Supplementary Information

Past aridity's effect on carbon mineralization potentials in grassland soils

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1. Supplementary Materials and Methods

1.1 Analysis of phospholipid fatty acids (PLFAs)

Briefly, a modified Bligh-Dyer extraction (Bligh and Dyer, 1959) was performed on ~5 g of freeze-dried soils using mixtures of methanol, dichloromethane and citrate buffer (30 mL; 1:2:0.8, v:v:v). Total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids with 10 ml chloroform, 20 mL acetone, and 10 mL methanol through a silica gel column, respectively. The polar lipid fraction containing phospholipids was evaporated to dryness under nitrogen gas and converted into fatty acid methyl esters (FAMEs) by a mild alkaline methanolysis reaction (Guckert et al., 1985). FAMEs were recovered with a mixture of hexane and chloroform (4:1, v/v). Solvents were evaporated under nitrogen and the extracts were re-dissolved in 200 μL hexane with cholestane added as an internal standard. FAMEs were analyzed on a Trace 1310 gas chromatograph (GC) coupled to an ISQ mass spectrometer (MS; Thermo Fisher Scientific, USA) using a DB-5MS column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) for separation. The GC oven temperature increased from 65°C (initial hold time 2 min) to 300°C at a rate of 6°C min^{-1} with helium as the carrier gas (1.2 mL min^{-1}). The mass spectrometer was operated in an EI mode with ionization energy of 70 eV and scanned from 50 to 650 daltons. Individual PLFAs were identified by comparison of mass spectra and retention times with authentic standards. Quantification was achieved by comparison with the internal standard in the total ion current (TIC).

Fatty acids are designated according to the standard PLFA nomenclature (Guckert et al., 1985). Because PLFA 18:3 (a commonly used indicator of plant lipids; Harwood and Russell, 1984) is not observed in our soils, contributions of plant-derived PLFAs are considered to be negligible. Gram-positive (G+) bacteria are represented by PLFAs *i*15:0, *a*15:0, *i*16:0, *i*17:0 and *a*17:0 while Gram-negative (G–) bacteria are represented by PLFAs 16:1ω7*c*, *cy*17:0, 18:1ω7*c* and *cy*19:0. PLFAs specific to fungi (18:2ω6,9*c* and 18:1ω9*c*) and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0) are also summarized (Harwood and Russell, 1984). Microbial biomass is represented by total PLFAs (Feng and Simpson, 2009), including the above and non-specific PLFAs (16:0, 17:0, 18:0, 20:0, 16:1ω5, 16:1ω11 and 19:1ω8).

The ^{13}C isotopic composition of individual FAMEs was analyzed on a Thermo Trace GC Ultra coupled to a stable isotope ratio mass spectrometry (Thermo MAT 253, Germany) via a combustion interface (GC-C-IRMS) using similar conditions as the GC/MS analysis. The $\delta^{13}C$ values of PLFAs were corrected for the methyl carbon added to FAMEs by methanolysis using a mass balance equation (Denef et al., 2009):

$$\delta^{13}C_{PLFA} = \frac{(N_{PLFA}+1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}}{N_{PLFA}} \quad (1)$$

where $\delta^{13}C$ is the isotope ratio for PLFAs, FAMEs or methanol (MeOH) used in methanolysis (–21.65‰) indicated by the subscripts, N_{PLFA} is the number of carbon atoms of individual PLFAs. For each soil sample, the $\delta^{13}C$ of total PLFAs was estimated by the abundance-weighted average of 10 most abundant PLFAs that on average represented ~42% of total PLFAs in all samples, including G+ (*i*15:0, *a*15:0, *i*16:0, *i*17:0 and *a*17:0), G– (16:1ω7*c* and *cy*17:0) and non-specific PLFAs (16:0, 18:0 and 20:0). The proportion of litter-derived carbon in

PLFAs was calculated as below (Fry, 2006):

$$PLFA - C_{litter} = \frac{\delta^{13}C_{PLFA} - \delta^{13}C_{control}}{\delta^{13}C_{litter} - \delta^{13}C_{control}} \times 100\% \quad (2)$$

where $\delta^{13}C_{PLFA}$, $\delta^{13}C_{control}$ and $\delta^{13}C_{litter}$ are the isotope value of total PLFAs in the litter-amended and control soils and of bulk litter, respectively.

1.2 Assay of extracellular enzyme activity

Briefly, 1 g of fresh soil was added into 91 ml MilliQ water and homogenized with a magnetic stirrer for 3 min. For the hydrolases, the resulting suspension (200 µl) was dispensed into 96-well microplates with 50 µl of 4-methylumbelliferone for α-glucosidase, β-glucosidase and alkaline phosphatase or 4-methylumbelliferone for leucine-aminopeptidase in pH buffers. The microplates were incubated in the dark at 25˚C for 4 h. For phenol oxidase analysis, 50 µl of L-3,4-dihydroxyphenylalanine (5 mM) in tris buffer solutions (pH of 8.2) were added to each sample well and incubated in the dark at 25˚C for 3 h. Eight replicate wells were used per sample per assay. Potential enzyme activity was quantified using Multi-Mode Microplate Reader (synergy Mx, BioTek Instruments Inc., USA). Fluorescence (tested for hydrolases) was measured with excitation at 365 nm and emission at 450 nm, while absorbance (for phenol oxidase) was measured at 450 nm.

1.3 Structural equation modelling (SEM)

Environmental variables were selected based on the results of bivariate correlations and hypothetical pathways and grouped into the following categories: past aridity (represented by aridity index of the sampling sites), soil pH, soil minerals (including Fe_d, Al_d and clay content), SOM properties (including SOC, SOC/N ratio and WEOC content), microbial biomass (total PLFAs), hydrolases (α-glucosidase, β-glucosidase, alkaline phosphatase and leucine-aminopeptidase activities) and phenol oxidase activity. As microbial CUE was correlated with mineralization potentials or aridity index, it was hence not included in the model. For the category encompassing more than one variable, a principal component analysis (PCA) was performed and the first principal component (PC 1) was used to represent the corresponding category of variables to avoid self-correlation. The Kaiser-Meyer-Olkin (KMO) test and Bartlett test of sphericity (BS) were used to assess the performance of PCA (Table S5).

Soil minerals are positively correlated with Fe_d, Al_d and clay ($p < 0.05$). SOM property is positively correlated with SOC content and SOC/N ratio but negatively correlated with SOC-normalized concentrations of WEOC ($p < 0.05$). Hydrolases are positively correlated with α-glucosidase, β-glucosidase, alkaline phosphatase and leucine-aminopeptidase activities ($p < 0.05$). All the variables used for PCA yielded KMO ≥ 0.5 and BS < 0.01, indicating that PCA was appropriate. In all cases, PCA results explained >54% of variations in variables.

A priori models for the SEM (Fig. S6) are developed based on knowledge and hypotheses involving three main groups of variables (SOM property, microbial biomass and enzyme activities) that are influenced by past aridity and soil minerals and directly regulate mineralization. Microbial community structures (i.e., F/B and G+/G– ratios) are not included due to their complex relationships with mineralization (Griffiths et al., 2001; Nannipieri et al., 2003; also see details in Results). The qCO_2 is excluded in the model due to its intrinsic strong correlation with mineralization. Microbial biomass and enzyme activities are directly linked to soil minerals (Bontti et al., 2009), SOM property and aridity index, while enzyme activities are also influenced by soil pH

(Sinsabaugh et al., 2008; Sinsabaugh, 2010) and microbial producers (PLFAs). Soil pH is affected by aridity index (van Breemen et al., 1984). For the added fresh litter, its mineralization potential is not directly linked to SOM property while all other pathways remain the same (Fig. S6b). Based on a priori models, step-wise exclusion of variables with non-significant regression weight and covariance (estimated by Akaike information criterion scores) was used until a minimal adequate model was achieved. The size of effect on substrate mineralization potentials for environmental variables is reflected by the standardized path coefficients (Milcu et al., 2013). The adequacy of the constructed SEM is indicated by non-significant chi-squared (χ^2) tests ($p > 0.05$), a low root mean square error of approximation index (RMSEA < 0.05), Tucker-Lewis Index (TLI ≥ 0.90) and a high comparative fit index (CFI ≥ 0.95; Schermelleh-Engel et al., 2003). The SEM is also modified with Satorra-Bentler correction to improve the chisquare approximation of goodness-of-fit test statistics for our relatively small dataset with non-normal distributions and validated with the Bollen–Stine bootstrap test ($p > 0.1$) (Hesterberg et al., 2005; Mancinelli et al., 2013).

2 Supplementary tables

Table S1. Mineral composition of the studied grassland soils (analyzed by X-ray diffraction).

Site	Quartz (%)	Feldspar (%)	Calcite (%)
OB	45.7	54.3	na
DQ	65.4	20.7	13.9
HLHT	72.9	17.5	9.6
XH	69.8	30.2	na
XG	84.9	15.1	na
HB	55.8	33.4	10.8

Table S2. The abundance-weighted average $\delta^{13}C$ of phospholipid fatty acids (PLFAs) in the litter-amended and control soils, litter-derived carbon in PLFAs (PLFA-C_{litter}) (mean ± standard error; n=1 for OB and XG subsoils; n = 3 for the rest). Difference levels are indicated by A, B or a, b in the topsoil and subsoil, respectively.

Site	Litter-amended soil PLFA-$\delta^{13}C$ (‰)	Control soil PLFA-$\delta^{13}C$ (‰)	PLFA-C_{litter} (%)
		Topsoil	
OB	–8.20 ± 5.21	–26.31 ± 0.18	1.40 ± 0.40B
DQ	–2.66 ± 1.38	–26.18 ± 0.16	1.82 ± 0.11B
HLHT	–11.37 ± 2.00	–25.38 ± 0.59	2.84 ± 0.16A
XH	–8.21 ± 5.90	–22.67 ± 0.35	1.12 ± 0.46B
XG	–0.26 ± 2.62	–25.47 ± 0.04	1.95 ± 0.20AB
		Subsoil	
OB	0.35	–18.80	1.49a
DQ	30.74 ± 13.97	–25.05 ± 0.38	4.31 ± 1.08a
HLHT	7.83 ± 3.34	–24.95 ± 1.27	2.53 ± 0.26a
XH	1.13 ± 5.14	–24.59 ± 0.52	1.99 ± 0.40a
XG	–5.95	–24.71	1.45a

Table S3. Information on the mineralization potential relative to soil organic carbon (SOC) in published incubation studies.

Reference	Location	Vegetation	Aridity index	Soil depth (cm)	Soil pH	SOC/ N	Incubation time (d)	OMP (%)
Čapek, et al. (2015)	Taymir Peninsula, Russia	Tundra	0.93	Oa	6.2	18.7	91	5.23
Feng and Simpson (2008)	Alberta, Canada	Grassland	0.45	0–16	6.8	NA	86	7.26
Gillabel, et al. (2010)	Termunck, Belgium	Cropland	1.10	5–15	NA	10.6	99	4.03
Guenet, et al. (2010)	Versailles, France	Cropland	0.82	5–20	7.2	10.8	80	1.15
Jia, et al. (2017)	Qinghai-Tibetan Plateau,China	Grassland	0.69	0–10	8.0	12.6	86	5.34
Lü, et al. (2015)	Chenda Town, China	Forest	1.41	0–10	NA	NA	90	2.95
Ma, et al. (2013)	Inner Mongolia, China	Grassland	0.41	0–10	6.3	11.1	100	2.89
Schimmelpfennig, et al. (2014)	Linden, Germany	Grassland	0.90	0–15	5.8	10.6	93	3.02
Thomsen, et al. (1999)	Lerbjerg, Denmark	Cropland	1.47	NA	NA	NA	105	2.95
Zheng, et al. (2012)	Colorado, America	Grassland	0.92	0–20	9.0	NA	93	6.41

Table S4. Litter decomposition rate in field litterbag or laboratory decay experiments.

Reference	Litter type	Location of experiment	Decomposition time (d)	Litter decay (%)
Davis, et al. (2003)	Tree leaves	Tropical forest	90	40
Guenet, et al. (2010)	Wheat-straw	Laboratory	80	39
Pascault, et al. (2013)	Alfalfa	Laboratory	120	28
	Wheat		120	17
Shaw and Harte (2001)	*E. grandiflorum*	Subalpine meadow	46	73
Sievers and Cook (2018)	Hairy vetch	Tropical cropland	84	96
	Cereal rye		84	88
Wang, et al. (2014)	*E. speciosus*	Temperate cropland	90	74
	S. viridis		90	58
	E. indica		90	60
Yahdjian, et al. (2006)	*S. speciosa*	Tropical shrubland	90	10

Table S5. Results of Kaise-Meyer-Olkin (KMO) test and Bartlett test of sphericity (BS) for variables used for principle component analysis (PCA) in the paper.

Parameter	Variables included	KMO value	BS test
Soil minerals	Fe_d (+), Al_d (+), Clay (+)	0.53	$\chi^2 = 40$; $p < 0.001$
SOM property	SOC (+), SOC/N (+), WEOC (–)	0.57	$\chi^2 = 21$; $p < 0.001$
Hydrolases (control)	AG (+), BG (+), AP (+), LAP (+)	0.50	$\chi^2 = 93$; $p < 0.001$
Hydrolases (litter-amended)	AG (+), BG (+), AP (+), LAP (+)	0.61	$\chi^2 = 99$; $p < 0.001$

Fe_d: dithionite-extractable iron; Al_d: dithionite-extractable aluminum; SOM: soil organic matter; SOC: soil organic carbon; N: nitrogen; WEOC: water-extractable organic carbon; AG: α-glucosidase; BG: β-glucosidase; AP: alkaline phosphatase; LAP: leucine-aminopeptidase. Soil minerals are positively correlated (indicated by "+") with Fe_d, Al_d and clay; SOM property is positively correlated with SOC contents and SOC/N ratios but negatively correlated (indicated by "–") with WEOC concentrations ($p < 0.05$). Hydrolases are positively correlated with all tested hydrolytic enzymes ($p < 0.05$).

3 Supplementary Figures

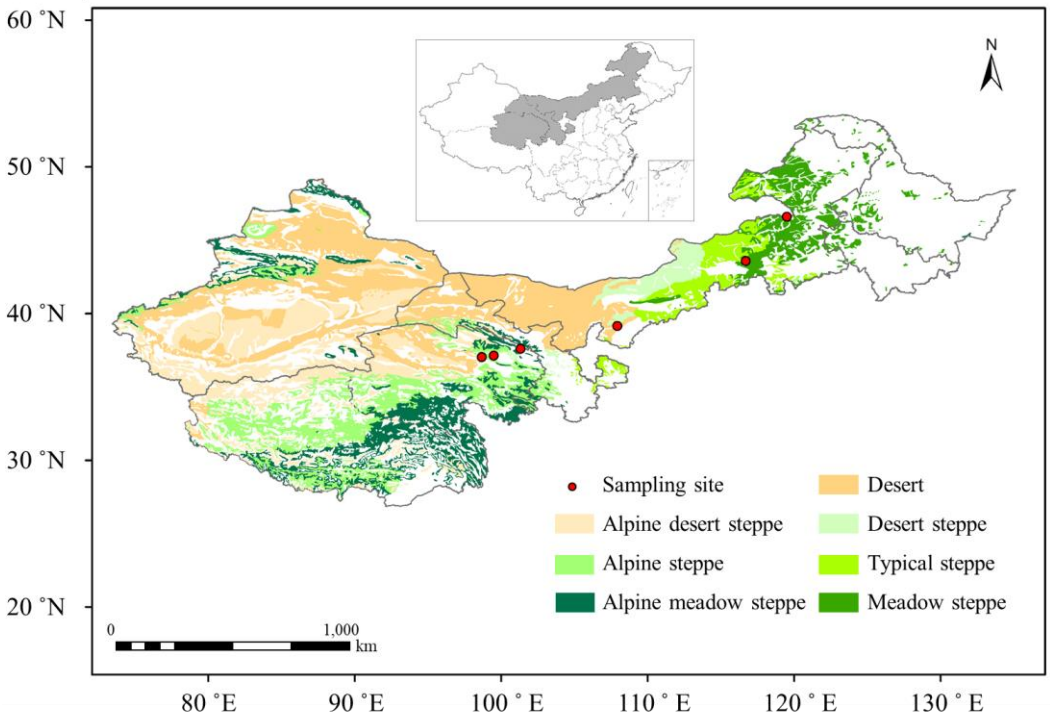

Figure S1. Map of study area and sampling sites.

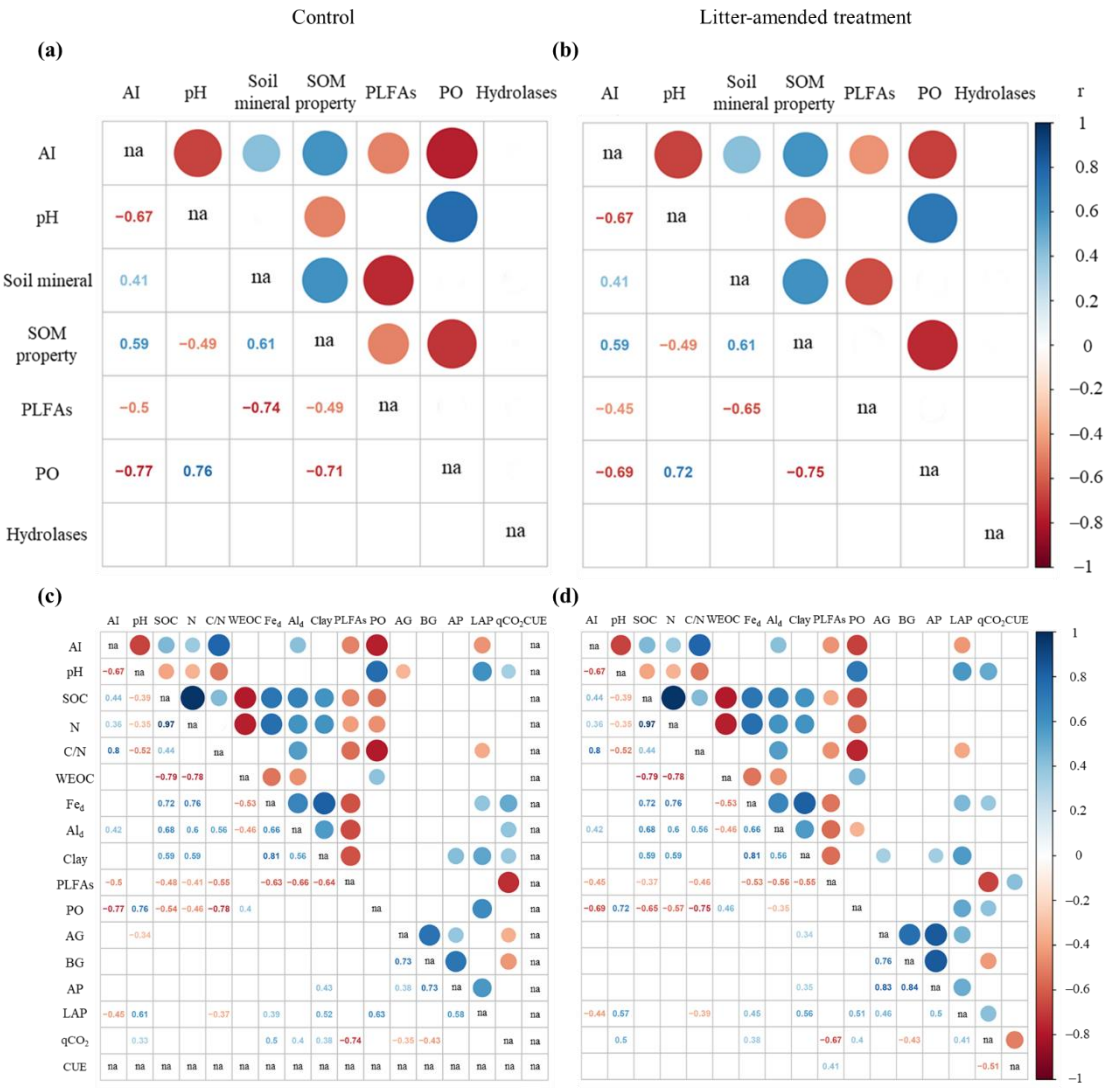

Figure S2. Correlation matrix of environmental variables in the control (a, c) and litter-amended soils (b, d). Numbers in the lower left triangle of the matrix indicate Spearman correlation coefficients (r) between corresponding variables ($p < 0.05$; n = 36), which is proportional to the size of the colored dots in the upper right triangle. Empty cells indicate non-significant correlations ($p > 0.05$). AI: aridity index; SOC: soil organic carbon; N: nitrogen; C/N: ratio of SOC/N; WEOC: water-extractable organic carbon; SOM: soil organic matter; Fe_d: dithionite-extractable iron; Al_d: dithionite-extractable aluminum; PLFAs: phospholipid fatty acids; PO: phenol oxidase; AG: α-glucosidase; BG: β-glucosidase; AP: alkaline phosphatase; LAP: leucine-aminopeptidase; qCO_2: microbial metabolic quotient; CUE: carbon use efficiency; na: not applicable. Soil minerals, SOM property and hydrolases are defined by a principle component analysis (Table S5).

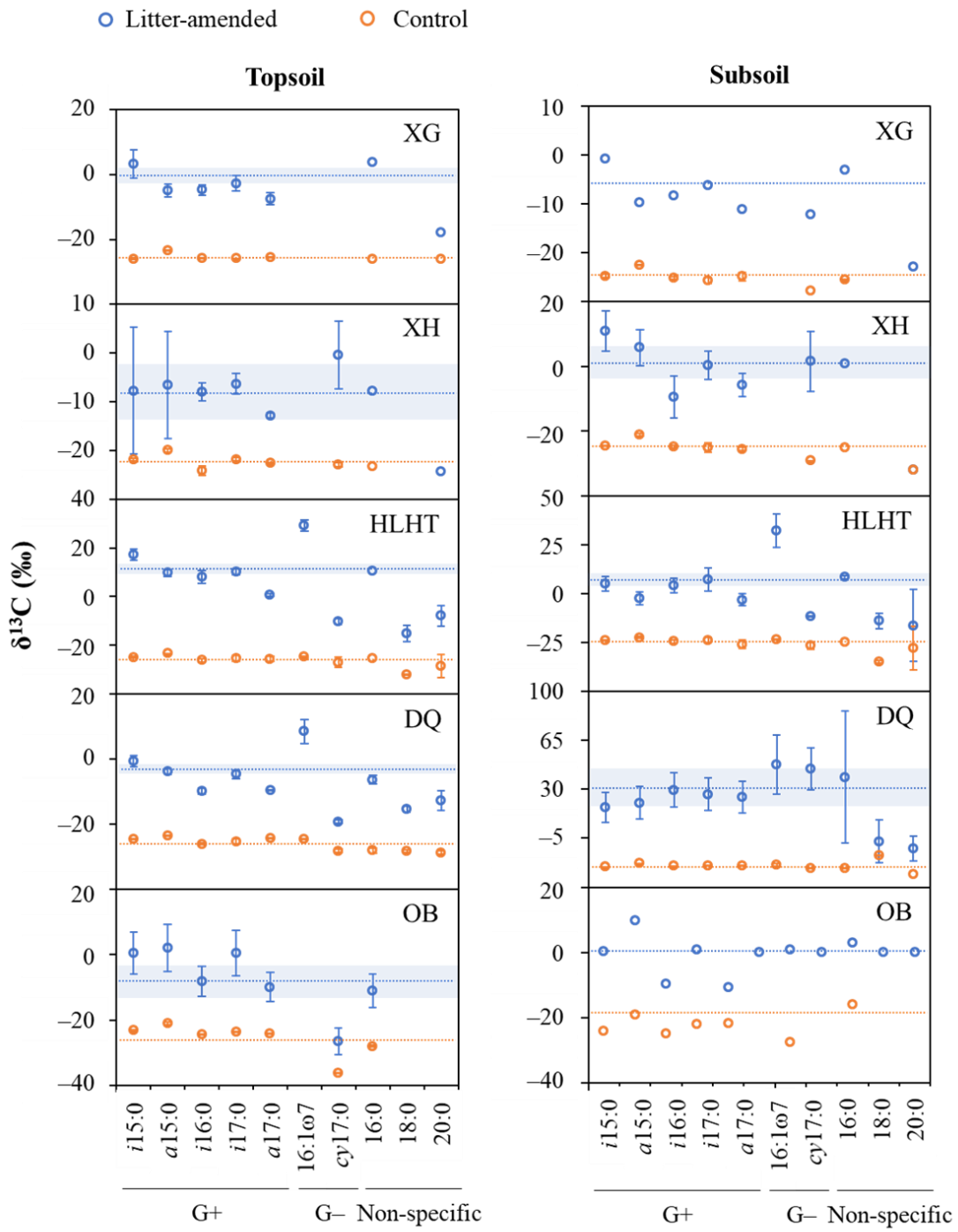

Figure S3. The ^{13}C composition of phospholipid fatty acids (PLFAs) in the control (orange dots) and litter-amended (blue dots) soils at the end of incubation. Mean values are shown with standard error (n=3) except the subsoils of OB and XG (litter-amended only) having only one replicate as low PLFA concentrations prevented accurate ^{13}C measurement. Lines refer to the abundance-weighted average $\delta^{13}C$ values for the corresponding treatments with shadows representing the associated standard error. Fatty acids are designated according to the standard PLFA nomenclature. G+ and G– refer to Gram-positive and Gram-negative bacteria, respectively.

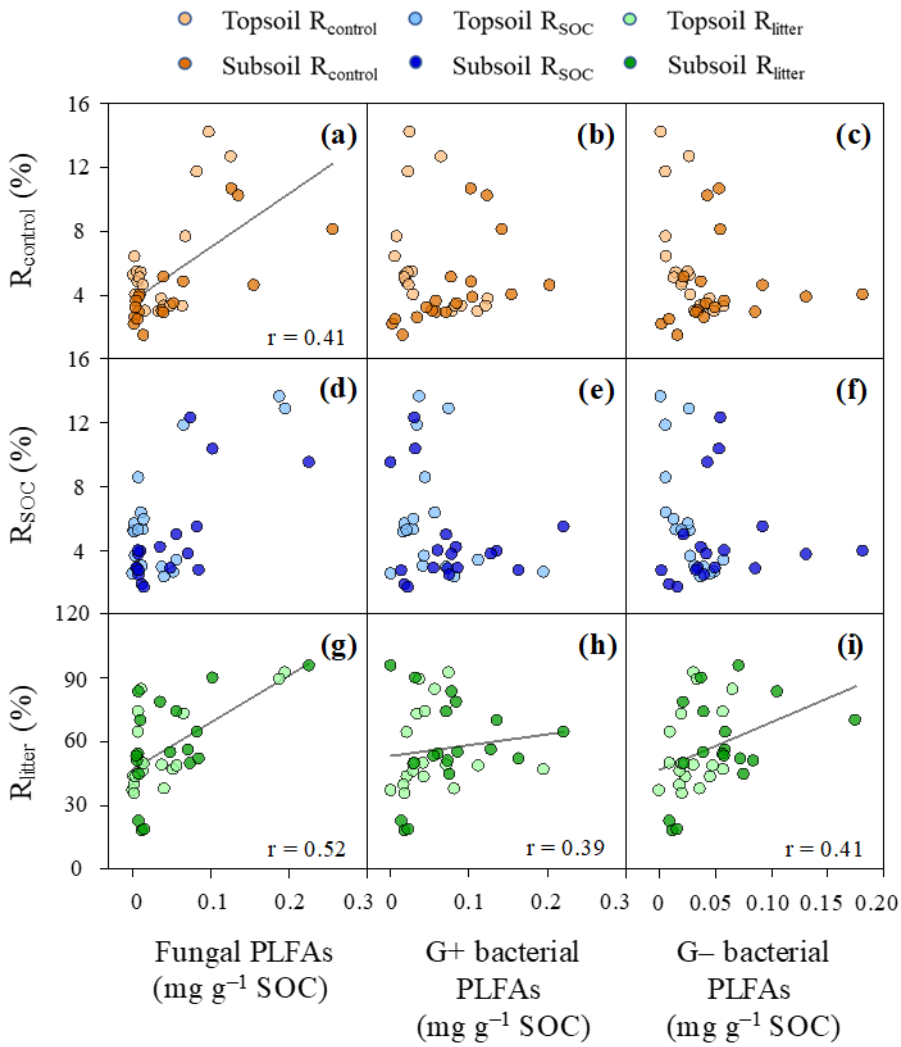

Figure S4. Spearman correlations between the mineralization potential and soil contents of phospholipid fatty acids (PLFAs) for different microbial groups at the end of the 91-day incubation. $R_{control}$ and R_{SOC} refer to the mineralization potential of soil organic carbon (SOC) in the control and litter-amended treatments, respectively while R_{litter} refers to the mineralization potential of litter. G+ and G– refer to Gram-positive and Gram-negative bacteria, respectively. Lines represent significant correlations at a level of $p < 0.05$.

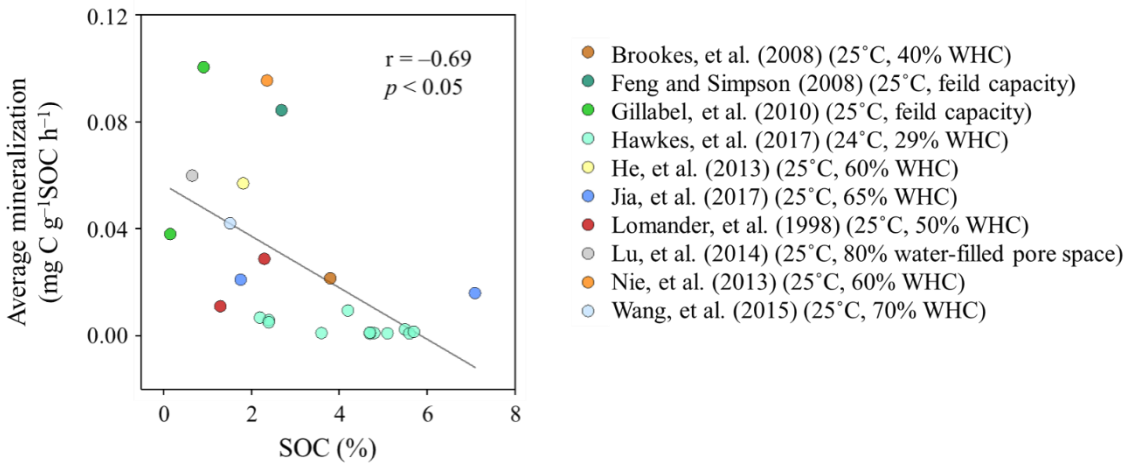

Figure S5. Spearman correlation between soil mineralization rate and soil organic carbon (SOC) content in published incubation studies at 24–25˚C with optimal moisture contents. Average mineralization rate is calculated for the first seven days of incubation for experiments of varied duration.

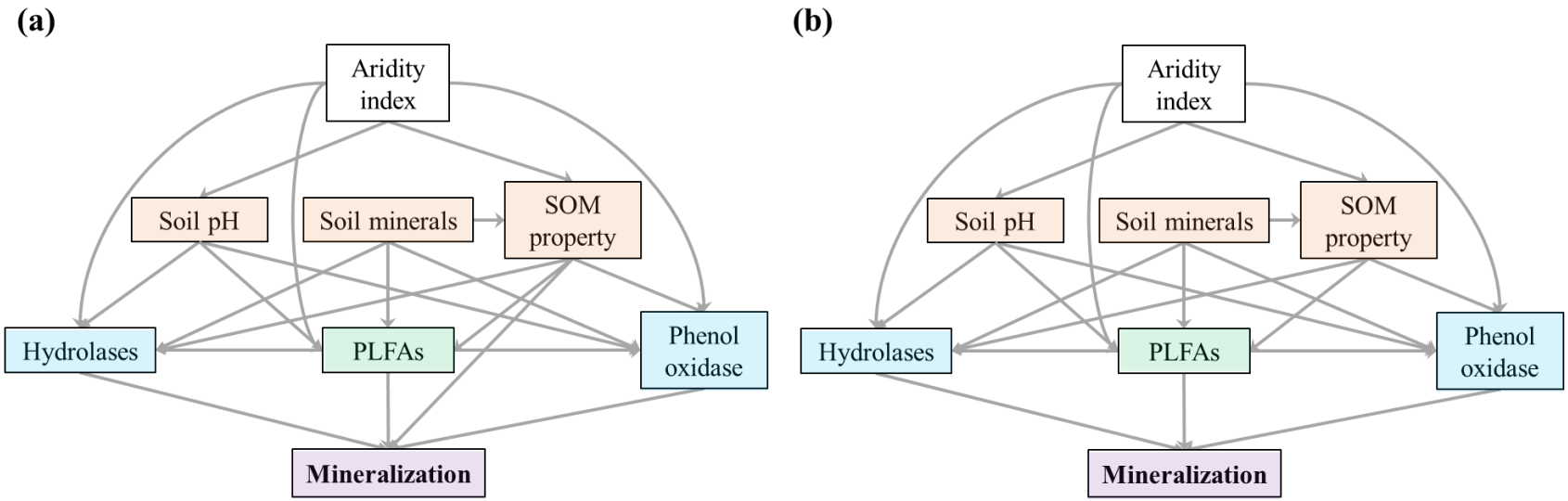

Figure S6. A priori models for the structural equation modeling of substrate mineralization potentials and environmental variables. Arrows indicate flows of causality. Environmental variables are categorized into past climate (i.e., aridity index of sites), extracellular enzymes (in blue) including hydrolyses and phenol oxidase, microbial biomass (in green) represented by phospholipid fatty acids (PLFAs) and soil properties (in orange) including soil pH, soil minerals and soil organic matter (SOM) property. Soil minerals, SOM property and hydrolases are defined by a principle component analysis (Table S5).

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