



## Preferential degradation of leaf- vs. root-derived organic carbon in earthworm-affected soil



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### ABSTRACT

Earthworms are integral parts of many ecosystems and may play a decisive role in determining whether soils function as carbon (C) sink or source. However, information on how earthworms affect the composition and stability of soil organic matter (SOM) is scarce. Particularly their effect on organic matter deriving from leaves and roots with distinct composition and, thus, susceptibility to decomposition and stabilization remains unclear. Here, we combine cutin- and suberin-derived lipids as specific markers for leaf- and root-derived SOM with their <sup>13</sup>C composition and physical fractionations of soil. We show that earthworms overprint the protective role of organo-mineral associations and aggregates to favor the accumulation of root- relative to leaf-derived SOM. This gradual accumulation contributes to the often-observed dominance of root-derived organic matter in soil and emphasizes the need to consider molecular level effects of earthworms on SOM dynamics.

Earthworms are major players in determining whether soil acts as a carbon (C) source or sink (Frouz et al., 2014; Jouquet et al., 2006; Lubbers et al., 2013). Their role in soil organic matter (SOM) dynamics, though, is often neglected despite their presence in many ecosystems worldwide (Hendrix et al., 2008). Earthworms affect SOM dynamics in two principal ways: (1) they stimulate soil microbial activity and biomass and, thus, enhance the mineralization of SOM (i.e., the release of CO<sub>2</sub> into the atmosphere; Ferlian et al., 2018; Groffman et al., 2015; Lubbers et al., 2019; McLean et al., 2006) and (2) they promote the formation of macro- and microaggregates, transferring SOM into a more stabilized form (Bossuyt et al., 2005; Pulleman et al., 2005). The net effect of these processes on the storage of soil organic C (SOC), though, is still subject to debate likely due to a focus on coarse-scale measures, such as the SOC contents of bulk soil. Instead, molecular level studies may reveal more intricate earthworm-effects on SOM (e.g., Angst et al., 2017b; Ma et al., 2013; Nguyen Tu et al., 2020; Vidal et al., 2019). Additional insights into the fate of SOM in the presence of earthworms may be generated by combining such molecular analyses with the physical fractionation of soils into SOM pools with different stability (e.g., Angst et al., 2019b; Lavallee et al., 2019; von Lütow et al., 2007). Specifically, the effect of earthworms on leaf- and root-derived organic matter calls for more attention. Due to differences in composition, roots decompose more slowly than leaves and root-derived SOM often represents a larger soil C pool as compared to leaf-derived SOM (Crow

et al., 2009; Rasse et al., 2005). Earthworms may unpredictably influence the proportion of these C pools with unknown consequences for the stability and amount of organic C in soil. This is of particular importance in light of the ongoing earthworm invasion of (North American) ecosystems (Hendrix et al., 2008) or shifting allocation of plant biomass to above- and belowground organs in the wake of climate change (Pendall et al., 2004). While some studies found short-term effects of earthworms on leaf- and root-derived SOM using molecular and isotopic methods (within the timeframe of several weeks; Vidal et al., 2019, 2016), no clear differences in the contribution of these sources to SOM have been found in the long-term. This is surprising given that earthworms, in the absence of mineral soil, commonly prefer more easily palatable substrates as a food source (Curry and Schmidt, 2007).

Here, we use cutin and suberin-derived lipids as specific markers for leaf- and root-derived SOM in combination with their <sup>13</sup>C composition and soil physical fractionations to provide clear indications as to whether the presence of earthworms results in a preferential degradation of leaf- relative to root-derived SOM.

We used material from a long-term incubation (33 weeks) of earthworm-affected and non-affected soil, where (3x) replicated treatments were mixed once at the start of the incubation with the same amounts of leaf and root material from *Alnus glutinosa* L. (European alder; 7 g plant material, 80 g mineral soil) crushed to a size of

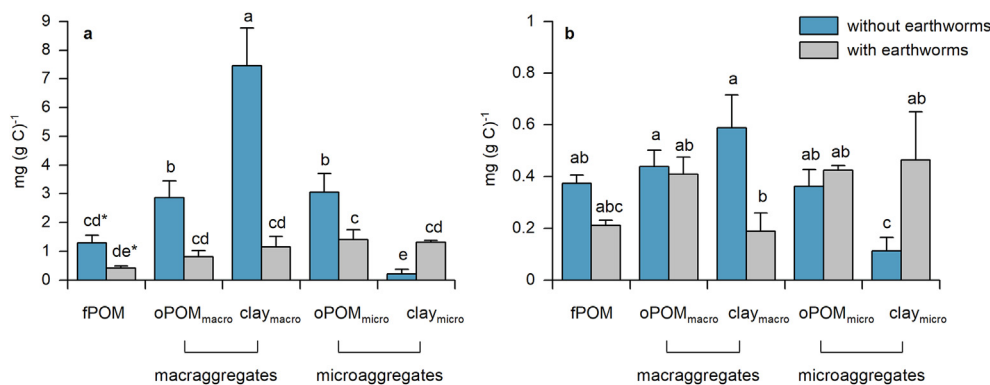
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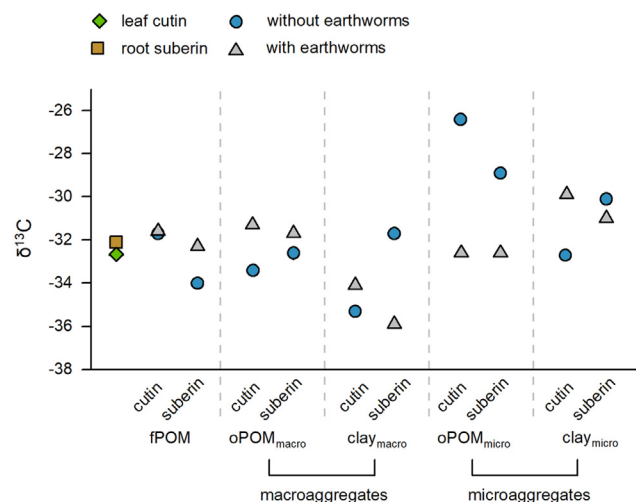
2 × 2 mm. Each treatment was incubated together with two specimens of *Aporrectodea caliginosa*, Savigny, an endogeic species (i.e., soil-dwelling), and one specimen of *Lumbricus rubellus*, Hoffmeister, an epigeic species (i.e., living in mineral soil and the forest floor). The soil samples and earthworms were collected from the same sites; the soil samples were taken from the subsoil that was devoid of earthworms, while the latter were collected from the topsoil. This approach guaranteed the absence of an earthworm legacy of the incubated soils, but a similar textural/mineralogical composition as compared to the topsoil in which the earthworms dwelled. After the end of the incubation, the soils from the treatments were physically fractionated into five fractions increasing in stability: plant fragments freely residing in the soil and most similar to the plant input (fPOM), organic matter occluded (i.e., stabilized) in macro- (oPOM<sub>macro</sub>) and microaggregates (oPOM<sub>micro</sub>), and organo-mineral associations from macro- (clay<sub>macro</sub>) and microaggregates (clay<sub>micro</sub>). Details about the physical fractionation procedure and the incubation experiment can be found in Angst et al. (2018) and Angst et al. (2019a).

Cutin and suberin-derived lipids were solvent extracted from the different soil fractions, followed by base hydrolysis and GC-MS/FID measurements to qualify and quantify the biomarkers in the extracts (Supplementary Material S1; Angst et al., 2018). Target lipids were plant-derived  $\alpha,\omega$ -dicarboxylic acids,  $\omega$ -hydroxy acids, and mid-chain substituted hydroxy acids, which were assigned to cutin and suberin depending on their occurrence in alder leaves or roots. We further analyzed these biomarkers for their  $^{13}\text{C}$  composition using GC-IRMS (see Supplementary Material S1 for analytical details). Because in most biochemical processes the lighter ( $^{12}\text{C}$ ) isotope is preferentially incorporated as compared to the heavier ( $^{13}\text{C}$ ) isotope (Hobbie and Werner, 2004), the decomposition of individual compounds, such as certain lipids, should be accompanied by a relative enrichment of  $^{13}\text{C}$  in the remaining compounds (cf. Chikaraishi and Naraoka, 2006). Thus, apart from changes in the concentration of cutin and suberin in a soil, their  $\delta^{13}\text{C}$  values should provide additional information on the fate of root- and leaf-derived SOM under the influence of earthworms.

The C-normalized concentrations of the cutin and suberin markers in the different soil fractions indicate that earthworm activity predominantly affected leaf-derived SOM. Compared with the unamended control soil, the content of cutin was substantially higher in the litter-amended treatments, indicating that most of the cutin extracted from the fractions derived from the newly added plant material (Supplementary Table S1; Fig. 1). Yet, the contents of cutin in the earthworm-affected treatments were substantially lower as compared to those in the non-affected treatments independent of the soil fraction focused on (from 54% in oPOM<sub>micro</sub> to as much as 80% in clay<sub>macro</sub>). An exception to this general pattern was clay<sub>micro</sub>, where the content of cutin markers was higher by 73% (Fig. 1). This increase may indicate a partial redistribution of cutin to clay<sub>micro</sub> (in which lipids might be particularly stable; cf., Angst et al., 2017a), which, however, does not fully compensate for the earthworm-induced loss of cutin in the other



**Fig. 1.** Cutin and suberin in differently stable soil fractions from macro- and microaggregates in earthworm-affected and non-affected soil. Statistically significant differences ( $p < 0.05$ ) are indicated by lower case letters, marginally significant differences ( $p < 0.1$ ) are indicated by asterisks. Results from factorial ANOVA with treatment (earthworm/no earthworm) and fraction as categorical variables;  $n = 15$ ,  $df = 4$ .



**Fig. 2.** Corrected  $\delta^{13}\text{C}$  values of cutin and suberin in leaves, roots, and the different soil fractions from earthworm-affected and non-affected soil. Cutin in this graph is represented by  $\alpha,\omega$ -dihydroxyhexadecanoic acids only because our other cutin monomer (9,10, $\omega$ -hydroxyoctadecanoic acid) had been partly methylated during base hydrolysis, for which we were not able to calculate the  $^{13}\text{C}$  introduced by the methyl groups. Delta  $^{13}\text{C}$  values for suberin are weighted  $^{13}\text{C}$  values (normalized to lipid concentrations) of its constituting lipids.

fractions ( $\sim 1$  vs.  $10 \text{ mg g C}^{-1}$ ). Through the ingestion of large quantities of organic matter and mineral soil, the disintegration and re-ingestion of aggregates, and the mixing of this material with mucus in their intestines (Drake and Horn, 2007; Jouquet et al., 2006), earthworms likely partly override the protective role of organo-mineral associations and aggregates and render most of the cutin susceptible to microbial decomposition or other transformation processes (cf., Angst et al., 2019a). This interpretation is supported by the relative enrichment in  $^{13}\text{C}$  of cutin in the soil fractions affected by earthworms, which likely results from the preferential decomposition of  $^{13}\text{C}$  depleted cutin, leaving the remaining cutin  $^{13}\text{C}$  enriched. Notably, the  $\delta^{13}\text{C}$  values of cutin (and suberin) in oPOM<sub>micro</sub> from soil without earthworms were much more positive than those of the same fraction from soil with earthworms and deviated from what could be expected from the  $^{13}\text{C}$  of cutin in the other fractions (Fig. 2). These increased  $^{13}\text{C}$  values in oPOM<sub>micro</sub> may perhaps derive from highly decomposed biomarkers previously present in this fraction (see also Supplementary Table S2), which might have been affected differently in the presence as compared to the absence of earthworms (e.g., via decomposition or re-distribution among fractions). In contrast to cutin, suberin was less severely affected by the activity of earthworms and its content compared to the treatments without earthworms changed significantly in organo-mineral associations only (Fig. 1). The decrease of suberin in clay<sub>macro</sub> corresponded to its increase in clay<sub>micro</sub> ( $0.4$  vs.  $0.35 \text{ mg g C}^{-1}$ ), indicating a

redistribution of suberin between organo-mineral associations from differently-sized aggregates in the presence of earthworms rather than a decrease, such as noted for cutin. The minor effect of earthworms on suberin is also mirrored by its  $^{13}\text{C}$  composition, where differences between earthworm-affected and non-affected soil fractions were either small ( $< 0.7\%$ , as for  $\text{oPOM}_{\text{macro}}$  and  $\text{clay}_{\text{micro}}$ ) or could not consistently be related to a preferential microbial utilization, such as was the case for cutin (Fig. 2). These ambiguous  $^{13}\text{C}$  values may also result from the fact that higher contents of suberin as compared to cutin were not derived from the added litter in some soil fractions (Supplementary Table S1).

When integrated over all soil fractions, the proportion of cutin remaining after the incubation in the earthworm-affected as compared to the non-affected soil was  $\sim 37\%$ , while that of suberin was  $\sim 86\%$ . We also estimated the amount of cutin- and suberin-derived C that remained after the incubation with respect to the initial, litter-amended soil, and calculated the ratio between this cutin- and suberin-derived C. This ratio was  $\sim 1.5$  for the non-affected treatments, while it was  $\sim 0.6$  for the earthworm-affected treatments, indicating a relative accumulation of suberin as compared to cutin in the presence of earthworms.

Combined, our data clearly indicate that earthworm activity favors the decomposition of leaf- over root-derived SOM, unrelated to the presumed stability of the soil fraction focused on. A possible explanation for these patterns is an active foraging by earthworms for more easily palatable substrates in mineral soil (i.e., leaf-derived SOM; see also Supplementary Fig. S1) or a weaker protection of leaf- as compared to root-derived SOM (cf., Angst et al., 2018). Independent of the reasons, our results suggest that the introduction of earthworms to soils overprints the protective role of aggregates and organo-mineral associations to “mix up” SOM composition (without necessarily affecting C contents; Angst et al., 2019a) and favors the accumulation of root- relative to leaf-derived compounds in soil. These results are of importance in predicting the consequences of earthworms on SOM dynamics and C sequestration, and highlight the need to study earthworm-effects on the molecular level.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2020.114391>.

### References

Angst, G., Mueller, C.W., Prater, I., Angst, Š., Peterse, F., Nierop, K.G.J., 2019a. Earthworms act as biochemical reactors to convert labile plant compounds into stabilized soil microbial necromass. *Commun. Biol.* 2, 1–7. <https://doi.org/10.1038/s42003-019-0684-z>.

Angst, G., Mueller, K.E., Eissenstat, D.M., Trumbore, S., Freeman, K.H., Hobbie, S.E., Chorover,

J., Oleksyn, J., Reich, P.B., Mueller, C.W., 2019b. Soil organic carbon stability in forests: distinct effects of tree species identity and traits. *Glob. Chang. Biol.* gcb.14548. <https://doi.org/10.1111/gcb.14548>.

Angst, G., Mueller, K.E., Kögel-Knabner, I., Freeman, K.H., Mueller, C.W., 2017a. Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter. *Biogeochemistry* 132, 307–324. <https://doi.org/10.1007/s10533-017-0304-2>.

Angst, G., Nierop, K.G.J., Angst, Š., Frouz, J., 2018. Abundance of lipids in differently sized aggregates depends on their chemical composition. *Biogeochemistry* 140, 111–125. <https://doi.org/10.1007/s10533-018-0481-7>.

Angst, Š., Mueller, C.W., Cajthaml, T., Angst, G., Lhotáková, Z., Bartuška, M., Špaldoňová, A., Frouz, J., 2017b. Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter. *Geoderma* 289, 29–35. <https://doi.org/10.1016/j.geoderma.2016.11.017>.

Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. *Soil Biol. Biochem.* 37, 251–258. <https://doi.org/10.1016/j.soilbio.2004.07.035>.

Chikaraishi, Y., Naraoka, H., 2006. Carbon and hydrogen isotope variation of plant biomarkers in a plant-soil system. *Chem. Geol.* 231, 190–202. <https://doi.org/10.1016/j.chemgeo.2006.01.026>.

Crow, S.E., Lajtha, K., Filley, T.R., Swanston, C.W., Bowden, R.D., Caldwell, B.A., 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Glob. Chang. Biol.* 15, 2003–2019. <https://doi.org/10.1111/j.1365-2486.2009.01850.x>.

Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms – a review. *Pedobiologia (Jena)* 50, 463–477. <https://doi.org/10.1016/j.pedobi.2006.09.001>.

Drake, H.L., Horn, M.A., 2007. As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. *Annu. Rev. Microbiol.* 61, 169–189. <https://doi.org/10.1146/annurev.micro.61.080706.093139>.

Ferlian, O., Eisenhauer, N., Aguirrebengoa, M., Camara, M., Ramirez-Rojas, I., Santos, F., Tanalogo, K., Thakur, M.P., 2018. Invasive earthworms erode soil biodiversity: a meta-analysis. *J. Anim. Ecol.* 87, 162–172. <https://doi.org/10.1111/1365-2656.12746>.

Frouz, J., Špaldoňová, A., Fričová, K., Bartuška, M., 2014. The effect of earthworms (*Lumbricus rubellus*) and simulated tillage on soil organic carbon in a long-term microcosm experiment. *Soil Biol. Biochem.* 78, 58–64. <https://doi.org/10.1016/j.soilbio.2014.07.011>.

Groffman, P.M., Fahey, T.J., Fisk, M.C., Yavitt, J.B., Sherman, R.E., Bohlen, P.J., Maerz, J.C., 2015. Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. *Soil Biol. Biochem.* 87, 51–58. <https://doi.org/10.1016/j.soilbio.2015.03.025>.

Hendrix, P.F., Callahan Jr., M.A., Drake, J.M., Huang, C., Sam, W., Snyder, B.A., Zhang, W., Hendrix, P.E., Callahan, M.A., Drake, J.M., Huang, C., James, S.W., Snyder, B.A., Zhang, W., 2008. Pandora's box contained bait: the global problem of introduced earthworms. *Annu. Rev. Ecol. Evol. Syst.* 39, 593–613.

Hobbie, E.A., Werner, R.A., 2004. Review and synthesis review Tansley and patterns isotope plants. *New Phytol.* 161, 371–385. <https://doi.org/10.1046/j.1469-8137.2004.00970.x>.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., Lepage, M., 2006. Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Appl. Soil Ecol.* 32, 153–164. <https://doi.org/10.1016/j.apsoil.2005.07.004>.

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2019. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Glob. Chang. Biol.* 261–273. <https://doi.org/10.1111/gcb.14859>.

Lubbers, I.M., Berg, M.P., De Deyn, G.B., van der Putten, W.H., van Groenigen, J.W., 2019. Soil fauna diversity increases  $\text{CO}_2$  but suppresses  $\text{N}_2\text{O}$  emissions from soil. *Glob. Chang. Biol.* 1–13. <https://doi.org/10.1111/gcb.14860>.

Lubbers, I.M., Van Groenigen, K.J., Fonte, S.J., Six, J., Brussaard, L., Van Groenigen, J.W., 2013. Greenhouse-gas emissions from soils increased by earthworms. *Nat. Clim. Chang.* 3, 187–194. <https://doi.org/10.1038/nclimate1692>.

Ma, Y., Filley, T.R., Johnston, C.T., Crow, S.E., Szlavecz, K., McCormick, M.K., 2013. The combined controls of land use legacy and earthworm activity on soil organic matter chemistry and particle association during afforestation. *Org. Geochem.* 58, 56–68. <https://doi.org/10.1016/j.orggeochem.2013.02.010>.

McLean, M.A., Migge-Kleian, S., Parkinson, D., 2006. Earthworm invasions of ecosystems devoid of earthworms: effects on soil microbes. *Biol. Invasions Belowgr.* Earthworms as Invasive Species 57–73. [https://doi.org/10.1007/978-1-4020-5429-7\\_7](https://doi.org/10.1007/978-1-4020-5429-7_7).

Nguyen Tu, T.T., Vidal, A., Quenea, K., Mendez-Millan, M., Derenne, S., 2020. Influence of earthworms on apolar lipid features in soils after 1 year of incubation. *Biogeochemistry*. <https://doi.org/10.1007/s10533-020-00639-w>.

Pendall, E., Bridgman, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M., Ryan, M.G., Wan, S., 2004. Below-ground process responses to elevated  $\text{CO}_2$  and temperature: a discussion of observations, measurement methods, and models. *New Phytol.* 162, 311–322. <https://doi.org/10.1111/j.1469-8137.2004.01053.x>.

Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. *Appl. Soil Ecol.* 29, 1–15. <https://doi.org/10.1016/j.apsoil.2004.10.003>.

Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269, 341–356. <https://doi.org/10.1007/s11104-004-0907-y>.

Vidal, A., Quenea, K., Alexis, M., Derenne, S., 2016. Molecular fate of root and shoot litter on incorporation and decomposition in earthworm casts. *Org. Geochem.* 101, 1–10. <https://doi.org/10.1016/j.orggeochem.2016.08.003>.

Vidal, A., Watteau, F., Remusat, L., Mueller, C.W., Nguyen Tu, T.-T., Buegger, F., Derenne, S., Quenea, K., 2019. Earthworm cast formation and development: a shift from plant litter to mineral associated organic matter. *Front. Environ. Sci.* 7, 1–15. <https://doi.org/10.3389/fenvs.2019.00055>.

von Lütow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.* 39, 2183–2207. <https://doi.org/10.1016/j.soilbio.2007.03.007>.