Warming accelerates the decomposition of root-derived hydrolysable lipids in a temperate forest ~~only in topsoil but not in subsoil~~ and ~~andi is is~~depth- and compound class-dependent

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Abstract.

Global warming could potentially increase the decomposition rate of soil organic matter (SOM), not only in the topsoil (< 20 cm) but also in the subsoil (> 20 cm). Despite its low carbon content, subsoil holds on average nearly as much SOM as topsoil across various ecosystems. However, significant uncertainties remain regarding the impact of warming on SOM decomposition in subsoil, particularly root-derived carbon, which serves as the primary organic input at these horizons. In a whole-soil field warming experiment at Blodgett Forest Research Station (California, USA), we investigated whether warming accelerates the decomposition of root-derived hydrolysable lipids ~~at three depths (10-14, 45-49, and 85-89 cm)~~in the top- (10-14 cm) and subsoil (45-49, 85-89 cm) by using molecular markers and *in-situ* incubation of [13]C-~~labelled~~labeled ~~root-litter~~root litter at each depth. Our results reveal that at compound-class level, ~~the decomposition of root-derived hydrolysable lipids was only accelerated in the topsoil (10-14 cm) but not in the subsoil (45-49 and 85-89 cm) with warming~~hydrolysable lipids presented compound-dependent responses. Warming consistently reduced fatty acid mass change across soil depths, particularly at 85-89 cm. In subsoil, ~~In subsoil, although the decomposition rate of hydrolysable lipids did not differ significantly between ambient and warmed plots, the underlying reasons for this similarity are distinct.~~ there was accumulation of fatty acids, which primarily originated from~~With molecular marker analysis, we found accumulation of several mid-chain fatty acids that are potentially~~ microbial-derived mid-chain

32 fatty acids such as octadecanoic acid ($C_{18:0}$ fatty acids), octadecenoic acid ($C_{18:1}$ fatty acids),

33 and hexadecanoic acid ($C_{16:0}$ fatty acids). Higher temperature attenuated this accumulation, ~~,~~

34 indicating ~~more~~ less microbial transformation of root-derived carbon under ~~ambient~~

35 ~~conditions~~warming. ~~At monomer level,~~ ~~With warming, the higher concentration of long-chain~~

36 ~~(carbon number > 20)~~ ω-hydroxy acids and diacids as suberin markers were more resistant to

37 decomposition than bulk root-derived carbon and their resistance increased with chain-length.

38 Moreover, warming accelerated decomposition of individual suberin monomers in the topsoil

39 but suppressed it in the subsoil. The slower decomposition in the subsoil was likely~~left after 3~~

40 ~~years of root incubation suggested a lower turnover rate and this could be~~ due to lower

41 microbial abundance and lower soil moisture induced by warming. Our study demonstrates

42 that the impact of warming on the decomposition of root-derived hydrolysable lipids in a

43 temperate forest ~~is~~ is compound class- and d~~d~~epth-dependent. ~~The slower turnover rate of long-~~

44 ~~chain ω-hydroxy acids and diacids shows that they are more persistent compared to bulk root~~

45 ~~mass and could be preserved in subsoil for longer time as long as the environmental conditions~~

46 ~~are unfavorable for decomposition with warming.~~The persistence of long-chain ω-hydroxy

47 acids and diacids may provide a potential way for long-term carbon stabilization in subsoil

48 under climate change. Nevertheless, due to the substantial heterogeneity of subsoil

49 environment, further studies are required to confirm and generalize this finding.

50

51 **Keywords**

52 Soil warming, decomposition, subsoil, hydrolysable lipids, suberin, fine roots, priming

53

54 **Highlights**

55 Warming effect on ~~accelerates the~~ decomposition ~~of root-litter in the topsoil but not subsoil~~of

56 root-derived hydrolysable lipids was compound class-dependent

57 Warming attenuated the accumulation of fatty acids in the subsoil

58 ~~Hydrolysable lipids are not resistant to warming in topsoil and could be preserved in subsoil~~

59 ~~for with warming~~Suberin markers are more resistant to decomposition and their resistance

60 increased with chain-length

61 Warming accelerated decomposition of suberin-derived monomers but inhibited it in the

62 subsoil

63 No priming effects on the pre-existing bulk soil carbon and hydrolysable lipids after 3 years

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1. Introduction

Global air temperatures are projected to increase between 2.6 °C and 4.8 °C by 2100 under Representative Concentration Pathway 8.5, according to the Intergovernmental Panel on Climate Change (IPCC, 2013). In synchrony with air temperature, soil temperature is also expected to increase, not only in topsoil (< 20 cm) but also in subsoil (> 20 cm) (Soong et al., 2020). Global soils hold the largest actively cycling terrestrial carbon pool and store between 2000 Pg and 3000 Pg of carbon in the top 3 m, with over 50% located in subsoil (Scharlemann et al., 2014). Previous studies demonstrated that warming could accelerate the decomposition of soil organic matter (SOM) in topsoil (Scharlemann et al., 2014), as well as in subsoil (Hicks Pries et al., 2017; Soong et al., 2021), potentially causing loss of CO_2 to the atmosphere. Moreover, enhanced temperature accelerated the decomposition of complex polymeric organic matter (OM) (Ofiti et al., 2023; Zosso et al., 2023), which had been regarded as comparatively recalcitrant to microbial decomposition.

Often, manipulative field warming experiments have focused on topsoil (Chen et al., 2022; van Gestel et al., 2018; Melillo et al., 2017; Verbrigghe et al., 2022), both in terms of the soil depths warmed by the manipulation and focus of the investigation. Therefore, it remains unclear from these experiments if the deeper soil horizons respond in similar ways to environmental changes as topsoil, since biotic and abiotic properties differ. Subsoil SOM has been assumed to be relatively insensitive to warming, because larger proportions of this deep SOM are more spatially inaccessible to microorganisms due to their associations to mineral surfaces compared to surface soil (Lützow et al., 2006). Furthermore, the microbial abundance varies throughout the soil profile (Rumpel et al., 2012; Zosso et al., 2021). Microbial biomass is substantially higher in topsoil than in subsoil (Naylor et al., 2022), by as much as two orders of magnitude (Fierer et al., 2003), leading to significantly slower turnover of carbon in the subsoil (Spohn et al., 2016). Also, microbial community structure changes with depth. Across different ecosystems, there is generally a proportional increase of Gram-positive to Gram-negative bacteria with depth (Eilers et al., 2012; Xu et al., 2021; Zosso et al., 2021) due to decreased carbon availability and quality (Fanin et al., 2019; Naylor et al., 2022). With warming, the difference in microbial abundance and composition between topsoil and subsoil could become more pronounced (Fontaine et al., 2007; Zosso et al., 2021), leaving large uncertainties of the impact on SOM decomposition at different depths.

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95   One of the most important biotic factors that could change the SOM dynamics in the
96   subsoil is root mass. Compared to topsoil, root mass is one of the major carbon sources in the
97   subsoil (Button et al., 2022; Rumpel and Kögel-Knabner, 2011), especially in seasonally dried
98   temperate evergreen forest, where root depth could be deep (Schenk and Jackson, 2005).
99   ~~Moreover, roots impact on SOM dynamics in subsoil in two way:~~The dual role of roots can
100 affect SOM dynamics in subsoil in contrasting ways. On one hand, root-derived carbon ~~They~~
101 ~~are~~is ~~more~~ more likely ~~than~~ ~~to form stable SOM~~aboveground plant biomass to associate with
102 minerals (Jackson et al., 2017; Rasse et al., 2005; Sokol and Bradford, 2019) ~~to aboveground~~
103 ~~plant biomass~~, thus promote carbon stabilization. On the other hand,~~and~~ they can also ~~could~~
104 stimulate ~~the~~ microbial ~~mineralisation~~mineralization of ~~preserved~~ previously stabilized SOM
105 ~~in subsoil~~, leading to loss of old ~~and pre-existing~~ SOM in the subsoil (Dijkstra et al., 2021;
106 Fontaine et al., 2007). However, ~~the scientific debate continues, how root and SOM interaction~~
107 ~~will alter under global warming, specifically in the subsoil~~it remains uncertain how root
108 biomass and its microbial decomposition will respond to warming, particularly in subsoil~~.~~.
109 Previous studies showed a variety of responses of root biomass in the surface soil to warming,
110 with either more fine root biomass (Kwatcho Kengdo et al., 2022; Malhotra et al., 2020; Wang
111 et al., 2021), less root biomass (Arndal et al., 2018; Ofiti et al., 2021), or no change in root
112 biomass (Wang et al., 2017). In subsoil, it is assumed that roots will forage in deeper soil
113 horizons under water stress induced by warming, (Wang et al., 2017; Wang et al., 2021) but
114 the opposite was observed in a warming experiment in a temperate forest at Blodgett forest
115 with a substantial loss of fine (< 2 mm) and coarse roots (2-5 mm~~;)~~ ~~(~~Ofiti et al., 2021) across
116 the soil profile after 4 years of warming. Additionally, it was also observed that warming (Parts
117 et al., 2019; Yaffar et al., 2021) or higher temperature induced drought (Meier and Leuschner,
118 2008) increased mortality of fine root biomass. Since this ~~root litter~~root litter could serve as
119 new substrates for carbon-limited subsoil horizons and fuel decomposition of pre-existing
120 carbon, it is important to understand how microorganisms will respond to this new input at
121 different depths under warming conditions.
122   ~~Besides,~~Although previous studies have provided~~exhibited~~ evidence of carbon loss
123 under~~with~~ warming at Blodgett Forest (Soong et al., 2021), mainly by rapid decomposition of
124 decadal-aged carbon (Hicks Pries et al., 2017), ~~but they did not provide information~~they have
125 not addressed ~~of~~ the transformation of new carbon input~~s~~ ~~and soil C~~into SOM formation at the
126 molecular level. Molecular proxies or markers such as plant-derived hydrolysable lipids
127 suberin, which mainly derive from woody tissues such as roots (Kolattukudy, 1980), could be

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128   used as quantitative and qualitative methods to follow alterations of root-derived carbon during

129   decomposition and determine their turnover rate when in combination with compound-specific

130   [13]C isotopic analysis (Feng et al., 2010). Moreover, i~~I~~t is ~~also very~~ important to know whether

131   these molecular proxies could be preserved under warming~~.~~ ~~since~~ Because applying genetic

132   modifications to improve plant traits associated with carbon sequestration (defined as

133   "harnessing roots")~~harnessing roots~~, specifically~~especially~~ to increase their hydrolysable lipids

134   content, is regarded as one of the solutions to mitigate climate change (Eckardt et al., 2023).

135   Many previous studies on the mechanisms of the interaction between root-derived

136   carbon and soil (decoupling carbon from mineral protection or formation of soil carbon) were

137   conducted as laboratory incubation experiments (Keiluweit et al., 2015; Sokol and Bradford,

138   2019). Such techniques with high replicability and controlled conditions contribute to

139   understanding certain soil carbon transformation or stabilization processes. However,

140   laboratory incubations are usually far from natural conditions and lack many of the biotic or

141   abiotic interactions that occur in soil *in-situ*.

142   Therefore, in our study, we used a long-term, whole-soil warming experiment, located

143   at Blodgett Forest Research Station (CA, USA), to study the effect of warming on the

144   decomposition of ~~root-litter~~root litter at different soil depths (10-14 ~~cm~~, 45-49 ~~cm~~, and 85-89

145   cm). We incubated [13]C-~~labelled~~labeled roots *in-situ* for three years, to understand how the

146   decomposition of root-derived hydrolysable lipids varies with depth and warming. Specifically,

147   we quantified different monomers in hydrolysable lipids that were released from polymeric

148   SOM. For each monomer we investigated stable carbon isotope values (δ^{13}C) to understand

149   how quickly root-derived carbon and molecular markers degraded at different soil depths with

150   warming. We hypothesized that: first, ~~first:~~ warming would accelerate the decomposition of

151   ~~root biomass and~~ root-derived hydrolysable lipids ~~across~~throughout the ~~whole~~entire soil

152   profile, regardless of soil depths. Second, hydrolysable lipids would accumulate relative to bulk

153   root-derived carbon~~we expect a relative accumulation of hydrolysable lip~~ due to their greater

154   chemical persistence compared to other carbon compounds~~ids in the root-derived carbon~~

155   ~~because they are commonly regarded as chemically persistent compounds~~ (Lorenz et al., 2007).

156   **2. Material and methods**

157   **2.1 Study site**

158   The whole-soil warming experiment at University of California Blodgett Experimental

159   Forest is located on the foothills of the Sierra Nevada near Georgetown, CA (120°39′40″W;

160 38°54′43″N) at 1370 m above sea level (Hicks Pries et al., 2018). The site has a Mediterranean

161 climate with a mean annual air temperature of 12.5 °C and a mean annual precipitation of 1774

162 mm (Bird and Torn, 2006). The experiment is situated in a thinned 80-year-old mixed

163 coniferous temperate forest, dominated by ponderosa pine (*Pinus ponderosa*), sugar pine

164 (*Pinus lambertiana*), incense cedar (*Calodefrus decurrens*), white fir (*Abies concolor*), and

165 douglas fir (*Pseudotsuga menziesii*;) (Hicks Pries et al., 2017). The soils are Holland series

166 and classified as fine-loamy, mixed superactive, mesic ultic Alfisol of granitic origin (mean

167 pH 5.5), which is equivalent to Dystric Cambisols (IUSS Working Group WRB, 2022).

168   Briefly, the whole-soil warming experiment consists of 6 plots in total arranged in three

169 replicated blocks, each having a pair of warmed and controlled circular plots 3 m in diameter.

170 Soils in warmed plots were 4 °C above ambient temperature to 1 m depth while the natural

171 temperature gradient with depth was maintained following the design described previously

172 (Hicks Pries et al., 2017). To maintain warming down to 1 m depth, twenty-two 2.4-m-long

173 resistance heating cables (BriskHeat, Ohio, USA) were vertically installed in metal conduits at

174 a radius of 1.75 m, surrounding each plot. Two concentric rings of surface heater cable were

175 installed at 1 and 2 m in diameter, 5 cm below the soil surface, to compensate for surface heat

176 loss. The average soil temperature was elevated by + 4°C in warmed plots relative to ambient

177 plots (except for at 0-20 cm only elevated by +2.6°C due to surface heat loss), while preserving

178 seasonality and natural temperature gradient with soil depth (Hicks Pries et al., 2017; Pegoraro

179 et al., 2025; in review). The setup of the control plots is identical to the warmed plots but

180 without heating cables placed inside the metal conduits (Hicks Pries et al., 2017).

181 **2.2 ^{13}C-labelledlabeled root-litterroot litter experiment and sampling**

182   Common wild oat (*Avena fatua)* is an annual grass, and its roots were used as a model

183 substrate whose fast growth enabled uniform ^{13}C-labelling in this experiment compared in

184 contrast to the slower-growing native coniferous trees. This made its roots a suitable model

185 substrate for decomposition studies (Hicks Pries et al., 2018). *Avena fatua* seedlings were

186 grown for 12 weeks in a greenhouse within an airtight chamber at the University of California,

187 Berkeley. Every 4 days the source of CO_2 was switched between ambient CO_2 and 10 atom%

188 $^{13}CO_2$ (Cambridge Isotope Laboratories, Inc., Massachusetts, USA)). (Castanha et al., 2018;

189 Hicks Pries et al., 2018). After this labeling phase, roots were excavated, dried and cut in 1-2

190 cm pieces (< 2 mm diameter). The root substrate was enriched by 5.6% atom% ^{13}C and had a

191 carbon concentration of 0.463 g C g root^{-1} (Castanha et al., 2018; Hicks Pries et al., 2018).

6

A total of six soil cores were extracted in each plot by using a perforated custom coring system made of polycarbonate tube (5.04 cm outer diameter and 4.41 cm inner diameter). Each core consisted of four sections (10 cm, 35 cm, 40 cm, and 10 cm in length, respectively) that were threaded on each end (male threads) and connected with female-to-female threaded polycarbonate connectors. Each section was threaded onto a sharpened aluminum cutting edge at the bottom for coring, and an aluminum tube with a weighted pounding head on the top. We cored the mineral soil based on the length of each polycarbonate section at the following depths: 0-10, 10-45, 45-85, and 85-95 cm. The top 4 cm of soil in each section (target experiment depth: 10-14, 45-49, and 85-89 cm) was marked and scooped into aluminum tins. Then a pre-weighed, 0.14 g aliquot of *Avena fatua* fine roots were added. The labeled *Avena* root litter was added to three soil depths (10-14 cm, 45-49 cm, and 85-89 cm) of four out of the six cores and these cores will be termed as *root litter* treatment in this paper. The same disturbance was applied to the rest of the two cores without addition of labeled root litter and will be denoted as *disturbance control* treatment. Mesh disks with 1mm aperture were positioned at the top and bottom of each target 4 cm soil increment to delineate the physical boundaries of the inserted root substrates. This setup was intended to retain the added root litter and prevent ingrowth of living roots into the cores and was also done in disturbance control treatment. The soil mixture was then added back to each polycarbonate section. The polycarbonate core sections were consequently connected by the female-to-female connectors and the resulting 95 cm long core was placed back in the soil. In July 2019, i.e., after three years of *in-situ* field incubation, two cores with root litter treatment and one core with disturbance control treatment were retrieved from each plot. The retrieved cores were wrapped in foil and transported to Lawrence Berkeley National Laboratory and stored in a −20°C freezer before further processing. The more detailed description of experimental setup and incubation methods is demonstrated in Pegoraro et al. (2025; in review). For brevity, DC will be used to refer to the disturbance control treatment in the rest of this paper.

225   Because prior work at this site concentrated on three depths (Hicks Pries et al., 2017;
226   Soong et al., 2021), we selected the same depths to facilitate cross-study, temporal and spatial
227   comparisons. These depths also capture major pedogenic zones: topsoil, a transitional mid-
228   depth between topsoil and subsoil, and the deep subsoil horizon.
229   (Hicks Pries et al., 2017; Soong et al., 2021)

2.3 Soil preparation and characterization

231   In the results and discussion for this paper, the topsoil denotes the soil depth at surface
232   (10-14 cm)), and the subsoil describes the soil depths at 45-49 cm (mid-depth) and 85-89 cm
233   (deep soil).

234   In the laboratory, we opened the polycarbonate cores to retrieve the 4 cm section where
235   labelledlabeled roots were added (same for disturbance control). We also sampled the 4 cm
236   section above and below the target depths. The soil samples were sieved < 2 mm. Roots were
237   picked off the top of the sieve by tweezers. Bulk soil < 2 mm was freeze-dried and re-weighed.
238   A subsample of bulk soil samples was ground by a ball mill (MM400, Retsch, Haan, Germany)
239   and analyzed for carbon and nitrogen concentrations, as well as stable carbon isotope
240   composition ($\delta^{13}C$) using an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS;
241   Flash 2000-HT Plus, linked by Conflo IV to Delta V Plus isotope ratio mass spectrometer,
242   Thermo Fisher Scientific, Bremen, Germany). The results are reported in the δ notation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000\text{0}$$

244   (1)

245   Where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the international
246   standard, Vienna Pee Dee Belemnite (VPDB, 0.01118), respectively. At least two analytical
247   replicates were measured for all samples. Calibration was carried out using IAEA-certified
248   primary standards (e.g., N600 caffeine) and caffeine (Merck) as secondary standard.

2.4 Analysis of hydrolysable lipids

250   All soil samples (< 2 mm) were pre-extracted by Soxhlet following an established
251   protocol (Wiesenberg and Gocke, 2017) to remove solvent-extractable lipids with
252   dichloromethane (DCM):: methanol (93:7; v/v) for 48 hours. The extraction residues were
253   dried until constant weight.

254   The extraction residues were homogenized with a ball mill (MM400, Retsch, Haan,
255   Germany) and then hydrolyzed according to (Zosso et al., (2023)(2023). Therefore, an aliquot

8

256 of each residue equivalent to > 20 mg carbon was weighed in a 250 mL round bottom flask.

257 The sample was mixed with the extraction solution methanol: deionized water (9:1; v/v) with

258 6% potassium hydroxide (KOH) and then saponified for 20 hours at around 85-88 °C in a water

259 bath under reflux. Subsequently, the solution was filtered and transferred to a separation funnel

260 for phase separation. The solution was acidified to pH 2.0 using 6 M hydrochloric acid (HCl)

261 and then extracted with dichloromethaneDCM. The collected fractions were volume-reduced

262 and remaining water was removed by water-free sodium sulfate (Na_2SO_4).

263 For quantification of hydrolysable lipids, deuterated eicosanoic acid ($D_{39}C_{20}$;

264 Cambridge Isotope Laboratories, Inc.) was added to the samples as an internal standard. The

265 samples were silylated at 80 °C for 1 hour with bis(trimethylsilyl)acetamide (BSA) ;

266 (Wiesenberg and Gocke, 2017). Individual compounds were quantified on an Agilent 7890B

267 gas chromatograph (GC) equipped with a multi-mode inlet and a flame ionization detector

268 (FID). Compound identification was performed on an Agilent 6890N GC equipped with

269 split/splitless inlet and coupled to an Agilent 5973 mass selective detector (MS). Compounds

270 were identified by comparison of mass spectra with those of external standards and from the

271 NIST and Wiley mass spectra library. Both instruments were equipped with DB-5MS columns

272 (50 m × 0.2 mm × 0.33 μm) and 1.5 m de-activated pre-columns, with helium as the carrier gas

273 (1 ml min^{-1}). Silylated fractions were injected in splitless mode at an initial GC oven

274 temperature of 50 °C that was kept isothermal for 4 min, then increased to 150 °C at a rate of

275 4 °C min^{-1}. Thereafter, the temperature ramped up to 320 °C at a rate of 3 °C min^{-1} and held for

276 40 min. The GC-MS was operated in electron ionization mode at 70 eV and scanned from m/z

277 60-650. The analysis of the data was processed with Agilent Chemstation software. The

278 concentration of each compound was finally normalized to the organic carbon concentration of

279 the respective sample (stated as μg g^{-1} OC). The weight of samples weighed in for

280 hydrolyzation is always corrected by accounting for the mass loss due to free lipid extraction:

281
$$M_{corrected} = \frac{M_{weighed}}{(1 - p_{free\ lipids})} \tag{2}$$

282 Where $M_{corrected}$ is the corrected weight of soil samples, $M_{weighed}$ is the weight of soil

283 samples weighed in for hydrolyzation, and $p_{free\ lipids}$ is the proportion of free extractable lipids

284 to the mass of soil samples weighed in for Soxhlet extraction.

285 In this paper, mid-length and long-chain monomers are the compounds with a carbon

286 chain length n between 14 and 20 ($14 \leq n < 20$) and length ≥ 20, respectively. For each

287 compound class, n-carboxylic acidsfatty acids are the synonymously used for for n-carboxylic

288 acids n-fatty acids, ω-hydroxy acids are short for ω-hydroxy carboxylic acids, diacids stand for

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289 *a, ω*-alkanedioic acids, alcohols are abbreviated for *n*-alcohols, and mid-chain acids stand for

290 mid-chain hydroxylated fatty acids, referring to fatty acids with functional groups or structural

291 modifications located in the middle of their carbon chain, typically at the C-9 and C-10 carbon

292 positions, such as x, *ω*-dihydroxyhexadecanoic acid (x = 9 or 10); (Graça, 2015).

293   Different monomers can be used as markers for leaf and needle (cutin) or woody and

294 root (suberin) biomass. However, there are no universal markers across a variety of studies

295 since the relative proportions of cutin and suberin markers could vary among plant taxa, plant

296 functional type or plant organ (Jansen and Wiesenberg, 2017; Mueller et al., 2012). Here, we

297 selected *ω*-hydroxy alkanoic acids and diacids as suberin markers since these monomers exist

298 in substantial amount in the roots of the dominating plant species around the experiment plots

299 and are 10 times higher in concentration compared to the same monomers in leaves or needles

300 in the same species (Supplementary *Fig. S2*). Cutin markers could not be distinguished since

301 all the mid-chain acids which were traditionally considered as cutin markers were present in

302 considerable amounts ~~both in leaves/needles and roots in the analyzed plants~~in added ^{13}C-

303 labeled roots (Supplementary *Table S1*).

304   To the best of our knowledge, there are no studies reporting the composition of mid-

305 chain fatty acids, *ω*~~ω~~-hydroxy or diacids from microorganisms in soil, although it was reported

306 that microorganisms can synthesize the compound classes mentioned above (Huf et al., 2011;

307 Kim and Park, 2019). These compound classes, however, are more regio~~n~~-specific (the

308 enzymes will target only one or a few defined carbon positions) and differ from those in plants

309 and animals (Kim and Oh, 2013) and usually have a carbon chain-length < 20 (Zhang et al.,

310 2024). Therefore, we hypothesize that all the compounds with a carbon chain length ≥ 20 and

311 mid-chain fatty acids are exclusively plant-derived.

312 **2.5 Compound-specific isotope analysis**

313   To determine the δ^{13}C of individual compounds, a Trace GC Ultra, coupled via GC

314 Isolink II and Conflo IV to Delta V Plus isotope mass spectrometer (Thermo Fisher Scientific)

315 was used to perform compound-specific δ^{13}C analysis of individual hydrolysable lipids. The

316 settings of the instrument and temperature program used here was the same as mentioned above.

317 Reproducibility and stability (<0.6‰) of δ^{13}C values were checked with pulses of CO_2

318 reference gas and *n*-alkane standard mixture (C$_{20\text{-}30}$; Sigma Aldrich) of known isotope

319 composition. The δ^{13}C values were presented in per mil (‰) relative to the Vienna-Pee Dee

320 Belemnite (V-PDB) reference standard. Every sample was measured with three analytical

10

321 replicates and the difference between measurements typically did not exceed 1.0 ‰ for natural
322 abundance samples and 10% of the measured isotope value for ^{13}C ~~labelled~~labeled samples.

323 **2.6 Calculations**

324 The isotope composition of individual hydrolysable lipids was corrected for the value
325 of the δ^{13}C value of each trimethylsilyl group that was added during silylation as:

326 $$\delta_{UD} = \frac{(n+3\times a)\times \delta_D - 3\times a\times \delta_M}{n} \tag{3}$$

327 Where n is the number of carbon atoms in the underivatized hydrolysable lipids and
328 δ_{UD} and δ_D are isotope ratios of the underivatized and the derivatized hydrolysable lipids,
329 respectively, a is the number of functional groups in individual compounds that were
330 derivatized by BSA. δ_M is the carbon isotope ratio of the added trimethylsilyl group (-44.3‰).
331 δ_M was determined by repeated measurement (n = 8, with 3 analytical replicates of each) of
332 derivatized standard FAs (C_{10} and C_{12} FAs with known δ^{13}C isotope composition).

333 The ^{13}C-excess, which can be expressed as percent atom excess, presents the
334 enrichment of ^{13}C in individual hydrolysable lipids. The value is defined as the ratio of the
335 relative abundance of the heavier stable isotope in a ~~labelled~~labeled sample to the natural
336 isotope abundance in the identical un~~labelled~~labeled sample (Epron et al., 2012). It was
337 calculated as followings (Speckert et al., 2023):

338 $$^{13}C - excess[\%] = \left(\frac{100}{^{13}C/\,^{12}C_{distcontrol}} \times\; ^{13}C/\,^{12}C_{labelled}\right) - 100 \tag{4}$$

339 Where $^{13}C/^{12}C_{distcontrol}$ is the atomic ratio of the stable isotopes in the compartments
340 (bulk soil carbon, and individual monomers of hydrolysable lipids) of the disturbance control
341 plots as natural abundance values, and $^{13}C/^{12}C_{~~labelled~~labeled}$ is the atomic ratio in the
342 corresponding compartments in the plots with added ~~labelled~~labeled ~~root litter~~root litter.

343 As individual isotope values can vary a lot in between different homologues for each
344 compound-class specifically in isotope labeling experiments, a more meaningful measure was
345 chosen to express the δ^{13}C values of the respective compound-classes that can be assigned to
346 the same carbon source (Wiesenberg et al., 2008). The δ^{13}C values and ^{13}C-excess for the most
347 abundant compound classes (*n*-alcohols, *n*-fatty acids, diacids, and ω-hydroxy acids) within
348 the hydrolysable lipid fractions were calculated separately as weighted means of individual
349 compounds within each compound class. This means within each compound class, weightings
350 will be given to each monomer in this compound class (e.g., ω-hydroxy acids) based on the
351 proportional contribution of individual monomer (e.g., C_{24} ω-hydroxy acids) to the total

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352   concentration of this compound class. Then for individual monomers, their weightings will be
353   multiplied by their $\delta^{13}C$ values, and we sum up all the monomers identified to get the weighted
354   mean $\delta^{13}C$ values for this compound class:

355           $\mu_c = \sum_{i=a}^{b} (x_{ci} \times w_{ci})$ (5)

356   Where μ denotes the average value and subscript c represents different compound
357   classes, x denotes the value of either $\delta^{13}C$ or ^{13}C-excess, a and b represent the lower and upper
358   limits of the respective carbon number range, w_i indicates the relative abundance of the
359   individual compounds within compound class c.

360   The amount of root carbon that was recovered in bulk soil was calculated by dividing
361   the amount of ^{13}C-~~labelled~~labeled root-derived carbon left in the soil by the amount of carbon
362   added with the original ~~labelled~~labeled roots. The proportion of root-derived carbon (f_{root}) was
363   estimated by using a simple mixing model (Hicks Pries et al., 2018):

364           $^{13}C\ atom\%_{sample} = {}^{13}C\ atom\%_{DC} \times f_{soil} + {}^{13}C\ atom\%_{root} \times f_{root}$ (6)

365           $f_{root} + f_{soil} = 1$ (7)

366           $Recovery_{root} = \frac{f_{root} \times M_{soil} \times C\%_{soil}}{0.14 \times 0.463}$ (8)

367   Where $^{13}C\ atom\%_{sample}$ is the ^{13}C atom% of soil samples where ~~labelled~~labeled ~~root-~~
368   ~~litter~~root litter was added; $^{13}C\ atom\%_{DC}$ is the ^{13}C atom% of soil samples in the disturbance
369   control plots; ^{13}C atom%$_{root}$ is the ^{13}C atom% of initial ^{13}C ~~labelled~~labeled roots; f_{soil} and f_{root}
370   are the proportion of carbon originally derived from native soil and ~~labelled~~labeled ~~root-~~
371   ~~litter~~root litter, respectively. M_{soil} is the mass of the soil sample and $C\%_{soil}$ is the carbon content
372   of the corresponding soil sample. 0.14 is the mass of ~~root-litter~~root litter (g) added at individual
373   soil depth and 0.463 is the carbon content of the added ~~root-litter~~root litter.

374   The decay rate, k, of initially added ^{13}C-~~labelled~~labeled roots and root-derived
375   hydrolysable lipids was calculated based on the following model (Olson, 1963):

376           $-kt = ln\ \frac{M_t}{M_0}$ (9)

377   where M_0 is the mass of original roots or root-derived hydrolysable lipids, M_t denotes
378   the mass of root-derived carbon or hydrolysable lipids at time t, and t is the duration of
379   incubation which is three years in our study. The model assumes that M is a well-mixed carbon
380   pool with first-order decay kinetics. The residence time is the reciprocal of k.

381   The priming effect of added ^{13}C ~~labelled~~labeled ~~root-litter~~root litter was calculated
382   using the mass of carbon in DC cores at individual depths as background values. Then, based

12

383 on the calculations shown in 2.6.4, the proportion of native SOM that is left in the
384 ~~labelled~~labeled cores within the same plot after three years of incubation was calculated:

385 $$Priming\ effect = \frac{MSOM_l - MSOM_{DC}}{MSOM_{DC}} \tag{10}$$

386 Where $MSOM_l$ and $MSOM_{DC}$ denote native SOM left in ~~labelled~~labeled cores and SOM
387 in DC cores, respectively. The priming effect for hydrolysable lipids was calculated with the
388 same approach. One outlier is excluded in priming effect calculation at mid-depth in ambient
389 plots. It is noteworthy that this is only an estimated proxy used for priming effect. The more
390 classical and straightforward way to calculate priming effect is described in (Schiedung et al.,
391 2023).~~.~~

2.7 Statistical analysis

393 All statistical analyses were performed in the RStudio interface 2024.12.1.563
394 (~~(Version 2024.09.1+394,~~ Posit team, 202~~5~~4) with R version 4.4.2 (R Core Team, 2024)~~(R:~~
395 ~~The R Project for Statistical Computing, 2025)~~. We used linear mixed effects models (LMEs)
396 in the '*nlme*' package (v3.1.166; Pinheiro et al., 2024) to test the fixed effects on responsive
397 variables. All the plots were created using the '*ggplot2*' package (v3.5.1; Wickham, 2016).
398 Homoscedasticity and normality were visually checked by residual plots and Q-Q plots. If
399 model assumptions were not met, the data was log-transformed.~~Shapiro-Wilk test and Levene~~
400 ~~test were used to assess the normality and heteroscedasticity of the data. To analyse the impact~~
401 ~~of main effects and their interactions on response variables, linear mixed effects models,~~
402 ~~lmerTest package was used (Kuznetsova et al., 2017). Normality and homoscedasticity were~~
403 ~~visually checked via residual plots. We used Akaike's information criterion (AIC) to test~~
404 ~~whether different fixed effects structures could improve model fit. If we observed a~~
405 ~~heteroscedasticity of variance of residuals, we log-transformed the data.~~

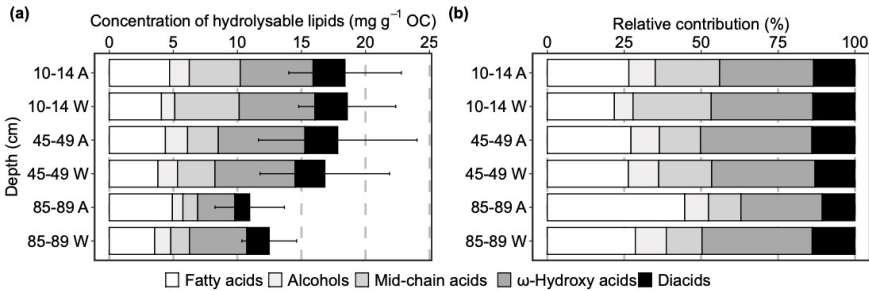**Bulk soil organic carbon concentration was tested in response to warming, root treatment, depth, and their interaction.** ~~Hydrolysable lipid concentrations in disturbance control plots were tested in response to warming treatment, depth, and their interactions. 13C excess of bulk soil was tested in response to carbon content, depth, warming, and their interactions. We examined the effect of warming, depth, and their interactions on root recovery. We investigated how different compound classes, depth, warming, and their interactions influence 13C excess and mass change of hydrolysable lipids. For the priming effect of bulk soil and hydrolysable lipids, we tested how they respond to warming, depth and their interactions. All analyses included paired plots nested in blocks as a random effect. We regarded results as significant when the *p* values are lower than 0.05.~~

^{13}C-excess was tested in response to warming, depth, and their interaction. The hydrolysable lipids concentration normalized to soil organic carbon (SOC; mg g^{-1} SOC) was tested in response to warming, root treatment, depth, and their interaction. We tested warming, depth, and their interaction on hydrolysable lipids mass change, and ^{13}C-exces of each compound class separately. To test whether chain length increases suberin-derived monomers resistance to decomposition, we used warming, depth, carbon number, and their interactions as fixed effects to evaluate their effects on mass change of individual ω-hydroxy acid and diacid monomers. For each statistical test, we regarded block as a random effect.

Model fits were evaluated by Akaike's Information Criterion (AIC; Akaike, 1998). We conducted a backward stepwise model selection to remove fixed effects that increased AIC values by 5 or more (Pegoraro et al., 2025; in review). The alpha level was set to α = 0.05 as significant in all statistical tests and a *p* between 0.05-0.1 was considered marginal.

> Commented [MS1]: Is "marginal" the correct technical term? Or could it be replaced with a word such as "non-significant"?

3. Results

3.1 Soils without ~~litter~~root litter incubation

Fatty acids ☐ Alcohols ☐ Mid-chain acids ☐ ω-Hydroxy acids ■ Diacids

~~After three years of *in-situ* incubation~~ In ambient plots, the normalized hydrolysable lipids concentration decreased from 18.4 ± 4.4 mg g^{-1} SOC in topsoil to 11.0 ± 2.7 mg g^{-1} SOC in the deep soil, whereas it decreased from 18.6 ± 3.8 mg g^{-1} SOC~~,~~ in topsoil to 12.5 ± 2.1 mg g^{-1} SOC in the deep soil in warmed plots (Fig. 1a). However, this average decrease of

14

431 hydrolysable lipids concentration with depth was not significant ($p = 0.460$; Table S10). ~~the~~

432 ~~average concentrations of hydrolysable lipids did not differ between ambient and warmed~~

433 ~~treatments~~Warming did not significantly alter the normalized hydrolysable lipids concentration

434 ($p = 0.472$; Table S10) across the soil profile.~~, but they decreased with depth by the same~~

435 ~~amount under both treatments. These cores did not receive any ^{13}C labeled root-litter, thus they~~

436 ~~tested how warming affected the abundance and composition of the pre-existing hydrolysable~~

437 ~~lipids at the experimental site. The concentrations of hydrolysable lipids were not different~~

438 ~~between ambient and warmed plots (LME; $p = 0.94$; $F = 0.006$) and depths (LME; $p = 0.17$; F~~

439 ~~$= 2.1$). with 18.4 ± 4.4 and 18.6 ± 3.8 mg g^{-1} OC at topsoil and 17.8 ± 6.2 vs. 16.8 ± 5.1 mg g$^-$~~

440 ~~^{1}OC at mid-depth in warmed and ambient plots, respectively. In the deep soil, the~~

441 ~~concentrations of hydrolysable lipids tended to be lower in the plots under ambient temperature~~

442 ~~(11.0 ± 2.7 mg g^{-1}OC) compared to warmed plots (12.5 ± 2.1 mg g^{-1}OC). We did not find~~

443 ~~interactions between warming and depths for concentrations of hydrolysable lipids (warming~~

444 ~~and depth, $p = 0.94$).~~

445 In general, warming did not significantly alter the relative contribution of each

446 compound class to the total hydrolysable lipids (%; $p > 0.1$; Table S21) and depth only

447 significantly decreased proportion of mid-chain acids ($p = 0.003$; Table S21). In ambient

448 topsoil, ω-hydroxy acids accounted for the largest proportion of total hydrolysable lipids by

449 31 %, followed by fatty acids (27 %), mid-chain acids (21 %), diacids (13 %), while alcohols

450 only make up 9 % of the total (Fig. 1; Table S22). At the same depth, warming did not

451 significantly affect the relative contribution of each compound class ($p > 0.1$; Table S22). At

452 mid-depth (45-49 cm), the proportion of each compound class to total hydrolysable lipids was

453 consistent compared to topsoil although contribution of mid-chain acids marginally decreased

454 by 7 % ($p = 0.072$; Table S22). In the deep soil (85-89 cm), the proportion of mid-chain acids

455 further significantly decreased by 10 % compared to its contribution in topsoil ($p = 0.011$;

456 Table S22). Albeit much higher average contribution of fatty acids (45 %) in ambient deep soil,

457 it did not significantly differ relative to topsoil ($p = 0.360$; Table S22). At this depth, warming

458 did not significantly alter the proportions of all the compound classes. However, elevated

Commented [MS2]: should there be more than "%"?

459 temperature marginally decreased fatty acids and increased diacids proportion by 16 % (*p* =
460 0.084; Table S23) and 3 % (*p* = 0.064; Table S23), respectively.
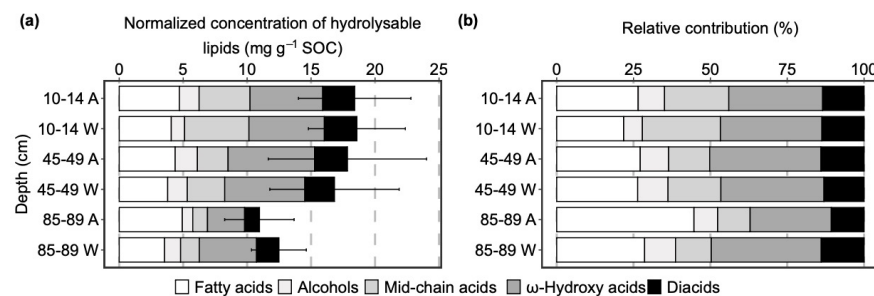

Figure 1: (a) Hydrolysable lipids normalized to soil organic carbon (SOC) concentrations (mg g^{-1} SOC) in disturbance control (DC) cores with ambient temperature (Aleft) and warming (Wright) in 2019 after 3 years *in-situ* incubation (mean ± SE, n = 3); (b) Proportions of individual compound classes to total hydrolysable lipids identified in disturbance control coresDC cores (mean, n = 3). For clarity of the visual presentation SE error bars are shown cumulatively.

461   To test how the hydrolysable lipid composition changed in between the three years of
462 warming (i.e., between year 2016 and 2019) in the cores without litter incubation, we calculated
463 the proportional contributions of individual compound classes to total hydrolysable lipids (Fig.
464 1b). The proportions of individual compounds in ambient temperature plots and warmed plots
465 at topsoil and at mid-depth were similar. In contrast, in the deepest soil horizon, the proportions
466 of fatty acids were much higher under ambient temperature (45%) than in the warmed treatment
467 (28%), leading to lower proportions of all the other compound classes in ambient plots.

468   With depth, the proportions of mid-chain hydroxy acids decreased regardless of
469 temperature difference. In ambient and warmed plots, the proportion of mid-chain hydroxy
470 acids consistently declined from topsoil (22% and 27% for ambient and warmed, respectively)
471 to deep soil horizon (11% and 12%, respectively). In the warmed plots, the proportions of ω-
472 hydroxy acids and diacids increased slightly from 45% in the topsoil to around 50% in mid-
473 depth and deep soil. In the ambient plots, these lipids increased between topsoil (44%) to mid-
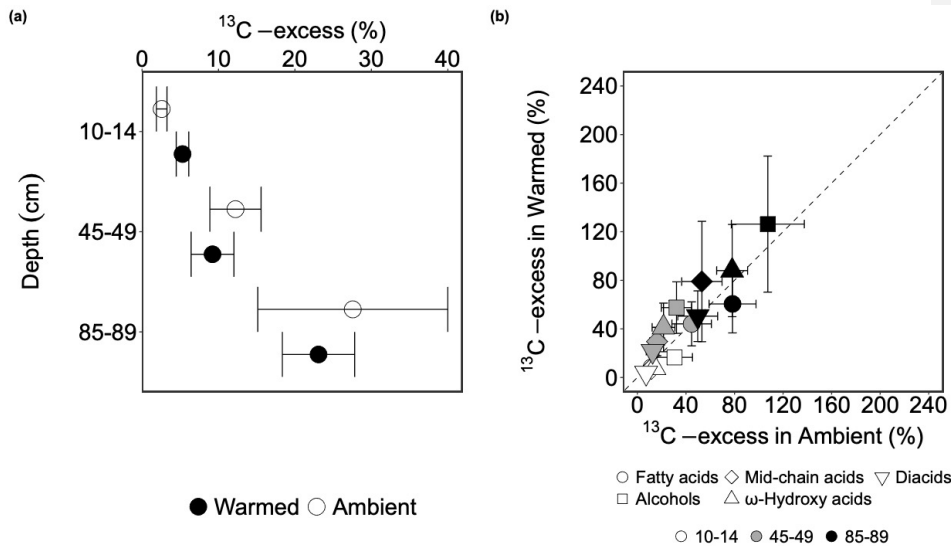474 depth soil (52%) but then decreased to deepest soil depth (37%).

475 **3.2 Soils with ~~root litter~~root litter incubation**

476 We estimated the amount of added ^{13}C-labeled root-litter remaining after three years *in-situ*
477 incubation as the ^{13}C-excess of bulk soil organic.

16

478

479 ~~Carbon concentration (LME; $p < 0.001$; $F = 78.54$), depth (LME; $p = 0.016$; $F = 13.83$) and their interaction (LME; $p = 0.005$; F~~

480 ~~$= 26.09$) had significant impact on 13C-excess,~~ ^{13}C-excess of ~~whe~~bulk SOC did not significantly change with

481 warming ($p = 0.527$; Table S8) but increased significantly with depth ($p = 0.001$; Table S8). At

482 10-14 cm, ~~reas warming treatment (LME; $p = 0.42$;~~the ^{13}C-excess of bulk SOC was on average

483 17.0 % lower in warmed than in ambient plots (Table S9), but this difference was not significant

484 ($p = 0.527$; Table S8). At 45-49 cm, ^{13}C-excess of bulk SOC on average increased by 2.7 times

485 from 3.8 % in topsoil to 10.5 % at this depth ($p = 0.016$; Fig. 2; Table S9). ~~$F = 0.81$) did not.~~

486 ~~In topsoil~~In the deep soil, the ^{13}C-excess~~, there were on average lower ^{13}C-excess values in~~

487 ~~warmed plots (2.6 ± 0.7%) than in ambient plots (5.3 ± 0.8%). In subsoil, ^{13}C-excess was on~~

488 ~~average higher in warmed than ambient plots. At mid-depth, ^{13}C-excess was lower in ambient~~

489 ~~plots (9.2 ± 2.8%) than in warmed plots (12.2 ± 3.3%), but these differences were not~~

490 ~~significant. In deep soil, warmed-plot values had large variability but averaged higher ^{13}C-~~

491 ~~excess (27.6 ± 12.4%) compared to ambient plots (23.1 ± 4.7%). The standard error increased~~

492 ~~significantly with depth, especially in warmed plots in the deep soil.~~ on average furthered

493 increased to 22.8 % (Fig. 2).

494 ⸺

495 ~~The recovery of root-derived carbon was similar, regardless of depth (LME; $p = 0.151$; $F =$~~

496 ~~2.29) or temperature treatment (LME; $p = 0.130$; $F = 2.72$), except for the warmed topsoil~~

497 ~~where the recovery was significantly lower in warmed (13.6 ± 1.9%) than the ambient-plot~~

498 ~~topsoil (24.9 ± 6.5%) (LME; $p = 0.011$). The interaction between warming and depth is close~~

499 ~~to the statistically significant threshold (LME; $p = 0.069$; $F = 3.53$), and this interaction is~~

500 ~~significant at mid-depth (LME; $p = 0.034$).~~

(a)

^{13}C −excess (%)

Depth (cm)

● Warmed ○ Ambient

(b)

^{13}C −excess in Warmed (%)

^{13}C −excess in Ambient (%)

○ Fatty acids ◇ Mid-chain acids ▽ Diacids
□ Alcohols △ ω-Hydroxy acids

○ 10-14 ◉ 45-49 ● 85-89

509 Similar to bulk ~~soil organic carbon~~SOC, the weighted ^{13}C-excess of each compound class was

510 not significantly altered by warming ($p > 0.05$; table S12) but significantly increased with depth

511 (~~*LME; p < 0.0001; F = 74.80) (Fig. 3a*~~$p < 0.05$; Fig. 2b; Table S12). ~~~~In topsoil, ~~all compound~~

512 ~~classes had lower ¹³C-excess in warmed plots than in ambient plots (LME; *p = 0.012*~~warming

513 on average decreased weighted ^{13}C-excess for all the compound classes between 3.2 % (diacids)

514 and 8.5 % (alcohols) compared to ambient conditions, however, this decrease was not

515 significant ($p > 0.1$; Table S12)). At 45-49 cm, warming non-significantly increased weighted

516 ^{13}C-excess for all the compound classes ($p > 0.1$; Table S14), except for fatty acids, where

517 warming on average decreased their weighted ^{13}C-excess ($p = 0.920$; Table S14). In the deep

518 soil, warming on average largely reduced the weighted ^{13}C-excess of fatty acids (28.0 %; $p =$

519 0.356; Table S14), ω-hydroxy acids (17.1 %; $p = 0.728$; Table S14), and diacids (8.2 %; $p =$

520 0.760; Table S14), and slightly lower the weighted ^{13}C-excess of alcohols (0.9 %; $p = 0.989$;

521 Table S14) and mid-chain acids (3.0 %; $p = 0.925$; Table S14), although none of them was

18

522   significant. The weighted ^{13}C-excess of fatty acids increased significantly from 10-14 to 45-49

523   cm by 25.5 % (p = 0.039; Table S13), whereas the weighted ^{13}C-excess all the other compound

524   classes non-significantly (p > 0.1; Table S13) increased between 2.5 % (alcohols; Table S13)

525   to 3.2 % (diacids; Table S13) at the same depth. At 85-89 cm, the weighted ^{13}C-excess of fatty

526   acids, ω-hydroxy acids, and diacids significantly increased compared to the values at 10-14 cm

527   by 62.2 % (p = 0.004; Table S13), 62.9 % (p = 0.033; Table S13), and 38.1 % (p = 0.015; Table

528   S13), respectively. The weighted ^{13}C-excess of alcohols and mid-chain acids marginally

529   increased by 73.3 % and 36.7 % correspondingly (p = 0.054 and 0.051, respectively; Table S13)

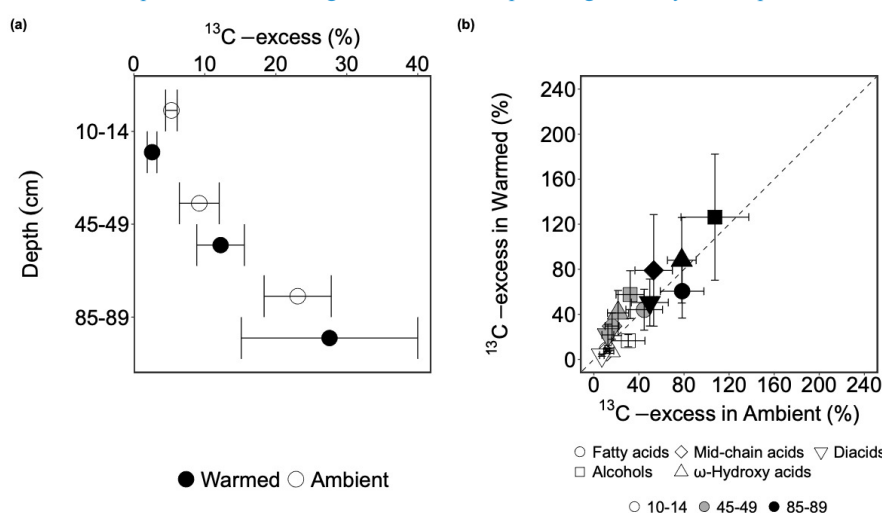530   at the same depth.Besides, the weighted ^{13}C-excess depends significantly on compound classes

Figure 2: (a) The presence of carbon derived from the ^{13}C-labeled root in the bulk soil after three years of incubation, expressed as ^{13}C-excess of bulk soil carbon (Mean ± SE, n = 3) at 10-14, 45-49, 85-89 cm depth in 2019 in ambient plots (white circles) and warmed plots (black circles). (b) Comparing the means of weighted ^{13}C-excess of each compound class from warmed plots (y-axis) and ambient plots at three depths (10-14, 45-49, 85-89 cm) with a 1:1 line (Mean ± SE, n = 3). Values above the 1:1 line indicate higher values in warmed plots than in ambient plots and values below the 1:1 line indicate the opposite.

531   (LME; p < 0.0001; F = 9.09).

532   The decomposition of different compound classes at mid-depth and in deep soil were

533   similar, except for fatty acids. The ^{13}C-excess of alcohols, mid-chain acids, ω-hydroxy acids,

534   and diacids was on average higher in the warmed plots than in the ambient temperature plots,

535   although the differences were not significant. The ^{13}C-excess of fatty acids was higher in

536   warmed than ambient plots in subsoil, particularly in the deep soil. In the latter, warming led

537 to, on average, much lower ^{13}C-excess of 60.5 ± 23.9 compared to ambient temperature (78.3

538 ± 19.3), although statistically not significant (LME; p = 0.219). Similar to ^{13}C-excess of the

539 bulk soil, the standard error was in general also larger in warmed than in ambient plots.

540 The differences of the absolute amounts of each compound class compared to added

541 root-litter allowed us to quantify the loss or accumulation of each compound class after three

542 years of incubation (Fig. 3b). The mass change of each compound class represents the

543 proportion of hydrolysable lipid-derived compound classes from the ^{13}C-labeled root litter that

544 remained after three years of incubation. This metric allowed us to evaluate whether these

545 compound classes experienced a net loss or accumulation during the incubation. Values above

546 and below 100 % indicates accumulation and loss of corresponding compound classes,

547 respectively.

548 The mass changes of different compound classes were affected differently by warming

549 and soil depth. Both warming (p = 0.035; Table S15) and depth (p = 0.008; Table S15) had

550 significant impacts on fatty acid mass change and the impacts of warming was independent

551 from depth (p = 0.409; Table S15). Fatty acids significantly accumulated across the soil profile,

552 resulting in an increased their mass change by 67 % (p = 0.044; Table S16) and 102 % (p =

553 0.006; Table S16) at mid-depth and in the deep soil, respectively. Although non-significantly

554 at 10-14 and 45-49 cm, warming lead to a consistent decreased fatty acid mass change across

555 the soil profile, particularly at 85-89 cm, where there was a significant lower (74 %; p = 0.029;

556 Table S17) recovered fatty acids with elevated temperature. The accumulation of fatty acids at

557 45-49 and 85-89 cm derived primarily from Hexadecanoic acid (n-$C_{16:0}$), Octadecanoic acid

558 (n-$C_{18:0}$), and Octadecenoic acid (n-$C_{18:1}$), where the mass change of these monomers exceeded

559 110 % or even 300 % (Table S2), e.g. Octadecanoic acid (n-$C_{18:0}$) at 85-89 cm in ambient and

560 warmed plot (348 ± 49 % and 181 ± 24 %; Table S2).

561 The effects of warming on the mass change of mid-chain acids were marginally depth-

562 dependent (p = 0.057; Table S15). In the topsoil, warming significantly decreased the mid-

563 chain acids recovered from hydrolysable lipids (p = 0.029; Table S17), whereas warming non-

564 significantly increased the mass change of this compound class at 45-49 (p = 0.257; Table S17)

565 and at 85-89 cm (p = 0.729; Table S17). Neither warming nor depth had significant effects on

566 the mass changes of all the other three compound classes (p > 0.1; Table S15). There was a

567 general trend that warming on average decreased the mass changes of alcohols, ω-hydroxy

568 acids, and diacids in the topsoil, but increased them at mid-depth and in the deep soil (Table

569 S17), however, none of these changes were significant (p > 0.1; Table S15).

20

570   For fatty acids with a carbon number ≥ 20, we did not observe significant warming
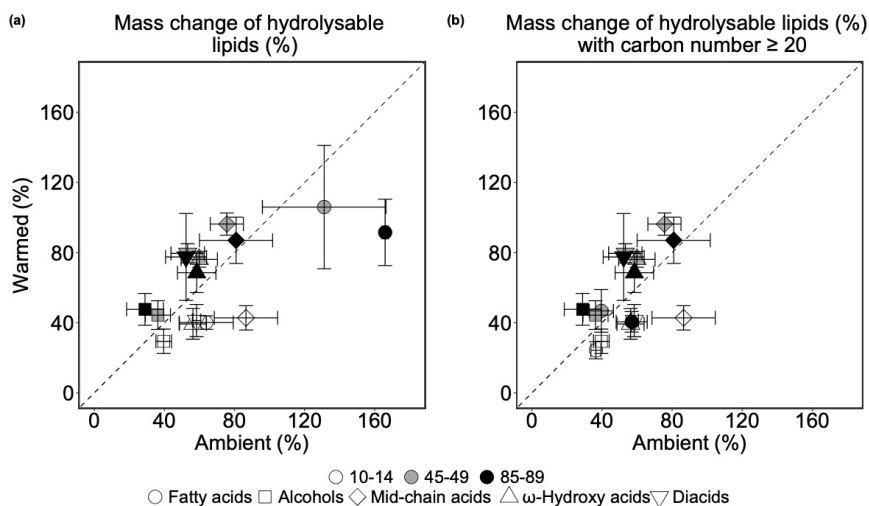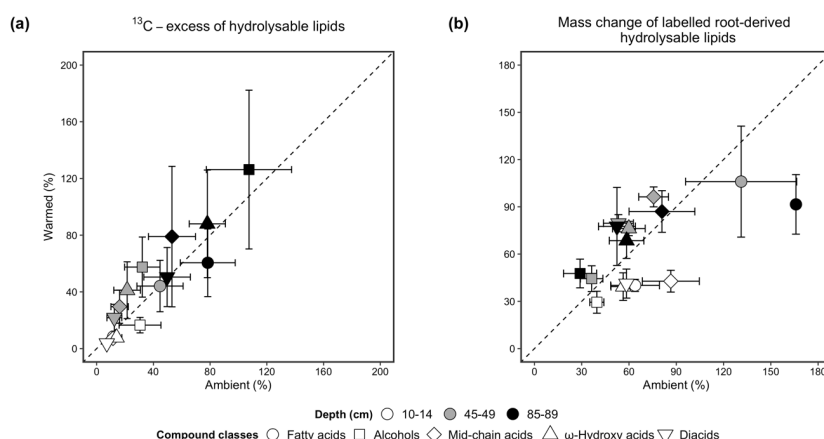571   effects on the mass change ($p = 0.255$; Table S18), but marginal depth effects ($p = 0.076$; Table

Figure 3: (a) Comparing mean of mass change (%) of each compound class in ^{13}C-labeled root-derived hydrolysable lipids (n = 3). Values above the 1:1 line indicate higher values in warmed plots than in ambient plots and values below the 1:1 line indicate the opposite. (b) Comparing mean of mass change (%) of each compound class in ^{13}C-labeled root-derived hydrolysable lipids (carbon number ≥ 20 for fatty acids, ω-hydroxy acids, and diacids; n = 3). In both plots, the error bars denote the standard error of each compound class in ambient and warmed plots in the individual soil depths. Values smaller than 100% indicate a loss and those larger than 100% indicate a gain of this compound class. Values above the 1:1 line indicate higher values in warmed plots than in ambient plots and values below the 1:1 line indicate the opposite.

572   S18). Warming on average reduced the mass change of fatty acids by 12 % at 10-14 cm, 7 %
573   at 45-49 cm, and 16 % at 85-89 cm, but these effects were non-significant ($p = 0.260$, 0.501
574   and 0.141, respectively; Table S20). Similar to fatty acids, there was no significant effects of
575   neither warming nor depth on the long-chain ω-hydroxy acids and diacids mass change, and
576   warming effects was not dependent on depth ($p > 0.1$; Table S18). At 10-14 cm, warming led
577   to non-significantly decreases in the ω-hydroxy acids and diacids mass change by 16 % and
578   22 % ($p = 0.334$ and 0.316, respectively; Table S20). In contrast, although warming on average
579   enhanced the mass change of the two compound classes at both 45-49 and 85-89 cm, none of
580   the changes were statistically significant ($p > 0.1$; Table S20).
581   On the molecular level, as in bulk soil, warming did not have a significant impact on the
582   decomposition of hydrolysable lipids (LME; $p = 0.518$; $F = 0.42$) across the whole soil profile

21

583 but in the topsoil, decomposition was faster of all the compound classes compared with deeper

584 soil depth (LME; $p = 0.001$). After three years of incubation the warmed topsoil had less

585 hydrolysable lipids that derived from added labelled roots remaining compared to ambient

586 temperature, indicated by higher loss of all the compound classes at this depth (Fig. 3b). After

587 three years of incubation, the warmed topsoil had less than half of the initial compound classes

588 left that was originally from the added as ^{13}C-labelled root-litter, i.e. $40.1 \pm 6.6\%$ of fatty acids,

589 $29.4 \pm 12.1\%$ of alcohols, $42.8 \pm 12.0\%$ of mid-chain acids, $39.4 \pm 15.2\%$ of ω-hydroxy acids,

590 and $41.3 \pm 16.0\%$ of diacids. More of each compound class was remaining in ambient plots,

591 i.e., $63.8 \pm 26.8\%$ of fatty acids, $39.6 \pm 7.7\%$ of alcohols, $86.5 \pm 31.3\%$ of mid-chain acids,

592 $56.3 \pm 13.4\%$ of ω-hydroxy acids, and $58.4 \pm 4.2\%$ of diacids, respectively (Fig. 3b).

593 Similar to the pattern observed for the ^{13}C-excess of individual hydrolysable lipids (Fig. 3a),

594 fatty acids and other compound classes showed an opposite trend (LME; $p < 0.001$; $F = 14.99$)

595 in subsoil when compared to topsoil (Fig. 3b). At both mid-depth and deep soil with ambient

596 temperature, there was a trend to a higher loss of each compound class due to microbial

597 decomposition compared to warmed plots except for fatty acids (Fig. 3b). On the contrary, fatty

598 acids seemed to accumulate more under ambient temperature in subsoil ($131.2 \pm 32.3\%$ at 45-

599 49 cm and $165.9 \pm 1.8\%$ at 85-89 cm), than with warming ($106.0 \pm 35.2\%$ for 45-49 cm and

600 $91.6 \pm 18.9\%$ for 85-89 cm) (Fig. 3b). Specifically, the values above 100% indicate an

601 enrichment of ^{13}C compared with the added root biomass over time, which was a surprising

602 finding.

(a) [13]C – excess of hydrolysable lipids   **(b)** Mass change of labelled root-derived hydrolysable lipids

Depth (cm) ◯ 10-14 ◕ 45-49 ⬤ 85-89

Compound classes ◯ Fatty acids ☐ Alcohols ◇ Mid-chain acids △ ω-Hydroxy acids ▽ Diacids
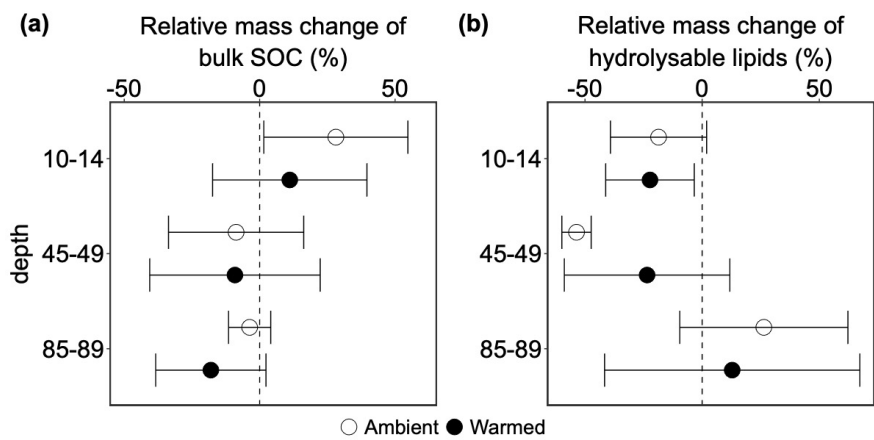
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3.3 Priming effect

Figure 4: Relative mass difference of: (a) pre-existing bulk soil organic matter and (b) hydrolysable lipids between cores with and without addition of ^{13}C-labeled root-litter (labelled DC cores). Negative values indicate the accelerated decomposition of pre-existing soil organic carbon (positive priming effect). Positive values
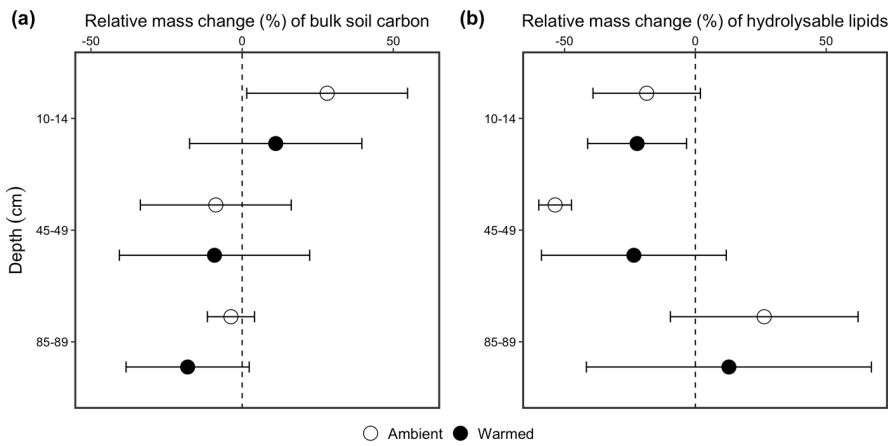
indicate inhibition (negative priming) of decomposition. Mean ± SE (n = 3).

Neither Both depth norand warming did not hadve significant impacts on the mass differences of pre-existing SOC (p = 0.558 and 0.422, respectively; Table S24). In topsoil, labeled root addition led to SOC accumulation on average by 28 % in ambient plots, but this accumulation was not significant (p = 0.273; Table S25). The effects of root addition decreased on average by 37 % at mid-depth and 32 % at 85-89 cm, resulting in an on average loss of pre-existing SOC (Fig. 4a),. hHowever, these effects were non-significant (p = 0.359 and 0.375, respectively; Table S25). Warming caused an average decline of the bulk SOC mass change at 10-14 and 85-89 cm by 17 % and 14 % (Fig. 4a), and no-change at mid-depth, but none of them were significant (p = 0.629, 0.684 and 0.991 for 10-14, 85-89, and 45-49, respectively; Table S26).

620   The similar trend was observed for the mass change of pre-existing hydrolysable lipids
621   as for pre-existing SOC, but the direction was opposite in the topsoil and the deep soil (Fig.
622   4b). ~~Both~~Neither depth ~~nor~~and warming ~~did not~~ significantly altered the relative mass
623   differences of pre-existing hydrolysable lipids ($p = 0.310$, and 0.987 for depth and warming,
624   respectively; Table S24). At 10-14 cm, labeled root addition led to a non-significant 19 % loss
625   of pre-existing hydrolysable lipids($p = 0.596$; Table S25). In the subsoil, the responses of
626   relative hydrolysable mass change were contrasting between 45-49 cm and 85-89 cm. At mid-
627   depth, labeled root addition caused a further non-significant decrease of the mass change of
628   hydrolysable lipids by 35 % (Fig. 4b; $p = 0.529$; Table S25), whereas in the deep soil, it caused
629   a non-significant increase of the hydrolysable lipids mass change by 45 % compared to topsoil
630   (Fig. 4b; $p = 0.372$; Table S25). In the topsoil, warming non-significantly decreased the mass
631   change of hydrolysable lipids by 4 % ($p = 0.941$; Table S25). In the subsoil, warming had also
632   contrasting effects on the mass change of hydrolysable lipids (Fig. 4b) between mid-depth and
633   deep soil. Warming on average increased the mass change of hydrolysable lipids at 45-49 cm
634   by 30 % and decreased it by 14 % compared to corresponding ambient conditions, but none of
635   them was significant ($p = 0.587$ and 0.784, respectively; Table S26). ~~Overall, there are no~~

Figure 4: Relative mass difference of: (a) pre-existing bulk soil organic matter and (b) hydrolysable lipids between cores with and without addition of ^{13}C-labeled root litter (labeled – disturbance control cores). Negative values indicate the accelerated decomposition of pre-existing soil organic carbon (positive priming effect). Positive values indicate inhibition (negative priming) of decomposition (Mean ± SE, n = 3).

636   ~~significant impacts of warming (LME; $p = 0.558$; $F = 0.37$) or depth (LME; $p = 0.422$; $F =$~~

637   ~~0.95) on the priming effectdepth and warminglabeled SOC accumulation on average by 28 %on~~

638   ~~average resulting in an on average loss of pre-existing SOC, (Fig. 4a), however, these effects~~

639 were non-significant (caused a average decline of bulk SOCchange at 10-14 and 85-89 cm by
640 17 % and 14 % (Fig. 4a), and no-change at mid-depth, but none of them were significant (*p*-
641 values = 0.629, 0.684 and 0.991 for 10-14, 85-89, and 45-49, respectively; Table S26). The
642 labeled root addition did not generate either a significant accumulation or loss of pre-existing
643 SOC.
644 The similar trend was observed for the mass change of hydrolysable lipids as for bulk SOC,
645 but the direction was the opposite in the topsoil and the deep soil (Fig. 4b). Both depth and
646 warming did not significantly alter the relative mass difference of pre-existing hydrolysable
647 lipids (*p*-values = 0.310, and 0.987 for depth and warming, respectively; Table S24). At 10-14
648 cm, labeled root addition led to an average of 19 % loss of pre-existing hydrolysable lipids, but
649 this effect was not significant (*p*-value = 0.596; Table S25). In the subsoil, the
650 For the hydrolysable lipids, similar to bulk soil, we did not find significant impacts of either
651 warming (LME; *p* = 0.987; *F* = 0.0002) or depth (LME; *p* = 0.310; *F* = 1.34) on the priming
652 effect. We observed a reverse trend at topsoil and deep soil compared to bulk soil (Fig. 4b). In
653 the topsoil, addition of root-litter led to the loss of pre-existing hydrolysable lipids in ambient
654 (-18.6 ± 20.5%) and warmed (-22.3 ± 18.9%) plots. Thus, positive priming occurred. This
655 positive priming happened also at mid-depth, where added labelled root-litter stimulated the
656 loss of pre-existing hydrolysable lipids in both ambient (-53.6 ± 5.1%) and warmed (-23.5 ±
657 35.3%) plots. However, in deep soil, there was negative priming, regardless of temperature
658 treatment (ambient, 26.3 ± 35.9%; warmed, 12.8 ± 54.5%). But the heterogeneity also increased
659 with depth as indicated by generally increasing standard errors.

660 **4. Discussion**

661 **4.1 Warming on hydrolysable lipids mass change was compound-dependent**

662 After three years of *in-situ* +4°C warming, we observed different trends for
663 decomposition of added ^{13}C-labelled root-litter between top- and subsoil both at bulk soil and
664 molecular level. Soil warming altered several factors that could govern the decomposition rate
665 of added root-litter such as microbial abundance, community structure (Zosso et al., 2021) and
666 soil water content (Pegoraro et al., submitted), and these changes could potentially further
667 affect microbial activity through reduced accessibility of substrates to exoenzymes or
668 microorganisms under warming conditions (Salomé et al., 2010). The warming effects further
669 interact with different abiotic and biotic conditions between top- and subsoil, leading to distinct
670 depth-dependent decomposition of added root-litter at different soil horizons.

25

4.1 Warming accelerated the decomposition of root-litter in topsoil, but not in subsoil **4.1.1 Warming significantly attenuated the accumulation of fatty acids in subsoil**

The same amount of root-litter was added to all depths, and thus initial ^{13}C-excess increased with depth, as soil organic carbon concentrations decreased. After three years of field incubation, this general trend was still preserved.

Root biomass decomposition responded differently to experimental warming in topsoil and subsoil The effects of soil warming on the decomposition of labeled root-derived hydrolysable lipids varied by compound class. Warming significantly decreased the mass change of fatty acids, independent of soil depth. In the topsoil (10-14 cm), although non-significantly, warming reduced mass change of fatty acids by 24 %, likely due to enhanced microbial decomposition. Similar acceleration of plant-derived input decomposition under warming has been reported at this site (Ofiti et al., 2021; Soong et al., 2021). In the topsoil, warming both increased carbon stocks and particulate organic matter, which is readily available substrates to microorganisms (Soong et al., 2021), and maintained microbial abundance while shifting microbial community structure toward taxa capable of degrading wider range of substrates (Dove et al., 2021; Zosso et al., 2021). Actinobacteria, known for breaking down complex carbon (DeAngelis et al., 2015; Goodfellow and Williams, 1983), increased in relative abundance under warming in our study (Pegoraro et al., 2025; in review).

In the subsoil (45-49, and 85-59 cm), fatty acid mass change exceeded 100 %, indicating accumulation of this compound class from other sources. This accumulation derived primarily from octadecanoic acid, octadecenoic acid, and hexadecanoic acid ($C_{18:0}$ fatty acids, $C_{18:1}$ fatty acids, and $C_{16:0}$ fatty acids, respectively; Table S2), which originate from both plant and microbial biomass. In contrast, long-chain fatty acids (carbon number $\geq$ 20), typically enriched in higher plant tissues (Harwood and Russell, 1984), did not accumulate. Therefore, the exceeding fatty acids originated likely from microorganisms, e.g. through phospholipid fatty acids synthesized during microbial growth (Joergensen, 2022; Zelles, 1997). ^{13}C-labeled substrate could be utilized by microorganisms and incorporated in microbial membrane lipids (Gunina et al., 2014; Yang et al., 2020). In our study, warming attenuated the accumulation of fatty acids in the subsoil, particularly at 85-89 cm, where there was a significant decline of the mass change of this compound by 74 %. This significant decrease implied a restricted decomposition of added root litter.

This pattern aligned with the concurrent observations from Pegoraro et al. (2025; in review), who observed higher phospholipid fatty acid concentrations and stronger ^{13}C-

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enrichment of fungal markers in ambient subsoil following root litter addition. Fungi preferentially utilized new plant-derived inputs (Lindahl et al., 2007) and incorporate these substrates into their biomass (Williams et al., 2006). Warming reduced fungal relative in the subsoil (Pegoraro et al., 2025; in review), potentially slowing~~dampening~~ root litter decomposition and lowering fatty acid mass change. Additionally, warming reduced soil moisture- by 18 % at mid-depth and 32% in the deep soil horizon in our study (Pegoraro et al., 2025; in review), which may have limited substrate and enzyme mobility, consequently restricting microbial access to fresh plan inputs, and thereby microbial decomposition (Manzoni et al., 2012b; Védère et al., 2020).

4.1.2 No significant change of suberin markers in subsoil with warming

In contrast to fatty acids, warming had no significant influence on the decomposition of alcohols, mid-chain acids, ω-hydroxy acids, and diacids, although the effect on mid-chain was marginally depth-dependent ($p = 0.057$; Table S15). This lack of~~a~~ warming effect persisted also when focusing on ω-hydroxy acids, and diacids with carbon numbers ≥ 20 (Table S20), which are typically identified as suberin markers (Angst et al., 2016b; Franke et al., 2005; Mendez-Millan et al., 2011). These results suggested that warming did not alter the decomposition of added ^{13}C-labeled root litter across the soil depths after three years of incubation ~~observation~~ (Pegoraro et al., 2025; in review).

Prior studies have reported contrasting hydrolysable lipid responses to warming. At the temperate forest site studied here~~our site~~, Zosso et al. (2023) have discovered a significant loss of hydrolysable lipids by 28 % in the subsoil after 4.5 years of warming, attributed primarily to increased fine root mortality caused by warming (Ofiti et al., 2021). The discrepancy between our findings may arise from methodological differences that our approach involved mixing ^{13}C-labeled root litter into the soil, which may have reduced the spatial accessibility of microorganisms to fresh substrates, an effect potentially further amplified by the warming-induced declines in subsoil microbial abundance (Zosso et al., 2021). In another study in a hardwood forest, 14 months of warming did not affect suberin markers (Feng et al., 2008), whereas in a mixed hardwood forest, warming significantly decreased suberin concentration after 4 years of warming (Pisani et al., 2015), however, this pattern was not significant in the long-term (vandenEnden et al., 2021). Divergent results indicate that the responses of hydrolysable lipids to soil warming are dependent on various factors, e.g. experimental methods, vegetation, and duration of the experiment.

The unchanged suberin markers, coupled with significantly lower recovery of fatty acids in warmed relative to ambient subsoil, indicates a potential shift in SOC processing pathways at these depths. In general, microbial carbon use efficiency (CUE) declined with soil depth (Pei et al., 2025; Spohn et al., 2016), a pattern also observed at our study site (Dove et al., 2021). Yet, the effect of warming on CUE remains uncertain (Zhang et al., 2022). If warming reduced microbial CUE in our incubation experiment, as observed elsewhere (Li et al., 2019; Li et al., 2024; Manzoni et al., 2012a), this could partly explain the greater fatty acid accumulation in the ambient subsoil. A decline in CUE under warming would favor microbial respiration over growth, thereby reducing the incorporation of substrates into microbial biomass (Manzoni et al., 2012a). Given the paucity of warming studies examining subsoil CUE, and its pivotal role in carbon storage (Tao et al., 2023), targeted experiments are needed to test this hypothesis in the future.

acceleratesslowdown observed Although tOurOn average, we found lower ^{13}C-excess of bulk SOM in topsoil under warming (Fig. 3a) compared to ambient conditions, indicating that warming accelerated the decomposition of the ^{13}C-labelled root-litter in topsoil ($p = 0.01$). This finding confirms previous observations from the same site, where warming accelerated decomposition of plant-derived inputs and increased soil respiration (Ofiti et al., 2021; Soong et al., 2021). There could be several reasons: First, at the same site, after 4 years of warming, there was an increase of carbon stock and free particulate organic matter in the topsoil (Soong et al., 2021), meaning there is sufficient easily decomposable substrate for microbial growth. This argues for co-metabolic decomposition of the added root litter, despite its lower decomposability than bulk carbon (Poirier et al., 2018). Therefore, microbial abundance was not significantly reduced by warming in topsoil (Zosso et al., 2021); Pegoraro et al., submitted). Second, warming enhanced microbial activity (Walker et al., 2018), potentially by activating a greater number of bacterial taxa (Metze et al., 2024). Third, because of more active bacteria and reorganization of microbial community towards more actinobacteria which could degrade more complex carbon sources (DeAngelis et al., 2015; Goodfellow and Williams, 1983), a wider range of complex C and N sources could be utilized by microbes (Dove et al., 2021) compared to ambient plots.

In the subsoil, we did not observe significant effects of depth (45-49 cm, $p = 0.81$; 85-89 cm, $p = 0.64$) on the decomposition of added root-litter. However, the interaction between warming and depth in subsoil is significantly more pronounced (warming x 45-49 cm, $p = 0.03$) or close to the threshold of statistical significance level (warming x 85-89 cm, $p = 0.06$). This

means that warming has depth-specific effects on the decomposition of added root-litter, highlighting the variability of subsoil compared to topsoil or even within subsoil between different depths. One reason could be the soil moisture. In ambient plots, the average annual soil volumetric water content increased with depth from 19.4 % (CI: 16.4, 22.2) to 90 cm by 10.5 % (CI: 8.1, 12.9) in ambient plots (Pegoraro, et al., submitted). Warming decreased soil moisture significantly at surface soil and at 90 cm, whereas soil moisture levels converged between warmed and ambient plots at 50 cm (Pegoraro, et al., submitted). Higher soil moisture increases the mobility of SOM, microorganisms, and exo-enzymes, which will allow the translocation of fresh carbon and decomposers (Védère et al., 2022) and then increases the connectivity among them (Védère et al., 2020), potentially resulting in higher decomposition rate. Another reason could be changes in microbial abundance and microbial community induced by warming. Warming decreases microbial abundance, especially in deep soil, and shifts microbial community towards relatively more actinobacteria and Gram+ bacteria which can utilize more complex carbon sources and adapt to environmental stress such as warming (Zosso et al., 2021). If bacteria could access the substrate, they could be more active under warming conditions. The cumulative effects of the two factors could compensate for each other and lead to on average no difference of root recovery in subsoil between warmed and ambient plots. However, they can only partially explain the results, since other factors such as oxygen, SOM protected by mineral-associations, pH could also have impacts on the variability between different depths.

Overall, our results reveal more complex responses of root-litter decomposition in subsoil under warming conditions in comparison to more straightforward acceleration of root-litter decomposition in topsoil.

4.2 Suberin markers' resistance to decomposition increased with chain length ~~Hydrolysable lipids are more resistant to decomposition than bulk roots~~

~~Root-derived hydrolysable lipids, especially those root-derived markers such as ω-hydroxy acids and diacids, degraded slower than root bulk carbon, indicated by higher proportions of hydrolysable lipids remaining in the soil than the bulk root recovery (Fig. 2b, Fig. 3b).~~ To further explore other factors governing the decomposition of suberin markers in our study, we examined the effects of chain length (number of carbon atoms in each monomer) on the mass change of ω-hydroxy acids and diacids after three years of incubation. Chain length had a significant effect on the mass change of suberin markers ($p < 0.001$; Table S27) that they

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decomposed more slowly with longer chain length. This pattern is independent of warming, depth, and compound class. Our results are in line with multiple previous studies, which also reported decreased decomposability of hydrolysable lipids with increasing chain length by examining either single compound class (Kashi et al., 2023), or suberin- and/or cutin-derived polymers (Altmann et al., 2021; Angst et al., 2016a; Suseela et al., 2017). Generally, molecules with a low oxidation state or low O/C ratio (i.e., more reduced compounds such as lipids) provide less favorable energy yields for microbial oxidation, which often translates into slower decomposition rates (Kleber, 2010; LaRowe and Van Cappellen, 2011). As chain length increases, the carbon atoms in respective compound classes exhibit progressively lower oxidation states (Chakrawal et al., 2020). Therefore, the microbial utilization of long-chain lipids requires more energy investment than that of mid-chain lipids (Schönfeld and Wojtczak, 2016), which may explain the slower decomposition of long-chain ω-hydroxy acid and diacid observed in our study.

It is noteworthy that when chain length was tested together with warming and depth on the mass change of ω-hydroxy acids and diacids monomers, warming emerged as a significant factor ($p < 0.001$; Table S27), and its effect was depth-dependent ($p < 0.001$; Table S27). Specifically, warming significantly enhanced the loss of suberin monomers in the topsoil ($p = 0.002$; Table S29), but reduced it at 45–49 cm and 85–89 cm ($p < 0.001$ and $p = 0.008$, respectively; Table S29). This pattern was also observed at compound class level, although with limited statistical power due to limited number of replicates. The stronger statistical significance at the monomer level reflects the greater number of observations (i.e., higher degree of freedom) and lower variability within individual monomers compared to the aggregated variability among monomers when grouped at the compound class level. Therefore, our results can confirm that the effect of warming on suberin marker decomposition was depth-dependent.

Root-derived hydrolysable lipids, especially those suberin markers, degraded more slowly than bulk root carbon, indicated by higher proportions of hydrolysable lipids remaining in the soil than the bulk root recovery (Pegoraro et al., 2025; in review). However, despite their higher resistance to decomposition compared to other root components, they resisted less to decomposition in warmed topsoil. One possible reason could be that the microbial population was well-adapted to the higher temperature and was able to harness complex biomass sources via hydrolytic processes (Dove et al., 2021; Zosso et al., 2021). The reported increased relative abundance of actinobacteria with warming in this experiment (Pegoraro et al., submitted) was

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834 similar to some other findings (DeAngelis et al., 2015; Pold et al., 2016). Since actinobacteria
835 are assumed to decompose complex carbon sources (Bhatti et al., 2017) and are a main group
836 of soil microorganisms producing hydrolytic enzymes (Mohan and El-Halwagi, 2007), the
837 relative increase of actinobacteria could have capitalized on the warmed conditions and have
838 accelerated the decomposition of added root litter. However, this acceleration of hydrolysable
839 lipids could be only traced in topsoil with warming. Although the amount of the roots added to
840 subsoil is proportionally more substantial compared to that added to topsoil, especially for deep
841 soil (proportionally contribution to pre-existing carbon ranging from 10% to 39%), this short-
842 term new substrate did not result in long-term faster mineralization of root-litter with warming
843 at depth. There may be an initial quick response of microbial mineralization to added root-litter
844 at the beginning of the experiment, but this faster decomposition could be slowed down over
845 time as the added litter becomes more fragmented and depleted at depth. This pattern of
846 decomposition with depth, which evolves over time, has also been observed in another root-
847 litter decomposition experiment (Hicks Pries et al., 2018). However, since we don't have time
848 resolved observations for our experiment, this argument cannot be confirmed. With continuing
849 warming, microbial capacity to utilize root-litter could further deteriorate by lower microbial
850 abundance, unfavorable environmental conditions such as lower soil moisture, and less spatial
851 accessibility of microorganisms to fragmented litter as mentioned above, leading to a slower
852 decomposition of root-litter in warmed than in ambient subsoil.

853 The faster and slower decompositiondepth-dependent effects of warming of
854 hydrolysable lipids at warmed top- and subsoil, respectively, lead to depth-specific mean
855 residence time (MRT) of these suberin molecular markers (chain length ≥ 20). such as ω-
856 hydroxy acids and diacids. In one previous study, hydrolysable lipids were revealed to have a
857 decadal MRT of 32 to 34 years (Feng et al., 2010). At In the topsoil, warming reduced the MRT
858 of ω-hydroxy acids and diacids due to accelerated decomposition from 5.8 and 5.6 years in
859 ambient plots to 3.4 years and 3.6 yearsin warmed plots. ,In the subsoil, slower decomposition
860 of suberin markers caused by warming enhanced MRT compared to ambient conditions,
861 particularly at 45-49 cm with warming, where the MRT for of ω-hydroxy acids and diacids
862 was 11.9 and 15.0 years, respectively only about 40% of ω-hydroxy acids and diacids
863 remained, whereas more (between 68% and 80% of these two markers) were left in subsoils
864 (Supplementary *Table S2*), leading to a shorter MRT of these compound classes in the topsoil
865 (3.4 years and 3.6 years, respectively) and longer in the subsoil (between 6.3 and 15.0 years)
866 (Supplementary *Table S3*). In ambient plots, the MRT of ω-hydroxy acids and diacids did not

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867 change much with depth (Supplementary *Table S3*), which has a similar trend as bulk root
868 recovery in ambient plots. The molecularThe suberin markers had a shorter makers we
869 analyzed had a shorter MRT in our study compared to a previous study, where Feng et al.
870 (2010) reported a decadal MRT of hydrolysable lipids between 32 to 34 years. whichThis
871 discrepancy could be related to the fact that they stemour model substrates arefrom grass roots
872 (*Avena fatua)* with less lignin and lower C/N ratios than the mostlylocal woody roots
873 originating from the local vegetation at the site (Hicks Pries et al., 2018; Silver and Miya,
874 2001). , aHnd hydrolysable lipids without association to lignin could be steadily decomposed
875 (Angst et al., 2016a). HoweverAdditionally, an underestimation of MRT could exist in our
876 study since we have a much shorter experimental duration compared to other studies (Feng et
877 al., 2010).
878 In prior study, Suseela et al. (2017) reported that elevated temperature and CO_2
879 increased the chain length of ω-hydroxy acids in grass roots (*Boutelou gracilis*). Together with
880 our finding that the decomposability of suberin markers decreases with chain length, this raises
881 the possibility that plant physiological adaptations to future warming could enhance the
882 persistence of root compartments against microbial decomposition and consequently mitigate
883 potential soil carbon losses under warming.
884 Another striking finding is that there is accumulation of fatty acids in subsoil in ambient
885 plots where the hydrolysable lipids were steadily decomposed (Fig. 4b). This accumulation of
886 fatty acids is also observed in deep soil in DC ambient plots where root-litter was not added
887 (Fig. 1b). The mass of fatty acids found in the soil after hydrolysis exceeds that in originally
888 added labelled roots, implying another source for these additional fatty acids. The accumulation
889 of this compound class is predominantly from an increase of octadecanoic acid ($C_{18.0}$ fatty
890 acids), octadecenoic acid ($C_{18.1}$ fatty acids), and hexadecanoic acid ($C_{16.0}$ fatty acids)
891 (Supplementary *Table S2*). It is noteworthy that there is no accumulation of fatty acids with a
892 chain length larger than 20 (Supplementary *Fig. S1*), which is also confirmed by an on-average
893 shorter carbon chain-length in DC ambient plots than in DC warmed plots Supplementary *Fig.*
894 *S2).* Long-chain fatty acids are typically enriched in higher plant biomass, while the mid-length
895 homologues are often enriched in microbial and plant biomass (Harwood and Russell, 1984).
896 These additional fatty acids originate likely from microbial biomass, e.g. phospholipid fatty
897 acids (PLFA) (Joergensen, 2022; Zelles, 1997), since these ester-linked microbial markers also
898 contain fatty acids which could be released during hydrolyzation as non-bound free-extractable
899 fatty acids were removed before hydrolysis. This is also confirmed by the presence of probably

900 ~~root-derived fatty acids, or other root-derived carbon has been metabolized by microorganisms~~
901 ~~and the ^{13}C signal is incorporated in microbial membrane lipids (Gunina et al., 2014).~~

902 ~~Another possible reason for the accumulation of fatty acids could be preferential loss~~
903 ~~of certain other compound classes in hydrolysable lipids during their decomposition. Previous~~
904 ~~studies suggested contradictory theories of how suberin and cutin can depolymerize. One~~
905 ~~theory is simultaneous and similar decomposition of the compound classes (Riederer et al.,~~
906 ~~1993). Alternatively, long-chain (>16) ω-hydroxy and diacids could be more prone to be~~
907 ~~released (Naafs et al., 2005; Nierop et al., 2003), or monomers with only one functional group~~
908 ~~such as *n*-alcohols could be preferentially decomposed due to their terminal position in~~
909 ~~polyesters (Mueller et al., 2013). Therefore, we cannot state whether fatty acids in our~~
910 ~~experiment were preferentially lost and taken up by microorganisms. However, the results of~~
911 ~~higher enrichment of ^{13}C signature in fatty acids in ambient plots support previous statements~~
912 ~~that microbial activity is higher in subsoil with ambient temperature than with warming, which~~
913 ~~is additionally validated by higher δ^{13}C values that are incorporated in PLFA (Pegoraro et al.,~~
914 ~~submitted.).~~

915 ~~Combining the results of root recovery in bulk soil and at molecular level we can notice~~
916 ~~that although the overall root-derived carbon recoveries are similar between warmed and~~
917 ~~ambient subsoil, a considerable amount of root-derived carbon is already incorporated in~~
918 ~~microbial biomass in ambient plots, meaning a slower decomposition of root-litter in warmed~~
919 ~~subsoil.~~

920 **4.3 ~~Minor~~ No priming effect of added ^{13}C-~~labelled~~labeled ~~root litter~~root litter after three**
921 **years of incubation**

922 Fresh biomass input can stimulate (prime) the decomposition of native, pre-existing
923 SOM. This is especially relevant in subsoils, where SOM might have existed for a long time,
924 from decades to millennia (Fontaine et al., 2007; Luo et al., 2019; Shahzad et al., 2018). Such
925 priming could offset long-term carbon sequestration, especially in subsoil where there is
926 usually substrate limitation (Bernard et al., 2022; Bingeman et al., 1953).

927 Three years after adding ^{13}C-~~labelled~~labeled root ~~tissues~~ at three different soil depths,
928 we found no evidence for significant priming across the whole soil profile, either~~both~~ for pre-
929 existing bulk ~~carbon or~~and hydrolysable lipids.~~ There could be several explanations. First,~~This
930 likely reflects the fact that ~~fresh~~ fresh carbon input was added only at the beginning of the
931 incubation. ~~Since priming~~Priming is ~~a temporary~~a transient response to fresh carbon input

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(Schiedung et al., 2023) and is commonly strongest at the beginning of the incubation (Fontaine et al., 2007; Tao et al., 2024), ~~this effect might became negligible after three years of incubation~~a pattern that has been also reported in lipid decomposition experiments (Angst et al., 2016a; Kashi et al., 2023). Therefore, any initial effect may have dissipated in the long-term.~~.~~ In addition, our calculations of priming effects showed large error bars, likely due to heterogeneous background SOC concentrations, particularly in the subsoil. For example, the mass of SOC in root litter added to the topsoil (10-14 cm) represented only 4.0-8.5% of pre-existing SOC, whereas at 85-89 cm, the same litter addition corresponded to 10.5-39.3% of pre-existing SOC. This wide range in the proportion of added to native SOC, alongside reduced microbial abundance under warming may have biased our estimates and masked potential priming effects. Therefore, although our back-of-the-envelope calculation provides a preliminary estimate, the strength of the interpretation is limited. Short-term monitoring of CO_2 fluxes and their partitioning between ^{13}C-labeled root- and native SOC-derived, would offer more robust insights into whether root litter addition primes native SOC decomposition, particularly in the subsoil. ~~Second, warming could suppress priming due to decreased N mining of microorganisms in native soil (Dong et al., 2024; Li et al., 2023; Sun et al., 2019). Blodgett Forest is limited in nitrogen and the added grass root-litter contains more nitrogen than the roots of native tree species (Hicks Pries et al., 2018; Silver and Miya, 2001). When native SOM is nitrogen deficient, and the added substrate contains nitrogen, as in our study, warming will stimulate the mineralization of the added substrate and release nitrogen from it for microbial growth (Feng and Zhu, 2021). Such a process could partially explain why we found negative priming (inhibition) for bulk subsoil SOM.~~

~~**4.4 Subsoil responds more heterogeneously than topsoil to warming**~~

~~One of the difficulties to implement an *in-situ* field warming experiment is the inherent heterogeneity and complexity of natural soil conditions, especially in subsoil.~~

~~Since the same amount of ^{13}C labelled root-litter was added at different depths, it could have different impacts on the microorganisms between top- and subsoil. Specifically, when the heterogeneous carbon concentrations between pairs of plots or even within the same plots were considered (Supplementary *Table S5*), the new input could contribute between 10% and 39% to the pre-existing carbon. This heterogeneity is partially reflected in ^{13}C-excess of bulk soil carbon (Fig. 2a), with larger error bars with depth. Although the root-litter was fully mixed with soil and the amount of added substrate is substantial for subsoil microorganisms, this does~~

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964 not mean that all the substrates could be accessed by microorganisms (Inagaki et al., 2023;
965 Salomé et al., 2010), especially in warmed subsoil due to less abundant microorganisms
966 compared to topsoil and their restricted mobility.

967 The different responses of root-litter decomposition between top- and subsoil with
968 warming highlight the fundamental importance for regarding depth as a rudimentary factor for
969 studying soil carbon dynamics. The large heterogeneity existing in subsoil spotlights the
970 uncertainties in predicting subsoil's responses to climate change, especially when subsoil is
971 considered for long-term carbon sequestration (Button et al., 2022; Sierra et al., 2024), and
972 more focus should be drawn on observation into subsoil. To reduce the large heterogeneity in
973 an experiment such as ours, a more realistic amount of root-litter added to individual depths
974 could be achieved by first assessing root distribution across the whole soil profile via
975 techniques such as minirhizotrons (Rahman et al., 2020), and then adjusting the added amount
976 of root litter.

977 **5. Conclusions**

978 Our experiment is among the first *in-situ,* long-term incubations to investigate the effect
979 of warming on the decomposition of simulated new substrates input across different soil
980 horizons under natural field conditions. Using compound-specific isotope analysis with
981 specific focus on root-derived molecular markers, we traced their fates through microbial
982 mineralization, assimilation and/or stabilization via e.g. aggregate occlusion, and organo-
983 mineral association (Pegoraro et al., 2025; in review). Distinct responses among compound
984 classes across soil depths and temperature treatments improved our understanding of how
985 warming shifts the fate of root-derived molecular markers. Therefore, we recommend applying
986 this technique or more advanced variants, such as position-specific ^{13}C-labeling, in the future
987 to better understand soil carbon dynamics under climate change. Position-specific ^{13}C-labeling
988 technique can pinpoint which substrates or carbon functional groups are more rapidly
989 mineralized, incorporated into microbial biomass, or associated with minerals (Apostel et al.,
990 2015; Kashi et al., 2023; Schink et al., 2021).

991

992 Three years of incubation enabled us to track the fate of ^{13}C-labeled root litter and assess
993 long-term dynamics of relatively stable compounds like hydrolysable lipids in the soil.
994 Collectively, at the compound class level, hydrolysable lipids showed compound class-
995 dependent responses to warming. Warming had the most pronounced effects on fatty acids,

consistently decreasing their mass change across the soil profile. However, the mechanisms were fundamentally different between top- and subsoil. In the topsoil, enhanced microbial decomposition accelerated fatty acid losses. In the subsoil, where there was accumulation of fatty acids, warming attenuated this accumulation, particularly at depth, coincident with lower microbial abundance and constrained hydrolysable lipids decomposition due to low soil moisture. At the monomer level, suberin markers, ω-hydroxy acids and diacids, were more resistant to decomposition than bulk root-derived carbon, with their resistance increasing with chain length. Moreover, warming exhibited depth-dependent effects on the decomposition of suberin-derived monomers–significantly accelerated their decomposition in the topsoil while suppressing it in the subsoil.

Substantial spatial heterogeneity was observed, especially in the subsoil, and this heterogeneity was partially reflected by wide range of added carbon to background SOC ratios. The disproportionate carbon inputs across depths may have biased our results, particularly in subsoil where higher soil moisture and microbial abundance under ambient conditions favored microbial decomposition. Along with the limited number of observations in our study, the capability to generalize our findings is constrained. Therefore, future *in-situ* warming experiments with greater emphasis on subsoil processes and enhanced replications are needed corroborate our findings.

Natural ecosystems are complex and their response to warming is intricate, depending on many biotic and abiotic factors and their interactions. Warming accelerated the decomposition of root-derived carbon in topsoil after three years of incubation, but surprisingly not in the subsoil. This difference could be attributed to the fundamental differences of biotic and abiotic factors between top- and subsoil, which are further affected by environmental stress, such as warming.

Suberin markers, such as ω-hydroxy acids and diacids, are relatively more resistant compared to other root components, but they are less resistant to decomposition with warming in topsoil. However, once the decomposition is slowed down in subsoil by warming due to unfavorable conditions such as lower microbial abundance and soil moisture, these molecular markers could be preserved in subsoil over one decade (Supplementary Table S3). Warming also altered the composition of hydrolysable lipids, especially in subsoil, with an accumulation of fatty acids. We interpreted the accumulation of fatty acids as potential evidence for the higher microbial activity and higher turnover rate of new OM in ambient than warming plots. It is noteworthy that the

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decomposition of added root-litter in natural subsoil with warming was heterogeneous, which obscured potentially existing trends with depth, and potential systematic differences between ambient and warmed plots.

Our experiment is one of the first in-situ long-term incubations on the effect of warming on the decomposition of simulated new substrates input at different soil depths in natural conditions at molecular level. The compound-specific isotope analysis leaves us a key message: Although after 3 years of incubation the recoveries of root-derived carbon are similar in subsoil regardless of temperature treatment, the carbon dynamics could be fundamentally changed by warming. The intrinsic chemical or thermodynamic properties may slow down the decomposition of chemically persistent molecules like hydrolysable lipids. However, in subsoil, the key factors influencing whether root-litter is transformed into long-term carbon storage are the decomposers' possibilities to access the substrates and their strategies to survive in harsh conditions with limited nutrients and substrates. The large natural heterogeneity of the investigated soil did not allow to identify a clear trend how subsoil responds to warming. Future in-situ observations should include both short-term (months) and long-term (several years to decade) observations to identify and quantify the time-resolved fate of new OM substrate and how the microbial community responds to warming.

Code/Data availability

The data used in this study are available from the ESS-DIVE repository.

Competing interests

The authors declare that they have no conflict of interest.

Author contributions

BS conducted lipids analysis and data interpretation, contributed to writing original draft, statistical analysis, and editing. EP shared resources and contributed to statistical analysis, writing review, and editing. MST applied for the funding, designed and maintained the warming experiment, contributed to statistical analysis, and writing review. CUZ conducted elemental analysis, introduced lipid analysis to me, and contributed to conceptualization, writing review and editing. GLBW supervised BS through the lipid analysis, contributed to methodology, conceptualization, data interpretation and validation, writing review, and editing. MWIS applied for funding and conceived DEEP C project, contributed to conceptualization, data interpretation, writing review, and editing. BS conducted lipids analysis and data interpretation, contributed to writing original draft, statistical analysis, and editing.

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