Temperature and Substrate Controls Woodchip Bioreactor Performance in Reducing Tile Nitrate Loads in East-central Illinois

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Abstract

Tile drainage is the major source of nitrate in the upper Midwest, and end-of-tile removal techniques such as wood chip bioreactors have been installed that allow current farming practices to continue, with nitrate removed through denitrification. There have been few multi-year studies of bioreactors examining controls on nitrate removal rates. We evaluated the nitrate removal performance of two wood chip bioreactors during the first 3 years of operation and examined the major factors that regulated nitrate removal. Bioreactor 2 was subject to river flooding and performance was not assessed. Bioreactor 1 had average monthly nitrate removal rates of 23 to 44 g N m⁻³ d⁻¹ in year 1, which decreased to 1.2 to 11 g N m⁻³ d⁻¹ in years 2 and 3. The greater N removal rates in year 1 and early in year 2 was likely due to highly degradable C in the woodchips. Only late in year 2 and in year 3 was there a strong temperature response in the nitrate removal rate. Less than 1% of the nitrate removed was emitted as N₂O. Due to large tile inputs of nitrate (729 to 2127 kg N) at high concentrations (~30 mg nitrate-N L⁻¹) in years 2 and 3, overall removal efficiency was low (3 and 7% in years 2 and 3, respectively). Based on a process-based bioreactor performance model, bioreactor 1 would have needed to be 9 times as large as the current system to remove 50% of the nitrate load from this 20 ha field.
Introduction

Nitrate loss to streams and rivers from tile drained corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) fields in the upper Midwest of the United States is a well-established environmental issue (e.g., David et al., 2010). Many in-field and edge-of-field techniques have been proposed and evaluated for reducing these nitrate losses, including fertilizer management, cover crops, perennial crops, water table management, constructed wetlands, buffer strips, two-stage ditches, saturated buffers, and bioreactors (e.g., USEPA, 2008; Schipper et al., 2010b; Skaggs et al., 2012; Jaynes and Isenhart, 2014; David et al., 2015; Groh et al., 2015). Effectiveness of these practices has been found to be highly variable, both from location to location as well as temporally due to differing tile flow amounts and nitrate concentrations. Edge-of-field techniques have particular difficulty reducing nitrate loads during high flow periods, and this is when most transport occurs (Royer et al., 2006; Ikenberry et al., 2014).

Wood chip bioreactors have been installed at the end of tile lines from many fields in the Midwest. A wide range of nitrate removal effectiveness (up to 98% nitrate reductions) in both laboratory and field studies has been reported in the literature (Greenan et al., 2009; Chun et al., 2009, 2010; Moorman et al., 2010; Verma et al., 2010; Woli et al., 2010; Christianson et al., 2012b; Bell et al., 2015). Most have been designed as plastic lined trenches, filled with mixed species wood chips, that receive tile flow from fields ranging from 1 to 20 ha in size (e.g., Woli et al., 2010; Christianson et al., 2012b). At low flow most of the tile water passes through the bioreactor, but at high flows they are designed so that water will bypass the wood chip bed (Christianson et al., 2012a). The published literature on the effectiveness of this technique is limited and additional results are needed as bioreactors have been proposed in two state nutrient...

One concern with any engineered denitrification technique is that nitrous oxide (N\textsubscript{2}O) could be released (Groh et al., 2015), trading a water quality problem for a greenhouse gas problem given the global warming effect of each N\textsubscript{2}O molecule (310 times that of carbon dioxide). Previous studies on wood chip bioreactors have found that N\textsubscript{2}O emissions are small, but these reports are limited (Moorman et al., 2010; Woli et al., 2010; Warneke et al., 2011a; Christianson et al., 2013b).

Studies are needed to evaluate the effectiveness of wood chip bioreactors under a range of tile flows, nitrate concentrations and loads, tile water temperature, retention times, and bioreactor and wood chip age. Each of these factors can interact in complex ways that may affect the overall effectiveness of a bioreactor in removing nitrate. Few studies to date have examined bioreactor performance past the first year or two of operation, and given that they are proposed to function for as many as 10-20 years, long-term studies are critically needed. Our objective was to evaluate the nitrate removal performance of two wood chip bioreactors installed at the end of subsurface drain lines during the first 3 years of operation, examining the major factors that regulated nitrate removal. An additional objective was to determine how much N\textsubscript{2}O was emitted from the bioreactors as nitrate was denitrified.

**Materials and Methods**

**Study Site and Bioreactors**

We installed two bioreactors in the Embarras River watershed in east-central Illinois, the site of extensive previous work on tile drainage and nitrate loss (e.g., David et al., 1997, 2015; Gentry et al., 2014). The watershed has extensive poorly and very poorly drained Mollisols that
are typically tile drained, and would be an excellent candidate watershed for using wood chip bioreactors to reduce riverine nitrate loads that average 30 kg N ha$^{-1}$ y$^{-1}$ (Gentry et al., 2014).

Daily precipitation data were obtained from two nearby locations located to the SW (Tuscola) and the NE (Philo) of the bioreactor field and averaged (MRCC, 2015). We installed bioreactor 1 in March of 2012 on a pattern-drained 20 ha field with Drummer series soils (fine-silty, mixed, superactive, Mesic Endoaquolls) in a corn and soybean rotation; year 1 results were reported in David et al. (2015). Seed corn fertilized with 168 kg N ha$^{-1}$ as anhydrous ammonia at planting was grown during 2012 but due to the severe drought and poor crop yield, the seed corn was not harvested. Hybrid corn was grown in 2013 (spring fertilization of 200 kg N ha$^{-1}$ as anhydrous ammonia), followed by soybean in 2014. The bioreactor was installed near the end of a 30 cm tile main within a riparian buffer, and was 6 by 15 m by 1.3 m deep and lined with 6 mil plastic sheeting. Wood chips were initially from two sources, with the bottom layer of chips having more fine material than the top layer with the bioreactor filled to just above the soil surface. No soil was placed over the wood chips, as they were left exposed for easy application of additional woodchips. The fine material in the bottom layer had some mulch like appearance along with wood chips, but leaves, twigs, and other identifiable plant parts were not observed. The upper chips were larger sized, fresh wood chips. Each source of wood chips was from local arborists who were cutting a variety of trees in the area. The source of the wood chips with fine material had stockpiled the wood chips for some period of time before we obtained them. Additional wood chips were added after two years as extensive settling (about 30 cm) and degradation had occurred. We used a four-chamber Agri Drain water level control structure fitted with three flashboards and V-notch boards, pressure transducers, and dataloggers to allow measurement of water that went through the bioreactor as well as bypass flow (tile water that
flows over the middle V-notch boards and does not pass through the woodchips and does not get treated). We used Motorola pressure transducer sensors (model MPX5010DP) combined with custom designed and built dataloggers during 2012 and 2013 to record water levels at 30 min intervals. In spring 2013 we installed Solinst Levelogger Junior Edge model 3001 water level pressure transducers and dataloggers, and found that they compared well with our previous system during a several month period where both were in operation. During 2014, only the Solinst instruments were utilized. During 2013 and 2014, an Onset Hobo temperature sensor/dataloggers were inserted in the tile inlet and bioreactor outlet to measure water temperatures at 15 min intervals.

Bioreactor 2 was installed during December 2012, and had the same dimensions as bioreactor 1 and the same type of Agri Drain structure and monitoring. It also drained a field of 20 ha. The major difference was that bioreactor 2 was installed in the main channel floodplain of the Embarras River, where river water often backs up and compromises the bioreactor outlet flow measurement. During major flood events the bioreactor can be completely submerged.

**Bioreactor Sampling and Analyses**

Weekly grab samples from bioreactor 1 were supplemented with ISCO automatic water samplers during high flow events to determine water quality. Nitrate, chloride, and sulfate concentrations on filtered samples (0.45 µm pore size) in all tile inlet and outlet samples were measured by ion chromatography (Dionex DX-120). Ammonium and dissolved reactive P (DRP) were analyzed colorimetrically on filtered samples by flow injection analysis with a Lachat QuikChem 8000 using the automated sodium salicylate and ascorbic acid methods, respectively. Samples for total P were digested with sulfuric acid (11.2 N) and ammonium persulfate (0.4 g per 50 ml sample) and then analyzed for DRP as described previously. Dissolved organic carbon
(DOC) was determined using a Shimadzu total organic carbon analyzer. All methods followed APHA (2005) procedures. Linear interpolation was used to estimate daily and annual nutrient loads. Nitrous oxide fluxes were measured using static chambers following Woli et al. (2010) and Groh et al. (2015) based on the GRACEnet protocol (Blowes et al., 2003). See supplemental material for further details. We did not measure dissolved N\textsubscript{2}O in water leaving the bioreactor.

**Hydraulic Conductivity Analysis and Process Model Development**

We used flow and depth measurements from 2013 in bioreactor 1 to determine the in-situ hydraulic conductivity in bioreactor 1. Assuming that flow in a bioreactor is Darcian, then it is described by

\[
q = \frac{Q}{A} = K_{\text{eff}} \times \frac{(h_u - h_d)}{L} = K_{\text{eff}} \times i \tag{1}
\]

where \(q\) is the specific discharge or Darcian velocity (m s\(^{-1}\)), \(Q\) is the flow rate through the bioreactor (m\(^3\) s\(^{-1}\)), \(A\) is the area of the cross section through which flow takes place (m\(^2\)), \(K_{\text{eff}}\) is the in-situ or effective hydraulic conductivity, \(h_u\) and \(h_d\) are the upstream and downstream flow depths (m), respectively, \(L\) is the length of the bioreactor (m), and \(i\) is the hydraulic gradient, the ratio of the head loss through the bioreactor to the length over which the head loss occurred. A plot of \(q\) against \(i\) should yield a straight line with slope \(K_{\text{eff}}\).

We also developed a process-based model with a daily time step to characterize bioreactor 1 performance. The model assumes that the bioreactor is a completely stirred reactor with constant concentration throughout. The flow rate through the system each day is the lesser of tile flowrate from the field and the capacity of the bioreactor given by Equation 1, but with \(h_u\) and \(h_d\) being the height of the upstream and downstream stop logs, respectively. If the tile flow rate exceeds the bioreactor capacity, the excess tile flow bypasses the system. If the nitrate concentration in
the bioreactor exceeds the threshold value for a first order reaction, set at 1 mg N L\(^{-1}\), based on the work of Robertson (2010), the mass of nitrate is the function of the woodchip volume and temperature dependent removal rate. The removal rate function was based on the relationship between temperature and observed nitrate removal rates during 2014 in bioreactor 1. If the concentration is less than the threshold value then the amount of nitrate removed is a percentage of the amount of nitrate in the bioreactor, in accordance with first order kinetics. The concentration is updated each day and was given by

\[
C_d = \frac{V_p \times C_{d-1} + I \times C_I - M_d}{V_p + I}
\]

where \(C_d\) is the concentration at the end of the day, \(C_{d-1}\) is the concentration at the end of the previous day, \(C_I\) is the inflow concentration, \(V_p\) is the pore volume, \(I\) is the inflow volume, and \(M_d\) is the mass of nitrate removed. The model was applied to 30 years of DRAINMOD (Skaggs, 1980, 1982; Sanoja et al., 1990) simulated daily drain flow for the 20 ha field, using historical weather data and the dominant soil type in the field (Drummer series).

**Results**

**Flow and Nutrient Balances**

Tile flow varied greatly during the three year study period, from 8.1 cm in 2012 to 33.3 cm in 2013 (Table 1) in response to precipitation (Figure 1). January through June precipitation was 310, 641, and 507 mm for 2012, 2013, and 2014, respectively. There were many large precipitation and resulting flow events during 2013, with about half the flow bypassing the wood chip bed (Figure 1). During 2012 and 2014, most of the flow (85-89%) passed through the bioreactor. Tile nitrate concentrations increased from about 16 mg N L\(^{-1}\) in 2012 to >30 mg N L\(^{-1}\) in 2013, remaining high throughout 2014. The high nitrate concentrations were a result of the
drought of 2012, which led to a failed seed corn crop that was not harvested and likely large residual soil N pools that fall. This nitrate was then leached from the field during the following two years, with nitrate yields of 106 and 36.5 kg N ha\(^{-1}\) yr\(^{-1}\) in 2013 and 2014, respectively. Bioreactor 1 had an input of 2127 kg N as nitrate in 2013, a large load that was reduced by only 3% (72 kg N) (Table 1). This was much less than the 81% removal in 2012, where 208 kg N were removed. In 2014, the percentage removal was 7%, with 47 kg N removed. The chloride budget was nearly balanced, indicating little leakage of water in the bioreactor or around the Agri Drain structure. Sulfate-S was nearly balanced in 2013 and 2014, but the tile input was reduced by 25% in 2012 suggesting much greater reducing conditions in the first year of operation. Both DRP and total P had much larger outputs from the bioreactor than the tile input each year, as did DOC. The flow weighted outlet DOC concentration in 2012 was 18.8 mg C L\(^{-1}\), which decreased to 3.7 and 3.0 mg C L\(^{-1}\) in 2013 and 2014, respectively. The nitrate removal rate was highly correlated with the DOC concentration (r=0.62, n=102, p < 0.0001).

**Tile Temperature and Bioreactor Nitrate Removal Rates, Nitrous Oxide Emissions**

Tile water temperature into bioreactor 1 was between 6-8°C from January through April of 2013, and then slowly increased to a maximum of 17°C just as tile flow stopped for the year in early July (Figure S1). In 2014, tile water was only 3-4°C in February and March, and then increased to 17°C by early July. The 2014 water temperatures lagged 2013 by approximately 2 weeks. Nitrate removal rates for wood chip bioreactors are typically expressed on a g N m\(^{-3}\) d\(^{-1}\) basis. Note: wood chip volumes used for removal rates were based on average depth of water in the bioreactor, not total bioreactor volume. During 2012, daily nitrate removal rates in bioreactor 1 were quite high, and decreased with increasing retention times, suggesting nitrate limitation (Figure 2). During 2013 and 2014, there was no relationship of retention time with nitrate
removal rates. We plotted both daily and average monthly nitrate removal rates versus tile water
temperature for 2013 and 2014, and observed that beginning in March of 2013 through July of
2013, and through all of 2014, removal rates increased linearly with increasing water
temperatures (Figures 3 and 4). Bioreactor 1 had January and February 2013 removal rates that
were well above subsequent months, and 2012 average monthly removal rates were not plotted
as we did not measure water temperature that year, but rates were much larger than 2013-2014
(23 to 44 g N m$^{-3}$ d$^{-1}$). Bioreactor 2 does function, but monitoring was not possible most days due
to either flooding or more typically, the outlet backing up due to high river water. We have not
reported any effectiveness data for this bioreactor given the partial record available.

Nitrous oxide fluxes from the surfaces of bioreactors 1 and 2 in 2013 and 2014 were nearly
all <0.2 mg N$_2$O-N m$^{-2}$ h$^{-1}$ (Figure S2). Rates were greatest during warmer months when there
was tile flow. For bioreactor 1, 0.32 and 0.42 kg N as N$_2$O was emitted in 2013 and 2014,
respectively. This was 0.44 and 0.89% of the nitrate N removed by bioreactor 1 in 2013 and
2014.

**Flow Analysis**

For bioreactor 1 in 2013 the plot of specific discharge versus the hydraulic gradient had
clusters of points that defined linear features, but there was not a unique linear relationship
between hydraulic gradient and specific discharge, except at hydraulic gradients greater than
0.008 (Figure 5). At lower hydraulic gradients, specific discharges between 0.1 and 0.3 cm s$^{-1}$
appear to be independent of hydraulic gradient. Specific discharges ranging from near 0 up to 0.3
cm s$^{-1}$ occur at a hydraulic gradient of approximately 0.006. Such a result could be due to short
circuiting, with water flowing along the sides and bottom of the bioreactor. Almost every
instance where specific discharge exceeded 0.1 cm s$^{-1}$ was during periods of bypass flow, that is,
when the tile flow exceed the transport capacity of the bioreactor. It would be during such events that short circuiting is more likely to occur.

The near parallel linear features at hydraulic gradients less than 0.006 may be indicative that the porosity in the bioreactor is not constant, as is likely with bioclogging, a decrease in the porosity of a porous medium due to the growth of microbial biomass. The serial cross correglogram of $Q$ versus $A \times i$, the product of area and hydraulic gradient, has a peak correlation at a lag time of approximately 16 days (Figure S3). This correlation, and the nature of the hydraulic gradient plot may be indicative that biofilm growth peaks in 16 days, and is reduced during periods of high flow. The peak cross correlation value was 0.11, so this process does not explain much of the variation in specific discharge. The highest hydraulic gradient in the bioreactor during 2013 was approximately 0.026. Ghane et al. (2015) found this value to be the upper limit for Darcian flow in fresh woodchips.

An analysis of the relationship between specific discharge and hydraulic gradient for the linear features in the plot yields in-situ hydraulic conductivity of 6.7 and 3.1 cm s$^{-1}$, respectively, for hydraulic gradients less than and greater than 0.015. These values are not dissimilar to the range of 2.5 to 5.5 cm s$^{-1}$ that Chun et al. (2009, 2010) obtained from the analysis of breakthrough curves from laboratory-scale and field-scale bioreactors in Illinois, and to values of 8.4 and 2.4 cm s$^{-1}$, reported by Ghane et al. (2015) for fresh and old woodchips, respectively. The reduction of the slope at higher hydraulic gradients is consistent with the deviation from Darcian flow reported by Ghane et al. (2015).
Discussion

Bioreactor Performance and Controls on Nitrate Removal

We installed two similar bioreactors on 20 ha fields in the Embarras River watershed of east-central Illinois. Bioreactor 1 was located along a small tributary of the Embarras River and was rarely flooded; however, bioreactor 2 was located along the main channel of the Embarras River and was often flooded. Bioreactor 2 did function much of the time, but accurate monitoring that would allow for an input and output budget was not possible on most days due to the outlet backing up with river water. A wetland would have been more suitable at this location, however the landowner was not interested in removing land from row crop production and the NRCS only allows bioreactors to be installed in riparian buffers and not constructed wetlands.

Bioreactor 1 had excellent removal during the first year of operation in 2012, removing 81% of the tile nitrate however this was during a year with little tile flow (8.1 cm). The nitrate removal percentage was similar to many reports in the literature such as Christianson et al. (2012b) where they reported a range of 12 to 76% for four bioreactors in Iowa, and Verma et al. (2010) who reported a range of 42 to 98% for three bioreactors in central Illinois. The percentage effectiveness and mass of nitrate removed decreased greatly in year 2 (2013), with only 72 kg N removed from a large tile input of 2127 kg N. Both the mass and tile concentration of nitrate-N in 2013 was high due to leftover nitrate on the field following the drought and failed seed corn in 2012. However, half of the flow bypassed the bioreactor in 2013, and the capacity was not enough to remove much of the substantial nitrate-N load, the largest load of nitrate we are aware of entering a bioreactor from a tile drained field in the Midwest.

Our results demonstrate that performance and response to drivers such as wood chip quality, wood chip age, and temperature can vary greatly during the first three years of bioreactor
operation. In addition, nitrate removal rates may be overestimated when tile flow temporarily ceases during dry weather and samples are collected during the overturn and flushing of old water out of the wood chips. Care must be taken to avoid using samples that do not represent nitrate removal but are indicative of the flushing of stagnant water out of the bioreactor during extremely low flow or no flow periods.

In bioreactor 1, the bottom layer of woodchips contained fine organic materials, and when combined with low flows in 2012 this led to large N removal rates (daily average of 43 g N m\(^{-3}\) d\(^{-1}\), range of 0.7 to 116 g N m\(^{-3}\) d\(^{-1}\)), much beyond those reported in the literature (e.g., Schipper et al., 2010ab). Dissolved organic C concentrations were high in 2012, which decreased rapidly through 2013. At times during year 1 (2012) the bioreactor bed had removed all nitrate and sulfate reduction occurred. Therefore, removal rates of nitrate were high throughout 2012, and nitrate load was the controlling factor. The relationship between nitrate removal and retention time was opposite what has been found in previous studies, where removal increases with longer retention times (Christianson et al., 2012a). For bioreactor 1, it was likely that there was so much available C in the first year, that nitrate limitation at low flows (and longer retention times) reduced the N removal rate.

At the start of year 2 when flow began in January, removal rates remained high as there likely was easily degradable C following the drought conditions of the summer of 2012 with the long dry period and limited flows. By March daily and average monthly N removal rates were responding to temperature, and from that month onward through 2014 N removal rates were related to tile water temperature (Figure 4). Due to the high nitrate concentrations in the tile inlet water and little removal, no sulfate was reduced in 2013 and 2014. Retention time was again not related to N removal rates, as temperature clearly was the regulating factor. Warneke et al.
272 (2011a) reported a $Q_{10}$ (the factor of the reaction rate increase with every 10°C increase in
273 temperature) of 2.0 for temperatures between 17 and 24°C, and Warneke et al. (2011b) reported
274 a $Q_{10}$ of 1.2 for experimental temperatures of 16.8 and 27.1°C. Our calculated $Q_{10}$ was 3.8
275 between 6 and 16°C for 2014. This suggests a strong response to temperature and rapid increase
276 in the denitrification rate with increasing temperature, and that at low tile water temperatures in
277 January through March, denitrification rates will be slow.

278 Currently, wood chip bioreactors are an NRCS interim standard practice (605) for removing
279 nitrate from tile water. The NRCS has sized some bioreactors using a 20°C water temperature
280 that was published by Chun et al. (2010). However, this was a two-day, pulse addition of nitrate
281 experiment conducted at the end of June at a temperature of 20 to 21.7°C, not representative of
282 typical tile water temperatures in east-central Illinois. Tiles in east-central Illinois typically flow
283 from January through July most years, and water temperatures will be low during much of this
284 period (Figure 3), reaching 10°C in May, while likely never reaching 20°C most years.

285 Nitrate Removal Rates in Comparison to Published Values

286 Overall, bioreactor 1 did a poor job (on a percentage basis) of reducing nitrate-N from a tile
287 with unlimited nitrate due to low water temperatures in years 2 and 3. However, the nitrate
288 removal rate per m$^3$ of wood chips was in the range of those reported in the literature. Many
289 studies report typical values of 5 to 10 g N m$^{-3}$ d$^{-1}$ when nitrate is non-limiting (Schipper et al.,
290 2010a), similar to rates we measured during May and June of 2013 and 2014. However, our
291 average monthly rates were only 1-3 g N m$^{-3}$ d$^{-1}$ when water temperature was colder in February
292 and March. Christianson et al. (2013c) reported rates of 0.38 to 1.06 g N m$^{-3}$ d$^{-1}$ for a bioreactor
293 in Iowa, and determined that internal hydraulics limited the removal rates. They found that
294 temperature was not a good predictor of removal rates, with measurements from May through
August and water temperatures varying from 8.9 to 17.1°C (Christianson et al., 2013c). Our results and those of Christianson et al. (2013c) suggest that bioreactors may have to be much larger to effectively remove nitrate from a typical tile line as the retention time would have to be substantially longer. On the same farm as bioreactor 1 we monitored two constructed wetlands intercepting tile lines, and found they were much more effective at reducing nitrate-N loads during 2012 and 2013 (Groh et al., 2015).

**Nitrous Oxide Emissions**

As had been previously reported (Moorman et al., 2010; Woli et al., 2010; Warneke et al., 2011a; Christianson et al., 2013b), we found small N$_2$O emissions from either bioreactor 1 or 2 wood chip beds. However, our rates were generally greater than those reported by Christianson et al. (2013b) for bioreactors with soil covers, but similar to those without. They suggested that soil surfaces limited N$_2$O losses and could be useful for mitigating losses (Christianson et al., 2013b). Less than 1% of the removed nitrate in 2013 and 2014 was as N$_2$O (0.44 and 0.89% of nitrate removed in 2013 and 2014, respectively), somewhat larger than the 0.4% N$_2$O emissions of nitrate removed reported by Christianson et al. (2013b). Christianson et al. (2013b) found more N$_2$O loss dissolved in the water leaving their bioreactors than the amount emitted from the wood chip or soil surfaces. Warneke et al. (2011a) also found substantial loss of dissolved N$_2$O from a denitrification bed that increased the fraction of nitrate lost as N$_2$O from 1% (loss from bed surface) to 4.3% overall. We did not measure dissolved N$_2$O in water leaving the bioreactor, which likely led to an underestimation of overall N$_2$O losses.

When N$_2$O losses are placed in the context of the overall field-bioreactor system, the losses are likely small and not an important source of N$_2$O. Bioreactor 1 had N$_2$O losses of 0.32 and 0.41 kg N in 2013 and 2014, respectively. A fertilized corn and soybean field in the watershed
had N$_2$O losses of 2.2 to 7.7 kg N ha$^{-1}$ yr$^{-1}$ during a three year study (Smith et al., 2013), an average of 4.4 kg N ha$^{-1}$ yr$^{-1}$. If our 20 ha corn field had a similar N$_2$O emission rate, it would average 88 kg N yr$^{-1}$, and the average of 0.37 kg yr$^{-1}$ emitted from the bioreactor would be unimportant. Even adding substantial losses of dissolved N$_2$O in the water leaving the bioreactor would likely still be small in comparison.

The decomposing wood chips did release substantial amounts of DOC, and with that C was both reactive and organic P. This is a potential water quality problem from installation of bioreactors, as P is an important freshwater nutrient that leads to algal blooms.

Implications of Nitrate Removal and Flow Analysis Results on Bioreactor Size

Our results demonstrate the importance of longer term monitoring of newly installed bioreactors. For bioreactor 1, the wood chips had substantial available C in the first year that led to high rates of nitrate removal, beyond those reported in the literature. Robertson (2010) also reported that fresh wood chips had nitrate removal rates about double those in years 2 through 7. By the middle of the 2nd year, our N removal rates were closer to what has been typically reported in the literature, but demonstrated that the bioreactor was too small for the large N load. No other study has had such a large nitrate-N load into a bioreactor. If the load in 2013, as an example, had been 15 kg N ha$^{-1}$ (300 kg N), the removal efficiency would have been 24% given the 72 kg N removed. This is similar to many removal rates previously published. Therefore, removal rate based on percentage is relative and not as useful as calculating total mass removed when comparing across other studies.

The bioreactors were sized using protocols and a routine developed by Cooke and Bell (2014). In this routine, hydraulic conductivity was assumed to be 4.5 cm s$^{-1}$. The empirical cumulative distribution function for in-situ hydraulic conductivity, assuming Darcian flow, in
bioreactor 1 in 2013, and fitted Gaussian and log-Gaussian distribution functions are given in Figure S4. The design value has an almost 99% probability of exceedance, and is, therefore, not representative of the performance of the system. The log-Gaussian distribution provides a better fit, and thus the median conductivity (17.9 cm s\(^{-1}\)) is more representative than the mean conductivity (25.1 cm s\(^{-1}\)). This median conductivity was used in the process model described in the methods. Based on this process model, the resulting long-term annual load reduction with the current bioreactor configuration was 9%. In order to have a 50% load reduction, the bioreactor would have to be nearly 9 and 3.7 times as large, if the inflow nitrate concentration were 30 and 12 mg N L\(^{-1}\), respectively.

**Conclusions**

During three years of operation bioreactor 1 demonstrated variable nitrate removal from the tile system, driven by age of wood chips, retention time, and water temperature. Year 1 and early year 2 results (January to February) had high nitrate removal rates, beyond those reported in the literature, likely due to a large amount of soluble C that was easily degraded. From March on in year 2, and in year 3, water temperature appeared to be the major factor determining nitrate removal rates and removal rates per m\(^3\) of wood chip were consistent with those in the published literature. Due to the large input of nitrate in year 2 (2127 kg N) and the amount of bypass flow, the removal rate was low (72 kg N, 3% of the tile nitrate load) following excellent removal in year 1 (208 kg N, 81% of the tile nitrate load). Although bypass flow was reduced in year 3, low water temperature with older wood chips led to only 47 kg of nitrate-N removed, 7% of the tile load. For this tile system where water temperatures average ~9°C for the year, the wood chip bed would need to be 9 times the current size to provide 50% removal of the measured tile nitrate loads. Little N\(_2\)O was emitted from bioreactor 1, equivalent to <1% of the nitrate removed.
Bioreactor 2 had no useable data to assess removal efficiencies due to its location in the floodplain of the Embarras River, where high water levels led to flooding and backup of the tile system.

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Table 1. Annual tile and bypass flow through bioreactor 1 during the 2012-2014 water years, along with input and output nitrate-N, Cl, sulfate-S, dissolved reactive P (DRP), total P, and dissolved organic carbon (DOC) loads.

<table>
<thead>
<tr>
<th>Tile flow (bypass) cm</th>
<th>Nitrate-N (% removal)</th>
<th>Cl In</th>
<th>Cl Out</th>
<th>Sulfate-S In</th>
<th>Sulfate-S Out</th>
<th>DRP In</th>
<th>DRP Out</th>
<th>Total P In</th>
<th>Total P Out</th>
<th>DOC In</th>
<th>DOC Out</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>8.1 (1.2)</td>
<td>257</td>
<td>49</td>
<td>627</td>
<td>603</td>
<td>0.1</td>
<td>1.3</td>
<td>15</td>
<td>305</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>33.3 (15.6)</td>
<td>2127</td>
<td>2055</td>
<td>1996</td>
<td>1975</td>
<td>1.0</td>
<td>4.7</td>
<td>1.9</td>
<td>6.4</td>
<td>83</td>
<td>247</td>
</tr>
<tr>
<td>2014</td>
<td>13.0 (1.5)</td>
<td>729</td>
<td>682</td>
<td>777</td>
<td>771</td>
<td>0.3</td>
<td>1.0</td>
<td>0.5</td>
<td>1.4</td>
<td>28</td>
<td>79</td>
</tr>
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</table>
Figure 1. Daily precipitation (a), tile flow intro bioreactor 1 along with daily bypass flow (b), and nitrate concentrations in the tile inlet and bioreactor 1 outlet (c) from 2012 through 2014.
Figure 2. Nitrate removal rates for bioreactor 1 plotted against retention time during 2012 through 2014.
Figure 3. Daily nitrate removal rates and tile water temperatures for bioreactor 1 in 2013 and 2014.
Figure 4. Average monthly nitrate removal rates and tile water temperatures for bioreactor 1 in 2013 and 2014.
Figure 5. Specific discharge versus hydraulic gradient for bioreactor 1 during 2013.
Supplemental Material

Temperature and Substrate Controls Woodchip Bioreactor Performance in Reducing Tile Nitrate Loads in East-central Illinois

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Nitrous Oxides fluxes

Three PVC rings (20 cm diameter, 10 cm height inserted ~ 5 cm into the wood chips) were installed along the flow path of bioreactors 1 and 2. End caps of PVC with weather stripping, septa, and ventilation tubes were made to fit tightly on the PVC rings to complete the enclosed chamber, which had overall had space volumes of 5 to 6 L (actual headspace was determined for each measurement on each ring). Fifteen mL samples were collected at 0, 10, 20, and 30 min with a syringe. The samples were placed into evacuated 10 mL glass vials with gray butyl septa. Gas samples were analyzed using a Shimadzu GC-2014 gas chromatograph with autosampler to determine N₂O concentrations. Linear regression was used to determine the rate of N₂O emissions during the 30-min incubation period. Linear interpolation was used to determine daily flux values between field measurements.
Figure S1. Daily temperature of tile water entering bioreactor 1 in 2013 and 2014.
Figure S2. Nitrous oxide fluxes of bioreactors 1 and 2 during 2013 and 2014.
Figure S3. Serial cross correlogram for flowrate versus the product of flow cross sectional area and hydraulic gradient for bioreactor 1 during 2013. The dashed lines are the upper and lower 95% confidence limits.
Figure S4. Cumulative distribution functions for in-situ hydraulic conductivity, based on the assumption of Darcian flow, in bioreactor 1 during 2013.