Support Information

Title: Soil bacterial communities present different levels of response to graphene oxide and reduced graphene oxide after 90 days'exposure

Junjie Du[1,3], Qixing Zhou[2]*, Jianhu Wu[1], Guifeng Li[1], Guoqin Li[1], Yongning Wu[3]

[1]College of Food Science, Shanxi Normal University, Linfen, 041004, China

[2]Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin, 300071, China

[3]NHC Key Laboratory of Food Safety Risk Assessment, China National Center for Food Safety Risk Assessment, Beijing, 100022, China

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*Correspondence atuhors:

E-mail: zhouqx@nankai.edu.cn

Soil bacterial community analysis (Materials and methods).

Bacterial DNA was extracted from 0.5 g of soil samples (in triplicate) with MP Biomedicals'Fast DNA SPIN Kit. The V3 and V4 regions of 16S rRNA were amplified by PCR with forward primer of 338 F and reverse primer of 806 R.

The PCR amplified products were purified using AMPure Beads (Agencourt) and quantified with PicoGreen dsDNA assay Kit (Invitrogen). Equal amounts of amplified products were pooled together after the quantification. High-throughput sequencing data were produced by Illumina MiSeq platform (2 × 300 cycles; MiSeq Reagent V3 Kit) at Personal Biotechnology Co., Ltd. (Shanghai, China). The raw reads were submitted to the NCBI Sequence Read Archive database (Accession codes: PRJNA552951).

The sequencing data were analyzed using the QIIME v 1.8.0 pipeline. The low-quality reads were filtered according to the following criteria: (1) sequence lengths less than 150 bp, (2) sequences with averag Phred scores less than 20, (3) sequences containing any ambiguous bases, and (4) sequences containing mononucleotide repeats (> 8 bp). The high-quality sequences were clustered into OTUs with a 97% similarity by using UCLUST (Edgar, 2010). The chimeric sequences were removed with the Uchime algorithm. Sequence data analyses were implemented by QIIME and R software (v 3.2.0). Taxonomic classification for each OUT was conducted with BLAST and Greengenes database. Alpha diversity indices (Chao1, ACE and Shannon) were calculated from the OTU table in QIIME (DeSantis et al., 2006). The structural variation of bacterial communities among samples was analyzed by using UniFrac distance metrics and visualized with principal coordinate analysis (PCoA). The taxa were classified to different functional groups using the functional annotation of prokaryotic taxa (FAPROTAX) database (Louca et al., 2016).

Desantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72, 5069-5072.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-2461.

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353, 1272-1277.

The characterizations of GO and GO (Materials and methods).

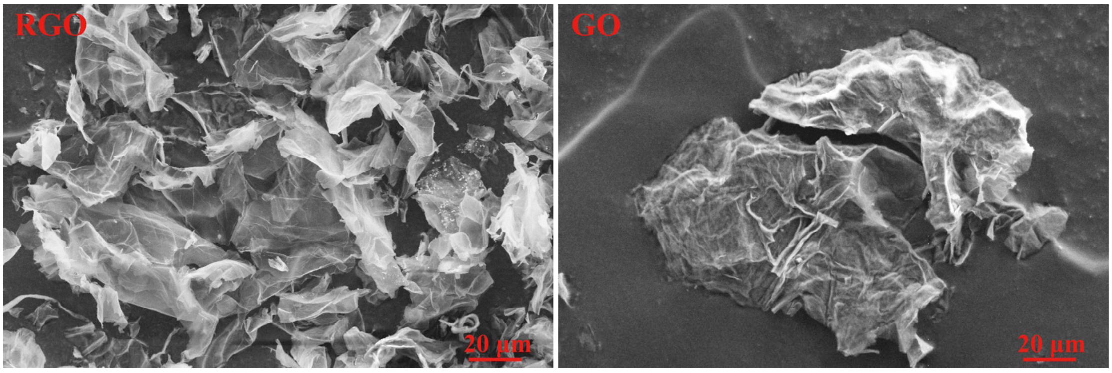

Fig. S1 Scanning electron microscopy (SEM) of RGO (reduced graphene oxide) and GO (graphene oxide).

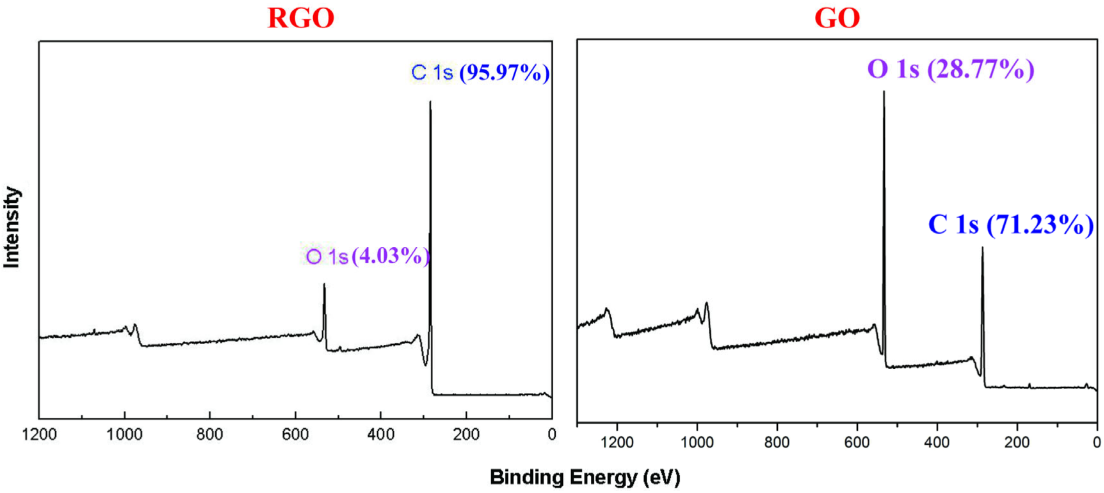

Fig. S2 X-ray photoelectron spectra (XPS) of RGO (reduced graphene oxide) and GO (graphene oxide).

The changes in soil bacterial community composition (Results and discussion).

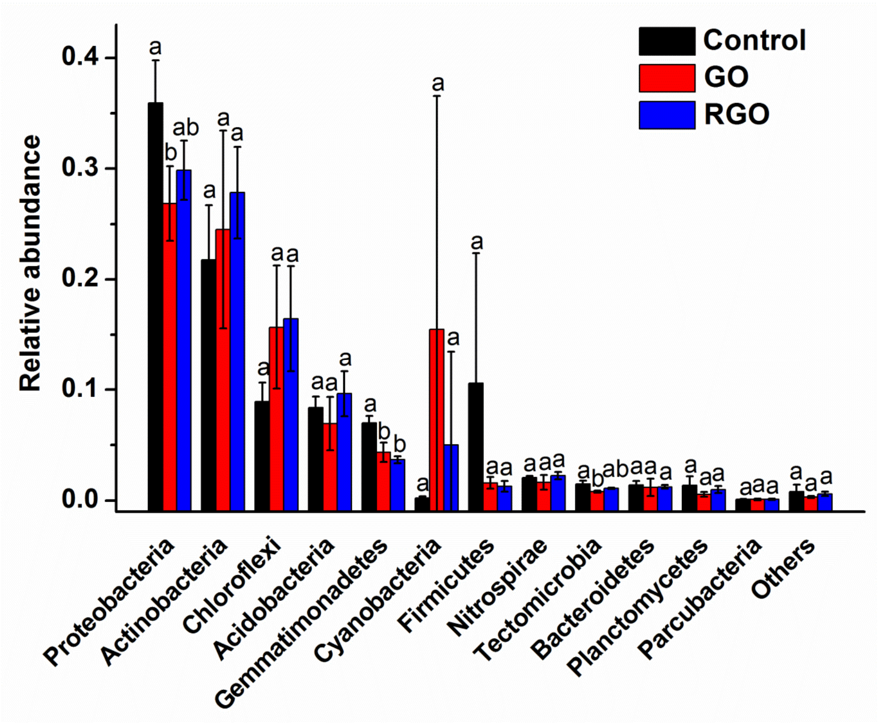

Fig. S3 Relative abundance of the dominant bacterial communities at the phylum level in soils with different treatments. The error bars on the results represent the means ± SD (standard deviation, $n = 3$). The different lowercase letters on the error bars indicate significant difference at $p < 0.05$ (based on LSD test). “Others” indicates that the phyla had mean relative abundance < 0.1% in at least on treatment. “Control” denotes the control treatment without graphene-based nanomaterials amendment. “GO” represents the treatment with a 50 mg/kg graphene oxide addition. “RGO” represents the treatment with a 50 mg/kg reduced graphene oxide addition.

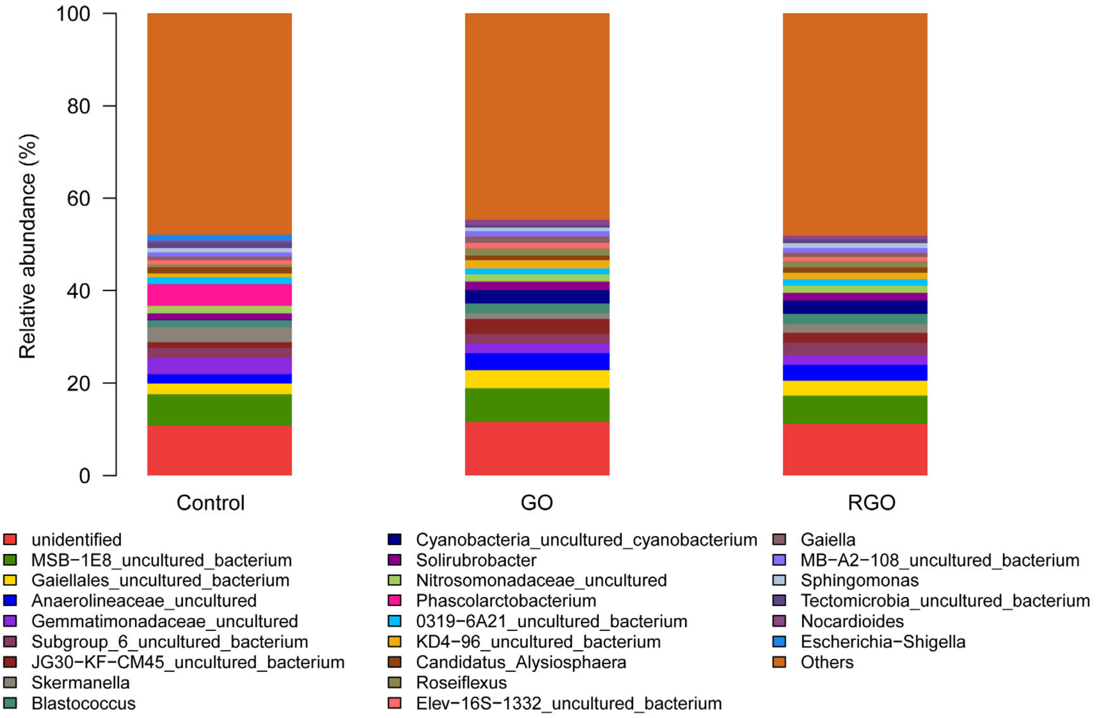

Fig. S4 Relative abundance of the dominant bacterial communities at the genus level in soils with different treatments. “Control” denotes the control treatment without graphene-based nanomaterials amendment. “GO” represents the treatment with a 50 mg/kg graphene oxide addition. “RGO” represents the treatment with a 50 mg/kg reduced graphene oxide addition.

The changes in functional groups of bacterial community (Results and discussion).

Table S1 The identified functional groups using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) software. Among the 90 functional groups in the FAPROTAX database, 74 groups were present in at least one of the samples.

Functional group	Control	GO	RGO
chemoheterotrophy	0.162 ± 0.028a	0.117 ± 0.005b	0.125 ± 0.015b
aerobic_chemohete	0.115 ± 0.028a	0.114 ± 0.005a	0.12 ± 0.014a

rotrophy			
fermentation	0.068 ± 0.048a	0.01 ± 0.002b	0.015 ± 0.002ab
nitrification	0.026 ± 0.003a	0.025 ± 0.003a	0.026 ± 0.003a
aerobic_nitrite_oxidation	0.014 ± 0.002a	0.014 ± 0.002a	0.015 ± 0.001a
aerobic_ammonia_oxidation	0.012 ± 0.002a	0.011 ± 0.001a	0.011 ± 0.003a
nitrate_reduction	0.012 ± 7E-04a	0.01 ± 1E-03b	0.01 ± 9E-04b
animal_parasites_or_symbionts	0.012 ± 0.005a	0.008 ± 7E-04a	0.009 ± 0.003a
respiration_of_sulfur_compounds	0.01±3E-04a	0.006 ± 5E-04b	0.007 ± 4E-04c
human_pathogens_all	0.008 ± 0.001a	0.008 ± 7E-04a	0.009 ± 0.003a
ureolysis	0.007 ± 0.002a	0.004 ± 7E-04b	0.006 ± 0.002ab
human_pathogens_pneumonia	0.006 ± 0.001a	0.007 ± 5E-04a	0.008 ± 0.002a
sulfur_respiration	0.007 ± 9E-04a	0.006 ± 8E-04b	0.006 ± 6E-04ab
nitrogen_fixation	0.006 ± 0.002a	0.003 ± 4E-04b	0.005 ± 0.001ab
predatory_or_exoparasitic	0.006 ± 0.001a	0.005 ± 4E-04a	0.004 ± 0.001a
manganese_oxidation	0.004 ± 0.001a	0.005 ± 8E-04a	0.004 ± 7E-04a
phototrophy	0.004 ± 0.001a	0.023 ± 0.017a	0.024 ± 0.036a
nitrogen_respiratio	0.003 ± 7E-04a	0.002 ± 2E-04b	0.003 ± 6E-04ab

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nitrate_respiration	0.003 ± 7E-04a	0.002 ± 2E-04b	0.003 ± 6E-04ab
photoautotrophy	0.003 ± 9E-04a	0.022 ± 0.017a	0.023 ± 0.036a
cellulolysis	0.002 ± 6E-04a	0.003 ± 3E-04a	0.002 ± 0.001a
photoheterotrophy	0.002 ± 5E-04a	0.002 ± 3E-04a	0.002 ± 5E-04a
aromatic_compound_degradation	0.003 ± 9E-04a	0.008 ± 0.001b	0.006 ± 0.002ab
sulfate_respiration	0.003 ± 6E-04a	5E-04 ± 3E-04b	0.001 ± 1E-03b
nitrite_respiration	0.002 ± 4E-04a	0.001 ± 2E-04a	0.001 ± 5E-04a
human_gut	0.004 ± 0.005a	2E-05 ± 3E-05a	8E-05 ± 4E-05a
mammal_gut	0.004 ± 0.005a	2E-05 ± 3E-05a	8E-05 ± 4E-05a
nitrate_denitrification	0.001 ± 5E-04a	0.001 ± 2E-04a	0.001 ± 4E-04a
nitrite_denitrification	0.001 ± 5E-04a	0.001 ± 2E-04a	0.001 ± 4E-04a
nitrous_oxide_denitrification	0.001 ± 5E-04a	0.001 ± 2E-04a	0.001 ± 4E-04a
denitrification	0.001 ± 4E-04a	0.001 ± 2E-04a	0.001 ± 4E-04a
anoxygenic_photoautotrophy	0.001 ± 3E-04a	0.001 ± 2E-04 a	0.001 ± 5E-04a
methylotrophy	0.001 ± 5E-04a	9E-04 ± 8E-05a	0.001 ± 4E-04a
anoxygenic_photoautotrophy_S_oxidizing	0.001 ± 3E-04a	0.001 ± 2E-04a	0.001 ± 5E-04a
iron_respiration	0.001 ± 5E-04a	0.001 ± 2E-04a	0.001 ± 1E-04a

hydrocarbon_degradation	8E-04 ± 5E-04a	7E-04 ± 1E-04a	0.001 ± 5E-04a
cyanobacteria	0.001 ± 7E-04a	0.021 ± 0.018a	0.022 ± 0.036a
oxygenic_photoautotrophy	0.001 ± 7E-04a	0.021 ± 0.018a	0.022 ± 0.036a
methanotrophy	7E-04 ± 5E-04a	5E-04 ± 5E-05a	7E-04 ± 3E-04a
chitinolysis	8E-04 ± 2E-04a	0.001 ± 2E-04a	8E-04 ± 3E-04a
chloroplasts	5E-04 ± 2E-04a	0.001 ± 6E-04a	0.001 ± 0.001a
intracellular_parasites	6E-04 ± 9E-05a	5E-04 ± 6E-05a	6E-04 ± 2E-04a
dark_oxidation_of_sulfur_compounds	4E-04 ± 1E-04a	3E-04 ± 2E-05b	4E-04 ± 1E-04ab
dark_hydrogen_oxidation	5E-04 ± 5E-04 ±a	9E-05 ± 9E-05 ±a	3E-04 ± 2E-04 ±a
nitrite_ammonification	5E-04 ± 4E-04a	4E-05 ± 4E-05a	3E-05 ± 4E-05a
dark_thiosulfate_oxidation	3E-04 ± 5E-05a	2E-04 ± 2E-05a	3E-04 ± 1E-04a
fumarate_respiration	5E-04 ± 5E-04a	3E-05 ± 3E-05a	0 ± 0a
xylanolysis	2E-04 ± 1E-04a	3E-04 ± 1E-05a	4E-04 ± 7E-05a
methanol_oxidation	3E-04 ± 7E-05a	4E-04 ± 1E-04a	5E-04 ± 1E-04a
nitrate_ammonific	1E-04 ± 9E-05a	4E-05 ± 4E-05a	3E-05 ± 4E-05a

ation			
human_pathogens_gastroenteritis	4E-04 ± 5E-04a	0 ± 0a	0 ± 0a
human_pathogens_diarrhea	4E-04 ± 5E-04a	0 ± 0a	0 ± 0a
knallgas_bacteria	8E-05 ± 6E-05a	3E-05 ± 3E-05ab	0 ± 0b
aromatic_hydrocarbon_degradation	1E-04 ± 4E-05a	2E-04 ± 2E-04a	4E-04 ± 2E-04a
aliphatic_non_methane_hydrocarbon_degradation	1E-04 ± 3E-05a	2E-04 ± 1E-04a	3E-04 ± 2E-04a
ligninolysis	9E-05 ± 7E-05a	6E-05 ± 2E-05a	8E-05 ± 4E-05a
plant_pathogen	5E-05 ± 2E-05a	6E-05 ± 2E-05a	2E-04 ± 2E-04a
aerobic_anoxygenic_phototrophy	3E-05 ± 3E-05a	0 ± 0a	1E-05 ± 2E-05a
dark_sulfide_oxidation	4E-05 ± 4E-05a	0 ± 0a	1E-05 ± 2E-05a
dark_iron_oxidation	2E-05 ± 3E-05a	2E-05 ± 3E-05a	3E-05 ± 2E-05a
invertebrate_parasites	5E-05 ± 2E-05a	9E-05 ± 1E-05a	1E-04 ± 5E-05a
thiosulfate_respiration	2E-05 ± 1E-05a	3E-05 ± 1E-06a	4E-05 ± 4E-05a
anammox	8E-06 ± 1E-05a	9E-06 ± 2E-05a	3E-05 ± 4E-05a
human_pathogens_	8E-06 ± 1E-05a	0 ± 0a	0 ± 0a

meningitis			
plastic_degradation	2E-05 ± 1E-05a	9E-06 ± 2E-05a	5E-05 ± 6E-05a
reductive_acetogenesis	2E-05 ± 1E-05a	9E-06 ± 2E-05a	0 ± 0a
dark_sulfite_oxidation	3E-05 ± 6E-05a	0 ± 0a	0 ± 0a
dark_sulfur_oxidation	3E-05 ± 6E-05a	0 ± 0a	0 ± 0a
human_pathogens_septicemia	2E-05 ± 3E-05a	0 ± 0a	0 ± 0a
human_pathogens_nosocomia	2E-05 ± 3E-05a	0 ± 0a	0 ± 0a
chlorate_reducers	2E-05 ± 3E-05a	0 ± 0a	0 ± 0a
sulfite_respiration	0 ± 0a	3E-05 ± 1E-06a	2E-05 ± 3E-05a
arsenate_detoxification	0 ± 0a	0 ± 0a	1E-05 ± 2E-05a
dissimilatory_arsenate_reduction	0 ± 0a	0 ± 0a	1E-05 ± 2E-05a

Mean values ± standard error (*n* = 3) followed by different lowercase letters within the same row indicate significant differences at $p < 0.05$ (based on LSD test). “Control” denotes the control treatment without graphene-based nanomaterials amendment. “GO” represents the treatment with a 50 mg/kg graphene oxide addition. “RGO” represents the treatment with a 50 mg/kg reduced graphene oxide addition.