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Effect of alternate dry-wet patterns on the performance of bioretention units for nitrogen removal

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ABSTRACT

The removal rate of NO_3^--N is a key indicator for the performance evaluation of nitrogen removal in bioretention units. At present, most studies show that setting the submerged area and adding carbon (C) source can improve denitrification effect and removal rate of NO_3^--N in bioretention units. However, experiments show that the dissimilatory nitrate reduction to ammonium (DNRA) has a significant impact on the N cycle and C/N ratio determines the reduction pathway of NO_3^--N. Based on earlier findings, current work aimed to investigate the DNRA process in bioretention system by alternate wetting and drying operation mode, considering Total Nitrogen, NO_3^--N and NH_4^+-N as assessment indices. Results show that after a prolonged drought period, bioretention unit was able to remove NO_3^--N mainly through DNRA with addition of C source. NH_4^+-N accumulation occurs in dry period and the removal rate of NH_4^+-N was lower than that of with C source. This phenomenon indicates that carbon is the main factor to determine the reduction pathway of NH_4^+-N. Results of the microbial sequencing analysis revealed the presence of six common DNRA bacteria in bioretention: *Pseudomonas* (Pseudomonas), *Bacillus* (Bacillus), *Thiobacillus* (Thiobacillus), *E. coli* (Escherichia), phosphorus *Vibrio* (Desulfovibrio) and *Desulfuvibibrio*, which proves the existence of DNRA process in bioretention units.

Keywords: Bioretention; DNRA; Nitrogen removal; Carbon source; Stormwater

1. Introduction

Fresh water resources, for example, ground water, lakes and rivers, are exposed to higher nitrogen and other pollutant inputs as a result of excessive use of organic chemical fertilizer usage in agriculture. This phenomenon results in the risk of eutrophication and public health concerns resulting from nitrate and nitrite exposure [1]. Previous studies reveal that elevated levels of nutrients in urban runoff are detrimental to urban waterways and bays [2,3]. Total Nitrogen (TN) in stormwater may contain up to 91% dissolved nitrogen, often dominated by NO_3-N (up to 47%) which is highly harmful to aquatic ecosystems [3]. In order to find the solution to this problem, bioretention plays an important role in nitrogen remediation from nonpoint source pollution in the same way as riparian zones [4,5].

Bioretention techniques, also known as biofiltration systems, are among the promising technologies that are capable to remove nutrients. Precipitation, filtration, adsorption and plant uptake are among the key processes in bioretention [2–6]. Henderson et al. [7] noted that vegetated biofiltration systems demonstrated higher TN removal by vegetated mesocosms in sand and loam than in unvegetated biofilter [7]. Earlier studies reveal that bioretention systems are efficient for NH_4^+-N removal but less efficient for NO_3^--N and NO_2^--N and suggested to enhance anaerobic processes (i.e., denitrification) in biofiltration systems in order to increase the nitrogen removal [8–10]. It has also been reported by researchers that creation of a submerged anoxic zone (SAZ) (that contains C source) at the bottom of biofilter could substantially improve NO_x removal [10,11]. In the biofiltration systems, the alternate wetting and drying condition and the leakage of O_2 from plant roots provide favorable environments, where many N transformation processes, for example, nitrification, denitrification,

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dissimilatory nitrate reduction to ammonium (DNRA), as well as assimilation and mineralization, can occur simultaneously [12–14]. In the DNRA process, reduction of NO_3^--N to NH_4^+-N can decrease the concentration of NO_3^--N, and concomitantly increase NH_4^+-N concentrations, which is significant to the availability and conservation of N in ecosystems [15–17]. It is also reported that NH_4^+-N is immobilized in the soil until nitrification, and plant uptake occurs. Conditions favoring DNRA are less understood than denitrification, although it can be a significant or even a dominant process in some ecosystems [15,16].

The potential importance of DNRA as an ideal NO_3^--N removal pathway in various ecosystems has been discussed by many researchers [17–19]. However, there is a lack of proper information on the specific gross N transformation rates (e.g., DNRA, nitrification, denitrification) occurring simultaneously rewetting/drying periods and estimation of the functional role of DNRA compared with other N transformation in the wet conditions. Earlier studies reveal that nutrients release from sediments upon rewetting because the prolonged sediment drying period promotes the release of significant amounts of bio-available N upon re-wetting conditions. It is believed, however, that heterotrophic denitrification supplies more free energy than DNRA [19–22]. Kraft et al. [16] noted that under nitrate limiting conditions, DNRA process could be favored because more electrons can be transferred per mole of nitrate. The role of microbial species in DNRA process and nitrogen removal in various treatment systems has been reported in earlier studies [17,19,22–26]. Significant loss of the sediment bacteria population upon desiccation has been reported by Baldwin et al. [19], and therefore, it may be hypothesized that the similar trend could happen in biofiltration systems, responsible for significant decrease in their pollutant removal performance during storm events that occur after prolonged drying periods. They have also suggested that this trend may even result in leaching of nitrogen from the biofilter [19].

Hence, understanding of N transformation and removal process in alternative drying and wetting conditions is of high importance. Considering the importance of DNRA and nitrate transformation process, and C/N ratio, the objectives of the current research work were as follows: (1) explore the impact of alternate wetting and drying pattern on nitrogen removal especially NH_4^+-N in bioretention units; (2) analyze the DNRA process in bioretention units; and (3) investigate the microbial sequencing through high-throughput sequencing (Miseq process) analysis and identify the microbial species capable of DNRA process in bioretention units.

2. Material and methods

2.1. Experimental device

Three experimental bioretention units were designed and constructed in the current work as shown in Fig. 1. These experimental units were created using 400-mm-diameter UPVC material columns and designed to control the flow rate. A synthetic transparent glass port was placed at 300 mm below the top of the column, with a size of 300 mm × 50 mm (length × width) in order to investigate the stuffing surface and permeation rate. Three experimental units installed in the laboratory were marked as units C1 (submerged and with C source), C2 (submerged and without C source) and C3 (unsubmerged). We have used old newspapers as an extra source of C in the current work. Water level in the experimental units was controlled through a riser pipe placed at the bottom of the each unit. SAZ was created by the switching valves set at the experimental unit walls. The thickness of the column wall was 10 mm, and the bottom of each unit was welded carefully to ensure no water leakage.

2.2. Filling material

The main consideration in the selection of filter media was to choose the locally available cost-effective materials that have significant pollutant removal capacity as well as good for plant growth. Other factors such as stability of the filter material to withstand stormwater runoff without substantial migration of sediment have also been considered in material selection process. As shown in Figs. 1(a) and 1(b), filling material configuration consists of mainly two layers: soil and sand layer. A transition layer was set below these

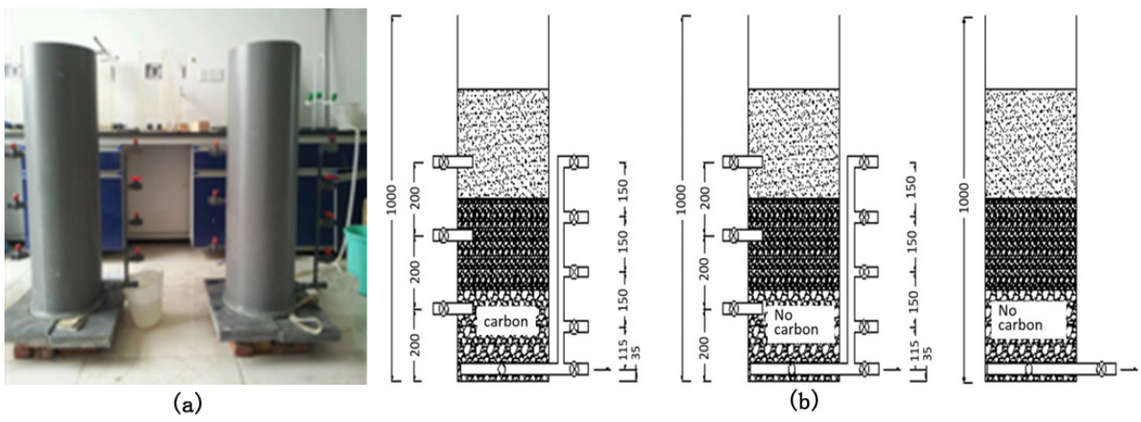

Fig. 1. Biofiltration column: (a) laboratory equipment and (b) schematic design.

Table 1
Physical properties of substrate filling materials

Media	Sandy soil	Fine sand	Coarse sand	Rock stone	Carpolite	Pebble
Size (mm)	1–3	0.4–0.6	1–2	2–4	4–8	8–32

Table 2
Substrate filling materials

Device No.	C1 (with SAZ* and C)	C2 (with SAZ; without C)	C3 (without SAZ)
Thickness	80 cm	80 cm	80 cm
Filling materials	Sandy soils (2 cm)	Sandy soils (2 cm)	Sandy soils (2 cm)
	Fine sand (26 cm)	Fine sand (26 cm)	Fine sand (26 cm)
	Coarse sand (26 cm)	Coarse sand (26 cm)	Coarse sand (26 cm)
	Rock stone (6 cm)	Rock stone (6 cm)	Rock stone (6 cm)
	Carpolite (6 cm)	Carpolite (6 cm)	Carpolite (6 cm)
	Pebble (14 cm)	Pebble (14 cm)	Pebble (14 cm)

layers to prevent the upper flushing. Filtration effect of filling materials is particularly important for reducing peak flow and removing contaminants. Filtration rate has been controlled between 45 and 120 mm h^{-1} for effective filtration and pollutant removal. Physical characteristics of filling material are presented in Tables 1 and 2.

2.3. Experimental procedure

2.3.1. Simulation of semi-synthetic stormwater

All the experiments in current work were conducted with semi-synthetic stormwater, since it is hard to collect and store the real stormwater without significant change to its quality. Semi-synthetic water was prepared with mixing sediments from a stromwater storage tank with tap water, and topping up sediments to reach typical stormwater concentrations (Table 3). It is also important to note that semi-synthetic stormwater with its sediment from the natural rainwater pond can well simulate the natural rain, to provide nutrients and heavy metal contents. Meanwhile, semi-synthetic rainwater with laboratory-scale chemical reagent was mixed with tap water to simulate the stormwater water properties. Table 3 shows the semi-synthetic water quality characteristics.

2.3.2. Denitrification impact of alternate dry-wet conditions

Semi-synthetic stormwater experiments were conducted for experimental units using semi-synthetic stormwater during the wetting period. During the wetting period, semi-synthetic water was added into the column once every 2 d, while there was no water inlet into the unit during the drying period. Drying period was designed to be 1 week, 2 weeks, 3 weeks and 7 weeks, respectively, in order to explore the desired wet-period length of units to recover the initial processing capability (Table 4). Water samples were collected from the experimental units prior to entering the dry period and after entering wet period to analyze pollutants in order to study the removal rate of pollutants.

Table 3
Concentration and source of water distribution

Index	Concentration (mg L^{-1})	Quantity (mg L^{-1})	Source
SS	300		Sediment
COD	200	187.5	Glucose
TN	10		N additive
ON	4	3.52	$C_6H_5O_2N$
NH_4^+-N	2	7.65	NH_4Cl
NO_3-N	4	24.28	KNO_3
FRP	0.3	1.32	KH_2PO_4
TP	1		Sediment and FRP

Table 4
Alternate wetting and drying pattern in bioretention units

C1	C2	C3
Wet		
Dry 1 week		
Wet 3 times	Wet 3 times	Wet 3 times
Dry 2 weeks		
Wet 10 times	Wet 10 times	Wet 10 times
Dry 3 weeks		
Wet 10 times	Wet 10 times	Wet 10 times
Dry 7 weeks		
Wet 10 times	Wet 10 times	Wet 10 times

At the first stage of the experiment, semi-synthetic stormwater was filled into experimental units C1 and C2 up to the height of 450 mm. In order to ensure the column operation under saturation state, the columns were filled with 36 L stormwater through inlet and kept for 5 h. Water head pressure was controlled by peristaltic pump. For the normal operation of column experiments, devices were dozed with semi-synthetic water for 2 to 3 times per week,

for 3 consecutive weeks. The water quality parameters/time changes of submerged area were investigated when the outlet water was stable. Sampling and chemical analysis of various N forms were conducted according to the standard methods described in American Public Health Association (APHA) manual [27].

2.4. Microbial analysis

Extracted DNA genome examination was carried out in the first step of microbial analysis. Agarose gel (1%) electrophoresis was used to examine the extracted DNA genome, and "5′ barcode-primer-barcode 3′" specific primers were compounded according to appointed order-checking range [24]. For accuracy and reliability of subsequent data analysis, two points were considered: first, tried to expand with low cycle data; second, made sure that the cycle data of every sample were identical. Representative samples were selected randomly to do pre-experiment in order to expand suitable product with lowest cycle data. Samples were tested in triplicate with formal experimental conditions, and rare field and polymerase chain reaction (PCR) from the same sample were tested with 2% agarose gel electrophoresis. Qubit method was used for the quantitative test of PCR products according to previous electrophoresis result, and then, mixed the samples with approximate proportion prior to Miseq order-checking (Fig. 2) [24].

The effect of TN removal was the high when experimental units with inundated areas and C source were running continuously in the wetting period. Therefore, objective of the Miseq test was to investigate experimental unit C1 with inundated areas and C source. Since the height of inundated area was 450 mm, the long-time running of biofiltration units forms an upper aerobic and lower hypoxia environment. In this case, microorganism and other dominant bacterial species in the upper and lower packing will be different. Samples were collected in duplicate from unit C1 for microbiological analysis to explore the mechanism of biological actions in biofiltration systems. Microbes on sand and gravel are hard to extract; therefore, it is necessary to prepare adequate filler (at least 2.5 Kg) for the extraction of DNA in the sequencing process. In addition, filling materials with particle size <4 mm were selected for microbiological analysis as big particle size cannot be used easily for Miseq test and pebbles filled in the lower filler area. The sampling of microbes from testing units was conducted at the end of the experiments because it is hard to get the filler samples due to the structure of column.

3. Results and discussion

3.1. Impact of different alternate dry-wet patterns on N removal performance

The impact of different alternate dry-wet patterns on the TN removal performance of bioretention units is presented in Figs. 3(a)–(c). Results reveal that after the drying period of 1 week, 2 weeks, 3 weeks and 7 weeks, the removal rate of TN by the bioretention unit of any structure has a different degree of decline trend (Figs. 3(a)–(c)). The bioretention unit without submerged area had the lowest removal rate on TN in dried period, but had no significant effect after the 3-week dry period. It is also clear from the results that in the early recovery

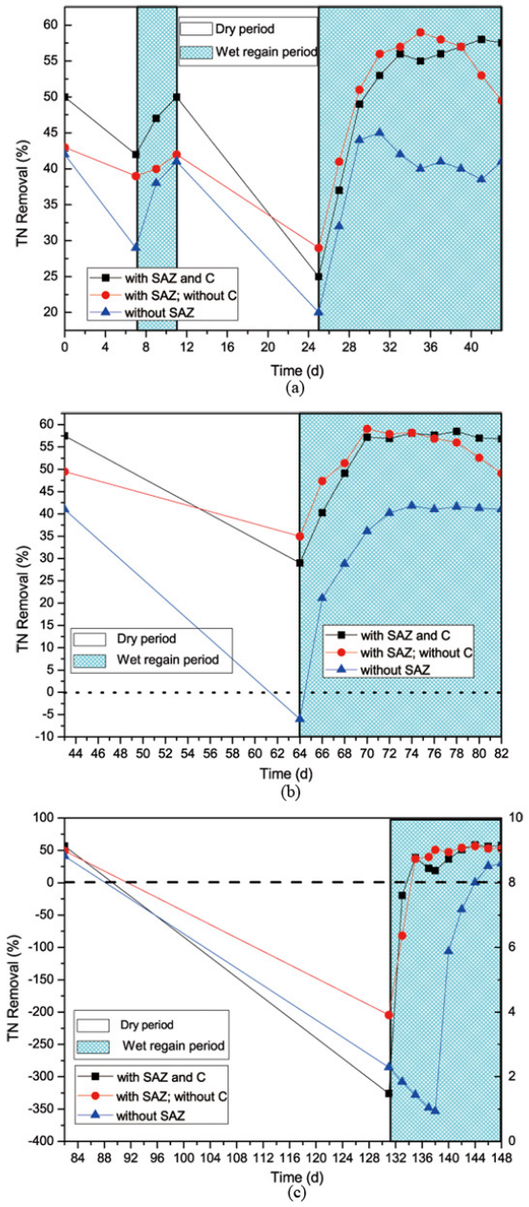

Fig. 3(a)–(c). Effect of alternate drying and wetting (1–2-, 3- and 7-week dry period) on removal of TN.

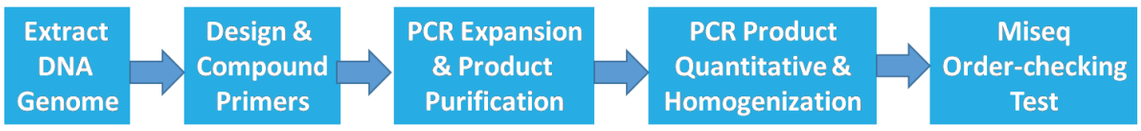

Fig. 2. Miseq test procedure for microbial analysis.

of humidity, the experiment unit C2 with submerged area without addition of C source showed better TN removal effect than unit C1. The result shows that the extra C source in early period of moist condition was not a restrictive factor for N removal, and the bioretention unit with submerged area after rewetting had a quick recovery in performance of N removal compared with nonsubmerged area.

The impact of different alternate dry-wet patterns on the NH_4^+-N removal performance of bioretention units is presented in Figs. 4(a)–(c). After the drying period, units C1 and C2 with submerged area had much larger removal rate of NH_4^+-N than that of the experimental unit C3 without submerged area. The removal rate of NH_4^+-N in the experimental units C1 and C2 was reduced to –196% and –135%, respectively, after 7-week drying period, whereas experimental unit C3 with no submerged area dropped from 87.6% to 37.7% (Fig. 4(c)). Results show that first ammonia leakage was appeared in experimental units with submerged area after 7-week drying period. Results also reveal that bioretention unit with submerged area was not conducive to the removal of NH_4^+-N.

Figs. 5(a)–(c) show the impact of different alternate dry-wet patterns on the NO_3^--N removal performance of bioretention units. After 1 week and 2 weeks of drying

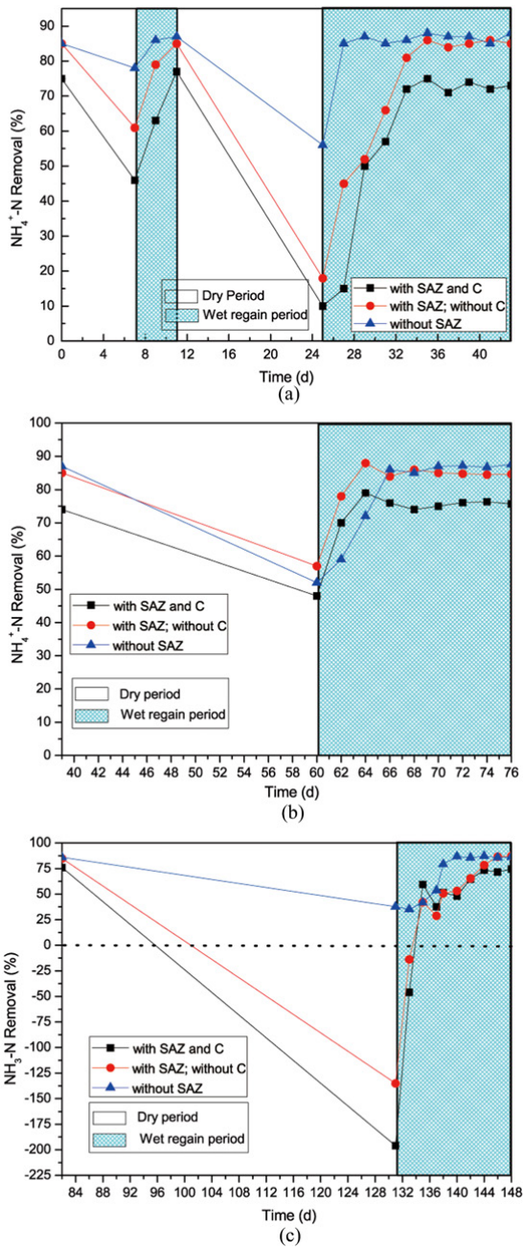

Fig. 4(a)–(c). Effect of alternate drying and wetting (1–2-, 3- and 7-week dry period) on removal of NH_4^+-N.

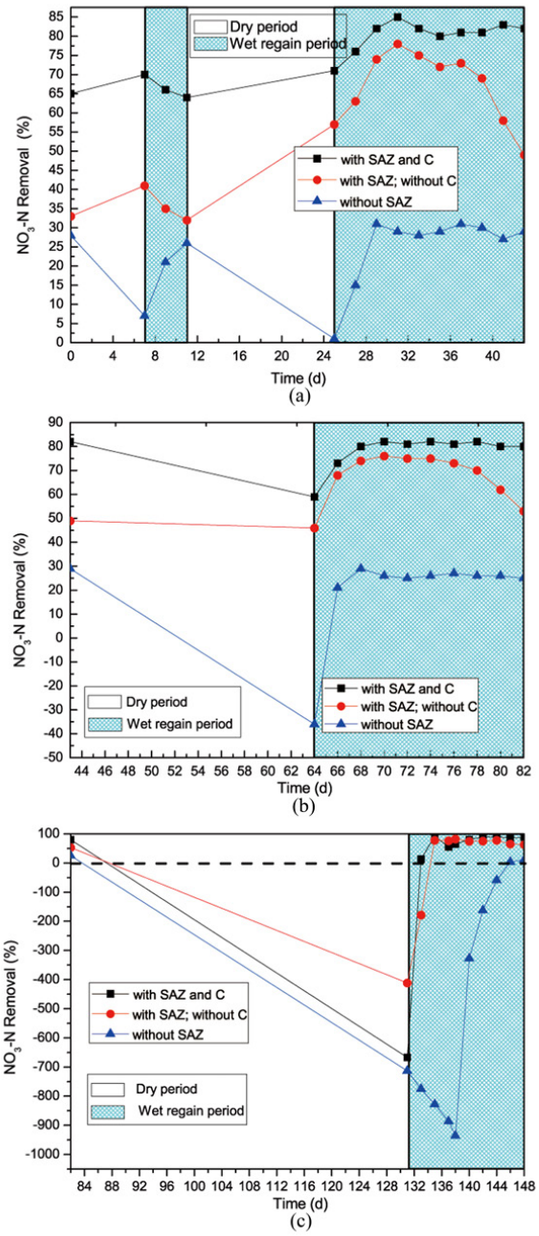

Fig. 5(a)–(c). Effect of alternate drying and wetting (1–2-, 3- and 7-week dry period) on removal of NO_3^--N.

period, the removal rate of NO_3^--N in the experimental units C1 and C2 with submerged area was elevated, whereas decreased in the experimental unit C3 (Fig. 5(a)). After the dry period of 3 weeks and 7 weeks, the removal effect of NO_3^--N on the experimental column C1, C2 and C3 was decreased Figs. 5 (a–c). However, the removal effect of NO_3^--N on the bioretention unit with submerged area recovered rapidly after the wetting period, and bioretention unit with the submerged area was not easy to recover after 7 weeks of drying period.

Bioretention unit experiencing drought shows remarkable decline in TN removal, because drought may result in the death of microorganisms. This trend is consistant with earlier finding [11–13]. Earlier findings reveal that organic matter released by dead microorganisms fixes the nitrogen compounds, resulting increase in TN concentration [11]. Current results show that with prolonged drying period, the TN removal rate decreased very fast (Fig. 3(c)). However, the bioretention units with submerged area have stronger resistance to drought than those without submerged area. The bioretention unit without submerged area after 3 weeks of drying period shows negative removal rate of TN, and the removal rate of TN on bioretention units C1 and C2 with submerged area was above 30%. TN removal performance recovered quickly after a longer drying period in the bioretention units with submerged area. When the drying period was less than three weeks, the nitrogen removal effect of the bioretention unit C3 without submerged area was reduced due to the drought period, but removal rate of TN rapidly improved after restoring water. After 7 weeks of drying period, the bioretention units with submerged area showed rapid recovery in TN removal efficiency, but removal rate of TN in ordinary bioretention unit C3 continued to decline after 5 times restoring water. After 3 weeks of wetting period, removal rate of TN recovered only to 29.4% and has not yet reached the removal effect before the drying period. Therefore, advantage of the bioretention unit with submerged area varied in different drying periods. Similar results have been observed by Baldwin et al. and Zinger et al. [11,19].

Compared to the effect of drying period on the removal rate of NO_3^--N in 1 week, 2 weeks, 3 weeks and 7 weeks (Figs. 5(a)–(c)), it is evident that the removal of TN in the bioretention unit with submerged area was higher than that of without submerged area (Figs. 3(a)–(c)). This trend occurred because it is possible that the denitrification and DNRA in bioretention units with the submerged area were more obvious than that of without submerged area, which reduces NO_3^--N to N_2 and NH_4^+-N [11]. The DNRA process occurs in mostly higher hypoxic environment with available carbon sources, and the studies have proved that the amount of carbon source is the key factor of DNRA, and when the C/N was ≥4, then NO_3^--N mainly reduced to NH_4^+-N [26]. Therefore, bioretention unit with the submerged area and carbon source has the highest NO_3^--N removal rate when the drought period was less than 3 weeks and the concentration of NH_4^+-N was the highest. This phenomenon is in consistence with earlier findings [11–14,18]. It also explains that denitrification was not the reduction pathway of NO_3^--N in the experimental unit C1, and DNRA process was most obvious in it where NO_3^--N reduced to NH_4^+-N. Zinger et al. [11] have reported that the removal rate of NH_4^+-N after 7 weeks of drying period was significantly higher in the bioretention unit with submerged area, but current results were inconsistent with it [11].

Bioretention unit with submerged area showed the phenomenon of NH_4^+-N leakage (i.e., effluent concentration greater than influent concentration) after 7 weeks of drying period, where concentration was dropped to –196% and –135% (Fig. 4(c)). Whereas, bioretention unit without the submerged area showed decline trend in NH_4^+-N removal (87.6%–37.7%) (Fig. 4(b)). This phenomenon also proves the existence of DNRA process, because the submerged areas and the carbon source are key factors to promote DNRA in the biological detention pool [11,16,18]. Results were in consistent with above-mentioned phenomenon as bioretention unit C3 without submerged area does not have the reaction conditions of DNRA. Therefore, the concentration of NH_4^+-N in C3 was lower than that of experiment units C1 and C2, which coincides with the experimental results. The dominant position of DNRA in experimental unit C1 showed that the water in bioretention unit with submerged area after drying period was stagnant and its quality in submerged area changes with time.

3.2. Submerged area with different time pattern

The influent concentration of TN, NH_4^+-N, NO_3^--N and COD is presented in Table 3. Whereas, the effluent concentration and removal performance of experimental units with submerged area are presented in Figs. 6(a)–(d). The submerged areas in the experimental units C1 and C2 were of 450 mm height, with and without C source. As shown in Figs. 6(a)–(d), the concentration of NO_3^--N decreases gradually with prolonged drying period, while the concentration of TN, COD and NH_4^+-N were increased. Effluent concentration and removal rates of TN in bioretention units C1 and C2 are presented in Fig. 6(a).

The submerged area of bioretention units has a prolonged hypoxic environment. Earlier findings revealed that is not easy to transform organic nitrogen in NH_4^+-N through ammonification in prolonged submerged conditions, so the concentration of NH_4^+-N at the bottom should not increase significantly, but the experimental results were inconsistent this phenomenon. According to the N cycling process in nature, the ammonification and DNRA can generate NH_4^+-N [11,13–16]. The ammonification generally occurs in the aerobic environment, and DNRA process is easy to occur in the available C source with anoxic environment relative to higher available nitrogen. Therefore, ammonification process in submerged area was not obvious, and the accumulation of NH_4^+-N in submerged areas occurs mainly by DNRA process. The DNRA process reduces NO_3^--N to NH_4^+-N, main cause for the decrease in NO_3^--N concentration and increase in NH_4^+-N concentration. It can be inferred that during the drying period, NO_3^--N might removed by denitrification and DNRA process. During the drying period, the DNRA process can be confirmed by comparing the concentrations of NH_4^+-N in the bioretention units with or without C source (Fig. 6(b)). NH_4^+-N concentration was consistently high in experimental units with carbon source, whereas the concentration of NO_3^--N was low. Dry condition increases the gap in NH_4^+-N concentration, indicates that the experimental column with carbon source produced more NH_4^+-N relatively

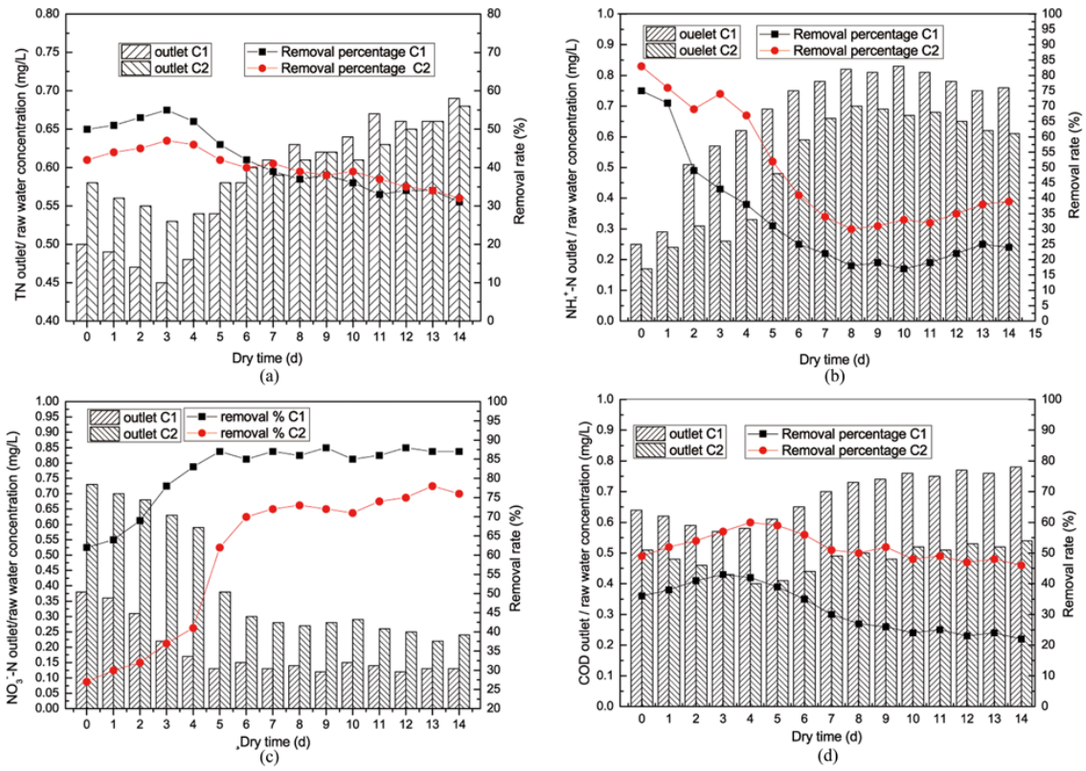

Fig. 6. Effluent concentration and removal rates of: (a) TN; (b) NH_4^+-N; (c) NO_3^--N; and (d) COD in bioretention units C1 and C2.

during the drying period. Addition of carbon source to the unit C1 promotes the reduction in NO_3^--N for NH_4^+-N reveal that C/N ratio are the key factors in DNRA process. Fig. 6(c) and 6(d) revealed that DNRA process was obvious in bioretention unit with the submerged area. Drought in bioretention units may cause the death of some microorganisms resulting in the increase of organic content and increase in the concentration of organic nitrogen and COD. Result shows that NO_3^--N in experimental unit with submerged area was mainly reduced to NH_4^+-N during the drought period, not by denitrification. Addition of organic nitrogen in the submerged area cannot remove TN effectively through ammonification during the dry period; therefore, the concentration of TN showed an increasing trend with the extension of the drying period. Similar results have been cited in the work of Zinger et al. [11].

3.3. Microbial analysis

Results obtained from Miseq test for the classification of species composition are presented in Fig. 7. Results show that microorganisms in the lower padding were mainly of Proteobacteria (24%), Actinobacteria (42%), Candidate divisionTM7 (23%) and Bacteroidetes (2%); microorganisms in the upper padding were mainly of Proteobacteria (42%), Actinobacteria (42%), Candidate divisionTM7 (4%), Acidobacteria, and Bacteroidetes (2%). As current work focus

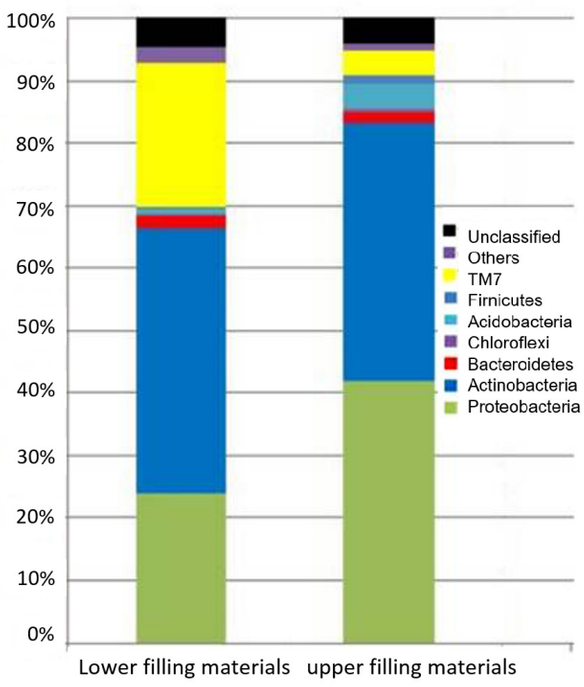

Fig. 7. Bacterial species in bioretention units through Miseq test.

on denitrification mechanism in biofiltration units through Miseq test, mainly on the reduction pathway of NO_3^--N, we concentrated on the denitrification bacteria and DNRA bacteria.

Earlier findings revealed that NrfA (nitrite reductase enzyme encoded by the nrfA operon) required as an enzyme to catalytically reduce NO_2^-–N to NH_4^+-N during DNRA process and both *Escherichia coli* [22] and *Desulfovibrio* [23] can generate NrfA. Smith et al. [24] also found NrfA from Proteobacteria and Bacteroidetes [24]. DNRA bacteria that have been investigated earlier can be categorized into four species: obligate anaerobe, facultative anaerobe, slightly aerobic bacteria, and aerobic bacteria [22–26]. The obligate anaerobe and facultative anaerobe bacterial species are presented in Table 5. It has been reported in previous investigation that there are some species of NrfA [24] in Proteobacteria and Bacteroidetes, and these dominant species in the biofiltration system can provide enzyme for the DNRA process. Based on the existing literature on the common anti nitrification bacteria and DNRA bacteria and the Miseq test, DNRA bacteria in biofiltration units with submerged area and C source were investigated in current study. Microbial species such as *Pseudomonas*, *Bacillus* and *Thiobacillus* (Table 6), can reduce NO_3^--N either by denitrification or by DNRA method, and C/N decides the reductive pathway of bacteria. The experimental results indicate that biofiltration unit with C source and submerged area has the negative effect on NH_4^+-N removal. This trend is similar to the earlier findings [11,17,25]. Therefore, extra C source was added to biofiltration units which help bacteria to reduce NO_3^--N into NH_4^+-N through DNRA process.

Table 5
Bacterial strains in DRNA process

Obligate anaerobe	Facultative anaerobe
Clostridium sp.	*Escherichia coli*
Veillonella alcalescens	*Citrobacter sp.*
Wolinella succinogenes	*Salmonella typhimurium*
Desulfovibrio gigas	*Klebsiella sp.*
Desulfovibrio sp.	*Enterobacter aerogenes*
Selenomonas ruminantium	*Serratia marcescens*
Aerobic bacteria	*Erwinia carotovora*
Pseudomonas sp.	*Photobacterium fischeri*
Neisseria subflava	*Vibrio sp.*
Bacillus sp.	*Slightly aerobic bacteria*
	Campylobacter sputorum

Table 6
Proportion of DNRA bacteria in biofiltration units

DNRA bacteria	Proportion (%)
Pseudomonas	3.28
Desulfovibrio	0.38
Bacillus	0.02
Escherichia	0.01
Desulfuvibibrio	0.009
Thiobacillus	1.46

DNRA process and high-flux sequencing evaluation result revealed the existence of DNRA bacteria in bioretention units. Therefore, it is obvious that NO_3^--N can reduced to N_2 through denitrification, which can further reduced to NH_4^+-N through DNRA process [24–26]. Hence, it can be concluded that DNRA has dominant effect in biofiltration units with additional carbon source. This conclusion draws a new idea for denitrification optimization in biofiltration.

4. Conclusions

Results reveal that NH_4^+-N leakage occurred after 7 weeks of alternate wetting and drying experiments (i.e., effluent concentration higher than influent concentration), and submerged area accumulates NH_4^+-N because of prolonged drying period. After a prolonged drying period, submerged area in hypoxia environment contains higher C and N source, which is favorable to the occurrence of DNRA process. Bioretention unit with submerged area shows an advantage of N removal performance in drying period, especially under more than 2 weeks drying period. Results reveal a negative TN removal trend (–6%) in 7-week drying period, while bioretention unit with submerged area had a certain removal effect (29%~35%) for TN. Removal rate of NO_3^--N reduced significantly in the bioretention unit without submerged area after a prolonged drying period. Removal rate of NO_3^--N was 36% in case of 3 weeks drying period, whereas negative change (–713%) observed after 7 weeks of drying period because denitrification and DNRA process in bioretention unit without submerged area may not be apparent. It is also evident from the results that NO_3^--N removal was higher in bioretention unit with C source, whereas NH_4^+-N removal was very low. This trend demonstrates that the DNRA process in bioretention can be stimulated by enough electron donors. The microbial examination result shows the presence of six kinds of DNRA bacteria species, viz. *Pseudomonas*, *Bacillus*, *Thiobacillus*, *E.coli* (*Escherichia coli*), dephosphorization Vibrio genus (*Desulfovibrio*) and *Desulfuvibibrio*, that fully proves the existence of DNRA process in bioretention. Current findings clearly suggest that the performance of bioretention units can be improved by addition of an extra C source, plants, saturated zone and varied microbial community. Further work on in-depth study on the role of microbes and quantification of loading, addition of C and denitrification is currently undertaken in order to improve the performance and design of bioretention systems.

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