

### Supplementary Information

**Manuscript title:** Doped carbon dots affect heavy metal speciation in mining soil:  
Changes of dissimilated iron reduction processes and microbial communities

**Authors:** Xinyi Huang,<sup>†</sup> Xuanming Liu,<sup>\*,‡</sup> ShaoBo Liu,<sup>\*,§</sup> Yunguo Liu,<sup>†</sup> Xiaofei  
Tan,<sup>†,¶</sup> Shuai Guo,<sup>‡</sup> Mingyang Dai,<sup>†</sup> Qiang Chen,<sup>†</sup> Gaobing Chen,<sup>†,¶</sup> Chenzhi  
Feng,<sup>†</sup>

<sup>†</sup> College of Environmental Science and Engineering, Hunan University and Key  
Laboratory of Environmental Biology and Pollution Control (Hunan University),  
Ministry of Education, Changsha 410082, China

<sup>‡</sup> Hunan Province Key Laboratory of Plant Functional Genomics and Developmental  
Regulation, College of Biology, Hunan University, Changsha 410082, China

<sup>§</sup> School of Architecture and Art, Central South University, Changsha 410083, China

<sup>¶</sup> Shenzhen Research Institute of Hunan University, Shenzhen 518055, China

\* Corresponding authors:

E-mail address: xmL05@hnu.edu.cn (Xuanming Liu)

liushaobo@csu.edu.cn (ShaoBo Liu)

## 1. The BCR extraction method used in this study

For the acid soluble fraction determination, weighed 1 g of air-dried soil were weighed in a 50 mL centrifuge tube, added 40 mL of 0.11 mol/L  $\text{CH}_3\text{COOH}$ , shaken for 16 h at 180 r/m, centrifuged at 5000 r/min for 10 min, passed the supernatant through a 0.45  $\mu\text{m}$  filter membrane and refrigerated at 4°C for further testing. Added 20 mL deionized water in the residue, shaken for 15 min and centrifuged at 5000 r/min. The residue was retained for subsequent extraction. For the reducible fraction determination, added 40 mL of 0.5 mol/L  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , shaken for 16 h at 180 r/min, centrifuged at 5000 r/min for 10 min, passed the supernatant through a 0.45  $\mu\text{m}$  filter membrane and refrigerated at 4°C for further testing. Added 20 mL deionized water in the residue, shaken for 15 min and centrifuged at 5000 r/min. The residue was retained for subsequent extraction. For the oxidizable fraction determination, added 10 mL  $\text{H}_2\text{O}_2$  and digested for 1 h at room temperature, then digested at 85°C until the volume was less than 3 mL. After cooling, added 10 mL  $\text{H}_2\text{O}_2$  and digested continued until the volume was 1 mL (do not evaporate). After cooling added 40 mL  $\text{NH}_4\text{OAc}$ , shaken for 16 h at 25°C, centrifuged at 5000 r/min for 10 min, pass the supernatant through a 0.45  $\mu\text{m}$  filter membrane and refrigerated at 4°C for further testing. Added 20 mL deionized water in the residue, shaken for 15 min and centrifuged at 5000 r/min. The residue was retained for subsequent extraction. For the residual fraction determination, weighed 0.100 g of air-dried residue, digested with a mixture of  $\text{HNO}_3\text{-HCl-HF-HClO}_4$  and the metal content was determined by ICP-MS.

## 2.The extraction of soil DNA

PCR raw products were gel-purified with a KOD-Plus-Neo DNA Polymerase (TOYOBO, Japan). The PCR amplification of 16S rDNA was performed as follows: initial denaturation at 94 °C for 1 min, followed by 25~35 cycles of denaturing at 94 °C for 20 s, annealing at 54 °C for 30 s and extension at 72 °C for 30 s, single extension at 72 °C for 5 min, and ending at 4 °C. TOYOBO (KOD-401B) .

**Table S1** Main properties of the soil used in this study

<b>Parameter</b>	<b>Value</b>
pH	3.67
Organic matter	1.395 mg/g
Conductivity	473 $\mu$ S/cm
Cation exchange capacity (CEC)	2.9 cmol/kg
Pb	317.83 mg/kg
Zn	55.60 mg/kg
Cu	91.27 mg/kg

**Table S2** Proportion of metal atoms on soil surface under different treatments

Time	Pb		Zn		Cu	
	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60
CK	0.06%	0.05%	0.34%	0.65%	0.46%	0.66%
0.5% N-CDs		0.02%		0.37%		0.56%
1.5% N-CDs		0.05%		0.42%		0.43%
0.5% N,P-CDs		0.04%		0.3%		0.29%
1.5% N,P-CDs		0.04%		0.23%		0.27%

**Table S3** Number of copies per gram of sample under different treatments

Time	Treatment				
	CK	0.5% N-CDs	1.5% N-CDs	0.5% N,P-CDs	1.5% N,P-CDs
Day 0	$2.4 \times 10^5 \pm 1.1 \times 10^5$				
Day60	$1.7 \times 10^4 \pm 2.4 \times 10^3$ <sup>b</sup>	$1.7 \times 10^4 \pm 5.5 \times 10^3$ <sup>b</sup>	$2.3 \times 10^5 \pm 6.7 \times 10^4$ <sup>b</sup>	$1.3 \times 10^5 \pm 1.4 \times 10^5$ <sup>b</sup>	$5.9 \times 10^8 \pm 1.1 \times 10^8$ <sup>a</sup>

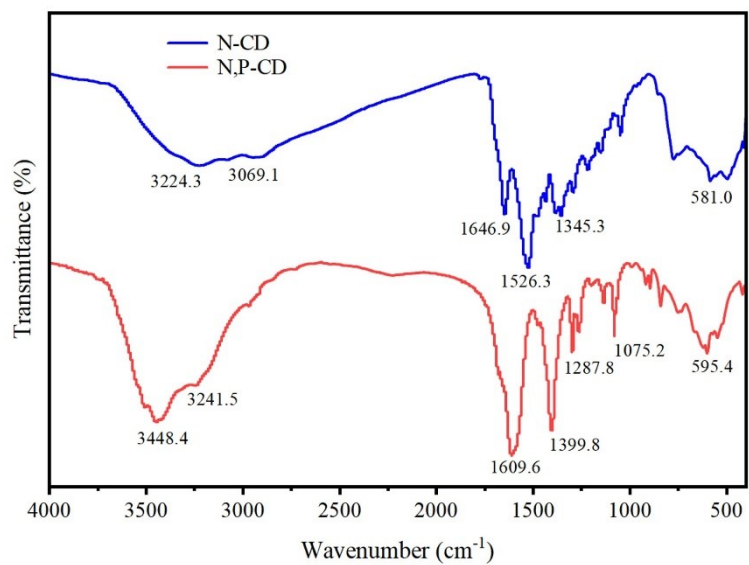


Fig. S1 FTIR spectrum of N-CDs and N,P-CDs

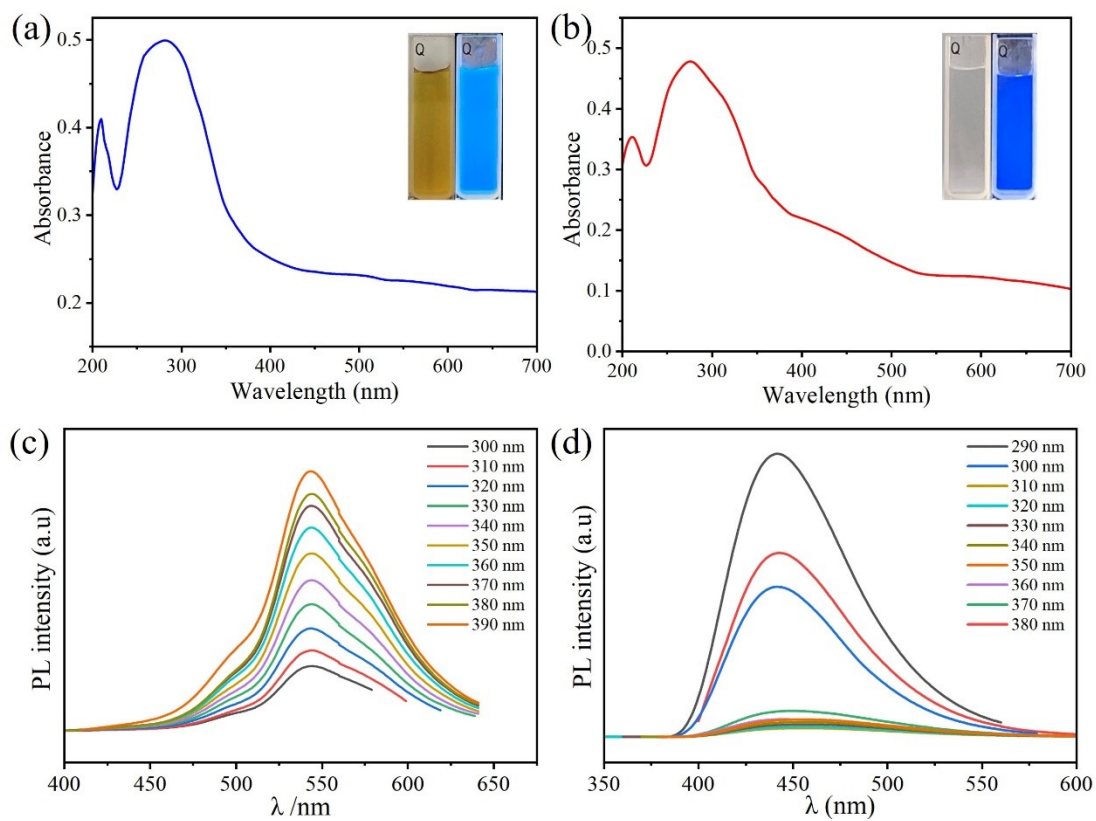


Fig. S2 UV-vis absorption spectrum of (a) N-CDs, (b) N,P-CDs and photoluminescence spectrum of (c) N-CDs, (d) N,P-CDs



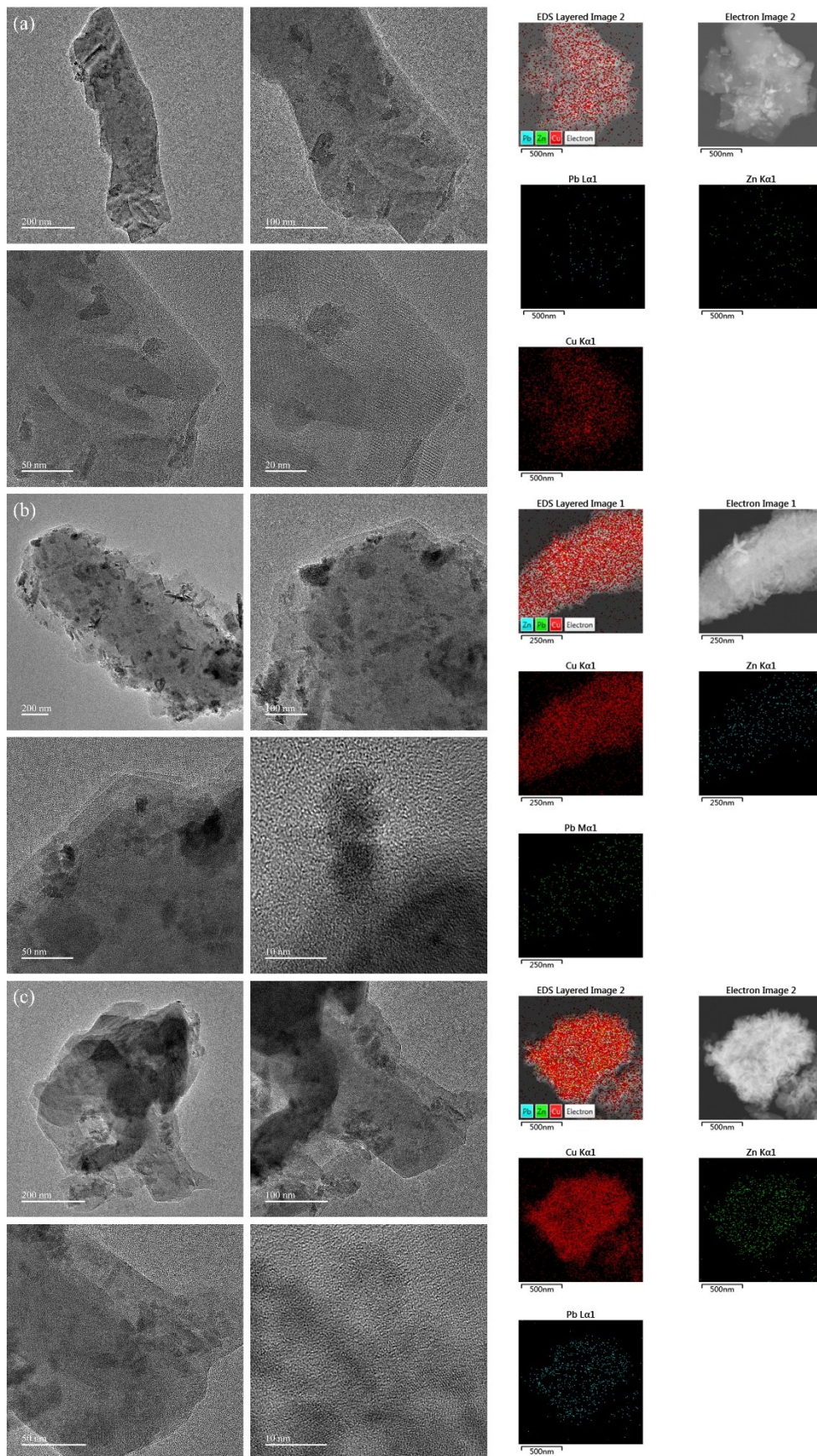


Fig.S3 Soil particles TEM (a) CK (b) N-CDs (c) N,P-CDs (red: Cu; green: Zn; blue: Pb)

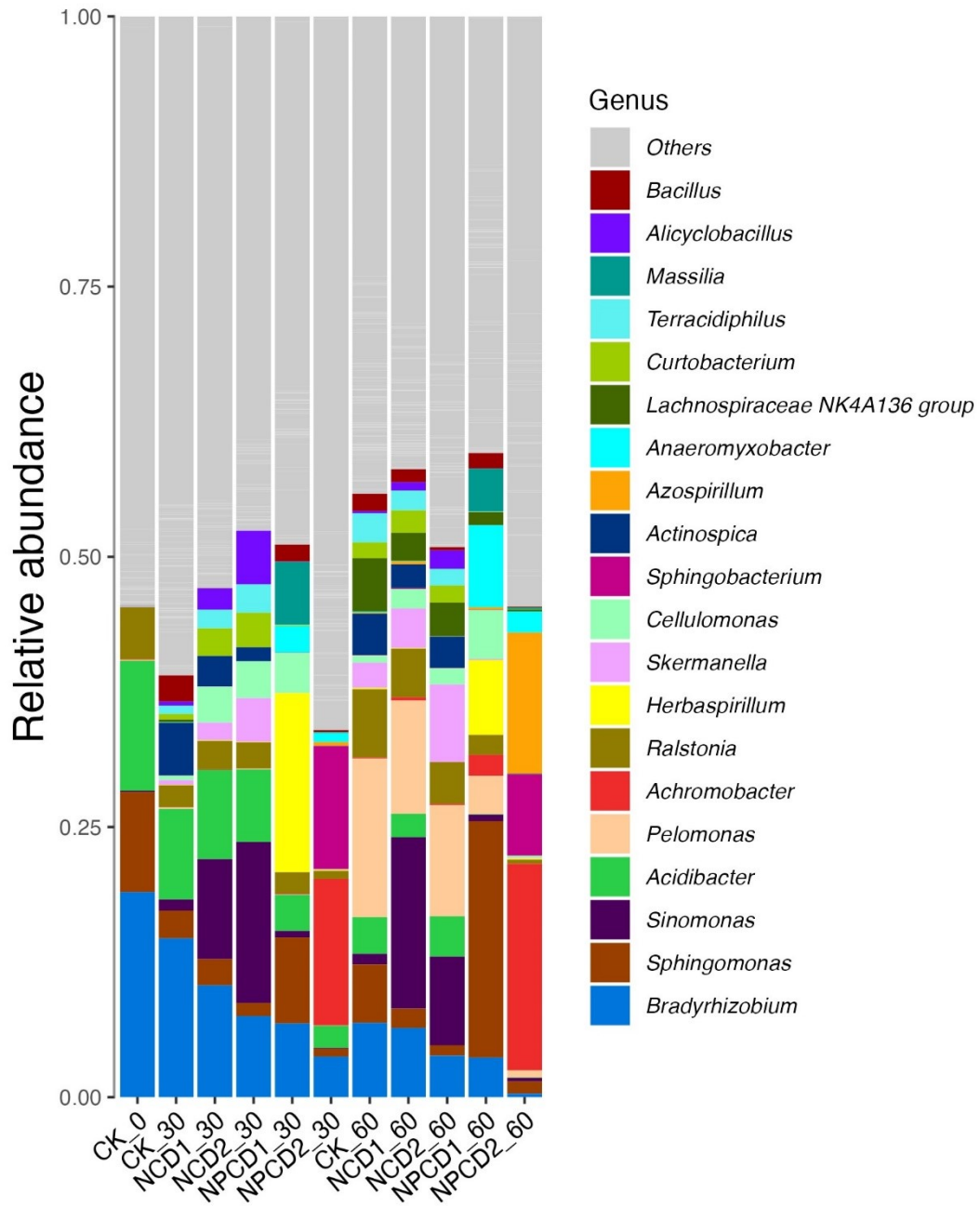


Fig. S4 Different treatment groups in soil at the genus level relative abundance of bacterial communities