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**Opportunities and limitations related to the application of plant-derived lipid  
molecular proxies in soil science**

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1 **Opportunities and limitations related to the application of**  
2 **plant-derived lipid molecular proxies in soil science**

3  
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10

11 **Abstract**

12 The application of lipids in soils as molecular proxies, also often referred to as biomarkers,  
13 has dramatically increased in the last decades. Applications range from inferring changes in  
14 past vegetation composition, climate and/or human presence to unraveling input and turnover  
15 of soil organic matter (SOM). Molecules used include extractable and ester-bound lipids as  
16 well as their carbon or hydrogen isotopic composition. While holding great promise, the  
17 application of soil lipids as molecular proxies comes with several constraining factors the  
18 most important of which are: i) variability in the molecular composition of plant-derived  
19 organic matter plant-internally and in between plant individuals; ii) variability in (relative  
20 contribution of) input pathways into the soil; and iii) transformation and/or (selective)  
21 degradation of (some of) the molecules once present in the soil. Unfortunately, the  
22 information about such constraining factors and their impact on the applicability of molecular  
23 proxies is fragmented and scattered. The purpose of this study is to provide a critical review  
24 of the current state of knowledge with respect to the applicability of molecular proxies in soil  
25 science, specifically focusing on the factors constraining such applicability. Variability in  
26 genetic, ontogenetic and environmental factors influence plant *n*-alkane patterns in the way  
27 that no unique compounds or specific molecular proxies pointing to e.g. plant-community  
28 differences or environmental influences, exist. Other components such as *n*-alcohols, *n*-fatty  
29 acids, cutin- and suberin-derived monomers have received far less attention in this respect.  
30 Furthermore, there is a high diversity of input pathways offering both opportunities and



1 limitations for the use of molecular proxies at the same time. New modelling approaches  
2 might offer a possibility to unravel such mixed input signals. Finally, transformation and  
3 turnover of SOM offer opportunities when tracing such processes is the purpose of applying a  
4 molecular proxy, whilst posing limitations when they obliterate molecular proxy signals  
5 linked to other phenomena. For *n*-alkanes several modelling approaches have recently been  
6 developed to compensate for (selective) degradation. Still such techniques are in their infancy  
7 and information about their applicability to other classes of components than *n*-alkanes is  
8 lacking yet. All constraining factors considered can have a significant influence on the  
9 applicability of molecular proxies in soil science. The degree of influence strongly depends  
10 on the type of molecular proxy as well as the environmental context in which it is applied.  
11 However, the potential impact of the constraining factors should always explicitly be  
12 addressed whenever molecular proxies are applied in a soil scientific context. More  
13 importantly, there is still a serious lack of available information in particular for compound  
14 classes other than the *n*-alkanes. Therefore, we urgently call for the consideration of more  
15 holistic approaches determining various parameters during sampling as well as using as many  
16 compound classes as possible.

17

## 18 **1 Introduction**

19 Since more than a century, various compounds deriving from the substance class of lipids,  
20 which are operationally defined as soluble in organic solvents, but not or to a limited degree  
21 in water, have been investigated in plant and soil science. Some of the earliest publications in  
22 plant science date back to the first half of the 19<sup>th</sup> century (Liebig et al., 1837; Wöhler F. and  
23 Liebig, 1839) and in soil science to the early 20<sup>th</sup> century as already reviewed by Stevenson  
24 (1966). One of the main interests to study lipids apart from the general understanding of the  
25 human diet was the large heterogeneity of compounds included in this substance class. Some  
26 of the individual compounds have been described as ‘biomarkers’ or ‘biogenic markers’, i.e.  
27 compounds that “*may be diagnostic of specific organisms, classes of organism, or general*  
28 *biota that contribute organic matter to the atmosphere, aqueous or sedimentary*  
29 *environment*” (Peters et al., 2005). In addition to these contemporary biogenic markers, also  
30 referred to as ‘geochemical fossils’ (Tissot and Welte, 1984), in environmental sciences also  
31 anthropogenic markers and petroleum markers were highlighted by Peters et al. (2005) that  
32 have the ability to be preserved with “no or only minor change” (Tissot and Welte, 1984).  
33 Eganhouse (1997) summarized the principal criteria for a specific marker as follows:



1 *"Molecular markers must be typical for specific sources and characterized by their*  
2 *conservative behavior in environmental archives"*. In other disciplines such as medicine and  
3 toxicology a variety of *"medical signs, symptoms, biomarkers, surrogate endpoints, clinical*  
4 *endpoints, validation"* is used under the umbrella biomarker (Strimbu and Tavel, 2010).  
5 Because *sensu strictu* the term biomarker has been used for the differentiation of biological  
6 tissues of different origin in environmental matrices, during the recent years the term  
7 'molecular proxy' has become more frequently used. This term allows for an inclusion of  
8 biomarkers *sensu strictu* as individual compounds characterizing specific biogenic sources,  
9 but also individual compounds acting as specific proxy e.g. for anthropogenic impact or  
10 thermal alteration. Furthermore, it accommodates the use of groups of compounds used in the  
11 before mentioned way. Finally, it implies the use of molecular ratios of compounds like the  
12 carbon preference index (CPI) or the average chain length (ACL) that could also be indicative  
13 for biogenic sources, alteration or overprint of organic matter. Therefore, in the present work  
14 we use the term molecular proxy rather than biomarker.

15 In its broadest sense, molecular proxies allow determination of the presence, absence, or  
16 certain characteristics of a (set of) molecule(s) that are indicative for a process in, or state or  
17 composition of a system of interest. For instance, in the clinical sciences molecular proxies  
18 among other applications are used as indicators of the presence of a disease or response to  
19 treatment (Brennan et al., 2013; Van Bon et al., 2014); in toxicology to assess the effect of  
20 toxicant exposure on biota (Clemente et al., 2014); in the forensic sciences to link suspects to  
21 a crime scene (Concheri et al., 2011); in limnology to examine past lacustrine environmental  
22 conditions (Castañeda and Schouten, 2011); and in organic geochemistry to follow oil  
23 formation and translocation in source and reservoir rocks (Curiale, 2002).

24 Also in soil science, molecular proxies have been used for decades, and their application has  
25 exponentially increased in the last decade as indicated by the number of related articles  
26 published in Web of Science indexed journals (Table 1). Compared to the overall timeframe  
27 covered by Scopus, between 23 % (pentacyclic triterpenoids) and 99 % (GDGTs = glycerol  
28 dialkyl glycerol tetraethers) of the publications using molecular proxies in soil science have  
29 been published in the last ten years (2006-2015). On average ( $\pm$  SEM)  $59 \pm 4$  % of the  
30 publications with the respective keyword selections have been published in the last decade.  
31 This clearly illustrates a strong increase associated by a diversification of the use of  
32 molecular proxies in soil science. The types of molecular proxies used are as diverse as the  
33 field of soil science itself. They range from the use of phospholipid fatty acids to estimate



1 bacterial and fungal biomass in soils (Frostegard and Bååth, 1996), to the application of  
2 preserved retene/caldalene ratios to infer palaeoecological vegetation shifts (Hautevelle et al.,  
3 2006). Also the archives of the molecular proxies in soil sciences that are used are diverse  
4 and, in addition to soils themselves, include lacustrine and terrestrial sediments, peat deposits,  
5 as well as paleosols (Zhang et al., 2006; Bai et al., 2009; Andersson et al., 2011; Berke et al.,  
6 2012). However, in spite of this large variety a limited number of scientific topics can be  
7 discerned that encompass the great majority of molecular proxy application in the soil  
8 sciences. These are:

- 9 • Changes in vegetation composition inferred from extractable and/or ester-bound lipids  
10 of plant origin, and/or their carbon isotopic composition (e.g. Huang et al., 1996; Zech  
11 et al., 2009; Le Milbeau et al., 2013).
- 12 • Changes in climate, i.e. mean annual temperature and/or precipitation inferred from  
13 bacterial membrane lipids and/or the hydrogen isotopic composition of plant-derived  
14 lipids (e.g. Weijers et al., 2006; Krull et al., 2006; Rao et al., 2009).
- 15 • Changes in palaeoelevation inferred from bacterial membrane lipids and/or the  
16 hydrogen isotopic composition of plant-derived lipids (e.g. Sachse et al., 2006; Bai et  
17 al., 2011; Ernst et al., 2013).
- 18 • Changes in human impact or settlement inferred from compound-specific N isotope  
19 analysis or transformation products of plant-derived lipids, e.g. through burning, or  
20 manure derived lipids (e.g. Bull et al., 1999; Eckmeier and Wiesenberg, 2009;  
21 Zocatelli et al., 2012).
- 22 • Contribution of fossil fuel-derived carbon to soil assessed by lipid molecular  
23 composition and compound-specific isotopes (e.g. Lichtfouse et al., 1995; Lichtfouse  
24 et al., 1997; Rethemeyer et al., 2004).
- 25 • Input, transformation and/or decomposition of soil organic matter inferred from or  
26 traced through extractable and/or ester-bound lipids of plant origin and/or bacterial  
27 membrane lipids and/or their carbon isotopic composition. (e.g. Nierop et al., 2001;  
28 Amelung et al., 2008; Hamer et al., 2012).

29 In Table 1 an overview is given of the classes of molecules frequently used as molecular  
30 proxies in soil archives in relation to their application as well as total and recent (last ten  
31 years) publications including the respective keywords.



1 When using molecular proxies to answer research questions in any of the areas identified, in  
2 particular when soils are used as an archive, several constraining factors have to be taken into  
3 account that vary with the type of application and research question to be answered. The most  
4 important ones are:

- 5 i) Variability in the source of plant-derived organic matter, i.e. abundance and  
6 composition of the molecular proxies in different plant species, plant specimens and  
7 plant parts as a result of genetic or life stage variations and/or external factors such as  
8 climate, seasonality or exposure to the sun (e.g. Nørdskov Giese, 1975; Lockheart et  
9 al., 1998; Shepherd and Griffiths, 2006).
- 10 ii) Variability in (relative contribution of) input pathways into the soil, in particular  
11 microbial versus vegetation input, and root versus aboveground biomass input (e.g.  
12 Jackson et al., 1996; Schefuß et al., 2003; Mambelli et al., 2011).
- 13 iii) Transformation and/or (selective) degradation of (some of) the compounds once  
14 present in the soil, when it is not the aim of the study to use the molecular proxies to  
15 study such transformations (e.g. De Leeuw and Baas, 1986; Nguyen Tu et al., 2004;  
16 Andreetta et al., 2013).

17 However, the information about such constraining factors and their impact on the  
18 applicability of molecular proxies is fragmented and scattered over different publications  
19 inside and outside the scientific discipline of soil sciences. For instance, much of the  
20 available information about variation of leaf wax lipid composition is presented in the plant  
21 physiological literature in studies that were not conducted with the application of such lipids  
22 as molecular proxy for past vegetation composition from soil archives in mind (e.g. Tulloch,  
23 1973; Avato et al., 1984; Kim et al., 2007). The fragmentation of the information makes it  
24 difficult for researchers to assess the potential influence of constraining factors on the  
25 application of molecular proxies. It also hinders the identification of hiatuses in the available  
26 knowledge about the constraining factors as well as the designation of potential strategies to  
27 compensate or correct for such constraints.

28 Therefore, the purpose of the present study is to provide a critical review of the current state  
29 of knowledge with respect to the applicability of molecular proxies in soil science,  
30 specifically focusing on the factors constraining such applicability. Based on this we will  
31 identify areas for future research both with respect to the application of molecular proxies in  
32 soil science as well as the constraints thereof.



1 The vastness of the field of molecular proxies forced us to restrict the scope of the present  
2 study. With respect to the molecules to consider, a first restriction was to focus on those  
3 related to the earlier mentioned main areas of application of molecular proxies in soil science.  
4 A second restriction was to focus on the main classes of components as used by several  
5 researchers. Finally, in spite of their common application, we explicitly excluded lignin and  
6 phospholipid fatty acids (PLFA) as lignin was subject of another recent review article  
7 (Thevenot et al., 2010) and PLFAs are considered in such a large set of studies (c.f. Table 1)  
8 that they would require a separate review. Finally GDGTs were excluded because their  
9 application is predominantly in aquatic sediments rather than soils and they have been  
10 recently reviewed (Schouten et al., 2013). This leaves the component classes labeled in bold  
11 in Table 1 to be considered in the present study. Our study is relevant to the application of  
12 compound-specific isotope analysis inasmuch that such analysis is directly affected by  
13 variability and transformation of the underlying molecules. However, we did not explicitly  
14 consider sources and effects of variation of the stable isotope signature of specific molecules  
15 themselves, this being a research area of its own and also subject of recent review by  
16 Diefendorf and Freimuth (2017). Furthermore, when considering application and preservation  
17 of molecular proxies we restricted ourselves to topsoils (i.e. surface soil horizons = A  
18 horizons as defined by the FAO in the Guidelines for soil description (2006)) as archives.  
19 Paleosols as well as pedogenesis have been excluded as their formation and influence on the  
20 preservation of molecular proxies forms an extensive research area in its own right that was  
21 already the subject of another recent review article (Wiesenberg and Gocke, under review).

22

## 23 **2 Source related variability of molecular proxies**

### 24 **2.1 Definition**

25 Source related variability of molecular proxies pertains to intra-species variation in the  
26 abundance of the molecules that are used as proxy. Such variability entails: i) variation in  
27 relative abundance of individual compounds that together constitute the proxy, e.g. of *n*-  
28 alkanes of different chain length in leaf waxes of a certain species; ii) variation in absolute  
29 abundance of the molecules used as proxy either between different specimens or between  
30 different parts of a single specimen. Depending on the research question, intra-species  
31 variability of molecular proxies may be desirable or not. For instance when preserved leaf  
32 wax lipids patterns are used to reconstruct past vegetation composition, the implicit



1 assumption is that the intra-species variability in the source vegetation is small compared to  
2 the inter-species variability. In opposite, when the  $\delta^2\text{H}$  signal of preserved leaf wax lipids is  
3 used to reconstruct past precipitation patterns, one assumes that the precipitation induced  
4 intra-species variability in the  $\delta^2\text{H}$  patterns is large.

5 There are two main causes of intra-species variability in molecular proxies: internal variation  
6 related to genetics and/or ontogeny; and external variation related to the growing  
7 environment. Both are related in the sense that differences in response to environmental  
8 factors are also often genetically determined (Shepherd and Griffiths, 2006). Here we discuss  
9 both causes separately with a third paragraph devoted to studies where combined effects were  
10 examined. For a detailed description of the biomolecular mechanisms of wax genesis and all  
11 potential sources of change, the reader is referred to the review provided by Shepherd and  
12 Griffiths (2006).

## 13 **2.2 Variation related to genetics and/or ontogeny**

### 14 **2.2.1 Wax lipids**

15 Many studies have indicated that the clear genetic control of leaf wax genesis leads to a  
16 significant and meaningful difference in their composition (Shepherd et al., 1995; Shepherd  
17 and Griffiths, 2006). For instance, prompted by the early works in this area (e.g. Eglinton et  
18 al., 1962; Herbin and Robins, 1968; Herbin and Robins, 1969), Maffei performed an  
19 extensive evaluation of the *n*-alkane patterns in several hundreds of plant species belonging  
20 to the Gramineae, Umbelliferae, Cruciferae, Leguminosae, Cactaceae, Pinales, Lamiaceae,  
21 Boraginaceae, Verbenaceae, Lolaneaceae and Scrophylariaceae (Maffei, 1994; Maffei,  
22 1996a; Maffei, 1996b; Maffei et al., 1997; Maffei et al., 2004). These studies were  
23 replenished by those on Styracacea (Li et al., 2013), Moraceae (Sonibare et al., 2005), and  
24 Clusiaceae (Medina et al., 2004; Medina et al., 2006). Further, Dove et al. (1996) described  
25 the alkane diversity among a grassland plant community, which enables tracing of the diet of  
26 grazing animals due to the different alkane compositions of the plants. Recently, Mueller-  
27 Niggemann and Schwark (2015) were able to differentiate rice from alternating crop plants  
28 based on their *n*-alkane patterns. The results support the chemotaxonomic discriminatory  
29 power of *n*-alkane patterns at family, sub-family and tribal level, which has been further  
30 examined by Diefendorf et al. (2017). Examining plant *n*-alkane and *n*-alcohol distribution of  
31 37 C<sub>4</sub> grasses, Rommerskirchen et al. (2006) also found chemotaxonomic differentiation was





1 possible at the sub-family level. Mongrand et al. (2001) examined the fatty acid composition  
2 of the leaves of over 137 species of gymnosperms belonging to 14 families and collected  
3 from different locations in France. They found a taxonomically meaningful clustering into  
4 four main groups, with the highest discriminatory power in the Pinaceae at the genus level  
5 (Mongrand et al., 2001). Additionally, Wiesenberg and Schwark (2006) determined  
6 differences in the fatty acid composition between temperate C<sub>3</sub>- and C<sub>4</sub>-crops. Within the  
7 same *Brassica* species of kale and swede Shepherd et al. (1995) observed a difference in  
8 chain length distribution of wax lipids between two genotypes of the same species, indicative  
9 of genetic control through variation in the enzyme system. Also for the isoprenoids, a  
10 genetically driven discriminatory power related to (groups of) plant species is attributed  
11 (Ohsaki et al., 1999; Jansen et al., 2007). However, an important issue is the phenotypic  
12 plasticity of the genetic variability in leaf wax lipid patterns found and the implications  
13 thereof for the stability of the patterns observed.

14 Maffei et al. (2004) concluded that phenotypic plasticity may overcome genetic variability,  
15 particularly when plant developmental stages are considered along with abiotic and biotic  
16 stress conditions. Several plant physiological studies have focussed on wax lipid composition  
17 related to plant life stage, and report different results. Avato et al. (1984) found that where the  
18 relative contribution of *n*-fatty acids, *n*-alcohols and *n*-alkanes differed between *Sorghum*  
19 seedlings and mature leaves, the chain-length distribution within a component class remained  
20 the same for the *n*-alkanes and *n*-alcohols. Giese (1975) observed a difference in homologue  
21 dominance of *n*-alkanes between leaves of seedlings and mature barley plants. Also Herbin  
22 and Robins (1969), Dyson and Herbin (1970), Baker and Hunt (1981), and Zhang et al.  
23 (2004) identified increasing chain length dominance of leaf wax alkanes with increasing leaf  
24 age. However, averaging of sampling over leaves of different age, position etc. within a stand  
25 of trees did allow for distinction from other stands, indicating that inter-species variation was  
26 larger than intra-species variation (Dyson and Herbin, 1970). Baker and Hunt (1981)  
27 observed differences between adaxial and abaxial parts of leaves for some of the plant  
28 species. Also Tulloch (1973) observed a variation of leaf waxes of several *Triticum* species  
29 with age. In particular the whole plant *n*-alkane predominance shifted from C<sub>31</sub> at 24 days  
30 after germination to C<sub>29</sub> at 100 days after germination (Tulloch, 1973). Furthermore,  
31 Wiesenberg et al. (2004; 2012) and Wiesenberg and Schwark (2006) observed changes in *n*-  
32 alkane and *n*-fatty acid compositions of a variety of temperate crop species with plant age.  
33 Other publications reported seasonal variations in the *n*-alkane composition for variety of



1 pasture and crop plants by Dove et al. (1996), Hellgren and Sandelius (2001), Moseley  
2 (1983), Shelvey and Koziol (1986) and various trees especially by Gülz and collaborators  
3 (Prasad and Gülz, 1990; Gülz et al., 1991; Gülz and Muller, 1992; Gülz and Boor, 1992).  
4 Variations in the alkane composition could be observed during the growing season among all  
5 investigated plants, but general trends of increasing or decreasing chain length and *n*-alkane  
6 contents have not consistently been determined. The *n*-alcohol predominance also varied but  
7 to a much smaller extent, not affecting the predominance of a specific *n*-alcohol (Tulloch,  
8 1973). Esters gradually showed an increase in esters of trans 2,3-unsaturated C<sub>23</sub> and C<sub>24</sub>  
9 acids with plant age (Tulloch, 1973). The variation was related to the development of the  
10 plant, in particular that of flag leaves and sheets between 55 and 66 days (Tulloch, 1973).  
11 Seldomly, also different source locations were analysed for their lipid composition, where the  
12 plants could have developed specific lipid patterns. Kreyling et al. (2012) described  
13 differences in the *n*-fatty acid and *n*-alkane composition of the same plant species originating  
14 from different regions across Europe with different climatic conditions most likely due to  
15 biosynthetic adaptation to the specific conditions.

16 In contrast to the previous, Li et al. (1997) studied the influence of ontogeny on leaf wax  
17 lipids (*n*-alkanes, *n*-aldehydes, *n*-alcohols, esters,  $\beta$ -diketones, flavonoids and triterpenoids)  
18 in several *Eucalyptus* species of the subgenus *Symphyomyrtus* on Tasmania, and found no  
19 significant effect of ontogeny on leaf wax composition, which they found to clearly and  
20 consistently differ between species (Li et al., 1997). Also Eglinton et al. (1962) observed that  
21 the *n*-alkane composition of leaf waxes of 74 species of *Crassulacea* from the Canary Islands  
22 showed no appreciable variation with respect to leaf position, age, size or specimen. Further,  
23 Bush and McInery (2013) found no influence of canopy position or sampling time on the *n*-  
24 alkane patterns of mature leaves from 24 tree species.

### 25 **2.2.2 Cutin and suberin monomers**

26 Cutin forms the molecular frame of the plant cuticle, whereas suberin is a cell wall  
27 component of cork cells (Kolattukudy, 1981; Kögel-Knabner, 2002). As a result cutin occurs  
28 mainly in the leaves of plants whereas suberin occurs on the outside of stems and roots of  
29 woody plants, as well as in the endodermis and bundle sheet cells of grasses (Kögel-Knabner,  
30 2002). Cutin and suberin monomers are mainly used as proxies to distinguish leaf from root  
31 input in soils (Schreiber et al., 1999; Bull et al., 2000; Mendez-Millan et al., 2011) or as  
32 proxy for related phenomena such as the degree of bioturbation in the topsoil (Nierop and



1 Verstraten, 2004). Therefore, the possible (onto)genetic effects on cutin and suberin  
2 composition are a concern if they were to alter the composition of the polyesters to such an  
3 extent that the separation between cutin and suberin is compromised.

4 Some general observations in literature are that long-chain even numbered  $C_{20}$ - $C_{30}$   $\omega$ -hydroxy  
5 fatty acids and  $\alpha,\omega$ -alkanedioic acids mainly originate from suberin, whereas shorter chained  
6  $C_{16}$  and  $C_{18}$   $\omega$ -hydroxy fatty acids mainly derive from cutin (Schreiber et al., 1999; Otto et  
7 al., 2005; Mendez-Millan et al., 2011). However, several publications challenge the universal  
8 applicability of such general observations, indicating instead that genetic variability results in  
9 many exceptions to such general rules. For instance, Hamer et al. (2012) found that  $\omega C_{22:0}$ ,  
10  $\omega C_{24:0}$  and  $\omega C_{26:0}$  hydroxy fatty acids were not exclusively associated to roots, but also  
11 occurred in the shoots of several species. In addition,  $\omega C_{16:0}$  and  $\omega C_{18:0}$  fatty acids were not  
12 exclusive to the leaves, but also occurred in the roots of several species.

### 13 **2.3 Variation related to environmental factors**

#### 14 **2.3.1 Effects of temperature**

15 Increased solar radiation levels are generally reported to lead to higher absolute amounts of  
16 waxes produced (Sanchez et al., 2001; Shepherd and Griffiths, 2006). In addition, the  
17 composition of the various component classes of wax lipids, i.e. the relative contribution of  
18  $n$ -fatty acids,  $n$ -alkanes,  $n$ -alcohols etc., has been reported to change. A shift towards lower  
19 chain lengths within different component classes was sometimes found (Shepherd and  
20 Griffiths, 2006). Thus, a positive correlation of long-chain odd  $n$ -alkanes with temperature  
21 was observed (Maffei et al., 1993; Zhang et al., 2004). Also, the abundance of membrane  
22 fatty acids with 16 and 18 carbons can change as a result of temperature (Maffei et al., 1993;  
23 Williams et al., 1995; Matteucci et al., 2011). Often, under heat stress the relative abundance  
24 of  $C_{16:0}$  fatty acid was found to increase and vice versa the abundance of polyunsaturated  
25  $C_{18:3}$  fatty acid to decrease (Larkindale and Huang, 2004; Bakht et al., 2006). Furthermore,  
26 effects of temperature were observed for mono- and sesquiterpenes, with compounds like  
27 limonene and myrcene having a close correlation with temperature, whereas others like 1,8-  
28 cineol were not affected by temperature (Maffei et al., 1993). As a cause, a different  
29 sensitivity of individual steps in the genesis of the wax lipid components is assumed  
30 (Shepherd and Griffiths, 2006). However, results were found to vary between different  
31 species and genotypes, indicating a species or genotype related sensitivity to changes in  
32 irradiation (Shepherd and Griffiths, 2006), whereas cold- or heat-acclimated plants respond



1 differently than those that are not acclimated (Larkindale and Huang, 2004). Thus, a  
2 dependency of temperature and lipid metabolism is widely observed, but especially in plants  
3 other factors such as humidity or greenhouse gas composition might coincide with a larger  
4 effect on the overall lipid composition.

### 5 **2.3.2 Effects of humidity**

6 With respect to the effects of water stress and/or high humidity, in their review Shepherd and  
7 Griffith (2006) reported mixed results, with respect to absolute amounts as well as chain  
8 length distribution. Bondada et al. (1996) reported an increase in absolute amounts of  
9 epicuticular wax production by 69% in the leaves of cotton (*Gossypium Hirsutum* L.) under  
10 water stress, which was confirmed by Hamrouni et al. (2001), Koch et al. (2006), Kim et al.  
11 (2007), and Bettaieb et al. (2010) for neutral lipids of other plant species. However, Kim et  
12 al. (2007) found that water stress had only a minor effects on chain length distribution. The  
13 relative contribution of different component classes to the wax composition remained  
14 unchanged except for *Brassica oleracea* at the highest relative humidity, which showed an  
15 increased contribution of ketones and primary alcohols and a reduction of secondary alcohols  
16 and aldehydes (Koch et al., 2006). Recently, Srivastava et al. (2017) determined that  
17 sustainable effects of drought on plant lipid composition are commonly missing with few  
18 exceptions for perennial plants. Thus, several months after exposure to drought the lipid  
19 biosynthesis and composition of leaves is resilient. The existing data shows that general  
20 effects of drought on plant lipid composition are difficult to draw.

### 21 **2.3.3 Effects of increased CO<sub>2</sub>**

22 Changes in greenhouse gases such as CO<sub>2</sub> have also been discussed to influence the lipid  
23 biosynthesis and thus the lipid composition of plants. Short-term exposure of several hours to  
24 elevated CO<sub>2</sub> concentrations e.g. during <sup>13</sup>CO<sub>2</sub> or <sup>14</sup>CO<sub>2</sub> labelling experiments has no or little  
25 effect on the lipid composition, especially if sampling occurs several days after labelling  
26 (Wiesenberg et al., 2009). In contrast a long-term rise in atmospheric CO<sub>2</sub> concentration has  
27 been investigated in laboratory or free air carbon dioxide enrichment (FACE) experiments  
28 (Ainsworth and Long, 2005). Although numerous such experiments have been maintained in  
29 the meantime, implication of investigations of lipid composition is limited. Greenhouse  
30 experiments showed that elevated CO<sub>2</sub> concentration affects the relative composition of  
31 saturated and unsaturated fatty acids in wheat plants (Williams et al., 1994; Williams et al.,  
32 1995; Williams et al., 1998). However, rising nitrogen fertilization and rising temperature can



1 lead to competing trends so that with elevated temperature and nitrogen fertilization  
2 (Williams et al., 1995; Griepentrog et al., 2016). Although specific abundances of individual  
3 long-chain alkanes and alcohols changed under elevated CO<sub>2</sub> concentration, the overall lipid  
4 composition expressed as ACL and CPI did not change (Huang et al., 1999). Nevertheless,  
5 concentration changes like an increase in *n*-alkane and *n*-alcohol abundances and a decrease  
6 in *n*-fatty acid abundance was determined under rising CO<sub>2</sub> concentration, whereas nitrogen  
7 fertilization led to a decrease in the effect (Huang et al., 1999), which was confirmed by  
8 Wiesenberg et al. (2008a) for *n*-alkanes, *n*-fatty acids and *n*-alcohols. In some forest FACE  
9 and open top chamber experiments, the effect of elevated CO<sub>2</sub> on plant lipid concentration  
10 were not identified (Feng et al., 2010; Griepentrog et al., 2015), but the <sup>13</sup>CO<sub>2</sub> labelling  
11 associated with the CO<sub>2</sub> enrichment was used for tracing turnover of lipids in soils as  
12 introduced by Wiesenberg et al. (2008b) for lipids.

#### 13 **2.4 Other or combined genetic, ontogenetic and/or environmental effects**

14 Many studies considered the effects of e.g. geographical location on wax amounts and/or  
15 composition without differentiating between individual genetic or environmental causes.  
16 Again the exact parameters investigated vary greatly between studies, as do the conclusions  
17 drawn. Cowlshaw et al. (1983) examined the *n*-alkane, *n*-alcohol, *n*-aldehydes and ester  
18 composition of composite samples of four species of *Chionochloa*, one of which was sampled  
19 at three different environmental locations to investigate environmental effects. They found  
20 distinct chain length patterns that allowed for chemotaxonomic identification, where variation  
21 between the three sampling sites did not alter dominant chain length patterns for any of the  
22 component classes (Cowlshaw et al., 1983). Similar observations were made by Herbin and  
23 Sharma (1969) for  $\omega$ -hydroxy fatty acid composition of *Pinus* species from Asia, Europe,  
24 North-America, Central America and the Caribbean. On the other hand, Piervittori et al.  
25 (1996) found that the distribution of C<sub>25</sub>, C<sub>27</sub>, C<sub>29</sub> and C<sub>31</sub> *n*-alkanes in *Xanthoria parietina*  
26 varied significantly between two different Piedmont valleys in Italy, and within those with  
27 altitude, reflecting a combined influence of elevation, water availability, radiation and  
28 temperature. For plaggen ecosystems Kirkels et al. (2013) also observed a significant  
29 variability in reported ratios of the dominant *n*-alkanes with chain lengths C<sub>27</sub>, C<sub>29</sub>, C<sub>31</sub>, C<sub>33</sub>  
30 most likely attributable to the causes examined here. However, in spite of this they found  
31 meaningful clustering of the three different plant groups grasses, shrubs and trees indicating  
32 that the variability did not obliterate the power of distinction (Kirkels et al., 2013). In a larger



1 study based on 2093 observations from 86 sources of plant material, Bush and McInerney  
2 (2013) concluded that the general observation that C<sub>27</sub> and C<sub>29</sub> *n*-alkanes are dominant  
3 markers for woody vegetation and C<sub>31</sub> for graminoids does not rigorously hold true. At the  
4 same time C<sub>23</sub> and C<sub>25</sub> *n*-alkanes do seem to be robust indicators of *Sphagnum* (Bush and  
5 McInerney, 2013) as already observed by Baas et al. (2000) and Pancost et al. (2002). Bush  
6 and McInery (2013) indicated that the lack of rigour of the mentioned proxies is likely caused  
7 by environmental conditions as indicated by a shift in patterns across African savannah and  
8 rainforest environments.

9 The distinction between African savannah and rainforest environments in general and C<sub>3</sub>  
10 versus C<sub>4</sub> vegetation in particular have been the subject of more detailed research. Vogts et  
11 al. (2009) studied the leaves and sometimes whole plants of 24 African rain forest and 45  
12 savannah species. They found that as a result of environmental influence, including  
13 temperature and aridity, chain length distributions of the *n*-alkanes and *n*-alcohols of some  
14 species shifted to different chain length predominance. The environmental influences  
15 overshadowed a taxonomic distinction at the order, family or sub-family level (Vogts et al.,  
16 2009). Patterns in grasses were more consistent and thus less dependent on environmental  
17 factors (Vogts et al., 2009). As a result, in spite of the environmental variability observed,  
18 Vogts et al. (2009) found that by averaging lipid patterns within a given environment a clear  
19 distinction between rain forest and savannah plants can be made, with a dominance of C<sub>29</sub> *n*-  
20 alkane representative of the average rain forest plant signal and a dominance of C<sub>31</sub> *n*-alkane  
21 of the savannah plants and C<sub>4</sub> savannah grasses. For the *n*-alcohols, C<sub>28</sub> dominated on  
22 average for savannah plants, C<sub>30</sub> for rain forest plants and C<sub>32</sub> for C<sub>4</sub> savannah grasses (Vogts  
23 et al., 2009).

24 Rommerskirchen et al. (2006) observed a generally higher content of C<sub>31</sub> and C<sub>33</sub> *n*-alkanes  
25 and therefore higher ACL value in African C<sub>4</sub> grasses with respect to C<sub>3</sub> grasses from the  
26 same area as a result of the genetic adaptation of C<sub>4</sub> grasses to warm, arid habitats. In  
27 addition, *n*-fatty acid patterns have also been shown to vary with C<sub>3</sub> and C<sub>4</sub> metabolism, with  
28 C<sub>3</sub> crops having relatively large proportions of C<sub>24</sub> *n*-fatty acid in leaves, stem and roots as  
29 compared to C<sub>22</sub> and C<sub>26</sub> *n*-fatty acids in C<sub>4</sub> crops (Wiesenberg and Schwark, 2006).

## 30 **2.5 Conclusions and implications regarding source related variability**

31 Already Herbin and Robins (1969) concluded that there is a basic genetic control on the  
32 composition of the wax components, including the alkanes, of plant leaves. However,



1 variable factors associated with age and environment can be superimposed upon the specific  
2 pattern in some cases, while in others the genetically controlled pattern appears to be stable  
3 and unaffected by external influences (Herbin and Robins, 1969). Now, 48 years later, a  
4 much more extensive database has been accrued, albeit with a large emphasis on leaf wax  
5 lipids in general and *n*-alkanes in particular. Nevertheless, the results are still equivocal. On  
6 the one hand, there is ample evidence that genetically driven variability of leaf wax lipid  
7 composition in principle leads to chemotaxonomically meaningful clustering that can form  
8 the basis of the application of leaf wax lipids as molecular proxies. On the other hand, it is  
9 clear that both ontogeny and environmental factors can have a significant and sometimes  
10 dominant influence on lipid composition like e.g. chain length distribution. Matters are  
11 complicated by the fact that much data with respect to the effects of environmental stress  
12 originates from studies where plants were studied for a limited period of time (typically one  
13 growing season), where extreme conditions were artificially imposed. In contrast, the lipid  
14 signal from soil or sediment archives as used in reconstructions typically represents a mixture  
15 of input of decades or longer from plants in various life stages of perennial plants, the  
16 induced diversity of plants by frequent changes of annual plants in managed ecosystems and  
17 the average of natural fluctuations in stress conditions during that time period.

18 In general from what is known to date, the conclusion seems justified that on the one hand  
19 because of genetic and environmental influences there are no unique compounds nor 'golden  
20 ratios' of different chain lengths of compounds that can always be linked to certain plants  
21 under all circumstances. On the other hand, there are many situations where the influence of  
22 genetic and environmental effects are small enough that they do not prevent the use of plant  
23 lipids as molecular proxies. The currently available data does not allow for objective,  
24 quantitative rules to be formulated in this respect. From the plant wax components, the *n*-  
25 alkanes are the dominant class studied. In addition, research attention has focussed to a lesser  
26 extent on *n*-alcohols and *n*-fatty acids. The other wax components such as isoprenoids and  
27 ester bound lipids received hardly any research attention to date with respect to source related  
28 variability in the context of their use as molecular proxies. Yet even for the *n*-alkane patterns  
29 in leaf waxes, only a tiny portion of dominant plant species on the planet have been examined  
30 in detail for the effects of genetics and environment on their amounts and patterns. It is clear  
31 that much more research is needed in this respect.

32 Based on the current insights it seems prudent to explicitly take the possibility of genetically  
33 and environmentally driven variability of lipid patterns into account when considering the use



1 of lipids as molecular proxies. For instance by considering plant species from the same  
2 climatic zone as where the reconstruction takes place, and by mixing plant material from  
3 different life stages to obtain the average molecular fingerprint to look for.

4

### 5 **3 Input pathway related variability of molecular proxies**

#### 6 **3.1 Definition**

7 Here we discuss differences in the amount and composition of molecules used as proxies,  
8 which is possible due to different input pathways of such molecules to the soil. A schematic  
9 representation of the different input routes of molecular proxies into the soil is provided in  
10 Fig. 1. The emphasis lies on potential effects for their use as molecular proxies. For a general  
11 description of the different molecular origins of organic matter in soil, the reader is referred  
12 to a dedicated review on this topic by Kögel-Knabner (2002).

#### 13 **3.2 Leaf versus root input**

14 Conservative estimates calculate roots to represent 33% of global annual net primary  
15 productivity (Jackson et al., 1997), whereas more recent studies highlight that the  
16 contribution of root-derived organic matter in soils can account for >70% of total plant-  
17 derived carbon (Rasse et al., 2005). As a result, roots form a considerable input of organic  
18 matter in soils and are proposed to improve carbon storage in soils (Kell, 2012). In addition,  
19 root input occurs to considerable depth in soils, ranging from an average depth of 0.5m in  
20 tundra biomes to 15.0m in tropical grassland/savannah (Canadell et al., 1996). But also in the  
21 temperate zone under certain circumstances such as the presence of nutrient rich fossil A  
22 horizons at depth, deep rooting can be very significant (Gocke et al., 2015). However, on  
23 average the majority of root biomass appears to be incorporated in the top 30 cm of the soil in  
24 most biomes, i.e. in the topsoil (Jackson et al., 1996). The ratio of root/shoot biomass input is  
25 also very variable across biomes, ranging from an average of 0.10 in cropland to 4.5 in  
26 deserts (Jackson et al., 1996). Table 2 represents an overview of the average maximum  
27 rooting depth, root biomass input in the first 30 cm of the soil and root/shoot biomass input  
28 for different biomes (see also Fig. 1).

29 Therefore, if the molecules to be used as proxy are present in both leaves and roots of plants,  
30 the possibility of root input is a factor that has to be considered depending also on the





1 purpose of the proxy. In the case of cutin and suberin monomers root input does not cause  
2 interference as discerning root from leaf input is the specific purpose of this molecular proxy  
3 (Mendez-Millan et al., 2011). However, this may be different for the wax lipids, i.e. *n*-  
4 alkanes, *n*-alcohols, *n*-fatty acids and isoprenoids, that have been found to occur in leaves as  
5 well as roots of species at varying concentrations (Jansen et al., 2007; Huang et al., 2011).  
6 Particularly when such wax derived lipids are applied as molecular proxies for vegetation  
7 cover in soil, root input can be an issue for two reasons: i) roots may contain a different wax  
8 lipid composition than leaves qualitatively and quantitatively, thereby clouding the leaf signal  
9 (Jansen et al., 2006; Martelanc et al., 2007); ii) young root input at depth may disrupt the  
10 chronology of a reconstruction in time by overprinting the originally present signal (Lavrieux  
11 et al., 2012; Gocke et al., 2014).

12 The main discussion with respect to the influence of root input in wax lipid based  
13 environmental reconstructions from soils therefore revolves around assessing the relative  
14 importance of root versus aboveground biomass input. Since plant wax lipids reside on the  
15 outer parts of leaves and roots, relative surface area and bioproductivity are important. On a  
16 global scale root surface area is almost always calculated to be higher than leaf surface area,  
17 more than an order of magnitude so in grasslands (Jackson et al., 1997). However, in many  
18 cases the absolute amount of lipids present per mass unit of root material is an order of  
19 magnitude or more lower than on leaf material (Marseille et al., 1999; Zech et al., 2011). The  
20 concurrent influence of such various factors makes the impact of root input a complex issue  
21 that still is subject of scientific debate (Wiesenberg and Gocke, 2013).

22 Given that different factors will have a highly variable influence in different situations, no  
23 general conclusion can be drawn. In some situations, the influence of roots as input pathway  
24 of extractable lipids to be used as molecular proxy may be limited (Quenea et al., 2006). In  
25 others, root input may be dominant (Van Mourik and Jansen, 2013). In addition, the relative  
26 degree of influence may vary greatly with depth leading to the concurrent presence of leaf  
27 lipid dominated and root lipid dominated zones at different depths in the same profile (Angst  
28 et al., 2016).

### 29 **3.3 Microbial input**

30 In general, microbial biomass can be a significant source of soil organic matter, with up to  
31 40% transformed to non-living soil organic matter, but is turned over much faster than plant  
32 residues (Miltner et al., 2012). Focussing specifically on lipids, isotopic studies show that



1 90% of fatty acids of microbial origin are turned over rapidly after cell death, whereas the  
2 majority of biomass derived residual bulk C was stabilized in the non-living soil organic  
3 matter pool (Kindler et al., 2009). In spite of the potentially shorter residence time, a  
4 concurrent faster production makes that microorganism derived molecules are a factor to  
5 consider when applying molecular proxies in soils except when such proxies are used to  
6 study microbial input.

7 For wax lipids generally *n*-alkanes, *n*-alcohols and *n*-fatty acids with longer chain lengths  
8 (>C<sub>20</sub>) and a distinct odd-over-even (*n*-alkanes) or even-over-odd (*n*-alcohols and *n*-fatty  
9 acids) chain length predominance are considered to be higher plant derived, whereas shorter  
10 chain length homologues are considered to be predominantly of microbial origin (Eglinton et  
11 al., 1962; Diné et al., 1990). Moreover, with the exception of an abundance of C<sub>16</sub> and C<sub>18</sub> *n*-  
12 alcohol and *n*-fatty acid, such microbial lipids are described to lack a specific chain length  
13 predominance (Stevenson, 1994; Lichtfouse et al., 1995). However, several researchers  
14 challenge the observation that higher chain length lipids in soils are exclusively of higher  
15 plant origin. Microorganisms have been shown capable of synthesizing higher chain length  
16 straight-chain lipids, albeit usually to a limited extent (Ladygina et al., 2006; Nguyen Tu et  
17 al., 2011). Jambu et al. (1978) indicated that while chain lengths >C<sub>20</sub> in soils are  
18 predominantly plant derived, particularly in acidic soils fungi may contribute such lipids as  
19 well. Furthermore, Marseille et al. (1999) observed an abundance of C<sub>25</sub> and C<sub>27</sub> *n*-alkanes  
20 that they also attribute to *in-situ* production by fungi. This was confirmed for an agricultural  
21 soil by Quenea et al. (2006), who observed old forest and fungi derived odd long-chain  
22 alkanes based on compound-specific isotope analysis and lipid distribution patterns. Possible  
23 pathways of *in-situ* genesis of *n*-alkanes in soils are reduction of *n*-alkenes and *n*-alcohols,  
24 decarboxylation of bacterial *n*-fatty acids as well as degradation of biopolymers containing  
25 aliphatic side chains (Lichtfouse et al., 1998). Nevertheless, based on the large number of  
26 studies where typical higher plant derived patterns of lipids are reported and used in soils  
27 (Table 1), including indicative ACL and CPI values, microbial input of longer chain length  
28 straight-chain lipids generally does not seem to be a major factor compared to direct plant  
29 derived input in the topsoil (Jansen and Nierop, 2009; Bai et al., 2009). In contrast, for  
30 steroids and triterpenoids such as camosterol, stigmasterol and lupeol, microbial input in  
31 soils can be considerable (Naafs et al., 2004). As another example, arbuscular mycorrhizal  
32 fungi derived  $\beta$ -sitosterol is by far the most abundant sterol identified in soils (Grandmougin-  
33 Ferjani et al., 1999).



1 With respect to cutin and suberin monomers, *in-situ* genesis in soils through microbial  
2 transformation of other precursor molecules can be an issue. For instance, oxidation of free  
3 fatty acids could be a source of  $\omega$ -hydroxy fatty acids, whereas microbial  $\beta$ -oxidation of  
4 unsaturated fatty acids and/or mid-chain hydroxy fatty acids may be a source of  $\alpha,\omega$ -  
5 alkanedioic acids, thus clouding the cutin/suberin signal (Naafs et al., 2004)

### 6 **3.4 Airborne input**

7 In addition to *in-situ* production and incorporation of soil lipids, airborne input must be  
8 considered. The distance of airborne transport of larger constituents such as leaves can be  
9 expected to be limited. However, smaller physical forms containing lipids, such as aerosols  
10 and dust particles, can travel substantial distances (Conte and Weber, 2002) thus causing  
11 input of alien molecules that may influence the local signal. This is of special importance  
12 where airborne sediments with low content of organic matter are investigated as in these  
13 environments already low inputs of foreign organic matter can significantly influence the  
14 molecular proxies. Liu et al. (2007) showed that the  $\delta^{13}\text{C}$  signature of sediment organic  
15 carbon in loess deposits of the western Chinese Loess Plateau corresponds to that of dust  
16 sources instead of the local vegetation. While in a study of marine sediment cores along the  
17 Southwest African continental margin, Rommerskirchen et al. (2003) revealed that aerosol  
18 derived input of higher chain-length *n*-alkanes and *n*-alcohols provides a significant signal,  
19 the  $\delta^{13}\text{C}$  signal of which corresponded well with continental C3/C4 plant distribution and  
20 fossil pollen input when prevailing wind patterns were taken into account. However, in this  
21 case, in contrast to vegetated soils, there was no *in-situ* input from higher plant vegetation.

22 Aerosol studies above plant canopies revealed a certain relationship of the plant wax  
23 composition of the present plants, but significant differences from the biomass were observed  
24 for *n*-alkanols and *n*-alkanes (Conte et al., 2003). While the wax molecular composition was  
25 not directly linked between biomass and aerosol, especially the compound-specific isotope  
26 composition ( $\delta^{13}\text{C}$ ) revealed a closer link of both. For Bermuda aerosols it could be shown  
27 that the aerosol compound-specific isotope composition of *n*-alcohols and *n*-acids reflects the  
28 plant wax compound-specific isotope composition as well as the course of the bioproductivity  
29 during the different seasons of the years (Conte and Weber, 2002).

30 In a study of PM<sub>10</sub> aerosols collected during a winter season in Baoij, China, Xie et al. (2009)  
31 found concentrations of C<sub>21</sub>-C<sub>33</sub> *n*-alkanes in the 10-600 ng/m<sup>3</sup> range as a result of intensive  
32 coal burning in the region. In a two year study of PM<sub>10</sub> and PM<sub>2.5</sub> aerosols in urban sites in



1 Nanjing, Wang et al. (2006) observed C<sub>21</sub>-C<sub>33</sub> *n*-alkanes present in the 10-100 ng/m<sup>3</sup> range.  
2 Concentrations of C<sub>21</sub>-C<sub>35</sub> *n*-alkanes in PM<sub>10</sub> aerosols in urban sites in Beijing sampled in all  
3 seasons were even lower (Zhou et al., 2009). In this study also *n*-fatty acids and hopanes were  
4 considered, but were found in small concentrations that, together with the *n*-alkanes,  
5 constituted ca. 3% of the total organic matter in the aerosols (Zhou et al., 2009). In all studies,  
6 the straight chain lipid patterns lacked the odd-over-even chain length predominance typical  
7 of higher plants (Wang et al., 2006; Xie et al., 2009; Zhou et al., 2009). Nevertheless, in a  
8 large survey a clear odd-over-even chain length predominance was found in spite of such  
9 potentially intense aerosol derived input (Rao et al., 2011). This indicates that even in areas  
10 under large aerosol deposition, as in the case of intensive anthropogenic pollution associated  
11 with fossil fuel burning, the effect of aerosol deposition on *n*-alkane patterns in the soil is  
12 limited as a result of the large *in-situ* input via roots and leaves of the local vegetation.

### 13 **3.5 Conclusions and implications regarding input pathway related variability**

14 The diversity of input pathways offers both opportunities and limitations for the use of  
15 molecular proxies. Opportunities arise when different sources can be elucidated using  
16 molecular proxies. Examples are the differences in molecular composition of leaf and root  
17 waxes as used to differentiate between their respective influences, or when aerosol associated  
18 lipids are used for source apportionment of terrestrial plant input in terrestrial or marine  
19 sediments. This can help budgeting organic matter input of different sources and thus  
20 improve (paleo-)environmental interpretations and reconstructions. Limitations are posed  
21 when input through multiple pathways clouds the linkage of a (set of) molecule(s) to a certain  
22 source for which it is to serve as proxy. For instance when linking a suite of straight-chain  
23 lipids to a particular group of plants at a certain site. When looking at the application of  
24 molecular proxies in soils, in particular the assessment of the influence of root derived input  
25 is a challenge that is not always acknowledged. The significance of root derived organic  
26 matter in soils and terrestrial sediments has been neglected for decades and has only been  
27 recently highlighted (Rasse et al., 2005; Rumpel and Koegel-Knabner, 2011). More research  
28 attention is needed to pinpoint how large possible interferences are and how the potential can  
29 be to compensate for them, e.g. through modelling approaches. For instance, the VERHIB  
30 model was designed to unravel the mixed *n*-alkane, *n*-alcohol and/or *n*-fatty acid signal  
31 observed in soils into the most likely combination of plant groups responsible for the original



1 lipid input, treating leaves and roots explicitly as separate entities (Jansen et al., 2010). This  
2 might form a starting point to disentangle leaf and root derived lipid input.

3 Although the aerosol studies so far provide useful information that plant wax components are  
4 transported via aerosols to remote places, other factors like degradation during transport and  
5 integration of regional vegetation patterns may hamper direct source-to-sink relationship of  
6 airborne molecular markers. Nevertheless the overall impact of aerosol borne molecules on  
7 molecular proxy based reconstructions seems to be limited whenever the total abundance in  
8 the soil is high.

9

#### 10 **4 Transformations and turnover in soil**

11 Transformations and turnover of soil organic matter are an important study area in their own  
12 right (Kögel-Knabner, 2002; Von Lützow et al., 2008). Important in the context of the  
13 application of molecular proxies is the recent paradigm shift to the attribution of external  
14 factors as drivers of soil organic matter turnover rates as opposed to inherent recalcitrance  
15 related to molecular structure (Schmidt et al., 2011; Lehmann and Kleber, 2015). Coupled to  
16 this are indications that microbial recycling of organic matter upon entering the soil  
17 decouples the molecules from their biological sources (Miltner et al., 2012; Gleixner, 2013).  
18 Here, we focus on the effects of (differences in) transformations/degradation of molecules in  
19 soils for their use as molecular proxies. This includes transformations during the stages of  
20 senescence or litter and covers attempts to estimate successive degradation processes of  
21 organic matter occurring after burial until stages of long-term preservation (see also Fig. 1).  
22 All of the attempts dealing with incorporation and preservation of organic matter deal with  
23 different assumptions and entail different problems in terms of uncertainties. Thus, in  
24 dependency of the environmental conditions, assumptions that are relevant for incorporation  
25 and burial of organic matter play a major role, as should the different aspects of degradation  
26 and preservation. However, currently much uncertainty exists regarding the influences of  
27 individual environmental and genetic factors concerning degradation and preservation.  
28 Therefore, the following paragraphs only provide the first insights tackling these issues,  
29 which need further attention in future research projects.

30 Molecular transformations and variations thereof of molecular proxies mostly offer  
31 complicate application of molecular proxies. However, in some instances they may also offer  
32 opportunities. For instance, *n*-alkanes can be degraded to *n*-methyl ketones through  $\beta$ -



1 oxidation (Chaffee et al., 1986; Ambles et al., 1993), which can be used to assess and trace *n*-  
2 alkane degradation in soils (Jansen and Nierop, 2009). Similarly, the presence of certain *seco*-  
3 acids formed through A-ring opening of 3-oxytriterpenoids under anaerobic conditions, may  
4 be used as proxy for the occurrence of such anaerobic episodes (Jaffe et al., 1996), e.g. under  
5 stagnant water conditions.

#### 6 **4.1 Differences related to incorporation pathway**

7 The incorporation pathway (Fig. 1) may influence subsequent turnover of molecular proxies.  
8 This includes (differences in) degradation during senescence and/or litter degradation stages,  
9 e.g. due to different input shapes (like root vs. leaf) offer a different degree of physical  
10 protection.

11 In a study of *Ginkgo biloba* leaf wax lipids during the senescence and litter stages, Nguyen  
12 Tu et al. (2003) found limited degradation that did not affect the dominant chain lengths of  
13 alkyl molecular proxies. When comparing different classes of wax lipids they found the *n*-  
14 alkanes to be the most resistant to degradation, followed by the *n*-fatty acids and then the *n*-  
15 alcohols (Nguyen Tu et al., 2003). Also, more in general, in a study of grassland and forest  
16 soils, Otto and Simpson (2005) determined that characteristic patterns of wax lipids and  
17 isoprenoids were preserved throughout the stages between fresh plant material and soil  
18 organic matter. They also determined preferential enrichment of suberin with respect to cutin  
19 monomers in particular in one of the grassland soils (Simpson et al., 2008). This indicated for  
20 example the fact that the former is embedded in woody tissue while the latter is exposed on  
21 leaf surfaces (Simpson et al., 2008) (see also 4.3.3).

22 When looking at bulk organic matter in soils, Rasse et al. (2005) estimated that the main  
23 residence time of root derived organic matter is on average 2.4 times that of shoot derived  
24 organic matter. When comparing cutin and suberin monomers, Andreetta et al. (2013)  
25 described selective preservation of leaf derived monomers in the more acidic and dryer soil,  
26 while in the more fertile soil root derived monomers were preferentially preserved. They  
27 attributed the former to inhibited microbial degradation due to drought and acidity, and the  
28 latter to protection within aggregates. In another study still small differences in degradation  
29 of the same *n*-alkanes that derived from different plants were found, with a slower  
30 degradation of *n*-alkanes derived from more woody roots (Nierop and Jansen, 2009),  
31 although lipids were generally well preserved. Killops and Frewin (1994) reported that



1 persistency of plant cuticles protected their composite isoprenoids from degradation in  
2 mangrove sediments. Similar preservation in soils is also perceivable.

3 More in general, Mambelli et al. (2011) observed root litter, including biomarkers, to be  
4 selectively preserved with respect to needle litter, which was confirmed by Mendez-Millan et  
5 al. (2010) for maize and wheat roots versus shoots. Using isotopic signatures, Mendez-Millan  
6 et al. (2011) were able to quantify and subsequently compensate for such differences in  
7 turnover rate. This further emphasizes the significance of root derived organic matter for  
8 turnover determinations as already discussed by Wiesenberg et al. (2004). In other words, the  
9 relative abundance of roots and the uncertainties in terms of root related overprint in the  
10 rhizosphere and rhizosphere extension entail large uncertainties and strong differences  
11 between different plant species and environmental settings, especially at a molecular level.  
12 Further research is required to enable extrapolations to or across ecosystem scales.

#### 13 **4.2 Differences between different soil compartments**

14 When soils are used as archives of molecular proxies, mostly bulk samples are used and  
15 replication per horizon or stratigraphic layer is often limited or absent. However, several  
16 studies indicate that preservation of molecules used as proxies can differ between different  
17 soil compartments (Flessa et al., 2008; Clemente et al., 2011; Griepentrog et al., 2014).  
18 Depending on the research question this may pose a problem, for instance it might obscure  
19 chronology when molecules are used as proxies to reconstruct changes over time.

20 Already Lichtfouse et al. (1998) showed that straight-chain lipids can become encapsulated in  
21 larger humic polymers, thus being protected against degradation. In addition, physical  
22 protection in (micropores of) aggregates and/or through association with clay minerals have  
23 been identified as important pathways for stabilization of soil organic matter in general,  
24 including molecules used as molecular proxies (Tonneijck et al., 2010). Using bulk and  
25 compound-specific  $\delta^{13}\text{C}$  analysis, Cayet and Lichtfouse (2001) showed that plant-derived *n*-  
26 alkanes in a soil under maize cultivation varied in average age per particle size fraction, with  
27 the  $\text{C}_{31}$  *n*-alkane from the 200-2000 $\mu\text{m}$  fraction being significantly younger than that from the  
28 50-200 $\mu\text{m}$  and 0-50 $\mu\text{m}$  fractions. A general trend of preferential preservation in smaller size  
29 fractions, in particular the clay fraction, is also reported in other studies. For instance, Quenea  
30 et al. (2004) and Flessa et al. (2008) observed longer turnover rates of soil organic matter in  
31 smaller size fractions. Clemente et al. (2011) studied the preservation of long chain aliphatic  
32 compounds in three soils with similar clay mineralogy but different carbon contents and



1 standing vegetation. Irrespective of these differences, they too found the aliphatic compounds  
2 to be preferentially preserved in the silt and clay fractions, and again linked this to strong  
3 interactions with the present clay minerals. In a recent study, Griepentrog et al. (2015, 2016)  
4 confirmed the higher residence time of organic matter in small sized density fractions when  
5 compared to macro-aggregates. This implies an improved preservation of organic matter  
6 associated with higher density and thus mineral association when compared to organic matter  
7 associated to lower density. However, physical fractionation techniques such as particle and  
8 density fractionation have a potential of creating analytical artifacts, especially when  
9 molecular proxies are investigated.

10 In addition, the effects of size or density fractions of soil on preservation of organic matter,  
11 including molecular proxies, are not uniform. For instance, Höfle et al. (2013) found size and  
12 density fraction related organic matter stabilization to be much less pronounced in the active  
13 upper layer than in the deeper soil horizons. This points to selective preservation of organic  
14 matter in the deeper soil because of more extensive aggregation and organo-mineral  
15 association. In a study of volcanic ash soils, Stewart et al. (2011) did not find differences in  
16 preservation of bulk soil organic matter in general or lipids in particular between different  
17 size fractions. They attributed this lack of differentiation to the presence of a large proportion  
18 of the soil organic matter that was not associated with mineral components as these were  
19 already saturated with previously incorporated soil organic matter (Stewart et al., 2011).

20 In general a combination of physical protection and sorptive preservation seems to be  
21 responsible for the observed differences (or lack thereof) in preservation of organic molecules  
22 in soils between different size or density fractions. This is corroborated amongst others by a  
23 study by Guggenberger et al. (1995), where they observed differences in the preservation of  
24 soil organic matter derived from tropical pastures compared to the preceding native savannah  
25 vegetation. They attributed this effect to a difference in interactions with the mineral phase,  
26 leading to physical protection of soil organic matter and molecular proxies contained therein.  
27 Similarly, differences in turnover rates between forest and grass derived molecules after land  
28 use change have been observed as a result of saturation of the adsorption sites on the mineral  
29 phase (Hamer et al., 2012).

30 In addition to heterogeneity in the effects of interactions with the mineral phase on  
31 preservation of molecular proxies, analytical artifacts cannot be completely excluded when  
32 physical and chemical fractionation techniques are applied to separate particle size or density  
33 fractions. To date systematic investigations addressing these issues are lacking, which





- 1 hampers the drawing of general conclusions with respect to processes that are relevant e.g.
- 2 under different climates and for different soil mineralogical composition.

### 3 **4.3 Selective preservation within or between classes of molecules**

4 Turnover rates of molecular proxies do not only vary between different compartments, but  
5 may also vary within the same compartment; between and even within different (classes of)  
6 molecules (Dinel et al., 1990; Bull et al., 2000; Amelung et al., 2008). For instance, Feng and  
7 Simpson (2007) found preferential enrichment of straight-chain lipids as well as cutin and  
8 suberin monomers with increasing depth with respect to bulk soil organic matter. In contrast,  
9 in a study of grain-maize and silage-maize cropped soils Wiesenberg et al. (2004) found  
10 turnover times in the sequence bulk soil organic matter > *n*-alkanes > *n*-fatty acids, with rate  
11 differences that varied substantially between the two cultivations. The differences could be  
12 related to the different biomass input on the one hand and large amount of lignite dust and the  
13 low biomass input on the other hand, thus hampering degradation at this site. The faster  
14 turnover of fatty acids than alkanes as also confirmed by Wiesenberg et al. (2008a) and  
15 Griepentrog et al. (2015; 2016). In contrast, it may also offer opportunities to apply such  
16 differences between molecular classes and their response to external factors to trace  
17 transformations and input of organic matter in soils (Feng and Simpson, 2007).

18 An important issue with respect to the application of straight-chain lipids as molecular  
19 proxies is also preferential degradation of certain chain lengths within a certain class of  
20 molecules, as molecular ratios of various (higher) chain lengths are often used as proxies for  
21 certain vegetation types (see paragraph 2). This issue is addressed in the following  
22 paragraphs.

#### 23 **4.3.1 Straight-chain lipids**

24 Already Moucawi et al. (1981) reported decreasing degradation rates with larger chain-length  
25 for *n*-alkanes in soils, which was confirmed by Lichtfouse et al. (1998) who determined a  
26 higher resistance of long straight-chain biopolymers in soil compared to their shorter chain  
27 counterparts. However, such preferential degradation was found in agricultural and acidic  
28 soils and in the absence of Fe(OH)<sub>3</sub> (Moucawi et al., 1981; Lichtfouse et al., 1998). Similar  
29 results were found for other lipid classes as well (Moucawi et al., 1981). More recently,  
30 several authors also indicate that such preferential degradation can occur in other soils  
31 (Jansen and Nierop, 2009; Cui Jingwei et al., 2010). However, the extent of the effect



1 questions the suitability of the compounds in question as molecular proxies. For instance,  
2 Jansen and Nierop (2009) found the overall effect of preferential degradation on higher plant  
3 derived *n*-alkane patterns in soils to be small and not of influence for their use as vegetation  
4 proxy. Similarly, Lei et al. (2010) determined that in spite of strong evidence of microbial  
5 degradation, relative abundance of long-chain *n*-alkanes could still be used to distinguish  
6 coniferous from broadleaf tree input in soils.

7 Within the group of straight-chain lipids, overall degradation rates of subclasses have been  
8 found to vary depending on soil physicochemical properties. For instance, *n*-alkanes have  
9 been reported to be better preserved in alkaline soils, whereas *n*-fatty acids accumulate in  
10 more acidic soils (Simpson et al., 2008).

#### 11 **4.3.2 Isoprenoids**

12 Isoprenoids are reported to have varying turnover rates both under oxic and anoxic conditions  
13 in soils (Jaffe et al., 1996; Amelung et al., 2008). Generally, sterols, diterpenes and  
14 pentacyclic triterpenes are reported to be turned over rapidly as compared to straight-chain  
15 lipids in grassland as well as forest soils, hindering their application as molecular proxies for  
16 their sources (Bull et al., 2000; Naafs et al., 2004; Jansen et al., 2007). However, Otto and  
17 Simpson (2005) observed the exact opposite trend, indicating a strong environmental control  
18 on the relative transformation rate of different classes of components. In an incubation study  
19 of derived triterpenols, Koch et al. (2005) highlighted marked differences between  
20 degradation rates of individual triterpenols, leading to a sharp relative increase in the  
21 proportion of taraxerol with respect to the other triterpenols.

22 In addition,  $\Delta^5$  sterols are transferred both aerobically and anaerobically to  $5\alpha$ - and  $5\beta$ -stanols  
23 (De Leeuw and Baas, 1986), which are reported to persist much longer in soils than their  
24 precursors (Bull et al., 2000). Simpson et al. (2008) suggest to use the ratio of precursor  
25 sterols to their stanol and stanone degradation products as measure for their degree of  
26 degradation.

#### 27 **4.3.3 Cutin and suberin monomers**

28 Bull et al (2000) observed different degradation rates for different components within the  
29 classes of free and ester bound lipids, depending on soil chemical and physical composition.  
30 However, Otto and Simpson (2006) found degradation of cutin and suberin to take place  
31 without preference for specific constituents. In general, Quenea et al. (2004) described cutin



1 and suberin to be more resistant to degradation than free lipids residing in the same particle  
2 size fraction.

3 In a study of hydrolysable lipids using compound-specific  $^{13}\text{C}$  analysis, Feng et al. (2010)  
4 described mean turnover times for cutin and suberin derived ester-bound lipids of 32-34  
5 years. While slower than for bulk soil organic matter in this system, it was much shorter than  
6 anticipated, leading them to conclude that a large portion of cutin and suberin derived  
7 compounds reside in the non-hydrolysable fraction (Feng et al., 2010).

8 As mentioned earlier (section 4.1), Simpson et al. (2008) observed preferential enrichment of  
9 suberin monomers with respect to cutin monomers, which was confirmed by Mendez-Millan  
10 et al. (2010). In addition to the physical location of suberin versus cutin as potential cause,  
11 Simpson et al. (2008) suggested a higher resistance of suberin to degradation than cutin  
12 owing to a larger content of phenolic units in the former. Mendez-Millan et al. (2010) argued  
13 that microbial degradation, potentially influenced by the access to degradation sites are other  
14 factors influencing the slower turnover of suberin vs. cutin monomers. Regardless of the  
15 mechanism, the general difference in root vs. aboveground biomass derived suberin and  
16 cutin monomers and their individual turnover would clearly influence the application of the  
17 cutin/suberin monomer ratio as proxy for leaf vs. root input.

#### 18 **4.4 Conclusions and implications regarding differences in transformations** 19 **and turnover of molecular proxies in soils**

20 Although available data is limited, it is clear that degradation of organic matter at a molecular  
21 level in terrestrial archives such as soils, paleosols and sediments can significantly influence  
22 the applicability of molecular proxies. As a result it seems useful to explore the possibility for  
23 a correction to improve the determination of paleovegetation and vegetation shifts and other  
24 paleoenvironmental information like paleotemperature and pH. The number of published  
25 approaches to compensate for the influence of degradation on paleoenvironmental  
26 reconstructions is still small. Zech et al. (2009) provided a simple two endmember model  
27 approach to improve paleovegetation reconstruction based on molecular ratios of different  
28 long-chain *n*-alkanes ( $\text{C}_{27}\text{-C}_{33}$ ). Assuming that forest vegetation is dominated by *n*- $\text{C}_{27}$  alkane  
29 and grass vegetation by *n*- $\text{C}_{31}$  and *n*- $\text{C}_{33}$  alkanes, high relative contributions of the respective  
30 homologues of the assumed source vegetation are used as end-members. At the same time the  
31 source vegetation is typically characterized by high odd-over-even predominance of long-  
32 chain *n*-alkanes. On the other hand, soils reveal a low odd-over-even predominance and



1 abovementioned molecular ratios with smaller differences between the different vegetation  
2 types. In theory, the degradation continuum from plant leaves to soils of the respective  
3 vegetation type thus enable the identification of the degradation intensity of an unknown  
4 sample, if the sample is mainly influenced by a single vegetation. If the unknown sample  
5 does not plot on the degradation continuum, but between the different lines of different  
6 vegetation types, the relative contribution of grass vs. tree derived vegetation might be  
7 estimated and also corrected for the vegetation.

8 A slightly different approach was established by Buggle et al. (2010) who also used long-  
9 chain *n*-alkane ratios and the odd-over-even predominance of alkanes for their correction.  
10 While Zech et al. (2009) used correlations and then graphical-based reconstructions, Buggle  
11 et al. (2010) used a calculation based approach. The degradation in the continuum from  
12 recent soils is taken as an analogy and the slope of the regression line is multiplied with the  
13 odd-over-even predominance and the addition of the intercept of a long-chain *n*-alkane ratio  
14 in the crossplot of the ratio with the odd-over-even predominance. By moving the regression  
15 line to an ancient sample set, the end of the regression line yields the former topsoil value of  
16 the molecular ratio and odd-over-even predominance. Variation in the corrected long-chain *n*-  
17 alkane ratio enable the assessment of fluctuations in palaeovegetation.

18 Both mentioned approaches rely on the general differentiation of grass vs. forest vegetation  
19 based on long-chain *n*-alkane composition. As mentioned above such clear distinction of  
20 vegetation types exclusively based on compounds deriving from one compound fraction such  
21 as alkanes might be hampered by various factors such as variability within and between plant  
22 species, thus leading to similar composition of e.g. alkane from coniferous trees and grass  
23 plants (Maffei, 1996b; Maffei et al., 2004). Thus, such simple approaches might be  
24 appropriate only in very well defined settings, where independent records such as pollen data  
25 confirm the composition of specific plant assemblages determined by molecular proxies.

26 The expansion of approaches like the ones mentioned here to a broader range of molecular  
27 proxies is required to receive more complete pictures and to acknowledge the different  
28 turnover and degradation of different substance classes. However, the availability of datasets  
29 on plant and soil chemical composition for substance classes other than the *n*-alkanes are  
30 quite limited, hindering such expanding approaches. Thus, further surveys are required for  
31 other molecular proxies than *n*-alkanes for a high diversity of plants and soils from different  
32 climates. Afterwards, combined studies of more than one substance class enable improved  
33 paleoenvironmental reconstructions, whereas cross-checking with other non-molecular



1 proxies, e.g. fossil pollen data, might be essential, especially if the paleorecord is targeted.  
2 Also the extrapolation of such approaches to different environmental and climatic settings  
3 might be limited as the effects of temperature, moisture, oxygen availability and others  
4 influence the degradation of organic matter as discussed above. Consequently, proper  
5 modelling approaches are required to assess not only palaeoenvironmental changes, but also  
6 to acknowledge and identify degradation of organic matter at a molecular scale.

7

## 8 **5 General conclusions**

9 In this review we considered the three most important constraining factors for the application  
10 of molecular proxies in soil science: i) variability in the molecular composition of plant  
11 derived organic matter as a result of genetic or life stage variations or external environmental  
12 factors; ii) variability in (relative contribution of) input pathways into the soil; and iii)  
13 transformation and/or (selective) degradation of (some of) the molecules once present in the  
14 soil. From the various studies done within and outside of soil science over the last decades  
15 the following general picture emerges. All constraining factors considered can have a  
16 significant influence on the applicability of molecular proxies in soil science. The degree of  
17 influence of the constraining factors strongly depends on the type of molecular proxy as well  
18 as the environmental context in which it is applied. In addition, the research question to be  
19 addressed by application of the molecular proxy has a strong influence. A factor that poses a  
20 constraining factor in one study might offer an opportunity in another. For instance microbial  
21 degradation may constrain the application of molecular soil organic matter composition as  
22 palaeo-vegetation proxy, but may offer the opportunity to study molecular transformation of  
23 soil organic matter in the context of a study of soil carbon cycling. Recently, the first  
24 modelling approaches to potentially compensate for some of the constraining factors,  
25 specifically variability in input pathways and degradation of molecular proxies once in the  
26 soil, have started to emerge. Based on the previous we strongly recommend that the potential  
27 constraining factors are always explicitly considered whenever studies are planned in which  
28 molecular proxies in soils play a role. This review may serve as starting point for gathering  
29 the necessary information to decide, which constraining factors may play a role and how they  
30 can be addressed best. At the same time, it became clear from available literature that much  
31 information about the mentioned constraining factors is still lacking. In particular for  
32 molecular classes other than *n*-alkanes, systematic information is often very scarce. We  
33 therefore strongly appeal to the soil scientific community to address this knowledge gap. Also



1 for this our review may serve as a starting point with future applicability in soil science and  
2 furthermore in paleopedology.

3

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## 1 Tables

2 Table 1: Compounds frequently used as molecular proxies in soils

| Compound (the ones considered in this review indicated in <b>bold</b> )                                      | Most commonly used as proxy for:   | Examples of recent publications <sup>a</sup> :  | Number of articles published until 2017 ( <i>publications 2007-2016</i> ) <sup>b</sup>   |
|--|--|---|--|
| Molecules of plant origin  |  |   |  |
| <b><i>n</i>-alkanes, <i>n</i>-alcohols (<i>n</i>-alkanol), <i>n</i>-fatty acids (<i>n</i>-alkanoic acid)</b> | (groups of) plant species  | (Zhang et al., 2006; Zeng et al., 2011; Jansen et al., 2013; Gocke et al., 2013)          | alkane: 1588 (1025)<br>alcohol: 1972 (1123);<br>alkanol: 18 (11)<br><i>n</i> -fatty acids: 43 (27); <i>n</i> -alkanoic acid: 67 (41) |
| <b><i>n</i>-methyl ketones</b>   | degradation/transformation of soil organic matter                                      | (Bai et al., 2006; Jansen and Nierop, 2009; Lei et al., 2010)                             | methyl ketone 104 (50)   |
| <b>plant sterols and pentacyclic triterpenoids</b>   | (groups of) plant species  | (Volkman, 2005; Jansen et al., 2007; Lavrieux et al., 2011)                               | plant sterol: 1682 (590)<br>pentacyclic triterpenoid: 25 (10)  |
| lignin monomers  | coniferous species vs. broadleaf species vs. grasses and organic matter transformation | (Dignac et al., 2005; Nierop et al., 2006; Heim and Schmidt, 2007; Thevenot et al., 2010; | lignin monomer: 115 (74)   |



|   |   |   |   |
|---|---|---|---|
|   |   | Simpson and Simpson, 2012)  |   |
| <b>cutin and suberin monomers</b>   | root vs. aboveground biomass input                        | (Mendez-Millan et al., 2011; Hamer et al., 2012)  | cutin monomer: 25 (17)<br>suberin monomer: 32 (18)                  |
| Molecules of animal or bacterial origin   |   |   |   |
| <b>Manure compounds such as coprostanol, 5<math>\beta</math>-stigmastanol, sitosterol and their epimers</b> | Human impact, animal husbandry                            | (D'Anjou et al., 2012; Birk et al., 2012)   | coprostanol: 35 (17)<br>stigmastanol: 12 (7)<br>sitosterol: 70 (47) |
| glycerol dialkyl glycerol tetraethers (GDGT)  | mean ambient air temperature, paleo-elevation and soil pH | (Luo et al., 2011; Weijers et al., 2011; Peterse et al., 2012; Ernst et al., 2013; De Jonge et al., 2014) | GDGT: 148 (144)   |
| phospholipid fatty acids (PLFA)   | microbial biomass   | (Kramer and Gleixner, 2006; Kindler et al., 2009; Ngosong et al., 2012; Malik et al., 2013)               | Phospholipid fatty acid: 2157 (1628)<br>PLFA: 1525 (1140)           |
| Compound-specific stable isotope signal of one or more of the   |   |   |   |



| above <sup>c</sup>                  |  |   |  |
|-------------------------------------|--|---|--|
| $\delta^{13}\text{C}$               | C <sub>3</sub> vs. C <sub>4</sub> plants and tracing carbon transformations e.g. by free air CO <sub>2</sub> enrichment (FACE) | (Sun et al., 2005; Feng et al., 2010; Mendez-Millan et al., 2012)               | <sup>13</sup> C: 13 (11)                       |
| $\delta^{15}\text{N}$               | (past) land management   | (Bol et al., 2005; Griepentrog et al., 2014)                                    | <sup>15</sup> N: 2 (2)                         |
| $\delta^2\text{H}$ (deuterium)      | precipitation and paleo-elevation  | (Peterse et al., 2009; Bai et al., 2011; Luo et al., 2011; Sachse et al., 2012) | <sup>2</sup> H: 6 (4)<br>deuterium: 9 (7)      |
| $\Delta^{14}\text{C}$ (radiocarbon) | Age and contamination determination  | Marschner et al., 2008; Mendez-Millan et al., 2014                              | <sup>14</sup> C: 3 (1)<br>radiocarbon: 35 (30) |

1 <sup>a</sup>Published from 2007 until 2017.

2 <sup>b</sup>According to ISI Web of Science, checked for ‘soil’ and ‘target compound’ in the topic of  
3 articles on 27th February 2017 included in all available databases.

4 <sup>c</sup>‘Compound-specific’ and the respective isotope (i.e. <sup>13</sup>C, <sup>15</sup>N, <sup>2</sup>H, and <sup>14</sup>C respectively) were  
5 used as separate keywords in addition to ‘soil’.



- 1 Table 2: average maximum rooting depth, biomass/depth distribution and root/shoot ratios in
- 2 different biomes (Canadell et al., 1996; Jackson et al., 1996)

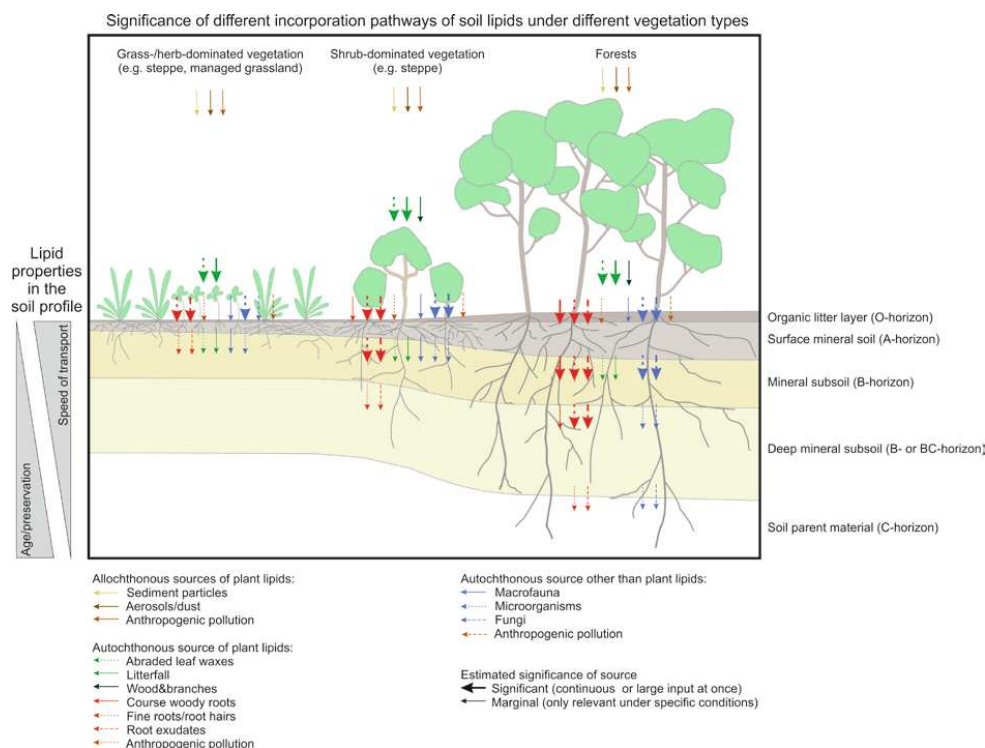
| Biome:                              | Average maximum rooting depth: | Average percentage of roots in the top 30 cm: | Average root/shoot ratio: |
|-------------------------------------|--------------------------------|---|---------------------------|
| Boreal forest                       | 2.0±0.3m                       | 83  | 0.32                      |
| Cropland                            | 2.1±0.2m                       | 70  | 0.10                      |
| Desert                              | 9.5±2.4m                       | 53  | 4.5                       |
| Sclerophyllous shrubland and forest | 5.2±0.8m                       | 67  | 1.2                       |
| Temperate coniferous forest         | 3.9±0.4m                       | 52  | 0.18                      |
| Temperate deciduous forest          | 2.9±0.2m                       | 65  | 0.23                      |
| Temperate grassland                 | 2.6±0.2m                       | 83  | 3.7                       |
| Tropical deciduous forest           | 3.7±0.5m                       | 70  | 0.34                      |
| Tropical evergreen forest           | 7.3±2.8m                       | 69  | 0.19                      |
| Tropical grassland/savannah         | 15.0±5.4m                      | 57  | 0.70                      |
| Tundra                              | 0.5±0.1m                       | 93  | 6.6                       |

3



1 **Figures**

2 **Figure 1**



3

4

5 **Figure caption**

6 Conceptual overview of different incorporation pathways of lipids in soils originating from  
 7 different biological sources and anthropogenic contamination. The different sources are  
 8 indicated by distinct colors and lines of the arrows. The line thickness is an estimated  
 9 significance of individual sources, without providing quantitative measure for different  
 10 sources. Autochthonous sources are further distinguished by their significance in different  
 11 soil depths or soil horizons, respectively. Further, the transport and age/probability of  
 12 preservation as general properties of lipids are given at the left side of the figure.

13