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Comparing the effect of carbon media on nutrient removal and greenhouse gas production in laboratory-scale bioreactors

Jacqueline Kohn [a,*], Nicole E. Seitz Vermeer[b], Janelle F. Villeneuve[b], Mark Kadijk[b,c] and Shanwei Xu[b,d]

[a] Alberta Agriculture and Irrigation, 7000 113 Street, Edmonton, Alberta T6H 5T6, Canada
[b] Alberta Agriculture and Irrigation, Lethbridge Research & Development Centre, Lethbridge, Alberta T1J 4V6, Canada
[c] Present affiliation: Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB T1J 4B1, Canada
[d] Present affiliation: Agriculture and Agri-Food Canada, Morden Research and Development Centre, Morden, MB R6M 1Y5, Canada
*Corresponding author. E-mail: jacqueline.kohn@gov.ab.ca

JK, 0000-0002-3731-1836

ABSTRACT

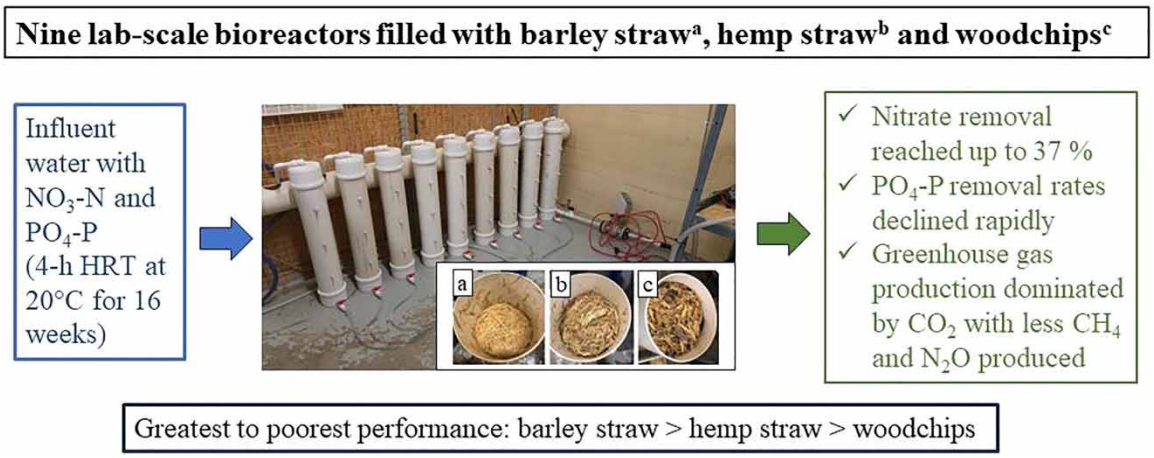
The performance of locally available agricultural carbon media (barley straw and hemp straw) was compared to woodchips for removing nitrate (NO_3-N) and orthophosphate (PO_4-P) in up-flow laboratory bioreactors. These media were tested in three replicates to quantify variability. The production of greenhouse gases nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) were quantified. Influent water with NO_3-N and PO_4-P flowed continuously through bioreactors at a 4-h hydraulic retention time at 20 °C for 16 weeks. Nitrate removal reached up to 37% across all carbon media after the fifth week, with a removal rate of 64 g N m^{-3} d^{-1}. Nitrate removal was affected by the type of carbon media in the order of barley straw > hemp straw > woodchips ($P < 0.05$). Most of the PO_4-P rates declined rapidly after the first week for all carbon media meaning none were superior. Greenhouse gas production was dominated by CO_2 with less CH_4 and N_2O produced. Production of N_2O relative to nitrate removal for the three media remained low at 0.16 to 0.75%. The findings suggest that agricultural residues could perform better than woodchips for NO_3-N removal although there was slightly higher N_2O and CO_2 production for these residues than woodchips.

Key words: agricultural residues, bioreactors, greenhouse gases, nitrate, orthophosphate, woodchips

HIGHLIGHTS

- Barley straw was more effective in reducing nitrate compared to hemp straw and woodchips.
- PO_4-P removal efficiencies and rates declined rapidly for the three carbon media.
- Greenhouse gas production was dominated by CO_2 with less CH_4 and N_2O produced.
- Production of N_2O relative to nitrate removal for the three media remained low.

GRAPHICAL ABSTRACT



INTRODUCTION

Natural overland flow is a process that occurs on the landscape when water cannot be absorbed by the soil. In agricultural landscapes, excess water can pool on the soil surface during times of high moisture such as snowmelt or periods of precipitation combined with high antecedent soil moisture conditions. Crop producers often rely on manual surface or subsurface drainage to remove excess water from the soil in order to improve soil moisture conditions and enhance crop production.

Subsurface drainage was introduced in the United States in the 1820s (Irwin & Spencer 1970). In Canada, subsurface drains were used in Alberta and Saskatchewan beginning in the 1970s to reduce soil salinity (Broughton & Jutras 2013). Since then, subsurface drainage has gained popularity in the Canadian prairies. While drainage provides benefits such as reduced soil salinity and improved soil moisture conditions, it also functions as a conduit for nutrient transport. Soils amended with chemical fertilizers or animal manure can contribute nutrients such as nitrogen (N) and phosphorus (P) to nearby streams and waterbodies through drainage water (Palliser Environmental Services Ltd., Alberta Agriculture and Rural Development 2008). The accumulation of nutrients in downstream waterbodies can cause eutrophication, which has increasingly become a water quality concern not only in North America but around the world (Trimbee & Prepas 1987; Turtola & Paajanen 1995; Paterson *et al.* 2006; Jaynes *et al.* 2008; Schindler *et al.* 2008; Jacquet *et al.* 2014; Bol *et al.* 2018).

While much of the research done to address nutrient loading to waterbodies has focused on P and its reduction (Schindler *et al.* 2016), N is also a major contributor to eutrophication. Nitrogen in the dissolved form of nitrate (NO_3-N) is flushed downward through the soil profile during periods of snowmelt, rainfall or when a crop is irrigated (Palliser Environmental Services Ltd., Alberta Agriculture and Rural Development 2008). Thus, a major concern regarding the use of subsurface drainage is the transport of NO_3-N to downstream waterbodies. N-fertilizer management and conservation methods can reduce NO_3-N transport, but additional methods of NO_3-N removal are needed (Jaynes *et al.* 2008). Nitrate in subsurface drainage water can be removed by denitrification processes under anaerobic conditions, however the natural occurrence of denitrification can be limited by the availability of an energy source, namely carbon, for denitrifying bacteria. Soils can be modified by adding solid organic carbon sources so that NO_3-N passes through carbon which supports denitrification. However, redevelopment of natural soils can take decades, so other in-field or edge-of-field treatment options for subsurface drainage water are needed for NO_3-N removal (Schipper & Vojvodic-Vukovic 1998; Jaynes *et al.* 2008).

Denitrifying bioreactors are a passive water treatment approach where water is routed through solid carbon substrates to remove dissolved nutrients. Bioreactors can be used to treat nutrient-laden agricultural drainage water for the protection of downstream water bodies.

The potential for carbon-based bioreactors to enhance nitrate removal via denitrification has been studied previously at the laboratory scale. Healy *et al.* (2012) found that among the four carbon source materials used within their lab-scale bioreactors, the highest NO_3-N removal efficiencies were for cardboard (~94%), followed by lodgepole pine needles (~75%), barley straw (~74%) and lodgepole pine woodchips (~70%). Woodchips are the commonly used fill media in bioreactor research. Woodchip bioreactors are typically designed to target removal

of nitrate, but some studies have included P removal as part of their testing under the assumption that success with P removal would make bioreactors an attractive option for combined N and P management. These studies, however, have had variable outcomes. Several lab-scale bioreactor studies have used woodchips containing other products (metals and water treatment residuals) and have shown reduction of orthophosphate concentrations by 5–10% (Goodwin 2012; Zoski *et al.* 2013). Sanchez Bustamente-Bailon *et al.* (2022) found promising results in their study that used discs filled with woodchips that naturally contained a range of metals known to sorb P and obtained P reduction of up to 87%. Rambags *et al.* (2016) reported reductions of P up to 14% in a full-scale woodchip bioreactor. Choudhury *et al.* (2016) reported that woodchip filters used in their study exhibited minimal ability to remove soluble reactive P.

One potential side effect for the application of denitrifying bioreactors is the production of the greenhouse gas (GHG) nitrous oxide (N_2O) (Greenan *et al.* 2009; Moorman *et al.* 2010; Davis *et al.* 2019). N_2O has a high global warming potential; 273 times that of CO_2 for a 100-year timescale (International Panel on Climate Change (IPCC) 2013). Incomplete denitrification within the bioreactors can lead to N_2O production (Elgood *et al.* 2010; Davis *et al.* 2019). However, several studies (Greenan *et al.* 2009; Woli *et al.* 2010; Feyereisen *et al.* 2016) have shown that the N_2O emissions account for a small percentage of the observed nitrate removal, suggesting little concern for N_2O release from in-field bioreactor systems.

Further, microbial decomposition of the organic carbon media (not quantified in this study) has the potential to release other GHGs such as methane (CH_4), which is the third largest contributor to global radiative forcing, and carbon dioxide (CO_2) which comprises 72% of global emissions (Woli *et al.* 2010; Healy *et al.* 2012; Davis *et al.* 2019).

As such, the objectives of this study were to assess the NO_3-N and PO_4-P removal efficiencies, and to quantify the dissolved N_2O, CH_4 and CO_2 production in laboratory scale bioreactors using three carbon media: woodchips and two locally available agricultural residues, hemp straw (*Cannabis sativa L.*) and barley straw (*Hordeum vulgare L.*). The purpose of this study was to contribute to the literature of quantifying GHGs produced by bioreactors using different carbon media and document whether agricultural residues were more suitable than woodchips for removing nitrate. The outcomes of this study were used to validate and implement in-field pilot-scale bioreactors (Kohn *et al.* 2023a, 2023b).

MATERIALS AND METHODS

Laboratory setting

In 2019, nine lab-scale bioreactors were constructed and tested at a Government of Alberta laboratory in Lethbridge, Alberta, Canada, to evaluate NO_3-N and PO_4-P removal from simulated drainage water. The bioreactors used a vertical-flow columnar chamber design and were constructed from polyvinyl chloride (PVC) pipe that was 15 cm in diameter and 92 cm in height (Figure 1(a)). To monitor the NO_3-N and PO_4-P concentrations at different locations within each bioreactor, four sampling ports were installed. One sampling port was located at the outlet (the highest point of the column, sampling port #4) and the other three sampling ports (bottom, port #1; middle, port #2 and top, port #3) were located along the side of the column at 23 cm intervals (Figure 1(b)). The inlet

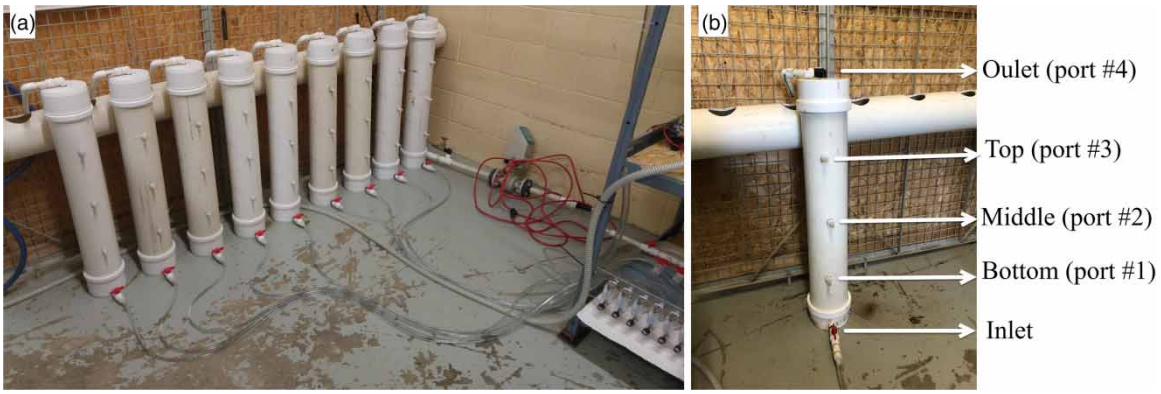


Figure 1 | (a) Lab-scale 92-cm vertical-flow columnar bioreactors, from A (left) to I (right), (A, E, I = woodchips; B, F, G = hemp straw; C, D, H = barley straw) (b) Lab-scale bioreactor inlet and ports.

(lowest point of the column) was used for dosing the simulated drainage water into the bioreactor (Figure 2). A 12 V recreational vehicle freshwater pump was used in conjunction with a pressure tank and float valve to continuously fill an elevated 250 L gravity feed tank from a large 6,000 L stock tank. This was done to maintain constant head pressure on the water inlet system. Simulated drainage water flowed from the constant head tank through a custom-made nine port manifold, then through individual needle flow control valves with flow meters before entering the inlet port of each bioreactor. The simulated drainage water flowed at an estimated 4 h hydraulic retention time (HRT), the average length of time the treated water remains in the bioreactor. Flow to each bioreactor was regulated by measuring outlet flow and adjusting the inlet needle valve. The outlet effluent flowed to a drainage pipe in the laboratory as shown in Figure 2 and then into the sewage system. During the study, efforts were made to hold the laboratory at a constant temperature of 20 °C since ambient temperature is known to affect denitrification and overall bioreactor performance (Soupir *et al.* 2018). Each bioreactor was packed with one of three different carbon media: woodchips, hemp straw or barley straw (Figure 3) resulting in three replicates per carbon media. The initial moisture content of all media was 3.5 ± 0.13%. All media were coarse textured with irregular pieces of carbon material generally ranging in size between 5 mm and 10 cm, based on visual observation (Figure 3). The dry bulk densities of the carbon media were 156.0 ± 1.5, 92.7 ± 0.7, and 80.4 ± 0.4 kg m^{-3} for woodchips, hemp straw and barley straw, respectively. The nine bioreactors were labelled from 'A' to 'I' to differentiate the media.

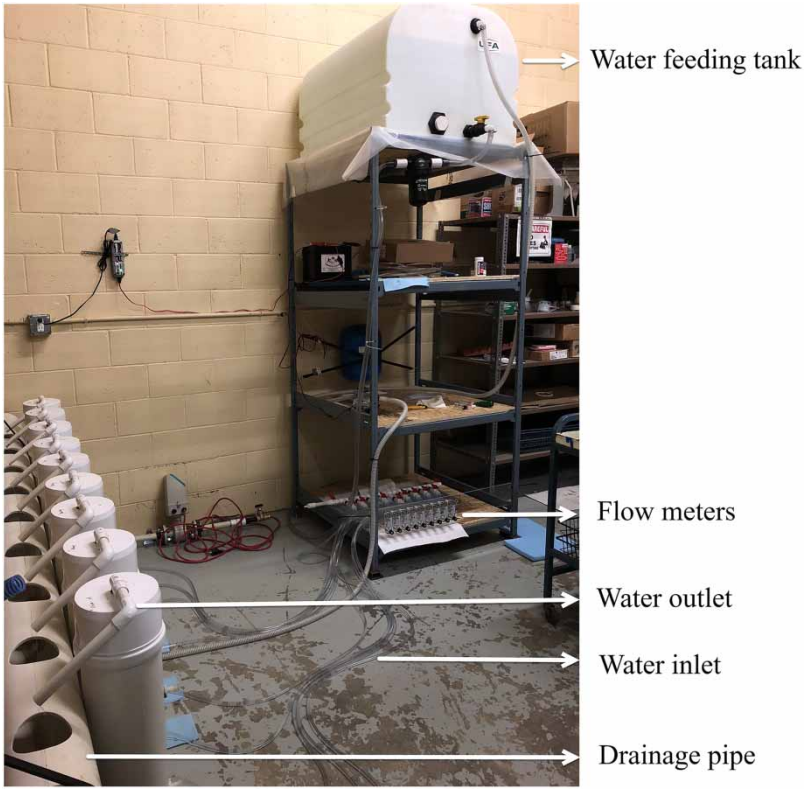


Figure 2 | Water flow design for the lab-scale bioreactors used in this study.

Tracer study

Tracer tests were done to evaluate the internal hydraulic properties of the bioreactors needed for subsequent removal calculations. Tracer tests are also useful for diagnosing poor performing systems (Hoover *et al.* 2017). In this study, the tracer was made from deionized water mixed with potassium bromide (KBr), a commonly used laboratory tracer. Although bromide occurs at low natural concentrations, it is considered to be a suitable tracer for water testing within a laboratory setting (Ghane *et al.* 2019).

Flow was maintained at an estimated 4 h HRT and approximately 100 mg of Br^- was introduced to the inlet port by injecting 3 mL of an approximately 35,000 mg L^{-1} Br^- solution using a syringe and a hypodermic needle. After the tracer injection, bromide concentrations at the outlet were assessed with 22 outlet water samples

Figure 3 | Lab-scale bioreactors filled with (a) barley straw (b) hemp straw and (c) woodchips (view from top, 15 cm in diameter).

collected over the following 24 h. The samples were analysed in the laboratory for Br^- using a Thermo Dionex ICS-5000 Ion Chromatograph (Thermo Fisher Scientific, Waltham, MA, United States).

Tracer residence time, $\bar{t}$ (h), is a measure of the time a molecule spends inside a bioreactor and was calculated as the sum of the incremental time steps, ti (h), multiplied by the incremental concentration values, Ci (mgL^{-1}), divided by the sum of the concentration values (Hoover *et al.* 2017):

$$\bar{t} = \frac{\sum tiCi}{\sum Ci} \tag{1}$$

The effective in-situ porosity,θ, was calculated using the tracer residence time, $\bar{t}$ (h), the flow rate, q ($mL\ h^{-1}$), and the saturated volume of the bioreactor, V_s (mL) (Ghane *et al.* 2019):

$$\theta = \frac{q\bar{t}}{V_s} \tag{2}$$

The results from the tracer test were used to evaluate the hydraulic performance of the bioreactors by calculating the hydraulic efficiency (λ), the Morrill Dispersion Index (MDI) and the short-circuiting indicator (S) (Christianson *et al.* 2013b) as follows:

$$\lambda = \frac{t_p}{HRT} \tag{3}$$

$$MDI = \frac{t_{90}}{t_{10}} \tag{4}$$

$$S = \frac{t_{16}}{t_{50}} \tag{5}$$

with t_p (h) as the time to reach peak outflow concentration (Persson *et al.* 1999), and t_{90}, t_{50}, t_{16} and t_{10} (h) representing the time for 90, 50, 16 and 10% of the tracer to pass through the bioreactor, respectively (Stover *et al.* 1986; Christianson *et al.* 2013b).

Hydraulic efficiency values range from 0 to 1.0. According to Hoover *et al.* (2017), a hydraulic efficiency greater than 0.75 is considered good, while an efficiency below 0.5 is poor. The Morrill Dispersion Index (MDI) is an indicator of dispersion and mixing of the tracer throughout the bioreactor, where 1 represents ideal plug flow (i.e., no mixing or back flow), and values from 1.0 to 2.0 indicate effective plug flow (Metcalf & Eddy 2003). A short-circuiting index (S) value nearer to zero indicates short circuiting is occurring and all water is not effectively being treated and an S value closer to 1.0 indicates more ideal flow.

Nutrient removal performance

Approximately 10 days after the tracer tests were completed, tests to determine NO_3-N and PO_4-P removal efficiency were initiated. Simulated drainage water was produced by mixing municipal water with KNO_3 and KH_2PO_4 in a 6,000 L cone-bottom tank to prepare a solution with a NO_3-N concentration of 30 mg L^{-1} and PO_4-P concentration of 1 mg L^{-1}. A constant head pressure gravity feed system was used to allow the treated water to flow continuously through the bioreactors for 16 weeks at a 4 h HRT. A pump was used to ensure the level of water in the gravity feed tank was maintained. Once a week, water samples were collected at each bioreactor outlet by placing a 125 mL sample bottle in the stream of water flowing between it and the drainage pipe (Figure 2); the bottom (#1), middle (#2), and top (#3) ports were sampled by momentarily removing the cap on the port and allowing the water to flow into a sample bottle. As sampling from these three ports was disruptive to the system flow, the outlet (port #4) was sampled first, followed by the top port then middle, and finally the bottom port. Inlet samples were taken at the nine-port manifold just before the needle flow meters. Samples were filtered through 0.45 μm cellulose membrane and analyzed for NO_3-N and PO_4-P by segmented flow analysis on Astoria Pacific Autoanalyzer2 (Astoria Pacific, Inc., Clackamas, OR, United States).

The NO_3-N and PO_4-P removal efficiency (ε) was calculated as follows:

$$\varepsilon(\%) = \frac{(C_{in} - C_{out})}{C_{in}} \times 100 \tag{6}$$

where C_{in} is the inlet concentration (g m^{-3}) and C_{out} is the concentration at the outlet (g m^{-3}) (Hassanpour *et al.* 2017).

The NO_3-N and PO_4-P removal rates were calculated as shown below:

$$\textit{Removal rate}\ (g\ m^{-3}\ d^{-1}) = \frac{q \times (C_{in} - C_{out})}{V} \tag{7}$$

where q is the flow rate (m^3 d^{-1}), (C_{in} – C_{out}) is the difference between the concentration at the inlet and outlet (g m^{-3}) and V (m^3) is the volume of the bioreactor.

Time series of NO_3-N and PO_4-P concentrations at different locations along the length of the bioreactors were calculated from samples taken from the bottom, middle and top and outlet ports along the side of the bioreactors.

A key determinant for nutrient removal is the availability of carbon. As such, total carbon (TC) mass was measured in the initial and final carbon materials to quantify the TC losses of each material.

Dissolved greenhouse gas extraction and analysis

A portion of the water samples collected each week over the experimental period were used to determine dissolved concentrations of CO_2, CH_4 and N_2O. A 30 mL subsample was drawn directly from the 125 mL sample bottle into a 60 mL closed syringe to prevent sample contact with air. Subsequently, 30 mL of helium was drawn into the syringe. Syringes containing the helium and water were placed on a reciprocal shaker and mixed at room temperature for 5 min at 180 rpm. Following mixing, the first 10 mL of air sample was expelled to ensure there were no water drops in the connector and needle before filling two replicate 5.9 mL evacuated exetainers (Labco) each with 10 mL. Gas samples were then analyzed for N_2O, CO_2 and CH_4 using a gas chromatograph (Varian 3800, Varian Instrument, Walnut Creek, CA) equipped with an electron capture detector (ECD), flame ionization detector (FID), and thermal conductivity detector (TCD).

The volume of CO_2, CH_4 and N_2O dissolved in 30 mL of water (V_{GHGt}, μL) was calculated as shown below:

$$V_{GHGt} = (C_t[V_h * (1 + \alpha)]) * \left(\frac{1\ \text{L}}{1{,}000\ \text{mL}}\right) \tag{8}$$

where C_t (μL gas L^{-1}) is the gas concentration in the gas phase at time t, V_h (mL) is the volume of the headspace (30 mL), α (mL gas mL^{-1} water) is the gas absorption coefficient of 0.901 for CO_2, 0.03464 for CH_4, 0.676 for NO_2 in water at 20 °C (Tiedje 1982).

The concentration of CO_2-C, CH_4-C and N_2O-N was then calculated as follows:

$$GHG - C \text{ or } N_t = \frac{V_{GHGt} * P * \begin{pmatrix} 28.0134 \text{ g } N_2O - N \text{ mol}^{-1} \text{or } 12.0107 \text{ g } CO_2 \\ -C \text{ mol}^{-1} \text{ or } 12.0107 \ CH_4 \\ -C \text{ mol}^{-1} \end{pmatrix}}{R * T * V_S} \quad (9)$$

where $GHG - C \text{ or } N_t$ (μg CO_2-C L^{-1}, CH_4-C L^{-1} or N_2O-N L^{-1}) is the concentration of dissolved GHG-N at the time t, V_{GHGt} (μL) is the volume of GHG dissolved in the water at time t as calculated in Equation (8), P is the pressure in kPa, R is the universal gas constant (8.31451 L kPa mol^{-1} K^{-1}), T is temperature in K at 20 °C, and V_S is the volume of sample water (i.e., 0.03 L).

Dissolved GHG production rates (g CO_2-N m^{-3} d^{-1}, CH_4-N m^{-3} d^{-1}, or N_2O-N m^{-3} d^{-1}) were calculated with the same method using Equation (7). Cumulative N_2O production (g N m^{-3}) was determined by trapezoidal integration of N_2O production rate versus time as described in Feyereisen *et al.* (2016). The cumulative N_2O production relative to the cumulative NO_3-N removal was calculated over the entire 16-week experimental period and expressed as a percentage (%).

Data analysis

Statistical analyses were performed using Microsoft® Excel® (Microsoft Corporation 2023) and SigmaPlot (2011). Specifically, Mann-Whitney Rank Sum Tests were used to determine significant differences ($P < 0.05$) in hydraulic characteristics, nutrient removal efficiencies and rates among the three different fill carbon media. These tests were also used to determine significant differences ($P < 0.05$) in the GHG production among the different carbon media for each GHG measured. Boxplots and scatterplots were created using the 'ggplot2' package (Wickham *et al.* 2023) in RStudio Team (2020) to provide visual displays of the nutrient concentrations.

RESULTS AND DISCUSSION

Bioreactor hydraulics

Concentrations of Br^- from the 24-h tracer study are presented in Figure 4. As expected, the curves exhibited the typical shape for a conservative tracer shown by a sharp rise in concentration followed by a sharp decline. The

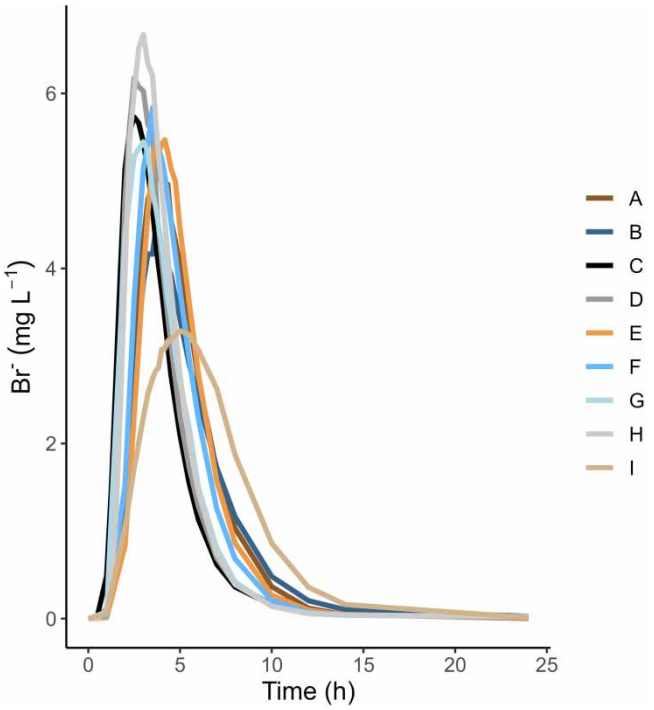


Figure 4 | Tracer response for the nine bioreactors (A, E, I = woodchips; B, F, G = hemp straw; C, D, H = barley straw) with Br^- concentrations (mg L^{-1}) vs time (h).

Br^- concentration peaked within 5 h of the injection for all the bioreactors except for one that was filled with woodchips. The percentage of the total mass Br^- recovery was 75–86%, which was a similar recovery to Ghane *et al.* (2019). A summary of calculated hydraulic properties for each lab-scale bioreactor is presented in Table 1.

Table 1 | Mean and standard deviation of the hydraulic properties for the lab-scale bioreactors

Bioreactor	A, E, I ($n = 3$)	B, F, G ($n = 3$)	C, D, H ($n = 3$)
Carbon media	Wood	Hemp	Barley
Flow rate, mL min^{-1}	67.43 ± 2.53	67.77 ± 1.01	64.73 ± 0.82
HRT calculated, h	4.01 ± 0.14	3.96 ± 0.05	3.93 ± 0.04
In situ porosity	0.76 ± 0.01	0.96 ± 0.03	0.98 ± 0.02
Recovery Br^-, %	76.4 ± 0.51	81.53 ± 3.92	79.17 ± 3.17
Hydraulic efficiency (λ)	0.79 ± 0.1	0.90 ± 0.02	0.97 ± 0.02
Short circuiting (S)	0.64 ± 0.03	0.61 ± 0.03	0.63 ± 0.04
MDI	3.02 ± 0.22	3.41 ± 0.40	3.04 ± 0.33
Time to peak, h	3.08 ± 0.42	3.56 ± 0.08	4.69 ± 0.28

Hydraulic efficiency was over 0.5 in all bioreactors and was highest for woodchips. There was a significant difference ($P < 0.05$) between the median hydraulic efficiency values for bioreactors with woodchips and barley straw fill media.

An MDI less than 2.0 is indicative of effective plug flow and most of the values reported in this study were between 2.7 and 3.9, indicating plug flow characteristics with low dispersion (Hoover *et al.* 2017). One of the bioreactors filled with hemp straw showed the highest dispersion rate (3.89) and one of the bioreactors filled with woodchips showed the lowest dispersion rate (2.66). As seen in Figure 4, bioreactor I, a woodchip bioreactor, showed a delayed tracer response compared with all other bioreactors. This may have been caused by a higher proportion of small wood fragments which affected flow dynamics within this bioreactor.

S values for all bioreactors were 0.58 or higher, indicating minimal short circuiting. Hemp straw showed lower S values and higher MDI values than the barley straw and woodchips, but there were no significant differences in the median MDI and S values among the three fill media ($P < 0.05$). Overall, the agricultural residues (hemp straw and barley straw) showed acceptable hydraulic properties and thus were determined to be suitable as carbon media for the bioreactors.

Nitrate removal performance

The NO_3-N inlet concentrations ranged from 27 to 30 mg L^{-1}. Flow rates were constant at each bioreactor ranging from 63.7 to 70.5 mL min^{-1} which achieved the desired HRT of 4 h.

Removal efficiencies at the outlet port varied from 0.5 to 36.9% for the three carbon media. After 16 weeks of operation, mean NO_3-N removal efficiencies were higher for barley straw (20.1 ± 6.1%) than hemp straw (13.8 ± 5.2%) and woodchips (6.8 ± 0.6%). The differences in the median removal efficiencies for NO_3-N among the three carbon media types were statistically significant ($P < 0.05$) with a performance rank of barley straw > hemp straw > woodchips. This is probably because agricultural straw media contains more soluble carbon than woodchips which can provide more carbon to promote the growth of denitrifying bacteria in the straw bioreactors.

Overall, nitrate removal efficiencies were highest at the top sampling port (#3) and outlet sampling port (#4) (Figure 5). This suggests that for a constant flow rate, the designed bioreactor length could be the critical factor affecting the performance of nitrate removal. This was consistent with the results from Saliling *et al.* (2007) who performed a study on 40-cm tall laboratory scale bioreactors filled with woodchips and wheat straw at a constant flow rate and three different influent NO_3-N concentrations. They found a nitrate reduction from 54 to 71% in the first 10 cm of the bioreactors, and also observed that at the end of the third section (30 cm), NO_3-N concentrations were reduced by more than 95%. Results from a mathematical model developed by Nečiporenko *et al.* (2022) suggested a linear dependence between the length of the bioreactor and the flow rate in order to achieve the desired outflow nitrate concentration. They found that when the rate of outflow is

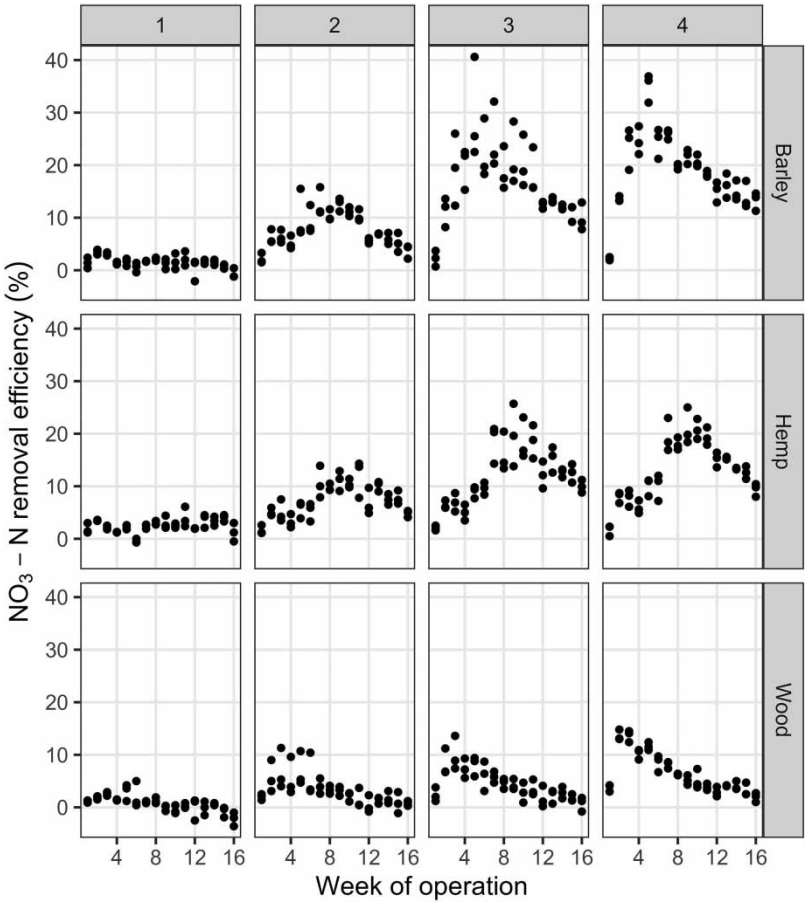


Figure 5 | Time series of NO_3-N removal efficiency (%) at different ports along the length of the bioreactors (sample ports: 1 = bottom, 2 = middle, 3 = top; 4 = outlet).

fixed, the targeted NO_3-N removal efficiency in the outflow can be achieved by adjusting the length of the bioreactor. Healy *et al.* (2014) performed a laboratory experiment on 1-m long bioreactors filled with different carbon rich media (woodchips, lodgepole pine needles, barley straw and cardboard) and found that most of the nitrate removal occurred within 0.4–0.6-m range from the inlet of all bioreactors for all the HRTs examined (ranging from 4 to 22 d). Healy *et al.* (2012) indicated that higher HRT leads to complete denitrification at shorter distances from the influent port of their bioreactors (1-m column). In their study, the HRTs were in the range of 8.5–21.8 days.

In our study, nitrate removal efficiencies peaked at different times for each carbon media and declined afterwards. This may be because different available carbon compositions (i.e., different cellulose, hemicellulose and lignin content) in three carbon media resulted in the denitrifying bacteria developing and predominating at different periods of time. However, quantification of these bacteria was out of scope of this study.

The nitrate removal efficiency in woodchip bioreactors peaked during the second week of operation (up to 14.8%) at the outlet sampling port (Figure 5, panel 4) and remained below 10% after the fifth week of operation at all sampling ports. For barley straw, removal efficiency peaked at 40.6% during the fifth week at the top sampling port (#3) and remained above 10% at the outlet sampling port (#4) after the second week of operation (Figure 5, panel 3). For hemp straw, the removal efficiency peaked during the ninth week of operation, up to 25.7% (Figure 5, panels 3 and 4).

Nitrate removal rates differed significantly ($P < 0.05$) between carbon media types and declined after the first 8 weeks for all carbon media. They were consistently higher for barley straw than the other two media over the 16-week experiment with values up to 64 g N m^{-3} d^{-1}. Nitrate removal rates for hemp straw showed values up to 42 g N m^{-3} d^{-1} while woodchips showed values no higher than 23 g N m^{-3} d^{-1}. These results show that agricultural residues are attractive alternatives as carbon media for denitrifying bioreactors. Moreover, under the conditions of this experiment and time frame of study, barley straw is the most effective at removing nitrate.

The results from this study are consistent with findings presented in the literature, which indicate lower performance for woodchip bioreactors compared to other carbon media. For example, Cameron & Schipper (2010) conducted a study over 23 months and reported nitrate removal rates ranging from 0 to 52 g N m^{-3} d^{-1} depending on carbon media and ranked their performance in the order of maize cobs > green waste > wheat straw > wood for treatments at 14 and 23.5 °C. In a laboratory study by Li *et al.* (2018), three hydraulic retention times ranging from 0.67 to 4.0 h were targeted, which resulted in total nitrate removal rates for woodchips between 40 and 49 g N m^{-3} d^{-1}, and 49–85% of the nitrate concentration being removed. Zoski *et al.* (2013) reported nitrate removal rates ranging from 0.11 to 0.29 g N m^{-3} d^{-1} for lab-scale woodchip bioreactors, which equate to removal efficiencies ranging from 8 to 60%. The authors suggest that the geometry of the woodchips could have created preferential flow conditions (i.e., carved out flow paths) within the bioreactors which resulted in fast flow through the chambers and consequently less opportunity for nitrate removal.

The calculated TC losses in water from the carbon materials were 5.7 ± 1.4, 27.1 ± 2.9 and 35.6 ± 1.1% for woodchips, hemp straw and barley straw, respectively. These results show that the agricultural residues have more carbon depletion than the woodchips, meaning that the agricultural residues would need to be replenished more often due to the higher depletion rate. These results are consistent with the findings reported by Schipper *et al.* (2010).

Orthophosphate removal performance

The inlet concentrations of PO_4-P ranged from 0.9 to 1 mg L^{-1}. Removal efficiencies at the outlet port varied from 1.1 to 53.5% for the three carbon media. Most of the PO_4-P removal occurred within the first week with average removal efficiencies ranging from 37.4 to 51.3% at the outlet sampling ports. This could be due to absorption of orthophosphate by the substrate or by microbial uptake as reported by Soupir *et al.* (2018). In our study, there was a rapid decline to less than 13% removal efficiency after the first week of operation (Figure 6), where the mean

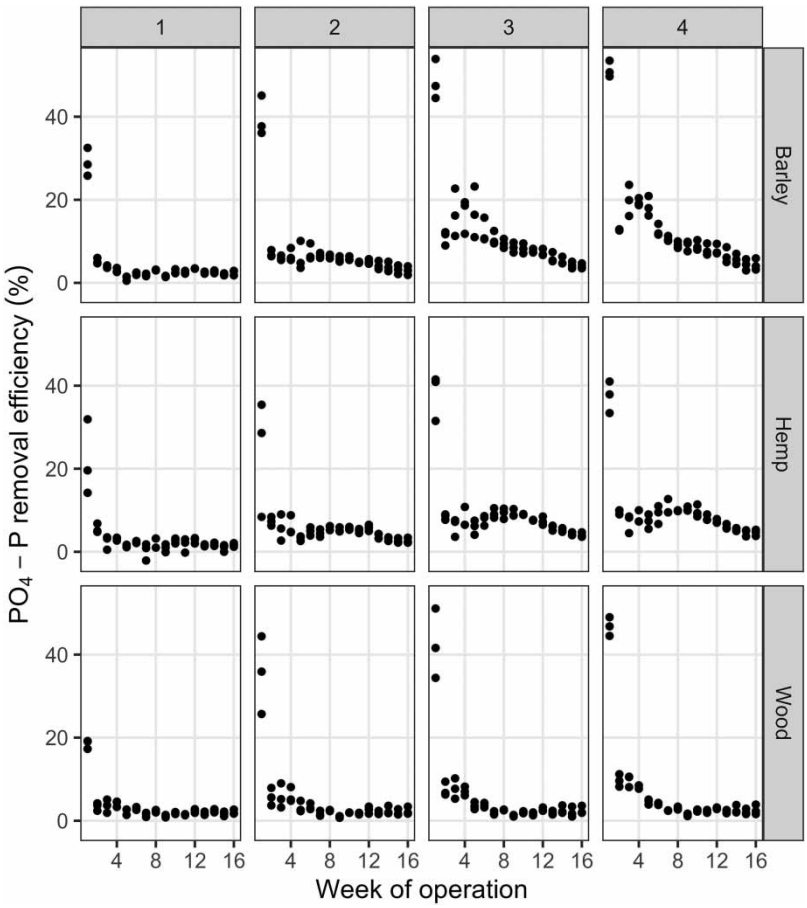


Figure 6 | Time series of PO_4-P removal efficiencies at different ports along the length of the bioreactors (sample ports: 1 = bottom, 2 = middle, 3 = top; 4 = outlet).

PO_4-P removal efficiencies were higher for barley straw (10.5 ± 5.2%) than hemp straw (7.9 ± 2.2%) and woodchips (4.0 ± 2.8%). Median values of PO_4-P removal efficiencies were significantly different between barley straw and woodchips and hemp straw and woodchips ($P < 0.05$).

Orthophosphate removal efficiencies in this study were lower than some of the values reported in the literature. Husk *et al.* (2018) found that a mixed-media bioreactor showed approximately 19 times more P removal than three woodchips-only bioreactors, however this study was also performed as a full-scale field experiment in the cold, humid climate of Quebec, Canada, during a three-year period. The soluble reactive P fraction concentration was reduced by 53% in the woodchip bioreactors, and by 97% in the mixed-media (activated alumina and gravel) bioreactor. Bock *et al.* (2015) performed a laboratory scale experiment with nine bioreactors – one woodchip control, and eight different woodchip-biochar treatments – and reported an average P removal of 65% by 18 h from the biochar treatments. Comparatively, Li *et al.* (2018) used a laboratory bioreactor design in which a 3-m long horizontal column was filled with woodchips and a 1-m long column was filled with fly ash pellets. The tests were performed for 28 h. The authors reported an average phosphate removal of 71.4% from the fly ash pellet column and an orthophosphate removal ranging from 10 to 13% from the woodchips column, despite their primary purpose being for nitrate removal. Further, Allred (2010) tested six different media fill materials to remove orthophosphate within lab-scale bioreactors and found that a high calcium oxide-high carbon fly ash material and surfactant-modified zeolite in combination had the greatest removal of PO_4-P at 50% or higher.

Orthophosphate removal rates differed slightly between the three carbon media with significant differences between both agricultural residues and woodchips ($P < 0.05$) and no significant difference between barley straw and hemp straw ($P < 0.05$). Removal rates were consistently higher for barley straw, with values up to 3.0 g P m^{-3} d^{-1}. Orthophosphate removal rates for hemp straw had values up to 2.3 g P m^{-3} d^{-1} while removal rates for woodchips had values no higher than 2.7 g P m^{-3} d^{-1}. Phosphate removal rates declined rapidly after the first week for the three carbon media making it difficult to recommend any for phosphate removal.

Greenhouse gas production

Bioreactors were a source of GHG production across the three different carbon media. In our study, woodchips generally produced the lowest GHG among the three media types (Table 2) which is consistent with the results from Bock *et al.* (2018) for laboratory scale bioreactors. The authors used three forms of woodchips as bioreactor fill media; woodchips, woodchips amended with 10% biochar – used as an agricultural soil amendment – and woodchips amended with 30% biochar. They found that biochar addition, particularly at 30% rate, was found to increase N_2O and CO_2 emissions.

Table 2 | Statistics (mean and standard deviation) of the GHG production rates in lab-scale bioreactors filled with woodchips, hemp straw and barley straw over 16 weeks

		GHG production rate (mean ± standard deviation)		
		N_2O	CH_4	CO_2
Carbon media	n	g m^{-3} day^{-1}		
Barley straw	96	0.18 ± 0.45 a	0.01 ± 0.01 a	24.88 ± 12.06 a
Hemp straw	96	0.13 ± 0.24 ab	0.01 ± 0.02 a	20.19 ± 11.28 b
Woodchips	96	0.11 ± 0.12 b	0.01 ± 0.02 a	18.24 ± 10.25 b

Presence of differing letters indicates significant differences at $P < 0.05$.

Greenhouse gas production was dominated by carbon dioxide (CO_2) with less methane (CH_4) and nitrous oxide (N_2O) produced (Figure 7). Methane production was lower compared to the other two gases but there were no statistically significant differences among the different carbon media (Table 2). These low values are consistent with findings from Davis *et al.* (2019) who reported less production of CH_4 (0.51 g m^{-3} day^{-1}) compared to N_2O (478.43 g m^{-3} day^{-1}) at 2 h HRT for woodchips. There were statistically significant differences for N_2O and CO_2 production among the different carbon media (Table 2).

Healy *et al.* (2012) reported high initial levels of CO_2 and a subsequent shift to CH_4 production under steady-state conditions in laboratory scale denitrification bioreactors using different media: lodgepole pine woodchips,

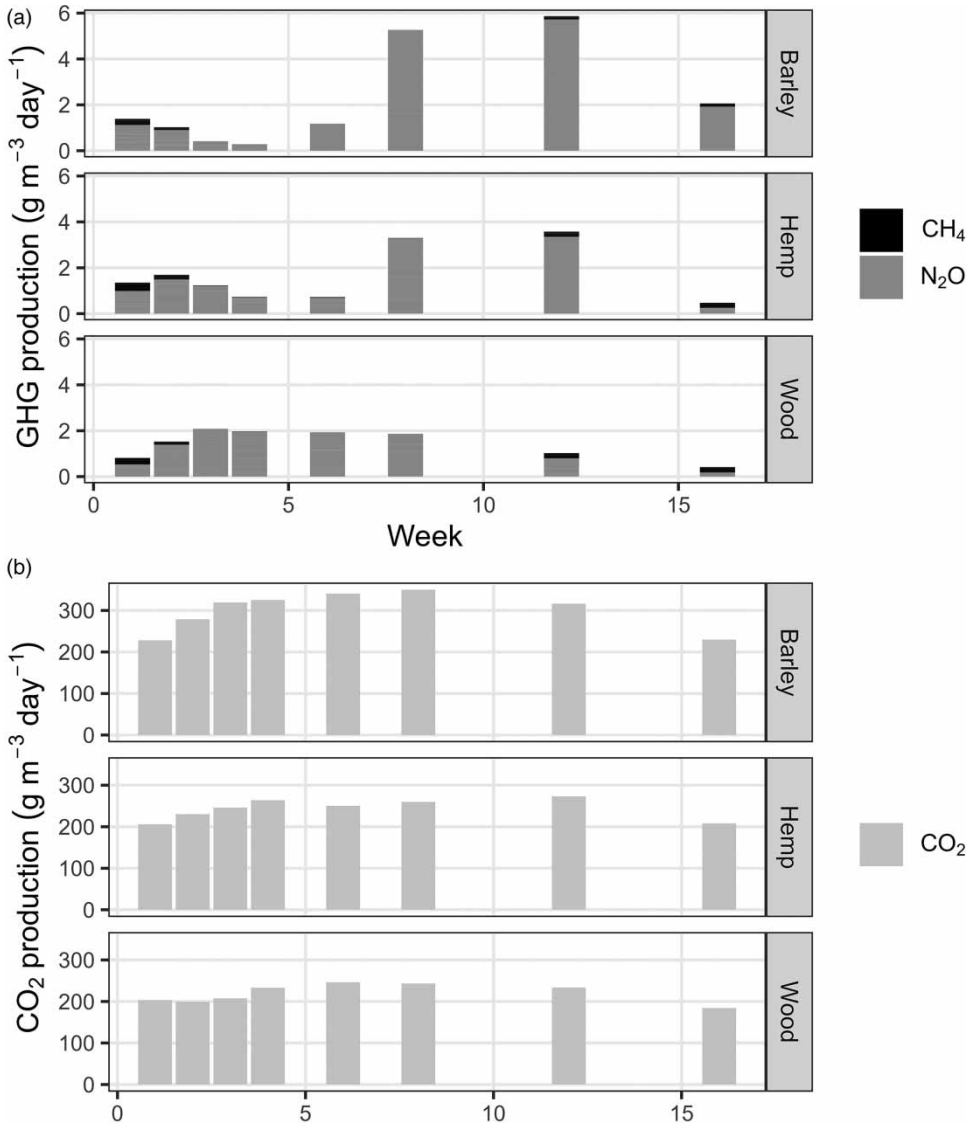


Figure 7 | GHG production measured in nine lab-scale bioreactors (three per carbon media) from four sampling ports over 16 weeks: (a) CH_4 and NO_2 (b) CO_2.

cardboard, lodgepole pine needles and barley straw. They recorded the highest emissions for the cardboard bioreactors, followed by barley straw and then by lodgepole pine woodchips.

After 16 weeks of bioreactor operation, the mean cumulative N_2O production in woodchip, hemp straw and barely straw bioreactors was 1.79, 8.03 and 24.16 g N m^{-3}, respectively (Table 3). Cumulative N_2O production was higher for barley straw than woodchips, although there was no significant difference ($P < 0.05$). After 16 weeks, the percentages of NO_3-N converted to N_2O while passing through the bioreactors were 0.16% for

Table 3 | Cumulative dissolved N_2O production and relative cumulative NO_3-N removal in lab-scale bioreactors filled with woodchips, hemp straw and barley straw over 16 weeks

		Cumulative N_2O production	Cumulative N_2O production relative to cumulative NO_3-N removal
Carbon media	**n**	**g N m^{-3}%**	
Barley straw	96	24.16 ± 41.8	0.75 ± 1.30
Hemp straw	96	8.03 ± 13.3	0.35 ± 0.58
Woodchips	96	1.79 ± 0.46	0.16 ± 0.05

woodchips, 0.35% for hemp straw and 0.75% for barley straw (Table 3), indicating that N_2O production was negligible.

Our results are consistent with the findings reported by Feyereisen *et al.* (2016) who found that cumulative N_2O production in lab-scale bioreactors filled with barley straw (56 g N m^{-3}) remained higher than those filled with woodchips (15.3 g N m^{-3}) over five months at a 12 h HRT. One difference, however, is that lower N_2O production rates and percentage of NO_3-N converted to N_2O were obtained in our study. This may have been because we used a shorter operation time and shorter HRTs which would not have allowed for as much opportunity for denitrification in comparison to the five months used in Feyereisen *et al.* (2016).

As mentioned, less than 1% of the NO_3-N removed was in the form of N_2O (0.16–0.75%). This is comparable with the values reported for woodchips by David *et al.* (2016) (0.44–0.89%) and by Christianson *et al.* (2013a) (<0.32%). Our values were much lower, however, than the values reported by Feyereisen *et al.* (2016) (9.7%). Woli *et al.* (2010) also reported negligible N_2O fluxes, suggesting that the denitrification process resulted in the production of N_2, rather than becoming N_2O and indicating little concern for N_2O release from their bioreactors. Greenan *et al.* (2009) also found in their laboratory study using columnar bioreactors with woodchips, that the N_2O released ranged from 0.003 to 0.033% relative to NO_3-N removal, indicating that near-complete denitrification occurred.

CONCLUSIONS

This study evaluated the performance of three carbon media including two agricultural residues – barley straw and hemp straw – and woodchips for removing NO_3-N and PO_4-P from simulated subsurface drainage waters. Bioreactor replication allowed for the estimation of the variability of the results. Findings from the two agricultural residues show adequate hydraulic properties for denitrification and thus were suited for field-scale bioreactor applications. Removal efficiencies varied from 0.5 to 36.9% for NO_3-N and from 1.1 to 53.5% for PO_4-P, with greatest to poorest performance shown by barley straw > hemp straw > woodchips, and the durability of carbon materials was in the order of woodchips > hemp straw > barley straw. Earlier peak time of NO_3-N removal occurred in woodchips than straw bioreactors, suggesting agricultural straw materials might need more flushing time after bioreactor installation to establish microbial populations for acceptable NO_3-N removal. This study shows that barley straw could remove twice as much nitrate as woodchips. PO_4-P removal efficiencies and rates declined rapidly after the first week for the three carbon media meaning none were superior. This could be a co-benefit for reduction of PO_4-P concentrations in tile drainage water considering the relatively low P concentrations as compared to N.

In this study, GHG production rates were dominated by CO_2, with less CH_4 and N_2O produced. Although the woodchip bioreactors produced the smallest quantities of N_2O, the respective production related to NO_3-N removal in all nine bioreactors were very low at 0.16–0.75%, so there was little concern of N_2O release from these bioreactors.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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