Gone or just out of sight? The apparent disappearance of aromatic litter components in soils

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Abstract

Uncertainties concerning stabilization of organic compounds in soil limit our basic understanding on soil organic matter (SOM) formation and our ability to model and manage effects of global change on SOM stocks. One controversially debated aspect is the contribution of aromatic litter components, such as lignin and tannins, to stable SOM forms. In the present opinion paper, we summarize and discuss the inconsistencies and propose research options to clear them.

Lignin degradation takes place step-wise, starting with (i) depolymerisation, followed by (ii) transformation of the water-soluble depolymerization products. The long-term fate of the depolymerization products and other soluble aromatics, e.g., tannins, in the mineral soils is still a mystery. Research on dissolved organic matter (DOM) composition and fluxes indicates dissolved aromatics are important precursors of stable SOM attached to mineral surfaces and persist in soils for centuries to millennia. Evidence comes from flux analyses in soil profiles, biodegradation assays, and sorption experiments. In contrast, studies on composition of mineral-associated SOM indicate the prevalence of non-aromatic microbial-

derived compounds. Other studies suggest the turnover of lignin in soil can be faster than the turnover of bulk SOM. Mechanisms that can explain the apparent fast disappearance of lignin in mineral soils are, however, not yet identified.

The contradictions might be explained by analytical problems. Commonly used methods probably detect only a fraction of the aromatics stored in the mineral soil. Careful data interpretation, critical assessment of analytical limitations, and combined studies on DOM and solid-phase SOM could thus be ways to unveil the issues.

1 Introduction

Storage and quality of soil organic matter (SOM) determine many crucial soil properties and the cycles of carbon (C) and essential nutrients through ecosystems. The storage of SOM is determined by plant litter inputs and decomposition processes. Decomposition of SOM is a significant source of atmospheric CO_2, thus, a critical parameter in climate models (Schlesinger and Andrews, 2000). Decomposition rates are sensitive to global change factors such as temperature, precipitation, and land use. However, our ability to understand and predict such responses is limited by uncertainties about pathways of organic matter transformation in soil. In particular, the question as to why some SOM components persist in soil for centuries (denoted as `stable SOM` from here on) while others turn over quickly is still puzzling (Schmidt et al., 2011).

Recent research challenges traditional theories presuming that stable SOM results from neoformation of complex humic polymers in soil (`humification`). Stable SOM rather seems to be composed of relatively simple organic compounds that are protected against biodegradation, e.g., because they are tightly bound to mineral surfaces (Schmidt et al., 2011; Kleber et al., 2015). Herein, we hold to this view but argue that, despite extensive research in the last years, the chemistry and source of compounds incorporated into stable SOM is still largely uncertain. In particular, the importance of aromatic compounds derived from abundant plant litter components, such as lignin and tannins, is controversially debated (Figure 1). One line of evidence suggests that they are important contributors to stable SOM. It bases primarily on data from research on fluxes and behaviour of dissolved organic matter (DOM) in soil, hence, we will denote it as the `dissolved phase line of evidence`. A contrasting line of evidence suggests a quick degradation of aromatic compounds in soil derives primarily from analyses of the composition of solid SOM (`solid phase line of evidence`). Herein, we sum up

and confront the arguments of the two views, discuss potential reasons for the controversies (including limitations in analytical methods and process understanding) as well as their implications for our basic understanding on SOM formation.

2 Dissolved phase line of evidence

The view that plant-derived aromatics are a major source of stable SOM is based on the following two main arguments:

(1) DOM produced during litter decomposition and leached into mineral soil is a main source of stable SOM adsorbed on mineral surfaces.

(2) Aromatic DOM components produced during litter decomposition are resistant to mineralization and preferentially sorb to mineral surfaces. Hence, they are preferentially stabilized in mineral soil.

2.1 Argument 1: DOM as source of stable SOM

Leaching of DOM is a major pathway for organic matter translocation from forest floor into the topsoil horizons. Estimates for acidic forest soils with permanent forest floor suggest that 25-89% of the SOM stored in mineral soils derives from DOM (Neff and Asner, 2001; Michalzik et al., 2003; Kalbitz and Kaiser, 2008), based on the typical observation of decreasing DOC fluxes with depth of the mineral soil (a large compilation of data from studies on forest and grassland soils is presented by Neff and Asner, 2001). Two processes can explain the decrease: mineralization and sorption.

Sorption of DOM to mineral surfaces likely is a major process forming stable SOM in many soils. Evidence for its importance comes from findings that the turnover and storage of SOM in mineral soil horizons is often related to the contents of reactive secondary minerals (e.g., Fe hydrous oxides, short-range ordered Al hydroxides). Such relationships have been found across a wide range of soil types (Kramer et al., 2012; Kleber et al., 2015). Also, higher radiocarbon age of SOM in heavy (i.e., mineral) fractions compared to light density (i.e. organic) fractions indicates that sorption stabilizes organic compounds (e.g., Marschner et al., 2008, Kleber et al., 2015). Density fractionation procedures indicate that the total soil C

associated with minerals in any given location can vary from 30% to 90% (see data compiled in Kleber et al., 2015). The relevance of sorptive stabilization depends on soil properties. Low soil pH enhances the formation of reactive secondary minerals and favors the formation of strong bonds between organic matter and the mineral surface (Kleber et al., 2015). Most studies cited herein (for both lines of evidence) examined acidic soils under temperate forests, in which sorptive stabilization clearly should play an important role.

2.2 Argument 2: Preferential stabilization of aromatic DOM components

Lignin, a macromolecule composed of phenyl propane units, is a major plant cell wall component (Kögel-Knabner, 2002). Typically, lignin concentrations negatively correlate with litter decomposition rates. They are the predominant control on litter decomposition within biomes worldwide (Cornwell et al., 2008), indicating that the lignin macromolecule is among the most persistent litter constituents. Nevertheless, results of recent studies suggest significant chemical alteration and losses of lignin already within the first months of litter decomposition (e.g., Preston et al., 2009; Klotzbücher et al., 2011). 'Degradation' of lignin has to be considered a step-wise process: (i) the first step is the depolymerization of the macromolecule, releasing (mainly aromatic) water-soluble depolymerisation products of varying molecular weight; (ii) these products can then be further transformed, and low-molecular weight compounds are eventually taken up by microorganisms to produce biomass or CO_2. Hence, losses of lignin-derived C during litter decomposition can occur due to leaching of water-soluble products of an incomplete degradation or as CO_2. Laboratory incubation tests on water-extractable organic matter from various forest floor materials suggest that aromatic components are more resistant to mineralization than non-aromatic components (Kalbitz et al., 2003 a,b; Hansson et al., 2010). This suggests that leaching is an important factor in loss of lignin-derived matter during litter decomposition. Consistent with this conceptual model, the typically high UV absorptivity of DOM leached from forest floors is indicative of a large contribution of aromatic components (e.g., Kalbitz and Kaiser, 2008).

Another factor for the export of aromatic DOM from forest floors is leaching of tannins. Tannins are water-soluble polyphenols of a molecular weight ranging from 500 to 3000 Daltons. Tannins rapidly leach from fresh litter; most studies suggest losses of ~80% within the first year of litter decomposition (Kraus et al., 2003).

It has been commonly found that the contribution of components likely derived from lignin and tannins to DOM decreases with depth of the mineral soil (summarized in Table 1), i.e., the decrease in fluxes of these compounds with depth is more pronounced than the decrease of bulk DOM. One explanation might be intensive biodegradation of aromatics in mineral soil. However, this would contradict results of the DOM biodegradation studies previously discussed. Hence, a more likely explanation is sorption to mineral surfaces. Laboratory sorption experiments support this view; a typical observation is that lignin-derived aromatic DOM components are preferentially sorbed by minerals and soils (e.g., Kaiser et al., 1996; Chorover and Amistadi, 2001; Hunt et al., 2007), and for some soils it has been shown they displace previously bound organic components from mineral surfaces (e.g., Kaiser et al., 1996). The degree of preferential sorption may depend on the composition of the soil mineral assemblage. Chorover and Amistadi (2001) observed that high molecular weight aromatic components preferentially sorbed onto goethite, while for montmorillonite no preference for aromatic moieties was observed. A likely reason for the preferential sorption is the large content of carboxyl groups linked to the aromatic rings, which bind to metals at mineral surfaces via ligand exchange reactions.

2.3. Roots as source of stabilized aromatic SOM?

We have so far focused on DOM leached from aboveground litter. However, roots might also be a crucial source of stable SOM. The contribution of root and aboveground litter as major source of SOM has been debated in numerous studies, but the available information allows no definite conclusions yet (see discussion in Lajtha et al., 2014). Presumably, the relative importance of the two types of organic matter input for SOM storage in topsoils differs between ecosystems (Crow et al., 2009) and the importance of root-derived matter increases with soil profile depth (Rumpel et al., 2015).

This raises issue of whether results from aboveground litter decomposition would also apply to root litter decomposition. Data by Crow et al. (2009) suggest that lignin concentrations of roots are in the range of those of leaf and needle litter. Hansson et al. (2010) showed that DOM production during root decomposition occurs in patterns that are similar to those of needle decomposition. Particularly during later decomposition stages, root-derived DOM is enriched in aromatics resistant to mineralization. Hence, available information suggests that root decomposition is just another important source of soluble aromatics in mineral soils.

However, to the best of our knowledge, studies to quantify the contribution of root-derived aromatics to DOM fluxes in the field have yet to be conducted.

3 Solid phase line of evidence

Many of the recent conceptual papers on SOM formation are built on the assumption that lignin-derived aromatics disappear quickly in soil, while SOM in mineral soils is dominated by non-aromatic and microbial-derived compounds (Grandy and Neff, 2008; Schmidt et al., 2011; Dungait et al., 2012; Miltner et al., 2012; Cotrufo et al., 2013; Castellano et al., 2015). Empirical support is provided by studies characterizing the chemical structure of solid SOM using a variety of analytical methods. In the following, we sum up the most widely cited work.

Numerous studies on a wide variety of soil types used the cupric oxide (CuO) method to analyse the distribution of lignin-derived phenols in profiles. Most of them reported decreasing phenol contribution to SOM from forest floor to A horizons and with depth of the mineral soil (reviewed in Thevenot et al., 2010). Measured phenols also decreases with increasing density of soil fractions, hence, are relatively small component in heavy (i.e., mineral-associated) and old soil fractions (e.g., Sollins et al., 2009; Kögel-Knabner et al., 2008; Cerli et al., 2012). Similar results are reported by studies using pyrolysis-gas chromatography/mass spectrometry (see e.g., data and references provided by Grandy and Neff, 2008 and by Buurman et al., 2007) and tetramethylammonium hydroxide (TMAH) thermochemolysis (Mason et al., 2012).

Analysis of heavy and clay-sized soil fractions using cross polarization and magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance spectroscopy (^{13}C-NMR) typically finds high peak intensities of alkyl and O/N alkyl C (mostly assigned to polysaccharides and proteins) and low peak intensities of aryl C (mostly assigned to lignin and tannins) (see reviews by Kögel-Knabner et al., 2008 and Miltner et al., 2012). In a comprehensive study on Ah horizons from 8 European forest sites, O/N alkyl C contributed up to 41-49% of total peak intensity in the <2-µm fraction, and the peak intensities were on average 10% higher than the those reported for bulk soil; the intensities of aryl C in the <2-µm fraction contributed 13-15% of total peak intensities, and they were on average 24% lower than values found for bulk soil (Schöning et al., 2005). Consistent with findings, the heavy soil fraction is characterized by low C/N values close to those of microbial tissues (Kögel-Knabner et al., 2008). Studies using

near-edge X-ray absorption fine structure (NEXAFS) spectra supported the conclusions drawn from ^{13}C-NMR analysis of a significant contribution of microbial-derived compounds to SOM at mineral surfaces (Lehmann et al., 2007; Kleber et al., 2011).

Lignin turnover (i.e., transformation into CO_2 or non-lignin products) in temperate arable, grassland, and tropical forest soils has been estimated using a combination of isotopic labeling and compound-specific isotope analysis of lignin-derived aromatics applying the CuO method. Most of the studies using this approach suggest that the turnover of lignin-derived aromatics is faster than the turnover of bulk SOM (Dignac et al., 2005; Heim and Schmidt, 2007; Heim et al., 2010). A modelling study based on the data by Dignac et al. (2005) suggested that about 90% of the lignin is mineralized as CO_2 or transformed into compounds devoid of lignin-type signatures within one year (Rasse et al., 2006). However, a study by Hofmann et al. (2009) suggests that after 18 years, approximately two-thirds of the initial lignin phenols remained in an arable soil. The authors concluded that lignin was preferentially preserved in the soil. It should be noted all of these land-use successional studies only determined turnover times in the top 10-30cm of the soil horizon and may not accurately represent lignin dynamics in the deeper soil.

4 Reasons for the controversies

4.1. Analytical limitations

The controversies in current literature might (partly) be due to difficulties in the analyses of aromatic matter in soils. Studies on DOM typically use bulk methods for inferring aromatic content, including UV absorbance and fluorescence spectroscopy. Limitations of this research include lack of identification of the source of aromatic compounds, and poor quantification of the fluxes. Also data on contribution of aromatic components to solid SOM are semi-quantitative or qualitative.

Commonly applied methods such as CuO oxidation, pyrolysis or TMAH thermochemolysis focus on few defined lignin-derived monomers to estimate the overall contribution of lignin. These estimates, however, can largely differ depending on the method applied (Klotzbücher et al., 2011). As outlined by Amelung et al. (2008), compound-specific isotope analysis of lignin-derived compounds with the CuO method presumably overestimates the turnover rates

of lignin as only part of the lignin-derived aromatics can be extracted from soil (incomplete extraction might also be a problem in all analyses of biomarkers, for which turnover times typically are estimated to be faster than turnover rates of bulk SOM). Firstly, CuO oxidation (as well as conventional pyrolysis or TMAH thermochemolysis) does not completely depolymerize lignin (Johansson et al., 1986; Goňi and Hedges, 1992; Filley et al., 2000). Secondly, lignin-derived aromatics bound to mineral surfaces are only partly assessed by the CuO method (Hernes et al., 2013). Thirdly, lignin-derived aromatics might be altered in a way that they escape the `analytical window` and cannot be ascribed to a lignin source anymore. For instance, the CuO method yields a number of aromatic monomers of unknown origin besides the lignin-derived monomers (Cerli et al., 2008). These compounds are typically not quantified, and thus, not considered in estimates of the SOM composition. Hence, monomer yield is a commonly used but uncertain measure of lignin concentration in soil. Our knowledge about how much lignin is `hidden` (Hernes et al., 2013) in soil is still insufficient.

The uncertainties related to quantification of SOM compositions with solid-state CPMAS ^{13}C-NMR have been intensively discussed in the literature (see Knicker, 2011). Mineral soil samples are commonly pretreated with hydrofluoric acid in order to remove paramagnetic minerals that disturb the analysis. The treatment can result in significant losses of SOM, and one might lose important information on SOM adsorbed onto minerals (e.g., SOM losses of 10-30% in topsoil samples and up to 90% in subsoil; Eusterhues et al., 2003). Further uncertainties arise from signal overlapping and a low sensitivity for aromatic C in soils (e.g., Skjemstad et al., 1996; Mao et al., 2000; Simpson and Simpson, 2012). It has been shown that the technique underestimates lignin vs. cellulose in ligno-cellulose isolated from wheat (Gauthier et al., 2002). By applying Bloch decay, another type of ^{13}C-NMR technique, one can overcome the problem of the reduced sensitivity for aromatic-C. The technique has been applied in studies on pyrogenic organic matter, for which CPMAS ^{13}C-NMR should be even less sensitive than for lignin as it is more condensed (Golchin et al., 1997; Simpson and Hatcher, 2004; Knicker et al., 2005). Bloch decay is, however, not routinely applied in SOM research as it is an extremely time-consuming experiment and the required instrument time is frequently not available (Simpson and Hatcher, 2004).

Pyrogenic organic matter is an important source of aromatic compounds in many soils. Despite extensive research efforts, rates and pathways of pyrogenic organic matter decomposition are still not well established (Schmidt et al., 2011; Kuzyakov et al., 2014).

Analyses of benzenecarboxylic acids as molecular markers suggest that aromatic compounds derived from pyrogenic organic matter are translocated within soil profiles and bind to mineral surfaces (Haumaier, 2010). The quantitative contribution of pyrogenic organic matter to DOM in soil is, however, still poorly studied (Smebye et al., 2016). Bulk analyses of aromatic matter used in most research on DOM fluxes cannot distinguish if the compounds derive from plant litter or from pyrogenic organic matter. This limits our understanding of the processes controlling turnover of aromatics. If a significant part of aromatic DOM in mineral soil derives from pyrogenic organic matter, the `loss` of plant litter-derived aromatics in mineral soil would be even more pronounced. Problems distinguishing sources of aromatic compounds can also occur in analysis of solid-phase SOM with CPMAS ^{13}C-NMR analysis (Simpson and Hatcher, 2004).

4.2. Limits in process understanding

Another source of uncertainty in quantifying the role of aromatics in SOM stabilization is that the timescales of the different processes affecting aromatic compounds vary considerably. Solubilization, leaching, and sorption of lignin-derived compounds might occur in a few days. In contrast, the composition of SOM as well as the mineral assemblage in soils is the result of years to centuries of biogeochemical processing. Possibly, one cannot simply extrapolate from patterns observed in ’short-term‘DOM dynamics to explain ’long-term‘SOM formation.

For instance, in their comprehensive review on organo-mineral interactions, Kleber et al. (2015) question the view that strong bonds between organic matter and mineral surfaces really guarantee slow turnover rates and hence long turnover times. Many factors that potentially determine the long-term fate of sorbed organic matter are still not well understood. They include exchange reactions between sorbed organic matter and new organic matter inputs or the impact of mineral weathering activity of roots on stability of sorbed organic matter. As proposed in Kaiser and Kalbitz (2012), the commonly observed increasing ^{14}C age of DOM with profile depth might be explained by temporal sorptive immobilization, followed by microbial processing and re-release of altered compounds into soil solution. Keiluweit et al. (2015) showed that root exudation of oxalic acid promotes the release of SOM sorbed onto mineral surfaces into soil solution through dissolution of mineral phases. Taken together, the view that DOM leached from litter decomposition sorbs onto mineral surfaces and then contributes to stable SOM might be too simple. Processing of sorbed material may exert a

significant effect on SOM dynamics. This possibly prevents the long-term storage of plant-derived aromatics in soil and explains the controversies outlined herein.

Besides oxalic acid, many other organic compounds are released from living roots (the so-called `rhizodeposition`). Estimates suggest that rhizo-deposition constitutes a loss term of 17% (on average) of the net C assimilated by plants (Nguyen, 2003). It comprises low-molecular weight compounds (organic acids, simple sugars, amino sugars, phenolics) as well as high-molecular compounds (exoenzymes, root cells) (Wichern et al., 2008). Most of the compounds are degraded quickly, but a smaller portion might contribute to stable SOM (Nguyen, 2003; Pausch et al., 2013). The rhizosphere is considered a `hot-spot` in soil, where microbial processes are accelerated as they are not C-limited (Kuzyakov and Blagodatskaya, 2015). Possibly, root activity not only fosters production, but also stabilization of microbial-derived compounds in soil. To date, root activity effects on DOM dynamics and stabilization of plant-derived aromatics at mineral surfaces are poorly studied. This is partly because traditional analyses are focused on mixed soil samples, while the rhizosphere may only comprise a small volume of bulk soil, and fluxes of DOM are averaged for a soil surface (Kuzyakov and Blagodatskaya, 2015).

Recent investigation at the submicrometer scale using Nano Secondary Ion Mass Spectrometry (NanoSIMS) or NEXAFS in combination with scanning transmission X-ray microscopy suggest that SOM associated with clay-sized minerals exists in small patches of varying chemical composition (Lehmann et al., 2008; Remusat et al., 2012; Vogel et al., 2014). Distinct patches of predominantly aromatic C can be differentiated from patches dominated by aliphatic C (Lehmann et al., 2008). Knowledge about processes controlling the submicrometer-scale distribution of SOM on mineral surfaces is still limited. Some of the patches are cell wall structures of microorganisms, which may contribute to stable SOM as they are composed of insoluble polymers and possibly sorb to the mineral surface (Miltner et al., 2012). Hence, stable sorbed organic matter might not only be derived from low-molecular weight compounds. On the other hand, it needs to be considered that microbial-derived compounds are continuously synthesized at the mineral surface. The microorganisms might use some of the older C (^{14}C age) for synthesis of relatively labile compounds. The age of the C atoms is, thus, decoupled from the stability of the organic matter, and microbial-derived compounds may `mimic` a similar or even higher stability than the plant-derived compounds

(Gleixner, 2013). Hence, concentrations or ^{14}C age of microbial-derived compounds at mineral surfaces do not per se allow for conclusions on their contribution to stable SOM.

5 Implications and future research strategies

The contradictions outlined herein limit our basic understanding on SOM formation, and our ability to model and manage effects of global change on SOM stocks.

For instance, elevated atmospheric CO_2 levels can induce increasing concentrations of aromatic components in plant litter (Cotrufo et al., 1994; Tuchman et al., 2002), and this raises the question whether this causes enhanced or decreasing storage of SOM in mineral soils. If aromatic matter is quickly degraded, and mineral-associated SOM primarily derives from microbial sources (as suggested by the solid SOM line of evidence), a `microbial filter` would control the built-up of stable SOM, which may then be determined by the microbial substrate use efficiency (i.e., the amount of organic C used by the microbial community to build biomass vs. the amount that is mineralized). As such, Cotrufo et al. (2013) hypothesized that input of labile substrates fosters the build-up of stable SOM. Available data on effects of litter quality and SOM formation are, however, inconsistent (Castellano et al., 2015), and we believe that understanding on these effects is in part limited by uncertainties about the incorporation of aromatics into stable SOM.

The issue is also related to the question of links between chemical structure of organic matter and its persistence. It is oftentimes assumed that structural properties of plant-derived matter do not determine stable SOM formation. This argument is based on data suggesting that specific compound classes (lignin, cellulose, alkanes, proteins etc.) turn over faster than bulk SOM (Schmidt et al., 2011). However, conclusions of DOM research imply that structure plays a role for the behavior of organic compounds in soil, and eventually their contribution to stable SOM: soluble aromatics may resist oxidation by microbes as they yield less energy than other structures; furthermore, they bind to mineral surfaces due to carboxyl groups attached to the rings.

How could we resolve the controversies? Based on our literature analysis we propose the following research strategies:

- More studies addressing links between microbial processes, composition/fluxes of DOM, and composition of solid-phase SOM are needed. The study by Kramer et al.

(2012) is a first attempt to relate the fluxes of aromatics to solid-phase SOM properties. In particular, the presumed microbial processing of sorbed material, causing desorption and subsequent mineralization or further transport in the soil profile is poorly studied. Eventually, these processes might cause loss of aromatic compounds. A related question is how root activity affects de-/sorption processes?

- Computer simulations could help to unravel the complex interrelationships between DOM fluxes and solid-phase SOM composition. Recently developed models integrate sorption, DOM transport, and microbial processes (Ahrens et al., 2015). In order to address the problems discussed herein, effects of molecular structure on behavior of the compounds in soil (e.g., differences in mineralization rate and affinity for sorption between aromatics and non-aromatics) could be implemented in the models, in order to develop novel hypotheses on turnover of plant-derived aromatics.
- Our knowledge is limited by the constraints in analysis of aromatics in soil. Quantification of total amounts and source of aromatics in soil are still problematic. Even if the problems cannot be fully solved with the currently available techniques, there might be strategies to obtain improved estimates. The work of Hernes et al. (2013) provides a first hint about how much lignin might be not accessible to CuO oxidation analyses. The authors evaluated the extraction efficiency for lignin-derived aromatics bound to different minerals. The size of the non-extractable fraction depended strongly on the mineral. Almost all of the aromatics bound to ferrihydrite were extractable, but for kaolinite the non-extractable fraction made >40%. But how about extractability in soil under field conditions? Possibly, a combination of tracking of C isotopes, DOM flux/ composition assessment, and analysis of solid-phase SOM composition could provide better estimates on the hidden aromatics in soil.

Acknowledgements

TK acknowledges the support of his research on lignin degradation from the German Research Foundation (DFG).

References

Ahrens, B., Braakhekke, M. C., Guggenberger, G., Schrumpf, M. and Reichstein, M.: Contribution of sorption, DOC transport and microbial interactions to the ^{14}C age of a soil organic carbon profile: Insights from a calibrated process model. Soil Biol. Biochem., 88, 390-402, 2015.

Amelung W., Brodowski, S., Sandhage-Hofmann, A. and Bo R.: Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Adv. Agron., 100, 155-250, 2008.

Buurman, P., Peterse, F. and Almendros Martin, G.: Soil organic matter chemistry in allophonic soils: a pyrolysis-GC/MS study of a Costa Rican Andosol catena. Eur. J. Soil Sci., 58, 1330-1347, 2007.

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E. and Six, J.: Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Glob. Change Biol., 21, 3200-3209, 2015.

Cerli, C., Celi, L., Kaiser, K., Guggenberger, G., Johansson, M.-B., Cignetti, A. and Zanini E.: Changes in humic substances along an age sequence of Norway spriuce stands planted on former agricultural land. Org. Geochem., 39, 1269-1280, 2008.

Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G. and Kaiser, K.: Separation of light and heavy fractions in soil – testing for proper density cut-off and dispersion level. Geoderma, 170, 403-416, 2012.

Chorover, J. and Amistadi, M.K.: Reaction of forest floor organic matter at goethite, birnessite and smectite surfaces. Geochim Cosmochim Acta, 65, 95-109, 2001.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Prerez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., van Bodegorn, P., Brovkin, V., Chatain, A., Callaghan, T.V., Diaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskala, N.A., Vaieretti, M.A. and Westoby, M.: Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett., 11, 1065-1071, 2008.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K. and Paul, E.: The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol., 19, 988-995, 2013.

Cotrufo, M.F., Ineson, P. and Rowland, A.P.: Decomposition of tree leaf litters grown under elevated CO_2: Effect of litter quality. Plant Soil, 163, 121-130, 1994.

Cronan, C.S.: Comparative effects of precipitation acidity on three forest soils: carbon cycling responses. Plant Soil, 88, 101-112, 1985.

Crow, S.E., Lajtha, K., Filley, T.R., Swanston, C.W., Bowden, R.D. and Caldwell, B.A.: Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Glob. Change Biol., 15, 2003-2019, 2009.

Dai, K.H., David, M.B. and Vance, G.F.: Characterization of solid and dissolved carbon in a spruce-fir Spodosol. Biogeochemistry, 35, 339-365, 1996.

Dignac, M.F., Bahri, H., Rumpel, C., Rasse, D.P., Bardoux, G., Balesdent, J., Girardin, C., Chenu, C., Mariotti, A.: Carbon-13 natural abundance as a tool to study the dynamics of lignin monomers in soil: an appraisal at the Closeaux experimental field (France). Geoderma, 128, 3-17, 2005.

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S. and Whitmore, A.P.: Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Change Biol., 18, 1761-1796, 2012.

Eusterhues, K., Rumpel, C., Kleber, M. and Kögel-Knabner, I.: Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Org. Geochem., 34, 1591-1600, 2003.

Filley, T., Hatcher, P.G., Shortle, W.C. and Praseuth, R.T.: The application of ^{13}C-labeled tetramethylammonium hydroxide (^{13}C-TMAH) thermochemolysis to the study of fungal degradation of wood. Org. Geochem., 31, 181-198, 2000.

Gallet, C. and Pellissier, F.: Phenolic compounds in natural solutions of a coniferous forest. J. Chem. Ecol., 23, 2401-2412, 1997.

Gauthier, A., Derenne, S., Dupont, L., Guilon, E., Largeau, C., Dumonceau, J. and Aplincourt, M.: Characterization and comparison of two ligno-cellulose substrates by ^{13}C

CP/MAS NMR, XPS, conventional pyrolysis and thermochemolysis. Anal. Bioanal. Chem., 373, 830-838, 2002.

Gleixner, G.: Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. Ecol. Res., 28, 683-695, 2013.

Golchin, A., Clarke, P., Baldock, J.A., Higashi, T., Skjemstad, J.O. and Oades, J.M.: The effect of vegetation and burning on chemical composition of soil organic matter in a volcanic ash soil shown by ^{13}C NMR spectroscopy. I. Whole soil and humic fraction. Geoderma, 76, 155-174, 1997.

Goňi, M.A. and Hedges, J.I.: Lignin dimmers: Structure, distribution, and potential geochemical applications. Geochim. Cosmochim. Acta, 54, 4025-4043, 1992.

Grandy, A.S. and Neff, J.C.: Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci. Tot. Environ., 404, 297-307, 2008.

Hansson, K., Kleja, D.B., Kalbitz, K. and Larsson, H.: Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots. Soil Biol. Biochem., 42, 178-185, 2010.

Hassouna, M., Massiani, C., Dudal, Y., Pech, N. and Theraulaz, F.: Changes in water extractable organic matter (WEOM) in a calcareous soil under field conditions with time and soil depth. Geoderma, 155, 75-85, 2010.

Haumaier L.: Benzene polycarboxylic acids – A ubiquitous class of compounds in soil. J. Plant Nutr. Soil Sci., 173, 727-736, 2010.

Heim, A. and Schmidt, M.W.I.: Lignin turnover in arable soil and grassland analysed with two different labelling approaches. Eur. J. Soil Sci., 58, 599-608, 2007.

Heim, A., Hofmann, A. and Schmidt, M.W.I.: Forest-derived lignin biomarkers in an Australian oxisol decrease substantially after 90 years of pasture. Org. Geochem., 41, 1219–1224, 2010.

Hernes, P.J., Kaiser, K., Dyda, R.Y. and Cerli, C.: Molecular trickery in soil organic matter: hidden lignin. Environ. Sci. Technol., 47, 9077-9085, 2013.

Hofmann, A., Heim, A., Christensen, B.T., Miltner, A., Gehre, M. and Schmidt, M.W.I.: Lignin dynamics in two ^{13}C-labelled arable soils during 18 years. Eur. J. Soil Sci., 60, 250-257, 2009.

Hunt, J.F., Ohno, T., He, Z., Honeycutt, W. and Dail, D.B.: Inhibition of phosphorus sorption to goethite, gibbsite, and kaolin by fresh and decomposed organic matter. Biol. Fertil. Soils, 44, 277-288, 2007.

Johansson, M.-B., Kögel, I. and Zech, W.: Changes in the lignin fraction of spruce and pine needle litter during decomposition as studied by some chemical methods. Soil Biol. Biochem., 18, 611-619, 1986.

Kaiser, K., Guggenberger, G. and Haumaier, L.: Changes in lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. Biogeochemistry, 70, 135-151, 2004.

Kaiser K., Guggenberger, G. and Zech, W.: Sorption of DOM and DOM fractions to forest soils. Geoderma, 74, 281-303, 1996.

Kaiser, K. and Kalbitz, K.: Cycling downwards – dissolved organic matter in soils. Soil Biol. Biochem., 52, 29-32, 2012.

Kalbitz, K. and Kaiser, K.: Contribution of dissolved organic matter to carbon storage in forest mineral soils. J. Plant Nutr. Soil Sci., 171, 52-60, 2008.

Kalbitz, K., Schmerwitz, J., Schwesig, D. and Matzner, E.: Biodegradation of soil-derived dissolved organic matter as related to its properties. Soil Biol Biochem, 35, 1129-1142, 2003a.

Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumeier, L., Glaser, B., Ellerbrock, R. and Leinweber, P.: Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biol. Biochem., 35, 1129-1142, 2003b.

Keiluweit, M., Bougoure, J.J., Nice, P.S., Pett-Ridge, J., Weber, P.K. and Kleber, M. Mineral protection of soil carbon counteracted by root exudates. Nature Clim. Change, 5, 588-595, 2015.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R. and Nico, P.S.: Mineral-Organic Associations: Formation, Properties, and Relevance in Soil Environments. Adv. Agron., 130, 1-140, 2015

Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swansten, C. and Sollins, P.: Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Glob. Change Biol., 17, 1097-1107, 2011.

Klotzbücher, T., Filley, T.R., Kaiser, K. and Kalbitz, K.: A study of lignin degradation in leaf and needle litter using ^{13}C-labelled tetramethylammonium hydroxide (TMAH) thermochemolysis: Comparison with CuO oxidation and van Soest methods. Org. Geochem., 42, 1271-1278, 2011.

Knicker, H.: Solid state CPMAS ^{13}C and ^{15}N NMR spectroscopy in organic geochemistry and how spin dynamics can either aggravate or improve spectra interpretation. Org. Geochem., 42, 867-890, 2011.

Knicker, H., Totsche, K.U., Almendros, G. and Gonzalez-Vila, F.J.: Condensation of burnt peat and plant residues and the reliability of solid-state VACP MAS ^{13}C NMR spectra obtained from pyrogenic humic material. Org. Geochem., 36, 1359-1377, 2005.

Kögel-Knabner, I., Guggenberger, G. and Kleber, M.: Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. J. Plant Nutr. Soil Sci., 171, 61-82, 2008.

Kögel-Knabner I.: The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem., 34, 139-162, 2002.

Kramer, M.G., Sanderman, J., Chadwick, O.A., Chorover, J. and Vitousek, P.M.: Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. Glob. Change Biol., 18, 2594-2605, 2012.

Kraus, T.E.C., Dahlgren, R.A. and Zasoski, R.J.: Tannins in nutrient dynamics of forest ecosystems – a review. Plant Soil, 256, 41-66, 2003.

Kuzyakov, Y. and Blagdodatskaya, E.: Microbial hotspots and hot moments in soil: Concenpts and review. Soil Biol. Biochem., 83, 184-199, 2015.

Kuzyakov, Y., Bogomolova, I. and Glaser, B.: Biochar stability in soil: Decomposition during eigth years and transformation as assessed by compound-specific ^{14}C analysis. Soil Biol. Biochem., 70, 229-236, 2014.

Lajtha, K., Crow, S.E., Yano, Y., Kaushal, S.S., Sulzman, E., Sollins, P. and Spears, J.D.H.: Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry, 76, 261-281, 2005.

Lajtha, K., Bowden, R.D. and Nadelhoffer, K.: Litter and root manipulations provide insights into soil organic matter dynamics and stability. Soil Sci. Soc. Am. J., 78, 251-269, 2014.

Lehmann, J., Kinyangi, J. and Solomon, D.: Organic matter stabilization in soil microaggegates: implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry, 85, 45-57, 2007.

Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S. and Jacobsen, C.: Spatial complexity of soil organic matter forms at nanometre scales. Nature Geoscience, 1, 238-242, 2008.

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L. and Wiesenberg, G.: How relevant is recalcitrance for the stabilization of organic matter in soils? J. Plant Nutr. Soil Sci., 171, 81-110, 2008.

Mao, J.D., Hu, W.G., Schmidt-Rohr, K., Davies, G., Ghabbour, E.A. and Xing, B.: Quantitative characterization of humic substances by solid-state carbon-13 nuclear magnetic resonance. Soil Sci. Soc. Am. J., 64, 873-884, 2000.

Mason, S.L., Filley, T.R. and Abbott, G.D.: A comparative study of the molecular composition a grassland soil with adjacent unforested and afforested moorland ecosystem. Org. Geochem., 42, 1519-1528, 2012.

Michalzik, B., Tipping, E., Mulder, J., Gallardo Lancho, J.F., Matzner, E., Bryant, C.L., Clarke, N., Lofts, S. and Vicente Esteban, M.A.: Modelling the production and transport of dissolved organic carbon in forest soils. Biogeochemistry, 66, 241-264, 2003.

Miltner, A., Bonbach, P., Schmidt-Brücken, B. and Kästner, M.: SOM genesis: microbial biomass as a significant source. Biogeochemistry, 111, 41-55, 2012.

Nakashini, T., Atarashi-Andoh, M., Koarashi, J., Saito-Kokubu, Y. and Hirai, K.: Carbon isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic relationship with soil carbon. Eur. J. Soil Sci., 63, 495-500, 2012.

Neff, J.C. and Asner, G.P.: Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. Ecosystems, 4, 29-48, 2001.

Nguyen, C.: Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie, 23, 375-396, 2003 .

Pausch, J., Tian, J., Riederer, M. and Kuzyakov, Y.: Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. Plant Soil, 364, 273-285, 2013.

Preston, C.M., Nault, J.R. and Trofymow, J.A.: Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. ^{13}C abundance, solid-state ^{13}C NMR spectroscopy and the meaning of "lignin". Ecosystems, 12, 1078-1102, 2009.

Qualls, R.G. and Haines, B.L.: Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. Soil Sci. Soc. Am. J., 55, 1112-1123, 1991.

Rasse, D.P., Dignac, M.-F., Bahri, H., Rumpel, C., Mariotti, A. and Chenu, C.: Lignin turnover in a agricultural field: from plant residues to soil-protected fractions. Eur. J. Soil Sci., 57, 530-538, 2006.

Remusat, L., Hatton, P.-J., Nico, P.S., Zeller, B., Kleber, M. and Derrien, D.: NanoSIMS study of organic matter associated with soil aggregates: advantages, limitations, and combination with STXM. Environ. Sci. Technol., 46, 3943-3949, 2012.

Rumpel, C., Baumann, K., Remusat, L., Dignac, M.-F., Barre, P., Deidicque, D., Glasser, G., Lieberwarth, I. and Chabbi, A.: Nanoscale evidence of contrasted processes for root-derived organic matter stabilization by mineral interactions depending on soil depth. Soil Biol. Biochem., 85, 82-88, 2015.

Sanderman, J., Baldock, J.A. and Amundson, R.: Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry, 89, 181-198, 2008.

Schlesinger, W.H. and Andrews, J.A.: Soil respiration and the global carbon cycle. Biogeochemistry, 48, 7-20, 2000.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D., Weiner, S. and Trumbore, S.E.: Persistance of soil organic matter as an ecosystem property. Nature, 478, 49-56, 2011.

Schöning, I., Morgenroth, G. and Kögel-Knabner, I.: O/N-alkyl and alkyl C are stabilized in fine particle size fractions of forest soils. Biogeochemistry, 73, 475-497, 2005.

Simpson, M.J. and Simpson, A.J.: The chemical ecology of soil organic matter molecular constituents. J. Chem. Ecol., 38, 768-784, 2012.

Simpson, M.J. and Hatcher, P.G.: Overestimates of black carbon in soils and sediments. Naturwissenschaften, 91, 436-440, 2004.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M. and McClure, S.G.: The chemistry and nature of protected carbon in soil. Aust. J. Soil Res., 34, 251-271, 1996.

Smebye, A., Alling, V., Vogt, R.D., Gadmar, T.C., Mulder, J., Cornelissen, G. and Hale, S.E.: Biochar amendment to soil changes dissolved organic matter content and composition. Chemosphere, 142, 100-105, 2016.

Sollins, P., Kramer, M.G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A.K., Wagai, R. and Bowden, R.D.: Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry, 96, 209-231, 2009.

Thevenot, M., Dignac, M.-F. and Rumpel, C.: Fate of lignins in soils: a review. Soil Biol. Biochem., 42, 1200-1211, 2010.

Tuchman, N.C., Wetzel, R.G., Rier, S.T., Wahtera, K.A. and Teeri, J.A.: Elevated atmospheric CO_2 lowers leaf nutritional quality for stream ecosystem food webs. Glob. Change Biol., 8, 163-170, 2002.

Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M. and Kögel-Knabner, I.: Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nature Commun., doi:10.1038/ncomms3947, 2014.

Wichern, F., Eberhardt, E., Mayer, J., Joergensen, R.G. and Müller, T.: Nitrogen rhizodeposition in agricultural crops. Soil Biol. Biochem., 40, 30-48, 2008.

Zech, W., Guggenberger, G., Schulten, H.-R.: Budgets and chemistry of dissolved organic carbon in forest soils: effects of anthropogenic soil acidification. Sci. Tot. Environ., 152, 49-62, 1994.

1

2 Table 1. Evidence from field studies suggesting that dissolved aromatics (products of lignin
3 depolymerization or tannins) disappear quickly once entering mineral soils.

4

Reference	Study site/ soil type	Result
Qualls and Haines 1991	Oak-hickory forest in mountain region of North Carolina; soil types: Umbric Dystrochrept, Typic Hapludult, Typic Dystrochrept	Selective removal of hydrophobic acids as DOM percolates through the mineral soil.
Cronan 1985	Forests, North-Western USA; soil types: Dystrochrept, Haplorthod	Selective removal of hydrophobic acids as DOM percolates through the mineral soil.
Zech et al. 1994	Spruce forest in Bavaria, Germany; Soil types: Typic Dystrochrepts, Entic Haplorthods, Typic Haplorthods	Selective removal of lignin-derived phenols (determined with the CuO method) as DOM percolates through the mineral soil.
Gallet and Pellissier 1997	Bilberry-spruce forest in Alps, France; soil type: Humoferric Podzol	Selective removal of lignin-derived phenols (as well as of total phenols) as DOM percolates through the mineral soil.
Kaiser et al. 2004	Spruce forest in Bavaria, Germany; Soil type: Haplic Arenosol	Selective removal of lignin-derived phenols, hydrophobic compounds, and total aromatic C as DOM percolates through the mineral soil.
Dai et al. 1996	Spruce forest, Maine; Soil type: Aquic Haplothods	Selective removal of hydrophobic acids and aromatics (^{13}C-NMR data) as DOM percolates through the mineral soil.
Lajtha et al. 2005	Coniferous forest, Oregon, USA; soil type: Typic Hapludands	Selective removal of hydrophobic acids as DOM percolates through the mineral soil.
Sanderman et al. 2008	Mediterranean climate; forest and grassland soils; soil types: Haplustols and Haplohumults	Decrease in UV absorbance (a measure for content of aromatics) as DOM percolates through the mineral soil.
Hassouna et al. 2010	Mediterranean climate; maize field; soil type: fluvic hypercalcaric cambisol	Decrease in contents of aromatic compounds (UV absorbance, fluorescence specroscopy) in water-extractable organic matter with depth of the mineral soil.
Nakashini et al. 2012	Beech forest, Japan; soil: “brown forest soil”	Decrease in contents of hydrophobic acids in water-extractable organic matter with depth of the mineral soil.
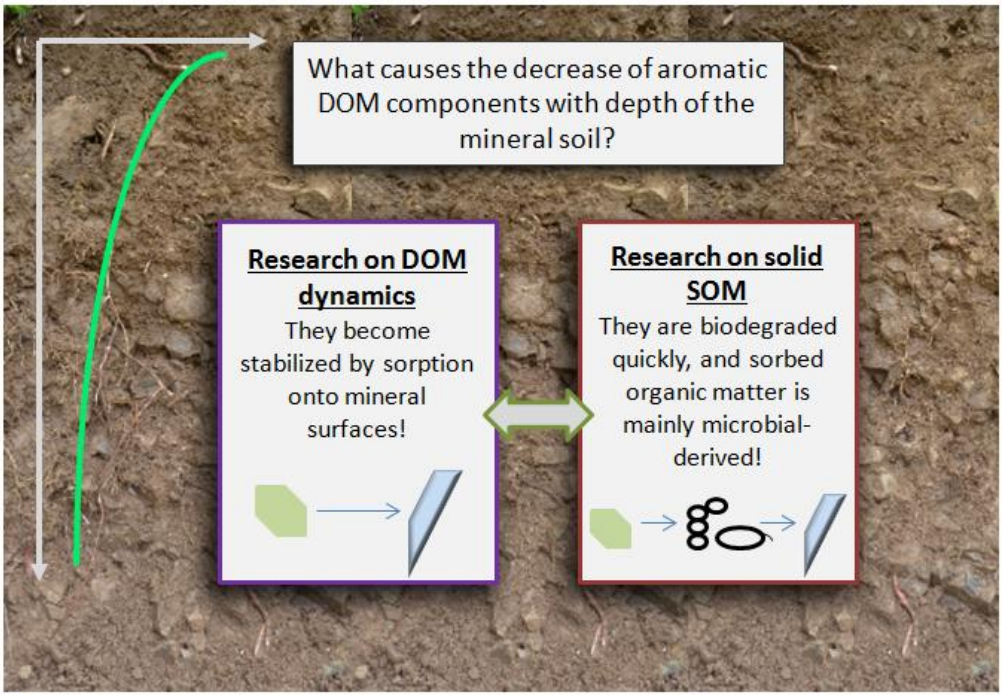
Figure 1. Conflicting views on the fate of soluble aromatics once they enter the mineral soil (see text for references).