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Treatment of Dairy Farm Runoff in Vegetated Bioretention Systems Amended with Biochar

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Abstract: Nitrogen and fecal indicator bacteria (FIB) in runoff from concentrated animal feeding operations (CAFOs) can impair surface and groundwater quality. Bioretention systems are low impact nature-based technologies that can effectively treat CAFO runoff if modified with an internal water storage zone (IWSZ) or amended with biochar. In this study, the performances of four pilot-scale modified bioretention systems were compared to assess the impacts of (1) amending bioretention media with biochar and (2) planting the systems with *Muhlenbergia*. The system with both plants and biochar amendment had the best performance, with an average of 5.58 log reduction in *E. coli* and 98% removal of total nitrogen (TN). All systems treated the first pore volume well as new runoff flushed the treated water from the IWSZ. Biochar improved TN and FIB removal due to its high capacity to adsorb or retain ammonium (NH_4^+), dissolved organic nitrogen, dissolved organic carbon, and *E. coli*. Planting improved performance, possibly by increasing rhizosphere microbial activity.

Keywords: agricultural runoff; biofilter; *E. coli*; fecal indicator bacteria; internal water storage zone; reactive nitrogen

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1. Introduction

Runoff from concentrated animal feed operations (CAFOs) carries organic matter, suspended solids, nutrients (nitrogen [N] and phosphorus [P]), and pathogens that can impair surface and ground water quality [1,2]. These contaminants can cause eutrophication, fish deaths, economic losses, and nitrate (NO_3^-) and pathogen contamination of drinking water supplies [3]. When used for irrigation, runoff from CAFOs has also been shown to contaminate vegetable crops with fecal indicator bacteria (FIB) [4]. For example, groundwater polluted by livestock wastes was responsible for contaminating vegetable crops with *E. coli* O157:H7, which is associated with food-borne illnesses [5]. Although FIBs, such as *E. coli* and *Enterococci*, are usually not pathogenic, their presence indicates potential fecal contamination of water [6].

Nature-based solutions (NBSs) for CAFO runoff include vegetated buffer strips, bioretention systems, lagoons, and constructed wetlands. These technologies are designed to remove organic matter and suspended solids from runoff; however, they often have low removal rates for dissolved nutrients due to high loading rates and limited hydraulic retention times [7]. Additionally, NBSs are not designed with alternating aerobic/anaerobic zones that promote biological nitrification and denitrification processes. Ibekwe et al. [8] reported that constructed wetlands removed only 16% of ammonium (NH_4^+), 25% of total nitrogen (TN), and 33% of phosphate (PO_4^{-3}). Similarly, increases in total kjeldahl nitrogen and chemical oxygen demand concentrations and leaching of nitrate (NO_3^-) (11–45 mg/L) were observed in vegetated buffer strips due to insufficient nitrification/denitrification [9].

A wide range of removal efficiencies have been reported for FIBs in NBSs. For example, in California's San Joaquin Valley (USA), log *E. coli* reductions ranged from 0.51 to 1.30 for four wetlands treating irrigation runoff [10]. Similarly, minimal retention of FIB (0.16 log removal) was observed for vegetative buffer strips treating dairy runoff [11]. Rusciano and Obropta [12] investigated the effectiveness of sand bioretention systems for the treatment of manure slurry runoff and found 0.37–2.7 log fecal coliform reductions of over 13 simulated runoff events. Although several studies have separately investigated FIB or nitrogen removal in NBSs, there is a lack of research comprehensively investigating both FIB and nitrogen removal in NBSs treating CAFO runoff.

Bioretention systems are a type of NBS consisting of a shallow depression containing the following layers of engineered porous media (top to bottom): (i) topsoil, mulch, or compost as surface layer with or without plants; (ii) sand or sand with alternative filtration medium; and (iii) a gravel drainage layer. A modified bioretention system design includes a saturated internal water storage zone (IWSZ), which is created using an elevated outlet pipe [13]. The IWSZ promotes the development of anoxic conditions to facilitate denitrification. For carbon limited runoff, an electron donor, such as woodchips or elemental sulfur pellets, may be added to the IWSZ [14] to enhance both nitrogen and FIB removal [13,15].

A wide variability in FIB removals has been reported for bioretention systems (conventional and/or modified) [16–18]. *E. coli* removal in bioretention systems is influenced by attachment, straining, predation, competition, and die-off [19,20]. Attachment is impacted by media properties and surface characteristics, FIB microbiology, and physico-chemical properties of the suspending fluid (e.g., pH and ionic strength), whereas straining is controlled by the pore and particle sizes of the porous medium [18,21–23]. Conventional sand media has a low specific surface area (SA) (0.1 m^2/g) [24] and narrow pore size distribution (10–150 μm) [25]. Therefore, amendment of sand with a material having a high SA and micro-porous structure could enhance FIB removal [26].

Biochar is a low-cost carbon-rich by-product of the pyrolysis of waste organic feedstocks, such as wood or animal waste, under oxygen-limited conditions. In crop studies, biochar amendment has been shown to help retain soil moisture, nutrients, and organic carbon and to enhance microbial activity, nitrogen fixation, and plant growth [27,28]. These improvements are due to the high cation exchange capacity (CEC), SA, and microporous structure of biochar.

Several prior studies have investigated biochar amendment of bioretention systems to enhance N removal. Rahman et al. [26] found that the high CEC of biochar increased NH_4^+ retention in bioretention columns treating urban runoff. Adsorbed NH_4^+ was oxidized to NO_3^- when aerobic conditions were present during the antecedent dry period (ADP) between runoff events. Berger et al. [29] found that biochar improved denitrification in woodchip biofilters used for stormwater treatment by reducing dissolved oxygen concentrations and increasing biomass density and water retention. Although biochar amendment improved nitrogen removal in modified bioretention systems treating urban runoff [26,30,31], there is limited information on its ability to enhance CAFO runoff treatment, where FIB and nitrogen concentrations can be an order of magnitude higher. In a prior study by our group [32], we investigated the effect of biochar type, biochar fraction, hydraulic loading rate (HLR), and ADP on nitrogen and organic carbon removal in laboratory columns treating CAFO runoff. However, this study did not include an IWSZ or plants and did not comprehensively analyze both FIB and nitrogen removal.

Prior research is conflicted about the effect of embedded vegetation on nutrient and FIB removal in bioretention systems [33–36]. Chandrasena et al. [37] found that vegetation had a net negative effect on *E. coli* survival in biofilters. Conversely, Skorobogatov et al. [38] stated that plant roots can negatively impact the media by forming macropores, preferential flow paths, and non-uniform breakthrough, leading to poorly treated runoff. Parker et al. [39] suggested that plants indirectly affected FIB removal, by influencing infiltration rate. Interactions between filtration medium and plants play an important role in water quality [40]. In constructed wetlands, the role of plant-root-associated microbes promote N transformation processes and the secretion of antimicrobial products for pathogen removal [41]. Biochar addition has been shown to dramatically increase plant biomass growth (by a factor of 24) in constructed wetlands systems treating landfill leachate, which contributes to nutrient uptake and rhizosphere microbial activity [42]. However, no prior studies have investigated the effect of vegetation on nutrient and FIB removal in modified bioretention systems amended with biochar.

The overall goal of this research was to comprehensively investigate the effect of biochar and plants on FIB and nitrogen removal in modified bioretention systems treating CAFO runoff. The specific objectives were to test pilot-scale modified bioretention units for their performance in FIB and nitrogen removal under varying ADPs (1) with and without biochar amendment and (2) with and without vegetation. This is the first study to investigate the use of modified biochar amended bioretention systems for managing both nutrients and FIB in CAFO runoff.

2. Materials and Methods

2.1. Porous Medium

Sand was purchased from Seffner Rock and Gravel (Tampa, FL, USA). The sand had a hydraulic conductivity of 13 cm/h, which was within the range suggested for bioretention systems [43]. It had a porosity (n) of 35% ± 0.95, moisture content at field capacity of 23.24% ± 0.96 (by wt.%), and a bulk density of 1.56 ± 0.14 g/cc. The sand contained 0.27% coarse-grain (>1 mm), 9.5% medium-grain (1−0.6 mm), and 90% fine-grain (0.6−100 µm) particles [44].

Biochar used in this study was donated by Biochar Supreme (Everson, WA, USA). The biochar was derived from wood that was pyrolyzed at 900–1000 °C and had a SA of 537 ± 60.15 m^2/g and a CEC of 10.57 cmol/kg. It had a low bulk density (0.10 g/cm^3) and high water-holding capacity (874 gH_2O/100 g biochar). The high pore volume of 0.36 cm^3/g included 0.19 cm^3/g micro-pore volume and 0.15 cm^3/g meso-pore volume. Additional characteristics of the biochar can be found in Rahman et al. [26].

2.2. Semi-Synthetic Dairy Runoff

Fresh dairy manure was collected from South Tampa Farm (Tampa, FL, USA). The manure was mixed with stormwater from Lake Benke, a stormwater pond on the University of South Florida Campus (Tampa, FL, USA). The solution was allowed to settle overnight, after which the supernatant was screened through a 0.25 mm mesh and stored until use. Semi-synthetic dairy runoff was prepared by mixing 60% of the screened supernatant with 40% additional pond water. The target concentrations for N-species were 35 mg/L NH_4^+-N, 1.0 mg/L NO_x-N, and 45 mg/L dissolved organic nitrogen (DON). Concentrations of FIBs were $8.63 \times 10^6 \pm 7.07 \times 10^6$ CFU/100 mL for *E. coli* and 5.43 × 106 ± 4.14×10^6 CFU/100 mL for *Enterococci*. These values were consistent with concentrations observed in prior field studies of CAFO runoff [14,45–47].

2.3. Pilot-Scale Bioretention Systems

Four pilot-scale modified bioretention systems were constructed in cylindrical polyethylene containers with a 77 cm diameter and a 100 cm height (Figure 1), containing (i) sand (S), (ii) sand with plants (S+P), (iii) biochar-amended sand (BC), and (iv) biochar-amended sand with plants (BC+P). A perforated PVC underdrain pipe with an upturned outlet elbow was used to create an IWSZ in each unit. From the bottom up, the units contained (i) 7.6 cm downgraded white river gravel (0.3 cm), (ii) 30.5 cm IWSZ filter medium, (iii) 46 cm unsaturated zone filter medium, (iv) 2.5 cm gravel (1.3 cm), and (v) 15 cm free board as a ponding layer at the top. For the S and S+P units, the IWSZ and unsaturated zone media consisted of the sand described above. Both sand and biochar were freshly collected prior to construction. For the BC and BC+P units, the IWSZ contained sand amended with biochar at a 45% fraction (by volume), and the unsaturated zone contained sand with biochar at a 35% fraction (by volume). Biochar fractions were selected to optimize water quality improvements without damaging hydraulic performance, based on results from our prior research [32]. A filter fabric was placed in between the drainage layer and IWSZ layer to avoid wash-out of fine particles. Note that the IWSZ did not contain wood chips due to the high dissolved organic carbon content (737 ± 200 mg/L) of the dairy runoff, which could promote denitrification.

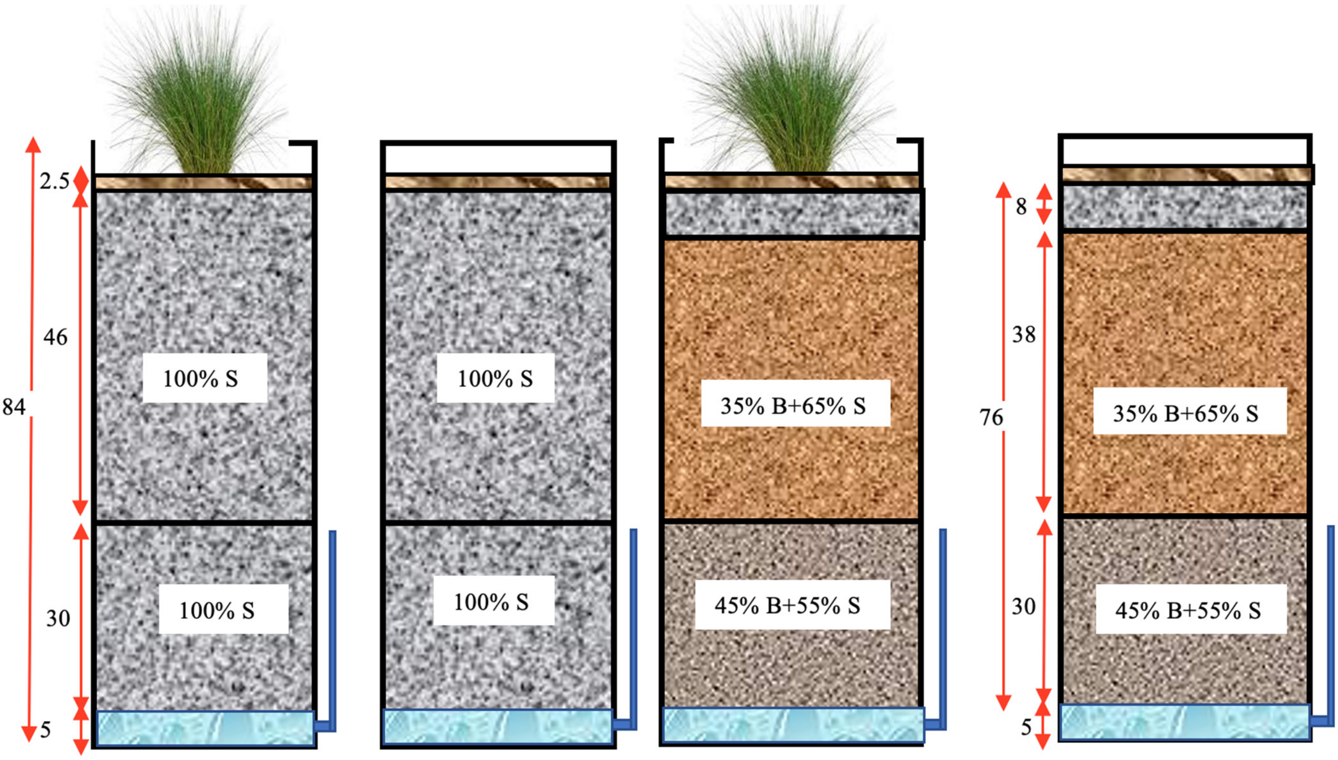

Figure 1. Schematic diagrams of (**left** to **right**) sand-modified bioretention cell with plants (S+P), sand-modified bioretention cell (S), biochar-amended modified bioretention cell with plants (BC+P), and biochar-amended modified bioretention cell (BC). Units are in cm.

S+P and BC+P units were planted with *Muhlenbergia capillaris* (Muhly Grass), which was purchased from a local nursery. *Muhlenbergia capillaris* is a native Florida perennial, that attracts wildlife and has favorable light and moisture requirements, growth rate, and mature plant density and spread. Each system had one *Muhlenbergia*, resulting in a plant density of one plant/1.2 m^2. After planting, the systems were watered with stormwater pond water periodically for three months to promote the growth of roots and biomass before performing dairy runoff experiments. After acclimation, seven semi-synthetic dairy runoff events were studied with varying ADPs between 2 and 21 days. During each event, all four systems were fed semi-synthetic dairy runoff at an HLR of 0.05 cm^3/cm^2/min (flow rate of 3.7 mL/s). In Events 1–5, the systems were fed runoff for a duration of 240 min, with a total influent flow volume of 0.89 L. In Events 6 and 7, the systems were fed runoff for a duration of 240 min, with a total influent flow volume of 1.0 L. Influent and effluent samples were collected every 15 to 30 min over the duration of the events.

2.4. Water Quality Analysis

E. coli were enumerated in duplicate at three dilutions using the EPA Method 1603 membrane filter method with modified membrane-thermotolerant *Escherichia coli* agar (m-TEC) [48]. Dilutions for microbial enumeration were performed in phosphate-buffered saline solution.

An Orion 5-Star meter (Thermo Scientific, Beverly, MA, USA) was used to measure pH and conductivity following Standard Methods 2510 B [49]. NH_4^+ and NO_x (NO_3^--N + NO_2^--N) were measured using a Timberline Ammonia Analyzer (Timberline Instruments, Boulder, CO, USA). TN was measured using a Shimadzu TOC-V CSH TOC/TN Analyzer

(Shimadzu Scientific Instruments, Columbia, MD, USA). DON was calculated by subtracting total inorganic nitrogen (TIN = NH_4^+ + NO_x) from TN. Method-detection limits for NH_4^+, NO_x, and TN were 0.05 mg/L, 0.05 mg/L, and 0.03 mg/L, respectively. Effluent flow rates were measured gravimetrically to assess hydraulic performance and calculate pollutant mass-load reductions.

2.5. Data Analysis

Concentrations of *E. coli* in feed solutions were measured at the start and end of each experiment to confirm that growth/death did not occur in the feed tanks during the experimental period. Log removal values were calculated using

$$E.\ coli \text{ logremoval} = -\log C/C_0 \quad (1)$$

where C_0 and C are the influent and effluent concentrations, respectively.

Mass removals of TOC, NH_4^+, DON, NO_x, and TN (R_{TOC}, R_{NH4+}, R_{DON}, R_{NOx}, and R_{TN}) were calculated using

$$R_x = \frac{\sum_1^N \frac{C_0V_0 - CV}{C_0V_0}}{N} \times 100\% \quad (2)$$

where N is the total number of effluent samples, C_0 and C are the influent and effluent concentrations (mg/L), and V_0 and V are the influent and effluent volumes (L). Duplicate influent samples were collected at the beginning of each event and C_0 concentrations were assumed to remain constant over each runoff event.

Statistical analysis was conducted using JMP 16.0.0 (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) with a significance threshold set at 95% (p = 0.05) was used to determine statistically significant differences between the datasets. Note that due to differences in water retention, the time for effluent to discharge from the units varied. Therefore, the total number of samples (n) collected within the first 90 min varied between systems. For statistical tests between systems, n values for BC, BC+P, S, and S+P were 54, 57, 59, and 56, respectively.

3. Results and Discussion

3.1. Hydraulic Performance

The average influent and effluent flow rate for each system during each storm event is shown in Figure 2. The influent was fed at a steady HLR of 0.05 cm^3/cm^2/min (flow rate = 3.7 mL/s) during each event, resulting in ponding at the top of the bioretention systems followed by slow drainage. The total effluent flow volumes observed from each event for each system are shown in Figure S2. Note that moisture losses between events are affected by both the presence of plants, which increase evapotranspiration, and the ADP (Figure 2). However, if moisture losses are disregarded, the difference between the influent and effluent flow rate gives the average rate of head build-up over time in the ponding layer. A lower flow rate implies slower infiltration through the medium, therefore a longer hydraulic residence time.

The hydraulic performance of the biochar-amended system that was planted with *Muhlenbergia* (BC+P) remained consistent through all seven events, while the performance of the other systems steadily declined over time. Although changes in hydraulic conductivity were not measured over time, plant roots have been shown to increase soil hydraulic conductivity [50]. *Muhlenbergia* has an extensive root system, which can provide additional flow pathways and pore space, potentially increasing the permeability and infiltration capacity of the media. Additionally, the sand and biochar mixture had a greater fraction of coarse- and medium-grained particles compared to sand alone. Although, Koivusalo et al. [51], reported that biochar amendment had no impact on the hydrologic performance of biofilters, Brown and Hunt [52] found that fine particles caused clogging and a reduction in permeability in modified bioretention systems. Results from these studies highlight the need to remove fine particles in bioretention media prior to construction.

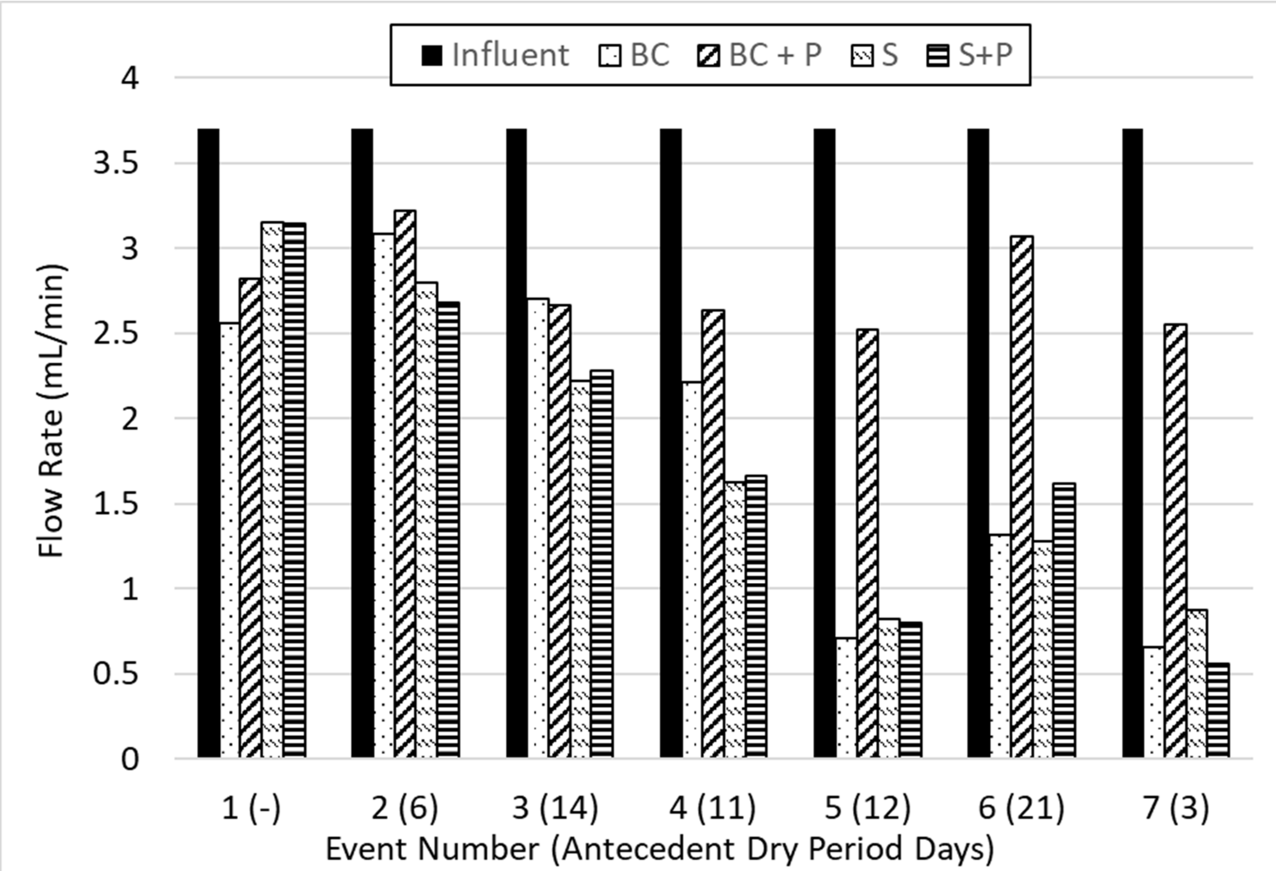

Figure 2. Average influent and effluent flow rates (mL/s) from each system during each runoff event. The antecedent dry period (ADP) for each event is shown in parentheses.

Excessive ponding and piling of water can diminish the feasibility of these systems in practice. Since the hydraulic head builds up over time, longer or more extreme storm events can lead to overflowing and poorly treated runoff. While overflow did not occur during these studies, additional research is necessary to understand the long-term feasibility of these systems under varying HLRs. In contrast, longer retention times due to low effluent flow rates can improve FIB and N removal, which is discussed further below.

3.2. Fecal Indicator Bacteria Removal

Average *E. coli* log removals for the pilot-scale systems are shown in Table 1. In order to elucidate removal mechanisms, log removals are shown as each "pore volume" (PV) of dairy runoff flushed through the system. The highest log *E. coli* removals were observed as the first PV exited the systems. This represented the water that was stored in the IWSZ during the ADP between stormwater applications, in which die-off was the major removal mechanism. Long retention times in the anoxic IWSZ over the ADP could have resulted in high *E. coli* die-off, explaining the best treatment performances during the first PV. After one PV, log *E. coli* removals reveal the systems' treatment during an event, when straining and attachment are the major removal mechanisms. Since lower log *E. coli* removals were observed after multiple PVs, larger runoff events will have poorer overall treatment.

As shown in Table 1, the best performance was observed in the bioretention system with both biochar and plant presence (BC+P). Note that plant presence only significantly improved performance when the system was amended with biochar. The quantity of microorganisms living in the rhizosphere is several orders of magnitude higher than that in bulk soils [53]. Plants can improve FIB die-off through predation and competition by rhizosphere microbes or inactivation by antimicrobial compounds from root exudates [54].

It is well known that the addition of biochar to soil aids in plant growth by retaining nutrients and providing a good habitat for beneficial microbes in the rhizosphere [55,56]. More biomass growth was observed in the BC+P system than the S+P system, as shown in Figure S1. The symbiotic relationship between plant presence and biochar amendment for biofilm and plant growth enhanced *E. coli* die-off.

Table 1. Average log *E. coli* removals for each system over different PVs. For each column, values with different letters represent statistically significant differences.

	Average Log Removals *			
System	**PV ≤ 1 (Time ≤ 90 min)**	**1 < PV ≤ 2 (90 min < Time ≤ 180 min)**	**PV > 2 (Time > 180 min)**	**Total**
BC	5.25 ± 1.81 (a, b)	3.64 ± 1.58 (b)	3.96 ± 2.02 (a, b)	4.24 ± 2.09 (b)
BC+P	6.22 ± 1.65 (a)	5.55 ± 2.19 (a)	4.82 ± 2.31 (a)	5.58 ± 1.89 (a)
S	4.60 ± 1.93 (b)	3.84 ± 1.69 (b)	3.50 ± 1.59 (b)	4.03 ± 1.79 (b)
S+P	4.84 ± 2.20 (b)	3.95 ± 2.05 (b)	3.02 ± 1.71 (a, b)	3.99 ± 2.11 (b)

Notes: * For statistical tests between pore volumes, n values varied according to the following: PV < 1, n = 17 (BC), 20 (BC+P), 22 (S), 19 (S+P); 1 < PV ≤ 2, n = 21 for all systems; PV > 2, n = 16 for all systems.

Plant roots have been shown to increase FIB transport in some studies by creating preferential flow paths through the media [57]. This may explain the slight decrease in FIB removal when *Muhlenbergia* was planted in sand media (Table 1). Even though the effluent flow rates of S and S+P systems were similar (Figure 2), preferential flow paths may have caused a greater fraction of the influent from the S+P system to bypass the media. The S+P system only decreased in performance after two PVs, which indicates treatment processes occurring during the runoff event rather than during the ADP. Therefore, these results suggest that plant presence decreased *E. coli* straining and attachment in the sand only medium. The addition of *Muhlenbergia* improved FIB die-off, yet preferential flow pathways led to a decrease in attachment and straining. Without biochar amendment to promote attachment and enhance plant growth, the addition of *Muhlenbergia* led to a poorer overall treatment.

Overall, FIB removal performance was improved with biochar amendment, even without the presence of plants. The BC system had higher average log *E. coli* removals than the S system for all PVs, suggesting that biochar increased die-off, attachment, or straining of *E. coli*. Biochar's ability to increase overall microbial biomass in the IWSZ [29] may have led to more competition with *E. coli* during the ADP. During the storm event, the high SA and biochar surface chemistry could have resulted in a greater affinity for *E. coli* attachment. A similar study by Mohanty and Boehm [58] at significantly lower influent *E. coli* concentrations suggested that biochar has a high affinity for *E. coli* attachment from biochar-amended sand filters treating urban runoff.

Although this study did not compare bioretention systems with and without an IWSZ, data were compared with our prior research [44] where the same CAFO runoff was treated in sand- and biochar-amended columns without an IWSZ. The modified systems with an IWSZ achieved a 40% higher *E. coli* removal for experiments conducted at the same ADP and HLR [44]. Similarly, Liu et al. [15] found a significant increase in *E. coli* removals when an IWSZ was included in bioretention systems treating stormwater runoff. The presence of an IWSZ lengthens the retention times and creates anoxic conditions that favor *E. coli* die-off. In a recent review study by Biswal et al. [59], a range of 0.78–4.23 log removals were reported from biochar-amended bioretention systems treating urban runoff. Although differences in influent concentrations, loading rates, and system design directly impact *E. coli* removal, the results in this study show promising improvements in FIB removal in systems treating CAFO runoff.

3.3. Nitrogen Removal

The average influent TN concentration of the semi-synthetic dairy farm runoff was 71.3 ± 18.7 mg N/L, consisting of 28.3 ± 7.69 mg N/L of NH_4^+, 43.4 ± 19.4 mg N/L of

DON, and negligible NO_x. The average influent TOC concentration of the runoff was 636 ±270 mg/L. Removal efficiencies of TN, NH_4^+, DON, and TOC for the four modified bioretention systems are shown in Figure 3. Effluent NO_x concentrations are shown in Figure 4 rather than as removal efficiencies since influent concentrations were below detection limits. Effluent NH_4^+ and TN concentration profiles over time for the four bioretention systems for a 4.5 h storm event (Event 5) are shown in Figures 5 and 6.

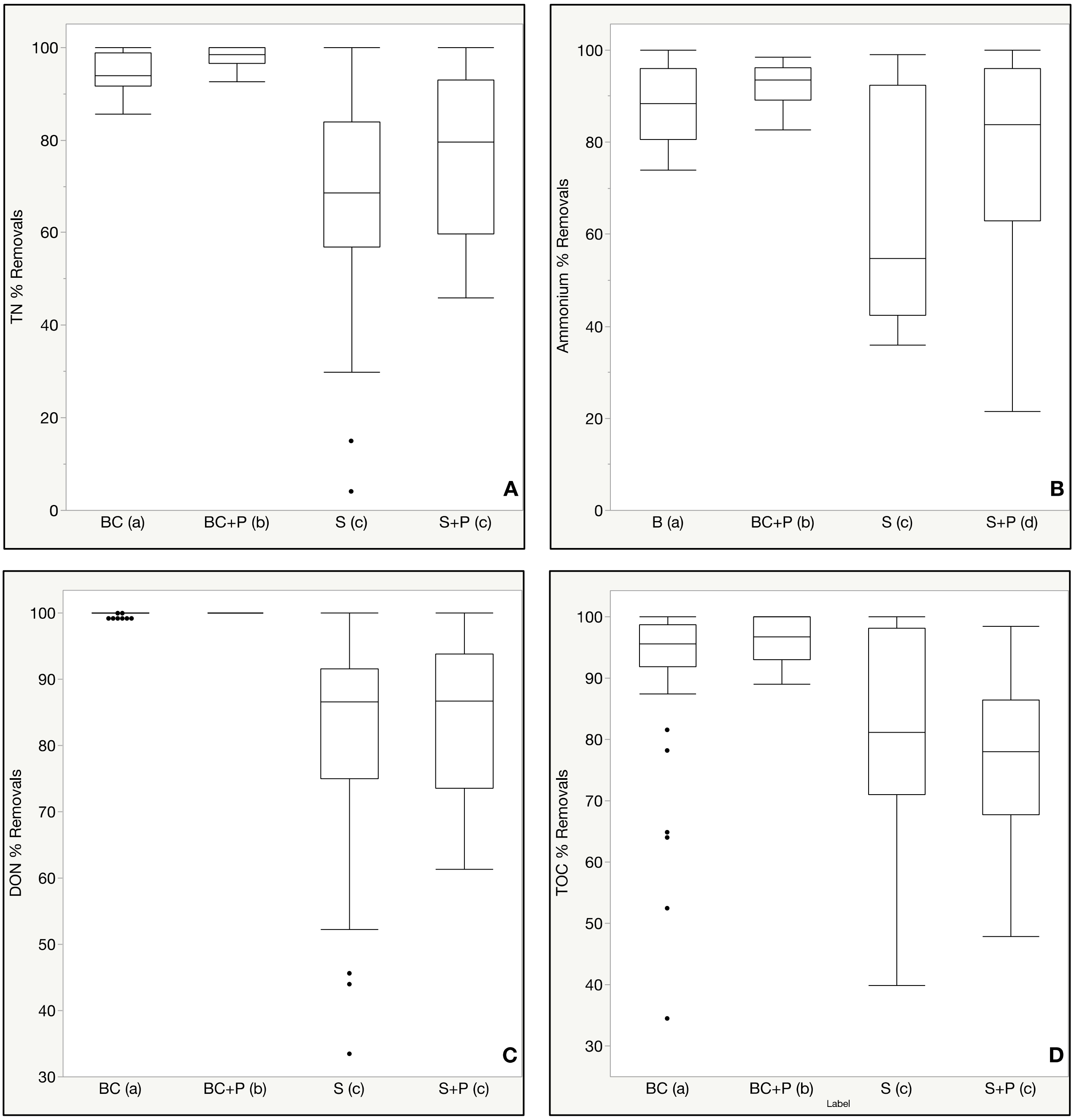

Figure 3. Box and whisker plots of N-species and TOC removal efficiencies for events 1–7: (**A**) TN, (**B**) ammonium (NH_4^+), (**C**) DON, and (**D**) TOC. Labels with different letters show statistically significant differences in mass removals.

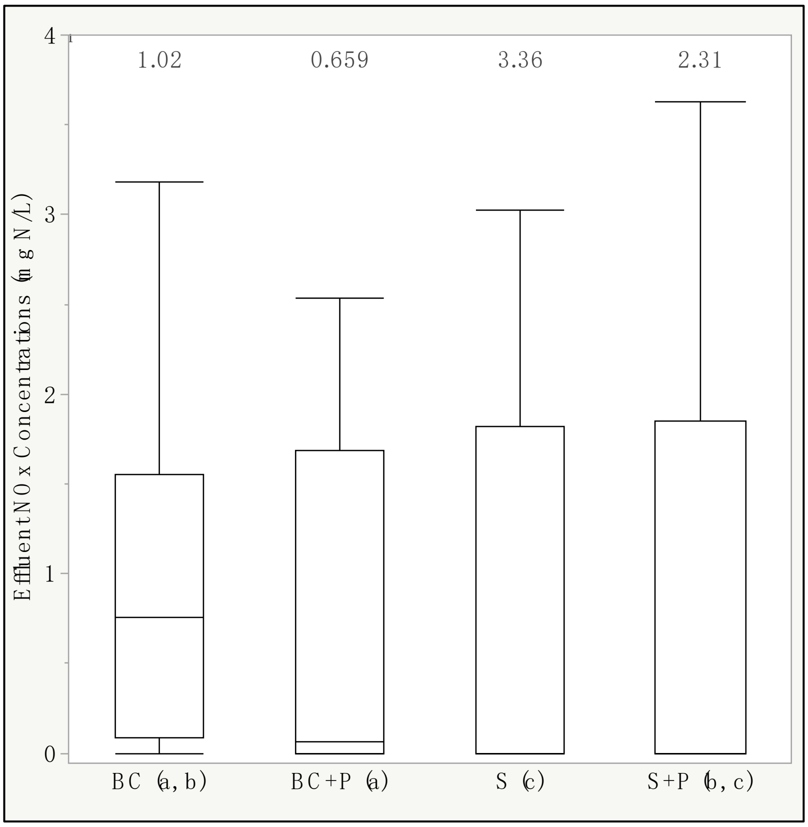

Figure 4. Effluent NO_x concentrations for Events 1–7 (outliers excluded). Mean values are displayed above each box. Labels with different letters represent statistically significant differences in effluent concentrations.

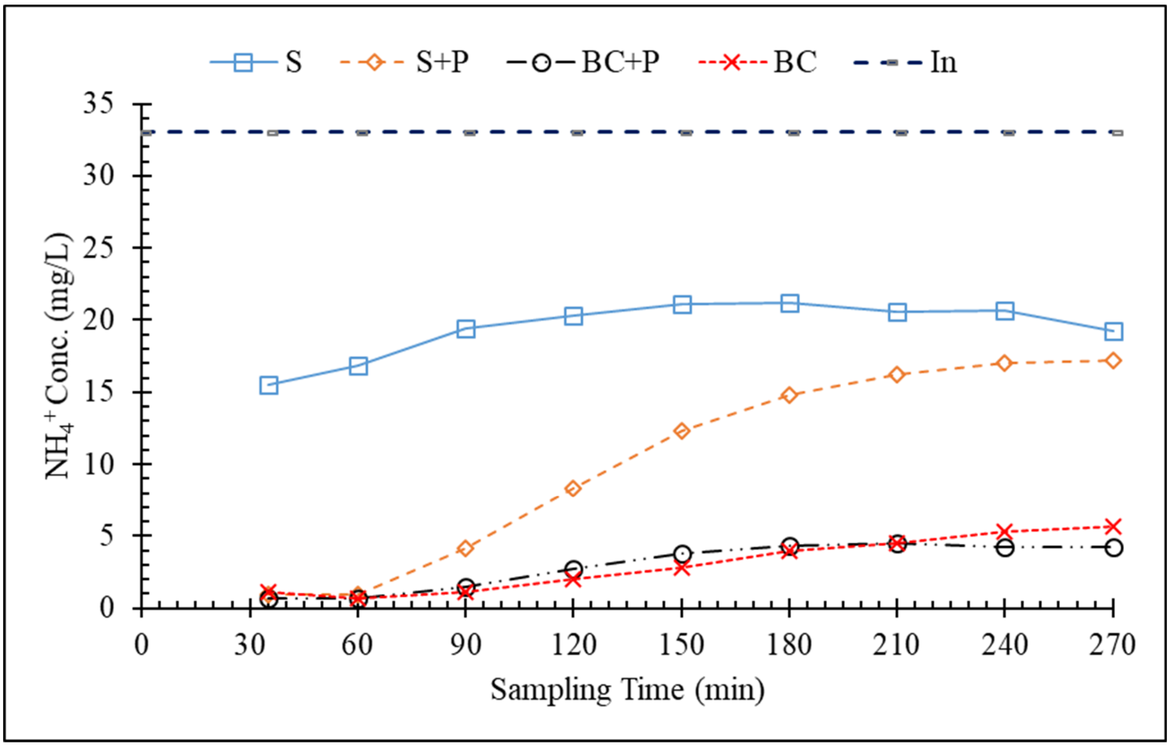

Figure 5. Pollutant breakthrough curve for NH_4^+ for four modified bioretention systems during Event 5.

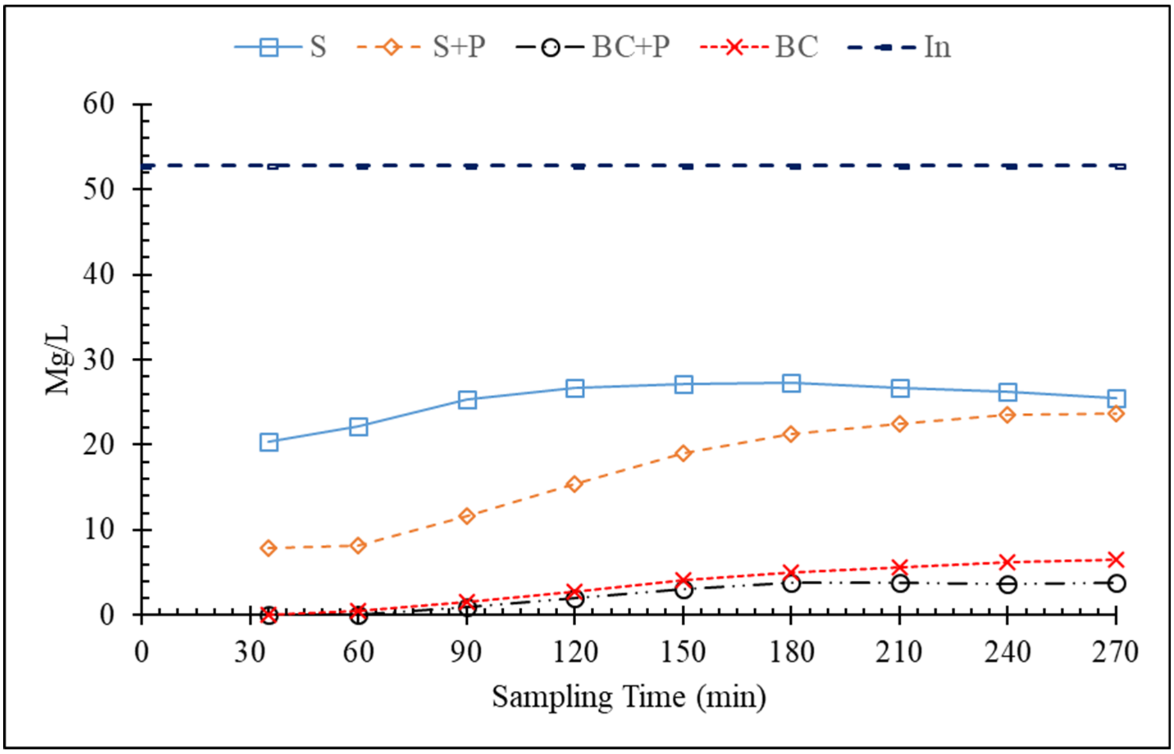

Figure 6. Pollutant breakthrough curve for TN for four modified bioretention systems during Event 5.

Median TN removals of >90% were observed in the biochar-amended systems, with the highest TN removal in the BC+P system at 98.5%. These removal efficiencies were higher than the typical range of TN removal for biochar-amended bioretention systems treating urban runoff (32–61%) [59]. Higher influent TN concentrations in the CAFO runoff most likely led to higher removal efficiencies. A regression analysis was carried out with average TN, TOC, and log *E. coli* removals for each runoff event vs. event number (see Figures S3–S5). TN removal was negatively correlated with event number (and therefore time) in the S (R^2 = 0.88) and S+P (R^2 = 0.92) systems; however, TN removal efficiency remained fairly constant in the BC (R^2 = 0.63) and BC+P (R^2 = 0.41) systems. There was no significant correlation of any other water quality parameter with event number. The results indicate that biochar addition helps to maintain TN removal performance over time in modified bioretention systems treating CAFO runoff.

Significantly higher NH_4^+ removals were observed in biochar-amended systems compared with un-amended systems (Figure 3B). The highest (92.5% ± 4.28) and lowest (65.7% ± 24.5) removal efficiencies were observed in BC+P and S systems, respectively. NH_4^+ removal mainly depends on (i) media adsorption, (ii) nitrification, and (iii) plant uptake. As shown in Figure 4, the S+P system had lower effluent NH_4^+ concentrations compared with the S system during the first 90 min. The lower initial NH_4^+ concentrations in the plant-amended systems suggests that plants and/or rhizosphere microbes assimilated NH_4^+ retained in the media during the ADP. Once the pore water in the IWSZ flushed through the S+P system (90–270 min), effluent NH_4^+ concentration gradually increased and was almost equivalent to concentrations in the S system by the end of the event. Both biochar-amended systems had significantly lower effluent NH_4^+ concentrations than the unamended systems (Figure 3B), suggesting that NH_4^+ was adsorbed to biochar in the unsaturated zone. In our previous laboratory-scale study with unmodified biofilters, the high CEC of biochar was able to increase NH_4^+ retention during the application period, resulting in higher nitrification activity during the oxic period between storm events [32].

As shown in Figures 5 and 6, effluent NH_4^+ and TN concentrations followed similar breakthrough trends. With low effluent TN concentrations from 0–90 min, the effluent N from the S+P unit consisted mostly of DON (Figure 3C) or NO_x (Figure 4). DON removal largely depends on either adsorption or ammonification followed by nitrification. As biochar enhances soil microbial activity due to its high surface area and porosity [60], enhanced adsorption and ammonification resulted in significantly higher average DON (<99%) removals in the BC and BC+P systems.

Significantly lower effluent NO_x concentrations were observed for biochar-amended systems compared to sand systems, as shown in Figure 4. As influent dairy runoff had high organic carbon content, it was hypothesized that TOC retained in the IWSZ due to adsorption onto biochar was utilized as an electron donor for denitrification. Significantly higher TOC removals were also observed in the systems amended with biochar (Figure 3D). In a similar study on bioretention systems treating commercial nursery runoff, biochar was found to increase NO_3^- removal, yet this study relied on woodchips as the carbon source, rather than adsorbed TOC [61]. In the S and S+P systems, a lack of adsorbed TOC in the IWSZ likely limited denitrification.

For all N-species, systems with plants achieved higher removal efficiencies compared to systems without plants (Figure 3). Prior research with planted and unplanted bioretention systems also showed that both NH_4^+ and NO_3^- are taken up by plants [62,63]. Denitrification is also favored by enhanced microbial activity and the availability of organic carbon in the rhizosphere due to the presence of root exudates and sloughed-off root tissues [64]. Planting the systems with *Muhlenbergia* increased median TN removals by an average of 7.5% due to enhanced microbial activity in the rhizosphere and plant uptake.

4. Conclusions

Fecal indicator bacteria (FIB) and N removal mechanisms were investigated in modified bioretention systems used for the treatment of dairy farm runoff, with and without biochar and with and without plants. Biochar increased FIB removal, most likely due to its high SA and affinity for *E. coli* attachment. The highest FIB removals were observed as the first flush of water retained in the IWSZ exited the systems and then declined over time as the biochar surface reached its adsorption capacity. The high SA and CEC of biochar also enhanced NH_4^+ and DON removal during infiltration, which facilitated ammonification and nitrification during the ADP. Higher TOC adsorption in the IWSZ in systems containing biochar favored denitrification, resulting in higher TN removal. Plants improved both FIB and TN removals in biochar-amended systems, most likely by increasing rhizosphere microbial activity and assimilation, facilitating predation and competition leading to FIB die-off and enhancing biological N removal mechanisms. This is the first study showing that biochar-amended bioretention systems treating CAFO runoff can effectively remove high loads of TN and FIB.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16101347/s1: Figure S1: Photographs of the two planted pilot-scale bioretention systems after 12 runoff experiments: (a) S+P system and (b) BC+P system. Figure S2: effluent flow volumes for each event. Figures S3–S5: Regression analysis of water quality data vs. event. Table S1: List of acronyms.

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References

1. Curriero, F.C.; Patz, J.A.; Rose, J.B.; Lele, S. The Association between Extreme Precipitation and Waterborne Disease Outbreaks in the United States, 1948–1994. *Am. J. Public Health* **2001**, *91*, 1194–1199. [CrossRef] [PubMed]
2. Holly, M.A.; Larson, R.A.; Cooley, E.T.; Wunderlin, A.M. Silage Storage Runoff Characterization: Annual Nutrient Loading Rate and First Flush Analysis of Bunker Silos. *Agric. Ecosyst. Environ.* **2018**, *264*, 85–93. [CrossRef]
3. Lian, Q.; Yao, L.; Uddin Ahmad, Z.; Lei, X.; Islam, F.; Zappi, M.E.; Gang, D.D. Nonpoint Source Pollution. *Water Environ. Res.* **2019**, *91*, 1114–1128. [CrossRef]
4. Bennett, S.D.; Sodha, S.V.; Ayers, T.L.; Lynch, M.F.; Gould, L.H.; Tauxe, R.V. Produce-Associated Foodborne Disease Outbreaks, USA, 1998–2013. *Epidemiol. Infect.* **2018**, *146*, 1397–1406. [CrossRef]
5. Heiman, K.E.; Mody, R.K.; Johnson, S.D.; Griffin, P.M.; Hannah Gould, L. Escherichia Coli O157 Outbreaks in the United States, 2003–2012. *Emerg. Infect. Dis.* **2015**, *21*, 1293–1301. [CrossRef]
6. *EPA Recreational Water Quality Criteria*; U.S. Environmental Protection Agency: Washington, DC, USA, 2012; pp. 1–69. Available online: https://www.epa.gov/sites/default/files/2015-10/documents/rwqc2012.pdf (accessed on 5 May 2024).
7. Mantovi, P.; Marmiroli, M.; Maestri, E.; Tagliavini, S.; Piccinini, S.; Marmiroli, N. Application of a Horizontal Subsurface Flow Constructed Wetland on Treatment of Dairy Parlor Wastewater. *Bioresour. Technol.* **2003**, *88*, 85–94. [CrossRef] [PubMed]
8. Ibekwe, A.M.; Grieve, C.M.; Lyon, S.R. Characterization of Microbial Communities and Composition in Constructed Dairy Wetland Wastewater Effluent. *Appl. Environ. Microbiol.* **2003**, *69*, 5060–5069. [CrossRef]
9. Larson, R.A.; Safferman, S.I. Field Application of Farmstead Runoff to Vegetated Filter Strips: Surface and Subsurface Water Quality Assessment. *J. Environ. Qual.* **2012**, *41*, 592–603. [CrossRef]
10. Díaz, F.J.; O'Geen, A.T.; Dahlgren, R.A. Efficacy of Constructed Wetlands for Removal of Bacterial Contamination from Agricultural Return Flows. *Agric. Water Manag.* **2010**, *97*, 1813–1821. [CrossRef]
11. Schellinger, G.R.; Clausen, J.C. Vegetative Filter Treatment of Dairy Barnyard Runoff in Cold Regions. *J. Environ. Qual.* **1992**, *21*, 40–45. [CrossRef]
12. Rusciano, G.M.; Obropta, C.C. Bioretention Column Study: Fecal Coliform and Total Suspended Solids Reductions. *Trans. ASABE* **2007**, *50*, 1261–1269. [CrossRef]
13. Lopez-Ponnada, E.V.; Lynn, T.J.; Ergas, S.J.; Mihelcic, J.R. Long-Term Field Performance of a Conventional and Modified Bioretention System for Removing Dissolved Nitrogen Species in Stormwater Runoff. *Water Res.* **2020**, *170*, 115336. [CrossRef] [PubMed]
14. Ergas, S.J.; Sengupta, S.; Siegel, R.; Pandit, A.; Yao, Y.; Yuan, X. Performance of Nitrogen-Removing Bioretention Systems for Control of Agricultural Runoff. *J. Environ. Eng.* **2010**, *136*, 1105–1112. [CrossRef]
15. Liu, J.; Yue, P.; He, Y.; Zhao, M. Removal of *E. coli* from Stormwater by Bioretention System: Parameter Optimization and Mechanism. *Water Sci. Technol.* **2020**, *81*, 1170–1179. [CrossRef] [PubMed]
16. Hathaway, J.M.; Hunt, W.F. Indicator Bacteria Performance of Storm Water Control Measures in Wilmington, North Carolina. *J. Irrig. Drain. Eng.* **2012**, *138*, 185–197. [CrossRef]
17. Mahmoud, A.; Alam, T.; Yeasir, A.; Rahman, M.; Sanchez, A.; Guerrero, J.; Jones, K.D. Evaluation of Field-Scale Stormwater Bioretention Structure Flow and Pollutant Load Reductions in a Semi-Arid Coastal Climate. *Ecol. Eng. X* **2019**, *1*, 100007. [CrossRef]
18. Zhang, L.; Seagren, E.A.; Davis, A.P.; Karns, J.S. The Capture and Destruction of Escherichia Coli from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-Coated Sand. *Water Environ. Res.* **2010**, *82*, 701–714. [CrossRef]
19. Li, Y.L.; Deletic, A.; Alcazar, L.; Bratieres, K.; Fletcher, T.D.; McCarthy, D.T. Removal of Clostridium Perfringens, Escherichia Coli and F-RNA Coliphages by Stormwater Biofilters. *Ecol. Eng.* **2012**, *49*, 137–145. [CrossRef]
20. Li, Y.; McCarthy, D.T.; Deletic, A. Escherichia Coli Removal in Copper-Zeolite-Integrated Stormwater Biofilters: Effect of Vegetation, Operational Time, Intermittent Drying Weather. *Ecol. Eng.* **2016**, *90*, 234–243. [CrossRef]
21. Grebel, J.E.; Mohanty, S.K.; Torkelson, A.A.; Boehm, A.B.; Higgins, C.P.; Maxwell, R.M.; Nelson, K.L.; Sedlak, D.L. Engineered Infiltration Systems for Urban Stormwater Reclamation. *Environ. Eng. Sci.* **2013**, *30*, 437–454. [CrossRef]
22. Rippy, M.A. Meeting the Criteria: Linking Biofilter Design to Fecal Indicator Bacteria Removal. *WIREs Water* **2015**, *2*, 577–592. [CrossRef]
23. Chen, G.; Walker, S.L. Fecal Indicator Bacteria Transport and Deposition in Saturated and Unsaturated Porous Media. *Environ. Sci. Technol.* **2012**, *46*, 8782–8790. [CrossRef] [PubMed]
24. White, R.E. *Principles and Practice of Soil Science: The Soil as a Natural Resource*; John Wiley & Sons: Hoboken, NJ, USA, 2005.
25. Minagawa, H.; Nishikawa, Y.; Ikeda, I.; Miyazaki, K.; Takahara, N.; Sakamoto, Y.; Komai, T.; Nairta, H. Characterization of Sand Sediment by Pore Size Distribution and Permeability Using Proton Nuclear Magnetic Resonance Measurement. *J. Geophys. Res. Solid Earth* **2008**, *113*, 1–9. [CrossRef]

26. Rahman, M.Y.A.; Nachabe, M.H.; Ergas, S.J. Biochar Amendment of Stormwater Bioretention Systems for Nitrogen and Escherichia Coli Removal: Effect of Hydraulic Loading Rates and Antecedent Dry Periods. *Bioresour. Technol.* **2020**, *310*, 123428. [CrossRef] [PubMed]
27. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar Effects on Soil Biota—A Review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
28. Omondi, M.O.; Xia, X.; Nahayo, A.; Liu, X.; Korai, P.K.; Pan, G. Quantification of Biochar Effects on Soil Hydrological Properties Using Meta-Analysis of Literature Data. *Geoderma* **2016**, *274*, 28–34. [CrossRef]
29. Berger, A.W.; Valenca, R.; Miao, Y.; Ravi, S.; Mahendra, S.; Mohanty, S.K. Biochar Increases Nitrate Removal Capacity of Woodchip Biofilters during High-Intensity Rainfall. *Water Res.* **2019**, *165*, 115008. [CrossRef]
30. Tian, J.; Miller, V.; Chiu, P.C.; Maresca, J.A.; Guo, M.; Imhoff, P.T. Nutrient Release and Ammonium Sorption by Poultry Litter and Wood Biochars in Stormwater Treatment. *Sci. Total Environ.* **2016**, *553*, 596–606. [CrossRef] [PubMed]
31. Nabiul Afrooz, A.R.M.; Boehm, A.B. Effects of Submerged Zone, Media Aging, and Antecedent Dry Period on the Performance of Biochar-Amended Biofilters in Removing Fecal Indicators and Nutrients from Natural Stormwater. *Ecol. Eng.* **2017**, *102*, 320–330. [CrossRef]
32. Rahman, M.Y.A.; Cooper, R.; Truong, N.; Ergas, S.J.; Nachabe, M.H. Water Quality and Hydraulic Performance of Biochar Amended Biofilters for Management of Agricultural Runoff. *Chemosphere* **2021**, *283*, 130978. [CrossRef]
33. Shrestha, P.; Hurley, S.E.; Wemple, B.C. Effects of Different Soil Media, Vegetation, and Hydrologic Treatments on Nutrient and Sediment Removal in Roadside Bioretention Systems. *Ecol. Eng.* **2018**, *112*, 116–131. [CrossRef]
34. Rycewicz-Borecki, M.; McLean, J.E.; Dupont, R.R. Nitrogen and Phosphorus Mass Balance, Retention and Uptake in Six Plant Species Grown in Stormwater Bioretention Microcosms. *Ecol. Eng.* **2017**, *99*, 409–416. [CrossRef]
35. Sarazen, J.; Hurley, S.; Faulkner, J. Nitrogen and Phosphorus Removal in a Bioretention Cell Experiment Receiving Agricultural Runoff from a Dairy Farm Production Area during Third and Fourth Years of Operation. *J. Environ. Qual.* **2023**, *52*, 149–160. [CrossRef]
36. Henderson, C.; Greenway, M.; Phillips, I. Removal of Dissolved Nitrogen, Phosphorous and Carbon from Stormwater by Biofiltration Mesocosms. *Water Sci. Technol.* **2007**, *55*, 183–191. [CrossRef] [PubMed]
37. Chandrasena, G.I.; Shirdashtzadeh, M.; Li, Y.L.; Deletic, A.; Hathaway, J.M.; McCarthy, D.T. Retention and Survival of *E. coli* in Stormwater Biofilters: Role of Vegetation, Rhizosphere Microorganisms and Antimicrobial Filter Media. *Ecol. Eng.* **2017**, *102*, 166–177. [CrossRef]
38. Skorobogatov, A.; He, J.; Chu, A.; Valeo, C.; van Duin, B. The Impact of Media, Plants and Their Interactions on Bioretention Performance: A Review. *Sci. Total Environ.* **2020**, *715*, 136918. [CrossRef]
39. Parker, E.A.; Rippy, M.A.; Mehring, A.S.; Winfrey, B.K.; Ambrose, R.F.; Levin, L.A.; Grant, S.B. Predictive Power of Clean Bed Filtration Theory for Fecal Indicator Bacteria Removal in Stormwater Biofilters. *Environ. Sci. Technol.* **2017**, *51*, 5703–5712. [CrossRef]
40. Muerdter, C.P.; Wong, C.K.; Lefevre, G.H. Emerging Investigator Series: The Role of Vegetation in Bioretention for Stormwater Treatment in the Built Environment: Pollutant Removal, Hydrologic Function, and Ancillary Benefits. *Environ. Sci. Water Res. Technol.* **2018**, *4*, 592–612. [CrossRef]
41. Thakur, T.K.; Barya, M.P.; Dutta, J.; Mukherjee, P.; Thakur, A.; Swamy, S.L.; Anderson, J.T. Integrated Phytobial Remediation of Dissolved Pollutants from Domestic Wastewater through Constructed Wetlands: An Interactive Macrophyte-Microbe-Based Green and Low-Cost Decontamination Technology with Prospective Resource Recovery. *Water* **2023**, *15*, 3877. [CrossRef]
42. Yang, X.; Arias, M.E.; Ergas, S.J. Hybrid Constructed Wetlands Amended with Zeolite/Biochar for Enhanced Landfill Leachate Treatment. *Ecol. Eng.* **2023**, *192*, 106990. [CrossRef]
43. Prince George's County. *Design Manual for Use of Bioretention in Storm Water Management*; Prince George's County: Landover, MD, USA, 1993.
44. Rahman, M.Y.A. Biochar Amended Bioretention Systems for Nutrient and Fecal Indicator Bacteria Removal from Urban and Agricultural Runoffs. Ph.D. Thesis, University of South Florida, Tampa, FL, USA, 2021.
45. Hu, Y.S.; Kumar, J.L.G.; Akintunde, A.O.; Zhao, X.H.; Zhao, Y.Q. Effects of Livestock Wastewater Variety and Disinfectants on the Performance of Constructed Wetlands in Organic Matters and Nitrogen Removal. *Environ. Sci. Pollut. Res.* **2011**, *18*, 1414–1421. [CrossRef] [PubMed]
46. Andrews, W.J. *Reconnaissance of Water Quality at Nine Dairy Farms in North Florida, 1990–1991*; US Geological Survey: Tallahassee, FL, USA, 1992.
47. Vadas, P.A.; Powell, J.M. Nutrient Concentrations in Leachate and Runoff from Dairy Cattle Lots with Different Surface Materials. *J. Soil Water Conserv.* **2019**, *74*, 613–621. [CrossRef]
48. EPA. *Method 1603:* E. Coli *in Water by Membrane Filtration Using Modified MTEC*; EPA: Washington, DA, USA, 2014.
49. American Public Health Association; American Water Works Association; Water Environment Federation. *Standard Methods for the Examination of Water and Wastewater*, 24th ed.; Lipps, W.C., Braun-Howland, E.B., Baxter, T.E., Eds.; APHA Press: Washington, DC, USA, 2023.
50. Lu, J.; Zhang, Q.; Werner, A.D.; Li, Y.; Jiang, S.; Tan, Z. Root-Induced Changes of Soil Hydraulic Properties—A Review. *J. Hydrol.* **2020**, *589*, 125203. [CrossRef]

51. Koivusalo, H.; Dubovik, M.; Wendling, L.; Assmuth, E.; Sillanpää, N.; Kokkonen, T. Performance of Sand and Mixed Sand–Biochar Filters for Treatment of Road Runoff Quantity and Quality. *Water* **2023**, *15*, 1631. [CrossRef]
52. Brown, R.A.; Hunt, W.F. Impacts of Media Depth on Effluent Water Quality and Hydrologic Performance of Undersized Bioretention Cells. *J. Irrig. Drain. Eng.* **2011**, *137*, 132–143. [CrossRef]
53. Mukerji, K.G.; Manoharachary, C.; Singh, J. *Microbial Activity in the Rhizosphere*; Varma, A., Ed.; Springer: Berlin/Heidelberg, Germany, 2006; ISBN 9783540291824.
54. Chandrasena, G.I.; Pham, T.; Payne, E.G.; Deletic, A.; McCarthy, D.T.E. Coli Removal in Laboratory Scale Stormwater Biofilters: Influence of Vegetation and Submerged Zone. *J. Hydrol.* **2014**, *519*, 814–822. [CrossRef]
55. Werner, S.; Kätzl, K.; Wichern, M.; Buerkert, A.; Steiner, C.; Marschner, B. Agronomic Benefits of Biochar as a Soil Amendment after Its Use as Waste Water Filtration Medium. *Environ. Pollut.* **2018**, *233*, 561–568. [CrossRef] [PubMed]
56. Ippolito, J.A.; Laird, D.A.; Busscher, W.J. Environmental Benefits of Biochar. *J. Environ. Qual.* **2012**, *41*, 967–972. [CrossRef] [PubMed]
57. Clothier, B.E.; Green, S.R.; Deurer, M. Preferential Flow and Transport in Soil: Progress and Prognosis. *Eur. J. Soil Sci.* **2008**, *59*, 2–13. [CrossRef]
58. Mohanty, S.K.; Boehm, A.B. *Escherichia coli* Removal in Biochar-Augmented Biofilter: Effect of Infiltration Rate, Initial Bacterial Concentration, Biochar Particle Size, and Presence of Compost. *Environ. Sci. Technol.* **2014**, *48*, 11535–11542. [CrossRef]
59. Biswal, B.K.; Vijayaraghavan, K.; Tsen-Tieng, D.L.; Balasubramanian, R. Biochar-Based Bioretention Systems for Removal of Chemical and Microbial Pollutants from Stormwater: A Critical Review. *J. Hazard. Mater.* **2022**, *422*, 126886. [CrossRef] [PubMed]
60. Anderson, C.R.; Condron, L.M.; Clough, T.J.; Fiers, M.; Stewart, A.; Hill, R.A.; Sherlock, R.R. Biochar Induced Soil Microbial Community Change: Implications for Biogeochemical Cycling of Carbon, Nitrogen and Phosphorus. *Pedobiologia* **2011**, *54*, 309–320. [CrossRef]
61. Richardson, N. *Nutrient Removal of Biochar Amended Modified Bioretention Systems Treating Nursery Runoff*; University of South Florida: Tampa, FL, USA, 2024.
62. Lea, P.J.; Morot-Gaudry, J.F. *Plant Nitrogen*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2001; Volume 162, ISBN 9783642087318.
63. Yao, Y.; Gao, B.; Zhang, M.; Inyang, M.; Zimmerman, A.R. Effect of Biochar Amendment on Sorption and Leaching of Nitrate, Ammonium, and Phosphate in a Sandy Soil. *Chemosphere* **2012**, *89*, 1467–1471. [CrossRef] [PubMed]
64. Havlin, J.L. *Module in Earth Systems and Environmental Sciences*; Elsevier: Amsterdam, The Netherlands, 2013.