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Tree species diversity increases soil microbial carbon use efficiency in a subtropical forest

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Abstract

Plant communities strongly influence soil microbial communities and, in turn, soil carbon (C) cycling. Microbial carbon use efficiency (CUE) is an important parameter for predicting soil C accumulation, yet how plant and soil microbial community traits influence microbial CUE remains poorly understood. Here, we determined how soil microbial CUE is influenced by plant and soil microbial community traits, by studying a natural gradient of plant species diversity in a subtropical forest. Our results showed that microbial CUE increased with increasing tree species diversity, suggesting a correlation between plant community traits and soil C storage. The specific soil properties that explained the greatest variation in microbial CUE were associated with microbial communities (biomass, enzyme activities and the ratio of oligotrophic to copiotrophic taxa); there were weaker correlations with plant-input properties, soil chemistry and soil organic C quality and its mineral protection. Overall, high microbial CUE was associated with soil properties correlated with increased tree species diversity: higher substrate availability (simple SOM chemical structures and weak mineral organic associations) and high microbial growth rates despite increased community dominance by oligotrophic strategists. Our results point to a mechanism by which increased tree species diversity may increase the forest C sink by affecting carbon use with the soil microbial community.

KEYWORDS
microbial community composition, microbial CUE, microbial growth strategies, mineral protection, plant species diversity, SOC quality

1 | INTRODUCTION

Soils are a critical component of the global carbon (C) cycle and constitute the largest reservoir of terrestrial organic C (~1500 Pg C in the surface 1 m) (Jackson et al., 2017). The dynamics of soil organic C (SOC) depends strongly upon microorganisms, which play key roles in both SOC formation and decomposition (Sokol et al., 2022). The balance of soil C inputs and outputs can shift under altered environmental conditions, in part due to changes in microbial community composition, activity, and physiology (Fanin et al., 2022; García-Palacios et al., 2021). Among the microbial traits that influence soil C balance, microbial carbon use efficiency (CUE), the proportion of C substrate that is retained in the microbial biomass compared with that respired as CO_2, is central to both SOC formation and decomposition (Kästner et al., 2021). As such, even small changes in CUE may have major implications for SOC storage and stabilization (García-Palacios et al., 2021; Tao et al., 2023). However, the connection between CUE and soil C dynamics is complex and mediated by multiple soil biological, ecological, and physicochemical factors. For example, high microbial CUE means relatively lower respiratory C losses during decomposition and higher accumulation of microbial biomass, which could enhance microbial-derived C storage in soils (Sokol et al., 2022). In contrast, a high CUE can also promote microbial proliferation, leading to more efficient plant litter exploitation with associated organic matter losses and lower plant-derived C storage (Craig et al., 2022). Therefore, the accurate evaluation of factors controlling CUE is crucial for more realistic projection of long-term soil C sequestration and climate-C cycle feedbacks.

There are many soil properties that can influence microbial CUE, and studies have shown effects of nutrient availability, substrate elemental composition (stoichiometry), substrate molecular structure, C accessibility, microbial community structure and depolymerization of plant matter (Feng et al., 2022; Kästner et al., 2021). In particular, substrate quality and stoichiometry have been shown to have important influences on CUE, by affecting microbial investment in resource acquisition (Li, Pei, et al., 2021). According to the theory of stoichiometric homeostasis, a higher soil C:nitrogen (N) or C:phosphorus (P) ratio is often linked to lower CUE as microorganisms maintain their homeostatic C:nutrient ratios through overflow respiration and investment of C into enzymes for nutrient acquisition (Feng et al., 2021). The microbial community composition may also influence CUE, which appears to be partially phylogenetically constrained (Hasby et al., 2021; Smith et al., 2021), thus suggesting that different communities have a different community-wide CUE. For example, Domeignoz-Horta et al. (2020) found that a shift in bacterial communities changed CUE, with CUE positively correlated with bacterial alpha and beta diversity but not with fungal diversity. However, studies have also reported neutral (Malik et al., 2018) and negative (Zhang et al., 2020) relationships between microbial alpha diversity and CUE. Another important influence on CUE is the accessibility of soil C to microbes, constrained by mineral protection and its influence on the microbial community (He et al., 2022; Kleber et al., 2021; Qin et al., 2021). For example, it has been reported that the presence of short-range order minerals, such as allophane, imogolite, and ferrihydrite can inhibit the growth of bacteria by restricting microbial mobility and access to organic matter (Finley et al., 2022). Plant community properties, that is, diversity or compositional difference resulting in variation in nutrient contents of plant-inputs to soil, can further modulate microbial CUE. For example, increased input quality can promote faster microbial growth, turnover and higher CUE (Craig et al., 2022). Collectively, how plant and soil properties, such as biotic diversity and community composition, chemistry, substrate availability and mineral protection of C substrate interact to affect microbial CUE remains unclear.

Plant species diversity promotes and stabilizes the provisioning of ecosystem functions and services, such as biomass production, nutrient supply, soil C storage, and climate change mitigation (Cantwell-Jones et al., 2022; Chen et al., 2023; Hua et al., 2022; Mori et al., 2021). Consequently, diversity loss due to global change and land-use change threatens ecosystem functioning (Redlich et al., 2022). Higher plant species diversity has been associated with increased primary production and plant-derived C inputs into the soil (Mori et al., 2020; Peng & Chen, 2021), in turn increasing the resource supply to soil microbial community and affecting soil C dynamics (Beugnon et al., 2021; Lange et al., 2015). Indeed, several studies have demonstrated the large influence of plant species diversity on soil microbial community composition, activity, and biomass (Gottschall et al., 2022; Lu & Scheu, 2021; Zhang et al., 2021). However, how plant species diversity modulates microbial physiology parameters, such as CUE remains poorly understood. To our knowledge, the response of CUE to plant species diversity has only been explored by two studies, which show contrasting results. In a temperate grassland diversity experiment, Prommer et al. (2020) showed that plant species richness had no significant effect on microbial CUE; whereas across a network of tree diversity experiments, Cesarz et al. (2022) showed that increased tree species diversity was associated with an increased metabolic quotient (i.e., the CUE decreased). These limited and contradictory insights demonstrate the lack of understanding of how plant–microbe–mineral interactions modulate the response of microbial CUE to plant species diversity.

Here, we explored how plant and soil properties determine soil microbial CUE and tested the overarching hypothesis that plant species diversity is positively related to CUE, by using a natural gradient of plant species diversity in a subtropical mature forest, southwest China. This region act as a hot spot of global greening with forest cover increasing over the past two decades (Tong et al., 2018), and accounts for about 32% of the terrestrial C sequestration across mainland China (Wang et al., 2020). To explore the key determinants of soil microbial CUE, we measured various biotic and abiotic factors including plant community traits, plant-input properties (litter and fine roots), soil chemical properties, SOC quality, mineral protection of SOC, and microbial properties (Figure S1, Table S1). We conducted partial correlation, hierarchical partitioning, variation

partitioning analysis (VPA), and structural equation modeling (SEM) to quantify the relative contribution of these factors in controlling microbial CUE. We aimed to answer the following questions: (i) how is microbial CUE related to plant species diversity, and (ii) what are mechanisms underlying the effects of plant species diversity on microbial CUE?

2 | MATERIALS AND METHODS

2.1 | Experimental design and soil sampling

The study site located in a subtropical karst forest in Mulun National Nature Reserve, southwest China (107°54′01″–108°05′51″E, 25°07′01″–25°12′22″N). Mean annual precipitation and air temperature of the region are 1500mm and 19.38°C, respectively. The climax vegetation is subtropical mixed evergreen and deciduous broad-leaved forest. The soils are calcareous soils with soil types being Cambisols, Luvisols or Leptosols underlain by a mixture of limestone and dolomite (Qian et al., 2023). The forest was naturally regenerated after clear-cut in the end of 1950s. Forty-five plots of 20m×20m each were established in the valley and on the lower slope in 2020 with the soil type being Cambisols. The horizontal interval between any two adjacent plots was 40m to avert any interference due to neighborhood effects and spatial autocorrelation. The elevation of the selected plots varied between 446.3 and 521.3m, to reduce climatic and topographic variation. Trees with a diameter at breast height (DBH) greater than 1cm were identified, measured, and tagged. Tree species diversity ranged from 4 to 61 (richness) and 0.15 to 3.57 (Shannon index). In addition, herbs and shrubs with a DBH <1cm were also surveyed in each plot. For each plot, the diversity indices for herbs and shrubs ranged from 7 to 27 (richness) and 1.03 to 2.39 (Shannon index). Tree species in each plot are presented in Data S1. All plant species diversity indices are presented in Table S2.

Soil sampling was conducted between July 28 and August 1, 2020. Sixteen sampling locations were randomly selected in each plot. Samples from the litter horizon (<2cm in depth) were collected at each location with a 10cm×10cm stainless frame. Mineral soil samples (0–10cm) were collected using a soil corer of 5cm in diameter after removing the possible organic layers. The 16 soil cores were mixed into one composite sample per plot. Fine roots (<2mm in diameter) were collected by hand and all the attached residues (soil, dead roots, stem materials and litter fractions) were carefully removed with tweezers. Fine roots were thoroughly rinsed in deionized water and oven dried at 60°C for 48h and weighed to determine fine root biomass and chemical properties. The soil samples were sieved to 2mm, and then divided into three portions. The first portion was stored at −20°C for DNA and phospholipid fatty acid (PLFA) extraction. The second portion was stored at 4°C for the determination of CUE, ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic C and N (DOC and DON), microbial biomass C, N, and P (MBC, MBN and MBP), and enzyme activities. The third portion was air dried for the determination of SOC molecular composition by ^{13}C nuclear magnetic resonance (NMR) spectroscopy, soil pH, texture, SOC, total N and P (TN and TP), available P (AVP), exchangeable Ca and Mg, and minerals of iron (Fe) and aluminum (Al).

2.2 | Measurements of root, litter, and soil physicochemical properties

Measurements of root, litter, and soil physicochemical properties were conducted following the protocols provided by Carter and Gregorich (2007). Soil pH (1:2.5 soil/water ratio) was measured with a pH meter (FE20K, Mettler–Toledo). Soil texture was determined by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments Ltd.) after removal of organic matter and carbonates. Sand, silt, and clay were defined as particle sizes of 2000–53μm, 53–2μm and <2μm, respectively. Litter, root and soil organic C were measured by dichromate redox colorimetric method. Litter, root, and soil total N were analyzed using an elemental analyzer (EA 3000; EuroVector). Litter, root, and soil total P were analyzed colorimetrically using the ascorbic acid molybdate method. DOC and total dissolved N (TDN) were measured by the wet oxidation and persulfate oxidation methods, respectively. Soil NH_4^+ and NO_3^- were analyzed by an auto-analyzer (FIAstar 5000, FOSS). Soil AVP was determined using the molybdenum blue colorimetric method. Soil MBC, MBN, and MBP were measured using the chloroform fumigation–extraction method. Total carbonate expressed as $CaCO_3$ equivalent was determined using a weak acid dissolution followed by measurement of pH of the extractant. The effective cation exchange capacity (CEC) was calculated by summing the charge equivalents of exchangeable Na, K, Mg, Ca, Mn, Al, Fe, and H. Soil physicochemical properties are given in Table S3. Litter and fine root properties are presented in Table S4.

2.3 | Determination of microbial carbon use efficiency

A short-term incubation experiment was conducted to determine microbial CUE (Spohn et al., 2016). Specifically, each soil sample in screw vials was enriched with $^{18}O–H_2O$ to reach 20% ^{18}O enrichment. A parallel set of control soil samples received the same volume of $^{16}O–H_2O$. All samples were incubated for 24h, post which gas samples were collected from vial headspaces for CO_2 determination via a gas chromatograph (Agilent GC 7890A, Agilent). The soil samples were stored at −80°C until DNA extraction. The ^{18}O abundance and total O content of DNA were measured using a MAT 253 isotope-ratio mass spectrometer coupled with a FLASH2000HT elemental analyzer (Thermo Finnigan MAT). CUE was calculated using equations presented by Zheng et al. (2019) using sample-specific conversion factors.

2.4 | Characterization of mineral protection of soil organic carbon

To explore the relationship between the mineral protection of SOC and microbial CUE, we determined exchangeable Ca^{2+} and Mg^{2+} (Ca_{exe}/Mg_{exe}) and three forms of Fe/Al oxides in soil (Carter & Gregorich, 2007). Exchangeable Ca and Mg were extracted with $1\,mol\,L^{-1}$ ammonium acetate (pH 7.0). Pedogenic Fe or Al (Al_d/Fe_d) were extracted using trisodium citrate, sodium bicarbonate, and sodium dithionate (referred to as CBD method). Poorly crystalline oxyhydroxides Fe or Al (Al_o/Fe_o) were extracted by oxalic acid–ammonium oxalate. Organically complexed Fe or Al (Al_p/Fe_p) was extracted by sodium pyrophosphate. Ca, Mg, Fe, and Al contents were analyzed by an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Agilent). The ratio of clay to SOC content (Clay:SOC) was used to assess the protection of SOC by clay particles (Fang et al., 2021). The ratio of Ca_{exe} to CEC (Ca_{exe}:CEC) was used to evaluate the Ca-related stabilization mechanisms in soil organic matter (Yang et al., 2020).

2.5 | Determination of microbial properties

Phospholipid fatty acids (PLFAs) were used to assess microbial biomass abundance. PLFAs were extracted following previously described procedures (Kaiser et al., 2010), and analyzed by gas chromatography (ThermoFisher Scientific) with a DB23 column (Agilent). Each fatty acid was quantified by comparing their individual peak areas with that of internal standard methyl-nonadecanoate (19:0). The fatty acids selected to represent bacterial biomass are the PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0, and the fatty acid representative of fungal biomass is the 18:2ω6, 9c, and 18:1ω9c.

Estimation of microbial activity was based on the maximum potential activity of extracellular enzymes, including hydrolytic enzymes (α-Glucosidase [AG], β-D-glucosidase [BG], β-xylosidase [BX], β-D-cellobiosidase [CBH], L-Leucine aminopeptidase [LAP], β-N-acetylglucosaminidase [NAG], and acid phosphatase [AP]) and oxidative enzymes (polyphenol oxidase [PPO], peroxidase [PER]; Saiya-Cork et al., 2002). Soil suspensions were prepared by homogenizing 1 g of fresh soil in 125 mL of 50 mM sodium bicarbonate (pH 5.0). Then, a mixture of soil homogenate, methylumbelliferyl (MUB), and MUB-linked substrate was placed in black polystyrene 96-well microplates and incubated in darkness for 4 h at 25°C. For oxidative enzymes, L-3,4-dihydroxyphenylalanine (LDOPA) was added to each well with soil aliquots. Half of the wells received additional H_2O_2 solution for PER measurement. Plates were incubated in darkness at 25°C for up to 18 h. The activities of extracellular enzymes are expressed as $\mu mol\,mg^{-1}\,MBC\,h^{-1}$. C-acquiring hydrolytic enzyme potential was calculated as the sum of the activities of AG, BG, BX, and CBH; N-acquiring enzyme activity was calculated as the sum of LAP and NAG activities; and oxidative activity was calculated as the sum of PPO and PER activities.

Soil DNA was extracted using the DNeasy PowerSoil® Pro kit (MoBio Laboratories) according to the manufacturer's instructions. Bacterial communities were assessed by amplifying and sequencing the V3–V4 region of the 16S rRNA gene with primers 338F/806R and the fungal communities were assessed by amplifying the internal transcribed spacer (ITS) region with primers ITS1F/ITS2R, respectively (Liu et al., 2018). Bacterial and fungal α (richness) and β (community composition) diversity were calculated based on 97% operational taxonomic units (OTUs) similarity of obtained bacterial and fungal sequences. Microbial composition was represented by the first component of nonmetric multidimensional scaling (NMDS) analysis (Wu, Chen, et al., 2021). The richness to biomass ratio for bacterial or fungal communities was calculated and used to explain changes in soil respiration (Bastida et al., 2021). For bacteria, *Actinobacteriota*, *Acidobacteriota*, and *Chloroflexi* were classified as the K-strategist (oligotrophs), and *Proteobacteria*, *Bacteroidota*, and *Firmicutes* were classified as the r-strategist (copiotrophs) of phylum level. For fungi, *Ascomycota* was classified as the K-strategist, and *Mortierellomycota* and *Basidiomycota* were classified as the r-strategist (Li, Yang, et al., 2021; Sterkenburg et al., 2015; Sun et al., 2021; Wu, Liu, et al., 2021). To calculate the ratio of K to r species, the oligotrophic and copiotrophic bacterial or fungal phyla were summed up, respectively.

2.6 | Analyses of soil organic carbon quality

An extracellular enzyme-based lignocellulose index and SOC molecular composition were used as indices of SOC quality. The lignocellulose index (LCI) was calculated as the ratio of lnPPO to the sum of lnPPO and lnBG, with higher LCI value denoting lower SOC vulnerability to microbial decomposition (Moorhead et al., 2013). ^{13}C NMR spectroscopy analysis was used to determine SOC molecular composition. Here, soils were pretreated with hydrochloric acid (HCl, 10 wt.%) and hydrofluoric acid (HF, 10 wt.%) to remove any calcium carbonate and mineral phases, respectively. After pretreatment, soil samples were subjected to solid-state magic-angle spinning NMR analysis (Bruker DSX 200). Quantification was performed by dividing the spectra into seven chemical shift regions after baseline correction with 0–45, 45–60, 60–95, 95–110, 110–145, 145–165, and 165–215 ppm being assigned to alkyl C, N-alkyl+methoxyl C, O-alkyl C, Di-O-alkyl C, aromatic C, phenolic C, and amide+carbonyl C, respectively. Two indices of SOC stability were calculated (Ye et al., 2019): (i) decomposability index, the ratio of alkyl C/(N-alkyl+methoxyl C+O-alkyl C+Di-O-alkyl C); and (ii) recalcitrance index, the ratio of (alkyl C+aromatic C+phenolic C)/(N-alkyl+methoxyl C+O-alkyl C+Di-O-alkyl C+amide+carbonyl C).

2.7 | Statistical analyses

Tree species diversity, rather than the diversity of herbs and shrubs, had a significant influence on each index of plant-input properties,

soil chemical properties, SOC quality, mineral protection, and soil microbial properties (Figures S2–S7). Therefore, only tree species diversity was used hereafter. Because Shannon index was more closely correlated with the above variables than tree species richness, we used Shannon index to represent tree species diversity in the following analyses. Prior to statistical analyses, we performed the Shapiro–Wilk test to examine data normality and conducted logarithmic transformation when necessary. We then processed the statistical analyses by the following four steps. First, given the strong connections and inter-correlations among the various factors, partial correlation analysis was used to explore the relationships of microbial CUE with five groups of factors, that is, plant-input properties (litter and fine root C, N, and P contents and fine root biomass), soil chemical properties (pH, $CaCO_3$, DOC, AVP, and TDN concentrations), SOC quality (LCI, decomposability and recalcitrance indexes), mineral protection (exchangeable Ca and Mg, Fe and Al oxides, ratios of Clay: SOC and Ca_{exe}: CEC), and microbial properties (biomass, activity, and richness: biomass ratio and growth strategies). Second, hierarchical partitioning method was employed to determine the contribution of different variables to microbial CUE via the "rdacca.hp" package in R (Lai et al., 2022). Third, variation partitioning analysis (VPA) was used to quantify the relative importance of plant-input properties, soil chemical properties, SOC quality, mineral protection, and microbial properties in controlling microbial CUE. A negative value in the variance explained by a group of factors was interpreted as zero, implying that the explanatory variables explained less variation than random normal variables.

Finally, structural equation modeling (SEM) was used to evaluate the direct and indirect effects of plant species diversity on microbial CUE. Owing to strong correlations among the factors within each group, principal components analysis (PCA) was performed to create a new index for plant-input properties, soil chemical properties, SOC quality, mineral protection, microbial biomass, activity, richness, and richness: biomass ratio and growth strategies. The first component, which accounted for >58.98% of the variance for each group (Table S5) was then used in the SEM. Model fitting criteria include Chi-square (0 ≤ CHI/DF ≤ 3), p value (>.05), root mean square

error of approximation (0 ≤ RMSEA ≤ 0.08), and comparative fit index (CFI >0.90). The SEM was constructed using AMOS 21.0 (Amos Development Corporation), and the other statistical analyses were implemented in R software 4.1.1 (R Development Core Team, 2022).

3 | RESULTS

The absolute values recorded for soil microbial growth and respiration significantly increased with tree species diversity ($p < .05$, Figure 1a,b). Moreover, along the tree species diversity gradient, microbial CUE increased significantly from 0.09 to 0.35 (Figure 1c), and was significantly and positively correlated with microbial growth, but was not significantly related to respiration ($p > .05$, Figure S8).

Partial correlation analyses indicated that without controlling any variables, microbial CUE was closely correlated with the five groups of potential predictors, that is, plant-input properties, soil chemical properties, SOC quality, mineral protection, and microbial properties (zero-order; Figure 2). However, after controlling microbial properties, the strength of the correlation coefficients between microbial CUE with plant-input properties, soil chemical properties, SOC quality, and mineral protection decreased by 71.86%, 81.45%, 77.53%, and 76.42%, respectively. In contrast, the ratio of K- to r-strategists was always significantly associated with microbial CUE ($p < .05$) irrespective of whether controlling plant-input properties, soil chemical, and mineral protection properties.

Hierarchical partitioning analysis revealed that microbial properties explained a much greater portion of the cumulative variance (61%) in microbial CUE than plant-input properties (13%), soil chemical properties (48%), SOC quality (37%), or mineral protection (45%) (Figure 3a). Briefly, microbial CUE was positively correlated with soil pH, $CaCO_3$, SOC quality, N:P ratio of enzymatic activities and fungal biomass, but was negatively correlated with $(Fe + Al)_d$, $(Fe + Al)_o$, and bacterial community composition (NMDS1) ($p < .05$, Figure 3b–j). Hierarchical partitioning analysis further revealed that fungal and bacterial taxa explained 56% in microbial CUE (Figure 4a). Microbial CUE was negatively correlated with the relative abundance of
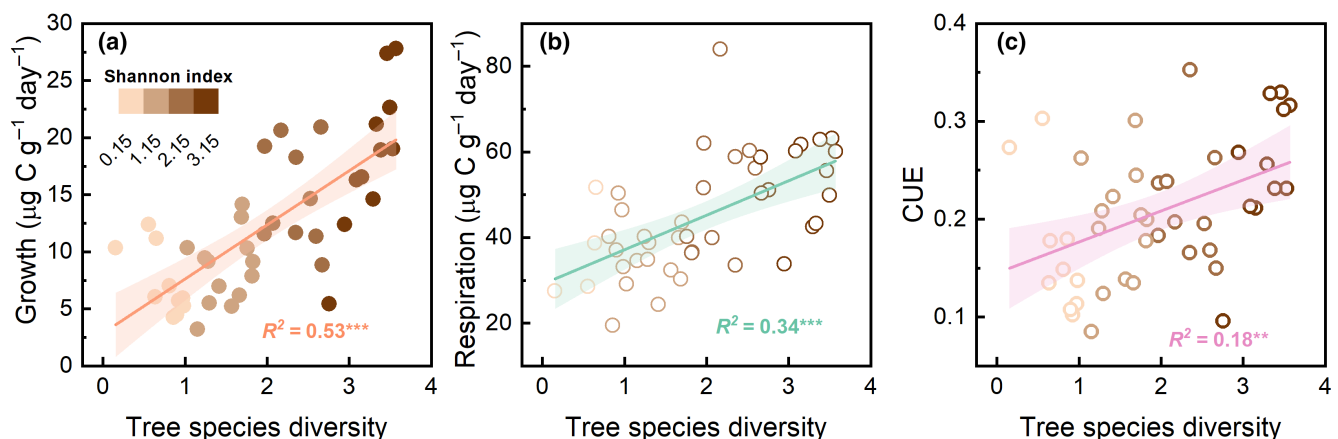
FIGURE 1 The correlations of tree species diversity with microbial growth (a), respiration (b), and microbial C use efficiency (CUE) (c). The linear regression lines with 95% confidence intervals reflect the predicted effects of fixed factors. *$p < .05$, **$p < .01$, ***$p < .001$.
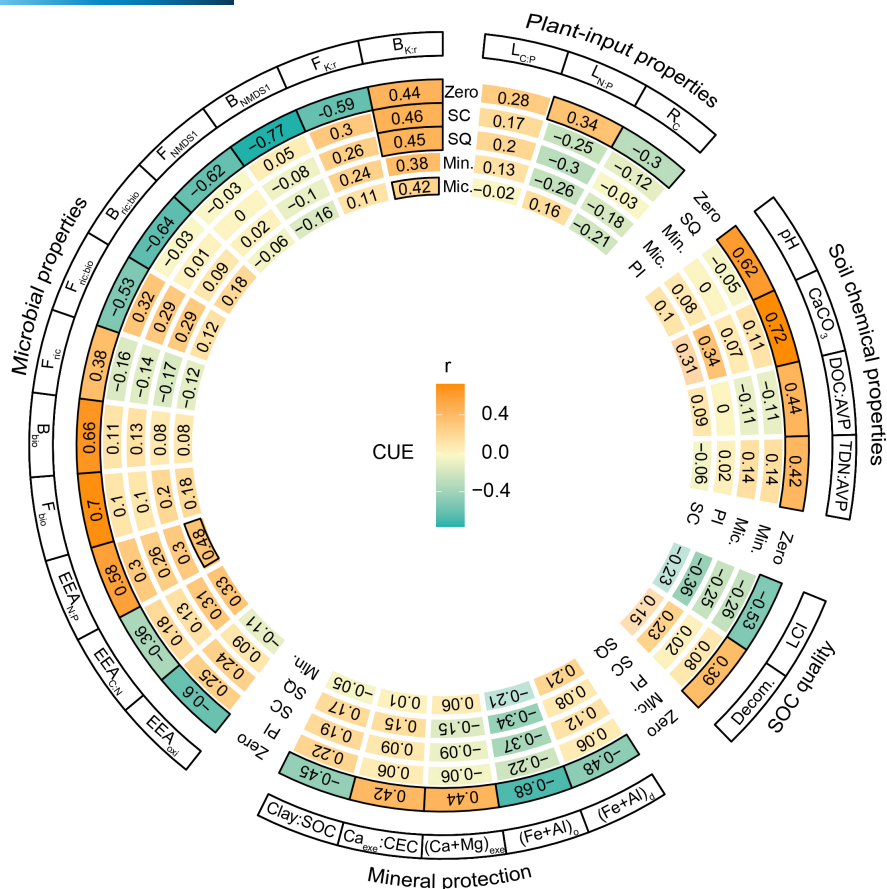
FIGURE 2 Partial correlations between soil microbial C use efficiency (CUE) and five types of predicting factors across the tree species diversity gradient. The outermost circle shows the factors (i.e., plant-input properties, soil chemical properties, soil organic carbon (SOC) quality, mineral protection, microbial properties) of which the correlations with the soil microbial CUE are examined. The color of the fan shapes indicates the strength and sign of the correlation, with black frame line representing the significant correlation at $p < .05$. Differences in color between the zero-order (Zero) and controlled factors indicate the level of dependency of the correlation between microbial CUE and the examined factor on the controlled variable (no change in circle color between the controlled factor and zero-order = no dependency; a decrease/increase in circle color intensity = loss/gain of correlation). The complete correlations between microbial CUE with potential predictor factors are shown in Figure S10. Representative variables for each types of predictors factors were selected at $p < .05$. PI, plant-input; SC, soil chemical; SQ, SOC quality; Min., mineral protection; Mic., microbial properties; CUE, carbon use efficiency; L and R, litter and root; C, carbon; N, nitrogen; P, phosphorus; CEC, cation exchange capacity; DOC and DON, dissolved organic carbon and nitrogen; AVP available phosphorus; LCI, lignocelluloses index; Fe and Al, iron and aluminum; $(Fe+Al)_d$, pedogenic Fe/Al oxides; $(Fe+Al)_o$, poorly crystalline Fe/Al oxides; $(Fe+Al)_p$, organically complexed Fe/Al oxides; $(Ca+Mg)_{exe}$, exchangeable Ca^{2+}/Mg^{2+}; EEA_{oxi}, oxidative enzyme activity, the sum of activities of polyphenol oxidase (PPO) and peroxidase (PER); EEA_C, C-acquiring hydrolase enzyme activity, the sum of activities of α-Glucosidase (AG), β-D-glucosidase (BG), β-xylosidase (BX), β-D-cellobiosidase (CBH); EEA_N, N-acquiring hydrolase enzyme activity, the sum of activities of L-Leucine aminopeptidase (LAP) and β-N-acetylglucosaminidase (NAG); AP, acid phosphatase; F and B, fungi and bacteria; $F/B_{ric: bio}$, the ratio of fungi/bacteria richness to biomass; F/B_{NMDS1}, the first component of nonmetric multidimensional scaling (NMDS) 1 analysis; $F/B_{K:r}$, the ratio of oligotrophic to copiotrophic members of fungi/bacteria phyla.

Mortierellomycota, *Basidiomycota*, and *Firmicutes*, but positively correlated with *Ascomycota* and *Acidobacteriota* ($p < .05$, Figure 4b–g).

Because plant-input properties only played a minor role in explaining the variation of microbial CUE, they were excluded in VPA analysis (Figure S9; Figure 5a). Compared to soil microbial properties, soil chemical properties alone explained lower variation in microbial CUE (Figure S9). SOC quality, mineral protection, and microbial properties explained 58.6% of microbial CUE variation in total, with the unique effects being 5.7%, 0.2%, and 14.6% for the three groups of factors, respectively (Figure 5a). The VPA result corroborated the key role of microbial properties in regulating microbial CUE.

SEM analysis revealed that tree species diversity indirectly enhanced microbial CUE through its roles in regulating soil chemical properties, mineral protection of SOC, plant-input properties, SOC stability, and microbial properties (Figure 5b). The increased microbial CUE was only directly related to higher microbial growth and SOC quality. Litter C:P and N:P ratios indirectly affected CUE via their positive effect on the ratio of K- to r-strategists. Fine root C content indirectly determines microbial CUE via changes in microbial K:r ratio. Soil chemical properties, such as pH, $CaCO_3$, DOC:AVP, and TDN:AVP ratios had an indirect influence on CUE through its positive effect on SOC quality and microbial growth. In addition,
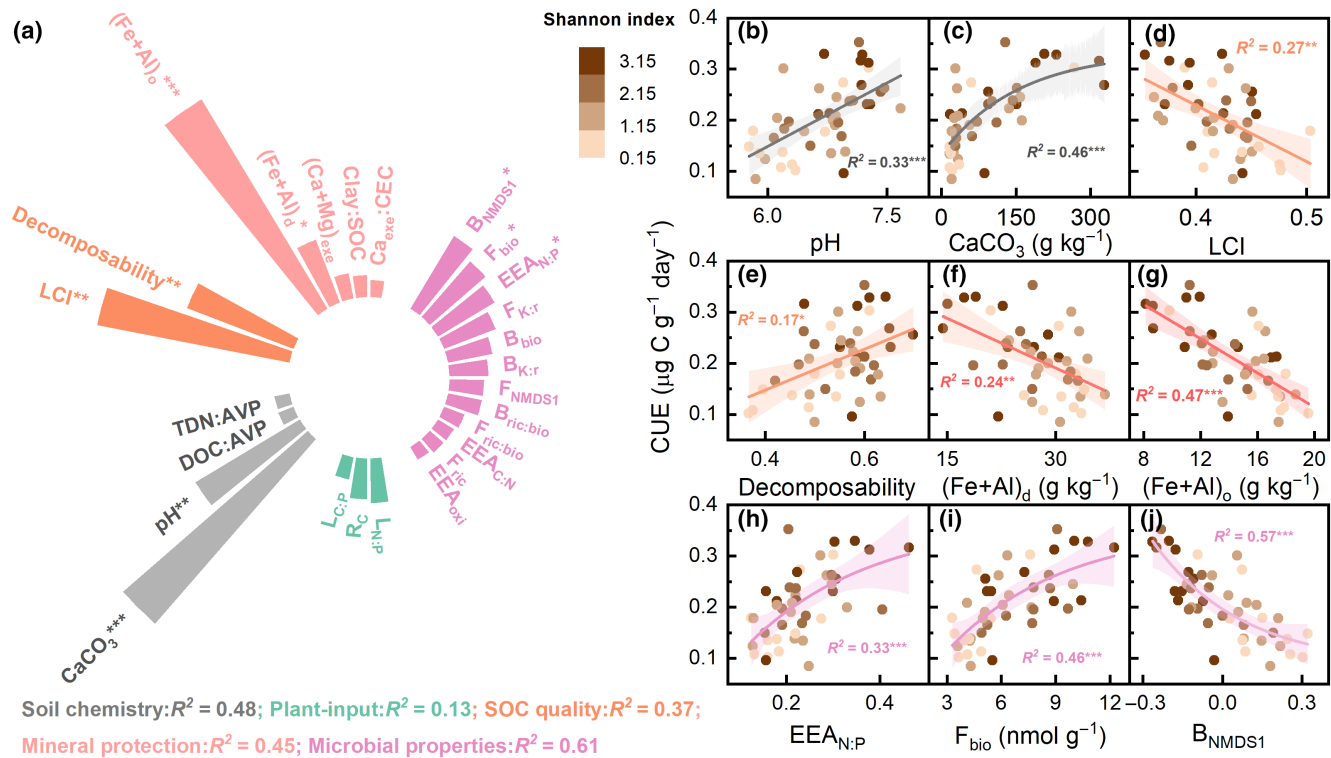
FIGURE 3 Relationships between microbial C use efficiency (CUE) and five types of predicting factors (plant-input properties, soil chemical properties, soil organic carbon (SOC) quality, mineral protection, microbial properties). Hierarchical partitioning analysis showing the relative importance of representative variables for each types of predicting factors in regulating microbial CUE (a). Relationships between microbial CUE and the significant predictors by hierarchical partitioning analysis (b–j). The linear regression lines with 95% confidence intervals reflect the predicted effects of fixed factors. $*p<.05$, $**p<.01$, $***p<.001$.

these soil chemical properties indirectly influenced the changes in the microbial K:r ratio via their negative effect on oxidative enzyme activities and ratio of C:N acquisition related enzyme activities and positive effect on ratio of N:P acquisition related enzyme activities. $(Ca+Mg)_{exe}$ and Clay: SOC indirectly increased CUE via its direct stimulation to microbial biomass or SOC quality. Nevertheless, $(Fe+Al)_d$, $(Fe+Al)_o$ and Ca_{exe}: CEC had negative effects on CUE via directly increasing microbial biomass or SOC quality. In addition, microbial biomass and K: r strategy positively affected microbial growth, therefore increasing CUE. Microbial biomass also showed a positive effect on CUE because it positively influenced microbial activity, K: r strategy, and growth.

4 | DISCUSSION

Consistent with our hypothesis, microbial CUE was positively related to tree species diversity in the current study (Figure 1c). The relationship between CUE and tree species diversity was largely driven by changes in microbial growth rather than respiration. As tree species diversity increased, there was an unproportionally increase in microbial growth rate compared to respiration, resulting in greater CUE under higher tree species diversity (Figure 1). This pattern suggests a shift in microbial growth strategies from greater resource allocation into maintenance respiration under

lower tree species diversity, to increased growth under higher tree species diversity.

Our results indicate that the influence of microbial properties (i.e., growth, biomass, enzyme activity) on CUE, was the result of an overall change in the microbial community structure and microbial taxonomic variation (Figures 2–4). This finding supports a previous study, which showed that community-level variations in physiological traits mostly reflect the change in microbial community composition and trait strategies (Domeignoz-Horta et al., 2020). In the current study, the positive relationship between the ratio of microbial K:r strategists on CUE (Figures 2 and 5), points toward higher CUE for communities with oligotrophic life strategies and a "frugal" metabolism. This relationship is consistent with observations that K-strategists might be superior competitors for resources at low resource concentrations due to their higher substrate affinity (Fierer et al., 2007) and, thus, can exhibit higher CUE than r-strategists due to their lower maintenance energy costs (Roller & Schmidt, 2015; Saifuddin et al., 2019; Zhong et al., 2020).

Microbial CUE was further influenced by tree species diversity via its indirect effect on soil nutrient availability and the microbial community composition. Soil P availability was lower with increased tree species diversity as indicated by the increased soil DOC:AVP and TDN:AVP ratio (Figure S4). An explanation for this pattern is that increased plant species diversity can increase the efficiency of P acquisition and uptake by plants (Oelmann et al., 2021), consistent
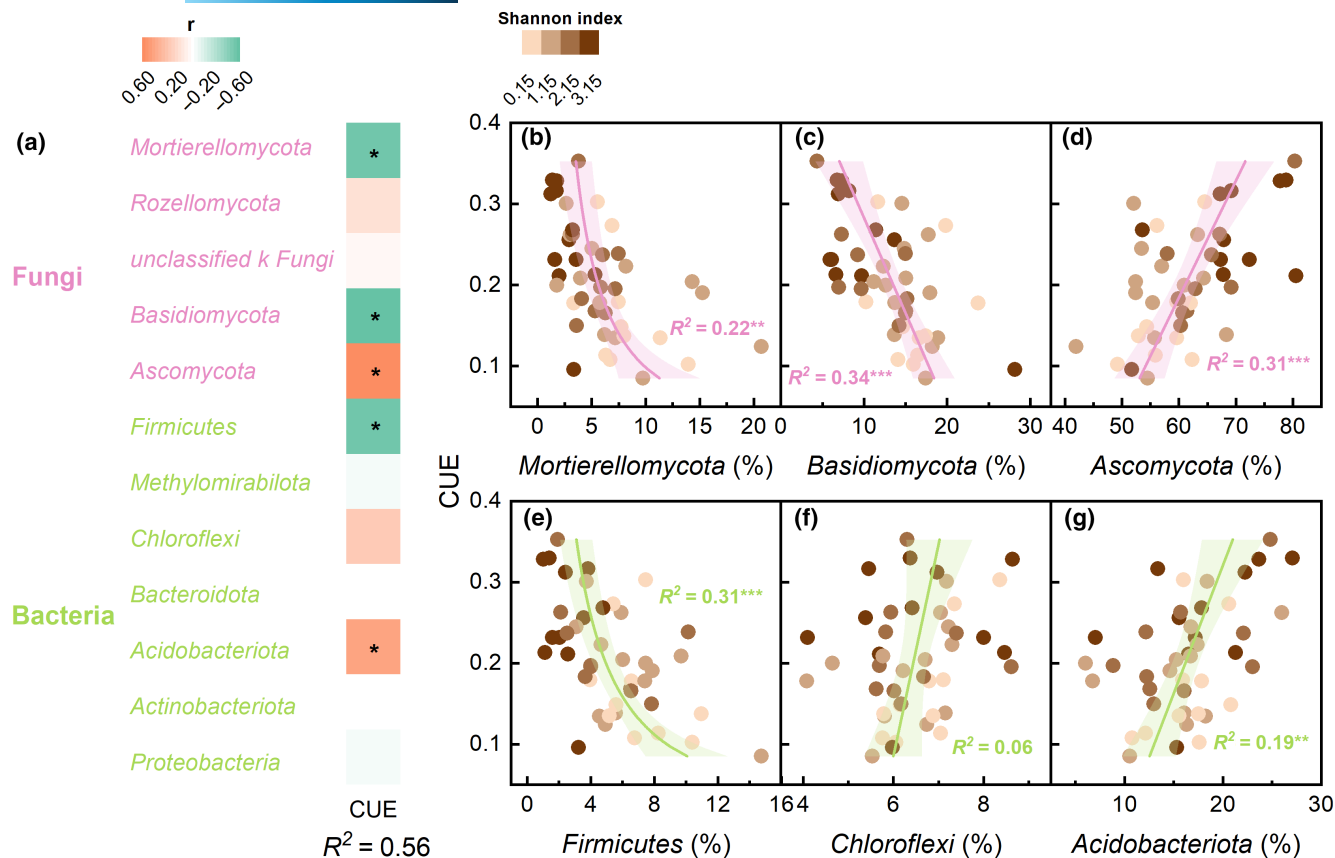
FIGURE 4 Correlations of microbial C use efficiency (CUE) with the relative abundances of major fungal and bacterial phyla. Hierarchical partitioning analysis showing the relative importance of representative phyla for fungal and bacterial in regulating microbial CUE, with the color indicating the strength and sign of the relationship (a). Relationships between microbial CUE and the significant fungal or bacterial phyla by hierarchical partitioning analysis (b–g). The linear regression lines with 95% confidence intervals reflect the predicted effects of fixed factors. *$p<.05$, **$p<.01$, ***$p<.001$.

with the observation of high resource partitioning resulting in increased plant productivity for high diversity plant communities (Chen & Chen, 2021), Together, these observations can explain lower soil P availability under high tree species diversity, because more P is retained in plant biomass (Figure 5). High tree species diversity was also found to decrease the return of P to soil from plants due to a reduction in litter P content, which occurs as a result of enhanced leaf nutrient use efficiency and increased leaf P resorption (Liu et al., 2022). With regard to the current study, the observed reductions in litter P availability, as indicated by the increased C:P and N:P ratios (Figure 5), therefore provide further support for the idea that increased tree species diversity can result in a decrease in soil P availability. Changes in dominant microbial taxa across our study sites are consistent with this pattern: under the combined effects of high tree species diversity, high CUE, and low soil P availability, we observed relative abundance increases for *Acidobacteriota* and *Ascomycota* but decreases for *Mortierellomycota*, *Firmicutes*, and *Basidiomycota* (Figure 4). The same taxon-level responses to soil P availability have been observed elsewhere, with *Acidobacteriota* associated with low soil P availability and *Mortierellomycota* and *Firmicutes* associated with high soil P availability (Feng et al., 2021; Khalid et al., 2021; Li et al., 2020; Liang et al., 2020). Decreased soil

P availability under high plant diversity may further feedback on microbial CUE, by affecting resource partitioning between r-strategists and K-strategists. Consistent with an influence of nutrient availability on microbial community growth strategies (and, consequently, CUE), we found that the ratio of microbial K:r strategists was positively correlated with enzymatic N:P ratios (Figure 5; Zheng et al., 2022). Overall, our findings highlight a potentially important role of microbial community growth strategies in regulating microbial CUE, with increased oligotroph growth under lower nutrient conditions, and subsequently increased microbial community CUE.

Our study reveals a further potentially important influence of soil mineralogy on the microbial CUE under changing tree species diversity and plant community traits. An influence of plant species diversity on the concentrations of soil Fe/Al oxides and exchangeable Ca/Mg could result from changes in root exudation (Dontsova et al., 2020). These organic acids can dissolve Ca and Mg from soil primary minerals or bedrock (Fang et al., 2023), which is a mixture of dolomite and limestone in the current study. Consistent with this idea, we found that under increased tree species diversity the proportion of reactive minerals decreased, such as Fe/Al$_o$ and Fe/Al$_d$ oxides, whereas Ca/Mg$_{exe}$ increased, suggesting decreased protection of soil organic C associated with Fe and Al minerals
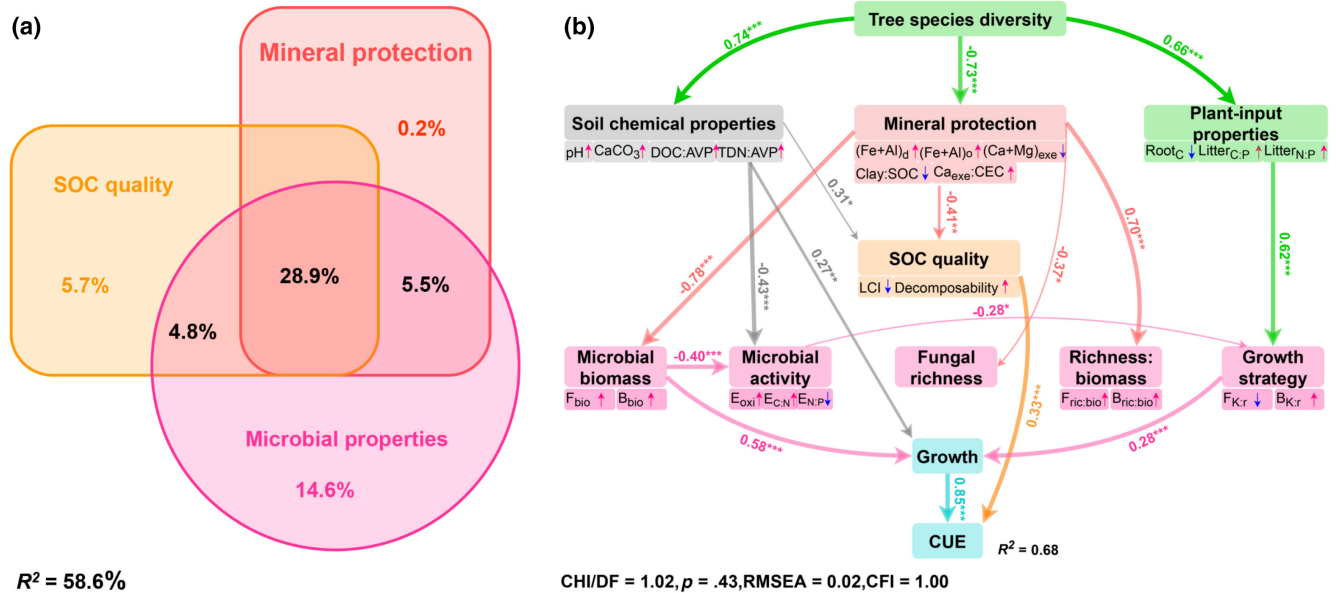
FIGURE 5 Effects of abiotic and biotic factors on microbial C use efficiency (CUE) across the tree species diversity gradient as revealed by variation partitioning analysis (a), and structural equation modeling (b). In panel (a), numbers in the overlapping area of two or three circles are the shared effects of the corresponding two or three factors, otherwise the unique effects of the corresponding factors. In panel (b), arrow width is proportional to the strength of the relationship, and the numbers adjacent to arrows denote standardized path coefficients. Plant-input properties, soil chemical properties, soil organic carbon quality, mineral protection, and microbial properties, that is, biomass, activity, biomass: richness ratio, and growth strategies are represented by the first components from the principal component analyses conducted for the corresponding groups of variables (Table S5).

(Figures 2 and 3f,g). The subsequent increase in soil assimilable C, released from mineral surfaces, can boost microbial growth (Finley et al., 2022; Wilhelm et al., 2022; Yang et al., 2022) and thus partially explain increased microbial CUE we observed under high plant species diversity. Elsewhere, several studies have reported high microbial community CUE in soils with high concentrations of labile C and nutrients (Soares & Rousk, 2019; Takriti et al., 2018). Indeed, a key mechanism by which CUE may decline under low resource availability (or quality) is that more energy is invested into enzyme synthesis for nutrient acquisition, as demonstrated in infertile (P-poor) tropical forest soils with high phosphatase activity and low CUE (Nottingham et al., 2015; Whitaker et al., 2014) and under highly controlled conditions (Domeignoz-Horta et al., 2020). Furthermore, the reduction in soil Fe/Al oxides and clay minerals may alleviate the risk of enzyme deactivation and microbial mobility restriction caused by mineral adsorption or co-precipitation (Chen et al., 2020; Finley et al., 2022). Consequently, this can facilitate the degradation of soil organic matter into smaller molecular compounds that are easily assimilated by microbes. Microorganisms allocate more of their metabolized C to the production of enzymes, enabling them to oxidize recalcitrant substrates and achieve a return on energy or C gain, ultimately resulting in lower CUE (Zhang et al., 2023). Therefore, the observed enhancement in SOC quality may contribute to an increase in microbial CUE, primarily by reducing mineral protection of soil organic matter (Figure 5b). Soil mineralogy, and specifically the abundance of iron-oxides and clay minerals, may further influence community CUE through its effect

on the microbial community composition following the observation that clay-rich soils may have greater dominance of r-strategist (e.g., *Basidiomycota*) than K-strategist (e.g., *Ascomycota*) microbes (Viscarra Rossel et al., 2022). Taken together, these results suggest that under higher tree species diversity (and associated high species–trait diversity including molecular diversity of litter and root-exudate inputs), although the soil mineral-associated C may decline, the total soil C may undergo a net increase as increased assimilable organic carbon C boosts microbial growth, biomass, and CUE (Figure 6).

Our result showing increased microbial CUE (and increased soil C sink potential), while compelling, requires consideration of the context of our study site. We used a natural plant species diversity gradient, rather a controlled plantation experiment. Under such conditions, the heterogeneity of the environment (soil and microclimatic conditions) and stand structure (tree species composition, productivity) across such large gradients can be substantial, and unconstrained variation can weaken the strength of the experimental approach (Jia et al., 2021; Leuschner et al., 2009). Furthermore, there are known exceptions to the patterns we find, where high species diversity occurs alongside low microbial CUE, such as in hyperdiverse tropical forests on strongly weathered soils where high microbial energy investment for enzymatic P acquisition may override any positive influence of species trait diversity on CUE (Nottingham et al., 2015, Whitaker et al., 2014). Hence, to better resolve the relationship between plant communities and soil microbial CUE, further study is required that combines manipulation
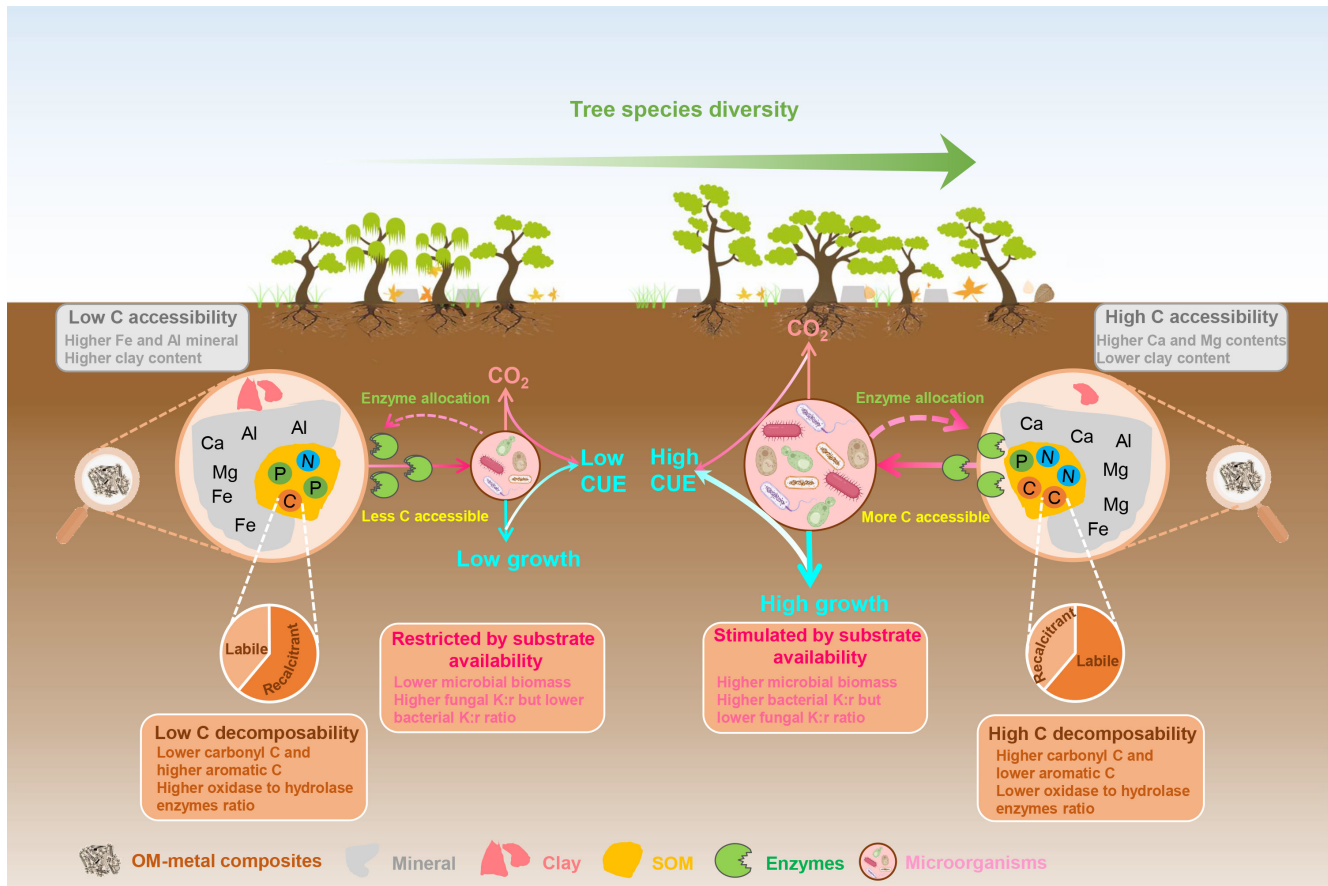
FIGURE 6 Conceptual model of how soil microbial properties (i.e., biomass, activity, and growth strategies), as regulated by soil organic carbon (SOC) quality and mineral protection, determines microbial C use efficiency (CUE). With the increase in tree species diversity, the increased substrate decomposition (low chemical recalcitrance) and the reduced clay and mineral protection (high C accessibility) could lead to the high substrate availability, and subsequently stimulate microbial growth and thus microbial CUE. Notably, this conceptual diagram mainly focused on the most important direct abiotic and biotic factors (i.e., SOC quality, mineral protection, and microbial properties) derived from the variation partitioning analysis and structural equation modeling.

studies and observational cross-site comparisons in existing forest stands of contrasting tree diversity, with particular focus on plant-trait distributions that likely drives this relationship (Boeddinghaus et al., 2019; Schoolmaster Jr. et al., 2020). Notwithstanding these caveats, our results clearly demonstrate a link between high species diversity and increased soil C sink potential for these subtropical forests, and go further to reveal the microbial community traits that may be associated with this increase.

5 | CONCLUSION

In summary, through a multiple approach-based analysis, this study used a tree species diversity gradient to show how plant–soil microbial traits (e.g., plant substrate inputs, soil chemistry and mineralogy and the microbial community composition) interact to influence microbial community CUE and the potential forest C sink (i.e., CUE increased under higher diversity). Our findings have two important implications for understanding subtropical forest C dynamics. First, the positive association between tree species diversity and microbial

CUE suggests that increased species diversity—and associated changes in productivity, species traits and plant-inputs to soil—contribute to more efficient microbial processing of C and the potential formation of persistent soil organic matter pools (Domeignoz-Horta et al., 2021; Rui et al., 2022). This important result, for a single study site in subtropical forest, highlights the need for more studies investigating the role of plant species diversity for the formation of microbial-derived persistent soil organic matter in other ecosystems. Second, the significant relationship between the microbial CUE and microbial community composition indicates that the shift in soil microbial community growth traits (e.g., fast-growth strategists to slow-growth strategists) under different plant communities and plant-inputs to soil can further influence microbial CUE. This finding highlights the importance of linkages between above and below-ground communities in affecting the feedback between terrestrial C storage and the climate (Canarini et al., 2021; Domeignoz-Horta et al., 2020; Nottingham et al., 2018). Thus, our results provide a mechanistic basis by which the maintenance of high tree species diversity can enhance soil C sequestration and contribute substantially to climate change mitigation.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.24192261.

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REFERENCES
Bastida, F., Eldridge, D. J., García, C., Kenny Png, G., Bardgett, R. D., & Delgado-Baquerizo, M. (2021). Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *The ISME Journal*, *15*(7), 2081–2091. https://doi.org/10.1038/s41396-021-00906-0

Beugnon, R., Du, J., Cesarz, S., Jurburg, S. D., Pang, Z., Singavarapu, B., Wubet, T., Xue, K., Wang, Y., & Eisenhauer, N. (2021). Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. *ISME Communications*, *1*(1), 1–11. https://doi.org/10.1038/s43705-021-00040-0

Boeddinghaus, R., Marhan, S., Berner, D., Boch, S., Fischer, M., Hölzel, N., Kattge, J., Klaus, V. H., Kleinebecker, T., Oelmann, Y., Prati, D., Schäfer, D., Schöning, I., Schrumpf, M., Sorkau, E., Kandeler, E., & Manning, P. (2019). Plant functional trait shifts explain concurrent changes in the structure and function of grassland soil microbial communities. *Journal of Ecology*, *107*, 2197–2210. https://doi.org/10.1111/1365-2745.13182

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C. W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M., & Richter, A. (2021). Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature Communications*, *12*, 5308. https://doi.org/10.1038/s41467-021-25675-4

Cantwell-Jones, A., Ball, J., Collar, D., Diazgranados, M., Douglas, R., Forest, F., Hawkins, J., Howes, M.-J. R., Ulian, T., Vaitla, B., & Pironon, S. (2022). Global plant diversity as a reservoir of micronutrients for humanity. *Nature Plants*, *8*(3), 225–232. https://doi.org/10.1038/s41477-022-01100-6

Carter, M. R., & Gregorich, E. G. (2007). *Soil sampling and methods of analysis* (2nd ed.). CRC Press. Taylor & Francis Group.

Cesarz, S., Craven, D., Auge, H., Bruelheide, H., Castagneyrol, B., Gutknecht, J., Hector, A., Jactel, H., Koricheva, J., Messier, C., Muys, B., O'Brien, M. J., Paquette, A., Ponette, Q., Potvin, C., Reich, P. B., Scherer-Lorenzen, M., Smith, A. R., Verheyen, K., & Eisenhauer, N. (2022). Tree diversity effects on soil microbial biomass and respiration are context dependent across forest diversity experiments. *Global Ecology and Biogeography*, *31*(5), 872–885. https://doi.org/10.1111/geb.13461

Chen, C., Hall, S. J., Coward, E., & Thompson, A. (2020). Iron-mediated organic matter decomposition in humid soils can counteract protection. *Nature Communications*, *11*(1), 2255. https://doi.org/10.1038/s41467-020-16071-5

Chen, X., & Chen, H. Y. H. (2021). Plant mixture balances terrestrial ecosystem C:N:P stoichiometry. *Nature Communications*, *12*(1), 4562. https://doi.org/10.1038/s41467-021-24889-w

Chen, X., Taylor, A. R., Reich, P. B., Hisano, M., Chen, H. Y. H., & Chang, S. X. (2023). Tree diversity increases decadal forest soil carbon and nitrogen accrual. *Nature*, *618*(7963), 94–101. https://doi.org/10.1038/s41586-023-05941-9

Craig, M. E., Geyer, K. M., Beidler, K. V., Brzostek, E. R., Frey, S. D., Stuart Grandy, A., Liang, C., & Phillips, R. P. (2022). Fast-decaying plant litter enhances soil carbon in temperate forests but not through microbial physiological traits. *Nature Communications*, *13*(1), 1229. https://doi.org/10.1038/s41467-022-28715-9

Domeignoz-Horta, L. A., Pold, G., Liu, X. J. A., Frey, S. D., Melillo, J. M., & DeAngelis, K. M. (2020). Microbial diversity drives carbon use efficiency in a model soil. *Nature Communications*, *11*(1), 3684. https://doi.org/10.1038/s41467-020-17502-z

Domeignoz-Horta, L. A., Shinfuku, M., Junier, P., Poirier, S., Verrecchia, E., Sebag, D., & DeAngelis, K. M. (2021). Direct evidence for the role of microbial community composition in the formation of soil organic matter composition and persistence. *ISME Communications*, *1*(1), 64. https://doi.org/10.1038/s43705-021-00071-7

Dontsova, K., Balogh-Brunstad, Z., & Chorover, J. (2020). Plants as drivers of rock weathering. In K. Dontsova, Z. Balogh-Brunstad, & G. L. Roux (Eds.), *Biogeochemical cycles* (pp. 33–58). John Wiley Sons, Inc. https://doi.org/10.1002/9781119413332.ch2

Fang, K., Chen, L., Qin, S., Zhang, Q., Liu, X., Chen, P., & Yang, Y. (2021). Mineral and climatic controls over soil organic matter stability across the Tibetan alpine permafrost region. *Global Biogeochemical Cycles*, *35*(12), e2021GB007118. https://doi.org/10.1029/2021GB007118

Fang, Q., Lu, A., Hong, H., Kuzyakov, Y., Algeo, T. J., Zhao, L., Olshansky, Y., Moravec, B., Barrientes, D. M., & Chorover, J. (2023). Mineral weathering is linked to microbial priming in the critical zone. *Nature Communications*, *14*, 345. https://doi.org/10.1038/s41467-022-35671-x

Fanin, N., Mooshammer, M., Sauvadet, M., Meng, C., Alvarez, G., Bernard, L., Bertrand, I., Blagodatskaya, E., Bon, L., Fontaine, S.,

Niu, S., Lashermes, G., Maxwell, T. L., Weintraub, M. N., Wingate, L., Moorhead, D., & Nottingham, A. T. (2022). Soil enzymes in response to climate warming: Mechanisms and feedbacks. *Functional Ecology*, *36*, 1378–1395. https://doi.org/10.1111/1365-2435.14027

Feng, J., Zeng, X. M., Zhang, Q., Zhou, X. Q., Liu, Y. R., & Huang, Q. (2021). Soil microbial trait-based strategies drive metabolic efficiency along an altitude gradient. *ISME Communications*, *1*(1), 71. https://doi.org/10.1038/s43705-021-00076-2

Feng, X., Qin, S., Zhang, D., Chen, P., Hu, J., Wang, G., Liu, Y., Wei, B., Li, Q., Yang, Y., & Chen, L. (2022). Nitrogen input enhances microbial carbon use efficiency by altering plant-microbe-mineral interactions. *Global Change Biology*, *28*, 4845–4860. https://doi.org/10.1111/gcb.16229

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, *88*(6), 1354–1364. https://doi.org/10.1890/05-1839

Finley, B. K., Mau, R. L., Hayer, M., Stone, B. W., Morrissey, E. M., Koch, B. J., Rasmussen, C., Dijkstra, P., Schwartz, E., & Hungate, B. A. (2022). Soil minerals affect taxon-specific bacterial growth. *The ISME Journal*, *16*(5), 1318–1326. https://doi.org/10.1038/s41396-021-01162-y

García-Palacios, P., Crowther, T. W., Dacal, M., Hartley, I. P., Reinsch, S., Rinnan, R., Rousk, J., van den Hoogen, J., Ye, J. S., & Bradford, M. A. (2021). Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming. *Nature Reviews Earth and Environment*, *2*(7), 507–517. https://doi.org/10.1038/s43017-021-00178-4

Gottschall, F., Cesarz, S., Auge, H., Kovach, K. R., Mori, A. S., Nock, C. A., & Eisenhauer, N. (2022). Spatiotemporal dynamics of abiotic and biotic properties explain biodiversity–ecosystem-functioning relationships. *Ecological Monographs*, *92*(1), e01490. https://doi.org/10.1002/ecm.1490

Hasby, F. A., Barbi, F., Manzoni, S., & Lindahl, B. D. (2021). Transcriptomic markers of fungal growth, respiration and carbon-use efficiency. *FEMS Microbiology Letters*, *368*(15), fnab100. https://doi.org/10.1093/femsle/fnab100

He, M., Fang, K., Chen, L., Feng, X., Qin, S., Kou, D., He, H., Liang, C., & Yang, Y. (2022). Depth-dependent drivers of soil microbial necromass carbon across Tibetan alpine grasslands. *Global Change Biology*, *28*(3), 936–949. https://doi.org/10.1111/gcb.15969

Hua, F., Bruijnzeel, L. A., Meli, P., Martin, P. A., Zhang, J., Nakagawa, S., Miao, X., Wang, W., McEvoy, C., Peña-Arancibia, J. L., Brancalion, P. H. S., Smith, P., Edwards, D. P., & Balmford, A. (2022). The biodiversity and ecosystem service contributions and trade-offs of forest restoration approaches. *Science*, *376*(6595), 839–844. https://doi.org/10.1126/science.abl4649

Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., & Piñeiro, G. (2017). The ecology of soil carbon: Pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 419–445. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jia, Y., Zhai, G., Zhu, S., Liu, X., Schmid, B., Wang, Z., Ma, K., & Feng, X. (2021). Plant and microbial pathways driving plant diversity effects on soil carbon accumulation in subtropical forest. *Soil Biology and Biochemistry*, *161*, 108375. https://doi.org/10.1016/j.soilbio.2021.108375

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., & Richter, A. (2010). Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytologist*, *187*(3), 843–858. https://doi.org/10.1111/j.1469-8137.2010.03321.x

Kästner, M., Miltner, A., Thiele-Bruhn, S., & Liang, C. (2021). Microbial necromass in soils—Linking microbes to soil processes and carbon turnover. *Frontiers in Environmental Science*, *9*, 597. https://doi.org/10.3389/fenvs.2021.756378

Khalid, M., Du, B., Tan, H., Liu, X., Su, L., Saeed ur, R., Zhao, C., Li, X., & Hui, N. (2021). Phosphorus elevation erodes ectomycorrhizal community diversity and induces divergence of saprophytic community composition between vegetation types. *Science of the Total Environment*, *793*, 148502. https://doi.org/10.1016/j.scitotenv.2021.148502

Kleber, M., Bourg, I. C., Coward, E. K., Hansel, C. M., Myneni, S. C. B., & Nunan, N. (2021). Dynamic interactions at the mineral–organic matter interface. *Nature Reviews Earth and Environment*, *2*(6), 402–421. https://doi.org/10.1038/s43017-021-00162-y

Lai, J., Zou, Y., Zhang, J., & Peres-Neto, P. R. (2022). Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods in Ecology and Evolution*, *13*(4), 782–788. https://doi.org/10.1111/2041-210X.13800

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., & Steinbeiss, S. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*, 6707. https://doi.org/10.1038/ncomms7707

Leuschner, C., Jungkunst, H. F., & Fleck, S. (2009). Functional role of forest diversity: Pros and cons of synthetic stands and across-site comparisons in established forests. *Basic and Applied Ecology*, *10*(1), 1–9. https://doi.org/10.1016/j.baae.2008.06.001

Li, F., Zhang, S., Wang, Y., Li, Y., Li, P., Chen, L., Jie, X., Hu, D., Feng, B., Yue, K., & Han, Y. (2020). Rare fungus, *Mortierella capitata*, promotes crop growth by stimulating primary metabolisms related genes and reshaping rhizosphere bacterial community. *Soil Biology and Biochemistry*, *151*, 108017. https://doi.org/10.1016/j.soilbio.2020.108017

Li, H., Yang, S., Semenov, M. V., Yao, F., Ye, J., Bu, R., Ma, R., Lin, J., Kurganova, I., Wang, X., Deng, Y., Kravchenko, I., Jiang, Y., & Kuzyakov, Y. (2021). Temperature sensitivity of SOM decomposition is linked with a K-selected microbial community. *Global Change Biology*, *27*(12), 2763–2779. https://doi.org/10.1111/gcb.15593

Li, J., Pei, J., Dijkstra, F. A., Nie, M., & Pendall, E. (2021). Microbial carbon use efficiency, biomass residence time and temperature sensitivity across ecosystems and soil depths. *Soil Biology and Biochemistry*, *154*, 108117. https://doi.org/10.1016/j.soilbio.2020.108117

Liang, J. L., Liu, J., Jia, P., Yang, T. T., Zeng, Q. W., Zhang, S. C., Liao, B., Shu, W. S., & Li, J. T. (2020). Novel phosphate solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *The ISME Journal*, *14*(6), 1600–1613. https://doi.org/10.1038/s41396-020-0632-4

Liu, X., Tang, X., Lie, Z., He, X., Zhou, G., Yan, J., Ma, K., Du, S., Li, S., Han, S., Ma, Y., Wang, G., & Liu, J. (2022). Tree species richness as an important biotic factor regulates the soil phosphorus density in China's mature natural forests. *Science of the Total Environment*, *845*, 157277. https://doi.org/10.1016/j.scitotenv.2022.157277

Liu, Y. R., Delgado-Baquerizo, M., Wang, J. T., Hu, H. W., Yang, Z., & He, J. Z. (2018). New insights into the role of microbial community composition in driving soil respiration rates. *Soil Biology and Biochemistry*, *118*, 35–41. https://doi.org/10.1016/j.soilbio.2017.12.003

Lu, J. Z., & Scheu, S. (2021). Response of soil microbial communities to mixed beech-conifer forests varies with site conditions. *Soil Biology and Biochemistry*, *155*, 108155. https://doi.org/10.1016/j.soilbio.2021.108155

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., van Agtmaal, M., Blaud, A., Clark, I. M., Whitaker, J., Pywell, R. F., Ostle, N., Gleixner, G., & Griffiths, R. I. (2018). Land use driven change in soil pH affects microbial carbon cycling processes. *Nature Communications*, *9*, 3591. https://doi.org/10.1038/s41467-018-05980-1

Moorhead, D. L., Lashermes, G., Sinsabaugh, R. L., & Weintraub, M. N. (2013). Calculating co-metabolic costs of lignin decay and their impacts on carbon use efficiency. *Soil Biology and Biochemistry*, 66, 17–19. https://doi.org/10.1016/j.soilbio.2013.06.016

Mori, A. S., Cornelissen, J. H. C., Fujii, S., Okada, K.-i., & Isbell, F. (2020). A meta-analysis on decomposition quantifies afterlife effects of plant diversity as a global change driver. *Nature Communications*, 11(1), 4547. https://doi.org/10.1038/s41467-020-18296-w

Mori, A. S., Dee, L. E., Gonzalez, A., Ohashi, H., Cowles, J., Wright, A. J., Loreau, M., Hautier, Y., Newbold, T., Reich, P. B., Matsui, T., Okada, K., Seidl, R., & Isbell, F. (2021). Biodiversity–productivity relationships are key to nature-based climate solutions. *Nature Climate Change*, 11, 543–550. https://doi.org/10.1038/s41558-021-01062-1

Nottingham, A. T., Fierer, N., Turner, B. L., Whitaker, J., Ostle, N. J., McNamara, N. P., Bardgett, R. D., Leff, J. W., Salinas, N., Silman, M. R., Kruuk, L. E. B., & Meir, P. (2018). Microbes follow Humboldt: Temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. *Ecology*, 99(11), 2455–2466. https://doi.org/10.1002/ecy.2482

Nottingham, A. T., Turner, B. L., Whitaker, J., Ostle, N. J., McNamara, N. P., Bardgett, R. D., Salinas, N., & Meir, P. (2015). Soil microbial nutrient constraints along a tropical forest elevation gradient: A belowground test of a biogeochemical paradigm. *Biogeosciences*, 12(20), 6071–6083. https://doi.org/10.5194/bg-12-6071-2015

Oelmann, Y., Lange, M., Leimer, S., Roscher, C., Aburto, F., Alt, F., Bange, N., Berner, D., Boch, S., Boeddinghaus, R. S., Buscot, F., Dassen, S., De Deyn, G., Eisenhauer, N., Gleixner, G., Goldmann, K., Hölzel, N., Jochum, M., Kandeler, E., … Wilcke, W. (2021). Above- and belowground biodiversity jointly tighten the P cycle in agricultural grasslands. *Nature Communications*, 12, 4431. https://doi.org/10.1038/s41467-021-24714-4

Peng, S., & Chen, H. Y. H. (2021). Global responses of fine root biomass and traits to plant species mixtures in terrestrial ecosystems. *Global Ecology and Biogeography*, 30(1), 289–304. https://doi.org/10.1111/geb.13205

Prommer, J., Walker, T. W., Wanek, W., Braun, J., Zezula, D., Hu, Y., & Richter, A. (2020). Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Global Change Biology*, 26(2), 669–681. https://doi.org/10.1111/gcb.14777

Qian, Z., Li, Y., Du, H., Wang, K., & Li, D. (2023). Increasing plant species diversity enhances microbial necromass carbon content but does not alter its contribution to soil organic carbon pool in a subtropical forest. *Soil Biology and Biochemistry*, 187, 109183. https://doi.org/10.1016/j.soilbio.2023.109183

Qin, S., Kou, D., Mao, C., Chen, Y., Chen, L., & Yang, Y. (2021). Temperature sensitivity of permafrost carbon release mediated by mineral and microbial properties. *Science Advances*, 7(32), eabe3596. https://doi.org/10.1126/sciadv.abe3596

R Development Core Team. (2022). *R: A Language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Redlich, S., Zhang, J., Benjamin, C., Singh Dhillon, M., Englmeier, J., Ewald, J., Fricke, U., Ganuza, C., Haensel, M., Hovestadt, T., Kollmann, J., Koellner, T., Kübert-Flock, C., Kunstmann, H., Menzel, A., Moning, C., Peters, W., Riebl, R., Rummler, T., … Steffan-Dewenter, I. (2021). Disentangling effects of climate and land use on biodiversity and ecosystem services—A multi-scale experimental design. *Methods in Ecology and Evolution*, 13, 514–527. https://doi.org/10.1111/2041-210X.13759

Roller, B. R. K., & Schmidt, T. M. (2015). The physiology and ecological implications of efficient growth. *The ISME Journal*, 9(7), 1481–1487. https://doi.org/10.1038/ismej.2014.235

Rui, Y., Jackson, R. D., Cotrufo, M. F., Sanford, G. R., Spiesman, B. J., Deiss, L., Culman, S. W., Liang, C., & Ruark, M. D. (2022). Persistent soil carbon enhanced in Mollisols by well-managed grasslands but not annual grain or dairy forage cropping systems. *Proceedings of the National Academy of Sciences of the United States of America*, 119(7), e2118931119. https://doi.org/10.1073/pnas.2118931119

Saifuddin, M., Bhatnagar, J. M., Segrè, D., & Finzi, A. C. (2019). Microbial carbon use efficiency predicted from genome-scale metabolic models. *Nature Communications*, 10(1), 3568. https://doi.org/10.1038/s41467-019-11488-z

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry*, 34(9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Schoolmaster, D. R., Jr., Zirbel, C. R., & Cronin, J. P. (2020). A graphical causal model for resolving species identity effects and biodiversity–ecosystem function correlations. *Ecology*, 101(8), e03070. https://doi.org/10.1002/ecy.3070

Smith, T. P., Clegg, T., Bell, T., & Pawar, S. (2021). Systematic variation in the temperature dependence of bacterial carbon use efficiency. *Ecology Letters*, 24(10), 2123–2133. https://doi.org/10.1111/ele.13840

Soares, M., & Rousk, J. (2019). Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. *Soil Biology and Biochemistry*, 131, 195–205. https://doi.org/10.1016/j.soilbio.2019.01.010

Sokol, N. W., Slessarev, E., Marschmann, G. L., Nicolas, A., Blazewicz, S. J., Brodie, E. L., Firestone, M. K., Foley, M. M., Hestrin, R., Hungate, B. A., Koch, B. J., Stone, B. W., Sullivan, M. B., Zablocki, O., Trubl, G., McFarlane, K., Stuart, R., Nuccio, E., Weber, P., … Pett-Ridge, J. (2022). Life and death in the soil microbiome: How ecological processes influence biogeochemistry. *Nature Reviews Microbiology*, 20(7), 415–430. https://doi.org/10.1038/s41579-022-00695-z

Spohn, M., Pötsch, E. M., Eichorst, S. A., Woebken, D., Wanek, W., & Richter, A. (2016). Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biology and Biochemistry*, 97, 168–175. https://doi.org/10.1016/j.soilbio.2016.03.008

Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2015). Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist*, 207(4), 1145–1158. https://doi.org/10.1111/nph.13426

Sun, Y., Wang, C., Yang, J., Liao, J., Chen, H. Y. H., & Ruan, H. (2021). Elevated CO_2 shifts soil microbial communities from K- to r-strategists. *Global Ecology and Biogeography*, 30(5), 961–972. https://doi.org/10.1111/geb.13281

Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy Alves, R. J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., & Richter, A. (2018). Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect. *Soil Biology and Biochemistry*, 121, 212–220. https://doi.org/10.1016/j.soilbio.2018.02.022

Tao, F., Huang, Y., Hungate, B. A., Manzoni, S., Frey, S. D., Schmidt, M. W. I., Reichstein, M., Carvalhais, N., Ciais, P., Jiang, L., Lehmann, J., Wang, Y.-P., Houlton, B. Z., Ahrens, B., Mishra, U., Hugelius, G., Hocking, T. D., Lu, X., Shi, Z., … Luo, Y. (2023). Microbial carbon use efficiency promotes global soil carbon storage. *Nature*, 618(7967), 981–985. https://doi.org/10.1038/s41586-023-06042-3

Tong, X., Brandt, M., Yue, Y., Horion, S., Wang, K., Keersmaecker, W. D., Tian, F., Schurgers, G., Xiao, X., Luo, Y., Chen, C., Myneni, R., Shi, Z., Chen, H., & Fensholt, R. (2018). Increased vegetation growth and carbon stock in China karst via ecological engineering. *Nature Sustainability*, 1(1), 44–50. https://doi.org/10.1038/s41893-017-0004-x

Viscarra Rossel, R. A., Yang, Y., Bissett, A., Behrens, T., Dixon, K., Nevil, P., & Li, S. (2022). Environmental controls of soil fungal abundance and diversity in Australia's diverse ecosystems. *Soil Biology and*

Biochemistry, 170, 108694. https://doi.org/10.1016/j.soilbio.2022.108694

Wang, J., Feng, L., Palmer, P. I., Liu, Y., Fang, S., Bösch, H., O'Dell, C. W., Tang, X., Yang, D., Liu, L., & Xia, C. (2020). Large Chinese land carbon sink estimated from atmospheric carbon dioxide data. *Nature, 586*(7831), 720–723. https://doi.org/10.1038/s41586-020-2849-9

Whitaker, J., Ostle, N., McNamara, N. P., Nottingham, A. T., Stott, A. W., Bardgett, R. D., Salinas, N., Ccahuana, A. J. Q., & Meir, P. (2014). Microbial carbon mineralization in tropical lowland and montane forest soils of Peru. *Frontiers in Microbiology, 5*, 720. https://doi.org/10.3389/Fmicb.2014.00720

Wilhelm, R. C., Lynch, L., Webster, T. M., Schweizer, S., Inagaki, T. M., Tfaily, M. M., Kukkadapu, R., Hoeschen, C., Buckley, D. H., & Lehmann, J. (2022). Susceptibility of new soil organic carbon to mineralization during dry-wet cycling in soils from contrasting ends of a precipitation gradient. *Soil Biology and Biochemistry, 169*, 108681. https://doi.org/10.1016/j.soilbio.2022.108681

Wu, M., Chen, S., Chen, J., Xue, K., Chen, S., Wang, X., Chen, T., Kang, S., Rui, J., Thies, J. E., Bardgett, R. D., & Wang, Y. (2021). Reduced microbial stability in the active layer is associated with carbon loss under alpine permafrost degradation. *Proceedings of the National Academy of Sciences of the United States of America, 118*, e2025321118. https://doi.org/10.1073/pnas.2025321118

Wu, X., Liu, P., Wegner, C. E., Luo, Y., Xiao, K. Q., Cui, Z., Zhang, F., Liesack, W., & Peng, J. (2021). Deciphering microbial mechanisms underlying soil organic carbon storage in a wheat-maize rotation system. *Science of the Total Environment, 788*, 147798. https://doi.org/10.1016/j.scitotenv.2021.147798

Yang, S., Jansen, B., Kalbitz, K., Chunga Castro, F. O., van Hall, R. L., & Cammeraat, E. L. H. (2020). Lithology controlled soil organic carbon stabilization in an alpine grassland of the Peruvian Andes. *Environmental Earth Sciences, 79*(2), 66. https://doi.org/10.1007/s12665-019-8796-9

Yang, Y., Shen, Z., Bissett, A., & Viscarra Rossel, R. A. (2022). Estimating soil fungal abundance and diversity at a macroecological scale with deep learning spectrotransfer functions. *The Soil, 8*(1), 223–235. https://doi.org/10.5194/soil-8-223-2022

Ye, G., Lin, Y., Kuzyakov, Y., Liu, D., Luo, J., Lindsey, S., Wang, W., Fan, J., & Ding, W. (2019). Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: Results of a 27-year field experiment. *Soil and Biochemistry, 134*, 15–24. https://doi.org/10.1016/j.soilbio.2019.03.018

Zhang, B., Cai, Y., Hu, S., & Chang, S. X. (2021). Plant mixture effects on carbon-degrading enzymes promote soil organic carbon accumulation. *Soil Biology and Biochemistry, 163*, 108457. https://doi.org/10.1016/j.soilbio.2021.108457

Zhang, K., Chen, L., Li, Y., Brookes, P. C., Xu, J., & Luo, Y. (2020). Interactive effects of soil pH and substrate quality on microbial utilization. *European Journal of Soil Biology, 96*, 103151. https://doi.org/10.1016/j.ejsobi.2020.103151

Zhang, Q., Qin, W., Feng, J., Li, X., Zhang, Z., He, J. S., Schimel, J. P., & Zhu, B. (2023). Whole-soil-profile warming does not change microbial carbon use efficiency in surface and deep soils. *Proceedings of the National Academy of Sciences of the United States of America, 120*(32), e2302190120. https://doi.org/10.1073/pnas.2302190120

Zheng, H., Vesterdal, L., Schmidt, I. K., & Rousk, J. (2022). Ecoenzymatic stoichiometry can reflect microbial resource limitation, substrate quality, or both in forest soils. *Soil Biology and Biochemistry, 167*, 108613. https://doi.org/10.1016/j.soilbio.2022.108613

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., & Wanek, W. (2019). Growth explains microbial carbon use efficiency across soils differing in land use and geology. *Soil Biology and Biochemistry, 128*, 45–55. https://doi.org/10.1016/j.soilbio.2018.10.006

Zhong, Z., Li, W., Lu, X., Gu, Y., Wu, S., Shen, Z., Han, X., Yang, G., & Ren, C. (2020). Adaptive pathways of soil microorganisms to stoichiometric imbalances regulate microbial respiration following afforestation in the loess plateau, China. *Soil Biology and Biochemistry, 151*, 108048. https://doi.org/10.1016/j.soilbio.2020.108048

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