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Supplementary material

Biochar amendment altered the molecular-level composition of native soil organic matter in a temperate forest soil

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Table S1. Biomarkers identified in the solvent extracts of the soil samples

Compound class/name	Molecular weight (g/mol)	Chemical formula
Phenols[A]		
p-hydroxybenzoic acid	138	$C_7H_6O_3$
p-coumaric acid	164	$C_9H_8O_3$
vanillic acid	168	$C_8H_8O_4$
Sugars[A]		
fructose	180	$C_6H_{12}O_6$
glucose	180	$C_6H_{12}O_6$
mannose	180	$C_6H_{12}O_6$
inositol	180	$C_6H_{12}O_6$
mannitol	182	$C_6H_{14}O_6$
sucrose	342	$C_{12}H_{22}O_{11}$
trehalose (mycose)	342	$C_{12}H_{22}O_{11}$
maltose	342	$C_{12}H_{22}O_{11}$
Long-chain *n*-alkanes ($\geq C_{20}$)		
n-docosane	310	$C_{22}H_{46}$
n-tricosane	324	$C_{23}H_{48}$
n-tetracosane	338	$C_{24}H_{50}$
n-pentacosane	352	$C_{25}H_{52}$
n-hexacosane	366	$C_{26}H_{54}$
n-heptacosane	380	$C_{27}H_{56}$
n-nonacosane	408	$C_{29}H_{60}$
n-hentriacontane	436	$C_{31}H_{64}$
Short-chain *n*-alkanols ($< C_{20}$)[A]		
n-pentadecanol	228	$C_{15}H_{32}O$
n-hexadecanol	242	$C_{16}H_{34}O$
n-octadecanol	270	$C_{18}H_{38}O$
Long-chain *n*-alkanols ($\geq C_{20}$)[A]		
n-eicosanol	298	$C_{20}H_{42}O$
n-heneicosanol	312	$C_{21}H_{44}O$
n-docosanol	326	$C_{22}H_{46}O$
n-tricosanol	340	$C_{23}H_{48}O$
n-tetracosanol	354	$C_{24}H_{50}O$
n-pentacosanol	368	$C_{25}H_{52}O$
n-hexacosanol	382	$C_{26}H_{54}O$
n-heptacosanol	396	$C_{27}H_{56}O$
n-octacosanol	410	$C_{28}H_{58}O$
n-nonacosanol	424	$C_{29}H_{60}O$
n-triacontanol	438	$C_{30}H_{62}O$
n-dotriacontanol	452	$C_{32}H_{66}O$
Short-chain *n*-alkanoic acids ($< C_{20}$)[A]		
n-tetradecanoic acid	228	$C_{14}H_{28}O_2$
n-pentadecanoic acid	242	$C_{15}H_{30}O_2$
n-hexadecenoic acid ($C_{16:1\omega9}$)	254	$C_{16}H_{30}O_2$
n-hexadecenoic acid ($C_{16:1\omega11}$)	254	$C_{16}H_{30}O_2$
n-hexadecanoic acid	256	$C_{16}H_{32}O_2$
n-heptadecenoic acid ($C_{17:1}$)	268	$C_{17}H_{32}O_2$

n-heptadecanoic acid	270	$C_{17}H_{34}O_2$
n-octadecadienoic acid ($C_{18:2\omega9,11}$)	280	$C_{18}H_{32}O_2$
n-octadecenoic acid ($C_{18:1\omega9}$)	282	$C_{18}H_{34}O_2$
n-octadecenoic acid ($C_{18:1\omega11}$)	282	$C_{18}H_{34}O_2$
n-octadecanoic acid	284	$C_{18}H_{36}O_2$
n-nonadecenoic acid ($C_{19:1}$)	296	$C_{19}H_{36}O_2$
n-nonadecanoic acid	298	$C_{19}H_{38}O_2$
Long-chain *n*-alkanoic acids ($\geq C_{20}$)[A]		
n-eicosanoic acid	312	$C_{20}H_{40}O_2$
n-heneicosanoic acid	326	$C_{21}H_{42}O_2$
n-docosanoic acid	340	$C_{22}H_{44}O_2$
n-tricosanoic acid	354	$C_{23}H_{46}O_2$
n-tetracosanoic acid	368	$C_{24}H_{48}O_2$
n-pentacosanoic acid	382	$C_{25}H_{50}O_2$
n-hexacosanoic acid	396	$C_{26}H_{52}O_2$
n-heptacosanoic acid	410	$C_{27}H_{54}O_2$
n-octacosanoic acid	424	$C_{28}H_{56}O_2$
n-nonacosanoic acid	438	$C_{29}H_{58}O_2$
n-triacontanoic acid	452	$C_{30}H_{60}O_2$
n-hentriacontanoic acid	466	$C_{31}H_{62}O_2$
Steroids[A]		
cholesterol (cholest-5-en-3β-ol)	386	$C_{27}H_{46}O$
ergosterol (ergost-5,7,22-trien-3β-ol)	396	$C_{28}H_{44}O$
campesterol (ergost-5-en-3β-ol)	400	$C_{28}H_{48}O$
stigmasterol (stigmasta-5,22-dien-3β-ol)	412	$C_{29}H_{48}O$
β-sitosterol (stigmast-5-en-3β-ol)	414	$C_{29}H_{50}O$
stigmastan-3β-ol	416	$C_{29}H_{52}O$
stigmastan-3-one	414	$C_{29}H_{50}O$
stigmasta-3,5-dien-7-one	410	$C_{29}H_{46}O$
sitosterone (stigmast-4-en-3-one)	412	$C_{29}H_{48}O$
Triterpenoids[A]		
diploptene (hop-22(29)-ene)	410	$C_{30}H_{50}$
lupenone (lup-20(29)-en-3-one)	424	$C_{30}H_{48}O$
α-amyrin (urs-12-en-3β-ol)	426	$C_{30}H_{50}O$
friedelin (friedelan-3-one)	426	$C_{30}H_{50}O$
oleanolic acid	456	$C_{30}H_{48}O_3$
ursolic acid	456	$C_{30}H_{48}O_3$

[A] Detected as trimethylsilyl (TMS) ether derivatives.

Table S2. Compounds identified in the base hydrolysates of the soil samples

Detected as trimethylsilyl (TMS) ether and methyl ester derivatives

Compound class/name	Molecular weight (g/mol)	Chemical formula
Benzyls		
p-hydroxybenzaldehyde	122	$C_7H_6O_2$
p-hydroxyacetophenone	136	$C_8H_8O_2$
m-hydroxybenzoic acid	138	$C_7H_6O_3$
p-hydroxybenzoic acid	138	$C_7H_6O_3$
3,4-dihydroxybenzoic acid	154	$C_7H_6O_4$
Phenols		
vanillin	152	$C_8H_8O_3$
acetovanillone	166	$C_9H_{10}O_3$
vanillic acid	168	$C_8H_8O_4$
acetosyringone	196	$C_{10}H_{12}O_4$
syringic acid	198	$C_9H_{10}O_5$
m-coumaric acid	164	$C_9H_8O_3$
p-coumaric acid	164	$C_9H_8O_3$
ferulic acid	194	$C_{10}H_{10}O_4$
α,ω-*n*-alkanedioic acids		
α,ω-heptanedioic acid	160	$C_7H_{12}O_4$
α,ω-octanedioic acid	174	$C_8H_{14}O_4$
α,ω-nonanedioic acid	188	$C_9H_{16}O_4$
α,ω-tetradecanedioic acid	258	$C_{14}H_{26}O_4$
α,ω-hexadecanedioic acid	286	$C_{16}H_{30}O_4$
α,ω-octadecenedioic acid ($C_{18:1}$)	312	$C_{18}H_{32}O_4$
α,ω-octadecanedioic acid	314	$C_{18}H_{34}O_4$
α,ω-eicosanedioic acid	342	$C_{20}H_{38}O_4$
α,ω-docosanedioic acid	370	$C_{22}H_{42}O_4$
α,ω-tetracosanedioic acid	398	$C_{24}H_{46}O_4$
ω-hydroxyalkanoic acids		
ω-hydroxynonanoic acid	174	$C_9H_{18}O_3$
ω-hydroxyhexadecenoic acid ($C_{16:1}$)	270	$C_{16}H_{30}O_3$
ω-hydroxyhexadecanoic acid	272	$C_{16}H_{32}O_3$
ω-hydroxyheptadecanoic acid	286	$C_{17}H_{34}O_3$
ω-hydroxyoctadecanoic acid	300	$C_{18}H_{36}O_3$
ω-hydroxyeicosanoic acid	328	$C_{20}H_{40}O_3$
ω-hydroxyheneicosanoic acid	342	$C_{21}H_{42}O_3$
ω-hydroxydocosanoic acid	356	$C_{22}H_{44}O_3$
ω-hydroxytetracosanoic acid	384	$C_{24}H_{48}O_3$
n-alkanols		
n-octadecanol	270	$C_{18}H_{38}O$
n-eicosanol	298	$C_{20}H_{42}O$
n-docosanol	326	$C_{22}H_{46}O$
n-tetracosanol	354	$C_{24}H_{50}O$
n-pentacosanol	368	$C_{25}H_{52}O$
n-hexacosanol	382	$C_{26}H_{54}O$
n-octacosanol	410	$C_{28}H_{58}O$
n-triacontanol	438	$C_{30}H_{62}O$

n-alkanoic acids		
n-tetradecanoic acid	228	$C_{14}H_{28}O_2$
n-pentadecanoic acid	242	$C_{15}H_{30}O_2$
n-hexadecenoic acid ($C_{16:1\omega7}$)	254	$C_{16}H_{30}O_2$
n-hexadecanoic acid	256	$C_{16}H_{32}O_2$
n-octadecadienoic acid ($C_{18:2\omega9,11}$)	280	$C_{18}H_{32}O_2$
n-octadecenoic acid ($C_{18:1\omega9}$)	282	$C_{18}H_{34}O_2$
n-octadecenoic acid ($C_{18:1\omega11}$)	282	$C_{18}H_{34}O_2$
n-octadecanoic acid	284	$C_{18}H_{36}O_2$
n-eicosanoic acid	312	$C_{20}H_{40}O_2$
n-heneicosanoic acid	326	$C_{21}H_{42}O_2$
n-docosanoic acid	340	$C_{22}H_{44}O_2$
n-tricosanoic acid	354	$C_{23}H_{46}O_2$
n-tetracosanoic acid	368	$C_{24}H_{48}O_2$
n-hexacosanoic acid	396	$C_{26}H_{52}O_2$
n-heptacosanoic acid	410	$C_{27}H_{54}O_2$
n-octacosanoic acid	424	$C_{28}H_{56}O_2$
n-triacontanoic acid	452	$C_{30}H_{60}O_2$
Branched alkanoic acids		
iso-pentadecanoic acid	242	$C_{15}H_{30}O_2$
anteiso-pentadecanoic acid	242	$C_{15}H_{30}O_2$
anteiso-hexadecanoic acid	256	$C_{16}H_{32}O_2$
10-methyl-hexadecanoic acid	270	$C_{17}H_{34}O_2$
iso-heptadecanoic acid	270	$C_{17}H_{34}O_2$
anteiso-heptadecanoic acid	270	$C_{17}H_{34}O_2$
10-methyl-heptadecanoic acid	284	$C_{18}H_{36}O_2$
α-hydroxyalkanoic acids		
α-hydroxydocosanoic acid	356	$C_{22}H_{44}O_3$
α-hydroxytetracosanoic acid	384	$C_{24}H_{48}O_3$
α-hydroxypentacosanoic acid	398	$C_{25}H_{50}O_3$
Sterols		
β-sitosterol	414	$C_{29}H_{50}O$
Mid-chain hydroxy and epoxy acids		
9,10-dihydroxyhexadecanoic acid	288	$C_{16}H_{32}O_4$
x,ω-dihydroxyhexadecanoic acid (*x* = 7, 8, 9 or 10)	288	$C_{16}H_{32}O_4$
x-hydroxyhexadecanedioic acid (*x* = 7 or 8)	302	$C_{16}H_{30}O_5$
x,ω-dihydroxyoctadecanoic acid (*x* = 7, 8, 9 or 10)	316	$C_{18}H_{36}O_4$
9,10-epoxyoctadecanedioic acid	328	$C_{18}H_{32}O_5$
9-oxo-octadecanedioic acid	328	$C_{18}H_{32}O_5$
9,10,ω-trihydroxyoctadecanoic acid	332	$C_{18}H_{36}O_5$

Table S3. CuO oxidation products identified in the soil samples

Detected as trimethylsilyl (TMS) ether derivatives

Compound class/name	Molecular weight (g/mol)	Chemical formula
Benzyls		
p-hydroxybenzaldehyde	122	$C_7H_6O_2$
p-hydroxyacetophenone	136	$C_8H_8O_2$
m-hydroxybenzoic acid	138	$C_7H_6O_3$
p-hydroxybenzoic acid	138	$C_7H_6O_3$
3,5-dihydroxybenzoic acid	154	$C_7H_6O_4$
Vanillyl phenols		
vanillin	152	$C_8H_8O_3$
acetovanillone	166	$C_9H_{10}O_3$
vanillic acid	168	$C_8H_8O_4$
vanillylglyoxalic acid	196	$C_{10}H_{12}O_4$
Syringyl phenols		
syringaldehyde	182	$C_9H_{10}O_4$
acetosyringone	196	$C_{10}H_{12}O_4$
syringic acid	198	$C_9H_{10}O_5$
syringylglyoxalic acid	226	$C_{11}H_{14}O_5$
Cinnamyl phenols		
p-coumaric acid	164	$C_9H_8O_3$
ferulic acid	194	$C_{10}H_{10}O_4$

Table S4. Solvent extractable biomarker total concentrations and calculated ratios for the control soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different superscript lowercase letters

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Phenols	2.3 ± 0.2^{a}	2.1 ± 0.2^{a}	1.0 ± 0.1^{b}	0.9 ± 0.1^{b}	2.2 ± 0.1^{c}	3.0 ± 0.2^{d}
Sugars	136.7 ± 5.4^{a}	96.1 ± 1.0^{b}	107.7 ± 3.8^{b}	95.5 ± 2.3^{b}	174.3 ± 4.4^{c}	92.1 ± 2.3^{d}
n-alkanes (C_{22}-C_{31})	14.0 ± 0.4^{a}	15.6 ± 0.9^{a}	10.8 ± 1.0^{b}	15.1 ± 0.8^{c}	17.3 ± 0.4^{c}	14.0 ± 2.0^{c}
n-alkanols						
Short-chain (< C_{20})	1.1 ± 0.1^{a}	0.7 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.2^{a}	1.0 ± 0.1^{a}	1.8 ± 0.2^{b}
Long-chain (≥ C_{20})	109.6 ± 8.3^{a}	113.2 ± 3.9^{a}	57.9 ± 1.2^{b}	53.8 ± 0.9^{b}	143.5 ± 6.1^{c}	102.9 ± 6.7^{d}
Total	110.7 ± 8.1^{a}	113.9 ± 3.9^{a}	58.4 ± 1.2^{b}	54.5 ± 1.0^{b}	144.5 ± 6.1^{c}	104.7 ± 6.7^{d}
n-alkanoic acids						
Short-chain (< C_{20})	30.6 ± 2.4^{a}	31.5 ± 2.0^{a}	38.9 ± 3.1^{a}	32.3 ± 1.5^{a}	34.6 ± 3.9^{a}	42.3 ± 0.2^{a}
Long-chain (≥ C_{20})	126.0 ± 5.2^{a}	149.9 ± 10.9^{a}	209.9 ± 5.9^{b}	219.5 ± 4.6^{b}	167.2 ± 1.9^{c}	136.7 ± 15.9^{c}
Unsaturated	11.8 ± 1.2^{a}	15.3 ± 1.3^{a}	10.8 ± 1.4^{a}	8.4 ± 0.6^{a}	9.8 ± 1.3^{a}	16.7 ± 0.8^{a}
Total	156.7 ± 7.5^{a}	181.5 ± 12.9^{a}	248.8 ± 8.5^{b}	251.9 ± 6.0^{b}	201.8 ± 4.5^{c}	179.0 ± 15.9^{c}
Steroids	22.8 ± 1.5^{a}	44.2 ± 2.6^{b}	22.2 ± 1.1^{c}	26.5 ± 1.0^{c}	34.1 ± 1.8^{c}	30.1 ± 2.0^{c}
Triterpenoids	8.3 ± 0.4^{a}	23.3 ± 3.0^{b}	10.0 ± 0.9^{c}	10.5 ± 0.2^{c}	17.7 ± 0.4^{d}	10.5 ± 1.1^{e}
Ergosterol	0.6 ± 0.1^{a}	1.9 ± 0.2^{b}	1.0 ± 0.1^{c}	1.5 ± 0.2^{c}	1.3 ± 0.1^{c}	1.2 ± 0.1^{c}
Total acyclic lipids[A]	281.6 ± 11.2^{a}	310.9 ± 17.6^{a}	318.0 ± 9.7^{a}	321.5 ± 6.1^{a}	363.7 ± 5.8^{a}	297.6 ± 24.4^{a}
Total cyclic lipids[B]	31.1 ± 1.6^{a}	67.5 ± 5.1^{b}	32.2 ± 1.7^{c}	37.0 ± 1.1^{c}	51.8 ± 1.7^{d}	40.7 ± 3.0^{d}
Ratios						
Acyclic/cyclic lipids[C]	9.1 ± 0.5^{a}	4.6 ± 0.1^{b}	9.9 ± 0.3^{c}	8.7 ± 0.4^{c}	7.0 ± 0.3^{c}	7.3 ± 0.1^{c}
Sterols/sterones[CD]	4.4 ± 0.1^{a}	2.8 ± 0.3^{b}	2.8 ± 0.3^{b}	2.6 ± 0.1^{b}	2.3 ± 0.2^{b}	3.2 ± 0.1^{c}
Sitosterol/sitosterone[C]	6.4 ± 0.2^{a}	4.8 ± 1.7^{a}	5.8 ± 0.6^{a}	3.9 ± 0.1^{a}	3.1 ± 0.2^{a}	8.8 ± 0.3^{b}
Stigmasterol/stigmasta-3,5-dien-7-one[C]	2.3 ± 0.2^{a}	1.6 ± 0.1^{a}	0.9 ± 0.1^{a}	1.1 ± 0.1^{a}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}
Stigmastanol/stigmastan-3-one[C]	6.0 ± 0.4^{a}	3.3 ± 1.1^{b}	4.0 ± 0.5^{b}	3.9 ± 0.1^{b}	2.1 ± 0.7^{b}	2.3 ± 0.2^{b}

[A] Σ total *n*-alkanes + total *n*-alkanols + total *n*-alkanoic acids.

[B] Σ total steroids + total triterpenoids.

[C] Otto and Simpson.[1]

[D] Σ (sitosterol + stigmasterol + stigmastanol) / Σ (sitosterone + stigmasta-3,5-dien-7-one + sigmastan-3-one).

Table S5. Solvent extractable biomarker total concentrations and calculated ratios for the 5 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different superscript lowercase letters

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Phenols	2.3 ± 0.2^{a}	2.0 ± 0.1^{a}	0.8 ± 0.1^{b}	1.0 ± 0.1^{b}	2.2 ± 0.1^{c}	2.8 ± 0.1^{d}
Sugars	136.7 ± 5.4^{a}	84.1 ± 1.2^{b}	96.5 ± 4.3^{b}	104.2 ± 5.5^{b}	159.8 ± 2.9^{c}	102.3 ± 2.8^{d}
n-alkanes (C_{22}-C_{31})	14.0 ± 0.4^{a}	12.0 ± 0.7^{a}	11.5 ± 0.8^{a}	15.9 ± 0.3^{b}	16.9 ± 0.2^{b}	18.3 ± 1.1^{b}
n-alkanols						
Short-chain (< C_{20})	1.1 ± 0.1^{a}	0.7 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	1.3 ± 0.1^{b}	1.8 ± 0.2^{b}
Long-chain (≥ C_{20})	109.6 ± 8.3^{a}	84.9 ± 3.8^{b}	46.2 ± 1.9^{c}	54.4 ± 1.9^{c}	127.8 ± 3.0^{d}	118.3 ± 3.0^{d}
Total	110.7 ± 8.1^{a}	85.6 ± 3.8^{b}	46.7 ± 1.9^{c}	55.0 ± 1.7^{c}	129.1 ± 3.0^{d}	120.1 ± 3.2^{d}
n-alkanoic acids						
Short-chain (< C_{20})	30.6 ± 2.4^{a}	24.7 ± 0.8^{a}	37.9 ± 2.1^{a}	36.9 ± 2.0^{a}	35.9 ± 0.3^{a}	48.9 ± 2.9^{a}
Long-chain (≥ C_{20})	126.0 ± 5.2^{a}	119.8 ± 7.5^{a}	187.3 ± 10.2^{b}	236.7 ± 9.7^{c}	159.2 ± 1.0^{d}	157.9 ± 9.4^{d}
Unsaturated	11.8 ± 1.2^{a}	10.2 ± 0.2^{a}	11.6 ± 0.9^{a}	10.2 ± 0.9^{a}	12.6 ± 0.8^{a}	21.0 ± 2.0^{b}
Total	156.7 ± 7.5^{a}	144.5 ± 8.3^{a}	225.3 ± 12.2^{b}	273.7 ± 11.7^{c}	195.1 ± 0.9^{d}	206.8 ± 9.5^{d}
Steroids	22.8 ± 1.5^{a}	38.5 ± 2.1^{b}	22.7 ± 1.1^{c}	33.7 ± 0.7^{d}	34.2 ± 1.6^{d}	33.9 ± 0.6^{d}
Triterpenoids	8.3 ± 0.4^{a}	17.5 ± 1.5^{b}	8.3 ± 0.6^{c}	11.0 ± 0.5^{c}	17.4 ± 0.1^{d}	13.9 ± 1.2^{d}
Ergosterol	0.6 ± 0.1^{a}	1.6 ± 0.1^{b}	1.0 ± 0.1^{b}	1.9 ± 0.1^{c}	1.5 ± 0.1^{d}	1.6 ± 0.1^{d}
Total acyclic lipids[A]	281.6 ± 11.2^{a}	242.1 ± 12.8^{a}	283.4 ± 14.7^{a}	344.5 ± 13.6^{a}	341.1 ± 3.0^{a}	345.2 ± 13.3^{a}
Total cyclic lipids[B]	31.1 ± 1.6^{a}	56.0 ± 3.4^{b}	31.0 ± 1.6^{c}	44.7 ± 1.1^{d}	51.6 ± 1.5^{d}	47.8 ± 1.0^{d}
<u>Ratios</u>						
Acyclic/cyclic lipids[C]	9.1 ± 0.5^{a}	4.3 ± 0.1^{b}	9.1 ± 0.1^{c}	7.7 ± 0.2^{c}	6.6 ± 0.3^{c}	7.2 ± 0.1^{c}
Sterols/sterones[CD]	4.4 ± 0.1^{a}	2.3 ± 0.1^{b}	3.1 ± 0.2^{b}	3.1 ± 0.1^{b}	2.5 ± 0.1^{b}	3.2 ± 0.2^{b}
Sitosterol/sitosterone[C]	6.4 ± 0.2^{a}	3.5 ± 0.2^{b}	4.1 ± 0.1^{b}	4.1 ± 0.1^{b}	3.7 ± 0.3^{b}	10.5 ± 0.5^{c}
Stigmasterol/stigmasta-3,5-dien-7-one[C]	2.3 ± 0.2^{a}	1.3 ± 0.1^{b}	1.4 ± 0.1^{b}	1.6 ± 0.1^{b}	1.7 ± 0.1^{b}	1.5 ± 0.2^{b}
Stigmastanol/stigmastan-3-one[C]	6.0 ± 0.4^{a}	2.2 ± 0.1^{b}	4.9 ± 0.7^{c}	5.1 ± 0.3^{c}	1.8 ± 0.1^{d}	2.1 ± 0.2^{d}

[A] Σ total *n*-alkanes + total *n*-alkanols + total *n*-alkanoic acids.

[B] Σ total steroids + total triterpenoids.

[C] Otto and Simpson.[1]

[D] Σ (sitosterol + stigmasterol + stigmastanol) / Σ (sitosterone + stigmasta-3,5-dien-7-one + sigmastan-3-one).

Table S6. Solvent extractable biomarker total concentrations and calculated ratios for the 10 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different superscript lowercase letters

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Phenols	2.3 ± 0.2^{a}	0.9 ± 0.1^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	1.7 ± 0.1^{c}	1.7 ± 0.2^{c}
Sugars	136.7 ± 5.4^{a}	59.6 ± 1.7^{b}	67.5 ± 7.8^{b}	52.0 ± 7.1^{b}	106.9 ± 6.2^{c}	136.0 ± 1.4^{d}
n-alkanes (C_{22}-C_{31})	14.0 ± 0.4^{a}	7.7 ± 0.3^{b}	7.7 ± 1.2^{b}	8.4 ± 0.8^{b}	11.5 ± 0.5^{b}	15.1 ± 0.3^{b}
n-alkanols						
Short-chain (< C_{20})	1.1 ± 0.1^{a}	0.6 ± 0.1^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	1.7 ± 0.1^{b}	1.0 ± 0.1^{c}
Long-chain (≥ C_{20})	109.6 ± 8.3^{a}	52.7 ± 1.0^{b}	31.7 ± 3.9^{b}	32.2 ± 3.5^{b}	100.7 ± 0.6^{c}	102.5 ± 0.7^{c}
Total	110.7 ± 8.1^{a}	53.4 ± 1.0^{b}	32.0 ± 3.9^{b}	32.6 ± 3.5^{b}	102.4 ± 0.6^{c}	103.5 ± 0.7^{c}
n-alkanoic acids						
Short-chain (< C_{20})	30.6 ± 2.4^{a}	20.1 ± 3.9^{a}	30.1 ± 6.3^{a}	29.1 ± 1.4^{a}	35.3 ± 2.2^{a}	35.5 ± 4.9^{a}
Long-chain (≥ C_{20})	126.0 ± 5.2^{a}	59.6 ± 2.2^{b}	114.7 ± 17.0^{c}	117.4 ± 14.6^{c}	117.0 ± 4.0^{c}	115.6 ± 1.3^{c}
Unsaturated	11.8 ± 1.2^{a}	6.7 ± 1.5^{b}	6.7 ± 1.2^{b}	6.7 ± 0.9^{b}	12.7 ± 1.4^{c}	14.2 ± 2.1^{c}
Total	156.7 ± 7.5^{a}	79.8 ± 5.9^{b}	144.8 ± 23.3^{c}	146.5 ± 15.8^{c}	152.3 ± 3.3^{c}	151.1 ± 5.8^{c}
Steroids	22.8 ± 1.5^{a}	21.8 ± 0.5^{a}	15.4 ± 2.4^{a}	17.9 ± 1.6^{a}	23.2 ± 1.6^{b}	25.6 ± 0.6^{b}
Triterpenoids	8.3 ± 0.4^{a}	9.8 ± 0.2^{a}	5.3 ± 0.7^{a}	5.8 ± 0.3^{a}	12.6 ± 1.3^{b}	9.1 ± 0.3^{b}
Ergosterol	0.6 ± 0.1^{a}	0.9 ± 0.1^{a}	0.7 ± 0.2^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	1.1 ± 0.1^{a}
Total acyclic lipids[A]	281.6 ± 11.2^{a}	140.8 ± 7.1^{b}	184.6 ± 28.4^{b}	187.5 ± 20.0^{b}	266.1 ± 4.3^{c}	269.7 ± 5.9^{c}
Total cyclic lipids[B]	31.1 ± 1.6^{a}	31.6 ± 0.5^{a}	20.8 ± 3.1^{a}	23.7 ± 1.9^{a}	35.8 ± 2.9^{a}	34.6 ± 0.9^{a}
Ratios						
Acyclic/cyclic lipids[C]	9.1 ± 0.5^{a}	4.5 ± 0.2^{b}	8.9 ± 0.3^{c}	7.9 ± 0.3^{c}	7.5 ± 0.5^{c}	7.8 ± 0.1^{c}
Sterols/sterones[CD]	4.4 ± 0.1^{a}	2.8 ± 0.1^{b}	2.9 ± 0.1^{b}	3.1 ± 0.1^{b}	2.7 ± 0.1^{b}	3.6 ± 0.2^{b}
Sitosterol/sitosterone[C]	6.4 ± 0.2^{a}	6.1 ± 0.2^{a}	4.1 ± 0.2^{a}	4.6 ± 0.1^{a}	4.6 ± 0.2^{a}	6.8 ± 0.6^{a}
Stigmasterol/stigmasta-3,5-dien-7-one[C]	2.3 ± 0.2^{a}	1.4 ± 0.1^{b}	1.2 ± 0.2^{b}	1.5 ± 0.1^{b}	1.5 ± 0.1^{b}	1.9 ± 0.1^{b}
Stigmastanol/stigmastan-3-one[C]	6.0 ± 0.4^{a}	1.9 ± 0.4^{b}	4.3 ± 0.5^{c}	3.7 ± 0.2^{c}	2.0 ± 0.3^{c}	2.6 ± 0.3^{c}

[A] Σ total *n*-alkanes + total *n*-alkanols + total *n*-alkanoic acids.

[B] Σ total steroids + total triterpenoids.

[C] Otto and Simpson.[1]

[D] Σ (sitosterol + stigmasterol + stigmastanol) / Σ (sitosterone + stigmasta-3,5-dien-7-one + sigmastan-3-one).

Table S7. Solvent extractable biomarker total concentrations and calculated ratios for the 20 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different superscript lowercase letters

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Phenols	2.3 ± 0.2^{a}	0.6 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	1.7 ± 0.1^{c}	2.4 ± 0.1^{d}
Sugars	136.7 ± 5.4^{a}	40.7 ± 1.4^{b}	45.6 ± 3.0^{b}	44.6 ± 1.0^{b}	129.1 ± 13.0^{c}	101.4 ± 1.5^{d}
n-alkanes (C_{22}-C_{31})	14.0 ± 0.4^{a}	5.7 ± 0.4^{b}	5.0 ± 0.4^{b}	6.1 ± 0.3^{b}	11.5 ± 0.9^{c}	14.7 ± 0.8^{c}
n-alkanols						
Short-chain (< C_{20})	1.1 ± 0.1^{a}	0.5 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	1.9 ± 0.3^{b}	1.5 ± 0.1^{b}
Long-chain (≥ C_{20})	109.6 ± 8.3^{a}	38.5 ± 1.6^{b}	20.6 ± 1.5^{b}	23.5 ± 1.4^{b}	98.8 ± 5.8^{c}	97.4 ± 4.1^{c}
Total	110.7 ± 8.1^{a}	39.1 ± 1.7^{b}	20.9 ± 1.6^{b}	23.8 ± 1.5^{b}	100.7 ± 6.1^{c}	98.8 ± 4.2^{c}
n-alkanoic acids						
Short-chain (< C_{20})	30.6 ± 2.4^{a}	13.2 ± 1.1^{b}	19.4 ± 0.8^{b}	20.8 ± 3.5^{b}	32.6 ± 6.7^{b}	41.5 ± 1.9^{b}
Long-chain (≥ C_{20})	126.0 ± 5.2^{a}	37.5 ± 2.8^{b}	65.9 ± 6.3^{b}	76.1 ± 4.7^{b}	120.1 ± 7.1^{c}	119.4 ± 7.1^{c}
Unsaturated	11.8 ± 1.2^{a}	4.2 ± 0.5^{b}	4.9 ± 0.2^{b}	4.3 ± 0.2^{b}	11.6 ± 3.6^{c}	17.1 ± 0.4^{c}
Total	156.7 ± 7.5^{a}	50.7 ± 3.8^{b}	85.3 ± 7.1^{b}	96.9 ± 7.5^{b}	152.7 ± 12.2^{c}	161.0 ± 5.1^{c}
Steroids	22.8 ± 1.5^{a}	15.1 ± 1.1^{a}	9.8 ± 0.4^{a}	12.6 ± 0.6^{a}	26.4 ± 5.0^{b}	23.7 ± 0.9^{b}
Triterpenoids	8.3 ± 0.4^{a}	5.9 ± 0.4^{a}	3.3 ± 0.3^{a}	4.2 ± 0.7^{a}	11.6 ± 1.0^{b}	8.6 ± 0.9^{b}
Ergosterol	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.1^{a}	0.9 ± 0.1^{a}	1.1 ± 0.1^{a}
Total acyclic lipids[A]	281.6 ± 11.2^{a}	95.5 ± 5.8^{b}	111.3 ± 8.9^{b}	126.8 ± 9.3^{b}	264.9 ± 18.9^{c}	274.5 ± 9.1^{c}
Total cyclic lipids[B]	31.1 ± 1.6^{a}	21.0 ± 1.2^{a}	13.1 ± 0.7^{a}	16.8 ± 1.3^{a}	38.1 ± 6.0^{b}	32.3 ± 1.6^{b}
Ratios						
Acyclic/cyclic lipids[C]	9.1 ± 0.5^{a}	4.6 ± 0.3^{b}	8.5 ± 0.5^{c}	7.6 ± 0.5^{c}	7.1 ± 0.6^{c}	8.5 ± 0.2^{c}
Sterols/sterones[CD]	4.4 ± 0.1^{a}	3.7 ± 0.3^{a}	3.3 ± 0.2^{a}	3.6 ± 0.2^{a}	2.6 ± 0.1^{b}	3.7 ± 0.4^{c}
Sitosterol/sitosterone[C]	6.4 ± 0.2^{a}	9.2 ± 0.6^{a}	4.9 ± 0.3^{b}	4.4 ± 0.1^{b}	5.1 ± 1.4^{b}	10.6 ± 0.5^{c}
Stigmasterol/stigmasta-3,5-dien-7-one[C]	2.3 ± 0.2^{a}	1.7 ± 0.2^{a}	1.6 ± 0.1^{a}	2.2 ± 0.2^{a}	1.7 ± 0.1^{a}	1.9 ± 0.2^{a}
Stigmastanol/stigmastan-3-one[C]	6.0 ± 0.4^{a}	2.2 ± 0.3^{b}	3.9 ± 0.5^{b}	4.4 ± 0.6^{b}	1.8 ± 0.5^{c}	2.3 ± 0.6^{c}

[A] Σ total *n*-alkanes + total *n*-alkanols + total *n*-alkanoic acids.

[B] Σ total steroids + total triterpenoids.

[C] Otto and Simpson.[1]

[D] Σ (sitosterol + stigmasterol + stigmastanol) / Σ (sitosterone + stigmasta-3,5-dien-7-one + sigmastan-3-one).

Table S8. CuO oxidation biomarker total concentrations and calculated acid/aldehyde (Ad/Al) ratios for the control soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different lowercase letters. No significant changes in Ad/Al ratios were observed during the study

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Benzyls	73.1 ± 12.5[a]	192.7 ± 16.5[b]	209.1 ± 25.5[b]	176.7 ± 10.6[b]	216.4 ± 19.3[b]	154.6 ± 13.6[b]
Vanillyl phenols	118.5 ± 21.6[a]	321.8 ± 25.5[b]	337.3 ± 43.0[b]	309.3 ± 3.3[b]	406.2 ± 30.8[b]	301.3 ± 25.6[b]
Syringyl phenols	80.2 ± 16.7[a]	148.3 ± 19.0[b]	167.9 ± 25.2[b]	159.7 ± 4.4[b]	246.8 ± 23.9[c]	178.2 ± 20.8[b]
Cinnamyl phenols	47.4 ± 7.0[a]	165.2 ± 12.9[b]	168.9 ± 21.2[b]	141.5 ± 10.2[b]	152.4 ± 11.8[b]	109.4 ± 8.1[c]
ΣVSC	229.1 ± 41.8[a]	583.6 ± 52.0[b]	621.0 ± 81.3[b]	565.9 ± 6.8[b]	744.8 ± 61.8[b]	548.1 ± 51.0[b]
Ratios						
vanillic acid/vanillin[A]	3.2 ± 1.0	4.6 ± 0.5	3.9 ± 0.6	3.8 ± 1.0	3.3 ± 0.6	2.1 ± 0.2
syringic acid/syringaldehyde[A]	1.3 ± 0.3	2.5 ± 0.2	2.1 ± 0.2	2.0 ± 0.4	1.7 ± 0.1	1.4 ± 0.1

[A] Ertel and Hedges.[2]

Table S9. CuO oxidation biomarker total concentrations and calculated acid/aldehyde (Ad/Al) ratios for the 5 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different lowercase letters. No significant changes in Ad/Al ratios were observed during the study

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Benzyls	73.1 ± 12.5[a]	190.0 ± 6.1[b]	190.7 ± 8.5[b]	171.4 ± 10.5[b]	178.0 ± 3.4[b]	140.2 ± 10.5[b]
Vanillyl phenols	118.5 ± 21.6[a]	349.1 ± 19.9[b]	377.6 ± 18.8[b]	340.7 ± 24.9[b]	368.8 ± 12.0[b]	284.0 ± 31.0[b]
Syringyl phenols	80.2 ± 16.7[a]	211.1 ± 11.9[b]	193.3 ± 6.7[b]	173.7 ± 13.6[b]	176.7 ± 5.4[b]	141.6 ± 19.8[b]
Cinnamyl phenols	47.4 ± 7.0[a]	135.8 ± 4.8[b]	152.0 ± 3.8[b]	134.1 ± 6.8[b]	139.9 ± 3.2[b]	112.1 ± 10.2[b]
ΣVSC	229.1 ± 41.8[a]	641.1 ± 25.4[b]	665.4 ± 24.4[b]	597.6 ± 42.5[b]	634.5 ± 16.2[b]	498.9 ± 56.1[b]
Ratios						
vanillic acid/vanillin[A]	3.2 ± 1.0	2.6 ± 0.5	2.5 ± 0.3	2.6 ± 0.4	2.5 ± 0.4	2.5 ± 0.2
syringic acid/syringaldehyde[A]	1.3 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1

[A] Ertel and Hedges.[2]

Table S10. CuO oxidation biomarker total concentrations and calculated acid/aldehyde (Ad/Al) ratios for the 10 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in micrograms per gram of soil extracted. Values are shown with standard error (n = 3). Statistically significant differences in concentration between consecutive time points are indicated by different lowercase letters. No significant changes in Ad/Al ratios were observed during the study

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Benzyls	73.1 ± 12.5[a]	123.7 ± 6.8[a]	146.1 ± 9.1[a]	117.4 ± 13.3[a]	147.6 ± 5.3[a]	100.8 ± 10.1[a]
Vanillyl phenols	118.5 ± 21.6[a]	210.5 ± 19.0[a]	263.1 ± 15.1[a]	216.7 ± 23.9[a]	287.6 ± 4.6[a]	174.4 ± 7.8[b]
Syringyl phenols	80.2 ± 16.7[a]	109.7 ± 10.5[a]	166.1 ± 15.3[a]	131.0 ± 20.8[a]	155.6 ± 15.1[a]	79.1 ± 3.2[b]
Cinnamyl phenols	47.4 ± 7.0[a]	89.4 ± 5.3[a]	87.4 ± 6.1[a]	69.9 ± 4.7[a]	104.4 ± 3.8[a]	75.3 ± 6.0[a]
ΣVSC	229.1 ± 41.8[a]	377.8 ± 30.3[a]	479.0 ± 25.7[a]	388.6 ± 46.2[a]	505.5 ± 19.0[a]	304.8 ± 13.2[a]
Ratios						
vanillic acid/vanillin[A]	3.2 ± 1.0	3.4 ± 0.9	2.2 ± 0.2	2.4 ± 0.5	2.4 ± 0.5	3.1 ± 0.3
syringic acid/syringaldehyde[A]	1.3 ± 0.3	1.8 ± 0.4	1.3 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	2.2 ± 0.3

[A] Ertel and Hedges.[2]

Table S11. CuO oxidation biomarker total concentrations and calculated acid/aldehyde (Ad/Al) ratios for the 20 t/ha biochar-amended soils as measured at the specified sampling time points during the biochar incubation study

All concentrations are in µg/g soil extracted. Values are shown with standard error (*n* = 3). Statistically significant differences in concentration between consecutive time points are indicated by different lowercase letters. No significant changes in Ad/Al ratios were observed during the study

Biomarker class	Week 0	Week 2	Week 8	Week 16	Week 24	Week 32
Benzyls	73.1 ± 12.5[a]	94.7 ± 11.6[a]	105.7 ± 6.8[a]	82.7 ± 7.7[a]	130.0 ± 11.4[a]	134.2 ± 4.5[a]
Vanillyl phenols	118.5 ± 21.6[a]	156.9 ± 30.7[a]	182.2 ± 7.4[a]	156.0 ± 14.6[a]	265.8 ± 26.9[a]	267.6 ± 6.4[a]
Syringyl phenols	80.2 ± 16.7[a]	78.3 ± 17.4[a]	90.5 ± 6.4[a]	76.8 ± 9.8[a]	101.9 ± 9.6[a]	108.6 ± 9.1[a]
Cinnamyl phenols	47.4 ± 7.0[a]	59.4 ± 8.1[a]	65.3 ± 3.5[a]	53.7 ± 2.7[a]	121.2 ± 11.5[b]	124.4 ± 3.0[b]
ΣVSC	229.1 ± 41.8[a]	274.1 ± 48.8[a]	311.2 ± 13.4[a]	266.4 ± 25.7[a]	452.8 ± 43.9[a]	463.1 ± 14.3[a]
Ratios						
vanillic acid/vanillin[A]	3.2 ± 1.0	3.8 ± 1.1	2.4 ± 0.3	2.6 ± 0.6	2.9 ± 0.4	3.0 ± 0.3
syringic acid/syringaldehyde[A]	1.3 ± 0.3	2.0 ± 0.4	1.4 ± 0.1	1.5 ± 0.2	1.7 ± 0.2	1.9 ± 0.1

[A] Ertel and Hedges.[2]

Table S12. Integration values for four major types of organic matter components in the solid-state ^{13}C cross-polarization magic-angle spinning nuclear magnetic resonance (NMR) spectra with total suppression of sidebands for the biochar and soil samples during the incubation study

Sample	Alkyl (0–50 ppm)	*O*-alkyl (50–110 ppm)	Aromatic and phenolic (110-165 ppm)	Carboxyl (165–200 ppm)
Biochar composite	6	12	77	5
Week 0 – control	36	43	15	6
Week 32 – control	36	42	15	7
Week 32 – 5 t/ha	34	42	18	6
Week 32 – 10 t/ha	33	40	20	7
Week 32 – 20 t/ha	32	37	24	7

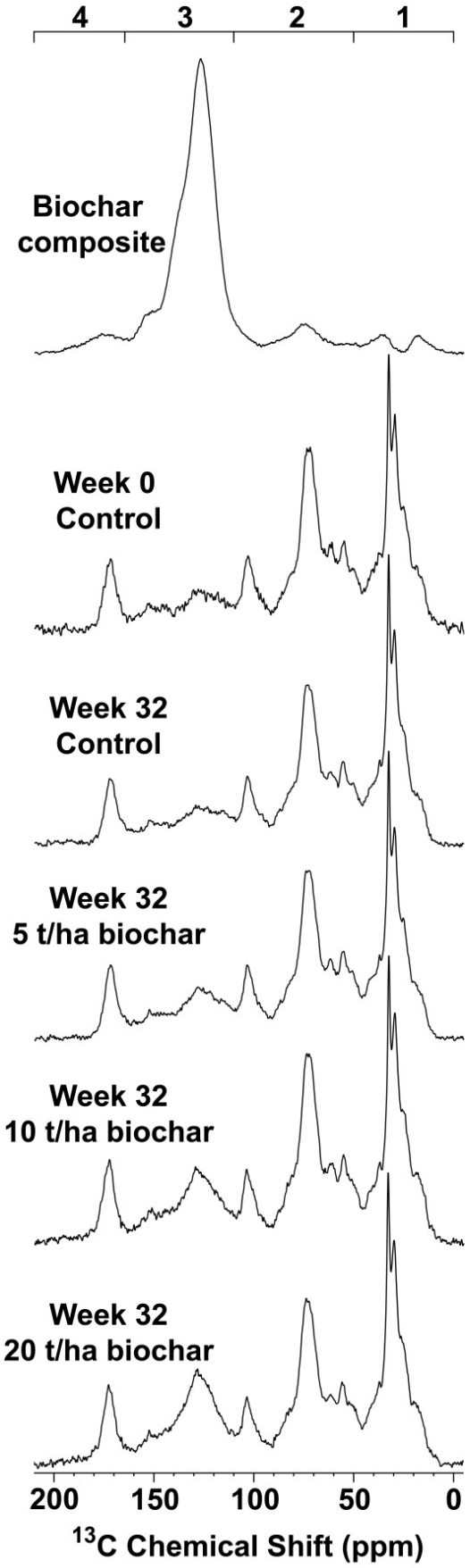

Fig. S1. Solid-state ^{13}C cross-polarisation magic-angle spinning nuclear magnetic resonance (NMR) spectra with total suppression of sidebands for the biochar composite and control and biochar-amended soils during the incubation study. The highlighted regions include: (1) alkyl carbon (0-50 ppm), (2) *O*-alkyl carbon (50-110 ppm), (3) aromatic and phenolic carbon (110-165 ppm) and (4) carboxyl carbon (165-200 ppm).

References

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