Environmental Science Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

Published on 07 July 2026
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Cite this: *Environ. Sci.: Adv.*, 2026, **5**, 2051

Sustained microbial reorganization after 30 years of variations in litter deposition underpins the persistence of soil organic matter molecular classes

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Forest soils store vast amounts of carbon (C), but the stability in part depends on the quality and quantity of above- and belowground C litter inputs, which are impacted by management decisions and global environmental change. We assessed soil organic matter (SOM) composition in forest floor and upper mineral soil (0–10 cm) after three decades of altering litter sources in a temperate deciduous forest at the Bousson Experimental Forest in Pennsylvania where treatments included leaf and/or root exclusion or doubled litter inputs. Elemental analysis, targeted compound analysis, solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, radiocarbon analyses, and measurements of microbial biomass and community composition were employed to characterize soil. Within the top 10 cm of mineral soil, C concentrations did not increase under the Double Litter treatment after 30 years of added litter. Instead elevated microbial biomass (+29%) supported by the availability of fresh substrates, indicated by radiocarbon signatures, suggest that microbes sustained decomposition of SOM and limited C accumulation in the upper mineral soil. The exclusion treatments (No Litter, No Roots, and No Inputs) also exhibited lower C concentrations in the upper mineral soil, with NMR analyses revealing advanced stages of SOM decomposition. Microbial community composition also shifted under exclusion treatments, with radiocarbon data suggesting greater reliance on older C pools when fresh inputs were limited. This indicates a microbial response to reduced preferred substrates, whereby community reorganization supports metabolic maintenance. Comparisons between 20 and 30 years of treatment demonstrate that microbial and biogeochemical responses have continued to shift over time with altered litter and root inputs. Together, our results underscore the dynamic interplay between microbial communities and SOM persistence under changing environmental conditions.

Received 7th October 2025
Accepted 26th June 2026

DOI: 10.1039/d5va00350d

rsc.li/esadvances

Environmental significance

Forest soils play a central role in global carbon (C) cycling, yet their capacity to store additional C under changing environmental conditions remains uncertain. Results from this 30 year detrital manipulation experiment show that even with continuous litter additions, microbial communities reorganized to sustain decomposition rather than promote long-term soil C gains in the top 0–10 cm of mineral soil. Conversely, when litter and root inputs were excluded, microbes relied on older C sources, further accelerating soil organic matter (SOM) degradation. These findings demonstrate that shifts in microbial community composition can counteract potential C accrual from increased litter inputs and underscore the challenge of managing forests to enhance durable soil C sequestration. Maintaining existing soil C stocks may therefore be a more reliable climate mitigation strategy than pursuing additional accumulation in temperate forest soils through greater C inputs.

1 Introduction

Soil organic matter (SOM) is derived from decomposing plant and microbial materials and contains more carbon (C) than the atmosphere and terrestrial vegetation combined. Forest ecosystems alone store as much as 40% of the global soil C.[1,2] Increasing C storage in forest soils has been suggested as a potential approach for sequestering atmospheric CO_2 and

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mitigating climate change.[3–5] Furthermore, observations revealed that more than half of the organic C in soil is chemically or physically associated with soil minerals[6,7] and thus increasing the amount of C in mineral soil may be instrumental to longer lasting C sequestration in soils.[8] However, soils also have a finite capacity to store C, and it is unclear whether the proximity to its C-storage limit for a given soil will impact the efficacy of soil C sequestration efforts.[9,10] Importantly, soil C is dynamic, in addition to being stabilized, SOM may follow other pathways as it is processed within the soil, such as being leached into surface waters as dissolved organic matter, transformed by soil fauna, or mineralized by soil microbes and returned to the atmosphere as CO_2.[11] Furthermore, the long-term fate of SOM accumulation and stabilization is hard to predict,[12–14] especially when global environmental changes are altering the net primary productivity of forests, which subsequently modifies the quality and quantity of plant inputs.[15] In addition to controlling litter decomposition and SOM formation processes, the quantity and source (above-*versus* belowground) of plant inputs also regulates microbial activity and the processing of plant-derived SOM.[16–19]

Microbes are the primary decomposers in soil systems, and are integral for stabilizing plant-derived SOM, decomposition of SOM, and soil respiration.[11,20] However, soil microbes are generally constrained by the availability of C and nutrients.[21] Not only do changes in litter availability (quantity) impact the abundance of preferred microbial substrates, but can also result in soil microbes altering their C use efficiency based on substrate accessibility.[21,22] Substrate chemistry may also drive C use efficiency indirectly by favoring microbes that are able to use the available resources most efficiently, leading to shifts in microbial community composition.[23,24] For example, in many observations from experiments in temperate forests from the Detrital Input and Removal Treatment (DIRT) network, the addition of extra litter inputs prompted the accelerated microbial decomposition of SOM, and resulted in decreases in soil C, even after 20 years of doubled litter additions.[25–27] These results contrast many models of ecosystem C balance[28,29] which predict a strong relationship between litter inputs and soil C accumulation, and results from other ecosystems, which observed increased soil C with elevated litter inputs.[30,31] These varying results demonstrate that the relationship between plant inputs, microbial processing and soil C stabilization are not directly linear, nor are they easy to predict. Therefore, long-term, molecular-level studies within diverse ecosystems may help elucidate how changes in litter deposition may alter microbial community responses and concomitant changes to SOM processing and stabilization.

DIRT is an international experimental network that conducts long-term litter manipulation experiments that are fundamental for understanding the roles that the quantity and quality of C inputs have on SOM chemistry in forests over time. Although one of the main motivations is identifying factors that contribute to long-term soil C sequestration in forests, important findings from other litter manipulation experiments have demonstrated that the removal of litter, roots or both have significantly decreased the organic C in soil pools in forests, suggesting that maintaining soil C requires the continuous input of exogenous C from fresh litter and/or roots.[32,33] Furthermore, without exogeneous C inputs from plants, there will be a continuous loss of soil C due to the microbial decomposition of SOM, especially in the long term.[31,34] Many observations from previous experiments within the DIRT network have shown that soil C has not increased in temperate or coniferous forest soils, even after 20 years of doubled litter inputs,[26,27,35,36] which was attributed to enhanced microbial decomposition of SOM prompted by the additional litter inputs. A previous meta-analysis[31] of detrital manipulation experiments in global forests suggested that the litter input quantity and experiment duration regulate the response of soil organic C pools to detrital treatments. Namely, the magnitude of soil organic C responses to litter alterations and no inputs became stronger over time. This is especially pertinent as the Bousson DIRT experiment[26,27,33] started in 1991, therefore making it one of the longest-running DIRT experiments, and a molecular-level study after 30 years of detrital manipulation could greatly improve our understanding of SOM persistence over time under changing environmental conditions.

Here, a suite of integrative analytical approaches was used to investigate the molecular-level changes to the SOM after 30 years of detrital manipulation at the Bousson Forest. Based on previous DIRT studies that were conducted at Bousson,[27] Harvard,[35] and H. J. Andrews Forests[36] after 20 years of treatment, we hypothesized that sustained microbial-induced decomposition with added litter over three decades would limit new soil C gains in this temperate forest. Additionally, we predicted that further declines in soil C and its molecular-level biogeochemical composition will continue to augment with time for exclusion treatments, through sustained microbial reorganization and use of altered substrates. To test these hypotheses, we used both targeted and non-targeted molecular-level approaches to respectively assess the composition of specific SOM components and the overall chemistry. These approaches included characterization by elemental analysis, targeted SOM compound measurements, nuclear magnetic resonance (NMR) spectroscopy, radiocarbon profiling and microbial biomass and community composition measurements. Ultimately, the objectives of this study were to identify the extent to which long-term litter manipulation alters SOM degradation and chemistry, providing insights into the long-term stability of forest soil C under changing plant input conditions.

2 Methods

2.1 Experimental site description and sample collection

The Bousson Experimental Forest, PA, USA (41°36N′, 80°3′W) is a temperate deciduous mixed hardwood forest dominated by black cherry (*Prunus serotina*) and sugar maple (*Acer saccharum*)[37] with a mean litter input rate of 249 ± 4 g C m^{-2} per years.[33] The mean daily temperature (1981–2010) was −4.4 °C in January and 20.8 °C in July.[38] Precipitation is seasonal, with a mean average precipitation of 1125 mm per year[39] The Bousson Forest is ∼100 years old and prior to this, was likely used as pasture. The soils are Cambridge series Alfisols, with a loamy sand texture comprised of 75% sand, 23% silt, and 2% clay on a 1% east-facing slope and are derived from glacial till overlying

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shale and sandstone. The DIRT experiment was started in 1991 and includes five treatments: Control, No Litter (NL), No Roots (NR), No Inputs (NI), and Double Litter (DL). The Control plots receive ambient above- (leaves, twigs, seeds, flowering parts and woody inputs (<1 cm diameter)) and belowground (roots and root exudates) inputs. Aboveground inputs were excluded from the NL and NI plots using a plastic mesh fabric. Belowground inputs were excluded from the NR and NI plots by severing roots with 1.4 m deep trenches, which were then lined with plastic barriers and refilled. Initial barriers were installed in 1991, and a second barrier was installed in 2006 to prevent root intrusion. For DL plots, litter was collected from the NL or NI plots and was transferred to DL plots within the same slope position such that there was twice the annual input of aboveground litter. There are three replicate plots per treatment, each 3 m by 3 m in size and are located under an intact forest canopy within a relatively uniform area (0.27 ha). No trees or saplings were included in the treatment plots and any seedlings or herbaceous materials were routinely removed. The plots are distributed in a stratified random design, with one plot placed in each of the upper, middle, and lower portions of the site for each of the treatments.

Soil samples were collected in August 2021, after 30 years of detrital manipulation. Soil samples were collected for the Control, DL and NR treatment plots from the O horizon (noted henceforth as "forest floor") by hand-removing 15 cm × 15 cm samples (NL and NI plots no longer have O horizons, due to the intrinsic nature of the treatment). The forest floor layer is clearly defined from the mineral layer in these plots. A gas-powered 9.62 cm diameter diamond-bit stainless steel soil corer was used to collect mineral soil samples from the top 0–10 cm of the soil profile for each of the plots. Three sampling locations were randomly selected within each plot and were pooled to create a single sample per plot for each soil depth. Forest floor samples were passed through a 5.6 mm sieve and sorted into roots, rocks, other organic matter, and soil.[40] Mineral soil samples were also passed through a 2 mm sieve and sorted. Bulk density for the mineral soil, as reported by Bowden *et al.*,[33] was not significantly different except for the NR and NL treatments. All soil samples were stored at −20 °C, then were freeze-dried, and finely ground, prior to SOM analyses.

2.2 Soil carbon (C) and nitrogen (N) concentration and content

A Thermo Flash 2000 Elemental Analyzer was used to measure soil C and N concentrations by combusting freeze-dried forest floor and upper mineral soil samples at 950 °C under a stream of oxygen gas. Duplicate analytical C and N measurements were made for each sample plot and averaged for each treatment. Soil C and N concentrations are reported as a percent based on the soil mass. To estimate soil C mass, we multiplied soil C concentrations by soil mass, wherein we used a site-wide estimate of mineral soil mass for each depth increment using all soil mass measurements across the 15 plots.[33] For forest floor soil C mass, we calculated the average forest floor mass separately for each of the DL, C, and NR treatments.

2.3 Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy

The overall SOM composition was characterized using solid-state ^{13}C cross polarization with magic angle spinning (CP-MAS) NMR spectroscopy. Due to the intensive effort required for analyses, soils from the three replicate plots per treatment were composited, by soil depth, which is consistent with the approach used in a previous study of this site.[27] To enhance the spectral quality of the mineral soil samples, 10% hydrofluoric acid (HF) was used to repeatedly treat the samples,[41,42] which concentrates the organic matter, and reduces the minerals and paramagnetic materials present in the samples that interfere with data acquisition. After pre-treating the samples with HF, the mineral soil samples were repeatedly rinsed with deionized water to remove excess salts, freeze-dried and then ground into a fine powder. Approximately 250 mg of sample was packed into a 4 mm zirconium rotor with a Kel-F cap. A 500 MHz Bruker BioSpin Advance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) with a 4 mm H-X MAS probe was used to acquire the spectra. A magic angle spinning rate of 11 k Hz, with a 1 ms ramp-CP contact time and a 1 s recycle delay were used to collect 21 k to 51 k scans, depending on the treatment.

TopSpin (version 4.1.3) was used to process all spectra with 100 Hz line broadening and manual baseline correction. Spectra were integrated into four chemical shift regions typically used for SOM: alkyl C (0–50 ppm); *O*-alkyl C (50–110 ppm); aromatic and phenolic C (110–165 ppm) and; carboxyl and carbonyl C (165–220 ppm).[43,44] Alkyl C components typically include polymethylene chains and terminal methyl groups of cutin, suberin side chains, and other aliphatic compounds.[43,44] *O*-alkyl C corresponds to structural groups found in carbohydrates, peptides, and methoxyl C in lignin resonate in the *O*-alkyl C region, while lignin, and aromatic amino acids found in peptides resonate in the aromatic and phenolic C region.[43,44] Resonances in the carboxyl and carbonyl C region are mainly from the fatty acid and amino acid side chains.[43,44] After integrating each chemical shift region, the signal was expressed as a percentage by normalizing each region to the total area to allow for the comparison of the relative amounts of each resonance region. The SOM decomposition state was assessed by comparing the proportion of alkyl to *O*-alkyl C (alkyl/*O*-alkyl C ratio).[41,43,45,46] Related, the SOM resistance to decomposition, which is the ratio of the (alkyl + aromatic and phenolic)/(*O*-alkyl + carboxyl and carbonyl) C, was used as an indication for the accumulation of more persistent forms of SOM with the higher use of preferred substrates.[47]

2.4 Targeted soil organic matter (SOM) extractions and analyses

Various plant- and microbial-derived compounds were extracted from the soil samples using an established three-step targeted compound sequential extraction.[48,49] Each sample was extracted in duplicate and complete details are provided in the Supplementary Information. Briefly, small molecules in soil were isolated *via* solvent extraction using 1 g of forest floor sample or 2 g of mineral soil and dichloromethane, then 1 : 1

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methanol : dichloromethane (v/v) followed by methanol.[48] The solvent-extracted soil residue (~0.5 g and 1 g for the forest floor and mineral soil samples respectively) was then extracted with 1 M methanolic potassium hydroxide in a Teflon-lined bomb and then heated at 100 °C for 3 hours for the alkaline hydrolysis of ester-bound lipids derived from cutin, suberin and microbial sources.[49,50] About half of the base hydrolysis residues (~0.3 g and 0.5 g for the forest floor and mineral soil samples respectively) were then oxidized to release lignin-derived phenol monomers and dimers in the last step of the sequential extraction. Copper(II) oxide, ammonium iron(II) sulfate hexahydrate and 2 M sodium hydroxide were added to the soil residues in Teflon-lined bombs and heated for 2.5 hours at 170 °C.[51,52] The lignin-derived phenols that were released through copper oxidation were then isolated and purified by solid phase extraction.[53] The extracts from each step of the sequential extraction were dried with N_2 prior to derivatization and analysis by gas chromatography-mass spectrometry (GC-MS).

Microbial biomass and community composition was measured *via* phospholipid fatty acids (PLFAs), which were extracted from the soil samples using a modified Bligh-Dyer method[54] with methanol, chloroform, and sodium citrate buffer (acidified to pH = 4). A silicic acid chromatography column was used to collect and fractionate the chloroform phase extracts into nonpolar lipids, glycolipids, and polar lipids by eluting with chloroform, acetone, and methanol respectively. The PLFAs in the methanol fraction were converted into fatty acid methyl esters using a mild alkaline methanolysis prior to analysis by GC-MS.

All targeted SOM compounds were quantified using GC-MS analysis (details in Supplementary Information). Each of the targeted SOM extracts and the external standards were derivatized to increase the volatility and thermal stability of the compounds prior to analysis by GC-MS.[55,56] The solvent extracts and CuO extracts were derivatized with *N*,*O*-bistrifluoroacetamide and pyridine, while the base hydrolysed extracts were methylated first using *N*,*N*-dimethylformamide dimethyl acetal before being derivatized in the same manner. Following derivatization, an Agilent 7890B gas chromatograph equipped with a 5977B mass spectrometer was used for compound identification and quantification. The ion source was operated in electron impact (70 eV) mode and all data was acquired using Agilent Mass Hunter GC-MS Acquisition software (version B.07.03.2129). Data processing for solvent extraction, base hydrolysis and PLFA data was conducted using the Agilent Enhanced ChemStation software (version F.01.03.2357) and identification of the compounds in mass spectra was performed using Wiley Registry (9th edition), an in-house mass spectral library and the NIST (2008) mass spectral databases, a published mass spectral library. For CuO data, Agilent MassHunter Workstation-Unknowns Analysis (version 10.1) was used for data processing and lignin monomer identification was obtained using an in-house customized library. Compound identification was also confirmed by peak retention times and concentrations were determined by comparing the peak areas to the peak areas of external standards.[49,52,57,58] To maintain consistency with other DIRT studies,[9,27,35,36,59–62] the targeted SOM compound concentrations were normalized to the mass of soil extracted (μg g^{-1} soil; Tables S1–S4 of the SI). Normalization of SOM compound concentrations to the soil C concentration (μg per g soil C) is also provided in the SI (Tables S5–S8).[63]

2.5 Radiocarbon analysis

The fraction of modern C ($F^{14}C$) was determined on composite, unesterified PLFA extracts (see Section 2.4) and composite samples of combined field replicates for the upper mineral soil (0–10 cm) samples using a 3 MV tandem accelerator mass spectrometer by the A.E. Lalonde AMS Laboratory in Ottawa, Ontario. Based on Reimer *et al.* (2004), the $F^{14}C$ was calculated as the ratio of $^{14}C/^{12}C$ in the sample to the concurrently measured standard $^{14}C/^{12}C$ ratio (Oxalic acid, Ox-II). By correcting the sample ratios by the radiocarbon decay of the standard up to the year of sample collection (2021), the absolute amount of ^{14}C, or $\Delta^{14}C$ values were determined.[64]

2.6 Statistical analysis

Statistical analyses of the soil samples were performed using the IBM SPSS Statistics software (version 28). The SOM and microbial indices and extractable compound concentrations of the treatment plots were compared to the control plots of their respective soil layer (NR and DL for the forest floor and NL, NR, NI, and DL for the upper mineral soil) using a two-factor analysis of variance (ANOVA) with Bonferroni post-hoc tests. The fixed factor was the field plot replication ($n = 3$), while the random factor was the analytical replicates ($n = 2$). The residual normality and homogeneity of variances were tested by Shapiro–Wilk and Lavene's tests to confirm that the assumptions of ANOVA were met. A confidence level of $p \leq 0.05$ was used to distinguish differences in the SOM and microbial indices and extractable compound concentrations between the treatments and control.

2.7 Comparison of 20 year and 30 year data

Data from a molecular-level study of SOM in the mineral soil (0–10 cm) conducted at the 20 year interval of this experiment[27] were compared to the 30 year results in this study to evaluate the magnitude of changes in SOM metrics over time from the treatments. The same analytical techniques outlined in Sections 2.2 and 2.4 had been used to generate the 20 year data.[27] The relative change was calculated for various SOM indices in the upper mineral soil for both the 20- and 30 year Bousson studies, and was determined as the ratio of the difference between each treatment SOM metric and the respective control SOM metric divided by the control SOM metric; or (B-A)/A; where A is the control SOM metric and B is the treatment SOM metric for each respective study.

3 Results

3.1 Soil carbon (C) and nitrogen (N) concentration and content

Soil C (Fig. 1A; Tables S1 and S2) and N concentrations were significantly different ($p \leq 0.05$) from the control for all forest floor and mineral soil (0–10 cm). The soil C and N concentrations were significantly lower for the forest floor NR treatment, while both soil C and N were significantly higher for the forest floor DL

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treatment. Comparatively, for the upper mineral soil, all treatments (NL, NR, NI, and DL) had significantly lower C and N concentrations relative to the control. Forest floor soil C mass (Fig. 1C) did not differ among the treatments. In contrast, however, total C (forest floor plus upper mineral soil) was lower in the NL and NI treatments ($p = 0.014$) than the control, with a 46% loss of C from the NL and a 43% loss from the NI (Fig. 1D).

3.2 Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy

The differences in the relative proportion of SOM structural classes (*i.e.*, alkyl, *O*-alkyl, and aromatic and phenolic C) were observed using solid-state ^{13}C NMR spectroscopy. Exclusion of above- and/or belowground inputs (NL, NR, and NI) increased alkyl C by 4–7% in the upper mineral soil. Alkyl C also increased by 2% in the mineral soil DL treatment. The *O*-alkyl C only decreased for the root exclusion treatments (NR and NI). Aromatic and phenolic C also decreased by 3–5% for all mineral soils (NL, NR, NI, and DL; Table S2). The SOM decomposition state (alkyl/*O*-alkyl ratio) was higher for all exclusion treatments relative to the control (Fig. 1B; Tables S1 and S2). The SOM resistance to decomposition ((alkyl + aromatic and phenolic C)/(*O*-alkyl + carboxylic and carbonyl C) ratio) was higher than the control only with root exclusion (NR and NI; Fig. 1B; Tables S1 and S2).

3.3 Targeted analysis of soil organic matter (SOM) compounds

3.3.1 Solvent-extractable compounds. Many changes to various lipid concentrations from plant and microbial origins were observed in the forest floor and upper mineral soil (0–10 cm) samples, which comprised of a series of aliphatic lipids (*n*-alkanes, *n*-alkanols, and *n*-alkanoic acids), cyclic lipids (terpenoids and plant-derived steroids), and simple sugars (Tables S1 and S2). Doubled litter inputs (DL) to the forest floor contributed to significant higher ($p \leq 0.05$) concentrations of all components typically associated with plants, such as long-chain ($\geq C_{20}$) aliphatic lipids (long-chain *n*-alkanes, *n*-alkanols, *n*-alkanoic acids),[65,66] plant-derived steroids, and triterpenoids (Table S1). The concentration of short-chain aliphatic lipids and sugars also increased ($p \leq 0.05$) with the doubling of litter at the forest floor (Table S1). For the upper mineral soil, higher

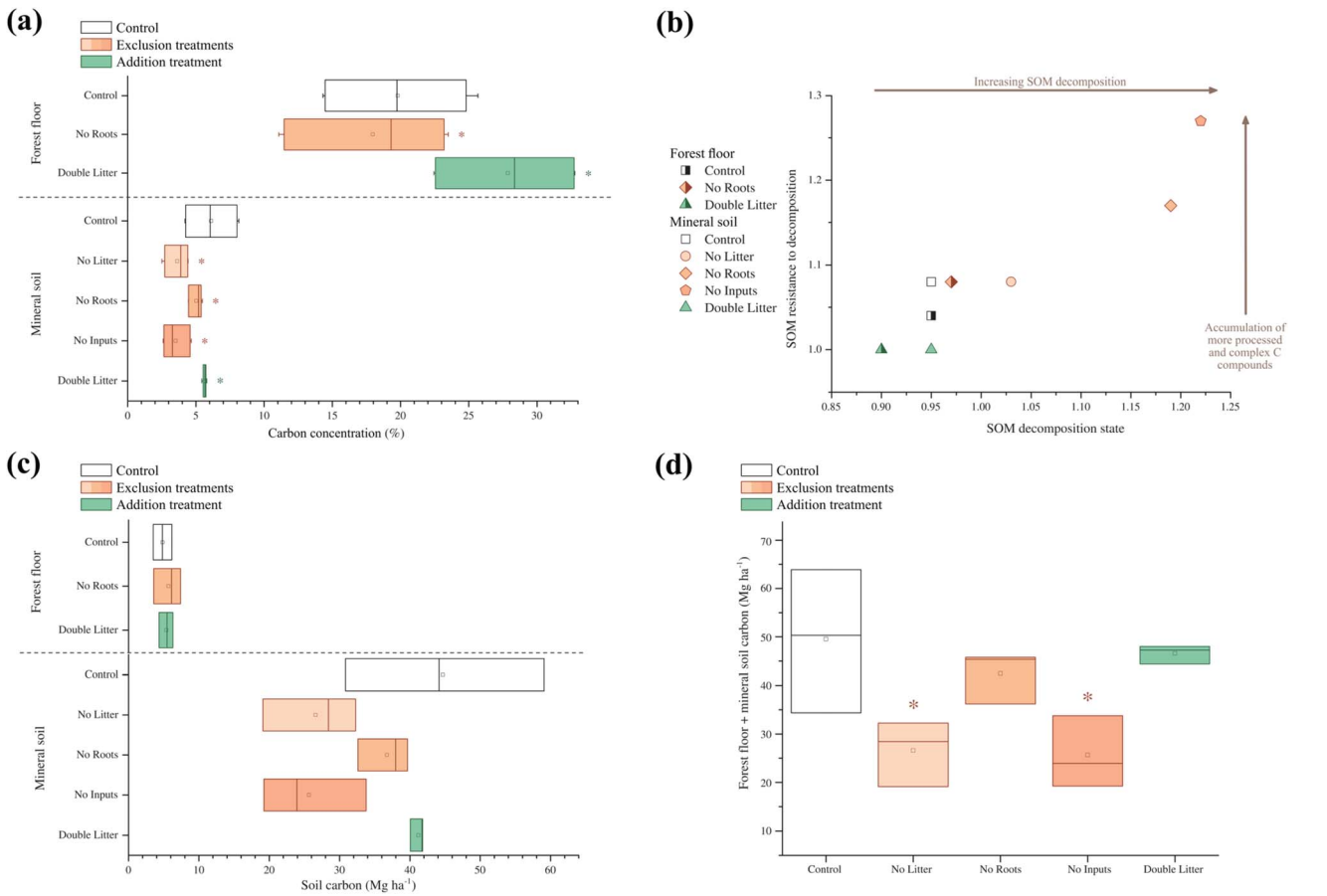

Fig. 1 (a) Soil carbon (C) concentration (%) (b) and soil organic matter (SOM) resistance to decomposition (ratio of (alkyl + aromatic and phenolic)/(*O*-alkyl + carboxyl and carbonyl) C) *versus* SOM decomposition state (ratio of alkyl/*O*-alkyl C) for forest floor and 0–10 cm mineral soil samples after 30 years of detrital manipulation at the Bousson Forest. Soil C mass (Mg ha^{-1}) for forest floor and mineral soil samples shown (c) separately (d) and combined after 30 years of treatments. Boxes show the 25–75th percentile, whiskers represent the 5–95th percentile; the line in the box indicates the median, and the square indicates the mean. An asterisk (*) denotes significant differences ($p \leq 0.05$) between treatments and the control.

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concentrations ($p \leq 0.05$) of plant-derived components (long-chain *n*-alkanoic acids; plant-derived steroids and triterpenoids) were also observed for the DL treatment, while there were significant reductions ($p \leq 0.05$) in these plant-derived components for the treatments that excluded litter (NL and NI; Table S2).

3.3.2 Cutin-, suberin-, and microbial-derived compounds. Exclusion of above- and belowground inputs (NI) in the upper mineral soil significantly reduced ($p \leq 0.05$) the number of cutin- and suberin-derived components (Table S2), which are respectively indicative of above- (leaf litter) and belowground (plant roots) inputs.[49,67] The number of cutin-derived compounds in the mineral soil was also lower ($p = 0.09$) for the litter exclusion treatment (NL) relative to the control.[68] Meanwhile for the forest floor, compared to the control, the concentration of cutin-derived components was significantly higher ($p \leq 0.05$) for both treatments (NR and DL; Table S1). Microbial-derived hydrolysable lipids (henceforth referred to as microbial-derived lipids) consist of branched C_{14}–C_{18} alkanoic acids and increased ($p \leq 0.05$) for the DL treatment in both the forest floor and mineral soil (Tables S1 and S2). The concentration of microbial-derived lipids was significantly lower ($p \leq 0.05$) relative to the control for the litter exclusion treatments (NL and NI) in the mineral soil (Table S2).

3.3.3 Lignin-derived compounds. While there were some changes in the proportions of lignin-derived phenols (% vanillyl, % syringyl, and % cinnamyl) in the mineral soil for the exclusion treatments (Fig. 2A and Table S2), the litter manipulation treatments across both the forest floor and top 10 cm of the mineral soil did not significantly alter the concentration of total lignin-derived compounds (Tables S1 and S2). Progressive lignin oxidation was assessed using acid-to-aldehyde ratios for vanillyl ($Ad/Al_{Vanillyl}$) and syringyl ($Ad/Al_{Syringyl}$) compounds and significantly increased ($p \leq 0.05$) in the upper mineral soil of the exclusion treatments (NL, NR, and NI) compared to the control (Fig. 2B; Table S2).

3.3.4 Microbial biomass and community composition. Microbial biomass, which encompasses Gram-negative bacteria (16:1ω7*c*, *cy*17:0, 18:1ω7*c* and *cy*19:0), Gram-positive bacteria (*i*14:0, α14:0, *i*15:0, α15:0, *i*16:0, *i*17:0, α17:0), actinobacteria (10Me16:0, 10Me17:0, 10Me18:0), fungi (18:2ω6,9*c*), and arbuscular mycorrhizal fungi (16:1ω5; Harwood and Russell 1984), were significantly higher ($p \leq 0.05$) for DL in both the forest floor and mineral soil (Fig. 3A; Tables S1 and S2). Comparatively, microbial biomass was significantly lower ($p \leq 0.05$) for the root exclusion treatments (NR and NI) in the mineral soil. Furthermore, the community composition was altered for all treatments in both the forest floor and mineral soil, with each treatment having significantly higher ($p \leq 0.05$) proportions of Gram (+) to Gram (−) bacteria, relative to their respective controls (Fig. 3B; Tables S1 and S2). The relative proportion of bacterial to fungal PLFAs was significantly lower for root exclusion treatments in the mineral soil (NR and NI), whereas the proportion of fungal PLFAs was higher for DL in the forest floor (Fig. 3B; Tables S1 and S2). The ratio of *cy*19:0/18:1ω7*c* PLFAs was used as an indicator for microbial stress and was significantly higher ($p \leq 0.05$) for the NR treatment in both the forest floor and mineral soil, and for all other treatments in the mineral soil (Tables S1 and S2). Microbial stress was also assessed through the ratio of monoenoic to saturated PLFAs- and was significantly lower ($p \leq 0.05$) for the DL treatment in the forest floor and the root exclusion treatments (NR and NI) in the mineral soil (Tables S1 and S2).

3.4 Radiocarbon analysis

Radiocarbon analysis of the isolated membrane lipids in the mineral soil (Fig. 4 and Table S2) reveal that there is selective substrate use based on the availability of above- and belowground inputs. The $\Delta\ ^{14}C$ signatures for both the whole soil and isolated membrane lipids increased (were more positive) for the DL treatment, while the ^{14}C content was lower for each of the exclusion treatments (NL, NR, and NI) relative to the control.

3.5 Comparison of 20 year and 30 year data

A comparison (fold change relative to the respective controls) of different SOM metrics between 20 years (Wang *et al.*, 2017) and this study (Fig. 5), shows that changes that ensued in the third decade of this experiment differed among biochemical and ecosystem markers. In addition to no net C gain in the mineral soil, the overall magnitude of the response for measured SOM components and microbial biomass exhibited different trajectories over time. Notably, the microbial biomass size and community composition for most of the treatments continued to respond to litter manipulation. Microbial biomass remained unchanged for NL but was higher in root exclusion treatments (NR and NI) and DL, which contrasts with what was observed after 20 years in Bousson, when there had been lower concentrations of microbial biomass. As well, lignin oxidation was higher after 30 years than previously reported for 20 years of treatment in all the exclusion treatments (NL, NR, NI).

4 Discussion

Despite that many models assume a direct linear relationship between litter inputs and soil C accumulation,[25,29] many of the observations from long-term DIRT experiments have demonstrated that gains in soil C concentrations from DL treatments are limited due to the enhanced microbial decomposition of SOM fueled by the additional litter.[25,27,35,40] Few DIRT experiments[25,69–71] have reported increasing soil C concentrations with litter addition treatments. There were increases in soil C for DL treatments at the Síkfőkút Forest DIRT experiment in Hungary, due to the site's unique ecological properties, such as the litter quality, mean annual precipitation, and history of elevated N deposition, resulting in unique changes to the SOM composition and microbial processing of SOM.[69,71] Soil C also increased for DL in the experiment at the Wisconsin DIRT site, however, this was only observed after an initial analysis of the soil 28 years into the experiment, and was attributed to low initial soil C from a history of agricultural land use.[25,70] Although we had hypothesized that soil C gains would be limited despite 30 years of additional litter inputs, many forest managers are still seeking to increase the amount of C in forest soils as a means to mitigate

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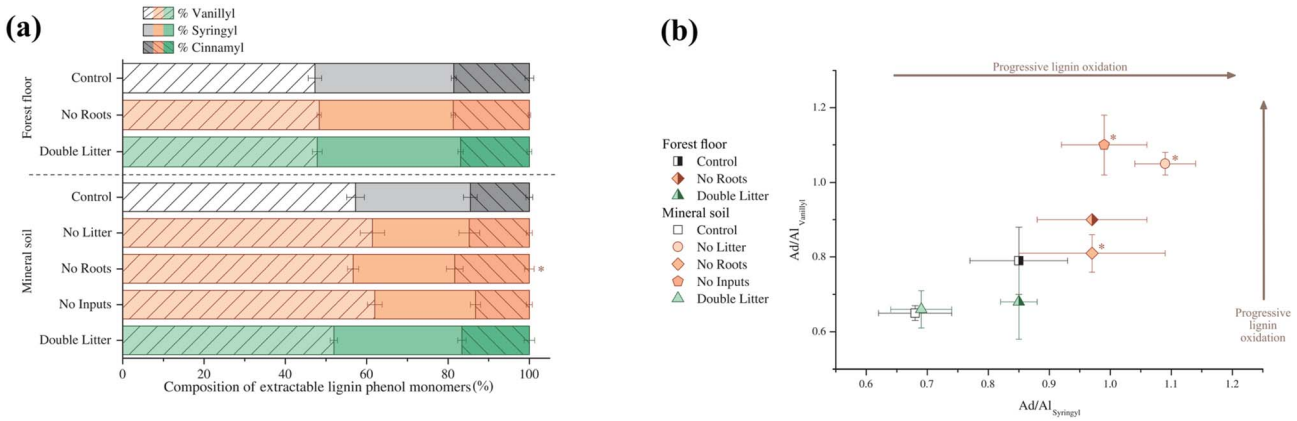

Fig. 2 (a) Lignin composition and (b) lignin oxidation indices (acid-to-aldehyde (Ad/Al) ratios for the vanillyl *vs.* syringyl monomers) for forest floor and 0–10 cm mineral soil samples after 30 years of detrital manipulation at the Bousson Forest. An asterisk (*) denotes significant differences ($p \leq 0.05$) between treatments and the control.

climate change, especially knowing that soil C can be protected through physical or chemical associations with soil minerals.[6,7] This can be especially important because physical disturbances that accelerate forest floor losses and reduce leaching of dissolved organic C from fresh aboveground litter may impact C in these mineral soil pools.[72,73] The main pathways by which aboveground litter is transported into the mineral soil as SOM are through the incorporation by soil fauna, leaching of dissolved organic matter, fragmentation and subsequent movement of particulate material.[11]

Interestingly, in our study, 30 years of doubled litter inputs increased soil C in the forest floor, but did not increase the upper mineral soil C concentration (Fig. 1A; Tables S1 and S2), which partially agrees with our hypothesis. The average litterfall at this site is 2.2 Mg C per ha per year, and despite doubled litter inputs over 30 years, the soil C mass did not significantly increase (Fig. 1C–D; Tables S1 and S2), demonstrating that only a very small fraction of this C accumulates in the topsoil over time. This was also supported by the accumulation of many

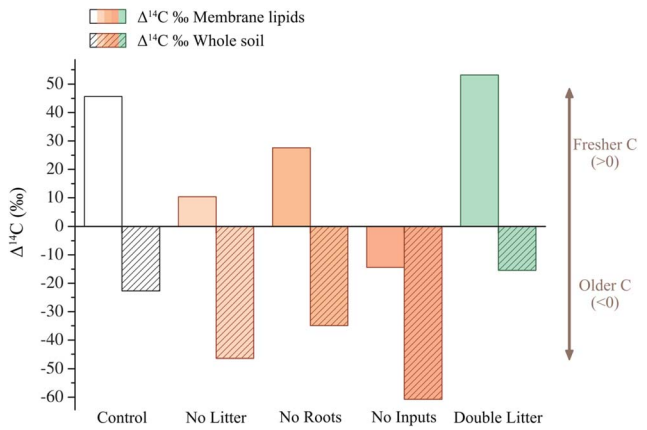

Fig. 4 Radiocarbon profiling ($\Delta^{14}C$ ‰) of membrane lipids (open bars) and whole soil (dashed bars) for 0–10 cm mineral soil samples after 30 years of detrital manipulation at the Bousson Forest.

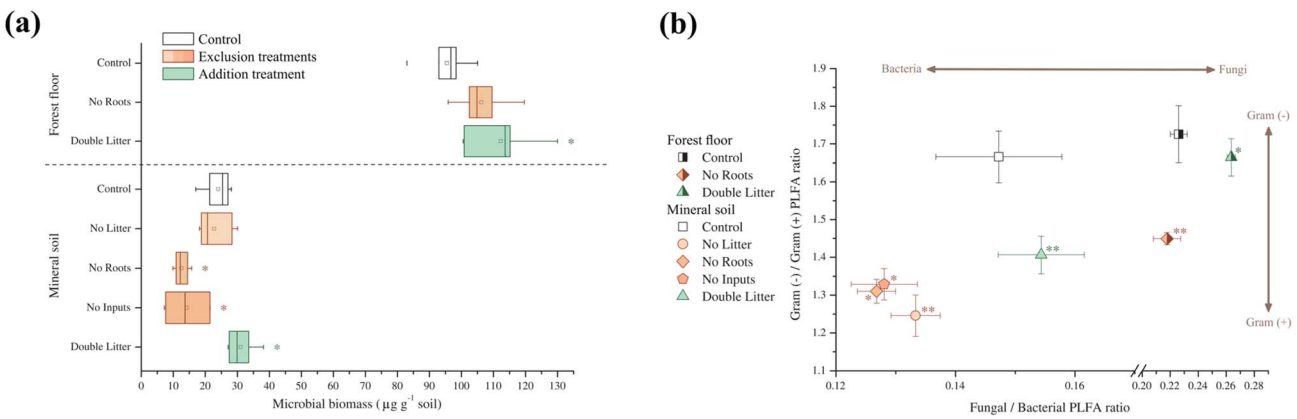

Fig. 3 (a) Microbial biomass (an asterisk (*) denotes significant differences ($p \leq 0.05$) between treatments and the control) and (b) microbial community composition, shown through the ratio of Gram (−) to Gram (+) PLFAs *vs.* the ratio of fungal to bacterial PLFAs for forest floor and 0–10 cm mineral soil samples after 30 years of detrital manipulation at the Bousson Forest. In (b) an asterisk (*) denotes significant differences ($p \leq 0.05$) between treatments and the control for both ratios, while a double asterisk (**) denotes significant differences ($p \leq 0.05$) between treatments and the control for the Gram (−)/Gram (+) ratio only. Boxes in (a) show the 25–75th percentile, whiskers represent the 5–95th percentile; the line in the box indicates the median, and the square indicates the mean.

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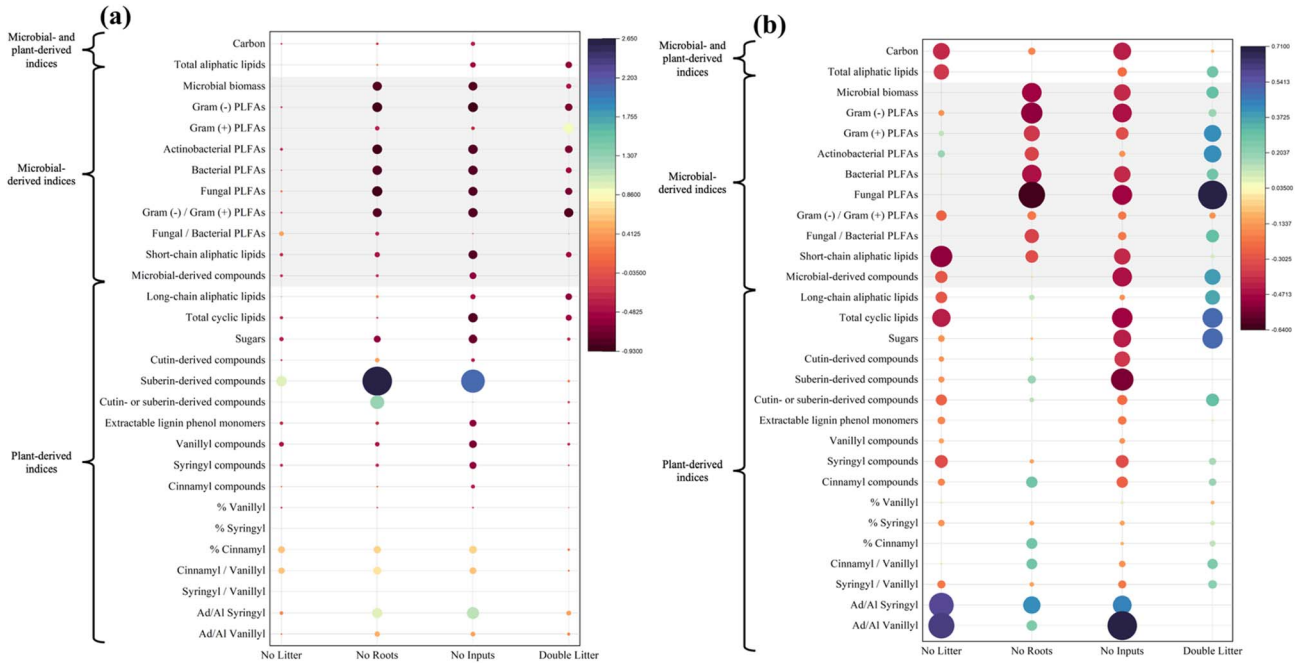

Fig. 5 The relative change between each of the treatments in the 0–10 cm mineral soil and their respective controls was determined for microbial and soil organic matter indices after (a) 20 years[27] and (b) 30 years of detrital manipulation at the Bousson Forest. Relative change was calculated as (B–A)/A, where A represents the control SOM metric and B represents the treatment SOM metric for each respective study. Positive values indicate an increase relative to the control, while negative values indicate a decrease.

SOM components associated with plant litter, such as long-chain ($\geq C_{20}$) aliphatic lipids,[65,66] cyclic lipids, sugars and cutin-derived compounds, which were elevated for the DL treatment at the forest floor (Table S1). Likewise, microbial biomass (Fig. 3A and Table S1) also increased in the forest floor under DL, likely supported by the additional C inputs. This is further corroborated by the increase in short-chain aliphatic lipids, which are characteristic of SOM derived from microbial origins,[74] as well as by the elevated contribution of microbial-derived lipids from both cellular and extracellular microbial sources[56,75,76] in the forest floor soil (Table S1). Despite the increases in C and accumulation of plant-derived SOM on the forest floor, our results demonstrate that the additional C from the elevated inputs are not sequestered within the 0–10 cm mineral soil. While deeper soil layers were not assessed here, complementary analyses at the same site have shown SOM accrual below 10 cm, however, these increases were not significantly different from the control.[33]

As hypothesized, there were observed changes to the microbial community in the upper mineral soil (0–10 cm), including elevated microbial biomass (Fig. 3A and Table S2) and microbial community structure reorganization (lower proportion of Gram (−)/Gram (+) bacteria; Fig. 3B and Table S2) in response to doubled litter inputs. The higher microbial biomass was likely due to increased availability of preferred substrates with DL, which concurrently limited new soil C gains within the top 10 cm of the mineral soil (Fig. 1A and Table S2). This is supported through the radiocarbon profiling of the membrane lipids of the microbes within the mineral soil for the DL treatment (Fig. 4 and Tables S2), as the $\Delta^{14}C$ signatures for these plots indicate that the microbial community was using fresher C inputs, such as the additional litter inputs received from this treatment. Furthermore, despite having doubled inputs of litter, there was a lack of accumulation of plant-derived compounds, such as long-chain aliphatic lipids,[65,66] cutin-, suberin-,[65,66] and lignin derived compounds,[52,77–79] which supports that the increased availability of fresher inputs from this treatment was fueling the microbial response in the mineral soil. Additionally, NMR analyses reveal that the SOM decomposition state (alkyl/*O*-alkyl ratio; Fig. 1B; Table S2) for the DL treatment in the upper mineral soil was the same as the control, which further demonstrates that the added litter did not accumulate due to continuous accelerated SOM decomposition by soil microbes. Elevated microbial biomass in conjunction with the ongoing degradation of specific SOM components also enhanced the contribution of microbial-derived lipids to SOM with added litter (Fig. 3A and Table S2) further indicating increased microbial decomposition.[24,80,81] Additionally, an accumulation of sugars for the DL treatment was also observed in the top 10 cm of the mineral soil (Table S2) and is indicative of the active breakdown of cellulose into smaller molecules by microbes.[82,83] As we had originally hypothesized, despite 30 years of double litter inputs, the reorganization of microbes in these plots allowed for the sustained decomposition of fresh substrates, restricting any soil C gains in the mineral soil in these plots.

In accordance with our hypothesis for the exclusion of above- and belowground inputs (NL, NR, and NI), soil C decreased (Fig. 1A, C and D; Table S2), especially in the absence of litter. On an ecosystem level, the losses are substantial, with losses over the 30 years averaging 0.76 Mg per ha per year in the NL

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treatment and 0.80 Mg per ha per year in the NI treatment, equivalent to more than a third of the site's average litterfall C input of 2.2 Mg C per ha per year. The higher SOM decomposition states (alkyl/*O*-alkyl ratio; Fig. 1B and Table S2) reveal that SOM in the mineral soil of the exclusion plots was more degraded than SOM in the control plots.[41,43,45,46] Furthermore, the SOM in the mineral soil of plots without root inputs (NR and NI) were more degraded than the exclusion of aboveground inputs alone (NL). Even with the exclusion of litter (NL), the microbial biomass (Fig. 3A and Table S2) remained at the same levels as plots receiving ambient inputs and suggests that the quantity of fresh litter is not a limiting factor for sustaining the microbial community in this forest. Other studies[84–86] have reported that the long-term exclusion of fresh roots and root exudates may reduce the number of available substrates (such as free sugars), restricting microbial growth and biomass maintenance, which is consistent with the lower microbial biomass observed for the root exclusion treatments (NR and NI; Fig. 3A and Table S2) in our study at the Bousson Forest. We also observed declines in *O*-alkyl C resonances, which originate from compounds such as carbohydrates,[43,44] and lower concentrations of free sugars (Table S2) for the root exclusion treatments suggesting that the long-term exclusion of roots contributed to a lower number of favorable substrates (such as free sugars), which aligns with observations made in previous studies.[84–86] Concurrently, the restricted microbial growth shown by the lower microbial biomass in the mineral soil for these treatments as a result of the reduced number of substrates is also consistent with these observations.[84–86]

In agreement with our hypothesis, we also observed shifts in the mineral soil microbial community composition for each of the exclusion treatments. The changes in C input availability led to the adjustment of microbial communities, such as the higher proportions of Gram (+) and actinobacteria bacteria (Fig. 3B and Table S2), which have higher capacities to process complex forms of C.[87,88] The findings from a study[89] using radiocarbon profiling to assess the microbial carbon sources for microbiota in an industrial site demonstrated that microbes were able to use almost all available pools of organic matter, including older pools of more complex and less easily degradable C. The $\Delta\ ^{14}C$ signatures of the membrane lipids in our study (Fig. 4 and Table S2) indicate that the microbes are indeed using older forms of C and suggests that the reorganization of their community structure facilitated the altered use of substrates by soil microbiota in the mineral layer to maintain their metabolism. Further evidence for substrate limitations and microbial community compositional shifts is observed through elevated microbial stress (higher *cy*19:0/18:1ω7*c* ratios and lower monoenoic/saturated PLFA ratios; Table S2), which is consistent for both microbial community reorganization and declines in microbial substrate quality and quantity.[90–95] Lignin oxidation also increased (higher Ad/Al ratios; Fig. 2B and Table S2) for both syringyl and vanillyl components[52,77,96,97] for each of the exclusion treatments, suggesting that with the lower amounts of preferred substrates, the microbes were using less desirable substrates such as lignin, which was more degraded for all exclusion treatments relative to the control.[98] Overall, in agreement with our hypothesis, these findings further demonstrate the resiliency of soil microbiota for sustaining their metabolic activity in response to substrate limitations, even with the restriction of fresh inputs.

Our results after 30 years of experiment were compared to a previous study[27] conducted at 20 years, and found that not only were microbial responses sustained over time, but that the extent of SOM compositional changes in the mineral soil varied markedly from 20 to 30 years (Fig. 5). After 20 years of treatment, above- and belowground inputs had contributed differently to SOM components, and shifts in SOM composition and microbial biomass and composition were more sensitive to root inputs but less to aboveground inputs at Bousson.[27] For example, suberin-derived components (Fig. 5A) were relatively more preserved for the root exclusion treatments after 20 years of experiment,[27] whereas the concentration of these components was not significantly different from the control after 30 years (Fig. 5B and Table S2). The exclusion treatments also exhibited a shift in both microbial biomass and community structure over time (Fig. 5B), such as through the changes in proportion of fungi to bacteria and the Gram (−) to Gram (+) bacteria (Fig. 3B).[87,88] The continued microbial reorganization from 20 years to 30 years in response to changes in litter deposition altered the use of different SOM components as substrates. This is further demonstrated through the relatively lower amounts of cutin- and suberin-derived compounds and the elevated lignin degradation (Ad/Al ratios)[52,77,96,97] for each of the exclusion treatments at 30 years compared to 20 years (Fig. 4), corroborating that these continued microbial responses further proceeded SOM decomposition, despite the lack of available preferred substrates. Additionally, soil C gains with litter addition were not observed over 20 years as well as 30 years in the mineral soil likely due to sustained microbial reorganization. Although microbial biomass and composition were not significantly different from the control after 20 years, both the total biomass and composition were significantly altered after 30 years. Coinciding with the elevated microbial biomass at 30 years compared to 20 years of doubled litter, components such as free sugars accumulated in the mineral soil, indicative of the active breakdown of cellulose into smaller molecules by microbes[82,83] with the concomitant incorporation of microbial-derived lipids to the soil.[24,80,81]

5 Conclusion

Our extensive integrative molecular-level analysis of forest soil samples after 30 years of litter manipulation at the Bousson forest demonstrated that the persistence and decomposition of SOM molecular classes are underpinned by the sustained long-term reorganization of the microbes in these soils. This study further emphasizes the critical importance of maintaining current soil C stocks in a changing world. Despite 30 years of doubled litter inputs, the mass of forest floor C did not increase, nor did mineral soil C mass. While soil texture and mineralogy are important for stabilizing soil C, other temperate forests with coarse-textured, loamy soil have demonstrated that mineral soil C gains are possible after 15 years of added litter.[99] The Bousson

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forest soils are also of a loamy sand texture, however, our work identified that microbial responses are more likely to determine the trajectory of new C inputs with respect to long-term soil C sequestration. Soil C in the forest floor pools are especially dynamic and incorporating this C deeper into the soil profile where it can be protected from microbial processing through physical or chemical association with soil minerals did not occur. Hence, trying to manage forests in ways to enhance long-lasting soil C sequestration to mitigate climate change will be challenging given the novel microbial responses identified here. These microbial and biochemical limitations to increasing the pool of mineral soil C which ultimately limited any soil C gains with new litter. Moreover, limiting above and belowground inputs altered C biogeochemistry and microbial substrate acquisition, resulting in reduced soil C demonstrating the sensitivity of temperate forest soils to litter deposition changes. In addition to lower soil C concentrations, there were observable shifts within the microbial community composition, and our radiocarbon profiling of the membrane lipids indicate that the microbes will use older C when preferred substrates are in low availability. Importantly, the microbial community is not static; SOM compositional changes observed after 30 years differed from those measured at 20 years, indicate that sustained reorganization of the soil microbial communities, in response to reduced litter input, makes all SOM vulnerable to decay and loss. Assuming simply that increased forest productivity will lead to soil C increases, or that protected mineral soil C is invulnerable to loss, will lead to incomplete or incorrect estimates of the abilities of forests to mitigate climate change. It is important to note that soils with a finer texture may be more resilient with respect to soil C losses with long-term litter changes and warrants further investigation over decadal time scales. Furthermore, another caveat is that our study was limited to the forest floor and the 0–10 cm mineral soil layer and future work investigating the molecular-level changes in deeper horizons will be critical to fully assess soil C accrual below the layers examined here.

Author contributions

Isla Wrightson: conceptualization, data curation, formal analysis, investigation, validation, visualization, writing – original draft, writing – review and editing. Richard D. Bowden: conceptualization, methodology, data curation, resources, writing – review and editing. Junjian Wang: resources, writing – review and editing. Kate Lajtha: conceptualization, methodology, writing – review and editing. Myrna J. Simpson: conceptualization, methodology, project administration, resources, supervision, writing – original draft, writing – review and editing.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The data supporting this article have been included as part of the supplementary information (SI).

Supplementary information: concentrations of carbon and nitrogen, soil organic matter compounds, and details of NMR results. See DOI: https://doi.org/10.1039/d5va00350d.

Acknowledgements

I. W. thanks the province of Ontario and the University of Toronto for the Queen Elizabeth II/F. E. Beamish Graduate Scholarship in Science and Technology and the ChemClub Graduate Scholarship. The Natural Sciences and Engineering Research Council (NSERC) of Canada supported this research *via* a Discovery Grant and Canada Research Chair in Integrative Molecular Biogeochemistry to M. J. S. J. W. thanks the support from the Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control (2023B1212060002). The authors thank Jennifer Walker, Sarah Murseli and Kayla Mckee from the A.E. Lalonde AMS Laboratory in Ottawa, Ontario for their assistance with radiocarbon data acquisition and Dr Ronald Soong (Environmental NMR Centre) for assistance with NMR data acquisition.

References

1 X. Wei, M. Shao, W. Gale and L. Li, Global pattern of soil carbon losses due to the conversion of forests to agricultural land, *Sci. Rep.*, 2014, **4**, 4062.
2 M. Mayer, C. E. Prescott, W. E. A. Abaker, L. Augusto, L. Cécillon, G. W. D. Ferreira, J. James, R. Jandl, K. Katzensteiner, J.-P. Laclau, J. Laganière, Y. Nouvellon, D. Paré, J. A. Stanturf, E. I. Vanguelova and L. Vesterdal, Tamm Review: Influence of forest management activities on soil organic carbon stocks: A knowledge synthesis, *For. Ecol. Manage.*, 2020, **466**, 118127.
3 R. Lal, Forest soils and carbon sequestration, *For. Ecol. Manage.*, 2005, **220**, 242–258.
4 T. A. Ontl, M. K. Janowiak, C. W. Swanston, J. Daley, S. Handler, M. Cornett, S. Hagenbuch, C. Handrick, L. Mccarthy and N. Patch, Forest management for carbon sequestration and climate adaptation, *J. For.*, 2020, **118**, 86–101.
5 L. Kaarakka, M. Cornett, G. Domke, T. Ontl and L. E. Dee, Improved forest management as a natural climate solution: A review, *Ecol. Sol. Evid.*, 2021, **2**, e12090.
6 I. Kögel-Knabner, G. Guggenberger, M. Kleber, E. Kandeler, K. Kalbitz, S. Scheu, K. Eusterhues and P. Leinweber, Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry, *J. Plant Nutr. Soil Sci.*, 2008, **171**, 61–82.
7 M. F. Cotrufo, M. G. Ranalli, M. L. Haddix, J. Six and E. Lugato, Soil carbon storage informed by particulate and mineral-associated organic matter, *Nat. Geosci.*, 2019, **12**, 989–994.

© 2026 The Author(s). Published by the Royal Society of Chemistry

8 J. M. Lavallee, J. L. Soong and M. F. Cotrufo, Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century, *Glob. Chang. Biol.*, 2020, **26**, 261–273.

9 M. M. Mayzelle, M. L. Krusor, K. Lajtha, R. D. Bowden and J. Six, Effects of detrital inputs and roots on carbon saturation deficit of a temperate forest soil, *Soil Sci. Soc. Am. J.*, 2014, **78**, S76–S83.

10 K. Georgiou, R. B. Jackson, O. Vindušková, R. Z. Abramoff, A. Ahlström, W. Feng, J. W. Harden, A. F. A. Pellegrini, H. W. Polley, J. L. Soong, W. J. Riley and M. S. Torn, Global stocks and capacity of mineral-associated soil organic carbon, *Nat. Commun.*, 2022, **13**, 3797.

11 C. E. Prescott and L. Vesterdal, Decomposition and transformations along the continuum from litter to soil organic matter in forest soils, *For. Ecol. Manage.*, 2021, **498**, 119522.

12 S. E. Trumbore and C. I. Czimczik, An uncertain future for soil carbon, *Science*, 2008, **321**, 1455–1456.

13 M. W. I. Schmidt, M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D. A. C. Manning, P. Nannipieri, D. P. Rasse, S. Weiner and S. E. Trumbore, Persistence of soil organic matter as an ecosystem property, *Nature*, 2011, **478**, 49–56.

14 J. Lehmann and M. Kleber, The contentious nature of soil organic matter, *Nature*, 2015, **528**, 60–68.

15 M. Wiesmeier, L. Urbanski, E. Hobley, B. Lang, M. von Lützow, E. Marin-Spiotta, B. van Wesemael, E. Rabot, M. Ließ, N. Garcia-Franco, U. Wollschläger, H.-J. Vogel and I. Kögel-Knabner, Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales, *Geoderma*, 2019, **333**, 149–162.

16 E. W. Sulzman, J. B. Brant, R. D. Bowden and K. Lajtha, Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest, *Biogeochemistry*, 2005, **73**, 231–256.

17 J. B. Brant, E. W. Sulzman and D. D. Myrold, Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation, *Soil Biol. Biochem.*, 2006, **38**, 2219–2232.

18 K. Lajtha, R. D. Bowden and K. Nadelhoffer, Litter and root manipulations provide insights into soil organic matter dynamics and stability, *Soil Sci. Soc. Am. J.*, 2014, **78**, S261–S269.

19 Z. Veres, Z. Kotroczó, I. Fekete, J. A. Tóth, K. Lajtha, K. Townsend and B. Tóthmérész, Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability, *Appl. Soil Ecol.*, 2015, **92**, 18–23.

20 B. Berg and C. McClaugherty, in *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*, ed. B. Berg and C. McClaugherty, Springer, Berlin, Heidelberg, 2014, pp. 1–10.

21 J. L. Soong, L. Fuchslueger, S. Marañon-Jimenez, M. S. Torn, I. A. Janssens, J. Penuelas and A. Richter, Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling, *Glob. Chang. Biol.*, 2020, **26**, 1953–1961.

22 S. Manzoni, P. Taylor, A. Richter, A. Porporato and G. I. Ågren, Environmental and stoichiometric controls on microbial carbon-use efficiency in soils, *New Phytol.*, 2012, **196**, 79–91.

23 B. R. K. Roller and T. M. Schmidt, The physiology and ecological implications of efficient growth, *ISME J.*, 2015, **9**, 1481–1487.

24 C. M. Kallenbach, S. D. Frey and A. S. Grandy, Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls, *Nat. Commun.*, 2016, **7**, 13630.

25 K. Lajtha, R. D. Bowden, S. Crow, I. Fekete, Z. Kotroczó, A. Plante, M. J. Simpson and K. J. Nadelhoffer, The detrital input and removal treatment (DIRT) network: insights into soil carbon stabilization, *Sci. Total Environ.*, 2018, **640–641**, 1112–1120.

26 L. Castañeda-Gómez, K. Lajtha, R. Bowden, F. N. Mohammed Jauhar, J. Jia, X. Feng and M. J. Simpson, Soil organic matter molecular composition with long-term detrital alterations is controlled by site-specific forest properties, *Glob. Chang. Biol.*, 2023, **29**, 243–259.

27 J.-J. Wang, O. Pisani, L. H. Lin, O. O. Y. Lun, R. D. Bowden, K. Lajtha, A. J. Simpson and M. J. Simpson, Long-term litter manipulation alters soil organic matter turnover in a temperate deciduous forest, *Sci. Total Environ.*, 2017, **607–608**, 865–875.

28 J. Liski, D. Perruchoud and T. Karjalainen, Increasing carbon stocks in the forest soils of western Europe, *For. Ecol. Manage.*, 2002, **169**, 159–175.

29 P. Gottschalk, J. U. Smith, M. Wattenbach, J. Bellarby, E. Stehfest, N. Arnell, T. J. Osborn, C. Jones and P. Smith, How will organic carbon stocks in mineral soils evolve under future climate? Global projections using RothC for a range of climate change scenarios, *Biogeosciences*, 2012, **9**, 3151–3171.

30 S. Xu, E. J. Sayer, N. Eisenhauer, X. Lu, J. Wang and C. Liu, Aboveground litter inputs determine carbon storage across soil profiles: a meta-analysis, *Plant Soil*, 2021, **462**, 429–444.

31 J. Feng, K. He, Q. Zhang, M. Han and B. Zhu, Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems, *Glob. Chang. Biol.*, 2022, **28**, 3426–3440.

32 J. W. Leff, W. R. Wieder, P. G. Taylor, A. R. Townsend, D. R. Nemergut, A. S. Grandy and C. C. Cleveland, Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest, *Glob. Chang. Biol.*, 2012, **18**, 2969–2979.

33 R. D. Bowden, M. J. Simpson, N. P. Saucedo, K. Brozell, J. DiGiacomo and K. Lajtha, Litter and root sources of soil organic matter in a temperate forest: Thirty years in the DIRT, *Soil Sci. Soc. Am. J.*, 2024, **88**, 593–601.

34 N. C. Dove, J. M. Stark, G. S. Newman and S. C. Hart, Carbon control on terrestrial ecosystem function across contrasting site productivities: the carbon connection revisited, *Ecology*, 2019, **100**, e02695.

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35 O. Pisani, L. H. Lin, O. O. Y. Lun, K. Lajtha, K. J. Nadelhoffer, A. J. Simpson and M. J. Simpson, Long-term doubling of litter inputs accelerates soil organic matter degradation and reduces soil carbon stocks, *Biogeochemistry*, 2016, **127**, 1–14.
36 M. Man, D. Pierson, R. Chiu, M. Tabatabaei Anaraki, L. vandenEnden, R. Ye, K. Lajtha and M. J. Simpson, Twenty years of litter manipulation reveals that above-ground litter quantity and quality controls soil organic matter molecular composition, *Biogeochemistry*, 2022, **159**, 393–411.
37 R. D. Bowden, G. Rullo, G. R. Stevens and P. A. Steudler, Soil fluxes of carbon dioxide, nitrous oxide, and methane at a productive temperate deciduous forest, *J. Environ. Qual.*, 2000, **29**, 268–276.
38 US Climate Data, Climate Meadville - Pennsylvania, https://www.usclimatedata.com, accessed 30 October 2024.
39 NOAA (National Oceanic and Atmospheric Administration), Annual Climatological Summary National Climatic Data Center, https://www.ncei.noaa.gov/, accessed 30 October 2024.
40 R. D. Bowden, L. Deem, A. F. Plante, C. Peltre, K. Nadelhoffer and K. Lajtha, Litter input controls on soil carbon in a temperate deciduous forest, *Soil Sci. Soc. Am. J.*, 2014, **78**, S66–S75.
41 M. W. I. Schmidt, H. Knicker, P. G. Hatcher and I. Kogel-Knabner, Improvement of ^{13}C and ^{15}N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid, *Eur. J. Soil Sci.*, 1997, **48**, 319–328.
42 C. N. Gonçalves, R. S. D. Dalmolin, D. P. Dick, H. Knicker, E. Klamt and I. Kögel-Knabner, The effect of 10% HF treatment on the resolution of CPMAS ^{13}C NMR spectra and on the quality of organic matter in Ferralsols, *Geoderma*, 2003, **116**, 373–392.
43 J. A. Baldock, J. M. Oades, A. G. Waters, X. Peng, A. M. Vassallo and M. A. Wilson, Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy, *Biogeochemistry*, 1992, **16**, 1–42.
44 M. J. Simpson, A. Otto and X. Feng, Comparison of solid-state carbon-13 nuclear magnetic resonance and organic matter biomarkers for assessing soil organic matter degradation, *Soil Sci. Soc. Am. J.*, 2008, **72**, 268–276.
45 C. M. Preston, Carbon-13 solid-state NMR of soil organic matter-using the technique effectively, *Can. J. Soil. Sci.*, 2001, **81**, 255–270.
46 M. J. Simpson, A. J. Simpson and W. L. Kingery, in *Reference Module in Earth Systems and Environmental Sciences*, Elsevier, 2017.
47 R. Ostertag, E. Marín-Spiotta, W. L. Silver and J. Schulten, Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico, *Ecosyst.*, 2008, **11**, 701–714.
48 A. Otto and M. J. Simpson, Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from Western Canada, *Biogeochemistry*, 2005, **74**, 377–409.
49 A. Otto and M. J. Simpson, Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada, *Org. Geochem.*, 2006, **37**, 385–407.
50 M. A. Goñi and J. I. Hedges, Potential applications of cutin-derived CuO reaction products for discriminating vascular plant sources in natural environments, *Geochim. Cosmochim. Acta*, 1990, **54**, 3073–3081.
51 J. I. Hedges and J. R. Ertel, Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products, *Anal. Chem.*, 1982, **54**, 174–178.
52 A. Otto and M. J. Simpson, Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil, *Biogeochemistry*, 2006, **80**, 121–142.
53 P. C. R. Pinto, E. A. B. da Silva and A. E. Rodrigues, Comparative study of solid-phase extraction and liquid−liquid extraction for the reliable quantification of high value added compounds from oxidation processes of wood-derived lignin, *Ind. Eng. Chem. Res.*, 2010, **49**, 12311–12318.
54 E. G. Bligh and W. J. Dyer, A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, 1959, **37**, 911–917.
55 *Practical Gas Chromatography: A Comprehensive Reference*, ed. K. Dettmer-Wilde and W. Engewald, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014.
56 A. Otto and M. J. Simpson, Analysis of soil organic matter biomarkers by sequential chemical degradation and gas chromatography – mass spectrometry, *J. Sep. Sci.*, 2007, **30**, 272–282.
57 S. Spielvogel, J. Prietzel, J. Leide, M. Riedel, J. Zemke and I. Kögel-Knabner, Distribution of cutin and suberin biomarkers under forest trees with different root systems, *Plant Soil*, 2014, **381**, 95–110.
58 G. Angst, S. John, C. W. Mueller, I. Kögel-Knabner and J. Rethemeyer, Tracing the sources and spatial distribution of organic carbon in subsoils using a multi-biomarker approach, *Sci. Rep.*, 2016, **6**, 29478.
59 L. L. Reynolds, K. Lajtha, R. D. Bowden, M. M. Tfaily, B. R. Johnson and S. D. Bridgham, The path from litter to soil: insights into soil C cycling from long-term input manipulation and high-resolution mass spectrometry, *J. Geophys. Res. Biogeosci.*, 2018, **123**, 1486–1497.
60 L. vandenEnden, S. D. Frey, K. J. Nadelhoffer, J. M. LeMoine, K. Lajtha and M. J. Simpson, Molecular-level changes in soil organic matter composition after 10 years of litter, root and nitrogen manipulation in a temperate forest, *Biogeochemistry*, 2018, **141**, 183–197.
61 D. Pierson, L. Evans, K. Kayhani, R. D. Bowden, K. Nadelhoffer, M. Simpson and K. Lajtha, Mineral stabilization of soil carbon is suppressed by live roots, outweighing influences from litter quality or quantity, *Biogeochemistry*, 2021, **154**, 433–449.
62 D. Pierson, H. Peter-Contesse, R. D. Bowden, K. Nadelhoffer, K. Kayhani, L. Evans and K. Lajtha, Competing processes drive the resistance of soil carbon to alterations in organic inputs, *Front. Environ. Sci.*, 2021, **9**, 527803.

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63 P. J. Reimer, T. A. Brown and R. W. Reimer, Discussion: Reporting and calibration of post-bomb ^{14}C data, *Radiocarbon*, 2004, **46**, 1299–1304.
64 *Radiocarbon and Climate Change*, ed. E. A. G. Schuur, E. Druffel and S. E. Trumbore, Springer International Publishing, Cham, 2016.
65 B. Jansen, K. G. J. Nierop, J. A. Hageman, A. M. Cleef and J. M. Verstraten, The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes, *Org. Geochem.*, 2006, **37**, 1514–1536.
66 R. T. Bush and F. A. McInerney, Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy, *Geochim. Cosmochim. Acta*, 2013, **117**, 161–179.
67 I. Kögel-Knabner, The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter, *Soil Biol. Biochem.*, 2002, **34**, 139–162.
68 J. L. Harwood and N. J. Russell, in *Lipids in Plants and Microbes*, Springer, 1984, pp. 35–70.
69 I. Fekete, Z. Kotroczó, C. Varga, P. T. Nagy, G. Várbíró, R. D. Bowden, J. A. Tóth and K. Lajtha, Alterations in forest detritus inputs influence soil carbon concentration and soil respiration in a Central-European deciduous forest, *Soil Biol. Biochem.*, 2014, **74**, 106–114.
70 K. Lajtha, K. L. Townsend, M. G. Kramer, C. Swanston, R. D. Bowden and K. Nadelhoffer, Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems, *Biogeochemistry*, 2014, **119**, 341–360.
71 I. Wrightson, M. Tabatabaei Anaraki, I. Fekete, Z. Kotroczó, K. Lajtha and M. J. Simpson, Ecological properties uniquely dictate molecular-level soil organic matter composition in a temperate forest in Central Europe with variation in litter deposition, *Environ. Sci. Process. Impacts*, 2025, **27**, 763–778.
72 G. Guggenberger and K. Kaiser, Dissolved organic matter in soil: challenging the paradigm of sorptive preservation, *Geoderma*, 2003, **113**, 293–310.
73 J. C. Neff and G. P. Asner, Dissolved organic carbon in terrestrial ecosystems: synthesis and a model, *Ecosyst*, 2001, **4**, 29–48.
74 É. Lichtfouse, G. Berthier, S. Houot, E. Barriuso, V. Bergheaud and T. Vallaeys, Stable carbon isotope evidence for the microbial origin of C_{14}-C_{18} *n*-alkanoic acids in soils, *Org. Geochem.*, 1995, **23**, 849–852.
75 Y. Cai, Z. Tang, G. Xiong, Z. Xie, Z. Liu and X. Feng, Different composition and distribution patterns of mineral-protected versus hydrolyzable lipids in shrubland soils, *J. Geophys. Res. Biogeosci.*, 2017, **122**, 2206–2218.
76 J. Jia, Z. Cao, C. Liu, Z. Zhang, L. Lin, Y. Wang, N. Haghipour, L. Wacker, H. Bao, T. Dittmar, M. J. Simpson, H. Yang, T. W. Crowther, T. I. Eglinton, J.-S. He and X. Feng, Climate warming alters subsoil but not topsoil carbon dynamics in alpine grassland, *Glob. Chang. Biol.*, 2019, **25**, 4383–4393.
77 J. I. Hedges and D. C. Mann, The characterization of plant tissues by their lignin oxidation products, *Geochim. Cosmochim. Acta*, 1979, **43**, 1803–1807.
78 M. A. Goñi and J. I. Hedges, Lignin dimers: structures, distribution, and potential geochemical applications, *Geochim. Cosmochim. Acta*, 1992, **56**, 4025–4043.
79 K. Kaiser and R. Benner, Characterization of lignin by gas chromatography and mass spectrometry using a simplified CuO oxidation method, *Anal. Chem.*, 2012, **84**, 459–464.
80 A. Miltner, P. Bombach, B. Schmidt-Brücken and M. Kästner, SOM genesis: Microbial biomass as a significant source, *Biogeochemistry*, 2012, **111**, 41–55.
81 W. R. Wieder, A. S. Grandy, C. M. Kallenbach and G. B. Bonan, Integrating microbial physiology and physiochemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model, *Biogeosciences*, 2014, **11**, 3899–3917.
82 D. L. Jones and D. V. Murphy, Microbial response time to sugar and amino acid additions to soil, *Soil Biol. Biochem.*, 2007, **39**, 2178–2182.
83 Y. Bao, J. Dolfing, B. Wang, R. Chen, M. Huang, Z. Li, X. Lin and Y. Feng, Bacterial communities involved directly or indirectly in the anaerobic degradation of cellulose, *Biol. Fertil. Soils*, 2019, **55**, 201–211.
84 S. Fontaine, S. Barot, P. Barré, N. Bdioui, B. Mary and C. Rumpel, Stability of organic carbon in deep soil layers controlled by fresh carbon supply, *Nature*, 2007, **450**, 277–280.
85 R. P. Phillips, A. C. Finzi and E. S. Bernhardt, Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation, *Ecol. Lett.*, 2011, **14**, 187–194.
86 L. Cheng, F. L. Booker, C. Tu, K. O. Burkey, L. Zhou, H. D. Shew, T. W. Rufty and S. Hu, Arbuscular Mycorrhizal fungi increase organic carbon decomposition under elevated CO_2, *Science*, 2012, **337**, 1084–1087.
87 R. Ghai, C. M. Mizuno, A. Picazo, A. Camacho and F. Rodriguez-Valera, Key roles for freshwater Actinobacteria revealed by deep metagenomic sequencing, *Mol. Ecol.*, 2014, **23**, 6073–6090.
88 J. Deng, Y. Gu, J. Zhang, K. Xue, Y. Qin, M. Yuan, H. Yin, Z. He, L. Wu, E. A. G. Schuur, J. M. Tiedje and J. Zhou, Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska, *Mol. Ecol.*, 2015, **24**, 222–234.
89 N. Mahmoudi, R. R. Fulthorpe, L. Burns, S. Mancini and G. F. Slater, Assessing microbial carbon sources and potential PAH degradation using natural abundance ^{14}C analysis, *Environ. Pollut.*, 2013, **175**, 125–130.
90 R. H. Findlay and D. C. White, Polymeric beta-hydroxyalkanoates from environmental samples and Bacillus megaterium, *Appl. Environ. Microbiol.*, 1983, **45**, 71–78.
91 J. B. Guckert, M. A. Hood and D. C. White, Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: increases in the trans/cis

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ratio and proportions of cyclopropyl fatty acids, *Appl. Environ. Microbiol.*, 1986, **52**, 794–801.
92 D. A. Bossio and K. M. Scow, Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns, *Microb. Ecol.*, 1998, **35**, 265–278.
93 Å. Frostegård, A. Tunlid and E. Bååth, Use and misuse of PLFA measurements in soils, *Soil Biol. Biochem.*, 2011, **43**, 1621–1625.
94 D. L. Wixon and T. C. Balser, Toward conceptual clarity: PLFA in warmed soils, *Soil Biol. Biochem.*, 2013, **57**, 769–774.
95 J. M. Melillo, S. D. Frey, K. M. DeAngelis, W. J. Werner, M. J. Bernard, F. P. Bowles, G. Pold, M. A. Knorr and A. S. Grandy, Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world, *Science*, 2017, **358**, 101–105.
96 J. I. Hedges, R. A. Blanchette, K. Weliky and A. H. Devol, Effects of fungal degradation on the CuO oxidation products of lignin: a controlled laboratory study, *Geochim. Cosmochim. Acta*, 1988, **52**, 2717–2726.
97 S. Opsahl and R. Benner, Early diagenesis of vascular plant tissues: lignin and cutin decomposition and biogeochemical implications, *Geochim. Cosmochim. Acta*, 1995, **59**, 4889–4904.
98 T. Klotzbücher, S. Strohmeier, K. Kaiser, R. D. Bowden, K. Lajtha, H. Ohm and K. Kalbitz, Lignin properties in topsoils of a beech/oak forest after 8 years of manipulated litter fall: relevance of altered input and oxidation of lignin, *Plant Soil*, 2013, **367**, 579–589.
99 I. Wrightson, M. Tabatabaei Anaraki, J. Den Uyl, K. J. Nadelhoffer, K. Lajtha and M. J. Simpson, Changes in litter and nitrogen deposition differentially alter forest soil organic matter biogeochemistry, *Geochem. Cosmochim. Acta*, 2024, **374**, 186–199.

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