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Research article

Biochar fails to enhance nutrient removal in woodchip bioreactor columns following saturation

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ABSTRACT

Denitrifying bioreactors are edge-of-field structures that remove excess nitrogen (N) from intercepted agricultural drainage by supporting the activity of denitrifying microorganisms with a saturated organic carbon substrate. Although these bioreactors successfully mitigate N export, the typical woodchip systems have little effect on phosphorus (P), which is also often present in environmentally harmful quantities in drainage waters. Currently, the evidence that amending woodchip bioreactors with biochar enhances both N and P removal rates is mixed, but more work is required to test this hypothesis under controlled conditions. To determine the effect of biochar amendment on nitrate (NO_3-N) and phosphate (PO_4-P) removal in woodchip bioreactors, three media types—aged woodchips (W), 10% (B_{10}) and 30% (B_{30}) biochar by volume—were tested under different operational conditions during five-day laboratory trials with horizontal, flow-through columns. Nutrient removal was observed under different flow rates yielding hydraulic residence times of 3, 6, and 12 hours with four formulations of simulated agricultural drainage, all combination of 16.1 or 4.5 mg L^{-1} NO_3-N and 1.9 or 0.6 mg L^{-1} PO_4-P. Each unique treatment with respect to media type, HRT, and influent formulation was tested in triplicate using independent columns. All treatments successfully removed NO_3-N, but PO_4-P removal was inconsistent. Cumulative NO_3-N removal efficiencies ranged 15–98% with an average removal rate of 11.0 g m^{-3} d^{-1}; biochar amendment enhanced removal only in response to sufficiently high loading rates. Cumulative PO_4-P removal efficiencies ranged from 66% removal to 170% export of the influent load; biochar addition was associated with increased export. These results indicate that pine-feedstock biochar poses a substantial increase to PO_4-P leaching risk and only modestly enhances NO_3-N removal given sufficiently high loading.

1. Introduction

Denitrifying bioreactors are increasingly accepted as agricultural best management practices that intercept and treat nutrient-enriched drainage waters, which can otherwise degrade downstream water quality (Christianson and Schipper, 2016). These edge-of-field structures typically consist of lined beds filled with organic carbon (C) substrate that intercept drainage waters transporting excess nitrogen (N) from agroecosystems. When the organic C substrate becomes saturated and anaerobic conditions develop, the bioreactor provides favorable habitat for ubiquitous soil microorganisms that convert reactive nitrate-nitrogen (NO_3-N) to inert dinitrogen gas (N_2) via denitrification, thereby removing bioavailable N. Traditionally, woodchips have been used as bioreactor substrate because of their low cost, widespread availability, hydraulic properties, and ability to support low-maintenance operation for over 10 years; woodchips strike a balance between providing sufficient C availability to sustain denitrification rates and recalcitrance to rapid degradation (Schipper et al., 2010). However, alternative substrates continue to be investigated for the purpose of enhancing nutrient removal or mitigating the production of harmful byproducts (e.g., Bock et al., 2018a, 2018b; Christianson et al., 2017; Feyereisen et al., 2016). Recently, the need to view the environmental impacts holistically and account for "pollution swapping" in addition to NO_3-N removal benefits has been emphasized (Christianson et al., 2017; Fenton et al., 2016; Healy et al., 2015). Pollution swapping refers to the unintended, negative environmental consequences that result from bioreactor implementation. Examples of pollution swapping include leaching of nutrients and dissolved organic carbon from fresh woodchips or incomplete denitrification resulting in the production of nitrous oxide (N_2O), a potent greenhouse gas (Schipper et al., 2010).

Recent studies have identified the potential of biochar, an organic C pyrolysis product, to both enhance orthophosphate (PO_4-P) and NO_3-N removal and mitigate N_2O production in woodchip bioreactors (Bock et al., 2015, 2016; Easton et al., 2015). However, these laboratory batch

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Abbreviations

B_{10}	woodchips with 10% biochar experimental treatment
B_{30}	woodchips with 30% biochar experimental treatment
BMP	best management practice
GHG	greenhouse gas
HRT	hydraulic residence time
i.d.	inner diameter
W	woodchip media experimental treatment

experiments (Bock et al., 2015; Easton et al., 2015) and a pilot-scale flood-and-drain study (Bock et al., 2016) are unlikely to capture the dynamics of flow-through, field-scale bioreactor systems or provide a sufficient basis to predict field performance. Furthermore, additional studies of the effect of biochar in denitrifying bioreactors have produced mixed results. A flow-through column study by Christianson et al. (2011) failed to find an effect of pine-feedstock biochar on NO_3-N removal when added to woodchips at rates of 7 or 14% by weight, but did not assess the effect on PO_4-P removal. In contrast, Pluer et al. (2016) found that a 10% volumetric addition of pine-feedstock biochar to woodchip bioreactors resulted in a statistically significant increase in NO_3-N removal (mean 6.8 g N m^{-3} d^{-1}) relative to woodchips alone (mean 6.6 g N m^{-3} d^{-1}); NO_3-N removal rates increased linearly with influent concentration for both media types. Pluer et al. (2016) also tested the effect of biochar amendment in a pair of field-scale bioreactors, one woodchip-only control and one with 10% biochar by volume, which were monitored over two years during the growing season and into the fall. Likewise, NO_3-N removal rates and concentration reductions were significantly greater in the biochar-amended bed; on average 5.3 mg L^{-1} for the biochar treatment bed and 4.0 mg L^{-1} for the control was removed, resulting in removal of 54% and 41% of influent NO_3-N, respectively (Pluer et al., 2016). Pluer et al. (2016) also observed PO_4-P export from the field bioreactors, which was more pronounced during the first growing season and from the biochar-amended bioreactor, though both bioreactors transitioned to low rates of PO_4-P removal by the second growing season. Hassanpour et al. (2017) noted the effect of bioreactor age on nutrient removal, finding that biochar ceased to enhance NO_3-N removal by the third year of operation in three treatment/control bioreactor pairs (including the pair monitored by Pluer et al. (2016)). Notably, considering average removal rates over the three years combined, the bioreactor treatment outperformed the woodchip control in the pair receiving high influent NO_3-N concentrations (mean ~17 mg L^{-1}) but not in the pairs receiving lower influent concentrations (means ~6 and 9 mg L^{-1}), despite the fact that the biochar amendment rate was 2.5% in the former and 10% in the latter (Hassanpour et al., 2017). Together, the experiments of Bock et al. (2016) and Hassanpour et al. (2017) suggest that biochar may only enhance NO_3-N removal in woodchip bioreactors with sufficiently high influent NO_3-N concentrations.

Despite the shared pine feedstock in these bioreactor studies, the mixed results are unsurprising given the variability in biochar properties and changes in these characteristics over time as biochar ages. Experiments with biochar as an agricultural soil amendment have produced conflicting results with respect to nutrient leaching and greenhouse gas emissions. These contradictory outcomes are often attributed to either to differences in biochar properties or the in mechanisms by which biochar affects N and P cycling, which are in turn dependent on environmental conditions (Cayuela et al., 2013; Ngatia et al., 2017). For example, biochar has been associated with low (Chintala et al., 2014) and high (Xu et al., 2014) PO_4-P sorption in acidic soils; increased (Anderson et al., 2014; Petter et al., 2016; Sánchez-García et al., 2014) and decreased (Dicke et al., 2015; Harter et al., 2017; Hüppi et al., 2015) N_2O emissions; and can affect N cycling in complex ways, such as indirectly by altering the microbial community structure and function (Anderson et al., 2011) and directly by altering the soil C:N ratio (Cayuela et al., 2014). Differences in biochar feedstock (e.g., lignocellulose, manure) and pyrolysis process parameters (e.g., temperature, oxygen content) result in differences in biochar properties (e.g., pH, surface area, and nutrient content, and surface charge density) that in turn affect nutrient cycling processes (Anderson et al., 2011; Brewer et al., 2012; Morales et al., 2013; Zhao et al., 2013). Of particular interest is recent work suggesting that biochars produced at higher temperatures have decreased P availability and increased P sorption, though the results were highly feedstock-specific (Ngatia et al., 2017). The P sorption capacity of biochar is largely dependent on adsorption to aluminum (Al) or iron (Fe) oxides, precipitation with calcium (Ca) or magnesium (Mn) at high pH, or precipitation with Al and Fe at low pH (Borno et al., 2018); as the quantities of these elements, the surface functional groups in which they occur, and biochar surface area vary with differing biochar feedstocks and pyrolysis conditions, so to do the P sorption capacities. However, the similarity between the wood-based feedstock biochars, which were selected due to relatively low nutrient content and thus lesser potential for leaching and tested in all reported bioreactor applications, reflect that environmental and operational conditions influence the effect of biochar on nutrient removal efficiency in addition to its material properties. Consequently, further research on the effect of biochar amendment on NO_3-N and PO_4-P removal in woodchip bioreactors under controlled but variable conditions is warranted. Indeed, the need for continued research on NO_3-N removal in concert with other relevant processes, such as PO_4-P removal and greenhouse gas emissions has been emphasized (Addy et al., 2016).

The objective of this experiment was to quantify the effect of biochar amendment on NO_3-N and PO_4-P removal in woodchip bioreactors under a range of operational conditions, focusing on the time period following the transition from prolonged dry to saturated conditions. The intermittent flow of agricultural drainage systems, yielding periodic dry conditions within bioreactors, has been hypothesized to select for a more resilient population of microorganism but potentially not the most efficient denitrifying population, which is thought to require sustained saturated conditions (Bock et al., 2016; Hathaway et al., 2015; Pluer et al., 2016). The importance of sustained saturated conditions was demonstrated by Christianson et al. (2011), who found that constant influent flow rates result in greater nitrogen removal in bioreactors than fluctuating flow rates. Since the transition from unsaturated to saturated bed conditions is a potential time of reduced bioreactor effectiveness, enhancing NO_3-N removal rates immediately following saturation would be particularly beneficial. The hypothesis that biochar enhances nutrient removal relative to woodchip media within two-weeks of saturation following a prolonged dry period was tested in laboratory-scale, flow-through columns across different hydraulic residence times (HRTs) and nutrient loading rates, which were controlled by adjusting the influent flow rate and nutrient concentrations. Simultaneously, the effect of biochar amendment on the production of greenhouse gases nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) was evaluated and is reported in a companion paper (Bock et al., 2018a).

2. Methods

2.1. Experimental design

The effect of biochar amendment at two rates, 10% and 30% (v/v), on NO_3-N and PO_4-P removal in woodchip bioreactors was tested using laboratory-scale, flow-through columns under different HRTs and nutrient loading rates. The columns were oriented horizontally and their dimensions were selected to represent field configurations. Twelve 6560 ± 30 cm^3 bioreactor columns were constructed from 10.2 cm (4 in) i.d. PVC pipe (Fig. 1). Endcaps with threaded plugs, which could be removed for the purpose of replacing media between trials, were affixed

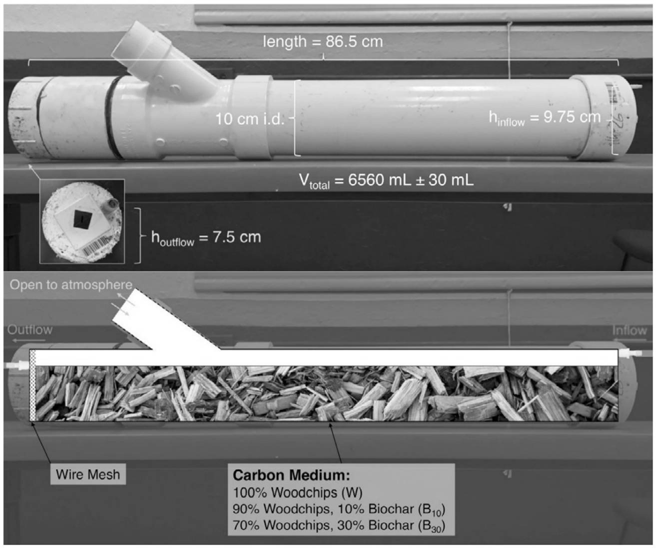

Fig. 1. Top: dimensions of horizontal flow bioreactor columns, where V_{total} is the total internal volume, and h_{inflow} and $h_{outflow}$ are the heights of the inlet and outlet. Bottom: flow of simulated agricultural drainage is right to left and column headspace freely exchanges with the atmosphere through the wye. Note that volume of woodchips was the same for each treatment and the percentage of biochar is volumetric.

with primer and cement. Couplings with barbed tubing adaptors for connection to the inlet and outlet lines were installed with Teflon tape into internally threaded holes tapped in the inlet and outlet plugs 9.75 and 7.5 cm above the bottom of the horizontally-oriented columns, respectively (Fig. 1). The outlet coupling position was just above the level of the fill media and controlled the volume of water contained in the column. During the trials, two peristaltic pumps (Masterflex®, Cole-Parmer®, Vernon Hills, IL) were used to pump the simulated agricultural drainage through inert tubing to the columns, and effluent samples were collected from short lengths of tubing attached to the outlet adaptors.

Three media types were tested, woodchips (W) and woodchips amended with pine-feedstock biochar at a volumetric rate of 10% (B_{10}) or 30% (B_{30}). Woodchips were locally sourced mixed hardwood species, approximately 0.5–5 cm on a side and 0.3–0.7 cm in width. The typical elemental composition of wood by mass is 45–50% C, 40–50% oxygen (O), ~6% hydrogen (H), < 1% N, and small percentages of other constituents, such as calcium (Ca), potassium (K), and magnesium (Mg) (Chandrasekaran et al., 2012). Prior to experimental use, the woodchips were aged for approximately one year outdoors, exposed to ambient temperatures and precipitation. The biochar (Biochar Now, Carbondale, CO) was produced from a pine feedstock via a two-stage pyrolysis process, during which the feedstock is briefly held for < 1 min between 500 and 700 °C under low oxygen conditions, and then the temperature is reduced to between 300 and 550 °C and held for up to 14 min. Two size fractions are produced by passing the biochar through an auger, yielding a biochar consisting of about 80% pieces approximately 1.5 cm long by 1 cm wide by 0.5 cm and 20% as a fine dust fraction on the order of 10–100 μm. This particular biochar was selected for success in enhancing NO_3-N and PO_4-P removal in prior experiments (Bock et al., 2015, 2016). Previously, initial evaluation of the nutrient retention capacity of the biochar was conducted by a professional soil laboratory. After soaking the biochar in a nutrient solution made from inorganic nutrients and organic compost extract for three days, the samples were drained and incubated at 36 °C for seven days to establish a microbial population. Subsequently, the biochar samples were saturated with a nutrient solution containing 3560 mg L^{-1} NH_4-N, 409 mg L^{-1} NO_3-N, 2219 mg L^{-1} PO_4-P, and 956 mg L^{-1} total P for 24 hours. The mass of nutrients retained by the biochar was determined by measuring nutrient concentrations in the extract from 20 sequential washes with deionized water, by which point < 0.15% of the initial nutrient concentrations was present in the extract. The biochar removed/retained 77% NH_4-N, 77% NO_3-N, 81% PO_4-P, and 22% total P from the original nutrient solution, indicating the potential for biochar to support cation sorption and denitrification. Although the elemental composition of the biochar was not determined in this study, pine feedstock biochar produced between 350 and 700 °C has been reported to range 72–81% C, 6.4–27% O, 1.4–4.6% H, 5.8–10.4% Ca, 2.3–3.7% K, 1.2–2.1% Mg, 0.47–0.84% P, 0.31–0.44%N, and similarly small percentages of sodium (Na), iron (Fe), and manganese (Mn) (Zhang et al., 2017).

The porosity of the woodchips was 0.66 cm^3 cm^{-3}, as determined by volumetric displacement, which falls within the range of 0.60–0.86 cm^3 cm^{-3} reported in bioreactor literature (e.g., Chun et al., 2009; Robertson, 2010; Woli et al., 2010). The porosities for B_{10} and B_{30} were estimated as 0.61 and 0.52 cm^3 cm^{-3}, respectively, by subtracting the biochar amendment volume from the woodchip porosity; the fine dust fraction of the biochar precluded porosity measurement by volumetric displacement. Each column received 5000 cm^3 of woodchips, and, for the B_{10} and B_{30} treatments, 500 cm^3 and 1500 cm^3 of biochar, respectively, were mixed with the woodchips. The biochar filled the interstitial spaces between the woodchips, so the total media volume was constant across treatments. Wire mesh and filter paper were installed between the media and outlet endcap in all columns to prevent media, especially biochar particulates, from washing out. A watertight seal was maintained using pipe thread sealant to reinstall the endcap plugs, and authenticity of the seal was verified prior to the start of each trial.

The performance of each media type was tested with representative moderate-high and low NO_3-N and PO_4-P concentrations selected based on reported values for agricultural subsurface (tile) and ditch drainage (Schmidt et al., 2007; Vadas et al., 2007). Four formulations of simulated agricultural drainage were used, each combination of high and low NO_3-N (16.0 and 4.5 mg L^{-1}) and PO_4-P (1.9 and 0.6 mg L^{-1}), so that the loading rate effects of each nutrient could be evaluated separately. The nutrient solutions were prepared in 114 L plastic bins by dissolving weighed quantities of granular calcium nitrate ($Ca(NO_3)_2$) and potassium phosphate monobasic (KH_2PO_4) in measured volumes of municipal water, which was found to have near neutral pH and consistently low NO_3-N and PO_4-P concentrations that did not result in significant variation to solution concentrations. Three HRTs were tested (3, 6, and 12 h) based on recommended targets to ensure adequate NO_3-N removal of 4–6 h (Damaraju et al., 2015; Greenan et al., 2009; Hoover et al., 2016; Lepine et al., 2016), 8+ h (Christianson et al., 2013), and 12 h (Moorman et al., 2015). Each unique combination of media type, influent formulation, and HRT was tested in triplicate using independent columns. The flow rates were assigned to achieve treatment HRTs assuming ideal plug-flow using the equation for theoretical HRT:

$$\tau = \rho\, V / Q$$

where τ is the HRT (h), ρ is the media porosity (cm^3 cm^{-3}), V is the saturated media volume (5000 cm^3), and Q is the flow rate (cm^3 h^{-1}). The time elapsed between solution entering and exiting the column was measured and assigned as the achieved HRT (Table 1), which varied slightly from the 3, 6, and 12 h targets. No tracer tests were performed to verify the uniformity of actual residence times, and nonuniform flow is acknowledged as a potential shortfall of the methodology.

Each of the nine trials tested a single combination of media type and HRT using three columns as replicates for each of the four influent formulations. To avoid confounding effects of the 'first flush' of labile nutrients and organic C associated with bioreactor startup (Abusallout and Hua, 2017; Sharrer et al., 2016), the woodchips had been aged outdoors exposed to ambient temperatures and precipitation for approximately one year; though the biochar was stored for the same duration, it was exposed only ambient temperature but protected from precipitation. Additionally, before use the woodchips were inoculated

Table 1
Flow rates, measured hydraulic residence times (HRTs), PO_4-P loading rates, and NO_3-N loading rates for each media type, woodchips (W), 10% biochar (B_{10}), and 30% biochar (B_{30}), for each target HRT.

HRT	3 h			6 h			12 h		
Media Type	W	B_{10}	B_{30}	W	B_{10}	B_{30}	W	B_{10}	B_{30}
Flow (mL min^{-1})	18.3	16.9	14.4	9.2	8.5	7.2	4.6	4.2	3.6
HRT (h)	3.3	3.1	3.4	6.0	5.8	6.6	11.8	12.5	13.1
High N (g m^{-3} d^{-1})	84.9	78.4	66.8	42.7	39.4	33.4	21.3	19.5	16.6
Low N (g m^{-3} d^{-1})	23.7	21.9	18.7	11.9	11.0	9.3	6.0	5.4	4.7
High P (g m^{-3} d^{-1})	10.01	9.25	7.88	5.03	4.65	3.94	2.52	2.30	1.97
Low P (g m^{-3} d^{-1})	3.16	2.92	2.49	1.59	1.47	1.24	0.79	0.73	0.62

with microbes by incorporating a small amount of native soil (< 2% by volume). Prior to the start each trial the columns were filled with a single substrate treatment and primed with the formulation of nutrient solution to be used during the experiment. Priming consisted of filling and draining the columns three times over one week to activate the microbial community of denitrifiers. The columns were then drained and washed three times with deionized water. All tubing was thoroughly rinsed between trials to prevent the buildup of precipitated material. During the trials the four formulations of simulated agricultural drainage were each pumped through triplicate columns at a constant rate corresponding to the treatment HRT (Table 1). Effluent samples were collected at 0, 2, 4, 6, 9, and 12 h after the first outflow occurred, every 6 h for the remainder of the first two days, every 12 h on the third day, and every 24 h for the remainder of each 5-day trial. Influent samples were collected periodically, during a subset of effluent sampling, to verify the stability of the influent concentration. All aqueous samples were collected in 50 mL polyethylene vessels, immediately filtered through 0.45-μm nylon filters, and stored at 4 °C until analyzed within the next several days. The concentrations of NO_3-N (more accurately NO_3-N combined with NO_2-N, but the NO_2-N concentrations were assumed to be low) and PO_4-P were quantified using flow injection analysis (QuikChem® 8500, Lachat Instruments, Loveland, CO) using the cadmium reduction method (Lachat #10-107-04-1-A, range 0.2–20.0 mg N L^{-1}) and the ascorbic acid method (Lachat #10-115-01-1-A, range 0.02–2.00 mg P L^{-1}), respectively. Ambient temperatures were relatively constant during the trials, but fluctuated between 19.5 °C and 22 °C.

2.2. Statistical analysis

All statistical analysis was conducted using the R language and environment (R Core Team, 2018). The first step of the statistical analysis was to determine if the columns reached stable nutrient removal rates during the trial. To test this, breakpoint analysis to detect a change in the slope of the effluent NO_3-N or PO_4-P concentration over time was conducted independently for each individual treatment replicate (n = 108). Breakpoints were detected using the 'segmented' package in R with 24 hours as the starting value for breakpoint estimation (Muggeo, 2008). The p-score test was used to determine if slopes for the two segments identified by a single breakpoint were significantly different. Subsequently, for those treatments determined not to have a statistically significant breakpoint, individual regressions on the entire time series of effluent nutrient concentrations for were fit by generalized least squares (GLS) with the 'nlme' package (Pinheiro et al., 2017); the GLS method enabled the inclusion of an autoregressive correlation structure to account for the non-independence of effluent samples from a single column. Alternatively, when the breakpoint was statistically significant, two separate GLS regressions were fit to each segment of the time series. Treatments were determined to have reached steady nutrient removal rates if the slope was found to be statistically insignificant ($p > 0.05$) for the second segment of the piecewise regression when a breakpoint was detected (p value of p-score test < 0.05) or for the entire time series when no statistically significant breakpoint occurred. Since data from each treatment replicate are independent, no error inflation was induced by conducting tests for slope significance on these multiple regressions. Subsequently, the effects of the treatment variables (media type, NO_3-N loading, PO_4-P loading, and HRT) on the likelihood of achieving pseudo steady-state removal rates was tested with logistic regression on the binary determination of effluent stability; the statistical significance of the independent variables was determined with the Chi-square test.

The effects of the treatment variables on column mean NO_3-N or PO_4-P removal rates were evaluated with linear regression models; the decision to use the time-weighted mean column removal rates was based on the determinations of steady state removal described above. The time-weighted mean removal rates were calculated as

$$WMR = \frac{QV^{-1}\sum_{i=1}^{n}(I - E_i)\Delta t_i}{\sum_{i=1}^{n}\Delta t_i}$$

where *WMR* is the time-weighted mean removal rate (g m^{-3} d^{-1}), Q is the flow (m^3 d^{-1}), t_i is the time interval between samples (d), V is the media volume (m^3), I is the influent concentration (mg m^{-3}), and E_i is the effluent nutrient concentration (NO_3-N or PO_4-P in mg m^{-3}) at time t_i. Only main effects and the interaction of media type by loading rate of the dependent variable were included in the models to limit the number of parameters to a level of complexity supported by the dataset created by calculating a single removal rate for each column (n = 108); the interaction effect was included to test the a priori hypothesis that biochar amendment induces greater nutrient removal at higher influent concentrations, and the same interaction between media type and loading was tested in the phosphorus removal model. Nutrient loading rates, rather than influent concentrations, were used as explanatory variables to account for the difference in flow rates among the media types required to achieve the same HRT due to their differing porosities (Table 1), whereas considering only influent concentration would mask these differences. Though HRT and loading are both dependent on the flow rate, they were not found to be problematically collinear, as determined by the variance inflation factor (< 2.5), since two influent concentrations of both analytes were tested at each flow rate. Due to appreciable differences between the target and measured HRT (Table 1), the latter was included as a continuous explanatory in the models. Model assumptions were validated by graphical analysis of model residuals, following the procedure recommended by Zuur et al. (2010). Examination of the quantile-quantile plot revealed no significant departures in the residuals from the normal distribution; equal error variance was indicated by the lack of an apparent relationship between residuals and predicted values; and, variance across media types was similar with respect to the distribution of nutrient removal rates. Statistical significance of fixed effects was evaluated with the F-test, and the significance of individual parameter coefficient estimates was evaluated with the *t*-test, both at the 95% confidence level. It should be emphasized that the purpose of these regression models is to support hypothesis testing and to describe the relationships between variables rather than to predict performance.

A secondary analysis was performed comparing nutrient removal rates between high and low influent treatments for each combination of media type and HRT (n = 27) to determine whether the nutrient removal rates varied with influent concentration across different HRTs/flow rates. The mean differences between removal rates for high and low nutrient loading rates and their statistical significance were calculated using simultaneous linear hypothesis testing with the 'multcomp' package in R (Hothorn et al., 2008).

3. Results and discussion

3.1. Data summary

All treatments successfully removed NO_3-N, with removal efficiencies ranging 15–98% and a median of 55%; time series of effluent concentration measurements are presented in Fig. 2. When considered separately, median removal efficiencies were 33% and 83% for high and low influent NO_3-N concentration, respectively, corresponding to median time-weighted effluent concentrations of 10.7 and 0.7 mg L^{-1}. Although the median effluent NO_3-N concentration from the 16.1 mg L^{-1} influent treatment exceeded the drinking water maximum contaminant level of 10 mg L^{-1} set by the US Environmental Protection Agency (USEPA, 2018), the median removal efficiency achieved the minimum of 30% specified by the Natural Resource Conservation Service Conservation Practice Standard Denitrifying Bioreactor Code 605 for field applications (USDA-NRCS, 2015). The NO_3-N mass removal rate among all treatments ranged 4.5–27.0 g m^{-3} d^{-1}, with a median of 10.8 g m^{-3} d^{-1}. Removal efficiencies were similar to the 18–95% range reported by Christianson et al. (2017), and removal rates were comparable to the commonly cited 2.0–22.0 g m^{-3} d^{-1} range reported by Schipper et al. (2010). However, observed NO_3-N removal rates were substantially higher than those reported in a recent meta-analysis, 4.7 and 3.5 g m^{-3} d^{-1} on average for woodchip beds and laboratory columns, respectively (Addy et al., 2016). Likewise, the columns supported higher removal rates than previous studies of aged biochar-amended woodchips, which yielded removal NO_3-N rates up to 6.1 g m^{-3} d^{-1} in a pilot experiment (Bock et al., 2016) and averaged 6.8 g m^{-3} d^{-1} over two growing seasons in a field study (Pluer et al., 2016). The relatively high removal rates of the columns are likely attributable to the freshness of the tested media, which, despite prior woodchip aging, had not previously sustained saturated conditions. The range of observed values corresponds with the 95% confidence interval of the mean NO_3-N removal rate of woodchip beds less than 13 mo old, 4.7–16.0 g m^{-3} d^{-1} (Addy et al., 2016). Denitrification was the most likely responsible for NO_3-N removal in the bioreactor columns. However, while it is well-established that microbial denitrification is the dominant NO_3-N removal mechanism in woodchip bioreactors (Ghane et al., 2015; Greenan et al., 2009; Warneke et al., 2011), a small portion of the removal may have been attributable to immobilization or sorption to biochar.

In contrast with N removal, the columns were largely unsuccessful in removing PO_4-P, as seen in the time series of effluent concentration measurements presented in Fig. 3. Across treatments, PO_4-P removal efficiency ranged from export of 138% of the influent load to 55% removal. The median effect of the bioreactor columns on PO_4-P resulted in export of 102% of the influent load. The corresponding removal/export rates ranged between -0.92–1.14 g m^{-3} d^{-1}, negative values representing export, with a median of -0.1 g m^{-3} d^{-1}. This fluctuation between removal and export was also observed by Sharrer et al. (2016) in another woodchip bioreactor column study where PO_4-P export averaged 104% of the influent load (-0.1 g m^{-3} d^{-1}) with a 24 h HRT, but periods of removal were observed. Overall, reports on P removal (PO_4-P or total phosphorus) in woodchip bioreactors have been mixed, with laboratory studies reporting 5–10% load reduction (Goodwin et al., 2015; Zoski et al., 2013) and a field study reporting over 18% reduction (Gottschall et al., 2016), while others observed mixed export and removal (Sharrer et al., 2016) or sustained export (Healy et al., 2012) in laboratory experiments. Overall, the columns failed to reduce PO_4-P concentrations below levels with the potential to cause environmental harm. Time-weighted median effluent concentrations were 0.54 and 2.00 mg L^{-1} for high and low influent treatments, respectively. Significant dilution would be required to reduce concentrations below EPA nutrient criteria for streams and rivers, which rages 0.010–0.076 mg L^{-1} total P across different ecoregions (USEPA, 2002).

3.2. Treatment effects

Although the nutrient removal rates observed in this experiment were similar to previous column studies, the short duration of the trial (seven days of priming followed by five days of testing) raised the question of whether stable removal rates were achieved. Breakpoint analysis and subsequent GLS regressions were conducted, as described in section 2.2, which indicated that at least 32 of the 108 columns demonstrated unstable NO_3-N removal rates and at least 33 demonstrated unstable PO_4-P removal rates (data not shown). Subsequently, logistic regression failed to demonstrate that the likelihood of achieving pseudo steady-state nutrient removal rates was dependent on any of the treatment variables (e.g., no treatment effects were determined to be statistically significant by the Chi-square test; data not shown). Given the absence of a predictive relationship between the treatment variables and achieving steady nutrient removal, in combination with the lack of a consistent trend in removal over time, column time-weighted mean NO_3-N and PO_4-P removal rates were determined to the be appropriate dependent variables for the linear regression models testing treatment effects. Retaining all the collected data for analysis, as opposed to dropping columns categorized as unsteady or the first so many hours of data, likely introduced noise, but not bias, to the linear regression models; the latter conclusion is supported by the logistic regression results. However, the lack of pseudo steady-state removal for a substantial proportion of the columns suggests that column mean removal rates could differ if the duration of the trials were longer. Therefore, the results of this experiment should be narrowly interpreted as indicative of relative treatment effects during a limited time following the transition from unsaturated to saturated conditions. For example, results are relevant to the period following saturation of a bioreactor after it

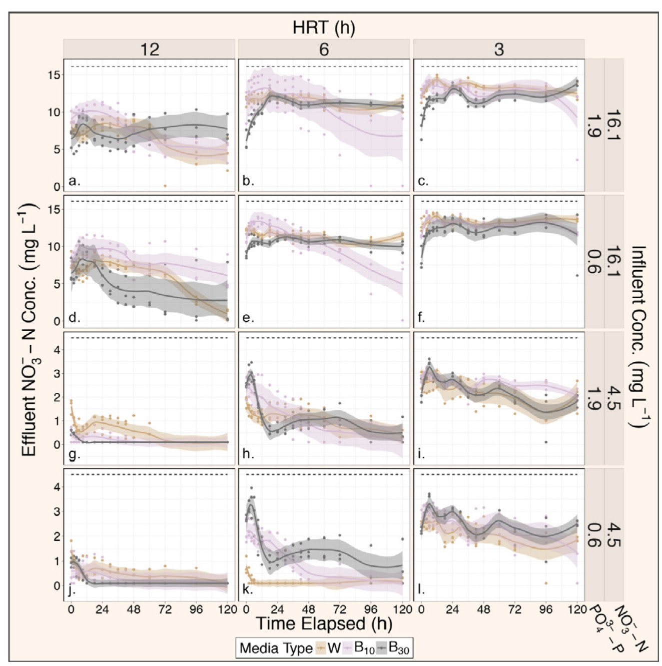

Fig. 2. Time series plot of column effluent NO_3-N concentrations (mg L^{-1}), with data from all three media types—woodchips (W), 10% biochar (B_{10}), and 30% biochar (B_{30})—in each panel representing a different combination hydraulic residence time (HRT) and influent NO_3-N and PO_4-P concentrations. Dotted lines on each panel represent influent NO_3-N concentrations, points represent individual effluent concentration measurements, solid lines represent the local regression (span = 0.35), and shading represents an estimate of the uncertainty associated with the local regression fit. Note that due to differences in media porosity and flow rates, for each combination of HRT and influent formulation, nutrient loading was slightly greater for W, followed by B_{10} and B_{30}.

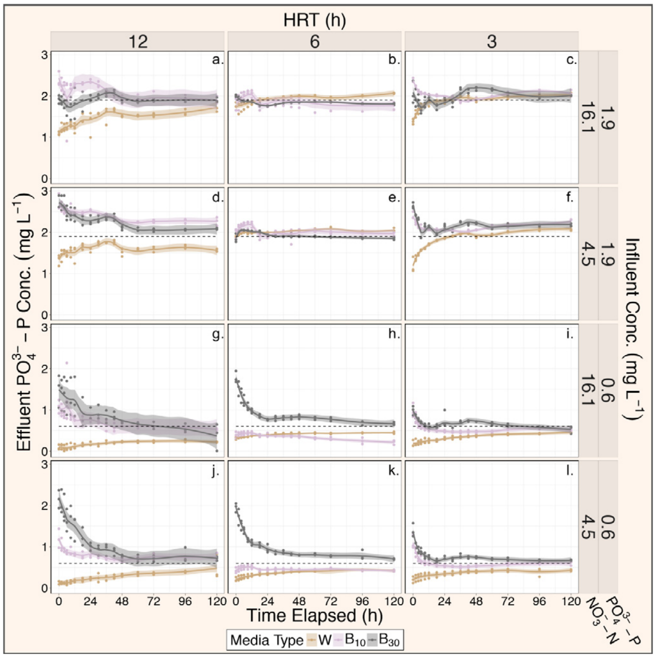

Fig. 3. Time series plot of column effluent PO_4-P concentrations (mg L^{-1}), with data from all three media types—woodchips (W), 10% biochar (B_{10}), and 30% biochar (B_{30})—in each panel representing a different combination hydraulic residence time (HRT) and influent NO_3-N and PO_4-P concentrations. Dotted lines on each panel represent influent PO_4-P concentrations, points represent individual effluent concentration measurements, solid lines represent the local regression (span = 0.35), and shading represents an estimate of the uncertainty associated with the loess fit. Note that due to differences in media porosity and flow rates, for each combination of HRT and influent formulation, nutrient loading was slightly greater for W, followed by B_{10} and B_{30}.

has received no flow for several weeks, as can commonly occur. Furthermore, the relative effects of the treatment variables are informative for hypothesizing field performance, but absolute removal rates are not recommended as a basis for prediction.

Both regression models of nutrient removal rates indicated that the effects of both media type and the loading rate of the dependent variable were statistically significant, as shown in Table 2. For NO_3-N removal, the interaction effect of NO_3-N loading and media type was significant ($p = 0.0134$), indicating that the effect of a change in the NO_3-N loading rate was dependent on media type (Table 2). While NO_3-N removal rates increase on average by 0.07 ± 0.02 g m^{-3} d^{-1} (plus or minus one standard error of the coefficient estimate, see Table 3) per unit increase in loading in the woodchip control, the biochar treatments increase by twice as much, 0.14 ± 0.03 g m^{-3} d^{-1}. These modeled relationships corroborate previous findings that biochar amendment enhances NO_3-N removal with sufficient NO_3-N loading, but that woodchips alone outperform given lower influent concentrations (Bock et al., 2016; Hassanpour et al., 2017). For example, model predictions for mean removal rates with a nutrient loading rate of 20.0 g m^{-3} d^{-1} are 11.1 ± 1.1 (expressing the 95% confidence interval), 10.4 ± 1.1, and 9.2 ± 1.1 g m^{-3} d^{-1} for W, B_{10}, and B_{30}, respectively; but if loading rates are increased to 60.0 g m^{-3} d^{-1}, the removal rates increase to 13.8 ± 1.5, 16.0 ± 1.6, and 14.8 ± 1.9 g m^{-3} d^{-1}, altering the relative performance of the media types.

The observed dependence of removal rates on NO_3-N loading indicated that N-limited conditions occurred within the columns, or, equivalently, the denitrification reaction rate was dependent on the NO_3-N concentration (Christianson et al., 2012). N-limitation has been observed in operational bioreactors and is often defined in as occurring when effluent NO_3-N concentrations are < 1 mg L^{-1} (Elgood et al., 2010; Robertson, 2010). Over 23% of effluent measurements, almost exclusively from columns receiving low NO_3-N influent (4.5 mg L^{-1}), met this definition. The NO_3-N removal rate would be expected to become independent of loading rate, transitioning from a first-order to zero-order reaction, given sufficient flow and influent concentration according to Michaelis-Menten kinetics, which govern NO_3-N removal in bioreactors (Ghane et al., 2015; Hua et al., 2016); since only two concentrations were tested, the threshold influent NO_3-N concentration for N-limitation in the bioreactor cannot be determined from this data, but would be expected to fall between the tested 4.5 and 16.1 mg L^{-1} concentrations within the range of HRTs tested.

With respect to PO_4-P, mean removal rates, estimated as 0.4 ± 0.3, −0.1 ± 0.1, and −0.3 ± 0.1 for W, B_{10}, and B_{30}, respectively, for mean nutrient loading rates and a 6 h HRT, decreased as the proportion of biochar increased, indicating net export by the biochar treatments. These findings are in agreement with those of Pluer et al. (2016), who observed PO_4-P export as a result of biochar addition to woodchip bioreactors, particularly during initial operation. Somewhat unexpectedly, the regression model indicated that removal rates decreased as PO_4-P loading increased, at an approximate rate of −0.15 ± 0.02 per unit increase in loading. This inverse relationship between PO_4-P loading and removal likely reflects that greater loading creates more opportunity for export. The PO_4-P removal rate and HRT were also inversely correlated, indicating that removal rates decreased as HRT increased (or, equivalently, as flow increased). One possible explanation for the effect of increasing HRT is the additional opportunity for remobilization of physiochemically sorbed PO_4-P, as the decrease in loading associated with a longer HRT would be expected to enhance rather than suppress PO_4-P removal according to the model. Note that although the coefficient estimate for NO_3-N loading was statistically different from zero ($p < 0.0001$, Table 3), the effect of NO_3-N loading rate on PO_4-P removal was not significant ($p = 0.1227$, Table 2). This simply reflects that inclusion of the NO_3-N loading rate as a parameter in the model improved the fit but was not determined to explain a sufficient amount of the variability in PO_4-P removal to be determined a significant effect. The coefficient estimate itself supports this conclusion, indicating an increase of only 0.007 ± 0.002 in PO_4-P removal per unit increase in NO_3-N loading, a small difference unlikely to be environmentally significant.

Although the short duration of the trial and lack of pseudo steady-state removal for a substantial proportion of the treatments limits what can be concluded from the results, the use of aged woodchips in this experiment allowed simulation of saturation onset in a previously dry bioreactor. Though the effect of this woodchip aging in a terrestrial environment differed from that which occurs in saturated bioreactor bed, use of woodchips and biochar stored outdoors exposed to ambient conditions provide a better comparison to bioreactor field applications

Table 2
ANOVA table for linear regression models of the NO_3-N and PO_4-P removal rates. The colon indicates the interaction between two variables. Starred p values indicate statistical significance at the 95% confidence level.

	Df[a]	Sum Sq[b]	F value	p value
NO_3-N removal rate				
Media	2	90.92	5.307	0.0065*
N loading rate	1	928.83	108.430	< 0.0001*
P loading rate	1	2.53	0.295	0.5884
HRT	1	20.86	2.435	0.1218
Media: N load	2	77.05	4.498	0.0135*
PO_4-P removal rate				
Media	2	7.48	40.509	< 0.0001*
N loading rate	1	0.224	2.422	0.1227
P loading rate	1	11.08	120.031	< 0.0001*
HRT	1	0.72	7.764	0.0064*
Media: P load	2	0.41	2.223	0.1136

[a] Degrees of freedom.
[b] Sum of squares.

Table 3
Summary statistics for fitted linear models of NO_3-N and PO_4-P removal rates. The units of nutrient loading and removal rates are g m^{-3} d^{-1} and HRT (hydraulic residence time) is in hours. Woodchips are the reference condition for media type, and B_{10} and B_{30} refer the 10% and 30% biochar treatments, respectively. The colon indicates the interaction between two variables. Starred p-values indicate statistical significance at the 95% confidence level.

Variable	Coefficient	Standard Error	t value	p value
NO_3-N removal rate				
Intercept	10.5627	1.3955	7.569	< 0.0001*
B_{10}	−2.1446	1.0794	−1.987	0.0407*
B_{30}	−3.2912	1.0830	−3.039	0.0030*
N loading rate	0.0682	0.0200	3.411	0.0009*
P loading rate	−0.0184	0.1292	−0.134	0.8867
HRT	−0.1294	0.1044	−1.239	0.2181
B_{10}: N load	0.0725	0.0273	2.656	0.0092*
B_{30}: N load	0.0720	0.0301	2.387	0.0189*
PO_4-P removal rate				
Intercept	0.8886	0.1453	6.115	< 0.0001*
B_{10}	−0.3799	0.1142	−3.298	0.0014
B_{30}	−0.7986	0.1156	−6.910	< 0.0001*
N loading rate	0.0065	0.0015	4.279	< 0.0001*
P loading rate	−0.1467	0.0181	−8.177	< 0.0001*
HRT	−0.0283	0.0108	−2.606	0.0106
B_{10}: N load	−0.0217	0.0244	−0.889	0.3759
B_{30}: N load	0.0366	0.0270	1.355	0.1786

than fresh media. Characterization of degradation of woodchips stored in piles for use as biofuels suggests chips are particularly susceptible to biodegradation due to their high surface area and that the most rapid decomposition occurs within the first six months to a year (Rex et al., 2016), similar to the time frame for leaching of dissolved organic C to stabilize in bioreactors (Abusallout and Hua, 2017). Thus, the aged woodchips used in this experiment may overcome the start-off effects of rapid carbon leaching that could constrain experimental findings as transferable only to bed startup conditions. However, the duration of priming and experimental observation in this study may have been inadequate for the establishment of a biofilm, and microbial community dynamics could have prevented stable removal rates. Therefore, the authors conclude that pine-feedstock biochar does not enhance PO_4-P removal and only has a modest effect on NO_3-N removal following saturation in woodchip bioreactors but recommend against inferring long-term treatment effects from these results.

Nonetheless, this study recommends against continued testing of woodchip bioreactors amended with biochar that has not specifically been engineered for its P sorption capacity. While modifying biochar by coating it with Mg or Ca has been shown to increase P sorption, high variability in biochar properties as result of feedstock and pyrolysis conditions (temperature, heating rate, holding time, gas type, gas flow rate) continue to produce inconsistent results, notably, with respect to whether the presence of NO_3-N enhances or interferes with PO_4-P sorption by Ca-rich biochar (Saadat et al., 2018). However, other P-sorption amendments have been tested in tile-drainage bioreactors to some degree of success. Husk et al. (2018) demonstrated that adding a mixture of activated alumina and gravel increased total P removal over three years from 0.019 g m^{-3} d^{-1} in the woodchip-only bioreactors to 0.363 g m^{-3} d^{-1} in the amended bioreactor. Gottschall et al. (2016) found that adding alum-based drinking water treatment plant residuals to woodchip bioreactors (tested at rates of 10 and 20% by volume) increased both NO_3-N and PO_4-P removal efficiency a during one-year trial; NO_3-N removal efficiency increased from 33% to 74% and PO_4-P removal efficiency increased from 35% to 89%. However, optimizing a single bioreactor for simultaneous NO_3-N removal via denitrification and PO_4-P removal via sorption has several challenges, including achieving homogeneous mixing, preventing preferential flow, and avoiding consuming P-sorption capacity before the woodchips require replacement, with the latter imposing a major yet incompletely understood constraint (Christianson et al., 2017). To avoid these challenges posed by amending woodchip bioreactors with P-sorption media, which can complicate design due to its differing particle size and hydraulic conductivity or introduce problematic contaminants, two-phase treatment systems have been proposed (Christianson et al., 2017; Hua et al., 2016). Laboratory-scale studies have shown pairing woodchip bioreactors with separate, downstream P-sorption filters to be promising when utilizing materials including Fe-based acid mine drainage treatment residuals (Christianson et al., 2017; Zoski et al., 2013), Fe-based steel byproducts (Goodwin et al., 2015; Hua et al., 2016), Ca-based steel slag (Christianson et al., 2017), and fly ash pellets (Li et al., 2018), all of which enhanced PO_4-P removal relative to woodchips alone. Consequently, engineering a biochar suitable for application in woodchip bioreactors may not be advisable with respect either to efficacy or cost-effectiveness given the active and productive research occurring with two-stage filters using, in many cases, industrial waste products.

3.3. Conclusions

Woodchips with and without biochar amendment were successful at removing NO_3-N from the simulated agricultural drainage following the onset of saturation but unsuccessful in removing PO_4-P. Although the applicability of this experiment is narrow due to the short duration of the trail, interesting questions remain about the effect of cycling between wet and dry conditions within bioreactors on nutrient removal in bioreactors. These findings advise caution in considering biochar amendment to woodchip bioreactors, as significant PO_4-P leaching can occur, and amendment only enhances NO_3-N removal given sufficiently high NO_3-N loading rates. However, other research at the field scale suggests that both the beneficial effect of pine-feedstock biochar on NO_3-N removal with high NO_3-N loading and the negative impact of exacerbated PO_4-P export are lessened as the media ages over several years, further complicating the calculation of the costs and benefits of biochar application in woodchip bioreactors. Further long-term monitoring would be required to determine if and when biochar becomes an effective PO_4-P sink, quantify uncertainty regarding the net effect on NO_3-N removal, and identify the operational conditions that affect biochar aging and resultantly altered properties. These efforts may not be justified given the advent of two-stage systems consisting of woodchip bioreactors and P-sorption filters in series that simplify design considerations and use materials that are more inexpensive and/or readily available than biochar.

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