

Comparative Nitrogen and Pesticide Removal with Sorption Media in Linear Ditch for Groundwater and Stormwater Treatment

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METRIC CONVERSION TABLE

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
LENGTH				
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
AREA				
in²	square inches	645.2	square millimeters	mm ²
ft²	square feet	0.093	square meters	m ²
yd²	square yard	0.836	square meters	m ²
ac	acres	0.405	hectares	ha
mi²	square miles	2.59	square kilometers	km ²

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
VOLUME				
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft³	cubic feet	0.028	cubic meters	m ³
yd³	cubic yards	0.765	cubic meters	m ³
NOTE: volumes greater than 1000 L shall be shown in m ³				

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
MASS				
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams	Mg (or "t")

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
TEMPERATURE (exact degrees)				
°F	Fahrenheit	5 (F-32)/9 or (F-32)/1.8	Celsius	°C

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
ILLUMINATION				
fc	foot-candles	10.76	lux	lx
fl	foot-Lamberts	3.426	candela/m ²	cd/m ²

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
FORCE and PRESSURE or STRESS				
lbf	poundforce	4.45	newtons	N
lbf/in ²	poundforce per square inch	6.89	kilopascals	kPa

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
LENGTH				
mm	millimeters	0.039	inches	in
m	meters	3.28	feet	ft
m	meters	1.09	yards	yd
km	kilometers	0.621	miles	mi

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
AREA				
mm ²	square millimeters	0.0016	square inches	in ²
m ²	square meters	10.764	square feet	ft ²
m ²	square meters	1.195	square yards	yd ²
ha	hectares	2.47	acres	ac
km ²	square kilometers	0.386	square miles	mi ²

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
VOLUME				
mL	milliliters	0.034	fluid ounces	fl oz
L	liters	0.264	gallons	gal
m ³	cubic meters	35.314	cubic feet	ft ³
m ³	cubic meters	1.307	cubic yards	yd ³

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
MASS				
g	grams	0.035	ounces	oz
kg	kilograms	2.202	pounds	lb
Mg (or "t")	megagrams	1.103	short tons (2000 lb)	T

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
TEMPERATURE (exact degrees)				
°C	Celsius	1.8C+32	Fahrenheit	°F

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
ILLUMINATION				
lx	lux	0.0929	foot-candles	fc
cd/m ²	candela/m ²	0.2919	foot-Lamberts	fl

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
FORCE and PRESSURE or STRESS				
N	newtons	0.225	poundforce	lbf
kPa	kilopascals	0.145	poundforce per sq. inch	lbf/in ²

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16. Abstract Performance of woodchip and B&G filter material were compared in a laboratory column study for nitrogen removal. Both performed well under a saturated condition. However, field construction along a highway ditch could not achieve saturated conditions. Field Sampling data were acquired for the comparison with the lab column study results. The performance of B&G in the field is consistent with the column study, but woodchip performs unsatisfactory for the removal of nitrogen in the field. The reasons involve media characteristics and saturation differences. In addition, the cost-benefit analysis was assessed based on both lab and field removal performance, B&G showed great potential when it was applied in the field while woodchip has negative nitrogen removal due to significant ammonia generation, plus, over 50% decomposition of woodchip was observed within one year of field operation.					
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In addition, the authors recognize the assistance and funding from the Florida Department of Transportation that was used for laboratory water quality analysis, analysis of microbial community information, and construction. The Suwannee River Management District also provided funding for the construction of the linear ditch and initial encouragement to document performance. Environmental Conservation Solutions provided media installation and evaluation of soil properties. The University of Central Florida faculty provided work space, lab data collection, analysis of results, and research coordination, using a team of faculty with water quality, microbial analysis, and water resources backgrounds. AECOM provided field design and construction as well as field sampling and operation.

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EXECUTIVE SUMMARY

The excessive nitrogen introduction into groundwater and surface water from stormwater runoff/agricultural discharge is regulated by the Florida Department of Environmental Protection. However, there is in most States including Florida a cost and space limitation adjacent to highways for construction of traditional best management practices. Biosorption activated media (BAM) shows the potential to achieve good performance within such limited space through a linear ditch design for co-treatment of groundwater and stormwater. Two recipes of BAM were selected in this project for a comparative study. One is the Bold & Gold, which is abbreviated as B&G in this report, containing sand, clay, and tire crumb. The other is the woodchip media, containing 1~3 inches woodchip from a local sawmill.

Joint funding support was made possible for this project by the Florida Department of Transportation in partnership with Suwanee River Water Management District for work in the laboratory at University of Central Florida (UCF) and at the Fanning Springs field study site. The National High Magnetic Field Laboratory located at the Florida State University helped the UCF research team to use the high-resolution Fourier transform ion cyclotron resonance mass spectrometry to freely assess the changes of dissolved organics nitrogen (DON).

The information in this report presents the background information, experimental design, and data collection/analysis regarding the nitrogen removal effectiveness of a linear ditch, from laboratory analysis to field study. Laboratory analysis was conducted under a variety of inlet total nitrogen (TN) concentrations and subsequent carbon addition effect and copper impacts on B&G and woodchip media mixtures. In this laboratory comparative study, the physical, chemical, and biological processes in both media were tested and compared for their treatment effectiveness under inlet TN concentrations and the presence or absence of carbon and copper in the inlet water of the two types of media mixtures to deepen understanding of the removal potential of the media. In addition, field-scale TN removal data were collected and compared with the laboratory study results to confirm the laboratory scientific evidence and validate the cost-benefit information of both media. In summary, woodchip and B&G performed equally well in the laboratory when saturating the columns continuously, and sometimes, woodchip medium performed even better due to its provision of assumed carbon sources (see the table below).

	B&G treat groundwater				Saturated Woodchip treat groundwater			
	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No Carbon</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>
	<u>Carbon</u>		<u>Carbon</u>				<u>Carbon</u>	
NO_x Removal	51.54%	99.92%	45.33%	54.11%	91.77%	96.56%	67.25%	98.65%
TN Removal	50.58%	87.98%	42.52%	51.90%	84.88%	89.86%	62.09%	92.83%
NH₃ Removal	7.33%	-960%	4.11%	-210%	79.34%	-167%	91.41%	-453%
	B&G treat stormwater				Saturated Woodchip treat stormwater			
	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No Carbon</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>
	<u>Carbon</u>		<u>Carbon</u>				<u>Carbon</u>	
NO_x Removal	99.80%	98.32%	73.13%	63.08%	99.79%	99.41%	92.93%	92.82%
TN Removal	77.54%	82.15%	70.49%	63.10%	68.68%	59.31%	79.65%	87.25%
NH₃ Removal	-8.55%	-16.5%	14.13%	-168%	97.96%	-6.3%	95.79%	38.14%
	B&G treat high TN stormwater				Saturated Woodchip treat high TN stormwater			
	<u>No copper</u>		<u>Copper</u>		<u>No copper</u>		<u>Copper</u>	
NO_x Removal	73.13%		71.90%		92.93%		92.26%	
TN Removal	70.49%		62.31%		79.65%		70.73%	
NH₃ Removal	14.13%		-127%		95.79%		34.08%	

In comparison with the field data, B&G performed consistently and even better than laboratory results in terms of TN removal. Woodchip removal of nitrogen did not appear in the field under un-saturated conditions. Woodchip in the field tends to generate a significant amount of ammonia through dissimilatory nitrate reduction to ammonium (DNRA) and shows mostly the generation of TN or negative removal efficiency of TN. This is mainly due to the inability of woodchip to screen out the leaching organic particles; additionally, increased oxygen in woodchip's large void space as a necessary condition for supporting the organic degradation and ammonification resulted in ammonia production that sustains the dissimilatory nitrate reduction to ammonium (DNRA) while depressing the denitrification (DNF) that releases nitrogen gas into atmosphere. Plus, more organics may come from the nearby farmland/highway via stormwater runoff or wind blow or the slow release fertilizer that was applied on the plants of the linear ditch. B&G has a small void space with a much larger surface area that accumulates the organics.

The cost-benefit comparison between the field and the laboratory study was conducted. Twenty-years of operation time is assumed for B&G but only 8 years for woodchip, due to its decaying issue. The evaluation was performed based on column study conditions (inflow rate, bed volume, nutrient concentrations, etc.) and field study conditions (pumping volume, bed volume, etc.). On average, the media cost are \$4.39 and \$4.48 for woodchip and B&G respectively to remove 1 pound of nitrogen in laboratory conditions (including carbon and non-carbon cases). When it comes to the field evaluation, the pumping volume and average groundwater nitrate concentration with 80% removal were adopted to calculate the result of B&G (estimation is not suitable for woodchip due to its negative removal efficiency of TN), with ~\$23,000 construction cost for 20 years operation, resulting in a cost of \$33/lb of nitrate removed. Note that the result considers only groundwater treatment, but there is also a nonnegligible amount of stormwater that shall be treated as well.

In conclusion, there are findings from the research project that are listed below:

- The co-treatment for nitrogen removal using both surface runoff and groundwater is possible. The dual-use of highway swales for surface and ground water treatment will remove pollution from the surficial aquifers. Also, when the surface water is infiltrated rather than directly discharged to a surface water, the quantity of water to the aquifers discharging to springs and estuaries will increase.

- In the field, B&G performs consistent with the lab performance or even better, but woodchip generates significant amount of ammonia in the field, and no such phenomenon was observed in the lab.
- The air can easily get into woodchip media in the field that depressed the denitrification for generating nitrogen gas, but the more available carbon source and high nitrate concentration from groundwater stimulated the second path way of denitrification, so called dissimilatory nitrate reduction to ammonia (DNRA), which converts nitrate into ammonia.
- It is beneficial to add carbon into groundwater for improving nitrogen removal effectiveness, but no need for adding carbon to stormwater as stormwater already contains enough carbon for both B&G and woodchip.
- Adding carbon enhances the microbial bioactivity and increases the bacteria population, which may result in promoting organic degradation and ammonification in those DON sources that generate more ammonia potentially.
- Copper has negative impact on the top section of B&G which resulted in decreased nitrogen removal efficiency initial treatment and less microbial population for AOB and NOB, but the overall nitrogen removal is not seriously impacted in the short-run (e.g., 1-2 days).
- Most copper was removed and accumulated in the top section of both B&G and woodchip, which means the top section are more vulnerable than the rest of the column.
- However, copper enhanced the population of denitrifiers because copper is the enzyme cofactor for the last denitrification step.
- Significant degradation of woodchip was noticed, about half the volume of woodchip was decayed within 6 months.
- The media cost \$4.39 and \$4.48 for woodchip and B&G respectively to remove 1 pound of nitrogen in laboratory conditions (including carbon addition and non-carbon cases). A twenty-year operation time for B&G and 8 years for woodchip were assumed. In the field, B&G unit removal cost was \$33/lb of nitrate removed. No unit cost was assigned to woodchip because of the negative removal.

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1. Project Background and Description

State Water Management Districts and the Florida Department of Environmental Protection (FDEP) have rules and regulations that require the Florida Department of Transportation (FDOT) to develop stormwater management systems that address excess nutrients in stormwater runoff. This project demonstrates that a treatment approach with two types of bio-sorption activated medium (BAM), of which one is called Bold & Gold (B&G hereafter) medium and the other woodchip media, can provide treatment for nutrient removal of stormwater from roadway systems, both in urban and rural areas. Implementation of a BAM-based treatment system can mitigate stormwater impacts, decrease transportation costs, and prevent water loss through evaporation.

Nutrients, particularly nitrate, are a rising concern in groundwater aquifers and springs throughout some areas in Florida. The nitrate-nitrogen concentration in many of Florida's aquifer springs has risen above 1 mg/L in recent years (Ritter et al. 2007). Hence, serious environmental issues, such as water body eutrophication (lakes, estuaries, streams, and springs), degradation of groundwater quality, and public health problems, raise the attention of both the public and scientists. This trend of increasing nutrient concentrations can be attributed to agricultural activities and urban land use practices near groundwater recharge zones. An example of sources for nutrient generation is rural land uses in the Fanning Springs area, located in North-Central Florida, east-northeast of the city of Fanning Springs in Levy County. Specifically, the study site is located in the southeast corner of SR-26 and 55th Ave. and extends along the southern FDOT right-of-way 1/2 mile west and up to 1 mile east (**Figures 1 and 2**). In this watershed, land use patterns include residential areas, a dairy farm, a wastewater treatment plant, and agricultural fields.

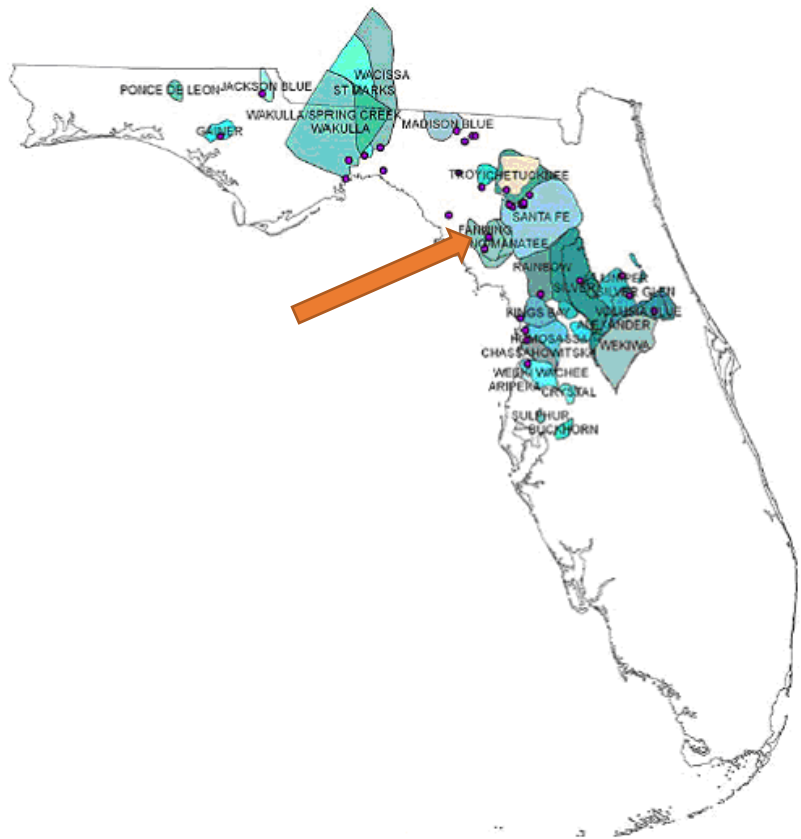


Figure 1. Spring and springshed distribution and the study site in Florida (Source: Wetland Solution Inc., 2010)



Figure 2. Aerial view of Fanning Springs and surrounding farm land

Due to land scarcity within the right-of-way of many roadways, the use of a linear ditch treatment system (i.e., similar to a bioswale), as a new Best Management Practice (BMP), is economically attractive when compared to traditional stormwater wet detention ponds or retention basins. A linear ditch requires much less area or footprint to achieve a similar removal efficiency, and it can be constructed within the right-of-way, which decreases the financial burden for buying additional land for construction. Moreover, a linear ditch with BAM is able to treat both groundwater and stormwater. This type of linear ditch can be used to remediate nutrient-laden groundwater during the non-storm period and treat stormwater runoff during storm events.

A BAM-based linear ditch may become a promising alternative BMP that is cost-effective for nutrient removal. B&G media has been shown to be useful in Florida BMPs when incorporated within infiltration basins as a soil amendment to mitigate nutrient impacts on groundwater resources. B&G demonstrated increased removal of nitrate and phosphorus relative to natural soil before infiltrating stormwater entered the groundwater (O'Reilly et al. 2012). The components in functionalized B&G are able to increase sorption capacity (more important to phosphorus removal) and soil moisture retention (more important for nitrogen removal) while providing sufficient infiltration ability for stormwater volume control (O'Reilly et al. 2014). Tire crumb and clay in B&G may behave as an effective sorption material to increase the sorption capacity, especially phosphorus adsorption (Wanielista et al. 2008), while the silt and clay provided high moisture retention capacity which made contributions to forming a more favorable anoxic condition that enabled the progression of biogeochemical processes toward denitrification (O'Reilly et al. 2012), and also provided porous space for maintaining the infiltration rate and biofilm growth (Hood 2012). Various green infrastructures could receive augments on nutrients removal efficiency from the application of B&G, such as off-line retention systems, underground retention, and exfiltration systems, indicating a wide range application of B&G and good compatibility to other BMPs (O'Reilly et al. 2012). Another benefit of B&G is the use of Florida's naturally occurring soils mixed with recycled materials, which leads to low capital investment in construction and the same maintenance cost as regular infiltration basins (Wanielista et al. 2011).

However, there are many potential BAM recipes to be considered for design; two media were chosen in this study, including B&G mixture and woodchip mixture. B&G mixture contains clay, tire crumb and sand, while woodchip mixture contains 100% small woodchip or shavings

(about 1/16 to 1-inch size); a picture of these two media is shown in **Figure 3**. Since nitrogen removal process is mainly a contribution from microorganisms as biological effects of nitrification and denitrification, different bacteria species formed biofilm so they could attach and reproduce on the surface of media. When enough oxygen exists, the nitrification process will transfer ammonia or ammonium into nitrite followed by nitrate, and nitrification usually happens at the beginning of infiltration when the dissolved oxygen is present (oxidation). Anaerobic condition is necessary for the denitrification process (reduction). During this reaction, multiple nitrogen species such as nitrate, nitrite, nitric oxide, and nitrous oxide can be transferred into nitrogen gas. With the cycle of nitrification and denitrification based on different bacteria species, nitrogen removal can be achieved. One crucial factor affecting the biological nitrogen removal process is the presence of toxic compounds from heavy metal, pesticide, herbicide or other chemical compounds, which are in all environments to some degree and not necessarily attributed to any specific land use. These toxic compounds may be inhibiting parameters for nutrient removal. It is desirable to conduct a comparative study for performance assessment of B&G and woodchip in terms of nitrogen removal with or without the presence of a toxic compound.



Figure 3. B&G mixture (left) and woodchip mixture (right)

Stormwater is a relatively untapped resource of water when it comes to meeting today's freshwater demand. Stormwater, if properly treated and managed, could provide an alternative source of water for integrated water management. Proper management to reduce nitrogen concentrations within groundwater aquifers, lakes, and springs is essential and can be accomplished in many ways, such as stormwater retention basins and/or injection wells with underground natural or artificial treatment systems. The addition of B&G to existing treatment strategies can provide effective treatment and storage of stormwater from a variety of roadway

systems. This research can help deepen the insight of removal mechanisms with regard to physical, chemical, and biological effects of the two types of media for some comparative properties. For example, the physiochemical adsorption capacity and biological effect related to the nitrification and denitrification processes, which may be influenced by different environmental factors such as the presence or absence of carbon or copper, for both types of media could affect the removal efficiencies of nutrients.

It is therefore worthwhile to conduct a series of rigorous column studies to prove the concepts of optimal design in the field. The data of this report provide scientific support for the use of a linear ditch BMP at the field scale to treat groundwater during non-storm periods and stormwater runoff during storm events alternately.

2. Experiment Design and Method

2.1 Column Study

Four columns were used. B&G mixture was placed in columns 1 and 2, while woodchip mixtures were placed in columns 3 and 4. Compaction was allowed to occur naturally when water flowed through the columns. The water comes from the top as down-flow for both BAM mixture columns and woodchip mixture columns, as shown in **Figure 7**. The experimental process of the BAM media in columns 1 and 2 can be found in **Figure 4**. In parallel with this design, the experimental process of woodchip mixture in columns 3 and 4 is described in **Figure 5**. There are three cycles for the main experimental process, including cycle 1 colored as brown, cycle 2 colored as blue, and cycle 3 colored as green.

In cycle 1, groundwater was provided within all columns for 3 days, then it was switched to stormwater and dosed for 1 day to simulate the field operation of the Fanning Spring study site. To evaluate the influence of different TN concentrations, the inlet TN concentrations of all columns were varied by adding standard nitrate solution to a theoretical concentration of 1.5 mg/L (low TN cases) and 5 mg/L (high TN cases), respectively. Water samples were taken (triplicates) from the inlet, outlet and each sample port of every column for groundwater and stormwater.

Cycle 2 had the same dosing process as cycle 1 with three days of groundwater and one day of stormwater; the only difference was that additional glucose was added in all inlet water (~ COD 40 mg/L) to evaluate the carbon source impact on the nutrient removal performance of B&G and woodchip. There are eight influent scenarios including high/low initial TN inlet with groundwater and stormwater, plus the carbon impact scenarios in cycle 2, which have been summarized in **Table 1** with corresponding acronyms. The tested low and high TN concentrations are around 6.7 and 9.7 mg/L for groundwater, and 2.0 and 5.5 mg/L for stormwater.

When the two cycles were finished, column 2 and 4 were chosen as the worst-scenario cases in cycle 3 for the copper toxicity test for both media (**Figure 6**). The inhibiting compound, copper, was used as a commonly recognized toxic compound at high concentration and was applied to the stormwater at a concentration of 50 $\mu\text{g/L}$, which is higher than the normal 15-30 $\mu\text{g/L}$ that can be found in stormwater runoff (Malmqvist 1983, TDC Environmental 2004). Copper was added to the stormwater and tested in cycle 3 with 5.0 mg/L spiked nitrate and additional copper. The running time lasted for 1 day before water and media samples were taken.

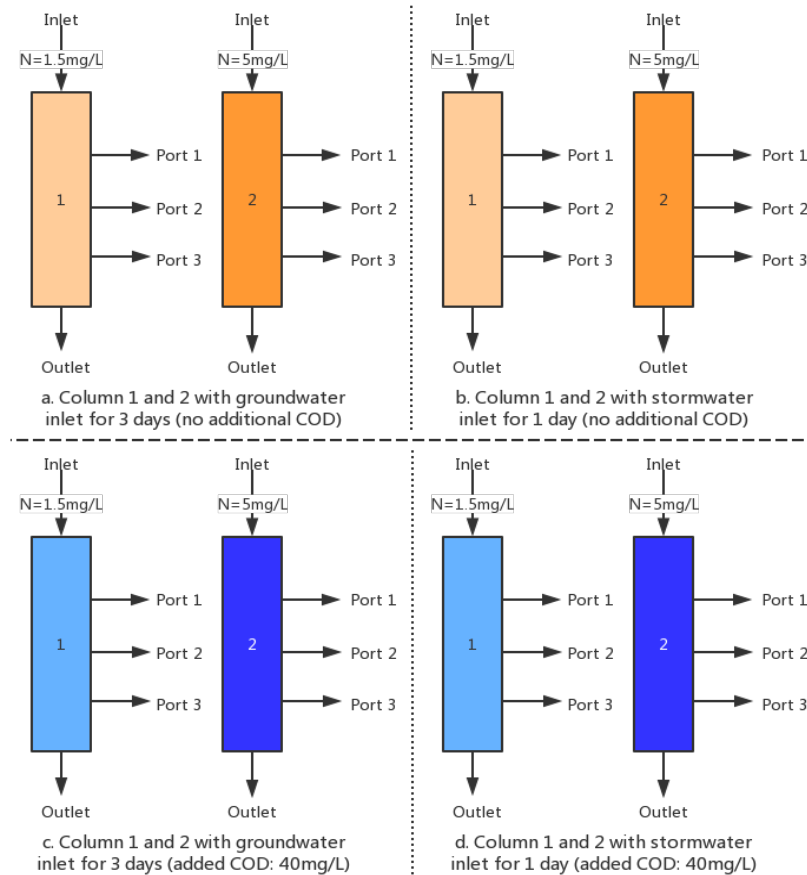


Figure 4. Schematic of experimental process for Cycle 1 (brown color, no COD addition) and Cycle 2 (blue color, with COD addition) with BAM medium (down-flow hydraulic pattern)

Table 1. Applied scenarios in the experiment

	Column 1 (B&G) and Column 3 (Woodchip)				Column 2 (B&G) and Column 4 (Woodchip)			
	Spiked with nitrate to 1.5 mg/L				Spiked with nitrate to 5.0 mg/L			
Process	Groundwater-3 days		Stormwater-1 day		Groundwater-3 days		Stormwater-1 day	
Cycle	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Scenario	LGN	LGC	LSN	LSC	HGN	HGC	HSN	HSC

LGN = Low TN Concentration Groundwater & No Carbon
 LGC = Low TN Concentration Groundwater & Carbon Added
 LSN = Low TN Concentration Stormwater & No Carbon
 LSC = Low TN Concentration Stormwater & Carbon Added
 HGN = High TN Concentration Groundwater & No Carbon
 HGC = High TN Concentration Groundwater & Carbon Added

HSN = High TN Concentration Stormwater & No Carbon
HSC = High TN Concentration Stormwater & Carbon Added

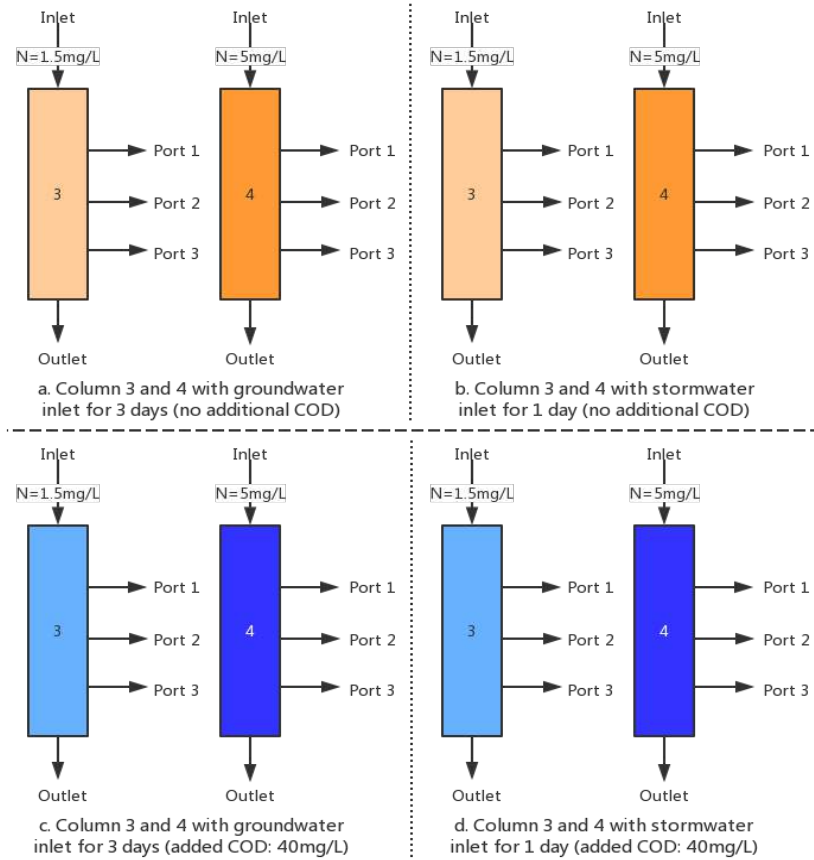


Figure 5. Schematic of experimental process for Cycle 1 (brown color, no COD addition) and Cycle 2 (blue color, with COD addition) with Woodchip mixtures (down-flow hydraulic pattern)

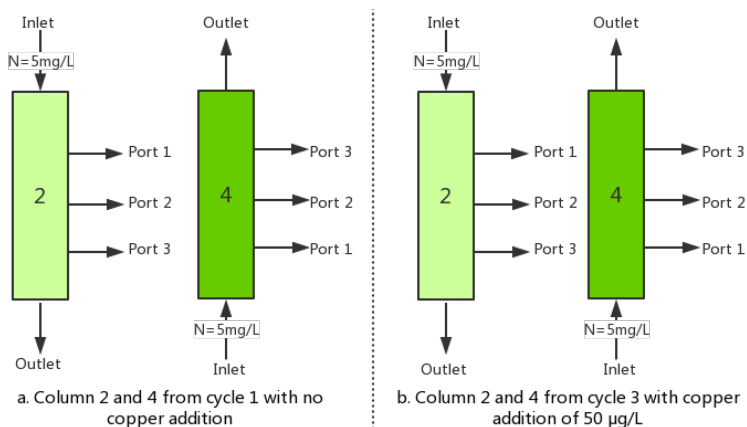


Figure 6. Schematic of experimental process for evaluation of BAM (down-flow) and Woodchip media (down-flow) before (cycle 1) and after (cycle 3) copper addition

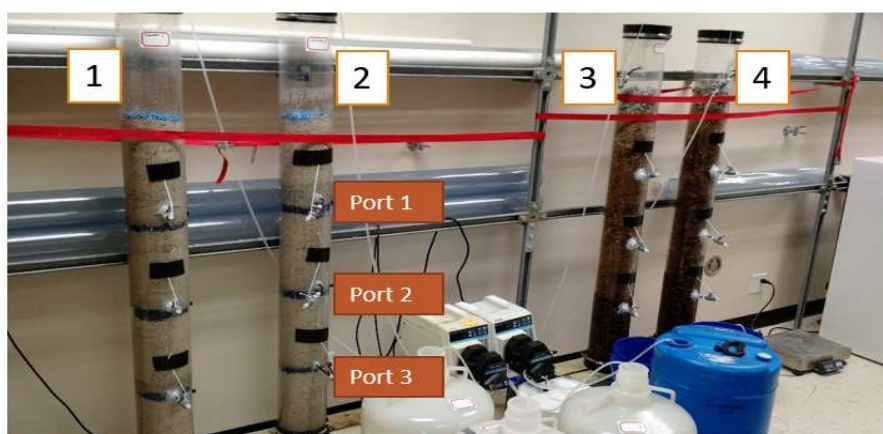


Figure 7. Column setup view in a laboratory at UCF

2.2 Water Collection for Column Study

Since two different water sources were used in our column study, i.e., groundwater and stormwater, they must be collected properly to ensure reliable experiment results. In order to better simulate the real-world condition in the field, groundwater was collected on 3/28/2016 in Fanning Springs, as shown in **Figure 8**. The collected groundwater was stored in the clean drums until its use in the column study. The stormwater was collected on the 4-B pond on the campus of University of Central Florida, as shown in **Figure 9**. Note that the stormwater was collected every few days to seed and cultivate the biofilm in B&G and woodchip for at least 6-8 weeks before the start of cycle 1.



Figure 8. Groundwater collection site in Fanning Springs on 3/28/2016



Figure 9. Stormwater collection pond 4-B on the main campus of UCF (left figure source: Google Maps)

2.3 Water Sample Analysis

The analysis of water samples from our column study was performed by a certificated laboratory named Environmental Research Design, Inc. (ERD), and all water samples were delivered to ERD within 24 hours after collection. The field samples were analyzed by another certified laboratory called Test America Laboratories, Inc. (TAL), and the sampling and delivery process was handled by Architecture, Engineering, Consulting, Operations and Maintenance (AECOM) staff. The analyzed parameters and methods are summarized in **Table 2**.

Table 2. Analysis method for lab and field water 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 FL-DEP = State of Florida Department of Environmental Protection, Florida Administrative Code. MCAWW = "Methods for Chemical Analysis of Water and Wastes", EPA-600/4-79-020, March 1983 and Subsequent Revisions. SM = "Standard Methods for The Examination of Water and Wastewater"</p>		

2.4 Media Sample Analysis

In order to better understand the bacteria evolution in both laboratory columns and field media critical for biological nitrogen removals in terms of nitrification and denitrification, certain bacteria were of interest, including ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifiers, and annamox (anaerobic ammonia oxidation; 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 densities were tested with qPCR in the Bioenvironmental Research Laboratory at UCF.

The media samples that were analyzed for our column study were collected in the end of each cycle for all columns, right after the stormwater sample collection. The locations (depth) of media samples were 0, 1 and 2 ft of the media in each column. For field media sample collection, the top soil layer was evacuated and then media samples were collected from the top, middle and

bottom depth of each media section on 5/3/2018, as shown in **Figure 10**. All media samples were stored at -80 °C right after collection until the extraction of DNA.



Figure 10. Media sample collection in field of linear ditch site on 5/3/2018

Collected media samples of B&G and woodchip media were stored at -80 °C until the gene extraction using the 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 3**. 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 3. 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 et al. (1997)
	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. 2002)
	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 et al. (2004)
	2050m2	CGTAGAAGAAGCTGGTGCT GTT		
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	(Tsushima et al. 2007)
	1066-R	AACGTCTCACGACACGAGC TG		

2.5 Bioactivity Analysis for Column Study

Bioactivity is one of the most important standards for evaluating the treatment performance of microorganisms. Bioactivity can be determined by measuring the concentration of a selected enzyme that closely relates to bacteria metabolism. Dehydrogenase is an enzyme that belongs to the group of oxidoreductases that oxidizes a substrate by a reduction reaction that removes one or more hydrogens from a substrate to an electron acceptor, usually NAD⁺/NADP⁺ or a flavin coenzyme such as FAD or FMN. This process is common in all creatures functioning as the fundamental step for biological reactions. Therefore, the detectable concentration of dehydrogenase can be measured through a chemical method as a practical way to determine the

bioactivity of microorganisms in B&G media. Woodchip mixture is not applicable in this kind of analysis due to its large particle size, which made it impossible to retrieve media sample in the column.

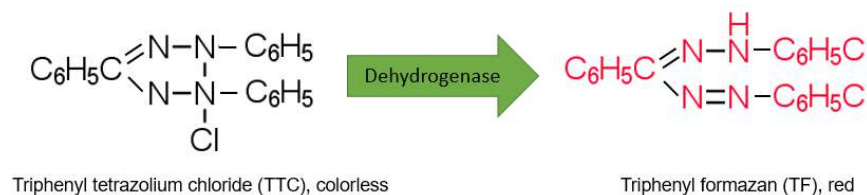


Figure 11. Mechanism showing the role of dehydrogenase in the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TF)

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 this context, docosaheptaenoic acid (*DHA*) measurement has been used to determine microbial activity in many studies for bioactivity assessment (Nanwen et al. 1996, Jin et al. 2005, Tian et al. 2006). *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 11**). *TF* can be extracted from cells using organic solvent and the concentration is determined through spectrometer by measuring the optical density at 492 nm. The protocol is as below:

Sample Preparation

1. Physical Method

10 g media was gently washed with distilled water twice, put into a conical flask with a small glass bead, and then vibrated for 20 min. 10 mL 0.9% NaCl solution was added and stirred as prepared biofilm solution.

2. Ultrasonic Method

5 g of media was washed with distilled water twice, and then mixed with 10 mL of 0.9% NaCl solution. Ultrasonic bath (40 W, 45 min) was applied, then the solution was stirred as prepared biofilm solution.

Sample Test

Two milliliters prepared biofilm solution was added into a 15 mL centrifuge tube, while 2 mL of Tris-HCl buffer, 0.1 mol/L glucose solution and 0.5% TTC solution were added to a separate tube. The tubes were put in a 37°C incubator for 6 hours, after which, two drops of sulphuric acid were added to terminate the enzymatic reaction. Then, 5 mL toluene was added to the tubes, and they were vibrated before allowing them to stabilize for 20 min, after which, they were tested with the color section in spectrophotometer.

2.6 Dissolved Organic Nitrogen (DON) Analysis

Dissolved organic nitrogen (DON) is a dynamic participant in aquatic ecosystems and a potential source of reactive N to the phytoplankton and bacteria that cause water quality degradation (Bradley et al. 2010). Various harmful algal species may use organic nitrogen for some or all of their N needs (Berggren et al. 2015). Moreover, DON is normally considered as a structurally complex mixture of materials that vary in chemical structure and composition, which contains thousands of molecules that are not easy to identify or measure. UCF researchers obtained permission from the National Science Foundation to cooperate with the National High Magnetic Field Laboratory in the Florida State University (FSU), and use the high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) to assess the changes of DON at the compound level in stormwater samples before and after B&G and woodchip treatment. FSU supported student training and travel expenditure to use FTICR-MS, which was useful in dealing with complex mixtures, such as biomass or dissolved organic matters, since the resolution allows the signals of two ions with similar mass-to-charge ratios (m/z) to be detected as distinct ions.

About 500 mL of influent and effluent from each column were collected by UCF researchers, and a pretreatment of solid phase extraction (SPE) was required prior to delivering those samples to FSU. The proper protocol for performing SPE is written below:

Preparation:

Water samples were filtered right after collection, and a volume of 500 mL was processed through a sequence of Whatman glass filter GMF (1 μm) and GF/F (0.7 μm); all the filters were pre-combusted at 450°C for 5 hours, wrapped in aluminum foil (pre-combusted at 450°C for 1 hour) in case of any contamination. All the aluminum foil was cut to a suitable size and combusted for 1 hour first; then the pieces were used to wrap filters (dull face inside with one side open to

allow gases out) and put in an aluminum tray in the muffle furnace. Following the cool down from combustion the open side was sealed and then the filters were put in a desiccator (or Ziploc bag) for a few days ahead of the sample collection.

All the Nalgene bottles, as well as the glassware and filtration kit were acid washed (10% Hydrochloric Acid, resin 4 times with DI water and ashed at 400°C for 4 hours), and then pre-combusted aluminum foil was used to cover over the glassware, and the Nalgene bottles were put in a plastic bin for safe storage in case of any contamination.

Procedure:

Using stainless steel forceps, place the filter onto the filtration kit and put the funnel on top of the filter and secure it. Resin funnel with sample water.

Be careful of the volume that has been filtrated.

Resin the first 100 mL of filtrated water in a flask and discard it, then start to collect.

Correct mark or label the Nalgene bottle.

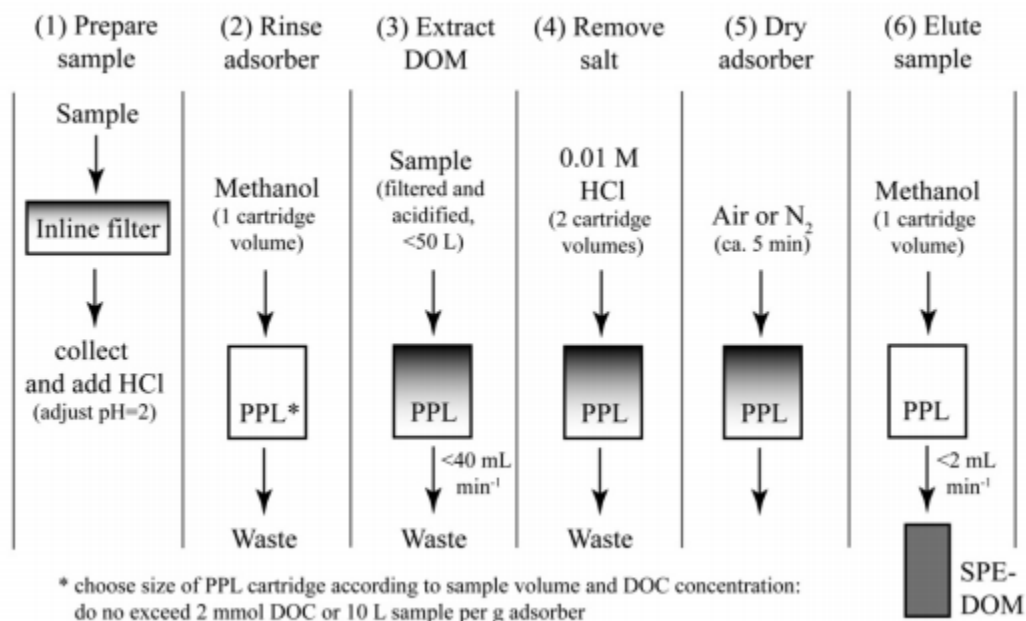


Figure 12. Scheme of isolation of dissolved organic matter (DOM) from sea water

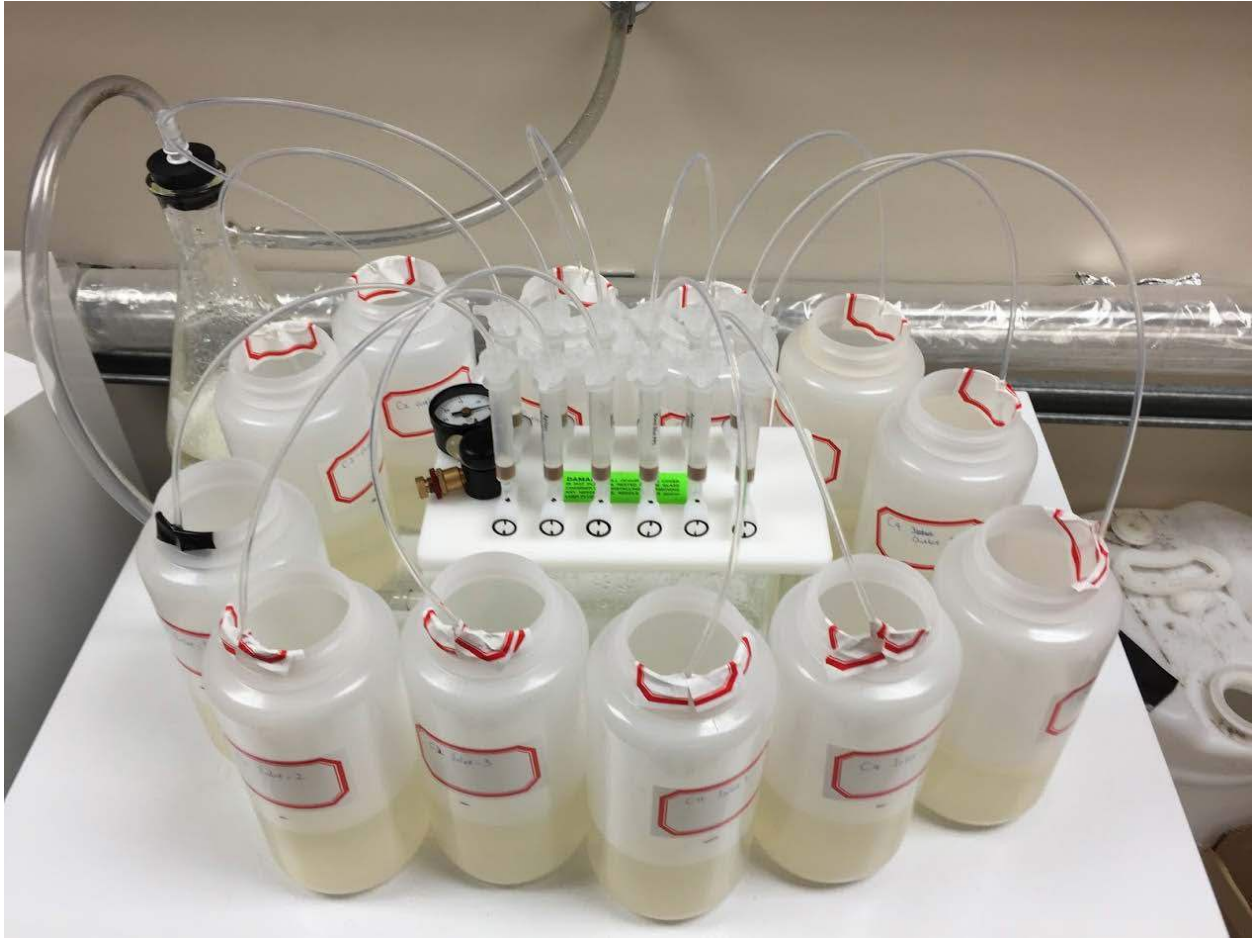


Figure 13. Performing SPE in UCF laboratory

3. Column Study Results

3.1 Hydraulic Retention Time

Tracer study is important for understanding the difference of hydraulic pattern for the two media recipes. The tracer study result is shown in **Figure 14** for both B&G media and woodchip media. Whereas the calculated tracer HRT is 77.92 and 113.10 minutes for columns 1 and 2 with B&G media, respectively, the corresponding HRT 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, which may cultivate more compacted/dense 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 a much larger void space such that the biofilm thickness can hardly impose any influence on HRT. The HRT differences between B&G and woodchip are also critical for their performance in the field application, because the inflow rate of stormwater is highly variable in the field when compared to the constant inflow rate in our column study.

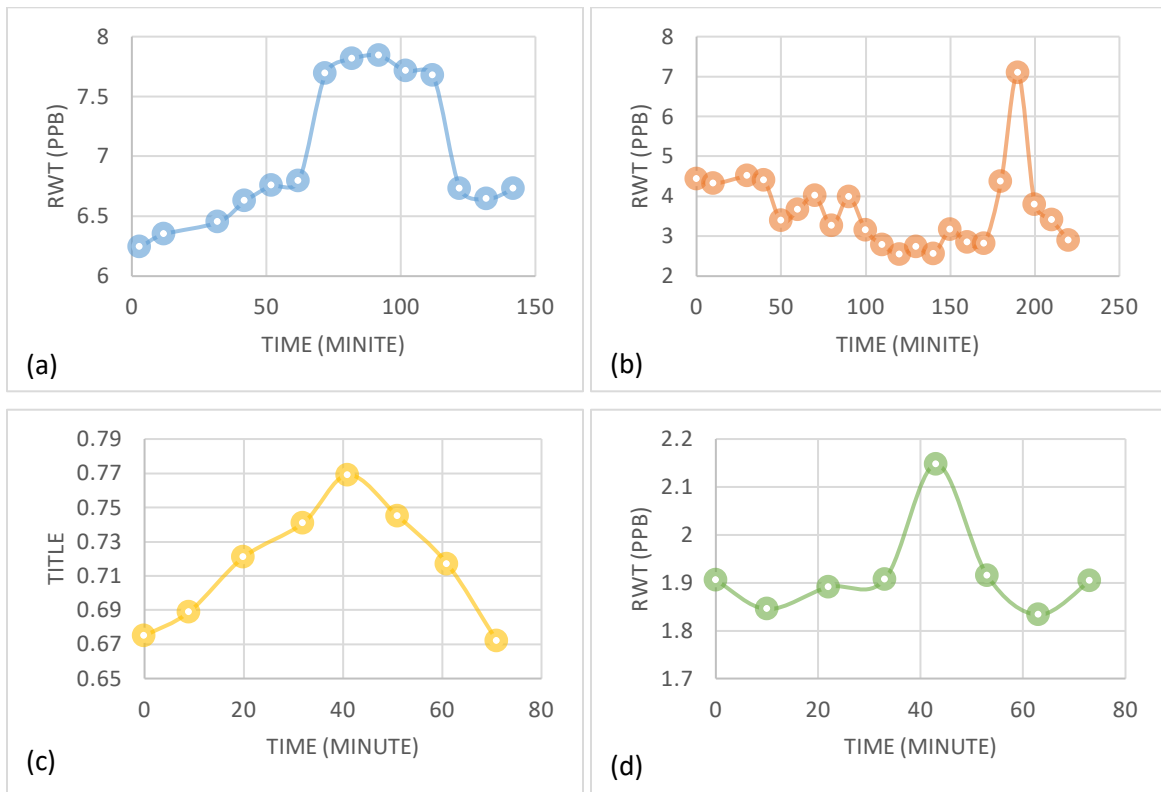


Figure 14. Tracer study results from column 1 to 4 as shown from (a) to (d)

3.2 Isotherm Study and Life Expectancy

To identify the nitrate sorption capacity of the B&G and woodchip mixture, isotherm tests were conducted. The preparation is straightforward; a volume of 300 mL of 2 mg/L N-nitrate solution was added to each of five 500 mL flasks with 30 g, 60 g, 90 g, 120 g, and 150 g of B&G and woodchip mixture, and another set of flasks with the same media weight distributions were filled with DI water as blank samples. The flasks were covered with Parafilm to separate the external disturbances when mixed thoroughly on a shaking platform for 2 hours at 200 rpm. The solution was obtained from those flasks and filtered through 0.45 μm filters and analyzed using

HACH Nitrate TNTplus Vial Test kits. Triplicates were prepared for each test. The results of isotherm tests were compared using the isotherm equations below:

$$\text{Langmuir isotherm equation:} \quad \frac{C_e}{q_e} = \frac{1}{q_m} C_e + \frac{1}{K_a q_m} \quad (1)$$

$$\text{Freundlich isotherm equation:} \quad \log q_e = \log K + \frac{1}{n} \log C_e \quad (2)$$

Where:

q_e = sorbed concentration (mg/g) [mass adsorbate/mass adsorbent];

q_m = maximum capacity of adsorbent for adsorbate (mg/g) [mass adsorbate/mass adsorbent];

K_a = measure of affinity of adsorbate for adsorbent (unitless);

K = capacity adsorbent (mg/g) [mass adsorbate/mass adsorbent];

C_e = aqueous concentration of adsorbate (mg/L);

n = measure of how affinity for the adsorbate changes with changes in adsorption density (unit less)

The Langmuir adsorption line of B&G was obtained by plotting C_e versus $\frac{C_e}{q_e}$ as shown in Figure 15 and the Freundlich adsorption line was obtained by plotting $\log C_e$ versus $\log q_e$ as shown in Figure 16. According to the isotherm tests, the highest loading rate was 0.0013 mg/g (adsorbate/adsorbent); suppose 1,500,000 g B&G was used, the maximum amount adsorbed is 300 mg, and assume that nitrate concentration in stormwater is 1 mg/L and the average stormwater flow is 378.5 L/day, the life expectancy of B&G for 100% removing nitrate would be 5.15 days. Because of the relatively short life expectancy for nitrate adsorption, the expected long-term TN removal would be mostly attributed to the biological removal processes (i.e., nitrification-denitrification process).

Since woodchip released a brown color into the solution, which worked as an inhibitor for testing the nitrate concentration, isotherm analysis for woodchip was not performed under current conditions.

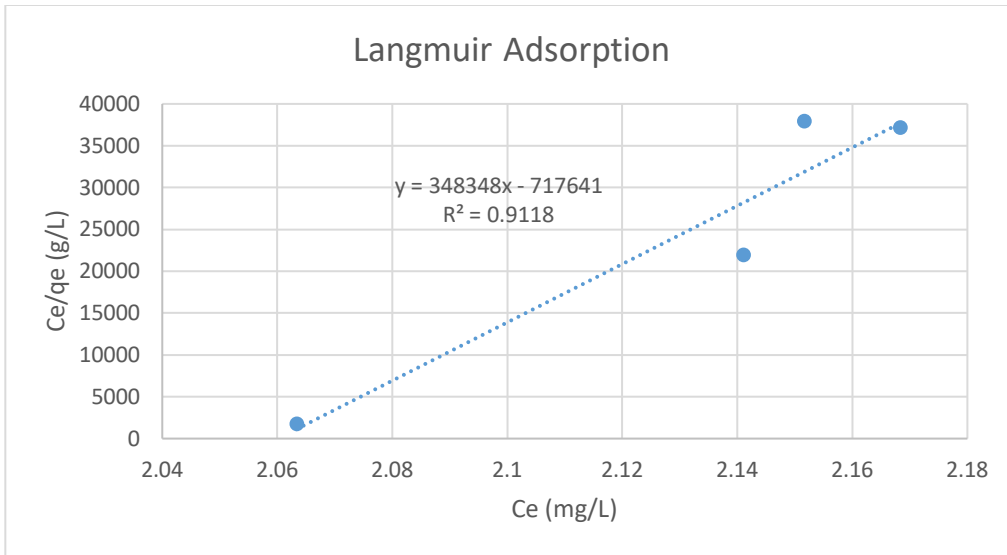


Figure 15. Langmuir adsorption line

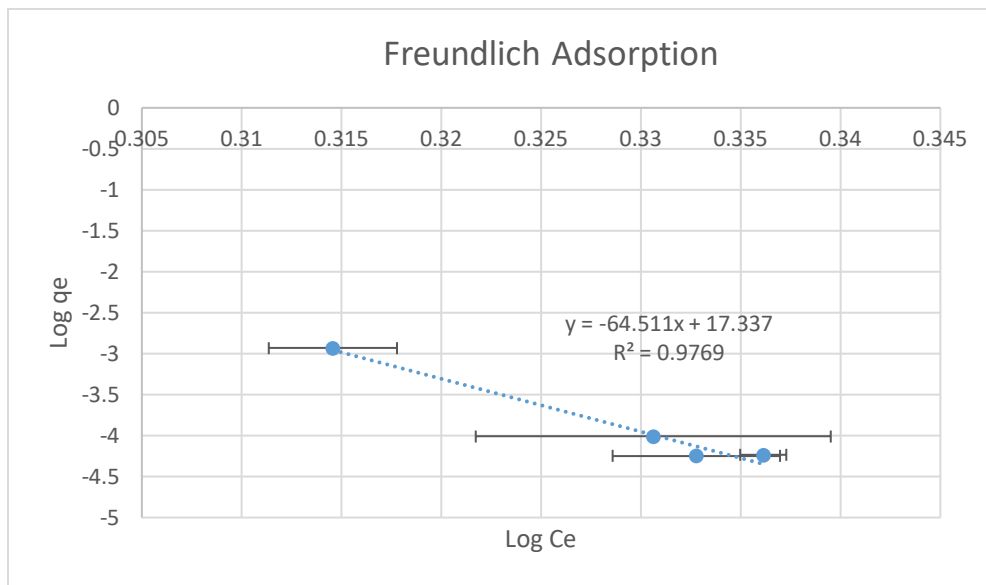


Figure 16. Freundlich adsorption line

3.3 Kinetic Study

The kinetic study plays an important role in the design of an optimized reactor to produce the desired nutrient removals as well as to predict a successful removal in a full-scale field application. It is common to assume first-order reactions in the beginning and to move on to second-order reactions if the first-order plotting cannot fit well. The first-order (Eq. 3) and second-order (Eq. 4) equations are as follow:

$$\ln(C) = -Kt + \ln(C_0) \quad (3)$$

$$1/C = Kt + 1/C_0 \quad (4)$$

Where:

C = the nutrient concentration at time t (µg/L)

C₀ = initial nutrient concentration (µg/L)

K = rate constant (h⁻¹)

The rates may be obtained from a linear regression for ln(C) versus reaction time for the reduction of TN in first-order reactions. If first-order cannot fit well, a second-order reaction may be assumed as the kinetics by a similar approach which graphs between 1/C versus time. The kinetic results of both media under four scenarios with and without carbon addition are summarized in Table 4 and Table 5. The removal rates, as measured by “K,” are all positive for both media, indicating removal will occur under the conditions of the test.

Table 4. Summary of kinetics of B&G in different scenarios

Inlet water	Condition	1 st Order equation	1 st order R ²	2 nd Order equation	2 nd order R ²
Low TN groundwater	No Carbon	y = 0.0076x + 0.0082	0.7783	y = 2E-06x + 0.0001	0.7582
	Carbon added	y = 0.0315x - 0.4555	0.944	y = 3E-05x - 0.0004	0.851
High TN groundwater	No Carbon	y = 0.0048x - 0.1061	0.7504	y = 8E-07x + 0.0001	0.7257
	Carbon added	y = 0.0069x - 0.066	0.9022	y = 1E-06x + 9E-05	0.8584
Low TN stormwater	No Carbon	y = 0.0153x + 0.2547	0.9782	y = 2E-05x + 0.0004	0.9327
	Carbon added	y = 0.0165x + 0.433	0.9826	y = 4E-05x + 0.0005	0.9636
High TN stormwater	No Carbon	y = 0.013x - 0.2926	0.7709	y = 6E-06x - 2E-05	0.7082
	Carbon added	y = 0.0078x + 0.1219	0.9772	y = 3E-06x + 0.0002	0.9863
High TN stormwater	Copper added	y = 0.0094x - 0.1524	0.9698	y = 3E-06x + 0.0001	0.9184
For 1st order equation: y = ln[C₀]/[C], x = t; for 2nd order equation: y = 1/[C], x = t					

Table 5. Summary of kinetics of woodchip in different scenarios

Inlet water	Condition	1 st Order equation	1 st order R ²	2 nd Order equation	2 nd order R ²
Low TN groundwater	No Carbon	$y = 0.0553x - 0.4889$	0.9742	$y = 3E-05x - 0.0002$	0.8525
	Carbon added	$y = 0.0549x - 0.2696$	0.8201	$y = 4E-05x - 0.0004$	0.7144
High TN groundwater	No Carbon	$y = 0.0286x - 0.2102$	0.9758	$y = 5E-06x + 5E-05$	0.9688
	Carbon added	$y = 0.062x - 0.6425$	0.5826	$y = 5E-05x - 0.0006$	0.5952
Low TN stormwater	No Carbon	$y = 0.0462x - 0.383$	0.8765	$y = 5E-05x - 5E-05$	0.8881
	Carbon added	$y = 0.0302x - 0.3786$	0.9811	$y = 3E-05x + 0.0002$	0.9379
High TN stormwater	No Carbon	$y = 0.0328x + 0.0831$	0.8387	$y = 2E-05x + 6E-05$	0.7791
	Carbon added	$y = 0.0467x - 0.2837$	0.6995	$y = 4E-05x - 0.0003$	0.6505
High TN stormwater	Copper added	$y = 0.0352x - 0.4833$	0.7911	$y = 1E-05x - 1E-05$	0.7151
For 1st order equation: $y = \ln[C_0]/[C]$, $x = t$; for 2nd order equation: $y = 1/[C]$, $x = t$					

3.4 Nutrient Removal in B&G

The TN, NO_x, and ammonia treatment results of B&G under four scenarios before and after carbon addition are shown in **Figure 17**, **Figure 18**, and **Figure 19**, respectively. The overall TN removal increased largely in the groundwater section after carbon addition, and it increased from 50.58% to 87.98% in low TN groundwater, and from 43.32% to 51.90% in high TN groundwater. Moreover, the TN removal increased at each sample port after carbon addition, and the additional carbon clearly enhanced the TN removal effectiveness at the initial treatment stage when treating groundwater. However, the overall TN removal seemed to be equivalent before and after carbon addition when treating stormwater with B&G mixtures. The overall TN removal for treating stormwater was 77.54% and 70.49% for the scenarios with low TN stormwater and high TN stormwater, respectively before carbon addition, while the values changed to 82.15% and 63.10% after the carbon addition. The different behaviors of B&G for treating groundwater and stormwater are due to the water quality difference in terms of carbon source availability. In this study, carbon source is defined as chemical oxygen demand (COD), and is an indicative measure of the amount

of oxygen that can be consumed by reactions in a measured solution. Groundwater contains less carbon source (4.10 mg/L COD of inlet groundwater) than stormwater (17.90 mg/L COD of inlet stormwater), which made the additional carbon source more valuable in groundwater than stormwater.

The NO_x removal results of B&G mixtures under four scenarios before and after carbon addition are shown in **Figure 18**. The overall NO_x removal increased largely when treating groundwater and after carbon addition; it increased from 51.54% to 99.92% in scenario LG, and from 45.33% to 54.11% in scenario HG. The overall NO_x removal was comparable in low TN stormwater (~98% removal) and high TN stormwater (~63-73% removal) before and after carbon addition. The reasons are the same as were stated for the TN removal. Moreover, the NO_x removal of B&G mixtures implied the importance of a carbon source as an electron donor for the denitrification process.

It is worth mention that the tire crumbs in B&G may leach out organics (dissolved organic carbon and nitrogen) inorganics (zinc, iron, aluminum, calcium, etc.), the leaching amount is closely related to the tire crumb sizes (the smaller of the tire crumb, the more leaching potential), but the leaching becomes minimum when the pH condition is neutral (Selbes et al. 2015). The author also discovered that the initial leaching amount is significant, but it quickly decreased the leaching speed within several days. Considering the implementation time frame of B&G is normally for years or decades, the leaching problem becomes negligible. Besides, the leached nutrients might be helpful for the growth of biofilm, because biological removal is the main pathway for the removal of nitrogenous species.

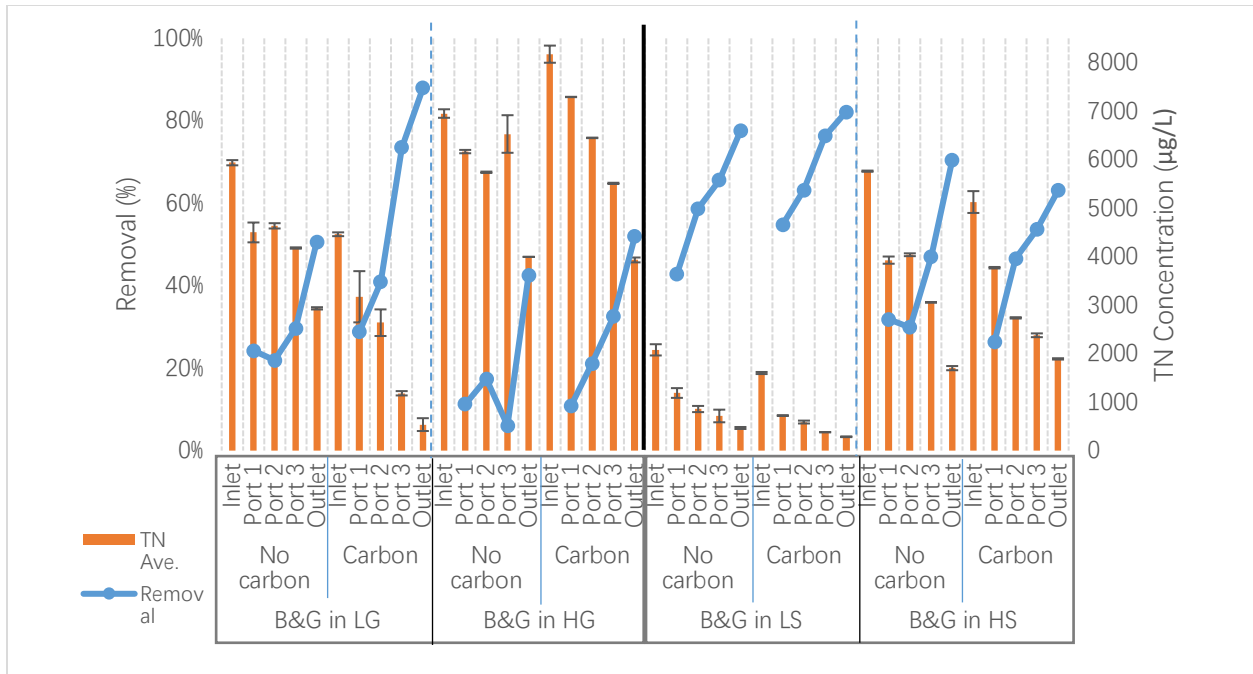


Figure 17. TN concentrations and removal in B&G for all scenarios

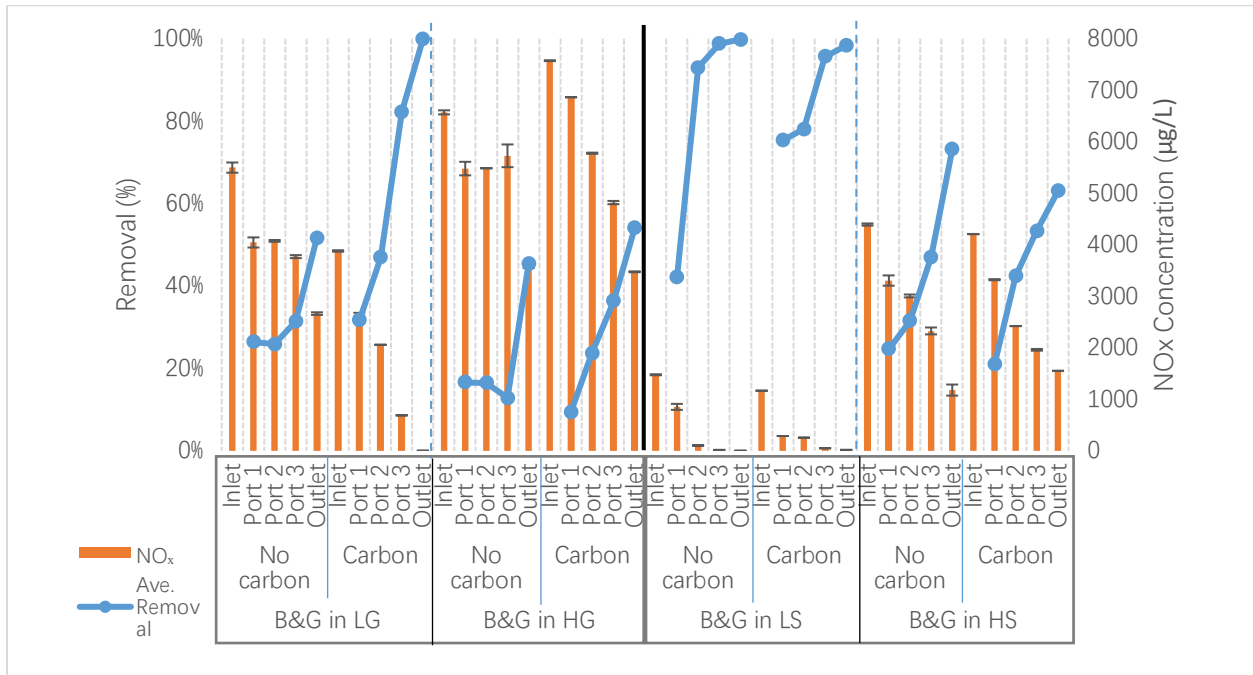


Figure 18. NOx concentrations and removal in B&G for all scenarios

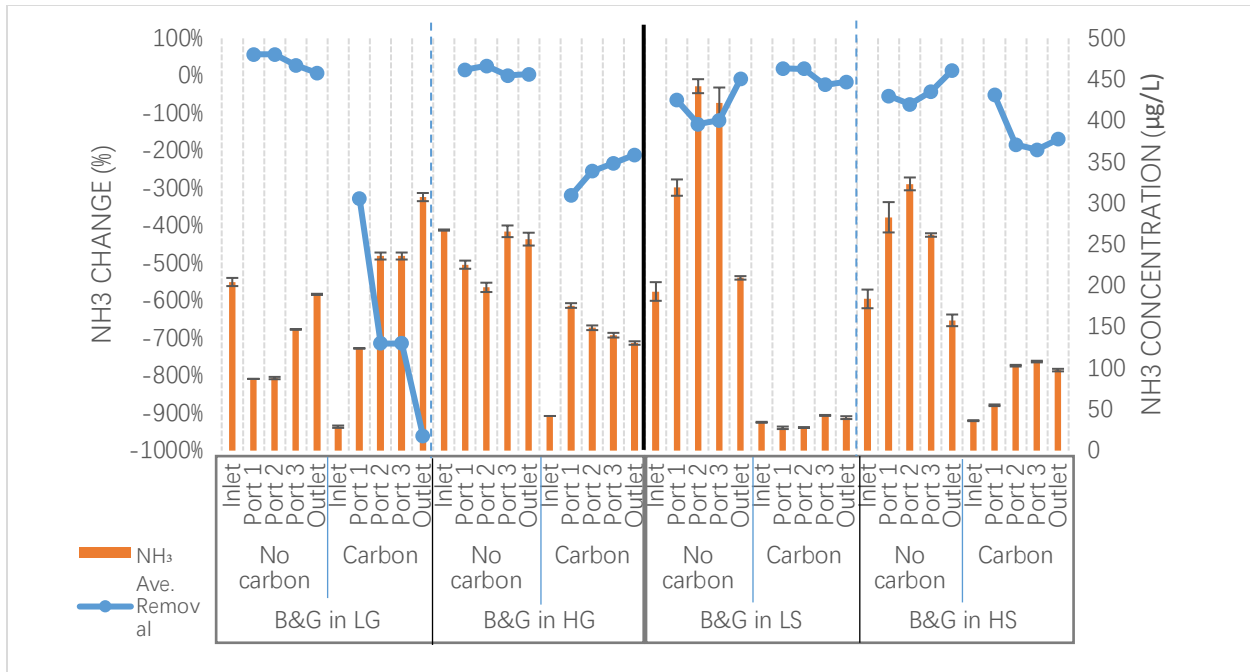


Figure 19. Ammonia concentrations and changes in B&G for all scenarios

3.5 Nutrient Removal in Woodchip

The TN removal results of woodchip mixtures under four scenarios before and after carbon addition are shown in **Figure 20**. The overall TN removal increased in high initial TN groundwater scenarios from 62.09% to 92.83% after carbon addition, while it showed equivalent removals in low initial TN groundwater scenarios with the carbon addition. This is indicative that when the nutrient concentration is high, the additional carbon may help the bacteria to consume more nutrient and increase the removal effectiveness; however, when the woodchip is treating water with lower concentrations of nutrients, there is not much room for nutrient removal, hence the importance of carbon addition is minimized because woodchip itself also may produce enough carbon source for treating low concentrated scenarios. When treating stormwater, woodchip mixtures showed comparable overall TN removals before and after carbon addition, mainly because carbon is not a rare element in stormwater and woodchip may also provide a carbon source.

The NO_x removal results of woodchip mixtures under each scenario before and after carbon addition are shown in **Figure 21**. The overall NO_x removal increased largely in high initial TN groundwater scenarios after carbon addition, in which it increased from 67.25% to 98.65%, while

it was comparable in low initial TN groundwater scenarios after carbon addition (~85-90% removal). The overall NO_x removal with carbon addition for treating stormwater was comparable with non-carbon conditions regardless of the inlet TN concentration level. The removal was ~99% for low initial TN stormwater scenarios and ~92% for high initial TN stormwater scenarios. The higher carbon availability of stormwater and woodchip itself ensured the NO_x removal effectiveness through denitrification as the carbon source may be regarded as an electron donor in denitrification; however, the removal efficiencies showed an obvious decrease in the middle sampling ports (port 1 to 3), which could be a sign of nitrification utilizing ammonia and nitrite.

The ammonia removal of woodchip was different when no carbon was added (**Figure 22**). High removal was achieved with no additional carbon: 79.34% for low initial TN groundwater, 91.94% for high initial TN groundwater, 97.96% for low initial TN stormwater, and 95.79% for high initial TN stormwater. After carbon addition, the ammonia removal dropped for all scenarios; the lowest drop was negative 453.73% in high initial TN groundwater. For the low and high initial TN stormwater, the ammonia removal was negative 6.25% and positive 38.14%, respectively. The reasons are the same as were stated for B&G mixtures. The additional carbon enhanced the bioactivity of bacteria on the biofilm surface, especially for heterotrophic bacteria, which are species that are more responsible for organic degradation and ammonification processes. As a result, the effluent ammonia concentration increased for all four scenarios.

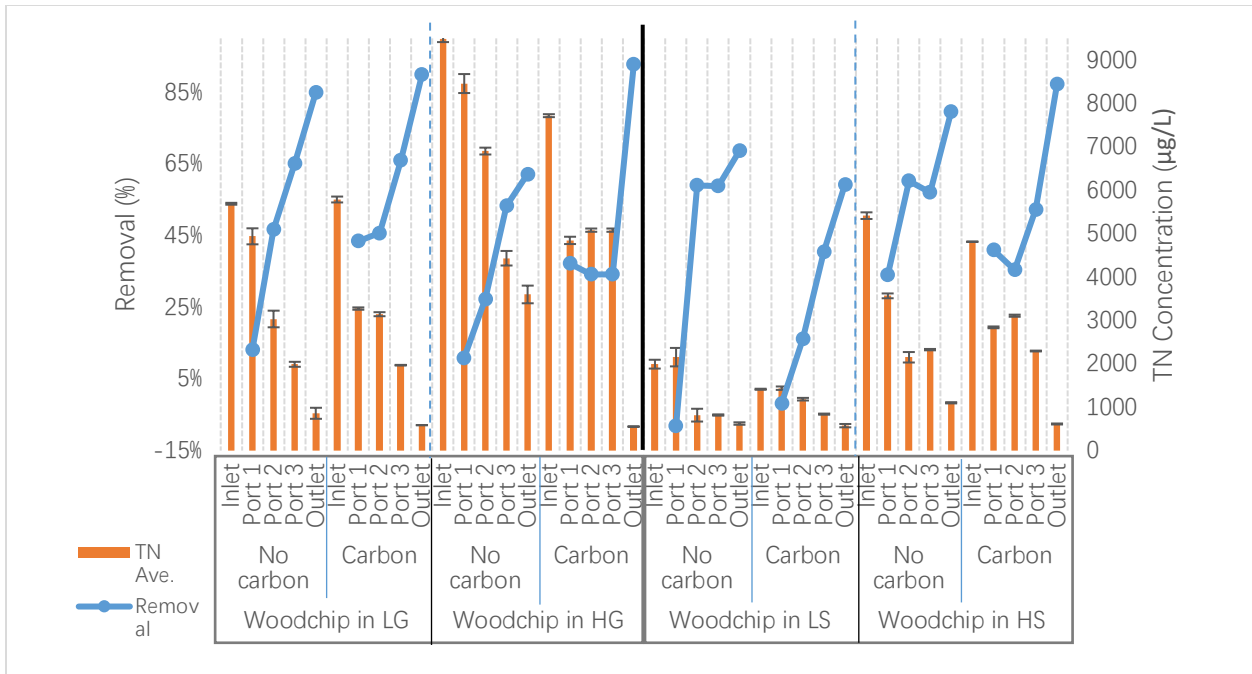


Figure 20. TN concentrations and removal in woodchip for all scenarios

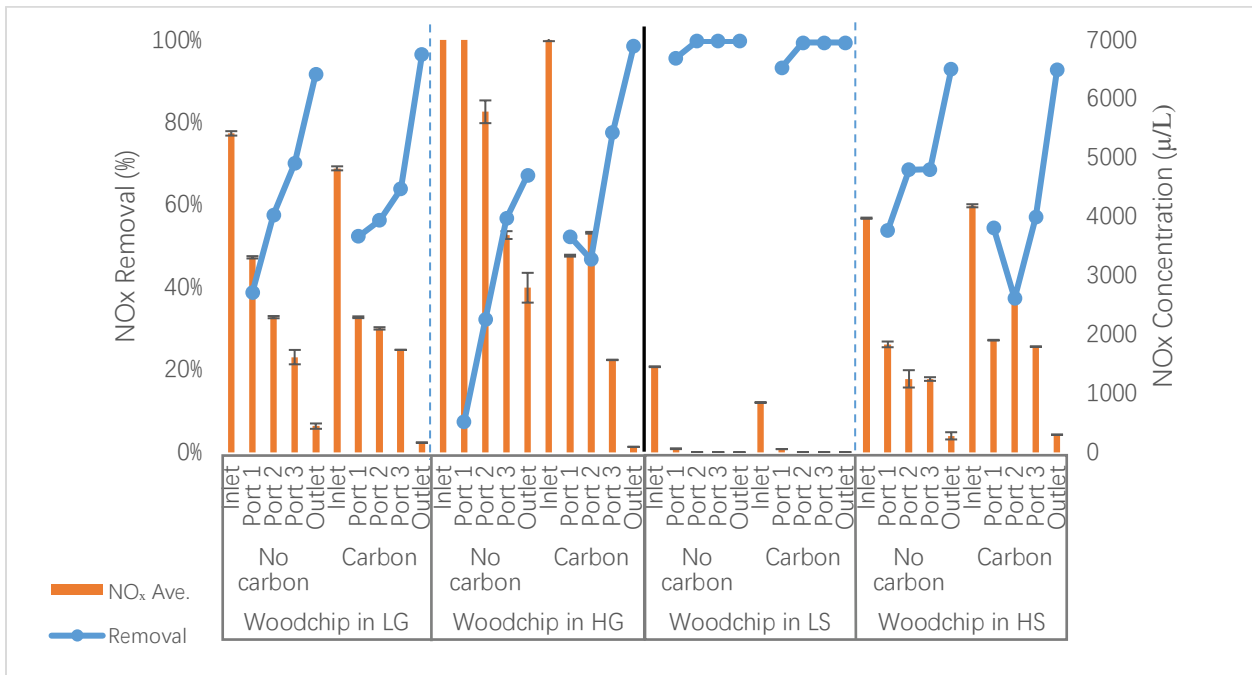


Figure 21. NOx concentrations and removal in woodchip for all scenarios

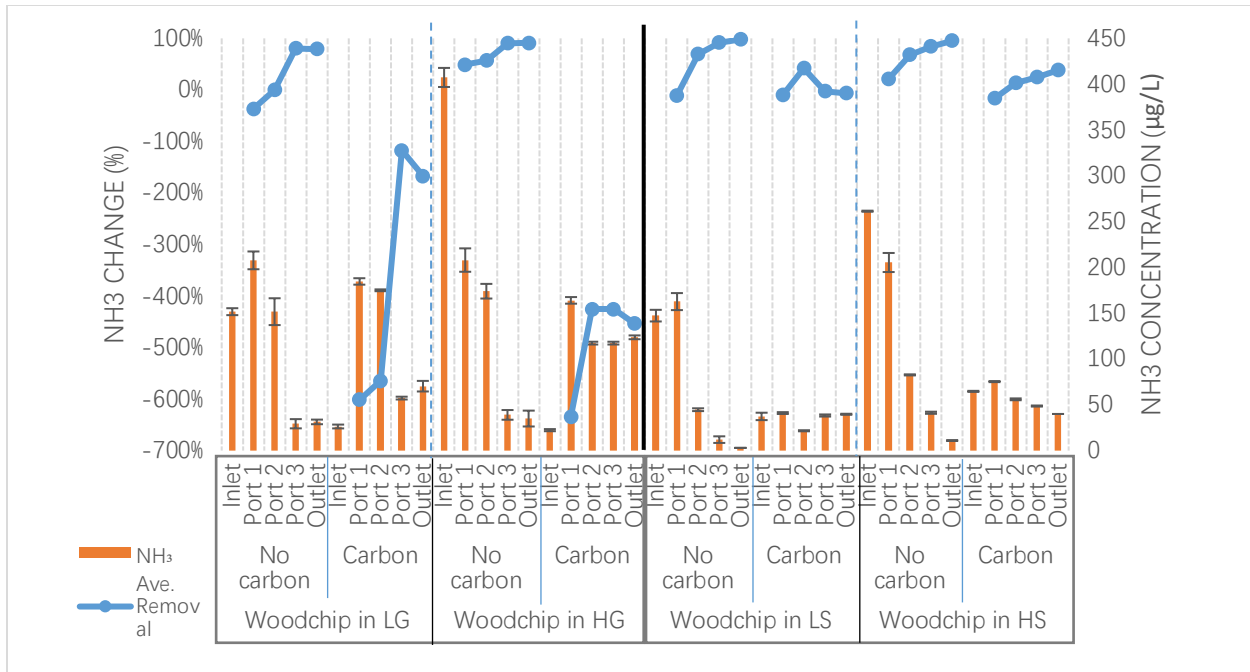


Figure 22. Ammonia concentrations and changes in woodchip for all scenarios

3.6 Copper Impact on Nutrient Removal

The nutrient concentration and removal comparison between the no copper and copper added cases (high initial TN stormwater) are shown in Figure 23, while the copper concentration and removals in B&G and woodchip are shown in Figure 24. The TN removal efficiency of column 2 decreased from 76% to 62% after copper addition, and from 80% to 71% in column 4. It seems the copper impact was not very severe or lethal to the treatment effectiveness. However, if we zoom in and focus on the TN removal efficiencies of port 1 or port 2 for both columns, the removal efficiencies all decreased after the copper addition. In port 1, the TN removal efficiency of column 2 decreased from 24% to 13%, whereas for column 4 it decreased from 34% to 5%. It is consistent with Figure 24 that most copper was removed from the top 1 ft section for both media, which means most of copper's negative impact happened at the top section. NO_x removal followed the same pattern as TN removal, since NO_x is the major contaminant in TN. However, ammonia removal was deeply affected by copper addition; it dropped from 17% to -88% in column 2, and from 96% to 34% in column 2. This indicates that AOB and NOB are more sensitive to copper and can be easily disturbed; more discussion about the bacteria evolution can be found in the following section.

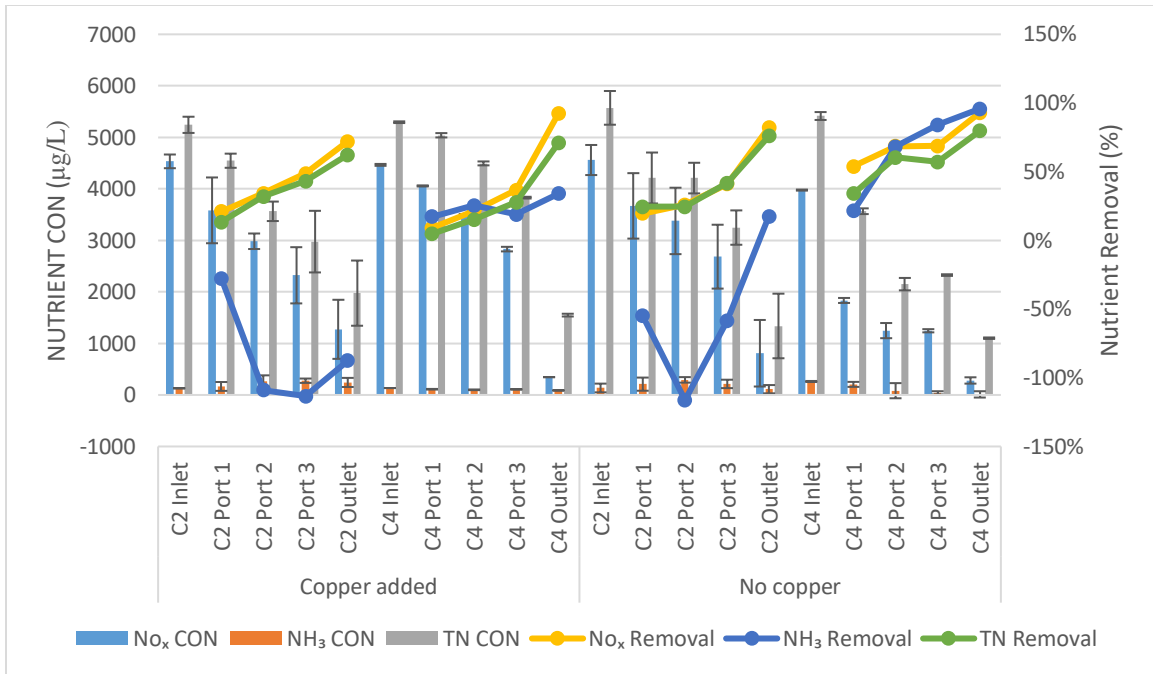


Figure 23. TN, NO_x, and ammonia concentrations and removals under high initial TN scenarios before and after copper addition in B&G and woodchip

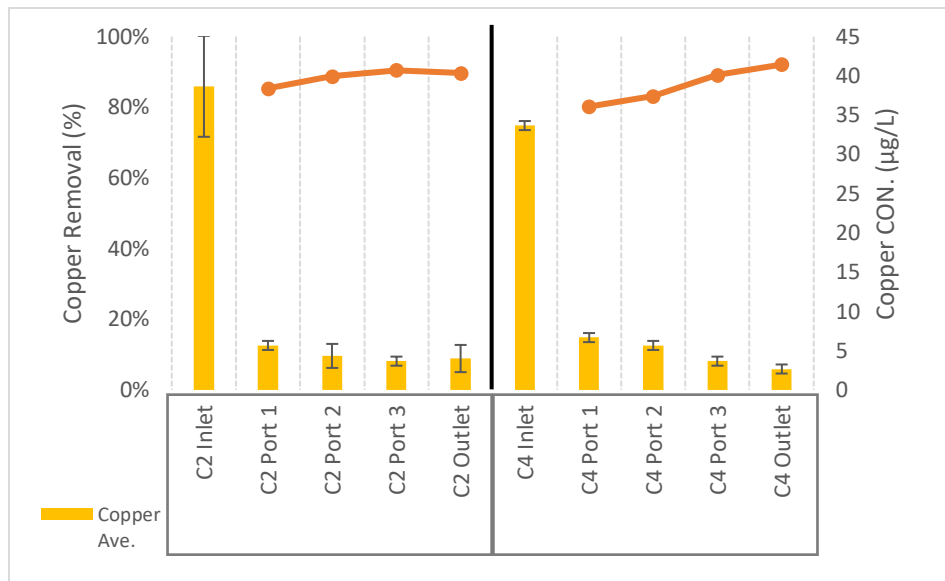


Figure 24. Copper concentrations and removals in B&G and woodchip columns under the high initial TN scenarios

3.7 qPCR Analysis in Column Study

By testing the density of target gene copies over different depths of media corresponding to key enzymes in nitrification and denitrification, the microbial ecology of AOB, NOB, denitrifiers and AMX are revealed for laboratory column study in **Figure 25** for the carbon impacts and **Figure 26** for copper impacts. The additional carbon seems to significantly increase the bacteria population, especially for AOB, NOB and denitrifiers in column 1 and column 2, as shown in Table 6. However, woodchip columns showed a different trend, as AOB and NOB were significantly decreased up to 90% for both columns 3 and 4. However, ~100% and 51-87% increase of denitrifiers and AMX was detected for columns 3 and 4. Since AOB and NOB normally accumulated at the surface layer of biofilm due to their high demands of oxygen availability, the restructure/changing processes caused by the carbon source would potentially disturb them more than denitrifiers and AMX, which normally stay at the bottom of the biofilm. However, this can hardly happen in B&G, as the changing of the biofilm structure is limited by the small porous space within the media. The microbial changes are consistent with the nutrient removal patterns, as stated in the previous section.

It is obvious that B&G also contains much more bacteria population than woodchip, due to its significantly higher surface area in unit volume, which would allow more biofilm to grow. It seems that in columns 1 and 2, the increased AOB would produce more nitrite, which enhanced NOB and generated more nitrate for denitrifiers as a cascade effect caused by carbon addition. Because carbon source would largely enhance the population and bioactivity of heterotrophic bacteria which have a much faster growth rate, it would increase the speed of decomposing organics and enhance the ammonification to produce more ammonia for AOB as the food source to start the cascade effect. The faster growth rate of the bacteria population in column 1 compared to column 2 (e.g., 3,989% AOB increased in column 1 and only 155% increase in column 2) indicates that the nutrient concentration level will shape the characteristics of microorganisms for the maximum thrive by using as many resources as possible. The average cell size in column 1 is smaller than that in column 2, which would benefit the bacteria extraction of nutrients from the low concentration liquid phase.

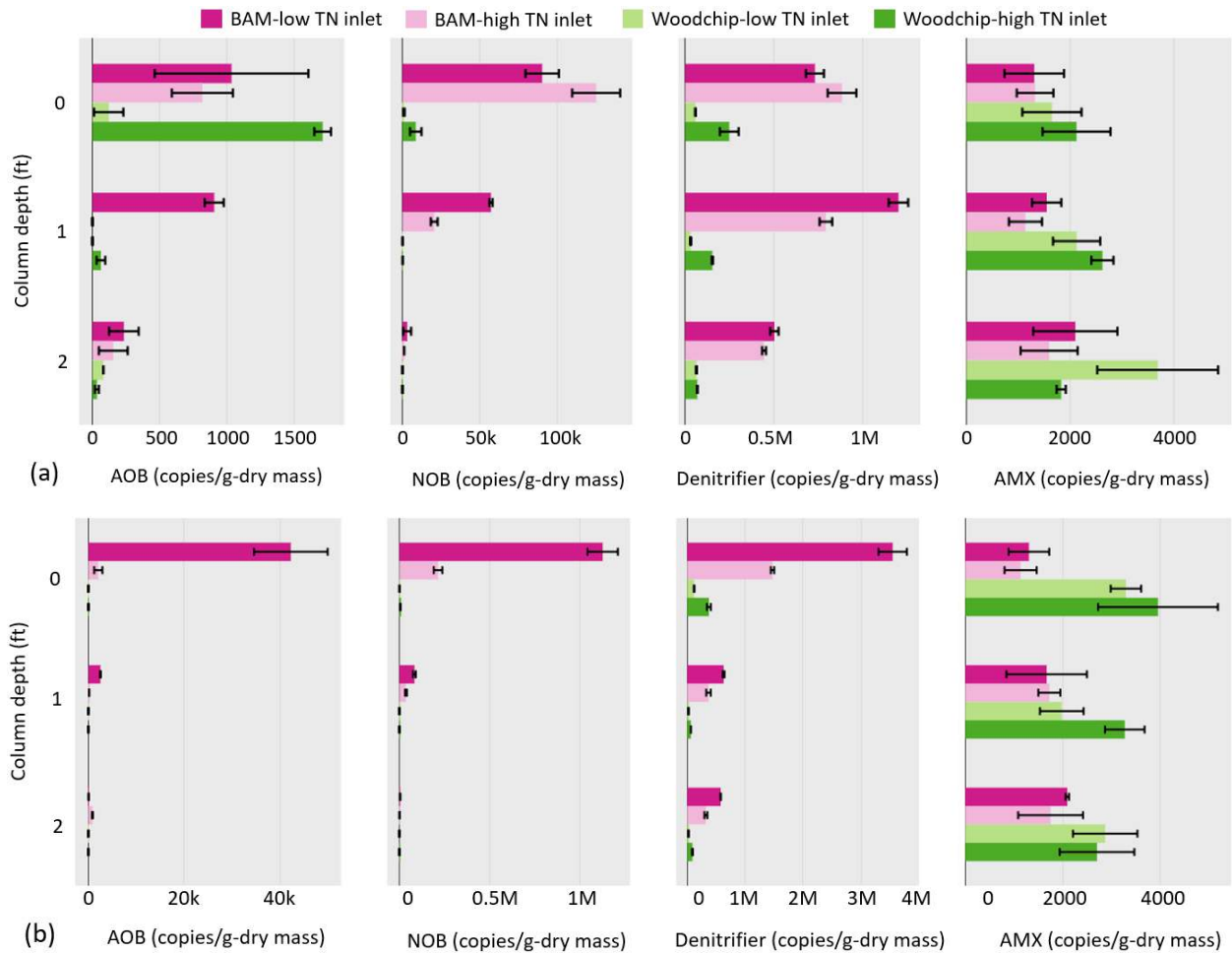


Figure 25. Gene copy density of AOB, NOB, denitrifiers, and AMX at different depths under low and high TN influent condition in B&G and woodchip columns (a) before and (b) after carbon addition

Table 6. Population change at the top layer after carbon addition

	Column 1	Column 2	Column 3	Column 4
AOB	3,989%	155%	-94%	-99%
NOB	1,142%	70%	-7%	-44%
Denitrifiers	386%	67%	105%	51%
AMX	0%	-14%	101%	87%

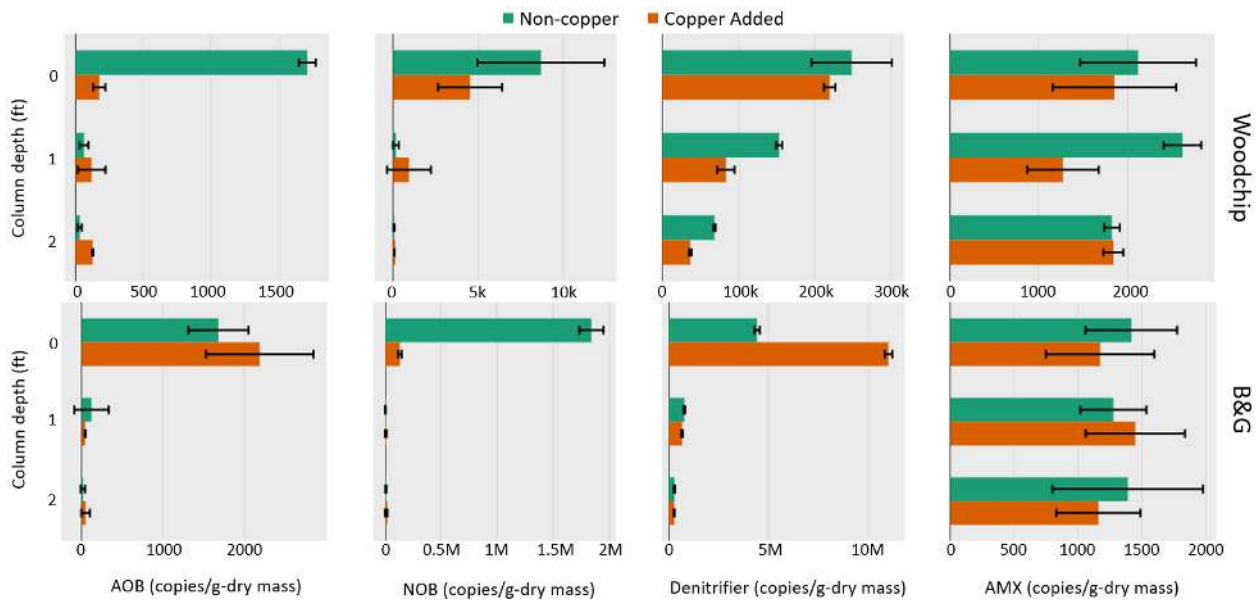


Figure 26. Gene copy density of AOB, NOB, denitrifiers, and AMX at different depths high TN influent condition in B&G and woodchip columns before and after copper addition

3.8 Bioactivity Analysis Results

The impact of copper on bioactivity is shown in Figure 27; with the copper addition, the bioactivity decreased by 54%. This is consistent with the AOB and NOB population results from the previous qPCR results. However, the decreased bioactivity could primarily be the result of heterotrophic bacteria that are very sensitive to copper presence. The carbon impact on bioactivity is shown in Figure 28, in which the bioactivity is shown to have increased 22 times in column 1 and 4 times in column 2 under low and high initial TN stormwater scenarios after carbon addition. This is also consistent with the microbial population from qPCR results which showed that microbial cell sizes are smaller in column 1 with much higher population enhancement.

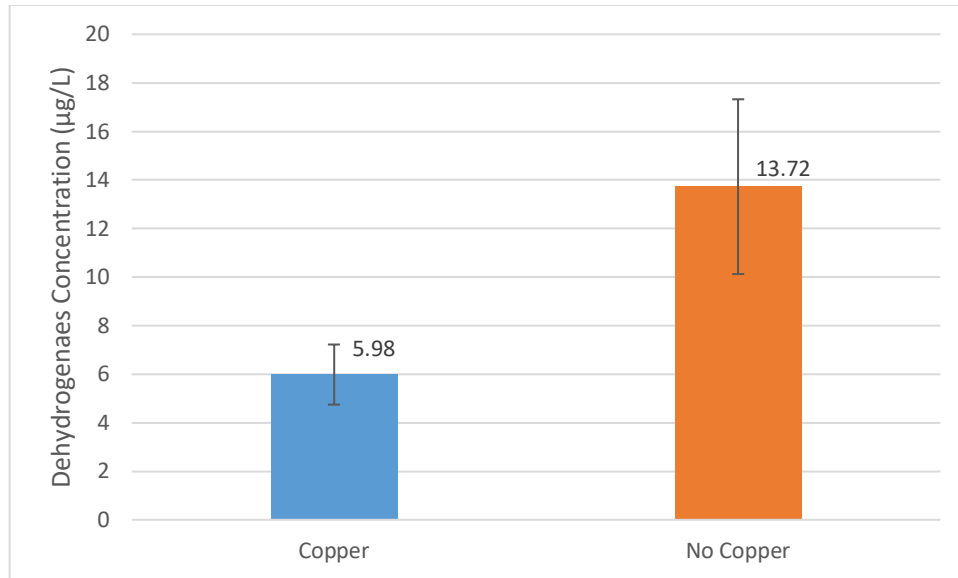


Figure 27. Concentration of detectable enzyme dehydrogenase of top B&G media in stormwater section before and after copper addition

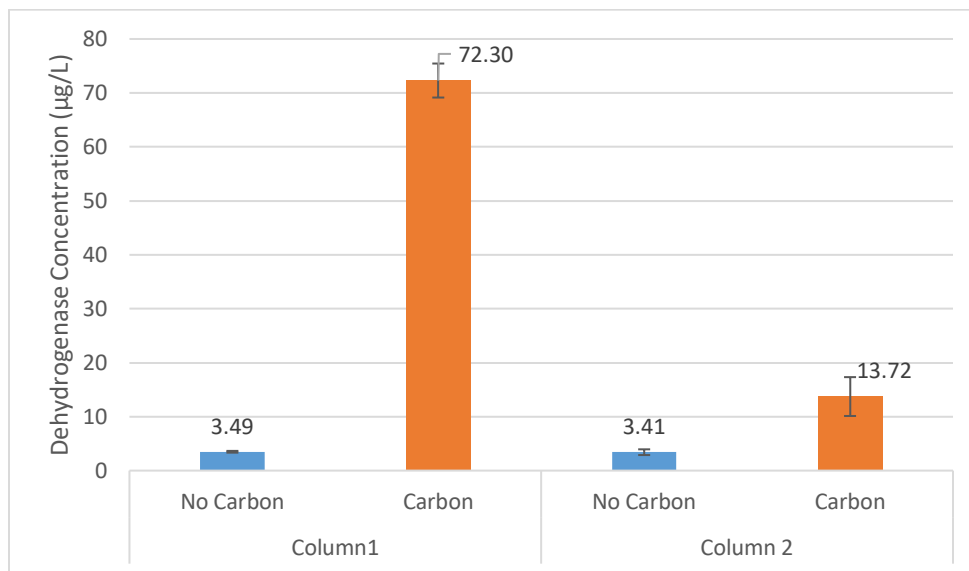


Figure 28. Concentration of detectable enzyme dehydrogenase of top B&G media in stormwater section before and after carbon addition

3.9 DON Analysis Results

Dissolved organic nitrogen (DON) is a dynamic participant in aquatic ecosystems and a potential source of reactive N for the phytoplankton and bacteria that cause water quality degradation (Bradley et al. 2010). Various harmful algal species may use organic nitrogen for some or all of their N needs (Berggren et al. 2015). Moreover, DON is normally considered a structurally

complex mixture of materials that vary in chemical structure and composition, which contains thousands of molecules that are not easy to identify or measure. UCF researchers obtained the chance to cooperate with the National High Magnetic Field Laboratory at the Florida State University (FSU), who provided access to the high-resolution Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) to assess the changes of DON at the compound level in stormwater samples before and after B&G and woodchip treatment. FTICR-MS is useful in dealing with complex mixtures, such as biomass or dissolved organic matters, since the resolution allows the signals of two ions with similar mass-to-charge ratios (m/z) to be detected as distinct ions.

The van Krevelen diagrams are graphical plots developed by Dirk Willen van Krevelen and used to assess the origin and maturity of kerogen and petroleum. The diagram cross-plots the hydrogen:carbon (hydrogen index), a function of the oxygen:carbon (oxygen index) atomic ratios of carbon compounds. It provides an effective and informative graphical method for displaying complex ultrahigh-resolution mass spectrometric data of complex DON from the FTICR-MS instrument. The location of different kinds of organic matter in the van Krevelen diagram are shown as an instructive guidance graph in Figure 29; it is possible to capture the overall changing direction of DON composition after treatment. The van Krevelen diagrams of DON in stormwater before and after the treatment with B&G and woodchip mixtures are shown from Figure 30 to Figure 33. The darkness of color and contour lines indicate the data point of identifiable DON formulas in the sample.

DON is the main food source for heterotrophic bacteria which convert high molecular weight DON (HMW-DON) to low molecular weight DON (LMW-DON) through degradation or convert LMW-DON into ammonia via ammonification. Degradation and ammonification can happen in parallel and can be affected by nutrient availability and carbon addition. For B&G, when the inlet is in low TN concentration (Figure 30), the microbial community tends to convert hydrocarbon into lignins as an indicator of bacteria's metabolism. After carbon addition, the enhanced microbial community tends to consume more DON as an indicator of microbial population growth. However, for high TN inlet concentration cases (Figure 31), the carbon addition is more helpful in digesting DON and converting it into lignins and proteins, which is an indicator of growth of microbial population and average cell sizes. Woodchip (Figure 32 and

Figure 33) in all cases produces condensed hydrocarbons, which means it may have a different microbial ecological structure than B&G, which tends to store DON as part of hydrocarbon. However, carbon addition can also enhance the microbial community in terms of the digestion capability of consuming more DON in lignins and proteins.

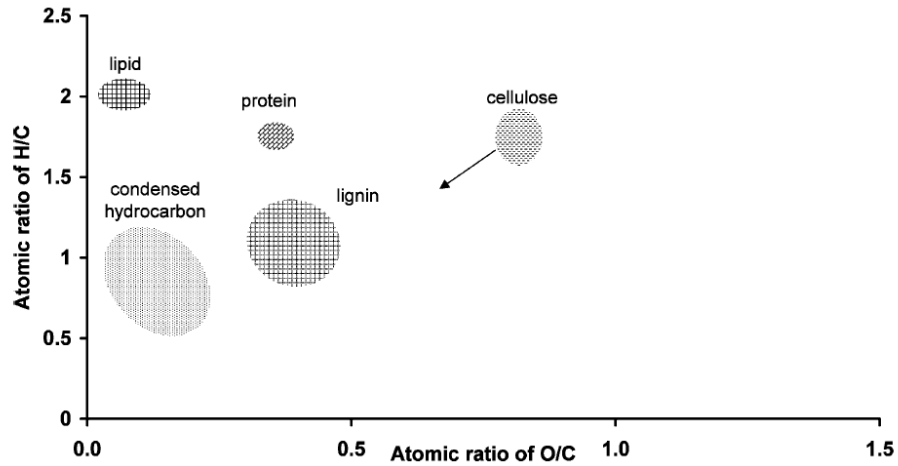


Figure 29. Regional plots of elemental compositions from some major biomolecular components on the van Krevelen diagram; the arrow designates a pathway for a condensation reaction (Kim et al. 2003)

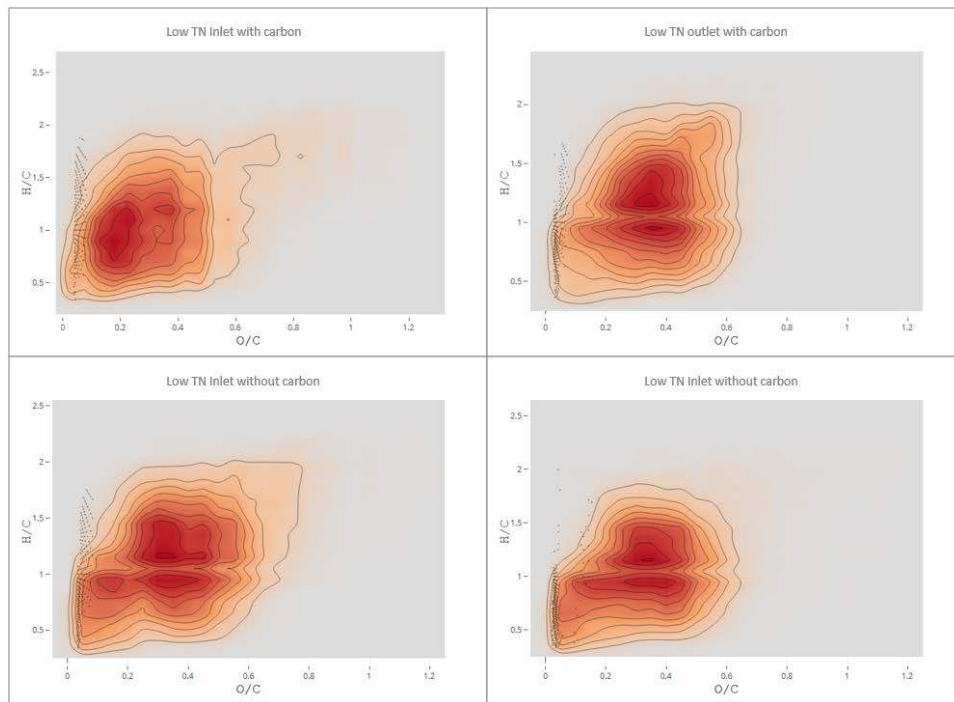


Figure 30. Inlet and outlet DON composition comparison for carbon and non-carbon scenarios in column 1

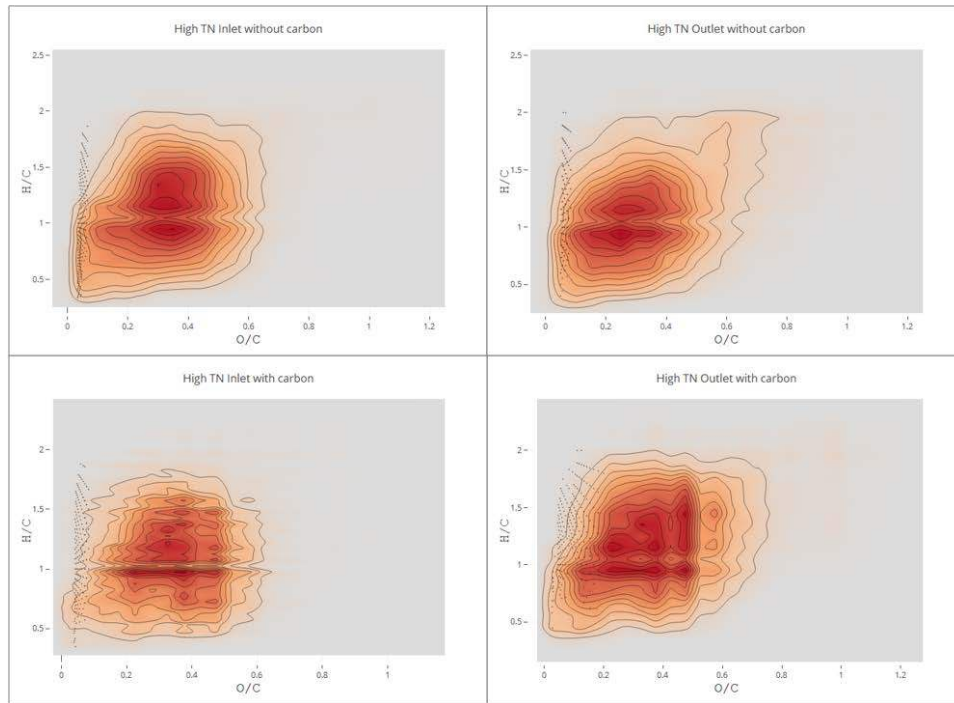


Figure 31. Inlet and outlet DON composition comparison for carbon and non-carbon scenarios in column 2

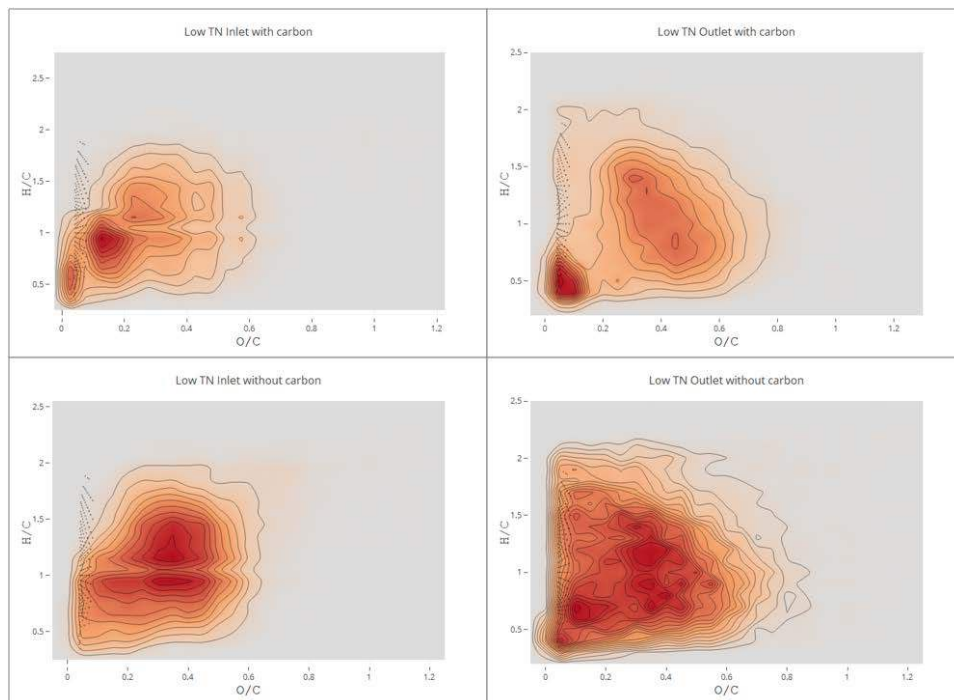


Figure 32. Inlet and outlet DON composition comparison for carbon and non-carbon scenarios in column 3

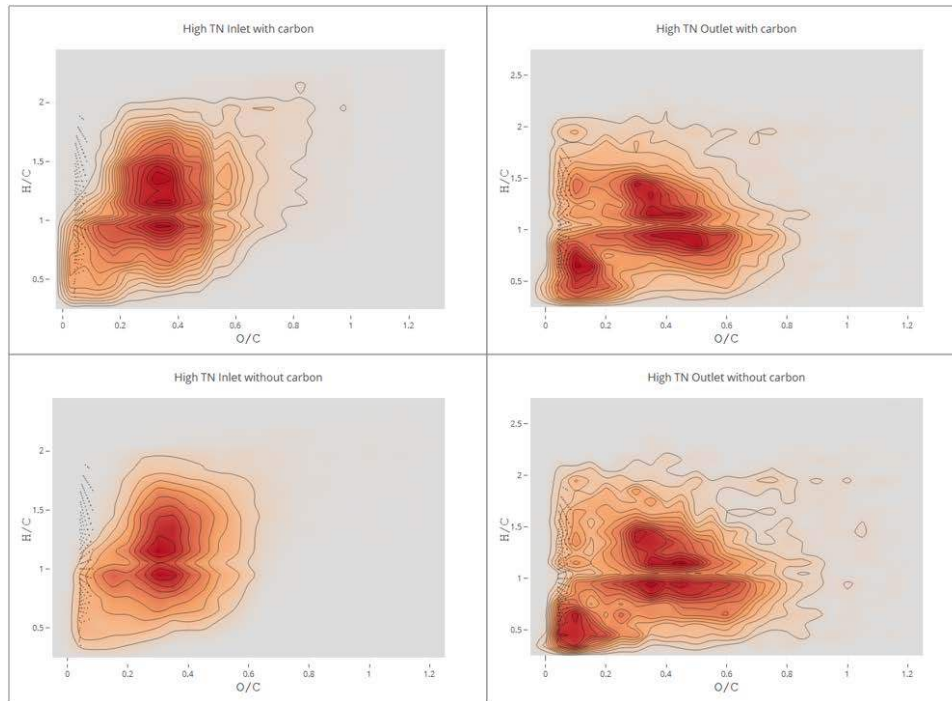


Figure 33. Inlet and outlet DON composition comparison for carbon and non-carbon scenarios in column 4

A summarized table is shown below for showcase all lab nutrients removal results. It includes TN, NO_x, and ammonia removal for B&G and woodchip when treat groundwater and stormwater. Carbon and copper impacts are also included.

Table 7. Summarized overall nutrient removal results in laboratory column study

	B&G treat groundwater				Woodchip treat groundwater			
	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	No <u>Carbon</u>	<u>Carbon</u>	No <u>Carbon</u>	<u>Carbon</u>	No <u>Carbon</u>	<u>Carbon</u>	No <u>Carbon</u>	<u>Carbon</u>
NO_x Removal	51.54%	99.92%	45.33%	54.11%	91.77%	96.56%	67.25%	98.65%
TN Removal	50.58%	87.98%	42.52%	51.90%	84.88%	89.86%	62.09%	92.83%
NH₃ Removal	7.33%	-960%	4.11%	-210%	79.34%	-167%	91.41%	-453%
	B&G treat stormwater				Woodchip treat stormwater			

	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	<u>No</u> <u>Carbon</u>	<u>Carbon</u>	<u>No</u> <u>Carbon</u>	<u>Carbon</u>	<u>No</u> <u>Carbon</u>	<u>Carbon</u>	<u>No</u> <u>Carbon</u>	<u>Carbon</u>
NO_x Removal	99.80%	98.32%	73.13%	63.08%	99.79%	99.41%	92.93%	92.82%
TN Removal	77.54%	82.15%	70.49%	63.10%	68.68%	59.31%	79.65%	87.25%
NH₃ Removal	-8.55%	-16.5%	14.13%	-168%	97.96%	-6.3%	95.79%	38.14%
	B&G treat high TN stormwater				Woodchip treat high TN stormwater			
	<u>No copper</u>		<u>Copper</u>		<u>No copper</u>		<u>Copper</u>	
NO_x Removal	73.13%		71.90%		92.93%		92.26%	
TN Removal	70.49%		62.31%		79.65%		70.73%	
NH₃ Removal	14.13%		-127%		95.79%		34.08%	

4. Field Study Results

4.1 Project Meeting and Site Visit

On 2/24/2016, a project meeting was held at the study site of Fanning Springs. Members who attended this meeting were AECOM, Suwanee River Water Management District, FDOT, and UCF. Two main discussion topics are summarized below:

- 1) The woodchip mixture may not be saturated in the condition of down flow, which would decrease the nitrogen removal efficiency since denitrification cannot occur without anaerobic conditions. However, the woodchip column in the laboratory at UCF was ponded to ease sample collection, rendering a different nutrient removal outcome. Two suggestions

were made during the meeting: the first one was to change the down flow to up flow, and the second one was to change it to horizontal flow.

- 2) Carbon source may be a critical factor for nitrogen removal, and some removal phenomenon has been assumed relevant to the presence of carbon because woodchip would provide carbon for microorganisms, which may enhance biological reactions. **Figure 34** shows the Fanning Springs site with recreation area, and **Figure 35** shows the linear ditch construction site, in which the red flags on the left side indicate public service lines, such as telephone lines and cables, and the flag on the right indicates the boundary of this construction site. The linear ditch was constructed between the left and right flags within the existing right-of-way swale.



Figure 34. View of Fanning Springs



Figure 35. Linear ditch construction site

4.2 Field Conditions

As shown in **Figure 36**, the linear ditch BMP was designed to fit parallel to the highway with trenches of woodchip and B&G media, respectively. During sunny daytime, the solar powered pump would pump the groundwater from the extraction well and distribute it along the pipeline at the top of both media. When storm comes, the pump would stop, and the linear ditch is fully switched to treat the runoff from the roadway and farmland. The continuous running of groundwater or stormwater in the media keeps moisture necessary for bacteria survival and maintains relatively higher nutrient biological removal capacity than a normal detention pond that would experience wet and dry conditions variously.

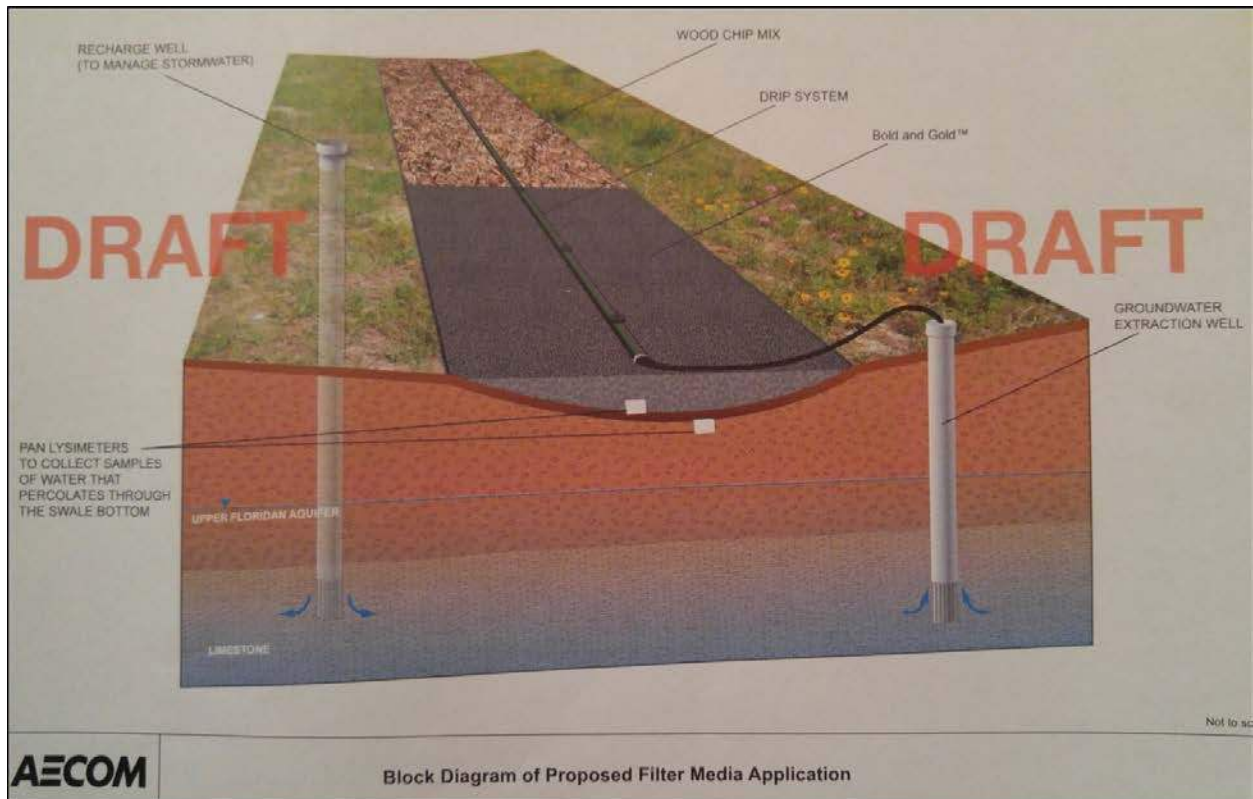


Figure 36. Schematic design proposed by AECOM

The schematic design and operation strategy is shown in **Figure 37**. The total length of the linear ditch is 600 ft; it was divided into two equal sections with 300 ft of each for B&G and woodchip, and the width is about 4 ft for both media sections. Furthermore, the woodchip section was divided into three subsections with depths of 2, 3 and 4 ft, and the length of each section is about 100 ft. The B&G section was divided into two subsections with depths of 1 and 2 ft, and the length of each subsection is about 150 ft. The various depths of each subsection in B&G and woodchip are designed to evaluate the impacts of different depths on the nutrient removal effectiveness. One solar pump was used to pump the groundwater during sunny daytime to distribute it on the surface of each media trench. The before-and-after site view is shown in **Figure 38**. After construction, there were lots of plants growing above each section of media, which can be a potential source for organic nitrogen such as dead leaves.

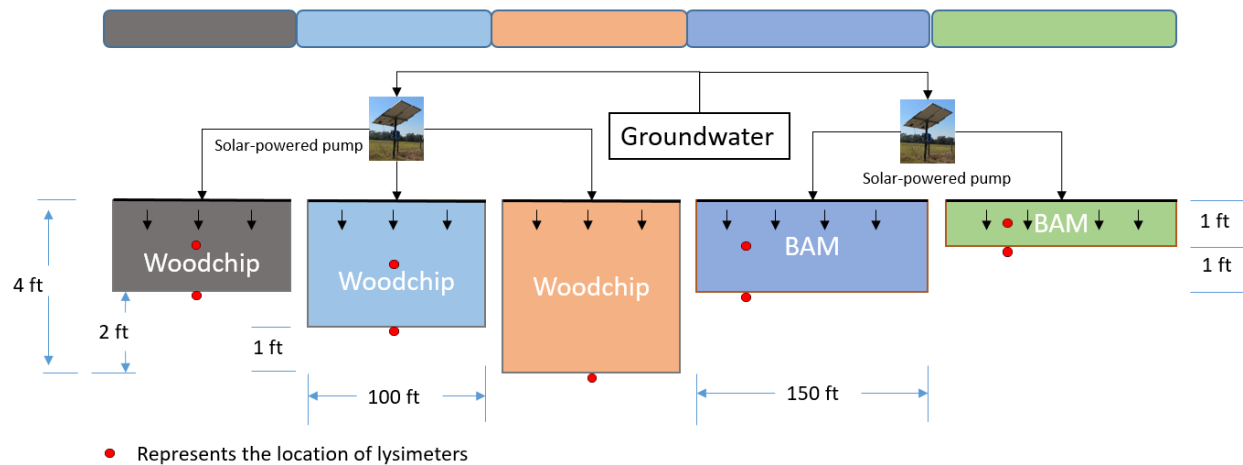


Figure 37. Schematic flowchart for design, construction, and operation strategy in the field



Figure 38. 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 media and woodchip sections; lower right: operational phase of B&G media and woodchip sections)

4.3 qPCR Analysis in Field

By testing the density of target gene copies over different depths of media corresponding to key enzymes in nitrification and denitrification, the microbial ecology of AOB, NOB, denitrifiers and AMX for field samples are revealed in **Figure 39**. Note that the field woodchip decomposed 50%

over the operational period of time. This means that the original woodchip of depth 4 ft is now a thinner layer of depth 2 ft, the original woodchip of depth 3 ft is now woodchip of depth 1.5 ft, and the original woodchip depth of 1 ft is now almost gone with less than a depth of 0.5 ft. This decay in depth may result in an unsafe operation. This decomposition makes it hard to separate the whole test site as top, middle, and bottom layers for the 0.5 ft depth section. Therefore, only media samples in the current woodchip depth of 1.5 ft and 2 ft were collected and analyzed for microbial ecology analysis as top, middle and bottom in **Figure 39**. The comparison between the lab (section 3.7, **Figure 25**) and field microbial information showed some common patterns. One is that NOB and denitrifiers had more population than AOB and AMX in the field, and that denitrifiers were the dominant bacteria of the four bacteria species in nutrient removal. The reason for this might be that nitrate/nitrite is one of the major chemical species in water. The other pattern is that B&G media were 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 (see section 3.1). Nevertheless, there were 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, which sometimes results in the most abundant bacteria in the middle or even the bottom layers. The main reason for this is the near constant environment in the laboratory settings (i.e., hydraulic loading, nutrient concentration, temperature, etc.), which is beneficial for bacteria to adapt and thrive. However, the uneven water distribution or preferential flow in the field may form very different micro-environments that make bacteria growth in various depths randomly possible.

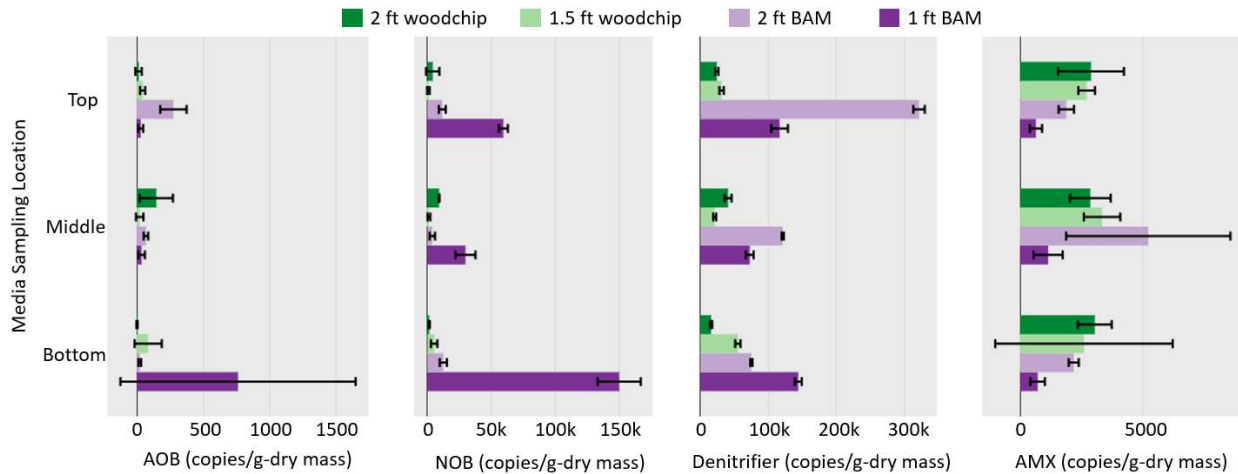


Figure 39. Gene copy density of AOB, NOB, denitrifiers, and AMX at the appropriate depth of each BAM and woodchip section in the field after operation

4.4 Nutrient Removal Results

4.4.1 Ammonification and Nitrification

Ammonification is the part of the nitrogen cycle that converts the organic nitrogen into ammonium and is followed up by the nitrification and denitrification processes. It requires the existence of organic matter and enough oxygen for bacteria to do the job. Then nitrification process consumes ammonia and generates nitrite and nitrate, which are also biological reactions that require oxygen. The two biological reactions can happen in parallel if the aerobic environment and other condition is suitable for corresponding bacteria. Since the linear ditch is designed to treat the discharge from a farmland with plenty of grass and other plants on it, 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 ammonia and organic nitrogen removal in the field are shown in **Figure 40** for B&G medium 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 medium and woodchip sections, which is consistent with the laboratory results, as the groundwater used in our column study was collected from Fanning Springs (see sections 3.4 and 3.5). In other words, almost all organic nitrogen was introduced from either the stormwater runoff (from road or farmland) or the microorganism activity in the media. For B&G medium, the highest organic nitrogen concentration (2.38 mg/L) was found at the middle

lysimeter at 1 ft depth. After that, the organic nitrogen concentration decreased rapidly and normally below 0.5 mg/L from the depth of 1 to 2 ft. Because of organic nitrogen intrusion, some ammonia was generated through ammonification at the bottom of the B&G section at 1 ft depth. However, there was only a mild ammonification process, with a small amount of ammonia generation due to the limitation of available oxygen. The holistic observation of the B&G medium section in the field was consistent with its performance in the laboratory column study. However, the woodchip performance was entirely different in the field. There was an enormous increase of ammonia concentration up to 9.1 mg/L at the middle lysimeter of the 2 ft depth section, and the rest ranged from 0.6 to 3.6 mg/L, which is significantly higher than the B&G medium section. This is because particulate organic nitrogen (PON) can more easily be transferred through woodchip than B&G medium and potentially triggers more intensive ammonia generation through ammonification, especially 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 no nitrate or nitrite. Again, because of 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 39**). There is another possible reason related to the second denitrification pathway of dissimilatory nitrate reduction to ammonium (DNRA), the details are discussed in the section 4.4.2. Also, there is an observation that some pocket gophers showed up only in woodchip sections, it is possible that animal activities could enhance the nutrient releasing process, which are further discussed in section 4.5.

The significant increased concentration of ammonia in the woodchip field sections showed a completely opposite trend when compared to the laboratory stormwater treatment results in the woodchip columns. There are two reasons to explain this phenomenon: one is that the stormwater used in our column study was 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. In addition, a significant number of plants were found in the field (**Figure 38**), which is a potential source of organic nitrogen as well. All those leaked organics supported more heterotrophic bacteria to decompose them and resulted in a large amount of ammonia generation. The other reason is related to the microbial community for nitrification. As explained in section 4.3, the microbial community (especially autotrophic bacteria) in the field is

much smaller and more 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 4.5), in addition to the higher concentration of organic nitrogen found in the field. The woodchip in the field had a small amount of AOB and NOB to deal with the highly concentrated ammonia, leading to the leakage of ammonia in high concentrations.

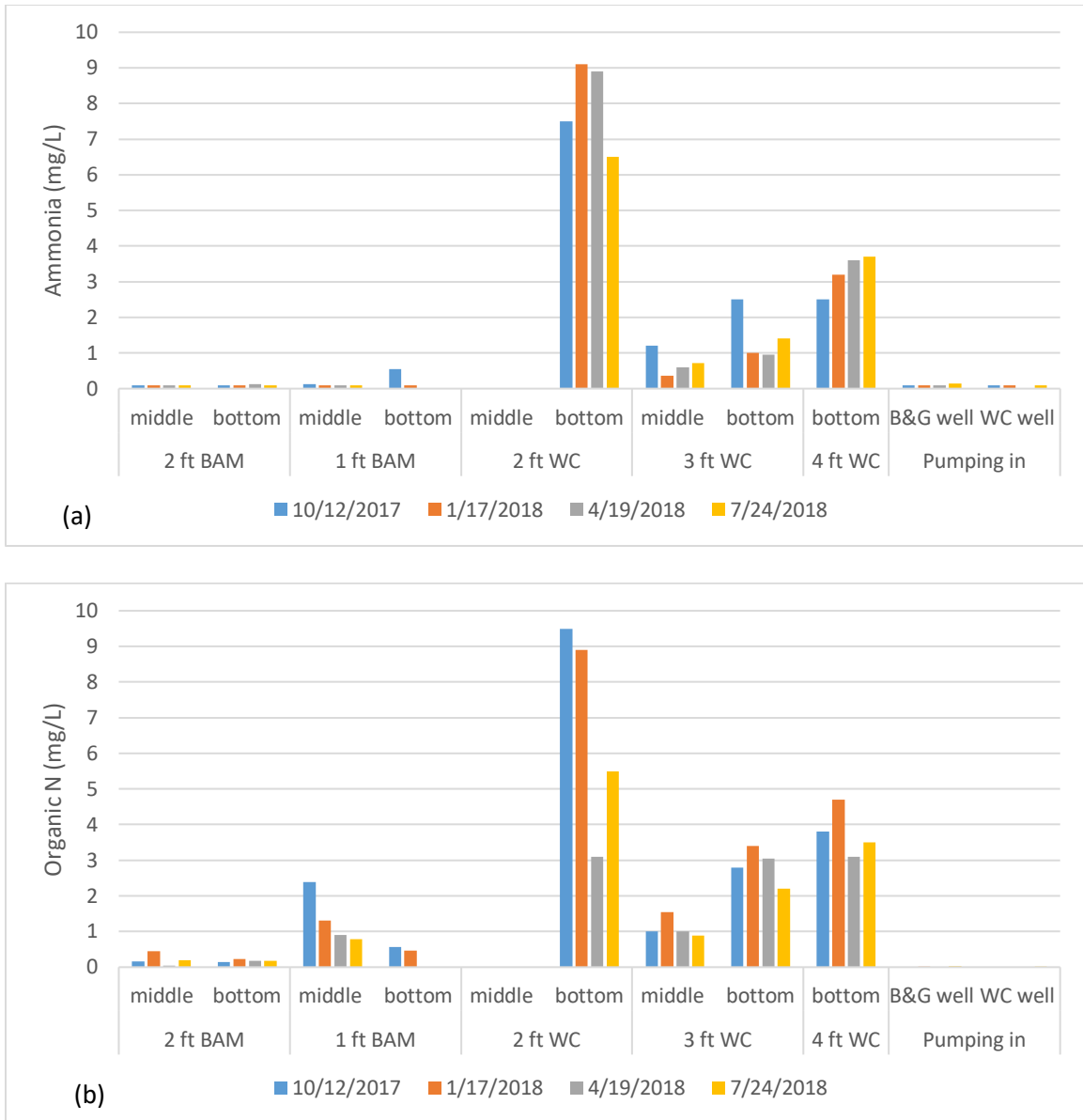
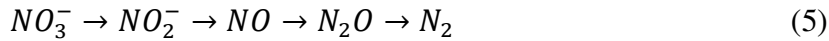


Figure 40. Field nutrient removal of (a) ammonia and (b) organic nitrogen (Note: No samples can be collected from the middle lysimeter of 0.6 m (2 ft) and 1.2 m (4 ft) woodchip sections.)

4.4.2 Denitrification

Denitrification (DNF) is normally considered as the critical step that converts dissolved inorganic nitrogen into atmosphere as nitrogen gas, its pathway is shown as Equation 5. However, there is a competing nitrate removal pathway that also plays an important role in the nitrogen cycle, which is dissimilatory nitrate reduction to ammonium (DNRA) as shown in Equation 6. Different from DNF, DNRA keeps nitrogen in the form of ammonia within the system instead of releasing

it as nitrogen gas to atmosphere via DNF. More than 30% of nitrate was reduced through DNRA in over half of investigated coastal site and DNRA dominates one-third of all investigated sites (Giblin et al. 2013). It is highly possible that DNRA is one of the two contribution processes of ammonia generation, and the other is ammonification as discussed in section 4.4.1.



As shown in **Figure 41**, B&G media in the field showed a trend similar to the one in the column study (**Figure 18**). 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 small porous size, as B&G media remains moist for at least 21 days (Naujock, 2008). This is also the reason that B&G media performed extremely well in removing organic nitrogen, since the PON was filtered at the B&G media surface. The woodchip in the field showed promising NO_x removal of over 97%, which is very similar to the result from the column study (**Figure 21**). The main denitrification process occurring in the woodchip is different from that in the B&G, where DNRA might be the dominant one in woodchip, while DNF is the main denitrification process in B&G. The most important reason is that the oxygen and electron donor (mostly carbon source) availability is entirely different in B&G and woodchip. DNF can only exist in anaerobic condition where B&G maintains the perfect environment with the help of moisture, but DNRA can exist in both aerobic and anaerobic conditions where the woodchip provides ideal situation for it with carbon source support (Yoon et al. 2015). More intensive DNRA is consuming nitrate and produce ammonia (Figure 40 and Figure 41), especially when their food nitrate is the main contaminant in groundwater that has been pumped through the pipeline during sunny time. So DNRA is also another contributor of ammonia other than ammonification. Compared to woodchip, B&G has limited carbon source to support the denitrification that generates nitrogen gas, which resulted in relatively lower NO_x removal. It is not quite clear why the woodchip performs differently in the lab, it could be that the stormwater quality is different on-site from the one collected at the UCF campus. The difference in water quality may be favored by the DNRA process within the woodchip.

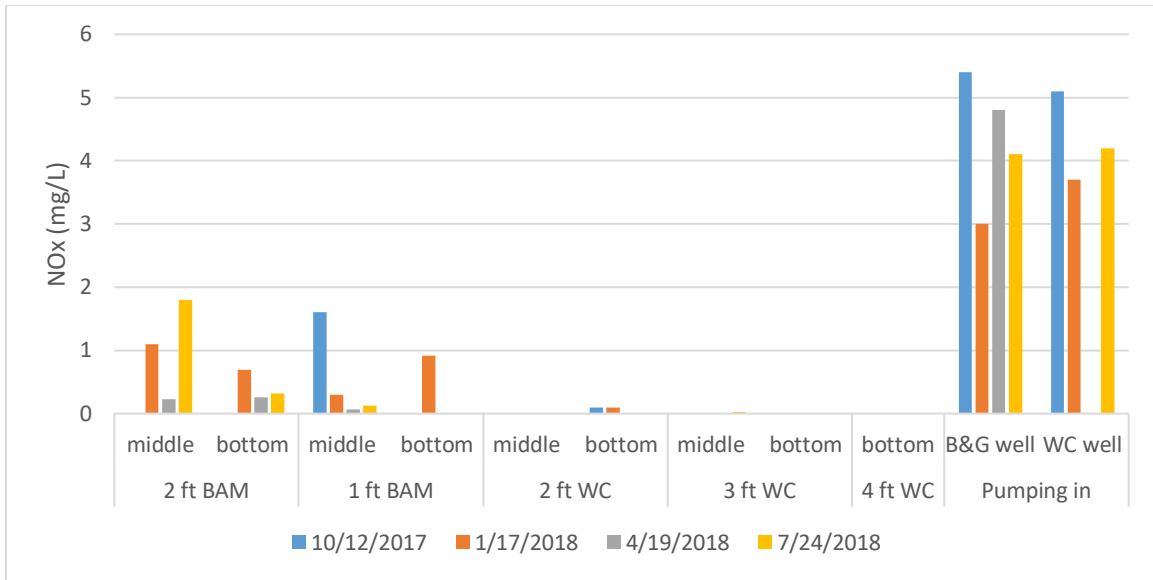


Figure 41. NOx concentration in the field lysimeters in the field

4.4.3 TN Removal

The field TN concentrations are shown in **Figure 42**, and the TN removal of B&G media is 52-80% and 68-95% for 1 ft and 2 ft depth sections, respectively. These values are very close or sometimes even better than the laboratory results. However, the woodchip in the field performed entirely differently from the one that was observed in the laboratory. It had almost no positive removal in the field except 16 -17% TN removal from the bottom lysimeter of the 3 ft depth section on 4/19/2018 and 7/24/18. 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 the 2 ft depth section on 1/17/2018. As mentioned in the previous section, the major reason why the B&G media performed better than woodchip is that B&G media can filter the sediments that also carry organic matter through the runoff. Woodchip, on the other hand, has no such capability due to its large void space and enhanced DNRA process that generates significant amount of ammonia. 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 flows into the linear ditch, the infiltration rate through the B&G media will not change more than a few percent from time to time because its HRT is limited by the small porous size. On the other hand, when it was dry, the B&G media was also able to maintain necessary moisture for bacteria survival. Therefore,

B&G media allowed sufficient contact time for the bacteria to do their job and cultivated more bacteria population than woodchip. 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. However, the TN removal would drop dramatically when the stormwater runoff is resulting in a larger quantity of water flowing through the woodchip with negligible contact time, minimizing the treatment effectiveness.

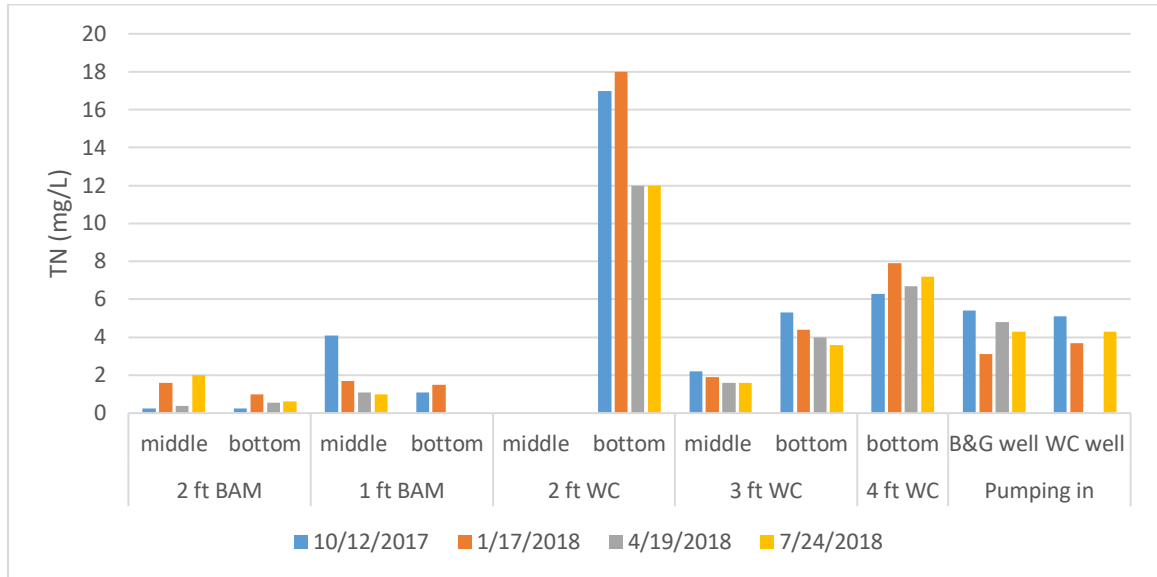


Figure 42. TN concentration from each lysimeter and influent (pumping well) for B&G media and woodchip

4.5 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 43** in which three sampling time points (10/12/2017, 1/17/2017, 4/19/2018, and 7/24/18) are identified. The rainfall data were collected from the Suwannee River Water Management District with an automatic rain gage located at a latitude of 29 40' 02" and a longitude of 82 52' 29". Figure 6 provides a general understanding of how often and how many storm events happened in this area, which is closely related to the treatment effectiveness of different kinds of nutrient species. Note that whenever the storm happens, the pump slows down or stops 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 records provide insightful information regarding 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 114.38 and 108.62 L/m²·day⁻¹ for B&G media and woodchip media, respectively.

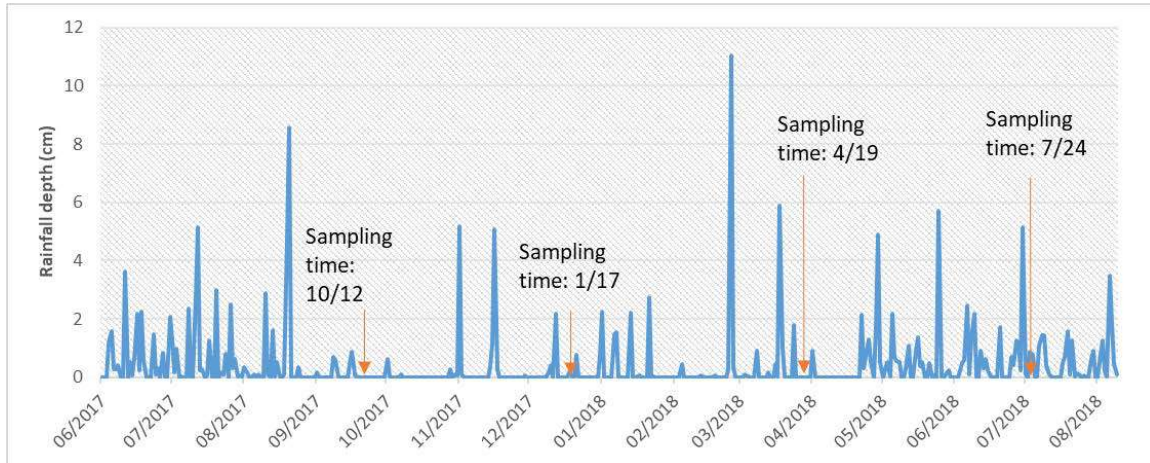


Figure 43. Rainfall depth during the linear ditch operation period and the corresponding sampling time point

Table 8. Pumped groundwater volume readings since the start of the linear ditch study

Date	Incremental 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	14.44	1,513	13.51
11/17/2017	36	1,872	7.06	1,748	6.53
12/7/2017	20	2,128	12.80	2,014	13.29
1/17/2018	41	2,582	11.06	2,450	10.62
2/1/2018	15	2,767	12.37	2,636	12.43
2/6/2018	5	2,823	11.13	2,687	10.27
04/19/18	71	3,789	13.60	3,574	12.48
6/5/2018	49	4,408	12.63	4,243	13.66
6/13/2018	8	4,501	11.63	4,333	11.33
7/24/2018	39	5,063	14.42	4,880	14.02

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 9**. Unlike the steady controllable environment in a laboratory in terms of temperature, inflow conditions, water quality, and hydraulic patterns, the field condition is much more complicated, with a 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 particularly affect the biological processes of ammonification, nitrification, and denitrification, all of which 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. Another interesting phenomenon is that almost all pocket gophers were found active in woodchip section in the field. Pocket gophers play an important role in soil ecological functionality, their extensive excavations impact on the generation of nutrients and change of soil conditions, which significantly accelerate erosion and downslope soil movement (Reichman and Seabloom 2002). The nutrient releasing is more intensive with such ecological activities, the more pocket gophers the more nutrients will be released into the woodchip (Huntly and Inouye 1988). But researchers found it differently that gophers’ activities reduce the nitrogen in surface soil in 50 years observation and increase point-to-point nitrogen variability in soil nitrogen (Inouye et al. 1987). However, the field condition is complicated especially when BMPs are implemented, it is hard to say that pocket gophers’ activities can impact so much on the whole woodchip section, since all samples in woodchip showed high ammonia concentrations while only couple of pocket gophers were found in there.

Table 9. Environmental and loading condition differences between lab and field operation

Condition	Laboratory	Field
Water source	Groundwater collected from Fanning Spring,	Groundwater pumping from the solar-powered pump, runoffs from highway

Condition	Laboratory	Field
	stormwater collected from the pond on UCF campus	stormwater runoff and farmland agricultural discharge
Oxygen availability	Relatively closed environment limits the oxygen availability in woodchip particularly	The open field would be more helpful for oxygen dispersion into the woodchip void space, which depresses the DNF while sustains the DNRA
Pollutant loads	Groundwater and stormwater spiked with nitrate standard solution	Highly variable in terms of pollutants species and concentrations. especially pesticide and fertilizers introduced from the farmland
Inflow rate	Consistent of 10 to 15 mL/min (use loading rate per square meter)	Highly variable when storm happens, and relatively variable when the pumps are working due to the availability of solar power (use average loading rate)
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
Animal activities	No such activities can happen in lab	Pocket gophers showed up mostly in woodchip section
Other disturbances	None	Uneven pumping rate along the pipe line system may occur as well as animal chewing the pipe line

5. Cost-Benefits and Practical Considerations

The cost of media and delivery to the site is used for comparing the performance cost of each media. The cost of the media based on the lab study is the actual sales and delivery cost and does not include installation and replacement costs. Installation can be a significant cost and is usually more than the media cost. The cost of B&G media delivered is \$5 per CF (actual documented product and delivery price), while there were no historical sales and delivery cost for the woodchip mix. However, for comparison purposes, a woodchip cost is assumed at \$2.00 per CF (bulk price plus delivery). The installation cost of B&G and woodchip is based on standard construction practice, essentially using a trench with parent soil cover. To reduce the flow rate in woodchip to obtain the infiltration rates used in the laboratory would take some type of flow-limiting construction containment area or a pump. It should be noted that the laboratory testing with the same loading rate for woodchip and B&G resulted in removal based on controlled hydraulic design. The benefits are the mass removal of nitrogen obtained from laboratory experiments and the field nutrient removal results (for B&G only). For the 4-foot columns used in the laboratory, a residency time of 95 minutes of B&G and 41 minutes of woodchip were adopted from the tracer study (section 3.1). Note that the residence time is closely related to the inflow rate, which was constant in the column study.

Cost and benefits are calculated based on lab treatment conditions, including 15 mL/min (1.96 inch/hour) flowrate and a bed volume of 1,357.16 in³. The cost and benefits of both media for treating stormwater and groundwater, based on the lab study result with and without carbon addition, are shown in Table 10 and Table 11. The benefits are estimated for the field; it was only conducted for B&G since woodchip is mostly showing negative removals in the field. The most recent pumping record (Table 8) was applied as the treated water volume that was evenly distributed into the two subsections in B&G, the inlet nitrate concentration in 5.2 mg/L, over a 20 year operation time, and the construction cost is about \$23,000. However, different assumptions are adopted in the column study; a 20-year period of treatment with media in column was assumed for B&G, but only 8 years operation period was assumed for woodchip, due to its decomposition problem. The average cost of the media for removing one pound of nitrogen is calculated as \$4.48. This is a non-weighted unit cost and a simple average of the test condition results. However, the

field results indicated an average cost of \$33 to remove one pound of nitrate in 20 years. The higher field cost is due to the inclusion of the construction of the filters in the field and the media cost while in the laboratory only the media cost is included. In addition, the field results only consider the pumping volume and ignore the stormwater that has been treated in the linear ditch. This means that the actual cost for removing one pound of nutrient would be much lower since the stormwater runoff volume is not considered. Also note that woodchip can only work well under saturated conditions. Recognizing that there is a probable decay of woodchip in a woodchip gravity filter (visual estimated at 50% in one year), the cost per pound of nitrogen removed was \$4.39 by assuming the life expectancy of the woodchip equal to 8 years based on lab condition. Note that this is an ideal condition in the lab with a fixed flow rate, saturated conditions and inlet water quality.

When treating groundwater, the addition of carbon reduces the cost per pound of removal. However, when treating stormwater, the addition of carbon does not significantly affect the cost per pound removed. It should be noted that the cost to introduce carbon compounds was not added to the cost estimates and furthermore the method of introduction was not included. At this time, it is not recommended to add carbon to increase removal. If added, a cost benefit analysis can be completed.

The cost and benefits of both media for treating stormwater with and without the impacts of copper are summarized in Table 11 based on lab conditions. When copper was added into the system for the scenario HS, the removal/mass of B&G mixtures decreased by 23% (from 0.052 g-TN/lb-media to 0.04 g-TN/lb-media), while the removal/mass of woodchip mixtures decreased by 13% (from 0.123 g-TN/lb-media to 0.107 g-TN/lb-media). The cost and benefits of B&G and woodchip in the presence of copper are approximately the same. Installation and operating costs are not included in this comparison.

Table 10. Cost-benefit analysis of B&G mixtures under multiple scenarios before and after carbon addition in lab and field treatment

Scenario	LG		HG		LS		HS		B&G in Field		
	NO	YES	NO	YES	NO	YES	NO	YES	1 ft depth	2 ft depth	
Carbon in Water											
Inlet TN ($\mu\text{g/L}$)	4461	5936	6059	8174	2077	1600	5761	5125	4433		
Overall TN Re.	87.98%	50.58%	42.52%	51.90%	77.54%	82.15%	70.49%	60.13%	70.67%	86.40%	
Flow rate (mL/min)	15								Highly variable		
Bed volume (in^3)	1357.16								1036800	2073600	
Treating Time (day)	7300								300		
Treating Volume (L)	157680.00								1899000		
Media Price ($\$/\text{ft}^3$)*	5								5		
Media Density (g/cm^3)	1.39								1.39		
Removal/Volume (g/m^3)	27826.6	21287.1	18265.8	30077.8	11418.4	9319.1	28791.9	21848.9	267518	327033	
Removal/Mass (mg/kg)	20019.2	15314.5	13140.9	21638.7	8214.7	6704.4	20713.6	15718.6	192459	235276	
Removal/Cost (mg/\$)	157613.3	120572.8	103459.6	170364.3	64675.4	52784.2	163080.5	123754.7	1515254	1852353.25	
Removal/Volume (oz/ft^3)	27.58	21.10	18.11	29.81	11.32	9.24	28.54	21.66	265.169446	324.161819	
Removal/Mass (g/lb)	0.05	0.04	0.03	0.05	0.02	0.02	0.05	0.04	0.46365137	0.5668001	
Removal/Cost (lb/\$)	0.35	0.27	0.23	0.37	0.14	0.12	0.36	0.27	3.334	4.075	
Cost/Removal ($\$/\text{lb}$)	\$2.88	\$3.77	\$4.39	\$2.67	\$7.03	\$8.61	\$2.79	\$3.67	\$0.30	\$0.25	
Non-weighted average cost in lab ($\$/\text{lb}$)			\$4.48	Non-weighted average cost in field($\$/\text{lb}$)						\$0.27	

* product and delivery cost, no installation or replacement cost, system operates for 20 years

* field cost estimation is based on most recent document of the actual total pumping volume by solar pump

Table 11. Cost-benefit analysis of woodchip mixtures under multiple scenarios before and after carbon addition with lab treatment

Scenario	LG		HG		LS		HS		
	NO	YES	NO	YES	NO	YES	NO	YES	
Carbon in Water									
Inlet TN ($\mu\text{g/L}$)	5,688	5,786	9,493	7,718	1,992	1,414	5,414	4,814	
Overall TN Re.	84.88%	89.86%	62.09%	92.83%	68.68%	59.31%	79.65%	87.25%	
Flow rate (mL/min)	15.00								
Bed volume (in^3)	1,357.16								
Treating Time (day)	2,920.00								
Treating Volume (L)	63,072.00								
Media Price ($\$/\text{ft}^3$)*	2.00								
Media Density (g/cm^3)	0.24								
Removal/Volume (g/m^3)	13,692	14,745	16,716	20,319	3,880	2,378	12,229	11,912	
Removal/Mass (mg/kg)	57,050	61,438	69,650	84,662	16,166	9,910	50,956	49,632	
Removal/Cost (mg/\$)	193,884	208,796	236,702	287,720	54,941	33,679	173,173	168,674	
Removal/Volume (oz/ft^3)	13.57	14.62	16.57	20.14	3.85	2.36	12.12	11.81	
Removal/Mass (g/lb)	0.14	0.15	0.17	0.20	0.04	0.02	0.12	0.12	
Removal/Cost (lb/\$)	0.43	0.46	0.52	0.63	0.12	0.07	0.38	0.37	
Cost/Removal ($\$/\text{lb}$)	\$2.34	\$2.18	\$1.92	\$1.58	\$8.27	\$13.50	\$2.62	\$2.69	
Non-weighted average cost ($\$/\text{lb}$) =			\$4.39						

* product and delivery cost, no installation or replacement cost, system operates for 8 years

Table 12. Cost-benefit analysis of B&G and woodchip mixtures with and without copper impacts and with lab treatment

Scenario HS	B&G		Woodchip	
	YES	NO	YES	NO
Copper in Water	YES	NO	YES	NO
Inlet TN ($\mu\text{g/L}$)	5,243	5,572	5,294	5,414
Overall TN Re.	62.31%	75.97%	70.73%	79.65%
Flow rate (mL/min)	15			
Bed volume (in^3)	1357.16			
Treating Time (day)	7,300		2,920	
Treating Volume (L)	157,680		63,072	
Media Price ($\$/\text{ft}^3$)*	5		2	
Media Density (g/cm^3)	1.39		0.24	
Removal/Volume (g/m^3)	23,164	30,012	10,620	12,230
Removal/Mass (mg/kg)	16,665	21,592	44,249	50,959
Removal/Cost ($\text{mg}/\text{\$}$)	131,202	169,993	150,380	173,184
Removal/Volume (oz/ft^3)	22.960	29.749	10.527	12.123
Removal/Mass (g/lb)	0.040	0.052	0.107	0.123
Removal/Cost ($\text{lb}/\text{\$}$)	0.289	0.374	0.331	0.381
Cost/Removal ($\text{\$/lb}$)	\$3.46	\$2.67	\$3.02	\$2.62
* product and delivery cost, no installation or replacement cost, system operates 20 years for B&G, 8 years for woodchip				

6. Conclusions

Laboratory analysis for the removal of nitrogen using two media has been completed using 6-inch diameter 4-feet long laboratory columns as well as the comparison between the lab and field nitrogen removal data. Constant flow rates were used for the laboratory media columns to compare the effectiveness. A lower flow rate of 1.96 inches per hour had to be used with the woodchip to obtain a contact (residency) time to remove nitrogen equal to that removed by B&G. This assumes that the flow rate using woodchip can be controlled in the field, however, the field study proved that such control is not possible in the field as stormwater and solar powered pumping rate for groundwater are not constant. The flow rate for B&G was the normal flow rate experienced in a non-flow restricted installation due to its small porous size. It is a challenge to obtain this low flow rate using woodchip in a linear ditch along a highway because of the need for constructing

treatment structures that may have safety concerns or additional land acquisitions. Nevertheless, the effectiveness of this report using woodchip shows completely different results for the laboratory and field study, which are profoundly affected by the inflow rate and the capability to adapt to highly variable environmental factors. An overall nutrient removal table (Table 13) was provided for holistic evaluation of both laboratory and field work. However, the operation of the laboratory column test procedure keeping a saturated condition makes it unlikely to fully compare the results with the field data. On the other hand, the condition in the field could not be duplicated easily in the laboratory.

Under the controlled constant inflow rate in the column study, both the B&G and the woodchip mixtures functioned to remove nitrogen. This was demonstrated for a wide range of influent nitrogen conditions, or concentrations ranging from 2- 9.7 mg/L as well as using both surface and groundwater. The cost for removal of one pound of nitrogen using B&G is about the same as the cost using woodchip with the assumed life expectancy of 8 and 20 years for woodchip and B&G, respectively. By visual observation, woodchip decomposed by 50% within 1 year while B&G did not decay. B&G contains mostly mineral materials that will not decay. B&G and woodchip mixtures have an unweighted average cost of about \$4.48 and \$4.39 per pound of nitrogen removed over an 8-year life expectancy for woodchip and over a 20-year life expectancy for B&G. With only purchase and shipping costs considered, no cost was added for the installation and operation of both systems. Also, the preliminary field data indicates that, unlike woodchip, the removal capacity of B&G can be enhanced significantly by increasing the inlet water volume. Moreover, the B&G mixture has been in operation for over 7 years at other locations and no replacement or maintenance activities were needed or recorded.

At the controlled laboratory constant flow rate, the woodchip mixture and the B&G mixture removed TN effectively with and without carbon addition for both stormwater and groundwater input. However, when treating groundwater with B&G, the removal increased with the addition of carbon. Carbon can actually enhance the nitrogen removal effectiveness in B&G but has very limited effects on woodchip since woodchip can produce carbon by itself. So, it could be more beneficial to add a carbon source to B&G when treating groundwater. The carbon addition can also largely increase the bacteria population/bioactivity for nutrient removal in B&G, while woodchip contains much smaller population/population enhancement of microbial community

since it has limited surface area for biofilm development when compared to B&G. Compared to the controlled lab environment, the microbial community is much smaller with higher dynamic changes in the field and also showed very different microbial community structure. Regardless, the woodchip is only capable of holding a small microbial community for DNF process that releases nitrogen gas when compared to B&G. In addition, woodchip produced a significant amount of ammonia from organics' degradation, ammonification, and DNRA that ended up with negative TN removal performance, while B&G kept similar or better nutrient removal performance when comparing the field and laboratory results that should be explored more about the second pathway of denitrification, namely DNRA pathway, in the future. This is mainly because woodchip cannot screen out the leaching organic particles and provide sufficient and necessary oxygen for heterotrophic bacteria to decompose the high molecular weight organics into low molecular weight ones. Also, ammonification requires oxygen to generate ammonia. The conversion to nitrogen gas is also inhibited with additional organic sources from the nearby farmland via either stormwater runoffs or wind blow particles and fertilizer on the linear ditch plants. It is also understood the oxygen also depressed the DNF process but sustaining the DNRA process with sufficient carbon and ended up with significant ammonia generation.

The copper addition decreases the nutrient removal effectiveness in both B&G and woodchip. This is especially true for the treatment at the top layer (initial stage), as most copper was removed/kept within the top layer, where it introduced negative impacts at the most population abundant location in both the B&G and woodchip columns. However, the overall removal capability is not seriously impacted, because copper was removed in an early stage and the rest of the column can contribute removal to the remainder of the nutrients. Surprisingly, the population of denitrifiers increased after copper addition in B&G, which could be a sign of the microbial community adapting to the toxic inlet water.

Table 13. Overall nutrient removal efficiencies in laboratory and field studies

Nutrient removal in the laboratory

	B&G treat groundwater				Woodchip treat groundwater			
	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>
	<u>Carbon</u>		<u>Carbon</u>		<u>Carbon</u>		<u>Carbon</u>	
NO_x Removal	51.54%	99.92%	45.33%	54.11%	91.77%	96.56%	67.25%	98.65%
TN Removal	50.58%	87.98%	42.52%	51.90%	84.88%	89.86%	62.09%	92.83%
NH₃ Removal	7.33%	-960%	4.11%	-210%	79.34%	-167%	91.41%	-453%
	B&G treat stormwater				Woodchip treat stormwater			
	Low TN inflow		High TN inflow		Low TN inflow		High TN inflow	
	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>	<u>No</u>	<u>Carbon</u>
	<u>Carbon</u>		<u>Carbon</u>		<u>Carbon</u>		<u>Carbon</u>	
NO_x Removal	99.80%	98.32%	73.13%	63.08%	99.79%	99.41%	92.93%	92.82%
TN Removal	77.54%	82.15%	70.49%	63.10%	68.68%	59.31%	79.65%	87.25%
NH₃ Removal	-8.55%	-16.5%	14.13%	-168%	97.96%	-6.3%	95.79%	38.14%
	B&G treat high TN stormwater				Woodchip treat high TN stormwater			
	<u>No copper</u>		<u>Copper</u>		<u>No copper</u>		<u>Copper</u>	
NO_x Removal	73.13%		71.90%		92.93%		92.26%	
TN Removal	70.49%		62.31%		79.65%		70.73%	
NH₃ Removal	14.13%		-127%		95.79%		34.08%	
Nutrient removal in the field								
	2 ft BAM	1 ft BAM	2 ft WC	3ft WC	4 ft WC			

NO_x Removal	77.00% to 99.81%	99.81% to 69.33%	97.30% to 99.76%	99.73% to 99.80%	99.73% to 99.80%
TN Removal	67.74% to 95.37%	51.61% to 79.63%	- 386% to - 179%	-18.92% to 16.28%	- 23.53% to - 114%
NH₃ Removal	≥ 71%	- 440% to 0%	- 6400% to - 9000%	- 860% to - 2400%	-2400% to - 3600%

Work to Be Performed in the Next Task

Waiting for additional field nutrient removal data to complete the full comparison between the lab and column study.

Research Impediments

The delay in field construction has affected the end date.

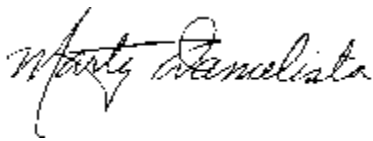
Updated Schedule

A no cost time extension has been granted.

Respectfully Submitted



Ni-Bin Chang, PhD, P.E.



Martin Wanielista, PhD, P.E.

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Appendix A: Column study nutrient analyzing data



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RESULTS OF SAMPLE ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT -- UCF Project No. 16607060

DATE COLLECTED	SAMPLE DESCRIPTION	ALKALINITY (mg/l)	COD (mg/l)	NO _x (µg/l)	NH ₃ (µg/l)	TOTAL N (µg/l)	COPPER (µg/l)
5/4/16	Column 1 Inlet	198	5.4	6,348	42	6,847	x
	Column 1 Port 1	210	x	5,426	52	5,645	x
	Column 1 Port 2	212	x	5,623	3	5,791	x
	Column 1 Port 3	207	x	4,352	3	4,635	x
	Column 1 Outlet	206	10.3	3,183	3	3,499	x
	Column 2 Inlet	206	1.0	8,628	14	9,710	x
	Column 2 Port 1	211	x	8,241	17	9,697	x
	Column 2 Port 2	204	x	7,436	80	8,313	x
	Column 2 Port 3	210	x	6,529	24	7,115	x
	Column 2 Outlet	212	7.2	5,491	81	6,055	x
	Column 3 Inlet	207	3.2	6,320	16	6,653	x
	Column 3 Port 1	188	x	1,586	1,242	3,210	x
	Column 3 Port 2	189	x	3,713	345	4,393	x
	Column 3 Port 3	198	x	5,036	3	5,805	x
	Column 3 Outlet	191	23.0	13	2,342	2,633	x
	Column 4 Inlet	200	4.6	8,695	232	9,602	x
	Column 4 Port 1	178	x	4,672	646	5,737	x
	Column 4 Port 2	192	x	6,612	255	6,925	x
	Column 4 Port 3	187	x	7,761	3	8,661	x
	Column 4 Outlet	202	27.9	2,941	1,062	4,279	x
5/5/16	Column 1 Inlet	45.8	12.9	1,536	32	1,949	x
	Column 1 Port 1	47.2	19.5	495	3	1,099	x
	Column 1 Port 2	52.4	16.9	218	79	786	x
	Column 1 Port 3	67.8	16.4	7	113	565	x
	Column 1 Outlet	76.2	13.4	3	6	452	x
	Column 2 Inlet	43.0	13.8	4,897	38	5,195	x
	Column 2 Port 1	42.5	15.1	4,399	63	4,779	x
	Column 2 Port 2	42.8	14.7	4,122	231	4,552	x
	Column 2 Port 3	54.0	15.6	3,398	120	3,634	x
	Column 2 Outlet	81.4	19.1	69	19	616	x
	Column 3 Inlet	45.0	12.9	1,580	43	1,955	x
	Column 3 Port 1	72.2	60.4	3	110	735	x
	Column 3 Port 2	57.9	29.2	331	61	888	x
	Column 3 Port 3	49.6	37.6	980	3	1,409	x
	Column 3 Outlet	87.4	77.2	3	220	800	x
	Column 4 Inlet	48.6	17.8	4,954	130	5,569	x
	Column 4 Port 1	85.6	38.4	1,121	228	1,968	x
	Column 4 Port 2	73.6	31.8	2,584	218	3,297	x
	Column 4 Port 3	62.8	54.3	2,929	210	3,537	x
	Column 4 Outlet	97.6	54.3	29	252	883	x

x: analysis of this parameter not requested for this sample Value measured is below detectable limits; listed value reflects 50% of the MDL

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted. The Analytical Results within these report pages reflect the values obtained from tests performed on samples as received by the laboratory unless indicated differently.


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RESULTS OF SAMPLE ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT -- UCF Project No. 16607060

DATE COLLECTED	SAMPLE DESCRIPTION	ALKALINITY (mg/l)	COD (mg/l)	NO _x (µg/l)	NH ₃ (µg/l)	TOTAL N (µg/l)	COPPER (µg/l)
5/9/16	Column 1 Inlet	211	4.6	4,290	3	4,416	x
	Column 1 Port 1	199	7.6	3,581	85	3,782	x
	Column 1 Port 2	213	8.5	2,796	19	2,951	x
	Column 1 Port 3	207	9.0	1,023	3	1,131	x
	Column 1 Outlet	220	9.8	527	3	689	x
	Column 2 Inlet	210	10.7	9,085	3	9,195	x
	Column 2 Port 1	218	8.1	6,201	76	6,313	x
	Column 2 Port 2	192	8.5	6,424	33	6,644	x
	Column 2 Port 3	211	9.8	5,308	3	5,353	x
	Column 2 Outlet	218	8.5	3,497	3	3,598	x
	Column 3 Inlet	210	6.8	4,394	3	4,499	x
	Column 3 Port 1	210	47.2	3	99	484	x
	Column 3 Port 2	217	23.5	87	214	607	x
	Column 3 Port 3	219	21.7	1,643	154	1,935	x
	Column 3 Outlet	211	62.6	3	47	449	x
	Column 4 Inlet	215	7.6	5,335	3	5,562	x
	Column 4 Port 1	206	46.8	3	88	428	x
	Column 4 Port 2	214	19.5	634	215	929	x
	Column 4 Port 3	208	13.8	2,094	153	2,391	x
	Column 4 Outlet	196	55.2	16	96	426	x
5/10/16	Column 1 Inlet	48.4	29.2	3	3	551	x
	Column 1 Port 1	51.4	27.4	3	3	389	x
	Column 1 Port 2	63.6	28.3	3	26	426	x
	Column 1 Port 3	63.0	26.1	3	221	656	x
	Column 1 Outlet	61.1	27.9	3	154	656	x
	Column 2 Inlet	48.4	34.0	3,461	3	4,069	x
	Column 2 Port 1	56.2	28.3	2,168	117	2,670	x
	Column 2 Port 2	62.0	27.4	1,106	129	1,732	x
	Column 2 Port 3	69.8	26.1	333	151	870	x
	Column 2 Outlet	75.8	28.3	3	156	506	x
	Column 3 Inlet	48.2	38.0	5	3	543	x
	Column 3 Port 1	75.6	57.4	3	8	539	x
	Column 3 Port 2	61.8	45.9	3	3	531	x
	Column 3 Port 3	55.0	28.8	3	3	472	x
	Column 3 Outlet	90.6	83.3	3	17	583	x
	Column 4 Inlet	49.4	31.0	4,331	3	4,887	x
	Column 4 Port 1	92.8	47.2	56	109	700	x
	Column 4 Port 2	81.4	31.4	856	114	1,558	x
	Column 4 Port 3	69.0	29.2	2,513	42	3,022	x
	Column 4 Outlet	104	62.2	3	50	553	x

x: analysis of this parameter not requested for this sample Value measured is below detectable limits; listed value reflects 50% of the MDL

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**RESULTS OF SAMPLE ANALYSES CONDUCTED ON SAMPLES
 COLLECTED FOR THE "LINEAR DITCH" PROJECT -- UCF Project No. 16607060**

DATE COLLECTED	SAMPLE DESCRIPTION	ALK. (mg/l)	COD (mg/l)	NO _x (µg/l)	NH ₃ (µg/l)	TOTAL N (µg/l)	TOTAL COPPER (µg/l)			DISSOLVED COPPER (µg/l)		
							1	2	3	1	2	3
5/13/16	Column 2 Inlet	47.2	23.9	4,687	3	5,062	44	44	46	41	41	41
	Column 2 Port 1	48.4	x	4,046	264	4,704	5	5	5	6	6	6
	Column 2 Port 2	53.6	x	3,157	397	3,770	6	6	6	6	6	6
	Column 2 Port 3	60.0	x	1,693	325	2,305	4	4	4	5	5	5
	Column 2 Outlet	58.2	26.1	612	141	1,246	3	3	3	3	3	3
	Column 4 Inlet	48.4	27.9	4,574	3	5,017	41	41	42	41	42	41
	Column 4 Port 1	69.8	x	79	3	898	3	3	3	4	4	4
	Column 4 Port 2	77.6	x	1,688	9	2,220	5	5	5	4	4	4
	Column 4 Port 3	31.0	x	3,510	3	3,883	12	11	12	24	25	25
	Column 4 Outlet	73.2	53.0	3	6	596	3	3	3	34	33	34

x: analysis of this parameter not requested for this sample Value measured is below detectable limits; listed value reflects 50% of the MDL

 Dissolved copper value is greater than total copper value

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted. The Analytical Results within these report pages reflect the values obtained from tests performed on samples as received by the laboratory unless indicated differently.


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RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING SEPTEMBER 2016 (UCF Project No. 16607060)

Sample Description	Date Collected	Alkalinity (mg/l)	COD (mg/l)	NO _x -N (µg/l)	NH ₃ -N (µg/l)	Total N (µg/l)
C1-Inlet-1	09/28/16	115	7.6	5,567	208	5,974
C1-Inlet-2	09/28/16	110	2.8	5,427	201	5,898
C1-SP1-1	09/28/16	109	x	3,975	87	4,643
C1-SP1-2	09/28/16	104	x	4,114	87	4,354
C1-SP2-1	09/28/16	106	x	4,087	87	4,673
C1-SP2-2	09/28/16	109	x	4,063	89	4,596
C1-SP3-1	09/28/16	109	x	3,790	147	4,168
C1-SP3-2	09/28/16	111	x	3,749	147	4,187
C1-Outlet-1	09/28/16	113	6.3	2,682	190	2,917
C1-Outlet-2	09/28/16	113	5.0	2,646	189	2,950
C2-Inlet-1	09/28/16	49.0	2.8	6,597	268	7,012
C2-Inlet-2	09/28/16	48.5	3.2	6,541	267	6,888
C2-SP1-1	09/28/16	46.4	x	5,385	222	6,141
C2-SP1-2	09/28/16	47.7	x	5,571	229	6,190
C2-SP2-1	09/28/16	48.2	x	5,486	202	5,749
C2-SP2-2	09/28/16	49.2	x	5,483	194	5,729
C2-SP3-1	09/28/16	46.3	x	5,882	271	6,802
C2-SP3-2	09/28/16	50.0	x	5,570	261	6,254
C2-Outlet-1	09/28/16	49.8	7.6	3,561	262	3,995
C2-Outlet-2	09/28/16	35.8	5.9	3,622	251	3,884
C3-Inlet-1	09/28/16	110	3.2	5,376	150	5,686
C3-Inlet-2	09/28/16	116	5.0	5,450	156	5,668
C3-Inlet-3	09/28/16	110	4.1	5,425	149	5,711
C3-SP1-1	09/28/16	111	x	3,293	197	4,735
C3-SP1-2	09/28/16	115	x	3,332	216	4,985
C3-SP1-3	09/28/16	114	x	3,314	210	5,095
C3-SP2-1	09/28/16	103	x	2,278	136	2,840
C3-SP2-2	09/28/16	111	x	2,316	154	3,037
C3-SP2-3	09/28/16	109	x	2,296	165	3,223
C3-SP3-1	09/28/16	96.2	x	1,758	24	1,968
C3-SP3-2	09/28/16	99.0	x	1,534	30	1,945
C3-SP3-3	09/28/16	97.0	x	1,562	34	2,054
C3-Outlet-1	09/28/16	89.2	17.3	405	29	892
C3-Outlet-2	09/28/16	93.2	30.5	438	34	720

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RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING SEPTEMBER 2016 (UCF Project No. 16607060)

Sample Description	Date Collected	Alkalinity (mg/l)	COD (mg/l)	NO _x -N (µg/l)	NH ₃ -N (µg/l)	Total N (µg/l)
C3-Outlet-3	09/28/16	91.1	24.4	495	31	968
C4-Inlet-1	09/28/16	70.4	5.0	7,944	404	9,427
C4-Inlet-2	09/28/16	98.0	6.3	8,835	399	9,472
C4-Inlet-3	09/28/16	99.8	3.7	8,832	419	9,582
C4-SP1-1	09/28/16	92.9	x	7,960	197	8,291
C4-SP1-2	09/28/16	95.9	x	7,912	205	8,706
C4-SP1-3	09/28/16	96.9	x	7,826	222	8,379
C4-SP2-1	09/28/16	106	x	5,574	182	6,941
C4-SP2-2	09/28/16	94.4	x	5,823	166	6,812
C4-SP2-3	09/28/16	98.2	x	5,954	174	6,955
C4-SP3-1	09/28/16	88.1	x	3,617	33	4,286
C4-SP3-2	09/28/16	87.9	x	3,708	41	4,398
C4-SP3-3	09/28/16	87.5	x	3,746	43	4,616
C4-Outlet-1	09/28/16	90.0	20.8	3,058	45	3,400
C4-Outlet-2	09/28/16	88.6	23.0	2,777	30	3,589
C4-Outlet-3	09/28/16	89.2	20.4	2,553	30	3,807
 						
C1-Inlet-1	09/30/16	43.8	20.0	1,470	201	2,174
C1-Inlet-2	09/30/16	45.2	20.8	1,484	185	2,110
C1-SP1-1	09/30/16	46.9	x	895	312	1,297
C1-SP1-2	09/30/16	47.8	x	813	326	1,168
C1-SP2-1	09/30/16	50.2	x	107	448	908
C1-SP2-2	09/30/16	50.6	x	102	436	879
C1-SP3-1	09/30/16	53.0	x	17	409	774
C1-SP3-2	09/30/16	55.4	x	20	435	804
C1-Outlet-1	09/30/16	55.9	17.8	< 5	208	458
C1-Outlet-2	09/30/16	55.0	18.2	< 5	211	490
C2-Inlet-1	09/30/16	44.0	19.5	4,377	192	5,770
C2-Inlet-2	09/30/16	43.0	17.3	4,407	176	5,753
C2-SP1-1	09/30/16	42.6	x	3,376	270	3,980
C2-SP1-2	09/30/16	43.9	x	3,235	296	3,875
C2-SP2-1	09/30/16	49.2	x	3,026	329	4,059
C2-SP2-2	09/30/16	49.5	x	2,986	318	4,018
C2-SP3-1	09/30/16	49.0	x	2,280	260	3,055

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted. The Analytical Results within these report pages reflect the values obtained from tests performed on samples as received by the laboratory unless indicated differently.

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RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING SEPTEMBER 2016 (UCF Project No. 16607060)

Sample Description	Date Collected	Alkalinity (mg/l)	COD (mg/l)	NO _x -N (µg/l)	NH ₃ -N (µg/l)	Total N (µg/l)
C2-SP3-2	09/30/16	53.6	x	2,375	263	3,058
C2-Outlet-1	09/30/16	59.2	14.7	1,104	153	1,670
C2-Outlet-2	09/30/16	57.6	19.5	1,256	163	1,731
C3-Inlet-1	09/30/16	43.2	20.8	1,459	151	1,924
C3-Inlet-2	09/30/16	44.7	22.6	1,459	140	1,944
C3-Inlet-3	09/30/16	43.9	16.9	1,447	151	2,109
C3-SP1-1	09/30/16	48.0	x	57	155	1,973
C3-SP1-2	09/30/16	50.0	x	70	173	2,388
C3-SP1-3	09/30/16	51.6	x	61	160	2,100
C3-SP2-1	09/30/16	50.2	x	< 5	45	855
C3-SP2-2	09/30/16	51.4	x	< 5	43	655
C3-SP2-3	09/30/16	52.2	x	< 5	46	942
C3-SP3-1	09/30/16	62.8	x	< 5	9	835
C3-SP3-2	09/30/16	64.0	x	< 5	11	806
C3-SP3-3	09/30/16	63.0	x	< 5	16	820
C3-Outlet-1	09/30/16	73.4	33.2	< 5	< 5	617
C3-Outlet-2	09/30/16	71.6	33.6	< 5	< 5	600
C3-Outlet-3	09/30/16	71.4	35.8	< 5	< 5	655
C4-Inlet-1	09/30/16	29.1	18.2	3,986	261	5,381
C4-Inlet-2	09/30/16	49.0	23.0	3,966	261	5,502
C4-Inlet-3	09/30/16	46.8	19.1	3,978	262	5,360
C4-SP1-1	09/30/16	37.2	x	1,781	197	3,504
C4-SP1-2	09/30/16	57.2	x	1,876	202	3,611
C4-SP1-3	09/30/16	57.0	x	1,846	217	3,584
C4-SP2-1	09/30/16	66.0	x	1,083	82	2,037
C4-SP2-2	09/30/16	61.4	x	1,301	83	2,139
C4-SP2-3	09/30/16	58.3	x	1,363	83	2,277
C4-SP3-1	09/30/16	55.6	x	1,276	42	2,345
C4-SP3-2	09/30/16	56.5	x	1,248	40	2,317
C4-SP3-3	09/30/16	77.8	x	1,214	42	2,319
C4-Outlet-1	09/30/16	79.0	40.6	224	11	1,086
C4-Outlet-2	09/30/16	78.7	37.6	346	11	1,108
C4-Outlet-3	09/30/16	77.8	36.2	274	11	1,112

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted. The Analytical Results within these report pages reflect the values obtained from tests performed on samples as received by the laboratory unless indicated differently.



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RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING OCTOBER 2016
(UCF Project No. 16607060)

Table with columns: Sample Description, Date Collected, Alkalinity (mg/l), COD (mg/l), NO3-N (ug/l), NH3-N (ug/l), Total N (ug/l), Copper (ug/l). Rows include C1-Inlet, C1-SP1, C1-SP2, C1-SP3, C1-Outlet, C2-Inlet, C2-SP1, C2-SP2, C2-SP3, C2-Outlet, C3-Inlet, C3-SP1, C3-SP2, C3-SP3, C3-Outlet, C4-Inlet, C4-SP1, C4-SP2, C4-SP3, C4-Outlet.

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted.

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Cassie Revell - Lab Director

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RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING OCTOBER 2016
(UCF Project No. 16607060)

Table with columns: Sample Description, Date Collected, Alkalinity (mg/l), COD (mg/l), NO3-N (ug/l), NH3-N (ug/l), Total N (ug/l), Copper (ug/l). Rows include C1-Inlet, C1-SP1, C1-SP2, C1-SP3, C1-Outlet, C2-Inlet, C2-SP1, C2-SP2, C2-SP3, C2-Outlet, C3-Inlet, C3-SP1, C3-SP2, C3-SP3, C3-Outlet, C4-Inlet, C4-SP1, C4-SP2, C4-SP3, C4-Outlet.

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted.

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**RESULTS OF ANALYSES CONDUCTED ON SAMPLES COLLECTED FOR THE "LINEAR DITCH" PROJECT DURING OCTOBER 2016
 (UCF Project No. 16607060)**

Sample Description	Date Collected	Alkalinity (mg/l)			COD (mg/l)			NO _x -N (µg/l)			NH ₃ -N (µg/l)			Total N (µg/l)			Copper (µg/l)		
		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
C2-Inlet	10/13/16	38.4	38.1	-	13.4	13.4	-	4,472	4,444	-	129	130	-	5,341	5,327	-	36	34	-
C2-SP1	10/13/16	41.2	40.9	-	-	-	-	3,849	3,855	-	107	125	-	4,485	4,451	-	8	8	-
C2-SP2	10/13/16	42.4	42.0	-	-	-	-	2,906	2,887	-	197	218	-	3,426	3,390	-	4	3	-
C2-SP3	10/13/16	48.4	48.9	-	-	-	-	2,651	2,624	-	249	255	-	3,172	3,152	-	4	3	-
C2-Outlet	10/13/16	45.3	45.7	-	12.9	11.6	-	1,610	1,600	-	203	205	-	2,324	2,350	-	6	3	-
C4-Inlet	10/13/16	49.4	49.0	49.7	12.0	13.4	11.6	4,462	4,408	4,450	135	133	134	5,313	5,297	5,293	34	34	33
C4-SP1	10/13/16	46.7	46.1	46.5	-	-	-	4,054	4,069	4,049	118	107	108	5,079	4,896	5,047	7	7	6
C4-SP2	10/13/16	41.4	41.0	41.5	-	-	-	3,501	3,512	3,503	93	101	106	4,521	4,512	4,451	6	6	5
C4-SP3	10/13/16	44.0	44.6	43.9	-	-	-	2,780	2,853	2,860	111	102	113	3,849	3,824	3,817	4	4	3
C4-Outlet	10/13/16	49.6	49.2	49.7	27.0	25.7	26.1	347	345	345	90	88	89	1,563	1,567	1,519	3	3	2

Analytical results presented in this report have been reviewed for compliance with the ENVIRONMENTAL RESEARCH & DESIGN, INC. (ERD) Quality Systems Manual and have been determined to meet applicable method guidelines and standards referenced in the July 2003 National Environmental Laboratory Accreditation Program (NELAP) Quality Manual unless otherwise noted. The Analytical Results within these report pages reflect the values obtained from tests performed on samples as received by the laboratory unless indicated differently.

Cassia Powell



Cassia Powell - Lab Director

NELAC No. E1031026

Appendix B: Gene density of AOB, NOB, denitrifiers, and AMX in copies/g-dry mass

		Column 1			
		No carbon		Carbon added	
		mean	std	mean	std
AOB	TOP	1034.854	571.8472	42315.64	7698.011
	PORT 1	906.7516	71.3574	2524.419	0
	PORT 2	234.2209	110.1618	66.79866	0
NOB	TOP	90457.64	10807.44	1123533	83767.14
	PORT 1	57319.35	1023.942	83639.14	7448.907
	PORT 2	3085.475	2589.951	3809.292	1982.059
Denitrifier	TOP	729847.7	50683.15	3548745	245437.3
	PORT 1	1199645	54862.66	628508.3	15395.66
	PORT 2	592109.1	23378.91	578121.8	4928.828
AMX	TOP	1307.184	573.7759	1308.944	416.845
	PORT 1	1547.374	281.0721	1673.602	828.0751
	PORT 2	2098.341	810.4638	1567.193	34.55738

		Column 2			
		No carbon		Carbon added	
		mean	std	mean	std
AOB	TOP	817.5319	227.0321	2081.564	858.4803

	PORT 1	0	0	162.8914	0
	PORT 2	154.5076	106.6569	854.3325	0
NOB	TOP	125397.5	15543.03	213747.4	23590.17
	PORT 1	20582.38	2217.108	36832.53	5042.444
	PORT 2	1136.634	0	1047.651	0
Denitrifier	TOP	882142.8	80624.17	1473386	29063.82
	PORT 1	791510.9	35709.77	367817.9	38792.15
	PORT 2	443899.7	10788.67	322317.4	25769.57
AMX	TOP	1322.57	353.333	1137.127	311.4087
	PORT 1	1137.133	319.3279	1728.931	226.3882
	PORT 2	1594.376	548.5346	1754.449	677.397

		Column 3			
		No carbon		Carbon added	
		mean	std	mean	std
AOB	TOP	121.1539	109.0042	7.138808	0.636672
	PORT 1	0	0	10.16464	0
	PORT 2	80.92476	0	4.977155	0
NOB	TOP	973.7657	494.2984	909.78	71.74952
	PORT 1	109.9509	98.85111	54.6499	0
	PORT 2	56.14603	0	25.40969	0
Denitrifier	TOP	57952.74	1493.152	118884.3	6088.161
	PORT 1	30386.77	975.1761	21317.29	848.089
	PORT 2	63561.05	1696.577	21263.85	459.9975
AMX	TOP	1647.723	570.886	3304.488	314.3777
	PORT 1	2123.306	454.6669	1984.026	449.248
	PORT 2	3685.108	1164.077	2877.407	663.2621

		Column 4			
		No carbon		Carbon added	
		mean	std	mean	std
AOB	TOP	1713.031	62.16602	24.92942	0
	PORT 1	63.40246	32.44249	0	0
	PORT 2	32.86776	15.15348	0	0
NOB	TOP	8664.002	3716.019	4888.137	2203.807
	PORT 1	166.6158	172.6565	69.609	85.7939
	PORT 2	48.27828	32.86092	46.48988	77.59552
Denitrifier	TOP	248303.8	52718.41	374567.7	33731.53
	PORT 1	153089.1	3913.745	62954.4	2497.107
	PORT 2	68292.54	1401.796	89040.35	5043.319

AMX	TOP	2120.536	653.6209	3964.34	1232.829
	PORT 1	2620.045	212.5328	3280.052	407.4022
	PORT 2	1825.666	88.33429	2709.696	768.1053

	Copper added			
	Column 2		Column 4	
	mean	std	mean	std
Top	2179.418	658.2839	176.2127	45.35693
Port 1	43.04567	5.11487	119.3419	103.1867
Port 2	50.33168	51.53411	127.9848	0
Top	129846.5	15380.3	4510.997	1876.853
Port 1	4670.937	6263.231	932.5205	1285.477
Port 2	7821.262	9973.532	85.39386	0
Top	11010856	191630.3	219343.9	7457.634
Port 1	672413	35215.53	83262.66	11341.7
Port 2	278221.7	12409.94	36527.45	1602.181
Top	1171.783	434.8451	1853.183	695.0245
Port 1	1448.469	388.7135	1274.178	403.3361
Port 2	1157.845	330.1964	1841.159	113.9052