Supplementary Material for:

Ecological properties uniquely dictate molecular-level soil organic matter composition in a temperate forest in Central Europe with variation in litter deposition

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Methods

Soil organic matter (SOM) targeted sequential extractions

Three extractions were conducted sequentially to extract specific soil organic matter (SOM) compounds. Each field replicate (n = 3) was extracted in duplicate (n = 2). Three field replicates and their analytical replicates (6 samples total) were extracted in one day of lab work (~8-12 hours). Unbound components of SOM were isolated using solvent extraction, and comprise of aliphatic lipids, cyclic lipids, and sugars. Approximately 2 g of freeze-dried soil underwent ultrasonication in Teflon tubes for 20 minutes in 30 mL each of dichloromethane (Optima® grade), followed by 1:1 dichloromethane: methanol (Optima® grade; v/v), and then methanol. After ultrasonication with each solvent, the samples were then centrifuged and filtered using glass fibre filters (Whatman GF/A and GF/F). The combined supernatants were then concentrated by rotary evaporation, before being transferred to 2 mL glass autosampler vials and dried under a stream of N₂ gas. The soil residues from the solvent extraction were air-dried and used in subsequent steps.

The second step of the sequential SOM extractions was an alkaline (or base) hydrolysis, and isolates lipids by cleaving the ester bonds found in cutin and suberin biomarkers. Half of the air-dried solvent-extracted soil residues (approximately 1 g) were placed in a Teflon-lined bomb with 15 mL of 1 M methanolic potassium hydroxide (ACS Grade). The samples were then heated at 100 °C for 3 hours, and then allowed to cool. Next, the supernatant was decanted, and the soil was washed and ultrasonicated with two 10 mL aliquots of 1:1 dichloromethane and methanol (v/v) for 15 minutes each. After each addition of the 1:1 dichloromethane and methanol (v/v) washings, the samples were centrifuged and added to the supernatant. Once the washings and supernatants were collected together, the samples were acidified to a pH of 1, with 6 M hydrochloric acid (reagent grade) and then concentrated by rotary evaporation. 30

mL of deionized water was added to the remaining concentrated extract. The samples then underwent liquid-liquid extraction, using three 30 mL aliquots of anhydrous diethyl ether (ACS Grade with butylated hydroxytoluene as a stabilizer). The organic diethyl ether layer was combined and dried over sodium sulfate (anhydrous) and concentrated by rotary evaporation. The extracts were then transferred to 2 mL glass autosampler vials and dried with N₂ gas. Once again, the soil residues from this step were air-dried and used in the proceeding step.

The last step of the sequential SOM extraction was used to isolate lignin phenol monomers and dimers by targeting the ether bonds in polymeric lignin via a cupric oxide oxidation reaction. About half (~0.5 g) of air-dried soil from the previous extraction was placed in a Teflon-lined bomb with 1 g of copper (II) oxide (97%), 0.1 g of ammonium iron (II) sulfate hexahydrate (ReagentPlus) and 15 mL of 2 M sodium hydroxide (reagent grade). The headspace of the Teflon-lined bombs was purged with N₂ gas before the samples were heated at 170 °C for 2.5 hours. Once cooled, the supernatant was decanted, and two 10 mL aliquots of deionized water were added to the remaining soil and sonicated. The samples were then centrifuged, and the deionized water washes were added to the supernatant and then acidified to a pH of 1, with 6 M hydrochloric acid. To prevent cinnamic acid polymerization, the extracts were kept in the dark for at least 1 hour. To isolate and purify the extracted lignin phenol monomers and dimers, solid phase extraction (Oasis HLB cartridges, 3 mL, 60 mg) was performed using 1.5 mL of 70:25:5 dichloromethane:methyl acetate:pyridine (v/v/v) and 1 mL of methanol. The extracts were passed through a sodium sulfate column and then transferred to 2 mL glass autosampler vials and dried under N₂ gas.

Phospholipid fatty acid (PLFA) extraction

A modified Bligh-Dyer method¹ was used to extract the phospholipid fatty acids (PLFAs), by adding 16 mL of methanol, 8 mL of chloroform, and 6 mL of 0.15 M sodium citrate buffer (acidified to a pH of 4) to approximate 5 g of freeze-dried soil. The sample mixtures were shaken in the dark for 24 hours using a reciprocal shaker and then centrifuged. The supernatant was removed and combined with 7 mL of chloroform and 7 mL of sodium citrate buffer and centrifuged again to separate the solvent layers. The top layer (aqueous) was discarded, while the bottom layer (chloroform) was dried under N2 gas. The extracts were redissolved using 1 mL of chloroform and then fractionated using silicic acid chromatography columns. The samples were fractionated into three fractions, using 10 mL of chloroform, 20 mL of acetone, and 10 mL of methanol respectively. These eluents isolate the nonpolar lipids, glycolipids, and polar lipids from the samples respectively. Only the methanol fraction underwent a mild alkaline methanolysis to convert the polar lipids into fatty acid methyl ethers. This was achieved by adding 1 mL of 0.2 M methanolic potassium hydroxide and 1 mL of a 1:1 mixture of toluene: methanol (v/v) to the extracts before heating the samples for 15 minutes at 37 °C. 2 mL of deionized water and 300 µL of 1 M acetic acid were added to the methanolized samples. Next, the samples were extracted using three 2 mL aliquots of 4:1 hexanes:chloroform (v/v), collecting the hexanes: chloroform layer. The extracts were then transferred to glass autosampler vial inserts and dried under N2 gas. Prior to analysis, extract residues were redissolved with hexanes.

Targeted soil organic matter (SOM) compound and PLFA extract quantification by gas chromatography-mass spectrometry (GC-MS)

Prior to identification and quantification by gas chromatography-mass spectrometry (GC-MS), all SOM compound extracts were derivatized to increase volatility. The solvent extraction and copper oxidation extracts were directly converted to trimethylsilyl derivatives by heating the dried extracts at 70 °C for 1 hour with 100 µL of N,O-bistrifluoroacetamide (>99.5%) with 10 µL of a pyridine (99.8%) catalyst. For the base hydrolysis extracts, they were first methylated heating the dried extract for 15 minutes at 60 °C with 500 μ L of N,Ndimethylformamide dimethyl acetal (97%), before derivatization with N.Obistrifluoroacetamide and pyridine. External standards were used for quantification of compounds for each step of the sequential extraction, and are listed as follows: for solvent extracts, tetracosane (analytical standard grade), 1-docosanol (approximately 98%), methyl tricosanoate (analytical standard grade) and cholesterol (minimum 99%) were used; for base hydrolysis, only methyl tricosanoate was used; and for copper oxidation, syringic acid (minimum 98%) and syringaldehyde (98%) were used. Before quantification, 1-docosanol, cholesterol, syringic acid, and syringaldehyde were converted to trimethylsilyl esters using the same derivatization method as described for the samples. Hexanes (HPLC grade) were used to dilute the sample extracts and external standards. 1 μ L of each sample and external standard were injected onto an Agilent 7890B gas chromatograph equipped with a 5977B mass spectrometer with an extractor ion source operated in electron impact (70 eV) mode. The samples and standards were injected into the inlet at a temperature of 280 °C, using the Agilent 7693A automatic sampler. An HP-5MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness) was used, with helium as a carrier gas with a flow rate of 1 mL min⁻¹. To achieve adequate separation, an oven temperature ramp program was used, staring at 65 °C for 2 minutes, followed by an increase of 6 °C min⁻¹ to 300 °C, and an isothermal hold at 300 °C for 20 minutes. Data acquisition and processing was conducted using the Agilent Mass Hunter GC-MS Acquisition (version B.07.03.2129) and Agilent Enhanced ChemStation (version E.02.02.1431) software respectively. Compounds were identified using the Wiley Registry (9th edition), which is an in-house mass spectral library, and the NIST (2008) published mass spectral library. All compounds were identified by comparing to the spectra of an external standard or by a major ion mass spectral pattern. Furthermore, some of the compounds have been verified using multiple external standards but have also been verified with known retention times.

Comparison between soil mass-normalized and soil carbon (C) concentration-normalized soil organic matter (SOM) compound concentrations

This study investigates the SOM compositional changes after 20 years of detrital experiments in a temperate forest in Central Europe. Soil mass and soil carbon (C) concentrations were both used to normalize the SOM compound concentrations ($\mu g g^{-1}$ soil; Tables S1-2 and $\mu g g^{-1}$ C; Tables S3-4 respectively). Soil C concentrations were determined using the Thermo Flash 2000 Elemental analyzer, which is able to determine concentrations of C and nitrogen between 0.01-100%. Previous Detrital Input and Removal Treatments (DIRT) network studies²⁻⁶ reported concentrations were also reported in this manner for this study. Normalization to soil mass was conducted with the intention to compare the changes in SOM concentration, the changes are instead proportional to the overall soil C pool. This may pose an issue, as these changes now may not reflect the actual changes occurring, since the additions or exclusions of C inputs are discernibly altering the soil C pool. The alteration of the soil C pool through detrital manipulation could distort the data and associated trends, potentially resulting in misinterpretations of changes in SOM compound abundance. Moreover, the

targeted extractions of SOM compounds conducted throughout this study are non-exhaustive, making it misleading to compare the concentrations of these compounds normalized to soil C concentration ($\mu g g^{-1} C$) when the addition or removal of C inputs alters the C concentration.



Figure S1 Diagram of the 18 treatment plots present at the Síkfőkút DIRT site. The 18 plots were randomly selected on a gentle 3-4 % slope with the same orientation. The farthest distance between two plots is 150 m, while the nearest neighbouring plots are 2 m apart.



blue) overlaid with the control (in black).



Figure S3 Solid-state ¹³C nuclear magnetic resonance spectra of the No Roots (in pink) overlaid with the control (in black).



Figure S4 Solid-state ¹³C nuclear magnetic resonance spectra of the No Inputs treatment (in green) overlaid with the control (in black).





Figure S6 Solid-state ¹³C nuclear magnetic resonance spectra of the Double Wood treatment (in purple) overlaid with the control (in black).

Table S1 Chemical and microbial indices of soils after detrital manipulation treatments at the Síkfőkút Experimental Forest. The concentrations (μ g g⁻¹ soil) and ratios are expressed as the mean±SE for both field (*n*=3) and analytical replicates (*n*=2). Bolded values denote significant differences (*p* ≤ 0.05) between the treatment and the control.

	Control	No Litter	No Roots	No Inputs	Double Litter	Double Wood
Nuclear magnetic resonance characteristics						
Alkyl C (%)	28	31	32	32	31	30
<i>O</i> -alkyl C (%)	48	48	47	48	49	51
Aromatic & phenolic C (%)	15	13	12	12	12	12
Carboxyl & carbonyl C (%)	9	8	9	8	8	7
Relative stage of SOM degradation ^a	0.58	0.65	0.68	0.67	0.63	0.59
Resistance to SOM decomposition ^b	0.75	0.79	0.79	0.79	0.75	0.72
Soil carbon and nitrogen (%)						
Carbon concentration	5.18 ± 0.19	3.51 ± 0.29	3.50 ± 0.05	3.24 ± 0.1	5.85 ± 0.1	5.72 ± 0.09
Nitrogen concentration	0.41 ± 0.02	0.29 ± 0.02	0.29 ± 0.01	$\boldsymbol{0.28\pm0.01}$	0.46 ± 0.01	0.44 ± 0.01
Phospholipid fatty acids (PLFAs; µg g ⁻¹ soil) and ratios						
Gram-negative bacterial PLFAs	5.31 ± 0.57	2.81 ± 0.63	2.23 ± 0.10	3.02 ± 0.15	6.02 ± 0.74	5.28 ± 0.16
Gram-positive bacterial PLFAs	4.70 ± 0.23	4.45 ± 0.92	2.99 ± 0.16	4.38 ± 0.15	4.57 ± 0.10	6.33 ± 0.18
Actinobacterial PLFAs	0.46 ± 0.03	0.51 ± 0.11	0.31 ± 0.02	0.50 ± 0.02	0.43 ± 0.01	0.72 ± 0.03
Bacterial PLFAs	10.01 ± 0.75	7.26 ± 1.54	5.22 ± 0.25	7.40 ± 0.30	10.59 ± 0.76	11.60 ± 0.34
Fungal PLFAs	1.09 ± 0.07	0.85 ± 0.17	0.63 ± 0.07	0.83 ± 0.06	1.11 ± 0.08	1.27 ± 0.03
Arbuscular mycorrhizal fungal PLFAs	0.13 ± 0.01	0.22 ± 0.13	0.07 ± 0.00	0.43 ± 0.03	0.12 ± 0.00	1.12 ± 0.03
Total PLFAs	11.24 ± 0.77	$\textbf{8.33} \pm \textbf{1.82}$	5.93 ± 0.28	8.65 ± 0.39	11.82 ± 0.83	13.99 ± 0.37
Gram-negative/gram-positive bacterial PLFA ratio	1.12 ± 0.09	$\boldsymbol{0.62 \pm 0.02}$	$\boldsymbol{0.75 \pm 0.03}$	0.69 ± 0.01	1.32 ± 0.16	$\textbf{0.83} \pm \textbf{0.01}$
Fungal/bacterial PLFA ratio	0.11 ± 0.1	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
<i>Cy</i> 17:0/16:1 <i>w</i> 7 <i>c</i> ratio	0.55 ± 0.01	0.55 ± 0.03	0.59 ± 0.02	0.65 ± 0.02	0.57 ± 0.01	0.66 ± 0.01
<i>Cy</i> 19:0/18:1 <i>w</i> 7 <i>c</i> ratio	5.92 ± 3.22	13.8 ± 0.48	14.58 ± 0.63	7.19 ± 0.44	6.19 ± 3.45	3.94 ± 0.09
Monoenoic to saturated PLFAs ratio ^c	1.48 ± 0.14	$\textbf{0.84} \pm \textbf{0.03}$	0.91 ± 0.06	1.57 ± 0.02	1.61 ± 0.19	2.01 ± 0.02
Solvent extractable compounds ($\mu g g^{-1}$ soil) and ratios						
<i>n</i> -Alkanes ($\geq C_{20}$)	14.85 ± 1.16	8.57 ± 0.91	7.1 ± 0.51	7.22 ± 0.51	17.85 ± 1.04	11.6 ± 0.75
<i>n</i> -Alkanols	23.66 ± 0.76	19.17 ± 1.17	14.86 ± 0.75	15.1 ± 0.45	$\textbf{27.14} \pm \textbf{0.66}$	22.76 ± 0.19
Short chain ($<$ C ₂₀)	1.91 ± 0.14	2.24 ± 0.16	1.75 ± 0.21	2.34 ± 0.16	2.07 ± 0.13	1.81 ± 0.22
Long chain ($\geq C_{20}$)	21.76 ± 0.7	16.93 ± 1.23	13.11 ± 0.56	12.76 ± 0.43	25.07 ± 0.66	20.94 ± 0.27
<i>n</i> -Alkanoic acids	73.67 ± 3.93	51.33 ± 4.01	40.46 ± 3.01	41.19 ± 2.42	90.35 ± 4.01	71.89 ± 2.22
Short chain acids ($)$	19.05 ± 0.86	13.23 ± 0.98	12.03 ± 0.39	12.19 ± 1.05	20.24 ± 1.07	19.75 ± 0.27
Long chain acids ($\geq C_{20}$)	47.21 ± 3.3	34.66 ± 2.98	$\textbf{25.54} \pm \textbf{3.03}$	26.57 ± 1.85	59.52 ± 3.66	41.93 ± 2.34
Unsaturated <i>n</i> -alkanoic acids	7.41 ± 0.66	$\textbf{3.44} \pm \textbf{0.48}$	$\textbf{2.89} \pm \textbf{0.23}$	$\textbf{2.42} \pm \textbf{0.16}$	10.59 ± 1.53	10.21 ± 0.51
<i>n</i> -Alkane- α , ω -dioic acids	0.79 ± 0.07	0.67 ± 0.11	0.62 ± 0.1	0.5 ± 0.06	1.08 ± 0.09	0.99 ± 0.05
Total Aliphatic Compounds ^d	112.97 ± 5.02	79.74 ± 5.85	63.05 ± 4.15	64.01 ± 2.59	136.42 ± 4.81	107.23 ± 2.83
Steroids	21.67 ± 1.5	11.6 ± 2.06	9.09 ± 0.26	8.75 ± 0.78	26.66 ± 3	21.17 ± 0.73

Plant-derived steroids	18.11 ± 1.23	10.02 ± 1.88	7.89 ± 0.19	7.48 ± 0.77	22.4 ± 2.38	18.31 ± 0.62
Ergosterol	1.6 ± 0.22	0.65 ± 0.09	0.49 ± 0.08	0.42 ± 0.03	1.7 ± 0.45	0.97 ± 0.1
Diterpenoids	0.44 ± 0.07	0.29 ± 0.04	0.1 ± 0.04	0.17 ± 0.03	0.29 ± 0.02	0.29 ± 0.05
Triterpenoids	37.05 ± 6.48	27.85 ± 3.56	16.64 ± 1.14	16.72 ± 1.69	49.77 ± 8.01	41.84 ± 1.62
Total Cyclic Compounds ^e	59.15 ± 7.85	39.74 ± 5.55	25.84 ± 1.2	25.65 ± 2.06	76.72 ± 10.71	63.3 ± 1.86
Aliphatic/cyclic compound ratio	2.07 ± 0.25	2.13 ± 0.18	2.45 ± 0.13	2.59 ± 0.26	1.95 ± 0.25	1.7 ± 0.06
Steroid degradation ratio	7.66 ± 0.62	8.17 ± 0.58	8.09 ± 0.44	10.36 ± 1.69	$\textbf{4.86} \pm \textbf{0.39}$	5.3 ± 0.3
Sugars	36.75 ± 1.47	29.79 ± 4.02	20.41 ± 3.45	29.15 ± 2.19	26.39 ± 4.12	29.46 ± 8.73
Cutin-, suberin-, and microbial-derived compounds (µg g ⁻¹	soil)					
Cutin-derived	60.59 ± 6.81	72.19 ± 3.27	61.34 ± 2.75	54.54 ± 3.45	42.07 ± 3.92	42.82 ± 2.15
Suberin-derived	142.31 ± 16.54	175.9 ± 16.56	158.81 ± 18.92	125.75 ± 17.55	158.46 ± 19.07	158.41 ± 13.89
Cutin- or suberin-derived	126.14 ± 23.38	62.85 ± 5.97	60.57 ± 7.97	50.43 ± 5.26	143.49 ± 27.86	189.82 ± 20.03
Microbial-derived	344.53 ± 41.84	207.77 ± 23.76	155.29 ± 12.67	172.67 ± 15.25	284.15 ± 20.8	341.35 ± 19.77
Lignin-derived compounds (µg g ⁻¹ soil) and ratios						
Vanillyls	315.03 ± 25.33	212.86 ± 37.42	258.76 ± 4.33	260.73 ± 29.09	336.58 ± 15.23	355.56 ± 16.27
Syringyls	220.59 ± 22.06	126.74 ± 31.28	157 ± 1.85	161.58 ± 19.92	238.5 ± 13.78	248.87 ± 11.74
Cinnamyls	104.66 ± 15.09	55.12 ± 11.25	72.3 ± 4.02	68.77 ± 5.58	113.72 ± 8.59	98.52 ± 3.04
Lignin-derived phenol monomers ^f	640.28 ± 62.11	394.73 ± 78.84	488.06 ± 7.42	491.08 ± 53.19	688.8 ± 36.86	702.95 ± 28.31
% Vanillyl	49.63 ± 0.96	55.19 ± 1.37	53.02 ± 0.45	53.01 ± 0.45	48.97 ± 0.53	50.53 ± 0.53
% Syringyl	34.4 ± 0.21	30.75 ± 1.43	$\textbf{32.18} \pm \textbf{0.33}$	32.59 ± 0.89	34.61 ± 0.44	35.35 ± 0.27
% Cinnamyl	15.98 ± 0.89	14.06 ± 0.84	14.8 ± 0.71	14.4 ± 1.13	16.42 ± 0.45	14.12 ± 0.64
Cinnamyl/Vanillyl	0.32 ± 0.02	0.26 ± 0.02	0.28 ± 0.02	0.27 ± 0.02	0.34 ± 0.01	0.28 ± 0.02
Syringyl/Vanillyl	0.69 ± 0.02	0.56 ± 0.04	0.61 ± 0	0.61 ± 0.02	0.71 ± 0.01	0.7 ± 0.01
(Ad/Al) _{Syringyl} ^g	1.3 ± 0.03	1.06 ± 0.06	1.07 ± 0.03	$\boldsymbol{0.97 \pm 0.03}$	1.22 ± 0.02	1.26 ± 0.03
(Ad/Al) _{Vanillyl} ^h	1.08 ± 0.02	$\boldsymbol{0.89 \pm 0.08}$	0.95 ± 0.02	0.87 ± 0.05	0.95 ± 0.01	0.98 ± 0.03

^aRelative stage of SOM decomposition: Alkyl/*O*-alkyl

^bResistance to SOM decomposition: Alkyl + aromatic & phenolic)/(*O*-alkyl + carboxyl & carbonyl) ^cMonoenoic to saturated PLFAs ratio: $(16:1\omega7 + 16:1\omega5 + 18:1\omega9 + 18:1\omega7) / (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 23:0 + 24:0)$

^dTotal Aliphatic Compounds: Sum of *n*-alkanes + *n*-alkanols + *n*-alkanoic acids + ω -hydroxyalkanoic acids + *n*-alkane- α, ω -dioic acids

^eTotal Cyclic Compounds: Sum of steroids + diterpenoids + triterpenoids

^fTotal extractable lignin phenol monomers: sum of vanillyl (acetovanillone, vanillic acid, and vanillin), syringyl (acetosyringone, syringaldehyde, and syringic acid), and cinnamyl (ferulic acid and *p*-coumaric acid)

^g(Ad/Al)_{Syringyl}: syringic acid/syringaldehyde ^h(Ad/Al)_{Vanillyl}: vanillic acid/vanillin

Table S2 Significance values for the carbon and nitrogen concentrations for detrital manipulation treatments compared to the control. If $p \le 0.05$ (bolded), the treatment was significantly different from the control. Refer to Table S1 for values that were tested.

	No Litter	No Roots	No Inputs	Double Litter	Double Wood
Soil carbon and nitrogen			•		
Carbon concentration	1.52E-11	1.40E-11	1.15E-12	7.67E-06	3.06E-03
Nitrogen concentration	5.00E-12	4.89E-12	4.05E-13	8.43E-06	1.67E-04
Phospholipid fatty acids (PLFAs) and ratios					
Gram-negative bacterial PLFAs	2.02E-08	7.12E-10	8.03E-08	7.82E-02	1.00E+00
Gram-positive bacterial PLFAs	1.00E+00	8.28E-04	1.00E+00	1.00E+00	3.20E-03
Actinobacterial PLFAs	1.00E+00	9.98E-02	1.00E+00	1.00E+00	1.60E-02
Bacterial PLFAs	1.05E-03	8.94E-07	1.60E-03	1.00E+00	2.20E-01
Fungal PLFAs	1.66E-01	5.74E-04	9.47E-02	1.00E+00	7.56E-01
Arbuscular mycorrhizal fungal PLFAs	1.00E+00	1.00E+00	9.57E-03	1.00E+00	6.64E-10
Total PLFAs	2.72E-03	2.25E-06	5.56E-03	1.00E+00	5.16E-02
Gram-negative/gram-positive bacterial PLFA ratio	3.36E-12	4.85E-10	4.56E-11	1.61E-05	5.19E-08
Bacterial/fungal PLFA ratio	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00
$Cy17:0/16:1\omega7c$ ratio	1.00E+00	3.30E-01	4.56E-04	1.00E+00	5.35E-05
$Cy19:0/18:1\omega7c$ ratio	4.90E-11	9.89E-12	2.61E-01	1.00E+00	1.02E-02
Monoenoic to saturated PLFAs ratio	1.86E-12	1.19E-11	2.27E-01	1.11E-02	3.68E-11
Solvent extractable compounds and ratios					
<i>n</i> -Alkanes ($\geq C_{20}$)	1.90E-06	8.41E-08	1.05E-07	9.98E-03	4.96E-03
<i>n</i> -Alkanols	1.69E-05	6.28E-10	9.88E-10	3.93E-04	7.08E-01
Short chain ($<$ C ₂₀)	3.04E-01	9.19E-01	1.10E-01	8.89E-01	9.90E-01
Long chain ($\geq C_{20}$)	1.31E-06	1.34E-10	6.92E-11	1.92E-04	7.08E-01
<i>n</i> -Alkanoic acids	5.53E-05	1.98E-07	2.77E-07	1.74E-03	1.00E+00
Short chain acids ($<$ C ₂₀)	8.43E-04	8.98E-05	1.20E-04	1.00E+00	1.00E+00
Long chain acids ($\geq C_{20}$)	1.60E-03	1.79E-06	3.58E-06	1.94E-03	3.63E-01
Unsaturated <i>n</i> -alkanoic acids	2.93E-07	3.95E-08	8.33E-09	7.05E-06	3.72E-05
<i>n</i> -Alkane- α , ω -dioic acids	6.80E-01	3.65E-01	2.33E-02	1.75E-02	1.80E-01
Total Aliphatic Compounds	4.44E-06	9.62E-09	1.31E-08	3.76E-04	7.55E-01
Steroids	3.99E-07	1.29E-08	8.44E-09	2.69E-03	9.97E-01
Plant-derived steroids	5.66E-07	1.57E-08	8.37E-09	1.73E-03	1.00E+00
Ergosterol	6.42E-05	9.02E-06	3.90E-06	9.82E-01	5.33E-03
Diterpenoids	1.17E-01	7.68E-05	1.02E-03	9.67E-02	1.03E-01
Triterpenoids	1.91E-01	4.51E-04	4.71E-04	3.30E-02	7.94E-01
Total Cyclic Compounds	7.07E-03	1.72E-05	1.60E-05	1.62E-02	9.46E-01
Aliphatic/cyclic compound ratio	9.96E-01	6.28E-02	5.75E-03	9.21E-01	7.83E-02
Steroid degradation ratio	9.47E-01	9.76E-01	2.53E-03	1.80E-03	8.80E-03

Sugars	8.00E-01	7.55E-02	7.38E-01	4.45E-01	7.69E-01
Cutin-, suberin-, and microbial-derived compounds					
Cutin-derived	8.65E-01	1.00E+00	9.91E-01	9.15E-01	9.53E-01
Suberin-derived	5.75E-01	9.61E-01	9.61E-01	9.64E-01	9.65E-01
Cutin- or suberin-derived	2.91E-04	1.92E-04	3.21E-05	6.40E-01	2.71E-04
Microbial-derived	6.62E-04	1.23E-05	4.37E-05	2.38E-01	1.00E+00
Lignin-derived compounds and ratios					
Vanillyls	3.47E-05	1.74E-02	2.27E-02	7.17E-01	1.32E-01
Syringyls	1.15E-06	2.02E-04	4.77E-04	5.85E-01	1.50E-01
Cinnamyls	4.18E-05	4.66E-03	1.72E-03	8.26E-01	9.60E-01
Lignin-derived phenol monomers	3.26E-06	1.22E-03	1.50E-03	6.21E-01	3.59E-01
% Vanillyl	2.44E-06	1.18E-03	1.22E-03	9.24E-01	7.69E-01
% Syringyl	1.29E-05	4.04E-03	2.18E-02	9.98E-01	4.30E-01
% Cinnamyl	3.91E-01	8.23E-01	5.95E-01	9.97E-01	4.23E-01
Cinnamyl/Vanillyl	5.05E-02	3.44E-01	2.04E-01	9.94E-01	3.54E-01
Syringyl/Vanillyl	5.26E-09	3.04E-06	1.09E-05	8.39E-01	9.95E-01
(Ad/Al) _{Syringyl}	1.74E-04	3.72E-04	3.30E-06	4.34E-01	9.37E-01
(Ad/Al) _{Vanillyl}	1.20E-04	4.64E-03	3.50E-05	7.21E-03	5.67E-02
Total extractable lignin phenol dimers	6.30E-01	1.00E+00	9.99E-01	9.98E-01	6.12E-01
Monomers/dimers	1.00E+00	6.71E-01	8.62E-01	1.00E+00	2.32E-01

Table S3 Chemical and microbial indices of soils after detrital manipulation treatments at the Sikfőkút Experimental Forest. The concentration of the second	rations ($\mu g g^{-1} C$) and ratios are
expressed as the mean \pm SE for both field (n=3) and analytical replicates (n=2). Bolded values denote significant differences ($p \le 0.05$) bet	ween the treatment and the control

	Control	No Litter	No Roots	No Inputs	Double Litter	Double Wood
Phospholipid fatty acids (PLFAs; $\mu g g^{-1}$ soil) and ratios						
Gram-negative bacterial PLFAs	101.42 ± 7.55	76.16 ± 10.45	63.82 ± 3.02	92.96 ± 3.29	102.26 ± 11.83	92.48 ± 3.49
Gram-positive bacterial PLFAs	90.76 ± 3.23	122.23 ± 14.81	85.31 ± 4.36	135.31 ± 3.94	78.11 ± 2.1	110.85 ± 3.99
Actinobacterial PLFAs	8.91 ± 0.41	13.83 ± 1.92	8.81 ± 0.58	15.48 ± 0.53	7.41 ± 0.24	12.62 ± 0.67
Bacterial PLFAs	192.18 ± 8.27	198.38 ± 25.08	149.13 ± 6.94	228.27 ± 6.98	180.37 ± 11.5	203.33 ± 7.44
Fungal PLFAs	21.24 ± 1.53	23.28 ± 3.23	17.92 ± 1.78	25.49 ± 1.6	18.97 ± 1.09	22.27 ± 0.62
Arbuscular mycorrhizal fungal PLFAs	2.6 ± 0.14	5.42 ± 2.78	2.1 ± 0.13	13.06 ± 0.63	2.11 ± 0.06	19.57 ± 0.64
Total PLFAs	216.02 ± 8.08	227.09 ± 30.02	169.15 ± 7.53	266.82 ± 8.89	201.45 ± 12.44	245.18 ± 8.43
Gram-negative/gram-positive bacterial PLFA ratio	1.12 ± 0.09	0.62 ± 0.02	0.75 ± 0.03	0.69 ± 0.01	1.32 ± 0.16	0.83 ± 0.01
Fungal/bacterial PLFA ratio	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0	0.11 ± 0	0.11 ± 0
$Cy17:0/16:1\omega7c$ ratio	0.55 ± 0.01	0.55 ± 0.03	0.59 ± 0.02	0.65 ± 0.02	0.57 ± 0.01	0.66 ± 0.01
$Cy19:0/18:1\omega7c$ ratio	5.92 ± 3.22	13.8 ± 0.48	14.58 ± 0.63	7.19 ± 0.44	6.19 ± 3.45	3.94 ± 0.09
Monoenoic to saturated PLFAs ratio ^a	1.48 ± 0.14	$\boldsymbol{0.84 \pm 0.03}$	0.91 ± 0.06	1.57 ± 0.02	1.61 ± 0.19	$\textbf{2.01} \pm \textbf{0.02}$
Solvent extractable compounds ($\mu g g^{-1}$ soil) and ratios						
<i>n</i> -Alkanes ($\geq C_{20}$)	286.6 ± 18.58	280.63 ± 34.58	$\textbf{202.12} \pm \textbf{12}$	223.72 ± 17.84	304.46 ± 14.49	$\textbf{202.83} \pm \textbf{12.04}$
<i>n</i> -Alkanols	461.11 ± 26.54	633.41 ± 66.69	423.39 ± 16.9	465.89 ± 8.14	464.33 ± 13.15	398.59 ± 7.47
Short chain (<c<sub>20)</c<sub>	37.05 ± 3.04	75.42 ± 9.69	49.78 ± 5.41	72.46 ± 5.44	35.51 ± 2.34	31.74 ± 3.91
Long chain ($\geq C_{20}$)	424.06 ± 24.75	557.98 ± 61.58	373.62 ± 11.91	393.43 ± 6.02	428.81 ± 12.59	366.85 ± 7.78
<i>n</i> -Alkanoic acids	1440.8 ± 117.33	1665.34 ± 111.23	1153.56 ± 79.9	1281.65 ± 100.09	1541.88 ± 54.56	1256.85 ± 27.71
Short chain acids ($)$	369.93 ± 19.95	429.28 ± 23.2	343.61 ± 11.88	379.91 ± 40.03	345.5 ± 16.73	345.87 ± 7.2
Long chain acids ($\geq C_{20}$)	926.71 ± 92.26	1127.18 ± 95.74	727.03 ± 81.8	827.34 ± 72.71	1017.07 ± 61.53	732.05 ± 33.37
Unsaturated <i>n</i> -alkanoic acids	144.16 ± 14.52	108.87 ± 9.62	82.92 ± 7.65	74.39 ± 3.04	179.31 ± 23.63	178.93 ± 9.21
<i>n</i> -Alkane- α , ω -dioic acids	15.39 ± 1.75	21.44 ± 3.04	17.72 ± 2.66	15.77 ± 2.17	18.43 ± 1.34	17.27 ± 0.98
Total Aliphatic Compounds ^b	2203.9 ± 152.65	2600.82 ± 207.95	1796.79 ± 104.91	1987.03 ± 116.85	2329.1 ± 61.71	1875.54 ± 37.6
Steroids	419.54 ± 27.93	377.57 ± 72.12	$\textbf{259.78} \pm \textbf{8.82}$	267.85 ± 15.92	452.7 ± 45.51	370.9 ± 14.49
Plant-derived steroids	350.12 ± 20.96	326.1 ± 65.48	225.52 ± 6.77	228.32 ± 16.71	380.54 ± 35.92	320.72 ± 12.64
Ergosterol	31.5 ± 5.26	20.81 ± 2.7	14.02 ± 2.36	13.06 ± 0.84	28.64 ± 7.27	16.91 ± 1.87
Diterpenoids	8.47 ± 1.33	9.5 ± 1.11	2.9 ± 1.21	5.3 ± 1.03	4.89 ± 0.33	5.06 ± 0.83
Triterpenoids	718.46 ± 131.74	903.36 ± 118.91	473.46 ± 27	514.96 ± 51.57	842.11 ± 126.59	733.12 ± 32.48
Total Cyclic Compounds ^c	1146.47 ± 158.04	1290.43 ± 189.84	736.13 ± 26.15	788.12 ± 54.09	1299.71 ± 166.33	1109.08 ± 39.8
Aliphatic/cyclic compound ratio	2.07 ± 0.25	2.13 ± 0.18	2.45 ± 0.13	2.59 ± 0.26	1.95 ± 0.25	1.7 ± 0.06
Steroid degradation ratio	7.66 ± 0.62	8.17 ± 0.58	8.09 ± 0.44	10.36 ± 1.69	$\textbf{4.86} \pm \textbf{0.39}$	5.3 ± 0.3
Sugars	711.1 ± 22.72	957.53 ± 105.4	578.18 ± 89.79	903.9 ± 75.81	450.42 ± 69.17	521.38 ± 159.75
Cutin-, suberin-, and microbial-derived compounds (µg g	' soil)					
Cutin-derived	1188.32 ± 151.41	2113.15 ± 151.61	1751.94 ± 79.96	1680.79 ± 91.84	847.53 ± 182.18	904.14 ± 166.08

Suberin-derived	2803.25 ± 401	5182 ± 576.89	4533.73 ± 531.47	3852.58 ± 513.05	2708.3 ± 323.41	2773.16 ± 245.58
Cutin- or suberin-derived	2387.23 ± 362.66	1848.59 ± 233.8	1719.06 ± 206.83	1541.59 ± 129.03	2428.07 ± 447.42	3308.35 ± 324
Microbial-derived	6598.07 ± 639.63	5975.68 ± 623.09	4433.33 ± 368.62	5282.29 ± 330.91	4852.3 ± 350.14	5967.16 ± 316.39
Lignin-derived compounds (µg g ⁻¹ soil) and ratios						
Vanillyls	6069.99 ± 380.26	5859.2 ± 547.55	7391.49 ± 144.52	7959.94 ± 698.01	5764.6 ± 311.21	6217.91 ± 260.87
Syringyls	4238.89 ± 334.07	3393.65 ± 561.67	4484.59 ± 69.75	4927.21 ± 499.72	4084.91 ± 267.12	4352.52 ± 191.37
Cinnamyls	1998.77 ± 233.86	1513.42 ± 205.94	2060.61 ± 99.32	2115.5 ± 140.5	1948.42 ± 160.85	1725.93 ± 62.49
	$12307.64 \pm$	$10766.26 \pm$	$13936.69 \pm$	$15002.65 \pm$	$11797.93 \pm$	$12296.35 \pm$
Lignin-derived phenol monomers ^d	931.19	1276.64	195.28	1274.96	726.19	459.77
% Vanillyl	49.63 ± 0.96	55.19 ± 1.37	53.02 ± 0.45	53.01 ± 0.45	48.97 ± 0.53	50.53 ± 0.53
% Syringyl	34.4 ± 0.21	30.75 ± 1.43	$\textbf{32.18} \pm \textbf{0.33}$	$\textbf{32.59} \pm \textbf{0.89}$	34.61 ± 0.44	35.35 ± 0.27
% Cinnamyl	15.98 ± 0.89	14.06 ± 0.84	14.8 ± 0.71	14.4 ± 1.13	16.42 ± 0.45	14.12 ± 0.64
Cinnamyl/Vanillyl	0.32 ± 0.02	0.26 ± 0.02	0.28 ± 0.02	0.27 ± 0.02	0.34 ± 0.01	0.28 ± 0.02
Syringyl/Vanillyl	0.69 ± 0.02	0.56 ± 0.04	0.61 ± 0	0.61 ± 0.02	0.71 ± 0.01	0.7 ± 0.01
(Ad/Al) _{Syringyl} ^e	1.3 ± 0.03	1.06 ± 0.06	1.07 ± 0.03	$\boldsymbol{0.97 \pm 0.03}$	1.22 ± 0.02	1.26 ± 0.03
(Ad/Al) _{Vanillyl} ^f	1.08 ± 0.02	$\boldsymbol{0.89 \pm 0.08}$	$\boldsymbol{0.95 \pm 0.02}$	$\boldsymbol{0.87 \pm 0.05}$	$\boldsymbol{0.95 \pm 0.01}$	0.98 ± 0.03

^aMonoenoic to saturated PLFAs ratio: $(16:1\omega7 + 16:1\omega5 + 18:1\omega9 + 18:1\omega7) / (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 23:0 + 24:0)$ ^bTotal Aliphatic Compounds: Sum of *n*-alkanes + *n*-alkanols + *n*-alkanoic acids + ω -hydroxyalkanoic acids + *n*-alkane- α , ω -dioic acids

^cTotal Cyclic Compounds: Sum of steroids + diterpenoids + triterpenoids

^dTotal extractable lignin phenol monomers: sum of vanillyl (acetovanillone, vanillic acid, and vanillin), syringyl (acetosyringone, syringaldehyde, and syringic acid), and cinnamyl (ferulic acid and *p*-coumaric acid)

°(Ad/Al)_{Syringyl}: syringic acid/syringaldehyde ^f(Ad/Al)_{Vanillyl}: vanillic acid/vanillin

Table S4 Significance values for the carbon and nitrogen concentrations for detrital manipulation treatments compared to the control. If $p \le 0.05$ (bolded), the treatment was significantly different from the control. Refer to Table S2 for values that were tested.

	No Litter	No Roots	No Inputs	Double Litter	Double Wood
Phospholipid fatty acids (PLFAs) and ratios			-		
Gram-negative bacterial PLFAs	1.12E-03	7.70E-06	1.00E+00	1.00E+00	1.00E+00
Gram-positive bacterial PLFAs	1.08E-02	1.00E+00	2.45E-04	1.00E+00	3.27E-01
Actinobacterial PLFAs	8.53E-04	1.00E+00	1.37E-03	9.74E-01	4.67E-01
Bacterial PLFAs	1.00E+00	2.84E-02	1.93E-01	1.00E+00	1.00E+00
Fungal PLFAs	1.00E+00	1.00E+00	8.16E-01	1.00E+00	1.00E+00
Arbuscular mycorrhizal fungal PLFAs	1.00E+00	1.00E+00	4.98E-05	1.00E+00	4.53E-08
Total PLFAs	1.00E+00	8.22E-02	7.58E-02	1.00E+00	1.00E+00
Gram-negative/gram-positive bacterial PLFA ratio	3.36E-12	4.85E-10	4.56E-11	1.61E-05	5.19E-08
Bacterial/fungal PLFA ratio	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00
$Cy17:0/16:1\omega7c$ ratio	1.00E+00	3.30E-01	4.56E-04	1.00E+00	5.35E-05
$Cy19:0/18:1\omega7c$ ratio	4.90E-11	9.89E-12	2.61E-01	1.00E+00	1.02E-02
Monoenoic to saturated PLFAs ratio	1.86E-12	1.19E-11	2.27E-01	1.11E-02	3.68E-11
Solvent extractable compounds and ratios					
<i>n</i> -Alkanes ($\geq C_{20}$)	1.00E+00	3.27E-02	2.40E-01	1.00E+00	3.50E-02
<i>n</i> -Alkanols	1.35E-02	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Short chain (<c<sub>20)</c<sub>	6.56E-05	6.88E-01	1.80E-04	1.00E+00	1.00E+00
Long chain ($\geq C_{20}$)	3.92E-02	1.00E+00	1.00E+00	1.00E+00	1.00E+00
<i>n</i> -Alkanoic acids	4.54E-01	1.13E-01	1.00E+00	1.00E+00	1.00E+00
Short chain acids (<c<sub>20)</c<sub>	9.97E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Long chain acids ($\geq C_{20}$)	2.03E-01	2.08E-01	1.00E+00	1.00E+00	2.41E-01
Unsaturated <i>n</i> -alkanoic acids	3.12E-03	3.46E-06	5.13E-07	3.25E-03	3.63E-03
<i>n</i> -Alkane- α , ω -dioic acids	1.69E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Total Aliphatic Compounds	2.76E-01	2.40E-01	1.00E+00	1.00E+00	6.89E-01
Steroids	1.00E+00	9.33E-04	1.64E-03	1.00E+00	1.00E+00
Plant-derived steroids	1.00E+00	2.37E-03	3.00E-03	1.00E+00	1.00E+00
Ergosterol	5.11E-02	4.70E-04	2.49E-04	1.00E+00	3.33E-03
Diterpenoids	1.00E+00	3.91E-03	2.86E-01	1.42E-01	1.88E-01
Triterpenoids	7.58E-01	1.86E-01	4.98E-01	1.00E+00	1.00E+00
Total Cyclic Compounds	1.00E+00	3.47E-02	9.35E-02	1.00E+00	1.00E+00
Aliphatic/cyclic compound ratio	1.00E+00	1.01E-01	7.66E-03	1.00E+00	1.29E-01
Steroid degradation ratio	1.00E+00	1.00E+00	3.26E-03	2.29E-03	1.20E-02
Sugars	8.36E-01	1.00E+00	1.00E+00	6.63E-01	1.00E+00
Cutin-, suberin-, and microbial-derived compounds					
Cutin-derived	1.85E-03	1.24E-01	2.76E-01	1.00E+00	1.00E+00

Suberin-derived	2.65E-03	4.55E-02	7.88E-01	1.00E+00	1.00E+00
Cutin- or suberin-derived	6.25E-01	2.10E-01	4.34E-02	1.00E+00	2.19E-02
Microbial-derived	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Lignin-derived compounds and ratios					
Vanillyls	1.00E+00	3.65E-02	1.28E-03	1.00E+00	1.00E+00
Syringyls	8.92E-02	1.00E+00	3.09E-01	1.00E+00	1.00E+00
Cinnamyls	2.57E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Lignin-derived phenol monomers	9.01E-01	7.22E-01	3.77E-02	1.00E+00	1.00E+00
% Vanillyl	2.78E-06	1.47E-03	1.53E-03	1.00E+00	1.00E+00
% Syringyl	1.49E-05	5.30E-03	3.16E-02	1.00E+00	1.00E+00
% Cinnamyl	9.52E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Cinnamyl/Vanillyl	7.90E-02	7.93E-01	4.03E-01	1.00E+00	8.25E-01
Syringyl/Vanillyl	5.80E-09	3.46E-06	1.25E-05	1.00E+00	1.00E+00
(Ad/Al) _{Syringyl}	2.08E-04	4.52E-04	3.76E-06	1.00E+00	1.00E+00
(Ad/Al) _{Vanillyl}	1.42E-04	6.11E-03	4.09E-05	9.71E-03	8.98E-02
Total extractable lignin phenol dimers	1.00E+00	2.84E-01	5.78E-01	1.00E+00	1.00E+00
Monomers/dimers	1.00E+00	1.00E+00	1.00E+00	1.00E+00	4.73E-01

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