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Contribution of macrophytes to nitrogen removal in free water surface and submerged bed constructed wetlands

Sean Matus

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Environmental Resources Engineering
With Honors

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APPROVED

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Abstract

In constructed wetlands, macrophytes and bacteria are considered the drivers of nitrogen removal. Understanding how well macrophytes contribute to nitrogen removal and the influencing factors can be used as a design consideration for the improvement of these systems. This study investigated the nitrogen removal through uptake by *Cyperus alternifolius* and *Typha angustifolia* in constructed wetlands. *Cyperus alternifolius* and *Typha angustifolia* contributed 2.87% – 15.9% of the total nitrogen removal under slightly stressed conditions in the constructed wetlands when the plants were slightly stressed by high concentrations of ammonia.

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Acknowledgements

Special thanks to Dr. Tao for his continued support throughout this project as my advisor. His contributions and guidance made this report possible. I would also like to thank the previous classes of ERE 475 whose data collection contributed to aspects of this report.

Additionally, I would like to extend my thanks to Dr. Murphy for his support.

1. Introduction

Constructed wetlands have proven to be effective in removing nutrients from wastewater (Vymazal, 2007). By utilizing the natural processes, constructed wetlands can improve water quality in a manner that is considerably less energy intense than traditional methods. This is possible through the combination of macrophytes and microbes. Macrophytes take up nitrogen and phosphorus as nutrients. Ammonia at low concentrations can serve as a nutrient to plants, but at increased levels can pose a stress (Britto and Kronzucker, 2002). Another mechanism of nutrient removal in wetlands involves nitrifying and denitrifying bacteria. Nitrification is a two–step aerobic process that oxidizes ammonium to nitrite by ammonium oxidizing bacteria (AOB), and then nitrite to nitrate by nitrite oxidizing bacteria (NOB) (He et al., 2012). A third mechanism is anaerobic ammonium oxidation (anammox) which oxidizes ammonium under anaerobic conditions using nitrite (He et al., 2012). A combination of partial nitrification with AOB and anammox is known to consume less oxygen than a nitrification–denitrification process, which makes it an appealing design consideration for constructed wetlands.

This study investigates primarily to what extent nitrogen assimilation in macrophytes contributes to improving water quality in constructed wetlands. The study took place over the course of seven weeks from September to December in 2015 on the SUNY ESF campus. That study was then compiled with trials run in the same pilot system for similar durations over the past three years as a means of comparison. To investigate macrophyte performance, *Cyperus alternifolius* and *Typha angustifolia* were studied to quantify nitrogen removal through uptake and to what extent this removal played in the total nitrogen removal of the system. Ammonia levels were observed along with this to compare the two species' tolerances to varying concentration. Additional physical and biological parameters were measured with the goal of being able to make inferences about the nutrient removal mechanisms occurring and to see how different types of constructed wetlands compared.

2. Methods

2.1 Constructed wetland set-up

Four constructed wetlands were operated in a greenhouse on the SUNY ESF campus. For the most recent trials in 2015 and 2014, each constructed wetland had dimensions of 42 cm x 45 cm x 53 cm. Two of these were vegetated submerged bed (VSB) wetlands and two free water surface (FWS) wetlands. The VSB wetlands had a 40.5 cm deep saturated layer of marble chips along with 0.5 L of Nucor electric furnace slag that served as the packing media. The FWS wetlands had a shallow sandy-loam rooting substrate with 1.0 L of Nucor electric furnace slag. VSB wetland #2 was planted with *Typha angustifolia* while the three other wetlands were planted with *Cyperus alternifolius*. Also, the FWS wetlands were in 68-L blue PP containers with dimensions of 53 cm × 36 cm × 37.5 cm with a 14.5 cm sand-loam rooting substrate and 2 L of Nucor slag placed on the surface. The four constructed wetlands operated in parallel in 2015. This differs from 2014 where a VSB wetland and FWS wetland were operated in series.

Seeding techniques are outlined in Table 1. Anaerobically digested dairy manure (ADDM) had 41 g VS/L and 1.8 g N/L of ammonium and sludge digestate had 10 g VS/L and 1.2 g N/L ammonium. All trials underwent batch operation with a cycle length of 7 days.

Table 1: Seeding Techniques for all trials

Year	Wetland	Seeding Technique
2015	FWS 1, VSB 1&2	1 L of ADDM, 0.5 L sewage
	FWS 2	4 L of ADDM, 0.5 L sewage
2014	FWS 1/VSB 1 Series	4 L of ADDM
	FWS 2/VSB 2 Series	4 L of ADDM

2.2 Development of macrophyte height and above ground biomass relationship

A relationship between plant height and dry-weight biomass was developed to be able to measure the increase in dry weight as the plants grew. At the beginning of the study in 2015 each constructed wetland had already been established for over a year and had a combination of mature and immature plants. The mature plants were harvested from each constructed wetland, except for VSB #2 where this was done at the end of the study due to there being fewer specimens. Plants that had developed seed-heads were deemed mature. Their height, measured as the length of the stem, and wet weight were recorded. These harvested specimens were placed on a bench in the greenhouse and left to dry for the duration of a week. Their heights and dry weights were then measured again. The heights and dry weights were plotted together and a regression model was applied to generate an equation to predict above ground dry weight, which is synonymous with above ground biomass, from measured plant height.

2.3 Determination of plant nitrogen uptake rate

After the mature plants were harvested from the constructed wetlands, the immature shoots were left. The heights of these specimens were measured in place at the beginning of the study on September 15, 2015 and twice more throughout the study on October 22, 2015 and November 12, 2015 to have height data initially, 37 days later, and 21 days after that (58 days from start).

The three series of height data were used to derive corresponding dry weight values with the regression equation developed from the mature plants. An above ground change in biomass could be found by subtracting dry weights from two consecutive measuring dates. Samples of air-dried plant tissues were acid digested for determination of nitrogen content using a Lachat QuickChem 8500 series flow injection autoanalyzer. An assimilation rate of nitrogen was calculated with the following equation:

$$R_P = \left(\frac{\Delta M}{A_s D}\right) \times N_P$$

Where R_p = rate of plant assimilation for nitrogen (g N/m^2/d); ΔM = increase of plant biomass (dry weight) between two dates recording plant growth (g); A_s = wetland surface area (m^2); D = number of days between two measurements of plant growth; and N_p = plant nitrogen content measured (g N/g biomass). From this the relative contribution of plant assimilation to TIN removal was calculated as the ratio of plant assimilation rate to areal nitrogen removal rate (Tao et al., 2012).

Similar measurements were considered for below ground assimilation. For *Cyperus alternifolius*, the biomass change from root growth was not directly measured though, so it was assumed from a ratio of above ground to below ground determined from a similar

VSB wetland in the greenhouse and relevant literature. With the ratio, the below ground biomass was found and similar calculations with Equation 1 provided a below ground assimilation rate. For *Typha angustifolia*, destructive methods were implemented at the end of the study to determine a ratio of above ground to below ground biomass. This involved harvesting the mature specimens at the end of the study and separating the stems from the roots. Once the specimens could dry sufficiently, the collective masses of the stems and roots were determined and a ratio was calculated.

2.4 Physical and biological parameters measured

With the batch operation occurring in seven day intervals, measurements were taken in situ and water samples collected weekly. Temperature, water volume, pH, oxidation reduction potential (ORP), dissolved oxygen (DO) were measured in both the influent and effluent to capture the intricacies of the constructed wetlands' performance. Concentrations of ammonia, nitrite, and nitrate were determined with the autoanalyzer to assess the treatment performance.

3. Results and Discussion

3.1 Development of height and above ground biomass relationship

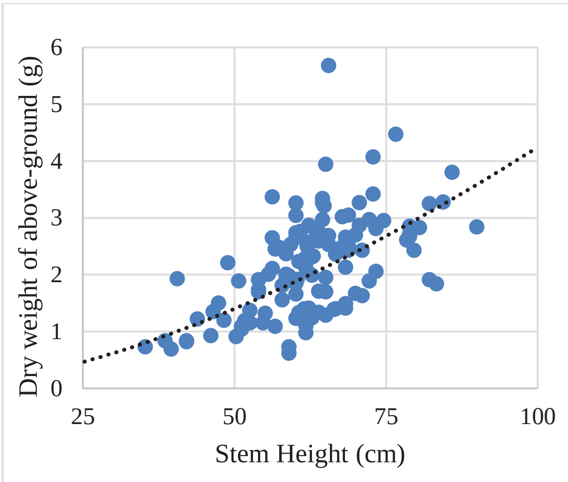

Figure 1: FWS #1 dry weight (g) vs height (cm); $R^2 = 0.39$

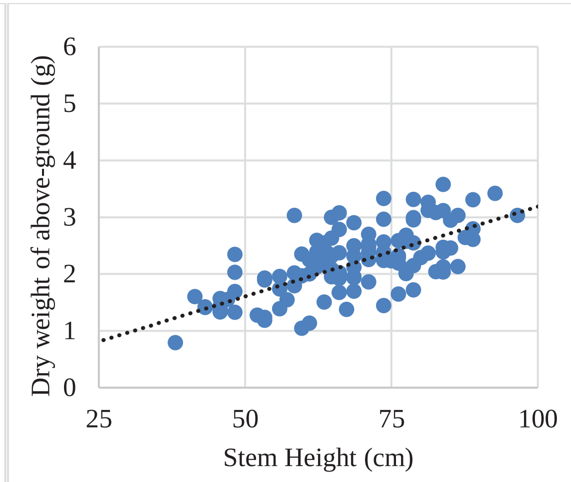

Figure 2: FWS #2 dry weight (g) vs height (cm); $R^2 = 0.47$

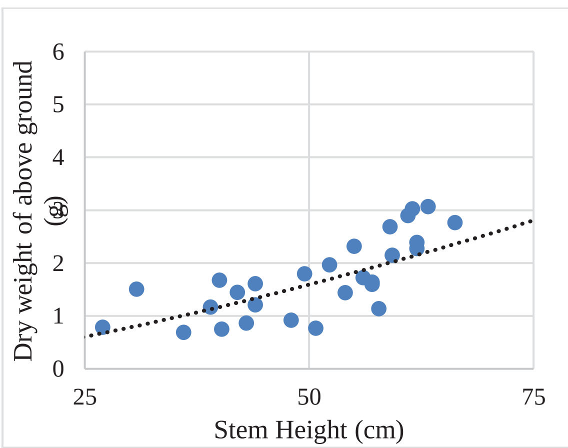

Figure 3: VSB #1 dry weight (g) vs height (cm); $R^2 = 0.49$

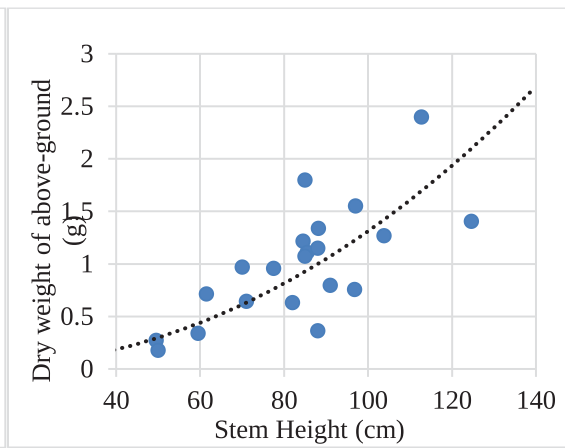

Figure 4: VSB #2 dry weight (g) vs height (cm); $R^2 = 0.62$

Figures 1–4 represent the above ground biomass verses height relationships that were developed for FWS #1, FWS #2, VSB #1, and VSB #2 respectively. The four above ground biomass prediction equations are:

$$FWS\ \#1: DW = 0.0027H^{1.6}$$

$$FWS\ \#2: DW = 0.0338H^{0.9874}$$

$$VSB\ \#1: DW = 0.0068H^{1.3938}$$

$$VSB\ \#2: DW = 0.00007H^{2.1347}$$

Where DW = dry weight in grams; H = stem height in cm

The four equations have weak adjusted R^2 and p values which introduces an aspect of uncertainty with the derived prediction values. Similar trends exist with other constructed wetland macrophytes though, such as *Phragmites australis* and *Typha latifolia* (Han and Tao, 2014; Tao et al., 2012). The weak relationship could be attributed to multiple factors. Of primary concern is the error that occurred during data collection. The materials used to collect height data were not precise enough to properly capture the trend. Errors also occurred from measurement devices not being long enough to capture the entire height of the stems at once. Because of this the stems had to be measured in parts and then those parts were summed. VSB #2 wetland had a combination of mature and immature plants for the duration of the study. At the end of the study, the mature plants were harvested and a more precise scale was utilized to acquire mass observations.

3.2 Finding above and below ground nitrogen assimilation rates

With the regression equations developed for above ground biomass, the change in biomass was calculated for every specimen measured for the two periods in 2015. The change in biomass was then divided by the 0.19 m^2 surface area and duration of period (1 = 37 days, 2 = 21 days) to find daily growth of above ground biomass. There was not a nondestructive way to measure the below ground biomass for this study for *Cyperus alternifolius*. Therefore, it was estimated based off a ratio between the above and below ground biomass. A below/above-ground ratio of 0.54 was determined from the destructive observation of a similar VSB in the greenhouse. This agrees with the ratio range of 0.44 –

0.83 presented for *Phragmites australis* (Vymazal et al., 2009) and 0.48 presented for *Typha latifolia* (Tao et al., 2012). For *Typha angustifolia*, destructive methods were utilized at the end of the study. A below/above-ground ratio of 2.73 was determined from this method. It is understandable that *Typha angustifolia* exhibits such as higher ratio because it can be characterized by long, narrow tubular stems compared to the woodier, denser stems observed with *Cyperus alternifolius* as seen by the images in Figure 5.

Figure 5: *C. alternifolius* in FWS #2 and *T. angustifolia in* VSB #2 at the beginning of the study

3.3 Plant species contribution to TIN removal

Table 2 shows the daily biomass accumulation values to find a per wetland value for the two periods in between plant height measuring. All of the values are on the low side or below the range of 2.5–15 g/m^2×day specified in Kadlec and Wallace (2009). This suggests that the macrophytes in these constructed wetlands were relatively less productive than other similar systems.

Table 2: Daily dry weight accumulation (g biomass/m^2×day) for constructed wetlands over the period of Sept to Dec 2015

	VSB1		VSB2		FWS1		FWS2	
Period	1	2	1	2	1	2	1	2
Above ground	2.84	2.90	0.41	0.60	2.66	3.07	3.73	5.29
Below	1.53	1.56	1.12	1.64	1.43	0.42	2.01	2.86

Table 3 shows the nitrogen plant assimilation rate (g N/m^2×day) for the constructed wetlands. These values were found by taking the values from Table 1 with Equation 1. Plant nitrogen content measured (g N/g biomass) for *Cyperus alternifolius* was 30.5 mg N/g biomass above ground and 18.2 mg N/g biomass below ground. For *Typha angustifolia*, nitrogen content was 24.5 mg N/g biomass above ground and 10.6 mg N/g biomass below ground. This coincides with the above ground average value 29.4 mg/g and below ground average value 26.3 mg/g presented by Tao et al. (2015) for *Cyperus alternifolius*.

Table 3: Nitrogen plant assimilation rate (g N/m^2×day) for constructed wetlands over the period of Sept to Dec 2015

	VSB1		VSB2		FWS1		FWS2	
Period	1	2	1	2	1	2	1	2
Above ground	0.087	0.088	0.006	0.008	0.081	0.094	0.114	0.161
Below	0.028	0.028	0.029	0.042	0.026	0.008	0.037	0.052

All of the values in Table 3 are very low relative to TIN removal (Table 4). The ratio of plant assimilation rate to areal nitrogen removal rate, 6.07 – 15.91%, was lower than the 11 – 47% *T. latifolia*, *C. prolifer*, and *C. papyrus* contribution to TIN removal as summarized by Tao et al. (2012). There seems to be no significant difference between wetland type and TIN removal rate with FWS #1 and VSB #1 having similar results.

Table 4: Relative contribution of plant assimilation to TIN removal for constructed wetlands over the period of Sept to Dec 2015

	VSB1		VSB2		FWS1		FWS2	
Macrophyte assimilation rate, g N/m2/d	0.114	0.117	0.035	0.051	0.107	0.101	0.150	0.213
Total inorganic N Removal rate, g N/m2/d	1.886	1.614	1.207	0.811	1.488	1.443	1.753	1.342
Relative contribution of macrophyte to TIN Removal, %	6.07	7.24	2.87	6.27	7.20	7.02	8.58	15.91

3.4 Impact of influent ammonia concentrations on plant productivity

The relatively low percent contributions could be attributed to multiple factors, and the influent concentration of ammonia could potentially be one of those factors. As seen by Tao et al. (2015), *C. alternifolius* assimilation can be significantly higher with a range of 30.0–96.8% total nitrogen removal when the plants' growth is not suppressed by a high concentration of ammonia. The average influent ammonia concentration was 153.5 mg N/L across the study in 2015. As seen in Tao et al. (2015), influent ammonia concentrations exceeding 147–236 mg N/L exerted stress on *Cyperus alternifolius* specimens, while specimens exposed to concentrations between 33–147 mg N/L experienced continued growth. This supports that the influent ammonia concentration could have posed stress on the plants resulting in lower productivity values than other systems.

Further investigation that isolates influent ammonia concentration as the sole independent variable is required to determine the significance of these levels on macrophyte production. It is important to recognize that the systems were not exposed to the influent concentration for the duration of batch operation. The ammonia concentration continually decreases during the 7 days of batch operation until the system is batched again with new influent. Thus, the macrophytes were only exposed to ammonia concentrations on the cusp of stress inducing for a very small percentage of the total study. It is seen by Tao et al. (2015) that lethal batch operating levels did not occur until weekly ammonia concentrations exceeded 300 mg N/L.

Table 4 shows that *Typha angustifolia* in VSB #2 had both lower percent contribution and absolute values of areal mass removal rate compared to the *Cyperus alternifolius* in VSB #1. These two set-ups were identical except for the plant species. This, combined with the high ammonia concentration, supports that *Cyperus alternifolius* has a higher tolerance to ammonia. Visual observations made at the end of the 2015 study provide further support. The majority of VSB #2 was showing signs of stress in the form of yellow/brown tips to the stems that were not apparent at the beginning of the study.

The trial conducted in 2014 had lower ammonia concentrations (Table 5). The 2014 trial was exposed to an average concentration of 85.8 mg N/L which coincided with the non-stress range reported by Tao et al. (2015). The plants in 2014 had a significantly higher contribution to the TIN removal (Table 6) while the TIN removal rates were roughly the same for both years. If the TIN remained constant across the two trials and macrophyte assimilation was higher in 2014, then nitrogen removal from bacterial mechanisms was less in 2014. One possible explanation relates to the effluent having a near 0 mg N/L concentration in 2014, but not in 2015. Nitrogen is a limiting nutrient. If all the nitrogen, in the form of ammonia, was consumed in 2014, then it is possible that the bacteria in the system were still capable of transforming higher concentrations of ammonia, but it was not available.

Table 5: Ammonia Concentration (mg N/L) in FWS wetlands in the fall of 2014 and 2015

	2014				2015		
	Influent		Effluent		Influent	Effluent	
Batch # of FWSs	FWS1	FWS2	FWS1	FWS2	FWS1 & FWS2	FWS1	FWS2
1	96.15	61.35	4.195	0.5	143.4	29.2	65
2	82.8	65.15	0.5	0.5	136.9	77.9	77.7
3	96.8	98.2	0.5	0.5	149.8	89.5	88.8
4	82	88.6	0.5	0.5	186.1	114	110
5	78.8	88.6	0.5	0.5	180.5	101	107
6	79	85.8	0.5	0.5	133.4	89	88.6
7					144.6	105.6	117.6
8						82.4	87.6
Mean	**85.9**	**81.3**			**153.5**		

Table 6: Macrophyte percent contribution in 2014 and 2015

	2014				2015			
	FWS1		**FWS2**		**FWS1**		**FWS2**	
Macrophyte assimilation rate, g N/m2/d	0.477	0.828	0.515	0.835	0.107	0.101	0.150	0.213
TIN Removal rate, g N/m2/d	1.521	1.241	1.093	1.322	1.488	1.443	1.753	1.342
Contribution of macriphyte assimilation to TIN Removal, %	31.4	66.7	47.1	63.2	7.2	7.0	8.6	15.9

To optimize the percent contribution of macrophytes, the influent ammonia concentration must be monitored. If concentrations are high enough to potentially induce stress on the plant specimens, then a potential solution is to further dilute the influent. This could ease the stress on the system, but can only be done if the constructed wetland is sized to be able to handle the additional water volume. With that macrophyte contribution to nitrogen removal is rarely the majority component, so optimizing this component may not be necessarily the most effective utilization of resources.

Table 7: Influent and effluent characteristics from 2014-2015. ND is representative of not detected.

		FWS1		FWS2	
		Influent	**Effluent**	**Influent**	**Effluent**
2015	Ammonia (mg N/L)	153.5 ± 21.1	86.1 ± 28.0	153.5 ± 21.1	92.8 ± 18.9
	Nitrite (mg N/L)	0.0 ± 0.0	0.7 ± 1.2	0.0 ± 0.0	0.4 ± 0.4
	Nitrate (mg N/L)	0.7 ± 0.1	2.3 ± 2.8	0.7 ± 0.1	2.4 ± 3.8
	pH	7.7 ± 0.1	7.3 ± 0.4	7.6 ± 0.1	7.0 ± 0.5
	Temperature (°C)	21.2 ± 3.2	20.7 ± 3.1	21.1 ± 3.2	20.3 ± 3.3
	DO (mg/L)			1.8 ± 0.6	1.6 ± 0.6
2014	Ammonia (mg N/L)	85.9 ± 8.3	1.1 ± 1.5	81.3 ± 14.6	0.5 ± 0.0
	Nitrite (mg N/L)	ND	ND	ND	ND
	Nitrate (mg N/L)	ND	ND	ND	ND
	pH	ND	ND	ND	ND
	Temperature (°C)	24.1 ± 2.1	23.2 ± 0.9	23.7 ± 2.1	22.8 ± 0.9
	DO (mg/L)	0.4 ± 0.3	1.9 ± 0.7	0.2 ± 0.4	3.3 ± 1.9

3.5 pH, DO, and temperature prioritization

The other major processes in nitrogen removal are nitrification-denitrification and simultaneous partial nitrification and anammox (SNA). Because anammox is an anaerobic process, the utilization of anammox bacteria is preferred over NOB as a treatment method. With that, NOB competes with AOB for oxygen in a treatment system, while also competing with anammox bacteria for nitrite (He et al., 2012). Thus, the limitation of NOB is necessary for the optimal implementation of SNA. DO was characteristically low/near zero for the FWS wetlands in this study because the water remained mostly stagnate, which could favor SNA. The optimum conditions for anammox are pH values in the range 7.5–8.0 and temperature values in the range 30–40°C (Strous et al., 1997; van de Graaf et al., 1996). Additionally, the optimum pH values to favor AOB over NOB are 7.8–8.5 (He et al., 2012). A tight optimum window is thus established, but is still achievable as seen by He et al. (2012) with the proper implementation of furnace slag. This introduces another potential explanation for the decreased bacteria productivity in the 2014 trial. Temperature is near impossible to maintain at optimum levels year round due to the northeast climate. This would just be an environmental constraint of utilizing constructed wetlands in this region.

It is seen from Table 7, that the other forms of inorganic nitrogen have a minimal impact to the study when these concentrations are compared to those of ammonia. These would also be very difficult to control from a design perspective. Further studies which maintain an optimum pH and minimal DO, while utilizing the warm season, could be used to isolate ammonia concentration as a lone independent variable. This would capture the role of macrophytes when the system is arguably operating as efficiently as possible.

4. Conclusion

As a whole, scientific literature appears to present a common theme that macrophytes play a relatively small role in nutrient uptake in constructed wetlands. That role can be inhibited by ammonia concentration posing stress on the macrophytes and further reducing their nutrient uptake. Macrophyte nitrogen assimilation was found to only contribute 2.87 – 15.91% of the TIN removal under conditions that were just within the range of stress inducing. This implies that other components of constructed wetlands should be prioritized with regards to improving nutrient levels. With that, it is important to recognize macrophytes in constructed wetlands serve more than just a role as a sink/source for nutrients and their importance with those regards is not diminished by their minimal role in nutrient uptake. Those roles include potentially supplying more oxygen to the substrate, serving as a mechanism for evapotranspiration, and playing ecological habitat roles.

It appears pH, temperature, and DO can all have impacts on both macrophyte and bacteria mechanisms. Future studies could look to affirming the optimal operating ranges of these variables, or hold them constant and try to maximize the system efficiency to see the true potential of constructed wetlands as an ammonia reduction method.

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