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Comparative Nutrient Removal with Innovative Green Sorption Media for Groundwater and Stormwater Co-treatment

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COMPARATIVE NUTRIENT REMOVAL WITH INNOVATIVE GREEN SORPTION MEDIA FOR GROUNDWATER AND STORMWATER CO- TREATMENT

by

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A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
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ABSTRACT

As indicated by the National Academy of Engineering, the understanding of nitrogen cycle has been deemed as one of 14 grand challenges in engineering of the 21st century. Due to rapid population growth and urbanization, the stormwater runoff increased in quantity as well as its nutrient concentrations, which may trigger serious environmental issues such as eutrophication in aquatic systems and ecosystem degradation. This study focuses on stormwater and groundwater quality control via Biosorption Activated Media (BAM) which can be applied to enhance the nutrient removal potential as an emerging Best Management Practices (BMPs). BAM was tested in this study with respect to two changing environmental factors including the presence of toxins such as copper and the addition of carbon sources that may affect the removal effectiveness. In addition, the impacts on microbial ecology in BAM within the nitrification and denitrification processes due to those changing environmental conditions were explored through the identification of microbial population dynamics under different environmental conditions. To further enhance the recovery and reuse of the adsorbed ammonia as possible soil amendment or even fertilizer, a new media called Iron Filing Green Environmental Media (IFGEM) was developed based on BAM, with the inclusion of iron filings as a key component for nitrate reduction. The functionality of IFGEM was analyzed through a series of column studies with respect to several key factors, including varying influent nutrient concentrations, pH values, and temperature. The results of the column studies demonstrate promising nutrient removal and recovery potential simultaneously under changing factors.

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CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Biofiltration Technologies

With population growth and migration, fast urbanization, and economic development, demand for water, transportation, waste management, and energy infrastructure in addition to regional food supply and housing need will increase rapidly and is expected to put more pressure on the environment.¹ One of the major consequences is the non-point source (NPS) pollution in the urbanized area, due to the change of landscape,² industrial emission³, and agricultural discharge.⁴ Biofiltration is one of the most cost-effective approaches to deal with such large-scale pollution caused by NPS in overland flows. Biofiltration mainly utilizes sand media bed with large surface area to support the microorganism growth in the form of biofilm. The contaminants in the fluid will be used as the food source by the microorganism in the biofilm, thus achieving a self-sustained system leading for contaminants removal. Biofiltration can be applied to remove multiple pollutants, including heavy metal⁵, volatile organic compounds (VOCs)⁶, nutrients in runoffs and discharges^{7, 8}, pesticides⁹, and pathogens¹⁰. Of course, for the different targeted contaminant, the biofilm in a biofiltration process appears in different forms and scales. This study mainly focuses on the advancements of traditional biofiltration technology with innovative sorption media for nitrogen and phosphorus removal from stormwater runoff and agricultural discharge toward the protection of groundwater and surface water quality and the promotion of sustainability of the aquatic ecosystem services.

1.1.2 Nutrient Removal in Stormwater Treatment

Many studies have been conducted since four decades' ago via the use of sorption media to remove nutrients in stormwater runoff and agricultural discharge with different media mixes leading to mitigate eutrophication issues in the receiving water bodies^{11, 12}. Back to the 1960s, people started to realize that urban stormwater runoff is an important source of contamination, such as organic compounds, nutrients, and pathogens, in the receiving water bodies, and the most commonly used method to reduce such impacts is sedimentation and disinfection through dosing disinfectants such as chlorine¹³. The removal effects of chemical oxygen demand (COD), nitrogen, and phosphorus ranged from 40% to 70%, depending on the settling/treatment time^{13,14, 15}. In the 1970s, more stormwater treatment technologies were developed to mitigate nutrient impact from stormwater runoff. With the aid of biomimetic philosophy, one of them is the use of wetland technology which achieved 77% total phosphorus (TP) removal and 94% total suspended solids removal¹⁶, although it requires using relative larger land area for implementation. In parallel with the development of natural and constructed wetland technologies, advancements in biofiltration technologies also came to help evaluate the performance of soil infiltration for possible pollutant removal, taking soil properties, infiltration rate, while taking storm characteristics into account for some of the stormwater detention basins in Central Florida¹⁷. However, it was soon discovered that the nutrient removal capacity of natural soil is relatively low and soil adsorptive capacity can be quickly saturated by those nutrients ending up leakage of the adsorbed nutrients ultimately^{18, 19}.

1.1.3 Development of Special Sorption Media

As mentioned in above, the most commonly used material is sand, and sand filters showed the removal of 61% of TN, 70% of TKN, and 53% of nitrate²⁷⁻²⁹. Varying sand characteristics, however, many results vary in response to different hydraulic conditions that in turn make the performance unstable³⁰. Encouraged by a variety of recycling effort for better waste management in the past, many filter media that mixed both recycled and natural materials were developed to remove excess nutrients and heavy metals from stormwater runoff in urban watersheds as an integral part of the low impact development (LID) strategies, but few of them became best management practices (BMPs)²⁰. One of these recycled materials is sawdust that can be effective only in the case when the influent concentration of nutrient is low.^{21, 22} However, sawdust may decay over time reducing the treatment capacity and increasing the concentration of COD, phenols, and color in the effluent which could introduce secondary contamination²³. Tire crumbs/chips were tested for its capability of nutrient removal from stormwater runoff due to its promising absorption potential²⁴, providing additional 23-58% nitrate removal when compared to green plots^{25,26}. Clays were tested as one of the key components for ammonia absorption³¹, and 61% removal was confirmed when the initial concentration of ammonia is low³². Zeolites were studied for its performance on phosphorus and ammonia removal, and 93% removal of phosphorus was achieved with a contact time of 120 minutes³³. Biochars were also tested with an average removal efficiency of 86% and 47% for nitrate and phosphorus, respectively.⁴⁰ Some commercially available mineral material such as limestone can also be applied in this regard, and limestones achieved 62% phosphorus removal with a contact time of 10 minutes³⁴. Other sorption media may include but are limited to activated carbon media^{35, 36}, crushed shells³⁴, and nanosized materials^{37,38}. In recent 10 years, more advanced media mixes including multiple components were developed to integrate

different functionalities with synergistic effect, and some of them even require pretreatment before applications. A salient example is the invention of bio-retention soil media amended with aluminum-based water treatment residuals and montmorillonites, which contain 85% sand, 10% silt, and 5% clay. The best removal capacity of these media is 313 mg/Kg for dissolved organic phosphorus³⁹.

The degradation of surface water and groundwater quality due to nutrient impact has resulted in big concern in public health and urban sustainability^{54, 55}. Particularly, excess nitrogen in stormwater runoff is one of the major issues in the nitrogen and phosphorus cycle that may triggers eutrophication in aquatic ecosystems, increased incidences and duration of harmful algal blooms in the receiving water bodies, and finally affect ecological structure and function⁵⁶. Particularly when considering areas with frequent storm events such as Florida or Washington states, nitrogen removal from stormwater runoff is critical to ensure urban sustainability. Stormwater retention basins and detention ponds are oftentimes used as traditional BMPs for nutrient removal as well as flood control⁵⁷. However, as the increased urban population converts more rural or suburban land into urbanized areas, nutrient concentrations in stormwater runoff also increase significantly; as a result, the traditional stormwater detention ponds or retention basins constructed with natural soil can no longer guarantee the essential level of treatment effectiveness.^{58, 59} For this reason, biosorption activated media (BAM) were developed and applied to stormwater detention ponds or retention basins as amendment of natural soil^{44, 45, 60-62} which provides a promising solution as best management practices (BMPs) to mitigate nutrient impact^{63, 64}. BAM contains clay, tire crumb, and sand, which are all environmentally friendly materials with no availability issues in the market. If necessary, BAM may include sawdust or other recycled

materials. Previous studies about BAM have revealed its potential in removal of inorganic nitrogen species, phosphorus, heavy metals, and pathogen from the stormwater runoff and wastewater effluent^{7, 41-47}. BAM were tested for phosphate removal^{45, 65}, copper removal⁶⁶, and nitrogen removal^{7, 41, 43, 60, 61, 67-69} under various conditions. Moreover, BAM has been applied to multiple LID sites in stormwater management, which have been proven effective as one of the most effective sorption media for pollutant removal.

1.2 Study Goals

However, previous studies only touch the base of microbial activities in BAM for nitrogen removal via nitrification and denitrification,⁴² which left some questions over in regard to how the microbial ecology varies under various influent conditions. In this study, BAM was developed and tested for advanced evaluation in a co-treatment endeavor of stormwater and groundwater along a section of road side. A direct comparison of results between the laboratory column study and the field pilot study was made possible. The pilot study site is close to the Fanning Spring, Florida as part of the state-wide initiative for spring protection. Two aspects have to be evaluated in order to fully understand the impacts on the structure and function of microbial ecosystem as its corresponding nitrogen removal can be achieved. One is related to the impact of carbon addition, as carbon can be used as electron donors in the denitrification process⁴⁸⁻⁵⁰. The other aspect is related to toxicity impact driven by the presence of heavy metal, such as copper that has been used as algicide for quite a long time because copper ion is toxic to bacteria and fungi⁵¹. As a consequence, copper concentration in stormwater runoff ranges from 20-30 µg/L normally, which may trigger unknown impact on nitrification and denitrification. Most importantly, all bacteria living in biofilm that attaches to the sorption media surface^{52, 53} may be collectively influenced by

the addition of carbon source and copper that can be nutritional or detrimental and that remains unclear in BAM till the present. These unknown impacts make engineers hard to fully realize the nutrient removal effectiveness when using BAM in different field environments.

In this study, emphasis was placed on microbiological reactions and the dominant nitrogen removal pathway, i.e., nitrification and denitrification associated with changing species of dissolved organic nitrogen (DON). DON species can potentially affect the microorganism as they can be used as carbon source to some extent^{70, 71}, although DON species are sometimes regarded as the inert components of the total nitrogen that can hardly be removed through microbiological reactions^{72, 73}. To gain more insight in this regard, the Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) was used in this study to investigate the DON composition at the molecular level, and help understand how BAM can impact DON removal via microbial activities. With the aid of real-time Polymerase Chain Reaction (PCR) technology, the microbial ecology can be further explored for evaluating the impacts of the presence of carbon sources (in chapter 2) and copper (in chapter 3).

Chapter 2 deepens the understanding of the interactions between carbon addition and DON decomposition through a linear-ditch best management practices for stormwater and groundwater co-treatment. By conducting a laboratory-scale column study for nitrogen removal using BAM as green sorption media, the variation in composition of DON and the population dynamics of microbial species that dominate the nitrification and denitrification processes can be linked together. With the different levels of influent concentration of total nitrogen, the efficacy of nitrogen removal via BAM may be fully realized at the molecular level with ultrahigh resolution FT-ICR-MS.

Chapter 3 focuses on investigating how the presence of copper impact the microbial structure and function, thus affecting the nutrient removal performance in the nitrification and denitrification processes through population dynamics and changing bioactivity⁷⁴⁻⁷⁶. In this chapter, effort was further directed to explore whether copper can stimulate denitrifiers' growth because copper could be a cofactor of the key enzyme in the last denitrification step (convert nitrous oxide to nitrogen gas) incase multi-enzymatic cascade reactions appear from the microbial ecology point of view. Additional media (woodchip) was included in the laboratory study as a baseline for comparison.

The main focus of chapter 4 is the comparison of results between the laboratory study and the field application of BAM and woodchip in a comparative approach. Bold & Gold[®] media (also known as BAM) and woodchip media were tested under various influent concentrations and flow conditions in a laboratory column study and a full-scale field application in terms of physical, chemical, and microbiological properties. BAM and woodchip were applied to construct a linear ditch in parallel to the state road 426 located close to the Fanning Springs, Florida as a pioneering examination to co-treating stormwater runoff and pumped groundwater for nutrient removal. The stormwater is primarily from both agricultural discharge and stormwater runoff given that there is an agricultural crop land nearby.

To explore possibilities for improvement of BAM, chapter 5 highlights the invention of Iron Filings-based Green Environmental Media (IFGEM) with the emphasis on the addition of iron filings into BAM. Iron was found to be active in nitrate reduction to ammonia or even nitrogen gas (for nanosized iron particles)⁸¹⁻⁸³. IFGEM was proven effective in chapter 5 through a thorough of study of isotherm, reaction kinetics, and microstructure examination under various inlet nutrient

concentrations. Ammonia generation and possible recovery in IFGEM were confirmed. The holistic observation supports the idea of using IFGEM as a promising nutrient removal media to treat stormwater runoff, wastewater effluent, and agricultural discharge. More importantly, the saturated IFGEM may become the source materials for soil amendment in cropland, gardens and yards, and green roofs. The reliability test results of IFGEM can be found in chapter 6 in regard to multiple changing environmental conditions driven by different pH values, influent concentrations, and temperatures. Two IFGEM recipes were selected to compare against one control (natural soil). Whereas IFGEM-1 is made of 96.2% fine sand and 3.8% iron filings (by volume), IFGEM-2 contains 80% sand, 10% tire crumb, 5% pure clay, and 5% iron filings (by volume).

CHAPTER 2: THE IMPACT OF CARBON SOURCE AS ELECTRON DONOR ON COMPOSITION AND CONCENTRATION OF DISSOLVED ORGANIC NITROGEN IN BIOSORPTION-ACTIVATED MEDIA FOR STORMWATER AND GROUNDWATER CO-TREATMENT¹

2.1 Introduction

The National Academy of Engineering has indicated that understanding and managing the nitrogen cycle is one of the 14 grand challenges for engineering in the 21st century.⁸⁴ Human activities have largely increased nitrogen consumption and distribution.⁸⁵⁻⁸⁷ Much of the residual nitrogen is normally carried out by stormwater runoff, wastewater effluent, or agricultural discharge.^{54, 55, 88} Within such an urban nitrogen cycle, dissolved organic nitrogen (DON) is a nonnegligible part since it occupies a large proportion, up to as much as 80%, of total nitrogen (TN) and is an important N source supporting many microbial processes.⁸⁹⁻⁹¹ Particularly in stormwater runoff and agricultural discharge, sediment release is the major source of DON, and the uptake of DON through bacterial degradation is the major sink of DON.^{73, 92} Understanding the processes responsible for DON production, behavior, and characteristics is thus critical for managing nutrient cycling with adequate nutrient control strategies. Previous studies tried to address the ecological significance of DON in various environments such as marine^{73, 89, 93} and freshwater systems.^{72, 91, 94} These studies revealed that DON is a structurally complex mixture of different kinds of organic molecules that are highly variable in chemical structure and composition and thus in bioavailability and ecological functioning. The inherent complexity of DON is a major barrier for understanding how different best management practices (BMPs) can change, modify, and remove DON through innovative stormwater treatment processes.^{8, 45, 46, 68, 95-101} One of the

¹ Authors: Ni-Bin Chang, Dan Wen, Amy M. McKenna, and Martin P. Wanielista; Publication: *Environmental Science & Technology* **2018** 52 (16), 9380-9390

promising media types, biosorption activated media (BAM), has been used in various BMPs in varying landscapes for effective nitrogen removal through biological nitrification and denitrification.^{24, 43-46, 60-62, 65, 102} However, the interaction between BAM and DON remains unclear, especially for the co-treatment of stormwater and groundwater in linear ditch (bioswale) facilities.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) has been applied to analyze complex dissolved organic matter (DOM) for marine organics,⁹³ surface water¹⁰³ and stormwater.¹⁰³⁻¹⁰⁵ The ultrahigh resolving power ($m/\Delta m_{50\%} > 2,700,00$ at m/z 400)¹⁰⁶ and mass accuracy (80-200 ppb) of FT-ICR-MS enables the resolution and confident identification of tens of thousands of unique elemental compositions in dissolved organic matter (DOM). This technique is promising for understanding the qualitative molecular interactions between DOM composition, nitrogen-containing compounds, and concentration of nitrogen species in the BAM-based stormwater treatment process. Previous studies have applied FT-ICR-MS to assess the biodegradability of DON from stormwater at the molecular level,¹⁰⁵ but they only evaluated the relative abundance of DON based on the DOM analysis. Moreover, the highly variable natural environment has many influential factors related to bacterial activities for nitrogen removal. One of these factors is a carbon source as part of the total organic carbon in a natural environment, which is a critical element for biomass formation and the electron donor for denitrification processes.⁵⁰ This impact on denitrification processes can be further realized by using real-time polymerase chain reaction (Real-Time PCR) to quantify the population dynamics of nitrifiers and denitrifiers.

Our objectives in this study were to evaluate the carbon source impact on nitrogen removal for co-treatment of groundwater and stormwater in a column study. By linking the results between

real-time PCR and FT-ICR-MS, the novelty of this study was the relationship between the change of DON composition and the response of the microbial community under scenarios with or without carbon addition in a low impact development engineering practice. The co-treatment process is an innovative strategy for treating the groundwater that was extracted and distributed by a solar powered pump during sunny days and the stormwater runoff during storm events. This co-treatment process helps maintain activity of the key bacteria for biological removal of nitrogen species before the water recharges to groundwater. Some scientific questions to be answered may include: 1) what is the effect of a carbon source on the nitrogen removal under various influent conditions? 2) How would the carbon addition affect the microbial species development in terms of population dynamics, metabolic rate, and cell conditions with respect to the changing inlet nutrient concentration? And 3) can the corresponding microbial community digest the DON concentration and reshape the DON composition efficiently given the available carbon source? We hypothesize that 1) carbon addition would enhance the nitrogen removal and make a difference in DON concentration and composition, 2) there is a cascade effect of the microbial species development in terms of population dynamics when digesting the DON concentration, and 3) there are different trends (changing directions) of DON concentration and composition when comparing the treated water under different influent conditions with untreated counterparts.

2.2 Material and Method

2.2.1 Experiment Setup

The groundwater used in this study was collected from Fanning Spring, Florida (linear ditch site), and the stormwater was collected from a stormwater retention pond on the University

of Central Florida (UCF) campus. The linear ditch on a road side in the watershed of Fanning Spring is the locale where the co-treatment of stormwater and groundwater with BAM was examined (Figure 1-c and d). To explore the feasibility of nitrogen removal performance as well as the impact on DON concentration and composition, two columns named column 1 and 2 were set up in a laboratory at the UCF for the study of nutrient concentration impacts in the influent; both columns are 15 cm in diameter and 1.2 m in height with 3 water sample ports at 30 cm intervals on the side (Figure 1-a and b). The BAM mixture used in this study contains sand (85%), tire crumb (10%) and clay (5%) by volume. There are two cycles for running the columns; in cycle 1 groundwater was pumped at 10 mL/min for 3 days followed by stormwater at 15 mL/min for one day and in cycle 2 the running method remained the same as cycle 1 except additional carbon source (40 mg/L COD of glucose) was added to both the groundwater and stormwater reservoir. For both cycles, the inlet was spiked with nitrate to the theoretical concentration of 1.5 mg/L for column 1 and 5 mg/L for column 2 for the study of nutrient concentration impacts due to the highly variable nutrient concentrations in stormwater runoffs (Table 1). Such operational strategies were thus set up to mimic the field conditions for dealing with the stormwater runoff in storm events, as well as for treating pumped groundwater in between two adjacent storm events for nitrogen removal since this area has been heavily polluted by excess nitrogen sources from stormwater runoff and agricultural discharge collectively. Triplicate water samples were collected from inlet, outlet and each sample port of the column at the end of the running section of stormwater and groundwater.

Water quality parameters of dissolved oxygen (DO) and pH were measured right after the water collection at UCF. Total nitrogen (TN), ammonia, nitrate and nitrite (NO_x), and alkalinity

were determined by an external certificated laboratory, the Environmental Research & Design (ERD), and all methods and instruments are listed in Table 2. The corresponding measurement unit is $\mu\text{g/L}$ for TN-N, $\text{NO}_x\text{-N}$, and $\text{NH}_3\text{-N}$, and the organic nitrogen concentration can be calculated by subtracting NO_x and NH_3 from TN. Water samples were collected only for the inlet and outlet of stormwater sections in each cycle to analyze the DON because stormwater contains the newly washed out DOM. The media samples were collected at the top, port 1, and port 2 from column 1 and 2 after running the stormwater section in each cycle.

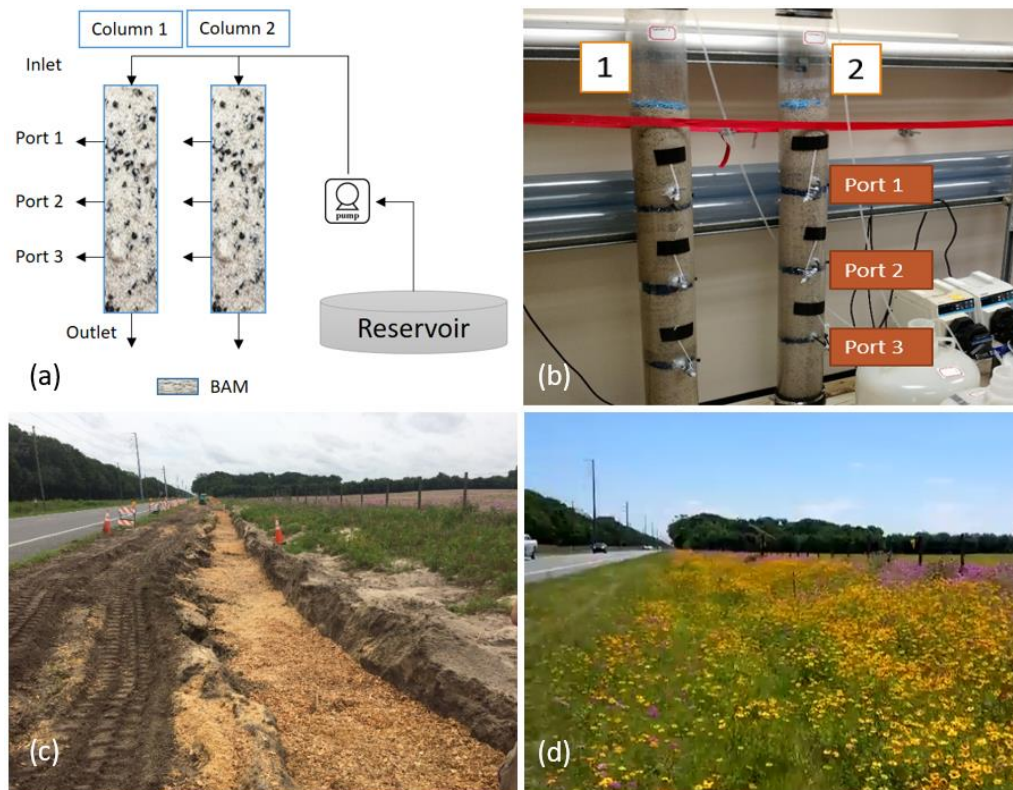


Figure 1. (a) Schematic diagram for column setup; (b) column study - laboratory view; (c) field construction with BAM deployment; and (d) after construction for co-treatment of groundwater and stormwater

Table 1. Inlet conditions for the column study

	Cycle 1 no carbon source				Cycle 2 with carbon source			
Water source	Groundwater (3 days)		Stormwater (1 day)		Groundwater (3 days)		Stormwater (1 day)	
Pumping rate	10 mL/min		15 mL/min		10 mL/min		15 mL/min	
Spiked Nitrate Concentration	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L
Scenario	LGN	HGN	LSN	HSN	LGC	HGC	LSC	HSC
<p>LGN = Low TN Groundwater inlet with no carbon addition; HSC = High TN Stormwater inlet with Carbon addition, etc.</p>								

Table 2. Methods and instruments for water sample analysis

Parameter	Analysis Method/Instrument
Total Nitrogen (TN)	SM-21, Sec. 4500 N C
Nitrate + Nitrite (NO _x)	SM-21, Sec. 4500-NO3 F
Ammonia	SM-21, Sec. 4500-NH3 G
Alkalinity	SM-21, Sec. 2320 B
Dissolved Oxygen (DO)	HACH HQ40D - IntelliCAL LDO101 LDO
pH	Waterproof Double Junction pHTestr® 30
SM = Standard Methods for the Examination of Water and Wastewater, 21 st Edition, 2005	

2.2.2 DON and DOM Analysis

The DON of interest is a part of DOM and we only focus on the N-bearing organic component as a subset of the DOM analysis. The water sample was first preserved with solid phase extraction (SPE) in the manner described by Dittmar, Koch et al.¹⁰⁷ After SPE, all final samples were kept under -20 °C until analysis. Sample analysis for DON was performed at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL. DOM extracts were analyzed with a custom-built FT-ICR-MS¹⁰⁸ equipped with a 9.4 T horizontal 220 mm bore diameter superconducting solenoid magnet operated at room temperature, and a modular ICR data station (Predator)¹⁰⁹ facilitated instrument control, data acquisition, and data analysis. Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry

(IUPAC) mass scale to the Kendrick mass scale¹¹⁰ to identify homologous series for each heteroatom class (i.e., species with the same $C_cH_hN_nO_oS_s$ content, differing only by their degree of alkylation). For each elemental composition, $C_cH_hN_nO_oS_s$, the heteroatom class, type (double bond equivalents, $DBE = \text{number of rings plus double bonds involving carbon}$) and carbon number, c , were tabulated for subsequent generation of heteroatom class relative abundance distributions and graphical abundance-weighted DBE vs. carbon number or $H:C$ ratio vs. carbon number images or van Krevelen diagrams. The full operation details of FT-ICR-MS can be viewed in an external link.¹¹¹

Due to the immense compositional polydispersity and polyfunctionality, ionization of DOM yields a range of ionization potentials and challenges all mass spectral techniques. Therefore, comparison of nitrogen-containing DOM compounds can be conducted between samples based on relative abundance differences between heteroatom classes.¹⁰⁵ However, it is possible to retrieve the absolute DON concentration of each heteroatom class with the help of the measurement of total DON from the water quality analysis (Equation 1). It is also necessary to understand that not all DON components are ionized equally in the ionization process, as oxygen-rich molecules are more efficiently ionized than DON. The absolute concentration of each heteroatom DON class is then calculated based on their relative abundance in DOM. Based on the sum of the relative abundance of DON, the relative abundance of each DON species becomes absolute when the whole DON can be divided only among DON species accounting for the total DON (Equation 2),

$$C_T^{DON} = C_{TN} - C_{NO_x} - C_{NH_3} \quad (1)$$

$$C_i^{DON} = C_T^{DON} \left[\frac{^{14}N_i}{AMW_i} RA_i^{DON} \right] \left[\sum_m^n \frac{^{14}N_i \cdot RA_i^{DON}}{AMW_i} \right]^{-1} \quad (2)$$

where C_i^{DON} is the absolute DON concentration of species i ; C_T^{DON} is the total DON concentration; RA_i^{DON} is the relative abundance of N-organic species i based on all DOM in the sample; AMW_i is the average molecular weight of N-organic species; N_i is the nitrogen atom number in each N-organic species i ; and C_{TN} , C_{NO_x} , and C_{NH_3} are the concentrations of total nitrogen, nitrate and nitrite, and ammonia.

2.2.3 Real-Time PCR Analysis

Identifying the gene copy number of corresponding microbial species in relation to nitrogen removal would be helpful in providing one more dimension for understanding the microbial community development in the media samples between ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and denitrifiers. Real-Time PCR, also known as quantitative polymerase chain reaction (qPCR), is a laboratory technique used in molecular biology for quantifying microbial species. The gene copy number of nitrifiers and denitrifiers was tested with real-time PCR in the Bioenvironmental Research Laboratory at UCF. The collected samples of BAM were stored at -80 °C until gene extraction by using the Mobio PowerMax Soil Kit; the extraction process followed the kit protocol provided by the vendor. All extracted DNA elutes were stored in Tris-EDTA buffer under -20 °C. The real-time PCR was performed with StepOne from Applied Biosystems, and PowerUp™ SYBR® Green Master Mix. The primer sets and running methods utilized are shown in Table 3.

Table 3. Primer sets and real-time PCR running conditions

Target bacteria	Primer name	Primer sequence	Running method	reference
AOB (Annealing at 60 °C)	amoA-1F	GGGGTTTCTACTGGTGGT	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	Rotthauwe, et al. ¹¹²
	amoA-2R	CCCCTKGSAAAGCCTTCTTC		
NOB (Annealing at 63.8 °C)	NSR1113f	CCTGCTTTCAGTTGCTACCG	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 63.8 °C for 45 cycles	Dionisi, et al. ¹¹³
	NSR1264r	GTTTGCAGCGCTTTGTACCG		
Denitrifier (Annealing at 60 °C)	1960m2f	TAYGTSGGGCAGGARAAAC	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	López-Gutiérrez, et al. ¹¹⁴
	2050m2	CGTAGAAGAAGCTGGTGCT		

2.3 Results

2.3.1 Carbon Impact on Inorganic Nitrogen Removal

The influent and effluent concentrations of TN, NO_x, and ammonia with the comparison of carbon influences is shown in Figure 2-a as well as the N-balance that evaluates the

transformation of different N species, which is shown in Figure 2-b. Note that in Figure 2-b, gray columns with positive values indicate the removed ammonia (with better nitrification), but those with negative values indicate the increased ammonia (with better ammonification). The inlet TN concentrations are about 7-8 mg/L and 5-6 mg/L for groundwater and stormwater in high TN cases, and 4.5-6 mg/L and 1.5-2 mg/L for groundwater and stormwater in low TN cases. Before carbon addition, the TN removals of groundwater and stormwater are 51% and 78%, respectively, for low TN case, while they were 42% and 70%, respectively, under high TN case. NO_x removal through denitrification seems to be the dominant removal mechanism, which is also evidenced by the dominant denitrifiers in the next section. After carbon addition, the impact on stormwater TN removal is negligible, however, there is a significant improvement for groundwater as it increased to 88% and 52% for the low and high TN inlet conditions, respectively. Denitrification is still the dominant removal mechanism for all columns, but it was significantly enhanced for treating groundwater. The enhancement of TN removal in groundwater at low TN is largely due to the removal of NO_x through denitrification, in which added carbon was used as electron donor, but the groundwater with high TN has less improvement of TN removal; this might be a sign of reaching the treatment capability. However, significant ammonia was generated in both stormwater and groundwater after carbon was added due to more complex decomposition of DON, which can be removed by including more clay contents in the green sorption media.

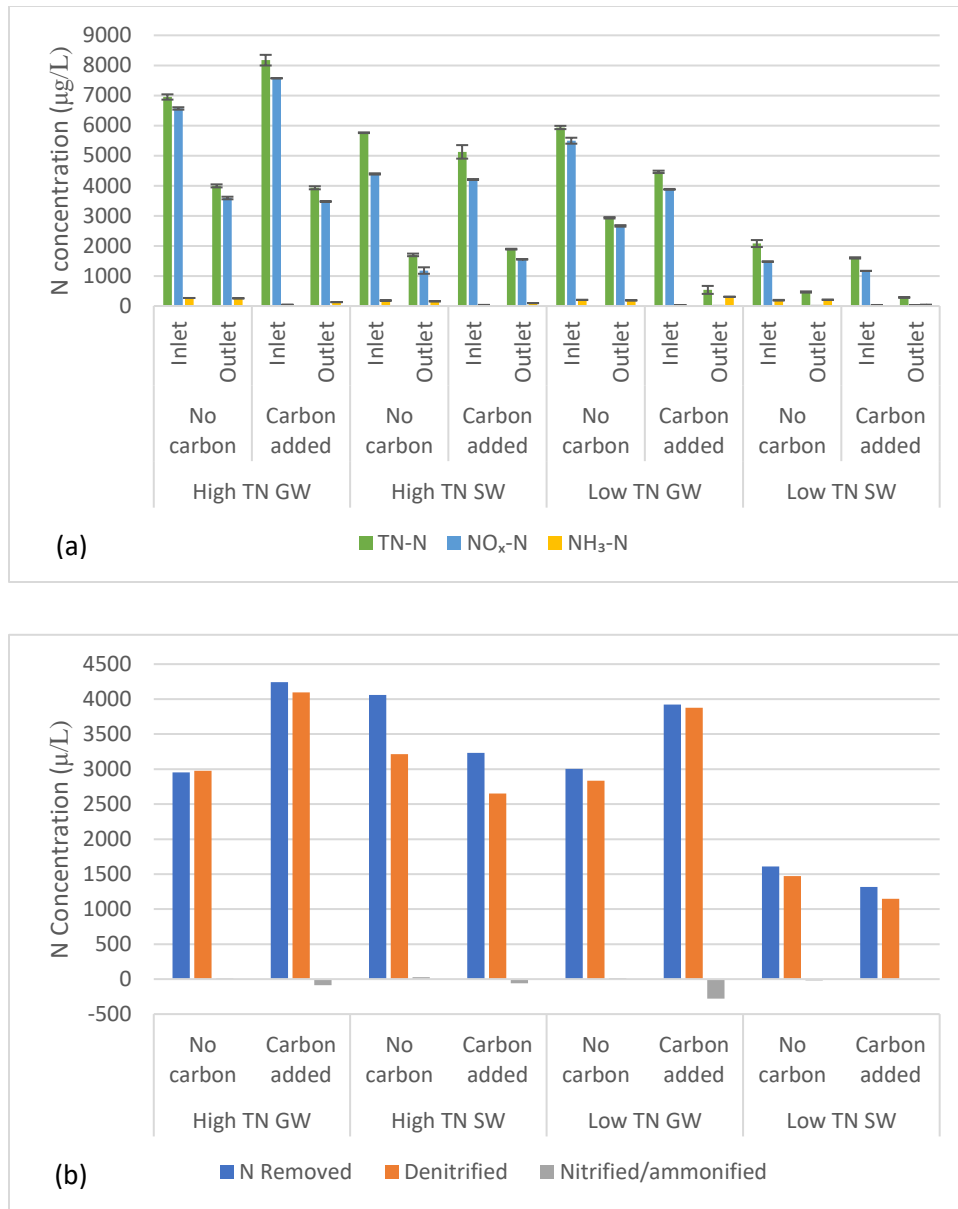


Figure 2. (a) Influent and effluent concentrations of TN, NO_x, and ammonia of BAM before and after the carbon source addition under low TN inlet conditions (column 1) and high inlet conditions (column 2) with groundwater (GW) and stormwater (SW) scenarios; and (b) Transformed N species for N-balance calculation based on the average concentration of nutrients from inlet and outlet under each scenario

2.3.2 Real-time PCR

The gene copy density of AOB, NOB, and denitrifiers for both columns with depths of 0, 30, and 60 cm and the relative abundance of all three species in each layer is shown in Figure 3. Despite carbon addition, the majority of the bacterial population stayed at the top layer for both columns, and denitrifiers were the dominant species in both columns (approximately 88% at the top and 99% at 60 cm depth). Before the carbon addition, column 2 had higher bacterial population density than column 1 due to its more nutrient-rich inlet condition; however, the population changes of the two columns are of different magnitudes after the carbon addition. The bacterial population density of the top layer in column 1 increased by 40 times for AOB, 12 times for NOB, and 4.8 times for denitrifiers, while it only increased by 2.5, 1.7, and 1.6 times for the top layer in column 2. However, the bacterial population densities of AOB, NOB, and denitrifiers in the column 1 top layer are 20, 5.2, and 2.4 times larger than those in column 2, respectively, after the carbon addition. Furthermore, the carbon addition had almost no impact on the relative abundance of each type of bacteria in the column 2 top layer, but it enhanced the AOB and NOB percentages in the column 1 top layer.

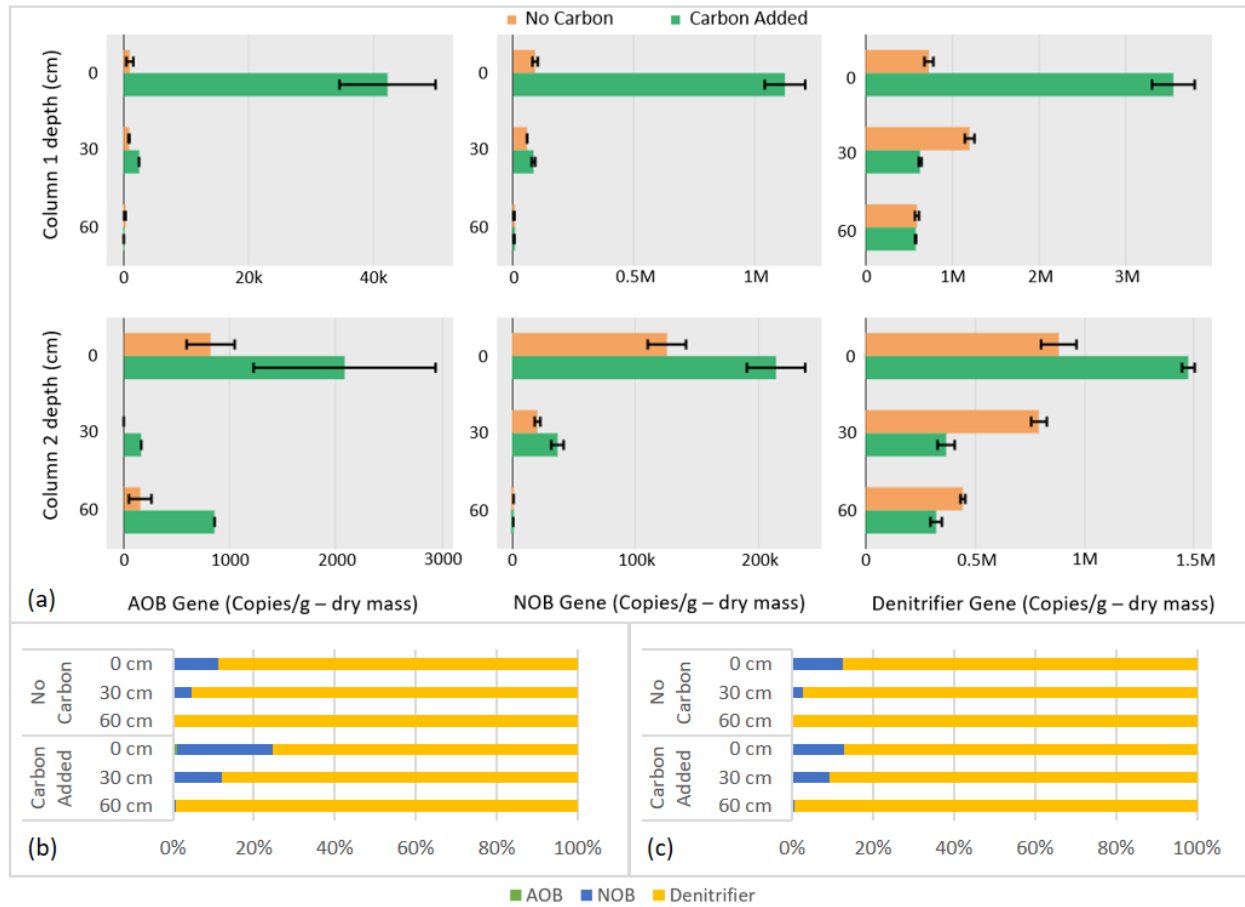


Figure 3. (a) Gene copy number of AOB, NOB, and denitrifiers from different depth in column 1 and 2; and population percentage of AOB, NOB and denitrifiers in different depths of (b) column 1 and (c) column 2

2.3.3 DON Compositional Changes

The total DON concentrations from the inlet and outlet are calculated through Equation 1 for both low and high TN scenarios before and after the carbon addition, as summarized in Table 4. Some acronyms are used in this paper to represent different scenarios. For instance, LSC represents low TN concentration (spiked 1.5 mg/L nitrate) stormwater with carbon addition while LSN represents low TN concentration (spiked 1.5 mg/L nitrate) stormwater with no carbon addition. Likewise, HSC represents high TN concentration (spiked 5.0 mg/L nitrate) stormwater

with carbon addition while HSN represents high TN concentration (spiked 5.0 mg/L nitrate) stormwater with no carbon addition. With the help of additional carbon, the total DON removal increased from 27% to 42% for stormwater treatment with low inlet TN, and from 52% to 73% for stormwater treatment with high inlet TN. This outcome is part of the reason why the ammonia concentration went up quickly as a result of carbon addition in the case with high inlet TN (Figure 2), because carbon addition may increase the ammonia concentration through the enhancement of ammonification for the treatment of both stormwater and groundwater.^{115, 116} At that moment, AOB was not abundant enough to decompose those ammonia (Figure 3), although more complex reactions may co-exist with ammonification toward ammonia generation.

The corresponding DON classes being removed can be seen through focusing on N-bearing formulas (CHON) in the analysis, and each of the heteroatom classes (e.g., N1O10 indicates the class of molecules containing 1 nitrogen atom and 10 oxygen atoms) can be quantified based on Equation 2 (Figure 4). High inlet TN showed generally better total DON removal than the counterpart with low inlet TN. This is indicative that carbon addition has limited impact on DON removal, which is not as significant as the change of initial TN concentrations at least; it did slightly enhance DON removal when compared to the overlapped DON portion, however, about 25% and 33% of new DON species were found after carbon addition in low and high TN influent scenarios, respectively.

Figure 4 shows the comparative inlet and outlet conditions of all N-bearing formulas found for stormwater treatment associated with either low or high inlet TN before and after carbon addition. In this figure, we further overlaid diagrams with rectangles to note where important classes of biomolecules are known to fall on a van Krevelen diagram^{37,51}. These biomolecules

include lipid-, protein-, tannin-, amino sugar-, and lignin- formulas as well as a group of uncharacterized hydrocarbons falling within O/C ratio < 0.4 and H/C ratios between 0.5 and 1.75. The scenario of stormwater treatment with low inlet TN shows a similar outlet DON composition pattern before and after carbon addition, which is also consistent with the observations from Figure 4a and Figure 4b. For the counterpart with high inlet TN, the outlet DON composition contains fewer and less dense molecular species than the low TN case, and the carbon addition seems to help remove more DON classes.

Table 4. Total DON concentration ($\mu\text{g/L}$) changes for stormwater treatment scenarios associated with low or high inlet total nitrogen (TN) with or without carbon addition

Carbon Dosage	Sample location	Low Inlet TN	High Inlet TN
No Carbon	Inlet	442	877
	Outlet	322	418
Carbon Added	Inlet	398	883
	Outlet	226	240

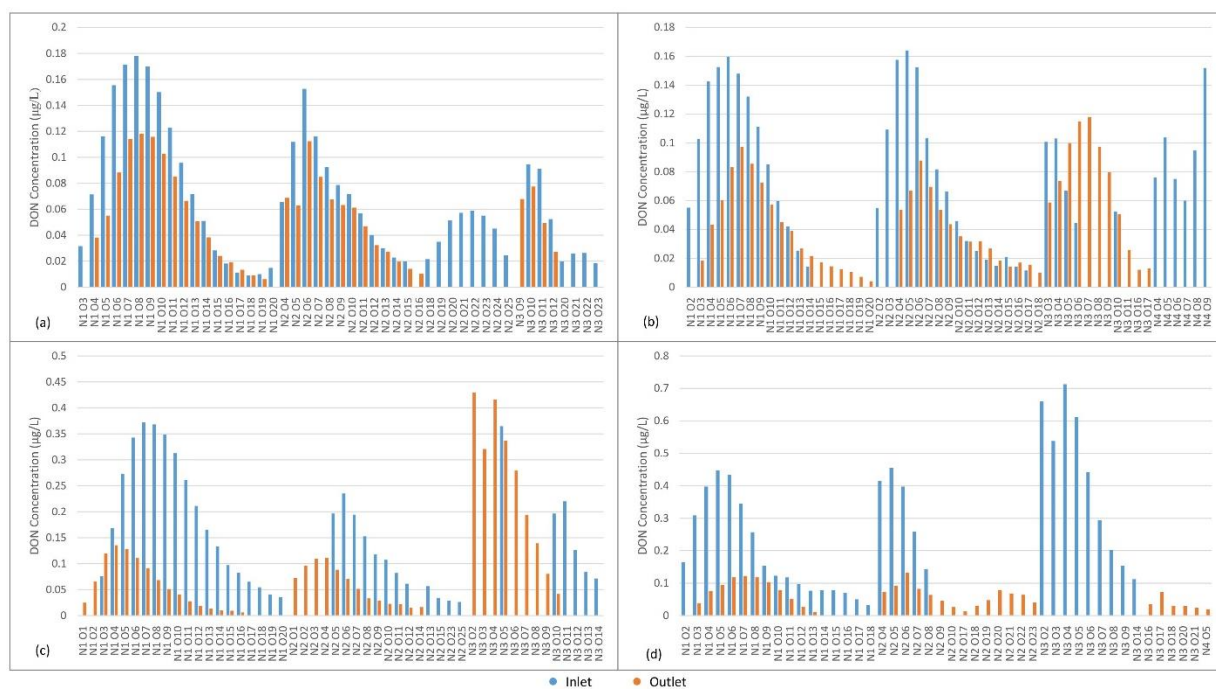


Figure 4. Calculated concentration of CHON classes in the low TN inlet for BAM with (a) no carbon addition (LSN), (b) carbon added (LSC), and in the high TN inlet with (c) no carbon addition (HSN), (d) carbon added (HSC)

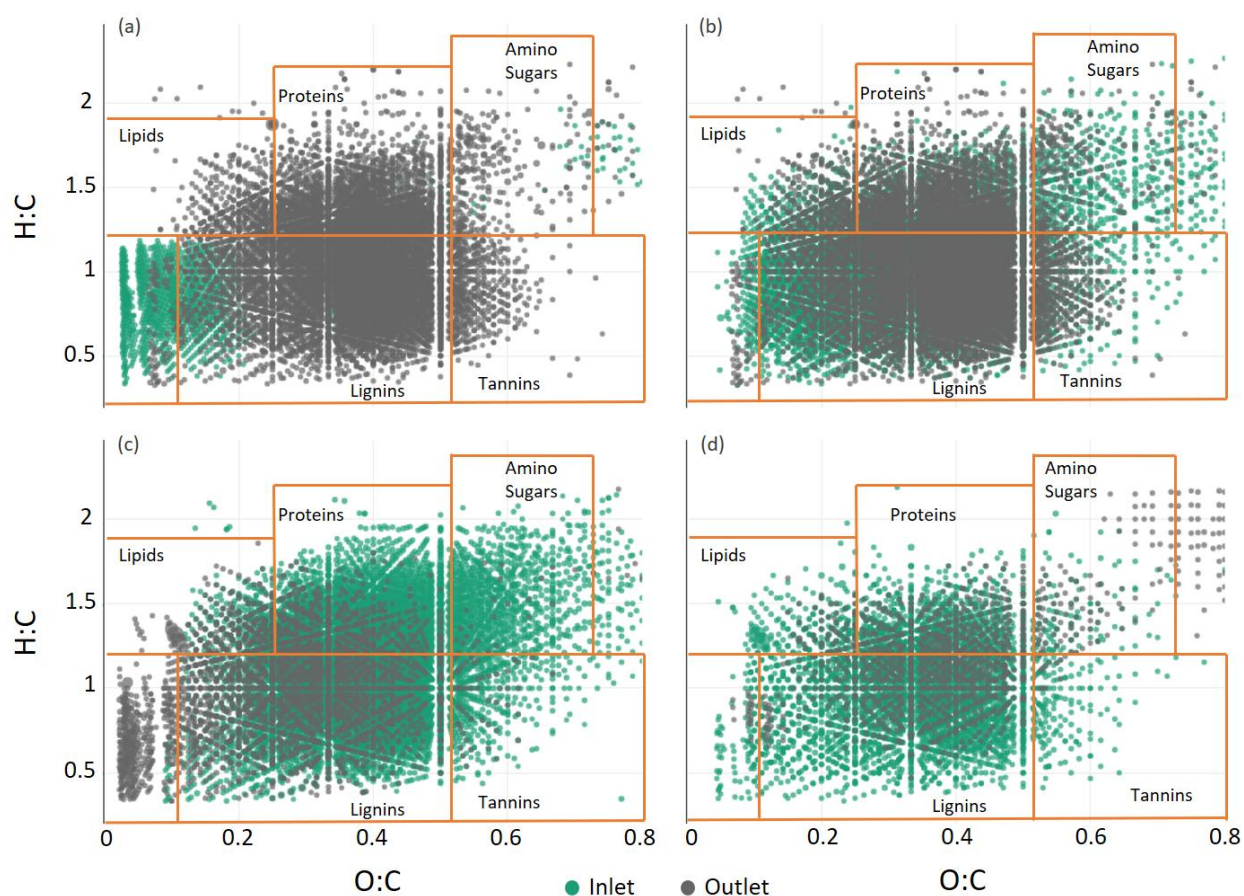


Figure 5. van Krevlen diagrams derived from negative-ion electrospray ionization FT-ICR mass spectral analysis for all N-bearing formulas in the mass spectra of the inlet and outlet with the low TN inlet for BAM with (a) no carbon addition (LSN), (b) carbon addition (LSC), and the high TN inlet with (c) no carbon addition (HSN), (d) carbon addition (HSC)

2.4 Discussion

2.4.1 Carbon Impact on Microbial Community Development and Nutrient Removal

Nitrogen removal within BAM is mainly attributed to a series of biological reactions involving ammonification, nitrification, and denitrification. Given the two inlet TN conditions, the microbial development can be seen in Figure 6, in which the addition of carbon boosted more bacterial growth in nutrient-poor conditions than in nutrient-rich conditions (Figure 3). One reason

is that the production of the initiation protein DnaA and other essential components of the replication machinery is proportional to carbon availability for nutrient-poor bacteria,¹¹⁷ but the DON can be utilized as a carbon source for bacteria in nutrient-rich environments, which makes carbon addition less valuable.⁷⁰

The difference from the inlet TN concentration can also affect the average cell size of bacteria that would substantially keep a certain surface-area-to-volume ratio (SA/V) favorable in response to nutrient availability. Given that column 1 was fed with low TN influent and column 2 with high TN influent, the bacteria cell size in column 1 has to be much smaller than that in column 2 to have a chance to survive. Harris and Theriot¹¹⁸ used the ratio of SA/V as a key indicator of the cell size because cells modify their size in order to achieve and maintain a specific, condition-dependent SA/V that benefits the species most. Schaechter, et al.¹¹⁹ also observed a similar phenomenon. In our study, the change of relative abundance of bacteria with depth after carbon addition is therefore meaningful (Figure 3). In column 1, the significant increase of the NOB percentage at the top section and 30 cm in depth indicates that the bacteria community had not developed to the maximum extent of its capability in terms of optimized community structure between different species before carbon addition. In column 2, the top layer has no significant change of NOB after carbon addition, which means it had already reached its optimal community structure, but the 30 cm in depth shows significant NOB percentage increase because the community had not reached its maximum capability and the additional carbon and other nutrients can push the community toward an even better community structure.

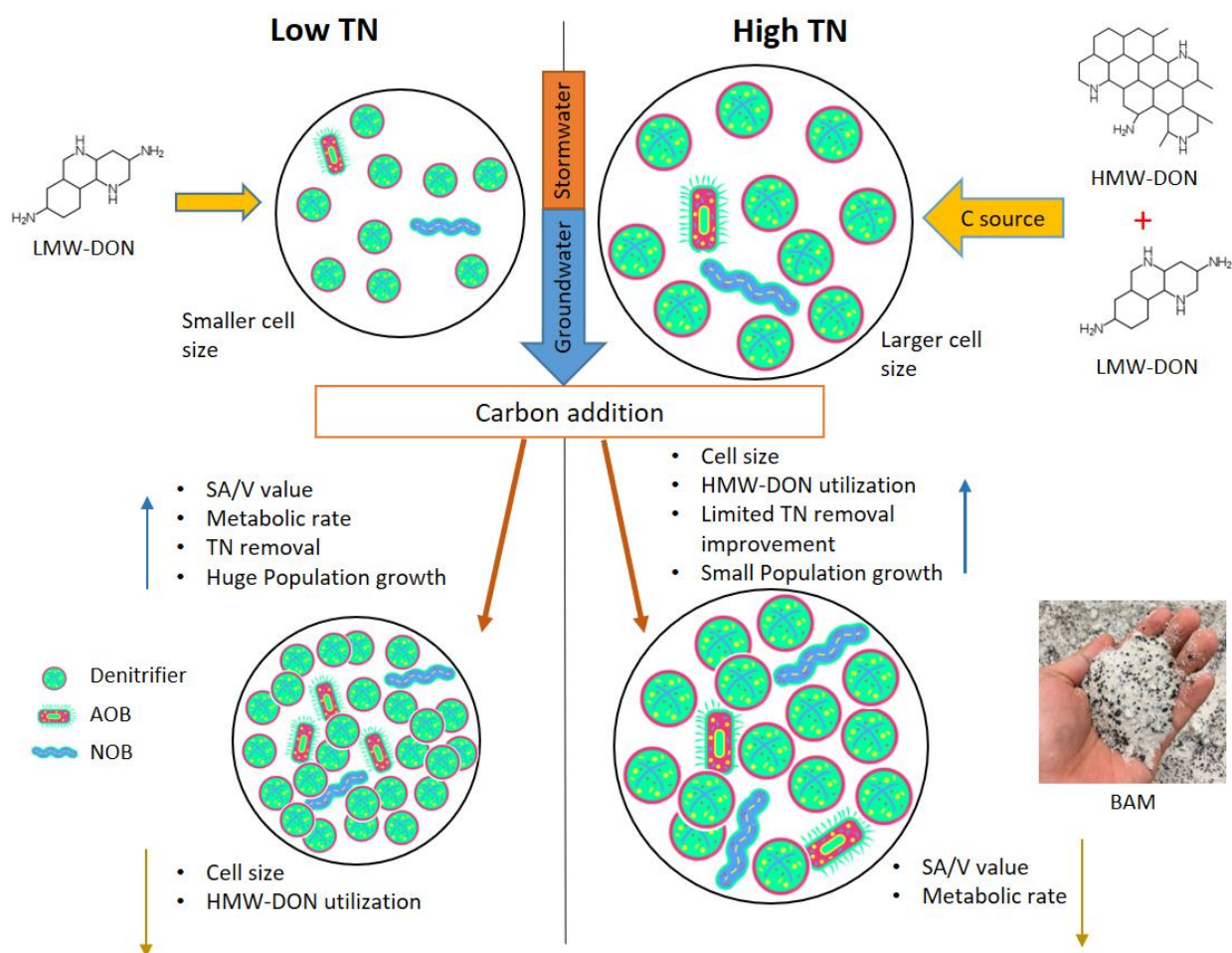


Figure 6. Schematic diagram of microbial community development (with different cell size) under different TN concentrations with carbon impacts (LMW: low molecular weight; HMW: high molecular weight)

The different microbial development in columns 1 and 2 also reflects the effectiveness of nitrogen removal under the impact of carbon addition given that the carbon source is important for denitrifiers in nutrient-poor condition regardless of whether the treatment is for stormwater (COD = ~15-20 mg/L) or groundwater treatment because the carbon source in groundwater is much lower (COD = 4~6 mg/L) (Figure 2 and Figure 3). For both columns, the top layer is the most active

section for nutrient removal since the majority of the bacterial population exists there rather than in the lower sections.

2.4.2 Carbon Impact on DON Concentration/Composition

Experimental studies and literature reviews have confirmed the general allometric pattern of an inverse relationship between cell size and biomass-specific metabolic rates.¹²⁰⁻¹²³ This pattern profoundly influences DON concentration and composition before and after treatment. The bacteria with smaller size in column 1 prefer to use low molecular weight DON (LMW-DON) and release the metabolic products faster because their higher metabolic rate cannot effectively utilize high molecular weight DON (HMW-DON) with the limited shorter reaction time within small-size bacteria. Thus, some of the DON classes are harder to be removed by column 1 (Figure 4) and column 2 seems able to remove more HMW-DON than column 1 (Figure 7). In column 1, the improvement of the total DON removal increased from 27% to 42% after carbon addition, a result that is probably driven by the cell synthesis and higher DON consumption with the population growth (Figure 2), but the effluent DON composition is quite similar before and after carbon addition (Figure 5), as some of the DON classes can hardly be removed (Figure 4). Conversely, the nutrient-rich environment in column 2 triggers a longer reproduction period because of the slower metabolic rate as DON provides a carbon source to fuel respiration, as demonstrated by Jones, Shannon, Murphy and Farrar⁷⁰ This outcome stimulates the bacteria in column 2 to absorb and accumulate more DON components within their cells (Figure 4 and Figure 5), resulting in larger cell size and slow release of metabolites back into the water that shows lower DON species/concentrations from the effluent (Figure 5) and promotes better DON removal performance for both DON concentration and composition.

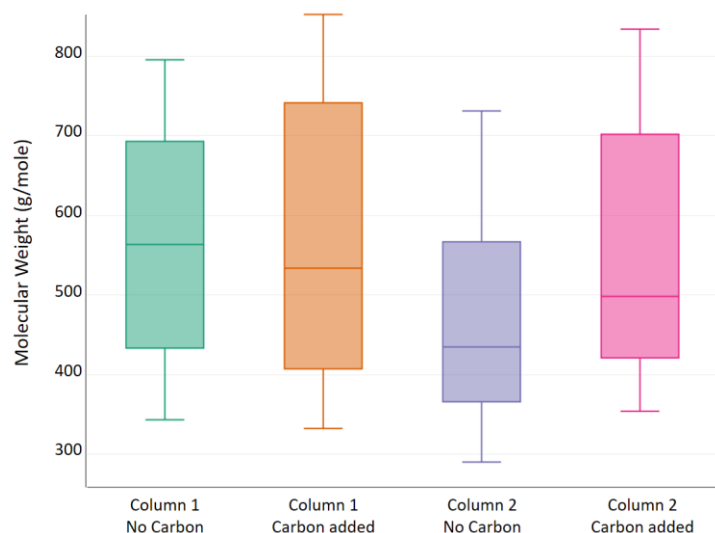


Figure 7. Comparison of outlet DON molecular weight for columns 1 and 2 with and without carbon addition

Other than comparing the qualitative differences between relative abundance differences in heteroatom class distribution (CHO, CHOS, CHON, etc.) before and after BAM treatment in Figure 4 and Figure 5, we also performed the class-wise analysis to address how the heteroatom classes changed under various conditions. Based on the FT-ICR-MS results (Figure 4 and Figure 5), the carbon addition decreased the % relative abundance of overlapped DON classes from 66% to 59% for low TN cases, and from 46% to 35% for high TN cases after treatment (Table 5). Additional carbon shows the potential to remove more DON compounds from the initial influent, due to the presence of more available carbon from the enhances bacterial population shown in Figure 3. Therefore, more DON classes were detected with a larger bacteria population in the effluent, and more bacteria population was observed indicating higher capability of consuming DON. Yet the average cell size, which has a direct relationship with inlet TN concentration as stated in section 4.1 (Figure 3), decides the ability of microbial community to utilize HMW-DON.

As shown in Table 5, after carbon addition, the relative abundance of new DON classes changed from 4% to 25% in low TN cases and from 28% to 33% in high TN cases, respectively. This could indicate that more carbon sources would increase the new DON classes as the microbial community with larger average cell size would produce more new DON classes due to consuming/converting more HMW-DON (evidenced by the amino corner of Figure 5-d). However, for most of the new DON compounds, the concentration is normally low, as shown in Figure 4 directly and Figure 5 indirectly as evidenced by the density of those smaller dots. This observation is consistent with the microbial ecology assessment from the previous statement in the sense that more abundant bacteria with larger sized cell are present that can potentially uptake more DON classes but can also generate more DON classes as microbial community exudates with such enhanced activity. Moreover, this microbial conversion process of DON is also noticed as a potential pathway to enhance the biodegradability of the initial DON from the inlet due to lower molecular weight from the outlet (Figure 7)¹⁰⁵.

From the holistic assessment point of view, the microbial community structure will evolve to an optimized status to utilize all resources as much as possible, such as nutrients, additional carbon, and growing space, etc. The utilization of DON is relatively difficult since some of them do not show appropriate biodegradability. However, more carbon and nutrient availability may further optimize the microbial community to evolve in terms of population, species structure, and cell status (size, bioactivity, etc.), as shown in Figure 3, which is also beneficial for DON consumption, as shown in Figure 4 and Figure 5, via such an enhanced microbial community. In general, the conversion from undissolved organics nitrogen to LMW-DON is known as

degradation whereby the further step of transferring LMW-DON to ammonia is called ammonification.

During the nitrification and denitrification processes, the most increased bacteria (in percentage) are in the order of AOB, NOB and denitrifiers in the top layer, as shown in Figure 8. Additional carbon can work as an electron donor for denitrifiers. Since NOB relies on AOB to provide nitrite as food, which is also a toxic product for AOB, they form an interactive mutualism relationship. That is why the NOB population is significantly enlarged (1142% and 70% in column 1 and 2) with the enhanced AOB population (3992% and 155% in column 1 and 2). This cascade effect resulted in denitrifiers (386% and 67% in column 1 and 2); more nitrate was provided by NOB to support the development of denitrifiers. Carbon source (glucose) cannot provide any ammonia, which is essential for AOB to thrive, but carbon can boost the heterotrophic bacteria to digest more DON via ammonification, as stated in the previous paragraph (optimized microbial community). In other words, the sequential enhancement of AOB, NOB, and denitrifiers happened once more DON could be consumed and converted into ammonia.

Table 5. Comparison of DON classes before and after treatment

	Low TN Inlet		High TN Inlet	
	No carbon	Carbon added	No carbon	Carbon added
Overlapped DON classes	66%	59%	46%	35%
New DON classes after treatment	4%	25%	28%	33%

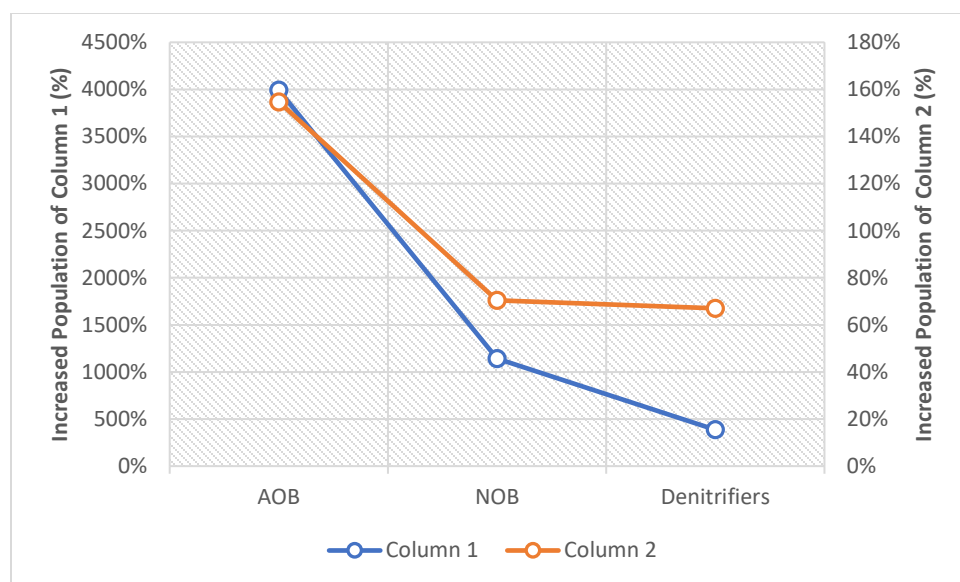


Figure 8. The population ratio of carbon added case to non-carbon case for AOB, NOB and denitrifiers at the top layer

2.5 Summary

The carbon addition impact on nitrogen removal for the co-treatment of groundwater and stormwater has been systematically evaluated in this study, in which the DON concentration/composition changes based on DOM for stormwater treatment were further realized with the help of FT-ICR-MS and qPCR together. The impact of carbon source is different for stormwater and groundwater; carbon is more important to TN removal in groundwater than in stormwater, as groundwater contains much less carbon in the first place, but carbon addition in this experiment did increase the ammonia concentration through the enhancement of ammonification for both stormwater and groundwater treatment. Nitrogen source variability resulted in different bacteria community development, in which low inlet TN cases tend to cultivate bacteria with smaller cell size while the high inlet TN cases end up favoring larger cell size bacteria that are quite different in terms of metabolic rate and population growth patterns.

Carbon works as the essential component for cell reproduction under the nutrient-poor environment, but DON can be utilized as a carbon source for bacterial respiration in the nutrient-rich environment. The DON utilization can be enhanced with additional carbon, but more DON classes would be generated because of the improved microbiological activities with a cascade effect over different microbial species from AOB to NOB to denitrifiers. This difference provides the foundation for understanding the different scale of SA/V that results in very different microbial structural functionalities since the smaller cell size bacteria tend to reproduce faster with a higher metabolic rate and maintain a larger SA/V value that is beneficial for absorbing nutrients more effectively. Therefore, LMW-DON is preferable for smaller cell size bacteria while more HMW-DON can be utilized by larger cell size bacteria. The most abundant bacteria exist at the top layer with denitrifiers as the dominant species, and the additional carbon has much less of an influence at the depth of 60 cm. For real-world applications, we suggest that the depth of BAM should be less than or equal to 60 cm (2 feet).

Overall, stormwater and groundwater are very important alternative sources of water in the urban water cycle. If cost-effective nutrient removal in heterogeneous landscapes and engineering conditions can be made possible with the aid of green sorption media, it may maintain the essential ecosystem service across many green urban infrastructures. These green infrastructures may include, but are not limited to green roof, bioswale or linear ditch, dry/wet pond, vegetated natural strip, exfiltration trench, lined underground piping networks with underdrain or reuse options, and bioswale. Our current study may lead to a deepened understanding of managing the nitrogen cycle in natural systems and the built environment as an integral part of the low impact development solution.

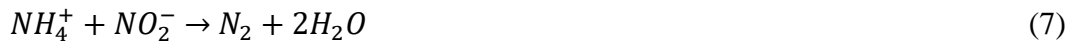
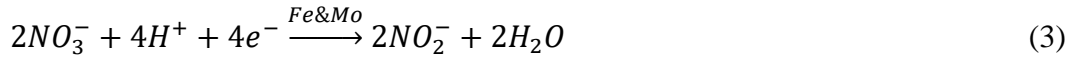
CHAPTER 3: COMPARATIVE COPPER TOXICITY IMPACT AND ENZYMATIC CASCADE EFFECT ON BIOSORPTION ACTIVATED MEDIA AND WOODCHIPS FOR NUTRIENT REMOVAL IN STORMWATER TREATMENT ²

3.1 Introduction

Biosorption Activated Media (BAM) and woodchip have been studied, modified, and applied to different stormwater Best Management Practices (BMPs) for nutrient removal before the stormwater recharges to groundwater aquifers or surface water bodies ^{7, 41, 44, 60-62, 80, 95, 124-126}. Nutrient removal, particularly removal of nitrogen, relies primarily on a series of biological reactions, namely ammonification, nitrification, and denitrification, all of which are substantially affected by the water quality of stormwater runoff ^{49, 127, 128}. Stormwater runoff may introduce substantial impacts on microbial ecology in terms of bacteria population and structure changes or soil degradation when encountering toxic/hazardous materials ^{129, 130}. One of the major impact factors is a toxicant such as heavy metal copper ^{131, 132} which commonly exists in stormwater runoff from urbanized areas ^{78, 79, 133, 134} with various concentrations normally around 15 – 30 µg/L ¹³⁵, but sometimes even in the magnitude of hundreds of microgram per liter ⁷⁷. Copper is also found to be more active in association with dissolved organic matter in the dissolved phase when compared with common metals like lead and calcium ⁷⁹. Although copper is an essential element for life on earth, an excess dosage of copper may have an inhibitory impact on microbial communities and, as related to this research, may cause a decrease in nutrient removal effectiveness ^{136, 137}. However, there are very limited studies regarding how copper may affect different involvement of the Nitrogen cycle (N-cycle) microbial community in BAM and woodchip.

² Authors: Dan Wen, Ni-Bin Chang, and Martin P. Wanielista; Publication: *chemosphere*.2018.09.062

This is related to the biological reactions for critical nitrogen removal as listed in Eqs. 3 – 9 ¹³⁸, in which four types of enzyme are needed in denitrification (Eqs. 1 - 4) to sequentially reduce NO_3^- to N_2 with the help of a redox metal cofactor in each of those enzymes; Eqs. 8 and 9 represent the sequential steps of nitrification. Eq. 7 is another pathway of denitrification performed by anammox (AMX) that requires an anaerobic condition and the existence of ammonium and nitrite as food/energy source. Eqs. 3 to 6 represent the involvement of different enzymes, and they are nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (NosZ), respectively. The four types of enzyme might have a possible enzymatic cascade effect for the denitrifying process when one or multiple enzymes are affected by copper.



The different characteristics of BAM and woodchip may result in entirely different outcomes on copper exposure. Some major differences between BAM and woodchip shall be noted, one of which is hydraulic difference due to very different porous structure. Another one relates to carbon source availability; woodchip itself can provide a carbon source through a slow decaying

process, while BAM cannot provide much by itself. These major differences may lead to interesting metabolic pathways driven by enzymatic cascade effect, which produce different nutrient removal efficiencies. Enzymatic cascade effect through confined multiple enzymatic reactions has been studied for decades in different fields such as medical and chemical science¹³⁹⁻¹⁴¹. One of its applications is for optimizing the over-all reaction process (velocity of product formation) through a series of reaction chains with multiple enzyme involvement in structural biochemistry. Even though many studies indicated that copper is toxic to microorganisms in general, including nitrifying and denitrifying bacteria^{74, 76, 136, 137, 142}, Black, et al.¹⁴³ found that additional copper in farmlands is surprisingly helpful in the conversion process of N_2O to N_2 , which is the last step of denitrification, as shown in Eq. 4. Black et al. (2016) may therefore have touched the base of *enzymatic cascade* catalysis for denitrification process initiated from the copper dosage. There is an acute need to investigate nutrient control and the potential of using BAM to treat stormwater runoff in order to deepen understanding of this unique phenomenon.

The objectives of this study were to evaluate the copper removal potential of BAM and woodchip and their toxic impact on changes of microbial ecology and enzymatic cascade effect for nitrogen removal from stormwater runoffs. Some science questions to be answered in this study include: 1) how the copper addition affects the nitrogen removal of BAM and woodchip holistically throughout the ammonification, nitrification, and denitrification processes, 2) how the copper addition influences the evolvement of the microbial community resulting in an enzymatic cascade effect on the denitrifying bacteria in BAM, and 3) how effective the two types of media are in copper removal. We hypothesized that 1) copper addition could decrease only some types of bacteria population in the N-cycle, 2) copper is an effective inhibitor for the whole microbial

community in terms of the effectiveness of nutrient removal, 3) BAM and woodchip should have different reactions on nitrogen removal performance with different copper removal potentials.

3.2 Material and Method

3.2.1 Experiment Setup

BAM contains sand, tire crumb, and clay, which are all environmentally friendly or recyclable materials. In the laboratory, one column 15 cm in diameter and 1.2 m in height with 3 water sample ports of 30 cm intervals on the side (Figure 1) was setup. BAM mixes applied by volume in this study contained sand (85%), tire crumb (10%) and clay (5%) by volume. In stage 1, stormwater was added to the column at 15 mL/min for two months with 5 mg/L spike of nitrate for cultivating the microbial communities. In stage 2, additional copper was added to the inlet with a concentration of 50 $\mu\text{g/L}$ which ran for 1 day, since most stormwater runoff usually ends within less than one day. Concentration of 50 $\mu\text{g/L}$ was chosen as the worst-case scenario when compared with 15-30 $\mu\text{g/L}$ copper in normal stormwater runoff. All stormwater was collected from the stormwater retention pond on the campus of the University of Central Florida (UCF). Water samples were collected from inlet, outlet, and three ports at the end of each stage. Water quality parameters of dissolved oxygen (DO) and pH were measured in the laboratory right after water sample collection. Total nitrogen (TN), ammonia, nitrate and nitrite (NO_x), and alkalinity were determined by a certificated laboratory, namely Environmental Research & Design, and all methods for water sample analysis are listed in Table 2. The BAM media samples were collected at the top, port 1, and port 2 in both stages 1 and 2.

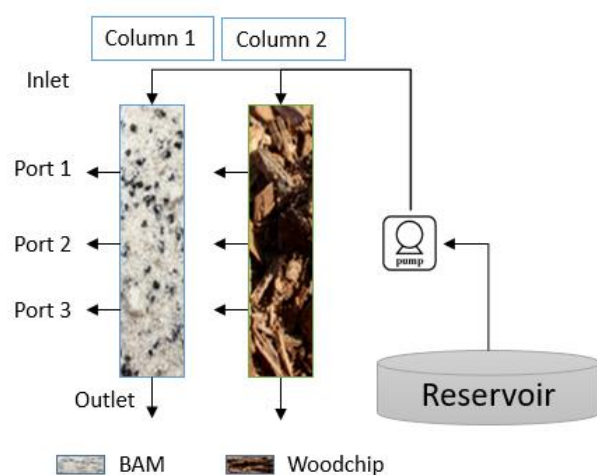


Figure 8. Schematic diagram for column setup

Table 6. Water sample quality analyzing methods and instruments

Parameters	Analyzing Methods/Instrument
Total Nitrogen (TN)	SM-21, Sec. 4500 N C
Nitrate + Nitrite (NO _x)	SM-21, Sec. 4500-NO3 F
Ammonia	SM-21, Sec. 4500-NH3 G
Alkalinity	SM-21, Sec. 2320 B
Dissolved Oxygen (DO)	HACH HQ40D - IntelliCAL LDO101 LDO
pH	Waterproof Double Junction pHTestr® 30
SM = Standard Methods for the Examination of Water and Wastewater, 21 st Edition, 2005	

3.2.2 Bioactivity Analysis

Filter sterilized solution of 2,3,5-Triphenyl tetrazolium chloride (TTC), recommended for the detection of microbial growth by means of TTC reduction in cells, was used to evaluate the holistic bioactivity in the microbial community (Nanwen et al. 1996). In particular, docosahexaenoic acid (DHA) measurement has been used to determine microbial activity in many studies of bioactivity assessment¹⁴⁴⁻¹⁴⁶. TTC is colorless in its oxidized form, but in the presence of dehydrogenase TTC is reduced to triphenyl formazan (TF), a red water insoluble compound (Figure 9). TF can be extracted from cells using organic solvent and the concentration is determined through spectrometer by measuring the optical density at 492 nm.

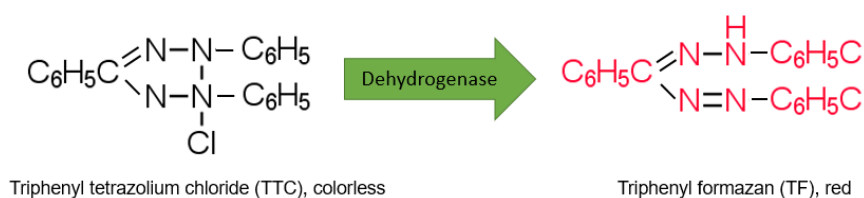


Figure 9. Mechanism showing the role of dehydrogenase in the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TF)

In the present study, TTC method was introduced and modified based on¹⁴⁷. Generally, 2 g of BAM media (woodchip cannot be analyzed due to its big size and the fact that it is floating in water) were taken from the top layer at the end of each stage, then the samples were transferred into 15 mL sterile centrifuge tubes with 2 mL TTC-glucose solution (0.1% of TTC and 1% of glucose by weight). Thereafter, the tubes were vigorously shaken in a water bathing vibrator with a constant temperature of 37 °C for 2 hours. The reaction was stopped by adding 1 drop of concentrated sulfuric acid to each tube. After that, the red color TF was extracted with toluene and followed by the use of a centrifuge for measuring the luminescence in spectrophotometer (492nm).

The final TF concentration was determined according to the standard curve that was prepared in advance. Note that 1 µg/L of TF produced in 1-hour equals 1 unit of DHA (unitless).

3.2.3 Real-Time PCR Analysis

In addition to bioactivity analysis, it is meaningful to retrieve the gene copy density information of key microbial species in relation to nitrogen removal since such information provides one more dimension for understanding the evolution of the microbial community in the media samples among ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifiers, and AMX. A *real-time PCR*, also known as quantitative *polymerase chain reaction* (qPCR), is a laboratory technique of molecular biology for identifying and quantifying microbial species. The gene copy density of nitrifiers and denitrifiers was tested with qPCR in the Bioenvironmental Research Laboratory at UCF. Collected media samples of BAM were stored at -80 °C until gene extraction by using the Mobio PowerMax Soil Kit, and the extraction process followed the kit protocol provided by the vendor. In particular, the woodchip samples were ground into smaller sizes before the DNA extraction for the purpose of obtaining more representative samples. All extracted DNA elutes were stored in the TE buffer under -20 °C. The real-time PCR analysis was performed with StepOne from Applied Biosystems, and PowerUp™ SYBR® Green Master Mix. The used primer sets and running methods are shown in Table 7. The qPCR assays are 20 µL reaction volume with 10 µL of master mix, 0.8 µL of each primer (10 µmole), 4 µL DNA template, and 5.2 µL of qPCR degree water for reactions.

Table 7. Primer sets and real-time PCR running condition

Target bacteria	Primer name	Primer sequence	Running method	Reference
AOB (Annealing at 60 °C)	amoA-1F	GGGGTTTCTACTGGTGGT	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	Rotthauwe, Witzel and Liesack ¹¹²
	amoA-2R	CCCCTKGSAAAGCCTTCTTC		
NOB (Annealing at 63.8 °C)	NSR1113f	CCTGCTTTCAGTTGCTACCG	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 63.8 °C for 45 cycles	Dionisi, Layton, Harms, Gregory, Robinson and Sayler ¹¹³
	NSR1264r	GTTTGCAGCGCTTTGTACCG		
Denitrifier (Annealing at 60 °C)	1960m2f	TAYGTSGGGCAGGARAAAC TG	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	López-Gutiérrez, Henry, Hallet, Martin-Laurent, Catroux
	2050m2	CGTAGAAGAAGCTGGTGCT GTT		

Target bacteria	Primer name	Primer sequence	Running method	Reference
				and Philippot 114
AMX (Annealing at 62 °C)	809-F	GCCGTAAACGATGGGCACT	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 62 °C for 45 cycles	148
	1066-R	AACGTCTCACGACACGAGC TG		

3.2.4 Statistic Analysis

Correlation analysis ($\alpha = 0.05$) was performed to determine whether the copper addition would make a difference in nutrient removal of TN, NO_x, and ammonia for BAM and woodchip. It can be used to compare the nutrient removal pattern along different sampling ports before and after copper addition. The mean nutrient concentration value from each sampling port was applied to Microsoft Excel for correlation analysis. The H_0 in copper has no significant impact on nutrient removal patterns (the correlation is statistically significant, where P – value < 0.05). In addition to that, one-way ANOVA analysis ($\alpha = 0.05$) was performed for the nutrient removal effectiveness between BAM and woodchip based on the replicates of overall nutrient removal efficiencies. The ANOVA analysis was conducted separately under no copper and copper added scenarios with

Microsoft Excel. The H_0 in BAM and woodchip have no significant differences from each other in terms of overall nutrient removal efficiencies ($P - \text{value} > 0.05$).

3.2.5 Scanning Electron Microscope (SEM) Analysis

SEM analysis was performed in the Advanced Materials Processing and Analysis Center at UCF. BAM media samples were collected for SEM analysis from the top layer of the column before and after the copper addition. All samples were pretreated in a 104 °C oven for 1 day to get rid of any moisture content before SEM analysis. The SEM equipment is the JEOL JSM-6480LV Scanning Electron Microscope, which provides ultrahigh resolution for observing biofilm on the surface of BAM particles.

3.3. Results

3.3.1 Copper Impact on Nutrient Removal

The detailed nutrient removals of BAM and woodchip are shown in Figure 10 and Figure 11 for TN, NO_x , and ammonia, respectively. For BAM, the overall TN removal (Figure 10-a) was 70% before copper addition, whereas it became 62% after copper addition. There was about 8% decrease in overall TN removal after the copper addition; however, more significant impacts were observed at the beginning section of the column as TN removal at port 1 decreased from 32% to 13% under copper impact. The overall NO_x removals (Figure 10-b) showed equivalent effectiveness (~72%) regardless of the copper addition. For port 1, NO_x removals changed from 25% to 21% after copper addition. Significant changes were observed for overall ammonia removals (Figure 10-c) from 14% to -127% removal before and after copper addition. In the case

of no copper addition, BAM tended to generate ammonia at the beginning section and remove it from the lower sections of the column. However, the ammonia generation process was delayed in the lower section under copper impact, which largely increased the ammonia concentration at the outlet of the column.

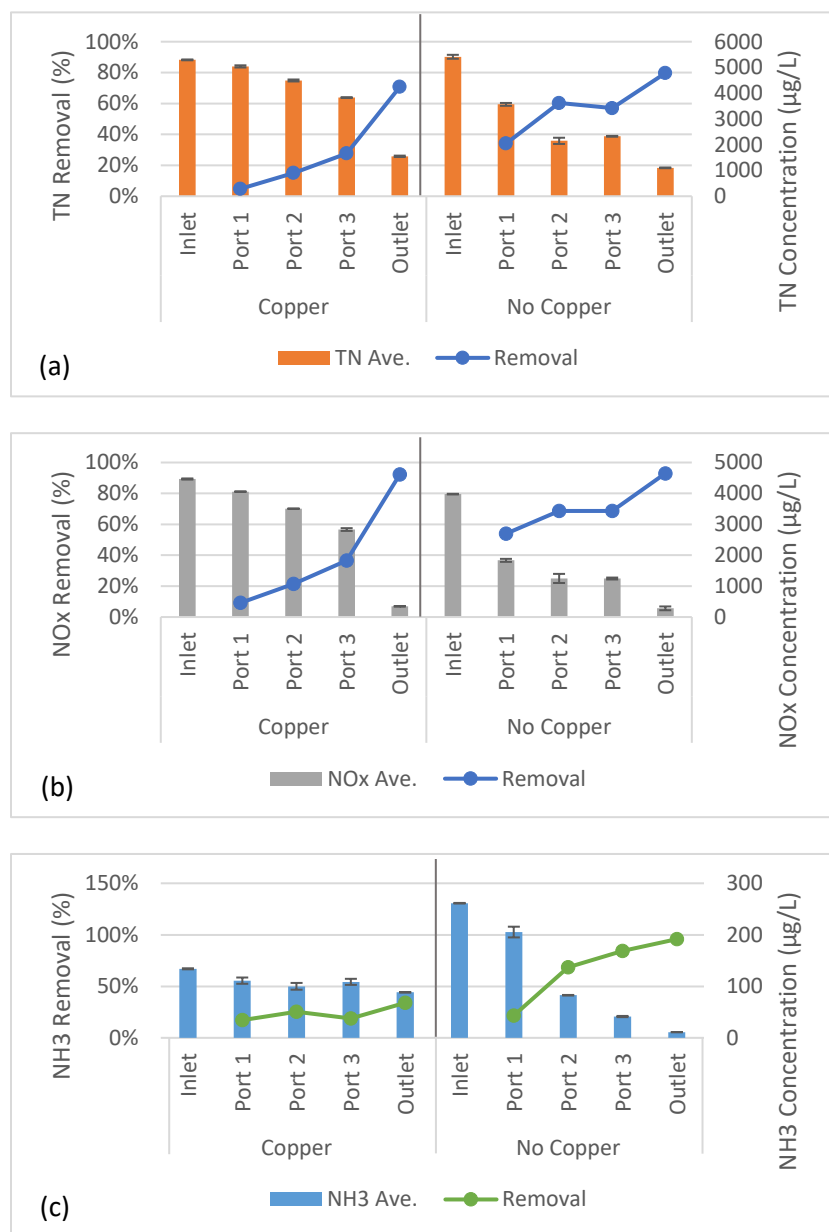


Figure 10. Nutrient removal in BAM before and after copper addition for (a) total nitrogen (TN), (b) nitrate and nitrite (NO_x), and (c) ammonia (NH_3)

For woodchip, the overall TN (Figure 11-a) removal decreased from 80% to 70% after copper addition, and the first three sample ports showed the trend of removal depression from copper when compared with the no copper case. For NO_x removal (Figure 11-b), the removal pattern was similar to TN removal in that the first three sample ports showed depressed removal efficiencies after copper addition, but the overall NO_x removal was equivalent in both scenarios, as it only dropped from 93% to 92%. However, the ammonia removal changed dramatically after copper addition in woodchip; not only did the overall removal decrease from 96% to 34% but also almost all sample ports showed depressed ammonia removal.

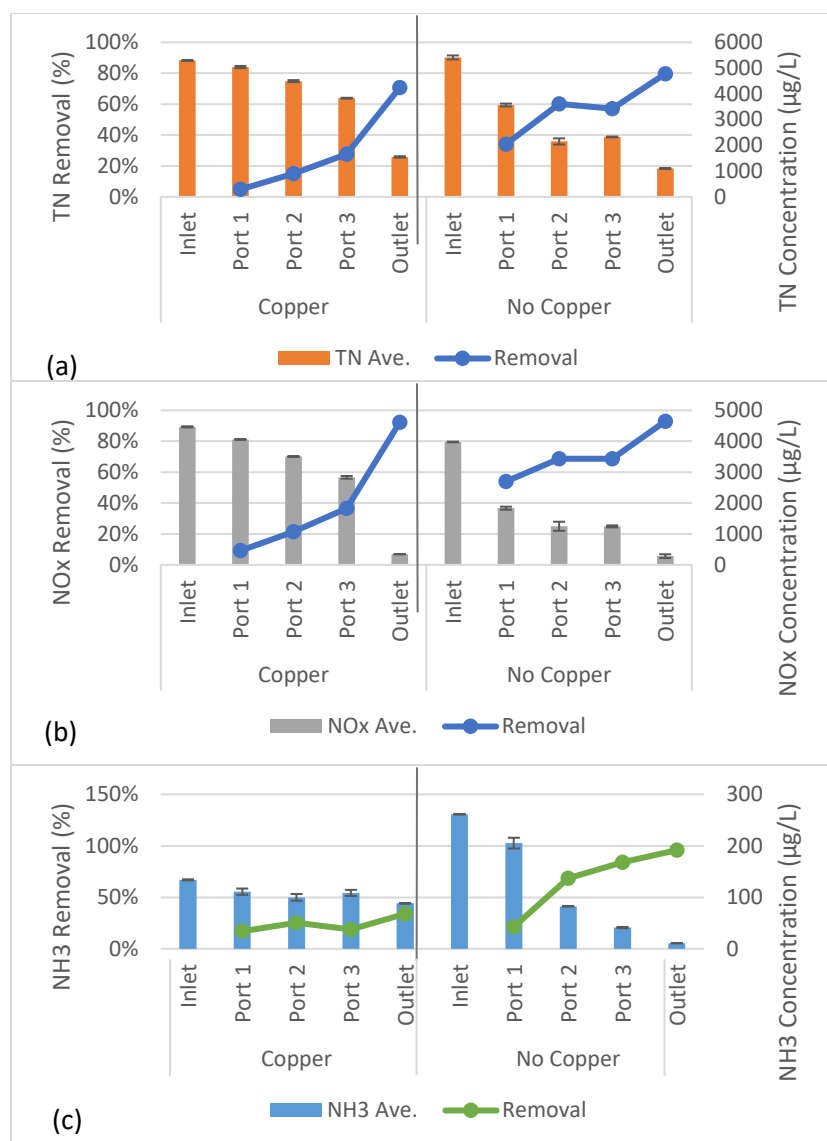


Figure 11. Nutrient removal in woodchip before and after copper addition for (a) total nitrogen (TN), (b) nitrate and nitrite (NO_x), and (c) ammonia (NH_3)

In addition to nutrient removal, copper removal was an important aspect to look into when considering the application of BAM or woodchip in copper contaminated areas. Figure 12 shows the corresponding copper concentration from different depths of the BAM and woodchip column. It can be concluded that both media performed well in copper removal, with a major removal of 85% in BAM and 80% in woodchip at the depth of 30 cm. Deeper depth of media cannot provide

further copper removal as the copper concentration varied around 3-5 $\mu\text{g/L}$ for the rest of the sample ports.

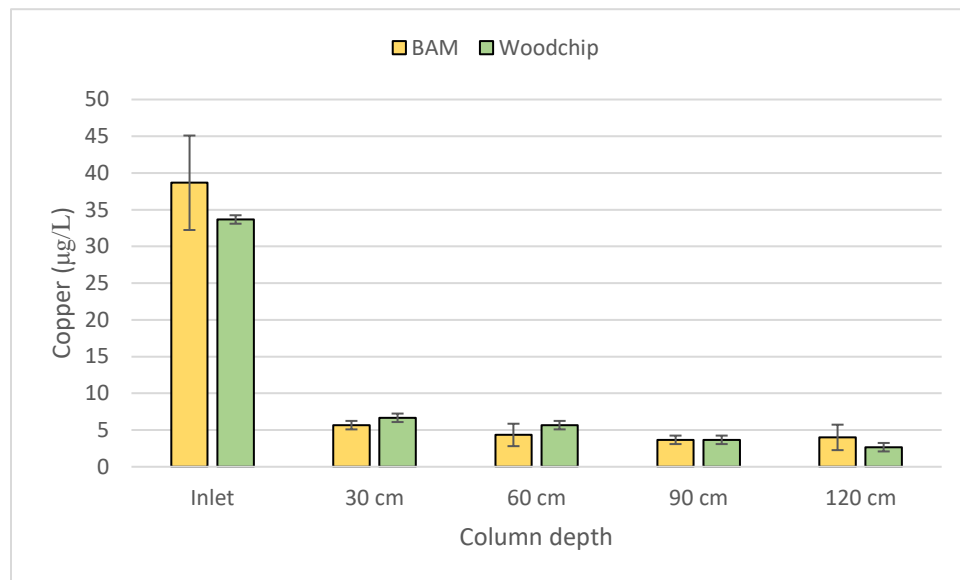


Figure 12. Copper concentrations from different depths of BAM and woodchip

3.3.2 Real-time PCR

The gene copy densities of AOB, NOB, denitrifier and AMX in BAM and woodchip are shown in Figure 13 with various depths of the column for copper and no copper comparisons. For BAM, the top section of the column contained the most abundant bacteria population, with denitrifier the most dominant among the four N-cycle related species. AOB and AMX are the minority here, with a minor scale around 1-2000 gene copies/g dry media while others have a magnitude of up to millions. Under such circumstances, copper addition seems to have no significant impact on AOB or AMX. AMX seemed to be evenly distributed along different depths of BAM and woodchip, however, NOB and denitrifier were different because NOB was largely depressed by the presence of copper while denitrifier was significantly enhanced. At the top section

NOB decreased significantly from 1.8 million copies/g to 130 k copies/g with ~92% population decrement under copper impact, but the denitrifiers increased 2.5 times from 4.4 to 11 million copies/g after copper addition. In the woodchip column, the total bacteria population was much lower when compared to the BAM column, and all bacteria species experienced population decrease after copper addition, especially AOB and NOB with 90% and 48% population reduction at the top layer, but the denitrifier had the strongest resistance with only 12% population reduction at the top layer.

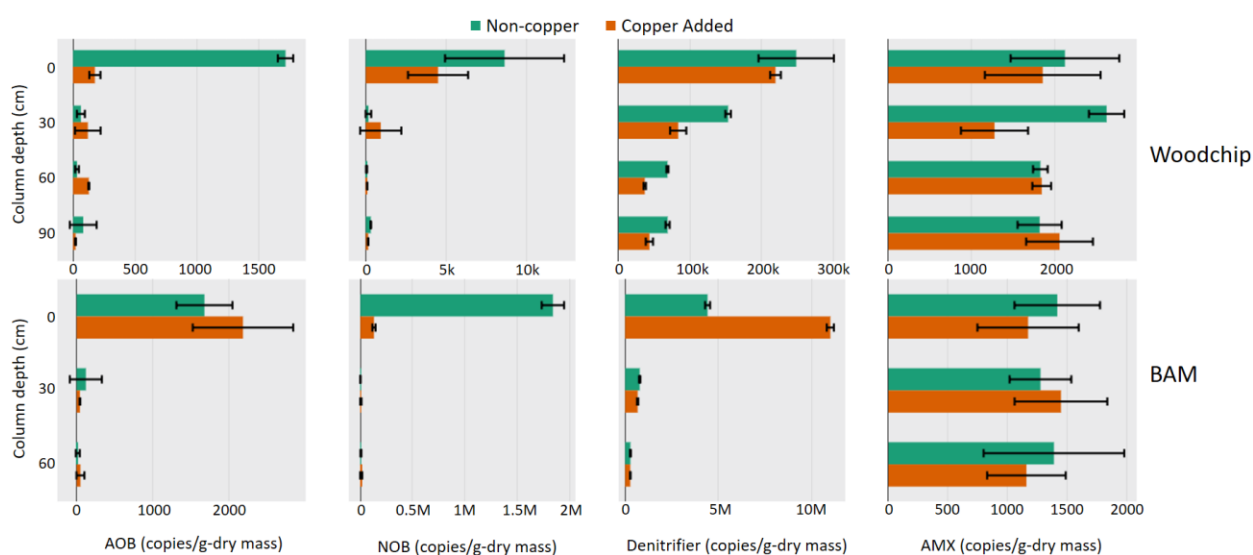


Figure 13. gene copy density of AOB, NOB, denitrifier, and AMX at various BAM and woodchip depths before and after copper addition

3.3.3 Statistical Analysis

The results of correlation analysis of each media for the copper impacts on nutrient removal patterns and one-way ANOVA analysis for media performance comparison are shown in Table 8. It can be seen that copper had no significant impact on TN and NO_x removal in BAM, but it did trigger a significant impact on the ammonia removal pattern of BAM. However, copper introduced

substantial impacts on all three parameters of TN, NO_x and ammonia removal in woodchip. For the nutrient removal performance comparison between BAM and woodchip, regardless of copper addition, only ammonia removal was significantly different between the two media.

Table 8. Correlation analysis results within each media for copper impact assessment and one-way ANOVA P-value for media comparison with and without copper impact

	Correlation Analysis				One-Way ANOVA	
	BAM		Woodchip		Media comparison	
	P-Value	Pearson's R	P-Value	Pearson's R	No copper	Copper added
TN	0.000343	0.995659	0.092958	0.814817	0.60842	0.271677
NO_x	0.000343	0.995659	0.112367	0.789289	0.258407	0.054364
NH₃	0.666965	0.264689	0.056591	0.867724	0.005189	0.022052

3.3.4 Bioactivity and SEM Analysis

The bioactivity test result of DHA from the top layer in BAM is shown in Figure 14 for the comparative copper impact assessment based on the whole microbial community given the copper concentration measured through each sample port. Copper has imposed negative influences on the holistic bioactivity within the top section of the BAM layer. The DHA decreased by 56% after copper addition. The SEM analysis of the BAM result is shown in Figure 15. It can be observed that there is more biofilm coverage area when copper is introduced than when copper is absent.

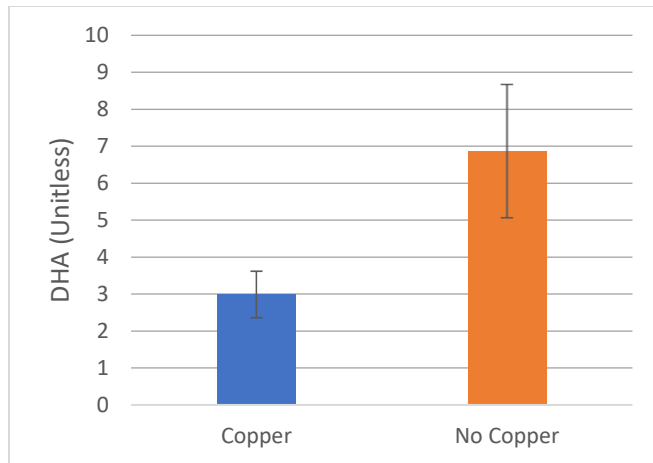


Figure 14. DHA of BAM media before and after copper addition

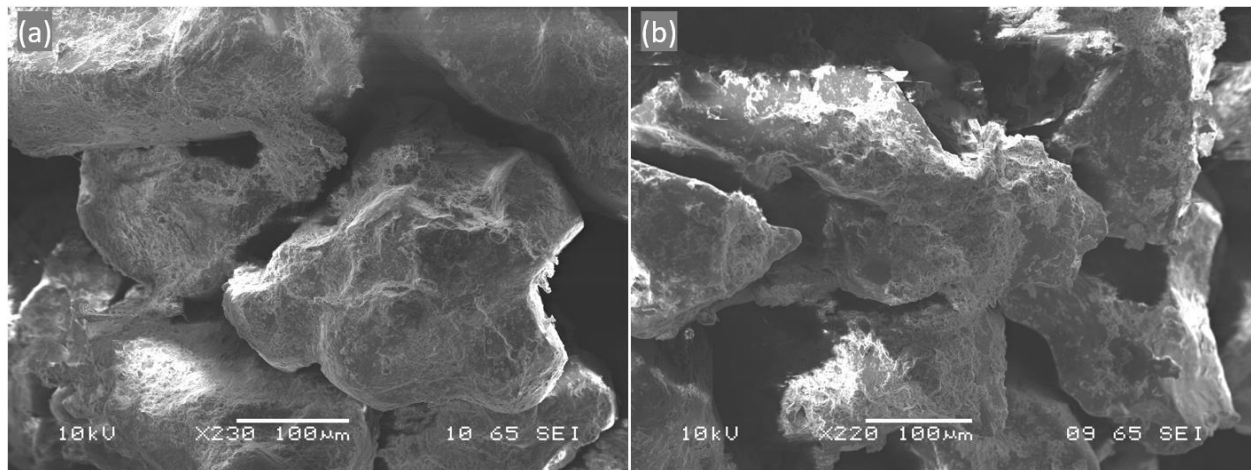


Figure 15. SEM analysis of BAM in cases of (a) copper addition and (b) no copper addition

3.4 Discussion

3.4.1 Copper Based Enzymatic Cascade Effect

The copper impact and enzymatic cascade effect on the evolvement of the N-cycle microbial community, particularly the denitrification process, can be clearly observed in the BAM column, which is summarized in Figure 16. After copper addition, all bacteria experienced substantial changes in population density and microbial structure, especially NOB and denitrifiers.

NOB population decreased, showing that copper has an inhibitory effect on N-cycle bacteria, as confirmed by many other studies^{74, 131}; however, in the current study, the population of denitrifiers showed an entirely different trend relative to NOB as it increased after copper addition, which supported hypothesis 1 (Figure 13).

Enzymatic cascade effect is the main reason behind the unusual increase in the population of denitrifiers within BAM. As shown in Figure 16, NosZ is the only enzyme that solely relies on copper as the enzyme cofactor that converts N_2O to N_2 in the last step of the denitrification process. The addition of copper resulted in enhanced gene expression, producing more NosZ and profoundly increasing the N_2 production efficiency. This influences the reaction equilibrium of Eq. 6, leading to the more efficient conversion of N_2O to N_2 . As a result, enzymatic cascade effect can be introduced from previous denitrification reactions (Eqs. 3 - 5) as their products are consumed faster, leading to the eventual enhancement of the overall reaction speed; thus, denitrifiers can obtain energy more effectively for reproduction, implying an inherent mutualism effect. Correlation analysis also revealed that copper had no significant impact on TN and NOx removal in BAM, where the ammonia removal pattern was significantly affected (Table 8), which may also be due to the enhanced denitrifiers population.

Moreover, Ochoa-Herrera, et al.¹⁴⁹ discovered that populations of nitrifier and denitrifier showed considerable recovery after a few days of dosage in sediment, probably because of the copper absorption or microbial adaptation. Black, Hsu, Hamonts, Clough and Condron¹⁴³ had similar findings, noting that 50 $\mu\text{g/L}$ of soluble copper caused maximum conversion of N_2O to N_2 via bacteria reduction in agricultural lands, and higher gene expression of denitrifier with copper dependent enzyme (NosZ) was confirmed. Therefore, the increased denitrifiers gene copy density

in the current study might be the early sign of microbial adaptation, but we only ran the stormwater column with copper addition for one day, which was an insufficient timeframe to see their full recovery status. The increase of denitrifiers was evidenced through the equivalent NO_x removal (Figure 10-b) and denser biofilm coverage in our SEM analysis (Figure 15-a).

At the same time, other bacteria species were deeply depressed because the copper addition inhibited some bacteria responsible for organic degradation and delayed the ammonification process. Those affected bacteria showed up more in the lower section of the column where the copper concentration was low (Figure 14-b, Figure 10-c). However, most copper was removed at the top layer of BAM and woodchip. BAM showed great copper removal potential in previous studies as well ⁶⁶, which is one of the major sinks for copper removal, because clay in BAM generally contains an abundance of negative charges that result in a strong surface electric field for capturing copper ions ¹⁵⁰. Natural materials such as sawdust and woodchip also showed promising heavy metal removal potential ^{151, 152}. Additionally, the other copper removal pathway is via biological reactions in denitrifiers, since copper is utilized as the cofactor of the critical nitrous oxide reductase as the key enzyme in last step of denitrification to convert N_2O to N_2 . Copper is captured by the dominant denitrifiers from the water and kept in biofilm. The enhancement of the last step of denitrification provides an additional 20% of energy and reduces the inhibitory products of nitrites to bacteria via the enzymatic cascade effects. However, the bioactivity (Figure 14-a) decreases after copper addition and so does the nutrient removal efficiencies (especially for ammonia). This means that even though the denitrifiers increased with copper addition, the whole microbial community was still under depression because bacteria species, other than denitrifiers, are more sensitive to copper toxicity. This finding echoes some

previous studies which also found that copper can effectively inhibit fermentative bacteria and aerobic glucose-degrading heterotrophs in biological wastewater treatment systems ¹⁴⁹. Those bacteria are also essential in breaking down the macro organic molecules for further utilization by nitrifiers and denitrifiers.

As a companion study, the woodchip column was different, as no enhancement of denitrifiers was observed after copper addition. Instead, it showed a reduction of all N-cycle bacteria, especially AOB and NOB (Figure 13). As a result, the NO_x and ammonia removal were largely limited at the first three sampling ports (Figure 11-b, c). However, denitrifiers were the least decreased species from the top layer, which could be a sign of enzymatic cascade effect for the BAM column in that denitrifiers showed the least vulnerability among all N-cycle bacteria (Figure 16). However, different media characteristics of woodchip profoundly restricted the benefits of enzymatic cascade effect on denitrifiers. Woodchip has much smaller surface area in unit volume and larger porous spaces when compared to BAM, which provides less living surface area for biofilm attachment and much less hydraulic retention time during treatment. This is the reason that water flow velocity in liquid bulk volume of woodchip is much faster than that in BAM, which led to more intensive shearing effects on biofilm in woodchip than BAM ^{153, 154}. Henriques and Love ¹⁵⁵ also found that biofilm loss is more severe under the same shearing forces when a toxic compound was introduced, especially for soluble, hydrophilic toxins, because the extracellular polymeric substances (EPS) cannot block them out of the biofilm. The loss of surface biofilm due to unstable biofilm structure caused by copper intrusion may increase the dissolved oxygen concentration at the biofilm bottom and oxygen is an inhibitor on the denitrification process. The last reason is that woodchip is not as good an absorbent for copper as BAM under

the blue line) but ammonia can only be obtained from inlet or organic matter degradation (ammonification). Normally, as shown in Figure 17, within a biofilm AOB and NOB are more likely to accumulate at the surface layer of the biofilm whereas denitrifier and AMX prefers the bottom layer due to the oxygen concentration, which decreases from the surface to the bottom of a biofilm.

In the BAM column, copper addition depressed NOB and enhanced the denitrifiers population (Figure 13) but there was not much change in the AOB population; copper triggers more available nitrite, as less NOB would consume it in nitrification and more denitrifiers may produce nitrite during the denitrification process. Moreover, in correspondence with the delayed ammonification process due to copper impact on the heterotrophic bacteria at top layer, the peak ammonia concentration showed up at the middle section of the BAM column (Figure 10-c). The more abundant food source for AMX led to more AMX population growth after copper addition at 30 cm depth (Figure 13). In woodchip, however, the enhanced shearing effects on biofilm when copper was added played a critical role in the microbial ecology of N-cycle bacteria. More AOB population was decreased proportionally than NOB, because it required AOB to obtain oxygen at the very top surface of biofilm for oxidizing ammonia as the first step of nitrification. However, NOB utilizes the product from AOB to produce nitrate in a deeper depth of the biofilm. This makes AOB more vulnerable to the hydraulic condition in woodchip, whereas the situation is entirely different in BAM; even though the copper inhibited other bacteria at the biofilm top layer, instead of losing biofilm surface layer it might become inert and maintain the necessary oxygen condition for AOB (Figure 17). For the AMX at the biofilm bottom with denitrifiers in woodchip, the shearing effects can hardly damage it and AMX can still obtain nitrite from the denitrification

process. However, as mentioned in the previous section, the decrease of biofilm thickness would lead to the increase of dissolved oxygen concentration at the biofilm bottom, which may inhibit the denitrification, as shown in Figure 11-b with less effective nitrate removal, and Figure 13 with decreased denitrifier population.

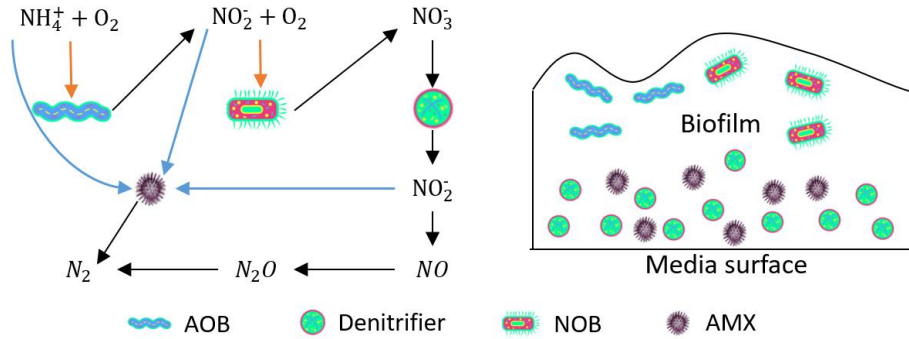


Figure 17. Schematic illustration of microbial interactions for N-cycling bacteria within the biofilm

3.4.3 Summary

The copper impact and enzymatic cascade effect on nutrient removal and the evolvement of microbial community deepen our understanding of the N-cycle process with microbial ecological evolvement in BAM and woodchip in the current study. The additional copper promotes denitrifiers with copper dependent nitrous reductase (NosZ) that converts nitrous oxide into nitrogen gas as the last step of denitrification. The enhancement of NosZ can initiate the enzymatic cascade effect on the early part of the denitrification process and result in faster enzymatic cascade reaction velocity. This enzymatic cascade effect ended up promoting the overall denitrification because more energy was gained for the reproduction of denitrifiers in BAM. This phenomenon was first discovered within BAM, which is a highly effective green sorption media for BMPs applications in stormwater management. The discovery of this study will alter the engineering design and strategy in certain cases for which the enzymatic cascade effects can be utilized to

achieve better nutrient removal. However, the cascade effects in woodchip are profoundly impacted due to different media characteristics in terms of void space, surface area, and size which affects oxygen availability. These factors resulted in thickened biofilm due to the enhanced shearing effects with copper addition. The understanding of microbial ecological interactions were deepened for both BAM and woodchip. Moreover, copper toxicity inhibited bioactivity on ammonification and nitrification processes as well as fermentative bacteria and so on, which substantially affected the corresponding part of the N-cycle before denitrification. However, we only evaluated the copper impact on a short-term basis since stormwater runoff usually ends within less than one day after the storm event. There are cases like wet stormwater detention ponds where long-term contact between BAM and copper might be phenomenal, which is critical for future research.

CHAPTER 4: COMPARISON OF NUTRIENT REMOVAL BETWEEN LABORATORY AND FIELD EXPERIENCE OF A LINEAR DITCH WITH TWO GREEN SORPTION MEDIA FOR SOTRMWATER AND GROUNWATER CO-TREATMENT³

4.1 Introduction

Driven by the rapid urbanization, economic development, and population growth worldwide, stormwater runoffs, wastewater effluents, and agricultural discharges have become more nutrient-laden streams altering the nutrient cycle and water cycle. Such impacts have gradually resulted in the changes *on urban* identity and infrastructure due to the transformation on *landform* configuration. As a result, groundwater aquifers and surface water bodies have been under jeopardizing conditions due to the presence of excessive nutrients through the overland flows and recharged water from the urbanized and sub-urban regions^{54-56, 88, 156, 157} and agricultural crop fields¹⁵⁸⁻¹⁶¹. Those stormwater runoffs, wastewater effluents, and agricultural discharges are an increasingly critical source of nitrogen to the receiving water bodies. Excess nitrogen in the ecosystem could result in eutrophication and algal bloom, which profoundly affects the community structure, degrades the habitat and biodiversity in an ecosystem⁵⁶. The National Academy of Engineering in the United States has indicated that “understanding and managing the nitrogen cycle” as well as “restoration and improvement of urban infrastructure” are two of fourteen grand challenges for engineering in the 21st century (Mote, 2016). Deepened understanding of nutrient cycling across natural systems and the built environment becomes critical for the continuation of life on the planet to make our world more sustainable.

³ Authors: Ni-Bin Chang, Dan Wen, William Colona, and Martin P. Wanielista; Pending publication: submitted to *Chemosphere*, anticipated publish date: Dec 2018

Stormwater and groundwater in the urban water cycle are relatively untapped resources of water when it comes to meet today's freshwater demand at the community scale ^{162, 163}. If the nutrient removal can be made possible by a cost-effective way with the aid of green sorption media to fit in heterogeneous landscapes and engineering conditions, it may maintain the essential ecosystem service across urban infrastructures. Low Impact Development (LID) facilities that are regarded as an integral part of green infrastructures may include, but are not limited to dry/wet pond, green roof, bioswale or linear ditch, vegetated natural strip, exfiltration trench, lined underground piping networks with underdrain or reuse options, and bioswale. Some Best Management Practices (BMP) are adopted widely to deal with the contamination of stormwater runoff and agricultural discharge at the field scale. To aid in nutrient removal through those LID facilities, the invention of green sorption media emphasizes direct and indirect benefits for providing ubiquitous ex-situ water treatment services for nutrient removal as well as cost-effective water reuse and possible nutrient recovery, which extend beyond stormwater harvesting in cities.

Recently, biosorption activated media (BAM) that is regarded as a new form of green sorption media has been implemented as an innovative BMP for enhancing nutrient removal from stormwater runoff ^{44, 45, 60-62, 65}. BAM is known as one of the green sorption media due to the inclusion of recycled materials providing better nitrogen removal efficiency and cost-effectiveness through the enhancement of the micro-environments and hydraulic patterns for biological reactions of nitrification and denitrification. This also implies BAM-based BMP requires less carbon footprint for construction relative to others but can still present promising results of nitrogen removal when compared to traditional biological nutrient removal (BNR) schemes. However, woodchip media can be regarded as a competitive green sorption media as well (Robertson, 2010;

Schipper, Robertson et al., 2010). Even though BAM and woodchip media have been tested and applied in various LID facilities as denitrifying bioreactors (Robertson, 2010; Schipper, Robertson et al., 2010; Xuan, Chang et al. 2013, O'Reilly, Chang et al. 2014), knowledge of using BAM and woodchip media to improve the performance of bioswales or linear ditches in terms of nitrogen removal is still unclear. This is especially true when investigating the difference between laboratory and field conditions for stormwater and groundwater co-treatment along a roadside corridor that is deemed very cost-effective. Co-treatment may be operated based on storm period and non-storm period. Whereas non-storm period is dedicated for groundwater treatment using pumps to withdraw groundwater, storm period is designed simply to treat the *in-situ* storm runoff from the road system to temper the nutrient impact on the groundwater system.

Two types of green sorption media are selected for comparison in this study, and they are called Bold & Gold (B&G) media and woodchip media. B&G media are composed of sand, clay, and tire crumb while the woodchip media are small chips and shavings collected from a wooden factory. This study therefore provides a holistic understanding of their performance from a laboratory scale to the field scale. It not only deepens the understanding of the nitrogen cycle but also aids in the green infrastructure design. The science questions to be answered in this study are: 1) can the co-treatment process achieve promising nitrogen removal under various nitrogen concentration levels with scales? 2) how would the difference of water quality in stormwater and groundwater affect the nitrification and denitrification process? 3) which media recipe is more appropriate in the future for better nutrient removal and cost-effectiveness? 4) will the nitrogen removal at the field scale follow the same pattern as it is at the laboratory scale? 5) how could the microbial ecology help explain the difference between the laboratory and field conditions and

justify the difference in their performance? With these science questions, the objectives of this study are to: 1) perform laboratory test of nitrogen removal in a column study with different nitrogen concentrations of groundwater and stormwater as the influent with respect to two types of green sorption media; 2) design, construct, and operate an innovative field-scale linear ditch BMP with two types of green sorption media; 3) measure the gene copy densities of key bacteria in the nitrification and denitrification processes in both the laboratory column study and the field applications; and 4) compare the scaling effect in terms of performance of nitrogen removal and hydraulic patterns between the field condition and the laboratory column study. Based on the science questions and objectives of this study, the hypotheses include: 1) the co-treatment strategy is suitable and effective for both recipes of green sorption media under various influent concentrations and flow conditions; 2) different water sources (groundwater and stormwater) might have impacts on the nitrogen removal due to their background constituents in the biogeochemical cycle; 3) the nitrogen removal and associated microbial ecology in the field follow a similar trend/pattern as it shows in the laboratory column study; 4) B&G media shall be more appropriate to be applied in the field as it exhibits a long-standing micro-environments and hydraulic patterns with respect to homogeneous hydraulic retention time and infiltration rate than the woodchip media. 5) the microbial community would be very different from the column study to the field conditions.

4.2 Study Site

The study site is rural land in Fanning Springs area, located in east-northeast of City of Fanning Springs in Levy County, North-Central Florida (Figure 18). Specifically, the study site is located in the southeast corner of SR-26 and 55th Avenue and extends along the southern right-

of-way of Florida Department of Transportation, 0.8 km (1/2 mile) west and up to 1.6 km (1 mile) east. As shown from the Figure 19(a), in this watershed, land use patterns include residential areas, a dairy farm, a wastewater treatment plant, and agricultural fields. More specific site view is shown in Figure 19(b), state road on one side and the other side is farmland. The linear ditch catches stormwater runoff from the road and agricultural discharge from the farmland next to the road corridor simultaneously. There are approximately 3.7 m (12 ft) gap between the private property boundary of the farmland and the local pipe/cable line indicated by the red flags on the ground

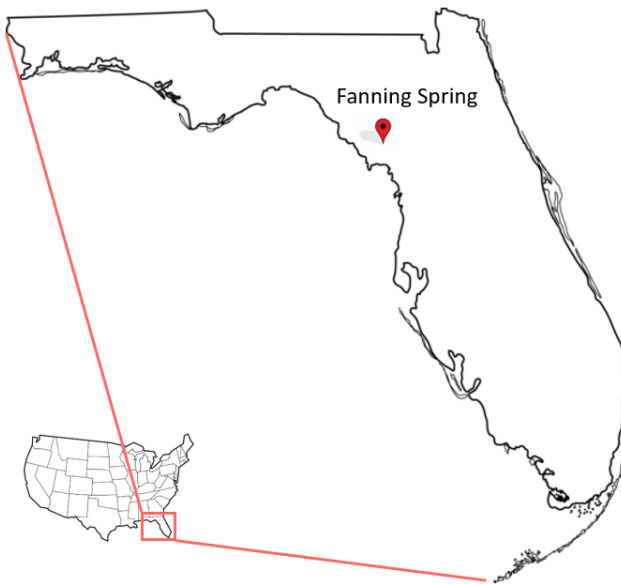


Figure 18. Location of the study site in Fanning Spring, Florida

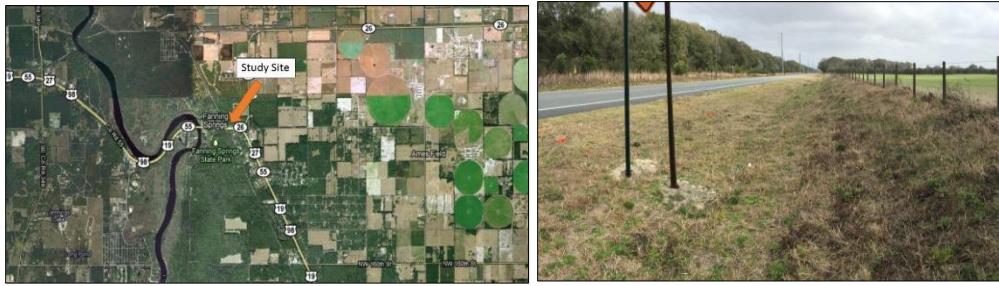


Figure 19. (a) Aerial view of Fanning Springs and surrounding and (b) construction site view

The schematic design for construction and its operation strategy are shown in Figure 20. The local site has very sandy native soils, and no nitrate removal is expected without using green sorption media. The length of the linear ditch being selected is 183 m (600 ft) in total, and half of it is filled with woodchip media and the other half is filled with B&G media for a side-by-side comparison. In order to investigate the impact of the media depths on nitrogen removal, the woodchip linear ditch is divided into three 30 m (100 ft) long sections with the depth of 0.6, 0.9, and 1.2 m (2, 3 and 4 ft), respectively whereas the B&G linear ditch is divided into two 45 m (150 ft) sections with the depth of 0.3 and 0.6 m (1 and 2 ft), respectively. Both have 1.2 m (4 ft) wide. Lysimeters are located in the middle and bottom of each section of B&G media and woodchip media except the middle part of 1.2 m (4 ft) depth in the woodchip section. There are two solar-powered pumps for the withdrawal of groundwater at the rate of about 132 L/h (35 gal/h) during sunny daytime (Figure 21). The water is distributed along the pipeline on the top of each section creating downflows. The pumping rate would be slow during a cloudy day or even become zero when a rain storm comes. During a storm event, the pumps are completely stopped and then the full treatment capacity of linear ditch is tuned for treatment of stormwater runoff from the road system and agricultural discharge.

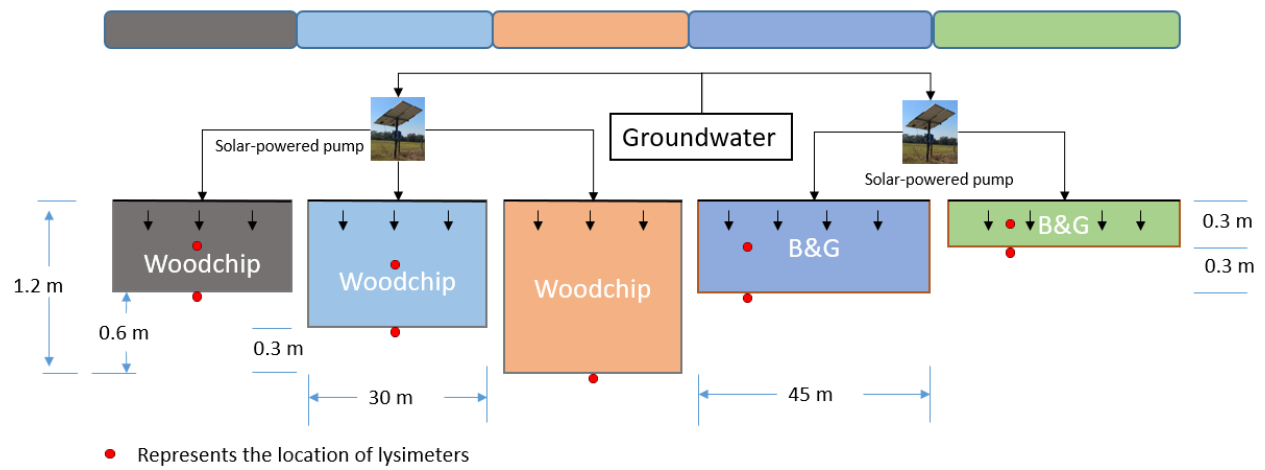


Figure 20. Schematic flowchart for design, construction, and operation strategy in the field



Figure 21. Schematic flowchart for design, construction, and operation strategy in the field (upper left: Construction phase; upper right: completion of construction of B&G[®] media section; lower left: operation of pumps with solar panel in the middle of B&G[®] med

4.3. Laboratory Study

4.3.1 Column Study

The laboratory experiment was set up through a suite of columns to mimic the field condition of the road side, and to simulate the co-treatment of stormwater and groundwater alternately. As shown in Figure 22, 4 columns with 15 cm (6 inches) diameter are noted from column 1 to column 4 hereafter. The columns' depth is 122 cm (4 ft), and 3 sample ports are marked on the side of the columns with 30 cm (1 ft) interval (Figure 4). Columns 1 and 2 are used for testing B&G[®] media and columns 3 and 4 are prepared for testing woodchip media. Different influent concentrations were chosen for both B&G[®] media and woodchip media systematically during the testing period in which columns 1 and 3 were running with lower concentrated influent (1.5 mg/L spiked nitrate) while columns 2 and 4 were running with higher concentrated influent (5.0 mg/L spiked nitrate). The running strategy of these four columns was schematically described for B&G[®] media in columns 1 and 2 and the same strategy applied for woodchip columns in the laboratory (Figure 23). All columns were running with stormwater spiked with nutrients to support the biofilm cultivation for at least one month before collecting any water sample. After the biofilm cultivation, the groundwater and stormwater were pumped into the columns alternately following a prescribed schedule. We initially ran groundwater for three days with the flow rate of 10-15 mL/min and then collected water samples from the inlet, port 1 to 3, and the outlet. Then we switched to operate the stormwater columns and ran them for 1 day and collected water sample by the same way again. Standard nitrate solution was spiked into the real-world groundwater and stormwater to the theoretical concentration of 1.5 mg/L for column 1 and 5 mg/L for column 2.

pH and dissolved oxygen (DO) were also measured right after the sample collection in the laboratory.

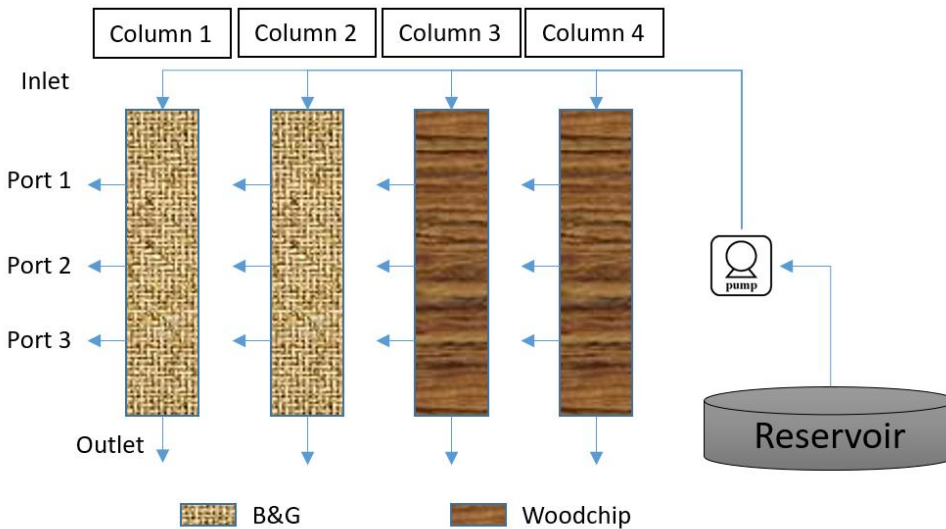


Figure 22. Schematic diagram of the column setup in the laboratory

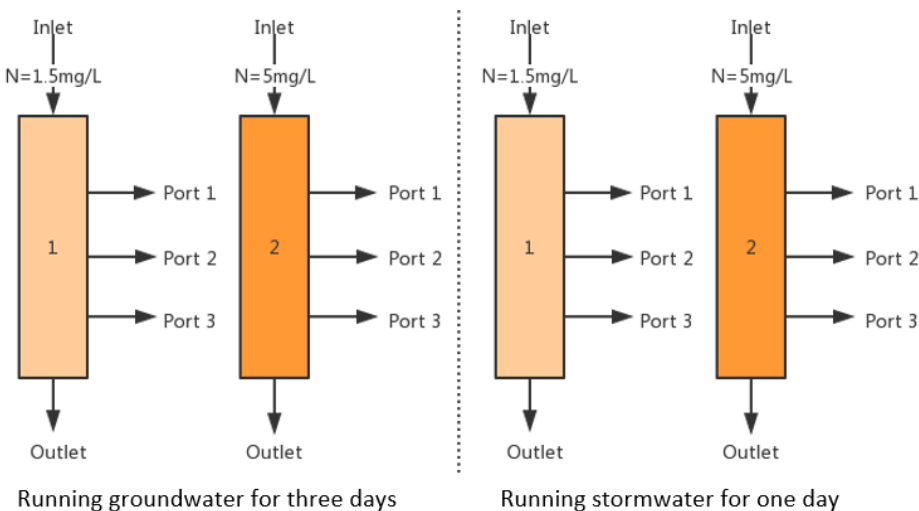


Figure 23. Schematic of the operational strategy for B&G[®] media columns and the same strategy applied for woodchip columns in the laboratory

The laboratory study was conducted at the University of Central Florida (UCF) under controlled room temperature that ranges from 22 to 23 °C. All samples were well preserved and

delivered to a certified laboratory named Environmental Research and Design, Inc. (ERD) within 24 hours after collection. All field samples were collected from the lysimeters and pumping wells located in the Fanning Spring linear ditch study site. The field water samples were delivered to another certified laboratory called Test America Laboratories, Inc. (TAL) for nutrient analysis. The analyzed parameters and methods are summarized in Table 9.

Table 9. Analysis method for lab and field samples

	ERD	TAL
Chloride	No Analyze	MCAWW 325.2
Ammonia	SM 4500 NH3 G	MCAWW 350.1
Nitrogen, Kjeldahl	No Analyze	MCAWW 351.2
Nitrate & Nitrite	SM 4500 NO3 F	MCAWW 353.2
phosphorus	No Analyze	EPA 365.4
Ortho-phosphate	No Analyze	SM 4500 P F
Nitrogen, Total	SM 4500 N C	EPA Total Nitrogen
Ammonium ion	No Analyze	FL-DEP Unionized NH3
<p>EPA = US Environmental Protection Agency</p> <p>FL-DEP = State of Florida Department of Environmental Protection, Florida Administrative Code.</p> <p>MCAWW = "Methods for Chemical Analysis of Water and Wastes", EPA-600/4-79-020, March 1983 and Subsequent Revisions.</p> <p>SM = "Standard Methods for The Examination of Water and Wastewater"</p>		

4.3.2 Tracer Study

The purpose of the tracer study is to determine the hydraulic retention time (HRT) of both BAM recipes (e.g., B&G[®] media and woodchip media). HRT is crucial for the estimation of

treatment time in the column and field study, which is an important factor for biological reactions such as ammonification, nitrification and denitrification. Rhodamine WT (RWT) dye (purchased from Ozark Underground Laboratory) was selected in this tracer study due to its advantages of low detection limits, zero natural background, low cost, and easy operation. The original RWT is 20% solution with a concentration of 200,000 ppb. To ensure the final concentration of RWT from the effluent is within the detection range of fluorometer (Aquafluor 8000-010) between 0.4 to 400 ppb, RWT was diluted to the concentration around 8000 ppb. The columns were running with a steady pumping rate of $49.36 \text{ L/m}^2 \cdot \text{h}$ for 3 hours to obtain the continuously stable hydraulic condition. Then a 5 mL of diluted RWT was dosed at the top of the column. The effluent samples were taken in 10 minutes interval for B&G[®] media columns and 1-minute interval for woodchip columns. The tracer HRT is calculated through the following equation (10), where τ is the tracer HRT, $C(t)$ is the RWT concentration at time t .

$$\tau = \frac{\int_0^{\infty} tC(t)dt}{\int_0^{\infty} C(t)dt} \quad (10)$$

4.3.3 Microbial Ecology Study

In order to better understand the bacteria involvement in both laboratory columns and field media critical for biological nitrogen removals in terms of nitrification and denitrification, those bacteria of interest include ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifiers, and annamox (AMX). A *real-time PCR*, also known as quantitative *polymerase chain reaction* (qPCR), is a laboratory technique of molecular biology for identifying and quantifying the microbial species. The gene copy densities were tested with qPCR in the Bioenvironmental Research Laboratory at UCF. BAM and woodchip media samples were collected from 0, 30 cm

(1 ft), 60 cm (2 ft) depth in column study whereas they were collected from top, middle and bottom of each media section in the field. Collected media samples of BAM and woodchip media were stored at -80 °C until the gene extraction by using Mobio PowerMax Soil Kit, and the extraction process followed the kit protocol provided by the vendor. In particular, the woodchip samples were grinded into smaller sizes before the DNA extraction for the purpose of obtaining more representative samples. All extracted DNA elutes were stored in TE buffer under -20 °C. The real-time PCR analysis was performed with StepOne from Applied Biosystems, and PowerUp™ SYBR® Green Master Mix. The used primer sets and running methods are shown in Table 10. The qPCR assays are 20 µL reaction volume with 10 µL of master mix, 0.8 µL of each primer (10 µmole), 4 µL DNA template, and 5.2 µL of qPCR degree water for reactions.

Table 10. Primer sets and real-time PCR running condition

Target bacteria	Primer name	Primer sequence	Running method	Reference
AOB (Annealing at 60 °C)	amoA-1F	GGGGTTTCTACT GGTGGT	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	Rotthauwe, Witzel and Liesack ¹¹²
	amoA-2R	CCCCTKGSAAA GCCTTCTTC		
NOB (Annealing at 63.8 °C)	NSR1113f	CCTGCTTTCAGT TGCTACCG	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 63.8 °C for 45 cycles	Dionisi, Layton, Harms, Gregory, Robinson and Sayler ¹¹³
	NSR1264r	GTTTGCAGCGCT TTGTACCG		
Denitrifier (Annealing at 60 °C)	1960m2f	TAYGTSGGGCA GGARAAACTG	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 60 °C for 45 cycles	López-Gutiérrez, Henry, Hallet, Martin-Laurent, Catroux and Philippot ¹¹⁴
	2050m2	CGTAGAAGAAG CTGGTGCTGTT		
AMX (Annealing at 62 °C)	809-F	GCCGTAAACGA TGGGCACT	2 min 50 °C and 95 °C; 15 s at 95 °C and 1 min at 62 °C for 45 cycles	¹⁴⁸
	1066-R	AACGTCTCACG ACACGAGCTG		

4.4. Difference between Laboratory and Field Study

The daily rainfall depth since the treatment started (6/23/2017) at the linear ditch site is shown in Figure 24 in which three sampling time points (10/12/2017, 1/17/2017, and 4/19/2018) were identified. The rainfall data were collected from the Suwannee River Water Management District with automatic rain gage located at latitude of 29 40' 02" and longitude of 82 52' 29". It provides a general understanding of how often and how much of storm events happened in this area, which is closely related to the treatment effectiveness of different kind of nutrient species. Note that whenever the storm happens, the pump would slow down or stop working completely due to diminished sunlight condition at that moment. In addition to the rainfall data, the total amount of pumped water since the start of the linear ditch treatment is shown within 7 recording time points and the corresponding average pumping rate for each media. Such record provides insightful information of the pumping speed which is strongly related to the weather condition. The hydraulic loading rate of groundwater to the BAM section along the length of the linear ditch was calculated as 115 and 108 L/m²·day⁻¹ for B&G[®] media and woodchip media, respectively.

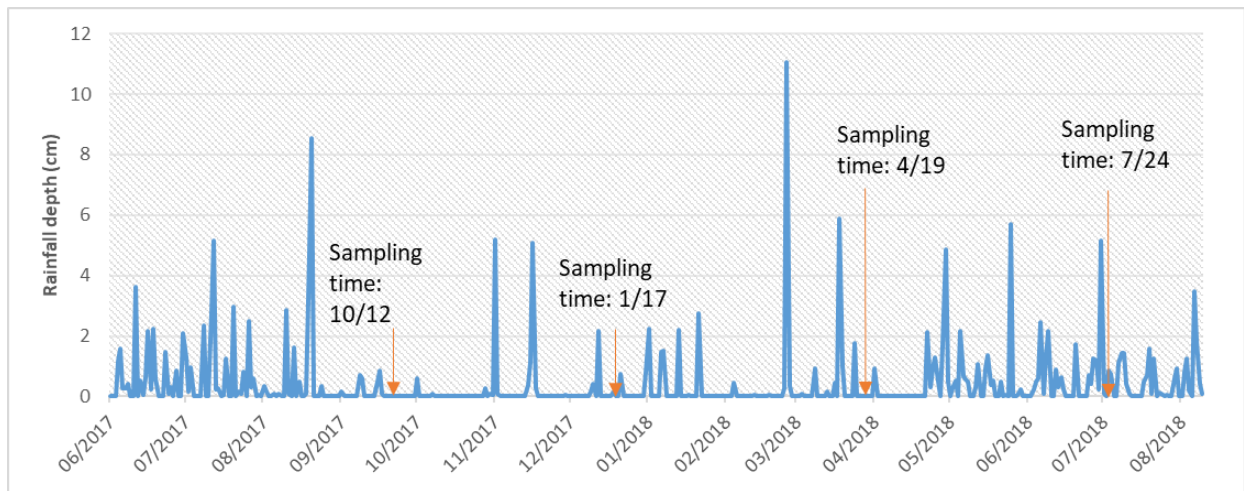


Figure 24. Rainfall depth during the linear ditch operation period and the corresponding sampling time point

Table 11. Pumped groundwater volume readings since the start of the linear ditch study

Date	Cumulative Days	BGW Meter Reading (m³)	BGW Average m³/day	WCW Meter Reading	WCW Average m³/day
6/23/2017	0	0	0	0	0
10/12/2017	112	1,618	14	1,513	14
11/17/2017	148	1,872	13	1,748	12
12/7/2017	168	2,128	13	2,014	12
1/17/2018	209	2,582	12	2,450	12
2/1/2018	224	2,767	12	2,636	12
2/6/2018	229	2,823	12	2,687	12
04/19/18	300	3,789	13	3,574	12

BGW = B&G[®] well – irrigation well for B&G[®] trench

WCW = Woodchip well – irrigation well for woodchip trench

There is a need to delineate the differences of environmental condition between the laboratory columns and the field condition of B&G[®] media and woodchip. The major differences are summarized in Table 12. Unlike the steady controllable environment in laboratory in terms of temperature, inflow conditions, water quality, and hydraulic patterns, the field condition is much more complicated, with highly variable inflow rate and varying levels of water quality during those storm events that may result in less efficient nutrient removal performance due to the disturbed

microbial community. This would affect particularly the biological processes of ammonification, nitrification, and denitrification that are closely related to the transformation of different nitrogen species for ultimate nitrogen removal. Unlike woodchip, the steady infiltration rate and finer micro-environment in BAM would certainly help the final performance.

Table 12. Environmental and Loading condition differences between lab and field operation

Condition	Laboratory	Field
Water source	Groundwater collected from Fanning Spring, stormwater collected from the pond on UCF campus	Groundwater pumping from the solar-powered pump, runoffs from highway stormwater runoff and farmland agricultural discharge
Pollutant loads	Groundwater and stormwater that spiked with nitrate standard solution	Highly variable in terms of pollutants species and concentrations. Especially pesticide and fertilizers that introduced from the farmland
Inflow rate	Consistent of $32.91 - 49.36 \text{ L/m}^2 \cdot h$	Highly variable when storm happens, and relatively variable when the pumps are working due to the availability of solar power with average loading of 115 and 108 L/m^2 per day for B&G [®] and woodchip trench

Condition	Laboratory	Field
Temperature	Consistent of 22 to 23 °C	Highly variable and should be hotter during summer and colder during winter
Water distribution	With consistent flowrate, the water was distributed with a pile of pebble above the media top	Water flows into the linear ditch, it is difficult to evenly distribute as the ditch is not perfectly flat. The infiltration rate would be different along the ditch due to the compaction difference during construction.
Other disturbances	None	Uneven pumping rate along the pipe line system may occur. Animal chewing the pipe line.

4.5. Results and Discussion

4.5.1 Tracer Study

Tracer study is important for understanding the difference of hydraulic pattern for the two media recipes. The tracer study result is shown in Figure 25 for B&G[®] media and woodchip columns. Whereas the calculated tracer HRT is 77.92 and 113.10 minutes for columns 1 and 2 with B&G[®] media, respectively, the value is 40.50 and 41.82 minutes for columns 3 and 4 with woodchip, respectively. It is noticeable that column 2 has longer HRT than column 1, mainly due to the higher TN concentration that may cultivate more compacted/denser biofilm within the

porous space of B&G[®] media. This can be evidenced from the qPCR results in the following sections. However, the woodchip columns showed very similar HRT under different TN influent concentrations, because woodchip has much larger void space such that the biofilm thickness can hardly impose any influence on HRT.

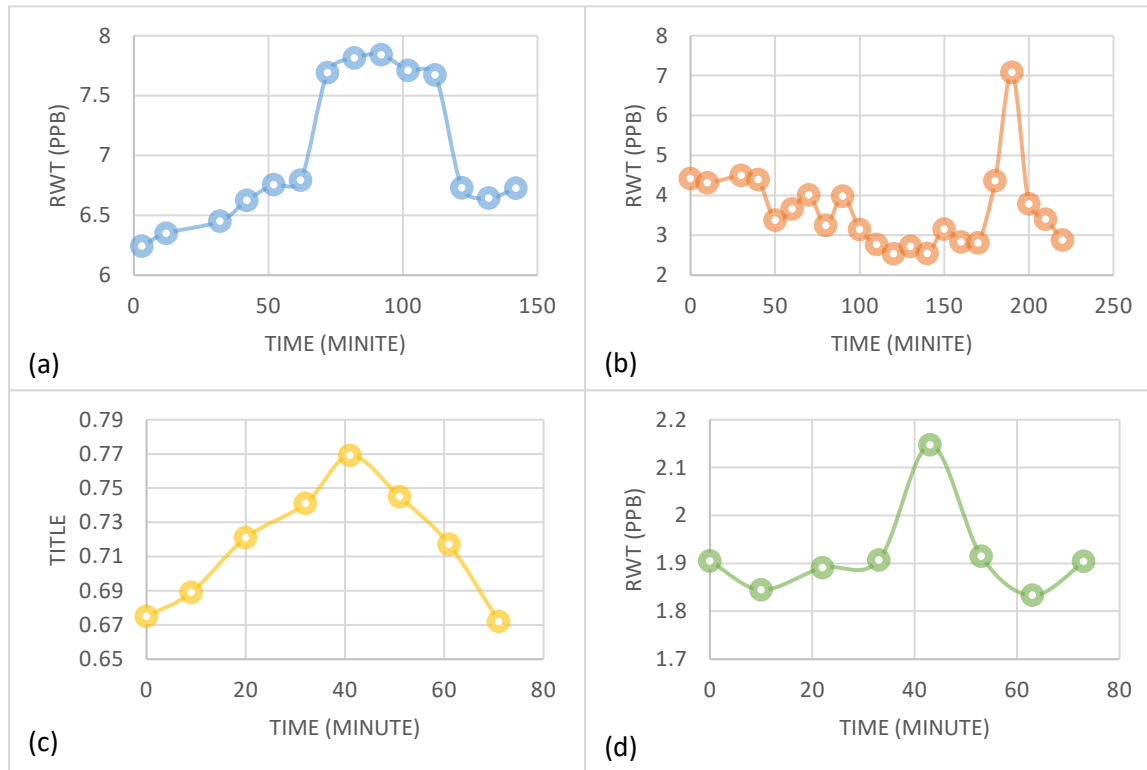


Figure 25. Tracer study results from column 1 to 4 as shown from (a) to (d)

4.5.2 Microbial Ecology and Nutrient Removal

4.5.2.1 Population Dynamics of Microbial Species

By testing the density of target gene copies over different depth of media corresponding to key enzymes in nitrification and denitrification, the microbial ecology of AOB, NOB, denitrifiers and AMX can be revealed for laboratory column study in Figure 26 and for field samples in Figure 27, respectively. Note that the field woodchip decomposed 50% over the operational period of time. This means that the original woodchip of depth 120 cm is now a thinner layer of depth 60

cm, the original woodchip of depth 60 cm is now woodchip of depth 30 cm, and the original woodchip depth of 30 cm is now almost gone with less than depth of 15 cm. This decomposition makes us hard to separate the whole test site as top, middle, and bottom layers. So only media samples in the current woodchip depth of 45 cm and 60 cm was collected and analyzed for microbial ecology analysis as middle and bottom in Figure 27. The comparison between the two figures showed some common patterns. One is that NOB and denitrifiers has more population than AOB and AMX, and that denitrifiers are the dominant one among the four bacteria species in nutrient removal. The reason might be that nitrate/nitrite is one of the major contaminants in water. The other one is that B&G[®] media are able to support more nutrient-related bacteria than woodchip media, probably due to the larger B&G[®] surface area with its more homogeneous and longer HRT. Nevertheless, there are some clear differences between the laboratory and field microbial ecology. One is that more bacteria were found at the top layer in the column study while the population density is more variable in the field that sometimes results in the most abundant bacteria in the middle or even the bottom layers. The second difference is that the overall bacteria population in the laboratory study is much larger than that in the field. The main reason is due to the steadier environment in the laboratory settings (i.e., hydraulic condition, nutrient concentration, temperature, etc.) that are beneficial for bacteria to adapt and thrive. But the uneven water distribution or preferential flow in the field may form very different micro-environment that triggers bacteria growth in various depth randomly possible.

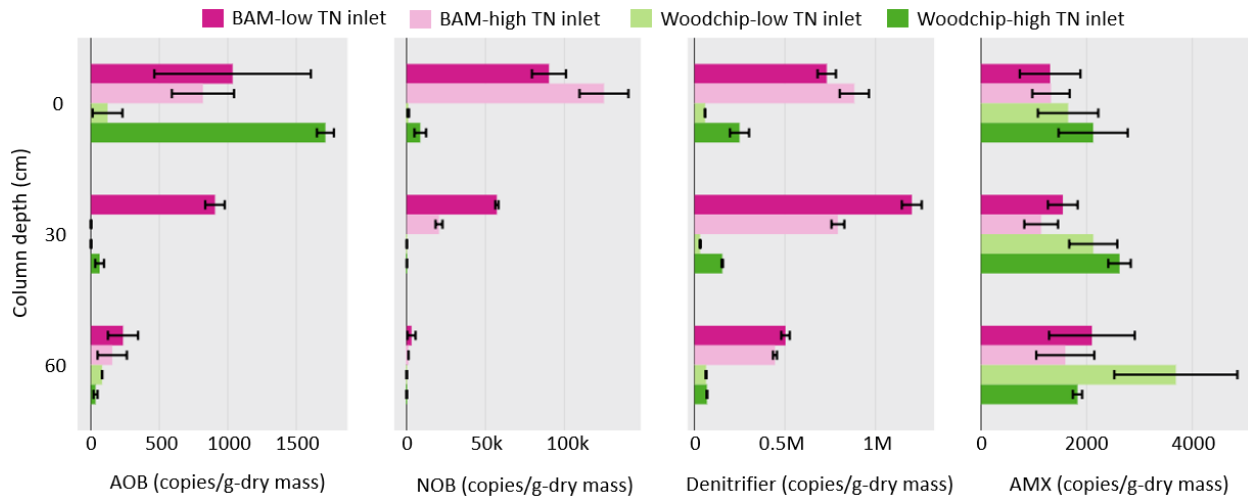


Figure 26. Gene copy density of AOB, NOB, denitrifiers and AMX at different depth under low and high TN influent condition in BAM and woodchip columns, respectively

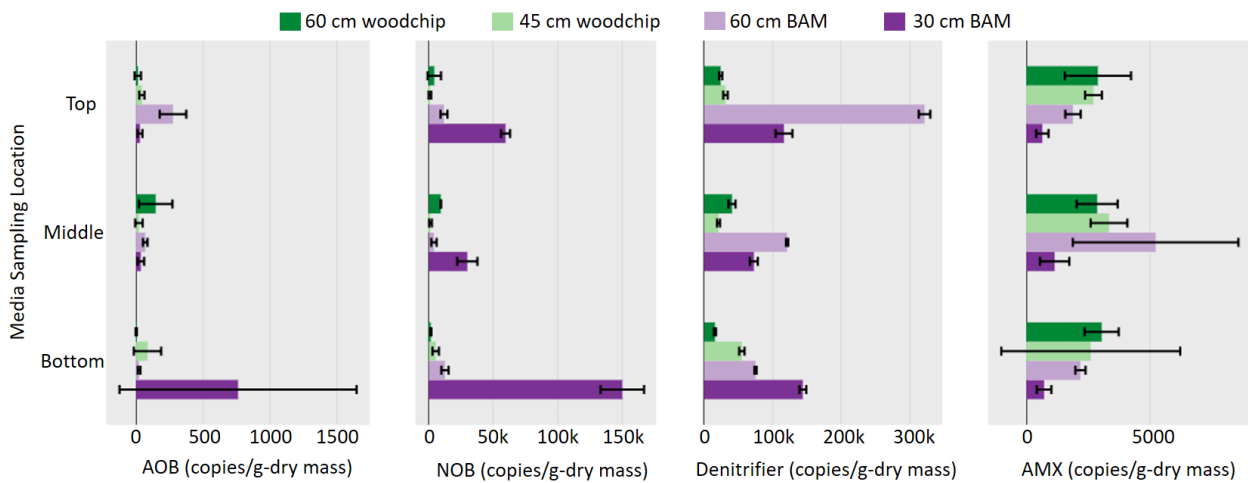


Figure 27. Gene copy density of AOB, NOB, denitrifiers and AMX at the appropriate depth of each BAM and woodchip section in the field after operation

4.5.2.2 Ammonification and Nitrification

After high molecular organics degrade into low molecular organics by heterotrophic bacteria, such as fermentative bacteria, the low molecular organics can be feed to next degradation step known as ammonification. It requires the existence of organic nitrogen and enough oxygen for bacteria to do the job. Ammonification is part of the nitrogen cycle that converts the organic nitrogen into ammonium followed up by the nitrification and denitrification processes. The nitrification process consumes ammonia and generates nitrite and nitrate, which are also biological

reactions that requires oxygen. The two biological reactions can happen in parallel as long as the aerobic environment is suitable for corresponding bacteria. Since the linear ditch is also designed to treat the discharge from a farmland with highly concentrated organic matters, it is expected to observe the increase of ammonia, ammonium or nitrite and nitrate in effluent water sample as the result of ammonification and nitrification. The column study results of ammonia removal are shown in Figure 28 and Figure 29 for the low (inlet is ~5.9 mg/L in groundwater and 2.0 mg/L in stormwater) and high (inlet is ~8.2 mg/L in groundwater and 5.5 mg/L in stormwater) total nitrogen (TN) scenarios. In low TN scenario, the ammonia removal in B&G[®] media and woodchip are 7% and 79% for groundwater treatment, -9% and 98% for stormwater treatment. In high TN scenario, the ammonia removal in B&G[®] media and woodchip are 4% and 91 % for groundwater treatment, and 14% and 96% for stormwater treatment. Note that B&G[®] media always has a rebound of ammonia concentration from the stormwater treatment section, which is the proof that ammonification always happens at the top section of the B&G[®] media followed with a nitrification process to lower down the ammonia concentration in the latter column section. This also indicates that the groundwater collected from the Fanning Spring may not contain as much organic nitrogen as the stormwater. However, for the case of either groundwater or stormwater treatment, ammonia removal of B&G[®] media is minor or negligible when compared to that of woodchip. This is probably because of more oxygen is available in woodchip than B&G[®] media that enhanced the nitrification process as well as the steady laboratory environment, with controlled flow rate and nutrient concentrations, cultivated well-adapted biofilm (more bacteria population in the column study as shown in Figure 26 and Figure 27) with different bacteria species distribution.

The ammonia and organic nitrogen removal in the field are shown in Figure 30 for B&G[®] media and woodchip, in which the organic nitrogen concentration was calculated by subtracting the ammonia concentration from the total Kjeldahl nitrogen (TKN) concentration. It seems that almost no organic nitrogen component was found in the influent groundwater samples collected from the pumping well location for B&G[®] media and woodchip section, which is consistent with the laboratory results as the groundwater used in our column study was collected from Fanning Springs. In other words, almost all organic nitrogen was introduced from either the road stormwater runoff or the farmland agricultural discharge, or possibly from the wind blow organic particles. For B&G[®] media, the highest organic nitrogen concentration (2.38 mg/L) was found at the middle lysimeter of 30 cm (1 ft) depth section. After that the organic nitrogen concentration decreases rapidly and normally below 0.5 mg/L from the depth of 30 to 60 cm (1 to 2 ft). Because of organic nitrogen intrusion, some ammonia was generated through ammonification at the B&G[®] section with 30 cm (1 ft) depth at the bottom. However, there was only a mild ammonification process with small amount of ammonia generation due to the limitation of available oxygen. The holistic observation of B&G[®] media section in the field is consistent with its performance in the laboratory column study. However, the woodchip performance is entirely different in the field. There is an enormous increase of ammonia concentration up to 9.1 mg/L at the middle lysimeter of 60 cm (2 ft) depth section, and the rest ranges from 0.6 to 3.6 mg/L, which is significantly higher than B&G[®] media section. This is because particulate organic nitrogen (PON) with high molecular weight organics can easily be transferred through woodchip than B&G[®] media and more likely ended up at the bottom of the media and triggers intensive ammonia generation through ammonification, because woodchip is able to allow more oxygen in the porous area. Moreover, nitrification is also insignificant in woodchip, as such a high ammonia concentration condition

triggers almost none nitrate or nitrite. Again, because the highly variable nutrient concentration and stormwater runoff volume, it is hard to form a steady and optimized biofilm for AOB and NOB which are bacteria that tend to utilize oxygen at the biofilm surface (Figure 27). Another possible organic source is from slow releasing fertilizer, which is mostly urea that can be converted into ammonia via ammonification.

The significant conversion from organic nitrogen to ammonia in woodchip field sections shows complete opposite trend when compared to the laboratory stormwater treatment results in woodchip columns. There are two reasons to explain this phenomenon; one is that the stormwater used in our column study is different from the actual runoff in the field. Because of the farmland, the organic nitrogen concentration is expected to be much higher due to the presence of animal waste and fertilizer leakage. Besides, significant number of plants were found in the field, which is a potential organic nitrogen source as well. All those leaked organics support more heterotrophic bacteria to decompose them and resulted in large amount of ammonia generation. The other reason is related to the microbial community for nitrification. As explained in section 6.2.1, the microbial community in the field is much smaller and unstable when compared to that in our column study. This is most likely due to multiple highly variable environmental factors and flow rates (mentioned in section 4), in addition to the higher concentration of organic nitrogen found in the field. The woodchip in the field have a small amount of AOB and NOB to deal with the highly concentrated ammonia, leading to the leakage of ammonia in high concentration.

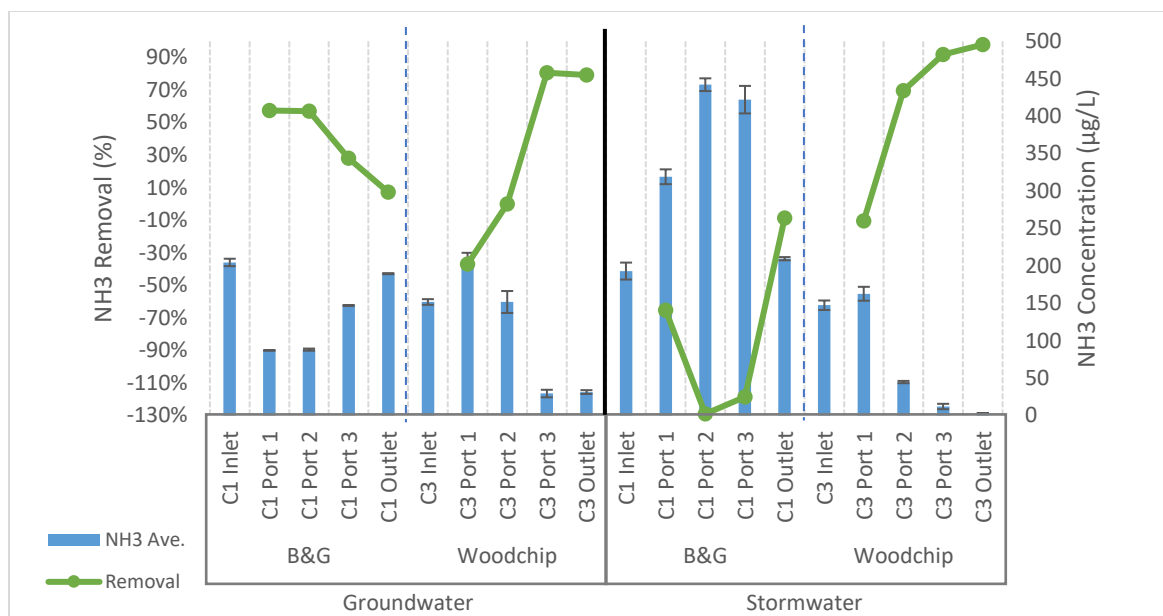


Figure 28. Ammonia concentration and removal for woodchip and B&G® media under low TN inlet scenarios for treating groundwater and stormwater in the columns (C1 = column 1, it applies to C2, C3, and C4)

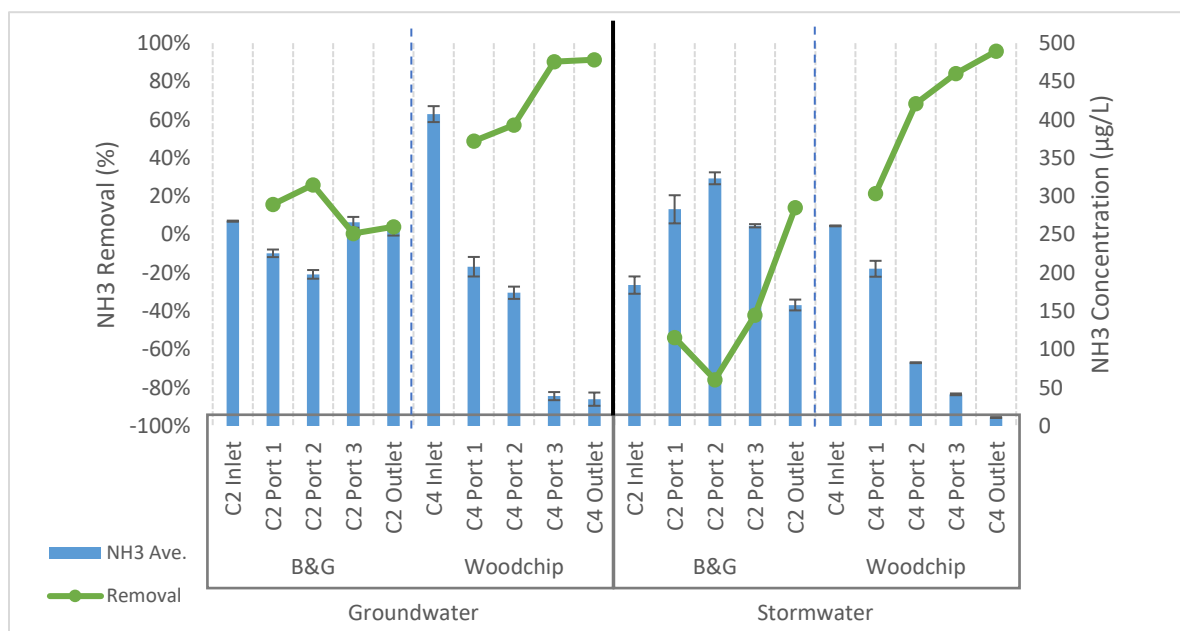


Figure 29. Ammonia concentration and removal for woodchip and B&G® media under high TN inlet scenarios for treating groundwater and stormwater in the columns

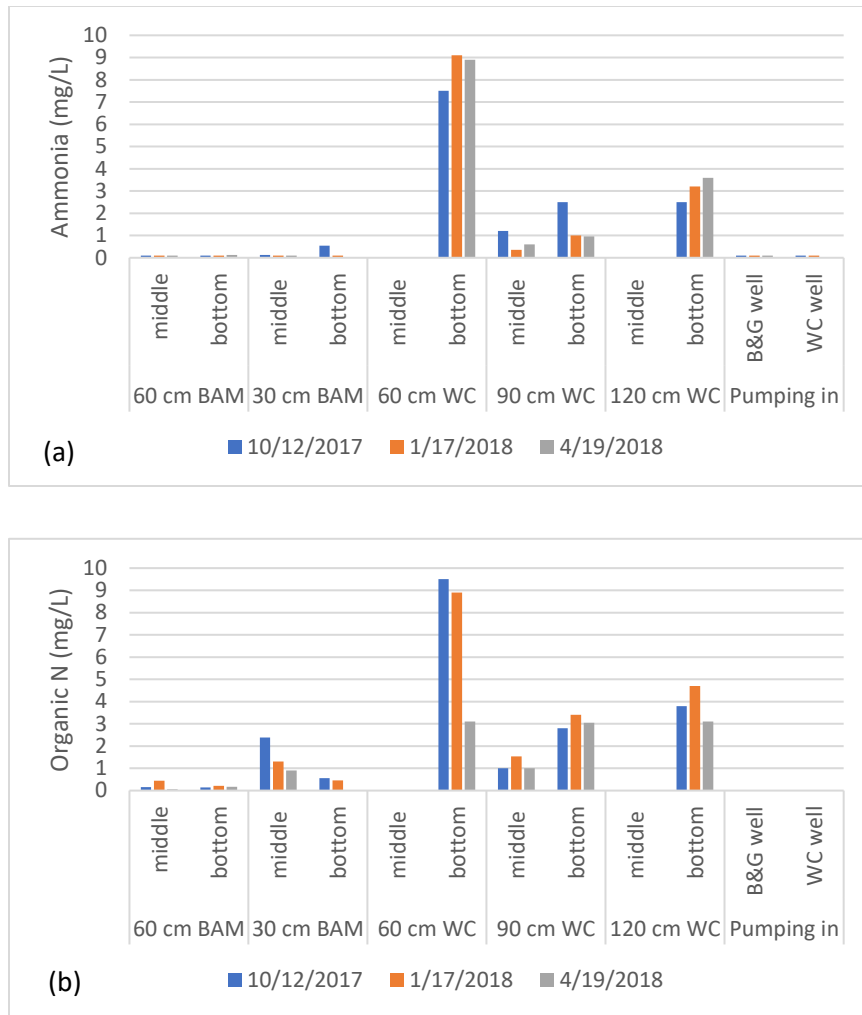


Figure 30. Field nutrient removal of (a) ammonia and (b) organic nitrogen (note: no samples can be collected from the middle lysimeter of 0.6 m (2 ft) and 1.2 m (4 ft) woodchip sections) in the columns

4.5.2.3 Denitrification

Denitrification is the crucial step to convert nitrate/nitrite (known as NO_x) into nitrogen gas as the last step of nitrogen cycle on earth which is performed by denitrifiers that only activate under anaerobic condition. The column study results of NO_x concentration and removals are shown in Figure 31 and Figure 32 for the low and high TN scenarios, respectively, while the field results of NO_x concentration are shown in Figure 33. In the column study of the low TN scenario, the NO_x removal of B&G[®] media and woodchip are 52% and 92% for groundwater treatment, while

both 99% for stormwater treatment. In high TN scenario, the NO_x removal of B&G[®] media and woodchip are 45% and 67% for groundwater treatment, respectively, and 73% and 93% for stormwater treatment, respectively. Both recipes achieved promising NO_x removal but woodchip outperformed B&G[®] media when treating the groundwater with low carbon concentration (~ 4 mg/L COD). Even B&G[®] media could maintain a suitable anaerobic environment for denitrifiers, the woodchip could provide sufficient carbon source as electron donor in the denitrification reaction. But the carbon scarcity is not a problem in stormwater treatment because there is enough carbon source in stormwater runoff (~ 15 to 20 mg/L COD). Moreover, the denitrification in woodchip happens within the bottom layer of the biofilm attached to the wood surface, which is also the best location for retrieving carbon source and maintaining anaerobic condition. Also, the inlet concentration has a significant impact on bacteria population densities in woodchip (Figure 26). Overall, it seems that nutrient availability is a more important factor for bacteria growth in woodchip.

In the field, B&G[®] media show similar trend as it is in the column study. Significant NO_x removal of 70-99% occurred from the bottom of each B&G[®] section. This is mainly because B&G[®] media can maintain a suitable anaerobic condition within the porous space when B&G[®] media is wet. It is also the reason that B&G[®] media perform extremely well in removing organic nitrogen since the PON was filtered at the B&G[®] media surface. For the woodchip in the field, it shows promising NO_x removal of over 97% that is very similar to the result from the column study. The denitrification process going at the bottom of the biofilm is relatively intensive with the support of ample carbon source from woodchip. The most important reason is that denitrifiers have been well cultivated in woodchip as well as B&G[®] media because both media are mainly prepared

for treating groundwater when there is no storm event and NO_x are available constantly to denitrifiers. With a relatively steady and continuous groundwater influent, denitrifiers could gain comparative advantages and stay reactive for nitrate/nitrite removal.

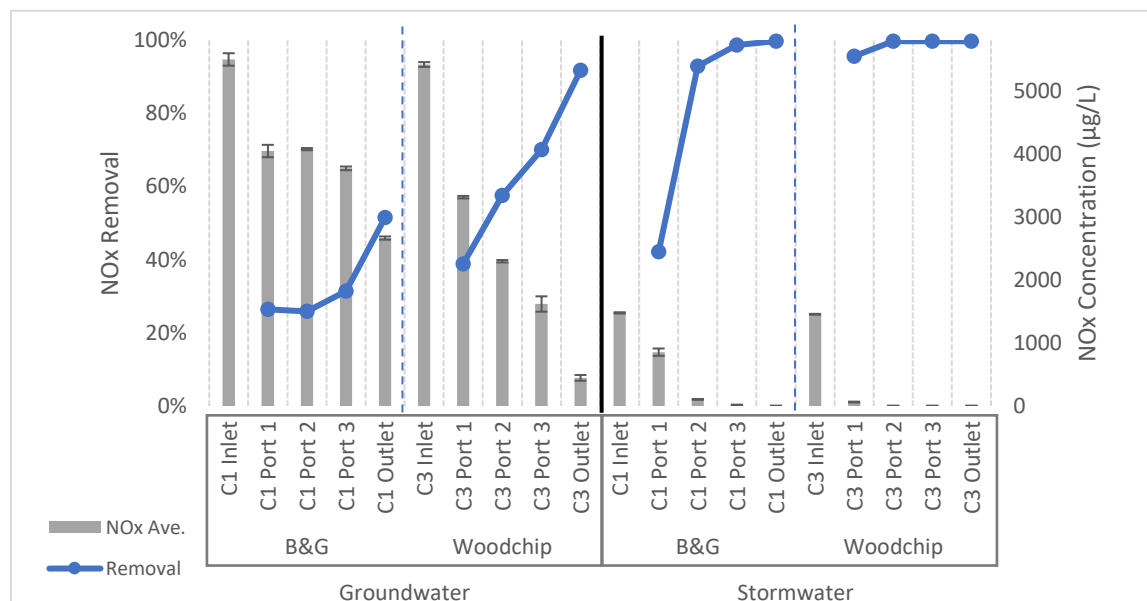


Figure 31. Nitrate and nitrite (NO_x) concentration and removal for woodchip and B&G media under low TN inlet scenarios for treating groundwater and stormwater in the field

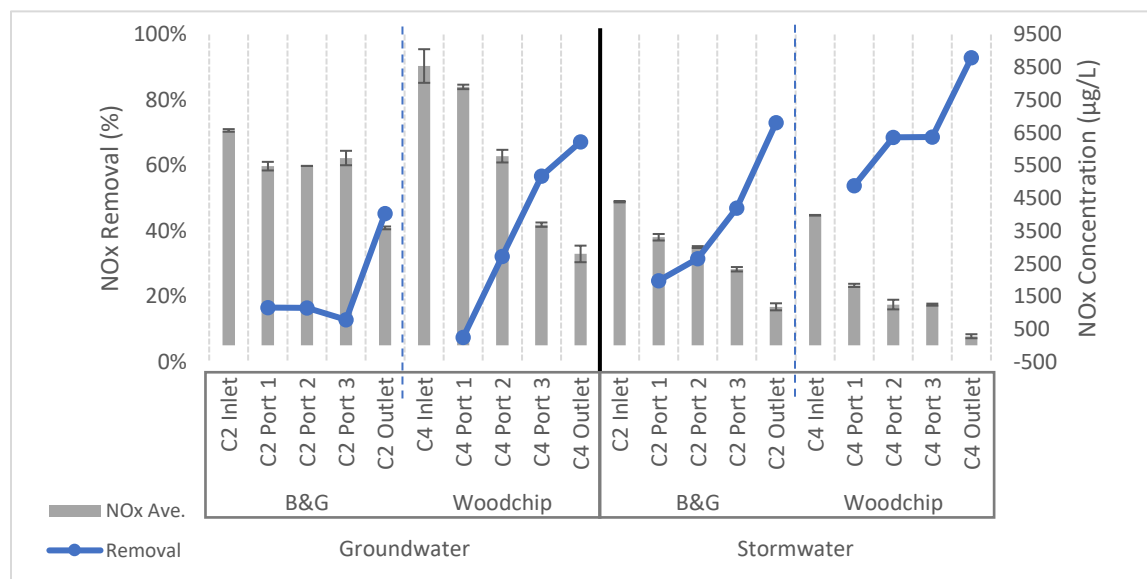


Figure 32. Nitrate and nitrite (NO_x) concentration and removal for woodchip and B&G[®] media under high TN inlet scenarios for treating groundwater and stormwater in the field

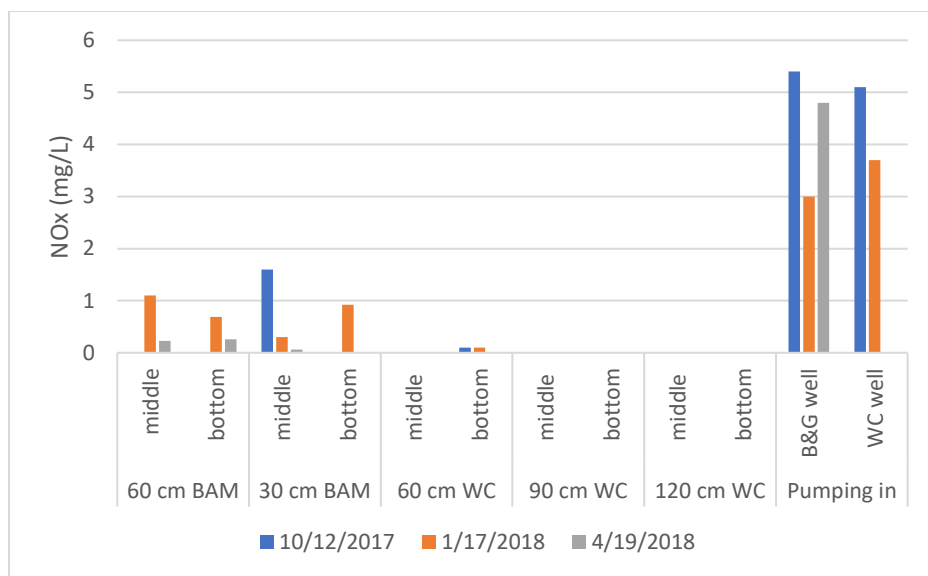


Figure 33. NOx concentration in the field lysimeters in the field

4.5.2.4 TN Removal

The TN concentration and removal from the laboratory columns of B&G[®] media and woodchip are shown in Figure 34 and Figure 35 for low and high TN scenarios, respectively. In low TN scenario, the TN removal of B&G[®] media and woodchip are 50% and 85% for groundwater treatment, while both are 78% for stormwater treatment. In high TN scenario, the TN removal of B&G[®] media and woodchip are 43% and 62% for groundwater treatment, respectively, while 70% and 80% for stormwater treatment, respectively. B&G[®] media and woodchip tend to show equivalent TN removal for stormwater treatment; however, the woodchip has a better performance than B&G[®] media when treating groundwater, because woodchip can provide carbon sources as electron donors in carbon deficient groundwater.

The field TN concentrations were shown in Figure 36, and the TN removal of B&G[®] media is 52-80% and 68-95% for 30 cm (1 ft) and 60 cm (2 ft) depth sections, respectively. These values are very close or sometimes even better than the laboratory results. However, the woodchip in the field performs entirely different from the one that has been observed in the laboratory. It has almost

no positive removal in the field except 17% TN removal from the bottom lysimeter of the 90 cm (3 ft) depth section on 4/19/2018. The TN concentration in the effluent increased as high as over 3 times of the influent value in the worst case from the bottom of 60 cm (2 ft) depth section on 1/17/2018. As mentioned in previous section, the major reason why the B&G[®] media perform better than woodchip is that B&G[®] media could filter the sediments that also carry highly contaminated organic matters through the runoffs. Woodchip, on the other hand, has no such capability due to its large void space. Hence a large quantity of sediments flowed through the woodchip and ended up in the lysimeter throughout different depths without proper treatment. Another reason why B&G[®] media perform better than woodchip is that the B&G[®] media have a much higher tolerance level for the fluctuation of the inflow rate. No matter how fast the stormwater runoff get into the linear ditch, the infiltration rate through the B&G[®] media would not change too much because its HRT is limited by the small porous size. On the other hand, when it was dry, the B&G[®] media are also able to maintain necessary moisture for bacteria survival. So, B&G[®] media would allow enough contact time for the bacteria to do their job and cultivated way more bacteria population than woodchip. However, it would be significantly different for woodchip when treating stormwater runoffs because storm's intensity is highly variable as it showed in Figure 24. For stormwater, woodchip might achieve acceptable TN removals from small storm events as the inflow rate is small, and there is enough contact time between the water flows and woodchip. But the TN removal would drop dramatically when the stormwater runoff is big enough that large quantity of water just flows through the woodchip with negligible contact time that minimizes the treatment effectiveness.

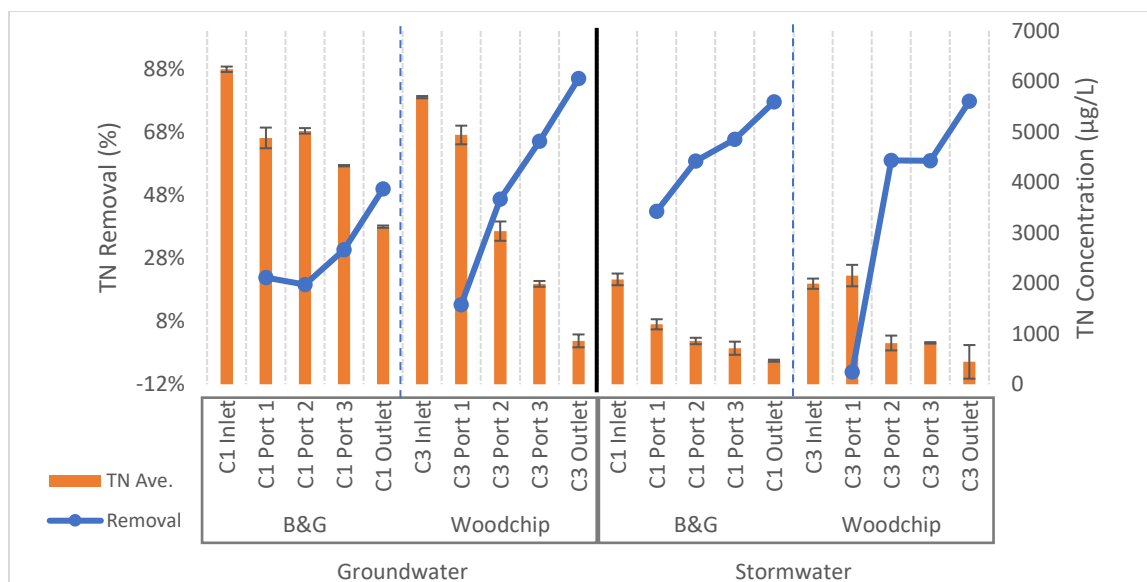


Figure 34. TN concentration and removal for woodchip and B&G[®] media under low TN inlet scenarios for treating groundwater and stormwater in the field

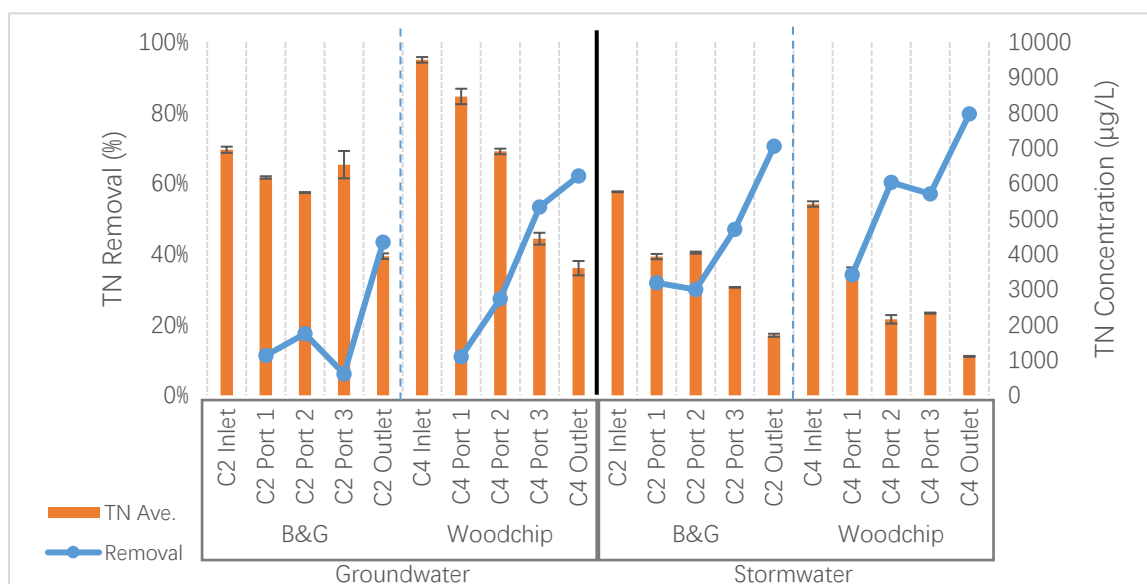


Figure 35. TN concentration and removal for woodchip and B&G[®] media under high TN inlet scenarios for treating groundwater and stormwater in the field

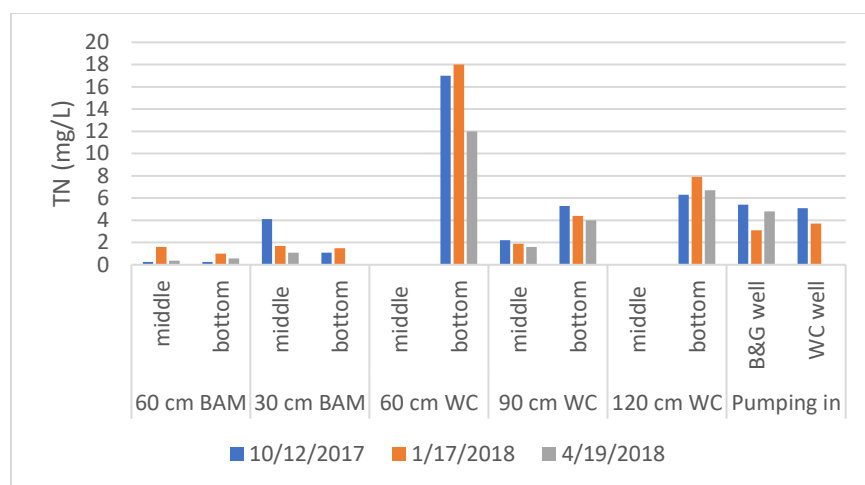


Figure 36. TN concentration from each lysimeter and influent (pumping well) for (a) B&G media and (b) woodchip

4.6 Summary

Two recipes of green sorption media including the B&G[®] media and woodchip were evaluated in the laboratory column study and the field test for the co-treatment of stormwater and groundwater. The laboratory results indicate that both recipes perform effectively for TN removal, in which woodchip showed better nitrification effects due to more oxygen available in the void space when compared to B&G[®] media. Both B&G[®] and woodchip media perform good denitrification reaction since both are able to sustain anaerobic environment in the biofilm. B&G[®] media eliminate the oxygen by holding moisture content within the small porous holes while the deeper layer of biofilm on the woodchip surface has low DO value. But B&G[®] media tend to hold denser bacteria population than woodchip by providing more surface area for biofilm development, and the constant loading in the laboratory column test condition is more beneficial for bacteria growth when compared to the field condition. Moreover, B&G[®] media performs even better in the field with similar conditions as they have in the column study. But the woodchip performs entirely different in the field as ammonification generates significant amount of ammonia from organic

nitrogen in the woodchip without sufficient nitrification to push the ammonia into the next step of N cycle. The larger pore size in woodchip fails to screen out the sediment from the runoff as well as keeping enough contact time between the water flows causing a diminishing treatment effectiveness. However, denitrification is relatively active in both B&G[®] media and woodchip in the field application, because the constant pumped groundwater flows have high concentration of nitrate. The nitrate is the main energy/food source for denitrifiers. In general, B&G[®] media are more appropriate for the co-treatment of stormwater and groundwater in space limited BMP under complicated natural environment and it has no decay issues as the woodchip would need to be replaced in a few years. Also, the woodchip is limited because they are suspect to attaining traffic bearing capacity while B&G[®] media is traffic bearing along the road side.

CHAPTER 5: SUSTAINABILITY EVALUATION OF NUTRIENT REMOVAL AND RESOURCES RECOVERY BY USING IRON FILINGS-BASED GREEN ENVIRONMENTAL MEDIA (IFGEM) FOR SOIL AMENDMENT⁴

5.1 Introduction

Stormwater runoff, wastewater effluent, and agricultural discharge are some of the major issues which cause ecosystem degradation such as eutrophication and loss of biodiversity through the delivery of excessive amounts of nutrients into receiving water bodies ^{19, 164-166}. For two decades, multiple types of green sorption media have been developed and applied for wastewater, groundwater, or stormwater treatment ^{7, 44, 45, 66, 80, 95, 167-169}, as summarized in Appendix C. Recently, green sorption media have been developed for wastewater and groundwater treatment ^{20, 41, 69, 80, 95, 170-172}.

However, as enhanced regulatory standards by the Environmental Protection Agency ¹⁷³ such as the Numeric Nutrient Criteria have appeared, the current green sorption media may be inadequate for achieving the desired managerial goals given the increased nutrient concentrations in the stormwater runoff and wastewater effluent ^{24, 99, 101, 174, 175}. It is even more difficult when dealing with agricultural discharge since the overdosed fertilizer eventually ended up in large quantity discharges and jeopardized surrounding aquatic ecosystems with relatively higher nutrient concentrations ¹⁷⁶⁻¹⁷⁸. On the other hand, as the mineral resource for making fertilizers will be quickly used up, traditional treatment processes for nutrient removal alone are not enough. This acute need has led to the development of more efficient green sorption media for nutrient removal

⁴ Authors: Dan Wen, Ni-Bin Chang, and Martin P. Wanielista; Pending publication: submitted to *Journal of Environmental Management*, anticipated publish date: Dec 2018

and recovery simultaneously, which is key to the success of sustainable development in the future^{179, 180}. Therefore, alternative technologies are of interest in seeking for potential solutions for nutrient removal and recovery/reuse in one effort when dealing with both point and nonpoint source pollution.

Nanoscale zero valent iron (NZVI) particles have been used for the remediation of a wide variety of contaminants, and nutrient removal using NZVI might have some public health concern^{81-83, 181-185}. Because of its high surface area to volume ratio, NZVI shows intensive reactivity for nitrate reduction (as electron donor) and phosphate adsorption. However, NZVI can hardly be applied in Best Management Practices (BMPs) for treating stormwater runoff, wastewater effluent, and agricultural discharge because the iron particles can be easily washed away due to its nanoscale sizes. In addition, the reaction would be so intensive that all the NZVI may lose its reactivity in a short period of time. In addition, the production of NZVI is relatively costly for large-scale BMP applications. Thus, NZVI is limited to applications in treating point source pollution with highly concentrated contaminants.

Iron filing is very different from NZVI in physiochemical properties due to the particle size difference. Chemically speaking, iron filing provides electrons in a slow-releasing process that is expected to show reasonable reaction rate and longer life expectancy when compared to NZVI. The formation of ferrous and ferric ion in the process of nitrate reduction is of great help in phosphorus precipitation due to the production of iron oxides known as good phosphorus adsorbent. Economically speaking, as a kind of recyclable from industry, iron filing is much more affordable for large-scale BMP implementation.

The use of iron filing to mix with green sorption media for nutrient recovery is of great interest. Yet, given the inclusion of iron filings, the unknown interactions among media components may trigger some potential for both nutrient removal and recovery. In this study, the mixture of iron filings with green sorption media is called Iron-Filing based Green Environmental Media (IFGEM). During the experimental design, influent concentrations of the adsorbent were prepared by *spiking distilled water* from a nutrient stock solution to mimic stormwater for testing at the laboratory scale. The following questions can be answered by this study: 1) how would the iron filings interact with different green sorption media components in the nitrate and phosphorus removal processes? 2) how would the different initial nutrient condition of the stormwater inflow influence the reaction kinetics and removal efficiencies between the multiple nutrient species using IFGEM? 3) will ammonia be generated in the treatment process due to the reduction effect provided by iron filings, and if so how would ammonia affect the performance of IFGEM? 4) what would the differences be in terms of microstructure and reaction products after the treatment? And 5) will the holistic observations in this study be able to confirm its potential for nutrient recovery? With a preliminary understanding, we hypothesize that: 1) Nitrate reduction would be significant due to the inclusion of iron filing as an electron donor; 2) Ammonia may be produced as a byproduct through nitrate reduction; 3) Phosphorus removal would be enhanced due to the generation of ferrous, ferric ion, and iron oxides; 4) nutrient removal is sensitive to the influent nutrient concentrations; 5) IFGEMs show the potential of cumulatively absorbing and recovering nutrients from stormwater inflows.

This study focuses on deepening the understanding of the fundamental characteristics of IFGEM, such as physical properties, reduction/absorption isotherm, reaction kinetics under room

temperature, and the morphological change in microstructure of IFGEM after treatment leading to the exploration of the nutrient recovery potential. The objectives of this study are to: 1) conduct an isotherm study for IFGEM to gain understanding of its absorption characteristics for nitrate and phosphorus separately under neutral pH and room temperature conditions; 2) carry out a column study to test the nutrient removal efficiencies under various influent concentrations for possible stormwater runoff, wastewater effluent, and agricultural discharge treatment in the future; 3) assess the holistic performance of IFGEMs with the aid of an imaging analysis technique for discussing its nutrient reuse/recovery potential.

5.2 Materials and Methods

The study plan for this paper is shown in Figure 37, and the flowchart is comprised of three main parts of this study, including isotherm study, material characterization, and column study. The material characterization section reveals the physical properties such as microstructure images, particle size distribution, density, surface area, and so on, which are crucial for understanding the hydraulic patterns to support other studies leading to answer question 4. In the isotherm study, part of questions 1 and 3 can be answered, which are closely related to the absorption capacity and working mechanism in a batch mode. Furthermore, in column study, questions 1, 2 and 3 can be answered collectively. By varying the influent nutrient concentrations, it mimics the water quality in real-world storm events from which the corresponding kinetic information may be retrieved as an important reference basis for engineering design of the corresponding BMPs. Finally, question 5 may be answered through the holistic evaluation of the performance of IFGEM.

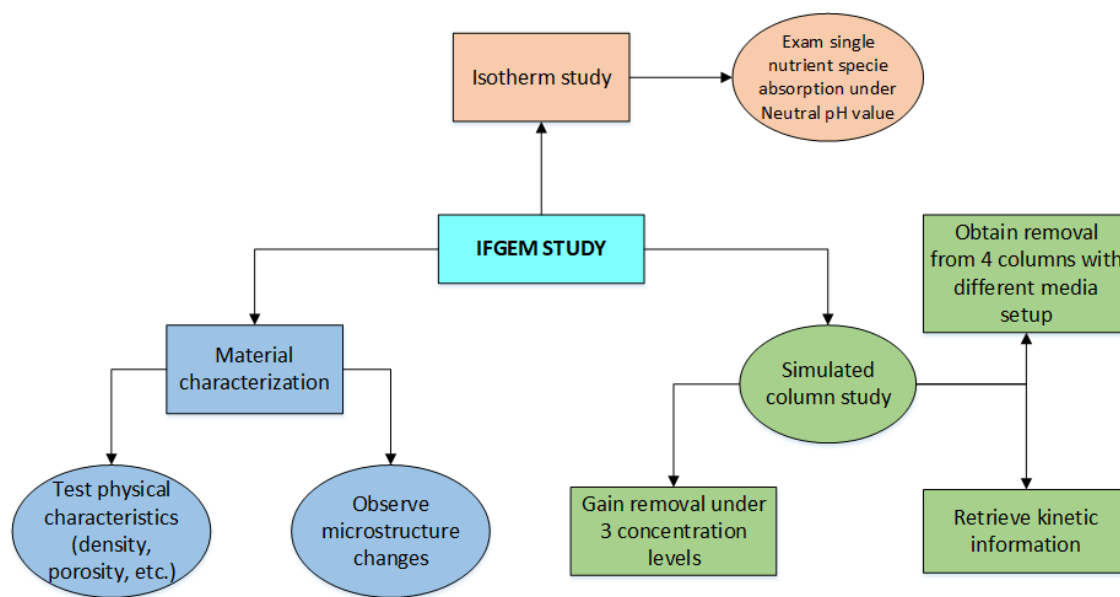


Figure 37. The flowchart for the current part of IFGEM study

5.2.1 Material Characterization

Material characterization is important not only for investigating question 4 but also for the understanding of basic physical properties that closely relate to hydraulic characteristics during treatment processes. Two IFGEM recipes and one green sorption media recipe were selected in this study; media mix 1 is named IFGEM-1, and it consists of 96.2% fine sand 3.8% grinded iron filings (by volume). Media mix 2 is named IFGEM-2, and it consists of 80% sand, 10% tire crumb, 5% pure clay, and 5% grinded iron filings (by volume). Media mix 3 is called bio-sorption activated media (BAM), and it is composed of 85% poorly graded sand, 10% tire crumb, and 5% clay (by volume), in which the tire crumb used are recyclable with no metal contents and mined clay has no less than 99% clay content. As the control media (media #4) in the experiment, natural soil was collected from SR 35 Basin 2 in Silver Springs watershed, located in Ocala, Florida.

ASTM Standard Practices are international standards that have been widely accepted and used for many materials, products, and systems. They were used to determine the particle size

distribution, specific gravity, and Brunauer–Emmett–Teller (BET) surface area. In this study, ASTM D 422 was adopted for particle size distribution, ASTM D 854 was applied for specific gravity and micrometrics, and ASAP 2020 was applied for BET surface area. Media mixes 1 to 4 were tested with the methods mentioned above by a certified laboratory (EMSL, Inc.). Olympus LEXT OLS 3000 Confocal Scanning Microscope was used to characterize the microstructure changes of media mixes before and after the nutrient adsorption. This was conducted at the Advanced Materials Processing and Analysis Center (AMPAC) at the University of Central Florida (UCF). The plane resolution could be as high as 0.12 μm , with simultaneous 3D and “true color” image acquisition. The media porosity and infiltration rate were tested in a laboratory at UCF, and the porosity was tested by pouring a known volume of water to a certain volume of media until the media was fully saturated. The infiltration rate was tested through constant head method; the media depth, density and cross-section area were documented, as well as the time consumed for a certain volume of water to flow through the media.

5.2.2 Isotherm and Kinetic Study

5.2.2.1 Isotherm Study on Individual Nutrient Adsorption

In order to understand the thermodynamic characteristics of IFGEM in terms of nutrient adsorption under neutral pH and address questions 1 and 3, the adsorption isotherm experiment was conducted separately for nitrate and phosphorus in IFGEM-1 and IFGEM-2 with deionized water. Then 30 – 120 g media mass was prepared in five 500 mL flasks with a 300 mL solution of 1.0 mg/L as total nitrate or phosphorus, and the experiment was carried out under room temperature on the rotary shaker with 250 rpm for 1 hour. Then the water sample from each flask was filtered through 0.45- μm membrane filters before the isotherm analysis. The parameters being

analyzed were nitrate and ammonia for the nitrate isotherm experiment, and total phosphorus for the phosphorus isotherm experiment. The Freundlich and Langmuir isotherm equations were adopted to analyze the data. The Freundlich isotherm was obtained by plotting $\log q$ versus $\log C$, and the Langmuir isotherm by plotting $1/q$ versus $1/C$. The following two equations were applied in this study.

Freundlich isotherm equation:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (11)$$

Langmuir isotherm equation:

$$\frac{1}{q_e} = \left(\frac{1}{K_L q_m} \right) \frac{1}{C_e} + \frac{1}{q_m} \quad (12)$$

where C_e is the aqueous concentration of adsorbate (mg/L), q_e is the sorbed concentration (mass of adsorbed adsorbate/mass adsorbent), q_m is the maximum capacity of adsorbent for adsorbate (maximum mass of adsorbed adsorbate/mass adsorbent), C is the aqueous concentration of adsorbent (mass/volume), K_L is the Langmuir equilibrium constant, K_F is a constant indicative of the relative adsorption capacity of the adsorbent ($\text{mg}^{1-(1/n)} \text{L}^{1/n} \text{g}^{-1}$), and n is a constant indicative of the intensity of the adsorption.

5.2.2.2 Kinetic Study

Performance in terms of filtration kinetics refers to the efficiency of the process and the concentration of the resulting effluent, which is crucial for the field design and BMP applications leading to answer question 2. Kinetic study for nitrate reduction and phosphorus adsorption in IFGEM was conducted in a continuous mode using a series of column tests. It assumes that the columns are in a steady state in terms of hydraulic condition and nutrient concentration from each

section after running them by 3 hours before sampling. The reaction time is recorded as hydraulic retention time (HRT) from each column section. Equation 13 is a general version of the zero, first, second, or higher order rate equations, which was applied to the kinetic study for determining the best fit reaction orders where C is the concentration of nitrate/phosphorus in solution, n is the reaction order, and k is the reaction constant.

$$\frac{dc}{dt} = k[C]^n \quad (13)$$

In zero order reaction, the reaction rate is independent of the concentration of reactants. The reaction speed will not change when the reactants' concentration is different. The first-order reaction is a reaction that proceeds at a rate that depends linearly on only one reactant concentration. That is, when the key reactant has a higher concentration, the reaction speed is faster than the lower concentrated case. Second order reaction proceeds at a rate that depends non-linearly on the power of 2 of the key reactant's concentration.

5.2.3 Design and Setup of Column Tests

Based on the goals of this study, a series of column experiments were designed to simulate the possible field conditions with a down-flow strategy which is critical for addressing a suite of absorption, adsorption, ion exchange, precipitation and oxidation/reduction reactions between sorption media and nutrients and leads to the answering of questions 1, 2 and 3. Four big columns (named from A to D) were constructed with 10 cm (4 inches) in diameter of PVC pipes, and each big column was divided into three equivalent sections (top, middle, and bottom) with each section 30 cm (1 foot) in length for the convenience of water sampling. As shown in Figure 38, IFGEM-1 was filled into all three sections of column A. In column B, IFGEM-1 was filled into the top

section and BAM was filled into the middle and bottom section. In column C, the natural soil was filled into all three sections. In column D, IFGEM-2 was filled into all three sections. All four big columns from A to D were attached to a wooden board; the outlet from one section is the inlet of the following one, and the joints between sections were wrapped with parafilm to eliminate outside impacts.

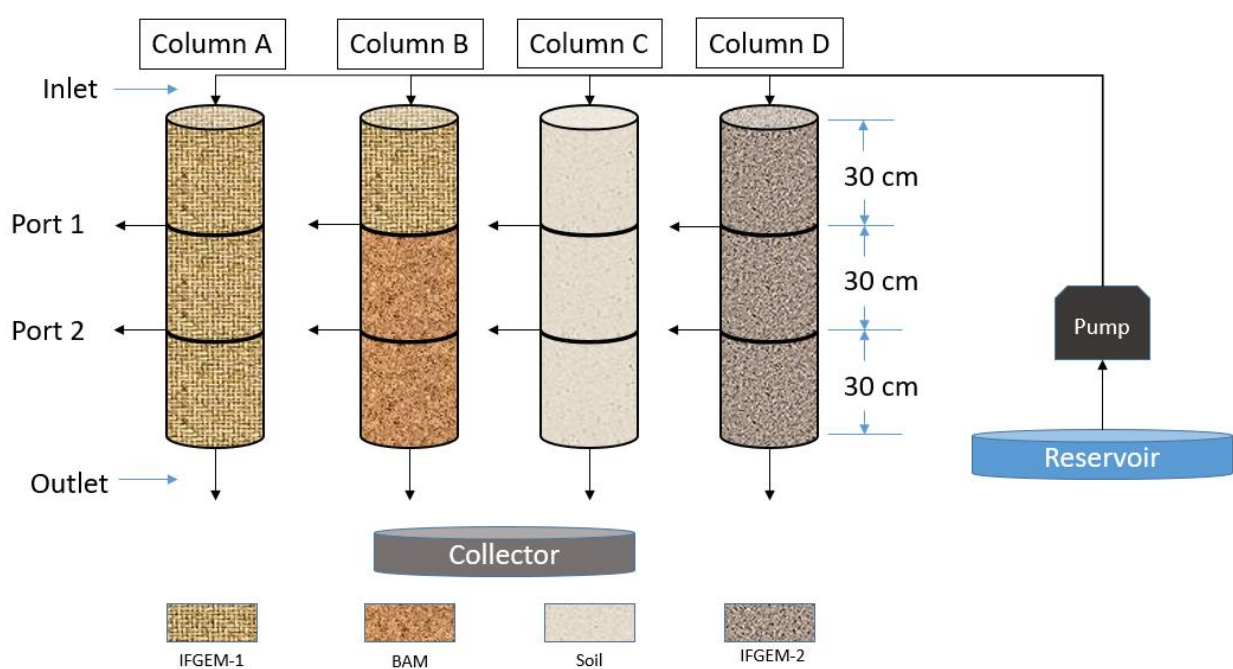


Figure 38. Diagram of column setup

Distilled water was spiked with nitrate and phosphate standard solutions in three concentration levels (nitrate = 0.6, 1.2, 1.8 mg/L; TP = 0.3, 0.5, 0.7 mg/L) to simulate the fluctuation of nutrient concentrations in real stormwater runoff, agricultural discharge, or wastewater effluent from a secondary wastewater treatment plant ⁵⁵. The columns were flushed with distilled water a couple times to wash off possible contaminants before and after running the columns under different influent conditions. It was expected that physiochemical

reduction/absorption would be the main mechanism for nitrate removal instead of biological effects, and the only exception was in column C, which was the control column with natural soil collected from SR35 Basin 2 located in Ocala, Florida. The experiment was conducted at room temperature from 22 to 23°C. A pair of peristaltic pumps were used to pump the influent water from the reservoir with a fixed flow rate of 8 mL/min, which is equivalent to the infiltration rate of 2.33 in/hr that was set up for the current column study in the beginning. With this rate, the columns were never saturated. The HRT and soil moisture from each section were recorded when the flow rate of the effluents could be stabilized after 3 hours' operation. Water samples were collected in triplicate from the reservoir and outlet of each section. The measurements of dissolved oxygen (DO), oxidation reduction potential (ORP), and pH values were conducted right after collection. DO measurement provides information to determine if the treating environment in each column is aerobic or anaerobic. The ORP values provide critical information regarding the existence and intensity of oxidation or reduction reactions in different sections during the column test. The decrement of ORP would indirectly affect the reaction spontaneity dynamically, which in turn could be influenced by changing pH values¹⁸⁶. IFGEM samples were collected before and after the experiment for the morphological comparison under confocal microscope. Nitrate concentrations were analyzed through HACH kit TN830, ammonia concentrations were analyzed through HACH kit TN835, and total phosphorus (TP) concentrations were analyzed with HACH Phosphorus (Total) TNT Reagent Set (summarized in Table 13). All water samples were analyzed within 24 hours after collection.

Table 13. Column study sample quality parameters and methods

Parameter	Method/instrument	Range
pH	Waterproof Double Junction pHTestr® 30	-10.0 to +15.0
Dissolved oxygen	HACH HQ40D - IntelliCAL LDO101 LDO	0.01 - 20 mg/L
ORP	HACH HQ40D - MTC101	± 1200 mV
Soil moisture	EC-5 SMALL SOIL MOISTURE SENSOR	0 - 100%
Nitrates	Method 10206	0.05 - 13.50 mg/L NO ₃ -N
Total phosphorus	DR/800 Method 8190	0.06 - 3.50 mg/L PO ₄
Ammonia	Method 10205	0.015 - 2.00 mg/L NH ₃ -N

Overall, this study involves absorption, adsorption, precipitation, ion exchange, and oxidation/reduction reactions. Absorption describes the assimilation of molecular species throughout the bulk of the solid or liquid, such as phosphorus and nitrate absorption to the IFGEM.

5.2.4 Statistical Analysis

In order to figure out if there are significant differences between overall nutrient removal efficiencies over different columns under various influent conditions, two-way ANOVA analysis was performed with Microsoft Excel packages. This analysis of variance may determine if manipulating the influent concentration and switching to different sorption media can create

significant differences in the nutrient removal. Each ANOVA analysis was considered statistically significant at a confidence interval of 95% ($\alpha = 0.05$).

The comparison was made possible in pairs of two columns each time. There are two independent variables, column number and influent concentration, since different columns have different media in them, with the impact factor of varying influent concentrations rendering nutrient removal via a triplicate analysis. The p-value results associated with the ANOVA analysis may indicate if there is a significant difference in nutrient removal when the columns or the inlet conditions vary, as well as whether or not there is significant interaction between the two variables. The first null hypothesis is H_0 : the means of nutrients removal grouped by the columns are the same; the second null hypothesis H_0 : the means of nutrients removal grouped by the inlet conditions are the same; the third null hypothesis H_0 : there is no interaction between columns and inlet conditions.

5.3 Results

5.3.1 Material Characterization

5.3.1.1 Physical Property

Figure 39 shows the particle size distribution curves of the four media mixtures. Two IFGEMs are closer to each other with smaller particle sizes but IFGEM-2 has finer particles than IFGEM-1, while BAM and natural soil are closer to each other with larger particle sizes than IFGEMs. The physical properties of the four media mixtures are shown in Table 14. BAM has the lowest density of 1.39 g/cm^3 and the density of the soil is 2.36 g/cm^3 , while the density of IFGEM-1 and IFGEM-2 is 2.73 and 2.60 g/cm^3 , respectively. Significant differences exist when we

compare BET surface area. Natural soil shows the highest value of $9.3712 \text{ m}^2/\text{g}$. The next one is IFGEM-2 with a value of $1.3963 \text{ m}^2/\text{g}$. Then BAM and IFGEM-1 follow behind with values of 0.7059 and $0.3142 \text{ m}^2/\text{g}$, respectively. In porosity comparison, there are two groups. Whereas IFGEM-1 and IFGEM-2 show similar results at 36.16% and 37.31% , BAM and natural soil exhibit almost the same results at 40.10% and 40.43% . Another obvious difference across the four media mixes comes with the infiltration rate. Natural soil shows the lowest value of 0.003 cm/s , while the values for IFGEM-1, BAM, and IFGEM-2 are 0.028 , 0.026 , and 0.017 cm/s , respectively.

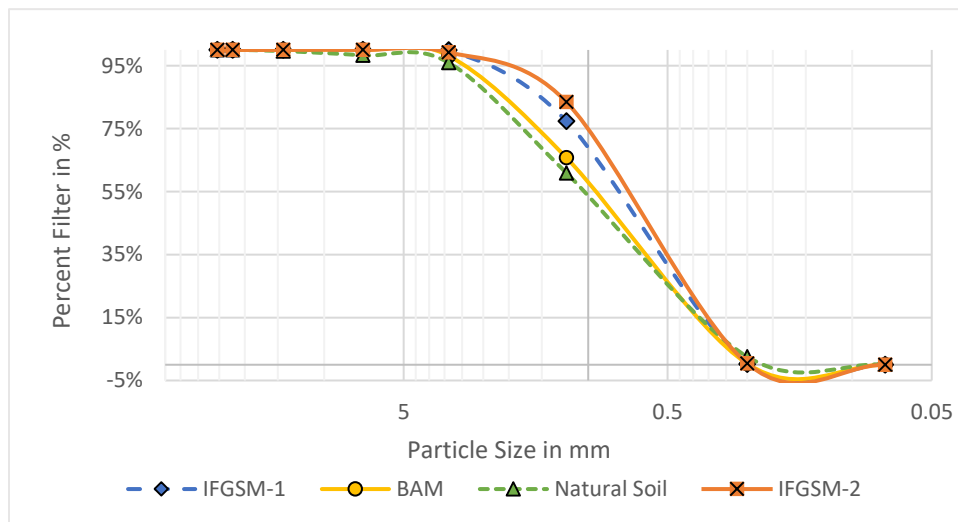


Figure 39. Particle size distribution for natural soil and media mixes.

Table 14. Material Characteristics

	IFGEM-1	BAM	Natural Soil	IFGEM-2
Density (g/cm ³)	2.73	1.39	2.36	2.60
BET Surface Area (m ² /g)	0.3142	0.7059	9.3712	1.3963
Porosity (%)	36.16	40.10	40.43	37.31
Infiltration Rate (cm/s)	0.028	0.026	0.003	0.017

5.3.1.2 Morphological Changes

The pre-treatment and post-treatment morphological images of IFGEM-1 and IFGEM-2 are shown in Figure 40. The iron filing pieces could be observed clearly before the treatment for both IFGEM recipes. Before treatment, the two main features of IFGEM-2 were the existence of tire crumb and the smaller sand particle size compared against the media mix of IFGEM-1. After treatment, the very first difference was the color, and both IFGEM-1 and IFGEM-2 tended to turn brown. In addition to the color changes, the iron filings could not be observed by the naked eye after treatment since they were coated by surrounding materials. When coated iron was exposed during the experimentation, it revealed that the size of the iron filing largely decreased as it was dissolved during the oxidation process.

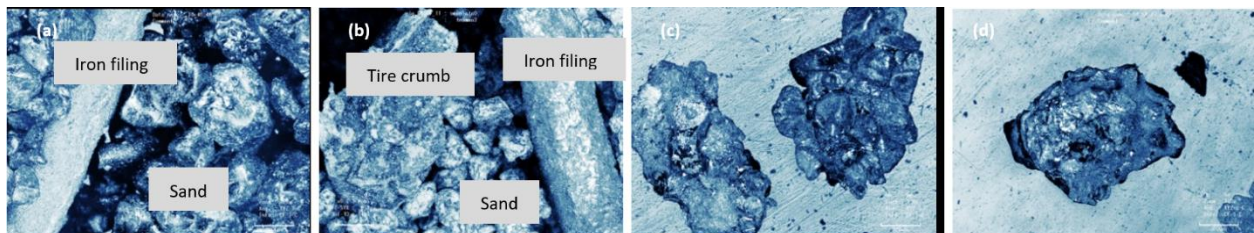


Figure 40. Confocal images of (a) raw IFGEM-1 shows iron filings and sand, (b) raw IFGEM-2 shows iron filings, smaller sand, and tire crumb, (c) used IFGEM-1 shows iron filing coated by surrounding materials, and (d) used IFGEM-2 shows iron filing coated by surrounding materials.

5.3.2 Results of Isotherm study

5.3.2.1 Phosphorus and Nitrate Absorption under Neutral pH

The isotherm study results of IFGEM-1 and IFGEM-2 for phosphate adsorption under neutral pH condition are shown in Figure 41. IFGEM-1 tends to achieve higher TP removal when the media mass is small while IFGEM-2 tends to absorb more TP when more media mass is available. The Langmuir and Freundlich isotherm equation parameters of IFGEM-1 and IFGEM-2 are shown in Table 15 to Table 16. As most $1/q_m$ values are negative in the Langmuir equation, it is inappropriate to apply for the calculation of maximum absorption capacity (q_m). So Freundlich relative absorption capacity is selected from the Freundlich section of Table 15 and Table 16.

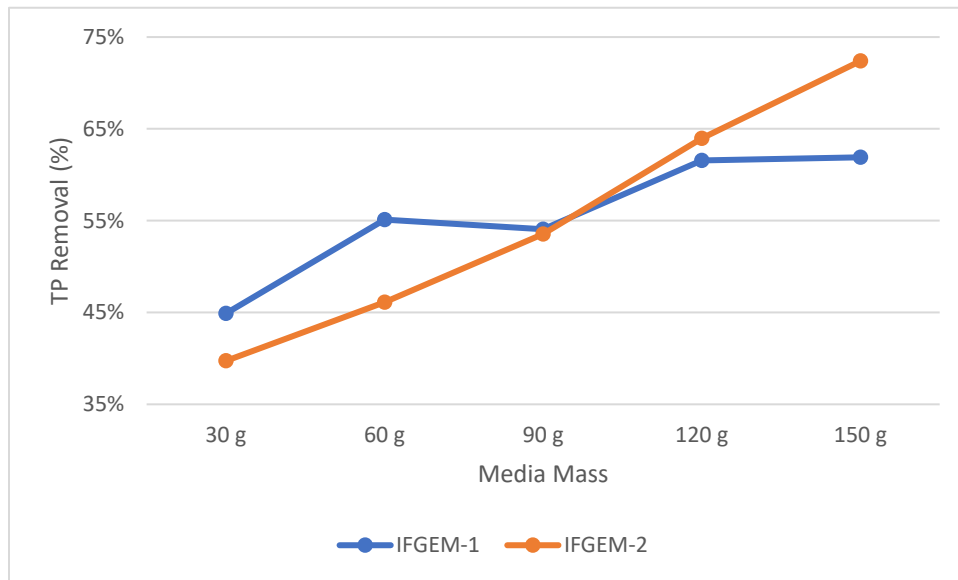


Figure 41. The TP removals from the isotherm study of IFGEM-1 and IFGEM-2 under neutral pH condition

Table 15. Phosphorus Absorption Parameters of the Langmuir and Freundlich isotherm for IFGEM 1 and 2 under neutral pH condition

IFGEM recipes	Isotherm equation for Langmuir	R-square value	$1/(q_m K_{ads})$	$1/q_m$ (mg/g)	
IFGEM-1	$y = 628.74x - 938.59$	0.8352	628.74	-938.59	
IFGEM-2	$y = 190.75x + 52.554$	0.7635	190.75	52.554	
IFGEM recipes	Isotherm equation for Freundlich	R-square value	$1/n$	Log K	$K (mg^{1-(1/n)} L^{1/n} g^{-1})$
IFGEM-1	$y' = 3.1346x' - 1.5403$	0.86	3.1346	-1.5403	0.0288
IFGEM-2	$y' = 1.0972x' - 2.2837$	0.7342	1.0972	-2.2837	0.0052
$x = 1/C_e$; $y = 1/q_e$. Where C_e is the aqueous concentration of phosphorus (mg/L) and q_e is the phosphorus concentration sorbed on the media (mg/g). $x' = \log(C_e)$; $y' = \log(q_e)$. Where C_e is the aqueous concentration of phosphorus (mg/L) and q_e is the phosphorus concentration sorbed on the media (mg/g).					

The isotherm study results of IFGEM-1 and IFGEM-2 for nitrate reduction under neutral pH condition, as well as the corresponding ammonia generation, are shown in Figure 42. The ammonia generation is confirmed for both IFGEMs, and IFGEM-1 seems to produce two to three times the ammonia produced by IFGEM-2. However, IFGEM-1 removes more nitrate (up to 35%)

while IFGEM-2 can only achieve approximately 10% nitrate removal. The Langmuir and Freundlich isotherm equation parameters of IFGEM-1 and IFGEM-2 are shown in Table 16. As most $1/q_m$ values are negative in the Langmuir equation, it is inappropriate to apply for the calculation of maximum absorption capacity (q_m). So Freundlich relative absorption capacity is selected from Table 16.

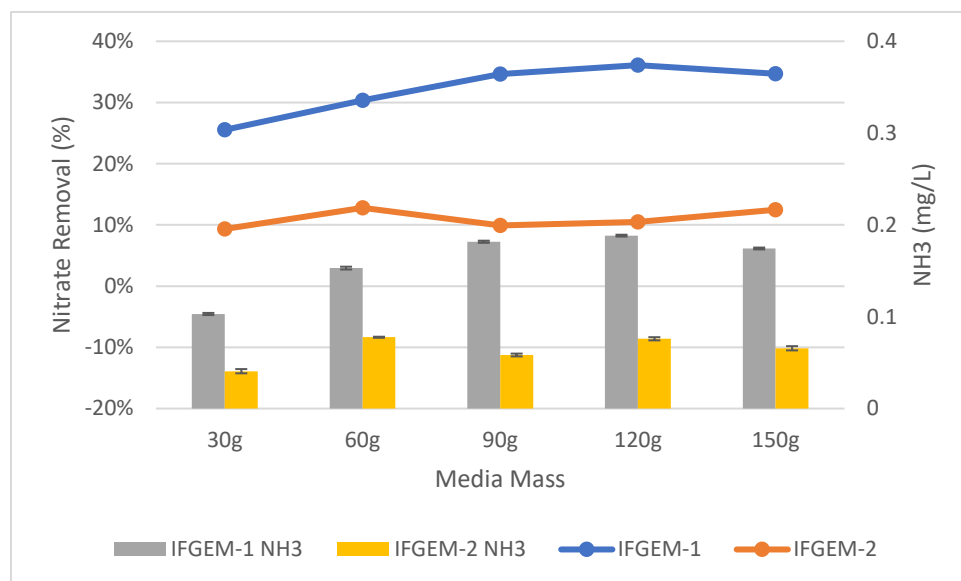


Figure 42. The nitrate removal and ammonia generation from the isotherm study on IFGEM-1 and IFGEM-2 under neutral pH condition

Table 16. Nitrate absorption parameters of the Langmuir and Freundlich isotherm for IFGEM 1 and 2 under neutral pH condition

IFGEM recipes	Isotherm equation for Langmuir	R-square value	$1/(q_m K_{ads})$	$1/q_m$ (mg/g)	
IFGEM-1	$y = 3754.5x - 4216.4$	0.7158	3754.5	-4216.4	
IFGEM-2	$y = 4924.3x - 4456.2$	0.1362	4924.3	-4456.2	
IFGEM recipes	Isotherm equation for Freundlich	R-square value	$1/n$	Log K	$K (mg^{1-(1/n)} L^{1/n} g^{-1})$
IFGEM-1	$y' = 7.3878x' - 1.9373$	0.8565	7.3878	-1.9373	0.0116
IFGEM-2	$y' = 7.7666x' - 3.2904$	0.0538	7.7666	-3.2904	0.0005
$x = 1/C_e$; $y = 1/q_e$. Where C_e is the aqueous concentration of nitrate (mg/L) and q_e is the nitrate concentration sorbed on the media (mg/g). $x' = \log(C_e)$; $y' = \log(q_e)$. Where C_e is the aqueous concentration of nitrate (mg/L) and q_e is the nitrate concentration sorbed on the media (mg/g).					

5.3.3 Column Tests

Column study is the only study that actually tries to mimic real-world conditions. The crucial parameters of pH, DO, and the oxidation-reduction potential (ORP) from the inlets and

each sampling port of the columns are summarized in Table 17. In general, the ORP values decreased at the top section, then slightly increased at the middle section and decreased again at the bottom section in columns B and C. However, it shows a straight decline of the ORP values over sections for column D. A similar trend happens to the DO test for column D. Obvious DO decrement occurred in columns A and D, and slight DO increment occurred in columns B and C. For pH changes, columns A and D exhibited a continuous increment of pH through each section, while column B tended to increase pH at the top section and keep a lower pH for the rest. Column C shows steady pH values across three sections. Additionally, the soil moisture and HRT for each section are summarized in Figure 43. Column A and the first section of column B showed lower moisture content, usually less than 20%. However, the rest of the media has a much higher moisture content, and the average moisture content is 35.50%, 35.66%, and 39.33% for BAM, IFGEM-2, and natural soil, respectively.

Table 17. Average ORP, dissolved oxygen, and pH values in the column study

Column	Port	Inlet = 0.6 mg/L nitrate			Inlet = 1.2 mg/L nitrate			Inlet = 1.8 mg/L nitrate		
		<u>ORP</u>	<u>DO</u>	<u>pH</u>	<u>ORP</u>	<u>DO</u>	<u>pH</u>	<u>ORP</u>	<u>DO</u>	<u>pH</u>
	inlet	327.50	8.76	6.98	316.40	7.58	7.10	320.17	8.35	6.76
A	Port 1	154.57	7.99	8.34	232.77	8.24	8.14	180.53	6.86	8.52
	Port 2	214.43	7.41	8.26	237.93	7.64	8.05	244.20	8.43	7.88
	Outlet	122.37	6.09	8.85	184.30	6.52	8.89	201.43	6.64	8.82
B	Port 1	117.53	8.21	9.67	165.70	8.51	9.37	99.53	7.01	9.44
	Port 2	243.33	8.57	8.00	256.87	8.59	7.87	240.23	8.52	7.85
	Outlet	235.07	8.26	7.96	265.03	8.64	7.99	296.27	8.07	7.81
C	Port 1	245.37	8.85	8.33	213.60	8.63	8.42	221.93	8.76	8.44
	Port 2	240.20	8.85	8.26	219.83	8.60	8.38	247.63	8.84	8.28
	Outlet	246.17	8.74	8.23	253.93	8.92	8.33	257.60	8.86	8.30
D	Port 1	246.83	8.54	8.08	268.80	7.79	7.67	271.23	8.32	7.59
	Port 2	-61.40	7.10	8.69	97.23	7.76	8.07	57.23	8.01	8.20
	Outlet	-102.90	4.98	7.77	56.97	5.28	8.47	0.03	7.01	8.39

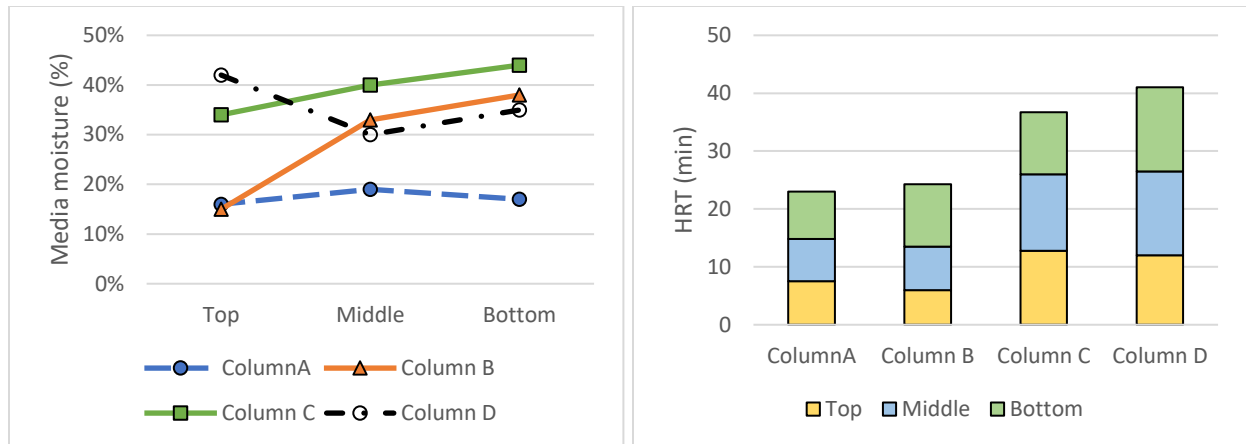


Figure 43. Soil moisture content (left) and hydraulic retention time (right) from each column section under operating condition

5.3.3.1 Nitrate Removal and Ammonia Generation

The cumulative nitrate removal at each sampling port of all columns is shown in Figure 44 given the three different influent nitrate concentrations (denoted as levels 1 to 3). Nitrate removals were observed in columns A, B, and D, while the control column C with natural soil showed negative or minor removal. When the inlet nitrate concentration was 0.6 mg/L, columns A and D exhibited the highest nitrate removal of 91.01% and 88.32%, respectively. Column B showed a moderate nitrate removal of 44.56%. When the inlet nitrate concentration became 1.2 mg/L, the overall removal of column A and D were 91.76% and 91.43%, respectively. Column B achieved 79.95% nitrate removal. When changing the inlet nitrate concentration to 1.8 mg/L, the overall removal of nitrate was up to 95.53% for column A, 94.49% for column D, and 75.85% for column B.

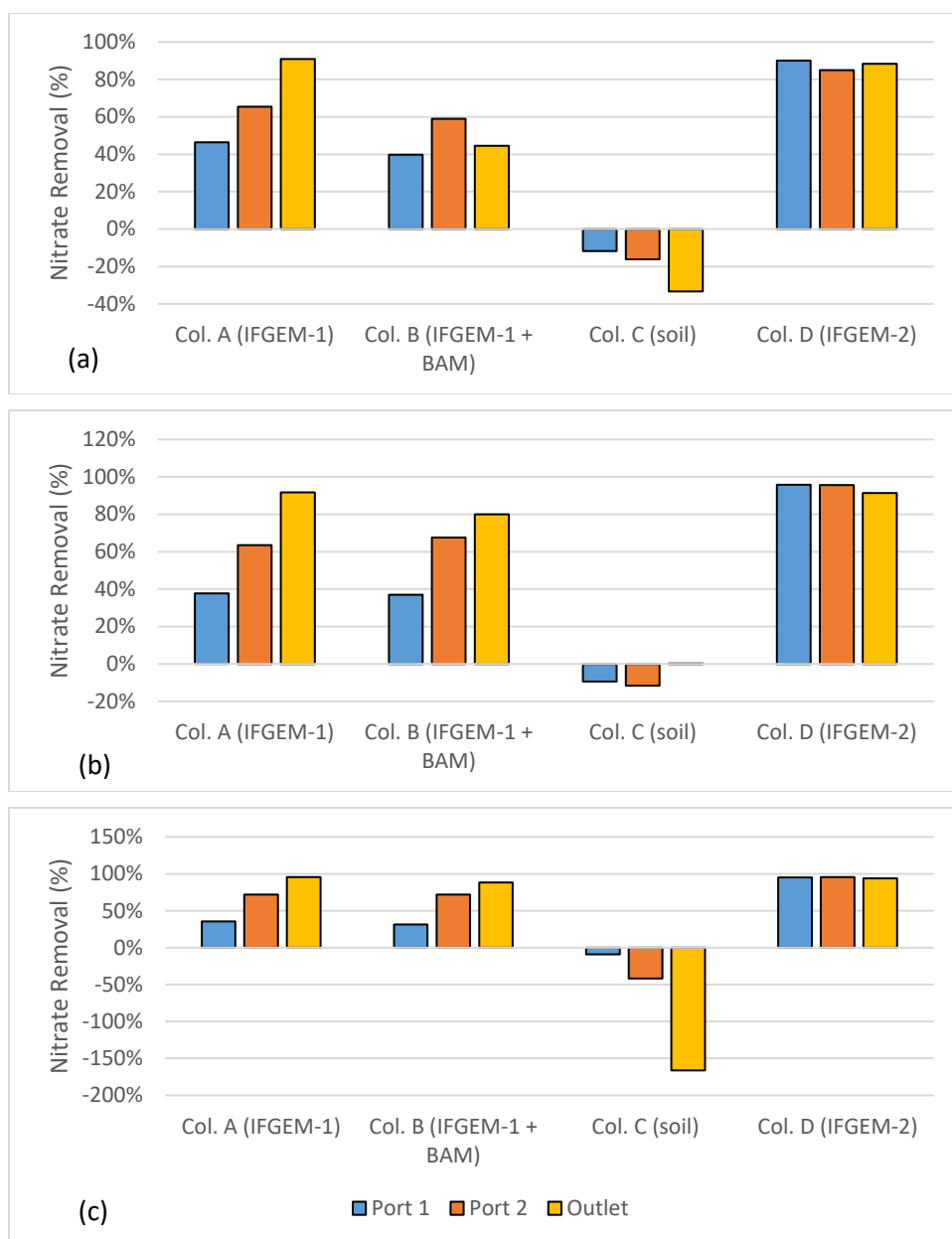


Figure 44. Cumulative nitrate removal at each section through columns when inlet nitrate concentration = (a) 0.6 mg/L, (b) 1.2 mg/L, and (c) 1.8 mg/L.

The generation of ammonia from the IFGEM treatment process is confirmed as the byproduct of nitrate reduction. The ammonia concentration from each sampling port for all columns given the three different inlet nitrate concentration levels is shown in Figure 45. Ammonia generation seems positively related to the nitrate removal in the two IFGEM columns, particularly

in the top sections. The higher the nitrate concentration, the more the ammonia being produced. It is noticeable that the ammonia concentration of the treated effluent was 7 to 23 times higher than that of the influent values from the column A test. Even though the first section of column D generated a significant amount of ammonia, the treated effluent at the outlet showed a negligible ammonia level. This is a strong evidence that IFGEM-2 in the middle and lower sections adsorbed most of the ammonia being produced in the first section.

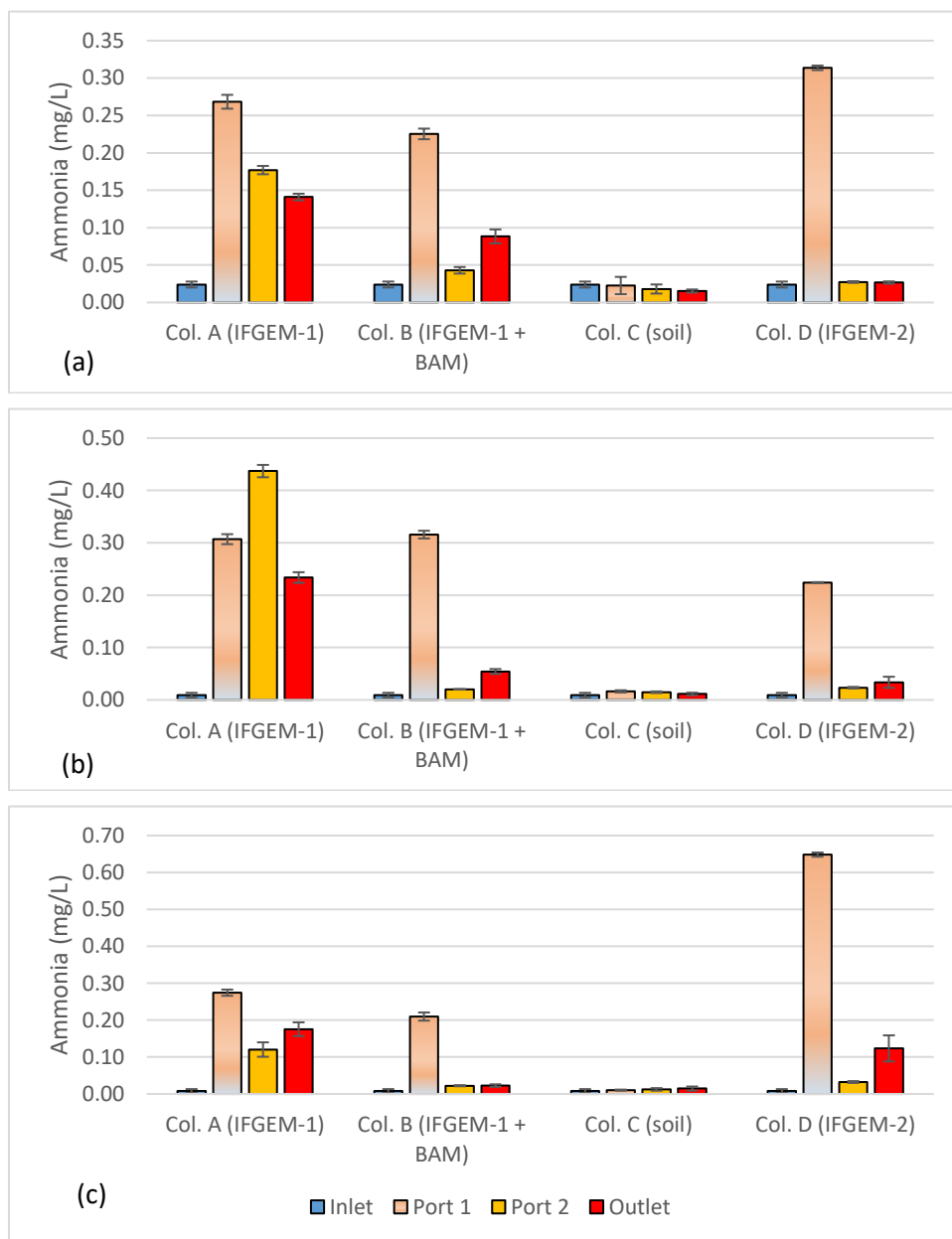


Figure 45. Ammonia concentrations at the outlet of each section through columns when inlet nitrate concentration = (a) 0.6 mg/L, (b) 1.2 mg/L, and (c) 1.8 mg/L.

5.3.3.2 Phosphorus Removal

When the inlet TP = 0.3 mg/L, the overall TP removal was 54.46% and 45.54% , respectively, for columns A and D. When the inlet TP concentration became 0.5 mg/L, the overall TP removal was 71.90% and 26.14%, respectively, for columns A and D. When the inlet TP increased to 0.7 mg/L, the overall TP removal changed to 82.53% and 62.45%, respectively, for columns A and D. For column B, the first section showed removal effects similar to column A, but the following two sections exhibited negative removal of TP under concentration level 1 and 2 (-168.32% and -29.41%), then a much higher TP removal as 59.39% was achieved under concentration level 3. However, when it came to column C, the TP removal was mostly negative or negligible.

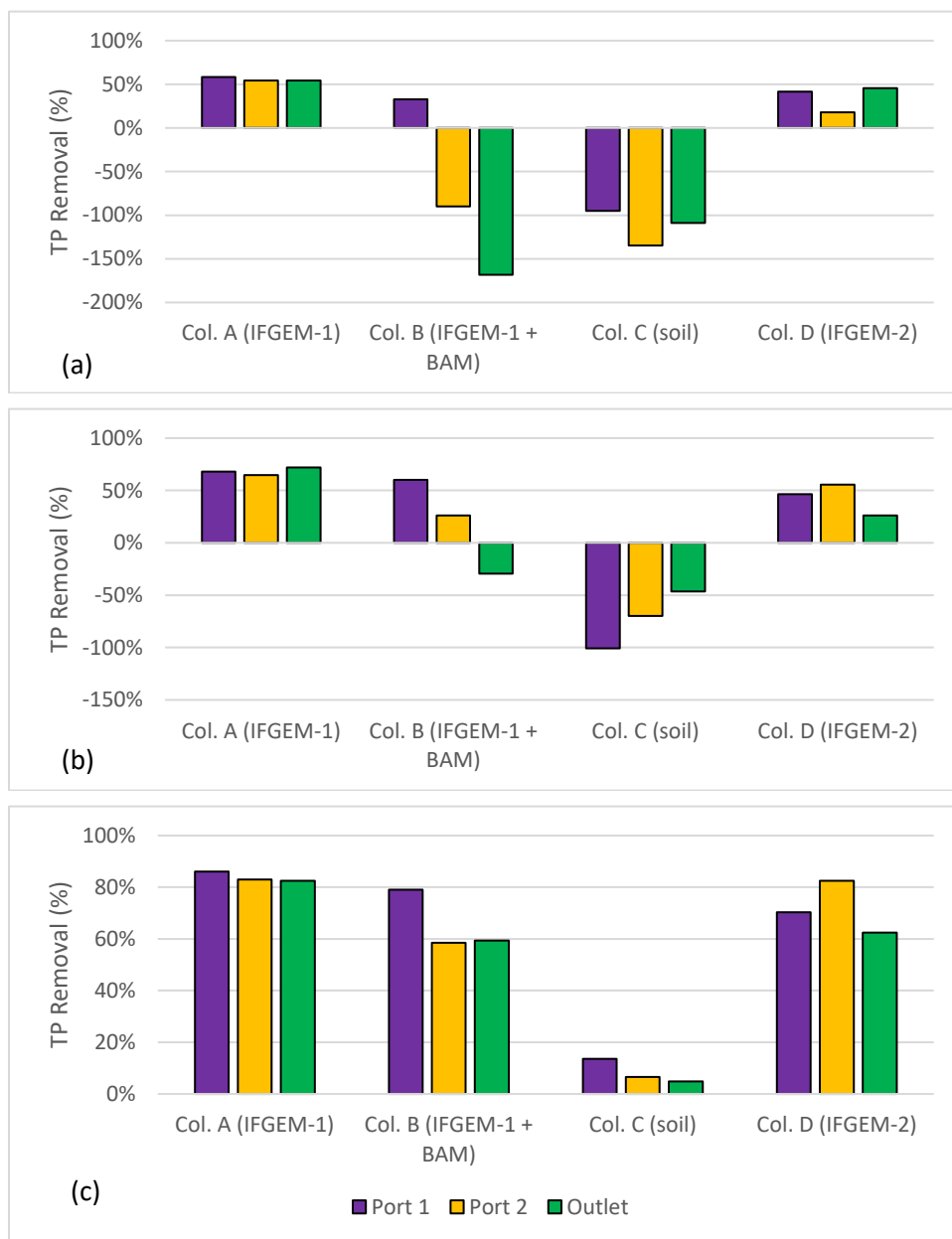


Figure 46. Total phosphorus removal when the inlet TP = (a) 0.3 mg/L, (b) 0.5 mg/L, and (c) 0.7 mg/L.

5.3.4 Results of Kinetics Study

Nutrients cannot flow through the flasks during the isotherm test, but the nutrients adsorption in the column test is a function of both time and space as the water flows through the column (Table 18). In column A, the kinetics analysis showed that IFGEM-1 mostly fits best in zero order, and the increase of influent nutrient concentration enhances the reaction rate constant from 0.0258 to 0.0809 for nitrate reduction and 0.0070 to 0.0242 for phosphorus removal. The situation was similar for column D, where zero order dominated reaction kinetic. As the influent nutrient concentration increased, the rate constant changed from 0.0135 to 0.0388 for nitrate reduction and 0.0027 to 0.0106 for phosphorus removal. In column B, the nitrate reduction mainly followed zero order, and the rate constant increased from 0.0124 to 0.0551. However, the phosphorus removal kinetics under various influent concentrations fluctuated and the R-squared values were relatively low in column B. The kinetics equation for column C may not have been precise because all R-squared values under three influent conditions were low for both nitrate reduction and phosphorus removal.

Table 18. Kinetics information of each column under various influent conditions

column	Nutrient species	Concentration level 1	R^2 / reaction order	Concentration level 2	R^2 / reaction order	Concentration level 3	R^2 / reaction order
A	Nitrate	$y = -0.0258x + 0.6898$	0.9593 / Zero	$y = -0.0486x + 1.1884$	0.9907 / Zero	$y = -0.0809x + 1.8616$	0.9744 / Zero
	Phosphorus	$y = -0.007x + 0.2746$	0.5301 / Zero	$y = -0.0141x + 0.4086$	0.632 / Zero	$y = -0.0242x + 0.5572$	0.5538 / Zero
B	Nitrate	$y = -0.0124x + 0.6138$	0.4775 / Zero	$y = 0.1372x + 0.6571$	0.9896 / 2 nd	$y = -0.0551x + 1.8511$	0.9602 / Zero
	Phosphorus	$y = 0.027x + 0.2311$	0.8537 / Zero	$y = 0.0097x + 0.3317$	0.2713 / Zero	$y = -0.0128x + 0.5274$	0.2632 / Zero
C	Nitrate	$y = -0.0085x + 1.3361$	0.9541 / 2 nd	$y = 0.0003x + 1.2911$	0.0049 / Zero	$y = -0.0041x + 0.5331$	0.8004 / 2 nd
	Phosphorus	$y = 0.0103x + 0.4281$	0.6830 / Zero	$y = -0.0142x + 1.6263$	0.2803 / 2 nd	$y = -0.0005x + 0.7253$	0.0347 / Zero
D	Nitrate	$y = -0.0135x + 0.5232$	0.5389 / Zero	$y = -0.0236x + 0.8296$	0.518 / Zero	$y = -0.0388x + 1.3747$	0.6689 / Zero

column	Nutrient species	Concentration level 1	R^2 / reaction order	Concentration level 2	R^2 / reaction order	Concentration level 3	R^2 / reaction order
	Phosphorus	$y = -0.0027x + 0.3015$	0.4374 / Zero	$y = -0.003x + 0.406$	0.1786 / Zero	$y = -0.0106x + 0.5627$	0.4470 / Zero
Zero, 1 st , and 2 nd represent zero, first, and second order reactions, x = reaction time, equivalent to HRT in column study; y = nutrient concentration (C) in effluent for Zero order reaction, ln(C) for 1 st order reaction, and 1/C for 2 nd order reaction.							

5.3.5 Results of ANOVA analysis

The two way ANOVA analysis¹⁸⁷ was applied to test the following null hypotheses for nitrate and TP removal separately. The following three hypotheses are employed in this study.

H₁: the average nutrient removals are the same between paired columns;

H₂: the average nutrient removals are the same among different inlet concentrations;

H₃: there is no interaction between columns and inlet concentrations in terms of nutrient removals.

From Table 19 the p values can be viewed with 95% confidence for each paired column. Most of the p values lie within the rejection region, which means there were significant differences between each paired column in terms of nutrient removal. However, there were several exceptions: for nitrate removal there were no significant differences of the overall removal between columns A and D, nor were there significant differences in the interaction between the inlet condition and

column type. Columns B and C also exhibited no significant differences in interaction from the column type and inlet conditions. For TP removal, the only exception was found between columns B and C, which showed they have no significant difference between their overall removals.

Table 19. ANOVA analysis between paired columns with three inlet conditions

compared aspects	A-B	A-C	A-D	B-C	B-D	C-D
	Nitrate removal analysis					
Columns	2.76 $\times 10^{-9}$	2.87 $\times 10^{-17}$	0.1208	9.30 $\times 10^{-14}$	7.22 $\times 10^{-9}$	5.64 $\times 10^{-17}$
Inlet conditions	1.37 $\times 10^{-6}$	1.91 $\times 10^{-7}$	0.0005	1.55 $\times 10^{-8}$	1.07 $\times 10^{-6}$	1.74 $\times 10^{-7}$
Interaction	5.67 $\times 10^{-6}$	1.05 $\times 10^{-6}$	0.7538	0.7332	1.58 $\times 10^{-5}$	3.44 $\times 10^{-6}$
	Phosphorus removal analysis					
Columns	4.67 $\times 10^{-10}$	7.62 $\times 10^{-9}$	2.35 $\times 10^{-5}$	0.6725	1.92 $\times 10^{-8}$	1.75 $\times 10^{-7}$
Inlet conditions	5.57 $\times 10^{-9}$	5.66 $\times 10^{-5}$	3.02 $\times 10^{-4}$	4.47 $\times 10^{-8}$	2.55 $\times 10^{-8}$	0.0002
Interaction	9.49 $\times 10^{-8}$	0.0039	5.35 $\times 10^{-3}$	0.0026	9.25 $\times 10^{-8}$	0.0014

5.4 Discussion

5.4.1 Nutrient Removal Interactions

The interactions between nutrient removal and sorption media are closely related to the nitrate reduction process through IFGEMs, given both IFGEMs in columns A and D exhibited promising removal efficiencies with varying influent nutrient concentrations. IFGEM-2 is more effective than IFGEM-1, as IFGEM-2 removes all the nitrate mainly through the top section while IFGEM-1 needs three sections to perform equivalent removal efficiencies (as indicated in Figure 44). The main reason for this outcome is that IFGEM-2 contains clay that can accumulate the nitrate concentration around the iron surface through adsorption, which enhances the nitrate reduction process since the reduction reaction requires close contact between iron surface and nitrate. Zhang, Li, Li, Hu and Zheng⁸³ observed the same enhancement with nano size iron particles and clay. Another reason is the longer HRT in IFGEM-2 versus IFGEM-1 (Figure 43) that caused over 4 times higher BET surface area of IFGEM-2 in unit weight (Table 14), which was mainly due to the existence of clay and tire crumb. This implies more contact time would be available for interactions between the nutrients and the sorption media on IFGEM-2 rather than that in IFGEM-1 in terms of nitrate reduction. Additionally, the faster decrement of ORP and DO concentration from each section in IFGEM-2 is another crucial piece of evidence indicating that the reaction intensity in IFGEM-2 is more severe (Table 17).

Not only the reactants but also the products are interacting with the sorption media. Ammonia is confirmed as one of the products of nitrate reduction reaction in IFGEMs (Figure 45). However, the ammonia generation pattern and removal efficiencies are totally different across the two media - IFGEM-1 and IFGEM-2. By following the nitrate reduction pattern, IFGEM-1

produces ammonia continuously throughout each section, while IFGEM-2 mainly produces ammonia through the top section. However, the ammonia removal performance of IFGEM-2 is much better than IFGEM-1, which can be explained by looking at ammonia concentration from each section of column B (IFGEM-1 on top, BAM at middle and bottom). When the newly produced ammonia flows from the IFGEM-1 section to BAM sections in column B, ammonia can be removed through BAM effectively due to unique components of BAM such as clay and tire crumb that also exist in IFGEM-2. Clay was found to be very effective for removing ammonia through ion exchange mechanism¹⁸⁸. Ammonia was removed as one of the reduction products that could shift the nitrate reduction reaction equilibrium to the direction of reducing more nitrate, which is another reason that IFGEM-2 has a higher reaction intensity than IFGEM-1 in ammonia removal.

When taking into account the phosphorus removal, the synergetic effects among media, nitrate, and phosphorus can be realized fully, as shown in Figure 47. One of the products of nitrate reduction reaction is ferrous/ferric iron ion, which can result in precipitation into FePO_4 given the presence of phosphate ion. As the influent nitrate concentration increases, more ferrous/ferric ion can be generated in IFGEMs through nitrate reduction. Due to the precipitation equilibration, the increased concentration of ferrous/ferric ion shifts the equilibrium to the direction of precipitating more phosphorus (Figure 46). The interaction can also be observed from reaction kinetics (Table 18), where rate constants of nitrate removal showed corresponding increment when the nitrate concentrations increased by 2 to 3 times, while the phosphorus removal rate constants showed similar or higher improvement when the TP concentration increased only up to 2.3 times at maximum. The better performance of TP removal in IFGEM-1 is also the result of its lower

intensity of nitrate reduction reaction when compared to IFGEM-2. Because ORP (Table 17) decreases through the oxidation reduction reactions, the change of ORP could be one of the major factors that affects the phosphorus adsorption to iron. Zhou, Tang and Wang ¹⁶⁹ also found that phosphorus absorption capacity would be depressed by low ORP conditions within certain pH ranges. It can be suggested that IFGEM-1 is suitable for places that require a faster infiltration rate and more phosphorus removal but are not sensitive to ammonia generation. IFGEM-2 is appropriate for applications with a higher standard for nitrogenous removal but less demands for infiltration speed and phosphorus removal.

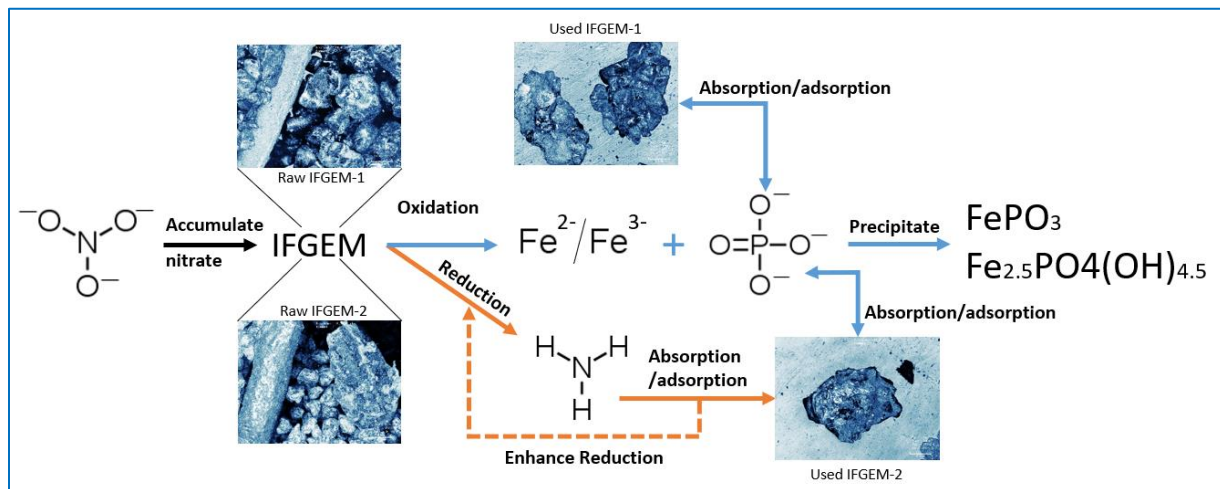


Figure 47. Nutrient removal interaction between nitrate and phosphate through IFGEM

The nitrate removal from the BAM layer (Figure 44) is significantly different from IFGEMs (Table 19), mainly because it has no iron filings that work as reactive electron donors. Thus, it is more about absorption rather than reduction with limited absorption sites ⁸³. When it comes to phosphorus removal, even in the best removal scenario with concentration level 3 in BAM (Figure 46-c), both IFGEMs can easily surpass column B, because the lack of iron filing in BAM means no contribution from the iron bonded phosphorus precipitation/absorption. So, the phosphorus removal of both column B (IFGEM-1 and BAM) and column C (natural soil) has no

statistical differences as none of the columns had salient removal effectiveness (Table 19). Natural soil, in particular, shows mostly negative or negligible removals for both nitrate and phosphorus as treatment failures. This indicates that natural soil has been saturated with nutrient contaminants from stormwater runoff and is no longer effective for removing nutrients from stormwater. The equivalent ORP values from each natural soil section (Table 17) also imply that soil has lost almost all its reactive sites for possible oxidation-reduction reactions and clarified the necessity of replacing the existing natural soil in the study site. However,

From Table 14, it is noticeable that IFGEM I rate is 0.028 cm/s or 40 inches per hour) and IFGEM II rate is 0.017 cm/s or 24 inches per hour. These differences exist due to compaction only with the same media. The filtration rate of our column testing for the purpose of demonstration of its feasibility is only 1.63×10^{-3} cm/s or 2.33 inches per hour. Thus, a greater rate of treatment was possibly, or the 0.028 will be a rate from a standard infiltration test in the future for field applications.

5.5 Summary

To deal with the treatment challenges under various influent conditions for stormwater runoff, wastewater effluent, and agricultural discharge, two IFGEM recipes were evaluated in parallel for nutrient removal in comparison against traditional green sorption media and natural soil from a study site. Both IFGEMs showed excellent nitrate reduction due to the existence of iron filings as the reactive electron donor, but IFGEM-1 generated more ammonia in the effluent while IFGEM-2 was able to remove and recover almost all generated ammonia. The phosphorus removal was enhanced by the nitrate reduction in IFGEMs given that the iron can bond phosphorus

during precipitation; however, IFGEM-1 showed better and more stable phosphorus removal than IFGEM-2 as the ORP decrement in IFGEM-1 is not as influential as that in IFGEM-2.

Overall, the nutrient reuse/recovery potential of IFGEMs has been confirmed through the treatment in this study due to the presence of both ammonia and phosphorus in the used media that may be applied for soil amendment directly even in a changing environment and condition. IFGEM-1 is preferred for treating relatively higher phosphorus removal albeit with lower achievement for ammonia removal and recovery. IFGEM-2 is appropriate for treating stormwater runoff, agricultural discharge, and wastewater effluents with the simultaneous removal of phosphorus and nitrate albeit with lower achievement for phosphorus removal. Moreover, both IFGEMs show possible nutrient recovery potential through the holistic assessment as tremendous amounts of nutrients can be captured through the field conditions in the future for reuse. Even though the nutrient removal effects of both IFGEMs are promising at room temperature and neutral pH conditions, the fixed values of both temperature and pH indicates limited applicability of those green sorption media in areas where significant temperature fluctuations might occur. Hence, the temperature and pH impacts on the IFGEMs with the inclusion of iron fillings for nutrient removal will be discussed in a companion study (Part II).

CHAPTER 6: IMPACT OF CHANGING ENVIRONMENTAL FACTORS AND SPECIES COMPETITION ON IRON FILINGS-BASED GREEN ENVIRONMENTAL MEDIA FOR NUTRIENT REMOVAL IN STORMWATER TREATMENT⁵

6.1 Introduction

Non-point source pollution impact is mainly attributed to agriculture discharges and urban stormwater runoff ¹⁸⁹. The major contaminants are nutrients, including nitrogen and phosphorus, which are key constituents that maintain the nutrient cycling or even trigger ecosystem degradation such as algal bloom, hypoxia, and eutrophication in receiving water bodies on some occasions ¹⁹⁰. Excessive nitrate contamination in drinking water may cause the occurrence of the serious disease blue baby syndrome ^{191, 192} in infants. Moreover, the toxicity of ammonia is fatal to many aquatic creatures even in a low concentration ¹⁹³. Different kinds of green sorption media with the inclusion of recycled materials have been adopted in various best management practices (BMPs) for nutrient removal from stormwater runoff ^{24, 44, 46, 56, 61, 63, 65, 68, 175, 194-196}. Although removal efficiency is fair, it is difficult to achieve a desirable level of removal recovery. Iron based sorption media were found to be effective for nitrogen and phosphorus removal ^{81, 184, 185, 197-201}. Nano technology-based sorption media are also emerging in research, showing that lanthanum-based nanocomposite sorbent is able to remove 116 mg/g and 138 mg/g phosphate and nitrate ion, respectively ²⁰². 0.11-0.16 mg/g phosphate removal was reported with nanoscale zero-valent iron (NZVI) under various temperatures ¹⁸¹. The nanoscale zero valent-iron supported on pillared clay (NZVI/PILC) reportedly achieved effective removal of at least 30% more nitrate when compared to NZVI alone

⁵ Authors: Ni-Bin Chang, Dan Wen, and Martin P. Wanielista; Publication: Pending publication: submitted to *Environmental Progress & Sustainable Energy*, anticipated publish date: Dec 2018

under the same condition ⁸³. Yet iron powders or nanoparticles are not cost-effective and environmentally friendly due to the energy and materials required in the production process.

In parallel to our previous study on green sorption media ⁶⁸, we propose to test the iron filing-based green environmental media (IFGEM) because of its great potential for nutrient removal and recovery of both nitrogen and phosphorus simultaneously. The inclusion of iron filings might result in varying performance on nutrient removal under different environmental conditions that deserve further attention. For example, whereas temperature is one of the most important factors which affect IFGEM's reaction kinetics, changing pH values may impact nutrient removal as well. Two new recipes of IFGEM were proposed in this study. IFGEM-1 is composed of 96.2% fine sand and 3.8% iron filings (by volume), while IFGEM-2 contains 80% sand, 10% tire crumb, 5% pure clay, and 5% iron filings (by volume). Note that iron filings were ground to small particles in advance to improve the chemical reactions. The aim of this study is thus to examine the potential of IFGEM in nutrient removal and recovery under changing environmental conditions with respect to temperature effect, influent concentration, pH value, iron filing content, and interaction (competition) between nutrient species. There are three study objectives. The first objective is to explore the difference in nutrient removal in terms of the presence and the changing level of iron-filings in the two IFGEM recipes. The second objective is to assess the sensitivity of nutrient removal to varying temperature and pH regimes. The third objective is to investigate the impact of species competition for adsorption/adsorption capacity between nitrogen and phosphorus on nutrient removal.

Some science questions to be answered based on the study objectives include: 1) How do different temperature regimes affect the nutrient species competition for adsorption capacity and

thus removal efficiency under various influent concentrations? 2) How does changing the pH values impact the nutrient removal process? 3) What are the interactions between the nutrient species during the treatment under competitive adsorption? 4) What is the role of iron filings and clay in the nutrient removal process? We hypothesize that: 1) different types of IFGEM have different responses to temperature changes, resulting in either positive or negative correlation to nutrient removal efficiency; 2) nutrient removal of IFGEM is sensitive to the changes of pH values; 3) competitive adsorption exists between nitrate and phosphorus removal in IFGEM; 4) iron filings and clay in IFGEM play complementary roles, as the presence of iron filings may help reduce nitrite to ammonia for better adsorption in clay which may signify the potential of nutrient reuse/recovery; and 5) the presence of iron filings may enhance phosphorus precipitation and hence nutrient reuse/recovery.

6.2 Material and Method

To answer these science questions, this study performed a comparative and comprehensive column test with various influent concentrations in four ambient temperature environments (4, 12, 23, 35°C) for assessing nutrient removal efficiency and reaction kinetics based on the two IFGEM recipes proposed (Figure 1). In addition to the two predetermined IFGEM recipes, one green sorption media (e.g., bioactivated sorption media, BAM) recipe was selected in this study to test the integrative effect when working with IFGEM in the same column. Natural soil was chosen as the reference basis (e.g., control) in this experimental setting. Hence, media mix 1, named IFGEM-1, was arranged in column A, and was made up of 96.2% fine sand and 3.8% iron filings (by volume). Media mix 2, IFGEM-2, was arranged in column D and contained 80% sand, 10% tire crumb, 5% pure clay, and 5% iron filings (by volume). Media mix 3 was BAM, composed of 85%

poorly graded sand, 10% tire crumb and 5% clay (by volume), in which the tire crumb used were recyclable with no metal contents and the mined clay had no less than 99% clay content. BAM and IFGEM-1 were arranged sequentially by depth in column B. Natural soil was collected from SR 35 Basin 2 in Silver Springs watershed, located in Ocala, Florida and was used to fill column C. These four big columns (named from A to D) were constructed with 10 cm (4 inches) in diameter PVC pipes, and each column was divided into three equivalent sections (top, middle, and bottom) with each section 30 cm (1 foot) in length for the convenience of water sampling.

The sensitivity of nutrient removal of the IFGEM recipes to the changes of pH values may be confirmed by the isotherm study with respect to three different pH values for individual nutrient species. Temperature ranges from 4 to 35 °C may cover the weather conditions in different geographical areas from high to low latitude, while three levels of nutrient concentration were adopted for the inlet to reflect the quality variations of stormwater runoff. The pH ranges from 2 to 10 simulate the extreme pH fluctuations that may affect the nutrient removal. Distilled water was spiked with nitrate and phosphate standard solutions in three concentration levels (nitrate = 0.6, 1.2, 1.8 mg/L; TP = 0.3, 0.5, 0.7 mg/L) to simulate the fluctuation of nutrient concentrations in real world stormwater runoff. Nitrate concentrations were analyzed through HACH kit TN830, ammonia concentrations were analyzed through HACH kit TN835, and total phosphorus (TP) concentrations were analyzed with HACH Phosphorus (Total) TNT Reagent Set (summarized in Table 20). All water samples were analyzed within 24 hours after collection.

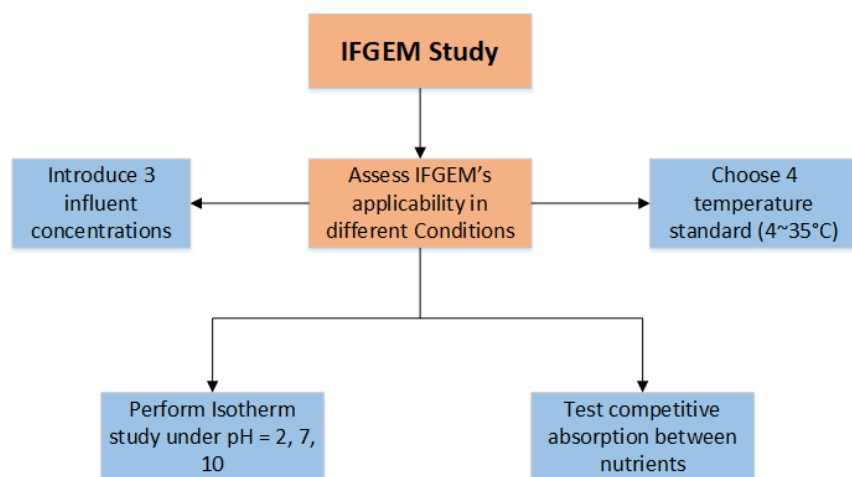


Figure 48. Flowchart of the current study

Table 20. Column study sample quality parameters and methods

Parameter	Method/instrument	Range
pH	Waterproof Double Junction pHTestr® 30	1 to 14
Dissolved oxygen	HACH HQ40D - IntelliCAL LDO101 LDO	0.01 - 20 mg/L
ORP	HACH HQ40D - MTC101	± 1200 mV
Soil moisture	EC-5 SMALL SOIL MOISTURE SENSOR	0 - 100%
Nitrates	Method 10206	0.05 - 13.50 mg/L NO ₃ -N
Total phosphorus	DR/800 Method 8190	0.06 - 3.50 mg/L PO ₄
Ammonia	Method 10205	0.015 - 2.00 mg/L NH ₃ -N

6.2.1 Kinetics Study under Temperature Impacts

It is crucial to understand how the impact of temperature changes on reaction kinetics profoundly affects nutrient removal in different geographical areas. The reaction kinetics study of nutrient removal effects in IFGEM was conducted under continuous mode using a series of column tests; these four columns can be operated under a steady state mode with respect to hydraulic conditions and inflow nutrient concentrations. Point measurement at each sample port located at different depths of the columns was conducted for kinetic study; the reaction time is presumably the same as hydraulic retention time (HRT) between sample ports. In our reaction kinetics study, the reaction time is thus defined by HRT from each column section with 30 cm (1 foot). For calculating the rate of a chemical reaction, it is assumed that the following simplified equation is used for the overall reaction in each column. Eq. 13 is a generalized form of the zero, first, or higher order rate equations.

$$\frac{dC}{dt} = k[C]^m \quad (14)$$

For further understanding of the temperature effect, the temperature correction factor model that relates the rate constant to temperature was adopted, as shown in Eq. 14. Where k_T is the rate constant at temperature T (°C), m is the reaction order, k_{23} is the rate constant at 23 °C, and θ is the temperature correction factor. The model is usually applied to a biological process that has low temperature dependence. To answer the first science question, the temperature range of around 23 °C was used as the reference point in support of the kinetic models that are related to differing temperatures in the subsequent column tests.

$$k_T = k_{23}\theta^{(T-T_{23})} \quad (15)$$

6.2.2 Isotherm Study on Individual Nutrient Adsorption

In order to understand the thermodynamic characteristics of IFGEM in terms of nutrient adsorption under various pH values, the adsorption isotherm experiment was conducted separately for nitrate and phosphorus removal in IFGEM-1 and IFGEM-2 with three initial pH values (2, 7, and 10) that are deemed as the only environmental factor of interest in this case. Then, 30 – 120 g media mass was prepared in five 500 mL flasks with 300 mL solution of 1.0 mg/L as total nitrate or phosphorus, and the experiment was carried out under room temperature on the rotary shaker with 250 rpm for 1 hour. The water sample from each flask was then filtered through 0.45- μ m membrane filters before the isotherm analysis. The analyzed parameters were nitrate and ammonia for the nitrate isotherm experiment, and total phosphorus for the phosphorus isotherm experiment. The Freundlich and Langmuir isotherm equations were adopted to analyze the data. The Freundlich isotherm was obtained by plotting $\log q$ versus $\log C$, and the Langmuir isotherm by plotting $1/q$ versus $1/C$. The following two equations were applied in this study:

- Freundlich isotherm equation:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (16)$$

where C_e is the aqueous concentration of adsorbate (mg/L), q_e is the sorbed concentration (mass of adsorbed adsorbate/mass adsorbent), K_F is a constant indicative of the relative adsorption capacity of the adsorbent ($\text{mg}^{1-(1/n)} \text{L}^{1/n} \text{g}^{-1}$) and n is a constant indicative of the intensity of the adsorption.

- Langmuir isotherm equation:

$$\frac{1}{q_e} = \left(\frac{1}{K_L q_m} \right) \frac{1}{C_e} + \frac{1}{q_m} \quad (17)$$

where q_m is the maximum capacity of adsorbent for adsorbate (maximum mass of adsorbed adsorbate/mass adsorbent), C is the aqueous concentration of adsorbent (mass/volume), and K_L is the Langmuir equilibrium constant.

6.2.3 Isotherm Study on Competitive Nutrient Adsorption

Singular nutrient specie isotherm study provides a fundamental understanding of the specific nutrient adsorption characteristics in a batch mode, but competitive adsorption more widely exists in real storm events as multiple nutrients are present. The competitive isotherm adsorption study follows exactly the same procedure as the individual nutrient isotherm study to answer science question 3, but with two conditions. One is that the solution used in the experiment contains both nitrate and phosphorus in the concentration of 1.0 mg/L for each nutrient, and the other is that only the neutral condition was selected as the initial pH value ($\text{pH} = 7$) in this experiment. The adsorption results are compared with their counterparts from the previous section (i.e., $\text{pH} = 7$ scenarios) to retrieve the competitive adsorption information when using IFGEM-1 and IFGEM-2.

6.2.4 Gibbs Energy Change

The Gibbs free energy change (ΔG^o) is applied in this study to demonstrate the degree of spontaneity of an adsorption or reaction process for phosphorus and nitrate removal. Note that adsorption is assumed for the calculation of Gibbs energy. A higher negative value is the reflection of a more energetically favorable adsorption/reaction, while a higher positive value indicates the adsorption/reaction tends to proceed in the opposite direction. Based on thermodynamic law and

isotherm study, ΔG° is calculated through the following equations, in which Eq. 18 was derived from literature review AL-HAKEIM, et al. ²⁰³.

$$\Delta G^\circ = -RT \ln K_C \quad (18)$$

$$K_C = \frac{q_e M}{C_e V} \quad (19)$$

where K_C is the equilibrium constant without units, T is the absolute temperature in Kelvin, M is the mass of adsorbent, V is the volume of solution, and R is the gas constant with a value of $8.214 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$.

6.2.5 Design and Operation of Column Tests

As shown in Figure 49, four columns were setup and named from column A to column D. Each column had three 30 cm (1 ft) sections from top to bottom. Column A and column D were filled with IFGEM-1 and IFGEM-2, and column C was filled with natural soil. However, to examine the integrate effect and differential performance, the first section of column B was filled with IFGEM-1 while the two lower sections were filled with BAM. Each section was attached to a wooden board and the joints between sections were wrapped with parafilm to eliminate outside impacts, with the outlet from one section serving as the inlet for the following one.

To answer science question 4, the columns were kept in a temperature controllable chamber (e.g., also called constant temperature room) in order to simulate the desired ambient temperature as 4, 12, 23, and 35 °C. Under each temperature condition, three nutrient concentration levels were introduced with the co-existence of nitrate and phosphorus. After at least 2 hours running, triplicate water samples were taken from the outlet of each section as well as the inlet from the reservoir.

Some parameters were measured immediately after collection, including oxidation reduction potential (ORP), dissolved oxygen (DO), and pH value. The nutrient concentrations were tested for nitrate and total phosphorus with HACH kits within 24 hours after the sample collection. The measurement methods are all summarized in Table 20.

6.3 Results

6.3.1 Nitrate Removal and Reaction Kinetics

The overall nitrate removals are shown in Figure 50 for columns A to D under three influent nutrient concentrations and four ambient temperature conditions. The overall nitrate removals in column A were generally over 85-90% throughout different temperature levels and influent concentrations. For column B, the highest nitrate removal happened at 12 °C with the removal range from 65% to 91%. Column C showed mostly negative or negligible nitrate removal when the influent concentration was low or middle, but extreme nutrients releasement was observed at 12 °C and 35 °C. Column D also showed the best removal (92% as average) under 12 °C, with the removal decreasing when the temperature either rose to 35 °C (76% as average) or decreased to 4 °C (61% as average). In addition to the temperature impacts, the increase of influent nitrate concentration may have enhanced the overall removals for both IFGEM recipes in columns A and D and the mixed configuration of IFGEM-1 and BAM in column C.

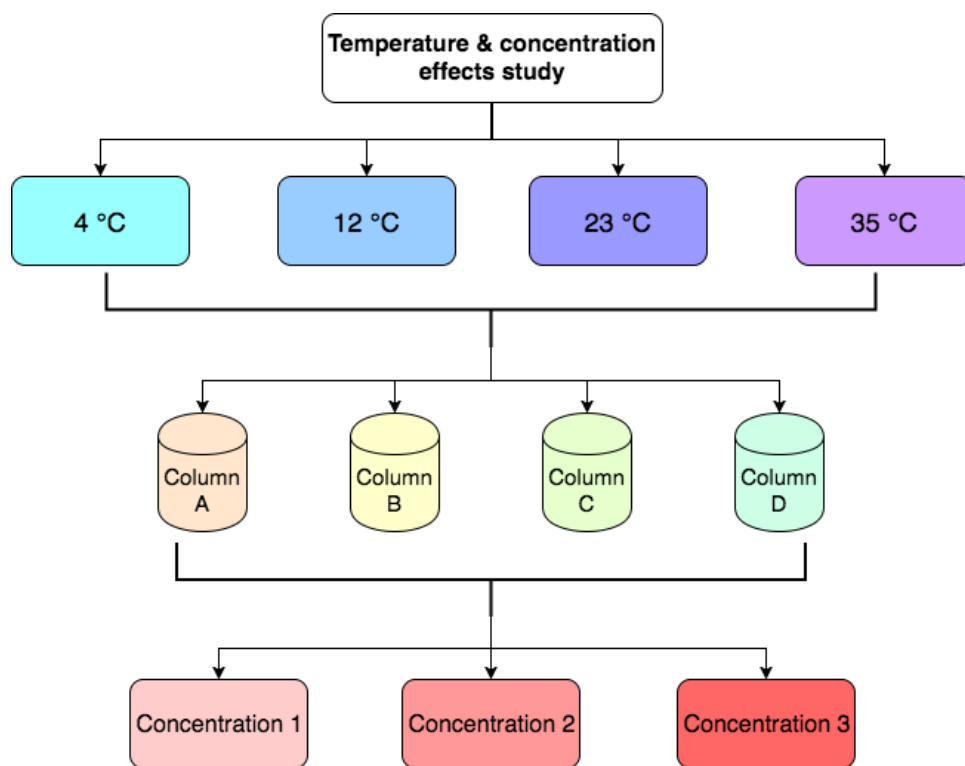


Figure 49. Diagram of Column Test Procedure under Various Temperature and Influent Concentrations (Column A is IFGEM 1, Column B is IFGEM-1 with BAM, Column C is natural soil, and Column D is IFGEM-2).

The nitrate removal rate constants (k value in Equation 1) of the four columns are summarized in Figure 51; the most suitable reaction orders were selected as zero order for columns A and B, second order for column C, and first order for column D. In column A, the impact of temperature on the rate constants was negligible when compared with the impact from influent concentration changes; in other words, the higher the influent concentration was, the faster the reduction appeared. Columns B and D, in addition to experiencing similar impacts from influent concentration changes, seemed to obtain the highest rate constant at 12 °C. In column C, it was hard to tell the impacts from temperature and influent concentration on the rate constants, as the diagram shows chaotic fluctuations due to the significant nutrient release during the column study.

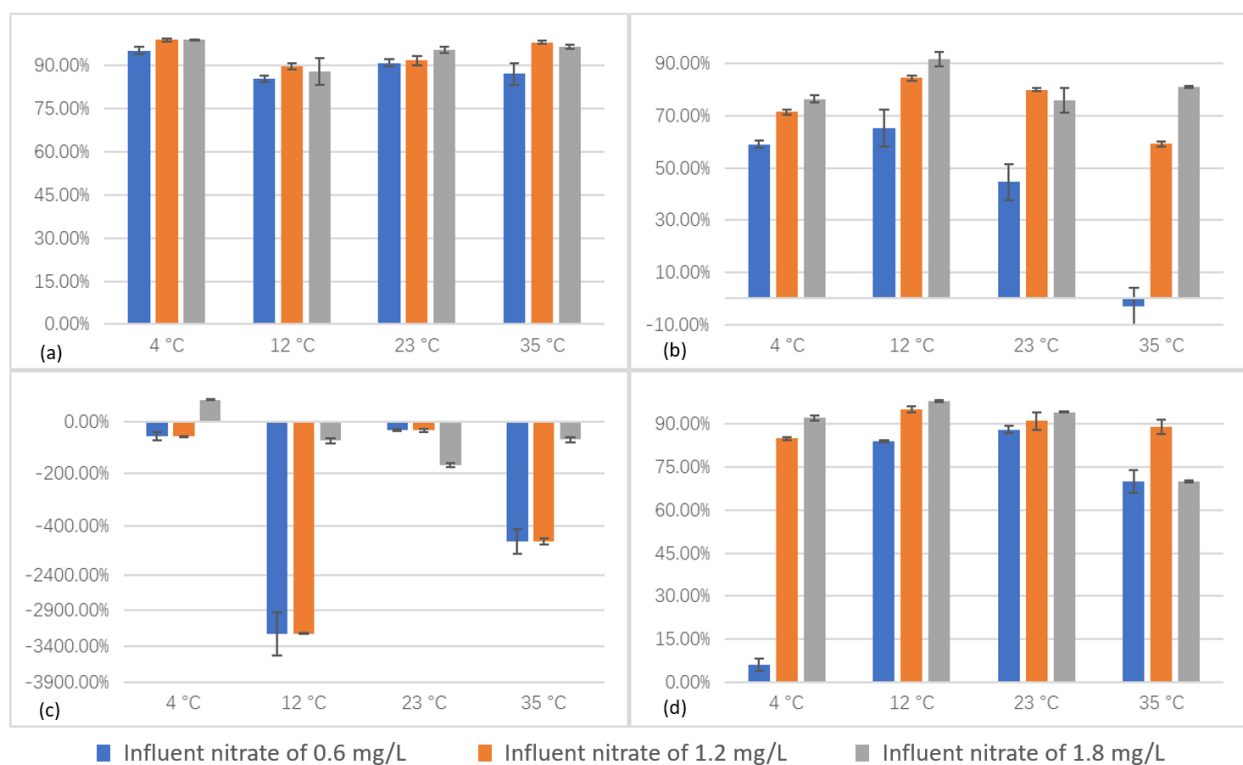
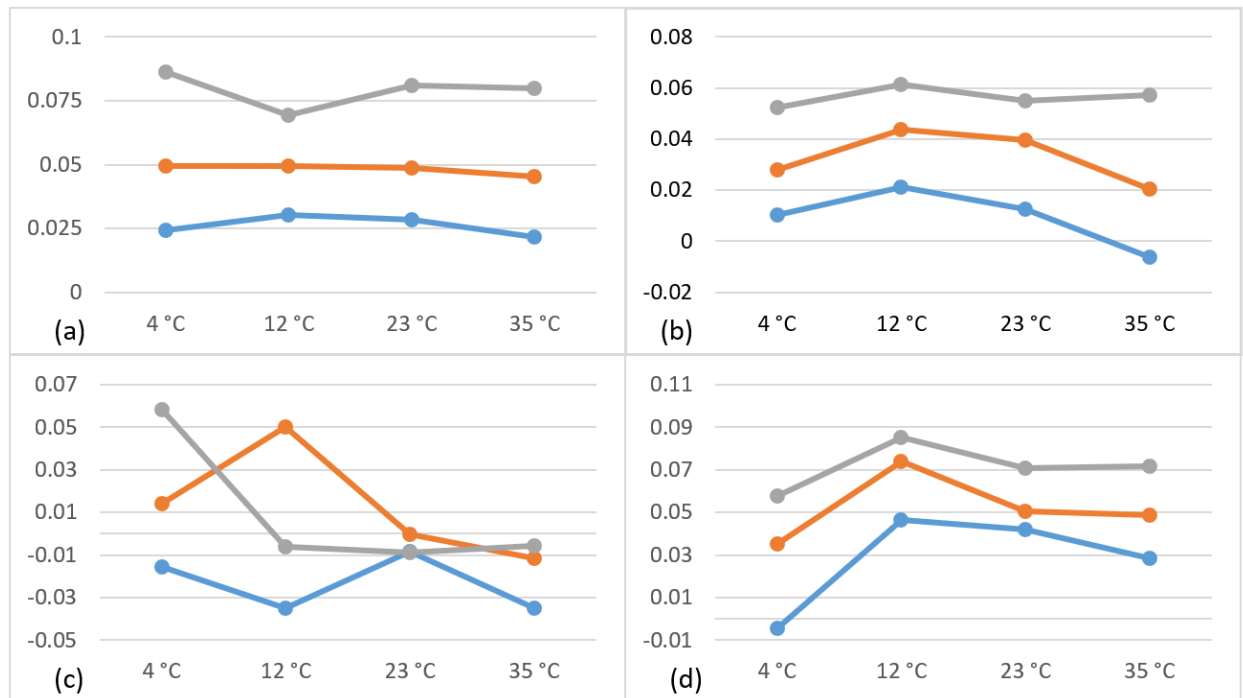


Figure 50. Overall nitrate removal from column A to D (Column A is IFGEM-1, Column B is IFGEM-1 with BAM, Column C is natural soil, and Column D is IFGEM-2) as shown from (a) to (d) under three influent nitrate concentration levels (0.6, 1.2, 1.8 mg/L) and four temperature conditions (4, 12, 23, 35°C)



■ Influent nitrate of 0.6 mg/L ■ Influent nitrate of 1.2 mg/L ■ Influent nitrate of 1.8 mg/L

Figure 51. Nitrate removal rate constants with the best associated reaction kinetics for (a) Column A: Zero order; (b) Column B: Zero order; (c) Column C: Second order; (d) Column D: First order (Column A is IFGEM-1, Column B is IFGEM-1 with BAM, Column C is natural soil, and Column D is IFGEM-2).

6.3.2 Phosphorous Removal and Reaction Kinetics

The overall total phosphorus (TP) removal from columns A to D are shown in Figure 52, which shows the TP removal under various temperatures for each influent concentration. The variation of influent concentration seems to affect TP removal effects more in column A, with the highest TP removal (over 80%) observed under higher influent nutrient concentration. For column D (IFGEM-2), both temperature and influent concentration were crucial to TP removal. The best temperature for TP removal in column D was 12 °C on average, while the highest influent concentration was favored for enhancing the TP removal. For column B, temperature impact was as significant as influent nutrient concentration levels. 12 °C was also the most appropriate

temperature for TP removal in column B and the highest removal, 64%, happened under the highest influent concentration. Column C, with natural soil, showed significant leaching of TP under most scenarios because the soil from the stormwater basin is highly contaminated with nutrients ¹⁵.

The phosphorus adsorption rate constants of the four columns are shown in Figure 53. The rate constants were selected, based on the most suitable reaction orders, as zero order for column A, B, and D, and first order for column C. For column A, the rate constants increased as the influent concentrations became larger, and the best temperature for influent concentration levels 1 and 2 was 12 °C. For column B, the rate constants were higher when the inlet TP was more concentrated under most temperature scenarios, and the best temperature was 12 °C. For column C, apart from the 4 °C scenarios, all cases exhibited negative removal as the phosphorus was released from the natural soil into the water instead of being adsorbed by the soil. This type of leaching phenomenon of phosphorus from natural soil is not uncommon in Florida. Column D showed generally lower rate constants when compared with those for column A; the influent concentration impacts were similar, although higher influent concentration at inlet triggered faster reaction rate. However, the temperature impact on these rate constants is negligible when compared to those results from columns A and B.

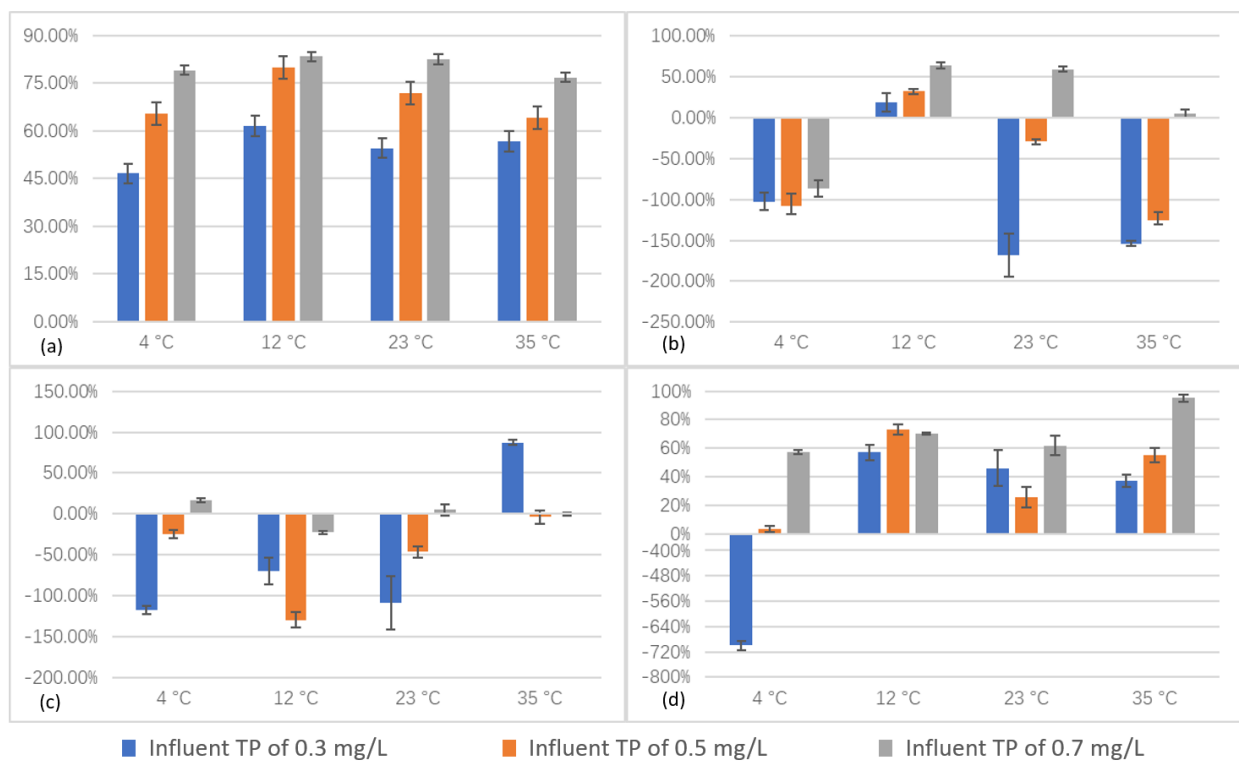


Figure 52. Overall TP removal from columns A to D as shown from (a) to (d) under three influent TP concentrations (0.3, 0.5, 0.7 mg/L) and four temperature conditions (Column A is IFGEM-1, Column B is IFGEM-1 with BAM, Column C is natural soil, and Column D is IFGEM-2).

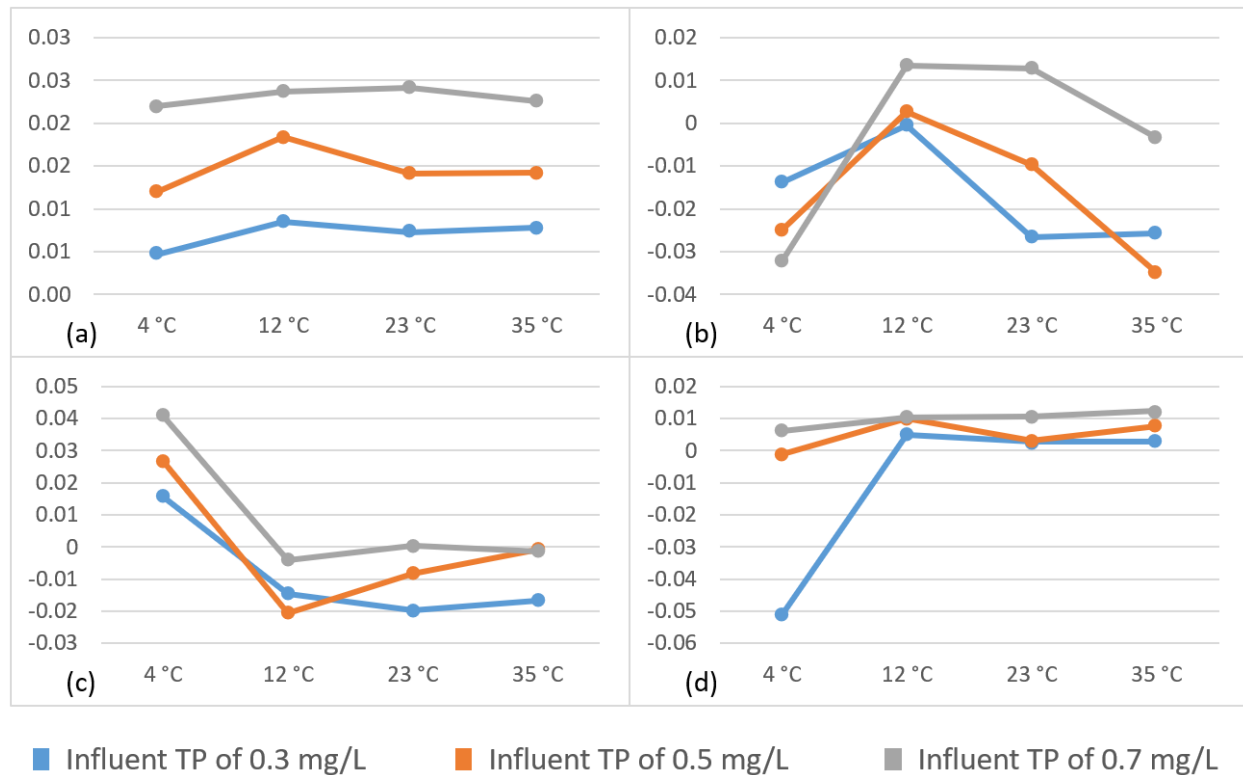


Figure 53. TP removal rate constants with the best associated kinetics for (a) Column A: zero order; (b) Column B: Zero order; (c) Column C: First order; (d) Column D: Zero order (Column A is IFGEM-1, Column B is IFGEM-1 with BAM, Column C is natural soil, and Column D is IFGEM-2).

6.3.3 Temperature Correction Factor

The temperature correction factor (θ) for each column under different nutrients and temperature conditions was calculated using the following methodology. The nitrate and phosphorus removals for each column were plotted separately. Since the initial influent concentrations were different from each other, the data from each run needed to be plotted individually. Then the linear regression was computed to select the reaction rates of best fit.

The temperature correction factor (θ) between each paired temperature can be obtained by inserting the values of rate constants and their corresponding temperatures. The average value of the temperature correction factor (θ) is also included. The values of 1, 2, 3, and 4 stand for the

temperatures 4, 12, 23, and 35 °C, respectively, along with the kinetic values associated with those temperatures. For example, θ_{12} represents the temperature correction factor determined with rate constant values under 4 and 12 °C. Based on the nutrient removal performance and the consistency of kinetic dynamics, column C is out of comparison here due to its poor removals and chaotic kinetic fluctuations. All the temperature correction factors for paired temperature scenarios under each concentration level for columns A, B, and D are summarized in Table 21.

Table 21. Temperature conversion factors under three concentration levels for Column A (IFGEM-1), Column B (IFGEM-1+BAM), and Column D (IFGEM2)

Column #	Factor	Concentration 1		Concentration 2		Concentration 3	
Nutrient species		Nitrate	TP	Nitrate	TP	Nitrate	TP
Column A	θ_{12}	1.0280	1.0769	1.0005	1.0549	0.9736	1.0093
	θ_{13}	1.0084	1.0234	0.9991	1.0085	0.9967	1.0048
	θ_{14}	0.9964	1.0165	0.9971	1.0054	0.9976	1.0009
	θ_{23}	0.9944	0.9863	0.9982	0.9761	1.0139	1.0015
	θ_{24}	0.9856	0.9963	0.9960	0.9888	1.0061	0.9979
	θ_{34}	0.9775	1.0055	0.9940	1.0006	0.9990	0.9947
Column B	θ_{12}	1.0951	0.6424	1.0572	N/A	1.0203	N/A
	θ_{13}	1.0103	1.0353	1.0184	0.9518	1.0028	-0.9525
	θ_{14}	-0.9836	1.0203	0.9901	0.9387	1.0030	0.9311

Column #	Factor	Concentration 1		Concentration 2		Concentration 3	
Nutrient species		Nitrate	TP	Nitrate	TP	Nitrate	TP
	θ_{23}	0.9528	1.4651	0.9911	-1.1233	0.9904	0.9945
	θ_{24}	-0.9475	1.1984	0.9678	-1.0112	0.9970	-0.9430
	θ_{34}	N/A	0.9968	0.9470	0.9183	1.0031	N/A
Column D	θ_{12}	N/A	N/A	1.0966	N/A	1.0498	1.0677
	θ_{13}	-1.1261	-0.8569	1.0188	-1.0595	1.0108	1.0290
	θ_{14}	-1.0620	-0.9118	1.0105	-1.0681	1.0069	1.0229
	θ_{23}	0.9906	0.9473	0.9657	0.8955	0.9833	1.0017
	θ_{24}	0.9787	0.9775	0.9883	0.9883	0.9925	1.0077
	θ_{34}	0.9679	1.0060	1.0817	1.0817	1.0009	1.0133
<p>N/A: mathematically unable to calculate;</p> <p>The negative values indicate one of the rate constants is negative.</p>							

6.3.4 pH Impacts on Nitrate and Phosphorus Adsorption

The isotherm study results of IFGEM-1 and IFGEM-2 for phosphorus adsorption under three initial pH conditions are shown in Figure 41. The lower the pH value, the more phosphorus can be adsorbed by both IFGEM-1 and -2. However, the impacts from the enhancement of pH was more significantly observed in IFGEM-1 rather than IFGEM-2. The Langmuir and Freundlich isotherm equation parameters of IFGEM-1 and IFGEM-2 are shown from Table 3 to Table 6. As most $1/q_m$

values are negative in the Langmuir equation, it is inappropriate to apply for the calculation of maximum adsorption capacity (q_m). Therefore, the Freundlich relative adsorption capacity is selected from Table 4 and Table 6. The phosphorus adsorption capacity of IFGEM-1 and IFGEM-2 exhibited higher levels in acidic solutions. When the pH value increased from 2 to 10, both IFGEM-1 and IFGEM-2 tended to lose part of their adsorption capacity.

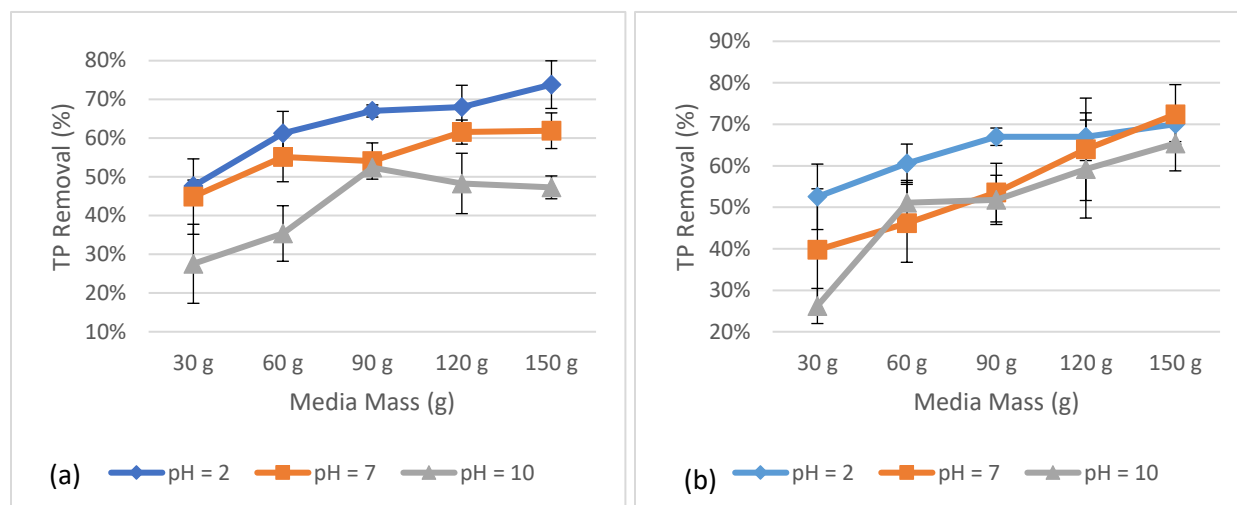


Figure 54. The isotherm study for TP removal based on IFGEM-1 (The same as the media in Column A) (a) and IFGEM-2 (The same as the media in Column D) (b) under various pH conditions

Table 22. Phosphorus Adsorption Parameters of the Langmuir isotherm for IFGEM-1 (The same as the media in Column A) and IFGEM-2 (The same as the media in Column D) under various pH conditions

pH value	IFGEM recipes	Isotherm equation for Langmuir	R-square value	$1/(q_m K_{ads})$	$1/q_m$ (mg/g)
2	IFGEM-1	$y = 259.09x - 305.7$	0.9336	259.09	-305.7
	IFGEM-2	$y = 409.28x - 700.71$	0.9145	409.28	-700.71
7	IFGEM-1	$y = 628.74x - 938.59$	0.8352	628.74	-938.59
	IFGEM-2	$y = 190.75x + 52.554$	0.7635	190.75	52.554
10	IFGEM-1	$y = 765.92x - 681.48$	0.7014	765.92	-681.48
	IFGEM-2	$y = 268.13x - 20.922$	0.7951	268.13	-20.922
$x = 1/C_e$; $y = 1/q_e$ in which C_e is the aqueous concentration of phosphorus (mg/L), and q_e is the phosphorus concentration sorbed on the media (mg/g).					

Table 23. Phosphorus Adsorption Parameters of the Freundlich isotherm for IFGEM 1 (The same as the media in Column A) and IFGEM-2 (The same as the media in Column D) under various pH conditions

pH value	IFGEM recipes	Isotherm equation for Freundlich	R-square value	1/n	Log K	K (mg ^{1-(1/n)} L ^{1/n} g ⁻¹)
2	IFGEM-1	$y' = 1.7809x' - 1.8089$	0.9564	1.7809	-1.8089	0.0155
	IFGEM-2	$y' = 50.563x' - 0.5833$	0.9534	50.563	-0.5833	0.2610
7	IFGEM-1	$y' = 3.1346x' - 1.5403$	0.86	3.1346	-1.5403	0.0288
	IFGEM-2	$y' = 1.0972x' - 2.2837$	0.7342	1.0972	-2.2837	0.0052
10	IFGEM-1	$y' = 2.1192x' - 2.2866$	0.7799	2.1192	-2.2866	0.0052
	IFGEM-2	$y' = 0.9594x' - 2.4206$	0.7306	0.9594	-2.4206	0.0038
$x' = \log(C_e)$; $y' = \log(q_e)$ in which C_e is the aqueous concentration of phosphorus (mg/L), and q_e is the phosphorus concentration sorbed on the media (mg/g).						

The isotherm study results of IFGEM-1 and IFGEM-2 for nitrate reduction under three different pH conditions are shown in Figure 42, in which the corresponding ammonia generation can be seen. For IFGEM-1, the best removal was achieved when initial pH = 7, and the ammonia generation was also optimized under neutral pH; on the contrary, both nitrate removal and ammonia generation decreased when the initial pH was off neutral. For IFGEM-2, the best nitrate removal happened when the initial pH = 2, with higher ammonia generation under most scenarios. When the pH increased, nitrate removal and ammonia generation decreased. The Langmuir and Freundlich isotherm equation parameters of IFGEM-1 and IFGEM-2 are shown in Table 5 and Table 6. As most $1/q_m$ values are negative in the Langmuir equation in Table 5, it is inappropriate

to apply for the calculation of maximum adsorption capacity (q_m). Therefore, the Freundlich relative adsorption capacity is selected from Table 6 for applications.

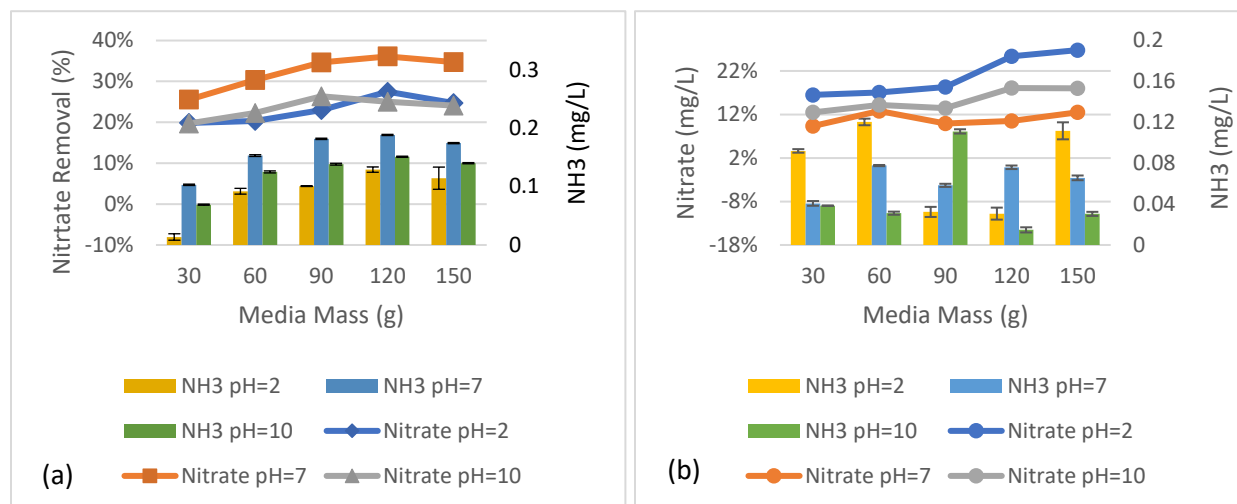


Figure 55. The nitrate removal and ammonia generation from the isotherm study on (a) IFGEM-1 (The same as the media in Column A) and (b) IFGEM-2 (The same as the media in Column D) under various pH conditions

Table 24. Nitrate adsorption parameters of the Langmuir isotherm for IFGEM-1 (The same as the media in Column A) and -2 (The same as the media in Column D) under various pH conditions

pH value	IFGEM recipes	Isotherm equation for Langmuir	R-square value	$1/(q_m K_{ads})$	$1/q_m$ (mg/g)
2	IFGEM-1	$y = 7554.6x - 7966.9$	0.5281	7554.6	-7966.9
	IFGEM-2	$y = 5358.5x - 4598.6$	0.2117	5358.5	-4598.6
7	IFGEM-1	$y = 3754.5x - 4216.4$	0.7158	3754.5	-4216.4
	IFGEM-2	$y = 4924.3x - 4456.2$	0.1362	4924.3	-4456.2
10	IFGEM-1	$y = 8491.2x - 8153.6$	0.3644	8491.2	-8153.6
	IFGEM-2	$y = 15821x - 15443$	0.5845	15821	-15443
$x = 1/C_e$; $y = 1/q_e$. Where C_e is the aqueous concentration of nitrate (mg/L), and q_e is the nitrate concentration sorbed on the media (mg/g).					

Table 25. Nitrate adsorption parameters of the Freundlich isotherm for IFGEM-1 (The same as the media in Column A) and IFGEM-2 (The same as the media in Column D) under various pH conditions

pH value	IFGEM recipes	Isotherm equation for Freundlich	R-square value	1/n	Log K	K (mg ^{1-(1/n)} L ^{1/n} g ⁻¹)
2	IFGEM-1	$y' = 9.5316x' - 2.2419$	0.5691	9.5316	-2.2419	0.0057
	IFGEM-2	$y' = 5.9821x' - 2.8225$	0.2768	5.9821	-2.8225	0.0015
7	IFGEM-1	$y' = 7.3878x' - 1.9373$	0.8565	7.3878	-1.9373	0.0116
	IFGEM-2	$y' = 7.7666x' - 3.2904$	0.0538	7.7666	-3.2904	0.0005
10	IFGEM-1	$y' = 12.039x' - 2.558$	0.5470	12.039	-2.558	0.0028
	IFGEM-2	$y' = 11.872x' - 2.7809$	0.5542	11.872	-2.7809	0.0017
$x' = \log(C_e)$; $y' = \log(q_e)$ in which C_e is the aqueous concentration of nitrate (mg/L), and q_e is the nitrate concentration sorbed on the media (mg/g).						

6.3.5 Competitive Adsorption

The study of competitive adsorption between nitrate and phosphorus was performed when the concentrations of each nutrient were equivalent (~ 1 mg/L) and co-existed in the solution. The results for IFGEM-1 and IFGEM-2 are summarized in Figure 56 and Figure 57. The competitive

adsorption effects between the two nutrient species were confirmed. In IFGEM-1, the average nitrate and phosphorus adsorption on unit mass of media decreased by 38.80% and 60.22%, respectively. For IFGEM-2, the average nitrate and phosphorus adsorption on unit mass of media decreased by 32.88% and 33.33%, respectively.

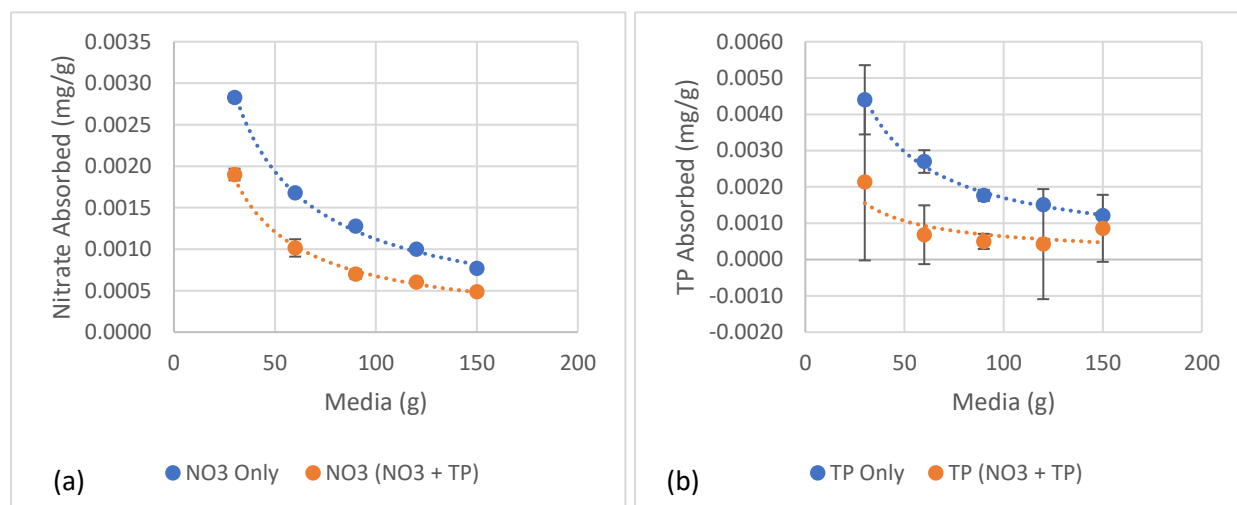


Figure 56. The isotherm study for competitive nutrient adsorption (The same as the media in Column A) on IFGEM-1 between (a) nitrate and (b) phosphorus

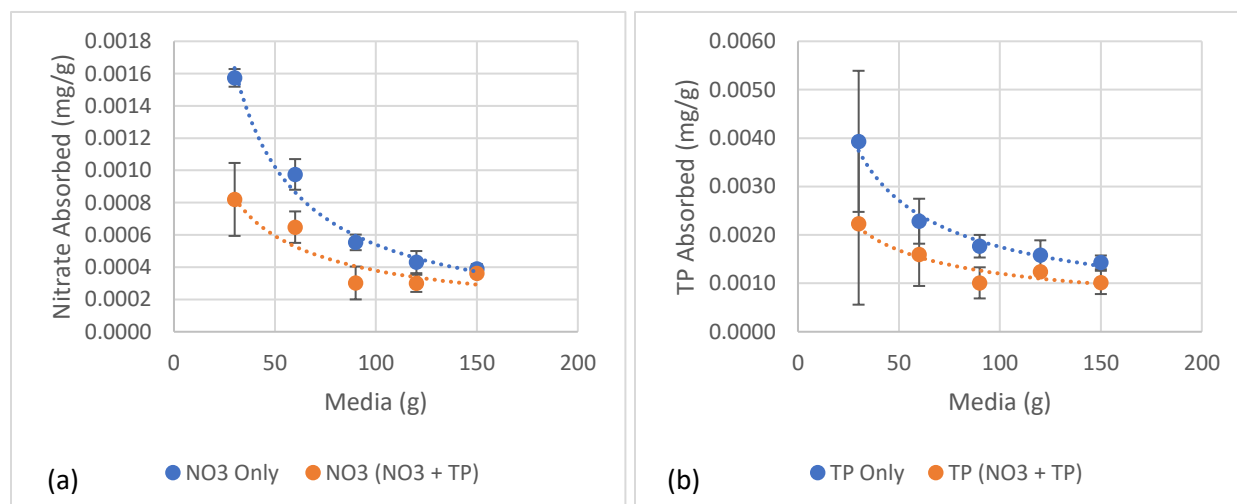


Figure 57. The isotherm study for competitive nutrient adsorption on IFGEM-2 (The same as the media in Column D) between (a) nitrate and (b) phosphorus

6.3.6 Adsorption Spontaneity

The changes of Gibbs free energy were quantified to examine the spontaneity of adsorption/reaction for nutrients removal in IFGEM-1 and IFGEM-2 under room temperature (~ 22 to 23°C). The values of Gibbs free energy from our isotherm study were calculated through equations 4 and 5 and are shown in Table 26 and Table 27. Both IFGEM recipes tended to enhance the adsorption/reaction spontaneity when the applied media mass increased for nitrate and phosphorus removal. In IFGEM-1, the nitrate removal/reduction was more likely to happen in neutral pH value, while the acidic and basic initial conditions required more energy to initiate the reaction. For phosphorus adsorption, the adsorption spontaneity increased with the decrement of pH value. For IFGEM-2, nitrate removal/reduction was less spontaneous since it required more energy to initiate the reaction under all pH levels. Nevertheless, the phosphorus removal achieved stronger spontaneity under acidic conditions than neutral or basic conditions.

Table 26. The change of Gibbs free energy (J/mole) with various media mass and pH values under room temperature in IFGEM-1 (The same as the media in Column A)

Initial pH	Nutrients	30g	60g	90g	120g	150g
2	Nitrate	3448.03	3371.85	2979.88	2395.71	2752.66
	Phosphorus	235.35	-1127.85	-1749.45	-1864.35	-2558.38
7	Nitrate	2642.86	2050.91	1569.03	1409.63	1559.18
	Phosphorus	505.69	-505.69	-404.04	-1163.29	-1198.84
10	Nitrate	3465.86	3080.30	2536.31	2707.32	2839.55
	Phosphorus	2387.38	1488.06	134.41	168.04	67.19

Table 27. The change of Gibbs free energy (J/mole) with various media mass and pH under room temperature in IFGEM-2 (The same as the media in Column D)

Initial pH	Nutrients	30g	60g	90g	120g	150g
2	Nitrate	4003.15	3908.96	3697.92	2667.20	5009.01
	Phosphorus	-249.63	-1063.71	-1749.07	-1749.07	-2096.16
7	Nitrate	4446.88	3817.29	4288.10	4177.18	3819.85
	Phosphorus	2184.32	1716.06	1424.94	968.05	603.72
10	Nitrate	4750.35	4385.02	4546.80	3690.56	3695.60
	Phosphorus	2555.43	-111.89	-175.88	-921.54	-1569.06

6.4 Discussion

6.4.1 Temperature and Influent Concentration Impacts on Nutrient Removal

Both IFGEM recipes showed good nitrate removal under various temperature and influent concentrations when compared with columns B and C (Figure 50). The main reason is that nitrate reduction is enhanced by iron filing, which is sufficient to overcome most negative influences from the changes of temperature and influent concentration. Column A with IFGEM-1 performed slightly better than column D with IFGEM-2 under the impact of temperature fluctuation as, unlike the direct contact between nitrate ion and iron filing in IFGEM-1, IFGEM-2 is deeply dependent on the clay content to enhance the nitrate removal by attracting nitrate ions to the surface of iron filing¹⁸². However, the increased temperature also enhances the escape probability of nitrate ions by providing more energy to them, as shown in Figure 58. As a result, nitrate equilibrium concentration (liquid phase) in IFGEM-2 increased when the temperature was changed from 4 to

35 °C. It is well known that increasing temperature usually causes the enhancement of reaction speed, such as the change from 4 to 12 °C for both IFGEM recipes in Figure 51. However, further temperature increase cannot promote the reaction speed from 12 to 35 °C, because the change of equilibrium concentration offsets the benefit from the temperature increase. Thus, the overall nitrate removal performance depends on finding the balanced temperature point that achieves the optimal configuration of equilibrium concentration and reaction speed. Based on the experiment, 12 °C seems to be the optimal temperature for highest reaction speed and acceptable nitrate equilibrium concentration. Similar results have been observed by other researchers when removing nitrate and nitrite with the help of Fe^0 ²⁰⁴, because the higher temperature also increases the pH value and inhibits the removal rate. The nitrate removal of column B was generally lower than columns A and D because the middle and bottom sections of column B were filled with BAM, which has no iron filing for enhancing the nitrate reduction reaction for the whole column. The chaotic performance of column C indicates that the natural soil was saturated with multiple contaminants, which can be released back into the treated water if the nutrient concentration is lower in liquid phase; basically, the collected natural soil has lost its potential for removing nitrate from the stormwater runoffs. Overall, IFGEM-1 is less vulnerable than IFGEM-2 in terms of the temperature impact, which makes it appropriate to apply in wide areas under different temperatures with less demand of ammonia removal because of the absence of clay. IFGEM-2 is preferable in areas with ambient 12 °C environment and a high expectation of simultaneous nitrate and ammonia removal.

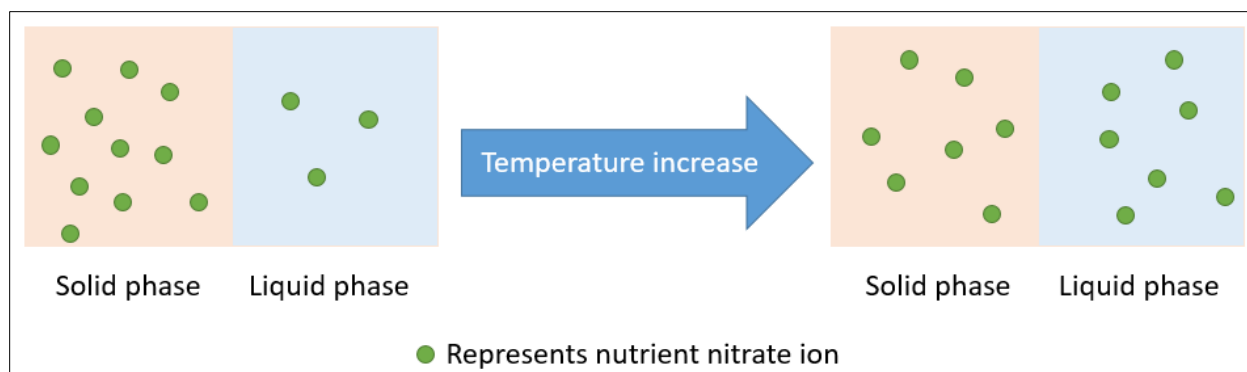


Figure 58. The schematic diagram of equilibrium concentration changes with temperature fluctuation in both IFGEM recipes

Similar to the nitrate removal, higher influent concentration triggers better TP removal for both IFGEM recipes (Figure 52). This is due to the increased chance for direct contact between the nutrient and the media when the solution is highly concentrated. Overall, IFGEM-1 performs better for gaining more stable TP removal than IFGEM-2 across different temperature regimes. There are mainly two reasons, as shown in Figure 59. One reason is closely related to the nitrate reduction process which produces Fe(II) and Fe(III) that can form precipitation with phosphorus as shown in Eqs. 7 - 10. The depressed nitrate reduction reaction under increased temperature, as stated in the previous section, may result in deficiency associated with ferric/ferrous ion for effective phosphorus removal through precipitation. The second reason is that increased temperature may trigger dissociation of phosphorus precipitates such as $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ ²⁰⁵. As a result, the TP equilibrium concentration increases and eventually the TP removal is depressed. Another possible reason might be the larger drop of ORP in IFGEM-2 (Figure 60) due to the more intensive nitrate reduction reaction, as ORP could exhibit a positive correlation with iron bonded phosphorus adsorption¹⁶⁹. Columns B and C showed mostly negative TP removal because of the absence of iron-related phosphorus in the latter two section of column B, and the phosphorus saturated natural soil that filled the entirety of column C. It can be observed that in most scenarios,

the temperature correction factors of both nutrient species are less than 1 when comparing any paired temperatures (Table 2). This indicates that the rate constant of nutrient removal under 12 °C is the highest one for each influent concentration. Overall, IFGEM-1 is preferable for TP removal under a larger temperature range from 4 to 35 °C, while IFGEM-2 is a better option for nitrogen and phosphorus removal at the same time when the ambient temperature is around 12 °C

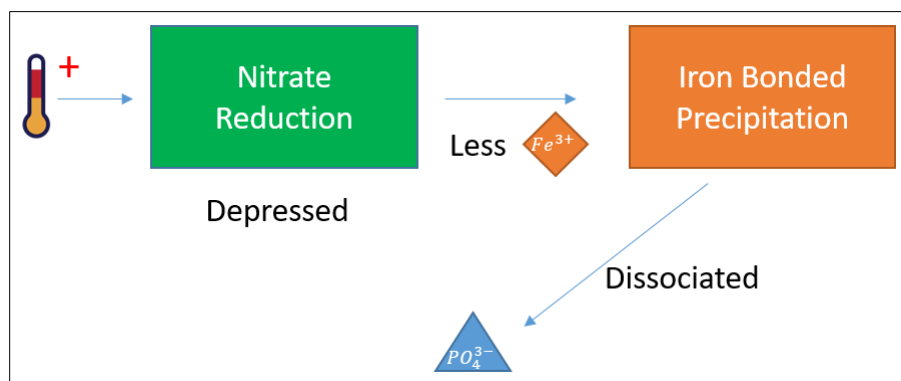


Figure 59. Schematic diagram of temperature impacts on TP removal in IFGEM

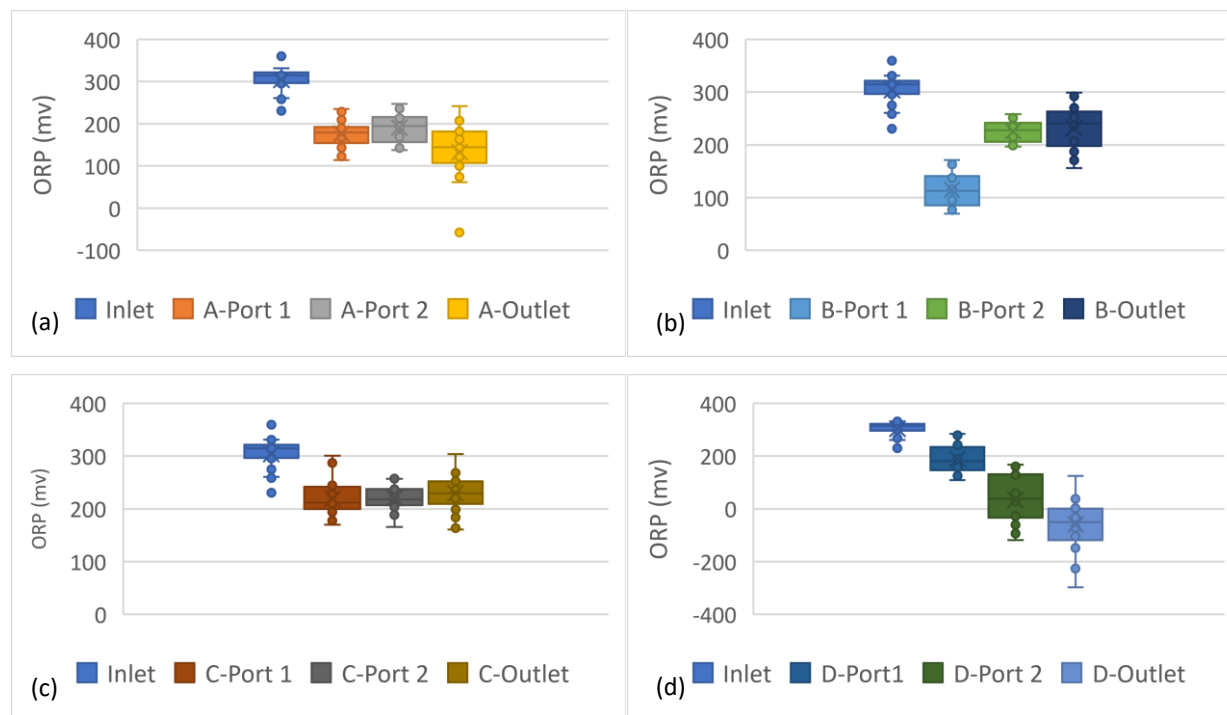
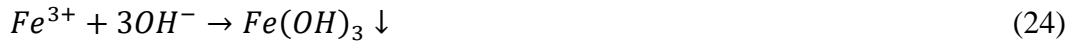
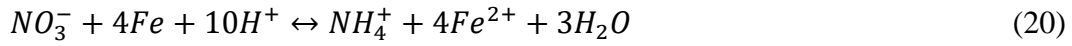


Figure 60. ORP values of each sample location from (a) column A to (d) column D in column study

6.4.2 pH Impacts and Competitive Adsorption

The impacts of pH variation on nutrient removal in IFGEM were evaluated using the isotherm study, in which nitrate and phosphorus were included in the test sequentially under pH values of 2, 7, and 10. Both the Langmuir and Freundlich models were applied to search for the best fit based on the collected data. However, Langmuir model parameters were not applicable due to its assumption of monolayer reactive sites that may lose reactivity once occupied, and this assumption is not suitable for both IFGEM recipes as iron filing can provide electrons layer by layer continuously. Both IFGEM recipes tended to show decrement in phosphorus adsorption as the pH value increased (Figure 41). The reason for such a trend might be the status differences of iron-based ion under different pH values. When the initial pH is acidic, the dominant dissolved iron ion is Fe(III) or Fe(II), which may strengthen the phosphorus precipitation (Eqs. 21 to 24) as the spontaneity of the phosphorus removal is enhanced due to the increased availability of Fe(III) or Fe(II) (Table 26 and Table 27). However, when the solution turns into the basic condition, the iron ion tends to form $\text{Fe}(\text{OH})_3$ with low solubility, resulting in less Fe(III) or Fe(II) available for phosphate precipitation. Another reason might be due to the competition between anions of OH^- and phosphate for adsorption sites. The higher the pH value is, the more OH^- there is to compete with the phosphate for the positive adsorption site¹⁶⁸. Other researchers have found that the phosphate and nitrate removal decreases continually when the pH increases using lanthanum based nanocomposite²⁰², and magnetic iron composites²⁰⁶. OH^- is the main competitor for the adsorption spots on the surface of sorption media. Equivalently, from the Gibbs free energy perspective, more energy is required for initializing the reaction (Table 26 and Table 27). Note that IFGEM-1 was generally better than IFGEM-2 in phosphorus removal under all pH conditions

in the isotherm study, and the same result can be observed from the column study as well (Figure 52 and Figure 53). The performance of IFGEM-1 was more effective and stable for phosphorus removal. This could be interpreted through the ORP values as well (Figure 60). In a certain range of pH, ORP could exhibit a positive correlation with iron bonded phosphorus adsorption¹⁶⁹. Since the ORP drops faster in IFGEM-2 than IFGEM-1, as shown in Figure 60, IFGEM-1 is more likely to be applied for phosphorus removal than IFGEM-2.



Comparatively, IFGEM-1 and IFGEM-2 act differently in nitrate reduction and ammonia generation under different pH values. As shown in Figure 42 (a), IFGEM-1 removes most nitrate and generates more ammonia under a neutral condition. Either the increase or the decrease of pH value in the solution would negatively impact its nitrate removal and ammonia generation. This process is graphically depicted in Figure 61 (a). Since nitrate reduction requires close contact between iron surface and nitrate ion, highly concentrated hydrogen ions in acidic solution can compete with the nitrate ion for obtaining electrons from zero valent iron, and the dominate reaction in this scenario would be Eq. 8. When it comes to basic solution, similar competition was observed between highly concentrated hydroxyl ion and nitrate ion, both of which are anion.

Conversely, when nitrate is attracted by clay on the surface, it is more about adsorption rather than reactions when compared to the acidic condition. Different from IFGEM-1, acidic solution is preferred by IFGEM-2, as shown in Figure 42 (b). Clay cannot adsorb a huge amount of nitrate, but it can adsorb nitrate to form a skinny layer with much higher nitrate concentration to interact with iron filing, which helped achieve excellent nitrate removal in our column study. The existence of clay in IFGEM-2 that interacts with pH fluctuations is graphically described in Figure 61 (b). When the initial pH = 2 in the solution, the hydrogen ions occupy the negative charged sites on clay which makes the surface of clay more positively charged, making clay become more attractive to nitrate ion and enhancing the nitrate reduction process²⁰⁷. However, the opposite happens when changing the initial pH to 10 in the solution. When the highly concentrated hydroxyl ions occupy the positive charged sites on the surface of clay, then the nitrate ion is repulsed as it is an anion, resulting in lower nitrate reduction.

Even though the two IFGEM recipes behave differently for nitrate reduction and removal when pH increases from 2 to 10, both follow the same trend for phosphorus removal. The reason for this outcome is that both IFGEM recipes share the same mechanisms of adsorption/adsorption and precipitation for phosphorus removal. Therefore, IFGEM-1 is preferable for treating water with neutral pH values for the purpose of optimizing the nitrate removal, but IFGEM-2 could be an alternative for treating acidic water to optimize both nitrate and phosphorus removal. Note that the more nitrate can be removed/reduced in both IFGEM recipes, the more ammonia shall be generated and even recovered as the spent IFGEM may have a lot of nutrients and may be used for soil amendment. Generally speaking, IFGEM-2 has a better ammonia generation and recovery capability due to the inclusion of the clay component.

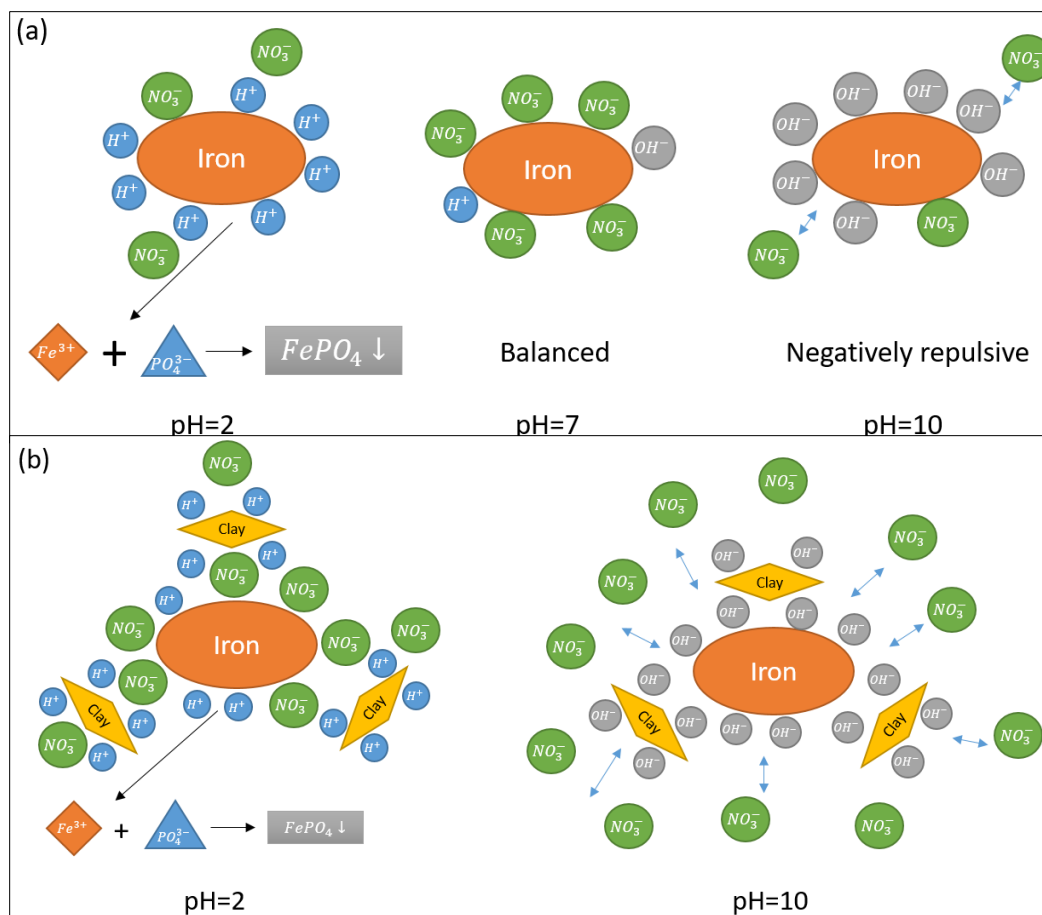


Figure 61. pH impacts on nitrate removal/reduction in (a) IFGEM-1 and (b) IFGEM-2

The competitive adsorption test was conducted in a batch-mode isotherm study for both IFGEM recipes, so that the nitrate and phosphorus removal capability could be compared in between scenarios containing singular nutrient species or multiple nutrient species. Note that the batch mode study has very different hydraulic conditions when compared with the continuous flow in a column study. In our isotherm study, the nutrients cannot flow in or out as the batch mode is basically a closed system. For IFGEM-1 (Figure 56), when both nutrients coexist in the solution it seems that phosphorus is less competitive than nitrate. This may be confirmed by the fact that the removal of phosphorus reduces by 60.22%, while the removal of nitrate only reduces 38.80%. As shown in Figure 62 (a), even though phosphorus is in competition with nitrate for reactive sites on

iron filings, phosphorus removal is partially dependent on the precipitation with ferric/ferrous ions which are produced through nitrate reduction reaction with iron filings. As a result, the decrement of nitrate reduction due to the competitive effects from phosphorus would cancel out the possibility for phosphorus removal limited by less precipitation reactions. However, nitrate and phosphorus show equivalent competition effects in IFGEM-2 (Figure 56), as it exhibits similar decrease of removal efficiencies for both nutrient species. As shown in Figure 62 (b), clay is the major factor that changes the balance in such a competition, as clay is very well known for phosphorus removal^{208, 209} that works as compensation to the decrement in phosphorus precipitation. Note that the batch-mode study provides one more aspect to examine the mechanism of competitive nutrients removal; however, it could be very different in continuous mode due to the significant change in hydraulic conditions, flow speed at the intersection between solid and liquid, nutrients concentration patterns, and so on.

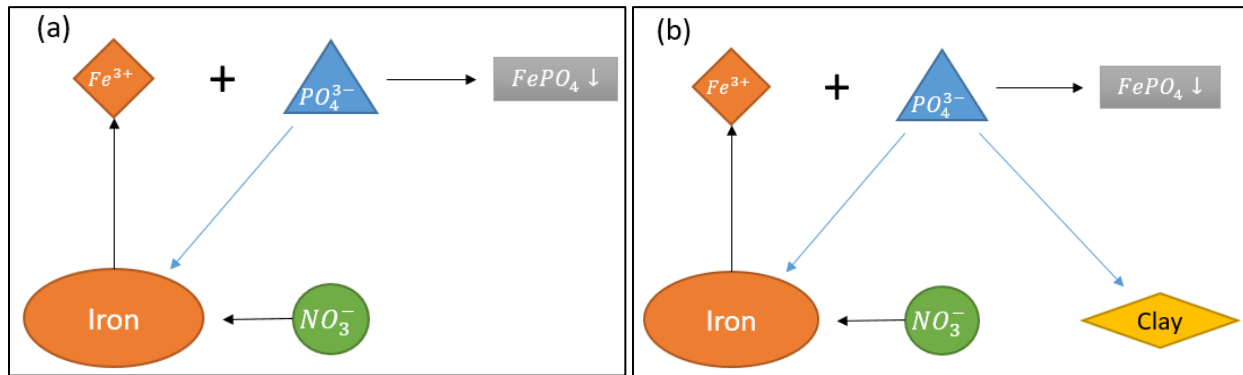


Figure 62. Competitive adsorption between nitrate and phosphorus in (a) IFGEM-1 and (b) IFGEM-2

6.5 Summary

This study deepened the understanding of the applicability of IFGEM for the treatment of stormwater runoff, which may be extended to treat wastewater effluent and agricultural discharge

in the future. Based on the previous study of nutrient removal effectiveness, two IFGEM recipes were evaluated for the impact factors of temperature, pH, and competition between nutrient species. Both IFGEM recipes showed great nitrate and phosphorus reduction due to the iron filing, as both the reactive electron donor and the precipitation reactant. The performance of IFGEM-1 is more stable than IFGEM-2 for nitrate removal when temperature increases from 4 to 35 °C, because IFGEM-2 is more sensitive to temperature fluctuations due to the decreased affinity between nitrate ion and clay, as the nitrate ion has more energy to escape. This introduced a cascade effect of the same trend of TP removal for both IFGEM recipes because TP removal is partially reliant on nitrate reduction to provide ferrous/ferric ions. Additionally, the increased temperature would trigger the dissociation of iron bonded phosphorus precipitation for both IFGEMs. The pH impacts on nitrate reduction in IFGEM-1 and IFGEM-2 are different. IFGEM-1 performs best under neutral pH conditions, while IFGEM-2 performs best under acidic conditions due to the existence of clay in IFGEM-2. However, both IFGEM recipes behave better for phosphorus removal under acidic conditions as more ferric/ferrous ions are available. The competition between nitrate and phosphorus does exist, but phosphorus removal is also partially dependent on the nitrate reduction reaction in IFGEM-1; however, clay in IFGEM-2 adsorbs additional phosphorus as compensation to the decrement of nitrate reduction.

Overall, the applicability of IFGEM recipes has been thoroughly evaluated through different environmental factors. IFGEM-1 is appropriate to be applied in different areas with wide temperature variations for nitrate and TP removal while not restricting standards for ammonia. IFGEM-1 is preferred for treating neutral pH water for optimizing nitrate removal/reduction process to achieve relatively higher phosphorus removal albeit lower achievement for ammonia

removal and recovery. IFGEM-2 is good for application in certain areas with suitable ambient temperature around 12 °C for simultaneous treatment of nitrate and ammonia, but less demand for phosphorus, because it is appropriate for treating stormwater runoff, agricultural discharge, and wastewater effluents with acidic or neutral pH condition for simultaneous removal of phosphorus and nitrate albeit lower achievement for phosphorus removal.

CHAPTER 7: CONCLUSION

The central contribution of this study is tied to deepen our understanding of nutrient removal and recovery potential via green sorption media (BAM and IFGEM) for stormwater and groundwater quality control in the built environment and improve the sustainability of nitrogen cycle in natural system. While both BAM and IFGEM are deemed as green sorption media, their performance is different in terms of the ability to remove both nitrogen and phosphorus simultaneously. The former presents amenable environment to form biofilm and remove nitrogen effectively with essential function and structure in a microbial ecosystem on a long-term basis, whereas the latter has better potential to adsorb/adsorb nitrogen and phosphorus on a short-term basis.

Through the test of those two types of media in terms of their treatability and reliability under changing environmental conditions and factors, BAM was proven more effective than woodchips in a linear ditch located at the Fanning Springs, FL. Such a pioneering effort for the co-treatment of both groundwater and stormwater simultaneously was proven successful. Yet IFGEM that is more suitable as a final polishing unit to receive the wastewater effluent have not yet gained an opportunity to carry out a field-scale pilot study.

The difference of nitrogen availability in BAM triggers different surviving strategies for microorganisms. In the case of low initial TN concentration, the bacteria tend to cultivate themselves with smaller cell size while in the case of high initial TN concentration the bacteria end up with larger cell size. Whereas the former present a larger surface area to volume (SA/V) ratio and metabolic rate, the latter exhibit a smaller surface area to volume (SA/V) ratio and metabolic rate. The ultimate goal is to extract nutrient from the flow as much as possible and makes

their community thrive. The high SA/V ratio is favored for small cells to extract more nutrients effectively in low nutrient-laden flows while the low SA/V ratio is beneficial for larger cells to store and slowly digest what they can absorb from the high nutrient-laden flows. Therefore, LMW-DON is prevalent when smaller cell size bacteria thrive, while more HMW-DON can be utilized by larger cell size bacteria, thus keeping bacteria with larger cells thrive.

When facing changing environmental conditions and factors, carbon addition triggers different growth patterns given different influent nutrient concentrations. The study of carbon impact was enhanced via the cooperation with National High Magnetic Field Laboratory at Florida State University which utilized the FT-ICR-MS characterize the changing distribution of DON in response to varying environmental conditions. When coupled with the information gained from the analysis of species population dynamics with qPCR, the impact of carbon addition on DON distribution can be further elucidated. In fact, the impact of the addition of carbon source on nutrient removal is different when treating stormwater and groundwater; carbon is more important to TN removal in groundwater than that in stormwater *per se*. However, it is noticeable that the carbon addition in this experiment did increase the ammonia concentration through the enhancement of ammonification for both stormwater and groundwater treatment. The most abundant bacteria exist at the top layer are denitrifiers that became the dominant species driven by having more electron donors, and the additional carbon has much less influence at the depth beneath 60 cm in our column.

In addition to carbon impact study, the toxic compound impact driven by the presence of copper was also evaluated. It is indicative that copper is able to enhance the population of denitrifiers, even though the AOB and NOB are depressed. Overall, the bioactivity of the whole

microbial community decreased over time. This observation reveals a key role of copper that plays a role as a cofactor of the enzyme required in the last step of denitrification, and the dosage of copper triggers the enzymatic cascade effects that provide additional 20% of energy to thrive. This discovery will alter the engineering design and planning strategy in future cases for which the enzymatic cascade effects can be utilized to achieve better nutrient removal. However, we only evaluated the copper impact on a short-term basis since stormwater runoff usually ends within less than one day after the storm event. There are cases like wet stormwater detention ponds where long-term contact between BAM and copper might be phenomenal, which is critical for future research.

The comparison between laboratory and field-scale application of the linear ditch study confirms the applicability and reliability of BAM both of which are much higher than those of woodchip. The complex field conditions leading to highly variable inflow rate and nutrient concentrations could compound the final assessment. On one hand, BAM with small porous sizes is easy to maintain a reasonable infiltration rate when compared to woodchip. BAM also exhibits even better nutrient removal capability in the field, because the waste load in field is much higher than the designed level in the laboratory experiment. On the other hand, woodchip cannot improve its hydraulic pattern to favor nutrient removal and the air can easily go into woodchip, which profoundly depressed the denitrification pathway for generating nitrogen gas in an aerobic environment with much less contact time or hydraulic retention time. However, the second denitrification pathway called “dissimilatory nitrate reduction to ammonia” can appear and prevail under such an aerobic condition that would generate a significant amount of ammonia. In general, BAM media are more appropriate for the co-treatment of stormwater and groundwater in space

limited BMP under complicated natural environment and it has no decay issues as the woodchip would in a few years after operation. Also, the woodchip is limited because they are unable to exhibit sustainable traffic bearing capacity while BAM media are traffic bearing along the roadside.

A set of new media mix was finally developed in this study as IFGEM including two recipes (i.e., IFGEM-1 and IFGEM-2). Both IFGEMs showed excellent nitrate reduction due to the inclusion of iron filings as the reactive electron donor. As IFGEM-1 generated more ammonia in the effluent, IFGEM-2 was able to remove and recover almost all generated ammonia. The phosphorus removal was enhanced by the nitrate reduction in IFGEMs given that the iron can bond phosphorus during precipitation; however, IFGEM-1 showed better and more stable phosphorus removal than IFGEM-2 as the ORP decrement in IFGEM-1 is not as phenomenal as that in IFGEM-2. The reliability of both IFGEMs was also evaluated under various temperatures, influent concentrations, and pH values. The removal and reaction involve complex cascade effects that are revealed with solid data support. IFGEM-1 is appropriate to be applied in different areas with wide temperature variations for nitrate and TP removal while no restriction for ammonia recovery. IFGEM-1 is preferred for treating neutral pH water for optimizing nitrate removal/reduction process to achieve relatively higher phosphorus removal albeit lower achievement for ammonia removal and recovery. IFGEM-2 is good for application in certain areas with suitable ambient temperature around 12 °C for simultaneous treatment of nitrate and ammonia, but less demand for phosphorus. It is more appropriate for treating stormwater runoff, agricultural discharge, and wastewater effluents with acidic or neutral pH condition for simultaneous removal of phosphorus and nitrate albeit lower achievement for phosphorus removal.

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Title:

The Impact of Carbon Source as Electron Donor on Composition and Concentration of Dissolved Organic Nitrogen in Biosorption-Activated Media for Stormwater and Groundwater Co-Treatment

Author:

Ni-Bin Chang, Dan Wen, Amy M. McKenna, et al

Publication:

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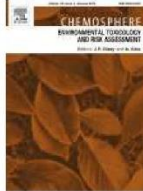
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APPENDIX B:
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Title: Comparative Copper Toxicity Impact and Enzymatic Cascade Effect on Biosorption Activated Media and Woodchips for Nutrient Removal in Stormwater Treatment

Author: Dan Wen, Ni-Bin Chang, Martin P. Wanielista

Publication: Chemosphere

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APPENDIX C:
OF PREVIOUS STUDIES OF NUTRIENT SORPTION MEDIA

Media used	Treated water	Nutrient removal	Limitation	Reference
Phosphate precipitation with $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Municipal wastewater with 0 – 5 mg/L $\text{OP}_4\text{-P}$	63% phosphate removal at 1:1 molar addition of Fe(III) at pH = 4	Excessive addition of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ is necessary for ideal removal; High maintenance cost	Fytianos, et al. ²¹⁰
Iron-aluminum hydroxyl (oxide) coated filter media	Artificial groundwater, tap water, treated wastewater with 0.5 mg/L $\text{OP}_4\text{-P}$	Over 90% phosphate removal	Lack of ability for nitrogen species removal; Special pre-treatment of the media is required	Ayoub, Koopman and Pandya ²⁰⁰
Bioretention system with soil, mulch, and plants	Urban stormwater runoff with 2-4 mg/L TN and 0.6 mg/L TP	> 90% heavy metal; 60 – 80% phosphorus;	Little nitrate was removed; Nitrate	Davis, et al. ²¹¹

Media used	Treated water	Nutrient removal	Limitation	Reference
		Moderate TKN	production confirmed	
Iron packed bed in columns	Groundwater spiked with nitrate solution to 5 mg/L	> 80% nitrate removal	By-products generation of ammonia and metal ions	Westerhoff and James ⁸¹
Soil, sand, mulch in bioretention system with layer configurations	Synthetic water with 2 – 3 mg/L of nitrate, ammonia and phosphorus	68% ammonia removal; 85% phosphorus removal;	Nitrate export was confirmed	Hsieh, et al. ²¹² ; Hsieh, Davis and Needelman ¹⁹⁵
Sand, tire crumb, sawdust, and limestone in different configurations	Stormwater with 0.38 - 2.5 mg/L nitrate, 0.125 – 0.785 mg/L OP ₄ -P	Over 90% nitrate removal; 50 – 90% orthophosphate removal	Ammonia export was confirmed	Moberg ⁹⁹
Expanded clay, tire crumb, sawdust, and limestone in wetland system	Septic tank effluent with 40 – 80 µg/L TN	75.4% TN removal; 94.9% TP removal	Lack of pathogen removal;	Xuan, Chang, Daranpob and Wanielista ⁹⁵

Media used	Treated water	Nutrient removal	Limitation	Reference
			Limited nitrification process	
Sand, limestone, sawdust, and tire crumb for stormwater dry ponds	Stormwater with 35 – 54 mg/L TN, 7.5 mg/L TP	65 – 95% nitrate removal; Completely ammonia removal	Certain requirement of media thickness for achieving proper HRT	Hossain, Chang and Wanielista ⁶⁹
Fine sand, tire crumb, and sawdust placed in vadose zone	Septic tank effluent with 1.76 mg/L ammonia, 0.352 nitrate, 0.116 nitrite, 1.498 OP ₄ -P	55% nitrate removal; 89% TP removal	Larger aerobic zone and longer HRT required for complete denitrification	Xuan, Chang, Wanielista and Hossain ⁸⁰
Nanoscale zero valent iron supported on pillared clay	Synthetic solution with 0.806 mmol/L nitrate	Over 90% nitrate removal within 40 min of HRT	End products of ammonium was observed	Zhang, Li, Li, Hu and Zheng ⁸³

Media used	Treated water	Nutrient removal	Limitation	Reference
Fine sand, tire crumb, sawdust, and limestone in various configurations	Stormwater under various temperatures	70 – 90% nitrate removal; 40 – 85% orthophosphate	Lower temperature may inhibit the nutrient removal	Chang, Wanielista and Henderson ⁶⁸
Nanoscale zero valent iron	Synthetic phosphorus solution with 1 – 10 mg/L OP ₄ -P	96 – 100% removal with ~ 78% phosphorus recovery	Too expensive to conduct economic implication	Almeelbi and Bezbaruah ¹⁸¹
Tire crumb, silt, clay, and sand in stormwater infiltration basin	Stormwater with 0.46 – 0.90 mg/L TDP	Minor nitrate removal; 70% phosphorus removal	Enhanced denitrification is required for better removal effects	O'Reilly, Wanielista, Chang, Xuan and Harris ⁴⁴
Cement sand, tire crumb, fine expanded clay, and limestone	Stormwater with 0.5 – 1.0 mg/L TDP	Maximum adsorption equilibrium is 0.0151 mg-P/g-absorbent	Lack of nutrients recovery potential	Jones, Chang and Wanielista ⁷

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