Supporting Material

Supporting Material for: First Demonstration of Nitrate Reduction Using Woodchip Bioreactor Technology at a Small Community Wastewater Treatment Plant

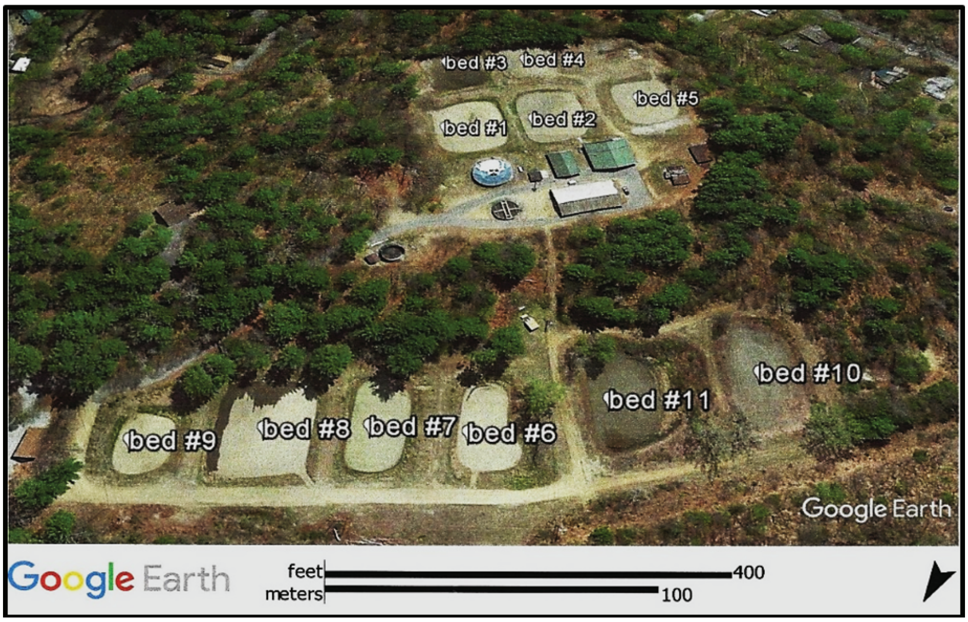

Figure S1: Google Earth™ view of the Bolton WWTP showing the location of the lower (#1-5) and upper (#6-11) sand infiltration beds and the plant components adjacent to the lower beds.

Figure S2: Google Earth™ view of the Bolton WWTP showing lower and upper sand bed location, the direction of groundwater movement toward the Mohican Road Tributary and Stewart Brook when effluent is discharged to the lower beds, and the direction of groundwater movement toward Stewart Brook when effluent is discharged to the upper beds.

2016-2017 Monitoring Program

Background

The near-shore littoral zone of Bolton Bay, Lake George (Warren County, New York) had experienced excessive algal blooms during the 2000s and 2010s to the extent that private beaches were unusable for recreation. Although the full extent of the problem was not known, two streams, the Mohican Road Tributary and Stewart Brook, discharge into Bolton Bay and were suspected of transporting nutrients into the lake from the Bolton WWTP which is located at a higher elevation in the watersheds of both streams. There had been several previous scientific investigations on both watersheds, so historical data were available to compare with current data.

Monitoring program components

A monitoring program was designed and initiated during April 2016 that included tracking certain treatment plant operations and extensive field sampling to determine the sub-surface direction and extent of groundwater flow from the Bolton WWTP, particularly from the region of the lower sand infiltration beds. The sites sampled included five wells, three emergent seepage streams, two locations on the Mohican Road Tributary and three locations along the channel of Stewart Brook. The sampling sites were selected to correspond with sites from previous investigations. An additional 'background' well was located in the region and included in the monitoring effort to characterize the chemistry of groundwater not impacted by subsurface groundwater flow from the Bolton WWTP.

Stewart Brook was sampled at three locations (Bradley Lane, Dula Place, Stewart Pond outlet) to segregate the segment of stream channel where groundwater from the upper sand infiltration beds at the Bolton WWTP enters the tributary. The infiltration area had been identified by an earlier Rhodamine dye study [1]. The sites sampled along the Stewart Brook channel had been studied previously [2,3].

The field effort included bi-monthly sampling of wells, groundwater seepage streams, the Mohican Road Tributary and Stewart Brook from April through September 2016 and monthly sampling from October 2016 through May 2017. A total of 196 water chemistry samples and corresponding field measurements were collected from 15 stations during the 14-month study.

Methods

Field measurements were determined in-situ and included temperature and dissolved oxygen concentration and saturation (YSI® Model 55 dissolved oxygen and temperature meter), conductivity, Total Dissolved Solids (TDS) and pH (Myron Ultrameter 4PII). Samples were collected in 1-L Polyethylene (PE) bottles and kept on ice until processing.

Ground water wells: All wells were sampled with protocol required for permit sampling, when possible, including well purging to remove stagnant water. In addition, the level of groundwater in each well was determined (Solinst Model #102M) along with the field measurements described above. Water was withdrawn from each well with the use of a standard well bailer.

Seepage streams, mohican road tributary and stewart brook: Upon arriving at each sampling station, field measurements were collected, and a 1-L PE bottle was used to collect a water sample for chemical analysis. Each site had its own dedicated PE sample bottle for the duration of the monitoring program. If stream level was too low to collect samples with the 1-L bottle, a separate 500 mL PE bottle was used to collect water and fill the sample bottle incrementally.

Manual gaging of seepage channels and streams was conducted using a cross-section technique [4] where the total channel width is divided into equal segments, depth is measured at the centerline of each segment, and velocity measured at the 0.6 depth above the bottom. The area, velocity profile and flow are calculated for each segment, and the segment flows are summed to determine total channel discharge. Flow measurements were made with a top setting wading rod and Marsh McBirney (Model 2000) Flow Meter (FlowMate).

Darrin Fresh Water Institute Laboratory: The Darrin Fresh Water Institute (DFWI) is a field station located in Bolton, New York, and affiliated with Rensselaer Polytechnic Institute (Troy, New York). At the DFWI Laboratory, the samples were analyzed for nitrate-nitrogen (NO_3-N), Total Nitrogen (TN), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP) and Chloride (Cl). Analytical results usually were reported by the laboratory within 3-4 weeks following collection.

Analytical lab techniques: The analytical techniques followed by the DFWI Laboratory for processing the chemistry samples are summarized below (Table S1).

All field sampling was conducted within a 2-3 hr window on the same day. The collected samples were processed at the Darrin Fresh Water Institute Laboratory in Bolton Landing immediately following collection and submitted for analysis. Raw water samples collected at the Bolton facility in association with the operating permit were picked up and delivered that same day, along with a completed Chain of Custody form, to the CNA Environmental, Inc. Laboratory in Ballston Spa, New York, a laboratory certified by New York State for analysis of wastewater samples.

At the CNA Laboratory, the samples were analyzed for nitrate-nitrogen (NO_3-N) in the influent, effluent and monitoring wells, ammonia-nitrogen and TKN in the effluent, total phosphorus (TP) in the influent, effluent and monitoring wells, 5-day Biochemical Oxygen Demand (BOD_5) in the influent and effluent and total suspended (non-filterable) residue (TSS) in the influent and effluent. According to the facility permit, effluent discharges shall be monitored monthly and nutrient limitations for effluent leaving the plant are limited as follows: nitrate-nitrogen, 20 mg/L, and phosphorus, 0.5 mg/L. A separate permit condition sets an upper limit of 10 mg/L of nitrate-nitrogen measured at the treatment plant monitoring wells.

Table S1: Summary of parameters and analytical methods followed by the DFWI Laboratory for processing water samples collected as part of the 2016-2017 monitoring program.

Parameter	Analytical Method
Anions	Ion chromatograph (US EPA Method 300)
Total nitrogen	Persulfate method (Standard Methods, 19th Edition, 4500-PE)
Soluble reactive phosphorus	Ascorbic Acid Method (Standard Methods, 4500-PE)
Total phosphorus	Persulfate Oxidation, Ascorbic Acid method (Standard Methods, 4500-PE)
Chlorophyll a	Fluorometric (Standard Methods, 10200)
Dissolved Oxygen	Membrane Electrode (US EPA Method 360.1)
Specific conductance	Wheatstone bridge type meter (US EPA Method 120.1)

Two separate special investigations were conducted during the study to establish the connectivity of the lower infiltration sand beds at the treatment facility and groundwater moving from these beds into the Mohican Road Tributary and Stewart Brook watersheds. Rhodamine-WT dye was added to the beds and traced into the watersheds using a field fluorometer and regularly sampling ground water emerging down-gradient of the Bolton WWTP as surface water. Both dye studies were successful in establishing the connectivity of effluent discharged to sand beds with the Mohican Road Tributary and Stewart Brook watersheds (Figure S3).

Woodchip Bioreactor Information

The bioreactor was constructed 30.5 m long by 6.1 m wide by 1.2 m deep. A volume of sand disposal bed #10 was excavated below ground level to include these bioreactor dimensions and the base carefully graded to a level condition. Plywood supports were installed to form the perimeter wall of the bioreactor unit and then 45-mil PVC pond liner was installed inside the entire woodchip containment area. The enclosed area then was filled with hardwood and softwood chips (size range=1.3-5.1 cm) provided by a local supplier. Filter fabric was installed over the entire unit to protect the woodchips from infiltration of overlying soil. Bioreactor construction utilized the Town of Bolton WWTP operations staff, the Town Highway Department, a private contractor, and the Town engineering consultant. The woodchip bioreactor became operational in October 2018.

The bioreactor received treatment plant effluent from a 7.57 m^3 concrete tank adjacent to the influent chamber of the bioreactor, with effluent pumped to the tank through a small capacity pump station with a new 10 hp Ebara submersible sewage pump (Model #100DLMFU67.5), installed in April 2018. The pump station sizing and operational characteristics of the pump necessitated that the concrete reservoir provide more consistent flow for the bioreactor. The concrete tank has an overflow to discharge tertiary effluent to the down-gradient infiltration sand beds during periods when the bioreactor cannot process all of the incoming flow. The bioreactor flow can be controlled by a gate valve.

Overview of 2019-2021 Woodchip Bioreactor Monitoring Program

Bioreactor monitoring began March 19, 2019 and concluded May 31, 2021; Stewart Brook was sampled until the end of September 2021. See table matrix below (Table S2).

Routine sample collection

The program included water chemistry sample and field data collection from the bioreactor influent and effluent, a series of three PVC wells installed to a depth of ~1.0 m at 7.6, 15.2, and 22.9 m along the length of the bioreactor from the influent end, treatment plant effluent discharge, and Stewart Brook. All bi-weekly field sampling was conducted within a 1-2 hr period with the bioreactor and associated sites sampled by Bolton WWTP personnel and Stewart Brook sampled by The Lake George Waterkeeper and The FUND for Lake George personnel. All samples for water chemistry collected in the field immediately were transferred to sample containers provided by the

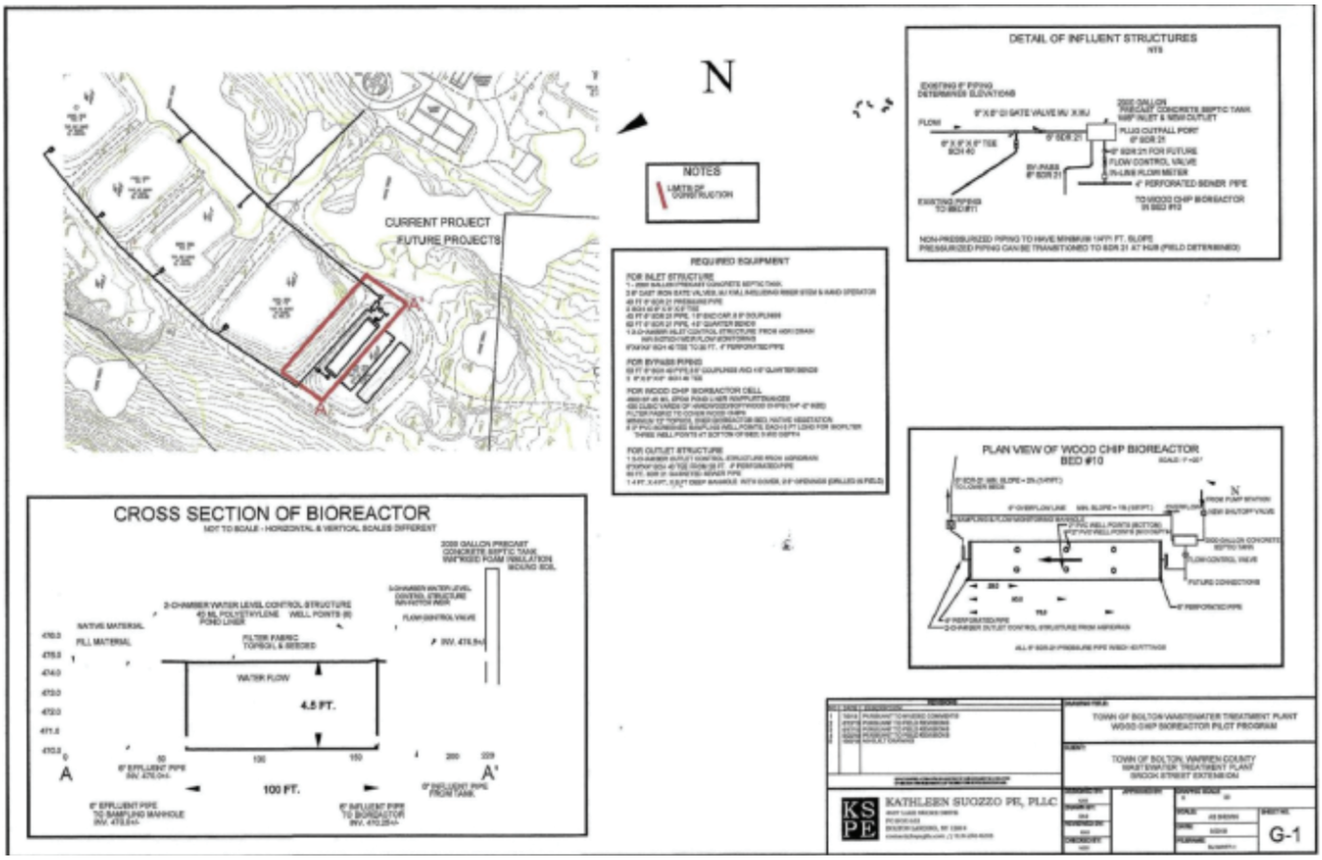

Figure S3: Design plans for the Bolton WWTP woodchip bioreactor constructed during 2018 in upper infiltration sand bed #10.

Table S2: Sampling sites, dates and samples collected as part of the 2019-2021 study.

Date	Bioreactor Sampling Sites								Bed Effluent	Monitoring Wells			Stewart Brook	
	Influent	MW1	MW2	MW3	MW4	MW5	MW6	Effluent	N	#3	#2	#4	Above	Below
3/19/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
4/2/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
4/16/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
4/30/2019										x	x	x	x	x
5/14/2019	x		x	x	x	x	x	x		x	x	x	x	x
5/28/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
6/11/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
6/25/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
7/9/2019	x	x	x	x	x	x	x	x		x	x	x	x	x
7/23/2019	x		x	x			x	x		x		x	x	x
8/6/2019	x		x	x			x	x		x	x	x	x	x
8/20/2019													x	x
9/3/2019	x		x	x			x	x		x	x	x	x	x
9/17/2019										x	x		x	x
10/1/2019	x		x	x			x	x					x	x
10/15/2019	x		x	x			x	x					x	x
10/29/2019	x		x	x			x	x		x	x	x	x	x
11/11/2019	x		x	x			x	x		x	x	x	x	x
11/26/2019	x	x	x	x	x	x	x	x	x	x	x	x	x	x
12/10/2019	x	x	x	x	x	x	x	x	x	x	x	x	x	x
12/23/2019	x	x	x	x	x	x	x	x	x	x	x	x	x	x
1/7/2020	x	x			x	x		x	x	x	x	x	x	x
1/21/2020	x		x	x			x	x	x	x	x	x	x	x
2/4/2020	x		x	x			x	x	x	x	x	x	x	x
2/18/2020	x		x	x			x	x	x	x	x	x	x	x
3/3/2020	x		x	x			x	x	x	x	x	x	x	x
3/17/2020													x	x
3/31/2020														
4/14/2020	x			x				x	x	x			x	x
4/28/2020	x			x				x	x	x			x	x
5/12/2020	x			x				x	x	x			x	x
5/26/2020	x			x				x	x	x			x	x
6/9/2020	x			x				x	x	x	x	x	x	x
6/23/2020	x		x	x			x	x	x	x	x	x	x	x
7/7/2020	x		x	x			x	x	x	x	x	x	x	x
7/21/2020	x		x	x			x	x	x	x	x	x	x	x
8/4/2020	x		x	x			x	x	x	x			x	x
8/18/2020	x		x	x			x	x	x	x	x		x	x
9/1/2020	x		x	x			x	x	x	x	x		x	x
9/15/2020	x		x	x			x	x	x				x	x
9/29/2020	x		x	x			x	x	x	x			x	x
10/13/2020	x		x	x			x	x	x	x			x	x
10/27/2020	x		x	x			x	x	x	x			x	x

11/10/2020	x		x	x			x	x	x	x			x	x
11/24/2020	x		x	x			x	x	x			x	x	x
12/8/2020	x		x	x			x	x	x	x	x	x	x	x
12/22/2020	x		x	x			x	x	x	x		x	x	x
1/5/2021	x		x	x			x	x	x	x	x	x	x	x
1/19/2021	x		x	x			x	x	x	x	x	x	x	x
2/2/2021	x		x	x			x	x	x	x	x	x	x	x
2/16/2021	x		x	x			x	x	x	x		x	x	x
3/2/2021	x		x	x			x	x	x	x	x	x	x	x
3/16/2021	x		x	x			x	x	x	x	x	x	x	x
3/31/2021	x		x	x			x	x	x	x	x	x	x	x
4/13/2021	x		x	x			x	x	x	x	x	x	x	x
4/27/2021	x			x			x	x	x	x	x		x	x
5/11/2021	x		x	x			x	x	x	x	x	x	x	x
5/25/2021	x			x			x	x	x	x	x	x	x	x
6/8/2021									x	x	x	x	x	x
6/22/2021										x	x	x	x	x
7/6/2021										x	x	x	x	x
7/20/2021													x	x
8/3/2021													x	x
8/17/2021													x	x
8/31/2021													x	x
9/14/2021													x	x
9/28/2021													x	x
Total collected	54	11	46	52	12	12	47	53	41	54	42	42	65	65
Color-code	bio down		pandemic		well dry		contamination		not in program yet					

contract laboratory, Phoenix Environmental Laboratories Inc., 587 East Middle Turnpike, P.O. Box 370, Manchester CT 06040, as follows:

- 1-250 mL Polyethylene (PE) bottle preserved as is for NO_3-N,
- 1-250 mL PE bottle preserved with H_2SO_4 for NH_3, soluble reactive phosphorus (SRP),
- 1-250 mL amber glass bottle preserved as is for DOC

Phoenix is certified to analyze chemistry samples collected as part of the New York State Department of Environmental Conservation SPDES (State Pollutant Discharge Elimination System) permit system which oversees wastewater treatment facilities. Collected samples were refrigerated, then placed on ice and delivered to the analytical laboratory on the same day as collected in the field, accompanied by a completed Chain of Custody form.

Bioreactor sites: Water samples for chemistry analysis were collected from bioreactor influent, monitoring wells and effluent using a well bailer to withdraw sample after the station had been flushed with twice the volume in the well to guarantee that a fresh sample was collected. The bed effluent was collected at end-of-pipe by holding the bottle under the discharge until just filled.

Stewart Brook: Both sites were sampled mid-channel for chemistry and field measurement by rinsing a PE container three times with tributary water and then filling the container which was used to fill the sample bottles and run field measurements. Tributary flow was measured using a cross-section technique [4] where the total channel width is divided into equal segments, depth measured at the centerline of each segment, and velocity measured at the 0.6 depth above the bottom. The area, velocity profile and flow are calculated for each segment, and the segment flows are summed to determine total channel discharge. Flow measurements were made with a top setting wading rod and Marsh McBirney (Model 2000) Flow Meter (FlowMate).

Field measurements: Water temperature and dissolved oxygen (concentration-saturation) were measured *in-situ* using a Yellow Springs Instrument (YSI) ProODO™ Optical Dissolved Oxygen meter. Subsamples of collected water were analyzed on-site for specific conductance, total dissolved solids and pH using an Ultrameter II™ (Myron L Company). Tributary flow was gaged using a top setting wading rod in combination with a Hach FH950 portable velocity flow meter with electromagnetic sensor.

Analytical laboratory methods: The analytical techniques for analysis of the chemistry samples are in Table S3 along with standard procedures for field measurements of dissolved oxygen and conductance (Figure S4).

Table S3: Summary of parameters and analytical methods followed by the Phoenix Laboratory for processing water samples collected as part of the 2019-2021 monitoring program.

Parameter	Analytical method
Nitrate as nitrogen	Colorimetric (US EPA Method 353.2)
Ammonia as nitrogen	Colorimetric (US EPA Method 350.1)
Soluble reactive phosphorus	Colorimetric (Standard Methods 4500-PE-99)
Dissolved organic carbon	Colorimetric (Standard Methods 5310B-11)
Iron	Colorimetric (US EPA Method 200.7)
Alkalinity	Titrimetric (Standard Methods 2320B-11)
Temperature	Thermometric (Standard Methods 2550 B-2000)
Total dissolved solids	Gravimetric (Standard Methods 2540-C)
Dissolved Oxygen	Optical (ASTM Method D888-09(C))
Specific conductance	Wheatstone bridge type meter (US EPA Method 120.1)

Woodchip Bioreactor Operational Gaps

See Table S4.

Woodchip bioreactor operational parameters

Flow: Until March 2019, flow through the bioreactor was gauged by the V-notched weir in the influent Agri Drain structure, the standard flow measurement method for agricultural applications. Flows were reported as wastewater depth over the influent V-notch weir, which could vary throughout the day depending on the pump cycle of the tertiary pump station supplying effluent to the 2000-gallon bioreactor influent reservoir.

This study required more exact flow measurement and a Greyline in-pipe flow meter was installed in the discharge pipe from the effluent Agri Drain flow control structure into the sampling manhole. This flow meter was operational on July 25, 2019, reported instantaneous and total flows, and was read daily. The influent reservoir was installed to provide a constant source of wastewater to the bioreactor, and influent flow was manually controlled with an in-line gate valve.

Alkalinity: Influent and effluent bioreactor alkalinities measured during the 2019-2021 study are summarized in Figure S5.

Nitrogen: Nitrogen removal from wastewater is a three-step process that includes ammonification, nitrification, and denitrification. Ammonification (mineralization) occurs in the processing tank with bacteria converting organic nitrogen in wastewater to ammonia. Nitrification occurs in the soil absorption system and oxidizes ammonia dissolved in the wastewater to NO_3-N with a specialized group of bacteria that require an inorganic source of carbon such as carbonate or carbon dioxide. The last step involves a bacteria-mediated reduction of NO_3-N to nitrogen gas (denitrification), which requires an organic carbon food source for the bacteria and also can occur in anoxic micro-zones of the soil absorption system.

Total nitrogen (TN) includes all forms of nitrogen found in water and consists of organic and inorganic forms including nitrate (NO_3^-), nitrite (NO_2^-), ionized ammonia (NH_4), un-ionized ammonia (NH_3^+) and nitrogen gas (N_2). The relationships of these forms are as follows:

Table S4: Summary of operational gaps in bioreactor flow due to shut down.

Bioreactor Down	Bioreactor Operational	Total Days	Reason
April 30, 2019	May 13, 2019	14	Snow melt, heavy rain – infiltration issues
August 16, 2019	August 26, 2019	10	Breach influent face due to plugged woodchips
September 17, 2019	September 19, 2019	3	Concern re: WWTP influent characteristics
November 13, 2019	November 14, 2019	2	
November 30, 2019	November 30, 2019	1	
December 10, 2019	December 10, 2019	1	Meter down
December 31, 2019	January 1, 2020	2	Dead battery
February 6, 2020	February 12, 2020	6	Dead battery – charging
February 20, 2020	February 20, 2020	1	Wiring issue; unplugged from meter
February 29, 2020	March 3, 2020	4	Meter down
March 27, 2020	March 31, 2020	4	Flow meter issue; recharging battery
April 8, 2020	April 9, 2020	2	Charging battery
May 3, 2020	May 5, 2020	3	Charging battery
June 10, 2020	June 13, 2020	4	Meter down
July 17, 2020	August 2, 2020	16	Battery out; system flushed; pump down
August 26, 2020	August 26, 2020	1	Flushing bioreactor
September 6, 2020	September 6, 2020	1	Loose wire on flow meter
September 15, 2020	September 23, 2020	8	Flow meter issues
October 8, 2020	October 8, 2020	1	
October 27, 2020	November 23, 2020	27	Flow meter sent out for repair
November 30, 2020	November 30, 2020	1	Dead battery
December 8, 2020	December 10, 2020	3	Battery charging
December 31, 2020	December 31, 2020	1	Dead battery
February 10, 2021	February 10, 2021	1	Flushing bioreactor
April 2, 2021	April 5, 2021	3	Dead battery, Cord issue
May 3, 2021	May 9, 2021	6	Flow meter not recording, wire issue
June 1, 2021		126	Bioreactor shut down due to surface ponding

Total nitrogen (TN)=Organic nitrogen (ON)+Ammonia-nitrogen (NH_3-N)+Nitrate-nitrogen (NO_3-N)+Nitrate (NO_2)

TKN is comprised of NH_3-N and ON. A municipal WWTP with an effluent wastewater TKN >5 mg/L is not fully nitrifying. NH_3-N is the first inorganic nitrogen product of organic decomposition by bacteria and is present in lake water primarily as NH_4^+ and NH_4OH. Ammonia (NH_3) is un-ionized; ammonium (NH_4^+) is ionized. pH is the major environmental variable that determines the proportion of NH_3 or NH_4^+ in water.

Bioreactor influent wastewater NO_3-N concentrations are summarized in Figure S6.

The mean annual NH_3-N concentrations measured at the bioreactor sites are shown in Figure S7. There was no pattern either within years or among years. The increase in effluent wastewater NH_3-Nconcentrations between 2019 (0.85 mg/L) and 2020 (1.45 mg/L) may be explained in two ways. First, WWTP operation staff noted seasonality of high NO_3-N effluent concentrations, which suggest that a seasonal influx of NH_3-N might be occurring within the treatment flow path. This influx would have to enter the system prior to the trickling filter because the Bolton trickling filter successfully nitrifies year-round. We suspected that accumulated sludge within the Imhoff tank was releasing NH_3-N back into the waste stream under anaerobic conditions during this time of year. The Bolton Imhoff tank acts as a primary clarifier as well as the repository for secondary clarifier solids and tertiary filtration reject water. To evaluate the hypothesis, operations staff monitored NH_3-N concentration and alkalinity through the wastewater treatment train. In September 2020, sampling indicated influent bioreactor alkalinity levels <20 mg/L, indicating extraordinary nitrification through the WWTP trickling filter.

Second, in December 2020, sampling showed significant NH_3-N production within the bioreactor, where influent NH_3-Nof 0.95 mg/L increased to a concentration of 8.96 mg/L in the bioreactor effluent. There also was a significant increase in alkalinity, which could not be correlated to the stoichiometric relationship of alkalinity recovery from denitrification (i.e., each mg/L of NO_3-N removed yields 3.57 mg/L of alkalinity). This unexpected event may have indicated ammonification as described by others [5]. However, subsequent sampling in January 2021 did not indicate any ammonification and bioreactor NH_3-N was reduced. Continued attention was directed toward the issue of ammonification or Dissimilatory Reduction of Nitrate to Ammonium (DRNA) in early 2021 but there was no evidence of DRNA through the bioreactor.

Heterotrophic denitrification and DRNA are two microbial processes competing for NO_3-N and organic carbon resources. Various environmental conditions (i.e., oxidation state of the media, carbon/nitrogen ratio, pH, temperature, and microbial species) favor DRNA over denitrification [5]. Whether the cause of this unusual ammonification event was due to suspended solids accumulation or microbial decomposition was not determined.

Denitrification: Denitrification is biologically driven and depends upon several factors. Facultative heterotrophic bacteria reduce nitrate (NO_3) to nitrogen gas (N_2) in the presence of an organic carbon source

Figure S4: Google Earth™ view of the Bolton WWTP showing the location of the woodchip bioreactor, the upper infiltration sand beds and the movement of groundwater down-gradient toward Stewart Brook where it emerges as surface water in the channel between the above and below sampling stations along the tributary.

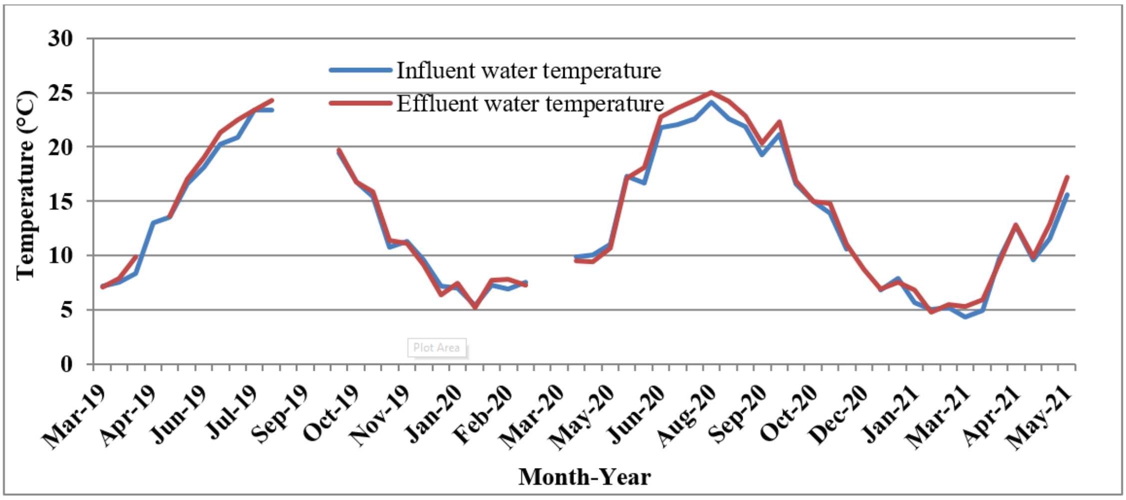

Figure S5: Woodchip bioreactor influent and effluent water temperatures, March 2019-May 2021.

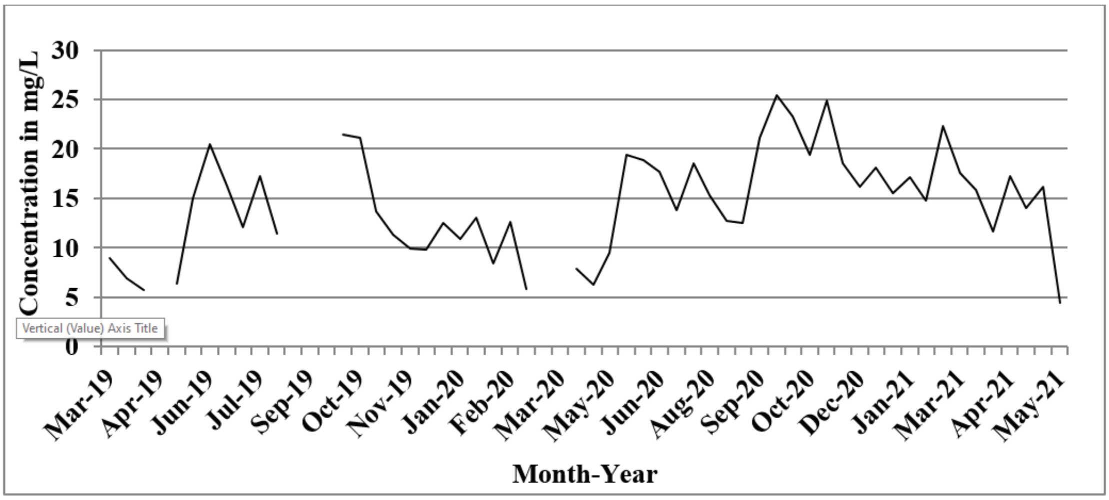

Figure S6: Bioreactor wastewater influent NO_3-N concentration, March 2019-May 2021.

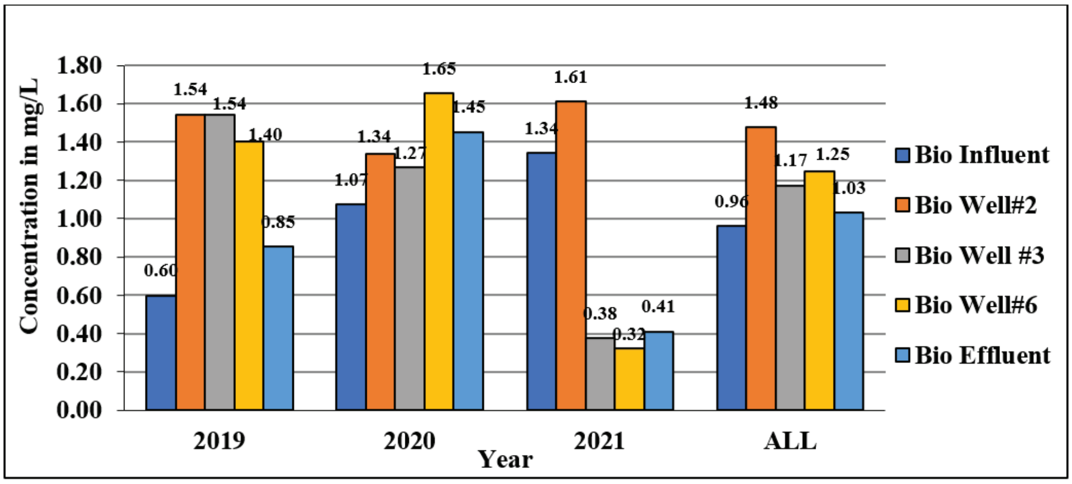

Figure S7: The mean annual NH3-N concentrations at the bioreactor sampling sites.

(acetate, methanol, and woodchips) and lack of oxygen. With anoxic conditions (i.e., DO concentrations <0.5 mg/L, ideally <0.2 mg/L), the heterotrophic bacteria break apart the NO_3 molecule to gain oxygen, with N_2O and then N_2 produced. N_2 escapes into the atmosphere as gas bubbles in the solution. The reaction also produces carbon dioxide gas, water, and alkalinity. The chemical reaction is as follows:

$$6(NO_3)+5(CH_3\text{-}OH)=3\,N_2+5\,CO_2+7(H_2O)+6(OH)$$

The optimum pH range for denitrification is 7.0-8.5 s.u. Denitrification is an alkalinity producing process. Denitrifying bacteria are facultative organisms and can use either DO or NO_3 as an oxygen source for metabolism and oxidation of organic matter. If both sources are present, bacteria will use DO first. Denitrification also requires a suitable carbon source. Conditions that affect denitrification efficiency include nitrate concentration, anoxic conditions, presence of suitable organic carbon matter, pH, temperature, and alkalinity. Temperature affects the growth rate of denitrifying organisms, with higher growth rates at higher temperatures. Denitrification occurs from 5-30°C, with increasing rates as temperature increases. The bacteria responsible for releasing carbon in the woodchips are even more sensitive to temperature variation.

Bioreactor treatment efficiency: The NO_3-N concentrations of WWTP effluent entering the bioreactor influent chamber and the corresponding effluent NO_3-N concentrations leaving the bioreactor and discharged to the effluent stream and upper sand beds are shown in Figure S8.

Operational Parameters affecting Denitrification

See Table S5.

Influent wastewater temperatures

This variable has a significant impact on the degree of denitrification, as documented in this study and by others [6]. Biological denitrification can occur from 5-30°C, with an increase in efficiency as water temperature increases. For the Bolton bioreactor, the summer seasonal high wastewater temperatures promoted increased removal efficiencies. During the cold Adirondack winters, efficiencies dropped to 20% or less, with wastewater temperatures decreasing to <6°C. The comparison between bioreactor influent wastewater temperature and NO_3-N removal efficiencies is summarized in Figure S9.

Low wastewater temperature during cold seasons significantly limits the bioreactor performance, probably related to the low metabolic activity of denitrifying microorganisms at low temperatures [6,7]. There is no practical method to increase these seasonally low wastewater temperatures. An operational modification to increase hydraulic residence time during cold weather does seem to be slightly more effective.

Hydraulic Retention Time (HRT)

HRT within the bioreactor has a significant impact on the extent of denitrification. Retention times of eight hours or more, especially in cold weather, improves efficiency [8]. During the eighth quarter of this study, the flows treated within the bioreactor were reduced from flows of the previous quarter to verify the extent of denitrification as the hydraulic retention time increased. The results varied (Table S6).

Other environmental factors contributing to the extent of denitrification include the availability of a suitable carbon source, coupled influent NO_3-N and DO concentrations, which all impact the process synergistically. From a theoretical perspective, longer retention times would improve efficiency. Excessive retention times can potentially exhaust the nitrate supply, driving methyl mercury production as a byproduct of further anaerobic biological processes.

Internal hydraulics

Internal hydraulics of the woodchip bioreactor also contribute to denitrification efficiency. As documented in later stages of this study, the woodchips in certain regions became plugged with biological and organic solids, affecting the internal hydraulics. Preferential flow paths developed, leading to short-circuiting of the wastewater flow, reduced retention times, and reduced removal efficiency. The development of preferential flow paths with tracer tests was researched and it was determined that short-circuiting can be indicated when tracer retention time was less than the theoretical HRT by >10% [9].

Bacterial assemblage

The bacterial assemblage in the woodchip bioreactor also impacts NO_3-N reduction. The bacterial species involved in denitrification favor anaerobic conditions, preferably with a DO concentration <0.2 mg/L. Many of the bacterial species involved in the cycling of nitrogen are facultative and can exist throughout a range of DO concentrations. In the front end of the bioreactor, the wastewater DO concentrations were well above denitrification thresholds, thus promoting aerobic biological processes and contributing to the eventual plugging of the initial 2-2.5 m of the woodchips. Another aspect of the bioreactor biological assemblage involves cellulolytic bacteria activity, those

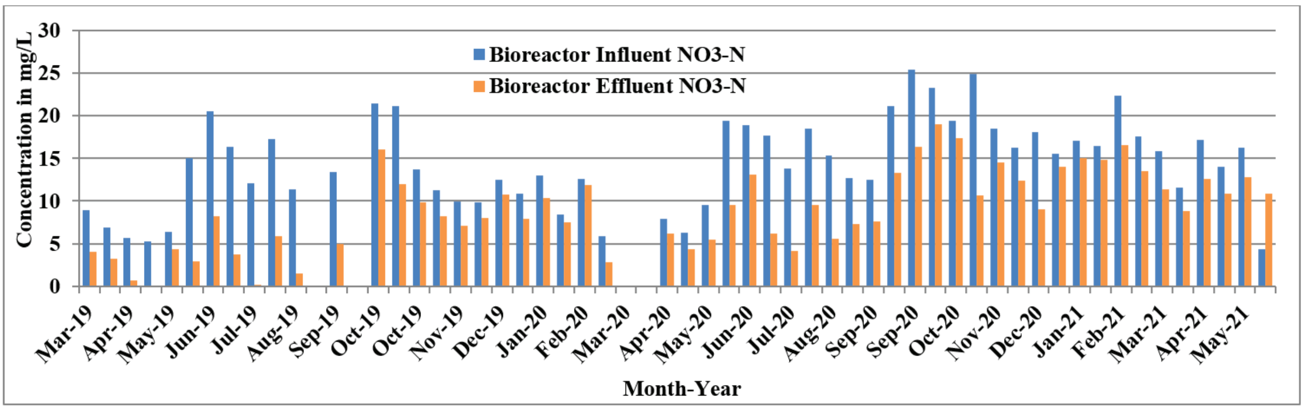

Figure S8: The mean annual NH3-N concentrations at the bioreactor sampling sites.

Table S5: Important factors affecting the operational efficiency of the Bolton WWTP woodchip bioreactor during the 2019-2021 study period.

	Bioreactor Influent [mg/L]	Bioreactor Effluent [mg/L]	Removal Efficiency [%]	Effluent Water Temperature [°C]	Flow (m^3d)	Estimated Residence Time [hrs.]	N Removal [g/day]	N Removal [g/ m^3/day]
8/6/19	11.4	1.5	87	24.3	339.9	5.8	3356.6	9.9
9/3/19	13.4	4.9	63.2	23.1	452.3	4.3	3810.2	8.5
10/1/019	21.4	16	25.2	19.5	368.91	5.3	1995.8	5.4
10/15/19	21.1	12	43.1	16.8	351.5	5.6	3220.5	9.1
10/29/19	13.7	9.9	27.8	15.9	331.8	5.9	1270.1	3.8
11/11/19	11.3	8.2	27.1	11.4	348.3	5.6	1043.3	3.1
11/26/19	9.9	7.1	28.1	11.1	322.3	8.6	907.2	2.8
12/10/19	9.9	8	19.4	9.1	340.22	6.7	635	1.9
12/23/19	12.5	10.8	13.6	6.4	316.2	8.3	544.3	1.7
1/7/20	10.9	7.1	27.1	7.4	329.1	8.2	952.5	3
1/21/20	13.1	8	20.8	5.2	289.1	9.3	771.1	2.7
2/4/20	8.4	7.5	10.8	7.7	278.2	10	272.2	0.9
2/19/20	12.6	11.9	5.6	7.8	331.4	8.4	226.8	0.7
3/3/20	5.9	2.8	51.6	7.3	337.63	8.2	1043.3	3
4/14/20	7.9	6.2	21.3	9.5	335.8	8.7	544.3	1.7
4/28/20	6.3	4.4	30.9	9.4	337.6	8.2	635	1.9
5/12/20	9.5	5.5	42.3	10.7	305.6	9.6	1224.7	4
5/26/20	19.4	9.5	51	17.1	235.6	11.1	2313.3	9.9
6/9/20	18.9	13.1	30.7	18.1	359.64	6.8	2086.5	5.8
6/23/20	17.7	6.2	64.9	22.8	334.8	6.8	3855.5	11.5
7/7/20	13.8	4.2	69.7	23.6	339	7.2	3265.8	9.6
8/4/20	15.3	5.6	63.5	25	309.3	6.8	2993.7	9.7
8/18/20	12.7	7.25	42.9	24.2	378.3	5.2	2041.2	5.5
9/1/20	12.5	7.56	39.5	22.9	244.8	8.7	1224.7	4.9
9/15/20	21.1	13.3	37	20.4	311.85	6.8	2449.4	7.8
9/29/20	25.4	16.3	35.8	22.3	298.4	6.6	2721.5	9.1
10/13/20	23.3	19	18.4	16.9	320	5.1	1360.8	4.3
10/27/20	19.4	17.4	10.3	15	321.56	7.6	635	2
11/10/20	24.9	10.7	57	14.8	208.27	14.1	2948.3	14.2
11/24/20	18.5	14.5	21.6	11	203.48	8.8	816.5	4
12/8/20	16.2	12.4	23.5	8.8	237.39	8.2	907.2	3.8
12/22/20	18.1	9.1	49.9	6.9	229.5	10.7	2086.5	9
1/5/21	15.5	14	9.68	7.5	205.6	14.3	317.5	1.5
1/19/21	17.1	15	12.3	6.8	262	8.7	544.3	2.1
2/2/21	16.51	14.8	10.3	4.8	146.3	13.4	226.8	1.7
2/16/21	22.3	16.6	25.6	5.5	160.5	13.2	907.2	5.7
3/2/21	17.6	13.5	23.3	5.3	220.4	5.9	907.2	4.1
3/16/21	15.8	11.4	27.8	5.9	148.1	13.2	635	4.411
3/31/21	11.6	8.9	23.7	9.3	114	20	317.5	2.7
4/13/21	17.2	12.6	26.7	12.8	232.3	7.7	1088.6	4.6

4/27/21	14	10.9	22.1	9.9	248	7.6	771.1	3.1
5/11/21	16.2	12.8	21	12.9	293	8.3	997.9	3.4

[1]No flow data on 10/1/19 so numbers from 10/8/19 were used.
[2]No flow data on 12/10/19 so numbers from 12/12/19 were used.
[3]Inaccurate flow meter readings in bioreactor from 3/3/20. Value in table is based on percentage of total WWTP flow going through Bioreactor (63.8%) from 3/5/20 and this was applied to 3/3/20 total WWTP to estimate bioreactor flow.
[4]No flow data on 6/9/2020, flow adjusted from 6/8/2020 was used.
[5]No flow data on 9/15/20 so numbers from 9/9/20 were used.
[6]No flow data on 10/27/20; numbers from 10/26/20 were used.
[7]No flow data on 11/10/20; manual measurement was conducted.
[8]No flow data on 11/24/20; numbers from 11/25/20 were used.
[9]No flow data on 12/8/20; numbers from 12/11/20 were used.
[10]On 2/2/21, we believe that the laboratory switched (or mis-read) labels on bottles because influent nitrate as 14.8 mg/L and effluent was 16.5 mg/L and alkalinity stoichiometric calculations showed that nitrification did occur in the bioreactor.
[11]On 2/16/21, the water level seems lower than normal; we suspect volumetric removal rate to be overestimated.

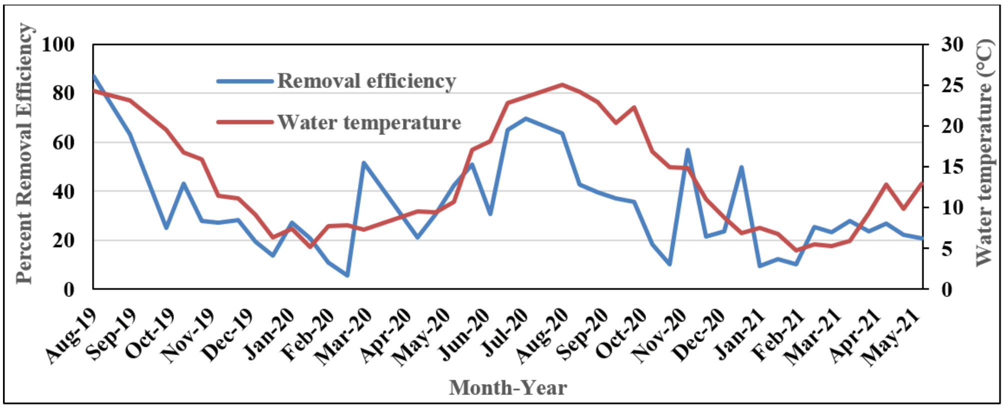

Figure S9: Relationship between bioreactor wastewater temperature and NO_3-N removal efficiency.

Table S6: 2021 experiment to evaluate percent denitrification based upon HRT.

Date	HRT (h)	Wastewater temperature (°C)	NO_3-N reduction (%)
November 10, 2020	14.1	14.8	57
March 2, 2021	5.9	530.00%	2330.00%
March 31 2021	20	930.00%	2370.00%

temperature-sensitive species that convert woodchip carbon into a soluble form for use by the denitrifying bacteria. The relationship between cellulolytic bacteria and the denitrifying bacteria, especially during cold wastewater temperatures, is thought to affect denitrification efficiency by impacting the carbon/nitrogen ratio [10].

Carbon/Nitrogen ratio

This ratio is another operational matrix variable that impacts removal efficiency. Soluble carbon, supplied by typical wastewater constituents or cellulolytic bacteria activity, is critical for proper denitrification. A ratio of 4.67/1 (C/N) has been reported as optimal for biological denitrification using glucose, sodium acetate and/or methanol [11]. However, more recent results identified C/N ratios of 2/3 for an Up-Flow Sludge Blanket (USB) reactor for domestic wastewater [12]. Even at these lower C/N ratios, it is obvious that during low temperatures and reduced metabolism of the cellulolytic bacteria, enhanced denitrification would be challenging. The 5-day biological oxygen demand (BOD_5) of the Bolton plant tertiary effluent is rarely >5 mg/L; chemical oxygen demand (COD) of the effluent is not measured directly. Bioreactor influent has a Dissolved Organic Carbon (DOC) concentration typically <1.0 mg/L.

The Bolton woodchip bioreactor was designed for tertiary treatment of municipal wastewater, which at the bioreactor influent was devoid of residual carbon sources. The BOD_5 of the bioreactor influent typically was <5 mg/L, coupled with low suspended solids. There were periods, however, when secondary clarifier solids were carried over to the influent to the bioreactor, and during these times the bioreactor was taken offline to protect its integrity.

Woodchip Bioreactor Maintenance

The original construction of the bioreactor included filter fabric around the influent and discharge collection headers. These headers were closed end 15.24 cm PVC pipe with 1.9 cm holes drilled 15.24 cm OC around the entire pipe. The pipes were originally wrapped in filter fabric as a protective measure. Within several months of operation, the discharge header failed to pass treated effluent out of the bioreactor and plugging of the filter fabric was suspected. The bioreactor was taken offline, and the effluent end of the bioreactor was excavated in November 2018. The filter fabric was verified to be plugged with woodchip fines. The filter fabric was removed, and accumulated effluent again flowed freely out of the bioreactor. Communications with other researchers indicated that similar effluent discharge header plugging issues were noted when filter fabric was used on agricultural applications [13].

In late August 2019, the Bolton WWTP woodchip bioreactor was taken offline due to surface accumulation of influent along the leading edge of the bioreactor. Plugging of the front end of the woodchip matrix was suspected. On August 23, 2019, Town personnel and a private contractor carefully excavated the front end of the bioreactor. The first 2 m of woodchips were removed, and replacement woodchips were installed. The removed woodchips had heavy organic accumulations and the integrity of the woodchips had broken down. At this same time the filter fabric around the influent pipe was removed. With the addition of new wood chips, the bioreactor heavy waterproof liner was reinstalled, and the bioreactor resumed its original condition. It was interesting to note that the degradation of the woodchips was only noted in the first 2 m or so of the woodchip matrix.

During the 28 months of bioreactor operation, routine flushing of the bioreactor took place. This maintenance program was designed to flush out any accumulated organic buildup within the woodchip matrix on a periodic basis. The operations staff flooded the bioreactor to its maximum capacity, allowed the water to saturate the bioreactor bed, and then the effluent stop logs all were removed to allow water to rush out of the bioreactor. This maintenance practice was completed when the operations staff noted that the influent flow was decreasing and/or that water began pooling on the bioreactor surface near the influent end. Thereafter, the maintenance flushing was done on a monthly basis, depending upon operations staff availability. The flushing program was successful in restoring operational efficiency, yet over time became less successful.

On June 1, 2021, the bioreactor experienced severe plugging issues and was shut down to prevent breaching of the structure. On June 23, 2021, the bioreactor bed was excavated the entire length to reveal the condition of the wood chips. The results of that investigation are reported below.

Woodchip Bioreactor Operational Challenges

Media clogging

The most challenging issue in this woodchip bioreactor demonstration was the periodic plugging of the woodchip matrix, which was due to several situations including buildup of fines in the media, woodchip decomposition, and possible accumulated suspended solids or microbial decomposition. The discussion of clogging events is presented in detail below.

Iron Contamination

During the latter months of 2019, all Bioreactor Monitoring Wells (MW) were exhibiting discolored water samples. Oxidation of the monitoring wells was suspected because the influent and effluent samples were not exhibiting discoloration.

In late December 2019, these monitoring well samples were analyzed for iron; levels as high as 339 mg/L were reported. The presence of iron in these samples prevented accurate characterization of the water relative to NO_3-N, alkalinity and DO, which resulted in short-term data disruption. The stainless-steel monitoring wells points were replaced by operations staff with custom 5.1 cm PVC wells in the same locations. The deep wells were replaced but not the shallow wells, which remained out of the sampling program due to the lower water levels.

Ammonia concentrations and release

Maintenance: Challenges that affected the operation of the bioreactor included the influent pump, flow meter, and bioreactor flushing. There were problems experienced with the pump station to the upper beds that prevented use of the upper beds and the bioreactor. Problems with the flow meter included dead batteries and loose wires, which sometimes allowed flow through the bioreactor but not the opportunity to collect data. The bioreactor flushing became a routine maintenance practice for operations staff, which took the bioreactor offline and temporarily reduced efficiency by requiring the reestablishment of microbes and bacteria.

Woodchip Bioreactor Shutdown Due to Plugging

Woodchip bioreactors have demonstrated their ability to use porous wood material to create an environment conducive for the process of denitrification to occur. However, there is concern demonstrated in various research papers for the potential of clogging of the material that will reduce the efficiency of denitrification and possibly lead to hydraulic failure. The following discusses bioreactor clogging during the 2019-2021 study.

Progression of events

The woodchip bioreactor pilot project began accepting effluent from the Bolton WWTP in October 2018. There was success from the beginning of the monitoring study (March 2019) with a 64% reduction in NO_3-N in the first quarter of the study. However, during the second quarter, the bioreactor was taken offline on August 16, 2019, due to breaching along the bioreactor's influent face. On August 23, 2019, the cause of this breaching was found to be waterlogged and plugged wood chips in the initial 1.5 m of the 30.5 m long bioreactor bed.

The area was filled with biological solids, likely from enhanced biological activity during the warm season, when wastewater temperatures were approaching 25°C. The compromised wood chips were removed and replaced with new wood chips from the original installation stockpile. The bioreactor was put back online August 26, 2019, and within a week, removal efficiency was at 63%.

During the warmer months of 2019 and the first year of the study (March 2019 through February 2020), when wastewater effluent temperatures at the Bolton WWTP reached 25°C, the level of water in the bioreactor had to be reduced to limit both the retention time and the complete consumption of the influent nitrate by the denitrifiers. To facilitate shorter retention time, several of the effluent weirs were removed, reducing the level of wastewater in the bioreactor to below the mid-level monitoring well depths. This situation created a larger zone that was not saturated, increasing the aerobic zone. Weir removal possibly created more hydraulic forces in the bioreactor that could have caused compacting or moving woodchips. This lower water level also occurred in the fourth quarter of the study, i.e., the shallow sampling wells in the bioreactor remained out of the sampling program due to seasonally lower water levels in the unit, resulting in a greater unsaturated zone in the top half of the bioreactor.

When low flows through the bioreactor were evident, possibly indicating plugging of the bioreactor material, the WWTP operation staff flushed the bioreactor (i.e., pulled all the effluent stop logs (weirs) to promote a rapid discharge from the bioreactor) and then a return operation with a slightly lower flow going through the bioreactor. This operational practice appears to have restored the bioreactor to its earlier operational efficiency. It then was decided that periodic flushing of the bioreactor would be practiced throughout the operating season. There is the potential, however, that this rapid discharge could result in dislodging woodchips in the bioreactor, possibly compacting them, and reducing porosity.

During the 10th quarter of the study (April 2021 through June 2021), the NO_3-N removal efficiency of the woodchip bioreactor consistently decreased from about 27% to 21% even as the water temperature increased and the flow through the bioreactor was reduced. On May 2021, there was an increase in NO_3-N concentration through the bioreactor, this event coincided with the malfunctioning of the Bolton WWTP trickling filter. NH_3-N was not being adequately nitrified through the trickling filter. NH_3-N remained in the wastewater influent to the bioreactor, where nitrification occurred, resulting in an increase in NO_3-N concentration in the bioreactor effluent. Additionally, wastewater solids were being carried over into the bioreactor, which ultimately plugged and was taken offline on June 1, 2021. A project meeting occurred on June 17, 2021, and it was decided to perform an exploratory investigation of the bioreactor.

Exploratory investigation

The exploratory investigation of the bioreactor occurred on June 23, 2021. Local contractor, Barry Kincaid, who provided the original bioreactor wood chip material and aided in construction, provided a rubber tract, small excavator to perform the forensic examination. After discussion, it was determined to excavate a trench down the center of the bioreactor to the full depth of the material (1.2 m) to see the condition of the woodchips. There had been no flow through the bioreactor for over three weeks and the bioreactor was dry. Excavation started about 1.5 m into the bioreactor to prevent the sidewalls from caving in due to the sandy sub-base material supporting the liner.

The following are notes from the exploratory investigation:

At the start of the excavation (Sta 0+1.5), the first 0.6 m of woodchip depth consisted of a very dense material with a low percentage of large wood chips and a high percentage of fine material. The material was a dark brown/black color, possibly indicating degradation. The bottom 15 cm was clean woodchips with a brighter tan/orange color; there was a higher percentage of whole wood chips and 10 cm of standing water in the bottom of the trench.

There was a change in the woodchips at Sta 0+4.6. There was more color in the woodchips, and less fines and dirt. The woodchips appeared to be smaller in size than original but were more intact. There was about 30.5 cm of clean woodchips at the bottom of the trench.

At Sta 0+15.24, the depth of the good woodchips started 30.5-38 cm from the surface, which was the greatest depth of good condition woodchips in the bioreactor.

There was a clear gradient of the boundary between the apparently degraded woodchips in the upper layer and the cleaner, intact woodchips in the lower layer of the trench; this started at a depth of 107 cm at Sta 0+1.5 and rose to a depth of 30.5 cm at Sta 0+15.24 then decreased to a depth to 91 cm at Sta 0+25.9.

Laboratory testing

With respect to the bioreactor plugging, the project research team had several meetings regarding the status of the project and the direction to take following the investigation and observations of bioreactor material. The project team made numerous contacts to various analytical laboratories and environmental service facilities to determine what type of testing could be done to determine the nature of the bioreactor plugging phenomenon and whether it was biological, organic from woodchip breakdown or a combination. Proposals included the use of mechanical sieve testing for determination and comparison of dirt-like material to woodchip material, which would speciate by size of materials only and not determine possible origin; SEM-EDS (scanning electronic microscope-energy dispersive x-ray spectroscopy) analysis to look at the elemental profile of the sample material and understand if a particular particle is carbon-based (assumed to be woodchips) or metal-based (assumed to be soils); and Raman Spectroscopy to identify the particles as either cellulose or a breakdown product of cellulose to determine if the woodchips were breaking down. Although these analyses would be very beneficial at evaluating the type of particles, it was determined to be very expensive, limited to specific particles and would not cover a wider range of samples. It was decided to proceed with a less complex analysis to focus on total and volatile solids, which would distinguish sediments and wastewater sludges, and sieve sizes.

Three separate locations along the 30.5 m length of the bioreactor were selected for the collection of woodchip samples for laboratory analysis, including Sta 0+7.6 (Sample Site A), Sta 0+15.24 (Sample Site B), and Sta 0+24.4 (Sample Site C). At each station, samples were collected at four different depths including (1) just below the filter fabric, (2) at a 0.6-m depth, (3) at 0.9-m depth and, (4) within the water-logged material at the bottom. This sampling strategy resulted in 12 samples collected. Samples were collected on September 2, 2021, by hand excavating the bioreactor, placing the material in gallon Ziploc bags, and storing the samples on ice. The woodchips were very compacted, and the samples were collected using a hand rake and some hand digging to extract the samples.

The collected woodchip samples were delivered the same day to the Darrin Fresh Water Institute in Bolton Landing, NY. The results of the Volatile Solids analysis are presented below (Table S7).

Table S7: Summary of percent solids and percent volatile solids for woodchip samples collected from the Bolton WWTP woodchip bioreactor (see text for explanation of Sample Location).

Sample Location	Percent Solids	Percent Volatile
A-1	0.712	0.613
A-2	0.254	0.007
A-3	0.28	0.006
A-4	0.27	0.007
B-1	0.319	0.026
B-2	0.294	0.004
B-3	0.245	0.004
B-4	0.264	0.011
C-1	0.506	0.542
C-2	0.236	0.003
C-3	0.256	0.003
C-4	0.257	0.012

The highest percent of solids at each station along the length of the bioreactor occurred just below the filter fabric at the top, indicating that these were the densest samples with the most material. The highest percent of volatile solids at each sample location were just below the filter fabric at the top also, indicating these had the most sediment/soil material. These results indicated that there was higher amount of soil/mineral material in the upper sample, possibly indicating migration into the bioreactor. It is unlikely there was any wastewater sludge material in this area as the water depth in the bioreactor never reached above 102 cm or approached the height of samples collected at depth (1).

The percent of solids for the 0.6 m depth, 0.9 m depth and the waterlogged bottom depth all were below 30%, indicating less dense samples consisting more of woodchips. The percent of volatile solids for depths at 0.6 m, 0.9 m, and the waterlogged depth were around 1.0% or below with the highest percentage of volatile solids of the three lowest samples being in the water-logged samples (4). This indicates there were very fewer sediments or sludge materials at these depths and most the material was wood chips but that there could be settling of finer soil material at the lowest level of the bioreactor. It should be noted that the percent of volatile solids in the upper sample (1) follows the clean woodchip gradient line with the higher percentages in Locations A and C with Location B having a lower percentage.

The results of the Manual Sieve analysis are presented below (Table S8).

It is important to note here that all samples were collected within the boundaries of the bioreactor liner/fabric and that the woodchips installed when the bioreactor was constructed ranged in size from 1.3-5.1 cm; therefore, all samples should have been classified as gravel under the sieve analysis. It is understood that a small amount of fines may be present but there should only be a very small percentage of fines present unless there was degradation of the woodchip material or deposition of material transported from the wastewater influent.

From the sieve analysis, the upper samples taken just below the filter fabric (1) exhibited the highest percentage of particles <2 mm (coarse sand or finer) with Sample Location A-1 showing the greatest percentage of fines at 51% <2 mm and Sample Location C-1 showing a percentage of fines <2 mm at 23.7%. It should be noted that Sample Locations A and C were the locations that exhibited the greatest depth of degraded woodchip material from the exploratory excavation discussed previously. The Sample Location B-1 percentage of fines <2 mm was 9.4%. It was evident from the sieve analysis that samples collected just below the filter fabric had the highest percent of fine particles at each Sample Location indicating that there was apparent breakdown of woodchips or migration of soil material into the bioreactor through the filter fabric. The sieve analysis also demonstrates the deeper the collected sample (from just below the filter fabric (1) to 0.6 m depth (2) to 0.9 m depth (3) to water-logged area (4)), there was a corresponding decrease in finer particles at each Sample Location A, B and C. This indicates the finer particles were originating either from the degradation of the upper woodchips or migration of soil material into the bioreactor.

There is consistency of results between the Volatile Solids analysis and the Sieve Analysis with regard to the higher percentage of apparent soil material being located in the upper samples taken just below the filter fabric (1) and decrease with depth of the sample taken with a slight percentage increase for the lowest sample (water-logged samples (4)), which still remains significantly lower than the upper sample (1). Since it is assumed that the woodchip/organic material would turn to ash during the heating, the volatile material remaining would be soil/mineral material.

It is of interest to note here is that in all three sampling locations along the length of the bioreactor, the uppermost woodchip matrix (i.e., the woodchips directly under the permeable filter fabric) exhibited the most extensive breakdown of the material. This correlates exactly with what was visually observed. The smell also indicated that the woodchips were decomposing, similar to what one would expect to see in a compost pile. And the influent portion of the woodchip matrix directly below the filter fabric showed the greatest degradation of the woodchips. Conversely, the bottom layer of woodchips at the influent end of the matrix (i.e., sample A-4) showed the least degradation,

Table S8: Summary of manual sieve particle analysis on woodchip samples collected from the Bolton WWTP bioreactor (see text for description of Sample Locations).

Sample Location	Particle Size				
	Gravel: >2mm	Coarse Sand: <2mm, >0.5mm	Medium to Fine Sand: <0.5mm, >0.25mm	Very fine Sand: <0.25mm, >0.125mm	Silt/Clay: <0.125mm, >0.063mm
A-1	45.50%	37.00%	13.80%	0.20%	0.00%
A-2	96.60%	2.30%	0.00%	0.00%	0.00%
A-3	97.60%	0.60%	0.00%	0.00%	0.00%
A-4	100.30%	0.40%	0.00%	0.00%	0.00%
B-1	82.90%	8.80%	0.50%	0.10%	0.00%
B-2	95.10%	4.10%	0.00%	0.00%	0.00%
B-3	94.90%	1.10%	0.00%	0.00%	0.00%
B-4	86.90%	0.60%	0.00%	0.00%	0.00%
C-1	70.20%	22.30%	1.20%	0.20%	0.00%
C-2	89.50%	3.70%	0.10%	0.00%	0.00%
C-3	96.20%	1.30%	0.00%	0.00%	0.00%
C-4	91.80%	0.70%	0.00%	0.00%	0.00%

verifying the fact that under anaerobic conditions the woodchips would retain their structure and could offer extended denitrification capacity.

This same degradation of the woodchips near the surface (i.e., samples B-1 and C-1) offers the premise that the upper matrix of the woodchip bioreactor tends to be impacted by surface precipitation and aerobic conditions, leading to the natural degradation of the wood. An alternative cover for the bioreactor, one that includes a more impermeable membrane and/or a deeper soil cover would alleviate this situation. Other bioreactor design modifications are discussed in the following section.

Potential causes of plugging

As detailed previously, there was evidence of clogging of the woodchip bioreactor through the pilot study. The WWTP operation staff was very aware of this and monitored the bioreactor daily to assess potential problems, being proactive to address this issue as demonstrated by the routine flushing of the bioreactor. The research team also was cognizant of this potential and contacted with Dr. Laura Christianson during the study to discuss observations and findings.

There is evidence in the literature of clogging potential of denitrifying bioreactors. Conventional knowledge indicates frequent woodchip replacement due to media clogging [9] and there is a need for better understanding of the potential for clogging, especially for wastewater application. It was noticed that influent wastewater took progressively longer to move into the woodchips, likely due to a combination of (1) woodchip settling, (2) clogging due to removed wastewater solids and/or accumulated bacterial growth and (3) pulsed flow system pushing the chips away from the inlet. There are references in the literature regarding the decomposition of woodchips as impacting the hydraulics of a bioreactor [7,14].

In review of the exploratory excavation and the sample analysis, there does appear to the degradation of woodchips in the upper layer of the bioreactor. The bioreactor was constructed with filter fabric over the woodchips as referenced in other research papers [15]. This material will allow the exchange of air and surface water infiltration with the surface as there was only a 15 cm cover of soil. During excavation, biological activity was observed in the bioreactor in the form of earthworms and root penetration. Others have recommended using a liner due to site conditions [16,17].

Bioreactor water level fluctuation could result in potential woodchip degradation by creating unsaturated conditions combined with the potential for oxygen exchange. One study [9] found that woodchips in the unsaturated top 15 cm of the bioreactor potentially were degrading more than the bottom woodchips. Another study [18] reported aerobic woodchips near the top of the denitrification wall had shortened life compared to deeper, more consistently anaerobic chips.

There was concern of wastewater solids decomposition and accumulation causing clogging, which was one of the reasons for routine flushing by WWTP operation staff.

2016-2017 and 2019-2021 Data Collected from Stewart Brook

Flow

Flow data collected during the 2019-2021 study at the above and below sampling stations on Stewart Brook and summarized in Figure S10 show the influence of continuous groundwater discharge into the tributary channel and elevated flow at the below station.

Temperature and DO percent saturation data collected during the 2019-2021 study also are presented here as additional evidence of the influence of groundwater entering the Stewart Brook channel between the above and below sampling stations.

Temperature

The effect of groundwater temperature on Stewart Brook was evident when comparing data collected during the 2019-2021 study at above and below stations (Figure S11).

The continuous inflow of groundwater between the two sampling stations cooled the tributary temperatures measured at the below station in the summer months and warmed the temperatures measured there during the winter months (Figure S11). The ambient temperature of groundwater emerging into the channel was about 13°C throughout the year.

Dissolved oxygen (DO) percent saturation

Bacteria can remove substantial amounts of DO and add carbon dioxide as wastewater effluent passes through the sand beds for recharge and moves toward Stewart Brook. We would expect that

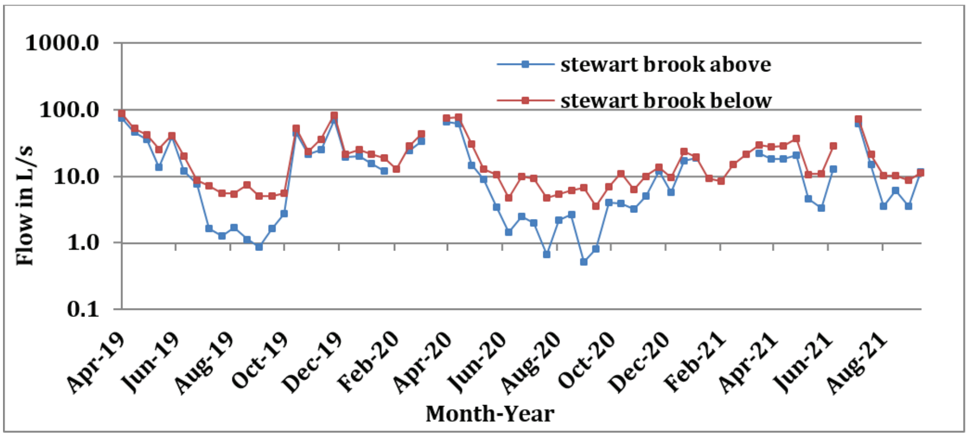

Figure S10: Stewart Brook flow measured at above and below stations, 2019-2021.

groundwater entering the tributary channel would exhibit some level of oxygen depletion compared with ambient DO in tributary channel flow. The average oxygen percent saturation values for samples collected from the Stewart Brook sampling sites during the two studies are shown in Figure S12.

Some oxygen depletion clearly is evident when comparing the mean values of percent saturation in tributary flow above and below the zone of groundwater influence for both studies being compared. Figure S13 presents the seasonal progression of percent saturation of dissolved oxygen above and below the zone of ground water influence for the 2019-2021 study.

The difference between the individual above and below values is apparent and due to the reduced DO percent saturation of groundwater emerging and mixing with the flow already in the channel. There

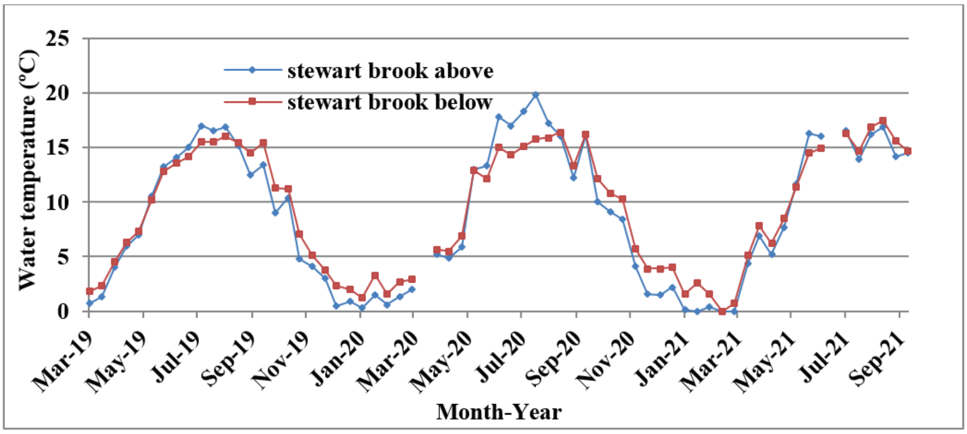

Figure S11: Seasonal patterns of water temperature measured in Stewart Brook at the above and below sampling stations, 2019-2021.

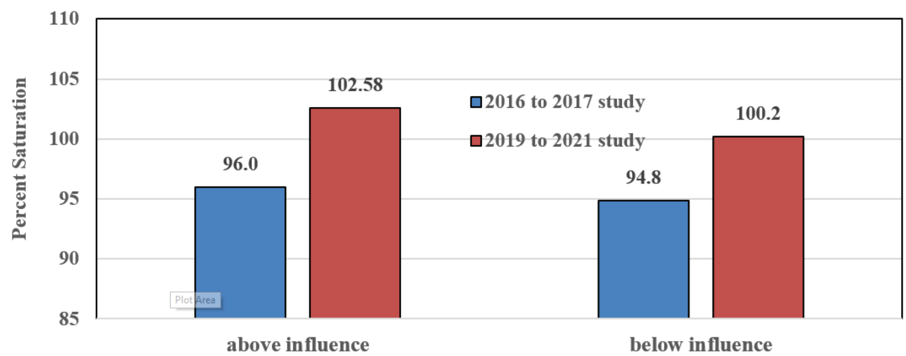

Figure S12: Mean DO percent saturation measured at the above and below sampling stations on Stewart brook during the 2016-2017 and 2019-2021 studies.

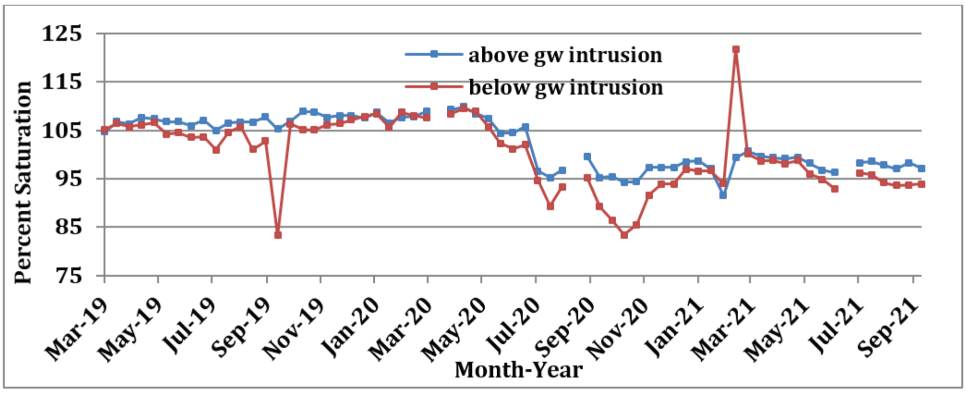

Figure S13: Seasonal pattern of DO percent saturation measured at the above and below sampling stations on Stewart Brook, 2019-2021.

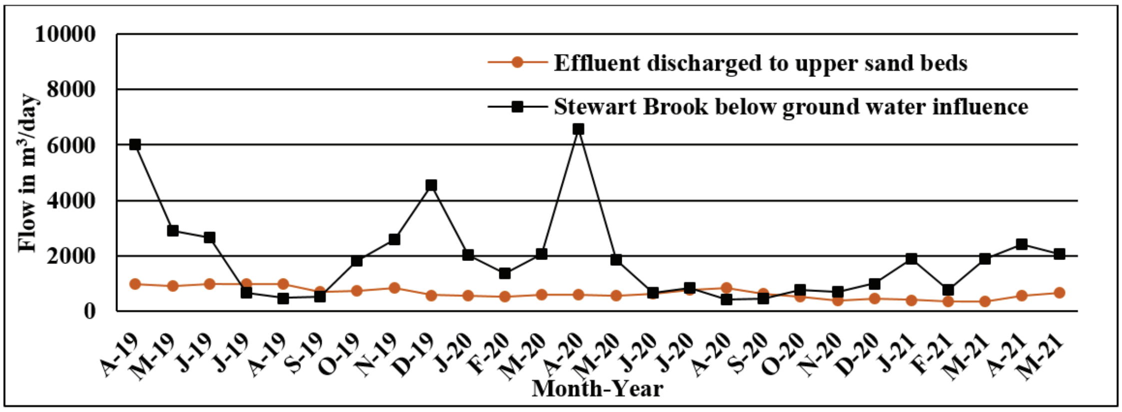

Figure S14: Mean monthly effluent discharge (m_3/day) to the upper sand beds and mean monthly flow (m_3/day) at the below station on Stewart Brook, April 2019-May 2021.

were occasions during the current investigation when the difference between the percent saturation above and below the area of ground water intrusion was as much as 20-30%.

Flow

The mean monthly discharge (m^3/day) of effluent to the upper sand beds after processing through the Bolton facility and the mean monthly flow (m^3/day) at the below station on Stewart Brook during the 2019-2021 study are summarized in Figure S14.

From April 2019 through May 2021, the mean monthly volume of plant effluent ranged from 358-996 m^3/day and the mean Stewart Brook flow below the groundwater influence ranged from 440-6564 m^3/day (Figure S14). During July, August, and September 2019, the mean volume of plant effluent discharged to the upper sand beds (991, 972, 709 m^3/day, respectively) exceeded the mean Stewart Brook flow (680, 478, 540 m^3/day, respectively), with a similar occurrence in August and September 2020. Explanations for the discrepancy between discharge to the upper sand beds and tributary flow include effluent evaporation from the sand bed surface and uptake by vegetation growing in the sand beds, both of which would reduce the effluent volume entering Stewart Brook through groundwater during the warm months.

References

1. Aulenbach DB, Fillip AJ (1983) The Bolton Landing rapid infiltration wastewater treatment plant. In: Carol Desormeau Collins (ed) The Lake George Ecosystem. Volume III, The Lake George Association, Lake George, New York, 101-109.
2. Keppler D (2008) 2007 Stream Assessment Report. The chemical, physical, and biological data collected in 47 stream sample sites throughout the Lake George watershed. Prepared for The FUND For Lake George, Lake George, New York, 2008.
3. Keppler D (2009) 2008 Stream Assessment Report. The Chemical, Physical, and Biological Data Collected from 52 Stream Sampling Sites throughout the Lake George Watershed. Prepared for The FUND For Lake George, Lake George, New York.
4. Rantz SE, et al. (1982) Measurement and computation of streamflow: Volume 1. Measurement of stage and discharge. U.S. Geological Survey Water Supply Paper 2175, United States Government Printing Office, Washington, 1982: 79-183.
5. Lepine C, Christianson LE, Sharrer ST, Summerfelt S (2016) Optimizing hydraulic retention times in denitrifying bioreactors treating recirculating aquaculture system wastewater. J Environ Qual 45: 813-821.
6. Christianson LE, Bhandari A, Helmers MJ (2012) A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. Appl Eng Agric 28: 861-874.
7. David, M.B., Addy, K., Gold AJ, Christianson LE, Schipper LA, et al. (2016) Denitrifying bioreactors for nitrate removal: A meta-analysis. J Environ Qual 45: 873-881.
8. Din Dar MU, Shah AI, Ali SR, Bhat SA (2021) Woodchip Bioreactors for Nitrate Removal in Agricultural Land Drainage. In: Rouf Ahmed Bhat, Khalid Rehman Hakeem, Humaira Qadri, Moonisa Aslam Dervash (eds) Agricultural Wastes: Threats and Technologies for Sustainable Management. 1st edition, Apple Academic Press, New York.
9. Christianson LE, Lepine C, Sharrer S, Summerfelt S (2016) Denitrifying bioreactor clogging potential during wastewater treatment. Water Res 105: 147-156.
10. Waugh S (2019) Personal communication, SUNY Stony Brook.
11. Sobieszuk P, Szewczyk KW (2006) Estimation of (C/N) ratio for microbial denitrification. Environ Technol 27: 103-108.
12. How SW, Ting CX, Yap JY, Kwang CY, Tan CK, et al. (2021) Effect of carbon-to-nitrogen ratio on high-rate nitrate removal in an upflow sludge blanket reactor for polluted raw water pre-treatment application. Sustain Environ Res 31: 16.
13. Christianson LE (2021) Personal communication, University of Illinois.
14. Hoover NL, Bhandari A, Soupir ML, Moorman TB (2016) Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. J Environ Qual 45: 803-812.
15. Sutphin T, Kult K (2010) Iowa bioreactor demonstration project. Minnesota/ Iowa Drainage Research Forum.
16. Woli KP, David MB, Cooke RA, McIsaac GF, Mitchell CA (2010) Nitrogen balance in and export from agricultural fields associated with controlled drainage systems and denitrifying bioreactors. Ecol Eng 36: 1558-1566.

17. Doheny A (2002) Amelioration of tile nitrate and atrazine using inline biofilters. M.S. Thesis, Urbana-Champaign, Ill.: University of Illinois at Urbana-Champaign, Agricultural & Biological Engineering.

18. Moorman TB, Parkin TB, Kaspar TC, Jaynes DB (2010) Denitrification activity, wood loss, and N_2O emissions over 9 years from a wood chip bioreactor. Ecol Eng 36: 1567-1574.