



# Comparison of Biological Nutrient Removal via Two Biosorption-Activated Media Between Laboratory-Scale and Field-Scale Linear Ditch for Stormwater and Groundwater Co-treatment

Ni-Bin Chang  · Dan Wen · William Colona · Martin P. Wanielista

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**Abstract** Excess nitrogen in the ecosystem could result in eutrophication and harmful algal bloom in an ecosystem. Low impact development (LID) facilities, regarded as an integral part of green infrastructures for flow control and water quality management may include, but are not limited to, dry/wet ponds, green roof, bioswale or linear ditch, vegetated natural strip, exfiltration trench, piping networks with underdrain or reuse options, and bioswale. This study presents a new approach using a linear ditch along a roadside for LID with the aid of two green sorption media that are designed for co-treatment of stormwater and groundwater for nutrient removal. The stormwater is primarily from agricultural discharge and transportation stormwater runoff. Two recipes of green sorption media, including the green sorption media and woodchip, were examined and compared through a laboratory-scale column study and a field-scale test bed media under various influent concentrations and flow conditions. The green sorption media were found more appropriate than the woodchip media for field-scale applications because the green sorption media may exhibit long-standing microenvironments and hydraulic patterns to provide a homogeneous hydraulic retention time and infiltration rate for

nutrient removal. Therefore, such a new LID practice may not only mitigate the impact from various surface stormwater runoffs but also co-treat groundwater and stormwater for nutrient removal.

**Keywords** Stormwater treatment · Groundwater treatment · Nutrient removal · Green sorption media · Linear ditch

## 1 Introduction

Driven by rapid urbanization, economic development, and population growth worldwide, stormwater runoffs, wastewater effluents, and agricultural discharges have become more nutrient-laden, altering the nutrient cycle and water cycle. Such impacts have gradually resulted in changes in *urban* metabolism and urban ecology due to the transformation of *landform* configuration and even earth surface processes. With such a long-term impact, many groundwater aquifers and surface water bodies are under critical conditions due to the presence of excessive nutrients through the overland flows and recharged water from the urbanized and sub-urban regions (Davis and McCuen 2005; Lee and Bang 2000; Li and Davis 2014; Page et al. 2010; Pitt et al. 2004; Taylor et al. 2005) and agricultural crop fields (Rawlins et al. 1998; Shortle and Abler 2001; Smith and Harlow 2011; Wauchope 1978). Excess nitrogen in the ecosystem could result in eutrophication and harmful algal bloom, affecting ecosystem structure and function, degrading habitats, and deteriorating biodiversity in an ecosystem

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(Li and Davis 2014). Nitrogen impact on receiving water bodies due to stormwater runoffs, wastewater effluents, and agricultural discharges have thus drawn global attention. On the other hand, stormwater and groundwater in the urban water cycle are relatively untapped resources of water when it comes to meeting today's freshwater demand at the community scale (Mekonnen and Hoekstra 2016; Postel 2000). The National Academy of Engineering in the United States has indicated that "understanding and managing the nitrogen cycle" as well as "restoration and improvement of urban infrastructure" are two of 14 grand challenges for engineering in the 21st century (Mote Jr et al. 2016). Deepened understanding of nutrient cycling across natural systems and the built environment is therefore critical for the continuation of life on the planet (Mote Jr et al. 2016).

Low impact development (LID) facilities are regarded as an integral part of green infrastructures and may include, but are not limited to, dry/wet pond, green roof, bioswale or linear ditch, vegetated natural strip, exfiltration trench, lined underground piping networks with underdrain or reuse options, and bioswale. Some best management practices (BMPs) have been widely adopted to deal with the contamination of stormwater runoff and agricultural discharge at the field scale. If the nutrient removal can be made possible cost-effectively with the aid of green sorption media adaptable for heterogeneous landscapes and engineering conditions, we should be capable of maintaining ecosystems through a better urban infrastructure system. To aid in nutrient removal through LID facilities, the invention of green sorption media emphasizes direct and indirect benefits for providing ubiquitous ex situ water treatment services for nutrient removal leading to cost-effective water reuse and possible nutrient recovery, which extend beyond the traditional goal of stormwater management in cities (Chang et al. 2016; Jones et al. 2015; Ryan et al. 2010).

Recently, biosorption-activated media (BAM), a new form of green sorption media, has been implemented as an innovative BMP for enhancing nutrient removal from stormwater runoff (Hood et al. 2013; Hood 2012; O'Reilly et al. 2012; O'Reilly et al. 2014; Salamah 2014; Xuan et al. 2013). BAM is known as one of the green sorption media normally mixed with tire crumb, clay, and sand which provides better nitrogen removal efficiency and cost-effectiveness through the enhancement of microenvironments and hydraulic patterns for

microbiological reactions of nitrification and denitrification. Previous studies have evaluated the impacts of multiple factors on the microbial community within BAM at a laboratory scale, including impacts from different water sources, nutrient concentration levels, and the addition of carbon sources and toxic compounds (Chang et al. 2018; Wen et al. 2018). BAM showed promising nutrient removal under various conditions with strong resistance to changes in key environmental factors. However, woodchip media can be regarded as a competitive green sorption media as well (Robertson 2010; Schipper et al. 2010). BAM-based BMP requires less carbon footprint for construction relative to others but can still present promising nitrogen removal when compared to traditional biological nutrient removal (BNR) engineering schemes. Even though BAM and woodchip media have been tested and applied in various LID facilities as in situ denitrifying bioreactors (O'Reilly et al. 2014; Robertson 2010; Schipper et al. 2010; Xuan et al. 2013), the use of BAM and woodchip media to improve the performance of bioswales or linear ditches in terms of nitrogen removal is still relatively unexplored. This is especially true when investigating the difference between laboratory- and field-scale conditions for stormwater and groundwater co-treatment along a roadside corridor that is deemed very cost-effective. Co-treatment operational strategies may be operated based on storm and non-storm periods. Whereas a non-storm period treatment uses pumps to withdraw groundwater, a storm period treatment is designed simply to treat the in situ storm runoff from the road system to temper the nutrient impact on the groundwater system.

Two types of green sorption media or BAM were selected for comparison in this study. They are green sorption media and woodchip media. Green sorption media are composed of sand (85%), tire crumb (10%), and clay (5%) by volume and were produced by the research team, while the woodchip media are small chips and shavings from 2.5 cm (1 in.) to 7.5 cm (3 in.) collected from a wood factory located near the study site. This study aims to provide a holistic understanding of their comparative performance from the laboratory to the field scale to address the variation in the nitrogen cycle to aid in the green infrastructure design. The science questions to be answered in this study are as follows: (1) can the co-treatment process achieve promising nitrogen removal under various nitrogen concentration levels with scales? (2) How would

the difference of water quality in stormwater and groundwater affect the nitrification and denitrification processes in the two green sorption media? (3) Which media recipe is more appropriate in the future for cost-effective nutrient removal? (4) Will the nitrogen removal at the field scale follow the same pattern as removal at the laboratory scale? (5) How could the microbial ecology help explain the difference between the laboratory and field conditions and justify the difference in their performance? With these science questions, the objectives of this study are to (1) compare the nutrient removal results and possible microbial ecology between a laboratory column study and a field-scale linear ditch BMP based on two types of green sorption media, and (2) investigate the scaling effect in terms of the performance of nitrogen removal and hydraulic patterns between the field condition and the laboratory column environment. Based on the science questions and objectives of this study, the hypotheses include the following: (1) the co-treatment strategy is suitable and effective for both recipes of green sorption media under various influent concentrations and flow conditions; (2) different water sources (groundwater and stormwater) might have impacts on the nitrogen removal due to their background constituents in the biogeochemical cycle; (3) the nitrogen removal and associated microbial ecology in the field will follow a trend/pattern similar to the laboratory column study; (4) B&G media shall be more appropriate to be applied in the field as they exhibit better long-standing microenvironments and hydraulic patterns with respect to homogeneous hydraulic retention time and infiltration rate than the woodchip media; and (5) the microbial community could be very different between the column study and the field conditions.

## 2 Study Site

The study site is rural land in the Fanning Springs area, located east-northeast of the city of Fanning Springs in Levy County, North-Central Florida. Specifically, the study site is located in the southeast corner of SR-26 and 55th Avenue and extends along the southern right-of-way of the Florida Department of Transportation, 0.8 km (0.5 mi.) west and up to 1.6 km (1 mi.) east. As shown in Fig. 1, in this watershed, land use patterns include residential areas, a dairy farm, a wastewater treatment plant, and agricultural fields. The state road is on one side and the other side is farmland. The linear

ditch catches stormwater runoff from the road and agricultural discharge from the farmland next to the road corridor simultaneously. There is a gap of approximately 3.7 m (12 ft) in between the private property boundary of the farmland and the local pipe/cable line, indicated by the red flags on the ground.

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

## 3 Laboratory Study

### 3.1 Column Study

The laboratory experiment was set up through a suite of columns to mimic the field condition of the road side, and to simulate the co-treatment of stormwater and groundwater alternately. The stormwater runoff was collected from a pond at the University of Central Florida (UCF) main campus, whereas the groundwater flows were collected from the study site—the Fanning Spring. As shown in Fig. 3a, four columns with 15 cm (6 in.) diameter are noted as column 1 to column 4 hereafter. The columns' depth is 122 cm (4 ft), and 3

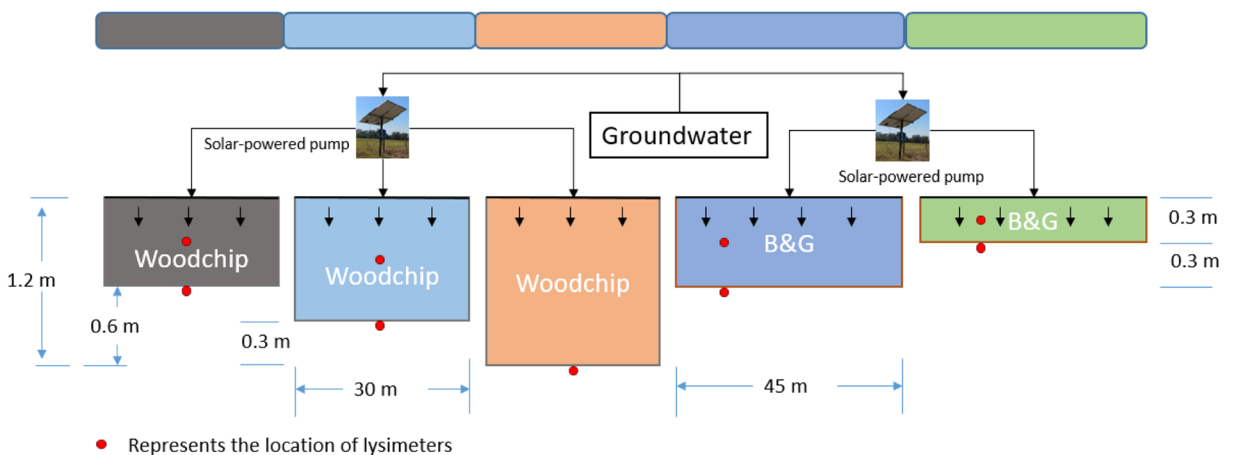


**Fig. 1** Schematic flowchart for design, construction, and operation strategy in the field including construction phase (upper left); completion of construction of B&G media section (upper right);

operation of pumps with solar panels in the middle of the B&G media and woodchip sections (lower left); operational phase of B&G media and woodchip sections (lower right)

sample ports were marked on the side of the columns at 30 cm (1 ft) intervals (Fig. 3a). Columns 1 and 2 were used for testing B&G media, and columns 3 and 4 were prepared for testing woodchip media. Different influent concentrations were chosen for both B&G media and woodchip media systematically during the testing

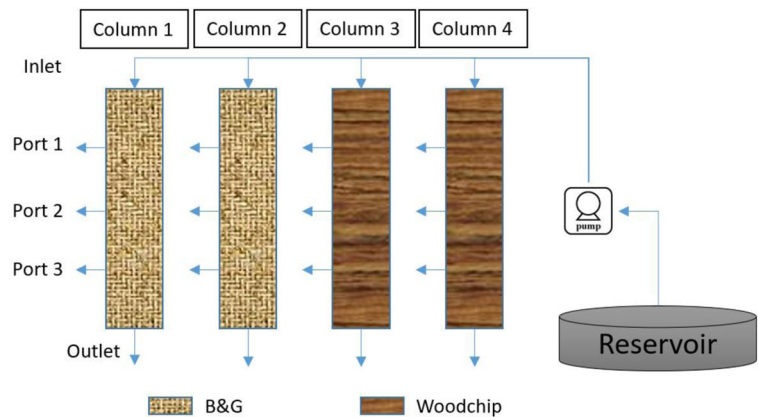
period in which columns 1 and 3 were running with lower concentrated influent (1.5 mg/L spiked nitrate) while columns 2 and 4 were running with higher concentrated influent (5.0 mg/L spiked nitrate). The running strategy of these four columns was schematically described for B&G media in columns 1 and 2 and the same



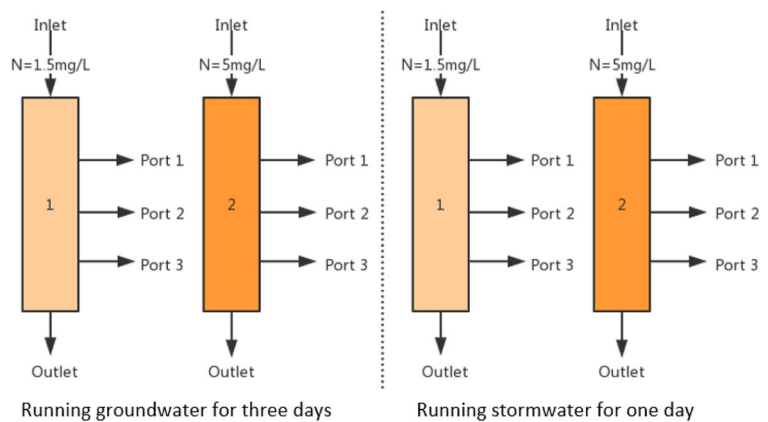
**Fig. 2** Schematic flowchart for design, construction, and operation strategy in the field



**Fig. 3** Laboratory setup. **a** Schematic diagram of the column setup in the laboratory. **b** Schematic of the operational strategy for B&G media columns and woodchip columns in the laboratory



(a)



(b)

strategy was applied for woodchip columns in the laboratory (Fig. 3b). All columns were running with stormwater spiked with nutrients to support biofilm cultivation for at least one month before collecting any water sample. After biofilm cultivation, the groundwater and stormwater were pumped into the columns alternately, following a prescribed schedule. We initially ran groundwater for three days with the flow rate of 10–15 mL/min and then collected water samples from the inlet, port 1 to 3, and the outlet. Then, we switched and operated the stormwater columns for one day and collected water samples in the same manner. Standard nitrate solution was spiked into the collected groundwater and stormwater to the theoretical concentration of 1.5 mg/L for column 1 and 5 mg/L for column 2. pH and dissolved oxygen (DO) were also measured right after the sample collection in the laboratory.

The laboratory study was conducted at UCF under a controlled room temperature that ranged from 22 to 23 °C. All samples collected from the column study were well preserved and delivered to the certified laboratory Environmental Research and Design, Inc. (ERD) within 24 h after collection. All field samples were collected from the lysimeters and pumping wells located in the Fanning Spring linear ditch study site. The field water samples were delivered to another certified laboratory called Test America Laboratories, Inc. (TAL) for nutrient analysis. The analyzed parameters and methods are summarized in Table 1.

### 3.2 Tracer Study

The purpose of the tracer study was to determine the hydraulic retention time (HRT) of both BAM recipes

**Table 1** Analysis method for lab and field samples

	ERD	TAL
Chloride	No analysis	MCAWW 325.2
Ammonia	SM 4500 NH <sub>3</sub> G	MCAWW 350.1
Nitrogen, Kjeldahl	No analysis	MCAWW 351.2
Nitrate and nitrite	SM 4500 NO <sub>3</sub> F	MCAWW 353.2
phosphorus	No Analysis	EPA 365.4
Ortho-phosphate	No Analysis	SM 4500 P F
Nitrogen, total	SM 4500 N C	EPA total nitrogen
Ammonium ion	No analysis	FL-DEP unionized NH <sub>3</sub>

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

(e.g., B&G media and woodchip media) in the laboratory-scale column study. Understanding HRT is crucial for treatment in the field study, which is highly related to biological nitrogen removal such as ammonification, nitrification, and denitrification. Rhodamine WT (RWT) dye (purchased from Ozark Underground Laboratory) was selected in this tracer test due to its advantages, specifically its low detection limits, zero natural background, low cost, and easy operation. The original RWT was 20% solution with a concentration of 200,000 ppb. To ensure the final concentration of RWT from the effluent was within the detection range of the fluorometer (Aquafluor 8000-010) between 0.4 to 400 ppb, RWT was diluted to around 8000 ppb. The columns were running with a steady pumping rate of 49.36 L/m<sup>2</sup> h for 3 h to obtain the continuously stable hydraulic condition. Then, 5 mL of diluted RWT was dosed at the top of the column. The effluent samples were taken in 10 min intervals for B&G media columns and 1-min intervals for woodchip columns. The tracer HRT can be calculated through the following Eq. (1), where  $\tau$  is the tracer HRT, and  $C(t)$  is the RWT concentration at time  $t$ .

$$\tau = \frac{\int_0^{\infty} tC(t)dt}{\int_0^{\infty} C(t)dt} \quad (1)$$

### 3.3 Microbial Ecology Study

In order to better understand the bacteria evolution critical for biological nitrogen removals in both laboratory

columns and the test bed in the field, those bacteria of interest included ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifiers, and annamox (AMX), all of which are related to nitrification and denitrification. A quantitative *polymerase chain reaction* (qPCR), also known as *real-time PCR*, is a laboratory analysis technique of molecular biology for identifying and quantifying microbial species. B&G and woodchip media samples were collected from 0, 30 cm (1 ft), and 60 cm (2 ft) depths in the column study, whereas they were collected from the top, middle, and bottom of each media section in the field. The gene copy densities were tested with qPCR in the Bioenvironmental Research Laboratory at UCF. Collected media samples of B&G and woodchip media were stored at  $-80^{\circ}\text{C}$  until the gene extraction was conducted by using Mobio PowerMax Soil Kit. The extraction process closely followed the kit protocol provided by the vendor. In particular, the woodchip samples were ground into smaller sizes before the DNA extraction for the purpose of obtaining more representative information. All extracted DNA elutes were stored in TE buffer under  $-20^{\circ}\text{C}$ . The real-time PCR analysis was performed with StepOne from Applied Biosystems, and PowerUp<sup>TM</sup> SYBR<sup>®</sup> Green Master Mix. The used primer sets and running methods are shown in Table 2. The qPCR assays are 20  $\mu\text{L}$  reaction volume with 10  $\mu\text{L}$  of master mix, 0.8  $\mu\text{L}$  of each primer (10  $\mu\text{mol}$ ), 4  $\mu\text{L}$  DNA template, and 5.2  $\mu\text{L}$  of qPCR degree water for reactions.

### 4 Difference Between Laboratory and Field Study

Since the treatment started (June 23, 2017) at the linear ditch site, the research team has recorded all the daily rainfall depths, as shown in Fig. 4, in which four sampling points (October 12, 2017, January 17, 2018, April 19, 2018, and July 28, 2018) were identified. The rainfall data were collected from the Suwannee River Water Management District close to the study site with an automatic rain gauge located at latitude of 29 40' 02" and longitude of 82 52' 29". They provide a general understanding of how often and how many storm events happened in this area during the study period, which is relevant to the treatment efficacy of different kinds of nutrient species. Note that whenever a storm happens, the pump slows down first and then stops working completely due to the diminished sunlight condition at that moment. In addition to the rainfall data, Table 3 shows

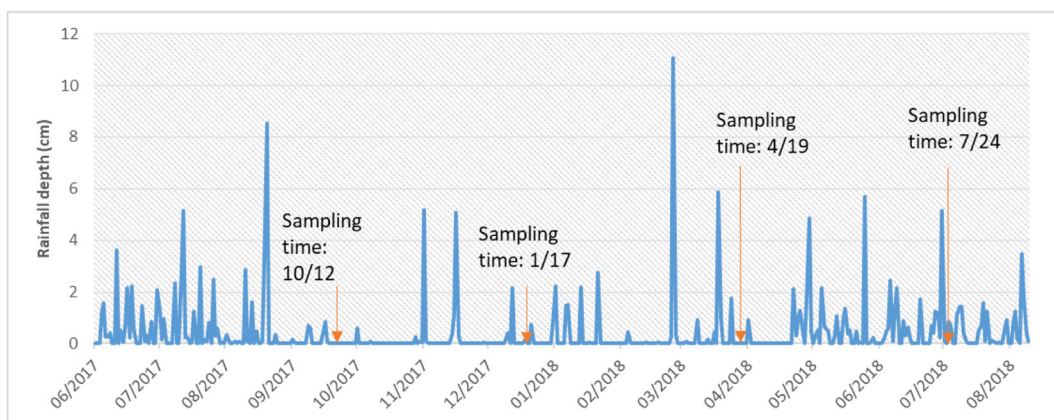
**Table 2** Primer sets and real-time PCR running condition

Target bacteria	Primer name	Primer sequence	Running method	Reference
AOB (annealing at 60 °C)	amoA-1F	GGGGTTTCTACTGG TGGT	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	CCCCTKGSAAAGCC TTCTTC		
NOB (annealing at 63.8 °C)	NSR1113f	CCTGCTTTCAGTTG CTACCG	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	GTTTGACGCGCTTT GTACCG		
Denitrifier (annealing at 60 °C)	1960m2f	TAYGTSGGGCAGGA RAAACTG	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	CGTAGAAGAAGCTG GTGCTGTT		
AMX (annealing at 62 °C)	809-F	GCCGTAAACGATGG GCACT	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	AACGTCTCACGACA CGAGCTG		

the total amount of pumped water since the start of the linear ditch treatment within 7 recording time points, and the corresponding average pumping rate for each media can be produced. This record provides insightful information about the pumping speed for groundwater treatment during clear days. Hence, the hydraulic loading rate of groundwater to the B&G section along the length of the linear ditch was calculated as 118 and 112 L/m<sup>2</sup>/day for B&G media and woodchip media, respectively.

There is a need to delineate the differences in environmental conditions between the laboratory columns and the field condition of B&G media and woodchips, as summarized in Table 4. Unlike the steady controllable

environment in the 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 storm events that may result in less-efficient biological nutrient removal due to the disturbance of the local microbial community. This would particularly affect the processes of ammonification, nitrification, and denitrification that are closely related to the transformation of different nitrogen species for ultimate nitrogen removal. Unlike woodchip, which has very small HRT, the steady and larger infiltration rate through the finer microenvironment in B&G would certainly help improve the final performance of nitrogen removal.

**Fig. 4** Rainfall depth during the linear ditch operation period and the corresponding sampling time points

**Table 3** Pumped groundwater volume readings since the start of the linear ditch study

Date	Cumulative days	Cumulative BGW meter reading (m <sup>3</sup> )	BGW average (m <sup>3</sup> /day)	Cumulative WCW meter reading	WCW average (m <sup>3</sup> /day)
6/23/2017	0	0	0	0	0
10/12/2017	112	1618	14	1513	14
11/17/2017	148	1872	13	1748	12
12/7/2017	168	2128	13	2014	12
1/17/2018	209	2582	12	2450	12
2/1/2018	224	2767	12	2636	12
2/6/2018	229	2823	12	2687	12
04/19/18	300	3789	13	3574	12
6/5/2018	349	4408	13	4243	12
6/13/2018	357	4501	13	4333	12
7/24/2018	396	5063	13	4880	12

BGW B&G well—irrigation well for B&G trench, WCW woodchip well—irrigation well for woodchip trench

## 5 Results and Discussion

### 5.1 Tracer Study

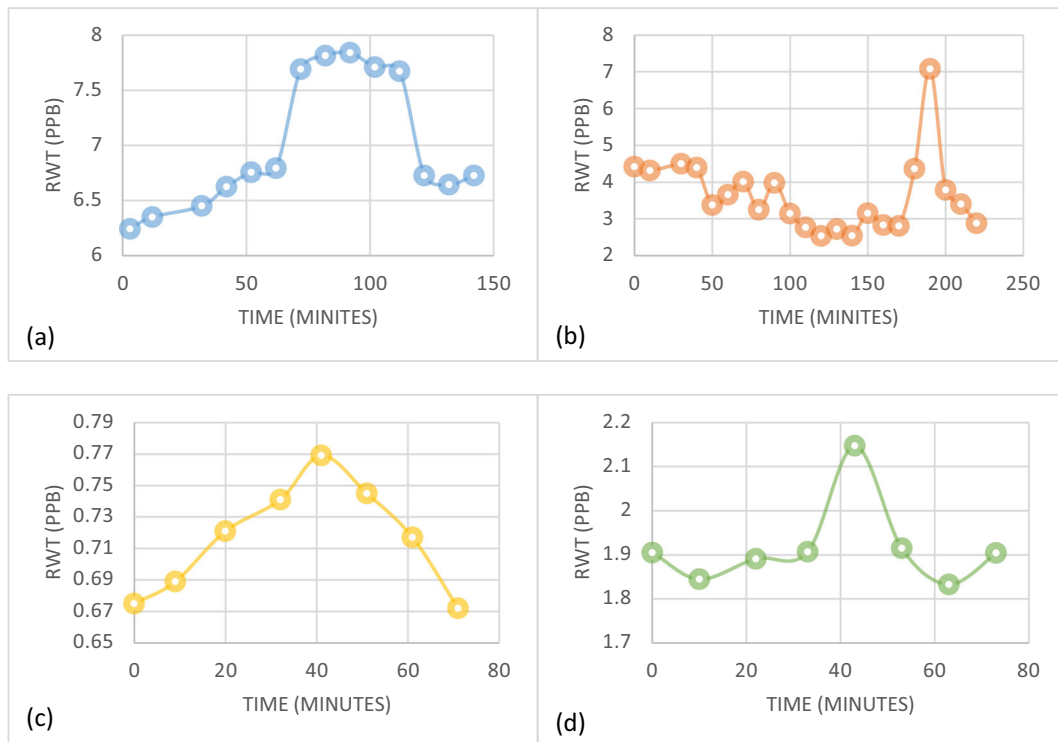
The tracer study was important for understanding the difference of hydraulic patterns for the two media recipes. The tracer study result is shown in Fig. 5 for the B&G media and woodchip columns. The HRT was determined from the breakthrough curves obtained for each column

at the point where the maximum RWT concentration was detected. Whereas the calculated tracer HRT is 77.9 and 113.1 min for columns 1 and 2 with B&G media, respectively, the values are 40.5 and 41.8 min for columns 3 and 4 with woodchip media, respectively. It is noticeable that column 2 has longer HRT than column 1, mainly because the higher TN concentration may cultivate more compacted and denser biofilm within the porous space of B&G media. This can be evidenced by the qPCR

**Table 4** Loading condition differences between laboratory and field operation

Condition	Laboratory	Field
Water source	Groundwater collected from Fanning Spring, stormwater collected from the pond on UCF campus	Groundwater pumping from the solar-powered pump, runoffs from highway stormwater runoff and farmland agricultural discharge
Pollutant loads	Groundwater and stormwater 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 32.91–49.36 $\frac{\text{L}}{\text{m}^2} \text{h}$	Highly variable when a storm happens, and relatively variable when the pumps are working due to the availability of solar power with an average loading of 115 and 108 $\text{L}/\text{m}^2/\text{day}$ for B&G and woodchip trench
Temperature	Consistent of 22 to 23 °C	Highly variable and should be hotter during summer and colder during winter
Water distribution	With consistent flow rate, the water was distributed with a pile of pebbles above the media top	Water flows into the linear ditch; it is difficult to evenly distribute as the ditch is not perfectly flat. The infiltration rate would be different along the ditch due to the compaction difference during construction.
Other disturbances	None	Uneven pumping rate along the pipeline system may occur. Animal chewing the pipeline.





**Fig. 5** The results of the tracer study from columns 1 to 4 as shown in a–d

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.

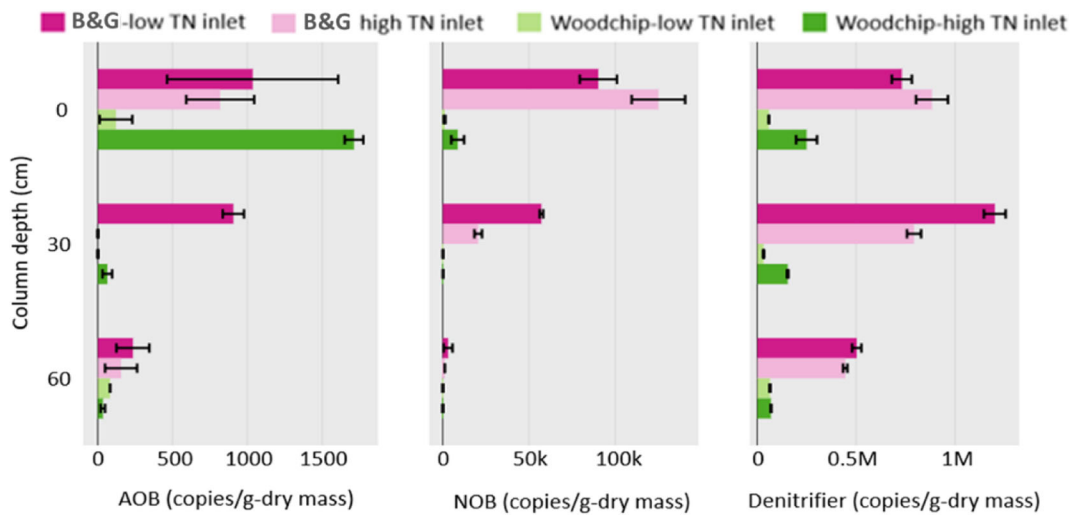
## 5.2 Microbial Ecology and Nutrient Removal

### 5.2.1 Population Dynamics of Microbial Species

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 can be realized from the laboratory column study in Fig. 6 and for field conditions in Fig. 7. Note that the field woodchip decomposed 50% over the operational period of time. This means that the original woodchip depth of 120 cm became a thinner layer of 60 cm later, the original woodchip depth of 60 cm became a thinner layer of 30 cm, and the original woodchip depth of 30 cm were almost gone with even less than a depth of 15 cm. This decomposition makes it hard to separate the top, middle, and bottom layers at the test site. So, only media samples in the final woodchip

depth of 45 cm and 60 cm were collected and analyzed for microbial ecology analysis at the middle and bottom locations in Fig. 7.

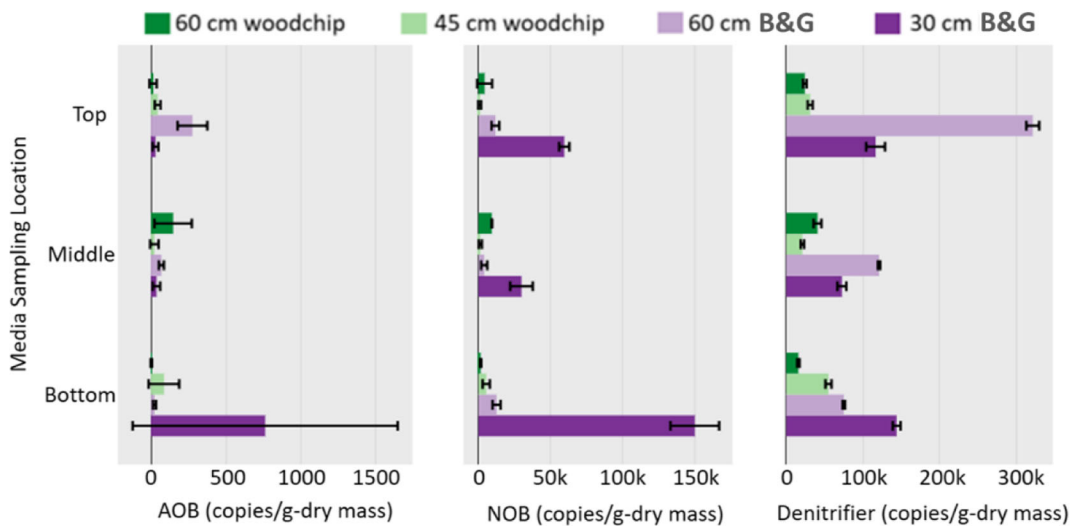
The comparison between the two figures (Figs. 6 and 7) showed some common patterns. AOB had a small population density while AMX was found to be under the detection limit; thus, AOB and AMX populations are negligible when compared to NOB and denitrifiers, in which denitrifiers are the dominant of the four bacteria species in the microbial community. The reason for this might be that nitrate and nitrite are the major contaminants in water. In addition, the overall bacteria population in B&G is much higher than that in the woodchips in both laboratory- and field-scale tests. This is because B&G is able to provide a better microenvironment, rendering the growth of more nutrient-related bacteria than woodchip media due to its larger surface area as well as more homogeneous and longer HRT. Nevertheless, there are some clear differences between the laboratory- and field-scale microbial ecology. One is that more bacteria were found at the top layer in the column study while the population density was more variable in the field condition, which sometimes results in the most abundant bacteria residing in the middle or



**Fig. 6** Gene copy density of AOB, NOB, and denitrifiers at different depths under low and high TN influent conditions in BAM and woodchip columns, respectively

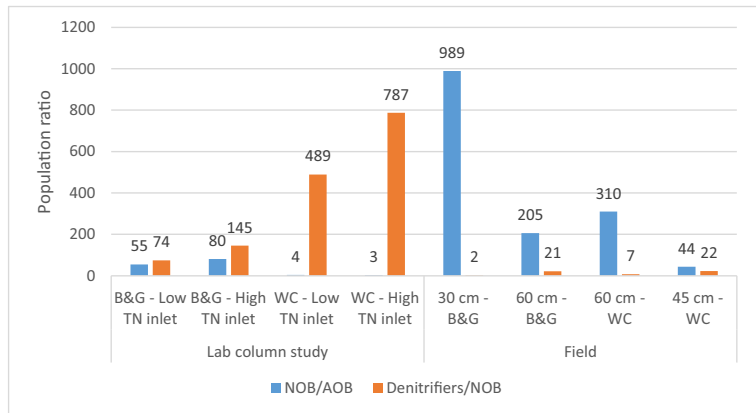
even the bottom layers. The second difference is that the size of bacteria population in the laboratory study was much larger than that in the field. The main reason is due to the steadier environment in the laboratory setting (i.e., hydraulic condition, nutrient concentration, temperature, etc.), which is beneficial for bacteria to adapt and thrive. But the uneven water distribution or preferential flow in the field may form a very different microenvironment that triggers bacteria growth in various depths randomly. The third difference relates to the microbial structural variances between the laboratory- and field-scale studies, as shown in Fig. 8. The denitrifiers/NOB

ratio is much higher in the well-controlled laboratory environment, indicating that the biofilm tends to utilize nutrients to the maximum extent by enhancing the denitrification process. Hence, more of the soluble nitrogen contaminants (ammonia and nitrate/nitrite) can be converted into nitrogen gas so that the cascade effects can be introduced and accelerated with a better reaction rate associated with nitrification and denitrification. However, the field condition resulted in a significantly decreased denitrifiers/NOB ratio while the NOB/AOB ratio increased for both media in the field condition. The increased NOB and decreased denitrifiers were driven



**Fig. 7** Gene copy density of AOB, NOB, and denitrifiers at the appropriate depth of each BAM and woodchip section in the field after operation

**Fig. 8** The comparison of mean population ratio of “NOB to AOB” and “denitrifiers to NOB” in the laboratory-scale column study and the field-scale application

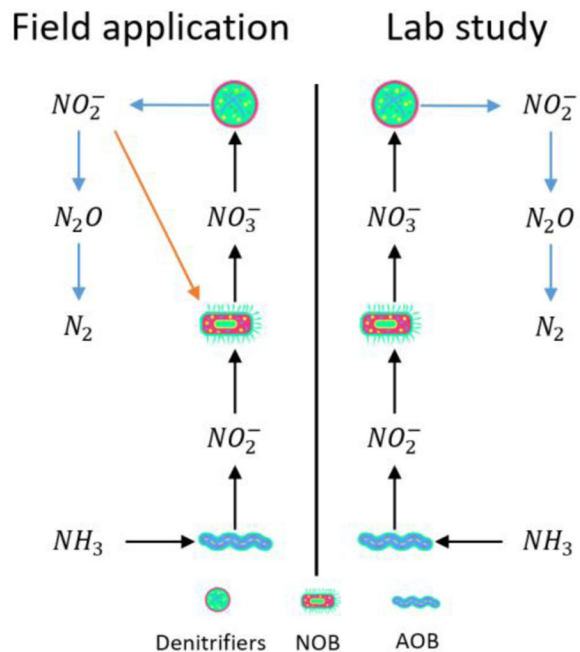


by the reuse of the nitrite (produced by denitrifiers) via the increased NOB population. As shown in Fig. 9, the closed circle of nitrogen recycling between denitrifiers and NOB carried out an energy recycling that maximized the survival opportunities of the two largest species in the harsh field environment.

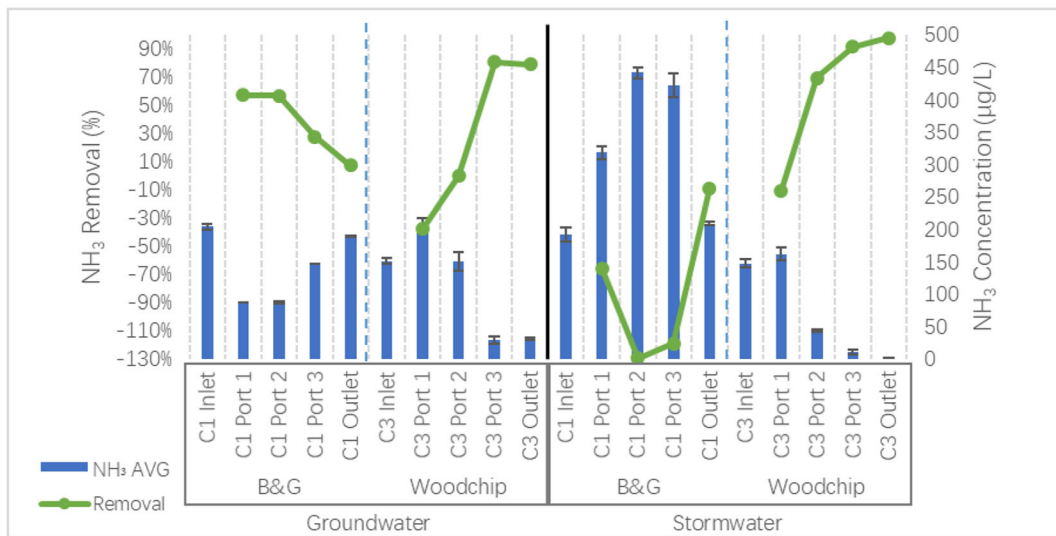
### 5.2.2 Ammonification and Nitrification

After high molecular weight organics degrade into low weight molecular organics due to heterotrophic bacteria, such as fermentative bacteria, the low molecular weight

organics can be fed to the next degradation step, known as ammonification. It requires the existence of organic nitrogen and enough DO for bacteria to carry out the work. Ammonification is the part of the nitrogen cycle that converts the organic nitrogen into ammonium and is followed by the nitrification and denitrification processes. The nitrification process consumes ammonia and generates nitrite and nitrate, which are also microbiological reactions that require oxygen supply. The two microbiological reactions can happen in parallel as long as the aerobic environment is suitable for corresponding bacteria. Since the linear ditch is also designed to treat the discharge from a farmland with highly concentrated organic matters, it is expected that ammonia, ammonium or nitrite and nitrate in effluent water samples will increase as the result of ammonification and nitrification. The column study results of ammonia removal are shown in Figs. 10 and 11 for the low (inlet concentration is ~ 5.9 mg/L in groundwater and 2.0 mg/L in stormwater, respectively) and high (inlet concentration is ~ 8.2 mg/L in groundwater and 5.5 mg/L in stormwater, respectively) total nitrogen (TN) scenarios. In the low TN scenario, the ammonia removal in B&G media and woodchip was 7% and 79% for groundwater treatment, and - 9% and 98% for stormwater treatment, respectively. In the high TN scenario, the ammonia removal in B&G media and woodchip was 4% and 91% for groundwater treatment, and 14% and 96% for stormwater treatment, respectively. Note that B&G media always have a rebound of ammonia concentration from the stormwater treatment section, which is the proof that ammonification always happens at the top section of the B&G media followed with a nitrification process to decrease the ammonia concentration in the latter column section. This also indicates that the groundwater collected from the Fanning Spring may not contain as much organic nitrogen as the



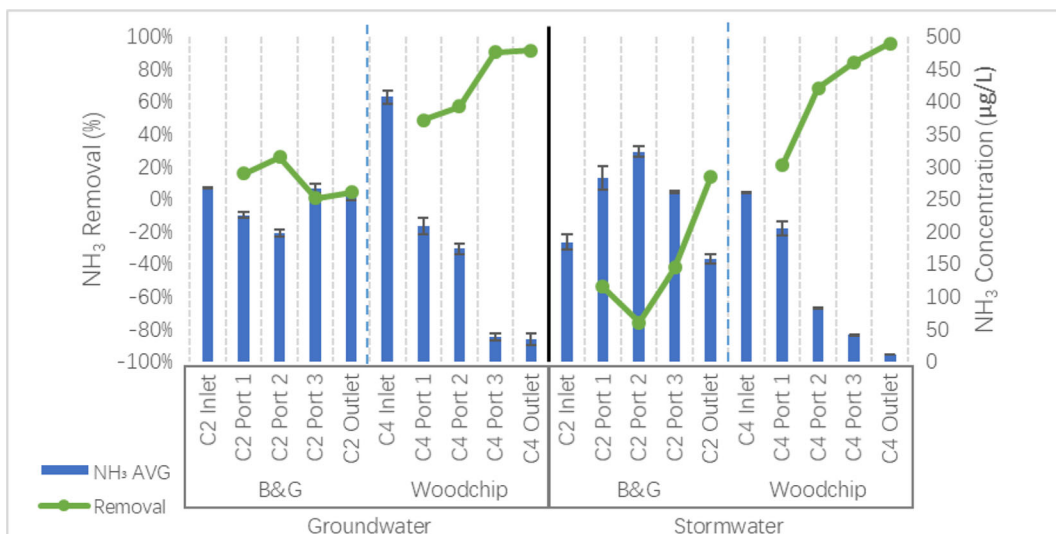
**Fig. 9** Difference of nitrogen removal pathways in the laboratory- and field-scale condition



**Fig. 10** Ammonia concentration (average with an error bar) and removal efficiency for woodchip and B&G media under low TN inlet scenarios for treating groundwater and stormwater in the columns (C1 = column 1, it applies to C2, C3, and C4)

stormwater. However, for the case of either groundwater or stormwater treatment, ammonia removal via the B&G media is deemed minor or even negligible when compared to that of woodchip. This is possibly due to more available oxygen in the woodchip than in the B&G media, which enhanced the nitrification process under the steady laboratory environment with well-controlled flow rate and nutrient concentrations and well-adapted biofilm (more bacteria population in the column study, as shown in Figs. 6 and 7) with different bacteria species distribution.

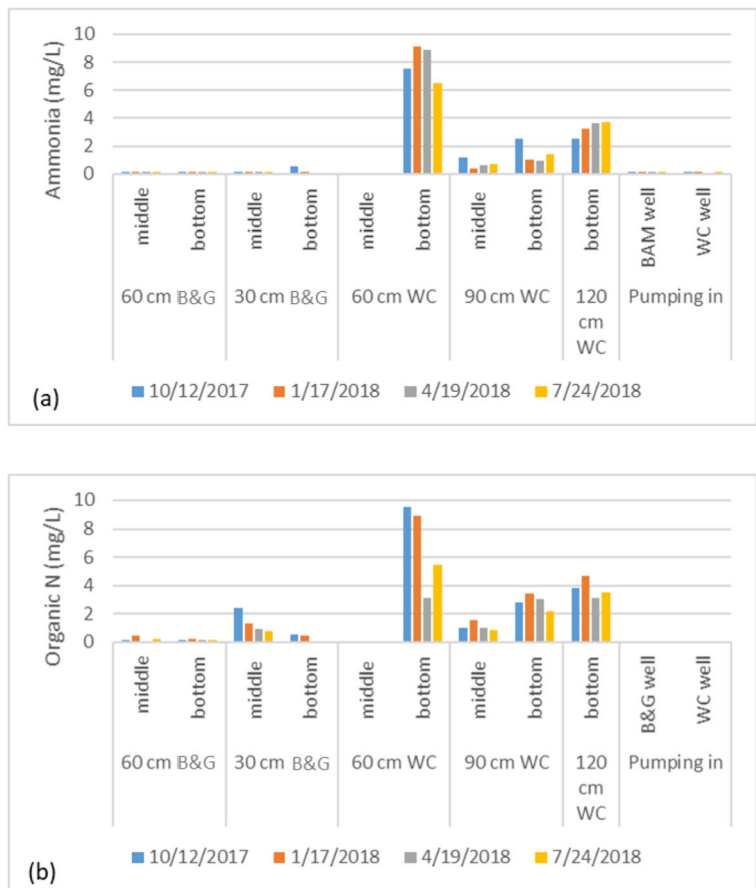
The removal of ammonia and organic nitrogen in the field is shown in Fig. 12 for B&G media and woodchip, in which the organic nitrogen concentration was calculated by subtracting the ammonia concentration from the total Kjeldahl nitrogen (TKN) concentration. Almost no organic nitrogen component was found in the influent groundwater samples collected from the pumping well location for B&G media and woodchip sections, which is consistent with the laboratory results, as the groundwater used in our column study was collected from



**Fig. 11** Ammonia concentration (average with an error bar) and removal efficiency for woodchip and B&G media under high TN inlet scenarios for treating groundwater and stormwater in the columns



**Fig. 12** The nutrient removal in the field of **a** ammonia and **b** organic nitrogen (note: no samples could be collected from the middle lysimeter of 0.6 m (2 ft) and 1.2 m (4 ft) woodchip sections)



Fanning Springs. In other words, almost all organic nitrogen sources were introduced from either the road stormwater runoff or the agricultural discharge from the farmland, or possibly from the dry or wet deposition of organic particles. For B&G media, the highest organic nitrogen concentration (2.38 mg/L) was found at the section with the middle lysimeter at 30 cm (1 ft) depth. After that, the organic nitrogen concentration decreased rapidly, normally dropping below 0.5 mg/L from the depth of 30 to 60 cm (1 to 2 ft). Because of organic nitrogen intrusion, some ammonia was generated through ammonification at the B&G section at 30 cm (1 ft) depth at the bottom. 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 media 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 section with the middle lysimeter of 60 cm (2 ft) depth, and the rest ranged from 0.6 to 3.6 mg/L, which is significantly higher than the B&G media section. This is because particulate organic nitrogen (PON) with high molecular weight organics can more easily be transferred through woodchip than B&G media and more likely ended up at the bottom of the media, triggering intensive ammonia generation through ammonification. Another possible organic source is slow releasing fertilizer, which is mostly urea that can be converted into ammonia via ammonification. This indicates that woodchip can allow more oxygen to be consistently present in the porous area. Moreover, nitrification is also insignificant in woodchip, as a high ammonia concentration condition with smaller HRT 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

biofilm for AOB and NOB, which are bacteria that tend to utilize oxygen at the surface of the biofilm for nitrification (Fig. 7).

For stormwater treatment, the significant conversion from organic nitrogen to ammonia in the woodchip section in the field shows a completely opposite trend when compared to that in the laboratory results of the woodchip columns. There are two reasons to explain this conflicting result; one is that the stormwater used in our column study was different from the actual runoff in the field because we collected the stormwater from UCF's main campus. The stormwater runoff from the farmland nearby has higher organic nitrogen concentration due to the presence of animal waste and fertilizer. Besides, a significant number of plants were found in the field, which is a potential organic nitrogen source 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 5.2.1, the microbial community in the field is much smaller and less stable when compared to that in our column study. This is most likely due to multiple highly variable environmental factors and flow rates (mentioned in Section 4), in addition to the higher concentration of organic nitrogen found in the field. The woodchips in the field have a small amount of AOB and NOB to deal with the highly concentrated ammonia, leading to the leakage of ammonia in high concentrations into the groundwater aquifer.

### 5.2.3 Denitrification

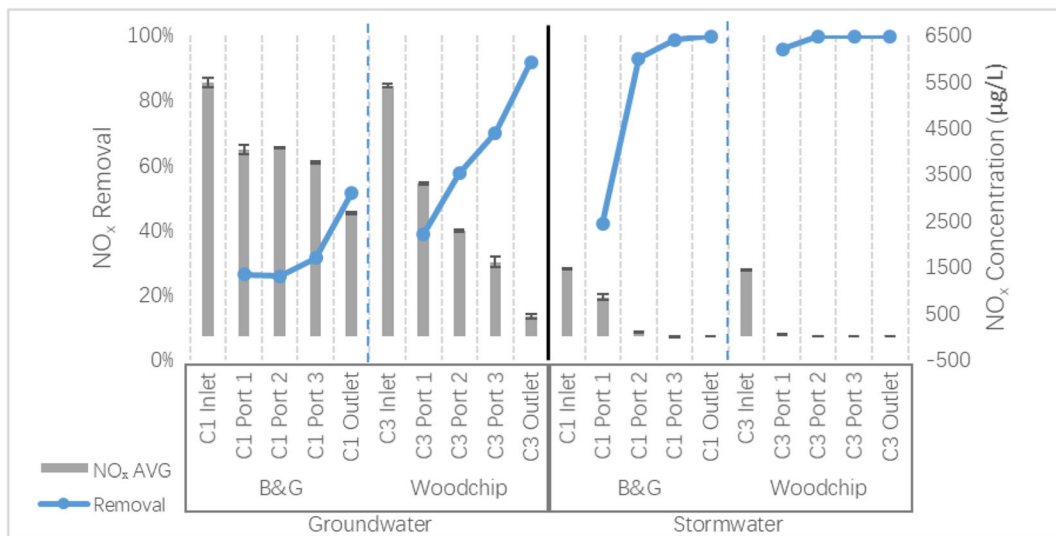
Denitrification is a crucial step to convert nitrate/nitrite (known as  $\text{NO}_x$ ) into nitrogen gas as the last step of the nitrogen cycle on Earth and is performed by denitrifiers that are only active under anaerobic conditions. The column study results of  $\text{NO}_x$  concentration and removal efficiency are shown in Figs. 13 and 14 for the low and high TN scenarios, respectively, while the field results of  $\text{NO}_x$  removal are shown in Fig. 15. In the column study of the low TN scenario, the  $\text{NO}_x$  removal efficiency of B&G media and woodchip was 52% and 92% for groundwater treatment, while both reached 99% of  $\text{NO}_x$  removal for stormwater treatment. In the high TN scenario, the  $\text{NO}_x$  removal efficiency of B&G media and woodchip was 45% and 67% for groundwater treatment, respectively, and 73% and 93% for stormwater treatment, respectively. Both media achieved promising  $\text{NO}_x$

removal but woodchip outperformed B&G media when treating the groundwater with a low carbon concentration ( $\sim 4$  mg/L COD). Even though B&G media could maintain a suitable anaerobic environment for denitrifiers, the woodchip could provide enough carbon sources as electron donors in the denitrification reaction. However, the carbon scarcity is not a problem in stormwater treatment because there is enough carbon source in stormwater runoff ( $\sim 15$  to  $20$  mg/L COD). Moreover, the denitrification in woodchip happened within the bottom layer of the biofilm attached to the wood surface, which is also the best location for retrieving carbon sources and maintaining anaerobic conditions. Also, the inlet concentration has a significant impact on bacteria population densities in woodchip (Fig. 6). Overall, it seems that nutrient availability is a more important factor for bacteria growth in woodchip.

In the field, B&G media show a trend similar to the column study. Significant  $\text{NO}_x$  removal efficiency of 70–99% occurred from the bottom of each B&G section. This is mainly because B&G media can maintain a suitable anaerobic condition within the porous space when B&G media are wet. It is also the reason that B&G media perform extremely well in removing organic nitrogen since the PON was filtered and trapped at the B&G media surface. The woodchip in the field showed promising  $\text{NO}_x$  removal of over 97%, which is very similar to the results from the column study. The denitrification process at the bottom of the biofilm was relatively intensive with the support of ample carbon sources from the woodchip. The most important reason for this result is that denitrifiers have been well cultivated in the woodchip as well as B&G media because both media are mainly prepared for treating groundwater when there is no storm event and  $\text{NO}_x$  are available constantly to denitrifiers. With a relatively steady and continuous groundwater influent, denitrifiers could gain comparative advantages and remain active for nitrate/nitrite removal.

### 5.2.4 TN Removal

The TN concentration and removal efficiency from the laboratory columns of B&G media and woodchip are shown in Figs. 16 and 17 for low and high TN scenarios, respectively. In the low TN scenario, the TN removal efficiency of B&G media and woodchip was 50% and 85% for groundwater treatment, while both were 78% for stormwater treatment. In the high TN scenario, the

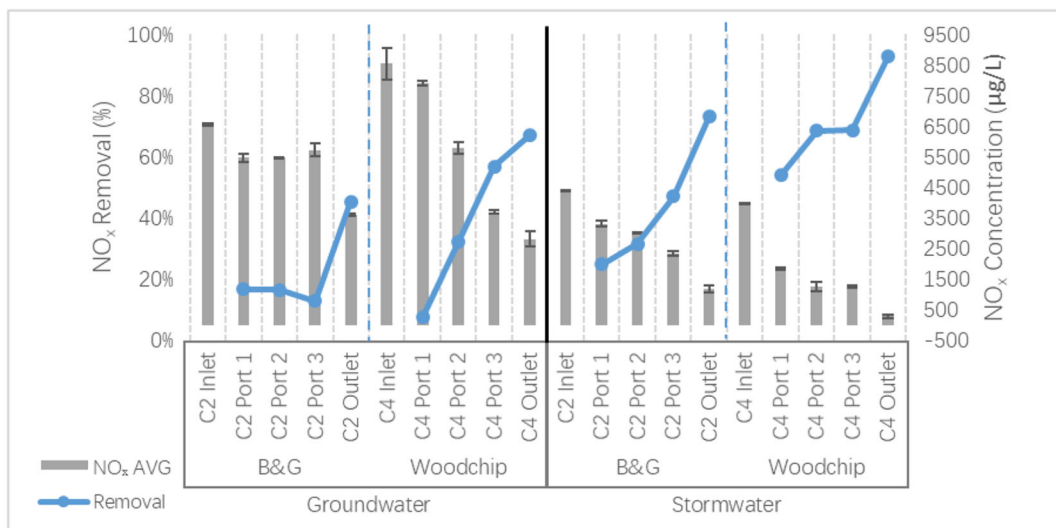


**Fig. 13** Nitrate and nitrite (NO<sub>x</sub>) concentration (average with an error bar) and removal efficiency for woodchip and B&G media under low TN inlet scenarios for treating groundwater and stormwater in the field

TN removal efficiency of B&G media and woodchip was 43% and 62% for groundwater treatment, respectively, while it was 70% and 80% for stormwater treatment, respectively. B&G media and woodchip tended to show equivalent TN removal for stormwater treatment; however, the woodchip had a better performance than B&G media when treating groundwater, because woodchip can provide carbon sources as electron donors in carbon deficient groundwater.

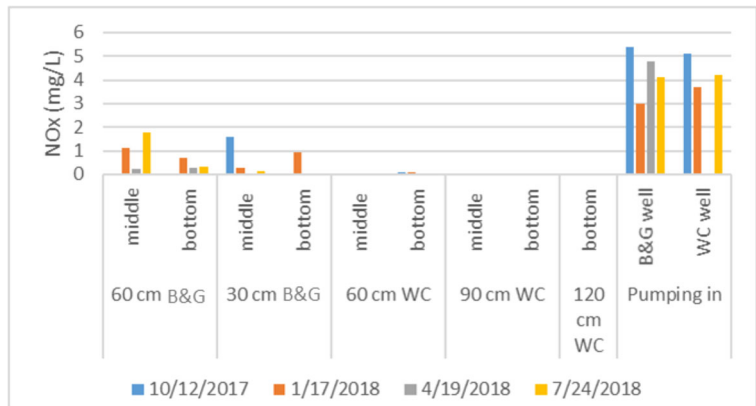
The field TN concentrations are shown in Fig. 18. The TN removal efficiency of B&G media was 52–80%

and 68–95% at 30 cm (1 ft) and 60 cm (2 ft) depth sections, respectively. These values are very close or sometimes even better than the laboratory results. However, the woodchip in the field performed entirely differently from that observed in the laboratory. It had almost no positive removal efficiency in the field except 16–17% TN removal from the bottom lysimeter of the 90 cm (3 ft) depth section on July 19, 2018, and July 4, 2018. The TN concentration in the effluent increased as high as over 3 times of the influent value in the worst case from the bottom of the 60 cm (2 ft) depth section on



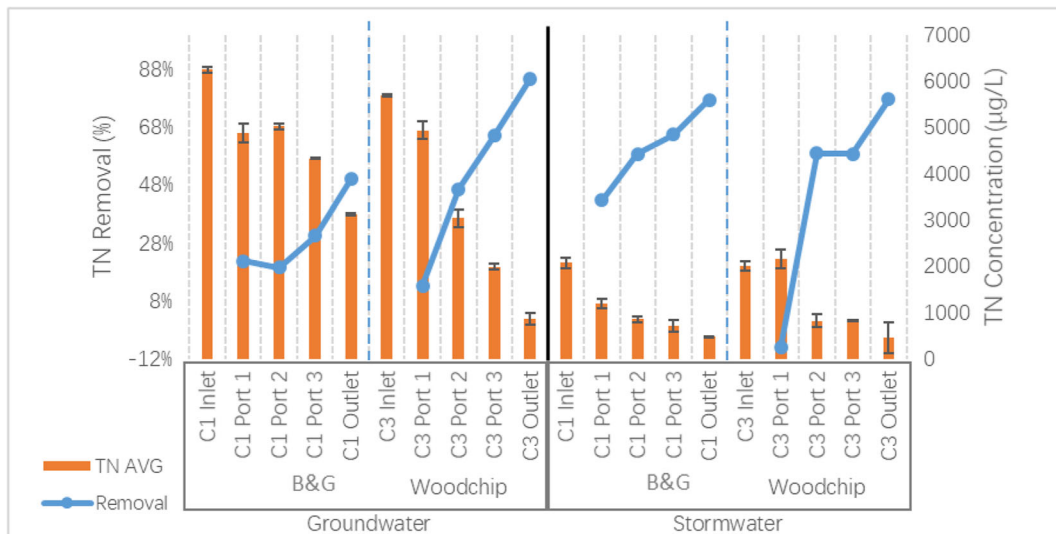
**Fig. 14** Nitrate and nitrite (NO<sub>x</sub>) concentration (average with an error bar) and removal efficiency for woodchip and B&G media under high TN inlet scenarios for treating groundwater and stormwater in the field

**Fig. 15** The results of NO<sub>x</sub> concentration at the lysimeter locations in the field



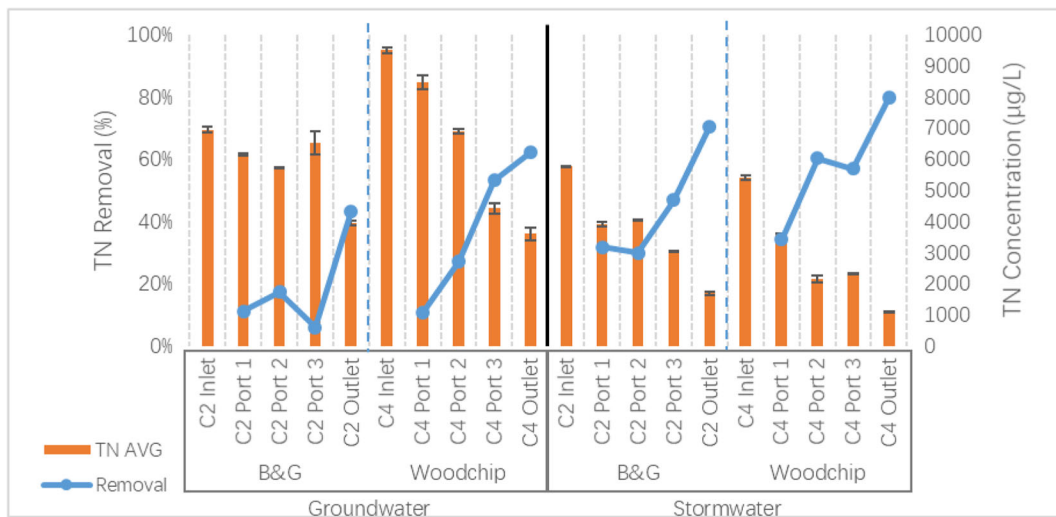
1/17/2018. As mentioned in the previous section, the major reason why the B&G media performed much better than woodchip is that B&G media could filter and trap the sediments that also carry highly contaminated organic matter through the runoffs. Woodchip, on the other hand, has no such capability due to its large void space. Hence, a large quantity of sediments flowed through the woodchip and ended up in the lysimeter throughout different depths without proper treatment due to insufficient HRT. Another reason why B&G media performed much better than woodchip is that the B&G media have a much higher tolerance level for fluctuation of the inflow rate. No matter how fast the stormwater runoff got into the linear ditch, the infiltration rate through the B&G media would not change too much because its HRT is limited by the small porous

size. On the other hand, when it was dry, the B&G media were also able to maintain necessary moisture for bacteria survival. So, B&G media allowed enough contact time for the bacteria to do their job and cultivated much more bacteria population than woodchip. However, it would be significantly different for woodchip treating stormwater runoffs because storm intensity is highly variable, as shown in Fig. 4. For stormwater treatment, woodchip might achieve acceptable TN removals from small storm events as the inflow rate is small, and there is enough contact time between the water flows and woodchip. But the TN removal would drop dramatically when the stormwater runoff is big enough so that a large quantity of water just flows through the woodchip with negligible contact time, minimizing the treatment effectiveness.



**Fig. 16** TN concentration (average with an error bar) and removal efficiency for woodchip and B&G media under low TN inlet scenarios for treating groundwater and stormwater in the field





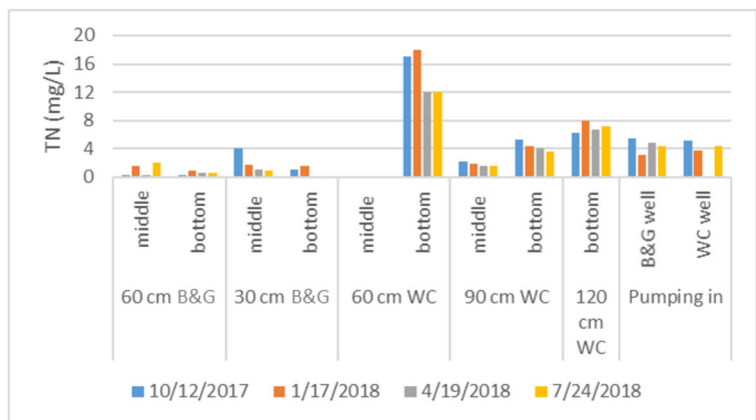
**Fig. 17** TN concentration (average with an error bar) and removal efficiency for woodchip and B&G media under high TN inlet scenarios for treating groundwater and stormwater in the field

**6 Conclusion**

Two recipes of green sorption media including the B&G media and woodchip were evaluated in the laboratory column study and the field study for the co-treatment of stormwater and groundwater. The laboratory results indicate that both green sorption media performed effectively for TN removal, in which woodchip showed better nitrification effects due to the increased amount of oxygen available in the void space when compared to B&G media. Both B&G and woodchip media performed denitrification since both can maintain an anaerobic environment in the biofilm, albeit at different thicknesses. B&G media can eliminate the oxygen by holding moisture content within the small porous holes while the deeper layer of biofilm on the woodchip surface has

low DO value. But B&G media tends to hold denser bacteria population than woodchip by providing more surface area for biofilm development, and the constant loading in the laboratory column test condition was more beneficial for bacteria growth when compared to the field condition. This observation has been evidenced by qPCR related data. Moreover, the pattern of microbial ecology differed across the laboratory and field applications resulting from population density in the biofilm. B&G media performed even better in the field under conditions similar to those in the column study, whereas the woodchip had better performance in the lab application. The woodchip performed entirely differently in the field as ammonification generated a significant amount of ammonia from organic nitrogen in the woodchip without enough nitrification to push the

**Fig. 18** The results of the TN concentration of influent (pumping well) and at each lysimeter location and for (a) B&G media and (b) woodchip in the field



ammonia into the next step of nitrogen cycle. The larger pore size of woodchip failed to screen out the sediment from the runoff as well as keep enough contact time between the water flows, which caused a diminishing effectiveness in treatment. However, denitrification was relatively active in both B&G media and woodchip in the field application, because the constant pumping rate of groundwater flows has a high concentration of nitrate. The nitrate is the main energy/food source for denitrifiers. But a higher percentage of NOB and lower percentage of denitrifiers were observed for both media in the field because the recycling energy circle between them helped them maximize the survival. In general, B&G media are more appropriate for the co-treatment of stormwater and groundwater in space limited BMP under a complicated natural environment and it does not have the decay issue evidenced by the woodchip. Also, the woodchip is limited because they cannot reliably maintain traffic bearing capacity while B&G media is traffic bearing along the road side.

However, we also understood the limitations of the current study, in which the external force impacts from traffic (compaction) and animal activities (conduit) are not considered. Future studies will evaluate the influence of those impacts on BMPs' performance. In addition to the external forces, carbon source availability is another important factor that could potentially impact microbial activities because carbon is widely available in urban and agricultural runoffs.

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