



Research article

Bioremediation potential of nutrient-rich effluent using aquatic vegetation in suburban watersheds

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Abstract: Nutrient loading in watersheds from anthropogenic sources has led to eutrophication and decreased biodiversity within water bodies globally. Denitrification via aquatic vegetation can assist with bioremediation; however, its effectiveness at fine spatial scales (i.e., <1 km) in stream systems is less understood. In this study, we used 50-m-scale longitudinal observations of changes in aquatic macrophyte abundance and frequency across more than 50 species. We related species abundance to changes in nitrate concentration and other water quality metrics along a low-order stream across an urban-suburban gradient in the Hudson River Valley, New York. Observations from a 2 km section of the Fall Kill stream corridor were conducted in a summer period of lower flows ($0.14 \text{ m}^3/\text{sec}$) and a fall period of higher flows ($0.24 \text{ m}^3/\text{sec}$). For statistical and predictive analyses, we aimed to establish potential relationships between biotic and abiotic variables to identify candidate species for future applied bioremediation studies and potential instream applications. Our findings indicated that low flow nitrate concentrations most strongly relate to the abundance of *Persicaria hydropiperoides* ($r = 0.35$, $p = 0.03$) and *Ludwigia palustris* ($r = -0.29$, $p = 0.07$). The strongest relationship analyzed with high flow nitrate concentrations was floating growth forms ($r = -0.33$, $p = 0.04$), such as *Lemna* sp. Machine learning (neural network modeling), also demonstrated the importance of *Viburnum* species in predicting in-stream nitrate concentrations, suggesting another potential candidate. The compounded effects of other correlated abiotic and biotic variables were also measured and considered, such as relations between macrophyte abundance and hydrologic conditions like depth and overhead light. Determining the specific relationships between nitrate concentrations and species-level aquatic macrophyte abundance and presence at these unique fine scales provides detailed suggestions for bioremediation in similarly eutrophicated low-order, urban-suburban watersheds regionally to globally.

Keywords: ecosystem biology; urban ecosystem; bioremediation; fine-scale; biometric observation; Hudson River watershed; aquatic macrophyte

1. Introduction

Monitoring water quality throughout watersheds is imperative, as anthropogenic activities continue to increase nutrient output, showing strong control over aquatic community compositions [1–3]. Agriculture, sewage, and fertilizer runoff direct terrestrial pollutants into water, producing eutrophic conditions and altering ecosystem functionality [4,5]. These effects often occur through nitrogen and phosphorus compounds that can shift the community structure of aquatic vegetation from rooted aquatic macrophytes to more floating forms (i.e., *Lemna* species), algae, and non-native species [6,7]. Evidence of these community structural shifts has increased quickly in the past half-century, with many suggesting changing climate conditions may be furthering the trend towards eutrophic water quality [6,8]. In addition to climate change, anthropogenic inputs produce their own localized impact on water quality and aquatic habitats, which are especially notable in urban and suburban environments close to waterways.

Eutrophication is problematic for aquatic species, and growths of cyanobacteria that often accompany eutrophicated water bodies are directly dangerous to humans [9–11]. In the absence of aquatic macrophyte growth (spatially or temporally) within areas experiencing nutrient loading, phytoplankton dominate [12,13], specifically cyanobacteria, which increase in their likelihood of toxicity with less competition and more nitrogen availability. Cyanobacteria accumulate at the surface and monopolize light availability within a water body [1,2,5]. The pairing of increasing nutrient inputs and increasing temperatures due to climate change enhances harmful growths (e.g., *Microcystis*, which are toxic cyanobacteria capable of surface dominance in eutrophic waters). These can release microcystins, toxins that are potential carcinogens and can damage the liver in mammals and amphibians and are a particular threat in warm water [10,14].

In contrast, aquatic macrophytes can potentially improve water quality as they utilize nutrients for photosynthesis, provide substrate for microorganisms at a variety of depths in the water column, create shelter for smaller organisms, and exhibit an inverse relationship with harmful algal blooms and cyanobacteria growth through competition for nutrients and light [1,7,15]. Through these mechanisms, macrophyte species can assist in regulating the nutrient levels in a body of water experiencing nutrient input from anthropogenic sources [16–19]. As opposed to the algal blooms and overgrowths seen in eutrophic ecosystems, a system with a diversity of aquatic macrophyte growths can function as nature-based solutions for ecosystem protection through the positive attributes listed, which has outsized importance in a changing climate.

Understanding the relationships between eutrophication and aquatic macrophyte growth in freshwater systems has primarily focused on lake systems [13,14,20], where macrophyte growth is common and often not impeded by the regular substrate shifts and movements characteristic of low-order fluvial systems. Researchers have also found an abundance of floating growth forms, especially *Lemna* species, and a lack of emergent or submerged growth forms to be indicative of eutrophication concentrated in temperate lake ecosystems [13,14,20] or large order fluvial systems [21,22]. There have been several studies completed globally exploring the relationship between aquatic macrophytes and water quality in larger fluvial systems [19,23–25]; however, the precise link between nutrient levels and vegetation growth at extremely fine-scales (<1 km) has not been investigated directly in low-order streams. Within low-order stream systems specifically, researchers have utilized methods to determine the longitudinal variation of macrophytes, but evaluated only relationships based on physical characteristics within a river network (i.e., flow patterns and depth variability) [4] or focused on characterizing unimpacted river networks [23]. Other studies completed in similar climates have also entailed longitudinal observations to relate abiotic and biotic conditions at various spatiotemporal

scales [6,19,26,27] or described spatial distributions of plant communities over time due to anthropogenic influence [3]. In recognizing that the most common fluvial systems, low-order streams, form the initial aquatic environments upstream of the thermally stratified lake and high-order systems (which are more regularly studied in eutrophication research because of their propensity for thermal stratification), it is imperative to determine the capabilities of aquatic macrophyte species acting as cost- and time- effective bioremediation strategies for nutrient overload at finer scales [17,23,28]. Researchers have also observed high spatial variation in nutrient levels, with nitrogen levels rapidly increasing and then rapidly decreasing by 200% or more at 100-m scales within a low-order stream in the U.S. Northeast [29].

Given the limited understanding of fine spatial relationships between aquatic macrophytes and nutrient concentrations in low-order streams, and the insight into the high heterogeneity in nutrient concentration at fine spatial scales [29], we aim to determine the aquatic plant species and fluvial growth forms within low-order stream systems that may relate most to hyper-localized eutrophic conditions. This will help build an understanding of the ecohydrological processes controlling how these observed rapid changes (<100 m) of in-stream nitrogen levels arise and dissipate, with nitrate serving as a proxy nutrient to larger eutrophication concerns in this study. Further, these initial observational relationships can lead to specific conclusions about potential options for aquatic macrophytes as a bioremediation tool to aid in the improvement of similar low-order urban-suburban watersheds.

In this study, we also aim to provide a case example of a low-order watershed that is characteristic of the heavily populated urban-suburban forested landscapes of the greater Boston-NYC-Philadelphia-Washington corridor of the U.S. Northeast. Considering the need for local entities in the region to better manage habitat deterioration and eutrophication, our applied goal of this study is to understand which aquatic macrophytes could be influencing nitrogen uptake, utilization, and denitrification capabilities by way of nutrient removal through atmospheric exchange or managed seasonal plant removal [17,19,30–32], and to corroborate these possibilities against findings from similar studies [1,25,18,32,33]. These species-level relationships can then be extrapolated to creeks spanning urban-suburban gradients in similar temperate climates globally. Therefore, our specific aims of this study are to: 1) Identify and map all aquatic and stream-connected riparian species at a m-scale resolution within a low-order urban-suburban stream corridor; 2) measure similar fine-scale variations in water quality at both high and low flow regimes; 3) perform an exploratory analysis of the fine-scale spatial relationships between vegetation and water quality to identify candidate species for future applied bioremediation studies and applications in low-order urban-suburban waterways; and 4) discuss and highlight previously known details of these determined candidate species, as they may apply to aquatic pollution bioremediation potential broadly in low-order urban-suburban watersheds.

2. Methods and data sources

2.1. Site description

This longitudinal study took place along the Fall Kill, focusing on a 1.73 km stretch in Hyde Park, NY (Figure 1), starting point at 41°45'58"N 73°54'07"W and ending point at 41°46'52"N 73°53'33"W. We chose a heavily-anthropogenically-influenced section of the Fall Kill watershed in Hyde Park, New York, a sub-watershed of the Hudson River. The Fall Kill is a “Class C” stream, which enables fishing but does not enable primary contact recreation (i.e., swim and play) [34]. Surveying for this study began at Roosevelt Road and ran upstream behind Haviland Road and alongside the mobile homes

behind Jennifer Court (Figure 1). This area was of interest because it contains 55% impervious land coverage via residential land usage, with the mobile homes utilizing shared septic systems in extreme proximity to the creek [34]. It is in a region populated by forested suburbs and uniquely high densities of urban-suburban populations dependent on individualized nutrient waste management via septic systems and backyard drain fields [34,35]. These waste management systems often reside in shallow soils overlain by glacially scoured, low-permeability crystalline bedrock. These wastewater systems' runoff and other anthropogenic impacts can alter water quality in the region, influencing aquatic plant and animal habitat viability, drinking water standards, and riparian ecosystem compositional changes [2,36–38]. Vegetative observations and nutrient sampling took place every 50 meters (Figure 1).

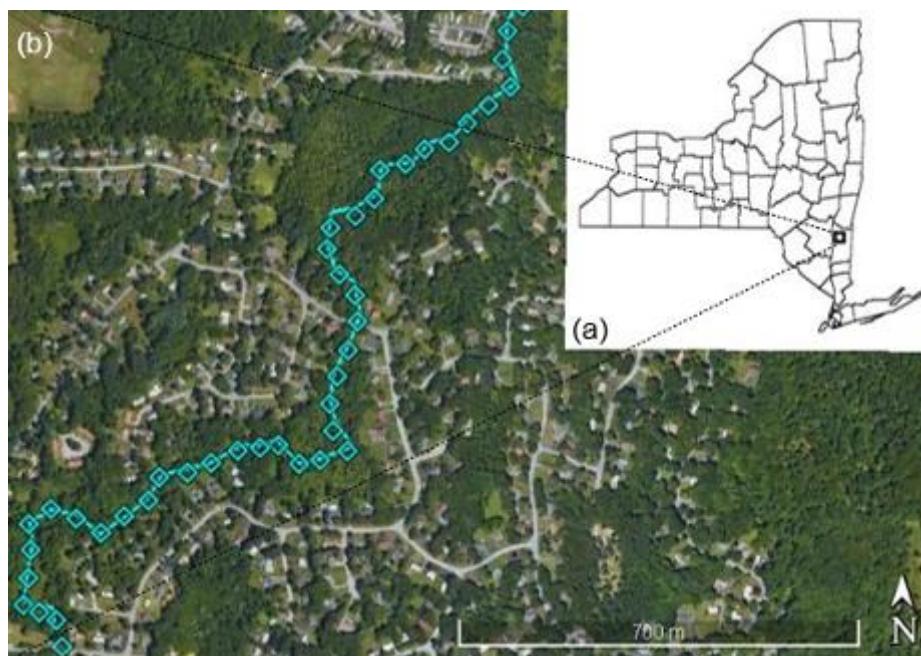


Figure 1. Location of the study area in Dutchess County, New York (a), showing the 1.73 km stretch of the Fall Kill within Hyde Park observed, with sampling every 50 meters shown through each diamond along the stream (b).

2.2. Field measurements

We included observation and data collection throughout September 2020 along the Fall Kill in Hyde Park during a low flow period ($0.14 \text{ m}^3/\text{sec}$), with additional data collection in November 2020 to quantify water quality conditions during a period of higher flows ($0.24 \text{ m}^3/\text{sec}$). These flow periods are intended to function as time frame references for the study. The length of the site area was surveyed at 50-m increments on day one in September (lower flow period) to take in-situ water quality metrics to control for time-variable water quality conditions (e.g., storms, etc.), and this was repeated for water quality during one day in November (higher flow period). Biometric surveys and environmental conditions such as depth, substrate, and canopy coverage were observed over four weeks between high and low flow water quality measurement periods to provide an average approximation across varying flow conditions.

Biometric observation for aquatic macrophyte growth followed the Braun-Blanquet [39] sampling method, which utilized transverse transects spanning the width of an observational area in the wetted channel with quadrats ($1 \times 1 \text{ m}$) along the transect at random intervals. Quadrats were given

a letter (A–J) and a number for the site observed (1–42), completing random sampling every 5 meters within one 50-meter reach (totaling 10 data points per 50 m reach). The 1 m² quadrats were used to measure percent areal abundance of each species at each of the 10 quadrat sub-sites (A–J) within each 50-meter reach. Species were initially identified using the *iNaturalist* application and corroborated with local identification guides and biology experts on campus [40], as well as determined to be floating, emergent, submerged, or algae. Species were identified through multiple avenues to confirm their identification. Samples of green algae and less conventional biological growths were collected directly from the Fall Kill and identified in the laboratory under a microscope with a biology professor proficient in plant identification.

Nutrient content (in the form of NO₃), as well as specific conductivity (SPC), temperature, pH, and dissolved oxygen (DO) were taken with an electronic, calibrated YSI ProOuatro Portable Multiparameter Water Quality Meter at 50 m increments. NO₃ was determined by an ion-selective electrode in the probe. This method requires frequent calibration, and calibration took place the day of each water quality measurement event. Nitrate was used as a proxy variable for nutrient input, chosen based on the limited resources provided for the study, and limitations of in situ electronic sensor data were noted as a shortcoming of the study. However, this method is useful in looking for relative changes over short increments of survey, which can show the same directional relationships between different forms of aquatic vegetation and spatial variation in water quality.

Depth was measured at each transect survey site with a meter stick. Stream light input was measured using a handheld spherical crown densiometer (Forestry Suppliers, Model-A, South Dakota, USA) at areas of macrophyte sampling. The dominant substrate size class was observed in areas of macrophyte sampling, and sieve size was estimated based on the Wentworth Scale, differentiating clay, silt, sand, pebble, or cobble. Relevant biotic and abiotic raw data collected at each reach for the high flow period and low flow period conditions can be seen in Tables S1–S3.

2.3. Statistical analysis

Frequency and abundance [39] for relevant biotic parameters were calculated using the scale from 0–100 based on percent coverage. Frequency of a species was calculated using Eq 1:

$$F_i = \frac{N_i}{n}, \quad (1)$$

where N_i is the number of quadrats at a site in which species (i) was present, and n is the total number of quadrats (10 for each reach). Abundance was calculated using Eq 2:

$$A_i = \sum(S_i/N_i), \quad (2)$$

where (S) signifies the sum of coverage from the percent cover chart (0–100) for species (i) across the 42 sites studied.

Biotic and abiotic variables were compared in Pearson's parametric correlational matrices using *R* open-source software (version 1.3.1093, base library collection) to determine the significance and strength of found relationships, further analyzing relationships determined to be significant based on the threshold of this exploratory study, where $\alpha = 0.2$. Due to the exploratory nature of this study, the threshold of statistical significance was left at a more liberal 80% confidence threshold ($\alpha = 0.2$), as opposed to the more common $\alpha = 0.5$. We do not account for normality because the n-size of the study was small. This is in line with other ecohydrological methods published investigating hydrologic variables in relation to species distribution [34]. Nitrate data for high and low flow periods were also

log transformed to analyze potential non-linear relationships. Significant variables related to high and low flow period nitrate concentrations were further analyzed to determine the strength of relationships and the ecological influence of each abiotic factor. Spatial analysis was completed in QGIS (version 3.16.0) to determine surrounding land use and compare nitrate concentrations along the studied section of the Fall Kill. Predictive modeling was completed through the *IBM—SPSS Modeler* application. In this analysis, using machine learning, we built a neural network model to predict the value of a target variable using all other numerical data collected (abundance, frequency, and abiotic data points). This established relationships between specific variables and their relative usefulness in the combined model in predicting a target (nitrate, species). All data (biotic and abiotic) were run against four target variables: Nitrate concentrations in (1) high flow and (2) low flow conditions, (3) *Lemna* sp. abundance, and (4) *Persicaria hydropiperoides* abundance. The target variables were chosen based on the most significant relationships found from the initial dataset. The program used machine learning to create neural network models for predicting each of the four target variables, with numerical predictive scores (from 0 to 1) for each predictor variable, where 0 was the least important predictor and 1 was the most important predictor from all other variables in the study. This quantified the relative importance of each variable in predicting the target compared to all other predictor variables (predictor variables included all other variables in the dataset besides the target variable). Note that though this analysis, we were able to only analyze one target variable at a time. As such, limitations to this methodology are noted because ecosystem facets show complex and interlinked relationships.

3. Results

Nitrate concentrations during high flow and low flow periods each had significant relationships with biotic and abiotic variables (Table 1, Figure S3). All nitrate concentrations are relevant to a collection period of one day per condition. High flow period mean nitrate concentrations were roughly double that of the mean concentrations during the low flow period. Spatial analysis exhibited a difference in nitrate concentrations as the Fall Kill flowed through a trailer park complex, impervious urbanized surfaces, and agricultural land (Figure 2).



Figure 2. Section of Fall Kill studied depicting low flow period nitrate concentrations (left) and high flow period nitrate concentrations (right) in 50-meter increments.

Table 1. Linearly and exponentially correlated variables to high and low flow period nitrate concentrations. Only select variables, where potentially significant relationships were observed, are included within the table. Bolded values indicate relationships significant at an 80% confidence threshold ($p < 0.20$), h annotations refer to high flow period conditions, and l annotations refer to low flow period conditions.

	Variable	Nitrate, h		Nitrate, l		\log_{10} (nitrate, h)		\log_{10} (nitrate, l)	
		p	r	p	r	p	r	p	r
Abiotic	temperature (h)	0.12	0.25	0.2	-0.21	0.23	0.19	0.2	-0.21
	temperature (l)	0.19	0.21	0.07	0.29	0.08	0.28	0.07	0.29
	SPC (h)	<0.01	-0.62	0.62	0.08	<0.01	-0.58	0.77	0.05
	SPC (l)	0.07	-0.29	0.28	0.17	0.06	-0.3	0.26	0.18
	DO (h)	0.1	-0.26	0.57	-0.09	0.05	-0.31	0.52	-0.11
	pH (h)	<0.01	0.9	0.46	0.12	<0.01	0.88	0.35	0.15
	pH (l)	<0.01	0.53	0.03	0.34	<0.01	0.53	0.02	0.38
	depth (l)	0.06	-0.31	0.72	-0.06	0.06	-0.3	0.69	-0.06
Biotic	<i>P. hydropiperoides</i>	0.3	0.17	0.03	0.35	0.26	0.18	0.03	0.35
	<i>Lemna</i> sp.	0.03	-0.34	0.56	0.09	0.08	-0.28	0.46	0.12
	floating species	0.04	-0.33	0.57	0.09	0.08	-0.28	0.47	0.12
	<i>L. palustris</i>	0.51	0.11	0.07	-0.29	0.71	0.06	0.05	-0.31
	<i>S. americanum</i>	0.18	-0.22	0.58	0.09	0.27	-0.18	0.55	0.09
	<i>C. foemina</i>	0.38	0.14	0.2	0.21	0.46	0.12	0.19	0.21

Table 2. Most abundant species of aquatic macrophytes and their corresponding frequencies, calculated by Eqs 1 and 2.

Aquatic macrophyte	Common name	Visual depiction	Abundance (%)	Frequency
<i>Cornus foemina</i>	swamp dogwood		4	0.67
<i>Lemna</i> sp.	duckweed		5.6	0.93
<i>Ludwigia palustris</i>	marsh seedbox		6.4	0.52
<i>Persicaria hydropiperoides</i>	swamp smartweed		9.5	0.45

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Aquatic macrophyte	Common name	Visual depiction	Abundance (%)	Frequency
<i>Sparganium americanum</i>	bur-reed		3.2	0.43
<i>Viburnum dentatum</i>	downy arrowwood		1.5	0.2

A summary of significant findings (Table 1) is visualized in Figure S3 through a matrix of Pearson's correlation coefficients (r) and p -values for each linear relationship. The most significant biotic relationships with high and low flow nitrate concentrations can be seen in Figures 3–6. The most significant positive relationship ($r = 0.35$) found between macrophyte species and nitrate is low flow period nitrate concentrations and *P. hydropiperoides* (Figure 3, Table 1). The most significant negative relationship ($r = -0.34$) is between high flow period nitrate concentrations and *Lemna* sp. (Figure 4, Table 1). The next most significant negative relationship ($r = -0.33$) is between high flow period nitrate concentrations and floating species (Figure 5). Another relevant potential relationship ($p = 0.05$, non-linear) is a negative relationship ($r = -0.31$) between the low flow period nitrate concentrations and *L. palustris* (Figure 6). Two other potential relationships with nitrate concentration also fall within the 80% confidence interval and are associated with *S. americanum* and *C. foemina*. Total species richness within each reach shows a negative relationship with low flow period nitrate concentrations (Figure S1), and a positive relationship with high flow period nitrate concentrations (Figure S2), though these relationships are not statistically significant ($p = 0.32$). Abiotic conditions have additional effects on biotic conditions (Figure S3) through similar p -values between closely related abiotic variables and their similar impact on biotic variables. The strongest relationships between abiotic conditions are high flow period nitrate and high flow period pH ($r = 0.90$, $p < 0.01$) and high flow period SPC ($r = -0.62$, $p < 0.01$), as well as low flow period nitrate and low flow period pH ($r = 0.34$, $p = 0.03$) (Figure S3). No causal relationships are inferred based on the exploratory design of this study.

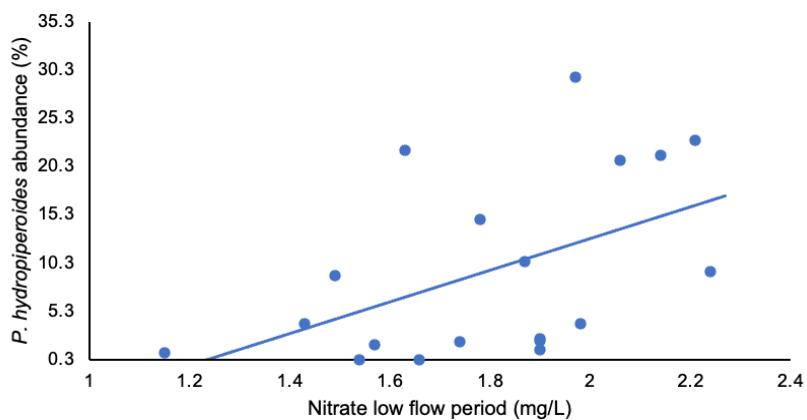


Figure 3. *P. hydropiperoides* (swamp smartweed) abundance is directly related to low flow period nitrate concentrations ($R^2 = 0.24$) and correlated ($p = 0.03$) along this stretch of the Fall Kill.

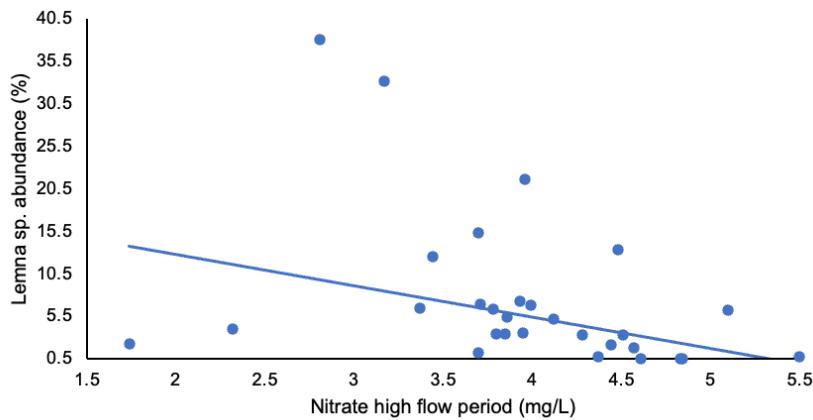


Figure 4. *Lemna sp.* (Duckweed) is inversely related to high flow period nitrate conditions ($R^2 = 0.12$) and correlated ($p = 0.03$) along this stretch of the Fall Kill.

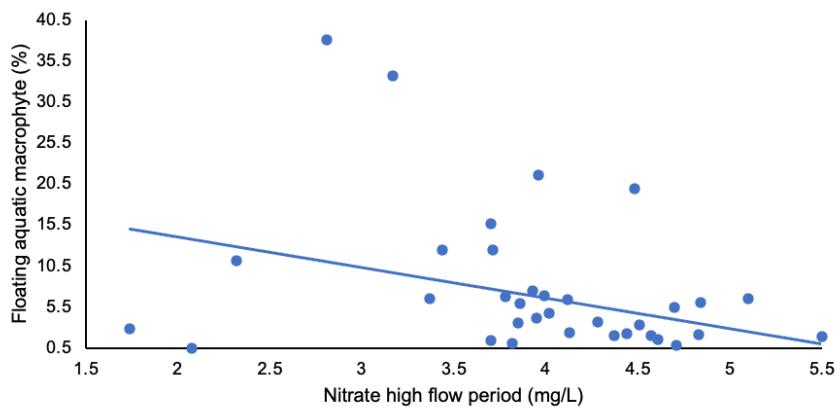


Figure 5. Floating species abundance is inversely related to high flow period nitrate concentrations ($R^2 = 0.12$) and correlated ($p = 0.04$) along this stretch of the Fall Kill.

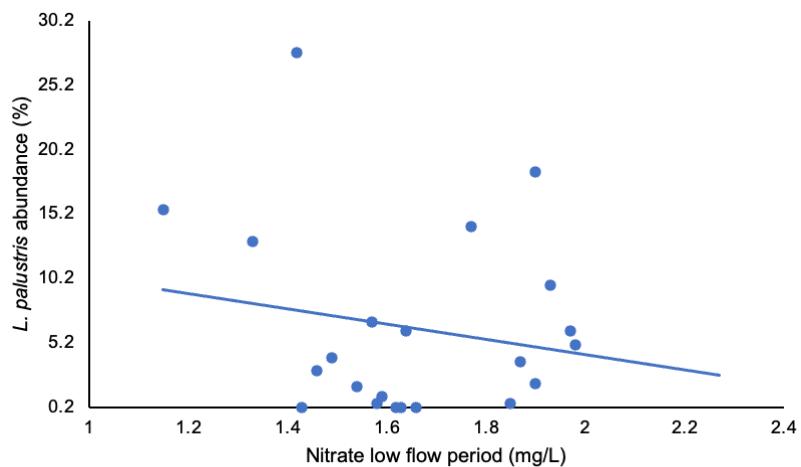


Figure 6. *L. palustris* (marsh seedbox) abundance is inversely related to low flow period nitrate concentrations ($R^2 = 0.03$) and correlated ($p = 0.07$) along this stretch of the Fall Kill.

In addition to statistical findings from the data, analysis was also completed using the *IBM—SPSS Modeler*. This software assisted in evaluating potential relationships based on the predictive capacity of a target variable, in this case, nitrate concentration (high and low flow period conditions), and *P. hydropiperoides* and *Lemna* sp. These biotic variables are chosen as target variables based on their strong correlation determination by the threshold of this study, as seen in Figures 3 and 4. The accuracy of the classification model system is demonstrated in Figure S4, visualizing the linear regression of predicted high flow period nitrate based on actual high flow period nitrate conditions. The strongest five predictor relationships for each target variable can be seen in Table 3.

Table 3. Most relevant predictive variables for each of the four target variables. Predictive numerical values range from 0–1, where *l* annotations refer to the low flow period and *h* annotations refer to high flow period. Predictor importance is derived from the model showing values closer to 1 as “more important” in relation to the target variable.

Target variable	Predictor	Predictor importance
Nitrate, <i>l</i>	<i>R. aquatilis</i>	0.27
	<i>P. sedoides</i>	0.16
	<i>Ranunculus</i> sp.	0.11
	<i>X. strumarium</i>	0.04
	<i>T. sagittatum</i>	0.03
Nitrate, <i>h</i>	<i>V. rafinesquianum</i>	0.4
	<i>P. longiseta</i>	0.2
	<i>I. verticillata</i>	0.09
	<i>P. arifolia</i>	0.07
	<i>M. laxa</i>	0.03
<i>P. hydropiperoides</i>	<i>N. advena</i>	0.09
	Canopy coverage	0.09
	<i>Elatine</i> spp.	0.07
	Temperature, <i>l</i>	0.05
	<i>P. cordata</i>	0.04
<i>Lemna</i> sp.	Canopy coverage	0.16
	<i>P. crispus</i>	0.16
	<i>Polygonum</i> sp.	0.12
	DO, <i>l</i>	0.08
	Temperature, <i>l</i>	0.07

4. Discussions

Our findings of this exploratory study suggest that various abiotic conditions in conjunction with fluctuating nitrate concentrations at two periods throughout the year may influence overall abundance and frequencies of several macrophyte species seen along the Fall Kill, and several relationships can have additive effects of multiple stressors [4,17,24,41]. These relationships can influence understanding the key ecological and hydrological drivers of many that are influencing the relationships between aquatic macrophyte species and in-stream nutrient concentrations (Sections 4.1–4.4). Despite the complexity in drivers, several possibilities have emerged from our findings that suggest certain

species that may be interacting more closely with nitrate and thus are strong candidates for additional research (Section 4.5). Overall, within this case example of a heavily impaired low-order watershed in the Northeastern U.S., our statistical exploration investigating potential linear and exponential correlations found *P. hydropiperoides* to have the strongest positive relationship, and *Lemna* sp. (as well as floating species in aggregate) to have the strongest negative relationship with nitrate (Table 2). From machine-learning-based analysis, we also found *Viburnum dentatum* and *Viburnum rafinesquianum* to be the two species most influential in predicting nitrate levels (Table 3).

4.1. Covariance of abiotic variables influencing major species-level outcomes

A lack of a clear ecological control for aquatic macrophyte species along the Fall Kill has led to conclusions about multiple controls for individual species (Table 2, Figures S1–S3). *Lemna* sp. related to high flow period pH ($r = -0.36$, $p = 0.02$) and high flow period nitrate ($r = -0.34$, $p = 0.03$) indicates that either of these variables could factor into its presence as they are also strongly correlated to one another ($r = 0.90$, $p < 0.01$). As seen in the supplementary materials (Figure S3), many species and growth forms exhibited notable relationships with multiple abiotic variables, corroborating with other studies that abiotic factors work in conjunction as ecological controls [4,23,42]. Multiple stressors on specific species can either reduce (antagonism) or amplify (synergism) individual effects of each condition, including co-variance between natural factors and anthropogenic factors, most notably nutrient concentrations, as seen in other studies [4,23]. Analysis of hydrologic conditions and environmental components together influence where macrophytes can grow [23,24], and when macrophytes can grow [19]. Because so many of these abiotic variables are dependent on one another, it cannot be stated which abiotic factor most heavily influences the presence or lack of species observed. The stronger correlations noted between relevant dependent variables support the intrinsic ecological relationships within a body of water, and the value in uncovering the most favorable conditions to influence bioremediation strategies at a specific location within a complex ecosystem [19,23,42].

4.2. Growth forms and emergence of algal growths influenced by eutrophic conditions

The most abundant growth form seen in this study is emergent aquatic macrophytes (17.98%), compared to only 6.61% floating species. This finding does not relate to other studies that have cited a structural shift towards floating species in eutrophic conditions, which have been present historically within the Hudson River watershed [1,6,13,14]. The previous studies were also not completed in similar environments (focusing on lakes, streams, and river basins outside the northeastern United States). However, it must be noted that the floating species observed are much smaller in size and more diffused (e.g., *Lemna* species) in comparison to dense beds of submerged/emergent aquatic macrophytes, and these smaller, floating species are more susceptible to faster moving water and environmental conditions (i.e., low temperature) that are common in fluvial systems. The lack of abundance of floating species, specifically that of *Lemna* species, could prove novel for this specific ecosystem, one that is in more constant motion (therefore, capable of dispersing small floating macrophytes more widely), and how it reacts to eutrophication and nutrient loading. In contrast, tolerant beds of emergent or submerged rooted species, as well as non-native species, could be the most prominent species in eutrophic low-flow streams within the northeastern United States. This gap is also notable because studies that have shown floating species, *Lemna* in particular, to be most effective in bioremediation of wastewater and toxic metals [16,18]; therefore, artificially increasing *Lemna* abundance could be an effective treatment in increasing water quality.

In addition, there was evidence of many algal growths, such as dense mats of green filamentous algae (*Ulothrix*, *Oedogonium*, and *Spirogyra*), diatoms, and multiple cyanobacterium species. These organisms are not considered aquatic macrophytes, so they are not included in abundance observations. However, it is worth noting because the New York State Department of Environmental Conservation does not define a numerical standard for nutrient concentrations forming eutrophic conditions; rather, unacceptable amounts are those that lead to algal growths, weeds, and slimes that impair water functionality, such as those observed along this stretch of the Fall Kill (New York State DEC Nutrient Criteria). In comparison, researchers concentrated on undisturbed and pristine environments have seen very little algal growth in their analyses [23]. The variety of findings specific to this ecosystem depicts the heterogeneity of responses to different stressors between environments, geographic locations, and hydrologic conditions.

4.3. Ecohydrologic functioning of individual species

The species found to have potential relationships with nitrate concentrations during both flow periods (Table 1) have been corroborated in other studies, particularly *Lemna* species [1], *Polygonum* spp. [18,28,32,33], *L. palustris*, and *S. americanum* [28,32]. The latter three aquatic macrophytes are obligate wetland species and generally contribute to mid-range denitrification potentials within a natural freshwater wetland [32]. Though not explicitly studied here, a proposed relationship was drawn between denitrification potential and plant structural productivity; this has also been widely studied globally [17,18,23,41,42]. Findings from this study suggest there are several species potentially linked to nitrate concentrations on the Fall Kill that may either benefit from increasing nutrient concentrations as anthropogenic input continues to increase (*C. foemina*, *P. hydropiperoides*), or they may be indicative of a threshold of nitrate concentrations being reached (*L. palustris*, *Lemna* sp., *S. americanum*, floating species). Preliminary knowledge that elicits further exploration into the mechanisms by which the more resilient species can withstand increasing nitrate concentrations.

4.4. Predictive modeling

Further analysis using machine learning conducted through automated AI modeling via the *IBM—SPSS Modeler* software establishes predictive strength, which is determined by the level of incorporation of a variable, relative to the other variables, in a neural network model created to predict a designated target variable. The strongest relationships are determined by the highest predictor importance values for each variable within each of the models created for each target value. When the high flow period nitrate values are targeted, the highest predictor values include *Viburnum rafinesquianum* (0.40) and *Persicaria longiseta* (0.20). The predictive capacity of the model for the high flow period nitrate values can be seen in Figure S4, and all top predictor values are listed in Table 3. When low flow period nitrate values were targeted, the highest predictor values include *Viburnum dentatum* (0.27), *Penthorum sedoides* (0.16), and *Ranunculus aquatilis* (0.11). Both high and low flow period nitrate concentrations are most strongly predicted by *Viburnum* species, an emergent species that is more aligned with a riparian tree than a macrophyte. This information is important for further exploration in future research to determine the relationship between the *Viburnum* species and nitrate utilization within this localized area.

In addition to the nitrate target values, analysis was completed for some of the most relevant species found through statistical analysis discussed previously, seen in Table 3 and supplementary Figures S5 and S6. In both models, species are most strongly predicted by the light availability (canopy

coverage) overhead of the observational area, which is a finding that could help direct future research and considerations as riparian trees continue to be at threat of removal from the expansion of urbanized areas. Studies have also corroborated this important link between overhead light availability and aquatic plant growth/nutrient uptake in areas under threat of deforestation [24,25]. The finding of the strongest predictor variables in relation to riparian trees (Table 3, Figure S4) is of interest considering the previous findings of predictors for nitrate concentration, as *Viburnum* species are an emergent woody species that have a large influence on riparian shading. After analyzing the data and realizing that riparian trees of various levels of growth were relevant in predictive conditioning, it is decided that their inclusion remains in the study although they are not technically an aquatic macrophyte. The species and process interactions listed above (e.g., the interplay of nutrient content, *Viburnum* species., shading, *P. hydropiperoides*, and *Lemna* species) should be studied further to determine the nature of their relationship with nutrients and one another and any potential benefit in using these factors together to influence stream conditions.

4.5. Considerations for future studies and potential for bioremediation

Findings from this system suggest that the species of highest interest for consideration in potential bioremediation efforts is *P. hydropiperoides*. This species shows the most strongly correlated positive relationship with increasing nitrate concentrations, and it would be beneficial to further examine the physical mechanisms by which this specific species can utilize nutrients (specifically nitrates) that are being loaded into the stream from upstream anthropogenic sources. Moreover, this species can then be viably extracted before senescence to artificially remove those nutrients from the aquatic system. However, it is important to also consider the 10 species of the 51 identified that are potentially influential for low-order, humid, temperate watersheds in relation to nitrate concentrations. Both *P. hydropiperoides* and *L. palustris* are considered relevant for the low flow nitrate period in the summer months, while *S. americanum* and *Lemna* sp. are considered relevant to the high-flow nitrate period in the fall months. Researchers have found similar importance for these specific species regarding nitrate utilization in various scales of watersheds and geographic locations globally, but applications must be tailored to low-order, temperate stream environments [1,23,28,32,33], and the viability of these aquatic macrophytes in bioremediation efforts needs to be further established as eutrophication within these ecosystems continues to be a threat [13,19–22,42]. While these preliminary findings lack exploration of chemical mechanisms by which specific species are successful in utilizing surplus nitrates within the water, as well as accompanying long-term flow pattern regimes associated with abiotic conditions, this knowledge provides clues towards species of interest. In addition, findings demonstrate possible ecohydrological interactions that are useful for directing additional studies that could inform remediation efforts in similar low-order watersheds, in corroboration with widely existing research focused on chemical mechanisms by which aquatic macrophytes can utilize nutrients at different periods in a year [17–19,42]. Mechanistic functionality of these important species and linkages to longer-term flow pattern data is a crucial next step in determining bioremediation efficacy specific to a certain ecosystem that can also be applied on a broad scale.

5. Conclusions

In conclusion, *P. hydropiperoides* is the most promising species for the studied niche in terms of bioremediation efforts in an ecosystem facing increasing nitrate inputs, as its abundance is most strongly linked to increasing nitrate concentrations. However, the 10 other species listed throughout

the article, all of which hold variable relationships with increasing nitrate concentrations and other abiotic conditions of a low-order stream, should also be researched further to develop time frames throughout a year period to best utilize the natural capabilities of these aquatic macrophyte species in specific bioremediation efforts. The physical mechanism of nutrient uptake by aquatic macrophytes is highly variable, and this study serves only as an exploratory basis for suggesting species that further work can explore more conclusively with a priori hypothesis-based deductive observation and experimentation. Through this further exploration, meaningful nature-based bioremediation can be a functional solution to increasing eutrophic conditions in streams and water bodies through bespoke outplantings, empowering watershed committees through community engagement. Although this study was localized to a small sub-section of the Hudson River Watershed in New York, findings of specific species and their respective relationships with relative nitrate levels have the potential to be applied to fluvial ecosystems and environments with similarly eutrophic conditions and aquatic macrophyte assemblages.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Kathryn Samarro was the primary researcher on this study. She participated in all aspects of the study, including designing the study, modeling the methodology, collecting data in the field, and organizing field work, as well as writing the manuscript and analyzing the data. P. Zion Klos participated in designing the study, formalizing methodology, assisting with data analysis, editing the manuscript, and supervising the study.

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