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ORIGINAL PAPER

Effects of cadmium addition on net nitrogen mineralization processes in the urban constructed wetland soils of a Chinese delta

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Abstract Heavy metal pollution is a serious problem in wetland ecosystems, and the toxicity of heavy metals affects microorganisms, thus influencing the biogeochemical process of nitrogen (N). To investigate the effects of heavy metal cadmium (Cd) pollution on N mineralization in urban constructed wetland soils of the Pearl River Delta, a 40-day aerobic incubation experiment was conducted under three Cd addition treatments [no Cd addition (control), low Cd addition (LCA) and high Cd addition (HCA)]. The results showed that compared with the control, the LCA treatment enhanced the soil N mineralization rate (R_M), while the HCA treatment inhibited R_M, with the average R_M values in the control treatment of 0.40 mg kg^{-1} day^{-1}, LCA treatments (0.66 mg kg^{-1} day^{-1}), and HCA treatments (0.21 mg kg^{-1} day^{-1}). The average ammonification rate values in the LCA treatments (− 3.15 to 2.25 mg kg^{-1} day^{-1}) were higher than those in the HCA treatments (− 2.39 to 0.74 mg kg^{-1} day^{-1}) and the control treatment (− 0.68 to 0.90 mg kg^{-1} day^{-1}) ($P < 0.05$). However, the nitrification values in the HCA treatments (− 0.37 to 3.36 mg kg^{-1} day^{-1}) were higher than those in the LCA treatments (0.42–1.93 mg kg^{-1} day^{-1}) and the control treatment (0.20–1.45 mg kg^{-1} day^{-1}) ($P < 0.05$). The net N mineralization accumulation generally increased over the entire incubation time in different Cd addition treatments. The percentage of NH_4^+–N to total inorganic N showed a decrease, while an increase was observed for NO_3^-–N over the incubation time. The urease activities were significantly inhibited in the LCA and HCA treatments and showed a "decreasing before increasing" trend. The abundance of ammonia oxidizing archaea (AOA) was higher in the two Cd addition treatments than the control treatment, and higher in the LCA treatments than in the HCA treatment. AOA was the dominant microorganism in the ammonia oxidation process of N mineralization in constructed wetland soils. The findings of this work indicate that Cd addition has a profound effect on the balance of N mineralization and may further impact the plant productivity and water quality of constructed wetlands.

Keywords Cadmium addition · Nitrogen mineralization · Nitrification · Ammonification · Constructed wetland soils

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Introduction

Wetlands play an important role in the biogeochemical cycles of elements, serving as sources, sinks and

transfers of carbon and nitrogen elements and other chemical contaminants (Bai et al. 2012; Craft 2008). Nitrogen is a vital biogenic element and is often considered to be a limiting factor determining primary productivity and plant community structure in wetland systems (Jia et al. 2017; Zaman et al. 2009). N mineralization is the critical process determining N availability, in which organic N (approximately 85–90% of total N) is converted to the inorganic form by soil enzymes and microorganisms (Chapin III et al. 2011; Chen et al. 2016), and the rate of N mineralization is an important indicator that reflects the soil N supply levels and the ability to impact the environment (Mishra et al. 2005). Ammonification and nitrification are the important processes of N mineralization in wetland soils, and urease is an important enzyme for ammonification, while AOA and AOB are the critical microbial groups that transform ammonium to nitrite in the nitrification process (Willm et al. 2009).

Kanat et al. (2018) studied that heavy metal concentrations (i.e., Zn, Cr, Cu, Pb, Cd, and Ni) originated from human activity in Golden Horn and they suggested that heavy metal concentrations were usually low, and these values indicated that the sediment was not too polluted presently. In contrast, the Pearl River Delta is a pioneer district with intense industrialization and urbanization in China. A large amount of heavy metal contaminants were discharged into the environment and have become a serious environmental problem (Li et al. 2007). In particular, Cd shows moderate to heavy pollution in rural and urban river sediments of the Pearl River Delta (Xiao et al. 2013). Heavy metals can not only impact the availability of soil mineral elements but also change the soil microorganism community structure and enzyme activity, which would change the N mineralization processes (Dar 1997; Giller et al. 2009). Vig et al. (2004) showed that the heterogeneity of Cd effects on soil microorganisms is largely due to different soil types. Constructed wetlands are created to retain and remove pollutants, which are more stable and easier to control than natural wetlands (Vymazal 2007). Leung et al. (2017) observed an excessive accumulation of heavy metals in the tissues of plants and fishes in constructed wetlands. Most of the previous studies of constructed wetlands focus on wastewater treatment and technology. However, little information is available on the effects of different Cd pollution levels on soil N mineralization (i.e., ammonification and nitrification) and N-related microbial activities in constructed wetlands affected by intense urbanization and industrialization.

The primary objectives of this study were: (1) to investigate the effects of different levels of Cd addition treatments on soil N mineralization processes in constructed wetlands, and (2) to evaluate the effects of Cd additions on urease activity and AOA/AOB (ammonia-oxidizing bacteria) abundances.

Materials and methods

Site description and soil collection

The study area is in the Panyu area of the Pearl River Delta, Guangdong Province (21° 17.6′–23° 55.9′ N, 111° 59.7′–115° 25.3′ E), which is a new urban agglomeration built in 2003. The original environment has been occupied by urban construction, and some constructed wetlands were created to control domestic discharge. In this study, we chose a constructed wetland in the university town.

Soil samples were collected in July 2014. Top soils (0–10 cm) were collected with three replicates using a plastic shovel. Each soil was fully mixed, sealed in a plastic bag and transported to the laboratory for further analysis. Some fresh soils were stored at 4 °C and used for the N mineralization incubation experiment. Some soil samples were air-dried for 3 weeks and sieved through a 2-mm sieve to remove coarse plant litter and stones. One part of the air-dried samples was used to determine the soil pH and salinity, and the remaining soil samples were ground and sieved through a 0.149-mm sieve for the determination of other soil properties. The soil properties of the constructed wetlands used for the incubation experiment are listed in Table 1.

Incubation experiment

The incubation experiment for nitrogen mineralization was conducted at three Cd addition levels: (1) A: no addition (control); (2) B: low addition (LCA, 15 mg kg^{-1} Cd); and (3) C: high addition (HCA, 100 mg kg^{-1} Cd). Cd was added to the soil as an aqueous solution of cadmium chloride ($CdCl_2$).

Before the incubation experiment, the soils were activated in an incubator at 25 °C for 3 days. Then,

Table 1 Soil physical and chemical properties of constructed wetlands

pH	SOM/g kg^{-1}	WC/%	TN/g kg^{-1}	TP/g kg^{-1}	Al/mg kg^{-1}	Fe/mg kg^{-1}	Cd/mg kg^{-1}
6.10 ± 0.15	40.01 ± 13.51	40.70 ± 4.17	1.96 ± 0.49	76.87 ± 51.1	107.67 ± 17.73	40.90 ± 21.05	0.92 ± 0.05

three replicates of fresh soils (100 g of dry soil) were placed in plastic jars. A total of 63 jars were incubated, with 21 jars for each Cd addition treatment, and all jars were covered with ventilated plastic film. The soil moisture was kept constant during the incubation period by the weighting subtraction method.

Three jars for each Cd addition treatment were taken out at days 0, 3, 6, 9, 15, 25, and 40, respectively, during the incubation period. The incubated soil in each jar was divided into two parts. One part was air-dried for 3 weeks and then ground and sieved with a 0.149-mm sieve to determine the basic physical and chemical indicators of the soil. One part was stored in a refrigerator at 4 °C to determine the contents of NH_4^+–N and NO_3^-–N and the urease activities. The remaining soil was stored in a refrigerator at − 20 °C to determine the abundances of AOA and AOB.

Soil sample analysis

Approximately 5 g of fresh soil was extracted with 25 mL of 2 mol L^{-1} KCl solution. After being mixed and filtered, the extracts were measured on the automated flow injection analysis AA3 (Bran + Luebbe, Germany) to determine the contents of NH_4^+–N and NO_3^-–N. Soil pH was measured using a pH meter (soil/water, 1:5). The soil moisture was measured by drying soils at 105 °C for 24 h. Soil total nitrogen was determined using an elemental analyzer (CHNOS Elemental Analyzer, Vario EL, German). Soil metals (Fe, Al) and total phosphorus (TP) were determined by inductively coupled plasma spectrometry (ICP-AES) (standard material is GBW07401 from the Chinese Academy of Measurement Sciences, 1 blank and 1 standard for each 10 samples). SOM was determined using the Nelson and Sommers method (Nelson and Sommers 1982). Urease activities were determined using the method of Schinner et al. (1996). The copies of *amoA* genes of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were estimated by quantitative real-time PCR (qPCR) in a StepOnePlus real-time PCR system (Huang et al. 2020).

Data calculation and statistical analysis

Net N mineralization (R_M), nitrification (R_N), ammonification (R_A) rates (mg kg^{-1} day^{-1}) and net mineralization accumulation (NMA) (mg kg^{-1}) were calculated by Eqs. (1–5):

$$M_i = A_i + N_i \quad (1)$$

$$R_A = (A_{i+1} - A_i)/(t_{i+1} - t_i) \quad (2)$$

$$R_N = (N_{i+1} - N_i)/(t_{i+1} - t_i) \quad (3)$$

$$R_M = (M_{i+1} - M_i)/(t_{i+1} - t_i) \quad (4)$$

$$NMA = M_i - M_0 \quad (5)$$

where t_i is the incubation time; M_i, A_i, and N_i are the contents of inorganic N, NH_4^+–N, and NO_3^-–N, respectively; and R_M, R_A and R_N are the net N mineralization rate, net ammonium rate and net nitrate rate, respectively.

One-way ANOVA was conducted to test the differences in R_M, R_A, R_N, NMA and urease activity among different incubation time or different Cd addition levels (Jia et al. 2019). Two-way repeated measures ANOVA was used to test the differences between groups of different levels of Cd addition on R_M, R_A, R_N and urease activities over the entire incubation period (Gao et al. 2014). The difference was considered to be significant if $P < 0.05$. Statistical analysis was conducted using the SPSS 21.0 software package, and bar and column plots were created using the OriginPro 2016 software package.

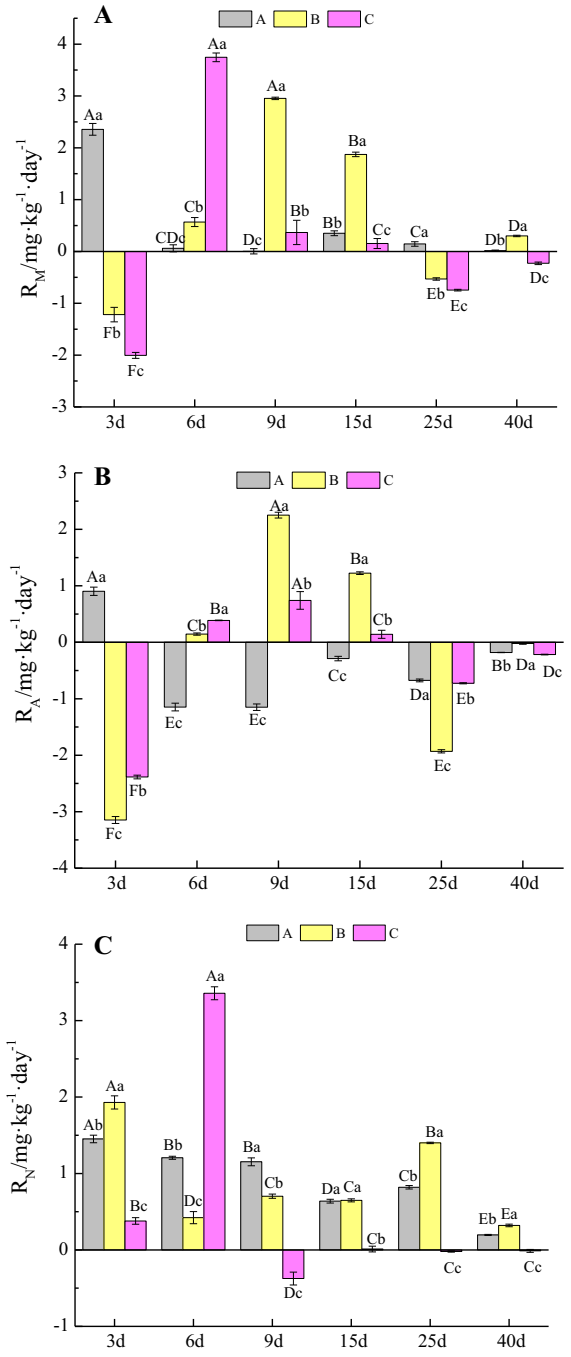

Fig. 1 Changes in net mineralization rates (**a**), ammonification rates (**b**) and nitrification rates (**c**) in constructed wetland soils under different levels of Cd additions (statistically significant differences are indicated by symbols as Aa, Bb, Cc, among them ABC represents the differences in the R_M, R_A and R_N values between different incubation time in the same treatment, abc represents the differences in the R_M, R_A and R_N values between different treatments at the same incubation time)

Results

Soil nitrogen mineralization processes

Figure 1 shows the variations in the soil net N mineralization rates (R_M), net ammonification rates (R_A) and net nitrification rates (R_N) over the 40 days of incubation. The R_M rates showed significant differences among the different Cd addition treatments ($P < 0.05$) (Table 2), and a higher R_M fluctuation was observed for the LCA (− 1.22 to 2.95 mg kg^{-1} day^{-1}) and HCA (− 2.01 to 3.75 mg kg^{-1} day^{-1}) treatments than that in the control treatment (0.01–2.36 mg kg^{-1} day^{-1}). The R_M in the control treatment decreased to 0 in the first 6 days of incubation and remained near 0 until the end. The R_M in the LCA and HCA treatments exhibited an "N" type tendency, with peak values at the 6th day and 9th day, respectively, while the minimal values occurred after 25 days of incubation. In the later stage of incubation, the R_M in the LCA treatments was higher than that in the HCA treatments, though both treatments had negative values.

The average R_A values ranged from − 3.15 to 2.25 mg kg^{-1} day^{-1} in the LCA treatments, which was higher than those in the HCA treatments (− 2.39 to 0.74 mg kg^{-1} day^{-1}) and the control (− 0.68 to 0.90 mg kg^{-1} day^{-1}) ($P < 0.05$). The R_A values in the LCA and HCA treatments showed a similar change, peaking after 9 days of incubation and declining to the low minimal values after 25 days of incubation, while no significant differences were observed between the treatments ($P > 0.05$) (Table 2). The R_A values in the control treatment first decreased and then increased, after which they remained stable at negative values.

Table 2 Two-way repeated measures ANOVA results among three Cd addition treatments during the incubation period

	R_M	R_A	R_N	UA
A × B	0.007*	0.001*	0.903	0.002*
B × C	0.007*	0.061	0.001*	0.061
A × C	0.007*	0.054	0.000*	0.005*

A, B, C represent the control, low Cd addition, high Cd addition treatments, respectively

*Represent significant level of $P < 0.05$

The R_N values in the HCA treatments varied from − 0.37 to 3.36 mg kg^{-1} day^{-1}, which were higher than those in the LCA treatments (0.42–1.93 mg kg^{-1} day^{-1}) and the control (0.20–1.45 mg kg^{-1} day^{-1}). The R_N generally decreased in the control treatment over the incubation time. The R_N values in the LCA treatments first decreased, then increased, and then decreased again, reaching maximum and minimal values at 6 days and 9 days of incubation, respectively. There was no significant difference in the R_N values in the control and LCA treatments ($P > 0.05$) (Table 2). The R_N values in the HCA treatments exhibited an "increasing before decreasing" tendency and then remained near 0 until the end of incubation.

The net N mineralization accumulation (NMA) was different in the different Cd addition treatments (Fig. 2). The NMA was reduced in the LCA and HCA treatments at the initial stage of incubation. In general, the NMA increased over the whole incubation time in the different Cd addition treatments. The NMA increased from 7.07 to 11.05 mg kg^{-1} in the control treatment and from − 3.65 to 17.34 mg kg^{-1} in the LCA treatments. In the HCA treatments, the NMA increased from − 6.02 to − 3.65 mg kg^{-1}.

The proportions of ammonium N (NH_4^+–N) and nitrate N (NO_3^-–N) showed significant differences

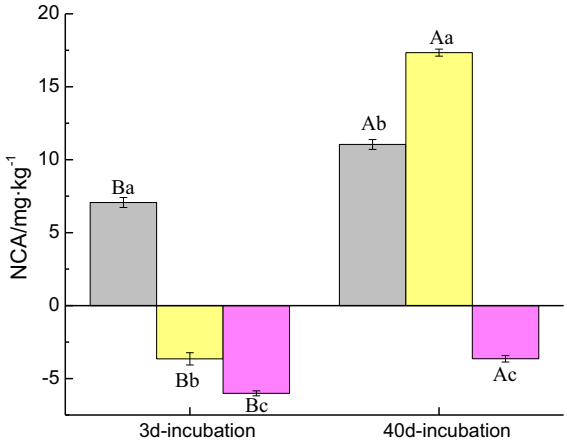

Fig. 2 Net N mineralization accumulation in constructed wetland soils under different levels of Cd addition treatments (statistically significant differences are indicated by symbols as Aa, Bb, among them AB represents the differences in the net N mineralization accumulation between different incubation time in the same treatment; ab represents the differences in the net N mineralization accumulation between different treatments at the same incubation time)

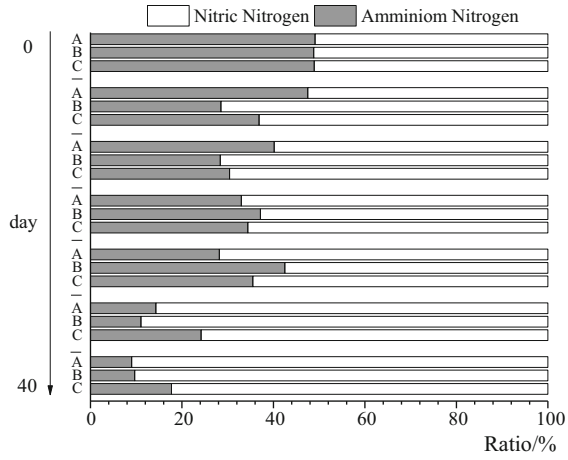

Fig. 3 Proportions of ammonium nitrogen and nitrate nitrogen with incubation time in different levels of Cd addition treatments

over the incubation time ($P < 0.05$) (Fig. 3). In the control treatment, the proportion of NH_4^+–N gradually decreased and was accompanied by an increase in the NO_3^-–N levels with incubation time. In the LCA and HCA treatments, the proportion of NH_4^+–N first increased and then decreased. In the initial stages of incubation, the percentages of NH_4^+–N accounted for approximately 50% of the inorganic N in the different Cd addition treatments. There was no significant difference among the different treatments ($P > 0.05$). The proportion of NH_4^+–N in the HCA treatment was significantly higher than that in the control and LCA treatments at the end of incubation ($P < 0.05$) (Table 2).

Changes in soil urease activities

The urease activities showed a "decreasing before increasing" tendency in the different Cd addition treatments (Fig. 4). In the first 6 days of incubation, there was a sharp decline in the urease activities in the control and LCA treatments, while in the HCA treatments, the urease activity first increased and then decreased. After 6 days of incubation, the urease activities generally increased with a fluctuation in the control and LCA treatments, while they maintained constant increase in the HCA treatments. There was a significant difference between the treatments without Cd additions and the treatments with Cd additions ($P < 0.05$); however, no significant difference was

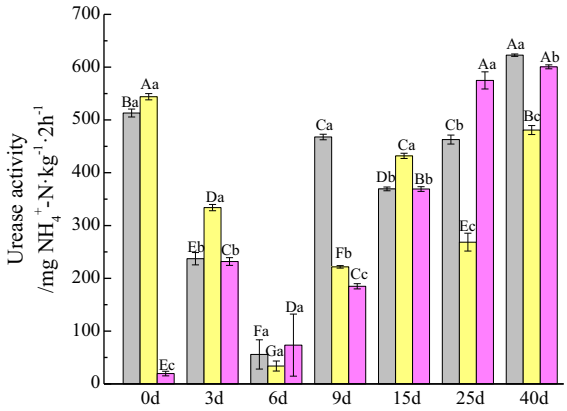

Fig. 4 Variations in urease activity in constructed wetland soils under different levels of Cd addition treatments (statistically significant differences are indicated by symbols as Aa, Bb, Cc, among them ABC represents the differences in the urease activity between different incubation time in the same treatment; abc represents the differences in the urease activity between different treatments at the same incubation time)

observed between the LCA and HCA treatments ($P > 0.05$) (Table 2).

Variations in AOA and AOB abundances

Figure 5 shows the variation in the initial and final proportions of AOA and AOB in the different Cd addition treatments. The abundances of AOA in the LCA treatment were higher than those in the HCA treatments, both of which were higher than that in the control treatment during pre-incubation and post-incubation. The proportions of AOA and AOB changed obviously with the incubation time. Compared with pre-incubation, the proportion of AOA increased in the three Cd addition treatments. In the control treatment, the proportion of AOA increased from 36.93 to 45.79%, while AOB was the dominant microbe of the ammonia oxidation process. The proportion of AOA increased from 61.93 to 81.06% and from 49.41 to 60.88% in the LCA and HCA treatments, respectively, and thus AOA was the dominant microbe of the ammonia oxidation process in these treatments.

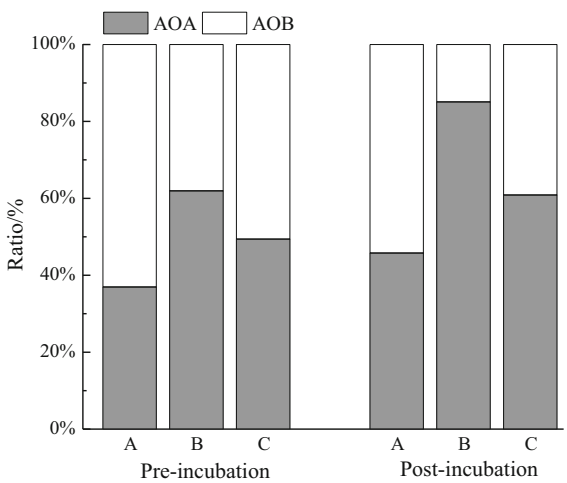

Fig. 5 Changes in the proportions of AOA and AOB copies under different levels of Cd addition treatments

Discussion

Effects of Cd on nitrogen mineralization processes

In general, heavy metals would poison soil microorganisms, thereby inhibiting the process of N mineralization (Vig et al. 2004), although Nwuche and Ugoji (2008) reported that the carbon and nitrogen mineralization rates were increased by copper or copper and zinc additions in clay loam soil. This result might be associated with the fact that copper and zinc are essential elements for plant and microbe physiological activities (Wyszkowska et al. 2013). However, the results on the effects of Cd addition on nitrogen mineralization were inconsistent. Some studies showed that Cd addition, even at low concentrations, would inhibit the N mineralization process (Raiesi et al. 2018; Ye et al. 2007), while some researchers observed that a low Cd addition promoted N mineralization, while a high Cd addition inhibited N mineralization (Chen et al. 2001; Dušek 1995; Hassen et al. 1998). In this study, the average rates of N mineralization and net nitrogen mineralization accumulation in the LCA treatments (0.66 mg kg^{-1} day^{-1}) were higher than those in the control treatment (0.49 mg kg^{-1} day^{-1}) and HCA treatments (0.21 mg kg^{-1} day^{-1}). This result might be because the toxicity of Cd changed the microbial community structures and activities. The low Cd addition was within the range of constructed wetland soil carrying capacity; therefore, the soil microbes transformed from being sensitive to Cd to being resistant to Cd (Atlas 1984). However, the high Cd addition was beyond the threshold of soil resistance to Cd toxicity,

resulting in a smaller microbial community than that in the low Cd addition; thus, very little inorganic N accumulated. In this study, the changes in AOA abundances also revealed a change in microbial number. Moreover, the tendency of N mineralization in the Cd addition treatments was consistent with the abundances of AOA at the initial and final stages of incubation (Fig. 5).

Compared with the control treatment, the time it took for the N cycle to reach equilibrium was extended from 6 to 25–40 days after Cd addition, and the N mineralization rates increased from 2.35 to 5.76 mg kg^{-1} day^{-1} after Cd addition. This result might be because the constructed wetlands had rich vegetation communities and many microbial species, with high quantities and activities; therefore, the system was relatively stable due to the complex community structure. Generally, an increase in heavy metal Cd could significantly increase the fluctuations in the N mineralization rates in constructed wetland soils. The effects of Cd addition were larger on the ammonification processes than on the nitrification process, and significant effects were observed at the initial stage of incubation; additionally, the high Cd addition treatments had larger effects than the low Cd addition treatments. However, previous studies indicated that the nitrification process was more sensitive to heavy metal additions (Bewley and Stotzky 1983; Dar 1997; Rother et al. 1982). This might be associated with the different soil properties, resulting in the different microbial communities related to N transformation.

At the late stage of incubation, the ammonification rates appeared negative values in the different Cd addition treatments, which could be ascribed to the following explanations. (1) As the incubation proceeded, the soil organic matter supply to the microbial carbon source was consumed, and the substances that easily ammoniate (amino, polypeptide) were reduced, thereby decreasing the ammonification rate later in the incubation period due to the insufficient soil organic matter (Dar 1997). (2) Ammonium was transformed into nitrate by nitrification, with an increase in nitrate and a decrease in ammonium (Fig. 3) due to the higher nitrification rates (Jia et al. 2017). (3) As the incubation proceeded, the microbe ability to adapt to the environment was enhanced, and the amount of microbes increased. Microbial immobilization might explain the decline in ammonium in constructed wetland soils because microbes reproduce using ammonium (Giller et al. 1998). Sakadevan et al. (1999) demonstrated that denitrification inhibition was linearly related to ammonium levels in the environment. In this study, the ammonium rates had negative values in the late stage of incubation, which might relieve the inhibition of Cd to denitrification; thus, the denitrification was enhanced. This process partly explains the decrease in nitrification rates in the late stage of incubation.

Effects of Cd on urease activities

The urease activity firstly decreased and then increased with the incubation process in the Cd addition treatments, and the lowest value appeared at 6 days of incubation, which was consistent with the result of Zhang et al. (2010). This might be related to the variations in Cd speciation. As a result of the strong availability of urease to the adverse environment (Sardans et al. 2008), another possible explanation for this trend was that the ammonification microbial community may have been greatly changed in the early stage of incubation to adapt to the new environment.

In this study, over the whole incubation time, the average urease activities in the control treatment (389.74 mg·NH_4^+–N·kg^{-1}·2 h^{-1}) were higher than those in the LCA (330.70 mg·NH_4^+–N·kg^{-1}·2 h^{-1}) and HCA (293.49 mg·NH_4^+–N·kg^{-1}·2 h^{-1}) treatments. This suggested that urease activities were inhibited by Cd toxicity. As one of the important enzymes in the ammonification process, urease activity is often closely associated with total N and N transformation (Zaman et al. 2009); however, in this study, there was no significant correlation among ammonification rate, mineralization rate and urease activity. Generally, inconsistent conclusions have been observed regarding the effects of Cd addition on urease activities. Tejada (2009) and Zheng et al. (2016) reported that urease activities decreased with increasing levels of heavy metal additions. Comparatively, Yan et al. (2013) demonstrated that low levels of Cd addition promoted urease activities, while high levels of Cd addition inhibited urease activities. However, Yang et al. (2015) found that Cd addition promoted urease activities more than the no Cd addition scenario, which might be attributed to different soil types and microbial communities. The

findings might be associated with the complexity and variety of enzymes involved in the ammonification process, and the specific reason for this association requires further research.

Effects of Cd on AOA and AOB

AOA had more tolerance to adverse stress than AOB (Chen et al. 2016). In this study, the proportion of AOA was higher in the LCA and HCA treatments than in the control treatment at the initial and final stages of incubation. This indicated that under heavy metal stress, the LCA treatment was more conducive than the HCA treatment to the growth of AOA, which might be due to the heavy Cd pollution stress caused by high Cd addition. Wang et al. (2017) reported that heavy metals had no significant effects on the ratios of AOA and AOB, while nitrogen conditions exhibited significant effects, with higher AOA abundance under low nitrogen conditions and higher AOB abundance under high nitrogen conditions. Gao et al. (2015) reported that the ratio of AOB was negatively correlated with the ammonium level. In this study, the proportion of AOA increased over the whole incubation time in the three Cd addition treatments, which was probably because of the decreased proportion of NH_4^+–N with incubation time (Fig. 3); hence, the growth of AOB was inhibited. Among the three Cd addition treatments, the proportion of NH_4^+–N in the low Cd addition treatments was lower than that in the HCA treatments, and AOB was severely inhibited in the LCA treatments, which might explain the higher proportion of AOA in the LCA treatments than in the HCA treatments. Overall, AOA was more adaptive to heavy metal stress than AOB, and AOA was the dominant microbe of ammonia oxidation in the N mineralization process in Cd-contaminated soils, which was consistent with previous studies (Anja et al. 2012; Vasileiadis et al. 2012).

Conclusions

Low concentrations of Cd addition promoted the N mineralization rates and net N mineralization accumulation in constructed wetland soils, while high concentrations of Cd addition inhibited these processes. The Cd addition enhanced the fluctuation of organic N mineralization, and the ammonification process appeared to be more sensitive than the nitrification process. Low and high Cd addition treatments inhibited urease activities and caused a higher proportion of AOA, while the proportion in the low Cd addition treatments was higher than that in the high Cd addition treatments. In addition, AOA was the dominant microbe in ammonia oxidation under Cd pollution stress. Therefore, Cd pollution has great effects on nitrogen transformation and will further affect constructed wetland productivity and ecological service functions. Proper actions should be taken to maintain the level of Cd pollution within ecologically safe levels to control nitrogen mineralization processes and keep the ecological service function and pollutant degradation process of constructed wetlands.

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