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The Influence of the Ratio of Nitrate to Ammonium Nitrogen on Nitrogen Removal in the Economical Growth of Vegetation in Hybrid Constructed Wetlands

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Abstract: Growing vegetables economically in the use of constructed wetland for wastewater treatment can play a role in overcoming water and food scarcity. *Allium porrum* L., *Solanum melongena* L., *Ipomoea aquatica* Forsk., and *Capsicum annuum* L. plants were selected to grow in hybrid constructed wetland (CW) under natural conditions. The impact of the ratio of nitrate to ammonium nitrogen on ammonium and nitrate nitrogen removal and on total nitrogen were studied in wastewater. Constructed wetland planted with *Ipomoea aquatica* Forsk. and *Solanum melongena* L. showed higher removal efficiency for ammonium nitrogen under higher ammonium concentration, whereas *Allium porrum* L.-planted CW showed higher nitrate nitrogen removal when NO_3–N concentration was high in wastewater. *Capsicum annuum* L.-planted CW showed little efficiency for both nitrogen sources compared to other vegetables.

Keywords: constructed wetland; NO_3–N/NH_4–N ratio; nitrogen removal; wastewater; CFW

1. Introduction

Wastewater can pollute receiving water bodies and thus need to be treated beforehand [1]. The conventional treatment processes are expensive to build especially for areas of low socioeconomic status [2]. Therefore, cost-effective and environmentally sound methods of treating wastewaters are needed. Constructed wetlands have gained popularity from the last several years and have been used as an alternative to conventional wastewater treatment methods [3] because of their easy maintenance and operation, low energy consumption, and water recyclability [4,5]. Constructed wetlands are successfully used to treat different types of wastewater [6–13]. Currently, the world population is on the edge of scarcity for water and food; therefore, the recycling of water and nutrients (in wastewater) are emerging as integral parts of water and food demand management [14]. Growing vegetation economically in the use of constructed wetlands for wastewater treatment is therefore needed and can help to reduce the gap between supply and demand [15].

Plants take up nitrogen in the form of NO_3–N (nitrate) or NH_4–N (ammonium); therefore, the total N (nitrogen) absorbed usually consists of a combination of these two forms [16,17]. The ratio of NO_3–N to NH_4–N is of a great significance and can impact plant growth. The optimum growth of plants species required a different ratio of nitrate to ammonium N. The best ratio to be applied also varies with other factors such as growth stage, temperature, pH, and soil properties [18]. NH_4–N in ionic form can compete with other forms (potassium, calcium, magnesium) for uptake by the roots [19].

An unbalanced NO_3–N to NH_4–N ratio may affect solubility and availability of other nutrients by changing the pH near the roots [20].

Several researchers have reported that NH_4–N as a sole source of N is deleterious to the growth of many higher plants [21], and a higher concentration than NO_3–N can limit the growth of plants [22,23]. In several crops, combinations of both forms (NH_4–N and NO_3–N) usually result in elevated growth compared to when either N form is used alone [24–26]. However, some plant species showed better growth when NH_4–N was the N source [27]. In a controlled environment, some plants absorb NO_3–N more rapidly [28], whereas other plants prefer NH_4–N [21]. The absorption rates of NO_3–N and NH_4–N are influenced by the ratio of NO_3–N to NH_4–N [29]. In several plant species, NH_4–N may compete with NO_3–N and inhibit NO_3–N absorption in the presence of both NO_3–N and NH_4–N [30]. However, there is no information available on the NO_3–N/NH_4–N ratio on plants species when grown in a constructed wetland with natural environment. The objective of this study was to improve the constructed wetland system for the economical growth of vegetation by examining the influence of the ratio of nitrate to ammonium N on N removal in wastewater post-treatment.

2. Materials and Methods

2.1. Experimental Site and Constructed Wetland

The research was conducted at the Southeast University campus, New District, Wuxi, China. The total area of constructed wetland is 100 m^2. The Wuxi has four distinct seasons and exist in a north subtropical humid monsoon climate zone, with rich rainfalls and sunshine. The average perennial temperature over 30 years (1981–2010) is 16.2 °C, and the average precipitation is 1121.7 mm, with 123 days of rain and 1924.3 h of sunshine [31]. Two pilot-scale hybrid constructed wetland systems were established for experimental plants. The hybrid system was a combination of Constructed Floating Treatment Wetlands (CFW) and horizontal flow constructed wetlands (HFCW). Each bed in each unit was 2.5 m × 0.3 m × 0.5 m (length × width × height) made of concrete and lined with epoxy. The first bed was designed for CFW without a substrate, whereas a second bed of each unit was packed with a 10 cm supporting layer of large gravel (30–40 mm), 25 cm of ceramsite (10–20 mm in diameter), and 10 cm of small gravel (10–20 mm). The wastewater entered in the first bed from the distribution channel, that was connected to a wastewater tank, and flow was controlled by value.

Four different types of plants were selected (Table 1) to grow in the constructed wetland, and selections were made keeping in mind the economic value of the vegetables, their easy availability in the local market, their aesthetic worth, and their ability to adapt in existing climatic conditions. Polyethylene foam boards were used for planting as floating mats in the CFW beds, and 2 cm holes were perforated for each plant. The systems were inspected on a daily basis. Special attention was paid to the inlet and outlet flows, as suspended solid-present wastewater can cause obstruction in the pipes.

Table 1. Selected plant species for experiments.

Number	Common Name	Scientific Name	Used Name
1	Leek	*Allium porrum* L.	*A. porrum*
2	Egg Plant	*Solanum melongena* L.	*S. melongena*
3	Water spinach	*Ipomoea aquatica* Forsk.	*I. aquatica*
4	Hot pepper	*Capsicum annuum* L.	*C. annuum* L.

2.2. Experimental Conditions

The experiment was carried out in the natural environment, and sewage was treated through A2O (anaerobic/anoxic/oxic) system and then artificially stimulated before being introduced into the constructed wetland. Two setups of the hybrid system were established. In the first setup, the CFW beds were planted with *A. porum*, and the HFCW beds were planted with *S. melongena*; in the second setup, *I. aquatica* and *C. annum* L were planted in the CFW and HFCW beds, respectively.

The selection of plants for each setup was random. Potassium nitrate (KNO_3) as NO_3–N, ammonium bicarbonate (NH_4HCO_3) as NH_4–N, potassium dihydrogen (KH_2PO_4) as total phosphorus, and glucose ($C_6H_{12}O_6$) as a source of chemical oxygen demand were used, whereas micronutrients were added according to Zhang et al. [32]. The pH was adjusted to 6.0 $\pm$ 0.2 with dilute NaOH or HCl. These constructed wetlands (CWs) ran for 20 days to achieve stabilization for further experiments, and the NO_3–N/NH_4–N ratio was adjusted and divided into four experimental runs (ERs) (Table 2). The hydraulic load was 0.2 $m \cdot d^{-1}$ and the hydraulic retention time was 1.25 d. The average DO in influent and effluent was 1–5 $mg \cdot L^{-1}$ and 0–2 $mg \cdot L^{-1}$, respectively. The quality of wastewater was measured at each step on a routine basis.

Table 2. Average influent stimulated wastewater quality of during experimental runs.

Experimental Run (ER)	NO_3–N/NH_4–N	NO_3–N	NH_4–N	TN	TP	COD
	mg·L^{-1}					
1	5:1	24.69	5.08	29.51	3.05	89.14
2	2:1	20.99	9.83	29.41	2.92	91.25
3	1:1	14.28	14.93	30.10	2.86	88.63
4	1:2	9.90	19.94	29.83	2.91	90.93

Here, TN = total nitrogen, TP = total phosphorus, COD = chemical oxygen demand.

2.3. Analytical Methods

Standard methods [33] were used to analyze ammonium (NH_4–N), nitrate (NO_3–N), total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) parameters in wastewater, whereas DO and pH was measured by DO200 and PH100 probes (YSI), respectively.

2.4. Statistical Method

MS Excel (Office package-16) and SPSS version-18.0 (SPSS incorporation, Chicago, IL, USA) were used for data analysis and presentation.

3. Results

3.1. NH_4–N Removal under Different NO_3–N/NH_4–N Ratios

The impact of the NO_3–N/NH_4–N ratio on the removal of NH_4–N is shown in Figures 1 and 2. For the first setup, the removal efficiency in the first bed was 76.93%, 63.94%, 30.60%, and 40.75%, and in the second bed, the removal efficiency was 90.03%, 87.88%, 73.64%, and 74.74%, whereas for the second setup, removal efficiency was 88.89%, 75.93%, 63.99%, and 58.79% and 89.23%, 82.84%, 69.72%, and 73.65% in Beds 1 and 2, respectively. NH_4–N removal efficiency was significant ($p > 0.05$) (Table 3). There was no significant difference between the two units for NH_4–N removal under different NO_3–N/NH_4–N ratios.

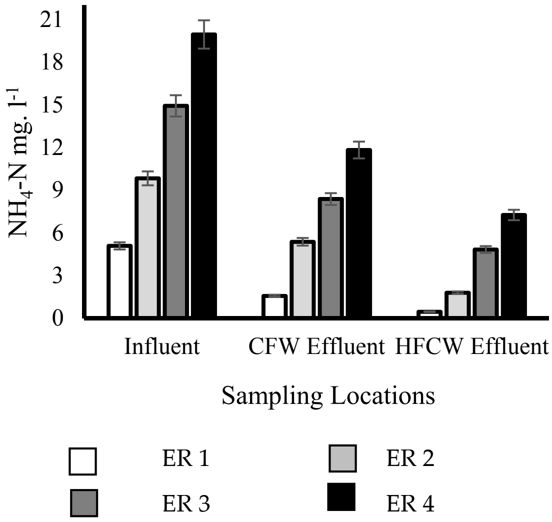

Figure 1. NH_4–N concentration during different experimental runs in the first setup.

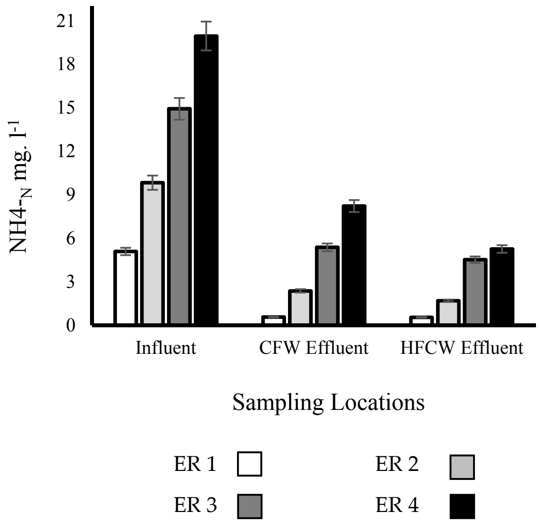

Figure 2. NH_4–N concentration during different experimental runs in the second setup.

Table 3. Estimated values of effluent during different ERs in Setups 1 and 2.

Set-up	Test	ER	Influent			CFW			HFCW		
			Min	Max	Mean ± SD **	Min	Max	Mean ± SD **	Min	Max	Mean ± SD **
			Mg·L^{-1}								
1	NH_4	I	4.20	5.90	5.061 ± 0.850	1.40	1.60	1.52 ± 0.106	0.40	0.05	0.442 ± 0.402
		II	9.70	9.90	9.8104 ± 0.1016	5.00	5.60	5.322 ± 0.302	1.58	1.98	1.782 ± 0.200
		III	14.60	15.20	14.909 ± 0.300	8.10	8.50	8.325 ± 0.205	4.71	4.91	4.8133 ± 0.1002
		IV	19.89	19.99	19.941 ± 0.050	11.70	11.90	11.806 ± 0.101	7.22	7.28	7.2521 ± 0.0302
	NO_3	I	24.40	24.80	24.630 ± 0.207	19.00	21.00	20.019 ± 1.001	8.42	8.82	8.622 ± 0.200
		II	20.80	21.20	20.997 ± 0.200	14.70	14.90	14.810 ± 0.101	6.47	6.67	6.5723 ± 0.1001
		III	14.00	14.60	14.295 ± 0.300	9.86	9.96	9.9110 ± 0.0500	6.31	6.51	6.4112 ± 0.1000
		IV	9.70	9.90	9.8036 ± 0.1002	6.37	6.77	6.571 ± 0.200	4.47	4.67	4.5703 ± 0.1000
	TN	I	27.00	29.00	28.002 ± 1.000	19.10	19.30	19.202 ± 0.100	10.64	10.84	10.741 ± 0.100
		II	29.70	29.90	29.800 ± 0.100	18.10	18.50	18.304 ± 0.200	9.04	9.08	9.0623 ± 0.0204
		III	29.05	29.15	29.100 ± 0.050	15.48	15.88	15.683 ± 0.200	12.10	12.52	12.315 ± 0.210
		IV	28.00	30.00	29.033 ± 1.002	16.58	16.78	16.682 ± 0.100	11.00	13.20	12.118 ± 1.100

** probability value > 0.001.

Table 3. *Cont.*

Set-up	Test	ER	Influent Min	Influent Max	Influent Mean ± SD **	CFW Min	CFW Max	CFW Mean ± SD **	HFCW Min	HFCW Max	HFCW Mean ± SD **
			Mg·L^{-1}								
2	NH_4	I	4.20	5.90	5.061 ± 0.850	0.30	0.80	0.555 ± 0.250	0.34	0.74	0.543 - 0.200
		II	9.70	9.83	9.8104 ± 0.1016	2.25	2.45	2.3553 ± 0.1004	1.40	1.80	1.629 ± 0.206
		III	14.60	15.20	14.909 ± 0.300	5.07	5.67	5.372 ± 0.300	4.30	4.70	4.507 ± 0.200
		IV	19.89	19.99	19.941 ± 0.050	8.10	8.30	8.2064 ± 0.1006	4.20	6.20	5.252 ± 1.000
	NO_3	I	24.40	24.80	24.630 ± 0.207	14.29	16.29	15.293 ± 1.000	9.70	11.70	10.709 ± 1.000
		II	20.80	21.20	20.997 ± 0.200	9.56	11.56	10.561 ± 1.000	6.60	8.60	7.623 ± 1.001
		III	14.00	14.60	14.295 ± 0.300	7.42	7.82	7.620 ± 0.200	6.00	6.41	6.209 ± 0.205
		IV	9.70	9.90	9.8036 ± 0.1002	3.10	5.10	4.103 ± 1.000	4.70	5.30	5.013 ± 0.301
	TN	I	27.00	29.00	28.002 ± 1.000	17.75	21.75	19.75 ± 2.00	7.95	8.64	8.276 ± 0.348
		II	29.70	29.90	29.800 ± 0.100	20.52	22.52	21.523 ± 1.000	6.48	7.00	6.743 ± 0.260
		III	29.05	29.15	29.100 ± 0.050	17.45	21.45	19.45 ± 2.00	10.50	11.62	11.082 ± 0.561
		IV	28.00	30.00	29.033 ± 1.002	18.12	22.12	20.12 ± 2.00	10.56	12.56	11.563 ± 1.000

** probability value > 0.001.

3.2. NO_3–N Removal under Different NO_3–N/NH_4–N Ratios

Figures 3 and 4 show NO_3–N removal in Setups 1 and 2 under different NO_3–N/NH_4–N ratios. In the first setup, the NO_3–N removal rate was 18.77%, 29.36%, 30.60%, and 33.01% in the CFW bed and 65.07%, 68.67%, 55.10%, and 53.41% in the HFCW bed. In Setup 2, removal efficiency was 38.04%, 49.68%, 46.31%, and 58.10% for the first bed and 56.55%, 63.47%, 56.48%, and 48.63% for the second bed. In both cases, there was a significant difference ($p < 0.05$) between the hybrid system beds in both setups.

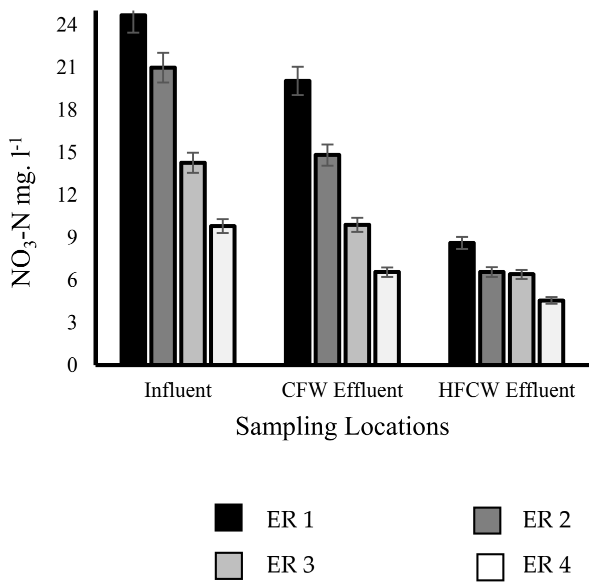

Figure 3. NO_3–N concentration during different experimental runs in the first setup.

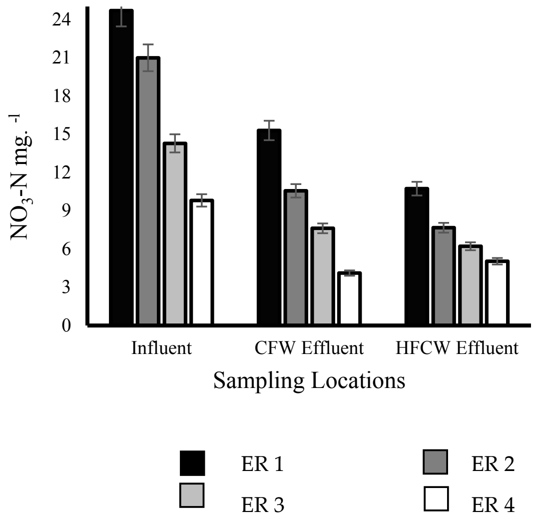

Figure 4. NO_3–N concentration during different experimental runs in the second setup.

3.3. Total Nitrogen Removal under Different NO3–N/NH4–N Ratios

Figure 5 reveals that there is no significant difference in the total nitrogen removal between both CW beds in both setups under different NO_3–N/NH_4–N ratios. The values were as follows: for Setup 1, 32.73%, 26.81%, 34.05%, and 32.65%, and 73.07%, 77.06%, 63.04%, and 61.22% for Beds 1 and 2, respectively; for Setup 2, 34.91%, 37.74%, 47.99%, and 45.18% and 63.59%, 69.17%, 59.05%, and 59.33% for Beds 1 and 2, respectively.

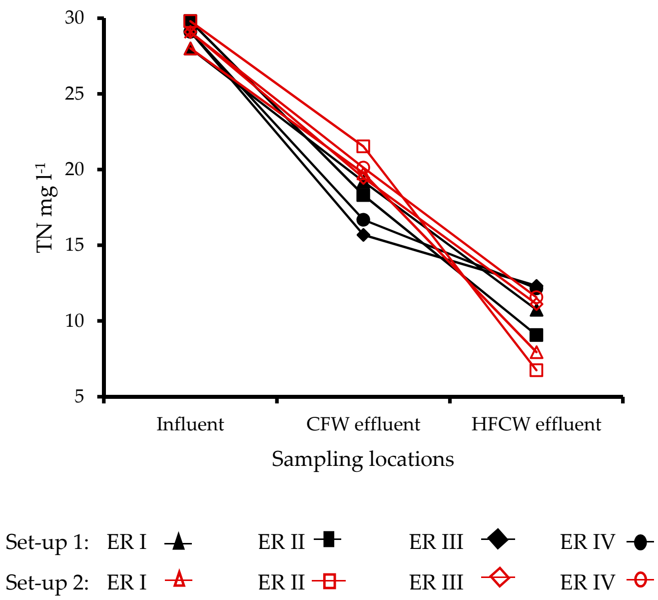

Figure 5. Total nitrogen concentration during different experimental runs in the first and second setups.

4. Discussion

The NO_3–N/NH_4–N ratio has great significance in constructed wetland systems by affecting plant growths [34]. For optimum uptake and growth, each plant species requires a different amount of NO_3–N/NH_4–N ratio [35]. Most of the plants grew well when they were provided by a mixture of NO_3–N and NH_4–N rather than either of these components alone [36,37]. *A. calamus*, *L. esculentum*, and *C. sativus* grew well and achieved the highest dry weight under a NO_3–N/NH_4–N ratio of 1:1, and higher amounts of NO_3–N suppressed growth [24–26]. Nitrogen removal in the constructed wetland system includes adsorption by the substrate, plant uptake, nitrification, and volatilization [38]. Many researchers stated that, in a constructed wetland system, little NH_4–N removal occurs through the direct absorption by plants [39] and it is mostly removed through microbial action [40], whereas on higher pH most of the NH_4–N is removed by volatilization [41]. The constructed wetland systems can consider a complex bioreactor, various biotic and abiotic factors interact with each other, and a number of physical, chemical and biological processes take place [42].

The effect of NH_4–N removal under different NO_3–N/NH_4–N ratios in hybrid constructed wetland systems was significant, and the removal rate reduces with the decrease in NO_3–N/NH_4–N ratio. When the ratio of NH_4–N/NO_3–N was 1:1, there was little impact on NH_4–N removal, a possible reason was which being that an insufficient amount of oxygen in the subsurface wetlands limits these processes [43]. For NO_3–N removal, there is variation between both experimental setups. In the first setup, the surface wetland removed a high concentration of NO_3–N compared to the subsurface constructed wetland, whereas the second setup showed quite the opposite result. The nitrification processes are effected by pH, temperature, inorganic carbon source, alkalinity, dissolve oxygen, and NH_4–N concentration [39]. NH_4–N uptake consumes more oxygen compared to NO_3–N. Ammonium breakdown occurs in roots and reacts with sugar, and this sugar is delivered from leaves to roots, whereas NO_3–N is transported to leaves and reduces to ammonium and then reacts with sugar [44]. At higher respiration, plants consumes more sugar, leaving less available for NH_4–N metabolism. *S. melongena* in the HFCW and *I. aquatica* in the CFW have well developed root systems, and their oxygen transfer ability is strong, rendering a good aerobic environment around the root system, which ultimately favors nitrifying bacteria and increases the removal ability of NH_4–N.

The rhizome and roots belowground are critical for the removal of nitrogen from wastewater; in the rhizosphere, they provide nutrients and exudates to fuel the microorganisms [45]. The plant root system is an important parameter to consider when selecting plant species for a constructed wetland, as a bigger root area can take up large amount of nutrients and thus improve N removal.

5. Conclusions

- *Ipomoea aquatica* Forsk. and *Solanum melongena* L. showed higher removal efficiency for NH_4–N under higher ammonium concentrations, whereas *Allium porrum* L. showed higher NO_3–N removal when NO_3–N concentrations were high in wastewater. Compared to other vegetables *Capsicum annuum* L. showed little efficiency for both N sources.
- The different plants may differ in their capacity to take in N from different nitrogen sources and therefore should select plants economically so that a constructed wetland can obtain optimum removal of nutrients as well as optimum growth.
- The gap between supply and demand for water and food can be reduced using a constructed wetland for the economical growth of plants, and this approach can broaden the application of a constructed wetland.

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