further analysis. The leachate was analyzed by the SEAL AQ300 Discrete Nutrient analyzer in triplicate (n = 3) and tested for $NH4^+$-N via the Salicylate method and NO_x^--N (NO_2^--N + NO_3^--N) via a cadmium coil reduction. *The resulting concentrations were given in a (mg/L) concentration.* The sample calculation is provided in Field Study Appendix A Equation 1.

Soil pH and Electrical Conductivity

pH and EC were measured in a 10 g soil solution of 1:2 with 20 mL of DI water, with a pH probe (Thermo-Fisher, Waltham, United States), and a conductivity probe (VWR, Radnor, United States) [52 ,53]. The measurements were performed according to the standard methods for pH and EC [80].

Statistics

In order to assess the similarities and differences between Drainfield 1 and Drainfield 2, a key assumption needs to be made. The key assumption is that the samples collected from the three depths for Drainfield 1 as illustrated in Figure 6 and four depths for Drainfield 2 as illustrated in Figure 11, will be a representative of what is occurring at that specific moment in time at the soil-water interface. In short, that while the drainfields sampled had different depth intervals collected and have different setups, it is assumed to be similar enough to compare each system against each other.

There were three samples collected from Drainfield 1 in accordance with Figure 9 (n = 3), there are (n = 9) samples to be analyzed statistically. For Drainfield 2 in accordance with Figure 12, (n = 4), there are (n = 12) samples to be analyzed statistically.

A t-test assuming equal variance was done for the tests: NO_x^--N samples collected (n = 9 for Drainfield 1 and n = 12 for Drainfield 2), and Organic Matter, and a t-test assuming *unequal* variance was done for the NH_4^+-N and Moisture Content samples (n=9 for Drainfield 1 and n = 12 for Drainfield 2). All statistics were done using Microsoft Excel. Field Study Appendix C contains the results from the statistics tests.

Results

The results from analysis of soil samples from the two drain fields are shown in Table 3 and 4, and there are marked differences in the pH and Moisture Content between the two drainfields.

Table 3: Physical Results from Drainfield cores at different depths

Drainfield	Soil Depth	Moisture Content (MC) Avg. % ± Std. Dev. %	Organic Matter (OM) Avg. % ± Std. Dev. %
Drainfield 1	0 - 12.7 cm	24 ± 5	2 ± 0.1
	12.7 - 25.4 cm	19 ± 1	3 ± 0.4
	25.4 - 38.1 cm	22 ± 0.6	5 ± 0.4
Drainfield 2	0 - 10.2 cm	34 ± 4	5 ± 0.9
	10.2 - 17.8 cm	7 ± 0.2	0.6 ± 0.1
	17.8 – 25.4 cm	13 ± 8	0.8 ± 0.2
	25.4 – 35.6 cm	14 ± 2	2 ± 0.4

Table 4: Chemical Results from Drainfield Cores at different depths

Drainfield Location	Soil Depth	pH	$EC_{1:5}$ (dS/m)
Drainfield 1	0 - 12.7 cm	4.90	0.020
	12.7 - 25.4 cm	6.58	0.022
	25.4 - 38.1 cm	6.83	0.019
Drainfield 2	0 - 10.2 cm	5.51	0.027
	10.2 - 17.8 cm	4.29	0.008
	17.8 – 25.4 cm	5.25	0.009
	25.4 – 35.6 cm	4.16	0.006

The moisture content levels in Drainfield 1, were higher than the Drainfield 2, apart from the first core sample from the Drainfield 2 system, which was due to the fresh wastewater effluent found in the Drainfield 2 system.

The elevated Total Organic Matter could be amplified by the wastewater organic compounds that were present in the soil, and not necessarily a true representation of the soil system itself.

It can be difficult to glean insight into the role of the buffering capacity of organic matter in the soil treatment area of both Drainfields 1 and 2, without taking more samples over a spatial and temporal basis, and it is important to note that there is no statistical difference for Organic Matter ($P > 0.05$) or Moisture Content ($P > 0.05$) between both Drainfields.

The Drainfield 1 system has a low pH when arriving to the distribution point of the soil-water interface, and this is validated by the first step of the two-step aerobic nitrification process, which produces protons from oxidation of NH_4^+ to NO_3^- by *Nitrosomonas* bacteria. As mentioned before with the Drainfield 2 system, there was effluent ponded when we arrived for sample collection. The low pH could be a result of the ponded effluent having had time to acidify in the soil environment from nitrification occurring. Another reason could be a lower buffering capacity due to the lower organic fraction in the soil. Although since the organic matter between drainfields is not statistically different, this could not be an actual phenomenon occurring in the soil environments. This could mean that the fraction of organic matter in the soil could not have an impact on the ability of the soil to withstand pH changes. Although more repeated sampling efforts should be done to ascertain if there is a correlation between levels of organic matter and pH fluctuations.

The electrical conductivities of both soil systems are almost zero, essentially being non-saline. In a table correlating the $EC_{1:5}$ method, with salinity classification and the various types of soil environments based on clay content, both soil systems rate as non-saline [66]. Non-saline systems are rated for sand systems a value of <0.15 dS/m, and for sandy clay loam systems the value is <0.25 dS/m [66]. The implications of a "non-saline" rating, could mean that the effluent entering the soil environment does not have a large impact on the soil salinity. Salinity increases in the soil environment can have impacts on the structural stability of the soil environment, as salt deposition can lead to clay swelling and shrinking.

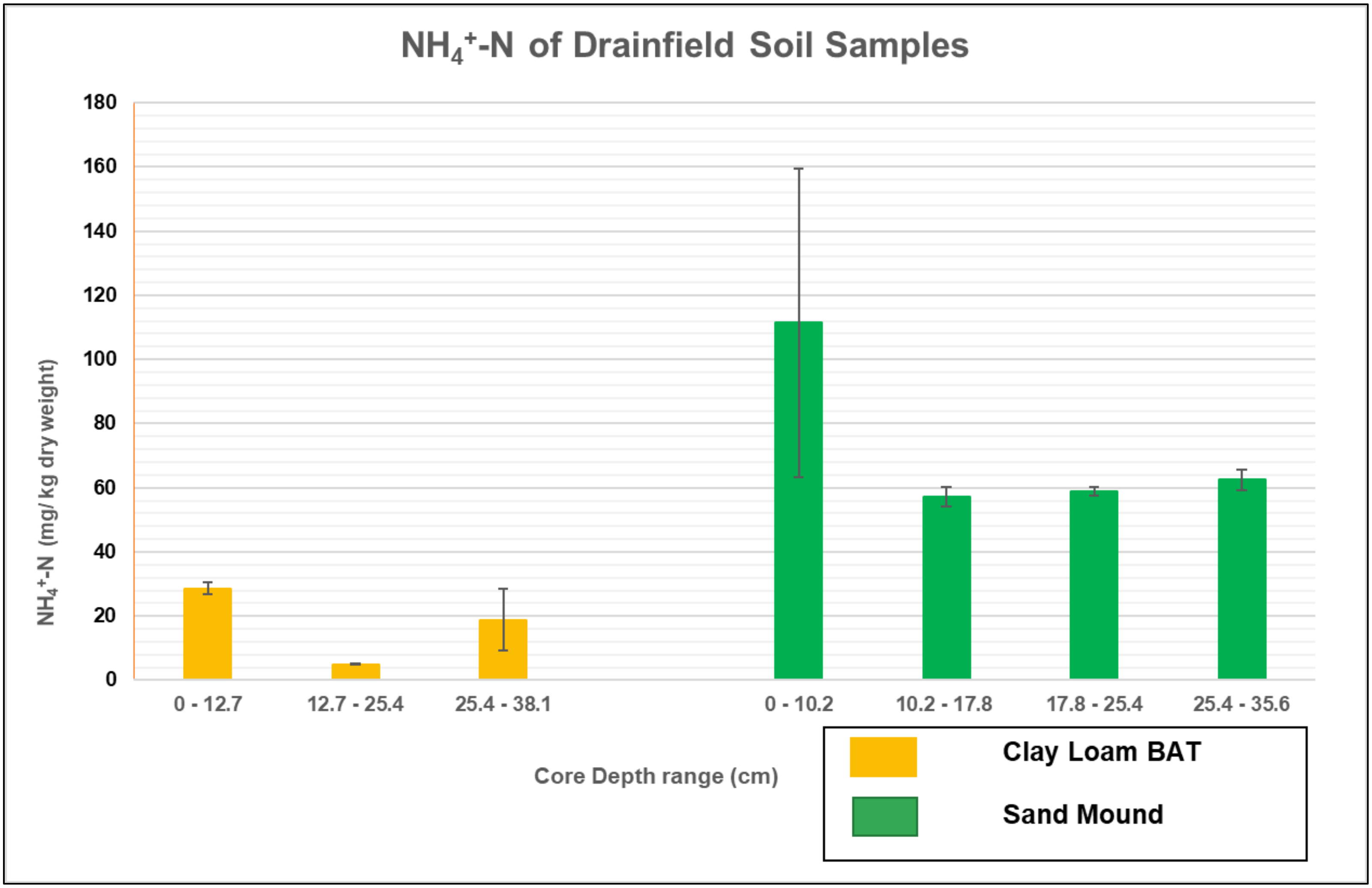


Figure 15: Inorganic Nitrogen NH_4-N from 2M KCl Extracted Soil Core Samples (mg / kg dry weight)

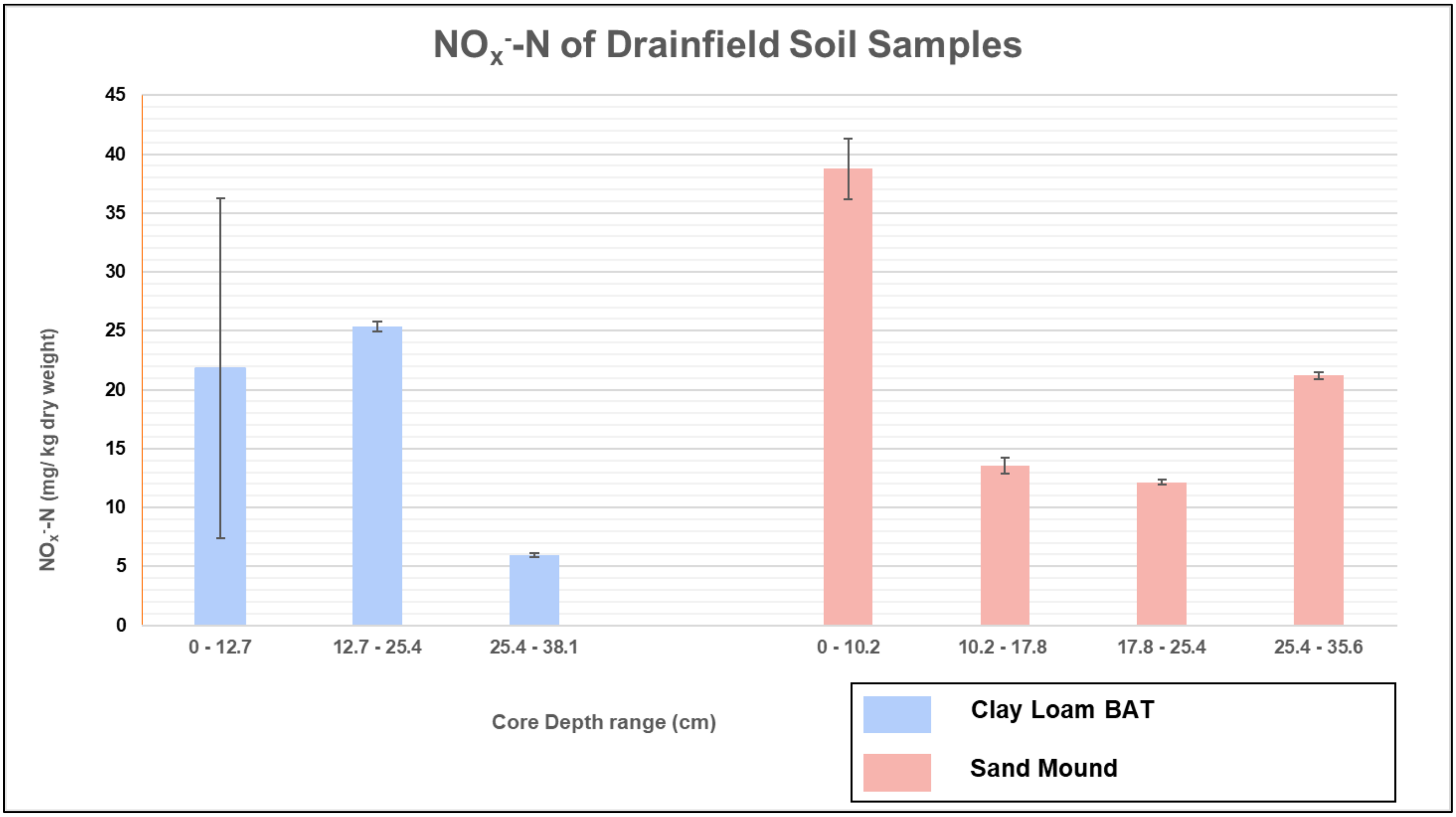


Figure 16: Inorganic Nitrogen NO_x-N from 2M KCl Extracted Soil Core Samples (mg / kg dry weight)

The values found in the field site were taken from extracted soil in Figures 15 and 16, and it can provide insight into inorganic nitrogen concentrations and transformations in the soil profile at the time of collection.

The NH_4^+-N concentrations found adsorbed to the soil samples from extraction have marked differences between the Drainfield 1 and Drainfield 2 systems. The Drainfield 1 values being much lower than the Drainfield 2 system might confirm that the aerobic pretreatment could be working to nitrify the effluent in the septic system before it reaches the soil-water interface. This also has an impact on the NO_x values entering the Drainfield 1 system, as they could be more elevated than without the aerobic pretreatment taking place.

In the Drainfield 2 system, the NH_4^+-N is elevated without treatment, but greatly decreases as the depth increases. This could be from the nitrification that is taking place and could be related to the higher Most Probable Number of Nitrifying bacteria in the Drainfield 2 system compared to the Drainfield 1 system.

There are two measurements with high error bars, namely the NH_4^+-N Drainfield 2 core 0 – 10.2 cm extraction, and the 0 – 12.7 cm Nitrate Drainfield 1 extraction. The NH_4^+-N Drainfield 2 error comes from one value of the replicate in the SEAL AQ300 being much lower than the other two replicates, and similarly with the Nitrate Drainfield 1 value, with one value of the replicates being much lower than the other two replicates.

There is a statistical difference for NH_4^+-N ($P < 0.05$) but conversely, NO_x^--N was not statistically different ($P > 0.05$) between both Drainfields. The NH_4^+-N difference could be due to the aerobic pretreatment that is occurring, while the NO_x^--N similarity could be due to the slow rates of denitrification that could be occurring, but it is difficult to ascertain without further testing.

Table 5: MPN Table Calculations Adapted from BAM Appendix 2: Most Probable Number from Serial Dilutions [36]

Significant Dilution						
Drainfield 1 (3,1,0)						
10^-2	+	+	+	**Value from BAM table**	43	MPN/g
10^-3	+	-	-	Factor of 10	430	MPN/g
10^-4	-	-	-	Dry Wt. Adjust	**549**	MPN / g dry weight
				Confidence Limit	Low	High
					111	1404
Drainfield 2 (3,2,1)						
10^-2	+	+	+	**Value from BAM table**	150	MPN/g
10^-3	+	+	-	Factor of 10	1500	MPN / g
10^-4	+	-	-	Dry Wt. Adjust	**1755**	MPN / g dry weight
				Confidence Limit	Low	High
					433	4914

There are more nitrifying bacteria in Drainfield 2 system (1755/ g dry weight), compared to the Drainfield 1 system (549 / g dry weight). These values come from BAM Appendix 2: Most Probable Number from Serial Dilutions, adjusting for the serial dilution and dry weight of the soil [72].

The results of this test do not align with the experiment conducted originally by the authors [44], as their values for a "Sandy Soil" ($7.0 * 10^5$) were approximately 13 times lower than compared to a "Clay Loam" ($93.8 * 10^5$) soil [44]. The researchers do not explicitly state their sample calculations, only stating they determined their number of nitrifying bacteria from an "*A Most Probable Number (MPN) table (Cochran 1950) was used to determine numbers of nitrifying bacteria",* and upon further review, Cochran's article provided an overview of the math models for the enumeration and steps for planning out an experiment for MPN analysis, but there is no distinct table to select a value from [73]. They could have used a table that was built upon from Cochran's 1950 paper, but it is unclear from reviewing their paper. The decision to use the FDA-BAM table came from a review of a paper studying the abundance of viable ammonia oxidizers in a wetland soil, where the researchers used an Excel spreadsheet developed by the author of the FDA-BAM table, which provides similar results between the table used here and the Excel spreadsheet [74]. It's important to note that the FDA would use a table such as this for enumerating coliform bacteria, as opposed to nitrifying bacteria.

This variability from the original research and the work done here, could be because the pH of the Drainfield 2 system's soil collected was lower than that of the Drainfield 1 system, influencing the drop in pH, making the color change from pink to yellow as illustrated in Figure 14, of the inoculated media. The samples were kept at field moist conditions, and the media could have been influenced by the effluent remaining in the soil during incubation, potentially leading to error and a biased result. In addition, the researchers conducted their studies in agricultural soils though, indicating that the conditions present in terms of nitrogen deposition and the non-engineered nature of their systems could play a part in the difference of results.

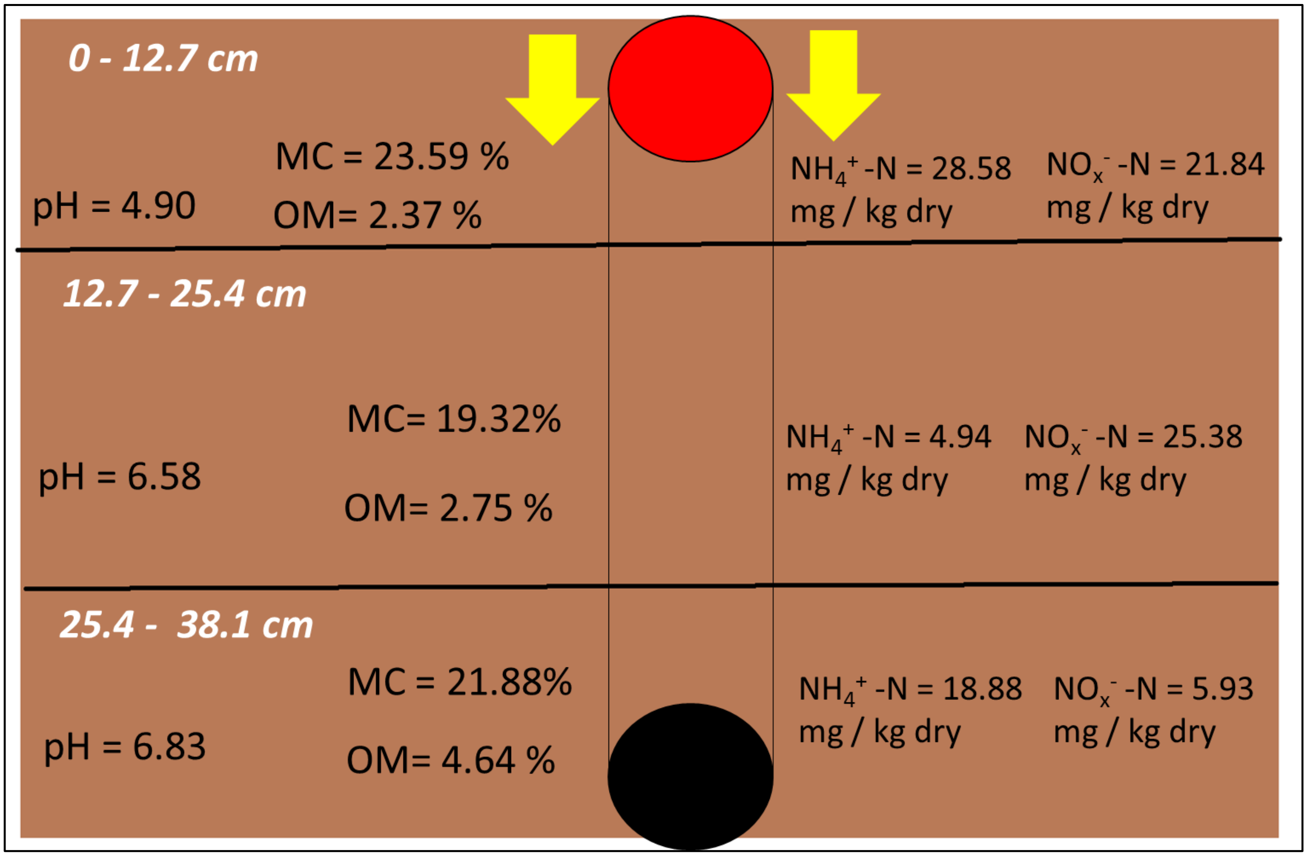


Figure 17: Selected Results from Drainfield 1

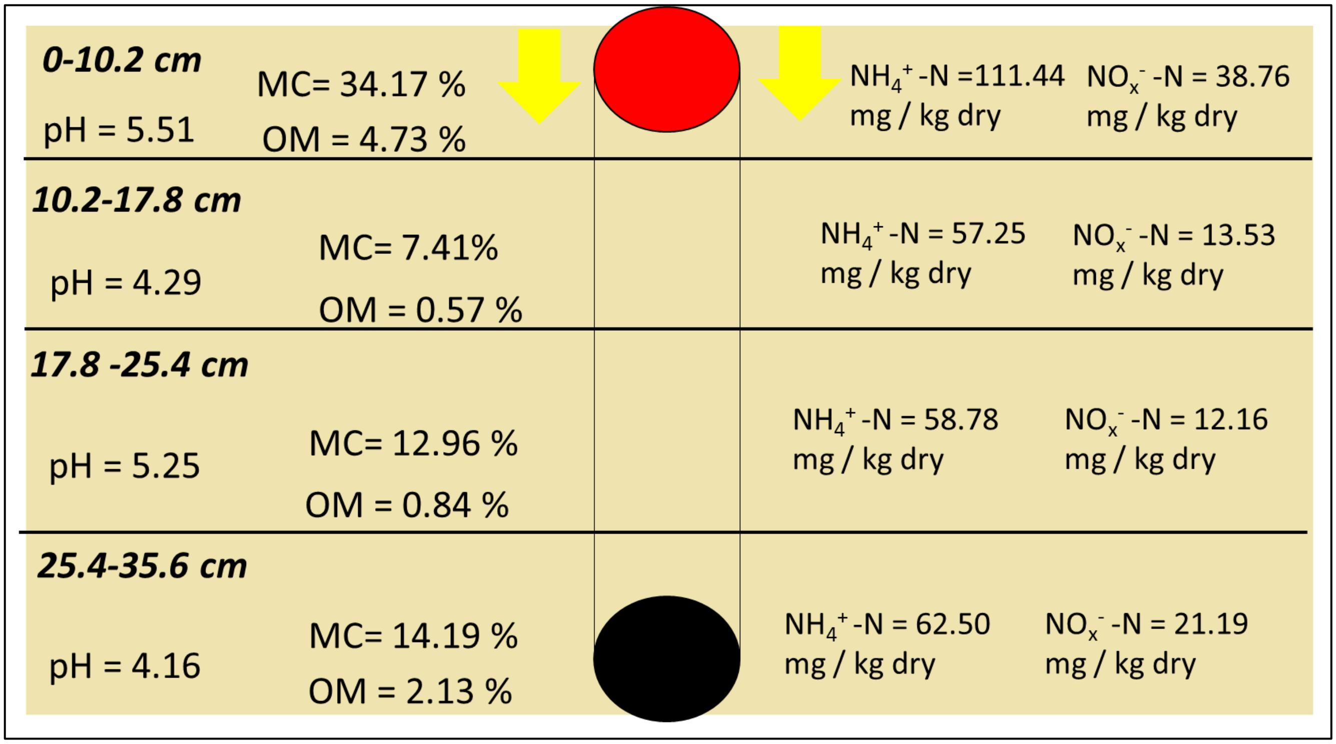


Figure 18: Selected Results from Drainfield 2

Discussion

Implications from Moisture Content to Hydraulic Movement through Soil Treatment Area

The Minnesota stormwater manual rates a simulated average saturated hydraulic conductivity of a sandy loam as 0.9 in/hr (2.3 cm/hr) (akin to Drainfield 2), and a sandy clay loam as 0.14 in/hr (0.36 cm/hr) (akin to Drainfield 1) [36]. These values, while derived from a simulation analysis, could provide insight into how quickly the water moves through the soil system. A study from North Carolina on the impacts of wastewater quality on the long-term acceptance rate of soil (defined as the ability of a soil to receive wastewater over an indefinite period), found that sandy soils had a decreased Infiltration Rate and Saturated Hydraulic Conductivity for simulated wastewaters, while clayey soils had mixed results [62].

The largely consistent moisture content percentage in the Drainfield 1 system gives an idea of the water moving slowly downward through the system, while the uneven percentage in the Drainfield 2 system could be an indicator the faster percolation rates in the system, with the water moving faster downward through the system upon contact with the soil. Although without further analysis of the hydraulic conductivity and infiltration rate of the wastewater through the soil treatment area, it can be difficult to determine how water moves through both drainfields. The systems do not show any indications of failure, as there was no ponding of effluent outside of the soil treatment area. In situations where the percolation rate is too slow, it can lead to a ponding of effluent with conventional systems, which can present a sanitation crisis, a recent example being Lowndes County in the Black Belt of Alabama [37].

Given both the older ages of the sites sampled, it would be a good comparison to find drainfield sites of a newer age and study their infiltration rates and average moisture content of cores over both a temporal and spatial range.

Soil pH, Buffering Capacity, and Implications of Wastewater movement in an Acidic Environment

Organic matter plays a role in the buffering capacity of a soil system, with one way being the humic substances produced by biochemical alterations of biopolymers, thought to comprise around 66% to 75% of the soil organic compartment [69]. Humic substances are very complex molecules, with high molecular weight, that can potentially contain phenolic groups. Humic substances contribute to wastewater treatment in soil, in such that phenolic groups can accept and release protons, potentially allowing for the soil pH to withstand changes via a buffering effect. The effects of humic substances have been studied and found to reside primarily in the biological zone of treatment, occurring after the formation of a biomat, found primarily in the top first few centimeters of the infiltration zone [69]. In future characterization studies of drainfields in the Maryland coastal areas, this could serve as a good parameter to investigate to observe if there is a buffering capacity occurring. Although, a study in North Carolina of a drainfield sandy soil, did not display any correlation between an acidic soil or organic matter [63].

In a meta-analysis of global forest systems studying the patterns of nitrogen deposition on soil pH, nitrogen deposition has been found to decrease soil pH globally by 0.26 on average, with the addition of urea (a major component of domestic wastewater) contributing more acidification than nitrogen in the form of fertilizer [40]. In addition, the depletion of the lower valence base ions can leave only the higher valence metal (Al^{3+} and Fe^{3+}) remaining [41].

This can have potential health and environmental effects as the water percolates down through the acidic soil, as a lower pH can lead to the leaching of toxic trace metals, such as arsenic or lead, that can become soluble in the soil environment, potentially depositing them into the groundwater [65]. A case study of septic tank effluent analyzing the concentration of metals, found that lead concentration was 2700 (µg/L) (n = 1, sample collected), and arsenic had a mean concentration was 37 (µg/L) (n=5, sample collected), indicating a public health concern with the possibility of metals being more readily available to leach in soil systems with a lower pH [27].

To understand better how metals and other contaminants are moving through the drainfields, downstream sampling efforts from both drainfields could be done in the future to see if observable concentrations are found, with a caveat that the water concentrations would be analyzed on a community or cluster level, as opposed to point source.

Nitrogen transformation in the Soil Treatment Area

Both drainfields analyzed do not seem to be failing to treat inorganic nitrogen as it moves through the soil profile. Since only nitrogen that was adsorbed to the soil was analyzed, it is difficult to conclude how much nitrogen is entering into receiving groundwaters. Both drainfields have an aggregate decrease in NH_4^+-N as it moves downward through the soil profile. In a simulated ArcGIS simulation of inorganic nitrogen loading from septic systems into local groundwaters of Jacksonville, Florida, it was found that NH_4^+-N was removed by about 16% in the soil treatment area, and NO_x^--N was removed by about 84% [85]. However, it was still modeled that there were higher mass loadings overall of NO_x^--N being delivered to coastal waters.

In their analysis, since it was a simulation, the nitrification and denitrification rates were chosen based on an advection-dispersion-reaction mathematical model with first-order kinetics. While mathematical models are important for large simulations, due to the natural variability of soil, it would be important to repeatedly test for inorganic nitrogen transformations over a spatial and temporal basis to better understand percentages of removal.

Soil texture plays a key role in how the nitrogen gets treated and delivered to the unsaturated zone of the soil. Studies have shown that 98% of TKN gets removed from septic tank effluent after being treated in 0.6 m of fine sand, and another study from the EPA manual found that while TKN can be greatly reduced in sandy soil, it delivers NO_3^- to the unsaturated zone [27].

Adsorption and biological action are thought to be the main factors that account for the movement of nitrogen through soils, and studies have indicated that other ions present in solution such as stronger base ions, Ca^{2+} for example, can influence the soil adsorption of NH_4^+-N [60]. Since NH_4^+-N nitrification occurs entirely on the surface of soil particles, this can decrease the overall rate of nitrification. However, when NO_3^- is formed, there is little to no adsorption taking place, making it highly mobile in soil systems [60].

Without further testing of nearby community groundwater wells or local costal waterways where the expected nitrogen plumes are to be, it can be difficult to measure the release of NO_3^--N into the water from the cluster of where these systems are.

In a study on coastal waterway plumes in a local residential neighborhood in southern Florida, water quality parameters were studied with one neighborhood was on sanitary sewer, and one neighborhood was on septic, over both seasonal high-water table and seasonal low water table events [61]. NO_3^--N levels were one of the water quality parameters analyzed, and their results suggested that there could be increased coastal pollutant loading during high water table events.

Biological Activity in the Soil Treatment Area

Reviewing the literature on the biological analysis of drainfield soils, there have been no attempts made to enumerate via MPN for nitrifying bacteria in soils, instead, focus is on enumerating via MPN on pathogenic coliform bacteria [71].

However, studies to analyze microbial communities responsible for biochemical transformations in drainfield soils approach methods with bio-computational techniques. An example was the use of qPCR to study drainfield soils mesocosms where researchers found that depth and soil texture are a significant factor for microbial community structure [50]. Bacterial communities were genetically sequenced after DNA extraction and amplified with general bacterial primers B27F, and they found that community richness and diversity was highest in sandy loam soils. Another study looking at drainfield soils via PCR-DGGE, found that aerated soil (sandy soil with a higher amount of macropores) had a larger and more diverse microbial community [51]. They summarized that this was due to the aerated soil having a higher number of unique substrates that are available to electron donors.

The slightly higher value of the Drainfield 2 site MPN/g from pH indicator as illustrated in Figure 14, could be explained by the sand having a higher community richness, with the soil microorganisms able to nitrify the NH_4^+ both present in the medium and soil that was inoculated.

It can be difficult to try and derive a nitrification reaction rate due to many different reasons including, variability of flow rates of effluent into the soil of these two drainfields, the concentrations of TKN in the septic tank effluent being highly variable, and that NH_4^+ adsorbs onto soil as a function of the soils cation exchange capacity, but the percentages of what is adsorbed versus in relation to what is then reacted is unknown from a literature search. If the values of Effective CEC from Table 1 and Table 2 are thought to be a representation of what is occurring in the systems, it could be said that NH_4^+-N could adsorb better into soils of the Drainfield 1 system, while proving to have a more difficult time in the Drainfield 2 soils.

A meta-analysis of literature values reported for nitrification rate constants, reported the range for first-order reaction constants are anywhere from 0.0768 to 211.2 day^{-1} with a median value of 2.9 day^{-1} [43]. In addition, there is not only *Nitrosomonas* that oxidizes NH_4^+ into NO_2, but there is also annamox bacteria that use both NH_4^+ and NO_x^- as reactants and create inert N_2. Other nitrifying organisms include heterotrophic nitrifying bacterias, ammonia-oxidizing archaea, and methanotrophs, which can oxidize ammonia in high concentration environments, making the soil environment a very diverse matrix, and isolation of rate constants can prove difficult without specific intentional methodology to solve for rates of nitrification [36].

Conclusion

A one-time sampling event of two distinct septic systems in Anne Arundel County resulted in marked differences in the biogeochemical parameters that comprise the soil treatment area of the onsite wastewater treatment system. Both soil systems are effective at reducing inorganic nitrogen as the depth increases. The moisture content difference between systems could indicate a difference in the hydraulic retention time of the soil. The pH is acidic in Drainfield 2 which could be a problem for dissolved metals to become more mobile. There seems to be a healthy activity of nitrifying bacteria, as represented by the enumeration of nitrifying bacteria that was cultured. In addition, both systems are not failing in accordance with the Maryland definition of “Failure” (discussed more in the Perspectives Paper chapter). Both drainfields could be functioning, as there appears to be nitrification occurring in the soil treatment area. Repeated sampling events over a spatial and temporal basis should be done to further ascertain what healthy and functioning soil could look like.

Chapter 3: Column Study

Introduction

Septic systems are used for domestic wastewater treatment by 20% of US households [76]. Each system can have its own setup and loading parameters, depending on the local conditions such as the soil matrix and proximity to water and depth to groundwater. Rising sea levels are threatening infrastructure along coastal areas in many areas including Maryland, where current projections are approximately one to two feet increase by 2050 and a potential to exceed more than four feet by 2100 [106]. This affects land usage and development, and since septic systems, and drainfields by extension, make use of the land via biochemical transformations in the soil, the need to mitigate flooding damage is becoming more pressing. In a report from Miami **Chapter 3: Column Study** -Dade County in 2018, they found that the number of septic systems that were periodically compromised during storms was at the time approximately 56% of parcels on septic, (58,349 parcels), and is expected to increase to 64%, (67,234 parcels) by 2040 [107].

Climate change adaptations have been studied for areas with varying levels of resources available for flood mitigation efforts [109]. Notable case studies reported by the EPA on centralized wastewater treatment flood mitigation efforts, stated that in Washington, D.C., their wastewater treatment plant, Blue Plains, constructed a 17.2 ft. (5.24 m) sea wall to mitigate the effects of a 500-year storm event by spending $13 million dollars [109]. Another wastewater treatment facility in Iowa City, Iowa, threatened by flooding impacts from the Iowa River, decommissioned their wastewater treatment plant, converting it into a green space, with a projected cost of $63 million dollars to decommission, demolish, and expand elsewhere [109]. With a variety of resiliency strategies to mitigate the impacts of flooding on community infrastructure, the need for solutions remains present for all communities that can be impacted by flooding.

In Maryland, sea level rise can have multiple impacts on the land application including the use of infrastructure systems in near-coastal areas. The use of septic systems for wastewater treatment is at risk from nuisance flooding as well as flooding due to more frequent and higher energy storms and hurricanes. These impacts on septic system drainfields can lead to increased periods of saturation as well as the impact of increased salinity in the soil.

A model system of a drainfield was developed in laboratory columns, where soil from an active drainfield was subjected to consistent flow of synthetic wastewater with intermittent tidal flooding. The system was set up with varying levels of salinity exposure mimicking different flooding scenarios. The results from the laboratory study will be used to assess how the increased salinity from tidal flooding can impact the biological treatment processes for nitrogen and dissolved organic solids.

Methods

Soil Collection

Soil was collected from an active residential drainfield on February 14th, 2023, and was stored in a 5-gallon plastic bucket with a lid until the columns were constructed. Certain soil parameters are displayed in Table 6. The soil was then sieved with a 9.5 mm sieve to remove rocks and debris, and the soil was scooped into the columns, held into place by the cheesecloth.

Table 6: Drainfield Selected Parameters from Web Soil Survey

Parameter	Reported Value
Effective Cation Exchange Capacity	5.3 meq/100 g
pH 1:1	5.0
Bulk Density	1.44 g/cm^3
Clay	20.3 %
Sand	30.5 %
Silt	44.5 %
Organic Matter	0.38 %
Depth to Water Table	More than 80 inches
Organic Matter Depletion	"Moderately High"

Column setup/usage

Columns were designed as modeled in Figure 18 with Polyvinyl Chloride (PVC) pipes, and Figure 19 and Figure 20 display the columns before and after the duration of the experiment. The cheesecloth was placed below the column, and was held into place by a flow reducer, which went from a 4 in. (10.2 cm) to a 2 in. (5.1 cm) diameter. The change in diameters allowed for the column to fit into the surface of the plastic bucket.

The constructed column as modeled in Figure 18, was held into place by cutting a hole into the surface of plastic bucket, in addition to having a foam insert placed around the bottom of the column for stabilization, to ensure that the column would not tip over if the weight became unevenly distributed. This was the setup during column normalization, when no effluent was collected, and before soil sampling. Prior to the actual start of the experiment, the initial layer of cheesecloth broke, with soil being lost from the column, and the foam insert was replaced with an additional layer of cheesecloth.

The synthetic wastewater was pumped from the synthetic wastewater carboy into the columns using Masterflex L/S 14 Tubing, with an inner diameter of about 0.063" (Masterflex, Radnor, PA) [110]. The tubes were taped onto the side of the column for stabilization. The flowrate into the column was designed to be at approximately 15 mL/hour. This was decided in accordance with the EPA Design Manual for Onsite Wastewater Treatment and Disposal, which states that an average Hydraulic Loading rate is 1.2 gal/day * sq.ft of soil surface area [27]. This was scaled down for the columns, in accordance with the given cross sectional of our column. The sample calculation is placed in Appendix A Equation 1.

The synthetic wastewater was adapted from OECD 2001 chosen based on readily available chemicals in the laboratory and ease of preparation to study the nitrification process [100]. Eight L of synthetic wastewater was prepared twice per week to limit biodegradation in the reservoir and refreshed every Monday and Friday of the week, for four weeks.

Table 7: Ingredients of Synthetic Wastewater in 1 L of DI Water

Chemical	**Concentration (mg/L)**
Peptone	160
Meat Extract	110
Urea	10
NH4Cl	20
K_2HPO_4	28
NaCl	7
$CaCl_2 \cdot 2H_2O$	4
$MgSO_4 \cdot 7H_2O$	2

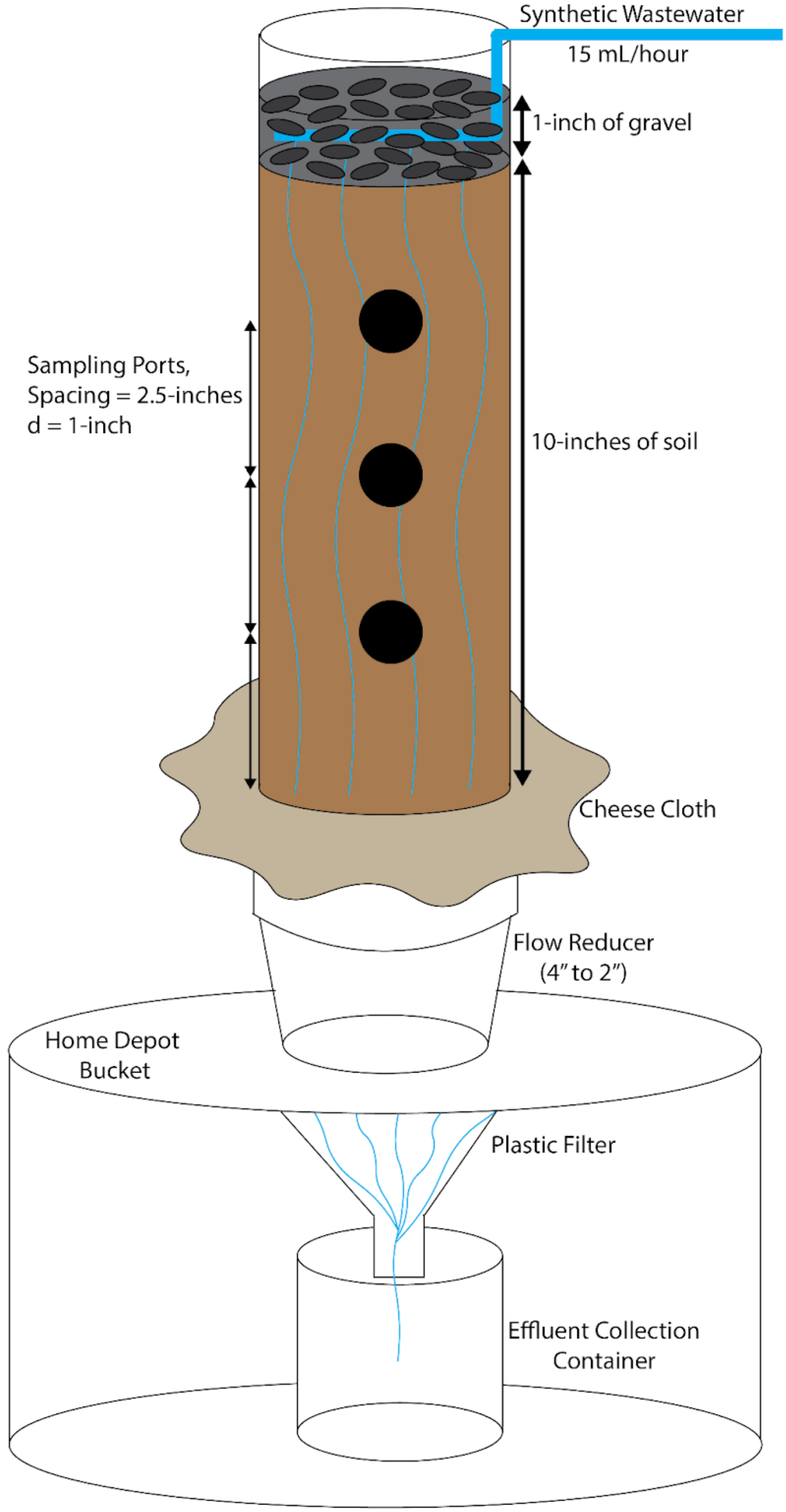


Figure 19: Individual Column Conceptual Model

Figure 20: Columns Before Experiment Start

Figure 21: Columns After Experiment Completion – Take note of the removal of the foam inserts, and then addition of cheesecloths under the flow reducers.

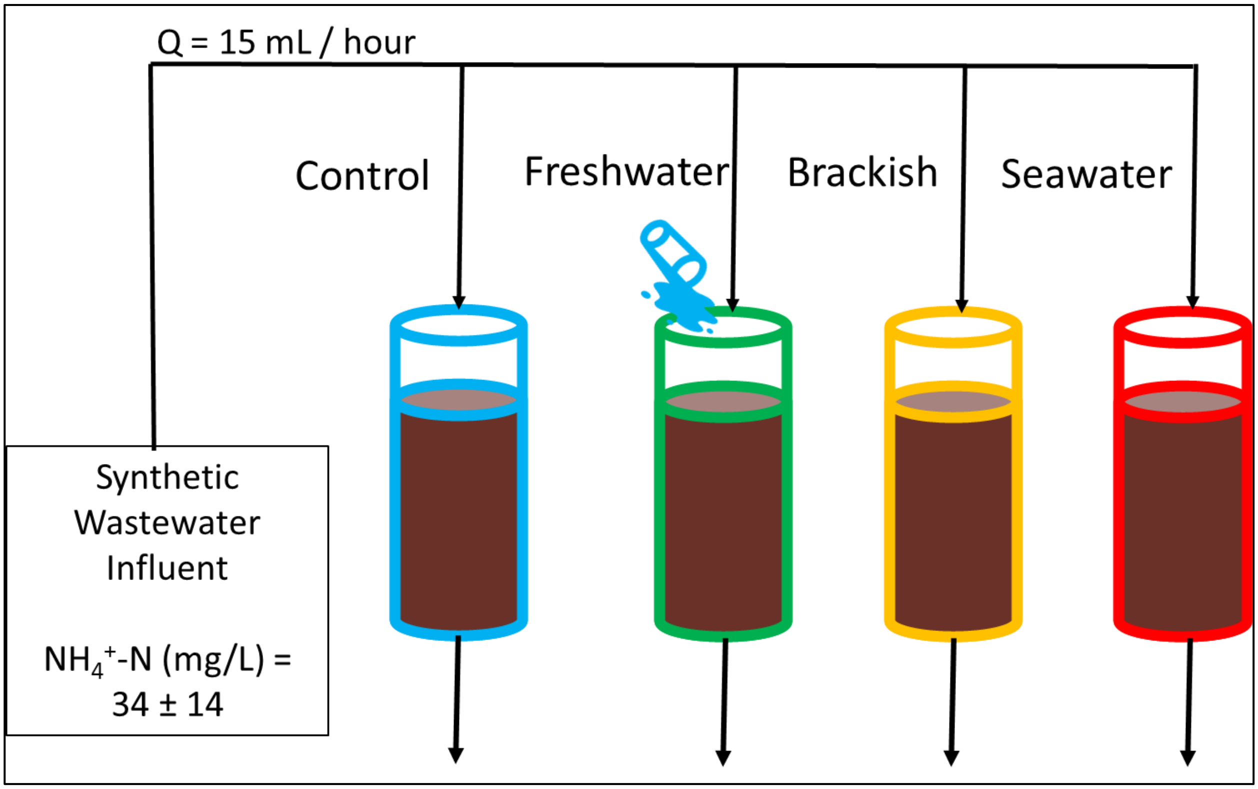


Figure 22: Conceptual Model of Columns

Soil Sample Collection

Each column had 3 sampling ports for measuring soil characteristics - at approximately 4.5 in. (11.4 cm) (Top), 7 in. (17.8 cm) (Middle), 10 in. (25.4 cm) (Bottom). Soil was collected using plastic straws, by way of making a readily available “soil corer”, prior to the start of the experiment on March 29, 2023, after two weeks of column normalization, from the Top and Bottom sampling ports in each column. The approximate mass of soil sample collected from each sampling port was about 30 g, which was then divided up into subsamples for pH, EC, Moisture Content, and Organic Matter, and the methods are detailed in further on in the paper.

An issue that occurred in collecting the soil was the subsequent heights of the column had decreased due to collection of soil and the cheesecloth ripping under the column, in such about 2.5 in. (6.35 cm) of height of soil was lost from the original design, making the Middle sampling port, to become the Top sampling port, and the bottom sample location became the bottom of the flow reducer. Figure 6 below shows the soil coming out of the flow reducer, where it was collected to be sampled.

After the experiment had concluded, soil was collected again on May 5^{th}, 2023, from the “Top” and “Bottom” sampling ports. The approximate mass of soil sample collected from each sampling port was about 30 g, which was then divided up into subsamples for pH, electrical conductivity (EC), moisture content, organic matter, and inorganic nitrogen. The methods are detailed further below.

Figure 23: Soil at the bottom of the flow reducer on top of the added cheesecloth. This soil was sampled as the "Bottom" of the column on May, 5th, 2023.

Column Effluent Collection:

Effluent was collected before each Flood event (Tuesday), on Monday (day before flood) and Friday (three days after flood), and was measured for pH, EC, chemical oxygen demand (COD), as well as inorganic nitrogen species. Effluent was collected using 15 mL centrifuge tubes (VWR, Radnor, PA) attached to the funnel under the column.

In addition, an aggregate over 24 hours was collected on Wednesday (a day after the flood), and a subsample of the aggregate was collected in 50 mL centrifuge tubes (VWR, Radnor, PA). This is to measure the potential flushing effect of nutrients directly after a flood occurs.

Column Normalization

Once the columns were set up with the soil in place, they were hydraulically top loaded via a pump with synthetic wastewater at a rate of 15 mL/hour constantly for from March 13th to March 27th, to homogenize both the microbial community to synthetic wastewater and potentially remove any variation that could be present in the soil matrix originally.

COD, NH_4^+-N, NOx-N, pH and EC as parameters were also measured every 4 days.

Table 8 includes both the “old” and “new” wastewater, designated “new” as fresh wastewater added, and “old” as a subsample of wastewater that was pulled after 4 days of constant usage. The range of NH_4^+-N indicates the incoming NH_4^+-N in the synthetic wastewater is increasing due to ammonification of the organic forms of nitrogen, which is urea and peptone.

Table 8: Parameters of Old and New Wastewater from 3/13 - 3/27

Parameter	**Range**	**Average**	**Standard Deviation**
COD (mg/L)	192 - 423.66	277.0	74.81
NH_4^+-N (mg/L)	23.77 – 58.25	32.19	10.44
NO_x^-–N (mg/L)	0.37 – 2.25	0.3711	0.7480
pH	6.64 – 7.41	6.956	0.2727
EC (uS/cm)	212 - 495	354.1	94.11

Mimicking flood events

In doing a literature review, there weren’t any relevant research articles that simulated tidal flooding in a soil treatment area, so a few assumptions need to be made to simulate what a tidal flood could be for the column study, and the resulting translation of that assumption to the impacts on the soil treatment area of a drainfield.

To simulate a high tide flooding, an approximation of flood water for the column was used based on NOAA prediction table of the depth of High Tide Flooding for Cambridge, MD - station # 8571892 [111].

From March 1st - 31st 2023, the average High Tide depth was about 1.565’. In accordance with the EPA Design Manual the depth of a drainfield can range anywhere from three to four feet in depth [27].

By way of using a ratio, a depth of four ft of the soil treatment area in a conventional drainfield, and in the column is mirrored by approximately six in. of soil, and if a hightide flood corresponds to 1.565 ft in a real-world application, it can then translate to a column flood depth of two in. The sample calculation is provided in Column Study Appendix A Equation 2.

This depth of 2 in. in the column corresponds to a volume of 400 mL, and this sample calculation is also provided in Column Study Appendix A Equation 3. The concentrations of salt water were developed to model what could be environmentally relevant in terms of salinity in and around Chesapeake Bay [19]. The salinity of 35 ppt was chosen as this represents the higher end of salinity in Chesapeake Bay. 15 ppt salinity was chosen based on the maximum value for the month of March 2023, 15.20 ppt, in the Choptank River monitoring gauge, the closest gauge to Cambridge, Maryland [112]. To better contextualize the flood categories, 0 ppt is categorized as “freshwater”, while 15 and 35 ppt are categorized as “brackish” and “saltwater” respectively.

The next assumption made is that the volume of tidal flood is not applied evenly in one instantaneous event. Instead, to try and better model a tidal flood, with increases and decreases in a volume of water being applied to a system, the volume added to the column was broken up into 3 increments. 100 mL on hour one, 200 mL on hour two, and 100 mL on hour three.

The saltwater flood solutions were created by using Instant Ocean (Instant Ocean, Blacksburg, United States) and was calculated to 15 ppt (brackish) and 35 ppt (saltwater) by way of using the manufacturers reported specific gravity of 1.022 for 35 ppt solution for a solution of 1.5 lbs in a 5-gallon mixture [113]. This was scaled down for 400 mL of flooding solution, and a ratio was applied for the 15 ppt solution. These sample calculations are in Column Study Appendix A Equation 4.

The various concentrations and their measured parameters of pH and Electrical Conductivity are in Table 9 below.

Table 9: Simulated Flood Parameters

Flood	**0 ppt - Freshwater**		**15 ppt - Brackish**		**0 ppt - Saltwater**	
Date Created and Applied	pH	EC (mS/cm)	pH	EC (mS/cm)	pH	EC (mS/cm)
Tue, Apr 4	7.91	0.389	7.91	16.49	7.95	35.7
Tue, Apr 11	7.6	0.295	7.47	14.83	7.8	34.1
Tue, Apr 18	6.83	0.374	7.68	17.91	7.77	39.6
Tue, Apr 25	7.51	0.651	7.69	17.52	7.81	42.2

Parameters analyzed and their methods.

Soil moisture content was determined according to ASTM D2216-19 where 10 g of wet soil was weighed and dried at 105°C for a minimum of 24 h or until the weight did not change when measured [45]. The dried soil was subsequently used for determination of the Total Organic Matter via the Loss on Ignition method, in accordance with ASTM D7348-21 [46]. This was done by placing the remaining soil samples in porcelain crucibles at 550°C for 2 h. The remaining mass was then determined.

The content of inorganic nitrogen in soil was determined according to UMD Agroecology's lab [48]. Briefly, the soil was leached with a 2 M KCl solution. The KCl solution was prepared by taking 1 L of DI water and dissolving 150 g of KCl salt (Fisher Chemical, Fair Lawn, United States). Soil (6 g) was mixed with 30 mL of 2 M KCl, the slurry was shaking for 60 minutes in a 50 mL centrifuge tube (VWR, Radnor, PA) after which the soil liquid was leached from the soil by filter paper by adding additional KCl solution. The leachate (30 ml) was frozen until further analysis. The leachate was analyzed by the SEAL AQ300 Discrete Nutrient analyzer in triplicate (n = 3) and tested for $NH4^{+}$-N via the salicylate method and NO_x^{-}-N (NO_2^{-}-N + NO_3^{-}-N) via a cadmium coil reduction. *The resulting concentrations were given in a (mg/L) concentration.* The obtain the concentration in a value that is commonly reported in literature (mg / kg dry weight), some conversions need to be made. These are listed in Column Study Appendix A, Equation 5.

pH and EC of the extracted column soil were measured in a 10 g soil solution of 1:2 with 20 mL of DI water, with a pH probe (Thermo-Fisher, city, United States), and a conductivity probe (VWR, city, United States) [52, 53]. The measurements were performed according to the standard methods for pH and EC [49].

Inorganic nitrogen samples were collected from the column effluent, in addition to "New" and "Old" wastewater, were run on the SEAL AQ300 Discrete Nutrient Analyzer in triplicate (n = 3) and tested for $NH4^{+}$-N via the Salicylate method and NO_x^{-}-N (NO_2^{-}-N + NO_3^{-}-N) via a cadmium coil reduction.

pH and EC of the collected effluent were measured in either 15- or 50-mL centrifuge tubes (VWR, Radnor, USA) according to the manufacturer's instructions [52, 53].

COD of the collected effluent was digested using 0-1500 ppm COD Vials (Chemetrics, Midland, United States) according to the manufacturer instructions [114]. The vials were analyzed using a Hach DR1900 Portable Spectrophotometer (Hach, Loveland, United States) according to the manufacturer instructions in triplicate (n =3) [115]. For the columns impacted by salt, the effluent was diluted [1:3] to minimize chloride interference, and the values were adjusted accordingly, by multiplying the value read by 4.

Statistics

The results from the column effluent, $NH4^{+}$-N, NO_x^{-}-N, pH, EC, and COD were analyzed using a one-way ANOVA test to compare the categorical variables of salinity to the continuous data values from each of those parameters. Pairwise t-tests were also done post-hoc to compare columns against each other for the given parameters. These tests were generated using the Real Statistics Resource Pack software (Release 7.6) for Microsoft Excel [116]. The full results of the statistical tests are placed in the Column Study Appendix.

Results

Biotransformation of Nitrogen

NOx⁻-N being produced in the Effluent

The average NO_x^--N concentration being delivered to the effluent for all the columns do not appear to be following a trend between columns, as the average concentrations over the experiment duration are very similar. This measured parameter does not provide reliable results. In addition to large peaks over the experiment duration there were also missing values of the columns impacted most by salt flooding (brackish and saltwater) in Figure 24 that contributed to standard deviations being close to 50% and 100% of the average values, respectively.

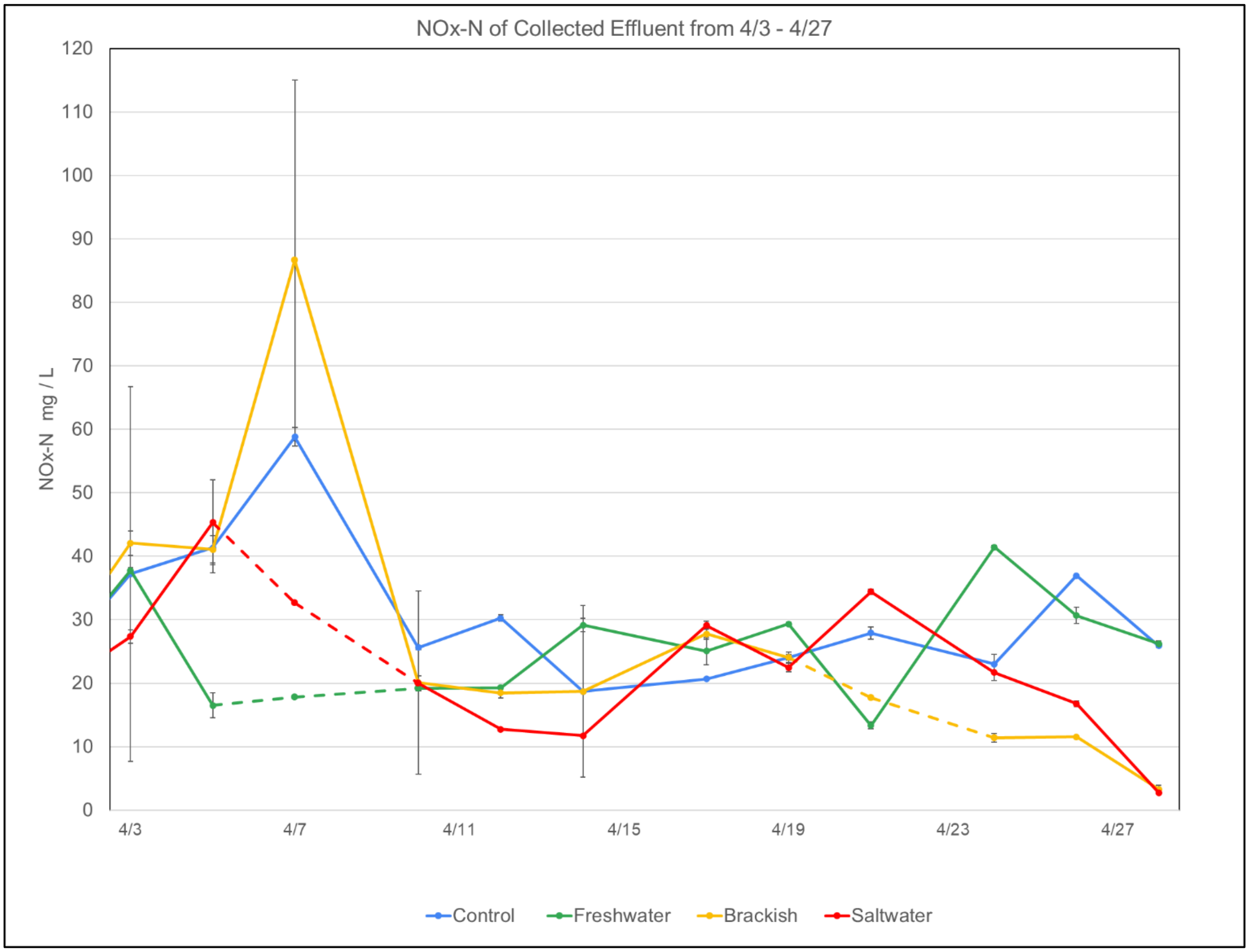


Figure 24: NOx-N of Collected Effluent

Table 10: NOx-N Parameters over the experiment duration

Column	Control	Freshwater (0 ppt)	Brackish (15 ppt)	Saltwater (35 ppt)
Average (mg/L)	28.11	24.26	25.67	19.22
Std Dev (mg/L)	12.43	10.60	25.63	10.06

The expectations in measuring NO_x—N should have been that there would have been a steady concentration in the control column, but yet it increases from roughly 15 mg/L to ~45 mg/L without any changes to the column during the experiment. This could have been from standards used to generate the calibration curves to measure the concentrations being out of range, which could also explain the high standard deviation from all the column measurements in the initial two weeks of the experiment.

Similarly, sharp increases and decreases in the salt columns occur as well, although missing data points for both salt columns increase the unreliability of the results.

The ANOVA P-Value for this parameter was ($P > 0.05$) in Column Study Appendix C, indicating that there was no statistical significance in the difference in NO_x^--N between all the columns. Table 13 displays the P-values for all pairwise t-tests for each of the parameters.

Removal Efficiency of $NH4^+$-N from Influent to Effluent

The NH_4^+-N removal efficiency has a marked difference between the control columns and the columns impacted by salt flooding. This could be from the salt being adsorbed to the soil surface, impeding nitrification by soil microorganisms.

In terms of a mass balance of NH_4^+ we can take the general form of a mass balance in Equation 1 and make a few assumptions.

Equation 1: General Mass Balance

$$In - Out + Net\ Generation = Accumulation$$

- Assuming steady state, means that the Accumulation term is equal to 0, as everything that comes in, either comes out or is reacted.
- Assuming that the Net Generation term is equal to both consumption and production of NH_4^+, which in this case is only consumption (i.e., nitrification as illustrated in Equation 4)
- The last assumption is that as NH_4^+ is adsorbed to the soil, it is reacted by soil microorganisms.

This simplifies the equation to:

Equation 2: Simplified Mass Balance

$$Net\ Generation = In - Out$$

Equation 3: NH4-N Mass Flow Removal Efficiency

$$[(NH^+_{4\,In} - NH^+_{4\,Out})\ /(NH^+_{4\,In}\ )\ ] *\ 100\ \%$$

Equation 4: Nitrification Two Step Reaction

$$2NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 4H^+ + 2H_2O$$
$$2NO_2^- + O_2 \rightarrow 2\ NO_3^-$$

Removal efficiency in Equation 4 can be thought of as nitrification occurring, as illustrated by Equation 3, as the NH_4^+ entering the column is adsorbed onto the soil particles, where it is then nitrified by bacteria on the soil surface [18]. If it is not adsorbed to the soil surface, it is freely mobile, being pushed downwards through the column by hydrological flow of the wastewater and/or flood water.

10 data points (n = 10) were used in constructing Figure 24 below, but only 9 data points (n = 9) were available for the 15 and 35 ppt flood due to a loss of sample during experiment. In addition, due to ammonification occurring, the conversion of organic nitrogen in the forms of urea and peptone in the synthetic wastewater, the concentrations of NH_4^+-N were inconsistent in their supply to the columns.

This led to in certain instances, more NH_4^+-N leaving the column than what the average concentration was over the duration of the experiment. These resulted in negative efficiencies, and these values were set to 0%. This occurred for one data point in each of the flooded columns, the zero values are displayed in a graph of efficiency over time, placed in Column Study Appendix B.

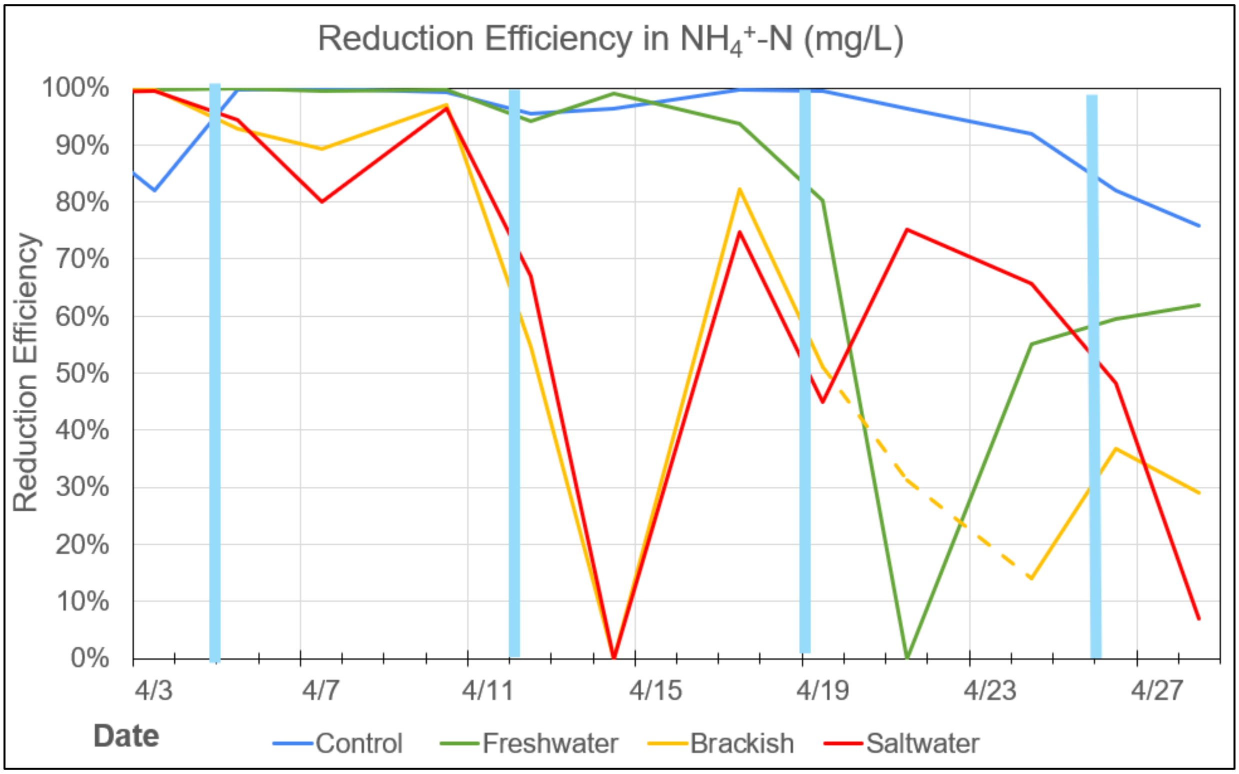


Figure 25: Removal Efficiency in NH4 over the experiment duration

Table 11: Average and Standard Deviation of NH4 removal Efficiency

Column	Control	Freshwater (0 ppt)	Brackish (15 ppt)	Saltwater (35 ppt)
Average	94.07%	89.74%	76.28%	77.44%
Std Dev.	8.45%	17.89%	34.54%	29.46%

The larger decrease in both columns impacted by salt could be from the sodium particles that have been adsorbed onto the soil, which can competitively block the ammonium from the wastewater from being adsorbed onto the soil, making it unable for nitrification to take place from soil microorganisms, instead passing through the soil column to be delivered to the effluent, untreated. The control column does not have this issue. The flooded column that does not have salt, shares a slight decrease, and this could due be due to a flushing effect from the increased hydraulic loading from the flood events.

The results from the column study could imply that the incoming NH_4^+-N from the synthetic wastewater is not being adequately nitrified in an event of a short-term saline tidal flood. The reasons for this could be from both substrate availability on the soil surface due to the competition from salt cations, in addition to the soil environment being determinantal to nitrifying activity taking place.

The ANOVA P-Value for the parameter of NH_4^+-N in the effluent was ($P < 0.05$), indicating that there was a statistical significance in the difference in reduction of NH_4^+-N between all the columns. The ANOVA output is in Column Study Appendix C.

Table 13 displays the P-values for all pairwise t-tests for each of the parameters. In comparing the columns that had been impacted by salt for reduction of NH_4^+-N, the P-Values < 0.05 compared to the columns that had not been impacted by salt. What this means is that the brackish and saltwater columns (salinity impact) when individually compared against the freshwater and control column (no salinity impact), showed a statistical difference in reduction of NH_4^+-N. This result could indicate that statistically, salinity can impact the reduction in NH_4^+-N compared to a soil treatment area that is not impacted by salinity.

Removal of Average Influent COD to Effluent

COD removal efficiency is being inhibited by the addition of salt flooding into the soil treatment area of the columns. The average removal being higher for the Control column and the column without salt influence could indicate that salt is a factor for poor COD removal.

Equation 3: COD Removal Efficiency

$$[(COD_{In} - COD_{out})/COD_{In}] * 100\ \%$$

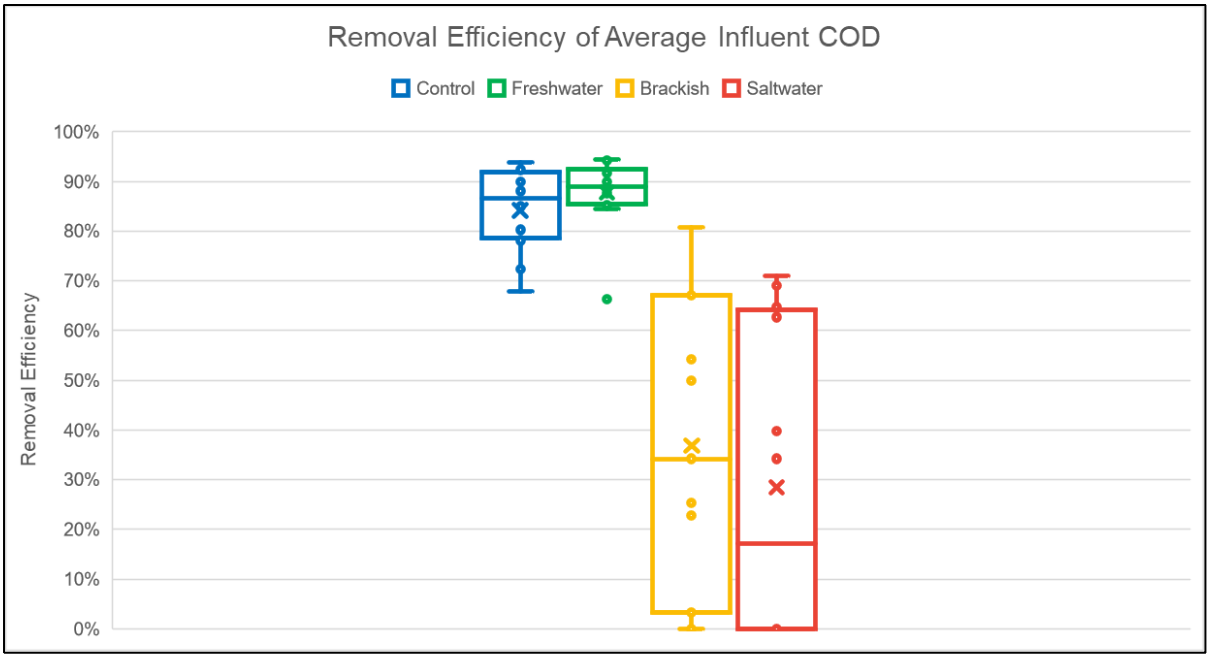


Figure 26: Removal Efficiency of Average Influent COD

Table 12: Average and Standard Deviation of COD Removal Efficiency

Column	Control	Freshwater (0 ppt)	Brackish (15 ppt)	Saltwater (35 ppt)
Average	84%	88%	37%	28%
Std Dev.	8%	8%	29%	32%

Twelve data points (n = 12) were used in constructing Figure 8 below, but only eleven data points (n = 11) were available for the column modeled after the "Cambridge" (15 ppt) tidal gauge, due to a loss of sample during the experiment. Although it is important to note that many of the efficiency values for the salt flood columns had to be set to 0%, this was due to more COD being received in the effluent than what was supplied on average in the synthetic wastewater effluent. This occurred twice in the brackish column and occurred six times in the saltwater column. This impacted the standard deviation of the results for the salt flooded, and resulted in standard deviations being well within the average, compared to the columns not impacted by salt.

From the results on an aggregate level, it could be that columns not impacted by salt are converting the organic material via soil mineralization into NH_4^+-N, which is then subsequently nitrified, while salt could have had a negative influence on soil microbial mineralization of the organic nitrogen.

An important factor influencing the conversion of organic nitrogen to inorganic nitrogen in soil is the Carbon to Nitrogen (C:N) ratio. If it is below 20:1, net mineralization occurs, indicating a conversion of organic to inorganic nitrogen. If it is above 20:1, net immobilization could occur, the opposite of mineralization, which is the conversion of inorganic nitrogen (typically nitrates) to organic nitrogen [90]. Both ratios are important for the soil treatment area, as mineralization can be important for treating the organic material in the soil from the wastewater, and immobilization, with the uptake of nitrate as inorganic nitrogen, can be important for denitrification. Further analysis of the C:N ratio's within the soil should be done to understand how the organic nitrogen is being mineralized in soil for use by chemotrophic nitrifying bacteria [90].

The ANOVA P-Value for this parameter was ($P < 0.05$), indicating that there was a statistical significance in the difference in reduction of COD between all the columns except for the brackish and saltwater columns (columns impacted by salt). Table 13 displays the P-values for all pairwise t-tests for each of the parameters. In comparing both the columns that had been impacted by salt for the reduction of COD, the P-Values < 0.05when comparing against columns that had not been impacted by salt. What this means is that the brackish and saltwater columns (salinity impact) when individually compared against the freshwater and control column (no salinity impact), showed a statistical difference in reduction of COD. This result could indicate that statistically, salinity can impact the reduction in COD compared to a soil treatment area that is not impacted by salinity.

pH and EC of the Collected Effluent

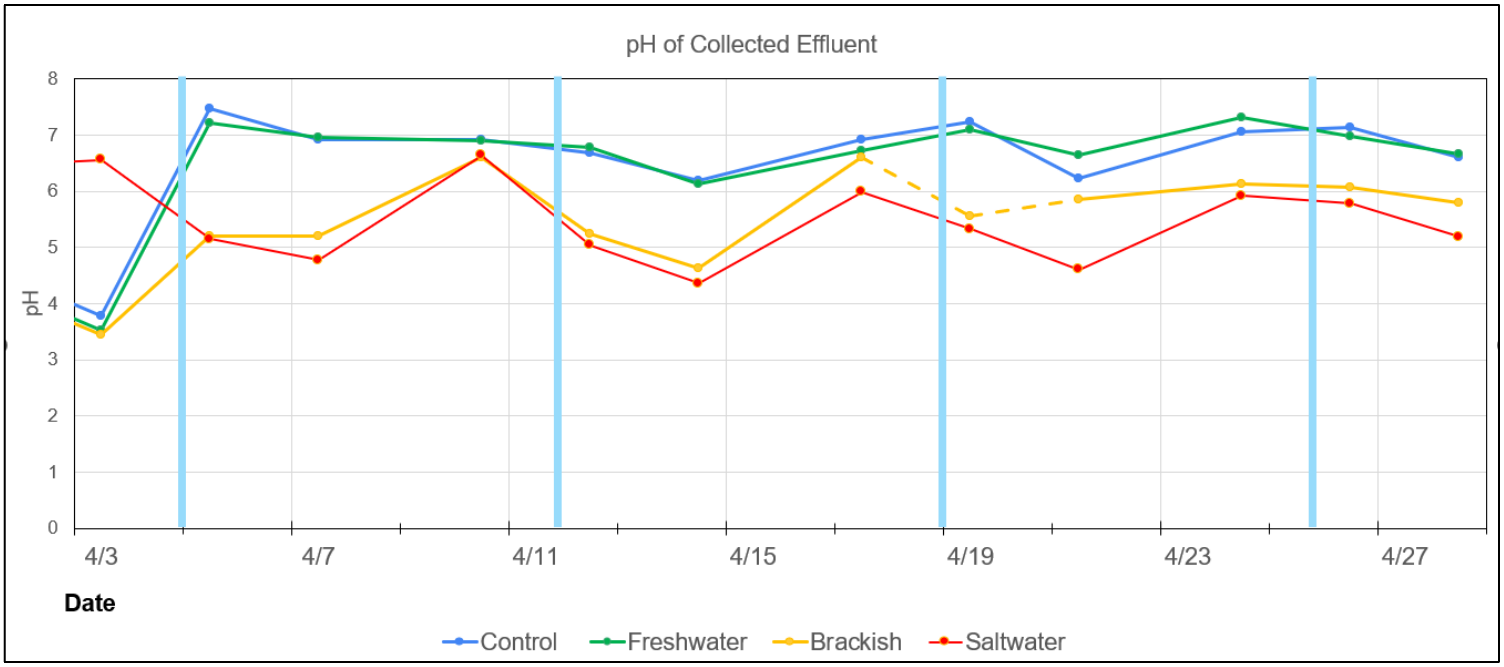


Figure 27: pH of Collected Effluent

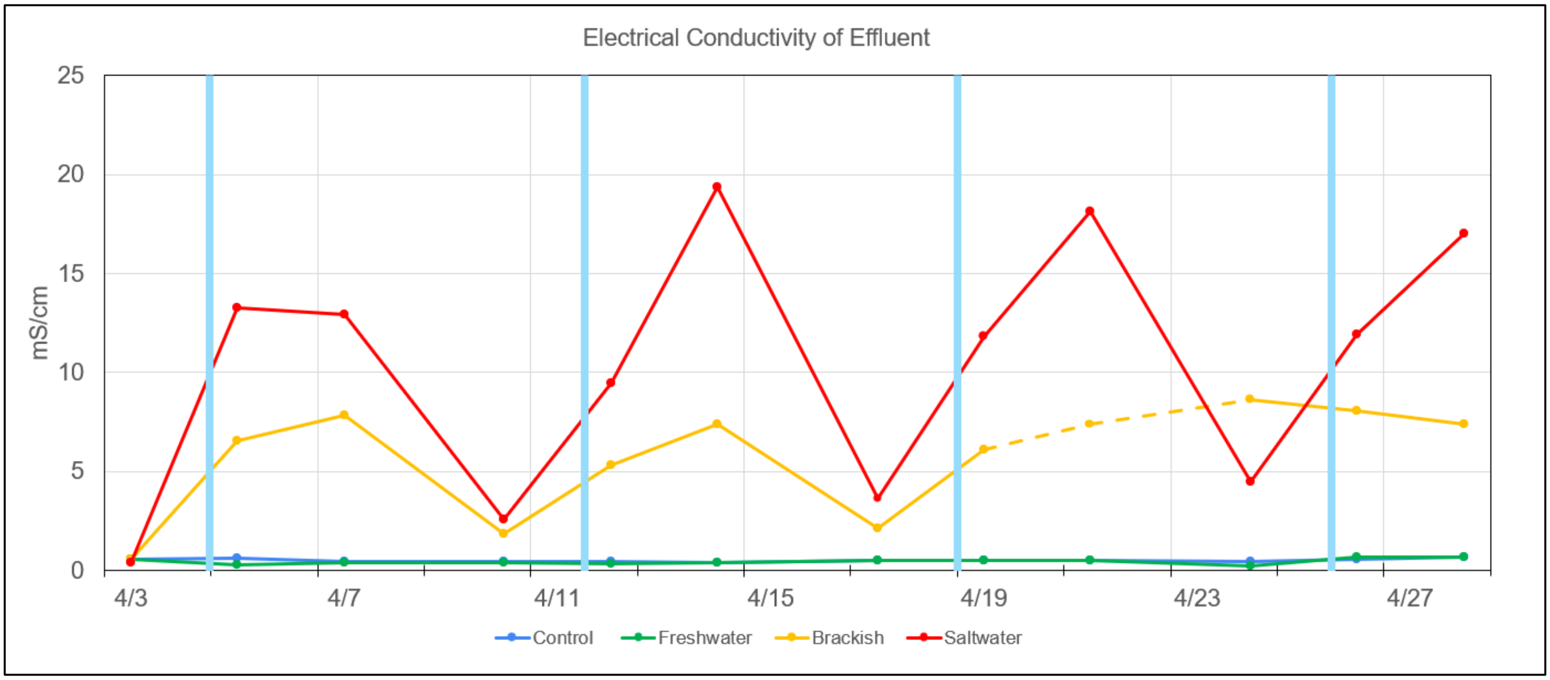


Figure 28: Electrical Conductivity of Collected Effluent

This difference in the lower EC of the effluent compared to the higher EC of the tidal flood waters in Table 4 could indicate that salt is becoming trapped in the column, adsorbing to the soil surface.

The data showed a marked trend in an overall decrease of the effluent's pH for the salt columns over time. From the first two flood events, it rebounded back to circumneutral (~7.0), but as the fourth flood occurred, it seemed to only rebound to around 6.0, potentially indicating that the increased loading of salt to the system over time could be affecting the effluent quality. Conversely, the EC of the effluent shared a marked increase as the experiment went on. Initially in the salt columns, the EC rebounded closer to 0 mS/cm after a flooding event, but as the experiment went on, the effluent settled at higher and higher values when collecting the effluent on the third days after a flood event occurred, potentially indicating the accumulated salt on the soil surface from the soil system to the receiving effluent.

The ANOVA P-Value for pH was ($P < 0.05$) in Column Study Appendix C, indicating that there was a statistical significance in the difference in the measured pH of the effluent between all the columns. In comparing the columns against each other for the effluent pH in Table 13, the P-Values < 0.05 when considering the addition of salt into the columns. This result could indicate that statistically, salinity can impact the effluent's pH compared to a soil treatment area that is not impacted by salinity.

The ANOVA P-Value for EC was ($P < 0.05$) in Column Study Appendix C, indicating that there was a statistical significance in the difference in the measured EC of the effluent between all the columns. Additionally, the result of the pairwise t-test in Table 13 for each of the columns indicate that the P-Values < 0.05 when considering the addition of salt into the columns as well as the amount of salt being added. This result could indicate that statistically, the amount of salinity from the modeled tidal flooding can impact the effluent being treated in the soil treatment area.

Ultimately what this could mean is that if the effluent pH and EC of the columns impacted by salt return to a different baseline over time than the control column, this could signal that their functionality could be changing. While this experiment was only done over four weeks, increasing repeated floods over a septic system's lifespan could prove to have similar effects.

Table 13: Results of Pairwise t-test between columns for Effluent Parameters measured.

Pairwise t-test table		Contextual Comparison	P-Value from pairwise t-test				
Group 1	Group 2		NH_4^+-N Removal Efficiency	NO_x—N Effluent	pH Effluent	EC Effluent	COD Effluent
Control	Freshwater	Impacts of tidal flooding overall	0.1365	0.1869	0.9265	0.2117	0.2910
Control	Brackish		0.0059	0.5184	0.0096	0.0001	0.0044
Control	Saltwater		0.0114	0.0539	0.0028	0.0002	0.0006
Freshwater	Brackish	Impacts of no-salt vs. salt tidal flooding	0.1356	0.8905	0.0138	0.0001	0.0031
Freshwater	Saltwater		0.2561	0.4140	0.0046	0.0002	0.0004
Brackish	Saltwater	Impacts of the levels of salt in the tidal flood waters	0.6834	0.5367	0.8513	0.0310	0.3994

Soil Parameters

From the one-time sampling event after the flooding experiment had been completed, there does seem to be a marked difference between the adsorbed NH_4^+ with regards to salt impacts to the column. Soil was extracted and analyzed in triplicate (n=3).

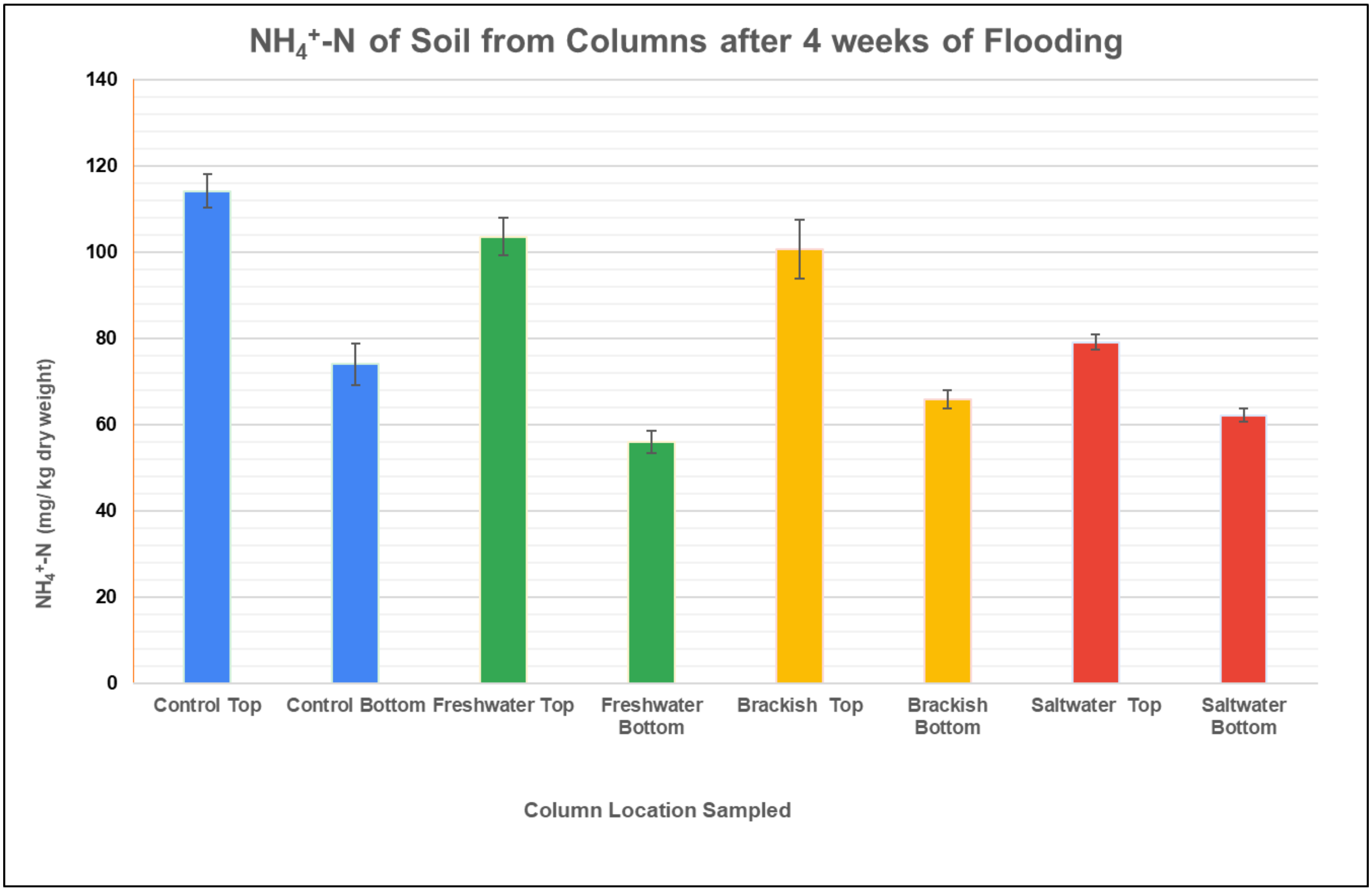


Figure 29: NH_4^+-N of Soil after Experiment

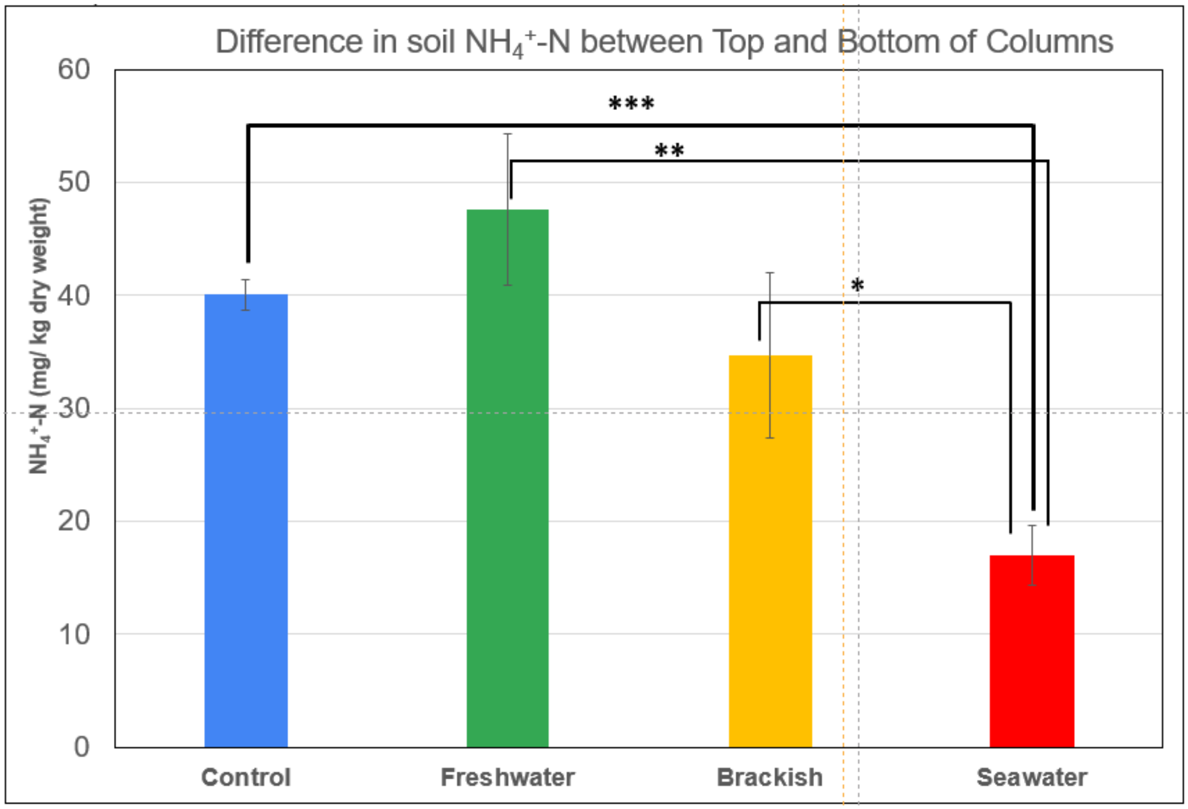


Figure 30: Difference in soil NH4 between columns

The differences in Figure 30 are the differences between column locations in Figure 29.

The difference in soil NH_4^+ between the top and bottom of the column as displayed in Figure 30 shows that the ability of NH_4^+ to be treated in the soil decreases with the addition of salt tidal floods, because the lower values in the brackish and seawater columns indicate a lower removal of NH_4^+ in the soil. This aligns with the expectations that salt can impede the adsorption of NH_4^+ onto the soil surface and could explain why more NH_4^+ was observed in the effluent of columns flooded with salt.

The implications of this could be that systems impacted by salinity can have a reduced function to treat nitrogen after a tidal flood.

There are marked differences in the adsorbed NO_x^- in the *bottom* of the columns not impacted by salt, indicating that this could be due to the activities of the nitrifying microorganisms unimpeded by the salt concentrations in the other columns.

Conversely the lower concentrations in the columns flooded by salt, could be an indication of the reduction in the activity of the nitrifying bacteria, due to the NH_4^+ substrate being less available on the soil surface from the impedance of the adsorbed salt, in addition to the potential physiological stress from the salt [89].

In addition, the differences in NO_x^- between the locations in the columns display an interesting trend in the production of NO_x^- through the soil column. The top location of the columns are relatively close to each other in terms of concentration. It is assumed that there is no NO_x^- present in the influent synthetic wastewater. At the time of sampling there is some nitrification activity occurring, and as the wastewater moves down through the column, it is expected that the NO_x^- will increase because of nitrification. In the columns not impacted by salt, it increases at the bottom of the column, however this is not the case for columns that are impacted by salt. This could relay the idea that nitrification activity, a function of the soil treatment area, is being impacted by salt.

Overall, from this one-time sampling event of soil from after the flooding events had occurred, no trends can be stated about the impacts of salinity on substrate usage, or nitrate production. In addition, there was no measure of how depth, (or the differences in “top and bottom”), impacted the activity of the nitrifying bacterias, i.e., the conversion of NH_4^+ to NO_x^-, and the results do not provide any insight into this.

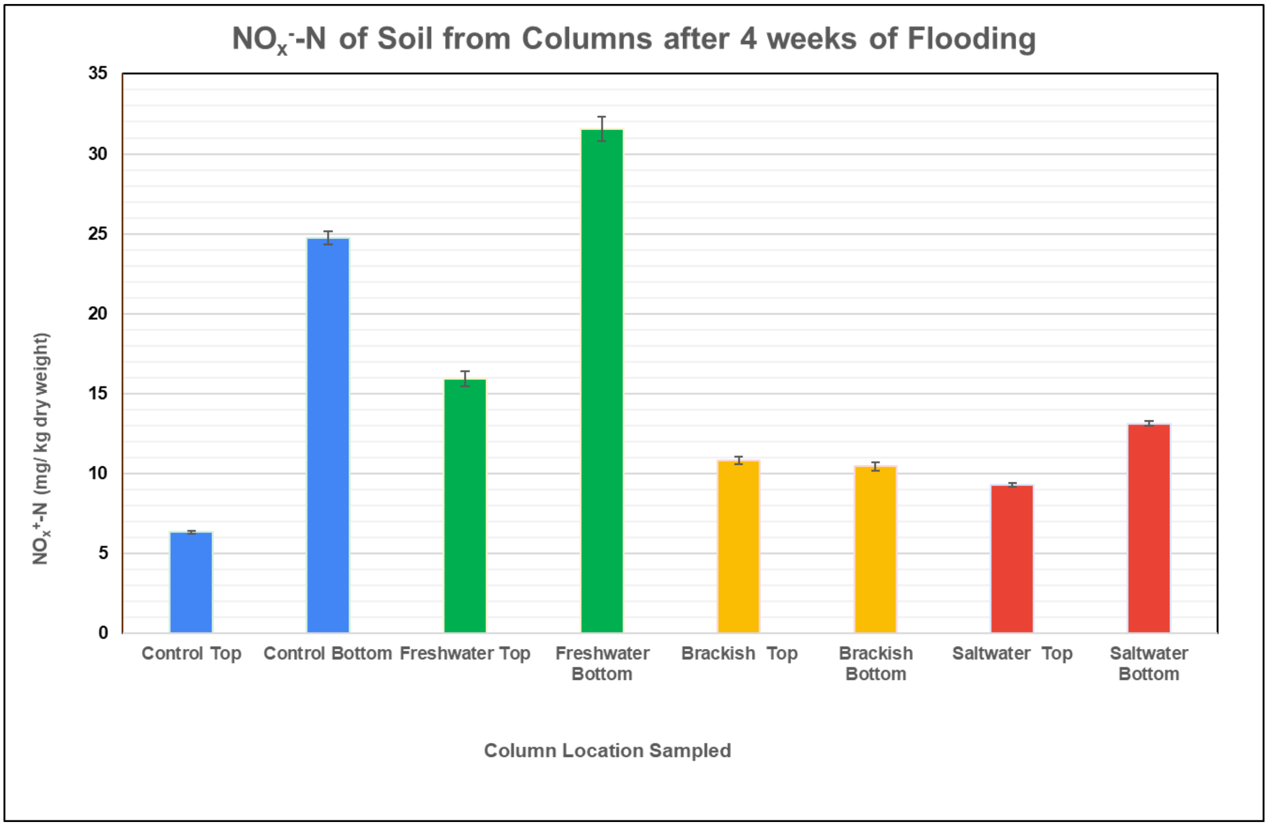


Figure 31: NO_x^--N of Soil after Experiment

Discussion

Effects of Salinity and Flooding on the Soil Treatment Process

In saline environments of a Yantgze river estuary in China, researchers found that the *amoA*-AOB and *amoaA*-AOA gene copies (responsible for expression of nitrification reaction in Bacteria and Archaea) had a negative correlation to salinity, indicating that as salinity increases, the expression decreases [96]. This could explain, at least partly, the reduction in nitrification activity taking place in the columns.

Based on Figure 29 and Figure 31, there is a possibility that salinity could be having an impact on the microbial nitrifying activities taking place in the soil matrix. A study of a Chinese riparian wetland found that rates of soil nitrification were impacted by both salinity and soil moisture [86]. Above an electrical conductivity of 4.05 mS/cm, there was an inhibitory impact on soil nitrification. The researchers concluded that this could be both from the NH_4^+ substrate not being able to be adsorbed to the soil surface, or that the higher salinities could reduce the permeability of the soil, creating a poor environment for nitrifying bacterias to grow and reside in.

In the column study undertaken, the columns effluent concentrations had a maximum electrical conductivity of 8.63 mS/cm and 19.39 mS/cm for the 15 ppt and 35 ppt columns respectively, as shown in Figure 27. The higher salinities in the effluent of our column, and the reduced efficiencies of NH_4^+ removal could be indicative of this effect.

The implications from our column study could be that in the soil treatment area where effluent first contacts the soil, salinity impacts can decrease the efficiency of nitrification needed to reduce nitrogen loading into local groundwaters. More treatment time would be needed in the unsaturated zone, and this zone could as well be compromised due to rising ground water levels from SLR impacts, decreasing the effective vertical separation needed from groundwaters, which is typically engineered to be at least four feet from the groundwater [27].

The ability of NH_4^+ to adsorb to soil sediments in increasing salinities was also observed in a study of soil cores of Danish estuarine sediments, and showed a marked decrease in the adsorption capacity when salinities were from increased from 0-10%, with a subsequent increase of NH_4^+ efflux from the cores due to this increase in salinity [89]. The researchers also found that even with an increase in available NH_4^+ substrate afforded to the saline soil cores, they found that nitrification activity decreased, indicating a potential for salinity to have detrimental physiological effects for nitrifying bacteria. There is no clear indication of the impedance nitrification activity with the results gathered from the soil sampling after the experiment concluded. Further intentional testing will need to be done to ascertain the effect of salinity on microbial nitrifying activity within soil.

The higher mass loadings of sodium into a soil system from tidal flooding events can lead to soil salinization, an issue already being seen in farms across the world, with at least 20% of the world's cultivated farmland being affected by salt [91]. A study done on the effects of the sodium adsorption ratio (SAR) on infiltration rates of water found a negative correlation between the two parameters. As the SAR increased, the infiltration rate decreased, indicating a potential of the soil aggregate shifting from clay shrinkage and/or swelling [97]. Other studies in literature have indicated that increased loading of salt can cause soil clogging, from the swelling and shrinking, changing the physical structure of the soil aggregate [98].

Septic systems are directly affected by soil properties, with the physical structure of the soil being a main factor for treatment. Seeing that infiltration rates can be affected by an increasing deposition of salts to the soil system, hazards from tidal flooding can cause detrimental effects to soil treatment. Another effect is that the ability of a soil to aerate decreases, as the salt can accumulate in available pore space, decreasing the ability of air to move through the soil pores [99]. The decreased aeration in turn can limit the potential of nitrification to occur, as it is an aerobic reaction.

In a study to determine the effects of salinity on nitrogen fixation in different soils, inorganic nitrogen was measured periodically for 102 days in soils of varying salinity with amendments of different organic nitrogen compounds. It was found that salinity impacts were observed on the processes of ammonification (conversion of organic nitrogen to inorganic nitrogen in the form of NH_4^+-N), as well as nitrification [103].

In a study on sand filtration columns with wastewater at different salinity ranges, from an electrical conductivity of 0.85 mS/cm to 3.5 mS/cm, the researchers found the effectiveness of biological treatment decreased, in that the degradation rate of organic matter decreased, and COD removal decreased [92]. This could suggest the idea that a volume of saline water with a much higher salinity found in the environments of the Chesapeake Bay (modeled as seawater with a corresponding average electrical conductivity of 37.9 mS/cm) could infiltrate the topsoil and the gravel backfill, mix with the septic tank effluent, and could result in a reduced efficiency of removing the organic material in the wastewater.

The implications of salinity impacts could be that in an event that a short-term flood occurs, the need for the filtration of incoming saline tidal waters via wetland plant buffers, or other nature-based solutions could help remediate the growing concerns of salinity on the soil treatment process [88].

Implications of incomplete nitrification in natural environments

Nitrification inhibition can lead to an excess of NH_4^+ that cannot be treated, as it cannot be adsorbed to the soil surface for treatment and is pushed further and further down into the soil environment. The ultimate fate of NH_4^+ could be variable due to a variety of factors, but there is a possibility of it moving so far that it meets the receiving groundwaters.

A study of shallow groundwaters underneath an agricultural region in Taiwan, found that there were high NH_4^+-N concentrations even with appropriate and well-managed fertilizer usage [94]. There are numerous studies that have shown that NH_4^+ toxicity can affect both plants and aquatic life. One such study found that high concentrations of NH_4^+ wastewater was shown to inhibit the nitrogen removal efficiency and COD removal in constructed wetlands, with the growth of certain wetland plants being inhibited at concentrations of total ammonium above 100 mg/L [93]. These types of nutrient enrichments can lead to eutrophication [8].

Challenges to column experiment

Challenges to the column study were numerous, in such that this was the first efforts to build, maintain, and experiment on a series of columns to model the soil treatment area of drainfields. A significant setback was the breakage of the cheesecloth that was holding in the soil of the columns. While it was good that the entire column did not break and lose too much soil, it did make the soil fall into the flow reducer. As illustrated in Figure 22, the soil being compacted by the flow reducer's change of diameter, and the added cheesecloth holding it in, could have elevated values such as moisture content and adsorbed inorganic nitrogen.

Another challenge was that soil was sampled five days afterwards from when the experiment had concluded. The synthetic wastewater that was being pumped into columns could have influenced the soil that was sampled due to the consistent flushing effect from the wastewater. The salt that had been adsorbed to the soil surface at the conclusion of the experiment could have been removed by the incoming wastewater. This could have affected inorganic nitrogen levels that could have been lower at the conclusion of the experiment, but thusly was potentially higher after five days of normal operation without flooding.

Another challenge to the experiment was the loss of sample on April 21st, 2023 (approximately three weeks into the duration of the experiment) for the column impacted by salt with a concentration modeled after the Cambridge tidal floods. This was because the tube that was connected to the synthetic wastewater had surfaced in the carboy, unable to draw in the wastewater to pump into the column. This resulted in no effluent production in the column, in addition to salt being adsorbed to the soil surfaces for longer than normal, leading to higher EC values when effluent collection resumed.

An additional challenge in analyzing the soil was that inorganic nitrogen was not analyzed before the experiment had started, leaving a large data gap that could have shown the impacts of sodium adsorption from the modeled tidal flooding, from before and after the experiment. By only having inorganic nitrogen data gathered from after the experiment, comparisons can be made on a spatial basis of "Top" and "Bottom" between columns, but not on a temporal basis.

Another challenge was from the synthetic wastewater not providing a consistent supply of NH_4^+-N from the synthetic influent due to ammonification of the organic nitrogen material. This made the mass balance performed in terms of the NH_4^+ reduction efficiency, difficult to ensure there was a consistent incoming value.

It would be very interesting to study the oxidation-reduction potential (ORP) of both locations in each of the columns. However, as I hypothesize that they could have differences based on the moisture content of the soil, in addition the salt potentially clogging the soil pores. The ORP is a measure of a species affinity to acquire electrons. NO_3^- in this case, would act as an electron acceptor. The water in the soil present could limit the diffusion of oxygen throughout the aggregate, and the salt present could be physically blocking the flow of oxygen through the aggregate as well, therefore changing the oxygen available conditions potentially for obligate aerobic species to conduct their biochemical transformations, such as nitrification, as illustrated by Equation 1.

In a study of a natural wetland that received secondary effluent in Australia, the ORP was +198 mV ± 7 mV (n =20) in soils upstream of effluent and was +112 ± 8 mV (n = 20) downstream of the effluent, indicating a decrease in voltage due to the effluent being received by the soil [105].

If the 100% organic cotton cheesecloth that was used to hold in the soil at the base of the column, as illustrated in Figure 2, could potentially be thought of as an organic carbon source, then it could also be thought of as an electron donor for heterotrophic denitrification. The net balanced redox denitrification reaction is illustrated below in Equation 4.

Potentially the difference in oxidation reduction potential and the usage of the cotton cheesecloth, could be a reason as to why the NO_x^- levels in the bottoms of the column salt flood columns could be markedly lower than compared to the bottom layers of the control and freshwater column. The limited diffusion of oxygen, due to salt clogging, and increased moisture content of the soil, could provide better conditions for heterotrophic denitrification.

More work will have to be done to ascertain the effects of salinity on denitrification processes in soil treatment areas.

Equation 4: Net Balanced Denitrification Reaction

$$2NO_3^- + 10e^- + 12\ H^+ \rightarrow N_2 + 6H_2O$$

All these challenges made running a column experiment for the first time over a short duration difficult, and while results were gathered and analyzed and made statistical and conceptual sense, changes can be made in the next iteration of a column study for stronger results.

Conclusion

It is evident that over a short-term column study, salinity impacts from tidal flooding to the soil treatment area can reduce the efficiency of NH_4^+-N and organic compounds such as peptone and urea to be treated by soil microorganisms. In addition, the sharp changes in the pH and EC of the effluent after a flood event in the columns impacted by salt, and the subsequent shift in the baselines of the columns, could indicate that the function of the soil treatment area will change as the flood events continue to occur.

With climate change increasing the frequency, duration, and depth of tidal flooding events in Maryland, this can be problematic for environmental managers in ensuring adequate treatment of septic tank effluent in the soils of drainfields if they are impacted by salinity from the tidal floods [117]. The number of drainfields that are in the critical area of Maryland (land within 1,000 ft of tidal waters) are approximately 52,000 [28]. These systems are at highest risk for tidal flooding impacts, and subsequently the receiving groundwaters that lead to the Chesapeake Bay can be at risk of increasing NH_4^+-N or dissolved organic solids deposition.

Chapter 4: Perspectives Paper

A variety of Geospatial Modeling Efforts

Onsite Wastewater treatment is used by over 1 in 5 households in the United States to treat their effluent [76]. Onsite wastewater programs and regulations are managed by local governments, typically on a county level, with federal programs such as the Environmental Protection Agency (EPA) only providing guidelines for implementing management programs [27]. This can lead to a data gap for state planners to assess septic system risk on a county basis, with the gaps in geospatial data making it difficult to model where upgrades or modifications for flood resilience need to be prioritized.

Efforts to integrate geospatial data of parcels on septic with various environmental parameters such as soil type, soil slopes, and distance to streams have been done to model environmental and public health risk have been undertaken, albeit with limited county data sets.

In a study in Athens-Clarke County (ACC), Georgia researchers were able to identify parcels on septic with limited county data resources, which was a combination of sewer line locations, tax records, septic geospatial layers [118]. They assessed those local layers in combination with national data sets, including socio-economic data from the US Census Bureau, distances of parcels to closest streams from the US National Hydrography Data Set, soil data from the US Department of Agriculture Soil Survey Geographic, and soil slope data derived US Geological Survey National Elevation Data Set.

Ultimately, what they concluded was that of the 9,083 parcels on septic in ACC (42,089 total parcels in the county), 6,259 systems were at risk from age (in use for >25 years). In addition, 2,524 parcels of the 9,083 total were above 45 years in age, and that 540 of the systems above 45 years of age were in predominantly non-white and impoverished areas. One of the researchers' conclusions, is that the age of the system can play a large risk to public health, and policies that can remediate infrastructure deficiencies such as consistent septic system maintenance can help to alleviate the public health risk from nutrient pollution. The takeaway from this study is that the age of septic systems should be a key component when assessing the risk of septic system failure.

In a separate study of Semarang City in Indonesia, a geospatial model was constructed for onsite wastewater epidemiology on a landscape scale [119]. Specific focus was studied with regards to explanatory variables including soil permeability, inundation risk, population density among others, and the relations they play with incidences of typhoid and diarrhea, two common diseases associated with poor sanitation. Their conclusions include that using the explanatory variables in relation to the target disease by way of Poisson regression (a statistics method used to model "count" data and the impact a variable (such as inundation) can have on an incident occurring), can be useful in landscape epidemiology. The outcome of their study was that they developed a hotspot analysis of Semarang City and indicated that in elevated areas prone to inundation with higher rainfall, there could be higher incidences of diseases from septic systems. Like the study in ACC, the researchers stress that data gaps can make modeling difficult and lead to uncertainties. With sea-level rise (SLR) affecting infrastructure in low lying coastal areas, the risk from septic systems to the natural environment and public health can increase, and climate change impacts from SLR can decrease efficiencies in treatment of nutrients and pathogen removal [120, 15].

In a separate study on coastal Rhode Island, researchers found that in an event of a hurricanes with increasing severity (category one to category four), that the number of systems to be at risk will range anywhere from 2,000 to 4,600 depending on the severity of the hurricane [121]. In addition, the number of systems at risk will increase by a count of 200 if 0.3 m of SLR increase is accounted for in their modeling.

Other studies have looked at risks to groundwater from seawater intrusion, such as a study of a coastal aquifer in Tunisia, the Mahdia–Ksour Essaf aquifer [123]. Nutrient loading from inorganic fertilizers and septic systems already contributed to poor water quality, and the researchers found that seawater intrusion can increase the vulnerability of the aquifer to the deposition of harmful pollutants, such as nitrate. In addition, highly vulnerable areas to saltwater intrusion had higher loadings of nitrate when groundwater wells were sampled. The implications of this can mean that in areas without robust water treatment facilities, drinking water wells that pull from groundwater can be at higher risk of ingesting contaminated water.

The uncertainty of "where" and "when" and "how often" these systems will be affected from sea level rise due to the lack of accurate and precise mapping can be a problem for local governments, interested homebuyers, insurance agencies, and natural environment stakeholders. SLR impacts to the real-estate market have already been noted for communities on the Chesapeake Bay [122]. The need for consistent and high-quality data to geo-spatially model parcels on septic in counties across the state of Maryland combined with an analysis of systems that are most at risk from the growing threat of sea level rise, is necessary and vital.

In reviewing papers that ranged from a socio-economic analysis of septic systems in a county in Georgia, risks to septic systems from coastal flooding and hurricanes in Rhode Island, creating a hotspot analysis in Indonesia to assess risk of disease from septic systems, to a study of saltwater intrusion in coastal aquifers, a common theme among those papers was that limited data made analysis difficult and that data gaps can exist to accurately and precisely assess the question the researchers set out to solve.

Inconsistent Definitions of Failure between states

In addition to geospatial modeling on a larger scale other than a single county or city being difficult, the EPA in their Onsite Wastewater Treatment Systems Manual, tabulated responses from a report commissioned by the Electrical Power Research Institute in 2000, in which a questionnaire was sent to regulators and managers of state onsite wastewater treatment programs [27, 124]. Questions included information to detail the number of systems that exist, the status of onsite wastewater treatment programs, changes in management or regulations, and interest in adoption of advanced technologies. A notable byproduct of their report was the large range of failure rates, and their definitions of what failure means for each state. It can be notable that for certain states such as Alabama and Louisiana, there was no definition given, with about 20% and 50% respective estimated rate of failure. Other states such as West Virginia estimate a 60% failure rate, but provide a definition of failure, such as effluent backing up, or surface and groundwater contamination. With specific regards to Maryland, the state definition of "Failing On–Site Sewage Disposal System" as defined by House Bill 190, concurs with Figure 30 [125].

The wide range of failure rates, and inconsistent definitions of what failure means between states, can make federal policy to prioritize onsite wastewater infrastructure difficult, and national efforts to assess risk almost impossible without consistent failure definitions.

On a state level, if permits and records are kept on a county level, and if, by chance, counties are not forthcoming with septic system permit data, it can lead to data gaps for spatial models and thus impedes policy makers to highlight where prioritization needs to be. Perhaps a recommendation could be that there should be a data collection scheme for states to prevent this issue. Specific focus on collected parameters could be information such as: age of the system, frequency of inundation events, and proximity to drinking water well. With these parameters in mind, if a system is older than 30 years, it could be recommended to prioritize a replacement. Note: it may be reasonable to identify older systems that may be due for upcoming replacement, but not to automatically replace an old system that is still functional. This is especially the case where there is no replacement area.

Table 1-3. Estimated onsite treatment system failure rates in surveyed states

State	Estimated system failure rate (percentage)	Failure definition
Alabama	20	Not given
Arizona	0.5	Surfacing, backup, surface or ground water contamination
California	1–4	Surfacing, backup, surface or ground water contamination
Florida	1–2	Surfacing, backup, surface or ground water contamination
Georgia	1.7	Public hazard
Hawaii	15–35	Improper construction, overflow
Idaho	20	Backup, surface or ground water contamination
Kansas	10–15	Surfacing, nuisance conditions (for installations after 1980)
Louisiana	50	Not given
Maryland	1	Surfacing, surface or ground water contamination
Massachusetts	25	Public health
Minnesota	50–70	Cesspool, surfacing, inadequate soil layer, leaking
Missouri	30–50	Backup, surface or ground water contamination
Nebraska	40	Nonconforming system, water quality
New Hampshire	<5	Surfacing, backup
New Mexico	20	Surfacing
New York	4	Backup, surface or ground water contamination
North Carolina	15–20	Not given
North Dakota	28	Backup, surfacing
Ohio	25–30	Backup, surfacing
Oklahoma	5–10	Backup, surfacing, discharge off property
Rhode Island	25	Not given
South Carolina	6–7	Backup, surface or ground water contamination
Texas	10–15	Surfacing, surface or ground water contamination
Utah	0.5	Surfacing, backup, exceed discharge standards
Washington	33	Public health hazard
West Virginia	60	Backup, surface or ground water contamination
Wyoming	0.4	Backup, surfacing, ground water contamination

[a] Failure rates are estimated and vary with the definition of failure.

Source: Nelson et al., 1999.

USEPA Onsite Wastewater Treatment Systems Manual

Figure 32: Various states and their definitions of failure and percentage of occurrence. Compiled from the Electrical Power Research Institute's market study state report.

Using ArcGIS, I was able to create a map of polygon parcels that could be on septic in Anne Arundel County, Maryland using a dataset from the Maryland Department of Planning [126]. By removing parcels that were on public sewer (PFUS = 1) and filtering the parcels to only include a land usage code of “Residential” (DESCLU = “Residential”), this was the first step to spatially mapping parcels on septic. Initially there were 223,548 polygons in the initial data set, and afterwards this left the number of parcels in the data set to (n = 37,048 parcels). This value is within 10% of the projected number of parcels (40,000) from the data report in the Field Study introduction.

After that, the FEMA Risk Index map by census tract was added in, specifically looking at an overlay of Coastal Flooding Expected Annual Loss Rating, which is defined as the dollar amount lost to damage to buildings, populations, or agriculture [127].

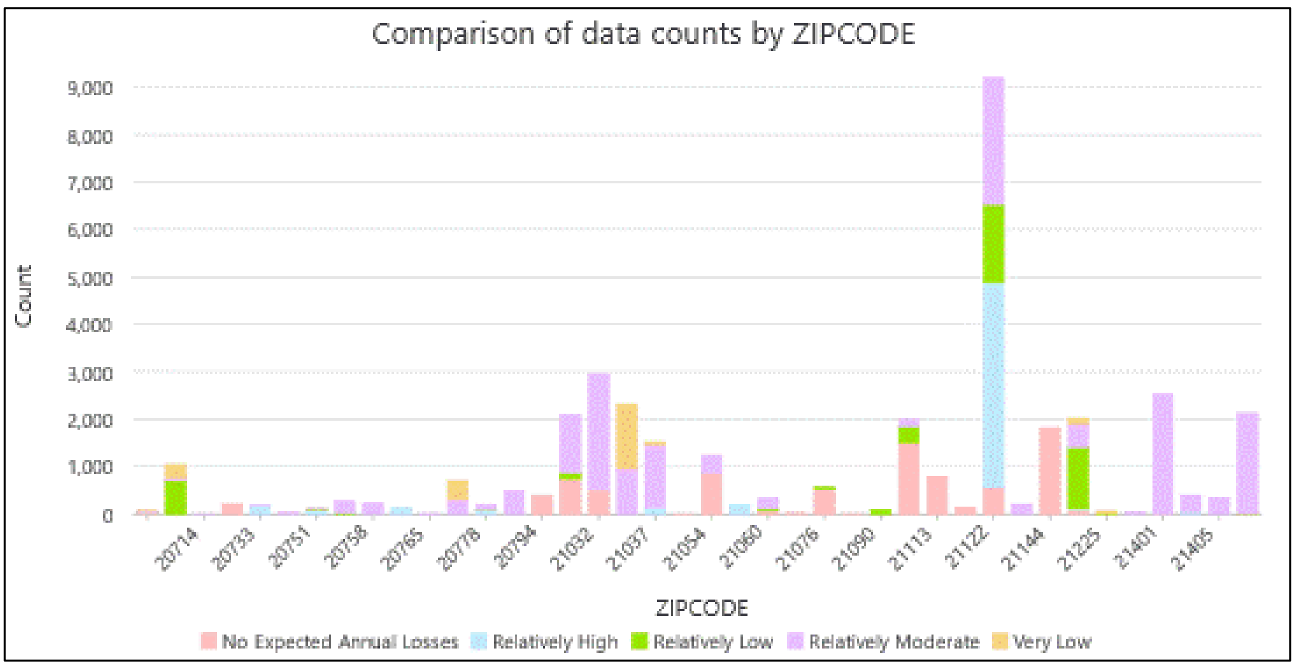


Figure 33: Residential Parcels in Anne Arundel County not on Public Sewer, in areas at risk of loss for coastal flooding – organized by zipcode.

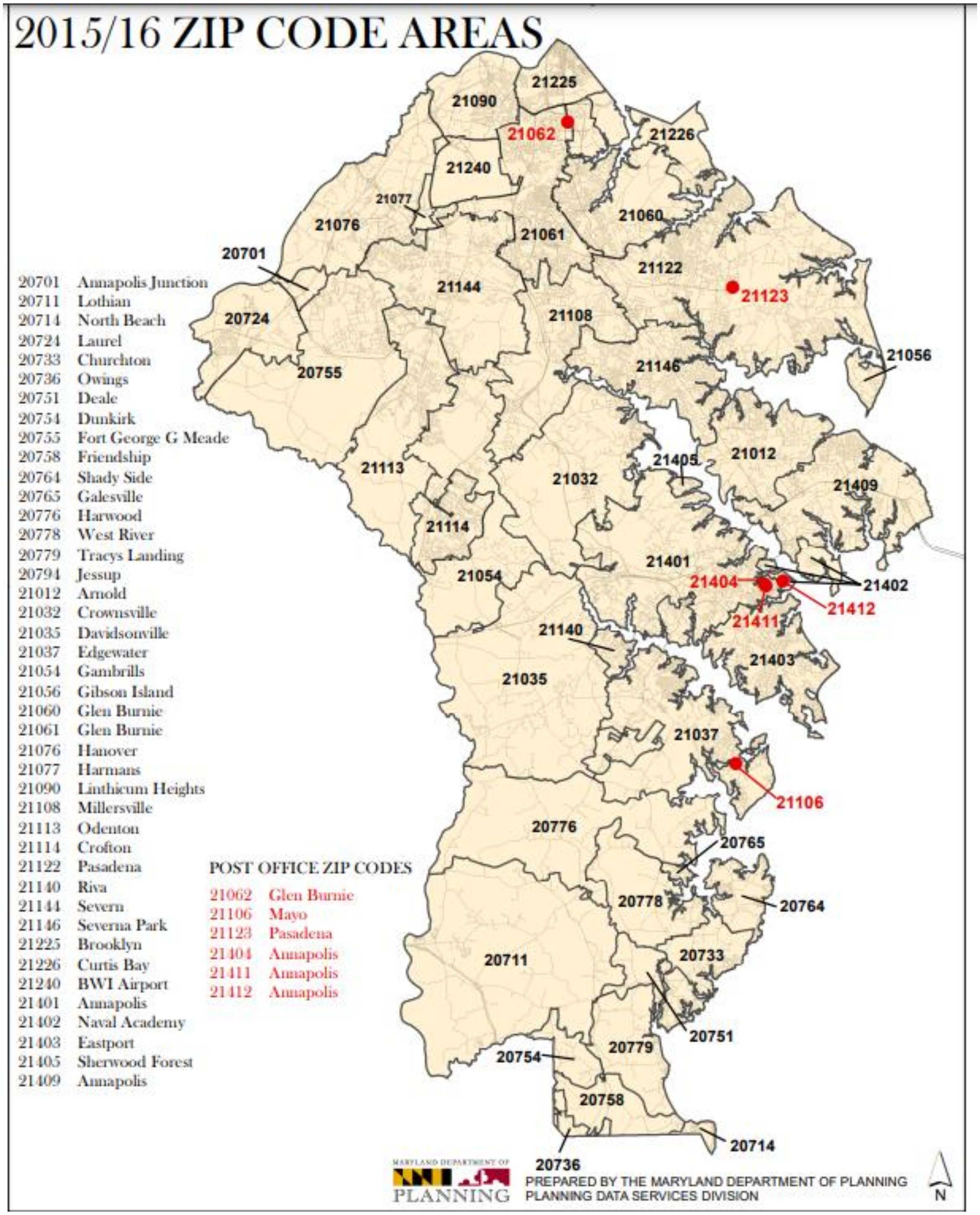


Figure 34: Zip codes of Anne Arundel County, red codes are for post offices.

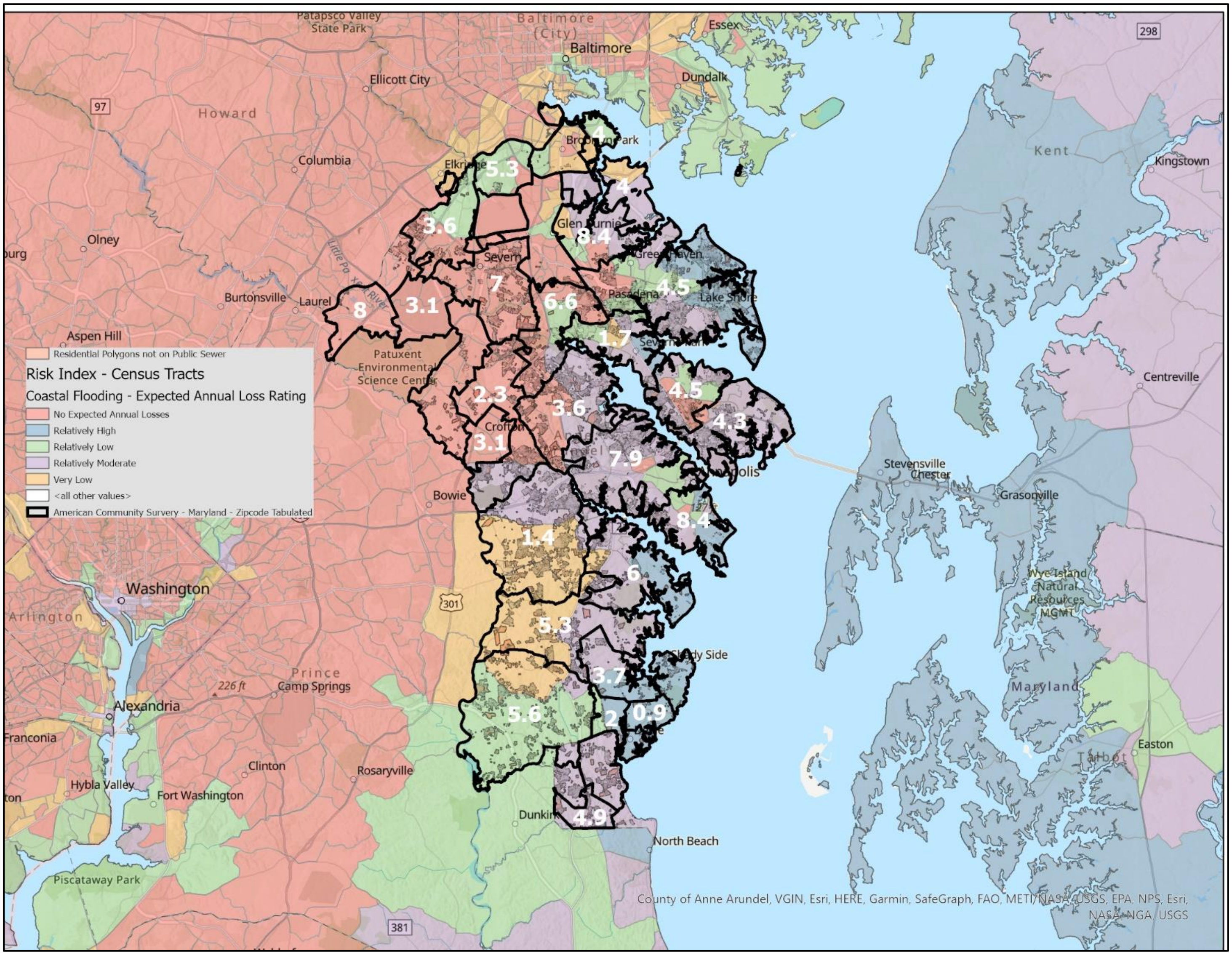


Figure 35: Map of Anne Arundel County with Residential Parcels not on Public Sewer - overlayed with FEMA Coastal Flooding Risk Index.

With the overlayed coastal flooding layer, a chart was generated to display which zip codes had septic parcels rated against the expected annual loss. With all this data, we can see those parcels in the zip code 21122, have approximately 4,000 parcels at risk of “Relatively High” loss. In addition, there are also data labels from the American Community Survey, with the label being percentage of residents without health insurance by zip code [128]. For example, 4.5% of residents in zip code 21122, do not have health insurance. This could also provide insight into another factor of risk from failing septic systems from a socio-economic perspective. While no analysis was done on the eastern shore of Maryland, it’s interesting to note that a wide swath of area directly across the Chesapeake Bay, is rated as “Relatively High”, indicated by the blue color.

From the initial analysis presented, it can be difficult to state which systems are at risk of failing, but potentially it can be used conjunction with county planners in Anne Arundel, to help to elucidate which parcels need to be prioritized to prevent flooding damage. While permits and records for septic systems are kept by the county health department, it could be worthwhile to request records and see if all the systems in areas at “Relatively High” have been upgraded to BAT systems, or have other infrastructure available to withstand flooding, such as bulkheads or natural riparian buffers [131].

Chapter 5: Conclusion

Soil treatment in septic systems is unique and can depend on treatment options. The systems sampled had unique soil conditions, technology upgrades, and loading conditions but the systems sampled are functioning and work well to treat wastewater. Both systems can treat nitrogen effectively in the soil, as cycling of nitrogen was seen throughout the soil profile. In addition, the activity of the nitrifying community in both systems was observed through the MPN analysis, by way of an inorganic chemical media that was inoculated with soil. Although this was a one-time sampling event, repeated sampling events over a spatial and temporal basis should be done to better grasp the processes that are occurring in these systems.

Salinity impacts to soil treatment area can reduce treatment of nitrogen and have impacts on effluent moving through columns of soil. It was found that there was a reduction in function in treating nitrogen when subjected to saline flooding. Repeated floods over time hindered the ability of the flooded saltwater systems to stabilize back to normal conditions in comparison to the control column. These results were only after four weeks of analysis, and it would be expected that the function of the soil treatment area would further decrease over the average lifetime usage of a drainfield which is about 20-30 years.

In terms of future work, there are many data gaps in terms of microbial processes related to septic system soil treatment that can be explored by researchers. In this project, mainly the activity of nitrifying bacteria was studied via two vectors, the MPN analysis from the field sites and the disappearance of NH_4^+ in the flooded columns. The analysis did not give much information with regard to the community composition of the nitrifying bacteria. A study on specific microbial communities via bioinformatic techniques such as qPCR using DNA extraction can give insight into how communities are being affected from flooding. This could be used to understand future problems that will arise from an inability to treat nitrogen in the soil from repeated flooding exposure. With an understanding of the composition of the microbial community, researchers could then find ways to make the soil treatment area more resilient to flooding. This could be done perhaps with the addition of certain bacteria that can undergo the “anammox” process, the ability of converting NH_4^+ and NO_2^- into N_2 and H_2O in a single step anaerobic process. Anammox bacteria has already been studied and applied in centralized wastewater treatment systems, with results showing that an annamox reactor can effectively treat domestic sewage [129]. If a soil treatment area becomes flooded and the dissolved oxygen levels make it such that aerobic reactions cannot occur, the addition of anammox bacteria could help to anaerobically oxidize the NH_4^+. Future work could also look at impacts to the nitrogen cycle via the addition of riparian wetlands near the soil treatment area. Riparian zones have been shown to prevent agricultural nitrogen pollution [130], and an analysis of the microbial ecology in the riparian wetland additions can help to characterize microbial interactions and manage the nitrogen pollution in coastal areas [131].

If I had to continue to keep working on this project, I would work with community partners such as SERCAP, to develop soil metrics for a “Healthy” and “Failed” drainfield, so I could better ascertain what it means for a soil treatment area of a drainfield to not work as intended in terms of their biogeochemical parameters.

Overall, I learned that nitrogen is a crucial nutrient to manage in terms of septic systems, which can have implications on the health of the Chesapeake Bay. In studying a variety of heterogeneous soil systems, I found that nitrification is a vital process for the treatment of nitrogen in drainfields. When a system becomes disturbed from flooding, it can reduce the efficiency of the soil system to undergo nitrification. I learned that consistent monitoring of drainfields is important to ensure that the systems are functioning and are working as designed. I learned a variety of analytical techniques that helped to quantity and classify the data gathered to reach my conclusions, which overall, was that drainfield function can be disrupted from flood events. Since drainfield repairs and maintenance are a burden placed almost entirely on homeowners themselves, low-income areas in coastal areas are at a higher disadvantage in terms of keeping their drainfield functioning after a flood occurs. Resources should be focused on reducing the maintenance burden for low-income residents using septic systems, in addition to mitigating flood exposure for all communities.

Appendices

DEI Assignment

1) *Identify topic of research and describe the field – research goals.*

The topic at large is how drainfields can be affected by salinity when it comes to nitrification, in addition to a comparative analysis on two distinct types of drainfields in the North and South of Anne Arundel County, Maryland.

The field of this research has been covered in a variety of ways, from discussion of microbial activity to transformation of nitrogen in areas receiving effluent, in addition to the "bigger-picture" effects of climate crisis induced sea-level rise on wastewater treatment systems. Not as much work has been done in comparison to research on septic tank systems/innovations.

The research goals for the summer were to develop methodology for the field sampling and lab tests to perform over Fall, which include chemical testing: pH, EC, ORP; soil classifications: organic matter, moisture content; and biological methods: MPN of nitrifying bacteria.

Table 14: Prominent Researchers in the Field

Most Cited Author/Group	Country / Funding	Gender, Career stage, Age
Jose A Amador	Department of Natural Resources Science – University of Rhode Island	Male, Professor, (older male)
George Loomis	Department of Natural Resources Science – University of Rhode Island	Male, Senior Program Advisor, New England Onsite Wastewater Training Program
Gurpal S Toor	University of Maryland, College Park	Male, Professor, Extension Specialist, & Associate Chair
Mriganka De	Minnesota State University, Mankato	Male, Assistant Professor
Jennifer Cooper	University of Nebraska-Lincoln	Female, Post-Doc,
Michael O'Driscoll	East Carolina University	Male, Associate Professor
Charles P Humphrey	East Carolina University	Male, Professor
US EPA	United States Government	Agency
Kathryn Lowe	Colorado School of Mines	Female, Senior Research Associate
Guy Iverson	East Carolina University	Male, Assistant Professor

Using Clarivate – Journal Citation Reports for 2021 for Impact Factor

% Gold Open Access is from Clarivate

Publication fee for OA is found from each individual journal website.

Table 15: Scientific Journals Citation Reports

Journal Name	Impact Factors Journal Impact Factor	Publishing Company	% Gold Open Access	Publication Fee ($) for Open Access
Journal of Environmental Quality	3.866	Wiley	33.54%	1150
Water Research	13.400	Science Direct	11.03	4220
Ecological Engineering	4.379	Science Direct	4.83%	3500
PLOS ONE	3.752	N/A	Open Access	N/A
Science of the total environment	10.753	Science Direct	9.07%	3680
US EPA Website	N/A	US Government	Open Access	N/A
Soil Research	1.878	CSIRO		2700
Environmental Technology	3.475	Taylor & Francis Online	1.72	3085
Ground Water	2.887	Wiley		3900
Water, Air, and Soil Pollution	2.984	Springer		3,280

Reflection

1) How difficult was it to find information about missing and underrepresented authors.

Since I focused the initial review around the biogeochemistry of drain fields, a very small field in and of its own with regards to septic tanks, the bulk of papers are found to be written by select authors that belong to the same research group, mainly at University of Rhode Island and East Carolina University. The base of the paper I started with to branch out using the website, Connected Papers, was “Hell and High Water: Diminished Septic System Performance in Coastal Regions Due to Climate Change”, published in 2016 and written by Jennifer Cooper, George Loomis, and Jose A Amador – which was a good starting point, admittedly though, written by the research group at University of Rhode Island, two men and one woman.

Using the derivative works from that starting point, the relevant papers were again written mostly by the same authors. There were a few papers that had lower amounts of citations, but they were again mostly by the same group.

There was one paper that a master’s thesis, “Establishing Failure Indicators for Conventional On-site Wastewater Treatment Systems: A thesis submitted in partial fulfilment of the requirements for the Degree in Master of Water Resources Management by Preston Junior Prince Waterways Centre for Freshwater Management” by T.D. Vries in 2017, but this was only a master’s thesis, which could potentially have varying levels of quality.

2) Did this assignment change the way you search for resources and literature sources? The way you cite and who you cite?

This assignment was instrumental in helping me learn about different resources for citing papers, mainly Connected Papers. I think it was insightful to look at what journals have their % OA, and how much they charge to do so, it makes me want to fully support journals like PLOS ONE, and retrieve as many citations I can from there, as opposed to something like Water Research.

3) How do you plan to integrate the tools (databases) etc. from this assignment into your research process?

I would like to start using the curated databases set forth for civil engineers by the engineering librarian more regularly, which I have used in the past, mainly the ASCE database.

From this assignment though, I have already gotten myself familiar with Boolean searching, and have gotten quite comfortable using “quotations” for specific searches with AND statements.

Connected Papers is an invaluable resource, and I would like to explore that more, although that is a subscription-based program to use.

Field Study Appendix

A. Sample Calculations

1. KCl Extraction Sample Calculation

In extracting the soil using a solution of 2M KCl, the SEAL AQ300 will report the values in (mg/L). This is not how it is typically reported in the literature, typically being reported in soil as (mg / kg dry weight).

To do this, take the 30 mL extraction solution used for the SEAL AQ300, and multiply it by the (mg/L) value. Then, take the weight of soil used (6 grams), and remove the moisture of the soil by taking the inverse of the moisture content value. Convert to g dry weight to kg dry weight, and that is the final value.

Sample = 0.13101 mg/L (Average of 3 samples from depth of 0 -5'' in Drainfield 1)

Average of 3 samples for Moisture Content of depth 0-5'' from Drainfield 1 = 23.59%

$$4.367\frac{mg}{L} * 30\ mL\frac{1\ L}{1000mL} = \ 0.13101\ mg$$

$$6\ g\ (1 - .2359) = 4.5846\ g\ Dry = \ 4.5846\ x10^{-3}\ kg\ Dry$$

$$\frac{0.13101\ mg}{4.5846x10^{-3}kg\ dry} = 28.58\frac{mg}{kg\ dry}$$

2. MPN Table Calculation and Dry Weight Adjustment

The FDA BAM manual [41] states to start with the largest dilution where all the tubes are positive.

The smallest dilution with all positive tubes is 10^{-2} and the largest dilution with all negative tubes is 10^{-4}. This is in accordance with the FDA BAM manual, in which the values from the table are done in accordance with Example B from the *"Selecting Three Dilutions for Table Reference"* as well as the *"Conversion of Table Units"* section of the manual.

1) Value from Table is (3,1,0) is 43 (for Drainfield 1) *(Selecting three Dilutions for Table Reference).*

Multiply by 10, since the first dilution series started at 10^-2, and not 10^-1 *(Conversion of Table Units).*

Value from Table 1 is now 430 MPN/g soil (wet weight)

Adjust for Dry Weight

The average moisture content across all samples from Drainfield 1 is 21.68%, meaning that 78.32% is dry.

$$\frac{430\,\frac{MPN}{g}}{78.32\%} = 549\,\frac{MPN}{g}\,dry$$

B. FDA-BAM Table

Table B-1: FDA BAM Table [41]

Pos. Tubes				Conf. lim.		Pos. tubes				Conf. lim.	
0.1	0.01	0.001	MPN/g	Low	High	0.1	0.01	0.001	MPN/g	Low	High
0	0	0	<>	–	9.5	2	2	0	21	4.5	42
0	0	1	3	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	–

C. Statistical Results from t-test

NH4+ between samples in both Drainfield at different depths

t-Test: Two-Sample Assuming Unequal Variances

	Drainfield 1	Drainfield 2
Mean	17.46615625	72.491857
Variance	145.3553137	1534.45535
Observatio	9	12
Hypothesiz	0	
df	14	
t Stat	-4.585126903	
P(T<=t) or	0.000212154	
t Critical o	1.761310136	
P(T<=t) tw	0.000424309	
t Critical tw	2.144786688	

NOx- between samples in both Drainfield at different depths

t-Test: Two-Sample Assuming Equal Variances

	Drainfield 1	Drainfield 2
Mean	15.2199566	28.0369132
Variance	180.428648	368.478674
Observatio	9	12
Pooled Va	289.299716	
Hypothesiz	0	
df	19	
t Stat	-1.7088862	
P(T<=t) or	0.05187831	
t Critical o	1.72913281	
P(T<=t) tw	0.10375662	
t Critical tw	2.09302405	

Moisture Content between samples in both Drainfield at different depths

t-Test: Two-Sample Assuming Unequal Varia

	Variable 1	Variable 2
Mean	0.216029	0.17183
Variance	0.001106	0.012693
Observatio	9	12
Hypothesiz	0	
df	13	
t Stat	1.286383	
P(T<=t) or	0.110374	
t Critical o	1.770933	
P(T<=t) tw	0.220747	
t Critical tw	2.160369	

Organic Matter between samples in both Drainfield at different depths

t-Test: Two-Sample Assuming Equal Variances

	Drainfield 1	Drainfield 2
Mean	0.0329267	0.02067199
Variance	0.0001122	0.00031253
Observatio	9	12
Pooled Va	0.0002282	
Hypothesiz	0	
df	19	
t Stat	1.8397637	
P(T<=t) or	0.0407404	
t Critical o	1.7291328	
P(T<=t) tw	0.0814808	
t Critical tw	2.0930241	

Column Study Appendix

A. Sample Calculations

1. Volume Flow of Synthetic Wastewater

*Equation = Hydraulic Loading Rate * Column Surface Area*
Diameter of Column = 4 in. = 0.333 ft.

$$1.2\frac{gal}{day * ft^2} * \frac{\pi(0.333\ ft)^2}{4} = .104\frac{gal}{day} \cong 16.5\frac{mL}{hour}$$

2. Flood Depth Sample Calculation

Equation = Ratio of Soil Treatment Areas (Column/Conventional) to Flood Depths (Column/Tidal Gauge)

0.5 ft = Approximate Depth of Soil Treatment area in Column

4 ft = Maximum Depth of Treatment in Conventional Drainfield

1.565 ft = Average Depth of High Tide Flood that occurs in Cambridge, MD Station #8571892 from March 1st to March 31st

X ft. = Unknown value to solve to obtain depth of tidal flood to simulate into column.

$$\frac{0.5}{4} = \frac{x}{1.565}$$

$$x \cong 0.2\ ft \cong 2.4\ in.$$

3. Flood Volume for Column from Flood Depth

*Equation = Flood Depth of Column * Column Surface Area*

0.2 ft = Approximate Value of Depth of Flood for column

Diameter of Column = 0.333 ft

$$0.2\ ft * \frac{\pi(0.333\ ft)^2}{4} \cong .0145 ft^3 \cong 410\ mL$$

4. Creating Saltwater Solutions

Manufacturer stated that for 1.5 lbs. of Instant Ocean Mix for 5 gallons will create a S.G. of 1.026, the approximate S.G. of Natural Sea Water (NSW) is 1.026. This will create a 35 ppt solution.

Equation = Ratio of Manufacturer Instructions (1.5 lbs. / 5 gallons) to Column Study (x grams / 410 mL)

$$\frac{1.5\ lbs}{5\ gal} = \frac{x\ g}{410\ mL}$$

$$x \cong 0.032\ lbs. \cong 14.7\ g$$

To create a 15 ppt solution, a ratio of the solved mass from the 35 ppt ratio will be done for a 15 ppt solution.

$$\frac{14.7\ g}{35\ ppt} = \frac{x\ g}{15\ ppt}$$

$$x = 6.2\ g$$

5. KCl Extraction Sample Calculation

In extracting the soil using a solution of 2M KCl, the SEAL AQ300 will report the values in (mg/L). This is not how it is typically reported in the literature, typically being reported in soil as (mg / kg dry weight).

To do this, take the 30 mL extraction solution used for the SEAL AQ300, and multiply it by the (mg/L) value. Then, take the weight of soil used (6 grams), and remove the moisture of the soil by taking the inverse of the moisture content value. Convert to g dry weight to kg dry weight, and that is the final value.

Sample = 17.9387 mg/L (Average of 3 samples of Control Top after flooding)

Moisture Content of Control Top after flooding = 21.40%

$$17.9387 \frac{mg}{L} * 30\ mL \frac{1\ L}{1000 mL} = 0.518\ mg$$

$$6\ g\ (1 - .2140) = 4.716\ g\ Dry = 4.716x10^{-3}\ kg\ Dry$$

$$\frac{0.518\ mg}{4.716x10^{-3} kg\ dry} = 114.11 \frac{mg}{kg\ dry}$$

6. NH_4^+-N Reduction Efficiency

Take the average value of NH_4^+-N from the synthetic wastewater influent into the columns, which was 33.84 mg/L, and multiply this by 15 mL/hour, converting it into a mass flow rate, as the incoming mass flow.

Then, by taking the average concentration reported by the SEAL AQ300, for example the Control column on March 27th, 2023, was 0.126 mg/L. By taking a flow rate of 15 mL/hour, convert into a mass flow rate, as the outcoming mass flow.

Then divide the difference of incoming mass flow and outgoing mass flow by the incoming mass flow to get an efficiency value of the reduction of NH_4^+-N (i.e., nitrification).

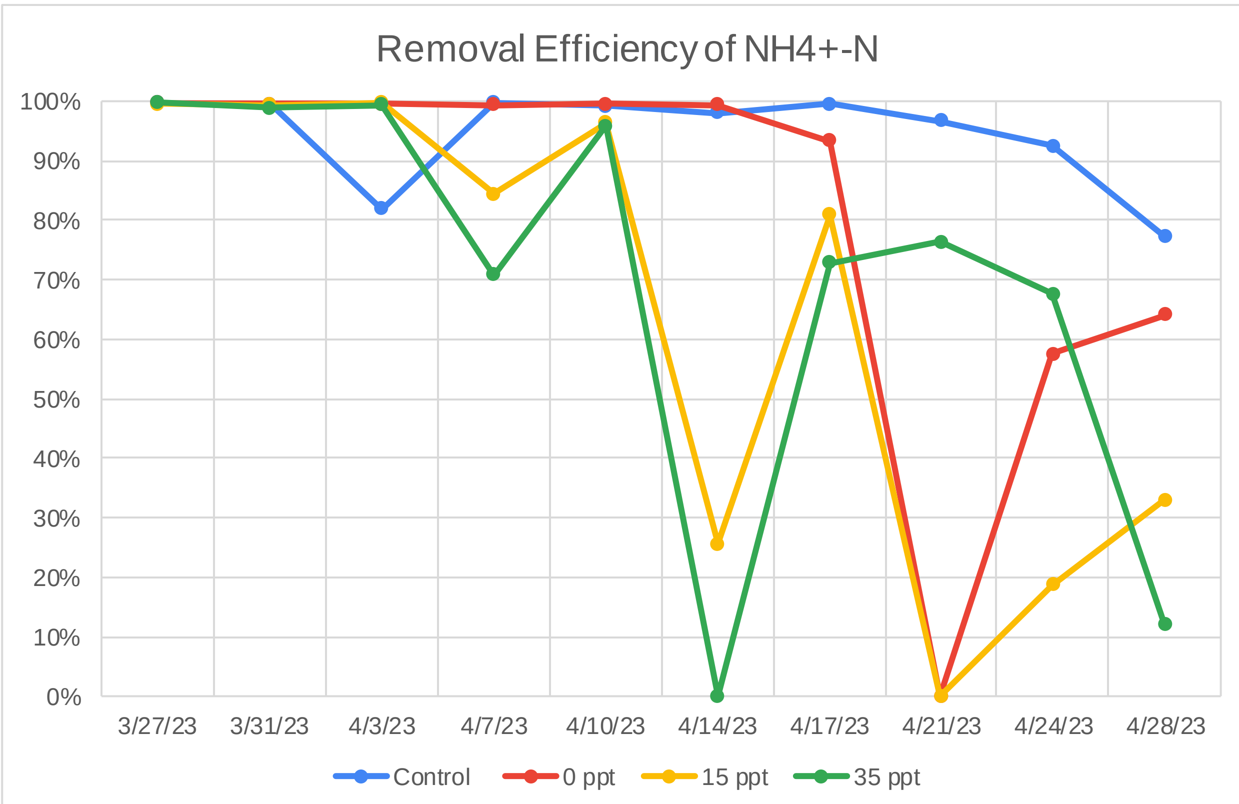
$$0.126\frac{mg}{L} * 15\frac{mL}{hour}\left(\frac{1L}{1000\ mL}\right) = 0.0019\frac{mg}{hour}(Incoming\ \mathrm{NH_4^+} - N)$$

$$33.84\frac{mg}{L} * 15\frac{mL}{hour}\left(\frac{1L}{1000\ mL}\right) = 0.51\frac{mg}{hour}\ (Outgoing\ \mathrm{NH_4^+} - N)$$

$$\frac{0.51\frac{mg}{hour} - 0.0019\frac{mg}{hour}}{0.51\frac{mg}{hour}} = 99.63\%\ Removal\ of\ \mathrm{NH_4^+} - N$$

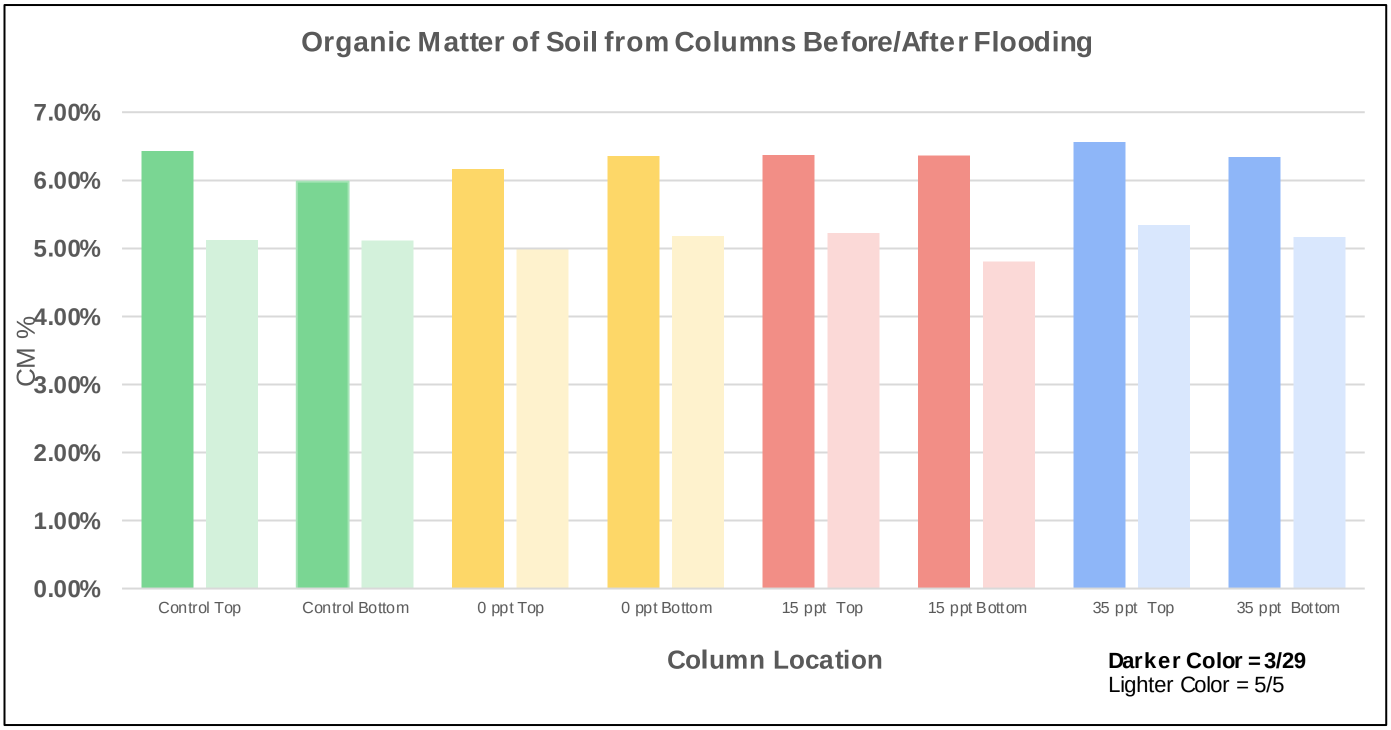
B. Supplemental Charts

NH_4^+ Reduction Over Time

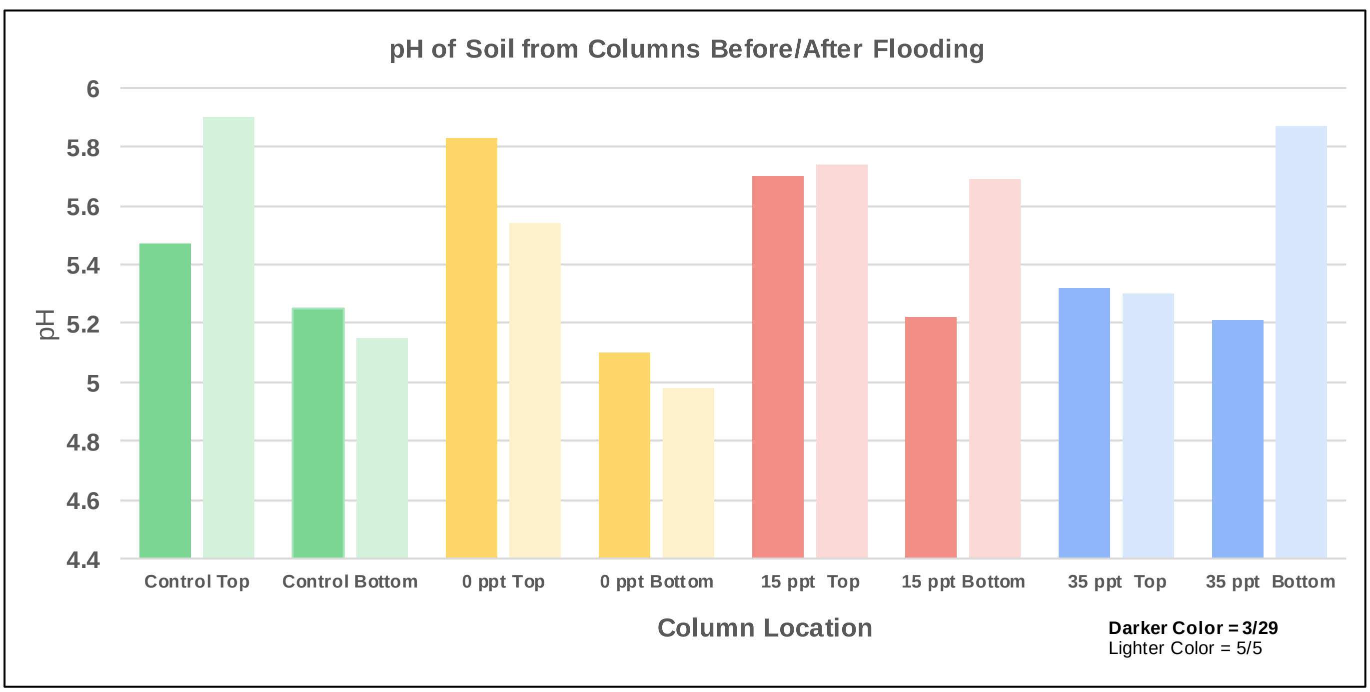


0% values are a result of more NH_4^+-N coming out then what is coming in, this was due to the synthetic wastewater ammonifying due to the organic nitrogen in the form of Urea and Peptone being broke down while the wastewater was being pumped into the columns.

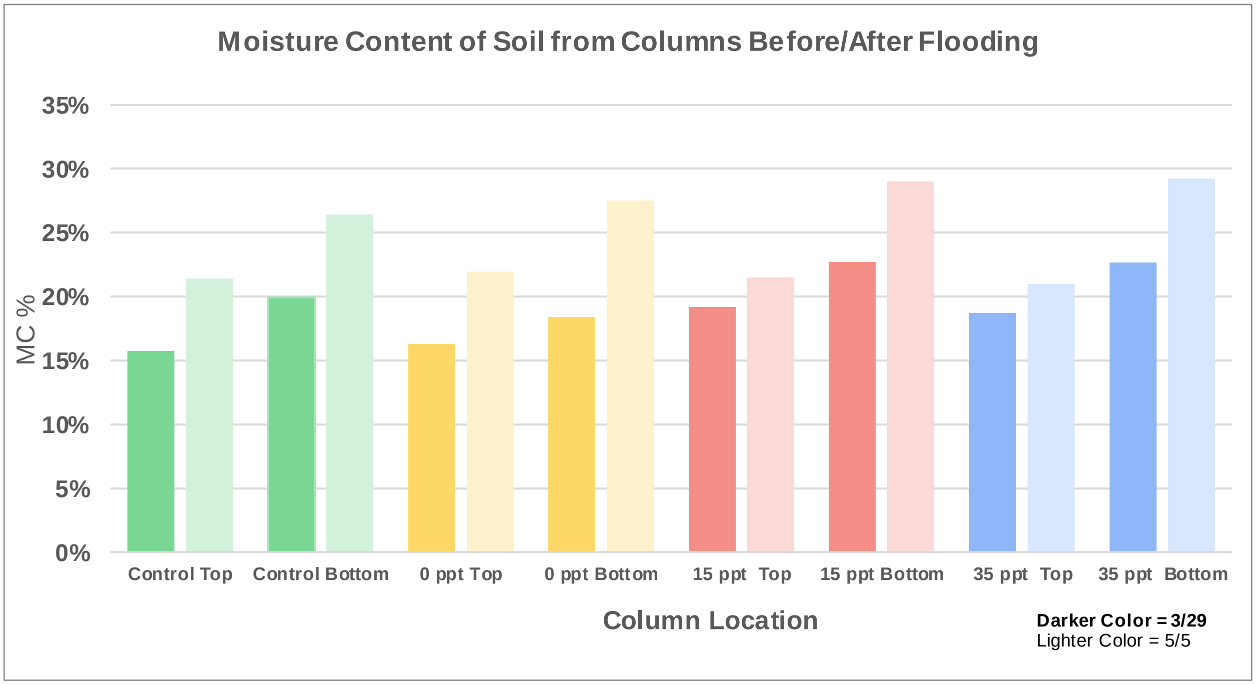
Soil Organic Matter



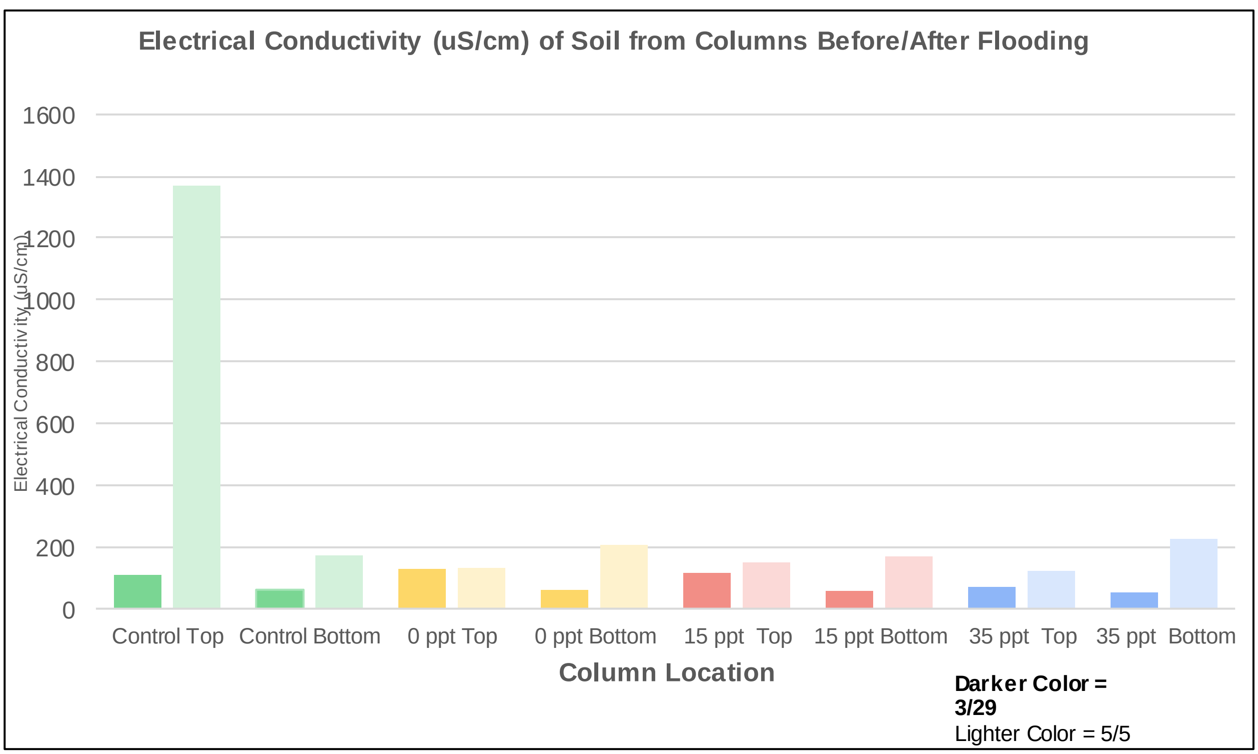
Soil pH



Soil Moisture Content



Soil Electrical Conductivity



C. Statistics Tables Output: Effluent and Soil

NH4-N Removal Efficiency

ANOVA: Single Factor

DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	14	#########	94.11%	0.006558	0.08524929	0.07608	0.788449	1.09378
Freshwate	14	#########	81.53%	0.083034	1.07944142	0.07608	0.662615	0.967945
Brackish	14	876.90%	62.64%	0.127022	1.65129005	0.07608	0.473692	0.779023
Saltwater	14	951.64%	67.97%	0.107522	1.39778197	0.07608	0.527079	0.832409

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	0.840464	3	0.280155	3.457255	0.02284678	0.166289	0.496937	0.116326
Within Grc	4.213763	52	0.081034					
Total	5.054227	55	0.091895					

Pairwise t tests

group 1	*group 2*	*p-value*	*mean*
Control	Freshwate	0.136514	0.125834
Control	Brackish	0.005982	0.314757
Control	Saltwater	0.011357	0.26137
Freshwate	Brackish	0.135601	0.188923
Freshwate	Saltwater	0.256069	0.135536
Brackish	Saltwater	0.683405	0.053387

NOx Concentration out

ANOVA: Single Factor

DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	14	413.67	29.55	125.8185	1635.64	3.807411	21.89624	37.1988
0 ppt	13	314.16	24.17	87.49866	1049.984	3.951137	16.22587	32.10608
15 ppt	13	326.15	25.09	478.3787	5740.544	3.951137	17.14817	33.02839
35 ppt	13	270.29	20.79	126.5291	1518.349	3.951137	12.85105	28.73126

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	528.9403	3	176.3134	0.868756	0.463676	0.050503	0.253274	-0.00748
Within Grc	9944.517	49	202.9493					
Total	10473.46	52	201.4126					

Pairwise t tests

group 1	*group 2*	*p-value*	*mean*
Control	0 ppt	0.186907	5.381549
Control	15 ppt	0.51841	4.459242
Control	35 ppt	0.053872	8.75637
0 ppt	15 ppt	0.890542	0.922308
0 ppt	35 ppt	0.414025	3.374821
15 ppt	35 ppt	0.536673	4.297128

pH								
ANOVA: Single Factor								
DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	13	85.84	6.60	0.853606	10.24328	0.246287	6.10761	7.098543
0 ppt	13	85.39	6.57	0.940881	11.29057	0.246287	6.072995	7.063928
15 ppt	12	66.82	5.57	0.820233	9.022567	0.256344	5.052636	6.084031
35 ppt	13	71.57	5.505385	0.54211	6.505323	0.246287	5.009918	6.000851
ANOVA								
Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	14.08852	3	4.696172	5.95547	0.001579	0.275434	0.682767	0.225705
Within Gro	37.06174	47	0.788548					
Total	51.15025	50	1.023005					
Pairwise t tests								
group 1	*group 2*	*p-value*	*mean*					
Control	0 ppt	0.926545	0.034615					
Control	15 ppt	0.00959	1.034744					
Control	35 ppt	0.00279	1.097692					
0 ppt	15 ppt	0.013802	1.000128					
0 ppt	35 ppt	0.004609	1.063077					
15 ppt	35 ppt	0.851304	0.062949					

EC

ANOVA: Single Factor

DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	12	5.97	0.50	0.005643	0.062073	1.009332	-1.5381	2.532929
0 ppt	12	5.27	0.44	0.018479	0.203274	1.009332	-1.59626	2.474762
15 ppt	11	61.68	5.61	7.958555	79.58555	1.054213	3.481432	7.733477
35 ppt	12	124.943	10.41192	40.52951	445.8246	1.009332	8.376405	12.44743

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	819.0689	3	273.023	22.33314	7.15E-09	0.609089	1.365811	0.576574
Within Grc	525.6755	43	12.22501					
Total	1344.744	46	29.23357					

Pairwise t tests

group 1	*group 2*	*p-value*	*mean*
Control	0 ppt	0.2117	0.058167
Control	15 ppt	0.00013	5.110038
Control	35 ppt	0.000218	9.9145
0 ppt	15 ppt	0.000118	5.168205
0 ppt	35 ppt	0.000208	9.972667
15 ppt	35 ppt	0.031049	4.804462

COD Removal Out

ANOVA: Single Factor

DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	12	629.67	52.47	769.3426	8462.769	38.27916	-24.7251	129.6695
0 ppt	12	489.33	40.78	631.7845	6949.63	38.27916	-36.4195	117.9751
15 ppt	11	2,758.00	250.73	32309.75	323097.5	39.98128	170.0973	331.3572
35 ppt	12	3815	317.9167	37961.98	417581.8	38.27916	240.7194	395.1139

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	698482.4	3	232827.5	13.24123	2.93E-06	0.480197	1.056012	0.43863
Within Grc	756091.7	43	17583.53					
Total	1454574	46	31621.18					

Pairwise t tests

group 1	*group 2*	*p-value*	*mean*
Control	0 ppt	0.290969	11.69444
Control	15 ppt	0.004387	198.2551
Control	35 ppt	0.000612	265.4444
0 ppt	15 ppt	0.003064	209.9495
0 ppt	35 ppt	0.000438	277.1389
15 ppt	35 ppt	0.399384	67.18939

Difference NH4 between soil ports for columns

ANOVA: Single Factor

DESCRIPTION					Alpha	0.05		
Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
Control	3	120.3	40.1	1.823212	3.646424	2.996726	33.18691	47.00784
Freshwate	3	142.7	47.6	44.96422	89.92843	2.996726	40.66769	54.48861
Brackish	3	104.1	34.7	53.9972	107.9944	2.996726	27.78047	41.6014
Seawater	3	50.9	17.0	6.979784	13.95957	2.996726	10.04102	23.86194

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between G	1529.764	3	509.9214	18.92727	0.000543	0.876509	2.51179	0.817579
Within Grc	215.5288	8	26.9411					
Total	1745.293	11	158.663					

Pairwise t tests

group 1	*group 2*	*p-value*	*mean*
Control	Freshwate	0.189081	7.480771
Control	Brackish	0.32987	5.406443
Control	Seawater	0.00091	23.1459
Freshwate	Brackish	0.088807	12.88721
Freshwate	Seawater	0.008224	30.62667
Brackish	Seawater	0.040231	17.73946

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