

Supplementary Materials for:

Long-term nitrogen addition suppresses microbial degradation, enhances soil carbon storage, and alters the molecular composition of soil organic matter

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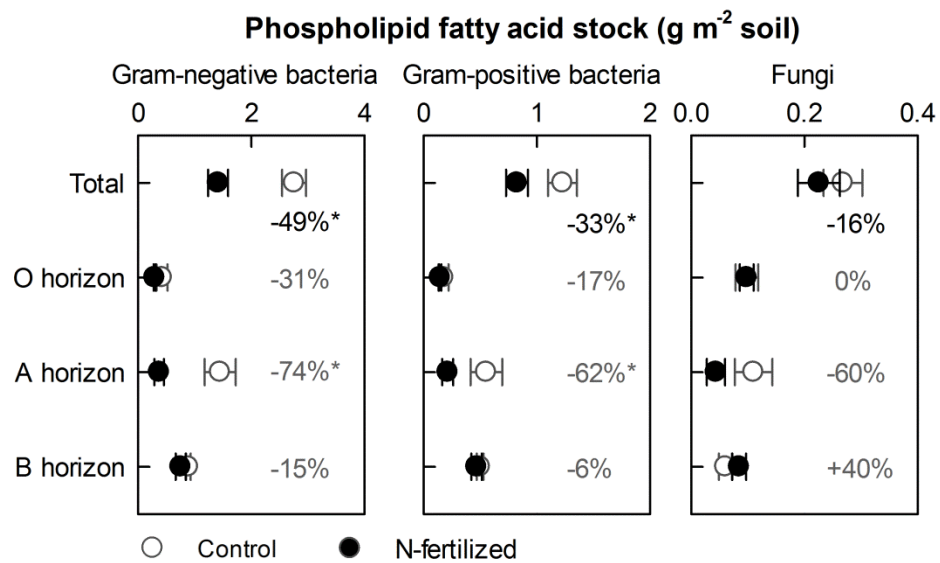


Fig. S1. Phospholipid fatty acid (PLFA) storage in control and N-fertilized plots (mean ± standard error; n=6). Percentages in figures show percent change compared to the control. “*” indicates significant difference between treatment and control at $P < 0.1$.

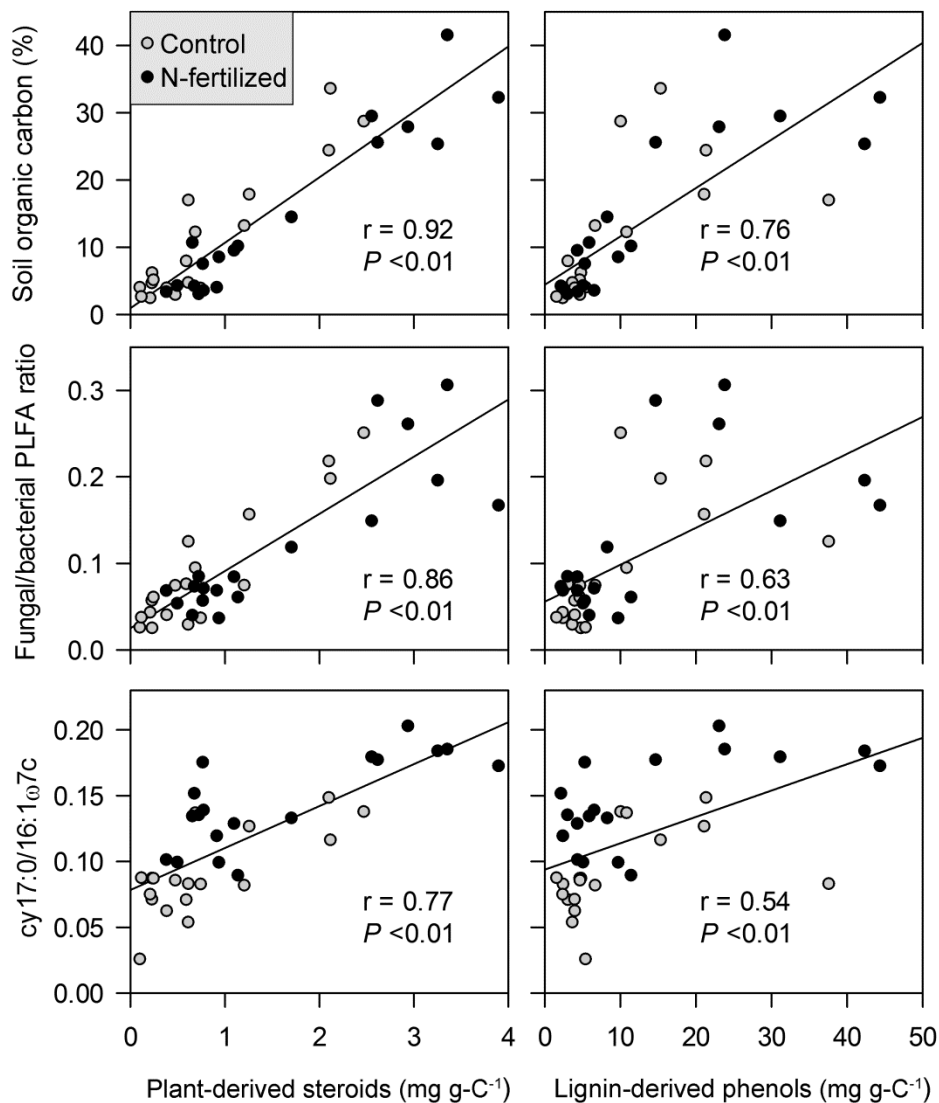


Fig. S2. Two-tailed Pearson's correlations of concentrations of plant-derived steroids and lignin-derived phenols versus soil organic carbon content, fungal/bacterial phospholipid fatty acids (PLFA) ratio, and cy17:0/16:1ω7c.

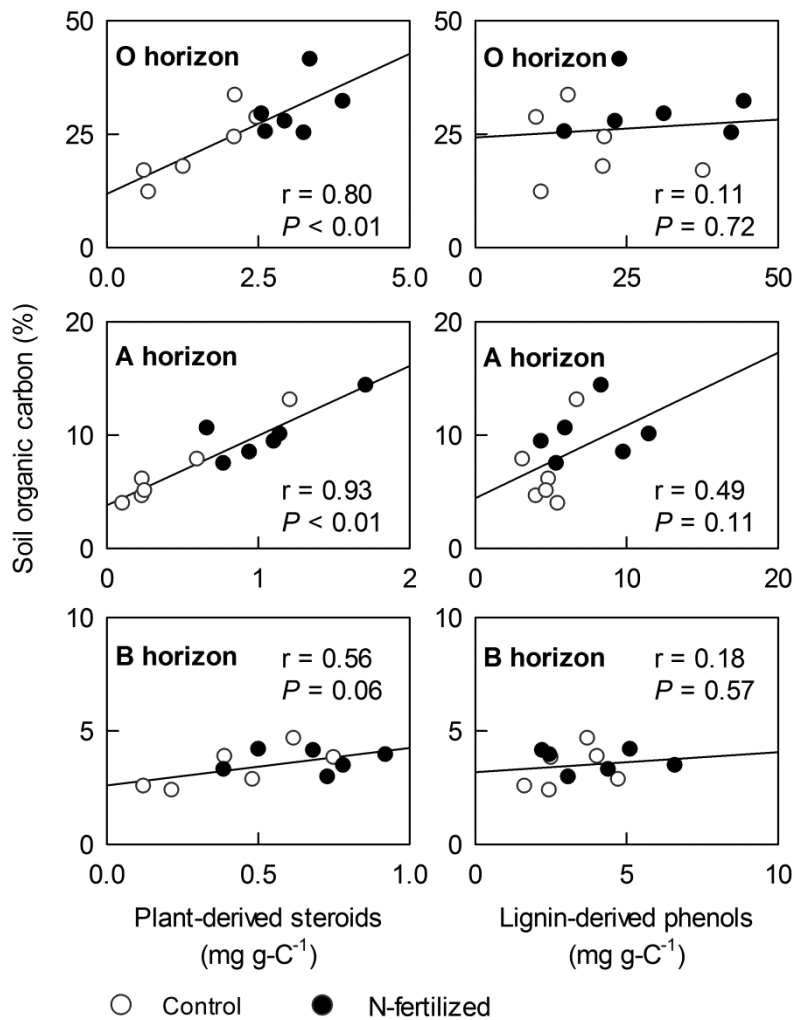


Fig. S3. Two-tailed Pearson's correlations of soil organic carbon content versus concentrations of plant-derived steroids and lignin-derived phenols for each of the soil horizons.

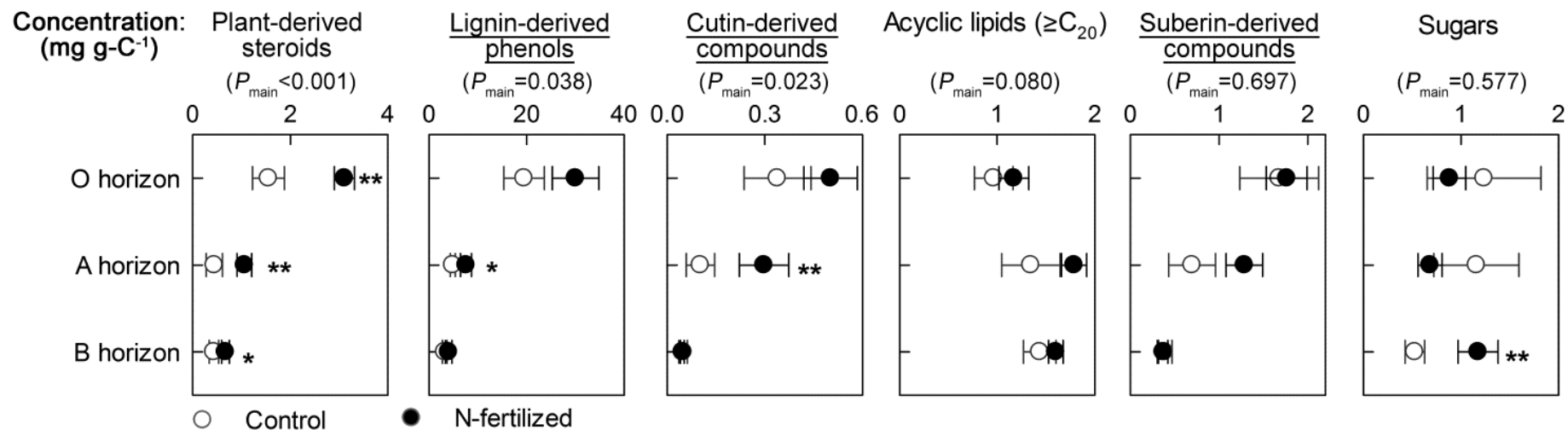


Fig. S4. Concentrations of soil organic carbon components in control and N-fertilized plots (mean \pm standard error; n=6). P_{main} refers to the main effect of N fertilization for all horizons based on two-way ANOVA; “*” and “**” indicate significant difference at $P_{\text{horizon}} < 0.1$ and $P_{\text{horizon}} < 0.01$, respectively, for the simple effect of N fertilization in a soil horizon. Biopolymer-derived compounds were underlined.

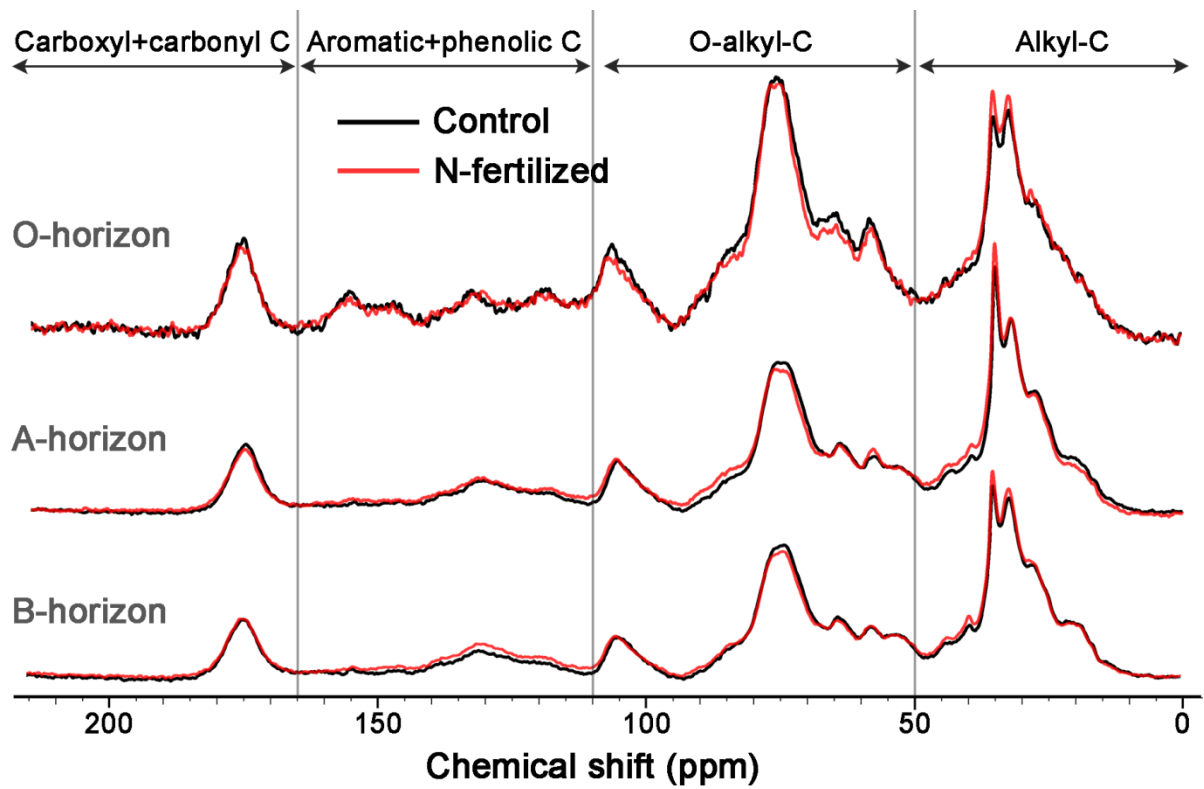


Figure S5. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectra of soil samples from control and N fertilized plots.

Table S1. Variation in average concentrations (carbon-normalized) and ratios of organic compounds in soils by N fertilization treatment. “~” indicates minor alteration within $\pm 10\%$; “-” and “—” show $>10\%$ and $>50\%$ decreases from Control, respectively; “+” and “++” show $>10\%$ and $>50\%$ increases from Control, respectively; *, and ** indicate statistically significant differences at $P < 0.1$ and $P < 0.05$, respectively.

Parameters	Unit	Indicator for	O horizon	A horizon	B horizon
Carbon content	%	C concentration	+*	+*	~
<i>Phospholipid fatty acids (PLFAs)</i>					
Gram-negative bacterial PLFAs	mg g-C ⁻¹	Bacterial biomass	—**	—**	-
Gram-positive bacterial PLFAs	mg g-C ⁻¹	Bacterial biomass	—**	—**	-
Fungal PLFA	mg g-C ⁻¹	Fungal biomass	-**	—**	+
Total PLFAs	mg g-C ⁻¹	Microbial biomass	—**	—**	-
Gram-positive/gram-negative PLFA ratio	-	Microbial composition	-**	—**	-
Fungal/bacterial PLFA ratio	-	Microbial composition	+	+	+++
Cy17:0/16:1 ω 7c PLFA ratio	-	Microbial stress	+++	+++	+++
Cy19:0/18:1 ω 7c PLFA ratio	-	Microbial stress	+++	+++	+++
Saturated/monounsaturated PLFA ratio	-	Microbial stress	+++	+++	+++
<i>Solvent-extractable compounds</i>					
Short-chain lipids (<C ₂₀)	mg g-C ⁻¹	Microbial lipids	-	-	+
Long-chain lipids (\geq C ₂₀)	mg g-C ⁻¹	Plant lipids	+	+	+
Plant-derived steroids	mg g-C ⁻¹	Plant-derived steroids	+++	+++	+++
Sugars	mg g-C ⁻¹	Simple sugars	-	-	+++
<i>Hydrolysable lipids</i>					
Cutin-derived compounds	mg g-C ⁻¹	Extractable cutin compounds	+	+++	~
Suberin-derived compounds	mg g-C ⁻¹	Extractable suberin compounds	~	++	~
Cutin/suberin ratio	-	Shoot/root contribution to soil C	+*	+	~
ω -C ₁₆ / Σ C ₁₆ ratio	-	Cutin degradation	~	-	~
<i>Lignin-derived phenols</i>					
Total lignin biomarker (VSC)	mg g-C ⁻¹	Extractable lignin phenols	++	+++	+
Syringyl/vanillyl (S/V)	-	Lignin source	-	-	-
Cinnamyl/vanillyl (C/V)	-	Lignin source	+	~	~
Acid/aldehyde for vanillyls (Ad/Al _v)	-	Lignin oxidation	~	+	~
Acid/aldehyde for syringyls (Ad/Al _s)	-	Lignin oxidation	-	+	~

Table S2. Carbon, nitrogen and organic compound storages in soils from control and N-fertilized plots (mean±SE; n=6). The unit is kg m⁻² for carbon and nitrogen storages and g m⁻² for organic compound storages. Significant difference between treatments within each horizon at $P<0.1$ are in **bold**.

Parameters	O horizon		A horizon		B horizon	
	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
Macro elements						
Carbon storage	0.60±0.10	1.13±0.05	2.18±0.45	2.43±0.24	2.55±0.28	2.69±0.15
Nitrogen storage	0.04±0.01	0.07±0.00	0.17±0.03	0.17±0.02	0.20±0.02	0.19±0.01
Phospholipid fatty acids (PLFAs)						
Gram-negative bacterial PLFAs	0.42±0.10	0.29±0.02	1.45±0.27	0.37±0.09	0.89±0.04	0.75±0.09
Gram-positive bacterial PLFAs	0.17±0.04	0.15±0.01	0.55±0.14	0.21±0.05	0.50±0.03	0.47±0.05
Total bacterial PLFAs	0.60±0.14	0.43±0.03	2.00±0.41	0.58±0.13	1.39±0.07	1.22±0.13
Fungal PLFA	0.10±0.02	0.10±0.01	0.11±0.03	0.04±0.02	0.06±0.01	0.08±0.01
Actinomycete PLFA	0.01±0.00	0.01±0.00	0.07±0.01	0.04±0.01	0.08±0.01	0.09±0.01
Total PLFAs	1.32±0.29	1.09±0.07	3.84±0.82	1.23±0.29	2.71±0.13	2.52±0.26
Solvent-extracted products/ratios						
Short-chain lipids (<C ₂₀)	0.18±0.05	0.20±0.04	0.32±0.16	0.24±0.03	0.69±0.12	0.85±0.08
Long-chain lipids (≥C ₂₀)	0.66±0.22	1.31±0.14	3.46±1.54	4.34±0.47	3.84±0.77	4.35±0.40
Plant-derived steroids	0.92±0.24	3.50±0.20	1.33±0.78	2.71±0.68	1.20±0.34	1.80±0.24
Sugars	1.09±0.42	1.55±0.23	5.34±1.99	3.40±0.93	3.95±0.76	5.63±0.39
Total solvent extractable compounds	3.02±0.91	7.40±0.54	11.04±4.68	11.17±2.20	9.83±1.99	12.82±0.65
Base-hydrolyzed products/ratios						
Short-chain aliphatics	13.64±2.47	24.86±2.95	80.65±29.4	102.78±15.79	96.30±20.26	89.48±15.1
Long-chain aliphatics	3.81±0.70	6.37±0.99	22.48±10.82	29.67±3.99	26.99±6.01	25.88±4.10
Suberin-derived compounds	3.90±0.86	6.61±0.83	22.47±8.52	31.05±4.96	29.30±6.38	26.33±4.48
Cutin-derived compounds	0.81±0.22	1.89±0.30	3.28±1.40	7.19±1.84	3.68±0.98	3.26±0.50
CuO depolymerized products/ratios						
Total vanillyls	7.66±3.11	21.35±3.69	7.08±1.62	13.14±2.58	5.86±1.13	8.02±1.60
Total syringyls	5.11±2.09	11.52±1.17	3.47±1.74	4.62±0.95	1.96±0.50	1.93±0.28
Total cinnamyls	0.13±0.02	0.62±0.05	0.54±0.22	0.88±0.20	0.54±0.14	0.69±0.06
Lignin-derived phenols	12.9±5.08	33.5±4.62	11.1±3.51	18.64±3.48	8.36±1.66	10.64±1.89

Table S3. Chemical properties of soils from control and N-fertilized plots (mean±SE; n=6). The unit is % for carbon and nitrogen contents, mg g-C⁻¹ for soil organic compound concentrations, and 1 for all ratios. Significant difference between treatments within each horizon at *P*<0.1 are in **bold**.

Parameters	O horizon		A horizon		B horizon	
	Control	N-fertilized	Control	N-fertilized	Control	N-fertilized
<i>Macro elements</i>						
Carbon content	22.20±3.27	30.24±2.47	6.77±1.38	10.06±0.98	3.34±0.37	3.65±0.20
Nitrogen content	1.45±0.20	1.89±0.14	0.52±0.08	0.70±0.07	0.27±0.02	0.26±0.01
C/N	0.07±0.00	0.06±0.00	0.08±0.00	0.07±0.00	0.08±0.00	0.07±0.00
<i>Phospholipid fatty acids (PLFAs)</i>						
Gram-negative bacterial PLFAs	0.71±0.08	0.26±0.02	0.68±0.10	0.15±0.03	0.37±0.05	0.29±0.04
Gram-positive bacterial PLFAs	0.29±0.03	0.13±0.01	0.24±0.03	0.09±0.02	0.21±0.03	0.18±0.03
Total bacterial PLFAs	1.00±0.11	0.39±0.03	0.93±0.12	0.24±0.05	0.59±0.08	0.47±0.07
Fungal PLFA	0.16±0.01	0.09±0.01	0.05±0.01	0.02±0.01	0.03±0.01	0.03±0.01
Actinomycete PLFA	0.02±0.00	0.01±0.00	0.03±0.01	0.01±0.00	0.04±0.01	0.04±0.00
Total PLFAs	2.21±0.19	0.97±0.07	1.75±0.19	0.50±0.11	1.14±0.15	0.97±0.14
Gram-negative/gram-positive	2.48±0.11	1.99±0.03	2.79±0.21	1.75±0.06	1.79±0.07	1.60±0.08
Fungi/bacteria	0.17±0.02	0.23±0.03	0.05±0.01	0.07±0.01	0.04±0.01	0.07±0.00
Cy17:0/16:1ω7c	0.12±0.01	0.18±0.00	0.07±0.01	0.13±0.01	0.07±0.01	0.12±0.01
Cy19:0/18:1ω7c	0.92±0.06	2.19±0.21	1.07±0.08	3.28±0.33	1.43±0.14	2.11±0.21
Saturated/monounsaturated	0.44±0.02	0.62±0.02	0.40±0.02	0.86±0.06	0.55±0.03	0.75±0.04
<i>Solvent-extracted products/ratios</i>						
Short-chain lipids (<C ₂₀)	0.28±0.04	0.18±0.04	0.12±0.03	0.10±0.01	0.27±0.04	0.32±0.03
Long-chain lipids (≥C ₂₀)	0.96±0.20	1.17±0.15	1.35±0.3	1.79±0.13	1.44±0.17	1.60±0.07
Plant-derived steroids	1.55±0.33	3.11±0.21	0.44±0.17	1.06±0.15	0.43±0.10	0.67±0.08
Sugars	1.72±0.53	1.36±0.18	2.10±0.56	1.33±0.23	1.49±0.13	2.13±0.20
Total solvent extractable compounds	4.78±1.07	6.54±0.37	4.19±1.02	4.45±0.47	3.68±0.40	4.79±0.18
<i>Base-hydrolyzed products/ratios</i>						
Bound short-chain aliphatics	5.69±1.36	6.59±0.76	2.50±0.91	4.26±0.65	1.27±0.27	1.27±0.18
Bound long-chain aliphatics	1.64±0.42	1.70±0.26	0.70±0.34	1.23±0.17	0.35±0.08	0.37±0.05
Suberin-derived compounds	1.68±0.45	1.76±0.23	0.70±0.26	1.29±0.21	0.38±0.08	0.37±0.05
Cutin-derived compounds	0.34±0.10	0.50±0.08	0.10±0.04	0.30±0.08	0.05±0.01	0.05±0.01
ω-C ₁₆ /ΣC ₁₆	0.34±0.02	0.33±0.02	0.34±0.02	0.31±0.01	0.34±0.01	0.34±0.00
Cutin/suberin ratio	0.20±0.03	0.28±0.03	0.15±0.03	0.25±0.05	0.12±0.01	0.13±0.01
<i>CuO depolymerized products/ratios</i>						
Total vanillyls	11.95±2.84	19.26±3.84	3.31±0.32	5.38±0.96	2.27±0.37	3.01±0.60
Total syringyls	7.30±1.81	10.19±1.00	1.31±0.32	1.84±0.21	0.72±0.13	0.72±0.09
Total cinnamyls	0.22±0.03	0.55±0.04	0.21±0.04	0.35±0.05	0.20±0.04	0.26±0.02
Lignin-derived phenols	19.47±4.14	30.00±4.76	4.83±0.50	7.57±1.14	3.19±0.48	3.99±0.70
Syringyl/vanillyl	0.75±0.22	0.59±0.06	0.41±0.09	0.37±0.04	0.32±0.04	0.26±0.02
Cinnamyl/vanillyl	0.03±0.01	0.03±0.01	0.07±0.02	0.07±0.01	0.10±0.03	0.10±0.01
Acid/aldehyde of syringyls	0.18±0.02	0.19±0.02	0.46±0.09	0.54±0.06	0.87±0.13	0.90±0.08
Acid/aldehyde of vanillyls	0.37±0.05	0.33±0.05	0.78±0.15	0.90±0.04	1.13±0.18	1.05±0.07

Table S4. Statistical analysis results of two-way ANOVA indicating the main and simple effects of N fertilization on soil carbon, nitrogen, and organic compound storages. Significant values at $P < 0.1$ are in **bold**.

Parameters	Total		O horizon		A horizon		B horizon	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
<i>Macro elements</i>								
Carbon	1.4	0.266	23.0	0.001	0.2	0.637	0.2	0.657
Nitrogen	0.2	0.650	21.5	0.001	0.0	0.969	0.3	0.587
<i>Phospholipid fatty acids (PLFAs)</i>								
Gram-negative bacterial PLFAs	23.6	0.001	1.8	0.211	14.0	0.004	2.0	0.193
Gram-positive bacterial PLFAs	6.3	0.031	0.4	0.533	5.3	0.044	0.3	0.574
Total bacterial PLFAs	16.5	0.002	1.3	0.289	11.0	0.008	1.3	0.283
Fungal PLFA	0.7	0.420	0.0	0.990	3.2	0.103	2.1	0.179
Actinomycete PLFA	2.6	0.141	0.2	0.677	6.7	0.027	1.6	0.235
Total PLFAs	11.1	0.008	0.6	0.452	8.9	0.014	0.4	0.546
<i>Solvent-extracted products/ratios</i>								
Short-chain lipids (<C ₂₀)	0.1	0.731	0.0	0.854	0.2	0.666	1.3	0.286
Long-chain lipids (≥C ₂₀)	0.8	0.379	6.4	0.030	0.3	0.598	0.3	0.574
Plant-derived steroids	8.2	0.017	68.5	<0.001	1.8	0.214	2.1	0.180
Sugars	0.0	0.949	0.9	0.359	0.8	0.397	3.9	0.076
Total solvent extractable compounds	1.0	0.335	17.3	0.002	0.0	0.981	2.0	0.183
<i>Base-hydrolyzed products/ratios</i>								
Bound short-chain aliphatics	0.2	0.664	8.5	0.015	0.4	0.522	0.1	0.793
Bound long-chain aliphatics	0.2	0.651	4.5	0.060	0.4	0.547	0.0	0.882
Suberin-derived compounds	0.2	0.652	5.2	0.046	0.8	0.405	0.1	0.711
Cutin-derived compounds	2.3	0.158	8.6	0.015	2.8	0.122	0.1	0.710
<i>CuO depolymerized products/ratios</i>								
Total vanillyls	8.4	0.016	8.1	0.018	4.0	0.074	1.2	0.296
Total syringyls	4.5	0.059	7.2	0.023	0.3	0.574	0.0	0.955
Total cinnamyls	5.0	0.050	73.1	<0.001	1.3	0.281	1.0	0.347
Lignin-derived phenols	8.8	0.014	9.0	0.013	2.3	0.158	0.8	0.386

Table S5. Statistical analysis results of two-way ANOVA indicating the main and simple effects of N fertilization on concentrations and ratios of carbon, nitrogen, and organic compounds. Significant values at $P < 0.1$ are in **bold**.

Parameters	Main effects		Simple effects					
	F-value	P-value	O horizon		A horizon		B horizon	
			F-value	P-value	F-value	P-value	F-value	P-value
<i>Macro elements</i>								
Carbon	6.8	0.014	3.8	0.079	3.8	0.081	0.5	0.478
Nitrogen	4.9	0.035	3.1	0.109	2.3	0.157	0.0	0.860
C/N	17.1	<0.001	1.8	0.209	9.7	0.011	6.2	0.032
<i>Phospholipid fatty acids (PLFAs)</i>								
Gram-negative bacterial PLFAs	52.7	<0.001	28.6	<0.001	27.1	<0.001	1.8	0.208
Gram-positive bacterial PLFAs	31.2	<0.001	21.8	<0.001	23.4	<0.001	0.6	0.443
Total bacterial PLFAs	48.4	<0.001	27.3	<0.001	27.9	<0.001	1.3	0.280
Fungal PLFA	21.3	<0.001	20.3	<0.001	10.3	0.009	0.5	0.496
Actinomycete PLFA	9.6	0.004	4.7	0.055	11.3	0.007	0.0	0.967
Total PLFAs	52.0	<0.001	35.7	<0.001	31.6	<0.001	0.7	0.423
Gram-negative/gram-positive	41.6	<0.001	18.8	<0.001	23.0	<0.001	3.3	0.100
Fungi/bacteria	5.4	0.027	2.2	0.166	0.7	0.429	12.0	0.006
Cy17:0/16:1 ω 7c	59.1	<0.001	31.8	<0.001	12.9	0.005	23.3	0.001
Cy19:0/18:1 ω 7c	74.8	<0.001	32.1	<0.001	41.7	<0.001	7.2	0.023
Saturated/monounsaturated	172.2	<0.001	59.8	<0.001	56.0	<0.001	19.6	0.001
<i>Solvent-extracted products/ratios</i>								
Short-chain lipids (<C ₂₀)	0.7	0.413	3.3	0.101	0.2	0.635	1.1	0.322
Long-chain lipids (\geq C ₂₀)	3.3	0.080	0.7	0.427	1.8	0.205	0.8	0.388
Plant-derived steroids	22.7	<0.001	16.4	0.002	7.4	0.021	3.6	0.087
Sugars	0.3	0.577	0.4	0.533	1.6	0.235	7.5	0.021
Total solvent extractable compounds	3.6	0.068	2.4	0.152	0.1	0.818	6.4	0.030
<i>Base-hydrolyzed products/ratios</i>								
Bound short-chain aliphatics	0.1	0.733	0.4	0.562	1.7	0.225	0.5	0.514
Bound long-chain aliphatics	0.4	0.546	0.7	0.408	2.5	0.145	0.1	0.711
Suberin-derived compounds	0.2	0.697	0.7	0.413	2.6	0.137	0.7	0.431
Cutin-derived compounds	5.8	0.023	0.7	0.426	8.4	0.016	0.4	0.567
ω -C ₁₆ / Σ C ₁₆	2.5	0.124	0.3	0.591	3.1	0.111	0.3	0.588
Cutin/suberin ratio	6.1	0.019	4.7	0.055	2.2	0.165	1.7	0.220
<i>CuO depolymerized products/ratios</i>								
Total vanillyls	4.2	0.049	2.3	0.157	4.2	0.068	1.1	0.315
Total syringyls	2.6	0.117	1.9	0.193	1.9	0.201	0.0	0.979
Total cinnamyls	33.9	<0.001	44.2	<0.001	5.2	0.046	1.9	0.200
Lignin-derived phenols	4.7	0.038	2.8	0.126	4.9	0.052	0.9	0.366
Syringyl/vanillyl	1.1	0.302	0.5	0.507	0.2	0.694	2.9	0.122
Cinnamyl/vanillyl	0.0	0.899	0.5	0.486	0.0	0.938	0.0	0.989
Acid/aldehyde of syringyls	0.4	0.530	0.1	0.814	0.6	0.461	0.0	0.838
Acid/aldehyde of vanillyls	0.0	0.993	0.3	0.614	0.6	0.475	0.2	0.695

Table S6. Statistical analysis results of two-way ANOVA indicating the main and interactive effects of N fertilization and soil horizon on concentrations and ratios of carbon, nitrogen, and organic compounds. Significant values at $P < 0.1$ are in **bold**.

Parameters	N fertilization		Horizon		N fertilization*Horizon	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Macro elements						
Carbon	6.8	0.014	86.3	<0.001	2.3	0.118
Nitrogen	4.9	0.035	86.8	<0.001	2.0	0.156
C/N	17.1	<0.001	16.3	<0.001	1.4	0.262
Phospholipid fatty acids (PLFA)						
Gram-negative bacterial PLFA	52.7	<0.001	3.2	0.053	7.8	0.002
Gram-positive bacterial PLFA	31.2	<0.001	1.5	0.242	4.0	0.028
Total bacterial PLFAs	48.4	<0.001	2.0	0.147	6.9	0.003
Fungal PLFA	21.3	<0.001	79.6	<0.001	11.0	<0.001
Actinomycete PLFA	9.6	0.004	10.9	<0.001	2.8	0.078
Total PLFA	52.0	<0.001	7.5	0.002	8.4	0.001
Gram-negative/gram-positive Fungi/bacteria	41.6	<0.001	17.7	<0.001	7.9	0.002
Cy17:0/16:1 ω 7c	5.4	0.027	50.6	<0.001	0.8	0.451
Cy19:0/18:1 ω 7c	59.1	<0.001	26.5	<0.001	0.1	0.873
Saturated/monounsaturated	74.8	<0.001	5.1	0.012	7.7	0.002
	172.2	<0.001	10.5	<0.001	16.7	<0.001
Solvent-extracted products/ratios						
Short-chain lipids (<C ₂₀)	0.7	0.413	15.3	0.000	2.7	0.080
Long-chain lipids (\geq C ₂₀)	3.3	0.080	4.5	0.019	0.3	0.719
Plant-derived steroids	22.7	<0.001	46.8	<0.001	7.2	0.003
Sugars	0.3	0.577	0.3	0.742	2.2	0.133
Total free compounds	3.6	0.068	2.8	0.077	0.6	0.548
Base-hydrolyzed products/ratios						
Bound short-chain aliphatics	0.1	0.733	6.9	0.004	1.6	0.221
Bound long-chain aliphatics	0.4	0.546	4.7	0.017	2.2	0.132
Suberin-derived compounds	0.2	0.697	6.6	0.004	2.5	0.097
Cutin-derived compounds	5.8	0.023	3.9	0.030	4.4	0.021
ω -C ₁₆ / Σ C ₁₆	2.5	0.124	0.5	0.607	0.5	0.599
Cutin/suberin ratio	6.1	0.019	7.3	0.003	1.2	0.300
CuO depolymerized products/ratios						
Total vanillyls	4.2	0.049	24.4	<0.001	1.5	0.243
Total syringyls	2.6	0.117	52.2	<0.001	1.6	0.219
Total cinnamyls	33.9	<0.001	9.4	0.001	6.7	0.004
Lignin-derived phenols	4.7	0.038	37.9	<0.001	1.9	0.169
Syringyl/vanillyl	1.1	0.302	7.1	0.003	0.2	0.839
Cinnamyl/vanillyl	0.0	0.899	9.4	0.001	0.0	0.955
Acid/aldehyde of syringyls	0.4	0.530	42.0	<0.001	0.1	0.883
Acid/aldehyde of vanillyls	0.0	0.993	25.2	<0.001	0.5	0.634