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Detrital Inputs Regulate Condensed Aromatic Carbon Persistence in Forest Soils

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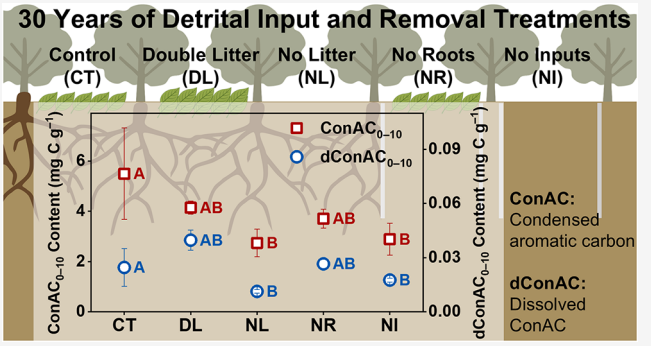
ABSTRACT: Condensed aromatic carbon (ConAC) is widely recognized as a chemically recalcitrant component of soil organic matter. However, its stock and potential transformation into dissolved condensed aromatic carbon (dConAC) in forest soils may be altered in response to environmental changes that alter forest litter production. Using three decades of detrital input and removal treatments in a temperate forest, we investigated bulk ConAC and dConAC in soil profiles with the benzene polycarboxylic acid method. Doubling litter inputs (Double Litter) did not increase ConAC content or stocks, but instead promoted ConAC oxidation to dConAC in surface soils (0−10 cm), evidenced by the higher benzene hexacarboxylic acid-to-benzene pentacarboxylic acid ratio and increased dConAC proportions in both ConAC and dissolved organic carbon pools. Litter removal (No Litter) and its combination with root exclusion (No Inputs) reduced surface-soil ConAC, consistent with decreased ConAC-carrying aboveground litter inputs; No Inputs lowered ConAC stocks throughout the 0−50 cm profile, likely due to intensified microbial utilization of ConAC under substrate limitation. Overall, forest soil ConAC stocks appear relatively resistant to detrital input changes, but enhanced ConAC-to-dConAC conversion highlights a pathway for increased terrestrial−aquatic ConAC fluxes with implications for ConAC cycling under future climate and land-use change.



KEYWORDS: *polycyclic aromatics, benzene polycarboxylic acids, detrital input and removal treatments (DIRT), forest ecosystems, microbial community*

1. INTRODUCTION

Forest soils store approximately 40% of the global terrestrial soil carbon pool and thus play a central role in regulating the global carbon cycle and climate feedbacks.[1,2] Among soil carbon fractions, condensed aromatic carbon (ConAC) represents a globally important and relatively stable component in soil organic carbon (SOC), accounting for approximately 13% of SOC on average.[3,4] It can originate from both pyrogenic and nonpyrogenic pathways. Pyrogenic carbon is typically produced by the incomplete combustion of fossil fuels or biomass,[4,5] whereas nonpyrogenic ConAC can form through the oxidative transformation of biomass.[6,7] Owing to its condensed aromatic structure, ConAC can persist in soils for millennia, making it a critical long-term carbon sink.[4,8] However, ConAC is not entirely inert and can undergo slow oxidative loss over time. Laboratory studies have shown that ConAC mineralization is limited but measurable, with cumulative losses of about 0.17% over 6 months[9] and estimated total losses of 3%−26% over centennial time scales.[10] Such oxidation is not uniform but depends on ConAC properties, with less condensed, more oxygen-rich, and more porous structures being more susceptible.[11,12] This process may contribute to the formation of dissolved condensed aromatic carbon (dConAC), a more mobile fraction that can facilitate carbon export and associated contaminant transport.[4,13,14] Despite recognition of ConAC persistence and importance, the processes governing its stabilization, partial oxidation to dConAC, and subsequent mobilization in forest soils remain poorly understood.

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Atmospheric deposition represents a continuous external input of ConAC particles to forest ecosystems, and vegetation can modulate this pathway.[4,15] Tree canopies intercept airborne ConAC particles, which are incorporated into aboveground litter (making it "ConAC-carrying aboveground litter") and transferred to soils.[16] Thus, aboveground litterfall quantity and composition directly influence soil ConAC pools. Greater canopy leaf volume may receive more deposition, whereas reductions in litter input may limit ConAC delivery to the forest floor and soils. Once in soil, ConAC stability is further influenced by microbial activity.[17] Studies show that altered litter and root inputs can restructure microbial communities.[18] For example, root exclusion usually increases the relative abundance of Gram-positive bacteria and actinobacteria, which are capable of degrading more persistent and recalcitrant compounds.[19,20] Shifted microbial community and carbon use strategies may therefore accelerate ConAC oxidation and increase the production of dConAC, raising potential concerns for water quality given the high mobility and affinity of dConAC for organic pollutants and metals. Together, these mechanisms suggest that both litter amount and source could play a pivotal role in regulating both the persistence of ConAC in soils and its conversion into dConAC.

The Detrital Input and Removal Treatment (DIRT) network[19,21,22] has provided an experimental framework to address the impacts of litter input amount and source on SOC by chronically altering above- and below-ground inputs. Past DIRT studies have demonstrated that litter addition can enhance[18] or destabilize[20,23] SOC pools through stimulated decomposition, whereas litter and root exclusion generally reduce SOC stocks and restructure microbial populations.[19,20,24,25] However, it is not yet clear whether altered litter inputs similarly regulate the ConAC content or its conversion to dConAC. Understanding ConAC responses to altered plant inputs in a temperate forest is particularly important for projecting the role of ConAC in the global carbon cycle. The Bousson Experimental Forest in northwestern Pennsylvania, U.S., exemplifies such a forest. Historically, the region experienced high particulate deposition from distant industrial sources and acidic deposition prior to air-quality regulations. The forest itself has no documented history of local fire disturbance and lies far from ongoing fossil fuel combustion sites,[26] so all plots are expected to receive broadly similar atmospheric ConAC deposition. Here, canopy-mediated deposition and plant inputs dominate ConAC delivery to soils, making the Bousson Forest a representative site to study long-term ConAC dynamics in temperate forests.

Here, we assessed how 30 years of DIRT manipulations regulated the ConAC and dConAC contents in soils from the Bousson Experimental Forest. ConAC was quantified using the benzene polycarboxylic acid (BPCA) molecular marker method, which provides insights into the ConAC structure, sources, and formation conditions.[27,28] It is based on the nitric acid oxidation of condensed aromatic structures under high-temperature and high-pressure conditions, producing a series of benzene polycarboxylic acids with varying numbers of carboxyl groups, including benzene hexacarboxylic acid (B6CA), benzene pentacarboxylic acid (B5CA), benzene tetracarboxylic acids (B4CAs), and benzene tricarboxylic acids (B3CAs). These BPCAs are subsequently separated and quantified using chromatographic techniques, and ConAC concentrations are calculated by using established conversion factors. We quantified the vertical distribution of ConAC and dConAC across a range of aboveground litter and root manipulation treatments. We hypothesized that (1) adding ConAC-carrying aboveground litter would increase soil ConAC and dConAC contents, elevate ConAC/SOC ratio, and lower dConAC/ConAC ratio (indicator of ConAC-to-dConAC conversion); (2) similarly, aboveground litter removal treatment would cause lower ConAC and dConAC contents and ConAC/SOC ratio and higher dConAC/ConAC in soils due to less ConAC-carrying aboveground litter inputs; and (3) excluding both aboveground litter and fresh root inputs would cause lower ConAC and dConAC contents and higher dConAC/ConAC due to the greater microbial degradation of ConAC in the absence of fresh plant carbon inputs. These findings aim to advance our understanding of ConAC responses to plant-derived inputs and inform projections of carbon sequestration potential and adaptive forest management under global change.

2. MATERIALS AND METHODS

2.1. Site Description and Soil Sampling

This study was conducted in Bousson Forest, a temperate deciduous forest in northwestern Pennsylvania (41°26′ N, 80°30′ W). The region has a temperate continental moist climate, with a mean temperature of −4.4 °C in January and 20.8 °C in July, and a mean annual precipitation of 1125 mm. Snow typically covers the ground for about 4 months.[22] The dominant tree species are black cherry (*Prunus serotina* Ehrh.) and sugar maple (*Acer saccharum* Marshall), accounting for approximately 60% and 28% of the aboveground biomass, respectively, with an annual litterfall of 2430 kg C ha^{-1} yr^{-1}.[29] Soils are classified as Cambridge series Alfisols, derived from glacial till deposits over shale and sandstone bedrock. The soil has a sandy loam texture, composed of roughly 75% sand, 23% silt, and 2% clay. The organic carbon content in 0−10 cm of soils is approximately 6% (Figure S1). The topography is relatively flat, with an average slope of about 5% and an elevation of about 390 m.[22,29]

The DIRT experiment was established in 1991 and comprised five treatments:[30] (1) Control (CT), maintaining natural litter and root inputs; (2) Double Litter (DL), doubling aboveground litter inputs by adding litter removed from No Litter plots; (3) No Litter (NL), regularly removing all aboveground litter; (4) No Roots (NR), excluding root inputs by installing impermeable plastic barriers in trenches around each plot to a depth of approximately 1.4 m (about 0.4 m below the maximum rooting depth); and (5) No Inputs (NI), excluding both aboveground litter and root inputs. Each treatment has three replicate plots (3 m × 3 m), with all plots located within a relatively small area (about 0.4 ha) and originally selected to be as uniform as possible in initial soil properties. This design helps reduce the potential influence of small-scale spatial heterogeneity.

In August 2021, soil samples were collected from depths of 0−10 cm, 10−20 cm, 20−30 cm, and 30−50 cm. Visible stones and fine roots were manually removed. Fresh subsamples were sieved (2 mm) and stored at −80 °C prior to microbial analyses. The remaining soil samples were air-dried, ground, and sieved through a 200-mesh screen (0.074 mm) to ensure uniformity. The resulting powders were stored in the dark at −80 °C for subsequent analysis.

2.2. Determination of Condensed Aromatic Carbon Content

ConAC content in soil samples was quantified using the BPCA method.[27,31] Dried soil samples (50.0 mg) were placed into glass ampules with 2 mL of 65% nitric acid (GR grade, Sinopharm, Beijing, China). The ampules were sealed and placed in a 250 mL Teflon-lined reaction kettle, with 1 mL of deionized water added to balance internal and external pressures and prevent explosion. The sealed system was heated at 170 °C for 8 h in an explosion-proof oven. After cooling to room temperature, samples were evaporated to dryness under a stream of high-purity nitrogen gas (≥99.999%) at 50 °C. The

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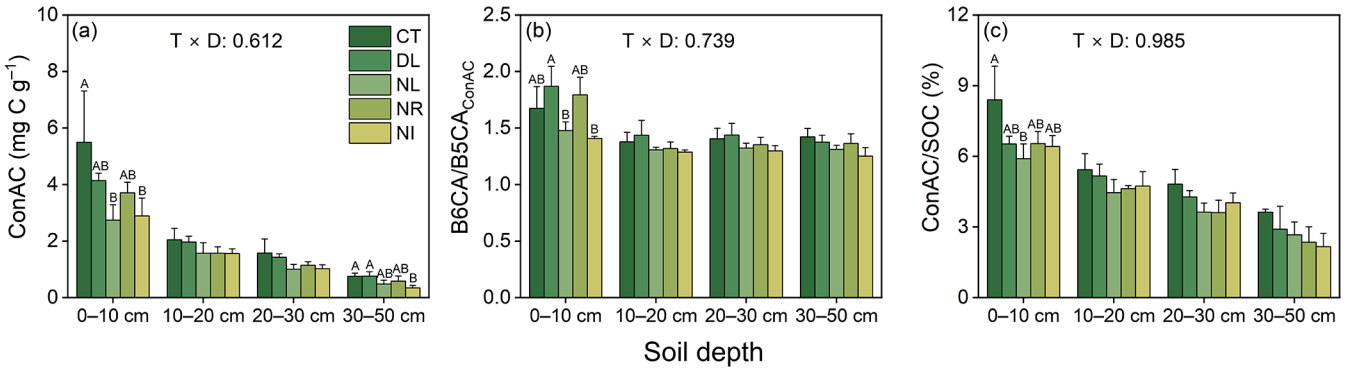

Figure 1. Effects of detrital input and removal treatments (DIRT) on soil condensed aromatic carbon (ConAC) content (a), the ratio of benzene hexacarboxylic acid-to-benzene pentacarboxylic acid of ConAC (B6CA/B5CA$_{ConAC}$) (b), and the ratio of ConAC to soil organic carbon (ConAC/SOC) (c). CT: Control; DL: Double Litter; NL: No Litter; NR: No Roots; and NI: No Inputs. SOC was estimated as 50% of soil organic matter,[45] sourced from Bowden et al.[22] Values represent means ± standard errors (n = 3). Different uppercase letters indicate significant or marginally significant differences among treatments within the same soil depth (p < 0.1). Numbers following "T × D" denote p-values from two-way analysis of variance testing the interaction between treatment and soil depth.

dried residues were then redissolved in 1 mL of ultrapure water and filtered through 0.22 μm polytetrafluoroethylene membranes.

BPCA compounds were separated and quantified using a high-performance liquid chromatography system with a photodiode-array detector (HPLC-DAD; U3000, Thermo Fisher, Waltham, MA) and a Poroshell 120 SB-C18 column (100 mm × 4.6 mm, 2.7 μm; Agilent Technologies, Santa Clara, CA). The column was maintained at 30 °C with a flow rate of 0.4 mL min^{-1} and an injection volume of 10 μL. The mobile phase consisted of solvent A (1.7% aqueous phosphoric acid solution, HPLC grade) and solvent B (acetonitrile, LC-MS grade), following a gradient elution program. Calibration curves were constructed using ultraviolet absorbance at 240 nm for B6CA, B5CA, one B4CA (1,2,4,5-B4CA), and three B3CAs (1,2,3-B3CA, 1,3,5-B3CA, and 1,2,4-B3CA), with standards from Aladdin, Shanghai, China. For 1,2,3,4-B4CA and 1,2,3,5-B4CA, which were not commercially available, quantification was performed using the calibration curve of their isomer, 1,2,4,5-B4CA.[32−34] Measured BPCA concentrations were all >1.5 μM, exceeding the instrumental detection limits (0.2−0.5 μM), with good analytical reproducibility (coefficients of variation <5%).[32] Final ConAC content was calculated by multiplying the total carbon content of the detected BPCAs by a conversion factor of 2.27.[27]

2.3. Extraction and Determination of Dissolved Condensed Aromatic Carbon

dConAC was extracted from soils using a 1:20 (w/v) soil-to-water ratio.[35,36] Briefly, 3 g of air-dried soil was mixed with 60 mL of ultrapure water, shaken at 180 rpm for 24 h at 20 °C. After shaking, the suspension was centrifuged and filtered through a 0.22 μm poly(ether sulfone) membrane (Millipore). The filtrate was then stored at 4 °C in the dark. The concentration of dissolved organic carbon (DOC) was determined using a total organic carbon analyzer (TOC-L CSH/CSN, Shimadzu, Tokyo, Japan).

dConAC concentration was quantified using the BPCA method. Specifically, 30 mL of soil extract was evaporated at 50 °C, and the dried residue was reconstituted in 1 mL of 65% nitric acid and transferred into an ampule. The subsequent oxidation and chromatographic steps followed the procedure described in Section 2.2. The dConAC concentration was calculated using the following equation.[37]

$$[\mathrm{dConAC}] = 0.0891 \times ([\mathrm{B6CA}] + \mathrm{B5CA}])^{0.9175} \quad (1)$$

where [dConAC] represents the concentration of dConAC in the liquid sample (μmol L^{-1}) and [B6CA] and [B5CA] denote the concentrations of B6CA and B5CA, respectively (nmol L^{-1}). The dConAC content in soil (in μg C g^{-1}) was calculated based on the concentration of dConAC in the liquid sample and the soil-to-water ratio for extraction. Based on the ConAC and dConAC contents in soil as well as the soil bulk density and soil mass in each soil depth, the ConAC and dConAC stocks (i.e., masses of ConAC and dConAC per square meter of soil) were also calculated (Figure S2). As the B6CA is mainly derived from ConAC with higher condensed aromaticity,[32,38] the B6CA to B5CA ratios for ConAC (B6CA/B5CA$_{ConAC}$) and dConAC (B6CA/B5CA$_{dConAC}$) were calculated to indicate the aromatic condensation degrees of ConAC and dConAC, respectively.[34,39]

2.4. Soil Enzyme Activities and Microbial Community Analysis

To characterize microbial functional responses to altered detrital inputs, the activities of four extracellular enzymes were determined using colorimetric assays with a SpectraMax 190 Microplate Reader (Molecular Devices, San Jose, CA), including catalase, polyphenol oxidase, urease, and acid protease. Catalase activity was determined by monitoring H_2O_2 decomposition at 240 nm.[40] Polyphenol oxidase activity was measured using pyrogallol as the substrate at 430 nm.[41] Urease activity was determined by the indophenol blue method at 578 nm following incubation at 37 °C for 24 h.[41] Acid protease activity was quantified using the Folin−Ciocalteu method at 680 nm.[42] Substrate-free controls were included for all assays to correct for background absorbance. Enzyme activities were expressed on a dry soil basis and further normalized to microbial biomass, which was estimated based on phospholipid fatty acid (PLFA) analysis, to account for variations in microbial abundance across treatments.

Soil genomic DNA was extracted using FastDNA SPIN Kit for Soil and quantified using a NanoDrop One spectrophotometer (Thermo Fisher, Waltham, MA). The V3−V4 region of the bacterial 16S rRNA gene and the fungal ITS1 region were amplified using universal primer pairs. PCR products were verified by 1.5% agarose gel electrophoresis, pooled in equimolar concentrations, and purified using the E.Z.N.A. Gel Extraction Kit (Omega, Norcross, GA). Paired-end sequencing (2 × 250 bp) was performed on an Illumina MiSeq platform at Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China). Raw sequences were quality-filtered, merged, and screened for chimeras prior to clustering into operational taxonomic units (OTUs) at a 97% similarity threshold. Taxonomic assignment was conducted against the SILVA database for bacteria and the UNITE database for fungi. α diversity indices, including Shannon diversity, Chao 1 richness, and Pielou's evenness, were calculated at the OTU level.

2.5. Calculations and Statistical Analyses

Two-way analyses of variance (ANOVA) were used to evaluate the interaction between detrital treatments and soil depth. One-way ANOVA combined with Duncan's post hoc test was applied to compare differences among treatments within the same soil depth. These analyses were used to assess overall effects and to further identify treatment-specific differences under each soil depth. Differ-

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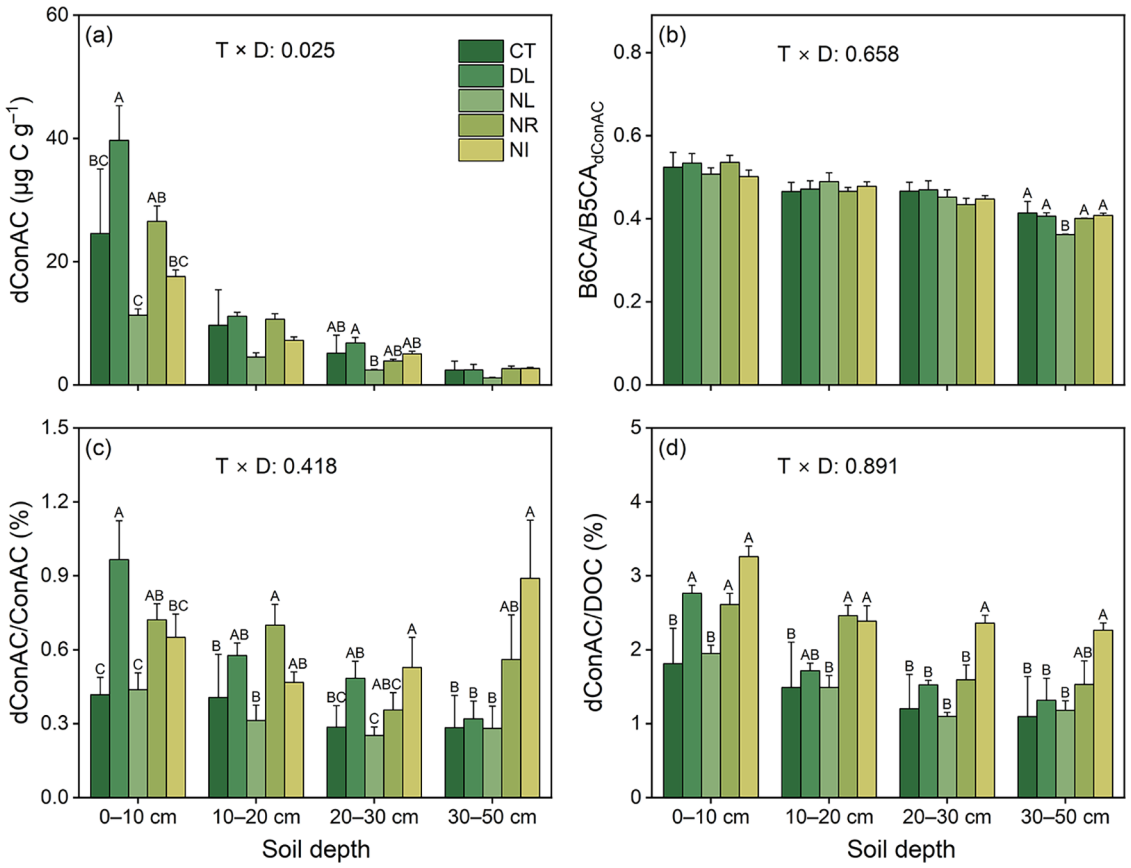

Figure 2. Effects of detrital input and removal treatments (DIRT) on dissolved condensed aromatic carbon (dConAC) content (a), the ratio of benzene hexacarboxylic acid-to-benzene pentacarboxylic acid of dConAC (B6CA/B5CA$_{dConAC}$) (b), the ratio of dConAC to condensed aromatic carbon (ConAC) (c), and the ratio of dConAC to dissolved organic carbon (DOC) (d). CT: Control; DL: Double Litter; NL: No Litter; NR: No Roots; and NI: No Inputs. Values represent means ± standard errors (n = 3). Different uppercase letters indicate significant or marginally significant differences among treatments within the same soil depth (p < 0.1). Numbers following "T × D" denote p-values from two-way analysis of variance testing the interaction between treatment and soil depth.

ences at p < 0.05 were considered significant, and those at 0.05 ≤ p < 0.1 were considered marginally significant. To quantify the effect size of treatments relative to controls, Hedges' g was calculated based on group means and variances. The calculation formula is as follows.[43,44]

$$\text{Hedges}'g = \left(1 - \frac{3}{4(n_T + n_C) - 9}\right) \times \frac{\overline{x}_T - \overline{x}_C}{\sigma_{pooled}} \quad (2)$$

$$\sigma_{pooled} = \frac{\sqrt{(n_T - 1)s_T^2 + (n_C - 1)s_C^2}}{n_T + n_C - 2} \quad (3)$$

where n_C and n_T are the sample sizes of groups (both three in this study), $\overline{x}_C$ and $\overline{x}_T$ represent the mean values of the control and treatment groups, respectively, and s_C^2 and s_T^2 denote their corresponding variances.

Spearman's rank correlation analysis was performed to assess the relationships between ConAC-related parameters and normalized enzyme activities, microbial community composition, and α diversity indices. Data analyses and visualization were performed using SPSS 27.0, R 4.3.2, and Origin 2024.

3. RESULTS

3.1. Decreased ConAC with No Litter but No Increase in ConAC with Doubled Litter

There was no interaction between detrital treatments and soil depth on soil ConAC content, the B6CA/B5CA$_{ConAC}$ ratio, or the ConAC/SOC ratio (all p > 0.1; Figure 1). Across all treatments, ConAC content and ConAC/SOC decreased with depth (p < 0.05; Figure 1a, c), showing patterns consistent with SOC content. However, the B6CA/B5CA$_{ConAC}$ remained relatively stable across soil depths (p > 0.05; Figure 1b). The intertreatment variations in ConAC content, B6CA/B5CA$_{ConAC}$, and ConAC/SOC were consistently greatest in the 0−10 cm surface soils (Figure 1). In this soil layer, the No Litter and No Inputs treatments had lower ConAC content compared to Control (50.1% and 47.4% lower, respectively; both p < 0.1; Figure 1a) and slightly lower B6CA/B5CA$_{ConAC}$ compared to Double Litter (p < 0.1; Figure 1b). Also, the No Litter treatment had a lower ConAC/SOC compared to Control and Double Litter (p < 0.1; Figure 1c). At depths below 10 cm, soil ConAC content, B6CA/B5CA$_{ConAC}$, and ConAC/SOC did not differ between treatments (p > 0.1), except at 30−50 cm, where ConAC content was lower in No Litter than in Control and Double Litter (p < 0.1; Figure 1). Interestingly, the Double Litter treatment did not significantly increase soil ConAC content compared to Control in any given soil depth (p > 0.1; Figure 1), consistent with SOC trends at this site (p > 0.1; Figure S1).

3.2. Effects of Detrital Manipulation on dConAC and Its Relevant Ratios

An interaction between detrital treatments and soil depth was observed only for dConAC content (p < 0.05), not for B6CA/B5CA$_{dConAC}$, dConAC/ConAC, or dConAC/DOC (Figure 2).

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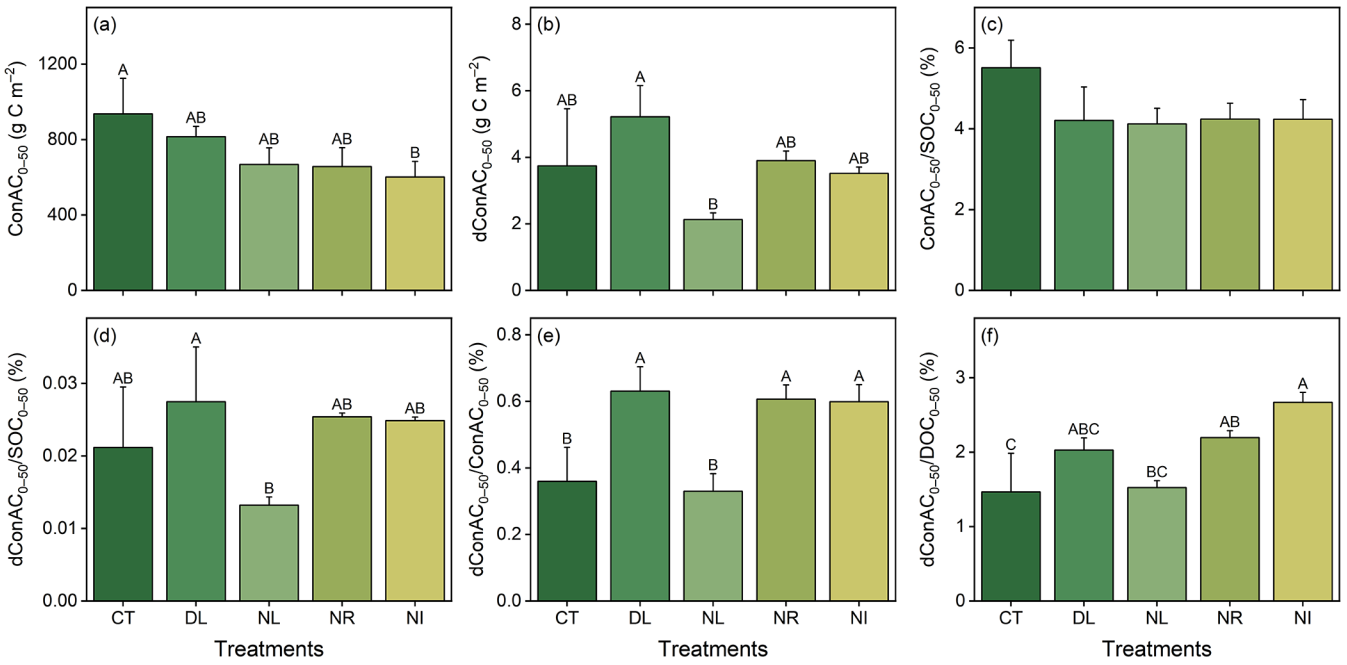

Figure 3. Effects of detrital input and removal treatments (DIRT) on condensed aromatic carbon stock and relevant ratios in the 0−50 cm soil profile. Parameters include condensed aromatic carbon stock ($ConAC_{0-50}$; panel a), dissolved condensed aromatic carbon stock ($dConAC_{0-50}$; panel b), proportion of dissolved condensed aromatic carbon in soil organic carbon ($ConAC_{0-50}/SOC_{0-50}$; panel c), proportion of dissolved condensed aromatic carbon in soil organic carbon ($dConAC_{0-50}/SOC_{0-50}$; panel d), proportion of dissolved condensed aromatic carbon in condensed aromatic carbon stock ($dConAC_{0-50}/ConAC_{0-50}$; panel e), and proportion of dissolved condensed aromatic carbon in dissolved organic carbon stock ($dConAC_{0-50}/DOC_{0-50}$; panel f). CT: Control; DL: Double Litter; NL: No Litter; NR: No Roots; and NI: No Inputs. Data represent mean ± standard error (n = 3). Different uppercase letters indicate significant or marginally significant differences among treatments within the same soil depth (p < 0.1).

This interaction was reflected in higher soil dConAC content in Double Litter than in Control (p < 0.1) and in No Litter (p < 0.05) at 0−10 cm, and higher soil dConAC content in Double Litter than in No Litter at 20−30 cm soils (p < 0.1), while there were no differences in dConAC content across treatments at any other soil depth (Figure 2a). Similar to ConAC content, the dConAC content, $B6CA/B5CA_{dConAC}$, dConAC/ConAC, and dConAC/DOC all decreased with increasing soil depth, except that dConAC/ConAC increased from 20−30 cm to 30−50 cm in the No Roots and No Inputs treatments (Figure 2).

Compared to Control, Double Litter had 131.2% higher dConAC/ConAC and 52.6% higher dConAC/DOC at 0−10 cm (both p < 0.05) but showed no differences on dConAC/ConAC or dConAC/DOC at deeper layers (p > 0.1; Figure 2c,d); No Litter did not have altered dConAC/ConAC or dConAC/DOC at any soil depths (p > 0.1; Figure 2c,d); No Roots had 72.8%−72.3% higher dConAC/ConAC and 44.3%−65.1% higher dConAC/DOC at 0−10 cm and 10−20 cm (p < 0.05) but no altered dConAC/ConAC or dConAC/DOC at deeper layers (Figure 2c,d); No Inputs had 85.0%−213.7% higher dConAC/ConAC at 20−30 cm and 30−50 cm and 60.1%−106.4% higher dConAC/DOC at all soil depths (Figure 2c,d). Variation in $B6CA/B5CA_{dConAC}$ was limited, with a lower value only at 30−50 cm in No Litter compared to Control (p < 0.05; Figure 2b).

3.3. Altered ConAC and dConAC Stocks in Soil Profiles by Litter Manipulations

Total ConAC and dConAC stocks (in g C m^{-2}) in the 0−50 cm soil profile followed trends similar to their contents (in g C g^{-1}) across treatments, with lower ConAC stock in No Inputs than in Control (p < 0.1; Figure 3a) and lower dConAC stock in No Litter than in Double Litter (p < 0.05; Figure 3b). There was no difference in ConAC/SOC stock ratio across treatments (p > 0.1; Figure 3c); whereas the dConAC/SOC stock ratio was lower in No Litter than in Double Litter (p < 0.05; Figure 3d). Compared to Control, the Double Litter, No Roots, and No Inputs treatments increased the dConAC/ConAC stock ratio across the soil profile by more than 60% (p < 0.05), while No Litter did not significantly alter this ratio (p > 0.1; Figure 3e). Furthermore, the No Roots and No Inputs treatment increased the dConAC/DOC ratio by 49.8% and 82.2%, respectively (p < 0.1 and p < 0.05, respectively; Figure 3f).

3.4. Microbial Associations with ConAC Transformation under Long-Term Detrital Manipulation

Long-term detrital manipulations significantly altered soil microbial community structure and functional potential. Microbial biomass-normalized enzyme activities showed significant treatment effects, with catalase activity increasing by more than 80% in the No Roots and No Inputs treatments (p < 0.1; Figure S4). Correspondingly, microbial community composition varied across treatments. At the phylum level, No Litter and No Inputs reduced the relative abundance of Proteobacteria while increasing that of Chloroflexi (p < 0.05; Figure S5). The Double Litter and No Roots treatments had higher Actinobacteria abundance compared to Control (both approximately 32% higher; p < 0.1; Figure S5). Fungal communities showed similarly pronounced compositional changes, with Mucoromycota increasing and Basidiomycota decreasing under No Inputs (p < 0.05; Figure S6). Beyond composition, No Inputs enhanced bacterial Shannon diversity,

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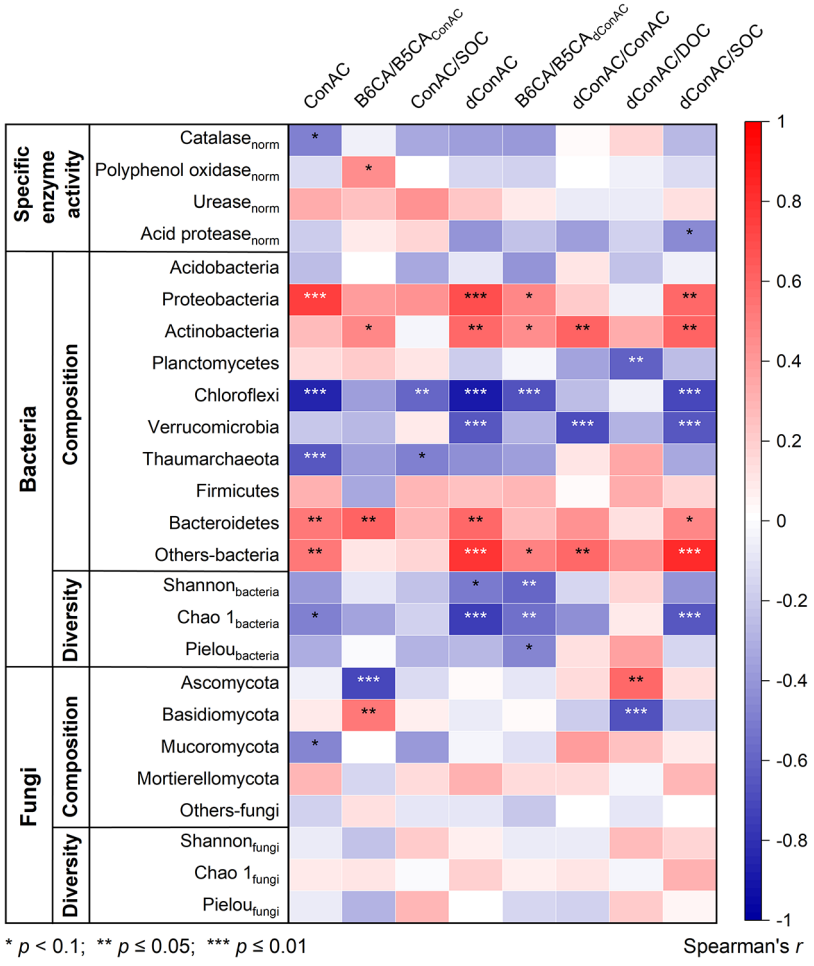

Figure 4. Spearman's correlations between condensed aromatic carbon (ConAC) and microbial properties under long-term detrital manipulation. *: 0.05 < p < 0.1; **: 0.01 < p ≤ 0.5; ***: p ≤ 0.01. B6CA: benzene hexacarboxylic acid, B5CA: benzene pentacarboxylic acid, SOC: soil organic carbon, dConAC: dissolved condensed aromatic carbon, and DOC: dissolved organic carbon.

Chao 1 richness, and Pielou's evenness compared to Control (p < 0.1), whereas No Litter significantly decreased both fungal Shannon diversity and Pielou's evenness (p < 0.05; Figure S7).

Spearman's correlation analysis revealed potential associations between microbial properties and ConAC-related variables (Figure 4). Overall, microbial-biomass-normalized enzyme activities showed weak relationships with ConAC-related variables. Only catalase activity exhibited a marginally significant negative correlation with ConAC content, while polyphenol oxidase activity was positively correlated with B6CA/B5CA$_{ConAC}$ (p < 0.1). In contrast, the bacterial community composition displayed clearer and more consistent associations with ConAC. Proteobacteria were positively correlated with both ConAC and dConAC (p < 0.01), whereas Actinobacteria showed significant positive correlations with dConAC and dConAC/ConAC (p < 0.05). Conversely, Chloroflexi were negatively associated with ConAC, dConAC, and ConAC/SOC (p < 0.05). Notably, bacterial α diversity also showed selective associations, with Chao 1 richness being negatively correlated with ConAC (p < 0.1) but strongly positively correlated with dConAC (p < 0.01). Compared to bacteria, fungal community attributes exhibited generally weaker relationships with the ConAC. Most fungal α diversity indices were not significantly correlated with these variables (p > 0.1). At the phylum level, Ascomycota and Basidiomycota showed contrasting patterns: Ascomycota were negatively correlated with B6CA/B5CA$_{ConAC}$ but positively associated with dConAC/DOC, whereas Basidiomycota exhibited the opposite trends (p < 0.05).

4. DISCUSSION

4.1. Asymmetric Impacts of Litter Addition and Removal on Soil ConAC Content and Stock

The ConAC accounted for ∼8.4% of SOC in this study site. This site has not experienced fires for at least decades, and we found no identifiable charcoal in the soils. Nevertheless, the ∼8.4% ConAC/SOC ratio indicates that even in fire-free forests, ConAC can represent a substantial fraction of SOC. Contrary to our first hypothesis, doubling litter input did not increase soil ConAC content at any soil depth or ConAC stock across the 0−50 cm soil profile. Litterfall over the past three decades is estimated to have contributed approximately 54 g C m^{-2} of ConAC, representing ∼15% of the current 0−10 cm ConAC stock in the Control plots (371 g C m^{-2}). Although the Double Litter treatment received roughly twice this ConAC input, its soil ConAC stock (298 g C m^{-2}) remains lower than that of the Control. Similarly, SOC content remained unchanged under the Double Litter treatment (Figure S1). This result aligns with previous research indicating that litter addition may not always increase SOC

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due to the fast decomposition of added litter or litter-induced microbial degradation.[20,23,46,47] In this study, microbial biomass in the Double Litter treatment (30.8 $\mu g\ g^{-1}$) was higher than in the Control (23.6 $\mu g\ g^{-1}$) based on phospholipid fatty acid analysis method (Figure S4). Enhanced microbial activity triggered by extra labile substrates in the added litter may have accelerated ConAC degradation, offsetting the additional ConAC supplied by litter. Although ConAC itself is not a biolabile carbon or energy source for microbes, extracellular enzymes produced during the microbial decomposition of other liable substrates can promote ConAC oxidation and subsequent breakdown.[48] Microbial community composition also showed marked shifts, including an increased relative abundance of Actinobacteria, suggesting an enhanced microbial potential for processing ConAC. Additionally, the higher dConAC/ConAC ratio (an indicator of greater ConAC-to-dConAC oxidation) under the Double Litter treatment compared to the Control further supports the enhanced ConAC decomposition.

Supporting our second hypothesis, aboveground litter removal (No Litter and No Inputs) decreased ConAC content in the 0−10 cm soil layer. As noted earlier, litterfall over the past three decades has contributed approximately 54 g C m^{-2} of ConAC, indicating that litter exclusion effectively reduces this external input pathway. This reduction in ConAC was accompanied by microbial community changes, including a significant increase in the relative abundance of Chloroflexi, a group associated with the degradation of recalcitrant carbon. These findings emphasize that the reduction in ConAC under litter removal is associated with lower external inputs and shifts in microbial community composition. Together, the lack of ConAC accumulation under litter addition and its clear decline under litter removal highlight the asymmetric impacts of detrital inputs on soil ConAC content.

Consistent with our third hypothesis, the combined exclusion of aboveground litter and roots (i.e., No Inputs) caused the greatest ConAC stock reduction compared to the Control. Previous works at this site[19] and other DIRT sites[18] reported that detrital removal treatments, particularly No Inputs, increase the relative abundance of Gram-positive bacteria and actinobacteria, which are more capable of degrading more persistent and recalcitrant organic compounds. Also, the strongest oxidation of lignin derivatives, another aromatic SOM component, was observed in No Inputs at this site[20] and in No Inputs in a temperate forest at the Harvard Forest (MA).[19] In this study, normalized catalase activity in the No Inputs treatment was more than 80% higher than that in the Control, indicating enhanced oxidative potential in the absence of litter and roots. Additionally, the relative abundance of Chloroflexi, a microbial group known for its ability to degrade recalcitrant carbon, nearly doubled in No Inputs. These microbial shifts, combined with previous research, suggest that microbial communities under substrate limitations may have an increased capacity to process more stable organic carbon (i.e., ConAC). Thus, the greatest ConAC losses under No Inputs likely reflect both reduced ConAC inputs and enhanced microbial decomposition of ConAC under substrate limitations.

In the 20-year DIRT experiment at this site, Wang et al.[20] reported significant increases in cutin and suberin derivative contents in soils under No Roots, whereas lignin derivatives remained relatively stable across treatments. Our 30-year DIRT experiment results here show that soil ConAC responses were most similar to those of lignin derivatives, showing substantially weaker responses than plant-derived compounds such as cutin and suberin derivatives (Figure S3). This pattern indicates that while ConAC is among the most chemically stable SOM fractions, its absolute amount and contribution to SOC can still decline when inputs are not maintained (e.g., No Litter, No Inputs).

4.2. Increased dConAC Content and dConAC/ConAC Ratio in Surface Soils with Litter Addition

Consistent with our first hypothesis, litter addition increased the dConAC content and the dConAC/ConAC ratio in surface soils. This likely reflects both extra inputs of ConAC-carrying aboveground litter and enhanced ConAC decomposition (indicated by the highest average $B6CA/B5CA_{ConAC}$ value under Double Litter) stimulated by extra fresh aboveground litter inputs, which enhanced dConAC release. Moreover, previous studies suggest that dConAC-like condensed aromatics can form through nonpyrogenic pathways, such as Fenton-like oxidation of lignin.[7,49−51] Therefore, a potential contribution of dConAC formation through oxidation of extra lignin in the aboveground litter could not be excluded. Indeed, our earlier study[20] reported higher lignin oxidation (indicated by acid-to-aldehyde monomer ratio) in Double Litter than in Control, supporting such a possibility. Nevertheless, no significant changes in the dConAC content or dConAC/ConAC were observed below 10 cm, likely due to no differences in microbial processes in deeper soils.

Supporting our second hypothesis, the average soil dConAC content and stock were consistently lowest under No Litter for all soil depths. This finding reinforces the critical role of aboveground litter inputs as a pathway for dConAC in soils. Aboveground litter not only incorporates atmospheric ConAC deposition but also serves as a precursor to dConAC formation. Although dConAC content in No Roots and No Inputs did not differ from Control, their dConAC/ConAC ratios were higher. As mentioned above, root exclusion treatments (No Roots and No Inputs) at this site caused strong shifts in microbial communities. Specifically, these treatments increased the relative abundances of Actinobacteria and Chloroflexi that preferentially degrade more persistent and recalcitrant compounds and also led to higher normalized catalase activity. Such microbial shifts likely accelerated ConAC decomposition, leading to higher dConAC production relative to ConAC stocks and supporting our third hypothesis. Furthermore, these findings further confirm that SOM degradation is advanced in the No Roots and No Inputstreatments.[19,20] Because root exudates, which typically include labile, less aromatic compounds such as organic acids, sugars, and enzymes,[52] were absent, the dConAC pool became less diluted by easily degradable substrates. This absence likely contributed to the elevated dConAC/DOC ratios observed in No Roots and No Inputs compared to the Control.

4.3. Environmental Implications

The DIRT experiment represents an extreme case of detrital change, yet detrital inputs in natural and urban forests can also be altered by changing climate (through shifts in plant productivity) and human disturbance (e.g., litter harvesting and understory clearing).[53] Compared to other studied SOM components such as sugars, cutin, suberin, and lignin derivatives (Figure S3), ConAC showed only marginal and relatively small changes even after 30 years of altered detrital inputs. This finding underscores the ConAC's critical role as a

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long-lasting SOM component with relatively low sensitivity to altered detrital inputs. Notably, doubling litter inputs for 30 years did not increase ConAC content or stocks and instead stimulated ConAC oxidation (indicated by elevated average $B6CA/B5CA_{ConAC}$, dConAC/ConAC, and dConAC/DOC) in surface soils.

Despite the relatively high stability of bulk ConAC, litter addition promoted ConAC oxidation, resulting in a higher dConAC content and greater proportions of dConAC in both the ConAC and DOC pools in surface soils. Because dConAC is more mobile and prone to leaching than particulate ConAC,[54,55] its production represents a potential loss pathway for ConAC. This enhanced mobilization of ConAC into dissolved forms may facilitate its export from soils, thereby reducing its long-term retention in forest ecosystems and influencing soil carbon storage over extended time scales. Such processes may partially offset the carbon sequestration gains associated with the increased plant inputs over time. Moreover, dConAC often exhibits much stronger (up to 10-fold) affinities for hydrophobic organic compounds (e.g., polycyclic aromatic hydrocarbons) and toxic metals compared with other DOM components.[56,57] Consequently, enhanced dConAC formation may facilitate pollutant transport from soils into downstream waters, thereby increasing contaminant loads in source water.

Overall, this study provides a systematic evaluation of how detrital input manipulations affect ConAC and dConAC stocks in forest soils, offering novel insights into the mechanisms governing soil ConAC recalcitrance and mobility. These findings improve our understanding of soil carbon pool responses under global change and inform more effective carbon-sink management strategies. However, capturing the temporal dynamics of ConAC and dConAC would further strengthen our understanding. Future studies should aim to integrate multitime-point data to better resolve soil ConAC and dConAC responses under shifting detrital inputs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.6c05973.

Include 4 tables and 7 figures; tables including the Two-way ANOVA results, condensed aromatic carbon- and dissolved condensed aromatic carbon-related parameters for individual soil depths and soil profiles; and condensed aromatic carbon content in litter; figures including soil organic carbon content; condensed aromatic carbon and dissolved condensed aromatic carbon stocks in different depths; comparison of condensed aromatic carbon changes with other organic compounds across treatments; and microbial biomass-normalized enzyme activities; and bacterial and fungal community composition and α diversity in surface soil (PDF)

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Author Contributions

This manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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