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

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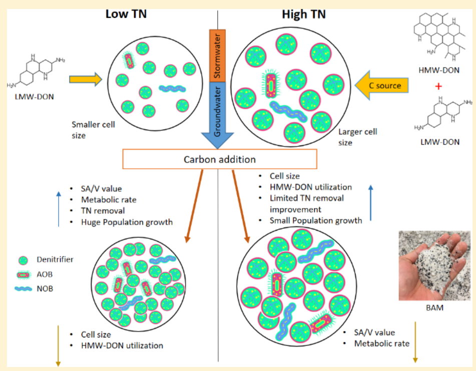
ABSTRACT: Eutrophication has been a long-term issue in aquatic environments, where dissolved organic nitrogen (DON) recalcitrance is important. Bioavailable nitrogen qualification and quantification for effluents from stormwater and wastewater are always a challenge. The information in this study deepens the understanding of the interactions between carbon addition and DON decomposition through linear-ditch best management practices for stormwater and groundwater cotreatment. By running a laboratory-scale column study for nitrogen removal using green sorption media, the variation in composition and concentration of DON can be further linked to the population dynamics of microbial species that dominate the nitrification and denitrification processes. With the varying levels of influent total nitrogen concentration, the efficacy of nitrogen removal via biosorption activated media may be realized at the molecular level with ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry.



1. INTRODUCTION

The National Academy of Engineering has indicated that understanding and managing the nitrogen cycle is one of the 14 grand challenges for engineering in the 21st century.[1] Human activities have largely increased nitrogen consumption and distribution.[2−4] Much of the residual nitrogen is normally carried out by stormwater runoff, wastewater effluent, or agricultural discharge.[5−7] Within such an urban nitrogen cycle, dissolved organic nitrogen (DON) is a nonnegligible part since it occupies a large proportion, up to as much as 80%, of total nitrogen (TN) and is an important N source supporting many microbial processes.[8−10] Particularly in stormwater runoff and agricultural discharge, sediment release is the major source of DON, and the uptake of DON through bacterial degradation is the major sink of DON.[11,12] Understanding the processes responsible for DON production, behavior, and characteristics is thus critical for managing nutrient cycling with adequate nutrient control strategies. Previous studies tried to address the ecological significance of DON in various environments such as marine[8,11,13] and freshwater systems.[10,14,15] These studies revealed that DON is a structurally complex mixture of different kinds of organic molecules that are highly variable in chemical structure and composition and thus in bioavailability and ecological functioning. The inherent complexity of DON is a major barrier for understanding how different best management practices (BMPs) can change, modify, and remove DON through innovative stormwater treatment processes.[16−26] One of the promising media types, biosorption activated media (BAM), has been used in various BMPs in varying landscapes for effective nitrogen removal through biological nitrification and denitrification.[21,22,27−34] However, the interaction between BAM and DON remains unclear, especially for the cotreatment of stormwater and groundwater in linear ditch (bioswale) facilities.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) has been applied to analyze complex dissolved organic matter (DOM) for marine organics,[13] surface water,[35] and stormwater.[35−37] The ultrahigh resolving power ($m/\Delta m_{50\%}$ > 270 000 at m/z 400)[38] and mass accuracy (80−200 ppb) of FT-ICR-MS enables the resolution and confident identification of tens of thousands of unique elemental compositions in dissolved organic matter (DOM). This technique is promising for understanding the qualitative molecular interactions between DOM composition, nitrogen-containing compounds, and concentration of nitrogen species in the BAM-based stormwater treatment process. Previous studies have applied FT-ICR-MS to assess the biodegradability of DON from stormwater at the molecular level,[37] but they only evaluated the relative abundance of DON based on the DOM analysis. Moreover, the highly variable natural environment has many influential factors related to bacterial activities for nitrogen removal. One of these factors is a carbon source as part of the total organic carbon in a natural environment, which is a critical element for biomass formation and the electron donor for denitrification processes.[39] This impact on

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denitrification processes can be further realized by using real-time polymerase chain reaction (Real-Time PCR) to quantify the population dynamics of nitrifiers and denitrifiers.

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

2. MATERIALS AND METHODS

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

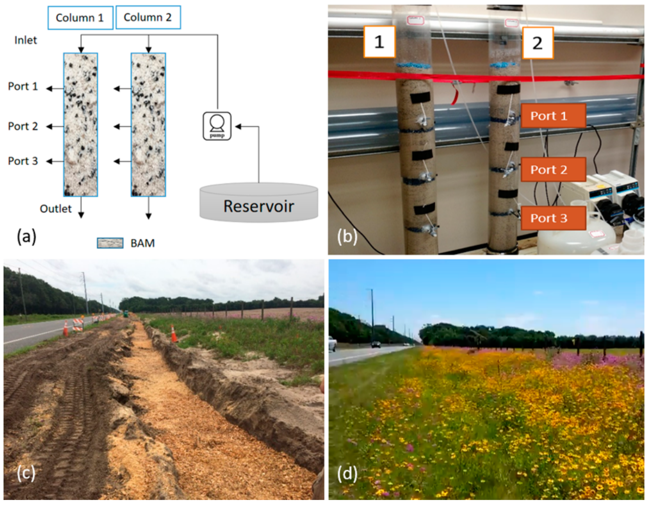

Figure 1. (a) Schematic diagram for column setup. (b) Column study—laboratory view, (c) field construction with BAM deployment, and (d) after construction for cotreatment of groundwater and stormwater.

Water quality parameters of dissolved oxygen (DO) and pH were measured right after the water collection at UCF. Total nitrogen (TN); ammonia, nitrate, and nitrite (NO_x); and alkalinity were determined by an external certificated laboratory, Environmental Research & Design (ERD), and all methods and instruments are listed in Table 2. The corresponding measurement unit is μg/L for TN−N, NO_x−N, and NH_3−N, and the organic nitrogen concentration can be calculated by subtracting NO_x and NH_3 from TN. Water samples were collected only for the inlet and outlet of stormwater sections in each cycle to analyze the DON because stormwater contains the newly washed out DOM. The media samples were collected at the top, port 1, and port 2 from columns 1 and 2 after running the stormwater section in each cycle.

2.2. DON and DOM Analysis. The DON of interest is a part of DOM, and we only focus on the N-bearing organic component as a subset of the DOM analysis. The water sample was first preserved with solid phase extraction (SPE) in the manner described by Dittmar et al.[40] After SPE, all final samples were kept under −20 °C until analysis. Sample analysis for DON was performed at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL. DOM extracts were analyzed with a custom-built FT-ICR-MS[41] equipped with a 9.4 T horizontal 220 mm bore diameter superconducting solenoid magnet operated at room temperature, and a modular ICR data station (Predator)[42] facilitated instrument control, data acquisition, and data analysis. Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale to the Kendrick mass scale[43] to identify homologous series for each heteroatom class (i.e., species with the same

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Table 1. Inlet Conditions for the Column Study[a]

water source	cycle 1, no carbon source				cycle 2, with carbon source			
	groundwater (3 days)		stormwater (1 day)		groundwater (3 days)		stormwater (1 day)	
pumping rate	10 mL/min		15 mL/min		10 mL/min		15 mL/min	
spiked nitrate concentration	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L	1.5 mg/L	5.0 mg/L
scenario	LGN	HGN	LSN	HSN	LGC	HGC	LSC	HSC

[a]LGN = low TN groundwater inlet with no carbon addition; HSC = high TN stormwater inlet with carbon addition, etc.

Table 2. Methods and Instruments for Water Sample Analysis[a]

parameter	analysis method/instrument
total nitrogen (TN)	SM-21, Sec. 4500 N C
nitrate + Nitrite (NO_x)	SM-21, Sec. 4500-NO3 F
ammonia	SM-21, Sec. 4500-NH3 G
alkalinity	SM-21, Sec. 2320 B
dissolved oxygen (DO)	HACH HQ40D - IntelliCAL LDO101 LDO
pH	Waterproof Double Junction pHTestr 30

[a]SM = Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005.

$C_cH_hN_nO_oS_s$ content, differing only by their degree of alkylation). For each elemental composition, $C_cH_hN_nO_oS_s$, the heteroatom class, type (double bond equivalents, DBE = number of rings plus double bonds involving carbon), and carbon number, *c*, were tabulated for subsequent generation of heteroatom class relative abundance distributions and graphical abundance-weighted DBE vs carbon number or H/C ratio vs carbon number images or van Krevelen diagram with PetroOrg software [Corilo, Y. E. PetroOrg Software; Florida State University, Omics LLC: Tallahassee, FL, 2014]. The full operation details of FT-ICR-MS can be viewed in an external link.[44]

Due to the immense compositional polydispersity and polyfunctionality, ionization of DOM yields a range of ionization potentials and challenges all mass spectral techniques. Therefore, comparison of nitrogen-containing DOM compounds can be conducted between samples based on relative abundance differences between heteroatom classes.[37] However, it is possible to retrieve the absolute DON concentration of each heteroatom class with the help of the measurement of total DON from the water quality analysis (eq 1). It is also necessary to understand that not all DON components are ionized equally in the ionization process, as oxygen-rich molecules are more efficiently ionized than DON. The absolute concentration of each heteroatom DON class is then calculated based on their relative abundance in DOM. On the basis of the sum of the relative abundance of DON, the relative abundance of each DON species becomes absolute when the whole DON can be divided only among DON species accounting for the total DON (eq 2):

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

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

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

2.3. Real-Time PCR Analysis. Identifying the gene copy number of corresponding microbial species in relation to nitrogen removal would be helpful in providing one more dimension for understanding the microbial community development in the media samples between ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and denitrifiers. Real-time PCR, also known as quantitative polymerase chain reaction (qPCR), is a laboratory technique used in molecular biology for quantifying microbial species. The gene copy number of nitrifiers and denitrifiers was tested with real-time PCR in the Bioenvironmental Research Laboratory at UCF. The collected samples of BAM were stored at −80 °C until gene extraction by using the Mobio PowerMax Soil Kit; the extraction process followed the kit protocol provided by the vendor. All extracted DNA elutes were stored in Tris-EDTA buffer under −20 °C. The real-time PCR was performed with StepOne from Applied Biosystems and PowerUp SYBR Green Master Mix. The primer sets and running methods utilized are shown in Table 3. The PCR assays are of 20 μL reaction volumes with 10 μL of master mix, 0.8 μL of each primer (10 μmole), 4 μL of DNA template, and 5.2 μL of PCR degree water for reactions.

3. RESULTS

3.1. Carbon Impact on Inorganic Nitrogen Removal. The influent and effluent concentrations of TN, NO_x, and ammonia with the comparison of carbon influences is shown in Figure 2a as well as the N-balance that evaluates the transformation of different N species, which is shown in

Table 3. Primer Sets and Real-Time PCR Running Conditions

target bacteria	primer name	primer sequence	running method	reference
AOB (annealing at 60 °C)	amoA-1F	GGGGTTTCTACTGGTGGT	2 min 50 and 95 °C, 15 s at 95 °C, and 1 min at 60 °C for 45 cycles	Rotthauwe et al.[45]
	amoA-2R	CCCCTKGSAAAGCCTTCTTC		
NOB (annealing at 63.8 °C)	NSR1113f	CCTGCTTTCAGTTGCTACCG	2 min 50 and 95 °C, 15 s at 95 °C, and 1 min at 63.8 °C for 45 cycles	Dionisi et al.[46]
	NSR1264r	GTTTGCAGCGCTTTGTACCG		
denitrifier (annealing at 60 °C)	1960m2f	TAYGTSGGGCAGGARAAACTG	2 min 50 and 95 °C, 15 s at 95 °C, and 1 min at 60 °C for 45 cycles	López-Gutiérrez et al.[47]
	2050m2	CGTAGAAGAAGCTGGTGCTGTT		

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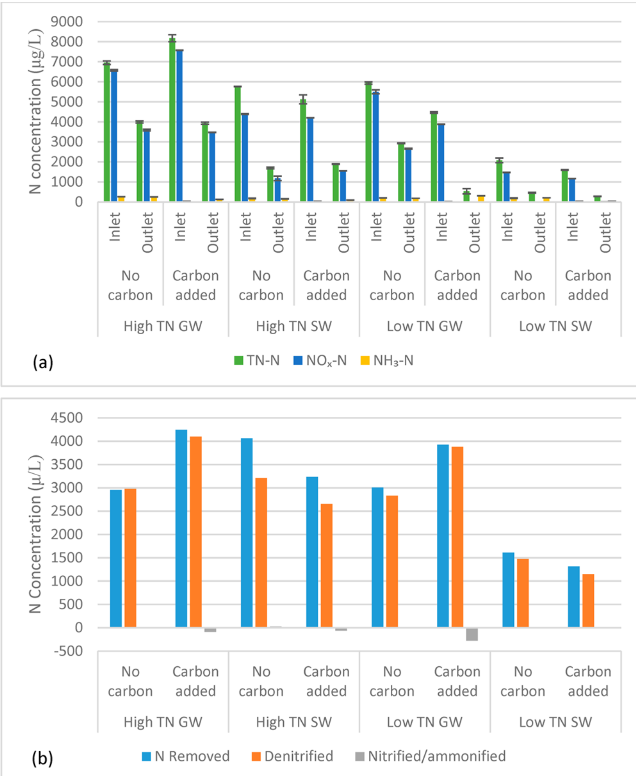

Figure 2. (a) Influent and effluent concentrations of TN, NO_x, and ammonia of BAM before and after the carbon source addition under low TN inlet conditions (column 1) and high inlet conditions (column 2) with groundwater (GW) and stormwater (SW) scenarios. (b) Transformed N species for N-balance calculation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

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

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

3.3. DON Compositional Changes. The total DON concentrations from the inlet and outlet are calculated through eq 1 for both low and high TN scenarios before and after the carbon addition, as summarized in Table 4. Some acronyms are used in this paper to represent different scenarios. For instance, LSC represents low TN concentration (spiked 1.5 mg/L nitrate) stormwater with carbon addition while LSN represents low TN concentration (spiked 1.5 mg/L nitrate) stormwater with no carbon addition. Likewise, HSC represents high TN concentration (spiked 5.0 mg/L nitrate) stormwater with carbon addition, while HSN represents high TN concentration (spiked 5.0 mg/L nitrate) stormwater with no carbon addition. With the help of additional carbon, the total DON removal increased from 27% to 42% for stormwater treatment with low inlet TN, and from 52% to 73% for stormwater treatment with high inlet TN. This outcome is part of the reason why the ammonia concentration went up quickly as a result of carbon addition in the case with high inlet TN (Figure 2), because carbon addition may increase the ammonia concentration through the enhancement of ammonification for the treatment of both stormwater and groundwater.[48,49] At that moment, AOB was not abundant enough to decompose those ammonia (Figure 3), although more complex reactions may coexist with ammonification toward ammonia generation.

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

Figure 5 shows the comparative inlet and outlet conditions of all N-bearing formulas found for stormwater treatment associated with either low or high inlet TN before and after carbon addition. In this figure, we further overlaid diagrams

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

Table 4. Total DON Concentration (μg/L) Changes for Stormwater Treatment Scenarios Associated with Low or High Inlet Total Nitrogen (TN) with or without Carbon Addition

carbon dosage	sample location	low inlet TN	high inlet TN
no carbon	inlet	442	877
	outlet	322	418
carbon added	inlet	398	883
	outlet	226	240

with rectangles to note where important classes of biomolecules are known to fall on a van Krevelen diagram.[37,51] These biomolecules include lipid-, protein-, tannin-, amino sugar-, and lignin- formulas as well as a group of uncharacterized hydrocarbons falling within O/C ratio <0.4 and H/C ratios between 0.5 and 1.75. The scenario of stormwater treatment with low inlet TN shows a similar outlet DON composition pattern before and after carbon addition, which is also consistent with the observations from Figure 4a and Figure 4b. For the counterpart with high inlet TN, the outlet DON composition contains fewer and less dense molecular species than the low TN case, and the carbon addition seems to help remove more DON classes.

4. DISCUSSION

4.1. Carbon Impact on Microbial Community Development and Nutrient Removal. Nitrogen removal within BAM is mainly attributed to a series of biological reactions involving ammonification, nitrification, and denitrification. Given the two inlet TN conditions, the microbial development can be seen in Figure 6, in which the addition of carbon boosted more bacterial growth in nutrient-poor conditions than in nutrient-rich conditions (Figure 3). One reason is that the production of the initiation protein DnaA and other essential components of the replication machinery is proportional to carbon availability for nutrient-poor bacteria,[50] but the DON can be utilized as a carbon source for bacteria in nutrient-rich environments, which makes carbon addition less valuable.[51]

The difference from the inlet TN concentration can also affect the average cell size of bacteria that would substantially keep a certain surface-area-to-volume ratio (SA/V) favorable in response to nutrient availability. Given that column 1 was fed with low TN influent and column 2 with high TN influent, the bacteria cell size in column 1 has to be much smaller than that in column 2 to have a chance to survive. Harris and Theriot[52] used the ratio of SA/V as a key indicator of the cell size because cells modify their size in order to achieve and maintain a specific, condition-dependent SA/V that benefits the species most. Schaechter et al.[53] also observed a similar phenomenon. In our study, the change of relative abundance of bacteria with depth after carbon addition is therefore meaningful (Figure 3). In column 1, the significant increase of the NOB percentage at the top section and 30 cm in depth indicates that the bacteria community had not developed to the maximum extent of its capability in terms of optimized community structure between different species before carbon addition. In column 2, the top

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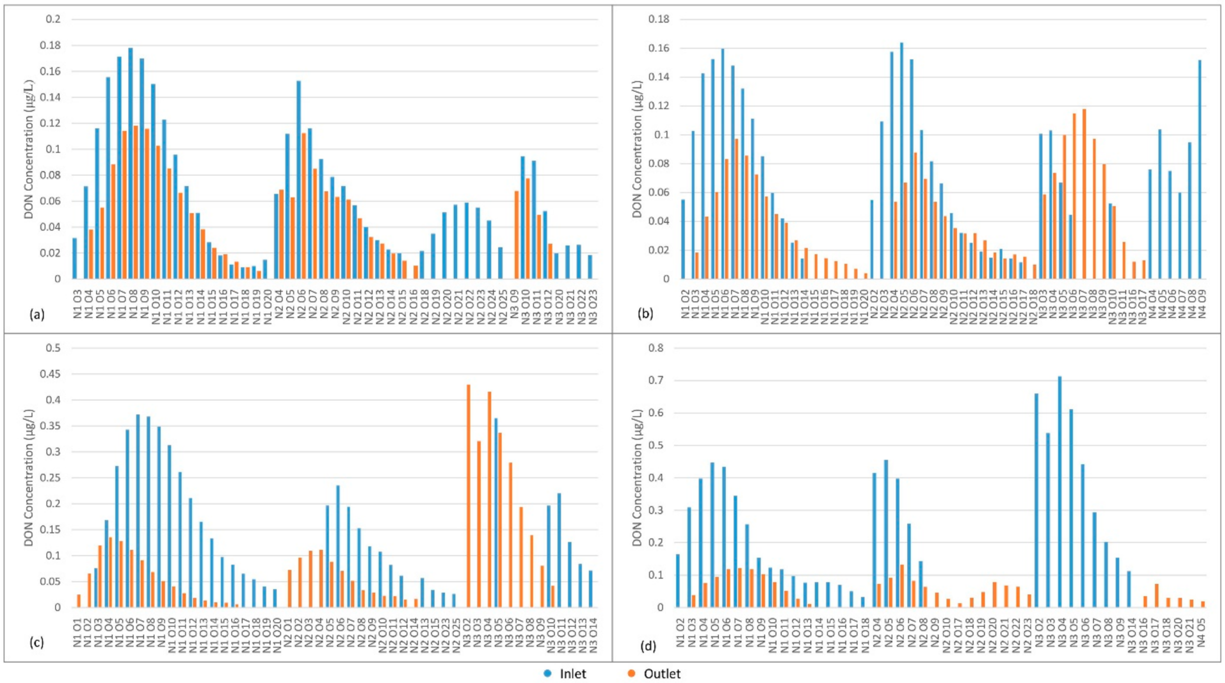

Figure 4. Calculated concentration of CHON classes in the low TN inlet for BAM with (a) no carbon addition (LSN), (b) carbon added (LSC), and in the high TN inlet with (c) no carbon addition (HSN) and (d) carbon added (HSC)

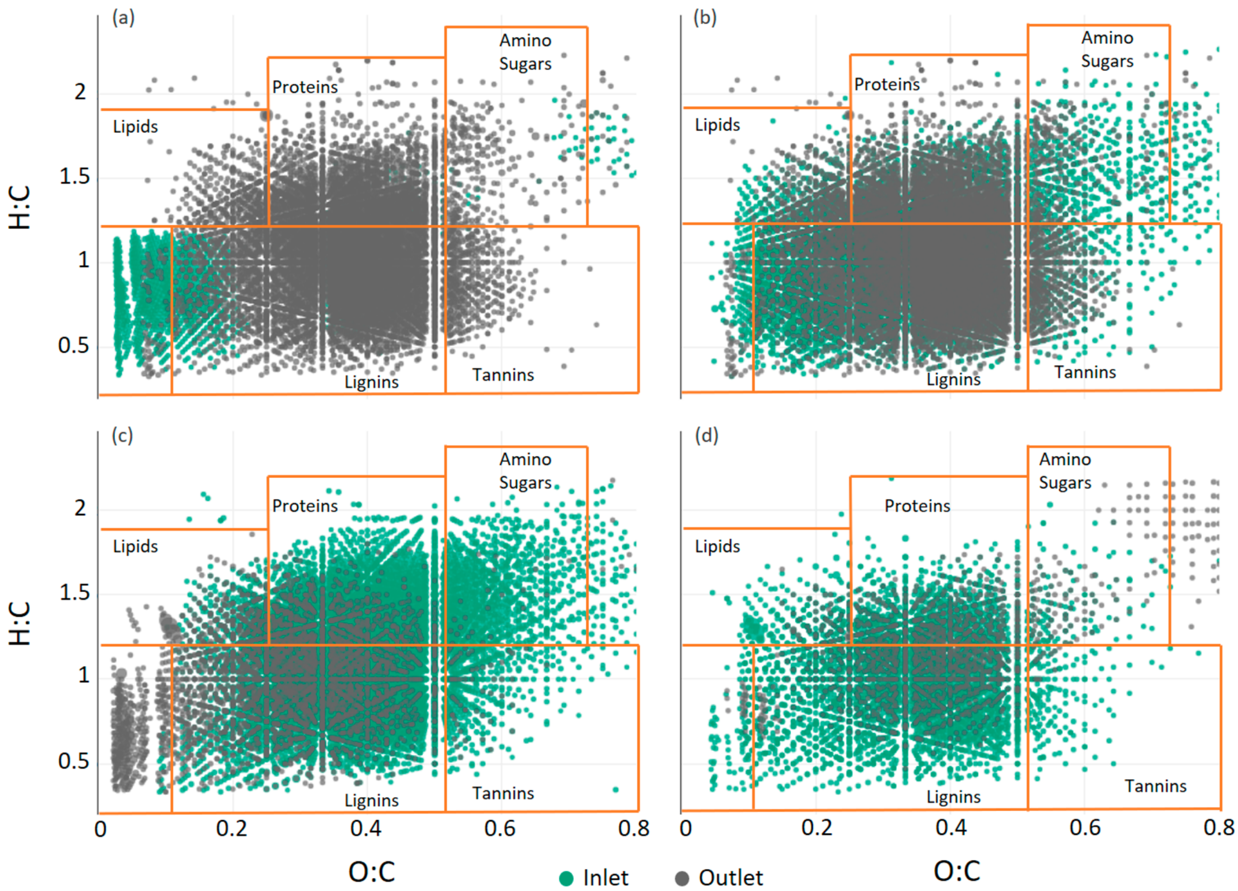

Figure 5. van Krevlen diagrams derived from negative-ion electrospray ionization FT-ICR mass spectral analysis for all N-bearing formulas in the mass spectra of the inlet and outlet with the low TN inlet for BAM with (a) no carbon addition (LSN), (b) carbon addition (LSC), and the high TN inlet with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

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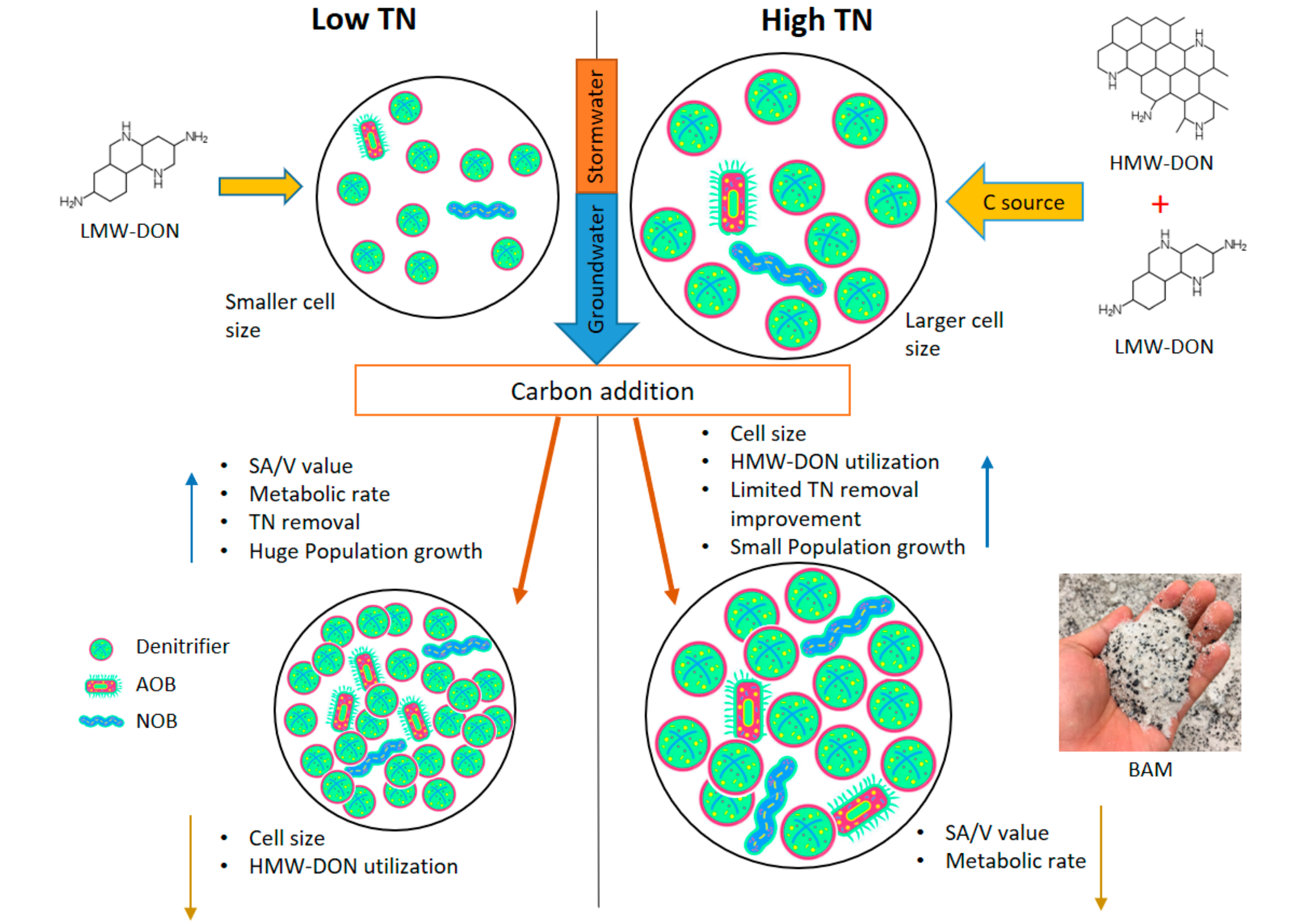

Figure 6. Schematic diagram of microbial community development (with different cell size) under different TN concentrations with carbon impacts (LMW, low molecular weight; HMW, high molecular weight)

layer has no significant change of NOB after carbon addition, which means it had already reached its optimal community structure, but the 30 cm in depth shows a significant NOB percentage increase because the community had not reached its maximum capability and the additional carbon and other nutrients can push the community toward an even better community structure.

The different microbial development in columns 1 and 2 also reflects the effectiveness of nitrogen removal under the impact of carbon addition given that the carbon source is important for denitrifiers in nutrient-poor conditions regardless of whether the treatment is for stormwater (COD = ~15−20 mg/L) or groundwater treatment because the carbon source in groundwater is much lower (COD = 4−6 mg/L; Figures 2 and 3). For both columns, the top layer is the most active section for nutrient removal since the majority of the bacterial population exists there rather than in the lower sections.

4.2. Carbon Impact on DON Concentration/Composition. Experimental studies and literature reviews have confirmed the general allometric pattern of an inverse relationship between cell size and biomass-specific metabolic rates.[54−57] This pattern profoundly influences DON concentration and composition before and after treatment. The bacteria with a smaller size in column 1 prefer to use low molecular weight DON (LMW-DON) and release the metabolic products faster because their higher metabolic rate cannot effectively utilize high molecular weight DON (HMW-DON) with the limited shorter reaction time within small-size bacteria. Thus, some of the DON classes are harder to remove with column 1 (Figure 4), and column 2 seems able to remove more HMW-DON than column 1 (Figure 7). In column 1, the improvement of the total DON removal increased from 27% to 42% after carbon addition, a result that is probably driven by

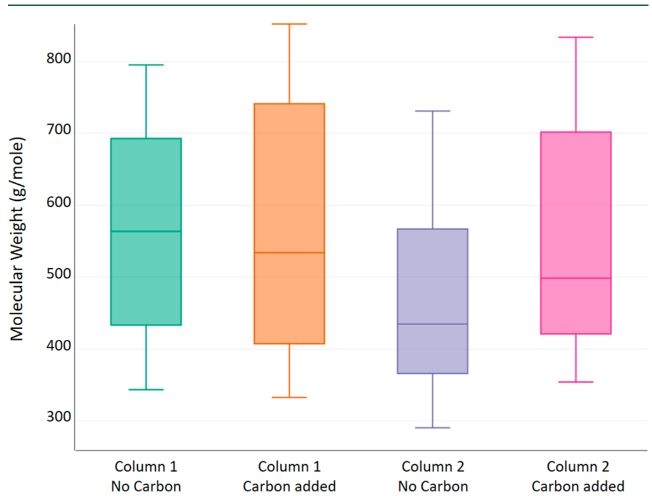

Figure 7. Comparison of outlet DON molecular weight for columns 1 and 2 with and without carbon addition.

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the cell synthesis and higher DON consumption with the population growth (Figure 2), but the effluent DON composition is quite similar before and after carbon addition (Figure 5), as some of the DON classes can hardly be removed (Figure 4). Conversely, the nutrient-rich environment in column 2 triggers a longer reproduction period because of the slower metabolic rate as DON provides a carbon source to fuel respiration, as demonstrated by Jones et al.[51] This outcome stimulates the bacteria in column 2 to absorb and accumulate more DON components within their cells (Figure 4 and Figure 5), resulting in larger cell size and slow release of metabolites back into the water that shows lower DON species/concentrations from the effluent (Figure 5) and promotes better DON removal performance for both DON concentration and composition.

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

Table 5. Comparison of DON Classes before and after Treatment

	low TN inlet		high TN inlet	
	no carbon	carbon added	no carbon	carbon added
overlapped DON classes	66%	59%	46%	35%
new DON classes after treatment	4%	25%	28%	33%

As shown in Table 5, after carbon addition, the relative abundance of new DON classes changed from 4% to 25% in low TN cases and from 28% to 33% in high TN cases, respectively. This could indicate that more carbon sources would increase the new DON classes as the microbial community with larger average cell size would produce more new DON classes due to consuming/converting more HMW-DON (evidenced by the amino corner of Figure 5d). However, for most of the new DON compounds, the concentration is normally low, as shown in Figure 4 directly and Figure 5 indirectly as evidenced by the density of those smaller dots. This observation is consistent with the microbial ecology assessment from the previous statement in the sense that more abundant bacteria with larger sized cells are present that can potentially uptake more DON classes but can also generate more DON classes as microbial community exudates with such enhanced activity. Moreover, this microbial conversion process of DON is also noticed as a potential pathway to enhance the biodegradability of the initial DON from the inlet due to lower molecular weight from the outlet (Figure 7).[37]

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

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

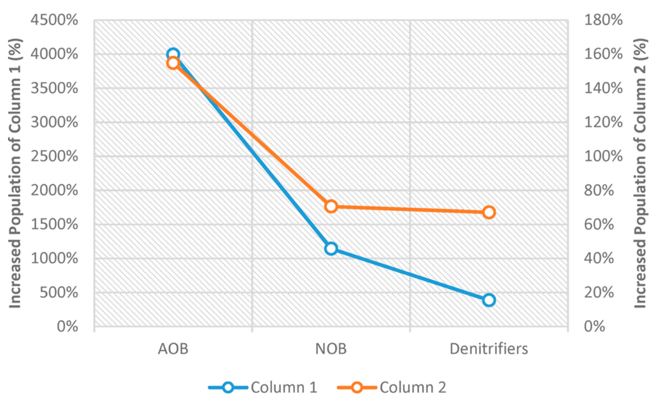

Figure 8. Population ratio of carbon added case to noncarbon case for AOB, NOB, and denitrifiers at the top layer

4.3. Final Remarks. The carbon addition impact on nitrogen removal for the cotreatment of groundwater and stormwater has been systematically evaluated in this study, in which the DON concentration/composition changes based on DOM for stormwater treatment were further realized with the help of FT-ICR-MS and qPCR together. The impact of carbon source is different for stormwater and groundwater; carbon is more important to TN removal in groundwater than in

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stormwater, as groundwater contains much less carbon in the first place, but carbon addition in this experiment did increase the ammonia concentration through the enhancement of ammonification for both stormwater and groundwater treatment. Nitrogen source variability resulted in different bacteria community development, in which low inlet TN cases tend to cultivate bacteria with smaller cell size while the high inlet TN cases end up favoring larger cell size bacteria that are quite different in terms of metabolic rate and population growth patterns. Carbon works as the essential component for cell reproduction under the nutrient-poor environment, but DON can be utilized as a carbon source for bacterial respiration in the nutrient-rich environment. The DON utilization can be enhanced with additional carbon, but more DON classes would be generated because of the improved microbiological activities with a cascade effect over different microbial species from AOB to NOB to denitrifiers. This difference provides the foundation for understanding the different scale of SA/V that results in very different microbial structural functionalities since the smaller cell size bacteria tend to reproduce faster with a higher metabolic rate and maintain a larger SA/V value that is beneficial for absorbing nutrients more effectively. Therefore, LMW-DON is preferable for smaller cell size bacteria while more HMW-DON can be utilized by larger cell size bacteria. The most abundant bacteria exist at the top layer with denitrifiers as the dominant species, and the additional carbon has much less of an influence at the depth of 60 cm. For real-world applications, we suggest that the depth of BAM should be less than or equal to 60 cm (2 feet).

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

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