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Soil organic matter molecular composition with long-term detrital alterations is controlled by site-specific forest properties

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Abstract

Forest ecosystems are important global soil carbon (C) reservoirs, but their capacity to sequester C is susceptible to climate change factors that alter the quantity and quality of C inputs. To better understand forest soil C responses to altered C inputs, we integrated three molecular composition published data sets of soil organic matter (SOM) and soil microbial communities for mineral soils after 20 years of detrital input and removal treatments in two deciduous forests: Bousson Forest (BF), Harvard Forest (HF), and a coniferous forest: H.J. Andrews Forest (HJA). Soil C turnover times were estimated from radiocarbon measurements and compared with the molecular-level data (based on nuclear magnetic resonance and specific analysis of plant- and microbial-derived compounds) to better understand how ecosystem properties control soil C biogeochemistry and dynamics. Doubled aboveground litter additions did not increase soil C for any of the forests studied likely due to long-term soil priming. The degree of SOM decomposition was higher for bacteria-dominated sites with higher nitrogen (N) availability while lower for the N-poor coniferous forest. Litter exclusions significantly decreased soil C, increased SOM decomposition state, and led to the adaptation of the microbial communities to changes in available substrates. Finally, although aboveground litter determined soil C dynamics and its molecular composition in the coniferous forest (HJA), belowground litter appeared to be more influential in broadleaf deciduous forests (BH and HF). This synthesis demonstrates that inherent ecosystem properties regulate how soil C dynamics change with litter manipulations at the molecular-level. Across the forests studied, 20 years of litter additions did not enhance soil C content, whereas litter reductions negatively impacted soil C concentrations. These results indicate that soil C biogeochemistry at these temperate forests is highly sensitive to changes in litter deposition, which are a product of environmental change drivers.

KEYWORDS

cutin, DIRT, lignin, nuclear magnetic resonance, phospholipid fatty acids, radiocarbon, soil carbon, suberin

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1 | INTRODUCTION

Forest ecosystems store as much as 40% of the global soil carbon (C) stocks (Janzen, 2004; Mayer et al., 2020; Wei et al., 2014) over 4 billion hectares of the Earth's land surface (FAO & UNEP, 2020). The future of these soil C reserves is threatened by many environmental changes that impact the dynamic balance of the soil C pool (Lal, 2005; Reichstein, 2007; Song et al., 2019), most of which occur via altered plant C inputs to the soil. For example, elevated atmospheric carbon dioxide (CO_2) levels are often associated with increases in plant biomass that generally occur at the expense of reduced soil C stocks (Terrer et al., 2021). Elevated CO_2 conditions can also impact plant C and nutrient allocation between plant shoots and roots, thus changing the quantity and quality of the above- and belowground litter inputs to the soil (Gifford et al., 2000; Pendall et al., 2004). Similarly, other environmental factors such as increasing temperatures (Kirschbaum, 2000; Smith et al., 2008; Zhao et al., 2017), and higher nutrient availability (Matson et al., 1999; Quinn Thomas et al., 2010; Yan et al., 2020) may alter plant productivity, plant litter decomposability and soil organic matter (SOM) decomposition with long-lasting impacts on the soil C pool of forests. Decreases in soil C with litter removal and increases with enhanced litter inputs seem to be a common response of most ecosystems across the globe (Feng et al., 2022; Xu et al., 2013, 2021). However, temperate forests from the detrital input and removal treatment (DIRT) network exhibit distinct responses to litter additions, showing instead decreases in soil C even after 20 years of doubled litter additions (Lajtha et al., 2018). The lack of C accumulation at these sites has been attributed to positive soil priming, referring to the microbial stimulation of SOM decomposition prompted by the fresh litter additions (Kuzyakov et al., 2000; Lajtha et al., 2018; Pisani et al., 2016; Wang et al., 2017). The conflicting results from DIRT temperate forests demonstrate that the relationship between ecosystem productivity, plant litter inputs, and soil C accumulation is not always linear nor easily predictable due to the many factors controlling SOM dynamics. Xu et al. (2021) list the dominant tree species and their mycorrhizal symbionts as well as soil type and N availability as potential drivers of the responses of temperate forests to litter additions. A recent metanalysis by Feng et al. (2022) suggested that the relatively higher initial soil C status of these forests before litter manipulations and the soil C saturation capacity along with comparably lower litter input rates at DIRT sites drive the lack of soil C increases with doubled litter inputs. Current global estimations by Georgiou et al. (2022) confirm the relatively high C saturation and low C accrual rates for these DIRT temperate forests in comparison with other temperate forests (Figure 1c). Although C is not accumulating in soil with doubled litter inputs in temperate DIRT forests, the SOM molecular composition and soil microbial communities might be responding to higher litter inputs. Similarly, decreases in soil C with litter removals can have an impact on SOM molecular composition and soil microbes in unique ways that are not captured in routine soil C measurements. Accounting for the complex nature of SOM can shed light on the mechanisms driving the observed responses to litter manipulations in these forests as distinct components of the SOM pool and soil microbial communities may respond differently to global climate change factors, ecosystem properties, and management (Feng & Simpson, 2011; Kögel-Knabner, 2017; Simpson & Simpson, 2012).

The DIRT network has experimental sites spanning across climatic conditions, forests, and soil types (Lajtha et al., 2018; Nadelhoffer et al., 2004). Here, we focus on three forests with different vegetation composition for which SOM molecular-level data sets and soil radiocarbon measurements in mineral surface soils (0–10 cm) after 20 years of litter manipulation are available. Two temperate deciduous forests, Bousson Forest (BF) in Pennsylvania and Harvard Forest (HF) in Massachusetts, and H.J. Andrews Forest (HJA), a coniferous temperate forest in Oregon (Figure 1b). Despite contrasting ecosystem and soil properties (Figure 1c), soil C responses were similar with double litter additions whereby soil C did not increase in comparison with the control plots (CTs) while it decreased with litter removal treatments (above-, belowground, or both) (Bowden et al., 2014; Lajtha et al., 2014; Pierson et al., 2021). Across all sites, the exclusion of aboveground C inputs led to slightly higher C losses, whereas the removal of belowground C inputs did not have impacts on soil C, which points toward a greater control of aboveground litter inputs on soil C dynamics (Bowden et al., 2014; Lajtha et al., 2014; Pierson et al., 2021) despite the general idea that root inputs are more persistent in soil, thus having a greater contribution to stable soil C (Angst et al., 2016; Lützow et al., 2006; Rasse et al., 2005; Spielvogel et al., 2014). Other lines of evidence suggest that root-derived compounds degrade in soil at rates similar to leaf-derived compounds (cutin) (Angst et al., 2016) or that suberin can decompose faster than cutin (Feng et al., 2008) and that the preferential use of root- versus leaf- derived compounds can be site-specific (Crow et al., 2009).

Our objective with this synthesis is to assess changes in the response of the molecular composition of SOM and soil microbial communities to altered litter inputs across temperate forests in the Northern hemisphere. These temperate forest sites exhibit a gradient of N availability, atmospheric N deposition rates, and aboveground litter chemistry, with higher N availability at BF, followed by HF and lastly, HJA (Figure 1c). This fertility gradient can influence the responses of soil microbial community composition, the molecular biogeochemistry of the SOM and their responses to litter manipulations. Despite the similarities in the response of soil C to litter additions and removals after 20 years of manipulations across sites, we postulate that SOM components as well as soil microbial communities will have unique responses to altered litter inputs that will be partly determined by the availability of N at each site. Generally, higher N availability is expected to favor bacterial growth over fungal growth as bacteria—as *r*-strategists—can more rapidly degrade high quality (low C:N) substrates (Feng et al., 2010; Hicks et al., 2021; Leite et al., 2017; Rousk et al., 2011; Rousk & Frey, 2015; Wallenstein et al., 2006). Thus, we expect that doubled high-quality (low C:N) litter additions in sites with higher soil N availability and bacteria-dominated microbial communities will lead to higher SOM decomposition, soil microbial biomass, and more significant decreases in

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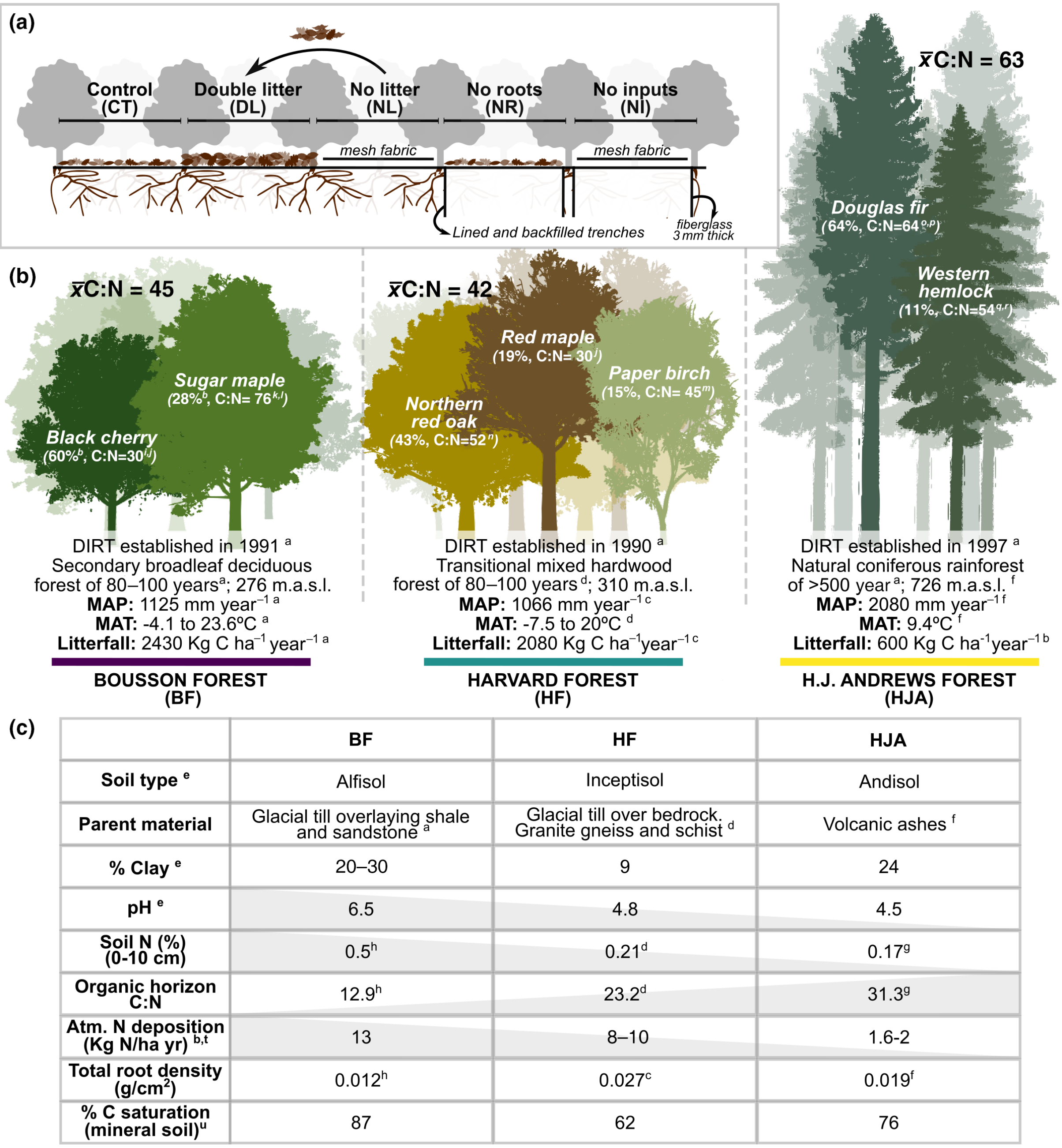

	BF	HF	HJA
Soil type [e]	Alfisol	Inceptisol	Andisol
Parent material	Glacial till overlaying shale and sandstone [a]	Glacial till over bedrock. Granite gneiss and schist [d]	Volcanic ashes [f]
% Clay [e]	20–30	9	24
pH [e]	6.5	4.8	4.5
Soil N (%) (0-10 cm)	0.5[h]	0.21[d]	0.17[g]
Organic horizon C:N	12.9[h]	23.2[d]	31.3[g]
Atm. N deposition (Kg N/ha yr) [b,t]	13	8–10	1.6-2
Total root density (g/cm^2)	0.012[h]	0.027[c]	0.019[f]
% C saturation (mineral soil)[u]	87	62	76

FIGURE 1 (a) Core experimental design of the detrital input and removal treatment network. Ecosystem (b) and soil properties (c) at the Forest sites. MAP: mean annual precipitation, MAT: mean annual temperature, N: nitrogen. In (b), percentages in parentheses represent the relative abundance of each species at each site and its litter C:N ratio, whereas $\bar{x}$C:N represents the weighted averages of leaf C:N of the dominant tree species. Data from [a]Bowden et al. (2014), [b]Crow et al. (2009), [c]Fanin et al. (2020), [d]Lajtha et al. (2014), [e]UC Davis Soil Resource Laboratory (2021), [f]Pierson et al. (2021), [g]Man et al. (2022), [h]BF 20 year harvest unpublished data, [i]Schwaner and Kelly (2019), [j]Adams and Angradi (1996) [k]Corrigan (2008) [l]King et al. (2001) [m]Parsons et al. (2004), [n]Stanek et al. (2020), [o]Perakis et al. (2012), [p]Yano et al. (2005), [q]Edmonds (1980), [r]Edmonds and Thomas (1995), [s]Acker et al. (2003), [t]Vanderbilt et al. (2003), [u] Georgiou et al. (2022).

concentrations of plant-derived compounds in soils in comparison with fungal-dominated sites with lower N availability and doubled low-quality (high C:N) litter inputs. Soil Δ^{14}C values reflect the time since C atoms were fixed by plants (Trumbore, 2009) and so, with doubled fresh litter inputs, Δ^{14}C values might increase as fresh litter accumulates in soil. However, congruent with the observed positive soil priming at these sites (Lajtha et al., 2018), soil Δ^{14}C values might instead decrease with doubled litter inputs as fresh litter is rapidly decomposed, a phenomenon that might be more marked in the high N sites where litter C:N is lower and litter inputs are processed more rapidly.

Litter exclusion treatments, on the other hand, would lead to the accumulation of more processed and complex C compounds, thus further increasing SOM decomposition state accompanied by

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decreases in cutin-, suberin-, and lignin-derived compounds as all litter inputs are reduced, along with lower microbial biomass and lower $\Delta^{14}C$ values. Yet, the impact of litter removal inputs should be stronger on high N availability sites as soil microbial communities at these sites can be more dependent on the low C:N plant-derived inputs than soil microbial communities from low N availability sites, more adapted to low availability of preferred C sources and low-nutrient substrates. Finally, although higher contributions to soil C accumulation from roots versus aboveground litter are generally expected, the available evidence from these DIRT sites shows that belowground litter reductions do not significantly alter soil C, whereas aboveground litter reductions decreased soil C (Lajtha et al., 2018). Therefore, this should be reflected in higher SOM decomposition state, increase degradation of lignin-, cutin-, and suberin-derived compounds and lower $\Delta^{14}C$ values for aboveground litter removal plots and not belowground litter removal plots across all sites. However, Crow et al. (2009) concluded from the 10 year DIRT analysis that root-derived C had greater contribution to stable C pools in deciduous forests, such as BF, whereas leaf-derived C defined in greater measure the stable C pools in coniferous forests, such as HJA. With our unique SOM molecular-level multiproxy approach, we aim to ascertain what other indicators point out to a preferential persistence of root-derived C in deciduous forests, whereas leaf-derived C persistence in coniferous forests.

2 | MATERIALS AND METHODS

2.1 | DIRT experimental design

The core DIRT experimental design includes the variation in above and belowground inputs via: doubled aboveground litter additions (DL), no aboveground litter (NL), no roots (NR) achieved by trenching, and no C inputs (NI) (Figure 1a). CTs receive ambient detrital inputs. Replicated plots (3 m × 3 m for BF and HF and 10 m × 15 m for HJA. $N = 3$) are located beneath the forest canopy. Aboveground litter is excluded in NL plots with a mesh fabric, and the collected litter is added to the DL plots. Root growth in NR plots is prevented by the installation of inert barriers, while the NI treatment is a combination of NL and NR plots (Nadelhoffer et al., 2004).

2.2 | Molecular composition of SOM determined by targeted compound analysis and nuclear magnetic resonance spectroscopy

Targeted analysis of numerous plant- and microbial-derived compounds from BF, HF, and HJA DIRT experiments collected after 20 years of experiment was compiled into one single database. The detailed protocols and original data sets for these analyses are published and discussed in detail in Pisani et al. (2016), Wang et al. (2017) and Man et al. (2022). Briefly, soil cores were collected from each experimental plot (two for BF and HF, and six for HJA) and combined into a composite sample, freeze-dried, sieved to 2 mm, and ground for sequential chemical extractions, microbial phospholipid fatty acid (PLFA) analyses, and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. For HF and HJA samples, sequential chemical extractions and PLFA analyses were conducted for two analytical replicates per composite sample and averaged for each plot ($n = 3$). For BF, there were no analytical replicates, and all analyses were performed on one composite sample per plot ($n = 3$). For all sites, NMR analyses were performed on a single composite sample prepared per treatment and CTs. Previous published articles and databases from all sites were consulted to obtain information about the ecosystems and soil properties (Figure 1b,c).

Sequential chemical extractions included solvent extraction followed by base hydrolysis and copper (II) oxide (CuO) oxidation (Otto et al., 2005; Otto & Simpson, 2006, 2007). Total solvent extractable compounds included microbial-derived short-chain aliphatic and plant-derived, long-chain aliphatic compounds, total acyclic and cyclic lipids as well as total sugars, total free lipids, and the plant-derived steroid ratio calculated as degradation products over their precursors with higher values indicating increased degradation (Otto & Simpson, 2005) (Table S1). Variables from base hydrolysis included leaf/needle-derived cutin, root-derived suberin, and microbial-derived lipids (Cai et al., 2017; Jia et al., 2019) as well as the suberin to cutin ratio, that increases with preferential degradation of cutin over suberin (Bahri et al., 2006; Wang et al., 2018); the cutin degradation ratio ($\omega C_{16}/C_{16}$) as the relative change of C_{16} ω-hydroxy-alkanoic acids to all hydrolysable C_{16} fatty acids, increasing with higher cutin degradation (Goñi & Hedges, 1990; Otto & Simpson, 2006); and the mid-chain-substituted acids to total suberin and cutin ratio (Σmid/Suberin + Cutin) that decreases with higher cutin and suberin degradation (Otto & Simpson, 2006) (Table S2). Lignin-derived compounds isolated from CuO oxidation include: vanillyls, syringyls, cinnamyls, total sum of vanillyls, syringyls, and cinnamyls, and ratios such as syringyls/vanillyls and cinnamyls/vanillyls that characterize the predominant botanical origin of lignin in soil. Finally, lignin oxidation ratios: acid-to-aldehyde ratio for lignin-derived vanillyls $[(Ad/Al)_V]$ and syringyls $[(Ad/Al)_S]$ increase with higher lignin decomposition (Hedges & Mann, 1979) (Table S3).

Microbial PLFAs were extracted using a modified Bligh–Dyer method (Bligh & Dyer, 1959; Frostegård & Bååth, 1996) and from this analysis, we compiled information of the total microbial biomass and key microbial groups including fungi, Actinobacteria, Gram-positive, and Gram-negative bacteria. Microbial community composition ratios were also compiled across all sites. These ratios included the fungi-to-bacteria ratio; and the Gram-negative to Gram-positive and Actinobacteria ratio. The Gram-negative to Gram-positive bacteria ratio is used as an indicator of C availability in soils (Fanin et al., 2019). Here, we added Actinobacteria and Gram-positive bacteria in the denominator as these microbial groups are known to preferentially access complex forms of C in soil (Deng et al., 2015; Ghai et al., 2014; Su et al., 2020). Finally, we also included the microbial stress ratio, a measurement of microbial stress, calculated as cyclopropane PLFA to its monoenoic precursor since

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higher cyclopropane PLFA is produced with substrate limitations or other stressors such as changes in temperature or moisture (Bossio & Scow, 1998). In this study, we compare stress ratios with ambient conditions (CTs) and anticipate that a higher microbial stress ratio is indicative of preferred substrate C availability (Table S4) or changes in community composition and structure (Frostegård et al., 2011).

Finally, NMR spectroscopy variables correspond to four main chemical shift regions: alkyl C (0–50 ppm), *O*-alkyl C (50–110 ppm), aromatic and phenolic C (110–165 ppm), and carboxyl and carbonyl C (165–215 ppm). The ratio of alkyl/*O*-alkyl C was calculated as a measurement of the SOM decomposition state, increasing with higher SOM degradation since *O*-alkyl C components are preferentially degraded over alkyl C components (Baldock & Preston, 1995). A second ratio was calculated with alkyl C + aromatic and phenolic C over *O*-alkyl C + carboxylic and carbonyl C as a complementary measurement of SOM decomposition. The resistance to decomposition ratio also increases with higher utilization of preferred substrates such as cellulose (*O*-alkyl) and carbonyl and carboxyl C versus less preferred substrates such as plant-derived lipids (alkyl C) and lignin (aromatic and phenolic C) (Ostertag et al., 2008). For consistency, all data and ratios were revised from raw data to obtain a homogenous database for the purposes of this study. All variables, except those from NMR analysis, are expressed in µg/g dry soil.

2.3 | Soil radiocarbon analysis and estimation of SOM turnover times

Bulk SOM radiocarbon data were obtained and estimated C turnover time calculated to examine changes in SOM dynamics. Fraction modern C ($F^{14}C$) was determined on composite samples of combined field replicates per treatment and forest site ($n = 15$) using a 3MV tandem accelerator mass spectrometer by the A.E. Lalonde AMS Laboratory in Ottawa, Ontario. $F^{14}C$ was calculated based on Reimer et al. (2013) as the ratio of the sample $^{14}C/^{12}C$ ratio to the standard $^{14}C/^{12}C$ ratio (Oxalic acid, Ox-II) measured concurrently with our samples. $\Delta^{14}C$ values were calculated by correcting this ratio by the radiocarbon decay of the standard up to the year of sample collection: 2011 (BF), 2010 (HF) and 2017 (HJA) (Schuur et al., 2016). The $^{14}C/^{12}C$ ratios of both samples and standards were background-corrected, and the result further corrected for spectrometer and preparation fractionation using AMS measured $^{13}C/^{12}C$ ratio and normalized to $\delta^{13}C$ (PDB) following Crann et al. (2017). To determine carbon turnover times (in years, equal to the inverse of the turnover rate, k), we assumed a steady state system and used the following equation from Torn et al. (2009):

$$C_t \times F'_{C,t} = I \times F'_{atm,t} + C_{t-1} \times (1 - k - \lambda) \times F_{C,t-1} \tag{1}$$

where C_t and C_{t-1} are organic carbon contents of the soil sampled in year t and 1 year before $t-1$. I is plant-derived carbon input during the sampling year, $F'_{C,t}$ is the corrected measured radiocarbon of each sample by radiocarbon decay ($^{14}C/1000+1$), $F'_{atm,t}$ is the atmospheric $F^{14}C$ values from 1950 to the year of sampling and λ is the radioactive decay constant of $^{14}C = 1.210 \times 10^{-4}$ year^{-1}. For a steady-state reservoir ($C_t = C_{t-1}$), k equals I divided by the inventory of carbon at year t (I/C_t) then Equation (2) is derived from Equation (1):

$$F'_{C,t} = k \times F'_{atm,t} + (1 - k - \lambda) \times F'_{C,t-1} \tag{2}$$

Based on Equation (2) a numerical model was constructed in MATLAB 2015 (MathWorks, Inc.) to calculate the optimal k to fit the measured $F^{14}C$ values of the soil samples. For this, atmospheric $F^{14}C$ values ($F'_{atm,t}$) of the Northern Hemisphere were retrieved from the literature (Hammer & Levin, 2017; Hua et al., 2013; ICOS, 2019; Levin et al., 2013; Levin & Kromer, 2004) considering 1 year time lag from the soil sampling at each site, based on the assumption that most C inputs to the soil come from the decomposition of litter in the previous year. In addition, the atmospheric $F^{14}C$ values used were an average of measurements from summer months (May, June, July, August) for each year. The optimal k value to calculate the most likely turnover time was then selected as the value representing the smallest difference between the corrected $F'^{14}C$ values and the measured $F'^{14}C$ in soil. Samples with $\Delta^{14}C$ above 20‰ yielded two possible turnover times (Table 1) and both values are reported, but for our interpretations, longer turnover times were used. Finally, error values (as standard deviation) were calculated using a Monte Carlo simulation (200 runs) of the corrected $F^{14}C$. The assumption of a steady-state system used to model the radiocarbon turnover times at these DIRT sites needs to be interpreted with caution as litter manipulations alter the assumed steady state of the system. However, we use this here as an exploratory tool to compare the relative change of the turnover times across treatments as previously done by Crow et al. (2009) for two of these DIRT sites.

2.4 | Statistical analyses

All statistical analyses were performed in R version 3.6.0 (R Core Team, 2019) unless otherwise stated. Analytical replicates, when available, were averaged per plot (field replicate) within each site ($n = 3$). We tested the impact of the DIRT treatments within each site and the impact of both treatment and site on all variables. For each case, two models were built: a simple linear effect model (using the package "stats," base R) and a linear mixed effect model where plot was added as a random effect (using the package "lme4"; Bates et al., 2021). Using the Akaike Information Criterion, the second model in which plot was included as a random effect, was deemed more appropriate in all cases to test the impact of treatment or treatment and site on the assessed variables. We performed a one-way and two-way analyses of variance (ANOVA) to test the impact of treatment within each site and the impact of treatment and site across the complete database using the ANOVA function from the "car" package (Fox et al., 2021) with Kenward–Roger degrees of freedom followed by multiple mean comparisons of all treatments using the Tukey test with the Bonferroni correction with the "glht" function from the "multcomp" package (Hothorn et al., 2021). Finally, as

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TABLE 1 Summary of soil C content, radiocarbon data, and turnover time estimates from mineral soil (0–10 cm) from the Bousson Forest (BF), Harvard Forest (HF), and H.J. Andrews Forest (HJA) for the control (CT) and litter manipulation treatments: double litter (DL), no litter (NL), no roots (NR), and no inputs (NI)

	BF			HF			HJA		
Treatment	%C†	Δ^{14}C (‰)	Turnover (years)	%C†	Δ^{14}C (‰)	Turnover (years)	%C	Δ^{14}C (‰)	Turnover (years)
CT	6.0 ± 0.6	−8.77 ± 2.58	378 ± 41	7.2 ± 0.7	38.53 ± 2.94	195 ± 8	5.0 ± 0.8	35.52 ± 2.85	199 ± 8^{b}
DL	5.7 ± 0.6	−14.69 ± 2.68	407 ± 25	6.9 ± 0.2	47.44 ± 3.02	172 ± 6^{a}	4.9 ± 1.0	20.83 ± 2.64	245 ± 9^{c}
NL	5.0 ± 0.7	−22.67 ± 2.70	453 ± 15	5.8 ± 0.8	16.22 ± 2.82	265 ± 10	4.7 ± 0.5	28.62 ± 2.75	218 ± 9^{d}
NR	5.7 ± 0.8	−14.66 ± 2.55	411 ± 25	6.8 ± 0.8	19.52 ± 2.95	254 ± 10	5.1 ± 0.5	8.00 ± 2.62	295 ± 11
NI	3.9 ± 0.6	−66.03 ± 2.70	763 ± 48	6.2 ± 0.5	27.36 ± 2.98	227 ± 10	4.1 ± 0.3	−5.08 ± 2.61	357 ± 12

Note: Turnover times were estimated using a limited steady-state model and are not intended to represent absolute turnover times but used to compare relative turnover shifts due to litter additions or exclusion relative to ambient conditions. Alternate turnover times were generated for samples with Δ^{14}C above 40‰ for HF: ^{a}3 ± 1; and above 20‰ for HJA: ^{b}7 ± 1, ^{c}4 ± 1, ^{d}6 ± 1. Errors for turnover years are standard deviation, for all other variables, error expressed as standard error.

†%C values taken from Wang et al. (2017) for BF, Pisani et al. (2016) for HF and Man et al. (2022).

a broader approach to capture differences between the treatments against the control within each site, we performed a Dunnett's test in paired comparisons using the "DunnettTest" function form the "DescTools" package (Signorell et al., 2021).

3 | RESULTS

3.1 | Long-term litter addition (double litter)

The lack of net increases in soil C after 20 years of DIRT at any of the three forests (Table 1) has been proposed as evidence for soil priming prompted by the doubled litter inputs and supported by observations of increased soil respiration in DL plots for all sites (Lajtha et al., 2018). Here, we also detected higher SOM decomposition state and higher SOM resistance to decomposition in DL plots from the NMR analysis across all sites (Figure 2a,b). Higher values of these ratios in DL plots further supports the idea of a positive soil priming by doubled litter inputs as fresh litter is accelerating soil microbial decomposition of preferred C components while more complex C substrates are accumulated. Aligned with this, increases in microbial biomass and microbial-derived lipids alongside decreases in cutin-, suberin- and lignin-derived compounds and lower Δ^{14}C values and longer turnover times in DL plots would collectively support the use of older, previously stabilized SOM, as unequivocal evidence of positive soil priming. We were also expecting these trends to be more significant for bacterial-dominated sites with higher N availability (BF and HF). However, only BF was dominated by bacteria, whereas the microbial community at HF was dominated by fungi instead (Figure 3c) likely due to the acidic soil conditions at HF that generally favor fungal growth (Silva-Sánchez et al., 2019) and act as an environmental filter facilitating fungal growth rather than bacterial growth. Increases in microbial biomass (Figure 3a) and microbial-derived lipids (Figure 4c) with DL were only detected in fungal dominated sites (HF and HJA). Moreover, increases in lignin oxidation for DL plots were observed at BF and HF (Figure 5a), whereas lignin oxidation decreased instead with doubled litter for HJA (Figure 5a). Cutin concentrations increased with DL only at HF (Table S2) while suberin concentrations were unaltered by doubled litter additions across all sites (Table S2). Finally, lower Δ^{14}C values and longer turnover times in DL plots were detected at BF and HJA but not at HF, were higher Δ^{14}C values and shorter turnover times in DL plots were observed instead (Table 1).

3.2 | Long-term above- and belowground litter exclusion

As expected, litter removal treatments (NR, NL, and NI) led to increased SOM decomposition, particularly for NI plots, which had more than 25% higher decomposition ratio than CTs across all sites (Figure 2a). A higher degradation ratio (more than 25% increase) was also detected in NL and NR plots at HF (Figure 2a). As expected, the exclusion of fresh litter inputs (above-, belowground, or both) led to the accumulation of more processed and complex C compounds o and thus, higher SOM resistance to decomposition was also detected in litter removal treatments for all sites (Figure 2b). In parallel, lower Δ^{14}C values and longer calculated turnover times were observed for all litter exclusion treatments in comparison with CTs across all sites (Table 1). The persistence of older SOM is traditionally associated with higher C substrate complexity, which is congruent with the higher SOM decomposition state and SOM resistance to decomposition (Figure 2a,b). A growing body of evidence also suggests that longer radiocarbon turnover times do not necessarily imply longer environmental persistence but instead relates to higher microbial processing of preferred C substrates (Gleixner, 2013; Kleber et al., 2011; Trumbore, 2009). Increases in alkyl C are likely associated not only with accumulation of complex C substrates like plant waxes, cutin, and suberin but also with newly synthesized C compounds that result from microbial processing of SOM (Baldock

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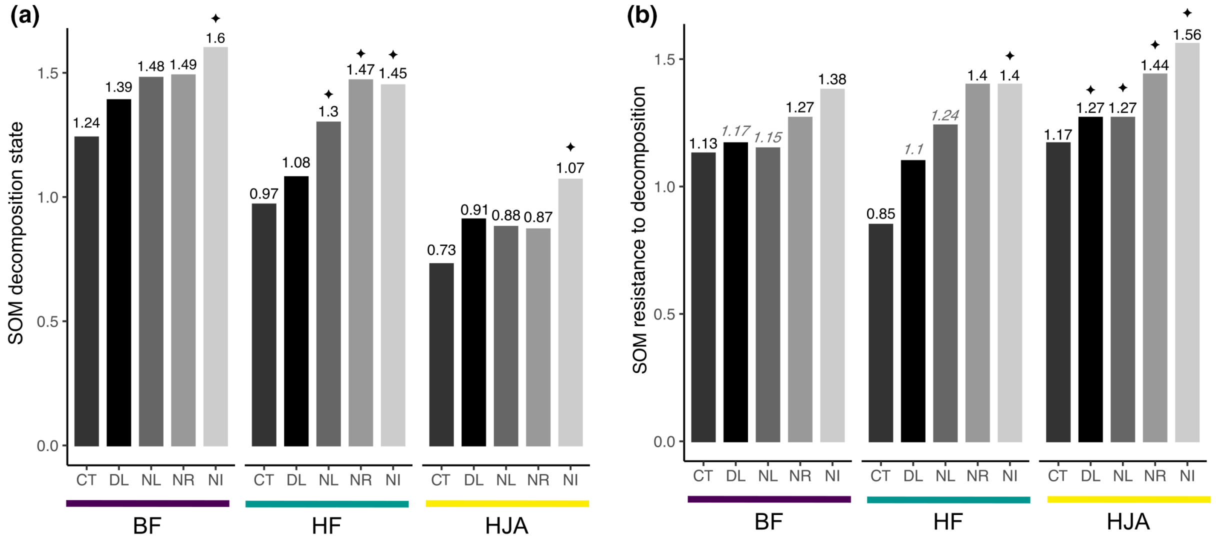

(c)

Site	BF					HF					HJA				
Treatment	CT	DL	NL	NR	NI	CT	DL	NL	NR	NI	CT	DL	NL	NR	NI
Alkyl	35	*38*	39 ↑	41 ↑	42 ↑	38	*39*	43 ↑	47 ↑	45 ↑	27	31 ↑	*29*	*26*	30 ↑
O-alkyl	28	*28*	*26*	*27*	*26*	52	39 ↓	36 ↓✦	33 ↓✦	32 ↓✦	37	*34*	33 ↓	30 ↓	28 ↓
Aromatic + Phenolic	18	16 ↓	14 ↓	15 ↓	16 ↓	14	*15*	*14*	12 ↓	*14*	27	25	*27*	33 ↑	31 ↑
Carboxyl + Carbonyl	19	*18*	*20*	17 ↓	16 ↓	9	10 ↑	10 ↑	*9*	10 ↑	9	*10*	11 ↑	11 ↑	11 ↑

FIGURE 2 (a) Soil organic matter (SOM) decomposition state ratio (alkyl/*O*-alkyl carbon), (b) SOM resistance to decomposition ratio (alkyl + aromatic and phenolic)/(*O*-alkyl + carboxyl and carbonyl carbon), and (c) associated data from solid-state ^{13}C nuclear magnetic resonance spectroscopy for the mineral soil (0–10 cm) from the Bousson Forest (BF), Harvard Forest (HF), and H.J. Andrews Forest (HJA) for the control (CT) and litter manipulation treatments: double litter (DL), no litter (NL), no roots (NR), and no inputs (NI). Gray numbers in italics represent less than a 10% difference compared with the control, whereas numbers in black represent more than a 10% difference compared with the control and ✦ denotes more than 25% compared with the control. Arrows pointing upward represent increases in comparison with the control, whereas arrows pointing downward represent decreases versus the control. As decomposition increases, higher use of preferred C and the accumulation of more persistent forms of SOM also increases. Therefore, higher SOM decomposition state is related to a higher resistance to decomposition.

et al., 1992; Kögel-Knabner et al., 1991; Marín-Spiotta et al., 2008). Therefore, our observation of higher SOM decomposition and thus higher resistance to decomposition (Figure 2a,b) along with longer radiocarbon turnover times at litter reduction plots may also indicate enhanced microbial processing of more available complex C sources via an adaptation to substrate limitations that facilitate sustain microbial metabolic activity. This also agrees with relatively higher microbial-derived lipids in litter reduction plots at both HF and HJA (Figure 4b), which is a proxy for microbially processed SOM residues (see section discussing these microbial-derived lipids in more detail).

The way each forest adapted to low C availability in litter exclusion plots differed according to the N status and the dominant microbial community at each site. We were expecting decreases in SOM compounds (i.e. lignin, cutin and suberin) as well as negative impacts on microbial biomass and would be stronger where N availability was higher. Indeed, we found that soil microbial biomass decreased in litter exclusion plots at the bacterial-dominated site (BF), whereas it increased at fungal-dominated sites (HF and HJA). Higher dependence of bacteria on labile C inputs (Butler et al., 2003; Chen et al., 2016; de Boer et al., 2005) was likely driving these decreases in soil microbial biomass, particularly at NR and NI treatments (Figure 3a), where the exclusion of readily decomposable C in leaf litter evidenced by significant decreases in solvent extractable short-chain aliphatic lipids and simple sugars (Table S1). Microbial community composition shifted at this site toward microbial groups with higher capacity to process complex C such as Gram-positive and Actinobacteria (Figure 3c) (Deng et al., 2015; Ghai et al., 2014; Su et al., 2020). These compositional changes facilitated the degradation of more complex C substrates, such as lignin, which presented higher oxidation state in litter removal plots (Figure 5a), consistent with lower aromatic + phenolic C from NMR analysis (Figure 2c).

Similar to BF, litter exclusion treatments at the fungal-dominated HF site led to decreases in fast-cycling C components (Table S1) and altered microbial community composition with increases in Gram-positive and Actinobacteria abundance as previously detected at this forest as a microbial adaptation strategy to reduced preferred C substrates, consequence of soil warming treatments (Melillo et al., 2017). Yet, soil microbial biomass tended to increase here rather than decrease (Figure 3a), and microbial community composition shifted to higher fungal abundance (higher fungi/bacteria ratio; Figure 3c) and

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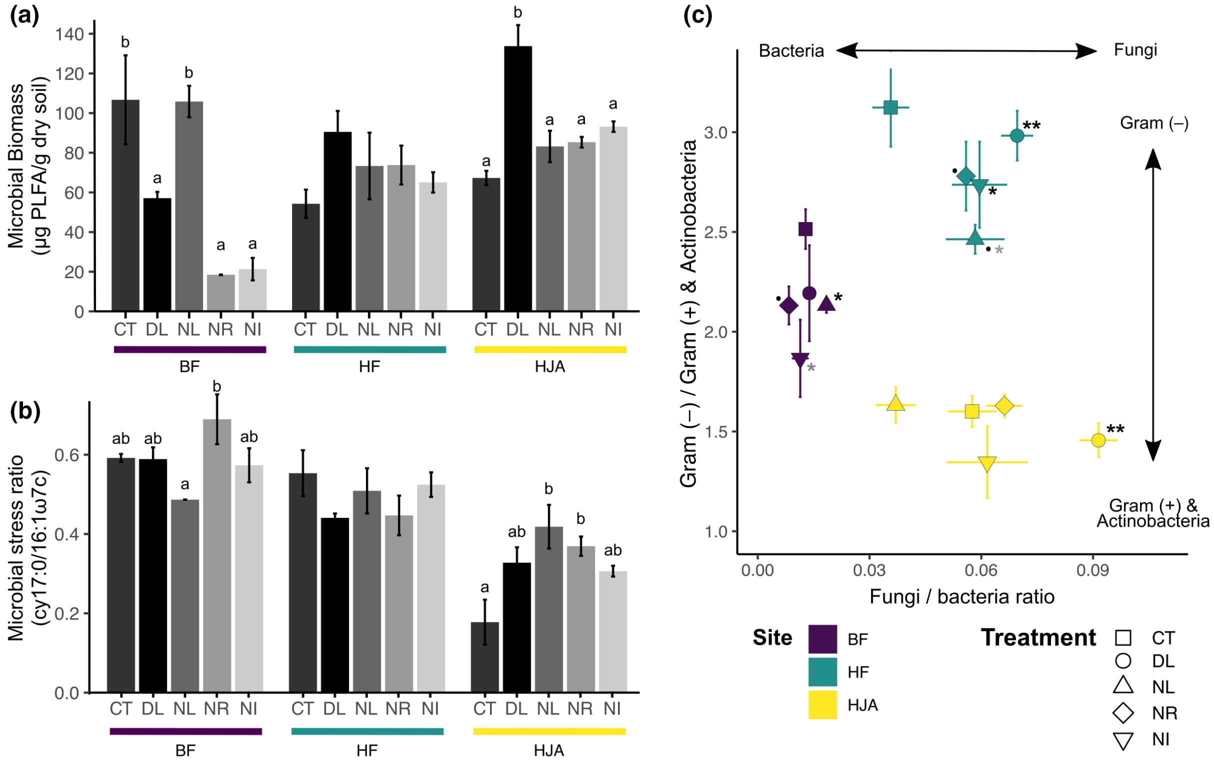

FIGURE 3 (a) Microbial biomass measured via phospholipid fatty acid (PLFA) profiling (µg PLFA/g dry soil), (b) microbial stress ratio and (c) microbial community composition as the ratio of Gram negative (Gram −)/ Gram positive (Gram +) and Actinobacteria on the *y*-axis and fungi-to-bacteria ratio. From mineral soil (0–10 cm) from the Bousson Forest (BF), Harvard Forest (HF), and H.J. Andrews Forest (HJA) for the control (CT) and litter manipulation treatments: double litter (DL), no litter (NL), no roots (NR), and no inputs (NI). For (c), significant differences between treatments and control (Dunett's test) on the *y*-axis shown in gray and significant differences on the *x*-axis shown in black. Significance levels: ≤.1 (•), ≤.05 (*), ≤.01 (**). For (b), different letters show significant differences $p < .05$ from Tukey's test, no letters mean no significant differences.

higher cutin degradation was observed instead of higher lignin decomposition (Figure 5a,b). At the HF site, overall higher cutin availability in comparison with the other sites (Table S2) points to the preferential use of this OM source by the fungal-dominated community. Selective preservation of cutin can occur in substrates with high lignin contents (Angst et al., 2016) and lower relative abundance of extractable lignin phenols relative to cutin at HF in comparison with BF (mean ± SE: extractable lignin/cutin_HF = 82.75 ± 10.30; extractable lignin/cutin_BF = 36.66 ± 4.75) could have also facilitated the preferential use of cutin at HF, whereas enhancing its protection from degradation at BF.

Litter exclusion treatments at the coniferous low N-availability forest (HJA) significantly increased microbial biomass (Figure 3a) and decreased cutin and lignin oxidation (Figure 5b). Substrate limitations at this site did not lead to changes in microbial community composition. Rather, the increases in microbial biomass of this fungi-dominated community were likely allowing for a more extended nutrient scavenging area of the fungal communities; thus increasing fungal growth and enhancing substrate exploration (Paustian & Schnürer, 1987) without the need to invest energy in degradation of available complex substrates such as cutin and lignin. Higher investment in hyphal length growth and fungal biomass production in litter removal plots at this site is also congruent with the lower efficiency of fungi to decompose lignin from coniferous needles than that in broadleaf dominated litter (Osono, 2007).

3.3 | Aboveground versus belowground controls of soil C accumulation

At the studied deciduous forests (BF and HF), we observed higher relative use of preferred C substrates over persistent C in NR plots rather than in NL plots as evidenced by the higher SOM decomposition state and higher SOM resistance to decomposition in NL (Figure 2a). Aboveground litter exclusion (NL) in these forests did not lead to significant decreases in leaf-derived cutin (Table S2), even though cutin degradation significantly increased in NL plots in both forests compared with the control (Figure 4b). Root-derived suberin was, however, significantly higher in NR plots for both BF and HF (Table S2), a sign pointing towards preferential preservation of root-derived C in these forests. Finally, lignin oxidation increased in NR plots rather than in NL plots at BF and HF, although this was not significant for HF (Figure 5a). Microbial biomass and the microbial stress ratio were not impacted in NL plots at either deciduous forest (Figure 3a), but significant decreases in microbial biomass accompanied by higher microbial stress ratio were detected in NR plots at BF

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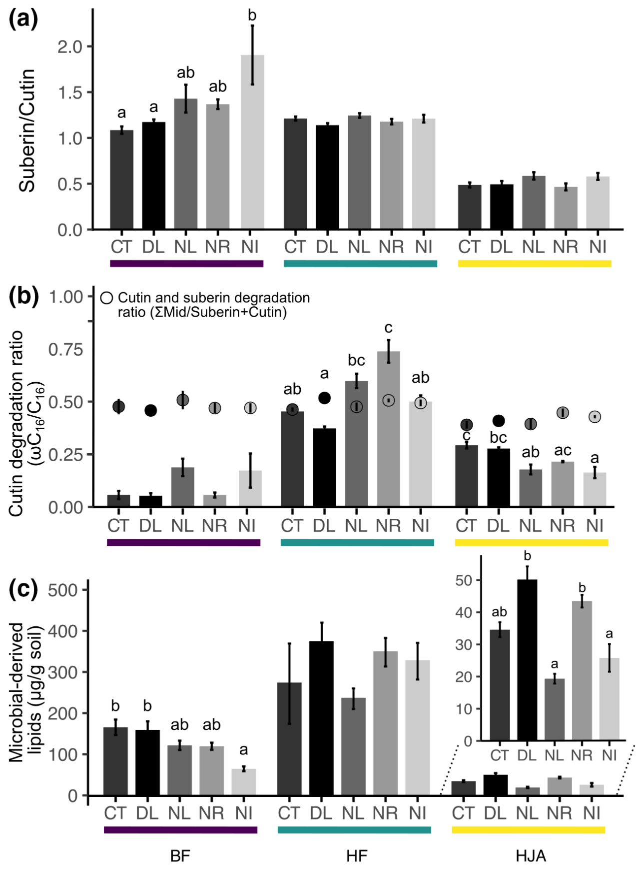

FIGURE 4 (a) Suberin and cutin ratio; (b) cutin degradation ratio (bars, $\omega C_{16}/C_{16}$), and cutin and suberin degradation ratio (circles, ΣMid/suberin + cutin) and (c) microbial hydrolysable-derived lipids (µg/g soil) from mineral soil (0–10 cm) from the Bousson Forest (BF), Harvard Forest (HF), and H.J. Andrews Forest (HJA) for the control (CT) and litter manipulation treatments: double litter (DL), no litter (NL), no roots (NR), and no inputs (NI). Different letters show significant differences $p < .05$ from Tukey's test, no letters mean no significant differences. For (b), significant differences across treatments and control for cutin degradation ratio ($\omega C_{16}/C_{16}$) in black and for the cutin and suberin degradation ratio (ΣMid/suberin + cutin) in gray.

(Figure 3a). Combined, this evidence suggests that belowground C inputs have indeed a greater impact on SOM dynamics than aboveground C inputs at the studied deciduous forests. At the coniferous HJA forest, we detected higher SOM decomposition state in NL plots than NR plots (Figure 2a), accompanied by a significant decrease in short-chain aliphatic lipids (Table S1), lower leaf-derived cutin (Table S2), and lower microbial-derived lipids (Figure 4b,c). On the other hand, root exclusion (NR) at HJA did not alter the concentration of root-derived suberin (Table S2) and only led to higher SOM resistance to decomposition (Figure 2b). This was congruent with the observed lower lignin decomposition (Figure 5a) and higher aromatic and phenolic C in NR plots at HJA (Figure 2c). The $\Delta^{14}C$ values and corresponding turnover times (Table 1) indicate a shift in soil C biogeochemical patterns. For BF and HF, litter exclusion resulted in longer turnover times compared with the control. HJA exhibited longer turnover times with NR and NI only but not with NL.

3.4 | Microbial-derived lipids: a DIRT perspective on microbial inputs to SOM formation

The quantity and quality of fresh plant litter inputs to soil is hypothesized to determine the substrate use of microbes and thus the quantity and chemical nature of the resulting microbial products (Microbial Efficiency-Matrix Stabilization—MEMS framework) (Cotrufo et al., 2013). Short-chain (C_{14}–C_{19}) branched alkanoic acids (*iso*, *anteiso*) (Cai et al., 2017; Otto & Simpson, 2006) as well as short chain (C_{10}–C_{18}) β-hydroxyalkanoic acids (Allard, 2006; Zelles, 1999) are lipids not commonly found in plants that have been useful as indicators of SOM decomposition and microbial inputs to the SOM pool (Cai et al., 2017; Jia et al., 2019). These lipids are generally extracted via an acidic or alkaline hydrolysis (Cai et al., 2017; Otto & Simpson, 2006) and although the relevance and source of these microbial-derived lipids is still cryptic, they have been found to respond to warming (Jia et al., 2019) and to be related with the organic C to N ratio of SOM rather than with soil mineralogical properties (Cai et al., 2017). The observed trends for these microbial-derived lipids in the studied forests agrees with recent proposed frameworks of stable SOM formation and its dependency on plant litter inputs chemistry and distinct microbial substrate use of contrasting microbial communities (Cotrufo et al., 2013). We detected overall higher quantities of these microbial-derived lipids at the broadleaf deciduous forests with low C:N litter (BF and HF), whereas HJA, the coniferous forest with high C:N litter, had the lowest amount of microbial-derived compounds (Figure 4c). Higher SOM decomposition state was concurrently observed at BF and HF, whereas HJA had the lowest SOM decomposition state (Figure 2a). Thus, the quantity of these microbial-derived lipids might be closely related with the C:N ratio of the plant inputs and could potentially be a by-product of microbial-mediated SOM decomposition and/or microbial biomass turnover. Another trend we observed is that the response of these microbial-derived lipids in DIRT varied depending on the dominant microbial community, regardless of the C:N ratio of the litter input. Although microbial-derived lipids in the bacteria-dominated site (BF) were generally unresponsive to doubled low C:N litter additions and reductions of above and belowground inputs (NL, NR, NI); fungal-dominated sites, particularly HJA did respond to DIRT treatments (Figure 4c), despite the different C:N ratios of the litter inputs. At the HF site, microbial-derived lipids tended to increase in DL and NR plots in comparison with the control, whereas at HJA, these lipids significantly increased in DL plots and NR but decreased in NL and NI plots (Figure 4c). Finally, the lack of responses of these lipids to DL in the bacterial-dominated deciduous forest (BF) could potentially be related to a generally lower substrate use efficiency of bacteria in comparison with that of fungi (Malik et al., 2016; Six et al., 2006) and the lower C:N litter inputs at this site that combined, led to a rapid and higher SOM decomposition state with minimal production of microbially derived compounds.

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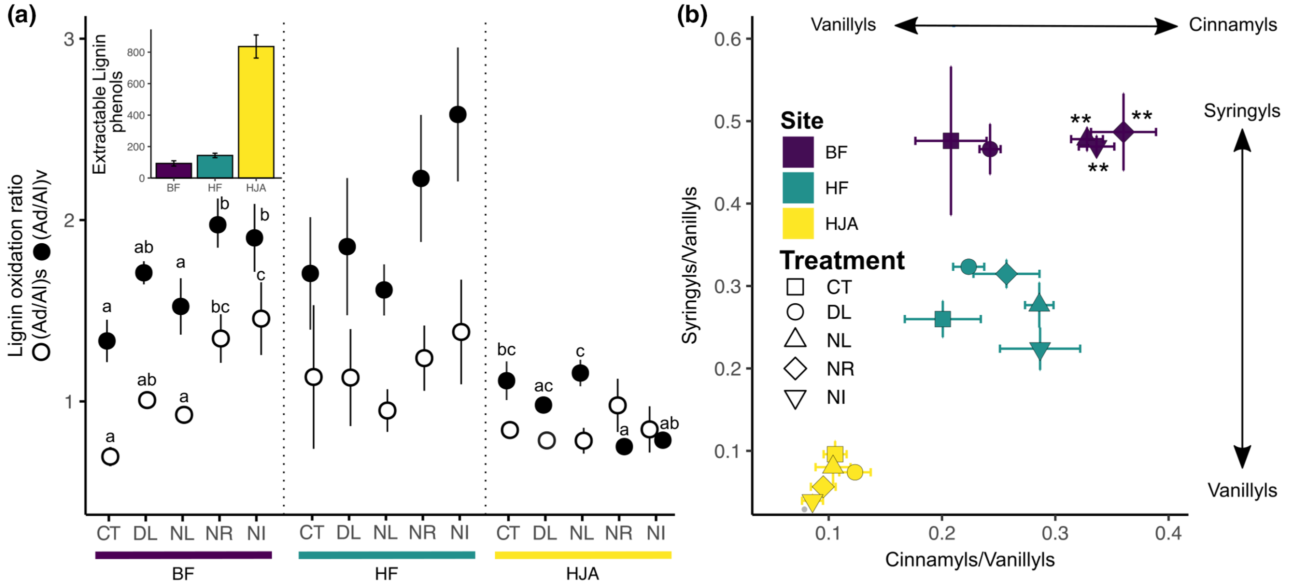

FIGURE 5 (a) Lignin oxidation ratios and (b) and lignin composition ratios from mineral soil (0–10 cm) from the Bousson Forest (BF), Harvard Forest (HF), and H.J. Andrews Forest (HJA) for the control (CT) and litter manipulation treatments: double litter (DL), no litter (NL), no roots (NR), and no inputs (NI). For (a), (Ad/Al)s or v: acid to aldehyde ratio for lignin-derived syringyl (s, white circles) and vanillyl (v, black) compounds. Inset (a) showing extractable lignin phenols as the sum of vanillyls, syringyls, and cinnamyls. Different letters show significant differences $p < .05$ from Tukey's test, no letters mean no significant differences. For (b), significant differences between treatments and control in the *y*-axis shown in gray and significant differences in the *x*-axis shown in black. Significance levels: ≤.1 (•), ≤.01 (**).

4 | DISCUSSION

With doubled litter inputs, sustained soil priming varied with forest N availability and soil microbial biomass and community composition. Although not all variables responded as expected to doubled litter inputs, the observed trends are consistent with the proposed long-term soil priming mechanism as the main reason for the lack of soil C accumulation at these sites with 20 years of annual DL. Instead, other lines of evidence from the collected molecular characterization of SOM from these sites can support the proposed soil priming although the driving mechanisms for this priming might differ in accordance with the N availability and the dominant microbial community at each site. For BF, doubled low C:N litter inputs may initially promote C mineralization of the plant material but as easily available C decreases, soil priming slows down and accumulation of processed, less preferred SOM is facilitated (Chao et al., 2019; Chen et al., 2014; Tian et al., 2019). The positive soil priming occurring at BF was, thus, likely the product of a stoichiometric balance between the low C:N litter at the site and the biomass C:N of fast-growing *r*-strategist microbes such as bacteria, which dominated the microbial community at BF (Figure 3c). Microbes, namely *r*-strategists mainly rely on easily available C (Butler et al., 2003; Chen et al., 2016) and the preferential use of more labile C components is supported by significant decreases in fast-cycling C compounds such as total sugars, short-chain aliphatic lipids and acyclic lipids as well as plant-derived long-chain aliphatic lipids for DL plots at BF (Table S1). As this easily accessible C is depleted, lignin is used as an alternate energy source as indicated by the marginally higher lignin oxidation ratio [(Ad/Al)s, v; Figure 5a]. Also, Gram-positive and Actinobacteria abundance in DL plots increased, facilitating the decomposition of more complex C substrates (Figure 3c). This stoichiometric decomposition theory may also explain the lack of increases in microbial biomass and microbial-derived lipids in DL plots at BF as pulses of microbial activity and growth after added doubled litter inputs might rapidly decrease and may have not been captured at the time of sampling (Wang et al., 2017) but were reported after 13 years of DIRT (Brant et al., 2006). Lack of more significant impacts on microbial biomass and microbial-derived lipids in the doubled litter treatment at BF could be also related with the rapid leaching of dissolved organic C from freshly dropped aboveground litter and its export out of the system or stabilization in mineral particles (Guggenberger & Kaiser, 2003; Hensgens et al., 2020; Neff & Asner, 2001), a flux of easily available C that is particularly relevant in high-N systems (Smemo et al., 2006).

Similarly, soil priming at HF was likely governed by the stoichiometric balance between doubled low C:N litter inputs that generated an immediate priming effect (Fanin et al., 2020) that combined with fungal-dominated communities at HF (Figure 3c) led to efficient decomposition of high-quality litter inputs. Although fungi are generally considered *K*-strategists with slower growth rates and preferential use of more complex C substrates (Chao et al., 2019; Chen et al., 2014; Fanin et al., 2020), fungal communities at HF challenge this notion as detrital fungal-dominated food webs at this site were found to associate with the use of easily available C substrates (Rousk & Frey, 2015). Therefore, doubled litter inputs at this forest, increased the relative abundance of fungi over bacteria (higher fungi/bacteria ratio) (Figure 3c) and tended to increase the microbial-derived lipids (Figure 4c) without significantly altering lignin oxidation (Figure 5a)

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or suberin concentrations (Table S2). Instead, cutin concentration increased (Table S2) and its degradation decreased (Figure 4b). HF had the highest litterfall rate across all sites (Figure 1b) and so, doubling litter inputs promoted microbial growth at this site and slightly increased SOM degradation state (Figure 2a) to a certain point where the nutrient and energy requirements of the soil microbial community were met followed by the accumulation of the litter surplus as evidenced by the higher $\Delta^{14}C$ values and shorter C turnover times at HF in DL plots. At HJA, soil priming is rather determined by active N mining by fungal-dominated microbial communities (Figure 3c) more adapted to process complex organic sources and with a typical *K*-selected strategy Thus, doubled low-quality (high C:N) litter inputs at HJA increased the relative abundance of fungi over bacteria (higher fungi/bacteria ratio) (Figure 3c), the microbial-derived lipids (Figure 4c) and more significantly increased the total microbial biomass (Figure 3a).

Across sites, litter exclusion led to the adaptation of microbial communities to preferred C-substrate limitations. As hypothesized, the negative impacts on microbial biomass were mostly detected at the bacteria-dominated high N-availability broadleaf forest (BF) while increases in soil microbial biomass were instead detected in mid and low N-availability fungal-dominated sites (HF and HJA). Contrary to our hypothesis, SOM components (i.e., cutin, suberin, and lignin) did not decrease at all sites but instead, specific shifts in microbial community composition at each site drove targeted decreases of preferred C substrates. At BF, higher Gram-positive and Actinobacteria abundance facilitated higher lignin degradation, whereas at HF, higher fungal abundance led to higher cutin degradation. Finally, at the fungal-dominated low N-availability site (HJA) increases in microbial biomass might have served as a mechanism for extended resource scavenging without further degradation of available complex C substrates. Taken together, these results suggest that reduction of litter inputs may ultimately reduce soil C storage, not only via decreases in C inputs into the soil C pool but also via changes in the composition and abundance of soil microbial communities to either decompose complex C substrates or to explore new sites in soil to find their preferred nutrient and energy sources.

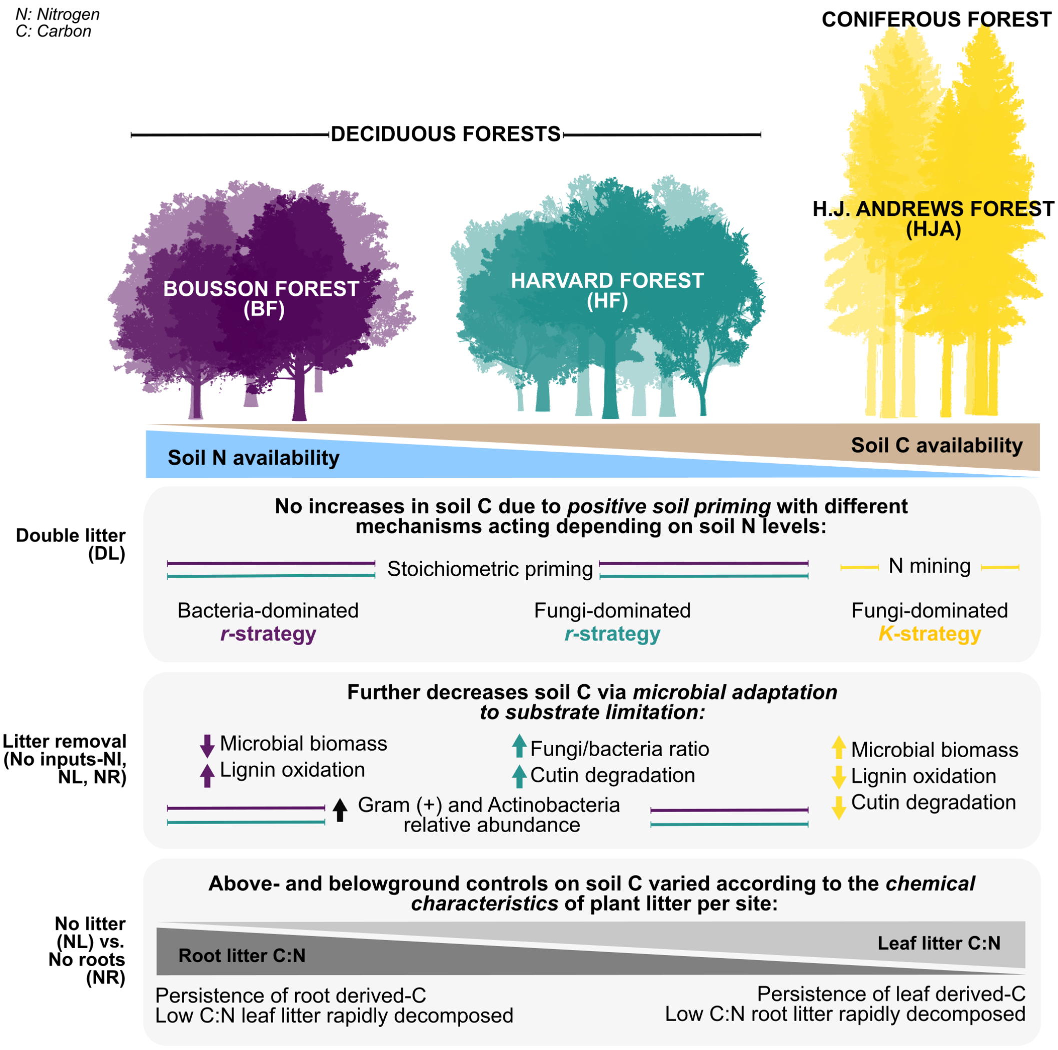

FIGURE 6 Summary of molecular composition of soil organic matter (SOM) and associated mechanisms defining the distinct responses of soil C across sites and treatments. SOM compositional data from Figures 2–5 are summarized in different groupings to better conceptualize relationships with mechanisms that control site-specific responses to long-term changes in litter quality and quantity.

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We found that belowground litter controls soil C accumulation in deciduous forests, whereas aboveground litter is more influential in coniferous forests. Given that aboveground litter reductions (NL) led to more significant decreases in soil C across these DIRT forests than belowground litter reduction (NR) (Lajtha et al., 2018), we were expecting to detect higher SOM degradation, increased degradation of lignin, cutin and suberin and lower $\Delta^{14}C$ values for litter removal (NL) plots and not NR plots across all sites. However, these expectations were only partially met in specific cases with the SOM molecular composition of each site responding differently and more in agreement with the observations by Crow et al. (2009) where the authors postulate a greater control of belowground litter in deciduous forests and aboveground litter being more influential for soil C accumulation at coniferous forest. Higher control of belowground litter in deciduous forests is supported here mainly by the lack of impacts in leaf-derived cutin for NL plots, whereas root-derived suberin was significantly higher in NR plots for both BF and HF. At the coniferous forest (HJA), higher control of aboveground litter on SOM dynamics is supported by the fact that leaf litter exclusions led to more significant changes in the SOM biogeochemistry than root exclusions. These trends were also observed by Crow et al. (2009) and confirmed here, and are likely explained by the contrasting chemistry of above and belowground inputs from coniferous and deciduous forests. For coniferous forests, aboveground litter tends to have higher C:N ratio in comparison with root litter, with fine roots being a major input of N in these systems (Chen et al., 2002; Yano et al., 2005) and having a faster decomposition than aboveground litter (Sulzman et al., 2005), whereas leaf litter decomposes slower with needle-derived compounds persisting in soil. For deciduous forests the opposite is true, given the higher C:N ratios of root litter in comparison with leaf litter, root litter decomposes slower than leaf litter in these forests (Kyaschenko et al., 2019; Sulzman et al., 2005) with leaf-derived compounds thereby persisting in soil.

The measured $\Delta^{14}C$ values and the estimated turnover times for litter exclusion did not agree with the observed trends in SOM decomposition. For coniferous forests, where aboveground litter seems more consequential for soil C accumulation, we were expecting to detect lower $\Delta^{14}C$ values, and longer C turnover times in NL plots as the lack of aboveground C inputs would accelerate SOM decomposition. Instead, we observed higher $\Delta^{14}C$ values, and shorter C turnover times in NL plots at HJA (Table 1). For deciduous forests, where roots were influencing soil C accumulation, we were expecting lower $\Delta^{14}C$ values, and longer C turnover times with belowground litter removal (NR) but instead, we observed higher $\Delta^{14}C$ values, and shorter C turnover times in NR plots at both BF and HF (Table S1). These seemingly contradictory trends are potentially explained by the production rates of above- and belowground litter in coniferous and deciduous forests. Coniferous forests have higher relative root production compared with aboveground litterfall, whereas deciduous forests have higher litterfall relative to total and fine root production (Anderson-Teixeira et al., 2021). Thus, the continued C inputs from roots in NL plots at HJA and of leaf litter in NR plots at BF and HF are driving the observed trends in the radiocarbon data and estimated turnover times.

5 | CONCLUSIONS

Our cross-site, comprehensive molecular-level comparison demonstrates that soil C responses to altered litter input quantity and quality as a proxy for shifts in ecosystem productivity with environmental change are contingent on site-specific ecosystem properties and respond uniquely to overall ecosystem N availability. Sustained annual doubled litter additions over 20 years of DIRT, either continuous (i.e., HJA) or seasonal (i.e., BF and HF), are unlikely to increase SOM in mature temperate coniferous and deciduous forests. The lack of increases in soil C suggests a long-term and sustained positive soil priming, yet the mechanisms for this priming might vary across sites, with a positive soil priming occurring at the N-rich, bacteria-dominated (BF), and fungal-dominated site (HF), characterized by a stoichiometric decomposition of the low C:N leaf litter. In contrast, the observed positive soil priming at the N-poor fungal-dominated site (HJA) may be determined by active N mining of available organic substrates. As expected, litter exclusion, either above- or belowground, led to even lower soil C across all sites with soil microbial communities adapting to the substrate limitation further degrading SOM in response to lower preferred C inputs. Finally, while root exclusion (NR) seemed more influential for soil C dynamics in both temperate broadleaf deciduous forests, litter exclusion (NL) altered soil C processes in the coniferous forest. Collectively, our results demonstrate that different mechanisms defined by inherent ecosystem properties are important determinants of altered soil C dynamics with litter manipulations. These mechanisms are conceptualized in Figure 6 and highlight the important drivers of these molecular-level biogeochemical processes. It is important to emphasize that the observed changes in the molecular biogeochemistry of the SOM for both litter additions and reductions ultimately had a negative impact on the capacity of these temperate forests to sequester C. Thus, climate change, environmental alteration, or management decisions that increase, reduce or alter C input sources have the potential to reduce soil C and increase SOM decomposition via different mechanisms determined by the inherent ecosystem properties of specific forest ecosystems. This outcome agrees with recent metanalyses (Feng et al., 2022; Xu et al., 2021) showing that the C sequestration potential of different ecosystems with increased litter inputs depends on factors such as the rate of the litter inputs as well as the initial soil C stocks. For these mature secondary temperate forests with already relatively high C stocks, increases in aboveground litter inputs will not lead to enhanced soil C stocks and instead, they might promote SOM decomposition, whereas the impacts of litter input reductions will further decrease soil C stocks. Our molecular-level synthesis also emphasizes the crucial role of microbial communities in SOM dynamics and how microbial community composition in combination with litter chemical

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properties and site-specific conditions can strongly determine the fate of soil C inputs. Consequently, forest management and climate change mitigation that enhances soil C sequestration and preservation of existing soil C stocks requires more refined measures based on dominant soil microbial groups and ecosystem properties to meet expected soil C enhancement goals or a point near soil C saturation in temperate forests.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Borealis at https://doi.org/10.5683/SP3/L4MYGR.

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