

1 **Gone or just out of sight? The apparent disappearance of** 2 **aromatic litter components in soils**

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14 15 **Abstract**

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

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

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

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

8

9 **1 Introduction**

10 Storage and quality of soil organic matter (SOM) determine many crucial soil properties and
11 the cycling of carbon (C) and essential nutrients through ecosystems. The storage of SOM is
12 determined by plant litter inputs and decomposition processes. Decomposition of SOM is a
13 significant source of atmospheric CO₂, thus, a critical parameter in climate models
14 (Schlesinger and Andrews, 2000). Decomposition rates are sensitive to global change factors
15 such as temperature, precipitation, and land use. However, our ability to understand and
16 predict such responses is limited by uncertainties about pathways of organic matter
17 transformation in soil. In particular, the question as to why some SOM components persist in
18 soil for centuries (denoted as `stable SOM` from here on) while others turn over quickly is
19 still puzzling (Schmidt et al., 2011).

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

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

4

5 **2 Dissolved phase line of evidence**

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

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

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

13 (3) Root decomposition in mineral soil could be another important source of aromatic
14 DOM components that contribute to stable SOM.

15

16 **2.1 Argument 1: DOM as source of stable SOM**

17 Leaching of DOM is a major pathway for organic matter translocation from forest floor into
18 the topsoil horizons. Estimates for acidic forest soils with permanent forest floor suggest that
19 25-89% of the SOM stored in mineral soils derives from DOM (Neff and Asner, 2001;
20 Michalzik et al., 2003; Kalbitz and Kaiser, 2008), based on the typical observation of
21 decreasing DOC fluxes with depth of the mineral soil (a large compilation of data from
22 studies on forest and grassland soils is presented by Neff and Asner, 2001). Two processes
23 can explain the decrease: mineralization and sorption.

24 Sorption of DOM to mineral surfaces likely is a major process forming stable SOM in many
25 soils. Evidence for its importance comes from findings that the turnover and storage of SOM
26 in mineral soil horizons is related to the contents of reactive secondary minerals (e.g., Fe
27 hydrous oxides, short-range ordered Al hydroxides). Such relationships have been found
28 across a wide range of soil types (comprehensive data sets have been presented in Torn et al.,
29 1997, Eusterhues et al., 2005, Kögel-Knabner et al., 2008, Kramer et al., 2012, and Kleber et

1 al., 2015). Also, higher radiocarbon age of SOM in heavy (i.e., mineral) fractions compared to
2 light density (i.e. organic) fractions indicates that sorption stabilizes organic compounds (see
3 data compilations by Marschner et al., 2008, Kögel-Knabner et al., 2008, and Kleber et al.,
4 2015). Density fractionation procedures indicate that the total soil C associated with minerals
5 in any given location can vary from 30% to 90% (data compiled in Kleber et al., 2015). The
6 relevance of sorptive stabilization depends on soil properties. Low soil pH enhances the
7 formation of reactive secondary minerals and favors the formation of strong bonds between
8 organic matter and the mineral surface (Kleber et al., 2015). Most studies cited herein (for
9 both lines of evidence) examined acidic soils under temperate forests, in which sorptive
10 stabilization clearly should play an important role for the long-term storage of organic matter
11 in soil.

12

13 **2.2 Argument 2: Preferential stabilization of aromatic DOM components**

14 Lignin, a macromolecule composed of phenyl propane units, is a major plant cell wall
15 component (Kögel-Knabner, 2002). Typically, lignin concentrations negatively correlate with
16 litter decomposition rates. They are the predominant control on litter decomposition within
17 biomes worldwide (Cornwell et al., 2008), indicating that the lignin macromolecule is among
18 the most persistent litter constituents. Nevertheless, results of recent studies suggest
19 significant chemical alteration and losses of lignin already within the first months and years of
20 litter decomposition (Kalbitz et al., 2006; Preston et al., 2009; Klotzbücher et al., 2011;
21 Duboc et al., 2014). 'Degradation' of lignin has to be considered a step-wise process: (i) the
22 first step is the depolymerization of the macromolecule, releasing (mainly aromatic) water-
23 soluble depolymerisation products of varying molecular weight; (ii) these products can then
24 be further transformed, and low-molecular weight compounds are eventually taken up by
25 microorganisms to produce biomass or CO₂. Hence, losses of lignin-derived C during litter
26 decomposition can occur due to leaching of water-soluble products of an incomplete
27 degradation or as CO₂. Laboratory incubation tests on water-extractable organic matter from
28 various plant and soil materials suggest that aromatic components are more resistant to
29 mineralization than non-aromatic components (Pinney et al., 2000; Kalbitz et al., 2003 a,b;
30 Marschner and Kalbitz, 2003; Don and Kalbitz, 2005; McDowell et al., 2006; Hagedorn and
31 Machwitz, 2007; Fellman et al., 2008; Hansson et al., 2010; Kothawala et al., 2012; Toosi et
32 al., 2012). This suggests that leaching is an important factor in loss of lignin-derived matter

1 during litter decomposition. Consistent with this conceptual model, the typically high UV
2 absorptivity of DOM leached from forest floors (i.e., higher than values found for DOM
3 leached from Oi and Oe horizons) is indicative of a large contribution of resistant aromatic
4 components (Kalbitz et al., 2007; Fröberg et al., 2007).

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

9 It has been commonly found that the contribution of components likely derived from lignin
10 and tannins to DOM decreases with depth of the mineral soil (summarized in Table 1), i.e.,
11 the decrease in fluxes of these compounds with depth is more pronounced than the decrease
12 of bulk DOM. One explanation might be intensive biodegradation of aromatics in mineral
13 soil. However, this would contradict results of the DOM biodegradation studies previously
14 discussed. Hence, a more likely explanation is sorption to mineral surfaces. Laboratory
15 sorption experiments support this view; a typical observation is that aromatic DOM
16 components are preferentially sorbed by minerals and soils (Davis and Gloor, 1981; Jardine et
17 al., 1989; McKnight et al., 1992; Dai et al., 1996; Kaiser and Zech, 1997, 2000; Chorover and
18 Amistadi, 2001; Guo and Chorover, 2003; Kalbitz et al., 2005; Kawahigashi et al., 2006; Hunt
19 et al., 2007; Mikutta et al., 2007; Jagadamma et al., 2012; Sodano et al., 2016), and for some
20 soils it has been shown they displace previously bound organic components from mineral
21 surfaces (Kaiser et al., 1996). The degree of preferential sorption may depend on the
22 composition of the soil mineral assemblage. Chorover and Amistadi (2001) observed that
23 high molecular weight aromatic components preferentially sorbed onto goethite, while for
24 montmorillonite no preference for aromatic moieties was observed. A likely reason for the
25 preferential sorption is the large content of carboxyl groups linked to the aromatic rings,
26 which bind to metals at mineral surfaces via ligand exchange reactions.

27

28 **2.3. Argument 3: Roots as source of stabilized aromatic SOM**

29 We have so far focused on DOM leached from aboveground litter. However, roots might also
30 be a crucial source of stable SOM. The contribution of root and aboveground litter as major
31 source of SOM has been debated in numerous studies, but the available information allows no
32 definite conclusions yet (comprehensive discussions on the topic can be found in Lajtha et al.,

1 2014, and Hatton et al., 2015). Presumably, the relative importance of the two types of
2 organic matter input for SOM storage in topsoils differs between ecosystems (Crow et al.,
3 2009) and the importance of root-derived matter increases with soil profile depth (Rumpel et
4 al., 2015).

5 This raises issue of whether results from aboveground litter decomposition would also apply
6 to root litter decomposition. Data by Crow et al. (2009) suggest that lignin concentrations of
7 roots are in the range of those of leaf and needle litter. Root-derived DOM shows higher
8 concentrations of aromatic compounds than DOM from foliar litter (Hansson et al., 2010;
9 Uselman et al., 2012). Hansson et al. (2010) showed that DOM production during root
10 decomposition occurs in patterns that are similar to those of needle decomposition.
11 Particularly during later decomposition stages, root-derived DOM is enriched in aromatics
12 resistant to mineralization. Hence, available information suggests that root decomposition is
13 just another important source of soluble aromatics in mineral soils. However, to the best of
14 our knowledge, studies to quantify the contribution of root-derived aromatics to DOM fluxes
15 in the field have yet to be conducted. Overall, the available information is limited to data from
16 laboratory experiments and refers to acid temperate forest soils, so that it is not yet possible to
17 draw general conclusions.

18

19 **3 Solid phase line of evidence**

20 Many of the recent conceptual papers on SOM formation are built on the assumption that
21 lignin-derived aromatics disappear quickly in soil, while SOM in mineral soils is dominated
22 by non-aromatic and microbial-derived compounds (Grandy and Neff, 2008; Schmidt et al.,
23 2011; Dungait et al., 2012; Miltner et al., 2012; Cotrufo et al., 2013; Castellano et al., 2015).
24 Empirical support is provided by studies characterizing the chemical structure of solid SOM
25 using a variety of analytical methods.

26 Numerous studies on a wide variety of soil types used the cupric oxide (CuO) method to
27 analyse the distribution of lignin-derived phenols in profiles. A typical observation is that the
28 phenol contribution to SOM decreases (i) from forest floor to A horizons, (ii) with depth of
29 the mineral soil, (iii) with decreasing soil particle size (reviewed in Thevenot et al., 2010) and
30 with increasing density of soil fractions, hence, are contributing little to heavy (i.e., mineral-
31 associated) and old soil fractions (Leifeld and Kögel-Knabner, 2005; Grünewald et al., 2006;
32 Sollins et al., 2009; Kögel-Knabner et al., 2008; Cerli et al., 2012). Similar results are

1 reported by studies using pyrolysis-gas chromatography/mass spectrometry (Gleixner et al.
2 2002; Nierop et al., 2005; Buurman et al., 2007; Grandy and Neff, 2008; Tonneijck et al.,
3 2010; Rumpel et al., 2012) and tetramethylammonium hydroxide (TMAH) thermochemolysis
4 (Nierop and Filley, 2007; Mason et al., 2012).

5 Analysis of heavy and clay-sized soil fractions using cross polarization and magic angle
6 spinning (CPMAS) ^{13}C nuclear magnetic resonance spectroscopy (^{13}C -NMR) typically finds
7 high peak intensities of alkyl and O/N alkyl C (mostly assigned to polysaccharides and
8 proteins) and low peak intensities of aryl C (mostly assigned to lignin and tannins) (see data
9 compilations by Mahieu et al., 1999, and Kögel-Knabner et al., 2008). For instance, in a
10 comprehensive study on Ah horizons from 8 European forest sites, O/N alkyl C contributed
11 up to 41-49% of total peak intensity in the $<2\text{-}\mu\text{m}$ fraction, and the peak intensities were on
12 average 10% higher than the those reported for bulk soil; the intensities of aryl C in the $<2\text{-}$
13 μm fraction contributed 13-15% of total peak intensities, and they were on average 24%
14 lower than values found for bulk soil (Schöning et al., 2005). Also studies using near-edge X-
15 ray absorption fine structure (NEXAFS) spectra supported the conclusions drawn from ^{13}C -
16 NMR analysis of a significant contribution of microbial-derived compounds to SOM at
17 mineral surfaces (Lehmann et al., 2007; Kleber et al., 2011).

18 Consistent with these findings, the heavy soil fraction is characterized by low C/N values
19 close to those of microbial tissues (Kögel-Knabner et al., 2008).

20 Analysis of microbial-derived polysaccharides by acid hydrolysis suggest an enrichment of
21 these compounds in fine and heavy soil fractions (Kiem and Kögel-Knabner, 2003; Rumpel et
22 al., 2010).

23 Lignin turnover (i.e., transformation into CO_2 or non-lignin products) in temperate arable,
24 grassland, and tropical forest soils has been estimated using a combination of isotopic labeling
25 and compound-specific isotope analysis of lignin-derived aromatics applying the CuO
26 method. Most of the studies using this approach suggest that the turnover of lignin-derived
27 aromatics is faster than the turnover of bulk SOM (Dignac et al., 2005; Heim and Schmidt,
28 2007; Heim et al., 2010). A modeling study based on the data by Dignac et al. (2005)
29 suggested that about 90% of the lignin is mineralized as CO_2 or transformed into compounds
30 devoid of lignin-type signatures within one year (Rasse et al., 2006). However, a study by
31 Hofmann et al. (2009) suggests that after 18 years, approximately two-thirds of the initial

1 lignin phenols remained in an arable soil. The authors concluded that lignin was preferentially
2 preserved in the soil.

3

4 **4 Reasons for the controversies**

5

6 **4.1. Analytical limitations**

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

13 Commonly applied methods such as CuO oxidation, pyrolysis or TMAH thermochemolysis
14 focus on few defined lignin-derived monomers to estimate the overall contribution of lignin.
15 These estimates, however, can largely differ depending on the method applied (Klotzbücher et
16 al., 2011). As outlined by Amelung et al. (2008), compound-specific isotope analysis of
17 lignin-derived compounds with the CuO method presumably overestimates the turnover rates
18 of lignin as only part of the lignin-derived aromatics can be extracted from soil (incomplete
19 extraction might also be a problem in all analyses of biomarkers, for which turnover times
20 typically are estimated to be faster than turnover rates of bulk SOM). Firstly, CuO oxidation
21 (as well as conventional pyrolysis or TMAH thermochemolysis) does not completely
22 depolymerize lignin (Johansson et al., 1986; Goñi and Hedges, 1992; Filley et al., 2000).
23 Secondly, lignin-derived aromatics bound to mineral surfaces are only partly assessed by the
24 CuO method (Hernes et al., 2013). Thirdly, lignin-derived aromatics might be altered in a way
25 that they escape the `analytical window` and cannot be ascribed to a lignin source anymore.
26 For instance, the CuO method yields a number of aromatic monomers of unknown origin
27 besides the lignin-derived monomers (Cerli et al., 2008). These compounds are typically not
28 quantified, and thus, not considered in estimates of the SOM composition. Hence, monomer
29 yield is a commonly used but uncertain measure of lignin concentration in soil.

30 Solid-state CPMAS ¹³C-NMR has been widely used in the last decades to study the
31 composition of SOM. Whether the results are quantitative has been subject of an intensive

1 debate (see Knicker, 2011). Mineral soil samples are commonly pretreated with hydrofluoric
2 acid (HF) in order to remove paramagnetic minerals that disturb the analysis. The treatment
3 can result in significant losses of SOM, and one might lose important information on SOM
4 adsorbed onto minerals (e.g., SOM losses of 10-30% in topsoil samples and up to 90% in
5 subsoil; Eusterhues et al., 2003). Eusterhues et al. (2007) attempted to assess the chemical
6 composition of HF-soluble SOM by comparing CPMAS ^{13}C -NMR spectra of untreated and
7 HF-treated soil samples. The data suggest that the composition of HF-soluble SOM varies
8 between soil type and soil horizons. The effect of HF treatment, thus, produces unpredictable
9 changes in composition and questions the meaning of spectra obtained on HF-treated samples.
10 However, the approach used comes with the uncertainty that in the untreated soil part of the
11 SOM attached to mineral surfaces might have been invisible due to the proximity to
12 paramagnetic material (Kinchesh et al. 1995), while in the HF-treated soil, the same SOM
13 might have become removed during the treatment, thus, was no more detectable. Further
14 uncertainties of CPMAS ^{13}C -NMR arise from signal overlapping and a general low sensitivity
15 for aromatic C in soils (e.g., Skjemstad et al., 1996; Mao et al., 2000; Simpson and Simpson,
16 2012). The common approach used to quantify the relative contribution of different C types to
17 SOM is to integrate and compare peak areas of different spectra regions without considering
18 any non-proportional signal responses (e.g., Kögel-Knabner 2002). It has been shown that this
19 approach underestimates lignin vs. cellulose in ligno-cellulose isolated from wheat (Gauthier
20 et al., 2002). Also, peaks of methoxyl C often tend to be larger than those of aromatic C.
21 Both, methoxyl C and aromatic C derive from lignin, but lignin has much less methoxyl C
22 than aromatic C. Spectra of wood samples (e.g., Bonanomi et al. 2014), needle litter (Preston
23 and Trofymow, 2015) and grass litter (McKee et al., 2016) all show that patterns of methoxyl
24 signals being larger than those of aromatic and phenolic C combined.

25 By applying Bloch decay, another type of ^{13}C -NMR technique, one can overcome the
26 problem of the reduced sensitivity for aromatic C. The technique has been applied in studies
27 on pyrogenic organic matter, for which CPMAS ^{13}C -NMR should be even less sensitive than
28 for lignin as it is more condensed (Golchin et al., 1997; Simpson and Hatcher, 2004; Knicker
29 et al., 2005). Bloch decay, however, also comes with problems, such as general low signal
30 intensity. It is not routinely applied in SOM research as it is an extremely time-consuming
31 experiment and the required instrument time is frequently not available (Simpson and
32 Hatcher, 2004).

1

2 **4.2. Limits in process understanding**

3 The contradictions outlined herein might also suggest gaps in the understanding of SOM
4 turnover processes. Here we argue that, in particular, knowledge about the turnover of SOM
5 at mineral surfaces is insufficient. This is due to the yet uncertain quantitative composition of
6 SOM. In addition, prevailing conceptual ideas and paradigms have been questioned in recent
7 years.

8 It has been frequently observed that the ^{14}C age of DOM increases with profile depth. Kaiser
9 and Kalbitz (2012) proposed that this can be explained by temporal sorptive immobilization,
10 followed by microbial processing and re-release of altered compounds into soil solution. That
11 would mean the assumption that aromatic compounds are stable after being sorbed onto
12 mineral surfaces could be erroneous. Hence, the microbial processing of sorbed compounds
13 might be the `missing argument` that proves the view of a fast disappearance of aromatic
14 compounds in mineral soil. However, these processes have hardly been studied yet, and
15 empirical evidence for their importance is missing.

16 Also, root activity might have significant effects on stability and composition of SOM sorbed
17 onto mineral surfaces. The recent study of Keiluweit et al. (2015) showed that root exudation
18 of oxalic acid promotes release of sorbed compounds into soil solution through dissolution of
19 mineral phases. Besides oxalic acid, many other low-molecular weight compounds,
20 including acids, simple sugars, amino sugars, phenolics, as well as high-molecular
21 compounds (exoenzymes, root cells) are released from living roots (Wichern et al., 2008).
22 Most of the compounds are degraded quickly, but a smaller portion seems to contribute to
23 stable SOM (Nguyen, 2003; Pausch et al., 2013). The rhizosphere is considered a `hot-spot` in
24 soil, where microbial processes are not C-limited (Kuzyakov and Blagodatskaya, 2015).
25 Hence, root activity possibly may accelerate the turnover of sorbed plant-derived aromatics,
26 and at the same time foster the production and stabilization of microbial-derived compounds.
27 Studies addressing these assumptions are not yet available.

28 Recent investigation at the submicrometer scale using Nano Secondary Ion Mass
29 Spectrometry (NanoSIMS) or NEXAFS in combination with scanning transmission X-ray
30 microscopy suggest that SOM associated with clay-sized minerals exists in small patches of
31 varying chemical composition (Lehmann et al., 2008; Remusat et al., 2012; Vogel et al.,
32 2014). Distinct patches of predominantly aromatic C can be differentiated from patches

1 dominated by aliphatic C (Lehmann et al., 2008). Knowledge about processes controlling the
2 sub micro meter-scale distribution of SOM on mineral surfaces is still limited. Some of the
3 patches are cell wall structures of microorganisms, which may contribute to stable SOM as
4 they are composed of insoluble polymers and possibly attach to the mineral surface (Miltner
5 et al., 2012). Hence, stable sorbed organic matter might not only be derived from low-
6 molecular weight compounds. On the other hand, it needs to be considered that microbial-
7 derived compounds are continuously synthesized at the mineral surface. The microorganisms
8 might use some of the older C (^{14}C age) for synthesis of relatively labile compounds. The age
9 of the C atoms is, thus, decoupled from the stability of the organic matter, and microbial-
10 derived compounds may `mimic` a similar or even higher stability than the plant-derived
11 compounds (Gleixner, 2013). Hence, concentrations or ^{14}C age of microbial-derived
12 compounds at mineral surfaces do not per se allow for conclusions on their contribution to
13 stable SOM.

14 Pyrogenic organic matter is an important source of aromatic compounds in many soils.
15 Despite extensive research efforts, rates, and pathways of pyrogenic organic matter
16 decomposition are still not well established (Schmidt et al., 2011; Kuzyakov et al., 2014).
17 Analyses of benzenecarboxylic acids as molecular markers suggest that aromatic compounds
18 derived from pyrogenic organic matter are transported within soil profiles and bind to mineral
19 surfaces (Haumaier, 2010). The quantitative contribution of pyrogenic organic matter to
20 DOM in soil is, however, still poorly studied (Smebye et al., 2016). Bulk analyses of aromatic
21 matter used in most research on DOM fluxes (i.e., UV absorption) cannot distinguish if the
22 compounds derive from plant litter or from pyrogenic organic matter. This limits the
23 understanding of the processes controlling turnover of aromatics. If a significant part of
24 aromatic DOM in mineral soil derives from pyrogenic organic matter, the `loss` of plant litter-
25 derived aromatics in mineral soil would be even more pronounced.

26

27 **5 Implications and future research strategies**

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

30 For instance, elevated atmospheric CO_2 levels can induce increasing concentrations of
31 aromatic components in plant litter (Cotrufo et al., 1994; Tuchman et al., 2002), and this
32 raises the question whether this causes enhanced or decreasing storage of SOM in mineral

1 soils. If aromatic matter is quickly degraded, and mineral-associated SOM primarily derives
2 from microbial sources (as suggested by the solid SOM line of evidence), a `microbial filter`
3 would control the built-up of stable SOM, which may then be determined by the microbial
4 substrate use efficiency (i.e., the amount of organic C used by the microbial community to
5 build biomass vs. the amount that is mineralized). As such, Cotrufo et al. (2013) hypothesized
6 that input of labile substrates fosters the build-up of stable SOM. Available data on effects of
7 litter quality and SOM formation are, however, inconsistent (Castellano et al., 2015), and we
8 believe that understanding these effects is in part limited by uncertainties about the
9 incorporation of aromatics into stable SOM.

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

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

- 21 • Knowledge on aromatics in soils is limited by the analytical constraints.
22 Quantification of total amounts and sources of aromatics in soil are still problematic.
23 Even if the problems cannot be fully solved with the currently available techniques,
24 there might be strategies to obtain improved estimates. The work of Hernes et al.
25 (2013) provides a first hint about how much lignin might be not accessible to CuO
26 oxidation analyses. The authors evaluated the extraction efficiency for lignin-derived
27 aromatics bound to different minerals. The size of the non-extractable fraction
28 depended on the mineral. Almost all of the aromatics bound to ferrihydrite were
29 extractable, but for kaolinite the non-extractable fraction made >40%. But how about
30 extractability in soil under field conditions? Possibly, a combination of tracking of C
31 isotopes, DOM flux/composition assessment, and analysis of solid-phase SOM
32 composition could provide better estimates of hidden aromatics.

- 1 • The causes of the commonly observed decreasing fluxes of aromatic DOM fluxes
2 with depth of the mineral soil need to be re-examined. Are they really mainly the
3 result of sorption to mineral surfaces (as proposed herein in Chapter 2), or do other
4 processes such as the binding of tannins to proteins or mineralization also play a
5 decisive role? Moreover, the presumed microbial processing of sorbed material,
6 causing desorption and subsequent mineralization or further transport in the soil
7 profile is poorly studied. Knowledge gaps also exist concerning the question whether
8 root activity affects de-/sorption processes. Eventually, these processes might cause
9 loss of aromatic compounds.
- 10 • Computer simulations could help to unravel the complex interrelationships between
11 DOM fluxes and solid-phase SOM composition. Recently developed models integrate
12 sorption, DOM transport, and microbial processes (Ahrens et al., 2015). In order to
13 address the problems discussed herein, effects of molecular structure on behavior of
14 the compounds in soil (e.g., differences in mineralization rate and affinity for sorption
15 between aromatics and non-aromatics) could be implemented in the models, in order
16 to develop novel hypotheses on turnover of plant-derived aromatics.

17

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21

1 **References**

2

3 Ahrens, B., Braakhekke, M.C., Guggenberger, G., Schrumpf, M. and Reichstein, M.:
4 Contribution of sorption, DOC transport and microbial interactions to the ¹⁴C age of a soil
5 organic carbon profile: Insights from a calibrated process model. *Soil Biol. Biochem.*, 88,
6 390-402, 2015.

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

10 Bonanomi, G., Capodilupo, M., Incerti, G. and Mazzoleni, S.: Nitrogen transfer in litter
11 mixture enhances decomposition rate, temperature sensitivity, and C quality changes. *Plant*
12 *Soil*, 381, 307-321, 2014.

13 Buurman, P., Peterse, F. and Almendros Martin, G.: Soil organic matter chemistry in
14 allophonic soils: a pyrolysis-GC/MS study of a Costa Rican Andosol catena. *Eur. J. Soil Sci.*,
15 58, 1330-1347, 2007.

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

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

22 Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G. and Kaiser, K.: Separation of light and
23 heavy fractions in soil – testing for proper density cut-off and dispersion level. *Geoderma*,
24 170, 403-416, 2012.

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

27 Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy,
28 O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Prezer-Harguindeguy, N., Queded, H.M.,
29 Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., van Bodegorn, P.,
30 Brovkin, V., Chatain, A., Callaghan, T.V., Diaz, S., Garnier, E., Gurvich, D.E., Kazakou, E.,

1 Klein, J.A., Read, J., Reich, P.B., Soudzilovskala, N.A., Vaieretti, M.A. and Westoby, M.:
2 Plant species traits are the predominant control on litter decomposition rates within biomes
3 worldwide. *Ecol. Lett.*, 11, 1065-1071, 2008.

4 Cotrufo, M.F., Ineson, P. and Rowland, A.P.: Decomposition of tree leaf litters grown under
5 elevated CO₂: Effect of litter quality. *Plant Soil*, 163, 121-130, 1994.

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

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

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

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

17 Davis, J.A. and Gloor R.: Adsorption of dissolved organics in lake water by aluminium oxide.
18 Effect of molecular weight. *Environ. Sci. Technol.*, 15, 1223-1229, 1981.

19 Dignac, M.F., Bahri, H., Rumpel, C., Rasse, D.P., Bardoux, G., Balesdent, J., Girardin, C.,
20 Chenu, C. and Mariotti, A.: Carbon-13 natural abundance as a tool to study the dynamics of
21 lignin monomers in soil: an appraisal at the Closeaux experimental field (France). *Geoderma*,
22 128, 3-17, 2005.

23 Don, A. and Kalbitz, K.: Amounts and degradability of dissolved organic carbon from foliar
24 litter at different decomposition stages. *Soil Biol. Biochem.*, 37, 2171-2179, 2005.

25 Duboc, O., Dignac, M.-F., Djukic, I., Zehetner, F., Gerzabrek, M.H. and Rumpel, C.: Lignin
26 decomposition along an Alpine elevation gradient in relation to physicochemical and soil
27 microbial parameters. *Glob. Change Biol.*, 20, 2272-2285, 2014.

28 Dungait, J.A.J., Hopkins, D.W., Gregory, A.S. and Whitmore, A.P.: Soil organic matter
29 turnover is governed by accessibility not recalcitrance. *Glob. Change Biol.*, 18, 1761-1796,
30 2012.

- 1 Eusterhues, K., Rumpel, C. and Kögel-Knabner, I.: Composition and radiocarbon age of HF-
2 resistant organic matter in a Podzol and Cambisol. *Org. Geochem.*, 38, 1356-1372, 2007.
- 3 Eusterhues, K., Rumpel, C. and Kögel-Knabner, I.: Organo-mineral associations in sandy acid
4 forest soils: importance of specific surface area, iron oxides and micropores. *Eur. J. Soil Sci.*,
5 56, 753-763, 2005.
- 6 Eusterhues, K., Rumpel, C., Kleber, M. and Kögel-Knabner, I.: Stabilisation of soil organic
7 matter by interactions with minerals as revealed by mineral dissolution and oxidative
8 degradation. *Org. Geochem.*, 34, 1591-1600, 2003.
- 9 Fellman, J.B., D'Amore, D.V., Hood, E. and Boone, R.D.: Fluorescence characteristics and
10 biodegradability of dissolved organic matter in forest and wetland soils from coastal
11 temperate watersheds in southeast Alaska. *Biogeochemistry*, 88, 169-184, 2008.
- 12 Filley, T., Hatcher, P.G., Shortle, W.C. and Praseuth, R.T.: The application of ^{13}C -labeled
13 tetramethylammonium hydroxide (^{13}C -TMAH) thermochemolysis to the study of fungal
14 degradation of wood. *Org. Geochem.*, 31, 181-198, 2000.
- 15 Fröberg, M., Berggren Kleja, D. and Hagedorn, F.: The contribution of fresh litter to
16 dissolved organic carbon leached from a coniferous forest floor. *Eur. J. Soil Sci.*, 58, 108-114,
17 2007.
- 18 Gallet, C. and Pellissier, F.: Phenolic compounds in natural solutions of a coniferous forest. *J.*
19 *Chem. Ecol.*, 23, 2401-2412, 1997.
- 20 Gauthier, A., Derenne, S., Dupont, L., Guilon, E., Largeau, C., Dumonceau, J. and
21 Aplincourt, M.: Characterization and comparison of two ligno-cellulose substrates by ^{13}C
22 CP/MAS NMR, XPS, conventional pyrolysis and thermochemolysis. *Anal. Bioanal. Chem.*,
23 373, 830-838, 2002.
- 24 Gleixner, G., Poirier, N., Bol, B. and Balesdent, J.: Molecular dynamics of organic matter in
25 cultivated soil. *Org. Geochem.*, 33, 357-366, 2002.
- 26 Gleixner, G.: Soil organic matter dynamics: a biological perspective derived from the use of
27 compound-specific isotopes studies. *Ecol. Res.*, 28, 683-695, 2013.
- 28 Golchin, A., Clarke, P., Baldock, J.A., Higashi, T., Skjemstad, J.O. and Oades, J.M.: The
29 effect of vegetation and burning on chemical composition of soil organic matter in a volcanic

1 ash soil shown by ¹³C NMR spectroscopy. I. Whole soil and humic fraction. *Geoderma*, 76,
2 155-174, 1997.

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

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

8 Grünewald, G., Kaiser, K., Jahn, R. and Guggenberger, G.: Organic matter stabilization in
9 young calcareous soils as revealed by density fractionation and analysis of lignin-derived
10 constituents. *Org. Geochem.*, 37, 1573-1589, 2006.

11 Guo, M. and Chorover, J.: Transport and fractionation of dissolved organic matter in soil
12 columns. *Soil Sci.*, 168, 108-118, 2003.

13 Hagedorn, F. and Machwitz, M.: Controls on dissolved organic matter leaching from forest
14 litter grown under elevated CO₂. *Soil Biol. Biochem.*, 39, 1759-1769, 2007.

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

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

21 Hatton, P.-J., Castanha, C., Torn, M.S. and Bird, J.A.: Litter type control on soil C and N
22 stabilization dynamics in a temperate forest. *Glob. Change Biol.*, 21, 1358-1367, 2015.

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

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

27 Heim, A., Hofmann, A. and Schmidt, M.W.I.: Forest-derived lignin biomarkers in an
28 Australian oxisol decrease substantially after 90 years of pasture. *Org. Geochem.*, 41, 1219–
29 1224, 2010.

- 1 Hernes, P.J., Kaiser, K., Dyda, R.Y. and Cerli, C.: Molecular trickery in soil organic matter:
2 hidden lignin. *Environ. Sci. Technol.*, 47, 9077-9085, 2013.
- 3 Hofmann, A., Heim, A., Christensen, B.T., Miltner, A., Gehre, M. and Schmidt, M.W.I.:
4 Lignin dynamics in two ¹³C-labelled arable soils during 18 years. *Eur. J. Soil Sci.*, 60, 250-
5 257, 2009.
- 6 Hunt, J.F., Ohno, T., He, Z., Honeycutt, C.W. and Bryan Dali, D.: Influence of decomposition
7 on chemical properties of plant- and manure-derived dissolved organic matter and sorption on
8 goethite. *J. Environ. Qual.*, 36, 135-143, 2007.
- 9 Jagadamma, S., Mayes, M.A. and Phillips, J.R.: Selective sorption of dissolved organic
10 carbon compounds by temperate soils. *PloS One* 7, e50434, 2012.
- 11 Jardine, P.M., McCarthy, J.F. and Weber, N.L.: Mechanisms of dissolved organic carbon
12 adsorption on soil. *Soil Sci. Soc. Am. J.*, 53, 1378-1385, 1989.
- 13 Johansson, M.-B., Kögel, I. and Zech, W.: Changes in the lignin fraction of spruce and pine
14 needle litter during decomposition as studied by some chemical methods. *Soil Biol. Biochem.*,
15 18, 611-619, 1986.
- 16 Kaiser K., Guggenberger, G. and Zech, W.: Sorption of DOM and DOM fractions to forest
17 soils. *Geoderma*, 74, 281-303, 1996.
- 18 Kaiser, K. and Kalbitz, K.: Cycling downwards – dissolved organic matter in soils. *Soil Biol.*
19 *Biochem.*, 52, 29-32, 2012.
- 20 Kaiser, K. and Zech, W.: Competitive sorption of dissolved organic matter fractions to soils
21 and related mineral phases. *Soil Sci. Soc. Am. J.*, 61, 64-69, 1997.
- 22 Kaiser, K. and Zech, W.: Dissolved organic matter sorption by mineral constituents of subsoil
23 clay fractions. *J. Plant Nutr. Soil Sci.*, 163, 531-535, 2000.
- 24 Kaiser, K., Guggenberger, G. and Haumaier, L.: Changes in lignin-derived phenols, neutral
25 sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic
26 Leptosols. *Biogeochemistry*, 70, 135-151, 2004.
- 27 Kalbitz, K. and Kaiser, K.: Contribution of dissolved organic matter to carbon storage in
28 forest mineral soils. *J. Plant Nutr. Soil Sci.*, 171, 52-60, 2008.

1 Kalbitz, K., Kaiser, K., Bargholz, J. and Dardenne P.: Lignin degradation controls the
2 production of dissolved organic matter in decomposing foliar litter. *Eur. J. Soil Sci.*, 57, 504-
3 516, 2006.

4 Kalbitz, K., Meyer, A., Yang, R., Gerstberger, P.: Response of dissolved organic matter in the
5 forest floor to long-term manipulation of litter and throughfall inputs. *Biogeochemistry*, 86,
6 301-318, 2007.

7 Kalbitz, K., Schmerwitz, J., Schwesig, D. and Matzner, E.: Biodegradation of soil-derived
8 dissolved organic matter as related to its properties. *Soil Biol Biochem*, 35, 1129-1142,
9 2003a.

10 Kalbitz, K., Schwesig, D., Rethemeyer, J. and Matzner, E.: Stabilization of dissolved organic
11 matter by sorption to the mineral soil. *Soil Biol. Biochem.*, 37, 1319-1331, 2005.

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

15 Kawahigashi, M., Kaiser, K., Rodionov, A. and Guggenberger, G.: Sorption of dissolved
16 organic matter by mineral soils of the Siberian forest tundra. *Glob. Change Biol.*, 12, 1868-
17 1877, 2006.

18 Keiluweit, M., Bougoure, J.J., Nice, P.S., Pett-Ridge, J., Weber, P.K. and Kleber, M.: Mineral
19 protection of soil carbon counteracted by root exudates. *Nature Clim. Change*, 5, 588-595,
20 2015.

21 Kiem, R. and Kögel-Knabner, I.: Contribution of lignin and polysaccharides to the refractory
22 carbon pool in C-depleted arable soils. *Soil Biol. Biochem.*, 35, 101-118, 2003.

23 Kinchesh, P., Powlson, D.S. and Randall, E.W.: ¹³C NMR studies of organic matter in whole
24 soils: I. Quantitation possibilities. *Eur. J. Soil Sci.*, 46, 125-138, 1995.

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

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

- 1 Klotzbücher, T., Filley, T.R., Kaiser, K. and Kalbitz, K.: A study of lignin degradation in leaf
2 and needle litter using ^{13}C -labelled tetramethylammonium hydroxide (TMAH)
3 thermochemolysis: Comparison with CuO oxidation and van Soest methods. *Org. Geochem.*,
4 42, 1271-1278, 2011.
- 5 Knicker, H.: Solid state CPMAS ^{13}C and ^{15}N NMR spectroscopy in organic geochemistry and
6 how spin dynamics can either aggravate or improve spectra interpretation. *Org. Geochem.*,
7 42, 867-890, 2011.
- 8 Knicker, H., Totsche, K.U., Almendros, G. and Gonzalez-Vila, F.J.: Condensation of burnt
9 peat and plant residues and the reliability of solid-state VACP MAS ^{13}C NMR spectra
10 obtained from pyrogenic humic material. *Org. Geochem.*, 36, 1359-1377, 2005.
- 11 Kögel-Knabner, I.: The macromolecular organic composition of plant and microbial residues
12 as inputs to soil organic matter. *Soil Biol. Biochem.*, 34, 139-162, 2002.
- 13 Kögel-Knabner, I., Guggenberger, G. and Kleber, M.: Organo-mineral associations in
14 temperate soils: integrating biology, mineralogy, and organic matter chemistry. *J. Plant Nutr.*
15 *Soil Sci.*, 171, 61-82, 2008.
- 16 Kothawala, D.N., van Wachenfeldt, E., Koehler, B. and Tranvik, L.J.: Selective loss and
17 preservation of lake water dissolved organic matter fluorescence during long-term dark
18 incubations. *Sci. Tot. Environ.*, 433, 238-246, 2012.
- 19 Kramer, M.G., Sanderman, J., Chadwick, O.A., Chorover, J. and Vitousek, P.M.: Long-term
20 carbon storage through retention of dissolved aromatic acids by reactive particles in soil.
21 *Glob. Change Biol.*, 18, 2594-2605, 2012.
- 22 Kraus, T.E.C., Dahlgren, R.A. and Zasoski, R.J.: Tannins in nutrient dynamics of forest
23 ecosystems – a review. *Plant Soil*, 256, 41-66, 2003.
- 24 Kuzyakov, Y. and Blagodatskaya, E.: Microbial hotspots and hot moments in soil:
25 Concepts and review. *Soil Biol. Biochem.*, 83, 184-199, 2015.
- 26 Kuzyakov, Y., Bogomolova, I. and Glaser, B.: Biochar stability in soil: Decomposition during
27 eight years and transformation as assessed by compound-specific ^{14}C analysis. *Soil Biol.*
28 *Biochem.*, 70, 229-236, 2014.
- 29 Lajtha, K., Bowden, R.D. and Nadelhoffer, K.: Litter and root manipulations provide insights
30 into soil organic matter dynamics and stability. *Soil Sci. Soc. Am. J.*, 78, 251-269, 2014.

- 1 Lajtha, K., Crow, S.E., Yano, Y., Kaushal, S.S., Sulzman, E., Sollins, P. and Spears, J.D.H.:
2 Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment.
3 *Biogeochemistry*, 76, 261-281, 2005.
- 4 Lehmann, J., Kinyangi, J. and Solomon, D.: Organic matter stabilization in soil
5 microaggregates: implications from spatial heterogeneity of organic carbon contents and
6 carbon forms. *Biogeochemistry*, 85, 45-57, 2007.
- 7 Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirrick, S. and Jacobsen, C.: Spatial
8 complexity of soil organic matter forms at nanometre scales. *Nature Geoscience*, 1, 238-242,
9 2008.
- 10 Leifeld, J. and Kögel-Knabner, I.: Soil organic matter fractions as early indicators for carbon
11 stock changes under different land-use. *Geoderma*, 124, 143-155, 2005.
- 12 Mahieu, N., Randall, E.W. and Powlson, D.S.; Statistical analysis of published carbon-13
13 CPMAS NMR spectra of soil organic matter. *Soil Sci. Soc. Am. J.*, 63, 2, 307-319, 1999.
- 14 Mao, J.D., Hu, W.G., Schmidt-Rohr, K., Davies, G., Ghabbour, E.A. and Xing, B.:
15 Quantitative characterization of humic substances by solid-state carbon-13 nuclear magnetic
16 resonance. *Soil Sci. Soc. Am. J.*, 64, 873-884, 2000.
- 17 Marschner, B. and Kalbitz, K.: Controls of bioavailability and biodegradability of dissolved
18 organic matter in soil. *Geoderma*, 113, 211-235, 2003
- 19 Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U.,
20 Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J.,
21 Schäffer, A., Schmidt, M.W.I., Schwark, L. and Wiesenberg, G.: How relevant is
22 recalcitrance for the stabilization of organic matter in soils? *J. Plant Nutr. Soil Sci.*, 171, 81-
23 110, 2008.
- 24 Mason, S.L., Filley, T.R. and Abbott, G.D.: A comparative study of the molecular
25 composition a grassland soil with adjacent unforested and afforested moorland ecosystem.
26 *Org. Geochem.*, 42, 1519-1528, 2012.
- 27 McDowell, W.H., Zsolnay, A., Aitkenhead-Peterson, J.A., Gregorich, E.G., Jones, D.L.,
28 Jödemann, D., Kalbitz, K., Marschner, B. and Schwesig, D.: A comparison of methods to
29 determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil
30 Biol. Biochem.*, 38, 1933-1942, 2006.

- 1 McKee, G.A., Soong, J.L., Caldéron, F., Borch, T. and Cotrufo, M.F.: An integrated
2 spectroscopic and wet chemical approach to investigate grass litter decomposition chemistry.
3 Biogeochemistry, 128, 107-123, 2016.
- 4 McKnight, D.M., Bencala, K.E., Zellweger, G.W., Aiken, G.R., Feder, G.L. and Thorn, K.A.:
5 Sorption of dissolved organic carbon by hydrous aluminium and iron oxides occurring at the
6 confluence of Deer Creek with the Snake River, Summit County, Colorado. Environ. Sci.
7 Technol., 26, 1388-1396, 1992.
- 8 Michalzik, B., Tipping, E., Mulder, J., Gallardo Lancho, J.F., Matzner, E., Bryant, C.L.,
9 Clarke, N., Lofts, S. and Vicente Esteban, M.A.: Modelling the production and transport of
10 dissolved organic carbon in forest soils. Biogeochemistry, 66, 241-264, 2003.
- 11 Mikutta, R., Mikutta, C., Kalbitz, K., Scheel, T., Kaiser, K. and Jahn, R.: Biodegradation of
12 forest floor organic matter bound to minerals via different binding mechanisms. Geochim.
13 Cosmochim. Acta, 71, 2569-2590, 2007.
- 14 Miltner, A., Bonbach, P., Schmidt-Brücken, B. and Kästner, M.: SOM genesis: microbial
15 biomass as a significant source. Biogeochemistry, 111, 41-55, 2012.
- 16 Nakashini, T., Atarashi-Andoh, M., Koarashi, J., Saito-Kokubu, Y. and Hirai, K.: Carbon
17 isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic
18 relationship with soil carbon. Eur. J. Soil Sci., 63, 495-500, 2012.
- 19 Neff, J.C. and Asner, G.P.: Dissolved organic carbon in terrestrial ecosystems: synthesis and a
20 model. Ecosystems, 4, 29-48, 2001.
- 21 Nguyen, C.: Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie,
22 23, 375-396, 2003 .
- 23 Nierop, K.G.J. and Filley, T.R.: Assessment of lignin and (poly-)phenol transformations in
24 Oak (*Quercus robur*) dominated soils by ¹³C-TMAH thermochemolysis. Org. Geochem., 38,
25 551-565, 2007.
- 26 Nierop, K.G.J., van Bergen, P.F., Buurman, P. and van Lagen, B.: NaOH and Na₄P₂O₇
27 extractable organic matter in two allophanic volcanic ash soils of the Azores Islands – a
28 pyrolysis GC/MS study. Geoderma, 127, 36-51, 2005.
- 29 Pausch, J., Tian, J., Riederer, M. and Kuzyakov, Y.: Estimation of rhizodeposition at field
30 scale: upscaling of a ¹⁴C labeling study. Plant Soil, 364, 273-285, 2013.

- 1 Pinney, M.L., Westerhoff, P.K. and Baker, L.: Transformations in dissolved organic carbon
2 through constructed wetlands. *Water Res.*, 34, 1897–1911, 2000.
- 3 Preston, C.M., Nault, J.R. and Trofymow, J.A.: Chemical changes during 6 years of
4 decomposition of 11 litters in some Canadian forest sites. Part 2. ^{13}C abundance, solid-state
5 ^{13}C NMR spectroscopy and the meaning of "lignin". *Ecosystems*, 12, 1078-1102, 2009.
- 6 Preston, C.M. and Tofymow, J.A.: The chemistry of some foliar litters and their sequential
7 proximate analysis fractions. *Biogeochemistry*, 126, 197-209, 2015.
- 8 Qualls, R.G. and Haines, B.L.: Geochemistry of dissolved organic nutrients in water
9 percolating through a forest ecosystem. *Soil Sci. Soc. Am. J.*, 55, 1112-1123, 1991.
- 10 Rasse, D.P., Dignac, M.-F., Bahri, H., Rumpel, C., Mariotti, A. and Chenu, C.: Lignin
11 turnover in a agricultural field: from plant residues to soil-protected fractions. *Eur. J. Soil*
12 *Sci.*, 57, 530-538, 2006.
- 13 Remusat, L., Hatton, P.-J., Nico, P.S., Zeller, B., Kleber, M. and Derrien, D.: NanoSIMS
14 study of organic matter associated with soil aggregates: advantages, limitations, and
15 combination with STXM. *Environ. Sci. Technol.*, 46, 3943-3949, 2012.
- 16 Rumpel, C., Baumann, K., Remusat, L., Dignac, M.-F., Barre, P., Deidicque, D., Glasser, G.,
17 Lieberwarth, I. and Chabbi, A.: Nanoscale evidence of contrasted processes for root-derived
18 organic matter stabilization by mineral interactions depending on soil depth. *Soil Biol.*
19 *Biochem.*, 85, 82-88, 2015.
- 20 Rumpel, C., Eusterhues, K. and Kögel-Knabner, I.: Non-cellulosic neutral sugar contribution
21 to mineral associated organic matter in top- and subsoil horizons of wo acid forest soils. *Soil*
22 *Biol. Biochem.*, 42, 379-382, 2010.
- 23 Rumpel, C., Rodríguez-Rodríguez, A., González-Pérez, J.A., Arbelo, C., Chabbi, N., Nunan,
24 N. and González-Vila, F.J.: Contrasting composition of free and mineral-bound organic
25 matter in top- and subsoil horizons of Andosols. *Biol. Fertil. Soils*, 48, 401-411, 2012.
- 26 Sanderman, J., Baldock, J.A. and Amundson, R.: Dissolved organic carbon chemistry and
27 dynamics in contrasting forest and grassland soils. *Biogeochemistry*, 89, 181-198, 2008.
- 28 Schlesinger, W.H. and Andrews, J.A.: Soil respiration and the global carbon cycle.
29 *Biogeochemistry*, 48, 7-20, 2000.

- 1 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A.,
2 Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.,
3 Weiner, S. and Trumbore, S.E.: Persistence of soil organic matter as an ecosystem property.
4 *Nature*, 478, 49-56, 2011.
- 5 Schöning, I., Morgenroth, G. and Kögel-Knabner, I.: O/N-alkyl and alkyl C are stabilized in
6 fine particle size fractions of forest soils. *Biogeochemistry*, 73, 475-497, 2005.
- 7 Simpson, M.J. and Hatcher, P.G.: Overestimates of black carbon in soils and sediments.
8 *Naturwissenschaften*, 91, 436-440, 2004.
- 9 Simpson, M.J. and Simpson, A.J.: The chemical ecology of soil organic matter molecular
10 constituents. *J. Chem. Ecol.*, 38, 768-784, 2012.
- 11 Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M. and McClure, S.G.: The chemistry and
12 nature of protected carbon in soil. *Aust. J. Soil Res.*, 34, 251-271, 1996.
- 13 Smebye, A., Alling, V., Vogt, R.D., Gadmar, T.C., Mulder, J., Cornelissen, G. and Hale, S.E.:
14 Biochar amendment to soil changes dissolved organic matter content and composition.
15 *Chemosphere*, 142, 100-105, 2016.
- 16 Sodano, M., Said-Pullicino, D., Fiori, A.F., Catoni, M., Martin, M. and Celi, L.: Sorption of
17 paddy soil-derived dissolved organic matter on hydrous iron oxide – vermiculite mineral
18 phases. *Geoderma*, 261, 169-171, 2016.
- 19 Sollins, P., Kramer, M.G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A.K., Wagai,
20 R. and Bowden, R.D.: Sequential density fractionation across soils of contrasting mineralogy:
21 evidence for both microbial- and mineral-controlled soil organic matter stabilization.
22 *Biogeochemistry*, 96, 209-231, 2009.
- 23 Thevenot, M., Dignac, M.-F. and Rumpel, C.: Fate of lignins in soils: a review. *Soil Biol.*
24 *Biochem.*, 42, 1200-1211, 2010.
- 25 Tonneijck, F.H., Jansen, B., Nierop, K.G.J., Verstraten, J.M., Sevink, J. and De Lange, L.:
26 Towards understanding of carbon stocks and stabilization in volcanic ash soils in natural
27 Andean ecosystems of northern Ecuador. *Eur. J. Soil Sci.*, 61, 392-405, 2010.
- 28 Toosi, E.H., Clinton, P.W., Beare, M.H. and Norton, D.A.: Biodegradation of soluble organic
29 matter as affected by land-use and soil depth. *Soil Sci. Soc. Am. J.*, 76, 1667-1677, 2012.

1 Torn, M.S., Trumbore, S.E., Chadwick, O.A., Vitousek, P.M. and Hendricks, D.M.: Mineral
2 control of soil organic carbon storage and turnover. *Nature*, 389, 170-173, 1997.

3 Tuchman, N.C., Wetzel, R.G., Rier, S.T., Wahtera, K.A. and Teeri, J.A.: Elevated
4 atmospheric CO₂ lowers leaf nutritional quality for stream ecosystem food webs. *Glob.*
5 *Change Biol.*, 8, 163-170, 2002.

6 Uselman, S.M., Qualls, R.G. and Lilienfein, J.: Quality of soluble organic C, N, and P
7 produced by different types and species of litter: Root litter vs. leaf litter. *Soil Biol. Biochem.*,
8 54, 57-67, 2012.

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

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

14 Zech, W., Guggenberger, G. and Schulten, H.-R.: Budgets and chemistry of dissolved organic
15 carbon in forest soils: effects of anthropogenic soil acidification. *Sci. Tot. Environ.*, 152, 49-
16 62, 1994.

17
18
19
20
21
22
23
24
25
26
27
28

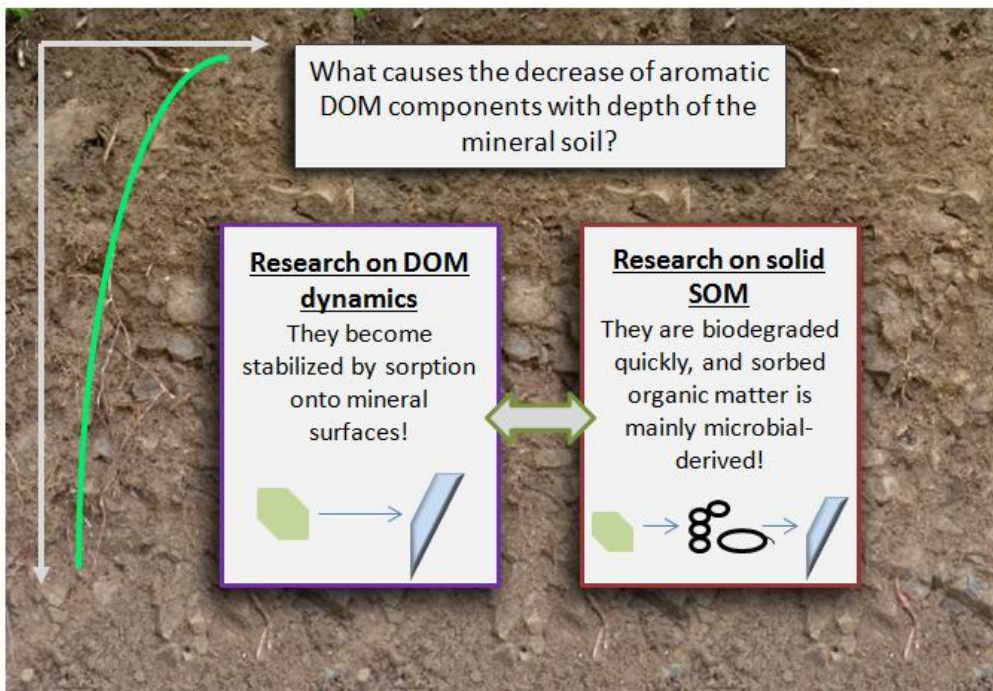
1 Table 1. Evidence from field studies suggesting that dissolved aromatics (products of lignin
 2 depolymerization or tannins) disappear quickly once entering mineral soils.

3

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 spectroscopy) 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.

4

- 1 Figure 1. Conflicting views on the fate of soluble aromatics once they enter the mineral soil
- 2 (see text for references).



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