Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.20938

96 (2017) 143–152
November

Effect of biochar on the migration and biodegradation of nitrogen during river-based groundwater recharge with reclaimed water: an indoor experimental study

Jiaji Sun[a], Yangyao Li[a], Zhupin Wang[b], Mengsi Ma[c], Weifang Ma[a,*]

[a]*College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China, emails: mpeggy@163.com (W. Ma), 602277877@qq.com (J. Sun), lyy18811371854@bjfu.edu.cn (Y. Li)*
[b]*Beijing Enterprise Water Group Limited, Beijing 100102, China, email: wzp@bewg.net.cn*
[c]*Graduate School of International Relationship, International University of Japan, Minamiuonuma 9497248, Japan, email: Catherinema917@163.com*

Received 25 January 2017; Accepted 14 May 2017

ABSTRACT

To investigate the effect of biochar on coupling migration–biodegradation of total nitrogen (TN) during river-based groundwater recharge with reclaimed water, lab-scale columns were used to simulate the recharging process. The packing medium of the three columns was mainly composed of biochar amended soil (BCS), silty clay (SC) and sterilized soil (SS). The results showed that the attenuation effect of TN was ordered as BCS > SC > SS, which showed that biochar could improve the decay rate. The migration trends of NH_4^+–N and NO_3^-–N were different because of the biotransformation among organic nitrogen, NH_4^+–N and NO_3^-–N. The main removal mechanism was sorption and biodegradation for NH_4^+–N, whereas it was denitrification for NO_3^-–N because the negative charge prevented sorption of NO_3^-–N into negatively charged aquifer soil. The key removal pathway was biodegradation in the recharge process, especially for the BCS system, because biochar amended improved the removal rate of nitrogen as well as denitrification enzyme activity. The 454 pyrosequencing 16s rRNA gene analysis showed that the ranking of the largest five relative abundances at the phylum level evidently changed and that the phylum related to denitrifying significantly increased with the biochar addition. Except for common and coexisting denitrifying bacteria, such as *Pseudomonas* and *Bacillus*, specific bacterial genera, such as *Caenimonas*, *Helicobacter*, *Halomonas*, *Lactobacillus* and *Flavisolibacter*, and the archaeal genus *Woesearchaeota_DHVEG-6* had previously been detected in the BCS system, which might be involved in nitrogen removal and should be studied in future research. Biochar has an intensification effect on denitrification by changing microbial living environmental factors, such as oxidation–reduction potential, pH and C/N, and providing a better biological attachment condition. These findings might provide a useful method to improve nitrogen biotransformation in groundwater recharge areas and could facilitate the prediction of different types of nitrogen in underground aquifers.

Keywords: Nitrogen migration and biodegradation; Biochar; Reclaimed water; Groundwater recharge; Microbial community and diversity; Indoor test

1. Introduction

In the face of water shortage and pollution, the reuse of reclaimed water has become a long-term and important strategy in the management of water resources in China. In 2016, nearly 716.2×10^8 tons of wastewater was treated and 85% was discharged into the river in China, and approximately 10×10^8 tons of wastewater was treated and 60% was recharged through river utilization in Beijing [1,2]. Many cities began to use recycled water to supplement rivers and lakes to alleviate the problem of drought. However, there were organic pollutants and

* Corresponding author.

Presented at the 9th International Conference on Challenges in Environmental Science & Engineering (CESE-2016), 6–10 November 2016, Kaohsiung, Taiwan.

inorganic nutrients, such as endocrine disrupting chemicals, nitrogen and phosphate, in reclaimed municipal wastewater, which could impact groundwater quality [3,4]. The total nitrogen (TN) concentration was between 15 and 20 mg L^{-1}, which was became effluent from the secondary wastewater treatment plant [5], and the concentration of nitrogen, even in the tertiary-treated reclaimed municipal wastewater, was also appreciable (approximately 5–15 mg L^{-1}). Therefore, the high content might cause nitrogen pollution, especially in the form of nitrate, which could be soluble in soil pore water and in artificially river-based recharge aquifers in this area and might pose a potential public health problem in the areas where drinking water comes from groundwater, such as northern China.

The transportation and transformation of nitrogen in the soil vadose zone is shown in Fig. 1, which indicated that ammonia absorption, nitrogen fixation, nitrification and denitrification occurred during the percolation process [6]. The main processes of nitrogen removal were denitrification and sorption. Among them, denitrification was mediated by denitrifying bacteria under anaerobic conditions [7]. However, recently, archaeal nitrification has been discovered [8]. Moreover, molecular biology technology and genome analyses provide new insights into the ecology of microorganisms responsible for the conventional denitrification process [9,10]. Thus, compared with the previous understanding, researchers are beginning to recognize that various microorganisms are involved in the nitrogen cycle.

Biochar could improve the utilization efficiency of nitrogen in agricultural production as an approach to alleviate nitrogen leaching [11–14]. Biochar is an organic material with a high carbon content, mostly an aromatic structure and a great stability resulting from high temperature thermal conversion of organic materials under complete or part anoxic conditions [15]. Because of its large surface area, negative surface charge and charge density, biochar has an excellent adsorption ability [16,17]. Some studies have revealed that adding biochar into the soil reduced the cumulative losses of NH_4^+–N because of the high adsorption capacity over the experimental period [18–20]. The physical and chemical properties of microbial colonization in the soil can be ameliorated by an attenuation of biochar and therefore affect soil microbial activity and community structure, which might be another mechanism for reducing N leaching because it could influence microorganisms that play an important role in the process of denitrification [21]. Therefore, to explore the variation of the microbial community after biochar amended and to study the effect of leaching into groundwater during the recharge process with reclaimed water is crucially important.

The purposes of this research were (i) to detect the difference of nitrogen migration and transformation during the artificial groundwater recharge process; and (ii) to investigate the effect of biochar on the bacterial community composition and diversity and nitrogen transformation. To achieve these objectives, a dynamic experiment was designed to simulate the recharge process with reclaimed water using a biochar amended soil (BCS) column, a silty clay (SC) column and a sterilized soil (SS) column. In the literature, it is noteworthy because only a few studies have focused on both the migration process and the shift of the microbial community structure throughout the whole denitrification process in continuous recharge column systems. The results of this study might reduce the risk of nitrate in reclaimed water leaching into the groundwater trough by providing practical information for guiding the exploitation of a novel amended.

2. Materials and methods

2.1. Raw water

Raw water was a tertiary effluent and was used for laboratory experiments from the YinWenJiChao Reclaimed Water Treatment Plant in Beijing whose coordinates are N 40.12°, E 116.50°. The processes of this treatment are primarily constituted by ozone preoxidation, a membrane bioreactor, chemical phosphorus removal, disinfection and a constructed wetland. The characteristics of the artificial recharge water are shown in Table 1.

2.2. Vadose zone soil samples and biochar materials

The filler of the recharge column was mainly composed of SC, which was collected from the southern section of the Chaobai River aquifer vadose zone in Beijing of the Huabei plain. The soil was air-dried before use and then uniformly distributed through a 2 mm nylon sieve and mixed to obtain a homogeneous soil sample [22]. The biochar was made from corn stalk using a furnace through pyrolysis under an N_2 environment at 400°C for 1.5 h. The biochar samples were milled and sieved through a 1 mm nylon sieve and then the 1% biochar was mixed with SC soil to make the BCS filler.

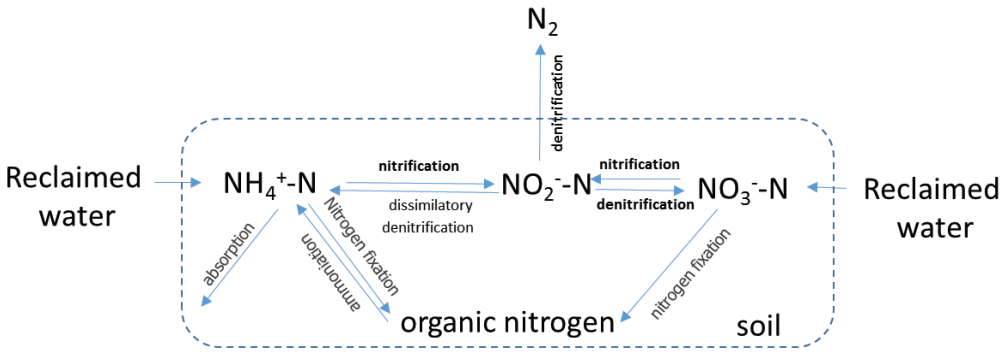

Fig. 1. A flow chart for nitrogen in the soil.

The physical and chemical properties of the tested soils and biochar are shown in Table 2.

The cation-exchange capacity (CEC) was determined by following the methods proposed by Chapman [23]. The total organic carbon (TOC) content was analyzed using a solid TOC analyzer (SCSH, TOC-VCPH, Japan) [24]. The Autosorb-iQ-C (Quantachrome, Boynton Beach, FL, USA) was utilized to determine the specific surface areas (SSAs). The elemental composition was determined by dry combustion using an EA3000 CHNS/O Analyzer (EuroVector, Italy).

2.3. Laboratory column replenishment systems' building and the dynamic experimental process of the nitrogen migration dynamic experiment

A schematic diagram of the laboratory scale is presented in Fig. 2. Three columns are contained in the recharge system. The filler media of the three columns were mainly composed of a BCS with 1% biochar, SC and sterile soil, which added 200 mg L^{-1} NaN_3 to inhibit the activities of the microorganisms (SS). The flow rate of each experiment that was operated in triplicates was approximately 0.5–0.7 m d^{-1}. The simulation recharge columns were designed in the same size: a diameter of 0.30 m and a height of 1.73 m with a water layer height of 0.5 m above the aquifer soil to simulate the water surface, a soil layer height of 1 m and a supporting layer height of 0.3 m. The whole system was covered with foil to avoid the light.

The temperature was 20°C ± 5°C in the room. Before starting the dynamic experiment, distilled water dissolved with 0.01 mol L^{-1} $CaCl_2$ was added in the water layers over a period of 60 d to create a homogeneously moist environment in the columns at the riverbed capacity. The water was sampled from each water sample port from day 3 and 7 and then sampled every 7 d according to the water quality changes. The total recharge period was 90 d. The information of the sample collection and nomenclature is shown in Table 3.

Table 1
The characteristics of the artificial recharge water (mg L^{-1})

NH_4^+–N	5.0
TN	25.0
NO_3^-–N	15.0
NO_2^-–N	1.5
Na^+	100.0
K^+	18.0
Ca_2^+	63.5
Mg_2^+	24.0
Cl^-	93.3
SO_4^{2-}	69.0
HCO_3^-	317.2

2.4. Sample analysis

The leachate water samples were stored in the dark at 4°C in the refrigerator. After centrifugation at 3,000 rpm for 3 min, a determination of the TN was conducted by alkaline potassium persulfate digestion ultraviolet spectrophotometry;

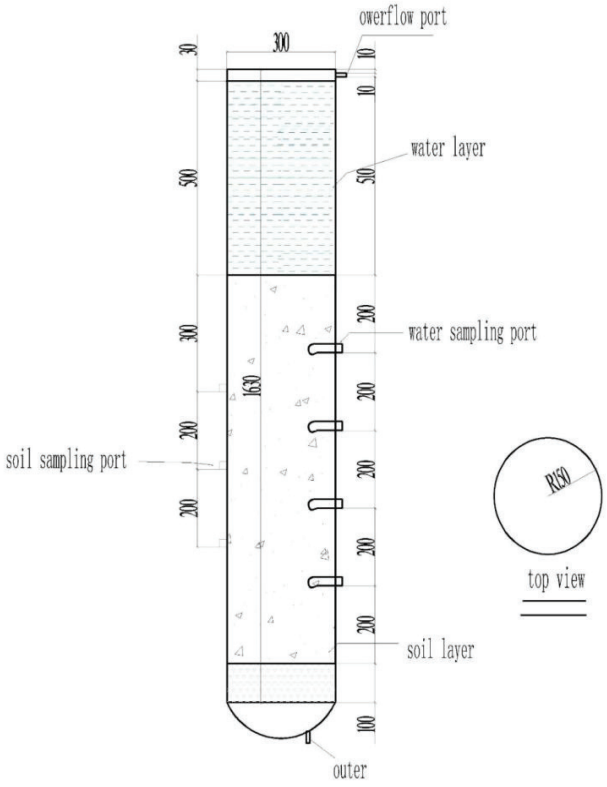

Fig. 2. Schematic diagram of the lab-scale column recharge system (mm).

Table 2
Basic physiochemical properties of the soil and biochar attended in the BCS

Soil		Sawdust biochar	
Total organic carbon (%)	0.86	Pyrolysis temperature (°C)	400
Total N (g kg^{-1})	0.54	pH	8.15
Ammonium N (mg kg^{-1})	3.50	C (%)	6.21
Nitrate N (mg kg^{-1})	1.72	H (%)	3.50
CEC (cmol kg^{-1})	8.30	O (%)	18.62
Clay (%)	32.38	Bulk density (g cm^{-3})	0.55
Bulk density (g cm^{-3})	2.10	Specific surface area (m_2 g^{-1})	380.00
Permeability coefficient (m d^{-1})	0.70	Water content (%)	5.90

Table 3
Soil sample collection and nomenclature information

Sample group	Sample nomenclature	Soil sample collection
Silty clay (SC)	SC1	30 cm depth of the silty clay column
	SC2	50 cm depth of the silty clay column
	SC3	70 cm depth of the silty clay column
Biochar amended soil (BCS)	BCS1	30 cm depth of the biochar amended soil column
	BCS2	50 cm depth of the biochar amended soil column
	BCS3	70 cm depth of the biochar amended soil column
Original soil (control)	Control	The original soil samples of the soil collection place

an ultraviolet spectrophotometry method was used for NO_3^-–N determination; and the Nessler's reagent spectrophotometry method was used for the NH_4^+–N test [25,26].

Measurements of denitrification enzyme activity, proposed by He et al. [27] and Peiffer et al. [28], have been widely used to evaluate the activities of existing denitrifying enzymes. A total of 1 g of soil was first placed in a 10-mL centrifuge tube, and then a 1 mL solution containing 10 g L^{-1} glucose and 10 g L^{-1} KNO_3 for 5 mL was added to each tube. The control sample was added with deionized water. Then, all of the samples were filled with deionized water to 10 mL. The samples were shaken at 37°C for 48 h and then the concentration of NO_3^-–N was measured. An analysis of the water quality and enzyme activity was performed in the Environmental Laboratory of Beijing Forestry University.

The bacteria and archaea community structure was analyzed using 454 pyrosequencing of the 16s rRNA genes. Soil samples came from the BCS and SC systems from depths of 30, 60 and 90 cm and the control, which was named BCS 1, 2 and 3, SC 1, 2 and 3 and the control, respectively, and the dry weight of each sample was 0.5 g. The DNA of the soil samples was extracted by Power Soil DNA extraction kits (OMEGA, CA, USA). The V4–V8 region's 16S rRNA gene was amplified through a broadly conserved primer set (515F/806R 50-GTGCCAGCMGCCGCGGTAA-30/ 50-GGACTACHVGGGTWTCTAAT-30). The primers of polymerase chain reaction (PCR) were established similar to the following: forward primer = 454 Titanium Lib-l Primer A/5-base barcode/forward 16S primer and reverse primer = 454 Titanium Lib-l Primer B/reverse 16S primer [28]. Each PCR was performed in triplicate using 30 μL reactions with 15 μL of the Phusion High-Fidelity PCR MasterMix (New England Biolabs, USA), 0.2 μmol L^{-1} of forward and reverse primers, and approximately 10 ng of the template DNA. The reactions were cycled with an initial denaturation at 98°C for 1 min, and followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, elongation at 72°C for 30 s and ultimately 72°C for 5 min.

Certified DNA-free PCR water was used in all dilutions. Pyrosequencing was performed at the Genome Center, Novogene, Beijing. A GeneJET Gel Extraction Kit (Thermo Scientific, USA) was used for mixing and the PCR products were purified and quantified on an Agilent Bioanalyzer 2100 system using an Agilent high sensitivity DNA assay. The QIIME software package was used for the analysis of the sequences [29], and both α- and β-diversity metrics were analyzed using in-house Perl scripts. In every soil sample the UniFrac distance metrics for comparing bacterial community diversity and structures by means of principal coordinate analysis and unweighted pair group method with arithmetic was used. Redundancy analysis (RDA) was selected and the significance of the total physicochemical factors was tested with the Monte Carlo permutations (permutations = 999). SPSS version 10.0 was used for data analysis and involved an analysis of variance (SPSS Inc., Chicago, IL, USA).

3. Results and discussions

3.1. Effect of biochar on spatial variation of nitrogen migration during the laboratory dynamic recharge process

3.1.1. TN migration in different depths of the three recharge systems

The concentration of TN in the leachate from different soil sampling ports showed high variance among different depths in the three recharging systems (Fig. 3). The concentration of TN gradually decreased with increasing depth, and the attenuation rates varied greatly in these three columns. The concentration of TN increased first and then decreased with recharge time in the SC and BCS system because the sorption capacity decreased with time and the microbial degradation ability gradually increased and stabilized. The TN content increased with increasing recharge time in the SS system because the NaN_3 inhibited the biodegradation effect. These results showed that biodegradation played an important role in the removal of TN. It is significant that biochar has a positive effect on nitrogen removal because biochar process has a large SSA and pore channels, which could enhance the adsorption capacity and provide a good living conditions for microbes. The concentration of TN in effluent was stable at 0.5 mg L^{-1} from every water sample port of the BCS column when the influent concentration was 25 mg L^{-1}. However, it gradually reached the saturated state at 4 mg L^{-1} on average in the SC system. TN includes ammonia nitrogen, nitrate nitrogen, nitrite nitrogen and organic nitrogen [26]. Therefore, the transformation between the different forms of nitrogen needs to be studied in detail.

3.1.2. NH_4^+–N migration at different depths in the three recharge systems

The variation of NH_4^+–N concentrations in the leachate during the recharge experiment is shown in Fig. 4. Compared

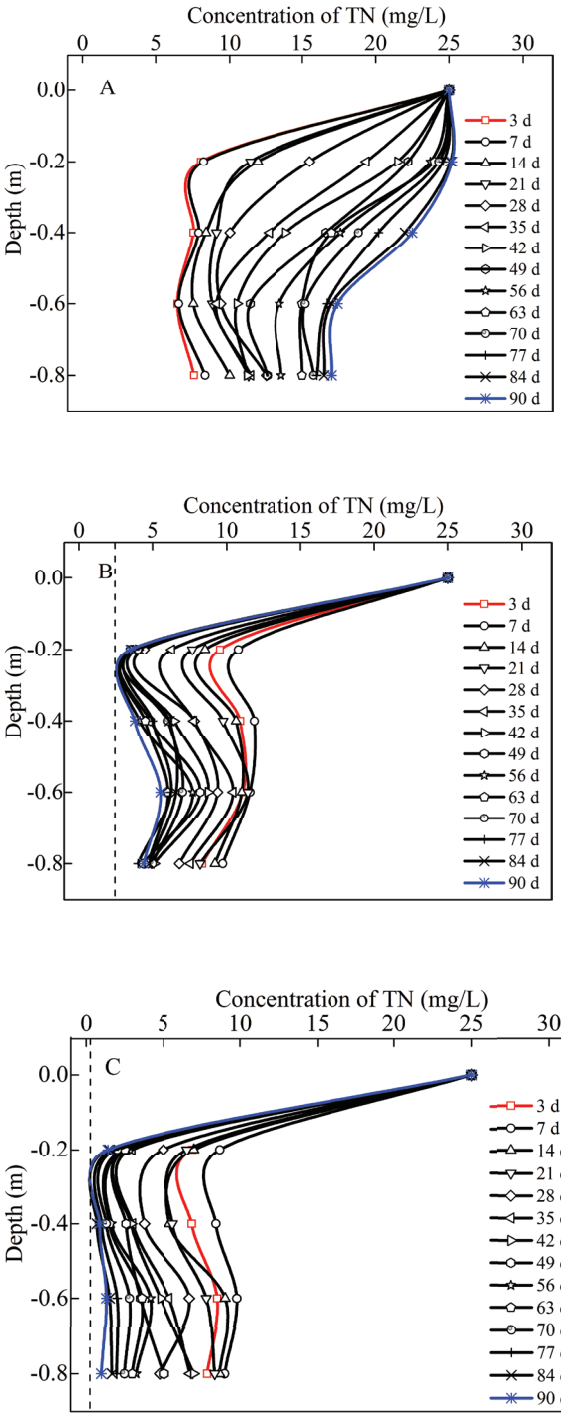

Fig. 3. Distribution of TN over time at different depths in three recharging systems: (A) SS, (B) SC, and (C) BCS.

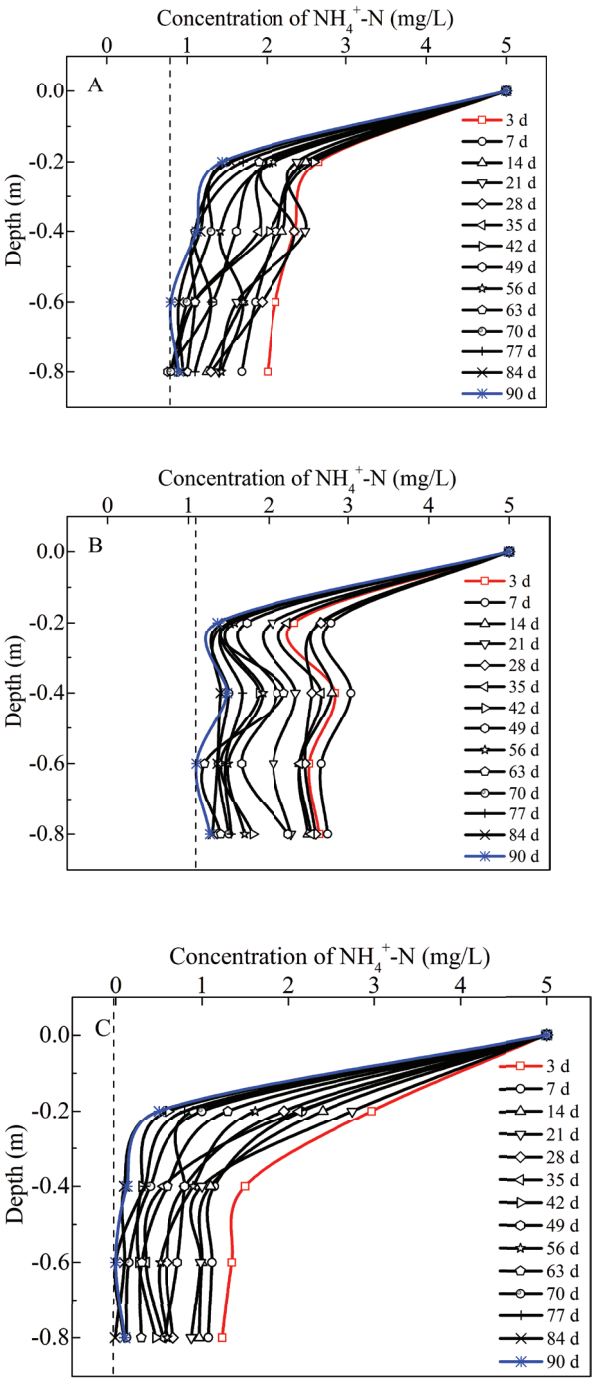

Fig. 4. Distribution of NH_4^+–N over time at different depths in three recharging systems.

with the BCS system, the NH_4^+–N concentration was higher in the SC system, which showed that biochar could reduce the chance of NH_4^+–N migration into groundwater because the biochar possesses higher affinity force and competition sorption sites than SC. The NH_4^+–N concentration in the SC system increased first and then decreased with the increasing recharge time because of the conversion from organic nitrogen to ammonia nitrogen under the activity of microorganisms.

The NH_4^+–N concentration in the BCS system decreased in the whole process because of the high sorption capacity of biochar, and the high microbial diversity in the soil profile prevented NH_4^+–N mitigation. The NH_4^+–N concentration in the SS system decreased in the whole process because NaN_3 inhabited the biotransformation of organic nitrogen and the aquifer soil medium has a high adsorption capacity for ammonia nitrogen. This result agreed with the research of Chen et al. [26] who proposed that the nitrogen removal rate improved by adding

peat in the deep soil of the subsurface wastewater infiltration system. Therefore, other forms of nitrogen removal, such as TN and NO_3^-–N, are worth considering.

3.1.3. NO_3^-–N migration at different depths in the three recharge systems

The variation of the NO_3^-–N concentration in the leachate during the recharge period is shown in Fig. 5. The TN and NO_3^-–N concentration in the recharge water was 25 and 15 mg L^{-1}, respectively. The decay rate of the three recharge system was BCS > SC > SS because the BCS system demonstrated stronger adaptability than the SC column. The NO_3^-–N concentration increased first and then decreased with the recharge depths, which might be because organic nitrogen and NH_4^+–N was transformed into NO_3^-–N under the aerobic condition by the microorganisms. The variation trend of NO_3^-–N was similar with TN, which proved that the key removal mechanism of TN might be denitrification of nitrate or nitrite to nitrogen gas [30]. At first, the NO_3^-–N concentration in the leachate had no obvious change, whereas it decreased with recharge time because the long generation cycle of denitrifying bacteria might influence the denitrification process. From the result shown in picture A of Fig. 6, the nitrate accumulation amount in the SS system increased with the experiment process, which indicated that the key removal pathway of NO_3^-–N removal was biodegradation compared with soil adsorption and chemical precipitation. In addition, the soil microbes played an important role in denitrification [31].

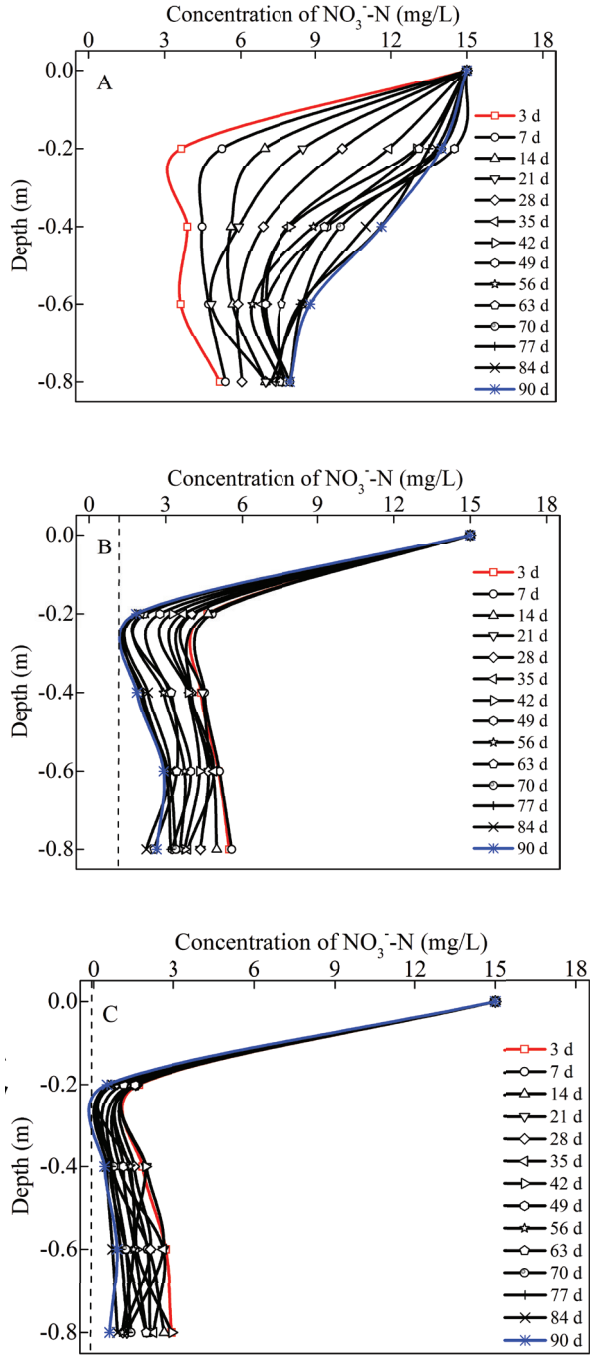

Fig. 5. Distribution of NO_3^-–N over time at different depths in three recharging systems.

The enzyme activity of denitrification for different depths of the SC and BCS systems is shown in Fig. 6. The denitrification enzyme activity was higher in BCS than in the SC column, which might have a close relationship with the change of nitrate concentration. The denitrification enzyme activity increased in the middle layer and decreased in the bottom layer, which directly affected the effect of denitrification.

3.2. Effect of biochar on the change of the microbial community structure

To analyze the effect of biochar on the change of the microorganisms, the microbial community structures were analyzed using 454 pyrosequencing of 16s rRNA genes. The microbial community structure at the same depths also greatly changed with the amended of the biochar. A clustering analysis according to the gene markers indicated that the soil samples tended to form separate clusters for different samples, which is shown in Table 4 from different depths of the different systems (Fig. 7). The most abundant genera were *Graclibacter*, *Geobacter* and *unidentified_WCHB1–69* in SC1,

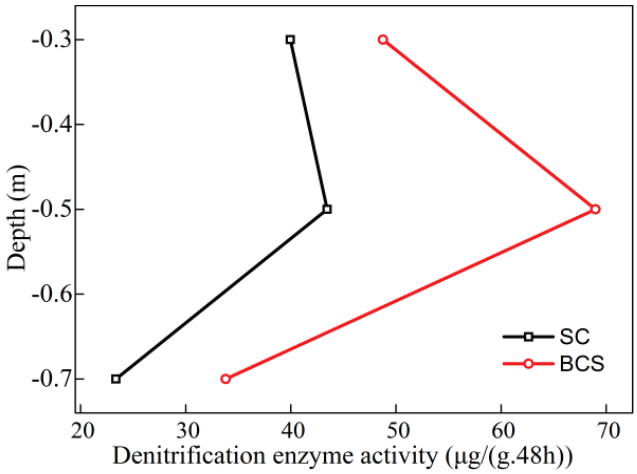

Fig. 6. Denitrification enzyme activity of the SC and BCS systems.

Table 4
The top 10 denitrifying bacteria and the number of sequences in the different systems on the genera level

Taxonomy	SC	BCS	Control
Bacillus	1,855	2,684	104
Pseudomonas	725	894	319
Xanthomonas	37	302	0
Bradyrhizobium	356	300	162
Acinetobacter	113	209	117
Rhizobium	163	199	232
Nitrosomonas	35	170	2
Paracoccus	20	123	22
Hyphomicrobium	252	133	76
Azospirillum	33	50	112

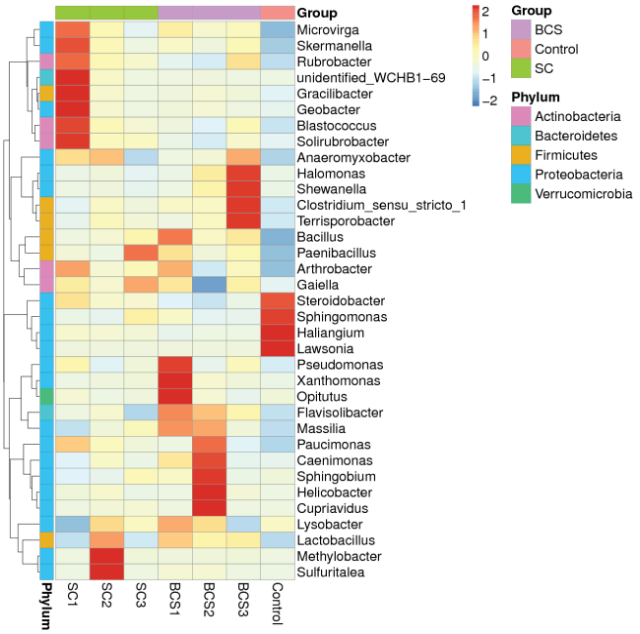

Fig. 7. Clustering analysis of the most abundant 35 genera vs. different sediment layers.

Methylobacter and *Sulfuritalea* were more abundant in SC2, whereas there were no significantly abundant genera in SC3. However, *Shingomonas, Haliangium* and *Lawsonia* showed the most diversity of genera in the control. The status of microorganisms in the BCS system was totally different, and the most abundant genera were *Pseudomonas*, *Xanthomonas* and *Opitutus* in BCS1, *Cupriavidus*, *Helicobacter* and *Sphingobium* in BCS2, and *Halomonas*, *Shewanella*, *Clostridium_sensu_stricto_1* and *Terrisporobacter* in BCS3. These results show that biochar probably affected the composition and distribution of the bacterial community by means of improving the physicochemical properties of the soil, and a similar viewpoint was reported that in an acidic soil biochar impacted the soil microbial community [31]. Furthermore, biochar might build up biogeochemical interfaces because of various functional groups and the high porosity. The compositional heterogeneity of biogeochemical interfaces could make the niche microhabitats

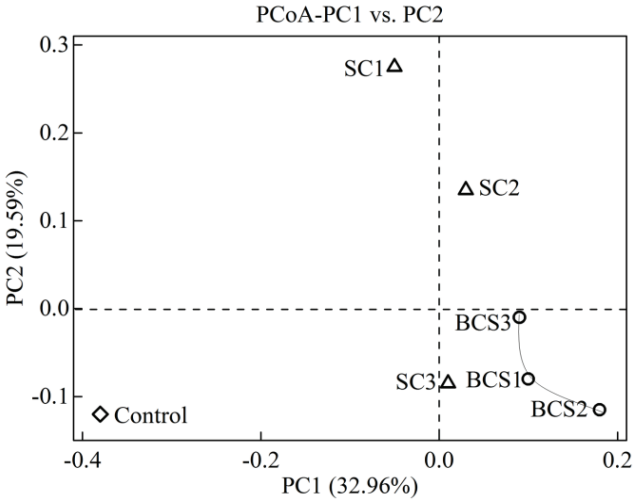

Fig. 8. Principal coordinated analysis (PCoA) based on the unweighted UniFrac metric.

more diverse and thus support the growth of high diversity bacterial communities [32], which indicated that the biochar amended could increase the bacterial diversity. The diversity of the microbial communities related to nitrogen transformation increased in the BCS system; therefore, nitrogen transformation was accelerated by nitrifying–denitrifying bacteria and archaea, which resulted in a lower residual ammonia and nitrate–N concentration in the leachate. This might be one of the reasons why the attenuation ratio in the BCS recharge system showed a higher similarity than the others. The bacterial diversity was depicted using a beta diversity analysis (Fig. 8). Two principal components accounted for 52.55% of the total variances with 32.96% and 19.59% for PC1 and PC2, respectively. The control was significantly separated from the other samples, but there was a close distance between BCS1, BCS2 and BCS3, which indicated that there was a lower difference and a higher similarity in the soil microbial community composition. The high removal efficiency of nitrogen and high enzyme activity of denitrification might be related to these dominant populations of the bacteria. The variety of different soil microbial phyla in the different depths had a certain dissimilitude. The microbial community is mainly composed of five phyla, including *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes* and *Verrucomicrobia*, which accounted for 82% on average from all of the soil samples. The dominant phylum in the BCS system was *Proteobacteria*, *Firmicutes* and *Actinobacteria*, which belong to the six major microbial community phyla in the denitrification process [33]. In Fig. 9, it is shown that these taxa accounted for more than 75% of the bacterial sequences. In addition, *Firmicutes* had a significant increase with biochar addition in the BCS column, whereas *Proteobacteria* and *Actinobacteria* had a corresponding decrease. *Firmicutes* has been reported as a predominant phylum in those biofilms with good performance in denitrifying [34]. Therefore, this might be one of the reasons that the nitrogen removal capability and denitrification enzyme activity was higher in the BCS system than the SC.

Many types of genera have been studied and reported in regard to the denitrification of microorganisms. Compared

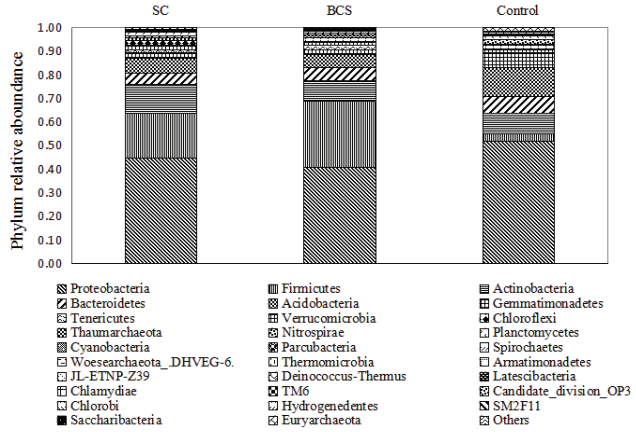

Fig. 9. Bacterial community composition of the three groups on the phylum level.

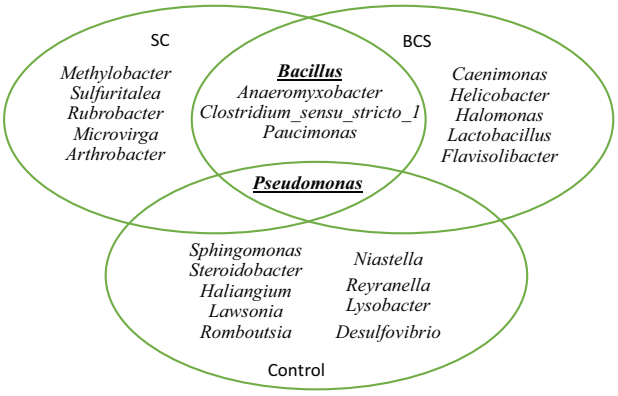

Fig. 10. A comparison of the bacteria of the top 10 genera in each system.

with the denitrifying bacteria genera summarized in "The denitrifying prokaryotes" [35], the top 10 genera of denitrifying bacteria in this study and among the SC, BCS and control systems are listed in Table 4. *Bacillus* is the largest denitrifying genus in the BCS and SC systems, especially for the BCS system. Most denitrifying bacteria were more numerous in the BCS column than the other systems as indicated in Table 3. Maybe these two aspects were also the reasons why the denitrification effect of the BCS system was better and the denitrification activity is higher than the other two columns.

The bacteria from the top 10 number of genera detected by 454 pyrosequencing of 16s rRNA genes was chosen for analysis (Fig. 10). As a result, *Pseudomonas* was the only genus in common in the three systems, which had been previously reported as a popular denitrifying bacterial genus. There was no other homogeneous genus for the control sample from the top 10 numbers of sequences compared with the other two systems, which indicated that the shift of microbial community structure occurred during the long time of the recharge process. There are four other homogeneous common genera between the SC and BCS systems, including *Bacillus*, which had been mentioned before as the largest denitrifying genus in the BCS and SC systems. For the BCS system, five uniquely detected

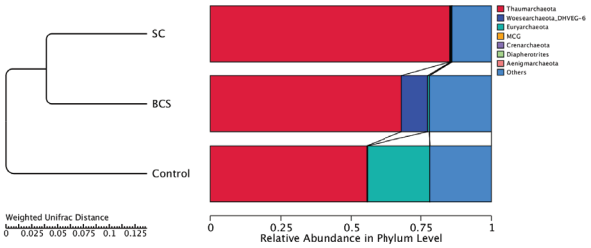

Fig. 11. Hierarchical clustering based on the unweighted UniFrac metric for archaea.

common species were *Caenimonas, Helicobacter, Halomonas, Lactobacillus* and *Flavisolibacter,* whose distributions with frequency were positively correlated with the biodegradation of nitrogen and their relative abundances increased compared with the control. These species might be involved in nitrogen removal and should be studied in future research.

Moreover, archaea had also been detected in the three groups, and the diversity of archaeal community was not as rich as the bacteria (Fig. 11). The archaeal community structure had some difference in SC and BCS, whereas the main phylum was *Thaumarchaeoca,* which has been reported as an ammonia-oxidizing archaeon [36]. *Woesearchaeota_DHVEG-6* was found to appear with high relative abundance in the BCS column, which was observed as the predominant uncultured archaeal community in wastewater treatment sludge related to nitrogen removal [37]. These genera reported that they might play an important role in the improvement of the capability of nitrogen removal and denitrification enzyme activity [38].

These results indicate that the soil environment was changed with increasing depth in the BCS and SC system and these four factors affected the community structure of both bacteria and archaea (Fig. 12). Aquifer depth, oxidation–reduction potential (ORP), pH and C/N (carbon and nitrogen ratio) were chosen as the major environmental factors on nitrogen removal [31,36,39,40] and these factors were analyzed through a RDA plot for the relationship of both bacteria and the archaeal community composition among the three groups and on the phylum level. The influence of these factors on the microbial community structure and the diversity between SC and BCS were different. The result of RDA for bacteria at the phylum level showed that the first axis explained 51.35% of the total microbial variance and the second axis explained 23.76% (Fig. 12(a)). The RDA plot analysis at the phylum level indicated that the depth and pH were important factors for the bacterial community in the BC and BSC. Moreover, the result of RDA for archaea showed that the first axis explained 41.19% of the total microbial variance and the second axis accounted for 31% (Fig. 12(b)). The result indicated that depth, pH and C/N were all important factors for the BCS system, whereas depth and C/N were important factors for the SC system. The archaeal community in both the BCS and the SC sediment was affected by the ORP but the influence was not significant. The change of the microbial community structure was affected by biochar because biochar not only improved the physical factors as the SSA and porosity but also changed the chemistry factors, such as the C/N, which are growth factors for the microbes.

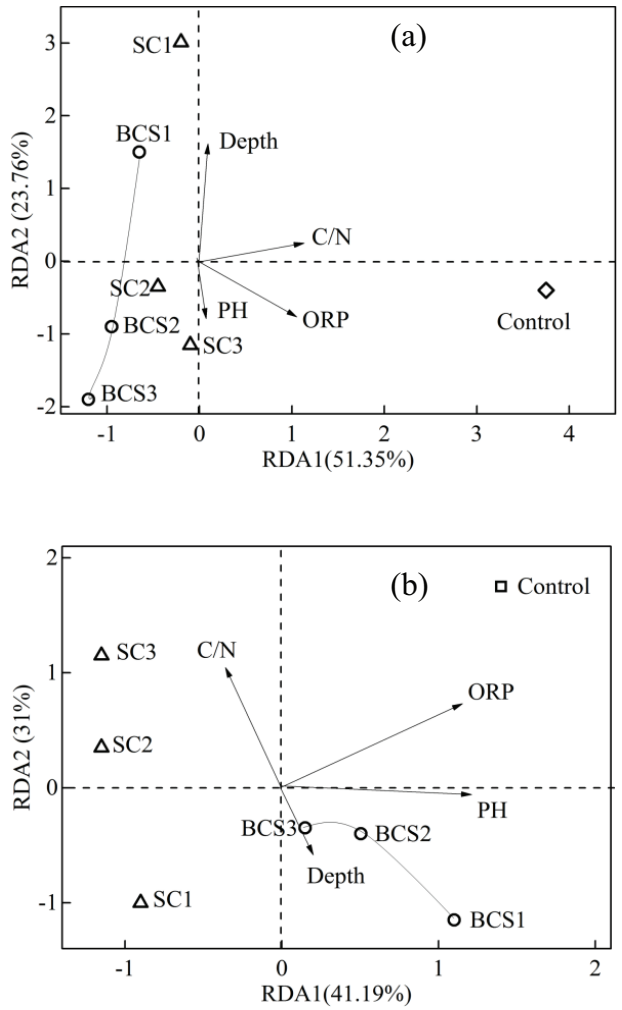

Fig. 12. The relationship of the microbial community composition among the three groups with the major environmental factors at the phylum level. A redundancy analysis (RDA) plot was drawn by RDA1 and RDA2. (a) For bacteria and (b) for archaea.

4. Conclusions

The attenuation effect of TN was BCS > SC > SS, and the consequence showed that biochar could improve the decay rate. The BCS for the BCS column improved the nitrogen removal capability and denitrification enzyme activity. It indicated that NO_3^-–N removal is the key pathway for nitrogen removal, which is mainly transformed through biological denitrification. After analyzing the change of the microbial community structure in the SC and BCS recharging modes, we found that the reasons of the nitrogen removal capability and denitrification enzyme activity's improvement in the BCS system and nitrogen transformation microorganisms related to biochar amended are related to the following four factors: (i) the relative abundance of the phylum *Firmicutes* increased significantly with biochar addition in the BCS column; (ii) the diversity of denitrifying microorganisms was very abundant in the BCS column compared with other systems, especially for *Bacillus*; (iii) *Woesearchaeota_ DHVEG-6* appeared in the largest archaeal abundance in the BCS column and (iv) five unique common species were detected in the BCS recharge system and were *Caenimonas, Helicobacter, Halomonas, Lactobacillus* and *Flavisolibacter,* which might be involved in nitrogen removal and should be studied in future research. Factors such as depth, ORP, pH and C/N also proved to be important factors influencing the shift of both bacteria and the archaeal community composition among the three groups affecting the change of microbial community structure in the BCS.

Acknowledgment

The authors gratefully acknowledge the financial support from the National Natural Science Foundation (51678052) and the Beijing Science and Technology Commission Foundation (D161100000216002).

References

[1] W.F. Ma, C. Nie, B. Chen, X. Cheng, X.X. Lun, F.G. Zeng, Adsorption and biodegradation of three selected endocrine disrupting chemicals in river-based artificial groundwater recharge with reclaimed municipal wastewater, J. Environ. Sci., 31 (2015) 154–163.
[2] W.F. Ma, Y. Yan, M. Ma, Y. Zhang, C. Nie, X. Lun, Effect of biochar on migration and biodegradation of 4-n-nonylphenol (NP) during river-based groundwater recharge with reclaimed water, Desal. Wat. Treat., 57 (2016) 29316–29327.
[3] S. Toze, Reuse of effluent water—benefits and risks, Agric. Water Manage., 80 (2006) 147–159.
[4] J. Wang, The reasonable arrangement of the Beijing' water resources in the early 21 century, Beijing Hydraul., 2 (2003) 33–35.
[5] W. Ma, X. Cheng, H. Guo, J. Sun, C. Nie, F. Su, Y. Ge, Nitrogen transport and transformation in artificial groundwater recharge with treated reclaimed municipal wastewater through river utilization, Sustai. Environ. Res., 25 (2015) 3–10.
[6] D. Keeney, W. Gardner, The Dynamics of Nitrogen Transformations in the Soil, Global Effects of Environmental Pollution, 1 (1970) 96–103.
[7] M. Hayatsu, K. Tago, M. Saito, Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification, Soil Sci. Plant Nutr., 54 (2008) 33–45.
[8] M. Konneke, A.E. Bernhard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, D.A. Stahl, Isolation of an autotrophic ammonia-oxidizing marine archaeon, Nature, 437 (2005) 543–546.
[9] L. Philippot, S. Hallin, Molecular Analyses of Soil Denitrifying Bacteria, Molecular Approaches to Soil, Rhizosphere and Plant Microorganism Analysis, 2006, pp. 146–165.
[10] H. Bothe, G. Jost, M. Schloter, B.B. Ward, K. Witzel, Molecular analysis of ammonia oxidation and denitrification in natural environments, FEMS microbiol. Rev., 24 (2000) 673–690.
[11] J. Lehmann, J.P. da Silva Jr., C. Steiner, T. Nehls, W. Zech, B. Glaser, Nutrient availability and leaching in an archaeological anthrosol and a ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments, Plant Soil, 249 (2003) 343–357.
[12] C. Steiner, W.G. Teixeira, J. Lehmann, T. Nehls, J.L.V. de Macêdo, W.E. Blum, W. Zech, Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil, Plant Soil., 291 (2007) 275–290.
[13] C. Steiner, B. Glaser, W. Geraldes Teixeira, J. Lehmann, W.E.H. Blum, W. Zech, Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal, J. Plant Nutr. Soil Sci., 171 (2008) 893–899.
[14] J.M. Novak, W.J. Busscher, D.L. Laird, M. Ahmedna, D.W. Watts, M.A. Niandou, Impact of biochar amended on fertility of a southeastern coastal plain soil, Soil Sci., 174 (2009) 105–112.

[15] S. Joseph, J. Lehmann, Biochar for Environmental Management: Science and Technology, Earthscan, London, 2009.
[16] B. Liang, J. Lehmann, D. Solomon, J. Kinyangi, J. Grossman, B. O'neill, E.G. Neves, Black carbon increases cation exchange capacity in soils, Soil Sci. Soc. Am. J., 70 (2006) 1719–1730.
[17] J. Lehmann, Bio-energy in the black, Front. Ecol. Environ., 5 (2007) 381–387.
[18] T. Asada, T. Ohkubo, K. Kawata, K. Oikawa, Ammonia adsorption on bamboo charcoal with acid treatment, J. Health Sci., 52 (2006) 585–589.
[19] Y. Ding, Y.-X. Liu, W.-X. Wu, D.-Z. Shi, M. Yang, Z.-K. Zhong, Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns, Water Air Soil Pollut., 213 (2010) 47–55.
[20] C.C. Rodrigues, D. de Moraes, S.W. da Nobrega, M.G. Barboza, Ammonia adsorption in a fixed bed of activated carbon, Bioresour. Technol., 98 (2007) 886–891.
[21] G. Yoo, H. Kim, J. Chen, Y. Kim, Effects of biochar addition on nitrogen leaching and soil structure following fertilizer application to rice paddy soil, Soil Sci. Soc. Am. J., 78 (2014) 852–860.
[22] N. Xu, G. Tan, H. Wang, X. Gai, Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure, Eur. J. Soil Biol., 74 (2016) 1–8.
[23] H.D. Chapman, Cation-exchange capacity, Chemical and microbiological properties, Methods of soil analysis, 9 (1965) 891–901.
[24] Ö. Gustafsson, F. Haghseta, C. Chan, J. MacFarlane, P.M. Gschwend, Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability, Environ. Sci. Technol., 31 (1996) 203–209.
[25] Q. Yu, Y. Chen, X. Ye, G. Tian, Z. Zhang, Influence of the DMPP (3,4-dimethyl pyrazole phosphate) on nitrogen transformation and leaching in multi-layer soil columns, Chemosphere, 69 (2007) 825–831.
[26] P. Chen, J. Cui, M. Zheng, S. Cheng, J. Huang, K. Mu, L. HU, Nitrogen removal enhancement by addition of peat in the depth of the subsurface wastewater infiltration system, J. Integr. Agric., 13 (2014) 1113–1120.
[27] W. He, Y. Wei, S. Cai, Study on assaying method and affecting factors of soil denitrification enzyme activity, J. Northwest Sci. Technol. Univ. Agric. For., 1 (2006) 025.
[28] J.A. Peiffer, A. Spor, O. Koren, Z. Jin, S.G. Tringe, J.L. Dangl, E.S. Buckler, R.E. Ley, Diversity and heritability of the maize rhizosphere microbiome under field conditions, Proc. Natl. Acad. Sci. USA, 110 (2013) 6548–6553.
[29] C. Luo, D. Tsementzi, N. Kyrpides, T. Read, K.T. Konstantinidis, Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample, PloS One, 7 (2012) e30087.
[30] J. Zhang, X. Huang, C. Liu, H. Shi, H. Hu, Nitrogen removal enhanced by intermittent operation in a subsurface wastewater infiltration system, Ecol. Eng., 25 (2005) 419–428.
[31] H. Xu, X. Wang, H. Li, H. Yao, J. Su, Y. Zhu, Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape, Environ. Sci. Technol., 48 (2014) 9391–9399.
[32] J. Hanzel, D. Myrold, A. Sessitsch, K. Smalla, C.C. Tebbe, K.U. Totsche, Microbial ecology of biogeochemical interfaces – diversity, structure, and function of microhabitats in soil, FEMS Microbiol. Ecol., 86 (2013) 1–2.
[33] Z. Shen, Y. Zhou, J. Hu, J. Wang, Denitrification performance and microbial diversity in a packed-bed bioreactor using biodegradable polymer as carbon source and biofilm support, J. Hazard. Mater., 250 (2013) 431–438.
[34] K.C. Wrighton, B. Virdis, P. Clauwaert, S.T. Read, R.A. Daly, N. Boon, Y. Piceno, G.L. Andersen, J.D. Coates, K.l. Rabaey, Bacterial community structure corresponds to performance during cathodic nitrate reduction, ISME J., 4 (2010) 1443–1455.
[35] R.M. Jeter, J.L. Ingraham, The Denitrifying Prokaryotes, The Prokaryotes, Springer, 1981, pp. 913–925.
[36] K. Kuroda, M. Hatamoto, N. Nakahara, K. Abe, M. Takahashi, N. Araki, T. Yamaguchi, Community composition of known and uncultured archaeal lineages in anaerobic or anoxic wastewater treatment sludge, Microb. Ecol., 69 (2015) 586–596.
[37] X. Fan, P. Xing, The Vertical Distribution of Sediment Archaeal Community in the "Black Bloom" Disturbing Zhushan Bay of Lake Taihu, Archaea, 2016.
[38] C. Beal, E. Gardner, N. Menzies, Process, performance, and pollution potential: a review of septic tank–soil absorption systems, Soil Res., 43 (2005) 781–802.
[39] B. Meek, L. Grass, Redox potential in irrigated desert soils as an indicator of aeration status, Soil Sci. Soc. Am. J., 39 (1975) 870–875.
[40] F. Ye, Y. Li, Enhancement of nitrogen removal in towery hybrid constructed wetland to treat domestic wastewater for small rural communities, Ecol. Eng., 35 (2009) 1043–1050.