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The negative impact of cadmium on nitrogen transformation processes in a paddy soil is greater under non-flooding than flooding conditions

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ABSTRACT

Nitrification and denitrification are two important processes in the nitrogen (N) cycle. Under heavy-metal pollution with water management of paddy soils, these two processes are not well understood. This study aimed to examine the effect of cadmium (Cd) on N transformation under flooding and non-flooding conditions. A paddy soil was incubated under two water regimes (flooding and non-flooding) and four Cd levels (0, 2, 5 and 10 mg kg^{-1}). The availability of Cd was higher in the non-flooding than flooding conditions. Cadmium contamination significantly ($p \leq 0.05$) decreased the copy number of archaeal and bacterial *amoA* genes, bacterial *nirS*, *nirK* and *nosZ* genes under both conditions with the decrease being greater under non-flooding. High level of Cd (10 mg kg^{-1}) was more toxic in non-flooding than flooding conditions to the nitrifiers and denitrifiers, which in turn decreased N transformation through microbially-mediated processes. Its contamination decreased N_2O emission initially under both water regimes but the effect was greater under the non-flooding condition. However, the non-significant stimulatory effect of Cd on N_2O emission was observed during the late phase. The microbial community structure was changed with time and water regimes. Irrespective of water regime, the dominated fungal phyla were *Ascomycota* and *Basidiomycota* while the dominated bacteria phyla were *Actinobacteria, Proteobacteria*, *Firmicutes* and *Acidobacteria*. In summary, water regimes and Cd bioavailability changed soil N transformations via microbial mediated processes.

1. Introduction

Agricultural lands are at risk of the contamination of cadmium (Cd) and other heavy metals. Cadmium in many agricultural soils has originated mostly from phosphate fertilizers and farmyard manures (Tóth et al., 2016). In China, Cd contamination is widespread in farmland; about 7% of the total soil survey sites were over-standard rate of Cd contamination (MEP and MLR, 2014). In the last decade, Cd contamination has been widely reported in rice paddies (Xiao et al., 2013). For example, due to mining activities in Yueyang county of Hunan Province, a large proportion of sampled rice grains had the Cd concentration exceeding the limit of 0.2 mg kg^{-1} (Du et al., 2013).

Previous studies reported that heavy-metal pollution decreased the microbial biomass (Liu et al., 2012a, 2012b) and abundance (Chen et al., 2014a, 2014b), and *amoA* genes abundance (Liu et al., 2018b) in the paddy soil. The change in microbial community structure and function due to metal contamination could alter the rates of soil microbial-mediated processes (Liu et al., 2012a, 2012b; Yu et al., 2016). For example, Cd salt such as $CdCl_2$ (200 mg kg^{-1}) suppressed the soil nitrification rate by up to 80% (Smolders et al., 2001). Similarly, after two weeks of soil incubation, 5 mg Cd kg^{-1} decreased the activities of microbial enzymes acid phosphatase and urea amidohydrolase, by up to 30.6% and 33.0%, respectively (Khan et al., 2010). A recent study showed that *amoA* gene abundance decreased by Cd in the soil which could decrease ammonium oxidation (Liu et al., 2018). Similar to nitrifiers, the denitrifying bacterial community was sensitive to Cd, Ni, and Cu with Cu and Ni having greater effects on the denitrifiers community structure than other soil factors and metals (Deng et al., 2018).

Autotrophic nitrification is the most important process in the soil in which ammonium is oxidized to nitrite and nitrate by autotrophic

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ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Pedersen et al., 1999; Norton et al., 2002; Leininger et al., 2006; Chen et al., 2008; Mukhtar et al., 2017). On the other hand, denitrification process is carried out by a diverse group of bacteria anaerobically in which nitrogen oxides (NO_3^- and NO_2^-) are reduced to gaseous forms (NO, N_2O, and N_2) by nitrite reductases and nitrous-oxide reductase (Ishii et al., 2011; Zumft and Kroneck, 2006). The *nirK* and *nirS* genes encode nitrite reductases and the *nosZ* gene encodes nitrous-oxide reductase (N_2OR) (Throback et al., 2004). The Nir and N_2OR have been reported to be sensitive to high concentrations of Zn, Pb and Cu in soils and sediments (Richardson et al., 2009; Sobolev and Begonia, 2008; Ruyters et al., 2010). Increasing contamination intensity of heavy metals was also reported to decrease the number of *nirK* and *nosZ* genes in paddy soils (Liu et al., 2016).

Different water management practices are used for rice production. Continuous flooding irrigation is the most common practice. However, water-saving irrigation for paddy fields has also been practiced for many years. Alternate drying-wetting and thin-shallow-wet-dry irrigation (TIR) methods are the water-saving practices and have been used in China since 1990s (Liang et al., 2016). Water management practices can affect the oxygen transfer into the soil, N transformation and redox potential of the soil (Hernandez-Soriano and Jimenez-Lopez, 2012). It has been reported that irrigation practices significantly affect soil microbial biomass C (MBC) and N (MBN) with intermitted irrigation decreasing MBC and MBN as compared to continuous flooding irrigation (Gordon et al., 2008; Liu et al., 2012a, 2012b). Other studies have reported that intermittent irrigation and mid-season drainage increased N_2O emissions of paddy fields (Cai et al., 1997; Yao et al., 2013).

Soil water regime also affects the bioavailability of Cd. The HCl-extractable Cd was higher in soil under the aerobic and intermittent than conventional and flooding conditions (Hu et al., 2013). Flooding and alternate wetting and drying have been used as effective methods to reduce the bioavailability of Cd in soil, and Cd concentration in rice grains (Arao et al., 2010; Lei et al., 2018). Saturated water conditions increased the complexes of metals with the potential binding sites in the soil (Hernandez-Soriano and Jimenez-Lopez, 2012). Several reduced organic substances such as acetate, formate and organic acid (formic, acetic, propionic and butyric acids) are produced after continuous flooding. These reduced organic substances change the surface charge properties of the soil, and increase the adsorption of metal ions in the soil (Davranche and Bollinger, 2000; Huang et al., 2010). For example, continuous flooding conditions are responsible for the conversion of SO_4^{2-} to S^{2-} and the formation of CdS to decreased Cd bioavailability (Bingham et al., 1976). Therefore, it is expected that heavy metals like Cd would be more bioavailable and toxic to nitrifiers and denitrifiers under the non-flooding than flooding conditions. However, it is unknown how these effects can be related to microbial-mediated N_2O emission from paddy soils.

Until now, the interactive effect of water management practices (flooding and non-flooding) and Cd bioavailability on N transformation has not been reported. This study aimed to elucidate how water management practices influenced Cd bioavailability which in turn affects N cycling by quantifying the abundances of most important, autotrophic soil nitrifier gene, *amoA,* and denitrifier bacterial genes, *nirS*, *nirK* and *nosZ,* under flooding and non-flooding conditions by real-time PCR. The study also examined the effect of Cd on N transformation and associated N_2O emission. Microbial community structure was studied by sequencing 16S *rRNA* and ITS1. Changes in the genes abundances and community structure were linked with N transformation and N_2O emission under the flooding and non-flooding conditions.

2. Materials and methods

2.1. Experimental design

The experimental soil was collected from the upper 10-cm layer of a paddy field, before rice planting in spring 2016, in Yuhang district (N 30° 43′ 13.49″ E 120°48′ 21.02″), Zhejiang Province, China. The soil was silty clay. The pH of the soil was 6.14, soil organic carbon (SOC) 38 g kg^{-1}, dissolve organic C (DOC) 208 mg kg^{-1}, total N 3.6 g kg^{-1}, total organic N 31.6 mg kg^{-1}, cation exchange capacity (CEC) 39.4 cmol kg^{-1}, clay 37.4%, silt 42.2% and sand 20.4%. The soil was air-dried and ground to pass through a 2-mm sieve. The experiment was in triplicate. Two sets of total 48 glass bottles (one liter in volume) were used in this study. Four Cd levels were used: Cd0, no Cd added; Cd2, 2 mg kg^{-1}; Cd5, 5 mg kg^{-1} and Cd10, 10 mg kg^{-1}soil. Cadmium was added as $CdCl_2$•$2.5H_2O$ (99%, Tianjin Fuchen Chemical Co., Tianjin, China). The experiment started immediately after the addition of Cd in the soil. Bottles were filled with 200 g of soil containing various amounts of added Cd. Two sets were kept with a moisture content of 60% water-holding capacity (Cabangon et al., 2004), and the other two in the flooding condition (3-cm water level above the soil surface). Deionized water was added to re-adjust and maintain soil moisture. The soils were incubated at a constant temperature of 25 ± 1 °C over 8 weeks.

2.2. Soil and gas sample collection

Soil samples from one set of bottles (flooding and non-flooding) were collected at Days 0, 14, 28 and 56 for measurements of pH, concentrations of NH_4^+-N and NO_3^--N, soil organic C (SOC), dissolved organic N (DON) and bioavailable Cd, and microbial DNA extraction. Soil pH was measured after shaking at 180 rpm for 30 min in water at a soil-to-water ratio of 1:2.5. Soil Eh was measured by making slurry of water-to-soil ratio of 1:2.5. The SOC was determined using the dichromate digestion (Kalembasa and Jenkinson, 1973). Total N concentration was determined by Kjeldahl digestion method. Nitrate (NO_3^-) and NH_4^+ were extracted in 1 M KCl and their concentrations quantified by a flow-injection analyzer (SAN^{++}, Skalar, Holland). After soil was air-dried and sieved (< 2 mm), bioavailable Cd was extracted in 0.01 M $CaCl_2$ and determined using ICP-MS (PerkinElmer, NexION 300X). Soil DON was extracted with water and then analyzed by Multi N/C TOC analyzer (Analytic Jena AG, Jena, Germany).

The other set of bottles were used to collect gas samples for N_2O determination at 0, 1, 6, 12, 18, 24, 30 and 36 days using a modified closed chamber method (Hutchinson and Mosier, 1981). After gas sampling, the lids of the bottles were replaced by new ones attached with a septum, three-way valve and a needle (Di et al., 2014). From each bottle, two gas samples were taken at 0 and 30 min and stored in 15-ml exetainers. Gas chromatography (GC-200 Plus SHIMADZU, Japan) was used to analyze the collected N_2O gas. The N_2O emission was calculated as the differences in N_2O concentrations in the gas samples between 0 and 30 min.

2.3. Total soil DNA extraction, q-PCR, and cloning of bacterial genes

The soil DNA was extracted from the fresh soil samples with help of FastDNA Spin Kit for Soil (MP Biomedicals, LLC., Solon, OH, USA), according to the manufacturer's protocol. The extracted DNA was stored at −20 °C and analyzed within 3 days. Nanodrop®ND-2000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify and check for purity of the DNA. To quantify the abundance of *amoA*, *nirS*, *nirK,* and *nosZ* genes, quantitative polymerase-chain-reaction (qPCR) assays were performed in triplicate by real-time PCR with LightCycler 480 (Roche Applied Science). The primers and conditions are given in Table S2. Standard curves were prepared for real-time PCR as previously described (Di et al., 2009). A plasmid containing 10^2–10^9 copies μL^{-1} was obtained by serial 10× dilutions. Then qPCR assay in triplicate was performed to plot an external standard curve for determination of unknown gene copy number. The efficiency for amplification of target genes in the assays ranged from 92.3 to 105.2% along with R values of 0.996–0.999.

2.4. Sequencing and phylogenetic analysis of 16S and ITS1

Total soil DNA of 34 samples was analyzed for 16S V3-V4 for bacterial, archaeal and ITS1 regions for fungal community analysis. From original DNA fragments, paired-end reads are merged by using FLASH (Magoč and Salzberg, 2011). According to the unique barcodes, paired-end reads were assigned to each sample. Then, these sequences were clustered at 97% similarity using UPARSE algorithm (Edgar, 2013), yielding operational taxonomy units (OTUs). Taxonomies of these OTUs were assigned against EzBioCloud database (Yoon et al., 2017) for 16S and Unite (Kõljalg et al., 2005) for ITS1. For each sample, the sequences were rarefied to the depth of 34,000 and 37,000 for 16S and ITS1, respectively. Representative sequences of each OTU were aligned using MUSCLE (Edgar, 2004) and phylogenies were constructed using FastTree (Price et al., 2010) to get an approximate maximum likelihood tree. These steps were performed, jointly using USEARCH (Edgar, 2010), VSEARCH (Rognes et al., 2016), and QIIME (Kuczynski et al., 2012) platforms.

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was performed for the abundance of functional genes using SPSS version 20, to test the effects of Cd level and water regime and their interactions on the measurements. The LSD ($p = 0.05$) was used to check for the differences in soil Cd bioavailability and other soil parameters, and abundance of functional genes between the treatments. The figures were prepared by Origin (Origin Pro 9.0 for Windows). R version (3.4.2) was used for correlation analysis and presentation of correlation analysis, and for statistical analyses of 16S and ITS1 *rRNA* (Mazucheli et al., 2017). Generalized UniFrac (Lozupone and Knight, 2005) distance was used to measure the dissimilarities between samples and visualized with non-metric multidimensional scaling technique (NMDS) (Kruskal, 1964). UniFrac is a metric weighing of the phylogenetic dissimilarities between microbial communities (Lozupone and Knight, 2005) and 3-axis were conserved when performing NMDS. Statistical significance was drawn by permutation multivariate analysis of variance (PERMANOVA) (Anderson, 2001). In PERMANOVA, Cd concentration and incubation time were regarded as categorical variables, and only the main effects were included (namely, higher ranks of interactive effects were mixed). ggplot2 (Kahle and Wickham, 2013) was used to prepare graphs. These steps were completed in R platform (Team, 2017) with packages “GUniFac”, “vegan” and “ggplot2” (Kahle and Wickham, 2013).

3. Results and discussion

3.1. Water regime and Cd bioavailability

The bioavailable Cd was recorded higher in the non-flooding than the flooding conditions (Fig. 1A and B, Table S1) at the end of the 56-d incubation experiment. In the non-flooding condition, a total 52% bioavailable Cd was recorded at 10 mg Cd kg^{-1} from the initial time of soil incubation till the end of 56-d incubation. While only 5% of Cd was bioavailable after 56-d of incubation in the flooding condition, the difference was noted in Cd bioavailability (Fig. 1A–B and Table S1) between flooding and non-flooding conditions. Soil Eh was higher under non-flooding than under flooding conditions whereas soil pH was lower under the non-flooding conditions (Table S3). Generally, Cd solubility decreases under flooding conditions (Vink et al., 2010). Saturated water conditions increase the binding of metals to the potentially-available sites (low- and high-affinity sites) in the soil solution to make metal–organic complexes (Hernandez-Soriano and Jimenez-Lopez, 2012). Low pH and high Cd concentration favor Cd binding with low-affinity sites of soluble organic matter while low Cd concentration and high pH promote Cd binding with high-affinity sites (Kinniburgh et al., 1999). Due to low Eh under the continuous flooding condition (Table S3), the conversion of sulfates to S^{2-} might have occurred, leading to the formation of insoluble CdS and decreased Cd bioavailability (Bingham et al., 1976; de Livera et al., 2011). In addition, average soil pH increased by one unit during flooding and 0.5 units under non-flooding conditions as compared to the initial pH at Day 0 (Table S3). Under reduced conditions, increased soil pH also promotes Cd adsorption by organic functional groups, decreasing Cd bioavailability (Antoniadis et al., 2008).

3.2. Effect of Cd on DON, greenhouse gas emission

Cadmium decreased the concentration of DON during incubation (Fig. 1C-D). In the flooding condition, the concentration of DON decreased significantly ($p \leq 0.05$) only at Cd level of 10 mg kg^{-1}, which was 18% lower after 28-d, as compared to the control. After 56-d, the DON concentration remained the same in all treatments and the control (Fig. 1C). In the non-flooding condition (Fig. 1D), DON after 28-d decreased by up to 6%, 35% and 40% at Cd levels of 2, 5 and 10 mg kg^{-1}, respectively, as compared to the control. The concentration of DON decreased by 32% at Cd level 10 mg kg^{-1} as compared to the control after 56-d of incubation under non-flooding conditions.

The decrease in DON concentration by Cd contamination could be caused by the decreased conversion (Schimel and Bennett, 2004) from organic polymers to monomers by Cd contamination in the paddy soil in our study. The production of DON can also be affected by moisture, temperature, pH, and microorganisms (Andersson et al., 2000). The DON in the soil is important to mediate microbial activities and to provide the electron donors and acceptors for microbial respiration and production of greenhouse gases like N_2O and others. It also plays a role in the transport and mobility of different metals and elements (Ullah and Zinati, 2006). During non-flooding condition, the DON concentration was lower in our study which decreased the electrons donors and accepters to nitrifiers and denitrifiers microbes to respire (Fig. S5), led to a decrease in the production of N_2O.

The N_2O flux was higher in the flooding than the non-flooding condition. The highest fluxes of N_2O in the flooding conditions after 24-h incubation were 0.42, 0.37, 0.23 and 0.20 mg m^{-2} h^{-1} at Cd levels 0, 2, 5 and 10 mg Cd kg^{-1}, respectively (Fig. 2A). In comparison, the peak N_2O fluxes in the non-flooding condition were lower than those in the flooding condition and occurred after 6-d of incubation (Fig. 2B). These were 0.27, 0.24, 0.12 and 0.10 mg m^{-2} h^{-1} at Cd levels 0, 2, 5 and 10 mg Cd kg^{-1}, respectively. Moreover, increasing Cd addition lowered the N_2O emission during the initial phase of incubation, i.e. in the flooding at Day 1 and in the non-flooding conditions at Day 6. In the late phase of soil incubation when the Cd bioavailability decreased, a non-significant increase in N_2O emission in Cd treatment was observed. Consistently, increasing Cd addition decreased the abundances of *nirS*, *nirK*, and *nosZ* (Fig. 4A–F).

These above findings suggest that denitrification is sensitive to Cd contamination, due to the toxic effect of Cd on *nirS*, *nirK*, and *nosZ*-carrying microbial community in the soil, particularly in the non-flooding soil where Cd bioavailability was higher (Table S1). With the passage of time, the bacterial community changed, and the composition of the community differed between the water regimes (Fig. 5A–6A). This change in community structure might be the cause of change in N_2O emission. In previous studies, pollution of multiple metals decreased the abundance of denitrifier community and decreased the emission of N_2O in paddy soils (Liu et al., 2016; Liu et al., 2018a). In another study, the addition of Cd, Cu, and Zn decreased N_2O production for more than two months in a sandy loam soil under anaerobic conditions (Holtan-Hartwig et al., 2002). The contamination of Cu up to 1333 mg kg^{-1} decreased total denitrifying activity by 78% and N_2O production by 88% in a clay loam soil (Liu et al., 2018a). In our experiment, the higher N_2O production in the flooding than non-flooding condition in the initial phase of soil incubation can be explained by the fast microbial activity initiation and labial N compounds mineralization

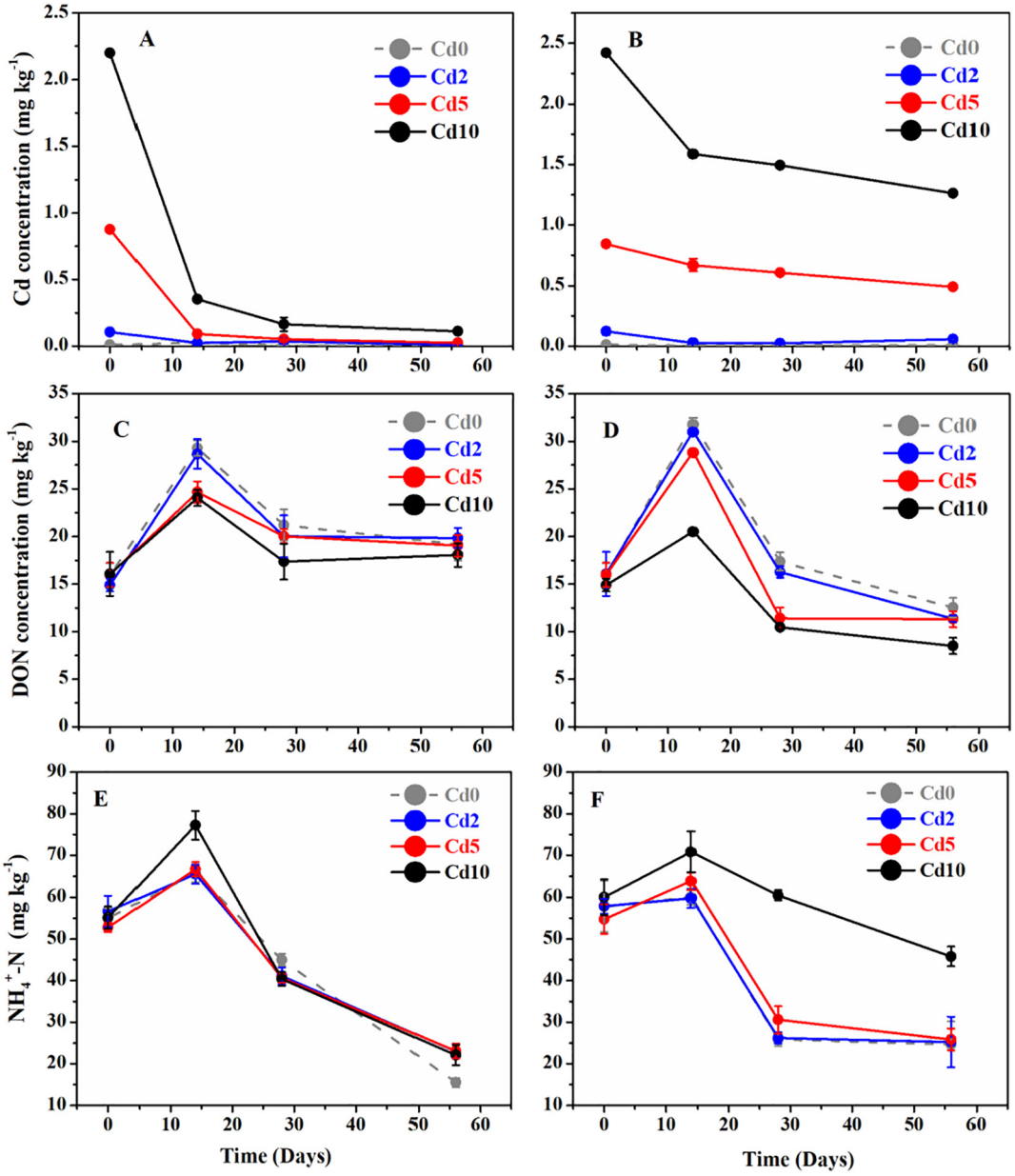

Fig. 1. Concentrations of $CaCl_2$-extractable Cd (A and B), dissolved organic N (DON) (C and D) and NH_4^+-N (E and F) in soil under flooding (A, C and E) and non-flooding (B, D and F) conditions at Cd levels of 0 (Cd0), 2 (Cd2), 5 (Cd5) and 10 (Cd10) mg kg^{-1} during 56-day incubation. Error bars indicate ± standard error of the mean of three replicates.

in the soil. In the late phase (18-d), there was no significant difference in N_2O production between water treatments or between Cd treatments. The similar emission of N_2O in the late phase may be due to the restoration of denitrifier abundances with the low availability of Cd in the soil.

3.3. The effect of Cd on nitrification under different water conditions

The concentration of NH_4^+-N increased over incubation time at the highest Cd addition in both flooding and non-flooding conditions with the concentration being higher in the non-flooding than the flooding condition (Fig. 1E–F). For example, NH_4^+-N concentration at level 10 mg kg^{-1} significantly increased by 77 and 70 mg kg^{-1} in flooding and non-flooding conditions, respectively, over the first 14-d of incubation. At the end of incubation, the concentration of NH_4^+-N was 1.5 fold higher in the Cd-added treatments than the no-Cd control in the flooding condition (Fig. 1E). In the non-flooding condition after 28-d and 56-d of incubation, the concentration of NH_4^+-N was the same between the control and at Cd levels 2 and 5 mg kg^{-1} (Fig. 1F). The concentration of NH_4^+-N was higher in non-flooding than flooding conditions (Fig. 1E) after 56-d of incubation with the soils at Cd level 10 mg kg^{-1}.

Ammonium oxidizers are sensitive to heavy metals like Cd. The decrease in the conversion of NH_4^+-N to NO_3^--N until 14-d of incubation (Fig. S4A—B) indicates that the increased bioavailability of Cd decreased the nitrification process. The result is consistent with a previous study showing that nitrification is negatively correlated to the concentration of free metal cations (Tessier and Turner, 1995). In our present study, the positive correlation of Cd bioavailability with NH_4^+-N show decreased NH_4^+-N oxidation by decreasing the nitrifier abundances (Fig. S1–3). The NH_4^+-N oxidation was decreased in the non-flooding condition more than the flooding condition due to the high bioavailability of Cd in the non-flooding condition. In a previous study, the rate of ammonium oxidation was not significantly influenced by

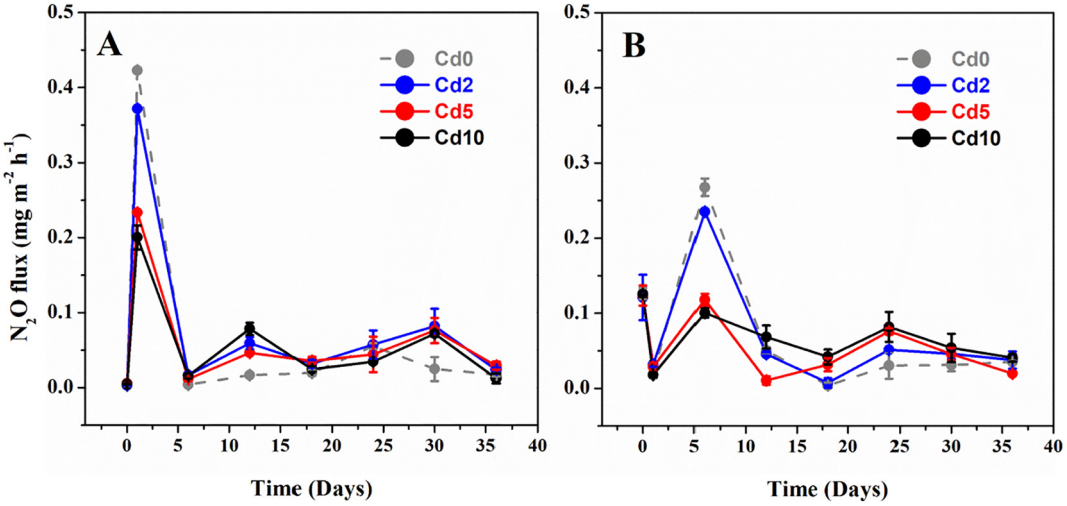

Fig. 2. Fluxes of N_2O (mg m^{-2} h^{-1}) from soil under flooding (A) and non-flooding (B) conditions at Cd levels of 0 (Cd0), 2 (Cd2), 5 (Cd5) and 10 (Cd10) mg kg^{-1} during 36-days incubation. Error bars indicate ± standard error of the mean of three replicates.

10 mg Cd kg^{-1} of dry soil while 100 and 500 mg Cd kg^{-1} decreased ammonium oxidation significantly during soil incubation experiment (Dušek, 1995). In our present study Cd10 showed a significant decrease in ammonium oxidation up to 14-d in the flooding and throughout incubation period in non-flooding conditions. This shows that ammonium oxidation is sensitive to Cd stress. High Cd might inhibit the activity and/or function of the microbes that are involved in the ammonium oxidation in the soil. In a batch reactor experiment, about 88% of ammonium oxidation was inhibited by Cd addition during incubation (Park and Ely, 2008). In addition, a recent study using a paddy soil reported 8-fold decrease in nitrification with 1.4 mg Cd kg^{-1}. However, their soil was contaminated with multiple heavy metals including Pb, Cu and Zn (Liu et al., 2014).

Increasing Cd levels decreased the abundances of all the nitrifier and denitrifier genes (Fig. S6). The abundances of archaeal and bacterial *amoA* genes in the no-Cd control were higher in the flooding (Fig. 3A) (4.14×10^7 and 4.37×10^7, respectively) than non-flooding conditions (8.38×10^6 and 2.65×10^7) (Fig. 3B). In the first 14-d of incubation, the archaeal *amoA* abundance was higher than the bacterial *amoA* abundance in both flooding and non-flooding conditions, and the ratio of archaeal *amoA* to bacterial *amoA* was 4.8–16.0 in the non-flooding as compared to 2.7–31.7 in the flooding condition. After 14-d, the opposite was true. Increasing Cd levels from 0 to 10 mg kg^{-1} decreased the abundance of archaeal *amoA* ($p < 0.05$) in the flooding condition from 1.46×10^8 to 1.70×10^6, respectively, after 56-d of incubation (Fig. 3A). The greatest decrease of 86% in the abundance of archaeal *amoA* was observed at Cd level 10 mg kg^{-1} under the non-flooding condition after 28-d of incubation, compared to the control. After 56-d, a significant difference was noted between the Cd-added treatments and the no-Cd control in the non-flooding condition (Fig. 3B). The abundance of nitrifiers negatively correlated with Cd bioavailability in both flooding and non-flooding conditions with the correlation coefficients (r) being higher in the non-flooding (Fig. S1-S3). Cadmium availability correlated negatively with the copy number of bacterial *amoA* gene ($p < 0.05$) but not with that of archaeal *amoA* gene under the flooding condition. In comparison, Cd availability correlated negatively with the copy number of both archaeal and bacterial *amoA* genes in the non-flooding condition. Increasing Cd addition significantly decreased the abundance of bacterial *amoA* gene in both flooding and non-flooding conditions after 14 and 28-d but not 56-d of incubation (Fig. 3C–D).

The decreased *amoA* copy number by Cd addition was associated with decreased nitrification under both water regimes after 14-d of incubation. The results suggest that the decreased oxidation of NH_4^+-N in the Cd treatments soil (Fig. 1E–F) might result from the decrease in the copy of the *amoA* gene after 14-d. In previous studies, Cd contamination decreased the abundance of nitrifier genes (Xing et al., 2015; Wang et al., 2018), and compared to AWD, continuous flooding of a paddy soil increased the abundance of AOA and AOB (Abid et al., 2018). In the present study, the greater effect of Cd on N transformation in the non-flooding than the flooding condition was inversely related to the abundances of AOA and AOB.

3.4. The effect of Cd on denitrification under different water conditions of soil

All the denitrifiers showed negative correlations with Cd bioavailability in both flooding and non-flooding conditions with correlations coefficient (r) are being higher in the non-flooding condition (Fig. S1-S3). Cadmium availability correlated negatively with the copy numbers of *nirS* gene, *nosZ* and *nirK* in the flooding ($p < 0.01$–0.05) and non-flooding ($p < 0.01$) conditions.

The average copy number of *nirK* gene in the no-Cd control during the entire incubation period was 2.62×10^6 and 7.65×10^6 for the flooding (Fig. 4A) and non-flooding (Fig. 4B) conditions, respectively. Increasing Cd addition consistently decreased the copy number of *nirK* in the non-flooding condition from 14-d (Fig. 4B). In the flooding condition, however, the copy number of *nirK* was significantly decreased only at Cd level of 10 mg kg^{-1} (Fig. 4A). The average copy number of *nirS* in the controls during 0–56-d was higher in the non-flooding (9.34×10^7) (Fig. 4D) than the flooding conditions (4.71×10^7) (Fig. 4C). Increasing Cd addition decreased the *nirS* copy number ($p < 0.05$) in both flooding and non-flooding conditions from 14-d of incubation (Fig. 4C-D). In the flooding condition, the copy numbers of *nirS* at Cd levels 2, 5, and 10 mg kg^{-1} were 2.98×10^7, 3.91×10^6 and 2.32×10^6, respectively (Fig. 4C). In comparison, in the non-flooding condition *nirS* copy number was 1.47×10^6 at Cd level 10 mg kg^{-1} (Fig. 4D). After 56-d of incubation in the flooding condition, no difference was noted in the copy number of *nirS* between the Cd level 5 and 10 mg kg^{-1}, while in non-flooding conditions, the copy number of *nirS* did not differ between the Cd-added treatments but was significantly lower than the no-Cd control.

The negative effect of Cd on the abundance of denitrifier genes shows that bacterial community containing *nirK*, *nirS,* and *nosZ* genes is sensitive to Cd. While DON provides the electron donors for denitrifier community in the soil, the decreased DON production by Cd contamination could lead to the inhibitory effect of Cd on the bacterial community containing the denitrifier genes, and hence inhibited the

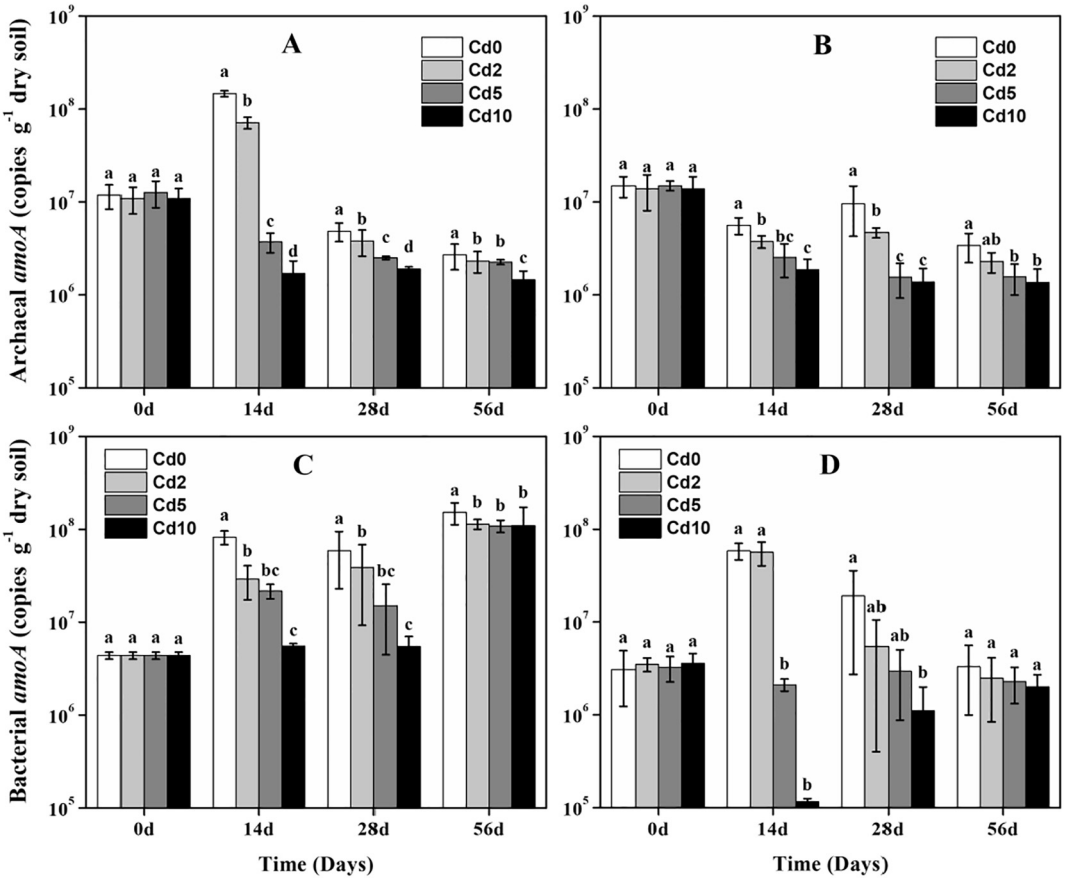

Fig. 3. Copy numbers of archaeal (A-B) and bacterial (C-D) *amoA* genes in soil under flooding (A and C) and non-flooding (B and D) conditions at Cd levels of 0 (Cd0), 2 (Cd2), 5 (Cd5) and 10 (Cd10) mg kg^{-1} during 56-days of incubation. Different lowercase letters indicate significant difference ($p \leq 0.05$) among the different Cd levels across the 56-days. Error bars indicate ± standard error of the mean of three replicates.

process of denitrification. Liu et al., (2014) showed that Cd contamination in a paddy clay loam soil did not affect the abundance of *nirK,* inconsistent with our finding of the significant ($p = 0.05$) decrease in copy number of *nirK* with increasing Cd levels (Fig. 4A-B). Our present study used silty clay soil with levels of 2, 5 and 10 mg kg^{-1} while the previous study by Liu et al. (2014) used a sandy loam with Cd level of 1.5 mg kg^{-1}. This could be a possible reason for the discrepancy in the effect on *nirK* gene abundance between two studies.

The overall average copy number of the *nosZ* gene was higher in the flooding than the non-flooding conditions. For example, the average copy number of *nosZ* in the control was 5.25×10^7 in the flooding (Fig. 4E) as compared to 4.32×10^7 in the non-flooding condition (Fig. 4F). Increasing Cd addition decreased *nosZ* copy number in both the flooding and non-flooding conditions from 14-d of incubation. Denitrifiers containing N_2O reductase prefer anaerobic conditions and therefore the copy number of the *nosZ* gene (8.71×10^7 g^{-1} dry soil) was higher in the flooding than non-flooding (4.32×10^7 g^{-1} dry soil) conditions. The present study also showed a strong interaction between the water regime and Cd bioavailability; the *nosZ* abundance was more affected by Cd in the non-flooding than the flooding condition.

The result is consistent with a recent study reporting that the flooding and waterlogged condition increased the microbial community containing the *nosZ* gene (Saeki et al., 2017). The decrease in abundances of the *nosZ* gene was 89% in the flooding and 96% in the non-flooding at 10 mg kg^{-1} of Cd as compared to the no-Cd controls during the entire 56-d incubation. We observed the restoration in abundances of *nosZ,* 11% in the flooding and 5% in the non-flooding condition during 56-d. This restoration in abundances also reflects in the N_2O emission after 18-d where no significant difference in the Cd treatments and no-Cd controls in the emission of N_2O we observed. In a previous study, two months of incubation after spiked Cu, Cd, and Zn in sandy loam soils, the denitrification restore up to the level of control (Holtan-Hartwig et al., 2002). This restoration of denitrification activity was probably due to a decrease in the bioavailability of heavy metals, a shift in metal tolerance of the microbial community and the horizontal transfer of heavy metals resistance genes in the plasmid (Holtan-Hartwig et al., 2002).

3.5. Microbial community abundance and composition

The structure of the bacterial community was changed during the water and Cd treatments. Under non-flooding conditions, the addition of 5 and 10 mg Cd kg^{-1}decreased the relative abundance of *Actinobacteria* by 10 and 13%, respectively, while 2 mg Cd kg^{-1} increased the relative abundance up to 4% (Fig. 5A). The addition of 2 mg Cd kg^{-1} decreased the relative abundance of *Proteobacteria* by 3% while 4 and 6% increases were observed at Cd levels of 5 and 10 mg Cd kg^{-1}. The relative abundance of phylum *Gemmatimonadetes* in non-flooding decreased with the increasing levels of Cd with 2% decrease at 10 mg Cd kg^{-1}. Under flooding conditions after 14-d of incubation, increasing Cd addition from 0 to 10 mg kg^{-1}increased the relative abundance of phylum *Actinobacteria* from 21% to 30%, but decreased that of phyla *Firmicutes* and *Chlorobi* from 38% to 26% and from 0.34% to 0.17%, respectively. The OTUs assigned to the nitrifier and denitrifier were altered by water and Cd treatments. For examples, irrespective of Cd treatment, OTUs of nitrifiers such as *Nitrosomonas,* decreased in the flooding whereas those of *Nitrosomonas* and *Nitrososphaera* decreased in the non-flooding condition (Fig. S5A). The OTUs of denitrifiers such as *Nitrosomonas* and *Pseudomonas* decreased in the non-flooding condition and those of *Nitrosomonas* decreased in the

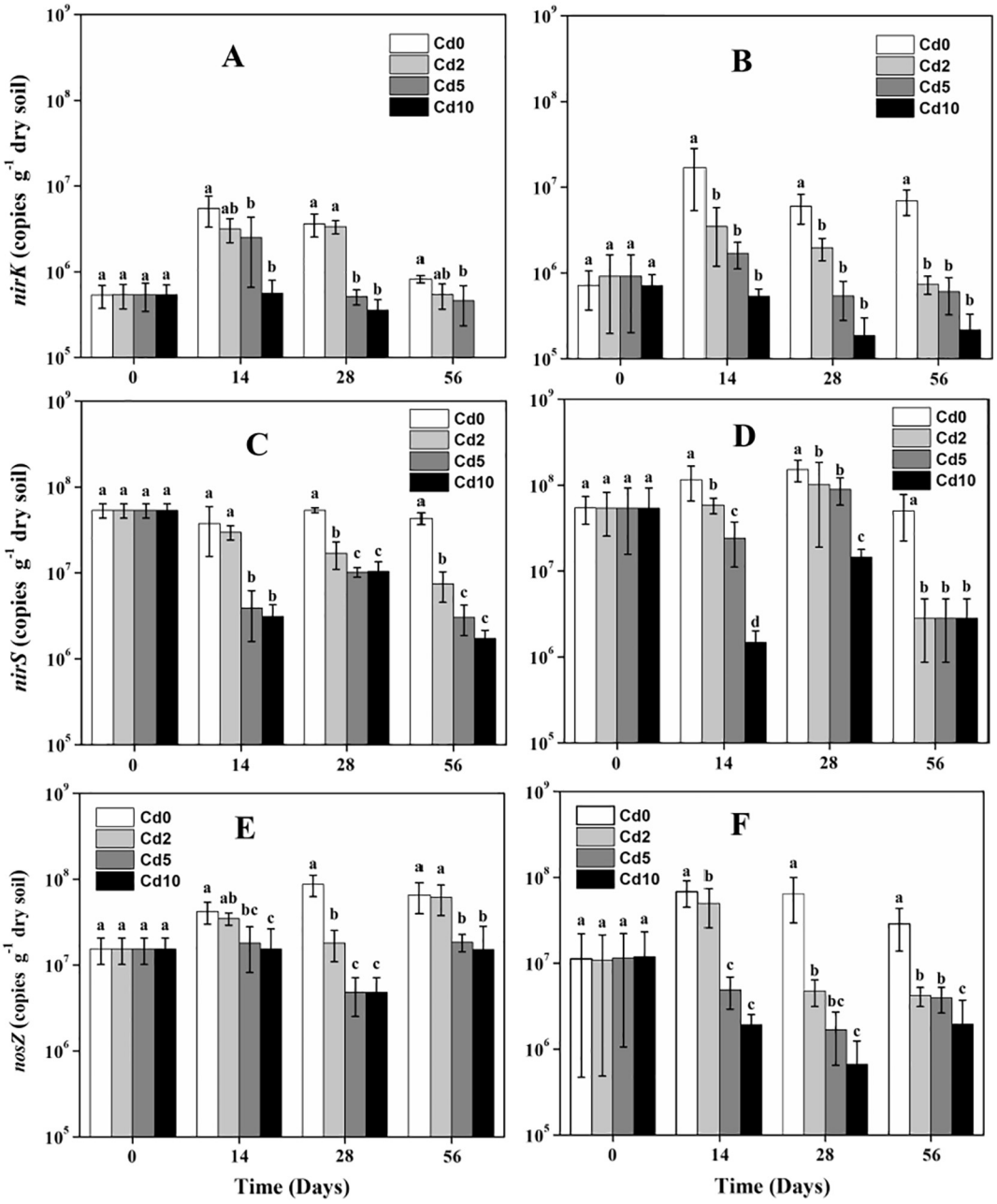

Fig. 4. Copy numbers of bacterial *nirK* (A and B), *nirS* (C and D) and *nosZ* (E and F) genes in soil under flooding (A, C and E) and non-flooding (B, D and F) conditions at Cd levels of 0 (Cd0), 2 (Cd2), 5 (Cd5) and 10 (Cd10) mg kg^{-1} over 56 days of soil incubation. Different lowercase letters indicate significant difference ($p \leq 0.05$) among the different Cd levels across the 56 days. Error bars indicate ± standard error of the mean of three replicates.

flooding condition at all Cd levels throughout the incubation period (Fig. S5B). Similarly, a Cd level ≥ 5 mg kg^{-1} was lethal for the archaeal community in the non-flooding condition. For example, 5 mg Cd kg^{-1} decreased the relative abundance of archaeal phyla *Euryarchaeota* and *Thaumarchaeota* by up to 96 and 99% respectively, in the non-flooding condition. In comparison, Cd addition increased the relative abundance of these phyla in the flooding condition (Fig. 5A).

In all the samples, 941,806 fungal sequences were not assigned to any phylum (Fig. 5B). The dominated fungal phylum in both flooding and non-flooding condition was *Ascomycota* with 332,876 sequences in total, while phyla *Basidiomycota* and *Zygomycota*-related OTUs were 81 and 20 in total, respectively. The addition of Cd did not affect the abundance of different fungal genera in the flooding condition.

A distinct bacterial community composition was observed with time and between water regimes (Fig. 6A) ($p < 0.004$ by PERMANOVA) but not between Cd levels (Fig. 6B). Consistent with 16S *rRNA* community, the structure of fungal community was influenced by water condition ($p = 0.035$, PERMANOVA) and incubation time ($p < 0.001$) but not by Cd level ($p = 0.445$).

The abundances of different bacterial and archaeal phyla decreased by Cd addition in the non-flooding condition at high Cd bioavailability. The effect of water regime on bacterial community composition led to changes in microbial community structure. Under continuous flooding conditions, oxygen and nitrate deplete rapidly due to the decrease in redox potential. Microbial communities adopting certain strategies to survive like a change in community structure which led to the change in biogeochemical cycling in the soil (DeAngelis et al., 2010). In the present study, the dominated phyla in the Cd treatment were *Actinobacteria, Proteobacteria*, *Firmicutes* and *Acidobacteria*. Similar results for metal-contaminated soils were also previously reported (Idris et al.,

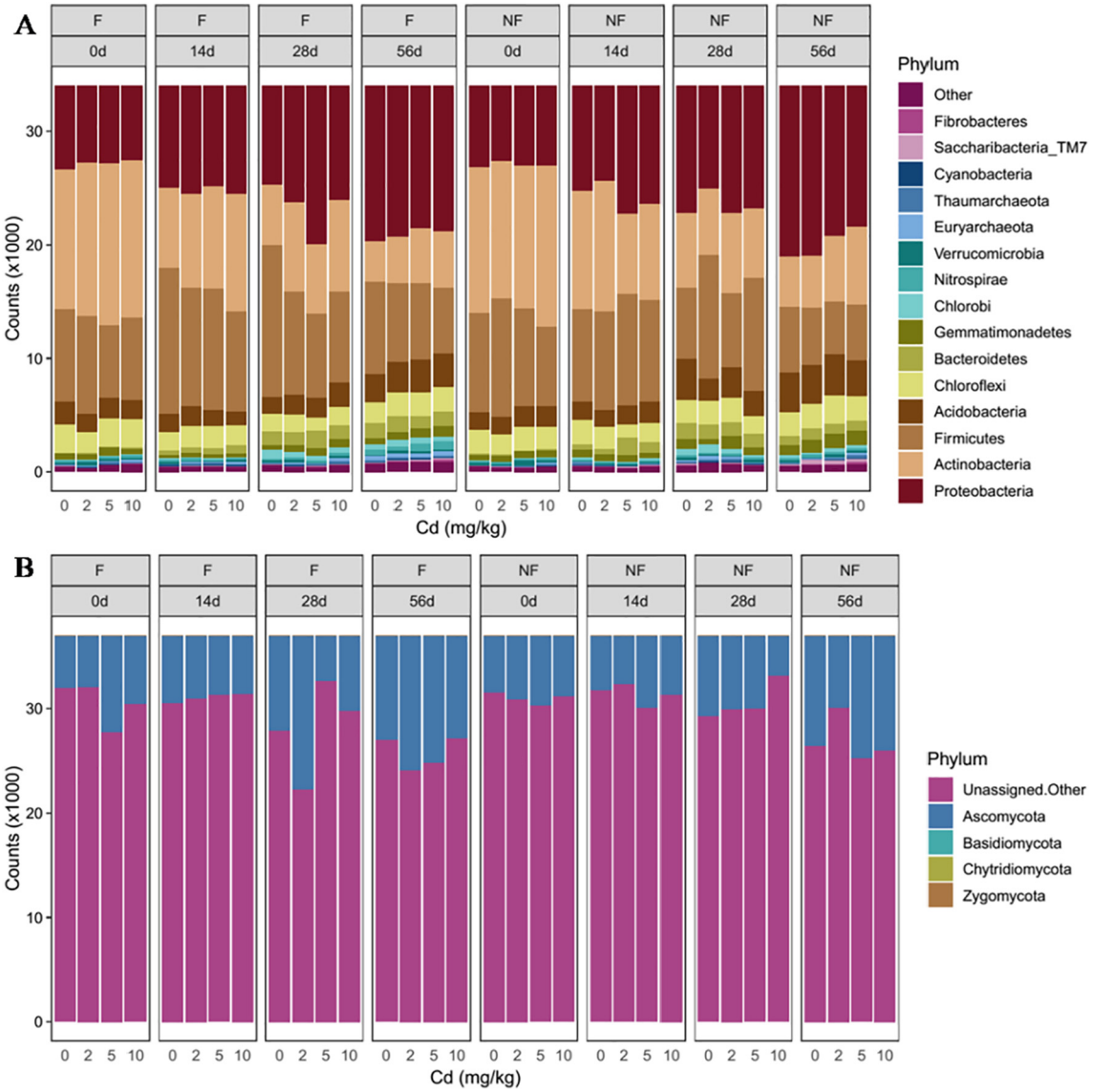

Fig. 5. Barplots of relative abundance of dominant phyla (relative abundance > 0.1%)of archaeal and bacterial (A) and fungal (B)in the flooding (F) and non-flooding (NF) conditions after soil incubation for 0, 14, 28 and 56 days at Cd levels of 0 (Cd0), 2 (Cd2), 5 (Cd5) and 10 mg kg^{-1} (Cd10).

2004; Ding et al., 2017). However, Idris et al. (2004) reported *Proteobacteria* in Ni contaminated soil as the dominated phylum. Although we reported that bacterial community was shaped due to water management practices, high Cd levels also decreased the abundances of certain phyla (Fig. 5A-S 5A-B). Contrast to our findings, previous studies reported that Cd changed the microbial structure in the soil (Akmal, 2005; Bell et al., 2015; Visioli et al., 2015; Roman-Ponce et al., 2017). The contradiction of these results is due to the difference in the concentration of Cd in the soil. The Cd concentrations in our study were lower than the mentioned studies.

We reported no change in the fungal community by water and Cd treatments with the dominated fungal phyla being *Ascomycota* and *Basidiomycota* in both flooding and non-flooding conditions. Similar results were reported on an increase in the abundance of *Ascomycota* in a soil of higher moisture content (Chen et al., 2014a, 2014b). Both these phyla were the main degrader of rice straw and other degradable material in the soil (Rousk et al., 2010). Clearly, much higher Cd levels are needed to change fungal community with the passage of time. We assume that too low and high moisture content may decrease the diversity of fungal community and lead to the dominancy of *Ascomycota* and *Basidiomycota*.

4. Conclusion

Our study demonstrated that Cd contamination of paddy soils slowed down the process of N transformation by decreasing the abundance of archaeal and bacterial *amoA* (nitrification), *nirS*, *nirK,* and *nosZ* (denitrification). The microbial communities containing these genes are the players of the N cycle. Cadmium toxicity is more severe under non-flooding than flooding conditions, and hence decreased N reduction and hence N_2O production to a greater extent under non-flooding conditions. The bacterial and fungal communities were changed by water treatment. While Cd did not affect the fungal community in the flooding conditions, Cd levels of 2 to 5 mg kg^{-1} increased the abundance of certain fungal phyla. Non-flooding conditions have been used as a farming practice of water reservation in rice production, and to decrease the concentrations of heavy metals in grains. However, this study showed that Cd bioavailability was higher under non-flooding than flooding conditions. Further studies are needed to explore the link of nitrification and denitrification activities with different water and nutrient management practices in contaminated paddy fields.

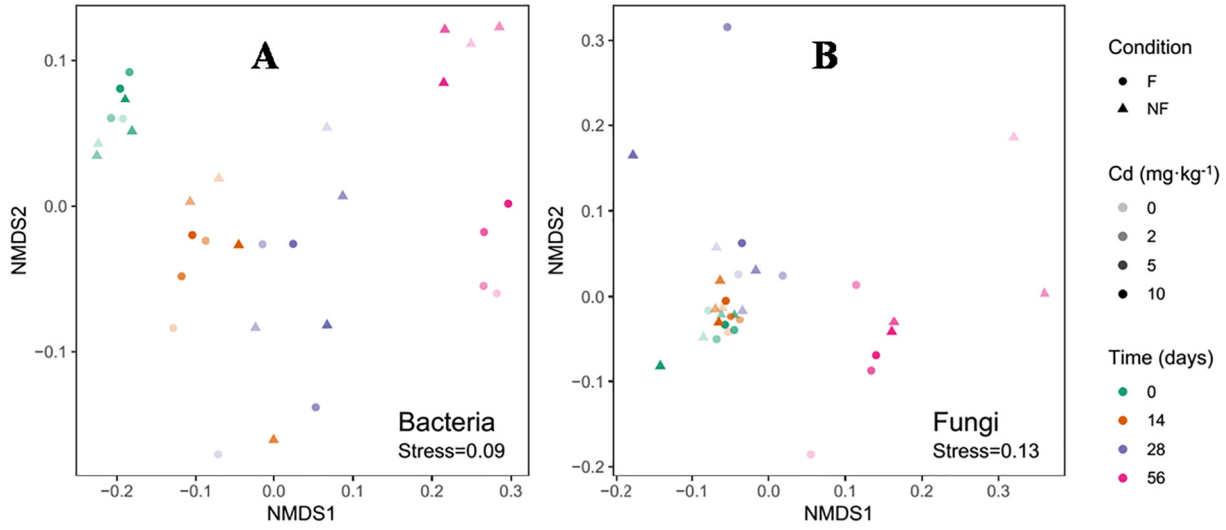

Fig. 6. Non-metric multidimensional scaling (NMDS) based on generalized UniFrac. Bacterial community was compositionally distinct between water treatments (p = 0.001, PERMANOVA). NMDS demonstrates bacterial (A) and fungal (B) community compositions in the flooding (F; circles), non-flooding (NF; triangles), Cd levels; (light to dark color), time days (0-d, green color; 14-d, red; 28-d, blue; 56-d, pink). Each point represents a specific community in one of the treatment plots. The close points are the similar community with each other than the far apart. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.05.058.

References

Abid, A.A., Gu, C., Zhang, Q., Wang, J., Di, H., 2018. Nitrous oxide fluxes and nitrifier and denitrifier communities as affected by dry-wet cycles in long term fertilized paddy soils. Appl. Soil Ecol. 125, 81–87.
Akmal, M., 2005. Changes in enzymes activity, substrate utilization pattern and diversity of soil microbial communities under cadmium pollution. J. Environ. Sci. 17, 802–807.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.
Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. Soil Biol. Biochem. 32, 1–10.
Antoniadis, V., Robinson, J., Alloway, B., 2008. Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. Chemosphere 71, 759–764.
Arao, T., Ishikawa, S., Murakami, M., Abe, K., Maejima, Y., Makino, T., 2010. Heavy metal contamination of agricultural soil and countermeasures in Japan. Paddy Water Environ. 8, 247–257.
Bell, T.H., Cloutier-Hurteau, B., Al-Otaibi, F., Turmel, M.C., Yergeau, E., Courchesne, F., St-Arnaud, M., 2015. Early rhizosphere microbiome composition is related to the growth and Zn uptake of willows introduced to a former landfill. Environ. Microbiol. 17, 3025–3038.
Bingham, F., Page, A., Mahler, R., Ganje, T., 1976. Cadmium availability to rice in sludge-amended soil under “flood” and “nonflood” culture 1. Soil Sci. Soc. Am. J. 40, 715–719.
Cabangon, R.J., Tuong, T.P., Castillo, E.G., Bao, L.X., Lu, G., Wang, G., Cui, Y., Bouman, B.A., Li, Y., Chen, C., 2004. Effect of irrigation method and n-fertilizer management on rice yield, water productivity and nutrient-use efficiencies in typical lowland rice conditions in China. Paddy Water Environ. 2, 195–206.
Cai, Z.C., Xing, G.X., Yan, X.Y., Xu, H., Tsuruta, H., Yagi, K., Minami, K., 1997. Methane and nitrous oxide emissions from rice paddy fields as affected by nitrogen fertilisers and water management. Plant Soil 196, 7–14.
Chen, X.-P., Zhu, Y.-G., Xia, Y., Shen, J.-P., He, J.-Z., 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? Environ. Microbiol. 10, 1978–1987.
Chen, J., He, F., Zhang, X., Sun, X., Zheng, J., Zheng, J., 2014a. Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. FEMS Microbiol. Ecol. 87, 164–181.
Chen, L., Zhang, J., Zhao, B., Yan, P., Zhou, G., Xin, X., 2014b. Effects of straw amendment and moisture on microbial communities in Chinese fluvo-aquic soil. J. Soils Sediments 14, 1829–1840.
Davranche, M., Bollinger, J.-C., 2000. Heavy metals desorption from synthesized and natural iron and manganese oxyhydroxides: effect of reductive conditions. J. Colloid Interface Sci. 227, 531–539.
DeAngelis, K.M., Silver, W.L., Thompson, A.W., Firestone, M.K., 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environ. Microbiol. 12, 3137–3149.
Deng, D., Hu, M., Li, L., Huang, Y., 2018. Denitrifying microbial communities in heavy-metal-contaminated paddy soils near electronic-waste processing centers. Water Air Soil Pollut. 229, 1–12.
Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat. Geosci. 2, 621–624.
Di, H.J., Cameron, K.C., Podolyan, A., Robinson, A., 2014. Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil. Soil Biol. Biochem. 73, 59–68.
Ding, Z., Wu, J., You, A., Huang, B., Cao, C., 2017. Effects of heavy metals on soil microbial community structure and diversity in the rice (*Oryza sativa* L. subsp. Japonica, Food Crops Institute of Jiangsu Academy of Agricultural Sciences) rhizosphere. Soil Sci. Plant Nutr. 63, 75–83.
Du, Y., Hu, X.-F., Wu, X.-H., Shu, Y., Jiang, Y., Yan, X.-J., 2013. Affects of mining activities on Cd pollution to the paddy soils and rice grain in Hunan province, Central South China. Environ. Monit. Assess. 185, 9843–9856.
Dušek, L., 1995. The effect of cadmium on the activity of nitrifying populations in two different grassland soils. Plant Soil 177, 43–53.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996.
Gordon, H., Haygarth, P.M., Bardgett, R.D., 2008. Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biol. Biochem. 40, 302–311.
Hernandez-Soriano, M.C., Jimenez-Lopez, J.C., 2012. Effects of soil water content and organic matter addition on the speciation and bioavailability of heavy metals. Sci. Total Environ. 423, 55–61.
Holtan-Hartwig, L., Bechmann, M., Høyås, T.R., Linjordet, R., Bakken, L.R., 2002. Heavy metals tolerance of soil denitrifying communities: N_2O dynamics. Soil Biol. Biochem. 34, 1181–1190.
Hu, P., Li, Z., Yuan, C., Ouyang, Y., Zhou, L., Huang, J., Huang, Y., Luo, Y., Christie, P., Wu, L., 2013. Effect of water management on cadmium and arsenic accumulation by rice (*Oryza sativa* L.) with different metal accumulation capacities. J. Soils Sediments 13, 916–924.
Huang, L., Hu, H., Li, X., Li, L.Y., 2010. Influences of low molar mass organic acids on the adsorption of Cd^{2+} and Pb^{2+} by goethite and montmorillonite. Appl. Clay Sci. 49, 281–287.
Hutchinson, G., Mosier, A., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes 1. Soil Sci. Soc. Am. J. 45, 311–316.

Idris, R., Trifonova, R., Puschenreiter, M., Wenzel, W.W., Sessitsch, A., 2004. Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl. Environ. Microbiol. 70, 2667–2677.
Ishii, S., Ikeda, S., Minamisawa, K., Senoo, K., 2011. Nitrogen cycling in rice paddy environments: past achievements and future challenges. Microbes Environ. 26, 282–292.
Kahle, D., Wickham, H., 2013. Ggmap: spatial visualization with ggplot2. R J. 5.
Kalembasa, S.J., Jenkinson, D.S., 1973. A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. J. Sci. Food Agric. 24, 1085–1090.
Khan, S., El-Latif Hesham, A., Qiao, M., Rehman, S., He, J.-Z., 2010. Effects of cd and Pb on soil microbial community structure and activities. Environ. Sci. Pollut. Res. 17, 288–296.
Kinniburgh, D.G., van Riemsdijk, W.H., Koopal, L.K., Borkovec, M., Benedetti, M.F., Avena, M.J., 1999. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. Colloids Surf. A Physicochem. Eng. Asp. 151, 147–166.
Kõljalg, U., Larsson, K.H., Abarenkov, K., Nilsson, R.H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol. 166, 1063–1068.
Kruskal, J.B., 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29, 1–27.
Kuczynski, J., Stombaugh, J., Walters, W.A., González, A., Caporaso, J.G., Knight, R., 2012. Using QIIME to analyze 16S *rRNA* gene sequences from microbial communities. Curr. Protoc. Microbiol. 27 (1E. 5.1-1E. 5.20).
Lei, L., Lv, H., Yu, Y., Hu, R., Wang, R., Xu, Y., Ding, Y., Feng, R., Fan, Z., 2018. Performances of water management, foliage dressing, and variation screening in controlling the accumulation of As and Cd and maintaining the concentrations of essential elements in the grains of rice plant. Front. Environ. Sci. 6.
Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809.
Liang, Y., Li, F., Nong, M., Luo, H., Zhang, J., 2016. Microbial activity in paddy soil and water-use efficiency of rice as affected by irrigation method and nitrogen level. Commun. Soil Sci. Plant Anal. 47, 19–31.
Liu, S., Li, F., Wei, X., Nong, M., 2012a. Effects of alternate partial root-zone irrigation on maize water use and soil microbial biomass carbon. Trans. Chin. Soc. Agric. Eng. 28, 71–77.
Liu, Y., Zhou, T., Crowley, D., Li, L., Liu, D., Zheng, J., Yu, X., Pan, G., Hussain, Q., Zhang, X., 2012b. Decline in topsoil microbial quotient, fungal abundance and C utilization efficiency of rice paddies under heavy metal pollution across South China. PLoS One 7, e38858.
Liu, Y., Liu, Y., Ding, Y., Zheng, J., Zhou, T., Pan, G., Crowley, D., Li, L., Zheng, J., Zhang, X., 2014. Abundance, composition and activity of ammonia oxidizer and denitrifier communities in metal polluted rice paddies from South China. PLoS One 9, e102000.
Liu, Y., Liu, Y., Zhou, H., Li, L., Zheng, J., Zhang, X., Zheng, J., Pan, G., 2016. Abundance, composition and activity of denitrifier communities in metal polluted paddy soils. Sci. Rep. 6.
Liu, J., Cao, W., Jiang, H., Cui, J., Shi, C., Qiao, X., Zhao, J., Si, W., 2018. Impact of heavy metal pollution on ammonia oxidizers in soils in the vicinity of a Tailings Dam, Baotou, China. Bull. Environ. Contam. Toxicol. 101, 110–116.
Liu, Y., Shen, K., Wu, Y., Wang, G., 2018a. Abundance and structure composition of *nirK* and *nosZ* genes as well as denitrifying activity in heavy metal-polluted paddy soils. Geomicrobiol J. 35, 100–107.
Liu, Y., Xue, C., Yu, S., Li, F., 2018b. Variations of abundance and community structure of ammonia oxidizers and nitrification activity in two paddy soils polluted by heavy metals. Geomicrobiol J. 1–10.
de Livera, J., McLaughlin, M.J., Hettiarachchi, G.M., Kirby, J.K., Beak, D.G., 2011. Cadmium solubility in paddy soils: effects of soil oxidation, metal sulfides and competitive ions. Sci. Total Environ. 409, 1489–1497.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Mazucheli, J., Menezes, A.F.B., Nadarajah, S., 2017. mle. tools: an R package for maximum likelihood bias correction. R J. 9, 268–290.
MEP, MLR, 2014. China National Soil Pollution Gazette. Ministry of Environmental Protection China Ministry of Land and Resources China.
Mukhtar, H., Lin, Y.-P., Anthony, J., 2017. Ammonia oxidizing archaea and bacteria in East Asian paddy soils—a mini review. Environments 4.
Norton, J.M., Alzerreca, J.J., Suwa, Y., Klotz, M.G., 2002. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177, 139–149.
Park, S., Ely, R.L., 2008. Candidate stress genes of *Nitrosomonas europaea* for monitoring inhibition of nitrification by heavy metals. Appl. Environ. Microbiol. 74, 5475–5482.
Pedersen, H., Dunkin, K.A., Firestone, M.K., 1999. The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by ^{15}N tracer and pool dilution techniques. Biogeochemistry 44, 135–150.
Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.
Richardson, D., Felgate, H., Watmough, N., Thomson, A., Baggs, E., 2009. Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle - could enzymic regulation hold the key? Trends Biotechnol. 27, 388–397.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.
Roman-Ponce, B., Reza-Vázquez, D.M., Gutierrez-Paredes, S., María de Jesús, D., Maldonado-Hernandez, J., Bahena-Osorio, Y., Estrada-De los Santos, P., Wang, E.T., Vásquez-Murrieta, M.S., 2017. Plant growth-promoting traits in rhizobacteria of heavy metal-resistant plants and their effects on *Brassica nigra* seed germination. Pedosphere 27, 511–526.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4, 1340.
Ruyters, S., Mertens, J., T'Seyen, I., Springael, D., Smolders, E., 2010. Dynamics of the nitrous oxide reducing community during adaptation to Zn stress in soil. Soil Biol. Biochem. 42, 1581–1587.
Saeki, Y., Nakamura, M., Mason, M.L.T., Yano, T., Shiro, S., Sameshima-Saito, R., Itakura, M., Minamisawa, K., Yamamoto, A., 2017. Effect of flooding and the *nosZ* gene in *Bradyrhizobia* on *Bradyrhizobial* community structure in the soil. Microbes Environ. 32, 154–163.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.
Smolders, E., Brans, K., Coppens, F., Merckx, R., 2001. Potential nitrification rate as a tool for screening toxicity in metal-contaminated soils. Environ. Toxicol. Chem. 20, 2469–2474.
Sobolev, D., Begonia, M.F.T., 2008. Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. Int. J. Environ. Res. Public Health 5, 450–456.
Team RC, 2017. Changes in R from version 3.4.2 to version 3.4.3. R J. 9, 568–570.
Tessier, A., Turner, D.R., 1995. Metal Speciation and Bioavailability in Aquatic Systems. Wiley Chichester.
Throback, I.N., Enwall, K., Jarvis, A., Hallin, S., 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. 49, 401–417.
Tóth, G., Hermann, T., Da Silva, M.R., Montanarella, L., 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environ. Int. 88, 299–309.
Ullah, S., Zinati, G.M., 2006. Denitrification and nitrous oxide emissions from riparian forests soils exposed to prolonged nitrogen runoff. Biogeochemistry 81, 253–267.
Vink, J.P., Harmsen, J., Rijnaarts, H., 2010. Delayed immobilization of heavy metals in soils and sediments under reducing and anaerobic conditions; consequences for flooding and storage. J. Soils Sediments 10, 1633–1645.
Visioli, G., D'Egidio, S., Sanangelantoni, A.M., 2015. The bacterial rhizobiome of hyperaccumulators: future perspectives based on omics analysis and advanced microscopy. Front. Plant Sci. 5, 752.
Wang, J., Wang, L., Zhu, L., Wang, J., 2018. Individual and combined effects of enrofloxacin and cadmium on soil microbial biomass and the ammonia-oxidizing functional gene. Sci. Total Environ. 624, 900–907.
Xiao, R., Bai, J., Huang, L., Zhang, H., Cui, B., Liu, X., 2013. Distribution and pollution, toxicity and risk assessment of heavy metals in sediments from urban and rural rivers of the Pearl River delta in Southern China. Ecotoxicology 22, 1564–1575.
Xing, Y., Si, Y.-X., Hong, C., Li, Y., 2015. Multiple factors affect diversity and abundance of ammonia-oxidizing microorganisms in iron mine soil. Arch. Environ. Contam. Toxicol. 69, 20–31.
Yao, Z., Zheng, X., Wang, R., Dong, H., Xie, B., Mei, B., Zhou, Z., Zhu, J., 2013. Greenhouse gas fluxes and NO release from a Chinese subtropical rice-winter wheat rotation system under nitrogen fertilizer management. J. Geophys. Res. Biogeosci. 118, 623–638.
Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S *rRNA* gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617.
Yu, H.Y., Li, F.B., Liu, C.S., Huang, W., Liu, T.X., Yu, W.M., 2016. Iron redox cycling coupled to transformation and immobilization of heavy metals: implications for paddy rice safety in the red soil of South China. Adv. Agron. 137, 279–317.
Zumft, W.G., Kroneck, P.M., 2006. Respiratory transformation of nitrous oxide (N_2O) to dinitrogen by Bacteria and Archaea. Adv. Microb. Physiol. 52, 107–227.