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Estimating the Importance of Hydrologic Conditions on Nutrient Retention and Plant Richness in a Wetlaculture Mesocosm Experiment in a Former Lake Erie Basin Swamp

Bingbing Jiang [1,2,3], William J. Mitsch [1,2,*] and Chris Lenhart [4]

[1] Everglades Wetland Research Park, The Water School, Florida Gulf Coast University, 4940 Bayshore Drive, Naples, FL 34112, USA; bjiang@fgcu.edu
[2] School of Geosciences, University of South Florida, 4202 E. Fowler Avenue, Tampa, FL 33620, USA
[3] The School of Fishery, Zhejiang Ocean University, No. 1 HaiDaNanLu Street, Dinghai District, Zhoushan 316022, China
[4] Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN 55108, USA; lenh0010@umn.edu
* Correspondence: wmitsch@fgcu.edu

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Abstract: The western basin of Lake Erie, the shallowest of the Laurentian Great Lakes in North America, is now plagued by harmful algal blooms annually due to nutrient discharges primarily from its basin. Water quality was impacted so significantly by toxic cyanobacteria in 2014 that the city of Toledo's water supply was shut off, affecting hundreds of thousands of residents. A new agricultural land management approach, 'wetlaculture (=wetland + agriculture)', has a goal of reducing the need for fertilizer applications while preventing fluxes of nutrients to downstream aquatic ecosystems. A wetlaculture mesocosm experiment was set up on agricultural land near Defiance, Ohio, on the northwestern edge of the former 'Great Black Swamp'. The mesocosms were randomly assigned to four hydrologic treatments involving two water depths (no standing water and ~10-cm of standing water) and two hydraulic loading rates (10 and 30 cm week^{-1}). Nearby agricultural ditch water was pumped to provide weekly hydraulic loading rates to the mesocosms. During the two-year period, the net mass retention of phosphorus from the water was estimated to have averaged 1.0 g P m^{-2} in the wetland mesocosms with a higher hydraulic loading rate, while the highest estimated net nitrogen mass retention (average 22 g N m^{-2}) was shown in the wetland mesocosms with 10 cm of standing water and higher hydraulic loading rate. Our finding suggests that hydrologic conditions, especially water level, contribute directly and indirectly to nutrient retention, partially through the quick response of the wetland vegetation community. This study provides valuable information for scaling up to restore significant areas of wetlaculture/wetlands in the former Great Black Swamp, strategically focused on reducing the nutrient loading to western Lake Erie from the Maumee River Basin.

Keywords: agricultural runoff; wetland vegetation; hydrological loading rate; wetland water level; soil nutrient; treatment wetlands; phosphorus; nitrogen

1. Introduction

Over the last few decades, significant expansion of agricultural land use has been widely recognized for leading to global and regional negative environmental impacts, especially reduced soil fertility and increased eutrophication of surface water systems [1–3]. Phosphorus and nitrogen are the two main elements in fertilizer and are the two key limiting factors of harmful algal blooms [4]. Wetlands have long been considered as an effective way to remove nutrients from stormwater/runoff to protect downstream rivers, lakes, and groundwater [5–7]. However, the influence of regional seasonality and hydrologic conditions are still poorly understood for agricultural runoff treatment wetlands [8].

Enriched nutrient loading from agricultural runoff in the Western Lake Erie Basin (WLEB) is identified as the major nutrient source for Lake Erie, which has frequently experienced harmful algae blooms for decades that can lead to serious public health issues, such as the water supply shutting down in 2014 in the city of Toledo, Ohio, USA, due to significant toxic cyanobacteria blooms that year [9–11]. Forty percent phosphorus reduction in the Maumee River watershed has been proposed by Ohio, Michigan, and Indiana state agencies to have an impact on these annual harmful algal blooms in Lake Erie; restoring historic wetlands is highlighted as a key strategy for achieving this target goal [12,13]. Much attention in the WLEB has focused on bringing back the Great Black Swamp, also called the Black Swamp, which lost over 90% of the historic wetland for agricultural development in the twentieth century [14,15]. In fact, it was recommended by Mitsch that restoration of 400 km^2 of wetlands in the Western Lake Erie Basin would decrease the phosphorus loading by 37%, close to the 40% reduction that has been recommended as a goal for nutrient control for western Lake Erie [15].

A new wetland–agriculture integration system model, referred to as 'wetlaculture' (wetland + agriculture), is aimed to reduce the need for fertilizer applications significantly and reduce nutrient discharges to downstream aquatic ecosystems in an agriculture-wetlands landscape [15–17]. Before practicing wetlaculture at a large landscape scale and long timescale, it is important to first conduct comprehensive wetland process studies, choose suitable wetland locations, provide appropriate wetland designs, and target appropriate nutrient retention goals. Our study investigates the first two years of a wetlaculture hydrologic experiment near Lake Erie in northwestern Ohio and has direct applicability toward decreasing the nutrient loading to the eutrophic western basin of Lake Erie.

Considering the positive or negative impacts of the hydrological conditions on the biogeochemical progress in various regions [18,19], few quantitative studies have been developed on agricultural land that was initially a forest swamp. By comparing different water levels, hydraulic loading rates, investigating plant community establishment and soil development in an agricultural runoff treatment wetland system, this research provides a valuable understanding of stormwater treatment wetland mechanisms and dynamics. Moreover, dynamics based on wetland mesocosm investigations can be viewed as "physical models" that allow us to predict the behavior of landscape-scale wetlands [20–23].

In this study, the capacity of wetland mesocosms to retain phosphorus and nitrogen over a two-year period of agricultural stormwater inflows from drainage ditches near Defiance, Ohio, is investigated. The goals were to 1. determine the time series distribution of phosphorus and nitrogen fluxes through the wetlaculture mesocosm system in the first two years of what is planned to be a 10-year study; 2. compare the effects of two distinct hydrologic loading rates and water levels on nutrient removal capacity of these wetland mesocosms; and 3. investigate the response of plant richness and community establishment to the two different hydrologic loading rates and water levels after two growing seasons.

2. Methods

2.1. Site Description

The Defiance mesocosm site is located on the northwestern edge of the former 4000 km^2 'Great Black Swamp' that once was the western extreme of Lake Erie (Figure 1). The Great Black Swamp was drained entirely in the period from 1850 to 1890 and is now mainly developed as agricultural land [16,24]. As one of the primary sources of sediments and almost 85% of the phosphorus loads for Lake Erie, the Maumee River drains 17,000 km^2 of Ohio, Michigan, and Indiana [25]. Considering the goal of 50% reduction in total phosphorus loading into Lake Erie under the US–Canada Great Lakes Water Quality Agreements of 1978 and 2016, controlling the nutrient loading to Lake Erie has focused on the Maumee River as the priority for nutrient runoff control [26,27].

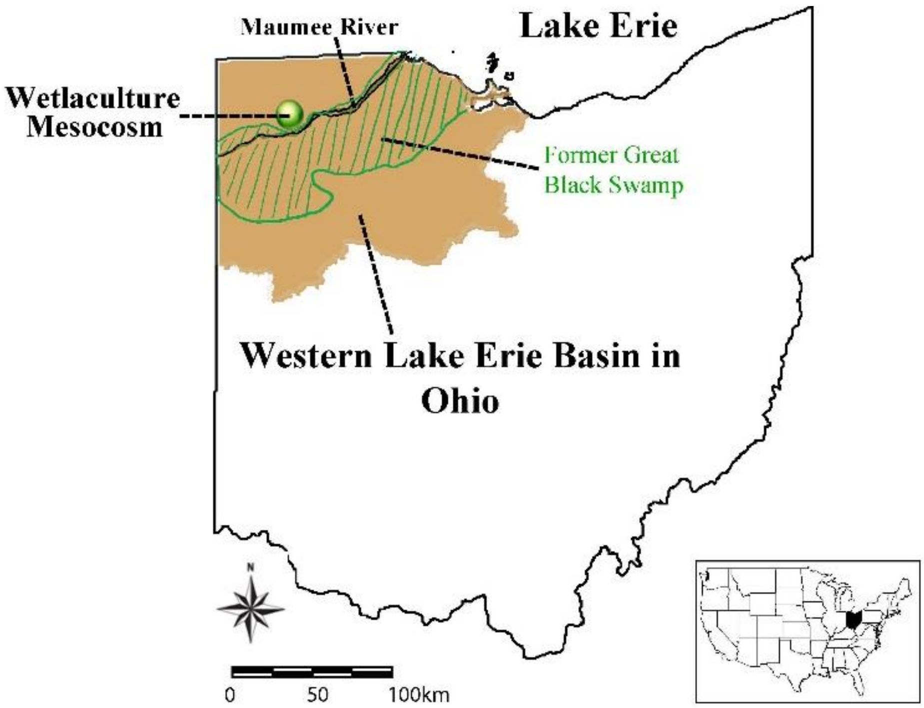

Figure 1. Location of experimental mesocosm site near Defiance in northwestern Ohio, USA.

2.2. Wetland Mesocosm Experiments

Our wetland mesocosm experiment is one of the three same wetlaculture mesocosm experiments that have already been conducted in Defiance County, Ohio (41°21′45.41″ N, 84°17′42.12″ W), Buckeye Lake, Ohio (39°55.84′ N, 82°30.07′ W), and Naples, Florida (41°21.71′ N, 84°17.95′ W) since 2016 [15,16]). All three location experiments use the same physical model designed with exactly the same hydrologic treatments [16,17]. The mesocosm compound consists of twenty-eight 380 L Rubbermaid tubs, sized 122 cm × 76 cm × 61 cm deep and constructed in Ohio. In each mesocosm, 5–10 cm of gravel was placed in the bottom to discourage clogging. Soil from the farm field used to fill the tanks was from the Hoytville Clay soil series, a typical lacustrine clay found underlying much of the former Black Swamp. Water levels were controlled by adjusting the length of vertical standpipes attached to the bottom of each tub. Local soil from the experimental site was used to fill the tubs. It consists of 20–25 cm of clay loam on top of clay and is considered prime farmland when drained by subsurface pipe. All mesocosm tubs were planted with *Schoenoplectus tabernaemontani* (bulrush), a native wetland sedge, on 16 October 2017. Water containing agricultural runoff and drainage flow from a 60-ha watershed was pumped weekly into the elevated water feed tank systems from a drainage ditch that flowed into the Maumee River via Barnes and Benien Creek in Defiance and was then fed to the mesocosms by gravity during the hydroperiod.

2.2.1. Hydrologic Experiment

The effect of hydraulic loading rate (HLR: 10 and 30 cm week^{-1}) and water level (0 cm and 10 cm water depth above the soil surface) was determined over a two-year hydroperiod. Half (14) of the mesocosms were fed weekly with a higher hydraulic loading rate (HLR_H) of 30 cm week^{-1}, and the other 14 were fed with a lower hydraulic loading rate (HLR_L) of 10 cm week^{-1}. A pump control system with NexSens G2-UW cloud-based data logger (NexSens Technology, Inc., Fairborn, Ohio, USA) was used for automatically releasing fixed amounts of water from the storage tank to each hydrological treatment mesocosm group on a weekly basis. Two different water levels were maintained for each: with standing water (SW) and without standing water (NSW) but with saturated soil that results in a 2 × 2 × 7 experiment with four different hydrologic control treatments: HLR_H

_SW, HLR_H _NSW, HLR_L _SW, and HLR_L _NSW. In the summer seasons, after flow in the ditches diminished, all the wetland mesocosms were filled with irrigation water to keep the plants alive during the dry season by maintaining a depth of 10 cm surface water. In the winter frozen season (temperature was lower than 0 °C), the vertical standing pipe of each wetland mesocosm was pulled out to drain the water from the system to protect the plants and pipes from freeze damage. The period of ditch water application to the mesocosms was determined by the ditch water level. The monthly ditch water level was recorded according to the staff gauge. Data of monthly precipitation and temperature at the Defiance, Ohio weather station, were downloaded from the NOAA database [28]. Monthly potential evapotranspiration rates were estimated by the Thornthwaite equation [29].

2.2.2. Sampling and Analysis of Water

Inflow and outflow samples were collected in acid-washed bottles every two other weeks during sampling hydroperiods. All water samples were preserved in a cooler with ice packs to keep the temperature < 4 °C and shipped to the Everglades Wetland Research Park lab in Naples, Florida. Samples filtered through 0.45 µm membrane filters were sent with overnight shipping so that they could be tested for soluble reactive phosphorus (SRP) and nitrate + nitrite (NO_x-N) within 48 h. Samples with pH adjusted to between 2 and 1 were sent to the same lab by ground shipping and analyzed for total Kjeldahl nitrogen (TKN) and total phosphorus (TP). All chemical analytical methods for TKN, SRP, NOx-N, and TP followed standard methods [30–33]. Total nitrogen (TN) is estimated as the sum of TKN and NO_x-N.

2.2.3. Vegetation Survey

A vegetation survey of the wetland mesocosms was conducted on 12 August 2019. All plants inside the mesocosms were identified to species level, and percent cover estimated Wetland indicator status for identified species was determined according to the National list for Region 1 (Northeast) [34]. Species not found in this National list were noted as NL.

2.3. Calculation and Statistics

2.3.1. Water Quality Removal Rates and Fluxes

(1) Removal rate formula:

$$RE = \frac{(C_{in} - C_{out}) \times 100}{C_{in}} \tag{1}$$

where RE is removal efficiency, %; C_{in} means the nutrient concentration of inflow, mg L^{-1}; and C_{out} means the nutrient concentration of outflow, mg L^{-1}.

(2) Nutrient flux formula:

$$LR = C_{in} \times HLR \times N/100 \tag{2}$$

$$ER = C_{out} \times HLR \times N/100 \tag{3}$$

$$RR = LR - ER \tag{4}$$

where C_{in} and C_{out} are nutrient concentrations of the inflow and outflow, mg L^{-1} = g m^{-3}; HLR is the hydraulic loading rate (10 cm $week^{-1}$ or 30 cm $week^{-1}$); N means the total weeks of the hydroperiod in 2018 or 2019; LR is the nutrient loading rate of the inflow, g m^{-2} $year^{-1}$; ER is the nutrient export rate of the outflow, g m^{-2} $year^{-1}$; and RR is the nutrient retention rate of the wetland system, g m^{-2} $year^{-1}$,

2.3.2. Statistical Analyses

Statistical analysis of water quality data was performed using ANOVA and MANOVA as the two multiple comparison methods. All tests were conducted at a 95% confidence interval ($p = 0.05$). The Student's *t*-test was used to test for a significant difference between

2018 and 2019 inflow and outflow water quality. The Newman–Keuls test was applied to determine the statistical significance of differences among water quality comparisons of different hydrological treatment groups. JMP 14.0 (SAS Institute, Cary, NC) was the software used for running statistical analysis.

3. Results

3.1. Hydrologic Regime and Budgets

The running time was based on the water level of the drainage ditch, which was profoundly impacted by local precipitation and evapotranspiration. From March 2018 to October 2019, the nearby ditch water was pumped into a storage tank and flowed into the 28 wetland mesocosms weekly, for a total of 13 weeks in 2018 and 18 weeks in 2019 (Figure 2). When rainfall was less than 50 mm per month and the potential evapotranspiration rate was higher than 100 mm per month, the ditch did have not enough water to feed the wetland mesocosms (Figure 2).

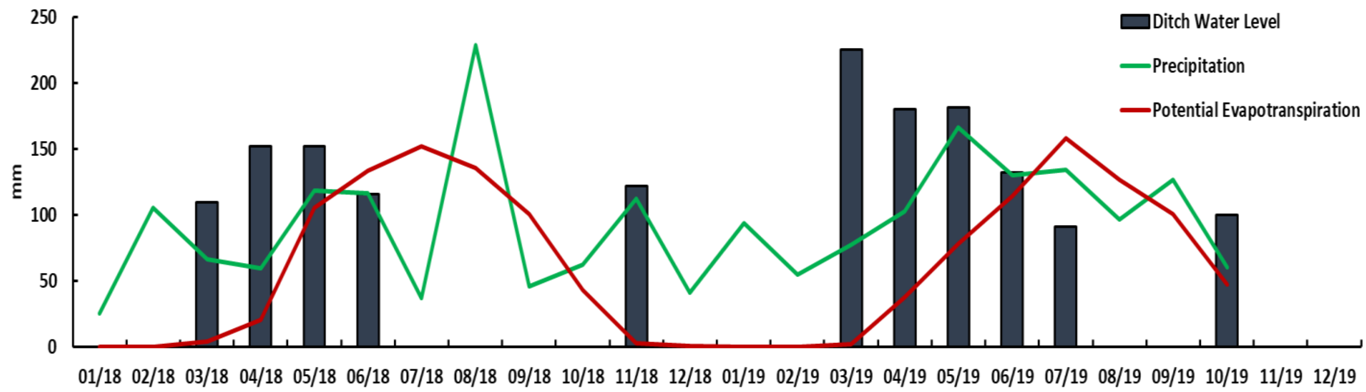

Figure 2. Monthly average potential evapotranspiration and precipitation (cm) in Defiance and monthly ditch water level (cm) from January 2018 to August 2019.

Precipitation totals during the period of sampling were approximately 270 and 540 mm, and potential evapotranspiration totals were approximately 470 and 280 mm in the 2018 and 2019 sampling periods, respectively. In total, 4030 and 5360 mm of ditch water was added to each of the high loading flow (300 mm week^{-1}) mesocosm wetlands, and 1340 and 1790 mm were added to each of the low loading flow (100 mm week^{-1}) mesocosm wetlands in 2018 and 2019, respectively. The amount of stormwater flowing through the wetlaculture system in 2019 was double the amount flowing in 2018, while the amount of water lost to evapotranspiration in 2019 was half the amount lost in 2018.

3.2. Temporal Patterns of Nutrients in the Wetland Mesocosms

3.2.1. Annual Nutrient Concentrations

Over the two-year study, the inflow total phosphorus concentrations varied from 23 to 361 μg·dm^{-3} (mean of 154 ± 10 (n = 52) μg·dm^{-3}), while the inflow total nitrogen varied from 2.26 to 7.41 mg·dm^{-3} (mean of 5.28 ± 0.18 (n = 64) mg·dm^{-3}). There was no annual statistical difference in inflow concentrations of TP and SRP (p = 0.3516 and 0.145, respectively) between the two years, while the inflow concentration of TN and NO_x-N showed a significantly lower means in 2019 compared to 2018 with decreases of 25% and 44%, respectively ($p < 0.0001$). The inflow concentration of TKN increased 52% from 2018 to 2019 (p = 0.0102) (Table 1).

Table 1. Average ± standard error (number of samples) concentration of nitrogen and phosphorus species at the mesocosm inflow and outflow and mean removal percentage in 2018 and 2019.

		2018	2019
TP	Inflow (µg·dm^{-3})	142 ± 14 (5)	162 ± 8 (8)
	Outflow (µg·dm^{-3})	57 ± 3 (139)	28 ± 2 (206) *
SRP	Inflow (µg·dm^{-3})	23 ± 5 (6)	35 ± 4 (8)
	Outflow (µg·dm^{-3})	11 ± 1 (168)	7 ± 1 (231) *
TN	Inflow (mg·dm^{-3})	6.137 ± 0.841 (7)	4.621 ± 0.494 (9) *
	Outflow (mg·dm^{-3})	4.403 ± 0.159 (195)	2.144 ± 0.097 (231) *
NOx-N	Inflow (mg·dm^{-3})	4.913 ± 0.845 (7)	2.757 ± 0.369 (9) *
	Outflow (mg·dm^{-3})	3.356 ± 0.16 (196)	0.963 ± 0.073 (233) *
TKN	Inflow(mg·dm^{-3})	1.224 ± 0.141 (7)	1.864 ± 0.184 (9) *
	Outflow(mg·dm^{-3})	1.055 ± 0.027 (195)	1.175 ± 0.036 (233) *

* indicates a significant difference in concentration between 2018 and 2019 at α = 0.05 level.

Through the two-year study, the outflow TP concentrations ranged between 2 and 177 µg·dm^{-3} (mean of 40 ± 2 (n = 344) µg·dm^{-3}), and the outflow TN concentrations ranged between 0.16 and 10.87 mg·dm^{-3} (mean of 3.19 ± 0.11 (n = 425) mg·dm^{-3}). The outflow concentrations of all phosphorus and nitrogen species in 2019 were significantly different compared to those in 2018 ($p < 0.05$) (Table 1). A 71% reduction in outflow NO_x-N concentrations from 2018 to 2019 is the highest difference; TN, TP, and SRP reduced by 51%, 51%, and 36%, respectively. However, TKN concentrations in the outflow were significantly higher in 2019 than in 2018.

3.2.2. Yearly and Monthly Nutrient Removal Efficiency

Overall, all the wetland mesocosms were net sinks of nutrients in both years. In 2018 and 2019, general nutrient concentrations were significantly lower in the outflow than in the inflow (Table 1). Moreover, the yearly mean removal efficiencies of nitrogen species in 2019 were approximately double those of 2018, while the yearly mean removal efficiency of TP and SRP increased by 38% and 59%, respectively.

Monthly removal efficiencies of nitrogen and phosphorus during the years 2018–2019 is shown in Figure 3. In 2018, the wetland mesocosm showed positive mean removal efficiencies of TP and SRP since April (40% for TP and 32% for SRP) and reached a peak in November (73% for TP and 78% for SRP). The removal efficiencies of TP and SRP in the second year were 80 ± 1 (n = 205) % and 70 ± 2 (n = 230) %.

While the removal efficiencies of TN and NO_x-N showed an increasing month-to-month trend in 2018, the removal efficiency of TKN was not stable (Figure 3). From March to July 2019, the removal percentages of TN and NO_x-N showed a strong seasonal trend (Figure 3). The results are consistent with the findings of Kadlec and Reddy, who found that nitrogen retention rates showed a significant seasonal, annual trend with an ideal water temperature range from 20 to 35 °C [6]. This is also consistent with results from the created wetlands at the Olentangy River Wetland Research Park in central Ohio, where nitrate-nitrogen concentrations were lowest and denitrification rates were highest in warmer months [35,36].

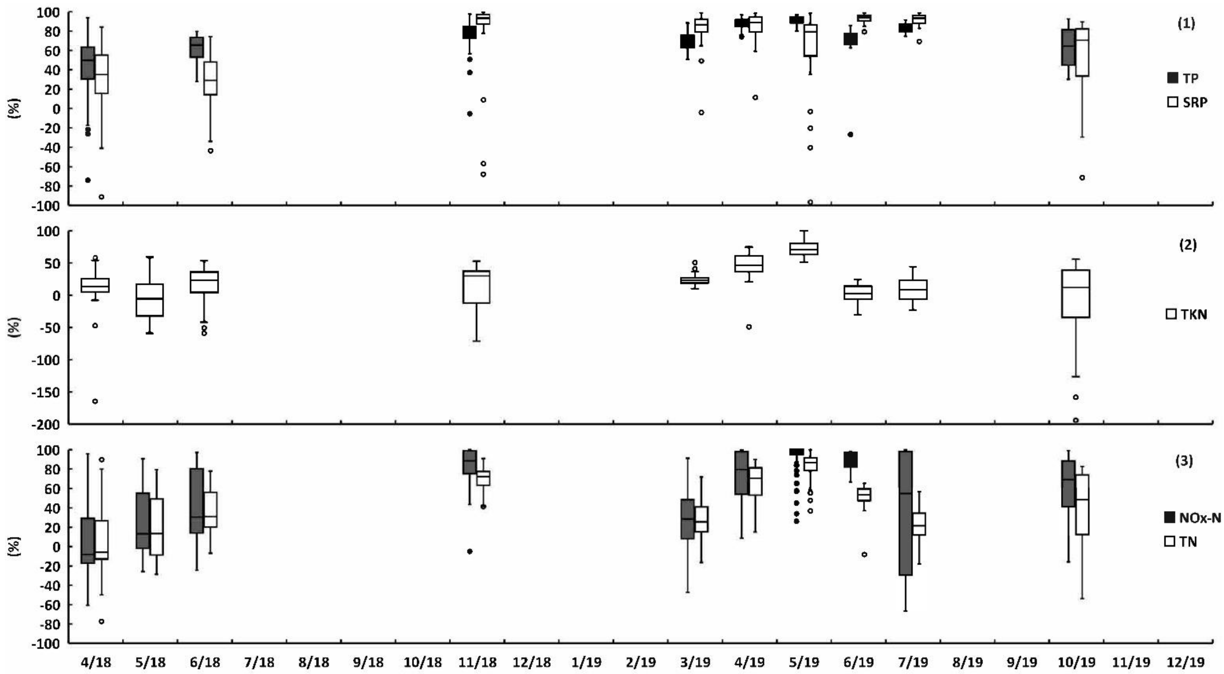

Figure 3. Removal efficiency of (**1**) TP and SRP; (**2**) TKN; and (**3**) NO_x-N (Nitrate + Nitrite) for April 2018–October 2019.

3.3. Effects of Hydrologic Conditions on Nutrient Retention

3.3.1. Removal Efficiency

Over the two years of measurements, all wetland mesocosms with different hydrologic conditions showed positive removal efficiencies of TP and TN (Table 2). The results revealed that the removal efficiencies of TN in wetlands with standing water were significantly higher than wetlands with no standing water (saturated soil) ($p < 0.05$) (Table 2). Moreover, the hydrologic treatment of HLR_L_SW showed significantly greater removal efficiency of TN compared with HLR_H_SW ($p < 0.05$). The highest removal efficiency of TP also occurred in the wetlands of HLR_L_SW ($p < 0.05$). However, there was no significant difference in TP and TN removal efficiency between wetlands of HLR_L_NSW and HLR_H_NSW.

Table 2. 2018–2019 Removal efficiency (average ± standard error (number of samples)) and mass retention of TP and TN during hydroperiod in 2018 and 2019 (total of 31 weeks) in four hydrologic treatments (HLR_H—high hydrologic loading rate = 30 cm week^{-1}; HLR_L—low hydraulic loading rate = 10 cm week^{-1}; SW—standing water: mesocosms with ~10 cm of surface water; NSW—no standing water: mesocosms with no standing water but with saturated soil). Lowercase letters indicate significant differences in the means of removal efficiency of four hydrologic treatments for TP and TN at $\alpha = 0.05$ ($p < 0.05$). Capital letters indicate significant differences in the means of mass retention of four hydrologic treatments for TP and TN at $\alpha = 0.05$ ($p < 0.05$).

		2018–2019 Removal Efficiency (%)		**2018–2019 Mass Retention (g m^{-2})**	
		HLR_H	**HLR_L**	**HLR_H**	**HLR_L**
TP	NSW	67 ± 3 (80) ^{b}	66 ± 4 (90) ^{b}	1.008 ± 0.036 (6) ^{A}	0.334 ± 0.009 (7) ^{B}
	SW	71 ± 2 (84) ^{ab}	76 ± 2 (90) ^{a}	1.076 ± 0.022 (6) ^{A}	0.373 ± 0.007 (7) ^{B}
TN	NSW	28 ± 3 (101) ^{c}	32 ± 4 (110) ^{c}	14.454 ± 0.933 (6) ^{B}	4.896 ± 0.313 (7) ^{C}
	SW	42 ± 3 (104) ^{b}	60 ± 3 (111) ^{a}	22.235 ± 1.135 (6) ^{A}	10.177 ± 0.238 (7) ^{B}

All the hydrological treatments showed significant improvement in nutrient removal ($p < 0.05$) from 2018 to 2019 except the removal efficiencies of TN and NO_x-N in HLR_L_SW (Figure 4). Although the wetlands with no standing water had relatively lower TP and TN removal efficiency than the ones with standing water, they showed a greater yearly difference in TP and TN removal efficiency between 2018 and 2019.

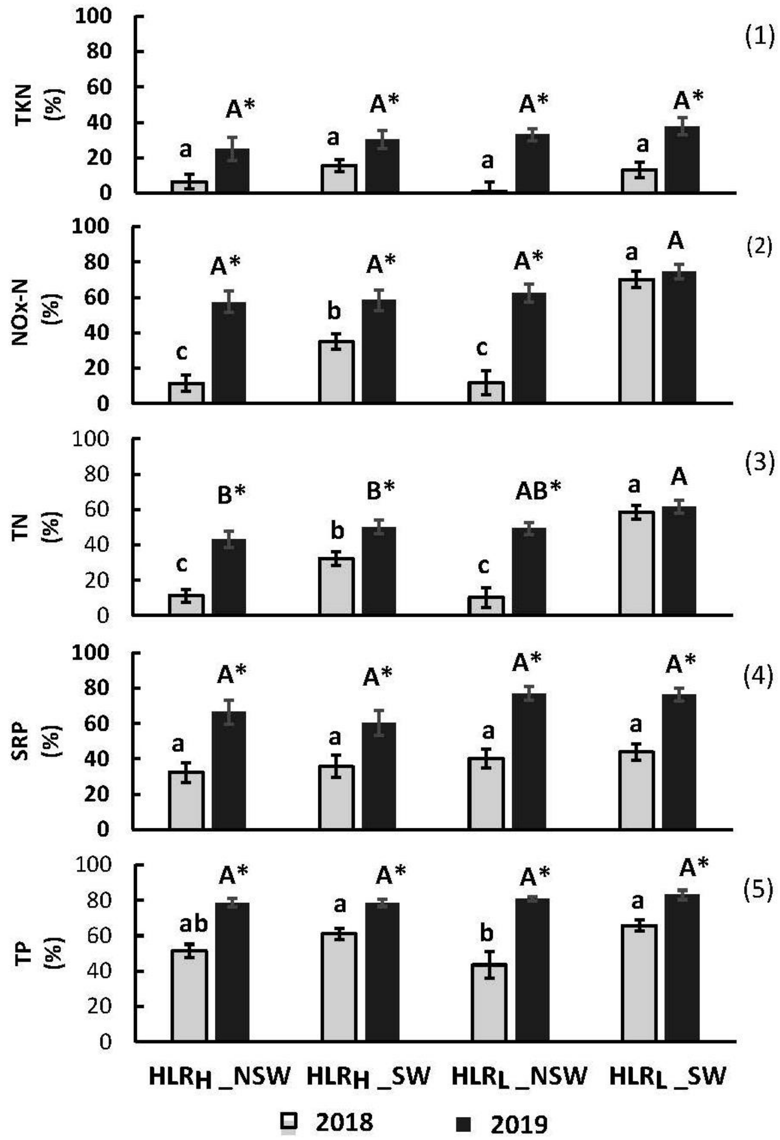

Figure 4. Mean removal efficiency (%) of (**1**) TKN; (**2**) NOx-N (Nitrate + Nitrite); (**3**) TN; (**4**) SRP; and (**5**) TP in four hydrologic treatments (HLR_H—high hydrologic loading rate = 30 cm week^{-1}; HLR_L—low hydraulic loading rate = 10 cm week^{-1}; SW—standing water: mesocosms with ~10 cm of surface water; NSW—no standing water: mesocosms with no standing water but with saturated soil) in 2018 and 2019 separately. Bars represent mean values with standard error lines and designated with different letters, which indicate a significant difference at $\alpha = 0.05$ level between the means ($p < 0.05$). The lowercase and capital letters relate to differences in removal efficiency between different hydrologic condition wetlands in 2018 and 2019, respectively. Lowercase and capital letters indicate significant differences in the means of removal efficiency of four hydrologic treatments for 2018 and 2019, respectively, at $\alpha = 0.05$ ($p < 0.05$). Mark of * indicates a significant difference between the means of removal efficiency of 2018 and 2019 for each hydrologic treatment at $\alpha = 0.05$ level ($p < 0.05$).

3.3.2. Mass Retention

Loading of phosphorus to the HLR_H and HLR_L were estimated to be 1.43 and 0.476 g P m^{-2}, while loading of nitrogen to the HLR_H and HLR_L were 49.2 and 16.43 g N m^{-2} over the two years. The mass retentions of TP in HLR_H had around three times the mass retention in HLR_L ($p < 0.05$) (Table 2), which makes sense as the flow rate for high loading rates is 200 percent higher than the low loading rates. However, there was no significant difference in phosphorus mass retention when comparing water level treatments. Listed from the highest magnitude of TN mass retention to the lowest, the treatments are ordered HLR_H_SW, HLR_H_NSW, HLR_L_SW, and HLR_L_NSW (Table 2). While the removal efficiency of TN is lower in HLR_H_SW, the total mass retention of N in HLR_H_NSW is approximately 142% of the retention in HLR_L_SW.

All the hydrologic treatments showed a significant increase from 2018 to 2019 in all nutrient mass retentions except NO_x-N mass retention in HLR_L_SW, which showed slight yet significant declines (Figure 5). In 2018, all the nutrient mass retentions in HLR_L_SW were significantly greater than HLR_H_SW, while in 2019, this was only true for the TN and NO_x-N mass retentions.

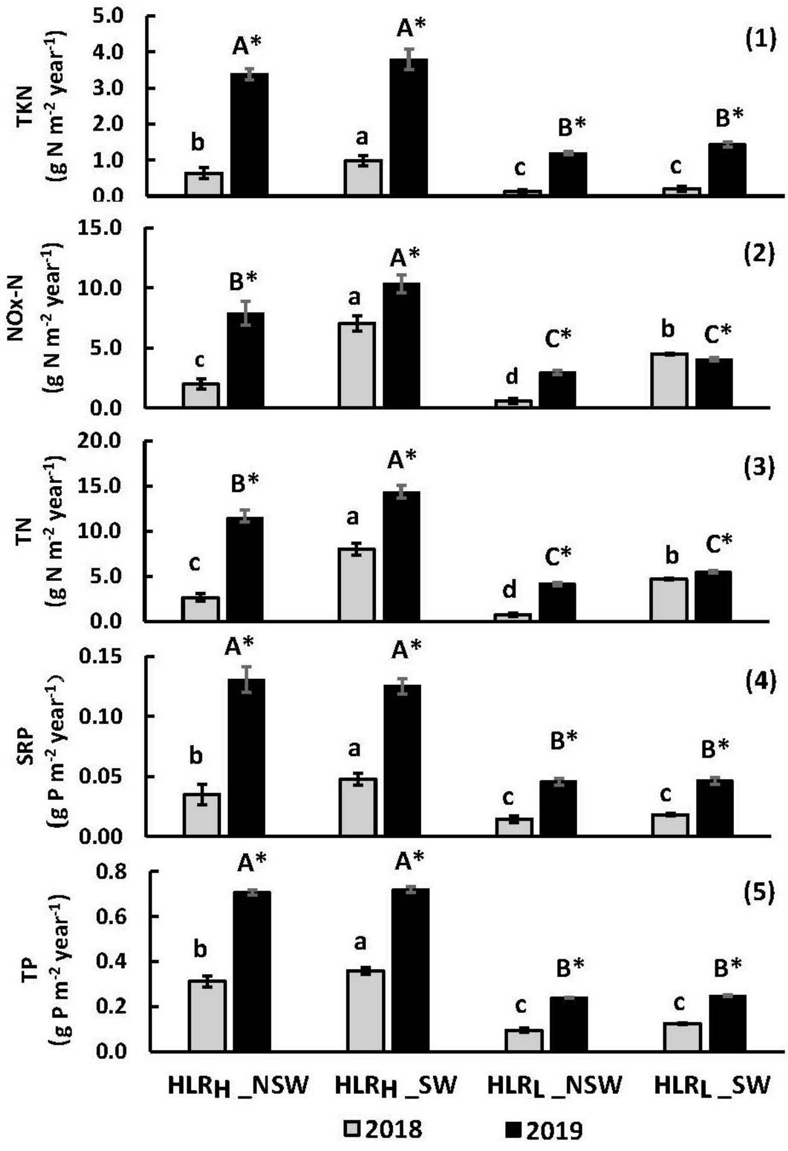

Figure 5. Nutrient retention in annual hydroperiod: 13 weeks in 2018 and 18 weeks in 2019 of (**1**) TKN; (**2**) NOx-N (Nitrate + Nitrite); (**3**) TN; (**4**) SRP; and (**5**) TP in four hydrologic treatments (HLR_H—high hydrologic loading rate = 30 cm week^{-1}; HLR_L—low hydraulic loading rate = 10 cm week^{-1}; SW—standing water: mesocosms with ~10 cm of surface water; NSW—no standing water: mesocosms with no standing water but with saturated soil) in 2018 and 2019 separately. Bars represent mean values with standard error lines and designated with different letters, which indicates a significantly difference at $\alpha = 0.05$ level between the means ($p < 0.05$). The lowercase and capital letters relate to differences of removal efficiency between different hydrologic condition wetlands in 2018 and 2019, respectively. Mark of * indicates a significant difference of each hydrologic condition wetland group between 2018 and 2019 at $\alpha = 0.05$ level.

3.4. Vegetation Cover and Species Richness

During the 2019 plant survey, a total of eleven new species were identified in the wetland mesocosms (Table 3); three and nine species were found in standing water and non-standing water mesocosms, respectively. Species richness in wetlands increased under all treatments; however, more OBL species had been found in SW than in NSW. A Kruskal–Wallis test indicated significant differences in species richness among four hydrological control treatments (p = 0.0003); however, a comparison for each pair stepwise using the Steel–Dwass method showed no significant difference between HLR_L_SW and HLRH_SW (p = 0.5712) or between HLR_L_NSW and HLR_H_NSW (p = 0.0790).

Table 3. Survey of vegetation coverage in four hydrologic treatments. HLR_H—high hydrologic loading rate = 30 cm week^{-1}; HLR_L—low hydraulic loading rate = 10 cm week^{-1}; SW—standing water: mesocosms with ~10 cm of surface water; NSW—no standing water: mesocosms with no standing water but with saturated soil). Plant species and coverage were recorded for each mesocosm, and the species were categorized as OBL—obligate wetland species; FACW—facultative wetland species; FACU—facultative upland species; UPL—obligate upland species; NL—not in the list.

Scientific Name	HLR	Water Level	Indicator	Coverage (%)
Schoenoplectus tabernaemontani	H	SW	OBL	98 ± 2 (6)
	L	SW	OBL	91 ± 3 (7)
	L	NSW	OBL	69 ± 4 (7)
	H	NSW	OBL	49 ± 8 (6)
Alisma plantago-aquatica	H	SW	OBL	10 ± 0 (1)
	L	SW	OBL	6 ± 1 (5)
Carex vulpinoidea	H	NSW	OBL	12 ± 1 (5)
	L	NSW	OBL	12 ± 1 (5)
Typha spp.	L	SW	OBL	9 ± 2 (4)
Polygonum pensylvanicum	H	NSW	FACW	15 ± 7 (2)
	L	NSW	FACW	8 ± 3 (3)
Cyperus esculentus	H	NSW	FACW	8 ± 1 (4)
	L	NSW	FACW	9 ± 1 (5)
Eupatorium perfoliatum	H	NSW	FACW	5 ± 0 (1)
Rumex crispus	H	NSW	FACU	20 ± 5 (3)
	L	NSW	FACU	13 ± 1 (3)
Echinochloa crusgalli	H	NSW	FACU	10 ± 2 (3)
	L	NSW	FACU	12 ± 1 (3)
Ambrosia artemisiifolia	H	NSW	FACU	15 ± 8 (5)
Setaria viridis	H	NSW	NL	5 ± 0 (2)
	L	NSW	NL	10 ± 0 (1)
Erigeron canadensis	H	NSW	NL	5 ± 0 (1)

By August 2019, two years after the mesocosms were planted, all the wetlands maintained with standing surface water were still dominated by *S. tabernaemontani* (bulrush), which had over 90% of coverage, but the treatments with no standing surface water had only around 50–60% cover of *S. tabernaemontani* (Table 3). Three new facultative wetland species (FACW) (*Polygonum pensylvanicum*, *Cyperus esculentus*, and *Eupatorium perfoliatum*) appeared in the drier (no surface water) mesocosms. Meanwhile, in the wetlands with only saturated soil, facultative upland species (FACU) became established. The obligate wetland species (OBL) *Typha* spp. species was only found in HLR_L_SW, while *Alisma plantago-aquatica* was present in both HLR_L_SW and HLR_H_SW. Moreover, *Carex vulpinoidea* (fox sedge) was the only OBL species present in NSW. After two growing seasons, the richness of plant communities showed a more significant difference in response to various water level conditions than hydrological loading rates.

Hydrologically open wetland ecosystems with continually feeding high-nutrient water sources showed a rapid succession of wetland vegetation development in community diversity in the first ten years at the experimental wetlands at the Olentangy River Wetland Research Park in central Ohio [37,38]. Plant community composition in a self-designed system could advance ecosystem functions such as nutrient uptake and carbon sequestration [39]. However, *Typha* spp. has been considered as an issue of invasion for wetland restoration, as their monospecific dominance could lead to the competitive exclusion of planted species like *S. tabernaemontani* [40].

4. Discussion

4.1. Nutrient Retention by Treatment Wetlands

Results of mass retention rate and removal efficiency in this study provide in situ evidence that restoring wetlands in the former swamp can potentially recover a natural ecosystem that would function as a great nutrient sink in the western Lake Erie basin [14,15]. Estimated nitrogen and phosphorus mass retention rates in our mesocosm wetlands fell in the value ranges suggested by Mitsch et al., which indicates that wetland systems can retain nitrogen and phosphorus at a rate of about 0.5–5 g-p m^{-2} $year^{-1}$ and 10–40 g-N m^{-2} $year^{-1}$ [40].

While nitrogen removal efficiencies in this study are consistent with previous studies of natural treatment wetland systems, we report a relatively higher phosphorus reduction than in other created wetland systems for agricultural runoff [8]. The capacity for nutrient removal in newly created wetlands normally increases gradually in the first couple of years [38]. Some studies even showed that agricultural wetlands might have negative removal rates in the beginning but gradually following an increasing trend in removal rates [8,22]. Mitsch et al. also reported that it took wetland mesocosms from 2 to 3 years to switch from being a nutrient source to a nutrient sink in the Florida Everglades [21]. Our mesocosm wetlands became sinks generally only after two months of operation (Figure 3).

Strong plant establishment, unique characteristics of soil, and implementing an appropriate hydrologic design for our system may explain the relatively high nutrient removal rates. The importance of hydrologic control for recovering the eco-services of a self-design wetland is due to the positive and negative feedback from other components and biogeochemical processes.

4.2. Role of Water Level in Treatment Wetlands

Significantly higher nutrient removal rates of nitrogen and phosphorus were observed in our mesocosm wetlands with standing water than in those with no standing water (Table 2). Standing water is important for designing treatment wetlands, as water level contributes both direct and indirectly impacts to nutrient cycling [29].

The soil with 10 cm standing water simulated shallow flooded conditions, while saturated soil with no standing water simulated moist soil conditions. A direct impact of soil being flooded is that anaerobic conditions increase and soil redox potentials decline [41]. There are two potential indirect impacts from inundated conditions. First, with standing water, the establishment of wetland plants should occur more quickly and with higher richness [42,43]. Studies show that higher species richness of wetland plants could lead to lower nutrient concentrations of the outflows [44]. Well-developed wetland plants had an advantage in the regulation of nutrient cycling due to their root zone development [45]. Second, surface water provides a habitat for submerged plants and algae, which both contribute significantly to nutrient uptake through their metabolic cycling [41].

4.3. Role of Hydraulic Loading Rate in Treatment Wetlands

The hydraulic loading rate was a relatively insignificant factor for phosphorus and nitrogen retention in no standing water mesocosms but had a significantly negative impact in standing water mesocosms. Our results are consistent with a previous study of HLR, which reported a negative relationship between HLR and nutrient removal percentage in wetlands [43,44]. In addition to the various impacts on nutrient retention, water level and HLR also influence plant growth and community diversity development. In return, plants can impact hydrology and biogeochemical processes. Restored wetlands may have natural hydrophyte seed banks from which wetland plants may germinate during the hydroperiod if the wetland has been drained for 50 years or less; plant roots advance oxygenation and nitrification/denitrification in the soil [46,47]. A sufficient period of inundated standing water (flooding) is important for the establishment of wetland plants and the targeted function of water quality improvement [48,49].

4.4. Response and Contribution of Wetland Plants in Treatment Wetlands

The annually increasing nutrient removal rates in the mesocosms may indicate a contribution from the continued development of vegetation communities. This increase advances the biological processes involved in nitrogen and phosphorus cycling and provides stress to plants [50–52]. Engelhardt and Ritchie found that wetlands with higher macrophyte species richness show higher algal and macrophyte coverage and higher phosphorus retention [44]. However, in practice, the selection and management of plant communities for treatment systems should be designed based on consideration of many other characteristics and factors of the wetland soils, such as clogging [53]. Moreover, different wetland plant species exhibit different tolerances to different levels of nutrient loading and water depth [54]. While plants could benefit from the bioavailable nutrients from water inflows, soil also plays a key role in wetland ecosystem development.

4.5. Nutrient Accumulation in Soils

Although all the wetland mesocosms showed significant mass retention of nutrients from water sampling of inflows and outflows, we did not find a positive nutrient accumulation in the topsoil when comparing soil tests from 2017 and 2019 (Figure 6). There are many potential factors and experiment operations that could explain this observed decrease in soil phosphorus over a period when water samples indicated great phosphorus mass retention. First, the nutrient uptake by plants could be significantly higher than we expected. Mitsch et al. reported that the phosphorus flux was estimated to be approximately 11.8 g P year^{-1} stored in *Cladium* and 18 g P year^{-1} in submerged/algae in a three-year mesocosm study in south Florida. Other studies have shown that biomass harvesting could be applied as a key management method to advance the water quality improvement function of treatment wetlands [55,56]. Second, the soil samples were from the topsoil (0–10 cm) and ignored the deeper soil, which may contain significantly more phosphorus than the newly accreted sediments above [57]. Vertical distributions of phosphorus concentrations can decrease sharply above 5 cm [58]. Third, even though the soil samples were collected in late October, plants probably still had not yet died and decomposed, especially vascular plants. Thullen et al. found that only 15% of the macrophyte mass (e.g., bulrush) may totally decay in one year, suggesting that our soil samples could have missed many of the nutrients maintained in the aquatic plant metabolic cycling [59]. Finally, the operation of draining the wetland in the winter for protecting plants and pipes could result in nutrients being flushed out by rainfall during the early spring.

Compared with the other wetlaculture study in Ohio (Buckeye Lake wetlaculture site, BLW) that has the same construction design, controlled hydrology conditions, initial planting with bulrush (*Schoenoplectus tabernaemontani*), using the original hydric soil due to a historic swamp, and inflowing with nutrient-rich agricultural runoff from nearby streams or ditches, the Defiance wetlaculture (DW) site has been applied for fertilizer as farmland for decades, unlike the BLW site has never been used for farmland. Before the beginning of the wetlaculture experiment, BLW soils had lower phosphorus concentrations (519 $\pm$ 12 µg/g), higher nitrogen concentrations (0.20 $\pm$ 0.01%), and higher carbon concentrations (2.05 $\pm$ 0.14%) than the phosphorus (561 $\pm$ 18 µg/g), nitrogen (0.16 $\pm$ 0.01%), and carbon (1.96 $\pm$ 0.06%) concentrations in DW soils [17]. There were no significant differences in soil phosphorus concentrations at both sites before and after this study period. However, at the Buckeye Lake site, soil carbon and nitrogen increased significantly from 2016 to 2019 by 39% and 19%, respectively. Moreover, a one-year following up study in 2020 at BLW showed a corn crop yield of 58 $\pm$ 9.5 bu/ac without applying additional fertilizer [60].

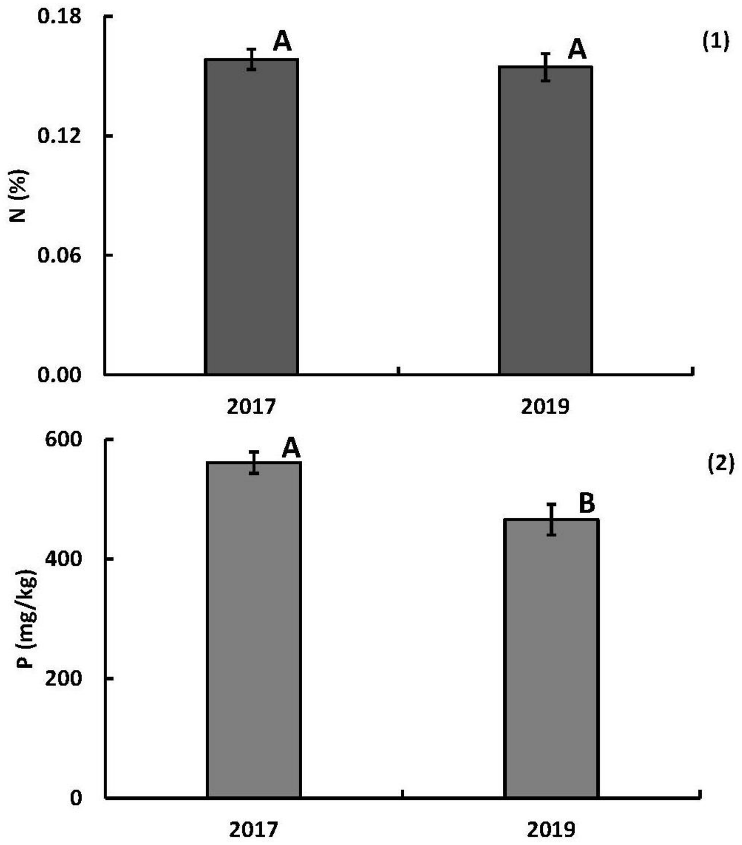

Figure 6. Concentration of (**1**) total nitrogen (%) and (**2**) total phosphorus(mg/kg) in the mesocosm soil in fall 2017 and fall 2019. Bars represent standard error. Different letters indicate significant difference at $\alpha = 0.05$ level between the two years.

4.6. Implication of Future Wetlaculture Research

This study investigated an in situ mesocosm experiment that restored wetlands' water purification functions under various hydrologic conditions. Our findings provide important evidence of the key role that wetlands can play in non-point nutrient source control; however, the results did not show significant nutrient accumulation in the soil after only two years of operation. Recovering the fertile soil conditions probably takes much longer than we initially expected. Therefore, for future wetlaculture studies, we recommend consideration of the following:

1. The below- and above-ground biomass of plants and nutrient concentrations in plants;
2. A profile of nutrient concentrations in the new accreted sediment of the topsoil;
3. The impact of draining the mesocosm wetlands in the winter;
4. Annual decomposition rates of plant detritus.

A deeper understanding of how hydrologic conditions can advance nutrient sequestration and accumulation in wetland systems will allow the model of wetlaculture approach to be applied on a landscape scale. In other words, ecological engineers can design the most efficient treatment wetland and sustainable agricultural system by selecting suitable locations, which will ultimately improve landscape water quality and public environmental health. Overall, restoring farmland to wetlands, such as a wetland already restored on this property [61] in this former swamp area, is a practical means of achieving nitrogen and phosphorus retention targets in the Western Lake Erie Basin.

Wetlaculture mesocosm experiments have been planned and conducted in the eastern USA since 2016 [15–17,48]. The first three-year results of positive nutrient retention by wetlands at the two Ohio sites (DW and BLW) allow continued study about the wetlaculture approach by converting from four to eight mesocosms from wetland to farmland in each following year. Eventually, these extensive nutrient recycling experiments will help develop a first-generation wetlaculture nutrient model that is applicable to various agricultural dominate watersheds. Moreover, long-term research using these physical models (mesocosms) will refine the creation, design, and management of an ecological landscape with a sustainable agriculture and wetland system.

5. Conclusions

Widespread eutrophication issues derived from agricultural nutrient loss impact freshwater and marine environments around the world. Wetlaculture^{TM} is a proposed landscape solution for landscape-scale pollution issues, especially harmful algal blooms, due to the over-loaded nutrient in agricultural-dominated watersheds. The wetlaculture approach to integrating agriculture and wetland ecosystems for the retention and recycling of nutrients at the local scale has the potential to not only improve water quality and decrease the need for external nutrient inputs to crop production but also to increase biodiversity, floodwater retention, and wetland habitat in increasingly homogenous agricultural landscapes. By comparing different water levels, hydraulic loading rates, vegetation community development, and soil accumulation in an agricultural runoff treatment wetland system, this research provides a better understanding of wetland mechanisms and dynamics. The wetlaculture experiments involving replicated mesocosms at Defiance, Ohio, which began in October 2017, are located at the former swamp area and have an identical design of construction and four hydrologic treatments.

a. These mesocosm wetlands created with hydric soils (Hoytville clay) left behind by the drained Black Great Black Swamp in northwestern Ohio and upstream of the western basin of Lake Erie became nutrient sinks almost immediately, with average removal efficiencies of TP and TN of 60% and 28% in 2018, and of 83% and 54% in 2019, respectively.
b. The combination of a high loading rate and 10 cm of standing water achieved the best phosphorus and nitrogen removal efficiencies through the two-year hydroperiod, averaging 76% and 60%, respectively.
c. During the two-year period, the average net mass retention of phosphorus from water sampling was estimated to be 1.0 g P m^{-2} in the wetland mesocosms with a higher hydraulic loading rate, while the highest estimated net nitrogen mass retention (average 22 g N m^{-2}) was shown in the wetland mesocosms with standing water and higher hydraulic loading rates. The mass retention of P in the high loading rate treatment was almost three times the retention of the low loading rate treatment.
d. A total of eleven new species were identified as volunteering in the wetland mesocosms to supplement the planted *S. tabernaemontani*. The coverage and species richness of wetland plants were both higher in the standing water treatments than in the no standing water treatment.
e. This study has established that the wetlaculture mesocosms under a variety of loading rates and water depths effectively removed phosphorus and nitrogen from agricultural runoff. Future wetland research in these or other mesocosm experiments should consider intersystem transformations such as plant decomposition, plant root nutrient retention, sediment retention, and other nutrient transformations, in addition to considering water quality changes.

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