

ISSN Online: 2327-4344 ISSN Print: 2327-4336

# Tile-Drain and Denitrification Bioreactor Water Chemistry for a Soybean (Glycine max (L.) Merr.)-Corn (Zea mays L.) Rotation in East-Central Missouri (USA)

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How to cite this paper: Aide, M., Braden, I., Mauk, D., McAlister, R. W., McVay, B., Murray, S., Siemers, S., Svenson, S., & Weathers, J. (2020). Tile-Drain and Denitrification Bioreactor Water Chemistry for a Soybean (*Glycine max* (L.) Merr.)-Corn (*Zea mays* L.) Rotation in East-Central Missouri (USA). *Journal of Geoscience and Environment Protection*, 8, 143-154. https://doi.org/10.4236/gep.2020.84010

Received: January 31, 2020 Accepted: April 23, 2020 Published: April 26, 2020

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#### **Abstract**

Nitrogen transport from agriculture production fields raises the specter of environmental degradation of freshwater resources. Our objectives were to document and evaluate nitrate-N, ammonium-N, phosphorus and other nutrients emanating from a 40-ha controlled subsurface irrigation drainage technology coupled in series with a denitrification bioreactor. The intent of the denitrification bioreactor is to create an environment for anoxic microbial populations to support denitrification. We monitored the tile-drainage effluent and denitrification bioreactor water chemistry under a corn-soybean rotation to estimate the nutrient concentrations and the competence of the denitrification bioreactor to foster denitrification. Nitrate-N bearing tile drainage effluents ranged from less than 1.5 to 109 mg NO<sub>3</sub>-N/L, with the nitrate concentration differences attributed primarily to the: 1) timing of nitrogen fertilization for corn, 2) soil mineralization and residue decomposition, and 3) intense rainfall events. The denitrification bioreactor was highly effective in reducing drainage water nitrate-N concentrations providing the rate of water flow through the denitrification bioreactor permitted sufficient time for equilibrium to be attained for the nitrate reduction reactions. The nitrate-N concentrations entering the denitrification bioreactor ranged from 0.4 to 103 mg NO<sub>3</sub>-N/L in 2018, whereas the outlet nitrate concentrations typically ranged from 0.3 to 5.2 mg NO<sub>3</sub>-N/L in 2018. Nitrate tile-drainage effluent concentrations in 2019 were marginal, given soybeans obtain nitrogen from biological nitrogen fixation. Nutrient uptake by corn reduced the soil nitrate leaching pool and created nitrogen-bearing biomass, features important for formulating best management practices.

## **Keywords**

Nitrogen, Edge of Field, Denitrification Bioreactor, Water Quality, Tile Drainage

#### 1. Introduction

Aide et al. (2016) provided a review of the Edge-of-Field technology involving denitrification bioreactors to reduce nutrient transport from croplands, thus the introduction of this manuscript concentrates primarily on significant literature from 2016 to the present. The USEPA maximum contamination level for nitrate in drinking water is 10 mg NO<sub>3</sub>-N/L; however, 5 mg NO<sub>3</sub>-N/L in groundwater and 1.5 mg NO<sub>3</sub>-N/L in freshwater may strongly support eutrophication (Billen et al., 2018; Faust et al., 2018). Sources supporting accumulation of nitrogen (N) in surface waters include: 1) agricultural and urban surface runoff, 2) soil erosion, and 3) subsurface drainage effluents and 4) baseflow from impacted aquifers (Hang et al., 2016; Lenhart et al., 2016, Amado et al., 2017; Faust et al., 2018). Elevated freshwater nitrogen concentrations, when present with elevated phosphorus concentrations, act synergistically to support eutrophication, with total P concentrations of 76 μg P/L cited as the threshold for P-induced eutrophication (Stackpoole et al., 2019).

In a major review Faust et al. (2018) documented that 70% of the nitrogen and phosphorus loads to the Gulf of Mexico were conveyed via the Mississippi and Atchafalaya Rivers, with the result that in 2017 the hypoxia zone in the Gulf of Mexico attained an areal extent of 22,760 km<sup>2</sup>. To mitigate the Gulf of Mexico hypoxia zone a 45% N and P reduction in the contributing rivers is necessary (Faust et al., 2018). Concentrating on the Lower Mississippi Alluvial Valley they observed management strategies for: 1) riser control structures (controlled drainage), 2) two-stage ditches (possessing floodplain water deposition in constructed wetlands), 3) vegetated ditches, 4) low-grade weirs to estimate real and predicted reductions in nitrogen, phosphorus and sediment delivery. Each management case showed positive effectiveness in mitigating nutrient and sediment conveyance with exceptions noted for more intense rainfall events. As examples, riser control structures reduced NO<sub>3</sub>-N loads -57%, a feature attributed primarily to 50% less water flow in the tile systems, whereas two-stage ditches reduced nutrient transport because of increased hydraulic retention times, improved riparian vegetation development and associated organic matter accumulation. In Iowa, Amado et al. (2017) documented that drainage-tile flow was the primary NO<sub>3</sub>-N transport pathway, delivering 80% of the stream N and 15% to 43% of the streamflow.

In a review, Hang et al. (2016) performed a meta-study of the effectiveness of commonly employed plant carbon sources for constructed wetlands and denitrification bioreactors to immobilize nitrate-N. Most plant-based materials are effective carbon sources as electron-donors for improving the efficacy of microbial nitrate reduction; however, optimization requires appropriate biomass dosing to

provide an effective C/N ratio and further requires an understanding of the frequency of the dosing to maintain long-term nitrate-N immobilization.

Few mid-western USA field-based studies involving denitrification bioreactors connected to tile-drainage effluent emanating from large agricultural fields exist. Therefore, this study is unique in its field-based scope and consists of a corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) rotation with typical fertilization practices. The objective of this manuscript was to evaluate a controlled subsurface drainage/irrigation technology coupled with a denitrification bioreactor. Specifically, our primary goals were: 1) to evaluate the nitrate-N concentrations in the tile drainage effluent and 2) to estimate nitrate-N concentrations after passage through the denitrification bioreactor. Water chemistry from tile-drains and the inlet/outlet ports for a denitrification bioreactor were evaluated during drainage intervals in 2018 and 2019. Routine water chemical analysis consisted of pH, NO<sub>3</sub>-N, NH<sub>4</sub>-N, HPO<sub>4</sub>-H<sub>2</sub>PO<sub>4</sub>, SO<sub>4</sub>-S, Ca, Mg, K, and Na. A corn-soybean rotation, with standard nitrogen fertilization rates based on population and yield goals, was selected to provide the typical agronomic practice of the study area.

## 2. Existing Physical Infrastructure

Located in Cape Girardeau County (Missouri, USA), the David M. Barton Agriculture Research Center hosts the 40 ha (100 acre) Crop Science Unit. The Crop Science Unit has a controlled subsurface drainage and irrigation system. The controlled drainage system consists of a series of parallel 10 cm (4 inch) subsurface conduits having parallel 10-meter (30 ft) spacing collecting into 20 cm (8 inch) conducts for transport of surplus drainage water to field ditches. Irrigation and drainage are monitored by stop-log boxes fitted with adjustable baffles strategically arranged in the field to permit the restriction of water flow, allowing irrigation/drainage water to be added/removed throughout the system by gravity flow. The irrigation pumping system consists of five wells, each with capacity to provide 265 L·min<sup>-1</sup> (70-gal·min<sup>-1</sup>).

The denitrification bioreactor was constructed June 2014. Sampling ports allow water sampling from the denitrification bioreactor at the influent and effluent tile lines. The denitrification bioreactor has dimensions of 10 meters width, 20 meters length and 0.7 meters thickness. The top of the denitrification bioreactor is approximately 0.6 meters below the soil surface. Oak (*Quercus* sp) wood chips having an approximately 5 cm (2 inch) equivalent circular diameter with 1 cm thickness constitute the denitrification bioreactor packed bed fill.

### 3. Soil Resources

The soils of the Wilbur series (USA Soil Taxonomy: Coarse-silty, mixed, superactive, mesic Fluvaquentic Eutrudepts) consist of very deep, moderately well-drained soils that formed in alluvium. Six pedons show uniform silt loam textures throughout their soil profiles and display Ap—Bw—Cg horizon sequences. Soil pH gen-

erally ranges from slightly acid (pH 6.1 to 6.5) to neutral (pH 6.6 to 7.3) in the near-surface horizons to strongly acid (pH 5.1 to 5.5) and very strongly acid (pH 4.5 to 5.0) in the Bw and upper Cg horizons, whereas the deepest Cg horizons have moderate to slight acidity (pH 5.6 to 7.0). The soil organic matter contents determined by loss on ignition are generally low (less than 2 percent) and decline with increasing soil depth. Soil phosphorus (extraction using Bray1-P) and sulfur (using Ba turbidity after extraction using 2M KCl) have their greatest concentrations in the near-surface horizons, showing a somewhat discontinuous P and S decline with increasing soil depth. The exchangeable cations are dominated by calcium (Ca), especially in the near-surface soil horizons. The total acidity is appreciable, particularly in the deeper soil horizons; however, some Wilbur pedons show reduced total acidity expressions in the deeper Cg horizons. The CEC is low (<12 cmol<sub>p(+)</sub>/kg) to medium (12 - 18 cmol<sub>p(+)</sub>/kg) and roughly corresponds with the clay and soil organic matter contents. All soil analysis was performed by the University Missouri soil testing laboratory using their laboratory protocols (https://extension2.missouri.edu/programs/soil-and-plant-testing-laboratory/spl -missouri-soil-accreditation-program, verified 11 Jan 2020).

### 4. Field Protocols

Corn (*Zea mays* L.) was no-till planted on 30 April to 1 May 2018 on 30-inch rows (76.2 cm) at an eventual population of 33,000 plants/acre (81,545 plants/ha). Nitrogen was split applied at one-day pre-plant at a rate of 100 lbs N/acre (112 kg N/ha) using urea (46-0-0) and then reapplied approximately three weeks later at 125 lbs N/acre (140 kg N/ha) using urea. Polysulphate [(0-0-14) with 48% sulfate, 6% MgO and 17% CaO] was applied at a rate of 1,000 lbs/acre (1120 kg/ha) just prior to planting in 2018. Phosphorus and potassium soil fertility was assessed using a 1-hectare grid-soil testing protocol with subsequent soil fertilizer applications applied using variable rate technologies.

Soybean was no-till planted 13 May 2019 on 30-inch rows (76.2 cm) to provide an eventual population of 144,000 plants/ac (363,000 plants/ha); however, heavy spring rains and issues of soil compaction resulted in some population variance across the study area.

### 5. Water Sampling and Analysis

Water sampling of tile-drains and the denitrification bioreactor influent and effluent ports (stop-log boxes) were conducted approximately weekly during drainage intervals. Water was collected in pre-cleaned plastic collection bottles and stored in refrigeration cabinets until analyzed. Nitrate concentrations were determined using an ion specific electrode and ammonium concentrations were determined using colorimetric indophenol blue. Water pH was determined using a combination pH electrode. Soluble Ca, Mg, K and Na were determined using atomic absorption spectrophotometry. Phosphorus was determined colorimetrically using ammonium molybdate and sulfate-S was determined using Ba

turbidity after 2 *M* KCl extraction. Samples were analyzed at the University Missouri Fisher Delta Research Center using their analytical protocols (<a href="https://extension2.missouri.edu/programs/soil-and-plant-testing-laboratory/spl">https://extension2.missouri.edu/programs/soil-and-plant-testing-laboratory/spl</a> -missouri-soil-accreditation-program, verified 11 Jan 2020).

## 6. Biomass and Plant Tissue Analysis

Corn plant tissues (four replicates) were collected at preharvest (17% grain moisture) and partitioned into the following yield components: 1) leaf sheaths, 2) leaf blades, 3) ear leaves, 4) stem (culm), 5) grain and 6) shank-cob. Soybean plant tissues (four replicates) were collected at R6 and partitioned into the following yield components: 1) leaf, 2) stem, 3) pods and seed. All yield components were oven dried at 70°C until constant weight, weighed and analyzed for N, P, K, Mg, Ca, S, Na, Fe, Mn, B, Cu and Zn. Plant analysis were performed by Midwest Laboratories in Omaha, NB. Analysis of the mid-season plant tissue analysis inferred that no nutrient was substantially yield-limiting. Plant yield component dry weights, coupled with population estimates and plant tissue analysis, were employed to provide estimates of the plant uptake per ha.

## 7. Rainfall Events during the Study Period

Rainfall was monitored using a digital weather station as part of the University Missouri Cooperative Extension service located at Delta, MO. The cumulative rainfall of the 2018 and 2019 months that experienced tile-drainage are presented in **Table 1**.

Substantial 2019 rainfall occurred on 13/14 April (4.44 cm, 1.57 inches), 18 April (3.71 cm, 1.46 inches), 24/25 April (5.21 cm, 2.05 inches), 21/22 May (6.10 cm, 2.4 inches), 4 to 6 June (5.49 cm, 2.34 inches) and 15/16 July (7.04 cm, 2.77 inches).

### 8. Soil Analysis of the Corn and Soybean Rotation

Soil analysis across four soil depth increments in July 2018 illustrates soil profile nutrient distributions (**Table 2**). Soil profile textural variations are very limited with all soil depths reporting silt loam textures. Soil pH ranges from 5.7 to 7.3. Soil organic matter contents range from 2.4% in the surface (Ap) horizon to 1.1% at 18 to 24 cm. Bray-1 P soil test values suggest surplus P in the Ap horizon. Total acidity, Ca, Mg, K and Na are appropriate for productive cropping. These soil chemical values are consistent with previous soil testing.

Soil profile sampling and analysis for mid-season and preharvest was performed to assess ammonium and nitrate concentrations. In 2018, mid-season ammonium-N and nitrate-N concentrations are appreciable, reflecting the previous urea applications for corn (Table 3). The greatest nitrate-N concentrations are observed in the Ap horizon. The 2018 pre-harvest ammonium-N and nitrate-N concentrations are substantially smaller, reflecting nitrogen uptake by corn, nitrate leaching, and possible denitrification during heavy rainfall events.

Table 1. Cumulative rainfall of the 2018 and 2019 months that experienced tile-drainage.

Months	2018 (cm)	2019 (cm)
January	5.6	11.6
February	29.0	27.6
March	41.1	38.9
April	54.3	54.9
May	69.3	73.7
June	91.2	88.5
July	98.6	104.6
August	108.7	114.3
September	123.7	

Table 2. Soil fertility assessment for the tile-drainage system at the on start of the project.

Depth	pН	SOM	P	SO <sub>4</sub> -S	Acidity	Ca	Mg	K	Na	CEC
cm		%	m	mg/kg cmol/kg						
6	5.7	2.4	56	12.6	2.5	5.28	0.81	0.36	0.06	9.0
12	7.3	2.0	13	13.5	0.0	10.05	0.73	0.19	0.10	11.1
18	7.2	1.3	16	13.1	0.0	6.95	0.59	0.09	0.08	7.7
24	6.9	1.1	22	14.0	0.0	6.43	0.56	0.10	0.13	7.2

Table 3. Ammonium-N and nitrate-N mid-season and pre-harvest soil concentration values.

	Mid-	Season	Pre-I	Harvest
Depth	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N
cm		<b></b> m	g/kg	
		2018 (	(Corn)	
6	10.1	98.1	2.1	2.9
12	7.2	7.3	0.1	3.4
18	5.6	6.0	0.2	1.8
24	6.5	5.0	0.1	0.9
		2019 (S	oybean)	
6	1.3	10.4	-	10.6
12	3.2	6.6	-	3.4
18	3.9	5.4	-	2.0
24	2.8	5.1	-	1.2

<sup>(-)</sup> indicates data not collected.

In 2019, mid-season soybean ammonium-N and nitrate-N soil concentrations are substantially smaller than those observed for the corresponding corn portion of the rotation in 2018, reflecting omission of nitrogen fertilization. The greatest

2019 nitrate-N concentrations are observed for the Ap horizon, reflecting soil organic matter and surface residue mineralization. Preharvest 2019 nitrate-N concentrations are like the 2019 mid-season nitrate-N concentrations.

# 9. Nitrate, Ammonium, Sulfate, pH and Other Nutrients from the Tile-Drainage Effluent

The time frames from March to September in each year were not continuous drainage intervals; rather, tile-drainage was episodic and corresponded with substantial rainfall events. Ammonium-N and nitrate-N concentrations from the tile drainage system indicate that there exists an environmental risk upon their discharge to freshwater resources (Table 4). In 2018 mean ammonium-N concentrations were 1.6 mg NH<sub>4</sub>-N/L and nitrate-N concentrations were 24.5 mg NO<sub>3</sub>-N/L, whereas in 2019 ammonium-N and nitrate-N exhibited mean concentrations of 0.5 mg NH<sub>4</sub>-N/L and 5.6 mg NO<sub>3</sub>-N/L respectively. The greater tile drainage nitrogen concentrations in 2018 reflect the greater soil profile 2018 nitrogen concentrations associated with urea fertilization. The maximum nitrate-N concentrations of 109 mg NO<sub>3</sub>-N/L in 2018 and 17 mg NO<sub>3</sub>-N/L in 2019 did occur following heavy rainfall events, which were conducive for stimulating nitrate soil profile leaching.

Phosphorus tile-drainage concentrations exhibited means of 0.25 mg P/L in 2018 and 0.19 mg P/L in 2019. Thus, phosphorus transported by tile drainage system support the loading of P into freshwater resources. Sulfate-S, Ca, Mg, K and Na tile-drainage concentration distributions over the drainage intervals similarly corresponded to rainfall events; however, their concentrations are not currently considered a significant environmental issue.

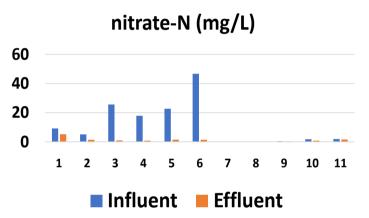
Table 4. Tile-drainage effluent nutrient concentrations and pH.

Statistic	NH <sub>4</sub> -N	NO <sub>3</sub> -N	P	K	Ca	Mg	Na	SO <sub>4</sub> -S	pН
					mg/L				
				2	018 (Corn	.)			
Max	9.8	109	0.6	3.1	64.5	13.8	15.9	25.6	8.1
Mean	1.6	24.5	0.25	1.4	46.1	11.1	9.6	8.3	7.0
STD	3.0	32.8	0.23	1.1	10.1	1.9	3.9	6.8	0.5
Min	0.1	1.5	0.05	0.1	30.5	8.1	2.7	2.4	6.5
				20	19 (Soybea	ın)			
Max	2.4	17.0	1.15	12.0	550	32.2	32.2	20.7	7.2
Mean	0.5	5.6	0.19	2.8	91.6	12.9	13.1	12.7	6.6
STD	0.6	5.1	0.30	3.1	141.2	7.2	7.3	6.0	1.9
Min	0.1	2.1	0.05	0.7	20.5	7.8	6.6	4.3	6.3

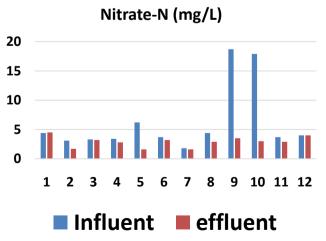
Max is the maximum value. Min is the minimum value and STD is the standard deviation. Sampling dates were: 1) for 2018 drainage interval 2-Feb, 16-Mar, 27 Apr, 15 and 25 May, 1 and 25 Jun, 16 Jul, 16 and 20 Aug and 2) for the 2019 drainage interval 21 Mar, 9 and 25 Apr, 6 and 20 and 29 May, 17 and 26 Jun, 3 and 10 and 17 and 27 Aug.

# 10. Denitrification Bioreactor Inlet and Outlet Nitrate and Ammonium Concentrations

Denitrification bioreactor effectiveness is integral to reducing nitrate migration to surface freshwater resources. The nitrate-N concentrations entering the denitrification bioreactor ranged from 0.4 to 103 mg NO<sub>3</sub>-N/L in 2018 and from 1.8 to 18.7 mg NO<sub>3</sub>-N/L in 2019, whereas the outlet nitrate concentrations typically ranged from 0.3 to 5.2 mg NO<sub>3</sub>-N/L in 2018 and from 1.6 to 4.5 mg NO<sub>3</sub>-N/L in 2019 (Table 4). Figure 1 shows that nitrate-N concentration reductions in 2018 occurred for every sampling time and Figure 2 shows that nitrate-N concentration reductions in 2019 either occurred at each sampling time or that the nitrate-N concentrations were sufficiently small that no effect was observed. The effect of the denitrification bioreactor on reducing the nitrate concentrations was significant with the paired t-test mean separation having a probability of 0.028 in 2018 and a probability of 0.033 in 2019.



**Figure 1.** Denitrification bioreactor influent and effluent  $NO_3$ -N concentrations during the 2019 drainage interval. Note: the time of sampling for #7 in 2018 was 103 mg  $NO_3$ -N/L for the influent and 1 mg  $NO_3$ -N/L for the effluent, whereas for the time of sampling 8 was 0.4 mg  $NO_3$ -N/L and 0.3mg  $NO_3$ -N/L.



**Figure 2.** Denitrification bioreactor influent and effluent NO<sub>3</sub>-N concentrations during the 2019 drainage interval.

The denitrification bioreactor ammonium-N influent concentrations were 0.1 to 3.4 mg NH<sub>4</sub>-N/L in 2018 and 0.1 to 1.3 mg NH<sub>4</sub>-N/L in 2019, whereas the effluent ammonium concentrations ranged from 0.1 to 37.1 mg NH<sub>4</sub>-N/L in 2018 and 0.1 to 2.2 mg NH<sub>4</sub>-N/L in 2019 (**Table 4**). The 2018 maximum influent concentration of 3.4 mg NH<sub>4</sub>-N/L and the maximum effluent concentration of 37.1 mg NH<sub>4</sub>-N/L suggests that microbial activity supported the liberation of ammonium from the oak wood chip packing material. The microbial reduction of nitrate to ammonium is thermodynamically permitted.

Denitrification bioreactor effluent pH levels of 6.5 in 2018 and 6.3 in 2019, promoting a slight but consistently more acidic reaction for the effluent water. Sulfate-S influent concentrations ranged from 8.8 mg SO<sub>4</sub>-S/L in 2018 and 13.1 mg SO<sub>4</sub>-S/L in 2019, whereas the effluent sulfate-S concentrations were 3.0 mg SO<sub>4</sub>-S/L in 2018 and 1.9 mg SO<sub>4</sub>-S/L in 2019. Microbial reduction of sulfate to sulfide is potentially the mechanism for the influent and effluent differences. Mean influent phosphorus concentrations were 0.26 and 0.24 mg PO<sub>4</sub>-P/L for 2018 and 2019, respectively. Mean effluent phosphorus concentrations were 0.42 and 0.27 mg PO<sub>4</sub>-P/L for 2018 and 2019, respectively, demonstrating that the phosphorus inlet and outlet concentrations were not significantly different. There is no evidence that Ca, Mg, K and Na concentrations were influenced because of bioreactor passage (Table 5).

Table 5. Denitrification bioreactor nutrient concentrations and pH.

Statistic	NH <sub>4</sub> -N	NO <sub>3</sub> -N	P	K	Ca	Mg	Na	SO <sub>4</sub> -S	pН
					mg/kg				
				Inflo	ow 2018 (C	Corn)			
Max	3.4	103	1.50	3.00	67.5	14.4	15.6	28.3	7.6
Mean	1.3	23.4	0.26	1.33	50.9	11.8	9.6	8.8	6.8
STD	1.4	31.5	0.44	0.99	12.7	1.7	3.3	7.8	6.4
Min	0.1	0.4	0.05	0.05	26.5	8.3	5.0	2.0	6.3
				Outfl	ow 2018 (	Corn)			
Max	37.1	5.2	0.95	7.55	65.5	15.7	13.3	12.1	7.8
Mean	4.5	1.5	0.42	3.43	47.1	10.0	6.7	3.0	6.5
STD	11.5	1.4	0.32	2.22	12.9	2.7	3.6	3.4	6.5
Min	0.1	0.3	0.05	0.35	29.0	6.2	0.5	0.2	6.2
				Inflov	v 2019 (So	ybean)			
Max	1.3	18.7	1.30	12.4	550	17.4	15.8	24.3	7.2
Mean	0.4	6.2	0.24	2.57	95.5	11.5	11.9	13.1	6.6
STD	0.4	5.7	0.36	3.17	143.8	3.3	2.9	5.0	6.3
Min	0.1	1.8	0.05	0.20	50	5.25	6.4	5.4	6.4
				Outflo	w 2019 (Sc	ybean)			
Max	2.2	4.5	0.55	6.25	404	9.2	10.1	3.7	6.8
Mean	0.5	2.9	0.27	3.89	60.3	6.2	6.4	1.9	6.3
STD	0.6	1.0	0.14	1.54	113.6	1.6	0.9	0.9	6.3
Min	0.1	1.6	0.10	2.15	1.5	3.5	1.7	0.6	5.9

Max is the maximum value. Min is the minimum value and STD is the standard deviation.

# 11. Nutrient Budgets Involving Corn Growth and Tile Drainage

The corn dry weights (grams/plant) are typical for mid-western USA corn production (data not shown), with seed (grain) having the greatest dry matter accumulation. Truck weights and weigh tickets suggest the corn yield was 13,181 kg/ha (11,760 lbs/acre or 210 bu/acre). The ratio of grain to total plant dry weight (Harvest Index) had a mean of 0.52. Plant tissue analysis of the plant parts (sheath, blade, culm, ear leaves, cob-shank and grain), dry matter partitioning and plant population were employed to estimate the nutrient partitioning into the mature corn crop. The total nitrogen plant uptake of 340 kg N/ha (Table 6). Of this nitrogen plant uptake quantity 231 kg N/ha are harvest removed from the soil resource. Difference between the grain yield to total plant nitrogen estimates indicates that the corn residue contained 109 kg N/ha.

Considering that the 2017 soybean residue would have provided approximately 84 kg N/ha (data not shown) and considering that the nitrogen fertilization program would have contributed 252 kg N/ha and soil mineralization (based on soil texture and soil organic matter content) would likely have provided approximately 45 kg N/ha, then a qualitative estimate of the available nitrogen would be 381 kg N/ha. Assuming that denitrification is minimal on the controlled drainage system, then approximately 40 to 170 kg N/ha is available for tile-drainage discharge because of nitrate leaching.

Soybean nutrient uptake patterns in 2019 at the R6 growth stage suggest that pods and seed collectively possessed 265 kg/ha nitrogen (**Table 7**). Total plant nitrogen uptake was estimated at 265 kg/ha. No fertilizer nitrogen was applied because of the reliance of biological nitrogen fixation to support the crop's nitrogen needs. Residue decomposition and soil organic matter mineralization supported to nitrogen economy.

Table 6. Corn 2018 nutrient accumulation (October 2018).

	N	P	К	Mg	Ca	S
Grain	231	68	80	g/ha 20	1.5	19
Total	340	93	280	40	54	29

Note: The coefficient of variation for the grain was 17% and the coefficient of variation for the residue was 13%.

Table 7. Soybean (2019) nutrient uptake/ha.

	N	P	K	Mg	Ca	S
			kg	g/ha		
Leaf	48	4	23	2	16	3
Stem	48	8	32	6	13	4
Pods	169	19	106	16	30	10
Total	265	31	161	24	59	17

#### 12. Conclusion

The denitrification bioreactor supports the immobilization of nitrate-N from tile drainage effluent. Factors that support denitrification include: 1) a nitrate source, 2) an anaerobic oxidation-reduction environment, 3) favorable pH and temperature, 4) an appropriate equilibration time interval, and 5) an effective carbon source. Data from the denitrification bioreactor suggests that nitrate is readily converted by anaerobic bacteria to dinitrogen gas  $(N_2)$ . The nitrate reduction is sufficient to mitigate nitrate transport to freshwater resources. Phosphorus transport was not sufficiently reduced and remains an environmental issue.

To improve the efficacy of the denitrification bioreactor the following agronomic best management practices seem prudent to apply: 1) apply nitrogen rates that are consistent with soil testing, the crop population and the yield goal, 2) support the split application of nitrogen fertilizer when the crop's root system may be optimally positioned to uptake nitrogen, while supporting plant growth and yield attainment, 3) consider cover crops to reduce early spring soil water contents and the conversion of soil legacy nitrates into organic biomass, 4) with controlled drainage, release drainage effluent flow volumes at sufficiently slow flowages to permit equilibrium attainment within the denitrification bioreactor, and 5) if land space is possible and suitable, release water into constructed wetlands prior to transit to freshwater resources.

#### **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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