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Population characteristics and influential factors of nitrogen cycling functional genes in heavy metal contaminated soil remediated by biochar and compost

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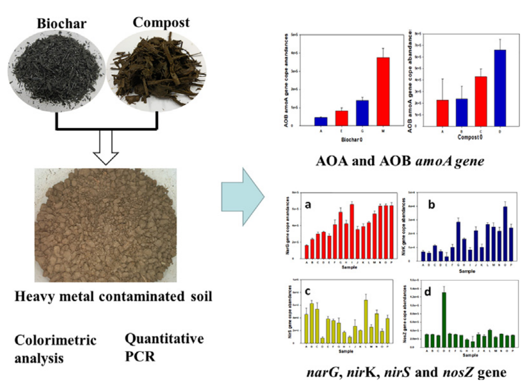
HIGHLIGHTS

- Denitrifying/ammonia-oxidizing gene and nitrite reductase activity were determined.
- The abundance of genes *nirK* and *narG* is most sensitive to the change of soil.
- 16S rDNA was significantly affected by NO_3^--N and S-NiR.
- Nitrifying genes were mainly related to water soluble organic carbon and S-NiR.
- Denitrifying genes were driven by pH, electrical conductivity, NO_3^--N and S-NiR.

GRAPHICAL ABSTRACT



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ABSTRACT

Sixteen treatments of soil contaminated by Cu, Pb, and Zn by the addition of a different percentage of biochar and compost were incubated for 120 days. The abundance of denitrifying genes such as *narG*, *nirK*, *nirS* and *nosZ* and the ammonia-oxidizing *amoA* genes of ammonia-oxidizing archaea/bacteria (AOA/AOB), soil nitrite reductase activity (S-NiR) and their shaping factors were also determined. The relationships between functional genes, S-NiR, and physico-chemical parameters were analyzed using the Pearson correlation method. The study found that the changes in physico-chemical parameters, including water-soluble organic carbon (WSC), nitrate (NO_3^-) and ammonium (NH_4^+), were predominant in different treatments. The abundance of *nirK* and *narG* genes is most sensitive to the changes in the properties of the soil sample. Bacterial 16S rDNA gene abundance was significantly affected by NO_3^- and S-NiR ($P < 0.05$). Nitrifying genes were mainly correlated to WSC and S-NiR, while denitrifying genes were associated with pH, electrical conductivity, NO_3^- and S-NiR. The systematic study for the relationship between the genes and the environmental parameters will help us to deep understand the biological mechanisms of nitrogen cycle in heavy metal contaminated soils remediated by biochar and compost.

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1. Introduction

Heavy metals are contributed to the agricultural soils by natural or anthropogenic activities (Alvarez et al., 2017; Ojedokun and Bello, 2016). Consequently, plants can accumulate heavy metals through plant root absorption and surface deposition, posing significant health risks to the consumers (Bolan et al., 2014). Therefore, the remediation of soils polluted by heavy metals is essential to reduce the adverse human and ecological health impacts.

Physical/chemical, phytoremediation, and biological techniques have been widely used for the remediation of soils contaminated by heavy metals (Thakur et al., 2016). Physical/chemical techniques are often used for in-situ management with rapid remediation effect. However, the in-situ management method could introduce new pollutants due to the application of chemical agents, while the ex-situ technology is generally expensive and has the potential to damage soil ecology (Kuppusamy et al., 2016). Further, Morillo and Villaverde (2017) found that the physical-chemical remediation technologies were harmful to the biological activities in soil media. Phytoaccumulation/phytoextraction, which uses the hyperaccumulator or metallophytes to remove heavy metals, is considered as an important path for in-situ depuration of moderately and slightly contaminated soils. However, these methods are effective for a specific heavy metal with a long repair cycle (Meier et al., 2017) and would require large area of the agricultural production land. Microbial remediation technologies are generally effective for the removal and degradation of organic compounds and could be used for the removal of large-scale inorganic contaminants (Varjani, 2017), including heavy metals from contaminated soil.

Furthermore, the new restoration materials could be efficient for the remediation of contaminated soil without secondary pollution. For example, compost, biochar, and red mud have excellent removal efficiency with little negative effect on the environment (Zhou et al., 2017). These stabilizing agents are widely regarded as effective alternatives to reduce and control the heavy metal pollution in soils. Biochar is a carbon-rich substance generated from the pyrolysis of organic materials under high temperature and anoxic conditions. Kim et al. (2016) found that biochar had changed soil condition properties such as binding of nutrients, enhancing cation exchange capacity, increasing the adsorption, and promoting nitrogen fixation, while Ahmad et al. (2014) reported that biochar has the potential for the remediation of soil contaminated with various heavy metals.

Compost is the product of artificially controlled microbial degradation of solid organic waste. Crop growth, yield, and quality of the soil are influenced by the application of compost by facilitating porous structure in soils for root penetration, enhancing water storage capacity and resistance to erosion. Additionally, the beneficial microbial symbionts and their habitat are introduced to the soils via compost to stimulate the microbial activity (Schmidt et al., 2014). The interaction between the biochar and the compost mixture can increase aeration conditions, while decreasing the bulk density and enhancing the yield of farmland soil (Wei et al., 2014). Biochar also keeps nutrients and improves the quality of the final compost product (Zhang et al., 2014). Effects of pH, dissolved total nitrogen (DTN), dissolved organic carbon (DOC), plant biomass on the uptake of heavy metals have been investigated in detail for the heavy metal contaminated soils remediated by biochar and compost (Karami et al., 2011). Nevertheless, studies on the microbial communities related to nitrogen cycling and their shaping factors in the heavy metal polluted soil are limited.

Inorganic and organic nitrogen compounds in soils are the crucial nutrient for crops. Additionally, nitrogen cycling is essential to maintain ecological balance and ensure the biological growth (Erisman et al., 2007). Nitrogen transformation, including nitrification, denitrification, ammonification and assimilation, is mainly driven by microorganisms and is affected by the heavy metal pollution due to the negative impacts on the functional genes in soils (Herrmann et al., 2017; Kuang et al., 2016; Kuppusamy et al., 2016; Li et al., 2009). Similarly, biochar remediation could affect the functional genes of nitrogen transformation in soil. Furthermore, biochar and compost had changed the soil physico-chemical properties and nutrients during the remediation of soils contaminated by heavy metals, resulting in a change in the population structure of nitrogen-cycling microorganisms and an increase in the community richness (Ahmad et al., 2014). Zhang et al. (2017) found that alfalfa planting with biochar applications significantly raised the abundance of *amoA* gene. Moreover, different amounts of biochar applications produced inconsistent effects on the abundances of functional genes in denitrification (Wang et al., 2013). The transformation of various forms of nitrogen, the process of selecting nitrogen and the effect of nitrogen conservation could provide an important insight into the transformation of nitrogen in soils polluted by heavy metals.

Hence, the primary aim of the current research was to determine the characteristics of functional genes for nitrogen cycling and their shaping factors in soil contaminated by heavy metals such as Cu, Pb, and Zn and remediated by biochar and compost. The abundance of *narG*, *nirK*, *nirS* and *nosZ*, and AOA, AOB *amoA* genes were evaluated using the quantitative PCR. The relationships between functional gene abundance, soil nitrite reductase activity (S-NiR) and physico-chemical parameters were determined using the Pearson correlation analysis.

Table 1
Characteristics of soil, biochar and compost in this experiment (mean ± standard deviation).

	Soil	Biochar	Compost
pH	6.33 ± 0.21	6.60 ± 0.10	6.63 ± 0.12
EC (μS cm^{-1})	13.60 ± 0.90	190.17 ± 5.76	974.07 ± 17.35
WSC (mg kg^{-1})	112.03 ± 10.42	–	604.71 ± 17.14
NO_3^--N (mg kg^{-1})	12.00 ± 0.95	0.30 ± 0.08	470.21 ± 12.97
NH_4^+-N (mg kg^{-1})	28.97 ± 1.22	0.40 ± 0.12	730.63 ± 37.75
Specific surface area (m^2 g^{-1})	0.80 ± 0.05	55.13 ± 5.70	–
Ash content (%)	–	50.52 ± 1.92	–
Total pore volume (cm^3 g^{-1})	–	0.04 ± 0.003	–
Organic matter content (%)	20.37 ± 1.81	–	57.14 ± 4.40
Cu (mg kg^{-1})	116.85 ± 13.07	–	–
Pb (mg kg^{-1})	81.27 ± 5.88	–	–
Zn (mg kg^{-1})	341.45 ± 10.56	–	–

Table 2
The amount of compost and biochar added for 16 different treatments and their corresponding numbers.

Soil:Biochar:Compost wet weight/g	Biochar addition amount increases direction ⟶			
Compost addition amount increases direction ↓	A 1800:0:0	B 1800:50:0	C 1800:100:0	D 1800:150:0
	E 1800:0:50	F 1800:50:50	G 1800:100:50	H 1800:150:0
	I 1800:0:100	J 1800:50:100	K 1800:100:100	L 1800:150:0
	M 1800:0:150	N 1800:50:150	O 1800:100:150	P 1800:150:0

2. Materials and methods

2.1. Soil preparation

The paddy soil samples were collected from Daxing Village, Huanghua Town, Changsha, Hunan, China. Soils in this area are polluted