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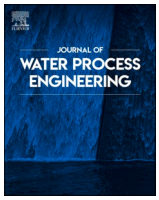

Biofiltration matrix optimization for efficient nitrogen removal from domestic onsite wastewater

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ABSTRACT

Bench-scale columns were packed with marble chip, zeolite and gravel to treat septic tank effluent at elevated hydraulic loadings (0.48–0.64 $m^3\ m^{-2}\ d^{-1}$) to challenge their hydraulic performance and ammonium (NH_4^+-N) removal performance. The results showed that higher NH_4^+-N removal efficiency was achieved in zeolite (75.8–94.1 %) and marble chip (54.9–83.9 %) columns than in sand (32.9–76.6 %) column without clogging during the experimental period (4 months). Biochar amendment (30 % *v*/v) resulted in a 23–29 % decrease of effluent NH_4^+-N in marble chip columns, while no impact was observed in the zeolite columns. Nitrification rather than adsorption contributed to the major NH_4^+-N removal (82–95 %) in zeolite, biochar amended zeolite and biochar amended marble chip columns. In addition, higher abundance of nitrifying microorganisms observed in the top layer of zeolite column (1.2×10^7 - 3.1×10^7 *amoA* copies g^{-1}, 3–10 times higher than marble chip) suggested NH_4^+-N was concentrated on zeolite surface by adsorption which then facilitated nitrification and promoted nitrifying biomass growth. Collectively, this study suggested that zeolite and biochar amended marble chip could serve as the filter materials for efficient NH_4^+-N removal from onsite wastewater at elevated wastewater loadings without clogging concerns.

1. Introduction

In the United States, around 20 % of households are served with conventional onsite wastewater treatment systems (OWTSs), such as a septic tank with the following leaching field or leaching pools, which can only provide primary treatment to remove suspended solids and chemical oxygen demand (COD) from wastewater [1–3]. Limited nitrogen removal (10–40 %) can be achieved by these systems [4,5]. The excessive nitrogen loading to the groundwater and coastal regions poses a high potential for eutrophication and harmful algal bloom (HAB) which threaten the drinking water quality and aquatic ecosystem balance [6,7]. Soil-based biofiltration systems such as engineered nitrogen removing biofilter (NRB), recirculating sand filter (RSF), and constructed wetland (CW) are economically feasible options to be easily integrated into a septic tank and significantly improve nitrogen removal efficiency (50–90 %) from onsite wastewater [8–13].

The major factor that limits the treatment capacity of soil-based biofiltration systems is the frequent clogging of the filter materials at high hydraulic loadings, which subsequently increases the maintenance frequency [14]. The clogging of biofiltration system was mainly caused by the accumulation of suspended solids, chemical precipitates, and microbial growth products beneath the surface of the soil-based biofiltration system [15]. Filter media particle size and porosity are major factors controlling the extent and frequency of clogging [16]. The smaller particle size and lower porosity decreased the void space for biofilm establishment and enhanced the suspended solids interception due to narrower pore diameters, increasing the clogging potential [15]. Sand, as the most extensively used material in biofiltration systems, showed high clogging potential due to its low particle size (0.2–1 mm) and porosity (0.2–0.4), significantly limited the hydraulic capacity of sand filters (0.01–0.04 $m^3\ m^{-2}\ d^{-1}$) [17,18]. For example, we developed a pilot-scale modular-based continuous flow biofilter (CFB) which could handle septic tank effluent (STE) at high hydraulic loadings (0.03–0.12 $m^3\ m^{-2}\ d^{-1}$, 2–4 times higher than the conventional nitrogen removing biofilters) while high nitrogen removal efficiency (> 80 %) was achieved [9]. In brief, the CFB comprises a pump tank, two nitrification sand

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modules and three denitrification woodchip modules. Part of denitrification module effluent was recirculated back to the pump tank and was continuously mixed with raw STE. The recirculation and continuous flow pattern of CFB increased the hydraulic detention time of contaminants, which was in favor of the growth of microorganisms and associated metabolic activities, thus achieved efficient nitrogen removal. However, the treatment capacity of CFB was restricted due to the high clogging potential of the nitrification module filled with sand, which received a mixture of STE and recirculated effluent. Clogging of nitrification modules was observed every 6–12 months when the sand nitrification module of the system was operated at high hydraulic loadings ($> 0.48\ m^3\ m^{-2}\ d^{-1}$) of mixed STE with recirculation flow. Although previous studies applied materials with high porosities and big particle sizes such as gravel, bio-ceramic, and zeolite in biofilters treating domestic wastewater at high hydraulic loading rates (HLR), the design hydraulic retention time (HRT) for these systems was high (18–100 h) to achieve efficient ammonium (NH_4^+-N) removal [19–22]. The low hydraulic capacity requires a large system footprint to accommodate the design loading and subsequently increases the construction and maintenance cost. Hence it is necessary to explore the alternative filter materials for biofiltration systems to overcome the hydraulic challenge and achieve efficient nitrogen removal from onsite wastewater with economical design.

Gravel, marble chip, zeolite, and biochar are ideal filter materials for soil-based biofiltration systems based on their physical and chemical properties, low cost, and local availability. Gravel has been extensively applied either independently or in combination with other materials such as sand and biochar in biofiltration systems for domestic wastewater, stormwater, and anaerobically digested effluent treatment [21,23,24]. Marble chip, which is majorly composed of calcium carbonate ($CaCO_3$), is a recycled material from the construction industry. Previous literatures have reported that the addition of marble chip to biofiltration systems can significantly recover the alkalinity loss caused by the nitrification process and maintain the pH level at around 7 [25,26]. However, the NH_4^+-N removal performance of marble chip in biofilters treating onsite wastewater has not been well evaluated in previous studies. Zeolite is an aluminosilicate mineral with a large specific surface area to support higher biomass and strong cation exchange capacity for NH_4^+ removal [27,28]. It was extensively applied as the sole media for biofiltration systems treating municipal wastewater, achieving 15–50 % higher NH_4^+-N removal efficiency compared with synthetic biocarrier and sand [29,30]. Biochar is a porous carbon-rich material produced from biomass under oxygen-free or oxygen-limited conditions [28,31]. It was commonly served as an amendment material, mixed with other materials such as gravel, clay, and sand in biofiltration systems with a ratio of 10–30 % (vol: vol) due to its low mechanical strength [32]. The addition of biochar in biofiltration systems increased NH_4^+-N removal efficiency by 10–40 % for industrial, agricultural, and domestic wastewater treatment [7,22,33,34]. While the nitrogen removal performance of biochar and zeolite in biofiltration systems has been comprehensively investigated, the major mechanism (adsorption, nitrification, and other physical/chemical/biological processes) of these two materials for NH_4^+-N removal from onsite wastewater remains unclear and was evaluated in this study.

In this study, the overall goal is to select the optimum filter materials for nitrification modules of an onsite wastewater biofiltration system that can overcome the hydraulic challenge of onsite wastewater while maintaining efficient NH_4^+-N removal performance. To achieve the goal, a two-stage bench-scale column experiment was conducted. At stage 1, the NH_4^+-N removal performance of columns packed with gravel, marble chip, and zeolite was investigated at increased HLRs ($0.48–0.64\ m^3\ m^{-2}\ d^{-1}$). At stage 2, the feasibility of biochar amendment (30 %, vol, vol) to further increase the NH_4^+-N removal performance of biofiltration systems was investigated. In addition, to understand the NH_4^+-N removal mechanism in zeolite and biochar amended filters, the NH_4^+-N adsorption kinetics by zeolite and biochar were evaluated using real wastewater and synthetic ammonium solution. The nitrifying biomass abundance on the media surface was analyzed and compared among various filter materials.

2. Methods and materials

2.1. Bench-scale column experiment

Clinoptilolite zeolite (ZMI, USA), biochar (Redbud Soil, USA), and marble chip (Vigoro, USA) were purchased from commercial vendors in the United States. C-33 sand and gravel were directly collected from the Water Research Innovative Facility (WRIF) of the New York State Center for Clean Water Technology (NYS CCWT). The major composition of the zeolite was SiO_2 (66.7 %) and Al_2O_3 (11.5 %) with 1.6–2.0 meq g^{-1} cation exchange capacity (CEC) [35]. The biochar was produced from beetle kill pine (pyrolysis temperature 500 °C).

At stage 1, four columns (diameter: 8 cm, height: 15 cm) filled with different combinations of filtration media (sand, gravel, zeolite, and marble chip) were set up (Fig. S1a). The sand column (S) was filled with 100 % C-33 sand (diameter: 0.2–1 mm) as an experimental control. The gravel/sand column (S + G) was filled with a 5-cm gravel (diameter: 10–15 mm) layer overlying a 10-cm C-33 sand layer to reduce the clogging potential at the surface of the sand filter. The zeolite column (Z1) was packed with zeolite (diameter: 10–15 mm) and gravel at a 50:50 (vol: vol) ratio and the marble chip column (M1) was packed with marble chip (diameter: 10–20 mm) and gravel at 50:50 (vol: vol) ratio. All columns were continuously fed with effluent collected from the pump tank of a pilot-scale CFB which was the diluted STE by recirculating denitrification effluent (NH_4^+: 14.6 ± 2.8 mg N L^{-1}, NO_3^-: 0.1 ± 0.1 mg N L^{-1}, COD: 36.2 ± 6.3 mg L^{-1} and pH: 7.4 ± 0.2) [9]. At the beginning of the experiment (stage 1a), the HLR of wastewater to all columns was $0.48\ m^3\ m^{-2}\ d^{-1}$, the same HLR as the nitrification modules of the CFB as described in the previous study (Table 1) [9]. Then the HLR was further increased to $0.64\ m^3\ m^{-2}\ d^{-1}$ at stage 1b to challenge the nitrification performance and the hydraulic capacity of various filtration materials.

At stage 2, 30 % biochar (vol: vol, diameter: 7–15 mm) amendment was applied to marble chip columns (M + B) and zeolite columns (Z + B), while 100 % zeolite (Z2) and 100 % marble chip (M2) columns were set up as the experimental controls (Fig. S1b). Duplicate columns were set up for each treatment. At the beginning of the experiment (stage 2a), the HLR of wastewater (NH_4^+: 16.9 ± 2.3 mg N L^{-1}, NO_3^-: 0.1 ± 0.1 mg N L^{-1}, COD: 42.6 ± 5.9 mg L^{-1} and pH: 7.4 ± 0.1) to all columns was the same as in stage 1b ($0.64\ m^3\ m^{-3}\ d^{-2}$) (Table 1). Then the HLR was reduced to $0.48\ m^3\ m^{-3}\ d^{-2}$ (stage 2b) to investigate whether the longer HRT has a positive impact on NH_4^+-N removal of biochar amended columns. Influent and effluent samples (50 mL) were collected every 2–5 days from different columns and were filtered by 0.45 μm membrane filters (Millipore Sigma, USA), then were acidified with concentrated sulfuric acid (H_2SO_4, 18.4 M), and were stored at 4 °C before further analysis.

2.2. Ammonium adsorption and desorption by zeolite and biochar

Synthetic ammonium solution and real influent wastewater were prepared for the adsorption kinetic experiment. Real influent wastewater (NH_4^+: 16.2 mg N L^{-1}, NO_3^-: 0.1 mg N L^{-1}, COD: 31.7 mg L^{-1} and pH: 6.8; Table S2) was collected from the pump tank effluent of CFB which was also utilized as the feeding wastewater for the bench-scale column experiment and was filtered by 0.22 μm membrane filters (Thermo Fisher Scientific, USA). Synthetic ammonium solution contained 100 mg NH_4^+-N L^{-1} and the pH was adjusted to 7 by 1 M sodium hydroxide (NaOH).

Both fresh and aged zeolite and biochar samples were used in the adsorption experiment. In brief, 1 g aged zeolite or biochar samples were collected from the surface (1–2 cm) of Z2 and the M + B columns on day

Table 1
Description of experimental stages in bench-scale columns.

Stage	Operational Period (days)	Column Depth (cm)	HLR (m^3 m^{-2} d^{-1})	HRT (hours)	Overall Objective	Tested Material
1a	48	15	0.48	3.9	Identify the optimum filtration material that can handle elevated hydraulic loadings	Sand, gravel, marble chip and zeolite
1b	80	15	0.64	5.2		
2a	89	15	0.64	5.2	Investigate the feasibility of biochar amendment to further increase NH_4^+-N removal performance	Marble chip and zeolite with 30 % biochar amendment
2b	48	15	0.48	3.9		

85 of the stage 2 experiment. The aged sample was added to a serum bottle containing 250 mL of real influent wastewater or synthetic ammonium solution. Then all serum bottles were purged with nitrogen gas for 5–10 min to remove dissolved oxygen (DO) in influent wastewater, inhibiting the nitrification activity of microorganisms attached to the aged zeolite and biochar. Finally, all serum bottles were sealed with rubber stoppers to maintain the anaerobic condition. The same protocol was performed for fresh zeolite or biochar samples in glass flasks containing 250 mL solutions (synthetic ammonium solution or real influent wastewater) without the addition of nitrogen gas. All groups of adsorption kinetic experiments were conducted in triplicates on a shaker at room temperature (20 °C) and 120 rpm for 48 h.

The desorption experiment was conducted in triplicates for aged zeolite and biochar samples. The aged zeolite and biochar were collected from Z2 and M + B columns at the end of the experiment. In brief, 1 g aged zeolite or biochar was added to a 50 mL Falcon conical tube (Corning, USA) containing 40 mL 1 M potassium chloride (KCl) solution and then the tube was incubated in a shaker at room temperature (20 °C) and 120 rpm for 40 h. Same protocol was also applied for fresh zeolite or biochar as the experimental control.

2.3. Chemical analysis

pH and temperature were measured onsite by a YSI Pro 10 m (YSI, USA). NH_4^+-N, NO_2^--N, and NO_x^--N were analyzed by a Lachat QuikChem 8500 autoanalyzer (Hach, USA) according to the manufacturer's instruction. NO_3^--N was calculated as the difference between NO_x^--N and NO_2^--N. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+ and NO_x^-. Chemical oxygen demand (COD) was measured by Hach COD kits (Hach, USA) and a Hach DR 6000 spectrophotometer (Hach, USA).

2.4. Microbial analysis

To assess the microbial density and functional microbial species abundance in different filter materials, the top 5 cm of sand, gravel, marble chip, and zeolite in the S, S + G, M1, and Z1 columns were collected, respectively at the end of stage 1. The top 5 cm of marble chip, biochar, and zeolite in M2, M + B, and Z2 columns were collected, respectively at the end of stage 2. All solid samples were stored at −80 °C before further microbial analyses.

For genomic DNA extraction, 10 g of each solid sample was first mixed with 20 mL phosphate buffer saline (PBS) in a 50 mL beaker on ice and was sonicated at 18 W for 2 min using a Fisherbrand model 120 ultrasonic processor with a 6.4 mm probe (Thermo Fisher Scientific, USA). Then, the mixture was filtrated with a 0.22 μm membrane filter to immobilize the microorganisms on the filter. Genomic DNA was extracted from the membrane samples using DNeasy PowerWater kit (Qiagen, Germany) according to the manufacturer's instructions. The abundance of total microbial biomass and nitrifying microorganisms were measured by the level of 16S rRNA and the functional gene (*amoA*) that encodes the ammonia monooxygenase for ammonium oxidation by ammonium oxidizing archaea (AOA) and ammonium oxidizing bacteria (AOB) via a QIAcuity One digital PCR system (Qiagen, Germany). The information on primers was reported in the previous study [9]. All dPCR reactions were performed in a total volume of 12 μL containing 4 μL of 3× EvaGreen PCR master mix (Qiagen, Germany), 0.48 μL of each primer (10 μM), 2 μL of DNA templates, and 5.52 μL of RNase-free water. The cycling conditions for dPCR of 16S rRNA were 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C, 15 s at 60 °C, 15 s at 72 °C, and 40 °C for 5 min. The cycling conditions for *amoA* genes were 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C, 1 min at 58 °C (*amoA* AOB)/ 53 °C (*amoA* AOA), 1 min at 72 °C, and 40 °C for 5 min. DNA templates, no template controls (NTCs), and plasmids containing the target genes were used as positive controls and analyzed in each dPCR assay.

2.5. Data analysis

The contribution of biological nitrification to NH_4^+-N removal in test columns was calculated as the mass ratio of NO_x^-- N production to NH_4^+-N removal during the column experiment. The contribution of adsorption to NH_4^+-N removal was calculated as the mass ratio of desorbed NH_4^+-N to NH_4^+-N removed by the filter materials in test columns. The calculation details were summarized in the supplemental material. One-way analysis of variance (ANOVA) was used to evaluate the impact of biochar amendment on the treatment performance of zeolite and marble chip columns at a significance level of 0.05. All analyses were performed in R (version 3.5.3) and OriginLab 2018 (OriginLab, MA).

3. Results

3.1. Ammonium adsorption and desorption by zeolite and biochar

The results of the batch adsorption experiment revealed greater NH_4^+-N adsorption capacities in fresh zeolite and biochar treating synthetic ammonium solution (8.5 mg NH_4^+-N g^{-1} zeolite and 3.1 mg NH_4^+-N g^{-1} biochar) compared with real wastewater (2.4 mg NH_4^+-N g^{-1} zeolite and 1.0 mg NH_4^+-N g^{-1} biochar) (Fig. 1 and Table S3). In addition, it took longer time for fresh zeolite and biochar to reach NH_4^+-N adsorption capacities with real wastewater (34 h for zeolite and 22 h for biochar) than with synthetic ammonium solution (26 h for zeolite and 11 h for biochar), suggesting higher adsorption rates of NH_4^+-N by zeolite and biochar in synthetic ammonium solution (Fig. 1). These results suggested that NH_4^+-N adsorption capacity and rate by zeolite and biochar were greatly impacted by the influent composition. Compared with fresh zeolite and biochar samples, both aged zeolite and biochar collected at the end of the entire experiment showed low NH_4^+-N adsorption capacities treating real wastewater (0.2 mg NH_4^+-N g^{-1} zeolite and 0.3 mg NH_4^+-N g^{-1} biochar), indicating adsorption contributed to a limited level of NH_4^+-N removal during long-term operation (Fig. 1 and Table S3). In addition, 2.1 mg NH_4^+-N g^{-1} zeolite and 0.7 mg NH_4^+-N g^{-1} biochar were desorbed from the aged zeolite and biochar collected at the end of the experiment, which accounted for 87 % and 70 % of real wastewater NH_4^+-N adsorption capacities in fresh zeolite and biochar (Fig. S2). This result confirmed that zeolite and biochar nearly reached adsorption capacity during long-term operation.

3.2. HLR impact on ammonium removal

At stage 1a (day 1–48), all bench-scale columns were fed with real wastewater containing 13.1 ± 2.5 mg NH_4^+-N L^{-1} at 0.48 m^3 m^{-2} d^{-1} (Fig. 2a). Z1 and M1 columns removed 94.1 ± 4.6 % and 83.9 ± 8.2 %

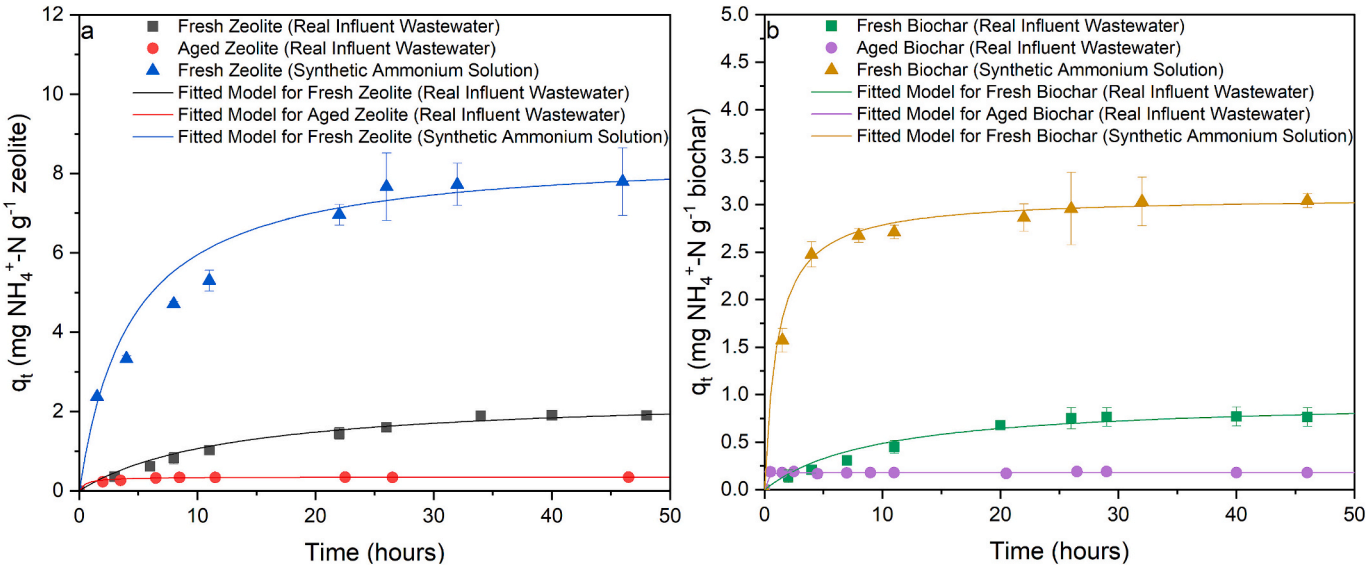

Fig. 1. Pseudo-second-order kinetic model for NH_4^+-N adsorption onto a) zeolite and b) biochar at different conditions. Error bars represent standard deviations of triplicate experiments.

NH_4^+-N from influent and achieved 0.9 ± 0.8 mg NH_4^+-N L^{-1} and 2.2 ± 1.4 mg NH_4^+-N L^{-1} in effluents, respectively (Fig. 2a and Table S4). On the contrary, significantly lower NH_4^+-N removal efficiency was observed in S (76.6 ± 21.2 %) and S + G columns (57.7 ± 17.8 %) with effluents containing 3.2 ± 2.6 mg NH_4^+-N L^{-1} and 5.7 ± 2.9 mg NH_4^+-N L^{-1}, respectively (Fig. 2a and Table S4). These results suggested marble chip and zeolite can achieve better NH_4^+-N removal performance than sand and gravel. At stage 1b (day 49–128), influent wastewater contained 15.7 ± 2.9 mg NH_4^+-N L^{-1}. The increased HLR (0.64 m^3 m^{-2} d^{-1}) at stage 1b resulted in reduced NH_4^+ removal efficiencies in S, S + G, and M1 columns to 32.9 ± 16.8 %, 36.3 ± 13.2 % and 54.9 ± 14.6 %, with increased effluent NH_4^+-N concentrations to 9.0 ± 2.3 mg NH_4^+-N L^{-1}, 8.9 ± 2.1 mg NH_4^+-N L^{-1} and 6.3 ± 2.5 mg NH_4^+-N L^{-1}, respectively (Fig. 2a and Table S4). However, less decrease of NH_4^+-N removal efficiency in the Z1 column was observed (75.8 ± 10.6 %) with 3.0 ± 1.5 mg NH_4^+-N L^{-1} in the effluent (Fig. 2a and Table S4), suggesting zeolite was more resistant to HLR change while maintaining high NH_4^+-N removal performance.

Biological nitrification was the major NH_4^+-N removal mechanism in S, S + G, and M1 columns which was supported by the similar levels of DIN observed in effluents from these columns (S: 21.4 ± 3.8 mg DIN L^{-1}, S + G: 21.2 ± 3.9 mg DIN L^{-1} and M1: 21.3 ± 3.7 mg DIN L^{-1}) and influent wastewater (21.5 ± 3.9 mg DIN L^{-1}) during stage 1 (Fig. 2c and Table S5). The Z1 column removed 4.8 ± 3.0 mg DIN L^{-1} from influent wastewater during the first 10 days of operation, then a similar level of DIN was observed in the effluent (20.7 ± 2.5 mg DIN L^{-1}) and the influent wastewater (21.5 ± 3.9 mg DIN L^{-1}) (Fig. 2c and Table S5). The results suggested NH_4^+-N adsorption capacity of zeolite was achieved after 10 days of filtration, then nitrification contributed to the majority of NH_4^+-N removal in the Z1 column.

The alkalinity concentration was reduced from 197.0 ± 16.4 mg $CaCO_3$ L^{-1} in influent to 141.8 ± 38.2–172.1 ± 28.6 mg $CaCO_3$ L^{-1} in effluents from all columns at stage 1a due to the consumption of alkalinity by nitrification process, respectively (Fig. S3c). Then at stage 1b, lower reduction of alkalinity from 211.0 ± 32.6 mg $CaCO_3$ L^{-1} in the influent to 169 ± 21.8–198.4 ± 33.6 mg $CaCO_3$ L^{-1} in effluents from S, S + G, and M1 columns were observed due to the inhibition of nitrification process by higher HLR (Fig. S3c). The effluent from the Z1 column still contained 130.0 ± 31.1 mg $CaCO_3$ L^{-1} alkalinity because the nitrification process in the Z1 column was less impacted by the increased HLR (Fig. S3c).

It is worth noting that the S and S + G columns experienced frequent clogging at high HLRs (0.48–0.64 m^3 m^{-2} d^{-1}), resulting in the replacement or remix of the top 2–3 cm of sand on a weekly basis (Fig. S4). However, no clogging was observed in the M1 and Z1 columns during the entire stage 1 experiment, indicating that the materials (zeolite and marble chip) with higher porosity (0.5) and bigger particle sizes (5–15 mm) can significantly reduce the clogging potential of biofiltration systems for onsite wastewater treatment. Therefore, zeolite and marble chip were selected for further investigations in stage 2.

3.3. The impact of biochar amendment on ammonium removal

At stage 2a (day 1–89), the Z + B and Z2 columns reduced NH_4^+-N concentrations from 16.2 ± 1.6 mg NH_4^+-N L^{-1} in the influent to comparably low levels at 2.6 ± 1.2 mg NH_4^+-N L^{-1} and 2.6 ± 1.3 mg NH_4^+-N L^{-1} with 83.5 ± 8.3 % and 83.6 ± 8.7 % NH_4^+-N removal efficiencies, respectively (Fig. 3a, Table S6 and S7). However, M + B columns achieved 23 % lower effluent NH_4^+-N concentration (4.9 ± 1.6 mg NH_4^+-N L^{-1}) with 69.6 ± 9.6 % NH_4^+-N removal efficiency, compared with M2 columns without any amendment (6.3 ± 1.7 mg NH_4^+-N L^{-1} in effluent and 61.1 ± 8.2 % NH_4^+-N removal efficiency) (Fig. 3a, Table S6 and S7). These results suggested biochar amendment has little impact on the NH_4^+-N removal efficiency of zeolite biofilters while it could enhance the NH_4^+-N removal performance in marble chip biofilters.

To investigate whether the lower HLR has a positive impact on NH_4^+-N removal by biochar amended columns, the influent HLR was reduced to 0.48 m^3 m^{-2} d^{-1} at stage 2b (day 90–138). The improved NH_4^+-N removal efficiency was achieved in all test columns, while comparable NH_4^+-N removal performance was still observed in the Z + B and Z2 columns. The NH_4^+-N concentration decreased from 17.0 ± 2.0 mg NH_4^+-N L^{-1} in influent to 0.9 ± 0.7 NH_4^+-N L^{-1} and 0.9 ± 0.3 mg NH_4^+-N L^{-1} in effluents from Z + B and Z2 columns with 94.1 ± 3.3 % and 94.1 ± 2.0 % NH_4^+-N removal efficiency, respectively (Fig. 3a, Table S6 and S7). On the contrary, effluent NH_4^+-N concentration in M + B columns (3.6 ± 1.2 mg NH_4^+-N L^{-1}) decreased by 29 % than M2 columns (5.1 ± 1.9 mg NH_4^+-N L^{-1}). That also contributed to increased NH_4^+-N removal efficiency observed in the M + B columns (78.6 ± 7.1 %) compared with M2 columns (69.7 ± 11.9 %) (Fig. 3a, Table S6 and S7).

The effluent DIN concentration in M2 columns (20.9 ± 6.9 mg DIN L^{-1}) was comparable to the levels observed in the influent wastewater (21.7 ± 6.5 mg DIN L^{-1}) (Fig. 3c). This result demonstrated that the

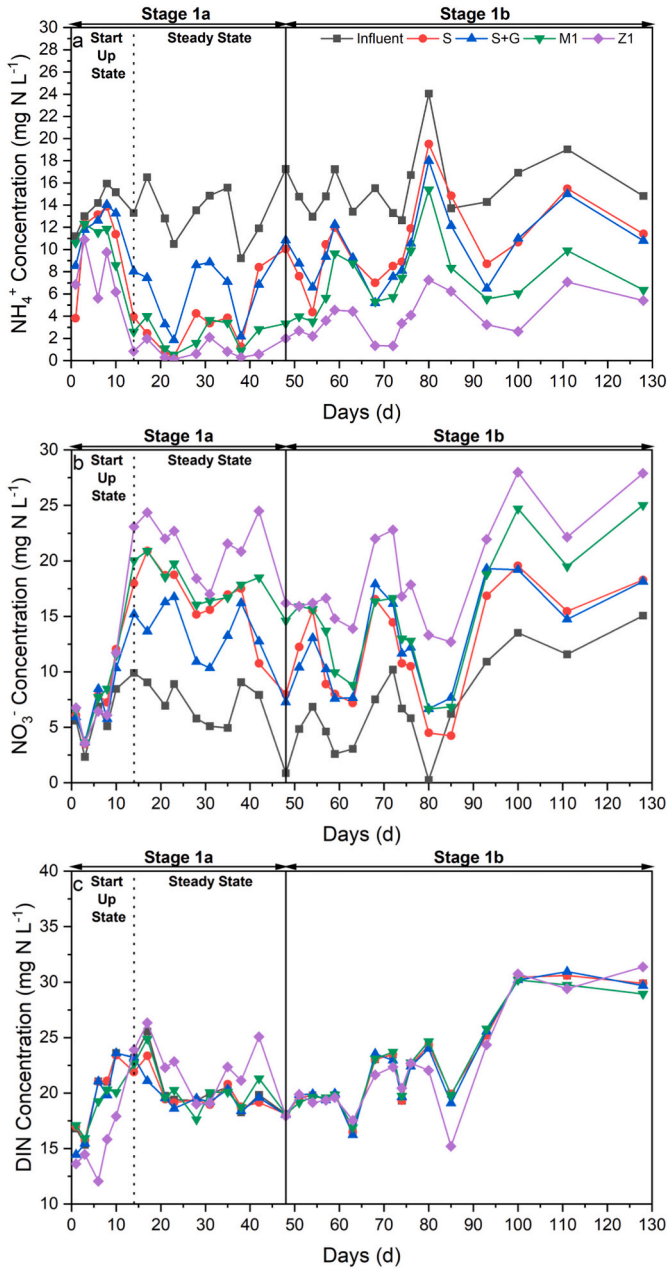

Fig. 2. Concentration of a) NH_4^+, b) NO_3^-, and c) DIN in influents and effluents from different experimental columns during stage 1 of bench-scale column experiment. Stage 1a was operated with 0.48 m^3 m^{-2} d^{-1} wastewater and stage 1b was tested with 0.64 m^3 m^{-2} d^{-1} wastewater. S: sand column, S + G: gravel/sand column, M1: marble chip column and Z1: zeolite column.

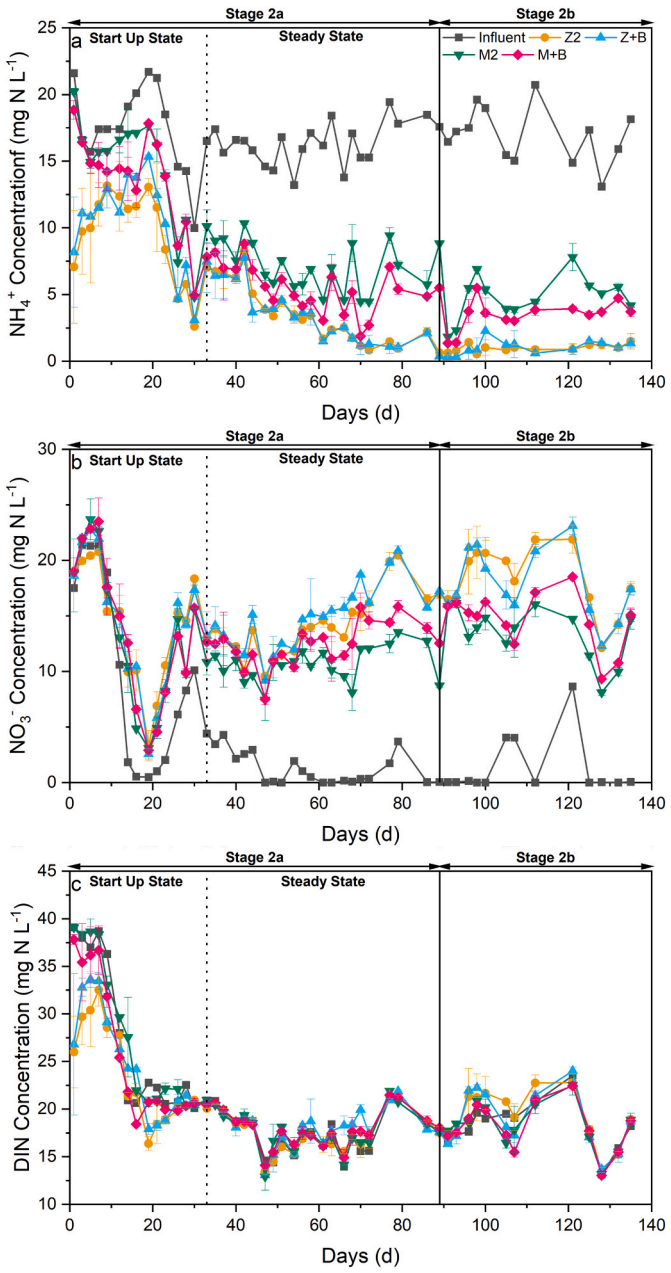

Fig. 3. Concentration of a) NH_4^+, b) NO_3^-, and c) DIN in influents and effluents from different experimental columns during stage 2 of the bench-scale column experiment. Stage 2a was operated with 0.64 m^3 m^{-2} d^{-1} wastewater and stage 2b was tested with 0.48 m^3 m^{-2} d^{-1} wastewater. Error bars represent standard deviations of duplicate columns. Z2: zeolite columns, Z + B: biochar amended zeolite columns, M2: marble chip columns and M + B: biochar amended marble chip columns.

marble chip had negligible NH_4^+-N adsorption capacity. However, Z2, Z + B, and M + B columns removed 4.3 ± 2.3, 3.1 ± 1.9 and 2.4 ± 1.9 mg DIN L^{-1} from the influent, respectively, during the first 28 days of the stage 2 experiment (Fig. 3c and Table S5). Then similar level of DIN was detected in effluents from these columns (Z2: 18.3 ± 2.4 mg DIN L^{-1}, Z + B: 18.5 ± 2.5 mg DIN L^{-1}, M + B: 18.1 ± 2.3 mg DIN L^{-1}) as influent wastewater (18.0 ± 2.4 mg DIN L^{-1}) during the remaining stage 2 experiment (Fig. 3c and Table S5). These results indicated that zeolite and biochar amended columns (Z2, Z + B, and M + B) reached NH_4^+-N adsorption capacities by the first 28 days of the experiment, then nitrification was the major NH_4^+-N removal mechanism.

At stage 2a, the alkalinity concentration decreased from 231.3 ± 40.5 mg $CaCO_3$ L^{-1} in the influent to 167.1 ± 30.0–185.6 ± 27.8 mg $CaCO_3$ L^{-1} in effluents from all columns, respectively (Fig. S5c). Then significantly higher reduction of alkalinity was observed in all columns due to the promoted nitrification process by lower HLR (0.48 m^3 m^{-2} d^{-1}) at stage 2b. All columns reduced alkalinity concentration from 250.8 ± 46.6 mg $CaCO_3$ L^{-1} in influent wastewater to 141.9 ± 28.2–169.9 ± 35.2 mg $CaCO_3$ L^{-1} in effluents at stage 2b, respectively (Fig. S5c).

3.4. Microbial abundance and distribution

At stage 1, total biomass in sand, gravel, marble chip, and zeolite were 7.2 ± 1.0 × 10^9, 2.0 ± 0.1 × 10^9, 3.3 ± 0.2 × 10^9 and 6.3 ± 0.4 × 10^9 16S rRNA copies g^{-1}, respectively (Fig. 4a). The abundance of

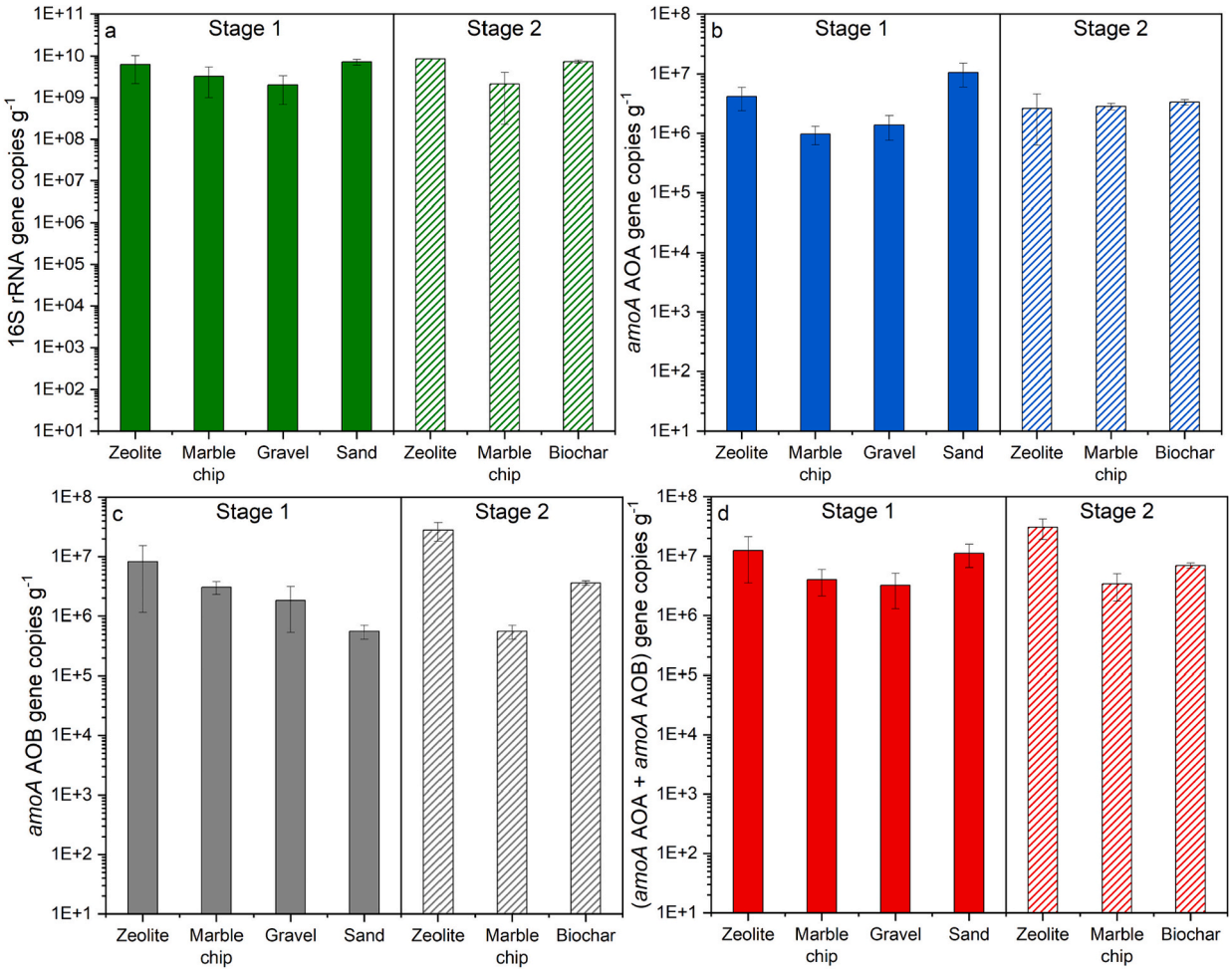

Fig. 4. Absolute abundances of a) 16S rRNA and functional genes: b) *amoA* AOA c) *amoA* AOB and d) *amoA* AOA + *amoA* AOB (AOM) in different materials collected at different operational stages. Error bars represent the standard deviations of experimental triplicates.

ammonium oxidizing microorganisms (AOM) was calculated as the sum of AOA and AOB. The results showed the levels of AOM in zeolite ($1.2 \pm 0.1 \times 10^7$ *amoA* AOM copies g^{-1}) and sand ($1.1 \pm 0.2 \times 10^7$ *amoA* AOM copies g^{-1}) were 64–73 % higher than that in marble chip ($4.0 \pm 0.3 \times 10^6$ *amoA* AOM copies g^{-1}) and gravel ($3.2 \pm 0.3 \times 10^6$ *amoA* AOM copies g^{-1}) (Fig. 4d and Table S8). The abundance of AOB in zeolite ($8.3 \pm 0.7 \times 10^6$ *amoA* AOB copies g^{-1}) was 62–93 % higher than that in sand ($5.6 \pm 0.3 \times 10^5$ *amoA* AOB copies g^{-1}), gravel ($1.8 \pm 0.1 \times 10^6$ *amoA* AOB copies g^{-1}), and marble chip ($3.1 \pm 0.2 \times 10^6$ *amoA* AOB copies g^{-1}) (Fig. 4c and Table S8). However, sand contained the highest level of *amoA* AOA ($1.1 \pm 0.2 \times 10^7$ *amoA* AOA copies g^{-1}) and 62–91 % lower abundance of AOA was observed in zeolite ($4.2 \pm 0.6 \times 10^6$ *amoA* AOA copies g^{-1}), gravel ($1.4 \pm 0.2 \times 10^6$ *amoA* AOA copies g^{-1}), and marble chip ($9.7 \pm 1.3 \times 10^5$ *amoA* AOA copies g^{-1}) (Fig. 4b and Table S8).

At stage 2, the abundance of 16S rRNA in zeolite, marble chip, and biochar were $8.5 \pm 0.4 \times 10^9$, $2.1 \pm 0.2 \times 10^9$ and $7.4 \pm 0.7 \times 10^9$ 16S rRNA copies g^{-1}, respectively (Fig. 4a and Table S8). The highest abundance of AOM was observed in zeolite ($3.1 \pm 0.1 \times 10^7$ *amoA* AOM copies g^{-1}) which was around one magnitude higher than that in biochar ($7.0 \pm 0.6 \times 10^6$ *amoA* AOM copies g^{-1}) and marble chip ($3.4 \pm 0.3 \times 10^6$ *amoA* AOM copies g^{-1}) (Fig. 4d and Table S8). Zeolite ($2.8 \pm 0.1 \times 10^7$ *amoA* AOB copies g^{-1}) and biochar ($3.6 \pm 0.3 \times 10^6$ *amoA* AOB copies g^{-1}) contained 1–2 magnitude higher levels of AOB than marble chip ($5.6 \pm 0.1 \times 10^5$ *amoA* AOB copies g^{-1}) (Fig. 4c and Table S8). However, zeolite ($2.6 \pm 0.4 \times 10^6$ *amoA* AOA copies g^{-1}), biochar ($3.4 \pm 0.3 \times 10^6$ *amoA* AOA copies g^{-1}), and marble chip ($2.8 \pm 0.3 \times 10^6$ *amoA* AOA copies g^{-1}) showed similar levels of AOA (Fig. 4b and Table S8).

4. Discussions

4.1. Ammonium removal mechanism in zeolite and biochar

Adsorption and nitrification were reported to be the major NH_4^+-N removal mechanisms in biofilters filled with zeolite and biochar [36,37]. However, adsorption can only temporarily remove NH_4^+-N from wastewater, while replacement or regeneration of the material is required after it reaches the adsorption capacity. On the other hand, nitrification is a biological process that can achieve stable NH_4^+-N removal in biofilters for long-term operation with low maintenance demand. Therefore, understanding the NH_4^+-N removal mechanism is critical for determining the sustainability of the filtration material for wastewater treatment. In this study, nitrification contributed to 82 %, 87 %, 92 %, and 95 % of NH_4^+-N removal in Z1, Z2, Z + B, and M + B columns during the entire experiment, respectively (Fig. S6). This observation was comparable with the contribution of nitrification to NH_4^+-N removal (79–87 %) in other biofilters amended with zeolite or biochar treating domestic wastewater or anaerobically digested swine wastewater [38,39]. Adsorption also contributed to a small portion of NH_4^+-N removal in Z1 (11%), Z2 (15%), Z + B (6 %), and M + B (4 %) columns during the entire column experiment (Fig. S6). The low adsorption capacities of aged zeolite (0.3 mg NH_4^+-N g^{-1} aged zeolite) and biochar (0.2 mg NH_4^+-N g^{-1} aged biochar) also suggested adsorption contributed to limited NH_4^+-N removal after long-term operation in zeolite and

biochar biofilters. Other processes such as precipitation and microbial assimilation contributed to <3 % of NH_4^+-N in zeolite and biochar amended columns (Fig. S6). These results demonstrated that nitrification was the major NH_4^+-N removal mechanism for long-term operation in zeolite and biochar biofilters.

Limited adsorption in zeolite and biochar may be attributed to the short HRTs in the column experiment and their low adsorption capacities for NH_4^+-N in real wastewater. The short HRTs (3.9 h and 5.2 h) may result in insufficient contact time between water flow and the filter material, decrease the extent of NH_4^+-N adsorption, and lead to a small contribution of adsorption to NH_4^+-N removal. However, when longer HRTs (>5 h) were applied, biological nitrification was likely still the major NH_4^+-N removal mechanism in zeolite or biochar amended filters for long-term treatment of onsite wastewater. Longer HRTs may increase the overall mass of NH_4^+-N being adsorbed by zeolite or biochar. However, after the materials reached their NH_4^+-N adsorption capacities, nitrification was still the major NH_4^+-N removal mechanism. This was supported by previous studies that nitrification was found to be the major NH_4^+-N removal mechanism in zeolite biofilters at long HRTs (66–158 h) after long-term (260–360 days) treatment of anaerobically digested swine wastewater and domestic wastewater [38,39]. In addition, the adsorption capacities of NH_4^+-N by zeolite and biochar were overestimated with adsorption kinetic experiments conducted at lab conditions using synthetic ammonium solution. Both zeolite and biochar showed significantly higher NH_4^+-N adsorption capacity and faster NH_4^+-N adsorption kinetics with synthetic ammonium solution (8.5 mg NH_4^+-N g^{-1} zeolite and 3.1 mg NH_4^+-N g^{-1} biochar) than real influent wastewater (2.4 mg NH_4^+-N g^{-1} zeolite and 1.0 mg NH_4^+-N g^{-1} biochar). The lower adsorption capacities and rates of real influent wastewater NH_4^+-N observed in zeolite and biochar may be attributed to the competition of other cations and organic matters in wastewater. Previous literature demonstrated that zeolite and biochar have high adsorption affinity for K^+, Ca^+, Na^+, and some heavy metals such as Cs^+, Rb^+, Fe^{3+}, and Al^{3+} in wastewater [36,37,40]. These cations may compete with NH_4^+ for the adsorption site on zeolite and biochar and reduce the NH_4^+-N adsorption capacities and kinetics. The organic matters in real influent wastewater can also be adsorbed by zeolite and biochar, occupying the adsorption site or causing pore blockage which makes the adsorption sites in small pores unavailable for NH_4^+-N [41]. This mechanism was confirmed by the reduced COD concentration from 31.7 mg L^{-1} at the initial stage of the batch adsorption experiment to 16.8 mg L^{-1} in zeolite and 12.3 mg L^{-1} in biochar at the end of the adsorption experiment (Fig. S7). The NH_4^+-N adsorption capacities of the fresh zeolite and biochar in synthetic ammonium solution were in the range reported for natural zeolite (7.1–16.8 mg NH_4^+-N g^{-1} zeolite) and biochar (0.2–14.3 mg NH_4^+-N g^{-1} biochar) in previous studies [32,34,42–46]. However, the NH_4^+-N adsorption kinetics and adsorption capacities of zeolite and biochar in real wastewater were rarely evaluated in the previous studies. The significant discrepancy in NH_4^+ adsorption performance observed in our experiment conducted with real wastewater provided valuable information when we evaluated filtration material adsorption capacity in practical conditions.

Previous studies reported dynamic equilibrium of NH_4^+ removal on the zeolite: firstly, the adsorption process adsorbs the NH_4^+-N at the surface of zeolite until equilibrium, then the nitrification process removes NH_4^+-N adsorbed at the surface of zeolite and breaks the adsorption equilibrium. Subsequently, the adsorption process occurs again to create a new equilibrium on the zeolite surface [39]. Since similar contributions of nitrification (82–95 %) and adsorption (4–15 %) for NH_4^+-N removal were observed in zeolite columns (Z1 and Z2) and biochar amended columns (Z + B and M + B) in this study, we speculated that the dynamic adsorption-nitrification process can also be the major NH_4^+-N removal mechanism in biochar amended columns (Z + B and M + B). The low but significant NH_4^+-N adsorption capacities in aged zeolite (0.2 mg NH_4^+-N g^{-1} zeolite) and biochar (0.3 mg NH_4^+-N g^{-1} biochar) suggested that the equilibrium of NH_4^+ adsorption in zeolite and biochar was broken and confirmed there was a dynamic process of NH_4^+-N removal on zeolite and biochar. The dynamic balance of nitrification and adsorption assisted zeolite and biochar to achieve better NH_4^+-N removal performance than other materials (sand, gravel, and marble chip). Meanwhile, the adsorption process can also concentrate the NH_4^+-N on the surface of zeolite and biochar which can increase the density of nitrifying microorganisms and then promote the nitrification process. It was confirmed by the significantly higher abundance of AOM observed in zeolite (3.1×10^7 *amoA* AOM copies g^{-1}) and biochar (7.0×10^6 *amoA* AOM copies g^{-1}), compared with marble chip (3.4×10^6 *amoA* AOM copies g^{-1}) at stage 2.

4.2. Compensation of alkalinity by zeolite and marble chip

Alkalinity plays an important role in maintaining optimal pH levels in water to achieve efficient NH_4^+-N removal in biofiltration systems because the nitrification process can release H^+ and reduce the pH in water. In this study, zeolite (Z1 and Z2) and marble chip (M1 and M2) columns consumed 6.2–6.7 and 5.7–6.7 mg $CaCO_3$ L^{-1} alkalinity to remove 1 mg NH_4^+-N L^{-1}, respectively, which were lower than the theoretical alkalinity consumption (7.1 mg $CaCO_3$ L^{-1}) to maintain the optimal pH (6.5–7.5) for nitrification [47]. This result demonstrated that the zeolite and marble chip can compensate for the alkalinity loss caused by the nitrification process.

The dissolution of $CaCO_3$, which is the major composition of marble chip, may contribute to the lower alkalinity consumption in marble chip (M1 and M2) columns [25]. Previous literature reported continuous dissolution of alkalinity from marble chip until the pH in water reached around 7–7.5 [26]. The supplementation of alkalinity in zeolite may be contributed by the hydrolysis process. The hydrolysis of zeolite involves the exchange of cation in zeolite and H^+ in water which can produce OH^- and neutralize the H^+ released by the nitrification process [48,49]. The nitrification process can continuously remove the NH_4^+-N and regenerate the cation exchange capacity at the surface of zeolite which promotes the OH^- released by hydrolysis and maintains a suitable pH range in water for the nitrification process [50]. The alkalinity compensation ability makes marble chip and zeolite ideal materials to keep the pH in wastewater at the optimal range (6.5–7.5) for the growth of nitrifying microorganisms and achieve efficient NH_4^+-N removal from wastewater with alkalinity deficiency [51].

Where Me is the exchangeable cation, Z is zeolite, n is the cation charge, s is the solid phase and l is the liquid phase.

4.3. Filtration material selection at elevated hydraulic loadings

Most conventional biofiltration systems designed for onsite wastewater treatment utilize sand as the filtration media due to its low cost, local availability, and acceptable treatment performance [9,18,52,53]. However, a 3-month to annual maintenance interval was recommended for most sand-based biofiltration systems to inspect the extent of clogging and replace the clogged sand as needed [9,54,55]. The clogging potential of sand-based biofilters was directly related to the HLR. Previous studies reported that HLRs exceeding 0.163 m^3 m^{-2} d^{-1} can result in biofilter clogging and HLRs higher than 0.326 m^3 m^{-2} d^{-1} can lead to bi-monthly (70 days) clogging problems [56,57]. Hence, to avoid frequent clogging, most sand-based biofilters can only accommodate low HLRs (0.01–0.04 m^3 m^{-2} d^{-1}) (Table 2) [13,17,18]. Materials with high porosities and big particle sizes such as zeolite and gravel have been used as filtration materials to treat wastewater at high HLRs (0.3–0.6 m^3 m^{-2} d^{-1}) and achieved 40–100 % NH_4^+-N removal (Table 2) [20–22,58]. However, the overall design for these biofilters was conservative, with deep depth (30–230 cm) to achieve high HRTs (11–96 h) for sufficient NH_4^+-N removal (40–100 %) [19–21,58].

In this study, zeolite and marble chip with higher porosities and bigger particle sizes can significantly alleviate the clogging problem of biofilters while achieving better treatment performance (54.8–94.1 %)

Table 2
A comparison of characteristics and treatment performance of different filtration materials.

Material	System	Wastewater Source	Depth (cm)	HRT[a] (hours)	HLR (m^{-3} m^{-2} d^{-1})	NH_4^+ Loading (g N m^{-2} d^{-1})	NH_4^+ Removal Efficiency (%)	Ref
Gravel	Trickling filter	Septic tank effluent	60	45	0.16	18.3	78	[21]
	Constructed wetland	Synthetic municipal wastewater	227	40	0.68	45.3	47	[76]
	Constructed wetland	Synthetic municipal wastewater	33	80	0.05	1.0	48	[77]
Zeolite	Trickling filter	Synthetic municipal wastewater	60	16–30	0.24–0.44	9.8–18.1	57–85	[27]
	Biofilter	Treated effluent from constructed wetland	43–49	11–27	0.22–0.48	5.2–11.3	78–85	[30]
	Constructed wetland	Anaerobic digestion effluent	40	96	0.1	40.0–50.0	96–98	[78]
	Biofilter	Diluted septic tank effluent	15	4–5	0.48–0.68	6.3–10.4	76–94	This study
Biochar	Constructed wetland (100 % amendment)	Synthetic municipal wastewater	227	40	0.68	45.3	77	[76]
	Trickling filter (100 % amendment)	Anaerobic digestion effluent	50	35	0.17	85.3–101.9	81–89	[79]
Marble Chip	Biofilter	Municipal wastewater	60	24–96	0.15–0.6	0.6–2.4	80–99	[25]
	Biofilter	Diluted septic tank effluent	15	4–5	0.48–0.68	6.3–10.4	55–84	This study
Sand	Biofilter	Septic tank effluent	46	110–147	0.03–0.04	1.1–1.4	93–96	[18]
	Biofilter	Municipal wastewater	50	60–120	0.01–0.02	0.51–1.54	75–76	[17]
	Biofilter	Diluted septic tank effluent	15	4–5	0.48–0.68	6.3–10.4	76–94	This study

[a] Hydraulic retention time was calculated based on the assumption that the porosity of biochar/zeolite/marble chip is 0.5 and sand porosity is 0.2.

than sand filters at the same treatment depth (15 cm) with short HRTs (3.9–5.2 h). The reduced clogging potential of marble chip and zeolite enabled the biofilters to accommodate 3–30 times higher HLR (0.48–0.64 m^3 m^{-2} d^{-1}) compared with conventional sand filters with lower maintenance demand and smaller footprint. However, in this study, COD/NH_4^+-N ratio in influent wastewater was around 2.5, which was lower than the typical COD/NH_4^+-N ratio in onsite wastewater (2–6) [9,12,18,59]. The higher COD/NH_4^+-N ratio may increase the competition between heterotrophic bacteria and nitrifying microorganism for oxygen and inhibit the NH_4^+-N removal. Hence, it is recommended to evaluate the hydraulic and NH_4^+-N removal performance of marble chip and zeolite biofilters for long-term STE treatment and at a higher COD/NH_4^+-N ratio. This is currently being tested in a field CFB system in the WRIF of CCWT.

When zeolite and marble chips are applied in onsite wastewater treatment, the effective biofilter depth is largely impacted by the influent wastewater strength. In this study, 15-cm column depth provided sufficient HRTs for zeolite to achieve efficient NH_4^+-N removal (0.8–3.8 mg NH_4^+-N L^{-1} in effluents) at NH_4^+-N loading rate of 7.0–10.8 g NH_4^+-N m^{-2} d^{-1} but lead to the high effluent NH_4^+-N concentration in marble chip columns (7.2 mg L^{-1} NH_4^+-N L^{-1}) at high NH_4^+-N loading rate (10.8 g NH_4^+-N m^{-2} d^{-1}). These results indicated that 15-cm zeolite column was able to effectively remove NH_4^+-N at the loading rate below 11 g NH_4^+-N m^{-2} d^{-1}. However, if higher NH_4^+-N loadings were applied or marble chips were used, the filter depth shall be increased to accommodate higher HRTs for efficient NH_4^+-N removal. In addition, the zeolite or marble chip can also be utilized in other biofiltration systems such as bioretention systems or trickling filters for stormwater treatment with elevated hydraulic loadings to reduce the clogging potential and increase the treatment performance. It is worth noting that the cost and local availability of materials should also be considered when selecting the biofiltration material. In our experiment, the zeolite (Z1 and Z2) columns showed an overall higher NH_4^+-N removal efficiency (75.8–83.6 %) than marble chip (M1 and M2) columns (54.9–61.1 %) at an HLR of 0.64 m^3 m^{-2} d^{-1}. The marble chip (M1 and M2) columns (69.7–83.9 %) and zeolite (Z1 and Z2) columns (94.1 %) achieved comparably high NH_4^+-N removal performance at a relatively low HLR (0.48 m^3 m^{-2} d^{-1}) and a high HRT (5.2 h). Zeolite is a kind of aluminosilicate mineral and its cost is highly related to the local availability [27,28]. Marble chip is a recycled waste generated from the construction industry, which makes marble chip available in most areas and reduces its overall cost [25]. When the biofilter is designed to treat wastewater containing low concentrations of NH_4^+-N or at relatively low HLRs, the selection of filtration materials from marble chip or zeolite shall be based on a comprehensive evaluation of cost, local availability, design treatment capacity, and discharge limit requirement.

4.4. The feasibility of biochar amendment to biofilters at elevated hydraulic loadings

Biochar amendment (30 % vol, vol) to marble chip column resulted in 23–29 % decrease of effluent NH_4^+-N at HLRs of 0.48–0.64 m^3 m^{-2} d^{-1}. This observation was consistent with previous studies that when 30 % biochar was amended to gravel and sand filters, 2–26 % further removal of NH_4^+-N was observed in the effluent [7,19,31]. The enhanced NH_4^+-N removal observed in M + B columns was majorly contributed by the higher surface area and effective NH_4^+-N adsorption capability of biochar [34]. The high surface area and NH_4^+-N adsorption capacity of biochar also increased the nitrifying biomass density and promoted the nitrification process. This was supported by the higher abundance of AOM observed in biochar (7.0×10^6 *amoA* AOM copies g^{-1}) than marble chip (3.4×10^6 *amoA* AOM copies g^{-1}).

Biochar amendment did not change the NH_4^+-N removal performance in Z2 columns (Fig. 3a) and the result was in agreement with a previous study that similar NH_4^+-N removal (81–83 %) was observed in lab-scale columns filled with 100 % zeolite and 100 % biochar treating anaerobic digestion effluents at a HLR of 0.17 m^3 m^{-2} d^{-1} [60]. In the batch adsorption experiment, the NH_4^+-N adsorption capacity by zeolite (2.4 mg NH_4^+-N g^{-1} zeolite) was higher than biochar (1.0 mg NH_4^+-N g^{-1} biochar) when real wastewater was applied. Considering the filtration material was packed for biofilters with volumetric consideration, the higher bulk density of zeolite (800–1100 kg m^{-3} zeolite versus 100–400 kg m^{-3} biochar) should result in better NH_4^+-N adsorption performance than biochar when utilized in biofiltration systems treating onsite wastewater [60–62].

In addition, the NH_4^+-N adsorption capacity of biochar may vary significantly due to its production conditions, such as pyrolysis temperature, feedstock, and modification method [34]. The increased

pyrolysis temperature can significantly increase the surface area, providing more adsorption sites for NH_4^+-N and subsequently enhancing the NH_4^+-N adsorption capacity [63,64]. Furthermore, biochar produced from woody materials such as straw, oak, and pine showed a higher NH_4^+ adsorption capacity (2.7–4.2 mg NH_4^+-N g^{-1} biochar) than non-woody-source (e.g., manure and sludge) biochar (1.2–1.8 mg NH_4^+-N g^{-1} biochar) probably because the low H/C ratio in non-woody-source biochar decreases the number of organic functional groups on biochar surface [44,65]. In this study, the biochar tested for column experiment was pyrolyzed from beetle kill pine at 500 °C and its NH_4^+-N adsorption capacity of synthetic ammonium solution (1.0 mg NH_4^+-N g^{-1} biochar) was in the lower range reported in other studies (0.2–4.3 mg NH_4^+-N g^{-1} biochar) [7,37,43,44,46,64]. The limited adsorption capacity of biochar used in this study may also lead to the insignificant change in NH_4^+-N removal performance observed in Z + B columns (Fig. 3a).

The pyrolysis process could alter the physical properties of biochar and reduce its mechanical strength [66]. The low mechanical strength of biochar may raise some concerns about its application as filter materials for onsite wastewater treatment. When wastewater was applied at high HLRs, the high shear stress of water flow may break down biochar and clog the biofilters. During the stage 2 experiment (0.48–0.64 m^3 m^{-2} d^{-1}), fine particles scraped from biochar were flushed out with water flow and clogged the effluent port of biochar amended columns (Z + B and M + B). A similar clogging issue, attributed to fragmented fine biochar particles, was also reported in another stormwater bioretention system amended with 30 % biochar at a high HLR (3.5 m^3 m^{-2} d^{-1}) [67]. In long-term operation, if a high HLR is applied to the biofilter, a significant amount of biochar amended in biofilters may be lost and negatively impact the treatment performance. Around 20–53 % of biochar in the agricultural soil was reported to be lost through intense rainfall [68]. In addition, a high amendment ratio (> 50 %) of biochar may even destroy the internal structure of the biofilter due to its poor mechanical properties [67,69]. The 30 % (volumetric) amendment ratio of biochar in this study was chosen based on the results of our previous study, 30 % of biochar amendment to the sand filters could maintain a stable internal structure of biofilter while achieving better ammonium removal performance [7]. For the application of biochar in the field onsite wastewater biofiltration systems, <30 % of biochar amendment should be considered to maintain the mechanical structure.

4.5. Microbial abundances in different materials

During the entire bench-scale column experiment, the abundances of AOB in zeolite, biochar, and marble chip were positively related to their NH_4^+-N removal performance. Zeolite with the highest level of AOB abundance achieved the most efficient NH_4^+-N removal at stage 2 (Figs. 3a, 4c, Tables S4, S6, and S8). Biochar contained 84 % higher AOB abundance than marble chip. The higher AOB level observed in biochar amended marble chip columns may explain the enhanced NH_4^+-N removal efficiency (i.e. 11.3–12.2 % higher) compared with pure marble chip columns at stage 2. However, no significant relationship was observed between AOA abundance and NH_4^+-N removal performance in zeolite, biochar, and marble chip. At stage 2, zeolite, marble chip, and biochar contained a similar abundance of AOA. However, Z2 columns showed similar NH_4^+-N removal performance with Z + B columns but 25.9–26.8 % higher NH_4^+-N removal efficiency than M2 columns (Figs. 3a, 4c Tables S4 and S7). The result may indicate that AOB was more functionally important in the biofilters treating onsite wastewater. Previous literature also reported that AOB was functionally more important to the nitrification process in municipal wastewater treatment plants even greater abundance of AOA was observed [70,71]. Approximately 80–90 % of nitrification in agricultural soil was also reported to be contributed by AOB rather than AOA [72].

The sand had a significantly higher *amoA* AOA/*amoA* AOB ratio (18.8) than gravel (0.7), marble chip (0.1), and zeolite (0.5) at stage 1. The frequent clogging observed in the S column can inhibit oxygen transport and distribution in the column, reducing the DO and limiting the nitrification process [55]. The low DO condition can promote the growth of AOA and inhibit the AOB because AOA has a higher affinity for oxygen than AOB [73,74]. Around 400 % increase of AOA and a 20 % reduction of AOB were reported in low DO condition (0.4 mg L^{-1}) compared with high DO condition (2 mg L^{-1}) in a lab-scale membrane reactor treating municipal wastewater [74]. While low oxygen environment can also suppress the nitrification activity of AOB but has no significant impact on AOA, further reducing the nitrification performance of clogged sand in sand columns [70–72,75]. That may explain the contradicted observation of high nitrifying AOA abundance and inefficient NH_4^+-N removal performance observed in sand columns at stage 1 (Figs. 2a and 4b).

5. Conclusions

Our study demonstrated that zeolite and marble chip with higher porosities (0.4–0.5) and bigger particle sizes (10–20 mm) can achieve significantly higher NH_4^+-N removal efficiencies (zeolite: 75.8–94.1 %, marble chip: 54.9–83.9 %) than sand (32.9–76.6 %) and gravel/sand (36.3–57.7 %) at high hydraulic loadings (0.48–0.64 m^3 m^{-2} d^{-1}) and short HRTs (3.9–5.2 h) with limited clogging concerns. Compared with marble chip, biochar contained 84 % more AOB, which resulted in 23–29 % lower effluent NH_4^+-N concentrations from the M + B column. However, the biochar amendment did not impact NH_4^+ removal in zeolite columns. Nitrification rather than adsorption contributed to the majority of NH_4^+-N removal (82–95 %) in all columns amended with zeolite or biochar (Z1, Z2, Z + B, and M + B) during a total of 266 days of operation. Marble chip and zeolite can compensate for the alkalinity loss caused by nitrification due to $CaCO_3$ dissolution and hydrolysis, respectively. Collectively, this study suggested that zeolite and marble chip are optimal filtration materials for biofilters treating onsite wastewater at high hydraulic loadings with less clogging concern while stable and efficient NH_4^+ removal could be retained. Biochar amendment may further improve NH_4^+ removal efficiency in marble chip biofilters.

CRediT authorship contribution statement

Siwei Chen: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mian Wang:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Danny Lin:** Methodology, Investigation, Formal analysis, Data curation. **Frank M. Russo:** Writing – review & editing, Project administration, Conceptualization. **Christopher J. Gobler:** Project administration, Funding acquisition. **Xinwei Mao:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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