Supporting Information for

Inducing inorganic carbon accrual in subsoil through biochar application on calcareous topsoil

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(16 pages, 7 Figures, 5 Tables)

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Supplementary Text

S1. Field Experiments

The field experiment was established in 2009 with four biochar application rates: (1) unamended control (Control, no biochar addition); (2) rick husk + cotton seed hull biochar (w/w 7:3, 400 °C) at 30 t ha^{-1} (B30, including 14.7 t ha^{-1} organic carbon (OC) and 0.3 t ha^{-1} inorganic carbon (IC)); (3) the same biochar at 60 t ha^{-1} (B60, 29.5 t ha^{-1} OC and 0.6 t ha^{-1} IC); and (4) the same biochar at 90 t ha^{-1} (B90, 44.2 t ha^{-1} OC and 0.9 t ha^{-1} IC) (Table 1 and Table S1). Biochar was applied once to the surface (0-0.2 m) of calcareous farmland soil (40° 08′ 21″N, 116° 10′ 52″E). Mineral fertilizers were applied with summer maize and winter wheat. Flood irrigation was used during the winter wheat growing season (~180 mm per year). Unamended (control) and biochar-amended soils were sampled to a depth of 2 m in 2019.

S2. Spectroscopic analysis

To obtain the semi-quantitative analysis for various functional groups of the original biochar and field-aged biochar samples (collected in 2019), X-ray photoelectron spectrometry (XPS, Thermo Fisher Scientific K-Alpha, Thermo Fisher, U.S.A.) and Fourier transform infrared spectroscopy (FTIR, Spectrum Spotlight 200, PerkinElmer, U.S.A.) over a wavelength range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} were used For FTIR analysis, the soil/biochar was homogenized with potassium bromide (KBr) at a ratio of 1:100 using an agate mortar and pestle, and carefully pressed into a uniform sheet for further analysis.[1,2]

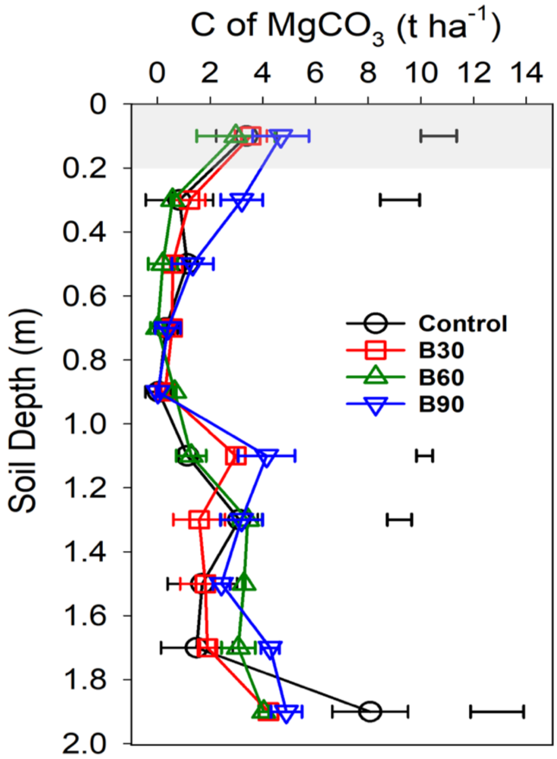

Figure S1. Effects of biochar on the carbon of magnesium carbonate (C of $MgCO_3$) through the soil profile 10 years after a single biochar application to top 20 cm soil. The shadowed area shows the depth (0-0.2 m) of biochar application. The data are the means ± standard deviation (n = 3). The error bars in c to e are the least significant differences (LSD) at a confidence level of 5%. Control, B30, B60, and B90 represent the biochar application rates of 0, 30, 60, and 90 t ha^{-1}, respectively.

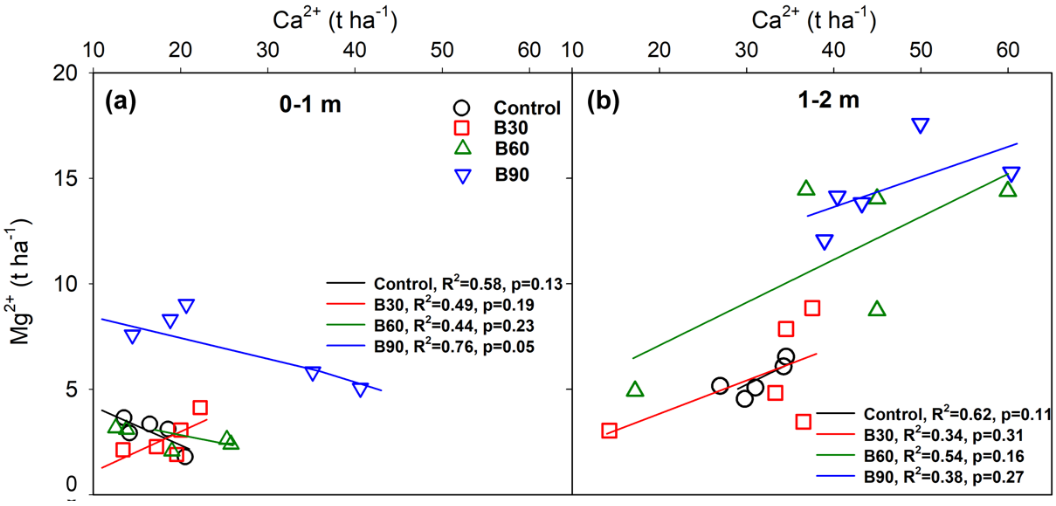

Figure S2. The ratio of soil Ca^{2+} and Mg^{2+} as a function of soil depth (a) 0-1 m and (b) 1-2 m 10 years after a single biochar application to top 20 cm soil. The lines in (a) and (b) are based on the linear regression analysis between the Ca and Mg for unamended control and biochar-amended soils.

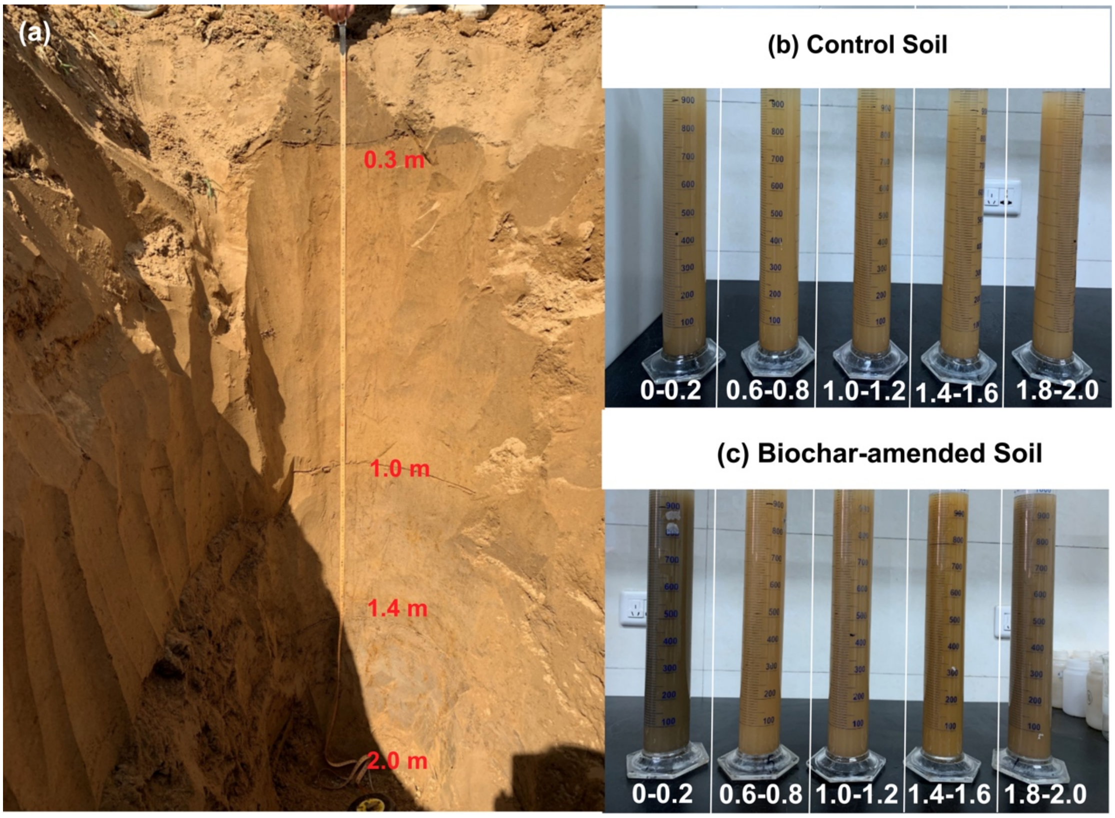

Figure S3. Effects of biochar application on the colors of soil colloidal suspensions using soils taken from various depths. (a) Biochar-amended soil profiles (60 t ha^{-1}) at depths 0-2 m. After settling for 24 hours, the soil suspensions of (b) unamended control and (c) 60 t ha^{-1} biochar-amended soil samples at various selected depths (0-0.2 m, 0.6-0.8 m, 1.0-1.2 m, 1.4-1.6 m, and 1.8-2.0 m).

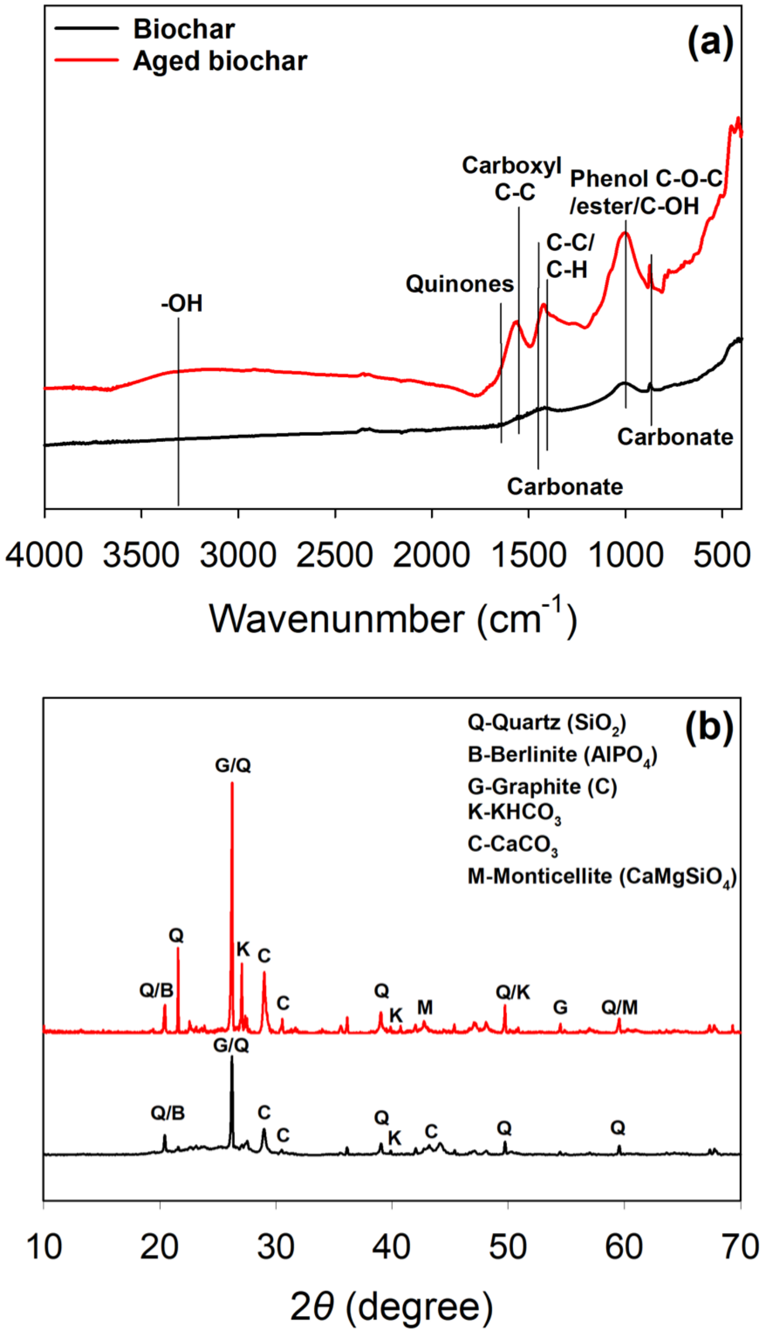

Figure S4. The surface functional groups and chemical composition of original and field-aged biochars collected from topsoil 10 years after a single biochar application to top 20 cm soil. (a) Fourier transform infrared spectra (FTIR) and (b) X-ray diffraction (XRD) patterns of original biochar (black line) and field-aged biochar (red line). The original biochar was prepared in 2009, and field-aged biochar was extracted from the biochar-amended topsoil (60 t ha^{-1}, 0-0.2 m) in 2019.

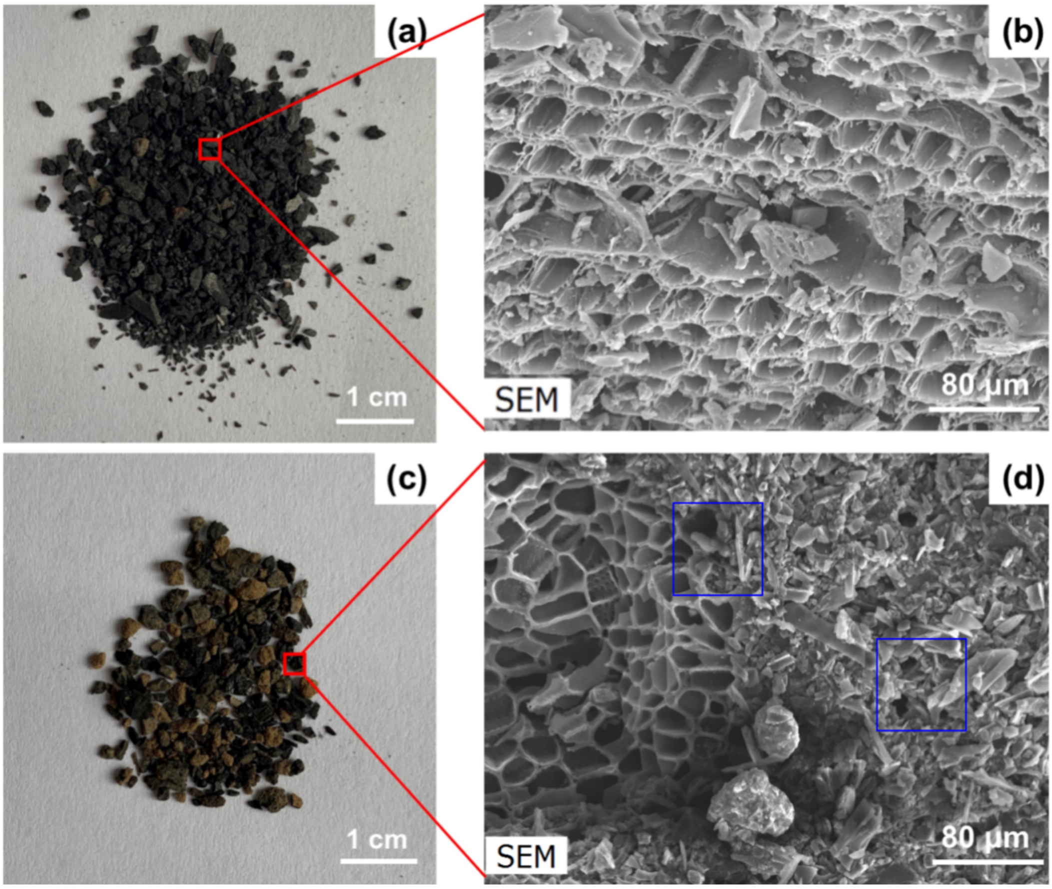

Figure S5. Images of original and field-aged biochar. (a) Photograph and (b) SEM image of original biochar. (c) Photograph and (d) SEM image of field-aged biochar particles collected from biochar-amended soil (60 t ha^{-1}) ten years after biochar application. The ruptured aged biochar was shown in the blue boxes on (d). The biochar particles obtained from the field were gently washed with ultrapure water 3 times and dried in an oven at 50 ℃.

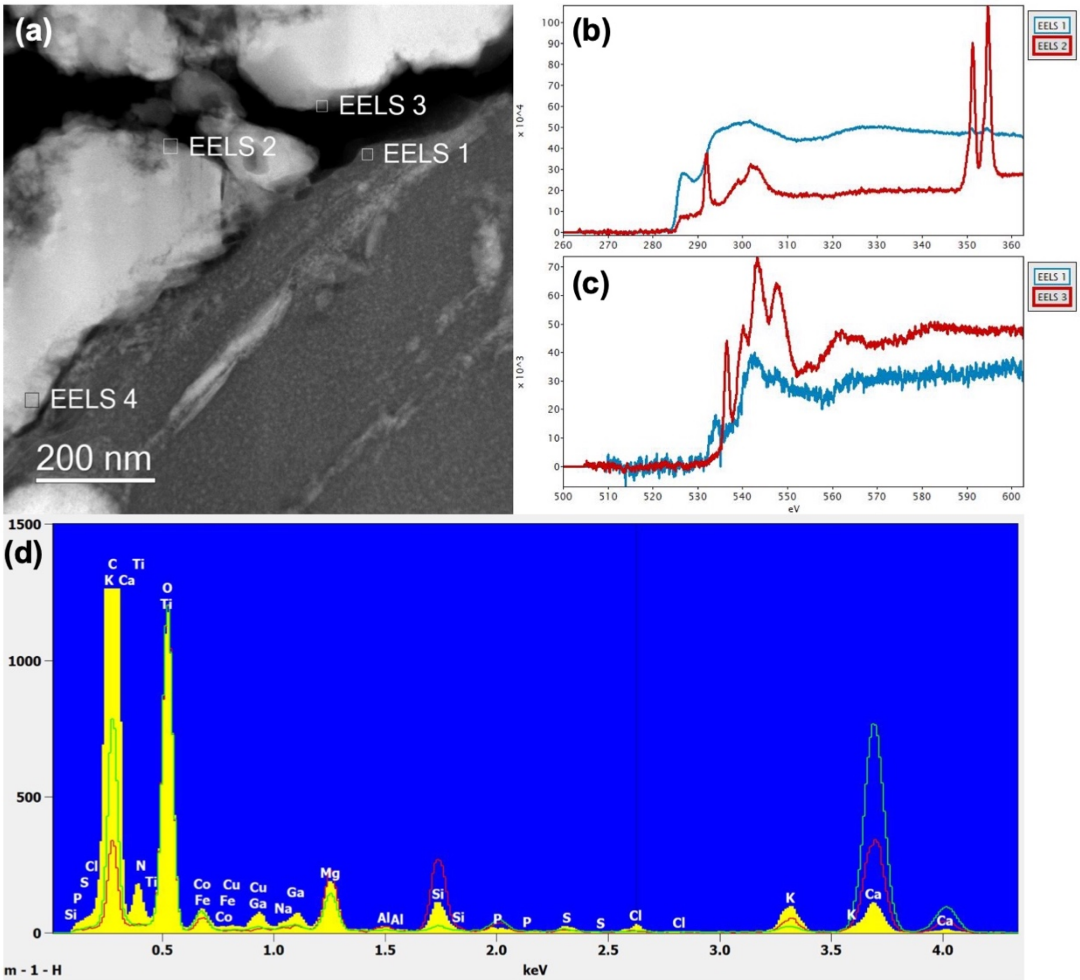

Figure S6. STEM imaging, EDS and EELS spectroscopic evidence. (a) HAADF image of the surface of the biochar (dark region, bottom right) with mineral phase (lighter color) on the surface of the biochar. (b) C K-edge EELS spectra of the biochar (EELS 1 blue) and calcium carbonate (EELS 2 red). (c) O K EELS spectra from the biochar (EELS 1 blue) and calcium carbonate (EELS 3 red). (d) Overlaid EDS spectra from regions from biochar (EELS 1 yellow) and carbonate particles (EELS 2, red and EELS 3 – cyan). The latter shows variable amounts of Mg and Ca. The sample was selected in biochar-amended soil at the depth of 1.4-1.6 m.

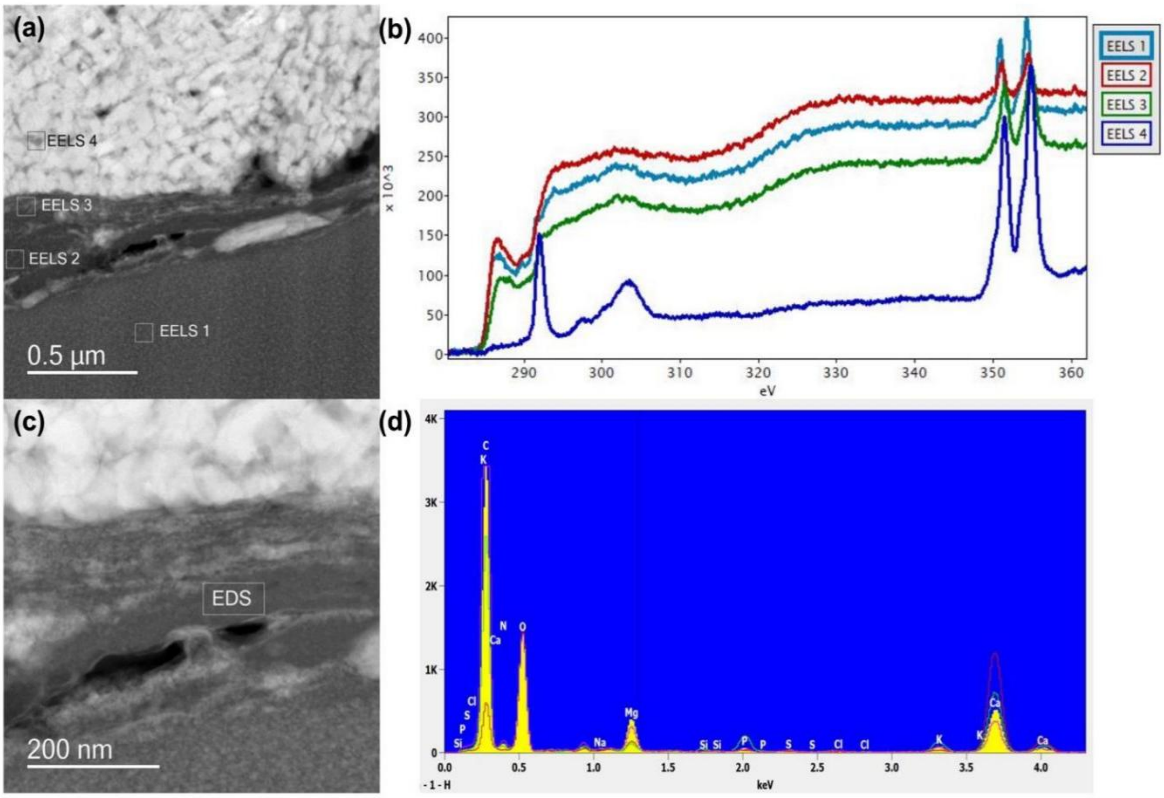

Figure S7. STEM imaging and spectroscopic evidence in biochar-amended soil at 1.4-1.6 m. (a) HAADF image of an agglomeration of mixed (Ca/Mg)CO_3 nanoparticles (bright region) adjacent to a micron-sized biochar particle coated with mineral-rich (Ca and Mg) organic compounds; (b) C K EELS spectra taken from the regions labelled in a). The EELS 4 spectrum from the carbonate shows a very different edge structure to the amorphous carbon of the biochar (EELS 1) and organo-mineral layer (EELS 2-3); (c) HAADF detail of a) showing the organo-mineral layer between the biochar (bottom) and the carbonate phase (top); (d) Overlaid EDS spectra from regions labelled in a) showing: EELS 1-yellow (typical of biochar), EELS 2-cyan (dolomite), EELS 3-green, EELS 4-red (mainly $CaCO_3$).

Table S1. The properties of the original biochar and unamended control surface soil (0-0.2 m).

Properties	Biochar	Unamended control soil
Soil Texture	—	Sandy clay loam
Bulk density (g cm^{-3})	—	1.42 ± 0.03
Water content (cm^3 cm^{-3})	—	0.10 ± 0.01
Particle Size (mm)	0.5-5	—
Organic carbon (g kg^{-1})	491 ± 3	6.28 ± 0.32
Inorganic carbon (g kg^{-1})	10.01	2.58 ± 0.18
Total nitrogen (g kg^{-1})	12.2 ± 0.8	1.75 ± 0.35
Ash content (%)	36.3 ± 0.2	—
pH	9.8 ± 0.2	8.10 ± 0.06
EC (mS cm^{-1})	1.0 ± 0.05	0.13 ± 0.27
CEC (cmol (+) kg^{-1})	12.5 ± 0.10	10.1 ± 0.13

Table S2. Bulk density, water content, soil fraction, and soil texture of the unamended control soil at 0-2 m.

Soil profile (m)	Bulk density (g cm^{-3})	Water content (g^3 g^{-3})	Soil fraction (mm)			Soil texture
			0.05-2	0.002-0.05	<0.002	
0-0.3	1.42 ± 0.03	0.10 ± 0.01	51.2	27.3	21.5	Sandy clay loam
0.3-1.0	1.56 ± 0.04	0.09 ± 0.03	23.8	44.8	31.4	Silty clay loam
1.0-1.4	1.73 ± 0.07	0.16 ± 0.01	41.2	34.6	24.2	Loam
1.4-2.0	1.57 ± 0.01	0.17 ± 0.03	15.8	62.7	21.5	Silt loam

Table S3. The size fractions and the physio-chemical properties of original biochar.

Properties	>2 mm	0.25-2 mm	0.075-0.25 mm	0.01-0.075 mm	<0.01 mm
Size fractions (wt, %)	0.09 ± 0.03	67.79 ± 2.04	20.26 ± 1.93	9.12 ± 0.91	2.31 ± 1.42
pH	9.10 ± 0.02	9.56 ± 0.04	10.08 ± 0.01	10.18 ± 0.01	10.17 ± 0.01
EC (μs cm^{-1})	432 ± 35.1	585 ± 4.4	770 ± 9.5	566 ± 1.0	1089 ± 3.2
ζ potential * (mV)	—	—	-19.8 ± 0.81	-19.7 ± 1.47	-27.9 ± 1.39

* The ζ potentials of various biochar particles sizes were measured in 1 mM NaCl solution at pH 7.

Table S4. Black carbon concentrations (mg C per g soil organic carbon) using benzene polycarboxylic acids (BPCA) method for original biochar, the unamended control soil and biochar-amended (60 t ha^{-1}) soils in the topsoil and selected subsoil (1.4-1.6 m).

Samples	Soil Depth (m)	B3CA (mg C g^{-1} OC^{-1})	B4CA (mg C g^{-1} OC^{-1})	B5CA (mg C g^{-1} OC^{-1})	B6CA (mg C g^{-1} OC^{-1})	SUM (mg C g^{-1} OC^{-1})
Original biochar	—	5.45 ± 0.35	21.09 ± 1.13	59.82 ± 0.04	144.52 ± 3.49	230.89 ± 1.97
Unamended control soil	0-0.2	1.73 ± 0.41	5.06 ± 1.08	11.90 ± 1.91	13.22 ± 0.73	31.91 ± 2.67
Biochar-amended soil	0-0.2	4.34 ± 0.29	12.16 ± 0.59	29.86 ± 1.50	53.65 ± 6.58	100.00 ± 8.96
Unamended control soil	1.4-1.6	0.64 ± 0.01	1.34 ± 0.20	1.27 ± 0.04	0.08 ± 0.02	3.33 ± 0.18
Biochar-amended soil	1.4-1.6	0.95 ± 0.06	3.19 ± 0.31	8.33 ± 0.73	10.14 ± 0.16	22.62 ± 0.94

Data are shown as means ± standard deviation (n=4)

Different lowercase letters indicate the significant differences among unamended control soil and biochar-amended soils using the least significant difference method (LSD) at a confidence level of 5% (n=4, $p<0.05$).

BPCA individual chemicals were summarized into different groups contained B3CAs (sum of trimellitic acid, hemimellitic acid and trimesic acid), B4CAs (sum of mellophanic acid, prehnitic acid and pyromellitic acid), B5CA (benzenepentacarboxylic acid), and B6CA (mellitic acid).

Table S5. XPS results of surface elemental composition and the atomic ratio of original and field-aged biochar.

		Position (eV)	Original biochar	Aged biochar
C1s	C—C/C—H/C=C	284.4	41.7%	47.7%
	C—O	286.2	—	13.3%
	C=O	287.2	14.7%	2.9%
	O=C—O	289.0	12.0%	4.1%
O1s	Chemisorbed oxygen	531.4	3.8%	6.1%
	Silicates	532.5	9.6%	8.2%
	O—C	533.2	8.8%	9.5%
N1s	Pyridine-N-Oxide	401.7	0.9%	0.5%
	Pyrrolic	400.0	1.0%	1.5%
Ca2p	$CaCO_3$	347.7/351.2	0.5%	2.2%
	$CaCl_2$	348.2	1.3%	0.1%
Fe2p	Fe^{2+}	710.9	0.0%	0.1%
	Fe^{3+}	713.3/714.2/725	0.3%	0.3%
Mn2p	Mn^{2+}	653.4	0.2%	0.1%
	Mn^{3+}	642.6	0.2%	0.2%
Mg1s	$Mg(OH)_2$	1303.9	0.4%	0.2%
	MgO	1304.3	1.2%	0.3%
Si2p	Si—O	102.1/102.7	0.7%	1.5%
	Si—O/Si	103.3	2.3%	0.8%
S2p		169.0	0.4%	0.4%

References

1. M. Lawrinenko, D.A. Laird, R.L. Johnson, D.P. Jing, Accelerated aging of biochars: Impact on anion exchange capacity. *Carbon* **103**, 217-227 (2016).

2. Y. Wang, W. Zhang, J.Y. Shang, C.Y. Shen, S.D. Joseph, Chemical aging changed aggregation kinetics and transport of biochar colloids. *Environ. Sci. Technol.* **53**(14), 8136-8146 (2019).