Effect of photosynthetically elevated pH on performance of surface flow-constructed wetland planted with Phragmites australis

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Abstract

Combination of emergent and submerged plants has been proved to be able to enhance pollutant removal effi- ciency of surface flow-constructed wetland (SFCW) during winter. However, intensive photosynthesis of submerged plants during summer would cause pH increase, which may have adverse effects on emergent plants. In this study, nitrogen transformation of lab-scale SFCW under pH gradient of 7.5, 8.5, 9.5 and 10.5 was systematically investigated. The results showed that total nitrogen (TN) removal efficiency decreased from 76.3 ± 0.04 to 51.8 ± 0.04 % when pH increased from 7.5 to 10.5, which was mainly attributed to plant assimilation decay and inhibition of microbe activities (i.e., nitrite-oxidizing bacteria and denitrifiers). Besides, the highest sediment adsorption in SFCW was observed at pH of 8.5. In general, the combination of submerged and emergent plants is feasible for most of the year, but precaution should be taken to mitigate the negative effect of high alkaline conditions when pH rises to above 8.5 in midsummer.

Keywords: Constructed wetland · Plant species combination · Photosynthetically elevated pH · Nitrogen transformation · Nitrification and denitrification

Introduction

Compared with conventional wastewater treatment technologies, constructed wetlands (CWs) are considered to be more environmentally friendly and economical due to their low construction and maintenance cost (Vymazal 2013). Generally, CWs can be categorized into two types, namely surface flow-constructed wetland (SFCW, also known as free water flow) and subsurface flow-constructed wetland (SSFCW) (Fonder and Headley 2013; Gikas et al. 2013). SFCW is an imitation of natural wetland as water flows over the surface of sediment (Wu et al. 2009). It is a typical eco- logical engineering system and can be easily transformed from an existing natural wetland. SFCW could promote the total nitrogen removal rate when it was collocated into sewage treatment CW line (Vymazal 2013). However, the further spread of SFCW is impeded by its low purification efficiency during winter when the temperature is low.

Some enhanced measurements have been proposed to solve the abovementioned problems (Kato et al. 2013; Meng et al. 2014; Cui et al. 2014). The most effective and economic one was plant species collocation (Liang et al. 2011). Liang et al. (2011) suggested that a SFCW with mixed plant species exhibited significantly better water quality than monoculture wetlands. This was because the mixed plant species in wetland led to complementary patterns of plant growth, root distribu- tion, and nutrition preference through time and space. However, a mixture of two emergent plants (Zhang et al. 2007) or a mixture of two submerged plants (Agami and Reddy 1990) was not a good choice, as fierce competition

would occur within similar species, resulting in decreased wetland performance. Thus, a modified SFCW form, influent→submerged plants→emergent plants→effluent, was introduced. Its enhancement for nitrogen removal has been theoretically proved by Weisner et al. (1994), and the system could withstand the impact of temperature change because some submerged plants still grew when emergent macrophytes went through declining during winter (Mjelde et al. 2012; Zhang et al. 2009). Besides, emergent plants′ final position at collocation line could guarantee the purification efficiency. Thus, a complementary ecological niche is established to avoid the decrease of purification efficiency.

However, prior to the application of plant species combination method in CWs, one problem needs to be solved. Generally, the pH of effluent from CWs planted with submerged plants raised sharply in summer (Mjelde et al. 2012; Rorslett et al. 1986), which was a stress for emergent plants. An investigation in Norway showed that the mass invasion of Elodea canadensis during summer time increased the water column pH inside E. canadensis stands to 8.5 and even 10.2 (Mjelde et al. 2012). Carter et al. (1991) also reported that overlying water of the submerged plant beds elevated pH to the maximum 9.5 in August. It was because strong photosynthesis could influence the carbonate ionization balance resulting in the increase in concentration of hydroxyl ions. Elevated pH values may adversely affect all CWs components. Firstly, pH could affect plants in the aspect of intracellular metabolic activity, cell growth, and biomass (Buchanan et al. 2000; Cosgrove 2000; Ranieri et al. 2013). Moreover, microbial processes were sensitive to pH in CWs (Meng et al. 2014). Previous studies showed that denitrification and nitrification process can be hampered at pH >8.0 (Vymazal 2007). Lastly, elevated pH caused the reduction of dissolved oxygen concentration in sediment (Rorslett et al. 1986) and changed the redox condition then influenced nitrification or denitrification.

Thus, the emphasis of this study was to evaluate the effect of photosynthetically elevated pH (caused by submerged plants) on the performance of SFCW with emergent plants. Variations of water quality and plant growth were intensively monitored, and microbial community structure was also investigated to better understand the mechanisms.

Materials and methods

Wetland microcosms

The study site was located at the Baihua Park in Jinan, China (36° 40′ 36″ N, 117° 03′ 42″ E) with annual average temperature of 14.7 °C, annual average rainfall of 671.1 mm, and distinct seasons. Lab-scale SFCWs were built outdoor under a transparent shed to prevent rainfall runoff. The detailed design of SFCWs was described by Wu et al. (2012). The wetland units were built in polyethylene buckets (50 cm in depth and 40 cm in diameter) with an outlet at the bottom. The buckets were filled with a 10-cm bottom layer of river gravel, above which was washed river sand with a depth of 25 cm. The average sediment bed porosity was 35 %. Each wetland was filled with 25 L water, with a depth of 25 cm above the media. Phragmites australis (reed) was chosen as the research object, and young reed plants were transferred from a freshwater wetland located in Dezhou, China. Healthy plants with similar size (approximately 1.2 m in height) were weighted and then transplanted into SFCWs. The planting density of reeds was 64 plants m^{-2}. After transplantation, plants were cultivated using the 10 % Hoagland nutrient solution for 2 weeks to acclimate to the new environment. To inoculate the microorganism, the system was seeded with active sludge at a density of 1.62 g m^{-2} (Zhu et al. 2011). The active sludge was obtained from the aeration tank of a full-scale artificial sewage treatment plant in Jinan, China.

Experimental procedure

Four different treatments were operated side by side from July 26 to October 25, 2014. In order to minimize the variability in the experiment, synthetic wastewater was used by mixing the following components into tap water: 44.5 mg L^{-1} sucrose, 28.2 mg L^{-1} NH$_4$HCO$_3$, 50.5 mg L^{-1} KNO$_3$, 4.4 mg L^{-1} KH$_2$PO$_4$, 10.0 mg L^{-1} MgSO$_4$, 10.0 mg L^{-1} CaCl$_2$, and 10.0 mg L^{-1} FeSO$_4$. At the first month, all treatments were operated around pH of 7.5. Afterwards, pH of three different treatments was adjusted to 8.5, 9.5, and 10.5, respectively, by adding sodium carbonate into the synthetic wastewater. The pH of the fourth treatment remained at 7.5, as a control. Each treatment had three replicates. Sequencing fill-and-draw batch mode was applied: wetlands were saturated with the wastewater for treatment and drained after an experiment cycle and then immediately refilled at the start of the next cycle. Hydraulic retention time (HRT) of each treatment was 5 days, with a hydraulic load of 0.0398 m day^{-1}.

Water sampling and analysis

Influent and effluent of each cycle were sampled to analyze the nitrogen transformation in the four treatments. Within an experiment cycle, water samples were taken from mixed depth below the water surface. At the end of every cycle, water inside the unit was drawn off from the outlet into an empty bucket. After mixing, the water samples were analyzed immediately for NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, and total nitrogen (TN), using methods described in APHA (2005). Water temperature, pH, and dissolved oxygen (DO) were measured in situ with a pH meter (PHS-3C, China) and a DO meter (HQ40d 53LED™, HACH, USA), respectively.

Plant sampling and analysis

The net photosynthetic rate of leaves was analyzed using a portable photosynthesis measurer (Li-6000, Li-COR Inc., USA) before the end of the experiment. Five measurements were carried out per pot between 10:00 and 12:00 a.m. under the same light intensity of 1000 µmol m^{-2} s^{-1} on a sunny day (Fan et al. 2013). Plants were sampled to estimate the biomass and plant uptake in the initial and at the end of the experiment. Every single plant's height and weight were measured, and the mean height and weight in each pH condition treatment were calculated. All plants were excavated, washed, and dried. Then, the harvested plant biomass was separated into root dry weight and total dry weight. The root weight ratios (RWRs) were calculated, and relative growth rate (RGR) was measured according to the equation

$$W_2 = W_1 e^{RGR \times (t2-t1)} \tag{1}$$

Then, the whole plants were ground and analyzed for TN with a CHNS/O analyzer (Elementar vario Macro cube, Elementar Analysensysteme GmbH, Hanau, Germany).

Sediment sampling and analysis

Before excavating and harvesting plants (on October 25), sediment samples from each treatment were taken from a 0–25 cm depth at five spots and then well mixed as one composite sample. One portion of each sample was air-dried and analyzed for TN change in sediments. The method was the same as plant samples. The other portion was placed in an ice incubator and immediately sent to the laboratory for RNA extraction and quantification of gene abundance. According to the method described by Zhi and Ji (2014), Total RNA Isolation Kit (RNA PowerSoil®) was used to extract total genomic RNA from 0.25-g samples. A quantitative analysis of ammonia oxidation bacterium (AOB, represented by its ammonia monooxygenase coding gene amoA), Nitrospira sp. (NOB, represented by its 16S rDNA), and denitrifiers (represented by the nirS gene) was conducted using primer pairs amo598f (5′ GAATATGTTCGCCTGATTG 3′)/amo718r (5′ CAAAGTACCACCATACGCAG 3′) (Dionisi et al. 2002), NSR1113f (5′ CCTGCTTTCAGTTGCTACCG 3′)/NSR1264r (5′ GTTTGCAGCGCTTTGTACCG 3′) (Dionisi et al. 2002), and nirScd3aF (5′ GT(C/G)AACGT(C/G) AAGGA(A/G)AC(C/G)GG 3′)/nirSR3cd (5′ GA(C/G)TTCGG(A/G)TG(C/G)GTCTTGA 3′) (Throback et al. 2004), respectively. Three standard curves were generated from a 10-fold serial dilution of plasmid DNA containing specific genes. The measurement of quantitative PCR (qPCR) was carried out in a MyiQ2 Real-Time PCR Detection System (Bio-Rad, USA) in the final 20 µL reaction mixtures. Data obtained from qPCR were exported to calculations in the unit of copies per gram of sediment.

Statistical analysis

All statistical analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, USA), and differences were considered to be significant at the level of $P < 0.05$. The experiments were measured in triplicate samples, and the results were displayed as mean ± standard deviation.

Results and discussion

Nitrogen mass balance

Nitrogen transformation in SFCW is a complex process, including plant uptake, sediment adsorption, and microbial metabolism. To better understand the nitrogen transformation in the wetland, proportions of different TN removal pathways in four pH treatments through the entire experiment period were calculated and are illustrated in Fig. 1, based on the results of daily monitoring.

In the present study, an influent was the only input source of TN, which was 742.4 mg N m^{-2} day^{-1} and TN content in the effluent under different pH stresses was 208.7, 268.6, 334.4, and 371.7 mg N m^{-2} day^{-1}, respectively. TN in the effluent showed an uptrend with the increase of pH, which was consistent with the downtrend of TN removal efficiency shown in Table 1.

TN delivered to plant was quantified to be 205.5, 71.8, −46.4, and −114.4 mg N m^{-2} day^{-1}, respectively. Within the ordinary pH condition (7.5) of wetland, 27.7 % of the input TN was harvested by plant, which was consistent with a report by Morvan et al. 1997. At pH 10.5, a severe decay occurred and the plant assimilated portion reduced, indicating that high
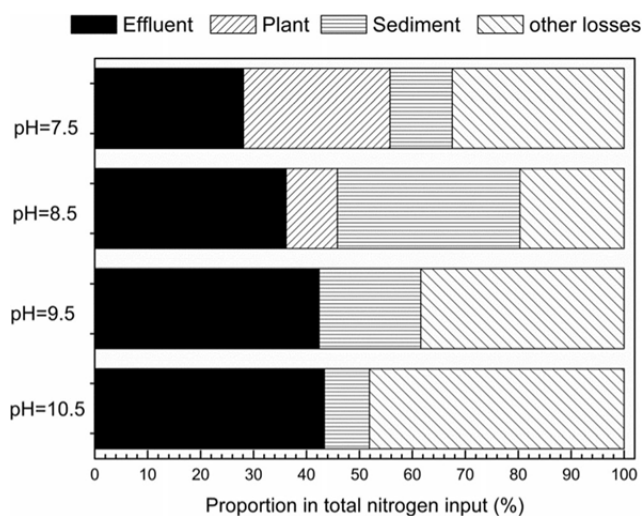
Fig. 1 Proportion in total nitrogen (TN) input through different pathways among wetlands after adding pH stress during the entire experiment period

Table 1 Results of average nitrogen removal efficiency before and after adding pH stress and plant growth during the study period

Treatment	Nitrogen removal efficiency (%)						Plant growth			
	NH_4^+ - N		NO_3^- - N		TN		RGR [a]	RWR [b]	ΔH [c]	P_n [d]
	Before	After	Before	After	Before	After				
pH = 7.5	92.5 ± 0.02	89.3 ± 0.04	78.7 ± 0.09	72.5 ± 0.10	83.5 ± 0.02	76.3 ± 0.04	0.00983	0.397	21.8	13.4 ± 3.26
pH = 8.5	94.0 ± 0.03	93.2 ± 0.03	79.5 ± 0.06	76.7 ± 0.10	80.5 ± 0.03	66.2 ± 0.04	0.00530	0.313	11.5	14.3 ± 3.04
pH = 9.5	96.0 ± 0.01	84.3 ± 0.03	71.5 ± 0.01	64.6 ± 0.14	78.0 ± 0.01	56.0 ± 0.07	0.00239	0.070	10.1	13.9 ± 2.19
pH = 10.5	90.5 ± 0.05	87.8 ± 0.03	75.2 ± 0.07	78.9 ± 0.07	78.0 ± 0.01	51.8 ± 0.04	−0.00223	0.192	18.8	12.4 ± 1.19

[a] Relative growth rate (day^{-1})

[b] Root weight ratio (g/g)

[c] Height increment (cm)

[d] Net photosynthesis rate ($\mu mol\ m^{-2}\ s^{-1}$)

alkaline conditions could directly damage plant organs and lead to an irreversible biomass loss. Further explanation was that damage happened in the molecular level. The value of 10.5 highly exceeded the pH requirement for normal cell, which varied from 5.0 to 8.0 at different parts of the cell (Marty 1999). Therefore, it affected adenosine triphosphate synthesis and cell elongation and destroyed the normal metabolism of the organism as well (Buchanan et al. 2000; Cosgrove 2000).

About 11.7, 34.4, 19.2, and 8.5 % of input TN was distributed to the sediment, respectively, standing for immobilization by sediment and microbial. Wu et al. (2012) reported that nitrogen accumulated in the sediment was 20.5−30.4 % of the overall input N, which was similar with the present study. Microbial immobilization provided 15−35 % of the total ammonium nitrogen removal (Morvan et al. 1997). Ion exchange function or physisorption was the mechanism of immobilizing ammonium or ammonia, respectively, since soil, the same with clinoptilolite, was taking silicon oxide as a basis, having negative reactive groups in the surface. However, not like ion exchange function for ammonium, the physisorption for ammonia was unstable (Jorgensen and Weatherley 2003). Therefore, when quantities of ammonium transformed into ammonia, immobilization was impaired. Du et al. (2005) investigated the effect of pH ranging from 4 to 10 on clinoptilolite exchange performance and demonstrated decreased removal efficiency as pH rose from 6 to 10.

The other TN losses mainly consisted of gas emissions (N_2, N_2O, and NH_3) and represented 32.4, 19.7, 38.4, and 48.1 % of the TN influent, respectively. At pH ≤8.5, gas losses were primarily denitrification gaseous products. Wu et al. (2012) reported that 0.6−1.9 % of the influent TN was lost as N_2O emission. At pH >8.5, denitrification was impaired, which would be supported by the results in Fig. 3, and ammonia volatilization strengthened to dominate. Emerson et al. (1975) illustrated that the percentages of NH_3 in aqueous

ammonia solution (25 °C) were 64.3 and 85.1 % at pH 9.5 and 10, respectively. While at pH around 7.5−8.5, there is no obvious volatilization (Vymazal 1999). Korner et al. (2001) reviewed that volatilization of ammonia varied from 20 to 70 % of the total ammonium nitrogen applied.

Wetland performance

The nitrogen removal efficiencies of different treatments from the entire experiment periods (before and after adjusting pH) are listed in Table 1. After applying pH stress, slight changes in NH_4^+ - N and NO_3^- - N removal efficiency were observed in four CWs. These changes were due to a shift of reed growth stages, as July is the end month for growing and September is the end month for flowering and setting seed. Accidental factors, such as weather variations and alga uptake, may also contribute to these variations. However, with pH rising from 7.5 to 10.5, TN removal efficiency dropped from 76.3 to 51.8 %. It was found that the plant RGR decreased from 0.00983 (at pH 7.5) to −0.00223 (at pH 10.5) (as shown in Table 1). A decline in plants which led to restricted assimilation had a crucial effect on removal efficiency decrease of TN. Another important reason is that elevated pH also affected microbiological removal routine, which played an important role in TN removal (Meng et al. 2014).

In addition, the intergroup dissimilarity of ammonia and TN removal efficiency gradually expanded with time (data not shown), indicating that the inhibitory effect of high pH strengthened with time. There were no significant intergroup differences for ΔH and P_n. The minimum RWR was found at pH 9.5 but not at pH 10.5. The reason was that although the root weights of these two treatments were almost equal, shoots and young stems decayed more severely at pH 10.5. These results indicated that alkaline conditions were more harmful to young stems than root base.

Nutrient removal in a typical cycle

Figure 2 presents the pH of effluents and the concentrations of NH_4^+ - N, NO_2^- - N, and NO_3^- - N in one typical cycle (the eighth cycle, from September 10 to September 15) after adding pH stress. The pH under different conditions showed the same tendency (except at pH 10.5) of decreasing at first followed by an increase, which may be due to microbial activities. At the first day of each cycle, the system was aerobic with abundant oxygen brought formed influent and was favorable for nitrification. During nitrification, the pH value decreased because alkalinity was consumed through ammonium oxidation at 7.14 g g^{-1} $N_{oxidized}$. Then, as DO was used up, pH increased because denitrification dominated and generated alkalinity at 3.57 g g^{-1} $N_{reduced}$ (Li and Irvin 2007). However, when pH exceeded 9.5, pH failed to return to normal or its recovery called for time longer than 5 days. To accelerate the pH recovery and reduce the adverse effect of alkalinity, direct buffering measurement such as CO_2 aeration, artificially adding organic acid (Mayes et al. 2009) and other indirect strategies, e.g., controlling the density of submerged plants within the constructed wetland or cutting HRT to a level to increase the water velocity, might need to be taken sometimes.

High ammonia removal was obtained under all pH conditions, with effluent NH_4^+ - N concentrations of 0.33−0.85 mg L^{-1}, as shown in Fig. 2. No significant difference ($P < 0.05$) was observed between treatments, indicating that high pH has no obvious effect on NH_4^+ - N removal. This was consistent with Dendooven et al. (2010). However, there is one opposite conclusion reported by Jones and Hood (1980). The explanation for the difference in results was that Jones and Hood (1980) experimented on flowing media (a freshwater marsh and an estuarine bay) in which ammonia volatilized in alkaline conditions, but they did not take this into account to conduct ammonia removal.

Nitrite concentration rose first and then declined, which could be observed generally in nitrogen transformation in many literatures (Zhi and Ji 2014). Nevertheless, significant nitrite accumulation was observed at pH of 10.5; i.e., in this condition, nitrite production rate was over nitrite consumption rate. Thus, at pH 10.5, the stress affected more on nitrite consumption approaches (oxidized to nitrate mediated by nitrite oxidoreductase or reduced to nitrogen gases mediated by nitrite reductase). Previous studies reported that nitrite reductase activity decreased to a negligible level at pH above 8 (Ellis et al. 2001), which was coherent with the results shown in Fig. 3; i.e., the generic (nirS) enzymatic activities were inhibited. And it was reported that the optimum pH of the membrane-bound nitrite oxidoreductase was estimated to be 8.0 (Sundermeyer-Klinger et al. 1984). Thus, in this experiment, at pH >9.5, the alkaline condition had a negative effect on these enzymes, causing nitrite accumulation.

Nitrate concentrations are on the decline under different pH values. The group at pH 10.5 decreased most quickly at 1.1 mg L^{-1} day^{-1}, while the other groups (pH 9.5 at 0.6 mg L^{-1} day^{-1}, pH 8.5 at 0.9 mg L^{-1} day^{-1}) were slower than the control group (pH 7.5 at 1.0 mg L^{-1} day^{-1}). At pH of 9.5 and 8.5, the slower nitrate decrease in rates was attributed to weaker plant assimilation as shown in Table 1. Besides, high pH caused a little depression in denitrifiers′ activities (as shown in Fig. 3) leading to an adverse effect on nitrate reduction by microbes. Moreover, as roots of P. australis transferred oxygen and released to their root, the root surface promoted the formation of an oxidized layer, which was

Fig. 2 Variation of wastewater quality parameters in mesocosm wetlands under different pH values (a pH; b NH_4^+-N concentration (mg L^{-1}); c NO_2^--N concentration (mg L^{-1}); d NO_3^--N concentration (mg L^{-1}))
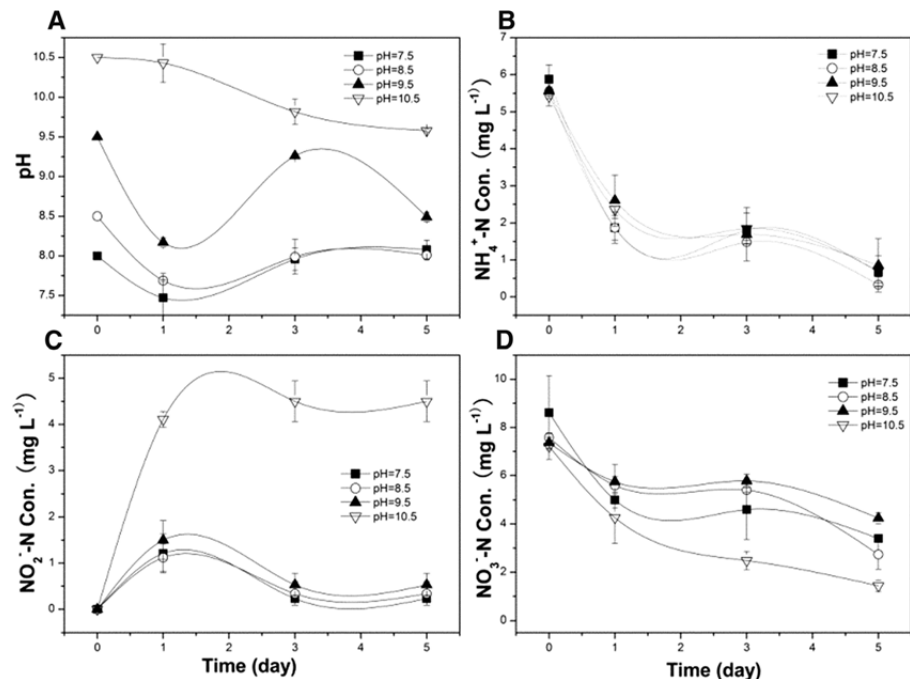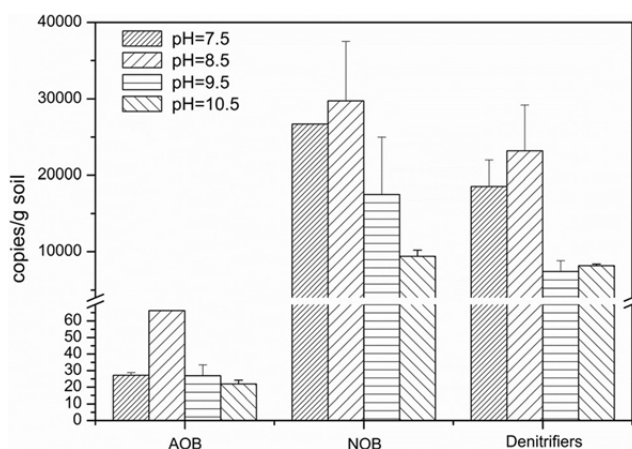
Fig. 3 Abundance of AOB, NOB, and denitrifiers

favorable for aerobic microbial (Münch et al. 2005). When the growth of plants was inhibited at pH above 8.5, such enhancement by creating redox gradients was gone. Another approach for nitrate consumption is dissimilatory nitrate reduction to ammonia (DNRA), the intensity of which reached maximum at around pH 8−8.9 (Nömmik 1956; Stevens et al. 1998). However, the results indicated that the consumption of nitrate by DNRA in the present study was limited to affect the nitrate concentration. At pH 10.5, nitrate concentration was bound up with nitrite when the accumulation of nitrate was observed significantly because nitrite was the direct intermediate of nitrate transformation.

Microorganism analysis

In the CW system, microbes play an important role in nitrogen transformation. It was reported that microbial metabolism contributed to 60−90 % of the TN removal in SFCW (Hsueh et al. 2014). Figure 3 shows the absolute abundance (copies per gram wet soil) of three physiological groups of bacteria involved in nitrogen transformation, i.e., AOB, NOB, and denitrifiers. Solution pH was one of the important factors that affect bacterial growth (Meng et al. 2014).

The abundance of AOB (function: $NH_4^+-N \rightarrow NO_2^--N$) in this experiment was low; however, NH_4^+-N accumulation was not observed and similar NH_4^+-N removal rates during degradation process were obtained in all four treatments. AOB was not in a large amount, and similar results were reported in Jones and Hood (1980), claiming 300 cells g^{-1} wet soil of AOB in natural wetland soil. Besides, the copy ratio of NOB/AOB in the present study was about 150−400, within the scope of 30−400 as reviewed in Dionisi et al. (2002). As for the similar efficiency of AOB under different pH conditions, this was because AOB could thrive well under alkaline conditions, even when pH was above 8.5 (Goloran et al. 2014). And it seems that the oxidation of NH_4^+-N functioned well even in wetland with the lowest AOB abundance (pH 10.5).

Regarding NOB (function: $NO_2^- \rightarrow NO_3^-$) and denitrifiers (function: denitrification), inhibiting effects were observed at pH of 9.5 and 10.5, as shown in Fig. 3. This was because nitrite reductase and membrane-bound nitrite oxidoreductase were both pH sensitive and had lower activities at pH ≥8 (Ellis et al. 2001; Sundermeyer-Klinger et al. 1984). It is worth noting that NO_2^--N accumulation was not obviously monitored in the effluent at pH 9.5 (the third treatment), although microorganism inhibition was detected. One reason was that weaker restriction of NOB resulted in a certain amount of nitrite oxidation, which could be seen in Fig. 3. The other reasons could be depression of membrane-bound nitrate reductase activity ($NO_3^--N \rightarrow NO_2^--N$) (Zhi and Ji 2014). Thus, the generation of nitrite was cut down, which was consistent with slower nitrate consumption at pH 9.5, as shown in Fig. 2.

Overall, all these inhibitory effects for microbial activity were originated from alkaline stress, which meant that proper strategies should be implemented to avoid alkaline arising from photosynthesis of submerged plants before the application of species combination. It was more complicated in a full-scale SFCW than the simulation of elevated pH in the present study. In a full-scale SFCW, high photosynthetic activity may lead to not only the direct alkaline stress but also the competition between submerged plants and nitrifying bacteria. They competed for oxygen for respiration, inorganic carbon for assimilation, and ammonia which was nutrition for plants or substrates for nitrification (Gieseke et al. 2005). Thus, in future studies, it might be worthwhile to consider a full-scale SFCW study.

Conclusions

In SFCW, TN removal efficiency decreased from 76.3 ± 0.04 to 51.8 ± 0.04 % when photosynthetically elevated pH increased from 7.5 to 10.5. This was mainly caused by plant assimilation decay and inhibition on NOB and denitrifiers′ activities. Nitrogen balance showed plant assimilation dropped from 27.7 % to minus with the increase of pH. And sediment adsorption was the maximum at pH 8.5 with 40.4 % of the input TN. The combination of submerged and emergent plants was feasible most of the year to be an alternative enhancement approach of constructed wetland. However, precautions were needed to be taken in midsummer to mitigate a negative effect of alkaline conditions when the pH rose to above 8.5. Besides, the present study supplied indirect evidence for the effect of photosynthetic activities on microorganism and the entire system of constructed wetland.

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