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RESEARCH ARTICLE

Multidimensional effects and mechanisms of micro-nano bubbles in regulating waterlogged soil microenvironments

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SUPPLEMENTAL MATERIALS

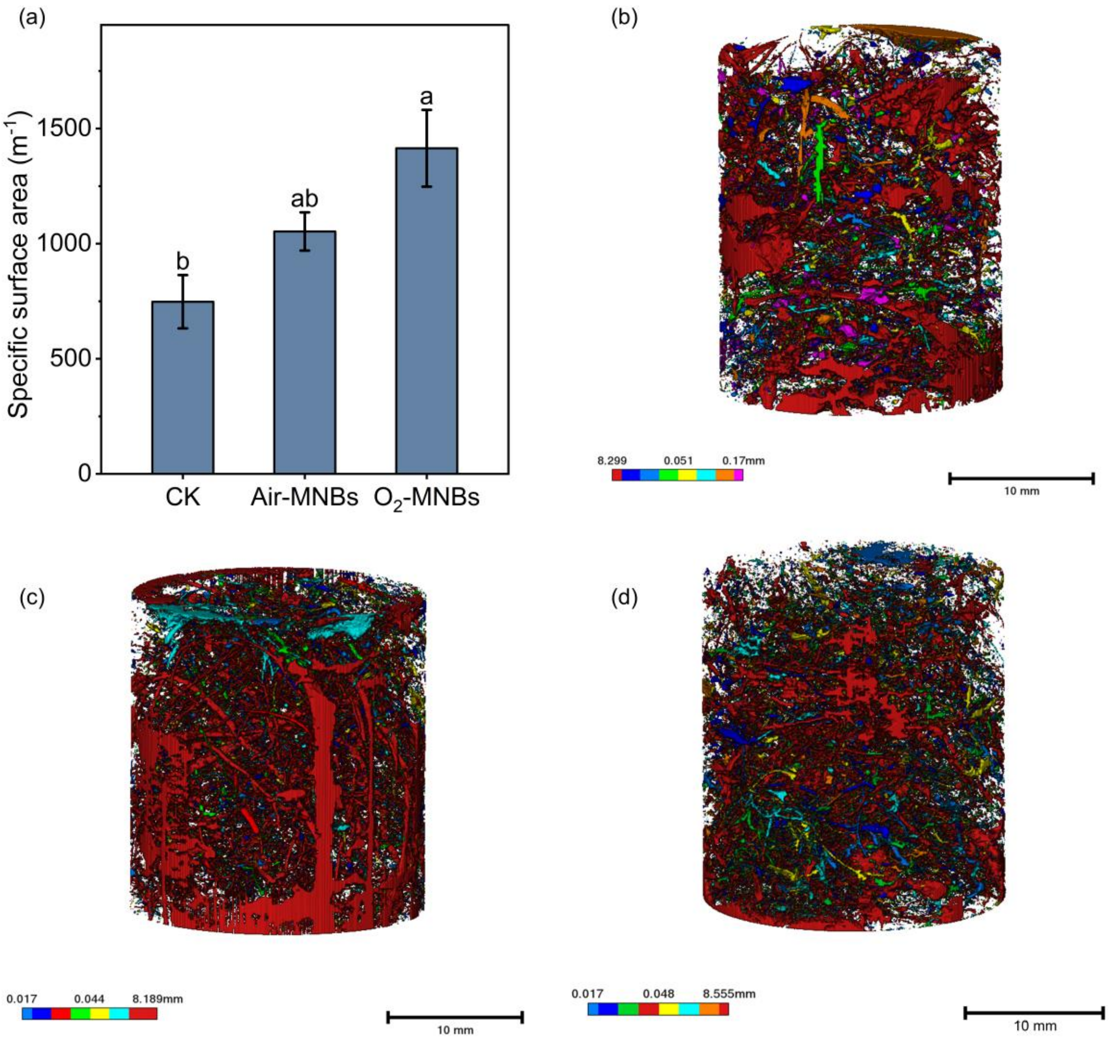

Fig. S1 Specific surface area and three-dimensional images of soil pores. (a) Specific surface area of soil pores in three treatments (standard error, *n* = 3). (b–d) Three-dimensional images of soil pores in CK, Air-MNBs and O_2-MNBs treatments, respectively. The color bar from blue to red represents the pore diameter (mm) from small to large.

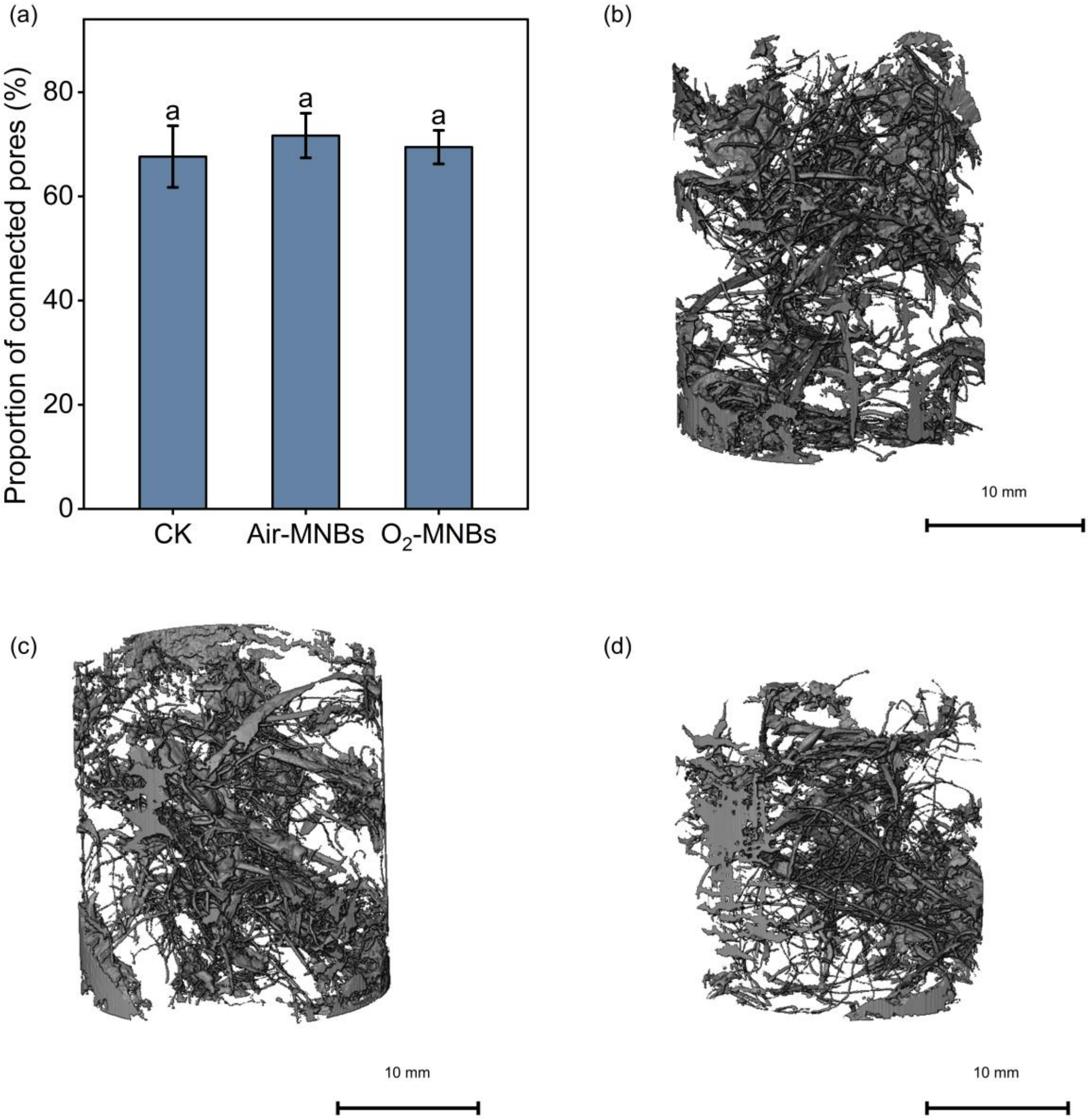

Fig. S2 Proportion and three-dimensional images of soil connected pores. (a) Proportion of connected pores in three treatments (standard error, *n* = 3). (b–d) Three-dimensional images of connected pores in CK, Air-MNBs and O$_2$-MNBs treatments, respectively.

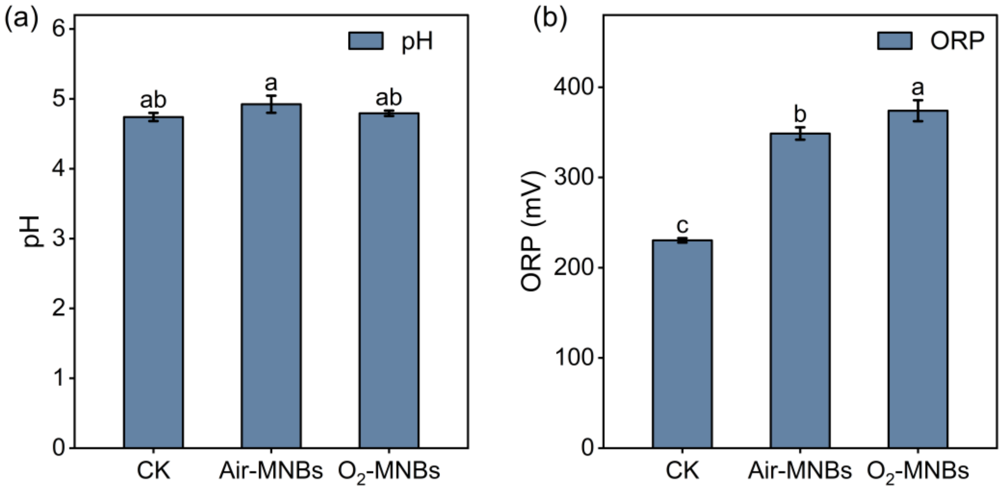

Fig. S3 Soil pH (a) and oxidation-reduction potential (b).

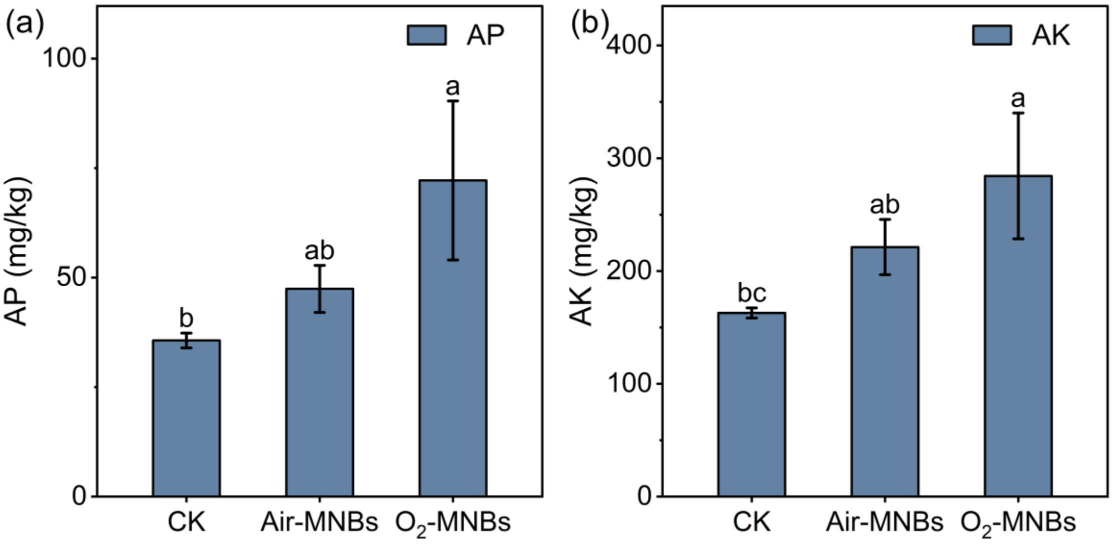

Fig. S4 Soil available phosphorus (a) and available potassium (b).

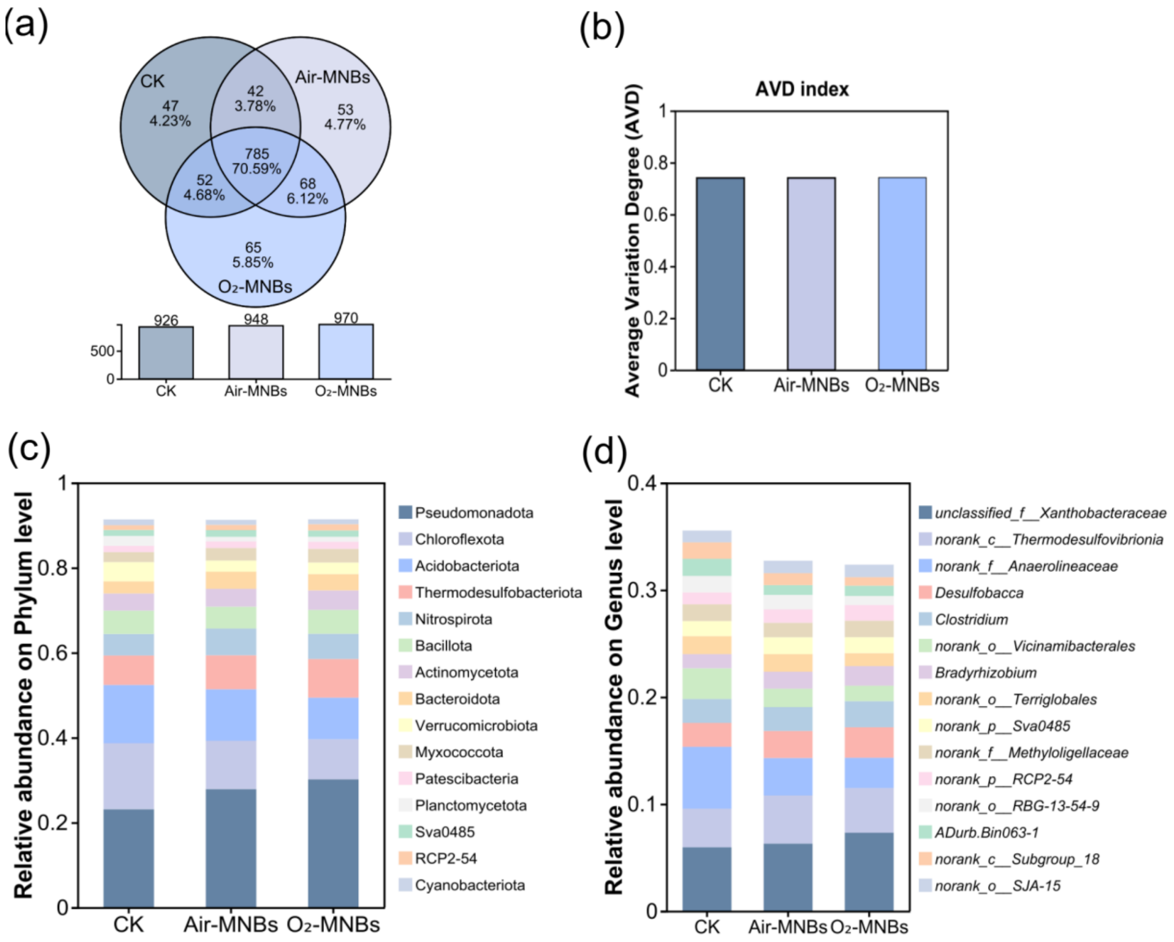

Fig. S5 Microbial composition of the soil in different treatments. (a) Venn diagrams demonstrated the overlaps among different treatments on genus level. (b) Average Variation Degree (AVD) demonstrated soil microbial community stability. AVD is calculated using the deviation degree from the mean of the normally distributed OTU relative abundance. Lower AVD value indicates higher microbiome stability. (c) Relative abundance of main microorganisms at phylum level. (d) Relative abundance of main microorganisms at genus level.

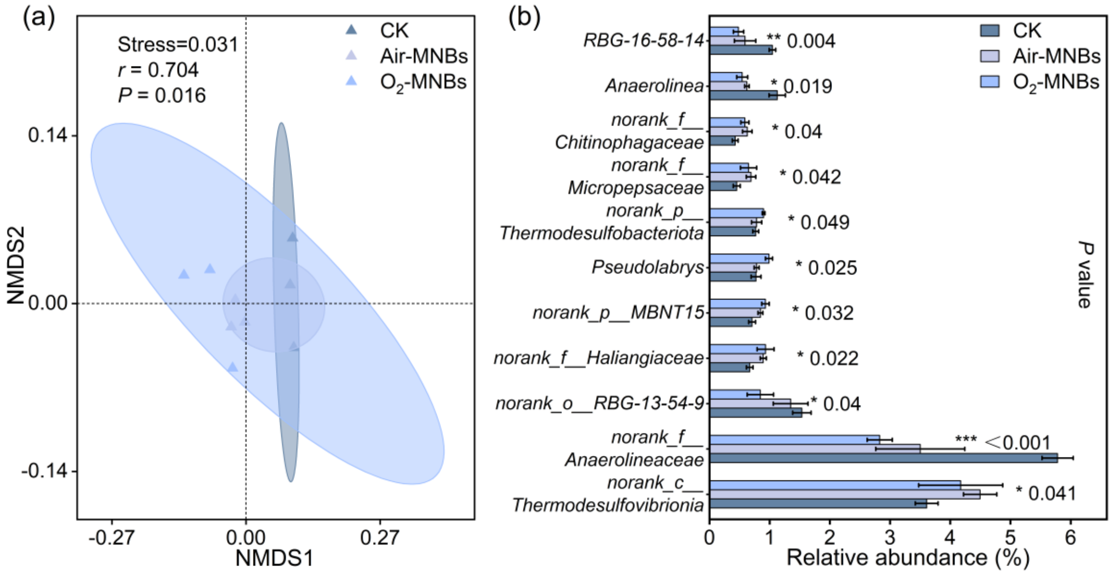

Fig. S6 Comparison of genus profile in different treatments. (a) Dissimilarities of microbial community represented by NMDS ordination plot. The closer two points are, the more similar their microbial composition; stress values represent the discrepancy between the original high-dimensional data and the reduced-dimensional space in NMDS. The group dissimilarity (*r*) and significance (*P*) values of the calculations are presented. (b) Differential abundance of genera in the three treatments. * P <0.05; ** P <0.01; *** P <0.001.

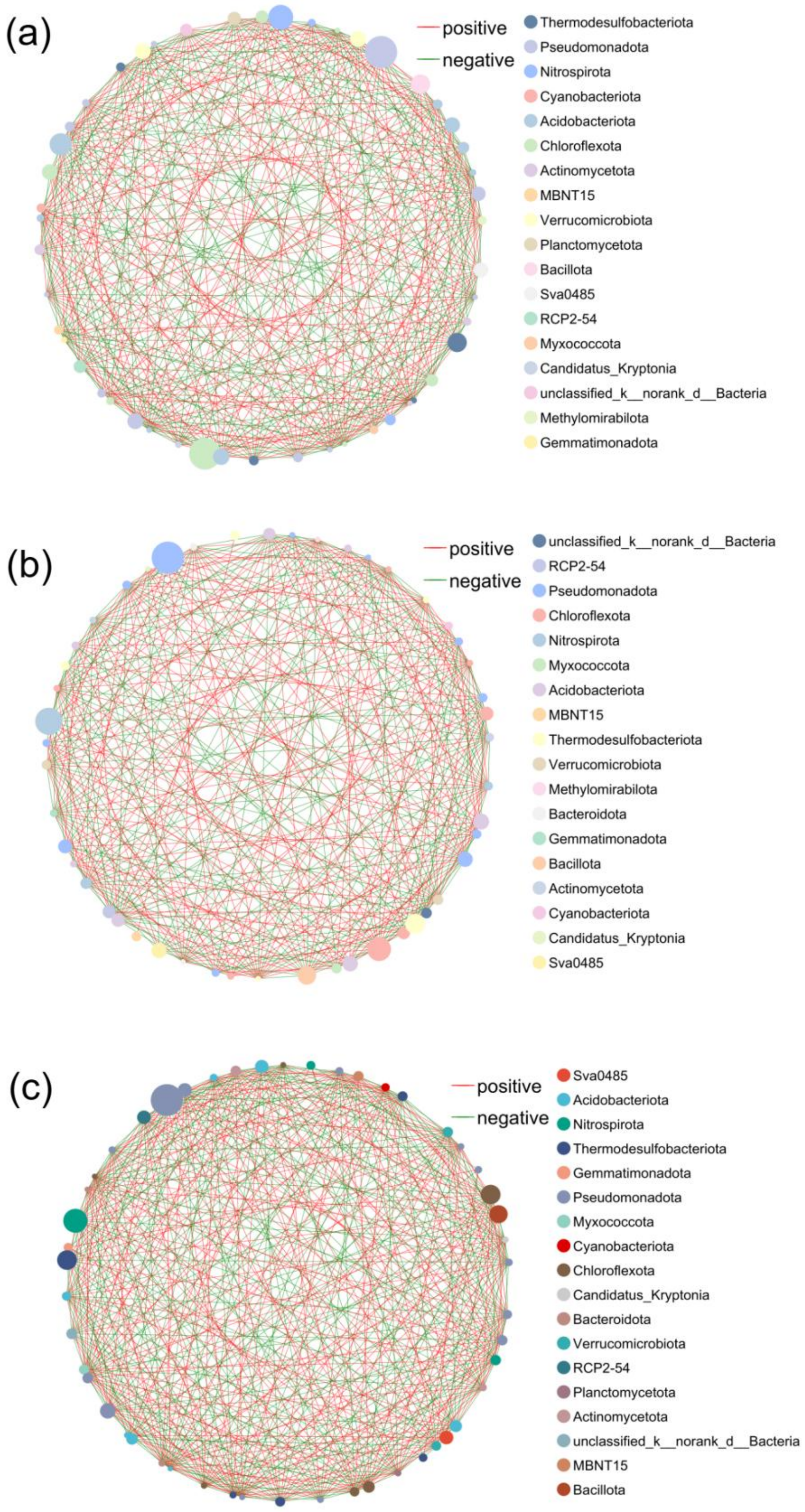

Fig. S7 Microbial community networks at the genus level. (a–c) The microbial community networks of CK, Air-MNBs and O_2-MNBs treatments, respectively.

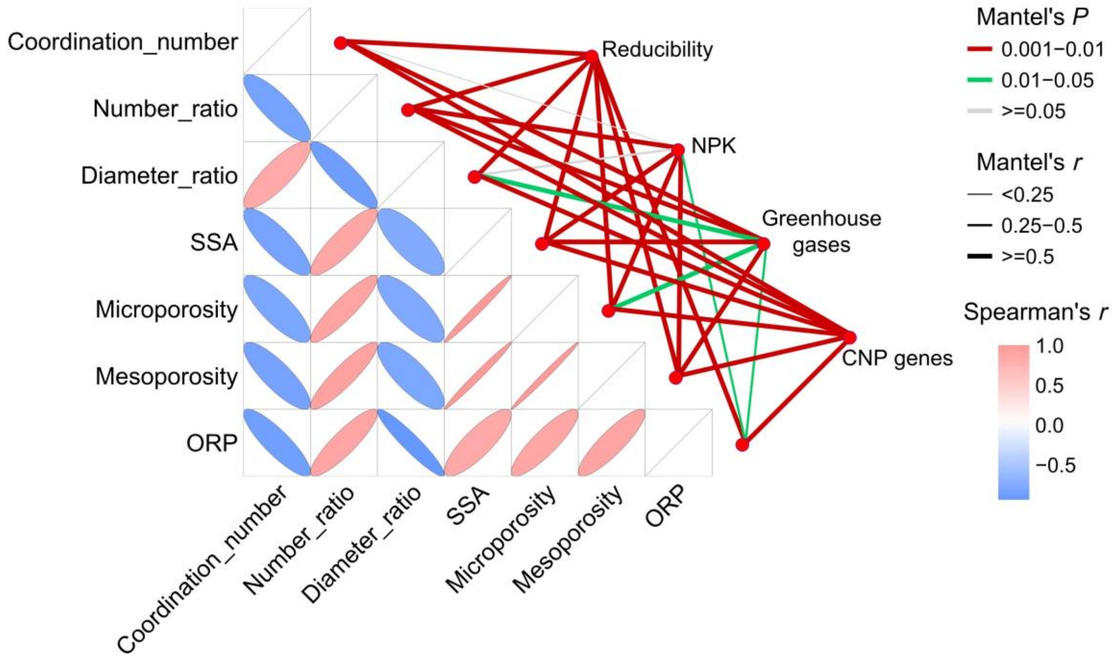

Fig. S8 Mantel test and Spearman correlation analysis between pore properties, ORP and soil biochemical properties. Coordination number: coordination number of pores and throats; Number ratio: number ratio of pores and throats; Diameter ratio: average diameter ratio of pores and throats; SSA: specific surface area of soil pores; Microporosity: porosity of pores with diameter <100 μm; Mesoporosity: porosity of pores with diameter 100–200 μm; ORP: oxidation-reduction potential; Reducibility: total reducing substances (TRS), ferrous content (Ferrous); NPK: total nitrogen (TN), alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK); Greenhouse gases: the emission flux of CO_2, CH_4, N_2O, global warming potential (GWP); CNP genes: absolute abundance of carbon, nitrogen and phosphorus cycle genes.

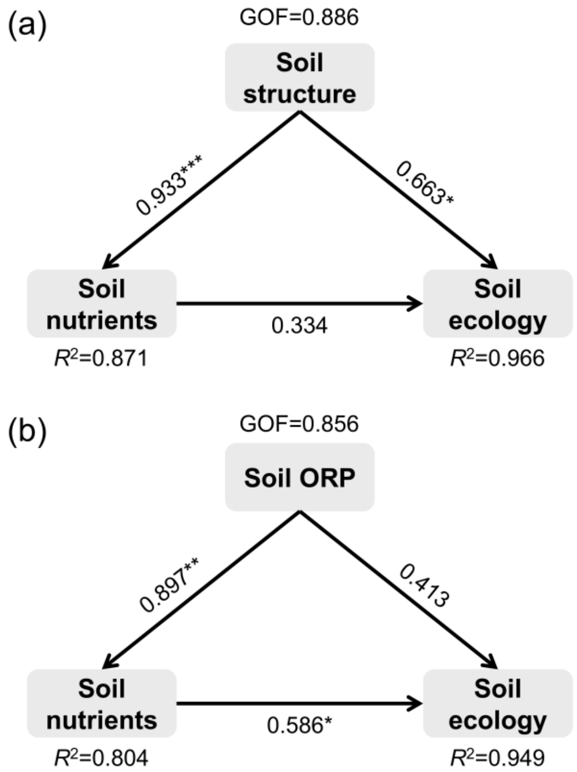

Fig. S9 Partial Least Squares Path Modeling (PLS-PM) between the soil properties and soil ecology. (a) Paths of soil structure, soil nutrients and soil ecology. (b) Paths of soil oxidation-reduction potential (ORP), soil nutrients and soil ecology. The soil structure included coordination number of pores and throats, average diameter ratio of pores and throats, specific surface area of soil pores, porosity of pores with diameter < 100 μm and porosity of pores with diameter 100–200 μm; The soil nutrients included total reducing substances, ferrous content, total nitrogen, alkali-hydrolyzable nitrogen, available phosphorus and available potassium; The soil ecology included absolute abundance of carbon, nitrogen and phosphorus cycle genes and global warming potential. Goodness-of-fit (GOF) is used to measure the effectiveness of the model fit. The number adjacent to the arrow is the path coefficient, which is the direct normalization effect size of the relationship. R^2 represents the proportion of variance explained by all predictor variables. The significance levels of each predictive factor are *: P <0.05; **: P <0.01; ***: P <0.001, respectively.

Table S1 Bubble size distribution of MNBs

Volume (%)	Pore size (μm)					
	< 2	2–50	50–100	100–200	200–500	> 500
Air-MNBs	3.3	7.9	15.6	17.7	42.5	13.0
O_2-MNBs	5.4	10.2	15.9	16.7	39	12.8

Table S2 Description of the C, N, P, S cycling genes analyzed

NO.	Gene name	Forward primer	Reverse primer	Class	Function
1	16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG	-	-
2	*abfA*	CGSTAYCCSGGCGGCAAYTT	TGCCASGGNCCGTCCATYTC	C degradation	arabinofuranosidase
3	*amyA*	YGGTTTTCGTCTTGACGCSG	MGGCTGMGTRTCATGRTTK		Alpha amylase
4	*amyX*	TATAAYTGGGGMTATGAYCC	CCCATYAAATCAAAWCGRAA		AmyX/pullulanase
5	*apu*	ACVTGGATAGGYGAGCCYCA	CCRTCSGGGAAGTAGTTKCC		Amylopullulanase
6	*cdh*	ATWRYCTWCCGMRTHGCCMT	GTKAGSGGRTTBYKGRYCAT		Cellobiose dehydrogenase
7	*cex*	YSTACGGSATGCACTGGMT	TANCGCAGRTAGTCVCCCAT		exoglucanase
8	*chiA*	TSAAGAARTACGCSGACAACG	ASGTCATCAGRCCCTTSAG		endochitinase
9	*exo-chi*	GATTGGTSVCAATATGAYRG	STCCARCCACCRAYRCTRAA		exochitinase
10	*gcd*	ATCGCGTTCGGGCCGGACG	ATSAGRTTSAGCTCGTCCCA		quinoprotein glucose dehydrogenase
11	*glx*	AACCAGTCGATCATCTACGA	RTGSACGAGCTCDGGCATGG		Glyoxal oxidase
12	*iso-plu*	GTCATYTACTTYGGNCC	CGNGCSACATCNGCCCA		Isopullulanase
13	*lig*	CCGCACACACTGTTGCTGC	CGAAGGATTGCCACTCGCA		Lignin peroxidase
14	*manB*	ATGCGCGGBGTCAACCA	TCGTTGSCGATGTTGABGA		β- mannanase
15	*mnp*	MACRCCSTTCGACTCSACC	ACGTCSGAGCAGTCRAYGA		manganese peroxidase
16	*naglu*	TVAAYTGGTAYCTGAAATAY	CCRTGYAGVGCCATCCAGTC		α-N-acetylglucosaminidase
17	*pgu*	ANCATTGGTGGCCSTGGAA	TTRAYGGCRATRCARTCRTC		pectinase/polygalacturonase
18	*pox*	ACYAGTATCCATTGGCACGGT	AGATGVGARTGATACCARAA		Phenol oxidase
19	*sga*	CGSAACTGGGAYTACCGS	TCCCACAGSCCSKCGTC		glucoamylase
20	*xylA*	TGGGGBGGTCGYGAAGG	ACTTTGGCRTCRAAGTT		xylose isomerase
21	*accA*	GAAGGCTAYCGCAARGC	CCTTCMGGSGARATMAC	C fixation	acetyl-CoA carboxylase carboxyl transferase
22	*aclB*	TGGACMATGGTDGCYGGKGGT	ATAGTTKGGSCCACCTCTTC		ATP-citrate lyase beta-subunit

NO.	Gene name	Forward primer	Reverse primer	Class	Function
23	*acsA*	GATACCTGGTGGCAGACCGA	TGATCACGTCGTCGACCCGG		carbon-monoxide dehydrogenase
24	*acsB*	CTYTGYCAGTCMTTYGCBCC	CCCATAAABCCYGGDGTYTG		acetyl-CoA synthase
25	*acsE*	TCATCGGCGAACGCATCAAC	AGRCCGGCTTCSATGGC		5-methyltetrahydrofolate corrinoid/iron sulfur protein methyltransferase
26	*cdaR*	CGARATGGTGGTGCTCAA	CARCGTRTTACGATGAATA		carbohydrate diacid regulon transcriptional regulator
27	*frdA*	MTGCTGCACACSCTGTW	CCGGTSGGGTGRWACTG		fumarate reductase flavoprotein subunit
28	*korA*	GCCGGCTACCCCATCACCCC	ATGATGGGATGGTCGCCATG		2-oxoglutarate ferredoxin oxidoreductase
29	*mct*	TGGGCGCSGASGTSATMCG	TTGACSGTRTARTCSAYSGC		mesaconyl-CoA C1-C4 CoA transferase
30	*mcrA*	GGTGGTGTMGGDTTCACMCARTA	CGTTCATBGCGTAGTTVGGRTAGT		methyl-coenzyme M reductase alpha subunit
31	*pccA*	GTGMTGATCAAGGCCWC	CGSGTGTTCATYTCSAGGAA		propionyl-CoA carboxylase alpha
32	*rbcL*	AAGGAYGACGAGAACATC	TGCAGSATCATGTCRTT		rubisCO large chain
33	*smtA*	TTTCTGGCCGGBTAYGCDGC	CGGTACGGHCCGGTYTGVCC		succinyl-CoA:(S)- malate CoA transferase
34	*mmoX*	ATGGAGGCGGTCAAGGACGA	CGCTTCATGCCCTTCCACAG	Methane metabolism	methane monooxygenase component A alpha chain
35	*mxaF*	GCGGCACCAACTGGGGCTGGT	GGGCAGCATGAAGGGCTCCC		methanol dehydrogenase (cytochrome c) subunit 1
36	*pqq-mdh*	TGTTCTATGTGCCGGCCAA	CTTCCACAGTTCCTTGCC		methanol/ethanol family PQQ-dependent dehydrogenase
37	*pmoA*	GGNGACTGGGACTTCTGG	GAASGCNGAGAAGAASGC		methane/ammonia monooxygenase subunit A
38	*amoA-1*	STAATGGTCTGGCTTAGACG	GCGGCCATCCATCTGTATGT	N Cycling	ammonia monooxygenase α-subunit
39	*amoA-2*	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTT		ammonia monooxygenase α-subunit
40	*amoB*	TGGTAYGACATKAWATGG	RCGSGGCARGAACATSGG		ammonia monooxygenase β-subunit

NO.	Gene name	Forward primer	Reverse primer	Class	Function
41	*gdhA*	GCCATCGGYCCWTACAAGGG	ATGTCRCCNGCCGGAACGTC		glutamate dehydrogenase
42	*hao*	TGTCACATGGGTGTAGACCA	ACCTGGAACATACCCAT		hydroxylamine oxidoreductase
43	*hzo*	AAGACNTGYCAYTGGGGWAAA	GACATACCCATACTKGTRTANACNGT		hydrazine oxidase
44	*hzsA*	WTYGGKTATCARTATGTAG	AAABGGYGAATCATARTGGC		hydrazine synthase subunit A
45	*hzsB*	ARGGHTGGGGHAGYTGGAAG	GTYCCHACRTCATGVGTCTG		hydrazine synthase subunit B
46	*napA*	CTGGACIATGGGYTTIAACCA	CCTTCYTTYTCIACCCACAT		periplasmic nitrate reductase
47	*narG*	TAYGTSGGGCAGGARAAACTG	CGTAGAAGAAGCTGGTGCTGT		nitrate reductase alpha chain
48	*nasA*	CARCCNAAYGCNATGGG	ATNGTRTGCCAYTGRTC		assimilatory nitrate reductase catalytic subunit
49	*nifH*	AAAGGYGGWATCGGYAARTCCACCAC	TGSGCYTTGTCYTCRCGGATBGGCAT		nitrogenase iron protein
50	*nirK-1*	GGMATGGTKCCSTGGCA	GCCTCGATCAGRTTRTGGTT		nitrite reductase (NO-forming)
51	*nirK-2*	ATGGCGCCATCATGGTNYTNCC	TCGAAGGCCTCGATNARRTTRTG		nitrite reductase (NO-forming)
52	*nirK-3*	TGCACATCGCCAACGGNATGTWYGG	GGCGCGGAAGATGSHRTGRTCNAC		nitrite reductase (NO-forming)
53	*nirS-1*	GTSAACGTSAAGGARACSGG	GASTTCGGRTGSGTCTTGA		nitrite reductase (NO-forming)
54	*nirS-2*	ATCGTCAACGTCAARGARACVGG	TTCGGGTGCGTCTTSABGAASAG		nitrite reductase (NO-forming)
55	*nirS-3*	TGGAGAACGCCGGNCARGTNTGG	GATGATGTCCACGGCNACRTANGG		nitrite reductase (NO-forming)
56	*nosZ-1*	CGCRACGGCAASAAGGTSMSSGT	CAKRTGCAKSGCRTGGCAGAA		nitrous-oxide reductase
57	*nosZ-2*	CGYTGTTCMTCGACAGCCAG	CGSACCTTSTTGCCSTYGCG		nitrous-oxide reductase
58	*nxrA*	CAGACCGACGTGTGCGAAAG	TCCACAAGGAACGGAAGGTC		nitrite oxidoreductase
59	*ureC*	AAGMTSCACGAGGACTGGGG	AGRTGGTGGCASACCATSAGCAT		urease
60	*bpp*	GACGCAGCCGAYGAYCCNGCNITNTGG	CAGGSCGCANRTCIACRTTRTT	P Cycling	β-propeller phytase
61	*cphy*	GTGGACCTRCGRMARGARWCICA	GTCCGACCATTGCCTGCYTCRCARTGRAMRTGIADCCA		cystein phytase

NO.	Gene name	Forward primer	Reverse primer	Class	Function
62	*phnK*	CATCGTCGGCGAATCCGG	TGCTGCATGCCGCCGGAAAA		putative phosphonate transport system ATP-binding protein
63	*phoD*	CAGTGGGACGACCACGAGGT	GAGGCCGATCGGCATGTCG		alkaline phosphatase D
64	*phoX*	GARGAGAACWTCCACGGYTA	GATCTCGATGATRTGRCCRAAG		alkaline phosphatase/Pho regulon
65	*ppk*	GACCCGAABGTRCTBGCSAT	TTATAATTNCCSGTNCCNA		polyphosphate kinase
66	*ppx*	TGCATCTGGCGGACGGCCT	AGATCCGCCGCCAATATCA		exopolyphosphatase
67	*pqqC*	AACCGCTTCTACTACCAG	GCGAACAGCTCGGTCAG		pyrroloquinoline-quinone synthase
68	*apsA*	GGGYCTKTCCGCYATCAAYAC	ATCATGATCTGCCAGCGGCCGGA	S Cycling	adenosine-5'-phosphosulfate reductase subunit A
69	*dsrA*	ACSCACTGGAAGCACG	GGTGGAGCCGTGCATGTT		sulfite reductase alpha subunit
70	*dsrB*	CAACATCGTYCAYACCCAGGG	GTGTAGCAGTTACCGCA		sulfite reductase beta subunit
71	*soxY*	ATCGATGACAACCCCGTGCC	AGCTGGTCCATCTGCATGCCG		sulfer oxidation protein
72	*yedZ*	CTGCTGATCACGCTGGCCAT	GCGATGCAGCTTCTTCCAGCG		sulfite oxidase

Table S3 Distribution of soil pore volume in different treatments

Pore size (μm)	Pore volume percentage (%)		
	CK	Air-MNBs	O_2-MNBs
< 100	1.05±0.51b	2.42±0.17ab	3.60±0.30a
100–200	2.28±0.91b	4.55±0.44ab	6.15±0.37a
200–500	5.99±1.25a	8.71±1.24a	9.76±0.62a
> 500	90.68±2.65a	84.32±1.76ab	80.49±0.82b
Sum	100	100	100

Table S4 Distribution of soil porosity in different treatments

Pore size (μm)	Porosity		
	CK	Air-MNBs	O_2-MNBs
< 100	0.05±0.02b	0.12±0.01ab	0.20±0.03a
100–200	0.10±0.03b	0.22±0.03ab	0.34±0.04a
200–500	0.27±0.05a	0.43±0.09a	0.54±0.05a
> 500	4.23±0.50a	4.02±0.25a	4.46±0.27a
Sum	4.65±0.00a	4.78±0.00a	5.55±0.00a

Table S5 Microbial alpha diversity indices in different treatments

α-Diversity index	CK	Air-MNBs	O_2-MNBs
Shannon	5.108±0.025a	5.230±0.020a	5.205±0.064a
Simpson	0.015±0.000a	0.013±0.000a	0.014±0.001a
ACE	820.1±19.0a	845.3±6.2a	861.0±19.3a
Chao1	821.9±13.1a	839.0±2.6a	861.2±15.8a

Table S6 Main topological properties of the microbial network in different treatments

Indicator	CK	Air-MNBs	O_2-MNBs
Total links	408	368	481
Positive correlation	202 (49.51%)	192 (52.17%)	237 (49.27%)
Negative correlation	206 (50.49%)	176 (47.83%)	244 (50.73%)
Average degree	16.7	15.0	19.2
Maximal degree	20	20	23
Average clustering coefficient	1	0.959	1