

Supplementary Information for
Calcium-rich parent materials enhance multiple soil functions and
bacterial network complexity

Running Title: Ca-rich bedrock enhances soil functions

Peilei Hu^{1,2}, Wei Zhang^{1,2*}, Wolfgang Wanek³, Ji Chen⁴, Diego Abalos⁵, Jie Zhao^{1,2},
Dan Xiao^{1,2}, Xinyu Hou^{1,2,6}, Juan Li^{1,2,6}, Hongsong Chen^{1,2}, Jun Xiao^{1,2}, Xionghui
Liao^{1,2}, Tiangang Tang^{1,2}, Hanqing Wu^{1,2}, Kelin Wang^{1,2*}

¹ Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha
410125, China

² Huanjiang Agriculture Ecosystem Observation and Research Station of Guangxi,
Guangxi Key Laboratory of Karst Ecological Processes and Services, Huanjiang
Observation and Research Station for Karst Ecosystems, Chinese Academy of
Sciences, Huanjiang 547100, China

³ Division of Terrestrial Ecosystem Research, Center of Microbiology and
Environmental Systems Science, University of Vienna, Vienna 1030, Austria

⁴ State Key Laboratory of Loess and Quaternary Geology, Institute of Earth
Environment, Chinese Academy of Sciences, Xi'an 710061, China

⁵ Department of Agroecology, Aarhus University, Tjele 8830, Denmark

⁶ University of Chinese Academy of Sciences, Beijing 100049, China

* These authors jointly supervised this work.

Corresponding authors: Wei Zhang (E-mail: zhangw@isa.ac.cn) and Kelin Wang (E-mail: kelin@isa.ac.cn)

Supplementary Materials

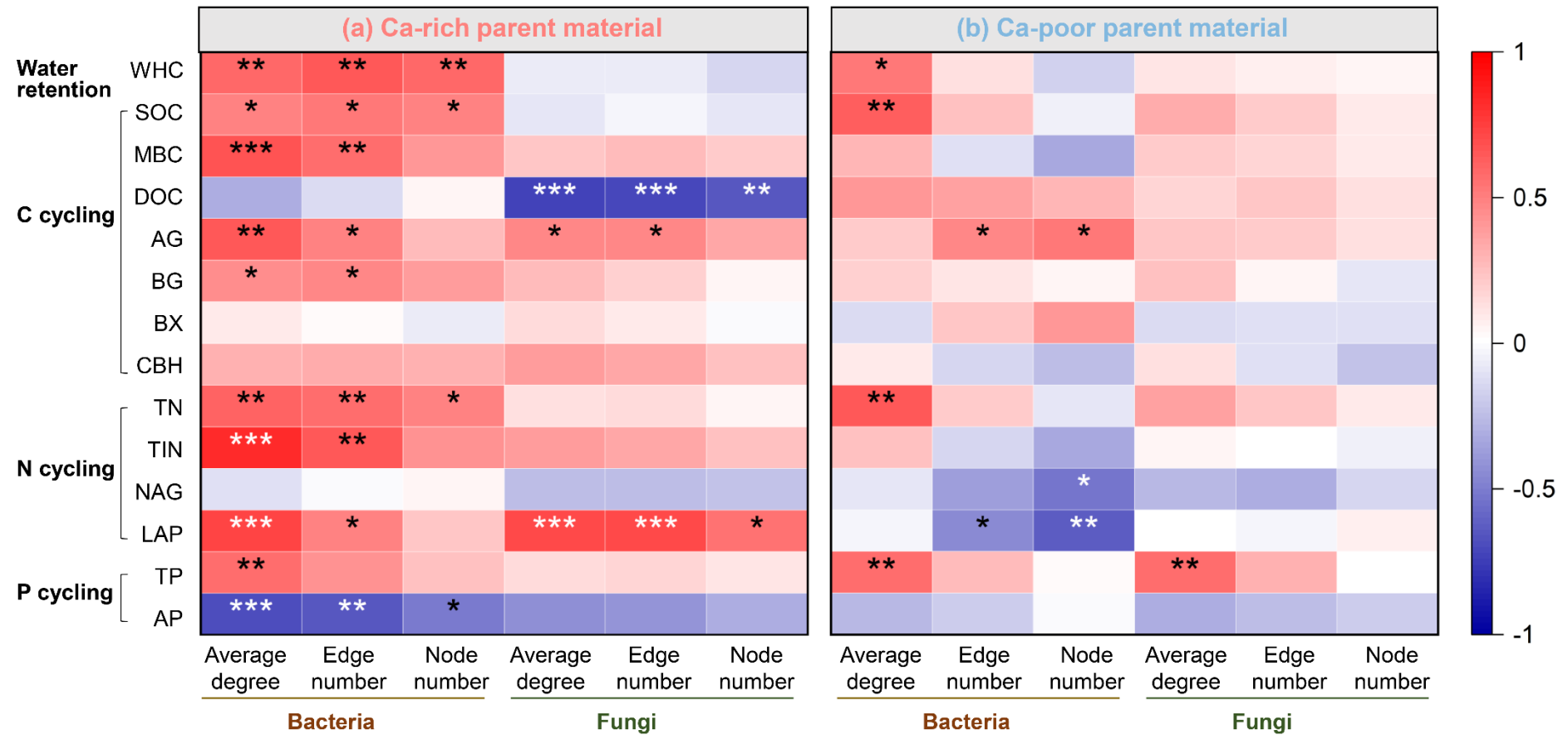
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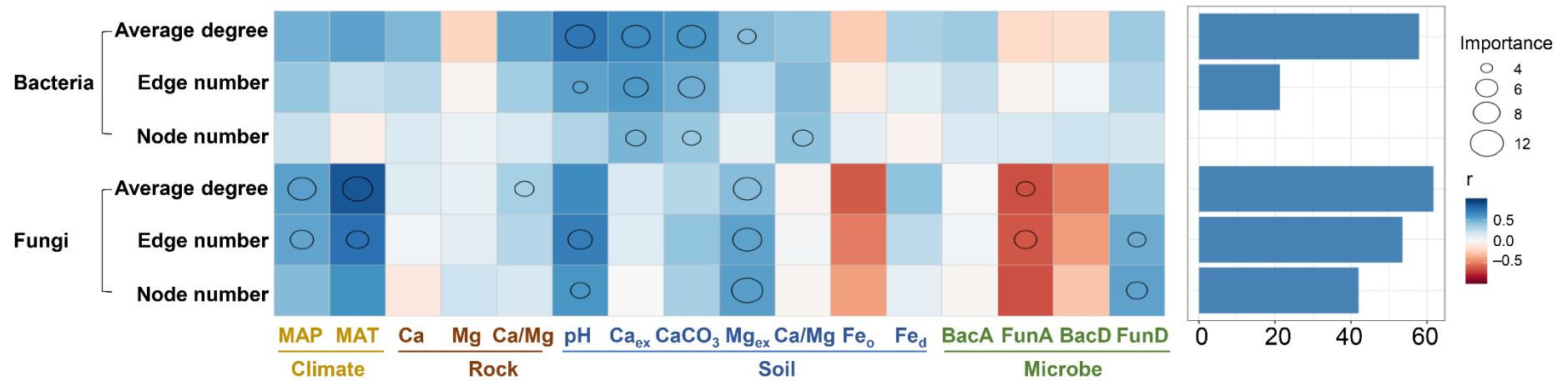
Supplementary Figures



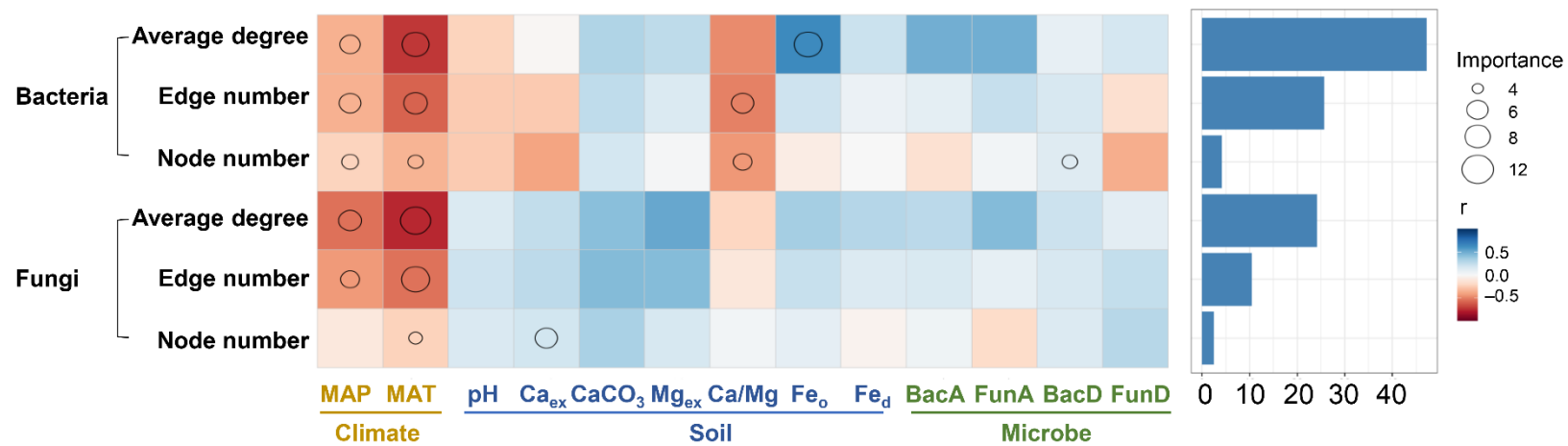
Supplementary Figure 1: Correlations between soil microbial network complexity and soil functions for the field sampling. a Correlations

in soils derived from Ca-rich parent materials. **b** Correlations in soils derived from Ca-poor parent materials. Box colors indicate Pearson's correlation coefficients between microbial network topological characteristics and soil parameters, with a gradient from blue (negative) to red (positive). C, carbon; N, nitrogen; P, phosphorus; WHC, water holding capacity; SOC, soil organic carbon; MBC, microbial biomass carbon; DOC, dissolved organic carbon; AG, α -glucosidase; BG, β -D-glucosidase; BX, β -xylosidase; CBH, β -D-cellobiosidase; TN, total nitrogen; TIN, total inorganic nitrogen; NAG, β -N-acetyl-glucosaminidase; LAP, leucine amino peptidase; TP, total phosphorus; AP, acid or alkaline phosphatase. Significant correlations are denoted by: * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

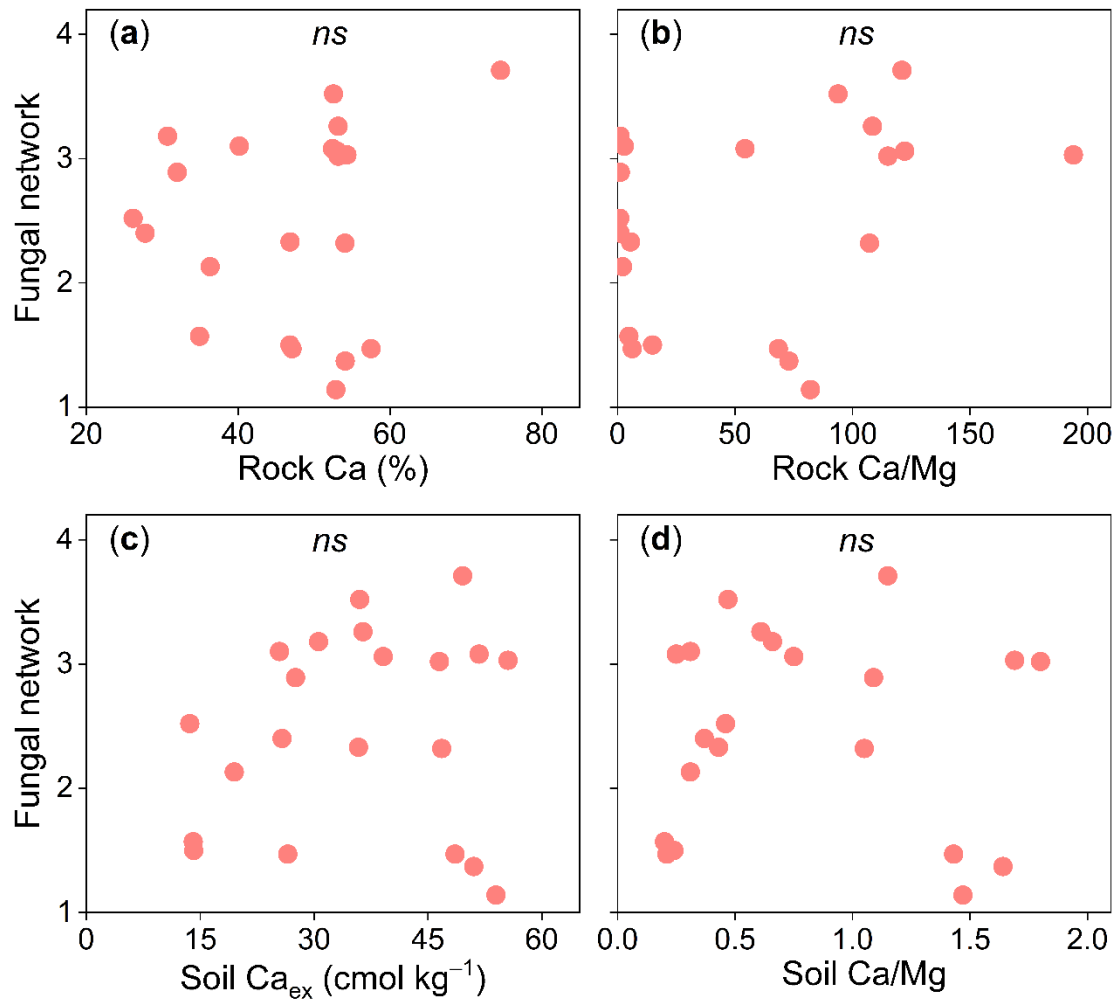
(a) Ca-rich parent material



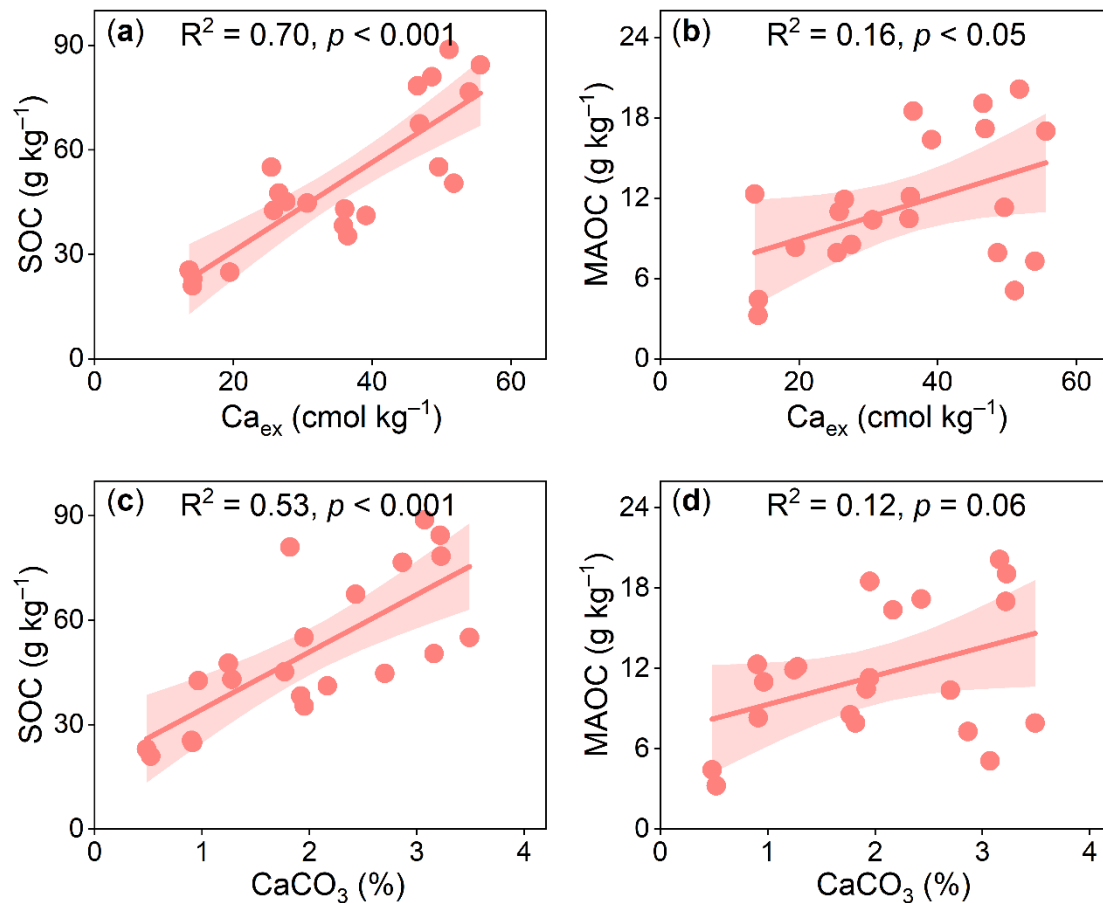
(b) Ca-poor parent material



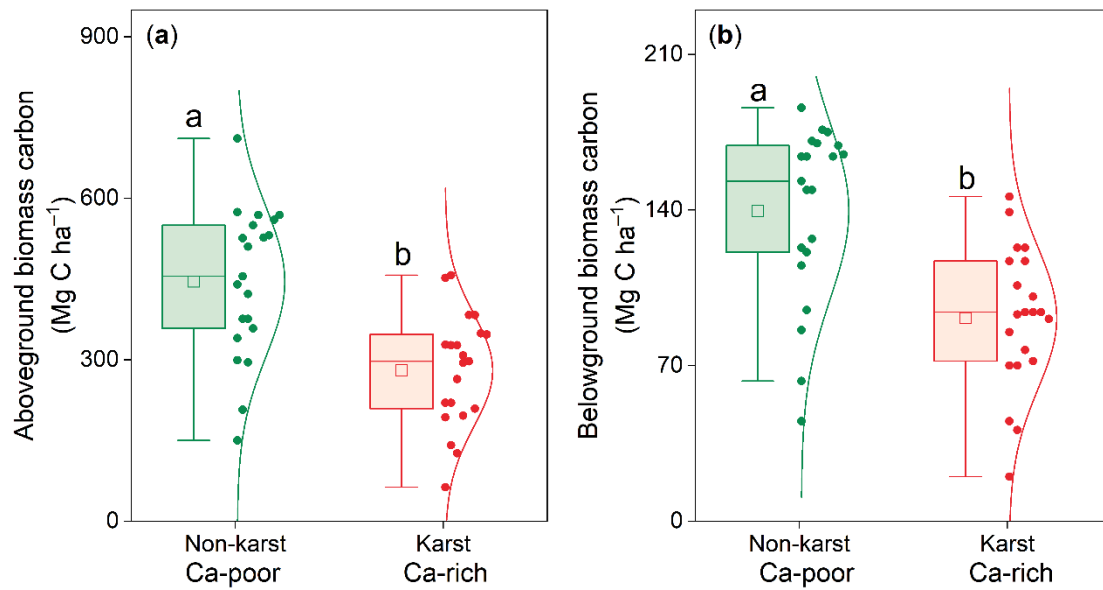
Supplementary Figure 2: Relative importance of climate, rock geochemistry, soil pH and minerals, and microbial properties in explaining the variation of soil microbial network complexity for the field sampling. **a** Contributions of these factors to microbial network complexity and their relationships in soils derived from Ca-rich parent materials. **b** Contributions of these factors to microbial network complexity and their relationships in soils derived from Ca-poor parent materials. The effect of rock geochemistry on microbial network complexity in Ca-poor parent materials was not evaluated due to the inaccessibility of these rocks. Circles within boxes indicate predictors that significantly explain the variation in corresponding network topological characteristics at $p < 0.05$ level, as determined by random forest analysis; the size of each circle reflects the predictor's explanatory power. Box colors represent the correlations between predictors and network topological characteristics based on Pearson's correlation coefficients, with a color gradient ranging from blue (positive) to red (negative). The combined use of random forest and Pearson's correlation analyses enables the assessment of both the significance and direction of each predictor's influence on microbial network topological characteristics. MAP, mean annual precipitation; MAT, mean annual temperature; Ca, total calcium; Mg, total magnesium; Ca/Mg, ratio of total calcium to magnesium; Ca_{ex}, exchangeable calcium; CaCO₃, calcium carbonate; Mg_{ex}, exchangeable magnesium; Fe_o, amorphous iron (hydr)oxides; Fe_d, pedogenic iron (hydr)oxides; BacA, bacterial abundance; FunA, fungal abundance; BacD, bacterial Shannon diversity; FunD, fungal Shannon diversity.



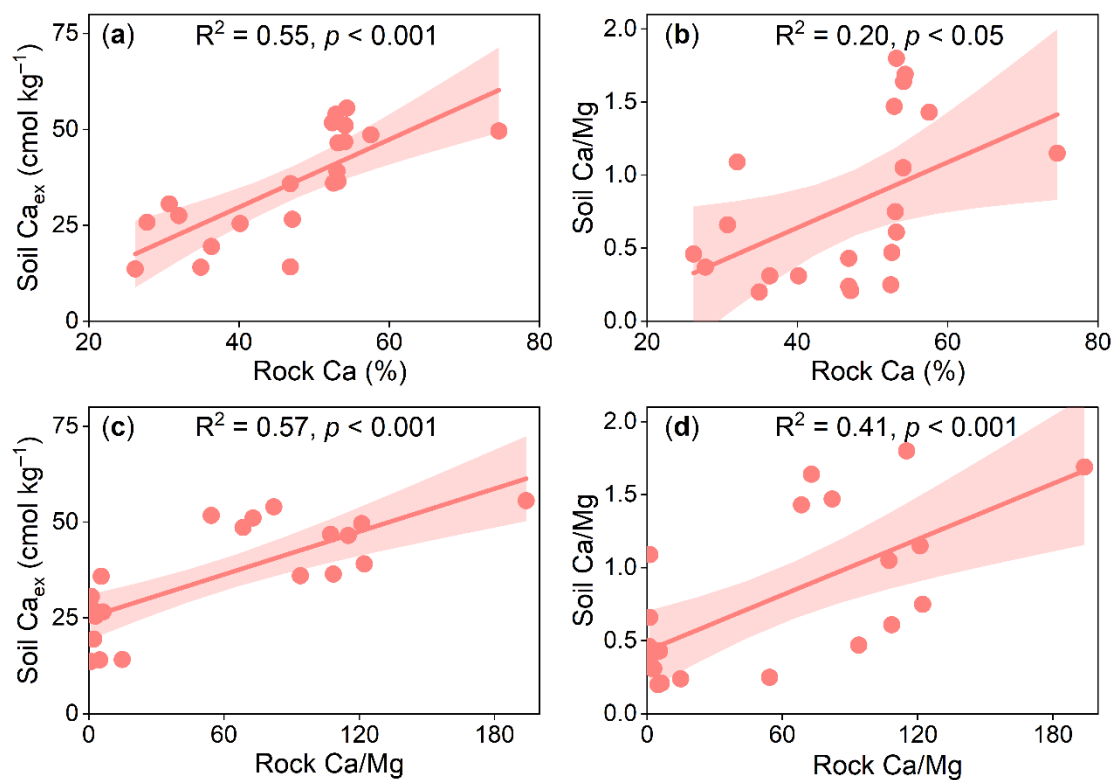
Supplementary Figure 3: Effects of rock and soil Ca on fungal network complexity within Ca-rich parent materials from the field sampling. a–b Effects of rock Ca (total Ca content, **a**; and the ratio of total Ca to magnesium, Ca/Mg, **b**) on fungal network complexity. **c–d** Effects of soil Ca (exchangeable Ca, Ca_{ex} , **c**; and Ca/Mg ratio, **d**) on fungal network complexity. Fungal network complexity was quantified by average degree, with higher values indicating more complex networks. *ns*, not significant ($p > 0.05$).



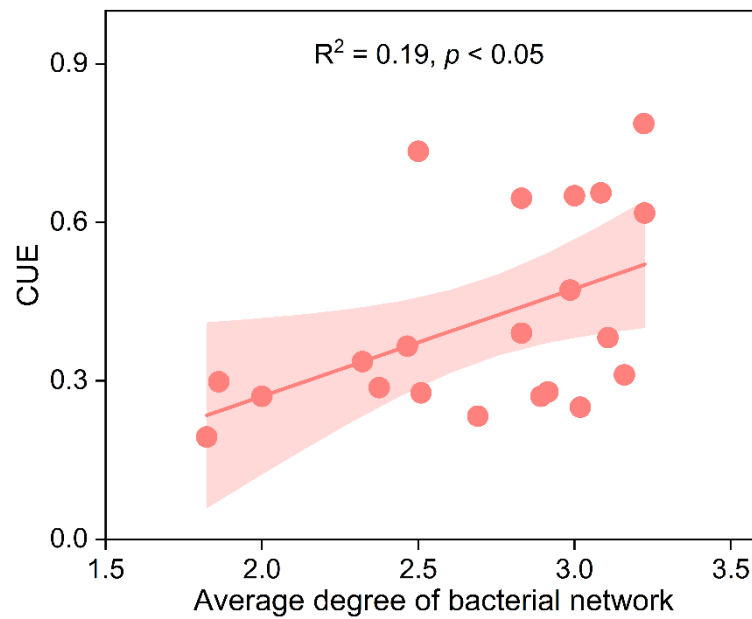
Supplementary Figure 4: Relationships of soil organic carbon and mineral-associated organic carbon with Ca in soils derived from Ca-rich parent materials from the field sampling. a Soil organic carbon (SOC) in relation to exchangeable Ca (Ca_{ex}). **b** Mineral-associated organic carbon (MAOC) in relation to Ca_{ex}. **c** SOC in relation to calcium carbonate (CaCO₃). **d** MAOC in relation to CaCO₃. Solid lines represent regressions, and shaded areas denote 95% confidence intervals. Higher MAOC content indicates a greater association of carbon with soil minerals, thereby enhancing SOC stability.



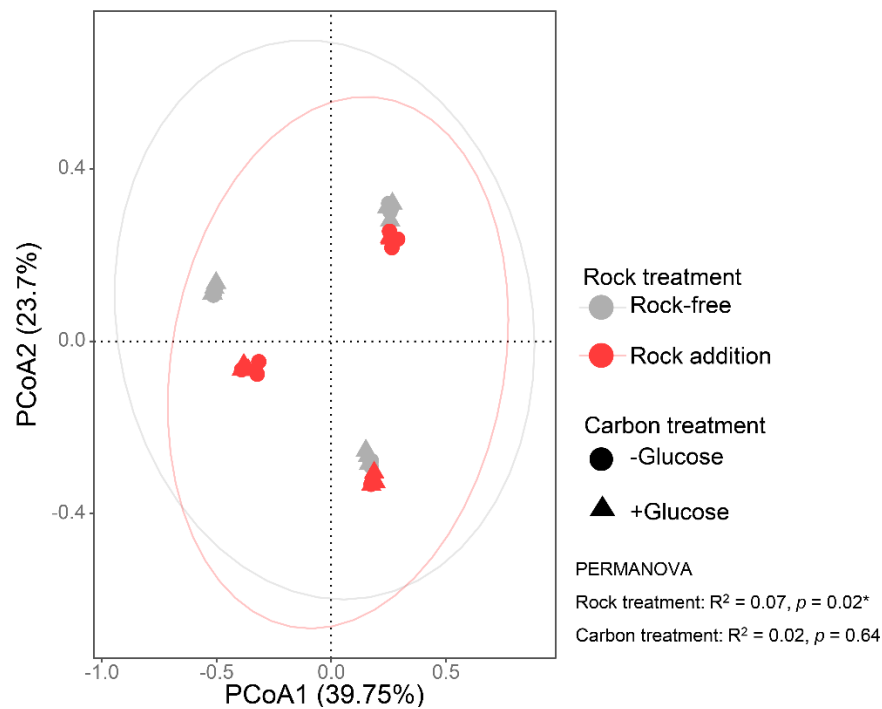
Supplementary Figure 5: Plant biomass carbon stocks from Ca-rich and Ca-poor parent materials. a Aboveground biomass carbon stock. **b** Belowground biomass carbon stock. The ends of the boxes represent the 25th and 75th percentiles, with whiskers extending to 1.5 times the standard deviation. The central line and square represent the median and mean, respectively. Lowercase letters represent significant differences at $p < 0.05$ between karst (Ca-rich) and non-karst (Ca-poor) regions.



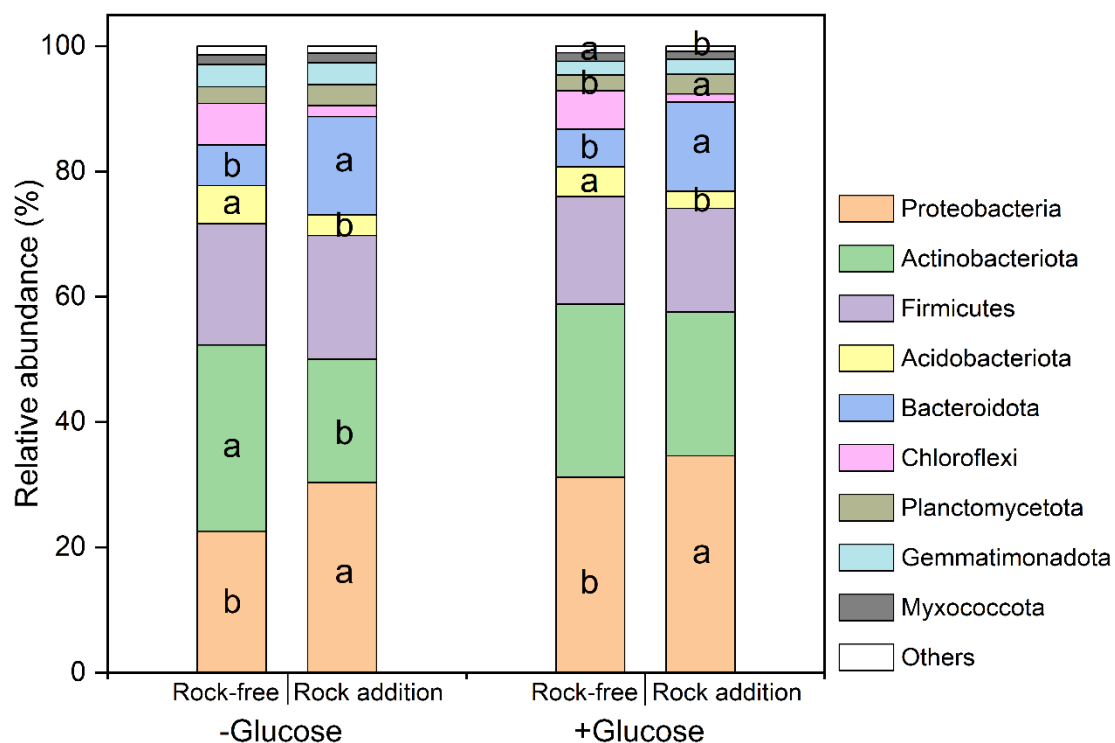
Supplementary Figure 6: Effect of rock Ca on soil Ca within Ca-rich parent materials from the field sampling. a Effect of rock Ca on soil exchangeable Ca (Ca_{ex}). **b** Effect of rock Ca on the ratio of soil total Ca to magnesium (Ca/Mg). **c** Effect of rock Ca/Mg ratio on soil Ca_{ex} . **d** Effect of rock Ca/Mg ratio on soil Ca/Mg ratio. Solid lines represent regressions, and shaded areas denote 95% confidence intervals.



Supplementary Figure 7: Relationship between soil bacterial network complexity and microbial carbon use efficiency within Ca-rich parent materials from the field sampling. Bacterial network complexity was quantified by average degree, with higher values indicating more complex networks. Solid lines represent regressions, and shaded areas denote 95% confidence intervals. CUE, carbon use efficiency.

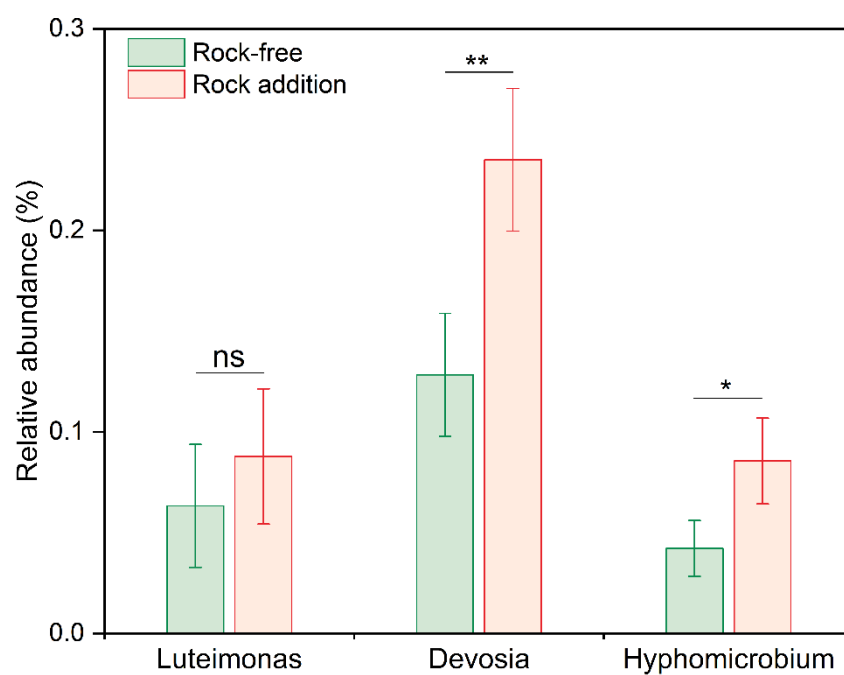


Supplementary Figure 8: Soil bacterial community structure in the microcosm experiment under treatments with and without rock addition, and with and without glucose addition. Bacterial community structure was assessed using principal coordinate analysis (PCoA) ordinations of Bray-Curtis dissimilarities and permutational multivariate analysis of variance ANOVA (PERMANOVA), based on 16S amplicon sequencing data. -Glucose: without glucose addition; +Glucose: with glucose addition.



Supplementary Figure 9: Relative abundance of bacterial taxa at the phylum level

in the microcosm experiment. The left panel compares treatments without and with Ca-rich rock addition under glucose-unamended conditions (-Glucose), while the right panel compares treatments without and with Ca-rich rock addition under glucose-amended conditions (+Glucose). Lowercase letters represent significant differences at $p < 0.05$ in the relative abundance of bacterial phylum between rock-free and rock addition within the same glucose treatment.



Supplementary Figure 10: Relative abundance of representative genes within the phylum *Proteobacteria* in the microcosm experiment, comparing treatments with and without Ca-rich rock addition. * $p < 0.05$, ** $p < 0.01$, and ns indicates non-significance ($p > 0.05$) in gene abundance between rock-free and rock addition treatments.

Supplementary Tables

Supplementary Table 1: Rock, soil, and microbial properties of the field study sites.

	Variable	Ca-poor	Ca-rich
Rock	Rock Ca (%)*	8.86±6.14	46.70±2.60
	Rock Mg (%)*	1.69±0.76	5.82±1.62
	Rock Ca/Mg*	7.99±7.31	56.22±12.56
Soil	pH	4.65±0.20 b	6.47±0.17 a
	Ca _{ex} (cmol kg ⁻¹)	6.75±2.18 b	35.65±3.05 a
	CaCO ₃ (%)	0.92±0.15 b	2.00±0.21 a
	Mg _{ex} (cmol kg ⁻¹)	2.20±0.41 b	9.39±1.78 a
	CaO (%)	0.36±0.08 b	1.87±0.51 a
	MgO (%)	0.80±0.12 b	1.74±0.23 a
	Ca/Mg	0.37±0.08 b	0.79±0.12 a
	Fe _o (g kg ⁻¹)	6.82±1.12 a	3.11±0.33 b
	Fe _d (g kg ⁻¹)	45.82±4.05	55.42±4.71
Microbe	Bacterial abundance (10 ⁷ copies g ⁻¹ soil)	485.7±134.2 b	799.3±120.4 a
	Fungal abundance (10 ⁷ copies g ⁻¹ soil)	12.31±2.74 a	5.08±1.19 b
	Bacterial diversity	4.73±0.08 b	5.10±0.05 a
	Fungal diversity	3.38±0.14 b	4.11±0.17 a

All values are presented as means ± standard errors. Lowercase letters denote significant differences ($p < 0.05$) between Ca-rich (carbonate rocks) and Ca-poor (clastic rocks) parent materials. * indicates that only 8 rock samples were collected from 21 Ca-poor sites, as clastic rocks are frequently buried at considerable depths, whereas all 21 Ca-rich sites provided samples. Due to the substantial disparity in sample sizes, an independent samples *t*-test was not performed. Nevertheless, the limited data demonstrate markedly lower rock Ca content in Ca-poor sites compared to Ca-rich sites, validating their classification as Ca-poor.

Supplementary Table 2: Lithology, climate, and dominant species of the field study sites.

Site (County)	Lithology	Latitude (N°)	Longitude (E°)	MAT (°C)	MAP (mm)	Altitude (m)	Dominant specie
Shuicheng	Carbonate rock	26.38–26.40	104.82	12.6	1208	2095	<i>Ulmus pumila</i> (L.); <i>Quercus variabilis</i> (Bl.); <i>Platycarya longipes</i> (W.); <i>Cyclobalanopsis glauca</i> (T.) Oerst.; <i>Daphniphyllum macropodum</i> Miq.
	Clastic rock	26.26–26.46	104.78–104.81	12.6	1208	2041	<i>Carpinus viminea</i> Wall.; <i>Symplocos sumuntia</i> Buch.-Ham. ex D. Don; <i>Quercus aliena</i> Bl.; <i>Quercus engleriana</i> Seem.; <i>Quercus fabri</i> Hance
Jinsha	Carbonate rock	27.52	106.07–106.08	14.6	1013	1035	<i>Celtis biondii</i> (Pamp.); <i>Cyclobalanopsis glauca</i> (T.) Oerst; <i>Cupressus funebris</i> (Endl.); <i>Lindera communis</i> Hemsl.; <i>Dendrobenthamia angustata</i> (Chun) Fang
	Clastic rock	27.38–27.54	105.99–106.29	14.6	1013	1088	<i>Carpinus viminea</i> Wall.; <i>Cyclobalanopsis multinervis</i> W.C. Cheng et T. Hong; <i>Camellia pitardii</i> Coh. St.; <i>Eurya oblonga</i> Yang; <i>Lyonia ovalifolia</i> (Wall.) Drude
Duyun	Carbonate rock	26.08–26.10	107.22–107.25	15.5	1256	1055	<i>Cyclobalanopsis glauca</i> (T.) Oerst; <i>Cinnamomum bodinieri</i> Levl; <i>Cinnamomum tamala</i> (Ham.); <i>Celtis biondii</i> Pamp.; <i>Clausena dunniana</i> Levl.
	Clastic rock	26.38	107.35–107.36	14.7	1220	1197	<i>Clethra kaipoensis</i> Levl.; <i>Machilus chienkweiensis</i> S. Lee; <i>Fagus longipetiolata</i> Seem.; <i>Rhododendron rivulare</i> Hand.-Mazz.; <i>Camellia pitardii</i> Coh. St.
Huanjiang	Carbonate rock	25.10–25.15	108.03–108.04	18.8	1371	398	<i>Mallotus japonicus</i> (Thunb.); <i>Liquidambar formosana</i> Hance; <i>Alchornea trewioides</i> (Benth.) Muell. Arg.; <i>Acer oblongum</i> Wall; <i>Pyracantha fortuneana</i> (Maxim.) Li.
	Clastic rock	25.10–25.21	108.10–108.65	18.6	1481	314	<i>Castanopsis hystrix</i> Miq.; <i>Macaranga henryi</i> (Pax et Hoffm.) Rehd.; <i>Lindera kwangtungensis</i> (Liou) Allen; <i>Corylopsis multiflora</i> Hance;

							<i>Neolitsea aurata</i> (Hay.) Koidz. var. <i>chekiangensis</i> (Nakai) Yang et P. H. Huang
Du'an	Carbonate rock	24.28–24.45	107.95–107.98	18.8	1516	422	<i>Cyclobalanopsis glauca</i> (T.) Oerst; <i>Acer oblongum</i> Wall.; <i>Phaseolus vulgaris</i> Linn.; <i>Ulmus pumila</i> (L.); <i>Koelreuteria paniculata</i> Laxm.
	Clastic rock	23.86–23.87	108.12	19.6	1607	176	<i>Schefflera delavayi</i> (Franch.) Harms ex Diels.; <i>Castanopsis hystrix</i> Miq.; <i>Macaranga henryi</i> (Pax et Hoffm.) Rehd.; <i>Daphniphyllum oldhami</i> (Hemsl.) Rosenth.; <i>Engelhardtia roxburghiana</i>
Mashan	Carbonate rock	23.66	108.33	21.0	1594	595	<i>Castanopsis hystrix</i> Miq.; <i>Pittosporum tonkinense</i> Gagnep.; <i>Platycarya longipes</i> Wu; <i>Cinnamomum bodinieri</i> Levl.; <i>Streblus tonkinensis</i> (Dub. et Eberh.) Corner
	Clastic rock	23.52–23.69	108.13–108.20	21.1	1513	239	<i>Castanopsis fargesii</i> Franch.; <i>Schima argentea</i> Pritz. ex Diels; <i>Corylopsis multiflora</i> Hance; <i>Skimmia arborescens</i> Anders.; <i>Engelhardtia roxburghiana</i>
Longzhou	Carbonate rock	22.48–22.67	106.77–106.91	21.8	1296	331	<i>Castanopsis hystrix</i> Miq.; <i>Ostrya japonica</i> Sarg.; <i>Ficus microcarpa</i> Linn.; <i>Strophoblachia fimbriicalyx</i> Boerl.; <i>Clausena dunniana</i> Levl.
	Clastic rock	22.37	106.67–106.69	21.3	1363	203	<i>Lasianthus appressihirtus</i> Simizu; <i>Streblus indicus</i> (Bur.) Corner; <i>Eurya subintegra</i> Kobuski; <i>Xanthophyllum hainanense</i> Hu; <i>Eurya subintegra</i> Kobuski

MAT, mean annual temperature; MAP, mean annual precipitation. Shuicheng, Jinsha, and Duyun are located in Guizhou Province, and Huanjiang,

Du'an, Mashan, and Longzhou in Guangxi Province. Carbonate rock is Ca-rich bedrock, while clastic rock belongs to Ca-poor bedrock.

Supplementary Table 3: Geographical and climatic characteristics of soil sampling sites for the microcosm experiment.

Site (County)	Lithology	Latitude (N°)	Longitude (E°)	MAT (°C)	MAP (mm)	Altitude (m)	Dominant species
Nanchuan	Carbonate rock	29.11	107.36	12.6	1272	1362	<i>Dendrobenthamia angustata</i> (Chun) Fang var. <i>angustata</i> ; <i>Symplocos sumuntia</i> Buch.-Ham.ex D. Don; <i>Cinnamomum wilsonii</i> Gamble; <i>Daphniphyllum macropodium</i> Miq.; <i>Machilus chienkweiensis</i> S.Lee
Dushan	Carbonate rock	25.98	107.53	15.3	1223	944	<i>Quercus phillyreoides</i> A. Gray; <i>Clausena dunniana</i> Levl.; <i>Platycarya longipes</i> Wu; <i>Celtis biondii</i> Pamp.; <i>Sageretia henryi</i> Drumm. et Sprague
Longzhou	Carbonate rock	22.47	106.94	21.7	1393	273	<i>Walsura robusta</i> Roxb. Hort.; <i>Diospyros siderophylla</i> Li; <i>Cleistanthus sumatranus</i> (Miq.) Muell. Arg.; <i>Fordia cauliflora</i> Hemsl.; <i>Strophoblachia fimbriicalyx</i> Boerl.

MAT, mean annual temperature; MAP, mean annual precipitation. Nanchuan is located in Chongqing Province, Dushan in Guizhou Province, and Longzhou in Guangxi Province.

Supplementary Table 4: Spearman correlation thresholds determined using the Random matrix theory method.

	Treatment	Bacteria	Fungi
Field study	Carbonate rock (Ca-rich bedrock)	0.778	0.686
	Clastic rock (Ca-poor bedrock)	0.743	0.686
Microcosm experiment	Control (no Ca-rich rock addition)	0.955	
	Ca-rich rock addition	0.930	

Supplementary Methods

Supplementary Method 1: Analysis of mineral-associated organic carbon

We measured mineral-associated organic carbon content due to its predominant stabilization by minerals and its contribution to long-term carbon storage in soils (Cotrufo et al., 2019). Briefly, 10 g of oven-dried soil was mixed with 50 mL of a 5 g L⁻¹ sodium hexametaphosphate solution and glass beads. The mixture was agitated on a reciprocating shaker for 18 hours to achieve complete soil dispersion. The dispersed solution was then rinsed multiple times with distilled water, and the fraction passing through a 53-μm mesh was collected as the mineral-associated organic matter fraction. The collected mineral-associated organic matter fraction was dried at 45°C until a constant weight was obtained. Organic carbon content of the mineral-associated organic matter fraction was determined using an Elementar Vario MAX cube analyzer (Elementar, Hanau, Germany) after treating the sample with phosphoric acid to remove inorganic carbon. Mineral-associated organic carbon content was calculated by multiplying the organic carbon content of the mineral-associated organic matter fraction by the proportion of mineral-associated organic matter in the total soil sample.

Supplementary Method 2: Analysis of microbial carbon use efficiency

Soil microbial carbon use efficiency (CUE) was determined using the substrate-independent method based on the incorporation of ¹⁸O-H₂O into microbial DNA (Spohn et al., 2016; Qu et al., 2020). Briefly, fresh soils were preincubated in darkness at 25 °C for 7 days, maintaining the soil moisture at 60% of the water holding capacity.

Subsequently, four replicates of each soil were prepared, with two tubes used for ^{18}O - H_2O labeling and the other two for unlabeled controls. For each replicate, 2 g of soil were placed into 2 mL centrifuge tubes. The tubes were then placed in 50 mL glass bottles, with four empty bottles serving as blanks. CO_2 -free air was then flushed into each bottle to ensure consistent initial gas conditions. The ^{18}O - H_2O (97 at% ^{18}O) was added to each of the two replicates, and the abundance was adjusted to 20 at% ^{18}O of the final soil water. The other two replicates received the same volume of unlabeled water to serve as natural abundance controls. After incubation at 25 °C for 24 hours, a 15 mL gas sample was collected from each bottle using a syringe, and the CO_2 concentration was determined using a gas chromatograph (GC-7890A; Agilent, Santa Clara, CA, USA). Microbial respiration (Respiration, $\mu\text{g C g}^{-1} \text{ soil h}^{-1}$) was calculated by determining the difference in headspace CO_2 concentration between the soil sample and blank bottles (Equation 1).

$$\text{Respiration} = \frac{(C - C_0) * M * V * 273}{V_m * DW * 298 * t * 1000} \quad (1)$$

where C and C_0 are the CO_2 concentrations (ppm) in the sample and blank, respectively; M is the molecular mass of the C (12 g mol^{-1}); V is the volume of the headspace in a bottle (mL); 273 is the absolute temperature (K) at standard atmospheric pressure; V_m is the standard molar volume of an ideal gas (L mol^{-1}); DW is the dry mass of soil (g); 298 is the incubation temperature (K); t is the incubation time (h); and 1000 is the unit conversion factor.

Microbial growth was calculated by tracking the incorporation of ^{18}O into microbial DNA through synthesis of new DNA. Total DNA was extracted from 0.5 g

soil after incubation using the FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's procedures. The quantity and quality of DNA were measured using a NanoDrop 2000 UV-VIS-spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The remaining DNA extract was transferred into a silver cup and dried at 45 °C, after which the abundance of ¹⁸O and the total O content were measured using a combined elemental analyzer-isotope ratio mass spectrometer (MAT253, Thermo Fisher, USA). During the 24-hour incubation, newly produced DNA ($DNA_{produced}$, µg) was quantified by the difference in ¹⁸O abundance between the labelled and unlabeled samples (Equation 2)

$$DNA_{produced} = O_{total} * \frac{at\%_{excess}}{100} * \frac{100}{at\%_{final}} * \frac{100}{31.21} \quad (2)$$

where O_{total} is the total O content (µg) of the dried DNA extract; $at\%_{excess}$ is the difference between ¹⁸O at% of the labelled and unlabeled sample; $at\%_{final}$ is the enrichment of ¹⁸O at% of the final soil solution; and the constant 31.21 is the average proportional mass of O content in DNA (C₃₉H₄₄O₂₄N₁₅P₄).

The corresponding conversion factor (f_{DNA}) was calculated based on the ratio of MBC (µg g⁻¹ soil) to soil microbial DNA content (µg g⁻¹ soil). Microbial growth rate (Growth, µg C g⁻¹ soil h⁻¹) was calculated based on the $DNA_{produced}$ and f_{DNA} (Equation 3).

$$Growth = \frac{f_{DNA} * DNA_{produced} * 1000}{DW * t * 1000} \quad (3)$$

where DW is the dry mass of soil (g), and t is the incubation time (h).

Finally, microbial CUE was calculated based on growth and respiration (Equation 4).

$$CUE = \frac{Growth}{Growth + Respiration} \quad (4)$$

Supplementary Method 3: Analysis of plant biomass carbon

Tree aboveground and belowground biomass carbon (Mg carbon ha⁻¹) of each forest site were obtained from the global biomass carbon density dataset developed by Spawn et al. (2020). This dataset is publicly available through the Oak Ridge National Laboratory DAAC repository (<https://doi.org/10.3334/ORNLDAAC/1763>). The data were imported into ArcGIS 10.5, where plant biomass carbon was extracted for each site based on the corresponding geographic coordinates.

Supplementary References

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