



Litter quality controls the contribution of microbial carbon to main microbial groups and soil organic carbon during its decomposition

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Abstract

A 163-day decomposition experiment with ¹³C-enriched leaf litter of *Populus davidiana* (low quality, with low N content, high C:N and high lignin content) and *Quercus wutaishanica* (high quality, with high N content, low C:N and low lignin content) was conducted to investigate the effects of litter quality on the microbial contribution to soil organic C (SOC). We used stable isotope probing (SIP) technology of phospholipid fatty acid (PLFA) and amino sugar, determined soil enzyme activities, and microbial C use efficiency (CUE) to study the microbial contribution to SOC formation as affected by litter quality. Gram-positive (G+) and Gram-negative (G-) bacteria rapidly assimilated the readily available C of high- and low-quality litter, whereas fungi selectively utilized more recalcitrant compounds. The ratio of ¹³C-fungal to ¹³C-bacterial necromass increased and then leveled off until the end of the incubation for both litters. Therefore, litter-derived C was first utilized by bacteria, then allocated presumably by the consumption of bacterial necromass to fungi, and, at the end, the litter C was mainly stabilized as fungal necromass. The addition of high-quality litter led to higher total necromass and SOC in comparison to the addition of low-quality litter. Likely this difference depended on the higher availability of easily available C compounds in the *Q. wutaishanica* than in *P. davidiana* litters. The efficiency of SOC formation, determined by the percentage of SOC content gain divided by the litter C content loss, correlated with the microbial incorporation of *P. davidiana* litter-derived ¹³C into PLFAs and amino sugars. However, it increased sharply in the late phases of *Q. wutaishanica* litter decomposition despite the decreased ¹³C incorporation in PLFAs and amino sugars, suggesting the dominance of physical litter C stabilization. Compared to the high-quality litter, the low-quality litter induced lower but steadier necromass accumulation, thus increasing the SOC content in the long term. Litter quality, litter-derived ¹³C in PLFAs, and microbial CUE are the main drivers of litter-derived C use pathways. Our findings underpin the microbial C pump-regulated SOC formation, whereby differences in litter quality shape the composition of main microbial groups, leading to differences in enzyme activities and CUE, which determine necromass turnover and thus SOC formation.

Keywords Leaf litter degradation · Main microbial groups · Enzymatic functions · Litter stoichiometry · Soil biomarker · Microbial residues

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Introduction

The decomposition of litter is one of the primary pathways affecting forest soil C formation (Aerts 2006) by inducing soil microbial growth, and then microbial death produces necromass, a key source of soil organic C (SOC) (Liang et al. 2017; Kravchenko et al. 2019; Sokol et al. 2019). The high-quality litter addition is assumed to induce a higher amount of SOC accumulation than low-quality litter (Cotrufo et al. 2015) due to its high N content but low C:N ratio and low lignin content. It is used by fast-growing microorganisms to form microbial necromass with the contribution to SOC formation (Lavallee et al. 2018; Fulton-Smith and Cotrufo 2019). High-quality litter has sufficient N to meet the microbial N demands required for its decomposition (Vesterdal et al. 2008), reducing the need to use N of the soil organic matter. In contrast, low-quality, slow-decomposing plant litter with high C:N ratio and high lignin content but low N content may decrease SOC storage as microbes have to decompose soil C mining N to satisfy their N demand as a prerequisite to degrade litter (Lyu et al. 2023). Indeed, soils under vegetation with high-quality litter show higher necromass and/or SOC formation than those exposed to low-quality litter (Cai and Feng 2023). However, opposite results have also been reported (Castellano et al. 2015; Craig et al. 2022; Lyu et al. 2023) because recalcitrant compounds are considered an important source of SOC. In this conceptual framework, low-quality litter addition leads to a small but continuous formation of soil C due to the presence of recalcitrant compounds and this may explain SOC formation in soils exposed to low-quality litter compared to those under high-quality litter (Lyu et al. 2023; Sarquis and Sierra 2023). Such contrasting results show that it still remains unclear how litter with contrasting qualities affects the microbial contribution to SOC and the effects of litter quality on SOC formation and accumulation need to be further studied.

Living soil microorganisms not only enzymatically degrade plant litter but also drive SOC formation (Don et al. 2017; Nannipieri 2020; Sokol et al. 2022) mainly through the *in vivo* pathway of substrate decomposition, which depends on the newly formed microbial biomass (Liang et al. 2017). ^{13}C -enriched plant residues have been used as substrates to gain precise quantification of C fluxes through different soil microbial groups (Cai and Feng 2023; Zheng et al. 2023). Most litter-derived ^{13}C is incorporated into bacterial phospholipid fatty acids and then into fungal PLFAs during decomposition (Heijboer et al. 2018). Interestingly, ^{13}C labeling-based experiments have revealed that fungi are major players in rapidly processing easily degradable C to gain energy for the release of the

efficient set of enzymes used to break down recalcitrant materials (Poll et al. 2006; Pausch et al. 2016). After cell death, the microbial necromass can accumulate in soil, constituting approximately 50% of the SOC pool (Liang et al. 2019; Wang et al. 2022). In the early stages of litter decomposition, the labile substrates (i.e., carbohydrates and amino acids) are utilized by soil microbes quickly and results in the rapid formation of high amounts of microbial metabolites (Cotrufo et al. 2015; Cai and Feng 2023). As litter decomposition proceeds, recalcitrant components (i.e., lignin) dominate the degradation process, leading to microbial energy and/or nutrient limitation (Bai et al. 2021). Microbial necromass can be recycled, i.e., partially decomposed and re-used by microorganisms for their growth under nutrient insufficient conditions, and this decreases necromass content (Poirier et al. 2018; Liang et al. 2019; Cui et al. 2020). Generally, necromass formed on substrates which are mainly decomposed by bacteria has a low content than that formed by fungi using recalcitrant C sources as the bacterial necromass is generally turning over faster than chitin-dominated fungal necromass (Liang and Balsler 2008). However, a comprehensive analysis of the temporal dynamics of main microbial groups and their contribution to the C flow into necromass and SOC as affected by litter quality is still poorly understood.

Microbial C use efficiency (CUE) indicates the efficiency of microorganisms to convert the C taken up into biomass (Kallenbach et al. 2016). A high CUE describes a rapid biosynthesis process, i.e., an efficient C incorporation by the microbial anabolism (Maynard et al. 2017). After cell death, this results in the accumulation of necromass, favoring SOC formation via the entombing effect. This C transformation pathway often leads to a positive relationship between CUE and SOC (Kallenbach et al. 2016; Liang et al. 2019). However, a high CUE can also result from an accelerated microbial biomass production on the basis of soil organic matter mining by soil enzymes, leading to the SOC loss. This process chain is often described as priming effect and implies a negative CUE-SOC relationship (Sinsabaugh et al. 2015; Liang et al. 2017). However, whether and under which circumstances CUE positively or negatively correlates with SOC formation is under debate. The effects of ^{13}C - or ^{14}C -labeled simple organic components (i.e., sugars and glucose) on the CUE have been explored (Bardgett and Sagar 1994; Jones et al. 2018). Less information is available on how decomposing complex plant residues (i.e., litter) with contrasting qualities affect CUE and downstream the SOC formation (Sauvadet et al. 2018). In addition, microbial use channels, that is, litter-derived C flows through the microbial groups into the necromass and even further levels of the soil food web are not yet understood. Therefore, it is

unknown how these used channels are linked to the initial substrate quality.

The objectives of this study were to (1) investigate the effects of litter quality on the microbial contribution to SOC and (2) shed light on how the main microbial groups, key soil enzyme activities involved in the selected reactions of C, N, and P transformation, CUE, and necromass regulate the formation and accumulation of SOC during litter decomposition. We hypothesized that (I) more necromass and SOC are formed in soil exposed to high-quality litter than those with low-quality litter because microbes would be forced to decompose necromass and/or SOC to get access to locked N in soil organic matter to degrade the low-quality litter. In contrast, the microbial nutritional demands would largely be met by the high-quality litter. (II) Litter quality is the major factor controlling the pathway of C through microbial process as it affects the microbial CUE, the C incorporation into PLFA, the C allocation to microbial anabolism (biomass formation), the soil enzyme activities degrading complex litter and also soil organic compounds, and the microbial products remaining after cell death affecting the necromass formation and accumulation.

Materials and methods

Leaf litter decomposition experiment

¹³C in situ labeling was conducted in the Ziwuling Mountains on the Loess Plateau, Yan’an City, Shannxi Province, China, to produce ¹³C-enriched litter. This region (36°03'52"–36°04'49"N, 109°09'54"–109°10'48"E) has a continental warm temperate monsoon and a mid-latitude semiarid climate, with a mean annual rainfall and temperature of 500–600 mm and 7.4–9.3 °C, respectively (Fig. S1). The soil type is Cambisol, classified according to the world reference base (WRB) soil taxonomy (IUSS Working Group WRB 2022). The primary forest species are *Robinia pseudoacacia*, *Platycladus orientalis*, *Populus davidiana*, and *Quercus wutaishanica*. *P. davidiana* and *Q. wutaishanica* were selected as study objects as they have distinctly different leaf N and lignin contents and C:N ratios, which are important indices for determining litter quality (Table 1).

Three sites dominated by *P. davidiana* and three dominated by *Q. wutaishanica* were selected. Four *P. davidiana* and four *Q. wutaishanica* specimens characterized by similar ages (5 years) and heights (3–4 m) were chosen for each site for ¹³C labeling. ¹³CO₂ was released by mixing Na₂¹³CO₃ (99 atom% ¹³C, Berlin, Germany) with 1 M H₂SO₄ in gas-tight low-density polyethylene labeling chambers. The leaves in the chambers were uniformly labeled because the selected *P. davidiana* and *Q. wutaishanica* were ¹³C multiple-pulse labeled (¹³C pulse labeling

Table 1 The litter and soil properties before the litter decomposition experiment

Plantation types	Site number	Leaf C content (g kg ⁻¹)	Leaf N content (g kg ⁻¹)	Leaf C:N	Lignin (g kg ⁻¹)	Cellulose (g kg ⁻¹)	SOC (g kg ⁻¹)		Soil N (g kg ⁻¹)		Soil P (g kg ⁻¹)		pH	
							0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
<i>Populus davidiana</i>	1	517 ± 12.1	15.2 ± 1.1	34 ± 2.8	192 ± 21	231 ± 42	17 ± 0.2	13 ± 0.4	1.7 ± 0.3	1.5 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	8.1 ± 0.1	8.2 ± 0.1
	2	529 ± 21.1	14.7 ± 0.1	35 ± 1.8	201 ± 32	236 ± 23	16 ± 0.1	14 ± 0.5	1.5 ± 0.6	1.3 ± 0.6	0.5 ± 0.1	0.5 ± 0.1	8.2 ± 0.1	8.2 ± 0.1
	3	535 ± 12.1	15.2 ± 1.1	35 ± 2.3	203 ± 23	241 ± 41	17 ± 0.5	13 ± 0.8	1.7 ± 0.4	1.5 ± 0.4	0.6 ± 0.1	0.5 ± 0.1	7.9 ± 0.1	8.1 ± 0.1
<i>Quercus wutaishanica</i>	1	424 ± 11.2	17.1 ± 1.1	25 ± 2.2	170 ± 12	201 ± 21	20 ± 0.4	14 ± 0.4	1.8 ± 0.6	1.7 ± 0.6	0.7 ± 0.1	0.6 ± 0.1	7.7 ± 0.1	7.9 ± 0.1
	2	425 ± 11.2	18.1 ± 1.1	27 ± 1.2	168 ± 16	204 ± 34	19 ± 0.5	14 ± 0.8	1.9 ± 0.3	1.8 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	7.9 ± 0.1	7.8 ± 0.1
	3	422 ± 11.2	17.3 ± 1.1	25 ± 1.5	167 ± 18	199 ± 12	21 ± 0.5	15 ± 0.4	1.9 ± 0.5	1.7 ± 0.5	0.8 ± 0.1	0.7 ± 0.1	8.1 ± 0.1	8.1 ± 0.1

was performed four times, and each pulse labeling was 2 days apart) (Kuzyakov 2006; Shahbaz et al. 2017; Lyu et al. 2023). Moreover, the sequential extraction method was used to detect whether ^{13}C was uniformly distributed in the labeled leaves (Kuzyakov et al. 2007). Five fractions (dissolved in hot H_2O , $\text{C}_2\text{H}_5\text{OH}$, NaOH , HCl , and non-dissolved residue) were obtained from nine replicates of each litters to analysis the $\delta^{13}\text{C}$ values. Except ethanol extract, the ^{13}C incorporation in the other four fractions ranged between 20 and 28% of total ^{13}C in plant residues, indicating the ^{13}C was evenly distributed in the plant residues (Fig. S2). Leaf samples were collected immediately after ^{13}C labeling, oven dried at 70°C about 1 week to constant weight in a small portable oven (Bao 2000). The oven-dried ^{13}C -labeled leaf samples were regarded as leaf litters as the $\delta^{13}\text{C}$ could decrease if we waited until they fell to the ground in autumn. This might result in insufficient ^{13}C enrichment to study the litter-derived C flows via main microbial groups into dead microbial necromass and monitor the long-term stabilization of microbial residues (Rubino et al. 2010; Lyu et al. 2023). After transported to the lab, leaf samples were cut into small pieces (approximately 2×2 cm). This resembled the process of litter fragmentation by soil animals, which was excluded by incubation as we used sieved soil. Thus, the cutting process may bring the rates of litter degradation closer to reality under unmanipulated conditions than by introducing artificial artifacts (Huang et al. 2023). Nine 5 g subsamples of *P. davidiana* and *Q. wutaishanica* labeled leaves were passed through a 1-mm sieve after grinding and used for the analysis of leaf C, N, and P, as well as $\delta^{13}\text{C}$ prior to lab incubation. The average $\delta^{13}\text{C}$ value of *P. davidiana* and *Q. wutaishanica* labeled leaves was 568‰ (1.7 atom %, the SE was 2.3‰) and 1040‰ (2.2 atom %, the SE was 3.8‰), respectively. The remaining samples were used for the decomposition experiments.

A 10×10 m site was selected to collect soil for *P. davidiana* and *Q. wutaishanica* litter decomposition experiments. We collected surface soils (0–20 cm) after clearing the top litter; it was passed through 2-mm sieve and pre-incubated in white glass wide-mouth bottles for 7 days at 25°C after adjusting the water content to 60%–70% of field capacity to provide suitable temperature and humidity to stabilize microbial activity. The decomposition device ($15 \times 15 \times 10$ cm) was made of low-density polyethylene with round holes on top for ventilation (Fig. S3). Soil (200 g) was placed at the bottom of the decomposition device, and 6 g of labeled/unlabeled leaf litter was spread evenly over the soil surface. The destructive sampling method was used to collect soil samples 4, 7, 14, 28, 56, and 163 days after the decomposition experiment started; there were nine replicates per sampling time. Thus, 108 (54 for labeled litter and 54 for unlabeled litter) decomposition devices were prepared for each litter

type. The water content was adjusted every 3 days following the gravimetric approach.

Plant and soil sampling

Litter and soil samples were collected 4, 7, 14, 28, 56, and 163 days after the start of decomposition. The mixture of litter and soil was passed through a 5-mm sieve to distinguish between the soil and litter samples (the litter detritus smaller than 5 mm but visible were collected with tweezers). The soil attached to the litter was removed using tweezers and a flow of distilled water. The litter samples were oven dried at 70°C about 1 week to constant weight, and passed through a 1-mm sieve after grinding before leaf C, N, P, and $\delta^{13}\text{C}$ analyses. The soil samples were divided into three parts: approximately 10 g of fresh soil samples were freeze dried for the analysis of PLFAs, and approximately 50 g of fresh soil samples were stored at 4°C for the analysis of soil microbial biomass C, N, and P and soil enzyme activities, which were conducted within 1 week after sampling. The remaining soil samples were air dried and ground before passed through 0.15-mm sieves for SOC, amino sugars, and $\delta^{13}\text{C}$ analyses.

Compound-specific isotope analysis

^{13}C -PLFAs and ^{13}C -amino sugars have been used as biomarkers to trace litter-derived C flows via living microbial groups into dead microbial necromass and to monitor the long-term stabilization of microbial residues (Liang and Balsler 2011; Bradford et al. 2016; Joergensen 2022). The identification and quantification of PLFAs, ^{13}C -PLFAs, amino sugars, and ^{13}C -amino sugars were performed as described by Peixoto et al. (2020). Briefly, fatty acids were extracted from 2 g of freeze-dried soil using a chloroform:methanol:citrate (1:2:0.8) buffer mixture. Mild alkaline methanolysis was used to methylate phospholipids and derivatize them into fatty acid methyl esters (FAMES). The FAMES were quantified, and their isotope enrichment was determined using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) (Thermo Finnigan, Bremen, Germany).

Three amino sugars, glucosamine (GlcN), galactosamine (GalN), and muramic acid (MurA) were determined as described by Zhang and Amelung (1996). Briefly, 3 g of freeze-dried soil (about 0.3 mg N) was hydrolyzed in 10 mL of 6 M HCl for 8 h at 105°C . The soil samples were then filtered through glass fiber filters, and HCl was removed via rotary evaporation to dry the filtrate. The filtrate was re-suspended in water and adjusted to a pH of 6.6–6.8 with a 0.4 M KOH solution and a 1 M KOH solution (corrected if necessary with 0.1 M HCl). The residue was freeze dried to remove residual liquid. The remaining residue was transferred to 5-mL reaction vials and dried under a flow of N_2 .

An internal standard was added and used to quantify amino sugar recovery. The residues were dissolved in 185 μL of ethyl acetate/hexane (1:1, v/v), 15 μL of a second internal standard, and tridecanoic acid methyl ester. All raw $\delta^{13}\text{C}$ values of the amino sugars were measured using a Delta V Advantage isotope ratio mass spectrometer via a ConFlo III interface (Thermo Fisher Scientific, Bremen, Germany).

^{13}C -PLFAs and ^{13}C -amino sugars were measured using a Delta V Advantage isotope ratio mass spectrometer with a ConFlo III interface (Thermo Fisher Scientific). Isodat software (version 3.0; Thermo Fisher Scientific) integrated the chromatogram peaks specific to the PLFAs and amino sugars of interest.

Analysis of soil enzyme activities

The potential activities of β -1,4-glucosidase (BG), β -1,4-xylosidase (XYL), cellobiase (CELLO), β -N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP) were determined using the fluorogenic methylumbelliferone-based substrate method (German et al. 2011) and the pH was adjusted using buffer before measuring. We used sodium acetate, citrate, and water as buffers. We prepared a standard curve with concentrations of 0, 0.5, 1, 1.5, 2, 2.5, 4, and 6 μM 4-methylumbelliferone (MUF) in soil suspension aliquots and buffer for each soil sample. Calibration curves in deionized water and buffer were obtained to determine the quenching factor (German et al. 2011). Sufficient amounts of substrates were tested to prevent the inhibition of soil enzyme activity. Finally, 100 μL of substrate solution was added at a concentration of 300, 300, 300, 200, 200, and 300 μmol substrate g^{-1} soil for BG, XYL, CELLO, NAG, LAP, and AP, respectively. We also determined the optimal incubation time (3, 4, 6, and 8 h) by fluorometrically measuring the potential enzyme activity after shaking at 25 $^{\circ}\text{C}$ every 30 min (excitation 360 nm; emission 450 nm) by an automated fluorometric plate reader (Synergy H4Bio Tek, USA). An incubation time of 3 h was optimal for our samples. Changes in fluorescence intensity were measured at 0, 12, and 24 h after incubation using a standard quenching curve, most of which increased linearly during incubation. The enzyme activities ($\text{nmol h}^{-1} \text{g}^{-1}$ dry soil) were then estimated by linear fluorescence intensity changes against time.

Analysis of litter C, N, and P contents and $\delta^{13}\text{C}$ of organic C

To measure the total litter P and N, 5 mL of concentrated H_2SO_4 was added to the samples and digested overnight at room temperature (Bao 2000). The samples were put in an electric furnace, digested until the color of the solution changed to black at 365 $^{\circ}\text{C}$, and cooled to room temperature.

Hydrogen peroxide (H_2O_2) was added, and the sample was digested at 365 $^{\circ}\text{C}$. This procedure was repeated until the solution became colorless. Total P and N were quantified using a colorimetric ultraviolet (UV) spectrophotometer and the micro-Kjeldahl method, respectively (Bao 2000). Organic C content and $\delta^{13}\text{C}$ values of soil and plant samples were analyzed by an elemental analyzer (Vario PYRO cube; Elementar, Langensfeld, Germany) coupled with an IsoPrime 100 Isotope Ratio Mass Spectrometer (Elementar).

Calculations

The $\delta^{13}\text{C}$ values of the FAMEs were calculated based on Glaser (2005), and the $^{13}\text{C}_{\text{amount}}$ of PLFAs was calculated as follows:

$$\text{nmol } ^{13}\text{C} - \text{PLFAs g}^{-1} \text{ soil} = \text{at}\%^{13}\text{C} \times \text{nmol PLFA g}^{-1} \text{ soil} \times \frac{M(\text{PLFAs} - \text{C})}{M(\text{PLFAs})} \quad (1)$$

where $\text{at}\%^{13}\text{C}$ is the $\%^{13}\text{C}$ excess, nmol PLFAs g^{-1} soil is the amount of PLFAs, $M(\text{PLFAs})$ is the molar mass of the PLFAs molecule, and $M(\text{PLFAs} - \text{C})$ is the molar mass of all C atoms within the PLFAs molecule.

The $^{13}\text{C}_{\text{atom}\% \text{ excess}}$ was calculated as

$$^{13}\text{C}_{\text{atom}\% \text{ excess}} = ^{13}\text{C}_{\text{atom}\% \text{ of labeled sample}} - ^{13}\text{C}_{\text{atom}\% \text{ of unlabeled samples}} \quad (2)$$

where $^{13}\text{C}_{\text{atom}\% \text{ of labeled sample}}$ and $^{13}\text{C}_{\text{atom}\% \text{ of unlabeled sample}}$ represent the percentages of ^{13}C in the labeled and unlabeled samples, respectively.

Chromatogram peaks of amino sugars were integrated using Isodat v. 3.0 (Thermo Fisher Scientific). The measured $\delta^{13}\text{C}$ values were corrected for amount (area) dependence, offset, and additional C atoms added during the derivatization reaction (Glaser and Gross 2005; Dippold et al. 2014). The ^{13}C incorporation into the amino sugars was calculated as follows:

$$\text{nmol } ^{13}\text{C} - \text{amino sugars g}^{-1} \text{ soil} = \text{at}\%^{13}\text{C} \times \text{nmol amino sugars g}^{-1} \text{ soil} \quad (3)$$

The ^{13}C incorporated into the soil and microbial pools ($^{13}\text{C}_{\text{content}}$) were estimated using Eqs. (4) and (5) (for each sampling):

$$F = \frac{^{13}\text{C}_{\text{atom}\% \text{ of labeled sample}} - ^{13}\text{C}_{\text{atom}\% \text{ of unlabeled samples}}}{^{13}\text{C}_{\text{atom}\% \text{ of litter}} - ^{13}\text{C}_{\text{atom}\% \text{ of unlabeled samples}}} \quad (4)$$

$$^{13}\text{C}_{\text{content}}(\text{g/kg}) = F \times C_{\text{content}}(\text{g/kg}) \quad (5)$$

where F is the fraction of the target pool (SOC, GlcN, GalN, and MurA) derived from the leaf litter; C_{content} refers to the

C content in SOC, GlcN, GalN, and/or MurA; $^{13}\text{C}_{\text{atom}\%}$ of litter is the $^{13}\text{C}_{\text{atom}\%}$ of litter before decomposition.

The soil microbial CUE was calculated based on formula (6):

$$\text{CUE} = \left(\frac{^{13}\text{C-MBC}}{^{13}\text{C-MBC} + \text{R}_{\text{cum}}} \right) \quad (6)$$

where $^{13}\text{C-MBC}$ is the ^{13}C content in MBC and R_{cum} is the accumulated soil mineralization at 4, 7, 14, 28, 56, and 163 days.

The mean residence time (MRT) of soil-living microbial groups (using PLFAs as the biomarker) was calculated as follows (Gunina et al. 2017):

$$^{13}\text{C}_{\text{enrichment}} = \frac{^{13}\text{C} - \text{PLFAs}}{\text{totalPLFAs}} \times 100\% \quad (7)$$

$$\text{C}_{\text{enrichment}(t)} = \text{C}_{\text{enrichment}(0)} \times \exp^{-kt} \quad (8)$$

$$\text{MRT} = \frac{1}{K} \quad (9)$$

where $^{13}\text{C-PLFAs}$ is the litter-derived C in PLFAs (nmol kg^{-1}), total PLFAs is the total PLFA-C (nmol kg^{-1}), $\text{C}_{\text{enrichment}(t)}$ (%) is the ^{13}C enrichment of PLFAs 7, 14, 28, 56, and 163 days after litter application, $\text{C}_{\text{enrichment}(0)}$ (%) is the ^{13}C enrichment of PLFAs at 4 days after litter application, k is the decomposition rate constant, and t represents the sampling time (days) after litter application.

The microbial necromass C content (fungal and bacterial necromass C) was calculated according to Eqs. (10) and (11) (Liang et al. 2019):

$$\text{Bacterial necromass C} = \text{MurA} \times 45 \quad (10)$$

$$\text{Fungal necromass C} = \left(\frac{\text{GlcN}}{179.17} - 2 \times \frac{\text{MurA}}{251.23} \right) \times 179.17 \times 9 \quad (11)$$

where 45 in Eq. (10) is the conversion factor from MurA to bacterial necromass C. In Eq. (11), 179.17 and 251.23 are the molecular weights of GlcN and MurA, respectively, and 9 is the conversion value of fungal GlcN to fungal necromass C. The microbial necromass C was estimated as the sum of fungal and bacterial necromass C.

The SOC formation efficiency (SOC_{FE}) is calculated as the percentage of SOC content gain divided by the litter C content loss (Cotrufo et al. 2015):

$$\text{SOC}_{\text{FE}} = \left(\frac{\text{SOC}_{\text{treatment}} - \text{SOC}_{\text{control}}}{\text{LC}_{\text{control}} - \text{LC}_{\text{treatment}}} \right) \times 100\% \quad (12)$$

where $\text{SOC}_{\text{treatment}}$ is the SOC content at the sampling time (4, 7, 14, 28, 56, and 163 days after litter application),

$\text{SOC}_{\text{control}}$ is the SOC content before the litter decomposition experiment, $\text{LC}_{\text{control}}$ is the litter C content before the litter decomposition experiment, and $\text{LC}_{\text{treatment}}$ is the litter C content at the sampling time (4, 7, 14, 28, 56, and 163 days after litter application).

Statistical data analysis

All data are presented as mean ($n=9$) and standard error (SE). All analyses were conducted using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to check for the normal distribution of the residues, and homogeneity was checked using Levene’s test. Any non-normal data were log-transformed to conform to the assumption of normality before further statistical analysis (no non-normal data were found in this study). A two-way analysis of variance (ANOVA) was used when considering the changes in ^{13}C in the soil for the two species at different sampling times. A t -test was used when considering the changes in ^{13}C in the soil between the two litter types. Differences at $p < 0.05$ were considered statistically significant. All charts were constructed using Origin Pro 2018 (Origin Lab Corporation, USA). To explore the cascading relationships among SOC formation, main microbial groups, the enzyme activities involved in the selected reactions of C, N and P, and amino sugars, partial least squares path models (PLS-PM) were used to identify possible pathways whereby variables control SOC formation during litter decomposition. The models were constructed by the “plsmpm” package of R.

Results

Dynamic of microbial groups and enzyme activities during litter decomposition

BG, XYL, and CELLO activities continuously increased with *P. davidiana* decomposition but only increased during the early stage of *Q. wutaishanica* decay and then decreased after that (Fig. 1). Until 14 days after litter addition, enzyme activities involved in few reactions of soil organic C breakdown were higher in high-quality litter (*Q. wutaishanica*) than in low-quality litter (*P. davidiana*); however, as litter decomposition progressed, the trend reversed. The transfer of litter C into total PLFAs depended on litter quality, with more *Q. wutaishanica* litter recovered in total PLFAs in the earlier decomposition stage but less at day 163 than of *P. davidiana* litter (Fig. 2). More litter-derived C incorporated into Gram + (G+) and Gram – (G–) bacteria than other microbial groups for both litters, and the ^{13}C content in G+ and G– bacteria PLFAs differed between high- and low-quality litter types. The *P.*

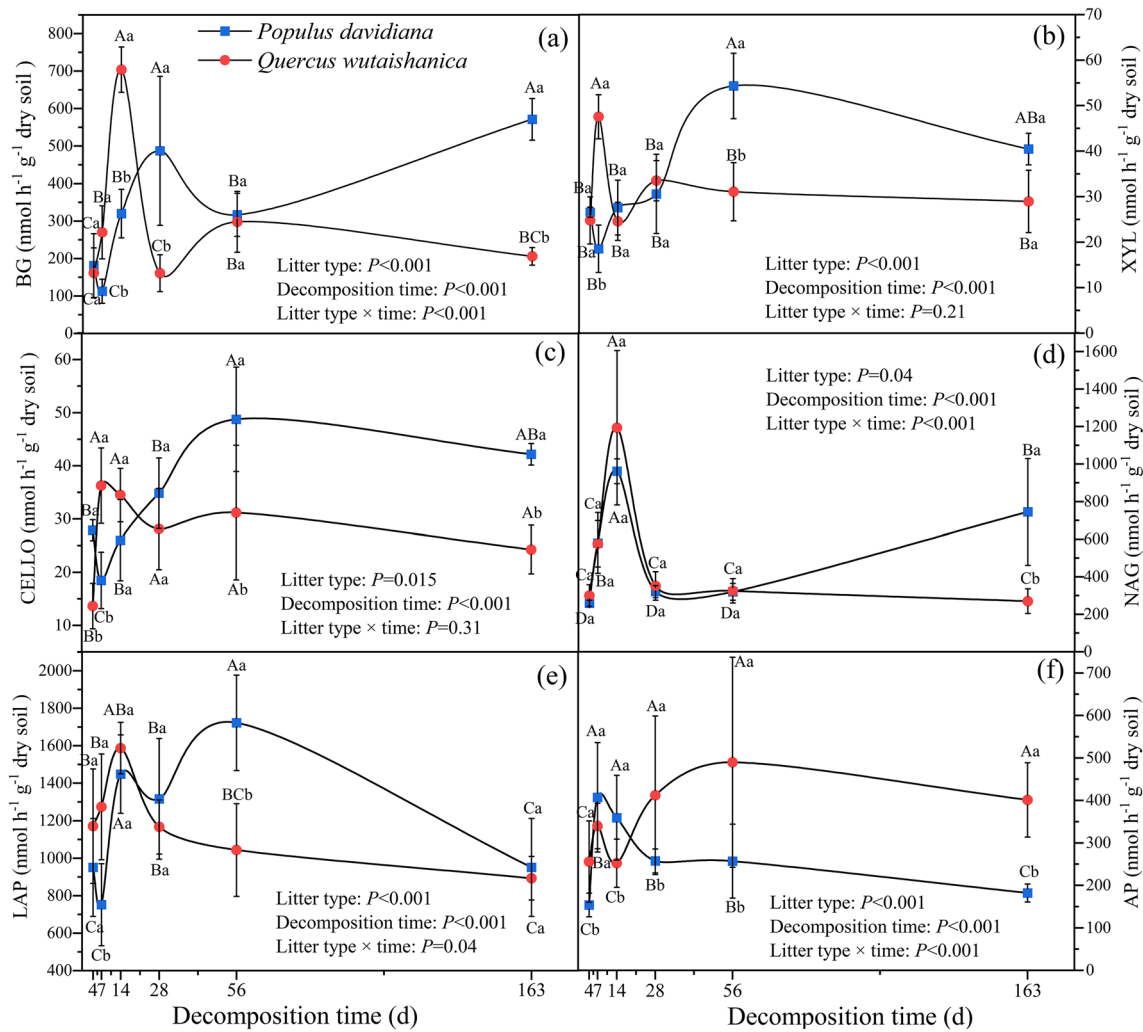


Fig. 1 Dynamic of soil enzyme activities during litter decomposition. Note: Figures represent **a** β -1,4-glucosidase (BG), **b** β -1,4-xylosidase (XYL), **c** cellobiase (CELLO), **d** β -N-acetyl-glucosaminidase (NAG), **e** leucine aminopeptidase (LAP), and **f** alkaline phosphatase (AP) activities, and colors indicate the two litter of contrasting quality. Values are the averages (\pm SE) of nine replicates. Uppercase letters

(A, B, and C) indicate the significant difference ($p < 0.05$) among sampling times for the same species. Lowercase letters (a, b and c) indicate the significant difference ($p < 0.05$) between two species at the sampling time. The P values from two-way ANOVA (litter types, decomposition times and their interaction) are shown

davidiana litter ^{13}C in bacteria PLFAs showed an almost continuous increase with decomposition but peaked on day 14 after *Q. wutaishanica* litter application. Regardless of the litter type, PCA analysis of ^{13}C in PLFA biomarkers indicated that litter-derived C was first incorporated into G+ and G- bacterial PLFAs and then into fungal and actinobacterial PLFAs.

^{13}C in necromass, SOC, and efficiency of SOC formation during leaf litter decomposition

High-quality litter addition accelerated microbial turnover (35 vs. 24 days turnover time of *P. davidiana* vs. *Q. wutaishanica* litter) (Fig. 2), which leads to a higher necromass

formation than that of lower-quality litter (Fig. 3). The ^{13}C in total necromass and SOC increased with progressing *P. davidiana* litter decomposition but reached a maximum at 14 days after *Q. wutaishanica* litter addition ($P < 0.05$). The ratio of ^{13}C -fungal to bacterial necromass increased before day 14 and then leveled off until the end of the *Q. wutaishanica* litter decomposition process. However, it increased before day 56 and remained stable until the end of the *P. davidiana* litter decomposition process.

A dissolved organic matter (DOM)-microbial path leads to the SOC formation because a continuous increase in SOC formation efficiency (the ratio of SOC gain to litter C loss) was observed throughout the *P. davidiana* litter decomposition process. The dynamic of SOC

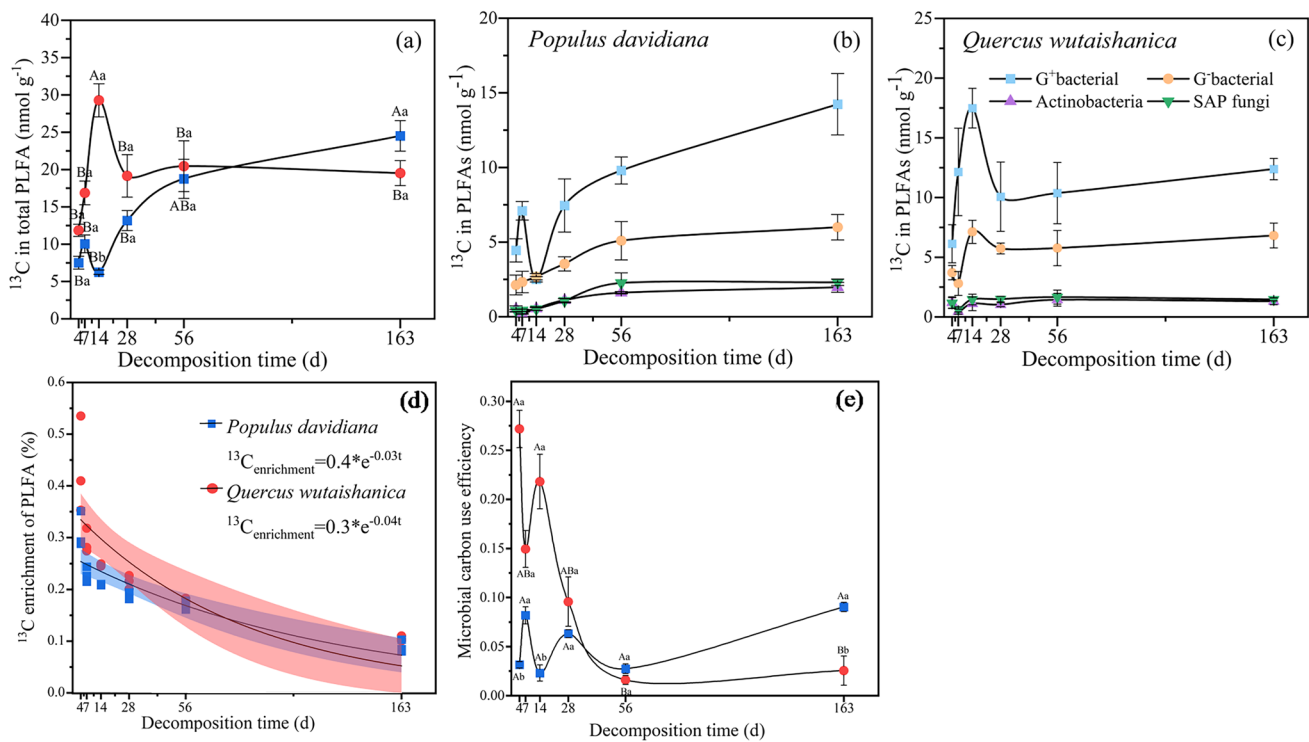


Fig. 2 Dynamic of ^{13}C amount in PLFAs, soil $^{13}\text{C}_{\text{enrichment}}$ in PLFAs, and soil microbial C use efficiency (CUE) during litter decomposition. Note: Figures represent **a** ^{13}C amount in total PLFAs; **b** ^{13}C amount in total bacterial, actinobacteria, and fungal PLFAs during *Populus davidiana* litter decomposition; **c** ^{13}C amount in total bacterial, actinobacteria, and fungal PLFAs during *Quercus wutaishanica* litter decomposition; **d** soil $^{13}\text{C}_{\text{enrichment}}$ in PLFAs during *Populus davidiana* and *Quercus wutaishanica* litter decomposition; **e** soil microbial CUE during *Populus davidiana* and *Quercus wutaishanica* litter decomposition and colors indicate the two litter of contrast-

ing quality; **f** shifts in ^{13}C in PLFA distribution pattern during litter decomposition as determined by fatty acid by principal component analysis (PCA). Values are the averages (\pm SE) of nine replicates. Uppercase letters (A, B, and C) indicate the significant difference ($p < 0.05$) among sampling times for the same species. Lowercase letters (a, b, and c) indicate the significant difference ($p < 0.05$) between two species at the sampling time. The P values from two-way ANOVA (litter types, decomposition times, and their interaction) are shown

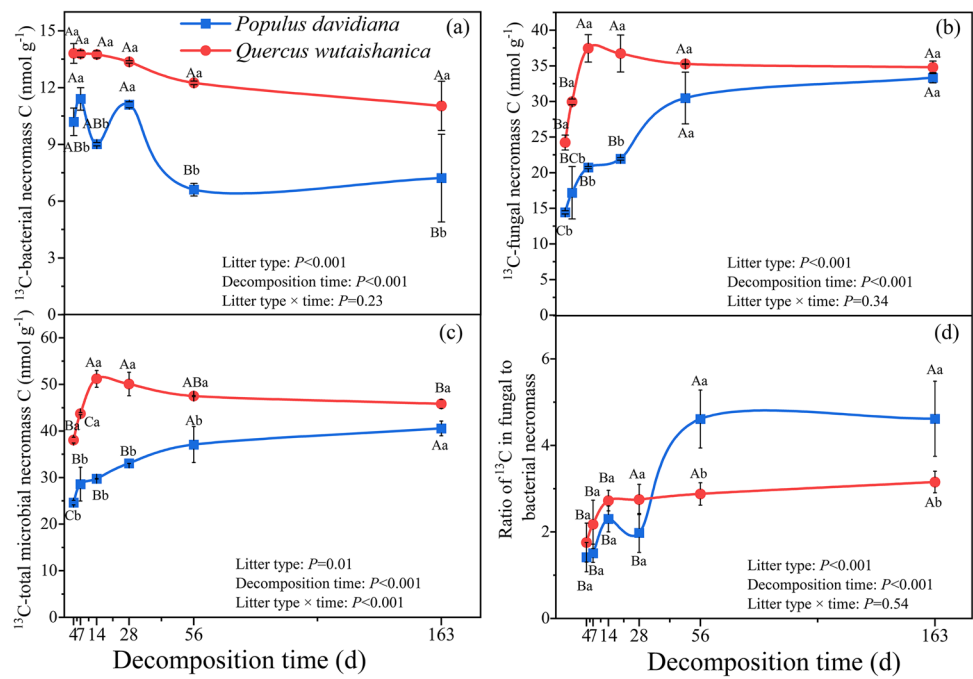
formation efficiency was consistent with litter-derived C incorporation into PLFAs and necromass and the ratio of ^{13}C in SOC to dissolved organic C (DOC) increased in the whole *P. davidiana* litter decomposition process (Figs. 4 and 5). As early as 14 days after *Q. wutaishanica* litter addition, the efficiency of SOC formation increased, which was consistent with the dynamics of ^{13}C in PLFAs and necromass. We observed increased SOC formation efficiency; however, the *Q. wutaishanica* litter-derived ^{13}C did not incorporate into PLFA from days 56 to 163, suggesting a pathway of SOC formation not mediated by soil microorganisms.

Key drivers and possible pathways of soil C accumulation

PLS-PM analyses identified potential pathways of SOC formation based on the possible contributions of living

microbial groups; selected enzyme activities involved in few reactions of C, N, and P transformation; microbial CUE; and litter-derived C in necromass and SOC (Fig. 6). SOC formation could be well predicted by the remaining litter mass and litter C:N ratio, regardless of litter quality. The decrease of *P. davidiana* litter remaining mass and litter C:N ratio induced enhanced XYL and BG activities and led to an increase in CUE after *P. davidiana* litter addition. The increase in enzyme activity further increased CUE, ultimately affecting ^{13}C in necromass formation and subsequently contributing to SOC stabilization. The remaining litter mass and C:N ratio consistently negatively affected SOC formation after *Q. wutaishanica* litter application. The increases of BG and CELLO activities lead to higher CUE and mainly the bacterial ^{13}C incorporation of litter-derived C, and affected necromass and SOC contribution.

Fig. 3 ^{13}C in soil bacterial necromass (a), fungal necromass (b), and total necromass (c), and the ratio of ^{13}C in fungal necromass to bacterial necromass during litter decomposition. Note: Values are the averages (\pm SE) of nine replicates. Uppercase letters (A, B, and C) indicate the significant difference ($p < 0.05$) among sampling times for the same species. Lowercase letters (a, b, and c) indicate the significant difference ($p < 0.05$) between two species at the sampling time. The P values from two-way ANOVA (litter types, decomposition times, and their interaction) are shown



Discussion

High-quality litter formed more necromass and SOC than low-quality litter

This study examined how litter C flows through main microbial groups into the soil food web, then forming microbial necromass, a key pool of SOC. We used litter with distinct C:N ratios and aimed to assess how the selected enzyme activities involved in some reactions of C, N, and P transformation and CUE regulate the process of SOC formation from litter C. Several studies claim that

high-quality litter addition induced higher necromass and downstream SOC formation than adding low-quality litter (Olagoke et al. 2022; Cai and Feng 2023). Results from Lyu et al (2023), however, state the opposite, i.e., that low-quality litter forms two times higher new SOC than high-quality litter. In our incubation study, soil microbes built biomass more efficiently with low C:N substrates than with high C:N because the turnover time of ^{13}C in PLFAs was longer with *P. davidiana* litter than with *Q. wutaishanica* (35 vs. 24 days), and thus, substrate quality directly affected the efficiency of growth and death of microorganisms. The ^{13}C in total necromass and SOC

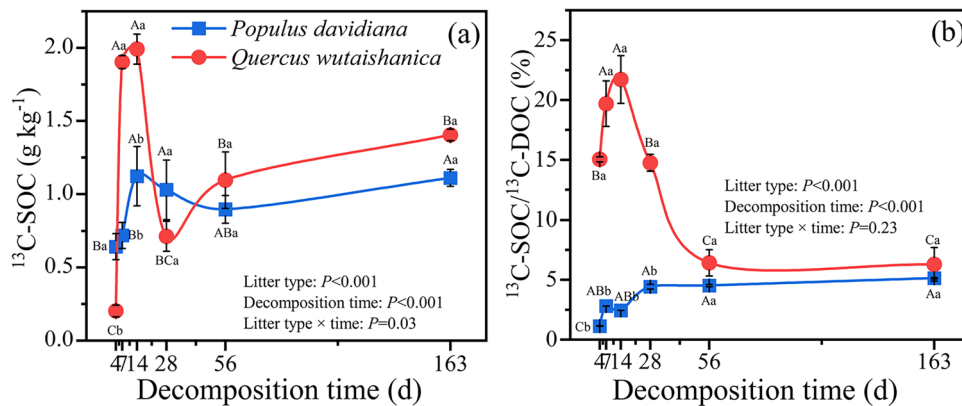
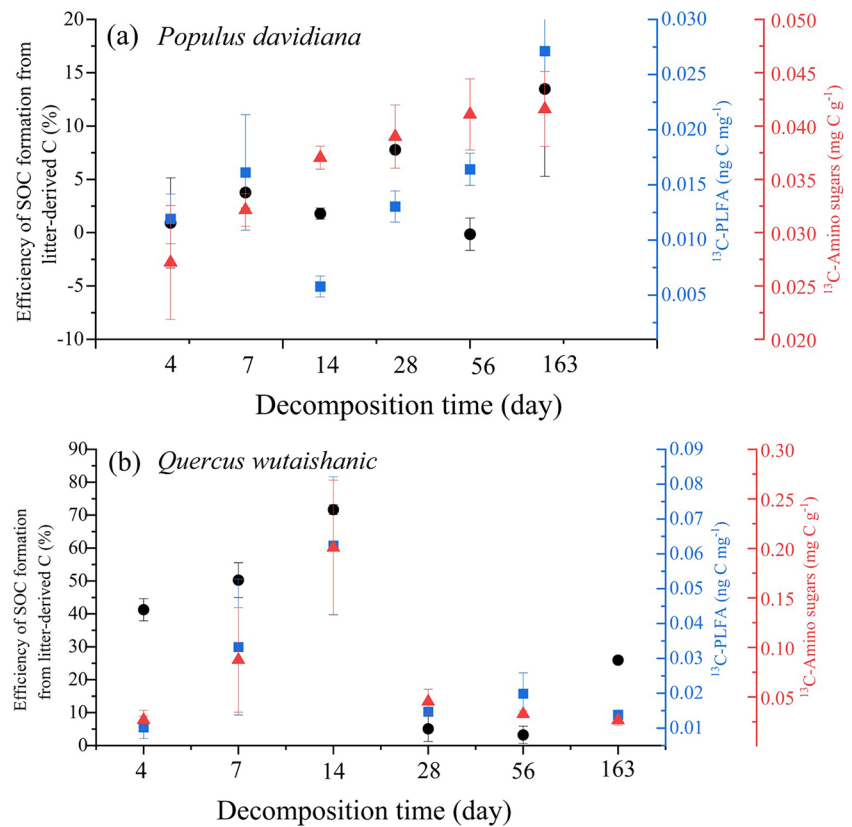


Fig. 4 Soil organic ^{13}C (a) and the ratio of ^{13}C in soil organic C to dissolved organic C (b) during litter decomposition. Note: Values are the averages (\pm SE) of nine replicates. Uppercase letters (A, B, and C) indicate the significant difference ($p < 0.05$) among sampling

times for the same species. Lowercase letters (a, b, and c) indicate the significant difference ($p < 0.05$) between two species at the sampling time. The P values from two-way ANOVA (litter types, decomposition times, and their interaction) are shown

Fig. 5 The efficiency of SOC formation and ^{13}C in soil microorganisms during litter decomposition. Note: SOC formation efficiencies (filled circle, left y-axis) are calculated as the percentage of SOC gain/litter C loss. Data are means with 95% confidence intervals. The blue squares are relative active microbial incorporation of litter-derived C, which is calculated as litter-derived PLFAs-C/litter-derived SOC (right y-axis). The red triangles are relative microbial necromass incorporation of litter-derived C, which is calculated as litter-derived amino sugars-C/litter-derived SOC (right y-axis)



increased with *P. davidiana* decomposition and the differences of ^{13}C -necromass between these two litter types was smallest 163 days after litter addition. There was far less available C in soils 14 days after *Q. wutaishanica* litter addition, based on the sharp increase in the proportion of soil alkyl C and aromatic C and activity of phenol oxidase and peroxidase (Figs. S4 and S5), potentially resulting in decreased necromass. Likely soil microorganisms decomposed either compounds of parts of their own biomass (i.e., cells get smaller) or necromass to maintain their energy metabolism during the decomposition of energetically unfavorable substances and consequently they have triggered SOC loss (Chen et al. 2022; Cai and Feng 2023). On the other hand, actinobacteria, which were present in greater abundance in the late decomposition stage of *P. davidiana* than in *Q. wutaishanica*, were likely the major contributors of the bacterial microbiome (Goodfellow and Williams 1983; Apostel et al. 2018). Furthermore, the decomposition process of *P. davidiana* was dominated by slow-growing microorganisms characterized by lower K_m values (i.e., the substrate concentration at half-maximal rate giving the enzyme affinity to the substrate) for all six soil enzymes than those of *Q. wutaishanica* (Table S1). The slow but steady C use results in a higher CUE than that observed for fast-growing taxa and likely this can increase

necromass accumulation (Deng and Liang 2021). This may confirm the greater ability of the microorganisms involved in *P. davidiana* litter decomposition to degrade recalcitrant components (Li et al. 2021). Thus, although temporal dynamics influence the efficiency of microbial C formation, a slow and steady decomposition process can lead to considerable accumulation of necromass C in soils with litter of low quality (Cai and Feng 2023). In the case of high-quality litter, SOC mainly originated from the utilization of easy-to-decompose substances during decomposition by bacteria in the early stages of litter degradation. In contrast, for low-quality litter, the SOC was mainly formed during later stages of the decomposition through the utilization of recalcitrant components by fungi.

Although bacteria reacted quickly to litter addition and accumulated ^{13}C in their PLFAs in a short time, this did not cause higher necromass accumulation than fungi, as supported by the observation that ^{13}C incorporation into bacterial necromass decreased, whereas fungal necromass increased during litter decomposition. This is likely due to the relatively low stability of peptidoglycan and its monomer MurA in soils, which has a faster turnover than chitin-derived GlcN (Liang and Balsler 2008) due to the less-easily degradable chitin (Paul and Clark 1989). The ratio of litter-derived ^{13}C in fungal necromass to ^{13}C in

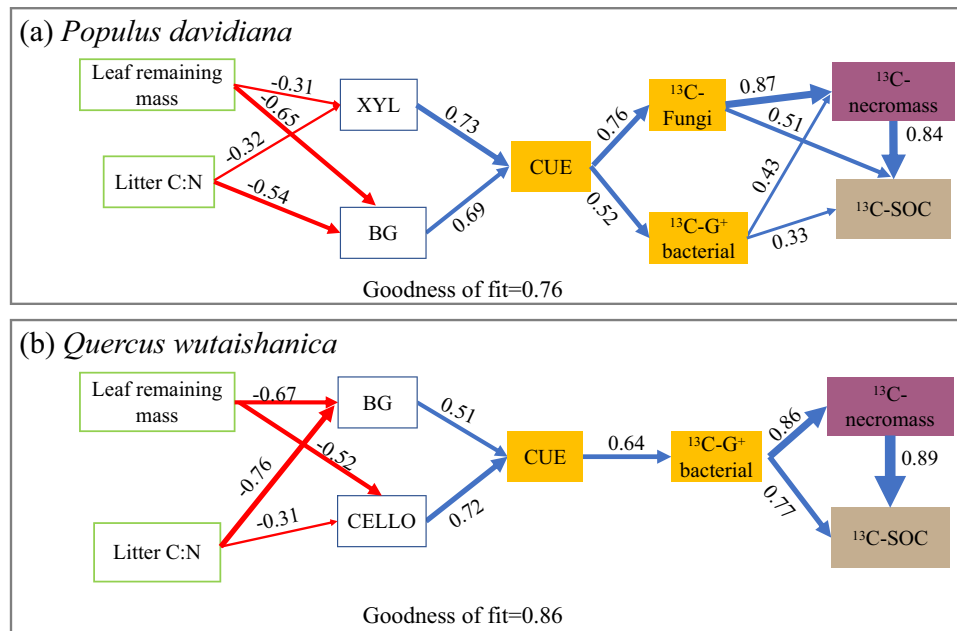


Fig. 6 Partial least squares path modeling (PLS-PM) disentangling major pathways of the SOC formation that influenced by soil microbial community, the selected enzyme activities, and microbial necromass during litter decomposition. Note: the VIF (variance inflation factor) for all variables in the model was smaller than 10 to remove the collinearity of a factor within the module. Loading in the outer models was chosen to be bigger than 0.7 to remove independent variables making small contributions. ^{13}C -fungi, litter-derived organic

C in fungi PLFAs; ^{13}C -G⁺ bacterial, litter-derived organic C in G⁺ bacterial PLFAs; ^{13}C -G⁻ bacterial, litter-derived organic C in G⁻ bacterial PLFAs; XYL, activity of β -1,4-xylosidase; CELLO: activity of cellobiase; BG, activity of β -1,4-glucosidase; CUE, soil microbial C use efficiency; ^{13}C -Amino sugars, litter-derived C in amino sugars; ^{13}C -SOC, litter-derived organic C in soil. Blue and red arrows indicate positive and negative flows of causality, respectively. The dashed gray line represents no relationship between the two variables

bacterial necromass first increased and then leveled off until the end of the decomposition process. This conversion process was faster in soils exposed to high-quality litter than under low-quality litter. Likely litter C was converted first to bacterial biomass in soils, then transformed to fungal biomass (probably following the death of bacterial cells and the necromass consumption) and finally stabilized as fungal-derived necromass C, which is more persistent in soil than the bacterial-derived one (Zheng et al. 2023).

Main microbial groups and enzyme activities during litter decomposition

For both litter types, G⁺ and G⁻ bacteria were mainly involved in the consumption of released litter C during the early decomposition process, and thus they prevailed over fungi and actinobacteria, confirming results from other studies regardless of the litter type (Wang et al. 2014; Pausch et al. 2016). The early decomposition of litter is characterized by the release of soluble C and N (Fanina et al. 2020), a hotspot with high growth and activity of bacteria (Nunan et al. 2018; Domeignoz-Horta et al. 2021). Fungi and actinobacteria can degrade highly polymerized compounds during the late litter decomposition

process because of their versatile ability to produce extracellular enzymes (Bonanomi et al. 2019; Pereira et al. 2019).

The microbial response to *P. davidiana* litter decomposition was slower than that following *Q. wutaishanica* addition. The activity of BG, XYL, and CELLO increased smoothly but continuously during *P. davidiana* litter decomposition, whereas it increased rapidly during the early stage of *Q. wutaishanica* litter decomposition. This pattern demonstrated that high litter N availability fosters the rapid decomposition of complex C compounds at early litter decomposition stages (Lyu et al. 2023). In parallel, soil microorganisms may activate a wide range of extracellular enzymes, such as chitinases and proteases, providing access to litter N, covering their C and N demands (Trivedi et al. 2016). This initial peak in *Q. wutaishanica* litter C and N decomposition was considerably slowed down in the second phase (≥ 28 days), where the production of the measured enzyme activities was reduced by $> 50\%$ of the previous activities. In contrast to these clear two-phase decomposition dynamics, soil microorganisms initially responded more slowly to *P. davidiana* litter application, demonstrating that litter with higher C:N values (Table 2) restricted microbial growth and induced a temporal delay in the decomposition of all litter constituents. Thus, litter quality is the key to the microbe's energy demand to break

Table 2 Variations of litter carbon, nitrogen, and phosphorus and their ecological stoichiometry during litter decomposition

Decomposition time (days)	<i>Populus davidiana</i>						<i>Quercus wutaishanica</i>						
	Leaf C content (g kg ⁻¹)	Leaf N content (g kg ⁻¹)	Leaf P content (g kg ⁻¹)	C:N	C:P	N:P	Leaf C content (g kg ⁻¹)	Leaf N content (g kg ⁻¹)	Leaf P content (g kg ⁻¹)	C:N	C:P	N:P	Mass loss rate (%)
4	517±14	14±0.3	1.5±0.1	35±2.4	343±32	9.6±0.8	449±39	17±0.6	2.4±0.3	26±3.6	191±40	7.2±0.9	2.2±0.1
7	512±35	15±0.4	1.7±0.1	36±3.1	304±31	8.7±0.7	431±21	16±1.3	2.2±0.3	27±1.6	200±26	7.3±0.6	6.2±0.7
14	449±36	12±2.0	1.6±0.3	37±1.9	273±28	7.4±0.5	427±22	16±0.5	2.0±0.2	26±1.6	219±31	8.3±0.8	13±1.3
28	482±51	15±0.8	1.7±0.1	33±1.8	277±29	8.5±0.7	416±17	18±0.2	2.0±0.2	24±0.7	212±28	8.9±1.2	21±2.4
56	475±66	15±0.9	1.8±0.1	31±0.9	270±32	8.6±0.8	465±34	19±0.2	1.9±0.2	24±1.6	247±31	10.2±1.1	27±3.2
163	524±26	18±2.6	1.7±0.3	29±0.7	302±21	10±1.2	375±21	19±1.3	1.8±0.1	20±1.4	212±8.3	10.5±0.8	41±4.2
<i>P</i> value													
Litter type	0.001	0.03	0.002	0.001	0.003	0.02	0.001	0.03	0.002	0.001	0.003	0.02	0.001
Sampling time	0.008	0.02	0.07	0.007	0.01	0.006	0.004	0.07	0.04	0.04	0.03	0.008	<0.001
Litter type × sampling time	0.06	0.21	0.02	0.001	0.32	0.48	0.06	0.21	0.02	0.001	0.32	0.48	0.32

down the complex litter polymers and by that the kinetics of monomer release contributing to the DOC pool. This rate of labile C release is the essential factor defining the microbial use and main microbial groups involved in and profiting from the litter degradation.

Pathways of C allocation through the microbial food web to form SOC during litter decomposition

The litter quality of *Q. wutaishanica* was higher than that of *P. davidiana*, triggering enhanced litter-derived C incorporation into the microbial biomass and subsequently necromass, especially during the early and intermediate stages of decomposition, when enzyme activity and microbial abundance peaked. This suggests a highly active “microbial C pump” and an enhanced flux through the soil C cycle into the SOC pool. The efficiency of this “microbial C pump” was largely determined by the quality, availability, and remaining mass of the litter, which were the main factors (Fig. 6) driving the pathway through the microbial C to SOC (Joergensen 2018). Microbial CUE was the primary factor affecting the litter-derived C transfer into microbial biomass and subsequent SOC formation during *P. davidiana* and *Q. wutaishanica* decomposition. CUE is related to litter-derived C incorporation into PLFAs. By that, it further determines necromass accumulation and SOC storage after cell death. CUE has been already identified as the most important factor in the contribution to SOC storage (Wang et al. 2021; Tao et al. 2023). The microbial CUE of soils with *P. davidiana* litter was lower than that with *Q. wutaishanica* until 28 days after litter addition but it was higher than that with *Q. wutaishanica* from day 28 to 163. A higher CUE promotes faster microbial growth (Tao et al. 2023), and more C sequestration, as highlighted in this study, underlining that the difference in the temporal dynamics in SOC accumulation depends on litter.

During the first 14 days of *Q. wutaishanica* litter incubation and throughout the 163 days of *P. davidiana* decomposition, an increase in SOC formation efficiency was evident (Fig. 5), which coincides with the observed C flux through PLFAs and amino sugars. One exception is the last sampling time of *Q. wutaishanica* decomposition, when the efficiency of SOC formation increased despite the ¹³C in PLFAs and amino sugars decreased. Likely chemically recalcitrant organic matter was stabilized at this late stage of litter decomposition by physical mechanisms and may have contributed significantly to the formation of SOC. Therefore, these mechanisms occurred when the initial rapid degradation structurally disintegrated the litter and litter particles most probably contributed to particulate organic matter with the low microbial activity (Cotrufo et al. 2015).

Conclusions

We demonstrated that when exposed to litter of contrasting quality, specific soil microbial groups produced different soil enzymes, that degraded the litter with a quality-dependent temporal dynamic. This defined the release of labile C compound from the litter, the major factor determining microbial CUE and biomass formation, which subsequently formed microbial necromass and SOC. This distinct microbial C channeling led to substantial differences in the fate of C from litter of contrasting qualities. In contrast to a clear peak with a rapid early phase of high-quality litter decomposition, we observed a continuous increase in the enzyme activities involved in the selected reactions of soil organic C transformations for low-quality litter decomposition. The dominant microbial groups in *P. davidiana* and *Q. wutaishanica* litter decomposition were consistent, but their temporal dynamics and biomass production differed significantly. Microbial necromass formation followed the pattern of litter-derived C incorporation into PLFAs, suggesting that the cell uptake, biosynthesis, growth, death of soil microbial groups, and necromass formation were correctly matching the concept of the microbial C pump. The temporal dynamics of SOC formation efficiency were very similar to the pattern observed for C allocation through microbial biomass and necromass. A physical transfer path, i.e., not depending on biochemical reactions, dominated when the easily available compounds were largely exhausted. This could occur by particulate transport of small litter pieces predominated by recalcitrant compounds to mineral surfaces, where litter pieces were stabilized. Subsequently, the SOC formation occurred by physical transfer of the litter residues during the last stage of *Q. wutaishanica* decomposition, which was characterized by the absence of almost any litter-derived ^{13}C incorporation into PLFAs. However, high-quality litter decomposition induced higher ^{13}C in PLFAs and CUE, resulted in higher SOC formation as long as sufficient amounts of easily degradable compounds remain in the litter than low-quality litter.

By this study, we provide detailed insight into the flux of ^{13}C through different microbial groups to necromass and SOC during degradation of labeled litters with different qualities. Further studies with higher temporal resolution are needed for a more detailed analysis of the succession of microbial species (i.e., using the DNA stable isotope probe technique) to reconstruct C pathways through the microbiome more precisely. Long-term (years to decades) in situ decomposition experiments with high- and low-quality litter addition are also needed to explore SOC turnover governed by soil microorganisms.

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Data availability The data will be available on request.

Declarations

Conflict of interest The authors declare no competing interests.

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