



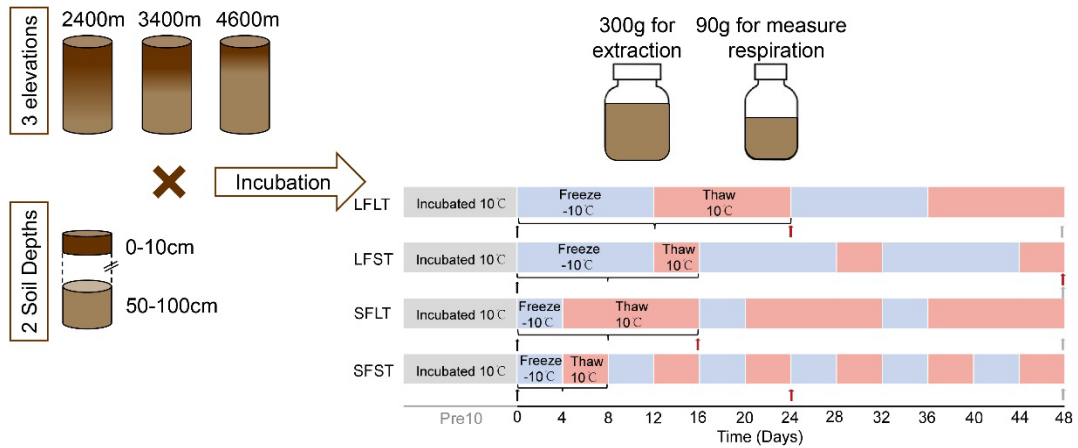
Supplement of

Dissolved organic carbon-mediated controls dominate soil carbon mineralization in response to freeze-thaw cycles

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5
6 **Fig. S1. Experimental design.** The experiment was conducted at a freezing
7 temperature of -10°C and a thawing temperature of 10°C . In the long freeze–long thaw
8 (LFLT) treatment, each cycle consisted of 12 days of freezing followed by 12 days of
9 thawing; in the long freeze–short thaw (LFST) treatment, freezing lasted 12 days with
10 4 days of thawing; in the short freeze–long thaw (SFLT) treatment, freezing lasted 4
11 days followed by 12 days of thawing; and in the short freeze–short thaw (SFST)
12 treatment, both freezing and thawing lasted 4 days. The curled bracket at the bottom of
13 the figure indicates one complete freeze–thaw cycle. The black arrow marks the
14 initiation of tests for dissolved organic carbon (DOC), soil organic carbon (SOC)
15 mineralization rate, and microbial activities (BG, CBH, PPO, POD). The red arrow
16 indicates the termination of DOC and microbial activity testing when the cumulative
17 thawing period reaches 12 days, and the gray arrow denotes the end of the soil organic
18 carbon mineralization test upon completion of 48 days of freeze–thaw cycling.

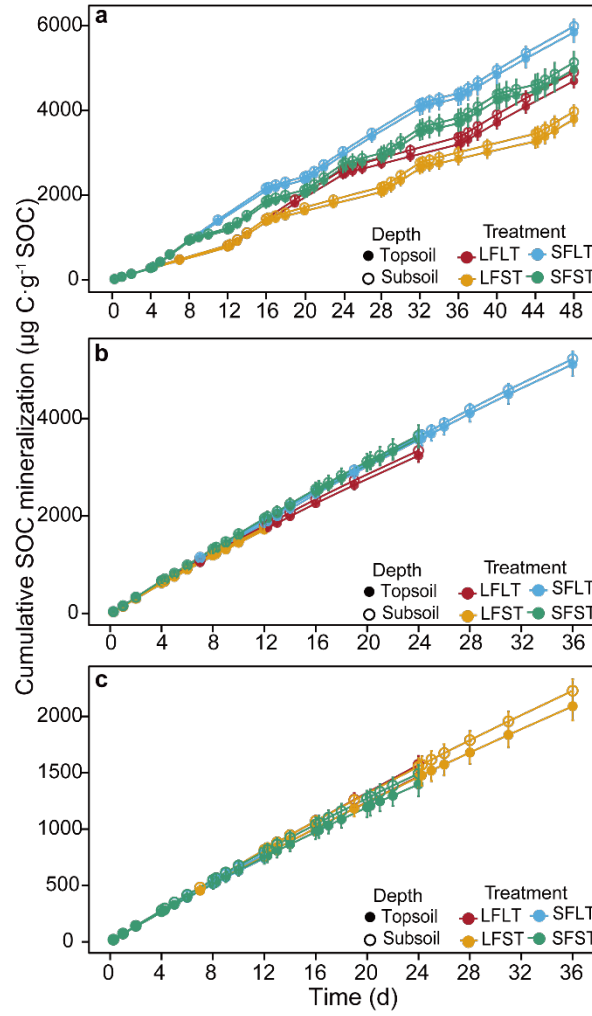


Fig. S2. Temporal changes in cumulative soil organic carbon (SOC) mineralization under different freeze-thaw cycles and soil depths. (a) SOC mineralization throughout the freeze-thaw cycle, with bars showing cumulative mineralization at incubation end. (b) Soil organic carbon mineralization during the thawing phase. (c) Soil organic carbon mineralization during the freezing phase. Different colours represent distinct freeze-thaw treatments and soil depths (topsoil: 0–10 cm; subsoil: 70–80 cm). The x-axis represents incubation time during each freeze-thaw cycle, while the y-axis displays cumulative soil organic carbon mineralization. Bars indicate means, with error bars representing one standard error ($n=18$).

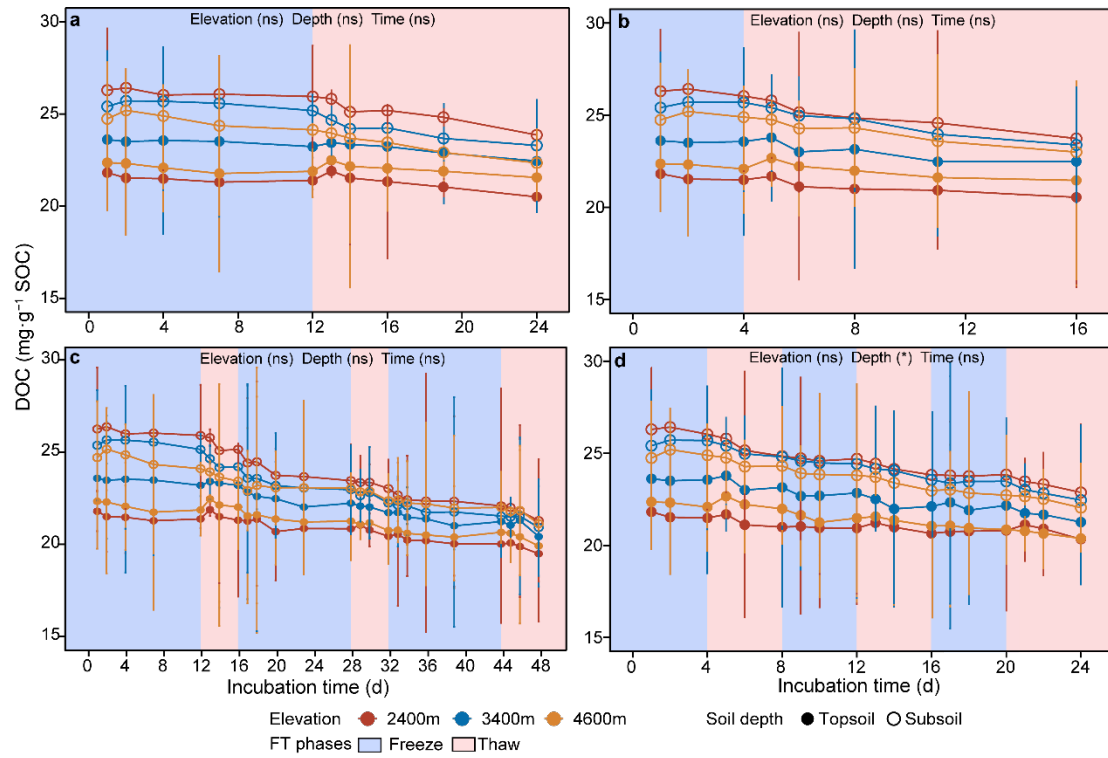


Fig. S3. Temporal dynamics of dissolved organic carbon (DOC) during freeze–thaw cycle incubation. Panels a–d show the temporal dynamics of DOC under the four freeze–thaw regimes: (a) long freeze–long thaw (LFLT), (b) short freeze–long thaw (SFLT), (c) long freeze–short thaw (LFST), and (d) short freeze–short thaw (SFST). The blue and red background areas denote freezing (–10 °C) and thawing (10 °C) phases, respectively. Data points represent treatment means $\pm$ 1 SE ($n = 3$). Significance tests compare elevations, soil depths, cycle number, and incubation time during thaw periods (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

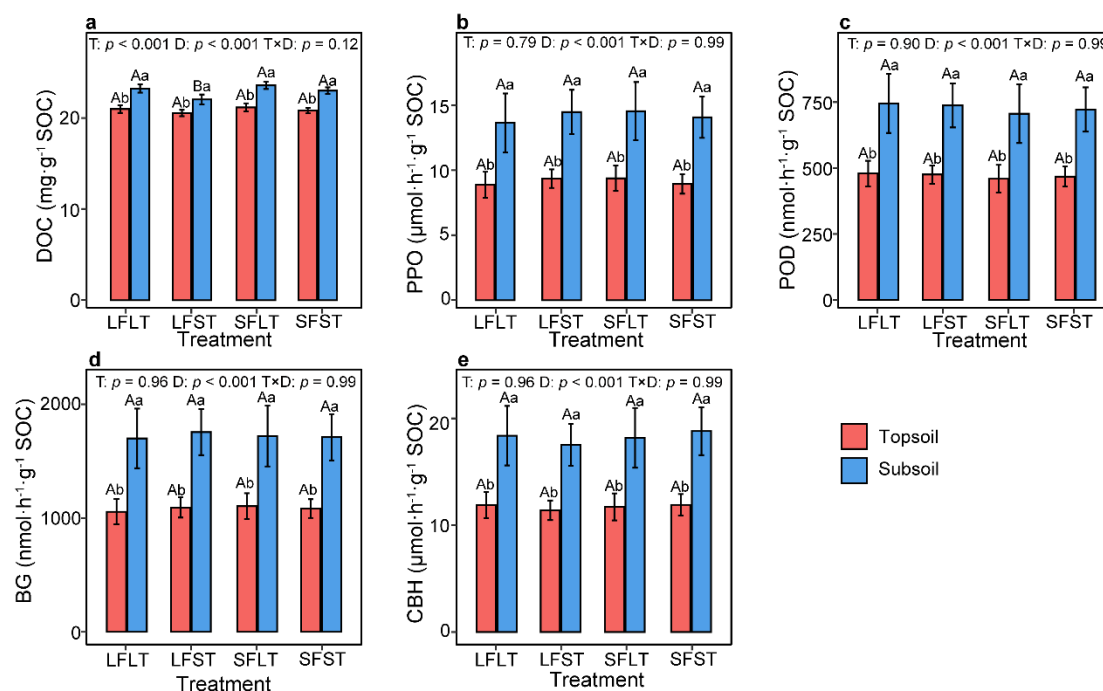


Fig. S4. Effects of freeze–thaw regimes on dissolved organic, enzyme activities, and microbial properties across two soil depths pooling all measurement together. Panels (a–e) show dissolved organic carbon (DOC), polyphenol oxidase activity (PPO), peroxidase activity (POD), β -glucosidase activity (BG), and cellobiohydrolase activity (CBH), respectively. Different uppercase letters indicate significant differences among treatments within the same soil depth, whereas different lowercase letters indicate significant differences between soil depths within the same treatment (Tukey’s multiple comparison test).

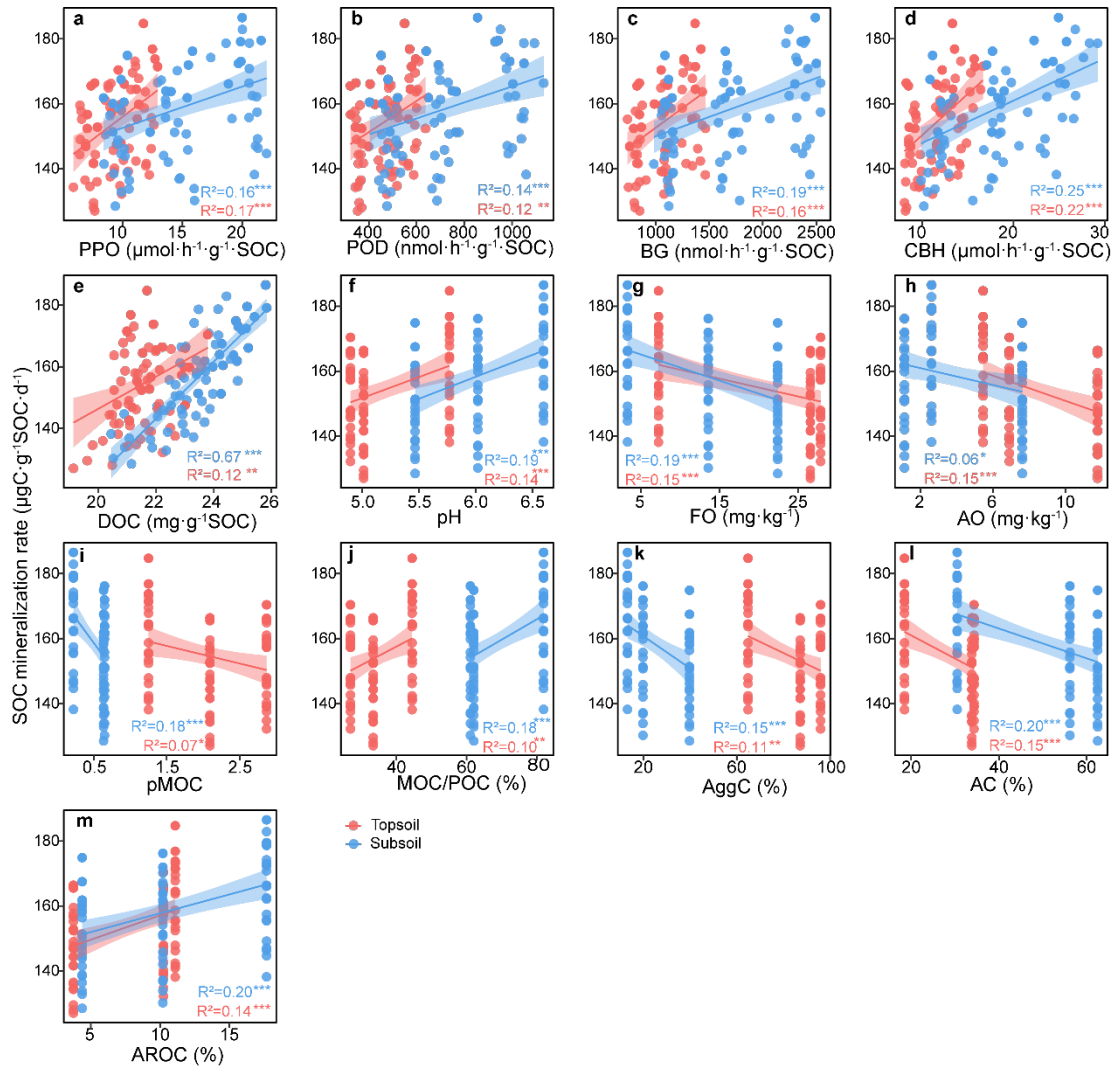


Fig. S5. Relationships between soil properties and soil organic carbon (SOC) mineralization rate during thawing phases. Red and blue symbols represent the topsoil and subsoil, respectively. Solid lines indicate significant linear regressions, whereas dashed lines indicate non-significant relationships. R^2 shows the determination coefficient of the regression. Abbreviations of soil properties are defined in Table S2. Asterisks denote levels of statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

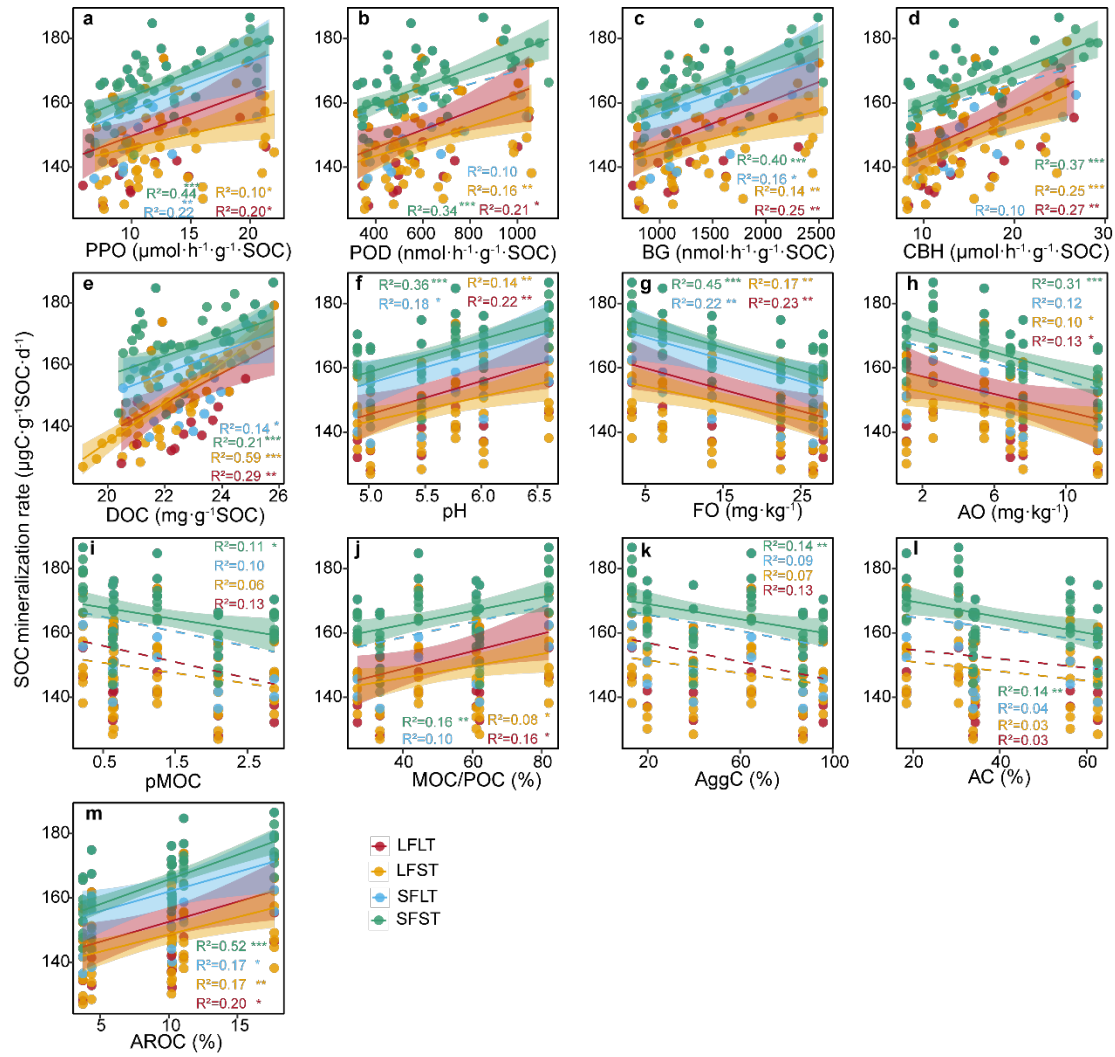


Fig. S6. Relationships between soil properties and soil organic carbon (SOC) mineralization rate during thawing phases. Red, yellow, blue and green symbols represent different treatments including LFLT, LFST, SFLT and SFST, respectively. Solid lines indicate significant linear regressions, whereas dashed lines indicate non-significant relationships. R² shows the determination coefficient of the regression. Abbreviations of soil properties are defined in Table S2. Asterisks denote levels of statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

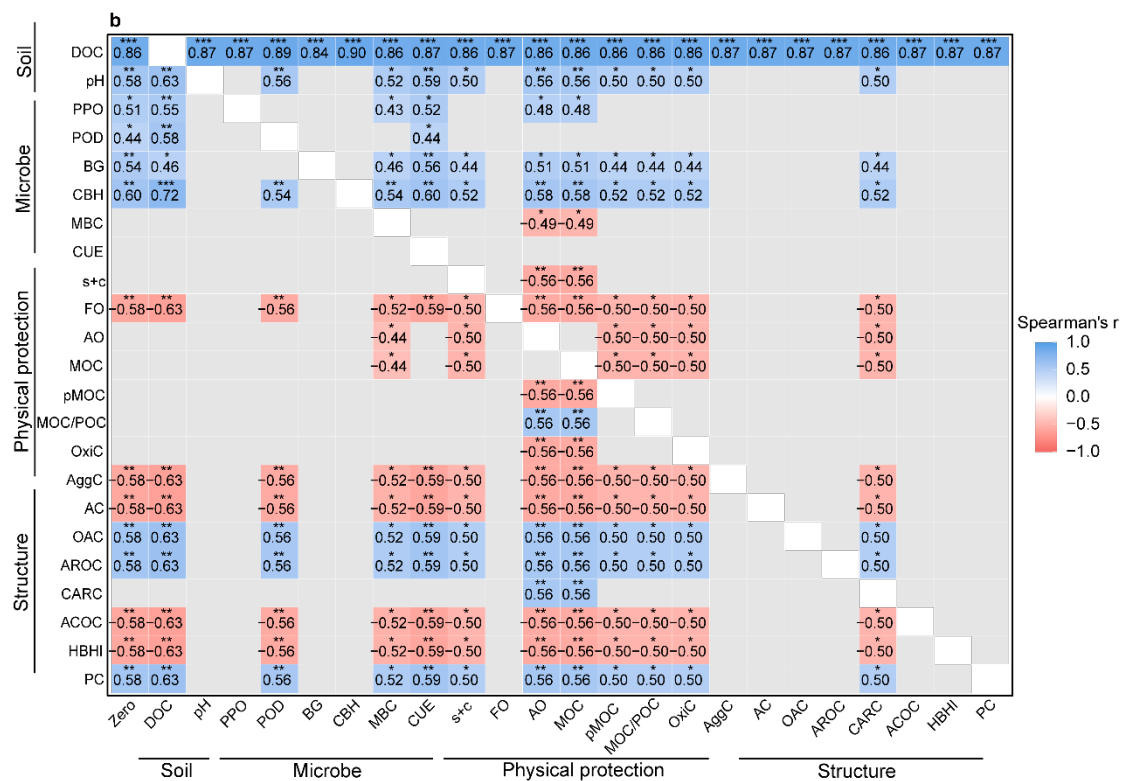
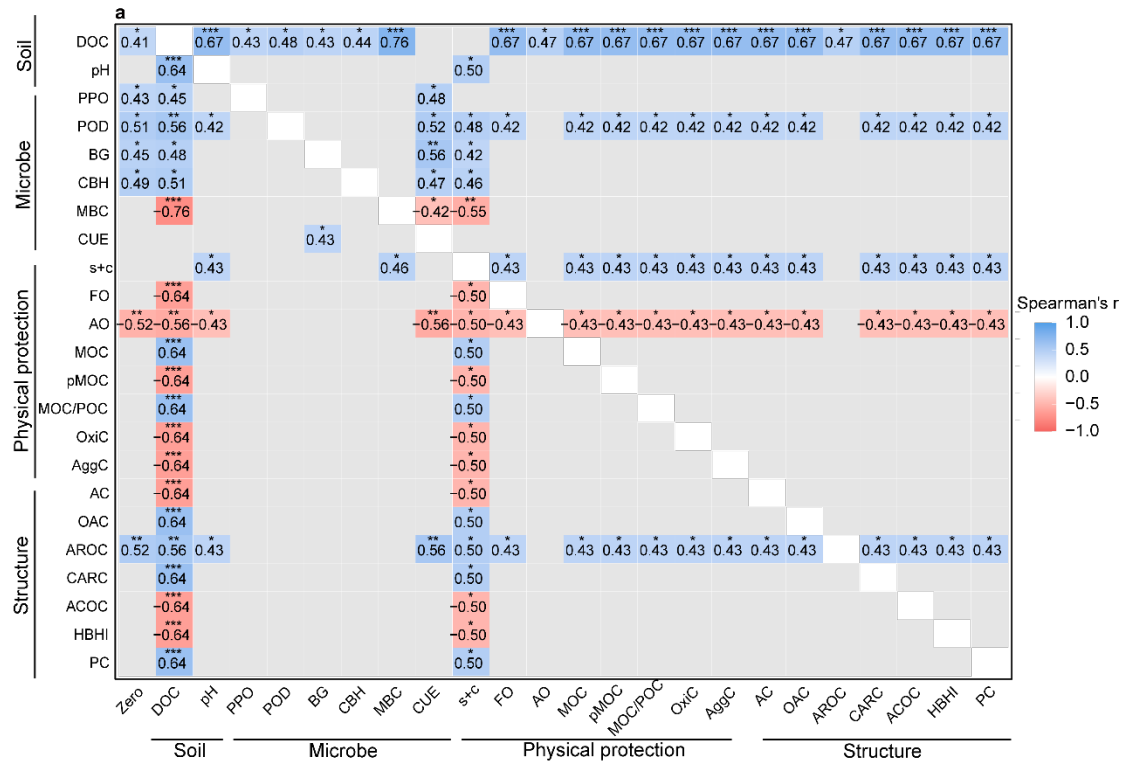


Fig. S7. Partial correlations (Spearman's r) of soil organic carbon (SOC) mineralization with soil physicochemical and biological properties across soil depths. Partial correlation relationships between topsoil (a) and subsoil (b) SOC mineralization rates and four categories of factors (microbial properties, soil properties, physical protection, and molecular structure). Correlation changes after controlling for

72 each soil property is presented. Difference between zero-order and partial correlations
73 indicates the level of dependency of the correlation between a given variable and the
74 corresponding fluxes. The color and numbers indicate the strength and sign of the
75 correlation. (No change in color between controlled variable and zero-order = no
76 dependency; decrease/increase of color intensity = loss of/gain of correlation.)
77 Statistical significance is indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p <$
78 0.001). Abbreviations of the variables are explained in Table S2.

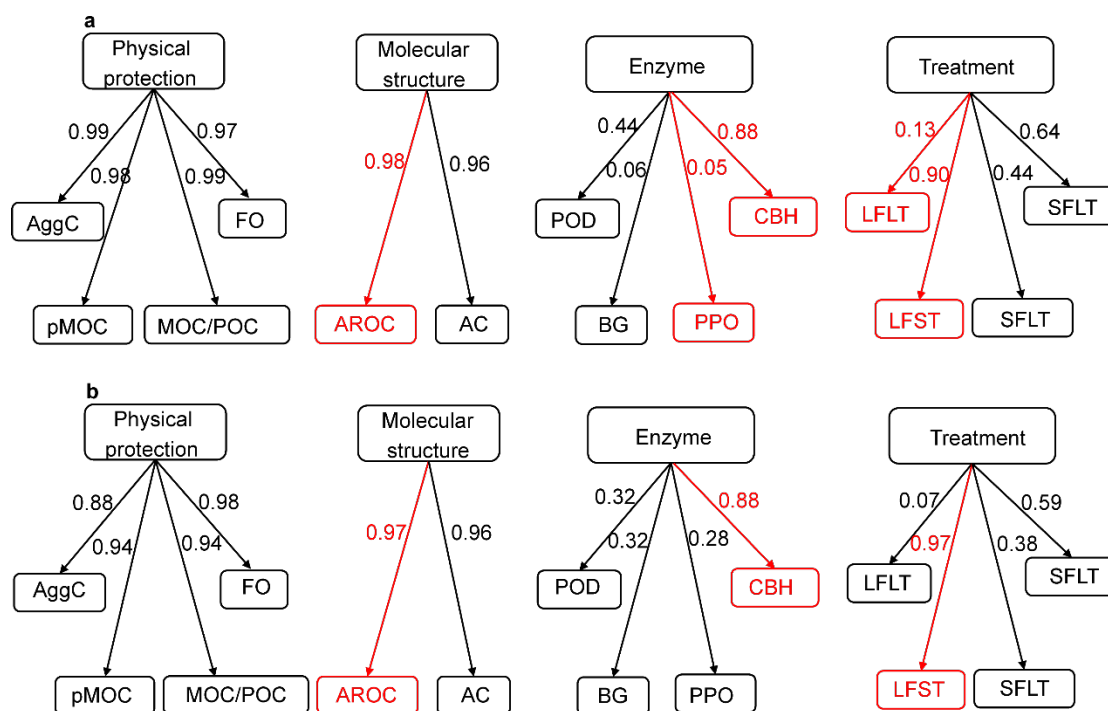


Fig. S8. Path analysis loadings of each individual variables to latent variables.

Variables marked in red have been reversed to ensure positive loadings. See Table S2

for variable abbreviations. (a) Topsoil, (b) Subsoil.

83 **Table S1. Soil sampling sites and soil properties**

Elevation (m, a.s.l.)	Variables	Soil depth	
		0-10 cm	70-80cm
2400	Coordinates	30°3'28"N 95°12'14"E	
	Ecosystem	Cropland	
	MAT (°C)	11.1	
	MAP (mm)	844	
	TC (g·kg ⁻¹ soil)	17.12 ± 1.38	10.11 ± 1.56
	SOC (g·kg ⁻¹ soil)	16.19 ± 1.42	9.28 ± 1.33
	Silt + Clay(%)	25.62 ± 0.1	27.88 ± 1.2
3400	Coordinates	29°46'7"N 94°44'18"E	
	Ecosystem	Pasture	
	MAT (°C)	3.4	
	MAP (mm)	985	
	TC (g·kg ⁻¹ soil)	20.79 ± 7.52	14.24 ± 4.8
	SOC (g·kg ⁻¹ soil)	19.62 ± 6.98	13.1 ± 4.57
	Silt + Clay (%)	64.48 ± 3.34	44.6 ± 3.1
4600	Coordinates	29°36'41"N 94°39'7"E	
	Ecosystem	Alpine meadow	
	MAT (°C)	-1.3	
	MAP (mm)	1057	
	TC (g·kg ⁻¹ soil)	27.89 ± 7.28	21.16 ± 0.47
	SOC (g·kg ⁻¹ soil)	26.37 ± 7.18	19.67 ± 1.06
	Silt + Clay (%)	50.4 ± 2.63	42.5 ± 6.01

84 Note: Values are given as mean ± one standard error ($n = 3$) if applicable.

85 **Table S2.** Explanatory variables used to explain the variance of SOC mineralization.

Category	Variable Code	Variable description	Unit
Rs	Rs	SOC mineralization rate	$\mu\text{g C} \cdot \text{g}^{-1}$ $\text{SOC} \cdot \text{d}^{-1}$
Climate	E	Elevation above sea level	m
Depth	D	Soil depth	cm
Microbial properties	BG	β - glucosidase	$\text{nmol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ SOC
	CBH	cellulase	$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ SOC
	PPO	Polyphenol Oxidase	$\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ SOC
	POD	Peroxidase	$\text{nmol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ SOC
	CUE	Microbial carbon use efficiency	-
Edaphic properties	MBC	Microbial biomass carbon	$\text{mg} \cdot \text{g}^{-1}$ SOC
	DOC	Dissolved organic carbon	$\text{mg} \cdot \text{g}^{-1}$ SOC
	pH	Soil pH	-
Physical protection	s+c	The percentage of silt and clay	%
	FO	The sum of free oxides	$\text{mg} \cdot \text{kg}^{-1}$
	AO	The sum of amorphous oxides	$\text{mg} \cdot \text{kg}^{-1}$
	MOC	Mineral-associated organic carbon	$\text{g} \cdot \text{kg}^{-1}$
	pMOC	The proportion of MOC in total SOC	-
	MOC/POC	The ratio of MOC to POC	%
	OxiC	The proportion of oxides-bond C in total SOC	%
	AggC	The proportion of macroaggregate C in total SOC	%
Molecular structure	AC	The proportion of alkyl C	%
	OAC	The proportion of O-alkyl C	%
	AROC	The proportion of aromatic C	%
	CARC	The proportion of carboxylic C	%
	ACOC	The ratio of alkyl C to O-alkyl	-
	HBHI	The ratio of hydrophobic C to hydrophilic C	-
	PC	The proportion of polysaccharide C	%

86

87 **Table S3.** Analysis of variance (ANOVA) of SOC mineralization rates during the thaw
88 phase as influenced by different variables.

Variables	df	Sum Sq	Mean Sq	F value	Pr(>F)
Time (t)	5	174015.28	34803.06	54.55	***
Cycle (C)	5	124502.98	24900.60	39.03	***
Elevation (E)	2	36885.08	18442.54	28.90	***
Treatment (T)	3	15084.01	5028.00	7.88	***
Soil depth (D)	1	3271.77	3271.77	5.13	*
T×C	5	9363.96	1872.79	2.94	*
T×E	6	1270.76	211.79	0.33	ns
C×t	19	1988.35	104.65	0.16	ns
E×C	10	955.68	95.57	0.15	ns
T×E×D	6	507.27	84.54	0.13	ns
D×C	5	288.12	57.62	0.09	ns
T×E×C	10	475.95	47.60	0.07	ns
T×D×C	5	223.32	44.66	0.07	ns
T×t	11	443.25	40.30	0.06	ns
T×E×D×C	10	366.97	36.70	0.06	ns
T×D	3	94.92	31.64	0.05	ns
T×C×t	17	301.62	17.74	0.03	ns
T×E×t	22	328.31	14.92	0.02	ns
E×D	2	27.14	13.57	0.02	ns
E×t	10	126.15	12.61	0.02	ns
E×D×t	10	100.16	10.02	0.02	ns
T×E×D×t	22	219.63	9.98	0.02	ns
E×C×t	38	339.28	8.93	0.01	ns
D×t	5	35.34	7.07	0.01	ns
E×D×C	10	68.23	6.82	0.01	ns
T×D×t	11	73.23	6.66	0.01	ns
E×D×C×t	38	251.55	6.62	0.01	ns
T×D×C×t	17	112.39	6.61	0.01	ns
T×E×C×t	34	180.75	5.32	0.01	ns
D×C×t	19	96.93	5.10	0.01	ns
T×E×D×C×t	34	140.79	4.14	0.01	ns
Residuals	792	505332.54	638.05		

89 Note: ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

90 **Table S4.** Analysis of variance (ANOVA) of Dissolved Organic Carbon (DOC) during
91 the thaw phase as influenced by different variables.

Variables	df	Sum Sq	Mean Sq	F value	Pr(>F)
Soil depth (D)	1	549.53	549.53	7.437	**
Cycle (C)	2	214.97	107.49	1.455	ns
E×D	2	68.37	34.19	0.463	ns
Elevation (E)	2	62.17	31.08	0.421	ns
Treatment (T)	3	86.99	29.00	0.392	ns
Time (t)	4	57.97	14.49	0.196	ns
D×C	2	27.99	14.00	0.189	ns
T×D	3	18.50	6.17	0.083	ns
T×C	2	6.24	3.12	0.042	ns
E×D×C	4	12.12	3.03	0.041	ns
T×E×D	6	14.58	2.43	0.033	ns
T×E	6	10.88	1.81	0.025	ns
T×D×C	2	3.38	1.69	0.023	ns
T×E×D×C	4	4.23	1.06	0.014	ns
D×t	4	3.49	0.87	0.012	ns
E×C	4	2.47	0.62	0.008	ns
T×E×C	4	1.90	0.48	0.006	ns
C×t	4	1.55	0.39	0.005	ns
E×t	8	1.64	0.21	0.003	ns
T×t	8	1.27	0.16	0.002	ns
D×C×t	4	0.45	0.11	0.002	ns
T×E×t	16	1.56	0.10	0.001	ns
T×D×t	8	0.58	0.07	0.001	ns
E×D×t	8	0.33	0.04	0.001	ns
T×C×t	4	0.29	0.07	0.001	ns
E×C×t	8	0.54	0.07	0.001	ns
T×E×D×t	16	0.83	0.05	0.001	ns
T×E×C×t	8	0.54	0.07	0.001	ns
T×D×C×t	4	0.34	0.09	0.001	ns
E×D×C×t	8	0.56	0.07	0.001	ns
T×E×D×C×t	8	0.38	0.05	0.001	ns
Residuals	336	24828.60	73.90		

92