CrossMark

RESEARCH ARTICLE

Nitrogen removal performance in planted and unplanted horizontal subsurface flow constructed wetlands treating different influent COD/N ratios

Wei Wang[1,2] · Yi Ding[1,3] · Jeffrey L. Ullman[3] · Richard F. Ambrose[2,4] · Yuhui Wang[1] ·
Xinshan Song[1] · Zhimiao Zhao[1]

Abstract Microcosm horizontal subsurface flow constructed wetlands (HSSFCWs) were used to examine the impacts of vegetation on nitrogen dynamics treating different influent COD/N ratios (1:1, 4:1, and 8:1). An increase in the COD/N ratio led to increased reductions in NO_3 and total inorganic nitrogen (TIN) in planted and unplanted wetlands, but diminished removal of NH_4. The HSSFCW planted with *Canna indica* L. exhibited a significant reduction in NH_4 compared to the unplanted system, particularly in the active root zone where NH_4 removal performance increased by up to 26 % at the COD/N ratio of 8:1. There was no significant difference in NO_3 removal between the planted and unplanted wetlands. TIN removal efficiency in the planted wetland increased with COD/N ratios, which was likely influenced by plant uptake. NH_4 reductions were greater in planted wetland at the 20- and 40-cm depths while NO_3 reductions were uniformly greater with depth in all cases, but no statistical difference was impacted by depth on TIN removal. These findings show that planting a HSSFCW can provide some benefit in reducing nitrogen loads in effluents, but only when a sufficient carbon source is present.

Introduction

Nitrogen enrichment in water bodies is the primary cause of eutrophication, which results in the algae blooms and deteriorating water quality (Xu et al. 2013). Horizontal subsurface

---

Responsible editor: Kenneth Mei Yee Leung

Highlights ● Nitrogen removal in horizontal subsurface flow wetlands was investigated.
● Treatments compared planted versus unplanted systems under varying COD/N ratios.
● Plants slightly enhanced NH_4 removal, but did not impact NO_3 mitigation.
● NO_3 removal was significantly higher in both systems under a high COD/N ratio.
● Total inorganic N removal was only enhanced by plants at a high COD/N ratio.

Wei Wang and Yi Ding contributed equally to this work.

✉ Xinshan Song
   newmountain@163.com; dingyi8866@gmail.com

[1] College of Environmental Science and Engineering, State Environmental Protection Engineering Center for Pollution Treatment and Control in Textile Industry, Donghua University, Shanghai 201620, People's Republic of China

[2] Department of Environmental Health Sciences, University of California, Los Angeles, Los Angeles, CA 90095-1771, USA

[3] Department of Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611, USA

[4] Institute of the Environment and Sustainability, University of California, Los Angeles, Los Angeles, CA 90095-1771, USA

flow constructed wetlands (HSSFCWs) can be used for treating various wastewaters and offer the advantages of moderate capital costs, low energy consumption, and maintenance requirements (Vymazal 2005; Wang et al. 2013; Fan et al. 2013a). They are appropriate to remove many kinds of pollutants, in particular nitrogenous compounds (Coban et al. 2015). Among all nitrogen removal mechanisms, nitrification-denitrification is widely acknowledged to be the most important nitrogen removal pathway in constructed wetlands (Bulc 2006; Ding et al. 2014), while other mechanisms, such as plant uptake, biomass assimilation, ammonia volatilization, and substrate adsorption, are generally less important (Vymazal 2007; Wang et al. 2015). The overall reaction process of nitrification is as follows: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$. Nitrification mainly depends on the dissolved oxygen concentrations of wetland systems (Langergraber 2005). The overall reaction process of denitrification is as follows: $NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$. Denitrification mainly depends on the carbon sources coming from influents, which provide electron donors for denitrification (Sirivedhin and Gray 2006). It is known that nitrification is indirectly attributed to nitrogen removal in effluent; however, nitrification providing essential nitrate for denitrification, which finally removes the nitrogen from water body by transforming nitrate into N_2 or N_2O (Bernat et al. 2011). Plants enhance oxygen transport throughout the HSSFCW by exuding oxygen from stems and rhizomes into the rhizosphere, which can stimulate nitrogen removal by promoting the conversion of ammonia to nitrate, which can then either undergo denitrification under anaerobic conditions or be taken up by plants (Lin et al. 2002; Langergraber 2005; Randerson 2006). Generally, it is reported that oxygen release rates from wetland plants range between 0.014 and 12 g m^{-2} day^{-1} (Nivala et al. 2013).

The COD/N ratio, which represents the relative amount of carbon source in a system related to nitrogen concentrations, can affect the nitrification and denitrification functions of microorganisms (Ding et al. 2012; Zhu et al. 2014). Organic carbon sources can act as a limiting factor in biological nitrogen removal (Liu et al. 2012; Feng et al. 2013). The dissolved oxygen and carbon concentrations are expected to vary with depth within a HSSFCW due to limited oxygen diffusion through the profile. Usually, the upper level is propitious to nitrification due to the effect of wetland plant and atmospheric reaeration while the bottom level is propitious to denitrification as its anoxic and anaerobic habitats. Correspondingly, depth variation will affect nitrification and denitrification. Recent studies have focused on the effect of influent COD/N ratios on the nitrogen removal in artificially aerated vertical flow constructed wetlands (Fan et al. 2013b; Liu et al. 2013). However, the lack of information about the effect of COD/N ratios and wetland plants on the nitrogen removal performance at different depths in non-aerated systems impedes an accurate understand about the nitrogen removal in HSSFCWs. This is a gap in knowledge that inhibits optimized designs for HSSFCWs to avoid unstable performance.

This study addressed the need to further examine the impact plants have on nitrogen removal in subsurface wetlands under varying COD/N regimes by adding synthetic wastewater in microcosm HSSFCWs. The objectives of this study were to: (i) determine the influence COD/N ratio, plants, and depth have on nitrogen species in HSSFCWs and (ii) evaluate the interactions these factors have on the nitrogen removal process in HSSFCWs.

Materials and methods

Configuration and operation of the wetland systems

Two microcosm HSSFCWs (W1 and W2) were used and the configuration is as shown in the schematic (Fig. 1). Each wetland was 200 cm in length, 60 cm in width, and 70 cm in depth, and divided lengthways into influent (20 cm × 60 cm × 70 cm), treatment (160 cm × 60 cm × 70 cm), and effluent (20 cm × 60 cm × 70 cm) zones. The influent and effluent zones were both filled with coarse gravel ($\Phi = 3$–4 cm) in order to facilitate the distribution and collection of the wastewater. The middle treatment zone contained a 55-cm layer of medium gravel ($\Phi = 1$–2 cm) with a 10-cm layer of washed sand ($\Phi < 0.2$ cm) placed over the surface to facilitate plant growth. Three sampling ports were located at depths of 20 cm (S1), 40 cm (S2), and 60 cm (S3) from the top.

W1 was unplanted while W2 was planted with eight *Canna indica* L. plants. Hoagland solution (Hoagland and Arnon 1950), a standard hydroponic nutrient solution, was added to the wetlands 2 months prior to the experiments to allow the plants and microbial communities to mature and adapt to the wetland conditions. After the first month, the Hoagland solution was replaced with fresh solution. Then, the fresh solution was supplied to the wetlands for the second month. The wetlands were located indoors where the air temperature was maintained at 25 ± 3 °C.

Three treatments consisting of COD/N ratios of 1:1, 4:1, and 8:1 were administered in parallel experiments to the planted and unplanted wetland. Synthetic wastewater solutions were prepared using tap water in a feed tank to simulate high nitrogen-content wastewater (Ong et al. 2010; Fan et al. 2013a). Nitrogen was added in the form of KNO_3 and NH_4Cl to achieve NO_3-N and NH_4-N concentrations of 20 and 25 mg L^{-1}, respectively. The TIN concentration was analyzed and found to be 46 mg L^{-1}, with the additional 1 mg L^{-1} coming from nitrogen impurities in the tap water. The COD concentrations were adjusted to 46, 184, and 368 mg L^{-1} by adding appropriate amounts of glucose to obtain COD/N ratios of 1:1, 4:1, and 8:1, respectively. In addition, 3.0 mg L^{-1} KH_2PO_4, 1.75 mg L^{-1} $MgCl_2$, 1.75 mg L^{-1} $CaCl_2$, and

Fig. 1 Schematic diagram (longitudinal cross-section) of the horizontal subsurface flow constructed wetlands (HSSFCWs)
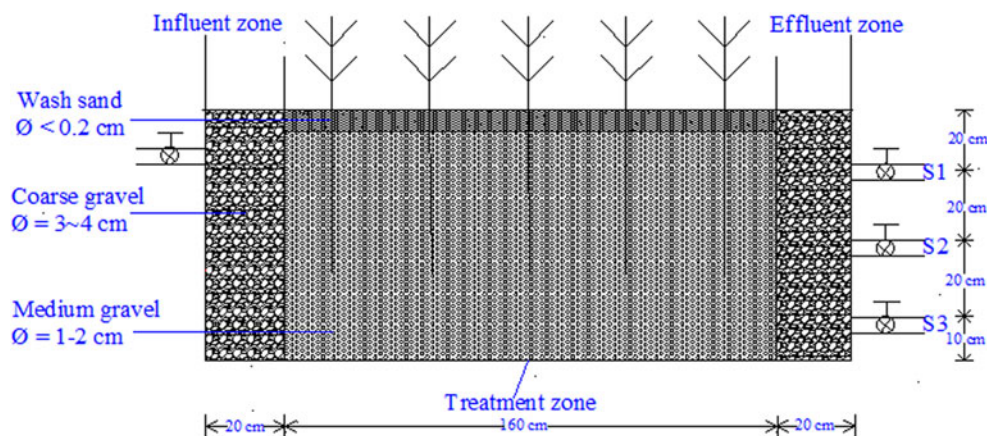
0.5 mg L^{-1} FeSO$_4$ were added to match constituents found in wastewater. Analysis indicated that the error range between design concentrations and actual concentrations were all within 4.85 %. The dissolved oxygen (DO) in the influent was 6.9 mg L^{-1}. The synthetic wastewater solution was added to the HSSFCWs using a peristaltic pump with a stable inflow rate of 1.0 ± 0.2 L min^{-1}. The total inflow of two microcosm systems was 120 L within 2 h. The wetlands were operated in an intermittent manner to obtain a hydraulic retention time (HRT) of 3 days according to previous studies (Fan et al. 2013a; Fan et al. 2013b; Ding et al. 2014).

Sampling collection and analysis

Each treatment was conducted in triplicate. Throughout one treatment, three water samples from the effluent were collected from the sampling ports (S1, S2, and S3) after the 3-day HRT to evaluate HSSFCW treatment performance. NO$_3$, NH$_4$, and TIN were analyzed using a gas-phase absorption spectrometer (GMA3202, China) based on established methods for nitrogen species (Anigbogu et al. 1983; Yang et al. 1997; Zhang et al. 2007; Zhang et al. 2011). Briefly, NO$_3$ was determined by the addition of titanium chloride (TiCl$_3$) at 70 °C to reduce NO$_3$ to NO, which was analyzed at $\lambda = 214.4$ nm on the spectrometer. Hypobromite (BrO$^-$) was added to the sample to oxidize NH$_4$ to NO$_2$, and aliquots of the resulting solution were added to a citric acid (0.5 mol L^{-1}) and alcohol (10 %) solution and the volatilized N oxides were analyzed at $\lambda = 213.9$ nm. TIN was measured by adding potassium persulfate (K$_2$S$_2$O$_8$) to oxidize all N species to NO$_3$ at 120 °C, after which TiCl$_3$ was added as described above. Nitrite in the samples was minimal because the second step of nitrification is rapid, and organic N was negligible since the synthetic wastewater only contained NO$_3$ and NH$_4$. COD was measured by using a HACH colorimeter (DR/890, USA) following standard methods. Temperature and DO were measured simultaneously by a HANNA DO meter (HI 9143, Italy) after the pore water was collected from the three

sampling ports located at depths of 20 cm (S1), 40 cm (S2), and 60 cm (S3) from the top.

Removal efficiency calculations were based on the following mass balance:

Concentration-based removal efficiency (%)

$$= \frac{\text{Influent concentration} - \text{Effluent concentration}}{\text{Influent concentration}}$$
$$\times 100$$

The effluent were collected from three sampling ports which were located at depths of 20 cm (S1), 40 cm (S2), and 60 cm (S3) from the top. The nutrient removal efficiency in different depths was calculated according to the above mass balance.

Built-in statistical functions of Origin 8.0 analysis software (OriginLab, MA, USA) were used for completing data analysis and visualization. All data are presented as mean $\pm$ SD of the nine samples for each treatment, which are conclusive. The statistical program MATLAB 7.0 software (Mathworks Corp. Inc, USA) were performed to conduct ANOVA and three-way ANOVA tests. Statistical significance was designated at $P < 0.01$ for all analyses, which can ensure the accuracy of statistical analyzes.

Results and discussions

Dissolved oxygen (DO) plays an important role in regulating microbial activity in wetlands and is a crucial environmental parameter that controls nitrification and organic biodegradation (Ding et al. 2012). Figure 2 illustrates that COD/N ratios had a significant effect on DO concentration in the wetland systems. As the COD/N ratio increased, oxygen levels decreased significantly in both the planted and unplanted wetlands at all depths. This finding can be attributed to the degradation of the influent carbon source by microbial activity,

Fig. 2 Dissolved oxygen concentrations at different depths in the unplanted and planted HSSFCWs treating different influent COD/N ratios
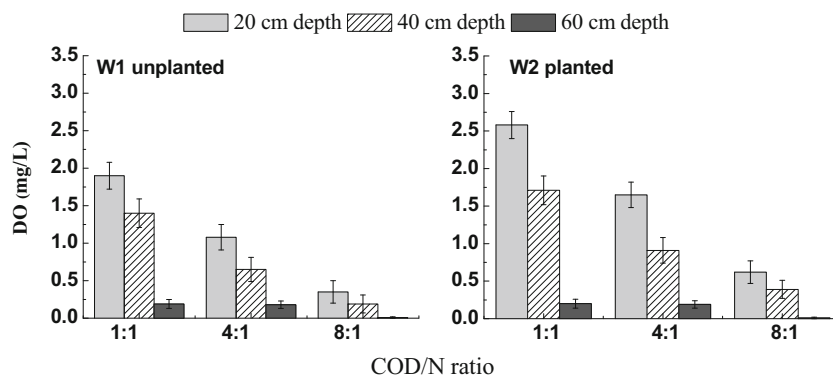
which depleted the available oxygen (Tanner and Kadlec 2003) and ultimately inhibited the nitrification process (Liu et al. 2013). Under oxygen limited conditions, wetland plants are assumed to play a central role by releasing oxygen from roots to create aerobic conditions, which facilitates the aeration of belowground organizations and induces growth of both heterotrophic and autotrophic aerobic bacteria in the rhizosphere (Brix 1997). The DO concentration in the planted system was significantly higher than in the unplanted system for all COD/N ratios at the 20- and 40-cm depths, but the difference was negligible at the 60-cm depth. The results indicate that oxygen release of plants can be a potentially significant source in supply of oxygen for wetland system. COD concentrations in outlet water ranged from 19.6 to 37.1 mg L^{-1} with the COD/N ratio of 8:1 for both systems, while it approached 0 mg L^{-1} with COD/N ratios of 1:1 and 4:1 in both wetlands. The effluent COD concentration indicated that carbon source was insufficient when COD/N ratio was less than 4. The results from Wu et al. (2009) also revealed that carbon source for denitrification was not sufficient when average COD/N ratio was less than 4.

Removal of NH$_4$, NO$_3$, and TIN in different depths of the unplanted (W1) and planted (W2) wetlands are presented in Fig. 3. Reductions in NH$_4$ were significantly greater in the planted wetland under all treatments at the 20- and 40-cm depths ($P < 0.01$) where the plant roots were the most established. This phenomenon could be explained by two facts: (1) plants release oxygen from the stems and rhizomes into the wetland profile to create the aerobic environment in favor of NH$_4$ reduction and (2) plants take up NH$_4$ for growing demand (Gabriel et al. 2009; Zhang et al. 2009; Konnerup and Brix 2010). Simultaneously, 40.2, 35.2, and 26.1 % of influent NH$_4$ were removed in the 60-cm depth when the COD/N ratio of influent in W1 was 1:1, 4:1, and 8:1, individually. Compare with W1, 42.8, 37.3, and 27.8 % of influent NH$_4$ were removed in the 60-cm depth when the COD/N ratio of influent in W2 was 1:1, 4:1, and 8:1, individually. There was no statistical difference in NH$_4$ removal at the 60-cm depth between W1 and W2, where anaerobic conditions would inhibit the transformation of NH$_4$ to other forms via

nitrification. An increase in the COD/N ratio led to a decline in NH$_4$ dissipation at all depths in both the planted and unplanted systems. An increase in the COD/N ratio corresponded with decreased DO content, which in turn inhibits nitrification. When the COD/N ratio of influent in W2 was 8:1, 36.7 % of influent NH$_4$ was removed in the 20-cm depth, which was significantly lower than the 53.8 % decline when the COD/N ratio of influent was 1:1 ($P < 0.01$). Results of three-factor analysis of variance for NH$_4$-N removal are presented in Table 1. The results indicated that the COD/N ratio had the greatest impact on NH$_4$ concentrations of the three factors considered (i.e., COD/N ratio, presence of plants, and depth), but that depth and the presence of plants also played a strong role in NH$_4$ dissipation ($P < 0.01$). Three-factor analysis of variance denoted that the interaction between depth and plants
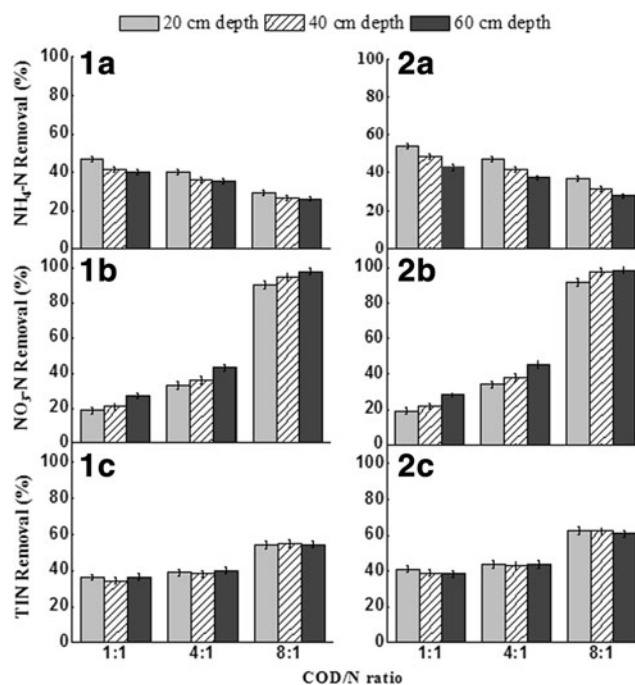
Fig. 3 Removal of *1a* NH$_4$-N, *1b* NO$_3$-N, and *1c* TIN at different depths in unplanted HSSFCWs treating different influent COD/N ratios and removal of *2a* NH$_4$-N, *2b* NO$_3$-N, and *2c* TIN at different depths in planted HSSFCWs treating different influent COD/N ratios

Table 1 Results of three-way ANOVA for NH_4-N removal at different depths in the unplanted and planted HSSFCWs treating different influent COD/N ratios

Source of variance	Sum of squares	df	Mean square	F ratio	P value
COD/N ratio (C)	781.48	2	390.74	1480.70	$P < 0.0001$
Depth (D)	168.69	2	84.35	319.63	$P < 0.0001$
Plant (P)	117.56	1	117.56	445.47	$P < 0.0001$
C × D	4.24	4	1.06	4.02	0.1033
C × P	0.57	2	0.28	1.08	0.4228
D × P	21.42	2	10.71	40.59	0.0022
Residual	1.06	4	0.26		

imparted a significant impact on NH_4-N removal ($P < 0.01$), but COD/N ratio interactions did not impart a significant impact on NH_4-N removal. When considering achieving optimal NH_4-N removal by controlling two variables of the three factors, depth and plants were the two most suitable choices.

Results of three-factor analysis of variance for NO_3-N removal are presented in Table 2. The results indicated that the COD/N ratio had the greatest impact on NO_3-N removal while the depth also played a strong role in NO_3 dissipation ($P < 0.01$). However, no statistical difference was found for NO_3 removal between the unplanted (W1) and planted (W2) wetlands for any of the treatments. Three-factor analysis of variance denoted that the interaction between the COD/N ratio and depth imparted a significant impact on NO_3 concentrations ($P < 0.01$), but plant interactions did not impart a significant impact on NO_3 concentrations. The presence of plants did not introduce enough oxygen into the system to disrupt denitrification at the 60-cm depth, and plant uptake was minimal over the hydraulic residence time considered to have an impact on NO_3 concentrations at the 1:1 and 4:1 COD/N ratios. Reductions in NO_3 were uniformly greater with depth in all cases, where anaerobic conditions would enhance denitrification. At a COD/N ratio of 1:1, NO_3 removal was 27.3 and 28.3 % at the 60-cm depth in W1 and W2, respectively,

which were significantly higher than 20.8 and 21.8 % at the 40-cm depth and 18.6 and 19.3 % at the 20-cm depth. At a COD/N ratio of 4:1, NO_3 removal was 43.1 and 45.6 % at the 60-cm depth in W1 and W2, respectively, which were significantly higher than 36.2 and 38.2 % at the 40-cm depth and 33.0 and 34.4 % at the 20-cm depth. Similarly, NO_3 dissipation increased with increasing COD/N ratios. NO_3 removal at all depths in both systems was over 90 % at a COD/N ratio of 8:1 due to sufficient carbon source supplement. The peak values of NO_3 removal was 97.9 and 98.8 % at the 60-cm depth in W1 and W2, respectively, which were significantly higher than 27.3 and 28.3 % at the COD/N ratios of 1:1 and 43.1 and 45.6 % at the COD/N ratios of 4:1 ($P < 0.01$). The additional carbon source caused comparatively complete denitrification and hence lowered the NO_3 concentrations. When considering achieving optimal NO_3-N removal by controlling two variables of the three factors, COD/N ratio and depth were the two most suitable choices.

Results of three-factor analysis of variance for TIN removal are presented in Table 3. The COD/N ratio and the presence of plants imparted a significant effect on TIN removal ($P < 0.01$), but no statistical difference was impacted by depth. Three-factor analysis of variance denoted that the interaction between the COD/N ratio and plants imparted a significant impact on TIN concentrations ($P < 0.01$), but depth interactions did not impart a significant impact on TIN concentrations. A slight increase in TIN removal occurred between the 1:1 and 4:1 COD/N ratios, while there was a significant increase at the COD/N ratio of 8:1. TIN removal for the planted wetland (W2) increased from 40.5 to 62.4 % between the 1:1 and 8:1 COD/N ratios. The TIN removal reached a maximum of 54.9 % and a minimum of 34.4 % in the unplanted wetland (W1) at COD/N ratios of 8:1 and 1:1, respectively. In this study, NH_4 and NO_3 were the main components comprising TIN. The transformations of NH_4 and NO_3 are important processes for the nitrification and denitrification (Li et al. 2012; Song et al. 2015). Higher TIN removal resulted primarily by enhanced NO_3 dissipation, which indicates that the carbon

Table 2 Results of three-way ANOVA for NO_3-N removal at different depths in the unplanted and planted HSSFCWs treating different influent COD/N ratios

Source of variance	Sum of squares	df	Mean square	F ratio	P value
COD/N ratio (C)	17469.70	2	8734.86	40944.67	$P < 0.0001$
Depth (D)	241.10	2	120.57	565.16	$P < 0.0001$
Plant (P)	10.10	1	10.12	47.46	0.0023
C × D	16.30	4	4.06	19.05	0.0072
C × P	0.90	2	0.45	2.09	0.2387
D × P	0.50	2	0.26	1.23	0.3842
Residual	0.90	4	0.21		

Table 3 Results of three-way ANOVA for TIN removal at different depths in the unplanted and planted HSSFCWs treating different influent COD/N ratios

Source of variance	Sum of squares	df	Mean square	F ratio	P value
COD/N ratio (C)	1454.09	2	727.05	5829.33	$P < 0.0001$
Depth (D)	2.65	2	1.33	10.64	0.0250
Plant (P)	119.61	1	119.61	959.00	$P < 0.0001$
C × D	4.60	4	1.15	9.23	0.0268
C × P	11.50	2	5.75	46.09	0.0017
D × P	3.52	2	1.76	14.13	0.0154
Residual	0.50	4	0.13		

supplement was beneficial to diminishing TIN concentrations. The lack of a carbon source has been shown to restrain the denitrification process (Fan et al. 2013b). Another study also examined that denitrification was the main process responsible for nitrogen removal (Bernat et al. 2011). Due to the large amount of NO_3 presented under experimental conditions, TIN removal was mainly dependent on the organic carbon source that acted as a major electron donor for denitrification (Lu et al. 2009). When considering achieving optimal TIN removal by controlling two variables of the three factors, COD/N ratio and plants were the two most suitable choices.

Conclusion

This study demonstrated influent COD/N ratios had a significant effect on the nitrogen removal performance in the HSSFCWs. The increase of COD/N ratio decreased the overall oxygen content and led to more efficient denitrification, even in the planted system. The presence of plants in the wetlands had a relatively minor impact on the overall nitrogen removal. There was significantly greater removal of NH_4 in the planted system compared to in the unplanted system, particularly in the root zone where W2 experienced up to a 26 % increase in NH_4 removal under a COD/N ratio of 8:1. However, there was no difference in NO_3 concentrations in W1 and W2 under any treatment. Overall, the planted system exhibited a slightly higher capacity for TIN removal than the unplanted system under the high COD/N ratio. However, compared to artificial aeration in other studies, the effect of plant oxygen release on N removal level in the planted system is limited. These findings indicate that plants can provide some benefit for reducing nitrogen levels based on a sufficient carbon source.

When controlling two variables of the three factors (i.e., COD/N ratio, presence of plants, and depth), the interaction between depth and plants imparted the greatest impact on NH_4-N removal, but COD/N ratio interactions did not impart a significant impact on NH_4-N removal. The interaction between COD/N ratio and depth imparted the greatest impact on NO_3-N removal, but plants interactions did not impart a significant impact on NO_3-N removal. The interaction between the COD/N ratio and plants imparted a significant impact on TIN concentrations, but depth interactions did not impart a significant impact on TIN concentrations.

References

Anigbogu VC, Dietz ML, Syty A (1983) Determination of ammonium, nitrate, and urea nitrogen in fertilizer by gas-phase molecular absorption spectrometry. Anal Chem 55:535–539

Bernat K, Kulikowska D, Zielińska M, Cydzik-Kwiatkowska A, Wojnowska-Baryla I (2011) Nitrogen removal from wastewater with a low COD/N ratio at a low oxygen concentration. Bioresour Technol 102:4913–4916

Brix H (1997) Do macrophytes play a role in constructed treatment wetlands. Wat Sci Tech 35(5):11–17

Bulc TG (2006) Long term performance of a constructed wetland for landfill leachate treatment. Ecol Eng 26:365–374

Coban O, Kuschk P, Wells NS, Strauch G, Knoeller K (2015) Microbial nitrogen transformation in constructed wetlands treating contaminated groundwater. Environ Sci Pollut Res 22:12829–12839

Ding Y, Song XS, Wang YH, Yan DH (2012) Effects of dissolved oxygen and influent COD/N ratios on nitrogen removal in horizontal subsurface flow constructed wetland. Ecol Eng 46:107–111

Ding Y, Wang W, Song XS, Wang G, Wang YH (2014) Effects of spray aeration on organics and nitrogen removal in vertical subsurface flow constructed wetland. Chemosphere 117:502–505

Fan JL, Liang S, Zhang B, Zhang J (2013a) Enhanced organics and nitrogen removal in batch-operated vertical flow constructed wetlands by combination of intermittent aeration and step feeding strategy. Environ Sci Pollut Res 20:2448–2455

Fan JL, Wang WG, Zhang B, Guo YY, Ngo HH, Guo WS, Zhang J, Wu HM (2013b) Nitrogen removal in intermittently aerated vertical flow constructed wetlands: Impact of influent COD/N ratios. Bioresour Technol 143:461–466

Feng HJ, Huang BC, Zou YQ, Li N, Wang MZ, Yin J, Cong YQ, Shen DS (2013) The effect of carbon sources on nitrogen removal performance in bioelectrochemical systems. Bioresour Technol 128:565–570

Gabriel ML, Roxane M, Jacques B, Florent C (2009) Nitrogen transformations and retention in planted and artificially aerated constructed wetlands. Water Res 43:535–545

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circ Calif Agr Exp Sta 347:1–39

Konnerup D, Brix H (2010) Nitrogen nutrition of Canna indica: effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. Aquat Bot 92:142–148

Langergraber G (2005) The role of plant uptake on the removal of organic matter and nutrients in subsurface flow constructed wetlands: a simulation study. Wat Sci Technol 51(9):213–223

Li HB, Li YH, Sun TH, Wang X (2012) The use of a subsurface infiltration system in treating campus sewage under variable loading rates. Ecol Eng 38:105–109

Lin YF, Jing SR, Wang TW, Lee DY (2002) Effects of macrophytes and external carbon source on nitrate removal from groundwater in constructed wetlands. Environ Pollut 119:413–420

Liu HB, Zhao F, Mao BY, Wen XH (2012) Enhanced nitrogen removal in a wastewater treatment process characterized by carbon source manipulation with biological adsorption and sludge hydrolysis. Bioresour Technol 114:62–68

Liu L, Zhao XH, Zhao N, Shen Z, Wang M, Guo YZ, Xu YB (2013) Effect of aeration modes and influent COD/N ratios on the nitrogen removal performance of vertical flow constructed wetland. Ecol Eng 57:10–16

Lu SL, Hu HY, Sun YX, Yang J (2009) Effect of carbon source on the denitrification in constructed wetlands. J Environ Sci 21:1036–1043

Nivala J, Wallace S, Headley T, Kassa K, Brix H, Afferden M, Müller R (2013) Oxygen transfer and consumption in subsurface flow treatment wetlands. Ecol Eng 61:544–554

Ong S-A, Uchiyama K, Inadama D, Ishida Y, Yamagiwa K (2010) Performance evaluation of laboratory scale up-flow constructed wetlands with different designs and emergent plants. Bioresour Technol 101:7239–7244

Randerson PF (2006) Constructed wetlands and vegetation filters: an ecological approach to wastewater treatment. Environ Biotechnol 2:78–89

Sirivedhin T, Gray KA (2006) Factors affecting denitrification rates in experimental wetlands: field and laboratory studies. Ecol Eng 26: 167–181

Song XS, Ding Y, Wang YH, Wang W, Wang G, Zhou B (2015) Comparative study of nitrogen removal and bio-film clogging for three filter media packing strategies in vertical flow constructed wetlands. Ecol Eng 74:1–7

Tanner CC, Kadlec RH (2003) Oxygen flux implications of observed nitrogen removal rates in subsurface-flow treatment wetlands. Wat Sci Technol 48:191–198

Vymazal J (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol Eng 25: 478–490

Vymazal J (2007) Removal of nutrients in various types of constructed wetlands. Sci Total Environ 380:48–65

Wang YH, Song XS, Ding Y, Niu RH, Zhao XX, Yan DH (2013) The impact of influent mode on nitrogen removal in horizontal subsurface flow constructed wetlands: A simple analysis of hydraulic efficiency and nutrient distribution. Ecol Eng 60:271–275

Wang XO, Tian YM, Zhao XH, Peng S, Wu Q, Yan LJ (2015) Effects of aeration position on organics, nitrogen and phosphorus removal in combined oxidation pond–constructed wetland systems. Bioresour Technol 198:7–15

Wu L, Peng CY, Zhang SJ, Peng YZ (2009) Nitrogen removal via nitrite from municipal landfill leachate. J Environ Sci 21:1480–1485

Xu DF, Li YX, Howard A (2013) Influence of earthworm *Eisenia fetida* on removal efficiency of N and P in vertical flow constructed wetland. Environ Sci Pollut Res 20:5922–5929

Yang F, Troncy E, Francœur M, Vinet B, Vinay P, Czaika G, Blaise G (1997) Effects of reducing reagents and temperature on conversion of nitrite and nitrate to nitric oxide and detection of NO by chemiluminescence. Clin Chem 43:657–662

Zhang L, Altabet MA, Wu T, Hadas O (2007) Sensitive measurement of NH_4^+ $^{15}N/^{14}N$ ($\delta^{15}NH_4^+$) at natural abundance levels in fresh and saltwaters. Anal Chem 79:5297–5303

Zhang ZH, Rengel Z, Meney K (2009) Kinetics of ammonium, nitrate and phosphorus uptake by *Canna indica* and *Schoenoplectus validus*. Aquat Bot 91:71–74

Zhang M, Zhang Z, Yan D, Feng S, Liu B (2011) An automatic gas-phase molecular absorption spectrometric system using a UV-LED photodiode based detector for determination of nitrite and total nitrate. Talanta 84:443–450

Zhu H, Yan BX, Xu YY, Guan JN, Liu SY (2014) Removal of nitrogen and COD in horizontal subsurface flow constructed wetlands under different influent C/N ratios. Ecol Eng 63:58–63