Supporting Information

Oxygen Nanobubble-Loaded-Biochars Mitigate Copper Transfer from Copper-Contaminated Soil to Rice and Improve Rice Growth

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Number of figures: 7

Number of tables: 2

Supporting information for materials and methods

Sequence processing for 16S rRNA and ITS rDNA. After sequencing, data were collected as follows: (1) The two short Illumina readings were assembled by PEAR (v0.9.6) software according to the overlap and fastq files were processed to generate individual fasta and qual files, which could then be analyzed by standard methods. (2) Sequences containing ambiguous bases and any longer than 480 base pairs (bp) were dislodged and those with a maximum homopolymer length of 6 bp were allowed [2]. And sequence short than 200bp were removed. (3) All identical sequences were merged into one.(4)Sequences were aligned according to a customized reference database. (5) The completeness of the index and the adaptor was checked and removed all of the index and the adaptor sequence. (6) Noise was removed using the Pre.cluster tool. Chimeras were detected by using Chimera UCHIME. For taxonomic analysis, the 16S rRNA gene sequences of each sample would be submitted to the RDP Classifier to assign archaeal and bacterial taxonomy to 97%. Shannon index was calculated using mothur. For functional analysis, PICRUSt [3] and KEGG (Kyoto Encyclopedia of Genes and Genomes) database [4] were used to generate a list of functional genes predicted to be present in the sample and to organize these genes into gene pathways. Finally, all effective sequences without primers were submitted for downstream analysis [5].

References

[1] S. Bao, Soil and Agricultural Chemistry Analysis, China Agriculture Press, 2000.

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[3] M.G.I. Langille, J. Zaneveld, J.G. Caporaso, D. McDonald, D. Knights, J.A. Reyes, J.C. Clemente, D.E.

Burkepile, R.L. Vega Thurber, R. Knight, R.G. Beiko, C. Huttenhower, Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences, Nat. Biotechnol. 31 (2013) 814–821. https://doi.org/10.1038/nbt.2676.

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[5] A. Rapin, C. Pattaroni, B.J. Marsland, N.L. Harris, Microbiota Analysis Using an Illumina MiSeq Platform to Sequence 16S rRNA Genes, Curr. Protoc. Mouse Biol. 7 (2017) 100–129. https://doi.org/10.1002/cpmo.29.

Supplementary Information:

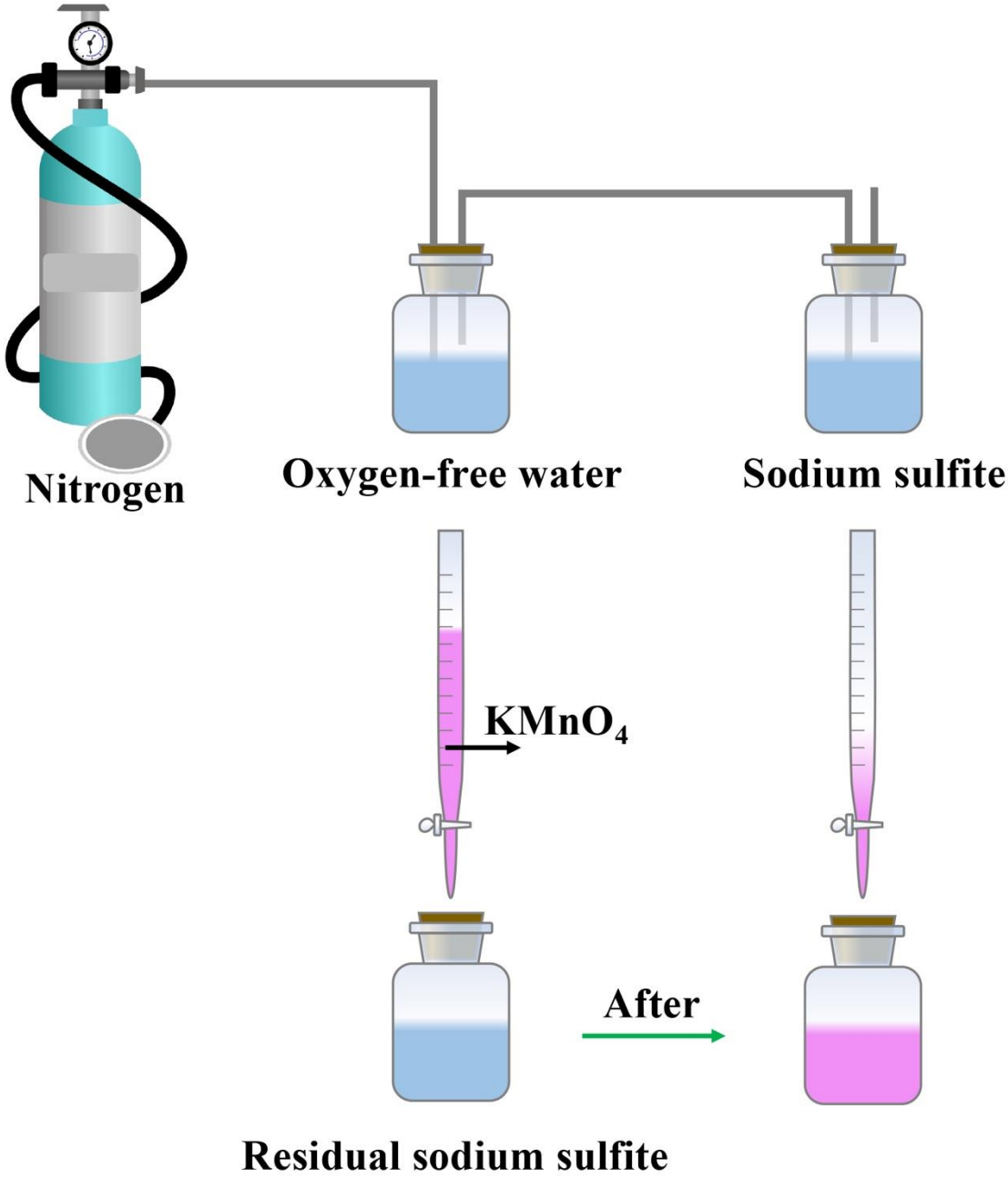

Fig. S1 The process of oxygen loaded to biochar and hydrochar. 1) preparing the oxygen-free water by filling the N_2 to Milli-Q water to remove the O_2; 2) placing the ONBC in the oxygen-free water under N_2 aeration to remove O_2 in ONBC; 3) the sodium sulfite and acidic potassium permanganate acidized by H_2SO_4 was applied to adsorb the oxygen and then titrated using sodium sulfite (**Fig. S1**). The oxygen content in ONBC was calculated by following chemical equation:

1) $2Na_2SO_3 + O_2 = 2Na_2SO_4$
2) $2KMnO_4 + 3Na_2SO_3 + H_2O = 3Na_2SO_4 + 2MnO_2 + 2KOH$

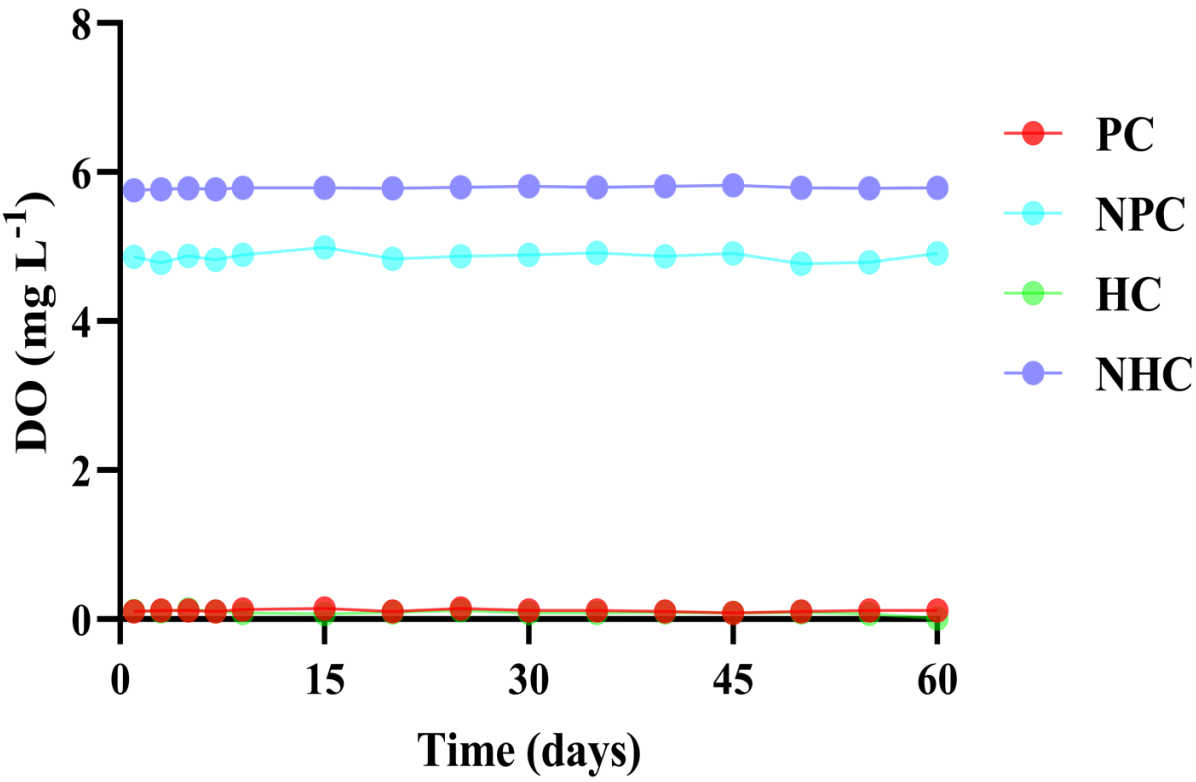

Fig. S2 The dynamics of DO in the water under different treatments for 90 days. PC: pyrochar; NPC: the pyrochar loaded with oxygen nanobubble; HC: hydrochar; NHC: the hydrochar loaded with oxygen nanobubble

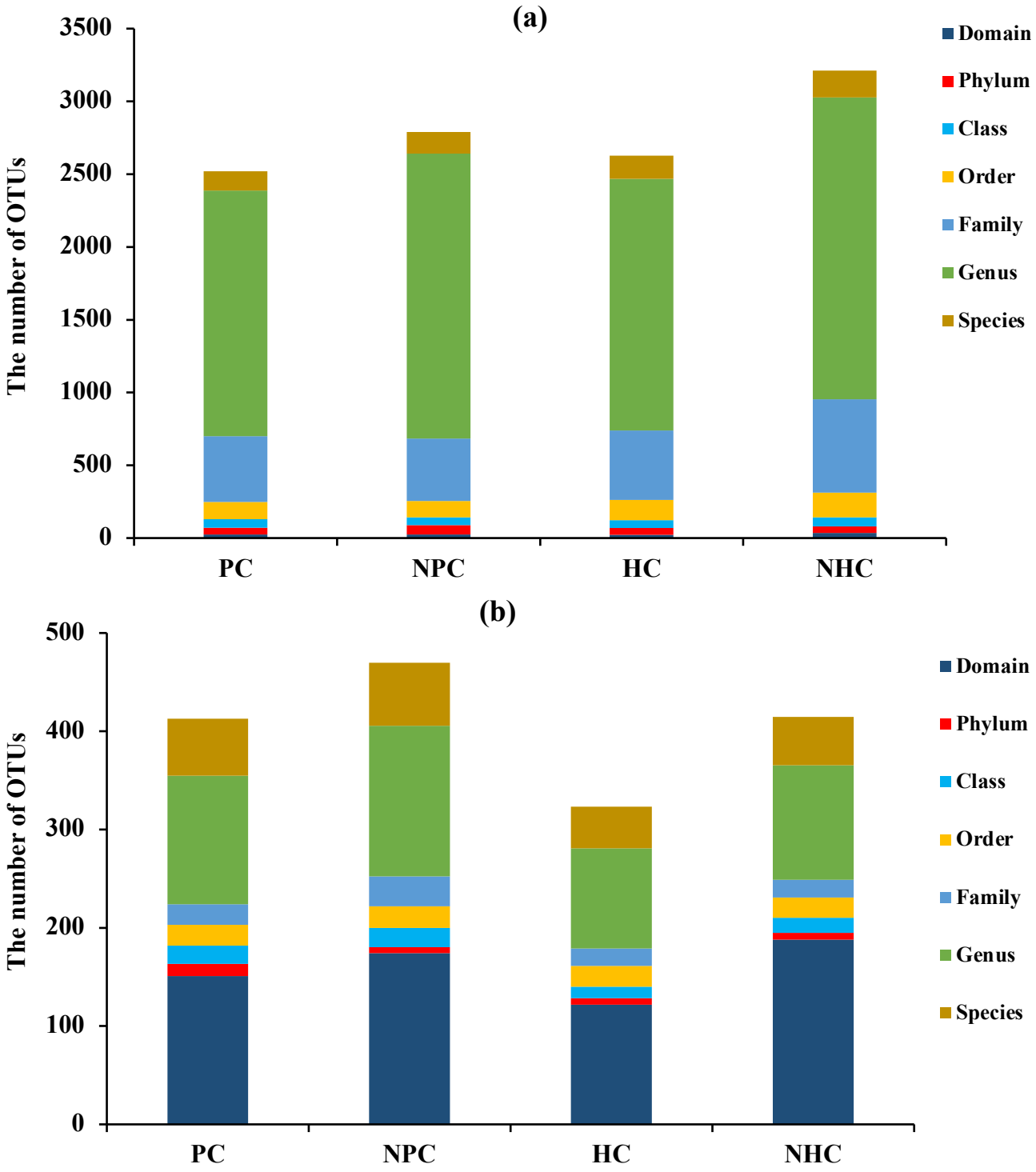

Fig. S3 The OTUs (operational taxonomic units) at the level of domain, phylum, class, order, family, genus and species for bacteria (a) and fungi (b) based on high-throughput sequencing at 3% sequence dissimilarity level in different treatments. PC: pyrochar (without oxygen nanobubbles); NPC: oxygen-nanobubbles-loaded-pyrochars; HC: hydrochar (without oxygen nanobubbles); NHC: t oxygen-nanobubbles-loaded-hydrochars.

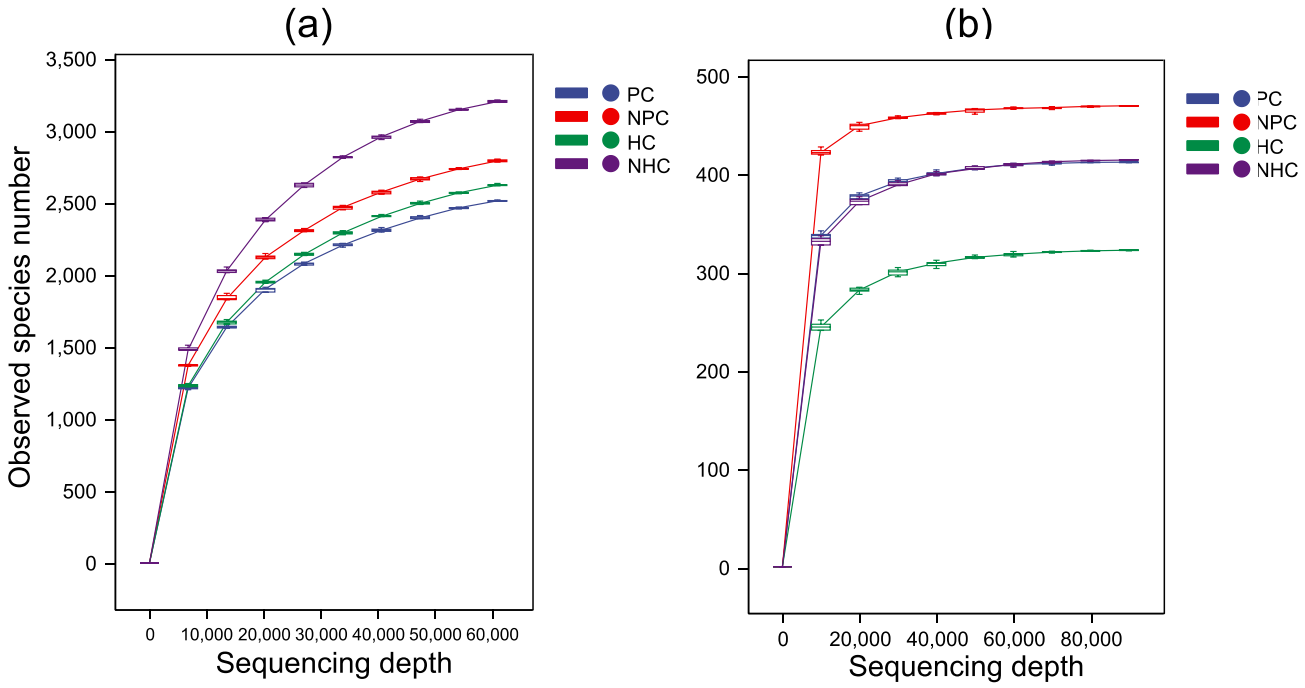

Fig. S4 Rarefaction analyses of bacterial (a) and fungi (b) in soils sequences following with different treatments. PC: pyrochar (without oxygen nanobubbles); NPC: oxygen-nanobubbles-loaded-pyrochars; HC: hydrochar (without oxygen nanobubbles); NHC: t oxygen-nanobubbles-loaded-hydrochars.

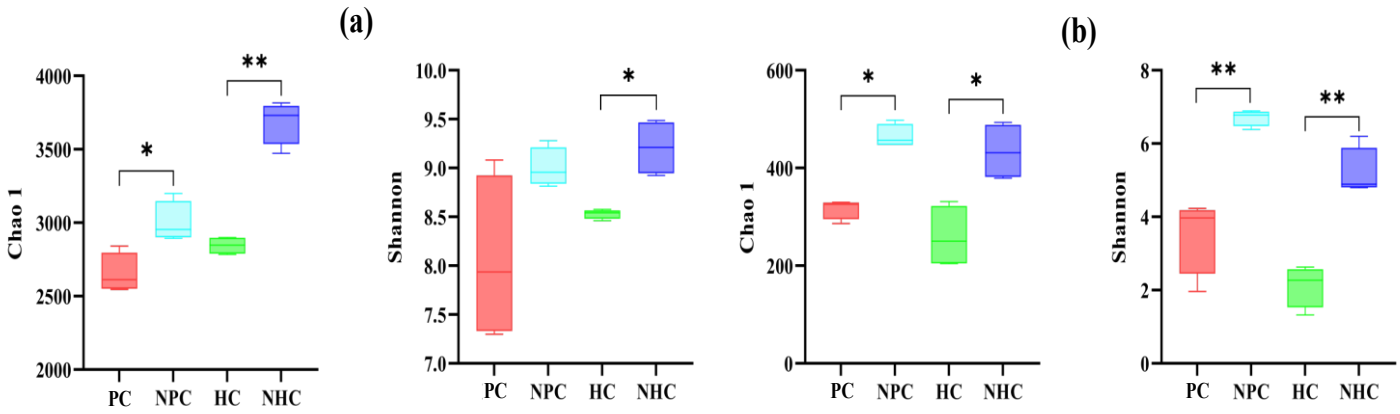

Fig. S5 Effects of oxygen nanobubble-loaded biochar application on diversity indices of bacterial (a) and fungal species (b). Mean values with standard errors in the error bars are shown (n = 4). The symbol "**" indicate significant difference between the different treatments at $P < 0.01$ and "*" indicate at $P < 0.05$.

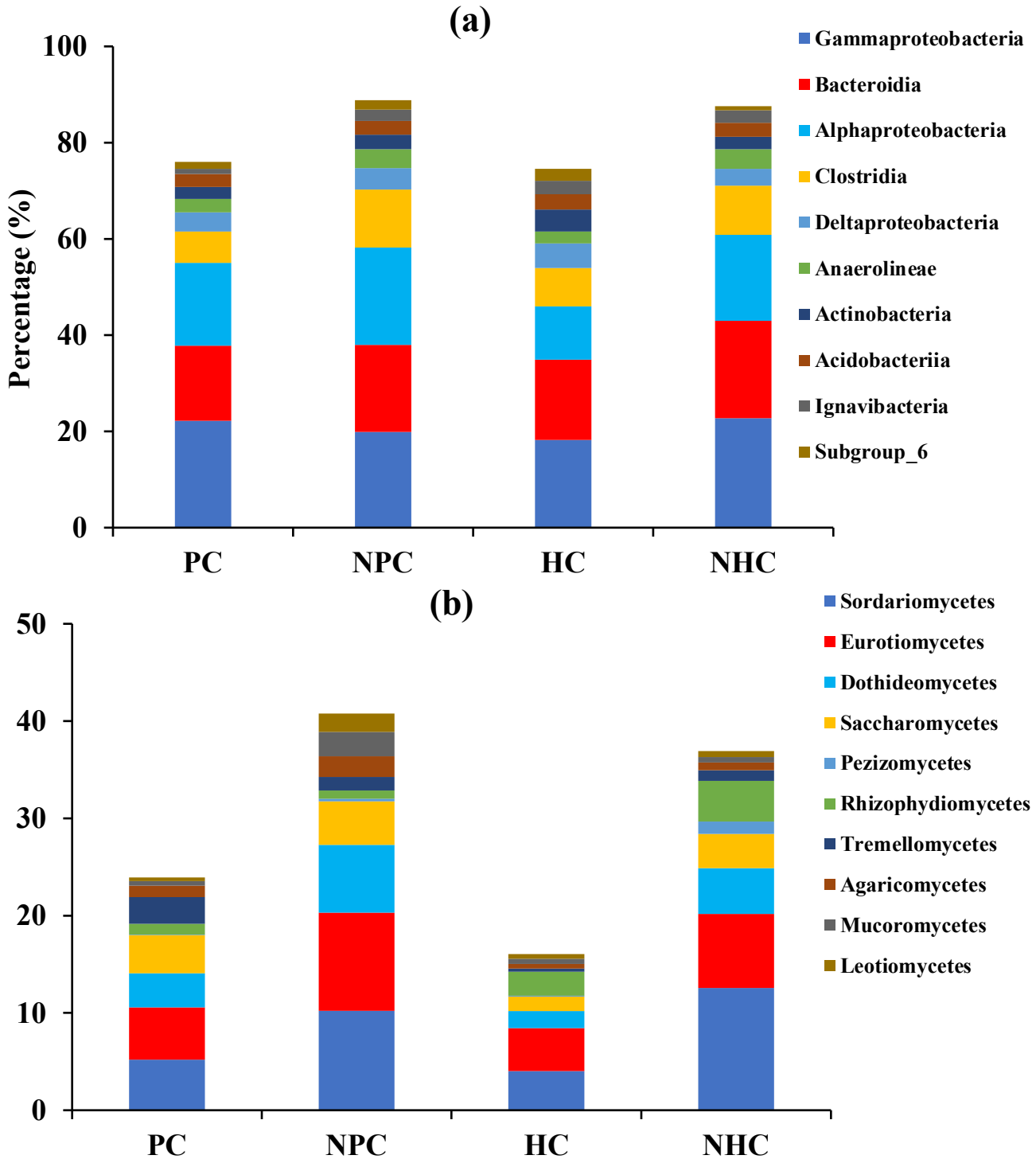

Fig. S6 Taxonomic classification of soil bacterial (a) and fungal (b) reads retrieved from different samples at class level.

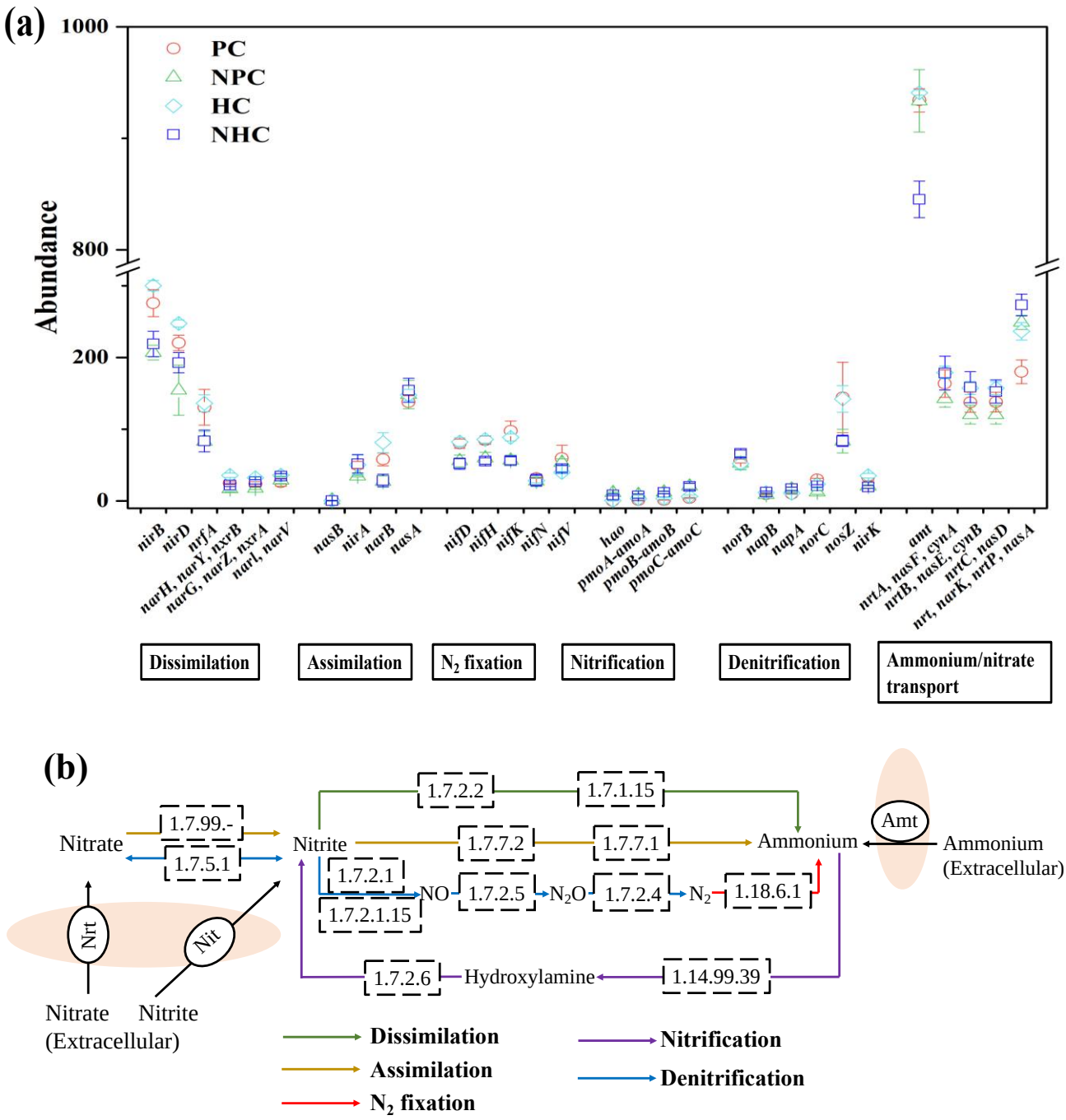

Fig S7. Effects of oxygen nanobubble-loaded biochar application on the genes of soil bacteria involved in soil N metabolism pathways (a). (b) is bacterial N-metabolism pathways. The different gene systems involved in N metabolism are boxed. N dissimilation includes EC 1.7.2.2, nitrite reductase (*nrfA*), and EC 1.7.1.15, nitrite reductase (*nirB*/*nirD*); N assimilation includes EC 1.7.99.-, assimilatory nitrate reductase (*nasA*/*nasB*), EC 1.7.7.1, ferredoxin-nitrite reductase (*nirA*) and EC 1.7.7.2, ferredoxin-nitrate reductase (*narB*); N_2 fixation includes EC 1.18.6.1 nitrogenase molybdenum-iron protein (*nifD*/*nifH*/*nifK*/*nifE*/*nifN*); nitrification includes EC 1.14.99.39, ammonia monooxygenase (*pmoA-amoA*/ *pmoB-amoB*/ *pmoC-amoC*) and EC 1.7.2.6, hydroxylamine dehydrogenase (*hao*);

denitrification includes EC 1.7.2.1 and EC 1.7.1.15, nitrite reductase (*nirK*, *nirB*, and *nirD*), EC 1.7.2.5, nitric oxide reductase (*norB*), EC 1.7.2.4, nitrous-oxide reductase (*nosZ*), and EC 1.7.5.1, nitrite reductase (*narG*/*narZ*/*narA*; *narH*, *narY*, *nxrB*); N transport includes *amt* (ammonium transporter), NO_3^-/NO_2^- transport substrate-binding protein (nirtA/nasF/cynA), permease protein (*nrtB*/*nasE*/*cynB*), ATP-binding protein (*nrtC*/*nasD*), and NO_3^-/NO_2^- transporter (*nrt*/*nark*/*natP*/*nasA*/*mfs*).

Table S1. Physiochemical characteristics of pyrochar and hydrochar.

Treatments	pH	C (%)	H (%)	O (%)	N (%)	S (%)	SSA (m^2 g^{-1})
Pyrochar	8.7	41.7	7.8	23.5	1.2	0.9	36.4
Hydrochar	6.4	64.2	4.9	18.6	1.8	0.4	10.1

The pH was determined by a pH meter using a solid/Milli-Q water ratio of 1:2.5 (w/v). The total elements concentration was measured by an Elemental Analyzer (EL III; Elementar Analysensysteme GmbH, Germany). The SSA (specific surface area), pore volume, and pore diameter were measured were measured using a NOVA 1200 analyzer, and the parameters were calculated using the Brunauer–Emmett–Teller (BET) method

Table S2. Sequential extraction procedure for Cu speciation analysis in paddy soil samples.

Fraction	Phase	Reagent	Extraction parameters	Soil/solution
EXC	Exchangeable	1 M $MgCl_2$	1 h shaking, 25 °C	1:8
CAR	Bound to carbonates	1 M NaAc	8 h shaking, 25 °C	1:8
HOX	Bound to iron (Fe) and manganese (Mn) (hydr)oxides	0.04 M $NH_2OH{\cdot}HCl$	4 h in water bath, 96 °C	1:20
OM	Bound to organic matter	0.02 M HNO_3 and 30% H_2O_2 (pH = 2)	2 h in a water bath at 85 °C	1:8
Residual	Residual	HNO_3 + HCl	Microwave digestion	1: 50