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Research article

Impact of hydraulic residence time on nitrate removal in pilot-scale woodchip bioreactors

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ABSTRACT

Nitrate (NO_3^-N) export from row crop agricultural systems with subsurface tile drainage continues to be a major water quality concern. Woodchip bioreactors are an effective edge-of-field practice designed to remove NO_3^-N from tile drainage. The NO_3^-N removal rate of woodchip bioreactors can be impacted by several factors, including hydraulic residence time (HRT). This study examined the impact of three HRTs, 2 h, 8 h, and 16 h, on NO_3^-N removal in a set of nine pilot-scale woodchip bioreactors in Central Iowa. NO_3^-N concentration reduction from the inlet to the outlet was significantly different for all HRTs ($p < 0.05$). The 16 h HRT removed the most NO_3^-N by concentration (7.5 mg L^{-1}) and had the highest removal efficiency at 53.8%. The 8 h HRT removed an average of 5.5 mg L^{-1} NO_3^-N with a removal efficiency of 32.1%. The 2 h HRT removed an average of 1.3 mg L^{-1} NO_3^-N with a removal efficiency of 9.0%. The 2 h HRT had the highest NO_3^-N mass removal rate (MRR) at 9.0 g m^{-3} day^{-1}, followed by the 8 h HRT at 8.5 g m^{-3} day^{-1}, and the 16 h HRT at 7.4 g m^{-3} day^{-1}, all of which were statistically different ($p < 0.05$). Significant explanatory variables for removal efficiency were HRT ($p < 0.001$) and influent NO_3^-N concentration ($p < 0.001$), ($R^2 = 0.80$), with HRT accounting for 93% contribution. When paired with results from a companion study, the ideal HRT for the bioreactors was 8 h to achieve maximum NO_3^-N removal while reducing the impact from greenhouse gas emissions.

1. Introduction

In the Upper Mississippi River Basin, crop fertilization and soil organic matter mineralization are major sources of nitrate (NO_3^-N) to surface waters because of subsurface tile drainage (Cordell et al., 2009). Nitrate in agricultural drainage is quickly exported to local surface waters (U.S. EPA, 2016), and eventually, the Mississippi River and the Gulf of Mexico (Goolsby et al., 2001). Excessive NO_3^-N contributes to harmful algal blooms (HAB), which causes hypoxic zones and leads to reduced aquatic integrity (Rabalais et al., 2002). When NO_3^-N is ingested by humans, it can be converted to nitrite (NO_2^-N), which can combine with amines to form carcinogenic nitrosamines. Ingestion at concentrations significantly above the drinking water standard set by the United States Environmental Protection Agency at 10 mg L^{-1}, can lead to several types of cancer (U.S. EPA NPDWR, 2017; McCasland et al., 2012; DeSimone et al., 2009).

Extensive subsurface tile drainage in the Upper Midwest typically combines flow from multiple ownerships into drainage district networks, making regulation and adoption of farm-level conservation practices challenging. In Iowa, over 40 million hectares of agricultural land are tile drained, with farms averaging 140 ha (Christianson et al., 2018). To reduce NO_3^-N export, multiple approaches are needed in the agricultural landscape. The Iowa Nutrient Reduction Strategy (INRS) estimates that a 41% reduction in NO_3^-N loading from non-point sources is needed to meet the 45% total load reduction goal set by the U.S. EPA (INRS et al., 2017).

Edge-of-field practices will play a critical role in meeting NO_3^-N reduction goals. In-field practices are estimated to remove from −3 to 41% of NO_3^-N by concentration, contributing to 10% of the total NO_3^-N load reduction needed to meet INRS goals (INRS et al., 2017). Edge-of-field practices have potential to remove 33–91% of NO_3^-N and are needed to account for the rest of the NO_3^-N load reduction. Woodchip bioreactors are a cost effective and minimally invasive strategy to remove NO_3^-N from subsurface drainage through microbial

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denitrification (Christianson et al., 2010) with an estimated NO_3^-N removal efficiency of 43% (INRS et al., 2017). Denitrification occurs under anaerobic conditions with a readily available carbon source (Schipper and Vojvodić-Vuković, 2001). The ideal final product of denitrification is N_2 (gas). Incomplete denitrification can result in the generation of nitrous oxide (N_2O), a volatile greenhouse gas (GHG) that contributes to positive radiative forcing (Moorman et al., 2010; IPCC, 2014).

As an edge-of-field practice, bioreactors have little to no impact on crop yield or soil quality, and they also require minimal land taken out of production (Christianson and Helmers, 2011). Bioreactors are designed with bypass flow, which allows for a fraction of the flow to be routed around the woodchip bioreactor during high flow conditions. This design feature helps prevent short-circuiting in the bioreactor, and also ensures that the bioreactor does not cause drainage to back-up into agricultural fields. While important for protecting crops, bypass flow allows for drainage water to go untreated during high flow conditions. Bioreactors require minimal management, with the inlet and outlet control structures needing to be adjusted approximately twice per year (Christianson and Helmers, 2011b). In Iowa, average bioreactor installation cost currently ranges from $10,000 to $20,000, with most installations being partially supported by cost sharing (Christianson and Helmers, 2011b; McKinney, 2018). With an average removal rate of 43%, bioreactors have an estimated cost per kg of nutrient removed of \$0.40 kg $NO_3^-N^{-1}$ to \$4.86 kg $NO_3^-N^{-1}$ (Christianson et al., 2013, 2018; Law et al., 2018). Woodchip bioreactors have a wide variety of reported NO_3^-N removal rates, but typically range from 13 to 100% depending on conditions and location (Christianson et al., 2012b, 2018; Greenan et al., 2009; Hassanpour et al., 2017). To maximize NO_3^-N removal, bioreactors must be engineered to optimize denitrification through consideration of landscape placement, shape, biomass source, carbon source, drainage treated, peak flow conditions, and hydraulic residence time (HRT).

Hydraulic residence time is an important factor impacting NO_3^-N removal within bioreactors. Ideal design for HRT is challenging based on studies in uncontrolled field settings because of high variability among sites. Christianson et al. (2012a) recommended that additional field-scale studies are needed to validate and enhance understanding of NO_3^-N removal in woodchip bioreactors. Previous research on bioreactors has not included in-reactor sampling, which could help identify specific processes besides denitrification (such as total ammonia nitrogen (TAN) production or nitrification) and where they occur within the bioreactor. This study focused on the role of HRT on overall NO_3^-N removal in controlled flow, triplicate pilot-scale woodchip bioreactors. Objectives were to (1) determine the effect of HRT on NO_3^-N removal, (2) compare NO_3^-N removal between in-reactor treatment zones, (3) compare each HRT by NO_3^-N removal when considering bypass flow. Pilot-scale bioreactors provided a unique opportunity to study these processes in a controlled system to improve woodchip bioreactor design to maximize NO_3^-N removal.

2. Materials and methods

2.1. System overview

The study was conducted in nine pilot-scale bioreactors at the Iowa State University Agronomy and Agricultural Engineering Farm, located west of Ames, Iowa (42.019861, −93.776872). The system was described in detail by Hoover et al. (2017) and a schematic is shown in Fig. 1. The bioreactors were installed in September 2014 and the water source was a 30.5 cm diameter tile drainage line. Water was pumped to three 11,356 L aboveground storage tanks, which held the water before entering the bioreactors. Water flowed by gravity to the reactors and was controlled by gate valves. The nine bioreactors had individual internal dimensions of 5.79 m × 1.0 m x 1.07 m with a concrete frame, which created a closed system that allowed for estimation of water

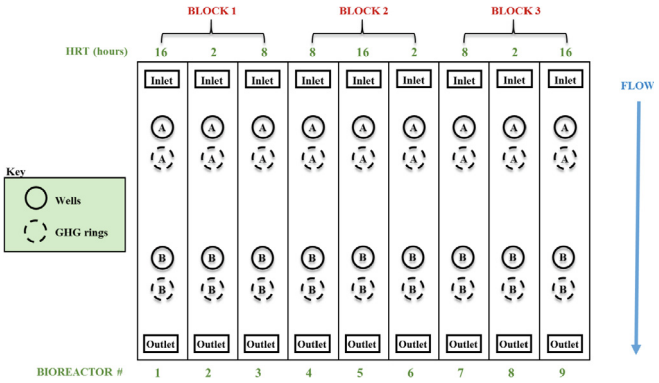

Fig. 1. The layout of the set of nine pilot-scale woodchip bioreactors used in the study located in Central Iowa. They were constructed in 2014 and ran for this study from 2016 to 2017. There are three blocks with three different hydraulic residence times (HRT) set at 2 h, 8 h, and 16 h for a total of three repetitions per HRT.

balance. The bioreactors were filled with local hardwood woodchips from Golden Valley Hardscapes (Story City, Iowa), which are described in detail by Christianson et al. (2010). Bioreactors were only active for three weeks in 2015, prior to this study.

The bioreactors had four main structures: an inlet port, two sampling wells, and an outlet structure (Fig. 1). Each bioreactor had two 1.8 m PVC sampling wells attached to the bottom of the bioreactor and positioned along the centerline lengthwise. The wells were slotted at 1.3 cm increments to a depth of 1.1 m that allowed the flow through of water for collecting samples. The two wells were considered sampling points “well A” and “well B” for each bioreactor, with well A located closest to the inlet port and well B located closest to the outlet structure (Fig. 1). Hydraulic residence times were randomly assigned in a complete randomized block design to include triplicates of 2 h, 8 h, and a 16 h HRTs.

2.2. System sampling and flow control

Hydraulic residence times were maintained using target flow rates. Target flow rates were determined for each HRT treatment using volume and media porosity for all nine bioreactors. Hoover et al. (2017) conducted potassium bromide tracer studies on each bioreactor shortly after system installation. The tracer residence time averaged among the nine reactors was 2.3 ± 0.3 h, which was similar to the estimated HRT (the method used to determine HRT for this study) of 2.1 ± 0.3 h. Media porosity was estimated as 0.7 (NRCS, 2016). Bioreactor volume was calculated using internal bioreactor dimensions. Inlet flow to each bioreactor was measured every three to four days and adjusted as needed to achieve target flows for 2, 8, and 16 h HRTs (Table 1). To measure the HRT of the bioreactors, inflow valves at the inlet ports were used to adjust flows weekly using a stopwatch and bucket. The initial flow was recorded by the bucket and stopwatch method, and then the flow was adjusted to the correct timing. Once the correct HRT was achieved, the bucket and stopwatch method was repeated twice to ensure accuracy.

Data was collected in 2016 from August to the end of October, but the system ran from June to the beginning of November. Boone County, Iowa experienced dry weather during June 2016, with precipitation 10.06 cm below annual averages (NOAA Climate Data, 2017). Precipitation was also below normal during 2017. Between June and July, rainfall totaled 15.37 cm below average, with drought conditions continuing through October (NOAA Climate Data, 2017). Valid data was collected from May through July for 2017. During the winter months of 2016 and 2017, bioreactors were left filled with water to prevent woodchip degradation. When temperatures reached above consistent freezing temperatures in Iowa, the bioreactors were turned on for the

Table 1
The summary data for the 2 h, 8 h, and 16 h hydraulic residence times from a set of pilot-scale woodchip bioreactors in Central Iowa, including flow and NO_3^-N removal rates. Standard deviation values are in parentheses. Letters indicate which values were significantly different from one another. MRR is mass removal rate. Removal efficiency is defined as the percent removal of NO_3-N from the inlet of the bioreactor to the outlet.

HRT	Flow				Nitrate-N Removal		Nitrate-N Concentration
	Target Flow		Observed Flow		Removal Efficiency	MRR	Concentration Removed
Hours	L min^{-1}	L day^{-1}	L min^{-1}	L day^{-1}	%	g m^{-3} d^{-1}	mg L^{-1}
2	31.4	45,187	31.7 a (± 4.84)	45,675 (± 6973) a	9.0 (± 3.69) a	9.0 (± 4.01) a	1.3 (± 0.58) a
8	7.9	11,298	8.2 b (± 0.71)	11,746 (± 1024) b	32.1 (± 5.18) b	8.5 (± 2.42) b	5.5 (± 1.32) b
16	3.9	5649	4.4 c (± 1.37)	6317 (± 1963) c	53.8 (± 17.55) c	7.4 (± 2.52) c	7.5 (± 4.11) c

season. Bioreactors flowed for one week before sample collection to allow for flushing of water held during the winter.

2.3. Sample collection

Samples were collected weekly for the duration of the study. Water samples were collected at the inlet, well A, well B, and the outlet. At the inlet, water samples were collected from the inlet valves. At well A, well B, and the outlet, a peristaltic pump was used to extract the water samples. Before collecting the water sample, wells were evacuated by pumping out 8.2 L of water, a volume equivalent to the well volume, and allowed to refill. Sample bottles were rinsed with the sample water before collection for analysis. A total of 125 mL was sampled from each location. Samples were stored on ice in the field before being taken to the laboratory. Nitrate and total ammonia nitrogen (TAN) samples were acidified with sulfuric acid (H_2SO_4). Samples were stored in the laboratory at 4 °C prior to analysis. Dissolved oxygen and temperature were measured in situ at all sampling points.

2.4. Sample analysis

Samples were analyzed using a Seal Analytical (Mequon, WI) AQ2 discrete autoanalyzer. NO_3^-N samples were measured as NO_3^-N + NO_2^-N using AQ2 method EPA-114-A, Rev. 7 (equivalent to U.S. EPA method 353.2, ver. 2 (1993)) where the NO_3^-N in the samples were reduced by copperized cadmium to NO_2. After reduction, NO_2 was measured spectrophotometrically at 520 nm with a detection limit of 0.03 mg N L^{-1}. If NO_3^-N concentrations were below 0.25 mg N L-1, the AQ2 method EPA-127-A, Rev. 7 (range 0.012–2.0 mg N L^{-1}) was used. TAN was measured as NH_3-N using AQ2 method EPA-103-A, Rev. 10 (equivalent to U.S. EPA method 350.1, Rev. 2.0) by allowing NH_3 to combine with hypochlorite (OCH) ions to form mono chloramine (NH_2Cl), which reacts with phenate, resulting in 5-aminophenate that oxidizes after being exposed to sodium nitroprusside. The reaction creates indophenol, a blue compound that is read in the spectrometer at 660 nm.

2.5. Data analysis

Weekly instantaneous flow values were converted to daily flows (L day^{-1}). Flows and NO_3^-N concentrations between collection days were linearly interpolated using R Studio statistical software (R Development Core Team, 2014). Flow rates were measured and adjusted as needed to respective target HRT flow rates approximately every three to four days. Linear interpolation of flow and NO_3^-N have been done in previous bioreactor studies (Herbstritt, 2014) and is a common practice with hydrologic flow data (Kratzer et al., 2006). Interpolated flows were used to calculate daily mass removal rate (MRR) of NO_3^-N using Equation (1), where V_{WB} is the volume of the woodchip bioreactor and t is time. Removal efficiency of NO_3^-N was calculated using Equation (2). Removal efficiency is defined as the percent removal of NO_3^-N from the inlet of the bioreactor to the outlet. Removal (MRR or concentration) was analyzed with a linear mixed model using R Studio with interactions between the treatment (HRT) and sampling location within and across the bioreactors as a time series (with repeated measures). Time, treatment, sampling location, and block all had fixed effects. Reactor and sampling location by reactor had random effects. An ANOVA was performed using the mixed model comparing sampling location and treatment (HRT), with a Tukey pairwise comparison post hoc analysis between treatment zones. Flow was compared among the three treatments in an ANOVA test with a Tukey Test as the post-hoc analysis in R Studio.

Temperature, dissolved oxygen (DO), influent NO_3^-N, and HRT were used as explanatory variables in a multiple linear regression model to test the dependency of each on removal efficiency and MRR using R Studio, and model performance was assessed using R^2 values. Temperature and DO values were averages of the outlet subtracted from the inlet values. All data was run together in the model to allow explanatory parameters only to be used if they were statistically significant ($p < 0.05$). A Durbin-Watson test was run at 5% significance to detect for correlated errors between observations that were temporally near each other. Multicollinearity diagnostics were also run to test for relationships between the explanatory variables. A leave-one-out cross validation was run using the same multiple linear regression model for removal efficiency to determine the percent contribution of HRT, temperature, DO, and influent NO_3^-N on removal efficiency. The percent contribution of each parameter was determined using Equation (3). The R-reference value was the R^2 value from the multiple linear regression model run using all four parameters. The R leave variable was the R^2 value from the multiple linear regression model without one parameter, which was repeated until each parameter had been removed one time.

An assessment was conducted to estimate NO_3^-N removal when bypass flow is included to represent typical field conditions. The total flow to all reactors was set to the 2 h HRT maximum flow (45,187 L d^{-1}), but the maximum flow delivered to each reactor was mediated by the average flow for each HRT (Table 1). Thus, the excess flow to the 8 and 16 h HRT reactors was classified as bypass, and that volume of water remained untreated. NO_3^-N influent to all reactors was calculated using the average influent NO_3^-N concentration for the 2016–2017 testing period while NO_3^-N removal was calculated using the fraction of flow treated and average removal efficiency for each HRT.

3. Results and discussion

3.1. Flow

In this unique experimental design, HRT was the controlled treatment within triplicate pilot-scale bioreactors. Target and achieved flow for the three HRTs are shown in Table 1. All flows were significantly different from each other ($p < 0.05$). Observed and target flow for all three HRTs were within < 1.0 L min^{-1} through the duration of 2016–2017. The 2 h HRT had the greatest standard deviation of ±

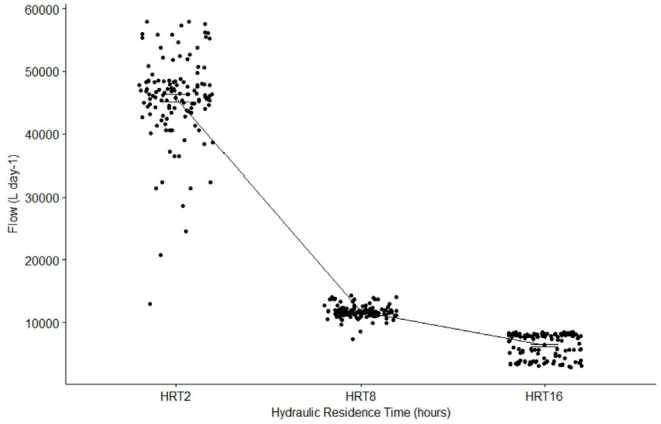

Fig. 2. The standard deviation in flow during the 2016–2017 testing season, grouped by HRT at 2 h, 8 h, or 16 h. The 2 h HRT had the greatest deviation at ± 6973 L day^{-1}, followed by the 16 h HRT at ± 1963 L day^{-1}. The 8 h HRT had the lowest deviation in flow at ± 1024 L day^{-1}. All mean flows were significantly different from each other at $p < 0.05$.

6973 L day^{-1}, followed by the 16 h HRT (± 1963 L day^{-1}) (Fig. 2), and the 8 h HRT, with the lowest flow standard deviation at ± 1024 L day^{-1}. The fast flow rate of the 2 h HRT and the slower flow of the 16 h HRT were difficult to maintain in the pilot-scale system.

3.2. Nitrate removal by concentration

All bioreactors at all HRTs exhibited a significant ($p < 0.05$) reduction in NO_3^-N concentration from the inlet to the outlet (Fig. 3). In the 2 h HRT, average NO_3^-N removal was 1.3 (± 0.58) mg L^{-1}. The average percent of NO_3^-N removed for the 2 h HRT was 9.0% (± 3.69). The 8 h HRT removed an average of 5.5 (± 1.32) mg L^{-1} NO_3^-N by concentration from the inlet to the outlet. Average percent NO_3^-N removal for the 8 h HRT was 32.1% (± 5.18). The 16 h HRT removed an average of 7.5 (± 4.11) mg L^{-1} NO_3^-N by concentration. The average percent of NO_3^-N removed for the 16 h treatment was 53.8% (± 17.55). As HRT increased, NO_3^-N removal efficiency and removal by concentration also increased. These results are in agreement

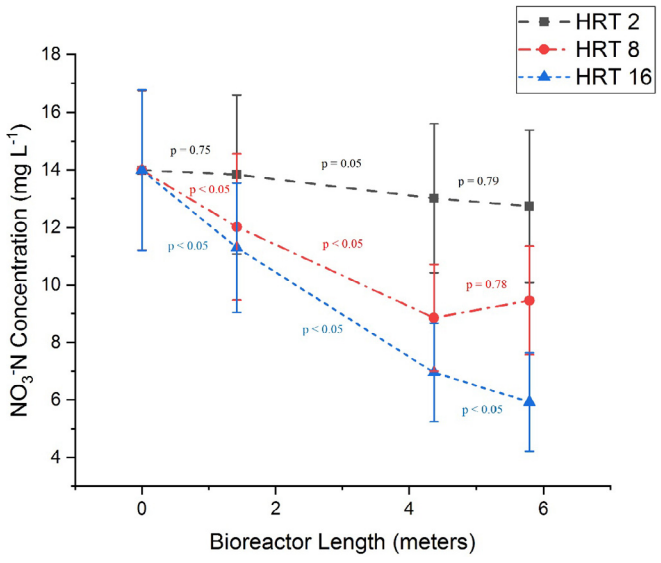

Fig. 3. The NO_3^-N removal rates by concentration over the length of the bioreactor, grouped by HRT. The significance is indicated by the p-values next to the corresponding line on the graph in the matching color indicated by the legend. All NO_3^-N concentrations for all HRTs were significantly lower ($p < 0.05$) from the inlet to the outlet.

with previously published studies that also found a positive relationship between increasing HRT and NO_3^-N percent removal (Nordstrom and Hebert, 2017; Hoover et al., 2015; Christianson et al., 2012a).

When evaluating NO_3^-N concentration removal within bioreactors, well A, well B, and the outlet were significantly different across all treatments ($p < 0.05$). The only exception was the inlet concentration, which was expected because the same water source was provided to all bioreactors. The majority of NO_3^-N removal in the 2 h HRT occurred from well A to well B with 66.2% of the total reduction. NO_3^-N concentration did not drop significantly between the inlet and well A ($p = 0.75$) or well B to the outlet ($p = 0.79$) (Fig. 3) in this HRT. In the 8 h HRT, 69.9% of the total NO_3^-N removal occurred between wells A and B. The only section of the bioreactors that did not have statistically significant NO_3^-N concentration removal was between well B and the outlet ($p = 0.78$). In the 16 h HRT, 53.8% of the total NO_3^-N removal occurred between wells A and B. Similar to the 8 h HRT reactors, the only area of the 16 h bioreactors that did not significantly differ in NO_3^-N concentration was well B to the outlet ($p = 0.09$).

For all HRTs, the majority of NO_3^-N removal occurred between wells A and B, which can be explained in several ways. First, this is the longest bioreactor length at 2.95 m versus the 1.42 m between all other sampling points. However, when normalizing for the extra length, the majority of NO_3^-N removal still occurred over the central portion of the bioreactors. Dissolved oxygen concentrations were still elevated in the first section of the bioreactors, potentially leading to less efficient denitrification. The first section of the bioreactors dropped, on average, from 7.1 mg L^{-1} to 2.4 mg L^{-1} in the 2 h HRT, 1.4 mg L^{-1} in the 8 h HRT, and 1.6 mg L^{-1} in the 16 h HRT (Fig. 4). Warneke et al. (2011a) found that the carbon substrate closest to the inlet mainly serves as the removal area for DO. Between well B to the outlet, NO_3^-N removal could be impeded by lower concentrations of NO_3^-N, switching from zero-order kinetics to first-order kinetics. Both have been observed in other bioreactors with a transition expected as NO_3^-N concentration decreases (Schipper et al., 2010).

A pattern of NO_3^-N increase from well B to the outlet was observed for differing time periods in almost every bioreactor (Fig. 3), though on

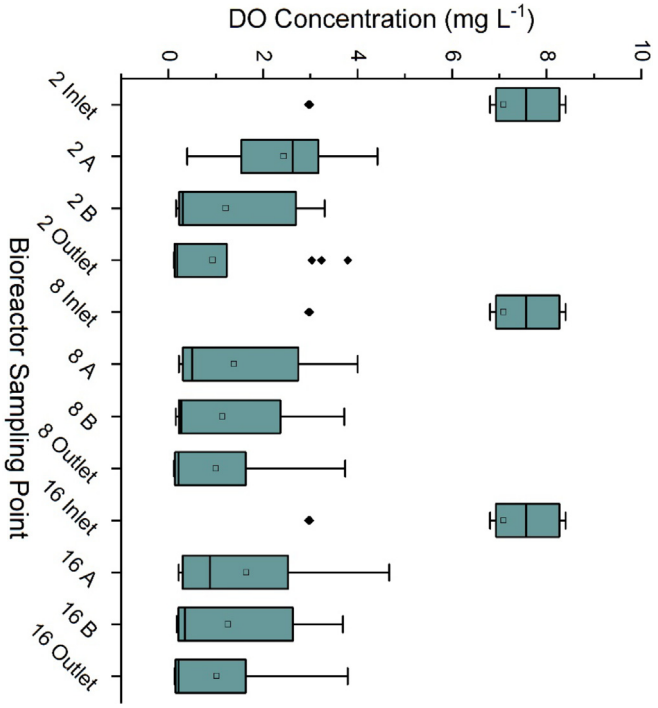

Fig. 4. The comparison of dissolved oxygen (DO) levels for the 2 h, 8 h, and 16 h HRTs. DO was not significantly different ($p > 0.05$) between any of the HRTs at any of the testing points.

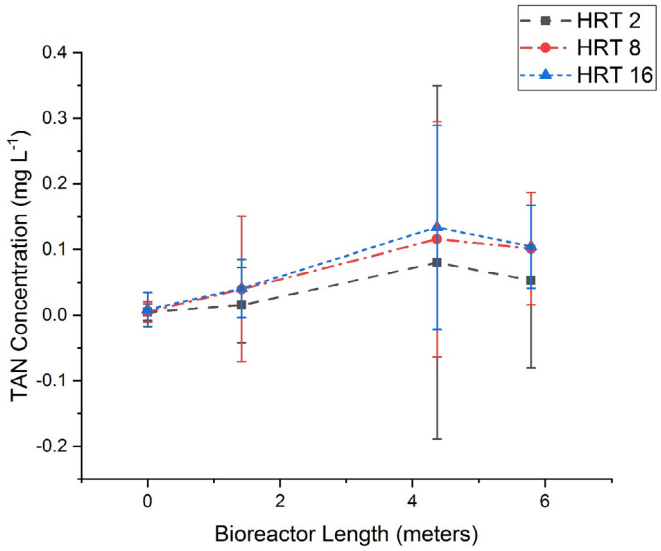

Fig. 5. The total ammonia nitrogen (TAN) concentrations over the length of the bioreactors for all HRTs. Over the course of the study period, each of the three HRTs had a significant net production of TAN ($p < 0.05$). TAN concentrations were not statistically different between any of the HRTs ($p > 0.05$).

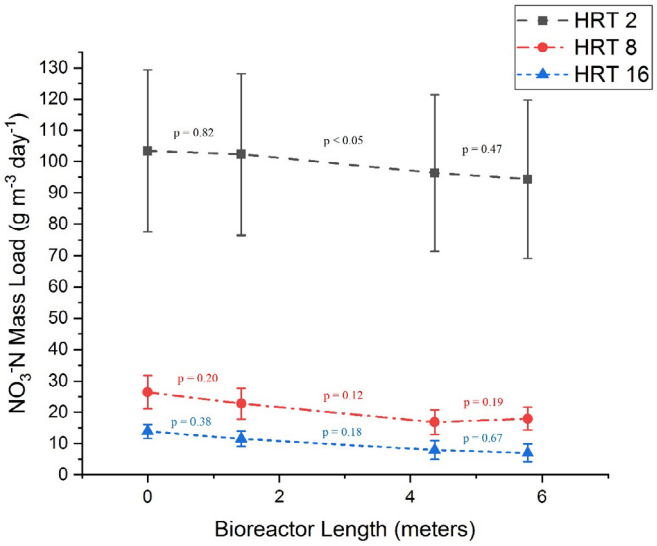

Fig. 6. The NO_3^-N mass load removal over the length of the bioreactors, grouped by HRT. Significance level is indicated as p-values next to lines with corresponding color as indicated by the legend. All NO_3^-N mass load removal rates for all HRTs were significantly lower ($p < 0.05$) from the inlet to the outlet.

average the increase in NO_3^-N was not statistically significant, except in one bioreactor (#4). This observation coincided with sampling points where there was also either TAN production, or TAN entered the bioreactor with the influent flow. Fig. 5 shows that at the same point from well B to the outlet there was a decrease in TAN. The increase in NO_3^-N during these periods can partly be explained by nitrification. Nitrification is a process carried out by chemoautotrophic bacteria in which ammonia or ammonium is oxidized to NO_3^-N (Chun et al., 2009). Nitrifying bacteria are obligate aerobes, but they are microaerophiles, which means they thrive under low oxygen conditions (Ward, 2008). Although maximum nitrification typically occurs at 3 mg L^{-1} DO and 30 °C, it only ceases once DO levels are < 0.5 mg L^{-1} and temperature is < 5 °C (Sharma and Ahlert, 1977). The DO levels in the bioreactors were never less than 0.5 mg DO L^{-1} at any point during the study (Fig. 4), and temperature never dropped below 11.5 °C, indicating that nitrification of TAN is a possible explanation for the observed increase of NO_3^-N between well B and the outlet. However, the mass of TAN removed (0.2 g m^{-3} day^{-1}) only accounts for 5.6% of the total NO_3^-N concentration produced (2.9 g m^{-3} day^{-1}) from well B to the outlet in the 8 h HRT. The source of the remaining N could also be due to a zone of unmixed flow at that location within the bioreactor, leading to an unrepresentative low NO_3^-N concentration at well B. While Hoover et al. (2017) found uniform flow among the pilot scale bioreactors, it is possible woodchip degradation and movement have created preferential flow around well B to the outlet.

In a review of denitrifying bioreactors, Christianson et al. (2012b) stated that the bioreactor denitrifying community can vary with time of year, flow direction, and depth of water within the bioreactor. The microbial community within the pilot-scale bioreactors could have adjusted throughout the year, possibly explaining the inconsistent timing of NO_3^-N increases between B and the outlet. Moorman et al. (2010) found that their bioreactor woodchip decomposition rates were controlled by sustained anaerobic conditions below the tile drainage line. Our woodchip bioreactors could have experienced similar variation with different areas within the bioreactors exhibiting different rates of processes or differing microbial communities.

3.3. Nitrate mass removal comparison

Average mass NO_3^-N removal rates were 9.0 (± 4.01) g m^{-3} day^{-1} (2 h HRT), 8.5 (± 2.42) g m^{-3} day^{-1} (8 h HRT), and 7.4 (± 2.52) g m^{-3} day^{-1} (16 h HRT) (Table 1). When comparing all points (inlet, A, B, and outlet) across HRTs, NO_3^-N mass load removal rates were significantly different ($p < 0.05$). Inlet mass NO_3^-N loads were significantly different between HRTs due to difference in flows.

In the 2 h HRT, a statistically significant NO_3^-N mass removal ($p < 0.05$) was observed between the inlet and outlet (Fig. 6). Nitrate mass removal was not significant between the inlet to well A ($p = 0.82$) and well B to the outlet ($p = 0.47$). However, NO_3^-N removal rate was significant between sampling points A and B ($p < 0.05$). In the 8 h HRT, when comparing the inlet to A, A to B, and B to the outlet, no statistically significant differences in NO_3^-N mass load removal rates were observed ($p > 0.05$). When comparing longer portions of the bioreactor, (inlet to outlet, A to outlet, and inlet to B) all had significant differences in NO_3^-N MRR ($p < 0.05$). For the 16 h HRT, the only points that were statistically different from each other were the inlet to the outlet and the inlet to point B ($p < 0.05$).

Our observed pattern in NO_3^-N mass load removal rate versus HRT is in contrast to previous studies. Hoover et al. (2015) found in a laboratory study that incrementally increasing HRT from 1.7 h to 21.2 h resulted in consistent NO_3^-N mass removal, not increasing. Christianson et al. (2011) studying pilot-scale bioreactors in Central Iowa also observed that NO_3^-N removal rates were consistent as HRT ranged from 1.3 to 11.3 h, despite the percent NO_3^-N reduction increasing with increasing HRT. Because this was a controlled study and flows did not vary as they do in the field, influent NO_3^-N mass loads were more consistent over time at each HRT. The mean influent load for the 2 h HRT was 103.3 g m^{-3} day^{-1} (± 25.98), the 8 h HRT was 26.4 g m^{-3} day^{-1} (± 5.34), and the 16 h HRT was 14.8 g m^{-3} day^{-1} (± 1.87). Despite its low NO_3^-N removal efficiency, the 2 h HRT removed the greatest NO_3^-N by mass ($p < 0.05$) because it treated a significantly greater volume of water in a 24-h period when compared to the reactors set at a higher HRT.

3.4. Total ammonia nitrogen production

Dissimilatory reduction of nitrate to ammonia or ammonium (DRNA) occurs under anaerobic conditions when NO_3^-N (the electron acceptor) is scarce, pH is above 7.0, carbon (the electron donor) is abundant, and microbes need to optimize the use of available oxidants

Table 2
The total ammonia nitrogen (TAN) concentrations across the bioreactors. Standard deviations are in parentheses. Over the course of the study period, each of the three HRTs had a significant net production of TAN ($p < 0.05$). TAN concentrations were not statistically different between any of the HRTs ($p > 0.05$). Letters indicate which values were significantly different from one another.

HRT	Total Ammonia Nitrogen Concentration			
	Inlet	A	B	Outlet
Hours	(mg L^{-1})			
2	0.01 (± 0.016) a	0.02 (± 0.060) ab	0.08 (± 0.277) ab	0.05 (± 0.085) b
8	0.01 (± 0.016) a	0.04 (± 0.114) ab	0.12 (± 0.182) ab	0.10 (± 0.085) b
16	0.01 (± 0.016)a	0.04 (± 0.044) ab	0.13 (± 0.157) ab	0.10 (± 0.061) b

to regenerate NAD+ (Mohan and Cole, 2007). DRNA is a two-step process, reducing nitrate to nitrite, and then reducing nitrite to ammonium. While the ideal final product of denitrification in bioreactors is N_2 (g), DRNA can alter the desired final product. TAN production and release can be directly toxic to aquatic ecosystems in high concentrations (Nordstrom and Herbert, 2017). TAN could have also been produced through the mineralization of N by the microbial decomposition of the woodchips or microbial biomass. Mineralization of N cannot occur at a C:N ratio that is higher than 16:1 (Enwezor, 1975). Woodchips have a high C:N ratio, sometimes as high as 513:1 (Nolan et al., 2011). This suggests that DRNA was the dominant TAN production process in the woodchip bioreactors.

Each of the three treatments had a statistically significant net production of TAN from the inlet to the outlet when averaged over the study period ($p < 0.05$). Concentrations were not significantly different between any of the HRTs. The 2 h HRT produced 0.05 (± 0.90) mg N L^{-1} TAN, the 8 h HRT produced 0.1 (± 0.09) mg N L^{-1} TAN, and the 16 h HRT produced 0.1 (± 0.06) mg N L^{-1} TAN (Table 2). The U.S. EPA has set ammonia standards at 1.9 mg TAN per liter at a pH of 7 at 20 °C over a 30-day average duration (U.S. EPA, 2017). The U.S. EPA also recommends that the highest four-day average in that period should not exceed 2.5 times the 1.9 mg TAN L^{-1} limit (USEPA, 2017). When taking these criterion into account, the woodchip bioreactors did not exceed the limits set by the U.S. EPA for any duration while they were running. Healy et al. (2012) found that ammonium (NH_4^+N) production increased in concentration along a lab-scale column, and that a shorter HRT had lower concentrations of NH_4^+N. Greenan et al. (2006) estimated that DRNA accounted for < 4% of NO_3^-N removal in bioreactors. Herbstritt (2014) had ammonium concentrations of 0.1 mg NH_4^+N L^{-1} in field-scale bioreactors, and they occasionally also saw increases from the inlet to the outlet.

3.5. Parameters that impact nitrate removal

In a multiple regression model analysis, NO_3^-N removal efficiency was found to be most strongly positively correlated with HRT and influent NO_3^-N concentration ($p < 0.001$) ($R^2 = 0.80$) (Table 3). The same relationships for HRT and influent NO_3^-N concentrations with removal efficiency were found in other bioreactors in Iowa (Christianson et al., 2012a). Temperature was also correlated with removal efficiency in this study but not as strongly as the other parameters ($p < 0.001$). This is in contrast to Christianson et al. (2012a), who observed that temperature was most strongly correlated with removal efficiency. The relationship between temperature and removal efficiency was negative due to large removal rates when influent NO_3^-N concentrations were high and temperatures were low. The only environmental parameter not significantly correlated with removal efficiency was DO ($p = 0.06$). In the leave-one-out cross validation, a percent contribution was found for each of the parameters (Table 4). These results showed that HRT had the greatest contribution to NO_3^-N removal efficiency (92.80%), followed by influent NO_3^-N concentration (6.19%), temperature (0.51%), and DO (0.19%).

When running the same multiple regression model analysis for MRR, all parameters were equally significant ($p < 0.001$) ($R^2 = 0.37$) (Table 3). All explanatory parameters except influent NO_3^-N concentration had a negative correlation with MRR. For HRT, this means that as HRT increased, the mass removal rate of NO_3^-N decreased. As DO increased, the MRR of NO_3^-N decreased in the woodchip bioreactors. Like removal efficiency, temperature had a negative correlation with MRR because of large removal rates when influent NO_3^-N were high and temperatures were low.

As found by Christianson et al. (2012a), this regression modeling approach is limited because of autocorrelated errors based on a time dependency. Their model failed the Durbin-Watson test ($p > 0.05$) because values that are closer in time dependency tend to be related. Their model passed the multicollinearity test, indicating that none of their variables were linear combinations of each other (Christianson et al., 2012a). Similarly, the regression model used in this study also failed the Durbin-Watson test and all variables passed the test for multicollinearity with variance inflation factors less than four.

Model results indicate that for every 1 mg NO_3^-N L^{-1} increase in influent concentration, there was a 0.7 g N m^{-3} day^{-1} increase in removal, resulting in a 1:0.7 ratio. A relationship of 1:0.9 strongly indicates first-order reaction kinetics, which were seen in other field bioreactors (Christianson et al., 2012a; Chun et al., 2010). Data were limited because the model assumed all other parameters were held constant, which may not be true under field conditions which can have a number of variables that affect kinetics. The influent NO_3^-N concentrations during the study period ranged from 10.7 mg N L^{-1} to 18.0 mg N L^{-1}. Other studies that were able to determine the impact of influent NO_3^-N concentrations on removal rates typically had larger ranges and higher concentrations (Ghane et al., 2015; Hoover et al., 2015). More variation in influent NO_3^-N concentration in the pilot bioreactors will help to better determine kinetics.

Temperature was a significant explanatory parameter for both NO_3^-N removal efficiency and MRR, but both were negative correlations. In-reactor water temperature ranged from 11.5 °C to 19.5 °C over the 2016–2017 testing period. Other lab-scale and field-scale studies have reported increasing removal rates with increasing temperature (Christianson et al., 2012a; Hoover et al., 2015; David et al., 2016). David et al. (2016) indicated that during the first three years of bioreactor operation, NO_3^-N removal performance can vary greatly in response to temperature, woodchip age, and woodchip quality. The pilot-scale bioreactors for this study were built in 2014 and run for the first

Table 3
N load reduction regression model environmental parameter estimates for independent factors of HRT, temperature, DO, and influent nitrate concentration for all 3 HRTs. Standard errors are in parentheses. Asterisk (*) indicates significance at $p < 0.05$, ** indicates significance at $p < 0.01$, *** indicates significance at $p < 0.001$, [a] indicates the factor was not significant and was dropped from the model.

	Model Intercept	HRT hours	Temperature °C	DO mg DO L^{-1}	Influent Nitrate Concentration mg NO_3^-N L^{-1}	R^2
Removal Efficiency	−8.68 (± 4.70)	3.10*** (± 0.09)	−0.51** (± 0.18)	[a]	1.98*** (± 0.21)	0.80
Mass Removal Rate	7.31*** (± 1.27)	−0.12*** (± 0.02)	−0.26*** (± 0.05)	−0.38*** (± 0.11)	0.66*** (± 0.06)	0.37

Table 4
Using the K-fold leave-one-out cross validation method, the multiple linear regression model was run multiple times, leaving one parameter out each time. This gave a ranking of which parameter contributed most to NO_3^-N removal efficiency in the bioreactors. The number in parenthesis after the Percent Contribution ranks the parameters from most contribution (1) to least contribution (4).

	HRT hours	Temperature °C	DO mg DO L^{-1}	Influent Nitrate Concentration mg NO_3^-N L^{-1}
Percent Contribution (%)	92.80 (*1*)	0.51 (*3*)	0.19 (*4*)	6.19 (*2*)
R^2	0.06	0.80	0.80	0.75

time for only a few weeks in 2015, which puts the 2017 testing season at the two to three year mark for operation time. Longer-term data in these systems will help elucidate the effects of temperature on NO_3^-N removal. A wider range of temperatures will also be necessary to perform a Q_{10} analysis.

In the pilot-scale woodchip bioreactors, DO concentrations were not significantly different between any of the HRTs at any point (Figure 3.5). The average starting DO concentration was 7.1 (± 1.48) mg L^{-1} and the DO level never fell below 0.5 mg DO L^{-1} from the inlet to the outlet for any duration of the study period. The majority of the DO was removed between the inlet and well A, which constitutes 24.5% of the total length of the bioreactor. Differences in DO from the inlet to the outlet were significant in all bioreactors ($p < 0.05$).

DO levels in a woodchip bioreactor can be an indicator of its denitrification efficiency. DO concentrations can inhibit denitrification rates at levels as low as 0.2 mg L^{-1} (Metcalf and Eddy, 2003). When DO is present, facultative aerobic microbes will utilize oxygen over NO_3^-N, making complete saturation of woodchip bioreactors necessary for complete denitrification to occur. Christianson et al. (2012b) reported that several field woodchip bioreactor sites were able to reduce DO concentrations to 0.5 mg DO L^{-1} within 25% of the length from the inlet. The pilot-scale bioreactors used in this study did not achieve similar DO concentration reductions. The NO_3^-N removal rates in this study could have been impacted by higher DO concentrations within the bioreactors, causing the microbial community to utilize DO instead of NO_3^-N over the entire length of the bioreactors (Christianson et al., 2011). Warneke et al. (2011b) studied lab-scale bioreactors and observed a DO reduction from 6 mg L^{-1} at the inlet to less than 2 mg L^{-1} at the outlet with an average NO_3^-N removal rate between 1.3 and 6.2 g N m^{-3} day^{-1}. They concluded that the substrate closest to the inlet serves to make conditions within the bioreactor anaerobic (Warneke et al., 2011b). In another study on a field-scale denitrification bed, Warneke et al. (2011a) observed removal rates of 7.6 g N m^{-3} day^{-1} with DO levels above 0.5 mg L^{-1}, which they concluded did not limit NO_3^-N removal.

3.6. Bypass flow

The pilot-scale bioreactors in this study did not have bypass flow, but as is typical under normal field conditions, excess flow would bypass the bioreactors and be discharged as untreated tile drainage (Christianson et al., 2012a). To account for this difference, the mass load removal rates were calculated by assuming each bioreactor received 45,675 L of tile drainage in a 24-h period, the maximum amount of flow that could be treated by the 2 h HRT. The average flow treatable by each HRT in a 24-h period was used, which is shown in the maximum flow treatable column in Table 5. Average influent NO_3^-N concentration from the study was used to calculate the amount of NO_3^-N by mass that needed to be treated in the 24-h period, which was 103.1 g m^{-3} day^{-1}. Average removal efficiency for each HRT was used to calculate how much NO_3^-N each HRT could remove of the total influent NO_3^-N mass load. Only the bioreactors set at 2 h HRT could treat 100% of the flow and removed a mass load of 9.3 g m^{-3} day^{-1} (Table 5). The 8 h HRT was able to treat 25.7% of the flow, removing 8.5 g m^{-3} day^{-1}. Finally, the 16 h HRT was able to treat 13.8% of the influent, removing 7.7 g m^{-3} day^{-1}. The mass load of NO_3^-N in the outflow for each treatment was 93.8 g m^{-3} day^{-1} (2 h), 94.6 g m^{-3} day^{-1} (8 h), and 95.5 g m^{-3} day^{-1} (16 h). With bypass flow, the 2 h HRT had a removal efficiency of 9.0%, the 8 h had 8.3%, and the 16 h had 7.4%.

In the field, HRT varies with season, precipitation, and management of flow within the bioreactors. Understanding the performance over the full range of expected HRTs under field conditions is therefore important. The 2 h HRT was able to remove the most NO_3^-N by mass when not accounting for bypass flow because it can treat the greatest volume of water in a 24-h period. Despite its low removal efficiency, it would still outperform the other two treatments in typical field conditions. The 8 h HRT had a removal efficiency of 32.1% and because of its lower retention time, it was able to treat almost twice the tile drainage as the 16 h HRT in a 24-h period (Table 5). Because a high removal efficiency is related to high HRT, bypass flow is increased, along with the mass of NO_3^-N that leaves untreated. Understanding the dynamics of bypass flow and HRT is critical to informing woodchip bioreactor design for use at the field-scale. There are also other important factors to consider beyond NO_3^-N removal rates. If removal rates were the only aspect to consider, a 2 h HRT would be the most ideal for field conditions without factoring in the difficulty in maintaining flow. Taking greenhouse gas (GHG) emissions into account, a companion study by Davis (2018) found that the 2 h HRT had the largest dissolved N_2O production (501.5 mg N_2O m^{-3} day^{-1}). On the other hand, the 16 h HRT had the highest CH_4 (1.69 g C m^{-3} day^{-1}) production with the 8 h HRT a close second (1.50 g C m^{-3} day^{-1}). When comparing the production of N_2O and CH_4 across all three HRTs, the 2 h HRT had the greatest ($p < 0.05$) global warming potential. When considering all factors, including GHG emissions, NO_3^-N MRR, NO_3^-N removal efficiency, and NO_3^-N removal rates with bypass flow, the optimal HRT for the woodchip bioreactors used in this study was 8 h.

4. Conclusions

This study was one of the first to collect samples at several points within bioreactors instead of only the inlet and outlet with controlled flows in a pilot scale setting. Substantial variability in NO_3^-N removal was observed under this increased spatial sampling, identifying the need for additional research from more bioreactors to better understand the drivers of the observed variability within bioreactors. Understanding the impact of HRT from a controlled pilot-scale system is crucial to optimizing woodchip bioreactor design under varied field conditions. Our results indicated that if MRR of NO_3^-N is the major design objective for woodchip bioreactors, a lower HRT could remove a large quantity of N and be relatively inefficient. When HRT was increased, the bioreactors removed more NO_3^-N by concentration and had higher efficiency, but the mass removal rate decreased. When considering the future of bioreactor data reporting, especially as it applies to policy planning, NO_3^-N mass removal rates will be more informational than NO_3^-N percent and concentration removal rates. For example, HRT restrictions could eliminate bioreactor installations on larger tile drains where a bioreactor may be inefficient but remove large quantities of N. NO_3^-N removal rates were not the only environmental impact that was taken into account in our study. Considering all of the factors discussed, the ideal HRT for the bioreactors used in this study was 8 h to achieve maximum NO_3^-N removal

Table 5
The bypass flow comparison for all treatments using the 2 h HRT as the base flow to be treated in a 24-h time period. Values were used from Table 1 to calculate NO_3^-N removal when bypass was accounted for.

HRT	Flow					Nitrate-N Removal				
	Flow to treat	Maximum flow treatable	Flow untreated	% flow untreated	% of flow treated	Amount of nitrate to be treated	Max. nitrate treatable	Nitrate removal efficiency	Final nitrate removal	Total nitrate untreated
Hours	L day^{-1}	L day^{-1}	L day^{-1}	%	%	g m^{-3} day^{-1}	g m^{-3} day^{-1}	%	g m^{-3} day^{-1}	g m^{-3} day^{-1}
2	45675	45675	0	0.0	100.0	103.1	103.1	9.0	9.3	93.8
8	45675	11746	33929	74.3	25.7	103.1	26.5	32.1	8.5	94.6
16	45675	6317	39359	86.2	13.8	103.1	14.3	53.8	7.7	95.5

while reducing the impact from GHG emissions (Davis, 2018).

$$MRR = \frac{V_t([NO_3^- - N]_{influent} - [NO_3^- - N]_{effluent})}{V_{WB} \times t} \quad (1)$$

$$Removal\ Efficiency = \left(\frac{V_t([NO_3^- - N]_{influent} - [NO_3^- - N]_{effluent})}{[NO_3^- - N]_{influent}}\right) * 100\% \quad (2)$$

$$Percent\ Contribution = \left(\frac{R\ reference\ value - R\ leave\ variable)}{R\ reference\ value}\right) * 100\% \quad (3)$$

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