

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

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Abstract

Eutrophication can be accelerated by excess amounts of reactive Nitrogen (Nr) entering aquatic ecosystems. Historically, the circa 1960 Bolton Wastewater Treatment Plant, Warren County, New York (USA), discharged plant effluent for final polishing to natural sand infiltration beds, which entered the groundwater and then tributaries to Lake George. The absence of a denitrification unit process at the Bolton facility resulted in the construction of a woodchip bioreactor and a corresponding demonstration project to evaluate denitrification of plant effluent prior to sand bed discharge. This Denitrifying Bioreactor (DNBR) installation was the first real time, in-situ application of this “green technology” for a small wastewater treatment plant world-wide. The Bolton DNBR reduced nitrate-nitrogen concentrations in the tertiary effluent by 38% when compared with untreated tertiary effluent. Here we show that wastewater denitrification using this passive, environmentally compatible technology offers a low-cost, effective tool for small community wastewater treatment plants where excess Nr is problematic. Combined with diligent plant operator attention, this innovative treatment should move beyond concept into full scale field applications for other small community wastewater treatment plants globally, using lessons learned at the Bolton facility.

Keywords: Woodchip bioreactors; Wastewater treatment; Reactive nitrogen; Nitrogen cascade



Introduction

Nitrogen (N) is an essential element for all plants, animals, and humans. N compounds in nature are classed into nonreactive and reactive [1]. N₂ is non-reactive and requires significant thermal or microbial energy to break the bond. All active N compounds in Earth's atmosphere and biosphere are labeled reactive N (Nr) and include

inorganic reduced forms (ammonia [NH₃], ammonium [NH₄]), inorganic oxidized forms (nitrous oxide [N₂O], nitric acid [HNO₃], nitrogen dioxide [NO₂], nitrate [NO₃⁻]), and organic compounds (proteins, amines, urea, nucleic acids).

Nr levels have increased significantly world-wide from increased fertilizer application to feed a rapidly expanding population and

the burning of fossil fuel [2]. Once the N_2 triple bond is broken, the created Nr distributes throughout the Earth's biogeochemical pathway and produces multiple effects, magnified in time, in atmospheric, terrestrial, freshwater, and marine ecosystems, termed the nitrogen cascade [1].

Eutrophication in aquatic ecosystems is defined as an increase in the rate of organic matter and simultaneous increases in primary production [3]. Although eutrophication is not necessarily problematic because nutrients are required for growth and reproduction, it becomes an issue when excessive nutrients are discharged to a receiving waterbody.

Nitrogen and phosphorus in aquatic systems usually are in limited supply. However, if discharged to receiving waters in excess, phytoplankton species most capable of nutrient assimilation will out-compete phytoplankton species that depend on other factors for successful growth and development [4]. Continuous nutrient delivery can result in specific selection of phytoplankton, such as cyanobacteria, that ultimately affect higher ecosystem levels [5].

The Bolton WWTP, located on the west side of Lake George (New York, USA), has two sets of sand infiltration beds for effluent disposal and two tributary watersheds are affected by groundwater movement from the facility depending upon which sand beds are used. Calculations performed using recent and historical data showed that 54 tonnes of nitrate-nitrogen (NO_3-N) was released to Lake George during 50 years of WWTP operation [6].

The Town of Bolton dealt with the excess nitrogen issue by constructing a woodchip bioreactor to treat tertiary plant effluent prior to sand bed discharge. Similar DNBRs have been evaluated successfully for several decades in agriculture [7,8] and aquaculture [9,10]. A thorough literature review did not find any examples of DNBR technology used to process effluent from a small community treatment plant, so this report was the first world-wide application of this technology. The successful use of this "green" technology for wastewater treatment in the Northeast US with its variable environmental conditions could lead to widespread application globally where Nr scenarios occur that are similar to Lake George.

Materials and Methods

The Bolton WWTP

The Bolton WWTP (circa 1960) was constructed with a grit chamber, Imhoff tank, trickling filter, secondary clarifiers, and sand infiltration beds ("lower" beds 1-5). In 1973, four additional infiltration beds were constructed above the plant ("upper" beds 6-9), and in 1984, two additional beds (10, 11) were constructed adjacent and south of the 1973 beds (SM Figure S1).

The Bolton WWTP was built on a series of stepwise deltaic glacial deposits to utilize the sand as infiltration substrate for treated wastewater effluent [11]. The area subsurface geology included a ridge of bedrock that bisected the lower sand beds so that groundwater flow derived from effluent disposed to these beds moved in two directions, either south toward the Mohican Road Tributary or north toward Stewart Brook (SM Figure S2). Effluent discharged to the upper sand beds entered the groundwater and moved down-gradient to Stewart Brook [11].

Impact of the Bolton WWTP on local tributaries

The effect of the Bolton WWTP on groundwater and local tributary watersheds was monitored from April 2016-May 2017 (see SM text)

with a Final Report issued in June 2017 [6]. The results corroborated findings from previous studies that treatment plant effluent discharged to sand beds for final polishing contained elevated NO_3-N concentrations which entered the local groundwater and flowed down-gradient into the two tributary watersheds [12-14].

Using historical [12-14] and 2016-2017 data, the NO_3-N load to Lake George *via* the Mohican Road Tributary during the 50-year period since the earliest tributary study [12] was estimated at 50 tonnes. With no historical data for Stewart Brook, the 2017 Final Report used mean tributary flow (4,073 m^3/day) and mean NO_3-N concentration (1.22 mg/L) to calculate a daily load of 4.97 kg of NO_3-N entering Bolton Bay (1.814 tonnes/yr).

Following release of the 2017 Final Report, the Town of Bolton passed a resolution that discontinued lower sand beds use for effluent disposal except for emergencies when the upper beds could not be used. This action immediately reduced the high NO_3-N load entering the Mohican Road Tributary and part of the load entering Stewart Brook from the lower beds. The Town of Bolton then applied a local grant to design, receive permit approval and install a woodchip bioreactor in an inactive upper sand bed.

Design and installation of the woodchip bioreactor

Co-author KS designed the bioreactor (SM Figure S3+SM text). Sand bed #10 was used as the bioreactor installation site (SM Figure S1). Construction occurred between July and October 2018 when the unit became operational. The unit was not designed to process the maximum daily flow permitted through the plant, so tertiary effluent, either treated or untreated by the bioreactor, is discharged to the upper sand beds for final disposal and enters the groundwater, moving down-gradient to Stewart Brook.

Evaluation of the denitrifying woodchip bioreactor

A monitoring program (SM text) was implemented to evaluate the effectiveness of DNBR technology to reduce the NO_3-N concentration in plant effluent. Sample collection and processing were carried out according to protocol (SM text) and submitted to a laboratory certified for the New York permit operating requirements that regulate this treatment facility.

Effect of the Bolton WWTP on the Stewart Brook watershed

The Stewart Brook watershed includes the upper sand bed portion of the Bolton WWTP and received continuous groundwater flow from this area during bioreactor operation. The March 2019-May 2021 study sampled Stewart Brook above and below the channel where groundwater from the upper sand beds emerges as surface water (SM Figure S4). The purpose of the study was to collect data to evaluate the impact of the bioreactor on reducing the NO_3-N load to Stewart Brook and Lake George.

Results and Discussion

Evaluation of woodchip bioreactor

The study period was 805 days, from March 19, 2019, through May 31, 2021. The bioreactor was in full operation for 756 days, and was offline and not treating wastewater for 49 days, or 6% of the study period. There were 77 days (~10%) of the 756 operational days during which the in-line flow meter was not recording, although the bioreactor was treating influent wastewater. The bioreactor flow data during the study are summarized (SM Table S4). Data reported here focused on the capacity of the woodchip bioreactor to (1) reduce Nr in

Bolton WWTP effluent, and (2) to reduce the loading of Nr to Stewart Brook.

Physical characteristics:

Temperature: This is an important property of wastewater influent entering the bioreactor due to its effect on NO₃-N removal efficiency. The mean temperatures of the bioreactor influent and effluent were 13.5°C and 13.9°C, respectively, and the bi-weekly data are plotted (Figure 1).

NO₃-N removal efficiency is greater with warm temperatures and lower with cold temperatures. Influent and effluent temperatures are similar to outside temperature because the Bolton WWTP process tanks are outside or in unheated shelters. Coupled with low temperature is the hypothesis that reduced activity by cellulolytic bacteria in the wood chips adversely impacts the removal efficiency by reducing the availability of a carbon source.

Flow: Flow was totaled daily and related to wastewater retention time in the bioreactor. The mean daily flow through the unit was 275.96 m³/day with a high flow of 450.26 m³/day on November 29, 2019, and a low flow of 114.04 m³/day on March 17, 2021.

A plot of daily wastewater flow from November 2019 through May 2021 is presented in Figure 2.

Beginning in November 2020, daily flows through the unit were reduced to optimize denitrification with greater retention time during a period when higher influent NO₃-N concentrations and colder temperatures were observed (Figure 1). The bioreactor was not designed to process all facility wastewater flow permitted for 1,135.62 m³/day.

A comparison of total plant wastewater flow and flow through the bioreactor during the period from November 2019 through May 2021 is summarized in Figure 3.

Bioreactor flow and retention time are indirectly related, and retention time influences the extent of denitrification. Retention time was manually adjusted to accommodate NO₃-N concentration and influent wastewater temperature. Data showed that high effluent NO₃-N levels could result from higher flows through the bioreactor, and bioreactor retention time was a focus of daily operation, having to factor in flows through the WWTP and variables such as influent NO₃-N concentrations and temperature.

Chemical characteristics: Chemical characteristics of the bioreactor influent and effluent are summarized in Table 1.

pH: All pH values were >6.0 s.u. for influent and effluent wastewater with an influent range from 6.1-7.7 s.u. and effluent range from 6.2-7.2 s.u. The mean pH for the bioreactor influent and effluent

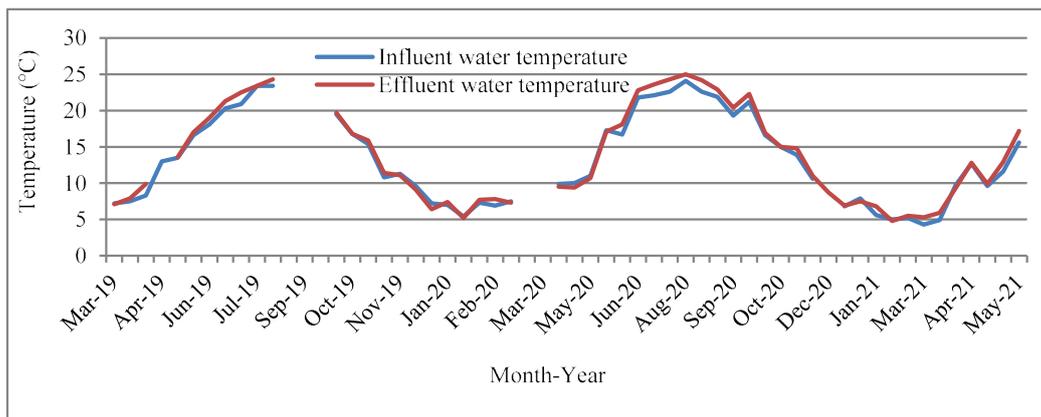


Figure 1: Woodchip bioreactor influent and effluent water temperatures, March 2019-May 2021.

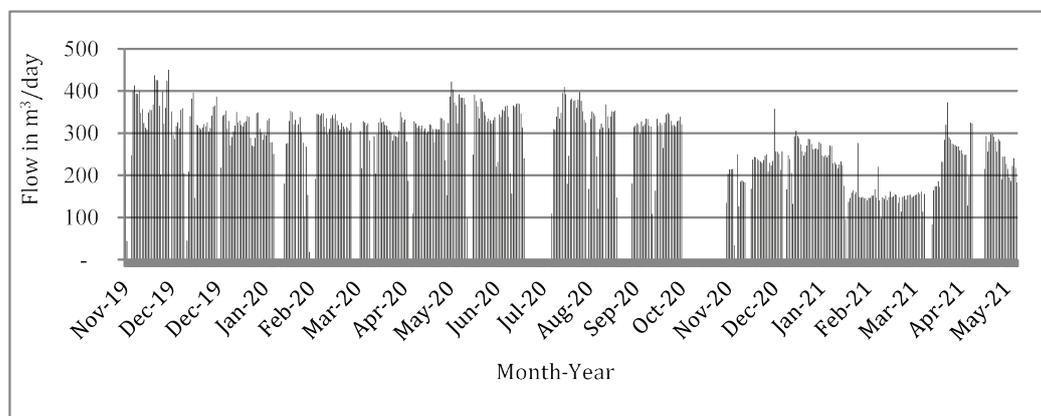


Figure 2: Daily wastewater flow through the bioreactor, November 2019-May 2021.

were 6.9 and 6.8 s.u., respectively. The optimum wastewater pH values for denitrification are 7.0-8.5 s.u. [15], and it was found that pH increases in denitrification as a result of the alkalinity produced [16].

Dissolved oxygen (concentration-percent saturation): Bioreactor denitrification is maximized by anoxic, or low Dissolved Oxygen (DO), conditions, preferably <0.2 mg/L [17]. Heterotrophic

Table 1: Chemical characteristics of influent and effluent wastewater of the woodchip bioreactor.

	pH	DO	DO	Alkalinity	NO ₃ -N	NH ₄ -N
	(s.u.)	(% sat)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Bioreactor Influent						
minimum	6.1	62.6	5.3	20	4.4	0.05
maximum	7.7	109.5	13.1	100	25.4	10.1
mean	6.9	85.0	8.9	56.3	14.5	1.1
n	54	54	54	54	54	54
Bioreactor Effluent						
minimum	6.2	0.2	0.02	21	0.01	0.025
maximum	7.2	75.0	8.03	128	19.0	8.960
mean	6.8	8.21	0.95	72.98	8.85	1.03
n	53	53	53	53	53	53

 =value reported is one-half the lowest detection limit

bacteria reduce NO₃-N to N₂ in the presence of an organic carbon source and low DO. Long retention time can deplete DO levels as laboratory tests have shown that the time required to deplete DO levels in DO-saturated water is about 1 h in aged, 2-yr-old woodchip media [18].

Bioreactor influent and effluent wastewater DO saturation levels are summarized in figure 4.

The influent wastewater DO concentration and saturation ranged from 5.3 mg/L and 62.6% to 13.1 mg/L and 109.5%, with mean values of 8.9 mg/L and 85.0%, respectively. The effluent wastewater DO concentration and saturation ranged from 0.02 mg/L and 0.2% to 8.03 mg/L and 75.0%, with mean values of 0.95 mg/L and 8.2%, respectively. The average reduction in wastewater DO concentration was just under 90%, from 8.9 mg/L to 0.95 mg/L and the average reduction in DO saturation was just over 90%, from 85.0% to 8.2%.

Higher influent wastewater DO concentration coincided with (1) greater bioreactor wastewater depth and was attributed to the surface being closer to the permeable filter fabric cover where oxygen exchange could occur, and (2) winter when wastewater temperature was low allowing increased DO concentrations.

Alkalinity: Wastewater usually is alkaline, and alkalinity concentration is important for this study where ammonia is removed by nitrification and converted to NO₃-N. Aerobic bacteria can use ammonia for food and DO to convert ammonia to NO₃-N.

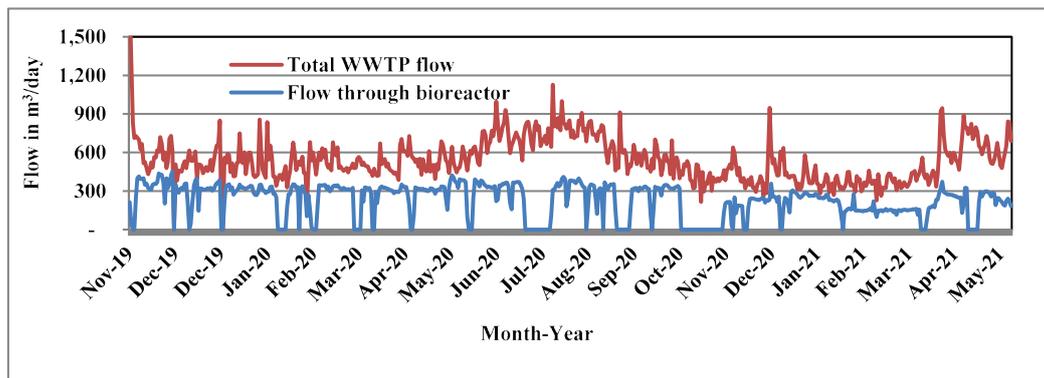


Figure 3: Total daily wastewater flow through treatment plant and bioreactor, November 2019-May 2021.

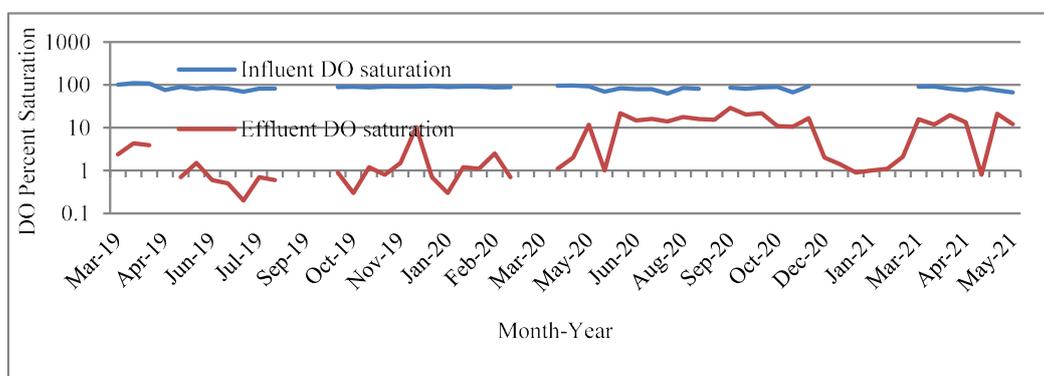


Figure 4: Bioreactor influent and effluent wastewater DO saturation, March 2019-May 2021.

Alkalinity concentrations should be at least eight times the ammonia concentration in wastewater to adequately nitrify.

In denitrification, alkalinity is created at 3.57 g of CaCO₃ (mg/L alkalinity) per gram of NO₃-N reduced to N₂. A good/poor nitrogen removal and short-cut nitrification/denitrification can be indicated or validated by alkalinity values and difference between influent and effluent concentrations [19]. Influent and effluent bioreactor alkalinities measured during the 2019-2021 study are shown (SM Figure S5). The mean annual alkalinity concentrations of the bioreactor influent and effluent are summarized in Figure 5.

The mean alkalinity concentration in the bioreactor influent decreased between 2019 and 2020 from 70.1 to 47.3 mg/L, then increased in 2021 to 51.5 mg/L. Bioreactor effluent alkalinity exhibited a constant decrease in mean concentration from 88.7 to 66.2 to 61.6 mg/L in 2019, 2020 and 2021, respectively. The difference between the mean alkalinity of the influent and effluent was +18.6 to +18.9 to +10.1 mg/L in 2019, 2020 and 2021, respectively.

Alkalinity is lost during nitrification (ammonia changed to NO₃-N) and produced during denitrification (NO₃-N changed to N₂). Parameters important for denitrification are anoxic conditions (DO concentration <1-2 mg/L; optimum concentration 0.2 mg/L [17]), pH (pH range 7.0-8.5 s.u. [15]), temperature (optimum 20-35°C; can occur as low as 3°C [20]), and available carbon. Other variables are NO₃-N concentration (high=greater availability), temperature and retention time. An increase in mean alkalinity from influent to effluent (Figure 5) indicated denitrification.

Nitrogen: Nitrogen removal from wastewater is a three-step process that includes ammonification, nitrification, and denitrification and is summarized (SM text). The nitrogen forms of concern in wastewater treatment are Total Nitrogen (TN), Total Kjeldahl Nitrogen (TKN), ammonia-nitrogen (NH₃-N), Organic Nitrogen (ON), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N) and nitrogen gas (N₂). The relationships of various nitrogen forms are presented (SM text). In this study, NO₃-N and NH₃-N were the only nitrogen forms chemically analyzed.

The mean annual NO₃-N concentrations measured at the bioreactor sampling sites from 2019-2021 are summarized in Figure 6.

A decrease of wastewater NO₃-N concentration from bioreactor influent through the well sites to the bioreactor effluent indicated successful denitrification. The difference between mean annual bioreactor influent and effluent NO₃-N concentration decreased over the study from 7.1 (2019) to 5.7 (2020) to 2.1 mg/L (2021), with an "All Years" mean reduction of 5.5 mg/L. The percentage of NO₃-N reduction decreased annually from 54.6% (2019) to 37.0% (2020) to 13.9% (2021) with an overall "All Years" reduction of 37.9% (Figure 6).

The possible conditions that influenced bioreactor denitrification reduction over time were higher NO₃-N concentrations in the wastewater influent during the study (SM Figure S8) indicating changes in wastewater characteristics. During 2020, the Bolton WWTP seasonality can be seen in the high influent NO₃-N concentrations entering the bioreactor starting in late May, into mid-September, and through February 2021 (SM Figure S8). At times, the 2020 and 2021

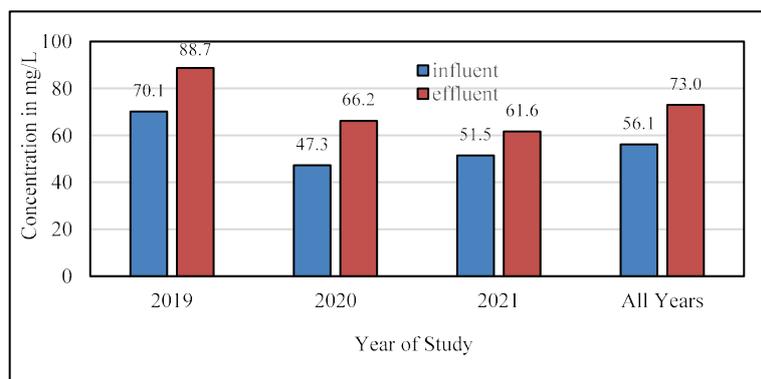


Figure 5: Mean annual bioreactor influent and effluent alkalinity concentrations, 2019-2021.

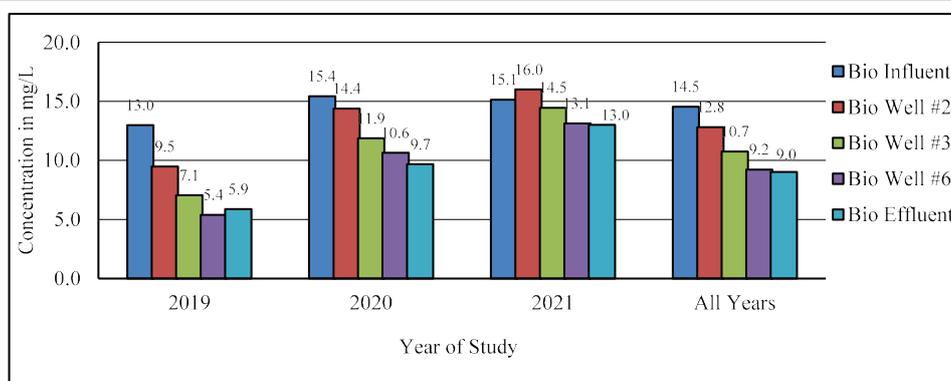


Figure 6: Mean annual wastewater NO₃-N concentrations measured at bioreactor sampling sites.

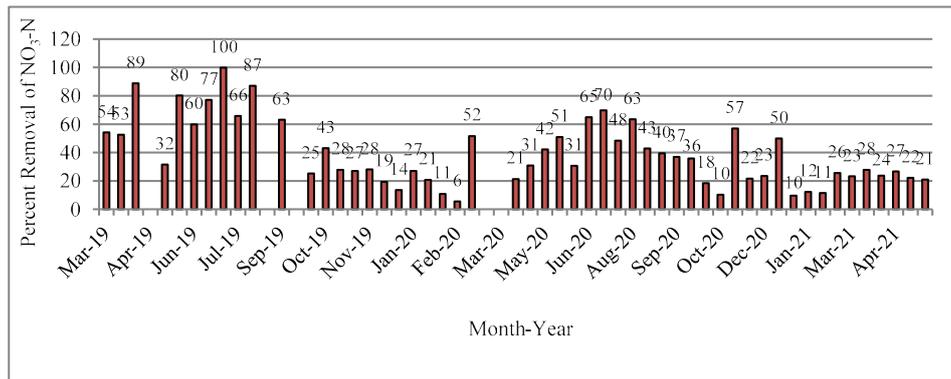


Figure 7: Percent removal of NO₃-N from wastewater entering the bioreactor influent and processed by the unit, March 2019-May 2021.

influent NO₃-N concentrations were twice the 2019 concentrations. Woodchip degradation, the carbon source, also could reduce denitrification.

We hypothesize that the decrease in NO₃-N removal efficiencies during cold weather is due to insufficient available woodchip carbon. Research indicates temperature sensitivity of cellulolytic bacteria [20-22]. The organic carbon/N ratio variability as it relates to microbial denitrification has been identified [23], and others [24] have defined a BOD/NO_x-N ratio of 2/3 to ensure 100% denitrification. The impact of lowered wastewater temperatures, reduced carbon source availability during cold weather due to temperature sensitivity of cellulolytic bacteria, and requisite C/N ratio for successful NO₃-N reduction present key areas for future research.

The bioreactor NH₃-N results are presented (SM text) with additional denitrification information.

Variability in bioreactor treatment efficiency: The percent removal of NO₃-N from effluent that entered the bioreactor is summarized (Figure 7) and the overall study removal was 38%.

The removal efficiency of NO₃-N and certain operational parameters influenced the degree of denitrification including water temperature, influent NO₃-N and DO concentrations, retention time, and suitable carbon source availability [25]. Factors that influenced the operational efficiency are summarized (Table S5+SM text).

Woodchip bioreactor maintenance: Bioreactor maintenance issues during operation are described in the SM text.

Woodchip bioreactor operational challenges: As a first-time, full-scale field installation, operational challenges related to the bioreactor were expected and encountered, and their resolution led to modified design, construction, operation, and monitoring of the process. See the SM section for further details.

Woodchip bioreactor shutdown due to plugging

There were several instances of bioreactor clogging of material that will reduce denitrification efficiency and possibly lead to hydraulic failure. Instances of clogging are discussed in the SM section,

Effect of the Bolton WWTP woodchip bioreactor on Stewart Brook water quality

Bolton WWTP effluent discharged to the upper sand beds enters the groundwater, moves down-gradient, and emerges as surface water in Stewart Brook (SM Figure S3). The 2016-2017 study evaluated the impact of effluent discharged to the upper beds on Stewart Brook water

quality with the beds used from May to October each year. Coincident with the installation and operation of the bioreactor in October 2018, the upper sand beds were used year-round for effluent disposal and the 2019-2021 study was conducted to evaluate the water quality impact on Stewart Brook.

Stewart Brook flow and NO₃-N data collected above and below the groundwater influence from the upper sand beds during the 2016-2017 and 2019-2021 studies are compared (Table 2). Temperature and DO percent saturation data collected during 2019-2021 also are presented (SM section) as additional evidence of the groundwater influence on Stewart Brook.

Flow: Flow variability between the two studies (Table S9+SM text) highlights the different annual precipitation amounts and patterns that occur. Bi-weekly flow measured during the 2019-2021 study demonstrates the seasonal nature of this tributary (SM Figure S12). The difference in flow between the above and below stations is attributed to the continuous discharge of effluent from the WWTP to the upper sand beds entering Stewart Brook. The greatest flow difference between these stations occurred during the late spring-summer-early fall each year when daily wastewater volume entering the plant increased due to local seasonal tourism.

Nitrogen: The mean NO₃-N concentrations measured above and below the groundwater intrusion into Stewart Brook during the 2016-

Table 2: Flow and NO₃-N concentrations measured during the 2016-2017 and 2019-2021 studies at the above and below stations on Stewart Brook.

	2016-2017 Study		2019-2021 Study	
	Flow (L/s)	NO ₃ -N (mg/L)	Flow (L/s)	NO ₃ -N (mg/L)
Above groundwater influence				
Minimum	0.283	0.005	0.525	0.01
Maximum	470.91	0.17	75.903	0.48
Mean	35.650	0.09	16.687	0.12
n	20	20	59	65
Below groundwater influence				
Minimum	0.963	0.02	3.506	0.43
Maximum	533.74	8.70	87.026	8.88
Mean	44.878	2.01	22.300	3.45
n	20	20	63	65

###value reported one-half lower limit of detection

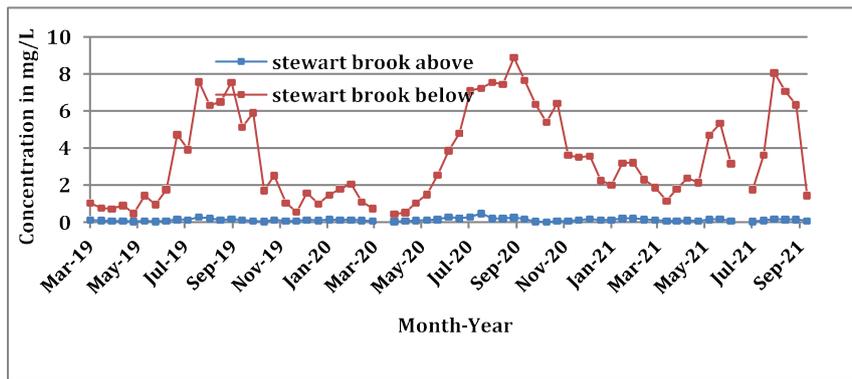


Figure 8: NO₃-N concentrations measured at above and below stations on Stewart Brook, 2019-2021.

2017 and 2019-2021 studies were summarized (Table 2). The mean NO₃-N concentration increased from 0.08 to 0.12 mg/L at the above site between the two studies. The below site exhibited a 72% increase (2.01 to 3.45 mg/L) when comparing the two studies, with the increase due to continuous effluent disposal to the upper sand beds.

The NO₃-N concentrations measured above and below on Stewart Brook during 2019-2021 are shown in Figure 8. The NO₃-N concentration at the below site increased in late spring, summer, and early fall when area tourism and wastewater volumes increased, followed by lower concentrations during winter when tourism and wastewater volumes decrease. The Bolton WWTP inability to achieve effective denitrification during high volume is evident (Figure 8).

Effect of the woodchip bioreactor on NO₃-N loading to stewart brook: Prior to the 2017 Town of Bolton mandate banning lower sand bed use, disposal of WWTP effluent alternated between lower and upper beds. After October 2018, the upper sand beds were used almost continuously for effluent disposal except for an occasional emergency.

Here, we characterize (1) NO₃-N loading to Stewart Brook from the upper sand beds prior to woodchip bioreactor construction, and (2) the woodchip bioreactor influence on reducing the NO₃-N concentration entering Stewart Brook compared with untreated effluent discharged directly to the upper beds with no treatment from the denitrifying bioreactor.

Table 3 presents the mean NO₃-N concentration in plant effluent and bioreactor effluent, mean plant flow, and duration of upper sand bed use in the 2016-2017 and 2019-2021 studies to demonstrate the ability of the woodchip bioreactor to reduce NO₃-N load to Stewart Brook.

The highest daily NO₃-N load (11.92 kg/day) occurred during the 2016-2017 study with a mean NO₃-N concentration of 17.5 mg/L in plant wastewater effluent. Post-2017 facility processing improvements achieved a 17% decrease in mean wastewater effluent concentration (to 14.5 mg/L) leading up to the 2019-2021 study. Woodchip bioreactor construction and treatment of plant effluent resulted in a 38% decrease in wastewater effluent concentration (to 9.02 mg/L), the lowest daily load of the various scenarios. Inability of the bioreactor to treat all daily plant flow resulted in the convergence of treated bioreactor effluent with tertiary treated plant effluent to form the “bed effluent” (Table 3) with a mean NO₃-N concentration (11.9 mg/L) intermediate between the untreated plant effluent and treated bioreactor effluent, and an 18% decrease from tertiary effluent leaving the plant untreated by the bioreactor.

These data clearly highlight bioreactor effectiveness in reducing the NO₃-N load to Stewart Brook even though all daily Bolton WWTP effluent could not be processed through this unit. During the 756-day period of woodchip bioreactor operation, effluent discharged from the Bolton WWTP without unit operation would have contributed a NO₃-N load of 9.63 kg/day, or 7.3 tonnes to Stewart Brook. The contribution of the bioreactor operating during this 756-day period reduced the NO₃-N load to Stewart Brook by 18% to 7.90 kg/day, or 6.0 tonnes.

Conclusions

The material presented here provides compelling evidence that denitrifying woodchip bioreactor technology has a beneficial application in small community wastewater treatment plants with are active nitrogen issue. This “green” sustainable technology can provide a low cost and effective treatment for situations similar to Lake George where continuous loading of Nr to local groundwater or tributaries occurs. Locations with climate similar to the Northeast US can expect better denitrification during warmer months and reduced denitrification during colder months. The biggest factor affecting the success of the current project was operator involvement on a continuous daily basis. Other installations similar to Lake George can take ‘lessons learned’ from the Bolton WWTP to guide efforts toward a successful outcome in wastewater management.

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Table 3: Summary of Bolton WWTP effluent characteristics during the 2016-2017 and 2019-2021 studies and estimates of various NO₃-N loading scenarios to Stewart Brook.

Study	Source of wastewater	mean [NO ₃ -N] in wastewater (mg/L)	# days upper sand beds used for disposal	mean plant flow to upper sand beds (m ³ /day)	Total NO ₃ -N Load		
					kg/day	kg/yr	kg during period (days)
2016-2017	plant effluent	17.5	119	681	11.92	4350	1419 (119)
2019-2021	plant effluent	14.5	756	664	9.63	3514	7279 (756)
	bioreactor effluent	9.02			5.99	2186	4528 (756)
	sand bed effluent	11.9			7.90	2884	5974 (756)

George Waterkeeper and Brea Arvidson, LGA Manager of Water Quality Research in the monitoring program. The Town of Bolton provided in-kind services, which allowed the participation of Bolton WWTP Chief Operator Matt Coon, Assistant Operator Justin Persons, and Town Engineer Kathleen Suozzo.

References

- Galloway JN, Aber JD, Erisman JW, Setzinger SP, Howarth RW, et al. (2003) The Nitrogen Cascade. *Bioscience* 53: 341-356.
- Erisman JW, Galloway JN, Seitzinger S, Bleeker A, Dise NB, et al. (2013) Consequences of human modification of the global nitrogen cycle. *Philos Trans R Soc Lond B Biol Sci* 368: 20130116.
- Nixon SW (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* 41: 199-219.
- Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal waters. *ICES J Mar Sci* 66: 1528-1537.
- Dolman AM, Rücker J, Pick FR, Fastner J, Rohrlack T, et al. (2012) Cyanobacteria and Cyanotoxins: the Influence of Nitrogen *versus* Phosphorus. *PLoS One* 7: e38757.
- Sutherland JW, Navitsky C (2017) Final Report. Bolton Bay (Lake George, Warren County) Water Quality Assessment. A Monitoring Program to Evaluate Current Water Quality Issues. Prepared for The Fund for Lake George and the Town of Bolton: Lake George, New York, 150 pp, + appendices.
- Christianson LE, Bhandari A, Helmers MJ (2012) A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. *Appl Eng Agric* 28: 861-874.
- Christianson LE, Schipper, LA (2016) Moving denitrifying bioreactors beyond proof of concept: Introduction to the special section. *J. Environ Qual* 45: 757-761.
- Von Ahnen M, Bovbjerg P, Dalsgaard J (2018) Performance of full-scale woodchip bioreactors treating effluents from commercial RAS. *Aquacult Eng* 83: 130-137.
- Lepine C, Christianson L, McIssac G, Summerfelt S (2020) Denitrifying woodchip bioreactors for wastewater treatment. *Hatchery Internat* 1-5.
- Aulenbach DB, Phillip AJ (1983) The Bolton Landing rapid infiltration wastewater treatment plant. In *The Lake George Ecosystem*, Volume III, Lake George, New York, Carol Desormeau Collins, Ed: The Lake George Association, Lake George, New York, 101-109.
- Fuhs GW (1972) *The Chemistry of Streams Tributary to Lake George*, New York. New York State Department of Health, Environmental Health Report No. 1; Albany, New York.
- Keppeler 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, 50 pp, + appendices.
- Keppeler 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, 69 pp, + appendices.
- Dangcong P, Yi W, Hao W, Xiaochang W (2004) Biological denitrification in a sequencing batch reactor. *Water Sci Technol* 50: 67-72.
- Mokhayerei Y (2010) Characterizing Denitrification Kinetics and Stoichiometry At Different Temperatures Using Various Carbon Sources. Ph. D. Dissertation, The School of Engineering & Applied Science, The George Washington University, Washington, D.C.
- Seitzinger S, Harrison JA, Bohlke JK, Bouwman AF, Lowrance R, et al. (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecol Appl* 2064-2090.
- Robertson WD, Merkley LC (2009) In-stream bioreactor for agricultural nitrate treatment. *J Environ Qual* 38: 230-237.
- Li B, Irvin S (2007) The comparison of alkalinity and PRP as indicators for nitrification and denitrification in a sequencing batch reactor (SBR). *Biochem. Eng J* 34: 248-255.
- Holt DM, Jones EB (1983) Bacterial degradation of lignified wood cell walls in anaerobic aquatic habitats. *Appl Environ Microb* 46: 722-727.
- Desvaux M (2006) Unravelling carbon metabolism in anaerobic cellulolytic bacteria. *Biotechnol Progr* 22: 1229-1238.
- Jang J, Anderson EL, Venterea RT, Sadowsky MJ, Rosen CJ, et al. (2019) Denitrifying bacteria active in woodchip bioreactors at low-temperature conditions. *Front Microbiol* 10: 635.
- Sobieszuk P, Szewczyk KW (2006) Estimation of (C/N) ratio for microbial denitrification. *Environ Technol* 27: 103-108.
- Narkis N, Rebhun M, Sheindorf CH (1979) Denitrification at various carbon and nitrogen ratios. *Water Res* 13: 93-98.
- Schipper L, Robertson W, Gold A, Jaynes D, Cameron S (2010) Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. *Ecol Eng* 36: 1532-1543.

Supporting Material

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

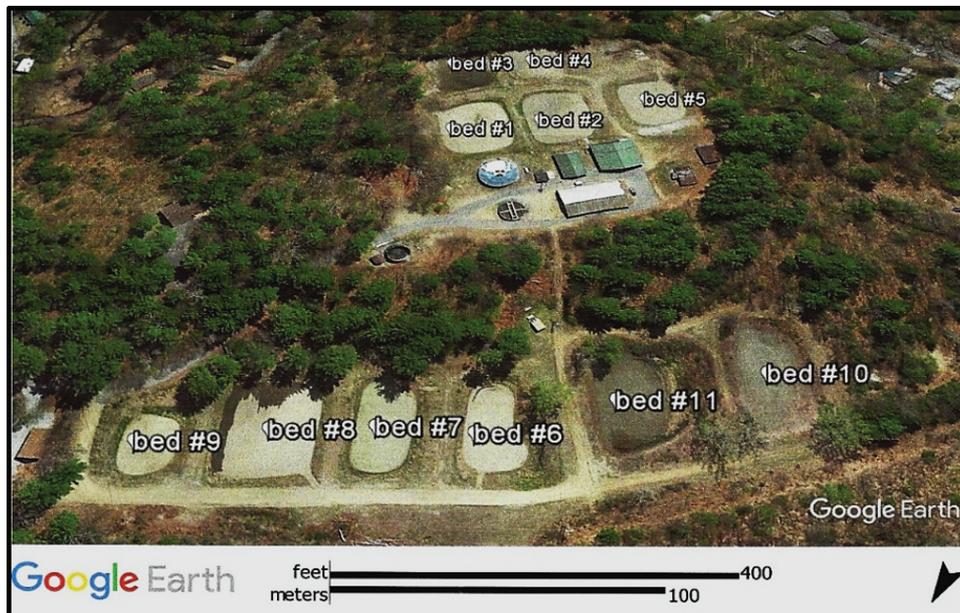


Figure S1: Google Earth™ view of the Bolton WWTP showing the location of the lower (#1-5) and upper (#6-11) sand infiltration beds and the plant components adjacent to the lower beds.

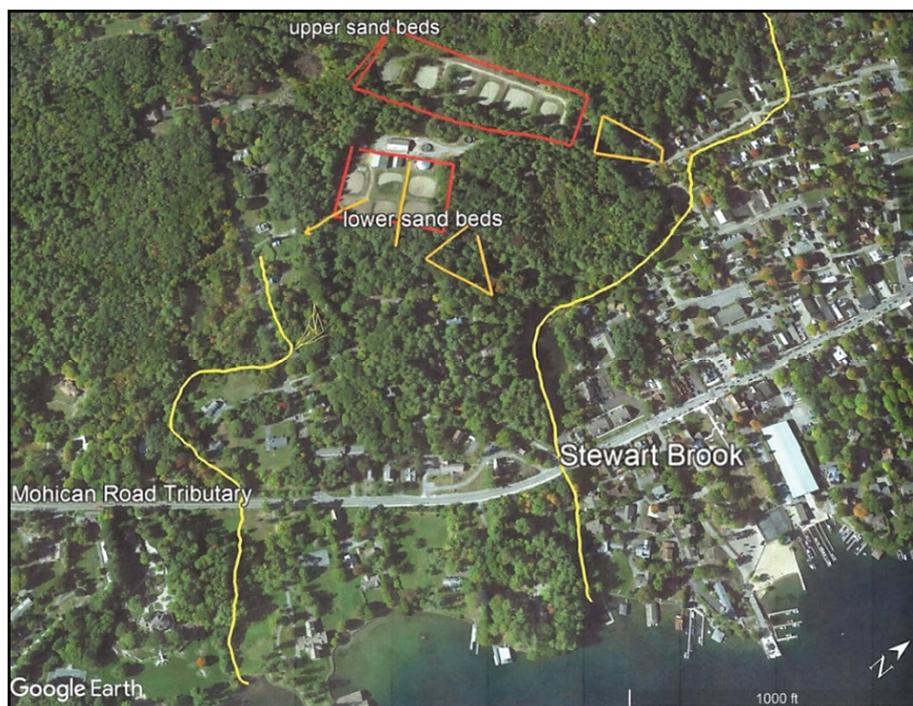


Figure S2: Google Earth™ view of the Bolton WWTP showing lower and upper sand bed location, the direction of groundwater movement toward the Mohican Road Tributary and Stewart Brook when effluent is discharged to the lower beds, and the direction of groundwater movement toward Stewart Brook when effluent 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 ($\text{NO}_3\text{-N}$), 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 ($\text{NO}_3\text{-N}$) 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 m³ 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

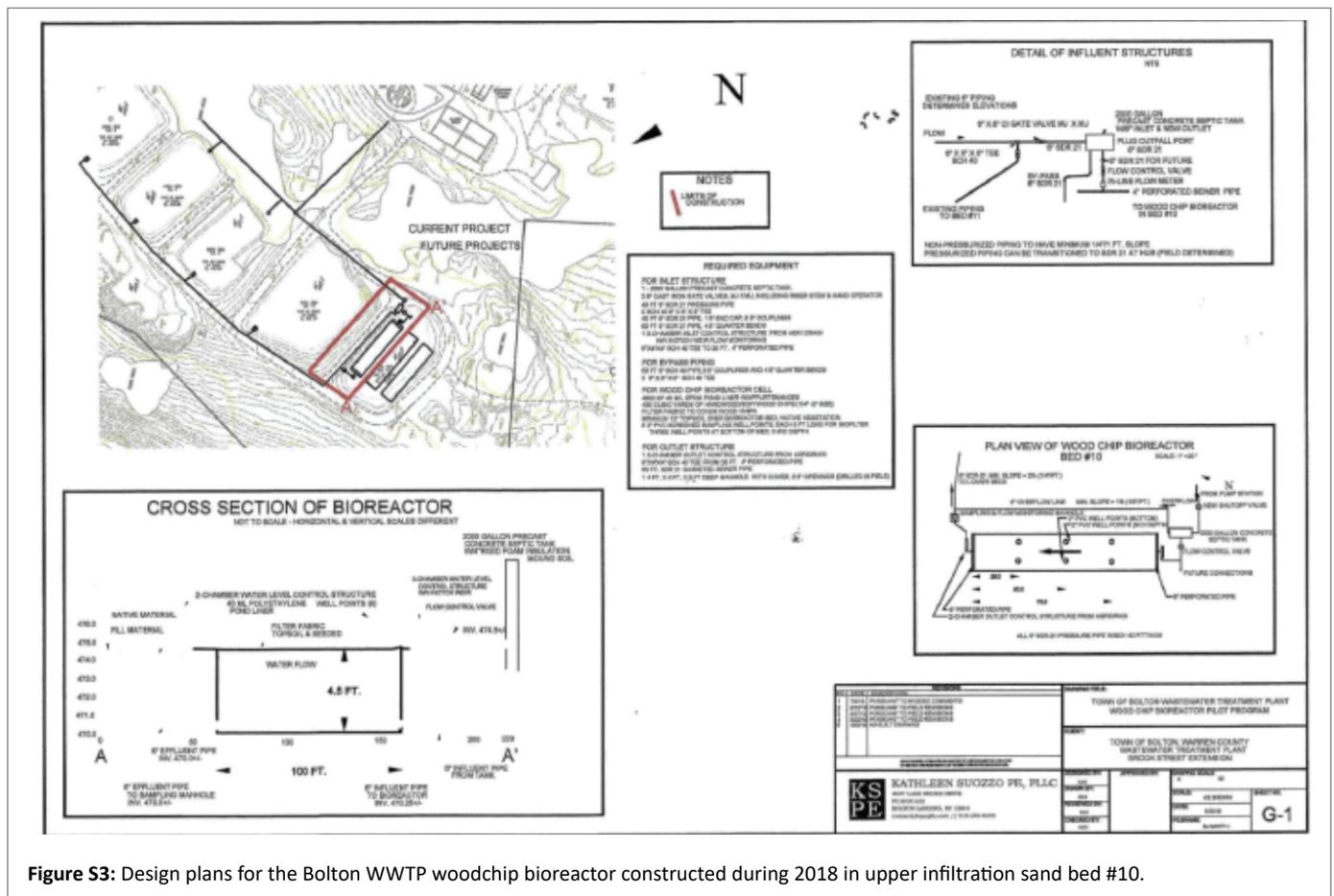


Figure S3: Design plans for the Bolton WWTP woodchip bioreactor constructed during 2018 in upper infiltration sand bed #10.

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	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 NO₃-N,
- 1-250 mL PE bottle preserved with H₂SO₄ for NH₃, 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 $\text{NO}_3\text{-N}$ 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 $\text{NO}_3\text{-N}$ 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 (NH₃-N)+Nitrate-nitrogen (NO₃-N)+Nitrate (NO₂)

TKN is comprised of NH₃-N and ON. A municipal WWTP with an effluent wastewater TKN >5 mg/L is not fully nitrifying. NH₃-N is the first inorganic nitrogen product of organic decomposition by bacteria and is present in lake water primarily as NH₄⁺ and NH₄OH. Ammonia (NH₃) is un-ionized; ammonium (NH₄⁺) is ionized. pH is the major environmental variable that determines the proportion of NH₃ or NH₄⁺ in water.

Bioreactor influent wastewater NO₃-N concentrations are summarized in Figure S6.

The mean annual NH₃-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 NH₃-N concentrations 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 NO₃-N effluent concentrations, which suggest that a seasonal influx of NH₃-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 NH₃-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 NH₃-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 NH₃-N production within the bioreactor, where influent NH₃-N of 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 NO₃-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 NH₃-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 NO₃-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 (NO₃) to nitrogen gas (N₂) in the presence of an organic carbon source

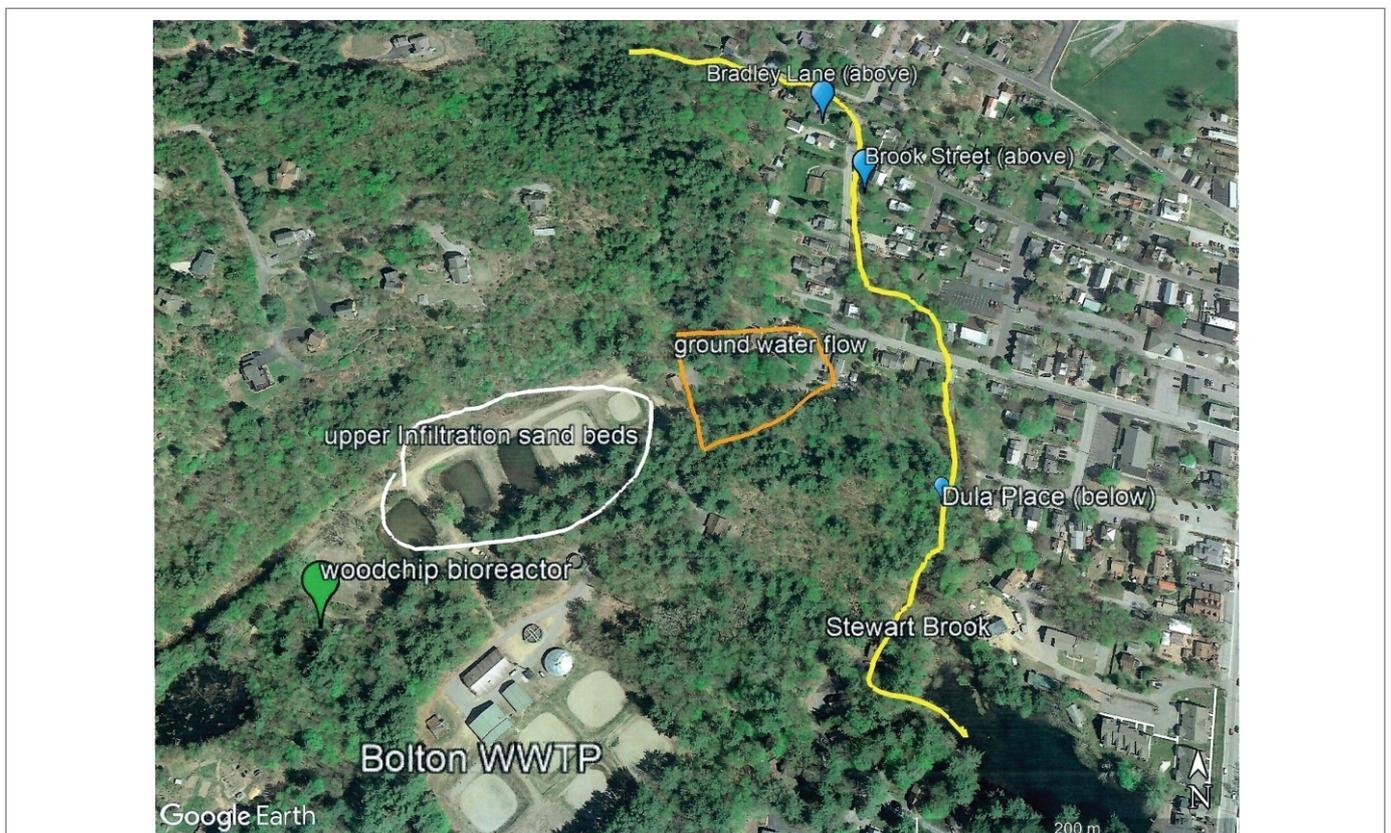


Figure S4: Google Earth™ view of the Bolton WWTP showing the location of the woodchip bioreactor, the upper infiltration sand beds and the movement of groundwater down-gradient toward Stewart Brook where it emerges as surface water in the channel between the above and below sampling stations along the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

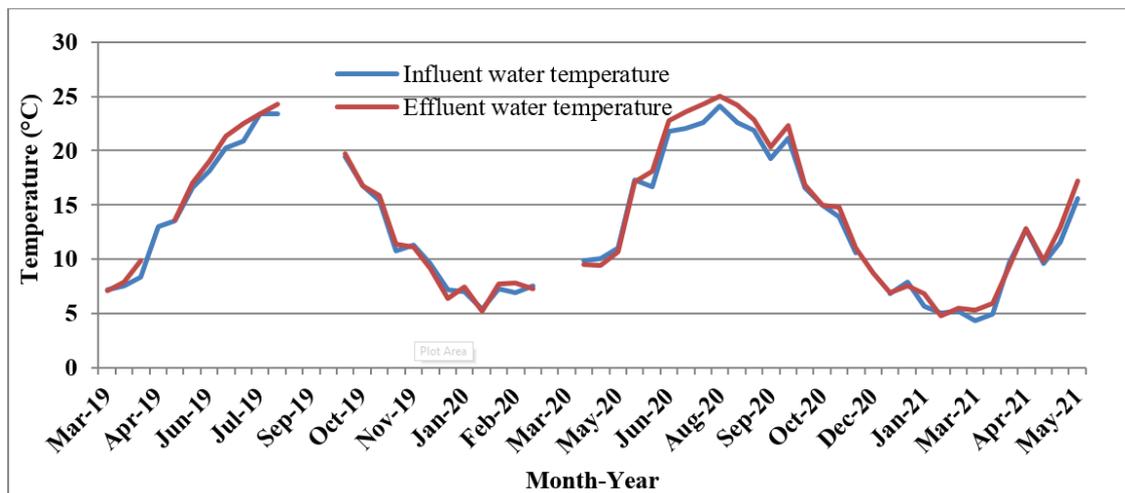


Figure S5: Woodchip bioreactor influent and effluent water temperatures, March 2019-May 2021.

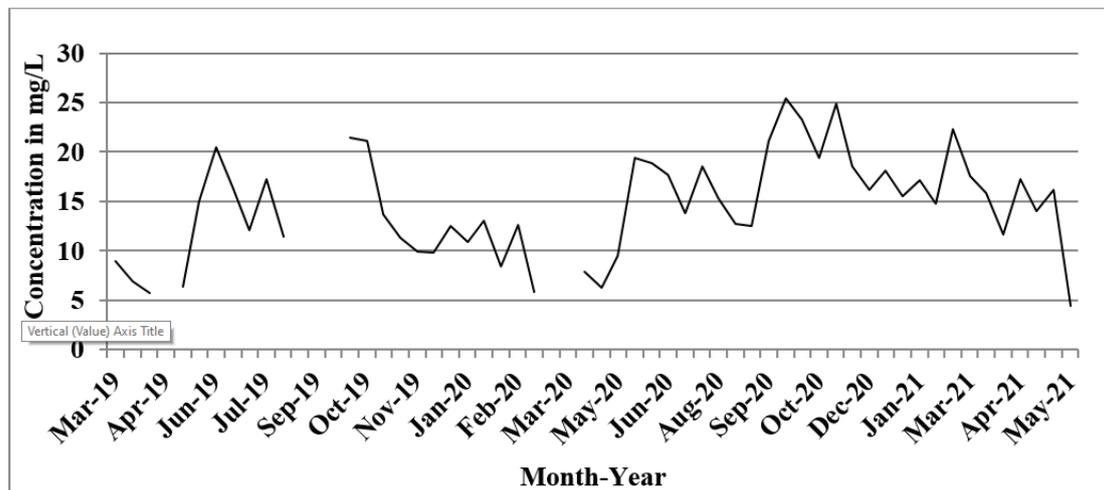


Figure S6: Bioreactor wastewater influent NO₃-N concentration, March 2019-May 2021.

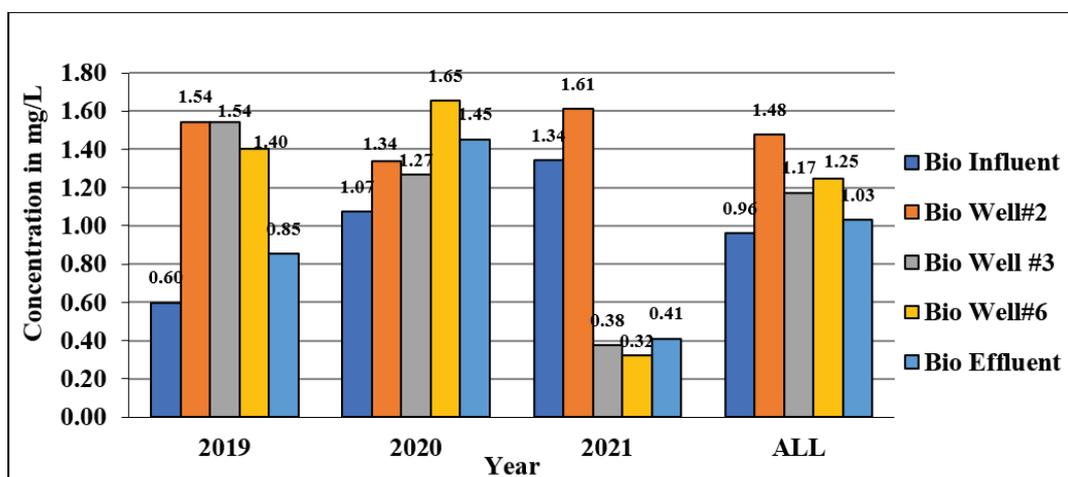
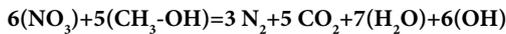


Figure S7: The mean annual NH₃-N concentrations at the bioreactor sampling sites.

(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 NO₃ molecule to gain oxygen, with N₂O and then N₂ produced. N₂ 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:



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 NO₃ 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 NO₃-N concentrations of WWTP effluent entering the bioreactor influent chamber and the corresponding effluent NO₃-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 NO₃-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 NO₃-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 NO₃-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

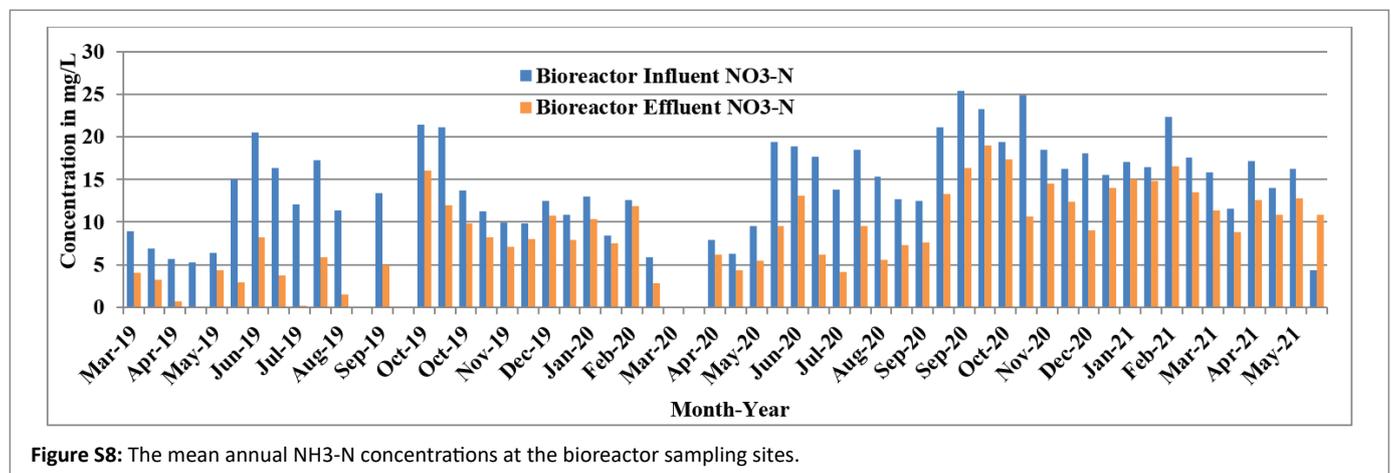


Figure S8: The mean annual NH3-N concentrations at the bioreactor sampling sites.

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 ³ d)	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

¹No flow data on 10/1/19 so numbers from 10/8/19 were used.
²No flow data on 12/10/19 so numbers from 12/12/19 were used.
³Inaccurate 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.
⁴No flow data on 6/9/2020, flow adjusted from 6/8/2020 was used.
⁵No flow data on 9/15/20 so numbers from 9/9/20 were used.
⁶No flow data on 10/27/20; numbers from 10/26/20 were used.
⁷No flow data on 11/10/20; manual measurement was conducted.
⁸No flow data on 11/24/20; numbers from 11/25/20 were used.
⁹No flow data on 12/8/20; numbers from 12/11/20 were used.
¹⁰On 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.
¹¹On 2/16/21, the water level seems lower than normal; we suspect volumetric removal rate to be overestimated.

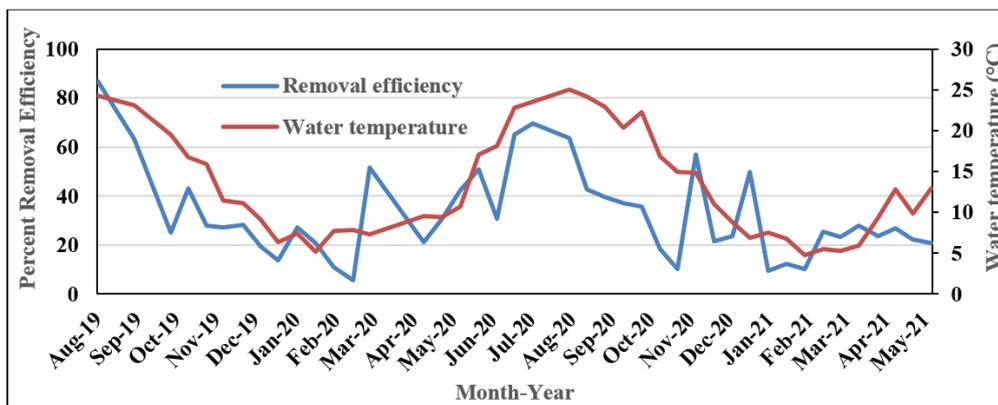


Figure S9: Relationship between bioreactor wastewater temperature and NO₃-N removal efficiency.

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 (BOD₅) 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 BOD₅ 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 $\text{NO}_3\text{-N}$, 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 $\text{NO}_3\text{-N}$ 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 NO₃-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 NO₃-N concentration through the bioreactor, this event coincided with the malfunctioning of the Bolton WWTP trickling filter. NH₃-N was not being adequately nitrified through the trickling filter. NH₃-N remained in the wastewater influent to the bioreactor, where nitrification occurred, resulting in an increase in NO₃-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 of 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 speculate 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

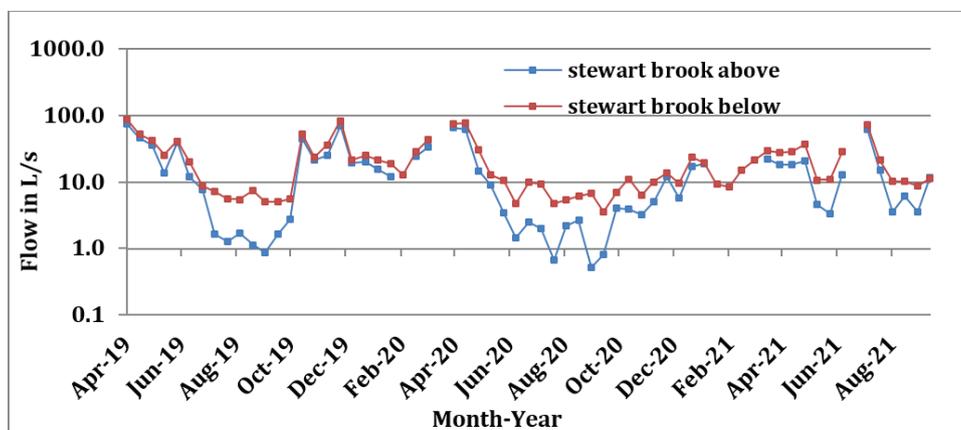


Figure S10: Stewart Brook flow measured at above and below stations, 2019-2021.

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

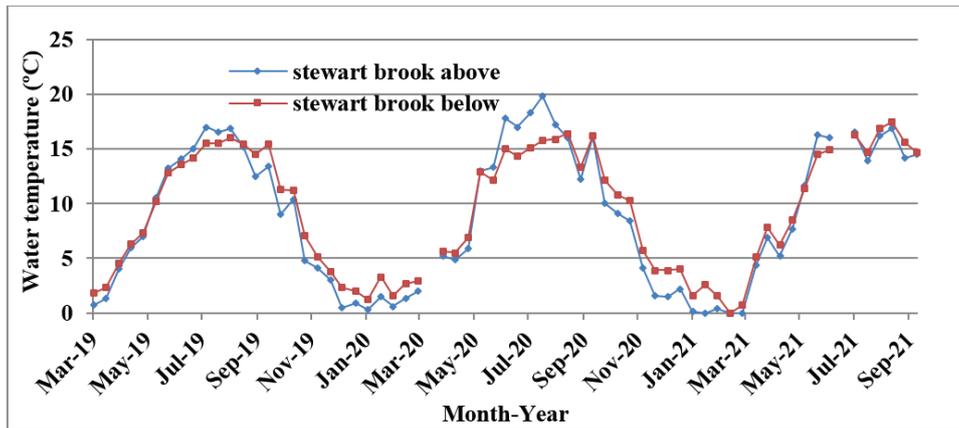


Figure S11: Seasonal patterns of water temperature measured in Stewart Brook at the above and below sampling stations, 2019-2021.

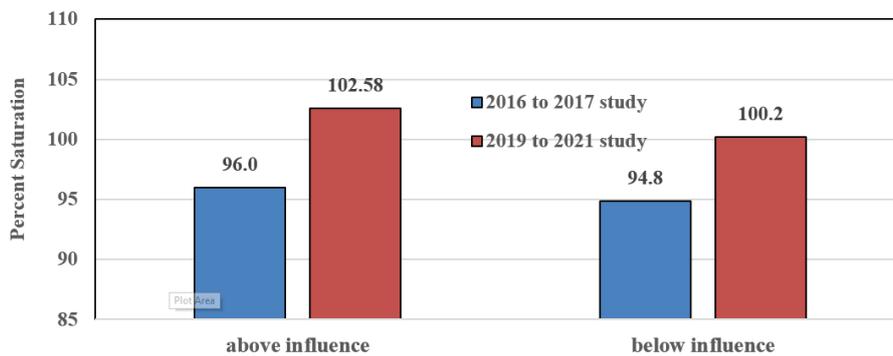


Figure S12: Mean DO percent saturation measured at the above and below sampling stations on Stewart brook during the 2016-2017 and 2019-2021 studies.

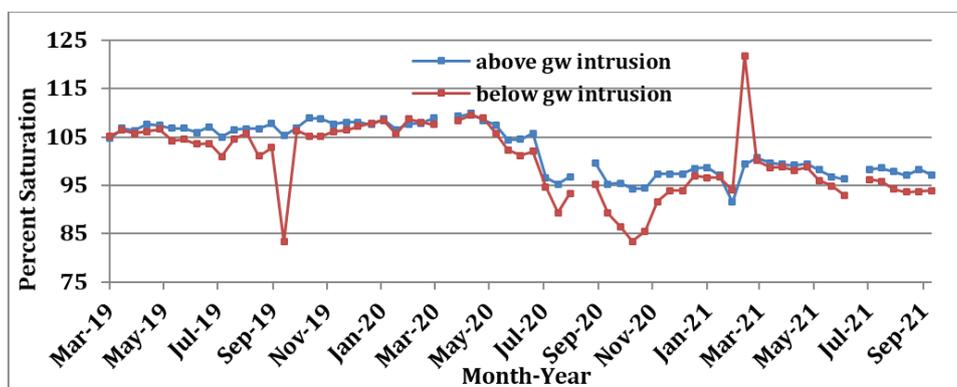


Figure S13: Seasonal pattern of DO percent saturation measured at the above and below sampling stations on Stewart Brook, 2019-2021.

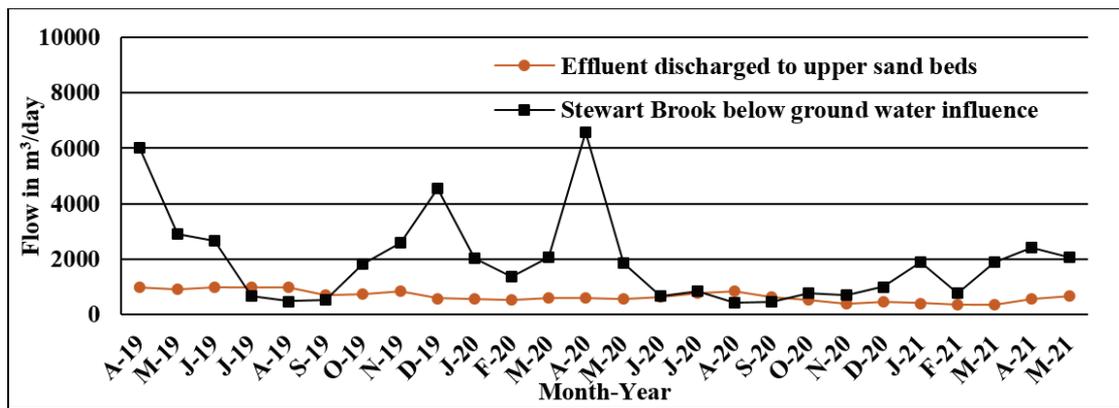


Figure S14: Mean monthly effluent discharge (m₃/day) to the upper sand beds and mean monthly flow (m₃/day) at the below station on Stewart Brook, April 2019-May 2021.

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

Flow

The mean monthly discharge (m³/day) of effluent to the upper sand beds after processing through the Bolton facility and the mean monthly flow (m³/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 m³/day and the mean Stewart Brook flow below the groundwater influence ranged from 440-6564 m³/day (Figure S14). During July, August, and September 2019, the mean volume of plant effluent discharged to the upper sand beds (991, 972, 709 m³/day, respectively) exceeded the mean Stewart Brook flow (680, 478, 540 m³/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

- Aulenbach DB, Phillip 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.
- 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.
- 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.
- 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.
- 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.
- Christianson LE, Bhandari A, Helmers MJ (2012) A practice-oriented review of woodchip bioreactors for subsurface agricultural drainage. *Appl Eng Agric* 28: 861-874.
- 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.
- 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.
- Christianson LE, Lepine C, Sharrer S, Summerfelt S (2016) Denitrifying bioreactor clogging potential during wastewater treatment. *Water Res* 105: 147-156.
- Waugh S (2019) Personal communication, SUNY Stony Brook.
- Sobieszuk P, Szweczyk KW (2006) Estimation of (C/N) ratio for microbial denitrification. *Environ Technol* 27: 103-108.
- 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.
- Christianson LE (2021) Personal communication, University of Illinois.
- Hoover NL, Bhandari A, Soupier ML, Moorman TB (2016) Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. *J Environ Qual* 45: 803-812.
- Sutphin T, Kult K (2010) Iowa bioreactor demonstration project. Minnesota/ Iowa Drainage Research Forum.
- 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 N₂O emissions over 9 years from a wood chip bioreactor. *Ecol Eng* 36: 1567-1574.