Earth Surface Processes and Environmental Sustainability in China

Increased copper levels inhibit denitrification in urban soils

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ABSTRACT: The consequences of urbanisation for Earth's biogeochemical cycles are largely unexplored. Copper (Cu) in urban soils is being accumulated mainly due to anthropogenic activities under rapid urbanisation. The increasing Cu concentrations may contribute to altering soil nitrogen (N) cycling in urban ecosystems through modulating denitrification processes. This research aims to identify how Cu impacts urban soil denitrification functions and denitrifier abundance. An urban park soil with a background total Cu concentration of 7.9 μg g^{-1} was incubated anaerobically with different Cu amendments (10, 20, 40, 80 and 160 μg Cu g^{-1} soil), similar to prevalent Cu contents in urban soils. We evaluated the soil denitrification functions using the acetylene (C_2H_2) inhibition method and assessed the denitrifier abundance by quantitative polymerase chain reaction (qPCR) analyses of denitrifying marker genes (*nirK*, *nirS* and *nosZ*). At the function level, we observed that both the potential soil denitrification activity and the N_2O emission rate due to denitrification were significantly ($P < 0.05$) inhibited by Cu; even the lowest Cu addition (10 μg Cu g^{-1} soil) drastically affected the denitrification function. Moreover, Cu significantly ($P < 0.05$) decreased the abundance of *nirK* and *nirS* genes at the additions of 160 μg Cu g^{-1} soil and 40 μg Cu g^{-1} soil, respectively, whereas it had no clear impact on *nosZ* gene copies. Further correlation analyses revealed that the potential denitrification activity was positively correlated to the copy numbers of *nirK* and *nirS* genes, but it was not correlated to *nosZ* gene abundance. These findings indicate that Cu additions inhibited soil denitrification function and decreased denitrifier abundance in the investigated urban park soil. Our results suggest that Cu accumulation in urban soils, resulting from urbanisation, may generally influence denitrification in urban ecosystems.

KEY WORDS: N_2O, *nirK*, *nirS*, *nosZ*, urbanisation.

Urbanisation is increasing globally and China's land surface in particular is undergoing a transition from rural to urban landscapes (Zhu *et al*. 2011). Under this rapid and dynamic urbanisation, intensively anthropogenic activities can lead to elevated contents of common heavy metals (lead, copper (Cu), zinc and cadmium) in urban soils compared to rural soils. In addition to natural geogenic sources, the increasing heavy metal concentrations in urban soils are mainly derived from the atmospheric deposition of coal combustion (Luo *et al*. 2015), followed by vehicle emissions. Among these so-called 'urban-metals', Cu could be the most critical one with respect to the microbial denitrification process through which nitrate (NO_3^-) can be sequentially reduced to gaseous nitrous oxide (N_2O) and dinitrogen (N_2) via nitrite (NO_2^-) and nitric oxide (NO). The intermediate N_2O is a powerful greenhouse gas with approximately 300-fold greater warming potential than CO_2 and also contributes to stratospheric ozone destruction (Ravishankara *et al*. 2009). In particular, denitrification is the dominant nitrogen (N)-loss pathway in the terrestrial biosphere, especially in urban soils where the primary form of N-input is NO_3^- (Zhu & Carreiro 2004; Pickett *et al*. 2011). Nitrate represents the most important substrate and electron acceptor for microbial denitrification. Moreover, Cu is known to function as an essential micronutrient, and, notably, the Cu-dependent nitrite reductase that converts non-gaseous to gaseous N as well as the N_2O reductase that destroys N_2O require Cu as a redox active metal cofactor (Felgate *et al*. 2012). Based on this, we expect a potential link between the soil Cu content and urban soil microbial denitrification.

For over three decades, Cu has been shown to be able to strikingly affect the activities of denitrifying reductases and to regulate the N_2O emission from denitrification (Matsubara *et al*. 1982; Bardgett *et al*. 1994; Sakadevan *et al*. 1999; Holtan-Hartwig *et al*. 2002; Granger & Ward 2003; Magalhães *et al*. 2011; Felgate *et al*. 2012; Moffett *et al*. 2012; Sullivan *et al*. 2013; Black *et al*. 2016). So far, quantitative DNA-based studies

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documenting how Cu impacts denitrification have been mainly conducted in microbial cultures (Felgate *et al*. 2012; Sullivan *et al*. 2013; Black *et al*. 2016) and sediments (Magalhães *et al*. 2011). Yet, very little is known about how Cu influences denitrification in soil environments at the gene level. Evidently, the potential effects have rarely been documented in urban soils, which are created by urbanisation and differ markedly from natural soils (Brown *et al*. 2005; Lehmann & Stahr 2007). Given that urban soils could receive nitrate and accumulate Cu due to urbanisation, Cu may profoundly affect urban soil denitrification. Genes encoding structurally different, yet functionally equivalent, nitrite reductases (*nirK* and *nirS*) and nitrous oxide reductase (*nosZ*) are commonly used to quantify different groups of denitrifying communities (Zumft 1997; Bru *et al*. 2011). Hence, this study evaluates the effects of Cu, a typical marker of urbanisation, on the abundance of *nirK*, *nirS* and *nosZ* genes in urban soils, which is a largely overlooked site but important hotspot for denitrification. Indeed, previous investigations exploring linkages between Cu and soil denitrification activity and N_2O emission mainly used considerably high Cu amendments that generally exceeded 80 μg Cu g^{-1} dry soil (Bardgett *et al*. 1994; Holtan-Hartwig *et al*. 2002; Wang *et al*. 2013). Here we focus on lower Cu concentrations at levels typical for urbanisation to obtain conclusive data at realistic environmental conditions.

Thus, the current study aims to: (1) assess the denitrification function indicated by N_2O emission and potential denitrification activity in a long-term undisturbed urban soil with distinct Cu amendments; and (2) investigate how Cu concentration affects denitrifier community abundance by detecting denitrification-related functional genes.

1. Materials and methods

1.1. Sampling

The topsoil (0–15 cm in depth) was collected from Xiamen Botanical Garden [24°27′N, 118°05′E], China. The local climate is subtropical oceanic, with an average annual temperature of 21 °C and a mean annual precipitation of 1200 mm. The construction of the botanical garden began in 1960. Vegetation in the sampling site consists of trees with little grass. The selected soil has not been disturbed since 1989. After sampling, the urban garden soil was sieved (<2 mm), then stored at 4 °C prior to incubation and partially air-dried for chemical analysis. The soil had a texture of 73.54 % sand, 25.22 % silt and 1.24 % clay, a pH of 5.8 and contained 28.3 g kg^{-1} total carbon, 2.1 g kg^{-1} total N and 0.4 g kg^{-1} total sulphur.

1.2. Microcosm incubation

Soil samples were uniformly sprayed with a gradient of $CuCl_2$ levels in a plastic valve bag and homogenised by hand-shaking for 30 min. The concentrations of added $CuCl_2$ were 0, 10, 20, 40, 80 and 160 μg Cu g^{-1} soil (denoted as Cu0, Cu10, Cu20, Cu40, Cu80 and Cu160, respectively). After Cu exposure at 25 °C in the dark for three days, the available total Cu concentrations were quantified, with values of 0.8, 7.4, 15.5, 29.5, 65.5 and 134.4 μg Cu g^{-1} soil, respectively.

Potential denitrification activity was measured using the acetylene inhibition method (Myrold & Tiedje 1985). Soils equal to 10 g of dry weight were transferred to 120 ml serum bottles, and 5 ml KNO_3 solution (50 mg N kg^{-1}) was added as denitrification substrate. A small volume of deionised water was supplemented to attain a uniform moisture of 60 % (w/w, absolute water content). For each treatment, three replicates were sampled to determine the initial nitrate concentrations, while the other nine replicates were sealed with butyl rubber stoppers and aluminium caps. All nine flasks were then evacuated and flushed with 99.999 % helium for four cycles and finally vented to atmospheric pressure. Three of the anoxic flasks were injected with C_2H_2 (10 % v/v of headspace) to inhibit nitrification and the reduction of N_2O to N_2 in denitrification. Immediately afterwards, all anoxic flasks were instantly incubated at 25 °C in the dark for 21 h using a robotised incubation system coupled to a gas chromatograph (see Section 1.3), as previously described (Molstad *et al*. 2007). The moment of acetylene injection was regarded as the start of incubation (denoted as 0 h). The flasks with C_2H_2 and three of the replicates without C_2H_2 were used to determine N_2O and CO_2 concentrations in the headspace at 0 h and subsequently at 5, 9, 13, 17 and 21 h of incubation; the remaining three replicates without C_2H_2 were only used for DNA extraction after 21 h incubation. Soils in flasks without C_2H_2, which were used for gas monitoring, were collected to determine NO_3^- concentrations at the end of incubation.

1.3. Soil and gas measurements

The total Cu concentration in soil was analysed using a strong acid (HNO_3–$HClO_4$) digestion method (Lee *et al*. 2006), and determined by inductively coupled plasma-optic emission spectroscopy (ICP-OES) (Optima 7000 DV, PerkinElmer, USA). The available Cu in soil was extracted in 0.1 M HCl (Aoyama & Nagumo 1996; Xu *et al*. 2013) and assessed by atomic absorption spectroscopy (M6, Thermo Fisher, USA). Soil pH was determined with a dual-channel pH-ion-conductivity-dissolved oxygen meter (X60, Fisher Scientific) using a soil–water suspension (1:2.5 w/v). Particle size distribution was analysed using a laser particle size analyser (Malvern Mastersizer 2000, UK). Total carbon, N and sulphur were determined using an element analyser (Vario MAX CNS, Germany). Soil NO_3^- was extracted in deionised water (Fang *et al*. 2012) and quantified by ion chromatography (Dionex ICS-3000, USA). Concentrations of headspace N_2O and CO_2 were analysed with an Agilent 7890 gas chromatograph (Santa Clara, CA, US). The robotised incubation system (Molstad *et al*. 2007) was coupled to this gas chromatograph through an autosampler, allowing the direct and sequential measurement of headspace gases.

1.4. DNA extraction and quantitative polymerase chain reaction (qPCR)

Soil DNA was extracted from approximately 0.5 g (wet weight) microcosm samples using the FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. The purity and concentration of DNA were checked using an ultraviolet–visible spectroscopy spectrophotometer ND-1000 (NanoDrop, USA). The DNA was then stored at −20 °C until real-time qPCR analysis.

Quantitative PCR amplifications were performed on a LightCycler Roche 480 instrument (Roche Molecular Systems). The bacterial *16S rRNA* gene and key denitrification functional genes *nirK*, *nirS* and *nosZ* were amplified and quantified using the primer pairs 341F/517R (Muyzer *et al*. 1993), FlaCu/R3Cu (Hallin & Lindgren 1999), cd3aF/R3cd (Throbäck *et al*. 2004) and *nosZ*-F/*nosZ*-1622R (Throbäck *et al*. 2004), respectively. Twenty microlitres of PCR reaction volume contained 2 μL of DNA (1–10 ng), 0.25 μM of each primer, 10 μL of SYBR Premix Ex TaqII (TaKaRa) and 0.2 μL of 0.1 % bovine serum albumin (BSA) (TaKaRa). For the bacterial *16S rRNA* gene, the thermal cycling condition was 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 56 °C for 34 s. For the *nirK* and *nirS* genes, the thermal profile was 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 57 °C for 1 min and 72 °C for 1 min. For the *nosZ* gene, the condition was 95 °C for 1 min,

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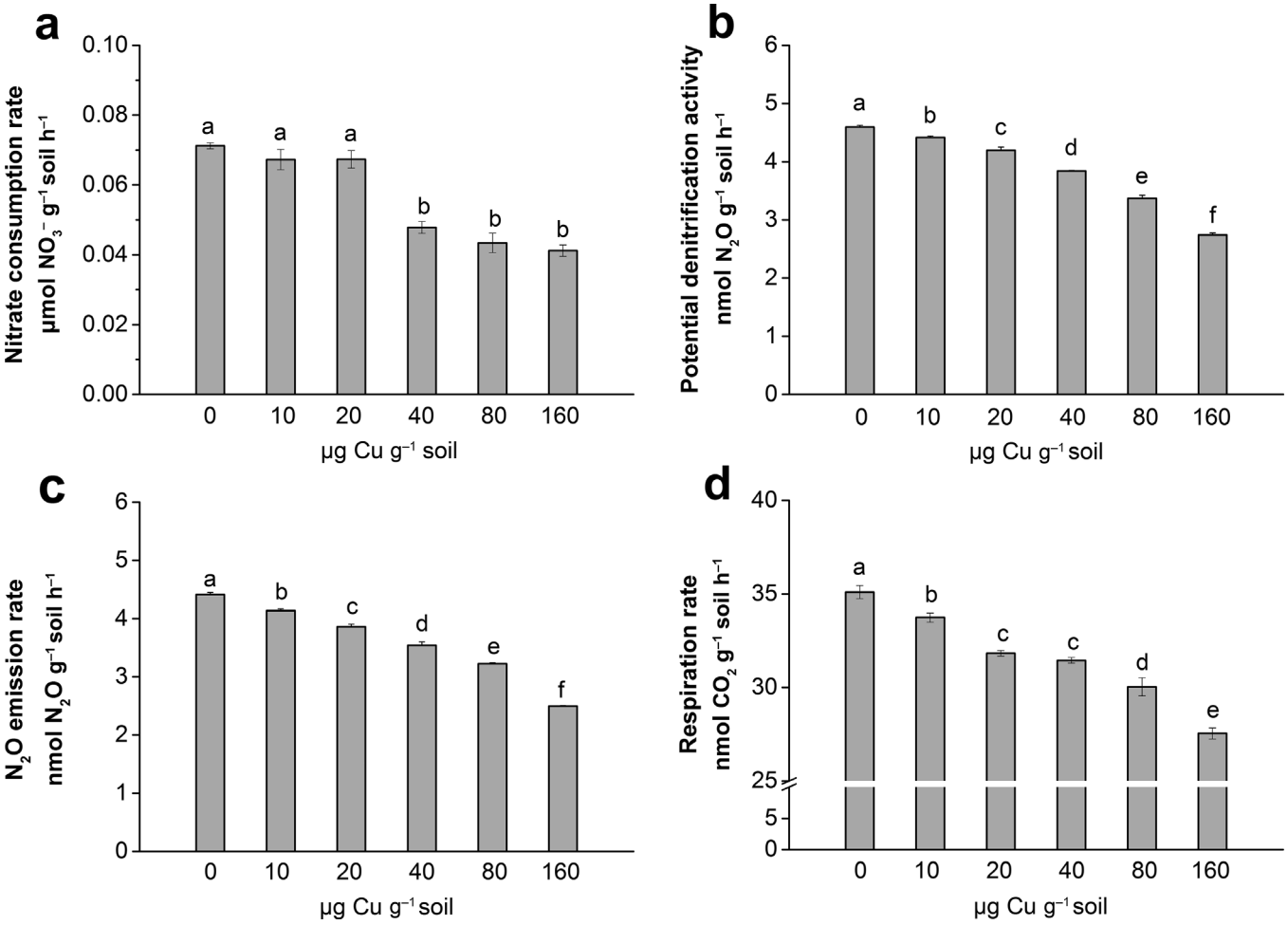

Figure 1 Mean (± standard error; $n = 3$) nitrate consumption rate (a), potential denitrification activity (b), N_2O emission from denitrification (c) and anoxic respiration rate (d) in soil microcosms with different Cu additions through the entire incubation. Matching letters above the bars indicate treatments without significant differences ($P < 0.05$).

followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 34 s. The PCR reaction mixtures without template DNA were used as negative controls. All real-time PCR standard curves were generated from gradient dilutions of plasmids with known copy numbers. The amplification efficiencies for all assays were above 90 %, with R^2 values of more than 0.99.

1.5. Statistical analysis

All experimental results are given on an oven-dry basis unless otherwise noted. Statistical analysis was conducted with SPSS software 18.0 for Windows. One-way ANOVA with Duncan's test was performed to determine the statistical significance of all data between treatments at $P < 0.05$. Pearson correlation analysis was used to evaluate the relationship among independent variables at $P < 0.05$ or $P < 0.01$.

2. Results

2.1. Potential denitrification activity and N_2O emission

The nitrate consumption rate decreased with increasing Cu amendments, and dropped to the lowest level (0.041 μmol NO_3^- g^{-1} soil h^{-1}) in the Cu160 treatment (Fig. 1a). The nitrate consumption rate in Cu-treated soils was found to be significantly lower ($P < 0.05$) than that in the control until the Cu addition reached the value of 40 μg Cu g^{-1} soil.

The N_2O emission in treatments with C_2H_2 represented the potential denitrification activity, due to the inhibitory properties of C_2H_2 on nitrification and N_2O reduction. In contrast, the N_2O release in C_2H_2-free treatments represented the N_2O emission via denitrification. We observed that the potential denitrification activity and denitrification-derived N_2O emission decreased progressively with evaluated Cu concentrations and differed significantly among all treatments. As shown in Figure 1b, in the highest Cu-amended soil (Cu160 treatment), the potential denitrification activity (2.74 nmol N_2O g^{-1} soil h^{-1}) was significantly ($P < 0.05$) reduced by 40 % compared with the non-amended control, and the corresponding N_2O emission of denitrification (2.49 nmol N_2O g^{-1} soil h^{-1}) decreased by 44 % (Fig. 1c). In general, the $N_2O/(N_2O + N_2)$ ratios of denitrification products ranged from 91 to 96 %, indicating that the dominant product of denitrification in the investigated soil was N_2O rather than N_2.

Anoxic respiration (CO_2 emission rate) was determined from soils incubated without C_2H_2, since C_2H_2 might serve as a carbon source for soil microbes. As shown in Figure 1d, the Cu additions significantly ($P < 0.05$) inhibited the CO_2 emission rate with increasing concentrations. The respiration rate was 27.55 nmol CO_2 g^{-1} soil h^{-1} in the Cu160 treatment – 22 % lower than that in the non-amended microcosm.

2.2. Abundance of denitrifying functional genes

The Cu additions exerted no significant effect on general bacterial abundance (quantified by *16S rRNA* gene) with the exception of the Cu160 treatment, in which the abundance

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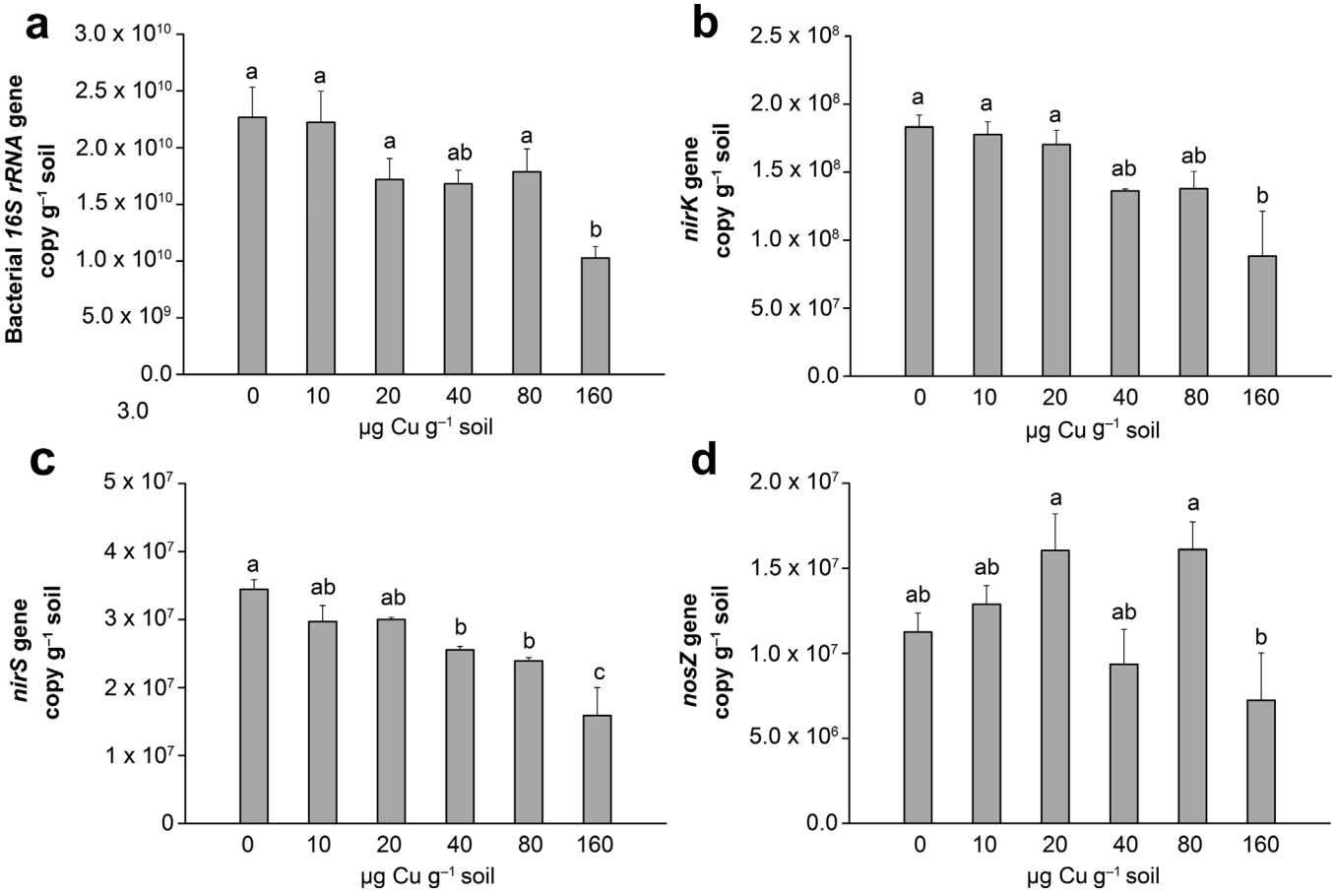

Figure 2 Mean (± standard error; $n = 3$) bacterial *16S rRNA* gene (a), *nirK* gene (b), *nirS* gene (c) and *nosZ* gene (d) abundance in soil microcosms with different Cu additions through the entire incubation. Matching letters above the bars indicate treatments without significant differences ($P < 0.05$).

Table 1 Pearson correlations between denitrification function and denitrifier abundance. Abbreviations: Cu = copper additions; NO_3^- = nitrate consumption rate; PDA = potential denitrification activity; D-N_2O = N_2O emission from denitrification; D-N_2 = N_2 emission from denitrification; CO_2 = anoxic respiration rate; NS = not significant at the 0.05 level (two-tailed).

Parameters	Cu	NO_3^-	PDA	D-N_2O	D-N_2	CO_2	*16S rRNA*	*nirK*	*nirS*	*nosZ*
Cu	1									
NO_3^-	−0.848[1]	1								
PDA	−0.981[2]	0.924[2]	1							
D-N_2O	−0.975[2]	0.911[1]	0.995[2]	1						
D-N_2	NS	NS	NS	NS	1					
CO_2	−0.943[2]	0.881[1]	0.975[2]	0.989[2]	NS	1				
16S rRNA	−0.900[1]	NS	0.903[1]	0.935[2]	NS	0.945[2]	1			
*nir*K	−0.963[2]	0.912[1]	0.971[2]	0.977[2]	NS	0.942[2]	0.930[2]	1		
*nir*S	−0.967[2]	0.900[1]	0.977[2]	0.986[2]	NS	0.963[2]	0.909[1]	0.977[2]	1	
*nos*Z	NS	NS	NS	NS	NS	NS	NS	NS	NS	1

[1] Correlation is significant at the 0.05 level (two-tailed).
[2] Correlation is significant at the 0.01 level (two-tailed).

(1.03×10^{10} copies g^{-1} soil) was significantly ($P < 0.05$) reduced by 55% compared to the control (Fig. 2a).

Further on, our results showed that Cu decreased the abundance of *nirK* and *nirS* genes with increasing concentrations. The significant difference on *nirK* gene abundance was only observed in the Cu160 treatment (8.83×10^7 copies g^{-1} soil) – 52% lower than that in the control (Fig. 2b). The abundance of the *nirS* gene in amended microcosms was not significantly affected compared to the control until the Cu concentration was as high as 40 µg Cu g^{-1} soil (Fig. 2c). In the Cu40 treatment, copy numbers of the *nirS* gene (2.56×10^7 copies g^{-1} soil) were reduced by 26% compared to the control. In the Cu160 treatment, the *nirS* gene abundance decreased to 1.59×10^7 copies g^{-1} soil – 54% lower than that in the non-amended control. No significant difference was found for the *nosZ* gene copies across all treatments (Fig. 2d).

Of the denitrification functional genes analysed in this study, the abundance of the *nirK* gene in the control and amended microcosms were one order of magnitude larger than that of *nirS* and *nosZ* genes in respective microcosms.

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2.3. Correlations between denitrification function and denitrifier abundance

The relationship between Cu additions, geochemical parameters indicating denitrification function, as well as denitrifier abundance, were examined using Pearson correlation analysis. As shown in Table 1, the Cu additions exhibited a strong and negative correlation ($P < 0.01$) with respectively potential denitrification activity, N_2O emission of denitrification, anoxic respiration and the abundance of *nirK* and *nirS* genes. On the other hand, there was no significant correlation with *nosZ* gene abundance. Nitrate consumption rate and bacterial *16S rRNA* gene copies were negatively correlated ($P < 0.05$) to Cu additions. Of the correlation between denitrification function (including both potential denitrification activity and denitrification-derived N_2O emission) and denitrifying gene abundance examined, the potential denitrification activity showed a clear and positive correlation with *nirK* ($P < 0.01$), *nirS* ($P < 0.01$) and bacterial *16S rRNA* gene copies ($P < 0.05$), yet was not found to be correlated to *nosZ* gene abundance.

3. Discussion

3.1. Effects of Cu on soil denitrification

Soil denitrification functions can be reflected by the potential denitrification activity and the N_2O emission from denitrification. Our study showed that Cu additions significantly inhibited potential denitrification activity and denitrification-derived N_2O emission, even at the lowest amendment (10 μg Cu g^{-1} soil). The observed inhibitory effects of Cu on denitrification function have also been described in soils and sediments in previous studies (Bardgett *et al*. 1994; Sakadevan *et al*. 1999; Holtan-Hartwig *et al*. 2002; Wang *et al*. 2013), where higher concentrations generally exceeding 80 μg Cu g^{-1} dry soil or 500 μg Cu g^{-1} dry sediment were required. In this investigation, Cu amendments exhibited suppression on denitrification functions at a level as low as 10 μg Cu g^{-1} soil. Similarly, Magalhães *et al*. (2011) reported that the potential denitrification rate in an estuarine sediment were inhibited when amended with 4 μg Cu per gram of wet sediment. However, we observed that the Cu additions had no significant effects on the N_2 emission rate, as calculated by subtracting denitrification-derived N_2O emission from corresponding potential denitrification activity in respective treatments (data not shown). Taking into account that N_2O was the prominent denitrification product in this urban park soil across all treatments, especially in non-amended microcosms, the dominant denitrification pathways in our study were the precedent reactions generating N_2O rather than the final step that reduces N_2O to N_2, and Cu decreased denitrification mainly by inhibiting the sequential reduction of NO_3^- to N_2O. Increased Cu levels were also observed to inhibit overall microbial activity, as indicated by the decreased soil respiration. Additionally, our study revealed that different reduction steps involved in denitrification differed in sensitivity to Cu amendments. By comparing responses of nitrate consumption and denitrification-derived N_2O emission to Cu additions, we at least can conclude that the nitrate reduction step was more tolerant than the entire two subsequent reduction steps by which NO_2^- was reduced to N_2O via NO. Of particular interest was the observation that Cu additions did not impact N_2 emission rate or stimulate N_2O accumulation, indicating that the N_2O reduction step was quite tolerant to Cu additions. This is not in line with the results of other studies (Holtan-Hartwig *et al*. 2002; Magalhães *et al*. 2007, 2011), suggesting that the N_2O reduction step was highly inhibited by Cu amendments, and thereby enhanced the N_2O emission from denitrification.

Our results showed that the copy numbers of the *nirK* gene detected were as high as 10^8 copies g^{-1} soil, while the abundance of *nirS* and *nosZ* genes were around 10^7 copies g^{-1} soil, respectively. Furthermore, across all treatments, the *nirS* gene was more abundant than the *nosZ* gene. The predominance of *nirK*-bearing denitrifiers and the greater abundance of *nirS*-bearing denitrifiers than *nosZ*-type denitrifiers could support our observation that the precedent reactions generating N_2O were the dominant denitrification pathways in this soil rather than the N_2O reduction. This was further supported by the significant and positive correlations between the abundance of *nirK* and *nirS* genes and the N_2O emission from denitrification, indicating that, also, the denitrifying-associated genes abundance could effectively predict the corresponding process (Hallin *et al*. 2009; Petersen *et al*. 2012; Guo *et al*. 2013). Correlation analysis revealed that Cu additions exhibited a clear and negative correlation with the abundance of *nirK* and *nirS* genes; such detrimental effects of Cu additions on *nirK*- and *nirS*-harbouring denitrifiers have also been reported by Magalhães *et al*. (2011). However, in our study, the overall abundance of the *nosZ* gene was generally not correlated to Cu amendments; this finding was contradictory to the study of Magalhães *et al*. (2011), reporting that the *nosZ*-bearing denitrifiers were highly sensitive to Cu, even at low concentrations. Furthermore, a possible toxicity of Cu to the entire bacterial community was found at 160 μg Cu g^{-1} soil, as indicated by the significantly decreased copies of the bacterial *16S rRNA* gene.

Taken together, our investigation revealed that soil denitrification functions and denitrifying community abundance behaved differently in sensitivity to Cu additions in different studies. This could be explained by the innate differentiations in denitrifier community abundance, diversity and composition between soils due to environmental heterogeneity (Giller *et al*. 1998; Bru *et al*. 2011; Guo *et al*. 2013). Moreover, it could be attributed to the differences in Cu availability resulting from various soil physico-chemical properties, as total metal content, pH, texture, cation exchange capacity, minerals, organic matter and redox potential can influence the sorption, mobility and speciation of metal in soils (Giller *et al*. 1998; Rooney *et al*. 2006; Li *et al*. 2010; Vega *et al*. 2010). Although the present study was limited to only one soil, the observed inhibition of the lowest Cu addition on denitrification functions underlines the resultant sensitivity and significance of the Cu to soil denitrification process and correlated N_2O emission.

3.2. Environmental implications

It is important to note that the native Cu concentration in this long-term undisturbed soil (7.9 μg Cu g^{-1} soil) was comparable to the local background soil value (9.7 μg Cu g^{-1} soil) of Xiamen City (China Environmental Monitoring Station 1990). In a recent study, Luo *et al*. (2015) reported an average Cu content of around 25 μg Cu g^{-1} in soils collected from six urban parks without specific point-sources of heavy metals in Xiamen City. Thereby, in our study, it was reasonable to add Cu as low as 10 μg Cu g^{-1} soil to simulate the increased Cu levels caused by continuous urbanisation. More broadly, the concentrations of Cu we used mainly ranged from 10 μg Cu g^{-1} soil to 80 μg Cu g^{-1} soil, corresponding well to the extent of urban soil Cu concentrations in Chinese cities based on their local background levels (China Environmental Monitoring Station 1990; Luo *et al*. 2012; Cheng *et al*. 2014). On the other hand, urban soils could receive large N inputs, mainly in the form of NO_3^- rather than NH_4^+, and have been recognised as a potential hotspot for denitrification and N_2O emission (Kaye *et al*. 2006; Li *et al*. 2014). In combination with the fact that at the national scale Cu was found to be the most significant and

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widespread pollutant metal in Chinese urban soils (Wei & Yang 2010; Luo *et al*. 2012), increased Cu may thus contribute to biasing urban soil denitrifier communities and profoundly alter urban soil denitrification functions.

4. Conclusions

To summarise, this study showed that Cu additions similar to increasing levels owing to rapid urbanisation can suppress soil denitrification function reflected by N_2O emission and potential denitrification activity, as well as minimise the abundance of the dominant denitrifying functional genes in the investigated urban park soil. Likewise, we suggest that a small amount of Cu increase caused by urbanisation, yet bellow the grade II limit of the Chinese Soil Quality Standards (300 mg Cu kg^{-1}; State Environmental Protection Administration of China, 2008), might be favourable for mitigating emissions of the greenhouse gas N_2O in urban soils. Nevertheless, how the soil denitrification process and its N_2O emission patterns respond to Cu amendments may differ in different urban soils due to discrepancies in the denitrifier community as well as variation of Cu availability. In addition, conclusions obtained from incubation experiments under ideally anoxic conditions are limited to reflect real natural environments. These warrant systematic investigations as well as *in situ* studies. Altogether, our study offers a valuable perspective for elucidating the role that the urbanisation process plays in altering urban N biogeochemical cycling.

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