Effects of biochar and its fractions on soil nitrogen forms and microbial communities under freeze-thaw conditions

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S1 DNA extraction, 16S rRNA gene amplification, and sequencing methods

Soil microbial DNA extraction was accomplished based on the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) description. The quality of extracted genomic DNA was confirmed by 1% agarose gel electrophoresis. DNA concentration and purity were determined using the NanoDrop2000 (Thermo Scientific, U.S.). PCR amplification of the V3-V4 variable region of the bacterial 16S rRNA gene was performed using the primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR products were recovered using a 2% agarose gel. Purification was performed with a DNA Gel Purification Kit (PCR Clean-Up Kit, China Yuhua) to isolate the recovered products. Quantification of the purified products was conducted using the Qubit 4.0 (Thermo Fisher Scientific, USA). Using the NEXTFLEX Rapid DNA-Seq Kit to library-prepare the PCR products. Sequencing was performed on the Illumina NextSeq 2000 platform (Illumina, San Diego,USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). To minimize the impact of sequencing depth on subsequent analysis, all sample sequences were rarefied to the minimum sequence count. The operational taxonomic unit (OTU) clustering was performed on the quality-controlled and assembled sequences using UPARSE v7.1 software (http://drive5.com/uparse/) based on 97% similarity, and chimeras were removed. Using the RDP classifier (http://rdp.cme.msu.edu/, version 2.11) to align against the Silva 16S rRNA gene database (v138) for OTU species taxonomic annotation, with a confidence threshold of 70%.

Table S1. Correlation coefficient between the cycle numbers and soil physicochemical factors.

	CTS	**FTS**	**S + PBC**	**S + DBC**	**S + UBC**
SM	-0.884**	-0.971**	-0.949**	-0.960**	-0.960**
pH	-0.926**	0.826**	-0.117	-0.291	-0.017
EC	0.676**	-0.436	-0.661**	-0.446	0.355
AP	0.666**	0.087	0.131	-0.185	0.224
AK	-0.273	-0.556*	-0.153	-0.164	-0.447
DOC	-0.556*	-0.251	-0.753**	-0.524*	-0.600*
SOC	-0.567*	-0.316	-0.578*	-0.273	-0.480
C/N	-0.535*	-0.327	-0.655**	-0.284	-0.611*
NH_4^+-N	-0.971**	-0.611*	-0.491	-0.535*	-0.578*
NO_3^--N	0.971**	0.873**	0.917**	0.917**	0.775**
IN	0.949**	0.807**	0.786**	0.720**	0.764**
MBN	-0.185	-0.982**	-0.884**	-0.949**	-0.917**
TN	-0.349	-0.104	-0.196	-0.196	-0.131

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

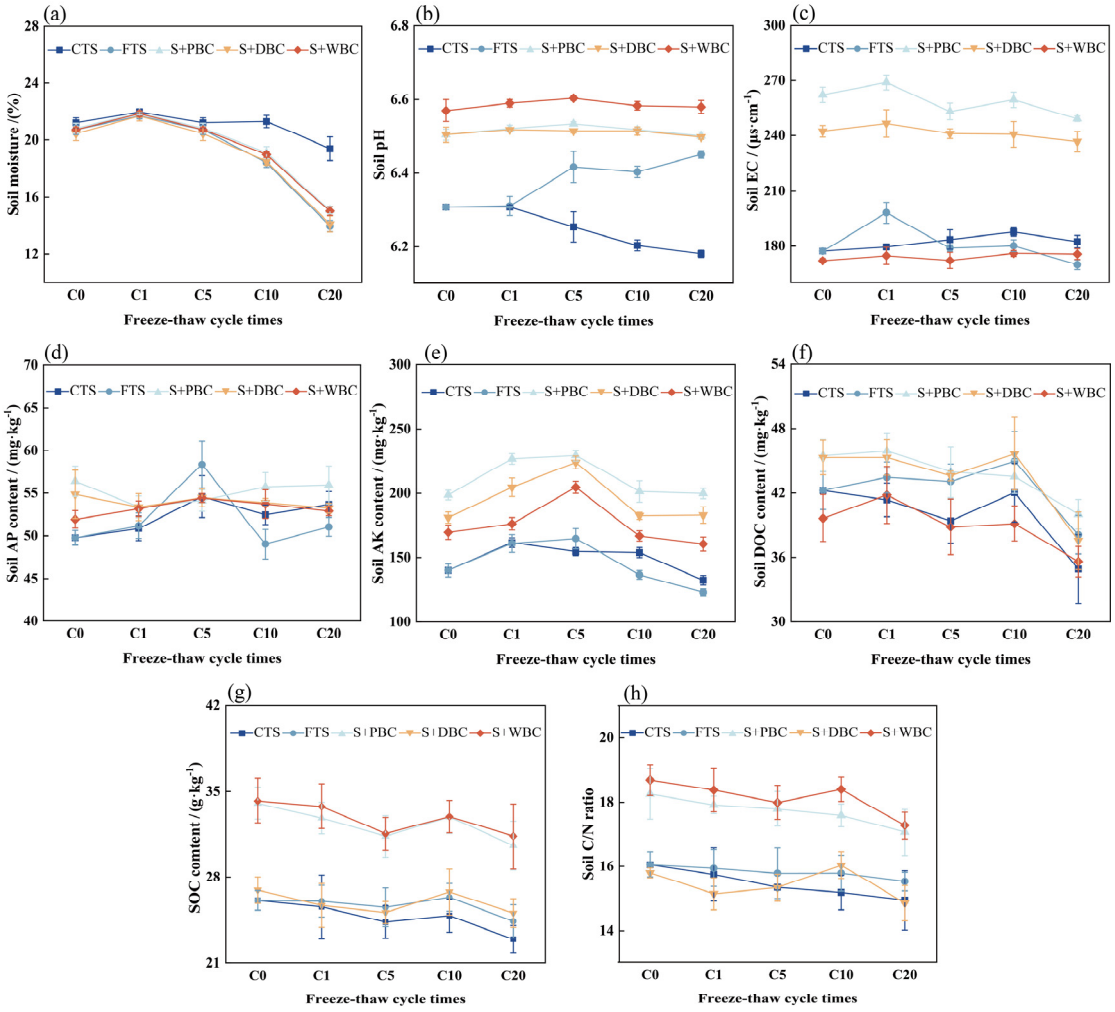


Figure S1. The changes in characteristics of soil physicochemical properties. (a) soil moisture content, (b) pH, (c) electrical conductivity (EC), (d) available phosphorus (AP) content, (e) rapidly-available potassium (AK) content, (f) dissolved organic carbon (DOC) content, (g) soil total organic carbon (SOC) con-tent, and (h) C/N ratio.

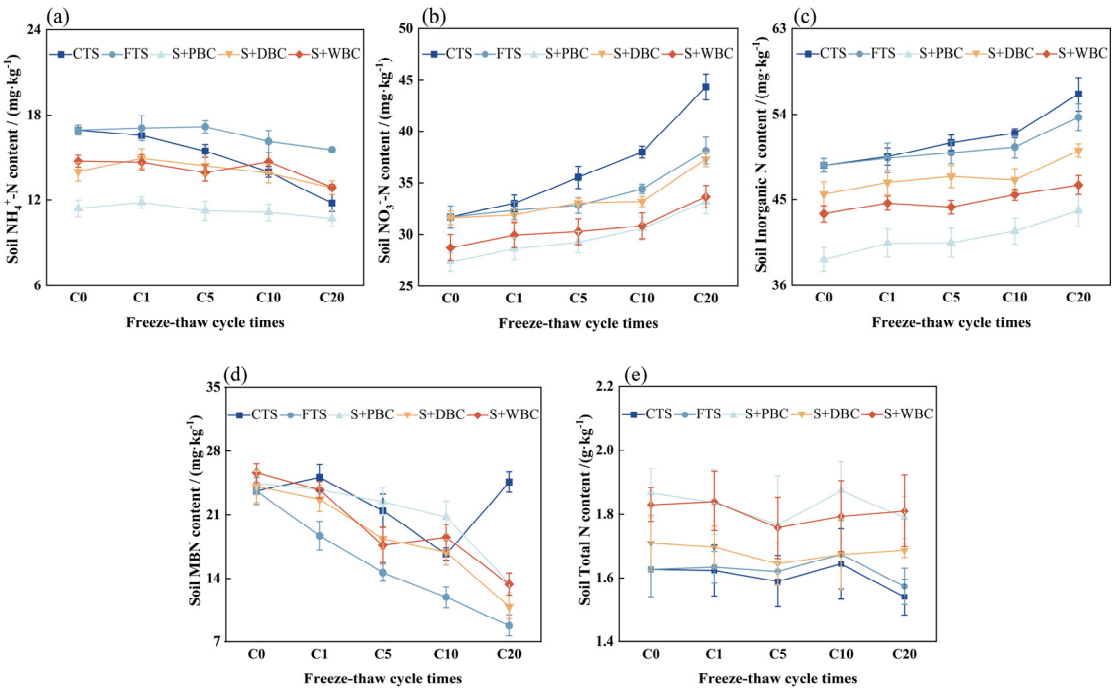


Figure S2. The changes in nitrogen form content in the soil across treatment groups. (a) ammonium nitrogen (NH_4^+-N) content, (b) nitrate nitrogen (NO_3^--N) content, (c) inorganic N content, (d) microbial biomass nitrogen (MBN) content, and (e) total N content.

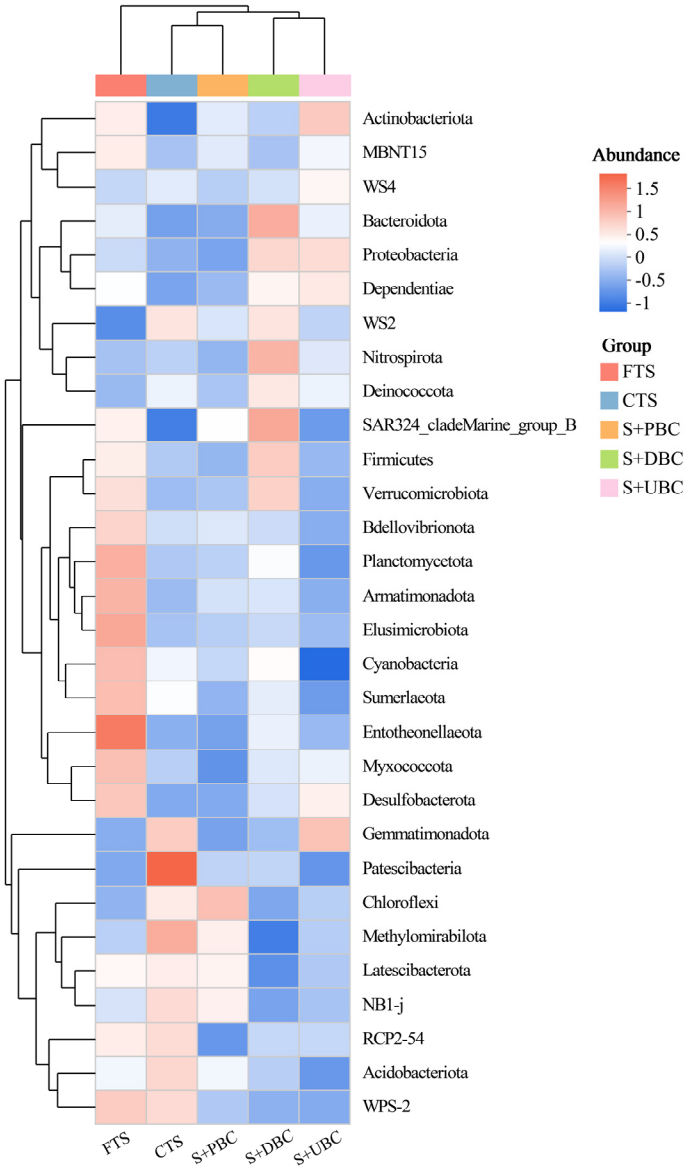


Figure S3. Clustering heatmap of community abundance at the phylum level across treatment groups.

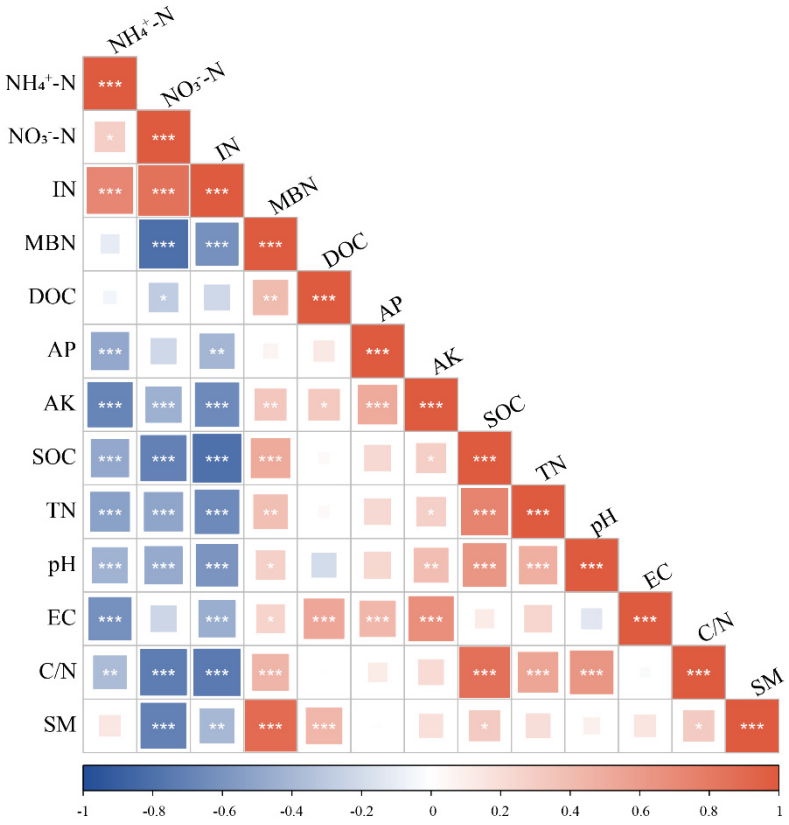


Figure S4. Heatmap of correlation between physicochemical factors across treatment groups under FTCs condition. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$