



## Research article

## Seasonal performance of denitrifying bioreactors in the Northeastern United States: Field trials



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## ABSTRACT

Denitrifying bioreactors are increasingly being used for nitrate removal from agricultural drainage water. Filled with carbon substrates, often woodchips, denitrifying bioreactors provide a favorable anaerobic environment for denitrification. Despite performing well in loess soils in the Midwestern United States, field bioreactors have not yet been evaluated in shallow soils over glacial till that are characteristic for the Northeastern United States. This study, therefore, investigates the performance of bioreactors and provides design criteria for shallow soil with flashy discharges.

Paired bioreactors, one filled with woodchips and one with a mixture of woodchip and biochar, were installed in tile drained fields in three landscapes in New York State. The bioreactors were monitored for a three-year period during which, the flow rate, temperature, nitrate ( $\text{NO}_3^- - \text{N}$ ), sulfate ( $\text{SO}_4^{2-} - \text{S}$ ) and dissolved organic carbon (DOC) were measured. Results showed that the average  $\text{NO}_3^- - \text{N}$  removal efficiency during the three years of observations was about 50%. The  $\text{NO}_3^- - \text{N}$  removal rate ranged from 0 in winter to  $72 \text{ g d}^{-1} \text{ m}^{-3}$  in summer. We found that biochar was only effective during the first year in enhancing denitrification, due to the ageing. An index for carbon availability related to  $\text{NO}_3^- - \text{N}$  removal was developed. During winter, availability of the DOC was a limiting factor in bioreactor performance. Finally, to aid in the design of bioreactors, we developed generalizable relationships between the removal efficiency and hydraulic retention time and temperature.

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## 1. Introduction

Anthropogenic nitrogen loading, especially from use of excess fertilizers in agriculture, contributes to coastal hypoxia such as the dead zone in the Gulf of Mexico (Burkart and James, 1999). Some nitrogen is subject to denitrification or can accumulate as organic nitrogen in the root zone (Van Meter et al., 2016), however, it leaches to the streams with groundwater fluctuations (van Verseveld et al., 2009). To decrease the nitrate load from agricultural fields, denitrifying bioreactors are an economic, practical, and ecologically-friendly remediation approach to remove nitrate from agricultural drains by providing an anaerobic environment and organic carbon for the denitrification process (Christianson et al., 2013a; Elgood et al., 2010; Schipper et al., 2010). Original studies

of this concept in Ontario, Canada (Blowes et al., 1994) and North Island, New Zealand (Schipper and Vojvodić-Vuković, 1998) formed the foundation for the use of denitrifying bioreactors, and the work in the United States during the last ten years has accelerated its implementation with a nearly exponential growth in published research (Addy et al., 2016; Christianson and Schipper, 2016).

Denitrifying woodchip bioreactors are made by routing drainage water through a buried trench filled with woodchips as a carbon source (Blowes et al., 1994; Bock et al., 2016). Efficiency of bioreactors on nitrate removal depends on carbon availability, temperature, hydraulic retention time (HRT), and  $\text{NO}_3^- - \text{N}$  availability (Warneke et al., 2011b). Increasing temperature accelerates the denitrifying activity (Elgood et al., 2010; Robertson et al., 2008; Warneke et al., 2011a). In addition, dry periods in bioreactors have been reported to make more carbon available (Woli et al., 2010). In some cases, biochar has been added to bioreactors to enhance denitrification, although Christianson et al. (2011a,b,c,d) found no significant difference in removal by adding biochar to woodchips. Biochar, a product of thermal decomposition of biomass (Lehmann et al., 2011; Singh et al., 2009), alters the

Abbreviations: HRT, hydraulic retention time;  $\text{NO}_3^- - \text{N}$ , nitrate as nitrogen; W, woodchips bioreactor; WB, Woodchips amended with biochar; DOC, dissolved organic carbon; DO, dissolved oxygen.

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nitrogen cycle by increasing the microbial population (Anderson et al., 2014a; Xu et al., 2014) through providing a “co-location” for carbon, microorganisms, and nutrients which leads to increased carbon use efficiency and microorganism activity (Lehmann et al., 2011). Hence, additional research is warranted on the effect of biochar on removal of nitrate in drainage water, especially under actual field conditions.

Although denitrifying bioreactors have been recommended for reducing nitrate losses in the Midwest (Christianson and Schipper, 2016), their efficiency is still being investigated in other regions with different soils and climatic conditions (Christianson et al., 2013b, 2012, 2011b; Chun et al., 2009; Schipper et al., 2010; Warneke et al., 2011a). Such as those of the northeastern United States, where the presence of a hardpan at shallow depth in the glacial till limits the amount of water that the soil can hold (French et al., 1978; Neeley et al., 1965; Pearson et al., 1973). Due to limited water holding capacity, drainage discharge from these soils increases rapidly during times of large precipitation events compared with the Midwest (Dahlke et al., 2012; Lesaffre and Zimmer, 1989), affecting hydraulic retention time (HRT) and nitrate inputs. In addition, the long winters limits the performance of the denitrifying bioreactors, but to what degree has not been studied well under field conditions in the Northeast US.

The design of denitrifying bioreactors should be fine-tuned to take the specific soil and climate factors into consideration. This study, therefore, field tests bioreactor performance for nitrate removal and discusses how the design should be tailored to soil and climatic conditions in the Northeast.

## 2. Materials and methods

Six (in pairs) denitrifying bioreactors were constructed on farms in three counties, Tompkins, Chemung and Steuben in upstate New York (Fig. A.1) to test their effectiveness in removing nitrate from agriculture tile lines.

### 2.1. Denitrifying bioreactor design

Bioreactors were excavated with the appropriate length, width, and depth dimensions to allow a retention time of 6 h for the estimated flow rate that would not be exceeded 80% of the time. This was followed by placing a plastic or geotextile liner around the bioreactors. Next, an AgriDrain<sup>®</sup> inline water level control structure with a weir were put in the inflow of the bioreactor to divert the flow to the bioreactor. In addition, the weir allowed the water to bypass the bioreactor (Fig. A.2). Thus, by measuring the water height above the gate, the bypass flow was calculated. The outflow structure was also used to maintain the water level in the bioreactor by setting the weir level. Similar to the inflow structure, a weir allowed measurement of the flow rate through the bioreactor. Solid plastic pipes connected the control boxes to the bioreactors where they transitioned to perforated pipes within the bioreactor (Fig. A.2). The bioreactors were then filled with wood chips or woodchips amended with biochar (Table 1) and covered with the liner, and then leveled with the surrounding area by soil.

The woodchips used for all sites were obtained locally from a lumber mill and were primarily from ash (*Fraxinus ornus* sp.) trees. The average woodchip length was about 3 cm. The biochar was obtained from Biochar Now<sup>®</sup> and was a woody feedstock blended chip mostly of pine (*Pinus* sp.) origin with a particle length typically around 1–2 cm. The biochar was produced by slow pyrolysis at temperatures of between 550 °C and 600 °C. To inoculate the media with denitrifying bacteria, the lower portion of the bioreactors was amended with 0.5 m<sup>3</sup> of soil taken from the hyporheic zones from the adjacent streambed. These zones are known to be enriched in

denitrifying bacteria (Anderson et al., 2014b).

### 2.2. Site description

#### 2.2.1. Tompkins County site

The pair of bioreactors were installed in October 2012 at the Homer C. Thompson Vegetable Research Farm, Tompkins County, New York. Soils in the drainage area were mostly gravelly loam which received only inorganic fertilizer during the period of investigation. The bioreactors were 19 m<sup>2</sup> and filled with 1 m depth of wood chips, and were enclosed with a polyethylene impermeable liner from North Plastics<sup>®</sup> (Table 1). In one of the bioreactors the woodchips were amended with 10% biochar (WB) (Table 1).

A 250 m long interceptor drain at the base of a sloping area, drained groundwater into the two denitrifying bioreactors, although it may have drained from the root zone when it was saturated. Fig. A.3 shows the schematic of the field and the drain. The inlet weir was set at a high elevation, therefore, high flows went to the bioreactor. Sampling began in March 2013 soon after the water level control gates and V-notch weirs were installed to divert tile drain flow into the bioreactors. The outlet weir depth was set to maintain a minimum water depth of 0.5 m within the bioreactor.

Bi-weekly sampling and manual readings of water levels of the inlet and outlet started on April, 2013. In 2014 (April to November), water temperatures at the inlet of the bioreactors were recorded every 5 min using a Watchdog<sup>™</sup> temperature logger fabricated by Spectrum Technologies, Inc. and water head was measured using a water level logger, Telog PR-31 from Telog Instruments Inc. Starting in April 2015 both temperature and water levels were recorded in the inlet and outlet structure with three HOBO<sup>®</sup> U20 water level loggers from Onset Computer Corporation. Water levels were used to calculate the flow rate with the standard weir equations (Table 1). Weather data was provided by a Cornell weather station on site.

#### 2.2.2. Chemung County site

Constructed in June 2013, the two bioreactors at the Chemung site received tile flow water from a corn field on a dairy farm (Fig. A.4). Manure was applied in spring and late fall on the silty loam soils of the drainage area. The bioreactors were identical in construction to those at the Tompkins site, using the same source of woodchips, biochar, and impermeable liner (Table 1). However, the WB surface area was 1.8 times greater than the W bioreactor. In addition, flow could bypass the bioreactor when the water level in the inlet control box exceeded the weir setting at 1 m above the bottom of the bioreactor. The weir in the outlet was set to maintain around 0.5 m flow depth within the bioreactor. Three HOBO<sup>®</sup> U20 water level loggers from Onset Computer Corporation were placed at the inlets and outlets of the bioreactors to measure temperature and water level in different periods (July to November 2014 and April to August 2015). At other times, the water head was measured during each bi-weekly sampling event. The discharge for the inflow and outflow was calculated using rating curves (Table 1). The water level at the inlet weir was used to calculate the bypass flow and the water level at the outlet weir was used for the discharge through the bioreactors. Precipitation was obtained from a station 16 km away.

#### 2.2.3. Steuben County site

The third set of two bioreactors was located at the outlets of two different drainage systems on the edge of a corn and forage field that received manure several times during the growing season. These two drainage systems collected water from a silt loam soil overlying shallow bedrock (Fig. A.5). The WB bioreactor at this site

**Table 1**  
Characteristics of the bioreactors.

Site	Installation date	Media+%biochar by volume <sup>a</sup>	Dimension L × U × D <sup>b</sup> (m <sup>3</sup> )	Liner specification	Drainage area (ha)	Number of Sampling events	Weir type and equation H (cm) Q (L s <sup>-1</sup> ) <sup>c</sup>
Tompkins	Oct 2012	W	6.1 × 3.1 × 0.5	North Plastics® 5 mil polyethylene	4	62	V notch weir
		W+10%B	6.1 × 3.1 × 0.5	polyethylene			Q = 0.0108H <sup>2.3151</sup>
Chemung	June 2013	W	6.1 × 3.1 × 0.5	North Plastics® 5 mil polyethylene	5	46	Rectangle weir
		W+10%B	7.6 × 4.5 × 0.5	polyethylene			Q = 0.3255H <sup>1.4856</sup>
Steuben	July 2013	W	6.7 × 3.6 × 0.5	Woven Geotextile Fabric 200 liners	6	45	Rectangle weir
		W+2%B	7.6 × 4.5 × 0.5	liners			Q = 0.3255H <sup>1.4856</sup>

<sup>a</sup> The W is the woodchips only bioreactors and WB shows the biochar amended members.

<sup>b</sup> Length (L), Width (U) and Depth (D) of the bioreactors. Depth of the trench for all bioreactors is 1.5 m and the average drain depth in the field is 1 m.

<sup>c</sup> H is the water head above the weir and Q is the discharge.

contained 2% biochar (Table 1). These bioreactors were lined with permeable W200 Geotextile from Granite Environmental® (Table 1). Therefore, surface water and shallow groundwater infiltrated into these bioreactors. High tile discharges bypassed the bioreactor when the water level was in excess of inlet weir height set at 1 m above the bottom of the bioreactor. Water level was recorded using Telog PR-31 from Telog Instrument Inc. at two different periods (June to November 2014 and April to November 2015). The weather data was obtained from a station 12 km away from the site. Manual water level recordings were taken during the biweekly sampling (Table 1).

### 2.3. Sampling and analysis

Bi-weekly duplicate samples were collected in 125 ml low-density polyethylene bottles from both the inlet and the outlet of the bioreactors except during the coldest winter periods when the bioreactors were frozen. Table 1 shows the number of sampling events. The samples were transported to the laboratory in an iced cooler and immediately filtered. The filtered samples were stored at 4 °C and analyzed for NO<sub>3</sub><sup>-</sup> - N, sulfate (SO<sub>4</sub><sup>2-</sup> - S) and DOC concentrations. The NO<sub>3</sub><sup>-</sup> - N and SO<sub>4</sub><sup>2-</sup> - S content was measured using a Dionex ICS-2000 Ion Chromatograph (Pfaff, 1993). The DOC was analyzed using an O.I. analytical® TOC Model 1010 analyzer (Potter and Wimsatt, 2009).

For most of the sampling period, probes were used to report the water temperature flowing to the bioreactors, as stated in the previous sections. However, since these probes were not present throughout the entirety of the sampling period or at all sites, a temperature model by McCann et al. (1991) as adapted by Brisson et al. (1998) was used to estimate missing data. Additional description is reported elsewhere (Hassanpour et al., 2016). The snowmelt was estimated using “EcoHydrology package” in R programming language (Fuka et al., 2015) and was added to the rainfall values obtained from the weather station data.

### 2.4. Data analysis

The NO<sub>3</sub><sup>-</sup> - N concentrations and the flow rate were used to calculate NO<sub>3</sub><sup>-</sup> - N removal efficiency, HRT, and NO<sub>3</sub><sup>-</sup> - N removal rate. The NO<sub>3</sub><sup>-</sup> - N removal efficiency, ε<sub>NO<sub>3</sub></sub>, was calculated as

$$\varepsilon_{NO_3-N} = \frac{C_{NO_3,in} - C_{NO_3,out}}{C_{NO_3,in}} \quad (1)$$

where C<sub>NO<sub>3</sub>,out</sub> is the NO<sub>3</sub><sup>-</sup> - N concentration in the effluent and C<sub>NO<sub>3</sub>,in</sub> is the NO<sub>3</sub><sup>-</sup> - N concentration in the influent.

Hydraulic retention time (HRT) was estimated as

$$HRT = \frac{A\rho d}{Q} \quad (2)$$

where A is the surface area of the bioreactor (m<sup>2</sup>), d is the active height of water in the bioreactor (m), Q is the discharge (m<sup>3</sup> h<sup>-1</sup>), and ρ is the effective porosity of the media (Christianson et al., 2011b). Effective porosity of woodchips is 0.6 (Robertson, 2010). This media porosity was verified using subsamples of the woodchips. Occasionally at the Tompkins and Steuben sites, the flow ceased during summer months. For those specific events, when no flow was observed, the number of days since the last day it rained was considered as the HRT. Such consideration was not pertinent for the Chemung site, since at this site, continuous flow was observed.

The removal rate of NO<sub>3</sub><sup>-</sup> - N, r<sub>NO<sub>3</sub></sub>, was calculated as

$$r_{NO_3-N} = \frac{C_{NO_3,in} - C_{NO_3,out}}{HRT} \quad (3)$$

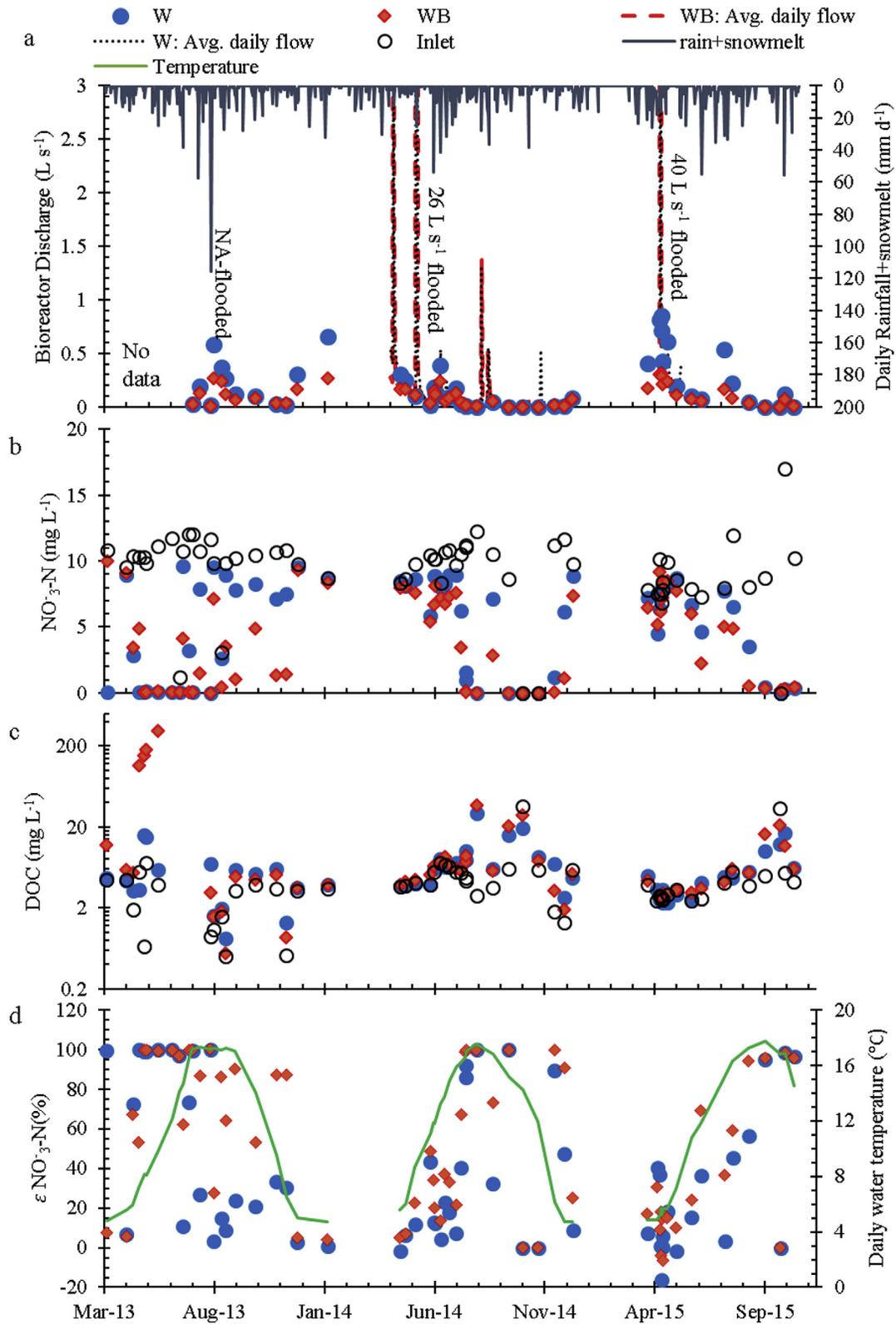
Student's t-tests were used to test for significant differences between NO<sub>3</sub><sup>-</sup> - N concentrations in the inlet and outlet of the bioreactors and between W and WB bioreactors. Confidence levels were set at 95%.

Water temperatures were calculated for the whole period of investigation for the Steuben site since temperature probes were not installed at this site. At the Chemung and Tompkins site, temperature probes were in place for two time frames during the last two years of sampling. For the rest of the period, the soil temperature model described by McCann et al. (1991) as adapted by Brisson et al. (1998) was used. The details were reported elsewhere (Hassanpour et al., 2016).

## 3. Results

### 3.1. Rainfall and discharge

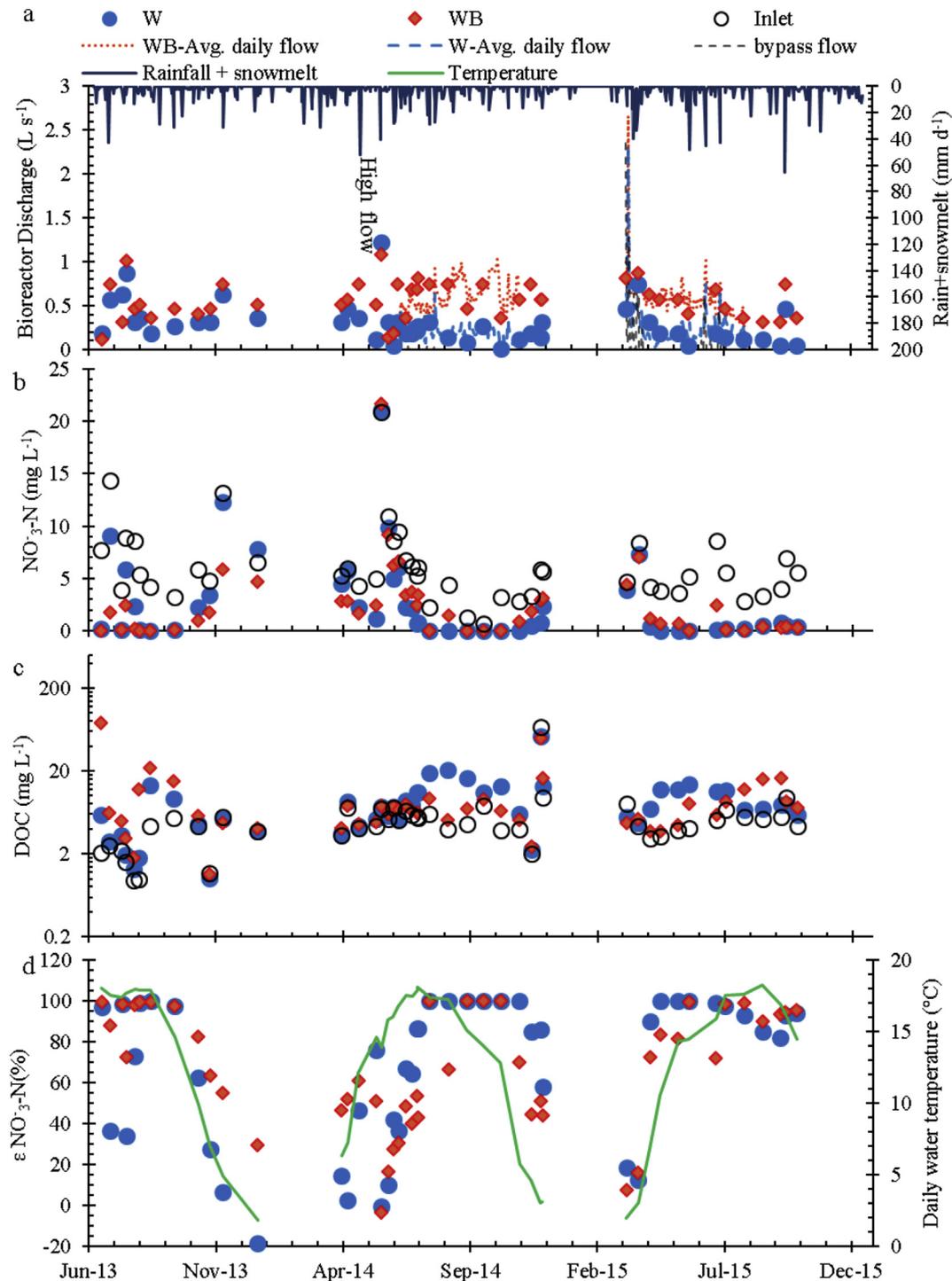
The records of three years of combined precipitation and snowmelt (Fig. 1), and discrete and available continuous discharge at the Tompkins site bioreactors are shown in Fig. 1a. The annual precipitation at the Tompkins site over the study period (2013–2015) varied between 858 and 990 mm y<sup>-1</sup> with 2013 being the wettest, especially during the summer. The annual rainfall in 2015 was 47 mm less than the 100-year annual precipitation average. At the Tompkins site, the tile inflow (Fig. 1a) was high during the spring and ceased in summer. Since the bypass weir in the inlet structure was set at a high elevation and the bioreactors were in a flood prone area next to the stream, this caused the bioreactors to be submerged on occasionally (referred to as “flooded” on Fig. 1a) every year despite being a wet or dry year.



**Fig. 1.** a) Discharge from the woodchips (W) and woodchips amended with biochar (WB) bioreactors and combined daily rainfall and snowmelt (blue hanging bars) at the Tompkins site. Both discrete sampling (symbols) and continuous observation (lines) are shown; b) NO<sub>3</sub><sup>-</sup>-N concentrations in the influent (open black symbols) and effluent (closed blue dots and red diamonds) of the bioreactors; c) DOC concentrations in the influent (open black symbols) and effluent (closed blue dots and red diamonds) of the bioreactors; d) temperature (green line) and NO<sub>3</sub><sup>-</sup>-N removal efficiency (ε<sub>NO<sub>3</sub><sup>-</sup>-N</sub>) of the bioreactors (closed blue dots and red diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The annual amount of rainfall at in the Chemung site ranged from 888 to 911 mm  $y^{-1}$  throughout the period of investigation (2013–2015), with 2014 being the wettest year. At this site the tile drained the regional groundwater and interflow from adjacent hillslopes resulting in a relatively continuous flow year around, which increased somewhat during spring (Fig. 2a). During spring,

some of the increased flow bypassed through the inlet thereby maintaining a more constant flow in the bioreactor. Thus, the amount of flow diverted into the bioreactors remained relatively uniform, typically less than 1 L  $s^{-1}$  (Fig. 2a). Although, in 2014, the wettest year, a flood event was observed. The flow through the larger bioreactor, WB (Woodchips amended with biochar), was

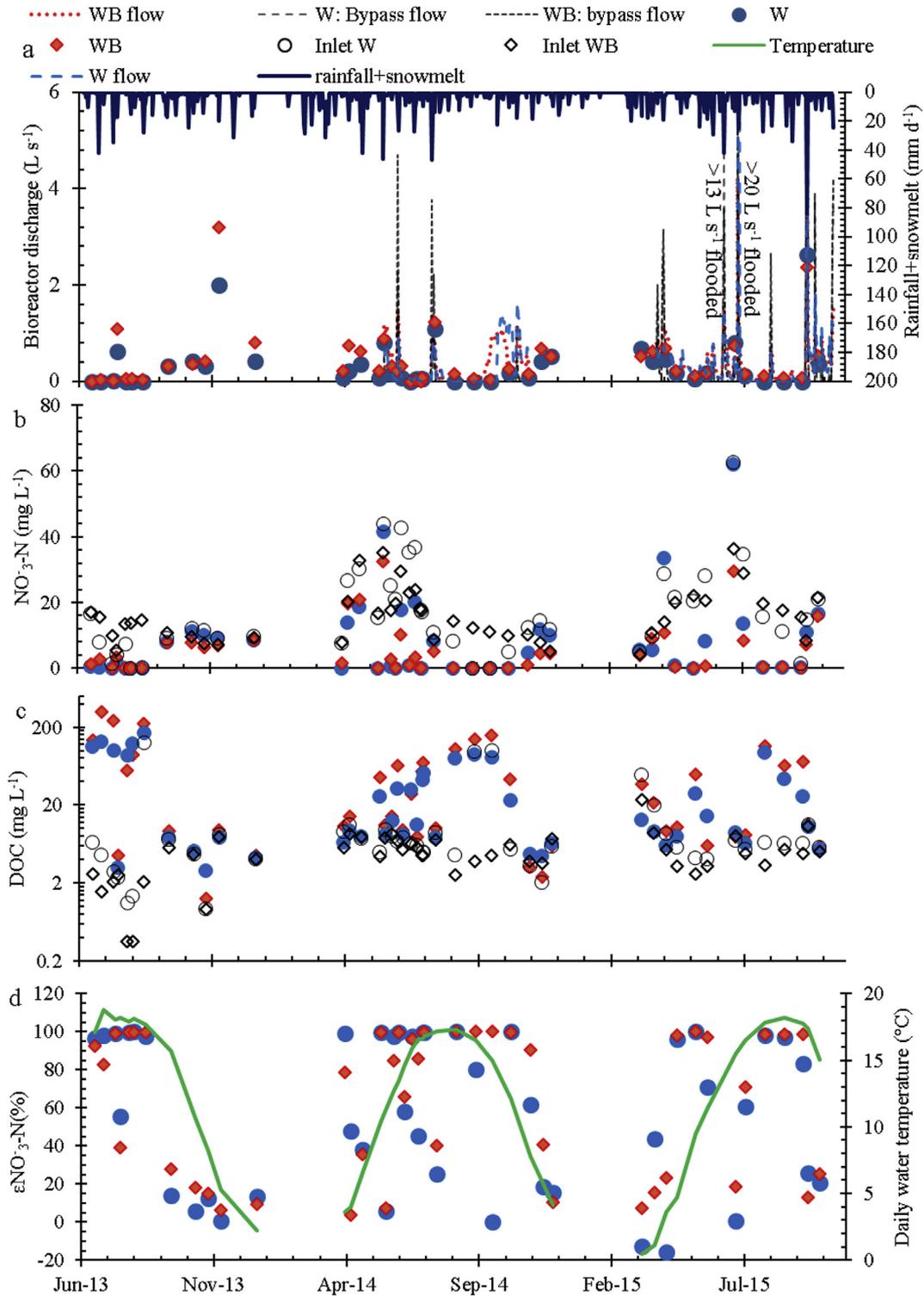


**Fig. 2.** a) Discharge from the woodchips (W) and woodchips amended with biochar (WB) bioreactors and combined daily rainfall and snowmelt (blue hanging bars) at the Chemung site. Both discrete sampling (symbols) and continuous observation (lines) are shown; b)  $NO_3^- - N$  concentrations in the influent (open black symbols) and effluent (closed blue dots and red diamonds) of the bioreactors; c) DOC concentrations in the influent (open black symbols) and effluent (closed blue dots and red diamonds) of the bioreactors; d) temperature (green line) and  $NO_3^- - N$  removal efficiency ( $\epsilon_{NO_3^- - N}$ ) of the bioreactors (closed blue dots and red diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

greater than that of the W (woodchips only) bioreactor but the average retention times for the WB and W bioreactors were 0.3 and 0.5 d, respectively.

At the Steuben site, the year with the least amount of rainfall

(933 mm y<sup>-1</sup>) was 2014 (Fig. 3a) and the wettest year was 2015, with 986 mm y<sup>-1</sup>. The inflow to the bioreactors was highly variable and bypass flow occurred more frequently than at the other two sites, bypassing 35% and 37% of the drain flow (Table 2 and Fig. 3a).



**Fig. 3.** a) Discharge from the woodchips (W) and woodchips amended with biochar (WB) bioreactors and combined daily rainfall and snowmelt (blue hanging bars) at the Steuben site. Both discrete sampling (symbols) and continuous observation (lines) are shown; b) NO<sub>3</sub><sup>-</sup>-N concentrations in the influent (black open symbols) and effluent (closed symbols) of the bioreactors; c) DOC concentrations in the influents (open symbols) and effluent (closed symbols) of the bioreactors; d) temperature (green line) and NO<sub>3</sub><sup>-</sup>-N removal efficiency (εNO<sub>3</sub><sup>-</sup>-N) of the bioreactors (closed symbols). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**  
Average discharge, bypass, average hydraulic retention time ( $\overline{HRT}$ ), saturated volume ( $\overline{SV}$ ),  $\text{NO}_3^- - \text{N}$  concentration and removal efficiency ( $\epsilon_{\text{NO}_3^- - \text{N}}$ ),  $\text{NO}_3^- - \text{N}$  removal rate ( $r_{\text{NO}_3^- - \text{N}}$ ), dissolved organic carbon ( $\text{DOC}$ ) and standard error (SE) at the inlets and outlets of the bioreactors.

Site and Bioreactor	Discharge <sup>a</sup> L s <sup>-1</sup> ± SE	Bypass <sup>b</sup> (%)	$\overline{HRT}$ <sup>c</sup> d	$\overline{SV}$ m <sup>3</sup>	$\text{NO}_3^- - \text{N}$ Concentration mg L <sup>-1</sup>			$r_{\text{NO}_3^- - \text{N}}$ <sup>d</sup> gNm <sup>-3</sup> d <sup>-1</sup>	$\text{DOC}$ mgL <sup>-1</sup>		
					In ± SE	Out ± SE	$\epsilon_{\text{NO}_3^- - \text{N}}$		In	Out	
Tompkins	W	0.2 ± 0.04	0	2.2	10.2	9.3 ± 0.4a <sup>e</sup>	5.1 ± 0.5b	42	3.8	4.9 ± 0.8a	20 ± 7b
	WB	0.1 ± 0.01		2.1	10.1		4 ± 0.4c	55	4.7		6.4 ± 0.7b
Chemung	W	0.29 ± 0.03	4	0.5	9.6	6.2 ± 0.5a	2.7 ± 0.6 b	68	13.5	6.05 ± 1.4a	8.8 ± 1.1b
	WB	0.56 ± 0.03		0.3	17.6		2.6 ± 0.6b	66	15.1		9.6 ± 1.8b
Steuben	W	0.33 ± 0.08	35	2.8	12.2	18.4 ± 2.7a	9.9 ± 2.7b	58	4.7	13.7 ± 3.9a	50.3 ± 10b
	WB	0.44 ± 0.09	37	2.3	17.4	16.6 ± 1.4a	6.4 ± 1.6b	62	6.7	5.3 ± 0.5a	34.1 ± 6b

<sup>a</sup> Values are from the separate sampling and do not include the flood events.

<sup>b</sup> From the continuous measurements.

<sup>c</sup> Average of the individual events.

<sup>d</sup> Removal rate values do not include flood and  $\text{NO}_3^- - \text{N}$  limited events (effluent  $\text{NO}_3^- - \text{N} < 0.5 \text{ mg L}^{-1}$ ).

<sup>e</sup> Different letters in each row indicate statistically distinct groups (Paired *t*-test at  $p < 0.05$ ).

The highly fluctuating flow and resulting bypassing of the bioreactor was caused by the limited water holding capacity of the surrounding area's shallow soil causing more of the rainfall to runoff. Furthermore, surface runoff occasionally ponded near the bioreactors and this may have also infiltrated through the permeable geotextile liner used at this site. There were two flood events in 2015, the wettest year.

### 3.2. Nitrate-N concentrations

The  $\text{NO}_3^- - \text{N}$  concentrations in the tile water flowing in the bioreactor at the Tompkins site were generally about  $10 \text{ mg L}^{-1}$ , the least variable of all sites (Table 2; Fig. 1b). They were insensitive to changes in temperature and discharge events. This implies that the source of the drain discharge at the Tompkins site was deep, old, and well-mixed groundwater (van Verseveld et al., 2008). The effluent  $\text{NO}_3^- - \text{N}$  concentrations of the bioreactor ranged from 0 to  $10 \text{ mg L}^{-1}$  (Fig. 1b). Both low temperatures (in winter) and early spring and increased discharge caused effluent  $\text{NO}_3^- - \text{N}$  concentrations to increase to around  $8 \text{ mg L}^{-1}$ . Only during the summer and early fall when both temperatures were elevated and flow was generally low, effluent  $\text{NO}_3^- - \text{N}$  concentrations were close to zero. Shortly after starting up, the bioreactors were less effective in removing the nitrate after a high flow event in mid-August 2013 even with temperatures over  $17 \text{ }^\circ\text{C}$  (Fig. 1b). Despite this, the bioreactors at this site significantly reduced the  $\text{NO}_3^- - \text{N}$  concentrations ( $p = 3.8 \times 10^{-14}$  for the Woodchips only(W) and  $2.4 \times 10^{-10}$  for the woodchips amended with biochar (WB)).

The average  $\text{NO}_3^- - \text{N}$  drain concentration at the Chemung location,  $6 \text{ mg L}^{-1}$ , was less than that of the other two sites (Table 2, Fig. 2b). The influent  $\text{NO}_3^- - \text{N}$  concentrations ranged from 0.7 to  $21 \text{ mg L}^{-1}$  and increased when the flow peaked (Fig. 2b). Regardless of the variation, the bioreactors at this site significantly reduced the  $\text{NO}_3^- - \text{N}$  concentrations ( $p = 1.7 \times 10^{-14}$  and  $6.6 \times 10^{-14}$  for the W and WB bioreactors, respectively; Table 2, Fig. 2b). The effluent  $\text{NO}_3^- - \text{N}$  concentrations ranged from 0 to  $21 \text{ mg L}^{-1}$  (Fig. 2b). The effluent concentrations were elevated at early spring when the low temperatures coincided with the high flow rates. They decreased with increasing temperatures except after a high flow event in June 2014.

Influent and effluent  $\text{NO}_3^- - \text{N}$  concentrations at the Steuben site were the greatest and the most variable among all bioreactors (Table 2, Fig. 3b). Inlet  $\text{NO}_3^- - \text{N}$  concentrations increased from  $4 \text{ mg L}^{-1}$  in March, peaked to  $62 \text{ mg L}^{-1}$  in late spring and early summer, and decreased to  $11 \text{ mg L}^{-1}$  at the beginning of fall varying with temperature, time of manure application, and storm events (Fig. 3b). The effluent  $\text{NO}_3^- - \text{N}$  concentrations especially for the WB

bioreactor were high when both the influent concentrations and the flow rates were high (Fig. 3b). Increased  $\text{NO}_3^- - \text{N}$  concentrations from the W bioreactor were rarely observed, as in April of 2015. One of the complicating features of this site was the surface ponding and water might have infiltrated from the surface into the bioreactor lowering the concentration. Similar to the other two sites, Steuben site bioreactors had significantly lower effluent  $\text{NO}_3^- - \text{N}$  concentrations than in the influent ( $p = 4.9 \times 10^{-8}$  and  $4.5 \times 10^{-12}$  for the W and WB bioreactors, respectively; Table 2, Fig. 3b).

### 3.3. Organic carbon concentration

For all bioreactors, the effluent DOC concentrations were significantly greater than influent DOC concentrations (Table 2, Figs. 1c, 2c and 3c), similar to the findings of Cameron and Schipper (2010) and Robertson (2010). Immediately after startup, the greatest DOC effluent concentrations were observed at all sites with the maximum concentrations of more than  $200 \text{ mg L}^{-1}$  at Tompkins and Steuben sites (Figs. 1c and 3c). Under such conditions, all nitrate was removed from water (corresponding events in Figs. 1d and 3d). For instance, at the Tompkins site on April 2013, when the water temperature was only  $7 \text{ }^\circ\text{C}$  and the DOC concentration in the effluent was  $200 \text{ mg L}^{-1}$ , the removal efficiency was 100% (Fig. 1c and d).

After high concentration at the startup period, a seasonal pattern in DOC concentrations was observed in the effluent at all three sites. At the Tompkins site, the influent DOC concentrations generally remained below  $5 \text{ mg L}^{-1}$ , although they increased to more than  $40 \text{ mg L}^{-1}$  in September of 2014 and 2015, about the time of the first frost (Fig. 1c). The effluent concentrations varied from 0.5 to  $22 \text{ mg L}^{-1}$  (Fig. 1c). In winter and early spring when temperatures were less than  $10 \text{ }^\circ\text{C}$  (Fig. 1d), inflow and outflow concentrations were nearly equal and the removal efficiencies were low (Fig. 1d). During the warm summer, the effluent DOC concentration were generally greater than the influent DOC concentrations and most nitrate was removed (Fig. 1b and d), except for after the high flow events which was especially obvious in 2013 (Fig. 1a and c).

Similarly, at the Chemung and Steuben sites, the amount of DOC released by the bioreactors was low in winter and early spring, peaked in the summer month, except during the times when the bioreactors were flooded (Figs. 2c and 3c). Despite having the same woodchip media for all the bioreactors, the released DOC from the bioreactors at the Steuben site was greater than the other two sites (Table 2, Fig. 3c).

### 3.4. Seasonal pattern of the Nitrate-N removal efficiency

The water temperature at the Tompkins site varied from 3 °C in early spring and late fall to 20 °C during the summer (Fig. 1d). Generally, the maximum  $NO_3^- - N$  removal efficiency ( $\epsilon_{NO_3^- - N}$ ; Eq. (1)) was near 100% when the temperatures were above 16 °C and usually below 30% when temperatures were below 5 °C (Fig. 1d). Moreover, especially in 2014 and 2015, the bioreactor efficiency increased with increasing temperature. However, reduced  $NO_3^- - N$  removal efficiencies were observed in spring and summer when the bioreactors were inundated, described as flooded in Fig. 1a. During some events in early spring 2015, high flow increased the  $NO_3^- - N$  coming out of the bioreactors, causing the  $\epsilon_{NO_3^- - N}$  to be less than zero. In summer 2013, after a high flow event, the woodchip (W) bioreactors' efficiency decreased and remained low until the following spring (Fig. 1d). In that period, the advantage of biochar amendment (WB bioreactor) was noticeable with over a 50% or greater  $\epsilon_{NO_3^- - N}$  (Fig. 1d). This difference between the W and WB reactor, however, did not occur in subsequent years.

At the Chemung site, the initial removal efficiency was 100% during the summer and decreased as temperature declined (Fig. 2d). In the following years  $\epsilon_{NO_3^- - N}$  increased with increasing temperature, peaked to 100% in summer, and dropped as temperature decreased (Fig. 2d). However, after a large storm in June 2014, the  $\epsilon_{NO_3^- - N}$  decreased. The data loggers were not installed at that time to measure the peak discharge through the bioreactors.

The  $\epsilon_{NO_3^- - N}$  of the bioreactors at the Steuben site (Fig. 3d), similar to the other two sites, usually followed the temperature pattern. In early spring,  $\epsilon_{NO_3^- - N}$  increased with rising temperature, reached the maximum in summer, and decreased in the following months. Like the other bioreactors, occasional reduced  $\epsilon_{NO_3^- - N}$  was observed when the flow increased and after the occasional flooding events.

## 4. Discussion

### 4.1. Temperature dependent Nitrate-N removal rate

The nitrate removal rates ( $r_{NO_3^- - N}$ ; Eq. (3)) for all six bioreactors are plotted as a function of the temperature of the input water in Fig. 4. We plotted the  $NO_3^- - N$  limited events separately, because in these cases, the rates were limited by the availability of  $NO_3^- - N$ .

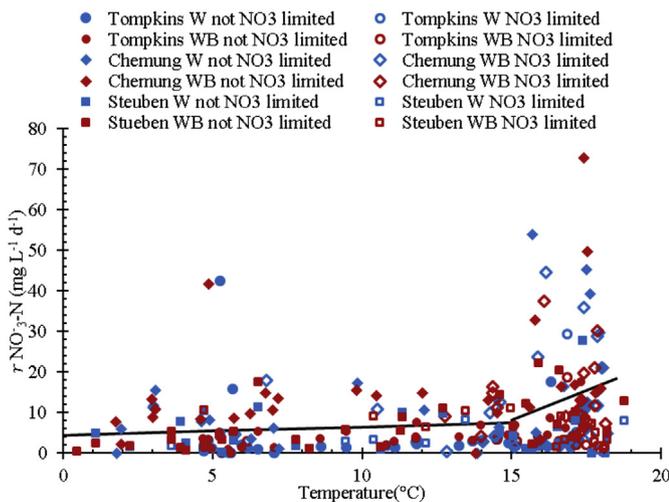


Fig. 4. Removal rate ( $r_{NO_3^- - N}$ ) with temperature at all sites. “ $NO_3^-$  limited events” (open symbols) are the events when the effluent  $NO_3^- - N$  concentration was less than 0.5 mg  $L^{-1}$ .

Removal rates for the woodchip substrate usually remains less than 10 g  $N d^{-1} m^{-3}$  (Schipper et al., 2010), although in the current study, the  $r_{NO_3^- - N}$  varied greatly from 0 to 73 g  $N d^{-1} m^{-3}$ . There are numerous events when the removal rates were greater than 10 g  $N d^{-1} m^{-3}$  at different temperatures. The maximum rates observed in this study were much greater than those for the woody media reported in a review by Schipper et al. (2010) of 22 g  $N d^{-1} m^{-3}$ . Nevertheless, the observed removal rates indicate the successful application of the denitrifying bioreactors.

Removal rates did not vary much below 16 °C, however, they increased sharply at temperatures above 16 °C at all bioreactors, despite having been constructed in different landscapes. The sharp increase in removal rates at 16 °C could be attributed to seasonal change in the bacterial community. The seasonal variation of the microbial community may be linked to the organic carbon availability which varies with temperature and moisture content of the bioreactors (Porter et al., 2015).

### 4.2. Carbon availability on Nitrate-N removal indices

Denitrification is controlled by three factors; presence of  $NO_3^-$ , presence of organic carbon as an electron donor and absence of oxygen (Schipper et al., 2010; Seitzinger et al., 2006). For denitrification to take place, at the first step, the aerobic microorganisms use organic carbon as an electron donor to reduce dissolve oxygen (DO) to obtain energy. By depleting DO, the anoxic environment is ideal for heterotrophic denitrification. Under these conditions, non-oxygenated electron acceptor such as  $NO_3^-$  breaks down to oxidize organic matter and to produce energy (Korom, 1992). In both processes, organic carbon plays an important role. Indeed, a labile carbon source has a profound effect on the performance of bioreactors (Cameron and Schipper, 2010; Greenan et al., 2006). Bioavailable carbon sources appear in the forms of amino acids, carbohydrate and other simple organic compounds (Zou et al., 2005). Regardless, a young carbon source, such as that in the studied bioreactors, was bioavailable (Chapelle et al., 2009). Therefore, DOC concentrations was used as an indicator of the bioavailable carbon.

Fig. 5 shows that  $NO_3^- - N$  removal efficiency ( $\epsilon_{NO_3^- - N}$ ) and DOC availability index are linked. In the abundance of nitrogen, DOC leachate decreased, whereas in nitrogen limited conditions, the DOC concentrations in the effluent were elevated (Fig. 5). This is in

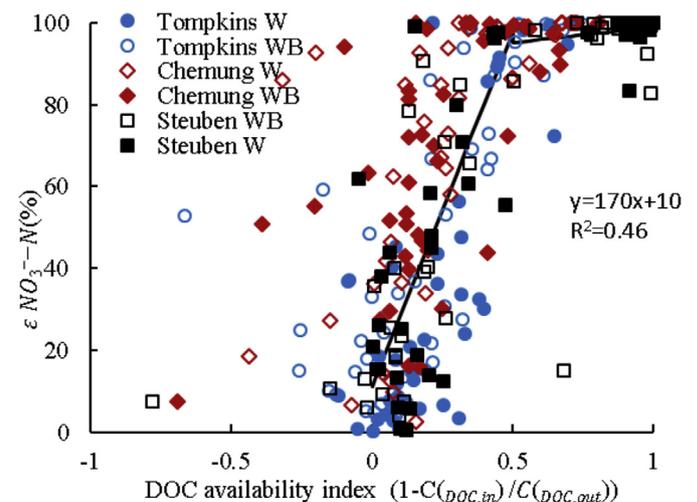


Fig. 5. The relationship between the DOC availability index and the  $NO_3^- - N$  removal efficiency ( $\epsilon_{NO_3^- - N}$ ) at all sites.

agreement with the observation previously made in landscapes (Lai et al., 2016). Since the DOC is generated in the bioreactors and concentrations in the effluent were generally greater than those at the influent (Table 2, Figs. 1c, 2c and 3c), the ratio of the DOC concentration in the inlet to that in the outlet was considered and the DOC availability index was acquired as:  $1 - C_{DOC.in}/C_{DOC.out}$ .

Similar to temperature dependent removal rate, all six bioreactors behave very much the same. When the DOC availability index was  $>0.5$ , the released organic matter exceeded the amount needed to complete denitrification, and  $\epsilon_{NO_3^- - N}$  was greater than 0.8. The  $\epsilon_{NO_3^- - N}$  decreased sharply when the DOC availability index dropped from 0.5 to 0. Finally, when the inlet concentrations were greater than those of the outlet, which occurred following manure applications, they sometimes boosted  $NO_3^- - N$  removal, as it might have been bioavailable to the denitrifying bacteria. This graph suggests that with sufficient carbon,  $NO_3^- - N$  was removed independent of other factors such as temperature. Indeed, at the start-up period at the Tompkins site, despite low temperature, a complete removal of  $NO_3^- - N$  occurred (section 3.3). Elevated DOC concentration at the start-up period is because of the rapid decay of young organic carbon (Janssen, 1984). However, over time, differentiating between the effect of temperature and DOC is difficult because the two factors are interconnected. The decomposition of organic matter increases with increasing temperature (Paré et al., 2006) and thus, the DOC availability index increased during warmer summer months.

It is also noteworthy that although the points from different sites overlapped closely, the Chemung site bioreactors (Fig. 5), in comparison with the other sites, experienced greater removal efficiencies relative to the DOC availability index. This is attributed to the lower inlet  $NO_3^- - N$  concentrations, and maintaining equilibrium due to the continuous flow in the bioreactors at this site. This suggests that the landscape characteristics of a site should be considered when designing the bioreactors.

#### 4.3. Effect of biochar amendment

The hydraulic properties of the bioreactors were influenced by the biochar since the particle size was finer than the woodchips, which reduced the conductivity of the medium (data not shown). Consequently, at the Tompkins site the discharge through the WB bioreactor was smaller, although both bioreactors had the same inlet water head (Table 2). Thus, the differences between the outflow concentrations may have been due to the slower flow rate and consequently longer HRT (Fig. 1a, Table 2). Therefore, the  $NO_3^- - N$  removal rate ( $r_{NO_3^- - N}$ ; Eq. (3)), was used to compare the W and WB bioreactors.

At all sites, the average  $r_{NO_3^- - N}$  of the reactors containing biochar was greater than that of the woodchips bioreactors (Table 2). These differences, however, were not significant at all sites or during the whole period of investigation (Table 3). At the Tompkins site (10% biochar added to the WB bioreactor; influent  $NO_3^- - N$  concentrations of  $9.3 \text{ mg L}^{-1}$ ) in the first two years of the bioreactor use, the  $r_{NO_3^- - N}$  of the WB bioreactor was greater than that at the W bioreactor (paired *t*-test *p* value  $< 0.002$ , Table 3). The differences were not significant during the third year and consequently, over

the whole period of investigation, suggesting that the positive effect of biochar may only have been temporal. At the Chemung site (10% biochar added; influent  $NO_3^- - N$  concentrations of  $6.3 \text{ mg L}^{-1}$ ) the difference was not significant during the years of investigation. However, at the Steuben site, where only 2.5% of biochar was added, the WB bioreactor had significantly greater  $r_{NO_3^- - N}$  than the W bioreactor (Table 1). This site has the greatest influent  $NO_3^- - N$  concentrations of 18.4 and  $16.6 \text{ mg L}^{-1}$ . In general, the sites with greater  $NO_3^- - N$  concentrations benefited more from the biochar amendment.

Our finding was consistent with that of a plot scale experiment of Bock et al. (2016) that biochar was only effective in removing  $NO_3^- - N$  when the effluent concentrations exceeded  $5 \text{ mg L}^{-1}$  at lower temperatures and  $10 \text{ mg L}^{-1}$  at higher temperature. According to Harter et al. (2014) biochar improves the  $NO_3^- - N$  removal in soils by altering pH, C:N ratio, and N and oxygen availability. The surface characteristics of biochar change in response to a series of reactions referred as “ageing” (Cheng and Lehmann, 2009; Harter et al., 2014), and evidently, this process diminished the beneficial effect of biochar amendment rather quickly. The ageing process involves decrease in carbon leachate from biochar coupled with the loss of ash and carbon content, decrease in pH and increase in cation exchange capacity (Cheng et al., 2006; Cheng and Lehmann, 2009; Heitkötter and Marschner, 2015). The abundance of organic matter in soil stimulates the ageing process, likely due to an increase in biological activity (Cayuela et al., 2013; Heitkötter and Marschner, 2015). Considering the abundance of organic matter due to the presence of woodchips in denitrifying bioreactors, the ageing process happened rapidly. However, Cheng et al. (2006) found that the ageing of black carbon was mostly abiotic. We cannot rule out the fact that some biochar was lost through leachate, since the bioreactors' effluent was dark after the initial start-up. Therefore, biochar's ash content decreased quickly after the start. Noting that the bioreactor performance was greatly influenced by the DOC concentrations and availability, the decrease in DOC availability due to the leachate of ash and ageing process negatively impacted the advantage of the biochar.

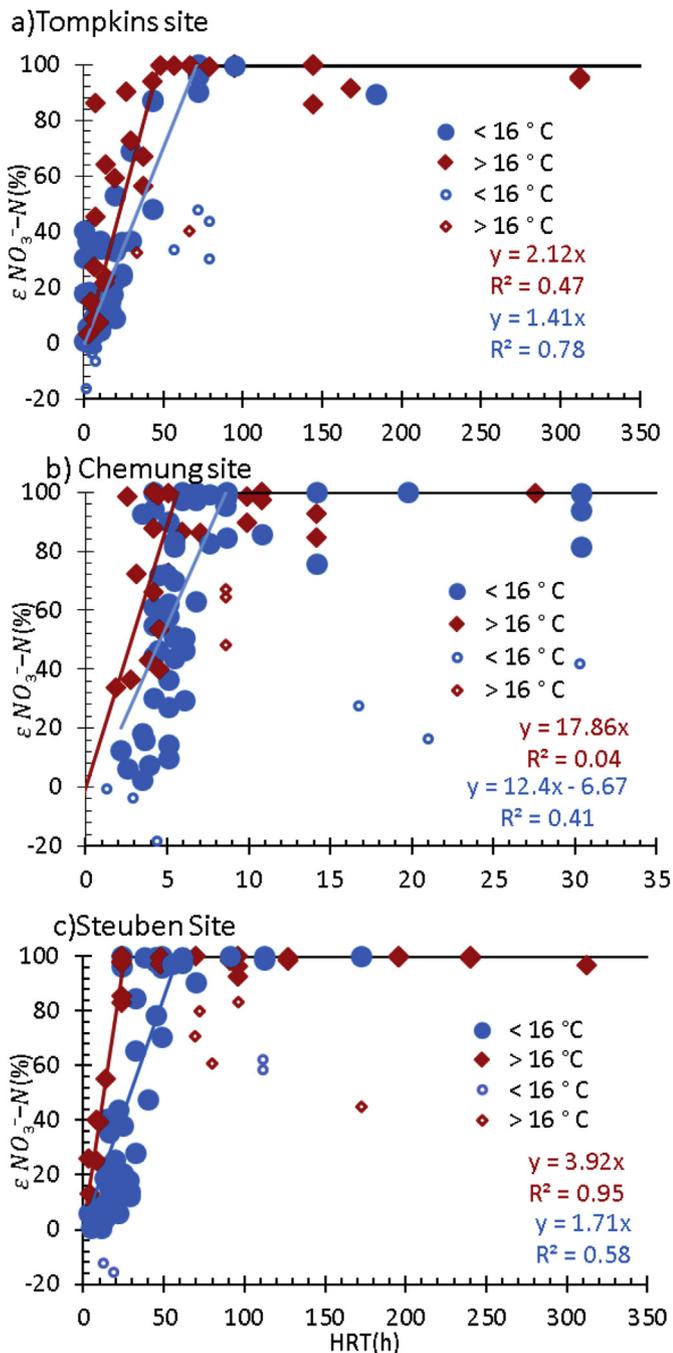
#### 4.4. Effect of hydraulic retention time on Nitrate-N removal efficiency

The relationship between  $NO_3^- - N$  removal efficiency ( $\epsilon_{NO_3^- - N}$ ) and hydraulic retention time, HRT, for the six bioreactors are shown in Fig. 6 for events when the water temperature was above  $16 \text{ }^\circ\text{C}$  and below  $16 \text{ }^\circ\text{C}$ . Since the difference due to the addition of biochar was barely significant - section 4.3 - the points from the two bioreactors at each site were not separated. Less than 10% of the data were excluded from the calculations (Fig. 6). These points comprised events when the bioreactors were flooded after which removal efficiencies were suppressed, and when some very high influent  $NO_3^- - N$  concentrations occasionally occurred. During the high and transient flow events, nitrification of organic nitrogen may have occurred in the top portion of the bioreactors which was usually unsaturated and did not contribute to its performance, which finally lead to the negative removal efficiencies (van Verseveld et al., 2009).

A critical HRT, the minimum HRT required for reaching 100%  $\epsilon_{NO_3^- - N}$ , was defined. Below this HRT, there is a linearly increasing relationship between HRT and  $\epsilon_{NO_3^- - N}$ , which was observed with previous studies (Chun et al., 2009; Greenan et al., 2009, 2006; Robertson, 2010). The critical HRT was greater for low temperatures than for temperatures above  $16 \text{ }^\circ\text{C}$ . The relationship between the  $\epsilon_{NO_3^- - N}$  and HRT was similar for the Tompkins and the Steuben site (Fig. 4a and c, both above or below  $16 \text{ }^\circ\text{C}$ ) with the Tompkins

**Table 3**  
P-values of the *t*-test analysis of the  $NO_3^- - N$  removal rates between the W and WB bioreactors during the period of investigation.

Site	Whole period	2013	2014	2015
Tompkins	0.69	0.002	0.001	0.66
Chemung	0.17	0.66	0.08	0.42
Steuben	0.02	0.006	0.03	0.71



**Fig. 6.**  $\text{NO}_3^- - \text{N}$  removal efficiency ( $\epsilon_{\text{NO}_3^- - \text{N}}$ ; %) at different HRTs at the bioreactors at a) Tompkins site, b) Chemung site, and c) Steuben site. The graphs include both W and WB bioreactors. The open circles were excluded from the calculations. Note that the scale of the X-axis for the graph b, representing the Chemung site, is one-tenth of the other two graphs for the other two sites.

site having a larger critical HRT to reach the 100% nitrate removal than the Steuben site. At the Tompkins site the critical HRTs were 48 and 79 h, for the temperatures above and below 16 °C, respectively. They were 24 and 61 h at the Steuben site for the temperatures above and below 16 °C, respectively. At the Chemung site, critical HRTs were much less than the other two sites (compare Fig. 4b with Fig. 4a and c, note the change of scale). This suggests that the uniform flow at the Chemung site provided more stable conditions and an equilibrium for the population of denitrifiers, which was accompanied with lower incoming nitrate

concentrations than those at the other two sites (Hoover et al., 2015). Consequently, a wide range of the removal efficiencies were observed within a narrow range of HRT of about 4 h. The critical HRTs (to reach 100% nitrate removal) were within the range of those in other studies, such as in laboratory experiments by Greenan et al. (2009) of 5 d, and by Chun et al. (2009) of 24 h, or that in a field experiment by Christianson et al. (2013b) of approximately 31 h.

Finally, the average  $\text{NO}_3^- - \text{N}$  removal efficiency that was between 42% and 68% (Table 2) were within the range observed in previous field studies (Christianson et al., 2012), indicating that the bioreactors in New York state were able to perform reasonably well.

#### 4.5. Performance evaluation of denitrifying bioreactors in the northeast

The design and application of denitrifying bioreactors requires an understanding of the landscape and hydrological setting of the field as well as understanding the relationship between the removal efficiency and HRT. For efficient removal of nitrate, the DO should be depleted from water. At short retention times, the DO may not be removed from water and thus, sporadic removal of  $\text{NO}_3^- - \text{N}$  occurs. Fig. 6 shows that at low retention times, a broad range of removal efficiencies were observed. At the Chemung site, when short retention times occurred, the sporadic  $\text{NO}_3^- - \text{N}$  removal was observed constantly (Fig. 6). In the Tompkins and Steuben sites, the sporadic  $\text{NO}_3^- - \text{N}$  removal only occurred at shorter retention times. Therefore, to have a constant and efficient removal of  $\text{NO}_3^- - \text{N}$ , a sufficient HRT is required.

At retention times that are longer than the critical HRT, complete  $\text{NO}_3^- - \text{N}$  removal occurs. Since sulfate is usually present in the drainage water, it is a substitute for  $\text{NO}_3^- - \text{N}$  as an electron acceptor only in the absence of it (Moorman et al., 2010; Schipper et al., 2010; Woli et al., 2010). In the current study, too, the concentration of sulfate in the outflow was less than that at the inflow when  $\text{NO}_3^- - \text{N}$  was limited (Fig. A6). In extreme reducing conditions, methane production may occur (Moorman et al., 2010). Moreover, the cost of the bioreactors increases with increase in HRT. Therefore, complete removal of  $\text{NO}_3^- - \text{N}$  is not intended.

Christianson et al. (2011a) suggested that the design procedure should use 10–20% of the peak flow at design HRT of 6–8 h, to ensure at least 30% removal at the time of peak flow. Previous to that, a HRT of 4 h was suggested as a design criteria (Christianson et al., 2011a). For The current research, the bioreactors were designed so that a discharge equal to 20% of the estimated peak flow would be contained using the hydraulic retention time of 6 h. This design criterion was met at the Chemung site with continuous flow and lower  $\text{NO}_3^- - \text{N}$  concentrations. The continuous drain flow in deep soils (such as in the Chemung site and often in the Midwest US), remove  $\text{NO}_3^- - \text{N}$  in the bioreactors throughout the growing season. Even at temperatures below 16 °C at the Chemung site, the critical retention time was less than 4 h. However, at the other two sites, variable HRTs were observed (Fig. 4). At the Steuben site, 30% of removal was acquired at 8 and 17 h at high and low temperatures, respectively. However, the steady removal of  $\text{NO}_3^- - \text{N}$  in colder temperatures occurred when the retention times were more than 21 h. At the Tompkins site, 14 and 38 h was required to achieve 30% removal. These retention times were more than the retention times in which sporadic removal of  $\text{NO}_3^- - \text{N}$  was observed. The shallow soils over an impermeable glacial till or bedrock layer have a low storage capacity which leads to flow variation, high discharge during wet periods and no flow during summer. The interceptor drain in the Tompkins site also received variable drain flows in response to seasonal fluctuations in the water table.

High flow events introduce dissolved oxygen to the bioreactors

and, therefore, decrease the removal efficiency (Christianson et al., 2011c). Furthermore, extremely large flows (small observed HRTs) could flush the biofilm, enzymes, and organic matter out of the media (Bradford et al., 2013; Chun et al., 2009; Soares and Abeliovich, 1998), which could have a long-term effect on the bioreactor performance. Thus, the removal efficiency was reduced for several days after high flow events (Figs. 1–3 after each flooded event; section 3.4).

As an edge of field technique, constructing larger bioreactors to accommodate such variable flow events and to remove a sufficient amount of  $\text{NO}_3^- - \text{N}$  may not be applicable. Therefore, more efficient bypass or storage systems are required to prevent damage to the bioreactors. However, more flow bypass means that the bioreactors will not be as effective overall for treating the drain discharge. This adds to the complexity of the design of the bioreactors in different landscapes. More research is required to quantify the threshold for the flow rate above which the flow rate would impair the performance of the bioreactors.

Seasonal variation of the bioreactor performance should be considered depending on the temperatures when the peak  $\text{NO}_3^- - \text{N}$  concentrations are observed. According to Figs. 1–3, and an earlier study by van Es et al. (2004), the maximum  $\text{NO}_3^- - \text{N}$  concentrations from drain flows in this region usually occur during early spring or early fall, and when the temperatures were above 16 °C. Therefore, designing the bioreactor in accordance to the higher temperature relationship should be sufficient.

## 5. Conclusion

This study investigated the application of paired denitrifying bioreactors in three field sites in New York State. One of each paired bioreactor was amended with biochar. The results showed that to some extent, bioreactors could benefit from the addition of biochar. However, the biochar did not influence the bioreactor with low influent  $\text{NO}_3^- - \text{N}$  concentrations, and its effect on the bioreactor with high  $\text{NO}_3^- - \text{N}$  concentrations was only temporal.

Despite many variables in the field settings, the extensive sampling during three years of investigation at six individual bioreactors made it possible to investigate seasonal variation of bioreactor performance. This study provided relationships between removal efficiency and HRT for the bioreactors at different temperatures and inflow nitrate concentrations in New York. Nevertheless, this study recommends more investigation on the performance of the bioreactors during and after high drainage flow.

During the study period, all the bioreactors maintained good  $\text{NO}_3^- - \text{N}$  reduction of about 50% and, therefore, the application of the denitrifying bioreactors was deemed successful. The bioreactors achieved a high removal rate and efficiency in summer, showing great potential to remove  $\text{NO}_3^- - \text{N}$  during the growing season. This could be attributed to the increased removal rate with temperature, seasonal variation in microbial community, and availability of DOC to the denitrifiers. Following a seasonal pattern, the availability of DOC was a major factor controlling bioreactor performance.

This study found a correlation between the removal efficiency and the HRT. The relationships between the removal efficiency and HRT at the Tompkins site were similar to those of the Steuben site, as they both had variable discharge and high inflow nitrate concentrations. Whereas, at the Chemung site, where the flow was constant at low rates and the inflow nitrate concentrations were low, the bioreactors achieved higher removal efficiency at lower HRTs. Due to these differences, a particular relationship was not found to govern all of the bioreactors. In addition, at temperatures above 16 °C, both the removal rate and efficiency in all bioreactors were elevated. Therefore, when designing new bioreactors for areas

with predominantly cold temperatures, the current design standard (NRCS, 2015) might not be sufficient, as they may overestimate  $\text{NO}_3^- - \text{N}$  removal at lower temperatures. In addition, when designing bioreactors in New York State, it is essential to consider the prevalence of extreme flow, due to the shallow soil profile and glacial tills, which overwhelms and flushes the bioreactor constituents out. This was observed after each flooding event when the bioreactors' performance declined, sometimes for several days. Therefore, bioreactor design for these conditions should consider an effective bypass or additional storage system. Further investigation is needed to quantify the threshold of velocity above which bioreactor performance declines.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jenvman.2017.06.054>.

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