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Long-term nitrogen deposition disrupts carbon cycling and enhances plant-derived carbon sequestration in a temperate forest

Biwei Yang, [a] Meiling Man, [a] Yuxuan Weng, [a] Richard D. Bowden, [b] Jun-Jian Wang [cde] and Myrna J. Simpson *[a]

Nitrogen (N) deposition alters carbon (C) dynamics in forests, but its long-term impact on soil C biogeochemistry remains unelucidated at the molecular-level. We collected soils after 27 consecutive years of N additions in a temperate forest and examined soil chemistry using molecular soil organic matter (SOM) compositional analysis to unravel mechanisms and impacts on soil biogeochemistry. N-addition increased soil C storage in the forest floor, where microbial stress increased and decomposition was suppressed, causing accumulation of aboveground plant inputs such as leaf litter and a shift toward less stable SOM. In the mineral soil, C storage did not change significantly, but decomposition of root and woody materials was reduced, and microbes exhibited stress. After nearly three decades, microbial stress persisted with a community shift toward fewer Gram-negative bacteria, which prefer labile C like cellulose. The rise in more labile C forms further supported accumulation of microbially preferred substrates. Overall, chronic N deposition thus impairs microbial decomposition and alters SOM composition, reducing C turnover and leading to accumulation of less persistent C forms that may be vulnerable to loss upon forest disturbance or environmental change. These findings emphasize the importance of integrating microbial and chemical composition in predicting long-term N deposition impacts on forest soil C sequestration and demonstrate the severe impacts on biogeochemical processes in forests.

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Environmental significance

In a high nitrogen world, increased atmospheric deposition in forest soils disrupts the biogeochemical cycling of carbon but the precise underlying mechanisms are poorly understood. In this study, we examined the molecular-level changes in soil carbon storage and soil organic matter chemistry with long-term nitrogen addition in a temperate forest. Chronic nitrogen addition suppressed microbial decomposition and reduced carbon turnover, leading to accumulation of vulnerable forms of carbon. The composition of soil organic matter reflected less decomposed plant inputs that are less durable and less persistent carbon forms. As such, the carbon sequestered through nitrogen-induced suppression of microbial decomposition processes is unlikely to be stable in a warmer world.

1 Introduction

Anthropogenic activity has dramatically increased nitrogen (N) deposition across the globe,[1,2] transforming the way ecosystems process carbon (C).[3] Soils, which store more C than the atmosphere and vegetation combined,[4] play a central role in the C cycle, yet the response of soil C to chronic N-enrichment is not fully understood. Field experiments and meta-analyses across forest biomes have reported mixed responses, with some studies finding enhanced C storage under N addition, while others detect no change or even C losses.[5–15] These disparities may arise from N-induced shifts in microbial decomposition and soil C stabilization pathways,[16] which vary across ecosystems and timescales.[6,10,17,18] In forest systems, accurately quantifying soil C feedback and microbial adaptation requires decadal-scale observations under chronic N enrichment, as both environmental recovery and microbial community restructuring operate on multi-year timescales.[8] Decadal N-addition studies suggest that soil C accumulation is more likely under continuous high N loads and extended durations, especially in temperate forests.[6,9,10,17,18] Over 15 years, intensive

[a]*Environmental NMR Centre and Department of Physical and Environmental Sciences, University of Toronto, Toronto, Ontario, M1C 1A4, Canada. E-mail: myrna.simpson@utoronto.ca*

[b]*Department of Environmental Science, Allegheny College, Meadville, PA, 16335, USA*

[c]*State Key Laboratory of Soil Pollution Control and Safety, Southern University of Science and Technology, Shenzhen, 518055, China*

[d]*State Environmental Protection Key Laboratory of Integrated Surface Water-Groundwater Pollution Control, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China*

[e]*Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China*

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monitoring at 102 plots in French temperate forests documented significant soil C increases in surface soil layers under N enrichment, likely due to the changes in soil microbial community composition and the accumulation of poorly decomposed soil organic matter (SOM).[19] Collectively, these patterns indicate that N-enrichment can alter microbial activity and SOM decomposition. However, the microbial and chemical underpinnings of this process, particularly over decadal timescales, remain unelucidated from a mechanistic perspective making it challenging to develop mitigation strategies for forests.

SOM is chemically diverse and is derived from multiple sources, including aboveground plant inputs (*e.g.*, leaf litter and woody tissues), belowground plant inputs (*e.g.*, roots), and microbial residues,[20–23] all of which exhibit distinct C turnover dynamics.[8,24–26] In forest ecosystems, fresh litter and root residues dominate the organic horizons (which contain more than 17% organic C or more than 30% organic matter) and introduce plant-derived SOM compounds such as long-chain lipids, cutin- and suberin-derived polymers, and lignin-derived phenols.[5] In parallel, microbial decomposition of plant litter produces rapidly cycled compounds (*e.g.*, phospholipid fatty acids, PLFAs, from living microbial biomass) alongside microbial-derived SOM compounds.[16,27] Therefore, the balance between SOM compounds and soil C preservation or degradation is highly dependent on soil microbial processing of plant-derived SOM.[28] While N-induced suppression of microbial activity and subsequent accumulation of soil C in soils are well-documented in forest ecosystems,[5,11] critical knowledge gaps remain regarding (i) microbial community responses, including biomass (*e.g.*, bacteria and fungi) shifts, microbial stress levels, and compositional changes and (ii) the molecular signatures of SOM composition and sources across soil layers (*i.e.*, the forest floor and mineral layer). These unresolved knowledge gaps limit our ability to accurately predict and manage long-term consequences of anthropogenic N deposition on soil C quantity and quality, with important implications for global C cycling. Recent modeling work demonstrates that SOM chemical composition and dynamics substantially affect predictions of forest C cycling under chronic N enrichment.[8] Addressing these gaps requires long-term experiments that combine decadal-scale N enrichment with advanced molecular characterization of SOM.

Here, we focus on changes in SOM composition and microbial decomposition after 27 years of continuous N-addition to temperate forest soils in Bousson, PA. The Bousson Experimental Forest N-addition experiment is one of the earliest long-term forest N-addition experiments globally, initiated in 1994 with an annual addition rate of 100 kg N ha^{-1} $year^{-1}$.[5,11] Previous work at the Bousson Experimental Forest found that N-additions increased soil C, decreased litter decomposition, and resulted in more preservation of less degraded SOM.[5,11] Consistent with prior findings across temperate forests,[5,12,13,29] we expected soil C accumulation. We hypothesize that long-term N enrichment continues to suppress microbial decomposition and selectively accumulates plant-derived compounds after 27 years of continuous N-addition, leading to greater C sequestration in the forest floor than in mineral soil. To test these hypotheses, two complementary molecular methods were applied to measure the molecular composition of SOM.[30] To assess the inputs and decomposition of source-specific compounds such as plant-derived lipids, cutin-derived and suberin-derived compounds, lignin-derived phenols, and bacteria- and fungi-derived phospholipid fatty acids (PLFAs), gas chromatography mass spectrometry (GC-MS) was applied for targeted organic compound analysis. Overall soil C character was also profiled by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Our overarching goal was to unravel the soil C mechanisms and how much C is accumulated in forest soils, why this accumulation occurred, and what it means for the long-term stability of soil C under chronic N deposition.

2 Materials and methods

2.1 Site description and soil sampling

The N-addition experiment at the Allegheny College Bousson Experimental Forest in northwestern Pennsylvania (41°36′ N, 80°2′ W) exists in a N-rich site (approximately 10 000 kg N ha^{-1}). The ~100 year-old temperate mixed deciduous forest is dominated by black cherry (*Prunus serotina*) and sugar maple (*Acer saccharum*).[31] Liquid forms (and occasionally pellet form) of N were applied six times per year from May to October for a total annual rate of 100 kg N ha^{-1} $year^{-1}$, beginning in 1994 and ending after the final addition in 2020. Forest floor (O, 1–3 cm) and the mineral (A, 3–8 cm) soil samples were collected from three control and three N-addition plots in 2021, following 27 years of N addition and one year of no fertilization to allow the system to stabilize. Soil samples were homogenized, sieved to 2 mm, and freeze-dried prior to chemical and molecular analyses.

2.2 Carbon and nitrogen analyses

Total organic C and N concentrations of soils were measured using a Thermo Flash 2000 elemental analyzer (oxygen gas combustion at 950 °C). C and N storage (with units converted from % to kg-C kg-$soil^{-1}$ and kg-N kg-$soil^{-1}$) for each soil layer were calculated using the C and N concentrations, bulk density of the soil layer (in kg-soil m^{-3}), and layer depth.

2.3 Solid-state ^{13}C NMR spectroscopy analysis

For NMR analyses, three field replicate subsamples were mixed evenly to create one composite sample. Soils were repeatedly treated with 10% hydrofluoric acid to concentrate the organic matter and reduce paramagnetic materials such as iron found in minerals.[32] Treated freeze-dried samples were ground into fine powder and characterized by solid-state ^{13}C cross polarization magic angle spinning NMR. The ^{13}C NMR spectra were collected using a 500 MHz Bruker BioSpin Avance III spectrometer (Rheinstetten, Germany) equipped with a 4 mm H–X MAS probe, using a 11 kHz spinning speed, a ramp-amplitude cross polarization pulse program (contact time was 1 ms) and a relaxation delay of 1s.[33] The spectra were integrated into four chemical shift regions and normalized to the total signal for comparison using Bruker BioSpin TopSpin (v 4): (1) alkyl carbon (0–50 ppm); (2) *O*-alkyl carbon (50–110 ppm); (3) aromatic +

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phenolic carbon (110–165 ppm); and (4) carboxyl + carbonyl carbon (165–210 ppm).[34,35]

2.4 Targeted organic matter compound analyses by GC-MS

Targeted SOM compounds with various microbial- and plant-derived sources were isolated by using sequential extraction and quantified using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Soil compounds were sequentially extracted in duplicate following our previous methods using solvent (dichloromethane/methanol), base hydrolysis, and copper(II) oxide (CuO) oxidation extractions.[20–23,36,37] We quantified concentrations for the free acyclic and cyclic lipids and sugars in the solvent extractable fraction, cutin- and suberin-derived compounds in the base hydrolyzed fraction, and the extractable lignin-derived phenols in the CuO depolymerized fraction. Furthermore, microbial-specific PLFAs were extracted by a modified Bligh–Dyer method and analyzed by GC-MS.[5,38,39] Details can be found in the SI.

2.5 Statistical analyses

Changes in the total organic carbon concentration and total nitrogen concentrations were assessed with paired *t*-tests. Repeated measures analysis of variance was used to determine the differences of targeted organic compounds between control and N-addition soil samples. For this analysis, two analytical replicates were considered the within-subject factor to account for variability within each plot, and the treatment (control *vs.* N-addition) was the between-subject factor. Statistical significance was considered at $p < 0.05$. Statistical tests were carried out using IBM SPSS Statistics (software version 28).

3 Results

3.1 Carbon and nitrogen analyses

To evaluate how chronic N-enrichment alters C biogeochemical cycling, we quantified soil C and N concentrations and storage, and C/N ratios, after 27 years of N-addition (100 kg N ha^{-1} $year^{-1}$) in a temperate forest (Fig. 1 and Table S1). Compared to the control, the forest floor under N-addition showed significant increases in the soil C concentration (by 84%) and C storage (by 73%, from 1.14 to 1.97 kg m^{-2}) (Fig. 1). The soil N concentration and storage also increased significantly in the forest floor. In contrast, the mineral layer showed no significant differences in the concentrations or storage of C or N. Across both layers, prolonged N-addition significantly increased soil C/N ratios by 22% in the forest floor and 17% in the mineral layer.

3.2 Solid-state ^{13}C NMR analysis

Solid-state ^{13}C NMR revealed differences in the proportion of different SOM structural components in the forest floor and mineral layers demonstrating a unique response to long-term N-addition (Fig. 2 and Table S2). For example, aliphatic molecules (alkyl C) associated with various lipids including plant waxes and microbial components decreased in the forest floor but increased in the mineral layer.[35] Preferred microbial substrates, such as cellulose and peptides (*O*-alkyl C), increased in the forest floor but decreased in the mineral horizon.[40] Furthermore, the broadness of the alkyl C resonances of the forest floor control sample indicates an increased diversity of molecules that was not observed with N-addition, suggesting increased preservation of plant-derived molecules with N-fertilization. Aromatic and phenolic C, which include resonances from components of lignin and suberin and aromatic moieties from some amino acids, increased in both the forest floor and mineral layers.[35,40] The region associated with side-chains from various components (carbonyl and carboxylic C) decreased in both layers.[35,40] These trends suggest an overall accumulation of plant-derived organic matter associated with leaf litter and wood in both soil layers.

3.3 Targeted organic matter compound analyses

Long-term N-addition did not significantly alter overall microbial biomass (PLFA analysis) in either the forest floor or mineral layers. However, significant changes in community composition and microbial stress were observed (Fig. 3). Gram-negative bacteria, which are *r*-strategist microbes, rely mainly on easily available and labile C,[41] significantly decreased by 21% after N-addition in the forest floor (Fig. 3A). Gram-positive and actinobacteria, *K*-strategists that typically degrade complex C such as lignin,[41] became more dominant in the mineral layer (Fig. 3B). No significant changes were observed in fungal biomass and the ratio of fungal to bacterial biomass between treatments in both layers (Tables S3 and S4).[41] Microbial stress (indicated by the ratio of total saturated to total mono-unsaturated PLFAs and the cy17:0/16:1ω7c ratio) increased with N-addition for both soil layers (Fig. 3C). Microbial contributions to SOM, the microbial-derived solvent-extractable lipids ($<C_{20}$; including *n*-alkanols and *n*-alkanoic acids) increased significantly in the forest floor (Tables S5 and S6). In contrast, the cellular and extracellular microbial-derived lipids (branched C_{16}, C_{17}, and C_{18} alkanoic acids and β-hydroxy C_{10}, C_{12}, and C_{14} alkanoic acids)[21] showed no significant differences in both layers (Tables S5 and S6).

Long-term N-addition selectively enhanced accumulation of plant-derived compounds in both the forest floor and mineral soil layers (Fig. 4A and S1). A series of plant-derived compounds were identified, including aliphatic lipids (*i.e.*, *n*-alkanes, *n*-alkanols, *n*-alkanoic acids, and ω-hydroxy alkanoic acid), phytosterols (campesterol, stigmasterol, sitosterol and their derivatives), triterpenoids (friedelin, oleanolic acid, and ursolic acid), cutin-derived lipids (*x*-hydroxy acids $C_{14:1}$, C_{15}, and C_{17}; *x*,ω dihydroxy acids C_{15} and C_{16}; and *x*-hydroxy α,ω-diacid C_{16}), suberin-derived lipids (ω-hydroxy acids C_{20}, C_{22}, and C_{24} and α,ω-diacids C_{20}, C_{22}, and C_{30}), and lignin-derived phenols (including vanillyls, syringyls, and cinnamyls). In the forest floor, N-addition significantly increased plant-derived SOM, associated with aboveground plant inputs, such as leaf waxes and cuticles.[20] Specifically, long-chain lipids, derived from plant waxes, increased by 123%, phytosterols increased by 95%, and cutin-derived lipids also increased by 81% (Tables S5 and S6). In the mineral layer, suberin-derived lipids (markers of roots) and

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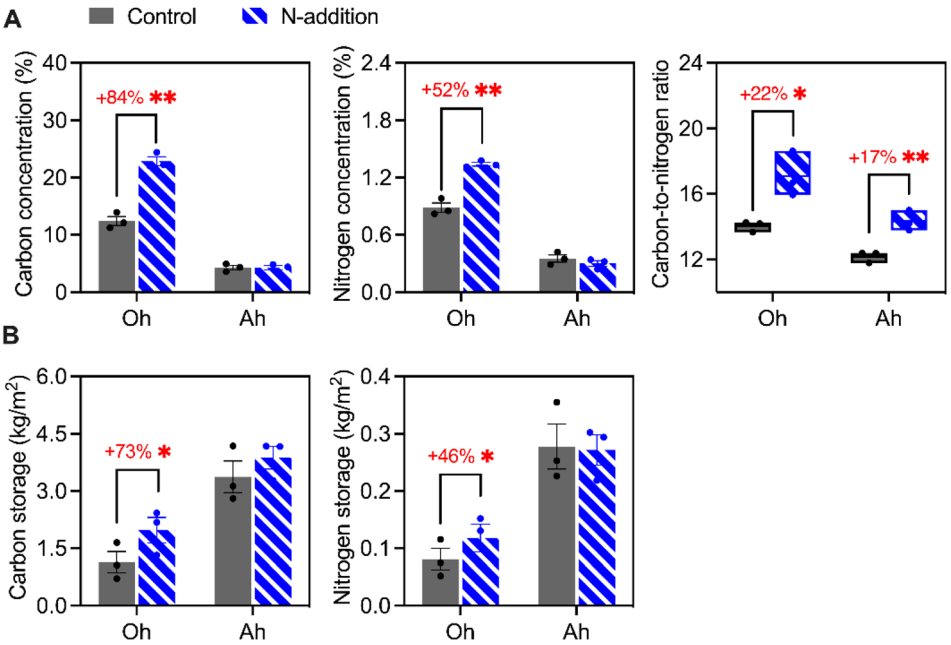

Fig. 1 Changes in carbon and nitrogen after long-term nitrogen-addition (shaded blue) compared to control (solid grey) of the forest floor (Oh) and mineral layer (Ah). (A) Total carbon concentration, total nitrogen concentration, and carbon-to-nitrogen ratio. (B) Storage (in kg m^{-2}) of carbon and nitrogen. Individual field replicates ($n = 3$) are shown as dots; means and standard errors are represented by bars and error bars. Significant differences between treatments (paired two-sample t-test) are highlighted by red asterisks (* and ** for $p < 0.05$ and $p < 0.01$, respectively), with percentage changes labeled.

lignin-derived phenols (indicative of woody tissues and vascular cell walls) increased by 39% and 181%, respectively.

The distribution of plant-derived SOM compounds (*n*-alkanes and *n*-alkanoic acid) also shifted under N-addition in the forest floor and mineral layers, demonstrating a dominant contribution from aboveground plant inputs (Fig. 4B). Long-chain *n*-alkanes (C_{29}, C_{31}, and C_{33}), commonly associated with leaf waxes,[42] increased significantly and became the three most abundant *n*-alkanes in N-addition forest floor soils (Fig. 4B). Notably, C_{29} and C_{31} *n*-alkanes were abundant in black cherry leaf litter (the dominant tree species at the study site) but were limited in sugar maple leaves and root litter (Fig. S2). A

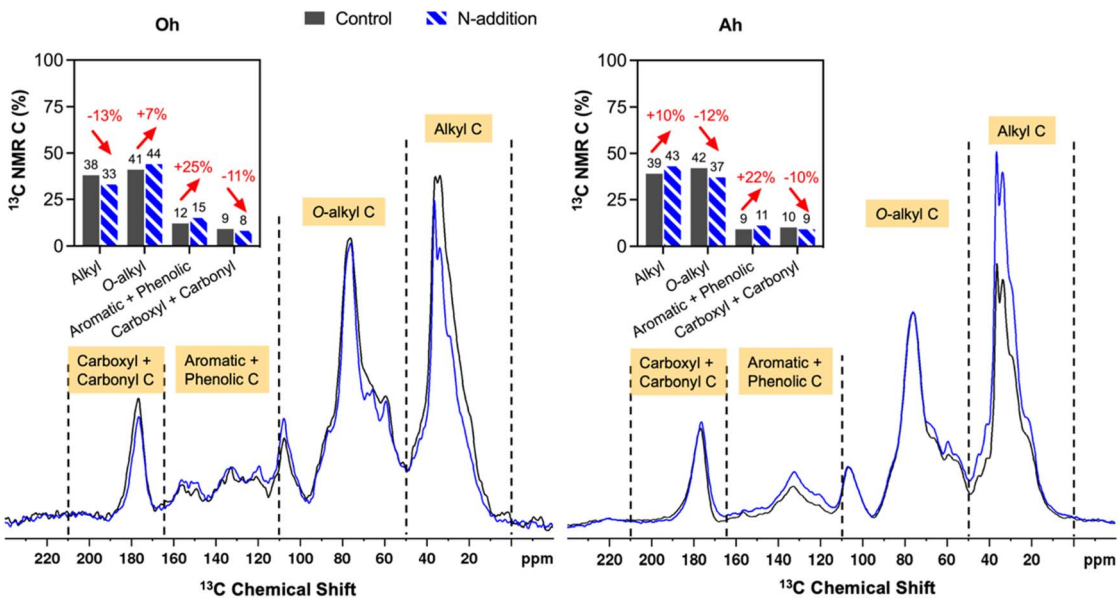

Fig. 2 Shifts in carbon (C) composition after long-term N-addition (blue line) compared to control (grey line) in the forest floor (Oh, left panel) and mineral layer (Ah, right panel), profiled by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The percentages of the four C groups are shown in bar plots, with percentage changes labeled in red numbers and arrows indicating changes after long-term nitrogen-addition.

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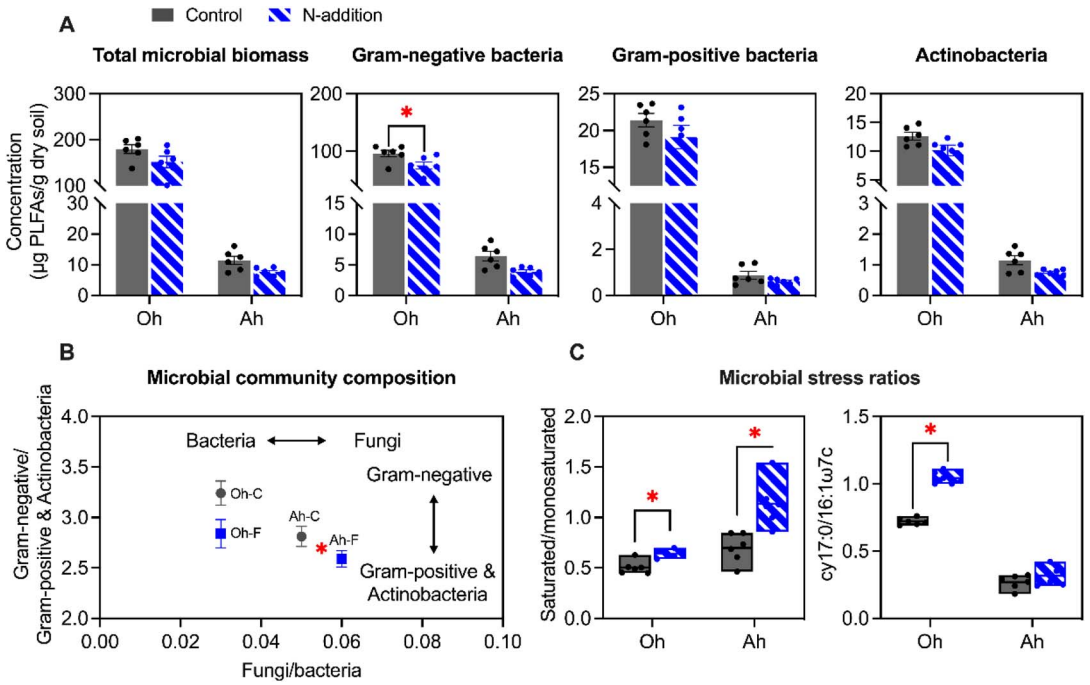

Fig. 3 Changes in the microbial biomass, community composition, and stress ratios under control (grey) and N-addition (blue). (A) Phospholipid fatty acid (PLFA) concentrations (μg PLFAs/g dry soil) of total microbial biomass, Gram-negative bacteria, Gram-positive bacteria, and actinobacteria in control (solid grey) and N-addition (shaded blue) plots are shown as bar charts. Individual replicates ($n = 6$) are shown as dots; means and standard errors are represented by bars and error bars. (B) The fungi-to-bacteria ratio (x-axis) and the ratio of Gram-negative bacteria to the sum of Gram-positive bacteria and actinobacteria (y-axis) are plotted for control (grey circles) and N-addition (blue squares); error bars indicate standard errors. (C) The ratio of total saturated to total monounsaturated PLFAs and the ratio of cyclopropane PLFAs to the monounsaturated PLFAs (cy17:0/16:1ω7c) are shown as floating box plots (min to max, individual replicates are shown as dots, and the line indicates the mean, $n = 6$). Significant differences between treatments were determined by repeated measures analysis of variance (ANOVA) and are indicated by red asterisks ($p < 0.05$).

significant increase was observed in C_{28} n-alkanoic acid (Fig. 4B), which mainly originated from aboveground plant inputs (leaf litter).[42] Declines were observed in the short-chain n-alkanoic acids ($C_{16:1}$, C_{16}, $C_{18:1}$, and C_{18}), typically of microbial origin.[20] In the mineral layer (Fig. 4C), $C_{18:1}$, C_{18}, and C_{22} n-alkanoic acids increased significantly, which are found in both the leaf and root litter (Fig. S2). Root-specific n-alkanoic acids ($C_{16:1}$ and C_{27}), which are dominant in root litter (Fig. S2B),[42] declined under N-addition in the forest floor and were not significantly different in the mineral layer.

4 Discussion

Long-term N-addition led to a substantial accumulation of soil C in the forest floor, as observed in our 27-year experiment at the Bousson Experimental Forest. Soil C concentrations increased by 84% in forest floor and are consistent with our hypothesis that chronic N-enrichment promotes soil C storage under chronic N-enrichment. Similar patterns were observed at this location in an earlier study at year 17, at this site,[5,11,12] and in other long-term field experiments,[43–45] where continuous N deposition slowed the decomposition of aboveground plant inputs, resulting in soil C accumulation. Likewise, at another temperate experimental forest (Harvard Forest), 14 and 20 years of N-addition (150 kg N ha^{-1} $year^{-1}$) led to 29% and 38% increases in soil C within 0–40 cm profiles, respectively.[12,43] Despite differences in forest types, 11 years of N-addition also resulted in soil C accumulation in a tropical forest.[46] Meta-analyses have reinforced this trend across forest biomes,[9,10,17,18] showing a mean 4% increase in soil C, with greater accumulation in temperate forests,[9,18] under higher N inputs (>100 kg N ha^{-1} $year^{-1}$) and longer durations (>10 years).[6] The C/N ratios increased significantly in both forest floor and mineral soils with N-addition suggesting that the composition of SOM was altered to more plant-derived materials that are C rich and N poor.[47] Our results extend this body of evidence by pairing one of the longest continuous N-addition experiments in temperate forest with detailed molecular-level SOM characterization, offering insight into how chronic N-enrichment alters soil C chemistry over decades.

The mechanism for increased soil C storage with long-term N-addition is likely tied to suppressed microbial activity and increased microbial stress, thereby limiting microbial decomposition and contributing to the specific accumulation of aboveground plant-derived SOM in the Bousson Forest. Alongside the increase in soil C, we observed higher C/N ratios and elevated microbial stress in both soil layers, indicating that microbes are facing limitations in preferred substrates or other stressors under chronic N enrichment.[5,41] Furthermore, the reduction of bacterial biomass suggests that microbial breakdown of plant inputs was also suppressed.[28] Specifically, Gram-negative bacteria, r-strategists that preferentially use labile C,

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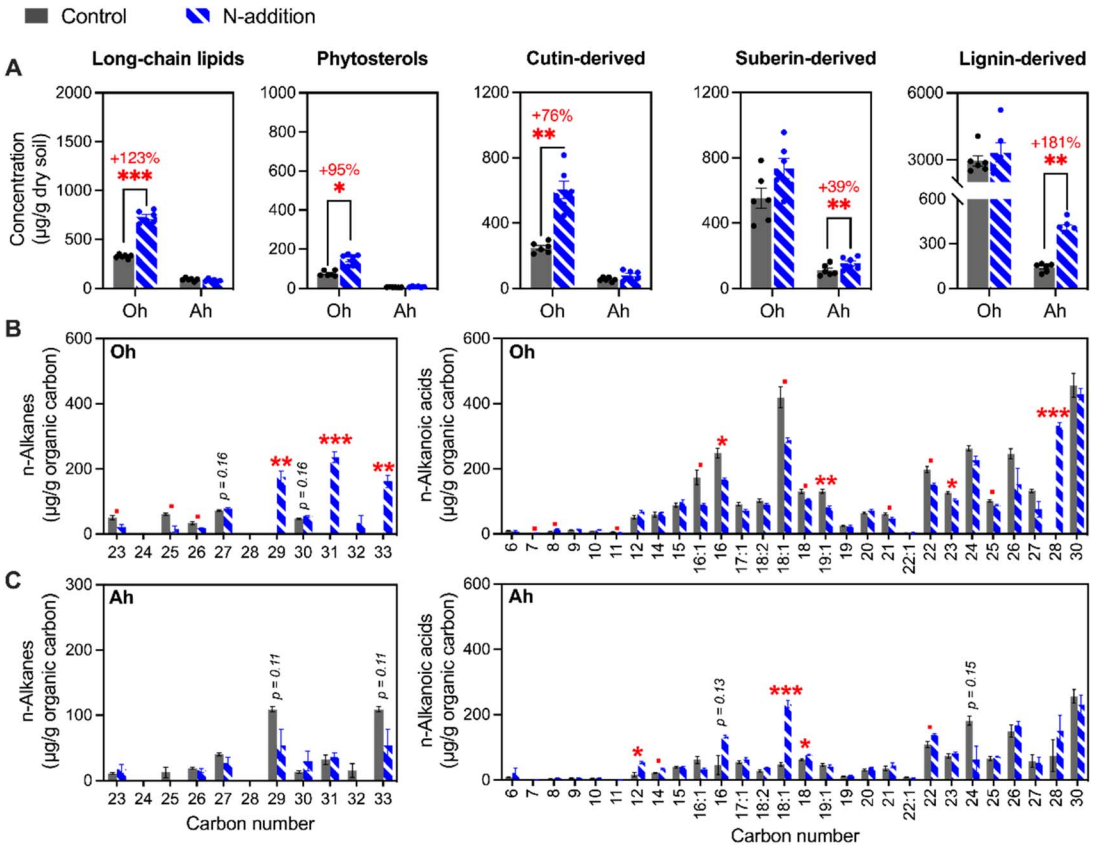

Fig. 4 Concentrations of soil organic matter compounds in control (solid grey) and nitrogen-addition (shaded blue) plots from the forest floor (Oh) and mineral layer soil (Ah). (A) Concentrations (μg g^{-1} dry soil) of long-chain lipids (≥C_{20}; *n*-alkanes, *n*-alkanols, *n*-alkanoic acids, and *ω*-hydroxy alkanoic acid; mainly of plant origin), phytosterols (campesterol, stigmasterol, sitosterol and their derivatives), cutin-derived lipids (*x*-hydroxy acids $C_{14:1}$, C_{15}, and C_{17}; *x*,*ω* dihydroxy acids C_{15} and C_{16}; and *x*-hydroxy *α*,*ω*-diacid C_{16}), suberin-derived lipids (*ω*-hydroxy acids C_{20}, C_{22}, and C_{24} and *α*,*ω*-diacids C_{20}, C_{22}, and C_{30}), and lignin-derived phenols (vanillyls, syringyls, and cinnamyls). Percent changes ($p < 0.05$) relative to control are labeled in red, and individual replicates ($n = 6$) are shown as dots. (B) Distributions (in μg g^{-1} organic carbon) of *n*-alkanes and *n*-alkanoic acids from Oh are shown, with (C) panels presenting Ah. Differences between control and N-addition plots were evaluated using repeated measures analysis of variance (ANOVA), with significance levels set at $p < 0.05$, 0.01, and 0.001 (*, **, and ***, respectively). $p \leq 0.1$ is denoted by a dot. Bars and error bars represent means and standard errors.

declined significantly in the forest floor, likely contributing to the accumulation of aboveground plant-derived SOM compounds such as cellulose (*O*-alkyl C).[16] In the mineral layer, the microbial community composition also shifted significantly toward fewer Gram-negative bacteria, indicating a deeper suppression of fast-cycling and labile-C decomposers in soils.[48] Although fungal biomass remained statistically unchanged between treatments, the increased aromatic and phenolic C percentages (revealed by NMR), along with the accumulation of lignin-derived phenols, imply that microbial breakdown of lignin biopolymers was also compromised.[49] Actinobacteria, another key decomposer of recalcitrant substrates, also declined in both layers but not significantly, which may have contributed to the persistence of undecomposed aboveground plant residues.[16,41] Earlier observations at this forest site after 22 years of N-addition showed reductions in both bacteria and fungi across soil layers.[5] After 27 years, bacterial suppression remained, while fungal biomass was no longer significantly different, potentially reflecting shifts in microbial community dynamics under chronic N stress rather than recovery of decomposition capacity. Furthermore, during active decomposition of plant-derived inputs, the production of microbial-derived hydrolysable lipids was enhanced.[21] In our study, the lack of significant change in microbial-derived hydrolysable lipid concentrations further supports that typical microbial processes involved in converting plant litter into SOM were suppressed in the forest floor.[47] The increase in C/N ratios with N-addition also supports that the SOM was more enriched with aboveground plant litter. Therefore, this research has identified that microbial communities do not recover or reorganize when faced with stress from chronic N-addition. This novel finding contrasts with other global change stressors or impacts where microbes acclimated to soil warming and changes in litter quality and quantity to maintain their biogeochemical function.[50,51] Overall, this work uniquely shows that chronic N-enrichment has long-lasting impacts on microbes and their ability to regulate C flows in soils which is further exacerbated by the accumulation of less durable forms of C, making these soils even more vulnerable to impacts of global environmental change.

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Microbial responses to long-term N-addition significantly altered SOM composition, primarily by increasing the proportion of aboveground plant-derived compounds in both the forest floor and mineral layers as reflected by the higher C/N ratios. Both NMR and targeted chemical analysis indicated selective accumulation of aboveground plant-derived compounds in N-enriched plots stemming from microbial suppression. In the forest floor, the relative abundance of labile compounds, such as cellulose (*O*-alkyl C), significantly increased, indicating that N-addition preserved plant-derived labile materials that are typically rapidly decomposed.[25,50] This pattern aligns with reduced microbial processing, particularly given the observed decrease in Gram-negative bacteria, fast-growing groups typically responsible for rapid turnover of labile C substrates.[48] In the mineral layer, more stable plant-derived SOM such as cutin, suberin, and lipids (alkyl C) increased, suggesting greater accumulation of more persistent plant-derived components and corresponding with the observed higher C/N ratios.[50] Since microbial decomposition was suppressed in both horizons, the transformation of plant inputs into microbially processed SOM, a necessary step for the formation of mineral-associated organic matter,[52–56] was likely limited. As a result, the additional C sequestered under long-term N-addition may be less biogeochemically stabilized, potentially making it more vulnerable to future C losses.[57] The accumulation of aromatic and phenolic C components in both horizons, along with elevated lignin-derived phenols, further highlights the persistence of plant residues under N-enriched conditions and corresponds to the increased C/N ratios observed.[58] The dominance of long-chain *n*-alkanes (C_{29} and C_{31}) in N-addition forest floor soil was markedly found in black cherry leaves (~60% of aboveground biomass), also confirming aboveground leaf litter as the primary source of plant inputs that were selectively accumulated over roots.[24,50] Altogether, these results indicate that long-term N enrichment suppressed microbial processing of aboveground plant inputs, leading to plant-derived SOM accumulation in the forest floor and surface mineral soil. Reduced microbial transformation likely limits the formation of more durable C, raising concerns about the long-term SOM stability of the new C in N-impacted forest soils.[54,57]

5 Conclusion

This study identified for the first time that 27 years of sustained impacts of chronic N deposition to forests have fundamentally shifted the composition of SOM. Long-term N enrichment has suppressed microbially driven transformation of soil inputs resulting in the enrichment of plant-derived organic matter, including less persistent forms that are susceptible to biodegradation. From a molecular biogeochemistry perspective, long-term N-addition led to the accumulation of mainly aboveground plant-derived compounds in soils. In the forest floor, this buildup was primarily due to the incomplete decomposition of aboveground litter. In the mineral layer, suppressed microbial activity likely also hindered the decomposition of plant inputs, such as root- and woody tissues indicated by the accumulation of suberin-derived and lignin-derived compounds.[21,59,60] At the same time, limited microbial turnover can slow the transformation of plant residues into biochemically altered forms that are typically more resistant to degradation.[27] Our findings are consistent with N saturation theory, which predicts ecosystem-specific thresholds beyond which added N suppresses decomposition and alters microbial function.[50] Consequently, while N-addition increased soil C storage in the forest floor, the accumulated material may be biased toward undecomposed or partially decomposed plant residues. Such soil C may be less stable over the long-term and vulnerable to changes in the climate, disturbance regimes[52,53] or reductions in N deposition that enable microbial populations and processing to return to undisturbed conditions.

Author contributions

Biwei Yang: data curation, formal analysis, validation, visualization, writing – original draft, writing – review and editing. Meiling Man: data curation, formal analysis, writing – review and editing. Yuxuan Weng: data curation, formal analysis, writing – review and editing. Richard D. Bowden: conceptualization, methodology, data curation, resources, writing – review and editing. Jun-Jian Wang: resources, writing – review and editing. Myrna J. Simpson: conceptualization, methodology, project administration, resources, supervision, writing – original draft, writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5va00352k.

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References

1 Y. Li, T.-M. Fu, J. Z. Yu, A. Zhang, X. Yu, J. Ye, L. Zhu, H. Shen, C. Wang, X. Yang, S. Tao, Q. Chen, Y. Li, L. Li, H. Che and C. L. Heald, Nitrogen dominates global atmospheric organic aerosol absorption, *Science*, 2025, **387**, 989–995.

2 P. Sanczuk, K. Verheyen, J. Lenoir, F. Zellweger, J. J. Lembrechts, F. Rodríguez-Sánchez, L. Baeten, M. Bernhardt-Römermann, K. De Pauw, P. Vangansbeke, M. P. Perring, I. Berki, A. D. Bjorkman, J. Brunet,

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M. Chudomelová, E. De Lombaerde, G. Decocq, T. Dirnböck, T. Durak, C. Greiser, R. Hédl, T. Heinken, U. Jandt, B. Jaroszewicz, M. Kopecký, D. Landuyt, M. Macek, F. Máliš, T. Naaf, T. A. Nagel, P. Petřík, K. Reczyńska, W. Schmidt, T. Standovár, I. R. Staude, K. Świerkosz, B. Teleki, T. Vanneste, O. Vild, D. Waller and P. De Frenne, Unexpected westward range shifts in European forest plants link to nitrogen deposition, *Science*, 2024, **386**, 193–198.

3 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.

4 R. S. Swift, Sequestration of carbon by soil, *Soil Sci.*, 2001, **166**, 858.

5 J.-J. Wang, R. D. Bowden, K. Lajtha, S. E. Washko, S. J. Wurzbacher and M. J. Simpson, Long-term nitrogen addition suppresses microbial degradation, enhances soil carbon storage, and alters the molecular composition of soil organic matter, *Biogeochemistry*, 2019, **142**, 299–313.

6 Y. Yang, J. Yang, Q. Dong, D. Li, B. Tan, Q. Wu, Z. Xu and H. Xu, Heavy nitrogen application rate and long-term duration decrease the soil organic carbon and nitrogen sequestration rates in forest ecosystems, *Forests*, 2024, **15**, 1585.

7 Q. Lin, J. Zhu, Q. Wang, Q. Zhang and G. Yu, Patterns and drivers of atmospheric nitrogen deposition retention in global forests, *Glob. Change Biol.*, 2024, **30**, e17410.

8 B. A. Eastman, W. R. Wieder, M. D. Hartman, E. R. Brzostek and W. T. Peterjohn, Can models adequately reflect how long-term nitrogen enrichment alters the forest soil carbon cycle?, *Biogeosciences*, 2024, **21**, 201–221.

9 M. J. Y. Ngaba, Y. Uwiragiye, R. Bol, W. de Vries and J. Zhou, Low-level nitrogen and short-term addition increase soil carbon sequestration in Chinese forest ecosystems, *Catena*, 2022, **215**, 106333.

10 K. Yue, Y. Peng, C. Peng, W. Yang, X. Peng and F. Wu, Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis, *Sci. Rep.*, 2016, **6**, 19895.

11 R. D. Bowden, S. J. Wurzbacher, S. E. Washko, L. Wind, A. M. Rice, A. E. Coble, N. Baldauf, B. Johnson, J.-J. Wang, M. Simpson and K. Lajtha, Long-term nitrogen addition decreases organic matter decomposition and increases forest soil carbon, *Soil Sci. Soc. Am. J.*, 2019, **83**, S82–S95.

12 S. D. Frey, S. Ollinger, K. Nadelhoffer, R. Bowden, E. Brzostek, A. Burton, B. A. Caldwell, S. Crow, C. L. Goodale, A. S. Grandy, A. Finzi, M. G. Kramer, K. Lajtha, J. LeMoine, M. Martin, W. H. McDowell, R. Minocha, J. J. Sadowsky, P. H. Templer and K. Wickings, Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests, *Biogeochemistry*, 2014, **121**, 305–316.

13 B. E. Propson, D. R. Zak, A. T. Classen, A. J. Burton and Z. B. Freedman, Gains in soil carbon storage under anthropogenic nitrogen deposition are rapidly lost following its cessation, *Ecology*, 2024, **105**, e4444.

14 T. Chen, R. Cheng, W. Xiao, L. Zeng, Y. Shen, L. Wang, P. Sun, M. Zhang and J. Li, Nitrogen addition enhances nitrogen but not carbon mineralization in aggregate size fractions of soils in a *Pinus massonia* plantation, *Front. For. Glob. Change*, 2024, **7**, 1240577.

15 J. Chen, W. Xiao, C. Zheng and B. Zhu, Nitrogen addition has contrasting effects on particulate and mineral-associated soil organic carbon in a subtropical forest, *Soil Biol. Biochem.*, 2020, **142**, 107708.

16 H. Hu, C. Qian, K. Xue, R. G. Jörgensen, M. Keiluweit, C. Liang, X. Zhu, J. Chen, Y. Sun, H. Ni, J. Ding, W. Huang, J. Mao, R.-X. Tan, J. Zhou, T. W. Crowther, Z.-H. Zhou, J. Zhang and Y. Liang, Reducing the uncertainty in estimating soil microbial-derived carbon storage, *Proc. Natl. Acad. Sci. U. S. A*, 2024, **121**, e2401916121.

17 H. Y. Liu, N. Huang, C. M. Zhao and J. H. Li, Responses of carbon cycling and soil organic carbon content to nitrogen addition in grasslands globally, *Soil Biol. Biochem.*, 2023, **186**, 109164.

18 M. J. Y. Ngaba, Y. Uwiragiye and J. Zhou, Patterns and controlling factors of soil carbon sequestration in nitrogen-limited and -rich forests in China—a meta-analysis, *PeerJ*, 2023, **11**, e14694.

19 A. Saenger, F. André, M. Jonard, M. Nicolas and Q. Ponette, Carbon sequestration and nitrogen loss drive the evolution of French forest soils, *Front. For. Glob. Change*, 2024, **7**, 1338239.

20 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.

21 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.

22 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.

23 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.

24 I. Wrightson, M. T. 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.: Processes Impacts*, 2025, **27**, 763–778.

25 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, *Geochim. Cosmochim. Acta*, 2024, **374**, 186–199.

26 T. R. Underwood, I. C. Bourg and K. M. Rosso, Mineral-associated organic matter is heterogeneous and structured

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by hydrophobic, charged, and polar interactions, *Proc. Natl. Acad. Sci. U. S. A*, 2024, **121**, e2413216121.
27 G. Angst, K. E. Mueller, K. G. J. Nierop and M. J. Simpson, Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter, *Soil Biol. Biochem.*, 2021, **156**, 108189.
28 X. Feng, A. J. Simpson, W. H. Schlesinger and M. J. Simpson, Altered microbial community structure and organic matter composition under elevated CO_2 and N fertilization in the duke forest, *Glob. Change Biol.*, 2010, **16**, 2104–2116.
29 F. S. Gilliam, N. T. Welch, A. H. Phillips, J. H. Billmyer, W. T. Peterjohn, Z. K. Fowler, C. A. Walter, M. B. Burnham, J. D. May and M. B. Adams, Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition, *Ecosphere*, 2016, **7**, e01250.
30 X. Feng and M. J. Simpson, Molecular-level methods for monitoring soil organic matter responses to global climate change, *J. Environ. Monit.*, 2011, **13**, 1246–1254.
31 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.
32 M. W. I. Schmidt, H. Knicker, P. G. Hatcher and I. Kögel-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, 319–328.
33 P. Conte, R. Spaccini and A. Piccolo, State of the art of CPMAS ^{13}C-NMR spectroscopy applied to natural organic matter, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2004, **44**, 215–223.
34 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.
35 M. J. Simpson and A. J. Simpson, The chemical ecology of soil organic matter molecular constituents, *J. Chem. Ecol.*, 2012, **38**, 768–784.
36 A. Frostegård and E. Bååth, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, *Biol. Fertil. Soils*, 1996, **22**, 59–65.
37 A. Otto, C. Shunthirasingham and M. J. Simpson, A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada, *Org. Geochem.*, 2005, **36**, 425–448.
38 E. G. Bligh and W. J. Dyer, A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, 1959, **37**, 911–917.
39 X. Feng and M. J. Simpson, Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality, *Soil Biol. Biochem.*, 2009, **41**, 804–812.
40 M. J. Salloum, B. Chefetz and P. G. Hatcher, Phenanthrene sorption by aliphatic-rich natural organic matter, *Environ. Sci. Technol.*, 2002, **36**, 1953–1958.
41 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. Change Biol.*, 2023, **29**, 243–259.
42 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.
43 S. D. Frey, M. Knorr, J. L. Parrent and R. T. Simpson, Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests, *For. Ecol. Manag.*, 2004, **196**, 159–171.
44 R. D. Bowden, E. Davidson, K. Savage, C. Arabia and P. Steudler, Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest, *For. Ecol. Manag.*, 2004, **196**, 43–56.
45 X. Lu, P. M. Vitousek, Q. Mao, F. S. Gilliam, Y. Luo, G. Zhou, X. Zou, E. Bai, T. M. Scanlon, E. Hou and J. Mo, Plant acclimation to long-term high nitrogen deposition in an N-rich tropical forest, *Proc. Natl. Acad. Sci. U. S. A*, 2018, **115**, 5187–5192.
46 X. Lu, P. M. Vitousek, Q. Mao, F. S. Gilliam, Y. Luo, B. L. Turner, G. Zhou and J. Mo, Nitrogen deposition accelerates soil carbon sequestration in tropical forests, *Proc. Natl. Acad. Sci. U. S. A*, 2021, **118**, e2020790118.
47 G. Zhou, S. Xu, P. Ciais, S. Manzoni, J. Fang, G. Yu, X. Tang, P. Zhou, W. Wang, J. Yan, G. Wang, K. Ma, S. Li, S. Du, S. Han, Y. Ma, D. Zhang, J. Liu, S. Liu, G. Chu, Q. Zhang, Y. Li, W. Huang, H. Ren, X. Lu and X. Chen, Climate and litter C/N ratio constrain soil organic carbon accumulation, *Natl. Sci. Rev.*, 2019, **6**, 746–757.
48 N. Fanin, P. Kardol, M. Farrell, M.-C. Nilsson, M. J. Gundale and D. A. Wardle, The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils, *Soil Biol. Biochem.*, 2019, **128**, 111–114.
49 X. Lu, F. S. Gilliam, G. Yu, L. Li, Q. Mao, H. Chen and J. Mo, Long-term nitrogen addition decreases carbon leaching in a nitrogen-rich forest ecosystem, *Biogeosciences*, 2013, **10**, 3931–3941.
50 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.
51 A. X. San Román, N. Srikanthan, A. A. Hamid, T. J. Muratore, M. A. Knorr, S. D. Frey and M. J. Simpson, Long-term warming in a temperate forest accelerates soil organic matter decomposition despite increased plant-derived inputs, *Biogeochemistry*, 2024, **167**, 1159–1174.
52 G. Angst, K. E. Mueller, M. J. Castellano, C. Vogel, M. Wiesmeier and C. W. Mueller, Unlocking complex soil systems as carbon sinks: multi-pool management as the key, *Nat. Commun.*, 2023, **14**, 2967.

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53 W. Yu, W. Huang, S. R. Weintraub-Leff and S. J. Hall, Where and why do particulate organic matter (POM) and mineral-associated organic matter (MAOM) differ among diverse soils?, *Soil Biol. Biochem.*, 2022, **172**, 108756.
54 Y. Chen, X. Liu, Y. Hou, S. Zhou and B. Zhu, Particulate organic carbon is more vulnerable to nitrogen addition than mineral-associated organic carbon in soil of an alpine meadow, *Plant Soil*, 2021, **458**, 93–103.
55 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. Change Biol.*, 2020, **26**, 261–273.
56 J. Lehmann and M. Kleber, The contentious nature of soil organic matter, *Nature*, 2015, **528**, 60–68.
57 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.
58 P. Zheng, R. Zhao, L. Jiang, G. Yang, Y. Wang, R. Wang, X. Han and Q. Ning, Increasing nitrogen addition rates suppressed long-term litter decomposition in a temperate meadow steppe, *J. Plant Ecol.*, 2023, **16**, rtac078.
59 J. K. Whalen, S. Gul, V. Poirier, S. F. Yanni, M. J. Simpson, J. S. Clemente, X. Feng, S. J. Grayston, J. Barker, E. G. Gregorich, D. A. Angers, P. Rochette and H. H. Janzen, Transforming plant carbon into soil carbon: Process-level controls on carbon sequestration, *Can. J. Plant Sci.*, 2014, **94**, 1065–1073.
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.

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